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EFFECT OF HEAVY METAL POLLUTION ON FRESH WATER FISHES *CYPRINUS CARPIO*

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ABSTRACT
This investigation was aimed to estimate the toxic impact of selected heavy metals such as Cadmium, Copper and Zinc on the biochemical changes in certain selected tissues of freshwater fish, *Cyprinus carpio*. The species of fresh water fish *Cyprinus carpio* was collected from local fresh water lake Kurukkupatti lake, Salem, District, Tamil Nadu. The measurement of heavy metls like, cadmimum, copper and zinc was done by absorption spectrophotometry. **Biochemical** changes in total protein, carbohydrate and lipid content was estimated by the method standard procedures. The results of quantitative analysis of cadmium, copper and zinc in different tissues such as gills, liver, kidney and muscle of both control and experimental fish. In the fishes exposed to cadmium, zinc and copper the level of carbohydrate in liver, muscle and kidney was found to be 1.64 ± 0.06, 1.38 ± 0.02, 1.33 ± 1.01%, 1.55 ± 0.09, 1012 ± 0.03 and0.68 ± 0.08% and was 1.22 ± 0.10, 1.18 ± 0.03 and1.05 ± 0.02% respectively. The total protein content of these tissues from the animal treated with cadmium, copper and zinc was in the order of 16.21 ± 0.20, 6.45 ± 0.04 and 3.33 ± 0.08%, 32.00 ± 0.00, 12.40 ± 0.05 and 6.60 ± 0.04% and 21.02 ± 0.08% in liver, 11.44 ± 0.06 in kidney and 9.62 ± 0.03% in muscle respectively.

KEYWORDS: Heavy metals, cadmium, copper, zinc *Cyprinus carpi*.

INTRODUCTION

Pollutions are chemical, physical or biological agents that exert undesirable effects on human health, environment or belongings. The air we breathe, the water we drink the food we consume and the land we live on are all contaminated with one form of pollutants (Pandian, 1987, and Mathivanan, 1988). In recent times, due to the population explosion, urbanization and industrialization, more and more freshwater bodies are becoming increasingly polluted with sewage water, as a consequence, there us a heavy demand for fishes. The impact of pollutants upon aquatic and terrestrial animals can be evaluated in terms of toxicity, stimulation, inhibition, destruction and alteration. (Mahalakshmi, 1997). Different kind of toxic pollution comes from heavy metals, such as cadmium, chromium, copper, iron, lead, zinc, magnesium and mercury. These metals particulates enter the aquatic medium through effluents discharged from tanneries, textiles, metal finishing, mining, dyeing and printing industries, ceramic and pharmaceutical industries etc. (Azmat and Talat, 2006). The toxicity of heavy metals has been studied extensively among fishes. These heavy metals act either synergistically or anatagonistically on the aquatic animals and in some cases may cause a disease in biotic diversity (Mathis and Cummings, 1973; Mathivanan, 1988 and Mathivanan *et al*., 2006).

The amount of heavy metal may be increased in the aquatic medium owing to environmental pollution. The aquatic animals, then tend to accumulate heavy metal in their body and us a consequence, several kinds of physiological disorders including many syndromes, could occur in human beings. Hence there is an immediate need to study the impact of heavy metal accumulation and its relative biochemical changes in the edible fresh water fish, namely a common carp, *Cyprinus carpio*. This is the fastest growing common carp fish in India, it is considered excellent for eating, especially when the fishes are of moderate size. The head is very fatty and those habituated to fish head preparation will appreciate its flavor. Hence, people prefer this fish for culturing freshwater ponds and lakes. It is one of the most productive food fishes in India. Since heavy metals find their entry into the environment around human habitat by various sources, it was felt to have a study about the effect of these metals on the major water inhabitants; the fish which also serve as an important source of food for mankind. An attempt has, therefore been made in the present investigation to study the toxic impact of selected heavy metals such as Cadmium, Copper and Zinc on the biochemical changes in certain selected tissues of freshwater fish, *Cyprinus carpio.*

MATERIALS AND METHODS

The species of fresh water fish *Cyprinus carpio* was collected from local fresh water lake Kurukkupatti lake, Salem, District, Tamil Nadu and were acclimatized to the laboratory conditions by keeping them in rectangular cement tanks. The fishes were fed regularly with boiled eggs on alternate days and the water was renewed daily. The fish tanks were kept free from fungal infection by washing with $KMNO_4$ solution. The fishes were acclimated for 20 days in fresh water medium.

