ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Environmental Impact of Heavy Metal Pollution on Metallothionein Expression in Nile Tilapia.

Iman MK Abumourad[1], Wafaa T Abbas[1], Mohammad MN Authman[1] and Shenouda M Girgis[2]*.

[1] Department of Hydrobiology, Veterinary Division, National Research Centre, 12622-Dokki, Cairo, Egypt.
[2]Department of Cell Biology, National Research Center, 12622-Dokki, Cairo, Egypt.

ABSTRACT

Heavy metal pollution is one of the most serious problems that face human with referring to people eating sea foods. Heavy metals may affect the aquatic organisms at the cellular level and possibly change some genes regulation. Metallothionein (MT) is a low molecular weight protein that binds to heavy metals in aquatic organisms and is considered as a biomarker of heavy metal pollution. Therefore, this study was undertaken to estimate the copper (Cu), cadmium (Cd), zinc (Zn) and lead (Pb) concentrations in water and fish tissues, the GOT, GPT and LDH enzymes value and the expression level of MT gene in Nile tilapia fish (*Oreochromis niloticus*) in Lake Manzala, Egypt. Elevated values of Cu, Cd, Zn and Pb concentrations were detected in water and fish tissues. Highly pronounced values of GOT, GPT and LDH enzymes were detected. Using RT- PCR, an evidence of increase in metallothionein expression in fish livers was detected, monitoring the fish response to heavy metal pollution in lake Manzala, Egypt.

Keywords: Heavy metal, pollution, metallothionein expression, Nile tilapia.

Corresponding author:

,

ISSN: 0975-8585

INTRODUCTION

Industrial development in the developing and developed countries has been resulted in heavy metal contamination of local waters. Heavy metals are non-biodegradable and once discharged into water bodies, they can either be adsorbed on sediment particles or accumulated in aquatic organisms. Pollutants like heavy metal act by changing the structural or biological functions of biomolecules [1]. Biomarkers for water pollution are considered as early diagnostic tools for biological effect measurement and environmental quality assessment [2]. They are defined as a change in biological response that differs from molecular to organismal level [3].

Fish are excellent organisms for the study of various effects of contaminants present in water samples since they can metabolize, concentrate, and store waterborne pollutants. Since fish often respond to toxicants in a similar way to higher vertebrates, they can be used to screen for chemicals that are potentially teratogenic and carcinogenic in humans [4].

Change in Metallothioneins gene expression is used as biomarker for exposure to pollutants. Metallothioneins (MTs) are a family of low-molecular-weight cytosolic proteins that contain highly conserved cysteinyl residues, these residues allow MT to bind, transport, and store various transition metals via thiolate bonding [5]. MT genes have been cloned and sequenced in several species of teleosts, including rainbow trout (*Oncorhynchus mykiss*), plaice (*Pleuronectes platessa*), winter flounder (*Pleuronectes americanus*), and stone loach (*Noemacheilus barbatulus*) [6]. The expression level of metallothionein gene in fish has been used as a biomarker for water pollution with heavy metals [7,8]. Quantification of fish metallothionein transcript levels in absolute units has been presented [9]. It also, considered as early warning for degradation of environmental quality and specific measures of the toxic, carcinogenic and mutagenic compounds in the biological materials [10].

Egyptian coastal lakes act as temporary reservoirs for drainage water and often contaminated with anthropogenic materials [11]. Economically Lake Manzala is the greatest and one of the most important lakes in Delta Egypt, it is connected to the Mediterranean Sea from the north-eastern coast and to the Nile Damietta branch at Damietta region. The lake has high inputs of pollutants from industrial, domestic, and agricultural sources from urban centers such as Cairo and others along the lengths of its drains and transports untreated or poorly treated wastewater to Lake Manzala.

Therefore, this study aimed to determine the level of heavy metals pollution in Lake Manzala and investigating the biochemical alterations as well as the expression of Metallothionein gene as a biomarkers for heavy metal pollution in Nile tilapia fish *O. niloticus*.

MATERIAL AND METHODS

Fish were collected from Lake Manzala, Egypt along the southern part as presented in fig. (1).

ISSN: 0975-8585

Figure 1: Map illustrating location of Lake Manzala, Egypt (Google Egypt map, 2008).

Water samples were collected from the study area and later on, the laboratory measurements of heavy metal concentrations were done by analysis of Cu, Zn, Cd and Pb by flame atomic absorption spectrophotometer according to [12].

