Enzymatic Biomarkers Of Oxidative Stress In Fish In Relation To Toxic Environmental Pollutants In Lake El Temsah, Ismalia, Egypt

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ABSTRACT

The potential of oxygen free radicals and other reactive oxygen species (ROS) damage tissues and cellular components, in biological systems has become a topic of significant interest for ecological toxicology studies. The balance between exogenous factors (i.e., environmental pollutants such as heavy metals) and antioxidant defenses in biological systems can be used to assess toxic effects under stressful environmental conditions. In our study superoxide dismutase (SOD) and catalase (CAT) activities has been used as a biomonitor for studying the effect of some heavy metals pollution of Lake El Temsah. Our results revealed Lead (Pb), Cadmium (Cd) and Nickel (Ni) accumulation in fish tissues (liver, gill and muscle) were over the permissible limits. Also results showed indirect negative correlation relation between heavy metals residues and enzymatic antioxidative stress biomarkers in all fish tissues as there were marked inhibition of SOD and CAT in all fish tissues concluding a state of oxidative stress. This study summarizes advances in the understanding of such oxidative processes in biological systems. This knowledge is extended to specific applications in fish because of their sensitivity to oxidative pollutants, their filtration capacity, and their potential for environmental toxicology studies.

INTRODUCTION

Environmental contamination by metals became intensively investigated in toxicological researches in recent years. Heavy metals such as Pb, Cd and Ni reach the aquatic systems as a consequence of industrial, agricultural and anthropogenic sources, such as an urban runoff, sewage treatment plants, domestic garbage dumps, and finally they can affect different ecosystems *(1)*. Metals are able to disturb the integrity of the physiological and biochemical mechanisms in fish that are not only an important ecosystem component, but also used as a food source *(2)*. Fish tissues, specifically the liver, gill and muscle tissues are endowed with an antioxidant defense systems to protect them from oxidative stress caused by metals. Elevated levels of metals can induce oxidative stress by generating highly reactive oxygen species (ROS), such as hydrogen peroxide, superoxide radical and hydroxyl radical via Haber–Weiss and Fenton reactions that can oxidize proteins, lipids and nucleic acids, often leading to damage in cell structure or even cell death *(3)*. Organisms have developed several protective mechanisms to remove ROS before the detrimental effects occur in cell. Antioxidant non enzymes glutathione (GSH), enzymes such as catalase (CAT) and superoxide dismutase (SOD) are of great importance in an oxidative stress to cope with free radicals leading several disturbances. GSH is synthesized in the liver and released to the blood for transferring to the other organs such as the kidney and muscle. Because metal exposures did not alter the levels of GSH in the blood, muscle and gill, it suggests that metals taken up from the gill were immediately transferred (via the blood) to the liver for the usage in the metabolism or sequestered *(4)*. SOD catalyzes the reduction of superoxide radical into hydrogen peroxide, which is eliminated by CAT into oxygen and water. Analysis of catalase (CAT) and superoxide dismutase (SOD) hepatic activities has been largely used as biomarkers of exposure to metals *(5)*, usually in combination with oxidative damages, as lipid peroxidation byproducts (MDA) *(6)*. Moreover, the measurements of MDA in muscles can be a useful tool to evaluate fish quality, as they indicate oxidation of constitute elements in nutritional supplies for human consumption. Various responses of enzyme have been observed in animals exposed to metallic contaminants in both field and laboratory experiments, which indicated an increase or a decrease in the activity depending on the dose, species and route of exposure *(7,8)*. The antioxidant mechanisms related with oxidative stress have gained considerable interest in the field of ecotoxicology. Therefore, antioxidant enzymes are considered as sensitive biomarkers in environmental stress before hazardous effects occur in fish, and are important parameters for testing water for the presence of toxicants *(9)*. In addition, tilapia (*Oreochromus niloticus*) is a widely distributed freshwater fish that can persist in a highly polluted habitat, and has the potential for the development as a biological monitor of heavy metal pollution.

