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EFFECT OF HEAVY METAL, NICKEL CHLORIDE ON PROTEIN AND AMINO ACID LEVELS IN GILL, LIVER AND KIDNEY OF FRESHWATER FISH, *Oreochromis mossambicus* (PETERS)

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Abstract

The universal problem is the environmental pollution and most important pollutants are the heavy metals in aquatic network because of their toxicity, accumulation and bio-magnification by aquatic animals. Domestic, industrial and anthropogenic activities are broadly become the source of natural aquatic systems contamination of heavy metals. Proteins occupy a unique position in the metabolism of cell because of the proteinaceous nature of all the enzymes which mediate at various metabolic pathways. The aim of the present study was to assess the protein and amino acid levels in gill, liver and kidney of *Oreochromis mossambicus* was exposed to sub-lethal concentration of nickel chloride for the period of 10, 20 and 30 days. The fish exposed to nickel chloride showed a decrease the protein and increase the amino acid levels for 10, 20 and 30 days in gill, liver and kidney. The objective of the present work was to observe the effect of nickel chloride on protein and amino acid levels in the gill, liver and kidney of freshwater fish, *Oreochromis mossambicus.*

Key words: Protein, amino acid, nickel chloride, sub-lethal concentration, *Oreochromis mossambicus.*

1. Introduction

Water pollution is one of the principal environmental and public health problems (Osman and Kloas, 2010). Water pollution is a cosmopolitan problem that needs urgent attention and prevention (Osman, 2007). It resulted from many sources, e.g. accidental spillage of chemical wastes, discharge of industrial or sewerage effluents, agricultural drainage, domestic wastewater and gasoline from fishery boots (Handy, 1994; Ali and Soltan, 1996). Agricultural, industrial and domestic effluents generally contain a wide variety of organic and inorganic pollutants, such as solvents, oils, heavy metals, pesticides, fertilizers and suspended solids (Pandey *et al.*, 2003) and are, invariably discharged into small rivers and streams, without proper treatment. Such contaminants change water quality and may cause many problems to fish, such as diseases, structural alterations (Chang *et al.*, 1998) and functional changes in the organs of the organisms.

Over the past few decades, heavy metal contamination of aquatic system has attracted the attention of several investigators both in the developed and developing countries of the world (Farombi *et al*., 2007). Many industrial and agricultural processes have contributed to the contamination of fresh water systems thereby causing adverse effects on aquatic biota and

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human health (Wang, 2002; Dautremapuits *et al*., 2004). The fact that heavy metals cannot be destroyed through biological degradation and have the ability to accumulate in the environment make these toxicants deleterious to the aquatic environment and consequently to humans who depend on aquatic products as sources of food. Heavy metals can accumulate in the tissues of aquatic animals and as such tissue concentrations of heavy metals can be of public health concern to both animals and humans (Kalay *et al*., 1999; Ashraf, 2005).

Heavy metals are ubiquitous in the biosphere, where they occur as part of the natural background of chemicals. Anthropogenic activities have also introduced substantial amounts of them into the environment by mobilization from their natural insoluble deposits or environmental sinks (Chiesa *et al.,* 2006). They represent a significant ecological and public health concern due to their toxicity and their ability to accumulate in living organisms. The heavy metals tend to concentrate in the body and cause damage in the kidneys, lungs, brain and nervous system and in the body metabolism (Fergusson, 1990). Nickel plays important roles in the biology of microorganisms and plants. In fact urease (an enzyme which assists in the hydrolysis of urea) contains nickel. Exposure to nickel metal and soluble compounds should not exceed 0.05 mg/cm³ in nickel equivalents per 40 hour work week. Nickel sulfide fume and dust is believed to be carcinogenic. Sensitized individuals may develop an allergy to nickel, affecting their skin, also known as dermatitis (Barceloux and Barceloux, 1999; Sigel *et al*., 2008).

