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### **RESEARCH ARTICLE**

# **EFFECT OF CADMIUM CHLORIDE ON GLYCOGEN CONTENT IN GILL, LIVER AND** KIDNEY OF EDIBLE EXOTIC FISH Hypophthalmichthys molitrix

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#### **ARTICLE INFO**

### ABSTRACT

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### **INTRODUCTION**

Human activities have led to accumulation of toxic metals in the aquatic environment (Yang and Rose, 2003; Heyvaert et al., 2000). The adverse input of diverse industrial wastes has aggravated the problem of contamination, and sewage disposal has greatly enhanced the addition of heavy metals into the aquatic ecosystem. Trace element pollution of the sediment in rivers, lakes, estuaries and bays caused by industrialisation has been reported by many researchers around the world (Karbassi et al., 2006; Al-Masri, 2002; Coker et al., 1995). The steady development of industrialization over the past six decades in the countries and developing regions of the world depend upon the expansions of more and more chemical industries and technology. It is true that such development is really necessary for the growing needs of an increasing human population and for improving our standard of living. This rapid industrialization and green revolution introduced a large variety of chemicals into the environment. These chemicals create serious ecological problems particularly aquatic pollution (Kharat et al., 2010). A great variety of pollutants affect the majority of water course which receive domestic. industrial and agricultural effluents. The complexity of this situation becomes apparent when toxicity is keenly considered in terms of its ramifications and environmental consequence. The contamination of freshwater with heavy metals such as cadmium and lead has become a matter of great concern over the past decades not only because of their threat to public water supplies but also because of the damage caused to aquatic life especially fishes (Tawari-Fufeyin et al., 2008).

Heavy metal contamination in the aquatic environment is a potential threat for aquatic organisms, when exposed to significant amounts of metals as consequences of industrial, agricultural and anthropological activities. Heavy metals at high concentrations can cause harmful effects on metabolic, physiological, and biochemical systems of fishes and it causes long-term ecotoxicological effects. The aim of the present study was to assess the glycogen content in gill, liver and kidney of the fish Hypophthalmichthys molitrix exposed to sublethal concentrations of cadmium chloride 1/5<sup>th</sup> (high), 1/10<sup>th</sup> (medium) and 1/15<sup>th</sup> (low) of the 96 hour LC<sub>50</sub> values for the period of 7, 14 and 21 days. The exotic fish, Hypophthalmichthys molitrix was exposed to sublethal concentrations of cadmium chloride for various exposure periods (7, 14 and 21days). Glycogen levels were measured both in control and experimental fish. During various exposure periods, the glycogen levels were (P<0.05) significantly decreased in the experimental fish over the control.

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Metals are commonly found in the environment, they are present as a natural elements or as a result of anthropogenic activities in different environmental media such as air, water and soil, which constitute an important factor of exposure to animals and human (Louis, 1993). Heavy metals are considered as one of the most important factors which affect fish population, reducing their growth, reproduction and/or survival rate (Mohamed and Saleh, 1996; Saeed, 2000). Cadmium belongs to the group of highly toxic heavy metals. Naturally, it occurs in water only in trace amounts, but recently its levels have increased due to anthropogenic activities (Papina, 2001; Kovarova et al., 2009). Most cadmium contamination comes from metal foundries, the dye industry, production of plastics and of accumulators. This exposure results in pathological changes in water ecosystems, mostly demonstrated in fishes, which are affected by heavy metals through the respiratory and digestive systems and through the skin. In general, toxic effects of all heavy metals are similar, including pathological changes in parenchymatous organs and the nervous system. Indeed, long-term exposure of cadmium has some specific effects like impairment of reproductive function and endocrine disruption. Current accepted opinion of cadmium action as well as other metals is related mainly to their influence on protein molecules, particularly enzymes. They have a strong affinity to bond with the aminoacid moieties of proteins and may cause changes in enzyme structures. The most obvious consequences of these changes are the inhibition of enzymes (Drastichova et al., 2004). The contamination of fresh waters with a wide range of pollutants has become a matter of concern over the last few decades (Vutukuru, 2005; Dirilgen, 2001; Voegborlo et al.,