MATERIAL AND METHODS

Fish samples

A total number of 50 fish samples *Oreochromus niloticus* (Tilapia Nilotica) 10-13 cm length and 35.70 ± 0.60 g were collected from five different regions of Lake El Temsah (El- Halloos drain, Lake of sayadeen, El Taawine region, the El Forsan Island, and El Mahsama drain.) in Ismailia province, Egypt. Fish were transferred in plastic bags filled with declorenated water to the laboratory. At laboratory, liver, gills and muscle were dissected from fish, washed with de-ionized water in which one part was wrapped separately in acid washed polyethylene bags for measurement of heavy metal residues and the other were kept in eppindorf tubes for assessment of antioxidant biomarkers. For determination of heavy metals, the tissue samples were transferred to a clean screw capped bottle and digested with 10 ml solution of nitric/sulfuric/perchloric acid (8:1:1) *(10)*. Initial digestion was made for 4 hours at room temperature, followed by heating at 40-45°C for one hour in water bath, then temperature was raised to 75°C until the end of digestion. After cooling at room temperature, the cold digest was diluted to 20 ml with de-ionized water and filtered through 0.45u Whitman filter paper. The clear filtrate of each sample was kept in refrigerator to avoid evaporation, and for enzymatic biomarkers assessment tissue homogenization was carried out.

Atomic Absorption Spectrophotometer analysis technique

Filtrated samples were analyzed for their heavy metal content by using UNICAM 969 Atomic Absorption Spectrometer. Instrumental analysis of cadmium, lead, copper, zinc and nickel was conducted by air/acetylene with flow rate of 0.9 to 1.2L./minute for lead and zinc, 1.0 to 1.3L./minute for cadmium and 0.8 to 1.1L./minute for copper.

Preparation of fish tissues homogenate for glutathione (GSH), catalase (CAT) and Malondialdehyde (MDA) activity assay

Tissue homogenization was carried out *(11)* and sample absorbance was measured against sample blank at 405, 510 and 534 nm respectively using Genway spectrophotometer

Quantitative determination of heavy metals

The concentrations of cadmium, lead, copper, zinc and nickel in the examined water samples were taken directly from digital scale reading of Atomic Absorption Spectrophotometer. The concentrations of cadmium, lead, copper, zinc and nickel in examined tissue samples were calculated according to following equation:

$$\text{ppm metal in sample} = \frac{A \times B}{W}$$

Where A: ppm metal in prepared sample from the digital scale reading of AAS.

B: final volume of prepared sample in ml. W: weight of sample in gm.

Preparation of fish samples homogenate for superoxide dismutase (SOD) activity assay

Tissue homogenization was carried out *(12)* and for determination of superoxide dismutase (SOD) level, absorbance of samples was measured against control at 560 nm at 25° C using Genway spectrophotometer.

Statistical analysis

The statistical analysis of data was carried out using spss 17.0 version (Chicago, USA) programs One-way ANOVA *(13)* to compare variables among different location along Lake Temsah. Significant differences ($P<0.05$) were reanalyzed by Duncan tests to determine which individual groups were significantly different from the other. Data concerning antioxidant enzymes was analyzed using bivariate correlation relationships among environmental variables to avoid redundancy using Pearson's correlation coefficient (one variables from each pair-wise with (r > 0.70 was removed). Principal component analysis (PCA) was then applied as an indirect ordination technique to describe the main sources of variation and relationships among the selected environmental variables. PCA reduces the dimensionality of the stressor variables to a few principal, synthetic gradients *(14)*.

