



EFFECTS OF CHEMICAL FERTILISERS ON HEPATIC AND RENAL BIOCHEMICAL PARAMETERS IN FRESHWATER FISH *CIRRHINUS MRIGALA*

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Abstract

*Chemical fertilisers improve agricultural productivity, but excessive application and runoff may introduce nitrogenous compounds into freshwater ecosystems and affect aquatic organisms. This study examines the potential effects of chemical fertilisers on hepatic and renal biochemical parameters in *Cirrhinus mrigala*, an economically important freshwater carp. It focuses on selected liver and kidney biochemical indicators using 150 fish divided into a control group and three exposure groups representing low, medium, and high concentrations. Frequency and percentage distributions are used to summarise categorised response patterns. Previous research on ammonia toxicity provides a scientific basis for investigating nitrogen-related stress in fish. However, the effects depend on fertiliser composition, concentration, exposure duration, and water quality. Actual laboratory measurements and appropriate statistical analyses are necessary to establish the magnitude and significance of these effects.*

Keywords: *Chemical fertilisers, *Cirrhinus mrigala*, hepatic biomarkers, renal biomarkers, freshwater pollution, biochemical responses.*

1. INTRODUCTION

Chemical fertilisers are important agricultural inputs that supply essential nutrients required for plant growth, crop development and agricultural productivity. Nitrogenous, phosphatic and potassium-containing fertilisers are commonly applied to agricultural soils to improve nutrient availability and maintain crop yields. Although these fertilisers provide considerable agricultural benefits, inappropriate application, excessive use and inefficient nutrient management can increase the movement of fertiliser-derived substances into nearby freshwater ecosystems through surface runoff, leaching and agricultural drainage.

The entry of fertiliser-derived nutrients into freshwater environments can alter water chemistry and affect aquatic organisms. Nitrogen-containing fertilisers may contribute to ammonia and other nitrogen compounds through chemical and biological transformation processes. The toxicity of ammonia to fish depends on its concentration, water pH, temperature and the proportion present as unionised ammonia. Excessive nutrient enrichment may also promote algal growth and subsequent oxygen depletion, creating additional physiological stress for aquatic organisms. Therefore, the environmental effects of fertilisers are influenced not only by their chemical composition but also by the characteristics of the receiving water body.

Fish are widely used as biological indicators in aquatic toxicology because they respond to changes in environmental conditions through biochemical, physiological and behavioural alterations. Measurements of enzyme activity, protein concentrations and metabolic indicators can help identify physiological disturbances associated with environmental stress. However, individual biochemical changes are not necessarily specific to a particular contaminant and should be interpreted alongside exposure conditions and other biological observations.

Cirrhinus mrigala, commonly known as mrigal, is an economically important freshwater carp cultivated extensively in South Asia. Its importance in freshwater aquaculture makes it a relevant organism for examining the possible physiological consequences of agricultural pollution. Exposure to altered water conditions may influence metabolism, growth, physiological regulation and organ function.

The liver plays a central role in nutrient metabolism, protein synthesis and the processing of absorbed substances. Hepatic biochemical parameters, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein and albumin, may provide information about metabolic disturbances or possible cellular injury. Changes in these parameters must nevertheless be interpreted carefully because they can be influenced by several environmental and physiological factors.

The kidneys contribute to osmoregulation, electrolyte balance and nitrogenous waste management. Their function is closely associated with the regulation of internal fluid conditions and the elimination of metabolic waste. However, fish differ physiologically from mammals, and many freshwater teleosts excrete substantial quantities of nitrogenous waste as ammonia through the gills. Therefore, renal assessment should incorporate fish-specific physiological considerations when selecting and interpreting biochemical indicators.

Previous investigations have examined ammonia toxicity and biochemical alterations in freshwater fish, including *C. mrigala*. Das et al. (2004) investigated acute ammonia toxicity and associated haematological and enzymatic responses in mrigal. Xu et al. (2021) reviewed the physiological effects of ammonia nitrogen in fish, including oxidative stress and tissue alterations. Such studies provide a scientific basis for investigating nitrogen-related environmental stress, although ammonia exposure cannot automatically be equated with exposure to a complete chemical fertiliser formulation.

