Assessment of metallothionein expression in *Biomphalaria alexandrina* snails and *Oreochromis niloticus* fish as a biomarker for water pollution with heavy metals.

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**ABSTRACT**

Metallothioneins (MTs) are small cysteine-rich proteins that play important roles in metal homeostasis and protection against heavy metals; therefore, they were used as biomarkers to evaluate metals pollution in the aquatic ecosystem. The present work aims to evaluate the tissue immunohistochemical expression of MT in *Biomphalaria alexandrina* snails and *Oreochromis niloticus* fish collected from Lake Manzala in the area of Port-Said, Dakahlia and Damietta governorates in response to the recorded levels of Cd, Cu, and Pb in their aquatic habitats. The levels of the heavy metals; Cd, Cu &Pb in water samples from Port Said were significantly exceeded those of Dakahlia (p<0.01), also, a similar pattern was recorded for Cu levels between Damietta and Dakahlia (p<0.01). Accordingly, the tissue expression of MT was significantly increased in snails and fish specimens from Port Said and Damietta compared to Dakahlia governorate specimens (p<0.01 and p<0.05 respectively). In general; MT immunopositivity in snails regarding the number of MT-positive cases and the number of positive cells, was observed as digestive gland> prostate tubules> ovotestis> head-foot area and in fish, it was as follows: liver & spleen> gills > ovary. Conclusion: There is a positive correlation between heavy metals levels and MT expression in snails and fish collected from Port Said, Damietta and Dakahlia, El-Manzala Lake (p<0.001 & r=0.636, p<0.001 & r=0.375 and p<0.05 & r=0.296, respectively), hence MT could be considered as a good potential biomarker for toxicological studies and for assessment of heavy metal stress in aquatic environment.

**INTRODUCTION**

Heavy metals are natural elements present in the environment (Wegwu *et al.*, 2010). They are usually present in trace amounts in natural waters but many of them are toxic even at very low concentrations (Herawati *et al.*, 2000). The accumulation of metals in an aquatic environment has a direct impact on the sustainability of the ecosystem. The bioavailability of any particular metal in the aquatic organisms or fish that are being
exposed to the polluted water are faced with various threats such as neurological damage, decreased immunity, disruption in metabolic function, defect in reproduction and offspring (M’kandawire et al., 2012).

Metallothionein (MT) is a metal-binding protein which can be found in many organisms (Roesijadi, 1996). MT has been documented to bind a wide range of metals including cadmium (Cd) (Freisinger and Vašák, 2013), lead (Pb) (Wong et al., 2017), mercury (Sutton and Tchounwou, 2007), copper (Stern, 2010) and arsenic (Tchounwou et al., 2004). The biological functions of MTs include homeostasis of many cellular processes, regulating the metal ions for cell function and in immunoregulation in the protection against heavy metals, oxidant damages, and inflammation. Li et al. (2007) explained that MTs bind to metal ions to dispel them out of the system and prevent cellular toxicity. Theoretically, when an organism is treated or exposed to heavy metals, the synthesis of MT will increase. Thus, a polluted area with high levels of heavy metals would induce an increase in MTs in the exposed organisms, hence they were used as a biomarker to detect heavy metal pollution and the bioavailability of any particular metal in the environment (Atli and Canli, 2007). However, the regulation of metal ions by aquatic organisms is up to a certain concentration to protect themselves from adverse effects; and continuous exposure of heavy metals into the aquatic body disturbs the regulatory processes and initiates metal accumulation (El-Khayat et al., 2015). Many studies proved that the polluted water caused a severe damage to the cells and different tissues of the fish inhabiting these places (Mohamed, 2009). Metals tend to accumulate in water and move up through the food chain; therefore, studies to quantify the levels of heavy metals in environment and determine potentially hazardous levels for human are necessary (Haram, 2015).

