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Physiological costs of combined cadmium and glyphosate exposure in sub-adult *Clarias gariepinus*: Evidence from endocrine, metabolic and renal biomarkers

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Abstract

The increasing occurrence of heavy metals and herbicides in aquatic environments raises concern regarding their combined effects on organisms. This study evaluated the physiological responses of sub-adult *Clarias gariepinus* exposed to fixed-ratio cadmium chloride–glyphosate binary mixtures using endocrine, metabolic, renal, and hepatic biomarkers. A static bioassay used six treatments including control (0.00/0.00 mg/L) and five mixture concentrations: 6.18/12.36, 12.36/24.72, 18.54/37.08, 24.72/49.44, and 30.90/61.80 mg/L cadmium chloride/glyphosate, respectively. Each treatment had three replicates containing ten fish. Blood samples were collected to determine cortisol, glucose, creatinine, urea, aspartate aminotransferase, alanine aminotransferase, calcium, and total protein concentrations. Results revealed significant treatment-dependent increases in cortisol ($F = 41.785$, $P < 0.001$), creatinine ($F = 17.135$, $P < 0.001$), and glucose ($F = 3.135$, $P = 0.049$). Cortisol increased from 12.47 ± 3.88 ng/mL to 66.93 ± 1.91 ng/mL, while creatinine rose from 0.45 ± 0.04 mg/dL to 0.86 ± 0.09 mg/dL. Glucose concentrations increased from 82.49 ± 6.05 mg/dL to 99.17 ± 2.81 mg/dL. Conversely, AST, ALT, urea, calcium, and total protein did not differ significantly ($P > 0.05$). Pearson correlation demonstrated strong positive relationships between mixture concentration and cortisol ($r = 0.845$, $P < 0.001$) as well as creatinine ($r = 0.768$, $P < 0.001$). Findings demonstrate that fixed-ratio cadmium chloride–glyphosate mixtures impose physiological costs on sub-adult *Clarias gariepinus*, primarily through activation of endocrine stress pathways, disruption of energy metabolism, and impairment of renal function. Cortisol and creatinine emerged as sensitive indicators for monitoring toxic impacts in ecosystems.

Keywords: *Clarias gariepinus*; Cadmium Chloride; Glyphosate; Cortisol; Creatinine; Ecotoxicology

1. Introduction

Aquatic ecosystems face unprecedented threats from anthropogenic contaminants, with heavy metals and agricultural pesticides posing severe risks to global freshwater biodiversity [1]. Among these chemical pollutants, cadmium and glyphosate are particularly problematic due to their environmental persistence and widespread distribution in surface waters. Cadmium enters aquatic habitats through mining activities, industrial effluents, and phosphate fertilizer runoffs [2]. Concurrently, glyphosate-based herbicides are applied globally on a massive scale, leading to frequent detection of their residues in nearby water bodies via agricultural runoff [3].

As a non-essential heavy metal, cadmium bioaccumulates rapidly within fish tissues, leading to severe osmoregulatory failure, oxidative damage, growth inhibition, and functional degradation of vital organs like the liver, kidneys, and gills [4, 5]. Similarly, emerging toxicological evidence indicates that glyphosate exposure causes systemic oxidative stress, endocrine disruption, haematological alterations, and histopathological damage in non-target aquatic species [6-8]. Because these two pollutants frequently co-occur in agricultural watersheds, evaluating them in isolation ignores the

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potential for complex mixture toxicities, which can manifest as additive, synergistic, or antagonistic biological interactions [9].

To detect these sublethal chemical impacts before irreversible tissue pathology occurs, researchers rely on sub-organismal biomarkers [10]. In teleost models, plasma cortisol serves as the primary endocrine stress index, while glucose reflects immediate metabolic and energy adjustments. Furthermore, fluctuations in blood urea and creatinine levels provide insight into renal clearance capacity, while changes in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) signify altered hepatic cellular integrity.

The African catfish, *Clarias gariepinus*, represents an ideal candidate for ecotoxicological research due to its ecological dominance in freshwater bodies, economic importance in regional aquaculture, and high tolerance to environmental stressors [11]. Despite its widespread use as a laboratory model, previous toxicological investigations on this species have heavily focused on single-toxicant dynamics or acute lethality endpoints [12]. Consequently, there remains a critical knowledge gap regarding how co-occurring heavy metals and herbicides interact to alter the complex internal physiology of sub-adult fish.

We hypothesized that simultaneous exposure to fixed-ratio mixtures of cadmium chloride and glyphosate potassium would induce a coordinated, concentration-dependent physiological strain in *C. gariepinus*, characterized by heightened endocrine stress, metabolic compensation, and multi-organ biomarker dysfunction. This study was developed to resolve the specific physiological costs associated with this multi-xenobiotic exposure. Quantifying these biochemical responses is highly critical for establishing realistic environmental risk assessment frameworks and safeguarding aquaculture production systems threatened by mixed chemical pollution. Therefore, the primary objective of this study was to evaluate the combined sublethal impacts of cadmium and glyphosate mixtures on the endocrine, metabolic, renal, and hepatic biomarkers of sub-adult *C. gariepinus*.

2. Materials and Methods

2.1. Experimental Location

The experiment was conducted in the Wet Laboratory of the Department of Fisheries and Aquaculture, University of Cross River State, Okuku campus, Cross River State, Nigeria. Laboratory analyses of serum biochemical parameters were performed in Keli Laboratory, Calabar, Cross River State, Nigeria.

