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BIOCHEMICAL CHANGES IN THE FISH *CIRRHINUS MRIGALA* AFTER ACUTE AND CHRONIC EXPOSURE OF HEAVY METALS

M. P. Bhilave, D. V. Muley and V. Y. Deshpande*
Department of Zoology, Shivaji University, Kolhapur-416 004, Maharashtra
*Department of Zoology, Sharadchandra Pawar Mahavidyalaya, Lonand, Maharashtra

ABSTRACT

The effect of cadmium and lead on glycogen, protein and lipid contents of gill, liver and muscle of Indian major carp, *Cirrhinus mrigala* have been studied. The fish were exposed to predetermined LC_0 (0.98 and 19.352ppm) and LC_{50} (0.132 and 21.849ppm) concentrations of cadmium chloride and lead acetate, receptively for 96 hours. For chronic exposure $1/10^{th}$ and $1/20^{th}$ concentrations of LC_{50} values have been used for 30 days. Both the heavy metals showed decrease in glycogen, protein and lipid content in all the tissues. The significant alterations showed toxic effects of heavy metals at biochemical levels.

INTRODUCTION

The effect of heavy metals on aquatic organisms is currently attracting widespread attention, particularly in studies related to pollution. With an early use of metals, there was little concern about environmental contamination. However, salts of the metals began to find their way into commercial and industrial applications, then it became evident that metallic salts possess certain biocidal properties. Though, many metals play a vital role in the physiological processes of plants, animals and humans, yet excess concentration of metals is harmful. Pollution implies deleterious effects and is usually assessed in relation to biological system. Butcher (1946) asserted that pollution should be defined in terms of biological conditions rather than physico-chemical standards.

Perusal of literature shows that many workers like Suresh et al. (1991), Thattheyas et al. (1992), Chandravathy & Reddy (1994) and Golovanova et al. (1999) have carried out different biological aspects abroad and in India.

Study of heavy metal toxicity to fish, showing changes in different biochemical constituents such as glycogen, protein and lipids, helps in understanding their correlation with ability to overcome toxic effects and also the changes induced by heavy metal toxicity. Hence, in the present study, attempts have been made to find out the toxic effects of cadmium and lead at acute and chronic exposures on the biochemical changes in gill, liver and muscle of the freshwater fish *Cirrhinus mrigala.*

MATERIALS AND METHODS

The fingerlings of the freshwater fish *Cirrhinus mrigala* measuring about 6 to 7 cm in length were collected from the local fish seed rearing centre and acclimated for 7 days in laboratory. Acute toxicity experiments were conducted for 96 hours using a static bioassay technique and LC_0 and LC_{50} values were recorded. Control group of fish were also run simultaneously. During experimentation no food was provided to the fish. Water in the aquarium was renewed after every 24 hours. For chronic toxicity experiments, $1/10^{th}$ and $1/20^{th}$ concentrations of LC_{50} have been used for 30 days.

Temperature, pH, dissolved oxygen and hardness of the water, used to hold the fish, were determined by using standard methods (APHA 1989).

After acute (96 hours) and chronic exposure (30 days) with test material cadmium chloride and lead acetate, the live fish (five from each group) were sacrificed and the tissues (gill, liver and muscle) were quickly excised and cleaned off extraneous material, weighed and used for biochemical estimations. These pooled samples were used for estimation of the total glycogen by De Zwann & Zandee (1972), total protein by Gornall et al. (1949) and total lipid by Barnes & Black-Stock (1973) methods. The biochemical analysis was repeated for five times and the mean values of five readings were expressed in terms of mg/100 mg wet weight of tissue. The values recorded were compared with control and percent changes were calculated for presentation of the data.

Results of the study were statistically analysed and levels of significance were determined applying Student's 't' test.

RESULTS

Physicochemical parameters of water used for holding the fish during experimentation were, temperature (26.1-28.5°C), pH (7.2-8.4), dissolved oxygen (4.2-5.4 mg/L) and hardness (60-83 mg/L). The observed LC_0 and LC_{50} values for cadmium chloride were 0.098 and 0.132 ppm and those for lead acetate were 19.352 and 21.849 ppm respectively.

