Egypt. J. Exp. Biol. (Zool.), 11(1): 23 – 36 (2015)

RESEARCH ARTICLE

Mohamed A. M. Hegazi*
Somya Z. Abd El Haleim*
Mahmoud M. S. Sirdah**
Saleh N. Mwafy**

OXIDATIVE STRESS IN LIVER AND WHITE MUSCLES OF NILE TILAPIA AS RESPONSES TO POLLUTED AREA IN THE NILE RIVER

ABSTRACT:

Oxidative stress in liver and white muscles of Nile tilapia, *Oeochromis niloticus* captured in Rosetta branch of Nile River at Kafr El-Zayat area, Al-Gharbiyah Governorate, Egypt was studied. The toxic effects of water in this area that, receive a lot of sewage and other industrial pollutants discharge, on the antioxidant system of Nile tilapia (62.4 ± 6.9 g) were investigated. Water samples were analyzed for physicochemical parameters including pH, turbidity, total dissolved solids, ammonia, nitrite and nitrate. The activities of antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST), glutathione reductase (GR) and the level of lipid peroxidation (LPO) in liver and white muscles were assayed. The examined physicochemical parameters found to be in excess than the maximum standard limits in water samples of the examined areas. The increase was more detectable during autumn and winter than other seasons. The alterations in the physicochemical parameters of water are consistent with the alteration reported in the enzymes activity of SOD, GST, GR, and GPx, as well as LPO, concentration which increased significantly ($P \leq 0.05$) as compared to the control. However, the increase was more detectable during autumn and winter than in summer and spring seasons. The physicochemical parameters considered as the most important principles in the identification of the nature, quality and type of the water and/or any aquatic system. Antioxidant enzymes and the oxidative stress biomarkers lipid peroxidation considered as the most important biomarkers used to identify polluted marine sites. This can be taken as a useful biomarker for environmental managers in investigating the exposure of fish to contaminated freshwaters. This study could be beneficial for ecotoxicological researches in freshwaters as it provides data about antioxidant system response for fish exposed to different water pollutants.

KEY WORDS:
Superoxide dismutase, catalase, glutathione-S-transferase, glutathione reductase, glutathione peroxidase, lipid peroxidation

CORRESPONDENCE:
Mohamed A. M. Hegazi*
Department of Zoology, Faculty of Science, Tanta, Egypt
E-mail: m_a_m_hegazi@hotmail.com

Somya Z. Abd El Haleim*
Mahmoud Mohamed S. Sirdah**
Saleh N. Mwafy**
*Department of Zoology, Faculty of Science, Tanta, Egypt
**Department of Biology, Faculty of Science, Al-Azhar University of Gaza, Gaza Strip, Palestine

ARTICLE CODE: 03.01.15

INTRODUCTION:

Pollution is one of the biggest global killers. Over the last three decades there has been increasing global concern over the public health impacts attributed to environmental pollution, in particular, the global burden of disease. Water pollution is one of the principal environmental and public health problems, of which Egypt and the Middle East region are facing (Anwar, 2003). Water pollution does not only greatly damage and threatened the aquatic ecosystems and the terrestrial organisms (Authman *et al.*, 2013). The natural resources of water polluted with a variety of solid and liquid wastes. Every waste ultimately dumped or emptied in natural water bodies (Garg *et al.*, 2009). Contamination of water can result from industrial and agricultural and sewage sources. Deficiencies in the treatment of wastewater, the disposal of untreated sewage, and inadequate operation and maintenance of treatment plants result in health risks (Anwar, 2003). About a quarter of the diseases facing humankind today occur due to prolonged exposure to environmental pollution (EPH, 2010; Azimi and Moghaddam, 2013). It was reported that the two biggest components of the global water crisis are the contamination of drinking supplies with

human discharge and the massive wastage of water that is inherent in prevailing agricultural practices (WHO, 1998).

River Nile from Aswan to El-Kanater Barrage receives wastewater discharge from 124-point sources, of which 67 are agricultural drains and the remainders are industrial sources (NWRC, 2000). Rosetta Branch, starting downstream of Delta Barrage receives relatively high concentrations of organic compounds, nutrients and oil and grease. Major sources of pollution are Rahawy drain (which receives part of Greater Cairo wastewater), Sabal drain, El-Tahrrer drain; Zawiet El-Bahr drain and Tala drain (Fig. 1).

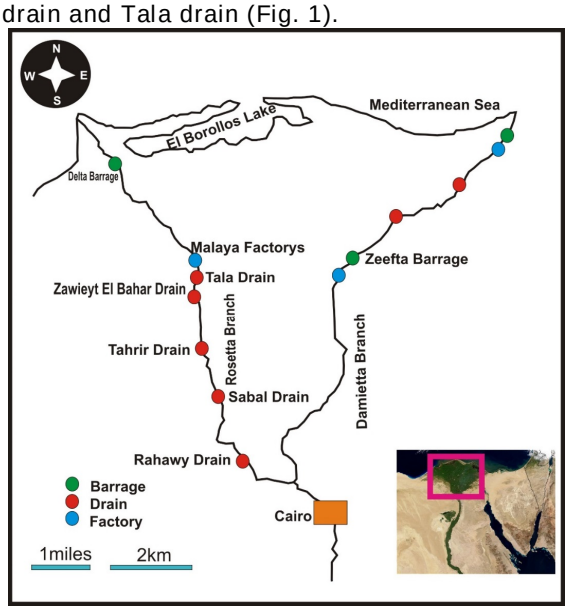

Fig. 1. Location Map of Rosetta Branch of the Nile River

Unfortunately, several industrial companies at Kafr El-Zayat City affect the Rosetta Branch. These industrial outfalls are El-Mobidat, El-Malyia, Salt and Soda companies, and Mashroa Eldalgamon for sewage and wastewater. Tayel *et al*. (2008) reported that these industrial outfalls are discharging directly at the east bank of the branch (Fig. 2). The Rosetta branch water serves for a wide range of functions including agricultural, industrial and domestic water supply, fisheries and recreation.

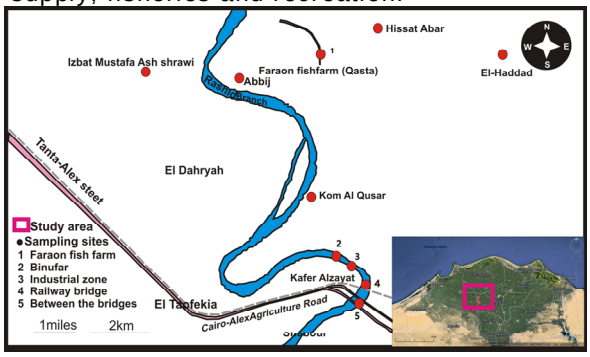

Fig. 2. Map of Nile Delta (Egypt) and Kafer El-Zayat city showing the experimental sites, 1 Firon fish farm, the reference site, 2 Binufar, 3 Industrial zone, 4 Railway bridge, 5 Between the bridges.

Previous studies have reported that exposure of fish to pollutants (agricultural, industrial and sewage) evolved antioxidative defense system which include enzymes such as glutathione-S-transferase, glutathione reductase, glutathione reductase superoxide dismutase and catalase that show general high activity in the liver, a major organ for xenobiotic (Freitas *et al*., 2007; Hegazi *et al*., 2010). These enzymes present in all organisms metabolize many pollutants in order to protect the organisms against the deleterious effects of xenobiotics. It 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). Kafr El-Zayat is one of the largest industrial cities in Egypt due to the presence of large number of factories and companies such as chemical, pesticides, fertilizers, pesticides, paper, and oil and soap factories, so it take rank in the global pollution. Therefore, the present study investigated the physiological changes in liver and white muscles of Nile tilapia, *Oeochromis niloticus*, exposed to polluted water of Rosetta Branch in Kafr El-Zayat City. This finding could open a new avenue of research in identifying and designing novel strategies, which could translate into better protection for fish and human.

MATERIAL AND METHODS:

Study area:

Nile Tilapia samples were collected from Rosetta branch of Nile River at Kafr El-Zayat Al-Gharbia Governorate, Egypt. Five sampling localities in this area were selected according to their position relative to municipal sewage effluents and other sources of pollution (Fig. 2). The first locality Faraon fish farm, Qasta (30° 53′ 26.55" N, 30° 47′ 50.49" E) was the cleanest of the four, and thus can be considered a reference locality. The second locality (30° 49′ 43" N, 30° 48′ 7" E) was located downstream from the effluent discharge from Rosetta branch, Binufar, Kafr El-Zayat. This locality is receiving extensive industrial wastewater discharge because it located near the fertilizer factory in Kafr El-Zayat. The third locality was located in the front of Soda and Salt factory (30° 49′ 35" N, 30° 48′ 25" E), and receive extensive industrial wastewater discharge. The fourth locality Railway bridge (30° 49′ 7" N, 30° 48′ 46" E) was the site from which we start our tour, receive extensive urban discharge. The fifth locality (30° 48′ 51" N, 30° 48′ 37" E) was located between the bridges and receive the contamination from El Dalgamon municipal effluents. Rosetta branch of Nile River at Kafr

El-Zayat area receives annually, sewage, agricultural, industrial drainage water without treatment from several drains. The main waste drains are shown in figures 1 & 2. Some Fisherman catch fish from these regions, sell it in the market and it may constitute health hazard for consumers.