In many species of Gastropoda (snails and slugs), Cd and Cu metabolism and detoxification are apparently linked to the expression of metal-specific MT, Biomphalaria glabrata is known to be sensitive to metal toxicity and has been proposed as an indicator of metal pollution in tropical environments (Ravera, 1977 and Allah et al., 1997). As well fish are considered to be sensitive pollution indicator because they are directly in contact with surface water and can store and metabolize the water borne pollutants (Ladhar-Chaabouni et al., 2012). MT can regulate heavy metals such as Zn and Cu in fish and protect aquatic biota from metal toxicity and oxidative stress (Roesijadi, 1996).

Lake Manzala is the largest of the northern deltaic lakes in Egypt that lies on the coast of Mediterranean Sea in the governorates Port Said, Damitta and Dakahlia. It is exposed especially in its southern part to high levels of pollutants from industrial, domestic and agricultural resources (Ibrahim et al., 1999). The present study aimed to assess the tissue expression of Metallothionein in Biomphalaria alexandrina snails and Oreochromis niloticus fish collected from Lake Manzala as a biomarker for heavy metal pollution in the lake.

**MATERIALS AND METHODS**

**Study area:**
This study was carried out in 12 different sites in Lake Manzala in three governorates; Port-Saïd (5 sites), Dakahlia (4 sites) and Damietta (3 sites), 2015
Sampling:
Snail samples were collected by a standard dip net (Jobin, 1970 and Yousif et al., 1992) from the water banks of Lake Manzala utilizing a motorized boat. The collected snails from each of the tested sites were placed in a plastic aquarium containing water from their habitat, transported to the laboratory and examined individually for natural trematode infections by the cercarial shedding test (McClelland, 1965). The negative (uninfected) Biomphalaria snails were used only where they removed from their shells gently then fixed in 10% buffered neutral formalin solution. On the other hand, fish specimens were collected by the local fishermen from each governmental site, anesthetized, dissected to obtain gills, liver, spleen and ovary that immediately fixed in 10% neutral-buffered formalin solution then transferred to the laboratory.

Water samples were collected in sterilized one-liter polyethylene bottles from each investigated site at 30 cm below the water surface. Samples were transported in an ice box to the laboratory where they were acidified with concentrated nitric acid 2ml/l, filtrated through filter papers then syringe-membrane filter pore size 0.45 mm and kept at 4°C till analysis.

Heavy metals analysis:
Metals stock standards of Cd, Pb & Cu were used in the determination of their respective heavy metals in the collected water samples using Atomic Absorption Spectrophotometry (AAS) (GBC AVANTA 3000, Australia) and expressed as ppb using Graphite furnace-AAS, in Environmental Research Laboratory, Theodor Bilharz Research Institute (TBRI).

Immunohistochemical technique
After fixation for 6 hrs snail tissues and fish organs were washed in 70% ethyl alcohol to get rid of excess fixative and then dehydrated through ascending grades of ethyl alcohol. The specimens were cleared in xylene for 15-20 min then embedded in paraffin wax. The paraffin wax block was sectioned at 5 μm, then Formalin-fixed paraffin sections (5μm in thickness) were cut undergo deparaffinization, rehydration and endogenous peroxidase was blocked with methanol containing 3% hydrogen peroxide. Sections were incubated overnight at 4°C in humid chamber with the primary antibodies: metallothionein polyclonal antibody (Biospes, Catalog no. YPA1550, China) at an optimal dilution of 1:100, with application of Power stain poly HRP DAB system (Genemed, Catalog no. 54-0017, USA). The antigen was detected by the addition of DAB substrate chromogen solution. Finally, slides were counterstained with hematoxylin and eosin, dehydrated in alcohol and mounted. Metallothionein stained sections were assessed by using light microscope (Scope A1, Axio, Zeiss, Germany). Photomicrographs were taken using a microscope-camera (AxioCam, MRc5, Zeiss, Germany). Metallothionein positivity was indicated by brownish cytoplasmic staining of cells.

