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Effects of Zinc Sulphateand Biochemical Profiles of the Fish Species rhohu (*Labeo rohita*), katla (*Catla catla*)

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ABSTRACT

Water is contaminated by various industrial aspects, Here the present study determine the high concentration of the metal content of ZnS, which may alters the biochemical element in fishes. Indian major carps (IMCs) – rohu (*Labeo rohita*), katla (*Catla catla*) were used as suitable fish models *invitro.* Total protein, glycogen, cholesterol concentrations were examined in Gills, muscles and liver in both the test fishes. Specimens were also exposed for 96hr toxicity of varying concentration of 0.1, 0.5mg/l. The accumulation of total zinc suphide content when comparing the condition acute, chronic and in the recovery stages of both the fishes were significantly higher in Gills, muscle than liver .The results indicated that the biochemical parameters varied by the exposure of fish in high metal contamination.

1. Introduction

Globally pollution in water bodies is very serious among industrial development in both developing and developed countries. Agriculture and industrial revolution such as textile, mining industry etc., are the main sources of pollutants of both air and water **(Khare and Singh, 2002).** The discharges from the industries contain toxic chemicals and hazardous substances, including heavy metals **(Woodling *et al.*, 2001)** which contribute tremendous pollution to the aquatic ecosystem. Heavy metals are particularly severe in their action due to the tendency of bio-magnification in the food chain. Heavy metals are non-biodegradable and once contaminated into water bodies, they can either be adsorbed on sediment particles or accumulated in aquatic organisms. The structural or biological functions of biomolecules are altered by the influence of heavy metal pollutants in water bodies (**Newman,1998)**.

The fish constitutes a valuable commodity from the stand point of human consumption. So heavy metal contamination of fresh bodies and aquatic biota becomes a serious concern from human health point of view. Heavy metal pollution of aquatic ecosystem poses a serious environmental hazard because of their persistence and toxicity.

Biomarkers for water pollution are considered as early diagnostic tools for biological effect measurement and environmental quality assessment **(Cajaraville *et al.*, 2000)**. They are defined as a change in biological response that differs from molecular to organism level **(Depledge, 1995)**.

2. Materials and Method

Sample:

Samples from various tissues like liver, muscles, gills were taken at 48, 72, 96 hrs for its accumulation and biochemical concentration analysis such as protein, lipid and carbohydrate from each group, the fish such *Labeo rohita, Catla catla* were taken for the study. Biochemical variations were analyzed from various stages such as acute, chronic and recovery stages of all tissues.

Test animals:

To calculate the 96 hr LC 50 values; acute toxicity tests are conducted on two fish species such as viz., *Labeo rohita* (4-9 cm), *Catla catla* (4-9 cm). The test animals are obtained from the local hatcheries. A total of 30 fishes each were introduced into the sub-lethal and median lethal concentrations of zinc sulphide. Biochemical alterations were conducted using standard methods. The fish are acclimatized in the laboratory at 30°C. If in any batch of fish, the mortality exceeded 5% during acclimatization, that batch of fish is rejected.

Zinc sulphide exposure: **96 – hours Toxicity Tests:**

Fourty fish were exposed to four concentrations of zinc sulphide (1.0, 0.5. 0.1 ppm) ten fish each. Other ten fish were kept in an extra tank as control. The experiment was continued for 96 hr. Water in all tanks was replaced daily. Fish were anaesthetized by putting on the dry ice; they were then dissected to obtain muscles, liver and gills. These organs were kept directly with dry ice and then kept in -70°C for the following assays. The 96 hr. LC50 values for both the pesticides were determined using bioassay methods proposed by Doudoroff et al.(1951) and Probit analysis proposed by Finny (1971). From the LC50 value a sub lethal concentration ($1/10^{th}$of LC50) and a median lethal concentration (1/2 of LC50) were calculated for each pesticide and were fixed as the experimental concentrations.

