Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 - 6131 Vol. 21(3): 11-28 (2017)
www.ejabf.journals.ekb.eg

Antioxidant Defense System Alternations in Fish as a Bio-Indicator of Environmental Pollution

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ARTICLE INFO
Article History:
Received: July, 2017
Accepted: July, 2017
Available online: July, 2017

Keywords:
Antioxidant defence system
Fish
Heavy metals
River Nile
Rosetta Branch

ABSTRACT

The present study carried out to clarify the impact of heavy metals (Fe, Zn, Cu, Mn and Cd) pollution of Rosetta Branch on the antioxidant defence system activities and lipid peroxidation indicator MDA levels in *O. niloticus* tissues (liver and white muscles) collected from three stations from Rosetta Branch of River Nile in summer 2014 and winter 2015. Rosetta Branch of River Nile exposed to high input of agricultural drainage water, sewage and industrial waste water which influence the living organisms especially fish. In the present study, results revealed that high concentrations of heavy metals (Fe, Zn, Cu, Mn and Cd) were detected in water and fish samples, especially in winter. In muscles of *O. niloticus*, the accumulation patterns of heavy metals were in the following order: Fe > Zn > Mn > Cu and Cd. The bioaccumulation factor (BAF) in winter was higher than summer. Antioxidant enzymes (SOD, CAT, GPx, GST and GR) activities and the indicator of lipid peroxidation MDA levels in liver and white muscles of *O. niloticus* were found to be significantly increased compared to the reference values, especially in winter. Moreover, the antioxidant enzymes activities and MDA levels were higher in liver than white muscles. These remarkable alterations in the activity of the selected enzymes in the liver and white muscles of the *O. niloticus* go in parallel with the elevation in the levels of heavy metals detected in the water of Rosetta branch, as a result of pollution stress in these areas. Thus we conclude that, the altered activities of SOD, CAT, GPx, GST and GR) and MDA levels could be useful biomarkers of water pollution.

INTRODUCTION

River Nile is the longest river in the world. It is the main source of water in Egypt that has a wide usage in different fields, drinking and domestic water supply agricultural, industrial investigation, fishery and others (**El-Sayed and Ouf, 2009**).

Pollution load in the Nile system (River Nile, canals, and drains) has increased in the past few decades due to population increases, several new irrigated agriculture projects, new industrial projects and other activities along the River Nile. Consequently, quality of Nile water worsened dramatically in the past few years (**Abdel-Dayem *et al*., 2007**). The most polluted part of River Nile is the two branches, Damietta and Rosetta (**NAWQAM, 2003**). Heavy metals pollution in fish has become a worldwide concern, not only because of the threat to fish, but also the health risks associated with consumption (**Lee *et al.*, 2011**).

Heavy metals are considered as serious pollutants of aquatic ecosystem because of their environmental persistence and toxicity effects on living organisms. Heavy metals enter the surroundings by natural means and through human activities (**Barbosa-Morais *et al.*, 2012**). Freshwater fish, occupying upper levels of trophic chains, it can accumulate non-degradable pollutants, like metals, in different tissues, including liver and white muscle (**Adeyeye *et al*., 1996**). The induced metabolic alternations in these tissues can serve as indicators of freshwater ecosystem contamination by heavy metals and constitute health hazard of fish and human health (**Ahmed *et al.*, 2015**).

Heavy metals can be taken up into fish either from digestion or contaminated food via alimentary track or through gills or skin after the absorption it transported through blood stream to the organs and tissues, where they are accumulated. Fish can regulate metal concentrations to a certain extent, after the occurrence of bioaccumulation.

Previous studies have reported that exposure of fish to pollutants (agricultural, industrial and sewage) affects the antioxidative defense system enzymes such as: SOD, CAT, GST, and GR (**Hegazi *et al.*, 2010**). It is suggested that some of these enzymes can constitute good molecular bioindicators for oxidative stress and can indicate the magnitude of response in vertebrate population chronically exposed to contaminants, such as metals and other xenobiotic (**Gad, 2009**).

Several circumstances promote the antioxidant defence response in fish, factors intrinsic to the fish itself such as: age, reproductive, metabolic status of fish and environmental conditions, that include food availability, oxygen level, temperature of water, salinity and photoperiod, toxins present in the water or pathologies, can either fortify or weaken antioxidant defences (**Melegaria *et al.* 2013**). The accumulation of heavy metals can produce increasing amount of (ROS) in fish by generating free radicals such as the hydroxyl radical ($OH^{\bullet}$), proxy radical ($RO_2^{\bullet}$) and superoxide ($O_2\bullet$) and some non-radical such as hydrogen peroxide (H_2O_2), this led to the induction of enzymatic antioxidants (SOD, CAT, GST, GR and GPx) and non-enzymatic antioxidant glutathione (GSH). These antioxidants scavenge free radicals and provide protection against this aspect of oxygen toxicity (**Kadar *et al.*, 2005**). Oxidative stress occurs when the equilibrium between ROS production and the antioxidant defenses is lost, ROS can be important mediators of damage to cell structures, including lipids and membranes, proteins and nucleic acids, that can seriously alter the cell membranes and other structures such as proteins, lipids, lipoproteins, and deoxyribonucleic acid (DNA) which lead to different pathologic processes, and fish diseases (**Pereira *et al.*, 2011**).

The first line of defense against oxidative stress is SOD and CAT. SOD catalyzes the superoxide anion radical ($O_2^{\bullet-}$) dismutation into hydrogen peroxide (H_2O_2) by reduction. The oxidant formed (H_2O_2) is transformed into water and oxygen (O_2) by catalase (CAT) or glutathione peroxidase (GPx) (**Stara *et al.* 2012**). GPx enzyme removes H_2O_2 by using it to oxidize reduced glutathione (GSH) into oxidized glutathione (GSSG). Glutathione reductase GR is a flavoprotein enzyme,

regenerates GSH from GSSG, using NADPH as a source of reducing power. Besides hydrogen peroxide, GPx also reduces lipid or non-lipid hydroperoxides while oxidizing glutathione (GSH) (**Genestra, 2007**).

