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Impact of Paper Mill Effluent on the Survival and Hatchability of Eggs of *Cyprinus carpio*

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ABSTRACT

Urbanization and industrial development have become responsible for deteriorating the aquatic environment through atmospheric deposition of waste and effluent discharge in the water bodies. The present study was aimed to analyze the effect of Paper Mill Effluent (PME) on the survival and hatchability of the fish eggs under laboratory condition. Four hundred fertilized eggs of *Cyprinus carpio* were exposed to different concentrations less than half of the LC_{50} (1, 2 and 4% v/v) of PME to investigate survival and hatchability. In control group percent mortality ranged between 0.375 (after 12 h) to 2.5 (after 40 h). In PME treated groups it ranged between 2.5-4.75 in 1% PME, 3.375 to 6.125 in 2% PME and 3.875 to 10.125 in 4% PME. Results showed that the PME entering the inland water is a serious environmental pollutant with high ecotoxical risks even in small concentration especially for fish species and their developmental stages.

Key words: Paper mill effluent, *Cyprinus carpio*, early developmental stages, mortality

INTRODUCTION

Urbanization and industrial development have become responsible for deteriorating the aquatic environment through atmospheric deposition of waste and effluent discharge in the water bodies (Ali and Sreekrishnan, 2001; Singh, 2007; Anbumani and Mohankumar, 2011; Moharram *et al*., 2011). After metals and the chemical industries, pulp and paper mills have their largest contribution in utilizing the huge amount of fresh water, wood and energy during paper production (Kallas and Munter, 1994). Thus pulp and paper industries are consuming the natural resources in production of paper on the other hand these industries are discharging their effluent in water bodies which is polluting the aquatic resources (Prabu and Udayasoorian, 2005; Nagasathya and Thajuddin, 2008; Muhamad *et al*., 2011). Approximately 350 paper mills with an installed capacity of 3.04×10^6 tonnes of paper per annum are present in India (Jayabalakrishnan, 2007). Chlorinated compounds produced during bleaching of paper, enter the aquatic environment through the effluent. Effluent also contains suspended solids including fibers, tannin, resin acid, lignin, sulfur compounds which leads to aquatic pollution (Ali and Sreekrishnan, 2001; Basibuyuk and Forster, 2003; Saquib and Muneer, 2003; Paasivirta *et al*., 2005; Ugurlu *et al*., 2005, 2008; Zahrim *et al*., 2007). These effluent constituents may induce clastogenic, carcinogenic as well as mutagenic effects on residing fishes (Owens, 1991; Ali and Sreekrishnan, 2001; Karrasch *et al*., 2006). Aquatic organisms are most vulnerable to environmental stress generated due to the industrial pollution during their ontogenetic development. Effects of metals such as zinc on the

early life stages (eggs) of *Clupea harengus* has been observed by Somasundaram *et al.* (1984). During early development, organ systems become sensitive at certain periods to chemicals present in surrounding environment (Ozoh, 1979).

Keeping in view, the xenobiotic effect of paper mill effluent on survival of fish, the present investigation on early developmental stages of *Cyprinus carpio* was undertaken. The present study was aimed to analyze the survived and hatchability of the eggs of *Cyprinus carpio* on exposure to PME under the laboratory conditions.

MATERIALS AND METHODS

The present study was conducted at Fish and Fisheries Laboratory, Department of Zoology, Kurukshetra University, Kurukshetra (29°58' N Latitude and 76°51' E Longitude) Haryana, India.

Test organism: The fertilized eggs of fresh water teleost, *Cyprinus carpio* (var. *communis*) Linnaeus, 1758 belonging to order cypriniformes and family Cyprinidae was used as test organism to investigate the effect of Paper Mill Effluent (PME) on the survival and, hatching performance in the eggs. Fertilized eggs of the fish were procured from National Fish Seed Farm, Jyotisar (Kurukshetra), Sultan Fish Seed Farm, Butana (Karnal) and Fish Seed Farm situated in village Mandheri (Kurukshera) during March, 2010.

Toxicant used: The effluent was collected in 20 L cane from the drain as it cross the boundary wall of the mill and was brought to the laboratory. The cane were left undisturbed overnight at environmental temperature so that all suspended particles settle down at the bottom. Supernatant of the effluent was mixed with dechlorinated tap water to obtain the required percentage (v/v) of the effluent.

Sixteen hundred eggs of *Cyprinus carpio* were divided into four sets A, B, C and D. Set A was considered as control and were reared in dechlorinated tap water. Sets B, C and D were respectively exposed to 1, 2 and 4% PME.