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1999; Canli *et al.*, 1998). The natural aquatic systems may extensively be contaminated with heavy metals released from domestic, industrial and other man-made activities (Velez and Montoro, 1998; Conacher, *et al.*, 1993). Heavy metal contamination may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms (Farombi, *et al.*, 2007; Vosyliene and Jankaite, 2006). Among animal species, fishes are the inhabitants that cannot escape from the detrimental effects of these pollutants (Olaifa *et al.*, 2004; Clarkson, 1998; Dickman and Leung, 1998). Fish are widely used to evaluate the health of aquatic ecosystems because pollutants build up in the food chain and are responsible for adverse effects and death in the aquatic systems (Farkas *et al.*, 2002; Yousuf and El-Shahawi, 1999; Vinodhini and Narayanan, 2008).

Carbohydrates are the main source of energy that is ingested by the human body (Caffall and Mohnen, 2009). Glucose is the major energy source in the body. Glycogen is the storage form of glucose and glycogen is stored in skeletal muscles and liver. If glucose intake exceeds than it is utilized in the body it is converted into fat (Asif et al., 2011). The effect of heavy metals on the alterations in the biochemical substances of the body is profusely studied by many investigators in fishes. Metal intoxication in fishes usually results in glycogen depletion and is reported in several species of fishes, such as Heteropneustes fossilis (Qayyam and Shaffi, 1977); Sarotheradon mossamibicus (Akhilender Naidu, 1982); Channa punctatus (Sastry and Sunita, 1983) and Labeo rohita (Bengery and Patil, 1986). Shukla and Sastry (1990) studied the effects of cadmium on some biochemical and physiological parameters in fish Channa punctatus. They showed that these fishes were, hypoglycemic, hypolactemic and the total plasma proteins, the levels of glycogen, lactic acid, pyruvic acid and total proteins in liver and muscles decreased significantly in both acute and chronic exposure. However, no information is on record concerning the different sublethal concentration of heavy metal cadmium chloride effect on the glycogen levels of Hypophthalmichthys molitrix.

## **MATERIALS AND METHODS**

The fish Hypophthalmichthys molitrix having mean weight 14-16 gm and length 12 – 14 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 seven days. They were fed on rice bran and oil cake daily. The cadmium chloride was used in this study and stock solutions were prepared. Cadmium chloride  $LC_{50}$  was found out for 96 h (28mg/L) (Sprague, 1971) and  $1/15^{\text{th}}$ ,  $1/10^{\text{th}}$  and  $1/5^{\text{th}}$  of the LC<sub>50</sub> values were 1.86, 2.8 and 5.6mg/L 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 cadmium chloride and served as the control. The other 3 groups were exposed to sub lethal concentration of cadmium chloride in 10 litre capacity aquaria. The 2nd, 3rd and 4th groups were exposed to cadmium chloride for 7, 14 and 21 days respectively. At the end of each exposure period, the fish were sacrificed and the required tissues were collected for glycogen estimation. The glycogen content of the tissues was estimated by the method of Kemp and Kits Van Heijininger (1954). The data so obtained were analyzed by applying analysis of variance DMRT one way ANOVA to test the level of significance (Duncan, 1957).

### RESULTS

Depletion of glycogen content in the gill, liver and kidney of *Hypophthalmichthys molitrix* exposed to the cadmium chloride for 7, 14 and 21 days in  $1/15^{\text{th}}$ ,  $1/10^{\text{th}}$  and  $1/5^{\text{th}}$  of the LC<sub>50</sub> values of sublethal concentrations were estimated. Among these, the maximum depletion of glycogen was observed in liver during 21 days. Generally, depletion in glycogen content is directly proportional to the exposure period of the toxicant. The obtained biochemical estimation values of the gill, liver and kidney were subjected to statistical analysis and showed significant values at P<0.05 (Table 1).

