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Temporal Toxicity Dynamics and Safe Exposure Thresholds of Fixed-Ratio Cadmium Chloride–Glyphosate Potassium Binary Mixtures in Sub-Adult Clarias gariepinus

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Abstract

Aquatic ecosystems face growing threats from heavy metals and agrochemicals, requiring assessment of impacts on important fish species. This study assessed the acute toxicity of fixed-ratio cadmium chloride–glyphosate potassium binary mixtures in sub-adult African catfish (*Clarias gariepinus*) using a 96-hour static bioassay. Fish with a mean body weight of approximately 93.05 ± 0.50 g were exposed to five concentration levels of a fixed-ratio (1:2) cadmium chloride–glyphosate potassium binary mixture comprising 6.18/12.36, 12.36/24.72, 18.54/37.08, 24.72/49.44, and 30.90/61.80 mg/L, alongside an untreated control, under triplicate experimental conditions. Mortality was recorded at 24, 48, 72, and 96 hours, and median lethal concentrations (LC_{50}) were estimated using Probit regression analysis. Mortality increased progressively with both concentration and exposure duration, with cumulative mortality ranging from 13.33% to 60.00% after 96 hours of exposure. The estimated LC_{50} values declined from 90.744 mg/L at 24 hours to 73.906 mg/L at 48 hours, 46.153 mg/L at 72 hours, and 28.100 mg/L at 96 hours, indicating a pronounced increase in toxicity with prolonged exposure. Probit analysis revealed strong concentration–response relationships and satisfactory model fit across all exposure periods. Preliminary safe exposure thresholds derived from the 96-hour LC_{50} were estimated at 2.81 mg/L and 0.28 mg/L using the Sprague and NAS/NAE application factors, respectively. Declining LC_{50} values indicated increasing cumulative toxicity of the binary mixture, highlighting ecological risks of combined heavy metal and herbicide exposure. These findings provide essential baseline screening data for environmental risk assessment and support mixture-based toxicity evaluations in aquatic ecosystems.

Introduction

Aquatic ecosystems are increasingly threatened by contamination from industrial, agricultural, and urban activities, with heavy metals and agrochemicals representing two of the most persistent and hazardous pollutant classes (Alengebawy et al. 2021; Liu et al. 2022). Their persistence, bioavailability, and cumulative effects pose significant risks to fish health, biodiversity conservation, and aquaculture productivity. Acute toxicity assessments remain a cornerstone of aquatic toxicology, providing baseline data for ecological risk evaluation and regulatory decision-making (Pillai et al. 2021).

Cadmium, a non-essential heavy metal, is widely recognized as a priority environmental contaminant due to its high toxicity and persistence in aquatic environments. It enters freshwater systems through industrial discharges, mining, battery manufacturing, and agricultural runoff, where it accumulates in sediments and aquatic organisms (Zhang et al. 2021). In fish, cadmium exposure has been linked to oxidative stress, impaired osmoregulation, enzyme inhibition, and tissue damage (Liu et al. 2022). These effects compromise physiological homeostasis and survival, making cadmium a critical focus of aquatic toxicology.

Additionally, glyphosate, one of the most extensively used herbicides worldwide, is frequently detected in aquatic environments due to agricultural runoff and spray drift (Lanzarin et al. 2023). Although often considered less persistent than heavy metals, glyphosate and its metabolites can exert significant toxic

effects on fish, including altered growth, reproduction, metabolism, and histopathological damage to organs such as the gills and liver (Alarape et al. 2023). Recent studies highlight oxidative stress and metabolic disturbances as key mechanisms of glyphosate toxicity in aquatic organisms (Lanzarin et al. 2023).

In natural aquatic environments, organisms are rarely exposed to single contaminants. Instead, they encounter complex mixtures of pollutants originating from multiple anthropogenic sources. Mixture toxicology has, therefore, emerged as a critical research area, as interactions between contaminants may produce additive, synergistic, or antagonistic effects (Heys et al. 2016; Ghafarifarsani et al. 2024). Heavy metal–herbicide mixtures, in particular, represent realistic exposure scenarios in freshwater ecosystems receiving both industrial effluents and agricultural inputs (Liu et al. 2022). However, despite growing recognition of mixture effects, data on combined cadmium–glyphosate toxicity in fish remain limited.

The African catfish (*Clarias gariepinus*) is one of the most economically important freshwater species in Africa, valued for its hardiness, rapid growth, and aquaculture relevance (Lucas et al. 2019; Langia et al. 2024). It serves as a model organism in aquatic toxicology due to its ecological importance and widespread use in aquaculture. Understanding contaminant effects on *Clarias gariepinus* is therefore essential for both ecological conservation and sustainable aquaculture development.

Although numerous studies have investigated the individual toxic effects of cadmium and glyphosate, little is known about their combined toxicity in *Clarias gariepinus*. This knowledge gap constrains accurate environmental risk assessment and the development of protective management strategies. The present study addresses this gap by evaluating the acute toxicity of fixed-ratio cadmium chloride–glyphosate potassium binary mixtures in sub-adult *Clarias gariepinus* using a 96-hour static bioassay. Specifically, the study aimed to determine concentration-dependent mortality responses, estimate median lethal concentrations (LC_{50}) at different exposure periods using probit analysis, examine temporal toxicity dynamics, and establish safe exposure thresholds for environmental risk assessment.

