**BIOCHEMICAL ASSESSMENT OF OXIDATIVE STRESS IN AFRICAN CATFISH (*CLARIAS HETEROBRONCHUS*) FROM OIL-POLLUTED RIVERS IN THE NIGER DELTA, NIGERIA.**

**ABSTRACT**

This study ascertained the biochemical state of the African catfish, *Clarias heterobronchus*, from the Oloibiri and Warri rivers in Nigeria's Niger Delta, both of which have been exposed to crude oil spills for 50 years. Fish samples (n=10 each) were obtained from the two rivers and also from a fish pond in Ibadan, Oyo State, Nigeria which served as checkpoint (reference location). The physicochemical properties of the two rivers were examined as well as the concentration of heavy metals in the fish samples. The catfish's muscle, liver, gut, and gills were also examined for the activities of catalase, superoxide dismutase, glutathione S-transferase, glutathione, and malondialdehyde production. Both rivers' pH values (6.35 for Oloibiri and 6.03 for Warri river) were below the threshold that various regulatory agencies have determined is acceptable (6.5-8.5 USEPA). The control water had a high dissolved oxygen (DO) concentration (6.69±1.15 mg/l), while the Oloibiri (4.50±0.50 mg/dl) and Warri (4.01±0.25 mg/l) rivers had lower DO concentrations. The two rivers had significantly greater amounts (p<0.05) of all the heavy metals evaluated than the control. Total hydrocarbon content (THC) concentrations in the control, Oloibiri, and Warri rivers were 9.52, 35.32, and 48.56 mg/l respectively. In every tissue analyzed, the MDA levels of *Clarias heterobronchus* were significantly (P <0.05) higher in the fish from the Oloibiri and Warri rivers than in the control group. When compared to the control, lipid peroxidation in the gills of fish from the Warri River increased by 222%, while those from the Oloibiri River increased by 184%. The organs of fish from the Oloibiri and Warri rivers had significantly higher (p<0.05) levels of expression of antioxidant enzymes. Overall, oxidative stress in key organs and tissues (muscle, liver, gut, and gills) indicated that *Clarias heterobronchus* from the Olobiri and Warrı rivers are physiologically stressed.

**Key Words:** *Clarias heterobronchus,* Oloibiri River, Warri rivers, Niger Delta, Nigeria, Oxidative stress, Crude oil pollution

**Introduction**

Nigeria is among the world's top producers of oil. It exports over 1.8 million barrels a day and is rated first in Africa and sixth globally. The Niger Delta, which has the biggest wetland in the world along with vast freshwater swamps, forests, and a wealth of biological diversity, is where the majority of oil exploration activities are focused. The Niger Delta region is situated on Nigeria's South-South geopolitical zone and near the tip of the Gulf of Guinea on Africa's West Coast [1]. It is made up of nine oil-producing states (Ondo, Bayelsa, Abia, Cross River, Edo, Rivers, Akwa Ibom, Delta, and Imo) and has a total land area of about 75,000 km². With a vast network of more than 900 producing oil wells and a large amount of infrastructure associated to petroleum production, the area spans more than 800 oil-producing villages [2].

The Niger Delta has been experiencing the detrimental environmental effects of oil extraction since the 1950s, when oil was discovered in Nigeria. The Niger Delta region's life expectancy is below 50 or 60 years due to the severe environmental and human damage caused by oil spills [1]. Pipeline leaks, ruptures, unintentional discharges, engineering errors, vandalism, oil theft, and artisanal crude oil refining can all result in oil spills [1]. According to assessments, over 2,567,960 barrels of crude oil were spilled in 5733 incidents in the Niger Delta region between 1976 and 2001; of these, approximately 549,060 barrels were successfully recovered, while 1,820 barrels were lost [2]. The Niger Delta has seen numerous oil blowout disasters. In the Niger Delta region, oil spills are a source of heavy metal contamination in both terrestrial and aquatic habitats [3]. In the Niger Delta, oil spills are common, and the pollution they cause has harmed the region for decades, endangering the freshwater marshes, the marine environment, the soil, as well as endangering the health and well-being of the host people [2, 4-5].

Fishing is one of the traditional livelihoods that used to support the local economy in the Niger Delta region. Oil spills offshore have had a devastating effect on fishermen, who have been left without a substitute source of income [1]. Researchers are looking into how environmental containments affect fish [6-7], and they are paying more attention to how dangerous crude oil or refined petroleum is to fish [8-9]. Fish life is either directly or indirectly impacted by heavy metals, a crucial component of crude oil [19]. It is well recognized that a number of contaminants, such as crude oil and its byproducts, can cause stressors that harm aquatic life [10-12].

The Niger Delta region of southern Nigeria is home to the significant Oloibiri and Warri rivers. Due to the high levels of pollution brought on by the widespread presence and operations of oil and gas industries, as well as the careless release of industrial effluents and household garbage, safety concerns have been raised about the rivers over time [13-14]. According to Oluowo and Omoregie [15], heavy metal contamination is one of the main contaminants that has been found in water, sediment, and biota at levels higher than those that are considered concerning. Due to their widespread presence in the ecosystem and their capacity to bioaccumulate in living things to potentially dangerous levels, heavy metal pollution has been considered a major pollutant in the Warri River [16]. The survival and well-being of non-target creatures, such fish, can be impacted by prolonged exposure to elevated concentrations [17-18].

