Original Research Article

Histopathological and Oxidative Stress Response of *Oreochromis niloticus* Exposed to Varying Concentrations of Sawdust Extract

ABSTRACT

Persistent dumping of sawdust on water banks and or directly into water bodies as a consequence of sawmilling activities is becoming alarming. This study therefore evaluated the effect of sawdust extract on some selected physiological parameters such as histopathology and oxidative stress response using *O. niloticus* as test organism. *O. niloticus* were divided into four groups based on exposure concentration to sawdust extract (0%, 1/10, 1/100 and 1/1000 of LC_{50} respectively) in triplicates. Heavy metals composition was evaluated using Atomic Absorption Spectrophotometer. Phytochemical analysis of sawdust, physico-chemical studies of the cultured water, histopathological and antioxidant enzyme activities were carried out using standard methods. Result showed the presence of metals such as iron, cadmium, zinc, copper and magnesium as well as the presence of alkaloids, flavonoids, steroid, phenols, terpenoid, saponin and anthraquinone in the sawdust extract. There was no significant difference in the water temperature and pH of the cultured water of *O. niloticus* exposed to the varying concentrations of the sawdust. Dissolved oxygen was however highest in the control water and reduced with increase in the concentrations of sawdust extract exposure. Disintegrated lamella was observed in the intestine and gills, and graded necrosis, in the liver of *O. niloticus* exposed to sawdust extract. There were inhibitions in the activities of superoxide dismutase (SOD) of both liver and kidney of *O. niloticus* exposed to sawdust extract. Activity of malondialdehyde (MDA) however, increased with increased extract concentration. This study has therefore shown that sawdust extract could cause tissue - organ architectural distortions and induce oxidative stress in *O. niloticus*.

Keywords: Physiological, Histopathological, Inhibitions

1. INTRODUCTION

Sawmill industries has dotted water body banks in various parts of the country due to ease to access logs and increased demand. This huge sawmilling operation has consistently been generating wood wastes of different forms. Wood shavings and sawdust have been implicated as sources of water pollution by reducing light penetration of water and weakening the immune system of fish due to inert solids and toxic substances associated to these wastes [1].

Sawdust leaches degrade into some compounds that can be toxic to life. These are phenols and methylated phenols, benzoic acid, terpenes and tripolenes [2]. Heavy metals being components of wood wastes at even low concentration can disrupt the ecological balance of the recipient environment [3]. There is clear scientific evidence that if sawdust as wastes is improperly managed, wood residue can negatively impact the environment, contaminate and destroy fish habitat [4]. However, there is dearth of information on the physiological effects of sawdust on aquatic lives in Nigeria. This study was conceived to scientifically evaluate the level of impairment that may result on exposing fish to wood waste, particularly sawdust. This would help authorities concerned with waste management to develop policies aimed at discouraging the direct dumping of sawdust into natural water bodies.

2. MATERIAL AND METHODS

2.1 SUBSTRATE COLLECTION AND ETHANOLIC EXTRACT PREPARATION

Sawdust was collected from Okobaba sawmill, Lagos State into clean plastic containers (20 x 12 x 10 cm^3) with stoppers. Immediately after they were air dried and returned to the stoppered plastic containers from Okobaba sawmill, Lagos State. "The collected sawdust was sieved using lab test shaker vibration (model GZ -200 with mesh size 7-600M) sieving machine to obtain fine homogenized particles and 0.3kg of the particles was weighed on an electrical weighing balance and soaked in 2.55L of 70% ethanol for 48 hours. The immersed sawdust was filtered using Whatmann's No. 1 filter paper to obtain a clear extract. The alcoholic sawdust extract was placed in a water bath at 80^oC for 2 hours to evaporate the ethanol content completely. The stock solution was kept in a sterile plastic container and stored at 4^0C" [25]. Thirty (30) ml of the sawdust extract was analyzed for its qualitative and quantitative phytochemical composition [5]. The work was carried out in the Biological Science laboratory of the Department of Biological Science, Yaba College of Technology.

2.2 PHYTOCHEMICAL SCREENING OF SAWDUST EXTRACT (QUALITATIVE)

Phytochemical tests were carried out on the alcoholic extract of the sawdust using a standard procedure to identify the constituents.