2.2. Experimental Fish and Acclimation

Healthy sub-adult African catfish, *Clarias gariepinus* (mean weight approximately 93.05 ± 0.50 g), were obtained from Federal College of Fisheries and Marine Technology, fish farm, Enugu campus, Nigeria, and transported to the laboratory in aerated containers. Upon arrival, the fish were stocked in holding tanks and acclimated for two weeks under laboratory conditions prior to the commencement of the experiment, [13].

During acclimation, fish were maintained in dechlorinated freshwater under continuous aeration. Water quality parameters were monitored regularly to ensure suitable environmental conditions. A thirty five percent Coppens commercial feed was used to feed five (5) percent body weight of the total fish during the acclimation period. Fish were not fed during the toxicity exposure period to minimize potential interference with toxicant uptake and biochemical responses.

2.3. Experimental Toxicants

Cadmium chloride ($CdCl_2$) and glyphosate potassium were used as test toxicants. Stock solutions were prepared using distilled water and subsequently diluted to obtain the desired experimental concentrations. A fixed-ratio binary mixture of cadmium chloride and glyphosate potassium was employed throughout the study. The mixture ratio of 1:2 (cadmium chloride: glyphosate potassium) was selected following preliminary range-finding tests designed to establish suitable exposure concentrations for the definitive experiment.

2.4. Experimental Design

The experiment consisted of six treatments arranged in a completely randomized design with three replicates per treatment.

The treatment concentrations were as follows:

Treatment	Cadmium Chloride (mg/L)	Glyphosate Potassium (mg/L)
T0	0.00	0.00
T1	6.18	12.36
T2	12.36	24.72
T3	18.54	37.08
T4	24.72	49.44
T5	30.90	61.80

Each treatment was replicated three times, with ten fish stocked per tank, resulting in a total of 180 fish used for the experiment. The exposure system was static, and fish were continuously observed throughout the exposure period. Dead fish, where observed, were removed immediately to prevent deterioration of water quality.

2.5. Water Quality Monitoring

Water quality parameters including temperature, pH, and dissolved oxygen were monitored during the experimental period using standard procedures, [14]. Mean temperature values ranged from 25.9 to 26.5°C, pH ranged from 6.11 to 7.55, while dissolved oxygen ranged from 3.54 to 6.91 mg/L across treatments. These parameters remained within ranges generally suitable for tropical freshwater fish culture.

2.6. Blood Collection and Serum Preparation

At the end of the exposure period, one fish was randomly selected from each replicate tank for blood collection. Consequently, three independent blood samples were obtained per treatment (n = 3), yielding a total of eighteen samples for serum biochemical analysis. 2ml blood samples were collected using sterile syringes and transferred into appropriately labeled tubes. Samples were allowed to clot and subsequently centrifuged to obtain serum, which was carefully separated and stored under refrigerated conditions until biochemical analysis.

2.7. Determination of Serum Biochemical Parameters

Serum biochemical analyses were performed using standard laboratory procedures and commercial diagnostic kits according to manufacturer instructions, [15].

The following biomarkers were determined:

2.7.1. Endocrine and Metabolic Biomarkers

- Cortisol (COR; ng/mL)
- Glucose (GLU; mg/dL)

2.7.2. Renal Biomarkers

- Creatinine (CREA; mg/dL)
- Urea (mg/dL)

2.7.3. Hepatic Biomarkers

- Aspartate aminotransferase (AST; U/L)
- Alanine aminotransferase (ALT; U/L)

2.7.4. Additional Physiological Biomarkers

- Calcium (Ca; mg/dL)
- Total Protein (TP; g/dL)

2.8. Statistical Analysis

Data were subjected to One-way Anova, [16], using Statistical Package for the Social Sciences (SPSS) version 25. Prior to analysis, data were examined for normality and homogeneity of variance. Treatment means were separated using Duncan's Multiple Range Test at a significance level of $P < 0.05$. Results are presented as mean ± standard deviation (Mean ± SD). Pearson correlation analysis was additionally performed to evaluate relationships between cadmium-equivalent mixture concentration and physiological biomarkers. Statistical significance was accepted at $P < 0.05$.

3. Results

3.1. Effects of Cadmium Chloride–Glyphosate Binary Mixtures on Stress Biomarkers

Exposure of sub-adult *Clarias gariepinus* to fixed-ratio cadmium chloride–glyphosate binary mixtures induced significant alterations in stress-related biomarkers (Table 1; Figure 1). Cortisol concentrations increased significantly ($P < 0.001$) with increasing mixture concentration, rising from 12.47 ± 3.88 ng/mL in the control group to peak values of 66.93 ± 1.91 ng/mL at T4. Duncan's multiple range test revealed significant differences between the control and exposed treatments, indicating activation of endocrine stress responses following mixture exposure.

Similarly, glucose concentrations increased significantly across treatments ($P = 0.049$), ranging from 82.49 ± 6.05 mg/dL in the control to 99.17 ± 2.81 mg/dL in the highest treatment group. The observed increase in glucose levels suggests enhanced metabolic activity associated with physiological stress and energy mobilization in exposed fish.

