GSJ: Volume 11, Issue 9, September 2023, Online: ISSN 2320-9186

www.globalscientificjournal.com

Toxicological Analysis of *Oreochromis niloticus* (Tilapia Fish) in Sallari Lake in Kano Metropolis, Nigeria

Tajudeen Afolayan Lawal[1]*, Qazeem Oyeniyi Sholadoye[2] and Olasumbo Adeola Mohammed[1]

[1]Biochemistry and Forensic Science Department, Nigeria Police Academy, Wudil. Kano, Nigeria
[2]Department of Chemistry, Nigeria Police Academy, Wudil. Kano, Nigeria

*talawal@polac.edu.ng; lawalat40@gmail.com

Abstract

Sallari Lake, popularly known by the fishermen as "Ruwan Kandamiy," is a polluted lake in the Kano metropolis. Municipal and household garbage are two different sources from which pollution is produced. Every day, residents of the area dump domestic and industrial waste, dead animals, human waste, faeces, and urine in Sallari Lake. A well-known lake in Nigeria's Kano metropolis is Sallari Lake, where fisherman capture fish to sell and eat. The water and fish in this lake, however, were found to be highly polluted, which raises the possibility that they are unfit for human consumption. In light of this, *Oreochromis niloticus* from this lake was evaluated biochemically, haematologically, and histopathologically, and the results were compared to those of another *Oreochromis niloticus* from an unpolluted pond. *Oreochromis niloticus* was sampled from various lake regions as part of the investigation. Glucose, total proteins, total lipids, cholesterol, AST, ALT, and ALP are among the blood biochemical markers that are examined. RBC, Hb, and Hct were the haematological parameters analysed. The liver and heart were examined histopathologically. According to the study's findings, there were differences between the data collected from the fish in Sallari Lake and the fish in the control pond. This implies that the fish in Sallari Lake have suffered some harm to their livers and other essential organs as a result of the pollution in the lake. Additionally, this might be harmful to the wellbeing of fish consumers.

Key Words: Sallari Lake; *Oreochromis niloticus*; Pollution; Haematology; Histopathology

INTRODUCTION

Ruwa Sallari is a lake that can be found in the Kano state capital city of Kano along Isah Waziri Road, Gyadi Gyadi. Latitude 11.965465 (11° 57' 55.674' N) and longitude 8.5362265 (8° 32' 10.4154' E) are the coordinates of this location (Google Map 2023). In the Kano metropolis of Kano State, it is situated between Hausawa-Danfulani, Gyadi-Gyadi, and Karkasara. The satellite image of Sallari Lake in Kano, Nigeria is depicted in figure a. The lake is actually a dump that has been there for more than 60 years. Everyday refuses like human waste and home and municipal trash are dumped on this site. Figure b shows a picture of a Sallari lake that has been polluted. The area is submerged under water, and everyday fishing operations are conducted there. Additionally, this lake's water is utilised to produce roads, building blocks, and other things. The lake's fish are sold for both commercial use and

human consumption. Within the parameters of our literature review, there have been no reports of any type on this lake. The current study examines the toxicological condition of fish taken in the "Ruwa Sallari" water. Because of the unwholesome activities taking place in Sallari Lake, the findings of this inquiry would notify the public about the safety of the water and the fish there. Additionally, it would make the authorities aware of the need to take good care of the lake or make it a major tourist destination. Water contamination is brought on by the presence of diseases, poisons, and heavy metals in amounts that are too high (Chiu *et al.*, 2011).

Human health is negatively impacted by contaminated water, either directly by ingesting the water or indirectly by consuming the fish or other foods that are present in the polluted water (Kadry *et al.*, 2015). Chemical and physical changes in the aquatic environment frequently cause the physiological problems that fish endure as a result of changes in blood and tissue parameters (Basha and Rani, 2003). Different biomarkers have been investigated, assessed, and approved as biomonitoring instruments to find toxicity in biological systems (Reddy and Kusum, 2013). In addition to evaluating the toxicants in the organism qualitatively and quantitatively, the biomonitoring technologies also look into how the organisms react to the toxicants (El-Serafy *et al.*, 2005).

Biochemical biomarkers are vital indicators used to assess fish health in the field (Haluzova *et al.*, 2011). Blood is an extremely effective standard medium for assessing the health of animals. It indicates the condition of the fish's internal organs both before and after any visible symptoms of a condition (Fernandes and Mazon, 2003). Any changes to a fish's environment could have a negative impact on the components of the fish's blood. Fish can tolerate these detrimental changes to some extent through to a few physiological processes (Adham *et al.*, 2002). The tissue biomarker, which has a variety of origins and offers a positive biological endpoint for previous exposure, is another significant indicator of environmental stress. In field investigations, histopathology is frequently the simplest method for determining both the short- and long-term effects of toxicants on animals (Liebel *et al.*, 2013). Therefore, histopathological evaluation continues to play a big role in the biomonitoring of toxicant effects in organisms. The primary metabolic functions of the liver in fish are detoxification, urea synthesis, and the biotransformation of toxins (from the intestine) into the blood (Yousuf *et al.*, 2012). Because it is one of the most damaged organs by water contaminants due to its multifunctional status, the liver is therefore recognised as an indicator to determine the impact of toxicants on fish (Reddy, 2017).

The liver can detoxify toxins at low to moderate quantities, but at large concentrations, its capacity can be overwhelmed, which can result in histopathological lesions (Gaber *et al.*, 2014). As a result of human activity on water bodies, many of them are currently becoming worryingly contaminated; this raises awareness of water quality issues (Carolin *et al.*, 2017). Groundwater quality is diminishing, which has negative effects on the water supply for drinking, irrigation, and industrial usage, all of which are important factors in determining the public's health (Ighalo *et al.*, 2020). According to a survey, levels of both chemical and natural pollutants are rising practically everywhere (Vardhan *et al.*, 2019). As urban living becomes more prevalent, environmental contamination becomes increasingly dangerous.

To be more specific, this issue has gotten worse alongside the growth of industry. Natural resources become more contaminated as a result of accelerated industrial development and human growth, which makes ecological devastation easier. Pharmaceutical and personal care products, polyfluoroalkyl and perfluoroalkyl substances, biocides (pesticides and herbicides), heavy metals, dyes, radionuclides,

plastics, nanoparticles, and pathogens are some of the main pollutants that harm both aquatic and terrestrial organisms and destroy ecosystems (Villarn and Merel, 2020).

Figure a: A satellite image of Sallari Lake

Figure b: Polluted Sallari Lake with domestic and industrial waste.

MATERIALS AND METHODS

MATERIALS

Study Area

For this experiment, Sallari Lake in Kano, Nigeria, in latitude 11° 57' 55.674' N and longitude 8° 32' 10.4154' E was employed. The lake was divided into four sections, labelled North (N°), South (S°), West (W°), and East (E°), based on the types of pollutants that were dumped into each section.