Estimation of Total proteins

Total protein content was estimated by the method Lowry et al.,(1951). 100 mg of tissue was homogenized in 5 ml of cold distilled water. 5 ml of 0% TCA was immediately added to precipitate the protein. Precipitate was collected by centrifugation at 3000 rpm for 15 minutes. The supernatant was discarded. The pellet was repeatedly washed with distilled water to remove the traces of TCA precipitated. Protein was re-

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dissolved in 0.1 N NaOH .0.5mL of the solution was transferred in to a test tube and 4 mL of alkaline copper sulphate (50 ml of 2% Na_2CO_3 and ml, 0.5% $CuSO_4 . 5H_2O$ in 1% sodium potassium tartrate) reagent was added followed by 0.4mL of diluted commercial Folin's reagent (diluted with distilled water in 1:1 ratio). The optical density of blue color developed was read at 750 nm after 30minutes on addition of the reagent using a spectrophotometer. Bovine serum albumin was used as standard. The protein content in the tissue was expressed micrograms/100mg wet weight of the tissue.

Estimation of Total Glycogen

The liver and muscle glycogen was estimated using the anthrone reagent method (Seifter et al., 1950). The sample was homogenized by adding 0.5 ml of 60% KOH and 1 ml of 30% KOH, both prepared in water. The mixture was incubated in a boiling water bath for 30 minutes. 4 ml of ethanol was added to the homogenate and it was kept in a refrigerator for 24 hours and then centrifuged at 3000 rpm. For 20 minutes. The pellet was resuspended in 1 ml of distilled water and from this 0.25 ml was taken and mixed with 1.75 ml of anthrone reagent and kept in boiling water bath for 15 minutes. The colour developed was read at 620 nm spectrophotometrically.

Estimation of Total Cholesterol

This test was carried out by Zlatkis et al.(1953) method. 0.1 ml of serum was taken and 10 ml of ferric chloride reagent was added.

Mixed well and kept for 10 minutes at room temperature. It was then centrifuged for 10 minutes at 3000 rpm. 5 ml of the supernatant was pipetted out into a test tube and 3 ml of concentrated sulphuric acid was added and mixed well. To prepare the standard, 10 ml of working standard was mixed with 0.1 ml of sodium chloride and kept for 10 minutes and centrifuged. 5 ml of the supernatant was taken and to this added 3 ml concentrated sulphuric acid. Both the tubes were kept for 30 minutes at room temperature. To prepare the blank, 5 ml of ferric chloride was mixed with 3 ml of concentrated sulphuric acid. This was also kept for 30 minutes. Read test and standard against the blank at 560 nm.

3. Results and Discussion

Biochemical Studies

The glycogen, total lipid and total protein levels in liver, muscle and gill of control fish and of *Labeo rohita* and *Catla catla* were exposed to the 96hr LC50 concentration of Zinc sulphide for 24 and 96 h were presented (Tables). It is clear from the results that there is an appreciable decline in different biochemical constituents of the fish under zinc sulphide stress.

Total Protein

Total Protein level in tissues common test animals exposed to sub-lethal concentration of zinc sulphide toxicity was found to be decreased moderately in experimental fish than the control (Table-1). The decrease of serum protein was directly proportional to the exposure periods. At the end of acute stage, minimum percent increase in chronic stage and a maximum percent decrease at the recovery were recorded.

Table1 shows the presence of Gill (*Catla catla* A: 8.74mg,C: 22.06mg, R: 16.96mg ;Gill (*Labeo rohita*) A:6.01mg, C:25.958mg , R: 19.84mg ;Liver (*C.Catla*) A: 6.79mg, C: 21.59mg, R: 16.34mg ; Liver (*Labeo rohita*) A: 11.18mg C: 19mg, R: 18.20mg ; Muscles (*C.Catla*) A:5.88mg C: 9.84mg R: 6.68mg; Muscles (*Labeo rohita*) A: A:11.88mg, C: 29.84mg, R:15.68mg

Acute: Muscle (C) 4.87 mg > Gill (R) 5.01mg > Liver (R) 6.68mg > Gill (C) 7.64 mg > Liver(C) 10.19 mg > Muscle (R) 12.86 mg. **Chronic** : Muscles (C) 8.74mg > Liver (C) 20mg >Gill (C) 23.06mg>Gill(R) 24.05m> Liver(R) 24.59 mg > Muscles (R) 28.83 mg. **Recovery**: Muscles (C) 5.59 mg> Muscles (R) 14.68mg > Gill(C) 14.86mg > Liver (R) 15.33mg > Liver (C)16.21mg > Gill (R) 17.85 mg.

