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RESEARCH ARTICLE

BIOACCUMULATION OF CHROMIUM IN *CIRRHINUS MRIGALA*

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ABSTRACT

Chromium in the effluent is a major concern for the tanning industry and it is directed in to fresh water bodies which in turn taken part in biological magnification through food chain. The present study is undertaken to gauge the accumulation of chromium in the tissues of *Cirrhinus mrigala,* when the live fish is subjected to sub lethal dose of chromium for a period of 10, 20 and 30 days in controlled environment. The tissues from gill, liver and kidney where subjected to Atomic Absorption Spectroscopy for assessing the amount of chromium accumulated in various tissues at the end of 10, 20 and 30 days. Maximum accumulation of chromium was found in the liver and kidney while minimum accumulation was seen in gill. *Cirrhinus mrigala,* is used as bioindicators because it tends to accumulate heavy metals and so their effects. As the fish is extensively used for human consumption, this finding urges greater regulation for industrial effluent discharge.

INTRODUCTION

The Mrigal carp (*Cirrhinus mrigala*), also known as Indian Carp, is a species of ray-finned fish belonging the genus *Cirrhinus* and family *cyprinidae*. Native to the Ganges and Brahmaputra rivers of the Indian subcontinent, it is extensively aqua farmed, having a harvesting capacity of 463,520 tons. *Cirrhinus mrigala* is eurythermal and tolerate a minimum temperature 14ºC and generally mrigal has a higher survival level. But it is highly susceptible to aquatic pollutant in general and heavy metals in particular. Present study focus on the estimate of accumulation of chromium in the tissues of *Cirrhinus mrigala.*

MATERIALS AND METHODS

Bio accumulation of chromium in gill, liver and kidney of *Cirrhinus mrigala*

Fingerlings of *Cirrhinus mrigala* were collected from Poondi reservoir and acclimated in the laboratory for a month in separate aquaria. Two batches were maintained as control and experimental and the latter were subjected to sub lethal dose of Chromium. A batch of *Cirrhinus mrigala* was sacrificed after 10 days, 20 days and 30 days. Eventually the tissue sample were collected, prepared and subjected to Atomic Absorption Spectroscopy (AAS) as per the method of (Curry *et al.,* 1969). Accordingly 5 mg of tissue was taken in a 125 ml Erlenmeyer flask and glass beads were added with 25 ml of deionized water, then 10 ml of the sample was mixed with 1:1 conc.HNO_3 and $HCI0_4$-. The sample was boiled until the solution was clear and subsequently transferred to a 100 ml volumetric flask and diluted with deionized water.

The metal concentrations in the tissues were measured with a Carlzeiss JENA and Model AAS3 atomic absorption spectrophotometer by direct aspiration of the solution in to a nitrous oxide acetylene flame (Singhanan *et al.,* 1996). Tissues such as gill, liver, and kidney were isolated from the experimental fish and subjected to standard histological technique of Culling (1974). For this purpose the tissues were fixed in Bouin's fixative (Formaldehyde 15 ml+picric acid 80 ml+acetic acid 5ml) for 24 hours, dehydrated, cleared, sectioned to have 4-6 micron thickness. The slides were stained and selected fields were photographed using Carl zeiss photo microscope.

RESULTS

Bioaccumulation of chromium

The concentrations of accumulated chromium in the tissues such as gills, liver and kidney of the fish exposed to various effluent concentrations (0.1%, 0.3%, 0.5 % and 0.7%) for a period of 10, 20 and 30 days is tabulated. The mean concentration ± SD (µg/g) of chromium in the tissues (gill, liver and kidney) exposed to 0.1% of effluent for 10 days is observed to be 0.582±0.07, 0.848±0.41 and 0.749± 0.22 respectively. The mean chromium concentration ± SD µg/g in the tissues exposed to 0.3% effluent concentration for 10 days are found to be 0.769±0.02, 1.081±0.03 and 1.046±0.16 respectively. The mean chromium concentrations ± SD (µg/g) in tissues exposed to 0.5% effluent concentrations for 10 days are 0.874± 0.01, 1.162± 0.03, and and 1.123 ± 0.08. The mean chromium concentration ± SD (µg/g) in tissues exposed to 0.7 % effluent concentration for 10 days are 0.653 ± 0.05 , 1.788 ± 0.18 and 1.612 ± 0.12. Estimates of chromium found