In the West Region (W°), dead animals, human garbage, and animal waste are discarded. "almagiri" come here to urinate and defecate every morning and evening. Due to its proximity to residential structures, the North Region (N°) is where municipal and domestic waste is disposed of. Block manufacturing industry is located in the East Region (E°), and garbage from that business is dumped here. Furthermore, domestic, and municipal trash are dumped here. The fishermen are primarily based in the South Region (S°), where they also take their baths and market the lake fish they catch.

Fish Sample Collection

For this experiment, a total of eight (80) *Oreochromis niloticus* (tilapia fish) with weights ranging from 28.70 g to 35.8 g were taken at Sallari Lake. With the help of fishermen, twenty (20) fish were

caught in each of the four zones. A weighing balance was used to take the weight of the fish that were captured.

Control Fishpond

After being acquired from a nearby commercial farm, *Oreochromis niloticus* fingerlings were reared in a fibreglass tank for six weeks in a natural photoperiod with continually aerated aged tap water. Commercial fish meal pellets were supplied to the fish three times per day. To get rid of any leftover feed, faeces, or other metabolic waste, the water in the fish tank was replaced every two days with aged tap water. The recommendations for producing high quality fish were dutifully followed.

METHODOLOGY

Fish Blood Collection

Through caudal venepunctures made in the fish's vertebral vein and artery, blood was drawn from the animal. The vertebral body was punctured with a needle, which was then, by providing negative pressure to the syringe, withdrawn a little laterally and ventrally. Blood was injected into the syringe during this operation. The needle was twisted slightly to ensure that the needle bevel was properly positioned in the blood vessel, which would help with the proper blood transfer into the syringe. To acquire serum and plasma for biochemical and haematological examination, the obtained blood was transferred into heparinized and nonheparinized tubes, respectively.

Histopathological Analysis

The liver and heart biopsies of tilapia fish collected from different Sallari Lake zones were fixed with 10% formaldehyde. The tissues were dehydrated with an ascending alcohol grade, cleaned with xylene, and injected with molten paraffin wax. Using a microtome, the treated tissue was divided into sections and stained using the haematoxylin and eosin method. A Leica ICC50HD camera and a Leica DM750 microscope were used to examine and photograph the stained tissue segment.

Haematological Analysis

Red Blood Cell Analysis

The Blaxhall and Daisley (1973) method was used to determine red blood cell (RBC) count. The RBC pipette was filled with an aliquot of blood up to the 1 mark on the pipette. The diluting solution was then drawn up to the pipette's 101-mark level, resulting in a 1:100 blood to diluting solution ratio. After that, the blood solution was thoroughly mixed for a further 1 – 2 minutes. After that, the haemocytometer (TL-2000A Blood Cell Counter) was filled with the diluted blood. From each dilution pipette, three replicate counts were performed, and the average and standard deviation of the RBC counts were determined. RBC count was calculated, using equation 1.

$$RBC\ (count \times 10^6\, mm^3) = \frac{C \times D \times 100 \times 4000}{S \times 80} \quad \ldots\ldots\ equation\ 1$$

Where C = number of cells counted; D = dilution factor; S = number of 1 mm² counted

Haemoglobin analysis

The basic idea behind this technique is that haemoglobin in a blood sample, with the exception of sulphhaemoglobin, is transformed to methaemoglobin by ferricyanide and subsequently reacts with cyanide to generate cyanomethemoglobin, which is then quantified spectrophotometrically at a wavelength of 540 nm. A 1000 mL volumetric flask that had previously held around 500 mL of distilled water was used to dissolve 1 g of sodium bicarbonate, 0.2 g of potassium ferricyanide, and 0.05 g of potassium cyanide to form a workable reagent. The solution was made up to 1000 mL with water and stored in an amber bottle. An aliquot of 4 mL of the reagent was put into a 5 mL volumetric flask, and 0.02 mL of blood was added and stirred properly. The mixture was then diluted with the reagent to a volume of 5 mL, thoroughly mixed, and incubated for 10 minutes at room temperature. The final result was then measured at 540 nm using the working reagent as a blank in a colorimeter. A blood sample with a known haemoglobin content that had been professionally analysed was used as the standard. Equation 2 was used to determine the amount of haemoglobin (Hb) in the blood.

$$Hb\ (g/100\ mL) = \frac{A_U \times C_S}{A_S} \quad \ldots\ldots\ equqtion\ 2$$

Where A_S is the absorbance of standard, $A_U$is the absorbance of unknown and C_S is the concentration of standard.

Haematocrit analysis

Haematocrit level was determined according to the method of Blaxhall and Daisley (1973). The packed red cell volume of the blood obtained in a heparinized capillary tube was quantified and recorded as a percentage after centrifugation under standard circumstances. Blood was poured into a capillary tube up to its two-third point, and the tube was repeatedly turned over to mix the blood with the heparin coating. One end of the tube was sealed with a plug of cristoseal, a clay sealant and centrifuged for 5 minutes at 12,000 rpm in a micro-haematocrit centrifuge. The haematocrit reader (Iris/StatSpin CritSpin Digital Haematocrit Reader, Model S120-22) was used to measure the content of the tube immediately following centrifugation.

Blood Glucose analysis

The Hyvarinen and Nikkila (1962) technique was used to calculate plasma glucose (mg/L). The principle behind it is that orthotoluidine in glacial acetic acid reacts with glucose in blood plasma to produce a blue-green colour that absorbs most strongly at 635 nm.

Glucose Standard Preparation

One hundred milligram of reagent-grade glucose were dissolved in a 100 mL standard volumetric flask with a little amount of water. The resultant solution was diluted with water to a maximum of 100 mL and refrigerated for storage.

Ortho-Toluidine Colour Reagent Preparation

Glacial acetic acid (94 mL) and O-toluidine (6 mL) were used to fill a 100-mL standard volumetric flask to its full capacity of 100 mL.

Blood Glucose Analysis Procedure

In a test tube, 3.5 mL of colour reagent and 0.05 mL of plasma were added and mixed. The colour reagent (3.5 mL) and glucose standard (0.05 mL) were combined and added to a second test tube. A blank reagent was prepared by combining 0.05 mL of water and 3.5 mL of colour reagent in a third test tube. After being heated in a water bath for ten minutes, the three test tubes were withdrawn and allowed to cool to room temperature. At 635 nm, the spectrophotometric absorbances of the three distinct contents were read. Equation 3 is used to calculate the glucose concentration (mg/L).

$$Glucose\ (mg/L) = \frac{A_U \times C_S}{A_S} \quad \ldots\ldots\ equation\ 3$$

Where A_S is the absorbance of standard, A_U is the absorbance of unknown and C_S is the concentration of standard.

Total Protein Analysis

According to Tietz (1995), total protein was evaluated using the Colorimetric Method-Biuret Reagent. The theory is based on the fact that when serum protein reacts with cupric ions in an alkaline medium, a violet colour is produced that absorbs maximally at 546 nm. The ratio between protein concentration and absorption intensity is linear.

Reagent Preparation for Total Protein Estimation

Normal saline (0.9% $^{w}/_{v}$ NaCl) solution

To prepare normal saline, 0.9 g of sodium chloride was dissolved in a little amount of water, and the resulting solution was then diluted to 100 mL.

