

“The Sublethal Toxicity of Cadmium on Glycogen Levels in the liver and Muscle Tissues of fish *Oreochromis mossambicus*”

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Abstract

The level of glycogen (polysaccharide) reserves in liver and muscles tissues were studied in fish, *Oreochromis mossambicus* exposed to sublethal concentrations of heavy metal cadmium compared with the levels measured in the controlled groups. A decrease in glycogen content was observed in both the liver and muscle tissues, indicates the possible effect of cadmium toxicity in fishes, subjected to increasing concentration of cadmium and the time period of exposure to different sub-lethal concentrations.

KEYWORDS: Glycogen, *Oreochromis mossambicus*, liver, muscles, Cadmium.

Introduction

Heavy metal accumulation in fish is matter of considerable practical importance since, in many parts of the world pollutants containing heavy metals are being discharged in to water bodies which fishes are taken for consumption. The natural waters have a high potential risk for receiving metals from anthropogenic sources, such as water runoff, sewage treatment plants and domestic garbage dumps which eventually cause adverse on biota. The filtration of toxic heavy metals into aquatic ecosystems is on the increase due to natural factors such as anthropogenic and geochemical. In aquatic ecosystems the toxic metals are carried via the food chain to the top tropic level and create important ecological imbalances and problems. The cadmium is one of biologically a non- essential heavy metals, it has a cumulative polluting effect and could cause toxicity to aquatic organisms even in low concentration. The high accumulation of heavy metals in abiotic and biotic components can lead to serious ecological consequence. In polluted environments concentrations can be considerably higher (USEPA, 2001)[23]. Aquatic organisms are affected by water hardness and some other characteristics of aquatic media. The toxic effect of cadmium in various aquatic animals, fishes are numerous like retardation of growth and development, pathological changes in organs such as liver and tissues. Lemaire, GS., Lmaire, P. 1992)[7]. These heavy metals tend to accumulate in metabolically active tissues and organs like liver. The accumulation rate of heavy metals such as cadmium in various aquatic animals including fishes depending upon sex, size, age, feeding status of the organisms (Witeska, M., Jezierska, B. Chaber J., 1995)[24]. In fish tissues and liver could change when exposed to cadmium, is more sensitive to physiological and biochemical parameters. (Sastry. K. V., Rao D.R. 1984)[15]. It has been found that cadmium could change glycogen reserve in fish by affecting liver activity that have roles in the carbohydrate metabolism glycolysis and gluconeogenesis (Levesque, H. M., T. W. Campbell, P.G. C. Hoentela, A. 2002) [8]. In fish muscles could be used as an indicator of heavy metal toxicity of cadmium on several biochemical parameters. (Togyani. A. Fauconneau. B., Boujard, T., Fostier., A. Kuhn, E. R. Mol. K.A. Baroiller. J. F. 1997)[21]. Due to heavy metal cadmium toxicity in an aquatic environment

exert an extra stress on fish, there must be some changes in glycogen reserves in fish when exposed to cadmium indicates the status of fish.

The heavy metals enter into the hydrosphere via many pathways. The different aquatic environment like rivers, lakes, river streams, estuaries and even ocean may thus be affected by heavy metals like cadmium either by concentration and degradation. The high concentration have been found to cause toxic effects to aquatic animal like fishes, lobsters, crabs, etc. which are economically important as food for human consumption of cadmium creates ecological and physiological imbalance. The toxicity of cadmium produces toxic effect on a wide range of animals. The life span condition factors and health are all functions of metabolic activities in fish exposed to heavy metals. This study the aimed to demonstrate effect of glycogen reserves in liver and muscles of *Oreochromis mossambicus* which has economic value in Kalwa creek area and Thane city premises in Maharashtra.

