Vol 3 Issue 8 Sept 2013 ISSN No : 2230-7850

Monthly Multidisciplinary
Research Journal

Indian Streams Research Journal

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RNI MAHMUL/2011/38595 **ISSN No.2230-7850**

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Indian Streams Research Journal
Volume-3, Issue-8, Sept-2013
ISSN 2230-7850

Available online at www.isrj.net

DOI : 10.9780/2230-7850/382013/3031

EFFECT OF CALCIUM ON CADMIUM INDUCED BIOACCUMULATION IN SELECTED TISSUES OF FRESH WATER TELEOST, OREOCHROMIS MOSSAMBICUS (TILAPIA)

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Abstract:Cadmium (Cd) is one of the most toxic, non-essential heavy metal and constitutes a real threat to fish because of its widespread occurrence in the aquatic environment. The present study is carried out to know the effect of calcium (Ca) in reducing the Cd bioaccumulation levels in the selected tissues of fresh water fish, Oreochromis mossambicus (Tilapia) exposed to Cd. The fish were exposed to cadmium chloride ($CdCl_2$) at a sub lethal concentration of 1/10th LC50/48 hrs i.e. 5ppm for 7, 15 and 30 days (d) time periods. After 15d Cd exposure, the fish were supplemented with trace element Ca (1ppm) and observed for again 7, 15 and 30d long sojourn. After the specified time periods, the test tissues like liver, kidney, muscle, brain and gill were isolated and tested for Cd bioaccumulation by Atomic Absorption Spectrophotometer (AAS – Schimadzu AA6300). There was a significant elevation in Cd concentrations in all the test tissues with increased period of exposure to the heavy metal. Maximum Cd accumulation was found in 30d Cd exposed fish kidney (22.353±0.41 µg / gm wet wt. of the tissue) than the other tissues. However there was significant reduction in Cd bioaccumulation with Ca supplementation suggesting their vital role in heavy metal detoxification. Maximum decrease in Cd accumulation was found in 30d fish kidney (6.996 ± 0.284 µg / gm wet wt. of the tissue) supplemented with Ca. Our findings clearly envisages that 30d Ca supplementation is more effective in reducing the Cd body burden when compared to other time periods in the fresh water fish, Oreochromis mossambicus.

Keywords: Cadmium, Bioaccumulation, Calcium supplementation, Fish.

INTRODUCTION:

The contamination of fresh waters with a wide range of pollutants has become a matter of concern over the few decades (Vutukuru, 2005; Omer et al., 2012). Natural aquatic resources are extensively contaminated with heavy metals like lead (Pb), cadmium (Cd), nickel (Ni) and copper (Cu) released from domestic, industrial and other man made activities. Among these metals Cd is a highly toxic, non-essential heavy metal and arising primarily from battery, electroplating, pigment, plastic, fertilizer industries and cigarette smoke.

The most dangerous characteristic of Cd is that it accumulates throughout a life time. It has a long biological half life 17 to 30 years in humans (Hideaki et al., 2008). Chronic exposure to inorganic Cd results in accumulation of the metal mainly in the liver and kidneys (Usha Rani, 2000; Bais and Lokhande, 2012) as well as in other tissues and organs causing many metabolic and histological changes, membrane damage, altered gene expression and apoptosis (Casalino et al., 2002; Waisberg et al., 2003; Soeginato, 2008). Cd caused swelling in the epithelium and chlorine cells of the gills (Usha Rani, 1999), hyperplasia, hypertrophy, determination and necrosis of the hepatocytes (Usha Rani and Ramamurthi, 1989) and vacuolization of the tubule cells of the kidney (Thophon et al., 2003; Fernandes et al., 2007).

Cd interacts with the essential micronutrients/ trace elements like zinc (Zn), iron (Fe), copper (Cu) and calcium (Ca) and influences the enzyme activities of metabolic pathways (Peraza, 1998; Strydom et al., 2006). Ca is one of the important major element which acts as a micronutrient. It contributes to various biochemical mechanisms in vertebrates including fishes (Galvez and Wood, 2007). Ca plays a major role in bone formation, muscle contraction, enzyme activities and hormonal secretion etc., in animals. Though fish is a vertebrate, literature on the effect of trace element Ca on Cd absorption, elimination and detoxification is scanty.

MATERIALS AND METHODS:

Chemicals: Cadmium as cadmium chloride ($CdCl_2$) and calcium as calcium chloride ($CaCl_2$) were purchased from Merck (Dormstadt, Germany). The other chemicals which were used in the present study were obtained from the standard chemical companies like Sigma Chemical Co. (St

Louis, Mo, USA), SD Fine Chemicals. The chemicals used for this study were of the highest purity.

