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Impact of Pulp and Paper Mill Effluent on Reproductive Behaviour of Fresh water Catfish, *Clarias batrachus* (Linn.)

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Abstract

The present study deals with the physic-chemical characteristic of pulp and paper mill effluent and their acute toxic impact on the reproductive behavior of fresh water cat fish, *Clarias batrachus.* In relation to water temperature, LC_{50} values was recorded at different exposure periods during pre-spawning, spawning and post spawning phases of reproductive cycle . The result of the present study showed that, LC_{50} of the *Clarias batrachus* was higher in post-spawning phase i.e. winter months when the temperatures were low and EC and COD were high.

Keywords: Pulp and Paper Mill Effluent*, Clarias Batrachus,* Spawning Phases.

Introduction

Rapid industrialization, urbanization and other developmental activities had led to water pollution by directly discharging industrial effluent and municipal waste into the water bodies. Any change in the natural conditions of aquatic ecosystem causes behavioural response in fish, ultimately leading to physiological adjustment. Chemical pollution has a great impact on aquatic organism. Due to chemical pollution, the normal functioning of cell is disturbed and this in turn, may result in alternation in the functional biochemical and physiological mechanisms of animal, therefore, in the present study, the toxicity of paper mill effluent has been studied with regards to the mortality of *Clarias batrachus.*

Review of Literature

Rapid industrialization in Indian has resulted in the substantial increase in the liquid waste which is directly discharged in open land or into nearby natural water, causing a number of environmental problem such as surface water logging, ground water contamination and salinizing good quality land due to presence of high quality salts (Ramona *et al*.,2001).

Discharge of effluent into freshwater system deplete the dissolved oxygen content and, by interfering with respiratory metabolism cause heavy mortality. Pollution of aquatic by domestic and untreated or partially treated industrial effluent greatly contributes to massive kill of fish and other important aquatic biota (Sarwade,2015).

Due to high chemical diversity of organic pollutants in pulp and paper mill effluent, a high variety of toxic effects on aquatic communities in recipient water have been observed (Sarwade,2015). The paper mill,when it is playing directly influx to the stream and rivers, without previous treatment of effluents it can change the native fish or biota (Mishra *et al*.,2011).

The pulp and paper Industry is one of the oldest industries in our country and there has been tremendous expansion of these industries during last 25 years. Controversially, the paper and pulp industry as it stand now, is one of the largest major Industries and contributes lot towards the pollution in our aquatic environment. Looking in to the serious nature of pollution the pulp and paper industries in India has been brought under 17^{th} categories, is highly polluting industries (Pathan *et al*.,2009).The effluent of this industry consists of various toxic and bioaccumulating compounds such as Chlorophenos, fatty acids and resin acid causing metabolic impartment in the aquatic organisms which could even lead to their death(Afroz and Singh,2013). The Indian pulp and paper industry is highly water intensive, consuming 100-250 m^3 of freshwater per tone of paper, and also generates the corresponding wastewater, i.e. 75-225 m^3 per tone of paper (Tewari *et al*, 2009). Fish are important members of

aquatic ecosystems and an important source of human food. Fish in ponds, lakes and rivers cannot avoid exposure to these chemicals that suspended or dissolved in water, being less than land animals to move to favorable regions to avoid unfavourable condition (Afroz and Singh, 2014).

Objective of the Study

The main objective and aim of this study is to evaluate the physio-chemical characteristics of pulp and paper mill effluent and to study its acute toxicity on the behavioral responses of fresh water cat fish, *Clarias batrachus* in context of annual breeding cycle.

Materials and Methods

Effluent samples were collected from pulp and paper mill in polyethylene container at the point of discharge into river Ghaghra. The collected samples of effluent were transported immediately to the laboratory for analyzed the physical and chemical characteristics. The pH effluent was measured on sampling site using digital portable pH meter. The electrical conductivity (EC) of the effluent was measured on the sampling site using a digital portable conductivity meter at 25°C. The BOD, COD, Chloride and Phenols were measured according to APHA (1998).