Table 1 Physiological Biomarker Responses of Sub-Adult *Clarias gariepinus* Exposed to Fixed-Ratio Cadmium Chloride–Glyphosate Binary Mixtures

Treatment	$CdCl_2$ (mg/L)	Glyphosate (mg/L)	Cortisol (ng/mL)	AST (U/L)	ALT (U/L)	Creatinine (mg/dL)	Urea (mg/dL)	Calcium (mg/dL)	Total Protein (g/dL)	Glucose (mg/dL)
T0	0.00	0.00	12.47 ± 3.88^{a}	121.92 ± 4.42	35.27 ± 4.57	0.45 ± 0.04^{a}	9.02 ± 0.37	7.84 ± 0.51	4.40 ± 0.45	82.49 ± 6.05^{a}
T1	6.18	12.36	41.48 ± 4.10^{b}	135.31 ± 7.26	38.60 ± 9.45	0.53 ± 0.07^{b}	11.25 ± 2.35	8.29 ± 0.89	4.74 ± 0.45	94.54 ± 4.61^{b}
T2	12.36	24.72	49.23 ± 9.92^{bc}	132.04 ± 6.03	38.11 ± 9.37	0.66 ± 0.11^{c}	10.35 ± 1.34	8.16 ± 0.92	4.57 ± 0.25	96.42 ± 5.14^{b}
T3	18.54	37.08	64.49 ± 5.69^{c}	137.46 ± 4.59	39.76 ± 2.91	0.86 ± 0.09^{d}	11.62 ± 0.63	8.29 ± 0.27	4.87 ± 0.33	94.39 ± 6.55^{b}
T4	24.72	49.44	66.93 ± 1.91^{c}	135.89 ± 10.18	43.08 ± 4.14	0.79 ± 0.01^{d}	11.43 ± 0.46	8.28 ± 0.70	4.69 ± 0.15	92.57 ± 7.31^{b}
T5	30.90	61.80	60.57 ± 3.67^{c}	138.46 ± 12.29	38.06 ± 3.63	0.74 ± 0.02^{cd}	11.05 ± 0.28	8.33 ± 0.54	4.81 ± 0.48	99.17 ± 2.81^{b}

Footnotes: Values are expressed as Mean ± SD (n = 3). Means within a column bearing different superscripts differ significantly at $P < 0.05$ according to Duncan's Multiple Range Test following One-way Anova.

One-way Anova revealed significant treatment effects for cortisol ($F = 41.785$, $P < 0.001$), creatinine ($F = 17.135$, $P < 0.001$), and glucose ($F = 3.135$, $P = 0.049$), whereas AST ($F = 1.737$, $P = 0.201$), ALT ($F = 0.498$, $P = 0.772$), urea ($F = 2.078$, $P = 0.139$), calcium ($F = 0.227$, $P = 0.944$), and total protein ($F = 0.639$, $P = 0.674$) were not significantly affected by treatment.

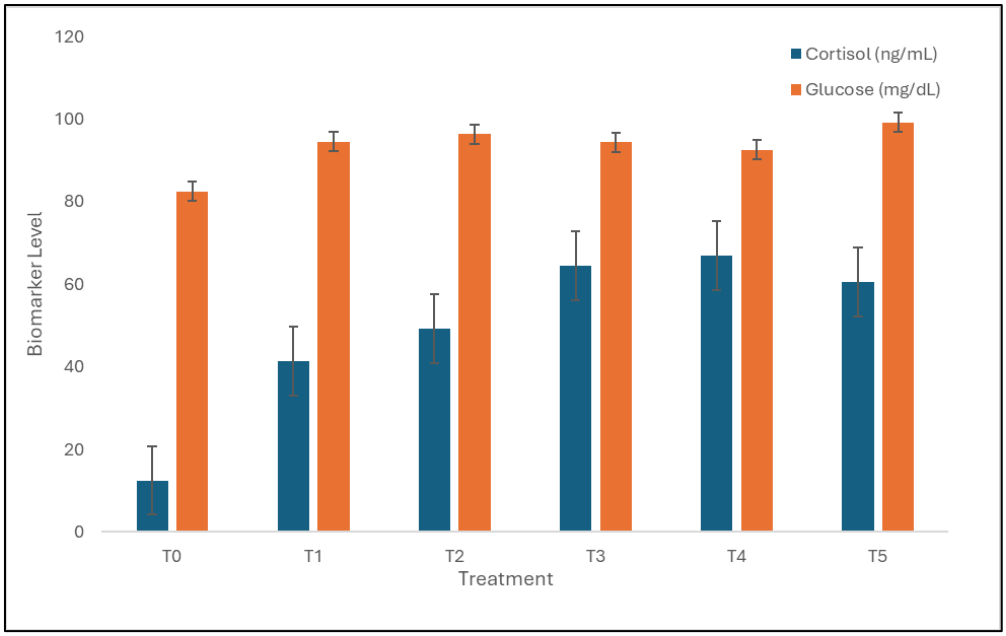

Figure 1 Effects of Fixed-Ratio Cadmium Chloride–Glyphosate Binary Mixtures on Stress Biomarkers (Cortisol and Glucose) in Sub-Adult *Clarias gariepinus*

3.2. Effects of Cadmium Chloride–Glyphosate Binary Mixtures on Renal Biomarkers

The binary mixture significantly affected creatinine concentrations ($P < 0.001$), whereas urea levels remained statistically unchanged ($P > 0.05$) (Table 1; Figure 2). Creatinine values increased progressively from 0.45 ± 0.04 mg/dL in the control group to 0.86 ± 0.09 mg/dL at T3, before declining slightly at higher concentrations. Duncan's test indicated significant differences between control and exposed groups.

