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BIOCHEMICAL INVESTIGATION ON FERTILIZER AMMONIUM SULPHATE EXPOSURE TO FRESHWATER FISH *Catla catla*

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ABSTRACT

The aquatic organisms are sensitive to environmental changes. They exhibit different degree of changes in the behavioral pattern when their habitat is polluted. To evaluate the toxic effect of ammonium sulphate on the biochemical parameters of *Catla catla*. The fish were exposed for 10, 20 and 30 days in 10% sublethal concentration of 96 h LC50 of ammonium sulphate (148mg/l). The fish exposed to sublethal concentration of ammonium phosphate showed mild alterations during 10 days of exposure, moderate alteration in 20 days exposure. However after 30 days, significant alterations were observed in carbohydrate, lipids and protein. These changes occurred predominantly in the 30 days exposure as compared to 10 and 20 days exposure.

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INTRODUCTION

Agrochemical fertilizers have been shown to have devastating effects on aquatic biota (Bobmanuel *et al*., 2006; Yadav*et al*., 2007). The aquatic organisms are sensitive to environmental changes. They exhibit different degree of changes in the behavioral pattern when their habitat is polluted. Fertilizers from nitrogen source are bound to pollute the fresh water ecosystem. Sub-lethal concentrations of fertilizers may cause ecological imbalance of these organisms after sufficiently long time of exposure probably as a result of cumulative impact of impaired metabolic functions (Cheng and Chen, 2002).

Fish are valuable sources of high grade proteins, mineral salts including calcium, phosphorus and iodine, essential amino acids, omega 3 fatty acids and vitamins A, B, D and E. Fish proteins occupy an important place and it constitutes about 17-20%. Moreover, carbohydrate content of the fish flesh is very low and hence, fish can make valuable contribution to any diet (Holt, 1967). Besides providing food to man, fishes are sources of numerous by products such as fish liver oil, fish flour, fish silage, fish glue, Isinglass etc. which have medical and economic importance. That's why it must be included in human diet at least 1.3 kg per week (FAO, 1989). However, the fish habitats are being contaminated alarmingly through a number of aquatic pollutants (Rajathy, 1991). Among these pollutants fertilizers are most injurious to fish. These pollutants have not only depleted the fish stock but also

have threatened the human health by incorporating into food chain (Thurston and Russo, 1983). In the present work, an attempt was made to evaluate the effect of ammonium sulphate on the biochemical alteration in freshwater fish *Catla catla*.

MATERIALS AND METHODS

Animal maintenance

Ninety juveniles of the fresh water fish Catla catla (Catla) were collected from the local fish pond at Thittai, Thanjavur district, Tamil Nadu. They were approximately weighed 4.27 ± 0.03 gram. These fishes were brought to the laboratory and acclimatized for 15 days glass aquaria containing aged tap water. Aged tap water (water stored for 24 hours) was used throughout the study to minimize mortality of the fishes during acclimatization; the aquarium water was maintained under standard conditions (Oxgen level of 6.00 – 6.50mg/L, PH 7.2-7.2 and temperature 27 –29 º C).

Experimental setup

The experiments were carried out with the help of small square type glass troughts of 10-liter capacity, which were covered with in iron wire gauge to avoid the jumping of the fish from the trough. To provide proper supply of oxygen an aerator was used. The test media was changed daily with fresh addition of the toxicant and sporolac.

Experimental Design

For sublethal toxicity tests 80 fishes were selected and divided into four groups (one control and three experimental) with 20 fish in each aquarium filled with water. The desired concentration (1/10 of 96h LC50 – 148 mg/l) of the toxicant was added directly in order to maintain constant concentration of the toxicant (Sheik Mohamed Salahudeen *et al*., 2014). The experiment was conducted for 30 days and sampled at 10 days interval and no mortality was observed during the above treatment period. After 10, 20 and 30 days, blood was collected and the fish were sacrificed. The tissues were removed and washed with saline and blotted. The tissues were homogenized using a glass homogenizer with chilled Tris HCl buffer (pH 7.4).

Tissue homogenate

End of the experimental periods, both experimental and control fish were anesthetized with 10 ppm Benzocain for 3 min. The fish were sacrificed and flesh was dissected out, washed with ice-cold physiological saline. The required amount was weighed and homogenized using a Teflon homogenizer. Tissue homogenate was prepared in 0.1 M Tris HCl buffer (pH 7.4) and used for the estimation of various biochemical parameters.

RESULTS

Group II fishes exposed ammonium sulphate shows mild alterations in protein, carbohydrate and lipids were observed as compared with group I control fish ($p < 0.05$). Group III fishes exposed ammonium sulphate shows significant ($p < 0.05$) alterations in protein, carbohydrate and lipids were observed as compared with group I control fish. Group II fishes exposed ammonium sulphate shows significant ($p < 0.05$) alterations in protein, carbohydrate and lipids were observed as compared with group I control fish. The alterations observed were directly proportional to the duration of the exposure.

Table 1 Effect of fertilizer ammonium sulphate exposure on proximate composition (Protein, Carbohydrate and lipids) in freshwater fish *Catla catla* with different days.

Parameters	Group I (Control)	Ammonium Sulphate Exposure		
		Group II (10 days exposure)	Group II (20 days exposure)	(Group III) (30 days exposure)
Protein (mg/gm)	5.19 ± 0.25	4.65± 0.23[a]	4.18 ± 0.21[b]	3.75 ± 0.23[c]
Carbohydrate (mg/gm)	2.14 ± 0.10	1.74± 0.08[a]	1.56 ± 0.06[b]	1.34± 0.08[c]
Lipids (mg/gm)	0.95 ± 0.06	0.75±0..05[a]	0.61 ±0..04[b]	0.52 ±0..03[c]

Values were expressed as Mean ± standard deviation (number of trials, 6)

Mean values within the row followed by different letters (Superscript) are significantly ($p< 0.05$) different from each other were comparison by Duncan's multiple range test (DMRT).

