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Acute Toxicity and Ethological Alterations in *Channa punctata* (Bloch) Exposed to Sugar Factory Effluent

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Abstract: The sugar factory effluent is recognized as a significant environmental pollutant and a major consumer of natural resources. This study investigates the impact of different concentrations and exposure durations of sugar factory effluent on the mortality and behavioural responses of the freshwater fish *Channa punctata* (Bloch). The LC_{50} values of sugar factory effluent for *Channa punctata* were determined as 23.85%, 18.63%, 14.97%, and 10.56% (v/v) for 24, 48, 72, and 96 h of exposure, respectively. Results indicated that both effluent concentration and exposure duration significantly influenced fish mortality. Behavioural abnormalities were also observed in effluent exposed fish, including increased surface activity, rapid fin and opercular movements, reddening of the eyes, excessive mucus secretion, and uncoordinated swimming or loss of equilibrium. These responses intensified with higher concentrations and longer exposure periods, clearly reflecting stress symptoms. Thus, the present study revealed that sugar factory effluent is potentially toxic to fish. Since *Channa punctata* is highly sensitive to this effluent, regular monitoring of natural water-bodies is essential to minimize ecological risks.

Keywords: Acute toxicity, LC_{50}, Mortality, Behavioural responses, *Channa punctata*, Sugar factory effluent

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Introduction

All industries discharge their effluents indiscriminately in the adjoining water bodies which cause serious hazards to aquatic life. Industrial effluents are the major sources of heavy metal pollution that is released into water bodies and causes water pollution worldwide. The environmental pollution issue arising from industrial growth mainly involves the disposal of industrial waste, which can be solid, liquid, or gaseous. Each of these waste types poses a risk of polluting water (Singh, 2021). Deterioration of water quality due to human activities has created a need for developing and adjusting methods for environmental quality. Due to urban, industrial and agricultural activities, freshwater sources are dumped with different kinds of chemicals that affect the inhabiting biota. In order to evaluate the adverse effects of these complex chemical mixtures on aquatic organism, there is a worldwide trend to complement chemical and physical parameters with biomarkers in aquatic pollution monitoring.

Human activities have further accelerated water quality deterioration, creating an urgent need for developing and adapting methods to assess and maintain environmental standards. Freshwater sources are increasingly contaminated with diverse chemicals from urbanization, industrialization, and agricultural practices, severely affecting aquatic biota. To better evaluate the adverse effects of these complex chemical mixtures on aquatic organisms, there is now a global shift towards integrating biological markers (biomarkers) alongside traditional chemical and physical parameters in aquatic pollution monitoring (Verma and Prakash, 2022).

India is recognized as the original home of sugar and sugarcane. The sugar industry ranks second among agro-based industries in the country, playing a vital role in supporting the rural economy while also contributing significantly to the national economy (Sahu *et al*., 2017). Sugar factories generally operate seasonally for about 120 to 200 days each year (Saranraj and Stella, 2014; Latha *et al*., 2018) and process more than 70% of the world's sugarcane to produce raw sugar (Patil and Kulkarni, 2022). Sugar manufacturing in India follows two major processes: carbonation and sulphitation. Among these, the double sulphitation process is the most widely used method for producing white sugar.

The sugar production process involves several stages: crushing sugarcane to extract juice, clarifying it to remove impurities, evaporating water to concentrate the juice, crystallizing to form sugar crystals, and centrifuging to separate the crystals from molasses. Together, these steps produce high-purity refined white sugar (Devi *et al*., 2025). Throughout this process, a substantial amount of water is consumed, leading to the continuous generation of wastewater. The effluents produced are predominantly organic in nature, with minor inorganic components. The major by-products and discharges include wastewater, bagasse, press mud, and molasses (Latha *et al*., 2018). Effluents from sugar factories, when discharged onto land or into surface water bodies, adversely affect water quality and soil health, while also contaminating groundwater through the percolation of water-soluble pollutants (Deshmukh, 2014). The release of untreated effluents into aquatic systems leads to a depletion of dissolved oxygen, causing serious harm to aquatic life.

