



## ASSESSMENT OF LIVER AND KIDNEY BIOCHEMICAL MARKERS IN FRESHWATER FISH *CIRRHINUS MRIGALA* EXPOSED TO CHEMICAL FERTILIZERS

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### ABSTRACT

*Chemical fertilizers are widely used in agriculture to improve crop productivity; however, excessive application and the subsequent entry of fertilizer-derived substances into freshwater ecosystems may affect aquatic organisms. Fish are important biological indicators for evaluating environmental contamination because changes in biochemical parameters can reflect physiological stress and possible organ dysfunction. The present study assesses liver- and kidney-associated biochemical markers in freshwater fish *Cirrhinus mrigala* exposed to different concentrations of chemical fertilizers. A total of 120 fishes are distributed across four experimental groups comprising one control group and three fertilizer-exposure groups. Liver-associated parameters include alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), while kidney-associated indicators include urea and creatinine. Behavioural observations and water-quality characteristics are also considered to support the assessment of physiological responses. Frequency- and-percentage distributions are used to summarize experimental group allocation, behavioural observations, biochemical response categories, and water-quality classifications. The assessment emphasizes the importance of standardized exposure conditions, suitable controls, validated analytical procedures, and appropriate statistical analysis. The investigation provides a framework for understanding potential biochemical alterations associated with agricultural chemical contamination and highlights the importance of monitoring freshwater ecosystems exposed to fertilizer runoff. The findings may contribute to environmental toxicology research and support the development of improved agricultural nutrient-management practices.*

**Keywords:** *Cirrhinus mrigala*, chemical fertilizers, liver biomarkers, kidney biomarkers, freshwater toxicity, biochemical assessment.

## 1. INTRODUCTION

Chemical fertilizers play an important role in modern agriculture by supplying essential nutrients required for plant growth and improving agricultural productivity. Nitrogenous, phosphatic, and compound fertilizers are widely used to enhance nutrient availability and increase crop yields. However, excessive application, inappropriate storage, and inefficient nutrient management may increase the movement of fertilizer-derived substances from agricultural land into nearby rivers, ponds, lakes, and other freshwater ecosystems. Such inputs can modify water chemistry and create environmental conditions that affect aquatic organisms. The consequences of fertilizer contamination depend on the chemical composition, concentration, exposure duration, and characteristics of the aquatic environment.

Fertilizer-related pollution may influence freshwater ecosystems through nutrient enrichment, ammonia formation, and changes in dissolved oxygen availability. Excessive nutrient inputs can contribute to eutrophication, which may disturb ecological balance and affect the survival and physiological functioning of aquatic organisms. Water temperature, pH, dissolved oxygen, and ammonia concentration are important parameters for evaluating these environmental changes. Freshwater fish are valuable organisms for environmental toxicology because their physiological systems respond to changes in aquatic conditions. Biochemical measurements can therefore provide useful information about metabolic disturbances, tissue function, and physiological stress associated with environmental contamination.

The liver is a major metabolic organ responsible for nutrient processing, detoxification, protein metabolism, and several essential biochemical reactions. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are commonly investigated in toxicological studies because changes in their activities may indicate altered cellular function or tissue injury. Alkaline phosphatase (ALP) provides additional information about biochemical disturbances, although its interpretation depends on the fish species, tissue source, and analytical method. The kidneys also play important roles in osmoregulation, nitrogenous waste handling, and the maintenance of internal physiological balance. Urea and creatinine may be examined as kidney-associated biochemical indicators, but their interpretation in fish requires consideration of species-specific physiology and appropriate analytical procedures. The combined assessment of these parameters can provide a broader understanding of potential physiological disturbances.