Different kind of nickel compounds present in the environment due to various industrial activities affect the health of human beings, animals and plants. Among the nickel compounds some of them soluble in water and insoluble in water. Nickel may occur as a soluble species, such as nickel sulphate and nickel chloride, or an insoluble species, such as nickel sulphide and nickel oxide. The dominant nickel species in urban air pollution is nickel sulphate, followed by nickel oxide. Some nickel species produced during refining and smelting, such as nickel sub-sulphide, are not usually found in the urban environment, but occupational exposures are composed of both soluble and insoluble components. Nickel fumes are also produced during stainless steel and alloy welding and soldering (Antonini *et al.*, 2004). Occupational exposures to nickel occur in the form of airborne dust and particles during mining, refining and smelting, as well as during work in factories and chemical plants (Klein and Costa, 2007). The present investigation was to assess the protein and amino acid content in gill, liver and kidney of *Oreochromis mossambicus* exposed to sub-lethal concentration of nickel chloride.

2. Materials and Methods

The fish *Oreochromis mossambicus* having mean weight 35 - 40 g and length 15 – 18 cm were collected from PSP fish farm, at Puthur and acclimatized to laboratory conditions. They were given the treatment of 0.1% $KMNO_4$ solution and then kept in plastic pools for acclimatization for a period of two weeks. They were fed on rice bran and oil cake daily. The nickel chloride was used in this study and stock solutions were prepared. Nickel chloride, LC_{50} was found out for 96 hrs (37 ppm) (Sprague, 1971) and $1/10^{th}$ of the LC_{50} value was 0.37 ppm taken as sub-lethal concentration for this study. Forty fish were selected and divided into 4 groups of 10 each. The first group was maintained in free from nickel chloride and served as the control. The other 3 groups were exposed to sub lethal concentration of nickel chloride, 10 litre capacity aquaria. The 2^{nd}, 3^{rd} and 4^{th} groups were exposed to nickel chloride, for 10, 20 and 30 days respectively. At the end of each exposure period, the fish were sacrificed and the required tissues were collected for protein and amino acid estimation. The protein and amino acid content in gill, liver and kidney of *Oreochromis mossambicus* were estimated by the method of Lowry *et al.* (1951) and Moore and Stein (1954) respectively. The data obtained were analyzed by

applying analysis of student 'T' test (Trivedy and Hoel, 1984).

3. Results

Protein level in Gill

The protein contents were observed to be 56.18 ± 4.28, 48.16 ± 3.67 and 39.57 ± 3.01 mg g^{-1} wet wt. of tissues in the fish *Oreochromis mossambicus* when exposed to sub-lethal concentration of heavy metal nickel chloride for the periods of 10, 20 and 30 days respectively. The decrease in gill protein is gradual between 10 to 30 days (Table - 1).

Protein level in Liver

The protein content in the liver of *Oreochromis mossambicus* exposed to sub-lethal concentration of heavy metal nickel chloride showed a decrease when compared to control fish. The per cent change over the control was -10.80, -23.49 and –45.11 respectively at 10, 20 and 30 days of exposure (Table - 1).

Protein level in Kidney

The protein content of the kidney of *Oreochromis mossambicus* exposed to sub-lethal concentration of heavy metal nickel chloride showed significant decrease when compared to control fish. The protein contents of sub-lethal concentration of heavy metal nickel chloride exposed fish were observed as 73.85 ± 5.62, 66.13 ± 5.04 and 58.35 ± 4.44 mg g^{-1} wet wt. of tissue for the periods of 10, 20 and 30 days respectively.

The decrease in kidney protein content is more pronounced at 30 days of exposure periods (Table - 1).

Amino acid level in Gill

The fish *Oreochromis mossambicus* when exposed to sub-lethal concentration of heavy metal nickel chloride showed an increase of amino acid contents in gill than the control fish. In sub-lethal concentration of heavy metal nickel chloride exposed fish, the amino acids showed a gradual increase from 10 to 30 days. The maximum of 288.71 per cent raise was noticed at 30 days of exposure periods (Table - 2).