RESULTS

Liver enzymes

The levels of oxidative stress biomarkers in liver samples of fish along Lake El Temsah were summarized in Table 1 and represented in Figure 1. The results concerning glutathione (GSH) level of liver samples showed highly significant level in lake of sayadeen 310.9±17.1 mg/g, El Taawine region 304.7±11.1 mg/g, El halloos drain 299.7±18.8 mg/g, El Forsan Island 281.6±13.2 mg/g, and meanwhile the lowest concentration was recorded in El Mahsama drain 263.7±14.8 mg/g. Liver superoxide dismutase enzyme (SOD) level of fish recorded highly significant values over the lake being 273 ± 4.26 mg/g in El Forsan Island while samples from El Taawine region 266.8 ± 6.85 mg/g, El Halloos Drain 259.3 ± 4.87 mg/g, liver of fish obtained from Lake of Sayadeen 258.3 ± 8.36 mg/g and the lowest level showed in liver samples of fish obtained from El Mahsama Drain 250.4± 9.08 mg/g. Whereas levels of catalase enzyme (CAT) of fish liver samples obtained from El Forsan Island revealed 8.04± 0.17 mg/g, El Mahsama Drain 7.84 ± 0.15 mg/g, Lake of Sayadeen showed 7.636± 0.15 mg/g, El Halloos Drain 7.35 ± 0.1 mg/g and 4.213± 0.32 mg/g in El Taawine region. Liver malondialdehyde (MDA) level of fish recorded highly significant values over the lake being 590± 37.1 mg/g in El Taawine region while samples from El Forsan Island 547.9 ± 54.7 mg/g, Lake of Sayadeen 481.9 ± 46.6 mg/g, liver of fish obtained from El Halloos Drain 335.1± 23.1 mg/g and the lowest level showed in liver samples of fish obtained from El Mahsama Drain 305.4 ± 19.4 mg/g.

Table 1. Levels of oxidative stress biomarkers of liver samples of fish (GSH, SOD, CAT and MDA) (MG/G tissue) from five areas (El- Halloos drain, Lake of sayadeen, El Taawine region, the El Forsan Island, and El Mahsama drain.) of Lake El Temsah. (Mean ± S.E).

Enzyme / Location	GSH mg/g tissue	SOD mg/g tissue	CAT mg/g tissue	MDA mg/g tissue
El Halloos Drain	299.7 ± 18.8	259.3 ± 4.87	7.35 ± 0.1	335.1 ± 23.1
Lake of Sayadeen	310.9± 17.1	258.3 ± 8.36	7.636± 0.15	481.9 ± 46.6
El Taawine region	304.7 ± 11.1	266.8 ± 6.85	4.213± 0.32	590± 37.1
El Forsan Island	281.6± 13.2	273 ± 4.26	8.04± 0.17	547.9 ± 54.7
El Mahsama Drain	263.7 ± 14.8	250.4± 9.08	7.84 ± 0.15	305.4 ± 19.4

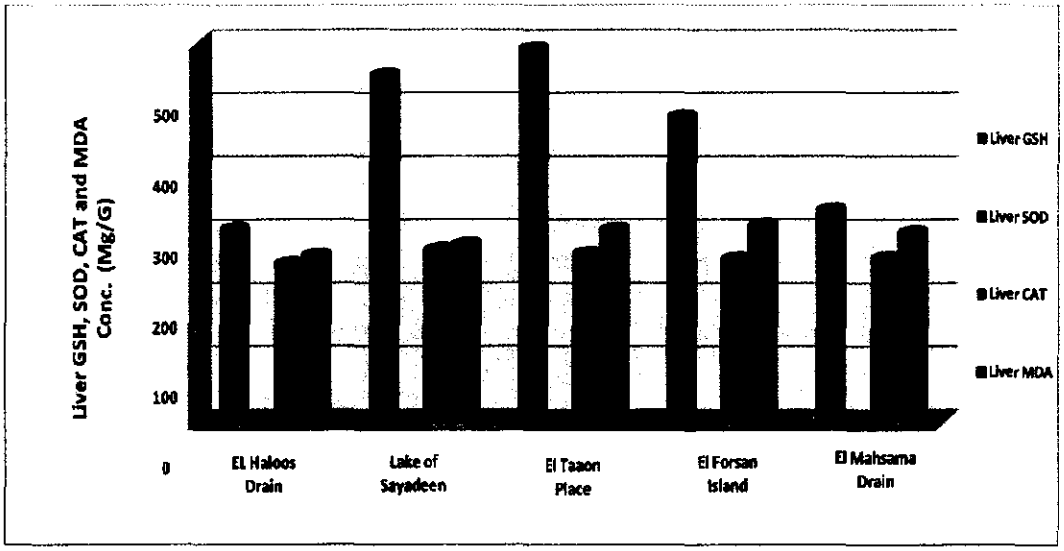

Fig. 1. Glutathione (GSH), Superoxide Dismutase (SOD), Catalase (CAT) and Malondialdehyde (MDA) concentration (mg/g tissue) of fish liver samples collected from five areas of Lake El Temsah (El Halloos Drain, Lake of Sayadeen, El Taawine region, El Forsan Island and El Mahsama Drain).