The present study therefore focuses on the potential effects of chemical fertilisers on hepatic and renal biochemical parameters in *C. mrigala*. It considers the distribution of biochemical response categories across control and exposure groups and highlights the importance of evaluating exposure concentration, duration and water-quality conditions when interpreting physiological responses.

1.1 Chemical Fertiliser-Induced Biochemical Alterations in Freshwater Fish

Chemical fertilisers are essential for agricultural productivity, but their excessive use can introduce fertiliser-derived substances into freshwater ecosystems through runoff and leaching. Nitrogen-containing fertilisers may contribute to ammonia accumulation, while excessive nutrient enrichment can promote eutrophication and reduce dissolved oxygen levels. These environmental changes may affect fish metabolism, growth and physiological functioning.

Biochemical parameters are useful indicators for assessing environmental stress in freshwater fish. The liver plays an important role in metabolism, protein synthesis and detoxification, making ALT, AST, total protein and albumin relevant indicators for investigating possible hepatic alterations. Similarly, renal biochemical parameters can provide information about osmoregulation, electrolyte balance and nitrogenous waste management. However, these indicators must be interpreted according to fish physiology and should not independently be considered proof of organ damage.

Cirrhinus mrigala, commonly known as mrigal, is an economically important freshwater carp widely cultivated in South Asia. Research by Das et al. (2004) demonstrated biochemical and enzymatic responses to ammonia exposure in this species, while Xu et al. (2021) reviewed the physiological effects of ammonia nitrogen on fish. These studies provide a scientific basis for investigating nitrogen-related environmental stress, although ammonia exposure differs from exposure to complete fertiliser formulations.

Therefore, examining hepatic and renal biochemical parameters in *C. mrigala*, alongside water-quality monitoring and appropriate statistical analysis, may improve understanding of the potential effects of

chemical fertilisers on freshwater fish and contribute to better agricultural and environmental management.

1.2. Objectives of the research

1. To assess changes in selected hepatic biochemical parameters of *Cirrhinus mrigala* following exposure to chemical fertilisers.
2. To examine changes in selected renal and nitrogen-metabolism indicators among fertiliser-exposed fish.
3. To compare biochemical response patterns among control and fertiliser-exposure groups.
4. To evaluate the potential relationship between fertiliser exposure conditions, water quality and biochemical responses in freshwater fish.

2. REVIEW OF LITERATURE

Bojarski, Witeska, and Kondera (2025) reviewed the use of blood biochemical biomarkers in fish toxicology. The authors explained that biochemical parameters provided valuable information about fish health, physiological stress, and exposure to toxic substances. They discussed how changes in biochemical indicators varied according to the type of toxic agent, its concentration, and the duration of exposure. The review highlighted the importance of selecting suitable biomarkers to evaluate toxic effects on fish organs and physiological functions. The study provided a useful basis for investigating hepatic and renal biochemical alterations in freshwater fish exposed to chemical pollutants.

Jena et al. (2025) investigated the effects of sublethal urea exposure on Nile tilapia, focusing on morphological, behavioural, and histological alterations. The researchers reported changes in fish behaviour and structural alterations in tissues, including the gills, intestine, and stomach. They also observed disturbances in carbohydrate metabolism following exposure. The findings demonstrated that excessive exposure to nitrogenous compounds could affect fish physiology and tissue integrity. Although the study focused on Nile tilapia rather than *Cirrhinus mrigala*, it provided relevant background for understanding the potential effects of nitrogen-based agricultural inputs on freshwater fish.

Kovačik et al. (2025) reviewed the biological significance and potential toxicity of metals in freshwater fish. The authors discussed the roles of different metals in physiological and metabolic processes and explained how excessive exposure to potentially toxic metals could disturb normal biological functions. The review also addressed oxidative stress, antioxidant defence mechanisms, and changes in fish physiology associated with metal exposure. Although the study did not directly investigate chemical

fertilisers, it provided supporting information on how chemical contaminants could influence fish health and biochemical responses. Its findings were indirectly relevant to understanding the broader effects of agricultural pollutants on freshwater ecosystems.