On the other hand, there is a few biochemical studies on the antioxidant defence system carried out on the fish collected from Rosetta Branch of River Nile. Therefore, this study was conducted to determine the level of some heavy metals in water and muscles of *O. niloticus* and evaluate the impact of such pollutants on antioxidant defence enzymes in liver and white muscles of fish. Moreover, the study trends to provide new data for further research in metal carcinogenicity that would help predicting the toxicological hazards to aquatic life and human.

MATRIALS AND METHODS

Area of Investigation

Rosetta Branch of River Nile is about 220 Km length and about 180 m width with an average depth of 2.0 to 2.3 m. It starts from EL-Qanater EL-Khayria and ends at Rosetta Estuary. It passed cutting six governorates; EL-Qalubia, EL-Menofiya, EL-Giza, EL-Gharbiya, Kafr El-Shiekh and EL-Boheira Governorates. Rosetta Branch selected to be the area of investigation, from El-Qanater El-Khayria to Kafr El-Zayat city; distance about 95.25 Km (Fig. 1).

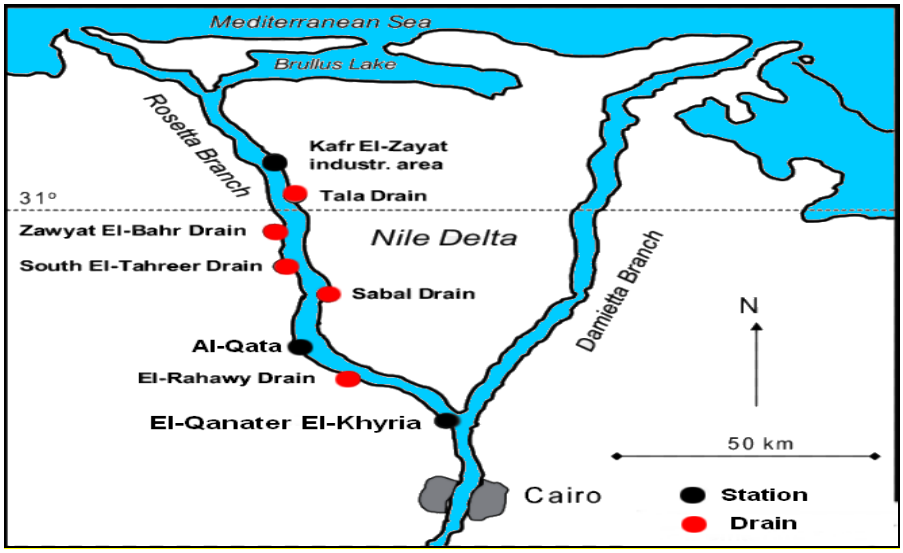

Fig. (1): A map of northern Egypt showing area of study and sampling stations of the Rosetta Branch.

There are three main sources of pollution discharged into Rosetta Branch which, potentially affects and deteriorates the water quality of it; (1) El-Rahawy drain, which discharged more than 1,9 million m^3/day of agricultural, domestic and sanitary wastes into Rosetta Branch. (2) Kafr El-Zayat industrial area, which includes the industrial effluents from the factories of super phosphate and sulfur compounds, oil and soap industries and pesticides factories. (3) Various small agricultural drains and sewage from several cities and its neighboring villages, which discharge their wastes directly into the branch without treatment.

Collection of Samples:

Water and fish samples were collected from three stations from Rosetta Branch of River Nile: (1) El-Qanater El-Khyria. (2) Al Qata. (3) Kafr El-Zayat, in spring 2014 and winter 2015. Water samples were collected in polyethylene bottles at two meter depth from selected stations, then acidified using nitric acid and transferred in ice box to laboratory to be analyzed.

Fish (*O. niloticus*) samples of were collected with the help of fishermen, the collected fish with an average body weight (150 ± 10 gm), and an average body length (19±5 cm). After dissection of fish, muscle tissues were separated for determination of heavy metal accumulation. Moreover, the right lobe of liver and white muscles tissues were carefully removed for evaluation of antioxidant enzymes activity and MDA levels.

Determination of Heavy metals in water and fish muscles:

Water samples of 500ml were acidified with conc. nitric and heated on a hot plat. Heating and addition of conc. Nitric acid were continued till complete digestion of heavy metals (Fe, Zn, Cu, Mn, and Cd) as described by (**APHA, 2012**). Fish samples were digested after drying according to the method described by **Ghazaly (1988**). The levels of heavy metals determined using GBC atomic absorption reader (Model SavantAA AAS with GF 5000 Graphite Furnace), results in water were expressed in (mg/L) and in mg/kg dry wt. in fish muscles.

Bioaccumulation Factor for Heavy Metals in Fish Muscle (BAF)

According to EPA guidelines, the BAF is defined as the ratio of chemical concentration in the organism to that in the surrounding water.

$$\mathbf{BAF = M_{(tissue)} / M_{(water)}).}$$

Where; M tissue is the metal concentration in fish tissue mg/kg and M water, metal concentration in water mg/L.

Biochemical analysis

Fish were carefully dissected on ice, excised pieces of white muscles or right lobe of liver tissue of *O. niloticus* fish were washed with isotonic saline, dried using filter paper and weighted (n= 8), tissues were quickly homogenized in ice-cold 50 mM phosphate buffer, 1% Triton X100 (pH 7.4) to give a 10% homogenate. then centrifuged at 6,000 xg in cooling centrifuge at 4°C for 15 min, the supernatant was saved for immediate assay of enzymes activity in liver and white muscles.

The activity of total superoxide dismutase (SOD) was determined in fish tissues using the NADH oxidation method of **Paoletti and Mocali (1990**). The unit of activity is defined as the amount of enzyme causing 50% inhibition of the rate of the superoxide driven NADH oxidation, the absorbance was monitored at 340 nm, enzyme activity was calculated using a calibration curve made from standard SOD. The specific activity was expresses as unit per gram wet weight tissue (U/mg).

Catalase activity was assayed according to the method of **Xu *et al.* (1997)** using H_2O_2 substrate solution (freshly prepared), absorbance readings at 240 nm, it was defined as the amount of extract needed to decompose of H_2O_2 per min. activity was expressed as µmol/min/g w.wt.

