

Di-Ammonium Phosphate-Triggered Toxicity and its Effect on the Morphology and Behavior of *Catla catla* Fingerlings Under Controlled Laboratory Settings

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ABSTRACT

Contamination of freshwater bodies by fertilisers is a significant issue due to their toxicity, persistence, and potential for bioaccumulation. This study focuses on the acute toxicity of diammonium phosphate (DAP) to the freshwater fish species *Catla catla*. This research aimed to understand the connection between mortality rates and the abnormal behavioural and morphological changes observed in *Catla catla* when exposed to cadmium chloride. Static bioassay tests were conducted to determine the LC50 value of diammonium phosphate (DAP) for *Catla catla*, as well as to observe behavioural responses and morphological alterations. After treating the fish with various concentrations of diammonium phosphate (DAP) over different exposure periods, the percentage of mortality was recorded. The lowest concentration of DAP that resulted in mortality was found to be 2 mg/l. The first death among the experimental fish was noted at a concentration of 4 mg/l after 24 hours of exposure. Following 96 hours, the LC50 value for diammonium phosphate (DAP) was determined to be 4.48 mg/l. Key behavioural changes observed during the experiment included restlessness, jumping, erratic swimming, air gulping at the surface, loss of balance, lethargy, increased opercular movements, and instances where the fish lay motionless on the water surface before death, along with morphological changes such as skin discoloration, pigmented patches on the body, scale shedding, chemical sedimentation on the body, increased mucous secretion, and ballooning in the exposed fish. The data collected indicate that *Catla catla* is a suitable bio-indicator for identifying heavy metal contamination in freshwater environments.

Key Words: Behavioural responses, *Catla catla*, diammonium phosphate, Morphological changes.

Introduction

Fertilisers stemming from nitrogen sources inevitably contribute to the pollution of freshwater ecosystems. Aquatic organisms are primarily impacted by these ecological changes. They exhibit varying degrees of modified behaviour when their habitats become polluted. Studies have shown that agrochemical fertilisers severely adversely affect aquatic life (Bobmanuel et al., 2006; Chukwu & Okpe, 2006; Yadav et al., 2007).

Farmers extensively rely on pesticides for crop protection. Unfortunately, these chemicals lack specificity for their targets, and prolonged exposure can lead to numerous abnormalities in various organisms, including humans (Zaluski et al., 2015; Gul et al., 2017). Nitrogen pollution resulting from agricultural activities is increasingly recognized as a significant problem in many parts of the world (Vidal et al., 2000; Haygarth & Jarvis, 2002). This situation is a consequence of human settlements, farming methods, and the presence of a large number of livestock, including pigs and cows (Randall & Tsui, 2002). Ammonia is released into the water as a result of fish excretion. Furthermore, untreated sewage and/or runoff from agricultural practices, such as the application of nitrogen fertilisers, contribute to nitrogen pollution. According to Clarkson et al. (1986), ammonium is the main inorganic type of nitrogen. The accumulation of ammonium in water can hinder growth (Thurston & Russo, 1983; Palanichamy et al., 1985), alter fish behaviour (Rani et al., 1997; Wicks & Randall, 2002), and increase vulnerability to diseases (Thurston & Russo, 1983).

Additionally, sublethal concentrations of ammonium in water have been demonstrated to inhibit fish enzyme activities (Hisar et al., 2004) and can lead to tissue degeneration (Erdogan et al., 2005). Composite fertilisers

(NPK) are not classified as hazardous substances under EEC Directive 67/548/EEC (EPC, 1999). Since these fertilisers contain phosphates, they can have detrimental environmental impacts such as eutrophication in enclosed surface waters. NPK fertilisers that do not include urea have a low potential for bioaccumulation and pose a low level of toxicity to aquatic organisms (EIFAC, 1973; EFMA, 2005). Composite fertilisers are used in aquaculture to promote the growth of algae and diatoms. Chemicals should not be directly introduced into freshwater bodies unless their toxicity and long-term sublethal effects on non-target species, such as fish in the same environment, have been comprehensively studied (Kabir & Ovie, 2011). Extended research is required to determine the long-term effects of fertilisation on water quality (Zachary & Martin Petrovi, 2004).

Diammonium phosphate (DAP) fertilizer leads to harmful effects and alters enzyme activity in tissues such as the kidneys, liver, and muscles at unpredictable times and levels of exposure. As a result, these changes in enzyme activities across various organs hint at the toxicity induced by the fertilizer. Further research revealed that the toxicity associated with DAP caused an unforeseen decline in hematological parameters, including erythrocyte count, hemoglobin levels, and hematocrit, while the total leucocyte count (TLC) showed an increase during DAP toxicity (Trivedi et al., 1990; Naqvi et al., 1993). Therefore, the present study investigated the acute toxicity of diammonium phosphate (DAP) on the fingerlings of the freshwater fish *Catla catla* using a static testing approach.

