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Full Length Research Paper

Acute toxicity and behavioral response of freshwater fish, *Mystus vittatus* exposed to pulp mill effluent

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The present study deals with the acute toxicity (LC_{50} evaluation) of paper mill effluent to a freshwater fish, *Mystus vittatus*. A monthly record covering all the spawning season (pre-spawning phase, spawning phase and post-spawning phase) was evaluated at different exposure periods (24-, 48-, 72- and 96-h) using the whole paper mill effluent. Annual variation in LC_{50} values in relation to annual breeding cycle and water temperature were also taken into consideration. A well marked variation in the LC_{50} values in different exposure periods as well as in various months of the spawning phases of the test fish were observed.

Key words: Acute toxicity, paper mill effluent, annual variation, spawning phases.

INTRODUCTION

The Bagridae family of fish is the richest and most important of the teleostei class and its members are distributed throughout the world (Day, 1878). In the Bagridae family, the fish *Mystus vittatus* (Smith, 1945) is economically important and distributed in the semitemporal freshwater system of South India (Perennou and Santharam, 1990).

Under laboratory conditions, toxicity testing procedures (mortality studies LC_{50} estimates) may provide information regarding the harmfulness of industrial stress for aquatic animals, including fishes (Marier, 1973). In the acute toxicity of contaminants in static bioassays, the use of 96-h, LC_{50} has been widely recommended as a preliminary step in toxicological studies on fishes (McLeay, 1976; Whittle and Flood, 1977; USEPA, 1973, 2005; APHA, 1998, 2005; Chapman, 2000; Ali and Sree-Krishnan, 2001; ASTM, 2002; Parrott et al., 2006; Moreira et al., 2008). The static bioassay procedures to study the toxicity of pulp and papermill effluent to variety of fishes have been reported like the Cohosalmon, *Onchorhynchus kisutch* (McLeay, 1973, 1975, 1976, 1977; Gordon and McLeay, 1977; McLeay and Howard, 1977; McLeay and Gordon, 1977; McLeay and Brown, 1979) the rainbow trout, *Salmo gairdneri* (McLeay, 1976; Gordon and McLeay, 1977; Whittle and Flood, 1977; McLeay and Gordon, 1978; Couillard et al., 1988) and other fishes (Gordon and Servizi, 1974; Davis, 1976; Walden, 1976; Hewitt et al., 2006).

The paper mill, when it is playing directly influx to the streams and rivers, without previous treatment of effluents it can change the native fish or biota. Therefore, in the present study, the acute toxicity of paper mill effluent has been studied with regards to the mortality of *M. vittatus.* The 24-, 48-, 72- and 96-h, LC_{50} values were determined during the different phases of the reproductive cycle of the fish, and the correlation of LC_{50} values with environmental temperature has also been established.

The main objective of this study is to evaluate the acute toxicity of paper mill effluent on the behavioral responses of freshwater fish *M. vittatus* in the context of annual breeding cycle and water temperature.

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Table 1. Characteristics of WPME (whole paper mill effluent), Magahar, Santkabeer Nagar (U.P.) India.

Characteristics	Variable constituents through the year (mean values)			Yearly average ± S. E.
	January-April	May-August	September-December	
Color	Dark brownish	Dark brownish	Dark brownish	-
Sodium, Na^+ (mg/L)	350	320	351	340±12.2
Cl^- (mg/L)	420	450	425.7	431.9±11.3
SO_4 (mg/L)	1.12	5.8	3.5	3.5±1.7
Nitrate (mg/L)	7.6	7.3	7.2	7.4±0.1
Total nitrogen (mg/L)	1.7	6.3	3.8	3.9±1.6
PO_4 (mg/L)	0.77	0.52	0.69	0.7±0.1
pH	7.3	7.3	7.4	7.3±0.04
Temp (°C)	23.5	28	26.5	26±1.6
Suspended solid (mg/L)	5021	4643	4256	4640±270
Dissolved solid (mg/L)	1111	1222	1013	1115±74
Total solid (mg/L)	6132	5865	5269	5755±312
BOD (mg/L)	552	538	498	526±19
COD (mg/L)	2379	2551	2326	2418±83
Fe (mg/L)	9.6	13.5	10.4	11.2±1.5
Mg (mg/L)	1.9	1.65	1.28	1.6±0.2
K (mg/L)	8.6	6.4	4.4	6.5±1.5
Cu (mg/l)	0.11	0.14	ND	0.1±0.1
Total Cr (mg/L)	ND	0.077	0.072	0.07±0.03
Mn (mg/L)	0.34	0.071	0.53	0.31±0.12
Co (mg/L)	-	0.001	0.0012	0.001±0.002
Cd (mg/L)	0.025	0.016	0.018	0.02±0.004
Zn (mg/L)	0.06	0.08	0.106	0.08±0.021