Materials and Methods

Experimental Fish and Acclimation

Ethical Statement

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).

Four hundred (400) sub-adult African catfish (*Clarias gariepinus*) with a mean body weight of approximately 93.05 ± 0.5 g were obtained from Federal College of Fisheries and Marine Technology, fish

farm, Enugu campus, Nigeria, and acclimated for 14 days in aerated holding tanks containing dechlorinated freshwater, in the Wet Laboratory of the Department of Fisheries and Aquaculture, University of Cross River State, Okuku Campus, Nigeria. Acclimation procedures followed standard guidelines for fish bioassays to ensure physiological stability before exposure (OECD 1992; Pillai et al. 2021). A thirty-five percent Coppens commercial feed was used to feed five (5) percent body weight of the total fish during the acclimation period. Only healthy fish showing no visible signs of disease or abnormal behavior were selected for the experiment.

Experimental Toxicants

Cadmium chloride ($CdCl_2$) and glyphosate potassium were used as the test toxicants in this study. Analytical-grade cadmium chloride and commercial glyphosate potassium formulations were prepared separately as stock solutions using distilled water and subsequently combined to produce a fixed-ratio binary mixture. The binary mixture was formulated at a cadmium chloride–glyphosate potassium ratio of 1:2 to simulate simultaneous exposure to heavy-metal and herbicide contaminants commonly encountered in freshwater environments affected by agricultural and anthropogenic activities (Reynel-Ávila et al. 2021).

Appropriate volumes of the stock solutions were measured and diluted with dechlorinated freshwater to obtain the required experimental concentrations for both the range-finding and definitive toxicity tests. The selected mixture ratio was maintained throughout the study to ensure consistency in exposure conditions and to facilitate assessment of the combined toxic effects of the two contaminants under controlled laboratory conditions.

Range-Finding Test

A preliminary range-finding test was conducted to establish suitable concentration ranges for the definitive acute toxicity bioassay. Fish were exposed to a series of fixed-ratio (1:2) cadmium chloride–glyphosate potassium mixtures under static conditions for 96 hours. Mortality responses obtained from the preliminary test were used to select concentrations that produced partial mortality without causing complete mortality, thereby ensuring reliable estimation of LC_{50} values during the definitive bioassay (Ghafarifarsani et al. 2024).

Definitive Acute Toxicity Bioassay

The acute toxicity bioassay was conducted using a static exposure system over 96 hours following OECD Guideline 203 for fish acute toxicity testing (OECD 1992). Six experimental treatments comprising one control (0/0 mg/L) and five concentrations of a fixed-ratio (1:2) cadmium chloride–glyphosate potassium binary mixture were established. The definitive test concentrations were 6.18/12.36, 12.36/24.72, 18.54/37.08, 24.72/49.44, and 30.90/61.80 mg/L ($CdCl_2$/Glyphosate Potassium, respectively). Each treatment was replicated three times with ten fish per replicate, resulting in a total of

thirty fish per treatment. No feeding was carried out during the exposure period to minimize interference with toxicant uptake and water quality (Gharib et al. 2022).

Mortality Assessment

Fish were observed regularly, and cumulative mortality was recorded at 24, 48, 72, and 96 hours. Mortality criteria followed the OECD (1992) guidelines, in which fish were considered dead upon the complete absence of opercular movement and a lack of response to external stimulation.

Determination of Median Lethal Concentration (LC_{50})

LC_{50} values were estimated using probit regression analysis, a widely accepted statistical method for quantifying dose–response relationships in aquatic toxicology (Finney 1971; Pillai et al. 2021). Mortality data were transformed into probit values and regressed against toxicant concentration to determine concentration–response relationships.

Water Quality Parameters

Water quality parameters (temperature, pH, dissolved oxygen) were monitored throughout the bioassay following APHA (2017) standard methods. These parameters were maintained within ranges suitable for fish survival, ensuring reliability of toxicity outcomes.

Estimation of Safe Exposure Thresholds

Safe exposure thresholds were derived from the 96-hour LC_{50} using established safety application factors. The Sprague safety factor ($0.1 \times LC_{50}$) and NAS/NAE safety factor ($0.01 \times LC_{50}$) were applied to estimate sub-lethal concentrations (Sprague 1971; NAS/NAE 1973; Lucas et al. 2019).

Statistical Analysis

All analyses were performed using IBM SPSS Version 27.0. Probit regression was used to estimate LC_{50} values, regression coefficients, and confidence limits. Model adequacy was evaluated using Pearson goodness-of-fit statistics (Lungu-Mitea et al. 2020).

Results

Mortality Response of *Clarias gariepinus* Exposed to Cadmium Chloride–Glyphosate Potassium Binary Mixtures

Cumulative mortality of sub-adult *Clarias gariepinus* exposed to fixed-ratio cadmium chloride–glyphosate potassium binary mixtures increased progressively with both concentration and exposure duration (Table 1). No mortality was recorded in the control group throughout the 96-hour exposure period.

