**Multiple Biomarkers Variability in Lead Exposed Fresh Water Fish (*Clarias gariepinus)***

**ABSTRACT**

**Background** Heavy metals are the most important pollutants of the aquatic ecosystems as they are present throughout the ecosystems. Freshwater environments are constantly being impacted by heavy metals mostly from untreated effluent discharged from industrial sites, some agricultural practices, and daily activities. Because it harms humans and aquatic biota, toxic heavy metal pollution of the water is a serious concern.

**Method** The current study examined the effects of sublethal lead levels (0.0, 0.4, and 0.8 mg/l) on the African catfish's serum metabolites, enzyme activity, and other factors following a 21-day exposure period. The activities of Glutamic Oxaloactic Transaminase (GOT), Glutamic Pyruvate Transaminase (GPT), Alkaline Phosphatase (ALP), Creatinine, and Urea in the fish's serum were examined in subadults of the African catfish *Clarias gariepinus*.

**Results** At the 0.05 probability level, statistical ANOVA results revealed a significant difference (P<0.05) between the experimental groups and the control for all parameters analysed.

**Conclusion** Organ dysfunction, enzyme inhibition, metabolic impairment, and organ damage were all brought on by the toxicant (lead). It is clear from the overall results that these biochemical parameters changed significantly on exposure to Lead. As a result, this study demonstrates that these biochemical measurements can be essential biomarkers for examining heavy metal pollution.

**Keywords**: Heavy Metal, *Clarias gariepinus*, Glutamic Oxaloacetic Transaminase (GOT), Glutamic Pyruvate Transaminase (GPT), Acid Phosphatase (ACP), and Alkaline Phosphatase (ALP)

**Introduction**

One of the most significant environmental issues of our time is heavy metal contamination (Elarabany & Bahnasawy, 2019). Fish may exhibit signs of stress if heavy metals infiltrate their aquatic environments (Ali et al., 2019). These metals are difficult for any biological degrading mechanism to remove from the environment because they are persistent. When released into the environment by industrial effluents, agricultural operations, and some life activities, they present a number of health dangers to the surrounding population and living organisms (Prakash & Verma 2020)

 The toxicity of these metals poses major threats to ecology, nutrition, and the environment. According to Authman et al. (2015), heavy metals can dissolve rapidly in water and then enter the bodies of aquatic creatures. As they ascend the food chain, these metals are subsequently absorbed by the bodies of larger animals.

 Lead (Pb), in contrast to other heavy metals, is one of the most dangerous pollutants in aquatic settings. The mining and smelting of lead ores, industrial effluents, fertilisers, pesticides, and municipal sewage wastes are the main sources of lead contamination (Needleman, 2006). Among other sites, a fish's skins, gills, and respiratory system can absorb lead. After ingestion, lead circulates throughout the fish's gonads, liver, kidney, heart, gills, and blood (ATSDR, 2005).

 Lead produces oxidative damage at greater quantities, which may immediately impact the cell membrane. *Clarias gariepinus* is now a frequently utilised test organism in fish research because of its extensive cultivation and availability in all freshwater settings. According to Jasmin et al. (2018), Onadje & Akalusi (2024), and Gupta and Sharma (2023), serum biochemical parameter analysis is widely recommended to provide early indicators of notable changes in stressed organisms. It is particularly helpful in determining the general health status of animals as well as the target organs of poisoning. The objective of this present study is to investigate how lead exposure affects the serum biochemical parameters of African catfish, *Clarias gariepinus.*

**Materials and Methods**

***Exposure medium / toxicant***

In this experiment, anhydrous lead chloride was used as the test material. The chloride form of the metal was selected because it is less toxic than other forms of lead. The concentrations for the experiment were 0.40 mg/l and 0.80 mg/l. The following formula was used to determine the molecular atomic weight of the lead chloride containing 1.0 g of lead.

$\frac{Molecular weight of Lead Chloride}{Atomic weight of Lead (Pb)}$ Equation 1

While the required concentrations were calculated thus:

$\frac{Weight of lead needed X Molecular weight of Lead}{Atomic weight of Lead (Pb)}$ Equation 2

***Experimental fish model***

The African catfish *Clarias gariepinus* sub-adults were obtained from a fish farm in Obiarukwu, Delta State, Nigeria. The fish were transported to the laboratory in an oxygen-filled bag. The fish were kept in bore-hole water-filled 60-litre polypropylene bowls. To prevent starvation and any impact on the fish's haematology, the fish were allowed to acclimatise to commercial fish pellets over 14 days. The health of the fish was carefully assessed for signs of disease and decline. The water was changed daily, and any dead fish was promptly removed to prevent ingestion. The fish were fed from 8:00 to 16:00 twice a day.

