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Biochemical Changes in estuarine fish *Mugil cephalus* exposed to industrial effluent

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Abstract

The present study deals with the toxicity of effluent from SIPCOT Industrial Estate on biochemical changes in muscle of an important estuarine fish *Mugil cephalus*. Overall reduction in total protein, total carbohydrates and total lipid of muscle was observed. The biochemical changes were higher in 96 hours effluent concentration in effluent treated fish when compared with control. The result of the present study recommends proper dilution of the effluent should be followed before its discharge.

Introduction

Pollution is the most burning problems of our age posing a potential threat to the health and well-being of our entire populations. The congregation of big communities in cities leads to the generation of waste quantities of liquid, gaseous and solid wastes. Industries also contribute to colossal quantities of wastes of different nature. Wherever the intensification of industries, the process of treatment of industrial discharges has lagged far behind the industrial growth. The toxic substances present in these wastes can affect the aquatic life thus disrupting the whole systems and pose a threat to human health directly or indirectly. Fish is a major source of food for human nutrition providing an important amount of dietary protein and lipid diet in many countries. Fish flesh is easily digestible because it contains long muscle fibers. A global scale, fish and fish products are the most important source of protein in the human diet.

In recent years, biochemical variables were used more when clinical diagnosis of fish physiology was applied to determine the effects of external stresses and toxic substances. Wepener (1997) suggested that haematology, biochemical changes, growth rate and oxygen consumption of fish can be used in determining the toxicity of pollutants. Therefore, biochemical evaluations are gradually becoming a routine practice for determining health state in fish. Biochemical and physiological biomarkers are frequently used for detection or diagnosing sub-lethal effects in fish exposed till different toxic substances. Sub-lethal effects are biochemical in origin as the most toxicants exert their effects at basic level of the organism with enzymes or metabolites and other functional components of the cell. Such effects might lead to irreversible and detrimental disturbances of integrated functions such as behavior, growth, reproduction, survival (Waldichuk, 1997). Proteins have a variety of functions and are especially important for the regulation of water balance in fishes. Lipids are an important metabolite for locomotory and reproductory and activities of fishes. Many environmental factors were reported to affect the seasonal variation in serum lipids (Fletcher, 1984). Hence, the present study was designed to investigate the biochemical changes in *Mugil cephalus* caused by effluent under laboratory conditions.

Materials and Methods

Samples of SIPCOT wastewater effluents were collected from chemical factory in Cuddalore. The samples were thoroughly mixed prior to refrigeration in the laboratory (Reish and Oshida, 1987). The physicochemical characteristics of industrial wastewater were determined by standard method (APHA, 1998) and the result are as follows: Colour- white; Odour- Pungent; Dissolved Oxygen- 1.2±0.02 mg, BOD- 2500±2.0 mg; pH- 4.68±0.2; Temperature- 28.2±2.0ºC; Salinity- 3±0.30 ppm.

Fish maintenance

Specimens of *Mugil cephalus* were collected from Vellar estuary, India and acclimatized to laboratory conditions at Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai for fifteen days. Water was changed daily and fish were fed with bivalves flesh and ground dried shrimp twice a day. For experimental studies, fish ranging from 9-10 cm in length and weighing 10-12 g were selected.

Acute Toxicity Test

Fish acute toxicity tests were conducted by exposing *Mugil cephalus* (N = 10 per aquarium) for 96 h to industrial effluent under static - renewal conditions. Five different concentrations of effluent (1.2, 2.3, 4.6, 9.2, and 18.4 %) for *Mugil cephalus was* diluted with seawater and control with sea water alone was tested to determine the LC_{50} values at 24, 48, 72 and 96h. Dead fishes were counted and removed immediately every day. All the experiments were conducted in triplicates. The acute toxic effects of effluent on *Mugil cephalus* was determined by the use of Finney's Probit Analysis LC50 determination method (Finney, 1971). The computer model (Probit Program Version 1.5 software) was developed by Environmental Protection Agency (EPA, 1999). In addition, the data were also assessed by S Dunnet's software.

Carbohydrate

Phenol-Sulphuric acid method followed by Dubois *et al.* (1956) was used to determine the total carbohydrate. Sample of about 5mg oven-dried tissue was taken in a test tube and 1ml of Phenol (5%) and 5ml of concentrated Sulphuric acid were added in quick succession. This tube was kept for 30 minutes at 30° C. Optical density of the colour was developed at 490 nm against the blank.

Protein

Protein estimation was determined by following the method of Lowry et al. (1952). To a 2 ml of sample solution, 2 ml of alkaline copper sulphate reagent (analytical reagent) was added to different test tubes. This was mixed well and the solution was incubated at room temperature for 10 minutes. Then to this solution, 2ml of Folin Ciocalteau reagent solution was added to each tube and incubated for 30 minutes. Finally the optical density was taken in Spectrophotometer (measure the absorbance) at 660 nm against blank.

Lipid

The analysis of lipid content was estimated by following the method of Folch et al. (1956). Using 500 mg of powdered oven dried tissue and 5ml of chloroform: methanol as 2:1 mixture was used to extract lipid. This extract is further filtered and taken in a pre-weighed beaker and subjected to oven dried. The beaker was weighed with lipids and the difference was taken as total lipid content to calculate the percentage.