In contrast, urea concentrations ranged from 9.02 ± 0.37 mg/dL in the control group to 11.62 ± 0.63 mg/dL at T3 (Figure 2B), although these variations were not statistically significant ($P = 0.139$).

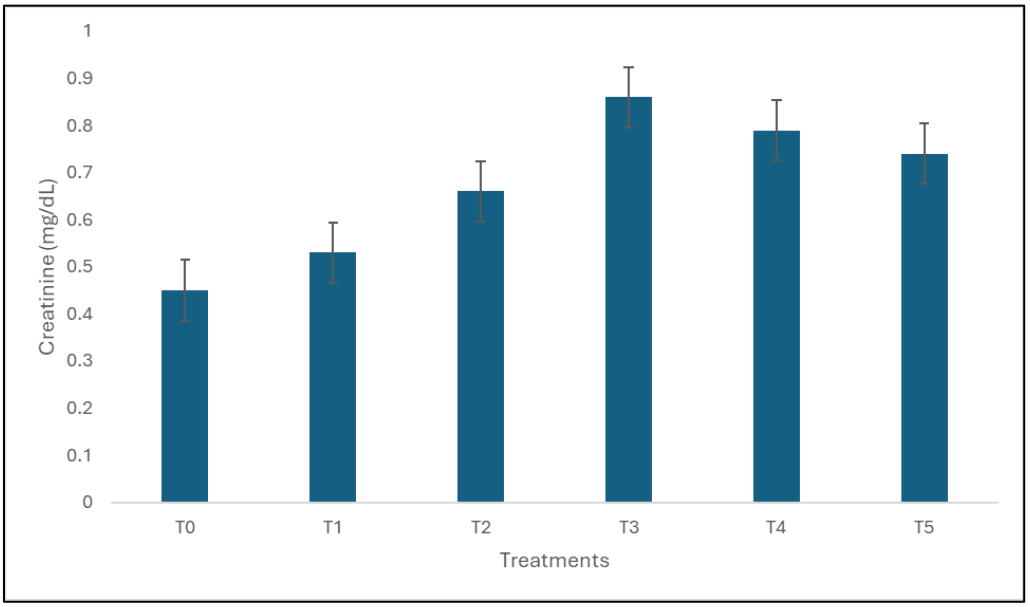

Figure 2A Effects of Fixed-Ratio Cadmium Chloride–Glyphosate Binary Mixtures on Serum Creatinine Concentration in Sub-Adult *Clarias gariepinus*

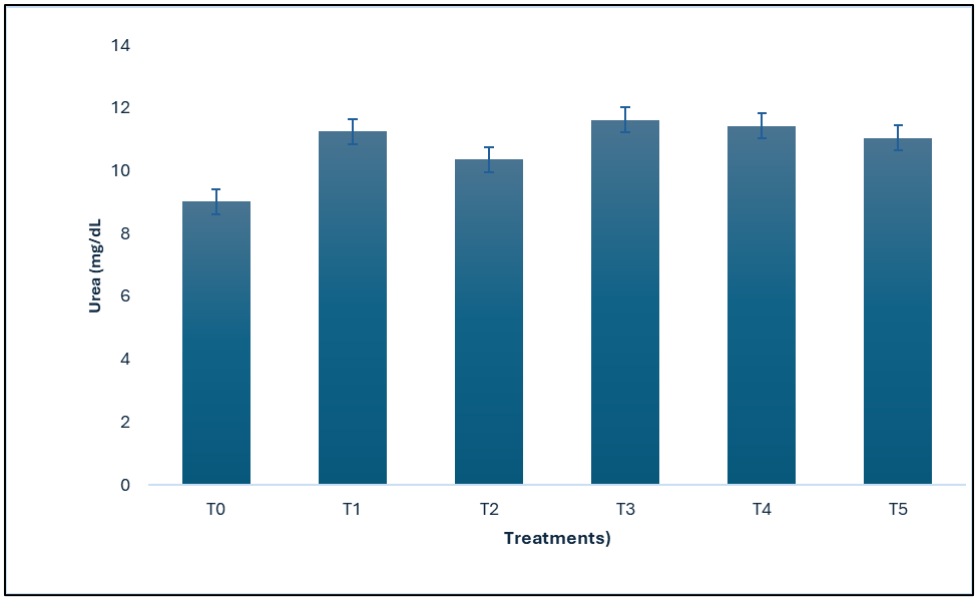

Figure 2B Effects of Fixed-Ratio Cadmium Chloride–Glyphosate Binary Mixtures on Serum Urea Concentration in Sub-Adult *Clarias gariepinus*

3.3. Effects of Cadmium Chloride–Glyphosate Binary Mixtures on Hepatic Biomarkers

The effects of cadmium chloride–glyphosate binary mixtures on hepatic biomarkers are presented in Table 1 and Figures 3A, 3B and 3C. Aspartate aminotransferase (AST) activity increased from 121.92 ± 4.42 U/L in the control group to 138.46 ± 12.29 U/L in the highest treatment group. However, the observed differences were not statistically significant (P = 0.201).

Similarly, alanine aminotransferase (ALT) activity ranged from 35.27 ± 4.57 U/L in the control group to 43.08 ± 4.14 U/L at T4 without significant treatment effects (P = 0.772). Total protein concentrations varied slightly among treatments, ranging from 4.40 ± 0.45 g/dL to 4.87 ± 0.33 g/dL, but did not differ significantly among groups (P = 0.674).

