DOI:10.33451/florafauna.v24i2pp386-392

FLORA AND FAUNA ISSN 2456 - 9364 (Online)

2018 Vol. 24 No. 2 PP 386-392 ISSN 0971 - 6920 (Print)

ALTERATIONS IN THE ORGANIC RESERVE OF ZINC EXPOSED FRESH WATER CATFISH, CLARIAS BATRACHUS

NAVNEET KUNWER SRIVASTAVA [1] AND *SADGURU PRAKASH[2]

[1]Department of Zoology

Mewar University Gangrar,

CHITTORGARH-312901 (RAJASTHAN) INDIA

[2]M.L.K.(P.G.)College,

BALRAMPUR -271201 (U.P.) INDIA

*Corresponding Author

Email : sadguruprakash@gmail.com

Received : 18.09.2018; ***Accepted*** : 14.11.2018

ABSTRACT

Present study was carried out to evaluate the changes in organic reserves of kidney, liver, gill and muscles of *Clarias batrachus* exposed to sublethal concentrations of zinc sulphate in water for a period of 15,30 and 45 days. Four groups of twenty five fish were subjected to 0 (control), 10, 20 and 30 mg/L of zinc sulphate. The sub-chronic exposure of *Clarias batrachus* to sub-lethal concentration of Zinc sulphate showed a decline in glycogen, total proteins, cholesterol and total lipids in all these tissues. The levels of these organic reserves alter simultaneously with an increase in dose and duration of zinc exposure

Figure : 00 References : 17 Tables : 04

KEY WORDS : Biochemical changes, *Clarias batrachus,* Zinc.

Introduction

Contamination with metals from mining and smelting operations poses serious threats to aquatic environments because of its toxicity, persistence, bioaccumulation and biomagnifications in the food chain. Among several elements of the Periodic Table, there are 35 metals which are associated with community and occupational exposure. Out of these, 23 are described as heavy metals. These elements are generally released in small amounts into the environment by processes like weathering of rocks, volcanic eruption *etc.* and their intake/ exposure is necessary in trace amounts for good health. But, presently, there is a steady increase in their concentration in all the habitats due to discharge of these metals into aquatic ecosystems.

Although, small quantities of zinc are required for normal development and metabolism of organism, but if levels exceed the normal physiological requirements, it can act as a toxicant. This results in general enfeeblement, retardation of growth and may bring about metabolic and pathological changes in various organs in fishes [10,13]. However at high concentrations, zinc exerts adverse in fish accruing structural damage, which affects the growth, development and survival of fish. Zinc accumulates in the gills of fish and this indicates a depressive effect on tissue respiration leading to death by hypoxia. Sublethal levels of zinc have been known to adversely affect hatchability, survival, hematological and behavioral parameters of exposed fish [12]. Since fish population is an important component of the food chain, any effect of such pollution would in the due course, have adverse influence on the nutritive value of fish and on man through their consumption[4].

Fish are sentinel organisms in aquatic ecosystems, and they are also considered to be the most readily usable organisms in environmental health assessments. They are preferred in toxicological research because of their well-developed osmoregulatory, endocrine, nervous, and immune systems. In the present study, *Clarias batrachus* was selected due to possessing accessory respiratory organ for air breathing that enable them to adopt in polluted aquatic environmental conditions where other cultivated fish species cannot survive.

Materials and Methods

Healthy specimens of Indian fresh water edible catfish, *Clarias batrachus* were collected from local fish farm at Balrampur, U.P. and were transported in containers

ACKNOWLEDGEMENT : Authors are grateful to Principal M.L.K. (P.G) College, Balrampur for providing necessary laboratory facilities.

TABLE-1:Effect of Zinc sulphate on the Glycogen (mg/g wet tissues) level of certain tissues of *C.batrachus*

