*International Journal Of*

**Recent Scientific Research**

**ISSN: 0976-3031**

**Volume: 7(1) January -2016**

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THE OFFICIAL PUBLICATION OF
INTERNATIONAL JOURNAL OF RECENT SCIENTIFIC RESEARCH (IJRSR)
http://www.recentscientific.com/ recentscientific@gmail.com

ISSN: 0976-3031

Available Online at http://www.recentscientific.com

*International Journal of Recent Scientific Research*
*Vol. 7, Issue, 1, pp. 8223-8225, January, 2016*

***International Journal of Recent Scientific Research***

**RESEARCH ARTICLE**

# HISTOPATHOLOGICAL STUDIES ON THE LIVER OF *CYPRINUS CARPIO FINGERLINGS* EXPOSED TO PULP AND PAPER MILL EFFLUENT

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**ARTICLE INFO**

***Article History:***

Received 05th October, 2015
Received in revised form 08th November, 2015
Accepted 10th December, 2015
Published online 28st January, 2016

***Key words:***

histology, liver, light microscopy

**ABSTRACT**

The pulp and paper mill are one of the highly polluting industries and hence the study of the effects of effluent exposure on organisms, populations or communities has high ecological relevance. The presents study uses the fingerlings of common carp, *Cyprinus carpio*, to analyse the effects of paper mill effluents on liver architecture at 7, 14, 21 and 28 days of exposure using light microscopic study and haematoxylin-Eosine stain. We present a survey on toxic effects, proposing a sequence of exposure duration dependent effects. Hepatocytes showed various anomalies such as vaculation, pycnotic nucleus, karyolysis, karyohexis, leucocyte infiltration, necrotic area, amongst others. We concluded that the paper mill effluent is extremely toxic to fish, even in a very low concentration. Such contaminant renders fish health by affecting them physiologically and ultimately subject to mortality thereby distressing the aquatic biota.



## INTRODUCTION

The paper industry has been one of the major sources of aquatic pollution in India as it often contains a variety of pollutants toxic to aquatic life (Pokhrel and Viraraghavan, 2004, Pathan *et al*., 2009). Paper mill effluent consists variety of toxic components such as heavy metals, high Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD), soda, legnin, chlorine, resin acid, dioxin, furan, etc. which might be responsible for causing metabolic impartment in the aquatic organisms which could even lead to their death. The paper mill effluent (PME), when it is directly discharged into the streams and rivers, without previous treatment, can affect the native fish or biota. Studies across the world revealed that effluents released from many of these industrial sites have a negative impact on fish life (Parrott *et al*., 2006). Therefore, the present study, aims at analysing the chronic affects of PME on the liver architecture of fingerlings of common carp, *Cyprinus carpio.*

## MATERIALS AND METHODS

### *Effluent Collection*

PME was collected from an effluent discharge site (24°51'47.9"N 92°36'20.8"E) of Cachar paper mill, situated in Southern Assam, India. Samples were collected with the aid of clean 1L water sampling cans. Collected samples were transported to the laboratory for physicochemical analysis (as per APHA, 2005) and stored for further use. The Physico-chemical parameters of effluent was as follows: pH (7.2-7.7), conductivity (283-286 µS), BOD (150-200mg/l), COD (1440-1450mg/l), TDS (181-183mg/l), total alkalinity (598-648mg/l), total hardness (66.7-81.66mg/l), chloride (600-683mg/l), nitrite (3245-4490mg/l), arsenic (0.69-3.42mg/l), cadmium (0.19-1.28mg/l), chromium (29.05-37.17mg/l), copper (2.4-5.4mg/l), iron (416.31-446.97mg/I), lead (2.1-4.62mg/l) and zinc (31.59-35.28mg/l).