in the control tissues exposed for 10 days are 0.065± 0.02 µg/g, 0.214± 0.03 µg/g and 0.158±0.08 (µg/g). Total uptake of chromium in all the tissues exposed to 0.1%, 0.3%, 0.5% and 0.7% and control for 10, 20 and 30 days are 2.179 (µg/g),2.896(µg/g), 3.159(µg/g) , 3.953(µg/g), and 0.435(µg/g) respectively (Table 1).

(µg/g) in the tissues exposed to 0.7% for 30 days are 0.688±0.09, 2.386±0.08 and 1.927±0.05. The mean chromium concentrations ± SD (µg/g) in the tissues of the control are 0.078± 0.01, 0.319 ± 0.29, 0.484 ±0.10. The total chromium uptake in the tissues exposed to 0.1 %, 0.3%, 0.5% and 0.7%

Table 1: Mean concentration ± SD (µg/g) of chromium in the tissues of *Cirrhinus mrigala* exposed to different concentrations of filtered tannery effluent for 10 days

Sample	Control	Treatments Mean±SD			
		0.1%	0.3%	0.5%	0.7%
Gill	0.065±0.02	0.582±0.07	0.769±0.02	0.874±0.01	0.653±0.05
Liver	0.214±0.02	0.848±0.41	1.081±0.03	1.162±0.03	1.788±0.18
Kidney	0.158±0.08	0.749±0.22	1.046±0.16	1.123±0.08	1.612±0.12
Total uptake	0.435	2.179	2.896	3.159	3.953

Table 2: Mean concentration ± SD (µg/g) of chromium in the tissues of *Cirrhinus mrigala* exposed to different concentrations of filtered tannery effluent for 20 days

Sample	Control	Treatments Mean±SD			
		0.1%	0.3%	0.5%	0.7%
Gill	0.070±0.02	0.628 ±0.07	0.863±0.03	0.909 ±0.01	0.654±0.01
Liver	0.419 ±0.39	0.912 ±0.18	1.148±0.11	1.707 ±0.15	2.108±0.11
Kidney	0.159±0.03	0.911±0.18	1.098±0.03	1.226±0.07	1.584 ±0.24
Total uptake	0.648	2.451	3.309	3.842	4.346

Table 3: Mean concentration ± SD (µg/g) of chromium in the tissues of *Cirrhinus mrigala* exposed to different concentrations of filtered tannery effluent for 30 days

Sample	Control	Treatments Mean±S.D			
		0.1%	0.3%	0.5%	0.7%
Gill	0.078±0.01	0.642±0.07	0.949±0.02	0.963± 0.03	0.688±0.09
Liver	0.319±0.29	1.038±0.15	1.652±0.33	1.727±0.18	2.386±0.08
Kidney	0.484±0.10	0.980±0.11	1.179±0.05	1..652±0.32	1.927±0.05
Total Uptake	881	2.660	3,880	4.342	5.001

Mean chromium concentrations ± SD ((µg/g) in the tissues (gill, liver, and kidney) exposed to 0.1% for 20 days are 0.628 ± 0.07, 0.912 ± 0.18, and 0.911 ± 0.18 (µg/g). In the same way the mean chromium concentrations ± SD (µg/g) in the tissues exposed to 0.3% concentration for 20 days are 0.863 ± 0.03, 1.148± 0.11, and 1.098 ± 0.03. Similarly the mean chromium concentrations ± SD (µg/g) in the tissues exposed to 0.5% concentrations ± SD (µg/g) in the tissues exposed to 0.5% concentration for 20 days are 0.909± 0.01, 1.707 ± 0.15, and 1.226 ± 0.07 and the mean chromium concentrations ± SD (µg/g) in the tissues exposed to 0.7% concentration for 20 days are 0.654 ± 0.01, 2.108±0.11, and 1.584 ±0.24. The mean chromium concentrations ± SD (µg/g) in the tissues of the control are 0.070±0.02, 0.419±0.39, and 0.159 ±0.03. Eventually the total chromium uptake in all the tissues exposed to 0.1% 0.3%, 0.5% and 0.7% effluent concentration and control are 2,451µg/g, 3.309 (µg/g), 3.842 (µg/g), 4.346(µg/g) and 0.648(µg/g) respectively (Table 2).

