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Impact of Distillery Effluent on Relative Growth Rate and Oxygen Consumption Rate of Freshwater Catfish, *Mystus vittatus* (Bloch)

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Abstract: Present investigation has been carried out to study the physico-chemical parameters of raw distillery effluent and also to determine the chronic impact of sublethal concentrations of raw distillery effluent on mortality or survival rate, relative growth rate and rate of oxygen consumption of effluent exposed fish, *Mystus vittatus*. The result of physico-chemical characteristics of raw distillery effluent revealed that pH was acidic and other parameters such as electrical conductivity, total dissolved solids, hardness, total alkalinity, biological oxygen demand and chemical oxygen demand were found to be beyond the permissible limits of ISI, CPCB India and WHO. On the basis of 96 h LC_{50} value (4.073 % v/v) of raw distillery effluent for *Mystus vittatus*, the selected sub-lethal concentrations 2.5% v/v (about1/10 of 96 h LC_{50}) and 5.0 % v/v (about 1/5 of 96 h LC_{50}) were selected for the study of chronic toxicity. There was no mortality i.e., 100% survival was observed in both sublethal concentrations of distillery effluent. The relative growth rate (RGR) decreases as the sublethal concentration and period of exposure of distillery effluent increases. A significant decreased (14.95 to 53.86%) in the rate of oxygen uptake was recorded in distillery effluent exposed fishes. The effect was more pronounced as the concentration and duration of exposure to distillery effluent increased. Thus, the chronic response of the fish towards toxicity was grossly dependent on concentration and length of exposure.

Keywords: Distillery effluent, Chronic toxicity, Mortality responses, Relative growth rate, Oxygen consumption rate, *Mystus vittatus*

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Introduction

Nowadays water scarcity becomes a growing problem and it has significant negative influence on economic development, human livelihoods and environmental quality throughout the world. Therefore, it has become crucial in today's climate to either create affordable remedial methods for water protection or to prevent it from becoming polluted. Rapid industrialization in India has led to a significant rise in liquid waste that is directly dumped into open space or into nearby natural waterbodies, creating a number of environmental issues like surface water logging, ground water contamination, and salinization of high-quality land due to the presence of high-quality salts (Ramona *et al*., 2001).

The natural resources of water bodies such as rivers, ponds, lakes and wetlands are polluted with a variety of solid and liquid wastes causing a great damage to the aquatic ecosystems. The two most important processes that affect the global environment are the resource production and the waste disposal. A proper balance between these two is necessary for a healthy biosphere. At present all human society either rural or urban are technologically or industrially advanced and poured certain waste products and by-products directly or indirectly into the environment. Therefore, the release of effluent without proper treatment into natural water bodies or aquatic ecosystems increases the risk to human health (Prakash, 2023).

Industrial growth is an important part of the evolution of human civilization and is vital for the development and prosperity of any country. However, industries also often prove hazardous to aquatic life when their toxic effluent are discharged without any proper treatment. Industrial effluents are a complex mixture of number of chemical constituents and are responsible for oxidation potential inside the aquatic ecosystems. Hence, effluents are capable to produce harmful effects even at sub-lethal concentrations. The effluent generated by industries are dumped along the bank of rivers. The discharge of spent wash, waste water of distillery into the surface water bodies with a high TDS adversely affects the aquatic life including fishes (ETPI, 2001). Approximately, 10-15 litres of spent wash is generated for the production of one litre of alcohol. Consequently, there has been a significant increase in the waste water generation from this sector. This massive quantity of effluent, if disposed untreated can cause considerable stress on the water resources. The industrial effluent alters the natural physico-chemical condition of aquatic medium which usually affects behavioural, haematological, biochemical, histological and physiological activities of the aquatic organisms including fishes (Prakash and Singh, 2020).

Discharge of industrial effluent directly or indirectly into inland fresh waterbodies depleted the dissolved oxygen content and, by interfering with respiratory metabolism causes heavy mortality. Pollution of freshwater aquatic waterbodies by domestic and untreated or partially treated industrial effluent greatly contributes to massive kill of fish and other important aquatic flora and fauna. The low pH, high biological oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS), chloride, sulphate, and organic load are major toxic constituents of distillery effluent. The high BOD causes depletion of dissolved oxygen and proves harmful to aquatic life.

The number of biological processes, including food intake, digestion, absorption, metabolism, and excretion are responsible for growth. The net energy is distributed among the activities such as metabolism, maintenance processes, reproduction and growth. Therefore, growth will be constrained if metabolism is raised unless food intake is increased (Ramesh and Nagarajan, 2014).