Gill enzymes

The levels of oxidative stress biomarkers in gill samples along Lake El Temsah were recorded in Table 2 and represented in Figure 2. The estimated oxidative stress biomarkers in gill samples were in the range of 467.6-573.9, 207.6-263.4 and 4.353-8.589 mg/g for glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) while oxidative damage biomarker malondialdehyde (MDA) was in the range 563.5-660.5 mg/g. The results concerning GSH level of gill samples showed highly significant level in El Taawine region 573.9±7.15 mg/g, Lake of Sayadeen (559.3 ± 3.83mg/g), El Mahsama Drain (537.7 ± 10.2mg/g) and El Halloos Drain (509.7 ± 35.7mg/g) meanwhile the lowest enzyme concentration was recorded in El Forsan Island (467.6± 10.6mg/g). Gill superoxide dismutase enzyme (SOD) level of fish recorded highly significant values over the lake being 263.4 ± 4.09 mg/g in El Halloos Drain while samples from Lake of Sayadeen were 253.6 ± 3.37 mg/g, El Mahsama Drain 248.8 ± 4.45 mg/g, gill of fish obtained from El Taawine region 245.1 ± 4.74 mg/g and the lowest level showed in samples obtained from El Forsan Island 207.6 ± 4.59 mg/g. Whereas levels of catalase enzyme (CAT) of fish gill samples were highly significant in all areas as El Halloos Drain revealed 8.589 ± 0.09 mg/g, El Taawine region 7.708± 0.19 mg/g, El Mahsama Drain showed 7.479 ± 0.07 mg/g, Lake of Sayadeen 6.125± 0.22 mg/g while significant value of CAT in gill of fish was recorded as 4.213± 0.32 mg/g in El Forsan Island. Gill malondialdehyde (MDA) level of fish recorded highly significant values over the lake being 660.5± 9.0 mg/g in El Forsan Island while that of gill samples from El Mahsama Drain 659.7 ± 9.0 mg/g, Lake of Sayadeen 655.6 ± 8.3 mg/g, gill of fish obtained from El Taawine region 653.6 ± 8.24 mg/g and the lowest level showed in gill samples of fish obtained from El Halloos Drain 563.5 ± 12.4 mg/g.

Table 2. Levels of oxidative stress biomarkers of gill samples of fish (GSH, SOD, CAT and MDA) (MG/G tissue) from five areas (El- Halloos drain, Lake of sayadeen, El Taawine region, the El Forsan Island, and El Mahsama drain.) of Lake El Temsah. (Mean ± S.E).

Enzyme / Location	GSH mg/g tissue	SOD mg/g tissue	CAT mg/g tissue	MDA mg/g tissue
El halloos drain	509.7 ± 35.7	263.4 ± 4.09	8.589 ± 0.09	563.5 ± 12.4
Lake of sayadeen	559.3 ± 3.83	253.6 ± 3.37	6.125± 0.22	655.6 ± 8.24
El Taawine region	573.9 ± 7.15	245.1 ± 4.74	7.708± 0.19	653.6 ± 8.1
El Forsan island	467.6± 10.6	207.6 ± 4.59	4.353 ± 0.3	660.5 ± 8.4
El Mahsama drain	537.7 ± 10.2	248.8 ± 4.45	7.479 ± 0.07	659.7 ± 8.1