According to research, fish species that consume pollutants from the environment may trigger reactions that generate reactive oxygen species (ROS), which could result in oxidative stress [19]. An imbalance between an organism's antioxidant defense system and ROS generation is known as oxidative stress [20-21]. Proteins, lipids, and DNA are examples of biological macromolecules that can be attacked by reactive oxygen species in an organism [22-23]. Oxidative damage mostly affects structural and enzymatic proteins, impairing a variety of cellular processes. Malondialdehyde (MDA), a byproduct of lipid peroxidation, used as a biomarker to indicate severe oxidative damage in an organism [24]. Antioxidant enzymes are utilized by biological systems to detoxify and break down the detrimental effects of ROS [25]. Fish have antioxidant defense mechanisms that are both enzymatic (superoxide dismutase, or SOD) and non-enzymatic (reduced glutathione, or GSH) and found in almost every tissue [26]. Antioxidant systems have been shown to be effective biomarkers of oxidative stress and are crucial for fishes to maintain their redox status [27]. Heavy metals can cause oxidative stress and valuating fishes' antioxidant defenses and oxidative damage can reveal heavy metal contamination in the aquatic environment, as well as the extent of the fish population's reaction to heavy metals [13, 28].

Understanding the existing level of water contamination is essential from an economic and environmental perspective [29]. This significantly aids in the development of laws, rules, and management recommendations to restrict the amount of wastewater contaminated by heavy metals and oil spills [4]. Thus this study determined the biochemical state of the African catfish, *Clarias heterobranchus*, in the Oloibiri and Warri Rivers, which have been chronically exposed to crude oil spills for more than 50 years.

**MATEIALS AND METHODS**

**Study Rivers**

The Warri River is located in Southern Nigeria's Niger Delta, between latitudes 50211 and 60001N and longitudes 50241 and 60211E. It begins in the vicinity of Utagba-Uno and runs through Warri City's oil exploration districts, passing past numerous marketplaces, residential neighborhoods, and the parking lots and waste dumps of petroleum refineries before draining into the Atlantic Ocean. According to Aghoghovwia et al. [30], this river is one of the most polluted coastal rivers in Southern Nigeria because of the regular oil spills brought on by deliberate vandalism, aging infrastructure, mishaps, and unlawful bunkering. On Sunday, January 15, 1956, crude oil was initially discovered in Nigeria at Oloibiri, which is considered to be the first renowned location for this discovery. At 4°41′30.12 N 6°21′33.3 E, it is situated in the Niger Delta, approximately 45 miles (72 km) east of Port Harcourt in the Ogbia Local Government Area of Bayelsa State, Nigeria.

**Chemicals and Reagents**:

Thiobarbituric acid, trichloroacetic acid, glutathione, epinephrine and additional chemicals were gotten from sigma, St. Louis, USA. All chemicals were of diagnostic grade.

**Determination of the physicochemical properties of the two Rivers (Oloibiri and Warri Rivers) and the Control**

The physicochemical characteristics of the two rivers (Oloibiri and Warri Rivers) and the control were analyzed using standard procedures (APHA 2006). pH, DO, BOD, THC, total dissolved solids, iron, zinc, copper, cadmium, lead, nickel, and manganese are among the physicochemical characteristics that were examined.

**Animals and treatment**

Ten samples (each) of catfish (*Claritias heterobronchus*) ranging in weight from 250 to 400 grams and measuring 25.8 to 30.5 centimeters in length were gathered from the Oloibiri and Warri Rivers in August and September of 2023. Fish from a pond in Ibadan, Nigeria, were used as control fish because they were free of any contaminants that would have an impact on their biochemical reactions. Every catfish sample was brought to the lab in ice-cold containers (0–4°C), and they were all identified using the Idodo–Umeh (2003) method. The muscle, liver, intestine, and gills were removed from the fish during medullary transection, which was used to sacrifice it [13]. Different tissue homogenates were made in a cold sodium phosphate buffer (0.1M pH 7.4). The homogenates underwent biochemical tests after being centrifuged at 10,000g for 19 minutes at 40C.

**Determination of Heavy Metal Concentration in the tissues of fish samples.**

To determine the concentration of heavy metals (Mn, Pb, Ni, Zn, Cu and Cd) in fish tissues, Atomic Absorption Spectrophotometer (AAS, 2000 series) was used.

**Biochemical Analysis**

**Determination of Catalase Activity**

Catalase activity was measured using the method of Aebi [31]. The technique is based on the observation that when dichromate in acetic acid is heated in the presence of H2O2, it is reduced to chromic acetate, with perchromic acid forming as an unstable intermediate. Colorimetric measurements of the resulting chromic acetate are made at 570–610 nm. After heating the reaction mixture, chromic acetate is measured colorimetrically to determine the specific tone of the catalase preparation, which is achieved by adding the dichromate acetic acid mixture and the leftover H2O.

**Determination of Superoxide Dismutase (SOD) Activity**

The Misra and Fridovich [32] approach was used to measure the amount of SOD activity. Because superoxide dismutase may prevent epinephrine from autoxidizing at pH 10.2, this reaction serves as the foundation for a straightforward assay for the dismutase. The oxidation of epinephrine to adrenochrome was caused by the superoxide (O2) radical produced by the santhine oxidase process. The yield of adrenochrome produced per O2' increased as the pH and epinephrine concentration increased. According to these findings, there are at least two different ways that epinephrine is autoxidized, but only one of them involves a free radical chain reaction involving superoxide (O2) radicals, making it susceptible to SOD inhibition. The quantity of SOD required to provide a 50% inhibition of the oxidation of adrenaline to adrenochrome in a minute was measured and expressed as one unit of SOD activity.

