

# Cellular stress response, induction of micronuclei and DNA strand breaks in two common fish species of Rivers and Reservoirs in Ilorin, North Central, Nigeria.

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## Research Article

**Keywords:** Rivers, Reservoirs, polluted water, Oxidative stress, *Tilapia zillii*, *Clarias gariepinus*.

**Posted Date:** June 24th, 2021

**DOI:** <https://doi.org/10.21203/rs.3.rs-172188/v1>

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# Abstract

Most rivers and reservoirs in the world are prone to pollution because of indiscriminate disposal of domestic, agricultural and industrial wastes into the water bodies. In this study, we investigated the ecotoxicological potential this could pose in fish species collected from major reservoirs and rivers in Ilorin, north central, Nigeria. Water samples were collected and the physicochemical parameters were examined from five different sites; Unilorin reservoir, Asa reservoir, Apodu reservoir, Asa river (Unity) and Asa river (Harmony). We determined serum biochemical (AST, ALT, ALP, serum ALB), histopathological (gill, lungs), serum antioxidant enzyme responses (SOD, CAT, GPx, GR, GST) which serves as a biomarker for evaluating oxidative stress while micronucleus and comet assays were used to detect level of DNA damage in *Tilapia zillii* and *Clarias gariepinus*. The physicochemical parameters and heavy metal analysed (Pb, Mn, Cu, Ni, N, P, Fe, Cl, and Ca) in the five different water bodies were below the permissible limits of WHO and USEPA except the DO, which was very low in the two rivers, indicating hypoxia. Our results showed significant increase in biochemical and hematological profiles, histopathological lesions in the gill and lungs, inductions of MN, NA and DNA single strand break in *Tilapia zillii* and *Clarias gariepinus* collected from Asa rivers compared to the Unilorin, Apodun and Asa reservoirs. This may be attributed to indiscriminate discharge of effluents from nearby industries, agricultural and domestic wastes into the rivers.

## 1. Introduction

Water is a universal solvent capable of dissolving many substances to some varying degree. It is an invaluable resource as it offers a major support to both all flora and fauna. Reservoirs and rivers are very useful ecological resources that serve numerous human needs such as water conservation (irrigation water supply for domestic needs), flood control, hydroelectric power generation, etc (Mustapha, 2010). There have been challenges of water conservation due to pollution. This could be attributed to the daily influx of pollutants from domestic agricultural waste and industrial wastes which have adverse effects on ecosystem health (Kushwaha et al. 2012; Anifowoshe et al. 2018; Oladipo et al. 2018). Water pollution is recognized globally as a potential threat to both human and other animal population which interact with the aquatic environment; this causes gradual degradation to the quality of the water body (Sevenson et al. 1995; Anifowoshe et al. 2018; Anifowoshe et al. 2019).

Fish are of ecological importance and commercially valued in the Nigerian fishing industries and around the world (Ita 1980; Oladipo et al. 2018). Thus, fishes like *Clarias gariepinus* and *Tilapia zillii* are frequently and widely cultured in ponds and they also occur freely in Nigerian's natural fresh water. Hence, aqueous environment variability as well as physicochemical changes have great effect on the fish physiology (Musa and Omoregie 1999) Fishes are good indicators of biocontamination because their biochemical stress responses are quite similar to those of mammals (Mishra and Shukla 2003; Anifowoshe et al. 2019), as they accumulate pollutants directly from contaminated water.

Fish have also been known as a good choice of animal for use in evaluating toxic potential of contaminants (Martin and Costa 2015) due to their ability to metabolize and store pollutants in water in various their organs. They also show fast responses to low concentration of toxicants and the responses are alike to that of vertebrates since fish possess similar vertebrate antioxidant enzymes used to nullify the harmful effects of ROS (Almeida et al. 2002; Klobucar et al. 2010).

Asa reservoir, Oyun reservoir and Apodu reservoir and the water bodies of the following rivers support a wide range of fish species and constitute the bulk of water being used in Ilorin metropolis, University of Ilorin and Malete town respectively in Kwara state (Omotosho 1998; Mustapha 2010; Oladipo et al. 2018). Similarly, fresh fish and fish products were supply to the populace through it resources. However industrial, agricultural and domestic waste discharges and practices are carried out along the bank of the river. These discharges contaminate the water with heavy metals and cause devastating effect on ecological balance of recipient environment (Anifowoshe et al. 2018; 2019).

This present study is an attempt to give an updated information on the current genotoxic status of major water bodies in Kwara state Nigeria by investigating the physicochemical parameters of the waterbodies, tissue lesions, oxidative stress and antioxidant response in the selected fish species and assessing the level of genotoxic damage induced in the selected fish collected from five different waterbodies using micronucleus and comet assays.

## **2. Materials And Methods**

### **2.1 Study Site**

In this study, we collected different species of fish from five different locations: These include Asa reservoir (ARe), Unilorin reservoir (URe), Apodu reservoir (APRe), Asa river (Unity) (ARiU) and Asa river (Harmony) (ARiH) as indicated in figure 1.

#### **2.1.1 Asa reservoir (ARe)**

Asa reservoir lies at a point 5 kilometers south of Ilorin across river Asa between latitudes 8°25'N-8°27'N and longitude 4°32'E-4°34'E. The reservoir was constructed between May 1975 and January 1977, with the aim of meeting the ever-increasing demand of the villagers and nearby community for drinking, irrigation, and as a source of income through commercial fishing for the rapidly growing population of Ilorin, the state capital of Kwara state. The major tributaries are rivers Iwonte, Jia, and Segbekuke. The reservoir is very large and wide with a maximum length of 20km, a breadth of 7km and a depth of 13m, with a storage capital of about 43 million cubic meters (Omotosho 1998).

#### **2.1.2 University of Ilorin Reservoir (URe)**

University of Ilorin reservoir is located within the university campus which lies entirely within the basement rocks in the Western part of Central Nigeria bounded by longitudes 4° 40'52" to 4° 41'0"E and

latitude 8° 27'5" to 8°28'5"N in Ilorin south local government of Kwara state. URe was established in 1975 while physical development of the structures commenced in 1979. It was refurbished between 2015 and 2016 with a depth of about 5m and a submerged area of about 650 km<sup>2</sup>. The main purpose of the dam is to supply the university communities water for dinking and irrigation. No industry is located on its course, thus, the water rarely received any wastes.

### **2.1.3 Apodu reservoir (APRe)**

Apodu reservoir in Apodu village, about 7 km away from Maleta Town in Moro local government area of Kwara State, North Central Nigeria. The dam was constructed in the year 1980 and the re-impoundment of the dam was carried out in 2016. It lies between the longitude 8°45'25.9" N, 45'27.7" N and latitude 4°27'41.4"E, 4°27'35.5"E with 560m long and 400m wide and has a depth of 8.2m with its surface area of about 15 hectares. It is characterized by two seasons' i.e. dry season in which water flow rate decreases and rainy season in which there is continuous flow of water, it falls within the guinea savannah region of Nigeria. The main purpose of the reservoir is to provide water for drinking and irrigation for the surrounding communities (Anifowoshe et al. 2018; Oladipo et al. 2018; Oladipo et al. 2019).

### **2.1.4 Asa River (Unity) (ARiU)**

Asa river is one off the major rivers in Ilorin Kwara state with the coordinate of 8°28'0"-8°31" N and 4°32'0"-4°34" E. Different companies like Dangote, Coca-cola, pharmaceutical and detergent industries are located near the river. Little agricultural activities like Ugwu and cassava plantation are also found near the river. The dumping of refuses, sewage disposal and indiscriminate discharge of effluents/chemicals from the above industries into the body of the river is common thereby making the water appear unfit for drinking and domestic purposes (Kolawole et al. 2011).

### **2.1.5 Asa River (Harmony)(ARiH)**

ARiH is a segment along Asa river with coordinates 8°30'0"-8°32" N and 4°33'0"-4°34" E. It is located along Harmony estate in Ilorin with little amount of waste entering into the waterbody.