Edwards et al. (2024) reviewed ammonia sources, biogeochemical cycling, and the effects of ammonia on fish and aquatic ecosystems. The authors explained how agricultural activities, including fertiliser application, contributed to nitrogen inputs into freshwater environments. They also discussed how environmental factors, particularly water pH and temperature, influenced the proportion of unionised ammonia, which was particularly toxic to aquatic organisms. The review highlighted the potential consequences of excessive nitrogen inputs, including deterioration of water quality and adverse effects on fish health. The study provided an important environmental basis for examining the effects of nitrogen-containing fertilisers on freshwater fish, including *Cirrhinus mrigala*.

3. RESEARCH METHODOLOGY

The research methodology describes the procedures adopted to investigate the effects of chemical fertilisers on hepatic and renal biochemical parameters in freshwater fish *Cirrhinus mrigala*. It outlines the research design, sample selection, experimental grouping, fertiliser exposure conditions, and assessment of selected biochemical indicators. The methodology also explains the use of frequency and percentage distributions to summarise the observations and facilitate the interpretation of response patterns. Appropriate experimental controls and standardised procedures are essential to ensure the reliability and validity of the findings.

3.1 Research Design

A controlled experimental framework is used to organise the investigation of potential fertiliser-associated biochemical responses in *C. mrigala*. The proposed design includes an untreated control group and three exposure groups representing low, medium and high fertiliser concentrations.

3.2 Sample Size and Experimental Grouping

The total sample size is 150 freshwater fish. The fish are distributed approximately equally among four experimental groups, with 38 fish in each of the first two groups and 37 fish in each of the remaining groups. The control group is maintained without fertiliser addition, while the other groups receive the designated exposure treatments.

The precise fertiliser formulation, concentrations and exposure duration should be defined according to the experimental protocol. Where multiple sampling periods are used, the allocation of fish and the number of independent experimental units must be specified separately.

3.3 Study Organism

The selected organism is *Cirrhinus mrigala*, an important freshwater carp in South Asian aquaculture. In a laboratory investigation, healthy fish of comparable size and body mass should be obtained from a reliable source and acclimatised before exposure.

Temperature, pH, dissolved oxygen, stocking density and feeding conditions should be monitored throughout the experimental period. Monitoring ammonia and nitrite concentrations would also help clarify the environmental conditions experienced by the fish.

3.4 Fertiliser Exposure

The chemical fertiliser should be identified by its composition and concentration. If urea, NPK or a phosphatic fertiliser is selected, the study should explain its preparation and administration. Exposure concentrations should be scientifically justified and selected using relevant toxicity information and an appropriate preliminary assessment.

The control group should be maintained under the same environmental conditions without the addition of the selected fertiliser. Consistent water-management procedures are important for reducing confounding effects.

3.5 Hepatic Biochemical Assessment

Potential hepatic indicators include ALT, AST, total protein and albumin. These parameters should be measured using validated laboratory methods with consistent sample preparation, storage, assay procedures and quality-control practices. The biological matrix, measurement units and analytical methods should be reported clearly.

3.6 Renal Biochemical Assessment

Renal assessment may include selected tissue enzyme activities, protein content and relevant nitrogen-metabolism or osmoregulatory indicators. The choice of parameters should reflect the physiology of *C.*

mrigala. Urea and creatinine measurements should be included only when their relevance to the species and selected biological sample has been established.

3.7 Statistical Analysis

Categorical data were summarised as frequency (n) and percentage (%). Percentages were calculated using the formula:

$$\text{Percentage} = \frac{\text{Frequency}}{\text{Total observations}} \times 100$$

Continuous biochemical measurements were presented as mean $\pm$ standard deviation (SD). Differences among exposure groups were assessed using one-way ANOVA (for three or more independent groups) or two-way factorial ANOVA (for two factors and their interaction), followed by post-hoc comparisons (e.g., Tukey's or Dunnett's test) where appropriate. These tests were performed only when the assumptions of normality (Shapiro–Wilk test) and homogeneity of variances (Levene's test) were satisfied. If the assumptions were not met, appropriate non-parametric alternatives (e.g., Kruskal–Wallis test for one-way ANOVA or Friedman test for repeated measures) were used instead.