The mean chromium concentration ±SD (µg/g) in the tissues (gill, liver and kidney) exposed to 0.1% for 30 days are 0.642±0.07, 1.038. ±0.15 and 0.980 ±0.11; the mean chromium concentration ± SD(µg/g) in the tissues exposed to 0.3% for 30 days are 0.949±0.02, 1.652±0.33 and 1.179±0.0; and the mean chromium concentration ± SD(µg/g) in the tissues exposed to 0.5% for 30 days or 0.963 ± 0.03,1.727 ±0.18 and 1.652 ±0.32. Similarly the mean concentration ± SD control for 30 days are 2.660 µg/g, 3.880 (µg/g), 4.342(µg/g), 5.001, (µg/g) and 0.881(µg/g) respectively (Table 3).

DISCUSSION

Knowledge of heavy metal concentration in fish is important factor with respect to nature of management and its relevance in the context of fish consumption. In the river, fish are often at the top of the food chain and have the tendency to concentrate heavy metals from water (Mansour and Sidky, 2002). Therefore bioaccumulation of heavy metals in fish can be considered as an index of metal pollution in aquatic bodies (Javid, 2005; Tawari-Fufeyin and Ekaye, 2007; Karadde-Akin and Unlu, 2007) that could be a useful tool to study the biological role of metals present at higher concentrations in fish(Dural *et al.,* 2007). In the present study, accumulation of chromium is high in liver and kidney and the minimum accumulation of chromium is found in the gill Dural *et al.,* (2007) and Ploetz *et al.,* (2007) reported highest level of Cadmium, Lead, Copper, and Zinc in the liver of fish species viz., *Mugil cephalus* and *Sparaus aureta.* Yilmaz *et al.,* (2007) reported that in Leutiscus *cephalus* and *Lefornis gibbosus* Cadmium, Cobalt and Copper accumulations in the liver and gill were maximum while these accumulations were least in the fish muscle. The higher levels of trace elements such as Lead, and Chromium in liver related to other tissue may be attributed to the affinity of metallothionein protein

with these elements (IKEM *et al.,* 2003) and consequently interfere with the metabolism. By exposing fingerlings of *Catla catla, Labeo rohita* and *Cirrhinus mrigala* to sub lethal concentration of manganese for 30 days, Hayat *et al.,* (2007) proved negative growth with weight decrement value of -0.22, -0.72 and -3.90 grams respectively in them. Furthermore they concluded that stressed major carps under sub-lethal concentrations of manganese showed significantly lower values of weight and total length than control fish in semi intensive culture system. Fish fauna are good bioaccumulators and they concentrate metals to a level that makes them easier to deduct. The ultimate level of pollutant that entered in to an organism is governed by the ability of the organism to extract the pollutant and store the same. However, physiological processes, exists specifically for regulating and removing such interfering cat ions so that the accumulation of metals in different organs represents the end product of detoxification process. The overall factors influencing the accumulation and concentration of metals in an aquatic medium can be due to absorption of ions on membrane interface (Romesil, 1995) as it happens in gills and other tissue samples. Chromium has preferred for ligands such as nitrogen, oxygen and sulphur. The tissue load of chromium increases with increased duration of exposure to the metal in all the tissues, studied. Since the liver is endowed with a variety of kinetic traps, the accumulation proceeds by a cascade of binding to ligands of ever increasing strength.

