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Research Article

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# Estimation of glycogen and protein contents of certain tissues of fresh water fish, *Clarias batrachus* (Linn) after exposure of Zinc Sulphate.

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**Keywords**
*Clarias batrachus*,
zinc sulphate,
glycogen,
protein,
fish tissues.

**Abstract**

The present study deals with the effect 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 concentration in the tissues- gills, liver, kidney, ovary and testis were found to be reduced during the exposure periods. The estimated protein concentration in the tissues-gills, liver, kidney, ovary and testis were found to be reduced during the exposure periods. Maximum reduction in protein level in the tissues was found at 96h.

## Introduction

Some of heavy metals are essential to living organisms and they are commonly found in natural waters but high concentrations and accumulation of them may become so toxic. 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. 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. Biomarkers of oxidative stress and heavy metal levels as indicator of environmental pollution in African (*Clarias gariepinus*) from Nigeria Ogun River (Farombi *et al* 2007). 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. 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). (Okacha and Adedeji 2011) overview of cadmium toxicity in fish. Toxicity of zinc on the biochemical contents of certain tissues of fresh water fish *Channa gachua* (Kawade and Khillare 2012). Effect of Cadmium Compound on the Biochemical Parameters of Fresh Water Fish in *Cirrhinus mrigala* (Prabhaker *et al* 2012). Raibole *et al* 2013 Impact of Chelating agent (Zinc) on Heavy metal (Arsenic) caused variations of Hexokinase in different Brain regions of fresh water Teleost. Ecotoxicology of Copper on Freshwater Fishes (Tokhun *et al*, 2014). 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, *Clarias batrachus*, for 24, 48, 72 and 96 hrs.

## Materials and Methods

Adult and live fish *Clarias batrachus* were collected from the farm Patra and Bhadbhada Bhopal brought to the laboratory, cleaned by using 0.1% $KMno_4$ to avoid dermal infection. Only healthy fishes (Length: 12-15cm, Weight: 50-60g) were taken for experiment. Fishes were acclimatized in glass aquaria for 15 days and were fed with fish food (earthworms) and water in the aquaria was replaced by freshwater at every 24h. The fish *Clarias batrachus* 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 *Clarias batrachus* 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).**

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

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

On exposure to $ZnSO_4$, fishes swim abnormally, try to leap (jump) out of water, finally lie on their sides and die.

### Zinc Sulphate Toxicity

The observed data of present study indicate that the fish *Clarias batrachus* survived well from 1 to 621 ppm for 24 hrs, 1 to 520 ppm for 48hrs, 1 to 419 ppm for 72 hrs, 1 to 331 ppm for 96 hrs of exposure. The mean $LC_{50}$ 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).

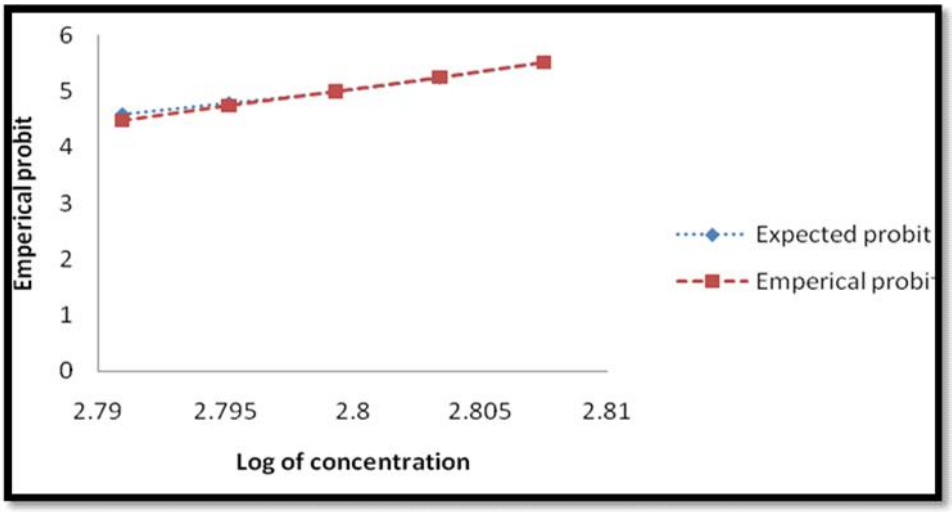


**Fig1A: Emperical and expected probit lines for *Clarias batrachus*, exposed to heavy metal zinc as ($ZnSO_4$) showing $LC_{50}$ values for 24hrs.**

