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ASSESSMENT OF BIOCHEMICAL INDICES IN CLARIAS GARIEPINUS TREATED WITH SUBLETHAL CONCENTRATIONS OF POTASH

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ABSTRACT
The intrusion of chemical substances in aquatic environments poses significant risks to fish health and overall ecosystem stability. Therefore this study evaluated the effects of sublethal concentrations of potash on the biochemical indices of Clarias gariepinus, a commercially and ecologically important freshwater species. Healthy juveniles of similar size and weight were acclimatized and exposed to graded sublethal concentrations of potash alongside a control group under laboratory conditions for 21 days. Tissue samples were collected for analysis of Biochemical indices. Results showed significant, concentration-dependent alterations in all measured parameters when compared with the control group. Biochemical analysis revealed elevated or increased ALT and AST activities compared to the control, pointing to possible liver dysfunction. Therefore, exposure to sublethal concentrations of potash induces pronounced physiological, metabolic, and ionic disturbances in Clarias gariepinus. These alterations may compromise fish health, growth, and survival over time. The findings underscore the need for careful monitoring and regulation of potash usage in aquatic environments to minimize its ecological impact and protect aquatic life.

KEY WORDS*: Biochemical, Clarias Gariepinus, Sublethal, Concentration, Potash*

1.0 INTRODUCTION

Aquatic ecosystems across the world are increasingly subjected to various anthropogenic stressors, including agricultural runoff, industrial discharge, and domestic waste, which introduce a wide range of chemical substances into water bodies (Bashir *et al.*, 2020; Thanigaivel *et al.*, 2023)**.** Amongst these chemical pollutants, Potash (primarily potassium carbonate and related alkaline compounds) is widely used in Nigerian communities for food processing, domestic applications, and traditional practices. When introduced inadvertently into aquatic ecosystems, potash has the potential to alter the pH, ionic balance, and chemical composition of water, which may interfere with the homeostatic mechanisms of aquatic organisms (Ogunyinka *et al.*, 2022; Olufemi *et al.*, 2021). These contaminants can alter water quality and negatively impact aquatic organisms, particularly fish, which serve as sensitive biological indicators of environmental health (Bashir *et al.*, 2020; Thanigaivel *et al.*, 2023). Fish are widely used in ecotoxicological studies because their physiological systems respond predictably to environmental stressors, making them suitable models for assessing aquatic pollution (Amaeze *et al.*, 2020). Among freshwater fish species, *Clarias gariepinus* (African catfish) is of significant economic and ecological importance due to its high adaptability, rapid growth, and widespread use in aquaculture and toxicological research (Bawa, 2024; Kwikiriza *et al.*, 2025; Chandra Segaran *et al.*, 2023).

Biochemical parameters in *C. gariepinus* serve as reliable diagnostic tools for evaluating fish health and environmental quality, as these indices reflect the internal physiological state of the organism. Biochemical indices such as liver enzymes (alanine aminotransferase [ALT], aspartate aminotransferase (AST]), total protein, cholesterol, urea, and creatinine are widely used to evaluate organ function and metabolic disturbances in fish (Bojarski *et al.*, 2025; Abdel-Tawwab *et al.*, 2025). Despite the established importance of these biomarkers in toxicity assessment, current literature lacks comprehensive investigations into how sublethal concentrations of potash affect biochemical, and electrolytic profiles in *C. gariepinus*. Consequently, aquaculturists, environmental managers, and policy makers are left with insufficient scientific evidence to guide safe potash usage and to mitigate its potential risks to fish health and aquatic ecosystems. Given the paucity of information on the toxicological effects of potash on fish, this study is designed to assess the biochemical, and electrolytic responses of *Clarias gariepinus* when exposed to sublethal concentrations of potash. The findings of this research will contribute to the existing body of knowledge on aquatic toxicology, provide baseline data for environmental monitoring, and inform regulatory policies on the safe use of chemical substances in aquaculture and aquatic ecosystems.

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2.0 METHODOLOGY

2.1 Source of Fish and Transport

A total of twenty-five (25) healthy juvenile *Clarias gariepinus* (African catfish) weighing between 100 g to 150 g per weight were used for the sublethal bioassay test in this study. These fish were obtained from Ikuligan Farm at Agudama Epie, within Yenagoa metropolis of Bayelsa State, Nigeria. Fish were transported under cool conditions in plastic cans covered with fishing nets to the wet laboratory of the Department of Biological Sciences, Faculty of Science, Niger Delta University, Bayelsa State, Nigeria.

