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Acute Toxicity and Behavioural Alterations in *Mystus Vittatus* (Bloch) Exposed to Paper Mill Effluent

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ABSTRACT:
The pulp and paper industry has been considered to substantial environmental polluter and user of natural resources. Globally it is sixth largest polluter industry, releasing a wide range of solid, liquid, and gaseous wastes into the environment. This study examines the effects of various concentrations and exposure times on the mortality and behavioural responses of freshwater catfish, *Mystus vittatus* (Bloch), caused by acute poisoning from paper mill effluent. The LC_{50} for 24, 48, 72 and 96 hours of paper mill effluent for *Mystus vittatus* were 14.405, 11.859, 9.682 and 8.223% (v/v), respectively. The outcome also showed that both exposure period and concentrations of effluent affect the mortality rate. Fishes exposed to effluent exhibited altered behaviour, including increased surface activity, faster fin and opercular movements, redness in eye, excessive mucus secretion, and unbalanced swimming or loss of equilibrium. It was shown that these activities were comparatively elevated, indicating signs of stress, as concentration and exposure period increased. Thus, the present study revealed that paper mill effluent is potentially toxic to fish. Because *Mystus vittatus* is sensitive to paper mill effluent, it is necessary to regularly monitor natural waterbodies to minimize.

Keywords:
Acute toxicity, Behavioural responses, *Mystus vittatus*, Paper mill effluent.

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1. INTRODUCTION

Paper mill is one of the most polluting industries and produce approximately 220-380 m³ of potentially toxic coloured wastewater for each tone of paper produced (Badar and Farooqui, 2012) which affect the life of aquatic organisms particularly fishes. Chlorophenols, fatty acids, and resin are among the acute hazardous and bioaccumulating substances found in its effluent. These substances are what cause changes in metabolic processes of aquatic organisms (Prakash and Upadhyay, 2023). The Indian paper industry consumes about 250 m3 of freshwater per ton of paper and generates the corresponding wastewater. For every ton of paper produced, the Indian paper industry uses roughly 250 m3 of freshwater and produces the wastewater. Every year, this industry in India uses about 905.8 million m3 of water and

releases about 695.7 million m3 of wastewater. In order to guarantee a sufficient supply of water, paper mills are typically situated along riverbanks (Prakash and Upadhyay, 2022). Paper mills are generally situated near the lotic waterbodies to ensure sufficient supply of water and to dispose of wastewater, which is characterized by elevated COD, BOD, TSS, pH, and temperature. Numerous harmful and bioaccumulating substances, including fatty acids, chlorophenols, and resins, are present in these effluents and can have an immediate impact on body tissue biomolecules and cell components (Prakash,2021). Several compounds that are known to be endocrine disruptive in addition to being carcinogenic, mutagenic, and teratogenic have been found in paper mill effluents that are produced at various phases of the paper-making process (Dey and Das, 2013).

Chlorophenols, fatty acids, and resins are examples of bioaccumulating substances that have the ability to directly impact bodily tissue macromolecules and cell components (Prakash, 2021). In addition to being carcinogenic, mutagenic, and teratogenic, a number of compounds that are known to disrupt hormones have been found in paper mill effluents produced at various stages of the paper-making process (Dey and Das, 2013). Industrial effluents exposed fishes accumulating harmful substances in its body tissues causes serious threat to human health (Jaiswal and Sharma, 2024).

Acute toxicity tests helpful to determine the possible toxicological effects of chemicals on an organism as well as the quantities of toxicants and the length of exposure required to produce them. Because different species react differently to the same dose of a harmful agent so, toxicity of any chemical is species-specific (Smith and Stratton, 1986). These tests offer a database that may be used to evaluate the danger of an environment in which the organisms live. The concentration required to kill the 50% of a large population of the test species is estimated by the LC_{50}. This is accomplished experimentally by giving a set of organisms a toxicant at varying concentrations and then tracking the subsequent mortality rates over a specific time frame, such as 24, 48, 72 and 96hr. The acute toxicity data are important to determination of sub lethal concentrations for chronic toxicity tests.