Data based on samples taken during the morning shift of the normal course of mil operation at 8 am.

MATERIALS AND METHODS

The freshwater teleost fish, *M. vittatus* were collected from a local freshwater river Ami of Sant Kabeer nagar district (U. P). They were transported to laboratory and washed with $KMNO_4$ solution (1 *mg*/L) for 5 min and then transferred to the acclimation tank. The fishes having an average body length of 7.6 ± 0.18 cm, and body weight of 73 ± 0.23 g. were selected for the present study.

The fishes were acclimatized in laboratory conditions for 3 to 4 weeks at room temperature (22 to 30°C) using dechlorinated tap water. The medium of the acclimation tanks was changed daily. The dissolved oxygen content of the medium was maintained at 60% to 100% by regular aeration. The whole paper mill effluent was collected in polyethylene container of 10 to 20 L capacity during the morning hours. The effluent samples were taken from two places, that is, the point where the effluent comes out of the mill and the point where it enters the collecting tank and the mixed unfiltered samples were used within 24 h of collection. The effluent samples were chemically analyzed per month and the characteristics are summarized in Table 1.

Acute toxicity of effluent on test fish was measured in terms of LC_0, LC_{50} and LC_{100}. Static bioassay procedures, as outlined by the USEPA (2005) were followed. A minimum of 8 concentrations of effluent and 20 animals were used for each concentration. The control set had the same number of fish kept in normal, dechlorinated tapwater. No food was provided to either the control or the test fishes during the period of the toxicity experiments. Experiments were carried out up to 96-h, and the observations were recorded after every 24-h. Experiments were conducted every month, and LC_{50} values for 24-, 48-, 72- and 96-h, were determined. The tested concentrations of effluent and the observed percentage of the fish mortality were subjected to linear regression for determining the LC_{50} values for the different exposure periods. The yearly average LC_{50} values were calculated, and the 95% confidence interval determined for this mean.

RESULTS

The mortality of test fish exposed to paper mill effluent is summarized in Table 2.

Yearly average LC_{50}

The yearly average LC_{50} value was found to be 60.04 ± 2.13; for 24 h, 53.6 ± 2.16, for 48 h; 48.6 ± 2.1, for 72 h, and 44.4 ± 2.2, for 96 h exposure periods (Table 2).

Annual variation in LC_0 and LC_{100}

The lowest LC_0 and LC_{100} values were recorded in the spawning phase of the fish, whereas the values were the highest in the post spawning phase (October to January)

Table 2. Seasonal variation of WPME toxicity (LC_{50}) to *M. vittatus* in different spawning phases.

Spawning phases	Month	Water temperature (℃)	LC_{50} % (v/v)			
			24 h	48 h	72 h	96 h
Pre spawning phase	February	23.5	64.5	54.0	49.5	45.6
	March	24.0	62.5	54.5	50.5	45.7
	April	25.0	60.4	53.5	46.05	41.5
	May	27.0	54.0	48.5	43.5	42.5
	Mean		60.35	52.6	47.8	43.8
Spawning phase	June	29.0	50.0	42.55	38.0	35.5
	July	28.0	50.5	42.55	38.0	37.0
	August	28.5	53.0	46.0	41.5	38.0
	September	27.0	57.25	53.75	49.0	44.25
	Mean		52.7	46.2	41.6	38.7
Post-spawning phase	October	26.5	64.5	59.5	50.5	46.0
	November	25.0	68.5	62.5	58.0	51.5
	December	23.0	71.75	64.0	59.0	53.0
	January	22.0	68.25	61.75	58.0	53.0
	Mean		68.2	62.0	56.4	50.8
Yearly average ±S. E.		25.7±0.7	60.4±2.13	53.6±2.16	48.6±2.1	44.4±2.2
95% Confidence interval		-	65.1-55.7	58.3-48.8	53.2-43.9	49.2-39.6