## **2.2 Experiment**

Two different fish species, *Tilapia zillii* and *Clarias gariepinus* were collected from the five sites namely Unilorin reservoir, Apodu reservoir, Asa reservoir, Asa river (unity) and Asa river (Harmony) in the early hours of the day (6:00-8:30am). They were caught with the help of fishermen using cast net, gill net and basket. The fishes caught were sorted and identified into species level following Idodo-Umeh (2003) and Olaosebikan and Raji (1998) and also with the help of a fish taxonomist. *T. zillii* (scaled fish) and *C. gariepinus* (scale-less fish) were the two common fish species caught at the various sites, however, *T. zillii* fishes were abundance and were used mostly in our experiment.

The sampled fishes were transported transparent 50-L plastic aquaria with net cover (to allow ventilation and prevent the fish from jumping out) filled with water two-third volume to the Department of Zoology,

University of Ilorin, Ilorin, Nigeria. The fish were allowed to recover from stress before the collection of blood through the tail region or by caudal vein puncture for analyses. For each analysis, two different fish species (n=2) were used for our experiments.

Water samples from the reservoirs and rivers were assessed for various parameters such as temperature, pH, electrical conductivity, dissolved oxygen (DO), total dissolved solids, total suspended solids, biochemical oxygen demand (BOD), chemical oxygen demand (COD) and heavy metals. Water parameters were measured using a Hanna portable waterproof tester, model HI 98129. The heavy metals were determined using a Hanna multiparameter bench photometer for laboratories, model HI 83200, and an AAS (Atomic Absorption Spectrophotometer) (model: Buck scientific ACCUS-IS 211) was used.

### **2.3 Oxidative stress and tissue lesions analysis**

To carry out the oxidative stress analysis, blood was taken from the *T. zillii* (n=3) from each site and kept in EDTA bottles prior to analysis and protocol by Reitman and Frankel, (1957) was followed with modification. The gills and liver tissue samples for lesion analysis were collected through dissection and fixed. These tissue samples fixed in buffered formal saline for a day (24 hours) were rinsed vigorously with distilled water and processed for paraffin tissue embedding using the methods of Drury and Wellington (1980) and Gobinath and Ramanibai, (2014). The sections obtained from the organs were stained with hematoxylin and eosin (H & E) and observed using an Olympus light microscope using the standard procedure of Bancroft and Gamble (2008).

### **2.4 Haematological Analysis**

Blood analysis was performed according to the methods described by Svobodova et al (2001), the blood samples collected were from *T. zillii* collected at the 5 sampling sites and taken to the laboratory for analyses such as red blood cells (RBC) profiles, white blood cells (WBC) profiles and platelets using standard method Sovio, and Oikari (2004)

### **2.5 Antioxidant Enzyme**

The use of 1-chloro-2,4-dinitrobenzene as substrate was used for determination of Glutathione-S-transferase (GST) activity (Habig et al. 1974). The specific activity of glutathione S-transferase was expressed as nmoles of GSH-CDNB conjugate formed/min/mg protein using an extinction coefficient of 9.6mM<sup>-1</sup>cm<sup>-1</sup>. The catalase activity (CAT) assay based on the breakdown of H<sub>2</sub>O<sub>2</sub> was performed as described by Clairborne (1995) and the absorbance was measured at 240nm (pH 7.0, 28°C) and expressed as unit/mg protein.

Superoxide dismutase activity (SOD) was determined by the method of Misra and Fridovich (1972) in which the assay depends on the auto-oxidation of adrenalin due to the presence of superoxide anion, measured spectrophotometrically at 420nm and expressed as a unit/mg protein. The method of Paglia and Valentine (1967) was used for the assay of glutathione peroxidase. About 0.2ml of tissue

homogenate was added to the mixture containing 0.2ml of buffer, 0.2ml of EDTA and 0.1ml of sodium azide. After well mixing, 0.1ml of reduced glutathione and 0.1ml of hydrogen peroxide were added before incubating in a water bath at 37°C for 10min. At the end of incubation period, 0.5ml of 10% TCA was added and centrifuged at 10000 rpm for 5min. 1.0ml of the supernatant was taken into a separate test tube and added 2.0ml Tris buffer and 50 µl DTNB. Immediately, the OD was measured at 412nm. While Glutathione reductase (GR, EC 1.6.4.2) activity was determined as described by Glatzle et al. (1974).

## 2.6 Micronucleus Assay

Blood was collected from the caudal region of the two fish species (*T. zillii* and *C. gariepinus*) [n=2] each using 2ml syringe and needle. The needle was inserted ventrally at the caudal region of the fish body until it pricks the vertebral column and then moved a little away so that the blood can flow into the syringe. The dispenser pulled about 0.5 ml of blood and immediately a drop of blood was placed on a clean, grease free microscope slides to make a thin blood smear. The smeared slides were allowed to air-dry overnight in a dust free environment. The air-dried slides were fixed in 70% absolute methanol for 20 minutes and air dried overnight. After drying the slide was stained with 10% Maygrunwald which was rinsed with distilled water and air dried overnight. Glass slide was subsequently stained with 5% giemsa, rinsed with distilled water and dried. The dried slide was then scored under light microscope by counting a total number of 2000 erythrocyte which were examined with oil immersion at 1000X magnification for micronucleus (MN) and nuclear abnormalities (NA) as biomarkers of cytogenotoxicity (Carrasco et al. 1990; Ergene et al. 2007).

## 2.7 Comet Assay

A fully frosted slides were pre-coated with 1% normal-melting-point-agarose overnight, which formed the first/base layer. A mixture of 75 µl of 0.7% low-melting-point agarose (LMA) and 25µl of lymphocyte suspension was applied as the second layer. Cover slips were immediately placed over the second layer, and the slides were chilled on ice for 10 min to solidify the agarose. The cover slips were removed and a third layer of 90 µl 0.5% LMA was applied, the cover slips replaced, and the agarose allowed to solidify over ice for 10 min. All samples were done in triplicate. The slides were immersed in cold alkaline lysis solution for 2 h at 4°C. Then, slides were placed in chilled buffer for 20 min at room temperature in a horizontal electrophoresis tank pre-filled with cold alkaline electrophoresis buffer to loosen the tight double-helical structure of DNA for electrophoresis. Electrophoresis was then performed at 25 V, 300 mA for 20 min in electrophoresis buffer at 4°C. After electrophoresis, Tris buffer (0.4 M Tris, pH 7.5) was gently added drop-wise to neutralize excess alkali; the buffer was allowed to remain on the surface of slides for 5 min. This neutralizing procedure was repeated three times. The slides were then stained with 80 µl propidium iodide (2 µg/ml) for 10 min. All of the above procedures were performed in the dark to avoid additional DNA damage (Bajpayee et al. 2005). The comets were viewed using a Nikon 90i fluorescence microscope, and images of 100 comets were collected for each concentration using a digital imaging system. Cells that overlapped were not counted. All the comet images were analyzed using

Comet Assay Software Project (CASP, Wroclaw University, Poland) and the tail length (TL), % tail DNA (%TDNA), and Olive tail moment (OTM) were recorded to describe DNA damage to lymphocytes.

## 2.8 Statistical Analysis

The SPSS software package version 21.0 (SPSS 21.0) was used to evaluate the micronucleus abnormalities and the differences between the test groups. The differences were analysed by comparing them with the use of one-way ANOVA, and the level of statistical significance was estimated at  $p < 0.05$  using the Duncan multiple range test (DMRT), the mean standard error was calculated.

## 3. Results

### 3.1 Physicochemical parameters of the water

The physicochemical parameters (COD, BOD, DO, TDS, pH, conductivity & temperature) and heavy metals analysed in the water samples (Manganese, Copper, Chloride, Nitrate, Phosphate, Iron, Nickel, and Calcium) were mostly within the maximum level approved by standard organization except lead (Pb) that was above the standard level approved, the value was high at 2.683 mg/l in Asa reservoir which also recorded high value of Cu at 1.29mg/l. Also, Ca, Ni, and Cl were observed to be highest at Unilorin reservoir (Table 1). High values of COD, BOD, TDS and Conductivity were detected in Unity, Apodu reservoir and Asa river Harmony. Here, we observed that Asa reservoir seems to have low values of all the parameters measured when compared to the other four sites.