Stock Biuret Reagent Preparation

To 400 mL of a 0.2 M sodium hydroxide solution, 45 g of Rochelle salt (potassium sodium tartrate) was added and dissolved. Then, 15 g of copper II sulphate and 5 g of potassium iodide were added to the solution and stirred. A 0.2 M sodium hydroxide solution was used to dilute the resulting solution up to 1000 mL.

Biuret Working Solution preparation.

With 0.2 M sodium hydroxide, which contains 5 g of potassium iodide, 200 mL of the stock Biuret reagent were diluted to 1000 mL.

Protein Standard Solution

The amount of bovine albumin in this solution was 6 g/dL.

Total Protein Estimation Procedure

In a test tube, 0.1 mL of serum was combined with 2.9 mL of normal saline solution, and 3 mL of the Biuret working solution was added. The technique was done twice more, each time substituting normal saline for the serum and the protein standard solution instead. The three test tubes were incubated for 10 minutes at 37 °C in a water bath to produce a violet colour that was read spectrophotometrically at 540 nm. From equation 4, a serum total protein estimation was calculated.

$$Total\ Protein\ (g/100\ mL) = \frac{A_U \times 6}{A_S} \quad \ldots\ldots\ equation\ 4$$

Where A_S is the absorbance of the Standard, and A_U is the absorbance of the Unknown.

Cholesterol Evaluation

The cholesterol level was determined according to the method of Zak (1957).

Cholesterol Estimation Reagent Preparations

Stock Solution of Ferric Chloride

Glacial acetic acid was used to dissolve 840.0 mg of iron II chloride hexahydrate ($FeCl_2.6H_2O$). The resultant solution was diluted to 100 mL with glacial acetic acid.

Precipitating Reagent

Using glacial acetic acid, the stock ferric chloride solution of 10 mL was diluted to 100 mL.

Diluting Reagent

With glacial acetic acid, 85 mL of the precipitating reagent were diluted up to 100 mL.

Cholesterol Standard Solution

Glacial acetic acid was used to dissolve 200 mg of pure recrystallized cholesterol, and the resulting solution was made up to 100 mL.

Cholesterol Estimation Procedure

In a test tube, 0.05 mL of serum and 2 mL of precipitating reagent were added and mixed. Two more test tubes were used, each containing 0.05 mL of water and 0.05 mL of a cholesterol standard solution. Two-millilitre of the diluting reagent was added to each test tube, which served as the standard and the blank, respectively. The three test tubes were stoppered and mixed thoroughly by inversion, centrifuged for 30 seconds, and 1.5 mL of each supernatant was transferred to a clean colorimeter tube. In the first test tube, 1 mL of concentrated H_2SO_4 was added, mixed, and incubated in a water bath at 80 °C for 5 minutes. This was repeated for the other two test tubes at 1-minute intervals. The test tubes were removed after 5 minutes of incubation in the water bath and allowed to stand for 10 minutes at room temperature. Using the blank to set the instrument to zero, the contents of the test tubes were read colorimetrically at 550 nm. Equation 5 is used to calculate the serum's cholesterol content.

$$Cholesterol\ (\mu g/L) = \frac{A_U \times 200}{A_S} \quad \ldots\ldots equation\ 5$$

Where A_S is the absorbance of the Standard, and A_U is the absorbance of the Unknown.

Total Lipid Analysis

Sulphuric acid reacts with lipids to produce the carbonium ion, which then reacts with vanillin phosphate ester to produce a purple complex. The quantity of total lipids contained in the sample is

proportional to the intensity of the purple colour, which can be determined spectrophotometrically at 540 nm.

Reagents Used in Lipid Analysis

1. *Vanillin Reagent* (0.04 M). A 1 L solution was produced by dissolving 6.1 g of vanillin in water. The resulting solution was stored in a brown bottle at room temperature.
2. *Phosphovanillin Reagent*. In 50 mL of water in a flask, 350 mL of the 0.04 M vanillin reagent were added. The solution was constantly stirred while 600 mL of concentrated (85%) phosphoric acid was added. This resulting solution was stored in a brown bottle at room temperature.
3. *Reagent-Grade Concentrated Sulphuric Acid (98%).*
4. *Lipid Standard Solution*. Olive oil (U.S.P. grade) was used as the standard. About 0.5 mL (500 mg) of the olive oil was pipetted into a previously weighed and zeroed 100 mL standard volumetric flask and then reweighed. Absolute ethanol was added to the flask to dissolve the oil. The resultant solution (500 mg/dL) was diluted to 100 mL by the ethanol and then refrigerated.

Total Lipid Analysis Procedure

In three separate tubes, 20 µL of each water, sample, and standard solution were added accordingly. In each tube, 200 µL of sulphuric acid were added. The resulting solutions were vortexed, incubated in a boiling water bath for 10 minutes, and then cooled to room temperature. Then, 10 mL of phosphovanillin reagent was added to the three tubes, swirled, and incubated at 37 °C for 15 minutes in a water bath. The final solutions were cooled, and the absorbance of the samples and the standard was read against the blank solution at 540 nm. The total lipid concentration was then calculated from equation 6. Twenty microlitre (20 µL) of water, the sample, and the standard solution were introduced to three different tubes in that order. Two hundred microlitre (200 µL) of sulphuric acid were added to each tube. The resulting mixtures were vortexed, heated for ten minutes in a boiling water bath, and then allowed to cool to room temperature. The three tubes were then filled with 10 mL of phosphovanillin reagent, stirred, and heated in a water bath at 37 °C for 15 minutes. After cooling the final solutions, the absorbance of the samples and the standard was read against the blank solution at 540 nm. The total lipid concentration was then calculated using equation 6.

$$Total\ lipid\ (mg/dL) = \frac{A_{Sample}}{A_{Standard}} \times 500 \qquad \ldots\ldots equation\ 6$$

AST and ALT Analysis

The determination of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) was performed by following the method of Reitman and Frankel, 1957. AST and ALT were measured by monitoring the concentration of intensely coloured hydrazones formed when 2,4-dinitrophenylhydrazine reacts with oxaloacetate and pyruvate from the transamination of L-aspartate and L-alanine, respectively, to α-ketoglutarates. This coloured complex absorbs maximally at 546 nm.

Reagents for AST and ALT Analysis

Reagent 1: This contains phosphate buffer (100 mmol/L, pH 7.4), L-aspartate (200 mmol/L), and oxoglutarate (2 mmol/L).
Reagent 2: It contains 2,4-dinitrophenylhydrazine (2 mmol/L).

Reagent 3: It contains 0.4 mol/L of NaOH.
For ALT analysis, the L-aspartate in Reagent 1 was replaced by L-alanine.