**Estimation of Reduced Glutathione (GSH) Level**

The reduced glutathione (GSH) level was estimated using the Beutler et al. [33] method. The majority of cellular non-protein sulfhydryl groups are typically found in the reduced form of glutathione. Thus, this technique is predicated on the fact that adding 5,5-dithiobis-12-airobentic acid (Ellman's rengen) to sulhydryl compounds results in the production of a rather stable (yellow) color. The chromophoric product of the Ellman reagent reaction with 2-tutro-5-thiobenzorc acid, the reduced glutathione, has a molar absorption at 412 nm, which was measured in a colorimeter at 430 nm.

**Estimation of Glutathione-S-Transferase Activity**

The activity of glutathione-S-transferase was measured in accordance with Habig et al. [34]. The basic idea is that all known glutathione-5-tranferases have a comparatively high level of activity when 1-chloro-2,4,dinitrobenzene is used as the second substrate. As a result, 1-enloro-2,4,dinitrobenzene is used as the substrate in the standard assay for glutathione-S-transfease activity. This substance's maximal absorption moves to a longer wavelength when it is conjugated with reduced glutathione. The enzymatic reaction may be directly measured thanks to the rise in absorption at the new wavelength of 340 mm.

**Protein Determination**

The Biuret approach, as outlined by, was used to do this. The reagent was mixed with potassium iodide to stop the Cu ions from precipitating. In an alkaline solution, proteins combine with cupric ions to generate colored complexes measured at 540nm [35].

**Assessment of Lipid Peroxidation (LPO)**

The thiobarbituric acid reactive (TBAR) products found in the test sample were measured using Vashney and Kale's [36] method to assess lipid peroxidation. The results were represented as micromolar of malondialdehyde (MDA)/g tissue. The chromogenic reagent (2-Thiobabituric acid) and malondialdehyde, an end product of LPO, reacted in an acidic environment to produce a persistent pink chromophore with maximum absorption at 532 nm, which served as the basis for the experiment. It is easily extracted into butan-1-01 and other organic solvents.

**Statistical Analysis**

ANOVA and the LSD multiple comparison test were used to compare the mean and standard error of the mean (SEM) for the different parameters in order to look for statistically significant differences. A P-Value of less than 0.05 was considered to be substantially different.

**RESULTS**

**Physicochemical properties of the two rivers (Oloibiri and Warri Rivers) and the Control**

Table 1 displays the physicochemical characteristics of the two rivers in this study (the Oloibiri and Warri Rivers) as well as the Control. Both rivers' pH values (6.35 for Oloibiri and 6.03 for Warri river) were below the threshold that various regulatory agencies have determined is acceptable (6.5-8.5 USEPA). The control water had a high dissolved oxygen (DO) concentration (6.69±1.15 mg/l), while the Oloibiri (4.50±0.50 mg/l) and Warri (4.01±0.25 mg/l) rivers had lower DO concentrations.

**Table 1: Physicochemical properties of the brewery effluent with standard permissible values of some regulatory bodies**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Parameter** | **Control** | **Oloibiri**  **River** | **Warri**  **River** | **NESREA**  **(2011)**  **Limit** | **USEPA**  **(2009)**  **Limit** |
| pH | 7.76±0.56a | 6.35±0.51b | 6.03±0.34b | 6-9 | 6.5.-8.5 |
| BOD (mg/L) | 78.26±2.20a | 24.42±1.68b | 20.26±1.28c | 50 | 250 |
| Conductivity (µS/cm) | 54.00±0.45a | 73.03±2.15b | 84.34±2.56c | 250 | 250 |
| Dissolved Oxygen (mg/l) | 6.69±1.15a | 4.50±0.50b | 4.01±0.25b | 5.0 | 5.0 |
| Total Dissolved Solids (mg/L) | 25.02±0.45a | 35.00±0.68b | 41.00±1.44c | 500 | 500 |
| THC (mg/l) | 9.52±1.68a | 35.32±3.58b | 48.56±3.74c | 10.0 | 11.0 |
| Iron (mg/L) | 0.23±0.01a | 0.45±0.02b | 0.50±0.03b | - | 0.3 |
| Lead (mg/L) | 0.1±0.01a | 0.30±0.01b | 0.42±0.05c | 0.05 | 0.02 |
| Copper (mg/L) | 0.32±0.14a | 1.32±0.26b | 1.44±0.34c | 0.5 | 1.3 |
| Zinc (mg/L) | 0.76±0.02a | 2.03±0.20b | 2.38±0.52c | - | 0.12 |
| Cadmium (mg/L) | 0.03±0.01a | 0.24±0.02b | 0.26±0.04b | 0.02 | 0.002 |
| Manganese (mg/L) | 0.10±0.001a | 0.50±0.03b | 0.70±0.04c | 0.2 | 0.05 |
| Nickel (mg/L) | 0.10±0.01a | 0.15±0.02b | 0.18±0.02c | 0.05 | 0.005 |

Values are displayed as Mean ± SEM of three determinations. Values with dissimilar superscripts differ significantly (p<0.05).

**Mean levels of heavy metals in the tissues of African catfish *Clarias heterobronchus* from Oloibiri and Warri Rivers**

The mean concentrations of heavy metals in the heart of *Clarias heterobronchus* from Oloibiri and Warri Rivers, as well as the control site, are displayed in Table 2.

Cadmium (Cd), iron (Fe), zinc (Zn), nickel (Ni), lead (Pb), and manganese (Mg) were the seven heavy metals that were examined. The two rivers had significantly greater amounts (p<0.05) of all the heavy metals evaluated than the control. The concentration of all the higher metals in the Warri River was the greatest, exceeding both the USEPA and NESREA allowable limits. Total hydrocarbon content (THC) concentrations in the control, Oloibiri, and Warri rivers were 9.52, 35.32, and 48.56 mg/l.