### 3.2 Biochemical and tissue lesions analyses

In table 2, there was a significant ( $p < 0.05$ ) increase the serum ALT, AST, ALP and albumin enzyme activities of *T. zillii* in Asa river Unity compared to other four sites. The AST enzyme activities in Asa river Unity and Asa river Harmony show higher activities compared to the reservoirs. Similarly, the ALT of Asa river Unity and Apodu reservoir also showing higher activities compare to other sites.

Figure 2I representing the photomicrograph of the gills of *T. zillii* from the sampling sites. A represents photomicrograph of the gill of *T. zillii* from Unilorin reservoir which shows a high-power magnification of the highly vascularized gill arch and outgrowing lamellae. Apodu reservoir (B) shows a high-power magnification of the highly vascularized gill arch and their adjoining primary and secondary lamellae. It appears normal with long and well vascularized lamellar system with no pathological alteration. The primary lamellae appear short and supported in the central part by a cartilage with appreciable vascular supply. Figure 2I (C) shows highly vascularized gill arch and out growing lamellae of *T. zillii* from Asa reservoir with no signs of pathological alterations. The primary lamellae appear short and supported in the central part by a cartilage with appreciable vascular supply. Figure 2I (D) represents the photomicrograph of *T. zillii* gill from Asa river in Unity which shows a high-power magnification (x400) of the gill arch and gill rakes. There appears to be some pathological alteration in the secondary lamella as they appear to be degenerating, distorted cartilage with epithelial lining. In Figure 2I (E) which represents

the photomicrograph of the gills of *T. zillii* from Harmony, shows the gill arch and their adjoining primary and secondary lamellae. It appears distorted with as in the gill arch and perturbation of gill filaments which appear to be degenerating and distorted.

Representative photomicrographs of the liver in Unilorin reservoir in Figure 2II (A) shows densely exhibited hepatocytes nuclei (black arrows) with normal staining characteristics and cellular disposition. Their general histomorphology appear characteristically normal with no apparent pathological alteration. In C; *T. zillii* liver from Asa reservoir shows densely distributed hepatocyte nuclei (black arrow). The micrograph appears characteristically normal with typical staining intensity and normal histoarchitectural manifestation. In D (Unity); the photomicrograph shows the central vein (black outline) and a high magnification of the hepatocytes (black arrow) revealing the disposition of the nuclei, staining intensity and general histomorphological presentation. Histomorphological presentation of the liver showing typically sized halo spaced central vein surrounded by densely distributed hepatocytes. In E (Harmony), the *T. zillii* liver shows densely distributed hepatocyte nuclei (black arrow). The micrograph appears characteristically normal with typical staining intensity and normal histoarchitectural manifestation.

### 3.3 Haematological analysis

Value of hematological parameters are presented in table 2 as mean value with  $\pm$  standard deviation. Data presented in table 3A indicates significant increase ( $p < 0.05$ ) in RBC (red blood cell), HGB (haemoglobin) MCV (mean corpuscular volume) and MCH (mean corpuscular haemoglobin) in *T. zillii* from Asa river (Unity) compared to other sampling sites. There was also a significant increase in MCH and MCHC (mean corpuscular haemoglobin concentration) in fish from Asa river (Harmony). The differences for HCT (haematocrit) and RDW (red blood cell distribution width) were insignificant across the 5 sites. In WBC profiles (WBC (white blood cell), LYM (lymphocyte), MID (mid-sized cells and GRAN (granulocyte)) measured, no significant difference ( $p < 0.05$ ) was observed across the 5 sites. However, *T. zillii* at Asa river (Unity) show a significant increase in GRAN when compare to others (Table 3B). PLT (platelet) MPV (mean platelet volume), PDW (platelet distribution width) PLCR (platelet larger cell ratio) and PCT (plateletcrit) measured show no significant difference ( $p < 0.05$ ) across the 5 sites. However, there was a significant increase in PLT and PDW in *T. zillii* at Asa river (Harmony) and PLT and PLCR at Asa river (Unity) (Table 3C).

### 3.4 Antioxidant production

The trend of antioxidant enzymes responses in blood of *T. zillii* across the five sampling sites were statistically different ( $p < 0.05$ ) in the order; SOD responses was highest in Unity river; followed by Unilorin; Harmony; Apodu and lowest response recorded at Asa reservoir; CAT responses was significantly highest in Unity; followed by Harmony; Unilorin; Apodu; and least significant in Asa reservoir; GPx responses was greatly induced in Apodu; followed by Asa reservoir; Unity; Harmony; and lowest in Unilorin; GR responses was significantly Unilorin; followed by Apodu; Harmony; Unity and Asa reservoir; GST responses was highest in Asa reservoir; followed by Apodu; Unity; Harmony and Unilorin (Table 4; Figure 3).

### 3.5 Micronucleus test

The peripheral blood erythrocyte of fish species (*T. zillii* & *C. gariepinus*) collected at different the sites show induction of micronuclei (MN) and other nuclear abnormalities (NA) which include; binucleated, nuclear bud, notched, lobed and blebbed. Site comparisons were conducted with each of the two fish species collected. The level of damage observed based on the frequency of MN & NA (Figure 4I) are in the following descending order: (ARiU) > (ARiH) ≥ (APRe) ≥ (URe) > (ARe) (Figure 4II). It was observed that *T. zillii* showed a higher level of NA when compared to *C. gariepinus* in this study (Figure 4II).

### 3.6 Comet assay

The level of DNA damage observed in the peripheral lymphocytes of fishes obtained at the various sites were measured using the alkaline comet assay with parameters such as %tail DNA, olive tail moment and tail length as shown in Figure 5a and b. Significant differences ( $p < 0.05$ ) were observed at some the sampling sites. The highest degree of DNA single strand break was detected in the blood cells of fishes collected from Asa river Unity. No significant difference in the level of DNA single strand break was observed in fishes collected at Unilorin and Asa reservoir. Over all the DNA damage observed between the two fish species can be said to be higher in *T. zillii* when compared to *C. gariepinus*. DNA damage was observed to be higher in fish species collected from the river sites when compared to the selected reservoirs. It was observed that at the reservoir sites, there is elevation in the level of DNA damage in Apodu reservoir. The level of DNA damage across the 5 sites goes in this order: Asa river unity > Apodu, > Asa river harmony, > Unilorin > Asa reservoir

## 4. Discussion

Most rivers and reservoirs in the world are prone to pollution because of indiscriminate disposal of domestic, agricultural and industrial wastes into the water bodies. The increased discharge of industrial effluents, domestic and agricultural wastes into waterbodies has raised a great concern as these effluents are toxic and can alter the ecology and genetic composition of aquatic animals. The physico-chemical parameters show low level of DO and high level of BOD in the rivers (Asa river Unity & Harmony) indicating hypoxia and slight nitrate content owing to runoff from agricultural farms around the area which employ the use of pesticides and fertilizers for farming. Low level of DO of the river and high BOD and COD values obtained from Asa river in Unity may be as a result of the industrial effluents as well as domestic wastes and run off some agricultural activities around the river bank. This a result of de-oxygenation caused by the influence of industrial effluents discharge into the water body. DO has been regarded as the most important parameter for assessing water quality because it influences the fauna and flora distribution (Morrison 2001). High BOD and COD values are principal indicators of the presence of organic and inorganic pollutants respectively. The higher value of COD and BOD correlate with the work of (Kolawole et al. 2011) on this river which are implicative of water pollution. Changes in the water quality parameters have been linked to the activation of the antioxidant defense system in fish and other aquatic organisms living there and this may further elevate antioxidant responses in these organisms in

the presence of environmental pollutants from agricultural, domestic, industries, landfill leachates among others leading to generation of reactive oxygen species (ROS) which result in oxidative stress in the biological organism like fish (Slaninova et al. 2009; Sevcikova et al. 2011).

The ALP, AST and ALT are enzymes produced in the liver and their increase in the blood indicates injury to the liver. The significant increased levels of oxidative enzymes in Asa river Unity indicate fish liver inflammation, injury, stress and disease and this might be attributed to high levels of conductivity, BOD, COD and decrease in DO which may have subjected the fish to undue stress. This correlates with findings of Oshode et al. (2008) that reported high level of AST in assessed fish samples as a result of pollution of the water sites.

