Impact of mercury chloride contamination on protein,carbohydrate and haemoglobin content of Heteropneustes fossilis.

Wanaz Nasreen Islam[1*] & Priyanka Phukan[2] & Dr.Aparajita Borkotoki.[3]
[1*]Assistant Professor,University of Science and Technology, Meghalaya Technocity, Killing Road :BariduaMeghalaya-793101
2Student of M.Sc. IV th Sem., Department of Zoology, University of Science and Technology, Killing Road:Baridua, Meghalaya-793101
[3]Professor, Gauhati University, Guwahati, Assam, India.

Received: June 10, 2018 Accepted: July 21, 2018

ABSTRACT *Aquatic pollution has become a matter of great concern over the last few decades and heavy metals can pose serious threat to aquatic life since they cannot be destroyed through biological degradation. Mercury is classified as a heavy metal and is third most dangerous metal according to ATSDR(Agency for Toxic Substances and Disease Registry). In the present study toxic effects of mercury chloride was tested on widely consumed catfish Heteropneustes fossilis. The study was conducted to measure the impact of toxicity on the fish within a short period of time. Protein, Carbohydrate and Haemoglobin content were determined by standard methods. Protein and carbohydrate content was determined after a period of 15 and 30 days of treatment with mercury chloride while haemoglobin content was determined after 7 and 15 days. It was found that mercury exposure has a strong potential to alter the biochemical constituents in various tissues of H. fossilis. Decrease in protein, carbohydrate and haemoglobin content was found in all exposure period and this may be due to excessive demands under toxic stress.*

Keywords: *Toxic, Protein, Carbohydrate, Haemoglobin, Heteropneustes fossilis*

INTRODUCTION:

Aquatic systems are exposed to a number of pollutants that are mainly released from effluents discharged from industries, sewage treatment plants and drainage from urban and agricultural areas. These pollutants cause serious damage to aquatic life. (Karbassi et.al, 2006) The fact that heavy metals cannot be destroyed through biological degradation and have the ability to accumulate in the ecosystem make these harmful chemicals to the aquatic system and consequently to humans who depend on aquatic products as sources of food. (Di Giulio et.al, 2008) Mercury is classified as a heavy metal and it is considered a toxic metal released into the environment. Mercury is the third most dangerous metal right after arsenic and lead according to the Agency for Toxic Substance and Disease Registry (ATSDR).

Fish are widely used to evaluate the health of aquatic ecosystem and physiological changes serve as biomarkers of environmental pollution. (Thakur Vinod and Kanhere RR, 2014). Blood is an important component for studying the effects of toxicants as it is highly susceptible to environmental fluctuations. (Pandey and Pandey, 2001) Protein and carbohydrate content in fishes is a dynamic factor that varies not only between the fishes of different kinds but also among the same species. In general fishes contain 16-20% of protein and <0.5% of carbohydrates. It is used in electrical industries, chlorine and caustic soda, in nuclear reactors, dental offices, and in gold mining or pharmaceutical and antifungal products. Mercury exists in multiple oxidative states, as inorganic salts and as organic complexes. Mercury ions produce toxic effects by protein precipitation, enzyme inhibition and generalized corrosive action. Mercury not only binds to sulfhydryl groups but also to phosphoryl, carboxyl, amide and amine groups. Proteins with such groups readily available are susceptible to reaction with mercury. Once bound to mercury most proteins are rendered inactive. Toxicity is a part related to the oxidative state and to the chemical form. Most fish, both freshwater and salt water contain methylmercury. While the GI tract is the primary route of absorption methylmercury can be absorbed through the skin and lungs as well. FDA advisory limit for methylmercury in commercial fish is 1ppm. By comparison concentration of 10-30ppm were present during the minamata epidemic. WHO provisional value of mercury (for inorganic mercury) is 0.006mg/ml. Fish contaminated with mercury suffer serious pathological changes, occurring inhibition of metabolic processes, blood disorders, reduced fertility and survival.

