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Oxidative stress biomarkers in liver of tilapia fish from New Gusau Reservoir, Zamfara State, Nigeria

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Abstract

Fish can be utilized as bio indicators of environmental contamination since they are frequently employed as model organisms for evaluating the quality of aquatic environments. Purposive sampling was used to gather data on the surface water physicochemical quality of the New Gusau reservoir (NGR) and Nagwamatse farm (control) from nine sampling stations. In-situ measurements of pH, temperature (°C), conductivity (EC), total dissolved solids (TDS), dissolved oxygen (DO) and biological oxygen demand (BOD5) were carried out using a multifunctional meter. Standard water and wastewater system testing technique was used to examine the results. This study investigated the activities of SOD, CAT, reduced glutathione (GSH) content and MDA production in the organs of tilapia, tilapia, from New Gusau Reservoir (NGR).The reservoir is said to be contaminated since it receives effluents discharged from many industries. A clean fish farm (Nagwamatse farm) was employed as the control, and Tilapia weighing between 200g to 300g were taken from NGR. The liver of Tilapia from NGR showed a notable rise in Thiobarbituric acid (TBARS), superoxide dismutase (SOD), and catalase when compared with control. The findings showed that fish from the new Gusau reservoir had large concentrations of contaminants accumulating in their livers. This could be as a result of the high concentration of industrial activity close to the reservoir reflecting the degree of pollution in NGR.

Keywords: *Malondialdehyde*, Tilapia, Oxidative stress, Antioxidant enzymes, Pollution.

Introduction

Fish are widely used to assess the quality of the aquatic environment. Therefore, they serve as bioindicators of environmental pollution. When heavy metals build up in fish tissues, they can catalyze reactions that produce reactive oxygen species (ROS), which can cause oxidative stress in the surrounding environment. Many species, especially aquatic ones like fish, have defense systems to offset the effects of ROS. Tilapia fish is of great commercial importance because it is the most common fresh water fish widely consumed in Gusau, Zamfara State capital, Nigeria. It can therefore be a good model to study responses to various environmental contaminants. Several studies have been carried out to investigate the presence of heavy metal pollutants in aquatic ecosystems in water bodies[1].

However, concentrations of metals dissolved in water may give a highly misleading picture of the degree of metal pollution and in some cases may significantly under-estimate the total metal concentration in the aquatic ecosystem[2].

Hence most researchers use benthic organisms as bio monitors of both the levels and long-term influences of pollutants within an ecosystem. Many aquatic organisms are capable of accumulating or bio-concentrating of contaminants in their tissues such as heavy metals and polycyclic aromatic hydrocarbons (PAHs)[3]. Biomagnification of heavy metals in edible tissues of fish species like *Tympanotonus* sp., *Pachymelania* sp., *Littorina* sp. and *Pugilina* sp. have revealed heavy burdens of trace metals[4] in soils, sediments, and aquatic systems, inorganic metal bioavailability and bioaccumulation are complicated processes. However, the amount that interacts with biological surfaces (such as blood, the gut or root tip epithelium, gills, etc.), as well as the kind of metals and the amount that binds to and is absorbed through these membranes, are determined by modifying factors such as metabolism, assimilation, and solubility of metals[5]. Metals differ from organic compounds in that they can be present in different species, with the parent element associated with different ligands, but never irreversibly transformed or metabolized. To better characterize the risk posed by metals in the environment to human and ecological receptors, the processes affecting metal speciation and the effects of speciation on metal bioavailability need to be better understood and quantified[6]. Furthermore, it is necessary to understand the influence of environmental characteristics on metal speciation as well as the speciation of metals within an organism. Once metals are taken up or assimilated into biota, they are subject to a variety of fate processes, including storage, metabolism, elimination and accumulation[7].

Metal species that are biologically accessible, absorbed or adsorbed by an organism (assuming they are bioactive upon contact), and having the capacity for distribution, metabolism, excretion, and bioaccumulation are all included in the notion of metal bioavailability. Nonetheless, the inorganic species are the primary subject of this investigation[8]. The metal salt and particle size, the receptor and its unique pathophysiological characteristics, the entry point, the length and frequency of exposure, the dosage, and the exposure matrix all affect a metal's bioavailability. Until solid evidence to the contrary is forthcoming and permits this default value to be lowered to a more realistic number, the United States Environmental Protection Agency (EPA) assumes a relative bioavailability of 100% for metals[5].

