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RESEARCH ARTICLE

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Induction of oxidative stress and DNA damage in two common fish species of rivers and reservoirs in Ilorin, Northcentral, Nigeria

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ABSTRACT

Rivers and reservoirs around the world are becoming dumping sites for liquid and solid wastes which are toxic to the fish. The physicochemical parameters and ecotoxicological potential of fish species in Ilorin's major rivers and reservoirs were investigated. Water and fish samples were collected from five sites: Unilorin Reservoir (A), Apodu Reservoir (B), Asa Reservoir (C), Asa River (Harmony) (D), and Asa River (Unity) (E). Excluding rivers (D and E) with low dissolved oxygen (DO) and a slight increase in lead (Pb), all physicochemical parameters were within the NSDWQ and WHO criteria for drinking water. Fish samples at D and E showed changes in the serum biochemical and haematological profiles and induction of micronuclei (MN), nuclear abnormalities (NA) and DNA single-strand break. Antioxidant enzymes were also increased in the fish. Our findings show that water from D and E can cause cytogenotoxic in fish due to hypoxic conditions triggered by Pb. This study will provide valuable health risks and future river water treatment strategies.

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Rivers; reservoirs; genotoxicity; oxidative stress; *Tilapia zillii*; *Clarias gariepinus*

1. Introduction

Water is a universal solvent that can dissolve a wide range of compounds to varying degrees. It is a priceless resource since it provides vital support to all organisms (plants and animals). In addition to being used for drinking and cooking, water is also used in the home, on the farm, and in the industrial sector. However, the majority of waste generated as a result of its uses is dumped into bodies of water without any regard for its toxicity. In recent years, the amount of these wastes has risen dramatically worldwide, posing a major health risk to humans. Genetics alone cannot explain two-thirds of human long-term illness risk; it may be influenced by environmental factors or gene-environment interactions [1]. Virus, bacterial, and parasitic diseases can all be spread through contaminated water. In aquatic and terrestrial animals, polluted water has been shown to cause neurological and respiratory disorders as well as kidney and liver damage, endocrine disruption, DNA damage and reproductive defects such as infertility in many studies. It can also weaken the immune system, reducing fertility, inducing developmental defects and even increasing the risk of some cancers [2–7] Effects of plant grown and/or aquatic organisms residing in polluted rivers have been shown to bioaccumulate different pollutants present in the water in their tissues and transfer these through the food chain to animals [8].

Reservoirs and rivers are valuable biological resources that serve a variety of human requirements, including water conservation, flood management, and hydroelectric power generation [9]. Water conservation has become a challenge due to pollution. This could be due to the daily input of contaminants from home, agricultural, and/or industrial wastes which could harm fish [10–13].

Fish are ecologically important and commercially valuable in Nigeria's and other countries' fishing industries. For example, *Clarias gariepinus* (The African sharptooth catfish, which belongs to the Clariidae family of airbreathing catfishes) and *Tilapia zillii* (an African native fish species is one of the dominant cichlids in reservoirs of Nigeria) are regularly and widely grown in ponds, and they also exist naturally in Nigerian freshwater. As a result, changes in the aquatic environment, as well as physicochemical alterations, have a significant

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impact on fish physiology [14]. Because fish absorb pollutants directly from contaminated water, their physiological stress responses are extremely comparable to those of mammals [13,15]. With the ability to digest and store toxins in water in their numerous organs, fish have also been shown to be an excellent organism for evaluating the hazardous potential of contaminants [16]. They also respond quickly to low concentrations of toxicants, and their responses are similar to those of vertebrates. As fish have antioxidant enzymes that are similar to those found in vertebrates and are utilized to counteract the detrimental effects of reactive oxygen species (ROS) [17,18].

There are various factors that influence water quality, but most past studies on the subject have focused on heavy metals. These factors such as Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Dissolved Oxygen (DO), precipitation, climate, soil type, vegetation, geology, flow conditions, groundwater, and human activities, all have an impact on water quality. Variations in BOD, COD and DO in rivers, as well as the harmful consequences they may have on aquatic life, are underreported, particularly in Nigeria. BOD is the quantity of oxygen taken up by microorganisms living on organic compounds present in the sample (e.g. water or sludge) when incubated at a specific temperature (typically 20°C) for a set period of time (usually 5 days, BOD_5) [19]. It acts as an indicator of water pollution since it offers information about the readily biodegradable percentage of the organic load in the water. If BOD_5 is greater than 5 mg/L, the water is considered contaminated. The amount of oxygen equivalent to the organic matter in a water sample is referred to as COD. It is a common metric for determining the susceptibility of organic and inorganic components in municipal, agricultural, and/or industrial wastes to oxidation. Higher COD values indicate that there is more oxidizable organic material in the sample, lowering DO levels. Anaerobic conditions can result from a decrease in DO, which is harmful to higher aquatic life forms. Hypoxia in water bodies has been shown to enhance ROS production and block the intracellular antioxidant system in aquatic animals, leading to physiological abnormalities and mortality [20]. Thus, because of the ability of fish to react to changes in dissolved oxygen levels when BOD and COD increase and their inclination to accumulate metals in their muscles [21], fish have been used as a biological indicator of water quality [22]. Alteration in water quality parameters could cause changes in physiological, biochemical, and genetic factors in the body of aquatic organisms [23], which have not been fully elucidated in most Nigerian water bodies.

In Nigeria, Asa, Oyun, and Apodu reservoirs, as well as associated water bodies, support a diverse range of fish species especially *T. zillii* and *C. gariepinus* (which are abundance/common) and provide the majority of water consumed in Ilorin city, University of Ilorin community, and Malete town in Kwara state [9, 24–26]. Fresh fish and fish items were also provided to the public via its resources. Industrial, agricultural, and domestic waste discharge and practices, on the other hand, are carried out along the river's bank. These discharges pollute the water and have a negative impact on the recipient environment's biological equilibrium [12,13, 27–29]. Most studies on the water bodies are always on their quality, there is a limited study on the toxicity impact posed by the polluted water in these regions.

The purpose of this study is to provide current information on the genotoxic status of major water bodies in Kwara State, Nigeria, by investigating the physicochemical parameters of the waterbodies, oxidative stress, and antioxidant response in selected fish species, and assessing the level of genotoxic damage induced in selected fish (*T. zillii* and *C. gariepinus*) collected from five different water bodies. Fish such as *T. zillii* and *C. gariepinus* is a suitable bioindicators to monitor water quality. The build-up of pollutants in these fish may affect their physiological processes due to their ability to generate ROS/free radicals that could result in DNA damage [23]. We observed that river water samples, in particular, triggered the generation of reactive oxygen species (ROS), altering the haematological profiles and inducing micronuclei (MN), nuclear abnormalities (NA), and DNA single-strand breaks in *T. zillii* and/or *C. gariepinus* in this study.