Fish sampling

Fifty fish weighting 50-150 gm and average length 15.5 ± 0.79 cm were collected along the southern part of Lake Manzala. Fish were anesthetized with Ethyl 3-aminobenzoate methane sulfonate salt (Sigma, St. Louis, MO, USA) for further analysis. Blood samples were taken from the caudal vein of each fish and collected in anticoagulant-free centrifuge tubes as described by [13].

Serum was obtained by centrifugation of blood at 3.000 rpm for 5 min. Serum samples were then stored at −20°C until the analysis. GOT and GPT activities were determined according to [14]. LDH activity was assayed by UV test technique [15] using biochemical analyzers (Geneway, Japan). Kits for all biochemical analysis were supplied from Spectrum diagnostics, Egypt.

Concerning to the organs and tissues damage related to the exposure of fish to pollutants, fish were dissected and because liver is the main organ for metabolism and a target organ for contaminants accumulation as reported by many authors such as [16], liver of each examined fish was rapidly excised, washed in DEPC-Water and was frozen in liquid nitrogen for further total RNA extraction and molecular analysis. Total RNA was extracted from fish livers and was purified using a spin column kit purchased from Fermentas life science Co., (Invitrogen Corporation, Van Allen Way, Carlsbad, Canada) according to the manufacturer's instruction. RNA samples were treated with DNase I (Ambion, UK) to remove contaminating genomic DNA and repurified by spin column. Then RNA samples were stored at –80 ^{o} C until the process of reverse-transcriptase.

Total RNA from liver tissues was treated with DNase, 2 µg RNA was reverse transcribed with reverse transcriptase using oligonucleotides (Invitrogen) to prim the reaction. The first strand cDNA was used as templates for RT-PCR with a pair of MT specific primers designated:

Forward: 5'-TTGGACACCCTGAAGT-3'and Reverse: 5'-GTGGCGGAAGTAAAGT-3'

The PCR cycling parameters were one cycle of 94 ° C for 5 min, 35 cycles of 94° C for 30 s, 48° C for 30 s and 72 ° C for 1 min, with a final extension step of 72° C for 10 min. The RT-PCR products were analyzed by electrophoresis on 2% agarose gel with PCR products derived from 18S rRNA of the tilapia as internal controls, documented with documentation system. Semi-quantitative assessment of mRNA levels was determined by quantifying the band's intensity of PCR products using GeneTools in the gene expression relative to 18S rRNA.

Muscle samples were separated, each composite sample of muscle tissues was weighed separately in clean, labeled Petri-dishes and dried for several days at 70ºC to constant weight. Homogenization were achieved by grinding the tissue samples in a Teflon mortar and analyzed for heavy metals according to [17]. An exact weight of dry sample (triplicate, each of 0.5 g) was placed in Teflon vessel and 4 ml of nitric acid was added. The vessels were tightly covered and allowed to predigest at room temperature over night. The digestion block was placed on a preheated hot at 80ºC for three hours. The samples were cooled at room temperature and then were transferred to 25 ml volumetric flask. All digested solutions were analyzed by Flame Atomic Absorption Spectrophotometer (Perkin-Elmer, Model 2380). Reagents of the analytical grade were utilized for the blanks and calibration curves. Metal concentrations were expressed in terms of µg/gm dry weight.

Statistical Analysis

Descriptive statistical analysis (Mean±SD) was performed for evaluating heavy metals in water, tissue samples and enzymes activity using SPSS (SPSS 17.0 Integrated Student Version for Windows). The Mann-Whitney U-rank test was used to test for the differences in MT/18S rRNA ratios among the tested groups of fish compared to the control group.

RESULTS

Heavy metal analysis of water from Lake Manzala showed highest pronounced values of contamination with Zn, Cu, Pb and Cd, respectively (Fig. 2).

