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ORIGINAL ARTICLE

Acute Toxicity of Detergent and Impact on Sublethal Concentrations on Dissolved Oxygen Consumption in Fresh Water Fish *Mystus vittatus* (Bloch)

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ABSTRACT
The present study examined the effects of a commercial household synthetic detergent on the mortality and behavioural changes of the freshwater fish Mystus vittatus. The median lethal concentration (LC_{50}) of Tide detergent was determined using probit analysis software (SPSS version 26) at 95% confidence limit. The LC_{50} value for Mystus vittatus at 96 hours was found to be 17.981 mg/L. Toxicity increased significantly with higher detergent concentrations and longer exposure periods. Morphological changes observed after 96 hours of exposure included body and gill discoloration, damaged fins, and excessive mucus secretion. Behavioural alterations in detergent-exposed fish included erratic swimming, restlessness, aggression, hyperactivity, and frequent movement near the bottom of the tank. Additionally, fish were exposed to sublethal concentrations (1/3rd and 2/3rd of the LC_{50} values) of Tide detergents for 96 hours. Oxygen consumption increased over time at the 1/3rd sublethal concentration. However, at the 2/3rd sublethal concentration, a significant decrease in oxygen consumption was observed after 48 hours of exposure. These findings suggest potential eco-toxicological risks associated with synthetic detergent exposure in aquatic environments. Further research is recommended to explore the physiological effects for a better understanding of environmental impacts.
Keywords: *Acute Toxicity, Ethological Responses, Oxygen Consumption, Tide, Mystus vittaus*

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INTRODUCTION

Detergents are categorized into three types: anionic, cationic, and non-ionic. However, based on their characteristics, they are broadly classified into two main types: phosphate detergents and surfactant detergents. Phosphate detergents are highly caustic, used to soften hard water and suspend dirt in water. The phosphates in these detergents significantly contribute to eutrophication and subsequent nitrogen imbalances in water bodies [1]. Detergents as surface-active agents or surfactants, commonly used in daily life for laundry, dishwashing, car cleaning, and carpet cleaning, contain various chemicals that can be harmful to living organisms upon prolonged exposure. Their impact is particularly severe on aquatic life, especially fish, when these chemicals enter riverine systems through household sewers and laundry waste [2].

Surfactant detergents, such as Linear Alkylbenzene Sulfonate (LAS) and Sodium Dodecyl Sulphate (SDS), are highly toxic to bacteria, microalgae, crustaceans, echinoderms, and fish. In commercial detergents, surfactant components make up 10% to 20% of the composition, with additional ingredients including bleach, fillers, foam stabilizers, builders, perfumes, soil suspending agents, enzymes, dyes, optical brighteners, and other substances that enhance cleaning efficiency [1]. The input of synthetic detergents in fresh waterbodies has reached a point of serious concern because of their adverse impacts on water quality by altering the pH, total alkalinity, free CO_2, dissolved oxygen, rate of photosynthesis, aquatic flora and fauna including fishes, eutrophication of natural water due to the presence of higher amounts of phosphates in detergents, reducing the primary and secondary productivity of freshwater ecosystem [3].

The studies reveal that almost all the detergent components are toxic to the aquatic organisms, especially the surfactant [4].
Detergents together with their ingredients and metabolites constitute the biggest groups of anthropogenic pollutants. The ability to these compounds to form foam is a serious problem because the organic contaminants or pathogenic micro-organisms are accumulated in the foam thereby posing an epidemiological threat. In addition, foaming also reduces aeration and causes hypoxia [5]. Even trace amounts of detergents weaken fish resistance, making them susceptible to parasitic fungi that damage their fins. Additionally, detergents impair their ability to locate food sources. Thus, contaminations of fresh waterbodies by detergents and their effects on flora and fauna have become a subject of scientific investigations. Fishes are good bioindicator as these are very sensitive to changes in the aquatic medium and thus, play an important role in the monitoring of water pollution. Therefore, the present study is meant to determine the LC_{50} value of commercial detergent and its effect on fresh water cat fish, *Mystus vittatus*, by using SPSS and also to study the ethological responses due to its toxic effects. The study will provide baseline data that could be a useful contribution in eco-toxicity risk assessment to the fish.

MATERIAL AND METHODS

Acclimatization of Test Fish: The freshwater catfish, *Mystus vittatus* (Bloch) were collected from local fresh waterbodies and the healthy fish of uniform length (8.5±0.2 cm) and weight (9.0±1g) were selected for the Acute toxicity test. Then these fishes were brought to the laboratory in plastic container to avoid injury during transportation. The collected fishes were washed with 1.0 % solution of $KMnO_4$ for 5 minutes to remove any dermal infection. Fish were acclimated to laboratory conditions for 10 days at room temperature (26 ± 1.5°C) prior to experimentation. For the first week, the acclimatized fishes were given artificial air by aerator. All of the fish were given commercial food pellets on a regular basis, and the medium (tap water) was changed every two days to get rid of food remnants and faeces. Feeding was stopped 24hr prior to the toxicity test.