This research is essential for freshwater pollution prevention as well as maintaining and increasing freshwater health [30,31]. As shown in Figure 1, when chromosomal breakage or chromosome loss occurs during anaphase damage, the micronucleus (MN) assay can be used to identify micronuclei [32,33]. In order to analyse cells' genotoxic response to complicated combinations of environmental pollutants, the frequency of MN can be used. To complement the MN assay, the comet assay is routinely used in a wide range of fish species and cell types for *in situ* genotoxicity assessments in freshwater environments [32,34]. Breakage of DNA strands is a highly sensitive sign of exposure to a wide range of genotoxic substances that may alter DNA in a number of different ways [35]. An important technique for detecting strand breakage in aquatic species is the single-cell gel electrophoresis (SCGE) or comet assay. This is the primary test in environmental biomonitoring investigations [36]. There are several advantages to using a comet assay, including the ability to detect early and nonspecific DNA damage in tissues, the ability to detect genotoxic damage at the single-cell level, and compatibility for most eukaryotic cell types [37]. The comet assay's greatest advantage is in detecting initial and/or acute DNA damage to target tissues without clinical signs of toxicity and helping to comprehend the significance of genotoxic pollution's influence on biota in local communities around it [34, 38]. This current

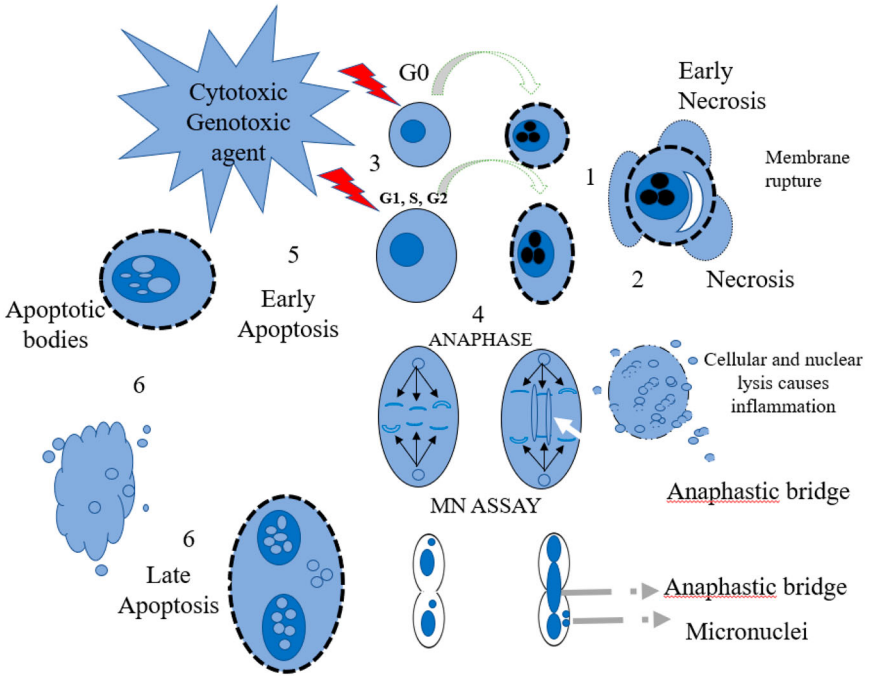


Figure 1. Mechanism of micronucleus (MN) assay.

study could potentially aid in assessing and identifying the ecotoxicological risk posed by pollutants present in these water bodies.

2. Materials and methods

2.1. Study location

We collected water and fish from five different locations in this study, including Unilorin reservoir (A), Apodu reservoir (B), Asa reservoir (C), Asa river (Unity) (D), and Asa river (Harmony) (E), as shown in Figure 2.

2.1.1. University of Ilorin reservoir (A)

The University of Ilorin reservoir is located in the western portion of Central Nigeria, bounded by longitudes 4° 40′52″ to 4° 41′0"E and latitudes 8° 27′5″ to 8° 28′5"N in the Ilorin south local government of Kwara state. It was founded in 1975, and the physical construction of the structures began in 1979. Between 2015 and 2016, it was restored to a depth of around 5 m and a submerged area of roughly 650 km^2. The dam's principal aim is to provide water to the university villages for drinking and irrigation. Because no industry is located along its path, the water rarely receives waste. Due to the result of the physicochemical parameters, this site was used as our control site.

2.1.2. Apodu reservoir (B)

Apodu reservoir is located in Apodu hamlet, some 7 kilometres from Malete Town in Kwara State's Moro local government region. The dam was built in 1980, and the re-impoundment was completed in 2016. It is located between longitudes 8°45′25.9″ N and 45′27.7″ N, and latitudes 4°27′41.4″E and 4°27′35.5″E, with a length of 560 metres and a width of 400 metres, and a depth of 8.2 metres, with a surface area of around 15 hectares. It has two seasons: a dry season during which the water flow rate declines and a rainy season during which the water flow rate remains constant. It is located in Nigeria's Guinea Savannah region. The reservoir's principal purpose is to supply water for drinking and agriculture to the surrounding villages [12,25,39].

2.1.3. Asa reservoir (C)

The Asa reservoir is located 5 kilometres south of Ilorin, across the Asa River, between latitudes 8°25′ and 8° 27′N and longitudes 4°32′ and 4°34′E. The reservoir was built between May 1975 and January 1977 with the goal of addressing the ever-increasing need for drinking water, agriculture, and a source of revenue through commercial fishing for the rapidly growing population of Ilorin, the state capital of Kwara state. The rivers Iwonte, Jia, and Segbekuke are the main tributaries. With a maximum length of 20 kilometres, a breadth of 7 kilometres, and a depth of 13 metres, the reservoir is unusually huge and wide, with a storage capacity of around 43 million cubic metres [24].

2.1.4. Asa River (Unity) (D)

With coordinates of 8°28′0"—8°31″ N and 4°32′0"—4°34″ E, the Asa river is one of the major rivers in Ilorin, Kwara state. The river is home to a number of businesses, including Dangote, Coca-Cola, pharmaceutical, and detergent industries. Near the river, there are a few small agricultural enterprises such as Ugwu and cassava plantations. The dumping of garbage, sewage disposal, and indiscriminate discharge of effluents/chemicals into the river's body is frequent, making

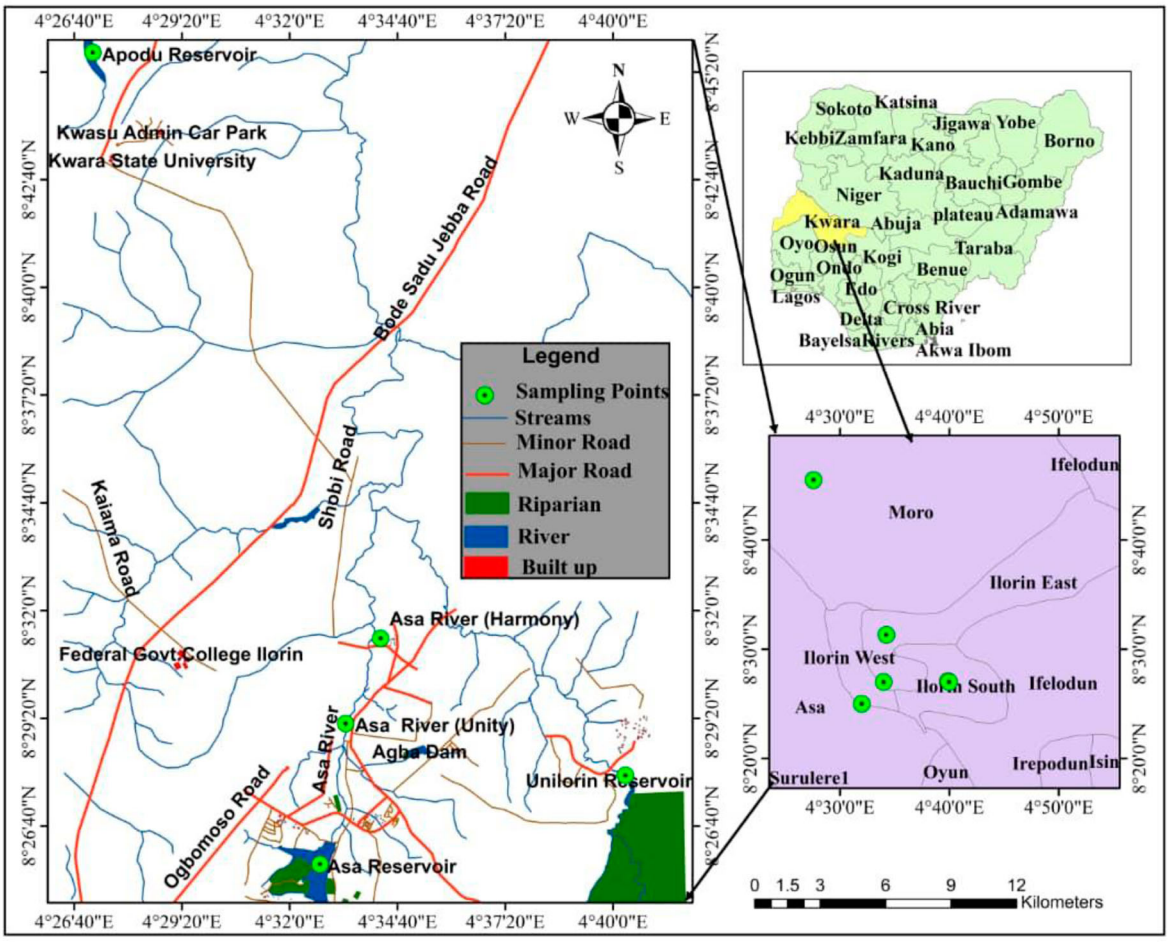


Figure 2. Map of the Study Area showing the Sampling points (Green circle).

the water appear unsafe for drinking and household purposes [40].

