

Effects of Some Heavy Metals in Different Organs and Some Hepatic Enzymes for European eel (*Anguilla anguilla*) at Lake Edku

Marwa M. Mazrouh

Department of Fisheries, Fish Physiology Laboratory, National Institute of Oceanography and Fisheries, Alex., Egypt

Abstract: The study was designed to determine the concentration of some heavy metals in liver, gills, and muscles and the levels of glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) and metallothionein (MT) in liver to estimate the efficacy of using metallothionein as a biomarker for heavy metal stress. Samples of European eel (*Anguilla anguilla*) collected from Lake Edku. Both agriculture and domestic effluents enter lake from different drains. Copper, Lead, Zinc and Nickel content were determined by atomic absorption spectrophotometer. The highest concentrations of metals were found in liver and gill tissues more than muscle tissues. Concentrations of Zn and Cu were significantly higher in the gills and livers of eels more than the concentration of Pb and Ni. The higher heavy metal concentrations accumulation in gills > liver > muscles respectively. Three enzyme (GOT), (GPT) and (MT) biomarkers were significant ($P < 0.05$) difference with detected heavy metals. It was concluded that alterations in *Anguilla* liver enzymes GOT, GPT and MT as well as increased may be a result of the tissue damage and dysfunction induced by the heavy metals and these parameters could be used as a rapid bioindicators for monitoring the impact of heavy metals on aquatic organisms.

Keywords: European eel, heavy metal, GOT, GPT, Metallothionein, (MT), liver, gill, muscle, Lake Edku

1. Introduction

Heavy metals have drastic environmental impact on all organisms. Heavy metal pollution is one of the most serious problems facing people who eating sea foods and affects their physiological state. 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[1]. The contamination of waters with a wide range of pollutants has become a matter of concern over the last few decades [2,3]. Studies carried out on fish have shown that heavy metals may have toxic effects, altering physiological activities and biochemical parameters both in tissues and in blood of fish[4,5,&6]. Aquatic organisms such as fish and shell fish accumulate metals to concentrations many times higher than present in water or sediment [7] they can take up metals concentrated at different levels in their different body organs [8] also certain environmental conditions such as salinity, pH, water accumulation in the living organisms up to toxic concentrations and cause ecological damage [9]. The essential metals also become toxic at high concentrations and the toxic effects of heavy metals have been reviewed, including bioaccumulation[10,11&12]. Lake Edku is a shallow lagoon situated on the western margin of the Nile delta. It is smallest in area comparing with Manzalah or Borollus lakes; it is connected to the Mediterranean through the lake-sea connection Bughaz El- Meadia. The lake can be divided into three ill-defined basins; eastern, central and western. It receives agricultural drain water at its eastern section where two main land drains pour their water through hydraulic pumps. Berzik and Edku-Bousily drains supply water to the lake. The European eel (*Anguilla anguilla* L.) is a catadromic fish species with its spawning grounds thousands of kilometers away in the ocean, possibly the Sargasso Sea. Young eels entered lake in elvers phase.

Heavy metals may affect the aquatic organisms at the

cellular level and possibly change some genes regulation. Measuring heavy metals in aquatic organisms may be a bioindicator of their impact on organism and ecosystem health[13], but a true evaluation of the damage inflicted by heavy metals should come from comprehensive biomarker studies. Essential metals, such as copper (Cu) and zinc (Zn), have normal physiological regulatory functions [14], but may also bioaccumulate and reach toxic levels [15]. Glutamic oxaloacetic (GOT) and glutamic pyruvic (GPT) transaminases are among many enzymes that play a vital role in protein and carbohydrate metabolism and they are commonly used as biomarkers of environmental pollution and act as an indicator for tissue damage [16,17]. Metallothionein was described by Bouquegneau *et al.*[18] in European eel. Mts are low molecular weight, cysteine-rich (20–30%) metal-binding proteins, whose neosynthesis represents a specific response of the organisms to pollution by heavy metals such as Cu, Zn, Cd and Hg. In particular, the metal-binding protein metallothionein (MT) is important for the intracellular binding and regulation of essential and nonessential metals [19]. The binding of toxic metals to MT represents a sequester function associated with the cellular protection against metal toxicity[20]. The studies on MT in fish species have consisted basically of the description of the protein and the relation of metallothionein with tolerance to metals, and quantification of MT, either under experimental or under environmental pollution conditions [21–24]. The expression level of metallothionein gene in fish has been used as a biomarker for water pollution with heavy metals [25,26]. Quantification of fish metallothionein transcript levels in absolute units has been presented by Evans *et al.*[27]. 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 [28]. The aim of work is to determine the levels of some heavy metals (Cu, Pb, Zn, and Ni) in liver, gills and muscles of *Anguilla anguilla* and GOT, GPT and MT concentrations in liver to assess the efficacy of using metallothionein as a biomarker for heavy

metal stress.

