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Research Article

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TOXIC EFFECTS OF PAPER MILL EFFLUENTS ON MORTALITY, BEHAVIOUR AND MORPHOLOGY OF SNAKE HEADED FISH, *CHANNA PUNCTATUS* (BLOCH.)

Sadguru Prakash¹ and Ashok Kumar Verma^{2*}

¹Department of Zoology, M.L.K. (P.G.) College, Balrampur (U.P.), India

²Department of Zoology, Govt. PG College Saidabad, Prayagraj (U.P.), India

*Corresponding author: akv.gdcz@gmail.com

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Abstract: Present study was undertaken to find out the physico-chemical characteristics of paper mill effluents and their impact on mortality, behavior and morphology of fish, *Channa punctatus*. The result of physico-chemical characteristics of treated effluents showed that pH, EC, chloride and phenolic compound are within the permissible limit but colour, order, BOD, COD, TSS do not meet the permissible standards set by Central Pollution Control Board of India. LC₅₀ values of paper mill effluents of both sites (Po and Pd) for *Channa punctatus* were found 15% and 20%, respectively after 96 hours. Authors recorded abnormal behaviour of the fish studied throughout 96 hours in both effluents. The fishes showed erratic, haphazard movement, rapid opercular movement and tendency to jump out of water. The effluent exposed fishes showed the excessive secretion of the mucus. Many changes were seen in gill, skin and fins of fishes due to exposure of effluent. The gill colour changes from dark red to dull red and skin become erupted. The response of the fish towards toxicity was grossly dependent on concentration of effluents and length of exposure. Thus, the present study revealed that fish, *Channa punctatus* is sensitive to paper mill effluent toxicity and can be used as biological indicator.

Keywords: Behavioral response, *Channa punctatus*, Effects, LC₅₀, Paper mill effluents.

INTRODUCTION

Rapid industrialization in India has resulted in the substantial increase in the liquid waste which is discharged directly or indirectly into nearby natural lotic water bodies such as river and streams, causing environmental pollution. The pollution is continuous and alarming influx to

aquatic environment worldwide from both naturally occurring and anthropogenic sources (Verma and Prakash, 2019a). The environmental pollutants or toxicant can induce physiological and biochemical changes in fish that lead to growth inhibition (Prakash and Verma, 2019a & 2020a; Verma and Prakash, 2019b). Many of the

toxic substances released from these industries are lipophilic and weren't adversely affected by water. These substances accumulate in fish fatty tissues or become protein bound, so it is significant to know the critical concentration above which human being is affected and the commercial fish species become unsuitable food. The paper industry is the largest industry in India and holds 20th ranks among paper producing country. These industries disturb the ecological balance of the aquatic environment by discharging the varieties of wastewater. The paper mill wastewater characteristically contains dark colour, very high level of Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD) due to presence of lignin and its derivatives from the raw cellulose materials, chlorinated compounds, suspended solids, fatty acids, tannins, resin acids, sulphur and sulphur compounds etc (Ali and Sreekrishnan, 2001).

The paper-making process requires large amount of water for the production processes, hence it is a water-intensive process. In India, around 905.8 million m³ of water is consumed and around 695.7 million m³ of wastewater is discharged annually by this sector (Srivastava *et al.*, 2019). Consumption of water depends upon the raw material used in industrial processes. The natural raw materials are used for the processes are wood, cellulose, vegetables, bagasses, rice husk, fibers, waste-paper and so on. This creates a high level of wastewater from processing. The world demand for paper has grown rapidly around 5-6% per year. The paper mills have large investment and provide employment to lakhs of people. It is estimated that the capacity of the mills increases from 8.3 million tons in 2010 to 14 million tons in 2020 (Kesalkar *et al.*, 2012).

The industrial effluent contains heavy metal and the accumulation of heavy metal becomes hazardous to the aquatic organisms and also causes various diseases in human such as cardiovascular, hypertension, chronic kidney disease, lung and prostate cancer because the fishes are the most important factors of food chain which have great nutritive value and source of all essential amino acids (Prakash and Verma, 2020b; Kumar *et al.*, 2019).

