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Effect of Paper mill effluent on Nucleic acid content in Vital Organs of Snake Headed Fish, *Channa punctatus* (Bloch, 1793)

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Abstract- Biochemical study provides an early warning to potentially damaging alterations in stressed animals. Animal's health is influenced by the fluctuations or alterations in metabolic processes taking place in its body's tissues. So, the objective of this study to investigate the impact of sub lethal concentrations of paper mill effluent on nucleic acids content in vital organs of fresh water snake headed fish, *Channa punctatus* (Bloch). A significant decrease in nucleic acids (DNA & RNA) content in heart, muscles, kidney, liver and gill of test fish after 15 days exposure to the effluent as compared with control. Although 10% paper mill effluent may not be lethal for *Channa punctatus* but it affects the growth and survival of entire population and community of fishes as the sublethal becomes lethal in the long term.

Keywords-Paper mill effluent, *Channa punctatus*, Nucleic acids, DNA, RNA.

I. INTRODUCTION

Substantial increase in the industrial wastewater in the developing country like India is the result of rapid industrialization that discharges it without proper treatment into freshwater waterbodies such as river and streams, causing tremendous aquatic pollution. The environmental pollutants or toxicant can induce alteration in metabolic processes occurring in the body of aquatic organisms that inhibit its growth [1]. Untreated or partially treated effluents of various industries have toxic substances in the form of inorganic and organic compounds and heavy metals which are protein bound or lipophilic which accumulate in fatty tissues of aquatic animals like fish. Fishes are the important source of nutrient rich animal protein for human being [2], so it is important to know the critical concentration above which human being are affected and the commercial fish species become unsuitable for human consumption.

The paper mill effluent is one of the major sources of water pollution in India. It uses large amounts of water during bleaching, scouring, rinsing, digesting, cortication, lime treatment, and other finishing processes. In India, around 905.8 million m^3 of water is used and about 695.7 million m^3 of wastewater is discharged per year by the paper industries [3]. Paper mills are normally located near the lotic waterbodies to ensure adequate supply of water and also for disposing the wastewater, which are characterized by high COD, BOD, TSS, pH and temperature. These effluents contain various toxic and bioaccumulating components such as chlorophenos, fatty acids and resins, which can directly affect the cell components as well as biomolecules of body tissues. The DNA content is constant in each tissue within the same species but the RNA content depends upon the protein synthesis process in that particular tissue, hence decline in ribonucleic acid levels causes the depletion in protein synthesis [4], thus the growth of organisms is based on the increase in protein content and increase in ribonucleic acid levels or ribosomal activities.

Nucleic acids are considered as important biomarkers in aquaculture as well as in eco-toxicology assessment because they directly or indirectly control the several metabolic activities in the tissues [5]. Toxicants can cause deformities in the cell organelles [6] so, any change in cellular organelles and nucleic acids (DNA & RNA) causes modulation in cell cycle and apoptosis [7] that are responsible for alterations in metabolic process of protein in aquatic organisms. Nucleic acid indices are sensitive to alteration in specific growth rate *i.e.* length and weight of an organism [4], hence they are used to detect early signs of stress physiology with respect to their habitat.

II. RELATED WORK

Nucleic acids act as biochemical indices and play a key role in all biological activities including growth and development and also regulate biosynthesis of proteins [8-10]. Ratio of RNA/DNA indicates the degree of synthesis of biomolecules especially proteins so it is an index of fish growth[11]. The maintenance of DNA integrity is vital for the protection of genetic diversity in natural population [12], so any structural and functional disturbance in the DNA and RNA helps in assessment of animal's health. There are several studies with reference to changes in DNA and RNA contents in the tissues of fish on exposure

to heavy metals, industrial effluents and several other pollutants or xenobiotics [1,4,10,13-22]. But the studies on the alterations in nucleic acids contents in vital tissues of industrial effluent exposed fishes are scanty. Hence the present investigation is designed to quantify the alterations in the nucleic acids in the vital organs of the snake headed freshwater fish, *Channa punctatus* exposed to sublethal concentration of paper mill effluent.

