



Effect of Pulp and Paper Mill Effluent on Blood Morphology of Fresh Water Fish in Tarai Region of Uttarakhand

V K Misra¹, M Das Trakroo², C D Sharma³, Shashank Singh⁴, N D Singh¹ and A N Tripathi⁵

Krishi Vigyan Kendra West Kameng, Dirang - 790 101 (Arunachal Pradesh)

ABSTRACT

The present paper deals with the toxicity of common mixed industrial effluent of Tarai Region of Uttarakhand on the blood morphology of an important fresh water fish (*Cyprinus carpio* var. *communis*). When fish was exposed to 3, 4, 5, 6 and 7 per cent effluent concentrations, various morphological changes were observed but the changes were less in the specimens treated with 3, 4 and 5 per cent effluent concentration compared to 6 and 7 per cent. In fish exposed to 3, 4 and 5 per cent concentrations, the formation of tear drop cell, binucleated cell, microcytes, spherocytes, rouleux cell, sickle cell, agglutinated cells and rupturing of donnanens membrane in the cells were observed but in fishes exposed to 6 and 7 per cent effluent concentration the agglutination of hemoglobin, membrane degeneration and formation of elliptocytes, hemoglobin crystal, stomatocytes, ovalocytes, and inclusion bodies were found.

Key Words: Blood morphology, *Cyprinus carpio*, Pulp and paper mill effluent, Toxicity.

INTRODUCTION

Rapid urbanization and industrialization in India has resulted in the substantial increase in the liquid waste (spent wash or effluent) which is traditionally discharged in open land or into nearby natural water, causing a number of environmental problems including threat to plants and animal lives present in that environment. These industrial pollutants alters the natural condition of aquatic medium that causes behavioural changes as well as morphological imbalance of aquatic organisms (Yadav *et al*, 2007). Hematology is used as an index of fish health status in a number of fish species to detect physiological changes following different stress conditions like exposure to pollutants, diseases, metals, hypoxia, etc. (Duthie and Tort, 1985). Therefore, hematological techniques are the most common method to determine the sub-lethal effects of the pollutants (Larsson *et al*, 1985). Blood of any

organism shows the direct relation between its health and in his surviving environment. There are three types of cellular elements presents in the blood i.e. RBCs, WBCs and platelets each of these cells have its own functions and differs morphologically from the others. In health, the destruction and production of cells is balanced and therefore the number of cells present in the blood at any particular time is relatively constant. The present work is based on the morphological studies of RBCs (erythrocytes). Erythrocytes are the most abundant cell type found in the peripheral blood and function in respiration by transporting oxygen to and carbon dioxide from body tissues (Michael and Stoskope, 1992). Many studies have indicated that heavy metal ions, such as copper, cadmium, and mercury ions, induce lysis of mammalian erythrocytes and may cause the accelerated destruction of erythrocytes (Kotsanis *et al*, 2000). The main functions of RBCs is to carry

Corresponding Author's Email: ndsingh62kvk@yahoo.com

1 Subject Matter Specialist (Fisheries) and Sr. Scientist cum Head K.V.K West Kameng, Dirang, Arunachal Pradesh.

2 Professor and Head (Aquatic Environment Management) College of Fisheries, G.B.P.U A & Tech. Pantnagar Uttarakhand.

3 Assit. Prof. (Zooloogy) Surajmal Kanya Mahavidyalaya Kichha, Distt U.S. Nagar, Uttarakhand.

4 Ph.D. Scholar CIFE Mumbai, Maharashtra.

5 Sr. Scientist cum Head K.V.K Lower Subansiri, Arunachal Pradesh.

Hb, which transports the oxygen to the tissues. The metabolic activities of RBCs also are capable of maintaining the Hb molecules in functional states. In normal morphological and cytological condition the RBCs have its own nucleus is extremely condensed. Erythrocytes morphology changes only in special external and internal conditions i.e. due to anemia and other pollutants of surrounding medium and in disease conditions. Hence, present study was conducted to see effect of pulp and paper mill effluent on blood morphology of fresh water fish in tarai region of uttarakhand

MATERIALS AND METHODS

Collection of Paper Mill Effluent.

Waste water from the Century pulp and paper mill Lalkuan (Nainital) is drained into a small rivulet called Gola Nala which after about 20 km is merged into the Gola river. The waste water samples were collected from Ghanshyam Dham Lalkuan. On the basis of LC50 six solutions of varying concentrations (0% control, 3%, 4%, 5%, 6% and 7%) of waste water were prepared to observe their effects on test organism.

Collection of Experimental Fish and design of experiment.

The experimental fish (yearlings) of *Cyprinus carpio*, used in this experiment was procured from Instructional Fish Farm, College of Fisheries Pantnagar. The range of Fishes were 15.10-20.60 cm in length and 132-153 gm in weight. Fishes were acclimatized in 1500 litre capacity cemented tank for one month before they were transferred to the test media. Cemented tanks were cleaned, sterilized and filled with water (temperature- $26.5^{\circ}\text{C}\pm 0.5$, pH- 8.2 ± 0.04 , dissolved oxygen $6.8\pm 0.5\text{mg/l}$). During acclimatization the fish were fed with conventional fish feed (rice polish and soya cake in 1:1 ratio) at the rate of 4% body weight. Water quality parameters were recorded following to APHA (2005).

