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# HEPATIC BIOTRANSFORMATION OF *OREOCHROMIS NILOTICUS* IN LAKE MARYÛT IN RESPONSE TO XENOBIOTIC EXPOSURE

BY

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## ABSTRACT

**For a long time, Lake Maryut has been recognized as the most productive fishery ground among the northern Delta Lakes. But because of man misuse, the lake has become highly eutrophic and overloaded with several pollution problems. The object of this study is to trace the effects of changes in water and sediments pollution at lake Maryut on hepatic biotransformation in *Oreochromis niloticus* and suggest some biomarkers to measure the pollution stress in fish in the lake. Fish were collected from all basins of the lake (five locations) and compared with those collected from the near by fish farm as a reference. Concentration of cytochrome P-450 can be used as a biomarker for exposure to polycyclic aromatic hydrocarbons (PAHs) since correlation was found between exposure to elevated levels of PAHs in water and sediments and hepatic cytochrome P-450. Data also exhibited that cytochrome b5 concentration was positively correlated with heavy metals content and can be also used as a sensitive bioindicator for environmental pollution with heavy metals. It was found also that high levels of pollution reduced the hepatic glutathione (GSH) concentration. In contrast, hepatic glutathione s-transferase (GST) and microsomal hepatic protein content drew a positive correlation with elevated levels of heavy metals in water and sediments.**

## INTRODUCTION

Environmental pollution of air, water, soil and food represents an essential threat to the existence of many plant and animal communities and may ultimately to the human

survival. Atmospheric deposition from human activities can be a significant contributor of toxic chemicals and nitrogen compounds to both water and sediment (USEPA, 2000).

Metal toxicity to fresh and brackish water organisms, has previously been reviewed by Spry et al., (1981); Campbell and Stokes (1985) and McDonald et al., (1989). The metals which are of great concern in fresh and brackish waters are Hg, Pb, Cd, Cu, Zn, Mn, and Cr. When they reach high levels in the body, heavy metals can be immediately poisonous, or can result in long-term health problems similar to those caused by pesticides and herbicides (Cunningham and Saigo, 2001). Admittedly, the disposal of untreated sewage, even in the open sea, is very harmful particularly its hygienic and aesthetical effects and its impact on fauna and flora of the aquatic environment. The basic way for tracing pollutants in water bodies is water analysis. Sediment analysis also became an important tool for tracing water bodies pollution since the bottom sediments are a "source" as well as a "sink" of contaminants in the aquatic environment (Johnson and Nicholas, 1988 and FRrstner et al., 1993).

Exposure of animals to xenobiotics brings disturbances in metabolic function, activating detoxifying enzymes and the antioxidant system, and also damage to genetic material. This could be used as potential biomarker to measure pollution stress in animals (Verlecar et al., 2006). Systems in which toxic metals can induce impairment and dysfunction include the blood and cardiovascular detoxification pathways (colon, liver, kidneys, skin), endocrine (hormonal), energy production pathways, enzymatic, gastrointestinal, immune, nervous (central and peripheral), reproductive, and urinary (ATSDR, 1999). Metals bound to membranes are likely to modify membrane function, for instance inhibiting trans-membrane transport of electrolytes, sugars, amino acids and other solutes (Shainskaya et al., 2000).

Numerous laboratory studies have shown that the prior sub lethal exposure to metals can result in enhanced lethal resistance (Duncan and Klaverkamp, 1983). The need for sensitive measures for assessing the early signs of chemical contaminants effects on environmental quality has led to study the biochemical and physiological mechanisms that govern contaminant-induced alterations in marine organisms at the sub-organismal level. Accordingly, over the last few years a suite of indices have been developed for monitoring such early responses induced by contaminants. These indices are frequently called biomarkers or bioindicators. The measurement of biochemical and physiological responses to chemical

contaminants in fish, serves to improve the assessment of biologically significant exposure to toxic anthropogenic chemicals, and enhances the ability to assess risk for its effects on the health and survival of contaminant exposed populations. The usefulness as biomarker has now been confirmed in many field studies worldwide (Broeg et al., 2005).