III. MATERIALS AND METHODS

Procurement of test fish: The snake headed fish, *Channa punctatus* (45±5 g & 12±5 cm) were collected with the help of fisherman from local fresh waterbodies and then kept in 1% potassium permanganate ($KMnO_4$) for one hour to remove any dermal infection. After these healthy fishes were kept in plastic jar containing 50L of clean tap water and were acclimatized for 15 days to the laboratory conditions, during that period they were fed on boiled egg yolk and commercial fish food.

Collection of paper mill effluent: For the present study, the treated paper mill effluent was collected from Yes paper mill Ltd. Darshan Nagar, Ayodhya in polyethylene container. The percent concentration of test solution has been calculated by using the formula [23].

$$\text{Volume Percent} = \frac{V_E}{(V_E + V_{DW})} \times 100$$

Where, V_E = Volume of Effluent , V_{DW} = Volume of Dilution water.

Plan of Experiment: The 96h LC_{50} of *Channa punctatus* for treated paper mill effluent was 15% [1] so, the fishes were exposed to sublethal concentrations (5% and 10%) for 15 days. A control group in dechlorinated water was also maintained in the same environment for same duration. The fishes of both groups were regularly fed with commercial food and the medium was exchanged daily. The fishes of both groups were sacrificed after 15 days and the desired tissues (Liver, kidney, gills and muscles) were dissected out for the estimation of nucleic acids. DNA and RNA was estimated by diphenylamine and orcinol methods described by Schneider [24].

IV. RESULTS AND DISCUSSION

The content of nucleic acid is considered as an index of capacity of any organism for protein synthesis because it regulates its biosynthesis [14], hence any alteration in nucleic acids content reflects on protein biosynthesis.

Table. Effect of Sublethal concentration of Paper mill effluent on Nucleic acids content of fresh water snake headed fish, *Channa punctatus* (Bloch) after exposure to 15 days

Experimental Group	Parameters (mg/gm wet wt of tissue)		
	DNA	RNA	RNA /DNA Ratio
Gills			
Control	5.58±0.34	5.36±0.12	1:1
5%	5.21±0.08 (-6.63%)	4.58±0.17(-9.51%)	0.9:1
10%	4.72±0.11*(-15.41%)	3.61±0.07*(-32.64%)	0.8:1
Muscles			
Control	3.51±0.16	3.39±0.24	1:1
5%	3.34±0.04(-4.84%)	2.87±0.15(-15.33%)	0.9:1
10%	3.11±0.14*(-11.39%)	2.21±0.08*(-34.80%)	0.7:1
Liver			
Control	2.14±0.04	1.05±0.08	1:2
5%	2.01±0.05(-12.01%)	0.95±0.08(-6.66%)	1:2
10%	1.77±0.09*(-17.29%)	0.74±0.11*(-29.52%)	0.8:1
Kidney			
Control	0.88±0.4	0.47±0.05	1:2
5%	0.84±0.3(-4.55%)	0.44±0.06(-6.38%)	1:2
10%	0.78±0.05*(-11.36%)	0.39±0.04*(-17.02%)	1:2
Heart			
Control	0.93±0.05	0.91	1:1
5%	0.89±0.2(-4.44%)	0.86±0.05(-5.49%)	1:1
10%	0.85±0.04(-8.60%)	0.76±0.05*(-16.48%)	0.9:1

*Significant at $p<0.05$

In the present investigation, the nucleic acids (RNA and DNA) contents in the vital tissues of *Channa punctatus* show a drastic alteration after being exposed to paper mill effluent. Similar observations have been observed in different industrial effluent exposed fishes [19, 21,22], in pesticides induced fishes [8,9,14] and also in heavy metal exposed fishes [10,17]. In the present study, alterations in nucleic acids (DNA & RNA) contents in all the vital tissues were more in 10% (v/v) paper mill effluent exposed fishes than the 5% (v/v) exposed fishes. The maximum decrease in nucleic acids content in fish exposed to 10% effluent concentration for the period of 15 days might be due to failure of the body's defence-mechanism and could have caused tissue degeneration. Thus, decrease in nucleic acids contents in all the tissues depends upon the concentration of industrial effluents.