Maintenance of animals (fish):

Fish O. mossambicus (Tilapia) weighing 10 ± 2 gm were collected from the local fresh water ponds and acclimatized to laboratory conditions for a week in separate troughs. The laboratory temperature was maintained at 28°C ± 2°C. The fish were feed ad libitum with ground nut cake and water was renewed for every 24 hrs with routine changing of troughs leaving no fecal matter.

Experimental design:

Fish were divided into two groups. First group served as control and other group as experimental. The experimental group was exposed to sub lethal concentration of $CdCl_2$ i.e., 5 ppm (1/10th of LC50 / 48 hrs) daily for 7, 15 and 30 days (d) time periods. Then 15d Cd exposed animals were subjected to Ca supplementation (i.e., 1 ppm) for again 7, 15 and 30d long sojourn. After specific time periods fish were sacrificed and tissues like liver, kidney, muscle, brain and gill were isolated and used immediately for bioaccumulation studies.

Bioaccumulation studies:

The Cd concentration levels in the selected tissues were measured by following the method of Kanno et al. (1994). After the specified time intervals the test tissues like liver, kidney, muscle, brain and gill were isolated and then immediately they were washed with saline (0.9%) and 50mg of each tissue was digested in acid mixture of Nitric acid : Perchloric acid (3:2 V/V) for overnight. The acid mixture was then subjected to evaporation and the residue obtained was dissolved in 5ml of double distilled water. From this 1 ml was withdrawn and analyzed for Cd concentrations by using Atomic Absorption Spectrophotometer (Schimadzu AA 6300).

DATA ANALYSIS:

The data was subjected to statistical analysis such as mean, standard deviation and Analysis of variance (ANOVA) using standard statistical software, SPSS (version 16) package. All values are expressed as Mean ± SEM of 6 individual samples. Significant differences were indicated at $P < 0.05$ level.

RESULTS:

The accumulation of Cd significantly increased in the selected tissues with the increased period of Cd exposure when compared to control (Table-1). Maximum level of Cd bioaccumulation was observed in 30d fish kidney (22.535 ± 0.41 µg / g). Further liver tissue showed high Cd concentrations when compared to other tissues (Fig. 1). Low level of Cd accumulation was found in the brain and muscle tissues of fish over a period of 30 days. Among all the selected tissues, the lowest concentration of Cd was observed in the 30d muscle tissue (4.962 ± 0.23 µg / g).

After supplementation with Ca, the levels of Cd accumulation were progressively decreased in all the test tissues (Table - 2). 30d Muscle tissue showed maximum percentage of depletion in Cd accumulation (0.865 ± 0.30 µg / g). The accumulation of Cd was less in gills and brain with moderate concentrations in liver and kidney (4.55 ± 0.41 µg / g and 6.99 ± 0.28 µg / g) of the fish respectively (Fig. 2).

DISCUSSION:

The results revealed that Cd concentrations were significantly increased in all the test tissues at all the exposure periods. Maximum accumulation of Cd was observed in kidney and liver of O. mossambicus (22.353 ± 0.41µg/g and 15.797 µg / g respectively). The increased accumulation of Cd in the liver and kidney over time could be due to the involvement of these organs in the detoxification and removal of toxic substances circulating in the stream. Moreover, since these organs are the major organs of metabolic activities including detoxification of xenobiotics (Klaassen et al., 2009). Cd might also be transported into these organs from other tissues like the gills and muscle for the purpose of subsequent elimination. The kidney is thus the final destination of all the Cd from various tissues as it has also been shown that Cd-MT is filtered through the glomerulus and is reabsorbed by the proximal tubular cells, possibly by endocytosis. Within these cells the complex is taken up by lysosomes and degraded by proteases to release Cd, which may result in renal accumulation of the metal. Thus, these factors might have accounted for the raised level of the heavy metal in the kidney during the exposure periods. These findings corroborates those of Asagba et al., (2008) studies on fresh water cat fish (Clarias gariepinus) and accumulation in fish can be proportionally higher through dietary exposure than through water borne exposure (Szebedinnsky et al., 2001; Baldisserotto et al., 2005; Omer et al., 2012).

Gill also accumulates a higher proportion of Cd (11.580 ± 0.314 µg/g). Several reasons have been proposed to justify the gills as the primary site for Cd uptake, such as proximity to toxicants due to its external position, it's highly branched structural and vascular nature with the resultant highly increased surface area through which large volumes of water pass through the gill surface amongst other tissues (Jayakumar and Paul, 2006).

In the brain, Cd inhibits enzymes such as Mg^{2+}-ATPase and Na^{+}- K^{+}- ATPase causing metabolic effects and disrupting neurotransmitter uptake (Beauvais et al., 2001). In several situations acetylcholine is not broken and accumulates within synapses causing physiologic impairment and alterations in fish swimming behaviour (Glusczak et al., 2006). The reason for the consistent low level accumulation of Cd in the brain (3.271 ± 0.40 µg/g) is offered with certainty. However, a possible reason is that the blood brain barrier restricts the entry of Cd into the brain (Crowe and Morgan, 1997).

