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Effect of Diammonium Phosphate (DAP) on Ecology, Behaviour, and Habitat Selection in *Channa punctatus* (Bloch, 1793)

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Abstract: ***Diammonium phosphate (DAP), a widely used nitrogen–phosphorus fertilizer, enhances agricultural productivity but also poses serious ecological risks when leached into freshwater ecosystems. Water, being one of the most essential natural resources, is highly susceptible to such contamination. In the present study, the effects of DAP on water quality, behavioural responses, and habitat selection of the freshwater fish Channa punctatus (Bloch) were evaluated. Prior to experimentation, all fish were acclimatized for 15 days under laboratory conditions to minimize stress and ensure uniform physiological stability. Fish were then exposed for 96 hours to four DAP concentrations: Control (0 g/L), Low dose (0.4 g/L), Moderate dose (0.8 g/L), and High dose (2.0 g/L) in separate 20 L aquaria. The LC_{50} value for DAP was estimated at 4 g/L. Water quality analysis revealed a dose-dependent decrease in dissolved oxygen and pH, along with an increase in salinity. Behavioural alterations such as erratic swimming, surfacing, restlessness, loss of equilibrium, and excessive mucus secretion were most pronounced in the high-dose group. Fish also exhibited habitat avoidance behaviour, preferring less contaminated zones. These results demonstrate that short-term DAP exposure causes marked deterioration in water quality and induces behavioural and ecological disturbances in Channa punctatus, indicating the potential ecological hazards of fertilizer runoff in freshwater ecosystems. At the same time, the findings contribute to developing strategies for effective management and conservation of the environment.***

Keyword: Toxicity, DAP, *Channa punctatus*, behavioural changes, freshwater ecology

1. Introduction

Chemical fertilizers are indispensable for sustaining global agricultural productivity; however, their excessive and unregulated use has emerged as a major environmental concern. Among commonly used fertilizers, Diammonium Phosphate (DAP) is extensively applied because of its high nitrogen and phosphorus content (Das & Sharma, 2022). When such fertilizers are transported into aquatic ecosystems through agricultural runoff, they promote nutrient enrichment, eutrophication, and oxygen depletion, thereby disturbing the ecological balance and threatening aquatic biodiversity (Yusuf & Kwayu, 2020). Fish are highly sensitive indicators of aquatic contamination, and their behavioural alterations provide early and reliable signals of environmental stress (Kumar, Gupta, & Tiwari, 2017; Verma & Mishra, 2021). Exposure to toxicants often manifests as irregular swimming, loss of equilibrium, hyperactivity, surfacing, and increased opercular movement (Rani & Datta, 2013; Das & Sharma, 2022). Such behavioural endpoints are valuable biomarkers for assessing sublethal toxicity and environmental quality (Suman, Prasad, & Chand, 2024). *Channa punctatus* (Bloch, 1793), a widely distributed freshwater fish in India, has been recognized as an efficient bioindicator owing to its ecological significance, wide adaptability, and sensitivity to pollutants (Yadav, Singh, & Thakur, 2020; Butle et al., 2024). Its feeding and habitat preferences are also influenced by water quality variations and contaminant exposure (Baghel, Prashad, & Toppo, n. d.). Previous research has primarily focused on the physiological and biochemical effects of fertilizers such as urea and ammonium nitrate on fish (Kumar et al., 2017; Verma & Mishra, 2021). However, limited information is available on the behavioural and ecological consequences of DAP exposure in freshwater species. Excessive DAP runoff contributes to nutrient enrichment, algal blooms, and ecological imbalance in freshwater habitats. Therefore, the present study aims to investigate the effects of varying concentrations of DAP on water quality, behavioural responses, and habitat preferences of *Channa punctatus* under controlled laboratory conditions. The findings will help elucidate the sublethal impacts of fertilizer pollution and strengthen the use of *C. punctatus* as a reliable model for aquatic ecotoxicological assessment.

2. Materials and Methods

Experimental Animal

Live specimens of *Channa punctatus* (Bloch) were obtained from local freshwater sources in Bhagalpur district and brought to the Cytogenetics Laboratory, Department of Zoology, T. M. Bhagalpur University, Bhagalpur. Fish averaged 15 ± 5 cm in length and 60 ± 5 g in weight. To prevent infections and remove external parasites, all fish were briefly immersed in 0.5% potassium permanganate ($KMnO_4$) solution for 1 minutes before the start of the experiment. After disinfection, fish were transferred to glass aquaria measuring 76 cm × 38 cm × 38 cm, each with a 20 L water holding capacity.

