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Cadmium Induced Metallothionein Synthesis In Liver Of *Oreochromis Niloticus* [Linnaeus, 1758]

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Abstract

Objective(s): Metallothionein (MT) are potential biomarkers that reflect the presence of heavy metals in the ecosystem/food chain. Their synthesis has been observed to be elevated after heavy metal exposure in aquatic organisms. OREOCHROMIS NILOTICUS (O. niloticus) is a globally important aquaculture species. Hence the objective of this study was to determine the Cadmium (Cd) levels and MT induction in liver tissue of O. niloticus, to study the relationship between tissue-specific Cd accumulation and MT induction and hence correlate MTs protective role against Cadmium
Method(s): Cd accumulation and MT induction levels were determined according to the methods of Ma et al., 2007. Cd concentration was determined using an Atomic Absorption Spectrophotometer (Perkin Elmer Optima-5300 DV).
Findings: At specific sub lethal Cadmium exposure, w.r.t time, MT induction levels were found to be increase correspondingly to Cd levels in liver tissue. The accumulation of Cd levels in liver tissue is seen to be time and dose dependent. A positive correlation between MT induction and Cd accumulation was observed. The results suggest that MT in the liver could play a role in trapping and binding Cd and its subsequent elimination. Hence, the present investigation reveals that heavy metal induced MT levels can be considered as a biomarker for waterborne Cd contamination. A positive correlation between cadmium accumulation and MT synthesis in the liver tissue of the experimental animal is an indicator of the role of MT in heavy metal sequestration and adaptation to heavy metal contaminated ecosystems.
Novelty: The results suggest that MT in the liver could play an important role by binding to Cd and its subsequent elimination. Hence, the present investigation reveals that heavy metal induced MT levels can be considered as a biomarker for waterborne Cd contamination.

Keywords: Oreochromisniloticus, hepatic cells, Cadmium, Metallothionein (MT), bioaccumulation

1. INTRODUCTION

Environmental pollution, today, has become a global problem. Heavy metals are major contributors to aquatic pollution. Toxicity, bioaccumulation and biomagnification of heavy metals is a serious threat to organisms in freshwater and marine ecosystems. Anthropogenic domestic and industrial activities are the source of heavy metals [1,2]. Heavy metals enter into aquatic food chains including fishes and gets accumulated in various trophic levels leading to bioaccumulation and biomagnification. In fishes, heavy metals cause impairment of metabolic, physiological and structural changes.
Cadmium is a toxic heavy metal. It occurs naturally in trace amounts in water. Due to anthropogenic activities like mining, smelting and electroplating industries, the levels of cadmium have increased [3]. In the aquatic ecosystem the biologic half-life of cadmium in the kidney is estimated to be between 6 to 38 years; the half-life of cadmium in the liver is between 4 and 19 years (ATSDR 1999) [4]. Excess amounts of cadmium in aquatic ecosystem have toxic effects on the aquatic organisms. Fishes being the primary consumers in the trophic level Cadmium accumulation in the tissues alters physiological process. Cadmium influence proteins and enzymes that changes enzyme structures [5]. Cadmium accumulates mainly in liver, kidney, gills of freshwater fish [6] hearts [7] and other tissues [8]. Cadmium alters morphological and histological nature of liver [9], carbohydrate metabolism [10], disrupts endocrine system [11], reproductive system and growth rate [12] in fishes. Cadmium bioaccumulates and biomagnifies and enters human through food chain and causes health risks.
Metallothionein (MTs) are low molecular weight (approximately 600-7000 Da) metal binding proteins. MTs are cystine rich proteins found in microorganism, plants and animals. MTs bring homeostatic control of essential metals such as Copper and Zinc. MTs also plays a role in detoxification of nonessential metals such as Cadmium [13]. Metallothionein was first discovered in kidney tissue of horse [14]. Metallothionein are also produced as a part of defence mechanism [15] against metal toxicity and oxidative stress. MTs detoxify metals by sequestering and reducing the amount of free metal ions [16]. MTs are inducible by heavy metals and binds to heavy metals tissues of fishes. In fishes it was found that metals induce MT synthesis [17]. Physiological stress in the liver of fish can be evaluated in terms of metal accumulation and metallothionein induction. It was suggested that MT plays a role in detoxification of heavy metals [18]. MT can be used as a potential biomarker to know the heavy metal pollution in aquatic ecosystem.
The present study aims to find the induction of MT due to heavy metal cadmium at sublethal concentrations and to conclude that MT can be used as biomarker for heavy metal (Cd) pollution.

