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Original Research Article

Impact of Paper Mill effluent on Protein Metabolism of Snake headed fish, *Channa punctatus*

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ABSTRACT:
The present investigation was designed to study the effect of sublethal concentration of paper mill effluent on the total protein and amino acid contents in muscles, liver and gill of *Channa punctatus* after exposure to 96 hours. A significant decrease in protein content with increase in amino acids content in muscles, liver and gill of effluent exposed fishes was observed. A reduction in the protein content and enhancement of free amino acids in the muscles, liver and gill of experimental fish suggests that the tissue protein might have undergone proteolysis, during the stressful situation. Thus, the present study concludes that the protein metabolism of fish *Channa punctatus* affected during effluent exposure and reduces the nutritive value of fish.

Keywords: Amino acids, Protein, Paper mill effluent, *Channa punctatus*

INTRODUCTION

The rapid growth in industrialization, urbanization, domestic sewage and tremendous increase in population causes tremendous environmental contamination that in turn create the huge problem of pure drinking water in the developing country including India (Prakash and Verma, 2021).The aquatic environment is affected by different types of chemicals released in the environment from both natural and anthropogenic sources (Verma and Prakash, 2019a).The discharge of untreated and partially treated effluents from various industries like chemical, pharmaceutical, fertilizer, pesticide, electronics, sugar, distillery and pulp and paper etc., have caused pollution both inlentic (ponds and ditches) and lotic (rivers and streams) water (Verma and Prakash, 2020). In India, about two tones wastewater is discharged into waterbodies annually from industries (Shaffi, 1981). The environmental pollutants or toxicants induce physiological and biochemical changes in fish that lead to growth inhibition (Prakash and Verma, 2019; Verma and Prakash, 2019b). Many of the toxic substances released from these industries are lipophilic and weren't adversely affected by water. These substances accumulate in fish fatty tissues or become protein bound, so it is of importance to know the critical concentration above which human beings are affected and the commercial fish species become unsuitable food.

The industrial effluents contain various heavy metals, which were accumulated in

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aquatic organisms and through food chain reaches into the human beings and causes various diseases such as cardiovascular, hypertension, chronic kidney disease, lung and prostate cancer because the fishes are the most important factors of food chain which have great nutritive value and source of all essential amino acids (Kumar *et al*., 2019).

Fishes are great sensitive to changing aquatic environment and play an important role in the monitoring of water pollution so they are considered as good bioindicator (Prakash and Verma, 2020a). Fishes are ideal organisms to work with in toxicogenomics studies due to the strong power of fish models to establish biomarkers of exposure (Prakash and Verma, 2020b).

Proteins are the polymers of aminoacids and play a vital role in the biological functions and serves as building blocks for cellular mass (Prakash and Verma, 2020a) and functioning of living matter. They are intimately connected with all phases of chemical and physical activity, that constitute the life of the cell. So, they are, essential to cell structure and cell function. They are also involved in physiological events to maintain the homeostasis of the cell. Therefore, the assessment of the protein content can be considered as diagnostic tool to determine the physiological process of the cell (Kapil and Ragothaman, 1999). Amino acids are essential intermediates in protein synthesis and its degradation products appear in the form of different nitrogenous substances. Thus the main objective of this investigation is to analyze the impact of paper mill effluent on protein metabolism of snake headed fish, *Channa punctatus*.

MATERIALS AND METHODS

Procurement of test fish: Healthy specimens of snake headed fish; *Channa punctatus* (45±5 g & 12±5 cm) were collected from local fish farm at Balrampur and were transported to the laboratory. In the laboratory, the fishes were carefully examined for any injury and then kept in 1% solution of $KMnO_4$ for few hours to get rid of dermal infection, finally they were kept in large plastic jar containing 50L of clean tap water and acclimatized for 15 days to the laboratory conditions, during which time they were fed on boiled egg yolk and commercial fish food.

Collection of paper mill effluents: For the present study, the treated effluent samples were collected from Yes Paper Mills Ltd. Darshan Nagar, Ayodhya (U.P.), India in polyethylene container. The percent concentration of test solution has been calculated by using the formula (FAO, 1984):

$$\text{Vol. percent} = \frac{V_E}{[V_E + V_{DW}]} \times 100$$

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

Plan of Experiment: The LC_{50} for treated effluent of paper mill for 96 hours was 15% (Prakash and Verma, 2020c). Based on 96 LC_{50}, fishes were exposed to sublethal concentrations (10%) for treated and control period of 24, 48, 72 and 96 hours. A control group was maintained in an identical environment. The fishes were regularly fed with commercial food and the medium was changed daily to remove faeces and food remnants. The fishes were sacrificed immediately at the end of 24, 48, 72 and 96 hours in both experimental and control groups. Tissues like muscles, gills and liver were excised rapidly and processed for the biochemical estimation. The protein and amino acids content were estimated by the methods of Lowry *et al*. (1951) and Rosen (1957) respectively.