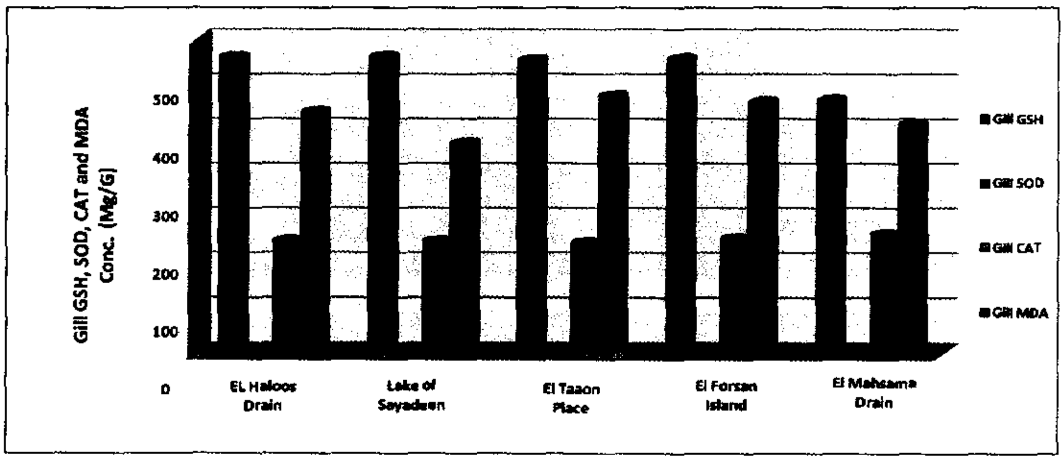

Fig. 2. Glutathione (GSH), Superoxide Dismutase (SOD), Catalase (CAT) and Malondialdehyde (MDA) concentration (mg/g tissue) of fish gill samples collected from five areas of Lake El Temsah (El Halloos Drain, Lake of Sayadeen, El Taawine region, El Forsan Island and El Mahsama Drain).

Muscle enzymes

The levels of oxidative stress biomarkers in muscle samples along Lake El Temsah were summarized in Table 3 and represented in Figure 3. The estimated oxidative stress biomarkers in muscle samples were in the range of 750-457 mg/g, 243.6-272.7 mg/g and 1.393-3.201 mg/g for glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) respectively while oxidative damage biomarker malondialdehyde (MDA) was in the range 126-191.3 mg/g. The results concerning (GSH) level in muscle samples along Lake Temsah showed highly significant values in El Taawine region 750± 33.1mg/g, and El Forsan Island 714.3± 2.53mg/g while El Mahsama drain 596.9 ± 11.2mg/g, Lake of Sayadeen 546.2 ± 8.11mg/g and El Halloos Drain 457± 36.6mg/g showed significant values of muscle GSH levels. Muscle superoxide dismutase enzyme (SOD) level of fish recorded highly significant values over the lake being 272.7 ± 2.36 mg/g in El Mahsama Drain while that of muscle samples from El Forsan Island 269.2 ± 2.92 mg/g, Lake of Sayadeen 262± 5.59 mg/g, muscle of fish obtained from El Taawine region 250.5 ± 2.6 mg/g and the lowest level showed in muscle samples of fish obtained from El Halloos Drain 243.6 ± 3.65 mg/g. Whereas levels of catalase enzyme (CAT) of fish muscle samples obtained from five areas along Lake El Temsah were highly significant in all areas as El Halloos Drain revealed 3.201 ± 0.3 mg/g, El Taawine region 2.808± 0.24 mg/g, El Mahsama Drain showed 2.776 ± 0.26 mg/g, El Forsan Island 1.513 ± 0.1 mg/g and 1.393± 0.11 mg/g in Lake of Sayadeen. Muscle malondialdehyde (MDA) level of fish recorded highly significant values as being 191.37± 3.77 mg/g in El Forsan Island while samples from El Mahsama Drain 179.25 ± 2.32 mg/g and El Halloos Drain 157.3 ± 8.92 mg/g while muscle samples of fish obtained from El Taawine region 134.4 ± 4.92 mg/g and Lake of Sayadeen showed significant values as follow 126± 5.87 mg/g. Tables 4, 5 and 6 revealed over all negative indirect relationship between liver, gill and muscle tissues metal content (lead, cadmium, nickel) and liver, gill and muscle tissues oxidative stress non enzymes (GSH) enzyme biomarkers (SOD and CAT) and biomarker of oxidative damage (MDA).