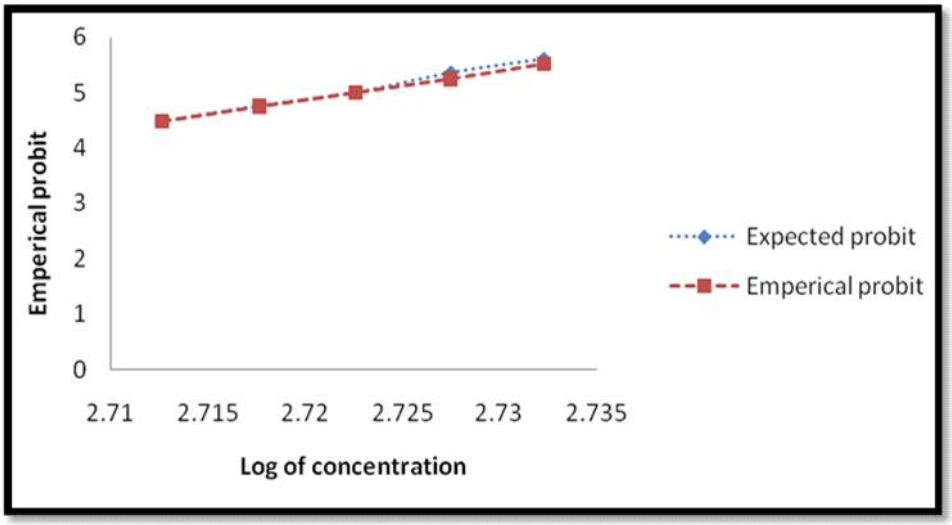


**Fig1B: Emperical and expected probit lines for *Clarias batrachus*, exposed to heavy metal zinc as ($ZnSO_4$) showing $LC_{50}$ values for 48hrs.**

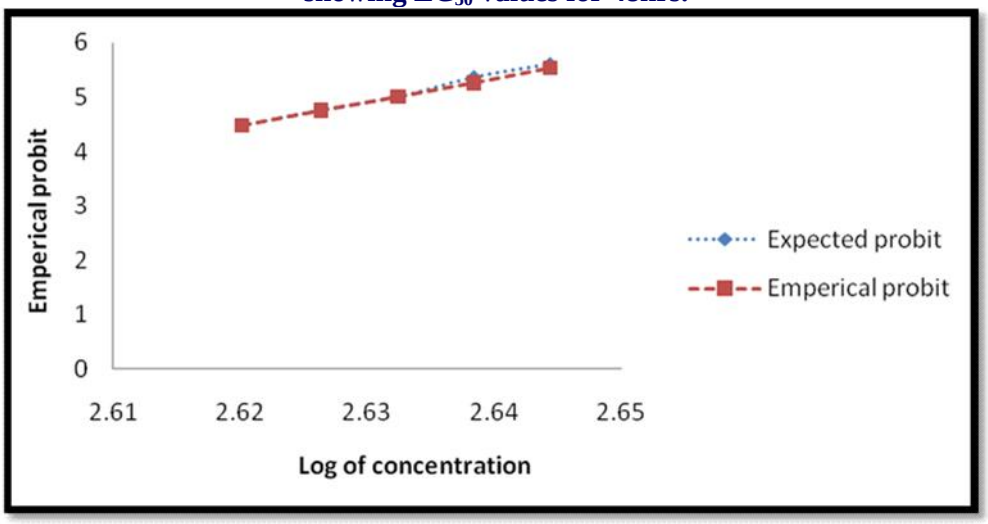


**Fig1C: Emperical and expected probit lines for *Clarias batrachus*, exposed to heavy metal zinc as ($ZnSO_4$) showing $LC_{50}$ values for 72hrs.**