Glutathione peroxidase (GPx) activity was assayed according to the method of **Paglia and Valentine (1967),** using glutathione reductase (GR) and NADPH, the reaction started by the addition of H_2O_2. Oxidation of NADPH to $NADP^+$ was monitored continuously at 25˚C, reading absorbance at 340 nm. every 15 seconds for 300 seconds. Data were expressed as (µM /min/g w.wt.).

Glutathione-S-transferase (GST) activity assayed according to the method of **Habig *et al.* (1974),** it based on the formation of CDNB (S-2,4-dinitrophenyl glutathione) adduct, it was monitored by the increase in absorbance at 340 nm. GST activity was expressed as µM /min/g w.wt.

The determination of Glutathione Reductase (GR) activity was measured by the method of **Smith *et al.* (1988),** The reduction of 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) to TNB was measured, the reaction initiated by the addition of oxidized

glutathione (GSSG) at 25˚C. The absorbance was recorded at 340 nm over a period of 5 min., GR activity was expressed as µM /min/g w.wt.

Lipid peroxidation is the degradation of Lipids by reactive oxygen species, resulting in a chain reaction with the production of end products, such as malondialdehyde (MDA), which is a useful marker for oxidative stress. The concentration of MDA was evaluated by the method of **Buege and Aust (1978)**. MDA reacts with TBA (thiobarbituric acid) to form a conjugate, absorbance was read at 532 nm against the reagent blank, and expressed as nmol/mg protein.

Statistical Analysis

The experimental data were subjected to statistical analysis by one-way analysis of variance (ANOVA), the significant of difference was analyzed by the Dunnett test (compare data of all vs. control), using a software program (GraphPad InStat Software, Inc.). The difference checked by one-way (ANOVA) was significance when ($P \leq 0.05$), each reading represents (Mean ± S.E) of 8 fish.

RESULTS

Concentration of heavy metals in water:

The mean concentrations of the selected heavy metals in water of Rosetta Branch of the River Nile are presented in table (1). The concentration of Fe, Zn, Cu, Mn and Cd in water of the selected stations ranged between (0.173-0.775), (0.093-0.124), (0.008-0.113), (0.016-0.078) and (0.011-0.023) mg/L in summer, and ranged between (0.223-0.952), (0.111-0.193), (0.022-0.177), (0.049-0.171) and (0.016-0.034) mg/L in winter respectively. The station (3) Kafr El-Zayat had the highest levels of heavy metals while, station (1) El-Qanater El-Khayria had the lowest values during period of sampling. The results showed that, Fe recorded the highest concentration (0.173-0.952 mg/l) among the tested metals, while Cd recorded the lowest one (0.011-0.034 mg/l) during the study period. Also, the concentrations of Fe, Zn, Cu, Mn and Cd in water were elevated in winter compared to summer at the three stations.

Table (1): Heavy metals concentrations (mg/L) in water from Rosetta Branch of River Nile during summer 2014 and winter 2015.

Heavy metal	Season	Station I	Station II	Station III	(Mean±S.E)	Law48/1982 Decree92/2013
Fe	**S**	0.173	0.603	0.775	0.517±0.18	0.5
	W	0.223	0.772	0.952	0.65±0.22	
Zn	**S**	0.093	0.119	0.124	0.112±0.01	0.01
	W	0.111	0.147	0.193	0.15±0.02	
Cu	**S**	0.008	0.011	0.133	0.05±0.03	0.01
	W	0.022	0.143	0.177	0.11±0.04	
Mn	**S**	0.016	0.062	0.078	0.052±0.02	0.2
	W	0.049	0.144	0.171	0.121±0.04	
Cd	**S**	0.011	0.019	0.023	0.017±0.003	0.001
	W	0.016	0.024	0.034	0.025±0.004	

(I) El-Qanater El-Khyria. (II) Al Qata. (III) Kafr El-Zayat. S: summer W: winter

Concentration of heavy metals and BAF in *O. niloticus* tissues:

In the obtained results Table (2) showed the mean concentrations of heavy metals Fe, Zn, Cu, Mn and Cd in muscles of *O. niloticus* during summer were (29.2±11.1), (4.97±1.21), (1.1±0.51), (0.817±0.30) and (0.106±0.06) mg/kg dry wt., respectively, while were (60.5±25.9), (11.1±3.1), (6.4±1.7), (3.56±1.6) and (0.252±0.08) mg/kg dry wt. during winter, respectively.

In muscles of *O. niloticus* the highest concentrations of Fe, Zn, Cu, Mn and Cd were 103.75, 16.13, 7.65, 5.94 and 0.36 mg/kg dry wt. respectively, at station III

in winter, while the lowest accumulation of Fe, Zn, Cu Mn and Cd were 8.25, 2.97, ٠.٠47, 0.22 and 0.032 mg/kg dry wt. at station I in summer, respectively. In the present study, station I recorded the lowest accumulation of the studied heavy metals in muscles of *O. niloticus*, while station III was the highest Table (2).

Table (2): Heavy metals concentrations (mg/ kg dry wt.) in *O. niloticus* muscles collected Rosetta Branch of River Nile in summer 2014 and winter 2015.

Heavy metal	Season	Station I	Station II	Station III	(Mean±S.E)	FAO(1983)
Fe	**S**	8.25	34.35	45.16	29.2± 11.1	30
	W	15.55	62.3	103.75	60.5 ± 25.9	
Zn	**S**	2.97	4.84	7.11	4.97 ± 1.21	30
	W	6.01	11.21	16.13	11.1 ± 3.1	
Cu	**S**	0.047	1.19	2.1	1.1 ± 0.51	30
	W	0.69	5.74	7.62	4.6 ± 1.7	
Mn	**S**	0.22	1.02	1.21	0.817 ±0.30	---
	W	0.62	4.11	5.94	3.56 ±1.6	
Cd	**S**	0.032	0.095	0.19	0.106 ± 0.05	0.5
	W	0.114	0.283	0.36	0.32 ± 0.03	

(I) El-Qanater El-Khyria. (II) Al Qata. (III) Kafr El-Zayat. S: summer. W: winter.