Determination of Acute toxicity (LC_{50}): The experiments were carried out in glass aquaria of 15liter capacity in laboratory conditions. The stock solution was prepared by dissolving Tide detergent (American brand of laundry detergent manufactured and marketed by Procter and Gamble) in tap water. The range finding tests or exploratory tests were conducted to ascertain the final concentrations to be used in definitive test by following the standard method [6]. Prior to the 96-h Static bioassay test, a range-finding test was carried out to determine the range of concentrations to be used. The range finding test was conducted using a broad concentration range (1, 10, 20, …. and 100 mg/L) and the test was terminated after 24 h. Concentrations of the detergent that caused death within 30 minutes were not considered for the further definitive toxicity test / LC_{50} test [7]. In the definitive test the concentrations selected were based on the mortality values obtained with the range-finding test and were in appropriate logarithmic dilution series. Three replicate test tanks for each concentration (5, 10, 15, 20, 25, 30, 35 and 40 mg/L) contained a total of 5.0 L of the test solution and 10 test fishes. The control experiment contained the tap water. LC_{50} value of detergent for fresh water cat fish, *Mystus vittatus* was determined by using probit analysis software (SPSS version 26) at 95% confidence limit.

Estimation of Oxygen Consumption: The experimental fishes were subjected to two different sub-lethal concentrations ($1/3^{rd}$ and 2/3rd of LC_{50} values) of Tide detergents as per suggestions for different exposure periods (24, 48, 72 and 96 hours). Similar number of fish was maintained in controls for similar duration of exposure8. After each exposure periods such as 24, 48, 72 and 96hours, Experimental weighed fishes were kept in various sublethal concentrations of detergents in an air tight glass jar for one hour. Before starting the experiment, the initial dissolved oxygen content of water in each glass jar was estimated with the help of digital Water Quality Meter Model: ISO-TECH SYSTEM (ITS)-901 using glass electrode. A healthy fish was allowed to respire for one hour in each glass jar. After one hour, again estimated the dissolved oxygen content in each glass jar was also determined in the same manner. The oxygen consumed by the fish was calculated by finding out the difference between the initial and final dissolved oxygen content in experimental glass jar which act as respiratory chamber. The difference in the dissolved oxygen content between initial and final water samples represents the amount of oxygen consumed by the fish. The rate of oxygen consumption per gram weight of fish per hour was calculated and the values were expressed as mg/litre/gram of body weight.

RESULTS AND DISCUSSION

Fish acute toxicity studies play a vital role in environmental risk assessment and hazard classification, as they provide preliminary estimates of the relative toxicity of different chemicals across various species. In the present study, acute toxicity tests usually provide estimates of the exposure concentration of 'Tide' detergent causing 50 % mortality (LC_{50}) to test species *Mystus vittatus* within 96 hours of exposure. The percentage of mortality of the test fish after 96 hours exposure to each concentration was calculated separately using the following formula: mortality (%) = no dead/ total number tested ×100%. The dead fishes were removed immediately from the aquaria. It is clear from the data that detergent toxicity was increased along with increasing the concentrations of detergents. At 96 h, when the test was terminated, mean mortality in response to 5mg/L, 10mg/L, 15mg/L, 20mg/L, 25mg/L, 30mg/L, 35mg/L and 40mg/L of 'Tide' detergent was 10%, 20%, 30%, 40%, 60%, 80%, 90% and 100%, respectively (Table 1). The figure 1 shows the increase in mortality percentage was significantly increased with increase in log concentration of Tide detergent and exposure. The same kind of toxicity effect was observed by some workers [8,9]. C_{50} value of the Tide detergent to *Mystus vittatus* was found to be 17.981 mg/L for an exposure period of 96 hr (Table 2). The 96h LC_{50} value of Surf excel and Nirma for fresh water fish *Mystus montanus* were 20.0 mg/litre and 23.5 mg/litre respectively [10]. The LC50 value of the common household synthetic detergent to *Catla catla* was found to be 14.20 ppm for an exposure period of 96 hr [11].

Table1: Survival of fish, *Mystus vittatus* at different test concentrations of detergent.