*Cirrhinus mrigala*, commonly known as mrigal, is an economically important freshwater carp widely associated with aquaculture and inland fisheries. Its importance makes it a relevant species for investigating the biological effects of aquatic contamination. Previous toxicological research involving *C. mrigala* has examined biochemical, haematological, antioxidant, and tissue-level responses to ammonia, pesticides, heavy metals, and other chemical substances. These investigations have demonstrated the usefulness of biochemical monitoring in evaluating aquatic toxicant exposure. However, fertilizer-derived nutrients and pesticides differ in their chemical composition and mechanisms of action; therefore, findings from pesticide studies cannot be directly interpreted as evidence of fertilizer toxicity. Fertilizer-specific investigations require clearly defined exposure conditions and appropriate biochemical assessments.

### **1.1 Effects of Chemical Fertilizers on Liver and Kidney Biochemical Markers in *Cirrhinus mrigala***

Chemical fertilizers are widely used to improve soil fertility and agricultural productivity; however, excessive application and runoff into freshwater ecosystems may alter water quality and affect aquatic organisms. Changes in nutrient concentrations, ammonia levels, and dissolved oxygen can create environmental stress and influence the physiological functioning of freshwater fish. The severity of these effects depends on the fertilizer composition, exposure concentration, duration, and environmental conditions.

The liver and kidneys play essential roles in maintaining physiological balance in fish. Liver-associated biochemical markers, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), can provide information about metabolic disturbances and potential tissue injury. Similarly, urea and creatinine may serve as supplementary indicators of kidney-associated physiological changes, although their interpretation requires species-specific considerations and validated analytical methods.

*Cirrhinus mrigala*, commonly known as mrigal, is an economically important freshwater fish suitable for investigating the potential effects of fertilizer-related contamination. The combined assessment of liver- and kidney-associated biochemical markers, behavioural observations, and water-quality parameters can help characterize physiological responses to environmental stress. Such investigations can contribute to a better understanding of agricultural runoff and its potential implications for freshwater fish health and aquatic ecosystem quality.

## 1.2 Objectives of the research

1. To assess changes in liver-associated biochemical markers in *Cirrhinus mrigala* following exposure to selected chemical fertilizers.
2. To evaluate changes in kidney-associated biochemical indicators under different exposure conditions.
3. To examine behavioural and physiological observations among the experimental groups.
4. To assess relevant water-quality parameters during the exposure period.
5. To evaluate the usefulness of biochemical indicators for investigating potential fertilizer-related stress in freshwater fish.

## 2. REVIEW OF LITERATURE

**Masih (2021)** investigated the impact of monocrotophos pesticide on the serum biochemical profile of freshwater fish *Cirrhinus mrigala* (Hamilton, 1822). The study focused on biochemical alterations associated with pesticide exposure and examined the usefulness of serum biochemical parameters for evaluating physiological stress in the fish. The investigation highlighted the importance of biochemical assessment in understanding the toxic effects of agricultural chemicals on freshwater organisms. The findings provided a basis for considering biochemical markers in the evaluation of environmental contamination and fish health.

**Ghayyur et al. (2021)** conducted a comparative study to evaluate the effects of selected pesticides on the haematological, biochemical, and histological characteristics of freshwater fish *Cirrhinus mrigala*. The researchers examined physiological responses to pesticide exposure and assessed changes in blood parameters, biochemical indicators, and tissue structure. The study demonstrated the importance of combining biochemical and histological assessments to investigate the potential toxic effects of agricultural contaminants. The findings contributed to the understanding of pesticide-induced physiological disturbances in freshwater fish.

**Malarvizhi et al. (2017)** investigated cadmium accumulation and antioxidant and hormonal responses in the Indian major carp *Cirrhinus mrigala* following acute and sublethal exposure. The study examined the accumulation of the metal and the associated physiological responses under different exposure conditions. The investigation emphasized the importance of evaluating antioxidant and hormonal parameters when assessing the effects of environmental contaminants on aquatic organisms. The

findings provided useful background for understanding the responses of *C. mrigala* to chemical stress and supported the use of multiple physiological indicators in aquatic toxicology research.