Keeping in view all the above facts,a study was planned to throw some light on the protein,carbohydrate and haematological changes brought about by mercuric exposure on one of the most commercially important fish *Heteropneustes fossilis*. *Heteropneustes fossilis* also known as stinging catfish has a very wide

distribution range(India, Pakistan, Srilanka, Nepal, Bangladesh, Myanmar, Thailand and Laos) and has been introduced elsewhere while it is heavily utilized for food and medicine in many parts of its range.

It is uniform grey-brown to olive brown in color. Body is elongate,compressed,abdomen rounded and it has 4 pairs of barbles and two elongated pulmonary sacs that run backwards from the gills through the muscles in the back. It inhabits freshwater rarely brackish water. Its air breathing apparatus enables it to exist in almost any kind of water. The females are stockier looking than the males. It attains sexual maturity at one year of age when the male is generally 5.5cm and female is 12cm.These fishes feed upon almost anything such as tablets and pellet forms.

MATERIALS AND METHOD:

Fish *H.fossilis* were collected and kept in aquarium for a period of 30 days. Fishes were daily fed with fish feed. Acute toxicity tests were conducted to measure the impact of toxicity on the fishes. Mercury level that was used was slightly higher (i.e., 0.05mg/ml) than the provisional value as prescribed by WHO (i.e., 0.006mg/ml for inorganic mercury). The sample was divided into 3 groups. For protein and carbohydrates Group I serve as control. Group II was exposed to sublethal concentration of mercury for 15days and Group III for 30days. For haemoglobin group I serve as control. Group II was exposed to sublethal concentration of mercury for 7 days and group III for 15days. The protein, carbohydrate and haemoglobin content were estimated by Lowry's method (1971), Anthrone technique for total carbohydrates and haemoglobin estimation by Sahli's haemoglobinometer (acid haematin method) respectively.

RESULTS AND DISCUSSION:

While treating the fish with mercury chloride it was found that mercury have significant toxic effect on the survivability of the exposed fishes. All the three factors i.e., protein, carbohydrate and haemoglobin content declined when the fish were treated with mercury. The weight of the fish both experimental and control was approx 50 grams.

Table 1: Effect of mercury chloride in the protein content of fish:

Fish	Weight(gram) approx	Protein (mg/ml)
Control	50	0.92
Experimental (15days)	50	0.75
Experimental (30days)	50	0.54

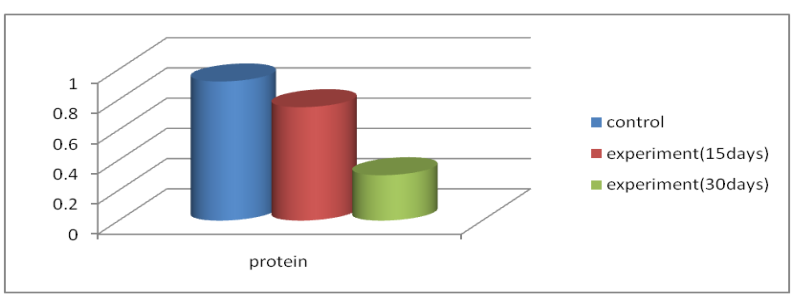

Fig 1: Comparison between control and experimental tests in protein estimation

In the present study protein content of muscles of fish were examined in control as well as mercury treated fish. It is evident from table 1 that protein content declined from its control value when it was treated with mercury chloride. Comparative diagram is shown in fig 1. In the present study the heavy metal mercury depleted the protein content of the muscle of the fish, suggesting a proteolytic effect of the heavy metals possibly to meet the excessive demands under stress. Proteins are involved in major physiological events therefore the assessment of the protein can be considered as a diagnostic tool to determine the physiological phases of organism. Proteins are highly sensitive to heavy metal poisoning (Jacobs et.al., 1977).