Statistical analysis:
Data were presented as mean ± SE. Multi-group comparison was performed (Kruskal-Wallis 1-way ANOVA for k samples. Spearman’s rho Correlation and Chi square test was followed using the computer software package SPSS 24.0. Differences among individual groups were determined using the least significant difference test. p<0.05 was considered as statistical significant.
RESULTS

Heavy Metals analysis:
Results of Cd, Pb & Cu in water samples collected from the Lake Manzala examined sites are presented in Table (1) in comparison with the levels of US Environmental Agency (USEPA), 2011. The Lake sites in Port Said and Damietta were highly polluted with Cu compared to Dakahlia, moreover the recorded mean value exceeded the permissible level by up to 8.2 times in Damietta and 4.4 times in Port Said (p<0.01), but in Dakahlia it was less than the level of USEPA. Cd was significantly increased in Port Said compared to Dakahlia with mean value 3.6 times over the USEPA level. Similarly, Pb was significantly increased in Port Said compared to Dakahlia and Damietta with mean value 1.7 times over the concern level of USEPA.

Immunohistochemical expression:
Expression of metallothionein reactivity in B. alexandrina snails
The sections of head-foot, prostate tubules, stomach, digestive gland and ovotestis of B. alexandrina snails collected from Lake Manzala in the area of Dakahlia, Port Said and Damietta governorates and control laboratory bred ones were examined for their expression of MT reactivity (Figure 1) using MT polyclonal antibody. The pattern of positive MT immunoreactivity was indicated by brownish cytoplasm of staining cells.

Table 1: Levels of Heavy Metals (ppb) in water samples collected from Lake Manzala referred to the studied Governments.

<table>
<thead>
<tr>
<th>Governments</th>
<th>Cadmium Mean±SE</th>
<th>Folds M/PL</th>
<th>Lead Mean±SE</th>
<th>Folds M/PL</th>
<th>Copper Mean±SE</th>
<th>Folds M/PL</th>
</tr>
</thead>
<tbody>
<tr>
<td>PL (ppb)</td>
<td>2</td>
<td>65</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dakahlia</td>
<td>2.32±0.62</td>
<td>1.16</td>
<td>23.99±4.62</td>
<td>0.369</td>
<td>6.60±0.36</td>
<td>0.50</td>
</tr>
<tr>
<td>Damietta</td>
<td>4.35±1.52</td>
<td>2.18</td>
<td>88.5±28.44</td>
<td>1.36</td>
<td>106±43.83ab</td>
<td>8.2</td>
</tr>
<tr>
<td>Port Said</td>
<td>7.20±3.31**</td>
<td>3.6</td>
<td>112±9.8**</td>
<td>1.7</td>
<td>57.51±6.63**a</td>
<td>4.4</td>
</tr>
</tbody>
</table>

*p<0.05 significant increase than Damietta; **p<0.01 significant increase than Dakahlia
*p<0.01 significant increase than Dakahlia; b*p<0.01 significant increase than Port Said and Dakahlia

PL: permissible limits according to EPA (2011).

Results (Table 2a and Fig. 1) show that tissues of control snails in head foot, prostatic tubules, stomach, digestive gland and ovotestis were negative for MT expression, while the expression of MT was recorded in snail specimens from Port Said and Damietta with high percentages compared to Dakahlia ones (p<0.01 and p<0.05, respectively. Positive MT immunostaining reactivity was seen in the unicellular glands in head-foot of Port Said (Fig. 2 a) and Damietta (Figure 2- b) specimens. MT positive expression was found in prostate tubules tissues in snail specimens from both of Port Said (Figure 3- a) and Damietta governorates (Fig. 3- b). Moreover, digestive gland showed more positive immunostaining reactivity for MT in snails from Port Said (Fig. 4 a) and Damietta (Fig.
The ovotestis of snail specimens from Port Said (Fig. 5-a) and Dakahlia (Fig. 5-b) governorates exhibited, also, positive expression of MT. In general; MT immunopositivity, regarding number of MT-positive cases and number of positive cells, was arranged as digestive glands> prostate tubules> ovotestis> head-foot of snails (Table 2b).