In addition to causing death, fish behaviours are altered by changes in physio-chemical and biological parameters of the water. Fish are more vulnerable to contaminants than other aquatic life, and modifications in behaviours in fish have been thought to be a sensitive sign of toxicity (Yadav *et al*, 2007). An organism may adapt to both internal and external stimuli through ethological responses, which helps it overcome the difficulties of existing in a changing environment. Because these fish behavioural anomalies might give appropriate indications regarding the environmental circumstances, so can be usage as biomarkers (Dhanalakshmi *et al.*, 2018). 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 (Srivastava *et al*. 2010). Therefore, the present study is meant to determine the LC_{50} value of paper mill effluent 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 might be utilized to determine the relative sensitivity of effluent and help further in determining the acceptable level concentration.

2. MATERIALS AND METHODS

The freshwater catfish, *Mystus vittatus* (Bloch) were collected from local fresh waterbodies in and around Ayodhya and the healthy fish of uniform length (8.8±0.20 cm) and weight (9.5±1g) were selected for the Acute toxicity test. Then these fishes were brought to the ichthyology lab 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 two weeks at room temperature (26 ± 1.4°C) prior to experimentation. For the first week, the acclimatized fishes were given artificial air by aerator. To keep the aquaria hygienic and free from mechanical disruptions, every effort was taken. 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.

The experiments were carried out in glass aquaria of 15 liter capacity in laboratory conditions. The stock solution was prepared by dissolving untreated paper mill in dechlorinated 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 method prescribed by APHA (1998).

After determining the range, fish were exposed to final concentration of 0.50, 0.60, 0.70, 0.80, 0.90, 1.00, 1.10, 1.20, 1.30, 1.40 1.50 and1.60 ml/L. Therefore, paper mill effluent was collected for and their percent concentration was prepared on volume to volume (v/v) ratio. The percentage concentration of each test solution is obtained by using the following formula (FAO, 1984):

$$\text{Volume percent} = \frac{V_E}{[V_E + V_{DW}]} \times 1000$$

Where, V_E = Volume of effluent, V_{DW} = Volume of Dilution water.

The appropriate amount of dechlorinated tap water was mixed with the calculated volume of wastewater, and the mixture was forcefully swirled to distribute the effluent.

3. RESULTS AND DISCUSSION

Bioassay Assessment: Acute toxicity test results, or LC_{50} values, indicate a fish's vulnerability to specific pollutants and their likelihood of surviving. The LC_{50} values for *Mystus vittatus* in paper mill effluent after 24, 48, 72, and 96 hours were found to be 14.405, 11.859, 9.682, and 8.223% (v/v), respectively. (Table 2). Fish exposed to dechlorinated tap water, however, were found to be normal and healthy.

Table 1: Percentage of Mortality of Fish, *Mystus vittatus* (Bloch) indifferent concentrations of Paper mill effluent (ml/L) at different Exposure Period

Concentration of Paper mill effluent (ml/L)	Log Conc. of Effluent	No. of Test Fish	Percent Mortality at different Exposure Period (Hours)			
			24	48	72	96
Control (0.0)	-	10	0	0	0	0
2	.301	10	0	0	0	0
4	.602	10	0	0	10	10
6	.778	10	0	10	20	30
8	.903	10	10	20	30	40
10	1.000	10	20	30	50	60
12	1.079	10	30	40	60	70
14	1.146	10	40	60	70	90
16	1.204	10	50	70	80	100
18	1.255	10	70	90	100	-
20	1.301	10	80	100	-	-
22	1.342	10	100	-	-	-

Table 2: LC_{50} values with 95% confidence limits for *Mystus vittatus* (Bloch) exposed to paper mill effluent at different Exposure Period

Exposure Period (hrs.)	LC_{50} Values of paper mill Effluent (v/v%)	95% Confidence limits (v/v%)		Regression Equation	Chi Square Value (P value)	Coefficient of determination (R^2Linea)
		Lower Limit	Upper Limit			
24	14.405	12.753	16.287	Y=-6.04+5.16X	2.833 (0.971)	0.971
48	11.859	10.276	13.564	Y=-5.33+4.95X	3.082 (0.929)	0.933
72	9.682	7.970	11.547	Y=-3.55+3.55X	2.456 (0.930)	0.981
96	8.223	6.734	9.715	Y=-3.95+4.30X	2.131 (0.907)	0.956

