Effect of heavy metal Cadmium sulphate on the toxicity and biochemical parameters of Reproductive cycle of *Colisa fasciatus*

Sangita Tripathi, Bhuwan Bhaskar Mishra and S.P. Tripathi

P.G. Department of Zoology, M.G.P.G. College, Gorakhpur, 273 001, India
b2mishra123@gmail.com

Abstract: Freshwater fish, *Colisa fasciatus* caught from lake was studied for toxicity and biochemical parameters in reproductive cycle and was compared with control fish. Fish liver was dissected out and analyzed for various biochemical parameters like total protein, total glycogen, nucleic acids (DNA and RNA). Cadmium Sulphate was lethal to 0, 50 and 100% of test fish which produce absolute mortality for four different time intervals. Sub-lethal exposure of Cadmium Sulphate for 30 days caused significant ($p>0.05$) alternation in total protein, total glycogen nucleic acids (DNA and RNA) and body weight in both testicular cycle and ovarian cycle of *C. fasciatus*.
[Sangita Tripathi, Bhuwan Bhaskar Mishra and S.P. Tripathi. **Effect of heavy metal Cadmium sulphate on the toxicity and biochemical parameters of Reproductive cycle of *Colisa fasciatus***. *Researcher*. 2012;4(4):65-68]. (ISSN: 1553-9865). http://www.sciencepub.net. 11

Key words: Heavy metal, cadmium sulphate, toxicity, biochemical parameters, reproductive cycle.

1. Introduction

Industrial growth is an important part of the evolution of human civilization and is vital for the development and property of any country. However, industries also often prove hazardous to aquatic life when their toxic effluents are discharged into water, more so when this is done without any pre-treatment.

Freshwater is vital for various aquatic animals and plants but the quality of freshwater has been abruptly change due to the introduction of different chemicals including pesticides, industrial discharges, metallic pollutants etc. Aquatic systems are exposed to a number of pollutants that are mainly released from effluents discharged from industries, sewage treatment plants and drainage from urban and agricultural areas. These pollutants cause serious damage to aquatic life (Karbassi et al., 2006; Al Masri, 2002).

Amongst the aquatic animals, fishes are chiefly utilized as food materials from the very ancient period. About 95% of the protein of fish flesh is easily digestible and fish flesh provides protein-rich food for rapidly growing world's population. A large part of these elements in the form of the heavy metal ions are toxic or carcinogenic in nature and pose a threat to human health and the environment (Damien et al., 2004; Farombi et al., 2007).

The tremendous increase in the use of heavy metals over the past few decades has inevitably resulted in an increased flux of metallic substances in the aquatic environment (Yang and Rose, 2003).

Cadmium is a nonessential heavy metal; however, it is considered as one of the most toxic water contaminant and could cause toxicity at each level in organisms, from populations and communities to cell elements (Rashed, 2001). The lethal and sub-lethal concentration, cadmium has a cumulative polluting effect and could cause serious disturbances in fish metabolism such as abnormal behavior, locomotor anomalies or anorexia (Woo et al., 1994; Bryan et al., 1995; Cicik and Engin, 2005). Cadmium may also affect the blood cells (Witeska, 1998).

Water born metals may alter the physiological and biochemical parameters in fish blood and tissues. The reaction and survival of aquatic animals depend not only on the biological state of the animals but also on the toxicity, and type and time of exposure to the toxicant (Brungs, 1977). Hematological profile in fish is proved to be a sensitive index for the evaluation of fish metabolism under metallic stress.

Therefore, in the present study various biochemical parameters like total protein, total glycogen and nucleic acids i.e. DNA and RNA were studies against heavy metal Cadmium Sulphate.

2. Material and Methods

The fresh water fish *Colisa fasciatus* (6.5 to 7.0 gm body weight) were collected from the Ramgarh lake district of Gorakhpur, Uttar Pradesh, India. They were acclimated to laboratory conditions (Table-1) for a week prior to the experiments. The collected fishes were maintained in glass aquaria containing 20 litres dechlorinated tap water for seven days under laboratory condition. During acclimatization the fishes were fed on alternate days at the rate of 5 % body weight with prawn powder and oil cake. Although the *Colisa fasciatus* is an air breathing fish, even then aeration facilities were provided for 3 hr. daily to the control and test fishes. No food was provided during the experiment.