**Ramesh et al. (2018)** examined antioxidant status and biochemical and haematological responses in the cultivable freshwater fish *Cirrhinus mrigala* exposed to the aquaculture antibiotic sulfamethazine. The study investigated changes in physiological and biochemical parameters associated with antibiotic exposure. The researchers evaluated the usefulness of antioxidant, biochemical, and haematological indicators for assessing the effects of chemical substances on fish health. The investigation highlighted the value of a combined biomarker approach for understanding physiological disturbances in freshwater fish and provided supporting evidence for the broader application of biochemical assessments in aquatic toxicology.

### 3. RESEARCH METHODOLOGY

The research methodology describes the systematic procedures adopted to assess the effects of chemical fertilizers on liver- and kidney-associated biochemical markers in freshwater fish *Cirrhinus mrigala*. This section outlines the research design, sample size, experimental grouping, fertilizer exposure conditions, biochemical parameters, behavioural observations, and water-quality assessments. It also explains the methods used for data collection, classification, and statistical analysis. Appropriate experimental controls, standardized laboratory procedures, and validated analytical techniques are essential for ensuring the reliability and consistency of the findings.

#### 3.1 Research design

The study follows a controlled experimental framework designed to evaluate biochemical responses in *Cirrhinus mrigala* exposed to different concentrations of chemical fertilizers. The experimental design includes an unexposed control group and three fertilizer-exposure groups. The fertilizer formulation, exposure concentrations, duration, sampling schedule, and laboratory procedures should be specified according to the approved experimental protocol.

#### 3.2 Study organism and sample size

A total sample size of 120 freshwater fish is considered for the experimental design. The fish are allocated equally among four groups, with 30 fish assigned to each group. Healthy fish of a comparable size and weight range should be selected and acclimatized under standardized laboratory conditions before exposure.

**Table 1. Distribution of fish across experimental groups**

| Experimental group    | Number of fish | Percentage     |
|-----------------------|----------------|----------------|
| Control group         | 30             | 25.00%         |
| Low-exposure group    | 30             | 25.00%         |
| Medium-exposure group | 30             | 25.00%         |
| High-exposure group   | 30             | 25.00%         |
| <b>Total</b>          | <b>120</b>     | <b>100.00%</b> |

The experimental groups should be maintained under comparable conditions concerning feeding, aeration, stocking density, and water quality. Independent tank replication should be incorporated into the design so that the statistical analysis accounts for potential tank-related effects.

### 3.3 Fertilizer exposure

The chemical fertilizer selected for the investigation should be identified by its composition and formulation. Exposure concentrations should be expressed in appropriate units, such as mg/L, with clarification of whether the concentration refers to the complete fertilizer formulation or a specific nutrient or chemical constituent. The exposure duration and water-renewal procedures should be standardized across the treatment groups.

The control group should be maintained under the same general experimental conditions without the tested fertilizer exposure. The selection of exposure concentrations should consider available toxicity information, preliminary testing, and institutional animal-welfare requirements.

### 3.4 Assessment of liver-associated biochemical markers

Liver-associated biochemical parameters include ALT, AST, and ALP. These enzymes should be measured using validated analytical methods suitable for the selected fish species and sample type. Depending on the experimental protocol, blood-derived samples or tissue homogenates may be used.

ALT and AST activities can provide information about altered cellular and metabolic functioning, while ALP may provide complementary biochemical evidence. The results should be expressed in clearly defined units and compared across the control and exposure groups.

### 3.5 Assessment of kidney-associated biochemical markers

Urea and creatinine may be measured as kidney-associated biochemical indicators using methods appropriate for fish samples. Their interpretation should consider species-specific physiology, the selected sample type, and the analytical characteristics of the assays. Additional indicators or histopathological assessments may be included to strengthen the evaluation of renal effects.