Table 2a: Metallothionein immunohistochemical reactivity in *Biomphalaria alexandrina* snails collected from Lake Manzala.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Governorates (n)</th>
<th>MT Immunohistochemical Reactivity</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive expression n (%)</td>
<td>Negative expression n (%)</td>
</tr>
<tr>
<td>Control snails</td>
<td>Dakahlia (5)</td>
<td>0</td>
<td>5 (100%)</td>
</tr>
<tr>
<td></td>
<td>Damiettaa (5)</td>
<td>0</td>
<td>5 (100%)</td>
</tr>
<tr>
<td></td>
<td>Port said (5)</td>
<td>0</td>
<td>5 (100%)</td>
</tr>
<tr>
<td>Lake snails</td>
<td>Dakahlia (15)</td>
<td>3 (20%)</td>
<td>12 (80%)</td>
</tr>
<tr>
<td></td>
<td>Damietta (30)</td>
<td>15 (50%)</td>
<td>15 (50%)</td>
</tr>
<tr>
<td></td>
<td>Port said (30)</td>
<td>24 (80%)</td>
<td>6 (20%)</td>
</tr>
</tbody>
</table>

*p<0.05* Significant difference compared to Dakahlia

*p<0.01* highly Significant difference compared to Dakahlia

Table 2b: Metallothionein immunohistochemical reactivity in different parts of *Biomphalaria alexandrina* snails.

<table>
<thead>
<tr>
<th>Governorates</th>
<th>no. of positive cases</th>
<th>Mean(%) of positive cells ± Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lake snails</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dakahlia</td>
<td>3</td>
<td>10±0</td>
</tr>
<tr>
<td>Damietta</td>
<td>15</td>
<td>38.57±2.60</td>
</tr>
<tr>
<td>Port said</td>
<td>24</td>
<td>46.00±3.05</td>
</tr>
</tbody>
</table>

Expression of metallothionein reactivity in fish organs

Metallothionein expression liver, spleen, gills and ovary of fish control specimens were negative (Table 3a). In the fish specimens collected from different governorates (Figure 1), positive MT expression 62.5% was seen in liver tissues of fish from both of Port Said and Damietta compared to 31% positivity in Dakahlia specimens. Similar pattern was recorded in spleen and gills of specimens from Port Said and Damietta compared to Dakahlia ones. Meanwhile, in ovary tissues, 50 % of fish from Damietta and Dakahlia were positive for MT compared to 25 % of fish from Port Said (Table 3a). The positive immunostaining expression was observed in liver and pancreatic acini in fish specimens.
from both Damietta (Figure 6-d) and in Port-Said (Figure 6-e,f). In spleen tissues, a high MT expression was found in scattered cells of specimens from Damietta (Figure 7-b), Dakahlia (Figure 7-c) and Port Said (Figure 7-d). In fish gills, the positive MT expression was seen in scattered cells of gill fish tissues of Damietta specimens (Figure 8-b). A marked up-regulation of MT was found in filaments of pillar system in of gills tissues from Dakahlia (Figure 8-c), and Port-Said specimens (Figure 8-d). The positive MT immunostaining was found in ovarian membrane and cytoplasm of premature ova in O. niloticus fish from Damitta and Port Said. According to positive number of cases and positive percentage of positive cells; MT positivity was higher in spleen & liver than that of gills, while the least positivity of MT was seen in ovary tissues (Table 3b).

Using Sperman's rho Correlation proved a positive correlation between heavy metals (Cd, Cu and Pb) in polluted water and MT expression in B. alexandrina snails and O. niloticus fish from Port-Said and Damietta governorates compared to those from Dakahlia (p< 0.001 & r=0.636, p< 0.001 & r=0.375 and p< 0.05 & r=0.296, respectively).

### Table 3a: Metallothionein immunohistochemical reactivity in studied Oreochromis niloticus fish collected from Lake Manzala.

