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TOXICITY AND BEHAVIOURAL CHANGES IN FRESHWATER FISH *PUNTIUS STIGMA* EXPOSED TO PAPER MILL EFFLUENT

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ABSTRACT

Many industries in India discharge their effluents directly or indirectly into inland water bodies leading to the nearby rivers. It is found that almost all industries are violating rule for discharging effluents and polluting nearby fresh water bodies and agricultural land. Present study was undertaken to determine toxicity of a paper mill effluent to fresh water fish *Puntius stigma* for 24 to 96 hours using renewal bioassay method. The estimate LC50 values, series of concentrations [% dilutions] were prepared for 24, 48, 72 and 96 hrs and expected mortalities were found at 40, 35, 30 and 25% concentrations respectively of the effluent.

Gross effects like erratic swimming, jerky movement, and rapid opercular movements leaping out of water and excess mucous secretion were observed during experiments. Therefore, adequate steps have to be taken towards compliance of all environmental quality standards for betterment of the environment and reduce health impact.

Key words: Paper mill effluent, Toxicity, Behavior*, Puntius stigma.*

INTRODUCTION

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 (Quasim and Siddque, 1960; David and Ray, 1966; Venkataraman, 1966; Hingoroni et al.1979). Pollution of aquatic by domestic and untreated or partially treated industrial effluent greatly contributes to massive kill of fish and other important aquatic biota (Kumari and RamKumari, 1997).

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 (Kim Oanha, 1996 and Yen et al., 1996)

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Van Horn (1961) reviewed the pulp and paper industry as it affects aquatic biology. Walden (1976) published an excellent review on the toxicity of effluents from pulp and paper mills. Reported toxicity of Kraft wastes to fish prior the work of Ebeling,(1931) in Sweden.

The present paper deals with the toxicity and behavioral changes Puntius stigma is exposed to lethal concentration of paper mill effluent

MATERIAL AND METHODS

In the present study, fish, *Puntius stigma* were collected from River Bhima at Bhigwan, and brought to laboratory without any mechanical injury. The fishes were maintained in glass aquaria and allowed to acclimatize for a period of four weeks before being used for the test. During the period of acclimatization the fishes were fed, after every 24 hours on pieces of live earthworm. Filtered and aged water was used to maintain the fish as well as test. All aquaria were uniformly illuminated by diffused sunlight through laboratory windows and tube lights. The aquaria were cleaned after every eight days and water changed every alternate day. All the necessary care was taken to keep the aquaria away from mechanical disturbance. The acclimatized healthy fishes showing normal activities were selected for chronic toxicity tests.

Glass aquaria were used as test containers. Artificial aeration during the test period was avoided. A group of ten healthy fishes of average weight (4 to 4.5 gm) and of average length (6.8 to 8.5 cm) were selected for the present study.

The physico-chemical parameters of the test water were analyzed on every alternate day during the toxicity test period of 30 days by the following methodology described by APHA (2000).

To determine the long-term effect of paper mill effluent on the survival and growth in fish, *Puntius stigma* two sub lethal concentrations were selected. Along with these two sub lethal concentrations, the control group also maintained. The rate of survival, feeding and growth were also recorded for control group.

The sub lethal concentrations were selected on basis of the result of acute toxicity studies. The LC_{50} value for 96 hours was estimated at 25 respectively. The Sub lethal concentrations for chronic test were selected at 5 % (1/5) and 2.5 % (1/10).

After exposing the test fish to two sub lethal concentrations of the paper mill effluent, the survival, feeding and growth of the test fish was studied for 30 days.

Survival Data

The test fishes were exposed to two sub lethal concentrations i.e. 5 % (1/5) and 2.5 % (1/10) of paper mill effluent; the observations were made at every week to note the number of fishes survived in particular concentration. Similar observations were continued for 30 days exposure. From this percentage of mortality in the two sub lethal concentrations were calculated at the end of the chronic exposure (30 days).

The study on the survival data is helpful to determine the toxicity of a paper mill effluent in relation to time.

Feeding

The effect of chronic exposure of paper mill effluent on the feeding was studied with a view to find out whether the appetite of the fish was affected any way. Observations on food consumption were also made at every week. Where, the experimental fish were fed with a definite weight of live earthworm pieces in the morning hours between 9 a.m. to 10 a.m. The unconsumed food (pieces of earthworm) were siphoned out, blotted with, blotting paper and weighed. The difference between the two weights is the quantity of food consumed by the fish. Food consumed is calculated and expressed in mg/gm body weight.