Collection of Samples:

Water samples:

Water samples were collected from the five selected sites of the Nile River from summer 2012 to spring 2013, using 500 ml sampling bottle. The samples for each site were composited for all analytical procedures conduct in the laboratory.

Fish Samples:

Nile tilapia samples were caught and collected at each sampling site with the help of fishermen. The fish were collected from five sampling localities in Rosetta branch of Nile River in Kafr El-Zayat. After collection, the fish taken to laboratory using an icebox at 4^{o}C and 10 fish were selected for examination. The selected fish weigh were from 62.4 ± 6.9 g. The fish weight and length were registered.

Water samples and physicochemical parameters:

The physicochemical analyses of water were done in the water analysis unit in the central laboratory, Tanta University using standard methods. The pH was measured at the sampling site using microprocessor based pocket pH meter (water proof pH scan WPI). The pH values were checked again in the laboratory using pH meter (Hanna instrument). Conductivity and total dissolved solid values were registered immediately after arrival to the laboratory using digital conductivity meter (Hanna instrument). Turbidity were measured using turbidity meter, titrimetric method was used for the determination of alkalinity, whereas EDTA titrimetric method was used for total hardness analysis. The level of ammonia, nitrite and nitrate of the water was determined using UV-Vis double beam pc scanning Spectrophotometer UVD 2950 following standard method of American Public Health Association (APHA, 1998).

Tissue sampling:

A small piece of tissue (white muscles or right liver lobe) was carefully excised on ice, avoiding squeezing the tissue, and washed in ice-cold isotonic NaCl saline, was blotted dry with filter paper and weighed. The tissue was homogenized in ice-cold phosphate buffer (50 mM, pH 7.4) 10% (w/v) using Omni international homogenizer (USA) at 22,000 rpm for 20 s each with 10 s intervals. The supernatant was freeze-thawed thrice to completely disrupt mitochondria. Then the supernatant was centrifuged at 6000×g in cooling centrifuge at 4^{o}C for 15 min and the yielded supernatant which contains the cytosolic and mitochondrial enzymes was saved for immediate enzyme assays. The UV/vis Spectrophotometer (JENWAY 6505, UK) used for the measurements of enzyme activities and oxidative stress parameters at 25^{o}C.

Antioxidant enzyme activities and lipid peroxidation assays:

Total superoxide dismutase (SOD, EC 1.15.1.1):

The activity assayed according to the method of Paoletti and Mocali, (1990), which monitors NADH oxidation. The SOD activity was assayed by assessing the inhibition of NADH oxidation by β-mercaptoethanol in the presence of EDTA and Mn as substrate. NADH solution made fresh daily, the assays run by adding sequentially to the cuvette: 0.8 ml of 50 mM phosphate buffer pH 7.4, 55 µl EDTA/Mn solution of 100/50 mM, 40 µl NADH solution of 7.5 mM and different volumes from sample tissue extract. The reaction then initiated by adding 100 µl β-mercaptoethanol solution. The changes in ΔE of NADH followed per minute for 15 minutes (Ex = 6.22/mM/1cm at 340 nm). One unit of SOD activity defined as the amount of cell extract required to inhibit the rate of NADH oxidation of the control by 50%. The specific activity was expresses as unit per gram wet weight tissue (wwt).

Catalase (CAT, EC 1.11.1.6):

Catalase activity assayed according to the method of Xu *et al*. (1997). Assay mixtures contained 10µl sample tissue extract added to 1.0 ml of H_2O_2 phosphate buffer pH 7.4. The changes in ΔE of H_2O_2 followed per minute for 15 minutes (Ex = 0.04/mM/1cm at 240 nm).

Glutathione reductase (GR, EC 1.6.4.2):

Glutathione reductase activity assayed according to the method of Smith *et al*. (1988). Ten µl of sample tissue extract were added to 1 ml of reaction mixture contained 50 mM phosphate buffer pH 7.4 containing 2 mM EDTA and 0.15 mM NADPH. The reaction initiated by the addition of 10 µl of 1 mM oxidised glutathione (GSSG). Specific activities were determined following the changes of ΔE per minute (Ex = 6.22/mM/1cm at 340 nm).

Glutathione S-transferase (GST, EC 2.5.1.18):

Glutathione-S-transferase activity assayed according to the method of Habig *et al*. (1974). The final reaction mixture contains 1.45 ml of (1.2 ml Phosphate buffer 50 mM, pH 7.4, 100 µl of 1 mM CDNB, 100 µl of 1 mM GSH). The reaction initiated by the addition of 50 µl sample tissue extract. Specific activities were determined following the changes of ΔE per minute (Ex = 9.6 /mM/1cm at 340 nm).

Glutathione peroxidase (GPx, EC 1.11.1.9):

Glutathione peroxidase activity assayed according to the method of Paglia and Valentine (1967). One milliliter of 50 mM

sodium phosphate pH 7.4 containing 1 mM GSH, 4 mM sodium azide, 2 units of GR, and 0.25 mM NADPH, add 10 µl sample tissue extract and 10 µl H_2O_2 (0.2 mM H_2O_2 in phosphate buffer pH 7.4). Specific activities were determined following the changes of ΔE per minute (Ex = 6.22/mM/1cm at 340 nm).

Lipid peroxidation (LPO):

Lipid peroxidation is the degradation of lipids that occurs, as a result of oxidative damage, is a useful marker for oxidative stress. Polyunsaturated lipids are susceptible to an oxidative attack, typically by reactive oxygen species, resulting in a well-defined chain reaction with the production of end products such as malondialdehyde (MDA). Malondialdehyde was quantified according to the method of Buege and Aust (1978). To 250 µl of sample tissue extract, 250 µl TCA (20%) and 500 µl thiobarbituric acid (0.67%) was added in a centrifuge tube. The mixture boiled for 15 min. After cooling, the sample to room temperature 2 mL butanol added and the sample centrifuged at 3000×g for 15 min. Absorbance of pink supernatant measured against the reagent blank by spectrophotometer (Ex =156/mM/1cm at 532nm).

Chemicals:

All chemicals used in this study were purchased from the following companies, Sigma Chemical Co. (St. Louis, MO, USA), Fisher bioreagent (USA), MP Biomedicals, ICN (USA) and Acros Belgium and were of analytical grade.

Statistical Analysis:

Data statistically analyzed and each reading represents means ± standard deviation. The statistical evaluations of all data were done using one-way analysis of variance (ANOVA) followed by Dunnett's test using a computer program (GraphPad InState Software, Inc). P values ≤ 0.05 regarded as statistically significant. Graphs and correlations between different parameters plotted using GraphPad Prism Software, Inc.

RESULTS:

Physicochemical analysis of water samples:

The pH, turbidity and total dissolved solids values of water at different localities during the four seasons in comparison with their respective control were tabulated (Table 1). The pH values of the samples ranged from 7.2 to 8.0, turbidity values ranged from 4.0 to 33.1 NTU and total dissolved solids values of the samples ranged from 112 to 430 ppm at different localities during the four seasons. The pH, turbidity and total dissolved solids values increased from lower values through summer to a maximum in the autumn in comparison with the other seasons.

Table 1. Some physicochemical parameters in water at different areas during the four seasons