At 24 hours, mortality ranged from 3.33% in fish exposed to 6.18/12.36 mg/L cadmium chloride–glyphosate potassium mixtures to 23.33% in fish exposed to 30.90/61.80 mg/L. Mortality increased with prolonged exposure, reaching 13.33%, 16.67%, 33.33%, 43.33%, and 60.00% in treatments T1, T2, T3, T4, and T5, respectively, after 96 hours.

The mortality-time relationship is presented in Figure 1, which demonstrates a clear increase in mortality with increasing exposure duration and concentration. The highest mortality was observed in fish exposed to the highest concentration of the binary mixture (T5), while the lowest mortality occurred in fish exposed to the lowest concentration (T1).

The concentration-dependent mortality response observed after 96 hours of exposure is shown in Figure 2. Mortality increased linearly with increasing mixture concentration, indicating a positive concentration-response relationship.

Table 1: Cumulative mortality of sub-adult *Clarias gariepinus* exposed to fixed-ratio cadmium chloride–glyphosate potassium binary mixtures

Treatment	Cd/Glyphosate (mg/L)	24 h	48 h	72 h	96 h	Cumulative Mortality (%)
Control	0/0	0	0	0	0	0.00%
T1	6.18/12.36	1	1	1	4	13.33%
T2	12.36/24.72	2	3	4	5	16.67%
T3	18.54/37.08	4	5	8	10	33.33%
T4	24.72/49.44	5	6	9	13	43.33%
T5	30.90/61.80	7	8	10	18	60.00%

Probit Analysis and Estimation of LC_{50} Values

Probit regression analysis revealed a progressive decline in LC_{50} values with increasing exposure duration (Table 2). The estimated LC_{50} values were 90.744 mg/L, 73.906 mg/L, 46.153 mg/L, and 28.100 mg/L at 24, 48, 72, and 96 hours, respectively. The corresponding regression slopes were 1.640 ± 0.664, 1.673 ± 0.626, 1.943 ± 0.589, and 2.027 ± 0.496 for the 24-, 48-, 72-, and 96-hour exposure periods, respectively. Pearson goodness-of-fit statistics indicated adequate model fit for all exposure periods, with p-values exceeding 0.05.

The relationship between log-transformed concentration and probit mortality at 96 hours is illustrated in Figure 3. The positive slope of the regression model indicates increasing mortality with increasing toxicant concentration.

Table 2: Probit regression parameters and LC_{50} estimates for fixed-ratio cadmium chloride–glyphosate potassium binary mixtures in sub-adult *Clarias gariepinus*

Exposure Time (h)	Slope ± SE	LC_{50} (mg/L)	95% Lower CL	95% Upper CL	χ^2	p-value
24	1.640 ± 0.664	90.744	43.596	34047.515	0.138	0.987
48	1.673 ± 0.626	73.906	39.497	3137.865	0.060	0.996
72	1.943 ± 0.589	46.153	31.032	177.456	0.611	0.894
96	2.027 ± 0.496	28.100	21.628	47.653	2.085	0.555

Temporal Toxicity Dynamics

The estimated LC_{50} values decreased consistently with increasing exposure duration (Figure 4). The highest LC_{50} value was observed after 24 hours of exposure (90.744 mg/L), while the lowest value occurred after 96 hours (28.100 mg/L). This decline indicates that prolonged exposure increased the toxicity of the cadmium chloride–glyphosate potassium binary mixture to sub-adult *Clarias gariepinus*. The temporal pattern demonstrates that lower concentrations were required to induce equivalent mortality responses as exposure duration increased.

Safe Exposure Thresholds

Safe exposure thresholds estimated from the 96-hour LC_{50} are presented in Table 3. Application of the Sprague safety factor yielded a safe concentration of 2.81 mg/L, while application of the NAS/NAE safety factor produced a more conservative threshold of 0.28 mg/L.

These values represent estimated sub-lethal exposure concentrations that may be used as reference points for future chronic toxicity studies involving cadmium chloride–glyphosate potassium binary mixtures in *Clarias gariepinus*.

Table 3: Estimated safe exposure thresholds for fixed-ratio cadmium chloride–glyphosate potassium binary mixtures

Parameter	Value (mg/L)
96-H LC_{50}	28.10
Sprague Safety Concentration (0.1 × LC_{50})	2.81
NAS/NAE Safety Concentration (0.01 × LC_{50})	0.28

Water Quality Parameters

The mean water quality parameters recorded during the acute toxicity bioassay are presented in Table 4. Temperature remained relatively stable across all treatments, ranging from 26.13 ± 0.21°C to 26.33 ± 0.21°C. In contrast, pH and dissolved oxygen exhibited concentration-dependent declines with increasing cadmium chloride–glyphosate potassium mixture concentrations. Dissolved oxygen decreased from 6.79 ± 0.13 mg/L in the control treatment to 3.74 ± 0.18 mg/L in the highest exposure group (T5), while pH declined from 7.45 ± 0.09 to 6.21 ± 0.10. Despite these reductions, water quality conditions remained within ranges capable of sustaining fish survival during the exposure period.

