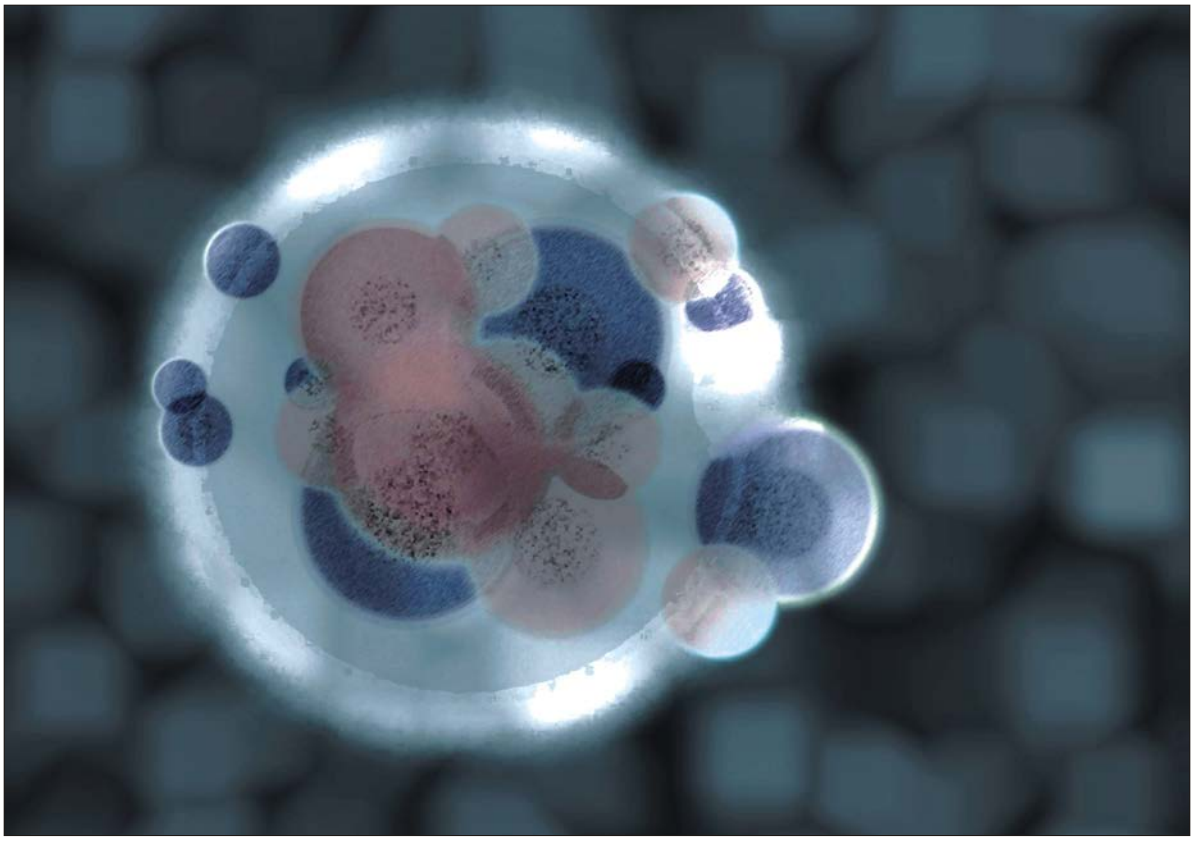

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Anomalies in *Cyprinus carpio* Larvae Exposed to Paper Mill Effluent

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Abstract: Water pollution due to urbanization and industrialization are the major cause of decline in aquatic animal populations all over the world. Keeping in view the present study deals with the teratogenic influence of paper mill effluent in *Cyprinus carpio* larvae under laboratory conditions. *Cyprinus carpio* larvae were exposed to different concentrations (less than half of the LC_{50}) of paper mill effluent to investigate the effects induced by the effluent. Control was run simultaneously along with all the experimental groups. Larvae were more sensitive after complete yolk sac absorption and increased mortality was observed during this phase. Common morphological abnormalities in hatchlings after exposure to the paper mill effluent include flexure of the larval axis, vertebral dysplasia, abnormalities in eyes such as single eye, microphthalmia, abnormality in the yolk sac and heart tube anomalies. Overall stunted growth was also recorded in the treatment groups depicting hazardous effect of paper mill effluent on larval stages of *Cyprinus carpio*.