Parameters	Seasons	Sampling sites					Standard max. permissible limit
		Site 1	Site 2	Site 3	Site 4	Site 5	
pH	Autumn	7.5 ± 0.05	7.6 ± 0.06	7.7 ± 0.06	7.8 ± 0.07	8.0 ± 0.07	6.5 – 7.8
	Winter	7.4 ± 0.04	7.4 ± 0.05	7.5 ± 0.05	7.6 ± 0.06	7.8 ± 0.08	
	Spring	7.4 ± 0.02	7.4 ± 0.03	7.5 ± 0.04	7.6 ± 0.05	7.6 ± 0.04	
	Summer	7.2 ± 0.02	7.3 ± 0.03	7.4 ± 0.04	7.5 ± 0.04	7.6 ± 0.04	
Turbidity (NTU)	Autumn	7.2 ± 1.4	20.4 ± 1.3	21.6 ± 1.5	27.2 ± 1.7	33.1 ± 2.0	< 10 NTU
	Winter	6.4 ± 1.1	18.3 ± 1.1	19.6 ± 1.3	22.7 ± 1.3	26.4 ± 1.7	
	Spring	6.3 ± 0.3	9.5 ± 0.5	11.3 ± 0.8	13.2 ± 0.6	15.6 ± 0.9	
	Summer	4.0 ± 0.1	4.2 ± 0.2	5.8 ± 0.3	6 ± 0.3	10 ± 0.6	
TDS (ppm)	Autumn	117 ± 8.2	321 ± 9.8	338 ± 11.2	358 ± 13.4	430 ± 12.7	<500 ppm
	winter	123 ± 10.7	268 ± 11.4	285 ± 13.7	291 ± 16.4	298 ± 20.4	
	spring	121 ± 8.9	213 ± 15.0	225 ± 17.0	227 ± 19.0	239 ± 21.0	
	Summer	112 ± 2.3	137 ± 3.5	143 ± 4.0	158 ± 5.4	179 ± 6.2	
Ammonia NH3 (mg/l)	Autumn	0.06 ± [illegible]	0.59 ± 0.008	0.62 ± 0.01	0.66 ± 0.02	0.73 ± 0.04	<0.1 mg/L N
	winter	0.062 ± [illegible]	0.376 ± 0.008	0.39 ± 0.01	0.423 ± 0.02	0.47 ± 0.02	
	spring	0.082 ± [illegible]	0.24 ± 0.006	0.28 ± 0.009	0.33 ± 0.01	0.36 ± 0.02	
	Summer	0.055 ± [illegible]	0.11 ± 0.007	0.13 ± 0.005	0.15 ± 0.008	0.16 ± 0.009	
Nitrite NO2 (mg/l)	Autumn	0.034 ± [illegible]	0.44 ± 0.02	0.51 ± 0.03	0.58 ± 0.03	0.74 ± 0.05	<0.03 mg/l
	Winter	0.032 ± [illegible]	0.144 ± 0.008	0.19 ± 0.007	0.272 ± 0.009	0.33 ± 0.008	
	Spring	0.028 ± [illegible]	0.092 ± 0.005	0.104 ± 0.007	0.109 ± 0.009	0.122 ± 0.008	
	Summer	0.025 ± [illegible]	0.038 ± 0.002	0.041 ± 0.003	0.048 ± 0.004	0.056 ± 0.005	
Nitrate NO3 (ppm)	Autumn	8.8 ± 0.6	63 ± 4.1	67 ± 3.7	71.3 ± 4.3	75.1 ± 4.8	<10 ppm
	winter	9.2 ± 0.7	26.1 ± 1.2	28.4 ± 1.9	31.5 ± 2.4	37.3 ± 3.3	
	spring	8.3 ± 0.4	18 ± 0.9	21.8 ± 1.4	24 ± 1.5	26 ± 1.6	
	Summer	8.2 ± 0.6	9.6 ± 0.7	11.1 ± 0.8	13.4 ± 0.7	15.6 ± 0.9	

The sampling sites: 1 Firon fish farm (control site), 2 Binufar, 3 Industrial zone, 4 Railway bridge and 5 between the bridges.

The values are presented as means ± S.E.M. (n = 10).

Standard maximum permissible limit of WHO (1998), USEPA (2009), and CFQGCI (2011).

Ammonia, nitrite and nitrate levels of water at different localities during the four seasons in comparison with their respective control were tabulated (Table 1). Ammonia levels of the samples ranged from 0.034 to 0.73 mg/l, nitrite levels ranged from 0.025 to 0.74 mg/l and nitrate levels ranged from 8.2 to 75.1 mg/l at different localities during the four seasons. Ammonia, nitrate and nitrite levels increased from lower values through summer to a maximum in the autumn in comparison with the other seasons.

Superoxide dismutase (SOD):

The SOD activity in liver and white muscles of fish increased significantly ($P \leq 0.05$) at different localities during the four seasons in comparison with their respective controls (Table 2). The increase in autumn was 2.1, 2.5, 2.5, and 2.7 -fold in liver and 23.8, 34.1, 41.1, and 53.5% in white muscles at each sampling sites, respectively. The increase in winter was 1.9, 2.0, 2.0, and 2 -fold in liver and 26.5, 33.4, 36.9, and 40.8% in white muscles at each sampling sites, respectively. The increase in spring was 50.5, 53.8, 58.7, and 63.1% in liver and 18.6, 25.7, 28.1, and 31.5% in white muscles at each sampling sites, respectively. The increase in summer was 31.4, 36.4, 38.1, and 39.9% in liver and 9.8, 14.1, 20.7, and 25.3% in white muscles at each sampling sites, respectively. The enzyme activity increased from lower values through summer to a maximum in the autumn in comparison with the other seasons.

Table 2. Superoxide dismutase activity (U/g/50% inhibition wet weight tissue) in liver and white muscles of Nile tilapia in different areas during the four seasons

Seasons	Organ	Sampling sites				
		Site 1	Site 2	Site 3	Site 4	Site 5
Autumn	Liver	95.8 ± 6.0	197.7 ± 5.6*	239.4 ± 6.1*	243.1 ± 10.5*	256.9 ± 13.4*
	White muscles	78.6 ± 2.4	97.3 ± 8.6*	105.4 ± 5.6*	110.9 ± 8.0	120.6 ± 10.9*
Winter	Liver	90.1 ± 1.9	173.6 ± 7.7*	179.6 ± 7.1*	183.0 ± 10.3*	187.5 ± 11.0*
	White muscles	76.6 ± 2.1	96.9 ± 4.1*	102.2 ± 4.8*	104.9 ± 5.1*	107.9 ± 7.1*
Spring	Liver	90.6 ± 1.6	136.4 ± 4.2*	139.4 ± 6.2*	143.8 ± 7.8*	147.8 ± 9.8*
	White muscles	76.0 ± 1.8	90.2 ± 3.5*	95.6 ± 5.6*	97.36 ± 5.5*	99.9 ± 7.9*
Summer	Liver	89.6 ± 2.3	117.7 ± 4.9*	122.2 ± 6.3*	123.7 ± 9.0*	125.3 ± 9.1*
	White muscles	74.8 ± 2.0	82.1 ± 1.9*	85.4 ± 6.0*	90.3 ± 5.9*	93.7 ± 6.7*

The sampling sites are: 1 Firon fish farm (reference site), 2 Binufar, 3 Industrial zone, 4 Railway bridge and 5 between the bridges.

Each reading represents Mean±SD of 10 fish.

The significant of difference was checked by one-way ANOVA and Dunnett test (compare all vs. control) using a computer program (GraphPad InState Software, Inc).

The difference checked by one-way ANOVA was significant at $P \leq 0.05$ and Dunnett test was significant at $^*P \leq 0.05$.

Catalase (CAT):

The CAT activity in liver and white muscles of fish increased significantly ($P \leq 0.05$) at different localities during the four seasons in comparison with their respective controls (Table 3). The increase in autumn was 2.0, 2.04, 2.1, and 2.14 -fold in liver and 1.9, 2.0, 2.0, and 2.2 -fold in white muscles at each sampling sites, respectively. The increase in winter was 1.6, 1.7, 1.9, and 2.0 -fold in liver and 67.5, 70.7, 78.4, 85.2% in white muscles at each sampling sites, respectively. The increase in spring was 42.6, 54.7, 57.3, and 66.3% in liver and 32.0, 35.6, 38.3, and 45% in white muscles at each sampling sites, respectively. The increase in summer was 19.7, 30.2, 34.6, and 37.5% in liver and 30.7, 31.5, 33.8, and 36.5% in white muscles at each sampling sites, respectively. The enzyme activity increased from lower values through summer to a maximum in the autumn in comparison with the other seasons.

Glutathione-S-transferase (GST):

The GST activity in liver and white muscles of fish increased significantly ($P \leq 0.05$) at different localities during the four seasons in comparison with their respective controls (Table 4). The increase in autumn was 58.1, 72, 85.5, and 92.6% in liver and 35.4, 53.1, 63.4, and 75.6% in white muscles at each sampling sites, respectively. The increase in winter was 33.6, 47.6, 63.2, and 86.1% in liver and 32.1, 38.9, 60.6, and 71.0% in white muscles at each sampling sites, respectively. The increase in spring was 28.2, 38, 43.6, and 52.4% in liver and 20.4, 31.1, 36.5, and 46.0% in white muscles at each sampling sites, respectively. The increase in summer was 17.7, 27.7, 36.3, and 40.5% in liver and 15.0, 20.1, 31.7, and 38.5% in white muscles at each sampling sites, respectively. The enzyme activity increased from lower values through summer to a maximum in the autumn in comparison with the other seasons.

Table 3. Catalase activity (µM/min/g/wet weight tissue) in liver and white muscles of Nile tilapia in different areas during the four seasons

Seasons	Organ	Sampling sites				
		Site 1	Site 2	Site 3	Site 4	Site 5
Autumn	Liver	718.2 ± 84.3	1445 ± 71.6*	1464.1 ± 99.9*	1475.8 ± 46.8*	1533.8±74.5*
	White muscles	90.5 ± 17.6	168.9 ± 25.5*	179.3 ± 27.5*	184.8 ± 38.2*	198.3 ± 47.5*
Winter	Liver	618.5 ± 72.3	1001.9 ± 93.6*	1049.4 ± 97.3*	1195.8 ± 96.0*	1251.9 ± 83.1*
	White muscles	80.1 ± 16.3	134.2 ± 27.3*	136.7 ± 38.4*	142.8 ± 41.7*	148.3 ± 22.4*
Spring	Liver	629.4 ± 46.8	897.2 ± 49.0*	973.7 ± 46.3*	990.1 ± 32.8*	1046.4 ± 80.0*
	White muscles	94.5 ± 13.9	124.8 ± 21.2*	128.2 ± 14.2*	130.7 ± 21.7*	137.1 ± 14.7*
Summer	Liver	662.5 ± 29.6	792.7 ± 72.6*	862.7 ± 75.0*	891.6 ± 34.9*	910.8 ± 51.4*
	White muscles	84.9 ± 8.7	111.0±7.1*	111.7±12.1*	113.7 ± 24.5*	115.9 ± 15.5*

The sampling sites are: 1 Firon fish farm (reference site), 2 Binufar, 3 Industrial zone, 4 Railway bridge and 5 between the bridges.