Healthy specimens of Indian fresh water catfish, *Clarias batrachus* (45±5 g & 12±5 cm) were collected from local fresh water river Ghaghra in pre-spawning(Summer), spawning(rainy) and post-spawning(Winter) phases/seasons and were transported in containers to the laboratory and washed with 1% solution of $KMnO_4$ for five minute and then transferred to the plastic jar containing 50L dechlorinated tap water for acclimatization. The medium of the acclimation jar was changed daily. The pulp and paper mill effluent was collected in polyethylene container. Acute toxicity of effluent on test fish was measured in terms of LC_{50} for the period of 24h, 48h, 72h and 96h to study the behavioral responses of fresh water cat fish, *Clarias batrachus* during pre- spawning(Summer), spawning(rainy) and post-spawning(Winter) phases/seasons in context of annual breeding cycle . No food was provided to either the control or the test fishes during the period of the toxicity experiments. The seasonal and yearly average LC_{50} values were calculated and 95% confidence interval determined for this mean.

Physico-Chemical Characteristics of Effluent

The results of physico-chemical analysis of effluent for different seasons are given in the Table1.The values for various physico-chemical characteristics obtained in the present study are in good agreement with the characteristics of pulp and paper mill effluents as reported by other workers.The dark brown colour of pulp and paper mill effluent might be due to the presence of low and high molecular weight chlorinated organic compounds generated from lignin degradation products during wood cooking, conventional bleaching and alkali extraction of the pulp. The characteristic pungent odour of pulp and paper mill effluent was due to the presence of a number of dissolved chemicals (specifically mercaptans and hydrogen sulphide) and the raw materials which were used during the manufacture of paper. Similar colour and order of pulp and paper mill effluent were observed by Afroz and Singh (2014). Similarly, most workers have reported the brown colour and pungent odour of pulp and paper mill effluent (Mahajan, 1985; Singh *et al*., 1996; Afroz and Singh,2014). In the present study the pH of the effluent was ranged 6.5-7.2 in all the seasons.Singh *et al.* (1996),Pathan *et al.,* (2009) Mishra *et al.*(2011) and Afroz and singh (2014) reported the pH of pulp and paper mill effluent were 6.9-8.6, 8.1, 7.3 and 8.06-9.22,respectively. The mean electrical conductivity values in the present study were ranged from 1.49-1.65 mD/cm in all seasons. This values of EC was within the maximum desirable limits (2.5mD/cm) of Central pollution control board of India. The minimum and maximum electrical conductivity were observed in rainy and winter seasons, respectively. Biological Oxygen Demand (BOD) values obtained in the present study ranged from 128.0-166.0 mg/L while Pathan *et al.,* (2009) Mishra *et al.*(2011) and Afroz and singh (2014) were found 276mg/L, 526 mg/L and 253-288mg/L of pulp and paper mill effluent, respectively.Chemical Oxygen Demand (COD) was in the present study was 273.02-341.22 mg/L while Pathan *et al.,* (2009) Mishra *et al.*(2011) and Afroz and Singh (2014) were found 1183 mg/L, 2418 mg/L and 313-681 mg/L, respectively. The chloride content in the present study was 145.25-174.00 mg/L where as Mishra et al.(2011) found 420.0- 450.0 mg/L in pulp mill effluent.

