

Original Research Article

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## Effect of Heavy Metal, Chromium on Protein and Amino Acid Contents in Gill, Liver and Kidney of Freshwater Fish, *Channa striatus* (Bloch)

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### ABSTRACT

#### Keywords

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Heavy metals are natural trace components of the aquatic environment, but their levels have increased due to industrial wastes, geochemical structure, agricultural and mining activities. All these sources of pollution affect the physiochemical characteristics of the water, sediment and biological components, and thus the quality and quantity of fish stocks. Fish is generally appreciated as one of the healthiest and cheapest source of protein and it has amino acid compositions that are higher in cysteine than most other sources of protein. The aim of the present study was to assess the protein and amino acid levels in gill, liver and kidney of *Channa striatus* was exposed to sublethal concentrations of chromium for the period of 10, 20 and 30 days. The fish exposed to chromium showed a decrease the protein and increase the amino acid levels for 10, 20 and 30 days in gill, liver and kidney. The objective of the present investigation was to observe the effect of chromium on protein and amino acid levels in the gill, liver and kidney of freshwater fish, *Channa striatus*.

### Introduction

Water contamination caused when an input from human activities cause an increase of a substance in fresh water, sediments and organisms above the natural background level for that area and for those organisms (Clark, 2001). Occurrence of potential toxicants in aquatic ecosystem causes a reduction in the quality of the aquatic environment that results in impaired level of dissolved oxygen (DO), pH, temperature, biological oxygen demand, and chemical oxygen demand (Robert, 2001; Ibrahim *et al.*, 2016).

Increases use of metal based fertilizers in agricultural revolution could result in the continued rises in the concentration of metal pollutants in fresh water due to the water run-off (Adefemi *et al.*, 2008; Prasath and Arivoli, 2008; Ibrahim *et al.*, 2016). The speedy urbanization and industrialization has led to increased disposal of pollutants like heavy metals, radio nuclides, and various types of organic and inorganic substances into the environment. Thus, the industrial wastes are the main source of metal pollution for aquatic organisms. It has

been cited that the heavy metals constitute the major pollutants in the environment. The heavy metals are important pollutants for fishes, because these are not eliminated from aquatic systems by natural methods, such as organic pollutants, and are enriched in mineral organic substances. The metal contaminants are mixed in the aquatic system through smelting process, effluents, sewage and leaching of garbage which cause severe harm to the aquatic system (Pandey and Madhuri, 2014).

The heavy metals are considered as critical toxic contaminants of aquatic ecosystems, due to their high potential to enter and accumulate in food chain (Olojo *et al.* 2005). The main sources of heavy metal pollution of the agriculture, industry and metropolitan cities, the bioaccumulation of toxic heavy metals in fish species from different aquatic systems is dependent on their foreign polluted substances. The distribution of heavy metals in water, sediments and fish play a key role in detecting sources of heavy metal pollution in aquatic ecosystem (Forstner and Wittman, 1981). The aquatic systems deposition of contaminants, including heavy metals, can lead to elevated sediment concentrations that cause potential toxicity of the aquatic biota (Yang and Rose, 2003; Heyvart *et al.*, 2000; Maurya and Malik, 2016).

Heavy metals are ubiquitous in the biosphere, where they occur as part of the natural background of chemicals. Anthropogenic activities have also introduced substantial amounts of them into the environment by mobilization from their natural insoluble deposits or environmental sinks (Chiesa *et al.*, 2006). They represent a significant ecological and public health concern due to their toxicity and their ability to accumulate in living organisms. The heavy metals tend to concentrate in the body and cause damage in the kidneys, lungs,

brain and nervous system and in the body metabolism (Fergusson, 1990). The Cr is present in the tannery effluent, and is known to cause various ill effects. Such health hazards are dependent on the oxidation state of Cr. Its hexavalent form is toxic than the trivalent form (Pandey and Madhuri, 2014). The present investigation was to assess the protein and amino acid content in gill, liver and kidney of *Channa striatus* exposed to sublethal concentrations of chromium.