Growth

The effects of two sub lethal concentrations of paper effluent on the growth have been measured. The growth was measured in terms of increase in the weight per fish. Observations were made once in a week and recorded till the end of the experiment.

A beaker with known quantity of water was taken, weighed and to this weighed beaker the test fish were added and weighed again. The difference in the two successive weights was taken as increase or decrease in weight in particular group during the every week, recorded in the present study. The data on growth analysis is as per the method of Webb and Brett (1972).

RESULTS

In the present investigation the test fish, *Puntius stigma* was exposed to two sub-lethal concentrations of paper mill effluent, the concentrations were 1/5 and 1/10 of the 96 hrs LC_{50} value of paper mill effluent. These values are 5% and 0 2.5% concentration of paper mill effluent.

The effect of two sub lethal concentrations i.e. 5% and 2.5 % of paper mill effluent on different aspects of fish, *Puntius stigma* like survival, feeding and growth were briefly summarized

Survival

During the present study, the number of fish died per week and total mortality and total survival at the end of 30 days exposure was observed. There was no mortality found in both the concentrations (5% and 2.5 %) of 96 hrs LC_{50} of paper mill effluent. Survival data of the test fish is given in table No (1).

Feeding

During present study, average food consumed by the fish every week was observed in term of mg/gm body weight and the percentage increase or decrease in the intake of food, during experiment have been recorded in the table No (2).

It was observed that the control fishes showed normal food intake and exposed fishes showed decrease in food consumption as compared to control.

Growth

Growth of *Puntius stigma* was studied in relation to the body weight in the control as well as in the treated fishes during chronic exposure period i.e. 30 days shown in Table No. (3).

In control group, the growth of fishes increased progressively (normal). But exposed fishes showed decrease in growth as compared to control.

DISCUSSION

The known sub lethal effects of pulp and paper mill effluents are attributed to coniferous fibers, hydrogen sulfide, and nonvolatile soluble toxic substances (Walden 1976). The last group is of major environmental concern.

It has been demonstrated that fish exposed to pulp and paper mill effluent evoked variety of adverse effects. These include liver dysfunction (Oikari and Nakari, 1982), reduced growth rate (Whittle and Flood, 1977). Warren (1973) observed the effect of various concentrations of Kraft mill effluent on growth of juvenile Chinook salmon, *Onchorhynchus tshawytscha* held in continuous flow system for 2 to 3 weeks. In aquarium, growth at 0.25 % percent by volume and stabilized bleached Kraft effluent reduced growth at 6 percent by volume. However, laboratory stream experiment showed that primary treatment of kraft effluent decrease growth at 1.5 percent by volume Kelso (1977) found that yellow perch, *Perca flavescens* were numerically dominant in areas uninfluenced by mill effluent in Nipigon Bay, Lake Superior, but were rare in areas where effluent was most concentrated. Juvenile Atlantic salmon, *Salmo salar* avoid BKME from 0.001 to 10% concentration (Sprague and Drury 1969). Walden and Howard (1968) described effects displayed by fish after exposure to lethal concentrations of kraft effluent: loss of schooling, respiratory distress, abnormal gill movement, reluctant to eat, loss of equilibrium, convulsion, coughing, excessive mucous production, and finally death. Schaumbrug *et al.,* (1967) studied the effects of kraft effluent on fish respiration. They found that stressed fish reversed the flow of water past their gills; this was designated as "coughing". Coughing increased with increasing concentration of effluents. Wong *et al.,* (1992) reported the exposure to paper and pulp mill effluent significantly reduced the weight of lymphoid organ Viz spleen, head kidney and total kidney. There was also a significant decrease in the total number of cells in spleen and pronephros of experimental fish as compared to control fish. Pulp mill effluent are Halogenated Aromatic Hydrocarbon (HAH's), such as PCDD's and PCDF's and heavy metal. The HAH's reported to induce reduction in lymphoid organ weight decrease resistance to infections and gross immunosupperssion.