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Results

Fishes were exposed to the effluents for 96 hours and then observed for their carbohydrate levels. The levels of carbohydrate, protein and lipid in *Mugil cephalus* was observed in both controlled and effluent exposed conditions. The biochemical composition in the muscles of the effluent exposed fish clearly shows that the glycogen reserves are being used to meet the stress caused due to the exposure. The levels of carbohydrate (4.23 mg/g), protein (0.99mg/g) and lipid (0.77 mg/g) were decreased in *Mugil cephalus* when compared with control (Figure 1).

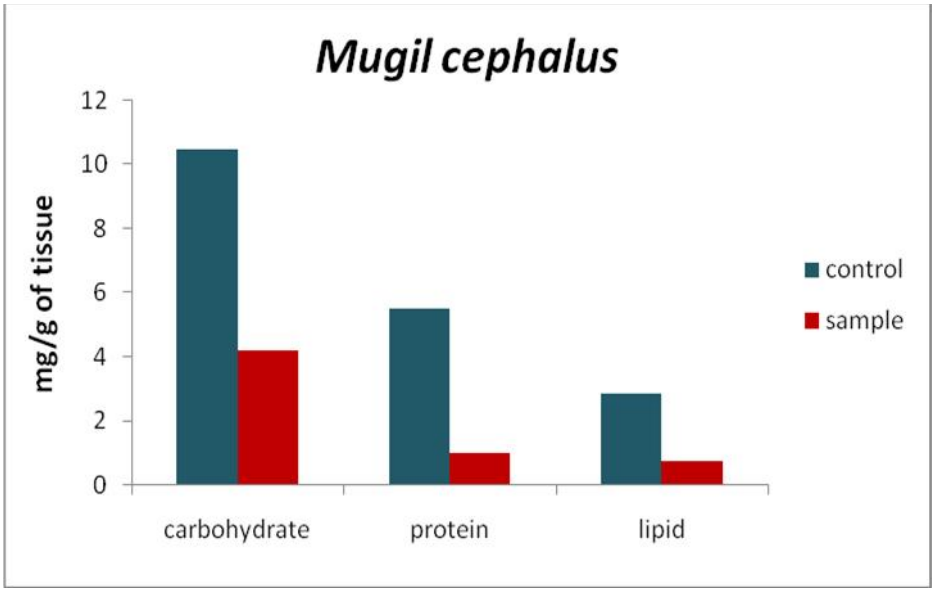

Fig.1 - Biochemical composition in tissues of *Mugil cephalus* at 96 hours under effluent treatment

Discussion

In the present study, carbohydrate, protein and lipid contents decreased in muscles of *Mugil cephalus* when exposed to 96h effluent concentration (Fig.1). Stored glycogen content in tissues is also released by anaerobic glycolysis and utilized to meet the energy requirement under pollutant stress as stated by Heath (1987). Hinson *et al*. (1973) remarked that maximum glycogen depletion corresponds to a dramatic increase in blood glucose level in the fish *Channa punctatus* exposed to industrial pollutants. They suggested that it might be due to some of the hepatic glycogen getting converted to glucose *via.* The intermediate glucose-1-phosphate getting and entering the circulation. The decreased glycogen content recorded in the present study may be due to the induced activation of adrenal pituitary glucocorticoid hormones which stimulate the hepatic glucose production thereby elevating the blood glucose level or it may be a physical response to meet the critical need of energy under effluent stress as suggested by the others.

Carbohydrates are considered to be the first degraded under the stress condition of animals. According to Dhavale and Masurekar(1986),the decreased level of carbohydrate constituents in tissues of toxicant exposed animals may be due to the prevalence of hypoxic condition in the tissues as a result of pollutant stress. The observed result in the present study is in accordance with the findings of Valarmathi and Azariah (2002), who have suggested that the decreased level of tissue carbohydrates in the toxicant exposed animals seemed to induce the glycogenolysis, possibly by increasing the activity of glycogen phosphorylase to meet the energy demand under stress condition or the toxicant may have an effect of glycogenesis by inhibiting the activity of carbohydrate metabolism Protein rich muscles form mechanical tissues that help mobility. In exposed fishes, muscles showed reduced levels of total protein content due to compound deposits from the effluent. *Mugil cephalus* seems to be more sensitive and had a drastic change in their protein levels when introduced to effluent Lipid content is an essential organic constituent of the tissues of all animals, and plays a key role in energy metabolism. The Lipids are best energy producers of the body next to carbohydrates. The decreased level of lipid content in the present study (0.77mg/g) may also be due to liver dysfunction or mobilization of glycerol or inhibition of oxidative phosphorylation. The above results revealed that the fluctuations of biochemical constituents were higher in the tissues of fish exposed to effluent concentrations. Considering the above facts, the present study concluded that the effect of common mixed effluent of SIPCOT Industrial Estate has heavy impact on the biochemical parameters of the fish. Hence, the present study recommends proper dilution of the effluent to be followed before its discharge.

Conclusion

Since the *Mugil cephalus* is considered to be one of the chief edible fishes in this region, a continuous assessment with respect to the discharge of effluents into the water bodies are need of the hour. If not treated properly, it will therefore affect the food chain including human. The present study recommends to avoid such impact in the aquatic environment, a proper dilution management is needed before they are discharged.

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