Table (3): levels of oxidative stress biomarkers of muscle samples of fish (GSH, SOD, CAT and MDA) (MG/G tissue) from five areas (El Halloos Drain, Lake of Sayadeen, El Taawine region, El Forsan Island and El Mahsama Drain) of Lake El Temsah. (Mean ± S.E).

Enzyme / Location	GSH mg/g tissue	SOD mg/g tissue	CAT mg/g tissue	MDA mg/g tissue
El halloos drain	457± 36.6	243.6 ± 3.65	3.201 ± 0.3	157.3 ± 8.92
Lake of sayadeen	546.2 ± 8.11	262± 5.59	1.393± 0.11	126± 5.87
El Taawine region	750± 33.1	250.5 ± 2.6	2.808± 0.24	134.4 ± 4.92
El Forsan island	714.3± 2.53	269.2 ± 2.92	1.513 ± 0.1	191.37± 3.77
El Mahsama drain	596.9 ± 11.2	272.7 ± 2.36	2.776 ± 0.26	179.25 ± 2.32

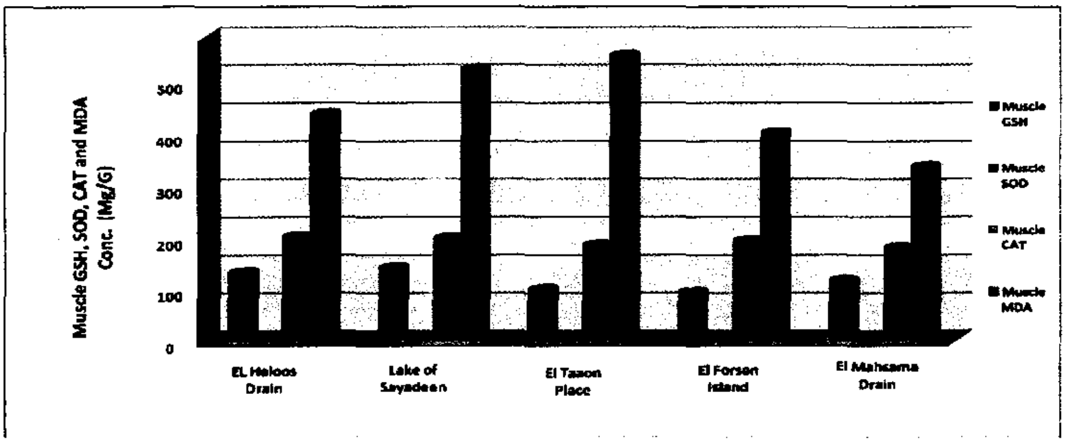

Fig. 3 Glutathione (GSH), Superoxide Dismutase (SOD), Catalase (CAT) and Malondialdehyde (MDA) concentration (mg/g tissue) of fish muscle samples collected from five areas of Lake El Temsah (El Halloos Drain, Lake of Sayadeen, El Taawine region, El Forsan Island and El Mahsama Drain).

Table 4. Pearson's correlation (r) between antioxidant biomarkers (GSH, SOD, CAT and MDA) and estimated heavy metals (Pb, Cd and Ni) of fish liver samples along Lake El Temsah.

		Lead	Cadmium	Nickel	Glutathione	Superoxide dismutase	Catalase	Malondi-aldehyde
Lead	Pearson Correlation	1	0.059	0.474	-0.032	-0.124	-0.451	0.177
Cadmium	Pearson Correlation		1	0.357	-0.314	-0.036	-0.408	0.170
Nickel	Pearson Correlation			1	-0.101	-0.127	-0.064	0.024
Glutathione	Pearson Correlation				1	0.321	-0.091	0.237
Superoxide dismutase	Pearson Correlation					1	-0.021	0.303
Catalase	Pearson Correlation						1	-0.274
Malondialde yde	Pearson Correlation							1

Table 5. Pearson's correlation (r) between antioxidant biomarkers (GSH, SOD, CAT and MDA) and estimated heavy metals (Pb, Cd and Ni) of fish gill samples along Lake El Temsah.