Many xenobiotics such as drugs and carcinogens as well as physiological substrates such as vitamins, hormones and lipids are primarily metabolized by cytochrome P-450 system, multigene superfamily of microsomal enzymes (Guengerich, 2004). Xenobiotic metabolizing enzymes have more recently been studied in fish, with particular emphasis on the cytochrome P-450 dependent mono-oxygenases (MO). The usefulness of MO induction in biomonitoring studies has been confirmed in a number of studies covering various species and pollutants (Monod et al., 1988 and Allen and Moore, 2004). The P-450s are highly inducible, especially in liver, and exists in multiple forms (Vlasuk et al., 1982 and Conney, 1986) that are structurally and biologically related and have distinct overlapping substrate specificity. Initial metabolism by P-450s results in both detoxification and metabolic activation of the parent compound. Cytochrome P-450 1A1 isoforms increase rapidly after exposure to different chemical contaminants. They are key enzymes in the activation of certain environmental contaminants to cell damaging intermediates. The cytochrome P-450 enzymes are collectively responsible for the bulk of oxidation of xenobiotic chemicals including drugs, pesticides, and carcinogens (Buhler and Williams, 1989).

Monod et al., (1988) studied the influence of chemical pollution on hepatic xenobiotic metabolizing enzymes of three fish species, roach (*Rutilus rutilus*), nase (*Chondrostoma nasus*) and grayling (*Thymallus thymallus*). They stated that, monooxygenases and conjugating activities were significantly higher in the liver of fish exposed to the effluents of a polychlorinated biphenyl (PCB) incineration plant than in these from reference areas. Conjugation with glutathione (GSH) catalyzed by glutathione S-transferase (GST) is one of the major pathways for the detoxication of metabolites generated by the P-450 system and also of endogenous compounds (Jakoby and Habig, 1980). In fish liver, GST activity is located primarily in the cytosol, but activity has also been detected in the microsomal fraction. Multiple hepatic glutathione S-transferase was significantly increased in caged Rainbow trout (*Oncorhyncus mykiss*), feral perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*) when exposed to water polluted with polycyclic aromatic hydrocarbons (PAHs), heavy metals and sulfates (Tuvikene et al., 1999). Rats dosed with 90 ppm

Trichloramine had significantly increased glutathione S-transferase (Nakai et al., 2000).

The interaction of metals with glutathione is an integral part of the toxic response to many metals. Glutathione (GSH) plays a central role in chemical detoxication. Howard and Billings (2000) provided a general review of the effects of several pollutants on various physiological aspects in aquatic organisms. Hepatic glutathione has an important role in billiary excretion of copper, zinc (Alexander and Aeseth, 1980), methylmercuric (Ballatori and Clarkson, 1983), cadmium and lead ions (Alexander et al., 1986). According to Kappus (1986), nickel administration to rats caused a transient fall in hepatic reduced glutathione (GSH) levels that were followed by a notable increase. Copper also was shown to cause depletion of GSH as well as inhibition of several enzymes associated with GSH metabolism. Mercury also has devastating effects on the glutathione content of the body, giving rise to the possibility of increased retention of other environmental toxins (Crinnon, 2000). GSH depletion is often associated with cytotoxicity and may promote tumor development through mechanisms that involve cytotoxicity and other different ways (Murray et al., 1987).

During the last decade, industrial activity at Alexandria, Egypt, has led to ecological disequillibrium and significant deterioration of the biological environment especially aquatic. Maryût lake receives agricultural drainage water in addition to major inputs of municipal and industrial wastes from Alexandria. The National Environmental Action Plan of Egypt (1992) has identified the north western Lake "Maryût" as the most polluted ecosystem in Egypt.

The purpose of this study is to trace the effects of changes in water and sediments pollution at lake Maryût on the hepatic biotransformation occurring in *Oreochromis niloticus* since it is the most abundant fish species in the lake. This could be achieved through the measurements of liver cytochrome P – 450, cytochrome b5, glutathione, glutathione S – transferase and microsomal protein concentration in the different locations of the lake since the work on biomarker research in Egypt is limited and does not receive sufficient attention.

## Materials and Methods

### *Sampling location:*

Fish were sampled from Maryût lake. The lake occupied a portion of the Mediterranean foreshore plain adjoining the city of Alexandria at latitude 31° 10" N and longitude 29° 55" E. Samples were collected from

six locations at the lake, location 1, 2, 3, 4, 5 and 6 represent Main basin (MB), East basin (E), Southeast basin (SE), Northwest basin (NW), Southwest basin (SW), and the fish farm (FH) which receives its water from the drainage water and used as a reference site, respectively (Fig 1).