The O_2 consumption is a very sensitive physiological process and the change in respiratory activity has been used as an indicator of stress in animals exposed to toxicants (Prasanna *et al*., 2020). It can be used as an early warning bioindicators of stress associated with pollution. Respiratory responses were found to be less sensitive, but also could be successfully used in bioassay testing of treated industrial and municipal effluents, before they are discharged into water bodies. In fishes, the gill epithelium tissue has intracellular and extracellular exchange of ions related to oxygen and chloride shift and any toxic substance having the capacity to alter this structure will affect the oxygen carrying capacity of haemoglobin and reduction in oxygen uptake. The gill damage observed in fish exposed to toxicant was due to impairment in the respiratory metabolism. This is also due to the fact that any pollutant or toxicant or xenobiotics can enter into the fish body, mainly through the gills only.

Fish reflect environmental conditions such as poor water quality and pollution since they are at the base of the aquatic food chain. They can accumulate toxic compounds or pollutants like pesticides and heavy metals present in the water body so, help in monitoring aquatic pollution in their environment. There is wide lacuna in this field of industrial effluent toxicity on freshwater fishes which prompted to take up this investigation further. The survey of literature reflects that the industrial effluent affects a wide range of non-target organisms such as fishes and other aquatic organisms. No information is available on the direct effect of the distillery effluent on the growth of freshwater fishes. The effluents of the factory leach and enter into neighbouring water bodies and contaminate these water bodies, where edible freshwater fishes are cultured. Therefore, the present investigation is projected in order to check the water quality and the effects of distillery effluent on the health of fish to maintain the health of fish consumers.

The Distillery unit of Balrampur Chini Mill Ltd. Balrampur, U.P. discharges its effluent and polluting the water of river Suwaon which is a tributary of river Rapti. The spent wash or wastewater of distillery industry is considered to be harmful to the fishes. The distillery effluents are discharged into Swan River through small open drains and are eventually fallen into the Rapti River. Hence, this investigation was designed for instant information on these issues. The present investigation was projected to understand the impact of sublethal concentration of distillery waste water on the survival rate, relative growth rate in terms of body weight, and oxygen consumption rate of freshwater catfish, *Mystus vittatus*.

Materials and Methods

With the help of fisherman live freshwater catfish, *Mystus vittatus* were collected from local water bodies of Balrampur. The collected fishes were washed with 1% solution of KMnO4 for 5 min to remove any dermal infection. Then these fishes were brought to the laboratory in plastic container. The fishes were again screened for any pathogenic infections and transferred to the plastic jar containing 50 L dechlorinated tap water. Diseased, injured and dead fishes (if any) were removed immediately in order to avoid the decomposition of body in the container. 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. All the fishes were regularly fed with commercially food pellets and medium (tap water) was renewed every two days to remove faeces and food remnants.

In the present investigation the sublethal concentration of untreated distillery effluent were selected on the basis of the acute toxicity test. The 96 h LC_{50} value of untreated distillery effluent was 4.073% (v/v) for *Mystus vittatus*. The sub-lethal concentrations for chronic test were selected (2.5% or 1/10 of 96 h LC_{50} and 5% or $1/5^{th}$ of 96 h LC_{50}). In this experimental period test medium was changed daily.

The raw distillery effluent collected directly from point of discharge and brought to the laboratory and kept at $4^{o}C$ in refrigerator. Characteristics of the raw distillery effluent and tap water such as temperature, pH, Electrical conductivity (EC), Dissolved oxygen (DO), Hardness (HA) and Total dissolved solids (TDS) were estimated with the help of digital Water Quality Meter (Model: ISO-TECH SYSTEM (ITS)-901) using glass electrode. Biochemical oxygen demand (BOD) and Chemical oxygen demand (COD) were determined by digital BOD and COD instrument. Total alkalinity (TA), chloride and sulphate were estimated by following the methods of APHA (2005). The physico-chemical

Table 1: Physico-chemical characteristics of tap water and raw distillery effluent