The maximum BAF of metals Fe, Zn, Cu, Mn and Cd in muscles of *O. niloticus* was (108.9, 83.4, 43.1, 34.5 and 12.3) at station III in winter, whilst the lowest BAF was (47.7, 31.1, 5.9, 8.14 and 3.3) at station I during summer, respectively. The results indicated that the BAF of heavy metals in muscles of *O. niloticus* were in the following order: Fe >Zn >Cu >Mn and Cd (Table 3).

Table (3): The bioaccumulation factor (BAF) In *O. niloticus* muscles collected from Rosetta Branch of River Nile in summer 2014 and winter 2015.

Heavy metal	Season	Station I	Station II	Station III
Fe	**S**	47.7	56.9	58.2
	W	69.7	80.7	108.9
Zn	**S**	31.1	40.6	57.1
	W	53.9	76.2	83.4
Cu	**S**	5.9	10.8	14.7
	W	31.7	40.1	43.1
Mn	**S**	8.14	15.4	15.6
	W	12.6	28.4	34.5
Cd	**S**	3.3	7.1	8.2
	W	6.9	11.1	12.3

(I) El-Qanater El-Khyria. (II) Al Qata. (III) Kafr El-Zayat**.** S: summer W: winter

Biochemical parameters:

The mean SOD activity in liver and white muscles of *O. niloticus* (Fig. 2) showed a highly significant increase ($P \leq 0.01$) at station III as compared with their respective control (site I).During the two sampling seasons, the increase in winter was 36.5 and 49.7 % in white muscles and 41.1 and 62.9 % in liver at each station, respectively. In summer the increase was 33.9 and 41.2 % in white muscles; in liver was 35.3 and 54.4 % at each sampling station, respectively. The CAT enzyme mean activity (Fig. 3) showed a highly significant increase ($P \leq 0.01$) in liver and white muscles in winter at stations II and III, The increase in winter was 39.6 and 59.1. % in white muscles, in liver was 41.71 and 57.1 % at each sampling station, respectively. In summer the CAT enzyme increase was 33.8 and 49.8 % in white muscles and 35.5 and 50.9 % in liver at each sampling station, respectively.

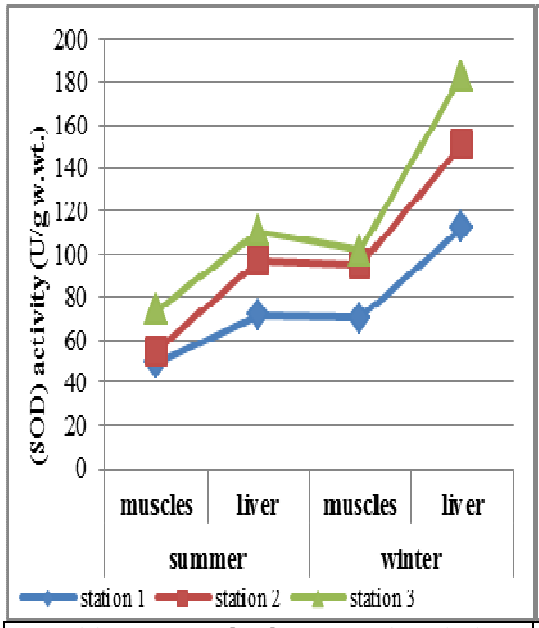

Fig. (2): Superoxide dismutase (SOD) (U/g w.wt.) activity in white muscles and liver of *O. niloticus* collected from Rosetta Branch of River Nile in summer 2014 and winter 2015 (Mean ± S.E).

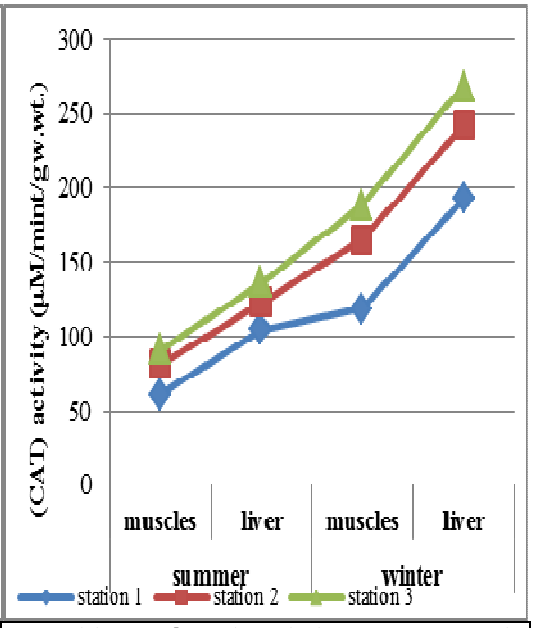

Fig. (3): Catalase (CAT) (µM/mint/g w.wt.) activity in white muscles and liver of *O. niloticus* collected from Rosetta Branch of River Nile in summer 2014 and winter 2015 (Mean ± S.E).

GPx activity of *O. niloticus* (Fig. 4) increased 1.4 and 1.6 fold in white muscles, and 1.6 and 1.8 fold in liver at stations II and III in winter, respectively. In summer the GPx enzyme increase was 1.3 and 1.6 fold in white muscles, and 1.8 and 2 fold in liver at each sampling station, respectively. GST activity (Fig. 5) in white muscles and liver of *O. niloticus* increased significantly at station III, liver highly significant increased ($P \leq 0.01$) in winter at station III compared to reference station I. GST activity in winter increase 1.8 and 2.1 fold in white muscles, and 1.9 and 2.4 fold in liver at stations II and III, respectively. In summer the GST enzyme increase was 1.2 and 1.7 fold in white muscles, and 1.5 and 2.1 fold in liver at each sampling station, respectively. GR mean activity (Fig. 6) showed a significant increase ($P \leq 0.05$) in white muscles and liver of *O. niloticus* at station III. GR activity increase in summer was 1.2 and 1.5 fold in white muscles and 1.4 and 2 fold in liver at each sampling station, respectively. In winter the GST enzyme activity increase was 1.4 and 1.6 fold in white muscles, and 1.7 and 1.9 fold in liver at each sampling station, respectively.