Test fishes were critically screened for the signs of disease, stress, physical damage and mortality. The injured, severely diseased, abnormal and mortality. The injured, severely diseased, abnormal and dead individuals were discarded. The fishes measuring 9 to 11 mm in length and 8 to 10 g in weight were employed for experimental studies. Feeding was discontinued 2 days prior to the commencement of the experiments to reduce the additive effects of animal excreta in the test trough (Arora *et al*., 1972).

Acclimatization to the laboratory conditions

The animals were reared in a large cement tank of 60 x 30cm with 30 cm depth and were disinfected twice with a formalin bath (25 ml of 40% formalin in 100 litres of water) for 30 minutes to prevents infection (Larsson *et al*., 1981 and Mathivanan 1988). After acclimatization, the fishes were transferes to the test chambers. The mean length and wet weight of the fishes were 80+- 10 mm and 45+-10gms respectively.

Metal accumulation

The fishes were exposed to 25ppm concentrations of Cadmium sulphate (CdSO4.8H2O – 78.105 mgs/1), Copper sulphate (CuSO4. 5H2O- 98.175 mgs/1) Zinc sulphate (ZnSO4. 7H2O – 96.134 mgs/1) separately for 120 hours. They were then dissected for obtaining various tissues such as gills, liver, kidney and muscles. The wet tissues each weighed (500 mgs) separately and digested in 5.0 ml of concentrated nitric acid at 60 c for 6 to 10 hours until to obtain a clear solution. The solution was diluted with 25 ml of deionized water and this solution was for the measurement of cadmimum, copper and zinc by Absorption Spectrophotometry (Ahsanullah *et al*., 1981).

Biochemical changes

Each tissue/organ was dried at 60˚C for 24 hours, finely powdered and used for the estimation of total protein, carbohydrate and lipid (Selvakumar, 1981).

Estimation of total carbohydrate

The total carbohydrate content was estimated by the method of Dubois *et al*., (1956).

Estimation of total protein

The total protein content was estimated by Biuret method as modified by Raymont *et al*., (1964).

Estimation of total lipid

The total lipid content was estimated by the method of Folch *et al*., (1956). The dried and powdered materials of gills, liver, kidney, and muscles (each 500 mgs) were taken separately to which 5.0 ml of chloroform methanol mixture were covered with an aluminum foil and allowed to stand overnight for digestion. The mixture was then filtered through Whatman No.1 filter paper and the filtrate was dried in an oven in a reweighed beaker. The percentage of the lipid was calculated by using the following formula:

Percentage of lipid = Weight of lipid / weight of the dried tissue (in mgs?) x 100

Where, Weight of lipid = Post weighed beaker – Preweighed beaker

Analyze of metal and biochemical estimation was carried out in the fishes similar to that of experimental ones.

RESULTS

The results of quantitative analysis of cadmium, copper and zinc in different tissues such as gills, liver, kidney and muscle of both control and experimental fish are presented in Table 4, 5 and 6.

Table 4: Accumulation of Cadmium in different tissues (Gill, Liver, Kidney and Muscle) of control and Experimental fishes, *Cyprinus carpio*, exposed to 120 hrs.

Tissues	Cadmium content (µg/gm wet weight of tissues)	
	Control fish	Experimental fish
Gills	4.16 ± 0.06	36.42± 0.25
Liver	15.22 ± 0.08	41.00 ± 0.94
Kidney	5.88 ± 0.12	7.38 ± 0.08
Muscle	8.33 ± 1.03	18.22 ± 1.01

Mean ± SE (Mean of 5 individual observations)

Table 5: Accumulation of copper content in different tissues (Gills, Liver, Kidney and Muscle) of control and Experimental fishes, *Cyprinus carpio*, exposed to 120 hrs.

Tissues	Copper content (µg/gm wet weight of tissues)	
	Control fish	Experimental fish
Gills	2.44 ± 0.02	18.24 ± 1.16
Liver	4.16 ± 0.08	28.00 ± 0.61
Kidney	3.08 ± 0.04	8.55 ± 1.02
Muscle	5.092 ± 1.05	45.28 ± 0.83

Mean ± SE (Mean of 5 individual observations).

Table 6: Accumulation of Zinc content in different tissues (Gills, Liver, Kidney and Muscle) of control and Experimental fishes, *Cyprinus carpio*, exposed to 120 hrs.

Tissues	Zinc content (µg/gm wet weight of tissues)	
	Control fish	Experimental fish
Gills	2.20 ± 0.05	32.33 ± 0.64
Liver	9.22 ± 0.96	62.45 ± 1.12
Kidney	4.24 ± 0.06	91.22 ± 0.08
Muscle	5.05 ± 0.02	36.33 ± 1.01

Mean ± SE (Mean of 5 individual observations).

