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Original Research Article

Toxicity and Ethological Responses of *Mystus vittatus* (Bloch) Exposed to Distillery Wastewater

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ABSTRACT:
This paper deals with the acute toxicity of distillery wastewater on freshwater catfish, *Mystus vittatus* (Bloch), at different concentration and duration of exposure on the mortality and ethological alterations. The LC_{50} for 96 hours of treated distillery effluent for *Mystus vittatus* was 3.38 % (v/v). The result revealed that mortality rate depends upon concentrations of distillery wastewater and duration of exposure. The effluent exposed test fish showed alterations in behavioural responses viz., hyperactivity (jumping and surface activity), convulsion, somersaulting activity, eye and fin movement and equilibrium status. It was observed that with the increasing concentration and exposure period these activities were relatively increased, expressing the sign of distress. Thus, the result of present study revealed that fish, *Mystus vittatus* are sensitive to distillery wastewater and can be used as biological indicators.

Keywords: Acute toxicity, Ethological responses, *Mystus vittatus*, Distillery effluent.

INTRODUCTION

Industrial effluents contain highly toxic chemicals that may pollute the aquatic environment so the safe disposal of waste water discharged from various industries is a serious problem worldwide. Water pollution by discharging of effluents from various industries like chemical, pesticides, fertilizer, pulp and paper, sugar and distillery etc. causes serious problems by altering the physical, chemical and biological characteristic of water in many waterbodies such as rivers, streams, reservoirs, lakes, wetlands, pounds and ditches (Tiwari and Prakash, 2021). The discharges of untreated and partially treated industrial effluent depleted the dissolved oxygen content of waterbodies so by interfering with respiratory metabolism they seriously affects aquatic biota and their production because they contain toxic substances detrimental to health (Prakash and Singh, 2020).

Discharges of industrial wastewaters in aquatic waterbodies cause serious

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consequence for inland fisheries resulting in impairment of important function such as respiration, reproduction and osmoregulation (Kumaraguru, 1995). The changes in physio-chemical and biological properties of water change the behavior of fishes besides causing mortality. The behavioural changes in fishes has been considered to be sensitive indicator of toxicity and among aquatic fauna, fishes are more sensitive to pollutants (Yadav *et al*, 2007). Behaviour allows an organism to adjust to external and internal stimuli in order to meet the challenges of surviving in the changing environment. The use of these abnormalities in behavior of fish as biomarkers has become more prevalent in recent years because they can provide suitable indication about the environmental conditions (Dhanalakshmi *et al.,* 2018).

Distilleries releases enormous amount of waste water known as 'spent wash' which has a typical unpleasant odour of fruity smell and dark brown colour. It has large amount of heavy metals, high COD, BOD and TSS with low pH and DO, causing pollution in the receiving water. Distillery wastewater also contain various types of organic pollutants like phthalates (endocrine disrupter) which causes the hormonal imbalance and disturb the reproductive fitness of living organism and ultimately leading to the carcinogenesis (Chowdhary *et al*., 2018). In a developing country like India, distilleries are one of the most polluting industries as 88% of its raw materials are converted into waste and discharged into the water bodies, causing water pollution (Ansari *et al*., 2012). Therefore, Government of India declared distillery industry as highly polluted industry (Thakkar, 2013) .These water pollutants accumulated in the body tissues of fish and via food chain reach the human body causing human affliction. Therefore, the main objectives of present investigation were to determine the 96 hours LC_{50} of distillery effluent and to study ethological changes in freshwater catfish, *Mystus vittatus.*

MATERIALS AND METHODS

Collection and maintenance of Test Fish: The freshwater catfish, *Mystus vittatus* were collected from local fresh waterbodies, washed with 1% solution of $KMnO_4$ for five minutes to remove any dermal infection and then transferred to the plastic jar containing 50L dechlorinated tap water for acclimatization. The collected fishes were acclimated to laboratory conditions for 15 days at room temperature (26 ± 1.4°C) and were fed daily on commercially available food pellets. After acclimation the only healthy fishes were used for experiments. Feeding was stopped 24 hours prior to the toxicity test, to minimize the contamination from metabolic wastes.

