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ORIGINAL ARTICLE

EFFECT OF NICKEL ON CERTAIN BIOCHEMICAL PARAMETERS IN SELECTED TISSUES OF CIRRHINUS MRIGALA

***P.Parthipanand M.Muniyan**

Department of Zoology, AnnamalaiUniversity,Annamalai Nagar-608002, Tamilnadu

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ABSTRACT

In the present study, the sub-lethal effects of nickel on various biochemical parameters of *Cirrhinus mrigala* were studied. The fish was exposed to sub- lethal concentration of nickel for 7, 14, 21, and 28 days for chronic toxicity studies. In the present study, total protein, amino acid, glycogen and glucose were observed in liver,gill,kidney and muscle tissues of fish. The present study showed the protein content was decreased and amino acid content was increased significantly in liver,gill,kidney and muscle of fish exposed with nickel and also glycogen was increased in the in liver,gill,kidney and muscle tissue of fish exposed with nickel.

Key words: Nickel, Biochemical parameters, *Cirrhinus mrigala*

1. INTRODUCTION

The rapid pace of industrialization and anthropogenic inputs have contaminated many ecosystems (Gayathri*et al*., 2008) especially the aquatic ecosystem, which receives a wide range of pollutants. Pollution of aquatic habitats seems to be an inevitable problem of universal nature and the intrusion of various pollutants into the aquatic environment affects the survival growth and reproduction of the biological organisms present in the environment. As fish being exclusively aquatic a number of potentially hazardous xenobiotics confront fish life in the sphere of their activities of which, a category of special interest is that of the heavy metals (Rajah, 1992).

Nickel is one of the heavy metals. The main sources of nickel come from hydrogenation of oil industry and paint factories, motor vehicle, aircraft industry, printing and in some cases the chemical industry. It is also used extensively in electroplating as nickel sulphate and nickel hydroxide is used in nickel–cadmium batteries (Nanda and Behera, 1996). In aquatic ecosystem, dissolved Nickel concentrations are generally between 0.005 and 0.01 mg l−1 (Galvin, 1996). The toxicityof Ni to aquatic life has been shown to vary significantly with organism species, pH and water hardness (Birge and Black, 1980). Nickel toxicity is generally low (Khangarot and Ray, 1990) but elevated concentration can cause sub lethal effects.

Nickel containing enzymes are ureas and methyl coenzyme reductase. Nickel inhibits acid phosphatase and this property is used to differentiate from nucleoside phosphatase. Nickel has a role in the production of pigments in fish, birds and insects. Some chocolate preparation may contain nickel more than the permitted level. Nickel in higher concentrations may be carcinogenic. Requirement of nickel is 500μg/day. Nickel is known to cause cancers of the nasal cavity, paranasal sinuses and lungs. The most common effect resulting from exposure to nickel compounds is the development of nickel itch.

Nickel is a nutritionally essential trace metal for at least several animal species, micro-organisms and plants, and therefore either deficiency or toxicity symptoms can occur when, respectively, too little or too much Nickel is taken up. Although a number of cellular effects of nickel have been documented, a deficiency state in humans has not been described (Barcelou, 1999; Klaassen, 1996). Nickel and nickel compounds have many industrial and commercial uses, and the progress of industrialization has led to increased emission of pollutants into ecosystems. Although Nickel is omnipresent and is vital for the function of many organisms, concentrations in some areas from both anthropogenic release and naturally varying levels may be toxic to living organisms.The present study was carried out to investigate the sub-lethal effect of arsenic in biochemical parameters in liver tissue of *Cirrhinus mrigala*

Corresponding author:
****Dr. P.Parthiban, Department of Zoology, AnnamalaiUniversity,Annamalai Nagar-608002***

2. MATERIALS AND METHODS

The fresh water fish *Cirrhinu smrigala* were collected from fish farm at Puthur, Tamil Nadu, India. The collected fish were acclimated to laboratory condition for 15 days. They were checked thoroughly for injury and disease conditions, and only healthy fishes were used for this study. After washing with 0.01% $KMnO_4$ solution for 15 min, they were placed in nine plastic pools (500 L) containing non-chlorinated water. Prior to the start of the experiment, the fishes were acclimatized to the food and laboratory conditions with 12 h dark and 12 h light cycles, pH range of 6.95 to 7.60 and temperature ranging from 16 to 24 °C for 15 days. Fishes were divided into five equal groups each comprising of 50 fishes. Each group was kept in separate plastic tanks. The first group was kept as negative control; the fishes were maintained in water containing normal water without any treatment. The fishes of two groups were exposed to a sub-lethal concentration of 2.82 concentration of nickel added in the water for 7,14,21 and 28 days respectively. Solutions were renewed once daily after exposure period, animals were sacrificed and the liver tissues were removed, homogenized and stored at −80 °C for further biochemical analyses.