According to Buchler and Shank (1970) median survival time was directly proportional to body weight in young coho salmon fed on diet containing technical DDT. Kamble (1983) also observed 100% survival in *L. thermalis* to two sublethal concentrations of BHC and samithion using chronic exposure period. Davne (1991) observed 100% survival and zero percent mortality in *T. sandkhol* to sublethal concentration of thiodon, diemethoate and rogar (carbaryl). Haithame (1999) observed mortality of the fish, *Channa gachua* was 10% in 1/15(0.0040ppm), 20% in 1/10 (0.0060ppm) and 30% in 1/5 (0.012ppm) concentrations of endosulfan and diemethoate showed the same effect, the overall mortality in each of these was 5% and break of the effects was zero in 1/15 (0.038), 10% in 1/10 (0.058) and 20% in 1/5 (0.116) concentration of pesticides. Similar finding was recoded by Gaikwad (2003). Ganeshwade (2002) observed 100% survival in *Puntius ticto* to two sublethal concentrations of dimethoate during chronic exposure period.

Webb and Brett (1972) observed, increased in growth rate of sokye salmon *Onchorhynchus nerka* .During endrin treatment *Salmo girdnerii* gained weight although a reduced growth rate was observed at high dose of endrin (145mg/kg) (Grant and Mehrle, 1973) where as the chronic exposure of the fish to endrin affect gonadal development.

Grant (1976) have reported that at higher concentration dose between (33-95ppm) decline in growth rate and abnormality in behaviour to endrin toxic action leading to mortality was observed. Exposure of *Gambusia affinis* and *Gymnocolymbus tarenetzi* to sublethal concentrations of thiodon and MEM shows significant rate of food consumption. Intermediate doses caused a significant fall in feeding rate (Joshi *et al.,* 1981). The growth rate in *Heteropneneustes fossilis* decreased from 18-69% on 8 weeks exposure (Choudhary *et al.,* 1981).

Manoharan and Subhaiah (1982) in *Barbus stigma* reported that the feeding and growth rate were declined due to exposure to endosulfan at 0.003 ppm. .Decrease in food consumption was observed in *Channa Punctatus* exposed to sublethal concentration of carbaryl (Aurnachalam *et al.,* 1985). Decrease in feeding and growth rate with increasing concentration of agricultural fertilizers were found in *Sarotherodon mossambicus*, might be because the fertilizers select as metabolic stressor at higher concentration like 0.4 ppm (Palanichamy *et al.,* 1985). Similarly *Macrones keletius* exposed to sublethal concentration of diemethoate showed decreased feeding rate (Hameed and Vadanli, 1986). James *et al.,* (1995) reported that fish *H. fossilis* exposed to copper and mercury showed decrease in food consumption and growth

Maria *et al.,* (2004) reported the effect of different concentration of B/UKME on physiological parameter of bass exposed to effluent for 28 and 56 days. The only significant changes observed were: a decline in the number of RBCs in largemouth bass exposed to 40% effluent for 28 days; an increase in the concentration of albumin in female and male exposed to ≥ 20% effluent for 56 days; and increase in HIS for males exposed to ≥20% effluent for either 28 or 56 days; an increases in the concentration of AKP in female exposed to 20% and 40% effluent for 28 days and 40% effluent for 56 days. The remaining physiological parameter Viz body weight and length; SSI; PCV; HB; glucose chloride; calcium; phosphorous; globulin; blood urea nitrogen; uric acid total bilirubin; ALT and AST were not affected by treatment.

Superanee Chinabul *et al.,* (1998) reported the sublethal effects of formalin to three freshwater fishes, *Puntius gonionotus* (Bleeker), *Cyprinus carpio* (Linn.), and *Channa striatus* (Flower). There were significant differences in percent weight gain among the control common carp fry and fish treated with 25, 50 and 75 ppm formalin had the lowest growth throughout the 8-week period.

Arunachalam *et al.,*(1980) found that over 27 day exposure to carbaryl, there was a decrease in growth rate and food conversion efficiency. A study by Kumari and Ramkumari (1997) on *Channa punctatus* revealed that significant decrease in carbohydrate content in the fish suggesting possibility of increased gycolgenolysis in fish under pollutant stress as compared to control fish: Further, Wendelaar (1997) have also reported that sublethal stress can induced growth rate in fish possibly as a result of energy reallocation. Changes in physiological activity and behaviour response recorded, by Kumar *et al.,* (1999) and Mishra *et al.,* (2002) in *H. Fossilis* on exposure to detamethrin and cypermethrin respectively. Burkhalter and Kaya (1977) reported the exposure of rainbow trout eggs and alevins to unionized ammonia caused mortality at or after hatching, retarded larval growth, inhibited yolk sac absorption.