The depletion in tissue proteins of *L.rohita, C.catla* may be due to impaired or low rate of protein synthesis under metallic stress. Further, direct and / or indirect utilization of proteins and lipids for energy needs was also reported (Nagai *et al*., 1971). Also, the utilization of proteins in cell repair and organization as causes of their depletion in the tissues cannot be ruled out.

Proteins were decreased significantly with exposure period of zinc. This could be attributed to renal excretion or impaired protein synthesis or due to liver disorder **(**Kori-Siakpere,1995). On the other hand, the observed decrease of protein could also result from the breakdown of protein into amino acids first and possibly into nitrogen and other elementary molecules. Similar reduction in protein has also been reported in *Saccobranchus fossils* following exposure to chlordane (Verma *et al.,* 1979**)**. Vutukuru, (2005) reported that there is an appreciable decline in different biochemical constituents in various tissues in fresh water fish *Labeo rohita* under zinc sulphate stress. Kori-Siakpere *et al,* (2006**)** reported that the plasma protein was lowered when *Heterobranchus bidorsalis and Clarias gariepinus* were exposed to sublethal effect of lead acetate toxicity.

Total Glycogen

Total Glucose level in of the common*L.rohita,C.catla* during exposure to zinc toxicity recorded overall elevations in its activity level over that of the control (Table-2). Gill (*C.catla*) **A**: 8.74mg **C**: 22.06mg **R**: 16.96mg ;Gill(*L.rohita*) **A**:6.01 mg **C**:25.958 mg, **R**: 19.84 mg; Liver(*C.Catla*) **A**: 7.79 mg **C**: 21.59 mg **R**: 16.34 mg Liver(*L. rohita*) **A** 11.18 mg **C**: 19 mg **R**: 18.20 mg; Muscles (*Labeo rohita*), **A**:5.88mg **C**: 9.84 mg **R**: 6.68 mg ; Muscles (*C.Catla*) **A**: 11.88 mg **C**: 29.84mg **R**:15.68mg.

The increase of glycogen is directly proportional to the exposure periods showing a maximum percentage increase in the chronic stage and maximum percent increase in recovery period were recorded in all the tissues taken for the study.

Acute: Muscle (R) 28.12mg > Liver (C) 36.5mg > Gill (R) 36 .16mg > Gill (C) 46.15 mg > Liver (R) 46.5 mg > Muscles (C) 48.2 mg.**Chronic**: Liver (R) 51.46 mg > Muscles 51.51 mg > Gill (C) 52.64mg > Gill (R) 56.63mg > Muscles (R) 60.52 mg > Liver (C) 61.41 mg.**Recovery:**Liver (R) 48.47mg > Gill (C) 50.11 mg > Muscles (C) 50.61mg > Liver (C) 51.18 mg > Gill (R) 52.11 mg > Muscles (R) 53.61mg.

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The decrease in the glycogen concentration of the tissues of *Labeo rohita and C.Catla* can be due to its enhanced utilization as an immediate source met energy demands under metallic stress. It could also be due to the prevalence of hypoxic or anoxic conditions, which normally enhances glycogen utilization **(Dezwaan *et al.*, 1973)**. Enhanced utilization of glycogen and its consequent depletion in tissues may be attributed to hypoxia, since it increases carbohydrate consumption. Under hypoxic conditions, the animal derives its energy from anaerobic breakdown of glucose, which is available to the cells by the increased glycogenolysis **(Chandravathy, 1995)**.

In the present study the metabolic rate of *Labeo rohita and Catla catla* was significantly dropped indicating hypoxia that probably have resulted in a shift to anaerobic glycolytic pathway by increased glycogenolysis. Depleted glycogen levels following chromium stress reported in *Cyprinus carpio* communis **(Ambrose *et al.*, 1994)** under hypoxic conditions also supports this view. A consistent decrease in tissue glycogen reserves observed in this study also suggests impaired glycogenesis. Further, the decline in glycogen might be partly due to its utilization in the formation of glycoproteins and glycolipids, which are essential constituents of various cells and other membranes. Decrease in tissue lipid and proteins were also observed in *Labeo rohita and C. Catla* exposed to zinc sulphate.