Estimation of tissue protein in tissue

Protein content in the tissue were estimated by the method of Lowry et al.(1951). The tissue was isolated and 2% homogenate was centrifuged at 3,000 rpm for 15 minutes. the supernatant was discarded and the reside was suspended in 1.0 ml of 0.1 N sodium hydroxide solution. 0.5 ml of this solution equivalent to 10 mg of tissue was transferred to a clean test tube and 4 ml of copper carbonate solution was added. The contents were mixed by lateral shaking and 0.4 ml of folin phenol (1:1 dilution) reagent was added. The thoroughly mixed contents were kept at room temperature for 30 minutes, the colour developed was read at 600 nm against a reagent blank in UV sisible spectrophotometer (Jasco Model-650). Bovine serum albumin (Sigma Chemical Co.) was used to construct expressed in mg/g wet weight of tissues.

Estimation of total amino acids in tissue

Total free amino acids in the tissues were estimated by the method of Moore and Stein (1954). The liver was isolated in ice, quickly weighed in an cold room and immediately homogenized in cold 10 per cent TCA. The homogenate contains 10 mg of tissues).One ml of the clear supernatant was taken into a clean test tube and 2.0 ml of ninhydrin reagent was added. The mixture was cooled immediately under running rap water and the intensity of the colour was read at 570 nm in a UN-visible spectrophotometer (Jasco, model 650). Tyrosine was used to construct the standard graph and the values were expressed mg/g wet weight of tissue.

Estimation of glycogen and glucose

Kemp and Kits van Heijningen (1954) were employed for the quantitative estimation of glycogen and glucose. The tissues were isolated and homogenized in 5.0 ml of 80% methanol and centrifuged at 3,000 rpm for 15 minutes. The supernatant containing free glucose was decanted into a calibrated test tube. The residue was set apart for the quantitative estimation of glycogen.

Estimation of Glycogen

The residue left after methanol extraction was homogenised in 5.0 ml of deprotenizing solution (5-0 ml of TCA and 100 mg of $AgS0_4$ in 100 ml of distilled water) and heated at 100° C over a water bath for 15 minutes. The mixture was cooled and made up to 5.0 ml with deproteinizing solution once again and later centrifuged at 2,000 rpm for 10 minutes. The clear supernatant was collected for the estimation of glycogen.

Estimation of glucose

To the decanted solution approximately 10.0 mg of activated animal charcoal powder was added. The methanol was allowed to evaporate by warming the solution over a water bath for 30 minutes. Deproteinizing solution (100 gm. of TCA in 100ml of distilled water) was added to the residual aqueous solution to bring the total volume to 5.0 ml. The suspension was centrifuged at 2,000 rpm for 15 minutes and the clear supernatant was used for the estimation of free glucose.

Quantitative estimation of glycogen and glucose

1.0 ml of the respective sample was taken in a separate test tube and 3.0 ml of concentrated sulfuric acid was added to it. The mixture was heated in a boiling water bath for 6.0 minutes and subsequently cooled in running tap water. The intensity of the colour developed was measured in a UV Spectrophotometer against the reagent blanks (3.0 ml of concentration sulfuric acid) at 520 nm. The quantitative of glucose and glycogen present in the respective samples were read form the standard graph drawn previously form known quantities of the sample. The glucose and glycogen values are expressed as mg/ g wet weight of tissues.

Statistical Analysis

The data obtained from the control and experimental parameters were subjected to determine the level of significance at exposure periods and metal concentrations by student ‘t’ test.

3. RESULTS

Level of protein content in the tissues

The amount of protein in the control liver tissue was 150.50 mg/g of wet weight of tissue and sublethal concentration metal treated fingerlings were 141.35; 136.76; 131.36; and 126.48 mg/g of wet weight of tissue. The amount of protein in the metal treated liver tissue shows the decreasing trends. The decreasing percentage were -6.08; -9.13; -12.72; and -15.96 at the 7,14,21,and 28 days of exposure respectively. The mean difference were statistically significant at $P<0.05$ level (Table 1). The amount of protein in the control gill tissue was 89.10

mg/g of wet weight of tissue and sublethal concentration metal treated fingerlings were 85.30; 83.35; 79.87; and 78.00 mg/g of wet weight of tissue. The decreasing percentage were -4.26; -6.45; -10.36; and -12.45 at the 7,14, 21 and 28 days of exposure respectively .The mean difference were statistically significant at P<0.05 level. (Table 1).