Depletion of protein content has been observed in muscles of the fish *H.fossilis* as a result of mercury chloride toxicity. When an animal is under stress, diversification of energy occurs to accomplish the impeding energy demands and hence protein level is depleted. (Neff, 1985). The depletion of total protein content may be due to breakdown of protein into free amino acid under the effect of mercury chloride. (Shakoori et.al, 1994)

Metals could alter the structure, permeability and integrity of lysosomal membranes resulting in the diffusion of their enzymes into cytosol. Hence high activity of protease, a lysosomal enzyme, in the organs of fish might be due to damage caused by mercury to lysosomes. (Sternlib et.al, 1976)

The decrease in protein level may be attributed to the stress condition and excess intermediate metabolism. (Ajaya Bikkini and Prasanta Nanda, 2016). There was also depletion in protein content of liver when H. fossilis is under metal stress. (Nanda, 1997). The increase in glucose and decrease in protein may be due to inhibition of glycolysis (Simons, 1979) and that leads to alternative pathways of aminoacid degradation used for energy source. (Gupta and Rai, 1995)

Table 2: Effect of mercury chloride on carbohydrate content of fish:

Fish	Weight (gram) approx	Carbohydrate (mg/ml)
Control	50	0.36
Experimental (15days)	50	0.28
Experimental (30days)	50	0.18

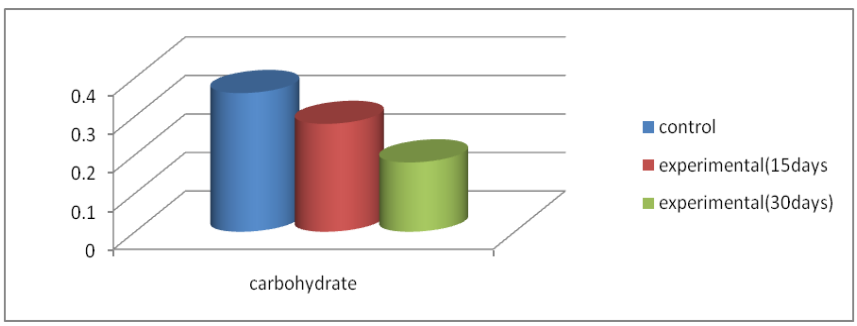

Fig 2: Comparison between control and experimental tests in carbohydrate estimation

Blood glucose levels have long been used as indicators of stress in fish. Under conditions of stress hyperglycemia may provide additional energy during the times of high metabolic need such as a "Fight" or "Flight" response. Reported that alteration of carbohydrate metabolism towards high circulating glucose level and gluconeogenesis are consistent responses of fish to acidic condition. (G.G Goss et. al, 1988)

In the present study it was found that although carbohydrate content is much less in control fish but that is also declined when the fish was treated with mercury chloride indicating its toxic effects on the fish. The values that were found during the tests is given in table 2 and comparative diagram is shown in fig 2 above.

The disturbance in the carbohydrate metabolism was considered as one of the most outstanding biological lesions due to actions of heavy metal (De Bruin, 1970). The decrease in carbohydrate content in the muscle may be due to glucose utilization to meet excess energy demand imposed by severe anaerobic stress of mercury intoxication. (Margarat et.al, 1999)

Another possible reason for depletion in tissue may be due to impairment of glycogen synthesis. Under hypoxic conditions fish derive energy breakdown of glucose which is available to the cells with increased glycogenolysis. The observed depletion of carbohydrate in the present study explains the increased energy demands of those molecules to provide energy for the cellular biochemical process under toxic manifestations. (Villalan et al., 1988)

Table 3: Effect of mercury chloride in the haemoglobin content of fish:

Fish	Weight (gram) approx	Haemoglobin (g/dl)
Control	50	10.8
Experimental (7days)	50	7.9
Experimental (15days)	50	5.2