2. Materials and Methods

A total of thirty (n=30) fresh European eels *Anguilla anguilla* were obtained from local fishermen during the period from June 2010 to March 2011 from Lake Edku (Fig.1). Lake Edku lies in the north of the Nile Delta, west of the Rosetta branch between longitudes 30°8'30" and 30°23'E and Latitudes 31°10' and 31°018'N. The lake is connected to the adjacent Abu Qir Bay through Boughaz El Maadiya. Lake receives huge inputs of terrigenous and anthropogenic nutrients discharge, sewage and agricultural runoff via three main drains, Edku, El-Boseily and Barzik situated at its eastern margins drains.

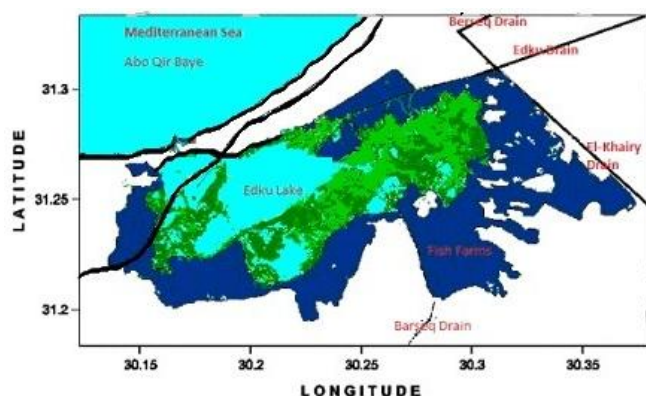


Figure 1: Map of Lake Edku

Samples were kept on ice during transport to physiology laboratory at NIOF, Alexandria. The total average length and weight were 32.4cm and 377.9g respectively. Heavy metal concentrations were estimated in liver, gills and muscles. These organs were collected and kept frozen at -80°C until analysis. One gram of each prepared samples were homogenized and digested in separate Teflon cup using 2ml of HNO₃ and 1ml of HClO₄. Teflon was kept at 100°C on hot plate until all materials dissolved and dried. Concentrations of heavy metals were measured using Atomic Absorption Spectrophotometer (Perkin- Elmer) 2280 and values were recorded in µg g⁻¹ dry weight. Glutamic oxaloacetic (GOT) and glutamic pyruvic (GPT) transaminases were estimated according to the method of Wooten *et al.* [29]. After removal of liver, excess water adhering to the tissues were removed by using Whatman filter paper. 100 mg of liver tissue homogenate were prepared in ice-cold glass distilled water with the aid of a chilled all glass homogenizer. The homogenates were centrifuged at 5000xg for 10 min and the clear supernatant was used as the enzyme source for enzyme analysis. The results were expressed as µg/g⁻¹ protein. Metallothionein determination was analyzed using a modification of the method described by Viarengo *et al.*[30]. Tissues were homogenized in a buffer solution of 0.15 M sucrose, 20 mM Tris-HCL (pH 8.6), and antiproteolytic agents. The homogenate was then centrifuged at 30000xg for 20 min at -4°C. The supernatant of that sample was purified with 1.05 ml cold ethanol and 80µl chloroform and centrifuged for 10 minutes at 6000xg at -4°C. The homogenate was then centrifuged at 3000xg for 20 min at -4°C. The 1 mL of supernatant was then treated with absolute cold ethanol

(1.05 mL) and 80µl of chloroform ethanol/chloroform and centrifuged at 6000xg for 10 min at -4°C. The sample was maintained and allowed the proteins to denature for one hour at -20°C and centrifuged for 10 minutes at 6000xg at -4°C and the pellet saved. Then discarded the supernatant and added 1 ml of the previously described buffer solution, 6ml cold ethanol and 80µl chloroform and centrifuged for 10 minutes at 6000xg at -4°C and dried under a nitrogen gas stream. A total of 150 µl of 1 N HCl containing 4mM EDTA was added to the sample. The concentration of Mt in the extract was quantified spectrophotometrically utilizing the Ellman's SH reagent. Results were compared to a GSH calibration curve.