The gills, one of the vital organs and considered the primary target of contaminant as it remains in close contact with external environment (Camargo and Martinez 2007). The histopathological lesions observed in the gills especially at Asa river Unity and Harmony indicate that the fish respond to the effects of toxic agents present in the water. This could cause disturbance in the blood flow in the gills as observed by Stentiford et al. (2003). The organ most associated with the detoxification and biotransformation process is the liver which most affected by contaminants in the water. The liver histological presentation in Asa river Unity, Asa river Harmony and Apodu reservoir showed abnormal liver with vacuolation in architecture. This alteration may be a result of pollutant in the ecosystem as observed by Oladipo et al. (2018; 2020).

Blood is a tissue that is sensitive to any changes in our environment. It is made up of RBC, WBC, PLT and plasma. They are good bioindicators for assessing the quality of water (Kopp et al. 2013; Khan et al. 2015; Parrino et al. 2018; Osman et al. 2018; Anifowoshe et al. 2018; 2019). Water contamination can cause changes in haematological parameters. In our study, a significant increase ( $p < 0.05$ ) in RBC, HGB, MCV, MCH, GRAN, PLT and PLCR in *T. zillii* from Asa river (Unity) was observed compared to other sampling sites. There was also significant elevation in MCH, MCHC, PLT and PDW in fish from Asa river (Harmony). This result is in line with Sahiti et al. (2018) wherein they reported slight differences in the values of HGB, MCHC and WBC and significant changes in RBC, MCV and MCH in blood of common carp (*Cyprinus carpio*) in two lakes of Kosovo. This difference in the RBCs may be due to low level of DO recorded in the rivers (Asa rivers (Unity/Harmony)). Blood parameters such as MCV, MCH and MCHC are important in diagnosing animal anaemia (Coles 1986). The increasing values of these indicators appear in the case of various anaemia.

The oxidative stress and antioxidants responses have been used as biomarkers to evaluate genotoxic potential of rivers in Nigeria like Ogun river, Eleyele river and Asejiri river (Farombi et al., 2007; Arojojoye and Adeosun (2016a); Arojojoye et al. 2016b) but there is little information about the oxidative stress and antioxidant responses of fish in major reservoirs and rivers in Kwara state which provide drinking water and fish to the populace of Kwara state. The antioxidant defense system is usually activated when there is an imbalance in the Reactive Oxygen Species (ROS) level and antioxidant level in fish and this defense is quickly activated to checkmate oxidative stress that may be induced by increased level of pro-oxidants

in the fish (Livingstone 2001). The SOD and CAT are usually the first line of defense against oxidative stress and their activities are easily noticeable as increase in enzyme activity (McCord 1996). Antioxidants may be depleted in cells during exposure to environmental pollutants, but sometimes antioxidants level may increase to compensate the imbalance caused by oxidative stress (Arojojoye et al. 2016a,b).

Increased SOD activities in Unity river and Harmony river may be a response to oxidative stress which could be caused by the low level of oxygen (low DO and high BOD) which may result in hypoxia. Decrease level of oxygen may have induced production of superoxide anions (which are the most dangerous free radicals) which was catalytically scavenged by SOD into  $H_2O_2$  and this a defense readily adopted by the antioxidant enzyme of the fish to reduce toxicity of oxygen. The results obtained in the SOD activities harmony and Unilorin reservoir correlate with Isamah et al. (2000) and Farombi et al. (2007) that attributes increased concentrations of SOD in fish to oxidative stress.

CAT is an important enzyme and acts on hydrogen peroxide in fish exposed to pollutants. CAT in conjunction with SOD are usually the first defense enzymes and CAT levels generally increase in fish exposed to pollutants but decrease in CAT levels has been obtained polluted water bodies as reported by (Farombi et al. 2007). The CAT levels were seen to decrease in representative fish species of Unilorin reservoir and Unity river and increased in Harmony river while the CAT values of Asa and Apodu reservoir were not statistically different. The reduced CAT may indicate that the fish is under oxidative stress as increased production of ROS is slowly beginning to overwhelm the CAT defense. The results in the CAT level is in line with Arojojoye et al. (2016) which established that increase in ROS production is correlated with decreased catalase activities and other antioxidant enzymes. The increased CAT activities in Harmony river is a typical response to environmental pollutants as CAT and SOD represents the first line of defense against oxidative stress.

The GPx levels was highest in blood of representative fish species of Apodu reservoir followed by Asa reservoir and Unity river. The activity of GPx was decreased in Asa river (Harmony) with the lowest values observed in Unilorin reservoir. Elevated GPx levels suggests an adaptive and protective role of this enzyme against oxidative stress induced by the heavy metals or organic pollutants. The elevated GPx level supports the work of Lenartova et al. (1997) that found out that GPx activity of fish was 1.8-fold higher in polluted river. The decreased level of GPx may indicate a low level of pollution in Unilorin reservoir and a condition of self-purification of the Asa river (Harmony).

GR plays a role of catalytically reducing oxidized glutathione in order to maintain GSH/GSSG ratio. GR and GPx work together as increased level of GR may be indicative of oxidative stress and may require an increased level of GPx to counteract the effect of stress (Akpakpan et al. 2014). GR was observed to be highest in the blood of representative fish species of Unilorin reservoir, followed by Asa river (Harmony), Apodu reservoir, Asa river (Unity) and the lowest GR values were obtained in Asa reservoir. Increased GR may portray a situation of oxidative stress in fish, this is in line with the work of Pandey et al. (2003) that observed increased GR in fish with higher level of pollution.

GST was observed to reduce in the blood of representative *T. zillii* of Unilorin reservoir, Asa river (Harmony), Asa river (Unity), Apodu reservoir and elevated in Asa reservoir. The reduction can be attributed to increase in ROS production and according to Arojojoye et al. (2016) antioxidant levels may get depleted in cells during exposure to environmental pollutants.

Next, we determined the level of DNA damaged using two different tests. In this study, micronucleus test which is a reliable and sensitive assay that is mostly consider as a biomarker of DNA damage showed induction of micronuclei and other nuclear abnormalities which increased mostly in *T. zillii* across all the five sites when compared with *C. gariepinus*. Our result is in contrast with Ali et al. (2008) reports where the peripheral blood of *C. gariepinus* was shown to be very sensitive in formation of MN when compared to *T. zillii* which was shown to be sensitive in MN formation compared with two other tilapia species; *Oreochromis niloticus*, and *Oreochromis aureus*. This may be attributed to the fact that *T. zillii* might have accumulated more metals in its body systems and also indicates that its genome well tolerates such type of cytogenetic damage without apoptosis. The obtained results support the fact demonstrated by Kligerman (1982) that fish inhabiting polluted waters as in ARiU site in this present study have greater frequencies of micronuclei. The micronuclei frequencies may vary according to the season, stress, type of pollution and heavy metals.

The comet assay has been used successfully to investigate the effects of genotoxic pollutants on the integrity of DNA. It confers several advantages as a tool for genotoxic studies such as: ability to detect genotoxic damage at the single-cell level, suitability for most eukaryotic cell types; a small number of cells is required, faster and more sensitive than other available methods for detecting strand breaks, relatively low-cost and ability to detect early exposure response of genotoxins (Dhawan et al. 2009; Frenzilli et al. 2009). Evaluation of genotoxicity of the five sampling sites using the comet assay in fish was performed for the first time in this present study across the five sites. The percentage tail DNA(%tail DNA) is the parameter of choice for DNA damage assessment because comets can be observed with the same length but different fluorescence intensities, this makes it ideal for description of DNA damage (Schnurstein and Braunbeck 2001). The (% tail DNA) in the five reservoirs in descending order; Asa river (Unity)>Apodu reservoir> Asa river (Harmony)>Unilorin reservoir>Asa reservoir shows the reservoir with the highest and lowest DNA damage (Asa river (Unity) and Asa reservoir) respectively.

The Asa river (the upstream) which has its course extended towards the Unity area (mid-stream) and Harmony area (downstream) shows a remarkable trend. The Asa river(upstream) has the lowest DNA damage when compared to its courses (Unity and Harmony rivers). The low DNA damage at the upstream and high DNA damage at the midstream could be associated with the level of exposure to genotoxic substances. The increasing extent of genotoxicity in the middle of Asa River is a typical example showing that DNA damage in peripheral erythrocytes of *C. gariepinus* and *T. zillii* are closely linked to contaminant's level when a gradient of pollution exists along the river. The trend observed on the Asa water course corroborate with Hariri et al. (2018) in which DNA damage was highest in the mid-stream (Asara) of the Karai river because of high influx of pollutants from sewage and industries and DNA damage was lowest at the upstream (Varangerud) because it was the region of minimum pollution.