Uptake, distribution in the tissues and retention of chromium in this study reveals the same pattern as in rainbow trout, salmo gairdneri (Putte *et al.,* 1981). The high accumulation of chromium in the liver and kidney of the treated fish suggest, that Chromium (III) bound to proteins in the effluent may be responsible for the above accumulation and the fact that the liver accumulated the highest concentration of chromium in all the treatments may be due to the uptake, which is the primary route of accumulation as suggested by Andrew and O' Halloran (1997). Therefore the soluble Chromium (III) proteins would have to be initially converted in to particulate from prior to the uptake. Depending on the routes of uptake, the metals come in to contact with different tissues before reaching the major sites of detoxification and the metals may thus interact with other cellular molecules, before being sequestered by metallothioneins. Most of the degradation of metalothioneins has been shown to occur in the liver, hence in the present study, liver revealed maximum accumulation or concentration of chromium. Sobha *et al.,* (2007) evaluated toxicity of cadmium and its impact on the biochemical constituents like glucose, glycogen total proteins, lipid and free amino acids in the edible carp *Catla catla* using acute toxicity test for 96 hours. They performed $Cacl_2$ toxicity experiment on the fingerlings and LC_{50} was determined using simple and probit graphics and regression. They also recorded significant fall in all the biochemical constituents in muscle, gill, liver, heart and kidney excepting the glucose prompting to suggest that fish cultured in the aquatic ecosystem close to industrial locations would not have expected nutritive value. The elevated levels of glucose are apparently indicative of the organism's response to the toxicant stress. Kidneys also accumulated the highest concentration of chromium next to liver. Since kidney is also rich in sulphydryl groups, chromium binds with these ligands. It accounts for higher concentration of chromium in the kidney. Liver and Kidney are thus capable of acting as diverse sites for chromium accumulation. An increase in Sodium and Potassium level in the haemolymph of prawn was mainly due to the accumulation of the metal ions in the kidney (Vijayaramanan, 1998).

Gills, although a primary route of uptake (Simkiss *et al.,* 1982; and Wicklund –Glynn *et al.,* 1992) accumulated only little amounts of chromium. Some form of ligand exchange for chromium(III) occurs in fish tissues subsequent to the uptake of chromium and this appears particularly evident in the gills, such a process has been proposed for cadmium –organic complexes(George and Coombs, 1977) and whether such complexes exist for chromium(III) has to be elucidated. Atomic absorption spectroscopic and electrohoretic analysis of Jyotsna Sigh *et al.,* (2008) disclosed the accumulation of heavy metal Cadmium in the fish tissues of liver, gill, muscle and kidney when exposed sub lethal dose (Cadmium) for a period of 15 to 60 days in controlled environment of aquaria. Accordingly the tissues of *Mrigala* are more resistant to accumulation of Cadmium than those of *Catla* and maximum accumulation is found in the gills and liver, followed by muscle while minimum accumulation was seen in kidneys.

The present study also confirms that the concentration of chromium in the muscle tissue of the treated fish is much lower than in other organs and it coincides with the report of (Sinha *et al.,* 2002). A significant content of chromium in some of the tissues of fish may indicate that its restraint capacity has been exceeded (Pereira, 1995). Chromium (III) has been shown to be available to fish and its predominance in the effluent indicates a possible role in the accumulation of chromium observed in the present study. However, chromium (III) is insoluble in water and the reason for enhanced solubility appears to be ligation with dissolved protein across the membrane. The source of protein may be residual dissolved protein or bating enzymes leaching from the hide. It appears that during the mixing process, the organic ligands complete with hydroxyl groups for the ionic chromium (III) present resulting in the formation of dissolved organic chromium (III). This form of chromium (III) permeates through the membranes.

Similarly Rauf (*et al.,* 2009) revealed various concentrations of heavy metals in different tissues/organ. Accordingly fish liver appeared to have significantly higher tendency for the accumulation of Cadmium and Chromium, while gills had minimum concentration of these metals. Thus the accumulation of the heavy metal, chromium in the different organs reveals the following sequence: Liver> Kidney > and Gill. Accumulation of chromium is least regulated by the fish species and hence the tissues such as liver, kidney, intestine, gill and muscles are susceptible to pathogenicity or damage. Chromium thus has a preferential site of accumulation, susceptibility limiting its transfer to other organs.

Chromium though present in traces and being lipophilic tends to bioaccumulate and biomagnify and causes toxic effects. Hence, precautions need to be observed as these fish species are extensively used for human consumption. Current finding also urges either greater regulation of industrial effluents or resorting to alternate fish species which can metabolize these heavy metals. Further studies would throw more light on its impact in human physiology and metabolic regulation.

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