Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 4, Issue, 11, pp.257-261, November, 2012

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

STUDIES ON BIOCHEMICAL CHANGES AND INDUCTION OF METALLOTHIONEIN IN FRESHWATER CATFISH (*CLARIAS GARIEPINUS*) EXPOSED TO LEAD

Esther Vanchhawng*, S. S. Jayaraj and S. Vincent

Center for Environment Research and Development, Department of Advanced Zoology and Biotechnology, Loyola Institute of Frontier Energy, Loyola College, Chennai- 600 034, India

ARTICLE INFO

Article History:
Received xxxxxxxxxxxxxxx
Received in revised form
xxxxxxxxxxxxxxx
Accepted xxxxxxxxxxxxxxxx
Published online xxxxxxxxxxxxxxx

Key words:
Acid phaspatase, Alkaline phosphatase, Metallothionein, Catfish.

ABSTRACT

In the present study effect of sublethal concentration of lead (0.08 and 0.10 mg/L of 96h LC_{50} value) on the tissue profile of *Clarias gariepinus* was studied. *Clarias gariepinus* when exposed to sublethal concentration of lead for 10 days showed various changes in the tissue parameters. The protein and carbohydrate levels were decreased in higher concentration of lead. The acid phosphatase was increased in muscle and gill but decreased in the liver. The alkaline phosphatase increased and decreased in different concentration of lead. The metallothionein levels increased in the liver, gill and muscle when compare to control samples of catfish.

INTRODUCTION

Heavy metals are known pollutants which inflict disorder in aquatic system with deleterious effects on associated organisms. Heavy metals compounds and other potentially hazardous materials are being discharged into the aquatic system. The concentration of heavy metals is significantly higher in the aquatic Biosphere (Waldichuk, 1947). The seriousness of heavy metals contamination is further compounded by the fact that they are generally water soluble, non-degradable and are vigorous oxidizing agent, strongly bound to many biochemical, inhibiting their function (Nammalvar, 1983). Heavy metals affect the biological active molecules such as lipids, amino acids, phosphorus, co-enzymes and other protein containing Sulphur, phosphorus, Nitrogen or oxygen (Devaraj and Devaraj, 1989).

The important action of heavy metals is poisoning of enzymes (Bowen, 1996). Further heavy metals effect growth and sexual maturity (Pierson, 1981) For centuries lead has been mined and used in industries and in household products. Modern industrialization, with the introduction of lead in mass produced plumbing, solder used in food cans, paints, ceramic wares and countless other products resulted in a marked rise in population exposure in the 20th century. The current annual worldwide production of lead is approximately 5.4 million tones and continues to rise. 60% of lead is used for the manufacturing of batteries (automobile batteries in particular) while the remaining is used in the production of pigments, glazes, solder, plastics, cables sheathing, Ammunition, weights, gasoline, addictives and a variety of other products. Moreover some segments of the population in developed countries remain high risk of exposure because of the persistence of Lead paint, Lead plumbing and lead contaminated soil and dust particularly in areas of old urban housing. Lead exposure in children and adults can cause a wide spectrum of health problems ranging from convulsion, comma, renal failure and death at the high end to subtle effect on metabolism and intelligence at the low end of exposure. In fish the symptoms of chronic lead intoxication include changes in the blood picture, with severe damaged to the erythrocytes and leucocytes, degenerative alterations of the parenchymatous organs and damaged to the nervous system. The highest admissible lead concentration in water is 0.004- 0.008 mg/litre for salmonids and 0.07mg/litre for cyprinids (Howard, 2002).
The MT's (the family of metal binding proteins) are one of the most promising biochemical indicators for exposure to heavy metals. These classes of proteins were first described (Marghoshes and Vallee, 1957) in the horse kidney.