MDA levels (Fig. 7) were increased at station III in liver 2.1 and 2 fold, while increased 1.6 and 1.5 fold in white muscles in summer and winter respectively. MDA level increase in summer was 1.6 and 2 fold in white muscles and 1.7 and 2.1 fold in liver at each sampling station, respectively. In winter the MDA level increase was 1.5 and 1.8 fold in white muscles, and 1.7 and 2 fold in liver at each sampling station, respectively.

The studied enzymes (SOD, CAT, GPx, GST and GR) activity and MDA levels in liver and white muscles increased from lowest values in summer to the maximum in winter, while station III were the highest in enzymes activity and MDA levels, in compare with station I and II.

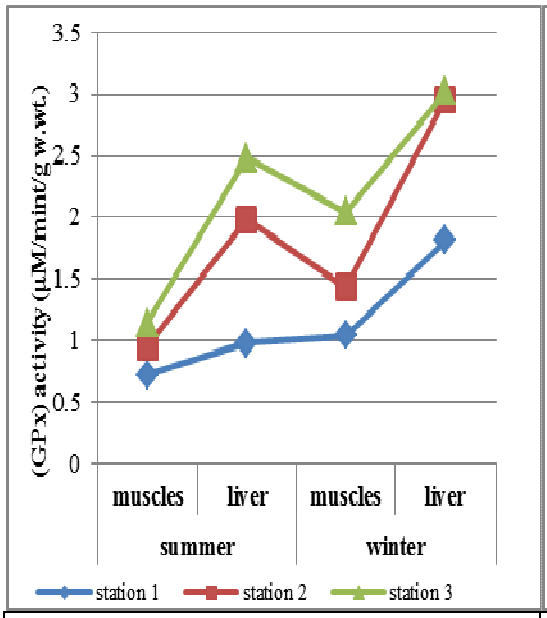

Fig. (4): Glutathione peroxidase (GPx) (µM/mint/g w.wt.) activity in white muscles and liver of *O. niloticus* collected from Rosetta Branch of River Nile in summer 2014 and winter 2015 (Mean ± S.E).

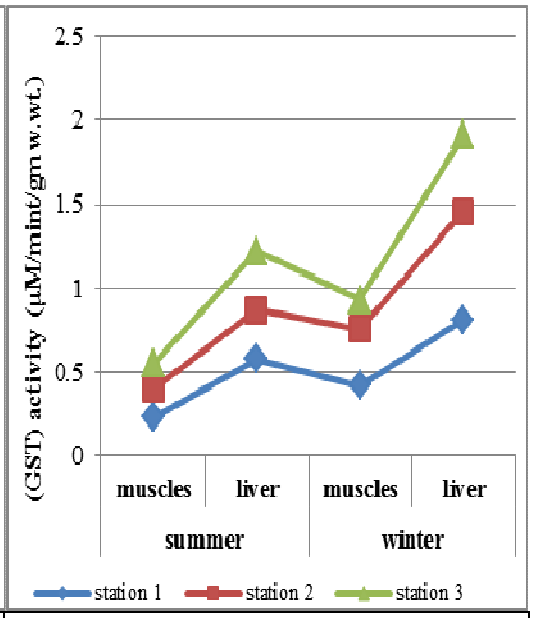

Fig. (5): Glutathione-S-transferase (GST) (µM/mint/g w.wt.) activity in white muscles and liver of *O. niloticus* collected from Rosetta Branch of River Nile in summer 2014 and winter 2015 (Mean ± S.E).

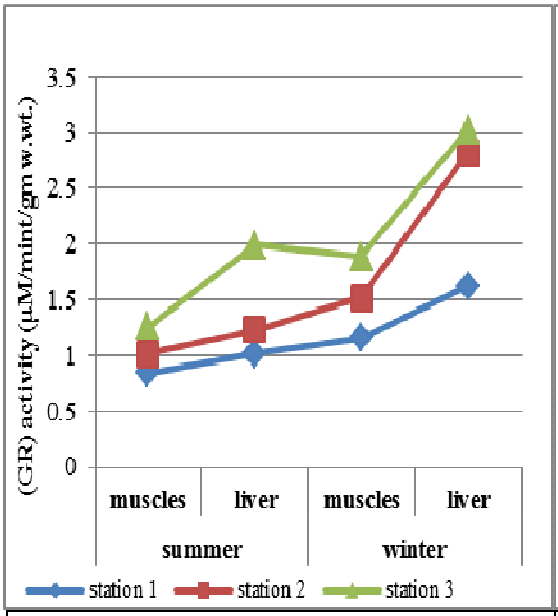

Fig. (6): Glutathione reductase (GR) (µM/mint/g w.wt.) activity in white muscles and liver of *O. niloticus* collected from Rosetta Branch of River Nile in summer 2014 and winter 2015 (Mean ± S.E).

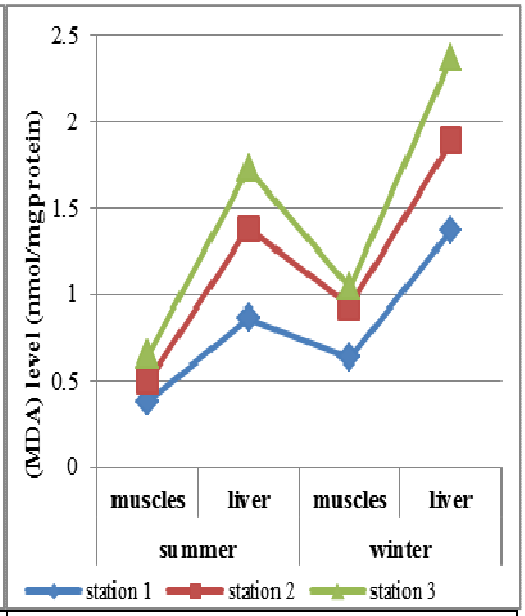

Fig. (7): malondialdehyde level (MDA) (nmol/mg protein) in white muscles and liver of *O. niloticus* collected from Rosetta Branch of River Nile in summer 2014 and winter 2015 (Mean ± S.E).