Ni had the lowest mean concentration (0.02 mg/kg) in the liver of fish from the Oloibiri River, whereas manganese had the highest mean concentration (7.45 mg/kg) in the tissues of fish from the control location when compared to fish from the two rivers. While t was found in every tissue of fish samples from the two rivers, cadmium was not found in any of the tissues of fish from the control site. When comparing the tissues of fish from the two rivers to the control, the mean levels of Pb and Ni were significantly higher (p<0.05).

**Table 2**: Mean levels of heavy metals in the tissues of African catfish *Clarias heterobronchus* from Oloibiri and Warri Rivers

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Heavy**  **Metals** | **Control** | | | | **Oloibiri** | | | | **Warri** | | | |
| Muscle | Liver | Intestine | Gills | Muscle | Liver | Intestine | Gills | Muscle | Liver | Intestine | Gills |
| Iron | 0.24 | 0.12 | 0.12 | 0.12 | 0.25 | 0.20 | 0.22 | 0.11 | 0.26 | 0.22 | 0.24 | 0.14 |
| Lead | 0.06 | 0.07 | 0.07 | 0.00 | 1.85 | 1.65 | 0.25 | 0.89 | 1.96 | 1.68 | 0.34 | 0.92 |
| Cupper | 0.22 | 0.23 | 0.09 | 0.37 | 0.33 | 0.25 | 0.26 | 0.36 | 0.22 | 0.18 | 0.35 | 0.16 |
| Cadmium | 0.00 | 0.00 | 0.00 | 0.00 | 0.15 | 0.24 | 0.06 | 0.36 | 0.19 | 0.11 | 0.08 | 0.96 |
| Manganese | 6.81 | 7.45 | 4.96 | 5.05 | 0.05 | 0.03 | 0.05 | 0.03 | 0.32 | 0.16 | 0.32 | 0.58 |
| Zinc | 0.12 | 0.10 | 0.07 | 0.09 | 0.74 | 0.23 | 0.77 | 0.16 | 0.94 | 0.24 | 0.80 | 0.98 |
| Nickel | 0.07 | 0.05 | 0.09 | 0.07 | 0.13 | 0.12 | 0.13 | 0.13 | 3.78 | 3.78 | 4.77 | 5.13 |

**Catalase activity in various tissues of African catfish *Clarias heterobronchus* from Oloibiri and Warri Rivers**

Table 3 displays the CAT activity in specific tissues of African Catfish (C. heterobronchus) from the Oloibiri and Warri Rivers.

Clarias gariepinus from the control location and the two rivers had CAT activity ranging from 1.20 to 3.48 µmol/mg protein/min in their muscle, liver, gut, and gills. Fish from the Warri River had the highest CAT activity (3.48) in their gills, while fish from the control site had the lowest (1.20). In comparison to the control, catalase activity was significantly higher (p<0.001) in all fish organs from the two rivers. The CAT activity in the gills, liver, and muscle of fish from the Warri River and Oloibiri was likewise significantly different (p<0.05), with the Warri River exhibiting the highest CAT activity.

**Table 3**: Catalase activity in various tissues of African catfish *Clarias heterobronchus* from Oloibiri and Warri Rivers

**Catalase µmol/mg protein/min**

|  |  |  |  |
| --- | --- | --- | --- |
| Organs | Control | Oloibiri | Warri |
|  |  |  |  |
| Muscle | 1.50 0.80a | 2.70 0.60b | 2.81 0.20c |
| Liver | 1.96 0.51a | 0.50b | 0.32c |
| Intestine | 1.20 0.41a | 0.80b | 0.20b |
| Gills | 1.80 0.52a | 2.70 0.40b | 0.70c |

Values are shown as meanSEM of five replicates (n=5). Values with different alphabets differ considerably (p<0.05).

**Superoxide dismutase activity in various tissues of African catfish *Clarias heterobronchus* from Oloibiri and Warri Rivers**

The SOD activity in the different tissues of African catfish Clarias heterobronchus from the Oloibiri and Warri Rivers is displayed in Table 4.

The intestine of fish from the Oloibiri River had the highest SOD activity (2.40), whereas the gills of fish from the control site had the lowest mean (1.02). Fish from the two contaminated rivers had significantly higher (p<0.05) SOD activity in their organs than fish from the control site.

**Table 4:** Superoxide dismutase activity in various tissues of African catfish *Clarias heterobronchus*

**SOD (units/mg protein)**

|  |  |  |  |
| --- | --- | --- | --- |
| Organs | Control | Oloibiri | Warri |
|  |  |  |  |
| Muscle | 1.10 0.60a | 1.68 0.23b | 1.80 0.31c |
| Liver | 1.20 0.35a | 1.80 0.18b | 0.60c |
| Intestine | 1.47 0.21a | 0.70b | 0.53c |
| Gills | 1.02 0.41a | 1.64 0.14b | 1.80 0.60a |
| Values are given as meanSEM of five determinations. Values with different alphabets differ significantly (p<0.05). | | | |

**Reduced glutathione levels in various tissues of African catfish *Clarias heterobronchus* from Oloibiri and Warri Rivers**

Table 5 displays the concentrations of reduced glutathione (GSH) in the different tissues of African catfish Clarias heterobronchus from Oloibiri and Warri Rivers. Clarias gariepinus organs from the control site had substantially lower GSH levels than fish samples from the two rivers. The gills of fish from the control site had the lowest GSH mean (0.41). Compared to samples from the Oloibiri River, fish samples from the Warri River had considerably (p<0.05) greater GSH levels in every organ.