Preparation of Stock Solution: The distillery wastewater was collected from the discharged point from distillery unit of Balrampur Chini Mills Ltd. The percent concentration of industrial wastewater was prepared on volume to volume (v/v) ratio. The percentage concentration of test solution is obtained by using the formula (FAO, 1984):

$$\text{Volume percent} = \frac{V_E}{[V_E + V_{DW}]} \times 100$$

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

Determination of Acute toxicity (LC_{50}): The acute toxicity experiments were repeated three times for a concentration with a batch of twelve fishes. Healthy twelve test fish *Mystus vittatus* (7.5±0.21cm and 7.5±1 gm) were placed in each aquaria containing 12 L of water (n=12/ aquarium). The distillery waste water samples were added as per following concentration- control, 0.75, 1.50, 2.25, 3.00, 3.75, 4.50, 5.25 and 6.00 % (v/v). In order to maintain the concentration of effluent in the test aquaria water was renewed every 24 hours and different concentrations of fresh effluents were added. Fish kept in effluent free medium served as the control. The test was performed for 96 hours treatment period and dead fish were removed as the experiment proceeded. The number of dead fish per group was recorded against the time of their mortality in a tabular form, as specified by Sprague (1969). The 96 hours LC_{50} value of effluent was calculated using arithmetic method of Karber as modified by Dede and Kaflo (2001).

$$LC_{50} = LC_{100} - \sum \frac{(\text{Mean mortality x Concentration difference})}{\text{Number of Fish in each set of experiment}}$$

RESULTS AND DISCUSSIONS

Toxicity Assessment

Acute toxicity test show susceptibility of fish to particular contaminants and reflect their survival potential. Therefore, LC_{50} is the most reliable and preliminary test for toxicity evaluation in fish (Shreelekshmy *et al.*, 2016). In the present investigation, the $LC_{50}$for 96 hours of distillery effluent for *Mystus vittatus* was determined as 3.38% (v/v)(Table2). However, fishes exposed to dechlorinated tap-water were observed to be healthy and normal.

However, reports regarding lethal toxicity in fishes exposed to distillery effluent are very scanty. LC_{50} values at 24, 48, 72 and 96 h for *Cyprinus carpio* exposed to untreated distillery effluent were 1.2%, 1.1%, 1.0% and 0.8, respectively (Ramakrishnan *et al.*, 2005) whereas LC_{50} values at 24, 48, 72 and 96 h for *Cyprinus carpio* exposed to treated distillery effluent were 2.8, 2.5, 2.3 and 2.0, respectively (Prakash and Singh, 2020) in *Cyprinus carpio* (2.0% v/v) exposed to distillery effluent. Accordingly, catfish were tolerant to industrial effluent than carps due to their physiological and ecological condition (Singh *et al*., 2019).

Table1: Survival of fish, *Mystus vittatus* at different concentrations and sampling intervals.

Concentra-tion % (v/v)	Number of alive fish at different time intervals (hours)				% Survival at 96 hours	% Mortality at 96 hours
	24	48	72	96		
Control	12	12	12	12	100	0
0.75	12	12	12	12	100	0
1.50	12	12	11	10	83.33	16.67
2.25	12	11	10	9	75.00	25.00
3.00	11	10	9	7	58.33	41.67
3.75	10	9	8	5	41.67	58.33
4.50	9	8	6	3	25.00	75.00
5.25	8	6	4	2	16.67	83.33
6.00	7	5	2	0	0	100

Table 2: Determination of LC_{50} value of distillery effluent for *Mystus vittatus* at 96 hours

Concentra-tion % (v/v)	Concentration difference	Number of Alive fish	Number of Dead fish	Mean mortality	Mean mortality x concentration difference
Control	0	12	0	0	0
0.75	0.75	12	0	0	0
1.50	0.75	10	2	1	0.75
2.25	0.75	9	3	2.5	1.875
3.00	0.75	7	5	4.0	3.000
3.75	0.75	5	7	6.0	4.500
4.50	0.75	3	9	8.0	6.000
5.25	0.75	2	10	9.5	7.125
6.00	0.75	0	12	11.0	8.250
					$\sum$=31.50
LC_{50} = 6.00 – (31.50/ 12)= 6.00- 2.625 = **3.375 % (v/v)**					