DISCUSSION

In the present study the increase of Fe in water is mainly due to liberation of Fe as ferrous ions from sediments and industrial wastes effluent from Kafr El-Zayat industrial area. However, the minimum values of Fe may be due to oxidation of (Fe^{+2}) to (Fe^{+3}), which precipitate as Fe (OH_3) to the sediment of the oxygenated water.

Zinc (Zn) concentration in water samples of the study area recorded the highest values in winter. This is mainly due to decrease in and Zn absorption with drop of water during winter and dissolution of clay containing zinc ions giving high content of zinc in water, or due to the decay of phytoplankton (**El-Hadad, 2005**).

The decrease in Cu concentration in water is mainly due to tendency to form complex with organic legends and humic matter, which leads to decreasing the penetration of free ions in water, where 90% of Cu in water were complexes by dissolved organic materials and suspended matters (**Mantoura *et al.*, 1978**).

Manganese concentration in water samples collected from Rosetta Branch within the permissible levels Table (1)**.** Cd concentration in water samples in present work exceeded the permissible limit.

Generally the concentrations of investigated heavy metals in water samples in present study were in the following order: Fe > Zn > Mn > Cu and Cd. Moreover, heavy metal concentrations in water samples showed seasonal variations. As elevated in cold season (winter), while decreased in hot season (summer). This in agreement with the results obtained by **El Bouraie *et al.* (2010)** and **Islam *et al.* (2015b)**, whom reported that, heavy metals concentration showed seasonal variations and increased in winter.

The observed decline in heavy metals concentration in present work during hot seasons may be attributed to: (1) phytoplankton growth which can absorb large quantity of heavy metals from water. (2) heavy metals are more likely attached to organic matter and clays, which have surface functional groups (negatively charged) that bind heavy metal (positively charged) and settled down in sediments. (3) dilution effect of river water for high rainfall (**Islam *et al.*, 2015a**). On the other side, the recorded elevation in heavy metals concentration in water samples in our study during cold seasons maybe due to: (1) winter closure period lead to water levels decrease, results in increase in concentration of the metals (**Abdel-Moati and El-Sammak, 1997**). (2) the fluctuation of the amount of agricultural drainage water, sewage effluents and industrial wastes discharged into water (**Zyadah, 1995**).

Heavy metals (Fe, Zn, Cu, Mn and Cd) concentration in water samples collected from Rosetta Branch at stations (II and III) recorded higher level of heavy metals than station (I), this may due to the impact of pollution sources in these sites, as sewage, domestic wastes and other pollutants that discharged in El-Rahawy drain and the industrial effluents at Kafr El-Zayat industrial area, that poured directly into the branch water without treatment as reported by **Authman *et al.* (2013)**.

Heavy metal concentrations in fish muscles

Contamination of aquatic environment with toxic element has seriously increased worldwide duo to the progressive industrialization. Studies carried out on fish have shown that, trace elements may have toxic effects, altering physiological activities and biochemical parameters both in blood and tissues (**Gad and Ibrahim, 2005**). Since the toxic effects of metals have been recognized, heavy metals affected directly or indirectly on aquatic organisms, this leads to hazardous effect on human health duo to, their biomagnifications over time (**Malik *et al.*, 2010**).

Thus, determination of accumulated metals in fish is extremely important form the human health point of view. Fish species mostly absorbed heavy metals from its feeding diets, sediments and surrounding waters resulting to their accumulation in reasonable amounts (**McCarthy and Shugart, 1996**). Moreover, bioaccumulation of heavy metals in fish critically influences the growth rate, physiological and biochemical status and consequently the meat quality of fish (**Elghobashy *et al.*, 2001**). The current study showed that, the highest concentrations of iron (Fe) in muscles of *O. niloticus* Table (2), was recorded at station III (Kafr El-Zayat), and the

lowest value at station I (relatively unpolluted site). The highest accumulation of (Fe) in fish muscles attributed to the large quantity of iron in water. The accumulated levels of (Fe) in *O. niloticus* muscles were higher in winter than in summer. The increase of (Fe) in water is mainly duo to liberation of Fe as ferrous ions from sediments and industrial wastes effluents from Kafr El-Zayat industrial area station III, however the minimum values in site (1) maybe duo to oxidation of Fe_2^+ to Fe_3^+ which precipitates as $Fe(OH)_3$ to the sediment of the oxygenated water. These results agree with the data obtained by **Gad and Yacoub (2009)** in the same sampling site, with **Gad and Mohamed (2010)** in *O. niloticus* and *lates niloticus* collected from Damietta branch of River Nile. The concentration of Iron in muscles of *O. niloticus* collected from Rosetta Branch of River Nile exceeded the permissible levels at stations II and III.

Zinc (Zn) is an essential micro nutrient required for normal growth and metabolic function for various fish species, zinc is known to be toxic to fish, it causes mortality, growth retardation, tissues alternation, respiration and cardiac changes, inhibition of spawning, destroys the gill epithelium and consequently causes tissues hypoxia (**Spear, 1981**). In the present study the concentration of Zn in muscles of *O. niloticus* collected from Rosetta Branch of River Nile (Tables 3 and 4) showed an elevation in winter at station II and III in winter. These results in accordance with those obtained by **Authman and Abbas (2007)** and **Gad and Yacoub (2009)**.

Cupper (Cu) is essential for good health but, a very high intake can cause adverse health problems, such as liver and kidney damage (**Ikem and Egiebor, 2005**). It is an essential component for numerous oxidation reduction enzymes (cytochrome oxidase, uricase and tyrosinase). The toxicity of copper depends on the hardness and pH of water, it is more toxic in soft water with low alkalinity (**Taha, 2004**). Copper concentrations in muscles of *O. niloticus* collected from Rosetta Branch of River Nile were increased at stations II and III especially in winter Table (2). The observed decrease in Cu concentration in muscles of *O. niloticus* is mainly due to, the decrease in Cu concentration in water, where 90% of Cu in water was complexes by dissolved organic materials and suspended matters (**El-Hadad, 2005**). Our results in the present study are in agreement with that, recorded by **Gad and Yacoub (2009) and Gad and Mohamed (2010)**.