Statistical Analysis:

Statistical Analysis of data was carried out using SPSS Inc. version (16) statistical package. One-way analysis of variance (ANOVA) and Duncan multiple range were used to assess whether metal concentrations varied as significantly among tissues. Correlation coefficients between the metals and Mt were computed. Data were expressed as means and standard deviation (M±SD) and the significance level was set at P<0.05.

3. Results and Discussion

The heavy metals contamination is one of the vital factors for decline of water quality that has an obvious impact on fish diversity. Consumption of fish from the contaminated site poses a higher health risk to human. The present study focused on the accumulation of heavy metals in *A. anguilla* and showed differences in heavy metals accumulation in the different tissues. The mean concentrations (±SD) of copper, lead, zinc and nickel in liver, gills and muscles of *Anguilla anguilla* are presented in Table (1). Data showed that there is a significant difference (P<0.05) between heavy metals among the studied organs. The result shows that Zn has the highest values in liver and gills (21.80±2.70 and 22.47±1.39 µg/g⁻¹) respectively while Pb concentration has the lowest values (1.20±0.22 µg/g⁻¹) found in muscles. Zn was the most abundant of all the metals considered. The Cu concentrations are significantly higher in gills than liver (11.04±1.32 µg/g⁻¹ and 8.84±1.53 µg/g⁻¹) respectively while the concentration of Cu is less in muscles (4.31±0.48 µg/g⁻¹). The Ni concentrations are significantly higher in gills than liver 6.65±0.58 µg/g⁻¹ and 5.97±1.15 µg/g⁻¹ respectively; these results are coincided with Bülent *et al.*[31]. The levels of metals accumulate mainly in liver and gills. The trend of accumulation of Zn > Cu > Ni > Pb were higher in gills > liver > muscles respectively. These results are in accordance with Camusso *et al.* [32] who reported that zinc accumulated mostly in the gills of Rainbow trout and Ishaq *et al.* [33] who indicated that Tilapia zilli gills contained the highest concentration (52.2%) of all the detected heavy metals and the muscle appeared the least concentration (21.5%). While other scientists found the highest zinc levels in the kidney, skin and in liver respectively [34-37]. The reason for high metal concentrations in the gills could be due to the metal complexing with the mucus that is impossible to remove completely from the lamellae before analysis[38] and also due to the gill is the primary site for uptake in fish [39,40].

Table 1: Mean concentrations ($\mu\text{g/g}^{-1}$) with standard deviations and values of metals in liver, gills and muscles of *Anguilla anguilla* in Lake Edku

Metals	Cu	Pb	Zn	Ni
Liver	8.84±1.53	2.27±0.39	21.8±2.70*	5.97±1.15
Gill	11.04±1.32	2.83±0.55	22.47±1.39*	6.65±0.58
Muscle	4.31±0.48**	1.20±0.22	17.84±1.18	4.8±0.48**

Mean values are significantly different at ($P < 0.05$) * superscripts are not significantly different ($P > 0.05$)

Liver and gills are metabolically active tissues and accumulate heavy metals of higher levels than muscles as it has been observed in this present study and in many experimental and field studies [41,42]. The levels of heavy metals in fish absorption through food depend upon many factors like the duration of exposure of fish to contaminants in the water, the feeding habits, water chemistry and any contamination of fish during handling and processing, and

fish sex, weight, season [43]. Those heavy metals accumulate in metabolic organs and stored are detoxified by producing metallothioneins. In the present study results showed that metal accumulation was higher in gills and liver, whereas it was lower in muscles. The measurement of suitable biomarkers in liver become useful and can give an idea about the health state of fish and more telling than bioindicators because they deal with chemical and physiological changes on the organism level and assess contamination based on a direct measure of change in the organism [44, 45]. Glutamic-oxaloacetic transaminase GOT and Glutamic-pyruvic transaminase GPT are two key enzymes considered as a sensitive measure to evaluate hepatocellular damage and some hepatic diseases [46]. Figure (2) shows the elevated values of enzymes in liver of *A. anguilla* compared to control. Changes in the enzymatic activities of aquatic organisms are widely used to demonstrate tissue damage and also diagnosis of fish diseases [17].