In the present study the result revealed that the mortality rate increased with increasing concentration of distillery effluent and for a particular concentration the mortality rate increase with increasing period of exposure. Similar observations were found by some researchers (Yadav *et al.*, 2007; Mishra *et al*., 2011; Singh *et al*., 2019; Prakash and Singh, 2020). This reflects the regular mode of action, due to harmful chemicals and toxicant of wastewater up to dangerous level that cause fish death. The death of fish could be due to the lethal action of distillery effluent that causes alterations in physiological and biochemical process related to cellular metabolic pathway. The toxicity of any toxicant for fish depends on species, sex, age, weight, dose or concentration, exposure period, organic or inorganic form etc. (Amsath *et al*., 2017). Yadav *et al.*, (2007) concluded that the mortality rate is rapid in fishes exposed to short duration in wastewater as a result of low dissolved oxygen and adverse effect of pollutants on their gills. Some worker reported that physicochemical factors of water are also responsible for mortalities in fishes such as BOD, COD, pH, Dissolved oxygen and temperature etc which were present beyond their prescribed standards (Hingorani *et al.*; 1979; Somanath, 2002; Singh *et al*., 2019; Prakash and Singh, 2020). Moreover, effluent released from paper mill, sugar factory, fertilizer industry etc are reported to have a large number of toxicants, resulting in mortality of aquatic animals (Edwards, 1973). Singh *et al*., (2019) concluded that inhibition of AChE, acetylcholinesterase enzyme (an acetylcholine hydrolyzing enzyme) by industrial effluents results in accumulation of acetylcholine in synaptic cleft, causing neuromuscular paralysis and asphyxiation, resulting finally in mortality. Thus, it can be concluded that presence of large number of heavy metals, high COD, BOD and TSS with low pH and DO in distillery effluent inhibit the AChE enzyme activity, resulting in mortality of stressed fishes.

The LC_{50} values of different industrial wastewaters for 96 hours exposure of different fish species have been reported by various researchers. These values were 70%(v/v) in *Channa striatus*, 2.35%(v/v) in *H. fossilis*, 0.80%(v/v) in *L. rohita* for fertilizer industry effluent, respectively (Singh *et al*., 2019); 20, 6 and 22%(v/v) in *Labeo rohita* for tannery, electroplanting and textile effluents, respectively (Muley *et al*., 2007); 4.21 and 2.5%(v/v) in *H. fossilis* and *Lebisstus reticulates*, respectively, exposed to galvanizing and raw bulk drug industrial effluents (Majumdar *et al*., 2007; Deshpande and Satyanarayan, 2011); 25% (v/v) in *Cyprinus carpio* exposed to textile dyeing industry effluent (Dhanalakshmi *et al*., 2008); 17 and 10% (v/v) in fresh water fish, *Poecilia reticulate* exposed to insecticide and pharmaceutical industrial effluents, respectively (Malik *et al*., 2012); 15% in fish, *Lepidocephalus thermalis* exposed to sugar factory effluent (Hyalij, 2013); 0.259% (v/v) in *Lebisstus reticulates* exposed to the untreated pesticide producing industrial effluent (Chavan *et al*., 2016);9.5%, 25% and 15% in fish, *Rasbora daniconius*, *Puntius stigma* and *Channa punctatus* exposed to paper mill effluent, respectively (Pathan *et al*., 2009; Sarwade, 2015; Prakash and Verma, 2020). These results suggested that the level of toxicity was dependent on nature of effluents discharged by the industries and the fish species exposed to these effluents. In general, it is concluded that catfish and air breathing fishes were more tolerant to industrial effluent than indigenous as well as exotic carps due to their physiological and ecological condition.

Ethological Responses

The changes in the behavioural responses are the most sensitive indication of potential toxic effects of any pollutant. The behavioural responses of the test fish, *Mystus vittatus* treated with distillery wastewater was found to depend on its concentration and duration of exposure period (Table 3.1-3.4). There were no behavioural alterations observed in the control group. In the present study, the behavioural alterations observed in test fish increased with the concentration of distillery wastewater (0.75 to 6.00 % v/v) and exposure periods (24 to 96 hours). The fish exposed at various distillery effluent concentrations showed abnormal behavioral responses in the form of hyperactivity (erratic swimming with enhance jumping, surface activity for gulping air on the surface), enhance convulsion, loss of equilibrium, reduced fin and eye movements. It was observed that with the increasing concentration and exposure period these activities were relatively increased, expressing the sign of distress. Later on fish struggled hard for breathing with increasing swimming rate and indicates poor response to external stimulant. Almost similar alterations in the behavioural parameters were observed in industrial effluent exposed fishes by some workers (Yadav *et al*., 2005 & 2007; Malik *et al*., 2012;Ikpi and Offem, 2013; Patro, 2017; Dhanalakshmi *et al*., 2018; Prakash and Singh, 2020). The loss of equilibrium status noticed in the present investigation may be due to non functioning of the brain (Yadav *et al*., 2007) and inhabitation of AChE enzyme activity.