### **Materials and Methods**

The fish *Channa striatus* having mean weight 18 - 22 g and length 13 – 15 cm were collected from PSP fish farm, at Puthur and acclimatized to laboratory conditions. They were given the treatment of 0.1% KMNO<sub>4</sub> solution and then kept in plastic pools for acclimatization for a period of two weeks. They were fed twice daily i.e. morning and evening on boiled chicken eggs approximately 4% of fish body weight divided into two equal meals daily. The chromium was used in this study and stock solutions were prepared. Chromium, LC<sub>50</sub> was found out for 96 h (34 ppm) (Sprague, 1971) and 1/20<sup>th</sup> (1.7 ppm), 1/15<sup>th</sup> (2.27 ppm) and 1/10<sup>th</sup> (3.4 ppm) taken as sublethal concentrations for this study. Forty fish were selected and divided into 4 groups of 10 each. The first group was maintained in free from chromium and served as the control. The other 3 groups were exposed to sub lethal concentration of chromium, 10 litre capacity aquaria. The 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> groups were exposed to chromium, for 10, 20 and 30 days respectively. At the end of each exposure period, the fish were sacrificed and the required tissues were collected for protein and amino acid estimation. The protein and amino acid content in gill, liver and kidney of *Channa striatus* were estimated by the method of Lowry *et al.*, 1951 and Moore and Stein (1954) respectively. The data obtained were

analyzed by applying analysis of variance DMRT one way ANOVA to test the level of significance (Duncan, 1957).

## Results and Discussion

### Protein level in gill

The protein contents were observed in the control gill to be  $119.63 \pm 8.74$ ,  $120.66 \pm 8.82$  and  $122.8 \pm 8.98$  mg g<sup>-1</sup> wet weight for 10, 20 and 30 days respectively. The protein contents were significantly decreased when the fish *Channa striatus* exposed with low ( $102.16 \pm 7.39$ ,  $96.02 \pm 6.95$  and  $92.53 \pm 6.68$ ), medium ( $95.00 \pm 6.86$ ,  $87.33 \pm 6.29$  and  $81.92 \pm 5.87$ ) and high ( $92.11 \pm 6.65$ ,  $83.19 \pm 5.97$  and  $76.71 \pm 5.48$ ) sublethal concentrations of heavy metal chromium for 10, 20 and 30 days respectively (Fig 1)..

### Protein Level in Liver

The protein contents were observed in the control liver to be  $133.70 \pm 9.82$ ,  $137.12 \pm 10.07$  and  $142.54 \pm 10.48$  mg g<sup>-1</sup> wet weight for 10, 20 and 30 days respectively. The protein contents were significantly decreased when the fish *Channa striatus* exposed with low ( $114.52 \pm 8.35$ ,  $117.88 \pm 8.61$  and  $106.64 \pm 7.75$ ), medium ( $105.44 \pm 7.66$ ,  $103.70 \pm 7.53$  and  $87.69 \pm 6.31$ ) and high ( $100.66 \pm 7.30$ ,  $97.04 \pm 7.02$  and  $78.65 \pm 5.62$ ) sublethal concentrations of heavy metal chromium for 10, 20 and 30 days respectively. The decrease in the protein content of the liver was more in 30 days of exposed fish compared to 10 and 20 days (Fig 1).

### Protein Level in Kidney

The protein contents were observed in the control kidney to be  $89.09 \pm 6.42$ ,  $89.95 \pm 6.48$  and  $89.33 \pm 6.49$  mg g<sup>-1</sup> wet weight

for 10, 20 and 30 days respectively. The protein contents were significantly decreased when the fish *Channa striatus* exposed with low ( $86.99 \pm 6.26$ ,  $85.21 \pm 6.13$  and  $80.92 \pm 5.79$ ), medium ( $83.51 \pm 5.99$ ,  $80.02 \pm 5.73$  and  $76.07 \pm 5.43$ ) and high ( $82.73 \pm 5.93$ ,  $78.59 \pm 5.62$  and  $70.61 \pm 4.51$ ) sublethal concentrations of heavy metal chromium for 10, 20 and 30 days respectively. The decrease in kidney protein content is more pronounced in high sublethal concentration of heavy metal chromium at 30 days of exposure periods (Fig 1).

