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RESEARCH ARTICLE

IMPACT OF HEAVY METAL CHROMIUM ON PROTEIN AND AMINOACID CONTENTS IN BRAIN AND MUSCLE OF FRESHWATER FISH *Oreochromis mossambicus* (PETERS)

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ABSTRACT

Numerous numbers of aquatic organisms are living in the aquatic medium which can majorly benefit to the man. However, due to the continuous rise in the development of industries, many of these aquatic flora and fauna are killed because of toxic metals. Heavy metals from natural and anthropogenic sources are continually released into aquatic ecosystem. The toxicity of heavy metal causes long persistence, bio accumulative and non-biodegradable in the food chain. Chromium is known to be a very toxic pollutant introduced into natural waters from a variety of sources including industrial wastes. The heavy metal, chromium can be toxic at even very low concentration when ingested over a long period of time. The aim of the present study is to assess the protein and amino acid content in brain and muscle of the fish *Oreochromis mossambicus* was exposed to sublethal concentrations of chromium 1/10th (high), 1/15th (medium) and 1/20th (low) of the 96 hour LC_{50} values for the period of 10, 20 and 30 days. The fish exposed to chromium showed a decrease the level of protein and increase the level of amino acid for 10, 20 and 30 days in brain and muscle. However, no information is on record concerning the three different sublethal concentrations of chromium on the protein and amino acid contents of freshwater fish *Oreochromis mossambicus*. The objective of the present work was to observe the effect of chromium on protein and amino acid contents in brain and muscle of freshwater fish, *Oreochromis mossambicus.*

INTRODUCTION

Water is an essential element for life. Freshwater comprises 3%of the total water on earth. Only a small percentage (0.01%) of this freshwater is available for human use (Hinrichsen and Tacio, 2002). Unfortunately even this small proportion of freshwater is under immense stress due to rapid population growth, urbanization and unsustainable consumption of water in industry and agriculture. According to a UNO report, the world population is increasing exponentially while the availability of freshwater is declining. Many countries in Africa, Middle East and South Asia will have serious threats of water shortage in the next two decades. In developing countries the problem is further aggravated due to the lack of proper management, unavailability of professionals and financial constraint (PCRWR, 2005; Azizullah *et al.,* 2011). The deliberate discharge and accidental release of harmful chemical compounds into the environment has the potential to disturb the structure and functioning of natural ecosystems. As industrial technology improves, the characteristics of industrial discharge sources become more complicated and the toxicity of industrial wastewater can also become more complicated and heavier. Since the aquatic environment is the ultimate recipient of the pollutants produced by natural and anthropogenic sources, accumulation and persistence industrial effluents in the aquatic environment constitute a threat to biological life (Fleeger *et al.*, 2003). The increasing worldwide contamination of freshwater systems with thousands of industrial and natural chemical compounds is one of the key environmental problems (Schwarzenbach *et al.,* 2006). Besides numerous organic compounds entering aquatic ecosystems, heavy metal input is still rising (Bopp *et al.,* 2008). The aquatic environment is continuously being contaminated with toxic chemicals from industrial, agricultural and domestic activities. Aquatic animals inhabiting polluted water bodies tend to accumulate many chemicals in high concentrations even when the ambient environmental contamination levels are low potentially hazardous situation for the entire food chain. Once a toxicant enters an organism, several biochemical and physiological responses occur which may be adaptive or may lead to toxicity. The biochemical processes represent the most sensitive and relatively early events of pollutant damage. Thus, it is important that pollutant effects be determined and interpreted in biochemical terms, to delineate mechanisms of pollutant action, and possibly ways to mitigate adverse effects. Tanneries are generally polluted the environment and intensive industrial complexes generating large volumes of high concentration of wastewater. These wastes have historically been discharged into rivers and waterways.

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Most of the objectionable components of a tannery effluent formerly discharged directly in to a nearby the river or any aquatic medium. Tannery effluents are ranked as the highest pollutants among all the industrial wastes. It is estimated that in India alone about 2000-3000 tones of chromium escapes into the environment annually from tannery industries, with chromium concentrations ranging between 2000 and 5000 mg/l in the aqueous effluent compared to the recommended permissible limits of 2 mg/l (Atlaf *et al.*, 2008).