The harmful effects produced by any toxicant or pollutant on an organism are referred to as toxicity (Prakash and Verma, 2021; Verma and Prakash, 2021). Toxicity tests are conducted to determine the concentrations or doses of a toxicant, along with the duration of exposure, required to elicit specific effects. Since organisms vary in their sensitivity, toxicity is species-specific, with individuals showing different responses to the same dose of a toxic substance (Verma and Prakash, 2022).

Toxicity bioassays are employed to detect and quantify the potential toxicological effects of chemicals on living organisms. The data obtained from these tests serve as a basis for assessing the risks associated with the environments in which organisms live. Several methods have been developed to evaluate chemical hazards, including acute, sub-acute, and chronic toxicity tests. Acute toxicity refers to the severe effects experienced by an organism following short-term exposure to toxic chemicals (Koprucu *et al*., 2006). Acute toxicity tests are particularly useful for determining the potential toxicological effects of chemicals, as well as the concentrations of toxicants and duration of exposure required to produce adverse outcomes.

Acute toxicity data are crucial for determining sub-lethal concentrations to be used in chronic toxicity tests. Since different species respond differently to the same dose of a toxicant, the toxicity of any chemical is considered species-specific (Jaiswal *et al*., 2024). These tests generate valuable datasets that help evaluate the potential risks organisms face in their environment. A common parameter derived from acute toxicity studies is the LC_{50}, which represents the concentration of a toxicant required to cause 50% mortality in a test population. This is determined experimentally by exposing groups of organisms to varying concentrations of the toxicant and recording mortality over specific time intervals, typically 24, 48, 72, and 96 h. Thus, acute toxicity data provide a scientific basis for identifying sub-lethal concentrations essential for conducting chronic toxicity assessments.

Probit analysis is a regression technique commonly used in toxicology to analyze binomial response variables and assess the relative toxicity of a toxicant or pollutant to living organisms (Singh and Zahra, 2017). The method involves exposing test organisms to varying concentrations of a toxicant and recording their responses, which are then compared to determine the concentration levels at which effects occur. The Probit method is widely recognized as the most accurate and reliable approach for calculating LC_{50} the concentration or dose required to cause 50% mortality in a test population. During toxicity tests, precautions are taken to minimize contamination from metabolic wastes to ensure accuracy (Tiwari and Prakash, 2021).

Fishes hold direct economic importance and are highly sensitive to the diverse pollutants discharged into aquatic ecosystems. Their sensitivity makes them valuable bio-indicators for assessing water quality. Water pollution can severely affect fish, often leading to lethal outcomes, and exerts harmful impacts on all aquatic flora and fauna. Much of this damage arises from human activities, highlighting the urgent need for focused attention and the implementation of appropriate corrective measures. To understand these effects, toxicity tests are commonly conducted on fish under controlled laboratory conditions to evaluate the impact of industrial effluents.

In addition to causing mortality, pollutants can alter fish behaviour by affecting the physico-chemical and biological parameters of water. Fish are more sensitive to contaminants than many other aquatic organisms, and changes in their behaviour are considered a reliable indicator of toxicity (Yadav *et al*., 2007). Through ethological responses, organisms adapt to both internal and external stimuli, helping them cope with environmental changes. Behavioural changes in fish due to effluent exposure include alterations in activity, movement, feeding, and social interactions caused by pollutants or waste released into water bodies (Devi *et al*., 2025). These behavioural anomalies can serve as effective biomarkers, providing valuable information about environmental conditions (Dhanalakshmi *et al*., 2018). Because of their sensitivity to changes in the aquatic environment, fish are excellent bio-indicators and play a crucial role in monitoring water pollution (Srivastava *et al*., 2010).