The fish's average weight was 127.02±89, and its average length was 17.54±0.62. A short-term static bioassay lasting 96 hours makes up the experimental setting. 50-litre plastic aquariums were utilised. Three 50-litre plastic aquariums with a capacity of 0.40 mg/l, 0.80 mg/l, and a control of 0.0 mg/l were used in a duplicated experiment. Six sub-adult fish were kept in each tank with bore-hole water, and the toxicant was replaced daily to preserve its efficacy and lessen the chance of the metal deteriorating. Feeding continued during the exposure time. During the exposure period, no fatalities were reported.

***Sampling and biochemical analyses***

To avoid stress, the experimental fish were anaesthetised with clove oil (4–4.5 mg/L) before blood collection and dissection. During days 7, 14, and 21 of the experiment, we used a 26-gauge hypodermic needle to caudally venipuncture. Approximately 2-3 mL of blood was collected in Ethylenediaminetetraacetic acid (EDTA) vacutainers for each of the five fish in each group. Additionally, the serum was separated from the obtained blood using centrifugation. The serum was created by centrifuging the blood for five minutes at 3000 rpm using a motor centrifuge. The serum was visible at the top and the plasma at the bottom. The serum obtained after the centrifugation was put in a plastic container containing anticoagulant properties. Biochemical parameters like Glutamate Pyruvate Transaminase(GPT), Glutamate Oxaloacetate Transaminase (GOT), Alkaline Phosphatase (ALP), Urea and Creatinine were evaluated using commercially prepared kits by the following methods:

Table 1: Some Serum Parameters and Methods Used

|  |  |  |
| --- | --- | --- |
| **S/N** | **Serum Parameters** | **Methods** |
| 1. | SGPT (U/L) | Bergmeyer (1974) |
| 2. | SGOT (U/L) | Bergmeyer (1974) |
| 3. | Alkaline Phosphate (U/L) | King and Armstrong (1934) |
| 4. | Creatinine (g/dl) | Tietz (1995) |
| 5. | Urea (g/dl) | Tietz (1990) |

***Data Analysis***

A one-way Analysis of Variance (ANOVA) was performed on the gathered data using Microsoft Excel 2000. Statistical differences were computed at the 95% level of freedom, and the results' displayed in tables.

**RESULTS**

***Serum Glutamate-Pyruvate Transaminase SGPT (U/L)*:** Table 2 illustrates how lead affects the African catfish's serum GPT activity. At day 7, the control (0.0 mg/l) had the lowest value of 28.29±0.21, while the highest concentration (0.8 mg/l) had the lowest mean value of 37.18±0.08. Throughout the 21-day exposure period, the data demonstrated a significant increase in SGPT values as concentrations increased.

Table 2: Mean Value of serum GPT activity in *Clarias gariepinus* exposed to sublethal concentrations of lead (Pb) for 21 days.

|  |  |
| --- | --- |
|  | **Days**  |
| **Conc. (mgPb/l)** | 7 | 14 | 21 |
| 0.0 | 28.29±0.21 | 29.22±0.40 | 29.05±0.01 |
| 0.4 | 29.69±0.29 | 29.93±0.01 | 29.99±0.58 |
| 0.8 | 34.85±0.17 | 36.58±0.27 | 37.18±0.08 |

***Serum Glutamate-Oxaloacetate Transaminase SGOT (U/L)*:** Table 3 documents the impact of lead on serum glutamate-oxaloacetate transaminase. According to the mean values, the concentration of 0–8 mg/l was the highest. At 0.8 mg/l, the highest and lowest concentrations were 44.05±0.38 and 20.49±0.23. The values varied, though, with day 14 showing the highest value at 34.26±0.23 for a concentration of 0.4 mg/l. However, there was a significant difference between the obtained and control values.

Table 3: Mean Value of serum GOT activity in *Clarias gariepinus* exposed to sublethal concentrations of lead (Pb) for 21 days.

|  |  |
| --- | --- |
|  | **Days**  |
| **Conc. (mgPb/l)** | 7 | 14 | 21 |
| 0.0 | 20.49±0.23 | 21.59±0.23 | 22.64±0.04 |
| 0.4 | 21.34±0.21 | 34.26±0.23 | 22.54±0.22 |
| 0.8 | 33.93±0.04 | 40.02±0.05 | 44.05±0.38 |

***Alkaline Phosphate (U/L)*:** Table 4 reveals how lead affected the alkaline phosphate activity over 21 days. The alkaline phosphate values in the control showed a fluctuating effect (0.0 mg/l), with the 14th day yielding the lowest value of 34.32±0.22. However, the alkaline phosphate values rose for concentrations of 0.4mg/l and 0.8mg/l. On day 21, at a concentration of 0.8, the highest value recorded was 44.48±0.39.

