



## TOXICITY AND BEHAVIORAL CHANGES IN FRESHWATER FISH *CLARIAS BATRACHUS* EXPOSED TO PAPER MILL EFFLUENT

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### ABSTRACT

The aim of the present study is to determine the lethal concentration for 50% mortality of paper mill effluent to freshwater fish *Clarias batrachus* (for 24 to 96 hours) using bioassay method. To determine the LC<sub>50</sub> value, dilutions were prepared viz 20, 40, 60, 80 and 100% and fishes were exposed for 24, 48, 72 and 96 hours. The LC<sub>50</sub> value was found to be 40% at 96 hours. At this concentration, erratic swimming, jerky movement, rapid opercular movement, leaping out of water and thick mucous covering over the whole body surface was observed during experiment.

### INTRODUCTION

Increase in industrialization is currently taking place continuously but its effect on mankind, domestic animals, fishes and wild life through its water is unpredictable. Acute toxicity tests have historically played an important role in assessing the effect of human activities on animals and such tests have wide applicability in evaluating toxicities of various types of pollutants on fish and other aquatic species (Carddock et al. 1977). Wastewater from pulping process known as 'black liquors' usually contain high concentration of chloride, lignin, sodium hydroxide and chlorinated compounds and Hg which are toxic to living organisms of rivers.

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. The paper and pulp industry as it stands now, is one of the largest major Industries and contributes a lot towards the pollution in our aquatic environment. Looking into the serious nature of pollution, the pulp and paper industries in India have been brought under 17th category as they are highly polluting industries. In India some work on toxicity of paper mill effluent has been done. Toxicity and behavioral changes in freshwater fish *Rasbora daniconius* exposed to paper mill effluent in Aurangabad were examined by Pathan (2009). Similar work on toxicity and behavior of fish *Labeo rohita* and *Channa punctatus* exposed to pulp and paper mill effluent was done

by Srivastava et al. (2007). Many workers since then have confirmed that kraft mill effluents are lethal to fishes at a concentration of 10 to 100%. The present paper deals with the toxicity and behavioral changes in *Clarias batrachus* exposed to different concentrations of paper mill effluent.

### MATERIALS AND METHODS

Living specimens of *Clarias batrachus* were collected from Gour River flowing near Jabalpur (M.P.). These were brought to the laboratory and kept in glass aquaria under normal conditions for a week. During acclimatization fishes were fed with standard fish food two times a day. The paper mill effluent was collected directly from site of effluent discharge in Son River, Shahdol (M.P.).

Physico-chemical characteristics of river water (control) and paper mill effluent have been analyzed. Samples were taken every month from July 2008 to June 2009. Samples from the sites were stored at 4°C and brought to the lab. and analyzed according to APHA guidelines (1992) within 24 hours. The bioassay method of Finney (1971) was followed to determine LC<sub>50</sub> values of effluent. 90 *Clarias batrachus* fishes (8.0 to 8.5 cm in length and 4 to 5.5gm in weight) were selected for LC<sub>50</sub> determination. 10 fish were kept in each aquarium, each aquarium contained 5L water. Preliminary experiment at 5 different concentrations of paper mill effluent was conducted to find out the percentage that resulted in 50% mortality in given time periods tested. For

experimentation, laboratory acclimatized fishes were exposed to 100%, 80%, 60%, 40% and 20% dilutions of paper mill effluent. The water in aquaria was changed every 24 hours and was supplied with full aeration. A batch of 10 fishes was also maintained along with experimental fishes as control group. Fishes exposed to lethal concentrations of paper mill effluent for a short-term exposure were studied in terms of general behaviour, rate of survival and mortality.



Fig. 1. Fishes in aquarium at different dilutions of effluent.

LC<sub>50</sub> values of freshwater fish *Clarias batrachus* exposed to effluent were calculated. Each experiment was run in triplicate.

