

RESEARCH ARTICLE

SEASONAL VARIATIONS OF ANTIOXIDANT ENZYME ACTIVITIES IN *T.ZILLII* FISH CAUGHT FROM LAKEQARUN.

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Abstract

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Lake Qarunis exposed to high inputs of agricultural drainage water, sewage and industrial wastewater through a system of twelve drains which influence the living organisms like fish. In the present study, antioxidant enzymes were chosen as bioindicators for pollution in lakeQarun. Fish samples, T.zillii were collected from Lake Qarun seasonally during (August 2014 to May 2015) to determine the changes in the activities of antioxidant defense enzymes (SOD and CAT) in the liver as well as performing meat quality of the collected fish through analysis of total proteins and total lipids in muscles. The present results showed that SOD activity in the liver of T. zilliifish collected from eastern and middle part of the lake was increased significantly ($p \le 0.05$) compared to the control value seasonallywhile, samples of the western part of the lake increased insignificantly in summer, autumn and winter season. Catalase activity in the liver of T. zilliifish collected from the eastern and middle part of the lake increased significantly compared with control value ($p \le 0.05$) in summer, autumn and spring. The levels of total protein in the muscle decreased significantly ($p \le 0.05$) reaching its minimum value in the muscle of fish from the eastern part of the lake. Total lipid content in the muscle was increased significantly ($p \le 0.05$) reaching its maximum value in the muscle of fish from the eastern part of the lake. It was concluded that pollution of the lake adversely affect enzymatic activities, physiological functions and meat quality of fish.

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Introduction:-

Today, most environmental problems are attributed to the production and release of toxic chemical capable of interacting with the environment and disrupting the ecosystem. The toxic chemicals produce stress on aquatic organisms like fish. Fish in ponds, rivers and lakes cannot avoid exposure to these toxic that suspended or dissolved in water, being less than land animals to move to favorable regions to avoid unfavorable conditions (EL-Ghobashy*et al.*, 2001). In Egypt, it has been found that many environmental pollutants reach the natural water (rivers, seas and lakes) through the industrial, agricultural and domestic effluents produced by human activities. This is true, particularly for Lake Qarun.

Corresponding Author:-Mohamed A. El- Desoky Address:-Faculty of Science, Cairo University. Nowadays, Lake Qarun suffers from several environmental problems. It receives agricultural and sewage drainage water from Fayoum Province. Therefore its salinity increase progressively which affects greatly the Lake biota. The Lake receives the agricultural and sewage drainage waters through a system of twelve drains, most of the drainage water reaches the Lake by two main drains, Al Batts and Al Wadi drain. The Lake received annually about 450 million cubic meters of agriculture drainage water. These pollutants have serious effects on the aquatic ecosystem, including fish, since these wastes contain a variety of toxic organic as well as inorganic compounds (Authman and Abbas 2007).

Fish have been proposed as indicators for monitoring land-based pollution because they may concentrate indicative pollutants in their tissue, directly from water through respiration and also through their diet. Fish are frequently subjected to prooxidant effects of different pollutants often present in the aquatic environment (Velkova-Jordanoska*et al.*, 2008).

Oxidative stress is more often used as a biomarker of the effects of exposure to environmental pollution in aquatic environments. Antioxidants are defined as compounds that can inhibit or prevent the oxidation of oxidizable materials by scavenging free radicals and diminishing oxidative stress. Many pollutants (or their metabolites) may exerttoxicity related to oxidative stress. The level of antioxidant enzymes have been extensively used as an early warning indicator of lake pollution (Lin *et al.*, 2001).

Superoxide dismutatase (SOD) and catalase (CAT) have been detected in a wide variety of mammalian cells. These enzymes play important roles in protecting the cell against the potentially toxic effects of environmental pollutants (Kuthan*et al.*, 1986). Superoxide dismutase catalyzes the dismutation of the superoxide ion (O_2 -) to hydrogen peroxide and oxygen molecule during oxidative energy processes. The reaction diminishes the destructive oxidative processes in cells. The liver in fish is an organ that performs various functions associated with the metabolism of xenobiotics (Jiminez and Stegeman, 1990).