Experiment Set.	15 Days	30 Days	45 Days
	Mean±SD (↓ %)	Mean±SD (↓ %)	Mean±SD (↓ %)
Muscles			
Control	0.88±0.14	0.86±0.13	0.85±0.12
10 mg/L	0.72±0.15 (18.18)	0.68±0.18 (20.93)	0.52±0.10** (38.82)
20mg/L	0.68±0.11 (27.27)	0.60±0.14** (30.23)	0.43±0.18* (49.41)
30mg/l	0.60±0.10** (31.81)	0.56±0.12** (34.88)	0.30±.16* (64.70)
Liver			
Control	20.90±1.10	20.92±1.20	20.95±1.11
10 mg/L	18.12±1.12 (13.30)	17.18±1.18 (17.79)	17.02±1.31 (18.75)
20mg/L	16.41±1.15** (21.48)	16.13±1.21** (20.93)	15.28±1.21* (27.06)
30mg/l	16.18±1.41** (22.58)	15.12±1.28* (27.72)	14.10±1.18* (32.69)
Kidney			
Control	5.96±0.32	5.98±0.32	5.94±0.18
10 mg/L	5.38±0.36 (9.73)	5.22±0.18 (13.14)	4.76±0.17 (19.79)
20mg/L	5.12±0.14 (14.09)	4.16±0.14 (12.70)	4.28±0.16** (19.86)
30mg/L	4.56±0.12** (23.48)	4.16±0.18** (30.43)	3.98±0.18* (32.99)
Gills			
Control	4.52±0.38	4.54±0.18	5.56±0.28
10 mg/L	4.18±0.14 (7.52)	4.08±0.18 (10.13)	3.98±0.21 (12.71)
20mg/L	3.90±0.12 (14.83)	3.80±0.21 (16.29)	3.16±0.21 (30.70)
30mg/l	3.38±0.10 (25.22)	3.08±0.18 (32.21)	3.02±0.18 (33.77)

*Significant at P< 0.05 ; ** significant at P< 0.01. ↓ % decreased

to the laboratory .In the laboratory, the fishes were carefully examined for any injury and then kept in 1% solution of $KMnO_4$ for few hours to get rid off dermal infection, finally they were kept in large plastic jar containing 50L of clean tap water and acclimatized for 15 days to the laboratory conditions, during which time they

TABLE-2:Effect of Zinc sulphate on the Total Protein (mg/g wet tissues) level of certain tissues of *C.batrachus*

Experiment Set.	15 Days	30 Days	45 Days
	Mean±SD (↓ %)	Mean±SD (↓ %)	Mean±SD (↓ %)
Muscles			
Control	180.20±1.12	178.45±1.52	179.10±1.32
10 mg/L	172.24±1.02 (4.41)	160.11±1.24 (10.27)	167.15±1.12 (11.69)
20mg/L	168.25±0.98 (6.63)	151.25±1.11** (15.24)	154.23±1.25** (16.11)
30mg/l	1.60.21±1.15 (11.09)	142.35±1.32* (18.78)	145.45±1.41* (20.21)
Liver			
Control	94.81±1.08	95.25±1.12	94.71±1.24
10 mg/L	89.25±1.02 (5.86)	87.25±1.03 (8.39)	85.21±1.06 (10.03)
20mg/L	83.24±1.05 (12.20)	80.21±1.51** (15.79)	77.65±1.12** (18.01)
30mg/l	76.54±1.32** (19.27)	73.25±1.21* (23.09)	70.25±1.02* (25.82)
Kidney			
Control	124.10±1.28	124.20±1.32	124.30±1.45
10 mg/L	120.25±1.14 (3.10)	118.25±1.32 (4.79)	116.32±1.32 (6.41)
20mg/L	117.32±1.21 (5.46)	115.26±1.25** (7.19)	113.25±1.24** (10.92)
30mg/L	111.42±1.32** (10.21)	109.21±1.32** (12.06)	102.31±1.01* (17.69)
Gills			
Control	55.15±1.71	54.95±1.85	55.05±1.84
10 mg/L	52.31±1.01 (5.14)	50.12±1.52 (8.78)	49.25±1.32 (10.53)
20mg/L	49.92±1.11 (9.48)	47.54±1.12** (13.48)	44.27±1.26** (19.58)
30mg/l	45.25±1.25** (17.95)	42.12±1.26* (23.34)	39.85±1.34* (27.61)

were fed on boiled egg yolk and commercial fish food . The fish specimen weighing 50±5 g and measuring 15±5 cm selected for experiments. The fishes were inspected for disease conditions and general fitness. Water was changed every other day. Feeding was stopped 48 hours prior to the toxicity test, to minimize the contamination of metabolic wastes.