**Table 5: Reduced glutathione levels in various tissues of African catfish *Clarias heterobronchus* from Oloibiri and Warri Rivers**

**GSH (mg/gram tissue)**

|  |  |  |  |
| --- | --- | --- | --- |
| Organs | Control | Oloibiri | Warri |
|  |  |  |  |
| Muscle | 0.30 0.10a | 1.10 0.22b | 1.50 0.55c |
| Liver | 1.60 0.12a | 1.80 0.54b | 1.10c |
| Intestine | 1.10 0.51a | 0.32b | 0.58c |
| Gills | 0.46a | 0.31b | 0.42c |

Values are stated as meanSEM (n=5). Figures with different alphabets differ considerably (p<0.05).

**Glutathione S-transferase activity in various tissues of African catfish *Clarias heterobronchus* from Oloibiri and Warri Rivers**

Table 6 shows the activity of GST in various tissues of African catfish *Clarias heterobronchus* from Olobiri and Warri Rivers. GST activities were highest in the intestine of fish from Warri Rver (4.62), while the gills of fish from the control site had the lowest GST activity (1.09). With the exception of the gills, GST activity was significantly higher (p<0.05) in all the organs of fish samples from Warri river compared to those from Oloibiri River.

**Table 6: Glutathione S-transferase activity in various tissues of African catfish *Clarias heterobronchus* from Oloibiri and Warri Rivers**

**GST (µmol/min/mg protein)**

|  |  |  |  |
| --- | --- | --- | --- |
| Organs | Control | Oloibiri | Warri |
|  |  |  |  |
| Muscle | 1.11 0.60a | 1.67 0.28b | 1.90 0.40c |
| Liver | 2.02 0.30a | 2.90 0.12b | 0.34c |
| Intestine | 2.23 0.28a | 0.51b | 0.56c |
| Gills | 1.09 0.35a | 2.25 0.24b | 1.95 0.21c |

Values are presented as meanSEM (n=5). Numbers with different alphabets differ significantly (p<0.05).

**Lipid peroxidation levels (MDA) in various tissues of African catfish *Clarias heterobronchus*****from Oloibiri and Warri Rivers**

The levels of lipid peroxidation in various tissues of African catfish *Clarias heterobronchus* from Oloibiri and Warri Rivers is shown in Table 7.

The malondialdehyde levels in the organs of *Clarias gariepinus* from the control site were between 10.53 (muscle), 19.32 (liver), 16.10 (intestine) and 14.00 (gills). These values were significantly increased (p<0.05) n all organs in the two rivers compared to the control. Malondialdehyde levels were highest in the gills of fish from site Warri river, while the muscle of fish from the control had the lowest MDA (10.53).

Table 7: **Lipid peroxidation levels (MDA) in various tissues of African catfish *Clarias heterobronchus*****from Oloibiri and Warri Rivers**

MDA µmol/mg protein

|  |  |  |  |
| --- | --- | --- | --- |
| Organs | Control | Oloibiri | Warri |
|  |  |  |  |
| Muscle | 10.53 2.89a | 18.10 5.60b | 25.05 7.30c |
| Liver | 19.32 3.75a | 28.15 4.30b | 32.20 3.85c |
| Intestine | 16.10 5.26a | 36.98 3.50b | 40.24 4.91c |
| Gills | 14.00 4.81a | 39.76 2.43b | 45.13 4.52c |
| Values are stated as meanSEM (n=5). Numbers with different alphabets differ significantly (p<0.05). | | | |

**Protein levels in various tissues of African catfish *Clarias heterobronchus*** **from Oloibiri and Warri Rivers**

The levels of Protein in various tissues of African catfish *Clarias heterobronchus* from Oloibiri and Warri Rivers is presented in Table 8.

Protein level in the muscle, liver, intestine and gills of *Clarias gariepinus* from control site and the two rivers ranged between 1.04 and 1.54 mg protein/ml. The highest concentration of protein (1/54) was in the liver of fish from Warri river while the least (1.04) was seen in the gills of fish from the control site. Protein levels was significantly increased (p<0.005) in all the organs (with the exception of muscle) of fish the two rivers compared to the control.

**Table 8: Protein levels in various tissues of African catfish *Clarias heterobronchus*** **from Oloibiri and Warri Rivers**

Protein levels (mg protein/ml)

|  |  |  |  |
| --- | --- | --- | --- |
| Organs | Control | Oloibiri | Warri |
|  |  |  |  |
| Muscle | 1.24 0.18a | 1.07 0.65b | 1.22 0.09a |
| Liver | 1.28 0.31a | 1.44 0.24b | 1.54 0.21c |
| Intestine | 1.12 0.01a | 1.18 0.06b | 1.12 0.47c |
| Gills | 1.04 0.05a | 1.06 0.08b | 1.05 0.16b |

Values are expressed as meanSEM (n=5). Values with different alphabets differ significantly (p<0.05).