Lake Qarun attracts the attentions of many authors because of its historical and scientifically importance to study its unique ecosystem, these studies dealt with different environmental aspects of the lake including ecological studies on zooplankton of the lake (Ahmed, 1994), phytoplankton composition (El-shabrawy and Taha, 1999), bacterial indices (Sabae and Ali, 2004), heavy metals in benthic invertebrate, water, fish and sediment (Ali and Fishar 2005), (Authman, and Abbas 2007), but the impact of the environmental pollution of lake Qarun on water quality and fish biochemistry (Mohamed and Gad2008), (Ibrahim and Ramzy2013) and (Sabae and Mohamed2015)are not enough . Also, no information was available on the effect of pollution on antioxidant defense system of fish collected from different sites of lake Qarun .Great efforts were needed for recovery of the purity and healthy of this lake and additional information were needed to provide a database for optimal fisheries and water quality status that help the proper management of the lake. So the main objectives of this study are to determine the changes in the activities of antioxidant defense enzymes (superoxide dismutase and catalase) as well as Performing meat quality of the collected fish through analysis of total proteins and total lipids in muscles.

Materials and Methods:-

Study Area:-

Lake Qarun is a closed elongated saline basin located between longitudes 30°24′ & 30°49′ E and latitudes of 29°24′ & 29°33′ N in the lowest part of El-Fayoum depression, about 80km Southwest of Cairo. It has an irregular shape of about 40km length and about 6 km mean width, with an average area of about 240 km. The lake is shallow, with a mean depth of 4.2 m and about 20% of the lake's area has a depth ranging between 5 to 8 meters. The water level of the lake fluctuatedbetween 43 to 45m below mean sea level (Sabae and Ali, 2004).

Sampling:-

Samples of fish *T.zilii*were collected from the east, middle and west of Lake Qarun. The fish measured about (12 to 15) intotallength and (40 to 67) in weight. They were collected seasonally during a period extending from (August 2014 to May 2015). After the dissection of fish samples, parts of liver and muscles were carefully removed and prepared for biochemical studies. Another fishs amples were collected from fish farm (unpolluted), to beusedasacontrolgroup.

Biochemical Measurements:-

The activity of superoxide dismutase (SOD) in the liver tissues was determined according to the method of Nishikimi et al., (1972). The activity of catalase (CAT) in the liver was determined according to the method of Aebi, (1984). Total protein in the muscle tissue was determined according to the method of Bradford, (1976). Total lipid concentration in the muscle tissue was determined according to themethod described by Lutzke and Brauler (1990).

Statistically analysis:-

The significant between control and results of the biochemical analysis will be analyzed statistically by Student's "t" Test to test the significance of the difference between the mean values of any two sets of observations using SPSS statistical package version 17. The data values were expressed as mean \pm standard error (M \pm SE).

Results:-

Biochemical analysis of fishTissues:-

Alterations in the biochemical parameters in *T. zillii* fish collected from different sites of lakeQarun during the study period (August 2014 to May 2015) were presented in Table (1-4).

SOD Activity:-

SOD activity in the liver of *T. zillii*fish collected from eastern and middle part of the lake was increased significantly ($p \le 0.05$) compared to the control value seasonally while, samples of *T. zillii* fish collected from the western part of the lake showed insignificant increase compared with control value in summer, autumn and winter season. The lowest value in the liver of *T. zillii* was recorded during winter in the western part of the lake. While, the highest value was recorded during summer in the eastern part of the lake.