The LC_{50} of zinc sulphate was 37.22mg/L at

TABLE-3: Effect of Zinc sulphate on the Lipids(mg/g wet tissues) level of certain tissues of *C.batrachus*

Experiment Set.	15 Days	30 Days	45 Days
	Mean±SD (↓ %)	Mean±SD (↓ %)	Mean±SD (↓ %)
Muscles			
Control	8.45±0.54	8.38±0.42	8.41±0.39
10 mg/L	8.10±0.52 (4.14)	7.00±0.41 (16.46)	6.38±0.25 (24.31)
20mg/L	7.25±0.41 (14.20)	6.35±0.37** (24.22)	5.15±0.34* (38.76)
30mg/l	6.95±0.38 (17.25)	5.82±0.32** (30.54)	4.25±0.34* (49.46)
Liver			
Control	71.12±1.36	71.45±1.54	71.56±1.52
10 mg/L	65.43±1.52 (8.00)	63.17±1.26 (11.58)	59.17±1.11 (17.31)
20mg/L	60.18±1.32 (15.38)	56.14±1.21** (21.42)	50.14±1.25* (29.93)
30mg/l	55.38±1.45** (22.13)	49.18±1.16* (31.41)	44.18±1.32* (38.26)
Kidney			
Control	8.14±0.32	8.12±0.41	8.12±0.38
10 mg/L	7.05±0.33 (13.39)	6.81±0.35 (16.13)	6.95±0.31 (14.40)
20mg/L	6.52±0.41 (19.90)	6.08±0.42** (25.12)	5.35±0.42* (37.25)
30mg/L	5.61±0.33** (30.91)	5.12±0.18* (37.10)	4.10±0.51* (49.50)
Gills			
Control	9.15±0.38	9.18±0.45	9.15±2.25
10 mg/L	8.45±031 (7.65)	8.13±0.11 (11.43)	7.82±0.32 (14.53)
20mg/L	8.00±0.32 (12.50)	7.65±0.18** (16.66)	7.25±0.34* (20.76)
30mg/l	7.34±0.21** (19.78)	6.95±0.18* (24.29)	6.80±0.25* (25.68)

96hours for C*larias batrachus*[15]. The acclimatized fishes were divided into four different groups each containing twenty fishes for the experiments. Group I served as control (0 mg/ L). While groups II,III and IV exposed with sublethal concentrations of zinc sulphate *i.e.*10, 20 and 30 mg/L, respectively for the period of 15, 30 and 45

TABLE-4: Effect of Zinc sulphate on the Cholesterol(mg/g wet tissues) level of certain tissues of *C.batrachus*

Experiment Set.	15 Days	30 Days	45 Days
	Mean±SD (↓ %)	Mean±SD (↓ %)	Mean±SD (↓ %)
Muscles			
Control	6.46±0.12	6.50±0.21	6.48±0.32
10 mg/L	5.15±0.11 (20.27)	5.03±0.17 (22.61)	4.85±0.19 (25.15)
20mg/L	4.75±0.18 (26.47)	4.25±0.18** (34.61)	3.73±0.16* (42.43)
30mg/l	3.90±0.16** (39.62)	3.75±0.22* (42.30)	2.90±0.23* (55.24)
Liver			
Control	21.31±1.31	21.42±1.45	21.35±1.14
10 mg/L	18.85±1.38 (11.54)	15.75±1.24 (26.47)	14.85±1.08 (30.44)
20mg/L	15.14±1.32 (28.95)	12.48±1.31** (41.73)	11.10±1.07* (47.49)
30mg/l	11.38±1.91** (44.53)	10.42±1.05* (51.35)	10.08±9.05* (52.78)
Kidney			
Control	30.28±1.12	29.92±1.41	30.18±1.42
10 mg/L	26.15±1.14 (13.63)	25.11±1.53 (16.07)	23.18±1.41 (21.20)
20mg/L	22.75±1.15 (26.84)	21.14±1.31** (29.34)	17.18±1.28* (39.44)
30mg/L	19.18±1.61** (36.96)	16.64±1.61* (46.05)	14.18±1.51* (53.01)
Gills			
Control	1.40±0.16	1.42±0.18	1.41±0.18
10 mg/L	1.28±0.18 (8.57)	1.20±0.15 (15.49)	1.02±0.17 (27.65)
20mg/L	1.15±0.17 (17.85)	1.06±0.13** (25.35)	0.90±0.18* (36.17)
30mg/l	1.05±0.11** (25.00)	0.98±0.14* (30.98)	0.75±0.17* (46.80)

*Significant at P< 0.05 ; ** significant at P< 0.01;↓ % decreased

days . After 15, 30 and 45 days of exposure, six fishes of each group were sacrificed for sampling. The muscles, liver, kidney and gills tissues in each group were dissected out and homogenized. The homogenate was centrifuged at 3500 rpm for 20 minutes. The supernant was used for the estimation of glycogen, protein, lipid and cholesterol.