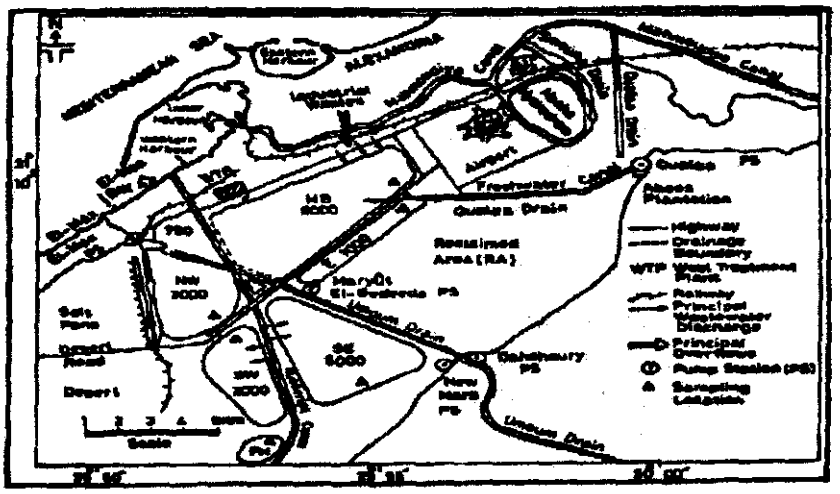

**Figure (1): Morphology of Lake Maryut**

***Sampling of fish:***

Nile tilapia, *Oreochromis niloticus*, was chosen in this study as an experimental model since it represents the main fishery in the lake. It is a standard bioassay fish and one of the most marketable fresh water species.

Random adult fish samples of uniform size nearly 18 cm in length and from 135 to 169 gm in weight (approximately 100 fish) were collected from the selected sampling locations. After capture, fish were transferred into large vessels filled up with aerated lake's water, where they quickly recovered. All fish collected were grouped according to the sampling locations and were maintained alive in separate glass aquaria (60×35×35cm).

***Preparation of liver for xenobiotic metabolizing enzymes:***

Liver of the collected fishes were immediately removed, washed with cold 0.1 M potassium phosphate buffer, pH 7.4, blotted dry, weighed and chilled on ice. All the following operations were carried out at 4c°. A 33% (w/v) crude homogenate was prepared (1gm liver + volumes of 0.1 phosphate buffer, pH 7.4) by homogenization with Teflon piston using five to eight strokes. The crude homogenate was centrifuged at

11,000 Xg for 20 minutes at 4c° to remove intact cells, nuclei, and mitochondria. The supernatant solution was subsequently centrifuged at 105,000 Xg for 60 minutes at 4c° to sediment microsomal pellets. The microsomal pellets were resuspended in 0.1 M phosphate buffer, pH 7.4, kept in ice bath and used as the enzyme source.

***Liver biotransformation tests:***

Liver microsomal cytochrome P-450 and b-5 were determined according to the method of Omura and Sato (1964). The hepatic content of glutathione was determined colourmetrically (Mitchel et al., 1973), while glutathione S-transferase was assayed according to the method of Chi-Yu et al. (1981). Total protein concentration in the hepatic microsomal fraction was determined as reported by Lowry et al. (1951)

***Data analysis:***

Statistical analysis was carried out using statistical package for social sciences (SPSS) software. Statistical parameters (Mean, SE, SD) as well as analysis of variance were calculated according to Turner (1970) and Snedecor and Cochran (1969).

## RESULTS

***Water and Sediment quality:***

Analysis of heavy metals content and polycyclic aromatic hydrocarbons (PAHs) in water and sediments were conducted by Ibrahim et al., (2006) and are shown in table (1) and fig. 2, 3. They reported that water and sediments are generally polluted with heavy metals and PAHs and exceeded the maximum contaminant levels (MCL) especially in the main basin (location #1).