Procedure for AST and ALT Analysis

Briefly, 0.1 mL of the diluted sample was mixed with Reagent 1, and the mixture was incubated for 30 minutes at 37 °C. Then, 0.5 mL of Reagent 2 was added to the reaction mixture and incubated for 20 minutes at 25 °C. Thereafter, 5.0 mL of Reagent 3 was added, and the absorbance was read at 546 nm against the reagent blank after 5 minutes. A reaction blank (blank reagent) was prepared as described above, replacing the sample with 0.1 mL of distilled water. The activity of AST and ALT in the serum was estimated from their respective calibration curves (Appendix I and Appendix II, respectively).

Serum Alkaline Phosphatase (ALP) Estimation

This investigation used Roy's (1970) colorimetric endpoint technique. AMP-buffered sodium thymolphthalein monophosphate is acted upon by alkaline phosphatase. While also producing a blue chromogen that may be detected spectrophotometrically at 590 nm, the addition of an alkaline reagent stops enzyme activity.

Preparation of Reagents for Serum Alkaline Phosphatase (ALP) Estimation

Reagent 1 (Alkaline Phosphatase Substrate): This contains 3.6 mM sodium thymolphthalein monophosphate in 0.2 M 2-amino-2-methyl-1-propanol buffer and 1.0 mM magnesium chloride (wetting agent, inactive ingredients, preservatives; pH 10.2).

Reagent 2 (Alkaline Phosphatase Colour Developer): This contains a mixture of equimolar amounts of 0.1 M NaOH and 0.1 M Na_2CO_3.

Reagent 3 (Alkaline Phosphatase Standard): This contains 0.5 mM thymolphthalein in n-propanol. This is equivalent to 50 U/L enzyme activity when used according to the alkaline phosphatase procedure.

Procedure for Estimating Serum Alkaline Phosphatase (ALP)

In a test tube, 0.5 mL of Reagent 1 (substrate) was added and equilibrated to 37 °C for 3 minutes. Thereafter, 0.05 mL of serum sample was added, mixed gently, and incubated for 10 minutes at 37 °C. Then, 2.5 mL of Reagent 2 (colour developer) was added and stirred. The absorbance of the resulting solution was taken at 590 nm. For the "standard" and the "blank," the procedure was repeated simultaneously at 30 second intervals, but Reagent 3 (alkaline phosphatase standard) and deionized water were used in place of the serum sample. ALP (U/L) activity was estimated from equation 7.

$$ALP\ Activity\ (U/L) = \frac{A_U}{A_S} \times 50 \qquad \ldots\ldots equation\ 7$$

Where A_U is the absorbance of the Unknown, and A_S is the absorbance of the Standard.

STATISTICAL ANALYSIS

Each test was run in triplicate, and the data obtained were analysed and expressed as the mean ± standard deviation ($\bar{x} \pm SD$) for five fish in each group ($n = 5$). The disparity in data obtained within and between the groups was authenticated by one-way analysis of variance (ANOVA) followed by the Tukey-Kramer Multiple Comparisons Test. The statistical scrutiny was carried out using GraphPad Instat software (version 3.05). The significant value was set as a P-value less than 0.05 ($p < 0.05$).

RESULT AND DISCUSSION

Results

The haematological, biochemical, and histopathological investigation results of the tilapia fish (*Oreochromis niloticus*) obtained from the Sallari lake, and the control pond are depicted in figures 1 to 12. Figure 1 shows the result of the red blood cell (RBC) count analysis: the RBC of the fish from the four regions of Sallari Lake are significantly ($p < 0.05$) lower than the control group. However, the differences observed among the serum RBC counts of the fish caught from the four regions of the lake are not significant ($p > 0.05$). The result of haemoglobin (Hb) analysis, as depicted in figure 2, shows that Hb of the fish from the Sallari lake is significantly ($p < 0.05$) lower than that of the control (pond) group. The Hb of the fish obtained from the W° and E° of the lake were significantly lower ($p < 0.05$) than that from N° and S°, and that from W° was significantly lower ($p < 0.05$) than that from E°. The results of the haematocrit (Hct), also known as packed cell volume (PCV) analysis, of the fish obtained from the four regions of the Sallari Lake, as depicted in figure 3, were significantly lower ($p < 0.05$) than the ones from the control group. However, the discrepancies observed among the serum RBC counts of the fish caught from the four regions of the lake are not significant ($p > 0.05$).

The outcomes of the serum glucose analysis of the fish caught in Sallari Lake, as shown in Figure 4, are significantly ($p < 0.05$) higher than those of the control group. That of the W° and E° regions of the lake was significantly higher ($p < 0.05$) than that of the N° and S° regions. However, no significant ($p > 0.05$) differences were observed between W° and E° and between N° and S° regions. Figure 5 shows the results of the serum total protein analysis of the fish obtained from Sallari Lake and the controlled pond. The results showed a significant decrease ($p < 0.05$) in the serum total protein of fish gotten from the Sallari lake when compared to the control group. Among the fish from Sallari, the group from the west region is significantly ($p < 0.05$) lower than that from the E°, N°, and S° regions. However, there is no significant ($p > 0.05$) difference among the values obtained for the E°, N°, and S° regions.

The results of total lipid analysis, as depicted in Figure 6, showed no significant ($p > 0.05$) difference in the fish gotten from the various regions of the lake in comparison with the control group. Figure 7 shows the results obtained from serum cholesterol analysis, which show a significant ($p < 0.05$) increase in all fish gotten from the lake when compared to the control group. However, no significant ($p > 0.05$) difference was observed among the values obtained in W°, E°, and N°, though S° is statistically ($p < 0.05$) lower than W°. Figure 8 shows the results obtained from serum AST analysis; the result shows a significant increase ($p < 0.05$) in serum AST of fish gotten from the Sallari lake when compared to the control pond. Region W° is significantly higher than E°, N°, and S°. However, no significant difference was observed between E° and N° regions, but the duo is statistically ($p < 0.05$) higher than S°.

Figure 9 shows the results obtained from serum ALT analysis of the tilapia fish obtained from Sallari Lake; a significant ($p < 0.05$) increase in serum ALT was observed in the fish caught from Sallari

Lake when compared to that of the control pond. However, no significant ($p < 0.05$) difference was observed among the serum ALT levels of the fish from the W°, E°, and N° regions of the lake. Also, no significant ($p < 0.05$) difference was observed among E°, N°, and S° or between W° and S° regions of the lake. Figure 10 shows the results obtained from serum ALP analysis of fish gotten from Sallari Lake. The results revealed a significant rise ($p < 0.05$) in the fish gotten from the four regions of the lake when compared to those from the control pond. Figure 11 shows the histopathological liver section of *Oreochromis niloticus* caught from the eastern (E°) shore of Sallari Lake, which depicts an area of degenerated hepatocytes. Figure 12 depicts the histopathological liver section of the fish from the control pond, which shows no pathological lesion. No pathological lesion was observed in the fish gotten from the other regions (W°, N°, and S°) of the lake.