Concentration of Detergent (mg/L)	Log Conc. of Detergent	No. of Test Fish	No. of Fish Alive	Number of Fish Dead	Percent Mortality at 96 hours Exposure
Control (0.0)	-	10	10	0	0
5	.699	10	9	1	10
10	1.000	10	8	2	20
15	1.176	10	7	3	30
20	1.301	10	6	4	40
25	1.398	10	4	6	60
30	1.477	10	2	8	80
35	1.544	10	1	9	90
40	1.602	10	0	10	100

In the present study, results indicated that mortality rates increased with higher concentrations of detergent. Similar findings have been reported by other researchers [12,13]. The toxicity of a given substance in fish depends on multiple factors, including species, sex, age, weight, concentration, exposure duration, and whether the toxicant is in an organic or inorganic form [14, 15].

Table 2: 96h LC_{50} values with 95% confidence limits for *Mystus vittatus* (Bloch) exposed to detergent

LC_{50} Values of Detergent (mg/L)	95% Confidence limits (*v/v* %)		Regression Equation	Chi Square Value (P value)	Coefficie-nt of determi-nation (R^2Linear)
	Lower Limit	Upper Limit			
17.981	13.870	22.390	Y= -3.63+2.89X	5.290 (0.507*)	0.878
*Since the significance level is greater than .150, no heterogeneity factor is used in the calculation of confidence limits.					

When the experimental fish were introduced into water containing detergent at higher concentrations, they exhibited signs of discomfort within minutes, moving rapidly. After 96 hours of exposure, careful observations revealed physical changes and damage in the test fishes. Symptoms included irregular fading of body and gill colouration, as well as gill and fin damage or rot during acute exposure to synthetic detergents. The severity of damage increased with higher detergent concentrations. The findings of the present study indicate that acute exposure to synthetic detergent significantly disrupted fish behaviour, leading to loss of balance, increased operculum beating, irregular swimming, disturbed equilibrium (Table 3), and additional effects such as loss of reflexes and coordination. A common physiological response to toxins is a change in breathing rate, accompanied by symptoms such as reddish patches on the skin, attempts to jump out of the aquarium, lying on one side, and frothing around the mouth, particularly at higher detergent concentrations and by the fourth day of exposure (Table 3). Additionally, bleeding in the gill filaments suggests that detergent exposure may cause significant gill damage. Such injury can severely impair the proper exchange of respiratory gases between the fish and the surrounding water. The increased opercular beating rate and visual examination of dead fish further indicate the lethal effects of detergent exposure [10,16,17].

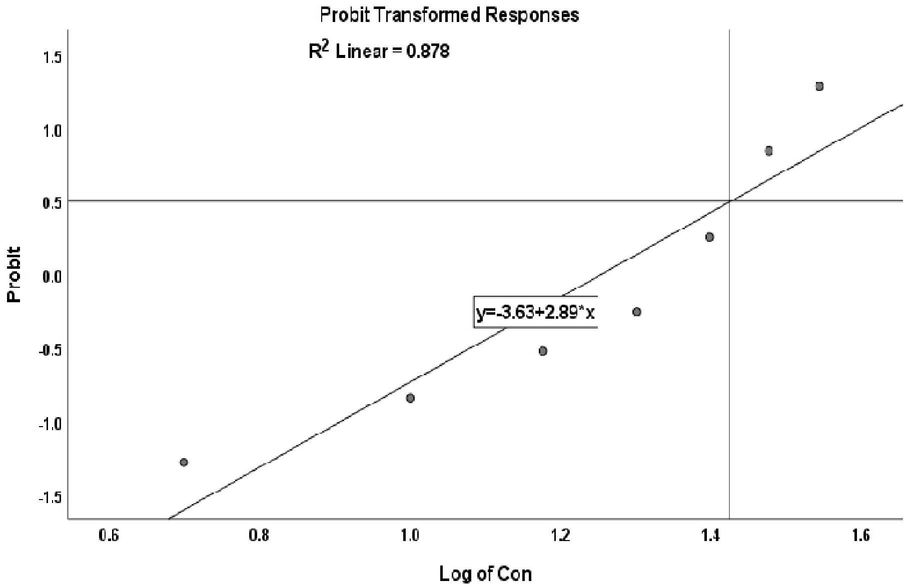

Figure 1: Regression Line (Based on Probit analysis) of Log concentration of Detergent *vs* % mortality of *Mystus vittatus* (Bloch) (at 96hrs).

Table 3: Ethological responses of *Mystus vittatus* to different concentration of Tide detergent for 96 hours.