Amino acid level in Liver

In the sub-lethal concentration of heavy metal nickel chloride exposed fish of liver, the amino acid contents were observed as 20.38 ± 1.55, 27.46 ± 2.09 and 40.73 ± 3.10 mg g^{-1} wet wt. of tissues respectively for the periods of 10, 20 and 30 days. The increase in the amino acid content of the liver was more in 30 days of exposed fish compared to 10 and 20 days (Table - 2).

Amino acid level in Kidney

The fish *Oreochromis mossambicus* when exposed to sub-lethal concentration of heavy metal nickel chloride showed increase in kidney amino acid level compared to control fish. The amino acid contents were observed as 12.16 ± 0.93, 22.65 ± 1.72 and 30.19 ± 2.30 mg g^{-1} wet weight of tissues for the periods of 10, 20 and 30 days respectively (Table - 2).

4. Discussion

The contamination of aquatic ecosystem with a wide range of pollutants has become a matter of great concern over the last few decades, not only because of the threat to public water supplies, but also with of the damage caused to the aquatic life (Mary Josephine Rani *et al.*, 2011). Gills represent a thin and extensive surface in intimate contact with water. They carry out three main functions, gas exchange, ion regulation and excretion of metabolic waste products. Due to the constant contact with the external environment, gills are the first targets of waterborne pollutants (Perry and Laurent, 1993). Heavy metals enter the fish mainly through the gills, thus being the first target of the metal. The effect of cadmium on fish gill Na+, K+, ATPase, as well as its sensitivity to the metal, differs from species to species and probably from organ to organ (Benson *et al*., 1988).

Table - 1: Protein changes (mg/g wet wt. of tissue) in gill, liver and kidney of *Oreochromis mossambicus* exposed to sub-lethal concentration of heavy metal nickel chloride

Tissue	Exposure period (days)					
	10		20		30	
	Control	Treated	Control	Treated	Control	Treated
Gill	72.35 ± 5.51	56.18 ± 4.28	73.59 ± 5.60	48.16 ± 3.67	74.27 ± 5.66	39.57 ± 3.01
% Over control		- 22.35		- 34.56		- 46.72
Liver	97.26 ± 7.41	86.76 ± 6.61	98.54 ± 7.50	75.39 ± 5.74	99.17 ± 7.55	54.43 ± 4.91
% Over control		- 10.80		- 23.49		- 45.11
Kidney	81.64 ± 6.22	73.85 ± 5.62	81.95 ± 6.24	66.13 ± 5.04	82.27 ± 6.26	58.35 ± 4.44
% Over control		- 9.54		- 19.30		- 29.07

All the values are mean ± SD of six observations
Values are significant at 5% level ($p < 0.05$)
+/- indicate for percent increase or decrease over control.

Table – 2: Amino acid changes (mg/g wet wt. of tissue) in gill, liver and kidney of *Oreochromis mossambicus* exposed to sub-lethal concentration of heavy metal nickel chloride

Tissue	Exposure period (days)					
	10		20		30	
	Control	Treated	Control	Treated	Control	Treated
Gill	4.25 ± 0.32	8.64 ± 0.66	4.79 ± 0.36	12.15 ± 0.93	4.96 ± 0.38	19.82 ± 1.51
% Over control		103.29		153.65		288.71
Liver	11.12 ± 0.85	20.38 ± 1.55	11.75 ± 0.89	27.46 ± 2.09	12.28 ± 0.94	40.73 ± 3.10
% Over control		83.27		133.70		231.68
Kidney	7.34 ± 0.56	12.16 ± 0.93	7.49 ± 0.57	22.65 ± 1.72	7.83 ± 0.60	30.19 ± 2.30
% Over control		65.66		202.40		285.57

All the values are mean ± SD of six observations
Values are significant at 5% level ($p < 0.05$)
+/- indicate for percent increase or decrease over control.