Key words: *Cyprinus carpio*, larvae, paper mill effluent, fish larval abnormalities, microphthalmia

INTRODUCTION

Water pollution due to urbanization and industrialization are the major cause of decline in aquatic animal populations all over the world (Maitland, 1995; Dan'azumi and Bichi, 2010; Singh, 2007; Anbumani and Mohankumar, 2011; Muhamad *et al*., 2011). The pollutants generated by anthropogenic activities including domestic sewage, pesticides, heavy metals, pulp and paper mill effluents, dye and dye products, pharmaceuticals etc. are known to affect aquatic organisms (Khan and Thulin, 1991; Tamburlini *et al*., 2002; Sekhar *et al*., 2003; Prabu and Udayasoorian, 2005; Saxena *et al*., 2006; Moharram *et al*., 2011). The pulp and paper mills rank high in terms of water use during paper production on the other hand they are contributing to pollution loads in rivers through effluent discharge (Jayabalakrishnan, 2007). Toxic dyes, bleaching agents, salts, acids and alkalis are present in effluents discharged from pulp and paper industries. Heavy metals such as cadmium, copper, zinc, chromium are present in the pulp and paper mill effluent that ultimately released into aquatic environment (Mathur *et al*., 2005; Zahrim *et al*., 2007). Dichloroguicol trichloroguicol, tetrachloroguicol and chlorinated phenols are major contaminants found in the effluent released from pulp and paper mill which are toxic to fish fauna (Leuenberger *et al*., 1985; Mellanen *et al*., 1996; Owens, 1991). Various subchronic effects including structural, physiological and biochemical abnormalities in fishes due to paper mill effluent have been documented (Lindstrom-Seppa and Oikari, 1989; Larsson *et al*., 1988; Pesonen and Andersson, 1992). Oxidative stress induced by effluent resulted in formation of hyperactive metabolites which may lead to mutations in DNA (Hodson *et al*., 1996; Oakes *et al*., 2005). Pulp and paper mill industries also alter the macroinvertebrate communities present in rivers (Flinders *et al*., 2009). Toxicants such as dioxins present in pulp and paper mill effluent, resulted alterations in reproductive performance and development of an organism (Kovacs *et al*., 1997; Parks *et al*., 2001). A number of the studies have been conducted on the effect of industrial effluents on fishes. However, very little work has been done on the larval stages of fishes. Therefore, the present study was designed to investigate the effect of paper mill effluent on larval stages of *Cyprinus carpio*.

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 during February 2010.

Two hundred hatchlings of *Cyprinus carpio* hatched from the eggs which were incubated in the sublethal concentrations of Paper Mill Effluent (PME). These hatchlings were further exposed to similar concentrations

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till they start feeding orally to study its survival and the abnormalities induced by the PME. Experimental group was divided in three sub groups: Set-A with 1% PME, Set-B having 2% PME and Set-C containing 4% PME. Whole experiment was performed in triplicates. Control was run simultaneously along with the experimental groups. Proper aeration was provided with the help of low pressure aerator. Physicochemical parameters such as DO, temperature, pH were recorded at regular interval during the experiment. No feeding was provided to the hatchlings during this experiment.

RESULTS

The embryos were examined throughout their prehatching development and up to sac fry stage of larvae to record morphological abnormalities and post hatchling development. *Cyprinus carpio* embryo test organism in control ones began to hatch after 45 h which was preponed by 3 h in 4% of PME and 2 and 1 h in 2 and 1% concentration of PME, respectively. Dose related increase in mortality of embryo was observed during the present study. Healthy normal embryos were observed in control group (Fig. 1). Significant decline in hatching success was observed in PME treated embryos (Tyor *et al*., 2010). Among the successful hatching embryos, teratism was clearly evident. Five major categories of developmental abnormalities including altered axial curvature, head and deformities, tail malformation were observed. In all 25-37% of the larvae were observed to have one or the other abnormality whereas in 8% of the larvae were found to be malformed. Most commonly encountered defects were flexure in the body axis along the whole length of the larvae or may be easily noticed as bent tails (Scoliotic embryo). In some cases bent was also observed in the pharyngeal region in all the treatment groups (Fig. 2). Malformation of the vertebral axis increased in dose-dependent manner. About 5% of the embryo in control was reported to have anomalies in the vertebral axis whereas in the experimental groups it increased up to 16% (4% PME). Besides bent tail, edema in pericardial region, microphthalmia, fused eye/single eye deformity in the fin region. Gills disorganized head region were also reported in the treated groups.