Materials and methods:-

For the present experiment the fish *Oreochromis mossambicus* have been selected as a model test species for the present experiment. With the help of local fisherman's the active and healthy specimen of fishes collected from Kalwa creek, commonly named as Thane creek in Maharashtra and brought to a controlled laboratory conditions in aquaria measures 20cm in width , 30cm in length and 15cm height. The mean weights, lengths of the fishes used in experiments. Because metabolic activities changes with size and in the experiments individuals of similar size and length were used and effects the parameters to be measured Canli, M., Furness, R. W. (1993) [1]. To avoid any fungal infection they were washed with 0.1% KMnO4 solution and examined for any pathological symptoms. The fishes were controlled laboratory environment and acclimatized to the laboratory condition for about four weeks to laboratory conditions in aquariae for being used for experiment. Fishes were regularly fed with dried tubifex worms and chironomus larvae. Feeding was stopped two days before being used for experiments. Chlorine free tap-water was used throughout the course of the experiment. Some of the physicochemical parameters of the cadmium free test water used in the experiment such as total alkalinity, total acidity, total alkalinity, total hardness, pH and temperature, were regularly reported and listed in table no.1.

Table 1: Physicochemical parameters of test water

| | |
|--|-------------|
| Temperature | 27 0C |
| PH | 7.3 |
| Do | 5.9mg/litre |
| Free chlorine | Nil |
| Total acidity | 3.5mg/litre |
| Total alkalinity | 44mg/litre |
| Total hardness (as CaCO ₃) | 31mg/litre |

After a general selection for healthy and same group of fishes (4.2±0.5) cm. and (0.850±0.5) in weight, they were transferred to glass aquaria containing de-chlorinated tap-water. After acclimatization, the healthy fishes were selected for experimental purpose without sex-discrimination. 10 such fishes were transferred to glass aquaria of 50 litres capacity, each

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Abstract

The fish *Mystus gulio* was exposed to sub-lethal concentrations of heavy metal cadmium. In the present study the evaluation of toxic effect of heavy metal cadmium on serum electrolyte profile on fish *Mystus gulio*. The sodium and potassium ion content decreases as the concentration of toxicants increases. The excretion of more loss ions through excretory organs increased because of induced stress.

KEYWORDS: *Mystus gulio*, Cadmium, Serum, electrolytes, Sodium, Potassium, ions, excretory organs.

Introduction

Natural waters have a high potential risk for receiving metals from natural, geochemical and anthropogenic sources such as an urban runoff, domestic garbage dumps and sewage water treatment plants which causes adverse effects on both biotic and abiotic ecosystem. Due to industrialization, transportation, urbanization there is tremendous dumping of various toxic heavy metals including cadmium in aquatic media. The toxic heavy metals are carried via the food chain to the upper trophic levels in aquatic ecosystem and create vital ecological issues. Cadmium is one of a non-essential heavy metal. Even in minute concentrations of cadmium could cause toxicity to aquatic organisms. The mechanism of heavy metal deposition, detoxification and excretion in specially fishes are not possible in short time hence heavy metal tends to accumulate in metabolically active tissues and organs Langston R. W. (1989). The accumulation of cadmium in fish depends upon the sex, age, size and feeding status. Pollution of water is responsible for a very large number of mortalities and incapacitation in the world. Polluted state of water resources has led to steady decline in fisheries. The insecticides which are liberated into the aquatic ecosystem have tremendous effect on fish and thereby a man. The poikilothermic animals have a tremendous effect on the blood even minute fluctuation in environmental conditions.

Fresh water bodies and adjacent environments are ecological systems that are subjected to continuous stress by natural and man induced perturbations. Fishes *Mystus gulio* have been reported to maintain a relatively narrow range for concentrations of ions in their serum. An effect of sub-lethal concentration of cadmium exposure on sodium and potassium level in blood of *Mystus gulio* has been analyzed in the present study. There are reports on impairment of osmoregulatory activity in various fish species under toxicant stress. Gopi (1992) observed an increase in Na^+ , K^+ , Ca^{++} and Mg^{++} ions and decrease in Cl^- ions in blood of *Cyprinus carpio* chronically exposed to sub-lethal concentration of Fenthion. Other reports on effect of pesticides on osmoregulatory activity of fish include by Bano (1986), Sastry and Dasgupta (1991), Sivaram Prasad *et al.*, (1985).