The aquatic organisms are susceptible to pollution effect of industrial effluent. But normally an organism tries to adapt itself to these changes by changing their metabolic activities, but at higher concentration these pollutant can cause damage to biochemical system by affecting the organism either organ level, cellular level or even at molecular level in turn causing changes in biochemical composition (Verma and Prakash, 2020). The toxicity tests are necessary in water pollution evolution because chemical and physical measurements alone are not sufficient to assess potential effects on aquatic biota (Prakash and Verma, 2018). Fishes are much sensitive to changing aquatic environment and play an important role in the monitoring of water pollution so they are considered as good bioindicator (Prakash and Verma, 2020c). Thus the main objective of this investigation was to analyze the physico-chemical characteristics of the effluents and their impact on mortality, morphology and behaviour of snake headed fish, *Channa punctatus* (Bloch.).

MATERIALS AND METHODS

Effluent samples were collected from Yes Paper Mill Ltd. Darshan Nagar, Ayodhya (U.P.) in polyethylene container at two sites i.e. point of origin of effluent from treatment plant (Po) and point of discharge of effluent into river Ghaghra /Saryu (Pd). The collected samples of effluent were transported immediately to the laboratory to analyze the physical and chemical characteristics. The pH and electrical conductivity (EC) of effluent was measured on sampling site using digital portable pH meter and conductivity meter. The BOD, COD, TDS, Chloride and Phenols were measured according to APHA (2005).

Healthy specimens of snake headed fish, *Channa punctatus* (45±5 g & 12±5 cm) were collected from local fish farm at Balrampur, Uttar Pradesh and were transported to the laboratory. In the laboratory, the fishes were carefully examined for any injury and then kept in 1% solution of KMnO₄ for few hours to get rid of dermal infection, finally they were kept in large plastic jar containing 50L of clean tap water and acclimatized for 15 days to the laboratory conditions, during which time they were fed on boiled egg yolk and commercial fish food.

Feeding was stopped 24 hours prior to the toxicity test, to minimize the contamination from metabolic wastes. To find out LC_{50} and sublethal concentration, a group of 10 acclimatized fishes were exposed to the effluent collected from both sites (Po and Pd) for 24, 48, 72 and 96 hours at different concentrations along with a control. The

water was changed every 24 hours and during that time aeration was given to aquarium. During the study, morphological and behavioural alterations were critically observed. The percent concentration of test solution has been calculated using the formula:

$$\text{Volume percent} = \frac{\text{Volume of Effluent}}{V_E + V_{DW}} \times 100$$

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

RESULTS AND DISCUSSION

Physico-chemical characteristics: The results of physico-chemical analysis of effluent of different

sites *i.e.* point of origin from treatment plant and point of discharge into river is given in the Table1.

Table1: Physico-chemical characteristic of pulp mill effluent collected from Point of origin (Po) from treatment plant and Point of discharge (Pd) of effluent.

Characteristics	Magnitude		Standard of CPCB of India for effluents
	Site-Po	Site-Pd	
Colour	Dark Brown	Brown	Disagreeable
Odour	Pungent	Pungent	Disagreeable
pH	6.5 ± 0.11	6.98 ± 0.42	5.5-9.0
EC (mS/cm)	2.32 ± 0.16	1.55 ± 0.51	2.25
BOD (mg/L)	425.25 ± 3.54	156.75 ± 4.62	30.0
COD (mg/L)	1105.32 ± 51.13	341.65 ± 6.24	250.0
Chloride (mg/L)	221.00 ± 5.68	154.15 ± 6.12	1000.0
TSS (mg/L)	1145.00 ± 15.14	824.00 ± 9.25	100.0
Phenol (mg/L)	0.52 ± 0.02	0.14 ± 0.06	1.0

The characteristic pungent odour of 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).