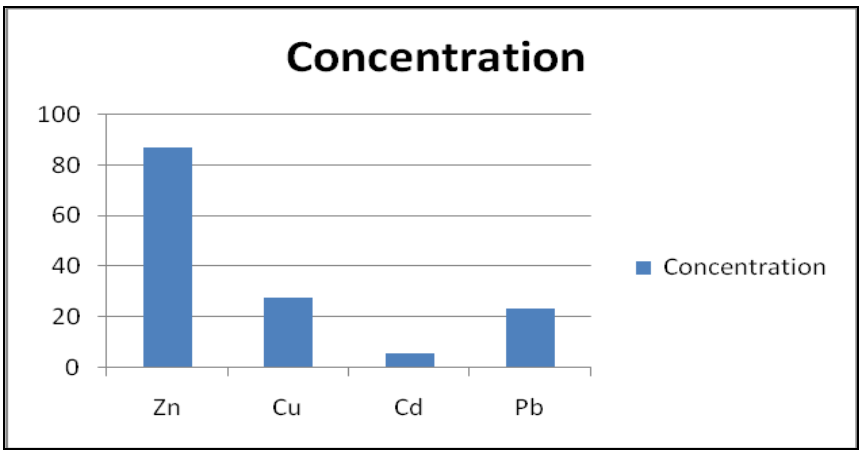

Figure 2: Columns represent the concentrations of heavy metals (Zn, Cu, Cd and Pb) measured in water (ug/L, microgram of metal/Liter of water).

ISSN: 0975-8585

Tilapia muscles analysis revealed pronounced high accumulation values of heavy metals as µg/g dry weight compared to the recorded by [18], (right column, Fig. 3).

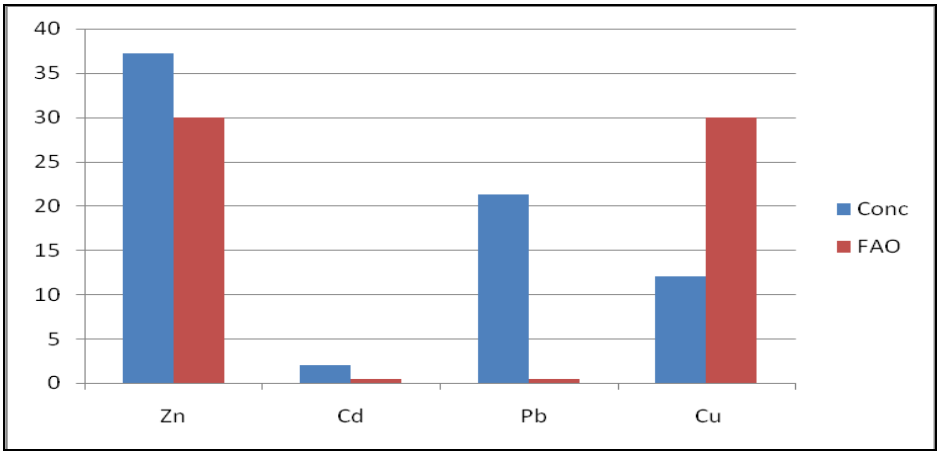

Figure 3: Bioaccumulation of heavy metals (Zn, Cu, Cd and Pb) expressed as µg/g dry weight (left column) in muscles of *O. niloticus* compared to the recorded by FAO, 1983 (right column).Data are expressed as means ± SD.

Enzymatic activities of GOT, GPT and LDH of *O. niloticus* showed pronounced elevated values in samples collected from Lake Manzala compared to control (right column, Fig. 4).

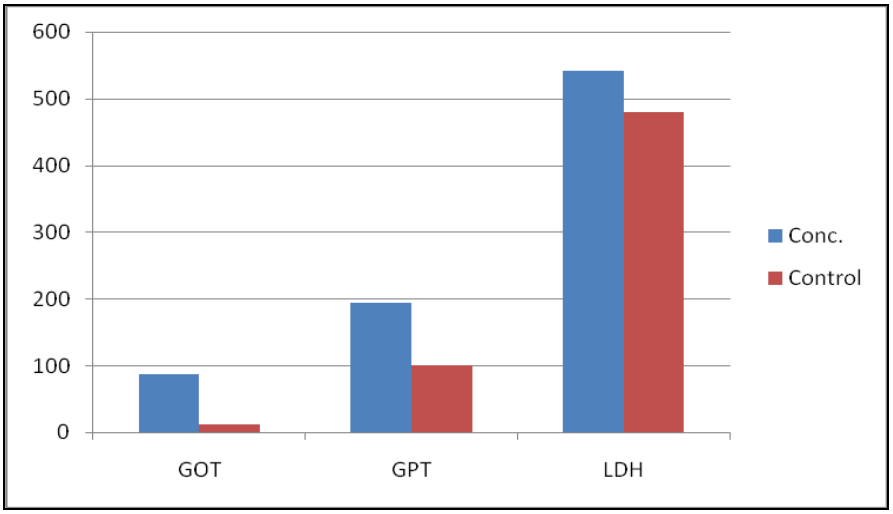

Figure 4: Biochemical parameters (GOT, GPT and LDH) in *Oreochromis niloticus* from Lake Manzala. Data are expressed as mean ± SD.