Total Cholesterol

Table: 3 reveals the presence of lipids in the tissues taken for the study. the results were Gill (*C.catla*):**A**: 50.48mg, **C**: 63.48mg,**R**: 52.46mg ; Gill (*L. rohita*) **A**: 40.13mg: 83.38mg, R: 53.46mg ; Liver(*C.catla*):**A**: 49.16mg **C**:63.38mg **R**:51.46mg ; Liver(*L. rohita*): **A**: 59.16mg, C:73.38mg, R:54.46mg; Muscles (*L. rohita*) **A**: 50.48mg **C**:53.68mg **R**:51.12mg ;Muscles (*C.catla*): **A**: 59.38mg **C**:65.68mg **R**:49.12mg.

The decrease in tissue lipid might be partly due to their utilization in cell repair and tissue organization with the formation of lipoproteins, which are important cellular constituents of cell membranes, and cell organelles present in cytoplasm **(Harper, 1983**. Decrease in the lipid concentration observed in the present study can also be attributed to its utilization in cell repair and tissue organization.

Acute: Gill (**R**) 38.10 mg > Liver(**R**) 47.16mg >Muscles (**R**) 47.36 mg > Muscles (**C**) 48.46 mg > Gill (**C**) 52.48mg > Liver (**C**) 57.16 mg. **Chronic**: Muscles (**C**) 52.68mg > Liver (**R**) 62.36 mg > Gill (C) 62.42 mg > Muscles (R) 64.65mg > Liver **(C)** 72.38 mg > Gill (**R**) 82.33 mg. **Recovery**: Muscles **(R)** 48.11 mg > Muscles **(C)** 49.11 mg > Liver **(R)** 50.45 mg ; Gill **(R)** 50.45 mg > Gill **(C)** 51.43 mg > Liver **(C)** 53.44 mg.

Cholesterol concentrations in the tissues of metal-exposed fish generally increased when compared to the control value **(Muazzez *et al.*, 2009)**. The report of many investigators **(Yang and Chen, 2003; Sing and Reddy; 1990; Canli, 1995)** support the increase of serum cholesterol concentrations in the metal-exposed fishes. The concentrations of cholesterol is an essential structural components of membranes and the precursor of all steroid hormones, may increase due to liver failure causing the release of cholesterol into the blood. Heavy metals are known to have hazardous effects on cell structure, especially on the membranes. Therefore, increase in cholesterol may be the indications of environmental stress.

4. Conclusion

The present study showed that zinc induced alterations at the biochemical level, more pronounced changes occurring at the end of 96h and thus it is time-dependent. Also, the metal induced alterations in the biochemical content in various tissues may probably affect the enzyme mediated bio defence mechanisms of the fish. Future research should focus on the effect of zinc sulphate toxicity on bio defence mechanisms of *L.rohita* and *Catla catla* at cellular and sub cellular levels. Hence direct contact with the contaminated water shed living shows the elevated biochemical changes and also as marker for heavy metal pollution.