Table 4. Water Quality Parameters Recorded During the Acute Toxicity Bioassay

Treatment	Temperature (°C)	pH	DO (mg/L)
T0	26.33 ± 0.21	7.45 ± 0.09	6.79 ± 0.13
T1	26.13 ± 0.21	7.23 ± 0.10	6.39 ± 0.16
T2	26.20 ± 0.20	7.04 ± 0.07	5.82 ± 0.16
T3	26.30 ± 0.20	6.75 ± 0.09	5.18 ± 0.18
T4	26.20 ± 0.20	6.51 ± 0.08	4.30 ± 0.14
T5	26.30 ± 0.20	6.21 ± 0.10	3.74 ± 0.18

Discussion

The present study demonstrated that fixed-ratio cadmium chloride – glyphosate potassium binary mixtures exert significant acute toxic effects on sub-adult *Clarias gariepinus*. Mortality increased progressively with both concentration and exposure duration, consistent with the principle that toxic responses intensify with dose and time (Pillai et al. 2021). Similar concentration-dependent mortality trends have been reported in fish exposed to glyphosate formulations (Alarape et al. 2023; Lanzarin et al. 2023) and heavy metals such as cadmium (Liu et al. 2022; Zhang et al. 2021), confirming mortality as a sensitive endpoint for acute toxicity assessment.

A key finding was the marked reduction in LC_{50} values from 90.744 mg/L at 24 H to 28.100 mg/L at 96 h, indicating cumulative toxic action. Temporal declines in LC_{50} have been observed in fish exposed to cadmium (Alengebawy et al. 2021) and glyphosate (Lanzarin et al. 2023), reflecting progressive physiological impairment under prolonged exposure. The observed pattern suggests that toxicants were absorbed and retained within tissues, gradually disrupting essential processes such as osmoregulation, respiration, and enzyme activity (Liu et al. 2022). This cumulative toxicity highlights the importance of considering exposure duration in ecological risk assessments, as short-term bioassays may underestimate long-term hazards.

The combined effects of cadmium and glyphosate likely imposed multiple physiological burdens. Cadmium is known to disrupt ion regulation and induce oxidative stress (Zhang et al. 2021), while glyphosate has been associated with metabolic disturbances and histopathological damage in fish (Alarape et al. 2023). Their simultaneous presence may have produced additive or synergistic interactions, reducing tolerance and increasing susceptibility to mortality. Mixture toxicology studies have shown that contaminant interactions can amplify toxic responses beyond those predicted from single exposures (Ghafarifarsani et al. 2024; Heys et al. 2016). The strong concentration–response relationships and satisfactory probit model fits observed here reinforce the predictability of mortality outcomes under mixture exposure.

Water quality monitoring revealed stable temperature but concentration-dependent declines in pH and dissolved oxygen. Although these changes may have contributed to physiological stress, the clear dose–response mortality pattern suggests that the binary mixture was the primary driver of toxicity. Similar water quality-mediated stress responses have been reported in fish exposed to pollutants in Nigerian freshwater systems (APHA 2017), underscoring the need to consider both chemical and physicochemical stressors in toxicity evaluations.

The safe exposure thresholds estimated from the 96-hour LC_{50} (2.81 mg/L using Sprague's factor and 0.28 mg/L using NAS/NAE) provide valuable baseline information for environmental monitoring and aquaculture management. These thresholds align with modern approaches to safe concentration estimation in aquatic toxicology (Lucas et al. 2019) and may serve as reference points for chronic toxicity studies. Given the widespread occurrence of cadmium and glyphosate residues in African freshwater environments (Lucas et al. 2019; Langia et al. 2024), the low safe concentrations observed here raise concerns about potential impacts on fish health, aquaculture productivity, and biodiversity.

From an applied perspective, the findings have direct relevance to aquaculture and fisheries management. *Clarias gariepinus* is a cornerstone of aquaculture in sub-Saharan Africa, contributing significantly to food security and livelihoods (Lucas et al. 2019; Langia et al. 2024). Contaminant-induced reductions in survival could therefore have ecological, economic, and social consequences. Effective regulation of contaminant discharge and continuous monitoring of vulnerable aquatic environments are essential to safeguard aquaculture sustainability.

Overall, this study provides additional evidence that cadmium–glyphosate mixtures pose substantial toxicological risks to *Clarias gariepinus*. The pronounced decline in LC_{50} values with exposure duration, coupled with strong concentration–response relationships and low safe thresholds, underscores the ecological significance of mixture toxicity. These findings highlight the need to incorporate mixture-based approaches into environmental risk assessment frameworks and to develop protective strategies for freshwater ecosystems increasingly burdened by combined chemical pollution.

Conclusion

The present study demonstrated that fixed-ratio cadmium chloride–glyphosate potassium binary mixtures exert significant acute toxic effects on sub-adult *Clarias gariepinus*. Mortality increased progressively with both concentration and exposure duration, resulting in a marked decline in LC_{50} values from 90.744 mg/L at 24 hours to 28.100 mg/L at 96 hours. These findings indicate that prolonged exposure substantially enhances the toxicity of the binary mixture and reduces the concentration required to induce lethal effects.