Biochemical characteristics of MT's include that: (1) they are of low molecular weight (about 6000 – 8000 Da) non-enzymatic proteins; (2) they are soluble, sulphydryl-rich proteins; and (3) they have peculiar cysteine-rich amino acid sequences such as Cys-X-Cy, Cys-XY-Cys where XY are amino acids different from cysteine (Kagi and Kogima, 1987). They have a high heavy affinity and binding capacity (7–9g atom per mole) and are able to chelate both essential (e.g. Zn, Cu) and nonessential metals (Cd, Hg, Ag etc) by cysteine tetrathiolate clusters (Viarengo *et al*., 1999). MT's show different affinities for heavy metals (Hg + 2 > Cu+2 > Zn+2). MT's are able to protect cell structures from non-specific interaction with heavy metal cations and to detoxify metal excesses penetrating into cell because of their biochemical and 12 functional characteristics (Viarengo *et al*., 1999 and Hammer, 1986) but they are thought to function in the

Correspponding author: ezzie7v@gmail.com

transport, storage and detoxification of heavy metals (Klaverkamp, 1999). Several possible functions of MT's have been proposed. They are thought to play an important role in essential metal homeostasis. For example, MT's might be essential in Zinc (Zn) homeostasis by regulating Zn absorption or as a donor of Zn to various enzymes and transcription factors during development or protein synthesis (Klaassen *et al*., 1999). It proposed that MT's in the cytosol might function in metal detoxification and protection from oxidative stress whilst those in the nucleus may provide protection against DNA damaging electrophiles (Woo and Lazo, 1997). Metallothioneins are usually considered an important specific biomarker of heavy metal exposure due to their inductibility by heavy metals (Viarengo *et al*., 1999). Bioindicator organisms commonly used in the application of MT's as biomarkers are fish (Hylland *et al*., 1996), molluscs (Viarengo, 1989) and crustaceans (Pedersen *et al*., 1997). The importance of MT's in biomonitoring is enhanced by their ubiquity which means they can be studied in most organisms[9].

Although MT's have been widely used in to identify specific responses to heavy metal pollution, there is new body of evidence demonstrating that in vertebrates (mammals and fish) MT synthesis is stimulated by different endogenous and exogenous agents (Kagi, 1993). The use of MT in biomonitoring should always be supported by knowledge about the physiology of stress response in bioindicator organisms. For example, MT induction in fish should be considered a general stress response, particularly sensitive to heavy metals but conversely in mussels and possibly other molluscs, MT's seem to represent a more selective biological response to heavy metals and emphasis that a correct approach in biomonitoring consist of use of battery of biomarkers such as acetylcholine esterase activity and DNA damage (Viarengo *et al*., 1999). Therefore, the present investigation was undertaken to evaluate the effect of lead on some biochemical parameters of *Clarias gariepinus* different tissue specifically protein, carbohydrate, alkaline phosphatase, acid phosphatase and metallothionein.

MATERIALS AND METHODS

Clarias gariepinus were obtained from the fish hatchery at Poondi, the fishes were transported from the form in oxygenated polythene bags to the laboratory and immediately transferred into glass aquaria of 50 L capacity containing well-aerated unchlorinated ground water .The water was changed every day. The healthy fish that showed active movements were only considered for the experiment. Fish weighing average of 6.8 gm and average length measuring 9.2 cm were acclimatized in the laboratory condition for 10 days. Fish were fed with fish food. After 10 days acclimatization of the fishes in laboratory condition, the fishes were exposed to two different concentrations of Lead 0.08 mg/L and 0.10mg/L (Olojo *et al*., 2005). After exposure, the control and experimental fishes were sacrificed and the tissues were isolated and kept in cold. SDS-PAGE was carried out according to the method (Laemmli, 1970) using 12% polyacrylamide gel. Protein estimation was done by the method (Lowry *et al*., 1951). Carbohydrate estimation was done by the method (Dubois *et al*., 1956). Acid and Alkaline Phosphatase activity was studied by the method (Wootton, 1964).

Metallothionein Quantification

The content of MT was quantified by the method of Silver-saturation (Martinez *et al*., 1993).

Statistical analysis

Data was statistically analyzed using Student t-test

RESULTS

Estimation of proteins

The current studies showed increased in the total protein of liver and muscle in low concentration of lead (0.08 mg/L) whereas it showed a significant decreased in the gill at high concentration (0.10 mg/L) of Lead as compared to control.

Estimation of Carbohydrates

The content of carbohydrates when compared to the control showed decline in the liver, muscle and gill from the lower concentration (0.08 mg/L) to the higher concentration (0.10 mg/L) of lead. The carbohydrate concentration was found highest in the liver then gill and lowest in the muscle.