MT cDNA PCR product yielded a 314-bp fragment, Agarose gel electrophoresis showed a single amplified PCR band , sequencing of the *O. niloticus* wasfound to be more than 90% similar to other teleost MT cDNA sequences.

Of the PCR-products (Fig.5), corrected values were obtained by dividing the measured value for MT transcript by that of 18S rRNA (control). Those showed significant increase ($P<0.05$) in the OD (Optical Density) indicating an increase in metallothionein gene expression compared to control.

ISSN: 0975-8585

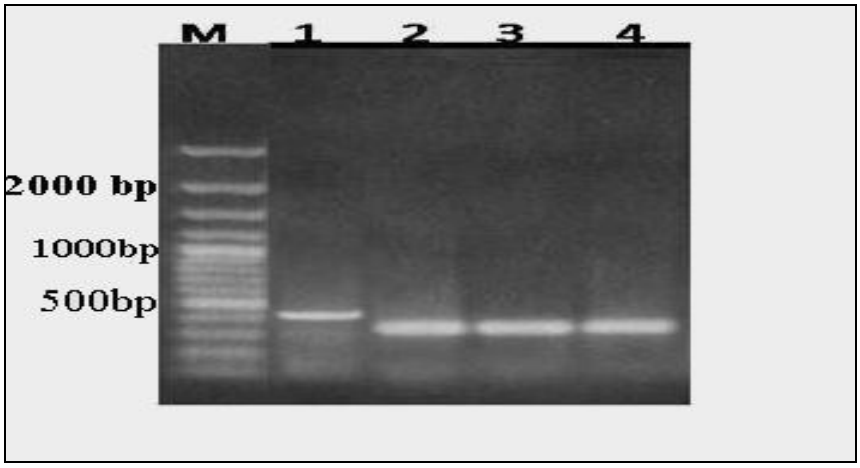

Figure 5: Metallothionein semi-quantitative PCR products (Lanes 2, 3 & 4) represents MT-gene expression (314 bp) compared to the internal control (Lane 1) of 18S rRNA gene (423 bp).

DISCUSSION

Human activities in industry and the increasing use of metals in industry has lead to serious environmental pollution through effluents and emanations of large quantities of metals to localized area of the water.

The present results revealed marked increase of heavy metals concentration in muscle tissues of the investigated fish exceeded the FAO permissible limit in 1983 and such increases were positively correlated with a corresponding increase in heavy metals concentration in water of Lake Manzala. In Egypt, most of lakes receiving agriculture drainage water mixed with industrial, herbicides and the phosphate fertilizers which are considered as the main source of heavy metals in the environment [19].

Biochemical profiles of blood can provide important information about the internal environment of the organism, as the unfavorable changes of the ambient environment are the first ones to earliest affect the blood. Several of soluble enzymes of blood serum have been considered as a relevant stress indicator. Therefore, activities of serum GOT, GPT, and LDH have been commonly used in the diagnosis of fish diseases as well as in the detection of tissue damage caused by environmental pollution and indicates stress-based tissue impairment [20].

An elevated values were shown in the serum enzymes activity exemplified by GOT, GPT and LDH of fish collected from Lake Manzala compared to controls, this could be regarded as the biochemical manifestation of the toxic actions of heavy metal contamination in water and fish tissues in this study. That coincide with [21,22], who concluded that blood levels of GOT and GPT, may increase due to the cellular damage in the liver and that high level of these enzymes in serum are usually indicative of disease and necrosis in the liver of animals.

It was observed that contamination by heavy metals resulted in increases in GOT, and GPT activities of plasma/serum of fish *Sparus aurata* [23] and *Cyprinus carpio* [24]. The present results are in agreement with the findings of [25] who noticed an increase in

activities of serum GOT, GPT, and LDH in Korean rockfish (*Sebastes schlegeli*) exposed to pollutants.

Therefore, in this study, the increase in enzymes activity of *O. niloticus* is mainly due to the leakage of these enzymes from the liver cytosol into the blood stream as a result of liver damage by heavy metals, which also gives an indication of the hepatotoxic effect of the toxicants.

Metallothionein was significantly highly expressed in liver tissue in response to heavy metal pollution in comparison to the 18S rRNA house keeping gene expression. That in accordance with [26], who found that MT-1 genes in adult mouse liver undergo a two-fold increase in average copy number within six hours of treatment with high levels of cadmium salts. As well, Montaser *et al*. (2010), reported that the extra MT gene copies are both transcriptionally competent and inducible by heavy metals [27].