The concentration-dependent mortality response and strong probit concentration–response relationships observed in the present study confirm the suitability of acute toxicity assessment for characterizing the hazard potential of cadmium–glyphosate mixtures in aquatic organisms. The estimated safe concentration thresholds derived from the 96-hour LC_{50} further provide useful baseline information for environmental monitoring, ecological risk assessment, and the development of regulatory guidelines for freshwater ecosystems.

Given the widespread occurrence of heavy metals and agrochemical residues in aquatic environments, the observed toxicity of the cadmium chloride–glyphosate mixture raises concerns regarding the potential impacts of combined pollutant exposure on fish health, aquaculture productivity, and aquatic biodiversity. The findings emphasize the importance of considering contaminant mixtures rather than individual toxicants when evaluating environmental risks in freshwater systems.

In total, the study provides valuable evidence that binary mixtures of cadmium chloride and glyphosate potassium can pose substantial toxicological threats to *Clarias gariepinus*, particularly under prolonged exposure conditions. Future studies should investigate the underlying physiological, biochemical, and histopathological mechanisms responsible for the observed toxicity and evaluate the long-term ecological consequences of chronic exposure to mixed contaminants in aquatic ecosystems.

Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by KIS and NAA. The first draft of the manuscript was written by KIS and NAA, and all authors commented on previous versions of the manuscript. Funding acquisition was secured by KIS. All authors read and approved the final manuscript.

Data Availability

The raw datasets generated and analyzed during the current study are fully integrated within the tables and figures of this manuscript. Additional statistical outputs from the Probit regression analysis are available from the corresponding author upon reasonable request.

References

1. Alarape SA, Adeogun AO, Olatunji OS, Fagbohun OA (2023) Assessment of glyphosate and its metabolites in cultured African catfish (*Clarias gariepinus*). Front Toxicol 5:1250137. https://doi.org/10.3389/ftox.2023.1250137
2. Alengebawy A, Abdelkhalek ST, Qureshi SR, Wang MQ (2021) Heavy metals and pesticides toxicity in agricultural soil and plants: Ecological risks and human health implications. Toxics 9(3):42. https://doi.org/10.3390/toxics9030042
3. APHA (2017) Standard methods for the examination of water and wastewater, 23rd edn. American Public Health Association, Washington, D.C.
4. Finney DJ (1971) Probit analysis, 3rd edn. Cambridge University Press, Cambridge
5. Ayoola SO (2008) Toxicity of glyphosate herbicide on Nile tilapia (*Oreochromis niloticus*) juvenile. Afr J Agric Res 3(12):825–834
6. Ghafarifarsani H, Rohani MF, Raeeszadeh M (2024) Pesticides and heavy metal toxicity in fish and possible remediation strategies. Annals Anim Sci. https://doi.org/10.2478/aoas-2024-0012
7. Gharib AA, Abdel-Hamid EA, Mousa MA, Naiel MA (2022) Improving water quality, growth performance, and modulating some stress physiological biomarkers in *Cyprinus carpio* using raw date nuclei as a zinc adsorbent agent. Appl Water Sci 12(7):159 doi.org
8. Heys KA, Shore RF, Pereira MG, Jones KC, Martin FL (2016) Risk assessment of environmental mixture effects: Advances and challenges. RSC Adv 6:47844–47857. https://doi.org/10.1039/C6RA05406D
9. Langia S, Maulu S, Hasimuna OJ (2024) Nutritional requirements and culture conditions of African catfish (*Clarias gariepinus*). Cogent Food Agric 10(1):2302642. https://doi.org/10.1080/23311932.2024.2302642

10. Lanzarin GAB, Félix LM, Monteiro SM, Venâncio C (2023) Effects of glyphosate-based herbicides during the zebrafish (*Danio rerio*) life cycle. Water 15(12):2276. https://doi.org/10.3390/w15122276
11. Liu Y, Chen Q, Li Y, Bi L, Jin L, Peng R (2022) Toxic effects of cadmium on fish: A review. Toxics 10(10):622. https://doi.org/10.3390/toxics10100622
12. Lucas JS, Southgate PC, Tucker CS (2019) Aquaculture: Farming aquatic animals and plants, 3rd edn. Wiley Blackwell, Hoboken
13. Lungu-Mitea S, Vogs C, Carlsson G, Montag M, Frieberg K, Oskarsson A, Lundqvist J (2020) Modeling bioavailable concentrations in zebrafish cell lines and embryos increases the correlation of toxicity potencies across test systems. Environ Sci Technol 55(1):447–457 doi.org
14. NAS/NAE (1973) Water quality criteria 1972. National Academy of Sciences/National Academy of Engineering, Washington, D.C.
15. OECD (1992) OECD guideline for testing of chemicals, No. 203: Fish, acute toxicity test. Organisation for Economic Co-operation and Development, Paris
16. Pillai SK, Kobayashi K, Mathai T, Michael M (2021) Use of LC_{50} in aquatic regulatory toxicology: Disharmony in global harmonization of hazard classification of chemicals. Ecotoxicol Environ Contam 16(1):91–96. https://doi.org/10.5132/eec.2021.01.12
17. Reynel-Ávila HE, Camacho-Aguilar KI, Bonilla-Petriciolet A, Mendoza-Castillo DI, González-Ponce HA, Trejo-Valencia R (2021) Engineered magnetic carbon-based adsorbents for the removal of water priority pollutants: an overview. Adsorpt Sci Technol 2021:9917444
18. Sprague JB (1971) Measurement of pollutant toxicity to fish. Water Res 5:245–266 doi.org
19. Tresnakova N, Stara A, Velisek J (2021) Effects of glyphosate and its metabolite AMPA on aquatic organisms. Appl Sci 11(19):9004. https://doi.org/10.3390/app11199004
20. Zhang X, Li J, Wang Y, Chen H (2021) The cascade of cadmium toxicity: From cellular damage to pyroptotic cell death in freshwater fish. Environ Sci Pollut Res 28:51234–51245 doi.org