Results and Discussion

Table1: Seasonal Variations in Physico-Chemical Characteristic of Pulp And Paper Mill Effluent

Characteristics	Variable constituents through the year (Mean Values)			Yearly Average ±S.E.
	Summer (Mar.-Jun.)	Rainy (Jul.-Oct.)	Winter (Nov.-Feb.)	
Colour	Brown	Brown	Brown	Brown
Odour	Pungent	Pungent	Pungent	Pungent
pH	6.5±0.01	7.2±0.01	7.2±0.02	6.98±0.48
EC(mS/cm)	1.51±0.07	1.49±0.1	1.65±0.02	1.55±0.51
BOD(mg/L)	166.00±4.57	128.00±3.21	146.25±5.12	146.75±5.62
COD(mg/L)	2.73.02±18.21	331.25±16.32	341.22±17.11	306.65±18.78
Chloride(mg/L)	174.00±5.54	145.25±6.41	154.00±7.12	157.92±8.58
Phenol(mg/L)	0.12±0.02	0.15±0.06	0.15±0.05	0.14±0.06

Seasonal variation in LC_{50}

The mortality (LC_{50}) of test fishes exposed to paper mill effluent during pre-spawning, spawning and post-spawning phases is summarized in Table 2. The yearly average LC_{50} values were found to be 59.28±1.98 for 24h; 52.56±1.76; 46.50±1.81 for 72h and 43.40±1.92 for 96h exposure periods (Table.2).The seasonal variation in LC_{50} values shows

an inverse relationship with water temperature. LC_{50} was higher in post-spawning phase i.e. in winter season with average temp.24.5^{0}C and lower in spawning phase i.e. rainy season with average temp. 27.8^{o}C. A well marked variation was recorded in the LC_{50} values for the different seasons of the year. The lowest LC_{50} values were recorded in the spawning phase (Rainy season) of the fish where as the values were the highest in the post spawning phase (winter season). The stressed fishes were found to be the most sensitive to effluent during the spawning phase of the reproductive cycle (Mishra *et al*., 2011). Increased sensitivity under pollution stress during the spawning season has also been reported by Korn *et al*,(1976). The result of the present study showed that, LC_{50} was higher in winter months when the temperatures were low and EC and COD were high. Mishra *et al*,(2011) also reported that LC_{50} was higher in winter season i.e. post spawning phase of *Mystus vittatus* exposed to pulp mill effluent. Toxicity of the effluent mostly depends on the uptake of the effluent by the body. The rate of uptake is determined by the ratio of the permeability of body surface in contact with the medium to volume or weight of exposed animal and similar with relationship persists between the rate of metabolism and weight of animal (Pathan *et al*., 2009).It is also noticed that the toxicity of the pulp and paper mill effluent is attributed synergistically to the physical factors of medium i.e. high EC and COD and low pH. Thus the death of exposed fishes may be results of severe physiological stress at cellular level.

Table2: Seasonal Variation of Pulp and paper mill effluent toxicity (LC_{50}) to *Clarias batrachus* in Different Spawning Phases

SpawningPhases (Season)	Water Temperature Range (^{o}C)	LC_{50} % (v/v)			
		24h	48h	72h	96h
Pre-Spawning Phase (Summer)	24.-28.5 (26.8)	59.25	51.3	46.2	42.4
SpawningPhase (Rainy)	26.0-29.0(27.8)	51.8	45.5	40.5	37.9
Post-Spawning Phase(Winter)	21.0-27.0(24.5)	66.8	60.9	55.8	49.9
Yearly average ±S.E.	24.8±0.6	59.28±1.98	52.56±1.76	47.50±1.81	43.40±1.92
95%Confidence Interval	-	64.2-54.6	57.1-47.7	52.2-47.8	48.1-38.5

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 become sluggish and lethargic. They also showed abnormal swimming and loss of equilibrium before death. Increased surface activity and opercular movement, erratic and rapid movements have been observed in *Channa punctatus* exposed to distillery effluent (Kumar and Gopal,2001) and in *Mystus vittatus* exposed to pulp mill effluent (Mishra *et al*.,2011). 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). Thus it can be concluded that the effluent of pulp and paper mill is not safe to non-target organism like fishes. Changes in behavior of fish, *Clarias batrachus* due to pulp and paper mill effluent stress can be used as a biological indicator of pollution.

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