The muscle of fish accumulated lowest concentration of Cd (2.654 ± 0.30 µg/g), even after 30 days of exposure. This may not be unconnected with the fact that the muscle is not concerned with detoxification and metals like Cd and Pb spread uniformly over the muscle tissue and this may be the reason for low level of Cd accumulation in the muscle (Vinodhini and Narayanan, 2008).

The current study revealed interesting interactions between Ca supplementation and the response to Cd exposure. Among all exposure periods for the 30 day Ca supplementation, there was maximum reduction in tissue Cd accumulation. It is indicated that the extra Ca present in aquatic media inhibited water born Cd accumulation in the selected tissues of the experimental animal (Fig. 2).

It is clear from the present study that the toxicity of metal is affected by Ca which in turn reduces the toxic effect of a metal through competitive inhibition at the gill surface. The non toxic Ca competes with the toxic metals for the same binding sites. If Ca occupies the sites, the lamellae are protected from deterioration. Increased Ca levels in the medium resulted in a slower transfer of Cd from the gills to the blood and the rate of Cd accumulation was lowered in liver, kidney and other tissues. Similar findings were also reported in rainbow trout by Hollis et al., (2000), Baldisserotto et al., (2006) and in Cirrhina mrigala by Ghosh and Adhikari (2006).

It could be therefore concluded that Ca supplementation might play a vital role in reducing the Cd tissue burden of fresh water fish thereby mitigating the risk of potential hazards to human health.

ACKNOWLEDGEMENTS:

The authors are highly thankful to CSIR, New Delhi for the financial support rendered with the award of Major Research Project (No. 37(1450)/10/EMR-II, dated 09-12-2010) to Prof. A. Usha Rani, Dept. of Zoology, Sri Venkateswara University, Tirupati.

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Table – 1. Cd accumulation (µ g / g wet weight of the tissue) in different tissues of O. mossambicus after Cd exposure.

S.No.	Tissue	Control	Cd treated		
			7d	15d	30d
1.	Kidney	1.282 ± 0.080	6.380 ± 0.520	14.063 ± 0.123	22.353 ± 0.410
2.	Liver	1.413 ± 0.028	4.025 ± 0.330	11.014 ± 0.263	15.797 ± 0.370
3.	Muscle	0.636 ± 0.015	1.719 ± 0.266	3.016 ± 0.124	4.962 ± 0.230
4.	Brain	0.836 ± 0.013	2.828 ± 0.353	4.076 ± 0.339	9.146 ± 0.357
5.	Gill	1.728 ± 0.018	3.841 ± 0.326	6.175 ± 0.258	11.580 ± 0.314

All values are expressed as Mean ± SD of 6 individual samples.
All values are significant at $P<0.05$ level.

Table – 2. Cd accumulation (µ g / g wet weight of the tissue) in different tissues of O. mossambicus after Ca supplementation.

S.No.	Tissue	15d Cd	Ca supplementation		
			7d	15d	30d
1.	Kidney	14.063 ± 0.123	11.954 ± 0.363	9.862 ± 0.414	5.996 ± 0.424
2.	Liver	11.014 ± 0.263	8.434 ± 0.451	6.651 ± 0.368	3.850 ± 0.468
3.	Muscle	3.016 ± 0.124	2.122 ± 0.466	1.240 ± 0.390	0.455 ± 0.196
4.	Brain	4.076 ± 0.339	2.763 ± 0.500	1.886 ± 0.458	0.988 ± 0.294
5.	Gill	6.175 ± 0.258	4.580 ± 0.418	2.920 ± 0.612	1.470 ± 0.254

All values are expressed as Mean ± SD of 6 individual samples.
All values are significant at $P<0.05$ level.

Fig. 1. Cd Bio-accumulation levels (µ g / g wet weight of the tissue) in selected tissues of Cd exposed O. mossambicus (Tilapia).

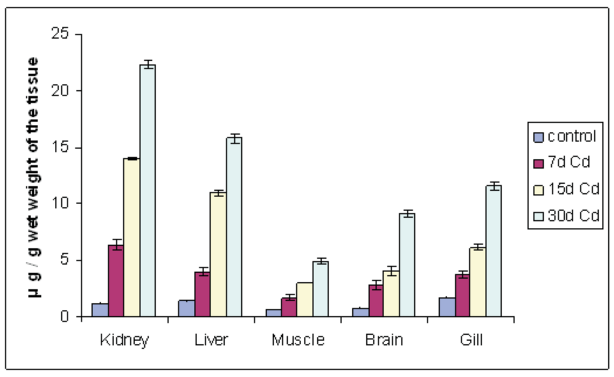

Fig.2. Cd Bio-accumulation levels (µ g / g wet weight of the tissue) in selected tissues of O. mossambicus (Tilapia) after Ca supplementation.