2.3 MEASUREMENT OF PHYSICOCHEMICAL PARAMETERS OF WATER

Water was collected from the tap source and allowed to dechlorinate over night before use. Water temperature pH, dissolved oxygen, electrical conductivity and nitrates, were measured every 4 days using a Bench top Multi-Analyzer (INE-DZS-708) with the accuracy of ±0.002.

2.4 TRACE/HEAVY METALS DETERMINATION OF SAWDUST SAMPLE

A total of 20g of oven-dried sawdust was weighed using a sensitive weighing scale into separate porcelain crucibles and ashed at 950^0c for 2hrs in a furnace and cooled. Out of the ashed samples, 2g of each was weighed into ten (10) 250ml separate beakers and digested using Aquaregia (a mixture of nitric acid and hydrochloric acid in the ratio 1:3). After which 75ml of distilled water was added to each sample and boiled for 10 minutes. Each sample was then filtered into 100ml volumetric flask and allowed to cool. The volume then made up (Fe, Cd, Zn, Cu and Mg) using an Atomic Absorption Spectrophotometer [6].

2.5 FISH COLLECTION

One hundred and twenty (120) *Oreochromis niloticus* of average weights 3.0g ± 1.81 were purchased from Nigeria Institute of Oceanography and Marine Research (NIOMR), Lagos, for acute toxicity. The fingerlings were acclimated for 14days in 20L carrying capacity rectangular tanks.

2.6 ACUTE TOXICITY STUDIES

Square glass tanks (volume 4.5 litres; 15 × 15 ×15 cm^3) were used as bioassay containers. In all bioassays for fish, test media was made up to two litres of water to hold 6fingerlings per bioassay tanks in triplicates. *O. niloticus* fingerlings were placed in sawdust extract of varied concentrations (6ml/l, 7ml/l, 8ml/l and 9ml/l) [7]. The concentrations needed to kill all *O. niloticus* fish were found to be in the range of 2ml/l, 3ml/l, 4ml/l and 5ml/l. The difference in concentrations of Sawdust extract used for acute toxicity was based on range findings.

2.7 CHRONIC TOXICITY STUDIES (SUBLETHAL TOXICITY STUDIES)

Rectangular glass tanks (50cm by 36cm by 34cm) were used as bioassay containers. In all test media, 8 litres of water with 10 juvenile *O. niloticus* each were used. Different concentrations of the extract being constituents of the test media were obtained in methods adopted by [8] after calculating the 96hr LC_{50} using the fractions 1/10, 1/100, and 1/1000. 96hr LC50. Period of sublethal studies lasted 42 days, and the choice of 42 days was to establish suitable duration period for sublethal studies which is usually minimum of 21 days. All bioassays were in 3 replicates.

2.8 HISTOPATHOLOGICAL ANALYSIS OF THE TISSUES/ ORGANS (INTESTINE, GILLS AND LIVER OF *OREOCHROMIS NILOTICUS*

Gill arch of the right side, liver and intestine of the fishes were collected and accessioned.

Tissues and organs were then grossly examined and fixed in Bouin's fluid for 24 hours, washed in 70% ethanol and dehydrated in graded (10%, 30% and 60%) alcohol [9]. Organs were later cleared in xylene to remove excess alcohol from the tissue. The tissues and organs were thereafter impregnated and embedded in paraffin wax and allowed to solidify. Sections (5μm of thickness) were cut using rotary paraffin microtone and stained using haematoxylin and eosin. Light microscopy was later done by compound microscope and film photographed by Olympus DP – 10 Digital cameras attached to the microscope through a C-connector.

2.9 ANTIOXIDANT AND NON-ENZYMES ACTIVITY OF LIVER, GILLS AND KIDNEY OF *Oreochromis niloticus* Antioxidant enzymes activities were determined by standard methods:

2.9.1 Determination of Superoxide Dismutase (SOD) Activity

Superoxide Dismutase activity was determined by its ability to inhibit the autooxidation of epinephrine determined by the increase in absorbance at 480nm as described by [10]. "The reaction mixture contained 2.95 ml 0.05 M sodium carbonate buffer pH 10.2, 0.02 ml of liver homogenate and 0.03 ml of epinephrine in 0.005 N HCl was used to initiate the reaction. The reference cuvette contained 2.95 ml buffer, 0.03 ml of substrate (epinephrine) and 0.02 ml of water" [10].