Manganese (Mn) is essential for aquatic organisms, its present below limited concentrations in their bodies due to its role in physiological metallo-enzymes, bone structure and for normal functioning of nervous system (**WHO, 1996**). In the present study the maximum Mn level was observed in muscles of *O. niloticus* collected from station III in winter. According to **FAO (1992)** and the **Egyptian standards (1993)**, there is no information on the carcinogenicity of manganese. **WHO (1994)** recommended that, manganese intake from food, water and dietary supplement should not exceeded the tolerable daily upper limit of 11 mg/ day.

Cadmium (Cd) is a non-essential element which has several toxic effects on fish. Cd can damage gills and disturbed calcium balance, Cd may replace Zn in certain enzymes, causing disease, severely limited oxygen metabolism of mitochondria in the liver of fish and know to accumulate in gills, liver and kidney of fish (**Wicklund *et al.*, 1992**) .

The concentration of cadmium in muscles of *O. niloticus* collected from the three sampling stations were higher at station III Table (2), this may duo to the impact of sewage and the industrial wastes. Cd concentrations in *O. niloticus* muscles were below the maximum permissible level. In the other hand Cd was not detected in muscles of *O. niloticus* collected from Rosetta Branch of River Nile at station I (relatively unpolluted site). Results of the present study revealed that, fish samples

collected from different sites of Rosetta Branch of River Nile displayed significant amount of heavy metals (Fe, Zn, Mn, Cu and Cd) in their muscles, where minimum values were recorded in summer and maximum values were in winter, these observations maybe attributed to seasonal variation in water temperature, with subsequent influence of detoxification rate and accumulation of toxicant (**Haggag *et al.*, 1999**).

The accumulation patterns of trace metals found in muscles of *O. niloticus* collected from Rosetta Branch of River Nile in the following order: Fe > Zn > Mn > Cu and Cd. The concentrations of heavy metals (Zn, Mn, Cu and Cd) in muscles of *O. niloticus* from stations II and III in the present study were within the permissible limits except (Fe). Consequently there was a public health risk from fish consumption especially that caught from Kafr El-Zayat station III, which contaminated by industrial effluents of El-Malyia Company which poured directly into the branch without treatments (**Yehia and Sebaee, 2012**).

Antioxidant defence system

in the present study the activates of antioxidant enzymes (SOD, CAT, GPX, GST and GR) and oxidative stress biomarker (MDA) in liver and white muscles of *O. niloticus* collected from Rosetta Branch of River Nile, were increased significantly ($P \leq 0.05$) at stations II and III, these sites are polluted by sewage and industrial effluents, Table (1). The observed increase in antioxidant enzymes activities indicates adaptive responses of fish to counteract the oxidative effect of generated ROS or due to resist the water pollutants toxicity against the damage caused by excessive amount of oxygen free radicals and oxidative stress (**Gad, 2009 and Carvalho *et al.*, 2012**). On seasonal bases the concentrations of antioxidant enzymes (SOD, CAT, GPX, GST and GR) as well as oxidative stress biomarker (MDA) levels in the present study, were increased in cold season (winter) compared to hot season (summer). This indicates that, low temperature induce more free radical, which led to oxidative stress damage in tissues and more antioxidant enzymes were produced to reduces the damaging effect of free radicals.

This response may be due to one or more of the following two reasons: (i) temperature decrease weakens the systems of ROS elimination (**Lushchak, 2011**). (ii) Fish at low temperatures also implies an increase in unsaturated fatty-acid in membrane lipids as a strategy to maintain membrane function as well as a higher risk of lipid hydrogen peroxide formation and oxidative injury (**Guderley and St-Pierre, 2002**). The critical effects of cold seasons (low temperature) are changes in the properties of cellular membranes, such as decreases in membrane fluidity. Compensatory responses to these effects of low temperature include changes in membrane phospholipid composition, causing changes in a variety of membrane properties such as membrane fluidity, membrane phase behavior, membrane thickness, and membrane permeability (**Crockett, 1998**). Another common response to cold seasons is an increase in mitochondrial enzyme amounts, which is often associated with an increase in the density of mitochondria in a tissue (**O'Brien, 2011**).

End-product of oxidative stress (MDA) may also increase at cold seasons simply because; oxygen is more suitable in aqueous fluids at low temperatures, increasing the oxygen concentration of cytoplasm. Although cold exposure has been associated with decreases in tissue oxygenation as a result of reduced cardiac performance (**Pörtner, 2010**). Consistent with this second hypothesis, cold seasons are associated with increases in the expression of the hypoxia inducible factors (HIF) and increases in the DNA-binding activity of this transcription factor, which ultimately result in increases in tissue capillarity that are thought to help restore

oxygen delivery as well as increases in the amount of myoglobin in critical tissues such as the heart (**Heise *et al.*, 2007**).

The observed increase in antioxidant defence enzymes and oxidative stress biomarker in fish tissues (white muscles and liver) were in agreement with the recorded in gold fish and *C.carpio* (**Lushck *et al.*, 2005**) and in *P. mesopotamicus* exposed to Cu and hypoxia (**Garcia-sampaio, 2008**).

In the present study the concentration of heavy metals Fe, Zn, Cu, Mn and Cd in water samples and their accumulation levels in fish muscles were higher at Kafr El-Zayat industrial area Tables (1 and 2), which causes alternations in the antioxidant defence enzymes and oxidative stress biomarker. Consequently the activities of SOD, CAT and GST were elevated in all *O. niloticus* white muscles and liver at polluted sites. The significant increase in these organs may be a response to oxidative stress caused by the presence of heavy metals in water samples at stations II and III.

The significant increase in (GPx and GST) activity in white muscles and liver of sampled *O. niloticus* at polluted stations II and III, suggests a protective and adaptive role against oxidative stress induced by the heavy metals present in water samples of the same sites. Our results are in agreement with the findings of **Farombi *et al.* (2007)**. While, fish trying to adapt to pollutants and oxidative conditions relatively higher (GR) activity was observed in present work in white muscles and liver of *O. niloticus* collected from polluted stations II and III. **Stephensen *et al.* (2000)** demonstrated that fishes from polluted sites have high GR activity due to higher peroxidative components in the polluted aquatic site.