The test fishes were also examined carefully for pathological symptoms. They were disinfected with 0.1% solution of potassium permanganate and then transferred one by one by small hand net from the acclimatization tank to the experimental containers. The experiments were conducted in two steps.

Experiment 1: (short term exposure or acute intoxication expeiments)

This group contains 20 fish, divided into two subgroups, one of which kept as control and the other experimental group of Cadmium sulphate for 24,48,72 and 96 hr. The LC_{50} value were obtained by plotting percent survival Vs.log pollutant concentration and finding at what point the straight line obtained crosses 50% of mortality.

Experiment 2: (Long term exposure or sub chronic intoxication and Biochemical exeriments)

In this experiment *Colisa fasciatus* were divided into two lots each of twenty fishes, for experimental and control purposes for long term exposure (30 days). After completion of treatment, fishes were removed from the aquaria and washed with water. The liver and muscle tissues were exacised and total protein (Lowry, 1951), Nuclic acid (DNA and RNA) (Schneider, 1957) and glycogen (Van der Vies, 1954) were measured. Dose- A 10% of 96 hr. LC_{50} concentration was selected for long term experiments. It was 1.86 mg/l for cadmium sulphate.

Data analysis- In the present study, mean and standerd error (Mean± S.E) were calculated for various phase. Student's 't' test (Campbell, 1974) was used to test significant different between experimental and their respective control groups with $p<0.05$ or less.

3. Results

The acute toxicity of cadmium sulphate to *C. fasicatus* in terms of LC_0, LC_{50}, LC_{100} have been observed for four time intervals. The concentration are in mg/l of Cadmium are lethal to 0, 50 and 100% of test fish which produce absolute mortality for four different time intervals (24,48,72,96 hr.) (Table-2). Length of *C. fasicatus* was also affected in control group comperision to experimental group (Table-3).

Exposure to sub-lethal dose of Cadmium sulphate for 30 days (Table-4) exposure periods cause significant reduction in protein,glycogen and nucleic acid (DNA and RNA). All these biochemical parameter progressively dicreases in both testicular cycle and ovarian cycle of *C. fasicatus*.

Table 1: Physico-chemical characteristics of the tap water used for various experiments

Parameters	Mean	Range	
		Min.	Max.
Hardness (mg/l) as $CaCO_3$	130.32	11.20	168.00
Dissolved oxygen (mg/l)	6.62	4.06	10.24
pH	7.28	7.00	7.78
Temperature	21.32	9.60	29.00
Electrical conductivity	296.62	90.00	480.00
Total dissolved solids (TDS)	720	665	786

Table 2: Acute toxicity of Cadmium sulphate of LC_0, LC_{50}, LC_{100} for the different time intervals (24,48,72,96 h)

Duraton	24h	48h	72h	96h
Acute Vllue(mg/l)				
LC_0	3.15mg/l	2.70 mg/l	1.80 mg/l	1.35 mg/l
LC_{50}	24.75 mg/l	22.50 mg/l	20.70 mg/l	18.60 mg/l
LC_{100}	50.40 mg/l	42.30 mg/l	32.40 mg/l	30.60 mg/l

Table 3: Length and body weight of control and heavy metal exposed group

Average wt.(in gm) at start of the experiment on first day		Average wt.(in gm) at the end of the experiment on 30^{th} day	
Control	Experimental	Control	Experimental
6.5±0.95	5.8±0.80 ^{a}	6.7±1.05	4.9±1.02 ^{a}

Data were analysed through student't' test. a significant ($p>0.05$),when treated groups compared with control

Table 4: Biological changes in tissues of *Colisa fasciatus* fish against Cadmium sulphate for 30 days exposure periods