Nocation	control	East	Alteration	Middle	Alteration	West	Alteration
season							
Summer	359±2.14	390±3.72*	+8.63	385±3.80*	+7.24	366±1.71	+1.94
Autumn	355±7.52	386±2.88*	+8.73	380±4.23*	+7.04	366±2.78	+3.09
Winter	351±2.69	381±4.03*	+8.54	379±4.44*	+7.97	359±2.78	+2.27
Spring	351±2.78	389±2.63*	+10.82	382±2.88*	+8.83	362±2.69*	+3.13

Table 1:-Superoxide dismutase (SOD) (U/g) (Means \pm SE)activity in the liver of *T. zillii* collected from lakeQarun.

-Data are presented as mean \pm SE of 6 fish, – SE: standard error,-% Alteration from control value

* significant difference from control at ($p\!\leq\!0.05$) .

Catalase Activity:-

Catalase activity in the liver of *T. zillii*fish collected from eastern and middle part of the lake was increased significantly ($p \le 0.05$) compared to the control value seasonally but *T. zillii*samples collected from western part of the lake showed an insignificant increase compared with control value seasonally. The lowest value in the liver of *T. zillii*was recorded during winter in the western part of the lake. While, the highest value was recorded in summer in the eastern part of the lake.

location	control	East	Alteration	Middle	Alteration	West	Alteration
season							
Summer	74±.816	82±1.50*	+10.81	79±1.23*	+6.75	76±.577	+2.70
Autumn	72±.930	80±1.73*	+11.11	79±.816*	+9.72	75±1.29	+4.16
Winter	70±.730	77±.730*	+10.00	76±1.46*	+8.57	73±1.09	+4.26
Spring	71±.930	78±1.34*	+9.85	78±1.29*	+9.85	74±1.06	+4.22

Table2:-Catalase activity (U/g) (Means \pm SE)in the liver of *T. zillii* collected from lakeQarun.

-Data are presented as mean± SE of 6 fish, – SE: standard error, -% Alteration from control value

* significant difference from control at ($p\!\leq\!0.05$) .

Total protein concentration:-

The total protein concentration in the muscle of *T. zillii* fish collected from east, middle and west of the lake was decreased significantly ($p \le 0.05$) in all seasons compared to the control fish samples collected from the unpolluted

site (fish farm) with percentage ranged between 17.24 - 34.48 %. Also, the lowest value was recorded in the eastern part of the lake in winter season.

location	control	East	Alteration	Middle	Alteration	West	Alteration
season							
Summer	17.7±0.21	11.7±0.12*	-33.89	12.3±0.24*	-30.50	12.7±0.29*	-28.17
Autumn	17.4±0.21	11.5±0.25*	-33.90	12.5±0.23*	-28.16	14.4±0.37*	-17.24
Winter	17±0.16	11.4±0.24*	-32.94	11.7±0.18*	-31.17	12.4±0.15*	-27.05
Spring	17.4±0.17	11.4±0.24*	-34.48	11.9±0.19*	-31.60	13±0.15*	-25.28

Table 3:-Total proteinconcentration(μ g/ml) (Means \pm SE) in the muscle of*T. zillii*collected from lakeQarun.

-Data are presented as mean± SE of 6 fish,-SE: standard error,-% Alteration from control value

* significant difference from control at ($p\!\leq\!0.05$) .

Total Lipid concentration:-

Total lipid concentration in the muscle of *T. zillii*was increased significantly ($p \le 0.05$) compared to the control value, reaching its maximum value (5.9±0.12) during winter in the eastern part of the lake.

Tocation	control	East	Alteration	Middle	Alteration	West	Alteration
season							
Summer	1.2 ± 0.17	5.3±0.17*	+341.6	4.3±0.37*	+258.3	3.4±0.23*	+183.3
Autumn	0.9±0.11	5.6±0.19*	+522.2	4.2±0.55*	+366.6	3.3±0.08*	+266.6
Winter	1.2 ± 0.09	5.9±0.12*	+391.6	4.4±0.12*	+266.6	3.7±0.05*	+208.3
Spring	0.8 ± 0.09	5.5±0.10*	+587.5	4.2±0.08*	+425.0	3.5±0.09*	+337.5

Table 4:- Total Lipid concentration (g/dl) (Means \pm SE) in the muscle of *T. zillii*collected from lake Qarun.