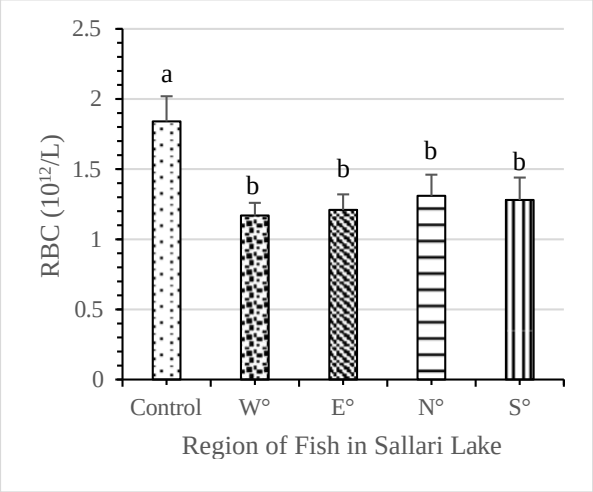

Figure 1: Status of the red blood cell (RBC) count of fish caught in various regions of Sallari lake in comparison to the control.

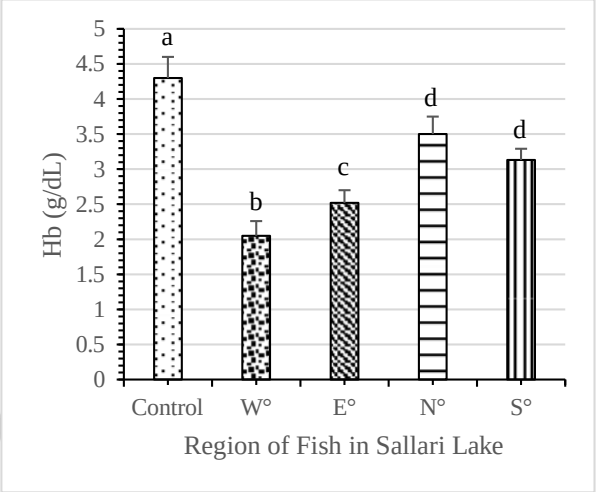

Figure 2: Status of haemoglobin (Hb) of fishes caught in various regions of Sallari lake in comparison to the control.

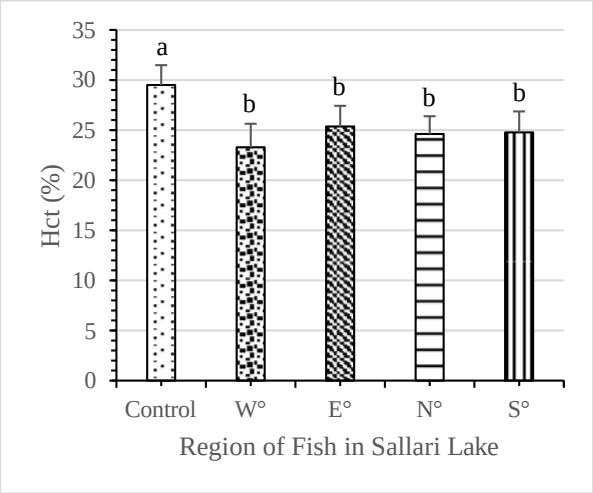

Figure 3: Status of haematocrit (Hct) of fish caught in various regions of Sallari Lake in comparison to the control.

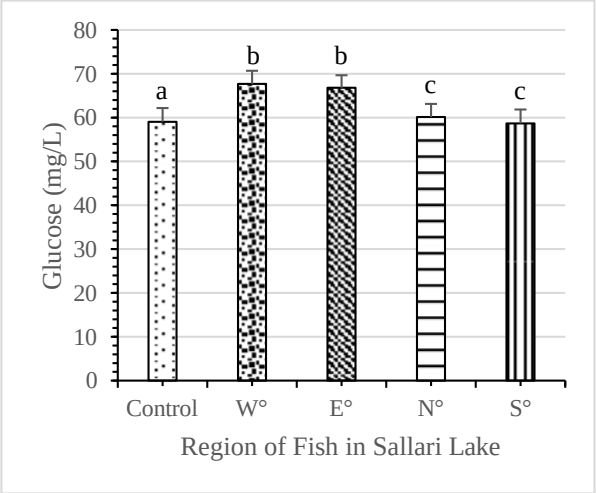

Figure 4: Status of serum glucose in fish caught in various regions of Sallari Lake in comparison to the control.

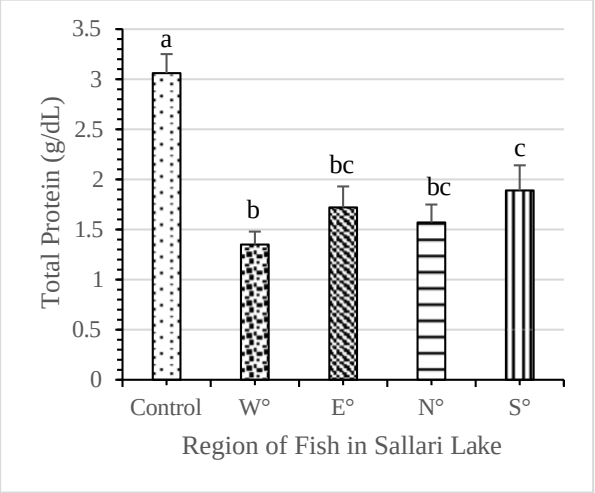

Figure 5: Status of serum total protein of fish caught in various regions of Sallari Lake in comparison to the control.

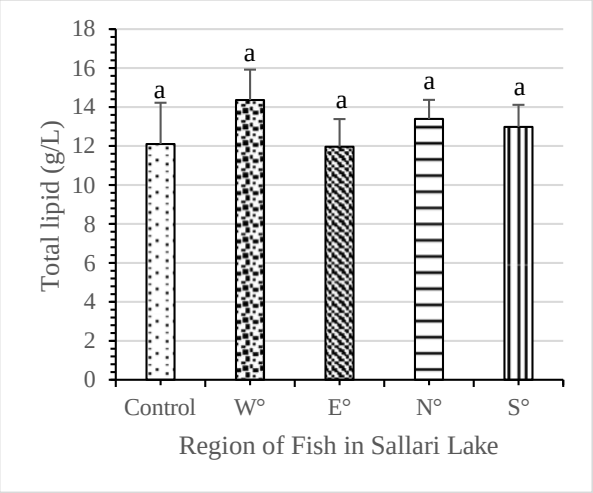

Figure 6: Status of serum total lipid of fish caught in various regions of Sallari Lake in comparison to the control.

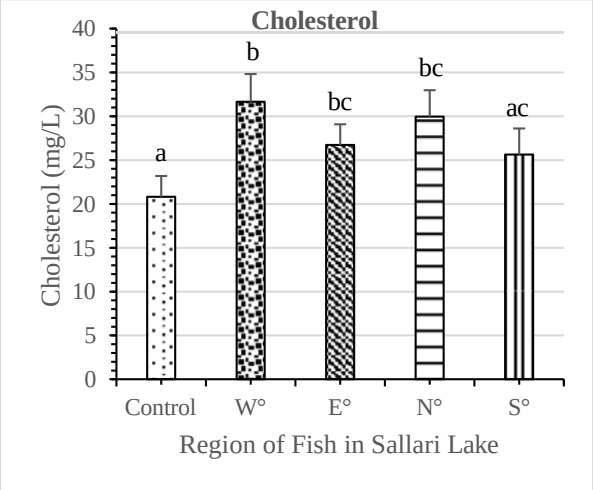

Figure 7: Status of serum cholesterol of fish caught in various regions of Sallari Lake in comparison to the control.