Table 3.1: Impact of Distillery wastewater on the Behavioural parameters of *Mystus vittatus* at various concentrations (Exposure Duration 24 hours)

Concentration % (v/v)	Hyperactivity	Equilibrium status	Swimming rate	Convulsions	Somersaulting activity	Fin movement	Eye movement
Control	-	+++	+	-	+++	+++	+++
0.75	-	+++	+	-	+++	+++	+++
1.50	-	+++	+	-	+++	+++	++
2.25	-	+++	+	-	++	++	++
3.00	+	++	++	-	++	++	++
3.75	+	++	++	-	++	++	++
4.50	+	++	++	-	++	++	+
5.25	+	+	++	+	++	+	+
6.00	++	+	+++	+	+	+	+

(-)Sign indicates no detection; (+) Sign indicates the increase / decrease the level of parameters

Table 3.2: Impact of Distillery wastewater on the Behavioural parameters of *Mystus vittatus* at various concentrations (Exposure Duration 48 hours)

Concentration % (v/v)	Hyperactivity	Equilibrium status	Swimming rate	Convulsions	Somersaulting activity	Fin movement	Eye movement
Control	-	+++	+	-	+++	+++	+++
0.75	-	+++	+	-	+++	+++	+++
1.50	+	++	+	-	++	++	++
2.25	+	++	++	-	+++	++	++
3.00	+	++	++	+	+	++	++
3.75	+	+	++	+	++	++	+
4.50	+	++	++	+	++	+	+
5.25	++	++	+++	++	+	+	+
6.00	++	+	+++	++	+	+	+

(-)Sign indicates no detection; (+) Sign indicates the increase / decrease the level of parameters

Table 3.3: Impact of Distillery wastewater on the Behavioural parameters of *Mystus vittatus* at various concentrations (Exposure Duration 72 hours)

Concentration % (v/v)	Hyperactivity	Equilibrium status	Swimming rate	Convulsions	Somersaulting activity	Fin movement	Eye movement
Control	-	+++	+	-	+++	+++	+++
0.75	+	+++	+	-	++	++	+++
1.50	+	++	+	+	++	++	++
2.25	+	++	++	+	+	+	++
3.00	+	+	++	+	++	++	++
3.75	++	++	++	+	++	+	+
4.50	++	++	+++	+	+	+	+
5.25	+++	+	+++	++	+	+	+
6.00	+++	+	+++	+++	+	+	-

(-)Sign indicates no detection; (+) Sign indicates the increase / decrease the level of parameters

Table 3.4: Impact of Distillery wastewater on the Behavioural parameters of *Mystus vittatus* at various concentrations (Exposure Duration 96 hours)

Concentration % (v/v)	Hyperactivity	Equilibrium status	Swimming rate	Convulsions	Somersaulting activity	Fin movement	Eye movement
Control	-	+++	+	-	+++	+++	+++
0.75	+	++	++	-	+++	++	+++
1.50	++	++	++	+	++	+	++
2.25	++	+	++	+	+	+	++
3.00	++	+	++	++	+	+	++
3.75	+++	+	+++	++	+	+	+
4.50	+++	-	++	+++	-	+	+
5.25	+++	-	+	+++	-	+	-
6.00	-	-	-	+++	-	-	-

(-)Sign indicates no detection; (+) Sign indicates the increase / decrease the level of parameters

Thus, the any kind of behavioural alterations in orientation and locomotion, as observed in the present study, can be related to the impairment of sensory organ systems particularly the mechano and chemo-receptor systems. Sensory organs like lateral line, olfactory organs and membranous labyrinth helps the fishes in maintaining harmony with their environments and also control their vital behaviours (Mishra *et al*., 2011). 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 in these stressed fishes.

CONCLUSION

To sum up from the observations made in the present study, we have concluded that the distillery wastewater possesses large amount of pollutant which are responsible for alterations in behavioural responses and mortality of fishes. It is therefore, needless to say, that the distillery effluent has large number of heavy metals, high COD, BOD and TSS with low pH and DO, which have adverse impact on aquatic organism like fish and therefore, they become potent abiotic factors adversely affecting the aquatic ecosystem. Thus it is concluded that the freshwater catfish, *Mystus vittatus* is sensitive to distillery wastewater stress.

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