Acclimatization

Fish were acclimatized for 15 days under controlled laboratory conditions to minimize stress and ensure proper adaptation. During acclimation:

- Water quality was maintained at 26 ± 2°C, pH 7.4± 0.2, and dissolved oxygen 7.86 ± 0.18 mg/L.
- Fish were fed boiled chicken eggs and mustard oil cake and soil mixed in 1: 2 ratio on alternate days.
- 50% of the aquarium water was replaced daily with fresh dechlorinated water.

- Any dead fish were immediately removed to maintain optimal water quality.

Experimental Design

After acclimatization, fish were exposed to Diammonium Phosphate (DAP) for 96 hours in four experimental groups:

Group	DAP Concentration (g/L)	Description
I	0.0	Control (no DAP)
II	0.4	Low dose
III	0.8	Moderate dose
IV	2.0	High dose

- Each group was housed in a 20 L glass aquarium, in triplicate.
- LC_{50} of DAP was determined to be 4 g/L.
- Water quality parameters (temperature, pH, dissolved oxygen, Free Carbon Dioxide (FCO_2), and Salinity) were measured every 24 hours.
- Behavioural responses (opercular movement, swimming pattern, surfacing, mucus secretion, equilibrium) were observed at 0, 24, 48, 72, and 96 hours.

Physicochemical properties of test water

Physicochemical parameters were measured before fish loading and during experimentation the temperature of test water varied from 28.4 ± 3°C and pH, dissolved oxygen (DO), and Free Carbon Dioxide (FCO_2) Salinity were monitored every 24 hours.

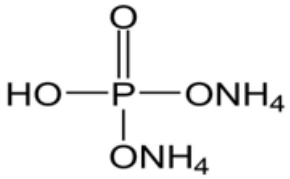

Figure 1: Structure of diammonium phosphate

Parameter	Description / Specification
Chemical / Product Name	Diammonium Phosphate
Common Name / Abbreviation	DAP
Chemical Formula	$(NH_4)_2HPO_4$
Molecular Weight	132.06 g/mol
Grade / Purity	Analytical grade (≥ 98%)
Supplier / Manufacturer	Commercial agricultural fertilizer obtained from a certified local supplier, Bhagalpur, Bihar (India)
Appearance	White to off-white crystalline or granular solid
Odor	Odorless
Solubility	Highly soluble in water
pH (1% aqueous solution)	7.2 – 8.0 (slightly alkaline)
Main Components	Nitrogen (as NH_4^+): 18%; Phosphorus (as P_2O_5): 46%
Functional / Alkyl Group	Contains ammonium (NH_4^+) and phosphate (HPO_4^{2-}) functional groups; no specific alkyl group present
Storage Conditions	Stored in airtight containers at room temperature to prevent moisture absorption
WHO Toxicity Class	Class III – Slightly hazardous (based on WHO classification of pesticides by hazard, adapted for fertilizer exposure limits)

3. Results

Exposure of *Channa punctatus* to different concentrations of diammonium phosphate (DAP) for 96 hours produced a clear, dose-dependent alteration in water quality and fish behaviour. In the control group, all physicochemical parameters—temperature, pH, dissolved oxygen, salinity, total dissolved solids, and free carbon dioxide (FCO_2) —remained stable and within normal freshwater limits, confirming suitable baseline conditions for fish survival (Table 2). At the low dose of 0.4 g/L, slight changes were detected, including a mild reduction in pH and dissolved oxygen, along with a small increase in salinity, conductivity, and FCO_2, reflecting the initial effect of DAP dissolution on the aquatic environment. Fish in this group exhibited minor behavioural deviations, such as slightly increased opercular activity and reduced feeding, but overall maintained normal swimming patterns.

At the moderate concentration of 0.8 g/L, changes in water quality became more pronounced. The pH declined further, indicating progressive acidification, while dissolved oxygen showed a substantial reduction, signifying elevated respiratory stress. Concurrently, salinity, conductivity, and FCO_2 increased noticeably, suggesting disruption of the water's carbonate equilibrium. Fish exposed to this concentration displayed distinct behavioural stress responses, including irregular swimming, frequent surfacing, elevated opercular movement, excessive mucus secretion, and intermittent loss of equilibrium, indicating impairment of normal physiological functioning.