The liver plays an important role in the synthesis of proteins. Gills are the vital organs in fish, which have direct contact with the medium through which pollutants enter into the body (Mount, 1962; Holden, 1972; Edwards, 1973). The impact of contaminants on aquatic ecosystem can be assessed by measurement of biochemical parameters in fish that respond specifically to the degree and type of contamination (Petrivalsky *et al.*, 1997). Tissue protein content has been suggested as an indicator of xenobiotic-induced stress in aquatic organisms (Singh and Sharma, 1998).

In the gill, liver and kidney protein content had decreased, whereas amino acids content had increased at all periods of exposure when *Oreochromis mossambicus* was exposed with sub-lethal concentration of nickel chloride. Similarly protein levels were decreased significantly in gill, liver and kidney of *Cyprinus carpio* exposed to sub-lethal concentration of pharmaceutical effluent (Muthulingam *et al.*, 2011). The protein content decreased in the liver, brain and kidney tissues of *Channa punctatus* during lihocin treatment (Abdul *et al.*, 2010). According to Sathyanarayana (2005), the physiological status of animal is usually indicated by the metabolic status of proteins. Jrueger *et al.* (1968) reported that the fish can get the energy through the catabolism of proteins. Proteins are mainly involved in the architecture of the cell, which is the chief source of

nitrogenous metabolism. Thus, the depletion of protein fraction in liver, brain and kidney tissues may have been due to their degradation and possible utilization for metabolic purposes. Increases in free amino acid levels were the result of breakdown of protein for energy and impaired incorporation of amino acids in protein synthesis (Singh *et al.*, 1996). The toxicants may have effect on hormonal balance, which could directly or indirectly affect the tissue protein levels (Murthy and Priyamvada, 1982; Khilare and Wagh, 1988). The protein content declined gradually in gill, liver and muscle tissues of *O. mossambicus* when exposed to deltamethrin and it was reported that it may be due to the utilization of protein controls to counteract the toxicant stress caused by pesticide (Rao and Rao, 1979; Rath and Mishra, 1980).

The sub-lethal concentration of nickel chloride caused a significant reduction in the liver protein content of *Oreochromis mossambicus* at all exposure periods. The liver is affected considerably when there is a disturbance in protein metabolism. The accumulation of toxic substance in liver may alter its function (Premdas and Anderson, 1963). Hori *et al.* (2006) have reported that liver protein decreased in phenol treated *Brycon cephalus*. Eva (1990) has reported a continuous reduction in protein content of the liver when *Anabas testudineus* exposed to sub-lethal concentration of Cuman L. Reduction in protein content of liver has been reported in *Sarotherodon mossambicus* exposed to lindane (Rajamanickam, 1985). Balaji and Chockalingam (1990) have reported that the protein content of liver decreased in an air-breathing fish *Channa punctatus* when exposed to sub-lethal concentration of dairy effluent. The level of protein content in the tissues of fresh water fish *Rasbora daniconius* became decreased on exposure to sub-lethal concentrations of pulp and paper mill effluent (Vijayaram and Vasugi, 1989).

Meenakshi and Indra (1998) have reported that the protein content of liver decreased in *Mystus vittatus* when exposed to sub-lethal concentration of distillery effluent. The different concentration of malathion, thiodon and ekalux significantly reduced the total protein in liver of *O. mossambicus* (Palanichamy *et al.*, 1986). Similar observations were noted when the fish were exposed to pollutants (Lone and Javaid, 1976; Shakoori *et al.*, 1976; Rath and Mishra, 1980; Ramalingam and Ramalingam, 1982). The protein contents in liver of *Catla catla* are depleted under the sublethal stress of chromium (Vincent *et al.*, 1995). Palanichamy and Baskaran (1995) have reported a decrease in muscle and liver protein in *Channa striatus* exposed to mercury, cadmium and lead for a period of 21 days. Karuppasamy (1990) has reported the decrease in protein content of liver, muscle and kidney in *Channa punctatus* when exposed to sub-lethal concentration of sugar mill waste.