The increase (%tail DNA and tail length) observed in Apodu reservoir compared to negative control can be attributed to the high nitrate pollution due to sewage disposal and runoff from neighbouring farms around the reservoir that utilize pesticides and fertilizers for its farming activities as reported by Anifowoshe et al. (2018). Exposure to contaminants of various sources may produce additive, synergistic or antagonistic interactive impacts on fish (Osman et al. 2012). Accumulation of DNA damage may occur either through an increase in the number of DNA-damaging events or a decrease in DNA repair (Cavas and Konen 2007). The results of the Apodu reservoir is in accordance with (Kushwaha et al. 2012) that revealed a significantly higher degree of DNA damage in comparison with baseline values in *Channa punctatus* and *Mystus vittatus* erythrocytes exposed to contaminated water of River Gomti, India. Unilorin reservoir has low %tail DNA when compared to negative control. This is probably because it is exposed to minimal pollutants as it is used for recreational activities and the location of the reservoir is not proximal to residential areas hence it receives little or no domestic waste, the only possible source of pollution would be runoff during rainy season or from its water course.

The results obtained from the integrated use of comet assay and antioxidant responses to evaluate genotoxic potential were in agreement in most of the sampling sites but contrasted in a few. The results of comet assay and antioxidant enzyme responses both pointed to the fact that Unity and Harmony rivers are polluted. This is similar to results obtained by Nwani et al. (2013) in which the comet assay and antioxidant enzyme responses were in agreement. There is a slight contrast in the Apodu and Unilorin reservoir. The DNA damage is low in Unilorin but the antioxidant responses were not as consistent and this may indicate that reservoir is probably exposed to minute pollutants that are triggering these responses. The Apodu reservoir which shows high DNA damage has moderate antioxidant responses. This is because the comet assay is a more sensitive test than the antioxidant response as it can detect toxins at low concentrations as reported by (Osman et al. 2012). Thus the comet assay in conjunction with the antioxidant enzyme responses helps to view the full picture of the genotoxic status of a water body.

## 5. Conclusion And Recommendations

The genotoxic potential and antioxidant response of five water bodies was investigated using the integrated use of comet assay and antioxidant enzymes responses. The water bodies investigated were observed to be currently at risk of pollution and if the anthropogenic activities on these water bodies remain unchecked, the ecology and the biotic life may deteriorate rapidly than being restored by self-purification. It is highly recommended that the government should enforce laws to eliminate indiscriminate dumping of wastes into water bodies, enlightened the society of the damages of the acts and risk of consuming contaminated fishes.

## Declarations

**Funding:** Not applicable

**Conflict of Interest:** None

**Data Availability:** The data sets generated during and/or analyzed during the study are available from the corresponding author on request.

**Animal Research (Ethics):** The care and use of experimental animals complied with Nigerian animal welfare laws, guidelines and policies

**Consent to Participate (Ethics):** Not applicable

**Consent to Publish (Ethics):** Not applicable

**Plant Reproducibility:** Not applicable

**Author Contribution:** AAT and OSO: designed the study; AAT, OSO, OAN, OA, OEO, AO and AYM: collected, preserved samples and conduct the laboratory analysis; AKM and IOA: provided technical assistance for the study; AAT, OSO, AKM and IOA wrote the initial draft of the manuscript; All authors read and approved the final manuscript.

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## Tables

**Table 1: Physiochemical parameters and metals of the water samples**

Parameter	Unilorin Reservoir	Apodu Reservoir	Asa Reservoir	Asa river Unity	Asa river Harmony	WHO	USEPA
pH	6.1	6.3	5.6	5.6	5.9	6.5-8.5	6.5-8.5
Conductivity (µs/cm)	140	120	80	850	540	-	-
Temperature(°C)	30	29	31	30	29	-	-
TDS (mg/dl)	70	60	40	80	270		500
DO (mg/L)	4.8	3.69	6.8	2.6	2.6	>2	-
COD (mg/L)	28	58.032	34.56	70	56		410
BOD (mg/L)	1.05	3.48	1.85	4.3	3.0	>6	250
Pb (mg/L)	NA	0.342	2.683	0.676	NA	<1	0.015
Mn (mg/L)	0.039	NA	0.029	NA	NA	5	0.05
Cu (mg/L)	0.134	0.002	1.29	0.142	0.036	<1	1.3
Ni (mg/L)	6.09	10.92	5.342	0.155	9.4	20	-
P (mg/L)	0.001	0.782	0.638	0.036	0.033	5	
Fe (mg/L)	0.277	0.483	2.784	1.394	0.288	20	0.3
Cl (mg/L)	7.087	1.049	5.332	0.885	4.664	25	-
Ca (mg/L)	13.196	4.209	0.028	0.396	0.375	75	-

Note: TDS—total dissolved solid, DO—dissolved oxygen, BOD—biochemical oxygen demand, COD— chemical oxygen demand, USEPA— WHO—World Health Organization, NA—Not available.

**Table 2:** Oxidative enzyme activities induced in *T. Zillii* across the five sampling sites represented as mean± standard error of mean (SEM)

ITE	Unilorin reservoir	Apodu reservoir	Asa reservoir	Asa river Unity	Asa river Harmony
ST/U/L	212.50±3.00 <sup>a</sup>	201.78±0.56 <sup>a</sup>	201.84±0.26 <sup>a</sup>	238.95±0.81 <sup>b</sup>	227.10±0.85 <sup>b</sup>
LT U/L	80.10±0.65 <sup>a</sup>	110.07±0.65 <sup>b</sup>	96.66±0.47 <sup>a</sup>	102.5±0.757 <sup>b</sup>	86.02±0.66 <sup>a</sup>
LP U/L	78.20±0.02 <sup>a</sup>	58.59±0.015 <sup>a</sup>	46.80±0.80 <sup>a</sup>	129.33±0.35 <sup>b</sup>	122.74±0.65 <sup>a</sup>
erum ALB µg/dl	2.46±0.05 <sup>a</sup>	2.29±0.01 <sup>a</sup>	2.28±0.01 <sup>a</sup>	9.88±0.77 <sup>b</sup>	2.27±0.01 <sup>a</sup>

Mean along the same row with different superscript are significantly different

**Table 3A:** Red blood cell differential parameters in *T. zillii* across 5 sampling sites

SITES	RBC( $10^6$ /ml)	HGB	HCT%	MCV	MCH	MCHC	RDW-SD	RDW-CV
Unilorin reservoir	1.41 $\pm$ 0.079	6.60 $\pm$ 3.4	27.90 $\pm$ 15.2	200.3 $\pm$ 4.85	48.10 $\pm$ 2.9	240 $\pm$ 9.0	37.80 $\pm$ 1.7	7.75 $\pm$ 0.05
Apodu reservoir	2.49 $\pm$ 0.020	10.50 $\pm$ 0.020	34.60 $\pm$ 0.02	143.1 $\pm$ 0.02	44.30 $\pm$ 0.020	309 $\pm$ 0.02	33.8 $\pm$ 0.019	9.50 $\pm$ 0.020
Asa reservoir	3.25 $\pm$ 0.02	14.40 $\pm$ 0.02	46.50 $\pm$ 0.02	18.15 $\pm$ 0.55	19.95 $\pm$ 18.15	37.10 $\pm$ 0.02	37.10 $\pm$ 0.02	16.40 $\pm$ 0.02
Asa river (Unity)	12.40 $\pm$ 2.30*	39.85 $\pm$ 5.15*	59.10 $\pm$ 5.00	777.1 $\pm$ 0.019*	14325 $\pm$ 0.019*	21.75 $\pm$ 0.25	14.80 $\pm$ 1.10	11.70 $\pm$ 0.92
Asa-river (Harmony)	3.01 $\pm$ 0.24	12.90 $\pm$ 1.90	41.95 $\pm$ 4.55	96.45 $\pm$ 38.65	309.10 $\pm$ 17.00*	141.50 $\pm$ 140.50*	34.05 $\pm$ 0.65	12.10 $\pm$ 1.80

RBC- Red blood cell, HGB- Haemoglobin, HCT- Haematocrit (Packed red blood cell), MCH- Mean corpuscular haemoglobin MCV- Mean corpuscular volume, MCHC- Mean corpuscular haemoglobin concentration, RDW- Red blood cell distribution width.