In the kidney tissue of control fish, the level of protein content was 124.88mg/g of wet weight of tissue. During the sublethal concentration nickel treated fingerlings were 119.35; 114.21; 110.66; and 108.34 mg/g of wet weight of tissue. The amount of protein in the nickel treated kidney tissue was decreased. The decreasing percentage were -4.43; -8.54; -11.39; and 13.24 at the 7, 14, 21 and 28 days of exposure respectively. The mean difference were statistically significant at P<0.05 level (Table 1). The amount of protein in the control muscle tissue were 145.18 mg/g of wet weight of tissue and sublethal concentration nickel treated fingerlings were 139.37; 136.98; 132.59; and 128.28 mg/g of wet weight of tissue. The amount of protein in the nickel treated muscle tissue shows the decreasing trends. The percentage change over the control were -4.00; -5.65; -8.67; and -11.64 at the 7, 14, 21 and 28 days of exposure respectively. The mean difference were statistically significant at P<0.05 level (Table 1).

Table 1. The amount of total protein in the selected tissues of *Cirrhinus mrigala* exposed to subleathal concentration of nickle chloride.

Organs	Control	Exposure period in days			
		7 days	14days	21 days	28 days
Liver	150.50±3.76	141.35±3.00	136.76±5.25	131.36±6.44	126.48±5.81
%COC		-6.08	-9.13	-12.72	-15.96
		1.8997*	2.1277*	2.5666*	3.4708*
Gill	89.10±1.50	85.30±1.20	83.35±1.45	79.87±1.64	78.00±2.05
%COC		-4.26	-6.45	-10.36	-12.45
		1.9782*	2.7561*	4.1529*	4.3697*
Kidney	124.88±2.87	119.35±4.06	114.21±4.86	110.66±5.20	108.34±3.79
%COC		-4.43	-8.54	-11.39	-13.24
		1.8155*	1.8996*	2.3942*	3.4792*
Muscle	145.18±2.05	139.37±2.10	136.98±3.18	132.59±3.22	128.28±3.37
%COC		-4.00	-5.65	-8.67	-11.64
		1.9797*	2.1699*	3.2982*	4.2847*

The values are mean ± S.E of six individual observations. **(**Values are expressed as mg/g wet wt. of tissue**).***Significance (P<0.05) of student't' test. %COC-percent change over the control

Table 2. Amino acid in the selected tissues of *Cirrhinus mrigala* exposed to subleathal concentration of nickle chloride

Organs	Control	Exposure period in days			
		7 days	14days	21 days	28 days
Liver	8.32±0.27	11.65±0.49	15.45±0.55	19.28±0.70	21.25±0.60
%COC		40.02	85.70	131.73	155.41
		5.9817*	11.6370*	14.6075*	19.6534*
Gill	1.98±0.07	2.39±0.09	2.77±0.11	4.65±0.20	4.67±0.19
%COC		20.64	-40.15	110.15	135.86
		3.5965*	6.0583*	12.6003*	13.2839*
Kidney	1.63±0.04	2.20±0.06	2.78±0.12	3.67±0.11	4.00±0.15
%COC		35.26	-70.56	-125.15	-145.60
		7.9057*	9.0909*	17.4359*	15.2706*
Muscle	4.31±0.17	4.85±0.16	5.02±0.17	5.98±0.18	6.09±0.16
%COC		12.53	16.47	38.75	41.36
		2.3136*	2.9534*	6.7447*	7.6264*

The values are mean ± S.E of six individual observations, values are expressed as mg/g wet wt. of tissue.*Significance (P<0.05) of student't' test. %COC-percent change over the control

Level of amino acids in the liver tissue

The amino acids in the control liver tissue were 8.32 mg/g of wet weight of tissue. During the sublethal concentration nickel treated fingerlings were 11.65; 15.45; 19.28 and 21.25 mg/g of wet weight of tissue. The amount of amino acids in the metal treated liver tissue shows the increasing trends. The increasing percentages were 40.02; 85.70; 131.73; and 155.41 at the 7, 14, 21 and 28 days of exposure respectively. The mean difference were statistically significant at P<0.05 level (Table 2). The amount of amino acids in the control gill tissue were 1.98 mg/g of wet weight of tissue and During sublethal concentration nickel, the amount of amino acids were; 2.39; 2.77; 4.65 and 4.67 mg/g of wet weight of tissue. The amount of amino acids in the nickel treated gill tissue shows the increasing trends. The increasing percentages were 20.64; 40.15; 110.15; and 135.86 at the 7, 14, 21 and 28 days of exposure respectively. The mean difference were statistically significant at P<0.05 level (Table 2).

The amount of amino acids in the control kidney tissue were 1.63 mg/g of wet weight of tissue and at sublethal concentration nickel treated fish, the amino acids content were 2.20; 2.78; 3.67 and 4.00 mg/g of wet weight of tissue. The amount of amino acids in the nickel treated kidney tissue shows the increasing trends. The increasing percentages were

35.26; 70.56; 125.15; and 145.60 at the 7, 14, 21 and 28 days of exposure of nickel respectively. The mean difference were statistically significant at P<0.05 level (Table 2).The amount of amino acids in the control tissue muscle were 4.31 mg/g of wet weight of tissue and sublethal concentration nickel treated fish the amino acids content were 4.85; 5.02; 5.98 and 6.09 mg/g of wet weight of tissue. The amount of amino acids in the metal treated muscle tissue shows the increasing trends. The increasing percentages were 12.53; 16.47; 38.75; and 41.36 at the 7, 14, 21, and 28 days of exposure respectively. The mean difference were statistically significant at P<0.05 level (Table 2).