### Amino Acid Level in Gill

The amino acid contents were observed in the control gill to be  $4.30 \pm 0.33$ ,  $4.38 \pm 0.33$  and  $4.34 \pm 0.33$  mg g<sup>-1</sup> wet weight for 10, 20 and 30 days respectively. The amino acid contents were significantly increased when the fish *Channa striatus* exposed with low ( $5.46 \pm 0.41$ ,  $6.26 \pm 0.47$  and  $7.57 \pm 0.57$ ), medium ( $5.98 \pm 0.45$ ,  $7.10 \pm 0.54$  and  $10.14 \pm 0.77$ ) and high ( $6.44 \pm 0.49$ ,  $6.64 \pm 0.65$  and  $13.28 \pm 1.01$ ) sublethal concentrations of heavy metal chromium for 10, 20 and 30 days respectively. In sublethal concentrations of heavy metal chromium exposed fish, *Channa striatus* the amino acids showed a gradual increase from 10 to 30 days. The maximum increase was noticed in high sublethal concentration of heavy metal chromium at 30 days of exposure periods (Fig 2).

### Amino Acid Level in Liver

The amino acid contents were observed in the control liver to be  $6.00 \pm 0.45$ ,  $6.03 \pm 0.45$  and  $5.98 \pm 0.45$  mg g<sup>-1</sup> wet weight for 10, 20 and 30 days respectively. The amino acid contents were significantly increased when the fish *Channa striatus* exposed with low ( $7.06 \pm 0.54$ ,  $7.46 \pm 0.56$

and  $9.67 \pm 0.73$ ), medium ( $7.98 \pm 0.61$ ,  $9.77 \pm 0.74$  and  $11.56 \pm 0.88$ ) and high ( $10.14 \pm 0.77$ ,  $13.58 \pm 1.03$  and  $16.30 \pm 1.24$ ) sublethal concentrations of heavy metal chromium for 10, 20 and 30 days respectively. In sublethal concentrations of heavy metal chromium exposed fish, *Channa striatus* the amino acids showed a gradual increase from 10 to 30 days. The maximum increase was noticed in high sublethal concentration of heavy metal chromium at 30 days of exposure periods (Fig 2).

### **Amino Acid Level in Kidney**

The amino acid contents were observed in the control kidney to be  $3.76 \pm 0.28$ ,  $3.82 \pm 0.29$  and  $3.80 \pm 0.29$  mg g<sup>-1</sup> wet weight for 10, 20 and 30 days respectively. The amino acid contents were significantly increased when the fish *Channa striatus* exposed with low ( $4.30 \pm 0.33$ ,  $4.91 \pm 0.37$  and  $6.12 \pm 0.46$ ), medium ( $4.94 \pm 0.37$ ,  $5.80 \pm 0.44$  and  $6.87 \pm 0.52$ ) and high ( $5.47 \pm 0.41$ ,  $7.54 \pm 0.57$  and  $9.16 \pm 0.69$ ) sublethal concentrations of heavy metal chromium for 10, 20 and 30 days respectively. In sublethal concentrations of heavy metal chromium exposed fish, *Channa striatus* the amino acids showed a gradual increase from 10 to 30 days. The maximum increase was noticed in high sublethal concentration of heavy metal chromium at 30 days of exposure periods (Fig 2).

Aquatic ecosystems are fragile and at risk primarily because the majority of contaminants derived from urban and industrial sources, abandoned military installations, and agricultural activities are released into rivers (Van der Oost *et al.*, 2003; Pinto *et al.*, 2015; Byrne *et al.*, 2015; Kaur and Dua, 2014). One of the major pollutants is heavy metals, which rapidly

accumulate in the body and are slowly metabolized in and excreted from animals. The main source of heavy metal contamination of water is industry which is located nearby water resources. The industrial waste water contain lead (Pb), chromium (Cr), zinc (Zn), and mercury (Hg) are the main polluting heavy metals (Outridge and Noller, 1991; Sabale *et al.*, 2012; Strbac *et al.*, 2015; Silva *et al.*, 2016).