Enzyme activity was calculated by measuring the change in absorbance at 480 nm for 5 min.

$$\frac{\Delta_A \times \Delta V_T \times 10^6}{\Sigma \times V_S \times \text{mg protein}}$$

where ΔA = Change in absorbance, V_T = Total volume (volume of sample reagent), Σ = Molar extinction, V_s= Volume 0f sample alone.

Σ= 4020M^{-1} cm^{-1}which is Molar extinction for SOD at 480 nm

2.9.2 Catalase Activity Determination

Catalase activity was determined according to [10]. It was assayed colorimetrically at 620nm and expressed as μmoles of H_2O_2 consumed/min/mg proteinat 25^{0}C. The reaction mixture (1.5ml) contained 1.0ml of 0.01M phosphate buffer (pH 7.0), 0.1ml of tissue homogenate and 0.4ml of 2M H_2O_2. The reaction was stopped by the addition of 2.0ml of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in 1:3 ratio). $\Sigma = 40M^{-1}\ cm^{-1}$

$$\frac{\Delta_A \times \Delta V_T \times 10^6}{\Sigma \times V_S \times \text{mg protein}}$$

2.9.3 Reduced Glutathione Determination

The reduced glutathione (GSH) content of liver, kidney and gill tissues as non-protein sulphydryls was estimated. Ten percent TCA was was added to the homogenate and centrifuged. One (1ml) of supernatant was treated with 0.5ml of Ellmans reagent (19.8mg of 5,5dithiobisnitro benzoic acid (DTNB) in 100ml of 0.1% sodium nitrate) and 3.0ml of phosphate buffer (0.2M, pH 8.0). The absorbance was read at 412nm and Σ = Molar extinction co-efficient for GSH = $1.34 \times 10^4\ M^{-1}\ cm^{-1}$.

$$\frac{\Delta_A \times \Delta V_T \times 10^6}{\Sigma \times V_S \times \text{mg protein}}$$

2.9.4 Determination of Malondialdehyde (MDA)

Malondialdehyde (MDA), an index of lipid peroxidation was determined using the method of [11]. "One ml of the supernatant was added to 2 ml of (1:1:1 ratio) TCA-TBA-HCl reagent (thiobarbituric acid 0.37%, 0.24N HCl and 15% TCA) tricarboxylic acid- thiobarbituric acidhydrochloric acid reagent boiled at 100^{o}C for 15 minutes and allowed to cool. Flocculent materials were removed by centrifuging at 3000 rpm (revolutions per minute) for 10 minutes. The supernatant was removed and the absorbance read at 532 nm against a blank. MDA was calculated using the molar extinction coefficient for MDATBA- complex of $1.56 \times 10^5\ M^{-1}CM^{-1}$" [11].

2.10 STATISTICAL ANALYSIS

Data were analyzed using the Statistical Package for Social Sciences (SPSS) version 20 [12]. Mean values were compared using Analysis of Variance (ANOVA). Post hoc test was done using the student-Newman-Keuls (SNK). Means were presented as Mean ± Standard deviation while P-value was set at 0.05 level of significance.

3. RESULTS

3.1 PHYTOCHEMICAL COMPOSITION

The result of qualitative phytochemical screening of the ethanol extract of sawdust revealed the presence of alkaloids, flavonoid, steroid, phlabotanin, phenols, terpenoid, anthraquinone and saponin (Table 1).

Table 1: Qualitative phytochemical composition of the sawdust extract obtained from Okobaba Sawmill Industry, Ebute
Metta Mainland, Lagos State

Phytochemical constituents	Status
Alkaloid	Positive

Flavanoid	Positive
Steroid	Positive
Philobatanin	Positive
Terpenoid	Positive
Anthraquinone	Positive
Saponin	Positive

3.2 Trace Metals composition

Five metals were detected in the sawdust extract used (Table 2). These metals include iron, cadmium, zinc, copper and magnesium. Of all these, iron was considerably highest in concentration. This was followed by zinc respectively. However, copper and cadmium had the lowest concentrations in the sawdust extract.