Related Work: Several studies have focused on oxidative stress biomarkers in the liver of Tilapia fish. Researcher[9] examined the impact of environmental pollutants on liver oxidative stress markers in *Tilapia*. Another research on investigation and protective effects of antioxidants on Tilapia liver against oxidative damage[10]. The influence of dietary factors on liver oxidative stress markers in *Tilapia* was analyzed[11]. The potential role of oxidative stress biomarkers in assessing the health of *Tilapia* populations in aquatic ecosystems was carried out[12]. These works collectively shed light on the importance of understanding oxidative stress biomarkers in the liver of *Tilapia* fish for environmental and health assessments. In terrestrial and aquatic organisms, where dietary intake is difficult to measure due to the presence of metals in the environment and the possibility of metal absorption through routes other than diet, bioavailability is not as straightforward as it is. In vertebrates, metal uptake is directly dependent on metal concentration in the diet. According to Ademoroti CMA[13] discussion, metal bioavailability could not be a quantitative metric in this instance, but rather a conceptual word. Since this reservoir was built and several human activities occurred in the industrial area of Gusau in Zamfara State, Nigeria, there is no biochemical data on fish exposed to pollution in their natural habitat for more than thirty years. Thus, the purpose of this study was to examine the effects of pollution on African catfish liver from the New Gusau Reservoir. This study sought to determine how contaminants from the NGR reservoir affected TBARS, SOD, and CATALASE levels in addition to conducting a biochemical examination of tilapia fish liver.

Methodology

Sampling Sites: The Gusau Local Government Area of Zamfara State, Nigeria, is home to the recently constructed Gusau Reservoir. Covering an area of 3,364 km , it is located between latitude 12°17'02.40"N and longitude 6°39'50.83" 6°66'41.20"E. There are 383,162 inhabitants living in the municipality of Gusau[14]. Situated within the Sokoto River's course, the new Gusau reservoir is made out of a sizable concrete weir topped by five steel gates that may be manually operated in an emergency. The gates are controlled by electric motors and link chains. There are also concrete walls at both ends of the reservoir to protect the ends of the reservoir from erosion[3]. Four sites were used for this research and have been previously described[3]: Site 1 (NGR/SP1), Site 2(NGR/SP2), Site 3 (NGR/SP3) and site 4 (NGR SP4) and Control.

Ethical Approval: Ethical approval was obtained from Federal University Gusau ethical committee for the care and use of animals. All experimental protocols were conducted with strict adherence to guidelines established by the committee for the care and use of animals.