Experiment set up: Whole experiment was performed in triplicates. The eggs were placed in inner hatching hapa fitted with in plastic tubs containing 35 L of different concentrations of the PME. Continuous water circulation inside the experimental tubs was maintained with the help of water pump. Physicochemical parameters such as Dissolved Oxygen (DO), temperature were recorded regularly during the experiment. Eggs were monitored regularly and dead eggs were removed and recorded immediately whenever observed. Time to start of hatching and end of hatching was noticed in each experimental tubs. Treated eggs were fixed in appropriate fixative for further embryonic study.

RESULTS

Percent mortality in the eggs of *Cyprinus carpio* after exposure of paper mill effluent was analyzed at two levels:

- At variable time in the same concentration (i.e., in time dependent manner)
- At variable sub lethal concentrations of effluent at constant time period (i.e., in dose dependent manner)

Mortality was measured as the number of dead eggs removed from the treatment tubs during the exposure period. To eliminate the effect of any environmental factor and variation in the

Table 1: Showing percent mortality after exposure of *Cyprinus carpio* eggs with different concentrations of paper mill effluent

	Percent mortality			
Exposure duration (h)	Control (Set A)	1% (Set B)	2% (Set C)	4% (Set D)
12	0.375	2.500	3.375	3.875
24	1.125	3.125	4.250	5.250
36	1.625	4.125	5.500	7.750
40	2.500	4.750	6.125	10.125

concentration of constituents of the PME the experiment was performed for the two consecutive years during the same months. Chi square analysis of the data recorded during the two years revealed no significant difference ($p \leq 0.05$) in percent mortality within the treatment groups of same concentration of PME. Therefore, the data of the two years was pooled and mean of the two years was taken as percent mortality in different groups at variable time of exposure till the hatching of the eggs (Table 1).

At variable time in the same concentration (i.e., in time dependent manner): In Set A percent mortality ranged between 0.375 (after 12 h) to 2.5 (after 40 h) through 1.125 after 24 h and 1.625 after 36 h. Chi square analysis of the data revealed insignificant difference in rate of mortality after different hours of exposure ($p \leq 0.05$).

In all the treatment set B, C and D the observed percent mortality was significantly high during the first 12 h of exposure ($p \leq 0.05$). Thereafter the mortality rate was insignificant. In Set B the observed percent mortality was 2.5 after 12 h of exposure durations, which increased to 3.125, 4.125 and 4.75 after the exposure of 24, 36 and 40 h, respectively. In Set C the observed percent mortality ranged between 3.375 (after 12 h) to 6.125 (after 40 h of exposure). Significantly high mortality was observed during first 12 h. After 24 h of exposure duration, mortality noticed was 4.25% which increased to 5.5% after 36 h and to 6.125% after 40 h of exposure (Table 1).

In Set D, similar trend in percent mortality was observed. Percent mortality recorded was significantly high during first 12 h of exposure ($p \leq 0.05$) and thereafter increase in percent mortality was insignificant as observed after 24 h and 40 h of exposure duration, however, significant increase in mortality was recorded from 24 h to 36 h of exposure. Percent mortality after 12 h of exposure was to be 3.875 which increased to 10.125 percent after 40 h of exposure duration through mortality percentage of 5.25 and 7.75 after 24 and 36 h of exposure duration, respectively (Table 1).

It is interesting to note that rate of mortality significantly increased during 24 to 36 h in all the experimental groups after a decline in mortality rate during 12 h to 24 h exposure and again insignificant increase was observed after 36 h of exposure.

At different concentrations of paper mill effluent (i.e., in dose dependent manner): After 12 h the per cent mortality was insignificant in the control group. Whereas in the treatment groups i.e., Set B, C and D it was significantly high ($p \leq 0.05$) as compared to control group. The observed per cent mortality was 0.375 in control, 2.5 in Set B, 3.375 in Set C and 3.875 in Set D (Table 1). However, the difference in the mortality was insignificant when group B, C and D were compared.

Similarly percent mortality after 24, 36 and 40 h was significantly high in the treatment group but was less than that was during the first 12 h of exposure. After 24 h of exposure percent mortality was 1.125 in the control group and in experimental groups it was 3.125, 4.25 and 5.25 in Set B, C and D, respectively (Table 1).

Similar trend of mortality was recorded after 36 h of paper mill effluent. After 36 h of exposure duration observed per cent mortality was 1.625 in control group, 4.125 in Set B, 5.5 in Set C and 7.75 in Set D (Table 1). This increase in mortality was calculated to be highly significant as compared to the control group.