Table 2: Metals composition of the sawdust extract from Okobaba Sawmill Industry, Ebute Metta Mainland, Lagos State

METALS	QUANTITY (mg/g)
Iron (Fe)	6.0492
Cadmium (Cd)	0.0136
Zinc (Zn)	2.2303
Copper (Cu)	0.010
Magnesium (Mg)	0.3349

Table 3: Physicochemical parameters of culture water for *Oreochromis niloticus exposed to different concentrations of sawdust extract*

	Control	0.003 ml/l	0.03 ml/l	0.3 ml/l
Temperature (^{0}C)	28.60±0.24^a	28.40±0.65^a	28.00±0.22^a	27.80±0.40^a
Conductivity (S/m)	600±3.25^d	800±0.82^c	970±0.14^b	1032±0.6^a
TSS (ppm)	86±0.83^d	128±1.03^c	382±0.02^b	420±1.25^a
BOD (ppm)	30±1.18^d	36±0.01^c	42±1.36^b	58±0.34^a
DO (ppm)	5.80±0.71^a	5.20±1.60^a	4.00±1.28^a	3.60±1.10^a
Nitrate (ppm)	10.50±0.13^d	18.20±1.87^c	20.50±1.28^b	23.40±0.18^a
Phosphate (ppm)	10.20±0.15^d	16.33±1.88^c	18.35±0.99^b	20.30±0.60^a
Turbidity (Ftu)	70±1.35^d	150±0.35^c	171±1.03^b	243±1.70^a
pH	7.80±0.96^a	7.30±0.50^a	6.90±1.40^a	6.10±0.61^a

^{abcd}Means (±Standard error of mean) in the same row having similar superscripts were not significantly different at P >0.05

3.3 PHYSICO-CHEMICAL PARAMETERS OF CULTURE WATER FOR *OREOCHROMIS NILOTICUS*

The physico-chemical parameters of the experimental treatment water are shown in Table 3. Temperature was relatively stable in all treatment water with the highest degree Celsius of 28 recorded at 0.3% extract concentration. Lowest levels of pH (6.1) and D.O (3.6ppm) were recorded at 30% extract concentration compared to values obtained at 3.0% and 0.3%. The highest conductivity was recorded at 0.3 ml/l (1032 ± 0.6 S/m) and this was significantly higher ($p<0.05$) than conductivity recorded at 0.003 ml/l and 0.03 ml/l extract concentrations. The Biological Oxygen Demand increased with increased concentration. The highest BOD (58 ± 0.34 ppm) was recorded at 0.3 ml/l extract concentration compared to BOD recorded at 0.003 ml/l and 0.03 ml/l extract concentrations. Turbidity was significantly higher (243 ± 1.7 ftu) compared to those recorded at 0.003 ml/l (150 ± 0.35 ftu) and 0.03 ml/l (171 ± 1.03 ftu).

3.4 MEAN WEIGHT OF *Oreochromis niloticus* EXPOSED TO DIFFERENT CONCENTRATIONS OF SAWDUST EXTRACT

Weight increase was also recorded in *O. niloticus* administered with the varying concentrations of sawdust extracts (0.003 ml/l, 0.03 ml/l and 0.3 ml/l) and the control over the six weeks study period (Figure 1). A gradual weight increase was observed between initial and week 2 in all fish exposed to varying concentrations of the extract. However, between week 2 and 3, there was weight gain in fish exposed to 0.03ml/l and 0.3ml/l. There was no significant difference ($p>0.05$) recorded in the mean weight gain of the control and those administered with 0.3ml/l of the saw dust extract (Table 4). The weight gain was significantly higher ($p < 0.05$) than those of the *O. niloticus* groups administered with 0.003 ml/l and 0.03 ml/l sawdust extracts respectively.

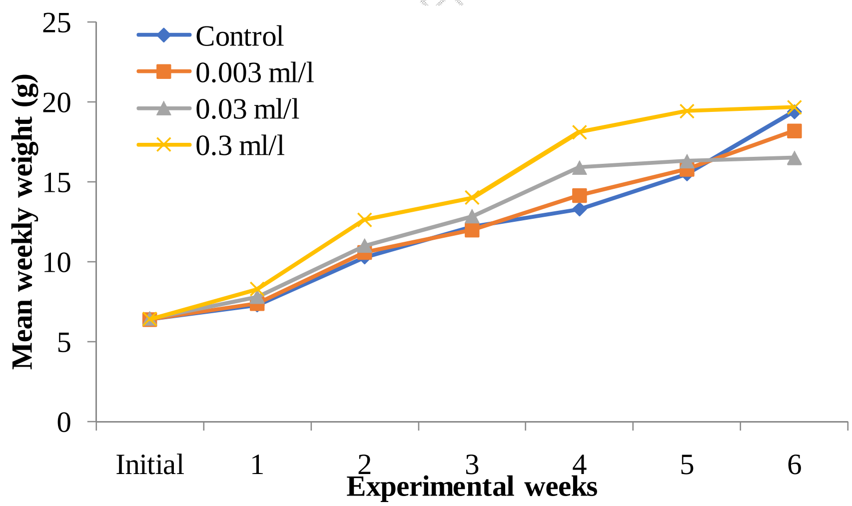

Figure 1: Mean weekly weight of *Oreochromis niloticus* on ethanolic extract of sawdust extract