2. MATERIALS AND METHODS

2.1 Sample Collection

Oreochromisniloticus (commonly called Nile tilapia), the experimental animal for the present study, was collected from Pulicat Lake in the Tiruvallur district of Tamil Nadu, India, with an average weight of 50±5 grams. The fishes were acclimatized for 15 days in 100-liter cement ponds. The water was maintained at 30°C ± 2°C with a photoperiod of 12 hours of darkness and 12 hours of light.

2.2 Cd Treatment

Experimental fish were subjected to a sub-lethal $CdCl_2$ concentrations of 2.5 ppm and 5 ppm for 0, 24, 48, 72 and 96 hours. Analytical grade $CdCl_2$ was used for the experiments. Fishes with similar size, length and weight (50±5 g) were selected and divided into 5 groups of 10 fishes each. The Control group and the other four group of fishes were kept in five aquaria each with a volume of 10 litre containing 0 ppm, 2.5 ppm, and 5.0 ppm. Uneaten food and dead fishes were removed every day. Tanks were continuously aerated, and water was periodically filtered.

2.3 Determination of Heavy Metal Content

The experimental water and the commercial fish food pellets used as feed were tested for cadmium (Cd) and other heavy metal using ICP-OES (Perkin Elmer Optima-5300 DV) at 228 nm and found to Below Detectable Level (BDL) before initiating the experiment. After 24h, 48h, 72 h and 96 hours, fish were euthanized with tricaine methane-sulfonate (MS-222), and liver tissue was aseptically extracted. The Cadmium levels was determined using the protocol of Ma *et.al.,* [19]. 0.25mg of the liver tissue was weighed, homogenised and dried in an oven at 80 °C for 48 hours. It was then digested in 10 ml HNO_3 and 5 ml H_2O_2 over a hot plate at 120 °C. Cd content in the processed liver tissue was measured by AAS. The accumulated Cd concentration was indicated in µg/g.

2.4 Estimation of Tissue MT

The liver tissue of the fish was saturated with Cadmium Chloride in vitro at the concentration of 6.4mg/kg (Onosaka et al., 1984). The excess Cadmium and all the Cd ligands other than MT in the sample was removed by the addition of RBS hemolysate in a subsequent heating step. Since it is known that 1 mole of thionein (MW 6050) binds with 6-7 atoms of Cd, the actual concentration of MT in the liver tissue is calculated after estimation of Cd^{2+} in the heated supernatant using AAS as presented above.

3. RESULTS AND DISCUSSION

The data obtained after analysing the liver tissues for Cd and MT with respect to dose and time using standard protocols were tabulated in Table 1. It is observed that the Cd treated group of fishes showed significant accumulation of cadmium compared to the control group in the liver tissue. The increase in cadmium concentration in the treated group over the observed time intervals indicates the impact of cadmium uptake on exposure to Cd. Statistical analysis validates the significance of the observed values which are in conformation with Suresh *et al.,* 2015, who observed a similar response to cadmium treatment in their study."

