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Evaluation of Morphological and Behavioral Alterations in *Clarias Batrachus* following Acute Zinc Exposure

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Abstract: Heavy metal contamination poses a significant threat to freshwater ecosystems. Zinc an essential trace element, exhibits toxicity at elevated concentrations, adversely affecting physiological, morphological, and behavioral responses in aquatic organisms. The present study aimed to investigate the dose-dependent morphological and behavioral alterations in freshwater fish *Clarias batrachus* during a 96-hour acute toxicity exposure to zinc sulphate ($ZnSO_4 \cdot 7H_2O$). Fish were exposed to graded concentrations ranging from 50 to 250 mg/L under static renewal conditions. Morphological changes observed included excessive mucus secretion, skin discoloration, fin erosion, and gill necrosis, which intensified with increasing concentrations. Behavioral anomalies such as hyperactivity, erratic swimming, surface respiration, spiralling, and loss of equilibrium emerged as early indicators of sublethal stress. The severity of both morphological and behavioral responses showed a direct correlation with zinc concentration and exposure duration. These changes suggest systemic physiological disturbances, including respiratory and neurological dysfunction. The study demonstrates that both morphological and behavioral parameters serve as reliable biomarkers of zinc toxicity, offering valuable insights into the health status of aquatic organisms under environmental stress. The findings contribute to environmental toxicology by supporting the use of *C. batrachus* as a sentinel species for monitoring heavy metal contamination and establishing safe ecological thresholds.

Keywords: *Acute Toxicity, Clarias batrachus, Zinc Sulphate, LC_{50}, Morphological Changes, Behavioral Responses, Sublethal Effects, Biomarkers.*

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I. INTRODUCTION

Freshwater ecosystems are increasingly under threat due to anthropogenic pollutants, particularly heavy metals which have long-term ecological consequences. Among these, zinc, although an essential micronutrient in trace amounts, can exhibit toxic effects when present in elevated concentrations. Excess zinc in aquatic environments, introduced through industrial discharges, agricultural runoff, and domestic effluents, leads to severe physiological stress in fish, affecting survival, reproduction, and behavior [1][2].

Fish are highly suitable bioindicators of aquatic pollution due to their direct interaction with the environment and sensitivity to toxicants. Toxic exposure can lead to a variety of sublethal responses, of which morphological and behavioral alterations are considered early warning indicators of environmental stress [3][4]. Morphological symptoms such as fin erosion, gill necrosis, mucus hypersecretion, and body discoloration indicate damage to the integumentary and respiratory systems [5][6]. These impairments can significantly compromise the fish's ability to maintain homeostasis, respiration, and osmoregulation [7]. In parallel, behavioral disturbances such as erratic swimming, surface respiration (piping), loss of equilibrium, and lethargy are commonly observed under sublethal heavy metal stress [8][9]. Such behaviors arise from neurophysiological and respiratory disruptions and are often the first observable responses to toxic insult [10]. In particular, surface gulping and spiralling behavior have been associated with impaired gill function and oxygen uptake in metal-exposed fish [11].

Clarias batrachus, commonly known as the walking catfish, is a hardy, air-breathing freshwater fish widely distributed across South Asia and extensively used in aquaculture due to its adaptability, fast growth, and high market value [12][13]. Its ability to survive in hypoxic and polluted conditions makes it particularly useful in ecotoxicological assessments [14]. Due to its ecological and commercial relevance, as well as its sensitivity to waterborne

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toxicants *C. batrachus* serves as an ideal model organism in toxicological studies [15]. Several investigations have documented hematological, histopathological, enzymatic, and behavioral alterations in this species following exposure to heavy metals such as cadmium, copper, lead, and zinc [16][17][18]. Zinc exposure, in particular, has been linked to gill tissue damage, skin discoloration, excessive mucus secretion, and behavioral anomalies such as hyperactivity, erratic swimming, and spiralling in *C. batrachus*, reinforcing its value as a sensitive bioindicator for sublethal aquatic toxicity [9][19].