**Table (1): Heavy metals and naphthalene concentration (μg $g^{-1}$) in sediments:**

| Element | Location #1 | Location #2 | Location #3 | Location #4 | Location #5 | Location #6 | SLC |
|---|---|---|---|---|---|---|---|
| Cd | 7.75* ± 0.23 | 7.10* ± 0.07 | 6.90* ±0.83 | 4.55* ± 0.48 | 5.90* ± 1.13 | 2.70 ± 1.78 | 0.60 – 10 |
| Cr | 7.28* ±0.36 | 6.25* ± 0.51 | 4.81* ± 0.22 | 4.18* ±0.24 | 3.49* ±0.69 | 1.54 ±2.03 | 26 – 110 |
| Cu | 24.15** ±1.77 | 19.15** ± 2.09 | 13.25** ± 0.78 | 11.05** ± 1.89 | 5.70 ± 1.89 | 5.35 ± 6.65 | 16 – 110 |
| Mn | 187.55** ±13.17 | 150.30** ± 9.33 | 123.90** ± 6.70 | 104.95** ± 6.35 | 87.00* ± 10.55 | 57.15 ± 46.10 | — |
| Hg (ppb) | 6.50 | 4.50 | 2.50 | 0 | 0 | 0 | 0.20 – 2 |
| Ni | 49.55** ± 0.90 | 47.00** ± 7.83 | 24.85** ± 1.79 | 19.80* ± 2.28 | 13.35* ± 1.04 | 10.40 ± 13.84 | — |
| Pb | 4.05* ± ,0.51 | 2.60* ± 2.49 | 9.65** ± 2.74 | 1.90 ± 0.23 | 1.25 ± 0.30 | 0.40 ± 1.29 | 31 – 250 |
| Zn | 40.90** ± 2.00 | 35.25** ± 0.48 | 33.90** ± 7.07 | 13.90* ± 1.20 | 10.50 ± 0.21 | 9.90 ± 10.96 | 120 – 820 |
| Co | 12.55* ± 2.09 | 9.35* ± 3.07 | 9.65* ± 2.65 | 4.09* ± 2.58 | 4.75* ± 0.53 | 6.15 ± 4.07 | — |
| Fe | 1998.50* ± 55.51 | 1290.50 ± 242.90 | 1561.50* ± 662.21 | 1615.00* ± 952.50 | 1682.50* ± 638.90 | 1253.00 ± 400.93 | 2100 – 4380 |
| Mg | 4347.50* ± 217.40 | 2470.00* ± 275.70 | 2317.50* ± 284.60 | 1927.50* ± 885.70 | 2447.50* ± 850.30 | 2866.70 ± 736.70 | — |
| Naphthalene | $5.66\times10^{-6}$* ± 3.50 $\times10^{-9}$ | $4.44\times10^{-6}$ ± 3.50 $\times10^{-9}$ | $5.61\times10^{-6}$* ± 3.30 $\times10^{-9}$ | $4.61\times10^{-6}$ ± 3.50 $\times10^{-9}$ | $5.06\times10^{-6}$ ± 3.50 $\times10^{-9}$ | $5.25\times10^{-6}$ ± 3.50 $\times10^{-9}$ | MCL = 0.01 |
| $\Sigma\overline{X}_1$ | 321.23** | 267.65** | 217.26** | 160.33** | 127.19* | 87.44 | |
| $\Sigma\overline{X}_2$ | 11.80* | 9.70* | 16.55** | 6.45* | 7.15* | 3.10 | |

**$\Sigma\overline{X}_1$ refers to the sum of means (ppm) of Cd, Cr, Cu, Mn, Hg, Ni, Pb and Zn in each location;**

**$\Sigma\overline{X}_2$ refer to the sum of means of Cd, Hg and Pb (the *most toxic* metals) in each location.**

**Asteriaks denote a significant difference from the control value (location #6). *P<0.05; **P<0.01; ***P<0.001. Presented data are means ± SE.**