Therefore, the present investigation aimed to evaluate the acute toxicity and determine the LC_{50} of sugar factory effluent on the freshwater fish *Channa punctata* using SPSS. Additionally, the study examined the ethological responses of the fish resulting from exposure to the effluent. The findings are expected to provide baseline data that can be used to assess the relative sensitivity of *Channa punctata* to the effluent and to help establish acceptable concentration levels for environmental management.

Materials and Methods

The freshwater fish *Channa punctata* (Bloch) were collected from local freshwater bodies in and around Ayodhya, Uttar Pradesh, India. Healthy individuals of uniform size (length: 8.8 ± 0.20 cm; weight: 9.5 ± 1 g) were selected for the acute toxicity test. The fishes were transported to the

Table 1: Mortality of *Channa punctata* (Bloch) at different time intervals after treatment with different concentrations of Sugar factory effluents

Concentration of Sugar factory effluent (ml/25L)	Log Conc. of Effluent	No. of Test fishes	Per cent Mortality at different Exposure Period (h)			
			24	48	72	96
0 (Control)	-	10	0	0	0	0
100	2.000	10	0	0	0	0
200	2.301	10	0	1	1	3
300	2.477	10	0	2	3	6
400	2.602	10	1	3	5	8
500	2.699	10	2	4	7	9
600	2.778	10	4	6	8	10
700	2.845	10	6	8	10	-
800	2.903	10	9	10	-	-
900	2.954	10	10	-	-	-

laboratory in plastic containers to prevent injury during transit. Upon arrival, they were disinfected with a 1.0% $KMnO_4$ solution for 5 min to eliminate any dermal infections. Before experimentation, the fishes were acclimated to laboratory conditions at room temperature for two weeks. During the first week, aeration was provided using an artificial aerator. Fishes were regularly fed with commercial food pellets, and the medium (tap water) was replaced every two days to remove leftover food and faecal matter. Feeding was discontinued 24 h prior to the toxicity test to minimize contamination from metabolic wastes.

The sugar factory effluent from Balrampur Chini Mills Ltd. was collected at the outlet of the effluent treatment plant in sterilized plastic containers and transported to the laboratory for experimentation. A series of dilutions were prepared by mixing the effluent with tap water in separate glass aquaria. Following acclimatization, ten individuals of the test fish *Channa punctata* were carefully introduced into aquaria containing different effluent concentrations. Mortality was recorded at 24 h intervals up to 96 h. The lethal concentration at which 50% of the test organisms survived after a specified exposure period (LC_{50}) was determined. In this study, LC_{50} values for 24, 48, 72, and 96 h were calculated, and statistical analysis was performed using SPSS software. The percentage concentration of each test solution is obtained by using the following formula (FAO, 1984):

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

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

Results and Discussion

Bioassay Assessment:

In order to estimate the LC_{50}, the fishes of different experimental sets have been treated with different concentrations of Sugar factory effluent. Ten concentrations 0 ml/25L, 100 ml/25L, 200 ml/25L, 300 ml/25L, 400 ml/25L, 500 ml/25L, 600 ml/25L, 700 ml/25L, 800 ml/25L and 900 ml/25L have been selected and for each concentration, the mortality of fishes at different time intervals viz. 24, 48, 72 and 96 h were observed. The mortality of fishes after exposure to sugar factory effluent is presented in Tables 1-3. The relation between number of fish died and concentration of sugar factory effluent are shown in Table 1, by Finnery's Probit analysis method and in Tables 2 and Figures 1-4 by SPSS Statistical Software.