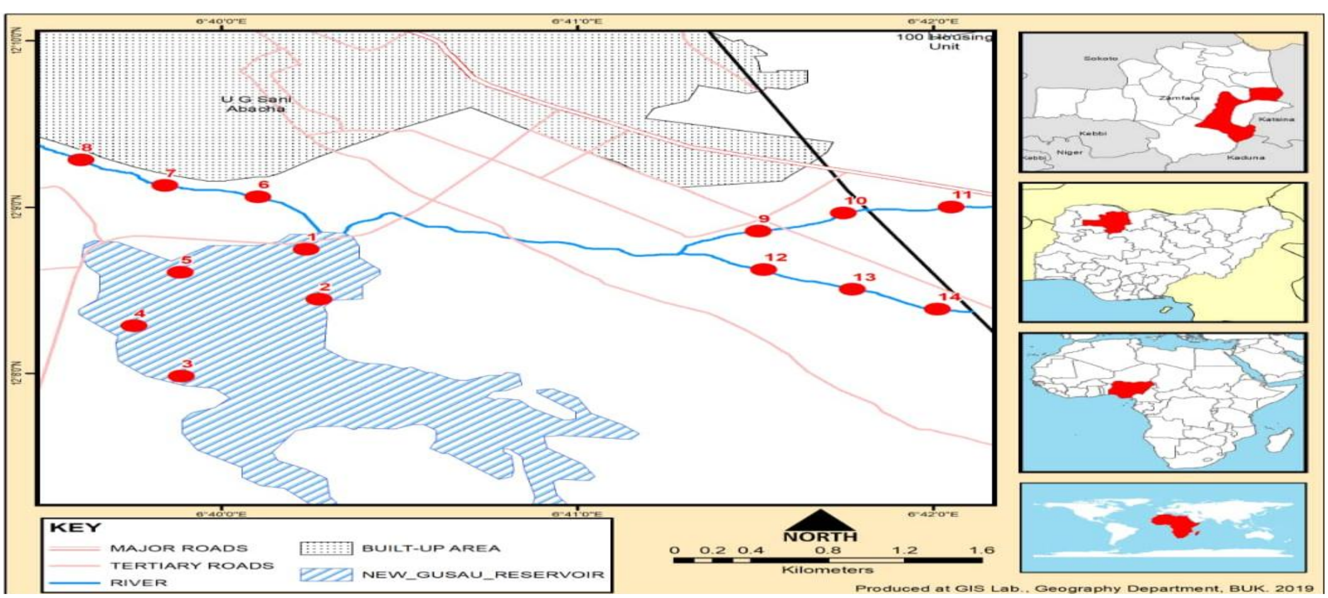

Figure-1: New Gusau reservoir sampling points.

Water Quality Assessment: According to established protocols, the following parameters were measured in the NGR and each tank: pH, temperature (^oC), dissolved oxygen (DO), biochemical oxygen demand (BOD), electric conductivity (EC), and total dissolved solid (TDS)[15,16]. The Multifunction pH/EC/Temp/TDS meter model/EZ9908 water quality checker was used to measure the following variables on-site: Temperature, pH, Total Dissolved Solids, Conductivity, Dissolved Oxygen, and Biological Oxygen Demand. Except for BOD5, where water samples were maintained in the icebox to keep them at a temperature below 4^oC during the transit from the field to the laboratory within 24 hours, the meter was allowed to equilibrate before the results were recorded. According to WHO[15], the biological oxygen demand (BOD5) was calculated by measuring the initial and final oxygen concentrations (DO) using an oxygen meter after five days of incubation at room temperature. The dissolved oxygen (DO) was measured on-site using an oxygen meter (model: DO 5510 M.R.C.) in compliance with the manufacturer's instructions. The calibrated meter's electrode was submerged in the reservoir's water and gently agitated with the probe before the value in milligrams/liter was recorded.

Study Animal: At each site, in November 2020, tilapia fish weighing an average of 123.30±2.20g were gathered. Local fishermen from NGR assisted in the catch of the fish using fishing net, and the control fish were taken from Nagwamatse farm near the new Gusau reservoir and brought to the Bayero University Kano Biological Sciences Laboratory on the same day in plastic containers along with the water from the collection site. Ten tilapia were transported in the same manner as the test fish to serve as a control. Nagwamatse is a clean fish farm devoid of infrastructure that could generate pollution or industrial effluents. After dying, the fish were dissected. The livers were taken right away, and 1.15% KCl rinsed them in freezing water. After that, they were homogenized in four liters of homogenization buffer, which contained 50 milligrams of Tris-HCl and 1.15% KCl with a pH adjustment to 7.4. The resulting homogenate was centrifuged at 12,500g for 10 minutes to obtain the post-mitochondrial fraction, which was used for biochemical analyses.

Biochemical Assays: Using the Varshney and Kale approach[2] lipid peroxidation was quantified by measuring the production of thiobarbituric acid reactive compounds (TBARS). Malondialdehyde (MDA), which is created when fatty acid membranes and food products oxidize, reacts with the chromogenic agent 2-thiobarbituric acid in an acidic environment to produce a pink complex with a maximum absorbance at 532 nm.

As stated Asaolu et al.[17], the suppression of adrenaline autoxidation at pH 10.2 was used to measure the activity of superoxide dismutase (SOD). The quantity of SOD required to achieve a 50% inhibition of adrenaline auto-oxidation is equal to one unit of SOD activity.

The Sinha approach was used to measure the activity of catalase (CAT)[8]. This process is based on the observation that, when heated in the presence of H_2O_2, dichromate in acetic acid is reduced to chromic acetate, with the creation of per chromic acid as an unstable intermediate. At 570nm, the amount of chromic acetate generated was determined using spectrophotometry.

Using 5,5-dithio-bis-2-nitrobenzoic acid (DTNB), reduced glutathione (GSH) was found in the postmitochondrial fraction of the liver, kidney, and gills of Clarias gariepinus at a wavelength of 412 nm, in accordance with the methodology reported[4].