The hydrogen ion concentration (pH) of the wastewater is an important factor because fluctuation in water pH affects the species composition and life processes of aquatic animal. Table 1 shows that the pH of effluent collected

from both sites *i.e.* effluent originates from treatment plant and effluent collected from point of discharge was 6.5 ± 0.11 and 6.98 ± 0.42 , respectively. The desirable limits of pH recommended by Central Pollution Control Board of India for effluent are 5.5-9.0. Low value of pH was due to metabolic production of acids by micro flora (Kesalkar *et al.*, 2012).

The mean electrical conductivity (EC) of effluent of both sites *i.e.* effluent originates from treatment plant and effluent collected from point of discharge was 2.32 ± 0.16 and 1.55 ± 0.51 mS/cm, respectively. These values of EC were

within the maximum desirable limits (2.5mS/cm) of Central Pollution Control Board of India. The values of electrical conductivity increased along with increasing concentration of effluents (Gagnetten *et al.*, 2007).

Biological Oxygen Demand (BOD) represents the amount of oxygen used by the microorganism to decompose the organic material. Mean BOD values of effluents collected from both sites *i.e.* Po and Pd were 425.25 ± 3.54 and 156.75 ± 4.62 mg/L, respectively. These values of BOD were higher the maximum desirable limit (30mg/L) of Central Pollution Control Board of India. Chemical Oxygen Demand (COD), represents the amount of oxygen required for oxidizing the all organic matters. The maximum permissible limit of COD recommended by Central Pollution Control Board of India is 250mg/L while the COD of effluents collected from treatment plant (1105.32 ± 51.13) and point of discharge (341.65 mg/L) into river effluents were found much greater than standard values. These values are higher than the range of 75 to 145 mg/L and 595 to 800 mg/L for BOD and COD, respectively as reported by Singh *et al.*, (1996). These differences may be due to variations in manufacturing processes, production capacity and efficiency of treatment plants as well as sites of effluent collection. In the present study, the levels of BOD and COD was reduced before discharge into river to certain extent due to oxidation of organic matter by biological treatment process, which provides energy for microbial metabolic process.

Chloride concentration in water indicates the presence of organic waste, primarily of animal origin. The maximum permissible limit of chloride recommended by Central Pollution Control Board of India is 1000.0 mg/L while maximum values of chloride in the present study was less than standard values.

Total suspended solid (TSS), the suspended matter consists of particles of different types of colloidal particles of various organic complexes (Afroz and Singh, 2014). The TSS of effluents collected from both sites was 1145.00 ± 15.14 and 824.00 ± 9.25 mg/L, respectively. These values of TSS were higher the maximum

desirable limit (100mg/L) of Central Pollution Control Board of India.

The maximum permissible limit of phenolic compound recommended by Central Pollution Control Board of India is 1.0 mg/L while maximum values of phenol were found within the standard values at both sites which varied between 0.12 and 0.5mg/L. Similar values of paper mill effluent was observed by (Srivastava *et al.*, 2019).

Thus, the physico-chemical characteristics of effluents collected from both sites of this mill revealed that pH, EC, chloride and phenolic compounds were within the permissible limit set by Central Pollution Control Board of India to discharge into water bodies. Whereas the other parameters *viz.* colour, order, BOD, COD and TSS were not within the permissible limits of Indian standard.

Determination of LC_{50}

Toxicity is a function of concentration and duration of exposure of an organism to a toxicant (Prakash and Verma, 2018). In the present study, during toxicity test fishes were exposed to various concentrations (5%, 10%, 15%, 20%, 25%, 30% and 35%) of paper mill effluent collected from both the sites *i.e.* Po and Pd for 96 hours and studied the general behavior and mortality rate of the fish studied. The fish, *Channa punctatus* was exposed to various concentrations from 24 to 96 hours and mortality rate in both effluents are shown in Table 2. For Po paper mill effluent, LC_{50} was observed at 15% effluent concentration after 96 hours, where as in Pd paper mill effluent LC_{50} was observed at 20% of effluent concentration after 96 hours. The toxicity study of mortality of *Channa punctatus* due to paper mill effluent shows that the mortality rates increase with increase in concentration and time of exposure. At 5% concentration of treated effluent, 90% of fish survival is not achieved which is the demand of WHO for final discharge of industrial wastewater. Thus, it can be concluded that the effluents are not safe to aquatic fishes.