Each reading represents Mean ± SD of 10 fish.

The significant of difference was checked by one-way ANOVA and Dunnett test (compare all vs. control) using a computer program (GraphPad InState Software, Inc).

The difference checked by one-way ANOVA was significant at $P \leq 0.05$ and Dunnett test was significant at $^*P \leq 0.05$.

Table 4. Glutathione-S-transferase activity (µM/min/g/ wet weight tissue) in liver and white muscles of Nile tilapia during the four seasons

Seasons	Organs	Sampling sites				
		Site 1	Site 2	Site 3	Site 4	Site 5
Autumn	Liver	0.94 ± 0.17	1.49 ± 0.26*	1.62 ± 0.22*	1.75 ± 0.35*	1.82 ± 0.27*
	White muscles	0.42 ± 0.02	0.57 ± 0.03*	0.65 ± 0.08*	0.69 ± 0.05*	0.74±0.04 *
Winter	Liver	0.91 ± 0.07	1.22 ± 0.05*	1.34 ± 0.19*	1.49 ± 0.18*	1.7 ± 0.17*
	White muscles	0.43 ± 0.02	0.56 ± 0.03*	0.59 ± 0.03*	0.68 ± 0.04*	0.73 ± 0.05*
Spring	Liver	0.88 ± 0.04	1.12 ± 0.08*	1.21 ± 0.04*	1.26 ± 0.07*	1.33 ± 0.12*
	White muscles	0.42 ± 0.02	0.51 ± 0.026*	0.55 ± 0.034*	0.57 ± 0.03*	0.61 ± 0.04*
Summer	Liver	0.82 ± 0.03	0.97 ± 0.6*	1.05 ± 0.14*	1.12 ± 0.45*	1.16 ± 0.06*
	White muscles	0.38 ± 0.019	0.43 ± 0.04*	0.45 ± 0.037*	0.49 ± 0.04*	0.52 ± 0.05*

The sampling sites are: 1 Firon fish farm (reference site), 2 Binufar, 3 Industrial zone, 4 Railway bridge and 5 between the bridges.

Each reading represents Mean ± SD of 10 fish.

The significant of difference was checked by one-way ANOVA and Dunnett test (compare all vs. control) using a computer program (GraphPad InState Software, Inc).

The difference checked by one-way ANOVA was significant at $P \leq 0.05$ and Dunnett test was significant at $^*P \leq 0.05$.

Glutathione reductase (GR):

The GR activity in liver and white muscles of fish increased significantly ($P \leq 0.05$) at different localities during the four seasons in comparison with their respective controls (Table 5). The increase in autumn was 1.9, 2.1, 2.2, and 2.3 -fold in liver and 23.4, 34.6, 38.8, and 52.5% in white muscles at each sampling sites, respectively. The increase in winter was 40, 70, 80.2, and 88.5% in liver and 17.8, 25, 30.0, and 38.4% in white muscles at each sampling sites, respectively. The increase in spring was 20.3, 30.0, 40.2, and 45.6% in liver and 13.5, 18.2, 23.7, and 27.6% in white muscles at each sampling sites, respectively. The increase in summer was 16.2, 20, 24.3, and 28% in liver and 13.7, 16.6, 20 and 21% in white muscles at each sampling sites, respectively. The enzyme activity increased from lower values through summer to a maximum in the autumn in comparison with the other seasons.

Glutathione peroxidase (GPx):

The GPx activity in liver and white muscles of fish increased significantly ($P \leq 0.05$) at different localities during the four seasons in comparison with their respective controls (Table 6). The increase in autumn was 1.7, 1.8, 1.9, and 2 -fold in liver and 28.0, 38.4, 48.2, and 54.8% in white muscles at each sampling sites, respectively. The increase in winter was 28, 44, 52, and 56% in liver and 25.2, 34.0, 38.9, and 53.1% in white muscles at each sampling sites, respectively.

The increase in spring was 20.5, 27, 32.1, and 36.5% in liver and 14.8, 22.4, 31.1, and 35.8% in white muscles at each sampling sites, respectively. The increase in summer was 10.7, 12.5, 15, and 18.6% in liver and 15.1, 17.0, 20.4, and 24.0% in white muscles at each sampling sites, respectively. The enzyme activity increased from lower values through summer to a maximum in the autumn in comparison with the other seasons.

Table 5. Glutathione reductase activity (µM/min/g/ wet weight tissue) in liver and white muscles of Nile tilapia during the four seasons

Seasons	Organs	Sampling sites				
		Site 1	Site 2	Site 3	Site 4	Site 5
Autumn	Liver	1.60 ± 0.13	3.1 ± 0.24*	3.3 ± 0.24*	3.57 ± 0.32*	3.68 ± 0.37*
	White muscles	1.11 ± 0.07	1.37 ± 0.06*	1.49 ± 0.08*	1.54 ± 0.13*	1.69 ± 0.09*
Winter	Liver	1.58 ± 0.2	2.21 ± 0.22*	2.67 ± 0.28*	2.84 ± 0.27*	2.97 ± 0.37*
	White muscles	1.10 ± 0.07	1.3 ± 0.07*	1.37 ± 0.11*	1.43 ± 0.16*	1.52 ± 0.12*
Spring	Liver	1.55 ± 0.07	1.86 ± 0.14*	2.01 ± 0.19*	2.17 ± 0.14*	2.25 ± 0.13*
	White muscles	1.11 ± 0.04	1.26 ± 0.05*	1.31 ± 0.12*	1.37 ± 0.08*	1.41 ± 0.08*
Summer	Liver	1.38 ± 0.11	1.61 ± 0.11*	1.66 ± 0.15*	1.72 ± 0.09*	1.77 ± 0.15*
	White muscles	0.95 ± 0.12	1.09 ± 0.08*	1.11 ± 0.04*	1.14 ± 0.05*	1.15 ± 0.05*

The sampling sites are: 1 Firon fish farm (reference site), 2 Binufar, 3 Industrial zone, 4 Railway bridge and 5 between the bridges.

Each reading represents Mean ± SD of 10 fish.

The significant of difference was checked by one-way ANOVA and Dunnett test (compare all vs. control) using a computer program (GraphPad InState Software, Inc).

The difference checked by one-way ANOVA was significant at $P \leq 0.05$ and Dunnett test was significant at $^*P \leq 0.05$.

Table 6. Glutathione peroxidase activity (µM/min/g/ wet weight tissue) in liver and white muscles of Nile tilapia during the four seasons

Seasons	Organs	Sampling sites				
		Site 1	Site 2	Site 3	Site 4	Site 5
Autumn	Liver	1.70 ± 0.080	2.8 ± 0.07*	3.09 ± 0.15*	3.20 ± 0.27*	3.37 ± 0.22*
	White muscles	1.11 ± 0.04	1.42 ± 0.05*	1.54 ± 0.18*	1.65 ± 0.13*	1.72 ± 0.30*
Winter	Liver	1.70 ± 0.08	2.17 ± 0.3*	2.45 ± 0.13*	2.58 ± 0.12*	2.65 ± 0.14*
	White muscles	1.09 ± 0.04	1.36 ± 0.03*	1.45 ± 0.07*	1.51 ± 0.05*	1.66 ± 0.06*
Spring	Liver	1.67 ± 0.052	2.01 ± 0.08*	2.12 ± 0.12*	2.21 ± 0.14*	2.28 ± 0.14*
	White muscles	1.06 ± 0.05	1.22 ± 0.04*	1.30 ± 0.07*	1.39 ± 0.03*	1.44 ± 0.06*
Summer	Liver	1.61 ± 0.12	1.8 ± 0.10*	1.92 ± 0.08*	1.96 ± 0.17*	2.1 ± 0.12*
	White muscles	0.99 ± 0.03	1.14 ± 0.07*	1.16 ± 0.04*	1.19 ± 0.01*	1.22 ± 0.09*

The sampling sites are: 1 Firon fish farm (reference site), 2 Binufar, 3 Industrial zone, 4 Railway bridge and 5 between the bridges.

Each reading represents Mean ± SD of 10 fish.

The significant of difference was checked by one-way ANOVA and Dunnett test (compare all vs. control) using a computer program (GraphPad InState Software, Inc).

The difference checked by one-way ANOVA was significant at $P \leq 0.05$ and Dunnett test was significant at $^*P \leq 0.05$.