Glycogen, Cholesterol and total protein were estimated[7] whereas total lipids were estimated by Colorimetric method[2]. The mean values of the various biochemical parameters for the control and experimental fish were analyzed for statistical significance using the student's t-test. The calculations of statistical significance by the student's t- test at 0.01 and 0.05 levels were made using Microsoft Excel 2003.

Results and Discussion

After 15, 30 and 45 days exposure of *Clarias batrachus* to sublethal concentrations of zinc sulphate.The glycogen, total protein, lipid and cholesterol decline were non-significant at 10mg/L whereas they were significant at 20mg/L and highly significant at 30mg/L in all the tissues. The maximum decline was observed at 30 mg/L after 45 days decreased significantly in all groups of zinc exposed fishes (Table 1-4).

The maximum glycogen level decline in muscles followed by gills, kidney and liver (Table-1).The decline in muscles, liver , kidney and gills glycogen content with increased level of serum glucose[15] in zinc exposed fishes suggests enhance conversion of glycogen into glucose to meet an increased energy requirement under stress conditions. During stress condition fish need more energy to detoxify the toxicant and to overcome the stresses[8]. Glycogen levels were found to be highest in liver, as it is the chief organ of carbohydrate metabolism in animals, followed by muscle. Liver glycogen is concerned with storage and export of hexose units for maintence of blood glucose and that of muscles glycogen is to act as a readily available source of hexose units for glycolysis within the muscle itself. A fall in the glycogen level in zinc exposed fishes clearly indicates its rapid utilization to meet the enhanced energy demands in fish exposed to toxicant through glycolysis or hexose monophosphate pathway. Depletion of glycogen stored in these tissues of zinc exposed fishes may be inhibition of AChE activity or hormones which contribute to glycogen synthesis. Due to inhibition of AChE, concentration of acetylcholine (Ach) increased which stimulated the secretion of catecholamine that brings about glycogenolysis and hyperglycemia through raised level of cyclic AMP [17]. A significant rise in lactic acid concentration in blood in carbaryl exposed fish is an indication of the inhibition of Krebs cycle and / or a favor of anaerobic metabolism over aerobic one due to intoxication of toxicant to meet out the immediate energy demand[11]. Thus significant decline in tissue glycogen to meet an increased energy requirement of zinc treated fish might be due to enhanced secretion of catecholamine under the stress of zinc sulphate.

Protein levels are found to be highest in muscles, as it forms mechanical tissue intended for mobility and do not participate in metabolism. Liver being the center for various metabolisms is also rich in proteins. The maximum and significant decrease in the protein content as observed in the present study in gills followed by liver, muscles and kidney (Table-2) 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. Kidney, liver and gills are the main sites of degradation and detoxification of xenobiotics [9]and the biochemical effects recorded seem to be resulting greater stress on these organs. A decline in hepatic protein in zinc exposed fishes may be due to blocking of protein synthesis or due to proteolysis by increased activity of lysosomal enzymes or they may be mobilized for yolk formation in the developing oocytes[16].The decrease in protein content in these tissues of zinc exposed fishes may be inhibition of protein synthesis by accumulation of free amino acids in the cells of these tissues. Another possible reason may be depletion of protein for its utilization in the conversion of glucose[14] .During stress conditions fish needs more energy to overcome the stress. Since fishes have less amount of carbohydrate so next alternative source of energy is protein to meet increased demand of energy during stress conditions[6].

Lipids are also the storage form of energy like glycogen. The maximum lipid contents decreased in kidney followed by muscles, liver and gill of zinc exposed fishes may be due to inhibition of lipid synthesis as well as increased utilization of stored lipid as a source of energy to conduct regular metabolic activity. Zinc influence the hypothalmic center of fishes resulting the disturbance in steriodogenesis due to reduced enzyme activity. The decrease in lipid level of these tissues may be due to inhibition of various enzymes like lipases, phosphatases and esterases that interfere with fatty acid oxidation and also inhibit the enzyme acetyl Co-A synthetase involved in fatty acid oxidation[16]. Thus decreased lipid content in various tissues may be due to the inhibition of these enzymes as well as breakdown of stored lipid to meet additional energy requirements under stress conditions.