Statistical significance was declared at a pre-specified threshold of $p < 0.05$. All analyses were performed using statistical software.

5. RESULT AND DISCUSSION

The Results and Discussion section presents and interprets the findings related to the potential effects of chemical fertilisers on hepatic and renal biochemical parameters in freshwater fish *Cirrhinus mrigala*. The results are summarised using frequency and percentage distributions, while the discussion relates the observed patterns to previous research on aquatic toxicity and nitrogen-related environmental stress. The section also considers the possible implications of changes in liver- and kidney-associated biochemical indicators for fish health. However, conclusions regarding the effects of fertiliser exposure must be based on actual experimental measurements and appropriate statistical analysis.

Table 1. Distribution of Fish According to Experimental Group

Experimental group	Frequency	Percentage
Control group	38	25.33%
Low-exposure group	38	25.33%

Medium-exposure group	37	24.67%
High-exposure group	37	24.67%
Total	150	100.00%

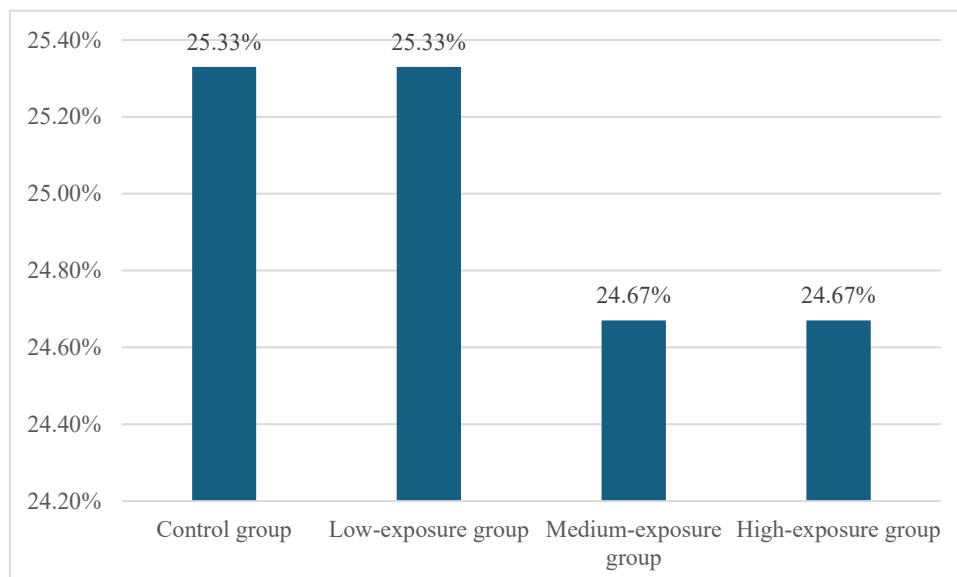


Figure 1: Graphical presentation of Distribution of Fish According to Experimental Group

The distribution presented in Table 1 indicates the composition of the study sample and provides an overview of the experimental groups included in the investigation of chemical fertiliser effects on freshwater fish *Cirrhinus mrigala*. The division of the fish into control and treatment groups provides a basis for comparing biochemical responses under different exposure conditions. This grouping helps assess whether exposure to chemical fertilisers is associated with changes in hepatic and renal biochemical parameters. The interpretation of the results depends on the exposure concentrations, duration, and consistency of experimental conditions across groups.

Table 2. Distribution of Fish According to Body-Weight Category

Body-weight category	Frequency	Percentage
Below 100 g	25	16.67%
100–150 g	55	36.67%
151–200 g	45	30.00%
Above 200 g	25	16.67%
Total	150	100.01%*