### *Fish maintenance and experimental design*

Fingerlings of *Cyprinus carpio* of similar length (4.5±0.8 cm) and weight (3.28±1.79 g) were collected from unpolluted, freshwater pond, near Assam University, Silchar campus in Assam state of India. They were acclimatized under laboratory conditions seven days prior to experimentation in 20L glass tanks and commercially available fish feed was given twice daily. The process of adaptability was performed in a temperature controlled room at $25^0$C, with a light: dark cycle of 12:12 h. The fingerlings of *C. carpio* were chosen due to their favourable experimental properties (small size, easy to collect and maintain in a laboratory environment), and also

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considering their widespread culture in various fisheries in this region. Based on preliminary study, 20 fish were exposed to a dose of 1.43% ($1/10^{th}$ 96 h $LC_{50}$ value) for 28 days. Control fish (n=20) were simultaneously run in tap water. Complete renewal of experimental and control water was performed every alternate day. At exposure duration of 7, 14, 21 and 28 days, 5 fish each were sacrificed from both effluent treated and control batches and liver tissues were dissected out, washed in saline and fixed using 10% formaldehyde for histological examination.

***Light microscopic study***

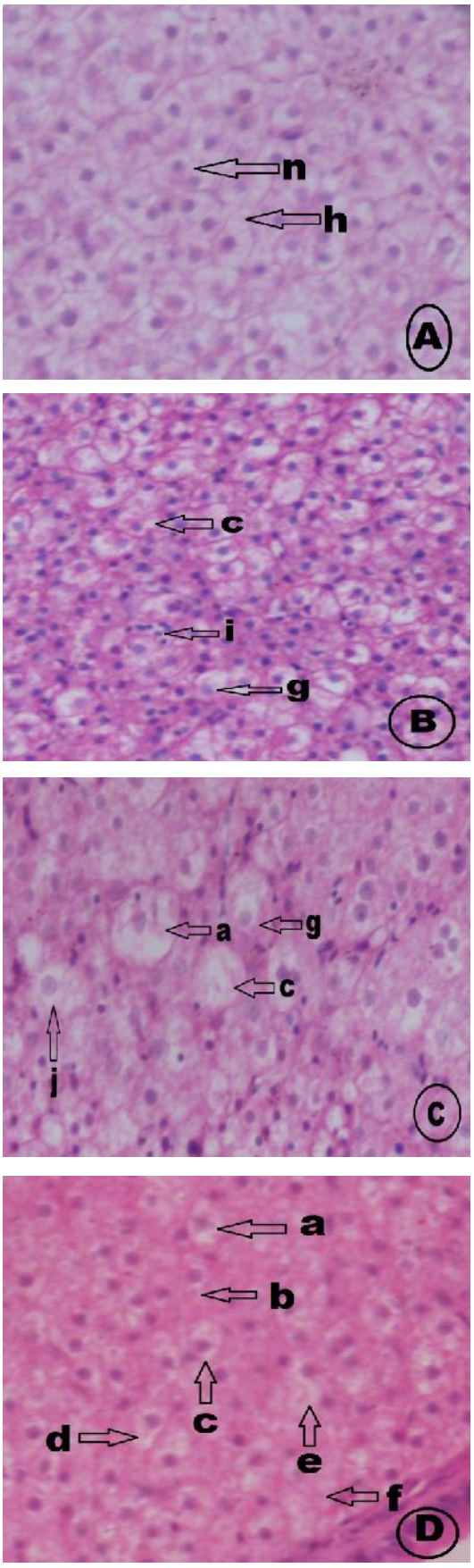
Fixed liver tissues were embedded in paraffin for microscopic examination. They were dehydrated through a graded series of ethanol, cleared in xylene, infiltrated in paraffin, and sectioned at a various sections of thickness 5µ were obtained, stained with haematoxylin-eosin and examined under a light microscope and then photographed (Olympus Microscope, model CX41RF with Olympus digital Camera, model: E-420). The histopathological lesions in the tissues were examined in the randomly selected five sections from 5 fish of each group. A search was carefully conducted to check for the presence of alterations.