**RESULTS**

Physico-chemical characteristics of river water and paper mill effluent are given in Table 1. The fish *Clarias batrachus*,

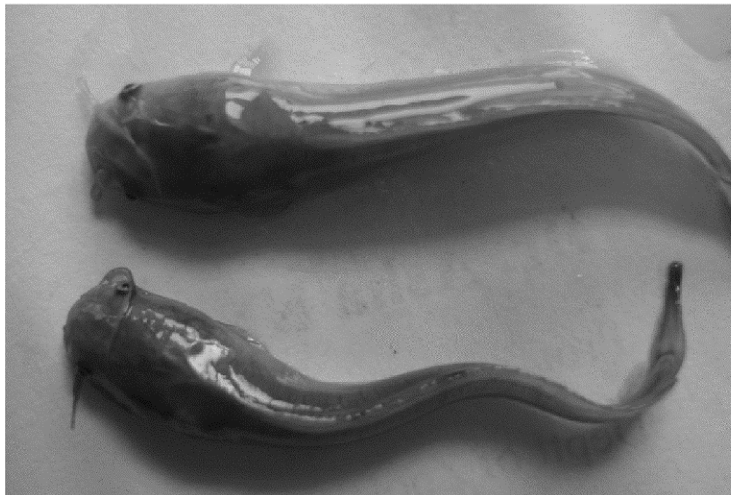


Fig. 2. *Clarias batrachus* exposed to the effluent having mucus covering (upper) fish and the normal (lower) fish

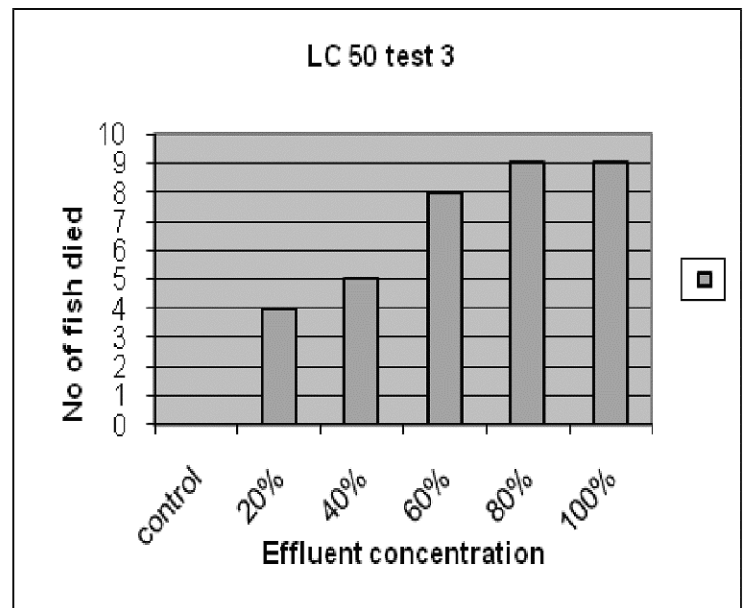
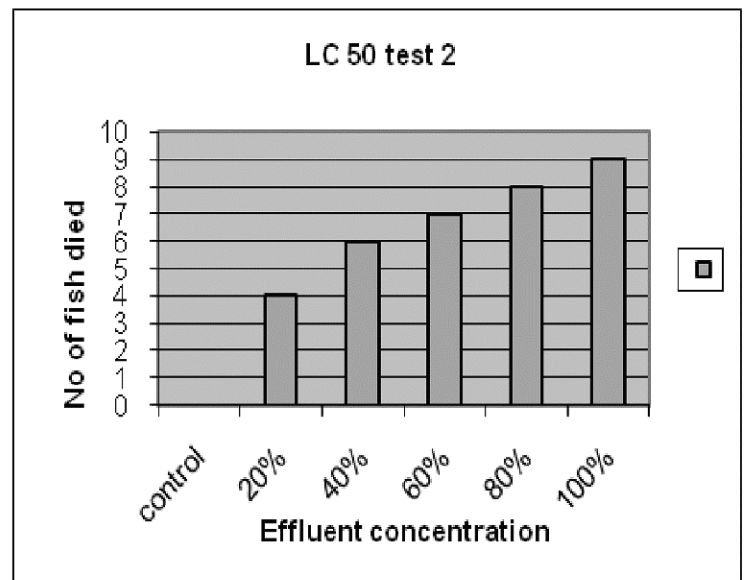
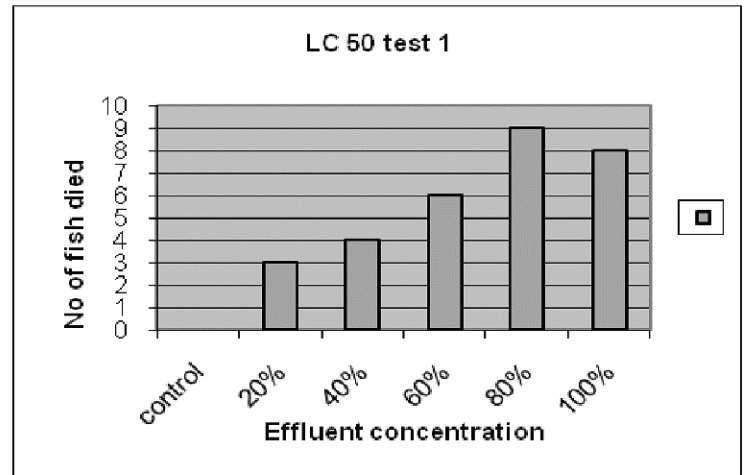