2.2 Source of Chemical (Potash)

The test chemical, potash (KCl), a white crystalline solid with a molar mass of 74.55mol^{-1} and density of 1.98 g/cm^3 was purchased from a chemical store at Swali market, within Yenagoa metropolis of Bayelsa State, Nigeria.

2.3 Fish Acclimatization

Experimental fish was acclimatized in four plastic aquaria for a period of two weeks (14 days).Within this period, a renewal bioassay was carried out, that is, water was changed daily (24 hours interval) and the plastic aquaria were properly aerated and cleaned, while maintaining equal light and dark conditions.

Fish were also fed with compounded fish feeds at 3 % body weight daily at 11:00 hours.. Fish were considered healthy and fit for the definitive test with mortality rate less than 5 %.

2.4 Experimental Design

2.4.1 Range Finding Test

The sublethal concentration needed for the definitive test was determined following a trial experiment, also known as range finding test. This is to determine the actual sublethal concentrations of the toxicant that would not kill experimental fish, but would produce sublethal effects in the exposed fish during the definitive test.

2.4.2 Definitive Experiment

The sublethal test was a completely randomized, static bioassay experiment. under room temperature following the method described by Inyang, (2008). During the sublethal test, Fish were randomly assigned into control and treatment groups. The sublethal experiment was made up of two main groups, that is, one (1) control group having four replicates with one (1) fish in each aquarium, labeled T_0R_1-T_0R_4 and three (3) treatment groups of four (4) replicates, having one (1) fish in each aquarium and was labeled thus: T_1R_1-T_1R_4, T_2R_1-T_2R_4 and T_3R_1-T_3R_4.

Fish in the treatment groups were exposed to three different concentrations (30.2 mg/L, 45.4 mg/L and 75.6 mg/L.) of the test chemical which was obtained from the range finder test for twenty-one (21) days (3 weeks), while the control group had no chemical exposure during the period of the sublethal experiment. Also, during the test, tap water (30 L) and test chemical concentrations were renewed daily at 11:00 hours.

A total of sixteen (16) plastic aquaria were used for the sublethal experiment with each aquarium having one (1) fish, making a total of sixteen (16) experimental fish, including the control. The holding plastic aquaria were properly aerated and regularly cleaned while equal light and dark conditions were maintained throughout the period of the experiment. Fish were also fed with compounded fish feeds at 5 % body weight daily at 11:00 hours during the period of the sublethal experiment.

2.5 SAMPLE COLLECTION

2.5.1 Collection of Organs

Organs such as liver, and kidney from experimental fish where collected by dissecting fish using a dissecting knife. A part of these organs were collected and crushed in a ceramic mortar. Crushed organ (s) was mixed with 0.5 ml of perchloric acid for metabolites, deionized water (distilled water) for electrolytes and physiological saline (normal saline) for enzymes respectively. Mixture (s) was centrifuged for 15 minutes at 3000 rpm and the supernatant (serum) was poured into labelled sample bottles and refrigerated until analysis.

2.6 Laboratory Analysis

Biochemical (enzymes) in the tissues of exposed Fish were analyzed in the chemical laboratory of Federal Medical Centre (FMC), Yenagoa to determine the sublethal effect of the toxicant (Punch with active ingredient 1.8% of abamectin**)** on experimental fish using standard procedures**.**

2.7 Statistical Analysis

Means and Standard Deviations were also calculated from the data derived from the analysis of the various experimental groups. One-way analysis of variance (ANOVA), conducted to see if significant differences exist between the measured parameters. Post Hoc Test (Turkey HSD test) was conducted to separate means between groups and determine their interrelatedness. All statistical analysis was done using the SPSS® (Statistical Package for Social Sciences) statistical tool kit version 20.

3.0 RESULT

3.1 Physico-chemical Parameters of test solution (water)

Table 1 show that water quality parameters viz: pH, Conductivity, and Temperature did not differ significantly ($p<0.05$) with the controls, except for Turbidity and Dissolved Oxygen (DO). The values of Turbidity slightly appreciated from 1.58±0.25 to 2.37±0.31, and later declined to 1.11±0.12 at the highest concentration. Generally, the values appreciated compared to the control, 0.10±0.21. Dissolved Oxygen on the other hand was seen to decline from 6.19±0.03 (control) to 5.22±0.02 in the treatment.