Table 4: Mean weight gain of *Oreochromis niloticus* administered with varying concentrations of sawdust extract

Treatment	**Live Weight**		
	Initial	**Final**	**Gain**
Control	6.41 ± 1.30[a]	19.40 ± 0.63[a]	12.99±0.58[a]
0.003 ml/l	6.39 ± 1.84[a]	18.20 ± 0.60[b]	11.81±0.01[b]
0.03 ml/l	6.45 ± 0.58[a]	16.53 ± 0.19[c]	10.08±0.01[c]
0.3 ml/l	6.40 ± 0.08[a]	19.69 ± 0.02[a]	13.29±0.06[a]

[abcd]Means (±Standard error of mean) in the same column having similar superscripts were not significantly different at P > 0.05

3.5 HISTOPATHOLOGY OF SOME BODY ORGANS EXPOSED TO DIFFERENT CONCENTRATIONS OF SAWDUST EXTRACT

3.5.1 Section of the Intestine of *Oreochromis niloticus* exposed to different concentrations of sawdust extract

The intestinal tissues of the control *O. niloticus* showed normal architecture with abundant mucosal folds, underlying submucosa folds and muscularis devoid of inflammatory cell infiltrates (Plate 1). On the other hand, the intestine of the *O. niloticus* groups administered with the varying concentrations of the sawdust extract showed disintegrating columnar epithelium with reduced mucosa folds thickness and gastric intestinal atrophy.

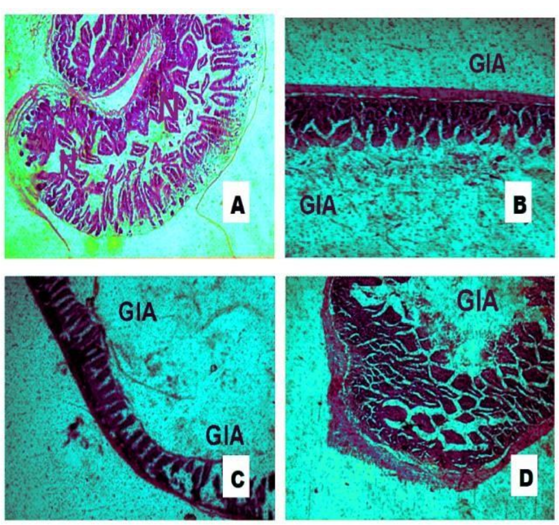

Plate 1: Section of experimental *Oreochromis niloticus* intestine; A = Control; B = 0.3 ml/l sawdust extract; C = 0.03 ml/l sawdust extract; D = 0.003 ml/l sawdust extract; N = Normal architecture; GIA = Gastric intestinal atrophy. (Mg = X40).

<u>3.5.2 Section of the Gills of *Oreochromis niloticus* exposed to different concentrations of sawdust extract</u>

The Histology of gills of *O. niloticus* administered with the varying concentrations of the sawdust extract is shown in Plate 2. Disintegrated columnar epithelium, increased lumen, increase in length of longitudinal muscle layer and increased mucosa were observed in the groups administered with the different concentrations of the sawdust extract. However, normal gill architecture consisting of the dense columnar epithelium, mucosa and submucosa lining, epithelial cells, longitudinal muscle layer and thin columnar cells was observed in the control group.