2.1.5. Asa River (Harmony) (E)

It is a river segment with coordinates of 8°30′0"—8°32″ N and 4°33′0"—4°34″ E along the Asa River. It is located in Ilorin's Harmony Estate, with very little garbage entering the waterbody. Effluents from multiple industries within the estate, as well as home (sewage) wastes and agricultural waste run-offs along the river's bank, are all contained in this river.

2.2. Experimental design

The physicochemical parameters such as the temperature, pH, electrical conductivity, dissolved oxygen (DO), total dissolved solids (TDS), total suspended solids (TSS), biochemical oxygen demand (BOD), chemical oxygen demand (COD), and heavy metals were all measured in water samples taken from reservoirs (A, B and C) and rivers (D and E). A Hanna portable waterproof tester, model HI 98129, was used to measure water parameters while Atomic Absorption Spectrophotometer (AAS) (model: Buck scientific ACCUS-IS 211) was used to determine the heavy metals. Winkler's method was used to determine the DO.

In the early hours of the day, two separate fish species, *T. zillii* and *C. gariepinus*, were collected from the five sites: A, B, C, D and E between 6:00-8:30 am in the period of March (dry season). They were caught with cast nets, gill nets, and baskets by fishermen. The fish were sorted and identified at the species level using the methods of Idodo-Umeh [41] as well as the assistance of a fish taxonomist. The two most common fish species captured at the various sites were *T. zillii* and *C. gariepinus*; however, *T. zillii* species were in large number and were used primarily in our experiment. The sampled fish were taken to the Department of Zoology, University of Ilorin, Ilorin, Nigeria, in transparent 50L plastic aquaria with a net cover (to enable ventilation and prevent the fish from jumping out). Before the blood was collected through the tail area or by caudal vein puncture for analysis, the fish were allowed to recuperate from stress. To avoid any trace of chemicals, the fish were anaesthetized on ice that covered the entire body for 10-15 min and immediately removed and placed the tail in a water bath (30°C) prior to blood collection. Our investigations used two separate fish species (both *T. zillii* and *C. gariepinus*) (n = 5 per site) for each analysis.

This was repeated three times (triplicate). This study was carried out following strictly the guidelines on the care and use of laboratory animals of the Ethical Committee of the University of Ilorin, Ilorin, Nigeria.

2.3. Oxidative stress, haematological analyses and and antioxidant enzymes activities

To carry out the oxidative stress analysis, blood was drawn from *T. zillii* (n = 5) from each site and stored in EDTA bottles prior to analysis, with the methodology of Reitman and Frankel (1957) being modified. For haematological analysis, blood was analysed using the methods outlined by Svobodova et al [42] and Sovio, and Oikari [43]. Blood samples were collected from the fish from each site for analysis, including red blood cell (RBC) profiles, white blood cell (WBC) profiles, and platelet profiles.

The activity of glutathione S-transferase (GST) was measured using 1 chloro 2, 4 dinitrobenzenes as a substrate [44]. Using an extinction coefficient of 9.6mM-1cm-1, the specific activity of glutathione S-transferase was expressed as nmoles of GSH-CDNB conjugate formed/min/mg protein. Clairborne [45] reported a catalase activity (CAT) assay based on the breakdown of H_2O_2, in which the absorbance was measured at 240 nm (pH 7.0, 28°C) and expressed as unit/mg protein. The activity of superoxide dismutase (SOD) was measured spectrophotometrically at 420 nm and expressed as a unit/mg protein using the Misra [46] method, which relies on the autooxidation of adrenalin due to the presence of superoxide anion. The assay for glutathione peroxidase was performed using Paglia and Valentine [47] technique. To a combination containing 0.2 mL of buffer, 0.2 mL of EDTA, and 0.1 mL of sodium azide, around 0.2 mL of tissue homogenate was added. After good mixing, 0.1 mL reduced glutathione and 0.1 mL hydrogen peroxide were added, and the mixture was incubated for 10 min in a water bath at 37°C. After the incubation period, 0.5 mL of 10% TCA was added and centrifuged for 5 min at 10,000 rpm. In a separate test tube, 1.0 mL of the supernatant was added to 2.0 mL Tris buffer and 50 µl DTNB. The OD was measured at 412 nm right away. While the activity of glutathione reductase (GR, EC 1.6.4.2) was measured according to Glatzle et al. [48].

2.4. DNA damage analysis

This was performed using micronucleus (MN) and comet assays. Blood was collected from the caudal region of the two fish species (*T. zillii* and *C. gariepinus*) [n = 5 per site] using a 2 mL syringe and needle. The needle was placed ventrally into the caudal portion of the fish's body until it pricked the vertebral column, then moved out a little to allow blood to flow into the syringe. For the MN assay, a drop of blood was immediately placed on a clean, grease-free microscope slide to form a thin blood smear after the dispenser drew around 0.5 ml of blood. In a dust-free environment, the smeared slides were allowed to air-dry overnight. The air-dried slides were fixated in 70% absolute methanol for 20 min before being air-dried overnight. The slide was then stained with 10% Maygrunwald, cleaned with distilled water, and air-dried overnight after drying. After that, the glass slide was stained with 5% Giemsa, rinsed with distilled water, and dried. Under a light microscope, the dried slide was scored by counting a total of 2000 erythrocytes, which were then analysed with oil immersion at 1000X magnification for micronucleus (MN) and nuclear abnormalities (NA) as cytogenotoxicity biomarkers [49,50].

For Comet assay, the first/base layer was generated by coating totally frosted slides with 1% normal-melting-point-agarose overnight. The second layer consisted of 75 µl of 0.7% low-melting-point agarose (LMA) and 25 µl of lymphocyte suspension. The slides were immediately covered with coverslips and refrigerated on ice for 10 min to harden the agarose. The coverslips were removed, and the third layer of 90 µl of 0.5% LMA was applied, followed by the coverslips being reinstalled and the agarose being allowed to harden for 10 min over ice. Triplicates of each sample were taken. For 2ï£¡ï£¡h at 4°C, the slides were submerged in a cold alkaline lysis solution. The slides were then refrigerated in a horizontal electrophoresis tank pre-filled with cold alkaline electrophoresis buffer for 20 min at room temperature to loosen the tight double-helical structure of DNA for electrophoresis. The electrophoresis was then carried out in an electrophoresis buffer at 4°C for 20 min at 25 V, 300 mA. After electrophoresis, a drop-by-drop application of Tris buffer (0.4 M Tris, pH 7.5) was used to neutralize excess alkali; the buffer was left on the slides for 5 min. This process of neutralization was carried out three times. After that, the slides were stained for 10 min with 80 µl propidium iodide (2 µg/ml). To avoid further DNA damage, all of the preceding operations were carried out in the dark [51]. A Nikon 90i fluorescence microscope was used to view the comets, and a digital imaging system was used to gather photographs of 100 comets for each concentration. Overlapping cells were not counted. Comet Assay Software Project (CASP, Wroclaw University, Poland) was used to examine all of the comet images, and the tail length (TL), per cent tail DNA (% TDNA), and Olive tail moment (OTM) were recorded to describe DNA damage to lymphoma cells.