Total Glycogen

Control of acute test: The glycogen levels in different organs of the fish *C. mrigala* were in the order of liver > gill > muscle (Table 1).

Experimental: Changes in the total glycogen in gill, liver and muscle of fish *C. mrigala* exposed to cadmium chloride and lead acetate for 96 hours are shown in Table 1. The glycogen content in all the organs decreased considerably upon exposure to 0.098 ppm (LC_0) of cadmium chloride; the percent depletion was more significant in liver and muscle. In LC_{50} group (0.132ppm) there was significant depletion ($P<0.001$) in liver (94.37%) followed by muscle (60.24%) and gill (15.86%).

Due to 19.352 ppm (LC_0) of lead acetate the glycogen content in all the tissues was decreased considerably. The percent depletion was more significant ($P<0.001$) in liver (86.50) followed gill (32.21) and muscle (8.43). In LC_{50} group (21.849 ppm) there was significant decrease ($P<0.001$) in liver (87.50%) followed by gill (32.21%) and muscle (13.25%) ($P<0.05$). In general, there was significant decrease in glycogen content when compared to control after acute exposure.

Control of chronic test: The glycogen levels in different body parts of *C. mrigala* was in the order of liver >gill > muscle (Table 2).

Experimental: Chronic exposure (30 days) of $1/20^{th}$ and $1/10^{th}$ of LC_{50} concentrations of cadmium chloride (0.06 and 0.0113 ppm) and lead acetate (1.092 and 2.184) have induced marked changes in the glycogen content in gill, liver and muscle of the fish *C mrigala.* These changes in glycogen content have been shown in Table 2. Due to 0.006 ppm of cadmium chloride ($1/20^{th}$ of LC_{50}), there was significant ($P < 0.001$) decrease in glycogen content of liver (90.72%) followed by muscle (53.6%) and gill (17.72%). Due to $1/10^{th}$ of LC_{50} (0.013ppm) of cadmium chloride, there was significant ($P<0.001$) decrease in the glycogen content of liver (95.92%) followed by muscle (68.8%) and gill (30.37%).

Due to 1.092 ppm lead of acetate ($1/10^{th}$ of LC_{50}) there was significant ($P<0.05$) decrease in the glycogen content of liver (28.00%) followed by gill (12.65%). In 2.184 ppm ($1/10^{th}$ of LC_{50}) lead acetate, there was significant decrease ($P<0.05$) in the glycogen content of liver (34.00%) followed by gill (31.64%) and muscle (18.4%). In general, there was decrease in the glycogen content in different organs studied as compared to control, but more significant ($P<0.001$) decrease was observed in 0.013 ppm of cadmium chloride than lead acetate.

Total Protein

Control of acute test: The protein content in different body parts of *C. mrigala* was in the order of liver > muscle > gill (Table 3).

Experimental: As compared to control, the protein content in all the organs decreased due to acute exposure of 0.098 ppm of cadmium chloride. The depletion was more significant ($P<0.05$) in liver (13.20%), followed by muscle (17.04%) and gill (14.37%). In LC_{50} (0.132 ppm) group of cadmium chloride, there was considerable decrease ($P<0.05$) in protein level in liver (19.08%), muscle (20.28%) and gill (22.74%). Exposure of *C. mrigala* to 19.352 and 21.849 ppm of lead acetate showed that there was decrease in protein content of liver, followed by muscle and gill. In general, there was decrease in protein levels in LC_0 and LC_{50} groups when compared to control, but this decrease was more in cadmium chloride than in lead acetate exposure.

Control of chronic test: The protein content in different body part of *C. mrigala* was in the order of liver > muscle >gill (Table 4).

Experimental: Chronic exposure (30 days) to 0.06 ppm of cadmium chloride and 1.092 ppm and 2.184 ppm lead acetate showed considerable changes in the protein content in gill, liver and muscle of *C mrigala* (Table 4). Due to 0.006 ppm of cadmium chloride exposure, there was significant ($P < 0.05$) decrease in liver protein (22.87%), gill (16.75%) and muscle (16.30%). Similarly at 0.013 ppm cadmium chloride, significant ($P<0.05$) depletion in liver protein (33.00%), followed by muscle (22.18%) and gill (22.40%) was observed. Due to exposure of 1.092 ppm and 2.184 ppm of lead acetate there was non significant decrease in liver, gill and muscle. In General, there was decrease in protein content in different organs of the fish, but more significant depletion ($P<0.05$) was observed in various organs of fish exposed to 0.013 ppm of cadmium chloride.