		Lead	Cadmium	Nickel	Glutathione	Superoxide dismutase	Catalase	Malondi-aldehyde
Lead	Pearson Correlation	1	0.729	0.611	-0.291	-0.117	-0.591	0.444
Cadmium	Pearson Correlation		1	0.181	-0.049	-0.050	-0.175	0.420
Nickel	Pearson Correlation			1	-0.038	-0.082	-0.559	0.502
Glutathione	Pearson Correlation				1	-0.006-	0.289	0.230
Superoxide dismutase	Pearson Correlation					1	0.133	-0.292
Catalase	Pearson Correlation						1	-0.488
Malondi-aldehyde	Pearson Correlation							1

Table 6. Pearson's correlation (r) between antioxidant biomarkers (GSH, SOD, CAT and MDA)and estimated heavy metals (Pb, Cd and Ni) of fish muscle samples along Lake El Temsah

		Lead	Cadmium	Nickel	Glutathione	Superoxide dismutase	Catalase	Malondi-aldehyde
Lead	**Pearson Correlation**	1	-0.865	0.066	-0.358	-0.611	-0.468	0.744
Cadmium	**Pearson Correlation**		1	0.072	-0.045	-0.677	-0.375	0.766
Nickel	**Pearson Correlation**			1	-0.263	-0.190	-0.028	0.076
Glutathione	**Pearson Correlation**				1	0.193	-0.207	-0.315
Superoxide dismutase	**Pearson Correlation**					1	-0.292	-0.588
Catalase	**Pearson Correlation**						1	0.296
Malondi-aldehyde	**Pearson Correlation**							1

DISCUSSION

This study was held in order to study the relation between residual accumulation of the measured heavy metals in fish tissues and its response through oxidative stress biomarkers glutathione (GSH) superoxide dismutase (SOD) and catalase (CAT) activities and oxidative damage biomarker malondialdehyde (MDA). Our results showed marked inhibition of GSH indicated that Metal exposure thus represents a situation of high glutathione demand as in metal-stressed fish the levels of GSH may decrease with an increase in ambient metal concentration, although other studies have reported no change in GSH content *(15,16)*. CAT in all fish tissues contaminated with heavy metal over the permissible limits especially lead, cadmium and nickel, and these results were in agreement with who reported that Cd-induced decrease in CAT activity in the mangrove killifish *(17)*. High concentrations of lead have also been reported to inhibit CAT in liver, gill and muscle. Also it has been reported that the decreased CAT activity may be due to the flux of superoxide radicals, which have been shown to inhibit CAT activity *(18)*. A decrease in the activity of CAT has been previously reported in Cyprinidae fish living in Seyhan dam Lake of Turkey and in starlet (Acipenser ruthenus L) from the Danube river of Serbia. Our study revealed marked inhibition of SOD in all fish tissues (liver, gill and muscles). The antioxidant activities were strongly inhibited in gill of fish exposed to higher concentrations of heavy metals, leading to accumulation of oxidative substances, suggesting inadequate compensation for the presence of environmental pollution *(19)*. Our results indicated that metals acts as a catalyst in the formation of reactive oxygen species and catalyzes peroxidation of membrane lipids. In this study, the higher values of an increased MDA rate indicate that the adaptive antioxidant response of fish living in this lake with different levels of pollution is enough to counteract the increased oxidative stress *(20)*.

The observation, together with some previous results, further demonstrated that gill, the first organ which contact with environmental pollutants, becomes the prime target to toxic chemicals because of not only its large surface area facilitates greater toxicant interaction but also its weak detoxification system. White muscle had a low content of mitochondria and low intensity of oxidative metabolism; hence, it is not surprising that the activities of all antioxidant enzymes in fish white muscle were not significantly induced under metal-stress. All antioxidant enzymes activities in muscles significantly inhibited after 42 day of exposure, indicating the serious damage caused by accumulation of ROS in this tissue. Our study conclude that the response of CAT activity in different tissues of O. niloticus exposed to different Ag+, Cd2+, Cr6+, Cu2+ and Zn2+ concentrations was found to be variable depending on tissues, metals and their concentrations. Bioaccumulation of metals in organisms in contaminated water is an important aspect of environmental awareness, because it may affect all members of the food chain, including fish. The CAT activity can be accepted as a sensitive biomarker for biomonitoring the aquatic environment before the detrimental effects occur for aquatic species. Our results also support the use of CAT activity as an indicator and may provide a useful data for future investigations; however, more detailed experiments will be required for better understanding the response mechanism of CAT to metal exposures.