Level of glycogen in the tissues

The level of glycogen content in the liver tissue control fish was 10.76±0.26 mg/g wet wt. of tissue. It exhibited remarkable changes in their level from a mean control level, when the fishes were exposed to sub-lethal concentration of nickel. The glycogen in liver tissue was found to be decreased 8.73±0.30, 6.96±0.31, 4.44±0.17 and 3.18±0.10 for 7, 14, 21 and 28 days respectively. The percent change over control was -18.87, -37.82, -58.74 and -70.45 respectively (Table 1).The level of glycogen content in gill tissue the control fish was 7.58±0.25 mg/g wet wt. of tissue. It exhibited remarkable changes in their level from a mean control level, when the fishes were exposed to sub-lethal concentration of nickel. The glycogen in gill tissue was found to be decreased 6.46±0.17, 5.37±0.19, 3.77±0.17 and 2.58±0.10 for 7, 14, 21 and 28 days respectively. The percent change over control was -14.77, -29.15, -50.26 and -65.96 respectively (Table 3). The level of glycogen content in the kidney tissue of control fish was 6.42±0.18 mg/g wet wt. of tissue. It exhibited remarkable changes in their level from a mean control level, when the fishes were exposed to sub-lethal concentration of nickel. The glycogen in kidney tissue was found to be decreased 5.67±0.22, 4.60±0.21, 3.32±0.12 and 2.25±0.10 for 7, 14, 21 and 28 days respectively. The percent change over control was -11.68, -28.58, -48.29 and -64.95 respectively (Table 3). The level of glycogen content in the control fish was 8.51±0.31 mg/g wet wt. of tissue. It exhibited remarkable changes in their level from a mean control level, when the fishes were exposed to sub-lethal concentration of nickel. The glycogen in muscle tissue was found to be decreased

Table 3. The level of glycogen in the selected tissue of fresh water fish *Cirrhinus mrigala* exposed with sub-lethal concentration of nickel chloride (Values are expressed as mg/g wet wt. of tissue)

Organs	Control	Exposure period in days			
		7 days	14 days	21 days	28 days
Liver	10.76±0.26	8.73±0.30	6.96±0.31	4.44±0.17	3.18±0.10
% COC		-18.87	-37.82	-58.74	-70.45
		5.1133*	9.3919*	20.3477*	27.2075*
Gill	7.58±0.25	6.46±0.17	5.37±0.19	3.77±0.17	2.58±0.10
% COC		-14.77	-29.15	-50.26	-65.96
		3.7049*	7.0382*	12.6034*	18.5735*
Kidney	6.42±0.18	5.67±0.22	4.60±0.21	3.32±0.12	2.25±0.10
% COC		-11.68	-28.35	-48.29	-64.95
		2.6389*	6.5799*	14.3319*	20.2525*
Muscle	8.51±0.31	7.10±0.23	5.68±0.21	3.68±0.10	2.72±0.12
% COC		-16.57	-33.25	-56.76	-68.04
		3.6528*	7.5588*	14.8296*	17.4188*

The values are mean ± S.E of six individual observations, values are expressed as mg/g wet wt. of tissue.*Significance (P<0.05) of student't' test. %COC-percent change over the control.

Table 4. The level of glucose in the selected tissue of fresh water fish *Cirrhinus mrigala* exposed with sub-lethal concentration of Nickel chloride

Organs	Control	Exposure period in days			
		7 days	14 days	21 days	28 days
Liver	8.45±0.27	9.86±0.42	11.00±0.45	12.53±0.49	14.15±0.57
% COC		16.69	30.18	48.28	67.45
		2.9842*	4.8589*	7.2922*	9.7286*
Gill	3.17±0.11	3.48±0.10	4.01±0.14	4.49±0.16	5.02±0.19
% COC		9.78	26.50	41.64	58.36
		2.0847*	4.7191*	6.7971*	8.4282*
Kidney	6.27±0.16	7.07±0.32	8.05±0.28	9.15±0.45	10.23±0.43
% COC		12.76	28.39	45.93	63.16
		2.2559*	5.5194*	6.0301*	8.6312*
Muscle	7.32±0.20	8.31±0.31	9.48±0.04	10.70±0.42	12.10±0.50
% COC		13.52	29.50	46.17	65.30
		2.7108*	4.7348*	7.2657*	8.8765*

The values are mean ± S.E of six individual observations (Values are expressed as mg/g wet wt. of tissue). * Significance (p<0.05) student 't' test . %COC-percent change over the control.