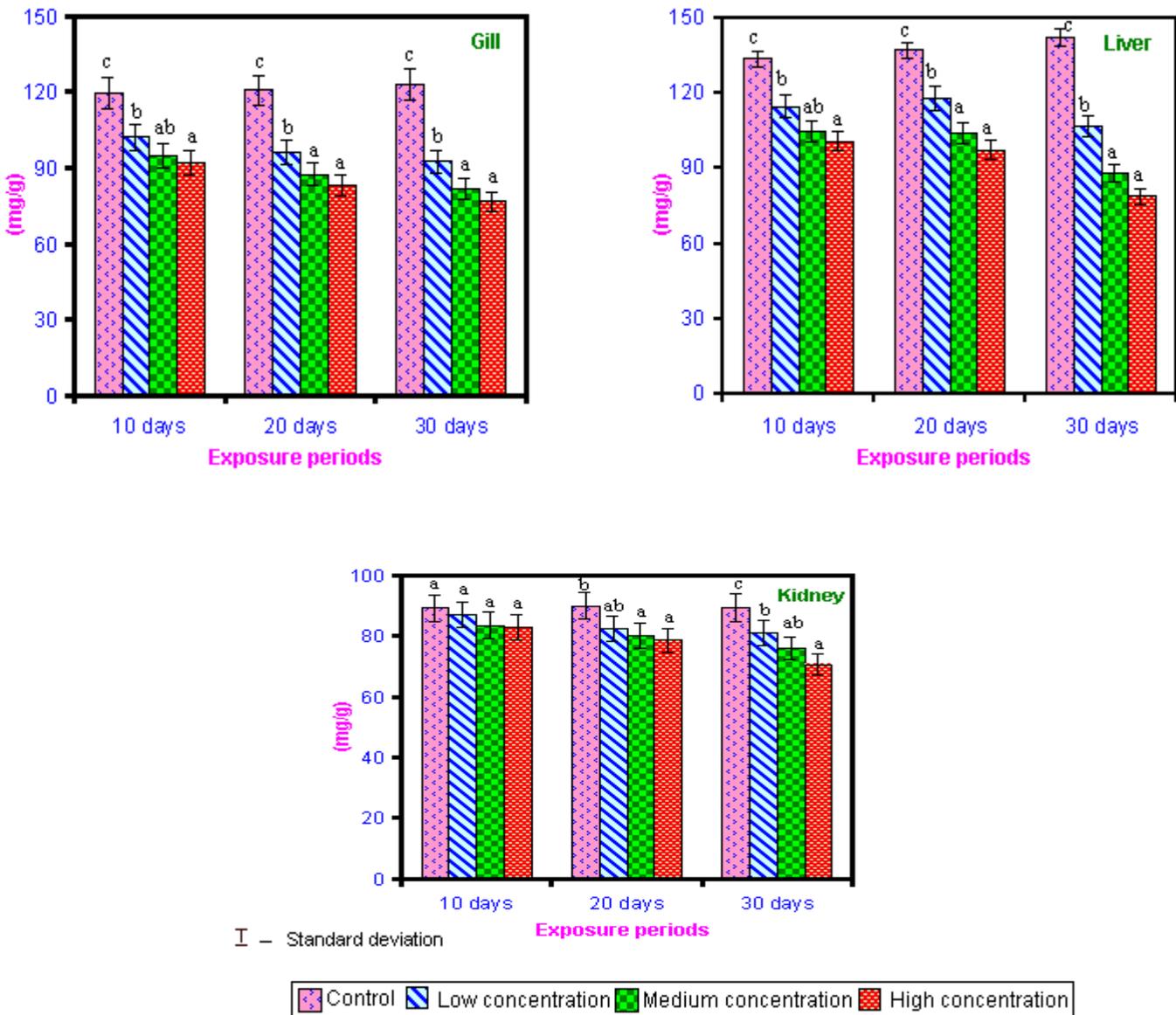
Gills represent a thin and extensive surface in intimate contact with water. They carry out three main functions, gas exchange, ion regulation and excretion of metabolic waste products. Due to the constant contact with the external environment, gills are the first targets of waterborne pollutants (Perry and Laurent, 1993). Gills are the vital organs in fish, which have direct contact with the medium through which pollutants enter into the body (Mount, 1962; Holden, 1972; Edwards, 1973). The liver plays an important role in the synthesis of proteins. The kidney, which is an important organ of excretion and osmoregulation, is indirectly affected by pollutants through blood circulation (Newman and MacLean, 1974). The impact of contaminants on aquatic ecosystem can be assessed by measurement of biochemical parameters in fish that respond specifically to the degree and type of contamination (Petrivalsky *et al.*, 1997). Tissue protein content has been suggested as an indicator of xenobiotic-induced stress in aquatic organisms (Singh and Sharma, 1998). Based on their known toxicological profiles in many animal models, it is certainly plausible that waterborne metals could alter physiological and biochemical parameters in fish (Abedi *et al.*, 2013).