| Organ                          | Concentration        | 7 Days                             | 14 Days                             | 21 Days                             |
|--------------------------------|----------------------|------------------------------------|-------------------------------------|-------------------------------------|
|                                | Control              | $30.12 \pm 2.29^{b}$               | $30.46 \pm 2.32^{\circ}$            | $31.28 \pm 2.38^{d}$                |
| Gill                           | Low concentration    | $29.64 \pm 2.25^{b}$               | $28.35 \pm 2.16^{\circ}$            | $26.62 \pm 2.02^{\circ}$            |
| %change over control<br>Gill   | Medium Concentration | -1.59<br>28.52 $\pm 2.17^{ab}$     | -6.93<br>25.08 ± 1.91 <sup>b</sup>  | -14.89<br>19.54 ± 1.48 <sup>b</sup> |
| %change over control<br>Gill   | High concentration   | -5.31<br>26.38 ± 2.01 <sup>a</sup> | -17.66<br>20.74 ± 1.58 <sup>a</sup> | -37.53<br>14.88 ± 1.13 <sup>a</sup> |
| %change over control           | -                    | - 12.42                            | - 31.91                             | - 52.43                             |
|                                | Control              | $78.32 \pm 5.97^{\circ}$           | $78.56 \pm 5.98^{\circ}$            | $79.22 \pm 6.03^{d}$                |
| Liver                          | Low concentration    | $75.46 \pm 5.74^{bc}$              | $73.24 \pm 5.57^{bc}$               | $71.06 \pm 5.41^{\circ}$            |
| %change over control           |                      | - 3.65                             | - 6.77                              | - 10.30                             |
| Liver                          | Medium Concentration | $71.28\pm5.42^{ab}$                | $67.82 \pm 5.16^{\circ}$            | $62.74 \pm 4.78^{b}$                |
| %change over control           |                      | - 8.99                             | - 13.67                             | - 20.80                             |
| Liver                          | High concentration   | $65.54 \pm 4.99^{a}$               | $56.94 \pm 4.33^{a}$                | $48.35 \pm 3.68^{a}$                |
| %change over control           | Control              | - 16.32 c                          | - 27.52 c                           | - 38.97 <sub>d</sub>                |
|                                |                      | $25.76 \pm 1.96$                   | $25.54 \pm 1.94$                    | $26.98 \pm 2.05$                    |
| Kidney                         | Low concentration    | $24.18 \pm 1.84^{\circ\circ}$      | $23.66 \pm 1.80^{\circ}$            | $22.82 \pm 1.73^{\circ}$            |
| %change over control           |                      | - 6.13                             | - 7.36                              | - 15.42                             |
| Kidney                         | Medium Concentration | $23.36 \pm 1.77^{b}$               | $20.72 \pm 1.57^{b}$                | $18.64 \pm 1.42^{\circ}$            |
| %change over control<br>Kidney | High concentration   | - 10.02                            | - 19.07                             | - 30.91 a                           |
| 2                              | righ concentration   | $20.64 \pm 1.57^{a}$               | $16.28 \pm 1.24^{a}$                | $12.44 \pm 0.94^{a}$                |
| %change over control           |                      | - 19.88                            | - 36.26                             | - 53.89                             |

Table 1. Glycogen levels changes (mg/g) in gill, liver and kidney of *Hypopthalmichthys molitrix* exposed to sublethal concentration of cadmium chloride

All the values are mean  $\pm$  SD of six observations; +/\_ indicates the % change over control; Values which are not sharing common superscript differ significantly at 5% level (n < 0.05): Duncan Multiple Range Test (DMRT).

### DISCUSSION

Glycogen, a large and branched polymer of glucose, is the storage form of carbohydrate for virtually every organism from yeast to primates. The major glycogen stores in mammalian vertebrates exist in liver and muscle, smaller amounts of glycogen being present in kidney, intestine and several other tissues. Classically, it is thought that the glycogen stored in liver, kidney and intestine can be made accessible to other organs by virtue of their possession of an enzyme glucose-6-phosphatase (Vornanen et al., 2011). Glycogen levels are found to be highest in liver, as it is the chief organ of carbohydrate metabolism in animals, followed by muscle. Liver glycogen is concerned with storage and export of hexose units for maintenance of blood glucose and that of muscle glycogen is to act as a readily available source of hexose units for glycolysis within the muscle itself. A fall in the glycogen level clearly indicates its rapid utilization to meet the enhanced energy demands in fish exposed to toxicant through glycolysis or Hexose Monophosphate pathway. It is assumed that decrease in glycogen content may be due to the inhibition of hormones which contribute to glycogen synthesis (Sobha et al., 2007).