A slight delay in the yolk absorption was noticed in the treated embryos. In 4% treatment group, the yolk sac was abnormally enlarged and protruded up to the head region which may resulted in death of the larvae. This yolk sac anomaly was observed in 1% of the control group larvae but reached to 5% in the higher concentration (4% PME). Malformation of eye included

Fig. 1: Normal larvae in control group (50X)

Fig. 2: Flexure of the larval axis, along whole body length (50X)

hypoplasia, microphthalmic and anophthalmia. Such abnormalities were observed in all the treatment groups ranging between 3-4% of the larvae (Fig. 3-5). No such abnormality was observed in the control group.

Irregularly shaped heart tube was also observed in the treatment groups. Edema of the pericardial cavity was observed in 2 and 4% effluent concentrations. The abnormality percentage in heart was 2, 1 and 1% in Set-A, B and C, respectively. No abnormality in heart was observed in control group. Red patches near the gill region were observed due to the effect of effluent. Deformities in fin region were recorded in 1% of larvae of control group which increased up to 3% in Set-A and B and 5% in Set-C, respectively. Disorganization in head and caudal region and reduced development of caudal fin

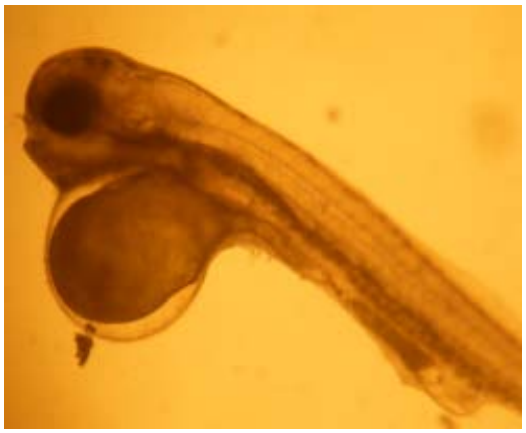

Fig. 3: Larva with abnormally large yolk sac extended up to the head region after exposure of 4% concentration of paper mill effluent (50X)

Fig. 5: Larvae with fused eye as well as abnormal caudal fin (50X)

Fig. 4: Microphthalmia or small eye after exposure to 2% concentration of paper mill effluent (50X)

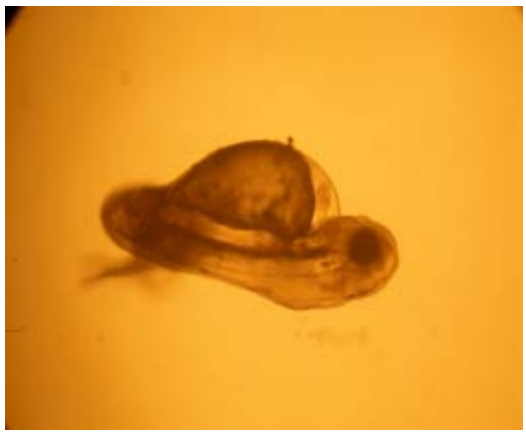

Fig. 6: Larvae showing overall stunted growth after exposure of 4% paper mill effluent (50X)

was noticed in PME treated larvae (Fig. 4). This type of abnormality was not observed in control group (Fig. 1) however, 2-4% of the larvae in treatment groups had abnormal head. Over all stunted appearance was observed in the larvae of the *Cyprinus carpio* (Fig. 6, 7). Occasionally, treated hatchlings showed twitching and erratic movement as compared with control group. Yolk sac absorption was delayed in PME exposed larvae. In control group yolk was completely absorbed in 4-5 days however, in PME treated group it lasted up to the sixth day. High larval mortality was in the treatment group after complete absorption of yolk sac and the young ones start feeding orally.