The degree of DNA and RNA contents in different tissues of both control as well as effluent exposed fish *Channa punctatus* are in order of Gill>Muscles>Liver>Kidney> Heart. The percent decrease in DNA in effluent exposed fish was found to be in the order of liver >gill> muscles >kidney > Heart where as in RNA was Muscles>Gill>Liver>Kidney> Heart (Table1). The variation in nucleic acid contents in these tissues shows the tissue specificity of the nucleic acids and their sensitivity to the pollutant or toxicant present in the paper mill effluent. The decrease in DNA and RNA contents in these tissues was the indication of malfunction and degenerative changes in the vital organs of stressed fish [3,17] discussed that decrease in nucleic acids content in the tissues of effluent exposed fish was due to genotoxic action of toxicant or pollutants that decreases the mitotic index and disturbed the cell division process or due to inhibitory action of toxicant on biosynthesis of nucleic acids or by apoptosis (cell death) due to focal necrosis.

Nucleic acids play the key role in all biological activities and also act as regulator of synthesis of all biomolecules as all the enzymes activities are controlled by the process of transcription. The nucleic acids content decreases in the tissues of fishes exposed to organophosphate pesticide [18, 25] due to inhibition of DNA synthesis in these tissues resulting the decrease in RNA contents [7,8]. The DNA content in the brain of pesticide induced fish may be attributed to the increased activity of enzyme DNAase or due to alterations in DNA replication [26,27]. DNA functions as primer in DNA and RNA polymerase reactions so inhibitions in these enzymes inhibit the synthesis of nucleic acids by inhabiting the DNA replication and RNA transcription processes. As RNA synthesis plays major role in protein synthesis so its inhibition at transcription step may decrease the protein content in the tissues. Thus, the toxicant present in the paper mill effluent appears as potential inhibitor of DNA synthesis, curtailing the transcription process that result in reduction of RNA content in the tissues.

The RNA/DNA ratio indicates the potential of protein biosynthesis of a cell and it is an index of fish growth because DNA content in a cell is constant within the same species, but RNA content is mainly ribosomic and depends on the rate of protein synthesis of that cell [12]. In the present study, RNA /DNA ratio in gill and muscles of control fish was approximately 1:1 where as in Liver and Kidney was 1:2 but in the effluent exposed fishes this ratio was declined as compared to control (Table1). Similar findings have been reported in carbaryl and copper sulphate exposed fishes [10]. The overall result reveals that the paper mill effluent disturbs the DNA synthesis that might have reflected in RNA synthesis. Hence any alteration in nucleic acids content reflects on protein biosynthesis. Thus, estimation of nucleic acids content can be used as a tool in ecotoxicological studies.

V. CONCLUSION

On the basis of aforementioned results and discussion it can be concluded that sublethal concentration of paper mill effluent causes deleterious effects on the nucleic acids content in vital organs of fish due to inhibition of enzyme, mitotic changes, impairment of nucleic acid metabolism, increased activity of phosphatases and cellular degradation. Although sublethal concentrations of paper mill effluent may not be lethal for *Channa punctatus* but it does affect the growth rate and reproduction resulting in disturbance of entire population and community as well as trophic levels of food chains or food webs and ultimately the entire aquatic ecosystem. Therefore, it is recommended that proper treatment of industrial effluent must be necessary prior to its discharge into aquatic ecosystem as the sublethal concentration becomes lethal in the long term.

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