Table 1. Cadmium uptake (mg/L) – dose – time table , values are given as Mean ± S.D, (n=8) and $p < 0.05$ level significance

Concentration of $CdCl_2$ (mg/L)	Concentration of Cd (mg/L) in Liver tissue				
	0 hours	24 hours	48 hours	72 hours	96 hours
Control	0.1±0.02	0.1±0.02	0.1±0.02	0.1±0.02	0.1±0.02
Treated with 2.5 ppm $CdCl_2$	0.11±0.03	0.98±0.06	1.19±0.03	1.31±0.03	1.55±0.07
Treated with 5 ppm $CdCl_2$	0.11±0.03	1.59±0.03	1.89±0.03	2.11±0.03	2.35±0.07

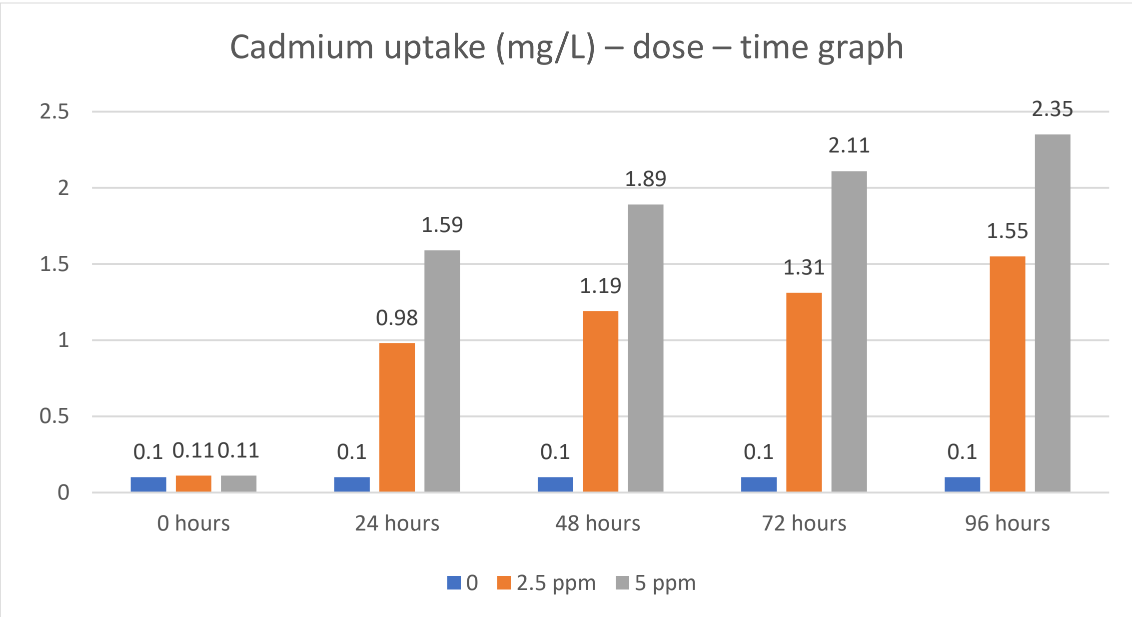


Fig 1 Graphical representation of Cd uptake between control and Cd exposed groups.

The data obtained after analyzing the liver tissues for MT after exposed to Cadmium with respect to specific concentration, dose and time using standard protocols are tabulated in Table 2. It is observed that the Cd treated group of fishes showed significant accumulation of cadmium compared to the control group in the liver tissue. The increase in cadmium concentration in the treated group over the observed time intervals indicates the cellular uptake of Cd on exposure.

The data in TABLE 2 suggests that Cadmium exposure had a substantial impact on metallothionein synthesis rates. The treated group exhibited a significant increase compared to the control group at each time point, indicating a significant response to the Cd dosage. The observed increase in Metallothionein concentration confirms with studies by [Sumit*et al.,* 2014) [20], highlighting the role of Metallothionein in metal detoxification."

Table 2. Metallothionein synthesis (mg/l) – dose – time table. Values are given as Mean ± S.D, (n=8) and p < 0.05 level significance

$CdCl_2$	Metallothionein concentration(mg/L) Mean ± SD				
	0 hours	24 hours	48 hours	72 hours	96 hours
Control	18.24±1.34	18.24±1.34	18.24±1.34	18.24±1.34	18.24±1.34
Treated with 2.5 ppm $CdCl_2$	18.24±1.34	81.24±12.24	104.13±10.34	138.24±10.54	180.46±11.34
Treated with 5 ppm $CdCl_2$	18.24±1.34	96.46±13.67	158.36±26.66	233.28±34.78	311.67± 45.4