of the annual reproductive cycle. Values observed in three phases are recorded in Table 3.

Annual variation in LC_{50}

A well marked variation was recorded in the LC_{50} values for the different months of the year. The values for the spawning phase were generally lower than those of other phases (Table 2).

Variation in relation to water temperature

The annual variation in LC_{50} values exhibited an inverse relationship with water temperature. The LC_{50} value was generally recorded to be higher in the colder months of the year and lower in the hotter months. Maximum LC_{50} values were observed in the month of December (water temperature 23℃), while lowest in the month of July (water temperature 28℃.).

The Cr and Cd contents of test effluent were found to be much higher; the total content is 0.497 mg/L for Cr and 0.0073 mg/L for Cd. Regarding Cu, the maximum permissible concentration is 0.006 ppm or alternatively 0.1 times the LC_{50} 96 h value. In case of the presently used papermill effluent, the Cu content was found to be 0.083 mg/L, which is about 2.5 times its estimated LC50 96 h value (that is, estimated concentration present in the LC50 96 h test effluent).

The matter suspended within the papermill effluent used in the present study could also be contributing to the mortality of the *M. vittatus* individuals maintained in this effluent. The concentration of suspended matter in WRPBILE ranged from 4256 to 5021 mg/L.

Behavioral responses

The surfacing behavior as well as the rate of opercular movement of the fishes was observed to be increased within an hour of the commencement of the toxicity experiments. With the continuation of the exposure, the fishes progressively became sluggish and lethargic. The majority of them became completely inactive after 72 h of exposure to 60 to 70% of test effluent, and all died after 96 h of exposure to 70% effluent. Prior to their death in the contaminated medium, they showed abnormal swimming and the loss of equilibrium.

DISCUSSION

The stressed fishes were found to be the most sensitive to effluent during the spawning phases of the reproductive cycle. Increased sensitivity under pollution stress during the spawning season has also been reported in pacific *herrings* exposed to benzene toxicity (Korn et al., 1976). Higher temperature nature has been shown to increase the toxicity of pulp and papermill

Table 3. Seasonal variation of WRPBILE toxicity (LC_0 and LC_{100}) to *M. vittatus* in different spawning phases.

Spawning phase	Months	Water temperature (°C)	LC_{50} % (v/v)							
			24 h		48 h		72 h		96 h	
			LC_0	LC_{100}	LC_0	LC_{100}	LC_0	LC_{100}	LC_0	LC_{100}
Pre- spawning phase	February	23.5	30.0	85.0	30.0	80.0	25.0	75.0	25.0	70.0
	March	24.0	30.0	80.0	30.0	75.0	25.0	75.0	25.0	70.0
	April	25.0	25.0	80.0	25.0	75.0	20.0	70.0	20.0	65.0
	May	27.0	25.0	75.0	20.0	70.0	20.0	65.0	20.0	60.0
		Mean	27.5	80.0	26.3	75.0	22.5	71.3	22.5	66.3
Spawning phase	June	29.0	20.0	75.0	20.0	70.0	20.0	65.0	20.0	60.0
	July	28.0	20.0	70.0	20.0	65.0	15.0	60.0	15.0	55.0
	August	28.5	25.0	75.0	20.0	70.0	20.0	65.0	15.0	60.0
	September	27.0	30.0	80.0	25.0	75.0	25.0	75.0	20.0	70.0
		Mean	23.7	75.0	21.3	70.0	20.0	66.3	17.5	61.3
Post-spawning phase	October	26.5	30.0	80.0	30.0	75.0	25.0	75.0	25.0	70.0
	November	25.0	40.0	85.0	35.0	80.0	35.0	80.0	30.0	75.0
	December	23.0	40.0	90.0	40.0	85.0	35.0	80.0	30.0	75.0
	January	22.0	40.0	85.0	40.0	80.0	35.0	75.0	30.0	70.0
		Mean	37.5	85.0	36.3	80.0	32.5	77.5	28.8	72.5
Yearly average ±S.E.		25.7±0.7	29.6±2.2	80.0±2.1	28.0±1.8	75.0±2.1	25.0±1.8	71.7±1.9	22.9±1.5	66.7±1.9
95% confidence Interval		-	34.4 -24.7	84.6 -75.3	31.9-24.0	79.6-70.3	28.9-21.0	75.8-67.5	26.2-19.6	70.8-62.5