DISCUSSION

Global fishery production has been reported to be 142 million tones in 2008 and the contribution of aquaculture was more than 60 %. The total fisheries production continued to grow rising from 34.50 % in 2006 to 36.90 % in 2008. It has been estimated that the total fish production will be 53.64 million metric tonnes in 2030, based on annual growth rate. In contrast to world capture fisheries, which have almost stopped growing since the mid - 1980s, the aquaculture sector maintains an average annual growth rate of 8.30 % worldwide. In aquaculture the contribution of inland fishery production is 4.66 metric tonnes of which almost 90 % is contributed from freshwater aquaculture. India now ranks second and third in world fishery production and freshwater aquaculture respectively (Umaa Rani *et al*., 2014).

Biochemical and physiological biomarkers have been used in order to prevent irreversible damage in whole organisms, communities and ecosystems (Lopez-Barea and Pueyo, 1998). Measurement of biochemical and physiological parameters is a commonly used diagnostic tool in aquatic toxicology and biomonitoring. The impact of a number of contaminants on aquatic ecosystems can be assessed by the measurement of their external levels in the surrounding water or sediments, or by determining some biochemical parameters in fish and other organisms that respond specially to the degree and type of contamination (Petrivalsky *et al*., 1997; Machala *et al*., 2001). Oner *et al*. (2009) reported that biochemical parameters assessed in fish may be a useful tool by providing quantitative measurement of metals impact as well as valuable information of ecological relevance on the effects of metals (Oner *et al*., 2009). Moreover, biochemical biomarkers are frequently used for detecting or diagnosing sublethal effects in fish exposed to toxic substances (Toguyeni *et al*.,

1997). Sublethal effects are biochemical in origin as the most toxicants exert their effects at basic level of the organism by reacting 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 and survival (Waldichuk, 1979).

Analysis of chemical substances in tissues and body fluids, toxic metabolites, enzymes activities and other biochemical variables have frequently been used in documenting the toxin interaction with biological systems. Components like carbohydrate, protein and lipid play a vital role as energy precursors for fish under stress conditions (Umminger, 1970). Glucose is a carbohydrate that has a major role in the bioenergetics of animals, being transformed to chemical energy (ATP), which in turn can be expressed as mechanical energy (Lucas, 1996). Changes in carbohydrate metabolism measured as plasma glucose (energy substrate whose production is thought to metabolically assist the animal to cope with an increased energy demand caused by stress) used as general stress indicators in fish (Teles *et al*., 2007). Glucose (or glucose 6-phosphate) is released through the degradation of glycogen by glycogen phosphorylase (GP) (Roach *et al*., 1998), and energy is mainly supplied by the oxidation of glucose and lactate as a result of carbohydrate metabolism (Morgan *et al*., 1997). The glucose concentration was proposed to be mediated by endocrine release such as cortisol (Hontela *et al*., 1996). Silbergeld (1974) stated that assay of this important parameter can serve as an indicator of environmental stress.

In a stress situation, glucose production provides energy substrates to tissues, in ordr to cope with the increased energy demand. Regardless of the wide use of glucose as an indicator of stress, some authors (Mommsen *et al*., 1999) emphasized that care has to be taken when using plasma glucose as the only indicator. It has been reported that glucose content is a less precise indicator of stress than cortisol (Pottinger, 1998). The storage or mobilization of metabolic substrates such as glucose, glycogen, lactate, lipid, and protein are disrupted by exposure to several trace metals, including cadmium (Fabbri *et al*., 2003), manganese (Barnhoorn *et al*., 1999), nickel (Sreedevi*et al*., 1992), and metal mixtures in a polluted habitat (Levesque *et al*., 2002). Many investigators have reported blood glucose levels under various toxicant exposure conditions; cadmium in *Oncorhynchus mykiss, Salmo salar Ctenopharyngodon idellus Cyprinus carpio* (Soenges *et al*., 1996; Joshi and Bose, 2002; Drastichova *et al*., 2004), copper in *Oncorhynchus mykiss* (Dethloff *et al*., 1999); endosulfan in *Salmo salar* (Petri *et al*., 2006) and cyfluthrin in *Cyprinus carpio* (Sepici-Dincel *et al*., 2009).

Proteins are important organic substance required by organisms in tissue building. They are intimately related with almost all physiological processes, which maintain a simple biochemical system in 'living condition' (Joshi and Kulkarni, 2011). Proteins are mainly involved in the architecture of the cell. Proteins occupy a unique position in the metabolism of cell because of the proteinaceous nature of all the enzymes which mediate at various metabolic pathways. During stress conditions fish need more energy to detoxify the toxicant and to overcome stress. Since fish have fewer amounts of carbohydrates so next alternative source of energy is protein and lipids to meet the increased energy demand (Singh *et al*., 2010).

The fish were exposed for 10, 20 and 30 days in 10% sublethal concentration of 96 h LC50 of ammonium sulphate (148mg/l). The fish exposed to sublethal concentration of ammonium phosphate showed mild alterations during 10 days of exposure, moderate alteration in 20 days exposure. However after 30 days, significant alterations were observed in carbohydrate, lipids and protein. These changes occurred predominantly in the 30 days exposure as compared to 10 and 20 days exposure.

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