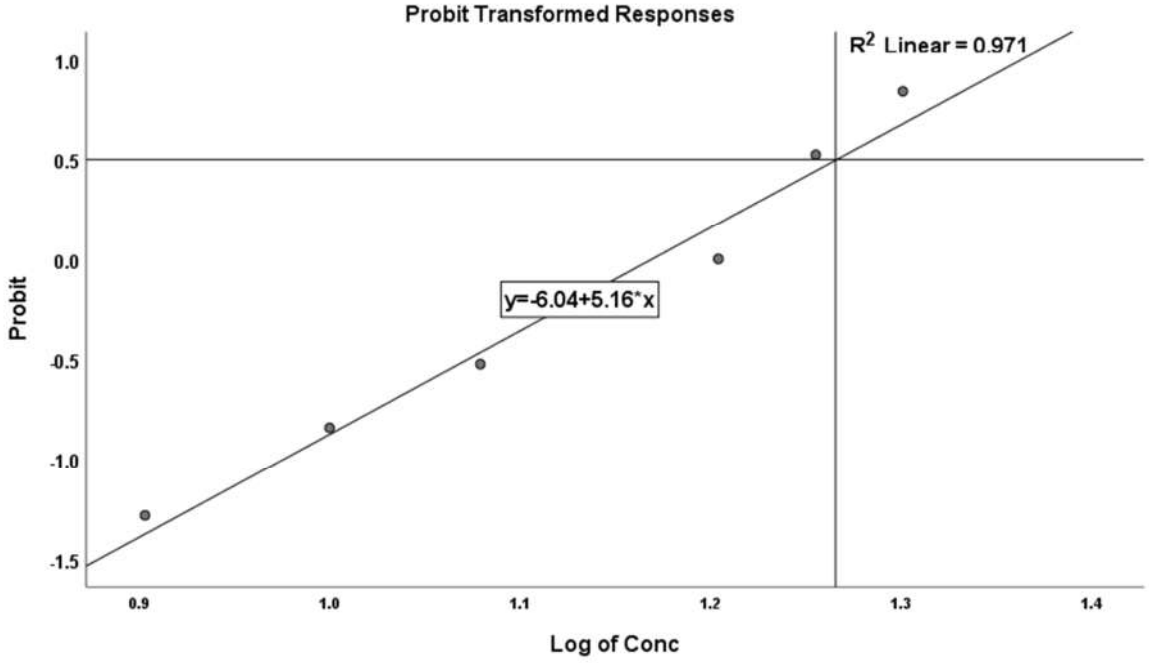

Fig. 1a. Regression Line (Based on Probit analysis) of Log concentration of Paper Mill Effluent Vs % mortality of *Mystus vittatus* (Bloch) (at 24hrs)

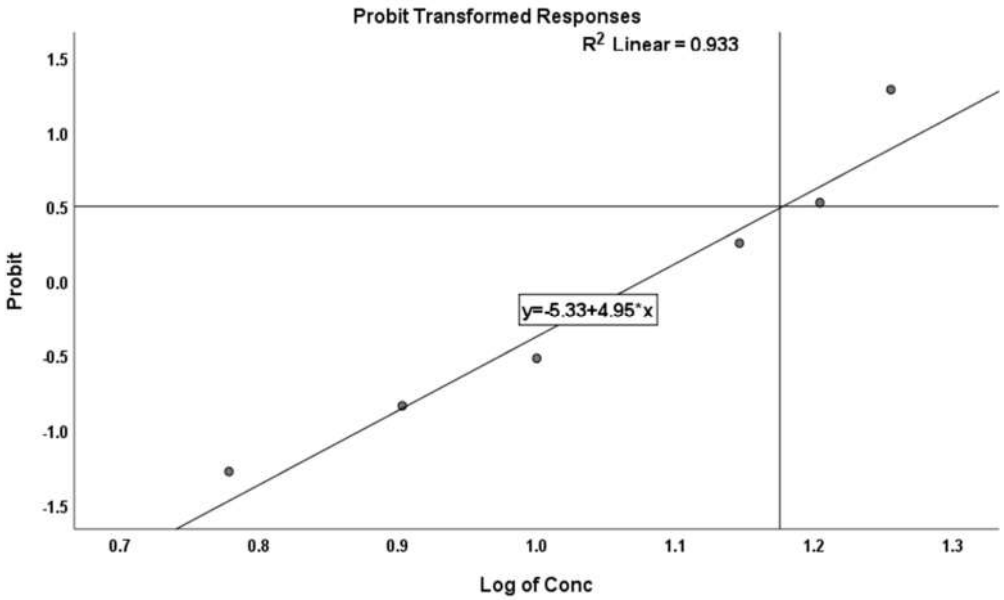

Fig. 1b. Regression Line (Based on Probit analysis) of Log concentration of Paper Mill Effluent Vs % mortality of *Mystus vittatus* (Bloch) (at 48 hrs)