Parameters (Unit)	Tap water*	Raw distillery effluent*	Standards Permissible Limit by		
			ISI	CPCB	WHO
Colour	Colourless	Dark Brown	-	Colourless	-
Odour	Odourless	Alcoholic	-	Odourless	-
Temperature (°C)	25.5 ±1.0	33 ±1.5	-	Shall not exceed 5°C above the receiving water temperature	-
pH	7.42 ±0.2	3.52 ± 0.3	6.0 -7.0	5.5-8.5	6.5-8.5
EC (mS/cm)	0.23 ±0.32	7.05 ± 0.70	-	4.0	4.0
DO (mg/L)	6.17 ± 0.8	0.7 ± 0.4	>5.0	4.0	-
TA (mg/L)	105 ± 5.0	2750 ± 50	-	600	300
HA (mg/L)	95 ± 5.0	3105 - 3209	600	600	500
COD (mg/L)		8550 ± 150	250	250	250
BOD (mg/L)		1700 ± 100	30	30	35
TDS (mg/L)		2400 ± 150	-	2100	1000
Chloride (mg/L)	0.85 ± 0.3	640 ± 35	600	750-1000	250
Sulphate (mg/L)	ND	3580 ± 25	2100	400	250

*Values are represented as mean (n=3) ± SD

characteristics of tap water and raw distillery effluent used for experiment are presented in Table 1.

After exposing the test fish to two sub-lethal concentrations of the raw distillery effluent, survival or mortality rate, relative growth rate and oxygen consumption rate of the test fish was studied for 30 days.

Survival or Mortality:

The test fishes were exposed to two sub-lethal concentrations i.e. 2.5% v/v (1/10 of 96h LC_{50}) and 5.0 % v/v (1/5 of 96h LC_{50}) of raw distillery effluent; the observations were made at 10 days exposure to note the number of fish survived in particular concentration. Similar observations were continued for 20 days and 30 days exposure. From this percentage of mortality in two sublethal concentrations were calculated at the end of the chronic exposure.

Relative Growth:

The weights of fishes were recorded by using electronic digital balance to the nearest 1.0 gram. Relative growth rate (RGR) of fish were calculated according to formula:

$$\text{RGR (\%)} = \frac{\text{Final weight} - \text{initial weight}}{\text{Initial weight}} \times 100$$

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. 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 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 ml O_2/g/hour.

Results and Discussion

In the present investigation to study the toxicity of raw distillery effluent, the test freshwater catfish, *Mystus vittatus* was exposed to sublethal concentrations of distillery effluent (2.5% v/v and

Table 2: Mortality of *Mystus vittatus* exposed to sub-lethal distillery effluent

Exposed Period (Days)	**Control**	**Distillery Effluent**	
		2.5% v/v (1/10 of LC_{50})	**5% v/v (1/5 of LC_{50})**
0 day	0	0	0
10th day	0	0	0
20th day	0	0	0
30th day	0	0	0
Total % mortality at the end of experiment	0%	0%	0%
Total % of survival at the end of experiment	100 %	100 %	100 %

Table 3: Relative Growth rate of *Mystus vittatus* exposed to sub-lethal distillery effluent

Exposed Days	**Control (Tap Water) Weight (g)**	**Distillery Effluent/Body weight**	
		Weight (g) 2.5% of LC_{50}	**Weight (g) 5% of LC_{50}**
1st day	8.70 ± 0.05	8.75 ± 0.05	8.80 ± 0.05
10th day	9.95 ± 0.08 **(14.36%)**	9.60 ± 0.04* **(9.71%)**	9.40 ± 0.07* **(6.82%)**
20th day	10.45 ± 0.04 **(20.11%)**	9.80 ± 0.06* **(12.00%)**	9.60 ± 0.04* **(9.09%)**
30th day	11.00 ± 0.05 **(26.43%)**	9.90 ± 0.07* **(13.14%)**	9.70 ± 0.05* **(10.22%)**

Figure in the brackets indicate Relative growth Rate of Fish; *The Value of ANOVA is significant at 0.05

5% v/v, 1/10th and 1/5th of 96 h LC_{50}, respectively) for the period of 30 days. The chronic effect of two sub-lethal concentrations i.e., 2.5% and 5.0 v/v of raw distillery effluent on different aspects of test fish, *Mystus vittatus* like survival or mortality rate, relative growth rate and oxygen consumption rate were briefly summarized.

In the present investigation, the number of fish died after 10 days, 20 days and 30 days exposure and total mortality along with total survival at the of experiment i.e., after 30 days exposure to both sublethal concentrations of distillery effluent were observed. There was no mortality found i.e., 100% survival was observed at both the concentrations i.e., 2.5% v/v and 5.0% v/v (1/10 and 1/5 of 96h LC_{50}) (Table 2). Similar finding was also noticed by Sarwade (2015) in *Puntius stigma* after chronic exposure period at two sublethal concentration of paper mill effluent. 100% survival and 0% mortality were also observed in pesticides exposed freshwater fishes, *Lepidocephalichthys thermalis* (Kamble, 1983) and in *Puntius ticto* (Ganeshwade, 2002).

