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Research article

TOXICITY OF ZINC ON THE BIOCHEMICAL CONTENTS OF CERTAIN TISSUES OF
FRESHWATER FISH, *CHANNA GACHUA* (HAM.)

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ABSTRACT: Industrial effluent containing heavy metals, on entering aquatic environment causes biochemical disturbances in the fish. The present study deals with the toxicity of Zinc as ($ZnSO_4$), as a component of industrial waste and its effect on tissue glycogen and protein level at 24, 48, 72 & 96 hrs respectively. The estimated glycogen and protein concentration in the tissues- gills, liver, kidney, ovary and testis were found to be reduced during the exposure periods.

Key words: *Channa gachua*, zinc, glycogen, protein, fish tissues.

INTRODUCTION

Industrial effluents contributing to aquatic pollution contain a vast array of toxic substances, which include heavy metals. It leads to alteration in physical, chemical and biochemical properties of water bodies as well as that of environment. The aquatic environment has always been subjected to different types of pollutants of industrial, domestic and agricultural wastes. (Mance, 1987; Farkas et al, 2000) and severely affect the aquatic organisms. Beaumont et al, 2000., reported that rapid industrialization in India has resulted into a substantial increase in the liquid waste (effluent), which is traditionally being discharged into open land or in nearby natural water, causing a number of problems like threat to plant and animal lives, surface water logging, ground water contamination and salinizing of land (Ramona et al, 2001). The problems of environmental pollution and its deleterious effects on aquatic biota, including fish is receiving focus during the last few decades. (Jagadeesan et al 2001. Zikic and Stajn, 2001). Industrial discharges containing toxic and hazardous substances, including heavy metals (Ghem et al, 2001; Woodling et al, 2001) contribute tremendously to aquatic ecosystem. Heavy metals are natural trace components of the aquatic environment, but their levels have been increased due to domestic, industrial and agricultural activities. It causes greatest threat to the health of Indian ecosystem (Rani et al, 2001; Desai et al, 2002; Joshi et al, 2002; Saxena, 2002). Level of trace elements in water and fish has been studied by Ikem et al, 2003. Discharge of heavy metals into the aquatic environment can change both aquatic species diversity and ecosystems, due to their toxicity and accumulative behavior. Aquatic organisms including fish accumulate metals many times higher than present in water or sediments (Madhusudan et al 2003; Surec, 2003; Olaifa et al, 2004), thus causing an adverse effect on the aquatic organisms (Ohe et al, 2004). These metals concentrate at different contents in organs of fish body (Khaled, 2004). Accumulation of trace metals in the benthic invertebrates and fish species have been studied by Ali and Fishar, 2005. Effluent of electroplating industry containing heavy metals, is an industry which exerts an impact on aquatic organisms (Kokila et al., 2005. Kaur and Kaur, 2006). Effect of different heavy metals on the aquatic organisms has been studied by many workers. Studies proved that, fish subjected to metals show biochemical and histopathological alterations in different target tissues. Fish population is generally considered to be very sensitive to all kinds of environmental stressors to which they are exposed. Gills, liver and kidney are the primary target organs. Histopathological lesions and increase in size of gills was reported in various fish exposed to heavy metals (Devlin, 2006). Histopathological lesions were observed in the gills and kidney of *Cirrhinus mrigala* (Ham.) fingerlings on exposure to mercury (Gupta and Kumar, 2006).