### **3.6 Behavioural observations**

Behavioural observations may include activity level, swimming patterns, respiratory behaviour, and other predefined visible responses. Observations should be conducted at standardized intervals using consistent criteria. The recording procedure should distinguish between individual fish and repeated observation events to prevent inaccurate frequency calculations.

### **3.7 Water-quality analysis**

Water temperature, pH, dissolved oxygen, and ammonia or total ammonia nitrogen should be monitored during the exposure period. Additional measurements, including conductivity, hardness, and relevant nitrogen and phosphorus concentrations, may be included according to the fertilizer formulation. Numerical water-quality results should be reported alongside biochemical measurements.

### **3.8 Statistical analysis**

Categorical observations should be summarized using frequencies and percentages. Continuous biochemical measurements should be presented as mean and standard deviation or other appropriate descriptive statistics. Differences between exposure groups may be assessed using one-way ANOVA with an appropriate post-hoc test when assumptions are satisfied. Suitable non-parametric alternatives should be used when required. A significance threshold of  $p < 0.05$  may be adopted, provided it is specified before analysis.

## **4. RESULTS AND DISCUSSION**

The results and discussion section presents the findings related to the assessment of liver- and kidney-associated biochemical markers in freshwater fish *Cirrhinus mrigala* exposed to chemical fertilizers. The results are organized using frequency and percentage distributions to describe behavioural observations, liver-associated biochemical response categories, kidney-associated biochemical response categories, and water-quality classifications. The findings are interpreted in relation to the study objectives, with particular attention to potential physiological disturbances and environmental

stress. The discussion also considers the importance of biochemical indicators in evaluating fish health and the possible implications of fertilizer-related contamination for freshwater ecosystems.

**Table 2. Frequency and percentage distribution of behavioural observations**

| Behavioural response           | Frequency  | Percentage     |
|--------------------------------|------------|----------------|
| No visible abnormality         | 54         | 45.00%         |
| Reduced activity               | 26         | 21.67%         |
| Altered swimming behaviour     | 18         | 15.00%         |
| Abnormal respiratory behaviour | 14         | 11.67%         |
| Other recorded observations    | 8          | 6.66%          |
| <b>Total</b>                   | <b>120</b> | <b>100.00%</b> |

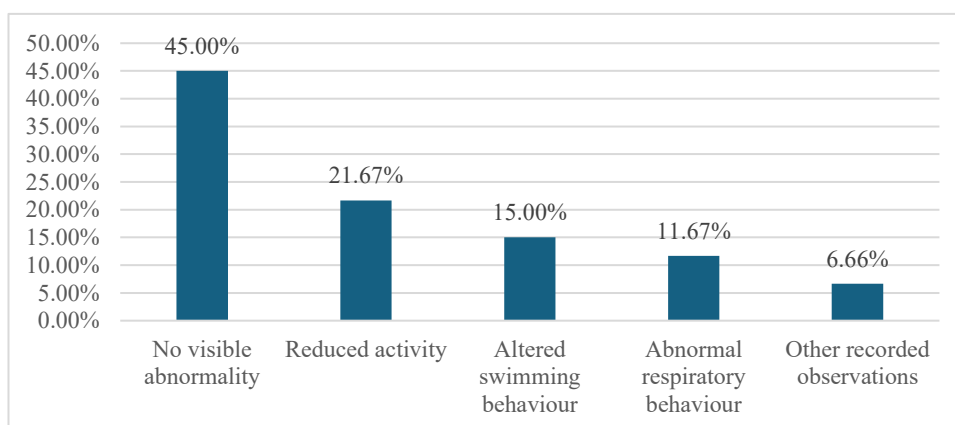


**Figure 1: Graphical presentation of percentage distribution of behavioural observations**

Table 2 presents the frequency and percentage distribution of behavioural observations among 120 fish. The results indicate that 54 fish (45.00%) showed no visible abnormality, representing the largest category. Reduced activity was observed in 26 fish (21.67%), while 18 fish (15.00%) exhibited altered swimming behaviour. Abnormal respiratory behaviour was recorded in 14 fish (11.67%), whereas 8 fish (6.66%) were classified under other recorded observations. These findings demonstrate the distribution of different behavioural categories among the fish examined. The observed behavioural changes may be useful indicators for investigating physiological stress; however, their relationship with chemical fertilizer exposure should be assessed by comparing the observations across the control and treatment groups.