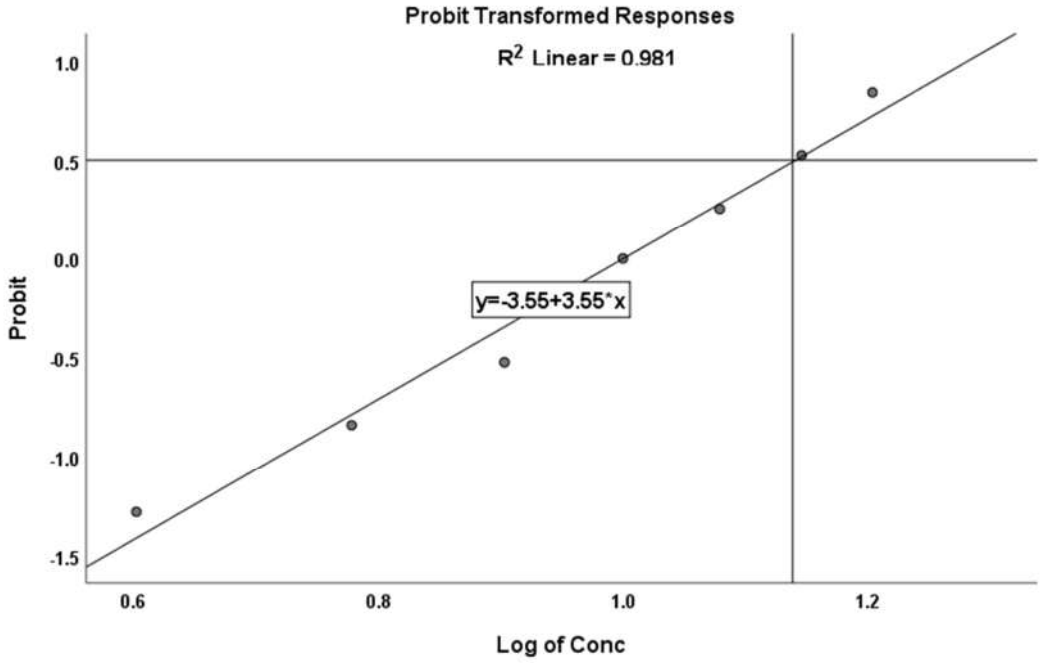

Fig. 1c. Regression Line (Based on Probit analysis) of Log concentration of Paper Mill Effluent Vs % mortality of *Mystus vittatus* (Bloch) (at 72 hrs)