Impact of heavy metal toxicity (cadmium, copper and zinc) on the selected tissues of fresh water fish, *Cyprinus carpio* in relation to total carbohydrate content

In the fishes exposed to cadmium, the level of carbohydrate in liver, muscle and kidney was found to be 1.64 ± 0.06, 1.38 ± 0.02, 1.33 ± 1.01% respectively. The total carbohydrate content was increased in all the three tissues. Of all the three tissues analyzed, the liver showed depletion in the level of carbohydrate and consequently there was an elevation of total carbohydrate in muscle at kidney (Table 7).

The result clearly indicates the decreased level of carbohydrate content in the liver and corresponding increase of this metal in muscle and kidney. The total carbohydrate content of liver, muscle and kidney of the animals exposed to zinc were in the order of 1.55 ± 0.09, 1012 ± 0.03 and 0.68 ± 0.08%. The level of total carbohydrate in each tissue was decreased to certain extend in the treated fishes when compared to the controls.

The total carbohydrate content was found to be 1.44 ± 0.04, 1.08 ± 0.03 and 0.94 ± 0.09% respectively in the liver, kidney and muscle of control *C. carpio* fishes. The total carbohydrate content of these tissues from the animal treated with copper was 1.22 ± 0.10, 1.18 ± 0.03 and1.05 ± 0.02%.

Table 7: Total carbohydrate content in different tissues of test fish Cyprinus carpio, exposed to 120 hrs.

Tissues	Total carbohydrate content (%)			
	Control fish	Experimental fish (treated)		
		Cd	Cu	Zn
Liver	1.44 ± 0.04	1.64 ± 0.06	1.22 ± 0.10	1.55 ± 0.09
Kidney	1.08 ± 0.03	1.38 ± 0.02	1.18 ± 0.03	1.12 ± 0.03
Muscle	0.94 ± 0.09	1.33 ± 1.01	1.05 ± 0.02	0.68 ± 0.08

Mean ± SE (Mean of 5 individual observations).

Impact of heavy metal toxicity (cadmium, copper and zinc) on the selected tissues of fresh water fish, *Cyprinus carpio* in relation to total protein content

In the animals treated with cadmium, the protein content was estimated in liver, kidney and muscle as 16.21 ± 0.20, 6.45 ± 0.04 and 3.33 ± 0.08% respectively. The protein content was less in the experimental fishes than that of the controls (Table 8).

The total protein content in the liver, kidney and muscle of control fish *Cyprinus carpio* was found to be 38.22 ± 0.62, 8.44 ± 0.05 and 5.08 ± 0.01% respectively copper the total protein content in the order of 32.00 ± 0.00, 12.40 ± 0.05 and 6.60 ± 0.04%. In animals exposed to zinc, the total protein content was 21.02 ± 0.08% in liver, 11.44 ± 0.06 in kidney and 9.62 ± 0.03% in muscle. The level of protein was elevated in the experimental fishes than in the control.

Table 8: Total protein content in different tissues of test fish *Cyprinus carpio*, exposed to 120 hrs.

Tissues	Total protein content (%)			
	Control fish	Experimental fish (treated)		
		Cd	Cu	Zn
Liver	38.22 ± 0.62	1.64 ± 0.20	32.00 ± 0.02	21.02 ± 0.08
Kidney	8.44 ± 0.05	6.45 ± 0.04	12.40 ± 0.05	11.44 ± 0.06
Muscle	5.08 ± 0.01	3.33 ± 0.08	6.60 ± 0.04	9.62 ± 0.03

Mean ± SE (Mean of 5 individual observations).

DISCUSSION

Heavy metals are known pollutants, which inflict disorders on any aquatic ecosystem and the concentration of heavy metal pollutants is significantly higher in the aquatic biosphere. Heavy metal uptakes in aquatic organism were reported from many investigations. Emmerson *et al*., (1997) and Geeta *et al*., (1998) have stated that the bioaccumulation of metal toxicants depends on availability and persistence of contaminates in water and food, physiochemical properties of the toxicants, quality of holding water uptake, transport of xenobiotics, bio-transformation and depuration. Further, it may be difficult to generalize uptake and accumulation of metals in aquatic organism because of species difference in trace metal concentration. Heavy metals, being non-biodegradable primarily necessitate knowledge on their uptake, distribution and persistence in the tissues of organisms.