Activity of Alkaline Phosphatase and Acid Phosphatase

The activity of alkaline phosphatase was found to increase in the lower concentration of lead (0.8 mg/L) but then there is a marked decreased in the higher concentration of lead (0.10 mg/L). Similar trend was observed in all the tissues i.e. liver, muscle and gill. Whereas the activity of Acid Phosphatase in the liver showed a decreasing the lower and higher concentration of lead when compared to control. The muscle and gill tissue showed an elevated activity in the lower concentration of lead and then decreased in the higher concentration of lead.

Metallothionein Quantification

The content of Metallothionein in the control tissue of liver was found to be 0.3 ± 0.05 and in the treatment of 0.08mg/L of lead it was found to be 0.9 ± 0.03 and at the treatment of 0.10mg/L it was found to be 1.6 ± 0.04. In the muscle tissue the control showed 0.25 ± 0.04 and at 0.08mg/L of treatment it was found to be 0.7 ± 0.03 and at 0.10 mg/l treatment it was found to be 1.2 ± 0.06. In the gill tissue of control the Metallothionein content was found to be 0.2±0.03 and at the treatment of 0.08mg/L it was found to be 0.5 ± 0.07 and at 0.10mg/L treatment it was found to be 0.8 ± 0.06. In all the tissues the Metallothionein content was found to increase from control to lower treatment and then to higher treatment of lead.

Protein Profile

The Protein profile showed there were polypeptides electrophorese ranging from 205,000 Da to 3000 Da. The electrophorogram revealed deeply stained polypeptide fraction of different region in the gel. The low and high concentration of lead (0.08 and 0.10 mg/L) treatment in the liver showed 9 and 8 protein bands respectively compared to 10 bands in the control. The treated gill showed 9 and 5 protein band in 0.08 and0.10 mg/L of lead while the control gill showed 8 bands. The muscle tissue at the treatment of 0.08 mg/L showed 12 bands and at 0.10mg/L concentration lead showed 11 bands while the control muscle showed 13 bands.

Table 1. Quantification of Protein (µg/g) in the tissues of catfish exposed to different concentration of Lead

Period of exposure	Tissues	Concentration of Lead (mg/L)		
		Control	0.08	0.10
10days	Liver	6500 ± 35	6940 ± 25	4440 ± 26
	Muscle	7000 ± 23	7633 ± 35	5453 ± 32
	Gill	4685 ± 33	4404 ± 33	2521 ± 34

Table 2. Quantification of Cabohydrates (µg/g) in the tissues of catfish exposed to different concentration of Lead

Period of exposure	Tissues	Concentration of Lead (mg/L)		
		Control	0.08	0.10
10days	Liver	2114 ± 26	1864 ± 15	710 ± 25
	Muscle	64 ± 27	50 ± 15	35 ± 13
	Gill	208 ± 45	71 ± 25	15 ± 20

Table 3. Quantification of Acid Phosphatase (µg/g) in the tissues of catfish exposed to different concentration of Lead

Period of exposure	Tissues	Concentration of Lead (mg/L)		
		Control	0.08	0.10
10days	Liver	6.2 ± 09	5.4 ± 13	3.2 ± 11
	Muscle	6.5 ± 15	18.4 ± 13	13.4 ± 10
	Gill	3.5 ± 12	14.5 ± 10	6.7 ± 21

Table 4. Quantification of Alkaline Phosphatase (µg/g) in the tissues of catfish exposed to different concentration of Lead

Period of exposure	Tissues	Concentration of Lead (mg/L)		
		Control	0.08	0.10
10days	Liver	183 ± 21	273 ± 33	182 ± 30
	Muscle	26 ± 20	28 ± 15	12 ± 11
	Gill	53 ± 15	126 ± 12	74 ± 09

Table 5. Quantification of Metallothionein (µg/g) wet wt in the tissues of catfish exposed to different concentration of Lead

Period of exposure	Tissues	Concentration of Lead (mg/L)		
		Control	0.08	0.10
10days	Liver	0.3 ± 0.05	0.9 ± 0.03	1.6 ± 0.04
	Muscle	0.25 ± 0.04	0.7 ± 0.03	1.2 ± 0.06
	Gill	0.2 ± 0.03	0.5 ± 0.07	0.8 ± 0.06