The protein contents were decreased may be due to breakdown of proteins in the fabrication of some amount of energy for organism (Tulasi and Jayantha Rao, 2013). Sathyanarayana (2005) suggested that the

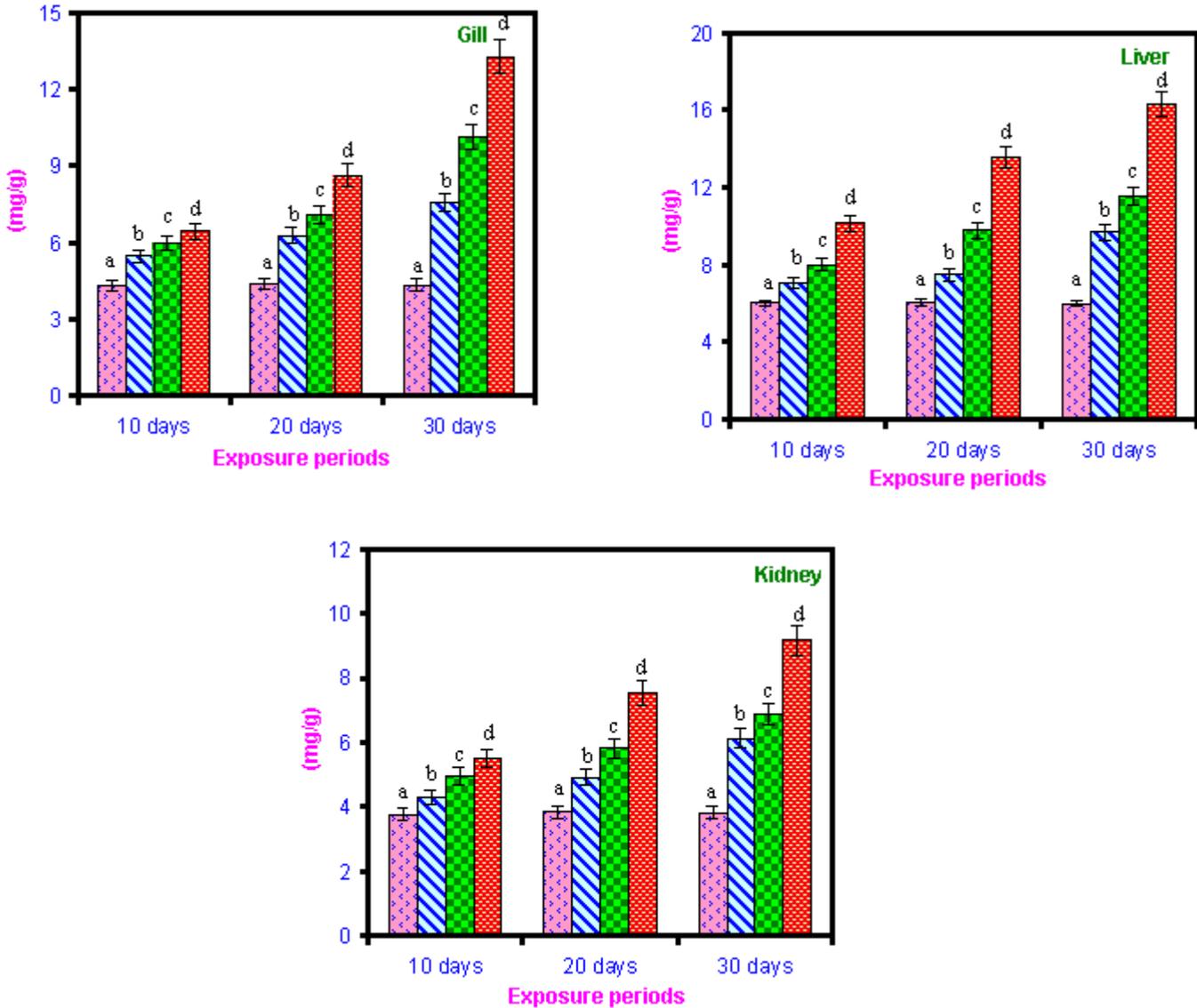
physiological status of animal is usually indicated by the metabolic status of proteins. Jrueger *et al.* (1968) reported that the fish can get the energy through the catabolism of proteins. Proteins are mainly involved in the architecture of the cell, which is the chief source of nitrogenous metabolism. Thus, the depletion of protein fraction in liver, brain