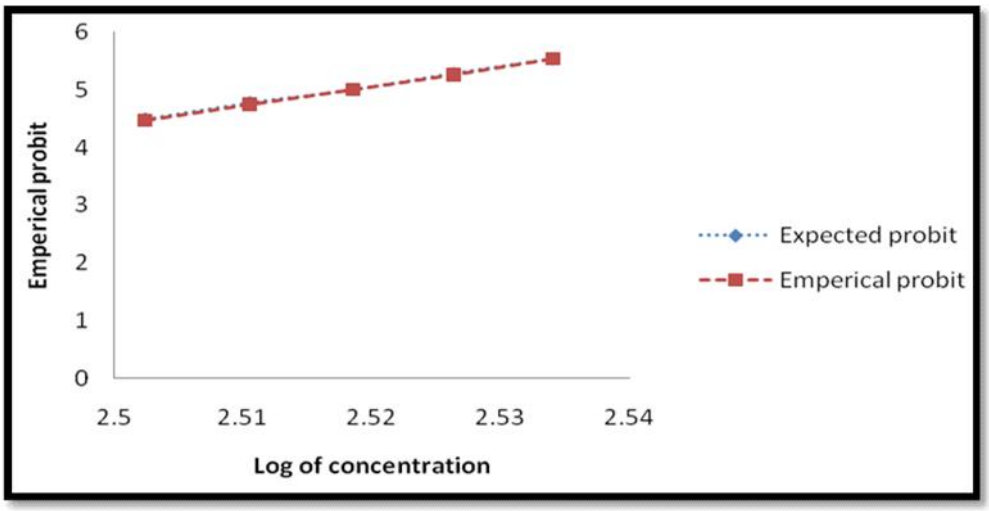


**Fig1D: Emperical and expected probit lines for *Clarias batrachus*, exposed to heavy metal zinc as ($ZnSO_4$) showing $LC_{50}$ values for 96hrs.**

**Table 1: $LC_{50}$ values, calculated and observed, of fresh water fish, *Clarias batrachus*, exposed to heavy metal zinc as ($ZnSO_4$) for a period 24, 48, 72 & 96 hrs**

| Exposure period (h.) | LC50 values upto 95% confidence (ppm) | Regression equation: Y'=(y-bx)+bx | Chi-square M1 | Variance M2 | Fiducial limits 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, *Clarias batrachus* was studied. The level of glycogen from control and exposed tissues of fish are presented in (table 5). 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 24hrs 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, *Clarias batrachus* was studied. The level of protein from control and exposed tissues of fish are presented in (table 4&6). A significant reduction in protein levels in all the tissues were observed as compared to the controlled fishes (Fig 2). 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 17.77mg/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 24hrs. 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 3: LC50 values, calculated and observed, for freshwater fish *Clarias batrachus*, after exposure to $ZnSO_4$ for a period of 24, 48, 72 and 96h.**

| Exposure period (h.) | LC50 values (ppm) | Regression equation: Y'=(y-bx)+bx | Chi-square M1 | Variance M2 | Fiducial limits upto 95% confidence M1 | M2 |
|---|---|---|---|---|---|---|
| 24 | 180 | 9.779977x - 17.003971 | 0.066881 | 0.000346 | 2.213541 | 2.286458 |
| 48 | 88 | 4.742416x - 4.158020 | 4.541213 | 0.0014843 | 1.854395 | 2.005404 |
| 72 | 40 | 2.090739x - 1.768394 | 0.387453 | 0.008330 | 1.397109 | 1.75489 |
| 96 | 20 | 3.445121 + 1.250568x | 0.328928 | 0.166447 | 0.282360 | 1.881639 |

**Table 4: Changes in protein levels in different tissues of *Clarias batrachus* after 24, 48, 72 and 96 h exposure to $ZnSO_4$.**

| Organs | Control | Experimental | | | |
|---|---|---|---|---|---|
| | | 4 h. (180ppm) | 48 h. (88 ppm) | 72 h. (40 ppm) | 96 h. (20 ppm) |
| Gills | 19.20 ± 0 | 12.06 ±0.12 (-37.16%)* | 10.96 ±0.43 (-42.91%)* | 8.74 ± 0.66 (-54.49%)* | 6.68 ± 0.88 (-65.20%)* |
| Liver | 16.34 ± 0.66 | 10 ± 0.81 (-38.77%)** | 9.84 ± 0.59 (-39.77%)** | 7.79 ± 0.38 (-52.32%)* | 5.88 ± 0.67 (-64.01%)* |
| Kidney | 14.60 ± 0.20 | 11.11 ± 0.67 (-23.87%)* | 10.16 ±0.66 (-30.38%)* | 7.94 ± 0.59 (-45.61%)* | 6.20 ± 0.45 (-57.53%)* |
| Ovary | 16.02 ± 0.31 | 10.95 ± 0.36 (-31.64%)* | 10.00 ±0.80 (-35.57%)* | 9.53 ± 0.29 (-40.51%)* | 7.15 ± 0.44 (-55.36%)* |
| Testis | 14.76 ± 0.43 | 12.06 ± 0.39 (-18.29%)*** | 9.85 ± 0.51 (-33.28%)* | 8.42 ± 0.59 (-42.95%)* | 6.68 ± 0.60 (-54.76%)* |