-Data are presented as mean \pm SE of 6 fish,- SE: standard error, -% Alteration from control value

* significant difference from control at ($p\!\leq\!0.05$)

Discussion:-

Lake Qarun is affected byagricultural drainage water, sewage and industrial wastewater through a number of drains. These pollutants have serious effects on the aquatic ecosystem, including fish, since these wastes contain a variety of toxic organic as well as inorganic compounds. Analysis of biochemical parameters could help to identify the target organs of toxicity as well as the general health status of animals. It may also provide an early warning signal in stressed organism (David, *et al* 2010). In the case of fish, foreign chemicals present in their environment may act as stressors threatening or disturbing their organism so a set of compensating and adaptive responses that readjust metabolic processes in order to cope with the effects of pollutants. These pollutants influence the growth rate, physiological and biochemical states and consequently the meat quality of fish.

Fish as all other aerobic organisms generate endogenous reactive oxygen species (ROS) and other oxidants during aerobic metabolisms and energy production in the mitochondria. Under normal physiological status the antioxidant defense systems including SOD, CAT and GST can be induced by a slight oxidative as a compensatory response and thus the reactive oxygen species (ROS) can be removed to protect the organism from oxidative damage (Livingstoone, 2001). Pollutants with redox potential can produce increasing amount of ROS in aquatic organisms in polluted sites. These ROS can trigger oxidative damage to proteins, nucleic acid and lipids (Atli and Canli 2007).

The activities of antioxidant may be increased or inhibited under chemical stress depending on the intensity and duration of stress applied as well as susceptibility of exposure species. The SODs are a group of metalo enzymes that catalyze the conversion of reactive superoxide anion (O_2 -) to yield hydrogen peroxide (H_2O_2). CATs are hematin-containing enzymes that facilitate the removal of hydrogen peroxide (H_2O_2), which is metabolized to molecular oxygen (O_2) and water, so CATs is the primary antioxidant defense component protect fish from oxidative stress. Heavy metal (pollutants) accumulated in the tissues of fish may catalyze reactions that generate reactive oxygen species (ROS) which may lead to environmental oxidative stress Deherty*et al.*, (2010).

The liver is found to be stronger in view of oxidative stress than the other tissues with the highest SOD and CAT

activities (Atli et al., 2006). This could be related to the fact that the liver is the site of multiple oxidative reactions and maximal free radical generation; therefore, liver tissue was thought to be the best to present the response of CAT activity to metal exposure (Avci et al., 2005). Antioxidant defense enzymes such as CAT and SOD have a remarkable importance for aquatic organisms because these enzymes protect them from free radicals that cause oxidative stress.Many environmental factors induce the production of reactive oxygen species (ROS). As temperature-dependent organisms, most fishes must routinely cope with fluctuations in environmental temperature and in the metabolic rate and consequently with oscillations in ROS levels. Therefore, ROS generation, oxidation rates and antioxidant status are directly related to ambient temperature or metabolic activity (Wilhelm Filho*et al.*, 2000).

In the present study, SOD and CAT activities in the liver of *T. zillii*fish were increased compared to the control value and the highest values were recorded in the eastern part of the lake during summer, indicating a high production of superoxide anion radicals and the high levels of CAT in the liver tissue could be attributed to high production of peroxide radicals. Increased SOD and CAT activities in the liver may be a response to oxidative stress.