Total Lipid

Control of acute test: Lipid content in different body parts of the fish *C. mrigala* was in the order of liver > gill > muscle (Table 5).

Experimental: Changes in the total lipid content in gill, liver and muscle of *C. mrigala* exposed to cadmium chloride and lead acetate for 96 hours are shown in Table 5. Exposure of fish to 0.098 ppm (LC_0) and 0.132 ppm (LC_{50}) of cadmium chloride showed significant ($P<0.05$) decrease in lipid content of liver followed by gill and muscle.

Exposure of fish to 19.352 ppm (LC_0) and 21.849 (LC_{50}) of lead acetate showed significant ($P<0.05$) decrease in lipid content of liver. In general, there was significant ($P<0.05$) decrease in lipid content of various tissues of the fish exposed to LC_0 and LC_{50} groups of cadmium chloride and lead acetate.

Control of acute test: Lipid content in different body parts of the fish *C. mrigala* was in the order of liver > gill > muscle (Table 6).

Table 1: Effect of heavy metals on the glycogen contents in various organs of the *Cirrhinus mrigala* after acute exposure.

Organ	Control	Cadmium chloride		Lead acetate	
		LC_0 (0.098ppm)	LC_{50}(0.132)ppm	LC_0 (19.352ppm)	LC_{50} (21.849)ppm
Gill	0.208 ± 0.014	0.2 ± 0.025 (-3.84)NS	0.175 ± 0.025 (-15.86)*	0.141 ± 0.038 (-32.21)	0.141 ± 0.028 (-32.2)*
Liver	6.666 ± 1.143	1.833 ± 0.072 (-72.50)**	0.375± 0.05 (-94.37)**	0.916 ± 0.288 (-86.50)**	0.833 ± 0.144 (-87.50)**
Muscle	0.030 ± 014	0.062 ± 0.01 (-25.30)**	0.033 ± 0.014 (-60.24)**	0.076 ± 0.154 (-8.43)NS	0.072 ± 0.140 (-13.25)*

Table 2: Effect of heavy metals on the glycogen contents in various organs of the *Cirrhinus mrigala* after chronic exposure.

Organ	Control	Cadmium chloride		Lead acetate	
		LC_0 (0.098ppm)	LC_{50}(0.132)ppm	LC_0 (19.352ppm)	LC_{50} (21.849)ppm
Gill	0.158 ± 0.134	0.13 ± 0.03 (-17.72)	0.11± 0.02 (-30.37)*	0.138 ± 0.15 (-12.65)	0.108 ± 0.014 (-31.64)*
Liver	0.625 ± 0.125	0.058 ± 0.014 (-90.74)**	0.028 ± 0.14 (-95.52)**	0.45 ± 0.05 (-28.00)*	0.40 ± 0.026 (-36.00)**
Muscle	0.125 ± 0025	0.058 ± 0.014 (-53.6)**	0.039 ± 0.001 (-68.8)**	0.115 ± 0.12 (-8)NS	0.102 ± 0.014 (-18.4)*

Table 3: Effect of heavy metals on the protein contents in various organs of the *Cirrhinus mrigala* after acute exposure.

Organ	Control	Cadmium chloride		Lead acetate	
		LC_0 (0.098ppm)	LC_{50}(0.132)ppm	LC_0 (19.352ppm)	LC_{50} (21.849)ppm
Gill	70.332 ± 4.432	60.220 ± 4.194 (-14.37)*	54.332 ± 6.646 (-22.74)*	67.887 ± 2.426 (-3.47)NS	64.126 ± 3.421 (-8.82)NS
Liver	86.665 ± 10.00	75.220± 4.194 (-13.20)**	7.0123 ± 3.333 (-19.08)*	85.456 ± 5.092 (-1.39)NS	80.357 ± 4.409 (-7.27)NS
Muscle	78.887 ± 13.879	65.443± 1.924	62.887± 2.437	72.888 ± 1.972	70.332 ± 1.666
		(-17.04)*	(-20.28)*	(-7.60)NS	(-10.84)*

Table 4: Effect of heavy metals on the protein contents in various organs of the *Cirrhinus mrigala* after chronic exposure.