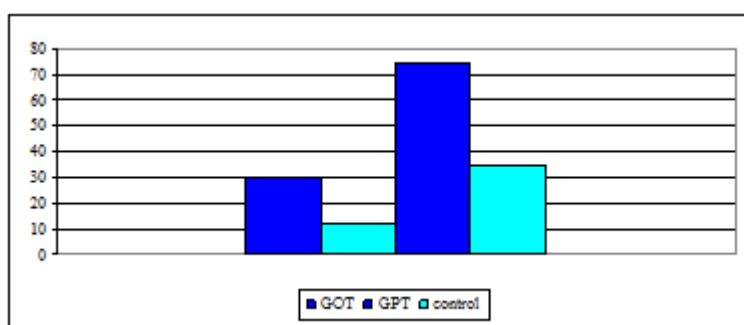


Figure 2: GOT and GPT in *Anguilla anguilla* from Lake Edku

According to statistical findings there were significantly higher ($P < 0.05$) differences between Cu, Zn, Ni, Pb and GOT ($29.73 \pm 2.75 \mu\text{g/g}^{-1}$), GPT ($74.77 \pm 8.51 \mu\text{g/g}^{-1}$) values in *Anguilla* liver than the controls. The increase was in response to heavy metals and might be attributed to tissue damage, particularly liver; this result was agreement with [47,48&49] in different fish species including *C. gariepinus*. When liver cells are damaged, GOT and GPT levels rise especially early in pollution also the enzyme levels are especially useful in assessing deformed cell and early changes in biliary obstruction and active cirrhosis. Metallothionein (MT) is a low molecular weight protein that binds to heavy metals in aquatic organisms. Metallothionein plays an important role in maintaining intracellular metal homeostasis, eliminating metal toxification and protecting against intracellular oxidative damages and sequesters these metals, thus functioning as a detoxification protein [50]. Moreover, there is a wide range of variation in MT and metal concentrations according to species and organs, depending on age, development status, and other physiological factors [51]. In the present result, the hepatic MT concentrations was ($18.20 \pm 3.77 \mu\text{g/g}^{-1}$) with significant difference ($P < 0.05$) between heavy metals. Metallothionein is often used to evaluate heavy metal contamination this foundation was coincided with [52] in the accumulation in Cu and Zn in liver and gill of eel and increased MT synthesis is associated with increased capacity for binding these metals and protection against metal toxicity. In the case of metal pollution, MT has been suggested to be suitable biochemical indicator this result was coincided with [53,54]. Abundance correlations between different

metals and metallothionein levels are presented in table (2). From the table more than one high correlations between the studies metals were observed. Zn is highly significant relation with MT ($r = 0.628$) and Cu correlations are significant ($r = 0.572$). Correlation between Ni & MT was ($r = 0.398$). Considered boardline statistically (with a pretty high probability of error (5%) these results are in agreement with Langston *et al.* (2002) [55]. No significant relationships was observed in Pb in the study however, other significant relations could be also noticed but with less significance.

Table 2: Correlation matrix between the four studies metals and MT in Lake Edku

	MT	Cu	Pb	Zn	Ni
MT	1				
Cu	0.573*	1			
Pb	-0.014	0.094	1		
Zn	0.628**	0.35	0.044	1	
Ni	0.398	0.237	0.021	0.191	1

** Correlations are highly significant at ($P < .005$) - * Correlations are significant at ($P < 0.01$)

4. Conclusion

The results of this study showed that the concentration of metals in livers and gills were considerably higher than in muscles and the most accumulation of Zinc was in gills. There were significant differences ($P < 0.05$) between metal studies among liver, gills and muscles and also between GOT, GPT liver enzymes. Significant positive correlations

were found between metallothionein and Zn, Cu and Ni in liver. No statistically significant differences were found between metallothionein and Pb. Based on the above results, metals bioaccumulation in fish organs did not exceeds the permissible limits set for heavy metals by FAO furthermore the use of enzymatic as biomarker are very important and realistic. Recommendation is the treatment of different wastes before discharging to the natural water sources to protect the fish and the public health from the danger of pollution.

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