After 40 h of rearing, in control group percent mortality was 2.5 and was 4.75, 6.125 and 10.125 percent in Set B, C and D, respectively (Table 1). Chi square analysis revealed significant difference in mortality in the treatment group as compared to the control.

Significant increase in mortality of eggs was observed with increase in concentration of the paper mill effluent and time duration. Mortality of the eggs increased with increase in PME concentration at particular time of exposure.

Another objective of the present study was to investigate any effect of PME on the hatchability duration of the fish eggs. It was observed that in the treatment groups hatching of the eggs initiated 2-3 h in advance in comparison to control. Hatching of the eggs started after 40 in Set-D, after 41 h in Set-B and C and after 43 h in the control group. The embryos were observed to be more active in the exposed sets as compared to the control.

During this investigation it was observed that paper mill effluent increased mortality in *Cyprinus carpio* eggs and the mortality percentage was enhanced with the increase in concentration of paper mill effluent and with increase in exposure duration. In the survived eggs development increased by 2-3 h which can be inferred by the early hatching in the treatment group. In control group the eggs were normal in control group (Fig. 1, 5) however, some depressions on the surface of eggs were observed and their number and dimensions enhanced with the increase in concentration of PME (Fig. 2-4). Mortality was enhanced with increased exposure duration (Fig. 6). Fully developed embryos were more active in the paper PME exposed groups.

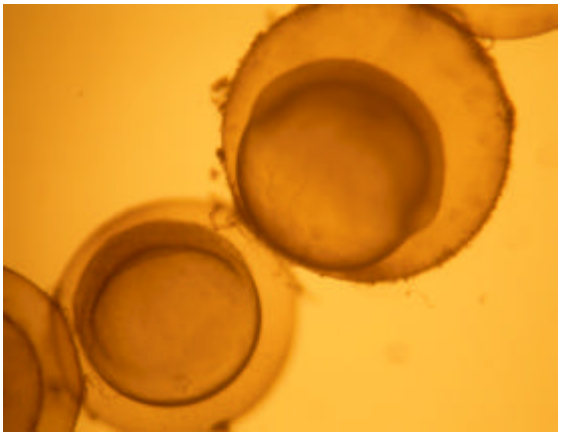

Fig. 1: The eggs of *Cyprinus carpio* with normal embryo with smooth surface after 4 h of culture duration in control group (X 50)

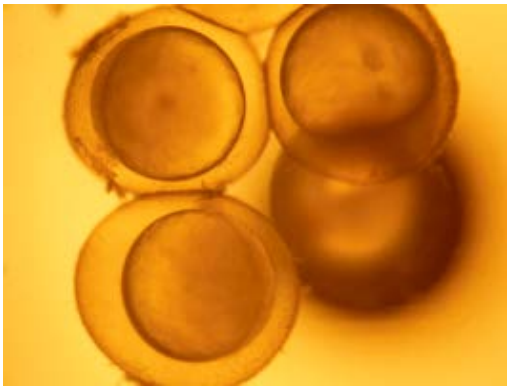

Fig. 2: *Cyprinus carpio* eggs treated with 1% paper mill effluent depicting the depressions on the yolk surface after 4 h of culture duration (X 50)

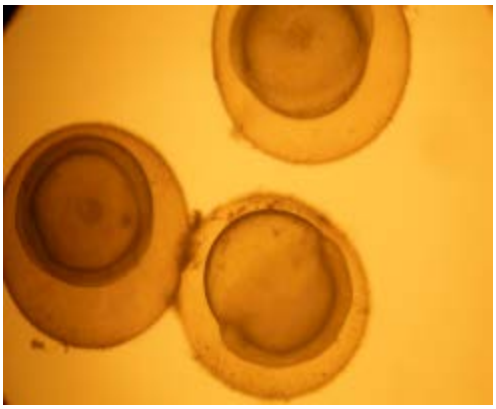

Fig. 3: *Cyprinus carpio* eggs exposed with 2% of paper mill effluent after 4 h showing depression in yolk surface (X 50)