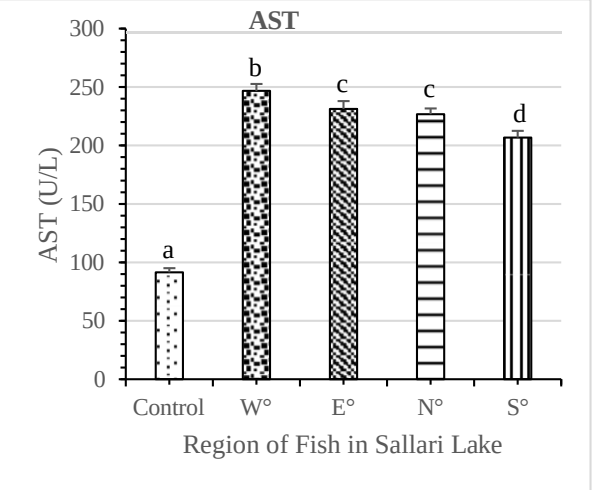

Figure 8: Status of serum AST of fish caught in various regions of Sallari Lake in comparison to the control.

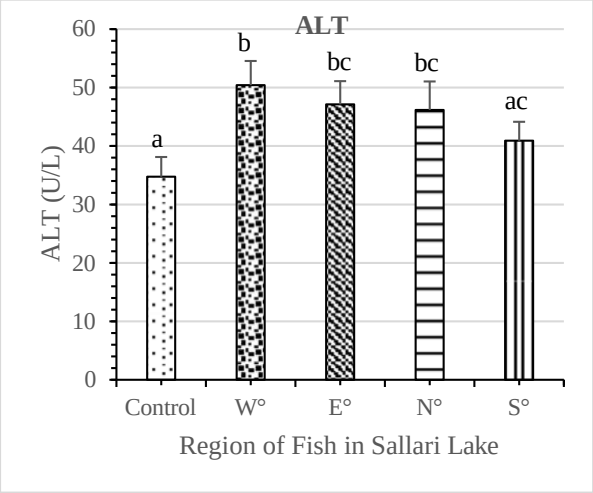

Figure 9: Status of serum ALT of fish caught in various regions of Sallari Lake in comparison to the control.

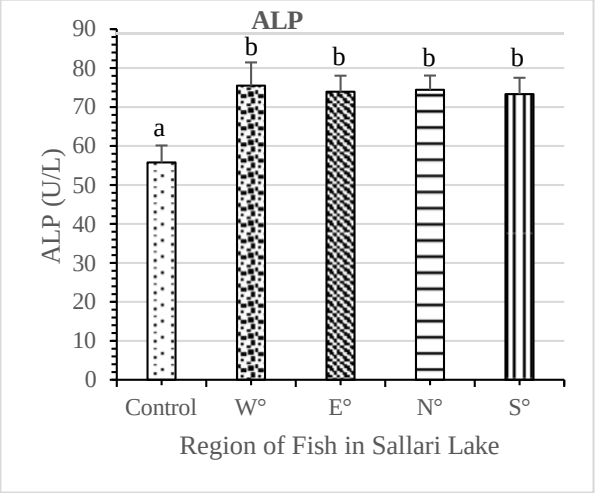

Figure 10: Status of serum ALP of fish caught in various regions of Sallari Lake in comparison to the control.

NB: All the data presented in figures 1–10 is in $\bar{x} \pm SD$ and $n = 5$. Bars with different superscript letters are significantly different from one another at $p < 0.05$.

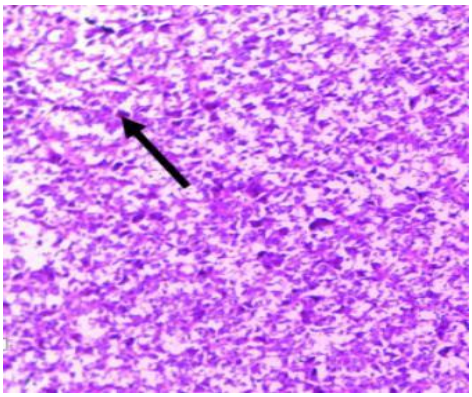

Figure 11: Liver section of a fish from the N° region of Sallari Lake showing degenerated hepatocytes (black arrowhead).

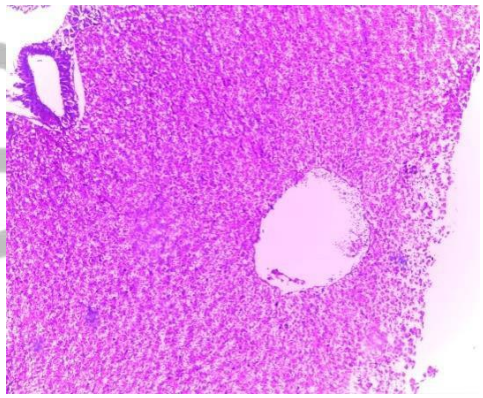

Figure 12: Liver section of a fish from the control pond showing no pathological lesion.

Discussion

When compared to fish from the control pond, fish in the four zones of the Sallari lake showed a significant decrease in the haematological indices RBC, Hb, and Hct. This significant decrease may have been caused by the destruction of mature erythrocytes or the prevention of their development. Consequently, fish species exposed to environmental pollutants may develop severe anaemia (Ucar *et al.*, 2020). A sensitive and accurate indicator of contaminants causing oxidative stress in fish is blood glucose (Ghayyur *et al.*, 2019). Alterations in the liver's carbohydrate metabolism may be responsible for the spike in serum glucose levels seen in fish from Sallari Lake. These changes may also cause hepatic glycogenolysis and glycolysis (Al-Otaibi *et al.*, 2019; Khan *et al.*, 2018). When compared to the controlled pond, the fish taken from Sallari Lake had less total protein, which could be a result of

the liver's inability to produce blood proteins or the breakdown of subcellular structures that produce proteins (Ullah *et al.,* 2019). The protein deficiency that has been detected could also be caused by protein loss from injured organs (Nara *et al.,* 2017; Jain and Batham, 2016).

It is possible that a disruption in the metabolism of lipids is the cause of the marginal rise in serum total lipids seen in some Sallari lake fish populations as compared to the control group. while impaired fatty acid oxidation may be to blame for the significant increase in serum cholesterol levels in Sallari Lake fish (Ghayyur *et al.,* 2019). A reliable indicator of stress is the aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzyme activity in serum. The large variations in these enzymes' serum activity suggest stress-related tissue damage (Al-Ghanim *et al.,* 2020; Fredianelli *et al.,* 2019). Increased serum AST and ALT levels in Sallari lake trout suggest that the liver is leaking these enzymes into the bloodstream (Ghaffar *et al.,* 2020; Galal *et al.,* 2018). Ghaffar *et al.,* (2016) claim that this is due to hepatocellular harm or cellular deterioration brought on by impurities or pollutants in the Sallari Lake.