Necrosis and rupture of gills of *Labeo rohita* on exposure to Copper was reported by Kalele and Dhande, 2006. Effects of sub lethal concentration of zinc on histological changes and bioaccumulation of zinc in kidney of fish *Channa punctatus* (Bloch) have been studied by Gupta and Srivastava, 2006. Zinc induced histological changes like enlarged pyramidal cells of brain and necrosis and degeneration of liver hepatocytes of *Labeo rohita* (Ham.) have been studied by Loganathan et al, 2006. Impact of cadmium on the biochemical constituents of *Oreochromis mossambicus* was studied by Hameed et al, 2006. Athikesavan et al, 2006, observed histopathological changes in the gills liver, intestine and kidney of nickel treated fresh water fish *Hypophthalmichthys molitrix* (Valenciennes). Satyaparameshwar et al, 2006, reported alterations in the protein levels in tissues of freshwater mussel, *Lamillidens marginalis*. Among heavy metals, Zinc is a group IIB heavy metal. Its great ability to protect steel by galvanizing makes it a must for all construction and building materials. It is used with other metals to form alloys such as brass, bronze, etc. Zinc compounds are widely used in pharmaceuticals and cosmetic products, from adhesive plasters to antiseptic creams and to sunscreen lotions. Zinc is an important trace nutrient for humans, plants and animals it is important for body growth and immunity. Even though Zinc is an essential element in low concentrations; it is discharged into the fresh water environment in higher concentrations as an industrial effluent and severely affects the freshwater fauna, especially fishes. It is listed among 25 hazardous substances that pose threat to human health. Excessive intake of zinc causes digestive problems and causes kidney damage. The mode of action of toxicants and cause for death of poisoned aquatic animals is better understood from biochemical investigations besides mortality studies. Since the stress condition caused alteration in metabolic cycles, it is necessary to understand the significance of these variations in the organic contents of tissues. Alterations in biochemical composition have been studied by many workers. Proteins are basic molecules to any living system. In cells they function as enzymes, structural materials, lubricants and carrier molecules. Carbohydrates play a structural role as well acts as a reservoir of chemical energy to be increased or decreased according to organisms need. Animals store homopolysaccharides in tissue as glycogen consisted of glucose and is considered to be the major source of energy and hence all metabolic events depend upon the breakdown of glycogen. Glycogen in the tissue is also considered to be the immediate source of energy to adapt to the environmental conditions. Several workers have reported the impact of various heavy metals on the carbohydrate metabolism of different aquatic organisms (Kharat et al, 2009). Heavy metal- copper is an osmoregulatory toxicant in gibel carp, *Carassius auratus* causing Na loss and glycogen depletion in liver (Boeck, 2010).

The present study deals with the toxicity of Zinc as ($ZnSO_4$) on the glycogen & protein levels of certain tissues like-gills, liver, kidney and gonads (ovary and testis) of freshwater fish, *Channa gachua*, for 24, 48, 72 and 96 hrs.

MATERIALS AND METHODS

Adult and live *Channa gachua* were collected from the local market and brought to laboratory. Only healthy fishes (Length: 12-15 cms, Wt.:50-56 gms) were taken for experiment. Fishes were acclimatized in glass aquaria for 15 day and were fed with fish food (earthworms) and water in the aquaria was replaced by fresh water at every 24 hrs. Stock solution of zinc sulphate was prepared by dissolving appropriate amount $ZnSO_4$ as Zn salt in distilled water. The fish *Channa gachua* were exposed to Zn ($ZnSO_4$) to know the acute toxicity at 24, 48, 72 and 96 hrs. For selection of test concentration, some pilot tests were carried out. The range of concentration was selected between 0 to 100% mortality. In order to maintain the concentration of zinc, the water in the aquaria was changed every 24 hrs during the exposure.

The mortality rate of *Channa gachua* was recorded at 24, 48, 72 and 96 hrs exposure to the heavy metal. The percentage for corrected mortality was calculated using the Abbott's formula (1952).

$$\text{Corrected mortality (\%)} = \frac{\text{Percentage living in control} - \text{percentage living in treatment}}{\text{Percentage living in control}} \times 100$$

The corrected mortality data was analyzed to determine the LC_{50} values for 24, 48, 72 and 96 hrs and were calculated by probit analysis method (Finney, 1971).