Lipid peroxidation (LPO):

The LPO concentration in liver and white muscles of fish increased significantly ($P \leq 0.05$) at different localities during the four seasons in comparison with their respective controls (Table 7). The increase in autumn was 2.1, 2.4, 2.9, and 3.3 -fold in liver and 1.6, 1.7, 2.0, and 2.2 -fold in white muscles at each sampling sites, respectively. The increase in winter was 1.9, 2.2, 2.3, and 2.6 -fold in liver and 48.8, 56.3, 66.2, 77.7% in white muscles at each sampling sites, respectively. The increase in spring was 45.0, 58.4, 67.3, and 76.4% in liver and 43.0, 56.2, 60.0, and 65.7% in white muscles at each sampling sites, respectively. The increase in summer was 25.8, 32.2, 37.1, and 49.8% in liver and 34.0, 36.6, 43.0, and 48.7% in white muscles at each sampling sites, respectively. The enzyme activity increased from lower values through summer to a maximum in the autumn in comparison with the other seasons.

DISCUSSION:

Rosetta Branch receives considerable amounts of pollutants from agricultural areas, sewages and industrial wastes at Kafr El-Zayat city on the banks of the branch (e.g. Soda, El-Malia and Kafr El-Zayat pesticides production) directly pour their effluents into the branch (Sohair and Aly, 1993; El Bouraie *et al.*, 2010). The impact of these toxic compounds on aquatic organisms can assessed through studying the antioxidant enzyme activities and some oxidative stress biomarkers in fish that respond to the degree and type of contamination.

The physicochemical parameters are considered as the most important principles in the identification of the nature, quality and type of the water for any aquatic ecosystem (Abdo, 2005). The increase in the activity of industry, agriculture, urbanization, tourism, and human activities contribute to these negative effects in the environment and aquatic ecosystems (Osman and Kloas, 2010). One of the most common types of freshwater pollutant is biodegradable organic material. When a high concentration of organic material such as raw sewage (human excreta) is discharged into a stream, the levels of dissolved oxygen in the water may fall so low that the water is completely deoxygenated. It is therefore necessary to know the standard quality required for each particular use in order to determine the degree of pollution control necessary and to forecast the probable effects of increased or new discharges of effluents. It understood that permissible standards limits for fresh water had been well defining (WHO, 1998; USEPA, 2009; CFQGCI., 2011).

The pH values of the samples markedly increased during autumn and winter season compared to other seasons and ranged from 7.2 to 8.0. The pH levels were within permissible standards limits of between 6.5 and 7.8 appears to provide protection for the life of fresh water fish. The pH values that depart increasingly from the normally found levels will have a more and more marked effect on fish, leading ultimately to mortality. The pH value is considering an important factor in the chemical and biological system of aquatic environment (Osman and Kloas, 2010). The relatively highest pH of water can be attributed to the large amounts of different pollution sources discharged. The pH has profound effects on water quality affecting the ability of bacteria which require slightly acidic pH to degrade toxic substances to less harmful forms (Adeogun, 2012).

Turbidity values of water sample found ranged from 4.0 to 33.1 NTU and the turbidity permissible standards limits less than 10 NTU. The maximum value was 33.1 ± 2.0 NTU followed by 26.4 ± 1.7 NTU that found at site 5 during autumn and winter respectively. The values increased during summer and autumn in comparison with the other seasons. The increase of turbidity (low transparency) may be due to the disposal of domestic and industrial effluent. Turbidity in water arises from the presence of very finely divided solids that are not filterable by routine methods. As turbidity can be caused by sewage matter in water there is a risk that pathogenic organisms could be shielded by the turbidity particles and hence escape the action of the disinfectant (Kumar and Puri, 2012).

TDS values of water sample found in ranged from 112 to 430 ppm the values are low as compared with the permissible standards limits. The maximum value of TDS 430 ± 12.7 followed by 298 ± 20.4 that found at site 5 during autumn and winter respectively. The values increased from the lower values than the standard maximum permissible limit during summer to a maximum in the autumn in comparison with the other seasons. Therefore, the palatability of water with TDS can be considered to be good. The lower values of suspended and dissolved solids are indicative of non-industrial pollution (Aggarwal and Arora, 2012).

Ammonia levels of water sample found in ranged from 0.034 to 0.73 mg/l. The maximum value was 0.73 ± 0.04 mg/l followed by 0.47 ± 0.02 mg/l that found at site 5 during autumn and winter respectively. These values are higher than permissible standards limits. The values increased from the lower values than the standard maximum permissible limit during summer to a maximum in the autumn in comparison with the other seasons. Ammonia is generally present in natural waters, though in very small amounts, because of microbiological activity that causes the reduction of nitrogen-containing compounds. The increase of ammonia may be due sewage and industrial contamination may indicated.

Nitrate and Nitrite are naturally occurring ions that are part of nitrogen cycle. Nitrite levels of water sample found in ranged from 0.025 to 0.74 mg/l. The maximum value was 0.74 ± 0.05 mg/l followed by 0.33 ± 0.008 mg/l that found at site 5 during autumn and winter, respectively. These values are higher than permissible standards limits. The values increased from the lower values than the standard maximum permissible limit during summer to a maximum in the autumn in comparison with the other seasons. Nitrite exists normally in very low concentrations and even in waste treatment plant effluents levels are relatively low, principally because the nitrogen will tend to exist in the more reduced (ammonia; NH_3) or more oxidized (nitrate; NO_3) forms. The higher level of nitrite may be indicating sewage pollution (Sakai *et al.,* 2013).

Nitrate levels of water sample found in ranged from 8.2 ± 0.6 to 75.1 ± 4.8 ppm. The maximum value was 75.1 ± 4.8 ppm, followed by 37.3 ± 3.3 ppm that found at site 5 during autumn and winter, respectively. These values are higher than permissible standards limits. The values increased from the lower values than the standard maximum permissible limit during summer to a maximum in the autumn in comparison with the other seasons. The higher level of nitrate may be due to excessive use of agriculture fertilizers, decayed vegetable water, domestic effluent, sewage disposal industrial discharges (Johnson and Kross, 1990).

The determination of the extent and severity of water contamination by pollutants is often difficult. Physicochemical analysis gives useful information on the levels of contamination, but alone may not sufficient to describe the adverse effects of the complex mixtures of chemicals present at contaminated sites. Bio-monitoring of aquatic environments by means of antioxidant enzymes activities and some oxidative stress biomarkers assessed as a useful tools for the early warning indicators to water pollutants exposure.

In many bio-monitoring studies, the liver is the main target organ for investigation because of its fast answer to environmental influences, high metabolic activity, and essential function in the organism. Although white muscles has a lower metabolic rate, its importance for investigation is of great significance to humans because of its nutritional importance, especially in the case of commercially important fish species such as Nile tilapia. The liver found to be stronger into the face of oxidative stress than the other tissues and a uniform organ with the highest activity (Zikić *et al*., 1996; Schlenk and Benson, 2001). Antioxidant defense system mechanisms have an important role for aquatic organisms because these mechanisms protect them from free radicals that caused oxidative stress and other factors.

The maximum activity of SOD and CAT in liver and white muscles of Nile tilapia was found at site 5 during autumn and winter respectively. The enzymes activities increased from lower values through summer to a maximum in the autumn in comparison with the other seasons. This increase may be due to its location so it receives annual contamination from El Dalgamon municipal effluents. Rosetta branch in autumn receives high concentrations of organic compounds, nutrients and oil and grease from Rahawy drain (which receives part of Greater Cairo wastewater). Furthermore, there are several industrial outfalls: El-Mobidat, El-Malyia and Salt, Soda companies, and Mashroa Eldalgamon for sewage and wastewater, which are discharging directly at the east bank of the branch. Consequently, the presence of the different sources of water pollutants, stimulate SOD to defend the body against the damage caused by excessive amount of oxygen free radicals. In addition, SOD showed more responses compared to the other enzymes, which was also in accordance with previous studies (Pandey *et al*., 2003; Atli and Canli, 2007). Trenzado *et al*. (2006) mentioned that CAT activity, found mainly in peroxisomes, is associated with elevated concentrations of H_2O_2. They detected higher activity of this enzyme in the liver and white muscles.

Hegazi *et al*. (2010) also found that chronic ammonia exposure significantly increased SOD activity. The increase was more predominant in high than low TAN exposure. Sampaio *et al*. (2008) also indicated the influence of acute Cu exposure in antioxidant enzyme protection based on increased ROS levels in the liver accompanied by enhanced SOD. The SOD-CAT system, the first line of defense system against oxidants, varied based on the response of fish antioxidant system to cope with the different environmental pollutants induced oxidative stress. SOD catalyzes the dismutation of the superoxide anion radical to water and hydrogen peroxide, which detoxified by the CAT activity (Zhang *et al*., 2007). Usually a simultaneous induction response in the activities of SOD and CAT observed when exposed to pollutants (Dimitrova *et al*., 1994; Zikić *et al*., 2001). Changes in enzyme activities can be used as biomarkers for contamination in different aquatic organisms (Di Giulio *et al*., 1995; Regoli and Principato, 1995).

The maximum activity of GST, GR, and GPx in liver and white muscles of Nile tilapia was found at site 5 during autumn and winter respectively. The enzymes activities increased from lower values through summer to a maximum in the autumn in comparison with the other seasons. The increase in this region suggests that GST, GR, and GPx activities could induced to resist the water pollutants toxicity such as sewage, agricultural and industrial. Such results are in agreement with previous studies (Matos *et al*., 2007; Gad, 2009; Carvalho *et al*., 2012).