Carbohydrates supply the major portion of the daily energy requirements. Apart from being oxidized as a source of energy, carbohydrate may be transformed to glycogen to supply the carbon chain for amino acids, or converted into fat. Of these various processes, glycogen formation and breakdown appears to occupy a central position. The carbohydrate metabolism is disturbed when the animals are subjected to toxic stress (Srivastava and Sing, 1980 and Metiev *et al*., 1983) by heavy metals (Mathivanan, 1988). The results of the present study reveal that the fishes *Cyprinus carpio* exposed to cadmium and copper and Zinc have showed an elevation in the levels of carbohydrates in muscles and liver. This was due to the transportation of glygogen in the form of glucose to muscle and kidney from the liver. Similar results were obtained by Bakthavathsalam (1980) who has reported the mobilization of glucose to muscle through blood in lindane exposed Anabas testuclineus.

The present study indicates that the fishes *Cyprinus carpio* exposed to cadmium have a marked increase in the total carbohydrate level in liver, muscle and kidney. This may be due to the processes of gluconeogenesis in liver and thus synthesized glucose might have been transported to muscle to meet the energy demand by the fishes under stress, when exposed to heavy metals. Another possible source for the increased level of carbohydrate in the fishes exposed to heavy metals might be from fat and protein, when they were converted to glucose through the process of deamination for obtaining energy to meet the energy required at times of steer due to metal toxicity. Grant and Mehrle (1973) noticed increased liver glycogen content in Salmo gairdneri when exposed to endrin. Kabeer *et al*., (1978) have reported that the amino acid can be terminated to keto acids to meet the extra energy demand through gluconeogenesis.

The result of the present study revealed that the liver constituted the highest level of protein since liver is the centre for metabolic activities, where, most of the metabolic transformations take place under the aegis of enzymes. The lower level of proteins in muscle than in liver was due to the fact that muscle was not engaged in the bio-synthesis of protein and only the structural constituents like myosin, action, trophomysin, tropinine etc. are responsible for the protein level.

In the present investigation, animals exposed to copper and zinc showed an elevated level of protein in liver, kidney and muscle. The increased level may be due to the boosting up of the protein and synthesis of aninoacids in liver, as these metals participate in many enzymatic reaction copper was an integrated part of ceruloplasmin which has ferroxidase effect and participates in the formation of haemoglobin.

Zinc forms a constituent of certain enzymes like carbonic anhydrase, carboxypeptidase and alkaline phosphatase etc. So, when the animal was exposed to these metals, these enzymes may be due to the mobilization of protein molecules from liver to muscles. Radhakrishanan (1980) reported that the level of protein in liver, kidney and muscle was elevated in the fresh water fish *Cyprinus carpio* exposed to lead nitrate. Bakthavasalam (1980) has reported that the high content of protein was due to the greater concentration of enzymes in the liver, since liver was the sited of metabolism (Harper *et al*., 1977). Lomte and Patil (1987) revealed that the stress caused an increase in protein level in the liver. It is thus suggested, that the increased protein level in the liver of *C. carpio* were perhaps due to the diminished utilization of proteins and their enhance synthesis under stress conditions, when the fishes were exposed at various metallic concentrations.

Ganapathy Raman (1987) reported the occurrence of sharp decline in protein level in the liver and muscle tissues of Channa punctatus exposed to monocrotophos. Subramanian and Chellappa (1989) have noticed a drop in protein level in liver and muscle of Sarotherodon mossambicus exposed to chromium. Amudhavalli *et al*., (1989) in their study reported that the animals (Tilapia mossambica) exposed to sublethal concentration of zinc have showed an increased protein content in the liver and muscle and a decreased protein content in the same organs when exposed to cadmium. Based on the above findings, in the present study stated that zinc and cadmium could have antagonistic effect on protein metabolism.

CONCLUSION

The ability of aquatic organism to accumulate Pollutant in their tissues to elevated level reaching concentration that are much higher than that of ambient water concentrations makes these biota useful for assessment purposes. The use of organism to monitor pollutants relies on the fact that a contaminant, if present in the water column, may not be recorded in the sediment, but may accumulate in biota, which become recorders of the contaminants.

The results of the present study showed interspecific variations in heavy metal body concentrations in biota of different trophic levels and this might be related to influence of specimen size, sensitivity to seasonal and water quality changes, feeding habits, trophic level and level of environmental contamination of the freshwater medium especially in the lake ecosystem.

Distribution of metals in isolated tissues of fish is useful to identify specific organs that may be particularly selectively and sensitive to accumulation of heavy metals.

Though the input of anthropogenic pollutants into the Karukkupatti lake, Salem. Salem district, Tamilnadu has not effected the levels of metals in fish gill, liver and kidney to an alarming extent, this baseline data can be used for regular ecological monitoring considering the industrial growth around this important freshwater lake ecosystem.

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