Fig. 3. Relation between effluent concentration and fishes died.

when exposed for 24 hours exhibited abnormal behaviour at all tested concentrations. These were under stress, which resulted in erratic swimming, convulsion, jerky movements and rapid opercular movement. The fish struggled hard for breathing sometimes engulfing atmospheric air and avoided toxic medium. The fishes tried to leap out of the toxic medium and a thick mucous covering over the whole body surface was observed (Fig. 2). The number of dead fishes were recorded (Fig. 3). The extrapolated  $LC_{50}$  value was found to be 40% at 96 hours.

## DISCUSSION

The freshwater environment is being polluted by various pollutants which have adverse effects on the aquatic organisms. The freshwater organisms particularly fishes are more susceptible to these pollutants, since their habitats are confined and escape from such polluted habitats is impossible. The effects of pollutants are generally seen as effects on survival, reproduction or growth due to physiological alteration in the animal. The physical and chemical factors have an important role in manifestation of biological response. This also depends upon many factors such as animal weight (Pickering 1968), developmental stages (Kamaldeep and Joor 1975), period of exposure and temperature, pH, hardness of water and dissolved content of the medium (Mc leese 1974). *Clarias batrachus* is a strong fish because it can survive in muddy water and also has a supportive air sac together with the gills to survive in stress conditions, but these fish have been seen to be affected by effluent and they show mortality. At the time of mortality, swelling in internal organs and bleeding in gills have been seen.

During present study fish, *Clarias batrachus* showed hyper excitation, erratic swimming, convulsions, jerky movement and rapid opercular movements and thick mucous covering over the whole body surface. Similar results were observed by Srivastava et al. (2007) when *Labeo rohita* and *Channa punctatus* were exposed to paper mill effluent. Kumar et al. (1991) reported the toxicity of paper mill effluent to fish *Puntius sophor*. Test was conducted in two groups. In first group dilutions were aerated. Whereas in group second dilutions were not aerated.  $LC_{50}$  for 96 hour was 1.5% whereas in second test it was recorded at 16.5%. Varadaraj and Subramanian (1991) reported the toxicity of paper and pulp mill effluent to fingerlings of *Oreochromis mossambicus* and reported  $LC_{50}$  for 96 hrs to be 6%. Nanda et al. (2002) studied toxicity of paper mill effluent. The  $LC_{50}$  values were found at 63.09, 80.35 and 81.28 % for *Anabus testudineus*, *Channa punctatus* and *Clarias batrachus* respectively. This indicates that *Anabus testudineus* is most susceptible, while *Channa punctatus* and *Clarias batrachus* were resistant.

The values for various physicochemical characteristics obtained in the present study are in good agreement with the characteristics of pulp and paper mill effluents as reported by other workers. Pathan (2009) reported temperature, pH and dissolved oxygen  $28\pm 2^{\circ}C$ , 8.1 and 0.98 mg/l respectively whereas in the present study it was  $32.26\pm 4.9^{\circ}C$ ,  $7.8\pm 0.25$  and  $1.5\pm 0.76$  mg/l. Mean BOD and COD values obtained in the present study ranged from 124 to 214 mg/l and 714 to 864 mg/l respectively. These values were higher than the ranges of 75 to 145 mg/l and 595 to 800 mg/l for COD and BOD respectively as reported by Singh et al. (1996) but lower than 147 to 254 mg/l and 224 to 1317 mg/l for BOD and COD respectively as reported by Malaviya and Rathore

Table 1. Physico-chemical characteristics of river water (control) and paper mill effluent