De Boeck *et al.,* (1997) reported copper, exposure at 0.80 µm affected both growth and feeding behaviour in common carp at 0.55 µm growth was affected despite normal food consumption. Even at the lowest copper concentration (0.20 µm), metabolic demand for the fish increased, challenging the carp with an increased demand for food. Watson and Mckcown (1976) reported the long term effect of the three sublethal concentrations of Zinc (0.214, 0.52 and 1.12 ppm) on growth and plasma glucose concentration in yearing rainbow trout *Salmon gairdneri*. Analysis of covariance of percent weight increase revealed that a significant inhibition of growth (P>0.05) in the 1.12 ppm zinc exposed fish had occurred. Plasma glucose showed a significant hyperglycemia (P>0.05) in all three zinc-exposed group of fish after 7 days exposure and in the 1.12 ppm zinc exposed group after 63 days.

Food consumption and average body weight are interrelated with each other and this may interfere when the fish exposed to the toxicant even at sub lethal concentration. Body is indispensably related to intake of food but other environmental factor play an important role in the proportionate growth.

In the present study, food consumption and growth of the fishes found to be reduced on treatment to paper mill effluent, and can be correlated with the inhibition of digestive enzyme secretions. Similar finding were recorded by Das there was significant decrease in carbohydrate content in the fish suggesting possibility of increased gycolgenolysis in fish under pollutant stress as compared to control fish: Further, Wendelaar (1997) have also reported that sublethal stress can induced growth rate in fish possibly as a result of energy reallocation. Changes in physiological activity and behaviour response recorded, by Kumar *et al.,* (1999) and Mishra *et al.,* (2002) in *H. Fossilis* on exposure to detamethrin and cypermethrin respectively. Burkhalter and Kaya (1977) reported the exposure of rainbow trout eggs and alevins to unionized ammonia caused mortality at or after hatching, retarded larval growth, inhibited yolk sac absorption.

Fig. 1: Relationship between probit of mortality and dose of paper mill effluent. Mortality of *Puntius stigma* is fitted in probit / concentration regression line after exposure for 24 (a), 48 (b), 72 (c) and 96 (d) hrs.