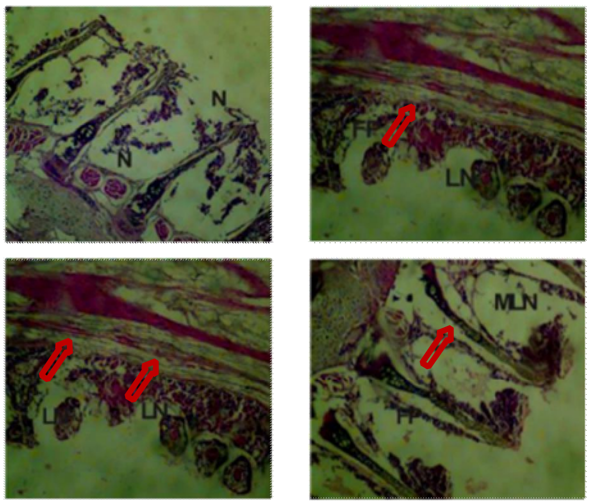

Plate 2:Section of experimental *Oreochromis niloticus* gill; A = Control; B = 0.3 ml/l sawdust extract; C = 0.03 ml/l sawdust extract; D = 0.003 ml/l sawdust extract; N = Normal architecture; MLN and LN = Lamellar necrosis; FP = Focal epithelial proliferations. (Mg = X40).

<u>3.5.3 Section of the Liver of *Oreochromis niloticus* exposed to different concentrations of sawdust extract</u>

The liver of the control *O. niloticus* showed normal architecture (Plate 3). Extensive proliferation of fibrous tissue (Hepathic fibrosis) with mild inflammation was however observed in the liver of those administered with the varying concentrations of the sawdust extract.

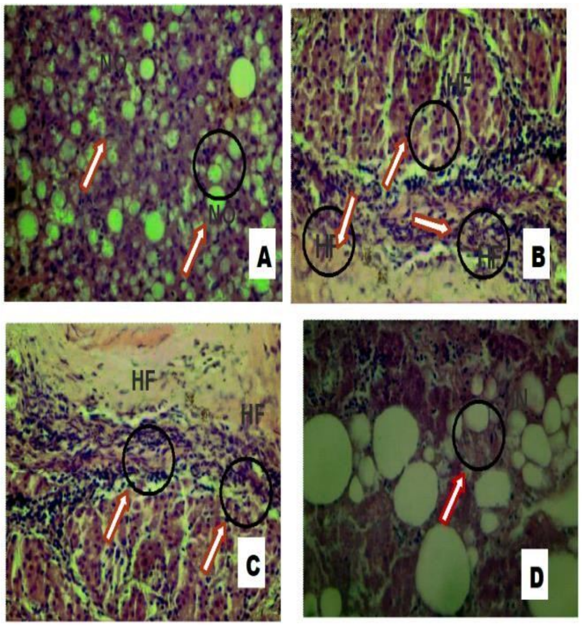

Plate 3: Section of experimental *Oreochromis niloticus* liver; A = Control; B = 0.3 ml/l sawdust extract; C = 0.03 ml/l sawdust extract; D = 0.003 ml/l sawdust extract; NO = Normal architecture; HF = Hepatic fibrosis. (Mg = X40).

3.6 Activities of Antioxidant enzymes level of the liver, kidney and gills of *Oreochromis niloticus* exposed to varying concentrations of sawdust extract

The activities of superoxide dismutase (SOD), reduced glutathione (GSH) and catalase in the liver, kidney and gills of the experimental *Oreochromis niloticus* is presented in table 5. In the liver, GSH activity was was highest at 0.003ml/l among the exposed concentrations with a value of 13.50nm/mg pro. GSH activity in the kidney among exposed concentrations of sawdust was highest at 0.003ml/l with a value of 10.12nm/mg pro. In the gills, the highest value (11.30nm/mg pro) of GSH activity among exposed concentrations was at 0.003ml/l. The liver recorded the highest value of GSH activity (13.50nm/mg pro) among the three organs exposed to the sawdust extract.

SOD activity in the liver was recorded highest at 0.003ml/l among the concentrations exposed to sawdust extract with a value of 110.11nm/mg pro. In the kidney, the highest SOD activity was recorded at the lowest concentration with a value of 98.15nm/mg pro. The gills recorded the highest value (122nm/mg pro) of SOD activity at the lowest concentration among the concentrations exposed to the extract. Among these three organs exposed to the extract, the highest value (122nm/mg pro) of SOD activity was in the gills at 0.003ml/l concentration.