RESULTS AND DISCUSSION

The total protein and amino acids content in the different tissues of control and effluent treated fish, *Channa punctatus*, after 24 hours, 48 hours, 72 hours and 96 hours exposure are presented in Table 1and 2.

Fish, *Channa punctatus* exposed to paper mill effluents showed initial increase in muscle protein during 24 hours and then goes on decrease up to 96 hours in treated group where as protein content in liver and gill of effluent exposed fish showed decreasing trend in 96 hours as compared to control ones (Table1). During experimentation, it was observed

that after 24 hours protein content increases in the muscles of toxicant exposed fish due to increase in protein synthesis by increase in the enzymatic activity involved in protein synthesis but after 24 hours it showed decreasing trends. This fall attributed to the constantly increasing content of the effluent with the biological system which ultimately resulted in protein breakdown. In the present study, after 96 hours, the decline in protein content in different tissues of effluent exposed fish might be due to stress caused by effluent which ultimately resulted in protein breakdown. During stress condition fish needs more energy to overcome the stress, since fishes have less amount of carbohydrate so next alternative source of energy is protein to meet increased demand of energy during stress condition (Prakash and Verma, 2018). Chandravathy and Reddy (1994) suggested that decline in the tissue protein content might be due to reduced protein synthesis, increased proteolysis and also due to utilization for metabolic process under lead toxicity.

Table 1: Effects of sublethal concentrations of paper mill effluent (10%) on protein content (mg/g wet wt of tissue) in different tissues of *Channa punctatus* at different period of exposure (N=6).

Tissue	Control	Experimental			
		24 hrs	48 hrs	72 hrs	96 hrs
Muscles	135.25±1.98	139.15±1.47*	134.54±1.85	133.41±1.49	130.15±1.38*
Liver	104.78±1.54	101.25±1.37	96.81±1.14	93.28±1.52*	89.27±1.65**
Gill	91.28±1.18	89.54±0.98	85.54±1.02	81.65±1.21*	75.24±1.35**

*Significant at p<0.05; ** Significant at p< 0.01

The free amino acid (FAA) content in the muscles of effluent treated fish, *Channa punctatus* fluctuated during 24, 48, 72 and 96 hours exposure but free amino acids content in liver and gill of effluent exposed fish showed increasing trend in 96 hours as compared to control ones (Table 2). In the present investigation, an increase in FAA level in tissues indicates stepped up proteases activity and fixation of ammonia into keto acid (Rao *et al.,* 1987) or due to depletion of reserved glycogen so that the fish can try to yield metabolic energy by gluconeogenesis process (Tripathi *et al*., 2003). Another reason of increase in free amino acids content was due to lesser use of amino acids in protein synthesis because stress condition induced elevation in the transamination pathway (Natarajan, 1985) and their involvement in the maintained of acid base balance (Moorthy *et al*., 1984). In the present study, the significant increase in the level of free amino acids and significant decreased in protein content of *Channabn punctatus* due to enhanced proteolysis (Prakash and Verma, 2020d) and an inverse relationship between protein and amino acids in the tissues to meet the demands for energy requisites in addition to the carbohydrate and fat during stress conditions (Prakash and Verma, 2020e). Kumar and Gopal (2001) concluded that in effluent treated fish, protein content was decreased in different tissues was due to conversion and degradation of protein into free amino acid, which is used for different metabolic activity during stress condition.

Table 2: Effects of sublethal concentrations of paper mill effluent (10%) on Free Amino Acid content (mg/g wet wt of tissue) in different tissues of *Channa punctatus* at different period of exposure (N=6).

Tissue	Control	Experimental			
		24 hrs	48 hrs	72 hrs	96 hrs
Muscles	38.39±1.21	37.11±1.32	38.49±1.11	39.39±1.22	41.16±1.45
Liver	30.45±2.03	32.21±1.24	34.09±1.05	36.17±1.22*	41.25±1.58**
Gill	35.07±1.98	38.54±1.35	40.11±2.01	42.14±1.94*	44.45±1.24**

*Significant at p<0.05; ** Significant at p< 0.01

CONCLUSION

Based upon the results of the present investigation, it can be concluded that alteration in protein and amino acid content in *Channa punctatus* indicates biochemical manifestation due to the toxic action of industrial effluents. Decrease in protein value might be due to proteolysis which leads into increase in free amino acid level in fish body. Effluent induces its effect at cellular or even at molecular level and ultimately causes biochemical alterations as evidenced in the present study. The changes in biochemical composition of fishes will naturally affect the nutritive value of aquatic fauna. Fish with low protein value is not used for nutritional purpose that in turn will also be great danger to human beings due to continuous consumption of such fish.

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