Although numerous studies have reported LC_{50} values for zinc sulphate in *C. batrachus*, most have primarily emphasized mortality-based endpoints without adequately investigating sublethal indicators such as morphological and behavioral changes. For instance, Kumar and Kumar (2013) reported a 96-hour LC_{50} value of 7.5 mg/L [15], while Joshi (2011) found a higher value of 17.22 mg/L using sigmoid curve analysis [9]. Gupta et al. (2009) observed an even lower LC_{50} of 4.1 mg/L, indicating variability across experimental setups [20]. Das and Das (2012) reported a significantly higher LC_{50} of 1.75 g/L under different physicochemical conditions [21]. Variations in these values can be attributed to differences in water quality parameters, fish age, size, health status, and methodological approaches used in each study. While LC_{50} assessments are valuable for identifying lethal thresholds, they often overlook early sublethal stress markers such as fin erosion, gill necrosis, excessive mucus secretion, erratic swimming, spiralling, and surface respiration all of which provide more sensitive and ecologically relevant indicators of toxicity [5][11]. Therefore, there is need for focusing on detailed morphological and behavioral changes during acute exposure, which can offer early diagnostic cues and improve ecological risk assessments for aquatic toxicants like zinc.

The present study was therefore conducted to evaluate the dose-dependent morphological and behavioral changes in *Clarias batrachus* during a 96-hour acute toxicity test with zinc sulphate ($ZnSO_4 \cdot 7H_2O$). Fish were exposed to a range of sublethal concentrations, and observable changes were systematically documented.

II. MATERIAL AND METHOD

➢ *Experimental Animal:*

Clarias batrachus with an average weight 18–22 g and length 10–12 cm was procured from a local fish farm in Patna, Bihar (India). Fish were acclimatized in laboratory conditions for 15–20 days prior to experimentation in glass aquaria containing dechlorinated tap water. Acclimatization tanks were maintained under continuous aeration, and fish were fed commercial pellets at 2% of their body weight until 24 hours prior to exposure.

➢ *Test Chemical and Water Quality Parameters:*

Zinc sulphate heptahydrate ($ZnSO_4 \cdot 7H_2O$) was selected as the test chemical for this study due to its solubility and widespread environmental relevance [22][23]. The physico-chemistry of water i.e., temperature, pH, dissolved oxygen was analysed as per the standard protocol of APHA (2017) [24].

➢ *Experimental Design and Acute Toxicity Exposure Protocol:*

The acute toxicity test was conducted over a 96-hour period using a static renewal system, following guidelines of OECD (2019) [25]. To determine the most suitable concentrations for zinc sulphate ($ZnSO_4 \cdot 7H_2O$) exposure, a range-finding test was initially performed in triplicate. Based on the observed mortality patterns across varying concentrations, five definitive doses were selected: 50, 100, 170, 190, and 250 mg/L. A control group was maintained in dechlorinated tap water without any chemical exposure.

Each experimental group consisted of 10 fish placed in 10 liters of test solution. To minimize confounding factors and ensure consistent toxicant levels, 50% of the test solution was renewed every 24 hours. Fish were not fed during the experimental period to prevent degradation of water quality. Mortality was recorded at 24-hour intervals, and all abnormal morphological and behavioral responses were monitored and documented systematically across each concentration group.

➢ *Morphological and Behavioral Assessments:*

A standardized observational chart was maintained to document morphological and behavioral responses in each test group.

- *Parameters Observed Included:*

✓ *Mucus Production:* Excessive mucus secretion on skin and gills was recorded as an indicator of epithelial irritation and gill dysfunction.
✓ *Gill Appearance:* Daily checks for pallor, hyperaemia, and necrosis were performed to identify structural damage.
✓ *Fin and Body Integrity:* Fin erosion, skin lesions, and body ulcers were noted as morphological indicators of stress.
✓ *Swimming Behavior:* Abnormal locomotion patterns such as hyperactivity, erratic swimming, spiralling, and lethargy were assessed.
✓ *Respiratory Response:* Increased opercular movements and surface respiration ("piping") were considered signs of respiratory distress.
✓ *Response to External Stimuli:* Reflex testing using gentle prodding assessed neuromotor coordination and alertness.