Methodology

Experimental Fish: Fingerlings of *Catla catla*

Fertilizer: Di-ammonium phosphate

Collection of Fish samples: Fish sample was collected from the pond in the local area of the District. Fish were held in recirculation systems (200L) for at least 15 days to acclimate to laboratory conditions prior to experiments. During the acclimation period, about 50% of the water in each recirculation system was replaced daily. During the acclimation period and succeeding periods of fertiliser exposure, fish were kept under a photoperiod of 12 hours of light and 12 hours of darkness. Fish were fed with commercial fish feed at 2.5% of their body weight twice a day until 2 days before exposure and not fed during the toxicity tests to evaluate the actual effect of the toxicant. Only commercial-grade of fertilisers were used to make it a meaningful study. The temperature, pH, salinity and dissolved oxygen of the water were found to be 29°C, 7.66, 0.85‰ and 6.54ml/l, respectively. Total ammonia was measured daily by the indophenols method (Boyd & Tucker, 1992).

LC50 value of di-ammonium phosphate: The LC50 value was calculated by the Finney method (1978). To determine the LC50 of Di-ammonium phosphate, different concentrations of toxicant, viz., 2 mg/L (Gr-2), 4 mg/L (Gr-3), 6 mg/L (Gr-4), 8 mg/L (Gr-5), 10 mg/L (Gr-6), were used to treat the fish under laboratory conditions. The fish of the control group (Gr-1) were not given any exposure throughout the study period. The number of exposed fish for both the treated and control groups was the same (n=10). Three repeats of each experiment were done. Probit regression analysis method was considered to determine the median lethal concentration (LC50) of Di-ammonium phosphate over a 96-hour exposure period.

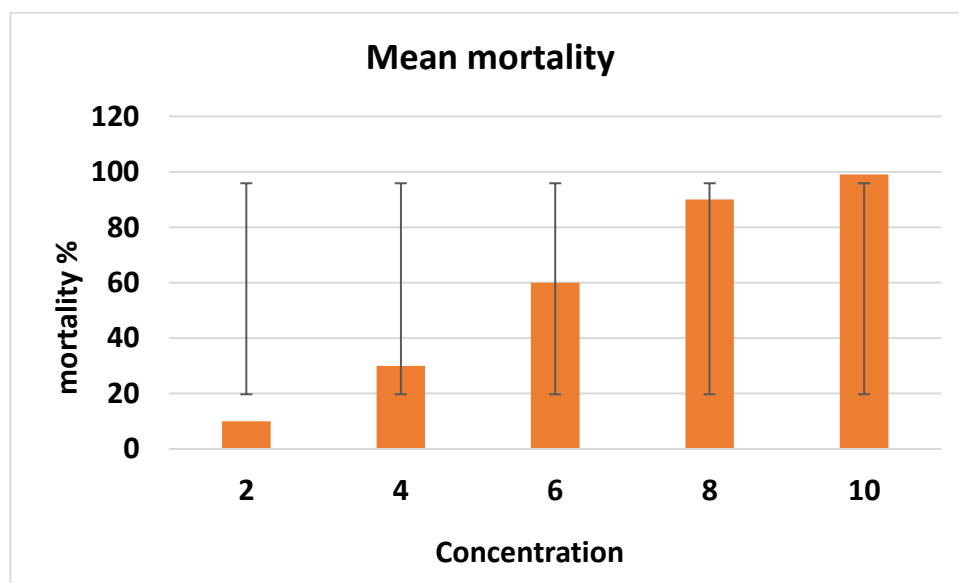
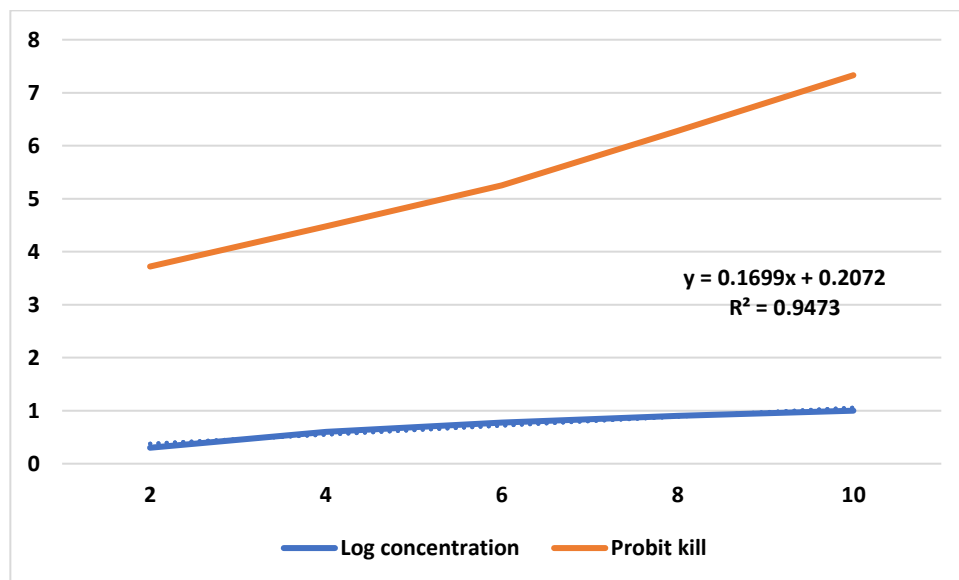
Results and discussion