Alkaline phosphatase (ALP) activity was found to be significantly greater in the blood of the fish *Oreochromis niloticus* from Sallari Lake when compared to controls. This data implies liver impairment in fish from the lake's most extensively polluted regions. This outcome is in line with previous studies that connected an increase in serum ALP activity to liver cell damage (Ghaffar *et al.,* 2018). Given that ALP is a multifunctional enzyme involved in the synthesis of nuclear proteins, nucleic acids, and phospholipids, increased ALP activity may be connected to tissue injury (Galal *et al.,* 2018). The hepatocyte injury of the fish in this region was corroborated by the histopathological findings of fish taken from the E° portion of the lake.

Conclusion

Fish from Sallari Lake show a decline in haematological indices, possibly due to adult erythrocyte destruction or stopped development. Liver carbohydrate and protein metabolisms change, leading to increased glucose levels and reduced total protein, respectively. Serum total lipids increase, while cholesterol rises due to decreased fatty acid oxidation. Stress-related tissue damage is indicated by elevated levels of the enzymes AST and ALT. These results showed that the fish are exposed to toxic pollution that affects their health status. Since these fish are consumed by the people who live around this lake or even sold to people far away, it can be harmful to the health of the people who consume them as a means of nutrition. There is a chance that contaminants could bioaccumulate in the tissues of these fish. Therefore, it is advised that the state legislature pass a law outlawing the disposal of trash in Sallari Lake.

List of Abbreviation

AST = Aspartate transaminase (Aspartate aminotransferase)

ALT = Alanine transaminase (Alanine aminotransferase)

ALP = Alkaline phosphatase

RBC = Red blood cell

Hb = Haemoglobin

Hct = Haematocrit, also known as packed cell volume (PCV)

PCV = Packed cell volume

N° = Northern region of the Sallari Lake

S° = Southern region of the Sallari Lake

W° = Western region of the Sallari Lake

E° = Eastern region of the Sallari Lake

DECLARATIONS

Ethical Approval

Not applicable

Competing Interests

All the authors of this work declare no conflict of interest.

Funding

The research is funded by the Federal Government of Nigeria through the Tertiary Education Trust Fund (TETFund).

Authors' Contribution

Tajudeen Afolayan Lawal developed and designed the methodology of the research. He carried out the supervision of the project. He also provided the resources and carried out the statistical analysis of the data.

Qazeem Oyeniyi Sholadoye prepared the published work; he specifically drafted the initial write-up of the work. He coordinated the work, and he was responsible for the research activity, planning, and execution.

Olasumbo Adeola Mohammed participated in the investigation process of the research. He participated in the conduction of the experiments and the collection of data for statistical analysis.

Acknowledgements

The authors of this article appreciate the efforts of all the laboratory technologists of the Nigeria Police Academy (POLAC) for their roles in this study.

References

Adham KG, Ibrahim HM, Hamed SS, Saleh RA (2002). Blood chemistry of the Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1757) under the impact of water pollution. *Aquatic Ecology*; 36:549-557.

Al-Ghanim KA, Mahboob S, Vijayaraghavan P, Al-Misned FA, Kim YO and Kim HJ, 2020. Sub-lethal effect of synthetic pyrethroid pesticide on metabolic enzymes and protein profile of non-target Zebra fish, Danio rerio. *Saudi Journal of Biological Sciences.* 27: 441-447. https://doi.org/10.1016/j.sjbs.2019.11.005

Al-Otaibi AM, Al-Balawi HF, Ahmad Z and Suliman EM, 2019. Toxicity bioassay and sub-lethal effects of diazinon on blood profile and histology of liver, gills and kidney of catfish, Clarias gariepinus. *Brazilian Journal of Biology*. 79: 326-336.

Basha P S, and Rani A U (2003). Cadmium-induced antioxidant defense mechanism in freshwater teleost *Oreochromis mossambicus* (Tilapia). *Ecotoxicological Environment*. Saf; 56:218-221.

Blaxhall, P., and Diasley K. 1973. Some blood parameters of the rainbow trout. I. The Kamloops variety. *Journal of Fish Biology*. 5:1-8.

Carolin, C.F., Kumar, P.S., Saravanan, A., Joshiba, G.J., Naushad, M., 2017. Efficient techniques for the removal of toxic heavy metals from aquatic environment: A review. *Journal of Environmental Chemistry and Engineering*. 5, 2782–2799. http://dx.doi.org/10.1016/j.jece.2017.05.029.

Chiu, HF, Chang CC, Chen CC, Yang CY, 2011. Calcium and magnesium in drinking water and risk of death from kidney cancer. *Journal of Toxicology and Environmental Health.* 74:6270.

El-Serafy SS, Ibrahim SA, Mahmoud SA, 2005. Biochemical and histopathological studies on the muscles of the Nile Tilapia (*Oreochromis niloticus*) in Egypt. *Egyptian Journal of Aquatic Biology and Fish.* 9:81-96.

Fernandes M N, and Mazon A F., 2003. Environmental pollution and fish gill morphology. In: Fish adaptation. Val AL, Kapoor BG. Eds. Enfield, Science Publishers., 203-231.

Fredianelli AC, Pierin VH, Uhlig SC, Amaral Gurgel Galeb L, Coatti Rocha DC, Ribeiro DR, Anater A and Pimpao CT, 2019. Hematologic, biochemical, genetic, and histological biomarkers for the evaluation of the toxic effects of fipronil for Rhamdia quelen. *Turkish Journal of Veterinary and Animal Sciences*. 43: 54-59.

Gaber HS, Ibrahim SA, EL-Kasheif MA, El-Ghamadi FA., 2014. Comparison of tissue lesions in two species of marine fish (*Solea solea* and *Mugil cephalus*) Inhabiting Bardawil Lagoon. *Research Journal of Pharmacology, Biology and Chemical Science*. 5:62-74.

Galal AA, Reda RM and Mohamed AA, 2018. Influences of Chlorella vulgaris dietary supplementation on growth performance, hematology, immune response and disease resistance in *Oreochromis niloticus* exposed to sub-lethal concentrations of penoxsulam herbicide. *Fish & Shellfish Immunology*. 77: 445-456.

Ghaffar A, Hussain R, Abbas G, Kalim M, Khan A, Ferrando S, Gallus L and Ahmed Z, 2018. Fipronil (Phenylpyrazole) induces hemato-biochemical, histological and genetic damage at low doses in common carp, Cyprinus carpio (Linnaeus, 1758). *Ecotoxicology*. 27: 1261-1271. https://doi.org/10.1007/s10646-018-1979-4

Ghaffar A, Hussain R, Aslam M, Abbas G and Khan A, 2016. Arsenic and urea in combination alters the haematology, biochemistry and protoplasm in exposed rohu fish (Labeo rohita) (Hamilton, 1822). *Turkish Journal of Fisheries and Aquatic Sciences.* 16: 289-296. https://doi.org/10.4194/1303-2712-v16_2_09

Ghayyur S, Tabassum S, Ahmad MS, Akhtar N and Khan MF, 2019. Effect of Chlorpyrifos on Hematological and Seral Biochemical Components of Fish Oreochromis mossambicus.