Acute toxicity test i.e. LC_{50} values show susceptibility of fish to particular contaminants and reflect their survival potential. The LC_{50} values

Table 2: LC_{50} values with 95% confidence limits for *Channa punctata* (Bloch) exposed to Sugar factory effluent at different exposure period

Exposure Period (h)	LC_{50} values of sugar factory effluent ml/25L (v/v%)	95% Confidence limits (v/v%)		Regression Equation	Chi Square Value (P value)	Coefficient of determination (R^2Linea)
		Lower Limit	Upper Limit			
24	610.700 (23.85%)	548.327	675.965	Y= -22.59+8.11X	2.307 (0.941)	0.935
48	474.576 (18.63%)	394.387	569.883	Y=-9.81+3.63X	3.960 (0.682)	0.925
72	380.019 (14.97%)	310.751	449.238	Y=-11.63+4.49X	1.391 (0.925)	0.998
96	266.770 (10.56%)	205.780	319.900	Y=-11.00+4.55X	0.757 (0.948)	1.000

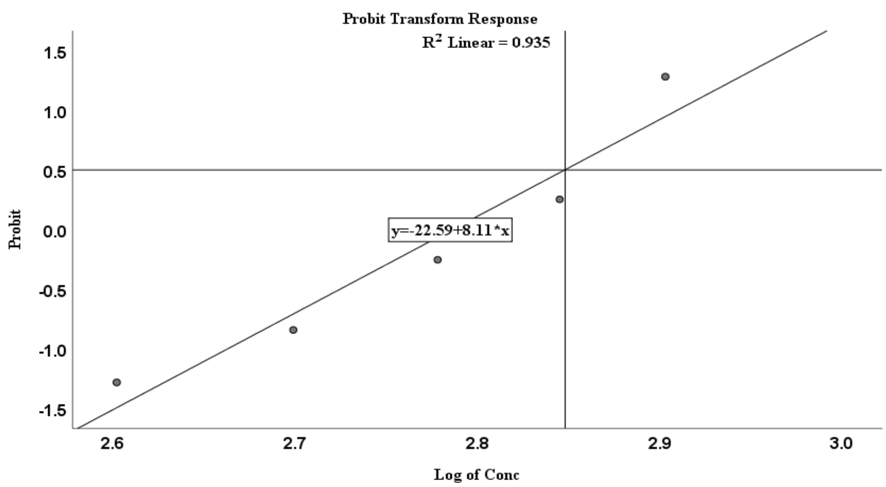

Fig. 1: Regression Line (Based on Probit analysis) of Log concentration of Sugar factory effluent Vs % mortality of *Channa punctata* (Bloch) (at 24 h).

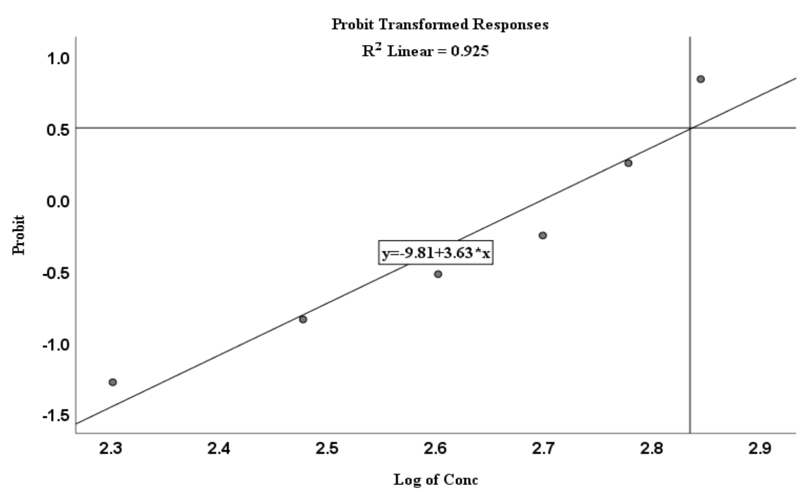

Fig. 2: Regression Line (Based on Probit analysis) of Log concentration of Sugar factory effluent Vs % mortality of *Channa punctata* (Bloch) (at 48 h).