DISCUSSION

Proteins are highly sensitive to heavy metals and hence indicators of heavy metals poisoning (Jacobs *et al*., 1997). The impairment of protein turn over may have an adverse impact on the synthesis of organic molecules reported moderate depletion of protein content in the liver and intestine of fish due to the invasion of heavy metals and similar observation (Shakoori *et al*., 1976 and Ramalingam, 1990). Incidentally the current pursuit disclosed a significant increase in the total protein of liver and muscle in low concentration and decreased in high concentration, while there is a decreased in the concentration of protein in the gills. The change in the protein concentration suggest an intestinal proteolysis in the respective tissues which in turn could contribute to the hike of free amino acids to be fed into the Tricarboxylic Acid Cycle (TCA) as ketoacid, and it support the hypothesis (Kabeer Ahmed Sahid, 1979). The decreased protein content in the fish is also in general agreement and the reported the conversion of protein into amino acids residues so as to increase amino acid pool (Omata *et al*., 1978). In the present study a significant change in the concentration of carbohydrates in the tissue of the fish was observed and are in general agreement with alterations noted in chronically exposed Rainbow trout in catfish, Heteropneustes fossils (Shastry and Subhadra, 1982). The decrease in the carbohydrate may be due to the decrease in glycogenesis (Szinciz and Forth, 1998). Increased ion in the breakdown of glycogen would suggest a higher energy demand in the animals; living under extreme stress conditions including the stress by toxicants. The increased in carbohydrates content in the liver and muscle could be a response to withstand the

toxicants which imposed stress conditions in the fish (Keller and Andrew, 1973). The Phosphatases are known to play an important role in energy crisis and serve as a marker for the evaluation of diseases or pathological conditions (De Duve, 1963). The Alkaline phosphatase in the present study was found to be inhibited in the treated muscle and liver tissue. This may be due to altered membrane permeability which is brought about by the binding of heavy metal ion present in the enzyme configuration which could hampered glycogen and lipid metabolism and disrupted the transfer of catabolites of the hepatic cells and it falls in line. The activity of Acid Phosphatase in the present study was also inhibited may be due to the alteration in the membrane structure caused by toxic metals or organic compounds which might have caused leakage in the lysosomal membrane thereby releasing all hydrolytic enzymes as reported (Hossain and Dutta, 1986). The significant inhibition of both alkaline and Acid Phosphatase possibly hampered the active transport across the muscle fiber leading to impaired cellular metabolism (Sahana *et al*., 1986).

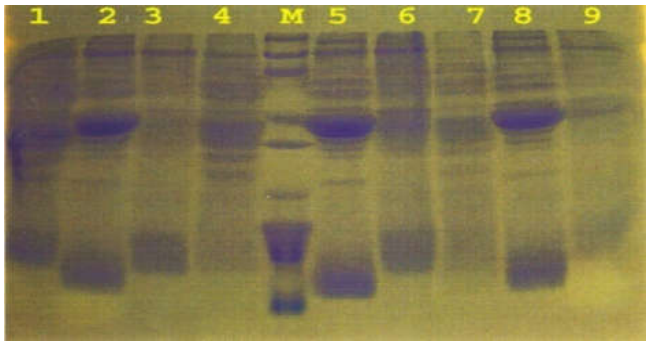

Figure 1. SDS-PAGE, Lane1: Control Liver; Lane2: Control Muscle; Lane3: Control Gill; Lane4: Liver (0.08mg/L); Lane5: Muscle (0.08mg/L); Lane6: Gill (0.08mg/L); Lane7: Liver (0.10mg/L); Lane8: Muscle (0.10mg/L); Lane9: Gill (0.10mg/L); M:Protein marker.