The sub-lethal concentration of nickel chloride caused a significant reduction in the kidney protein content of *Oreochromis mossambicus* at all exposure periods. Rao *et al*. (1980) and Devi (1981) have reported that the kidney was the site of degradation and detoxification of toxic substances. The decreased protein level in the kidney tissue at sub-lethal concentration of lead may be due to the enhanced proteolysis. The kidney, which is an important organ of excretion and osmoregulation, is indirectly affected by pollution through blood circulation (Newman and Maclean, 1974). A reduction in the protein content in the kidney could possibly be due to protein breakdown leading to increased amino acid pool of tissue (Radhaiah *et al.*, 1987). Rao (1989) has reported decreased protein content in kidney of *Catla catla* after exposure to endosulfan. The decreased protein level was observed in the kidney tissue of *Catla catla* at sub-lethal concentration of chromium (Vincent *et al.*, 1995). Ambrose *et al*. (1994) have observed a decrease in carbohydrate, protein and lipid contents of gill, liver and kidney in *Cyprinus carpio* exposed to sub-lethal concentration of tannery effluent. The protein content of the fresh water fish was decreased with increasing concentration of textile mill effluent (Nisha and Shukla, 1986).

Manoharan and Subbiah (1982) have reported that depletion in protein level was due to diversification of energy to meet the impending energy demand when the animals were under toxic stress. The reduction in protein content in the present study indicates that the tissue protein undergoes proteolysis resulting in the production of free amino acids. Many investigators have also recorded such a reduction in protein content in different tissues when the animals were exposed to different pollutants (Shah and Dubale, 1983; Palanichamy *et al.*, 1986; Palanichamy *et al.*, 1989).

When the fish, *Oreochromis mossambicus* was exposed to sub-lethal concentration of nickel chloride, the gill, liver and kidney amino acid level rapidly increased at all exposure periods. Similarly amino acid levels were significantly increased in gill, liver and kidney of *Cyprinus carpio* exposed to sub-lethal concentration of pharmaceutical effluent (Muthulingam *et al.*, 2011). The

free amino acid (FAA) pool was increased in the tissues of the fish during exposure to lihocin (Abdul *et al.*, 2010), while the elevated FAA levels were utilized for energy production by supplying them as keto acids into TCA cycle through aminotransferases to contribute energy needs during toxic stress. Increases in free amino acid levels were the result of breakdown of protein for energy and impaired incorporation of amino acids in protein synthesis (Singh *et al.*, 1996).

Eva (1990) has observed that an increase in amino acid content both in liver and intestine of *Anabas testudineus* when exposed to sub-lethal concentration of Cuman Balaji and Chockalingam (1990) have reported that the increase in amino acid content in liver of *Channa punctatus* when exposed to sub-lethal concentration of dairy effluent. The elevated amino acid levels in the kidney of sub-lethal treated *Cirrhinus mrigala* during effluent intoxication indicate a high turnover of amino acids, which should normally lead to increased deamination and oxidation of amino acids. Ravichandran *et al*. (1994) have attributed the decrease in protein content and an increase in amino acid content in the liver, kidney and muscle of *O. mossambicus* exposed to phenol.

Many investigators have also recorded such a reduction in protein content in fishes exposed to different toxicants (Karuppasamy, 1990; Rao, 1989; Vincent *et al*., 1995; Nisha and Shukla, 1986). A reduction in the protein content in the present investigation in *Oreochromis mossambicus* suggests that the tissue protein undergoes proteolysis, which results in an increase in the production of free amino acids. These amino acids are utilized for energy production during stressful situation in the intoxicated fishes. Moorthikumar and Muthulingam (2010) addressed that reduction in the protein and enhance the level of amino acid contents in liver, kidney and brain of *Labeo rohita* under heavy metal, nickel chloride stress. Senthil Elango and Muthulingam (2014) noticed that declining trends of protein and elevated levels of amino acid contents in brain and muscle of *Oreochromis mossambicus* was exposed to sub-lethal concentrations of chromium. It is evident that proteins are degraded to meet the energy requirements during nickel chloride exposure. It can be concluded that in *Oreochromis mossambicus* exposed to nickel chloride at sub-lethal concentration causes energy crisis and alter protein metabolism.

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