7.10±0.23, 5.68±0.21, 3.68±0.10 and 2.72±0.12 for 7, 14, 21 and 28 days respectively. The percent changes over control were -16.57, -33.25, -56.76 and -68.04 respectively (Table 3).

Level of glucose in the tissues

The level of glycogen content in the control fish was 8.45±0.27 mg/g wet wt. of tissue. It exhibited remarkable changes in their level from a mean control level, when the fishes were exposed to sub-lethal concentration of nickel. The glucose in liver tissue was found to be increased 9.86±0.42, 11.00±0.45, 12.53±0.49 and 14.15±0.57 for 7, 14, 21 and 28 days respectively. The percent change over control was 16.69, 30.18, 48.28 and 67.45 respectively (Table 4).The level of glycogen content in the control fish was 3.17±0.11 mg/g wet wt. of tissue. It exhibited remarkable changes in their level from a mean control level, when the fishes were exposed to sub-lethal concentration of nickel. The glucose in gill tissue was found to be increased 3.48±0.10, 4.01±0.14, 4.49±0.16 and 5.02±0.19 for 7, 14, 21 and 28 days respectively. The percent changes over control were 9.78, 26.50, 41.64 and 58.36 respectively (Table 4).The level of glycogen content in the control fish was 6.27±0.16 mg/g wet wt. of tissue. It exhibited remarkable changes in their level from a mean control level, when the fishes were exposed to sub-lethal concentration of nickel. The g glucose in kidney tissue was found to be increased 3.48±0.10, 4.01±0.14, 4.49±0.16 and 5.02±0.19 for 7, 14, 21 and 28 days respectively. The percent changes over control were 9.78, 26.50, 41.64 and 58.36 respectively (Table 4).The level of glycogen content in the control fish was 6.27±0.16 mg/g wet wt. of tissue. It exhibited remarkable changes in their level from a mean control level, when the fishes were exposed to sub-lethal concentration of nickel. The g glucose in kidney tissue was found to be increased 7.07±0.32, 8.05±0.28, 9.15±0.45 and 10.23±0.43 for 7, 14, 21 and 28 days respectively. The percent change over control was 12.76, 28.39, 45.93 and 63.16 respectively (Table 4).The level of glycogen content in the control fish was 7.32±0.20 mg/g wet wt. of tissue. It exhibited remarkable changes in their level from a mean control level, when the fishes were exposed to sub-lethal concentration of nickel. The glucose in muscle tissue was found to be increased 8.31±0.31, 9.48±0.04, 10.70±0.42 and 12.10±0.50 for 7, 14, 21 and 28 days respectively. The percent change over control were 13.52, 29.50, 46.17and 65.30 respectively (Table 4).

4.DISCUSSION

Heavy metal poisoning induced physiological and biochemical changes in the liver of an animal can be regarded as an index for the identification of pollutant stress (Rao*et al.,* 1995; Shambhunath Bose *et al.,* 1994). Metals are known to change the physiology of the organism by affecting important aspects of the cellular metabolism such as transport across the membrane, mitochondrial function and lysosomal stability (Reddy *et al.,* 1998). They have a special affinity for sulfhydryl groups, which are essential for the activity of many enzymes

In the present study, the level of protein content in the gill, liver, kidney and muscle tissues were significantly decreased due to the treatment of nickel chloride in fish. Similar observation was also made by Gayatri, (1998). She also reported that the reduction in total protein content after the exposure of heavy metal may be due to reduced protein synthesis. It has been reported that heavy metal treatment would reduce the binding of phenylalanyl and lysiltRNA to ribosome leading to protein depletion.

The reduction in the protein content after exposure to nickel chloride may be due to protein synthesis, which is considered the primary biochemical parameter for early indication of stress. This synthesizing is influenced by a large number of exogenous substances. They reduce the protein synthesizing capacity of the endoplasmic reticulum in the cell. In the present investigation, a sub lethal concentration of nickel-exposed fish, *Cirrhinus mrigala* showed a decrease in the protein content of gill, liver, kidney and muscle at the end of 28 days. This may be due to proteolysis, lack of protein biosynthesis or inhibition of translation. A significant decrease in protein content of gill, brain, intestine, liver, kidney and muscle was observed in nickel-treated fish (Joseph Thatheyus*et al.,* 1992).

The amino acids are the building blocks of protein. There are twenty four naturally occurring amino acids and proteins vary in accordance with the number and sequence of amino acids (Linder, 1985). The experimental animal body synthesized its known protein from the free amino acids that are produced as a result of proteolysis of the dietary proteins. The present study showed that the increased level of amino acid content in the gill, liver kidney and muscle tissues due to the treatment of nickel chloride in fish. Increase in the free amino acid level due to heavy metal stress is mainly a consequence the higher catabolic activity of protein to meet the high energy demand by breaking down the protein into free amino acids. The incorporation of amino acid in the protein may also be suppressed by heavy metal exposure (Dhar and Banerjee, 1983).