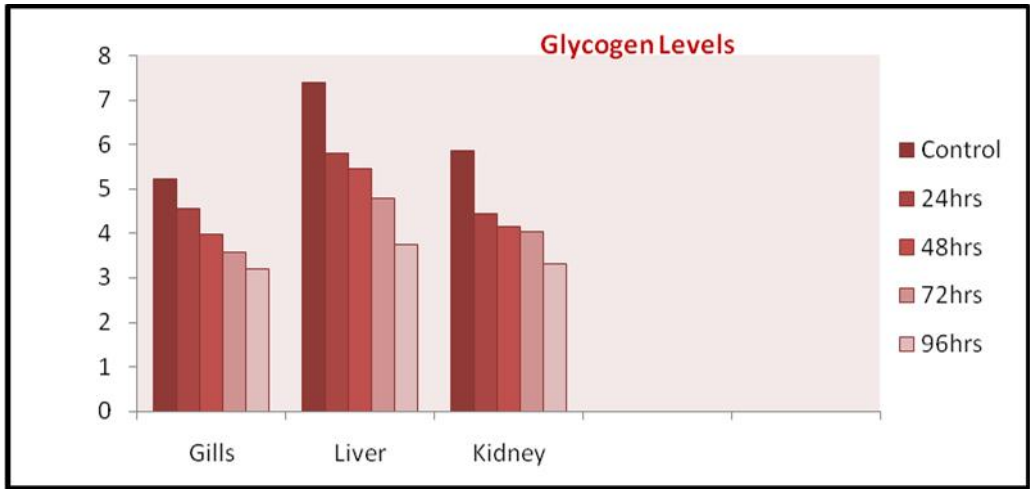


**Fig 1: Graph showing changes in the glycogen levels of certain tissues of freshwater fish *Clarias batrachus*, after 24,48,72, and 96 hrs exposure to $ZnSO_4$**

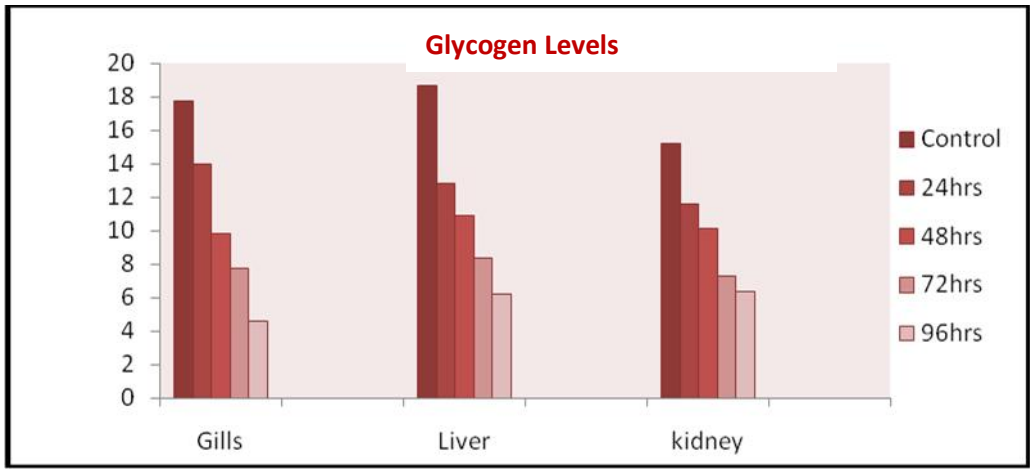


**Fig 2: Graph showing changes in the protein levels of certain tissues of freshwater fish *Clarias batrachus*, after 24,48,72, and 96 hrs exposure to $ZnSo_4$**

**Table No. 5: Changes in glycogen level in certain tissues of freshwater fish, *Clarias batrachus* after 24, 48, 72 and 96 hrs exposure to $ZnSO_4$.**

| Organs | Control | Experimental | | | |
|---|---|---|---|---|---|
| | | 24 hrs (528 ppm) | 48 hrs (630 ppm) | 72hrs (429 ppm) | 96hrs (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. 6: Changes in protein level in certain tissues of freshwater fish, *Clarias batrachus* after 24, 48, 72 and 96 hrs exposure to $ZnSO_4$.**

| Organs | Control | Experimental | | | |
|---|---|---|---|---|---|
| | | 24 hrs (528 ppm) | 48 hrs (630 ppm) | 72hrs (429 ppm) | 96hrs (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 earths' 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. 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 $LC_{50}$ value, lethal concentration, variance, etc. have been calculated. Regression line and regression equation have been calculated. An attempt has been made to simplify this intractable 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, reductions of glycogen in all the tissues were found at 24, 48, 72 and 96 hrs. Similar results were obtained by many workers. 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 catecolamine and adenocortical harmones (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.

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). Jeebu kumar *et al,* 2014 histopathological changes in the gills of *Channa gachua.* 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, *Clarias batrachus*, which may cause severe to fatal physio-metabolic dysfunction.

## Conclusion

After the above discussion it had been concluded that zinc causes deleterious effects on fishes and much alters the biochemical and protein characteristic of certain tissue. In sub lethal concentration it may fatal for an individual organism but it also affects the growth rate and reproduction resulting in disturbance to whole community and tropic levels of food chains, ultimately the ecosystem.

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 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).

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