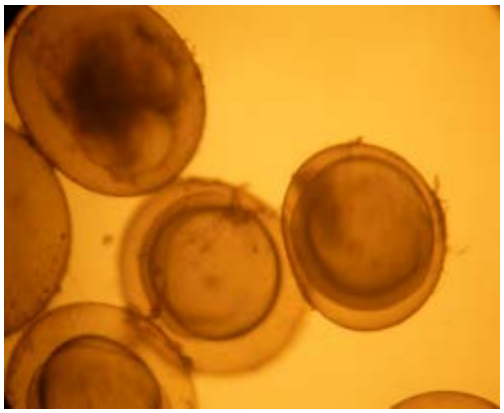

Fig. 4: *Cyprinus carpio* eggs exposed with 4% of paper mill effluent exhibiting dead embryo after 4 h (X 50)

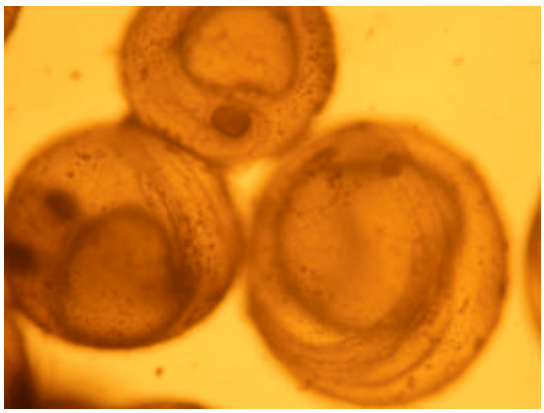

Fig. 5: The 40 h old normal embryos in control group (X 50)

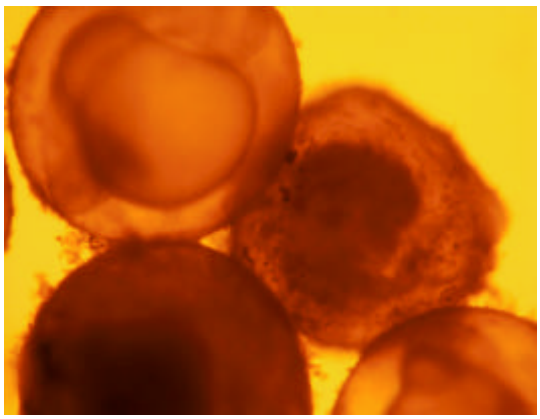

Fig. 6: The increasing mortality in embryos after 40 h of exposure duration (X 50)

DISCUSSION

During the present study it was observed that the paper mill effluent affected the survival of the *Cyprinus carpio* eggs prior to their hatching. Higher mortality rates in the eggs after the exposure to different concentrations of PME and at varied time duration supports the earlier studies conducted on most fish species wherein high juvenile mortality rates have been demonstrated (Roff, 1992). Paper mill pollutants have already been established to kill fish or affect their reproductive physiology (Van den Heuvel and Ellis, 2002), or may induce male-biased sex ratios among fish embryos (Larsson and Forlin, 2002). Egg hatchability has been documented a more sensitive indicator of toxicological analysis as compared with other parameters studied (Henderson *et al*., 1981; DeGraeve *et al*., 1982; Lewis and Wee, 1983). The difference in hatching duration during the present investigation is possibly because of the fact that hatching is the extremely sensitive period in early fish ontogenesis and is influenced by the combined effects of the hatching enzyme, osmotic and mechanical mechanisms (Denuce, 1984; Yamagani, 1988). The

paper mill effluent has possibly interfered with any of these factors leading to precocious hatching. The results of the present investigation that paper mill effluent induce hazardous effects on early developmental stages strongly supports the earlier findings of Milston *et al.* (2003) wherein short-term exposure of very early life-stage, to organic contaminants lead to long-term effects on immune competence without any effect on growth and reproduction of the animal. The present results are inconsistent with the earlier study depicting embryonic tissue may be partly sensitive to damage by xenobiotic metabolism (Binder and Lech, 1984). It provides the tangible support that although morphologically all eggs were not affected yet this exposure at embryonic life might have resulted in major complications and impairment in physiological functioning leading to early hatching.

McMaster *et al.* (1992) demonstrated that Bleached and Kraft Mill Effluent exhibited no difference in mean survival or developmental times in white sucker (*Catostomus commersoni*) but grew at slower rate as compared to the control larvae. However in the eggs exposed with PME exhibited difference in mean survival and hatching at the faster rate compared to controls in contrast to earlier study conducted by McMaster *et al.* (1992). This variation is possibly due to difference in effluent chemistry, potency or suspectivity of the stage of development and the species.

The results of present investigation revealed that the paper mill effluent entering the inland water is a serious environmental pollutant with high ecotoxical risks even in small concentration especially for fish species and their developmental stages.

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