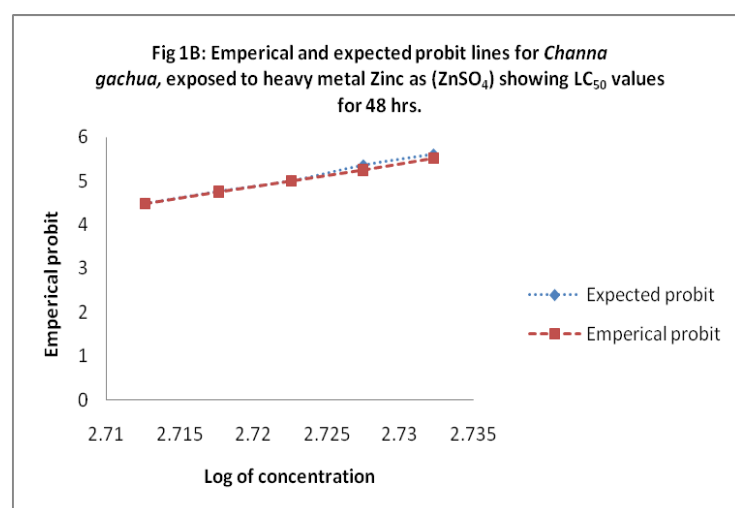
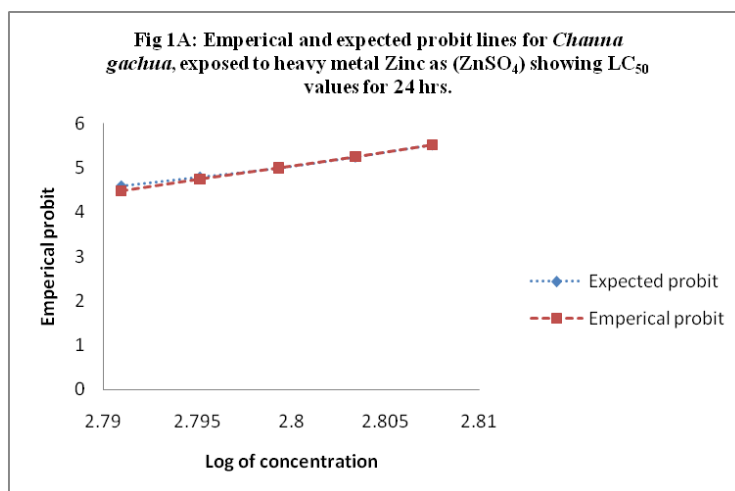
For studying the protein & glycogen levels in the gills, liver, kidney and gonads, fishes were divided in two groups as control and experimental. After exposure, both control and experimental fishes were sacrificed. The fishes were dissected and gills, liver, kidney and gonads were processed for protein estimation by Lowry's method (Lowry et al, 1951). Glycogen estimation was done by Anthrone reagent method of Van der Vier, (1954) as modified by Mahendru and Agrawal, (1982).

RESULTS AND DISCUSSION

On exposure to ZnSO₄, fishes swim abnormally, try to leap (jump) out of water, finally lie on their sides and die.

Zinc Sulphate Toxicity

The mean LC₅₀ values of zinc sulphate toxicity for 24 (fig.1A), 48 (fig. 1B), 72 (fig 1C), 96 (fig 1D) hrs of exposure were estimated at 630 ppm, 528 ppm, 429 ppm and 330 ppm respectively. (Table 1, fig 1). The observed data of present study indicate that the fish *Channa gachua*, survived well from 1 to 621 ppm for 24 hrs, 1 to 520 ppm for 48 hrs, 1 to 419 ppm for 72 hrs, 1 to 331 ppm for 96 hrs of exposure.