The yearlings of *Cyprinus carpio* after proper acclimation in the test tanks with daily feeding and replenishment of fresh water for one week

were exposed to six different concentrations i.e. 0% (control) and (3%, 4%, 5%, 6% and 7%) in duplicate.

Morphological study of blood

After blood removal, blood smears were made immediately. Blood samples were collected at 7, 15 and 30 days interval on the well neat and clean slides from the caudal vein of the fishes by cutting the peduncle. The slides were air dried for 1 h at room temperature and then fixed in 95% methanol at 4°C (Michael and Stoskope, 1992). After that slides were stained with dyes following suitable standard methods and a cover slip was placed on top using glycerol. Slides were examined under electronic microscope attached with camera.

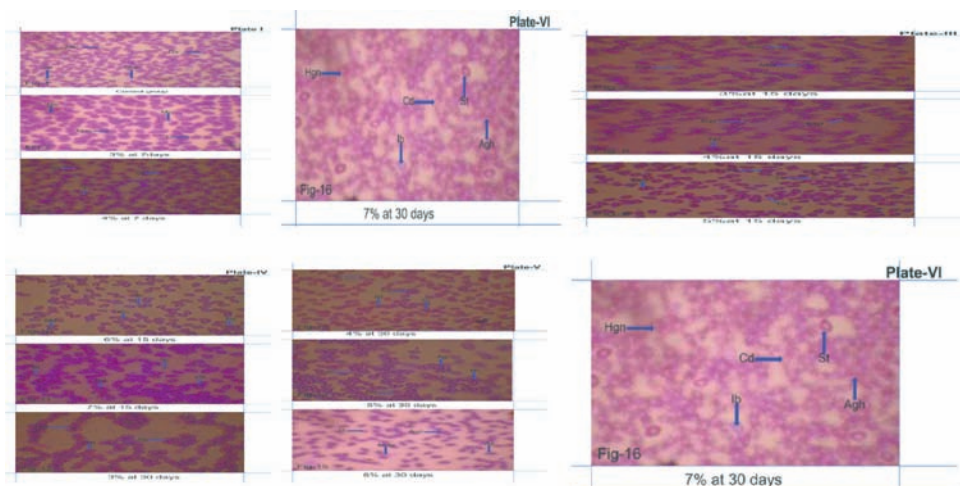
RESULTS AND DISCUSSION

Normal erythrocytes of carp have an oval shape with a rounded to oval central nucleus with densely packed chromatin (Fig. 1). The present study shows erythrocytes morphological alterations in carp exposed to paper mill effluent (Figs. 2 and 16). A high percentage of red blood cells were in the process of losing their normal outline and cytoplasm, according to the peripheral blood smear examination at higher exposure levels.

After 7days, treatment effect of 3 per cent effluent concentration on erythrocytes morphology were present in plate-I, Fig-2 indicates some morphological changes i.e. Presence of macrocytes, microcytes, discocytes and tear drop cells. Tear drop cells indicates anoxia condition which may be due to low formation of RBCs. In Fig-3 at 4 per cent effluent concentration sickle cell and diffusion of cell membrane directly indicates low intensity of heavy metals toxicity. Due to increase in effluent concentration at 7 days dramatic morphological changes were found. It was observed that at 5 per cent effluent concentration in plate- II (fig-4) donnan's membrane disruption and diffusion of cells and presence of elliptocytes are the main characters which clearly indicates the slow formation of RBCs.

Fig 5 and 6 at 6 and 7 per cent effluent

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concentration shows the cell diffusion, formation of elliptocytes and stomatocytes and membrane diffusion. Presence of these all characters indicates about anemic condition of the test organism. In plate -III (Fig-7, 8 and 9 at 3, 4, 5% concentration of effluent respectively at 15 days exposure) indicates sickle cell, membrane diffusion, ovalocytes, elliptocytes and target cell that indicates towards metal toxicity and anemic condition. Plate -IV (Fig-10,11 at 6 and 7% effluent concentration) shows membrane diffusion, sickle cell, ovalocytes, stomatocytes and target cell and hemoglobin crystals that may be due to the increase in concentration of heavy metals in blood along with deteriorate water conditions. In same plate fig-12 at 3% effluent concentration for 30 days exposure shows cell agglutination and tidal bodies indicates toward anoxic condition. Plate-V fig-13,14 and 15 (at 4,5,and 6% effluent concentration respectively) shows that cell diffusion, agglutination, hemoglobin crystal, acanthocytes, membrane degeneration, hemoglobin agglutination and cabot rings are main symptoms that indicates the pernicious anemia and lead poisoning. Formation of hemoglobin network, almost cell degeneration, hemoglobin agglutination and inclusion bodies were observed in plate-VI fig-16 at 7% effluent concentration for 30 days exposure, may be due to heavy metal toxicity.

CONCLUSION

The present study clearly indicates that the

effluent from the various industries should be properly treated and diluted before discharging in natural ways as the various toxic materials are found in the effluents which may leads various detrimental effects on fish diversity as well as on the human being which are consuming these affected fish organisms, through food chain.

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Received on 30/08/2016 Accepted on 12/09/2016