To investigate the chronic effect of distillery effluent, the Relative growth rate (RGR) of *Mystus vittatus* was studied in relation to the body weight in the control as well as in the effluent exposed fishes. The growth of test fish in terms of average body weight was noticed for different period. The result of the present investigation revealed that in control group, the relative growth rate of fishes increased progressively (normal), but in effluent exposed fishes, relative growth rate decreased as

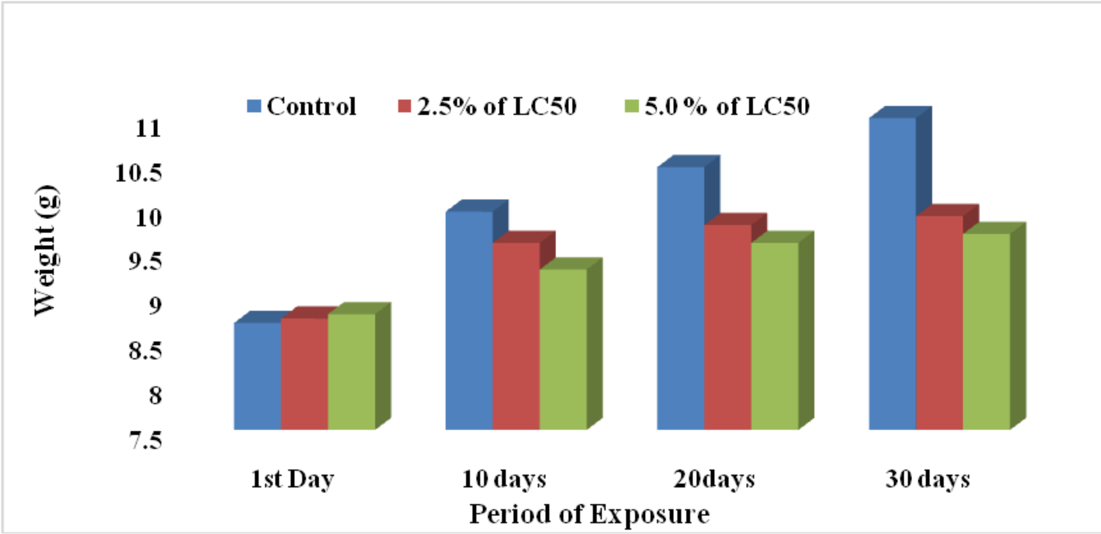

Fig. 1: RGR of *Mystus vittatus* exposed sub-lethal concentration of distillery effluent.

Table 4: Changes in the Oxygen uptake of *Mystus vittatus* at different sublethal concentrations of distillery effluent (O_2 ml/g/h)

Experimental group	Exposure Periods (Days)		
	10	20	30
Control (Tap water)	0.535 ± 0.003	0.532 ± 0.003	0.531 ± 0.002
2.5% or 1/10th of LC_{50}	0.455 ± 0.005* (-14.95%)	0.375 ± 0.005* (-29.24%)	0.328 ± 0.003* (-38.22%)
5.0% or 1/5th of LC_{50}	0.342 ± 0.069* (-36.07%)	0.289 ± 0.005* (-45.47 %)	0.245 ± 0.002* (-53.86 %)

Each values corresponds to mean ± SD;Figures in the brackets indicate %age decrease in the oxygen consumption or uptake; * Significant at P<0.001