During the acclimation phase, only a small number of *C. catla* fingerlings died, and there was no mortality observed in the control group during the toxicity assessments. In this study, fish that were subjected to fertilizers displayed quick opercular movement, instability, erratic swimming patterns, and frequent surfacing. They also stayed still for extended periods. Subsequently, a slanted posture in the fish was noted, accompanied by occasional movements toward the surface for gasping. At the moment of death, both the operculum and mouth were found to be open. The surviving fish were moved to flow-through tanks to monitor their behavior following the 96-hour acute toxicity tests. Death among the fish persisted for an additional day. The survival rates varied depending on the type of fertilizers used. Most of the fish that were exposed to toxicants showed quicker recovery. Toxic substances induce various physiological and biochemical alterations in freshwater species by impacting their activities. Changes in the chemical makeup of the aquatic environment typically influence the behavioral and physiological processes of the organisms living there, especially fish (Radhaiah et al., 1987).

The results showed that the mortality rate is lowest at a concentration of 2 mg/L, and no marked behavioural changes have been recorded. But at the doses of 4 mg/L, 6 mg/L and 8 mg/L of di-ammonium phosphate exposure, fish showed morphological changes as well as behavioural changes. Major morphological changes were observed on the skin, resulting in depigmentation of the skin, muscular bleeding and shedding of scales. 100% mortality

was recorded at the concentration of 10 mg/L. The result reveals that 50% mortality was recorded at 4.1 mg/L in 96 hrs of exposure. Therefore, the LC50 of di-ammonium phosphate for *Catla catla* was found to be 4.48 mg/L as per probit analysis.

LC50 value of di-ammonium phosphate



Concentration	Log concentration	Mean mortality	Probit kill
2 mg/ L	0.30103	10	3.72
4mg/ L	0.60206	30	4.48
6mg/ L	0.778151	60	5.25
8mg/ L	0.90309	90	6.28
10mg/ L	1	99	7.33

Fish death resulting from toxicant exposure largely hinges on the fish's vulnerability to the toxicant, along with the concentration and length of exposure (Ram et al., 2009). Identifying unusual behavior involves comparing the reactions of exposed fish to those recorded during a baseline or pre-exposure phase, or to the observations of fish in a control group (Richmond & Dutta, 1992). The reactions observed in this study align with findings reported by various researchers under different stress conditions (Paul & Banerjee, 1996; Rani et al., 1997; Palanivelu et al., 2005; Ufodike & Onusiriuka, 2008; Sangeetha et al., 2011).

Control fish tend to stay at the bottom of the aquarium and exhibit minimal disturbance. Immediately after being placed in the test solution, fish displayed increased swimming, surfacing, and hyperactive behavior. After 24 hours of exposure, signs of agitation, rapid surfacing, skin peeling, and noticeable color fading became apparent. Following 48 hours of exposure, there was a slight decrease in activity and a continued decline in coloration. By this point, gill adhesion and a thin layer of mucus were observed on the gills, operculum, and the body surface. After 72 hours of exposure, fish showed more frequent surfacing and air gulping. At this stage, they demonstrated a loss of balance and erratic movements while swimming. After 96 hours, ulceration on the trunk and the bases of the caudal and pectoral fins was evident in 95% of the fish. Almost all test fish exhibited a thick layer of mucus covering their bodies and gills. The test fish also lost their natural coloration. A loss of balance prior to death was a common symptom exhibited by all tested fish.

Fertilizers can have positive or negative impacts on ecosystem quality, which may benefit or harm aquatic life, including fish (Yaro et al., 2005). The effects of pollutants on aquatic organisms can be lethal or allow for quick recovery (Kumar & Krishnamoorthi, 1983; Yaro et al., 2005). In the current study, nearly 10% of the survivors from the 96-hour toxicity test died after being transferred to flow-through tanks, a finding consistent with previous studies (Xu & Oldham, 1997; Little et al., 2002).

Conclusion

The outcome of the present investigation also showed that the fish exposed to these fertilisers recovered quickly when they were moved to unpolluted freshwater. It is inferred that application of fertilisers to a larger extent may have toxic potential to the adjacent shallow water bodies, posing a threat to their inhabiting freshwater fish fauna. The study recommends monitoring and regulating fertiliser use, especially DAP, to prevent heavy metal contamination, and exploring sustainable alternatives to minimise environmental and health risks.

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