The tanning industries are especially large contributors of chromium pollution in India. Two types of effluents are discharged during the tanning process: vegetable tanning, which does not contain chromium, and chrome tanning, which contains chromium (Manivasagam, 1987). Leather processing requires large amount of chemicals like sodium chloride, chromium sulphate, calcium salts, ammonium salts, sodium sulphide, acids, alkalis, fat, liquor, and organic dyes. However, one of the major emerging environmental problems in the tanning industry is the disposal of chromium contaminated sludge produced as a by-product of wastewater treatment (Amita *et al.*, 2005). Fish is generally appreciated as one of the healthiest and cheapest source of protein and it has amino acid compositions that are higher in cysteine than most other sources of protein (Akan *et al.*, 2012). Fish is a commodity of potential public health concern because it can be contaminated by a range of environmentally persistent chemicals, including heavy metals (Soliman, 2006; El-Morshedi *et al*., 2014). The present investigation was to assess the protein and amino acid content in brain and muscle of *Oreochromis mossambicus* exposed to three different sublethal concentrations of chromium.

MATERIALS AND METHODS

The fish *Oreochromis mossambicus* having mean weight 23-28 g and length 11 – 13 cm were collected from PSP fish farm, at Puthur and acclimatized to laboratory conditions. They were given the treatment of 0.1% KMNO4 solution and then kept in plastic pools for acclimatization for a period of two weeks. They were fed on rice bran and oil cake daily. The $K_2Cr_2O_7$ (Potassium dichromate) was used in this study and stock solutions were prepared. Chromium, LC_{50} was found out for 96 h (25 ppm) (Sprague, 1971) and 1/20th, 1/15th and 1/0th of the LC_{50} values were 1.25, 1.66 and 2.5 ppm respectively taken as sublethal concentrations for this study. Forty fish were selected and divided into 4 groups of 10 each. The first group was maintained in free from chromium, and served as the control. The other 3 groups were exposed to sub lethal concentration of chromium, 10 litre capacity aquaria. The 2nd, 3rd and 4th groups were exposed to chromium, for 10, 20 and 30 days respectively. At the end of each exposure period, the fish were sacrificed and the required tissues were collected for protein and aminoacid estimation. The protein and amino acid content in brain and muscle of *Oreochromis mossambicus* were estimated by the method of Lowry *et al.*, 1951 and Moore and Stein (1954) respectively. The data obtained were analyzed by applying analysis of variance DMRT one way ANOVA to test the level of significance (Duncan, 1957).

RESULTS

The protein and amino acid contents in brain and muscle of *Oreochromis mossambicus* exposed to low, medium and high sublethal concentrations of chromium showed significant decrease in the level of protein whereas increase the levels of amino acids when compared to control fish. The decrease the level of protein and increase the level of amino acid in brain and muscle of *Oreochromis mossambicus* were more pronounced at 30 days of exposure period (Table 1 and 2).

Table 1. Protein level changes (mg/g) in brain and muscle of *Oreochromis mossambicus* exposed to sublethal concentrations of chromium

Treatments	10 days	20 days	30 days
	Brain		
Control	78.36 ± 5.96^{c}	79.64 ± 6.06^{c}	79.83 ± 6.07^{c}
Low concentration	74.11 ± 5.64^{bc}	68.43 ± 5.21^{b}	64.35 ± 4.90^{b}
Medium concentration	71.55 ± 5.44^{b}	64.19 ± 4.88^{ab}	59.76 ± 4.55^{b}
High Concentration	64.12 ± 3.53^{a}	60.38 ± 4.59^{a}	49.62 ± 4.31^{a}
	Muscle		
Control	70.19 ± 5.34^{b}	71.36 ± 5.43^{c}	71.47 ± 5.44^{d}
Low concentration	68.55 ± 5.21^{b}	65.28 ± 4.96^{b}	60.13 ± 4.57^{c}
Medium concentration	65.22 ± 4.96^{ab}	62.55 ± 4.76^{ab}	55.73 ± 4.24^{b}
High Concentration	61.11 ± 4.65^{a}	58.46 ± 4.45^{a}	49.38 ± 4.13^{a}