In the liver, CAT activity was recorded highest at 0.003ml/l among the exposed concentrations with a value of 604.88nm/mg pro. CAT activity in the kidney among exposed concentrations of sawdust was highest at 0.003ml/l with a value of 466.55nm/mg pro. In the gills, the highest value (495.96nm/mg pro) of CAT among exposed concentrations was at 0.003ml/l. The liver recorded the highest value of CAT activity (604.88nm/mg pro) among the three organs exposed to the sawdust extract. There was no significant difference ($p > 0.05$) in the activities of reduced glutathione (GSH) and superoxide dismutase (SOD) recorded in the liver of the control *O. niloticus* and those administered with 0.003 ml/lof the sawdust extract (Table 5). These were significantly higher ($p < 0.05$) than those administered with 0.03 ml/l and 0.3 ml/l concentrations of the sawdust extract. Catalase activity of the liver was also significantly higher in the control group and observed to significantly reduce with increasing concentration of the sawdust extract administration.

In the kidney of the experimental *O. niloticus*, GSH and catalase activities were significantly higher in the control group. These were observed to significantly reduce in the experimental *O. niloticus* with increase in the concentration of the sawdust extract administration. Kidney SOD activity was not significantly different ($p > 0.05$) between the control group and those administered with 0.003 ml/l and 0.03 ml/l of the sawdust extract. Kidney SOD activity was however significantly lower ($p < 0.05$) in the *O. niloticus* group administered with 0.3 ml/l of sawdust extract. Similarly, GSH and catalase activities of the gills were significantly higher ($p < 0.05$) in the control group those administered with the varying concentrations of the sawdust extract. These were however observed to reduce with increase in the concentration of the sawdust extract administration. On the other hand, SOD activity of the gill was significantly lower in the *O. niloticus* group administered with 0.3 ml/l of the sawdust extract. SOD activity was however not significantly different ($p > 0.05$) between the control group and those administered with 0.003 ml/l and 0.03 ml/l of the sawdust extract.

Table 5: Antioxidant enzymes activity of *O. niloticus* administered with varying concentrations of sawdust extract

	Conc. (ml/l)	GSH (nm/mg pro)	SOD(nm/mg pro)	Catalase(nm/mg pro)
Liver	**Control**	16.14±0.75[a]	114.03±1.44[a]	615.02±0.31[a]
	0.003	13.50±0.43[a]	110.11±0.42[a]	604.88±0.29[b]
	0.03	6.82±0.91[b]	104.00±1.70[b]	594.06±0.01[c]
	0.3	3.81±0.94[b]	99.60±1.06[b]	573.17±0.25[d]
Kidney	**Control**	16.80±0.68[a]	103.41±1.86[a]	501.70±1.85[a]
	0.003	10.12±1.42[b]	98.15±1.15[a]	466.55±0.31[b]
	0.03	4.01±0.34[c]	96.71±1.93[a]	457.62±0.17[c]
	0.3	3.21±0.07[c]	82.31±1.19[b]	432.11±0.1[d]
Gill	**Control**	18.32±1.08[a]	125.00±1.36[a]	506.33±3.18[a]
	0.003	11.30±0.22[b]	122.00±0.50[a]	495.96±0.24[b]
	0.03	3.86±0.13[c]	120.63±0.04[a]	455.01±2.83[c]
	0.3	3.01±0.55[c]	116.3±0.48[b]	461.63±0.93[c]

[abcd]Means (±Standard error of mean) in the same column for each of the organs having similar superscripts were not significantly different at $P > 0.05$

3.6.1 Lipid peroxidation in the liver, kidney and gills of *Oreochromis niloticus* cultured on varying concentrations of sawdust extract

The level of lipid peroxidation recorded in the liver, kidney and gills of the *O. niloticus* were not significantly different ($p > 0.05$) between the control group and those administered with the varying concentrations of the sawdust extract (Figure 2). Lipid peroxidation was also observed to be lowest in the control group and increase with increase in the concentration of the sawdust extract. Lipid peroxidation was therefore highest in kidney, followed by liver and gills of the *O. niloticus* at 0.3 ml/l concentration of the sawdust extract.