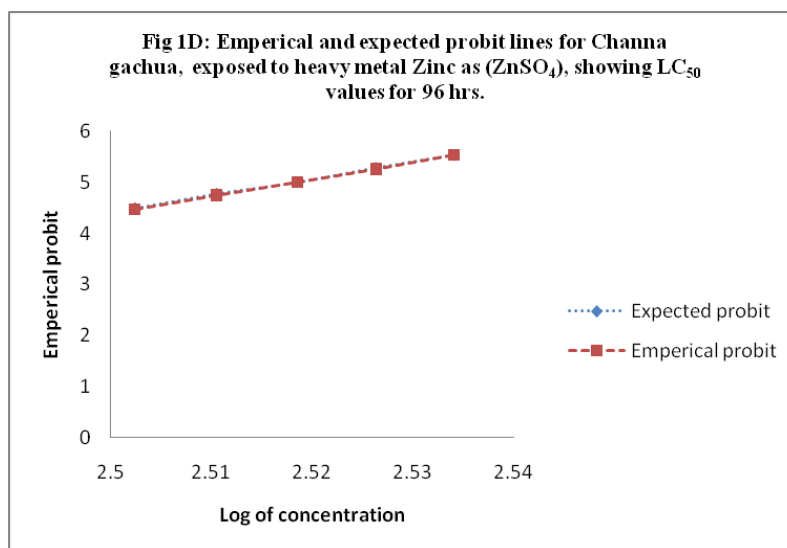
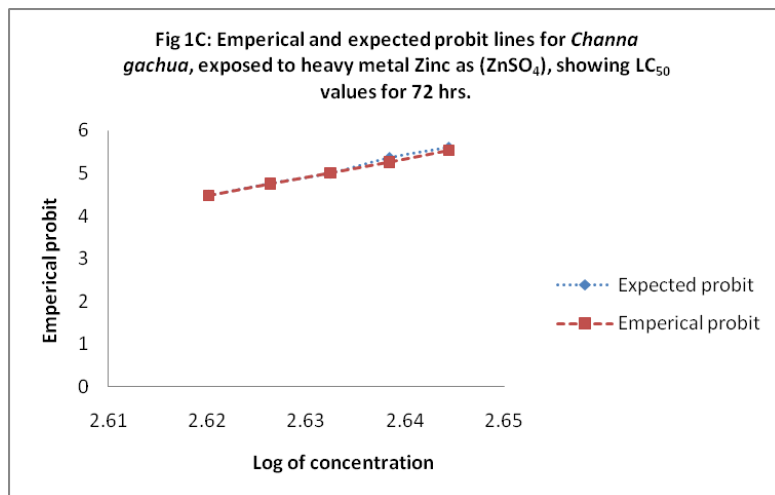


Table 1: LC50 values, calculated and observed, of fresh water fish, *Channa gachua*, exposed to heavy metal zinc as (ZnSO₄) for a period 24, 48, 72 & 96 hrs

Exposure period (hrs.)	LC50 values (ppm)	Regression equation	Chi-square	Variance	Fiducial limits upto 95% confidence	
					M1	M2
24	630	8.9540 X - 20.0639	3.3618	0.000403	2.76	2.8384
48	528	9.5268 X - 20.9329	3.0644	0.000365	2.6850	2.7599
72	429	3.8744 X - 5.1841	0.4102	0.002215	2.5401	2.7244
96	330	12.3394 X - 26.0704	2.3377	0.000213	2.4897	2.5469

Glycogen content

The impact of zinc on glycogen levels of different tissues like gills, liver and kidney of freshwater fish, *Channa gachua* was studied. The level of glycogen from control and exposed tissues of fish are presented in table 2. A significant reduction in glycogen levels in all the tissues were observed as compared to the controlled fishes (Fig 2). A reduction in glycogen values seen in the initial stages altered and this reduction increased at 48, 72 and 96 hrs.