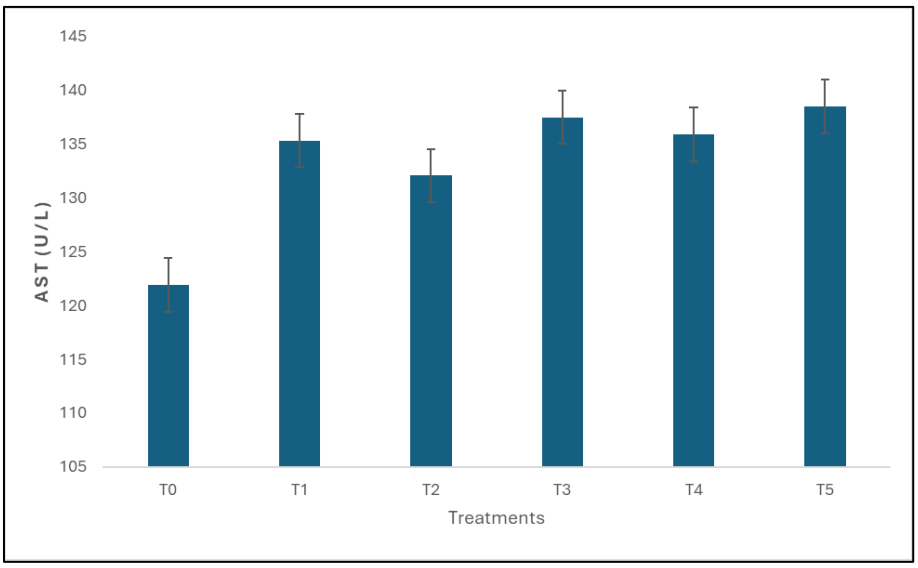

Figure 3A Effects of Fixed-Ratio Cadmium Chloride–Glyphosate Binary Mixtures on Serum AST Activity in Sub-Adult *Clarias gariepinus*

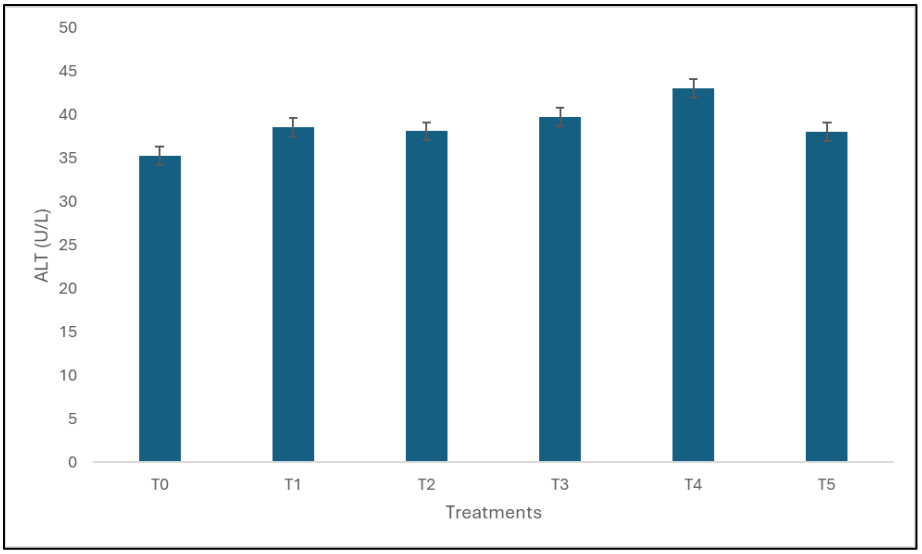

Figure 3B Effects of Fixed-Ratio Cadmium Chloride–Glyphosate Binary Mixtures on Serum ALT Activity in Sub-Adult *Clarias gariepinus*

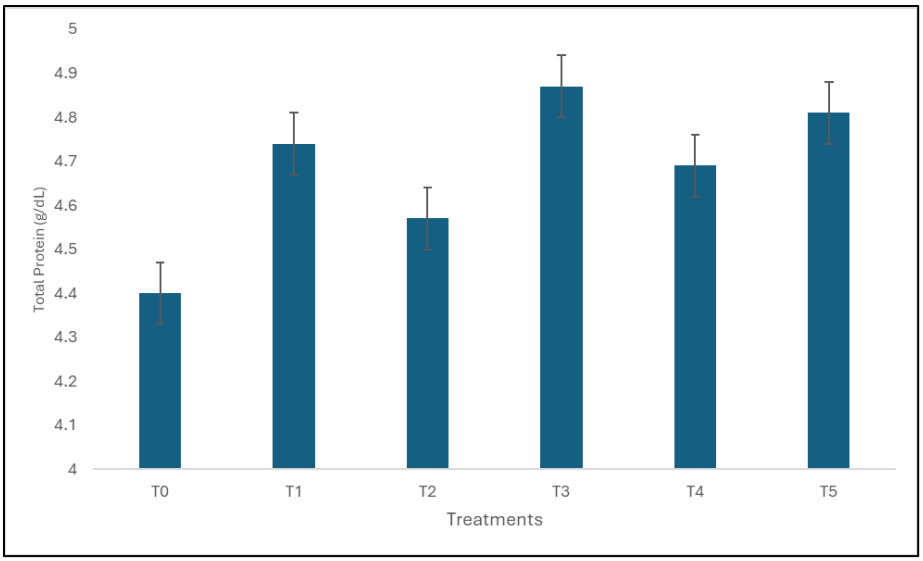

Figure 3C Effects of Fixed-Ratio Cadmium Chloride–Glyphosate Binary Mixtures on Serum Total Protein Concentration in Sub-Adult *Clarias gariepinus*