Materials and Methods

Test fish *Mystus gulio* is selected for the study are more common edible fish species that has been used as test fish by number of researchers earlier. In the laboratory conditions both fish species can be easily collected, breed and maintained. *Mystus gulio* are collected and brought from kalwa creek or Thane creek in which are easily available. Both these fish were acclimatized in the laboratory for more than two weeks before actual use of bioassay test. These fish were maintained in the large glass aquaria containing aged tap water. The aged tap water needs to stabilize constituents of water and more important in the elimination of chlorine. The water in tank was about 2 liter/gm wet weight of the body of the test fish. The water was continuously aerated to maintain the dissolved oxygen relatively constant. Fish were feed with live tubifex worms and daphnia available in the market. Strictly avoided malnutrition and over feeding. To avoid any type of contamination and infection dead fishes was immediately removed. After every eight days entire water was replenished to keep metal concentrations constants. The aquaria were well aerated and dissolved oxygen levels were kept around $7.5 \pm 1.03 \text{mg}^{-1}$ through the experiment. They were kept free from any disturbances and mechanical shocks, injuries and overcrowding was avoided. The fish were exposed to diffused sunlight, the photoperiod being 10-12 hours. For bioassay test the disease free fish which were found to have acclimatized satisfactorily were used. Prior to the experimentation the fish were not fed for 24 hours. During experiment, Healthy fishes of uniform size and weight were selected. Ten fish of approximately same size were used for each concentration as test. Ten fish were also kept in toxicant free water under analogous conditions.

Acclimated fish of almost equal size (4.5 ± 0.5 cm) were exposed to three sub-lethal doses of cadmium for a period of 4 weeks. The tests were carried out on adult fish and the test period is often restricted to 4 weeks. The sub-lethal concentrations selected for cadmium is 0.021ppm, 0.014, 0.007 ppm.

The experimental and control tanks were maintained in duplicate. The fish were washed with tap water and dried using drying paper before collecting the blood samples. Blood sampling was done by incising the caudal peduncle. At the end of 4 weeks, blood was removed from control and exposed fish using hypodermic syringe. Blood was withdrawn by cardiac puncture or incision of tail Wedemeyer, G. A., Yasutake, W. T. (1977). The samples were centrifuged and Na^+ and K^+ content were estimated from clear supernatant. Na^+ and K^+ ion content was done by using a flame photometer as advocated by Hawk et al., (1965). Na^+ and K^+ ion concentration was expressed as meq/liters in blood of both fishes *Mystus gulio*.

Table No.1

Na^+ content in mmol/litre in the blood of fish, *Mystus gulio* during chronic cadmium exposure for a period of 4 weeks.

| Days of exposure | Control | Concentrations of Cadmium mg/litre | | |
|------------------|---------|------------------------------------|---------|---------|
| Initial | | 0.001 | 0.002 | 0.003 |
| 0 Days | 67.423 | 60.525 | 56.218 | 52.135 |
| SD | | 3.451 | 5.6025 | 7.644 |
| PV | | 10.7825 | 18.1251 | 25.5742 |

Discussion

In blood plasma Sodium is the main cation and is associated with maintenance of acid base balance along with Cl^- and HCO_3^- ions. It also deals with maintenance of osmotic pressure of body fluids, permeability of cells and normal irritability of muscles. In the present study may be attributed to inhibition of ATPase activity the decrease in Na^+ content of blood observed (Nilima Naik, 1991). It may also reveals to possible increase in permeability to Na^+ and K^+ ions as a result of which, these ions might have been lost from blood.

Potassium ions play vital role for maintenance of osmotic pressure, transport of carbon dioxide, muscle and nerve function and acid-base balance. These ions are also essential for protein biosynthesis by ribosomes. Even in case of K^+ ions of blood, inhibition of enzyme ATPase and increased ions or water permeability might have been the possible because of depletion. Inhibition of Na^+ and K^+ dependent ATPase in *Periophthalmus dipus* exposed to Fenvalerate is noted by Sivaram Prasad *et al.*, (1995).

Conclusion

In experimental fish *Mystus gulio* chronically exposed to sub-lethal doses of heavy metal cadmium significant depletion in Na^+ and K^+ ions in blood has been observed. The decrease in ion content of blood may either be due to stress induces loss of ions due to increased excretion through excretory organs.

The regulation of ionic composition of body fluids in animals is presumed to have adaptive significance (Burton, 1973).