Parameters	Reproductive cycle	Control	Experimental
Total protein (mg/g)	Testicular cycle	94.23±0.38^a mg/g	84.12±0.43 ^{a} mg/g
	Ovarian cycle	93.10±0.46 ^{a} mg/g	83.37±0.62 ^{a} mg/g
Total glycogen (mg/g)	Testicular cycle	23.32±0.46 ^{a} mg/g	12.48±0.32 ^{a} mg/g
	Ovarian cycle	42.04±0.72 ^{a} mg/g	31.34±0.38 ^{a} mg/g
DNA µg/100 mg	Testicular cycle	66.82±0.24 ^{a} µg/100 mg	59.12±0.26 ^{a} µg/100 mg
	Ovarian cycle	55.42±0.12 ^{a} µg/100 mg	50.24±0.18 ^{a} µg/100 mg
RNA µg/100 mg	Testicular cycle	96.62±0.44 ^{a} µg/100 mg	82.18±0.28 ^{a} µg/100 mg
	Ovarian cycle	92.14±0.32 ^{a} µg/100 mg	80.22±0.38 ^{a} µg/100 mg

Values are mean ±SE of ten replicates. Values in percentages are level with control taken as 100%. Data were analysed through student't' test. a significant ($p>0.05$),when treated groups compared with control.

4. Discussion

Water pollutant is commonly defined as any physiologival, chemical or biological changes in water quality which adversely impacts on living organisms in the environment. Fishes from control group is free from such behavioral changes, which indicate that cadmium sulphate is responsible for above altered behavioral response and fish mortality. Shukla et al., (1986) also suggested that the aquatic medium in which the fishes live when contaminated with endosulfan results in change in oxygen contents, pH and also the physiochemical factors of water. Probably these toxicants thus interfere with reproductive mechanism in fishes. In the acute toxicity of cadmium sulphate the fish showed profuse mucus secretion which is aparently a protective device against the tolerence of toxicity. Such observations were also made in the media polluted by heavy metals (Skidmore and Tovell, 1972; Pandey and Shukla, 1980). The present study has demonstrated that the effects of lethal and sublethal concentration of heavy metal for the exposure period of 4 days foe lethal and 30 days for sublethal proved to be toxic to Colisa fasciatus. The effect of the metal also depends on the size of the animal, salinity of water, temperature and the type of the animal.

The levels of various biochemical constituents in mg/g of the wet tissue in control fish and fish exposed to sub-lethal doses of cadmium sulphate are presented in Table 4 and the changes in terms of percent increase or decrease are given in this table. From the data presented, it is clear that at the end of sub-lethal concentration for 30 days, glycogen, nucleic acid and total protein content showed decreases. The increase in the glucose level of the tissue while decrement in tissue glycogen in exposed fish makes it clear that the glycogen reserves are being used to meet the stress caused. Increase in serum glucose level in fish under stress condition are also observed (Bedii and Kenan, 2005; Chowdhury et al., 2004; Almeida et al., 2001). 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 kinase that decrease the capacity of glycolysis (Almeida et al., 2001).

Glycogen levels are found to be highest as it is the chief organ of carbohydrate metabolism in animals. 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 glycogen levels is in corroboration with the reports of earlier workers (Bedii and Kenan, 2005; Dubale and Shah, 1981; Sastry and Subhadra, 1984).

The protein content in fish *Colisa fasciatus* was noted. Many markers suggested that continuous stress due to toxicant poisoning could reduce the protein markedly (Baskaran.1980). Similar types of results were observed in *Cyprinus carpio* and *Catla catla* when exposed to imidane and cadmium respectively (Jana and Bandyopadhya, 1987; Chadravathy and Reddy, 1994) suggested that increased proteolysis and possible utilization of the products and their degradation for metabolic purpose cause depletion of protein. Decreased protein may be due to proteolysis which result in production of free amino acid and utilization of this acid to TCA cycle for energy production in mercury exposed fishes. The quantitative estimation of nucleic acid (DNA and RNA) revels that cadmium produce much significant decline in the nucleic acids contents in the testicular and ovarian cycle.

5. Conclusion

The observations from the present study showed that, these effluents at sub-lethal and lethal concentrations altered the biochemical composition (glycogen, protein and nucleic acid) of the test fish, due to utilization of biochemical energy to counteract the toxic stress caused due to heavy metals present in effluents.

Acknowledgement
Authors are grateful to the authorities of Mahatma Gandhi P.G. College, Gorakhpur for providing facilities.

Corresponding Author:
Dr. Sangita Tripathi
P.G. Department of Zoology
M.G.P.G. College, Gorakhpur
Uttar Pradesh, 273 001, India
Email: b2mishra123@gmail.com

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