

Single Superphosphate Induced Toxicity and Behaviour of *Channa striata* Under Laboratory Conditions

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ABSTRACT

Recent concerns have arisen regarding the dangers of fertiliser contamination, particularly the impact of high concentrations of diammonium phosphate and single superphosphate (SSP) on aquatic ecosystems. This study examined the mortality rates and behavioral changes in *Channa striata* exposed to SSP. Static bioassay tests indicated an LC50 value of 4.48 mg/l for SSP after 96 hours, with the lowest observed mortality concentration at 2 mg/l and the first recorded death at 4 mg/l after 24 hours. Notable behavioral changes included restlessness, erratic swimming, and lethargy, while morphological changes involved skin discoloration, scale loss, and increased mucus secretion. These findings suggest that *Channa striata* serves as an effective bio-indicator for detecting heavy metal contamination in freshwater environments.

Keywords: *Channa striata*, single superphosphate (SSP), Behavioural responses, exposure time

Introduction

The rapid industrial expansion occurring globally, particularly in India, driven by a concerning increase in the human population, has resulted in substantial environmental pollution. A variety of harmful chemicals, stemming from both natural and human-made sources, have adversely affected the aquatic ecosystem (Kopecka, 2006). The integration of agrochemicals—including fertilizers, insecticides, herbicides, and fungicides—has become a fundamental component of modern agricultural practices; however, their excessive usage can lead to significant environmental contamination (Despommier, 2009). Many of these pollutants ultimately find their way into aquatic systems, either through direct discharge or via runoff and other mechanisms (Stegeman & Hahn, 1994). These synthetic chemicals and their breakdown products contaminate water ecosystems, notably impacting aquatic organisms, particularly fish (Altinok & Capkin, 2007).

While agriculture is regarded as the backbone of the Indian economy, farmers tend to apply 70% of synthetic chemicals as insecticides and herbicides, often opting against biocides in their farming practices. During application, farmers are exposed to these chemicals through inhalation or dermal absorption, especially when skin lesions or wounds are present. Furthermore, synthetic chemical contaminants infiltrate water ecosystems from agricultural activities through leaching into groundwater and nearby water bodies. Fish serve as critical bioindicators of the aquatic environment, reflecting its ecological integrity (Faggio et al., 2014a, b; Gobi et al., 2018; Bartoskova et al., 2013). They can provide quantifiable data on ecological health and integrity, exhibiting various behavioral changes in response to environmental pollution. Research indicates that agrochemical fertilizers have a significantly detrimental effect on aquatic life (Bobmanuel et al., 2006; Chukwu & Okpe, 2006; Yadav et al., 2007).

Farmers rely heavily on pesticides to protect their crops. Unfortunately, these substances do not specifically target their intended organisms, and prolonged exposure can lead to various abnormalities in multiple species, including humans (Zaluski et al., 2015; Gul et al., 2017). Nitrogen pollution from agricultural practices is increasingly recognized as a significant issue in many regions around the world (Vidal et al., 2000; Haygarth & Jarvis, 2002). This problem arises from human settlements, farming techniques, and the high density of livestock, such as pigs and cows (Randall & Tsui, 2002). Ammonia can enter aquatic systems due to fish waste, as well as untreated sewage and runoff from farming, particularly the use of nitrogen fertilizers. According to Clarkson et al. (1986), ammonium is the primary inorganic form of nitrogen. The accumulation of ammonium in bodies of water can hinder growth (Thurston & Russo, 1983; Palanichamy et al., 1985), alter fish behavior (Rani et al., 1997; Wicks & Randall, 2002), and increase vulnerability to diseases (Thurston & Russo, 1983).