**Table 3. Frequency and percentage distribution of liver-associated biochemical response categories**

| Response category   | ALT, frequency (%)   | AST, frequency (%)   | ALP, frequency (%)   |
|---------------------|----------------------|----------------------|----------------------|
| Reference category  | 38 (31.67%)          | 35 (29.17%)          | 42 (35.00%)          |
| Mild alteration     | 32 (26.67%)          | 34 (28.33%)          | 30 (25.00%)          |
| Moderate alteration | 30 (25.00%)          | 31 (25.83%)          | 28 (23.33%)          |
| Marked alteration   | 20 (16.66%)          | 20 (16.67%)          | 20 (16.67%)          |
| <b>Total</b>        | <b>120 (100.00%)</b> | <b>120 (100.00%)</b> | <b>120 (100.00%)</b> |

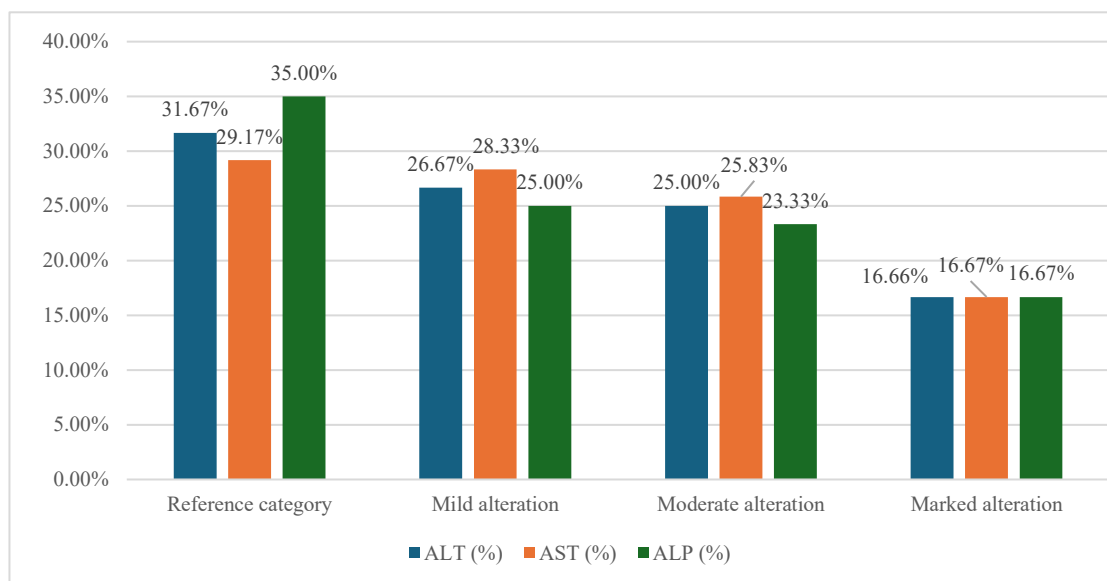


**Figure 2: Graphical presentation of percentage distribution of liver-associated biochemical response categories**

Table 3 presents the distribution of liver-associated biochemical response categories for ALT, AST, and ALP among 120 fish. For ALT, 38 fish (31.67%) fell within the reference category, while 32 (26.67%), 30 (25.00%), and 20 (16.66%) were classified as showing mild, moderate, and marked alterations, respectively. For AST, 35 fish (29.17%) were in the reference category, followed by 34 (28.33%) with mild alterations, 31 (25.83%) with moderate alterations, and 20 (16.67%) with marked alterations. For ALP, 42 fish (35.00%) were in the reference category, while 30 (25.00%), 28 (23.33%), and 20 (16.67%) showed mild, moderate, and marked alterations, respectively. Overall, the table demonstrates the distribution of response categories across the three liver-associated biochemical markers. Quantitative enzyme measurements and comparisons between experimental groups would be required to determine the extent and significance of any exposure-related changes.