Figures

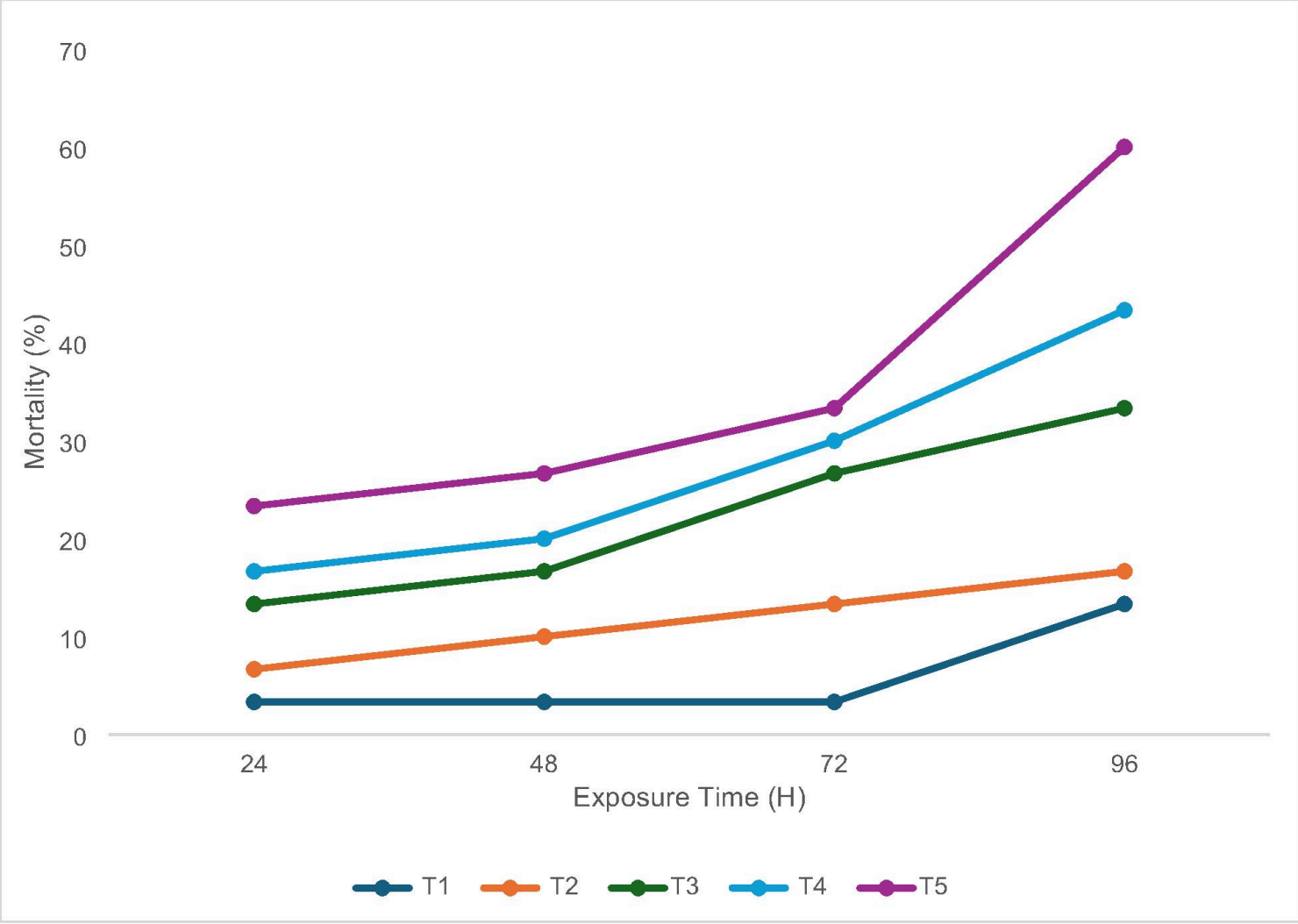


Figure 1

Mortality-time relationship of sub-adult *Clarias gariepinus* exposed to fixed-ratio cadmium chloride–glyphosate potassium binary mixtures