The fishes were exposed to zinc as ($ZnSO_4$) at 630 ppm, 528 ppm, 429 ppm and 330 ppm for a period of 24 hrs, 48 hrs, 72 hrs and 96 hrs respectively. In the gills of control fishes, the glycogen content was 5.24 mg/50 mg of wet weight, which was reduced to 4.55 mg, 3.98 mg, 3.59 mg and 3.20 mg at 24, 48, 72 and 96 hrs respectively. This showed a non-significant reduction ($p < 0.05$) of (-13.16 %) at 24 hrs, a significant reduction ($p < 0.01$) of (-24.04%), (-31.48%) at 48 hrs and 72 hrs respectively and a highly significant reduction ($p < 0.001$) of (-38.93%) at 96 hrs. respectively, as compared to the controlled fishes. In the liver of control fish, glycogen content of 7.39 mg/50 mg of wet weight of tissue was reduced to 5.80 mg, 5.46 mg, 4.78 mg and 3.76 mg at 24, 48, 72 and 96 hrs respectively. Here, a significant reduction ($p < 0.01$) of (-21.51%) was found at 24 hrs with a highly significant reduction ($p < 0.001$) of (-26.11%), (-35.32%) and (-49.12%) at 48, 72 and 96 hrs. In the control fishes, the glycogen content in kidney was 5.86 mg/50 mg of wet weight of tissue. After an exposure of 630 ppm, 528 ppm, 429 ppm and 330 ppm at 24, 48, 72 and 96 hrs the glycogen content was reduced to 4.44 mg, 4.15 mg, 4.04 mg and 3.31 mg at all the four concentrations respectively. A significant reduction ($p < 0.01$) of (-24.23%), (-29.18%) was found at 24 and 48 hrs. and a highly significant reduction ($p < 0.001$) of (-31.06%), (-43.51%) occurred at 72 and 96 hrs respectively. During this acute toxicity test, liver and kidney were the most affected organs followed by gills. Minimum reduction in the tissue protein level occurred at 24 hrs and maximum reduction occurred at 96 hrs indicating that % reduction is related with exposure period.

Protein content

The impact of zinc on protein levels of different tissues like gills, liver, kidney, ovary and testis of freshwater fish, *Channa gachua* was studied. The level of protein from control and exposed tissues of fish are presented in table 3. A significant reduction in protein levels in all the tissues were observed as compared to the controlled fishes (Fig 3). A reduction in protein values seen in the initial stages altered and this reduction increased at 48, 72 and 96 hrs. The fishes were exposed to zinc as ($ZnSO_4$) at 630 ppm, 528 ppm, 429 ppm and 330 ppm for a period of 24 hrs, 48 hrs, 72 hrs and 96 hrs respectively. In the gills of control fishes, the protein content was 17.77 mg/100 mg of wet weight, which was reduced to 13.97 mg, 9.85 mg, 7.77 mg and 4.62 mg at 24, 48, 72 and 96 hrs respectively. This showed a non-significant reduction ($p < 0.05$) of (-21.38 %) at 24 hrs, a significant reduction ($p < 0.01$) of (-44.56%), (-56.27%) at 48, 72 respectively, and a highly significant reduction ($p < 0.001$) of (-74%) at 96 hrs as compared to the controlled fishes. In the liver of control fish, protein content of 18.72 mg/ 100 mg of wet weight of tissue was reduced to 12.86 mg, 10.96 mg, 8.42 mg and 6.20 mg at 24, 48, 72 and 96 hrs respectively. Here, a highly significant reduction ($p < 0.001$) of (-31.30%), (-41.45%), (-55.02%) and (-66.88%) at 24, 48, 72 and 96 hrs, respectively, was observed. In the control fishes, the protein content in kidney was 15.23 mg/100 mg of wet weight of tissue. After an exposure of 630 ppm, 528 ppm, 429 ppm and 330 ppm at 24, 48, 72 and 96 hrs the protein content was reduced to 11.59 mg, 10.16 mg, 7.31 mg and 6.36 mg at all the four concentrations respectively. A significant reduction ($p < 0.01$) of (-23.90%) was found at 24 hrs. and a highly significant reduction ($p < 0.001$) of (-33.29%), (-52%) and (-58.24%) occurred at 48, 72 and 96 hrs respectively. During this acute toxicity test, liver and kidney were the most affected organs followed by gills. Minimum reduction in the tissue protein level occurred at 24 hrs and maximum reduction occurred at 96 hrs indicating that % reduction is related with exposure period.