CONCLUSION

This study concluded that Lake Manzala contributes to be one of the most heavy metal contaminated lakes in Egypt. Alterations in tilapia liver enzymes GOT, GPT and LDH as well as increased in gene expression of metallothionein in liver tissue may be a result of the target tissue damage and dysfunction induced by the heavy metals and these parameters could be used as a rapid and sensitive bioindicators for monitoring the impact of heavy metals on aquatic organisms and ultimately whole of the ecosystem.

Conflict of Interest

The authors declare that there are no conflicts of interest.

REFERENCES

[1] Newman MC. Fundamentals of Ecotoxicology. Ann Arbor Press, Chelsea, USA 1998; pp. 25-39.
[2] Cajaraville MP, Bebianno MJ, Blasco J, Porte C, Sarasquete C. and Viarengo A. Sci Total Environ 2000; 247:295-311.
[3] Depledge MH, Aagaard A. and Gyorkos R. Mar Pollut Bull 1995; 31:19-27.
[4] Sarangi PK. Int J of Res in BioSci 2012; 1(2):32-37.
[5] Dunn SE, Putallaz M, Sheppard BH. and Lindstrom R. J Educat Psychol 1987; 79: 467-473.
[6] Kille P, Stephens PE. and Kay J. Biophys Acta 1991; 1089:407-410.
[7] Tom M, Chen N, Segev M, Herut B. and Rinkevich B. Mar Pollu Bull 2004; 48:705-710.
[8] Sturve J, Berglund A, Balk L, Broeg K, Bohmert B, Massey S, Savva D, Parkkonen J, Stephensen E, Koehler A. and Forlin L. Environ Toxicol Chem 2005; 24:1951-1961.
[9] Evans CW, Wilson DA. and Mills GN. Biomarker 2001; 6:7-14.
[10] Verlecar XN, Pereira N, Desai SR, Jena KB. and Snigdha. Curr Sci 2006; 91(9-10): 1153-1157.
[11] Gad NS. Global Veterinaria 2009; 3(1):37-44.
[12] APHA (American Public Health Association). Standard Methods for the Examination of

Water and Wastewater. 18th ed., Greenberg, A.E.; Clesceri, L.S. & Eaton, A.D. (editors). APHA, WEF & AWWA, Washington DC, USA 1992.
[13] Congleton JL. and La Voie WJ. J Aquat Anim Health 2001; 13:168-172.
[14] Bergmeyer HU, Horder M. and Rej R. J Clin Chem Clin Biochem 1985; 24:481- 495.
[15] Wacker A, Heyl W, Biiechl H. and Holthoff HJ. ArzneimForsch 1956; 6:712.
[16] Khan R A. Arch Environ Contam Toxicol 2003; 44:485-492.
[17] UNEP/FAO/IAEA/IOC. Rev 1984; 2:19.
[18] FAO. Compilation of legal limits for hazardous substances in fish and fishery products. Food and Agriculture Organization. FAO Fishery Circular No. 464, 1983; pp. 5–100.
[19] Osman HAM, Ibrahim TB, Ali AT and Derwa HIM. World Applied Sci J 2009; 6:1569-1575.
[20] Palanivelu V, Vijayavel K, Ezhilarasibalasubramanian S. and Balasubramanian MP. J Environ Biol 2005; 26:191–196.
[21] Harvey RB, Kubena LF. and Elissalde M. Am J Vet Res 1994; 55: 572-577.
[22] Svoboda M. Stress in fish-review. Bull VURH Vodnany 2001; 37: 69-191.
[23] Vaglio A. and Landriscina C. Ecotoxicol Environ Saf 1999; 43B:111–116.
[24] Karan V, Vitorovic S, Tutundzic V. and Poleksic V. Ecotoxol Environ Saf 1998; 40:49-55.
[25] Jee JH, Masroor F. and Kang JC. Aquat Res 2005; 36:898-905.
[26] Koropatnick J. and Leibbrandt MEI . Effects of metals on gene expression. In: Goyer R R, Cherian MG (Eds.), Toxicology of Metals. Springer, Berlin 1995; pp. 93-120.
[27] Montaser M, Mahfouz ME, El-Shazly SAM, Abdel- Rahman GH and Bakry S. World J Fish Marine Sci 2010; 2(3): 174-185.