All the values mean ± SD of six observations Values which are not sharing common superscript differ significantly at 5% ($p < 0.05$) Duncan multiple range test (DMRT)

Table 2. Amino acid (mg/g) in brain and muscle of *Oreochromis mossambicus* exposed to sublethal concentrations of chromium

Treatments	10 days	20 days	30 days
	Brain		
Control	3.08 ± 0.23^{a}	3.10 ± 0.23^{a}	3.05 ± 0.22^{a}
Low concentration	3.45 ± 0.26^{b}	4.06 ± 0.30^{b}	5.54 ± 0.42^{b}
Medium concentration	3.82 ± 0.29^{c}	4.40 ± 0.33^{b}	6.06 ± 0.46^{b}
High Concentration	4.56 ± 0.34^{d}	5.25 ± 0.39^{c}	7.42 ± 0.56^{c}
	Muscle		
Control	2.40 ± 0.18^{a}	2.38 ± 0.17^{a}	2.41 ± 0.18^{a}
Low concentration	2.94 ± 0.22^{b}	3.42 ± 0.26^{b}	3.96 ± 0.30^{b}
Medium concentration	3.38 ± 0.25^{c}	3.75 ± 0.28^{c}	4.82 ± 0.33^{c}
High Concentration	3.55 ± 0.26^{c}	4.49 ± 0.34^{d}	5.94 ± 0.43^{d}

All the values mean ± SD of six observations Values which are not sharing common superscript differ significantly at 5% ($p < 0.05$) Duncan multiple range test (DMRT)

DISCUSSION

Heavy metals are stable and persist in environmental contaminants of aquatic environments and their organisms. They occur in the environment both as a result of natural processes and as pollutants from human activity (Jordao *et al.*, 2002). According to World Health Organization (1991), metal occur in less than 1% of the earth's crust, with trace amounts generally found in the environment and when these concentrations exceed a stipulated limit, they may become toxic to the surrounding environment. The presence of metals in aquatic ecosystems originates from the natural interactions between the water and atmosphere (Sankar *et al.*, 2006). Heavy metals may enter an aquatic ecosystem from different natural and anthropogenic sources including industrial or domestic sewage, storm runoff, leaching from landfills, shipping and harbor activities and atmospheric deposits (Nair *et al.*, 2006). Tanning industry contributes significantly towards exports, employment generation and occupies an important role in the Indian economy while on the other hand, tannery wastes are ranked as the highest pollutants among all the industrial wastes (Soyaslan *et al.,* 2007). Damage to the environment by the hazardous tannery effluent is becoming an acute problem in Indian (Taju *et al.,* 2012). Chromium is one of the most common elements in the earth crust and water. For drinking water WHO described its maximum allowable concentration at 0.05mg/L. In water sources of Pakistan it has been documented in a wide concentration range by different studies. Exceed the safe limits for chromium in the ground water of 23 major cities in Pakistan (PCRWR, 2005). In contrast, individual studies reported chromium concentrations ranging above the safe limits of WHO (0.05 mg/L) in most of the cases. Analysis of drinking water samples from the residential area of Kasur showed chromium concentrations reaching 9.80 mg/L. In general, chromium had 21–42 times higher concentrations than the recommended quality value (Tariq *et al.,* 2008). Similarly, 75% samples from various sources in Khyber Pukhtoonkhwa and 25% samples from Karachi (Sindh) exceeded the maximum acceptable value for chromium in drinking water (Midrar-Ul-Haq *et al.,* 2005). In most cases chromium concentrations are lower in surface than in groundwater; however, in some cases they exceeded the recommended safe WHO limits 0.05 mg/L. Frequently higher concentrations of chromium in drinking water in cities like Lahore, Gujrat and Sialkot have been traced to the leather industry and tanneries (Ullah *et al.,* 2009). Chromium itself is not toxic and plays an important role in the carbohydrate metabolism in the body (Cefalu and Hu, 2004). But some of its compounds especially in its hexavalent status cause skin diseases, cancers, irritants and diseases related to the digestive, excretory, respiratory and reproductive system (Anonymous, 2008; Azizullah *et al.,* 2011).