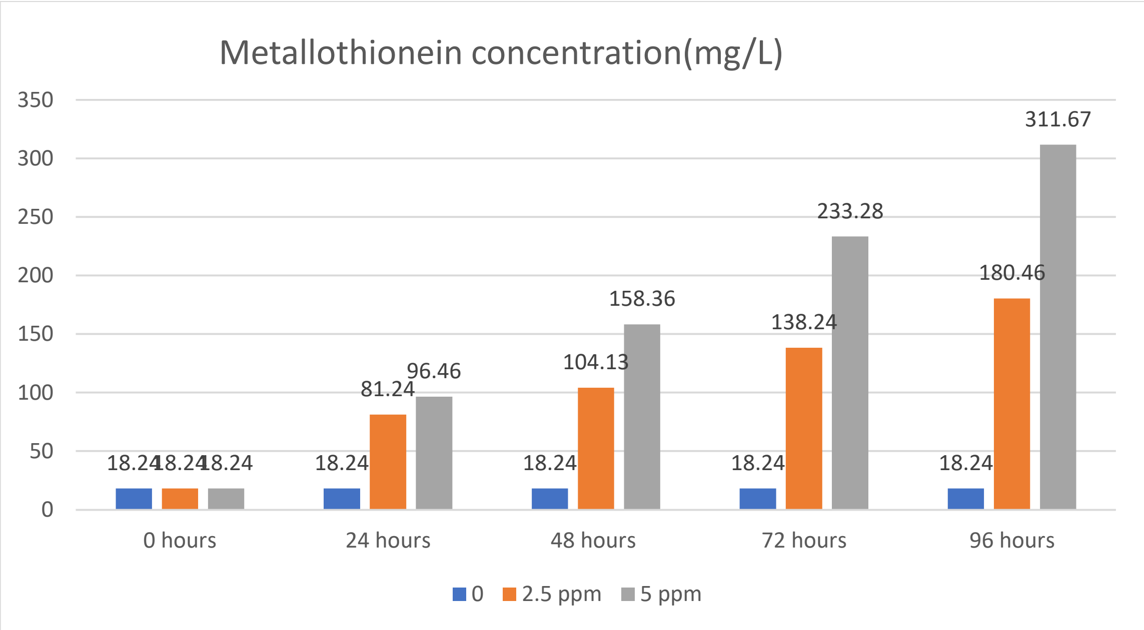
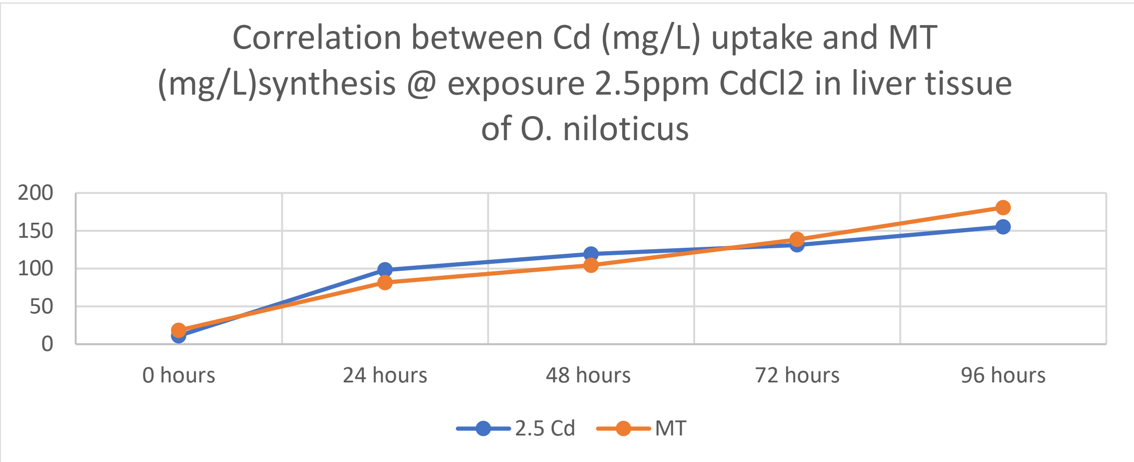


Fig 2 Graphical representation of MT synthesis in control and Cd exposed groups.