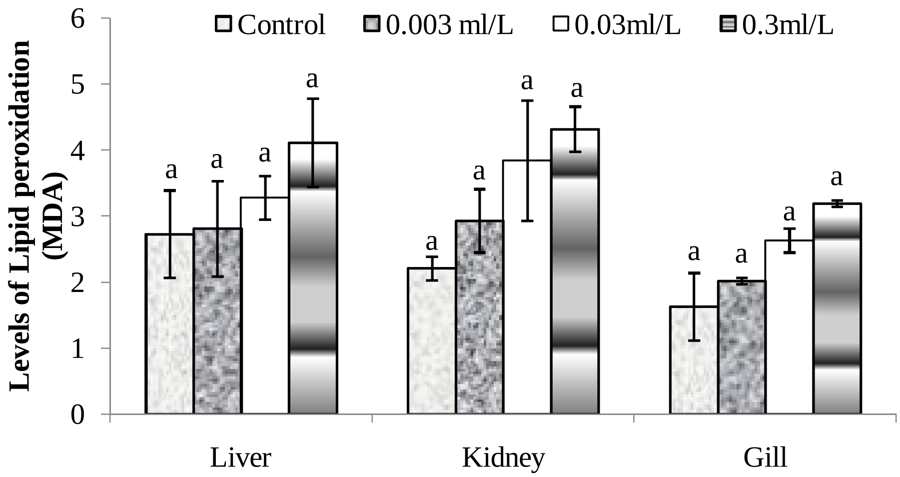

Figure 2: Levels of lipid peroxidation (Malondialdehyde – MDA) in *O. niloticus* administered with varying concentrations of sawdust extract; [abcd]Means in the same cluster having similar superscripts are not significantly different at $p < 0.05$; Error bars represents Standard error of mean.

3.7 DISCUSSION

The study has shown that exposure of *O. niloticus* culture water to sawdust extract reduced the level of dissolved oxygen with increased concentration of the sawdust extract exposure. Reduction in the dissolved oxygen of fish culture tank has been shown to be detrimental to fish health. According to [22], reduction in water oxygen level could result in direct mortality or reduction in the rate of fish growth and therefore has been a major parameter of concern in the aquatic environment. Hence, reduced level of dissolved oxygen in the culture water of this present study could be an indication that sawdust extract could deplete water oxygen level, thereby exposing the fish to reduced growth or mortality.

Reduced level of dissolved oxygen recorded in the culture water of *O. niloticus* exposed to varying concentrations of sawdust extract could be responsible for the histopathological alterations observed in the gills and liver tissues. Histological responses have been pointedly reported as valuable markers of toxicology. For instance, the severity of gill damage as observed in the experimental *O. niloticus* corresponds with exposure concentration of sawdust extract. Previous study has shown that impairments of fish gill cause direct destruction of oxygen–carbon dioxide exchange [18]. Some of the histopathological changes observed in the liver of *O. niloticus* include increased cytoplasmic vacuolation, definitive necrosis and hepatocellular alteration. Increased vacuolation of the hepatocytes is a signal of degenerative process that suggests metabolic damage. This agrees with the findings of [13] [14] [15]. The intestine of *O. niloticus* exposed to sawdust extract also showed some alterations such as abnormality and elongation of the intestinal wall with disintegrating columnar epithelium. Disintegrated columnar was earlier observed in the intestine of fish exposed to toxicants [16] [17].

This study also recorded reduction in the activities of reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) in the livers and kidneys of the fish (*Oreochromis niloticus*) after 42 days exposure to sawdust extract. Reduced activity of these enzymes could be a clear indication of increased oxidative stress. In the antioxidant system, SOD plays active role in cell defense and protection against free radical-induced damage [23]. This SOD attacks the superoxide radicals of the reactive oxygen species and thus, converting it to hydrogen peroxide [24]. Catalase also acts on the hydrogen peroxide to produce oxygen and water. Some studies have associated the presence of phytochemicals in fish culture medium with increased oxidative stress. Alkaloids have been linked with stress in fish [19]. Similarly, anthraquinone content has also been linked with the inhibition of SOD, CAT and GSH [20].

Reduced levels of antioxidant enzymes could have resulted in increased level of lipid peroxidation (MDA) in the liver of sawdust extract exposed fish. Significant increase in MDA in the liver, kidney and gills of *Oreochromis niloticus* agreed with the findings of [21] that reported MDA increase in the tissues of fish exposed to varied concentrations of hydrocarbons. The increased MDA indicates that ROS formed may be associated with the metabolism of sawdust leading to peroxidation of membrane lipids of the respective organs.

4. CONCLUSION

The results from this study have shown that sawdust extract could result in reduced dissolved oxygen levels of *O. niloticus* culture medium and thus inducing oxidative stress and histological alterations of the gills, liver and intestine of the fish.

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