The GSH was probably the most abundant cellular thiole, that was detected virtually in all tissues but, its concentration, in general is higher in liver (as a major organ for antioxidant defense in fish) (Otto *et al*., 1999). Apparently, GSH is important in protecting against deleterious effects of the cell exposed to ROS by reacting with them to form GSSG. This antioxidant effect occurs spontaneously through GSH or may also catalyzed by GST (El-Wakf, 1998).

Glutathione-S-transferase is an enzyme that important in the detoxication of many

different xenobiotics in mammals. The enzyme protects cells against toxicants by conjugating the thiol group of the glutathione to electrophilic xenobiotics, and thereby defends cells against the mutagenic, carcinogenic, and toxic effects of the compounds. Glutathione-S-transferase activity found to be present in plants, insects, yeast, bacteria, and in most mammalian tissues, especially in the liver, which plays a key role in detoxification (Mannervik and Danielson, 1988; Wilce and Parker, 1994). glutathione-S-transferase enzymes generate less toxic and more hydrophilic molecules (Olsen *et al*., 2001) and play a role preventing oxidative damage by conjugating breakdown products of lipid peroxides to GSH (Barata *et al*., 2005; Fernandes *et al*., 2008). This metabolic pathway allows the protection of nucleophilic groups in macromolecules such as proteins and nucleic acids. Other functions, not associated with detoxification, include repair of macromolecules oxidized by ROS, regeneration of S-thiolated proteins, and biosynthesis of physiologically important metabolites (Freitas *et al*., 2007), indicate the presence of various xenobiotics like polycyclic aromatic hydrocarbons (PAHs) (Ahmad *et al*., 2005), mercury chloride (Monteiro *et al*., 2009) and pesticides (Printes *et al*., 2011). The present results indicated that these enzymes could be crucial and show peroxidase activity towards ROS in the cells under oxidative stress in the Nile tilapia related to a great input of pollutants into the Nile River. These results show the vulnerability of these organs and, the Nile tilapia were undergoing oxidative stress pollutant-induced industrial.

Glutathione reductase (GR) catalyzes the reduction of GSSG to GSH. This enzyme, which found in many tissues, enables the cell to sustain adequate levels of cellular GSH. Reduced glutathione is a substrate for the glutathione peroxidases, which provide a mechanism for the detoxification of peroxides, and GST, which are involved in the conjugation and elimination of xenobiotics from the organism (García - Alfonso *et al*., 1993). Reduced glutathione also acts as an antioxidant, reacting with free radicals and organic peroxides.

Glutathione peroxidase (GPx) is another propagation inhibitor in the aqueous phase of fish muscles that is located in the mitochondria and cytosol of skeletal muscles cells. Glutathione peroxidase catalyzes the reduction of hydrogen of lipid peroxides with GSH. GSSG then reduced back to GSH by GR at the expense of NADPH. Glutathione reductase (GR) differs from catalase in that it is capable of reacting with both lipid and hydrogen peroxides (Halliwell and Gutteridge, 1989). GPx provides a mechanism for detoxification of peroxides in living cells (Scholz *et al*., 1981). This reaction plays a crucial role in protecting cells from damage by free radicals, which formed by peroxide decomposition. Lipid components of the cell are especially susceptible to reactions with free radicals, resulting in lipid peroxidation. The GPx enzymes reduce peroxides to alcohols using glutathione, thus preventing the formation of free radicals.

Biomarkers of oxidative stress directly connected with changes of ROS concentration in organism (Barker *et al*., 1994; Ahmad *et al*., 2004). When antioxidant defenses are impaired or overcome, oxidative stress may produce lipid peroxidation (LPO), protein carbonyl formation (PCO), and enzymatic inactivation (Halliwell and Chirico, 1993; Shacter, 2000).

The LPO is the degradation of lipids that occurs because of oxidative damage and is a useful marker for oxidative stress. Polyunsaturated lipids are susceptible to an oxidative attack, typically by reactive oxygen species, resulting in a well-defined chain reaction with the production of end products such as MDA. The LPO may contribute to the pathology of many diseases including atherosclerosis, diabetes, and Alzheimer.

The maximum levels of LPO in liver and white muscles of Nile tilapia was found at site 5 during autumn and winter respectively. The enzymes levels increased from lower values through summer to a maximum in the autumn in comparison with the other seasons. This increase may be attributed to the large amounts of different pollution sources discharged. The degree of LPO was sensitive to seasonal difference and metabolic products including wastes and toxicants in the environment. LPO in fish, measured as TBARS. It has frequently used as a marker of oxidative stress in response to different water pollutants in a number of studies (Gabrzelak and Klekot, 1985; Ando and Yanagida, 1999; Choi and Oris, 2000; de Lafontaine *et al*., 2000; Oakes and Van Der Kraak, 2003; Roméo *et al*., 2000; Almroth *et al*., 2005).

This situation may be associated with an increased influx of free radicals. Fish become more sensitive to diseases and lose adaptation capabilities to different water conditions (Ozmen *et al*., 2004). The LPO is a well-established mechanism of cellular injury in animals, and used as an indicator of oxidative stress in cells and tissues. Malondialdehyde (MDA) is widely used as an indicator of lipid peroxidation (Esterbauer *et al*., 1991). Increased levels of lipid peroxidation products have been associated with a variety of chronic diseases in humans, other vertebrates and model systems (Altura and Altura, 1985; Allen-Gil and Martynov, 1995). The MDA reacts readily with amino groups on proteins and other biomolecules to form a variety of adducts (Adeyeye *et al*., 1996), including cross-linked products (Arabi

and Alaeddini, 2005). The MDA also forms adducts with DNA bases that are mutagenic (Becker *et al*., 2008) and possibly carcinogenic (Canli and Atli, 2003). In another experiment, the authors have shown that the exposure to TAN causes the plasma level of ammonia in the Nile tilapia plasma to be 2-fold higher than the surrounding habitat level (Hegazi and Hasanein, 2010). Despite these parameters used as biomarkers for contaminants, since the fish response depends on several variables such as the species, tissue, the antioxidant parameter itself, time of exposure, and contaminant concentration, besides the other physiological and environmental changes.

CONCLUSION:

The continuous and annual discharge of different pollutants into Nile River can caused physiological changes in liver and white muscles of Nile tilapia. The Nile water at the area of study is therefore of prime health concern because there is no known treatment system for it, and the contaminants possibly transferred from polluted drain waters by fish to the consumers. Alteration in the activities of examined enzymes can provide a useful biomarker for environmental managers in investigating the exposure of fish to contaminated waters. Furthermore, to protect the fish from pollution and reduce environmental risk, it could recommended that treatment of the agricultural, industrial and sewage effluents should be carried out before their discharge to the Nile River. Regular evaluation of pollutants in the Nile River is also very important. This study could be beneficial in eco-toxicological researches in freshwaters as it provides data about antioxidant system response of fish exposed to different water pollutants. Nevertheless, further researches are necessary to shed more light on characterization of biological responses, such as antioxidant enzymes as sensitive biomarkers in fish.

REFERENCES:

Abdo MH. 2005. Physico-chemical characteristics of Abu Za'baal Ponds. Egypt. Egypt. J. Aquat. Res., 31(2): 1–15.

Adeogun AO. 2012. Impact of industrial effluent on water quality and gill pathology of *Clarias gariepinus* from Alaro stream, Ibadan, Southwestern Nigeria. Eur. J. Sci. Res., 76(1): 83-94.

Adeyeye EI, Akinyugha NJ, Fesobi ME, Tenabe VO. 1996. Determination of some metals in *Clarias gariepinus* (*Cuvier and Vallenciennes*), *Cyprinus carpio* and *Oreochromis niloticus* fishes in a polyculture fresh water pond and their environments. Aquacultulture, 147(3): 205-214.

Aggarwal R, Arora S. 2012. A study of water quality of Kaushalya River in the submountaneous Shivalik region. Int. J. Sci. Technol. Res., 1(8): 52-68.

Ahmad I, Oliveira M, Pacheco M, Santos MA. 2005. *Anguilla anguilla* L. Oxidative stress biomarkers responses to copper exposure with or without bnaphthoflavone pre-exposure. Chemosphere, 61(2): 267–275.

Ahmad I, Pacheco M, Santos MA. 2004. Enzymatic and nonenzymatic antioxidants as an adaptation to phagocyte-induced damage in *Anguilla anguilla* L. following in situ harbor water exposure. Ecotoxicol. Environ. Saf., 57(3): 290–302.

Allen-Gil SM, Martynov VG. 1995. Heavy metal burdens in nine species of freshwater and anadromous fish from the Pechora River, northern Russia. Sci. Total Environ., 160-161: 653-659.

Almroth BC, Sturve J, Berglund A, Förlin L. 2005. Oxidative damage in eelpout (*Zoarces viviparus*), measured as protein carbonyls and TBARS, as biomarkers. Aquat. Toxicol., 73(2): 171–180.

Altura BM, Altura BT. 1985. New perspectives on the role of magnesium in the pathophysiology of the cardiovascular system. I. Clin. Aspects. Magnesium, 4(5-6): 226-244.

Ando S, Yanagida K. 1999. Susceptibility to oxidation of copper-induced plasma lipoproteins from Japanese eel: protective effect of vitellogenin on the oxidation of very low density lipoprotein. Comp. Biochem. Phys. C., 123(1): 1-7.