2.5. Statistical analysis

The micronucleus abnormalities and differences between the test groups were assessed using the SPSS software package version 21.0 (SPSS 21.0) and/or

Table 1. Physiochemical parameters and metals of the water samples.

Parameter	Unilorin Reservoir (A)	Asa Reservoir (B)	Apodu Reservoir (C)	Asa river Harmony (D)	Asa river Unity (E)	NSDWQ	WHO
pH	6.1	5.6	6.3	5.9	5.6	6.5-8.5	6.5-8.5
Conductivity (ds/m)	0.14	0.08	0.12	0.54	0.85	1.0	0.9
Temperature(°C)	30	31	29	29	30	–	30
TDS (mg/L)	70	40	60	270	80	500	500
DO (mg/L)	4.8	6.8	3.69	*2.6*	*2.6*	7.50	–
COD (mg/L)	28	34.56	58.032	56	70	–	–
BOD (mg/L)	1.05	1.85	3.48	3.0	4.3	–	–
Pb (mg/L)	NA	0.683	0.342	*2.523*	*2.676*	0.01	0.01
Mn (mg/L)	0.039	0.029	NA	NA	NA	0.20	0.40
Cu (mg/L)	0.134	1.29	0.002	0.036	0.142	1.00	2.00
NO_3^- (mg/L)	6.09	5.342	10.92	9.4	0.155	50	50
PO_4^- (mg/L)	0.001	0.638	0.782	0.033	0.036	–	–
Fe (mg/L)	0.277	2.784	0.483	0.288	1.394	0.50	0.50
Cl^- (mg/L)	7.087	5.332	1.049	4.664	0.885	250	–
Ca (mg/L)	13.196	0.028	4.209	0.375	0.396	–	75

Note: NSDWQ: Nigerian Standard for Water Quality (NSDWQ); WHO—World Health Organization, NA—Not available.

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

SITE	Unilorin reservoir	Asa reservoir	Apodu reservoir	Asa river Harmony	Asa river Unity
AST/U/L	212.50 ± 3.00^a	201.84 ± 0.26^a	201.78 ± 0.56^a	227.10 ± 0.85^b	238.95 ± 0.81^b
ALT U/L	80.10 ± 0.65^a	96.66 ± 0.47^a	110.07 ± 0.65^b	86.02 ± 0.66^a	102.5 ± 0.757^b
ALP U/L	78.20 ± 0.02^a	46.80 ± 0.80^a	58.59 ± 0.015^a	122.74 ± 0.65^a	129.33 ± 0.35^b
Serum ALB mg/dl	2.46 ± 0.05^a	2.28 ± 0.01^a	2.29 ± 0.01^a	2.27 ± 0.01^a	9.88 ± 0.77^b

Mean along the same row with different superscripts are significantly different at $p < 0.05$.

Graph-pad prism (version 5.0). The differences were compared using one-way ANOVA, and the Duncan multiple range test (DMRT) was used to evaluate the degree of statistical significance at $p < 0.05$. The mean and standard error were calculated.

3. Results

3.1. The water samples' physicochemical properties demonstrate a drop in DO and an increase in Pb when compared to the permitted limit in drinking water

Except for DO, which was very low in the two rivers (D and E), all of the physicochemical parameters and heavy metals (Mn, Cu, Ni, N, P, Fe, Cl, and Ca) evaluated in the five separate water bodies were within the NSDWQ and WHO permitted levels (Table 1). When compared to the limit set by the two organizations, all five locations saw a modest rise in Pb (mg/L) (Table 1).

3.2. Biochemical and tissue lesions studies of Tilapia zillii fish show elevated ALT, AST, ALP, and albumin levels

Table 2 shows that the serum ALT, AST, ALP, and albumin enzyme activity of *T. zillii* in the E site were significantly higher ($p < 0.05$) than in the other four sites (A-D). In comparison to the reservoirs (A-C), the AST enzyme activity of fish in the D and E sites is higher. Similarly, the ALT of fish in B and E sites show higher activity when compared to other sites.

3.3. Rbc, WBC, and PLT levels have increased in the haematological study in Tilapia zillii

Table 3 shows the mean value of haematological parameters along with the standard deviation. Table 3 shows that RBC (red blood cell), HGB (haemoglobin), MCV (mean corpuscular volume), and MCH (mean corpuscular haemoglobin) in *T. zillii* from E sites are significantly higher ($p < 0.05$) than in other sampling sites. MCH and MCHC (mean corpuscular haemoglobin concentration) levels of fish at the D site also increased significantly. The changes in HCT (haematocrit) and RDW (red blood cell distribution width) between the 5 sites were insignificant. There was no significant difference ($p > 0.05$) between the 5 sites in WBC profiles; LYM (lymphocyte), MID (mid-sized cells), and GRAN (granulocyte). When compared to other species, *T. zillii* at the E site has a considerable rise in GRAN (Table 4). PLT (platelet), MPV (mean platelet volume), PDW (platelet distribution width), PLCR (platelet larger cell ratio), and PCT (plateletcrit) measurements across the five sites indicate no significant variation ($p < 0.05$). However, PLT and PDW in *T. zillii* at the D site and PLT and PLCR at the E site increased significantly (Table 5).

3.4. The release of antioxidants indicates that the cells are under oxidative stress in Tilapia zillii.

The order of antioxidant enzyme reactions in *T. zillii* blood was statistically different ($p < 0.05$) across the five sampling sites; SOD responses were highest in the fish at the E site, followed by A, D, C and lowest at B (E > A > D > C > B). CAT responses were significantly highest in fish from the E site, followed by D,

Table 3. Red blood cell differential parameters in *Tilapia zillii* across 5 sampling sites.

SITES	RBC (10^6ml)	HGB	HCT%	MCV	MCH	MCHC	RDW-SD	RDW-CV
A	1.41 ± 0.079	6.60 ± 3.4	27.90 ± 15.2	200.3 ± 4.85	48.10 ± 2.9	240.0 ± 9.0	37.80 ± 1.7	7.75 ± 0.05
B	2.49 ± 0.020	10.50 ± 0.020	34.60 ± 0.02	143.1 ± 0.02	44.30 ± 0.020	309 ± 0.02	33.8 ± 0.019	9.50 ± 0.020
C	3.25 ± 0.02	14.40 ± 0.02	46.50 ± 0.02	18.15 ± 0.55	19.95 ± 1.815	37.10 ± 0.02	37.10 ± 0.02	16.40 ± 0.02
D	3.01 ± 0.24	12.90 ± 1.90	41.95 ± 4.55	96.45 ± 3.865	309.10 ± 17.00*	141.50 ± 14.05*	34.05 ± 0.65	12.10 ± 1.80
E	12.40 ± 2.30*	39.85 ± 5.15*	59.10 ± 5.00	777.1 ± 0.019*	1432.5 ± 0.019*	21.75 ± 0.25	14.80 ± 1.10	11.70 ± 0.92

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.
*Show differences at $p < 0.05$ level compared to the reservoirs. Values are given as mean ± SE

Table 4. White blood cell differential parameters in *Tilapia zillii* across the 5 sampling sites.