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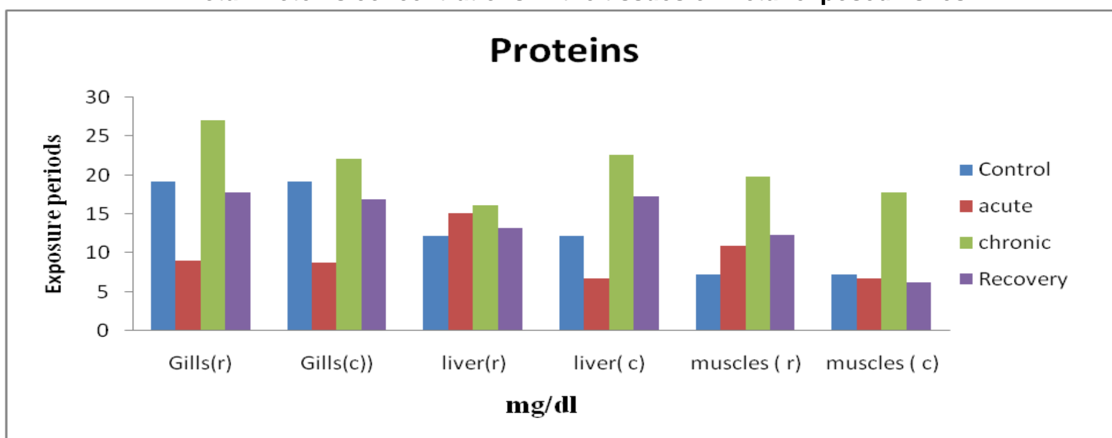
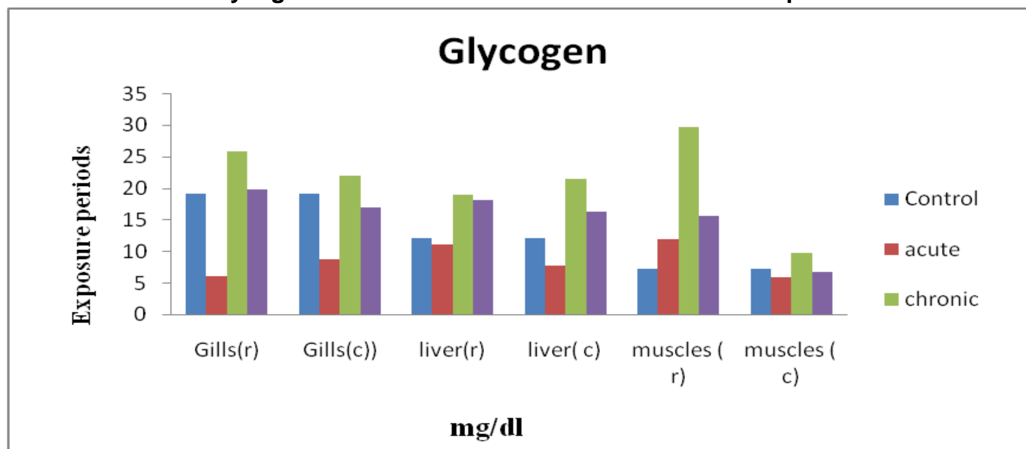
Graphs

1. Total Proteins concentrations in the tissues of metal-exposed fishes



2. Total Glycogen concentrations in the tissues of metal-exposed fishes



3. Total Cholesterol concentrations in the tissues of metal-exposed fishes

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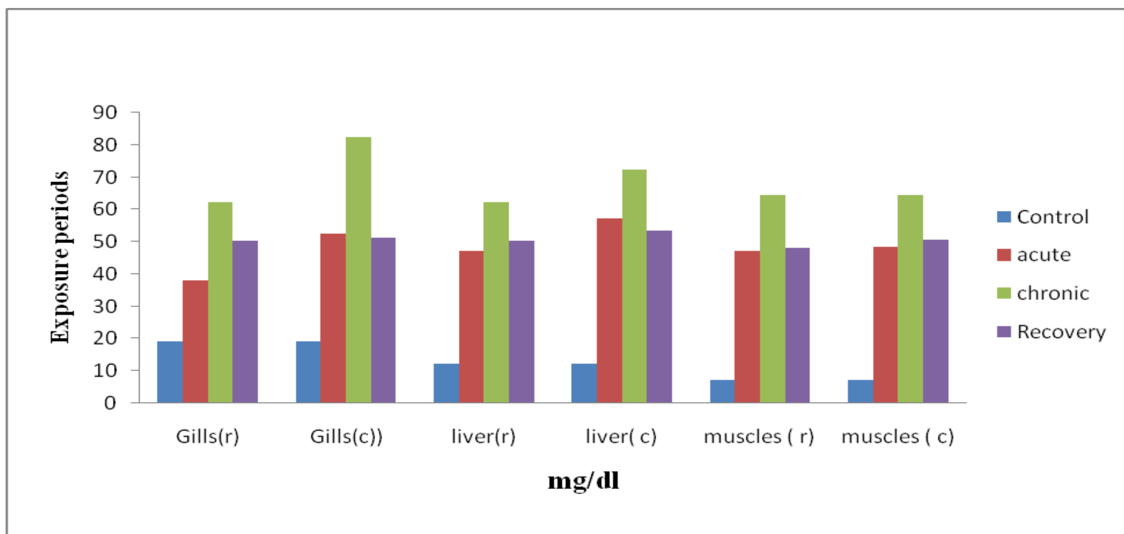
90
80
70
60
50
40
30
20
10
0
Exposure periods
Gills(r)
Gills(c))
liver(r)
liver(c)
muscles (r)
muscles (c)
mg/dl
Control
acute
chronic
Recovery