The increase in amino acids level has been reported in different tissues of fish when treated with nickel chloride (Seshagiri Rao*et al.,* 1983; Radhiah *et al.,* 1987; Rajamanickam, 1988b; Singh and Srivastava, 1992; MalIa Reddy and Philip, 1991). Jagadeesan (1994) has observed that the mercuric chloride exposed to *Labeorohita*shows an increased level of amino acid content in kidney, gill and liver tissues. He also suggests that the increase in amino acid may be due to proteolysis and it is routed through gluconeogenesis for increasing the energy supply to cope with the heavy metal stress. Sivaramakrishna and Radhakrishnaiah, (1998) have also observed the increased free amino acids content in liver, kidney and muscle on mercury. *Cypnnus carpio* exposed to sublethal concentration of mercury. According to Sahib *et al.,* (1978) an enhanced level of free amino acids were observed due to proteolysis and the derived amino acids were fed into the TCA cycle in the form of ketoacid.

Parthiban and Muniyan, (2011) suggested that the higher levels of the toxicant affect the kidneys while lower levels affect the liver. The decrease in the protein content as observed in the present study in most of the fish tissues may be due to metabolic utilization of the ketoacids to gluconeogenesis

pathway for the synthesis of glucose, or due to directing the free aminoacids for the synthesis of proteins, or for the maintenance of osmo and ionic regulation (Schmidt Nielson, 1975). Desmet and Blust (2007) has reported that proteolysis is intended to increase the role of proteins in the energy production during cadmium stress. The decrease in protein level observed in the present study may be due to their degradation and also to their possible utilization for metabolic purposes. According to Nelson and Cox, (2005) and Sathyanarayana (2005), the physiological status of animal is usually indicated by the metabolic status of proteins. Jruege*et al*. (1968) reported that the fish can get the energy through the catabolism of proteins. Singh *et al.* (1996) observed the decreased protein level resulted in marked elevation of free amino acid content in the fish tissues. The free amino acid pool was increased in the tissues of the fish during exposure to arsenic, while the elevated amino acid 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). It is also attributed to lesser use of amino acids (Seshagiri*et al.,* 1987) and their involvement in the maintenance of an acid-base balance (Moorthy*et al.,* 1984).

Kapila and Ragothaman (1999) have also reported decreased tissue proteins followed by increased level of proteins in the fish, *Boleopthalmusdussumieri*exposed to mercury, copper and cadmium for prolonged periods. The initial drop in the protein content during mercury toxicity may be on account of reduced protein synthesis and an enhanced proteolysis in the various organs of fish (Jagadeesan and Mathivanan, 1999). Reduction in protein content is a cause of decrease in RNA in the tissues of fish or amino acid incorporation and disaggregation of polysomes (Holbrook, 1980). The induced proteolysis by the heavy metal in the respective tissues contributes to the increase in the amino acids (Radhaiah*et al.,* 1987; Ravichandran*et al.,* 1994). Jebakumar*et al.* (1990) observed decrease in protein content of *Lepidocephalichthys thermalis* exposed to sublethal concentrations of fenvalerate. A significant decrease was reported in the protein content of almost all tissues in *Ctenopharyngodon idellus* when exposed to sublethal and lethal concentrations of fenvalerate (Tilak*et al.,* 2001). Decreased protein content in the kidney could be possible due to protein break down leading to increased amino-acid pool of tissue (Radhaiah*et al.,* 1987). The decrease in the protein content of the kidney of *C. batrachus*after exposure to malathion is attributed to the impairment of protein synthesis and/or increase in the rate of their degradation to amino acids which may be fed to the TCA cycle through aminotransferase probably to cope with the stress condition (Aruna*et al.,* 2000). Kasturi and Chandran (1997) observed decreased level of protein content in the tissues of *Mystus gulio* exposed to sublethal concentration of lead. Sen*et al.,* (1992) also observed decrease in protein content of brain and liver of *Channa. Punctatus* exposed to toxic effects of zinc.