DISCUSSION

The paper mill effluent exposure induced abnormalities including conditions wherein, the yolk sac get protruded up to the head region, deformities in eyes, spinal curvature, abnormal head and overall stunted growth were observed during the present study. Woodworth and Munday (2000) observed similar abnormalities in eyes and yolk sac due to the effect of paper mill effluent in larval stages of Tasmanian blennies (*Parablennius tasmanianus*). According to Van Leeuwen *et al.* (1985), accumulation of environmental toxicants in protein which may result in enhancement of

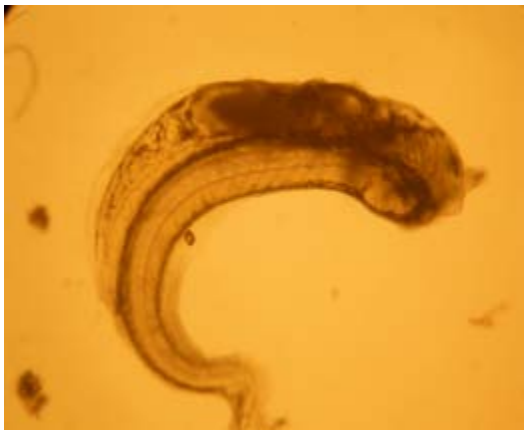

Fig. 7: Stunted growth and disorganized head region due to exposure of 4% concentration of paper mill effluent (50X)

toxicant concentration as well as the accessibility of the toxic compound to larvae during yolk sac absorption. 2,3,7,8-tetrachlorodioxin (TCDD) is a component of paper mill effluent which have been reported to induce anomalies in yolk sac and head region in larvae (Walker *et al*., 1994). The observation of the present investigation showed that paper mill effluent induced abnormalities in body structure and function which may possibly result in death of deformed larvae. In the present study mortality was also observed during yolk sac absorption strongly support the observations of Helder (1980) in which yolk sac absorption process has the major contribution in redistribution of toxicants such as TCDD which may result in alteration in physiological functions that ultimately lead to death of aquatic organisms Helder (1980). The paper mill effluent induced the morphological alterations such as single eye, microphthalmia, abnormality in fin region, abnormality in heart in *Cyprinus carpio* larvae observed during the present study are similar to the morphological abnormalities observed in fish exposed with effluents released from other plants such as sugar-beet processing plant (Hegrenes, 1999). Curvature in the body axis as observed in the present study was also documented by Boudreau *et al*. (2005) in the larvae of the estuarine mummichog, *Fundulus heteroclitus*, induced by androgenic and anti-androgenic compounds present in the environment. Boudreau *et al*. (2005) observed that gross morphological abnormalities in embryonic, larval and juvenile stages of fish are the sensitive indicator of exposure of toxicant. Bleached Kraft mill effluent have been documented to induce the alteration in Gonadosomatic Index (GSI) and the decline in number of germ cells which may lead to complete voiding of germ cells in the organism with the passage of time (Jobling *et al*., 1996; Leatherland, 1992). Pryce-Hobby *et al*. (2003) observed that chemical constituents present within pulp mill effluent bind to steroid binding proteins, hence alter the properties of steroid binding proteins. Effluent exposure induced masculinization of females in fish populations (Howell *et al*., 1980). Earlier studies have been demonstrated that discharges of wastewater from papers mills have various substances with androgenic or anti-estrogenic activities (Karels *et al*., 1999; Hegrenes, 1999; Larsson and Forlin, 2002). Fathead minnows exposed with paper mill effluent over a full life cycle induced depression in sex steroid production, delay in sexual maturity, reduced egg production as well as changes in the secondary sex characteristics (Munkittrick *et al*., 1998).

CONCLUSION

From the present study it becomes clear that pulp and paper mill effluent have deleterious effects in larval stages of *Cyprinus carpio*. Despite lower doses of exposure, prolonged duration paper mill effluent lashed environment leads to serious development problems associated with the survival of aquatic organisms. Further research is needed to better understand of mechanism involved in toxicity caused by paper mill effluent. The present investigation will help in formulating policies and strategies to circumvent the toxic effects of paper mill effluent.

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