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1. Materials and Methods

Test fishes *Mystus gulio* are selected for the study as more common edible fish species that has been used as test fish by number of researchers earlier. In the laboratory conditions both fish species can be easily collected, breed and maintained. *Mystus gulio* were collected and brought from Kalwa Creek or Thane Creek in which are easily available. Both these fish were acclimatized in the laboratory for more than two weeks before actual use of bioassay test. These fish were maintained in the large glass aquaria containing aged tap water. The aged tap water needs to stabilize constituents of water and more important in the elimination of chlorine. The water in tank was about 2 liter/gm wet weight of the body of the test fish. The water was continuously aerated to maintain the dissolved oxygen relatively constant. Fish were fed with live tubifex worms and daphnia available in the market. Strictly avoided malnutrition and over feeding. To avoid any type of contamination and infection dead fishes were immediately removed. After every eight days entire water was replenished to keep metal concentrations constant. The aquaria were well aerated and dissolved oxygen levels were kept around $7.5 \pm 1.03 \text{ mg}^{-1}$ throughout the experiment. They were kept free from any disturbances and mechanical shocks, injuries and overcrowding was avoided. The fish were exposed to diffused sunlight, the photoperiod being 10-12 hours. For bioassay test the disease free fish which were found to have acclimatized satisfactorily were used. Prior to the experimentation the fish were not fed for 24 hours. During experiment, healthy fishes of uniform size and weight were selected. Ten fish of approximately same size were used for each concentration as test. Ten fish were also kept in toxicant free water under analogous conditions.

Acclimated fish of almost equal size ($4.5 \pm 0.5 \text{ cm}$) were exposed to three sub-lethal doses of cadmium for a period of 4 weeks. The tests were carried out on adult fish and the test period is often restricted to 4 weeks. The sub-lethal concentrations selected for cadmium are 0.021 ppm, 0.014, 0.007 ppm. The experimental and control tanks were maintained in duplicate. The fish were washed with tap water and dried using drying paper before collecting the blood samples. Blood sampling was done by incising the caudal peduncle. At the end of 4 weeks, blood was removed from control and exposed fish using hypodermic syringe. Blood was withdrawn by cardiac puncture or incision of tail (Wedemeyer, G. A., Yasutake, W. T. (1977)). The samples were centrifuged and Na^+ and K^+ content were estimated from clear supernatant. Na^+ and K^+ ion content was done by using a flame photometer as advocated by Hawk et al., (1965). Na^+ and K^+ ion concentration was expressed as meq/liters in blood of both fishes *Mystus gulio*.

Table-7.7

Na^+ content in mmol/litre in the blood of fish, *Mystus gulio* during chronic cadmium exposure for a period of 4 weeks.

| Days of exposure | Control | Concentrations of Cadmium mg/litre | | |
|------------------|---------|------------------------------------|---------|---------|
| | | 0.001 | 0.002 | 0.003 |
| Initial | | 0.001 | 0.002 | 0.003 |
| 0 Days | 67.423 | 60.525 | 56.218 | 52.135 |
| SD | | 3.451 | 5.6025 | 7.644 |
| PV | | 10.7825 | 18.1251 | 25.5742 |
| 7 Days | 68.127 | 59.029 | 56.105 | 51.504 |
| SD | | 4.549 | 6.011 | 8.3115 |
| PV | | 14.31 | 19.3541 | 27.7905 |
| 14 Days | 68.136 | 58.103 | 52.303 | 50.214 |
| SD | | 5.0165 | 7.9165 | 8.961 |
| PV | | 15.8952 | 26.2921 | 30.2864 |
| 21 Days | 68.142 | 57.064 | 51.503 | 50.032 |
| SD | | 5.539 | 8.3195 | 9.055 |
| PV | | 17.6956 | 27.8139 | 30.6497 |
| 28 Days | 68.175 | 57.002 | 50.116 | 49.002 |
| SD | | 5.5875 | 9.0295 | 9.5865 |

depletion in Na⁺ and K⁺ ions in blood has been observed. The decrease in ion content of blood may either be due to stress induces loss of ions due to increased excretion through excretory organs.

The regulation of ionic composition of body fluids in animals is presumed to have adaptive significance (Burton,1973).

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7. References

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