**DISCUSSION**

The aquatic ecosystem's biodiversity and productivity are impacted by water pollution [12]. A river or lake's physical and chemical characteristics are impacted by pollution, which then cascades down to harm the community, disturb the intricate food web, and make it harder to use the lake or river [4]. When values are compared to standard values, a physicochemical parameter analysis provides an accurate picture of the water's quality [37]. Both rivers' pH values (6.35 for Oloibiri and 6.03 for Warri river) were below the threshold that various regulatory agencies have determined is acceptable (6.5-8.5 USEPA). The solubility of heavy metals and other compounds is impacted by the acidic nature of the contaminated rivers. Low pH also slows down the rate of photosynthetic activity and the absorption of carbon dioxide and bicarbonates. The amount of cations and anions in water is closely correlated with its conductivity, which is a measurement of its capacity to carry electric current [38]. The two rivers' water had higher electrical conductivity than the control. The concentration of total dissolved solids (TDS), or salts, which often consist of anions like sulfate, chloride, and bicarbonates as well as cations like potassium, magnesium, calcium, and sodium, has also been linked to electrical conductivity. The total hydrocarbon content (THC) of the water from the two rivers and the control clearly shows a significant difference (p<0.05). The presence of oil contamination is confirmed by the high THC levels in the rivers, which are greater than allowed limits.

Some trace elements, known as heavy metals, are necessary for the body's normal maintenance processes. However, due to their non-biodegradable nature, their presence in excess causes them to bioaccumulate and become poisonous [39]. The study's findings demonstrated that, in comparison to the control water, the two rivers had noticeably higher concentrations of heavy metals. The high concentrations of heavy metals in both rivers are concerning since they are not biodegradable but instead move up the food chain [40]. They also consistently confirm levels of crude oil pollution [41]. Heavy metals have the potential to harm fish critical organs at the organism level and reduce aquatic life's biodiversity at the population level in receiving water bodies [42, 43]. Numerous cellular processes, including growth, apoptosis, differentiation, proliferation, and damage-repairing mechanisms, have been demonstrated to be hampered by them. They produce toxicity by inactivating enzymes, producing ROS, and weakening antioxidant defense [21, 43, 44]. Certain macromolecules are selectively bound by some heavy metals [16]. For instance, ferrochelatase and aminolevulinic acid dehydratase interact with lead to reduce the biological activity of these macromolecules. Genomic instability has also been connected to cadmium [16].

The amounts of heavy metals in the fish tissues in this investigation provide proof of contamination in the Oloibiri and Warri Rivers. Because fish gills serve as respiratory organs that absorb metal ions, the gills of fish captured at the two rivers acquired noticeably larger quantities of the heavy metals. Since the gills have the thinnest epithelia and are in close touch with the contaminated water, metals can easily pass through them, unlike other fish organs [41]. Heavy metals may have accumulated in other heart problems through metal-binding proteins [45].

Fish from two rivers had higher levels of CAT in all of their organs than at the control location. This was most likely caused by the high concentrations of heavy metals in the organs, which activated antioxidant defenses to lessen biological stress. Other writers [27, 46] have reported similar findings. Fish organs from the two rivers had significantly higher SOD activity in this investigation; this is again related to the biological reaction to crude oil contamination [47]. Fish from the two rivers had considerably higher amounts of GSH and malondialdehyde (MDA) in their tissues, indicating that the heavy metals in the fish were successful in causing oxidative stress in the organs.

Crude oil and its byproducts are among the many contaminants that are known to cause stress conditions that harm aquatic life [10]. Following a spill, contaminants in the water may enter the bloodstream, travel through the body, and be absorbed by the gills. Additionally, the sensitive cells of the gill secondary lamellae may be harmed by excessive concentrations, which would negatively impact the essential processes of respiration and salt management. Because the majority of heavy oils and greases are insoluble in water, they stay on the water's surface and prevent oxygen from dissolving, which has an adverse effect on aquatic life. Because oil converts organic components into inorganic ones, it depletes the dissolved oxygen in water [48]. And because the respiratory system is unable to get enough oxygen from the water to meet their fundamental metabolic demands, the fish experience increased stress at reduced dissolved oxygen levels caused by the oil spill [11].

Although both rivers are experiencing crude pollution due to a series of crude oil spills that have occurred in the two areas over the last 50 years, the Warri River may have higher levels of pollution, which explains the significant difference in lipid peroxidation in the various tissues of *Clarias heterobranches* obtained from the Oloibiri and Warri rivers. .

**CONCLUSION**

The study concludes that the catfish *Clarias heterobronchus* from the two rivers are physiologically stressed, which is relevant to the study's goal. Significant organs and tissues (muscle, liver, gut, and gills) have elevated levels of lipid peroxidation, indicating the production of oxidative stress. The impacted organism is at risk for a variety of disease processes due to this lipid peroxidation process. In comparison to catfish from unpolluted areas, the data from this study generally support the claim that catfish living in environment exposed to a 50-year crude oil pollution still exhibit symptoms of cellular physiological stress.

**Availability of data and materials**

Data presented in this study are available on request from the corresponding author.

**DECLARATIONS**

**Ethical approval**

The research ethics committee of the Delta State School of Marine Technology, Burutu, Nigeria, approved the experimental protocol for this study.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