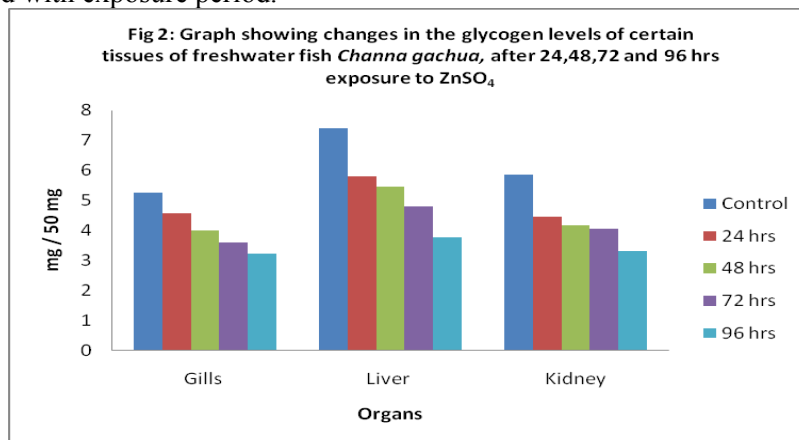


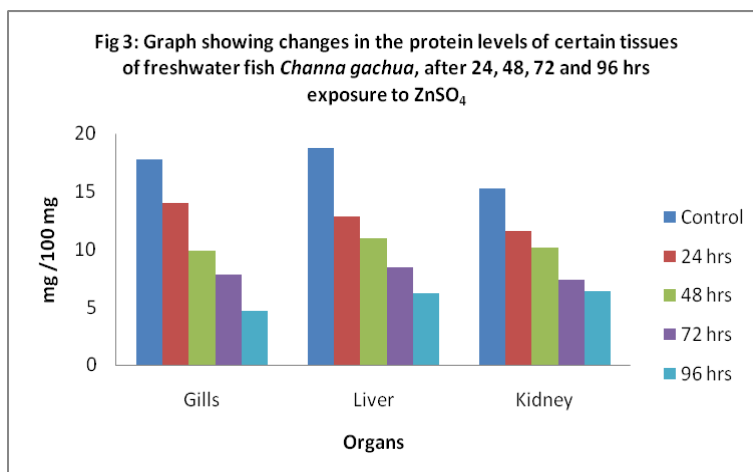
Table No.-2 : Changes in glycogen level in certain tissues of freshwater fish, *Channa gachua* after 24, 48, 72 and 96 hrs exposure to ZnSO₄

Organs	Control	Experimental			
		24 hrs (630 ppm)	48 hrs (528 ppm)	72 hrs (429 ppm)	96 hrs (330 ppm)
Gills	5.24± 0.29	4.55±0.21 (-13.16%) ***	3.98±0.07 (-24.04%) ***	3.59± 0.14 (-31.48%) **	3.20±0.15 (-38.93%)*
Liver	7.39± 0.21	5.80±0.27 (-21.51%) **	5.46± 0.11 (-26.11%)*	4.78± 0.13 (-35.32%)*	3.76±0.16 (-49.12%)*
kidney	5.86± 0.22	4.44± 0.35 (-24.23%) ***	4.15± 0.15 (-29.18%) **	4.04± 0.19 (-31.06%) **	3.31± 0.14 (-43.51%)*

Table No.3: Changes in protein levels of certain tissues of freshwater fish, *Channa gachua* after 24, 48, 72 and 96 hrs exposure to ZnSO₄

Organs	Control	Experimental			
		24 hrs (630 ppm)	48 hrs (528 ppm)	72 hrs (429 ppm)	96 hrs (330 ppm)
Gills	17.77 ± 0.67	13.97 ± 0.78 (-21.38%) ***	9.85 ± 0.59 (-44.56%) **	7.77 ± 0.67 (-56.27%) **	4.62 ± 0.89 (-74%)*
Liver	18.72 ± 0	12.86 ± 0.81 (-31.30%) *	10.96 ± 0.43 (-41.45%) *	8.42 ± 0.59 (-55.02%) *	6.20 ± 0.98 (-66.88%)*
Kidney	15.23 ± 0.22	11.59 ± 0.67 (-23.90%) **	10.16 ± 0.66 (-33.29%)*	7.31 ± 0.39 (-52%) *	6.39 ± 0.67 (-58.24%)*