Behavioral and morphological anomalies were evaluated in reference to the control group to determine dose-dependent variations.

➢ *Data Documentation:*

Photographic documentation was performed daily to visually record changes in fish appearance, gill structure, and swimming posture. These visual records were used alongside written logs to enhance the objectivity of the morphological assessments.

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III. RESULTS AND DISCUSSION

During the 96-hour LC_{50} exposure with varying concentrations of zinc sulphate, *C. batrachus* exhibited significant morphological and behavioral changes. These changes serve as early indicators of zinc toxicity and align with existing research on heavy metal exposure in fish species. Specific observation has been mentioned in the Table 1.

Table 1 Behavioural and morphological effects of zinc sulphate *C. batrachus* (Linn.)

Dose (mg/L)	**Behavioural changes**	**Morphological changes**
Upto 50	No visible changes	No visible changes
Upto 100	Startle response, hyperactive, increased swimming rate, tendency to jump out of the tank	Slight discoloration
Upto 170	Hypoactive, erratic swimming, frequent surfacing	Mild discoloration, body appeared covered with thin white film
Upto 190	Significant reduction in movement, loss of equilibrium	Severe discoloration, pronounced fin erosion
Upto 250	Immobile, loss of reflexes, gasping at the surface	Extensive haemorrhaging, fin erosion, bloated abdomen

Fig 1 Morphological and Behavioural Changes in Freshwater Fish *Clarias batrachus* upon Zinc Sulphate Exposure over a 96-Hour Acute Toxicity Test.

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Acute exposure to zinc sulphate induced marked morphological and behavioral alterations in *C. batrachus*, increasing in severity with concentration and time. Excessive mucus secretion on skin and gills was one of the earliest responses, consistent with Fafioye et al. (2004) [6], likely serving as a barrier but impairing respiration. Skin discoloration and pigment loss, observed at higher doses, aligned with stress-induced dyspigmentation reported by Mishra and Mohanty (2008) and Hoseini et al. (2016) [17][26].

Gill damage, including discoloration, swelling, and necrosis, was concentration-dependent and confirmed gill sensitivity to metal exposure [1][5][27]. Fin erosion and body ulceration observed at 170 mg/L and above indicated progressive epithelial damage [7].

Behaviourally, fish initially exhibited hyperactivity, erratic swimming, and surface respiration, indicative of acute stress and gill dysfunction [2][11]. At higher doses (≥190 mg/L), hypoactivity, loss of equilibrium, spiralling, and vertical floating emerged, suggesting neurotoxicity [9]. Final stages showed immobility, haemorrhaging, and bloating symptoms of systemic failure similar to those reported by Kumar and Kumar (2013) and Nikam (2012) [15][28].

The progressive shift from initial alarm behaviors to exhaustion phases reflects classic acute toxicity stress [10]. The dose-dependent responses validate that both morphological and behavioral changes serve as sensitive, early indicators of zinc toxicity in aquatic environments.

IV. CONCLUSION

The current investigation demonstrated that *C. batrachus* exhibits distinct and progressive morphological and behavioral disturbances during acute exposure to zinc sulphate. The responses ranged from mucus hypersecretion and gill damage to hyperactivity, surface respiration, and neurological dysfunction. These changes intensified with increasing concentration and duration of exposure, reinforcing their value as early biomarkers of heavy metal toxicity. The study emphasizes the necessity of incorporating both morphological and behavioral endpoints in environmental monitoring protocols to ensure early detection of aquatic contamination and to protect freshwater biodiversity.

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➢ *Conflict of Interest*

The authors declare no conflict of interest.

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