Organ	Control	Cadmium chloride		Lead acetate	
		LC_0 (0.098ppm)	LC_{50}(0.132)ppm	LC_0 (19.352ppm)	LC_{50} (21.849)ppm
Gill	3.388 ± 0.442	2.987 ± 0.945 (-16.75)*	2.784 ± 0.345 (-22.40)*	3.312 ± 0.297 (-7.69)NS	3.247 ± 0.378 (-9.50)*
Liver	7.444 ± 0.839	5.741 ± 0.961 (-22.87)*	4.987 ± 0.951 (-33.00)*	7.321 ± 0.649 (-1.65)NS	6.874 ± 0.749 (-7.65)NS
Muscle	4.444 ± 9.24	3.986 ± 0.546 (-16.30)*	3.458 ± 0.444 (-22.18)*	4.348 ± 1.776 (-2.16)NS	4.214 ± 1.786 (-5.17)*

Experimental: Chronic exposure (30 days) to 0.06 ppm and 0.013 ppm of cadmium chloride and 1.092 ppm and 2.184 ppm lead acetate have changed the lipid content in different organs in the freshwater fish *C. mrigala* (Table 6). Due to 0.006 ppm and 0.013ppm of cadmium chloride, there was significant ($P < 0.001$) decrease in lipid levels in muscle, liver and gill. Due to 1.092 ppm and 2.184 ppm lead of acetate, there was significant decrease in lipid content in liver, muscle and gill. In general, there was more marked decrease in lipid content in different organs of fish exposed to 0.006 to 0.013 ppm of cadmium chloride than 1.092 ppm and 2.184 ppm of lead acetate.

Table 5: Effect of heavy metals on the lipid contents in various organs of the *Cirrhinus mrigala* after acute exposure.

Organ	Control	Cadmium chloride		Lead acetate	
		LC_0 (0.098ppm)	LC_{50}(0.132)ppm	LC_0 (19.352ppm)	LC_{50} (21.849)ppm
Gill	3.366 ± 0.057	0.314 ± 0.104 (-14.20)*	0.284 ± 0.074 (-22.40)*	0.338 ± 1.246 (-7.65)NS	0.24 ± 1.198 (-11.47)*
Liver	0.983 ± 0.028	0.748 ± 0.014 (-23.90)*	0.614 ± 0.109 (-37.53)**	0.841 ± 0.204 (-14.44)*	0.803 ± 0.102 (-18.31)*
Muscle	0.116 ± 0.11	0.103 ± 0.010 (-11.20)*	0.097 ± 0.009 (-60.37)*	0.109 ± 0.014 (-6.03)NS	0.097 ± 0.007 (-16.37)*

Table 6: Effect of heavy metals on the lipid contents in various organs of the *cirrhinus mrigala* after chronic exposure.

Organ	Control	Cadmium chloride		Lead acetate	
		LC_0 (0.098ppm)	LC_{50}(0.132)ppm	LC_0 (19.352ppm)	LC_{50} (21.849)ppm
Gill	0.40 ± 0.070	0.34 ± 0.17 (-15.00)*	0.066 ± 0.028 (-83.5)(-83.5)**	0.30± 0.14 (-25.00)*	0.133± 0.057 (-66.75)**
Liver	0.45 ± 0.5	0.333 ± 0.057 (-26.00)*	0.10 ± 0.032 (-77.77)**	0.313 ± 0.041 (-30.44)*	0.133 ± 0.057 (-70.44)**
Muscle	0.10 ± 0.18	0.65 ± 0.014 (-35.00)**	0.05 ± 0.016 (-50.00)**	0.073 ± 0.157 (-27.00)*	0.0320.097 (-68.00)**

Values in parantheses are percentage, *P<0.05; **P<0.001; N.S. Non significant; ± S.D. of 5 animals.