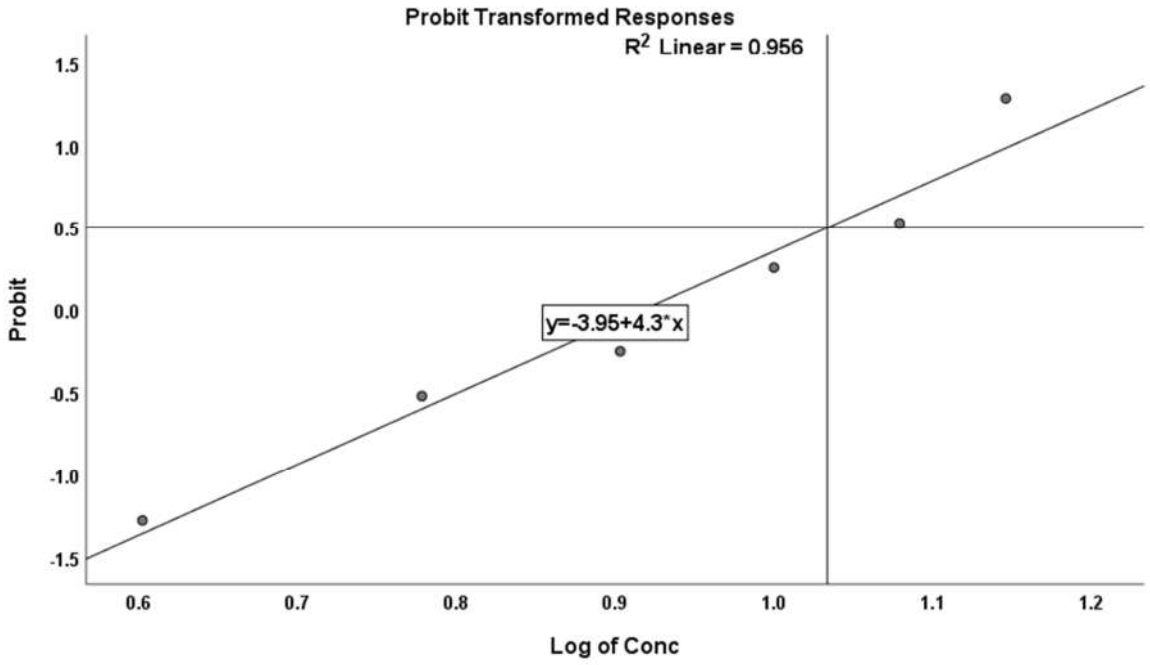

Fig. 1d. Regression Line (Based on Probit analysis) of Log concentration of Paper Mill Effluent Vs % mortality of *Mystus vittatus* (Bloch) (at 96 hrs)

However, few literatures related to acute toxicity of fresh water fishes exposed to paper mill effluent are available. LC_{50} values at 24, 48, 72 and 96 h for *Rasbora daniconius* exposed to paper mill effluent were 11.0, 10.5, 10.1 and 9.5%, respectively (Pathan *et al*., 2009); LC_{50} values at 24, 48, 72 and 96 h for *Puntius stigma* were 40,35, 30 and 25% concentrations respectively (Sarwade, 2015); LC_{50} values at 24, 48, 72 and 96 h for *Tilapia* were 11.0,10.6, 10.2 and 9.6% respectively (Pandey *et al*., 2018), 96 h LC_{50} value for Channa punctatus was 15% (Prakash and Verma, 2020); whereas LC_{50} values at 24, 48, 72 and 96 h for pre-spawning, Spawning and post-spawning phases of *Mystus vittatus* exposed to paper mill effluent were 60.35, 52.70 and 68.2 % (v/v), respectively at 24 hours exposure; 52.60, 46.20 and 62.0 % (v/v), respectively at 48 hours exposure, 47.80, 41.6 and 56.4% (v/v) respectively at 72 hours exposure, 43.8, 38.7, 50.8 % (v/v), respectively at 96 hours exposure to paper mill effluents (Mishra *et al*., 2011). Accordingly, catfish were tolerant to industrial effluent than carps due to their physiological and ecological condition (Singh *et al*., 2019). The findings of the current study revealed that the mortality rate increased as the concentration of paper mill effluent increased and for a given concentration the mortality rate increases as the duration of exposure increased. Similar observations were found by some researchers (Mishra *et al*., 2011; Sarwade, 2015; Pandey *et al*., 2018). The results of this study reflect that due to presence of harmful chemicals or toxicant in the effluent up to dangerous level cause death of fish. The mortality of fish may result from the toxic effects of effluent discharged from paper mills, which disrupts physiological and biochemical processes associated with cellular metabolism. Any chemical's toxicity to fish is contingent upon a number of factors, including species, sex, age, weight, exposure duration, dose or concentration, and organic or inorganic form (Tiwari and Prakash, 2021). Physicochemical parameters such as BOD, COD, pH, Dissolved oxygen and temperature etc. of water if beyond the permissible limits causes fish mortality (Singh *et al*., 2019; Tiwari and Prakash, 2021) due to large number of toxicants.

The LC_{50} of industrial wastewaters for 96 hours exposure of different fish species for different effluents has been reported by various scientists. These values were 70% (v/v) for *Channa striatus*, 2.35%(v/v) in *Heteropneustes fossilis*, 0.80 %(v/v) for *Labeo rohita* exposed to fertilizer industry effluent, respectively (Singh *et al*., 2019); 20, 6.0, and 22%(v/v) for *Labeo rohita* exposed to tannery, electroplanting and textile industrials effluents, respectively (Muley *et al*., 2007); 4.21 and 2.5%(v/v) for *Heteropneustes fossilis* and *Lebisstus reticulates*, respectively, on exposure to galvanizing and drug industries effluents (Majumdar *et al*., 2007; Deshpande and Satyanarayan, 2011); 25% (v/v) for *Cyprinus carpio* exposed to textile dyeing industry effluent (Dhanalakshmi *et al*., 2008); 17 and 10% (v/v) for *Poecilia reticulate* exposed to insecticide and pharmaceutical industrial effluents, respectively (Malik *et al*., 2012); 15% for fish, *Lepidocephalus thermalis* exposed to sugar factory effluent (Hyalij, 2013); 0.259% (v/v) for *Lebisstus reticulates* exposed to industrial effluent of pesticide producing industry (Chavan *et al*., 2016); 0.8% and 2.0 % for *Cyprinus carpio* exposed to untreated (Ramakrishnan *et al*, 2005) and treated(Prakash and Singh, 2020) distillery effluent; 8.223% (v/v) for *Mystus vittatus* exposed to distillery effluent (Tiwari and Prakash, 2021).