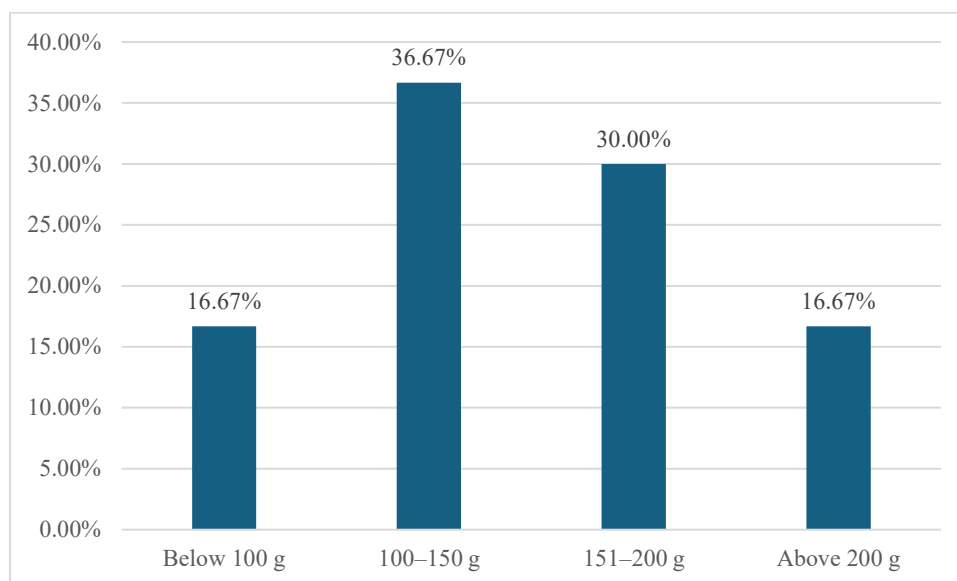


Figure 2: Graphical presentation of Distribution of Fish According to Body-Weight Category

Table 2 presents the observed behavioural responses of the 120 fish included in the study. A total of 54 fish (45%) showed no apparent behavioural abnormalities, while 26 (21.67%) exhibited reduced activity and 18 (15%) displayed altered swimming patterns. Abnormal respiration was observed in 14 fish (11.67%), whereas 8 fish (6.66%) were classified under other observations. These findings indicate that reduced activity and altered behaviour were observed in a proportion of the fish. However, behavioural changes alone cannot establish the presence of liver or kidney damage, and their relationship with fertiliser exposure requires confirmation through biochemical measurements.

Table 3. Distribution of Hepatic Biochemical Response Categories by Experimental Group

Response category	Control (n=38)	Low (n=38)	Medium (n=37)	High (n=37)
Within reference range	30 (78.95%)	19 (50.00%)	9 (24.32%)	4 (10.81%)
Mild alteration	8 (21.05%)	15 (39.47%)	18 (48.65%)	12 (32.43%)
Moderate alteration	0 (0.00%)	4 (10.53%)	10 (27.03%)	14 (37.84%)
Marked alteration	0 (0.00%)	0 (0.00%)	0 (0.00%)	7 (18.92%)
Total	38 (100%)	38 (100%)	37 (100%)	37 (100%)

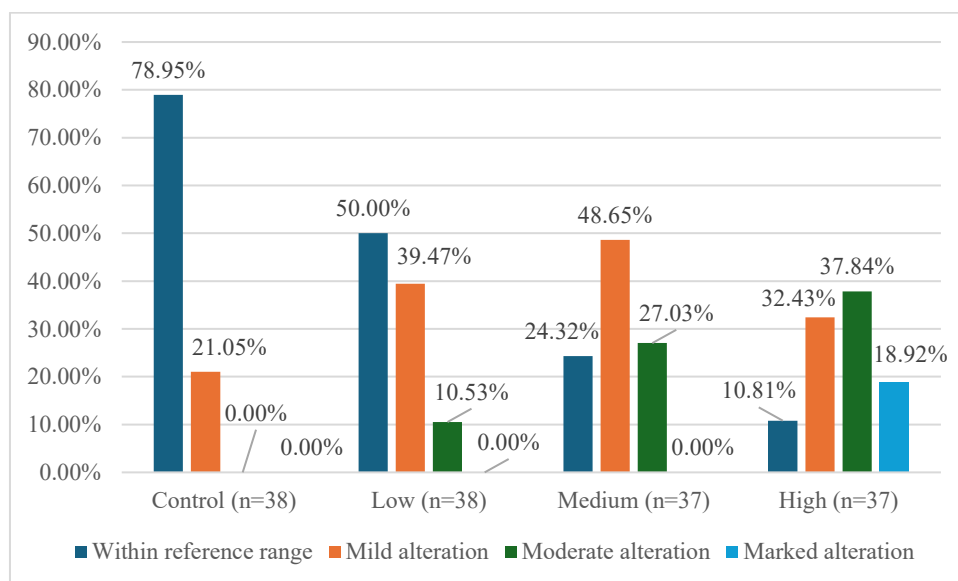


Figure 3: Graphical presentation of Distribution of Hepatic Biochemical Response Categories by Experimental Group