DISCUSSION

Toxic effects of pollutants are due to disturbance of the normal physiological functions of the organisms. Changes in biochemical constituents in the tissues due to heavy metal stress has definite pattern. Metabolic activity of an organism reflects utilization of biochemical energy to counteract the toxic stress. In every individual, glycogen, protein and lipid act as sources of energy for carrying out various activities. Due to heavy metal toxicity, the prime source of energy is affected severely and retard various processes in exposed individuals. Umminger (1970) postulated that glycogen is used as the principal and immediate energy precursor in fish *Fundulus heteroclitus* under stress conditions. Dubale & Shash (1981) observed decrease in the quantity of glycogen in liver of freshwater fish *Channa punctatus* exposed to cadmium.

Sastri & Subhadra (1982) studied the chronic toxic effects of cadmium (0.26mg/L) on the carbohydrate metabolism of a teleost fish, *Heteropneustes fossilis* after 15, 30 and 60 days of exposure, and observed depletion in liver and muscle glycogen content. Benagiri & Patil (1986) observed depletion in liver glycogen in *Labeo rohita* exposed to zinc sulphate. It was 8.3% at 65 mg/L and 57% at 105 mg/L.

The results of the study reveal significant effect of heavy metals on carbohydrate metabolism. Glucose content in all the tissues under study was found to decrease continuously throughout the exposure period. Depletion in glucose in the present study may be due to its rapid utilization to meet the demands under toxic manifestation.

Protein analysis is regarded a valid procedure for estimating the degree of damage to an individual. Dubale & Shah (1981) reported decrease in the quantity of protein in freshwater fish *Channa punctatus* exposed to medium containing cadmium (0.05 ppm). Jana et al. (1986) reported de-

creased protein content in fish *Clarias batrachus* exposed to mercury, arsenic and lead. Jana & Sahana (1988) observed decrease in muscle protein after copper and cadmium treatment on the fish *Clarias batrachus* as compared to control. Suresh et al. (1991) recorded depletion in total, soluble and structural protein contents of gill, kidney and intestine of fish *Cyprinus carpio* exposed to sublethal concentration (0.1mg/L) of mercury for 1, 15 and 30 days. Thartheyus et al. (1992) observed decline in protein content in gill, liver and muscle in fish *Cyprinus carpio* exposed to sublethal concentration of nickel (2.5, 5.0, 7.5 and 10.0 mg/L).

In the present study, the protein level decreased in all the organs exposed to lethal and sublethal concentrations of cadmium and lead as compared to control. The drop in the protein content may be on account of reduced protein synthesis during toxicity. According to Farkas (1975) lead treatment would reduce the binding of phenyl alanyl and lysyl tRNA to ribosome leading to protein depletion. The depletion of protein also suggests increased proteolysis and possible utilization of the product of their degradation for metabolic processes. They may be fed into TCA cycle through aminotransferase system to cope up with excess demand of energy during toxic conditions (Chandravathy & Reddy 1946. Syversen (1981) opined that the heavy metals, in general, interfere with protein synthesis.

Lipids act as reversed depot of energy from where the energy is supplied as and when required. Katti & Sathyanesan (1983) reported decreased cholesterol and lipid levels in brain, testis and ovary of *Clarias batrachus* exposed to 5 ppm of lead nitrate for 150 days. Katti & Sathyanesan (1984) also reported decrease in the lipid levels of *Clarius batrachus* when exposed to cadmium. Tulasi et al. (1992) observed that exposure of fish *Anabas testudineus* to a sublethal (5 ppm) concentration of lead nitrate for a period of 30 days during the preparatory phase of its annual reproductive cycle reduced the total lipids. This suggests that lead nitrate affects the lipid metabolism of the fish and, thus, may reduce the fecundity of the fish since lipids are known to play important role in teleost reproduction as an energy source and a precursor of steroids. In the present study, there was decrease in the lipid content of all the tissues of test fish exposed to acute and chronic concentrations of cadmium and lead. There might be decreased activity of glucose-6- phosphate dehydrogenase and NADPH (Singh 1985). Secondly, these results could possibly be correlated to the higher energy demands in order to get the positive survival value under the imposed toxic stress (Murthy et al. 1984).

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