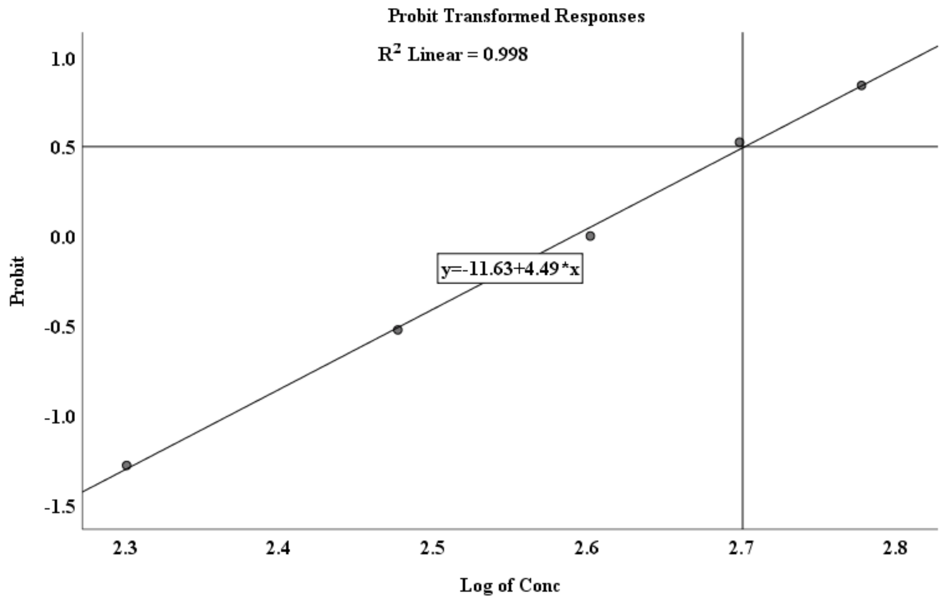

Fig. 3: Regression Line (Based on Probit analysis) of Log concentration of Sugar factory effluent Vs % mortality of *Channa punctata* (Bloch) (at 72 h).

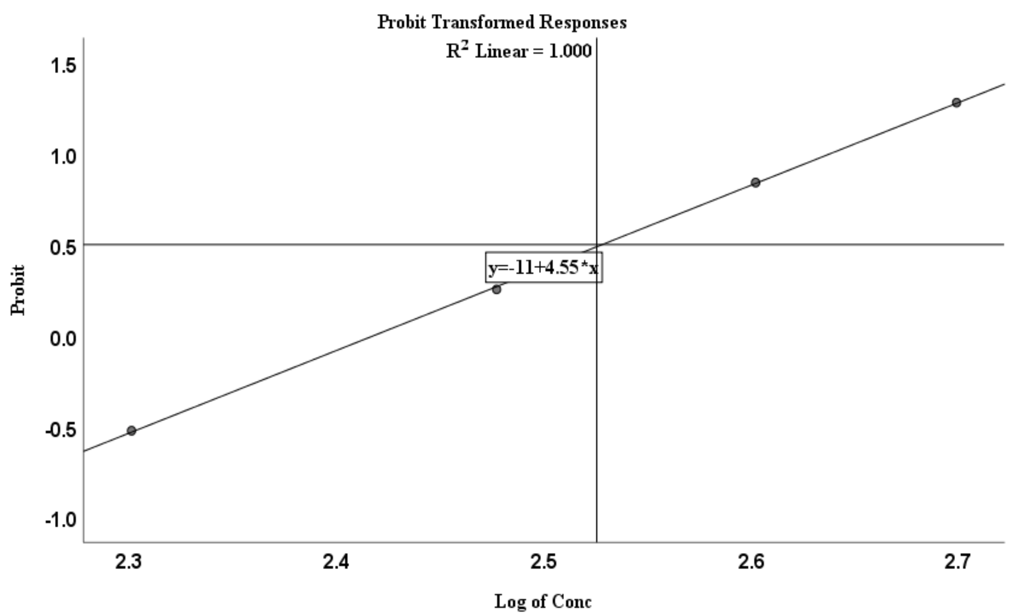

Fig. 4: Regression Line (Based on Probit analysis) of Log concentration of Sugar factory effluent Vs % mortality of *Channa punctata* (Bloch) (at 96 h)

for *Channa punctata* after exposure to Sugar factory effluent after 24, 48, 72, and 96 h were 23.85%, 18.63%, 14.97% and 10.56% (v/v), respectively (Table 2; Figs. 1-4).