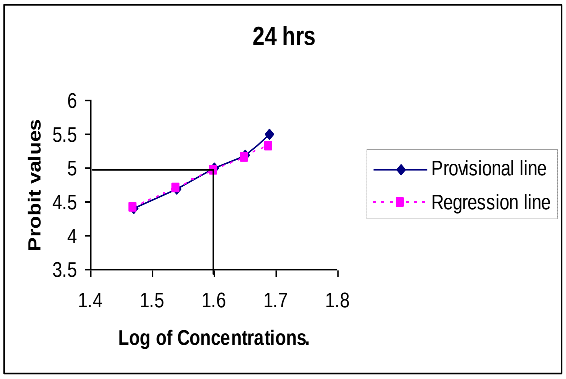

Fig. a

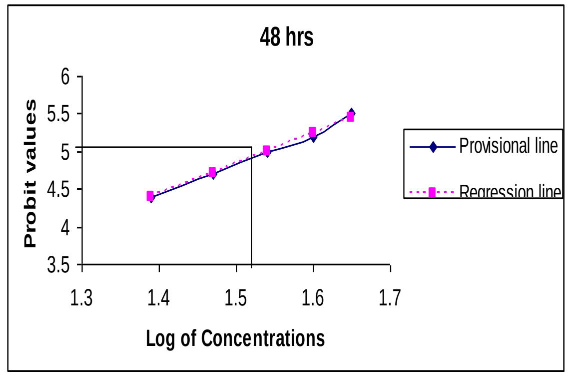

Fig. b

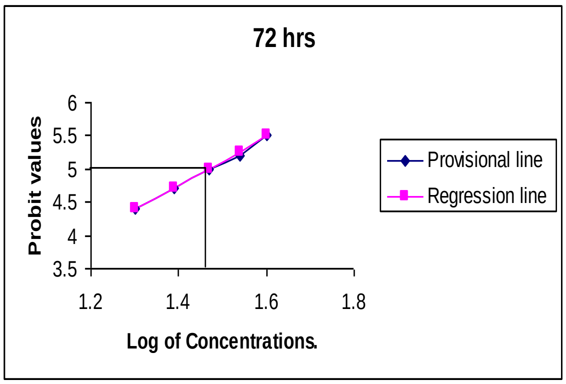

Fig. c

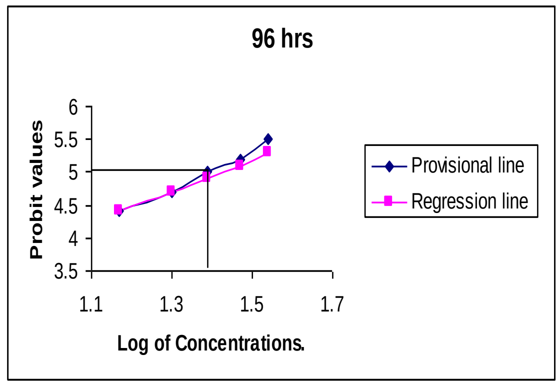

Fig. d

De Boeck *et al.,* (1997) reported copper, exposure at 0.80 µm affected both growth and feeding behaviour in common carp at 0.55 µm growth was affected despite normal food consumption. Even at the lowest copper concentration (0.20 µm), metabolic demand for the fish increased, challenging the carp with an increased demand for food. Watson and Mckcown (1976) reported the long term effect of the three sublethal concentrations of Zinc (0.214, 0.52 and 1.12 ppm) on growth and plasma glucose

Test Fish :- *Puntius stigma*

Table No. 1 Mortality of Puntius *stigma* chronically exposed to Paper mill effluent

Week	Control	Paper Mill Effluent 2.5 % (1/10)	Paper Mill Effluent 5 % (1/5)
I^st	0	0	0
II^nd	0	0	0
III^rd	0	0	0
IV^th	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 No. 2 Food intake (g/week) in fish, *Puntius stigma* exposed to the paper mill effluent

Week	Control	Paper Mill Effluent	
		2.5 % (1/10)	5 % (1/5)
I st	1.52	1.45	1.50
II nd	1.57 (3.28)	1.40 (-3.44)	1.43 (-6)
III rd	1.60 (5.26)	1.38 (-4.82)	1.40 (-6.66)
IV th	1.63 (7.23)	1.35 (-6.89)	1.39 (-7.33)

***The figures in brackets show the percentage increased/decreased in the intake of food by the fish**.

concentration in yearing rainbow trout *Salmon gairdneri*. Analysis of covariance of percent weight increase revealed that a significant inhibition of growth (P>0.05) in the 1.12 ppm zinc exposed fish had occurred. Plasma glucose showed a significant hyperglycemia (P>0.05) in all three zinc-exposed group of fish after 7 days exposure and in the 1.12 ppm zinc exposed group after 63 days.

Food consumption and average body weight are interrelated with each other and this may interfere when the fish exposed to the toxicant even at sub lethal concentration. Body is indispensably related to intake of food but other environmental factor play an important role in the proportionate growth.

In the present study, food consumption and growth of the fishes found to be reduced on treatment to paper mill effluent, and can be correlated with the inhibition of digestive enzyme secretions. Similar finding were recorded by Das and Konar (1974), Khillare (1990,1992), Srivastava [2007]and *Pathan et al.,* (2010) Contrary, the reserved food of the body might be utilized during the stress condition. The growth of the fish on food intake and normal movement totally depends upon the healthy condition of the fish.

CONCLUSION

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 LC50 values and to derive safe concentration

Table No. 3 Growth (average weight in gms) of fish, *Puntius stigma* chronically exposed two sub lethal concentrations of the, paper mill effluent

Week	Control	Paper Mill Effluent	
		2.5 % (1/10)	5 % (1/5)
Initial Weight	4.28	4.30	4.50
I st	4.30 (0.46)	4.25 (-1.16)	4.43 (-1.55)
II nd	4.33 (1.16)	4.23 (-1.62)	4.41 (-2)
III rd	4.40 (2.80)	4.20 (-2.32)	4.38 (-2.66)
IV th	4.45 (3.97)	4.19 (-2.55)	4.33 (-3.77)

*** Figures in the brackets indicate percentage increase/decrease in the growth of fish.**

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