Sastry and Dasgupta (1991) reported that decrease in total protein level in liver and muscle of *Channa punctatus*exposed to monocrotophos for 15, 30 and 60 days. Monocrotophos reduced the protein content of fish brain, *Tilapia mossambica* (Joshi and Desai, 1983; Richardson, 1981). A significant decrease was reported in the protein content in almost all tissues in *Channa punctatus*when exposed to sublethal and lethal concentration of fenvalerate (Tilak*et al.,* 2003).Similar findings by Malla Reddy, (1988); Kale *et al.* (2006), proteins are the main source of energy there degradation is to cope with high energy demand augmented during malathion stress in *Cyprinus carpio*.Also the total protein level showed decreased trend in Nile Tilapia (*Oreochromis niloticus*) in response to the treatment of cypermethrin by Korkmaz*et al.* (2009). In *Clarius gariepinus* exposed to cyhalothrin decreased protein observed by Ogueji and Auta, (2007). Decreased in protein level may be attributed to impaired synthetic machinery due to cypermethrin effect David *et al.* (2004). These alterations may be due to utilization of amino acids through transamination, and deamination which might have supplied necessary keto acids to act as precursors for the maintenance of carbohydrate metabolism to meet the energy requirements during zinc stress (Palanisamy*et al.,* 2011). The decrease in protein might be due to their degradation and also to their possible utilization for metabolic purposes (Digvijay Singh and Ajay Singh, 2002). Bradburgy*et al*. (1987) have pointed out that the decreased protein content might also be attributed to the destruction or necrosis of cells and consequent impairment in protein synthesis machinery.

The heavy metals are known to elicit changes in the biochemical constituents of fish there by altering the metabolic pathway (Sarkar and Medda, 1993). Toxic exposure of organisms interferes with organ integrity at the biochemical level and unlimitedly gives rise to affect at the individual levels (Smolders *et al*., 2002). The heavy metal and pesticides are found to influence the biochemical composition of fishes (Shakoori*et al*., 1997; Kaviraj*et al*., 2000).

*Cirrhinusmrigala*treated with nickel chloride shows reduction in the level of glycogen at 7, 14, 21, and 28 days. The glycogenolysis in liver and muscle is the major cause of hyperglycemic in blood. The depleted content of liver and muscle glycogen in *Cirrhinusmrigala*may be due to glycogenolysis. A remarkable depletion in liver glycogen shows an extensive utilization of energy stores under toxic stress. The stopped up utilization is to meet the extra energy demands necessitated by the quick and brisk movements, which the animal shows in its abnormal behavioural response due to the effluent stress. Reduction in the glycogen content of liver and muscle indicates the utilization of carbohydrate as the principle and immediate precursor of energy production under effluent stress. The glycogen content of the muscle has decreased significantly indicating that it might be the immediate energy fuel necessary for muscular activity. In fishes, it is known that the carbohydrate reserve is in general utilized under unfavorable conditions (Metalev *et al*., 1983). Earlier studies have shown that the stress of acute hypoxia and physical disturbance accompanied with rapid depletion of liver

and muscle glycogen when the fishes are exposed to pollutants (Murthy and Priyamada Devi, 1982).

The present study showed the level of glycogen decreased and glucose increased in the liver, gill, kidney and muscle tissue of *Cirrhinus mrigala* exposed to nickel chloride. This results indicates and extensive utilization of energy stores. Perhaps this stopped up titillation is to meet the extra demands of energy necessitated by the quick and brisk movement which shows in the behavioral pattern of the fish during stress. The reduction in stored glycogen content in the respective tissues of fish treated with arsenic metals. The liberated glucose mobilized form livers glycogen are transported to other organs through blood to meet the energy requirements necessitated by the allele rated movements of the fish under the toxicity or arsenic (Metleve*et al*., 1983).

Al-Ekel (1994)studied the toxic effect of lead in *Cyprinu scarpio* and reported that the glycogen content in liver has been decreased, Exposure of carbamate pesticide in *Channapunctatus*caused decrease of glycogen content in liver and muscle. Muscle glycogen was decreased when the fish *Nephrops norvegicus* exposed to copper and manganese. Radhakrishnaiah*et al*., (1992) reported that the muscle and liver glycogen contents were decreased when *Labe orohita* exposed to copper which may be due to the utilization of glycogen through anaerobic glycolysis to meet extra requirement under hypoxia caused by chemical stress and physiological dysfunctions. Bash (2002) reported that the glycogen content was decreased in *Clariusgariepinus*due to exposure to lead. Likewise, glycogen content was decreased when *Anabas testudineus* exposed to lead nitrate. Similar effects have been described by Radhakrishnaiah (1993); Al-Akel*et,al.,* (2000) Showed that the increasing of glucose level is due to high secretion of hormones like catecholamines, glucocorticoids and that lead to increasing of glycolysis and this lead to high glucose level in blood.

Abou EL-Naga *et al*., (2005) observed that glucose recorded high values than control group level; also muscle glycogen content was increased at the same time intervals. This high level was explained through glucogenesis, which mean formation of glucose and glycogen from non-carbohydrate source. Heath (1987) reported that muscle glycogen was increased in the same fish organ without other organs. The reduction in the availability of carbohydrates for energy was partially compensated by increasing the activity of glutamate dehydrogenase and amino oxidase; which are the enzymes of controlling the utilization of amino acids for energy.