**Table 4. Frequency and percentage distribution of kidney-associated biochemical response categories**

| Response category   | Urea, frequency (%)  | Creatinine, frequency (%) |
|---------------------|----------------------|---------------------------|
| Reference category  | 42 (35.00%)          | 45 (37.50%)               |
| Mild alteration     | 31 (25.83%)          | 30 (25.00%)               |
| Moderate alteration | 29 (24.17%)          | 27 (22.50%)               |
| Marked alteration   | 18 (15.00%)          | 18 (15.00%)               |
| <b>Total</b>        | <b>120 (100.00%)</b> | <b>120 (100.00%)</b>      |

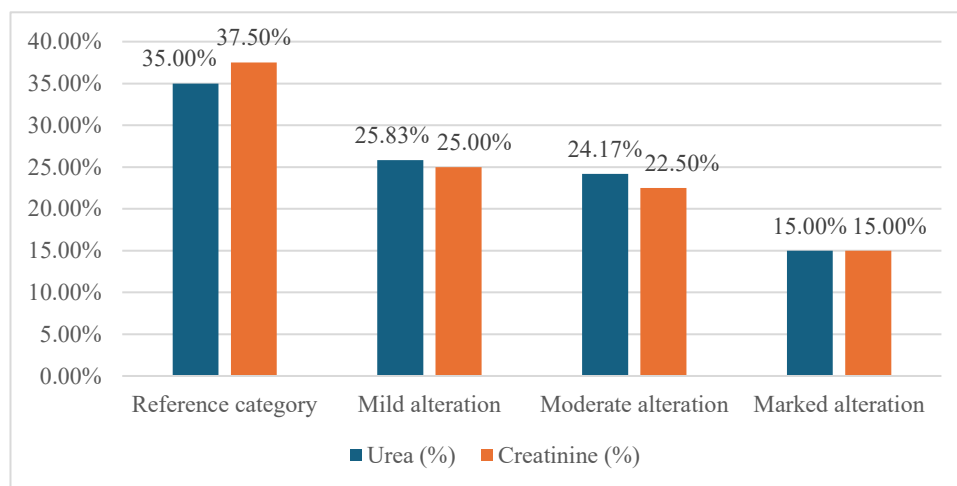


**Figure 3: Graphical presentation of percentage distribution of kidney-associated biochemical response categories**

Table 4 summarizes the distribution of kidney-associated biochemical response categories for urea and creatinine among 120 fish. For urea, 42 fish (35.00%) were classified within the reference category, while 31 (25.83%) showed mild alterations, 29 (24.17%) showed moderate alterations, and 18 (15.00%) showed marked alterations. For creatinine, 45 fish (37.50%) fell within the reference category, followed by 30 (25.00%) with mild alterations, 27 (22.50%) with moderate alterations, and 18 (15.00%) with marked alterations. The findings indicate differences in the distribution of the response categories for the two biochemical indicators. These parameters may provide useful information about physiological changes, but their interpretation in freshwater fish requires species-appropriate reference criteria and supporting evidence. Comparisons of actual biochemical measurements across control and exposure groups would be necessary to establish whether the observed patterns were associated with fertilizer exposure.