<table>
<thead>
<tr>
<th>Governorates</th>
<th>Liver</th>
<th>Spleen</th>
<th>Gills</th>
<th>Ovary</th>
<th>Control</th>
<th>Total (64)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dakahlia (16)</td>
<td>5 (31%)</td>
<td>11 (69%)</td>
<td>8 (50%)</td>
<td>8 (50%)</td>
<td>4 (25%)</td>
<td>12 (75%)</td>
</tr>
<tr>
<td>Damietta (16)</td>
<td>10 (62.5%)*</td>
<td>6 (37.5%)</td>
<td>12 (75%)*</td>
<td>4 (25%)</td>
<td>9 (56.3%)</td>
<td>7 (43.7%)</td>
</tr>
<tr>
<td>Port Said (16)</td>
<td>10 (62.5%)*</td>
<td>6 (37.5%)</td>
<td>13 (81%)*</td>
<td>3 (19%)</td>
<td>12 (75%)*</td>
<td>4 (25%)</td>
</tr>
</tbody>
</table>

*p<0.05 Significant difference compared to Dakahlia

### Table3b: Metallothionein immunohistochemical reactivity (Percentage of positive cells) in Oreochromis niloticus fish organs. Mean(%) of positive cells ± Standard Error

<table>
<thead>
<tr>
<th>Governorates</th>
<th>Liver</th>
<th>Spleen</th>
<th>Gills</th>
<th>Ovary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dakahlia (16)</td>
<td>5 92.5±4.27 8 70.00±6.55</td>
<td>4 35±2.88 8 6.25±0.82</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Damietta (16)</td>
<td>10 72.00±7.82 12 86.67±1.42</td>
<td>9 34.44±2.27 8 6.87±0.92</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Port Said (16)</td>
<td>10 73.00±7.0 13 90±0.00</td>
<td>12 47.5±3.05 4 7.5±1.45</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fig. (1): Metallothionein immune histochemical reactivity in snails and fish samples collected from Lake Manzala.

![Graph showing metallothionein immune histochemical reactivity](image)

**Fig. (2)**. Light photomicrograph for transverse sections in the head foot area of *Biomphalaria alexandrina* snails from Manzala Lake.

a) MT positivity in the unicellular glands of head food area in Port Said (MT x200).
b) MT positivity of head food area in Damietta (MT x100).
c) Negative stain for MT in head foot area in Dakahlia (MT x50).

![Images of photomicrographs](image)

**Fig. (3)**. Light photomicrograph for transverse sections in the prostate tubule area of *Biomphalaria alexandrina* snails from Manzala Lake.

a) Positive expression in prostate tubules from Port Said (MT X200).
b) MT positive expression in prostate tubules from Damietta (MT X200).
Metallothionein expression in *Biomphalaria alexandrina* and *Oreochromis niloticus*

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**Fig (4).** Light photomicrograph for transverse sections in the digestive gland and stomach in *Biomphalaria alexandrina* snails from Manzala Lake.

a) MT positivity in the digestive gland from Port Said (MT x200)
b) MT positive in the digestive gland from Damietta (MT x200)
c) MT negative in the digestive gland from Damietta (MT x200)
d) MT negative expression in the stomach from Damietta (MTX100)
e) MT negative expression in the stomach from Dakahlia (MTX100)
f) MT negative in the digestive gland from Dakahlia (MT x100)

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**Fig (5).** Light photomicrograph for transverse sections in the ovotestis in *Biomphalaria alexandrina* snails from Manzala Lake.

a) MT positivity in the ovotestis from Port Said (MT x200)
b) MT positivity in the ovotestis from Dakahlia (MT x200)
Fig (6). Light photomicrograph for transverse sections in the liver tissues in *Oreochromis niloticus* fish from Manzala Lake.

a) MT negative expression in control fish liver cells (MTX200)
b) MT negative expression in liver cells and pancreatic acini in fish from Dakahlia (MTX200)
c) MT negative expression in cytoplasm of cells of liver and MT positive expression in cytoplasm of pancreatic acini cells in fish from Port Said (MT X200)
d) MT positive expression in cytoplasm of cells of liver and pancreatic acini in fish from Damietta (MT X200)
e) MT positive expression in cytoplasm of pancreatic acini cells in fish from Port Said (MT X100)
f) MT positive expression in cytoplasm of liver cells and pancreatic acini cells in fish from Port Said (MT X200)
Fig (7). Light photomicrograph for transverse sections in the spleen of *Oreochromis niloticus* fish from Manzala Lake.