Additionally, low concentrations of ammonium in water can inhibit enzyme function in fish (Hisar et al., 2004) and lead to tissue damage (Erdogan et al., 2005). Chemicals should not be introduced directly into freshwater ecosystems unless their toxicity and long-term sublethal effects on non-target organisms, such as fish in the same habitat, have been thoroughly assessed (Kabir & Ovie, 2011). More extensive studies are needed to determine the long-term impacts of fertilization on water quality (Zachary & Martin Petrovi, 2004). Fertilizers can have harmful effects and disrupt enzyme activity in vital organs such as the kidneys, liver, and muscles, depending on varying exposure times and levels. These differences in enzyme activity across tissues indicate the toxicity caused by fertilizers. Further research has shown that the toxic effects of fertilizers can lead to a significant reduction in hematological parameters, including erythrocyte counts, hemoglobin concentrations, and hematocrit levels, while the total leukocyte count (TLC) tends to increase during fertilizer exposure (Trivedi et al., 1990; Naqvi et al., 1993). Therefore, the current study aimed to investigate the acute toxicity of fertilizers on the freshwater fish *Channa striata* using a static testing method.

Methodology

Experimental Fish: *Channa striata*

Fertilizer: Single superphosphate (SSP).

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).

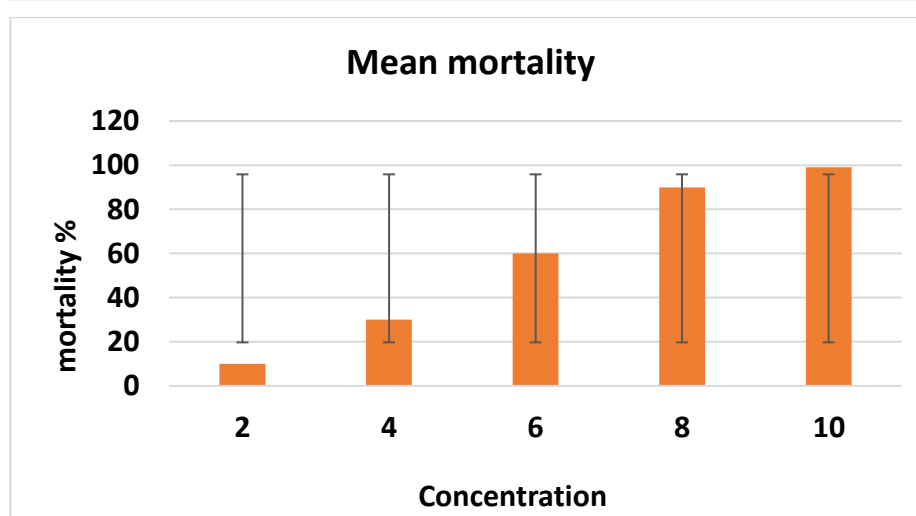
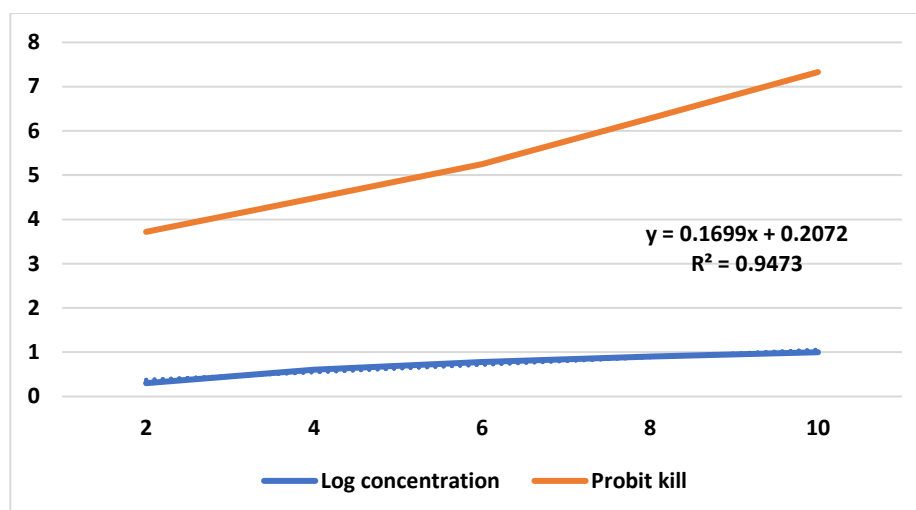
LC50 value of Single superphosphate (SSP): The LC50 value was calculated by the Finney method (1978). To determine the LC50 of Single superphosphate (SSP), different concentrations of toxicant, viz., 2 mg/L (Gr-2), 4 mg/L (Gr-3), 6 mg/L (Gr-4), 8 mg/L (Gr-5), 10 mg/L (Gr-6), were used to treat the fish 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. The probit regression analysis method was considered to determine the median lethal concentration (LC50) of Single superphosphate (SSP) over a 96-hour exposure period.