**SLC refers to standard level criteria as reported by USEPA (1996).**

**MCL refers to the maximum contaminant level as reported by USEPA (1996).**

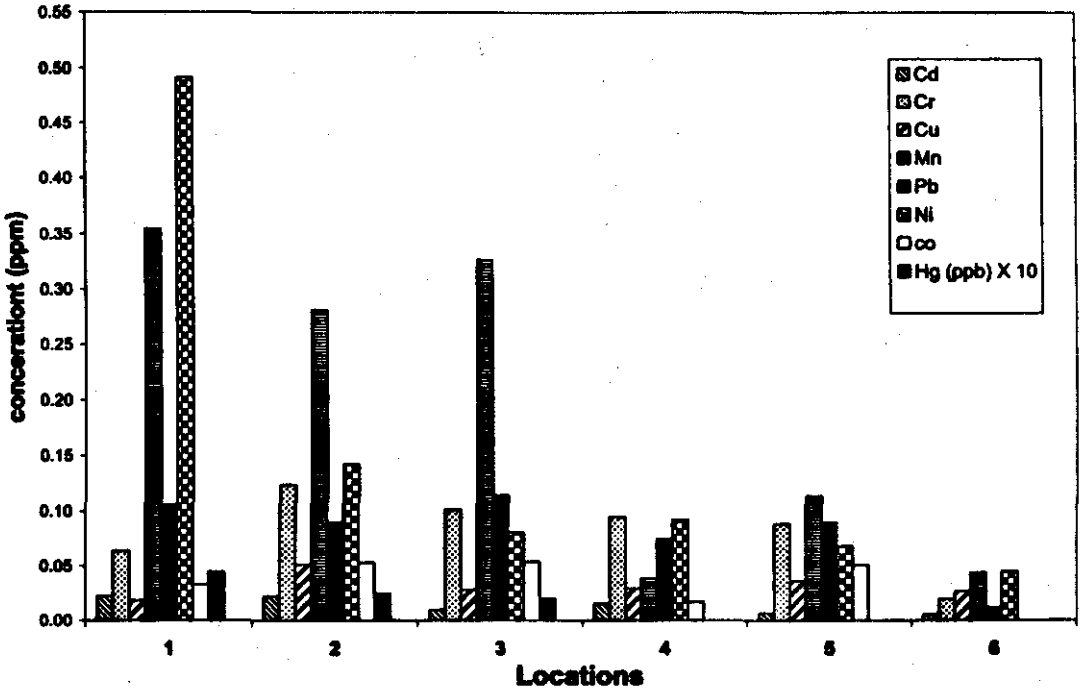


**Figure (1): Heavy metals concentration in water**

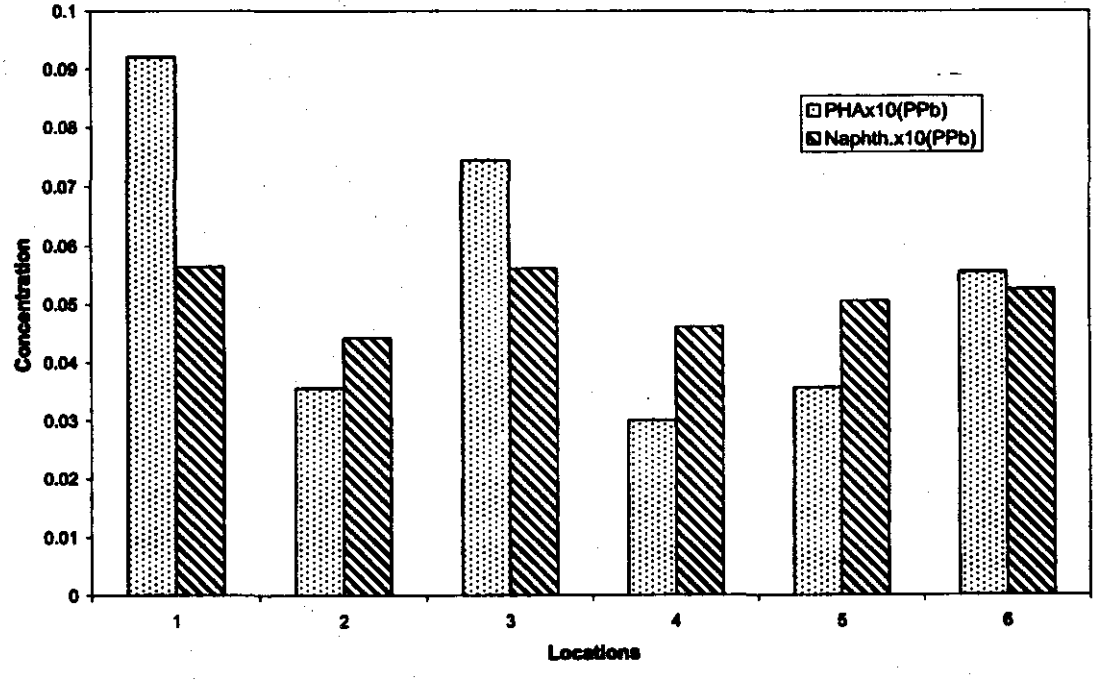


**Figure (2): Concentration of Polycyclic aromatic hydrocarbons (PAHs) in water and Naphthalene in sediments**

***Liver biotransformation tests:***

***1. Cytochrome P-450:***

Table (2) and Fig. (4) Illustrate the values of cytochrome P-450. High significant values for cytochrome P-450 were recorded in locations #1 and 3 (10.1 ± 0.59, 9.5 ± 0.56 nmoles $mg^{-1}$ protein, respectively) as compared to the reference site. On the other hand, lower significant values were observed in locations #4 and 5 (2.17 ± 0.29 & 2.53 ± 0.72 nmoles $mg^{-1}$ protein, respectively).

**Table (2): Liver cytochrome P-450 concentration (nmoles $mg^{-1}$ protein) of *O. niloticus*:**

| Locations | Range | Mean | SD | SE | % Change | Probability |
|---|---|---|---|---|---|---|
| #1 | 8.33-12.25 | 10.10*** | 1.33 | 0.59 | +40% | <0.005 |
| #2 | 2.80-7.98 | 4.20*** | 1.07 | 0.53 | -42% | <0.002 |
| #3 | 2.28-10.40 | 9.50** | 1.13 | 0.56 | +32% | <0.010 |
| #4 | 1.10-3.17 | 2.17*** | 0.66 | 0.29 | -62% | <0.001 |
| #5 | 1.21-4.90 | 2.53*** | 1.43 | 0.72 | -65% | <0.001 |
| #6 | 6.03-8.50 | 7.20 | 0.89 | 0.39 | | |

Asterisks denote a significant difference from the control value. *P<0.05; **P<0.01; ***P<0.001.