Anwar WA. 2003. Environmental health in Egypt. Int. J. Hyg. Environ. Health, 206(4-5): 339-350, Review.

APHA. 1998. Standard method for examination of water and wastewater 20th Edition. Amer. Publ. Health Assoc., Washington, D.C., USA, pp. 541.

Arabi M, Alaeddini MA. 2005. Metal-ion-mediated oxidative stress in the gill homogenate of rainbow trout (*Onchorhynchus mykiss*): antioxidant potential of manganese, selenium, and albumin. Biol. Trace Elem. Res., 108(1-3): 155-168.

Atli G, Canli M. 2007. Enzymatic responses to metal exposures in a freshwater fish *Oreochromis niloticus*. Comp. Biochem. Phys. C., Toxicol. Pharmacol., 145(2): 282–287.

Authman MMN, Abbas HH, Abbas WT. 2013. Assessment of metal status in drainage canal water and their bioaccumulation in *Oreochromis niloticus* fish in relation to human health. Environ. Monit. Assess., 185(1): 891-907.

Azimi S, Moghaddam MS. 2013. Effect of Mercury Pollution on the Urban Environment and Human Health. Environ. Ecol. Res., 1(1): 12-20.

Barata C, Varo I, Navarro JC, Arun S, Porte C. 2005. Antioxidant enzyme activities and lipid peroxidation in the freshwater cladoceran *Daphnia magna* exposed to redox cycling

compounds. Comp. Biochem. Phys. C., 140(2): 175–186.

Barker CW, Fagan JB, Pasco DS.1994. Down regulation of P4501A1 and P4501A2 mRNA expression in isolated hepatocytes by oxidative stress. J. Biol. Chem., 269(6): 3985–3990.

Becker JS, Füllner K, Seeling UD, Fornalczyk G, Kuhn AJ. 2008. Measuring magnesium, calcium and potassium isotope ratios using ICP-QMS with an octopole collision cell in tracer studies of nutrient uptake and translocation in plants. Anal. Bioanal. Chem., 390(2): 571-578.

Buege JA, Aust SD. 1978. Microsomal lipid peroxidation. Methods Enzymol. 52: 302–310.

Canli M, Atli G. 2003. The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species. Environ. Pollut., 121(1): 129-136.

Carvalho CS, Bernusso VA, de Araújo HS, Gaeta Espíndola EL, Fernandes MN. 2012. Biomarker responses as indication of contaminant effects in *Oreochromis niloticus*. Chemosphere, 89(1): 60–69.

CFQGCI. 2011. Canada's Freshwater Quality in a Global Context Indicator, 2011. Data Sources and Methods, ISBN: 978-1-100-17978-0.

Choi J, Oris JT. 2000. Evidence of oxidative stress in bluegill sunfish (*Lepomis macrochirus*) liver microsomes simultaneously exposed to solar ultraviolet radiation and anthracene. Environ. Toxicol. Chem., 19(7): 1795–1799.

de Lafontaine Y, Gagné F, Blaise C, Costan G, Gagnon P, Chan HM. 2000. Biomarkers in zebra mussels (*Dreissena polymorpha*) for the assessment and monitoring of water quality of the St Lawrence River (Canada). Aquat. Toxicol., 50(1-2): 51–71.

Di Giulio RT, Benson WH, Sanders BM, Van Veld PA. 1995. Biochemical mechanisms: metabolism, adaptation and toxicity. In: "Fundamentals of aquatic toxicology: effects, environmental fate, and risk assessment, (Rand GM. Ed.)", 2nd Edition. Taylor and Francis, Washington, D.C., pp. 523-561.

Dimitrova MST, Tsinova V, Velcheva V. 1994. Combined effect of zinc and lead on the hepatic superoxide dismutase-catalase system in carp (*Cyprinus carpio*). Comp. Biochem. Phys. C., 108(1): 43–46.

El Bouraie MM, El Barbary AA, Yehia MM, Motawea EA. 2010. Heavy metal concentrations in surface river water and bed sediments at Nile Delta in Egypt. Suoseura, 61(1): 1–12, Research notes.

El-Wakf AM. 1998. Modulation of bromobenzene induced hepatotoxicity in rate by post toxicant treatment with glutathione. J. Egypt. German. Soc. Zool. (Comp. Physiol.), 27: 99-111.

EPH. 2010. Environmental Pollution and Impacts on Public Health. Report Summary, Implications of the Dandora Municipal Dumping Site in Nairobi, Kenya, Based on a study by Njoroge G. Kimani in cooperation with United Nations Environment Program and the St. John Catholic Church, Korogocho.

Esterbauer H, Schaur RJ, Zollner H. 1991. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radical Biol. Med., 11(1): 81-128.

Fernandes C, Fontainhas-Fernandes A, Ferreira M, Salgado MA. 2008. Oxidative stress response in gill and liver of *Liza saliens*, from the Esmoriz–Paramos coastal lagoon, Portugal. Arch. Environ. Contam. Toxicol., 55(2): 262–269.

Freitas DR, Rosa RM, Moraes J, Campos E, Logullo C, Da Silva Vaz I Jr, Masuda A. 2007. Relationship between glutathione S-transferase, catalase, oxygen consumption, lipid peroxidation and oxidative stress in eggs and larvae of *Boophilus microplus* (Acarina: Ixodidae). Comp. Biochem. Physiol. A., 146(4): 688–694.

Gabrzelak T, Klekot J. 1985. The effect of paraquat on the peroxide metabolism enzymes in erythrocytes of freshwater fish species. Comp. Biochem. Physiol. C., 81(2): 415–418.

Gad NS. 2009. Determination of glutathione related enzymes and cholinesterase activities in *Oreochromis niloticus* and *Clarias gariepinus* as bioindicator for pollution in Lake Manzala. Global Vet., 3(1): 37-44.

García-Alfonso C, Martínez-Galisteo E, Llobell A, Bárcena JA, López-Barea J. 1993. Horse-Liver glutathione reductase: purification and characterization. Int. J. Biochem., 25(1): 61-68.

Garg S, Gupta RK, Jain KL. 2009. Sublethal effects of heavy metals on biochemical composition and their recovery in Indian major carps. J. Hazard. Mater., 163(2-3): 1369-1384.

Habig WH, Pabst MJ, Jakoby WB. 1974. Glutathione-S-transferase. The first enzymatic step in mercapturic acid formation. J. Biol. Chem., 249(22): 7130-7139.

Halliwell B, Gutteridge JMC. 1989. Free radicals in biology and medicine. 2nd ed. Clarendon Press, Oxford, pp. 543.

Halliwell B, Chirico S. 1993. Lipid peroxidation: Its mechanism, measurement, and significance. Am. J. Clin. Nutr., 57(5 Suppl): 715S-724S.

Hegazi MM, Attia ZI, Ashour OA. 2010. Oxidative stress and antioxidant enzymes in liver and white muscle of Nile tilapia juveniles in chronic ammonia exposure. Aquat. Toxicol., 99(2): 118-125.

Hegazi MM, Hasanein SS. 2010. Effects of chronic exposure to ammonia concentrations on brain monoamines and ATPases of Nile tilapia (*Oreochromis niloticus*). Comp Biochem Phys. C., 151(4): 420-425.

Johnson CJ, Kross BC. 1990. Continuing importance of nitrate contamination of groundwater and wells in rural areas. Am. J. Ind. Med., 18(4): 449-456.

Kumar M, Puri A. 2012. A review of permissible limits of drinking water. Indian J. Occup. Environ. Med., 16(1): 40–44.

Mannervik B, Danielson UH. 1988. Glutathione transferases - structure and catalytic activity. CRC Crit. Rev. Biochem., 23(3) 283-337.

Matos P, Fonta´ınhas-Fernandes A, Peixoto F, Carrola J, Rocha E. 2007. Biochemical and histological hepatic changes of Nile tilapia *Oreochromis niloticus* exposed to carbaryl. Pestic. Biochem. Phys., 89: 73–80.

Monteiro SM, Rocha E, Mancera JM, Fontaínhas-Fernandes A, Sousa M. 2009. A stereological study of copper toxicity in gills of *Oreochromis niloticus*. Ecotoxicol. Environ. Saf., 72(1): 213–223.

NWRC. 2000. National Water Research Centre: WL/DELFT Hydraulics. National water resources plan for Egypt, Water Quality and Pollution Control. Techical Report No. 5.

Oakes KD, Van Der Kraak GJ. 2003. Utility of the TBARS assay in detecting oxidative stress in white sucker (*Catostomus commersoni*) populations exposed to pulp mill effluent. Aquat. Toxicol., 63(4): 447–463.

Olsen T, Ellerbeck L, Fisher T, Callaghan A, Crane M. 2001. Variability in acetylcholisnesterase and glutathione S-transferase activities in *Chironomus riparus* Meigen deployed in situ at uncontaminated field sites. Environ. Toxicol. Chem., 20(8): 1725–1732.

Osman AGM, Kloas W. 2010. Water Quality and Heavy Metal Monitoring in Water, Sediments and Tissues of the African Catfish, *Clarias gariepinus* (Burchell, 1822) from the River Nile, Egypt. J. Environ. Protect., 1(4): 389-400.