SITES	WBC	LYM%	MID%	GRAN%
A	56.65 ± 3.945	84.45 ± 5.350	10.30 ± 4.100	5.250 ± 1.250
B	102.6 ± 0.02	74.90 ± 0.02	16.60 ± 0.02	8.500 ± 0.02
C	122.2 ± 0.02	62.20 ± 0.02	20.80 ± 0.02	17.00 ± 0.02
D	123.15 ± 0.95	59.20 ± 3.00	20.55 ± 0.25	20.25 ± 3.25
E	70.55 ± 4.495	48.65 ± 12.55	16.55 ± 3.15	34.80 ± 9.40*

WBC- White blood cell, LYM- Lymphocyte, MID- Mid-Sized Cells GRAN- Granulocyte.
*Show differences at $p < 0.05$ level compared to the reservoirs. Values are given as mean ± SE

A, C, and least significant in B (E > D > A > C > B). GPx responses were greatly induced in fish from the C site, followed by B, E, D, and lowest in A (C > B > E > D > A). GR responses significantly increased in fish from the A site, followed by C, D, E and B (A > C > D > E > B). GST responses were highest in B, followed by C, E, D, and A (B < C > E > D > A) (Table 6; Figure 3).

3.5. Water samples from the rivers induced DNA damage in both Tilapia zillii and Clarias gariepinus

An increase in micronuclei (MN), nuclear abnormalities (NA), and DNA single-strand break are all signs that the fish are suffering from DNA damage. The peripheral blood erythrocytes of two fish species (*T. zillii* and *C. gariepinus*) obtained at various locations demonstrate induction of micronuclei (MN) and other nuclear abnormalities (NA), such as binucleated (BN), nuclear bud (NB), notched (N), lobed (L), and blebbed (BLB). With each of the two fish species obtained, site comparisons were made (E > D ≥ C ≥ A > B) indicating that the two rivers (E and D) showed the highest frequency of MN and NA compared to the reservoirs (C, A and B) (Figure 4(a)). The frequencies of abnormalities per fish are shown in the supplementary information (Supplementary Figure S1-S2) while the level of significance is presented in supplementary Table (S1-S2). In this investigation, it was discovered that *T. zillii* had a significantly increased (**$p < 0.01$ and *$p < 0.05$) in MN/NA than *C. gariepinus* (Figure 4(b-d)).

As illustrated in Figure 5(a and b), the level of DNA damage detected in the peripheral lymphocytes of fish collected at various places was assessed using the alkaline comet assay with parameters such as per cent tail DNA, olive tail moment, and tail length. The blood cells of fish obtained from the Asa river Unity (E) had the highest degree of DNA single-strand break. In fish collected at A and B, there was no significant variation in the level of DNA single-strand break. When comparing the two fish species, the DNA damage reported in *T. zillii* is higher than in *C. gariepinus*. In comparison to the selected reservoirs, DNA damage was found to be higher in fish species taken from river sites (Asa rivers Harmony (D) and Unity (E)). It was observed that at the reservoir sites, there is an elevation in the level of DNA damage in Apodu reservoir (C). The order of DNA damage across the 5 sites: Asa river unity > Apoduï£¡ï£¡ > Asa river harmony > Unilorin > Asa reservoir (E > C > D > A > B).

4. Discussion

The *Tilapia* fish sampled from five distinct sites; Unilorin reservoir (A), Asa reservoir (B), Apodu reservoir (C), Asa river (Harmony) (D) and Asa river (Unity) (E) in Ilorin, North Central Nigeria, show induction of oxidative stress which resulted in cellular and DNA damages in the study. The Nigerian Standard for Drinking Water Quality (NSDWQ) is the legally recognized standard for drinking water quality in Nigeria. This specifies the allowed values (which must not be exceeded) for certain water parameters before it may be regarded safe to drink [52]. Because of the indiscriminate discharge of domestic, agricultural, and industrial wastes into water

Table 5. Platelets count in *Tilapia zillii* across 5 sampling sites.

SITES	PLT	MPV	PDW	PLCR	PCT
A	74 ± 2.500	7.600 ± 0.200	9.850 ± 0.1500	16.65 ± 8.550	0.0550 ± 0.1500
B	38.00 ± 0.0200	7.700 ± 0.0200	10.30 ± 0.0200	22.50 ± 0.0200	4.070 ± 0.0200
C	125.50 ± 6.050	11.60 ± 0.0200	6.700 ± 0.0200	38.10 ± 2.670	0.13 ± 0.09
D	2599.00 ± 25.3700*	9.10 ± 1.60	41.95 ± 4.55*	96.45 ± 3.865	5.67 ± 0.17
E	6083 ± 0.0200*	9.15 ± 2.45	8.55 ± 0.95	69.40 ± 0.200*	7.050 ± 0.0200

PLT-Platelet, MPV-Mean Platelet Volume, PDW-platelet distribution width, PLCR-Platelet larger cell ratio, PCT-plateletcrit. **Show differences at $p < 0.05$ level compared to the reservoirs. Values are given as mean ± SE

Table 6. Antioxidant enzyme activities induced in *Tilapia zillii* across the five sampling sites represented as mean ± standard error of mean (SEM).

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

Mean along the same row with different superscripts are significantly different at $p < 0.05$.
$F = 25.78; p = 0.0000273$.

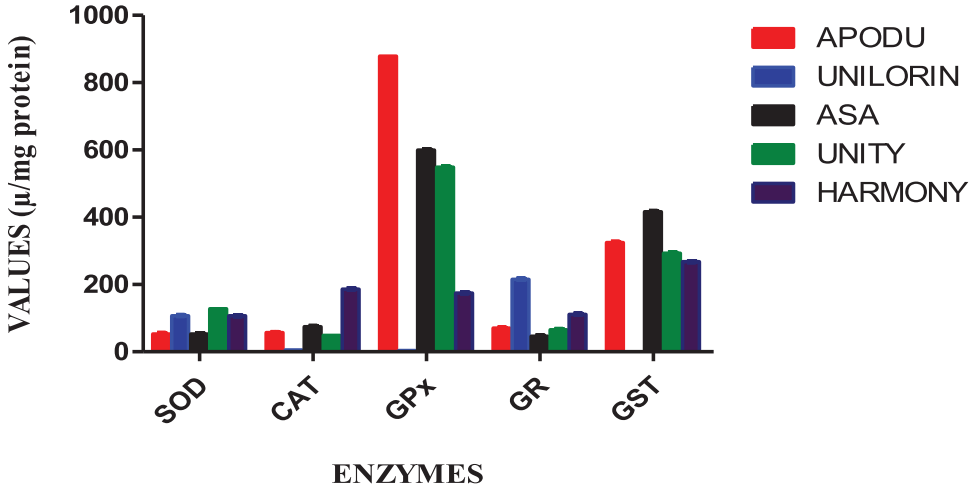


Figure 3. Antioxidant enzyme activities induced in *Tilapia zillii* across the five sampling sites.

bodies, most rivers and reservoirs around the world are polluted. These wastes are hazardous, and they have the potential to change the ecosystem and genetic makeup of aquatic organisms. The physicochemical characteristics in the rivers (D and E) show a low level of DO and a slight increase in BOD, indicating a hypoxic condition of the rivers and a slight nitrate content due to runoff from agricultural fields in the vicinity that utilize pesticides and fertilizers for farming. If BOD_5 is greater than 5 mg/L, the water is considered contaminated. Increased Pb, low DO levels in the rivers, and a modest increase in BOD and COD values obtained from the E site could be due to industrial effluents, home wastes, and runoff from some agricultural operations along the river's edge. This is due to de-oxygenation brought on by the discharge of industrial effluents into the water body. Because DO determines the distribution of animals and flora, It has been recognized as the most essential parameter for measuring water quality [53]. Local streams in Uyo, Akwa-Ibom state, have also been found to be polluted by home sewage and waste, resulting in low DO and high nitrate levels [54].