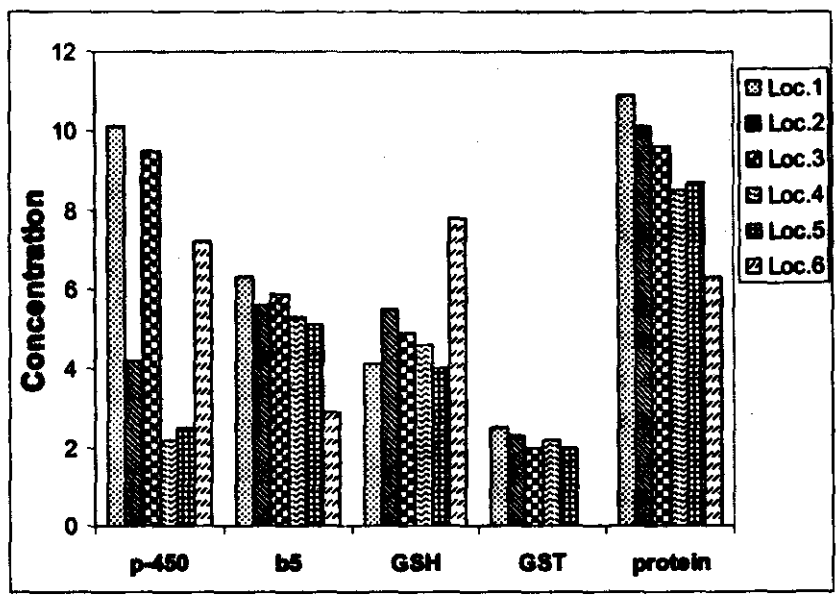


**Figure (4): Concentration of cytochrome P-450, b5 (nmole $mg^{-1}$ protein), GSH (µmole $g^{-1}$ liver), GST (unite $mg^{-1}$ protein) and microsomal protein (mg $g^{-1}$ liver)**

### *2. Cytochrome b5:*

Data revealed that Cytochrome b5 values (Table 3 and Fig. 4) were significantly higher in all locations as compared to the reference site (location #6). It fluctuated in the order #1 > #3 > #2 > #4 > #5 > #6 (6.26 ± 0.38 > 5.93 ± 0.51 > 5.60 ± 0.81 > 5.33 ± 0.55 > 5.10 ± 0.78 > 2.92 ± 0.36 n moles $mg^{-1}$ protein, respectively).

### *3. Glutathione (GSH):*

High significant value of hepatic glutathione (Table 4 and Fig. 4) was found in location #6 (7.80 ± 0.62 µmole $g^{-1}$ liver). In other locations, (the polluted areas) GHS values were relatively low and fluctuated between 4.00 ± 0.58 in location #5 and 5.48 ± 0.72 µmole $g^{-1}$ liver in location #2.

**Table (3): Liver cytochrome b5 concentration (nmoles $mg^{-1}$ protein) of *O.niloticus*:**

| Locations | Range | Mean | SD | SE | % Change | Probability |
|---|---|---|---|---|---|---|
| #1 | 5.01-7.20 | 6.26*** | 0.85 | 0.38 | +114% | <0.001 |
| #2 | 2.55-8.87 | 5.60* | 1.80 | 0.81 | +92% | <0.02 |
| #3 | 4.77-7.55 | 5.93*** | 1.02 | 0.51 | +103% | <0.002 |
| #4 | 4.18-7.70 | 5.33*** | 1.33 | 0.55 | +82% | <0.005 |
| #5 | 2.70-7.29 | 5.10* | 1.74 | 0.78 | +75% | <0.05 |
| #6 | 2.05-4.40 | 2.92 | 0.79 | 0.36 | | |

Asterisks denote a significant difference from the control value. *P<0.05; **P<0.01; ***P<0.001.

### *4. Glutathione S-transferase (GST):*

Table (5) and Fig. (4) show that values of GST were significantly higher in all locations as compared to the control site (location #6). The highest significant value was observed in location #1. Values fluctuated as follows #1 > #2 > #4 > #5 > #3 (2.54 ± 0.02 > 2.27 ± 0.10 > 2.17 ± 0.09 > 2.02 ± 0.09 > 2.02 ± 0.15 units $mg^{-1}$ protein). The lowest value of GST was found in location #6 (1.17 ± 0.11 units $mg^{-1}$ protein).