Reddy *et al.,* (2008) observed reduction in the glycogen levels in the tissues of fry of common carp, *Cyprinuscarpio*(Linn). This may be due to generalized disturbances in carbohydrate consumption (Simon *et al*, 1983). These alterations may be due to rapid utilization of glycogen to meet the energy demands under stress condition and supply energy demand in the form of glucose which undergoes breakdown to produce energy rich compound ATP through glycolytic pathway as suggested by Omkar*et al*. (1984), Muley*et al*. (2007).

The present study shows the level of glucose in liver, gill, kidney and muscle of *Cirrhinusmrigala* for 7, 10, 21 and 28 days. The increase in the glucose level of the tissue while decrease in tissue glycogen in exposed fish makes it clear that the glycogen reserves are being used to meet the stress caused. This can be attributed to several factors and one of them is the decrease in the specific activity of some enzymes like phosphofructokinase, lactate dehydrogenase and citrate kinas that decrease the capacity of glycolysis (Almeida *et al*., 2001).

A fall in the glycogen level clearly indicates its rapid utilization to meet the enhanced energy demands in fish exposed to toxicant through glycolysis or Hexose Monophosphate pathway. It is assumed that decrease in glycogen content may be due to the inhibition of hormones which contribute to glycogen synthesis. Decrease in liver and muscle glycogen levels is in corroboration with the reports of earlier workers (Bedii and Kenan, 2005; Sastry and Subhadra, 1984). The order of depletion of glycogen in liver, gill, kidney and muscle exposed to sub-lethal dose of the toxicant. This could be because the gills utilize glycogen reserves rapidly to meet the respiratory stress when exposed to the lethal concentration.

Kawade and Khillare, (2012) reported that the reduction of glycogen in all the tissues were found at 24, 48, 72 and 96 hrs. Reddy *et al*. (2008) observed reduction in the glycogen levels in the tissues of fry of common carp, *Cyprinuscarpio*(Linn). The alteration in the tissue glycogen suggests disturbance in the physiological activity. Decrease in the glycogen content may be due to enhanced breakdown of glycogen to glucose through glycogenolysis in the fish tissues to withstand the existing stress condition, mediated by catecolamine and adenocorticalharmones (Gluszak*et al.,* 2007). Colley *et al*. (2001) reported that the glycogen content reduced in the liver tissue of *Oncorhynchus mykiss* exposed to dietary effluent. Ramakrishnan *et al*., (1997) have reported that glycogen content decreased in muscle and liver tissue of *Cyprinus carpio* exposed to distillery effluent. Alkakhera *et al*, (2005) have reported the depletion of glycogen reserved of liver in atrazine treated animal. Patil and Dhande, (2000) reported that a fall in glycogen in the fishes exposed to heavy metal. Black *et al*., (1960) reported that reeducation in glycogen level is thought to be the result of detoxification of act vive moieties of muscular exercise on liver glycogen energy reserves in fish. Dezwann and zendee, (1989) have observed the reduction in tissue glycogen content due to decrease in synthesis or break down as consequence of toxic stress. Samuel and Satry, (1989) reported the level of glycogen decreased in *Channa punctatus* exposed to moncrotophos. Bakthavathsalam and Srinivasa Reddy. (1985) have reported the similar fluctuation in *Anabas testudineus* exposed to disyston.

The decreased glycogen concentration in the liver of common carp could be due to its enhanced utilisation as an immediate source to meet the energy demand under metallic stress.

Depleted glycogen level under chromium stress reported in *Labeo rohita* (Vutkuru, 2005) also supports our research findings. The decreased glycogen content as a result of hypoxic or anoxic condition activates the glycolytic enzymes via catecholamines that initially enhance glycogen concentration. It was also found that cadmium could decrease glycogen reserves in the liver and muscle tissues of *Cyprinus carpio*(Cicik and Engin, 2005).

In the present study, the level of glucose increased in the liver, gill, kidney and muscle tissue to *Cirrhinus mrigala* exposed nickel chloride. This result indicates that the glycogenolysis take place in the liver, where by the reserved glycogen is being slowly converted into glucose. Koundinya (1979) has reported the increase in glucose level in *Saratherodon mossambicus* exposed to pesticide. The present study suggests that glycogen is being a ready source of energy, reduction in glycogen is probably due to more rapid breakdown, when releases glucose into circulatory system to meet the increased energy requirement in a stressful condition. Bakthavathsclam and Srinivasa Reddy (1985) noticed similar increase of glucose in *Anabas testudineus* exposed to disyston. Chowdhury (2004) reported the similar result in *Orecorhychus mykiss* exposed to cadmium. Radhakrishnaiah *et al*., (1992) reported that the level of glucose increased in the blood of *Labe orohita exposed* to copper.

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