The enzymes ALP, AST, and ALT are produced in the liver, and their presence in the blood signifies liver damage. A significant level of oxidative stress enzymes observed in *Tilapia* fish at the E site indicates fish liver inflammation and oxidative stress which could be linked to a minor rise in conductivity, BOD, COD, and a decline in DO that could have put the fish under undue stress. This is consistent with the findings of Oshode et al. [55], who found significant levels of AST in examined fish samples as a result of water pollution.

Blood is a tissue that reacts to changes in our surroundings. It consists of RBCs, WBCs, PLTs, and plasma. They are useful bioindicators for determining water quality [56–59]. Changes in haematological parameters can be caused by contaminated water. In comparison to other sampling locations, we found a significant increase ($p < 0.05$) in RBC, HGB, MCV, MCH, GRAN, PLT, and PLCR in *T. zillii* from Asa river (Unity). MCH, MCHC, PLT, and PDW levels in *Tilapia* fish at Asa river Harmony were likewise significantly higher. This finding is consistent with that of Sahiti et al. [60], who found slight alterations in HGB, MCHC, and WBC levels, as well as significant changes in RBC, MCV, and MCH in blood of common carp (*Cyprinus carpio*) in two lakes in Kosovo. This disparity in RBCs could be related to the rivers' low DO levels at D and E sites and a slight increase in Pb. Blood parameters such as MCV, MCH and MCHC are important in diagnosing animal anaemia [61]. The increasing values of these indicators appear in the case of various anaemia. Our result is in line with a study from Vergolyas et al. [62] who showed water samples from the Desna and Dnieper rivers indicated a change in blood formula and an increase in the number of cells with nuclear abnormalities in fish.

Changes in water quality parameters have been linked to the activation of the antioxidant defence system in fish and other aquatic organisms living there, which may further elevate antioxidant responses in

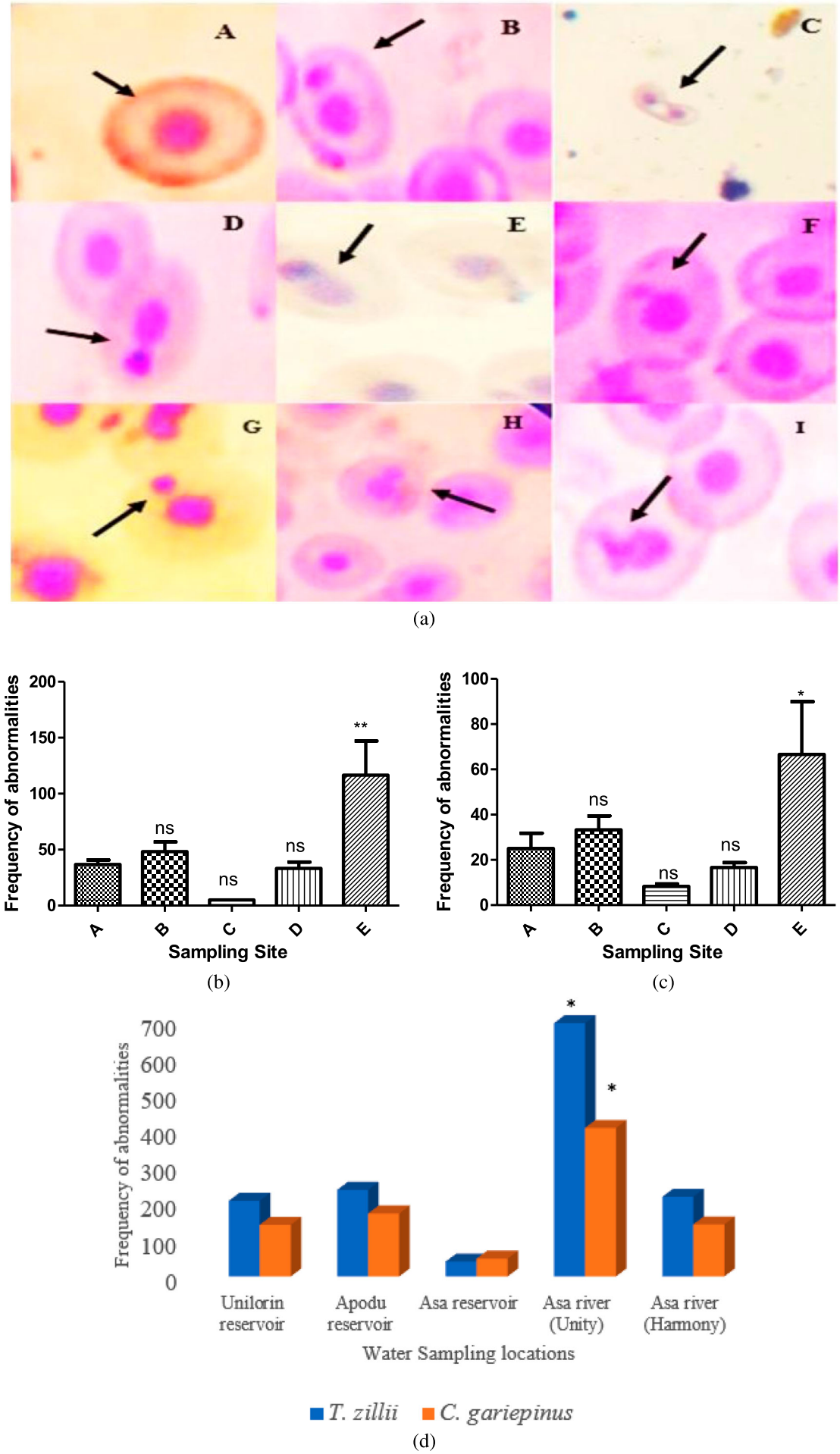
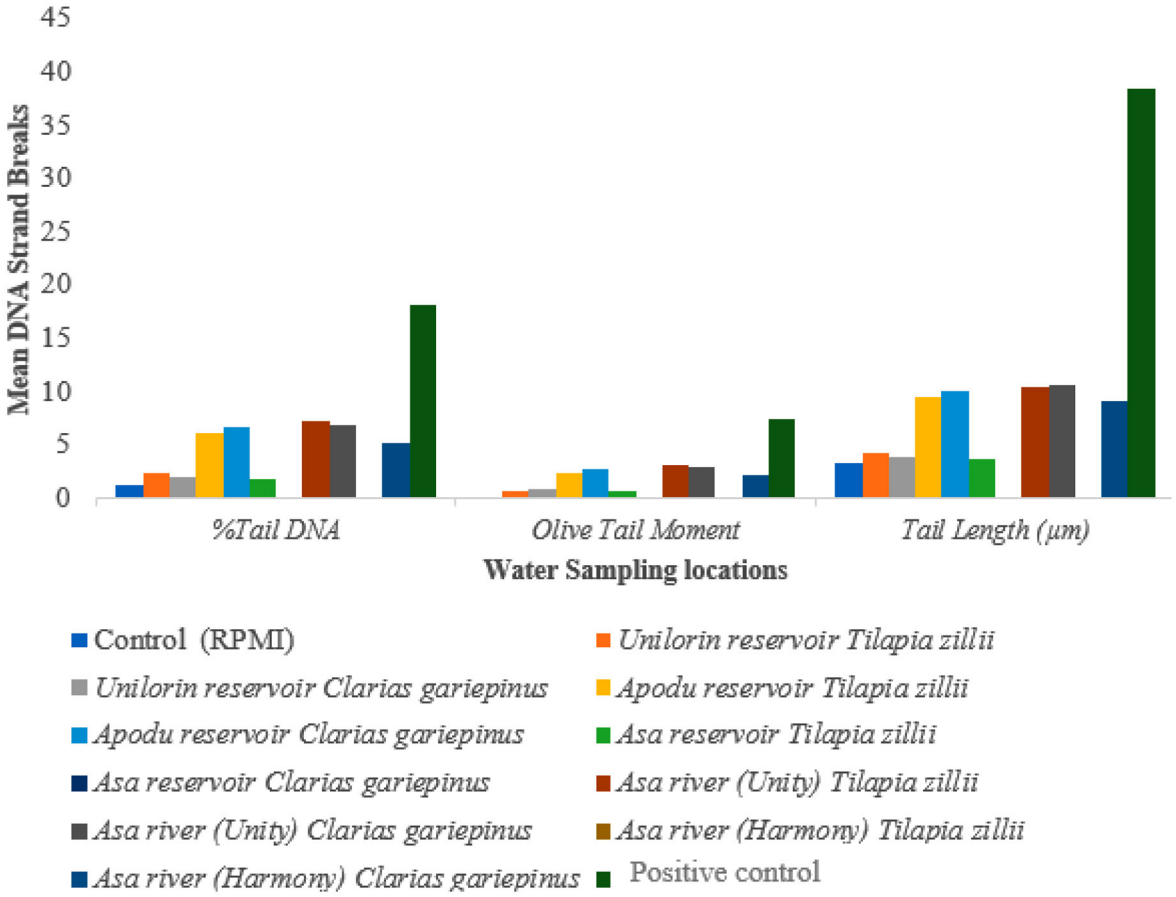
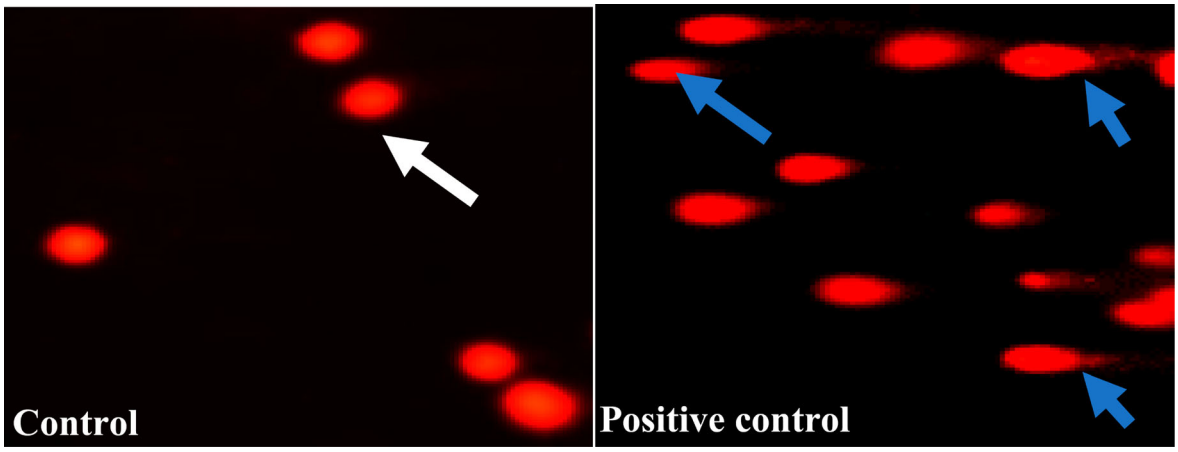