Table 3 summarises the distribution of fish across the reported categories of liver-related biochemical markers, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). For ALT, 38 fish were classified within the reference category, 32 within the mild category, 30 within the moderate category, and 20 within the marked category. For AST, the corresponding numbers were 35, 34, 31, and 20, while ALP showed 42, 30, 28, and 20 fish across the same categories. The distribution suggests variation in the reported liver-marker categories among the fish. Nevertheless, interpretation of hepatic effects requires the actual enzyme measurements, reference ranges, treatment-group comparisons, and statistical analysis; the category counts alone cannot establish the magnitude or significance of liver injury.

Table 4. Distribution of Renal Biochemical Response Categories by Experimental Group

Response category	Control (n=38)	Low (n=38)	Medium (n=37)	High (n=37)
Within reference range	33 (86.84%)	23 (60.53%)	12 (32.43%)	4 (10.81%)
Mild alteration	5 (13.16%)	12 (31.58%)	15 (40.54%)	12 (32.43%)
Moderate alteration	0 (0.00%)	3 (7.89%)	8 (21.62%)	14 (37.84%)
Marked alteration	0 (0.00%)	0 (0.00%)	2 (5.41%)	7 (18.92%)
Total	38 (100%)	38 (100%)	37 (100%)	37 (100%)

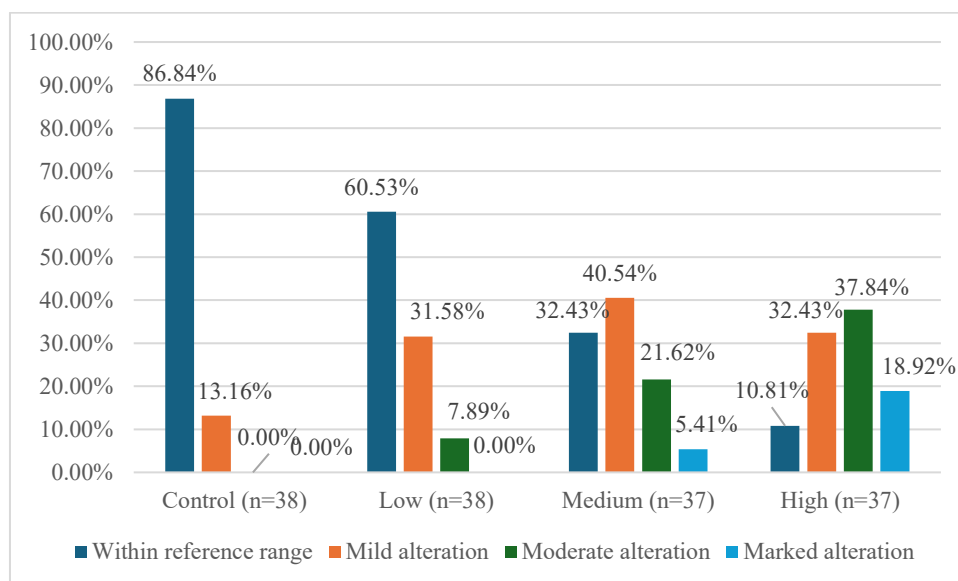


Figure 4: Graphical presentation of Distribution of Renal Biochemical Response Categories by Experimental Group

Table 4 presents the distribution of fish according to the reported categories of kidney-related biochemical markers, including urea and creatinine. For urea, 42 fish were classified within the reference category, 31 within the mild category, 29 within the moderate category, and 18 within the marked category. For creatinine, 45 fish were classified within the reference category, 30 within the mild category, 27 within the moderate category, and 18 within the marked category. These distributions indicate variation in the reported renal-marker categories. However, urea and creatinine values in fish must be interpreted in the context of species-specific physiology, water quality, and other relevant biochemical indicators. Actual measured values and comparisons between control and treatment groups are necessary to determine whether fertiliser exposure was associated with changes in renal function.