Concentration (mg/L)	Morphological changes & Ethological Responses
0, 5, 10	Swimming, and Opercular beating normal and the mortality 20%
15, 20, 25	Random Swimming, Up and down jumping from the aquarium quickly, floating at surface, schooling, lethargy, immobility, irregular beating of operculum, gulping, dullness of body colour, and mortality 60%
30, 35, 40	Loss of balance, Loss of Schooling, excess mucous secretion, reddish patches on the skin, irregular fading of body and gill colour, dullness eye and rotten gill and fins; fish become lethargic, inactive and mortality 100%

Detergent molecules can penetrate and solubilize the lipid content of the cell membrane, reducing its permeability. In fish, the gills serve as osmoregulatory organs and are the primary sites for the uptake of waterborne pollutants. Swelling of the gill epithelium caused by pollutants decreases gas exchange efficiency and reduces oxygen consumption. In *Mystus vittatus* (Bloch), oxygen consumption increased with exposure to a $1/3^{rd}$ sublethal concentration of detergent over time. However, at a $2/3^{rd}$ sublethal concentration, a significant decrease in oxygen consumption was observed after 48 hours of exposure (Table 4).

Table 4: Effects of Tide detergent on Oxygen Consumption in *Mystus vittatus* at various sublethal concentrations.

Concentration (mg/L)	Exposure Period (in Hours)			
	24 hrs	48 hrs	72 hrs	96 hrs
Control	0.10422 ±0.002543	0.10445 ±0.002544	0.10454 ±0.002551	0.10463 ±0.002614
6.0 ($1/3^{rd}$ of LC_{50} value)	0.110753* ±0.003342	0.111218* ±0.003425	0.112031* ±0.002674	0.110724* ±0.002137
12.0 ($2/3^{rd}$ of LC_{50} value)	0.110361* ±0.003125	0.105966** ±0.0028214	0.091028** ±0.002682	0.0881604** ±0.001882
Values expressed as –mg/litre/gram of body weight; ±= SD of three observations; *= insignificant; **= significant at 0.5 %				

Similar findings have been reported in detergent-exposed fish by other researchers [10, 18]. When *Mystus vittatus* was exposed to detergent-contaminated water, dissolved oxygen consumption initially increased. As detergent concentration rose, the fish exhibited increased breathing activity and signs of distress.

Despite being highly resilient to environmental stress, *Mystus vittatus* was negatively affected by detergent exposure, while less sturdy aquatic organisms would likely succumb more easily to increased detergent concentrations [10, 18]. The presence of toxic substances in water often triggers increased surfacing activity in aquatic animals due to heightened oxygen demand for respiration [12]. Detergent exposure can lead to gill epithelium degeneration, disrupting respiratory gas exchange and resulting in hypoxia. This oxygen deficit, coupled with impaired carbon dioxide removal from the bloodstream, contributes to "histotoxic anoxia," where gill tissues suffer from oxygen deprivation and lose their efficiency in eliminating carbon dioxide. Anoxia or hypoxia increases carbohydrate metabolism, placing additional respiratory stress on the organism, even at sublethal levels, and leading to increased energy expenditure [12]. Furthermore, reduced diffusional efficiency may contribute to hypoxia, impeding carbon dioxide efflux and causing hypercapnia [10]. Thus, the toxic elements in detergents can trigger excessive mucus secretion over the gill filaments and irritate the gill epithelium, disrupting respiration and reducing gill diffusing capacity. This interference can lead to either an increase or decrease in oxygen consumption. Oxygen consumption generally decreases with higher pollutant concentrations and prolonged exposure, likely due to the penetration of pollutants at the sub-cellular level and damage to gill epithelium.

CONCLUSIONS

The environmental damage caused by chemical pollution is often overlooked, making it one of the major obstacles to inland fish production in India. Addressing this issue is crucial for sustainable development of fisheries. Chemical pollution not only harms the environment but also poses both short-term (acute) and long-term (chronic) health risks to humans and other living organisms. Rapid urbanization and high population density further complicate waste management, leading to increased pollution levels. Detergents, which contain high concentrations of nitrate and phosphate compounds, are significant pollutants that negatively impact water bodies. Additionally, detergents contain oxygen-depleting substances that can severely harm fish and other aquatic life. Previous studies have documented extensive fish mortality caused by detergent pollution and other contaminants. To safeguard the environment, regular monitoring of chemical pollutants, including detergents, is essential to prevent and mitigate their harmful effects. Completely eliminating detergent use in households and businesses is unrealistic; however, improving the safe disposal of wastewater is essential. This study highlights the harmful effects of detergents on *Mystus vittatus*, ultimately leading to mortality. Detergents are considered hazardous to all aquatic life, including fish. If they continue to enter aquatic ecosystems at current levels without regular monitoring and regulation, the risk to aquatic organisms will be severe. Therefore, the development and implementation of innovative technologies are crucial to reducing the toxicity of detergents and minimizing their environmental impact.

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