3.4. Correlation Between Mixture Concentration and Physiological Biomarkers

Pearson correlation analysis revealed significant positive relationships between cadmium-equivalent mixture concentration and several physiological biomarkers (Table 2; Figure 4). Cortisol exhibited the strongest positive correlation with concentration (r = 0.845, $P < 0.001$), followed by creatinine ($r = 0.768$, $P < 0.001$). Glucose also demonstrated a significant positive correlation with concentration (r = 0.529, $P = 0.024$).

AST showed a moderate positive correlation with concentration ($r = 0.511$, $P = 0.030$), whereas ALT, urea, calcium, and total protein exhibited weak to moderate non-significant correlations ($P > 0.05$). These findings indicate that cortisol and creatinine were the most responsive biomarkers to increasing cadmium chloride–glyphosate mixture concentrations.

Table 2 Pearson Correlation Between Cadmium-Equivalent Mixture Concentration and Physiological Biomarkers in Sub-Adult *Clarias gariepinus*

Biomarker	Pearson Correlation (*r*)	*P*-value	Interpretation
Cortisol	0.845	<0.001	Strong positive
AST	0.511	0.030	Moderate positive
ALT	0.251	0.315	Weak positive
Creatinine	0.768	<0.001	Strong positive
Urea	0.448	0.062	Moderate positive
Calcium	0.220	0.381	Weak positive
Total Protein	0.315	0.203	Weak positive
Glucose	0.529	0.024	Moderate positive

Footnote: Correlation analysis was performed using cadmium-equivalent mixture concentrations (0.00, 6.18, 12.36, 18.54, 24.72 and 30.90 mg/L), corresponding to the fixed-ratio (1:2) cadmium chloride–glyphosate binary mixture. Positive coefficients indicate increasing biomarker responses with increasing toxicant concentration. Significant relationships were accepted at $P < 0.05$.