and kidney tissues may have been due to their degradation and possible utilization for metabolic purposes. Increases in free amino acid levels were the result of breakdown of protein for energy and impaired incorporation of amino acids in protein synthesis (Singh *et al.*, 1996).

**Fig.1** Protein levels changes (mg/g) in gill, liver and kidney of *Channa striatus* exposed to sublethal concentrations of chromium



**Fig.2** Amino acid content changes (mg/g) in gill, liver and kidney of *Channa striatus* exposed to sublethal concentrations of chromium



I – Standard deviation

Control Low concentration Medium concentration High concentration

The present investigation, in the gill, liver and kidney protein content had decreased whereas amino acids content had increased at all periods of exposure when *Channa*

*striatus* was exposed with sublethal concentrations of chromium for the periods of 10, 20 and 30 days exposure. The free amino acid (FAA) pool was increased in the

tissues of the fish during exposure to lihocin (Abdul *et al.*, 2010), while the elevated FAA levels were utilized for energy production by supplying them as keto acids into TCA cycle through aminotransferases to contribute energy needs during toxic stress. Increases in free amino acid levels were the result of breakdown of protein for energy and impaired incorporation of amino acids in protein synthesis (Singh *et al.*, 1996). Similarly protein levels were decreased and amino acid contents increased significantly in gill, liver and kidney of *Cyprinus carpio* exposed to sublethal concentration of pharmaceutical effluent (Muthulingam *et al.*, 2011).

The protein content decreased in the liver, brain and kidney tissues of *Channa punctatus* during lihocin treatment (Abdul *et al.*, 2010). A reduction in the protein content in the kidney could possibly be due to protein breakdown leading to increased amino acid pool of tissue (Radhaiah *et al.*, 1987). Satyaparameshwar *et al.*, (2006) suggested that *Lamellidens marginalis* exposed to sublethal concentration of chromium showed decrease the protein and RNA where as amino acid level was elevated. The decreased protein level was observed in the kidney tissue of *Catla catla* at sublethal concentration of chromium (Vincent *et al.*, 1995). Rao *et al.* (1980) and Devi (1981) have reported that the kidney was the site of degradation and detoxification of toxic substances. The decreased protein level in the kidney tissue at sublethal concentration of lead may be due to the enhanced proteolysis.

Many investigators have also recorded such a reduction in protein content in fishes exposed to different toxicants (Karupphasamy, 1990; Rao, 1989; Vincent *et al.*, 1995; Nisha and Shukla, 1986). A reduction in the protein content in the present investigation in *Channa striatus* suggests that the tissue protein undergoes

proteolysis, which results in an increase in the production of free amino acids. These amino acids are utilized for energy production during stressful situation in the intoxicated fishes. Moorthikumar and Muthulingam (2010) reported that decline in the protein and elevated contents of amino acid in liver, kidney and brain of *Labeo rohita* under heavy metal, nickel chloride stress. Senthil Elango and Muthulingam (2014) suggested that decreasing patterns of protein and increased levels of amino acid contents in brain and muscle of *Oreochromis mossambicus* was exposed to sublethal concentrations of chromium. It is evident that proteins are degraded to meet the energy requirements during sublethal concentrations of chromium. The depletion in protein level was due to diversification of energy to meet the impending energy demand when the fish *Channa striatus* exposed to sublethal concentrations of heavy metal chromium stress. The reduction in protein content in the present study indicates that the tissue protein undergoes proteolysis resulting in the production of free amino acids leads to disturbances in the physiological activity of the fish *Channa striatus*.

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