Prakash and Capoor (2018) have reported LC_{50} values as 257.59 ml/25L (or 10.21% v/v) at 96 h for sugar factory effluent exposed *Channa punctatus*) that is more or less similar to present finding. LC_{50} values at 96 h of sugar factory effluent were observed for *Mystus vittatus* as 3.10% v/v (Verma and Prakash, 2022); for *Rasbora daniconius* as 11% v/v (Kakade, 2017) and for *Lepidocephalus thermalis* as 15% v/v (Hyalij, 2013).The findings of the present study revealed that the mortality rate of fish increased with increased concentrations of sugar factory effluent, and at a given concentration, mortality rate also increased with longer exposure period. Similar patterns have been reported by other researchers (Yadav *et al*., 2007; Mishra *et al*., 2011; Pandey *et al*., 2018; Prakash and Singh, 2020; Prakash and Verma, 2020; Tiwari and Prakash, 2021; Verma and Prakash, 2022). The results suggest that the presence of harmful chemicals or toxicants in the effluent, at dangerously high levels, leads to fish mortality. Such mortality may arise from the disruption of physiological and biochemical processes involved in cellular metabolism. The toxicity of any chemical to fish depends on several abiotic factors, including species, sex, age, weight, exposure duration, dose or concentration, and whether the toxicant is organic or inorganic (Tiwari and Prakash, 2021).

The 96 h LC_{50} values of industrial effluents for different fish species have been investigated by several researchers. Reported values include 70% (v/v) for *Channa striatus*, 2.35% (v/v) for *Heteropneustes fossilis*, and 0.80% (v/v) for *Labeo rohita* exposed to fertilizer industry effluent (Singh *et al*., 2019); 20%, 6.0%, and 22% (v/v) for *Labeo rohita* exposed to tannery, electroplating, and textile industry effluents, respectively (Muley *et al*., 2007); 4.21% and 2.5% (v/v) for *Heteropneustes fossilis* and *Lebistes reticulatus*, respectively, exposed to galvanizing and drug industry effluents (Deshpande and Satyanarayan, 2011); 25% (v/v) for *Cyprinus carpio* exposed to textile dyeing industry effluent (Dhanalakshmi *et al*., 2008); and 17% and 10% (v/v) for *Poecilia reticulata* exposed to insecticide and pharmaceutical industry effluents, respectively (Malik *et al*., 2012). Similarly, 0.259% (v/v) was reported for *Lebistes reticulatus* exposed to effluent from a pesticide-producing industry (Chavan *et al*., 2016); and 0.8% and 2.0% (v/v) for *Cyprinus carpio* exposed to untreated (Ramakrishnan *et al*., 2005) and treated (Prakash and Singh, 2020) distillery effluent, respectively. In addition, 8.223% (v/v) was recorded for *Mystus vittatus* exposed to distillery effluent (Tiwari and Prakash, 2021).

Ethological Responses:

The behavioural responses of fishes to effluent exposure 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. The behavioural responses are the most significant parameter for determining the toxicity of any toxicant (Tiwari and Prakash, 2021). In the present investigation, the detrimental behavioural alterations were observed in test fish after 96 h exposure period at sub-lethal concentration of sugar factory effluent. The fishes kept in tap-water (control) behaved as normal but the fishes exposed to sub-lethal concentration of effluent showed abnormal behavioural responses. The behavioural activities such as equilibrium status, operculum movement, swimming rate, surfacing activity, movement of fin and mucus secretion were altered in effluent exposed fishes in comparison to control (Table 3).