Biochemical responses of aquatic organisms to contaminants usually represent the first measurable effects of contaminant exposure, and accordingly are advantageous for use in monitoring programs (Hinton, 1994). Proteins are the primary structural and functional polymers in living systems. They have a broad range of activities, including catalysis of metabolic reactions and transport of vitamins, minerals, oxygen, and fuels. Some proteins make up the structure of tissues, while others function in nerve transmission, muscle contraction, and cell motility, and still others in blood clotting and immunologic defenses, and as hormones and regulatory molecules. The degradation of proteins was mainly brought about by protein hydrolyzing enzymes which cleave proteins into peptides and amino acids. Proteins are the most important and abundant biochemical constituent present in the animal body, particularly in fish. Protein generally contains a sequence of amino acid residues of the polypeptide chains. Fish, a common source of protein contains a greater quantity of protein than any other living organism (Karthikeyan, 2012). Proteins are highly sensitive to heavy metals and hence indicators of heavy metals poisoning. The liver is also much in protein because of metabolic potential being oriented towards it and is the seat for the synthesis of various proteins besides being the regulating center of metabolism. The present study in the brain and muscle were justifiable in the wake of mechanical tissue of muscle intended for mobility and does not participate in metabolism. The decreased trend of the protein content as observed in the present study in most of the fish tissues may be due to metabolic utilization of the keto acids to gluconeogenesis pathway for the synthesis of glucose, or due to the directing of free amino acids for the synthesis of necessary proteins for the maintenance of osmotic and ionic regulation. A dynamic equilibrium exits between proteolysis and synthesis which is mainly responsible for protein turnover and homeostasis in any tissues (Tulasi and Jayantha Rao, 2013).

In the present investigation freshwater fish, *Oreochromis mossambicus* exposed to sublethal concentrations of chromium for the periods of 10, 20 and 30 days shows decrease the levels of protein and elevated levels of amino acid in brain and muscle. The present work agrees with Tulas and JayanthaRao (2013) reported that total protein content is decreased and it may be due to breakdown of proteins in the fabrication of some amount of energy for organism. The degree of increase in free amino acids was resulted by the decreased protein level. Bhaskaran (1980) and Manoharan and Subbiah (1982) noticed that depletion in protein level was due to diversification of energy to meet the impending energy demand when the animals was under toxic stress. Protein contents were decreased in gill, liver, muscle and heart of *Oreochromis niloticus* exposed to sublethal concentration of cadmium chloride (Faheem *et al.,* 2012). Similar observations were noted when the fish were exposed to pollutants (Lone and Javaid, 1976; Shakoori *et al*., 1976; Rath and Mishra, 1980; Ramalingam and Ramalingam, 1982). The protein contents in liver of *Catla catla* are depleted under the sublethal stress of chromium (Vincent *et al*., 1995). Palanichamy and Baskaran (1995) reported a decrease in muscle and liver protein in *Channa striatus* exposed to mercury, cadmium and lead for a period of 30 days. Meenakshi and Indra (1998) addressed that the protein content of liver decreased in *Mystus vittatus* when exposed to sublethal concentration of distillery effluent. Karuppasamy (1990) reported the decrease in protein content of liver, muscle and kidney in *Channa punctatus* when exposed to sublethal concentration of sugar mill waste. The change in the protein concentration suggest an intestinal proteolysis in the respective tissues which in turn could contribute to the like of free amino acids to be fed into TCA (Tricalboxylic acid cycle) as keto acid, and it support the hypothesis (Kabeer Ahmed Sahid, 1978). The decreased protein contents in the gill and muscle of Zebra fish, *Danio rerio* exposed to mercuric chloride (Vutukuru and Kalpana, 2013). It is evident that proteins are degraded to meet the energy requirements during heavy metal chromium exposure. It can be concluded that in *Oreochromis mossambicus* exposed to sublethal concentrations of chromium causes energy crisis and alter protein metabolism.

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