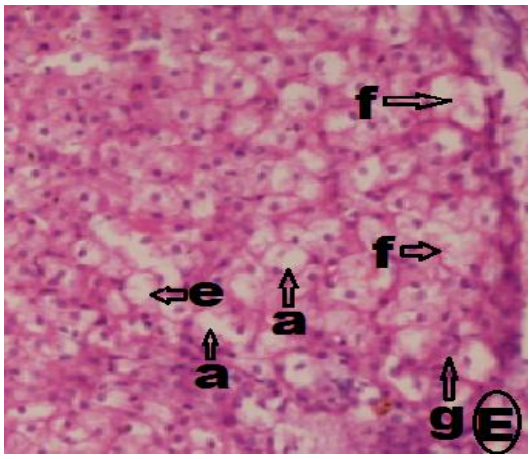
## RESULTS AND DISCUSSION

The liver architecture under light microscopy revealed ovoid hepatocytes with centrally placed, well developed nucleus. In the PME treated fish, several anomalies were observed, with severity of response progressively appearing as the duration of exposure increased (Fig 1). The liver is known to be one of the major organs that not only acts as a storage organ, but is also the primary site for detoxification and biotransformation processes (Dutta *et al*., 1993, Van der Oost *et al*. 2003), thus being one of the principal targets of pollutants present on the aquatic environment.

Alterations in the liver may be useful as markers that indicate prior exposure to environmental stressors. In the present study the liver of control *C. carpio* showed normal architechture with hepatocyte and nucleus (Fig.1). The histopathological alterations in the liver of *C. carpio* after 7 days observed were vacuolation with nucleus towards periphery and leucocyte infiltration (Fig 1 B); after 14 days of exposure, hepatocyte showed vaculation with nucleus towards periphery. Karyohexis was prominent and some of the nucleus showed enlargement (Fig 1 C). After 21 days, hepatocytes showed abnormal shapes, vaculation with peripheral nucleus, pycnotic nucleus, nuclear karyohexis and karyolysis. Some of the necrotic areas were prominent (Fig. D). After 28 days, hepatocytes with extreme vaculation and necrotic areas were prominent. Karyolysis and karyohexis were also evident (Fig. E).

Fig. 1 (A-E) T.S of liver of *C. carpio* fingerlings control (A) showing hepatocytes (h) and nucleus (n), After (B) 7d (C) 14d (D) 21d and (E) 28d of 1.43% PME exposure, showing: (a) karyohexis (b) pycnotic nucleus (c) vaculation (d) abnormal shape (e) karyolysis (f) necrotic area (g) nucleus towards periphery (i) leucocyte infiltration and (j) nucleus enlargement. H&E, (400x).






Almost similar alterations have been described in other species of fish living in contaminated environments (Teh *et al*., 1997; Nero *et al*., 2006) suggesting that these alterations might be related to the exposure to environmental chemicals present. Necrosis indicated degeneration of structural proteins in the hepatocyte membrane and the hepatocyte pycnosis signified altered protein synthesis. Several investigators previously related these cellular deteriorations to PME exposure (Santos *et al*., 1990; Axelsson and Norrgren 1991; Pacheco and Santos, 2002). Similar hepatic damage was also observed in gudgeon (*Gobio gobio*) and mullet (*Mugil cephalus*) exposed to contaminants from wastewater treatment plant (Pinto *et al*., 2010). In another study, Cengiz *et al*. (2001) found that there were hepatic lesions including degeneration, hypertrophy, sinusoids enlargement, hemorrhage, pycnosis position of nuclei, vacuolization of cell cytoplasm, infiltration of mononuclear lymphocyte.

## CONCLUSION

From the present study, it can be concluded that the paper mill effluent is extremely toxic to fish, even in a very low concentration. Such contaminant renders fish health by affecting them physiologically and ultimately subject to mortality thereby distressing the aquatic biota. Therefore, the better treatment of effluent before release into environment is recommended.

**Acknowledgement**

We thank the Biotech Hub, Life Science, Assam University, Silchar for providing microscopic facilities.

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**How to cite this article:**

Sangeeta Dey, Manabendra Dutta Choudhury and Suchismita Das.2016, Histopathological Studies On The Liver Of Cyprinus Carpio Fingerlings Exposed To Pulp And Paper Mill Effluent. *Int J Recent Sci Res.* 7(1), pp. 8223-8225.

TSSN 0976-3031