Behavioural Responses:

The behavioural responses in effluent exposed in fishes have been found to be an extremely sensitive indicators of industrial effluent toxicity (Yadav *et al*., 2007). The alterations in behavioural activities indicate disturbance of body function. So, the behavioural responses are the most significant parameter for determining the toxicity of any toxicant (Doving, 1992). In the present study, the detrimental behavioural alterations observed in test fish with the exposure period and sub-lethal concentration of paper mill effluent at $1/20^{th}$, $1/15^{th}$ and $1/10^{th}$ of LC_{50}. The fishes kept in tap-water (Control) behaved as normal but the fishes exposed to sub-lethal concentration of papermill effluent showed abnormal behavioural responses. The behavioural activities such as equilibrium status, operculum movement, swimming rate, surfacing activity, movement of fin, redness of eyes and mucus secretion were altered in effluent exposed fishes in comparison to control are presented in Table 3.

Table 3: Impact of Sub-lethal concentration of Paper Mill Effluent on the Behavioural parameters of *Mystus vittatus* (Bloch) exposed to 96 hours

Conc. of Paper mill Effluent	Equilibrium status	Operculum Movement	Swimming Rate	Surfacing activity	Fin Movement	Redness In Eye	Mucus Secretion
Control	N	N	N	N	N	N	N
$1/20^{th}$ of LC_{50}	N	+	+++	+	+	-	+
$1/15^{th}$ of LC_{50}	-	++	++	++	++	+	++
$1/10^{th}$ of LC_{50}	--	+++	+	+++	+++	++	+++

(N) Normal; (-) decrease; (+) slightly increase; (++) moderately increase; (+++) much increased

It was observed that with the increasing concentration these activities were relatively altered, expressing the sign of stress. Later on, fish struggled hard for breathing with increasing swimming rate and indicates poor response to external stimulant. The detrimental effect of toxic components resided in industrial effluent in behavioural parameters is accountable in effluent exposed fishes by some workers (Yadav *et al*., 2007; Srivastava *et al*., 2007; Malik *et al*., 2012; Ikpi and Offem, 2013; Dhanalakshmi *et al*., 2018; Srivastava *et al*., 2019; Prakash and Singh, 2020; Singh and Pandey, 2020; Tiwari and Prakash, 2021).

The alteration in equilibrium status in effluent exposed fishes noticed in the present study may be due to nonfunctioning of the brain (Yadav *et al*., 2007) or due to variation in enzymatic

activities and biogenic amine (Yadav *et al*., 2005). The operculum beating and surfacing activity of effluent exposed fish rises with increasing the concentrations of effluent. The physiological adaptation of fish to hypoxic conditions may be the cause of the increase in operculum movement. Fishes' opercular activity, or breathing rate, rises in hypoxic environments to compensate the low pO_2 level in blood. In an effort to prevent breathing in the contaminated water, there is an increase in surfacing activity and gulping of surface water. The fish eventually wrap their body, gills, and buccal cavity with a substantial amount of mucus as a defence mechanism to counteract the poisonous effects of paper mill effluent. Another possible explanation for fishes' frequent surfacing is mucus-induced gill shock. The increase in surfacing activity for gulping the surface water was observed in case of test fishes exposed to maximum sub-lethal concentration i.e., $1/10^{th}$ of LC_{50} of untreated effluent might be due to greater oxygen requirement in the effluent exposed fishes (Singh and Pandey, 2020). Therefore, the impairment of sensory organ systems, specifically the mechano and chemo-receptor systems, can be linked to any behavioural abnormalities in orientation and locomotion that were seen in the current study. Hence, any impairment of these organs would produce behavioural changes in the fishes. Therefore, the ethological responses observed in distillery effluent exposed fish, *Mystus vittatus* might be contributing to the mortality during acute toxicity test in these stressed fishes. These findings implied that the type of effluents released by the industries and the species of fish exposed to them determined the degree of toxicity. Based on their physiological and ecological conditions, it is considered that catfish and airbreathing fishes were more tolerant of industrial effluent than indigenous fish species.

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