Our results indicate a significant elevation of lipid peroxidation end-product (MDA) in *O. niloticus* white muscles and liver. The apparent increase in MDA may be attributed to the accumulation of the heavy metals, as our data indicate significant concentration of heavy metals accumulation in muscles of sampling fish from polluted stations II and III. Same results were recorded in *C. gariepinus* collected from heavily polluted River nearby major industries in Nigeria (**Farombi *et al.*, 2007**), in *Oreochromis niloticus, Tilapia rendalli*, and *Geophagus brasiliensis* collected from metal contaminated site (**Ruas *et al.*, 2008**), in *Catostomus commersoni* collected from Québec River (Canada), which impacted by agricultural chemicals (**Dorval *et al.*, 2005**), also in (*Cyprinus carpio* fish from rural and industrial sites in Western Ukraine (**Falfushynska and Stoliar, 2009**).

In present study all antioxidant enzymes and MDA level recorded in liver were higher than white muscles, especially in cold seasons. This may be referring to the strong constitutive antioxidant potential in liver, to resist ROS generation. Liver is a very active tissue metabolically, white muscle has a limited set of physiological functions and anaerobic metabolism dominates during burst swimming activity. Because of this, it appears that muscle is equipped with much lower activities of the main antioxidant enzymes **(Kubrak *et al.*, 2012).** Moreover, the several potential sources of ROS in liver, as liver contains high levels of cytochromes P450, which are involved in fatty acid metabolism and detoxify xenobiotics, generating ROS as a by-product of these reactions, this high levels of cytochromes, liver contains more iron than muscle (**Canli and Atli, 2003**), and free iron reacts with hydrogen peroxide via the Fenton reaction, producing hydroxyl radicals, the most damaging form of ROS (**McCord, 1998**).

CONCLUSION

In conclusion, this study revealed that enzymatic responses of *O. niloticus* to heavy metal exposures lead to a significant increase in activity of antioxidant defense system enzymes (SOD, CAT, GPx, GST and GR) and lipid peroxidation indicator MDA in white muscles and liver of *O. niloticus* from polluted Rosetta Branch of River Nile. Levels of heavy metals in the present study elevated at station II (Al-Qata) because of the extended negative effect of El-Rahawy drain; station III (Kafr El-Zayat) recorded the highest concentration of metals, because of the industrial effluents. This enzymatic response can serve as biomarkers for early detection of pollution during biomonitoring programs. There is a need for extensive evaluation and comparison of data obtained from field studies and those obtained from laboratory studies.

From economical prospect, fish health management is a critical aspect of fish farming and production, especially during the last five years, farmers have been confronted with disease outbreaks, which have occasionally led to the death of all fish in ponds, and hence considerable economic losses. Diseases also affect the quantity, quality and prices of fish in the market. Moreover, low water quality is due to the presence of agriculture, industrial, municipal wastewater and discharges from drains along the course of Nile River. So, more attention should be paid to pollutants discharge into the River Nile without any treatment especially heavy metals. As known, free radical involved in pathogenesis of many immune disease and organs malfunction, so adding some dietary antioxidant (beta-carotene, vitamin A, vitamin E and vitamin C) to fish diet could help in disease resistance, improve fish health and mortality control.

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ARABIC SUMMARY

تغيرات في النظام المضاد للأكسدة فى الأسماك كمؤشر حيوي للتلوث البيئي

مجدي توفيق خليل[١] - ناهد شفيق جاد[٢] - نصر أحمد محمد أحمد[٢] - سالي صلاح الدين مصطفي[٢]

١- قسم علم الحيوان– كليه العلوم – جامعه عين شمس- مصر.

٢- المعهد القومي لعلوم البحار والمصايد- مصر.

تم تقيم مستويات العناصر الثقيله مثل (حديد- الزنك- منجنيز- النحاس- الكادميوم) في مياه فرع رشيد من نهر النيل و معدل تراكم هذه العناصر في عضلات أسماك البلطى النيلي *O.niloticus* ، من ثلاثة مواقع (الموقع ١) القناطر الخيرية قبل التفريعة (موقع غير ملوث نسبيا)، (الموقع ٢) أمام القطا (ملوثه من مصرف الرهاوي) و (الموقع ٣) أمام المنطقة الصناعية بكفر الزيات (ملوثة بالنفايات الصناعية) في فصلي (صيف ٢٠١٤ و شتاء ٢٠١٥)، و إيضاح مدى تأثيرها علي إنزيمات الجهاز المضاد للأكسده (SOD, CAT, GPx, GST, GR) و الجهد التأكسدي للدهون (MDA) في العضلات البيضاء والكبد لأسماك البلطي النيلي.

لوحظ أن: تركيزات المعادن الثقيلة (الحديد، الزنك، النحاس، المنجنيز و الكادميوم) في المواقع (٢ و ٣) ارتفعت بشكل ملحوظ مقارنة بالموقع (١)، نظرا لتأثير ملوثات مصرف الرهاوي و المنطقة الصناعية بكفر الزيات. أيضا شهدت معدلات تراكم المعادن الثقيلة في عضلات الأسماك التي تم جمعها من فرع رشيد نهر النيل زيادة كبيرة في المواقع (٢ و ٣) خاصة في فصل الشتاء، وكانت تركيزاتها بالترتيب التالي:
حديد> الزنك> منجنيز>النحاس و كادميوم. كما أظهرت جميع الانزيمات المضادة للأكسدة و المنتج النهائي لاكسدة الدهون (MDA) مستويات مرتفعة في أنسجة سمك البلطى النيلي *O.niloticus* تزامنا مع إرتفاع مستويات العناصر الثقيله في المياه ، و سجل الكبد تركيزات أعلى من العضلات البيضاء في جميع المواقع ، خاصة في المواسم الشتاء. وأخيرا، فإن الدراسة قد خلصت أنه من الممكن إستخدام التغيرات في إنزيمات الجهاز المضاد للأكسده كمؤشر بيولوجي للتلوث البيئي الذي تتعرض له اسماك البلطي النيلي *O.niloticus*.