Figure 4. (a) Various micronuclei and nuclear abnormalities observed in both fish species. Normal nucleus, (B and C) Binucleated, (D) Nuclear bud, (E) Notched, (F and G) Micronucleus, (H) Lobed, (I) Blebbed. Mag.x1000. (b) Frequency of abnormalities observed in *Tilapia zillii*. (c) Frequency of abnormalities observed in *Clarias gariepinus*. (d) Micronuclei induction and nuclear abnormalities observed in different species of fish present at the various sites per 2000 cells. ($*p < 0.05$).



(a)



(b)

Figure 5. (a) Mean DNA strand breaks in *Clarias gariepinus* and *Tilapia zillii* induced by the sampling water. Positive control (H_2O_2). (b) Cellular DNA damage. Negative control shows the normal shape of a nucleus without a comet tail (white arrow). Positive control shows the abnormal shape of a nucleus with a comet tail (blue arrow).

these organisms in the presence of environmental pollutants from agricultural, domestic, and industrial sources, as well as landfill leachates, resulting in the production of reactive oxygen species (ROS) [63,64]. Although oxidative stress and antioxidant responses have been used as biomarkers to assess the genotoxic potential of rivers in Nigeria such as the Ogun, Eleyele, and Asejiri rivers [65–67], little is known about the oxidative stress and antioxidant responses of fish in major reservoirs and rivers in Kwara state. When there is an imbalance in the level of reactive oxygen species (ROS) and antioxidants in fish, the antioxidant defence system is triggered, and this defence is swiftly activated to checkmate oxidative stress that may be generated by elevated levels of pro-oxidants in the fish [68]. The initial line of defence against oxidative stress is usually SOD and CAT, and their activities are easily evident as an increase in enzyme activity [65]. Antioxidants may be reduced in cells as a result of exposure to environmental contaminants, but antioxidant levels may rise to compensate for the oxidative stress imbalance [69,70]. Increased SOD activities in *T. zillii* fish at D and E could be

a reaction to oxidative stress produced by low oxygen levels (low DO and a slight increase in Pb), which could lead to hypoxia. Reduced oxygen levels may have triggered the generation of superoxide anions (the most damaging free radicals), which were catalytically scavenged by SOD and converted to H_2O_2, a defence readily adopted by the fish's antioxidant enzyme to limit oxygen toxicity. The findings in the SOD activities at D, E and A sites are consistent with the findings of Farombi et al. [65], Isamah et al. [70], that linked higher SOD concentrations in fish to oxidative stress.

In fish exposed to contaminants, CAT is a key enzyme that works on H_2O_2. CAT, along with SOD, is normally the first line of defence in fish exposed to pollutants, and while CAT levels increase in fish exposed to pollutants, its levels have been found to decrease in contaminated water bodies, as reported by Farombi et al. [65]. *Tilapia* fish at the A site (reservoir) and E site (river) had lower CAT levels than those at the D site, but the CAT values at the B and C sites were not statistically different. Increased generation of ROS is slowly beginning to overpower the CAT defence, which could suggest that the fish is under oxidative stress. The CAT level findings are consistent with those of Arojojoye et al. [66], who found that an increase in ROS generation is associated with lower catalase activity and other antioxidant enzymes. Increased CAT activity in *T. zillii* at the D site is a common response to environmental contaminants, as CAT and SOD are the first lines of defence against oxidative stress. The blood of representative fish species from the C site had the greatest GPx levels, followed by the B and E sites. GPx activity was reduced in *T. zillii* fish from the D site, with the lowest values found at the A site. Increased GPx levels show that this enzyme has a preventive and adaptive role against oxidative stress caused by heavy metals or organic contaminants. The increased GPx level backs up the findings of Lenartova et al. [71], who discovered that fish GPx activity was 1.8-fold greater in contaminated rivers. The lower GPx level could suggest a low amount of pollution at the A site and a circumstance where the D site is self-purifying. In order to maintain the GSH/GSSG ratio, GR catalytically reduces oxidized glutathione. GR and GPx operate together because an increased level of GR may indicate oxidative stress, which may necessitate an increase in GPx to offset the stress impact [72]. The highest GR values were found in the blood of representative fish (*T. zillii*) from the A site, followed by D, C, E, and B sites with the lowest GR values. Increased GR may indicate oxidative stress in fish, which is consistent with the findings of Pandey et al. [73], who found increased GR in fish exposed to higher levels of pollution. GST levels were found to be lower in the blood of *T. zillii* at A, D, E and C sites and higher at the B site. The decrease can be linked to an increase in ROS production, and antioxidant levels in cells may be depleted in cells during exposure to environmental contaminants [70].