**Table (4): Liver hepatic glutathione (GSH) concentration (µmoles $g^{-1}$ liver) of *O. niloticus*:**

| Location | Range | Mean | SD | SE | % Change | Probability |
|---|---|---|---|---|---|---|
| #1 | 2.79-4.70 | 4.12*** | 0.68 | 0.30 | -47% | <0.001 |
| #2 | 4.09-8.66 | 5.48* | 1.61 | 0.72 | -30% | <0.05 |
| #3 | 3.13-8.00 | 4.93* | 1.66 | 0.74 | -37% | <0.02 |
| #4 | 3.43-4.70 | 4.59*** | 1.01 | 0.45 | -41% | <0.005 |
| #5 | 2.76-5.77 | 4.00*** | 1.17 | 0.58 | -49% | <0.001 |
| #6 | 6.30-9.63 | 7.80 | 1.38 | 0.62 | | |

Asterisks denote a significant difference from the control value. *P<0.05; **P<0.01; ***P<0.001.

### *5. Microsomal hepatic protein concentration*

In general, microsomal protein content was significantly higher in all the tested locations than that in the reference site. The highest value was found in location #1 (10.86 ± 1.03), while location #2 (10.12 ± 0.58 mg protein $g^{-1}$ liver) comes next. The lowest significant value was recorded in location #6 which represents the reference site (6.29 ± 1.64 mg protein $g^{-1}$ liver) as compared to other sites as shown in table (6) and fig. (4).

**Table (5): Liver glutathione – s-transferase (GST) concentration (Units $mg^{-1}$ protein) of *O. niloticus*:**

| Locations | Range | Mean | SD | SE | % Change | Probability |
|---|---|---|---|---|---|---|
| #1 | 2.52-2.64 | 2.54*** | 0.04 | 0.02 | +120% | <0.001 |
| #2 | 1.87-2.50 | 2.27*** | 0.22 | 0.10 | +94% | <0.001 |
| #3 | 1.51-2.28 | 2.02*** | 0.33 | 0.15 | +72% | <0.001 |
| #4 | 2.10-2.43 | 2.17*** | 0.21 | 0.09 | +85% | <0.001 |
| #5 | 1.78-2.15 | 2.02*** | 0.18 | 0.09 | +73% | <0.001 |
| #6 | 0.74-1.23 | 1.17 | 0.25 | 0.11 | | |

Asterisks denote a significant difference from the control value. *P<0.05; **P<0.01; ***P<0.001.

**Table (6): Liver protein concentration (mg protein $g^{-1}$ liver) in the hepatic microsomal fraction of *O. niloticus*:**

| Locations | Range | Mean | SD | SE | % Change | Probability |
|---|---|---|---|---|---|---|
| #1 | 7.50-13.40 | 10.86* | 2.31 | 1.03 | + 72% | <0.05 |
| #2 | 8.94-12.08 | 10.12* | 1.30 | 0.58 | + 60.8% | <0.05 |
| #3 | 8.50-11.44 | 9.60* | 1.19 | 0.40 | + 52.6% | <0.01 |
| #4 | 7.83-11.16 | 8.45* | 1.37 | 0.61 | + 34% | <0.01 |
| #5 | 9.53-7.50 | 8.70* | 0.84 | 0.38 | +38.3% | <0.02 |
| #6 | 2.17-11.99 | 6.29 | 3.67 | 1.64 | | |

Asterisks denote a significant difference from the control value. *P<0.05; **P<0.01; ***P<0.001.

## DISCUSSION

The physical and chemical qualities of water, are important to a great extent and determine the success or failure of aquaculture operations (LaDon, 1990). Moreover, prediction the bioavailability and toxicity of metals in aquatic sediments should be a critical component in developing sediment quality criteria (USEPA, 1996). Significant impact to pollution exposure at cellular level are the specific biochemical responses, such as production of cytochrome P-450 mediated system of mixed function oxygenation of organic compounds, antioxidants and metal-binding proteins.