**Table 3B: White blood cell differential parameters in *T. zillii* across the 5 sampling sites**

SITES	WBC	LYM%	MID%	GRAN%
Unilorin reservoir	56.65 $\pm$ 39.45	84.45 $\pm$ 5.350	10.30 $\pm$ 4.100	5.250 $\pm$ 1.250
Apodu reservoir	102.6 $\pm$ 0.02	74.90 $\pm$ 0.02	16.60 $\pm$ 0.02	8.500 $\pm$ 0.02
Asa reservoir	122.2 $\pm$ 0.02	62.20 $\pm$ 0.02	20.80 $\pm$ 0.02	17.00 $\pm$ 0.02
Asa river (Unity)	70.55 $\pm$ 44.95	48.65 $\pm$ 12.55	16.55 $\pm$ 3.15	34.80 $\pm$ 9.40*
Asa-river (Harmony)	123.15 $\pm$ 0.95	59.20 $\pm$ 3.00	20.55 $\pm$ 0.25	20.25 $\pm$ 3.25

WBC- White blood cell, LYM- Lymphocyte, MID- Mid-Sized Cells GRAN-Granulocyte

**Table 3C: Platelets count in *T. zillii* across 5 sampling sites**

SITES	PLT	MPV	PDW	PLCR	PCT
Unilorin reservoir	74.00 $\pm$ 25.00	7.600 $\pm$ 0.2000	9.850 $\pm$ 0.1500	16.65 $\pm$ 8.550	0.0550 $\pm$ 0.1500
Apodu reservoir	38.00 $\pm$ 0.02000	7.700 $\pm$ 0.02000	10.30 $\pm$ 0.02000	22.50 $\pm$ 0.02000	4.070 $\pm$ 0.02000
Asa reservoir	125.50 $\pm$ 60.50	11.60 $\pm$ 0.02000	6.700 $\pm$ 0.02000	38.10 $\pm$ 26.70	0.13 $\pm$ 0.09
Asa river (Unity)	6083 $\pm$ 0.02002*	9.15 $\pm$ 2.45	8.55 $\pm$ 0.95	69.40 $\pm$ 0.02000*	7.050 $\pm$ 0.02000
Asa-river (Harmony)	2599.00 $\pm$ 2537.00*	9.10 $\pm$ 1.60	41.95 $\pm$ 4.55*	96.45 $\pm$ 38.65	5.67 $\pm$ 0.17

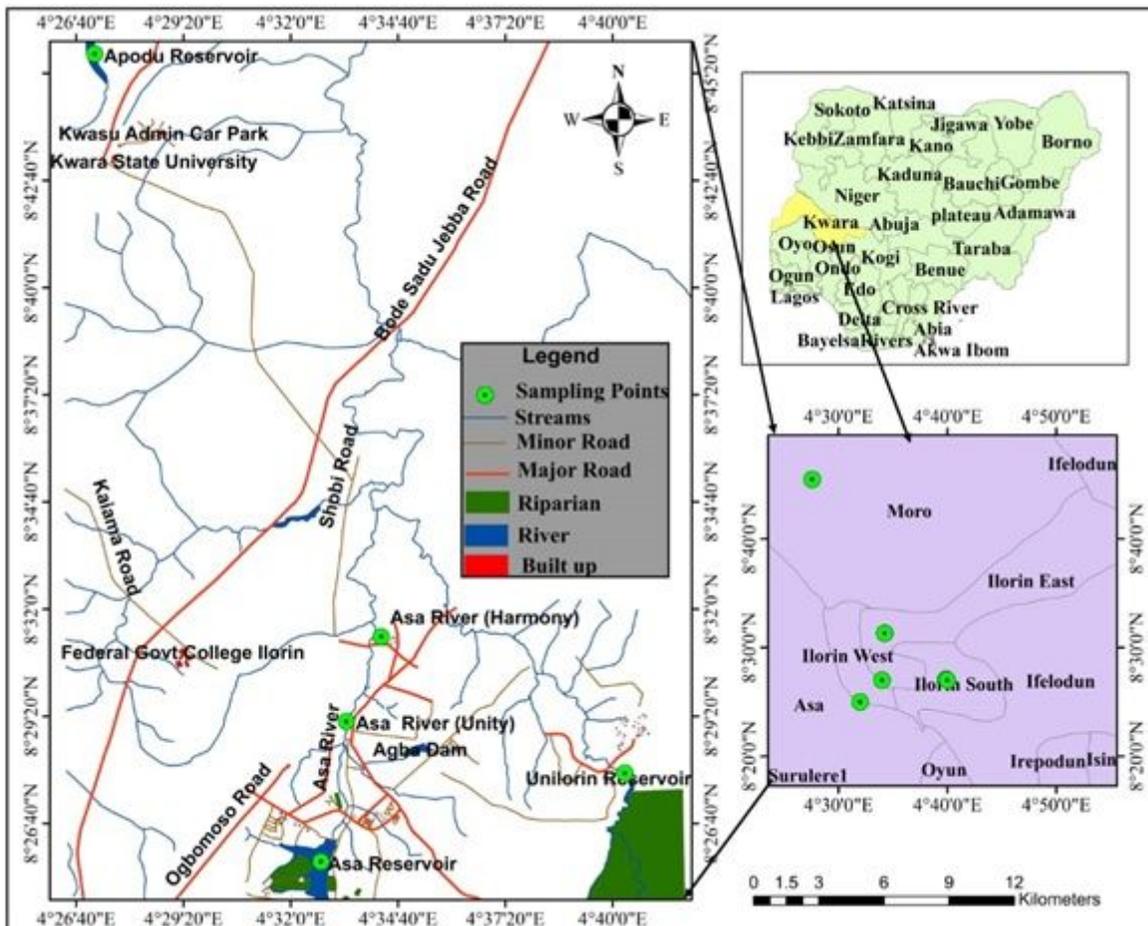
PLT-Platelet, MPV- Mean Platelet Volume, PDW- platelet distribution width, PLCR- Platelet larger cell ratio, PCT- plateletcrit

**Table 4:** Antioxidant enzyme activities induced in *T. zillii* across the five sampling sites represented as mean± standard error of mean (SEM)

Antioxidant Enzymes	Unilorin reservoir	Apodu reservoir	Asa reservoir	Asa river (Unity)	Asa river (Harmony)
SOD	107.14±0.47 <sup>b</sup>	53.23±1.0 <sup>a</sup>	53.20±0.57 <sup>a</sup>	128.021±0.05 <sup>c</sup>	106.74±0.71 <sup>b</sup>
CAT	5.48±0.23 <sup>b</sup>	56.70±0.44 <sup>a</sup>	74.93±0.18 <sup>a</sup>	48.80±0.36 <sup>c</sup>	186.30±0.53 <sup>b</sup>
SP <sub>x</sub>	3.6±0.15 <sup>a</sup>	878.90±0.15 <sup>e</sup>	599.82±1.0 <sup>d</sup>	548.92±0.7 <sup>c</sup>	174.73±0.28 <sup>b</sup>
SR	215.8±0.47 <sup>e</sup>	70.09±0.24 <sup>c</sup>	46.56±0.31 <sup>a</sup>	65.70±0.25 <sup>b</sup>	111.26±0.7 <sup>d</sup>
GST	0.15±0.005 <sup>a</sup>	324.93±0.0075 <sup>d</sup>	415.86±0.80 <sup>e</sup>	293.2±0.55 <sup>c</sup>	267.66±0.60 <sup>b</sup>