a) MT negative expression in control fish spleen (MTX50)  
b) MT positive expression in scattered cells of spleen from Damietta (MTX50)  
c) MT positive expression in scattered cells of spleen from Dakahlia (MTX100)  
d) MT positive expression in scattered cells of spleen from Port Said (MTX100)

Fig (8). Light photomicrograph for transverse sections in gills of *Oreochromis niloticus* fish from Manzala Lake.

a) Negative MT in Fish Gills in fish from Damietta (MTX100)  
b) MT positivity in scattered cells in filaments in fish from Damietta (MTX200)  
c) MT positivity in filaments of pillar system in fish from Dakahlia (MTX100)  
d) MT positivity in filaments in fish from Port Said (MTX100)

Fig (9). Light photomicrograph for transverse sections in ovary of *Oreochromis niloticus* fish from Manzala Lake.

a) MT negative expression in Yolk of ova, mature oocytes in fish from Port Said (MTX50)  
b) MT positive expression in ovarian membrane and cytoplasm of premature ova in fish from Dakahlia (MTX100).
DISCUSSION

Metallothionein was used by many investigators as a biomarker for assessment of heavy metal stress in the field of applied biosciences for environmental pollution in snails (Ravera, 1977 and Allah et al., 1997), fish (Rosesijadi, 1996 and Wu et al., 2000), Calonectris diomedea (Stewart et al., 1996), Major Carp Species (Singha et al., 2011). The present work used B. alexandrina snails and O. niloticus fish as bioindicators through determining their MT immunopositivity tissue expression as an evidence for presence of the heavy metals Cd, Cu & Pb in Lake Manzala. The Lake sites in Port Said and Damietta governorates showed significantly increased heavy metal levels compared to Dakahlia and were over the concern levels of USEPA , 2011. Alonso et al. (2005) reported that heavy metals play a crucial role in various biological functioning of aquatic organisms; the severity of metal toxicity is different between species because it depends on what kinds of metal and the species dexterity to synthesize metallothionein naturally in their system. El-Khayat et al. (2018) used the same aquatic organisms; B. alexandrina snails and O. niloticus fish, as bio-indicators for the presence of pollutants in Lake Burullus nearby waste water discharge areas and recorded that water quality adversely affect their physiological and hematological parameters. The present results detected immunopositivity in different examined parts of snails; head foot, prostate tubules, stomach, digestive gland and ovotestis and fish organs; liver, spleen, gills & ovary. Positive correlation was confirmed between higher levels of Cu, Pb & Cd recorded in Port Said and Damietta sites and the pronounced MT immuno-reactivity in their samples of both B. alexandrina and O. niloticus compared to those from Dakahlia, indicating that the increased level of MT was parallel to the increased pollution levels of these metals. These findings consistent with Dallinger et al.(2004) who found that there is a strong correlation between heavy metals, particularly Cd accumulation, and MT concentration. As well, Pedrini-Martha et al. (2016) reported that, MT genes can be up regulated in response to exposure to the corresponding metal ion. In addition, Roch et al. (1982), Olsson and Haux (1986), Sulaiman et al. (1991) and Hylland et al. (1992) evidenced that an elevated level of MT in fish indicates that the fish has been exposed to heavy metals Pb,Cu and Cd. Furthermore, Stewart et al. (1996) reported positive correlation between MT expression and Cd & Cu accumulation in kidney and liver of black-backed gulls.