In the present investigation, concerning cytocrome P-450, it was found that it reached its maximum levels (10.1 ± 0.59, 9.5 ± 0.56 and 7.2 ± 0.39 nmoles $mg^{-1}$ protein) in fish caught from locations #1, #3 and #6, respectively). This elevation of cytochrome P-450 is mostly due to the relatively high levels of PAHs and naphthalene in water and sediment in these locations. This explanation is supported earlier by reports of LindstrRm-Seppä and Oikari (1990a, b), Soimasuo *et al.* (1995). Cytochrome P-450 proved, in this instance, is a very important biomarker for exposure to PAHs in fish environment. Compared to reference fish group, cytochrome-P450 in fish groups #2, #4 and #5 of Lake Maryût was significantly reduced (4.2 ± 0.53, 2.17 ± 0.29 and 2.53 ± 0.72 nmoles $mg^{-1}$ protein, respectively). Data also showed that water concentrations of cadmium and lead exceeded the MCL recommended by USEPA (1987) in locations #2, #4 and #5, which could

be one of the causes of the depression of cytochrome P-450 in these fish groups. This result agreed with the results obtained by Maines and Kappas (1977), and FRrlin et al. (1986).

The possible explanation of the above-mentioned data could be based on the fact that exposure to trace metals results in a variety of biochemical alterations in liver and kidney. Many of these effects result from the capacity of metals to bind to nucleophilic sites in the cell, which primarily occur in the form of free sulfhydryl groups (Maines and Kappas, 1977). Hepatic microsomal heme oxygenase is a sulfhydryl-dependent enzyme which is responsible for the oxidative degradation of heme to the tetrapyrrol, biliverdin (Tenhunen *et al.*, 1968). Although many metal ions *in vitro* inhibit heme oxygenase activity, *in vivo* studies have demonstrated that *de novo* synthesis of heme oxygenase is enhanced by the presence of metal ions, which act directly on the enzyme regulatory site. Associated with the increase in heme oxygenase activity is a depression in hepatic microsomal cytochrome P-450 content and a concomitant decrease in mixed-function oxidase activity (Maines and Kappas, 1977). Thus, in addition to the direct inhibition of sulfhydryl containing enzymes, some metals also decrease hepatic xenobiotic biotransformation capacity by increasing the rate of hemoprotein degradation. Also, Korashy and El-Kadi (2005) showed that treatment with heavy metals decreased the degradation rate of protein of the cytochrome m-RNA.

The present data of cytochrome b5 showed nearly two-fold increase in fish of all locations of Lake Maryût compared to control. This results are in agreement with those reported by Ikeda et al.(1991). Hepatic microsomal cytochrome b5 concentration is positively correlated, in a very sensitive manner, to heavy metal concentrations in both water and sediment in fish environment, therefore, cytochrome b5 can be used as a sensitive bioindicator of aquatic environmental pollution.

Glutathione (GSH) is one of the most abundant intracellular thiols, aids in protection of cells from the lethal effects of toxic and carcinogenic compounds (Arrick and Nathan, 1984). It has been shown to be an important determinant of cellular sensitivity to a wide variety of drugs and other cytotoxic compounds (Orrenius and Moldeus, 1984). Hepatic glutathione concentrations in liver of *Oreochromis niloticus* of Lake Maryût showed a significant depletion in all locations as compared to control. This reduction in the levels of glutathione may be due to the high levels of toxic metals in these locations where values of most of the toxic heavy metals exceeded the MCL (USEPA, 1987). It seems that GSH depletion may promote cytotxicity (Murray *et al.*,1987). It

was reported that the toxicity of chemical carcinogens might be a result of GSH depletion (Gopalan *et al.*, 1994). The toxicity of certain xenobiotics can be enhanced either by depleting glutathione level or by blocking the reduction of oxidized form of glutathione (GSSG) to its reduced form (GSH) through inhibition of glutathione reductase (GSH-R).

There was a significant increase of glutathione S-transferase in fish collected from all locations of Lake Maryût as compared to the control fish. The presesnt results are in agreement with the results obtained by Sheweita, (1998). Also, Goksøyr et al., (1987) found that the activity of GST increased two to three bold. This increase in GST refers to some adaptations to the toxic effects in the lake's environment and that cytotoxicity in these fish groups has not completely occurred (George, 1994).

The increase in microsomal hepatic protein in the treated birds was in agreement with that previously reported by Davison et al. (1976) when they fed rats with mirex in the diet. This increament is correlated positively with those took place in cytochrome b5 and GST,while it has a negativ correlation with GSH content. However, Folmer et al. (1993) and Adham et al. (2001) stated that as a stress response, hypoproteinemia as well as hyperproteinemia may be observed in fish.

In conclusion,the determination of cytochrome P450,cytochrome b5,GSH and GST as biomarkers for envirnmental pollution could serve as an early warning to the serious environmental changes in biodiversity that may be occurring as a result of water pollution in Lake Maryut.

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