On a seasonal basis, the increase of these enzymes in summer comparing to other seasons is probably related to the higher ambient water temperature and therefore to the oxygen consumption and reactive oxygen species generation during the warm period followed by enhanced antioxidant capacity in fish (Wilhelm Filho*et al.*, 2001). We suggest that the accumulation of pollutants in the liver of fish may lead to the induction of antioxidants enzymes (SOD and CAT) particularly in the eastern part of the lake. Also, the induction of SOD and CAT in the liver of *T.zillii*collected from the studied area in lakeQarun suggests that oxidative stress response still works well under the current conditions, and the increase of anti-oxidative enzymes may be a physiological adaptation for the elimination of ROS generation (Gad, 2011).

Proteins are the building units of the body and are also the most abundant macromolecules in the cells constituting half of their dry wt.Present data indicated marked depletion of total protein concentration in muscle of *Tilapia zillii* fish samples collected from different sites of lake Qarun when compared to the control samples reaching its minimum value in the winter season. The lower value of total protein in winter comparing other season suggest the different metabolic activity of the tissue with respect to season and probably depend on food availability and feeding behaviour. The lower temperature (onset of winter) may have affected feeding activity or caused a decrease in the protein assimilation efficiency.

This protein depletion could be attributed to the change in the lake water quality as a result of the discharged effluents from different sources. Bioaccumulation of pollutants in fish may critically influence tissue protein level and fish quality (Zaghloul*et al.*, 2002). This may be explained as follows: exposure to metal as (Cu and Zn) may lead to high accumulation in the gills that may cause damage in their structure and a reduction in the rate of oxygen consumption, causing sharp reduction in the metabolic rate of fish and consequently decreased protein content in tissues. Moreover, the decreased tissues protein in fish living in the polluted environment may be a result of the decrease in insulin level caused by metal toxicity as in the case of Cu and Zn (Zaglool*et al.*, 2002). Insulin is known to have profound effects on the proteogenic pathways in fish; it stimulates the inward cellular transport of amino acid particularly in muscles, leading to intracellular accumulation of amino acids with a subsequent decrease in the protein content.

Another possible reason for the decreased total protein in the muscles of *Tilapia zillii* in the present study is the over secretion of glucocorticoids in response to heavy metals stress and other pollutant. This promotes degradation of endogenous proteins, particularly in muscles to provide substances required to apply fish extra source of glucose to control energy required during stress (Gad, 1999). Also, The decreased of total protein in muscle of studied fish showthattheprotein wastaken as an alternative source of energy, due to high energy demand that induced bydifferent pollutants inLakeQarunas previously reported by Vutukuru, (2005) and Durmaz, (2006). Also, the depletion intissue proteins may be due to impairor low rate of protein synthesis (Rajamanickamand Muthuswamy2008), due totheir utilization incellrepair and organization (Vutukuru, 2005) and/orthedecrease in uptake ofamino acidsintothepolypeptide chain.

Lipid play an important role in metabolic activities of animals because they are a source of energy and are involved in the building of cellular components. They are stored in the form of metabolites and provide energy when an organism faces adverse conditions. In the present study, total lipid in fish tissues of *T. zilli* collected from different sites of lakeQarun markedly sensitive to the environmental pollution of the lake. There is a highly significant increase (p < 0.05) in all regions. The observed increased in total lipid in fish tissues may be due to the fact that excess energy reserves (as glucose, triglycerides and cholesterol) are required by organisms to mediate the effects of stress (Sayed*et al.*, 2011). There is a number of way by which toxic materials can elevate levels of total lipid in fish blood or tissues: 1) increase production by liver and other tissues, 2) release oflipids constituents from damagedcell membranes, 3) thyroid dysfunction 4) decreased activity of cytochromeP450 enzymes (Metwally, 2009).

Conclusion:-

The present data showed an increase of superoxide dismutase and catalase activities in the liver samples. On the other hand, total lipid increased significantly ($p \le 0.05$) but total protein decreased in the muscle samplesreaching its minimum value in the muscle of fish from the eastern part of the lake. Our results display that it is needed more studies in Lake Qarun, to resolve the question, about pollution impact of this lake.

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