The highest concentration, 2.0 g/L, caused the most severe deterioration in water quality. This group exhibited the lowest pH values, the sharpest decline in dissolved oxygen, and the highest levels of salinity, conductivity, and total dissolved solids. FCO_2 also increased markedly, indicating a significant reduction in buffering capacity and a buildup of carbon dioxide in the water. Under these conditions, fish displayed extreme behavioural distress, including rapid and laboured opercular movement, erratic and uncoordinated swimming followed by periods of lethargy, persistent attempts to surface, severe loss of balance, continuous mucus secretion, and complete cessation of feeding. The intensity of these symptoms increased consistently with higher DAP concentrations and corresponded closely to the deterioration of water quality parameters.

Overall, the findings confirm that DAP exposure produces a strong dose-dependent decline in water quality—most notably reduced pH and dissolved oxygen, accompanied by increased salinity, and FCO_2. These physicochemical alterations triggered progressive behavioural disturbances in

Channa punctatus, demonstrating that the species is highly sensitive to fertilizer contamination and can serve as an effective ecological indicator of pollution-induced stress in freshwater ecosystems.

Table 2: Effect of different concentrations of DAP on water quality parameters and behavioural responses of Channa punctatus

Parameters / Observations	Control (0.0 g/L)	Low Dose (0.4 g/L)	Moderate Dose (0.8 g/L)	High Dose (2.0 g/L)	Units
Temperature	28.3 ± 1.2	-	-	26 ± 2	°C
pH	7.4 ± 0.2	7.0 ± 0.1	6.6 ± 0.2	6.2 ± 0.2	—
Dissolved Oxygen (DO)	7.86 ± 0.18	5.4 ± 0.3	4.2 ± 0.2	3.1 ± 0.3	mg/L
Free Carbon Dioxide (FCO_2)	0.81 ± 0.13	-	-	2.3 ± 0.2	mg/L
Salinity	1.2 ± 0.12	1.3 ± 0.13	-	1.5 ± 0.13	ppt

Behavioural Observation

Behavioural responses including swimming activity, opercular movement, surfacing frequency, mucus secretion, feeding activity, and equilibrium were observed visually at 0, 24, 48, 72, and 96 hours.

Control

Exposed

Figure 1: A photograph of experimental design

Overall, the behavioural alterations observed in this study demonstrate that DAP exposure disrupts normal physiological functions, interferes with respiration, reduces feeding efficiency, and forces fish to modify their habitat use within the tank to avoid contaminated zones. Such responses serve as sensitive, early-warning ecological indicators of fertilizer-induced toxicity in freshwater ecosystems.

4. Discussion

The study reveals that DAP exposure induces significant alterations in water chemistry and fish behaviour. The observed decrease in pH and dissolved oxygen, along with increased salinity concentration indicates oxygen stress and chemical toxicity. Similar findings were reported by Verma and Mishra (2021) and Das and Sharma (2022), who observed fertilizer-induced behavioural stress in freshwater fishes. The erratic swimming, hyperventilation, and mucus secretion recorded in this study are adaptive mechanisms aimed at reducing toxin uptake and mitigating hypoxia.

Habitat avoidance behaviour suggests that DAP contamination disturbs ecological balance and may affect species distribution. These results align with earlier reports emphasizing the adverse impact of agricultural runoff on aquatic life (Yusuf & Kwayu, 2020; Yadav et al., 2020). Overall, even short-term exposure to DAP significantly affects the physiology and ecology of *Channa punctatus*, which could have cascading effects on aquatic biodiversity. Therefore, regulating fertilizer use and promoting eco-friendly agricultural practices are essential to preserve freshwater ecosystems**.**

5. Conclusion

DAP exposure for 96 hours significantly affected water quality, behaviour, and habitat selection in *Channa punctatus*. These findings provide strong evidence that fertilizer runoff poses ecological risks and that *C. punctatus* can serve as a sensitive bioindicator of freshwater contamination.

Significance of the Study

This study highlights the ecological consequences of sewage discharge originating from domestic, municipal, and industrial activities, with particular emphasis on its role in degrading freshwater environments. The findings offer important insights into the ways sewage effluents influence fish health, especially in species like *Channa punctatus*, which play a vital role in aquatic food chains. Owing to their high sensitivity to environmental fluctuations, such fish serve as reliable bioindicators of water quality deterioration. By examining behavioural alterations under varying DAP concentrations, the present investigation aids in identifying safe exposure limits for aquatic organisms. These outcomes possess both academic relevance and practical value, contributing to improved environmental monitoring, pollution assessment, and freshwater conservation strategies.

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Conflict of Interest

No conflict of interest exists in relation to this study. All relevant support and collaborative efforts have been transparently disclosed, and the research has been performed ethically and honestly.

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