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الملخص العربى

المؤشرات الحيوية الأنزيمية للأكسدة فى الأسماك نتيجة للتعرض للملوثات السامة فى بحيرة التمساح

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قسم الطب الشرعى والسموم- كلية الطب البيطرى- جامعة قناة السويس

التلوث البيئى ظاهرة مقلقة للعالم بأسره خاصة فى السنوات الاخيرة لزيادة انواع التلوث البيئى وعدم وجود حدود محدودة تمنعه من الانتشار السريع فى كل انحاء العالم.

تعتبر المعادن الثقيلة من أخطر انواع هذة الملوثات لما لها من خطورة عالية على الاحياء المائية خاصة الاسماك التى تشكل غذاء بروتينى هام للانسان فى كل ارجاء العالم وبالتباعية مدى التأثير الصحى الضار الذى يعود على الانسان من خلال السلسة الغذائية فى كل الكائنات الحية و على رأسهم الانسان.

انطلاقا من هذا جاءت فكرة هذة الدراسة التى اعتمدت على عملية مسح شامل لمنطقة هامه جدا فى محافظة الاسماعيلية والتى تعتبر مصدر رئيسى للحصول على الاسماك التى يتم اصطيادها من بحيرة التمساح والتى تمتد الى مسافة تقدر ب 14كم2. وقد تم الاختيار خمس مناطق على امتداد تلك البحيرة كل منها لها خصائص تختلف عن الاخرى وهى كالاتى :

- منطقة الحلوس وهذة مصدر التلوث بها الصرف الصحى
- بحيرة الصيادين وهذة معروفة بأنها المصب الاساسى لمياه الصرف الصحى بالاسماعيلية.
- مصرف المحمسة والمعروف بكونه المصرف الصناعى والزراعى لمنطقة المحسمة.
- منطقة التعاون
- جزيرة الفرسان وهاتان الاخيرتان هما الممر الرئيسى لسفن قناة السويس.

بناء على هذا تم أخذ عينات من كل من المياه والرواسب والاسماك الموجودة فى كل منطقة من المناطق المختارة لقياس نسب المعادن الثقيلة الموجودة باستخدام جهاز الامتصاص الذرى.

والجزء الثانى من الدراسة اختص بدراسة تقييم بعض الدلالات الحيوية مثل الجلوتاثيون المختزل (Reduced Glutathione) ، فوق الأكسيد ديسموتاز (Superoxide Dismutase)، الكاتلاز (Catalase) و المالونديالديهيد (Malondialdehyde). والتى تم قياسها بواسطة جهاز التحليل الطيفى وقد أسفرت النتائج عن الآتى :

اسفر وجود نسب اعلى من السموح بها للرصاص والكادميوم والنيكل فى أنسجة الاسماك المختلفة (الكبد والخياشيم والعضلات) التى تثبيط ونشوء علاقة سلبية عكسية مع مضادات الاكسدة مثل الجلوتاثيون المختزل (Reduced Glutathione)، فوق الأكسيد ديسموتاز (Superoxide Dismutase)، الكاتلاز (Catalase) فى كل انسجة الاسماك كما أدى الى تحريض المالونديالديهيد (Malondialdehyde). والذى يعنى التضرر التأكسدى لانسجة الاسماك مع وجود علاقة طردية تركيزات المعادن العالية.وهذة النتائج توضح انه يمكن استخدام الاسماك كدليل حيوى على التلوث بالمعادن الثقيلة فى النظم الايكولوجية المائية كما ان استخدام انزيمات التأكسد ودلالات الاكسدة هى الاختيار الاول حاليا للكشف عن التسمم ببعض المعادن الثقيلة.