effluent for fishes like *O. kisutch* and *S. gairdneri* (McLeay, 1976; Gordon and McLeay, 1977). Increase in temperature reduces the solubility of oxygen in water and hence, could raise the metabolic rate (oxygen demand) of the fish, thus limiting the oxygen carrying capacity of the blood (Gordon and McLeay, 1977). But in the present study, the LC_{50} played the higher toxicity in the colder months, when the temperatures were very low. The result indicates that toxicity can occur independent of the high or low temperatures. Simultaneously, the higher BOD (Biological oxygen demand) and COD (Chemical oxygen demand) could also contribute to the mortality (Polak and Palmer, 1977; Whittle and Flood, 1977; McLeay et al., 1979a, b; McLeay and Brown, 1979).

It is known that many pollutants, such as heavy metals, like Mg, Cu and Zn, and industrial wastes become more harmful at higher water temperatures. In view of this, it may be presumed that the toxic metals, especially Cu and Zn, present in the tested effluent would acquire greater toxicity during the warmer months of the year and thus contribute to the overall increased mortality during these months in *M. vittatus*. As regard the contribution of the different constituents of effluent to *M. vittatus* mortality, the content of many of these constituents is noticeably higher than the upper limits considered as safe for fish health (Wedemeyer, 1976). For example, among metals, the upper limit for Cr is 0.03 ppm, and that for Cd 0.0004 ppm. The total suspended solids, absorbable organic halogens and also certain other toxic compounds like wood extractives, present in pulp and papermill waste water are also known to affect fishes (Sprague and McLeese, 1968; Kelso,1977; Hewitt et al., 2006; Parrott et al., 2006). The concentration of the suspended matter in WRPBILE is much above the TSS concentration considered (Wedemeyer, 1976) optimum for fishes (that is, 80 to 100 ppm), and falls within that range of suspended matter of papermill effluents (that is, 530 to 17900 mg/L approximately) which has earlier been considered injurious to fishes.

The opercular movement of the dying fishes became extremely slow and they were seen to secrete thick coat of mucus profusely around their opercular region. Similar types of increased opercular movement, erratic and rapid movements have been observed by Kumar and Gopal (2001) in *Channa punctatus* exposed to distillery effluent. Clotfelter et al., (2006) have observed aggressive behaviour in fighting fish, increased surface breathing and opercular movement in the stressed *M. vittatus* would point a sustained respiratory discomfort in the toxic WPME (whole paper mill effluent) (Lioyd, 1961). The stressed fishes were observed to secrete mucus around their opercular region which has been considered to be a symptom of the inflammatory reaction of gill towards the pollutants (Durve and Jain, 1980 ; Pandey and Pandey, 1988), while the abnormal swimming and disturbed orientation has been considered as a generalized sign of diseases in fishes (Sindermann, 1970).

The kinds of behavioral faults 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 babyrinth helps the fishes in maintaining harmony with their environments and also control their vital behaviors (Gardner, 1975). Hence, any impairment of these organs would produce behavioral faults in the fishes. Therefore, the behavioral changes, particularly those concerned with respiratory insufficiency, observed in *M. vittatus* exposed to mill effluent, might be contributing to the mortality in these stressed fishes.

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