Otto, D.M., Sen, C.K, Casley, W.L. and Moon, T.W., 1999. Regulation cytochrome P501 a metabolism by glutathione. Pharmacol. Toxicol., 48: 201–210.

Ozmen I, Bayir A, Cengiz M, Sirkecioglu AN, Atamanalp M. 2004. Effects of water reuse system on antioxidant enzymes of rainbow trout (*Oncorhynchus mykiss* W., 1792). Vet. Med.-Czech, 49(10): 373.

Paglia DE, Valentine WN. 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med., 70(1): 158–169.

Pandey S, Parvez S, Sayeed I, Haque R, Bin-Hafeez B, Raisuddin S. 2003. Biomarkers of oxidative stress: a comparative study of river Yamuna fish Wallago attu (Bl. & Schn.). Sci. Total Environ., 309(1-3): 105-115.

Paoletti F, Mocali A. 1990. Determination of superoxide dismutase activity by purelychemical system based on NADPH oxidation. Methods Enzymol., 186: 209–220.

Printes LB, Fernandes MN, Espíndola EL. 2011. Laboratory measurements of biomarkers and individual performances in *Chironomus xanthus* to evaluate pesticide contamination of sediments in a river of southeastern Brazil. Ecotoxicol. Environ. Saf., 74(3): 424–430.

Regoli F, Principato G. 1995. Glutathione, glutathione-dependent and antioxidant enzymes in mussels, *Mytilus galloprovincialis*, exposed to metals under field and laboratory conditions: implications for the use of biochemical biomarkers. Aquat. Toxicol., 31: 143–164.

Roméo M, Bennani N, Gnassia-Barelli M, Lafaurie M, Girard JP. 2000. Cadmium and copper display different responses towards oxidative stress in the kidney of the sea bass *Dicentrarchus labrax*. Aquat. Toxicol. 48(2-3): 185–194.

Sakai H, Kataoka Y, Fukushi K. 2013. Quality of Source Water and Drinking Water in Urban Areas of Myanmar. Sci. World J., 2013: 854261.

Sampaio FG, de Lima CB, Oba ET, Santos LRB, Kalinin AL, Rantin FT. 2008. Antioxidant defenses and biochemical changes in pacu (*Piaractus mesopotamicus*) in response to single and combined copper and hypoxia exposure. Comp. Biochem. Phys. C., 147(1): 43–51.

Schlenk D, Benson WH. 2001. Target Organ Toxicity in Marine and Freshwater Teleosts. Taylor and Francis, London, UK.

Scholz RW, Cook LS, Todhunter DA. 1981. Distribution of selenium-dependent and nonselenium-dependent glutathione peroxidase activity in tissues of young cattle. Am. J. Vet. Res., 42(10): 1724-1728.

Shacter E. 2000. Quantification and significance of protein oxidation in biological samples. Drug Metab. Rev., 32(3–4): 307–326.

Smith IK, Vierheller TL, Thorne CA. 1988. Assay of glutathione reductase in crude tissue homogenates using 5,5'-dithiobis(2-nitrobenzoic acid). Anal. Biochem., 175(2): 408-413.

Sohair IAE, Aly NE. 1993. Pollution in the River Nile; Assessment and control. A case of study - Nile 2002 Conference, Aswan, Egypt. 2: 1–15.

Tayel SI, Yacoub AM, Mahmoud SA. 2008. Histopathological and haematological responses to freshwater pollution in catfish *Clarias gariepinus*. J. Egypt. Acad. soc. Environ. Dev., 9: 43-60.

Trenzado C, Hidalgo MC, García-Gallego M, Morales AE, Furné M, Domezain A, Domezain J, Sanz A. 2006. Antioxidant enzymes and lipid peroxidation in sturgeon Acipenser naccarii and trout *Oncorhynchus mykiss*. A comparative study. Aquaculture, 254: 758–767.

USEPA. 2009. National Recommended Water Quality Criteria. Office of Water, Office of Science and Technology 4304T, pp. 21.

WHO. 1998. Guidelines for drinking water. Health criteria and other supporting information. WHO, Geneva.

Wilce MCJ, Parker MW. 1994. Structure and function of Glutathione S-Transferases. Biochem. Biophys. Acta, 1205(1): 1-18.

Xu JB, Yuan XF, Lang PZ. 1997. Determination of catalase activity and catalase inhibition by ultraviolet spectrophotometry. Chin. Environ. Chem., 16: 73-76.

Zhang KF, Zhang ZP, Chen Y, Lin P, Wang YL. 2007. Antioxidant defense system in animals. Chin. J. Zool., 42: 153–160.

Zikić RV, Stajn A, Saicic ZS, Spasic MB, Ziemnicki K, Petrovic VM. 1996. The activities of superoxide dismutase, catalase and ascorbic acid content in the liver of goldfish (*Carassius auratus gibelio* Bloch.) exposed to cadmium. Physiol. Res., 45(6): 479–481.

Zikić RV, Stajn AS, Pavlović SZ, Ognjanović BI, Saićić ZS. 2001. Activities of superoxide dismutase and catalase in erythrocytes and plasma transaminases of goldfish (*Carassius auratus gibelio Bloch*.) exposed to cadmium. Physiol. Res., 50(1): 105–111.

دراسة الاجهاد التأكسدي في الكبد والعضلات البيضاء لسمكة البلطي النيلي كمؤشر على تلوث بعض المناطق في نهر النيل

محمد عبد المنعم حجازي*، سمية زكي عبد الحلي*، محمود محمد سفيان سرداح، صالح نظمي موافي****

*قسم علم الحيوان، كلية العلوم، جامعة طنطا، طنطا، جمهورية مصر العربية

**قسم الأحياء، كلية العلوم، جامعة الازهر- غزة، قطاع غزة، فلسطين

دراسة الاجهاد التأكسدي في الكبد والعضلات البيضاء لسمكة البلطي النيلي التي تم تجميعها من فرع رشيد، مدينة كفر الزيات، محافظة الغربية، مصر. حيث تم دراسة تأثير الملوثات المائية في هذه المنطقة والتي تتعرض لصرف كميات كبيرة من مياه الصرف الصحي والملوثات الصناعية والزراعية على نظام مضاد الأكسدة في أسماك المياه العذبة البلطى النيلي (62.43 ± 6.86 جم). تم دارسة بعض الخصائص الفيزيوكيميائية لعينات المياه مثل الرقم الهيدروجيني، التوصيل الالكترونى، العكارة، المواد الصلبة الذائبة، العسر الكلي للماء، القلوية، الأمونيا والنتريت والنترات. كما تم قياس أنشطة الإنزيمات المضادة للأكسدة سوبراكسيد دسميوتيز، كاتاليز، جلوتاثيون بيروككسيديز، جلوتاثيون-اس-ترانسفيريز، جلوتاثيون ريدكتيز والليبيد بيروكسيد في الكبد والعضلات البيضاء. أظهرت النتائج ان الخصائص الفيزيوكيميائية زادت عن الحدود القياسية العالمية للمياه خلال فصلي الخريف والشتاء مقارنة بفصلي الصيف والربيع. حيث ان تلك الزيادة كانت من المستوى الأقل في فصل الصيف الى الاعلى في الخريف مقارنة بباقي الفصول. كما ان التغير في الخصائص الفيزيوكيميائية جاء متزامنا مع التغير في نشاط الانزيمات سوبراكسيد والكاتاليز، دسميوتيز، جلوتاثيون-اس-ترانسفيريز، جلوتاثيون ريدكتيز، جلوتاثيون بيروككسيديز والليبيد بيروكسيد حيث أظهرت النتائج زيادة ذات دلالة إحصائية عند القيمة $P \leq 0.05$. ووجد أن هناك زيادة ملحوظة خلال فصلي الخريف والشتاء مقارنة بفصلي الصيف والربيع. بحيث يزداد نشاط الانزيمات من المستوى الأقل في فصل الصيف الى الاعلى في الخريف مقارنة بباقي الفصول. تعتبر الخصائص الفيزوكيميائية من اهم المبادئ في تحديد طبيعة ونوعية ونوع من الماء و / أو أي نظام مائي. كما تعتبر الإنزيمات المضادة للأكسدة ومؤشر الاجهاد التأكسدي الليبيد بيروكسيد مؤشرات حيوية تستخدم للتعرف على تلوث البيئة المائية للكائنات البحرية ولتقييم المخاطر البيئية المتكاملة. وعليه فان تلك التغيرات تعتبر كدلالات بيولوجية مفيدة لمديري الشؤون البيئية للكشف عن تعرض الأسماك للمياه الملوثة. أخيرا تعتبر هذه الدراسة ذات اهمية كبرى في الأبحاث السمية البيئية في المياه العذبة، حيث أنها توفر بيانات عن استجابة النظام المضاد للأكسدة في الأسماك المعرضة لملوثات المياه المختلفة.

المحكمون:

أ.د. عزه أسماعيل عثمان قسم علم الحيوان، علوم المنصوره

أ.د. زينب إبراهيم عطيه قسم علم الحيوان، علوم طنطا