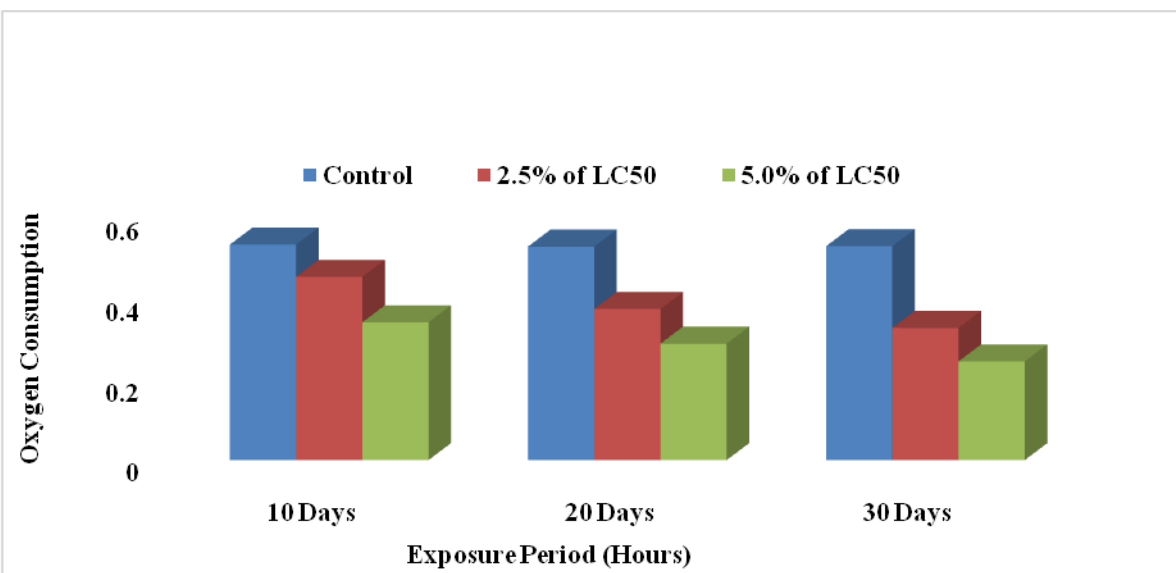

Fig. 2: Alterations in the Oxygen uptake of *Mystus vittatus* at different sublethal concentrations of distillery effluent (O_2 ml/g/h).

compared to control (Table 3; Fig.1). This may be due to presence of toxic substance in the distillery effluent. Similar finding was also noticed in industrial effluent exposed fishes, *Heteropneustes fossilis* (Prakash, 2023), *Oreochromis mossambicus* (Kaveri *et al*., 2018 ; Varadaraj *et al*., 1997), *Puntius stigma* (Sarwade, 2015), *Tilapia mossambica* (Noorjahan and Rohini, 2015) and *Catla catla* (Ramesh and Nagarajan, 2013).The result of the present investigation also showed that the relative growth rate of fishes exposed to sublethal concentrations of distillery effluent was dose and exposure period dependent (Table 3; Fig.1) i.e., RGR decreases as the concentration of distillery effluent and days of exposure increases. The higher RGR was observed in 2.5% distillery effluent treated fish sample compared to 5.0% distillery effluent treated fish sample. This is because of presence of less amount of toxic substances in diluted effluent as compared to concentrated effluent.

Rate of Oxygen consumption of fish, *Mystus vittatus* exposed to sublethal concentrations of distillery effluent is given the Table 4 and Figure 2. Decrease in O_2 consumption rate was observed in *Mystus vittatus* exposed to two sublethal concentrations i.e., 2.5% v/v (1/10 of 96h LC_{50}) and 5 % v/v (1/5 of 96h LC_{50}) of distillery effluent for the period of 10, 20 and 30 days. In the present investigation, the rate of oxygen consumption was gradually decreasing with increasing the exposure periods and concentrations of distillery effluents. Maximum decline (-53.86%) over control in the oxygen uptake by experimental fish was noticed at 5.0% v/v sublethal concentration on 30 days of exposure (Table 4; Fig. 2). Similar finding was observed in distillery effluent exposed *Cyprinus carpio* (Prakash and Singh, 2020). The decrease in oxygen consumption rate in pesticide and heavy metal exposed fishes were observed by some investigators (Sornaraj *et al*., 1995; Ray and Kumar, 2013).

In the present investigation the decrease in oxygen consumption in distillery effluent exposed fish was due to degeneration of the gill epithelium that causes oxygen debt and also loses its effective mechanisms for "hypoxia anoxia" in which gill tissue not only suffers from oxygen debt but also loses its effective mechanism for removing carbon dioxide from blood. Anoxia or hypoxia increases carbohydrate consumption and thereby induces a sort of respiratory stress on organisms even at a sublethal level resulting in additional expenditure of energy (Prakash and Verma, 2019, 2020).

Conclusion

The results of the present investigation suggest that freshwater catfish, *Mystus vittatus* can suitably be used as a bioindicator of water pollution. distillery effluent depicted terrible impact on survival, relative growth rate and oxygen uptake of the exposed fishes. Our findings indicate that distillery effluent has a physiological impact on fish, and thus observation of relative growth rate and respiratory of fishes inhabiting in the polluted waterbodies are helpful tools for identifying the impact of pollution. Therefore, it can be concluded that the effluent is not safe to non-target aquatic animals like freshwater fishes. This type of investigation can be useful to compare the sensitivity of various aquatic animals and potency of effluent using LC_{50} values and to derive safe concentration.

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