Behavioural responses in effluent-exposed fishes are highly sensitive indicators of industrial effluent toxicity (Yadav *et al*., 2007). Alterations in these activities reflect disturbances in normal body functions, making behavioural responses one of the most important parameters for assessing the toxicity of any toxicant (Tiwari and Prakash, 2021).

It was observed that with increasing concentrations of sugar factory effluent, the behavioural activities of the fishes were progressively altered, indicating signs of stress. As exposure intensified, the fishes exhibited laboured breathing, increased swimming rates, and a diminished response to external stimuli. Such detrimental effects of toxic components on behavioural parameters of fishes have also been reported by other researchers (Yadav *et al*., 2007;

Table 3: Impact of Sub-lethal concentration of Sugar factory effluent on the behavioural parameters of *Channa punctata* (Bloch) exposed to 96 h

Conc. of Sugar factory Effluent	Equilibrium status	Operculum Movement	Swimming rate	Surfacing activity	Fin Movement	Mucus Secretion
Control	N	N	N	N	N	N
1/15th of 96 h LC_{50}	N	+	+++	+	+	+
1/10th of 96 h LC_{50}	N	++	++	++	++	++
1/5th of 96 LC_{50}	--	+++	+	+++	+++	+++

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

Srivastava *et al*., 2007; Srivastava *et al*., 2010; Malik *et al*., 2012; Dhanalakshmi *et al*., 2018; Prakash and Singh, 2020; Singh and Pandey, 2020; Tiwari and Prakash, 2021).

The alteration in equilibrium status observed in effluent-exposed fishes in the present study may be attributed to impaired brain functioning or variations in enzymatic activities and biogenic amines (Yadav *et al*., 2007; Tiwari and Prakash, 2021). Opercular beating and surfacing activity increased with rising concentrations of effluent, likely as a physiological adaptation to hypoxic conditions. In such environments, the rate of opercular movement rises to compensate for the reduced pO_2 level in the blood. Similarly, increased surfacing and gulping of surface water reflect attempts to avoid breathing in contaminated water. As a further defense mechanism, the fishes secreted excessive mucus covering the body, gills, and buccal cavity to counteract the toxic effects of the sugar factory effluent.

Another possible explanation for the frequent surfacing of fishes is mucus-induced gill shock. The increased surfacing activity, particularly for gulping surface water, was most evident in test fishes exposed to the highest sub-lethal concentration (1/5th of LC_{50}), which may be attributed to a greater oxygen demand under effluent stress (Singh and Pandey, 2020). The observed behavioural abnormalities in orientation and locomotion can be linked to impairment of sensory organ systems, especially the mechano- and chemo-receptors. Any damage to these systems is likely to induce significant behavioural alterations in fishes. Thus, the ethological responses observed in *Channa punctata* exposed to sugar factory effluent may have contributed to mortality during the acute toxicity test in these stressed individuals.

Conclusion

It is concluded that aquatic animals are sensitive to toxicant present in the industrial effluent or in aquatic ecosystem and that cause deleterious effects to them. The findings of the present investigation demonstrate that sugar factory effluent is highly toxic and capable to disturb the normal life of aquatic animals. Therefore, effective treatment of effluents prior to their discharge is necessary to prevent harmful effects on aquatic ecosystems. These findings suggest that the degree of toxicity depends both on the type of industrial effluent released and on the fish species exposed. Thus toxicity studies help to determine possible limit of a toxicant in an ecosystem and helps to propose policies to protect the aquatic ecosystem.

Ethical Statement

The authors confirm that all ethical issues have been dealt with the research ethics guidelines provided by the Department of Zoology, Shri L.B.S. Degree, College, Gonda, Uttar Pradesh, India.

Author Contributions

Each author has contributed equally to the study's planning, analysis, writing, and editing. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare no conflicts of interest.

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