Pakistan Journal of Zoology. 51: 1047-1052. http://dx.doi.org/10.17582/journal.pjz/2019.51.3.1047.1052

GM (2023). Google map application; https://maps.google.co/uk

Haluzova I, Modra H, Blahova J, Havelkova M, Siroka Z, Svobodova Z., 2011. Biochemical Markers of Contamination in Fish Toxicity Tests. *Interdisciplinary Toxicology.* 4:8589.

Hyvarinen, A., and Nikkila E. 1962. Specific determination of blood glucose with o-toluidine. *Clinica Chimica Acta.* 7:140-143.

Ighalo, J.O., Adeniyi, A.G., 2020. A comprehensive review of water quality monitoring and assessment in Nigeria. *Chemosphere.* 260, 127569.

Jain S and Batham D, 2016. Acute toxicity and biochemical studies of lead nitrate on the liver and kidney of freshwater fish Mystus cavasius. *Journal of Global Biosciences*. 5: 4590-4597.

Javed M, Usmani N, 2015. Stress response of biomolecules (carbohydrate, protein and lipid profiles) in fish Channa punctatus inhabiting river polluted by Thermal Power Plant effluent. *Saudi Journal of Biological Sciences*. 22:237–242.

Kadry SM, Tayel SI, Afify MFH, El-Sayed RA., 2015. Ecohistopathological Studies on *Oreochromis niloticus* fish living in Damietta Branch in Egypt. *World Journal of Pharmaceutical Sciences.* 3:815-825.

Khan N, Ahmad MS, Tabassam S, Nouroz F, Ahmad A and Ghayyur S, 2018. Effects of sub-lethal concentration of cypermethrin on histopathological and hematological profile of rohu (Labeo rohita) during acute toxicity. *International Journal of Agricultural Biology.* 20: 601 – 608 .

Liebel S, Tomotake MEM, Ribeiro CA, 2013. Fish histopathology as biomarker to evaluate water quality. *Ecotoxicology and Environmental Contamination.* 8:9-15.

Perrier H, Perrier C, Peres G, Gras J (1979) The lipoproteins of the plasma of the rainbow trout (Salmo gairdnerii Richardson): immunoelectrophoresis, selective precipitation and lipid composition. *Comparative Biochemistry and Physiology.* 62B:245–248.

Reddy PB (2017) Evaluation of malathion induced oxidative stress in Tilapia mossambica. *Trend in Fisheries Research*. 6: 2319 – 4758.

Reddy PB, Kusum W (2013). Using histopathology of fish as a protocol in the assessment of aquatic pollution. *Journal of Environmental Research and Development* 8:371-375.

Ucar A, Özgeriş FB, Yeltekin AÇ, Parlak V, Alak G, Keleş MS and Atamanalp M 2020. The effect of N-acetylcysteine supplementation on the oxidative stress levels, apoptosis, DNA damage, and hematopoietic effect in pesticide-exposed fish blood. *Journal of Biochemical and Molecular Toxicology*. 33: e22311. https://doi.org/10.1002/jbt.22311

Ullah R, Zuberi A, Ullah S, Ullah I and Dawar FU, 2019. Cypermethrin induced behavioral and biochemical changes in mahseer, Tor putitora. *Journal of Toxicological Sciences.* 39: 829-836. https://doi.org/10.2131/jts.39.829.

Vardhan, K.H., Kumar, P.S., Panda, R.C., 2019. A review on heavy metal pollution, toxicity and remedial measures: Current trends and future perspectives. *Journal of Molecular Liquids*. 290, 111197. http://dx.doi.org/10.1016/j.molliq.2019.111197.

Villarín, M.C., Merel, S., 2020. Assessment of current challenges and paradigm shifts in wastewater management. *Journal of Hazardous Materials.* 390, 122–139. http://dx.doi.org/10.1016/j.jhazmat.2020.122139.

Yousuf R, Mir SH, Tanveer S, Chishti MZ, Darzi MM, Mir MS (2012). Comparative Bio-chemical Evaluation of *Schizothorax niger* and *Cyprinus carpio* from River Jhelum of Kashmir Valley. *Research Journal of Pharmaceutical, Biological and Chemical Sciences.* 3:116-131.

Zak, B. (1957). Simple rapid microtechnique for total serum cholesterol. *American Journal Clinical Pathology*. 27: 583 – 588.

Appendix I: AST Calibration Curve

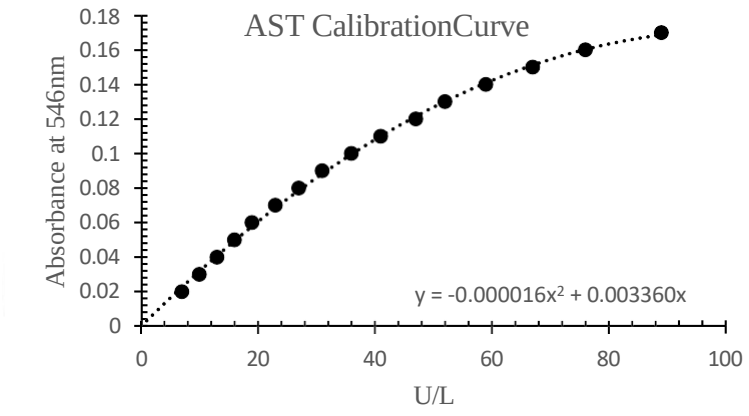

Figure A1: AST Standard Calibration Curve

Appendix II: ALT Calibration Curve

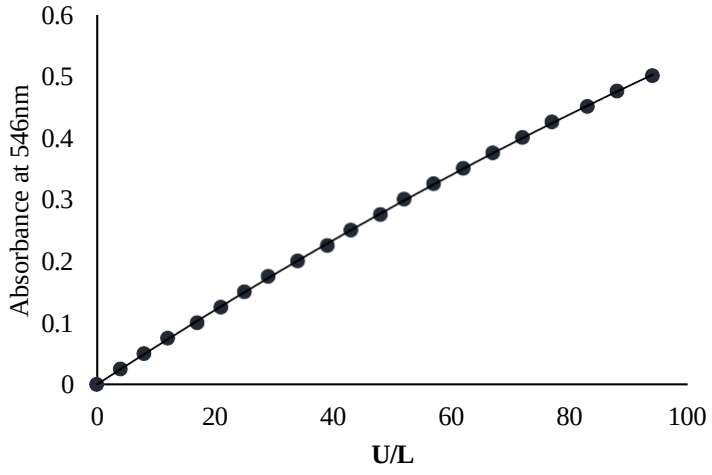

Figure A2: Standard curve for ALT