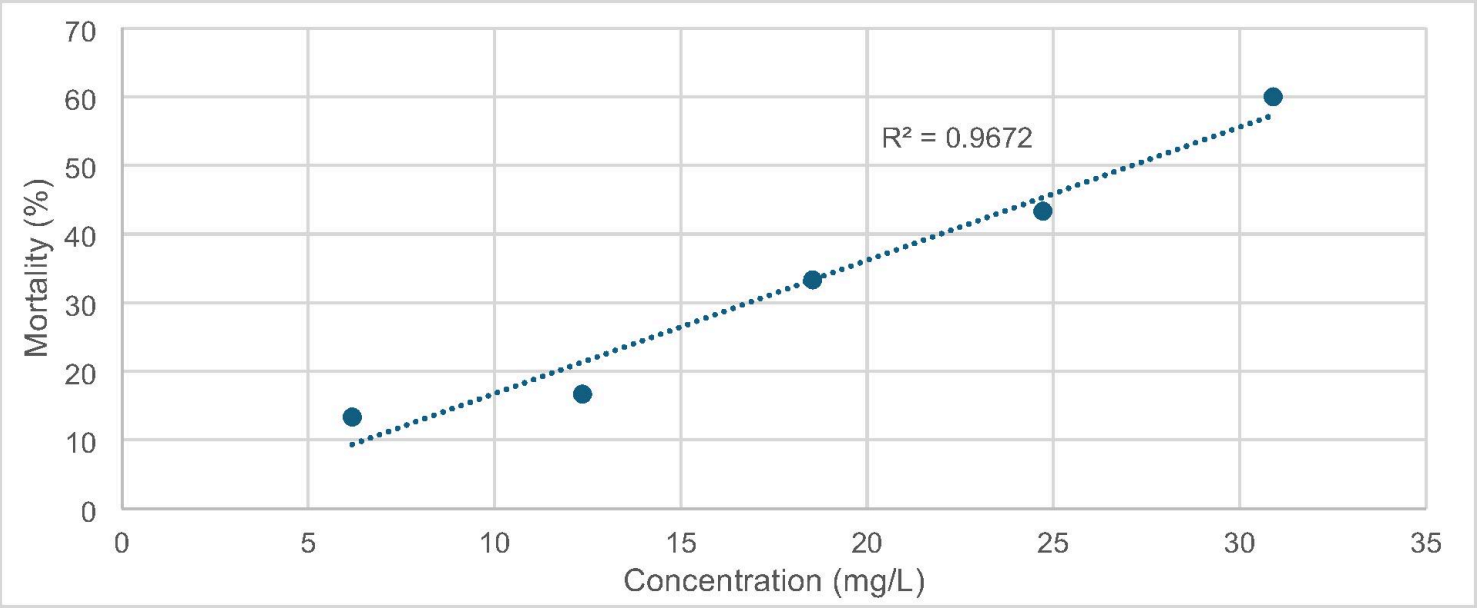


Figure 2

Concentration-dependent mortality response of sub-adult *Clarias gariepinus* exposed to cadmium chloride–glyphosate potassium binary mixtures for 96 h