Statistical Analysis: The data from the biochemical analyses and the water quality analyses were evaluated using analysis of variance (ANOVA), with both values given as the mean (SD). The Duncan's Multiple Range Test (DMRT) and the approved WHO water quality standard were used to compare group means values. At a 5% chance level, values were deemed statistically different. SPSS version 23.0 was used for all statistical calculations.

Results and Discussion

For all of the composite sampling sites (Nagwamatse farm and new Gusau reservoir), the research's results revealed a wide range of variation. pH value, temperature, electrical conductivity, total dissolved solids, dissolved oxygen and biochemical oxygen demand were the six physico-chemical parameters measured.

The results obtained in this research (Table-1) were within the maximum permissible limit (MPL) sets[17,18]. In relation to electrical conductivity and total dissolved solid and DO, the water is considered safe for fish production.

The pH results that were found in this study are comparable to those found in studies[19]. Aquatic creatures, particularly fish, are negatively impacted by pH levels below 4.8 and over 9.2. Similar findings in water samples from Ondo State's coastal waters were also reported[20]. The two locations' pH significantly varied from one another, according to the analysis of variance (Table-1; p 0.05). The mean temperature was 27.26°F (0.184°C) at the control location and 27.76°F (0.1112°C) at the NGR site. There was no discernible difference between the temperatures at the sampling sites, according to an analysis of variance at the 0.05 level of probability (p=.282). According to Magami et al.[21], the temperature swings in the lake were normal (29°C) for the metabolic processes of creatures like fish and won't have an impact on the organisms. The water temperature recorded during this study, when compared with other tropical water was within acceptable ranges for aquatic life in tropical ecosystems. Another investigation[22], found the surface water temperature for major rivers of Kainji Lake National park, Nigeria range between 22.6–31^0C while[23] observed at a mean temperature range of 23.2–25.2^0C in Kisian and Kisat Rivers in Lake Victoria drainage basin, Kenya.

Table-1: Mean ±SE and Range Values of Composite Sites Surface Water Physicochemical Parameters Recorded July, 2022.

Parameters/ site	Control (Nagwamatse farm)	NGR	P-Value	NSDWQ	WHO
pH	6.59^{b} ±.006	6.85^{a} ±.035	.052	6.5-8.5	
Range	5.79-8.18	6.62-8.6			
Temp. (^{o}C)	27.26^{a}±.184	27.76^{a}±.112	.282	30	
Range	24.20-30	24.10-30			
EC (µS cm^{-1})	205.09^{a}±39.810	518.95^{b}±27.234	.019	1000	250
Range	63-923	57-2330			
TDS (ppm)	137.61^{a}±17.467	283.82^{a}±11.993	.015	500	1000
Range	38-754	44-1170			
DO (mg L^{-1})	6.98^{b}±.083	6.35^{a}±.042	.000	10	
Range	5.90-9.76	4.60-7.54			
BOD_5 (mg L^{-1}	3.49^{b}±.062	2.20^{a}±.151	.000	10	
Range	1.55-4.13	1.24-3.90			

Note: Mean values in the same row with different superscripts are significantly different at $p < 0.05$. P – Value= One way NOVA for comparison between the mean values of the individual locations.

The types and quantity of chemicals dissolved in water affect electrical conductivity. The rainy season was found to have much lower levels of EC and total dissolved solids, which may be a result of dilution as volume increases and continued water release to maintain a specific reservoir capacity or volume to prevent flooding[24]. The outcome is consistent with what[23,25,26] found in their studies of the Shagari Reservoir, Kankariya, Vastrapur, Malav, and Chandola Lake in India, as well as the River Kubbani and Makwaye Lake in Zaria. Long-term eutrophication can result from high TDS levels in surface waters[27]. Water should ideally have a TDS of less than 500 ppm for home use, while water with a greater TDS is not always dangerous[27]. In order to protect delicate crops, water used in agriculture must have a TDS of less than 1200 ppm. Pure water is necessary for industry and particularly for the electronics industry[28]. The results obtained fell within the boundaries of the MPLs established[17,18]. The water is deemed safe for fish production based on its total dissolved solids and electrical conductivity.