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Table 1: Physio-chemical Parameters of Test Solution (water) Mean±SD

Conc. of Potash (mg/L)	pH	Conductivity	Temperature	Turbidity	Dissolved Oxygen
0.00	5.25±0.06[a]	87.39±20.45[a]	22.00±0.00[a]	0.10±0.21[c]	6.19±0.03[a]
30.2	5.34±0.05[a]	87.72±19.06[a]	22.00±0.00[a]	1.58±0.25[b]	5.48±0.02[b]
45.4	5.32±0.09[a]	87.08±12.02[a]	22.11±0.01[a]	2. 37±0.31[a]	5.75±0.01[b]
75.6	5.21±0.03[a]	87.02±14.10[a]	22.09±0.05[a]	1. 11±0.12[b]	5.22±0.02[b]

Superscripts in a column indicate significant difference (ANOVA + Tukey HSD test, P < 0.05).

3.2 Enzymes in the Liver

The relative activity of enzymes (AST, ALT and ALP and ACP) in the Liver of *Clarias gariepinus* after 21 days of exposure are presented in Table 2. The results shows significant (P<0.05) appreciation in the values of AST, ALT and ACP in the liver from 435.33±44.96 to 707.93±224.16, 12.56±1.59 to 36.35±6.41 and 1.28±1.04 to 786.41±110.78 compared to the control (277.46±8.16, 7.17±1.02 and 1.90±0.19). This indicates an elevation of the activities of enzymes in the liver, thus increasing its capacity to degrade poison in exposed fish as toxicant concentration increases.

On the other hand, the values of ALP significantly depreciated in the liver from 88.97±0.77 to 8.15±0.88 compared to the control (117.67±6.33).

Table 2: Activities of Enzymes in the Liver of *Clarias gariepinus* Exposed to Potash for 21 days (Mean ± SD)

Conc. of Potash (mg/L)	AST (µ/L)	ALT (µ/L)	ALP (µ/L)	ACP (µ/L)
0.00	277.46 ± 8.16[c]	7.17 ± 1.02[c]	117.67 ± 6.33[a]	1.90 ± 0.19[b]
30.2	435.33 ± 44.96[b]	12.56 ± 1.59[b]	88.97 ± 0.77[b]	1.28 ± 1.04[b]
45.4	680.80 ± 3.57[a]	33.11 ± 1.34[a]	7.46 ± 0.68[c]	705.45 ± 316.43[a]
75.6	707.93 ± 224.16[a]	36.35 ± 6.41[a]	8.15 ± 0.88[c]	786.41 ± 110.78[a]

Values are Mean ± SD. Different superscripts in a column indicate significant difference (ANOVA + Tukey HSD, p < 0.05

3.3 Enzymes in the Kidney

The relative activity of enzymes in the kidney (AST, ALT, ALP and ACP) of *Clarias gariepinus* after 21 days of exposure are presented in Tables 3 of this study. From the results obtained, values of AST, ALT and ACP in the kidney significantly (P<0.05) appreciated from 103.40±19.78 u/L to 146.33±39.22 u/L, 21.73±1.41 u/L to 21.13±2.31 u/L, and 22.58±18.92 u/L to 27.05±6.46 u/L, compared to the controls (137.29±3.74 u/L, 6.78±3.05 u/L and 7.79±9.95 u/L). On the other hand, values of ALP declined significantly from 200.32±71.55 u/L to 176.30±6.33, compared to the control, 1219.87±84.66 u/L as presented in Tables 3.

Table 3: Activities of Enzymes in the Kidney of *Clarias gariepinus* exposed to Potash for 21 days (Mean ± SD, n = 3)

Conc. of Potash (mg/L)	AST (µ/L)	ALT (µ/L)	ALP (µ/L)	ACP (µ/L)
0.00	137.29 ± 3.74[a]	6.78 ± 3.05[c]	1219.87 ± 84.66[a]	7.79 ± 9.95[c]
30.2	103.40 ± 19.78[b]	21.73 ± 1.41[b]	200.34 ± 71.55[b]	22.58 ± 18.92[b]
45.4	107.47 ± 18.01[b]	22.15 ± 2.63[b]	201.32 ± 20.93[b]	26.73 ± 15.66[b]
75.6	146.33 ± 39.22[a]	21.13 ± 2.31[b]	176.30 ± 6.33[b]	27.05 ± 6.46[a]

Different superscripts indicate significant difference (ANOVA + Tukey HSD, p < 0.0

3.4 Discussion

3.4.1 Enzymes in the Liver

In Table 2 of this study, values of AST, ALT and ACP in the liver of potash treated fish recorded significant (p<0.05) increase, while that of ALP declined significantly (p<0.05) compared to the control. The appreciation of AST, ALT and ACP was dose dependent as shown in Table 2, compared to the controls. This trend generally shows that, an increase in concentration of toxicant would result in increase in the values of AST, ALT and ACP in the liver of exposed fish. The observed alterations in the values of enzymes in the liver of potash treated fish is a proof of hepatoxicity, and this could trigger distortion in gene expression in exposed fish with prolonged exposure to toxicant.