Electrophoretic technique remains a promising procedure for identifying the protein in response to stress or sub lethal levels of pollution. The present study revealed an alteration in the protein profile in the liver, muscle and gill tissues which may be attributed to the protein denaturation and arises out of exposure to adverse physical and chemical conditions. Living organism response to cellular level to unfavorable conditions by synthesizing an additional class of proteins which repairs and protect cellular organization against stress-induced damage (Ellis, 1990). High molecular weight protein from the muscle and liver tissues may be due to continuous influx of lead and hence there is a partial loss of high molecular weight protein. However, the fish were able to survive in the polluted water which might be due to the presence of Metallothionein. Minor polypeptides were seen in the muscle and gill tissue and it might be due to the fact that there are very little proteins in these tissues. Since liver tissues a detoxifying organ, the total protein variations and the protein fractions are distinctly observed and may be due to the accumulation of metals in the renal tissues. Gill and intestine are the major routes of uptake of heavy metals with the other cellular molecules before being sequestered by the Metallothionein. In invertebrates, generally the relationship between concentration of trace elements and Metallothionein levels in tissues of various taxa of terrestrial and aquatic organisms has been reported by researchers (Shearer and Fletcher, 1984; Hamza-chaffai, 2003). In the present study Metallothionein has been synthesized more in the liver than in gills and muscle of the fish exposed to Lead but was not inducted in the control sample. The early studies on Metallothionein in fish showed that the levels of Metallothionein increased after administration of heavy metals. It was concluded that the increased rate of Metallothionein synthesis matched the rate of Lead influx and that this offered protection against Lead toxicity. In this study Lead induced synthesis of Metallothionein in the Liver, Muscle and gill of catfish. The above result clearly showed that Metallothionein involved in detoxification of toxic metals. The lead ions are quite toxic to fish in various functional levels when environmental concentrations of lead are increased (Infante *et al*., 2003). The protective role of Metallothionein against the toxicity of cytosolic Lead has been demonstrated in fishes and this protein acted as biomarker to reflect on Lead toxicity.

REFERENCES

Bowen HJM. 1996. Trace elements in Biochemistry, Academic Press, London., Pg; 78-90.

De Duve C. 1963. In: lysosomes. De Reuck, S. V. S and gameron. M.P (Eds). Churchill Ltd., London, pp 257-284.

Devaraj H, Devaraj M.1989. Rat intestinal changes in Biochemical due to toxicity. Ind. J. Exp. Biol., 25:637-638.

Dubois M, Ciles KA, Hamildon JK, Rebers PA, Smith F. 1956. Calorimetric determination of sugar and related substances. Anal. Chem., 28:351-356.

Ellis RJ. 1990. The molecular chaperone concept . Se. Cell. Biol., 1:1.

Hammer DH. 1986. Metallothioneins. Ann. Rev. Biochem., 55:913-951.

Hamza-chaffai A, Pellarin J. Amiard J. C. 2003. Health assessment of a marine bivalve *Reditapes decussates* from the gulf of gabes (Tunisia). Environ. Inter., 28: 609-612.

Hossain. A and Dutta. H.M. 1986. Metallothionein. Ann. Rev. Biochem. 55:913-951.

Howard Hu MD, MPH, Sc.D. 2002. Human health and heavy metal exposure in two subtropical fish species. J. Exp. Marine. Biol. Ecol., 138:69-84.

Hylland K, Sandvik M, Utne-Skare J, Beyer J, Egaas E, Goksoyr A. 1996. Biomarkers in flounder (*Platichthys flesus*): an evaluation of their use in population monitoring. Mar. Environ. Res., 42: 223-227.

Infante, H. G, Cuyckens F, Vaeck, KV, Adams FC. 2003. Characterization of metal complexes with Metallothioneins in the Liver of the Carp (Cyprinus carpio) by reverse phase HPLC with ICP-MS and Electrospray Ionisation (ESI). J. Anal. At. Spectrom., 19:159-16.

Jacobs JM, Carmichael, Cavanagh JB. 1997. Ultrastructural changes in the nervous system of rabbit poisoned with methylmercury. Toxicol. Appl. Pharmacol., 39: 249-261.

Kabeer Ahmed Sahid J. 1979. Studies on some aspects of protein metabolism and associated enzyme system in the freshwater *teleost Tilapia*, to malathion exposure. Ph.D thesis, Sri Venkateshwara University, Tirupati., Pg 136-184.

Kagi JHR. 1993. Evolution, structure and chemical activity of class I metallothioneins: an overview. In: Suzuki, K.T., Imura, N. and Kimura, M. (eds) Metallothionein III. Birkhauser Verlag. Basel, Switzerland., Pg.29-55.

Keller LF, Andrew M S. 1973. Copper metabolism and gut morphology in rainbow trout during chronic sublethal dietary copper exposure Can. J. Fish. Aquat. Sci., 58: 293-305.