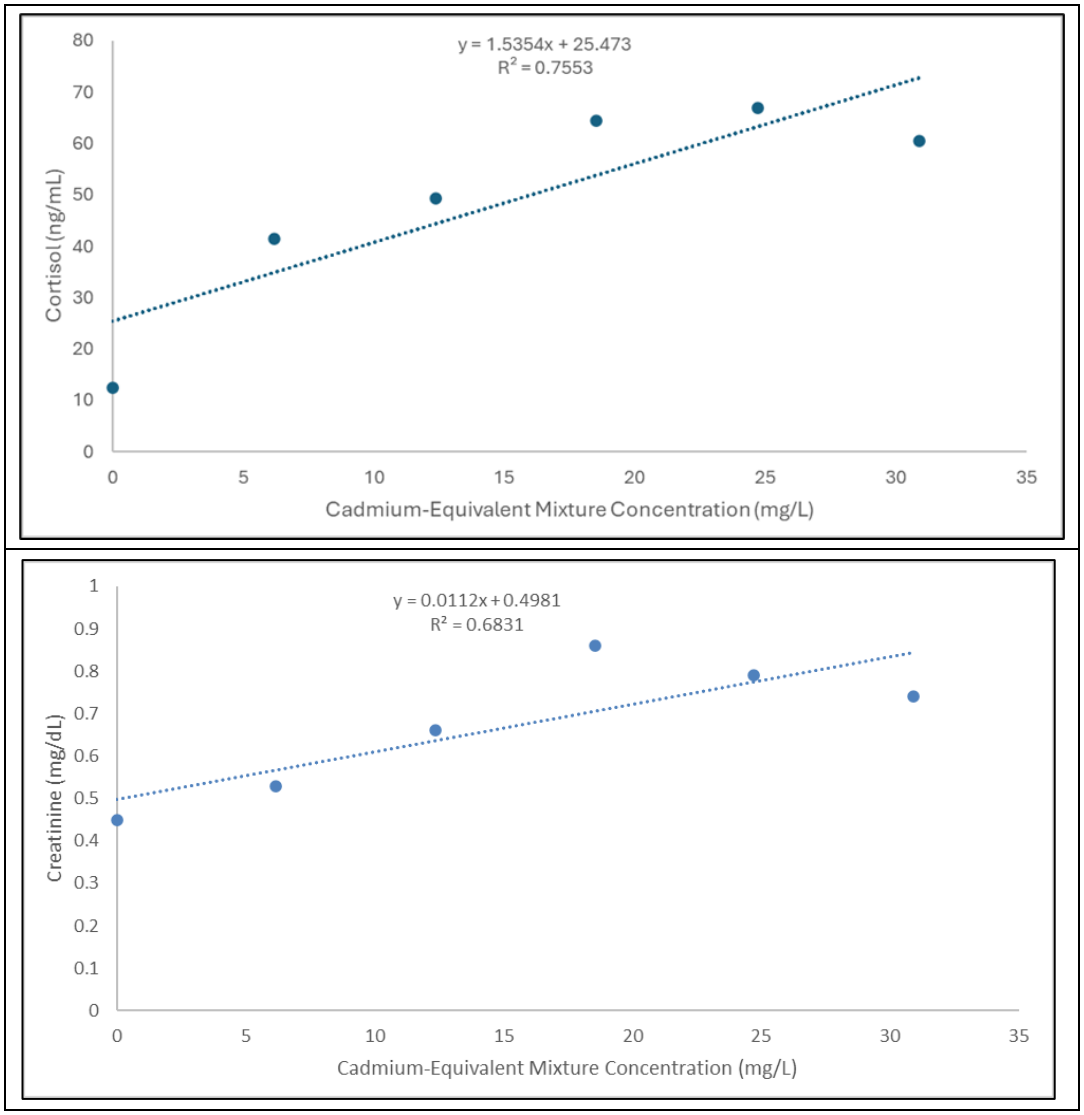

Figure 4 Relationships between cadmium-equivalent mixture concentration and the two most responsive biomarkers, cortisol (A) and creatinine (B), in sub-adult *Clarias gariepinus* exposed to fixed-ratio cadmium chloride–glyphosate binary mixtures

4. Discussion

The present study demonstrated that cadmium–glyphosate mixtures induced significant alterations in cortisol, glucose, and creatinine concentrations in *Clarias gariepinus*, while hepatic biomarkers and total protein remained relatively stable. These findings highlight the differential sensitivity of physiological systems to contaminant mixtures and emphasize the importance of biomarker-based approaches in ecotoxicology.

Cortisol elevation across treatments reflects activation of the hypothalamic–pituitary–interrenal (HPI) axis, a hallmark of teleost stress physiology [17]. Cadmium exposure is known to disrupt osmoregulation and induce oxidative stress, leading to cortisol release [4]. Glyphosate formulations similarly trigger endocrine disruption, with documented increases in plasma cortisol in exposed fish [7]. The strong correlation between mixture concentration and cortisol observed here underscores its utility as a sensitive biomarker of contaminant stress. Interestingly, the decline at the highest concentration suggests endocrine exhaustion or negative feedback regulation, consistent with chronic stress models where prolonged HPI activation suppresses cortisol synthesis [17]. This biphasic pattern highlights the complexity of endocrine responses under mixture exposure.

Glucose elevation indicates secondary stress responses, mediated by cortisol induced glycogenolysis and gluconeogenesis [18]. Hyperglycemia has been widely reported in fish exposed to metals and pesticides, including *Clarias gariepinus* and *Oreochromis niloticus* [12]. Elevated glucose provides energy for coping with stress but prolonged hyperglycemia may impair growth and immune function. The simultaneous elevation of cortisol and glucose in this study suggests coordinated endocrine–metabolic activation, consistent with adaptive stress physiology. However, sustained metabolic disruption could compromise aquaculture productivity by reducing feed efficiency and disease resistance.

Creatinine exhibited the most pronounced response, increasing significantly with exposure concentration. Elevated serum creatinine is a well-established indicator of impaired renal function, reflecting reduced clearance of metabolic waste [19]. Cadmium accumulates in renal tissues, causing oxidative stress, tubular degeneration, and impaired filtration [5]. Glyphosate formulations also contribute to renal dysfunction through oxidative damage and inflammatory responses [8]. The strong correlation between creatinine and mixture concentration suggests that renal physiology is particularly vulnerable to combined cadmium–glyphosate exposure. In contrast, urea remained stable, consistent with reports that creatinine is a more sensitive early biomarker of renal impairment in fish [19]. This differential sensitivity highlights the importance of selecting appropriate biomarkers for environmental monitoring.

AST and ALT activities showed modest increases but were not statistically significant. These enzymes are markers of hepatocellular integrity, with elevations typically indicating membrane damage or leakage [7]. The absence of significant changes here may reflect the acute exposure duration, which was insufficient to induce overt hepatocellular injury. Chronic studies have reported significant elevations in AST and ALT following cadmium or glyphosate exposure [12], suggesting that longer exposure periods may reveal more pronounced hepatic effects. The upward trends observed may represent early disturbances that could progress under chronic conditions.

Total protein concentrations remained stable across treatments, consistent with compensatory mechanisms maintaining protein synthesis under sublethal stress [10]. Some studies have reported reductions in total protein under prolonged contaminant exposure due to impaired hepatic function [8]. The stability observed here suggests that protein metabolism was not substantially disrupted under the experimental conditions, further supporting the conclusion that renal and endocrine systems were more sensitive to acute mixture exposure.

The combined effects of cadmium and glyphosate highlight the importance of mixture toxicology in ecological risk assessment. Traditional single contaminant studies may underestimate risks under real world conditions where multiple pollutants co-occur [9]. The additive or potentially synergistic effects observed here underscore the need for integrated approaches that consider contaminant interactions. From an environmental perspective, agricultural runoff and industrial discharge frequently introduce metals and herbicides simultaneously, creating exposure scenarios similar to those tested in this study. The demonstrated sensitivity of cortisol and creatinine suggests that these biomarkers could serve as early warning indicators in monitoring programs.

For aquaculture, contaminant induced stress responses have direct implications for productivity and fish health. Elevated cortisol and glucose can impair growth, reproduction, and immune function, while renal impairment compromises waste excretion and osmoregulatory balance. In *Clarias gariepinus*, a species central to African aquaculture, such physiological disturbances could reduce survival and market value, threatening food security and

livelihoods. Monitoring biomarkers such as cortisol and creatinine in cultured fish could therefore provide practical tools for managing water quality and minimizing contaminant impacts.