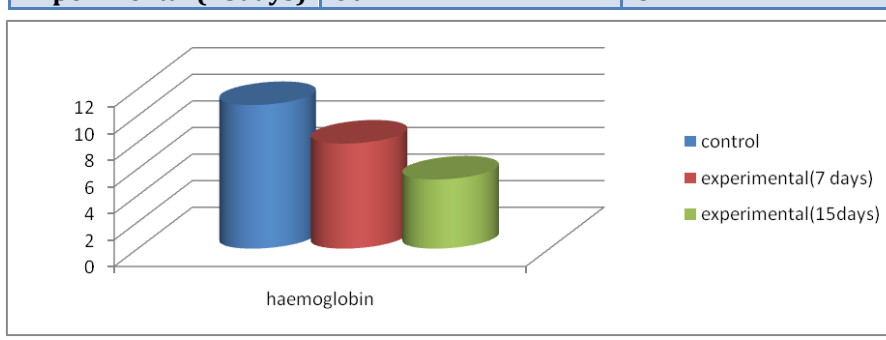

Fig 3: Comparison between control and experimental test in haemoglobin estimation

The present study showed decrease in haemoglobin content in H.fossilis from its control value when treated with mercury chloride. Thus mercury chloride has significant toxic effects on haemoglobin content as well which is evident from the values shown in table 3.

Normally anaemic conditions are marked when fishes are exposed to heavy metals (Nanda et al 1996) .Fish could not absorb enough iron from the intestine to produce necessary amount of haemoglobin. It may be due to anemia developed in the fish because of the interference of mercury chloride with haemopoesis (Verma et al., 1982) and impaired absorption of iron by intestinal wall. (Joshi, 2002)

Any changes in the environment affect the physiology of the fish. Blood is said to be the mirror in which all vital processes taking place in the organism are reflected. The blood properties are indispensable for understanding the biological process, taking place in the fish species (Hawkns and Thomasi, 1971).

Fish hematology is an essential tool for the biologists as a frontline sensitive indicator of vital physiological and biochemical functions as well as status of nutrition, health diseases and stress in response to changing environmental conditions.

The haematological assessment is the rising motive to prove any disturbances of environmental influences on the fishes, fishes show abnormal effects due to food, habitat temperature, pH and many other elements of environment. (Fange, 1994)

Fish blood is being studied in toxicological research and environmental monitoring as a possible indicator of physiological and pathological change in fishery management (Gupta and Gupta, 1981)

The lowering of RBC count may be due to destructive action of mercury on the red blood cells. The damaging effects of erythrocytes may be secondary, resulting from a primary action of the toxicant on the erythropoietin tissue due to which there exists a failure in red cell production. (Rupam Kumari et al., 2017).

CONCLUSION:

From the present study it is concluded that mercury exposure has a strong potential to alter the biochemical constituents in various tissues of *H.fossilis*. Proteins and carbohydrate being vital biochemical constituents for growth and development are directly affected with subsequent exposure for mercury. Depletion of protein was observed at all exposure periods. The decline in the level of proteins after exposure to mercury chloride may be due to extensive proteolysis in muscle of the fish. The decrease in carbohydrate content in the muscle may be due to glucose utilization to meet excess energy demand imposed by severe anaerobic stress of mercury intoxication.

Haemoglobin content also decreased when the fish was exposed to mercury chloride. This decrease in haemoglobin levels of *H.Fossilis* indicates stressful condition of fish. This suggests that mercury chloride can negatively affect the physiology of fish.

These effects of the heavy metals can be attributed to the excessive demands under the toxic stress and require further study to demonstrate the possible effect in higher animals including humans.

REFERENCE:

1. Ajaya bikini and Prasanta Nanda (2016) Vanadium Toxicity to fish Heteropneustes fossilis
2. Bhalerao SN (2016) Herbal protection against mercury uptake and histological damage in gills of H.fossilis.
3. Di Giulio RT, Hinton DE. The toxicology of fishes, Tylor and Francis, 2008)
4. Fange (1994) reviewed the blood cell, haemopoesis and lymphoid tissues in fish and taking into consideration the species and seasonal variation, evaluated the health of wild and cultured fish.
5. GG Goss, CM Wood (1988) the effects of acid/aluminium exposure on circulating plasma cortisol levels and other blood parameters in rainbow trout.
6. G.Jayaprakash, N.Raju,T.Bheem Rao and Y.Venkaiah(2015) effect of melathion on biochemical constituents of fresh water cat fish Heteropneustes fossilis
7. Gupta BK, Gupta RB. (1981) haematological observation on the Channa punctatus .
8. Gupta S, Rai V. Effect of Vanadium on phosphatases and transaminases in certain organs of Clarius batrachus.
9. Hameed and Muthukumavel (2006) effect of cadmium on the biochemical constituents of fresh water fish Oreochromis mossabicus.
10. Hawkns M, Thomasi T (1979) fish haematology bibliography.
11. Holeton, GF (1970) Oxygen uptake and circulation by haemoglobinless Antarctic fish
12. Karbassi R, Bayati I, Moattar F, (2006) origin and chemical portioning of heavy metals in riverbed sediments.
13. Kumudini Mishra, Amrita Khatri (2015) Alteration in protein and glucose level in the ovary of a freah water cat fish H.fossilis exposed to textile dyes.
14. K.V.Shastry and Rao D.R (1981) Enzymological and biochemical changes by mercuric chloride in Channa punctatus.
15. Lalit Choudhary and Seema Bharadwaj (2015) Effect of heavy metal pollution on survival and protein changes in liver and intestine of fresh water fish Tilapia mossambica
16. Lowry, O.H., Rosenberbrough, N.J. and Randall, R. J. (1951) Protein measurements with folin phenol reagent.

17. Margarat, A., Jagadeesan G, (1999). Abtodotal recovery ion carbohydrate metabolism of mercury intoxicated mice.
18. Mayank C, Radha C, Keerikkattil P. (2015) Toxic effects of Lead nitrate on Testis of Catfish H.fossilis
19. Mosammat Salma akter (2009) Arsenic and mercury induce death of Anabus testudineus involving fragmentation and chromosomal DNA.
20. M.V.Raghavendra Rao (2017) Study of effects of heavy metals on the biochemical constituents of protein metabolism and total ninhydrin positive substances using alternate animal model: Heropmetus Fulvipes
21. Nanda P (1997) Nickel toxicity to liver and brain of a cat fish Heteropneustes fossilis.
22. P.Martin deva prasath, S.Arivoli (2008) Biochemical study of freshwater fish Catla catla with reference to mercury chloride.
23. Pandey AK, Pandey G.C Thiram and Ziram fungicides indues alteration on some haematological parameters of freshwater catfish H.fossilis.
24. Qayyum and Shaffi (1977) Changes in the tissue glycogen on a freshwater catfish Heteropneustes fossilis due to mercury intoxication
25. Rupam kumara, Raj Kumar, Arjun Pratap Singh (2017) Impact of sublethal concentration of aquatholon some haematological parameters of Indian catfish Heteropneustes fossilis
26. Sastry, K.V and Rao, D.R. (1981) Enzymological and biochemical changes produced by mercuric chloride treatment on the activities of a few digestive enzyme in teleost fish Channa punctatus
27. Shakoori, A.R., Javed Iqbal, Latif Mughal, Sayed Shahid, (1994) Biochemical changes induced by inorganic mercury on the blood, liver and muscle of fresh Ctenopharyngodon idella.
28. Simons TJB. Vanadate a new tool for biologist. (1979)
29. Sternlib.I and Goldfisher, S (1976) heavy metals and lysosomes. In: Lysosomes in biology pathology.
30. S.Gupta , R.Shrivastava (2006) Environmental Toxicology.
31. Thakur Vinod, Kanhere RR (2014) Comparative study on the protective role of vitamin C and A on mercury induced toxicity in H.fossils
32. Verma SR, Tonk IP (1983) Effect of sublethal concentration of mercury composition of liver, muscle and ovary of Notopterus notopterus.
33. Villalan, J.B (1969) Measurement of pollutant toxicity to fish I. Bioassay methods for acute toxicity.
34. V.Sethuraman, Badhul Haq, A Chezhian, S.Shanker, D.Senthamil Selvan (1852) changes due to the effect of the heavy metal concentration on the marine fish, Tilapia Mossambica
35. Vutukuru SS (2013) acute effects of mercuric chloride on glycogen and protein content of Zebra fish, Danio rerio