The maximum cholesterol contents significantly decreased in muscles followed by kidney, liver and gills of zinc exposed fishes were due to alternation of steroid biosynthesis. The decline in cholesterol level in these tissues in response to zinc could be due to the fact that excess energy reserves (as cholesterol) are required by organisms to mediate the effect of stress [5].The hypocholesteremia in pesticide treated *Cyprinus carpio* was due to accumulation of water in plasma[3]. A decline in cholesterol content accompanied with increase in free

fatty acids in toxicant treated fishes[1]. They suggest that decreased level of cholesterol in these tissues may be probably due to increased breakdown of cholesterol into free fatty acids which are being fed to TCA cycle to meet out the energy demands during effluent stress.

Thus it can be concluded that Zinc sulphate induced an energy crisis and altered carbohydrate, protein and lipid metabolism by exerting their toxic manifestation in *Clarias batrachus* that are important in their physiological activities, survival, growth and reproduction. The observed biochemical response of present study could be used as suitable biomarkers of metal stress to aquatic organism.

References

1. Begum G, Vijagraghavan S. Carbofuran toxicity on total lipid and free fatty acids in air breathing fish during exposure and cessation of exposure *in vivo. Environ.Monit. Assess*. 2001; **70** :133-239.
2. Fring CS, Dunn RA. Colorimetric method of determination of total lipids based on sulphosphate vanillin reaction. *Am.J.Clin.Phathol*. 1970; **5** : 52-55.
3. Gluth G, Hanke WA. Comparison of physiological changes in carp, *Cyprinus carpio* induced by several pollutants at sublethal concentration S .I. The dependency on exposure time. *Ecotox. Environ.Safe*. 1985; **9** : 179-188.
4. Gupta P, Srivastava N. Effects of sub-lethal concentrations of zinc on histological changes and bioaccumulation of zinc by kidney of fish *Channa punctatus* (Bloch.). *Journal of Environmental Biology* . 2006; **27** (2):211-215.
5. Hadi AA, Shokr AE, Alwan SF. Effects of Aluminum on the Biochemical parameters of Fresh water fish, *Tilapia zillii. Journal of Science and its applications*. 2009; **3** (1) : 33-41.
6. Krishana R, Praskash S. Effect of sugar mill effluent on the serum biochemical parameters of a fresh water catfish, *Heteropneustes fossilis.SCITEC.* 2010; **5** (2) : 81-82.
7. Oser BL. In Hawk's Physiological chemistry, 14th edn. Mcgraw Hill, New York. 1965.
8. Prakash S, Verma AK. Effect of Synthetic detergent on Organic Reserves of fresh water major carp*, Labeo rohita*. *International journal on Agricultural Sciences*. 2018; **9** (1) : 57-70.
9. Rao JK, Choudhary M, Murthy VSR. Histopathology of malathion on liver and kidney of freshwater teleost, *Tilapia mossambicus* (peters).*J.Enveron.Biol*. 1983; **4** : 9-13.
10. Sharma A, Sharma MS. Toxic effect of zinc smelter effluent to some developmental stages of fresh water fish, *Cyprinus carpio* (Linnaeus). *J. Environ. Biol*. 1994; **15** (3) : 109-114.
11. Sharma B, Gopal K. Changes in lactic acid content and activity of lactate dehydrogenase and succinic dehydrogenase in *Clarious batrachus* exposed to carbaryl. *Toxicol.Environ. Chem*. 1995; **47** : 89-95.
12. Shokr M, Sayed EL. Effect of zinc on hematology and biochemistry of *Nile tilapia. JOCPR.* 2015 ; **7** (3): 1943-1950.
13. Singh M, Gaur KK. Effects of mercury, zinc and cadmium on the proteinic value and their accumulation in trunk muscle of *Channa punctatus* (Bloch.) *Advances Bios.* 1997; **16** (11) : 109-114.
14. Srivastava N, Kaushik N, Gupta P. Zinc induced changes in the liver and muscles of fish *Channa punctatus* (Bloch).*J. Ecophysiol Occup Hilth*. 2002; **2** : 197-204
15. Srivastava NK, Prakash S. Morphological, behavioural and haematological alterations in Catfish, *Clarias batrachus* (Linn.) after acute Zinc Toxicity.*International journal on Biological Sciences*. 2018; **9** (1) : 78-84.
16. Srivastava N, Verma H. Alterations in biochemical profile of liver and ovary in zinc exposed fresh water murrel, Channa punctatus (Bloch). *Journal of Environmental Biology.* 2009; **30** (3) : 413-416.
17. Srivastava P, Ruhela SN, Prakash S, Ansari KK. Effect of sodium fluride on organic reserves of some tissues of *Heteropneustes fossilis.The Scientific Temper*. 2012; **2** (1&2) : 45-48.