[Each value indicate the mean (X ± SD) of three estimations] [Values in the parenthesis indicate percent change over control] [*p<0.001, **p<0.01, ***p<0.05] [*Highly significant, **Significant, ***Non-significant]



DISCUSSION

Heavy metals are natural components of earth's crust. Large doses of these heavy metals can enter the water and thus affect the aquatic organisms. Extensive studies have been carried out on effects of heavy metals on aquatic organisms. Bioaccumulation and gill damage due to mercury in tropical fresh water fish was reported by Pelletier et al, 2000., Adami et al, 2002, reported bioaccumulation of cadmium and zinc in certain tissues of *Mytilus galloprovincialis*. Mercury induced histopathological changes in certain tissues of Arctic charr (*Salvelinus alpinus*) was reported by Oliveira et al, 2002, Gallium affects the serum biochemistry and erythrocyte morphology in the Common carp, *Cyprinus carpio* (Yang et al, 2003). Hematological changes induced by mercuric chloride in *Cyprinus carpio* was studied by Masud, 2005., Kulkarni et al, 2005 observed arsenic and iron induced toxic effects on *Tilapia mossambica*. Lead induces hematological and behavioral abnormalities in *Cirrhinus mrigala* (Kumar et al, 2005). Copper induces biochemical alterations in freshwater fishes (Hatai et al, 2005.) In the present study, the toxicity of Zn increases with increasing exposure time, at 24, 48, 72, 96 hrs recorded at 630, 528, 429, 330 ppm respectively. Through **toxicity tests**, mean LC₅₀ value, lethal concentration, variance, etc. have been calculated. Regression line and regression equation have been calculated. An attempt has been made to simplify this interactable process for a biologist to understand, since it alone provides the basis of calculation of LC₅₀, chi-square values for reliability of data, etc.

In the present study, a reduction of glycogen in all the tissues were found at 24, 48, 72 and 96 hrs. Similar results were obtained by many workers. Vutukuru, (2005) studied changes in oxygen consumption rate and biochemical profiles induced by chromium. Shoba et al, 2007, observed biochemical changes in freshwater fish, *Catla catla* on exposure to heavy metal toxicant cadmium chloride. Reddy et al, 2008 observed reduction in the glycogen levels in the tissues of fry of common carp, *Cyprinus carpio* (Linn.). Initially a decrease at 24 hrs may be observed due to Zn stress. But this decrease continued with an increase in exposure period .i.e., 48, 72 and 96 hrs. The alteration in the tissue glycogen, in the present study 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 catecholamine and adenocortical hormones (Gluszak et al, 2007). Depletion of glycogen in the liver and kidney suggests that these tissues do not contribute much anoxia resulting from resulting from pollution stress, since anoxia and hypoxia are known to increase carbohydrate consumption or 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). Similar results were obtained by Muley et al, 2007. Apart from Chromium, other heavy metals and pollutants like pesticides also alter the biochemical composition of different organs. Martin, 2008 reported biochemical alterations induced by mercuric chloride in *Catla catla*. Parvathi et al, 2011 observed alteration in the biochemical composition in different tissues of freshwater fish, *Cyprinus carpio*. Similar alterations in the biochemical composition were observed in the freshwater Snail, *Indoplanorbis exustus* on exposure to heavy metals, mercury and zinc by Patil et al, 2011. The alteration in the tissue protein, in the present study suggests disturbance in the physiological activity. Decrease in the level of tissue protein may be due to excessive proteolysis to overcome the metabolic stress, as deposited protein in the cytoplasm can easily be used to replace the loss of proteins that occur during physiological stress (Patil, A. G, 2011). 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 contamination of heavy metals is a serious threat to aquatic organisms because of their toxicity, long persistence, bioaccumulation and biomagnifications in the food chain. Toxicity of heavy metals is time dependant and on nature of heavy metal. The present study reveals that zinc has a tangible effect on the glycogen and protein level of certain tissues of freshwater fish, *Channa gachua*, which may cause severe to fatal physio-metabolic dysfunction.

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