TBARS, SOD and Catalase: The result of liver pollutants from *Tilapia* fish in New Gusau reservoir was found the content of Thiobarbituric acid (TBARS), Superoxide dismutase (SOD) and Catalase was elevated as shown in; (Figure-1, 2 and 3). There was no significant difference in reduced glutathione concentration in the liver of the test and control group (Table 2).

Table-2: The concentration of reduced glutathione (nmol/ mg protein) in the liver of tilapia from NGR compared to the control.

Group	Liver
Tested	$248.3±0.01^{a}$
Control	$245.0±0.01^{a}$

Anthropogenic metal input can come from metallurgical processes, mining operations, industrial effluents, home wastewater, and combustion pollutants. Heavy metals like Pb, Cd, Cu, and Zn are released as a result of these processes[29]. State that rising industrial and agricultural output in Nigeria is known to have contaminated a number of the country's waterways. The findings demonstrate a substantial increase in hepatic lipid peroxidation. Our research's findings suggest that the liver contains a significant quantity of contaminants, which may be the cause of the apparent rise in lipid peroxidation. It is widely acknowledged that pollutants increase ROS generation, which can damage cellular components like lipids, proteins, and DNA.

Important indicators in fish toxicity testing and for on-site monitoring of aquatic pollution are biochemical markers of contamination. They confirm that the material came into contact with particular chemical compound groups and explain their subsequent metabolic destiny. Aquatic environmental contamination assessment indicators require sensitive and predictive diagnostic tools (biomarkers) for assessing animal exposure and toxic effects of chemical contaminants. Biochemical investigations enable cause-effect relationships to be established at an early stage of pollution. According to studies[30-32] exposure to a variety of environmental contaminants, such as petroleum and heavy metals, can cause oxidative stress in aquatic animals, including fish. Oxidative stress occur when the production of reactive oxygen species (ROS) overwhelms theendogenous protection afforded by antioxidant enzymes like catalase, superoxide dismutase, glutathione S-transferase and redox sensitive thiol compound, reduced glutathione etc.

Similar to in mammals, antioxidant enzymes such as Superoxide dismutase (SOD), Catalase, and Glutathione S-transferase (GST) aid in the neutralization of ROS' damaging effects on fish. One of the most important enzymes that serves as the first line of defense against pro-oxidants and catalyzes the conversion of superoxide radicals into H_2O_2 and O_2 is superoxide dismutase[33]. A heme-containing enzyme called catalase makes it easier to remove H_2O_2, which is converted into oxygen and water. Reduced glutathione (GSH) and glutathione S-transferase (GST) ward off reactive oxygen species (ROS) and shield the cells from oxidative damage. As seen in Figures 1, 2, and 3, the liver of tilapia from the Nagwamatse farm (control) had lower activity levels of SOD, catalase, and GST than treated C. gariepinus from the New Gusau reservoir. Oxidative stress can cause cellular component peroxidation, enzymatic inactivation, and damage to DNA when antioxidant defenses are compromised or overpowered. When aquatic species are exposed to contaminants, which enhance the generation of ROS and can eventually overwhelm antioxidant enzymes, oxidative stress is the result. This increased oxidative damage happens in these creatures. The high concentration of pollutants in NGR, as reported[34] and other researchers, may be the cause of the observed increase in these enzymes' activity. These researchers reported that NGR has high levels of pollutants because various industries discharge their effluents into the water body through the Yar Dantse and Yar Dole sewers. The significant degree of lipid peroxidation in the liver of the NGR fish may have resulted from the release of free radicals from these effluents, which also overtaxed the antioxidant enzymes.

Conclusion

The research suggests that concentration of the selected physicochemical parameters from New Gusau Reservoir and Nagwamatse farm did not exceed[17,18] international accepted standard for fish and fish product. Therefore, the water is of good quality for human consumption and to support aquatic life. The changes observed in the markers of biotransformation and oxidative stress (SOD, CAT and MDA) showed that the fish in their natural habitat were relatively stressed compared to the fish from the Nagwamatse farm (control). The results show that enzymatic and non-enzymatic biomarkers of oxidative stress can be sensitive indicators of water pollution. They need to be carefully checked to ensure that unnecessarily high concentrations of some toxic heavy metals are not transferred to humans through fish consumption. Therefore, the null hypothesis is rejected and the alternative hypothesis is upheld.

Feature Scope: Future research should also be carried out using different species and other animals from this important reservoir to determine pollution load.

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