5. Conclusion

This study successfully determined the physiological impacts of fixed-ratio cadmium chloride and glyphosate potassium mixtures on sub-adult *Clarias gariepinus*. Our findings reveal that combined exposure to these common aquatic contaminants triggers immediate, systemic physiological strain. This is clearly demonstrated by pronounced increases in blood cortisol, glucose, and creatinine levels, proving that the chemical mixture concurrently activates corporate endocrine stress pathways, accelerates energy mobilization, and compromises renal clearance systems.

A key finding of this research is the high sensitivity of cortisol and creatinine as early-warning indicators. Their robust, concentration-dependent tracking of mixture levels makes them superior monitoring tools compared to hepatic markers like AST and ALT, which remained stable under the tested conditions. This proves that mixed environmental pollution can inflict serious physiological costs on aquaculture species even before visible liver damage or mass mortality occurs. Consequently, environmental agencies and fish farmers can utilize cortisol and creatinine profiles to detect sublethal chemical stress early, preventing widespread production collapses.

Ultimately, these insights advance our fundamental understanding of multi-xenobiotic mixture toxicology in freshwater ecosystems. They provide regulators with empirical data needed to establish more realistic ecological risk assessments and sustainable aquaculture management guidelines in regions prone to mixed agrochemical and heavy metal pollution. Moving forward, future ecotoxicological research should focus on long-term, chronic exposure trials and integrate molecular or histopathological techniques to fully map the deep cellular mechanisms driving this combined toxic pathway.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that there are no known competing financial interests, personal relationships, or conflicts of interest that could have influenced the work reported in this study.

Statement of ethical approval

All experimental protocols involving sub-adult African catfish (*Clarias gariepinus*) were conducted in strict accordance with institutional guidelines for the care and use of laboratory animals. The study protocols were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Cross River State, Nigeria (Approval No: UNICROSS/FAEC/2006-F001).

References

[1] Schwarzenbach RP, Egli T, Hofstetter TB, von Gunten U, Wehrli B. Global water pollution and human health. Annu Rev Environ Resour. 2010; 35(1): 109-36.

[2] Järup L, Åkesson A. Current status of cadmium as an environmental health problem. Toxicol Appl Pharmacol. 2009; 238(3):201-8.

[3] Benbrook CM. Trends in glyphosate herbicide use in the United States and globally. Environ Sci Eur. 2016; 28(1):3.

[4] Atli G, Canli M. Response of antioxidant system to heavy metal (Cd, Cu, Zn) exposures in a freshwater fish *Oreochromis niloticus*. Comp Biochem Physiol C Toxicol Pharmacol. 2010; 153(1):91-8.

[5] Liu Y, Chen Q, Li Y, Bi L, Jin L, Peng R. Toxic effects of cadmium on fish. Toxics. 2022; 10(10):622.

[6] Lee JW, Jo AH, Lee DC, Choi CY, Kang JC, Kim JH. Review of cadmium effects on fish: Oxidative stress and immune responses. Environ Res. 2023; 236(Pt 2):116600.

[7] Modesto KA, Martinez CBR. Effects of glyphosate on the blood of fish: Hematological parameters and oxidative stress. Comp Biochem Physiol C Toxicol Pharmacol. 2010; 151(3):369-74.

[8] Nwani CD, Nagpure NS, Kumar R. Oxidative stress and genotoxic effects of glyphosate on *Channa punctatus*. Environ Toxicol Pharmacol. 2013; 36(2):539-47.

[9] Cedergreen N. Quantifying synergy: A systematic review of mixture toxicity studies within environmental toxicology. PLoS One. 2014; 9(5):e96580.

[10] Van der Oost R, Beyer J, Vermeulen NPE. Fish bioaccumulation and biomarkers in environmental risk assessment: A review. Environ Toxicol Pharmacol. 2003; 13(2):57-149.

[11] Akinrotimi OA, Abu OMG, Ansa EJ. Environmental implications of aquaculture development in Nigeria. J Appl Sci Environ Manage. 2011; 15(3):447-53.

[12] Sani A, Idris MK. Acute toxicity of herbicide (glyphosate) in *Clarias gariepinus* juveniles. Toxicol Rep. 2016; 3:513-5.

[13] OECD. OECD Guidelines for the Testing of Chemicals: Fish Acute Toxicity Test. Paris: Organization for Economic Co-operation and Development; 1992.

[14] APHA. Standard Methods for the Examination of Water and Wastewater. 23rd ed. Washington, DC: American Public Health Association; 2017.

[15] Trinder P. Determination of glucose in blood using glucose oxidase. Ann Clin Biochem. 1969; 6(1):24-7.

[16] Zar JH. Biostatistical Analysis. 5th ed. Upper Saddle River, NJ: Pearson; 2010.

[17] Barton BA. Stress in fishes: a diversity of responses with particular reference to stressors of farmed fish. Integr Comp Biol. 2002; 42(3):517-25.

[18] Vijayan MM, Moon TW. Cortisol-induced changes in plasma glucose, protein, and hepatic metabolism in rainbow trout (*Oncorhynchus mykiss*). Can J Zool. 1994; 72(3):379-86.

[19] El-Sayed YS, Saad TT. Subacute intoxication of cadmium in Nile tilapia (*Oreochromis niloticus*): Biochemical and histopathological evaluation. Environ Toxicol Pharmacol. 2007; 24(2):178-87.