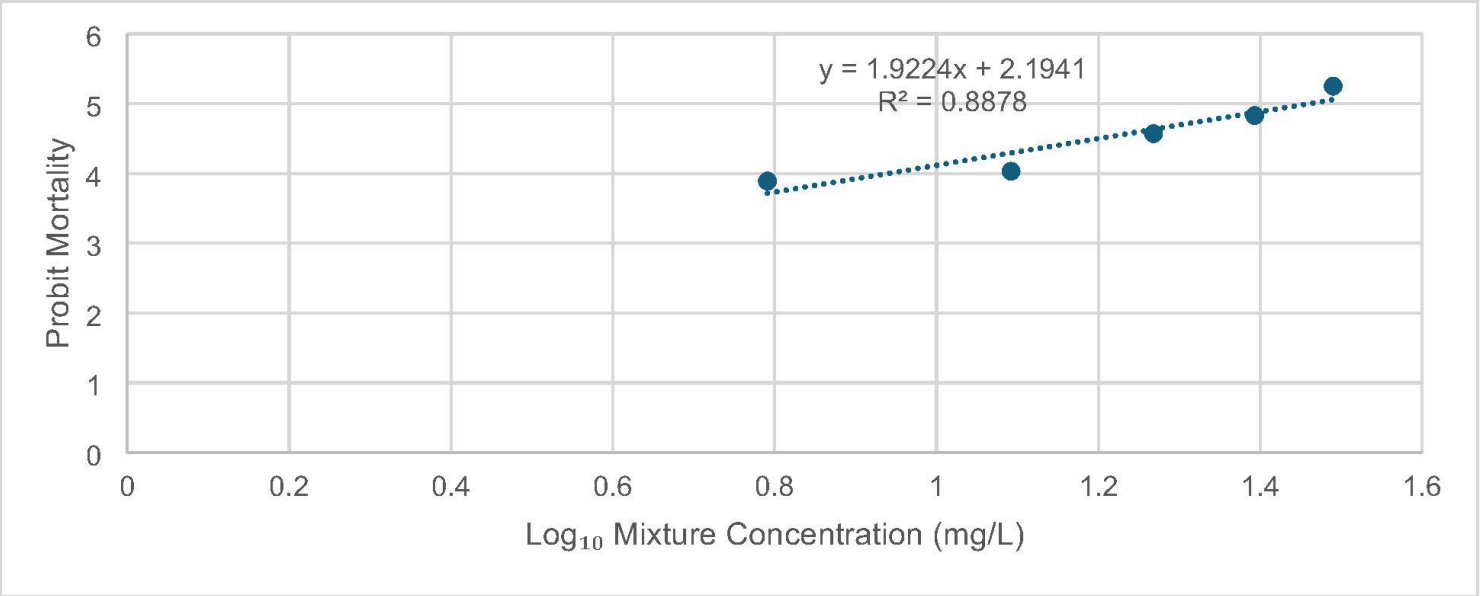


Figure 3

Probit concentration–response relationship for 96-hour acute toxicity of fixed-ratio cadmium chloride–glyphosate potassium binary mixtures in sub-adult *Clarias gariepinus*

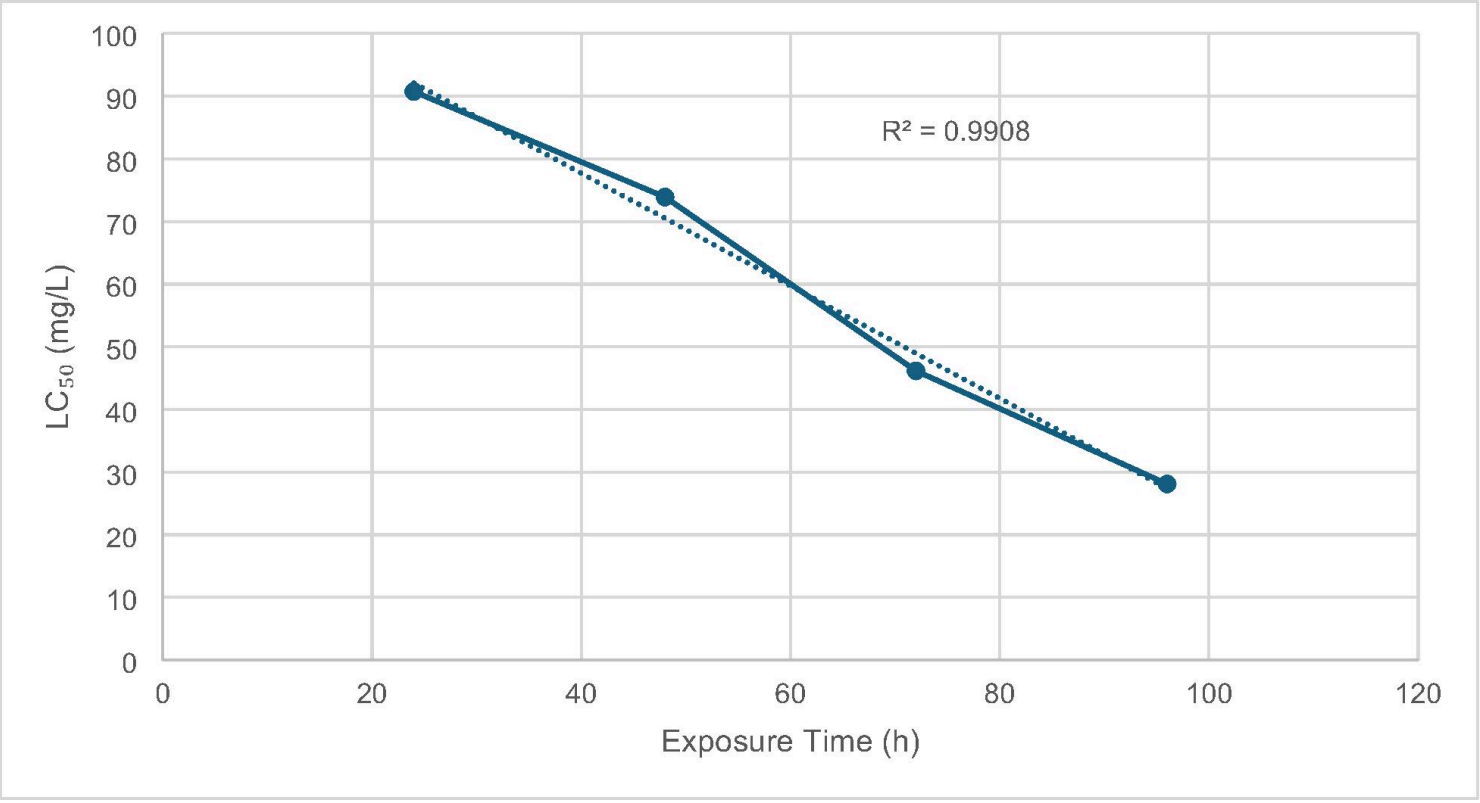


Figure 4

Temporal decline in LC_{50} values of fixed-ratio cadmium chloride–glyphosate potassium binary mixtures in sub-Adult *Clarias gariepinus*