Klaassen CD, Choudhuri S, Liu. 1999. MT an extracellular protein against Cd toxicity. Ann.Rev. Pharmacol. Toxicol., 39:267-294.

Klaverkamp JF, Dutton MD, Majewski HS, Hunt RV, Wesson LJ. 1999. Evaluating the effectiveness of metal pollution controls in smelters by using metallothionein and other biochemical responses. In: Newman, C.M. and Mc Intosh, A.W (eds) Metal Ecotoxicology: Concepts and Applications. Lewis Publishers. Michigan., Pp 33-59.

Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227:680-685.

Lowry OH, Rosenborough N, Farr AL, Randall RJ. 1951. Protein measurement with the Folin-phenol reagent. J. Biol. Chem., 193: 265-275.

Marghoshes M, Vallee BL. 1957. A Cadmium Protein from equine Kidney cortex. J. Am. Chem. Soc., 79:4813-4814.

Martinez M, Torreblanca A, Del Ramo J, Pastor A, Diazmayans J. 1993. Cadmium induced metallothionein in hepatopancreas of *Procambrus clarkia*. Quantification by a silver saturation method. Comp. Biochem. Physiol., 105C:263-267.

Nammalvar.1983. Heavy metal pollution in the Marine Environment. Sci. Rep., 20: 158-160.

Olojo EAA, Olurin KB, Mbaka G, Oluwemimo A D. 2005. Histopathology of the gill and liver tissues of the African catfish *Clarias gariepinus* exposed to Lead. Afr. J. Biotech., 4: 117-122.

Omata S, Sakimura K, Tnubaki H, Sugano H. 1978. *In vivo* effect of methyl mercury on protein synthesis in brain and liver of rat. J. Environ.Biol., 44:367-378.

Pedersen SN, Lundebye AK, Depledge M H. 1997. Field application of metallothionein and stress protein biomarkers in the shore crab (*Carcinus meneas*) exposed to trace metals. Aquat. Toxicol., 37:183-200.

Pierson, K.B. 1981. Effect of Chronic Zinc exposure on the growth, sexual maturity, Reproduction and accumulation of the *Poecilia reticulate*. Can. J. Fish. Agnat., 12: 343-348.

Ramalingam, K. 1990. Biochemical constituents as stress indicators in fishes. J. Zool., 10:152-157.

Sahana, F. F, Jana S, Choudhari M A, Choudhari D K. 1986. Hg (II) induced changes in some biochemical parameters in the freshwater fish *Clarias batrachus*. Physiol. Bihemoslov., 35: 81-85.

Shakoori AR, Saleem AZ, Mohammed SA. 1976. Effect of malothion dieldrin and andrin on blood serum proteins and free amino acids pool in *Channa punctatus* (Bloch). Pak. J. Zool., 8: 124-134.

Shastry K V, Subhadra K. 1982. Effect of cadmium on some aspect of carbohydrates metabolism in a freshwater catfish Heteropneuster Fossils. Toxicol. Lett., 14: 45-55.

Shearer MA, Fletcher G I. 1984. The relationship between metallothionein and intestinal zinc adsorption in the winter flounder. Can. J. Zool., 62: 2211-2230.

Szinciz L, Forth W. 1998. Effect of As2O3 on gluconeogenesis. Arch. Toxicol., 61: 444-449.

Viarengo A, Burlando B, dondero F, Marro A, Fabrri R. 1999. Metalothionein as a tool in biomonitoring programmes. Biomarkers., 4:455-466.

Viarengo A. 1989. Heavy metals in marine invertebrates: mechanisms of regulation and toxicity at the cellular level. CRC Crit. Rev. Aquat. Sci., 1:295-317.

Waldichuk M. 1947. Some biological concerns in heavy metal pollution. In: Pollution and Physiology of marine organism (Eds). Vernberg F. J; W. B Vernberg. Academic press NY., 1-57.

Woo E S, Lazo J S. 1997. Nucleocytoplasmic functionality of metallothionein. Cancer Res., 57: 4236-4241.

Wootton IDP. 1964. Microanalysis in Medical Biochemistry. London: Churchill Ltd, pp. 101–118.

****