Table2. LC₅₀ of fish exposed to various concentration of paper mill effluents

Paper mill Effluent Conc. (%)	No. of Fishes Exposed	Site of Effluent collection	24hrs	48hrs	72hrs	96hrs	Mortality % age
5	10	Po	-	-	-	2	20
	10	Pd	-	-	-	1	10
10	10	Po	-	-	1	3	40
	10	Pd	-	-	1	2	30
15	10	Po	-	-	2	3	50
	10	Pd	-	-	1	3	40
20	10	Po	-	1	2	4	70
	10	Pd	-	-	2	3	50
25	10	Po	-	2	2	4	80
	10	Pd	-	1	2	3	60
30	10	Po	1	2	3	4	100
	10	Pd	-	2	3	3	80
35	10	Po	-	-	-	-	100
	10	Pd	1	2	3	4	100

Behavioural changes: The fish *Channa punctatus* when introduced to 5% concentration of effluent showed abnormal swimming pattern. At such low concentration this abnormal swimming behavior continued up to 5-6 hours. and then comes to normal conditions. By increasing the % of effluent concentration from 15% at both the effluent sites, fishes were abnormal throughout 96 hours. and showed erratic haphazard movements and try to jump out of water and rapid opercular movement to get atmospheric air. Similar observation in heavy metal exposed fishes has been reported some researchers (Srivastava and Prakash, 2018 & 2019; Prakash and Verma, 2019b). During the study, at and above 10% all fishes form a mucous covering on body to resist the toxic chemicals in the effluents. These activities are directly related to complex biochemical and physiological responses of fishes. There was a positive

correlation between the effluent concentrations, time of exposure and degree of abnormal behaviour. Thus changes in behavior of fish to effluents stress can be used as biological indicator of pollution as early alarm system of effluents.

Morphological changes: The morphological changes in fish, *Channa punctatus* exposed to different concentrations of paper mill effluent for 96 hours are shown in Table 3. The effluent exposed fishes show the excessive secretion of the mucus. The blood clotting was observed on the gill and body surface of fish. Many other changes recorded in the body of fishes due to exposure of effluent such as change in gill colour from dark red to dull red and skin got eruptions. Thus from the present study, it may be concluded that air breathing fish, *Channa punctatus* is sensitive to paper mill effluent and can be used as indicators of effluent related stress in the water as

Table 3: Effect of paper mill effluent (Po) on morphology of *Channa punctatus* exposed for 96 hours.

Body Parts	Concentration of paper mill effluent						
	Control	10%	15%	20%	25%	30%	35%
Gill	Dark Red	Red	Red and Light slimy	Red and Moderate slimy	Dull Red	Blood Clotted	Blood Clotted
Skin	Normal grey	Gray	Grey and moderate slimy	Dark grey and slimy	Dull Black and slimy	Few Black & white patches appears and covered with mucous	More Black & white patches appear and covered with thick mucous
Fin	Grey	Gray	Light Slimy grey	Moderate slimy grey	High Slimy grey	Grey and wounded	Grey and wounded

CONCLUSION

From the physico-chemical studies of effluents and bio-assay tests, it can be concluded that (1) even treated effluents are not safe to aquatic animals including fishes, (2) the mortality rate was more pronounced as the concentration and duration of exposure of effluent increased, (3) change in behavior and morphology of fish is biological indicator of pollution, (4) rapid, erratic and haphazard swimming and rapid opercular movement of the fishes, (5) there was formation of a thick mucus covering over the whole body surface and (6) changes in gill and body colour. This study can be useful to compare the sensitivity of various species of aquatic animals and potency of effluents.

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