S. No.	Parameter	Unpolluted river water	Paper mill effluent
1	Temperature	$28.79 \pm 4.8^{\circ}C$	$32.26 \pm 4.9^{\circ}C$
2	pH	$6.8 \pm 0.22$	$7.8 \pm 0.25$
3	Dissolve oxygen	$7.98 \pm 1.0$	$1.5 \pm 0.76$
4	Total hardness	$139.2 \pm 13.84$	$486.1 \pm 30.99$
5	Alkalinity	$91.39 \pm 8.821$	$37.2 \pm 37.19$
6	BOD	$22 \pm 2.71$	$177 \pm 26.09$
7	COD	$187 \pm 18.77$	$798 \pm 48.91$
8	Lignin	-	$727 \pm 61.21$
10	TSS	-	$166.6 \pm 19.52$
11	Chloride	$4253 \pm 6.19$	$1175 \pm 49.3$

Except Temperature and pH, all values are expressed in mg/L

(2007) in Century Pulp and Paper mill, Lalkuan (Distt. Nainital). The value of lignin content was  $727 \pm 61.21$  mg/l that was lower than 4830.18, 318.87 and 2298.97 mg/l reported by Malaviya and Rathore (2007) at 3 sites. These differences may be due to variation in manufacturing processes, production capacity and efficiency of mill treatment plants as well as site of effluent collection.

The fish death may be the result of severe physiological stress at cellular level. It was also noticed that, the toxicity of the paper mill effluent is attributed synergistically to the high COD and BOD values besides low pH and low dissolved oxygen (DO).

Thus it is concluded that the effluent is not safe to non-target organisms like fishes. This type of study can be useful to compare the sensitivity of various species of aquatic animals and potency of effluent using  $LC_{50}$  values and to derive safe concentration. Changes in behaviour of fish *Clarias batrachus*, due to paper mill effluent stress can be used as a biological indicator of pollution and as biological early alarm system of the paper mill effluent.

## REFERENCES

- APHA. 1992. Standard Methods for the Examination of water and wastewater 18th Edition.
- Craddock, D.R. 1977. Use and limitations of acute toxicity test- a review. In Malins, Dc (ed), Effect of petroleum on arctic and sub arctic marine environment and organism. Academic press, New York, pp: 1-93.
- Finney, D.J. 1971. Probit analysis. Cambridge University press London.
- Kamaldeep, K. and H.S. Joor. 1975. Toxicity of pesticides to embryonic stage of *Cyprinus carpio* (Linn.) Indian Journal of Experimental Biology 15: 193-196.
- Mc leese, D.W. 1974. Response of lobster, *Homarus americanus* to odour solution in the presence of bleached kraft mill effluent. Journal of the Fisheries Research Board of Canada 30: 279-283.
- Nanda, P., S. Panigrahi, B. Nanda and B.K. Behera. 2002. Toxicity of paper mill effluent to fishes. Environmental Economics 20(2): 496-498
- Pathan T. S. 2009. Toxicity and Behavioural Changes in Freshwater Fish *Rosbora deniconious* Exposed to Paper Mill Effluent. Botany Research International 2(4): 263-266.
- Pickering, Q.H. 1968. Some effects of dissolved oxygen concentrations upon the toxicity of zinc to the blue gill *Lepomis macrochirus*. Water Research 2: 187-194.
- Malaviya, P. and V.S. Rathore. 2007. Seasonal variation in different physico-chemical parameters of the effluents of Century pulp and paper mill, Lalkuan, Uttarakhand. Journal of Environment Biology 28(2): 219-224.
- Kumar, R., M. Chouhan, Sunita and K.D. Mishra 1991. Toxicity of paper mill effluent to fish, *Puntius sophor*. Journal of Cell and Tissue Research 1(1 and 2): 41-48.
- Singh, R.S. Marwaha and P.K. Khanna. 1996. Characteristics of pulp and paper mill effluents. Journal of Indian Pollution Control 12 (2): 163-172.
- Srivastava, S., Prabhakar, P. Singh and B.C. Srivastava 2007. Toxicity and behaviour of the fish *Labeo rohita* and *Channa punctatus* exposed to pulp paper mill effluent. Journal Ecotoxicology Environmental Monitoring 17(3): 241-244.
- Varadaraj, G and M.A. Subramanian. 1991. Toxic effect of paper and pulp mill effluent on different parameters of bioenergetics in the fingerlings of *Oreochromis mossambicus*. Environment and Ecology 9(4): 857-859.