Next, the degree of DNA damage was then evaluated using two separate tests in two different fish species (*T. zillii* and *C. gariepinus*). When compared to *C. gariepinus*, the micronucleus test, which is a reliable and sensitive assay that is commonly used as a diagnostic of DNA damage, revealed induction of micronuclei and other nuclear abnormalities in *T. zillii* at all five sites. Our findings contradict those of Ali et al. [74], who found that the peripheral blood of *C. gariepinus* was particularly sensitive to the formation of MN when compared to *T. zillii*, which was sensitive to the production of MN as compared to two other tilapia species; *Oreochromis niloticus*, and *Oreochromis aureus*. In another study, *Labeo rohita* was the most sensitive to DNA damage and micronucleus formation, followed by *Cirrhinus molitorella*, red tilapia, and *Oreochromis niloticus* [75].

Thus, this could be due to the fact that *T. zillii* has accumulated various metals in its body systems, such as Pb, and it also shows that its genome can withstand cytogenetic damage without apoptosis in our study. The findings support Kligerman [76] report that fish living in contaminated waters, such as the E site in our study, have higher micronuclei frequencies. MN frequencies can change depending on the season, stress, pollutant type, and heavy metals [77,78].

The comet assay has been used to evaluate the impact of genotoxic contaminants on DNA integrity with great success. It has several advantages as a tool for genotoxic studies, including the ability to detect genotoxic damage at the single-cell level, suitability for most eukaryotic cell types; only a small number of cells are needed, faster and more sensitive than other available methods for detecting strand breaks, low cost, and the ability to detect early genotoxic exposure-response [78,79]. Because comets might be observed with the same length but varying fluorescence intensities, the percentage tail DNA (per cent tail DNA) is the parameter of choice for DNA damage assessment [80]. The (% tail DNA) in the five sites in descending order; $E > C > D > A > B$, indicating that the reservoir with the highest and lowest DNA damage at the E and B sites respectively.

The Asa river (upstream), which has extended its path towards the Unity area (mid-stream) and Harmony area (downstream), exhibits a striking pattern. When compared to its courses, the Asa river (upstream) has the least DNA damage (Unity and Harmony rivers). Low DNA damage in the upstream and high DNA damage in the midstream could be linked to the amount of genotoxic chemical exposure. When a pollution gradient exists along the river, the rising amount of genotoxicity in *C. gariepinus* and *T. zillii* peripheral erythrocytes is directly linked to the level of pollutants. The trend observed on the Asa water course agrees with Hariri et al. [78], who found that DNA damage was highest in the mid-stream (Asara) of the Karai river due to a high influx of pollutants from sewage and industries, and DNA damage

was lowest in the upstream (Varangerud) due to low pollution levels.

According to Anifowoshe et al. [12], the increase (per cent tail DNA and tail length) observed in Apodu reservoir compared to the negative control can be attributed to a slight increase in Pb and nitrate pollution due to sewage disposal and runoff from nearby farms that use pesticides and fertilisers for their farming activities. Exposure to pollutants from diverse sources can have additive, synergistic, or antagonistic interactions with fish [31]. DNA damage can accumulate through an increase in the number of DNA-damaging events or a decrease in DNA repair [31,81]. The Apodu reservoir's results are consistent with those of Kushwaha et al. [11] who found a considerably higher degree of DNA damage in *Channa punctatus* and *Mystus vittatus* erythrocytes exposed to contaminated water from the Gomti river in India when compared to baseline values. When compared to the negative control, the A site shows a low percentage of tail DNA. This is likely due to the fact that it is utilized for leisure activities and the reservoir is not close to residential areas, thus it receives little or no domestic garbage. The only conceivable source of contamination would be runoff during the rainy season or from the reservoir's water channel.

In most of the sampling sites, the results from the combined use of comet test and antioxidant responses to determine genotoxic potential were in accord, but in a few, they differed. Both the comet assay and antioxidant enzyme reactions indicated that the Unity and Harmony rivers were contaminated. This is comparable to the findings of Nwani et al. [82], who found that the comet assay and antioxidant enzyme responses matched. The Apodu and Unilorin reservoirs have a little difference. Although DNA damage is slight in the Unilorin reservoir, antioxidant responses were inconsistent, which could imply that the reservoir is likely exposed to minute contaminants that induce these reactions. The antioxidant responses of the Apodu reservoir, which has substantial DNA damage, are mild. This is because, according to some studies, the comet assay is a more sensitive test than the antioxidant response because it can identify toxins at low doses [31]. As a result, combining the comet assay with antioxidant enzyme responses allows for a more complete picture of a water body's genotoxic condition. Sources of lead (Pb) compounds are found in nature when Pb is mixed with two or more additional elements. Anthropogenic sources include mining and smelting, soldering, battery production, ammunition, metal water pipes, paint, and fuel. Oxidative stress (OS) is a known mechanism for lead poisoning, and lead has been shown to cause OS via the formation of reactive oxygen species (ROS). ROS overpowers the antioxidant system, allowing oxidized molecules to enter the cell [83]. Tests on animal models have shown that continuous exposure to Pb damages the kidneys and increases the risk of cancer (inorganic lead has been found to be carcinogenic to humans—Group 2A) via increasing DNA strand breaks (genotoxicity) [84]. Thus, an increase in DNA strand breaks as observed in this study in the two rivers may be attributed to the presence of Pb. Pb molecular toxicity is well-understood and occurs at blood levels as low as 5 micrograms per deciliter [85]. The implications of these findings in our present study are that, because the fish are stressed as a result of increased ROS, this could affect their physiology and result in mortality. As a result, in the future, we will determine the current ichthyofauna diversity of rivers/reservoirs and compare it to previous reports to see if pollutants are causing a decline in fish populations, as well as evaluate how pollutants like Pb enter water bodies, their hosts, and possible developmental defects they may cause over time. However, the seasonal influx of contaminants could be one of this study's limitations.

5. Conclusions and recommendations

Using various tests and antioxidant enzyme reactions, the cytogenotoxic potential and antioxidant response of five water bodies were studied. The water bodies surveyed were found to be currently polluted, and if anthropogenic activity on these water bodies continues unabated, the ecology and biotic life may deteriorate more quickly than self-purification can repair. It is strongly advised that the government enact legislation to prohibit indiscriminate dumping of rubbish into water bodies, educate the public about the consequences of such activities, and warn people about the dangers of eating infected fish; otherwise, what goes around comes around. Therefore, in order to understand the overall quality of the rivers that have been sampled, future monitoring programmes should be improved and more analyses, including heavy metals and microplastic contamination, should be considered.

Abbreviations

Ast: Aspartate aminotransferase, ALT: Alanine transaminase, ALP: Alkaline phosphatase ALB: Albumin, WBC: White Blood Cell RBC: Red Blood Cell, PLT: Platelet, SOD: Superoxide dismutase, CAT: Catalase GPx: Glutathione peroxidases, GR: Glutathione reductase GST: Glutathione S-transferase DO: Dissolved oxygen, NSD WQ: Nigerian Standard for Drinking Water Quality WHO: World Health Organization, MN: Micronuclei NA: Nuclear abnormalities.

Declarations

No funding is available.

There are no conflicts of interest.

Data Availability

The corresponding author can provide data sets generated and/or analysed during the study upon request.

Animal Research (Ethics)

Experimental animals were cared for and used in accordance with the guidelines on the care and use of laboratory animals of the Ethical Committee of the University of Ilorin, Ilorin, Nigeria.

Participant Consent (Ethics)

Not applicable

Consent to Publish (Ethics)

This does not apply.

Plant Reproducibility

This isn't the case.

AAT and OSO designed the study; AAT, OSO, OAN, OA, OEO, AO, and AYM gathered, stored, and analysed materials in the lab. Technical assistance was provided by AKM, ASI, and IOA for the study. AAT and OSO wrote the first draft of the manuscript, which was then reviewed and edited extensively by AKM, ASI, and IOA. The final manuscript was read and approved by all authors.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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