Table 4: Mean Value of Alkaline Phosphate (u/l) activity in *Clarias gariepinus* exposed to sublethal concentrations of lead (Pb) for 21 days.

|  |  |
| --- | --- |
|  | **Days**  |
| **Conc. (mgPb/l)** | 7 | 14 | 21 |
| 0.0 | 34.44±0.23 | 34.32±0.22 | 34.59±0.18 |
| 0.4 | 34.73±0.13 | 34.67±0.03 | 35.79±0.29 |
| 0.8 | 31.12±0.37 | 40.02±0.05 | 44.48±0.39 |

***Creatinine (g/dl)*:** The mean values found in Table 5 show how lead affects the creatinine levels of exposed fish. Concentration and days led to a significant increase in creatinine levels. On day 21, at 0.8 mg/l, the highest value recorded was 1.94±0.05; on day 7, the control had the lowest value, 1.53±0.01.

Table 5: Mean Value of Creatinine (u/l) activity in *Clarias gariepinus* exposed to sublethal concentrations of lead (Pb) for 21 days.

|  |  |
| --- | --- |
|  | **Days**  |
| **Conc. (mgPb/l)** | 7 | 14 | 21 |
| 0.0 | 1.53±0.01 | 1.56±0.01 | 1.58±0.01 |
| 0.4 | 1.54±0.01 | 1.58±0.00 | 1.68±0.12 |
| 0.8 | 1.55±0.01 | 1.82±0.14 | 1.94±0.05 |

***Urea (g/dl):*** The effect of lead on the urea levels of the exposed fish is displayed in Table 6. The findings demonstrated that there was a considerable variation in the mean values. As days and concentration grew, values climbed as well. The control value was 9.48±0.15 on day 7, and the maximum value, 16.28±0.13, was recorded on day 21 at a concentration of 0.8 mg/l.

Table 6: Mean Value of Urea (g/dl) activity in *Clarias gariepinus* exposed to sublethal concentrations of lead (Pb) for 21 days.

|  |  |
| --- | --- |
|  | **Days**  |
| **Conc. (mgPb/l)** | 7 | 14 | 21 |
| 0.0 | 9.48±0.15 | 9.19±0.10 | 12.67±0.33 |
| 0.4 | 9.60±0.22 | 9.50±0.09 | 14.65±0.16 |
| 0.8 | 9.45±0.09 | 9.30±0.15 | 16.28±0.13 |

**Discussion**

Variations in blood biochemical levels often indicate changes in the fish's physiological state. Although no death was seen in this investigation, we found physiological alterations in the fish after they were exposed to lead. In particular, there is a correlation between fish performance and the quality of their environment. Heavy metals like lead enter the aquatic ecosystem through various human activities, including industrial, agricultural, and others. Fish must adjust by altering their physiological and behavioural habits to deal with the oxidative stress they encounter due to the toxicity induced by heavy metals (Onadje & Akalusi, 2024).

Numerous studies have employed changes in biochemical parameters to forecast possibly detrimental consequences in fish under stress (Prakash & Verma 2020; Abdel-Warith et al. 2020). This work investigates the toxicological consequences of sub-lethal Pb exposure to African catfish (*Clarias gariepinus*).

 The metabolism of proteins and amino acids depends on transaminases like SGOT and SGPT, which can be released into the plasma after tissue damage or dysfunction. A higher amount of SGOT and SGPT indicates tissue damage and fish metabolism impairment. According to Onadje and Akalusi (2024), fish *Clarias gariepinus* exposed to lead had higher levels of GOT and GPT. The authors suggested this could be because the liver, kidney, heart, and other tissues were damaged during the metal-induced stress. Protein and amino acid metabolism depends on the liver-produced serum glutamate pyruvate transaminases (SGOT) and SGPT. The result of this study is in tandem with those of Alshkarchy et al., 2021. Elevated SGOT and SGPT levels indicated liver dysfunctions in lead-induced *Clarias gariepinus* (Table 2). Similar increases in transaminases were reported in response to arsenic (Prakash & Verma, 2020), zinc (Srivastava & Prakash, 2018), and fluoride (Srivastava et al., 2012). Therefore, any change in the concentration of these enzymes reflects the state of the fish's liver. Thus, when metal intoxication like lead destroys the hepatic tissue, phosphatases and transaminases are released into the bloodstream, potentially increasing their blood content.