The data of snails and fish samples showed MT immunopositivity. Regarding the number of MT-positive cases and number of positive cells, the immunopositivity was arranged in snails as: digestive gland> prostate tubules> ovotestis> head-foot area. The pattern for MT expression for fish organs was: liver & spleen> gills> ovary. These findings were also observed by Wu et al. (2006) and Çoğun et al.(2003)who stated that liver is the most common organ that principally responsible for accumulation of environmental xenobiotics in order to metabolize them. The present data showed a high MT expression in tissues of fish from liver, pancreatic acini and spleen in O. niloticus fish from Damietta and Port Said. Also, Metallothionein positivity was significantly more expressed in the filamental epithelium of gills in O. niloticus fish specimens from Port Said and Damietta compared to Dakahlia, and it is known that the toxic impact of metals is primarily as a result of accumulation in the tissues of fish gills which lead to their damage. Similarly, Dang et al. (1999) found more MT positivity in gills of O. niloticus fish. In ovary tissues, half of O. niloticus fish from Damietta and Dakahlia were positive for Metallothionein compared to quarter of fish from Port Said.
Ghedira et al. (2010) found that MT induction following intraperitoneal injection of cadmium. In addition, Ahmad et al. (2000) reported that induction of MT biosynthesis after exposure to Cu. Furthermore several studies described MTs to play a major role in the detoxification of Cd, and this process is clearly organ-specific (De Smet et al., 2001) and also MTs play a role in the oxidative defense against chronic copper exposure in the liver of a fresh water catfish *Channa punctatus*. These findings match our results that indicate expression of MT correlates with increased heavy metals especially Cu that the most high metal over the concern level of USEPA,2011 followed by Cd.

### Conclusion

Positive correlation was proved between heavy metal levels (Cd, Cu and Pb) in Lake Manzala and MT expression in snails and fish from Port-Said and Damietta governorates compared to those from Dakahlia. Therefore, there is a need to periodic monitor of the aquatic environment to avoid contamination and its impacts on human health and ecosystem. MTs can play a role as a potential biomarker for toxicological studies and for assessment of heavy metal stress in aquatic environment.

### References


Metallothionein expression in Biomphalaria alexandrina and Oreochromis niloticus


**ARABIC SUMMARY**

*Oreochromis* and *Biomphalaria alexandrina*: تقييم تعبير الميثالوثيونين في قواقع الاسماء البلطي النيلي و الأسماك البلطي النيلي كمؤشر بيولوجي لتلوث المياه بالمعادن الثقيلة. niloticus

البحث في البحوث البيئية والرخويات الطبية - قسم البيولوجيا - معهد تيودور بارس- جيزة - مصر.

الميثالوثيونين هي بروتينات صغيرة غنية بالسيستين تلعب أدوار مهمة في التوازن المناعي والحماية من المعادن الثقيلة في الكائنات الحية. وذلك تم استخدامها كمؤشرات أو دليل بيولوجي لتقني تلوث المعادن في النظام الإيكولوجي المائي. يهدف هذا البحث إلى تقييم التعبير المناعي للأنسجة للميثالوثيونين في قواقع بِيوغلاريا الكسندرنا وأسماك البلطي النيلي التي تم جمعها من بحيرة منزلة في منطقة من محافظات بورسعيد والدقهلية ودمياط استجابة للمستويات المسجلة من المعادن الثقيلة الرصاص والكادميوم والناحاس في عينات المياه الخاصة بها.

ونلاحظ أن التعبير النسيجي للميثالوثيونين قد زاد بشكل ملحوظ في القواقع وأسماك البلطي النيلي من بورسعيد ودمياط مقارنة بعينات محاكاة من المياه الجوفية في هذه الأثناء تجاوزت بورسعيد الدقهلية بشكل كبير في جميع المعادن الثقيلة التي تم قياسها لمستويات الرصاص والكادميوم والنحاس. ودمياط تجاوزت بشكل كبير الدقهلية مستوي النحاس كما أن مستويات التعبير المناعي للميثالوثيونين في عدد الحالات الإيجابية وعدد الخلايا الإيجابية في القواقع كانت مرتفعة في حين كان في أجهزة الأنسجة الكبدية والسائل النظيرين. 

بالترتيب التالي: digestive glands> prostate tubules> ovotestis> head-foot> liver&spleen> gills> ovary

الأسماء على النحو التالي: 

الاستنتاج: لو حظ ارتباط إيجابي بين مستوى المعادن الثقيلة والتعبير الميثالوثيونين في القواقع وأسماك وبالتالي يمكن أن تصبح كمؤشرات حيوية جيدة لوجود الملوثات في النظم المائية وأن تكون ضمنية لدراسات السمية ولتقييم الإجهاد المعادن الثقيلة في البيئة المائية.