Correspondingly the development of MT was induced following the Cd uptake and it was found that the MT level in the treated group has increased from 18.24 mg/l to 311.67 mg / l of the liver tissue after four days of exposure to Cd toxicity. There is a time dependent positive correlation between the Cd uptake and the induction of MT protein in the liver of the fish. The trend line graph between the Cd concentration and MT levels in the liver of the fish is shown in the fig below.



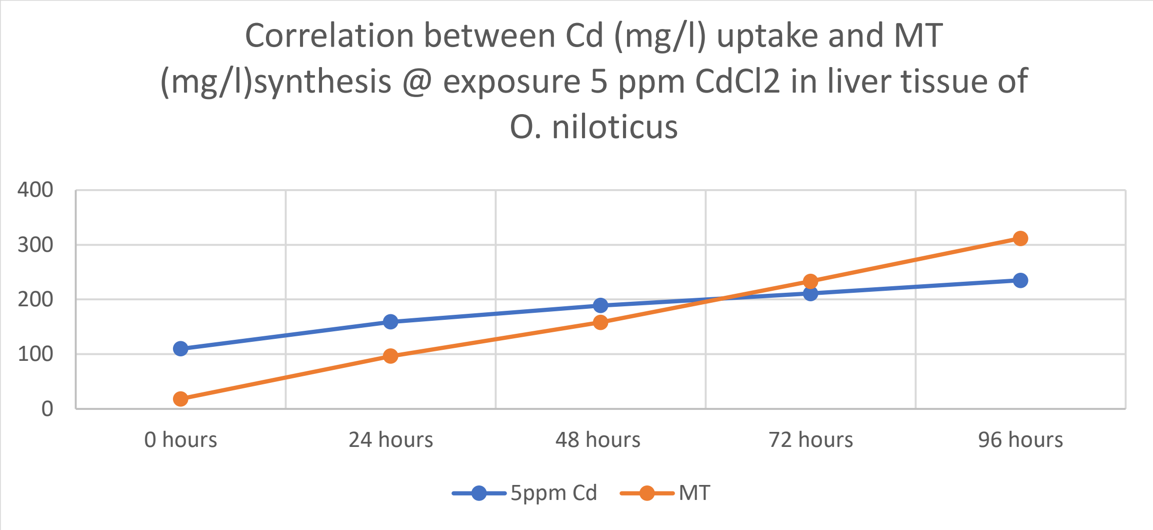


Fig 3: Graph showing correlation between Cd uptake and MT levels in the liver tissue of the fish.

4. CONCLUSIONS

In the present study fish exposed to the metal developed MT protein over a period of 24h, 48h, 72h and 96h. Since liver is the organ of detoxication, MT levels were increased on prolonged exposure to the heavy metal in water especially hitting the peak on the fourth day. This indicates that the MT protein has given protection and provided a means of survival to the fish in the toxic environment.

The observed increase in cadmium concentration in the treated group may have profound environmental implications. Cadmium is a known toxic heavy metal with detrimental effects on aquatic ecosystems. The elevated levels could potentially disrupt the balance of the ecosystem, affecting various trophic levels. If the study area is in proximity to human populations, there might be concerns about water quality for consumption and potential contamination of aquatic food sources. Elevated cadmium levels in water bodies can enter the food chain, leading to bioaccumulation in edible aquatic organisms. The synthesis of MT is an indicator of the evolution of detoxification mechanisms and survival adaptations in organisms vulnerable to exposure to heavy metals.

The findings confirm with the study by Suresh *et al., (*2015)[21], indicating a consistent response to cadmium treatment across different research contexts. Comparisons with other relevant literature could highlight common patterns or disparities in cadmium accumulation.

CONFLICT OF INTERESTS

Declared None

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