The alkaline phosphatase (ALP) is a metalloenzyme that is bound to the cell membrane by a glycosylphosphatidylinositol (GPI) (Atkins et al., 2011). ALP is a glycoprotein with an exterior and catalytic domain based on serine. ALP cannot work until two Zn+2 ions and one Mg+2 ion bind to the enzyme's active site. The serine base may reduce the activity of the ALP enzyme (Banaee & Taheri, 2019). Alkaline phosphatase is a non-specific indicator for diagnosing liver and bone injury. Additionally, ALP is a tremendous biological indication for detecting cellular stress, according to Banday et al. (2019). It plays a critical function in detoxifying xenobiotics by hepatocytes (Vaziryan et al. Hatami et al., 2017). Tumour formation in the liver and bone tissue, hyperthyroidism, bile duct obstruction, liver nodules, liver cysts, liver failure, increased ALP biosynthesis rates in hepatocytes, and intestinal dysfunction can lead to increased  ALP activity in the serum or plasma. The elevated levels of ALP in this study support an earlier study by Prakash Verma (2020). Thanga Malathi Anuradha (2020) reported higher levels of ALP in Lithium-induced *Channa punctatus* and *Oreochromis niloticus*. Similar outcomes have been shown following exposure to diazinon (Abdelkhalek et al., 2017) and deltamethrin (Abdel-Daim et al., 2015; Abdelkhalek et al., 2015) in *Oreochromis niloticus*. Onadje & Akalusi once reported increased levels of ALP in *Clarias gariepinus* exposed to sublethal concentrations of lead chloride for 96 hours. However, the result of this study contradicts an earlier report by Alshkarchy et al. (2021). These authors reported decreased ALT levels with increasing concentrations.

Creatinine is a waste product from creatine phosphate that breaks down in muscular tissues. Fish mostly eliminate creatinine through their gills and kidneys. Fish kidney health and function can be inferred from creatinine levels. A higher creatinine level could indicate renal disease or injury in fish, creatinine levels can be a good indicator of muscle mass and protein metabolism (Hafez & Hassan, 2019) The increased creatinine levels observed in this study support earlier works by Onadje & Akalusi (2023), Zeyad et al. (2021) and Hafez & Hassan (2019). This study's outcome aligns with those of Abdel-Moneium et al. (2008), who showed that after exposure to dyestuff and chemical effluent, *Clarias gariepinus* had higher serum creatinine, a symptom of renal failure. Thus, while Zaki et al. (2014) discovered that chronic exposure to metals led to a progressive rise in serum creatinine levels of *Clarias gariepinus*, which may have been brought on by glomerular insufficiency, increased muscle tissue catabolism, or impaired carbohydrate metabolism, Mahmoud et al. (2013) found that creatinine levels had risen in *Clarias gariepinus* after lead exposure.

The breakdown of fish protein and amino acids results in the waste product urea. Fish kidney health and function can be extrapolated from urea levels. Raised urea levels could indicate an infection or damage to the kidneys. Environmental variables like pollution, temperature, and water quality might affect fish urea levels. The data recorded in this study is contrary to the decreased urea levels reported by Hafez & Hassan (2019) but supported by studies by Khalifa et al. (2024), El-Khadragy et al. (2019) and Elarabany et al. (2019).

**Conclusion**

Significant concentrations of heavy metals in the aquatic environment due to anthropogenic, agricultural, and industrial activities are thought to threaten aquatic life. The current findings and discussion support the conclusion that lead, a heavy metal, caused noticeable changes in the serum biochemistry of *Clarias gariepinus*. This study offers succinct proof that varying Pb concentrations caused stress in the physiological and metabolic processes of the fish, which in turn caused biochemical dysfunction in this species. Furthermore, the data demonstrate that both enzymatic and nonenzymatic oxidative stress biomarkers can be employed as sensitive indicators of aquatic pollution.

**List of Abbreviations**

ALP: Alkaline Phosphatase

ANOVA: Analysis of Variance

EDTA: Ethylenediaminetetraacetic acid

GOT: Glutamic Oxaloactic Transaminase

GPT: Glutamic Pyruvate Transaminase

SGPT: Serum Glutamic Pyruvate Transaminase

SGOT: Serum Glutamic Oxaloactic Transaminase

**Availability of data and materials**

All data collected by us and analyzed during this study have been provided in this manuscript.

**Competing interests**

There is no conflict of interest among the authors neither financially nor non-financial

**Declaration**

We declare that “Guidelines for Use of Fish in Field Research” were followed as outlined in the AFS Policy Statement #16 in conjunction with the specific laws of the land as it applies to this study

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1.

2.

3.

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