**REFERENCES**

1. Flourizel I, Tatah G, Odekina MU (2024) A Review on the Effects of Crude Oil Spill on Aquatic Life (Fish) in The Niger Delta, Nigeria. Int J Environ Pollution Res 12(1),75-94.
2. Denny AD, Jacob EA (2022) Petroleum Spills and Accidental Discharges in the Niger Delta- A Literature Review. Int J Demo Deve Studies 5(3):20-29.
3. Onyena AP, Sam K (2020) A review of the threat of oil exploitation to mangrove ecosystem: Insights from Niger Delta, Nigeria. Glob. Ecol. Conserv. e00961. <https://doi.org/10.1016/j.gecco.2020.e00961>
4. Efekemo O, Davies IC, Orororo OC (2024) Water Quality Assessment and Heavy Metal Levels in Mudskipper (*Periophthalmus papilio*), Sediments and Water of Mangrove Swamps, Rivers State, Nigeria. Afr J Environ Natural Sci Res 7(1): 128-145.
5. Efekemo O, Orororo OC, Davies IC (2024) Evaluation of Water Quality and Heavy metal Concentrations in Shrimp (*P. Monodon*), Sediment and Surrounding Water of the Mangrove Swamps, Rivers State, Nigeria. Scientia Afri 23 (2): 251-262
6. Olorunfemi DI, Oteri O, Orororo OC, Enize TB, Pere C, Osioma E (2024). Genotoxic Effects of Cassava Effluent on the Expression of Selected Genes in the African Catfish, *Clarias gariepinus.* J Adv Bio Biotech27(5):352-364.
7. Olorunfemi DI, Orororo OC, Iloduba NE, Osioma E, Kpomah ND, Osio OL (2024) Evaluation of Genotoxicity by Comet assay in tissues of Clarias gariepinus exposed to cassava Effluent. Asian J Biochem Gen Mol Bio 16 (7): 98-108.
8. Dighiesh HS, Eldanasoury MA, Kamel SA, Sharaf SM (2019) Toxicity of Water-Soluble Fractions of Petroleum Crude Oil and its Histopathological Alterations Effects on Red Tilapia Fish. Egypt Soc Environ Sci 18:25–31.
9. Monteiro L, Traunspurger W, Roeleveld K, Lynen F, Moens T (2018) Direct toxicity of the water-soluble fractions of a crude and a diesel-motor oil on the survival of free-living nematodes. Ecol Indic 93:13–23.
10. Eriegha OJ, Omitoyin BO, Ajani EK (2019) Water soluble fractions of crude oil deteriorates water quality parameters and alters histopathological components of juvenile *Clarias gariepinus*. Animal Res Int 16(2): 3308 – 3318.
11. Eriegha OJ, Sam K (2020) characterization of crude oil impacts and loss of livelihood in the niger delta, nigeria: a fisheries perspective. Int J Maritime Interdis Res 1(1): 255-273.
12. Olorunfemi D, Nkemneme GO, Afolabi O, Ebisintei P, Okunoja HB, Orororo OC, Kpomah ED, Tesi JN (2024) Biochemical, histological and molecular investigation of fish exposed to a Brewery Effluent. J Basic Appl Zoology 85: 64: 1-12.
13. Iyorah I, Enagbonma BJ, Abu O, Biose E (2023) Oxidative stress responses to heavy metal burden in African catfish (*Clarias gariepinus*) from Warri River, Niger-Delta, Southern Nigeria. Sokoto J Vet Sci 21(2): 74 - 82.
14. Obafemi AA, Eludoyino OS, Akinbosola, BM (2012) Public perception of environmental pollution in Warri. J Appl Sci Environ Manage 16(3): 233 – 240.
15. Oluowo EF, Omoregie IE (2016) Assessment of heavy metals in surface water and bottom sediment of Ekpan Creek Effurun, Delta state Nigeria. J Appl Life Sci Inter 8(4)1-10.
16. Balali-Mood M, Naseri K, Tahergorabi Z, Khazdair MR, Sadeghi M (2021) Toxic mechanisms of five heavy metals: Mercury, lead, chromium, cadmium and arsenic. Frontier Pharmaco doi.10.3389/fphar.2021.643972.
17. Achuba FI, Osakwe SA (2004) Petroleum-induced free radical toxicity in catfish (*Clarias gariepinus*). Fish Physiol Biochem 29:97-103
18. Hamilton PH, Cowx IG, Olekiak MF, Griffiths AM, Grahn M, Stevens JR, Carvalho GR, Nicol E, Tyler CR (2015). Population level consequences for wild fish exposed to sublethal concentrations of chemicals - A critical review. Fish Fisheries 17(3): 545-566.
19. El-Agri AM, Emam MA, Gaber HS, Hassan EA, Hamdy SM (2022) Integrated use of biomarkers to assess the impact of heavy metal pollution on Solea aegyptiaca fish in Lake Qarun. Environ Sci Europe, doi.10.1186/s12302-022-00656.
20. Hoseinifar SH, Van Doan SVH, Ashouri G, Gioacchini G, Maradonna F, Carnevali O (2020) Oxidative stress and antioxidant defense in fish: The implications of probiotic, prebiotic and synbiotics. Rev Fisheries Sci Aqua 29 (2): 198-217.
21. Orororo OC, Asagba SO (2022) Treatment with Hibiscus sabdarrifa L Anthocyanins Improve Hematological Parameters in Rats Exposed to Cadmium. J Expl Res Pharma 3(2):7-15. DOI: 10.14218/JERP.2022.00003
22. Efekemo O, Orororo OC (2022) Effect of mixture of leaf extract of *Ocimum gratissimum* and *Vernonia amygdalina* on cadmium induced hepatotoxicity in wistar rats. J Biochem Int 9(4):73-81
23. Valon M, Valbon A, Fahri G, Qenan M, Dhurat K, Fatmir C (2013) Evaluating environmental pollution by applying oxidative stress biomarkers as bioindicators of water pollution in Fish. Polish J Env Studies 22 (5): 1519-1523.