Similar reports of enzyme distortions in the liver induced by toxicants have been established. One of such reports is that of Inyang et al. (2016). The authors measured the effect of Dimethoate on Lactate Dehydrogenase, Creatinine Kinase and Amylase in *Clarias lazara* and recorded appreciation in the values of biochemical parameters with few declining trend in enzymes of exposed fish. The recorded appreciation in the value of ALT, AST and ACP, as well as decline in ALP in the liver of exposed fish in this study thus, predict a liver damage, musculoskeletal disorders; myocardial infarction, anaemia, as well as liver susceptibility to cellular damage. Dewan et al. (2004) and Ncibi et al. (2008), in similar reports have also confirmed that pesticide exposure to probe animals, could cause damage of the liver and seepage of cytosolic enzymes from hepatocyte cells and other body organs into blood. Also, the findings of this study is in tandem with the report of Mohammed et al. (2017), who recorded elevation in the values of AST in the liver of rabbits exposed to Dimethoate pesticide after 6 weeks of exposure.

The susceptibility of liver tissues to stress, resulting from exposure to xenobiotics like potash used in this study is a function of cellular balance between the extent of oxidative pressure and the antioxidant ability of test organ in exposed fish (Kalender, et al., 2010). Therefore, the elevation of AST, ALT and ACP, as well as depreciation of ALP levels in the liver of potash treated fish as presented in Table 4.4 in this study is an indication of liver inflammation and injury induced by toxicant.

The observed decline in the values of ALP in the liver of potash treated fish in this study compared to the control shows an interruption in the secretion and flow of bile in the liver. This condition also defines the inability of the liver to breakdown or metabolizes compounds of the toxicant, thus revealing the sublethal impact of potash on biochemical activities in the liver of exposed fish.

This observation is also in line with the findings of Arellano *et al.*, 2004, who also observed similar result in his study. The decrease in ALP values in the liver of exposed fish to potash in this study could also be attributed to decline in the rate of synthesis caused by lowered metabolic demands, and also due to electrolytic imbalance caused by tissue over hydration.

Furthermore, reduction in the values of ALP, as well as the alterations recorded in other enzyme values in the liver of exposed fish is indicative of disturbance in the membrane transport system. This aligns with the reports of Begum, 2004; Inyang *et al.*, 2009 as well as Semanta *et al.*, 2014, who also reported alterations of liver enzymes in fish treated with organophosphate pesticides at different times.

3.4.2. Enzymes in the Kidney

The results obtained shows significant ($p<0.05$) appreciation in the values of AST, ALT and ACP in the kidney of potash treated fish, while that of ALP significantly ($p<0.05$) declined, compared to the controls.

It was also observed that there was an initial sharp reduction in the values of AST in the kidney at first and second intoxication with potash, compared to the control. This sudden reduction in the value of AST in the kidney of treated fish could be an indication of the toxic effect of potash to enzymes in exposed fish and fish response to the introduction of sudden toxicant. This result is similar to the report of Li et al. (2009), when they exposed rainbow trout to Carbamazepine for 42 hrs and recorded similar appreciation in the biochemical parameters of exposed fish.

The recorded alteration in the values of biochemical indices in the kidney of fish treated with potash in this study demonstrates the nephrotoxic effect of toxicant on the function of the kidney, thus resulting in the appreciation of the intracellular enzymes like AST and ALT and ACP in the kidney of exposed fish. The significant ($p<0.05$) increase in the values of AST, ALT and ACP compared to the control in this study also shows distorted or alteration in transamination and oxidative deamination in the kidney of exposed fish.

The elevation of transferases in this study also possibly suggests the diversion of the alphamino acids in the Trichloroacetic Acid (TCA) cycle as keto acids to augument energy production. Findings of this research is also in tandem with the report of Das et al. (2004), who reported elevation in the activity of AST as well as reduction in ALP of Indian major Carps exposed to Nitrite toxicity. In effect, the result shows the toxic effect of potash on enzyme activities in the kidney of exposed fish and further exposure to higher concentrations of toxicant can lead to renal failure.

4.0 CONCLUSION

4.1 Conclusion

The obtained results presented in this study simply reveals the toxicological potentials of sublethal concentrations of potash to juveniles of *Clarias gariepinus*. Specifically, the results further reveals the impact of the toxicant onmbiochemical indices of exposure fish at higher concentrations.

It is therefore imperative that potash and its related toxicants are potentially harmful to aquatic lives and fish in general, and should be used cautiously and in a safe manner around rivers, oceans, lakes, swamps and even flood prone environments to avoid continuous contamination and pollution of the aquatic food chain.

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