The results of the present study showed that the sublethal concentrations of heavy metal cadmium chloride significantly altered the glycogen levels in gill, liver and kidney of Hypophthalmichthys molitrix after 7, 14 and 21 days exposure. The glycogen levels were decreased in the gill, liver and kidney of Hypophthalmichthys molitrix when exposed to sublethal concentrations of cadmium chloride may be glycogenolysis takes place by the action of heavy metal cadmium chloride. A fall in glycogen levels clearly indicates its rapid utilization to meet the enhanced energy demands in pesticide treated individuals through glycolysis or hexose monophospahte pathway (Cappon and Nicholes, 1975). Decreased glycogen synthesis is attributed to inhibition of enzyme glycogen synthesis (Stamp and Lesker, 1967). The decreased carbohydrate level is also attributed to the conversion of carbohydrates into aminoacids as observed by Gaiton et al., (1965). Alteration of carbohydrate metabolism is observed in Tilapia mossambicus exposed to arsenic toxicity (Shobha Rani et al., 2000) in Labeo rohita exposed to arsenic trioxide (Pazhanisamy, 2002) and in Mystal guili exposed to lead (Kasthuri and Chandran, 1997).

Carbohydrates are stored as glycogen in fish tissue and organs like the muscle and liver in order to supply the energy needs when there are hypoxic conditions, intensive stocking and a lack of food (Cicik and Engin, 2005; Wendelaar-Bonga, 1997). It has been demonstrated that liver glycogen levels decreased in Oncorhynchus mykiss as a result of the activation of glycolytic enzymes via catecholamines under lack of food and hypoxic conditions (Vijayan and Moon, 1992). The carbohydrate metabolism of the fish used in the present experiment might also have been affected by the lack of food since they were not fed during the experiments. It was also found that heavy metals could create stress in fish (Richard et al., 1998) and that cadmium could decrease glycogen reserves in the American eel (Anguilla rostrata) by increasing the production of catecholamines from the adrenomedulla (Gill and Epple, 1993). Prolonged environmental stress in fish makes adaptation difficult and creates weakness in fish.

Weakness is characterised by decreases in liver glycogen and serum cortisol levels, which subsequently create a series of alterations in the metabolism and shorten the life span of organisms (Heath, 1995). Some investigations also showed that heavy metals could decrease the glycogen reserves in fish (Levesque *et al.*, 2002) by affecting the activities of enzymes that play a role in the carbohydrate metabolism. Cadmium decreased the glycogen reserves in Heteropneustes fossilis by stimulating glycolytic enzymes like lactate dehydrogenase, pyruvate dehydrogenase and succinate dehydrogenase (Sastry and Subhadra, 1982). The decrease in glycogen reserves in the muscle and liver tissues of fish under heavy metal toxicity has been demonstrated to change with species (Sastry and Rao,1984; Naidu *et al.*, 1984).

Decrease in carbohydrates is probably due to glycogenolysis and utilization of glucose to meet increased metabolic cost as suggested by Viswarajan et al. (1988) in Oreochromis mossambicus under the stress of tannic acid. Decrease in liver glycogen may also be due to acute hypoxia (Heath and Pritchard, 1965). Decrease in glucose and glycogen content in gill tissue has been observed in Anabas testudineus fingerlings when exposed to mercuric chloride (Jagadeesan, 1990). The decreased level of glucose and glycogen contents in the liver, muscle, intestine, kidney and brain of Channa punctatus exposed to phenyl mercuric acetate (Karuppasamy, 2000). Shoba Rani et al. (2000) have also observed the decline in gill glycogen content in Tilapia mossambica exposed to sodium arsenite. Stressful situation in fish elicits neuroendocirine response which in turn induces disturbances in carbohydrate metabolism (Mazeand et al., 1977) and this lend support to the present results in declined glycogen levels in the gill, liver and kidney of Hypophthalmichthys molitrix when exposed to sublethal concentration of cadmium chloride. In conclusion, this study showed that cadmium chloride altered the carbohydrate metabolism in Hypophthalmichthys molitrix by affecting the levels of glycogen in gill, liver and kidney due to impairments in energy requiring vital processes.

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