Results and discussion

During the acclimation phase, a few *Channa striata* fish succumbed, while the control group showed no mortality. The fish exposed to fertilizers displayed symptoms such as rapid opercular movement, instability, erratic swimming, and frequent surface surfacing, along with prolonged stillness and a slanted posture. Upon death, both the operculum and mouth were found to be open. After 96 hours of acute toxicity testing, the surviving fish were transferred to flow-through tanks for behavioral monitoring, with additional mortality noted for another day. Survival rates varied depending on the type of fertilizer, and most fish exposed to toxic substances exhibited a quicker recovery. The presence of these toxic agents induced physiological and biochemical changes in the freshwater species. Typically, alterations in the chemical makeup of the aquatic environment impact the behavioral and physiological processes of its inhabitants, particularly fish (Radhaiah et al., 1987).

The results indicated that the mortality rate was lowest at a concentration of 2 mg/L, with no significant behavioral changes observed. However, at concentrations of 4 mg/L, 6 mg/L, and 8 mg/L of Single Superphosphate (SSP), fish exhibited both morphological and behavioral alterations. Notable morphological changes included skin depigmentation, muscular bleeding, and scale loss. A complete mortality rate of 100% was recorded at a concentration of 10 mg/L. The findings further revealed that 50% mortality occurred at 4.1 mg/L after 96 hours of exposure. Consequently, the LC50 of Single Superphosphate (SSP) for *Channa striata* was determined to be 4.1 mg/L based on probit analysis.

LC50 value of Single superphosphate (SSP).



The mortality of fish due to exposure to toxicants is primarily influenced by the fish's susceptibility to the toxic substance, as well as the dose and duration of exposure (Ram et al., 2009). Detecting atypical behavior necessitates a comparison between the reactions of exposed fish and those documented during a baseline or pre-exposure period, or against the behaviors observed in a control group (Richmond & Dutta, 1992). The behaviors recorded in this investigation align with findings from various researchers under different stress scenarios (Palanivelu et al., 2005; Ufodike & Onusiriuka, 2008; Sangeetha et al., 2011).

Control fish typically remain at the bottom of the aquarium and exhibit minimal disturbance. However, when placed in a test solution, they initially display increased swimming and hyperactivity. Over the course of 24 hours, signs of agitation, rapid surfacing, skin peeling, and color fading were observed. After 48 hours, a decrease in activity was noted alongside gill adhesion and mucus buildup. By 72 hours, the fish demonstrated more frequent surfacing, air gulping, and loss of balance. After 96 hours, 95% of the fish exhibited ulcerations on their bodies and fins, accompanied by a thick mucus covering and loss of natural coloration. Loss of balance prior to death was consistently observed in all tested fish.

Fertilizers can influence ecosystem quality in both beneficial and detrimental ways, ultimately affecting aquatic life, including fish (Yaro et al., 2005). While the impact of pollutants on aquatic species can be fatal, it may also allow for rapid recovery (Kumar & Krishnamoorthi, 1983; Yaro et al., 2005). In this study, nearly 10% of the organisms that survived the 96-hour toxicity assessment perished after being relocated to flow-through tanks, a result consistent with findings from previous research (Xu & Oldham), 1997; Little et al., 2002).

Conclusion

The findings of the current study suggest that fish exposed to these fertilizers exhibited a rapid recovery when transferred to unpolluted freshwater. This leads to the conclusion that excessive fertilizer application poses toxic risks to nearby shallow water bodies, threatening the freshwater fish species that reside there. The research underscores the importance of monitoring and regulating fertilizer use, particularly potassium phosphate (SSP),

to prevent heavy metal pollution. It also advocates for exploring sustainable alternatives to mitigate environmental and health risks.

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