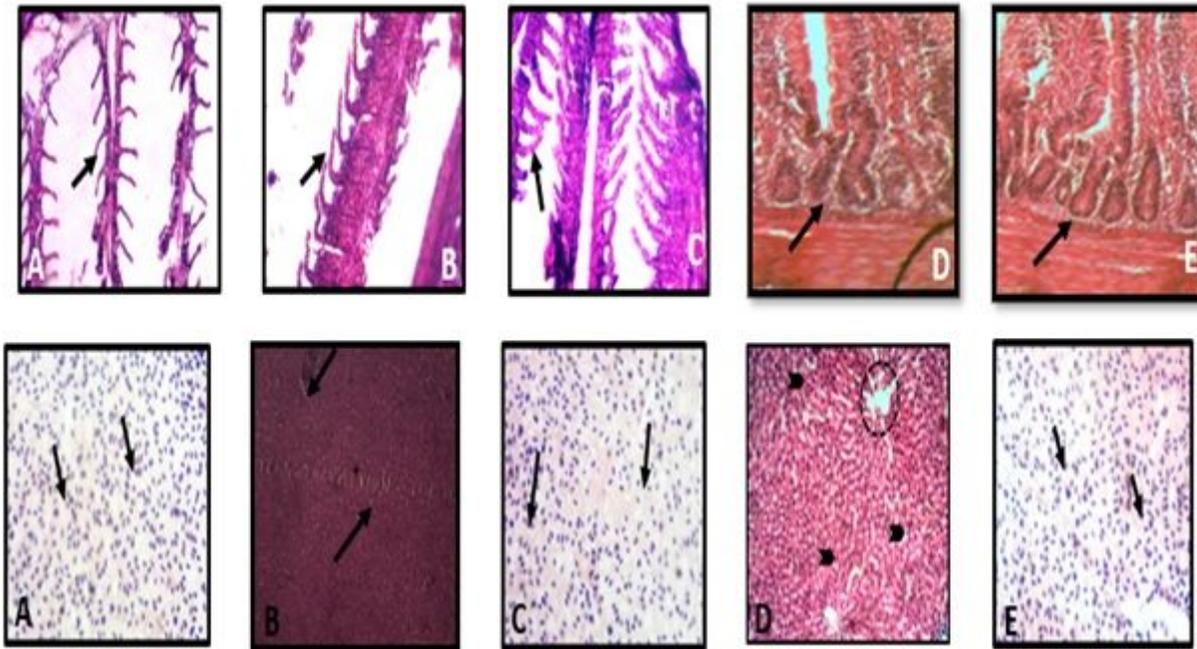
Mean along the same row with different superscript are significantly different

## Figures



**Figure 1**

Map of the Study Area showing the Sampling points (Green circle).



**Figure 2**

2I: Histopathological Analysis of the gills of *Tilapia zillii* across the five sampling sites. Mag x100 (ABC), x 400 (DE). Figure 2II: Histopathological Analysis of the Liver of *Tilapia zillii* across the five sampling sites. Mag x100 A. Unilorin reservoir: shows densely exhibited hepatocytes nuclei (black arrows) with normal staining characteristics and cellular disposition. Their general histomorphology appear characteristically normal with no apparent pathological alteration B. Apodu reservoir: shows mild distortion of hepatic tissue with interstitial congestion scan. C. Asa reservoir: shows densely distributed hepatocyte nuclei (black arrow) The micrograph appears characteristically normal with typical staining intensity and normal histoarchitectural manifestation D. Asa river (Unity): the photomicrograph shows the central vein (black outline) and a high magnification of the hepatocytes (black head arrow) revealing the disposition of the nuclei, staining intensity and general histomorphological presentation. Histomorphological presentation of the liver showing typically sized halo spaced central vein surrounded by densely distributed hepatocytes E. Asa river (Harmony): the *T. zillii* liver shows densely distributed hepatocyte nuclei (black arrow). The micrograph appears characteristically normal with typical staining intensity and normal histoarchitectural manifestation.

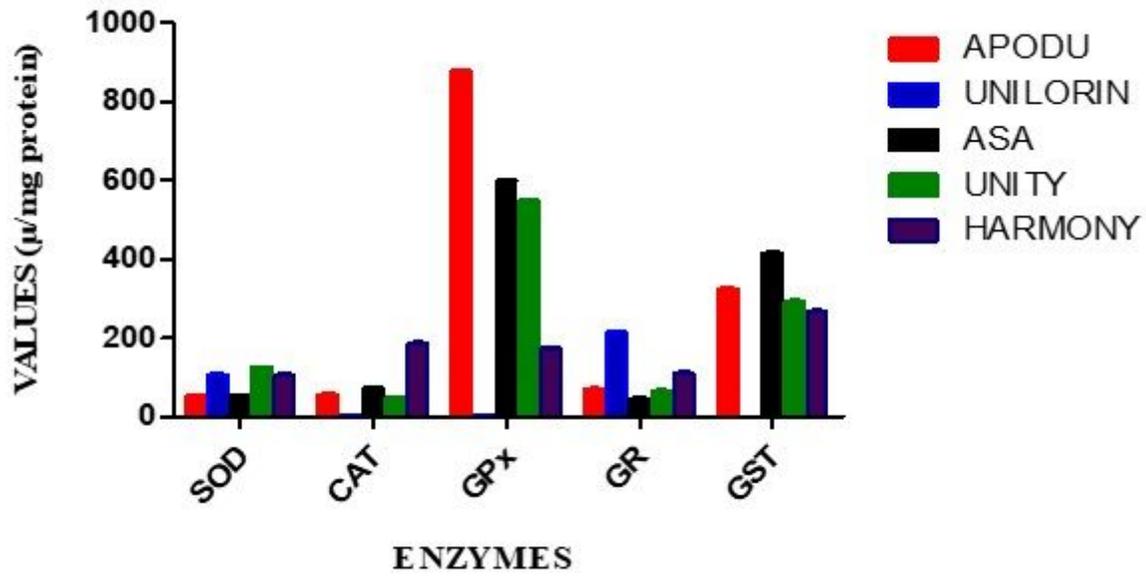
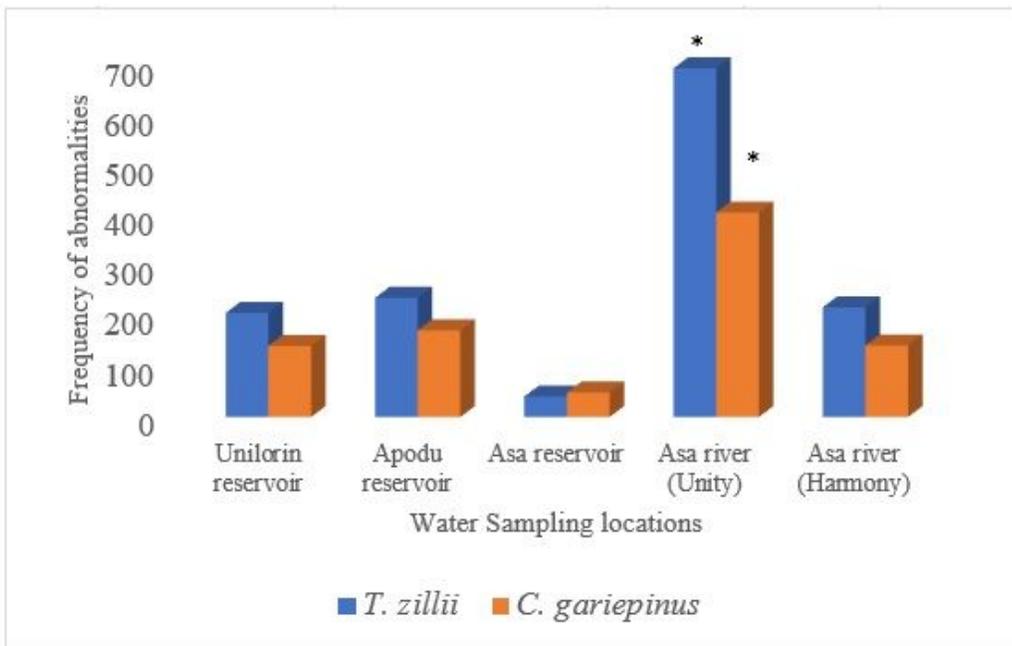
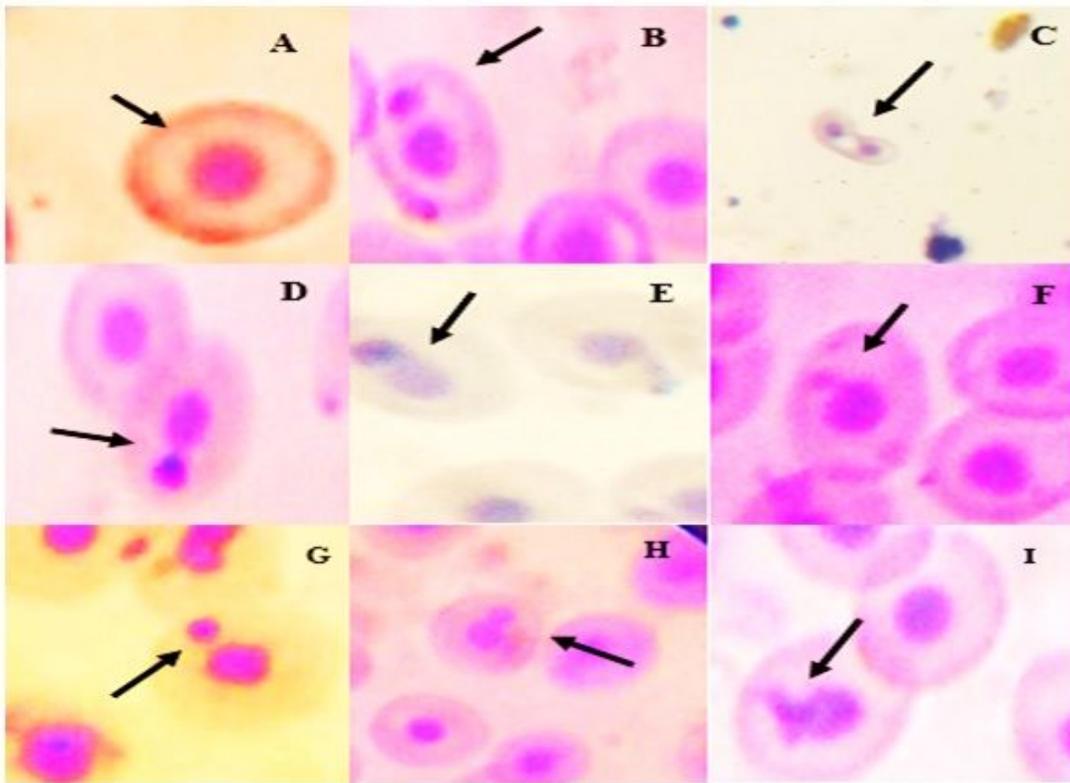