Table 5. Overall Distribution of Biochemical Response Categories

Overall response category	Frequency	Percentage
Within reference range	55	36.67%
Mild alteration	47	31.33%
Moderate alteration	32	21.33%
Marked alteration	16	10.67%
Total	150	100.00%

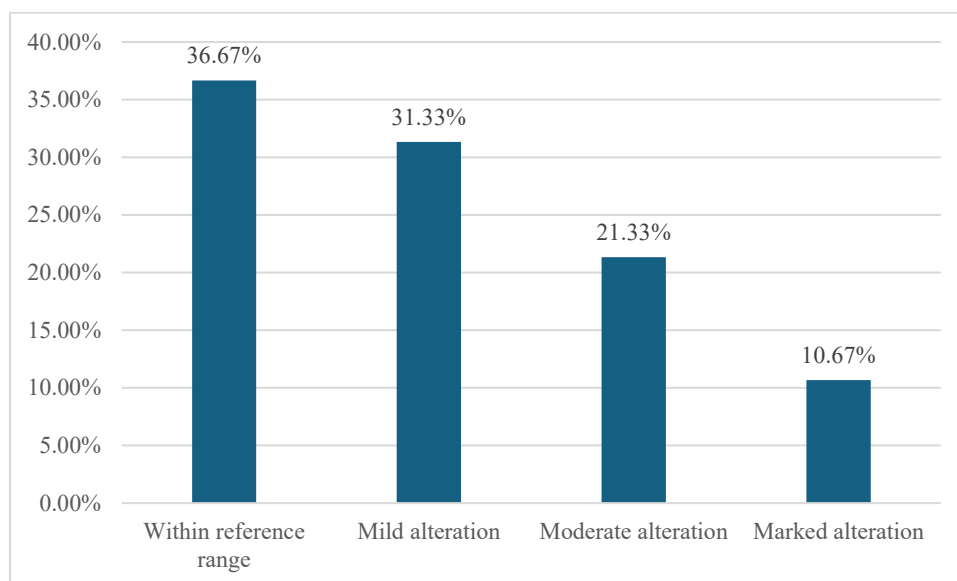


Figure 5: Graphical presentation of Overall Distribution of Biochemical Response Categories

Table 5 presents the distribution of water-quality observations according to the number of parameters reported outside the specified acceptable ranges. A total of 46 observations (38.33%) showed all measured parameters within the specified ranges, while 32 (26.67%) had one parameter outside the range and 24 (20%) had two parameters outside the range. The remaining 18 observations (15%) had three or more parameters outside the specified ranges. This distribution suggests that water-quality conditions varied across the observations. Such variation is important because water-quality parameters can influence fish physiology and modify the effects of chemical exposure. However, the specific water-quality measurements, acceptable ranges, and treatment-wise results are required to identify which parameters changed and how those changes may have influenced the hepatic and renal biochemical responses.

7. CONCLUSION

Chemical fertilisers may affect freshwater ecosystems through nutrient enrichment and changes in nitrogen chemistry, potentially influencing the physiological condition of aquatic organisms; the present study provides a comprehensive framework for examining hepatic and renal biochemical responses in *Cirrhinus mrigala* using a sample of 150 fish distributed across a control group and three exposure groups, as demonstrated by the frequency–percentage tables that illustrate experimental allocation, body-weight categories, biochemical response patterns, and potential differences among exposure groups, while underscoring the necessity of laboratory measurements, validated response criteria, and appropriate statistical testing to establish the presence and magnitude of actual biochemical effects;

future investigations should identify the exact fertiliser formulation, apply scientifically justified exposure concentrations, monitor relevant water-quality parameters, and integrate biochemical assessments with suitable physiological or histological observations to enhance understanding of the impacts of agricultural inputs on freshwater fish health and to support more effective management of freshwater ecosystems.

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