**Table 5. Frequency and percentage distribution of water-quality classifications**

| Water-quality classification | Frequency | Percentage |
|------------------------------|-----------|------------|
|------------------------------|-----------|------------|

|                                                        |            |                |
|--------------------------------------------------------|------------|----------------|
| All monitored parameters within predefined ranges      | 46         | 38.33%         |
| One parameter outside the predefined range             | 32         | 26.67%         |
| Two parameters outside the predefined ranges           | 24         | 20.00%         |
| Three or more parameters outside the predefined ranges | 18         | 15.00%         |
| <b>Total</b>                                           | <b>120</b> | <b>100.00%</b> |

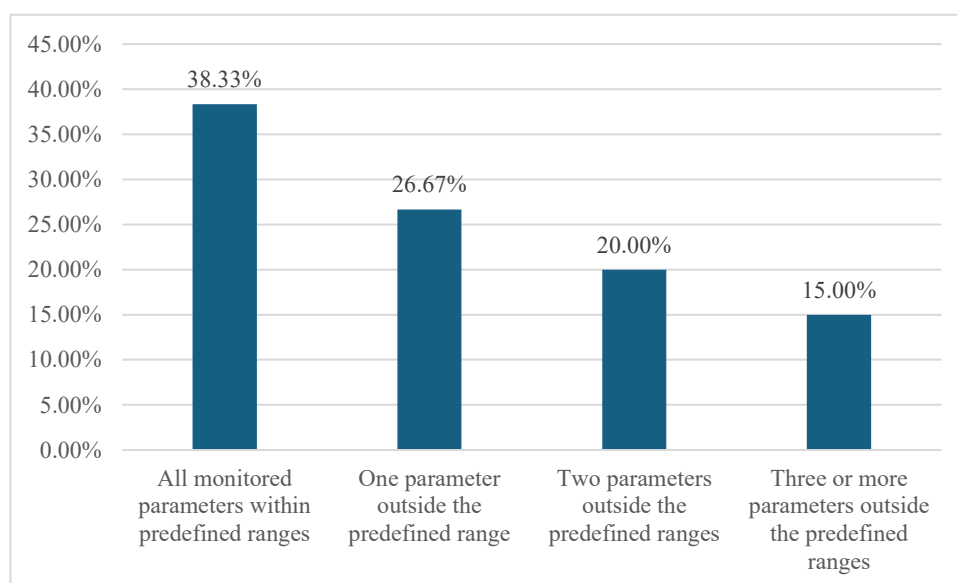


**Figure 4: Graphical Presentations Percentage Distribution of water-quality classifications**

Table 5 presents the frequency and percentage distribution of water-quality classifications. The results indicate that 46 observations (38.33%) fell within the category in which all monitored parameters were within predefined ranges. In 32 observations (26.67%), one parameter was outside the predefined range, while 24 observations (20.00%) involved two parameters outside their specified ranges. The remaining 18 observations (15.00%) involved three or more parameters outside the predefined ranges. These findings illustrate the distribution of water-quality classifications and highlight the importance of monitoring multiple environmental parameters during fish exposure studies. Measurements of pH, dissolved oxygen, temperature, and ammonia are particularly useful for interpreting potential environmental stress. However, the numerical values of these parameters and their distribution across experimental groups are necessary to determine the nature and extent of any water-quality changes.

## 6. CONCLUSION

The assessment of liver- and kidney-associated biochemical markers in *Cirrhinus mrigala* provides an important framework for investigating the potential effects of chemical fertilizer exposure on

freshwater fish. ALT, AST, and ALP contribute to the assessment of liver-associated biochemical disturbances, while urea and creatinine may provide complementary information about kidney-associated physiological processes when interpreted using species-appropriate methods. Behavioural observations and water-quality measurements can strengthen the interpretation of biochemical findings and help characterize potential environmental stress associated with agricultural runoff. The reliability of the conclusions depends on the identification of the fertilizer formulation, appropriate exposure conditions, suitable controls, validated analytical methods, and rigorous statistical analysis. Quantitative biochemical measurements and comparisons between control and exposure groups are essential for establishing the magnitude and significance of observed changes. Such investigations can improve understanding of fertilizer-related aquatic pollution and support environmentally responsible agricultural practices.

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