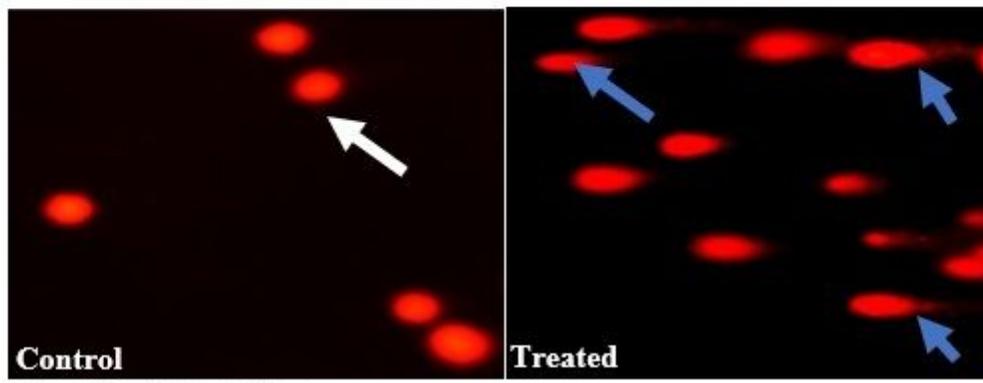
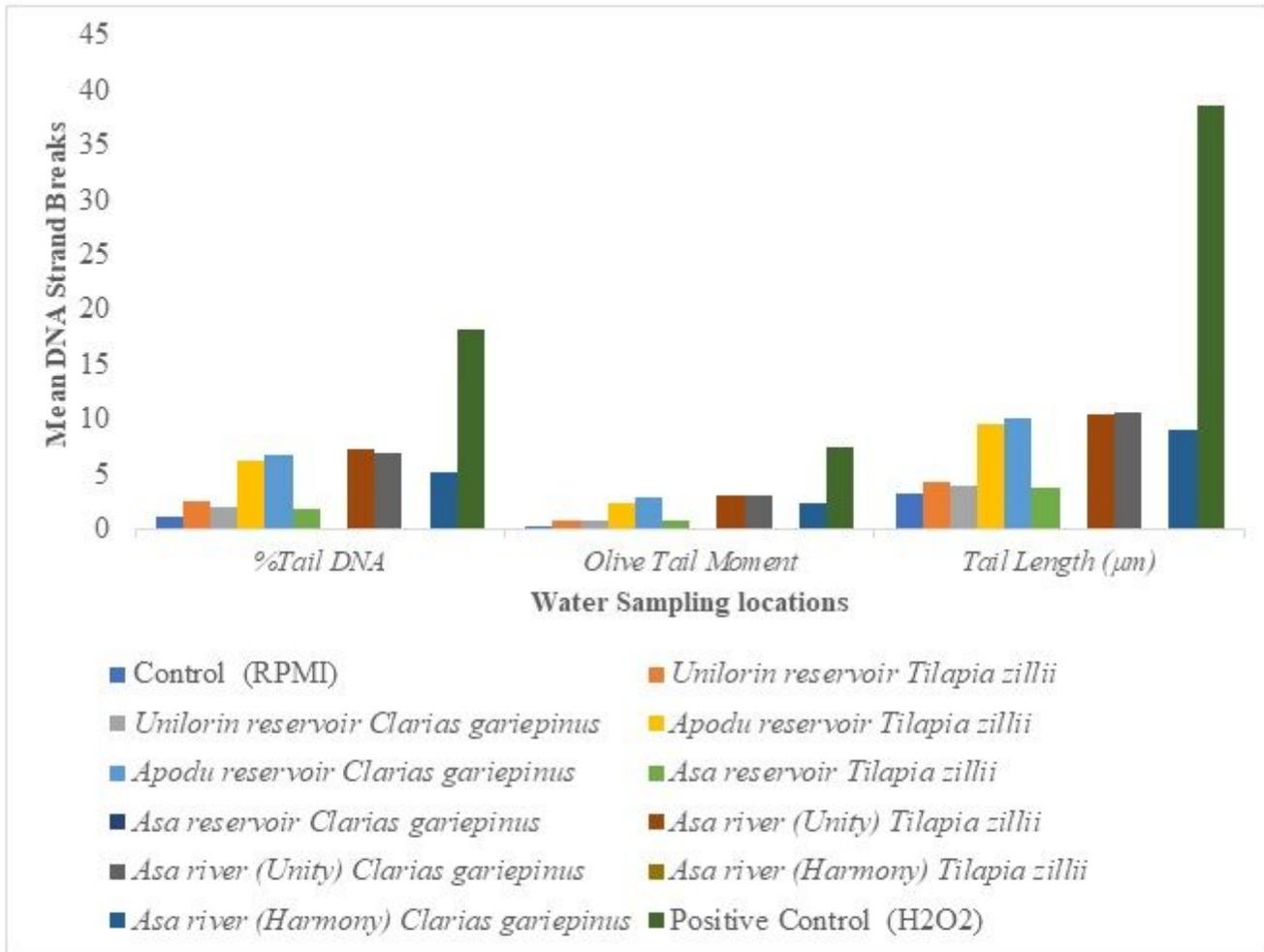
Figure 3

Antioxidant enzyme activities induced in *T. zillii* across the five sampling sites.



**Figure 4**

4I: (A) Normal nucleus, (B and C) Binucleated, (D) Nuclear bud, (E) Notched, (F and G) Micronucleus, (H) Lobed, (I) Blebbed. Mag.x100 4II: Micronuclei induction and nuclear abnormalities observed in different species of fish present at the various sites per 2000 cells.



**Figure 5**

5a: Mean DNA strand breaks in *Clarias gariepinus* and *Tilapia zillii* induced by the sampling water. 5b: Cellular DNA damage Control showing normal shape of a nucleus without comet tail (white arrow) Treated showing abnormal shape of a nucleus with comet tail (blue arrow)