24. Busari AA, Orororo OC, Opute UA, Efejene IO, Badmos K, Obadiah CC, Akinshipo WA, Egbune EO (2023) Effects of Black Seed Oil on Oxidative Stress Parameters and Gingival Expression of Inducible Nitric Oxide Synthase in Diabetes and Periodontitis-Induced Rats. Afr Scientist 24(3): 376-386.
25. Esiekpe GT, George BO, Orororo OC, Akpede AA, Okevwe GE, Onotugoma E, Oguntola J (2020) Monodora myristica seed extracts ameliorate crude oil contaminated diet-induced cardiotoxicity in rats. Thai J Pharma Sci 44 (3): 145-151
26. Abhijith BD, Ramesh M, Poopal RK (2016). Responses of metabolic and antioxidant enzymatic activities in gills, liver and plasma of Catla catla during methyl parathion exposure. J Basic Appl Zoology, doi.10.1016 /j.jobaz.2015.11.002.
27. Aly MY, El-Gaar DM, Salaah SM, Mohammed H (2020) Evaluation of heavy metals and oxidative stress with biochemical parameters as bio-indictors of water pollution and fish in Lake Burullus Egypt. J Marine Sci Res Oceano 3(1): 30-34.
28. Akinsanya B, Ayanda OI, Fadipe A, Onwuka B, Sali JK (2020) Heavy metals, parasitolgic and oxidative stress biomarker investigations in Heterotis niloticus from Lekki Lagoon, Lagos, Nigeria. Tox Report, doi.10.1016/j.toxrep.2020.08.010
29. Busari AA, Efejene IO, Olayemi SO, Orororo OC, Egbune EO (2024) Evaluation of antibiotic use and analysis of ciprofloxacin and gentamicin residue in fish samples from farms in Lagos, Nigeria. Environ Mon Assess 196:127:1-10.
30. Aghoghovwia OA, Ohimain EI, Izah SG (2016). Bioaccumulation of heavy metals in different tissues of some commercially important fish species from Warri River, Niger Delta, Nigeria. Biotech Res J 2(1): 25-32.
31. Aebi H (1984) Catalase in vitro. Methods Enzy 105:121-126.
32. Misra HP, Fridovich I (1972). The role of superoxide ion in the auto-oxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 247:3170-3175.
33. Beutler E, Duron O, Kelly BM (1963) Improved method for the determination of blood glutathione. J Lab Clin Med 61:882-888.
34. Habig WH, Pabst, MJ, Jakoby WB (1974). Glutathione S-transferases, The first enzymatic step in mercapturic acid formation. J Biol Chem 249 (22): 7130-7139.
35. Gornall AG, Bradawill CJ, David MM (1949) Determination of serum protein by means of biuret reaction. J Bio Chem 177(2): 751-766.
36. Varshney R, Kale RK (1990) Effects of calmodulin antagonists on radiation-induced lipid peroxidation in microsomes. Int J Radiat Biol 58(5):733–743.
37. Arojojoye OA, Adeosun AM (2016) Effect of environmental pollution on oxidative stress biomarkers in African Cat fish (*Clarias gariepinus*) from Asejire River in Oyo State, Nigeria. J Environ Occup Sci doi.10.5455/jeos.20161122030636.
38. Enegide C, Chukwuma KC (2018) Oil Spillage and Heavy Metals Toxicity Risk in the Niger Delta, Nigeria. J Health Poll 8(19): 1-8
39. Orororo OC, Asagba SO Tonukari NJ, Okandeji OJ, Mbanugo JJ (2018) Comparative Assessment of the Antioxidant Properties of *Hibiscus sabdarrifa* L Anthocyanins and its Aqueous Extract in Cadmium-exposed Rats. Res J Pharma Bio Chem Sci9(3): 836-843
40. Kpomah ED, Orororo OC (2023) Evaluation of Some Heavy Metals in Selected Canned Sardine Sold in YEnagoa Metropolis, Bayelsa State, Southern Nigeria. Nig J Sci Env 21(1):224 – 236.
41. Onyegeme-Okerenta BM, Oharisi AO, Wegwu MO (2017) Impact of Crude oil spillage on Water and African Catfish (*Clarias gariepinus*) in Uzere, Isoko South LGA of delta state Nigeria. Eur J Earth Environ 4(1):7-18
42. Farombi EO, Adelowo OA, Ajimoko YR (2007) Biomakers of oxidative stress and heavy metals levels as indicators of environmental pollution in African Catfish (*Clarias ariepimus*) from Nigeria Ogun River. Int J Environ Res Public Health 4(2): 158-165.
43. Ekakitie LI, Orororo OC, Okpoghono J (2021) Changes in haematological parameters of aluminium-exposed rats treated with natural bee honey. IOSR J Env Sci Toxico Food Tech 15(5):22-25
44. Orororo OC, Asagba SO, Egbune EO, Efejene OI (2022) Sperm parameters and histological changes in testes of cadmium-exposed rats treated with *Hibiscus sabdarrifa* L*.* anthocyanins. Sokoto J Med Lab Sci 7(3):114-122.
45. Rajeshkumar L, Xiaoyu L (2018) Bioaccumulation of heavy metals in fish species from the Meiliang Bay, Taihu Lake, China. Tox Report 5: 288-295.
46. Osioma E, Akanji MA, Arise RO (2013) Hepatic enzyme markers and proteins in serum and some selected tissues in *Clarias gariepinus* from swamp around Kokori- Erhoike oil field, Nigeria. J Res Bio 3(4): 984-992.
47. Thitiyan T, Pongdontri P, Tengjaroenkul B, Neeratanaphan L (2021) Bioaccumulation and oxidative stress in Barbonymus gonionotus affected by heavy metals and metalloid in municipal landfill reservoir. Int J Environ Studies 79(1): 98-113.
48. Ogbeibu AE, Edutie LO (2002) Impact of Brewery Effluent on the Water Quality and Rotifers of Ikpoba River, Southern Nigeria. Afri J Environ Poll Health 1:1-12.