BioBacta

Journal of Bioscience and Applied Research
www.jbaar.org

Oxidative stress and DNA damage in Nile Tilapia (*Oreochromis niloticus*) as biomarkers of aquatic pollution

EL-Hassan Mokhamer, Eman H. Radwan and Moataz Elsaka

Zoologlogy Department, Faculty of science, Damanhour university,Egypt
(Email: elhassan.gaber@sci.dmu.edu.eg)

Abstract

The main purpose of this study is to evaluate the impact of heavy metals (Pb, Zn, Cu, Fe, and Cd) aquatic pollution of EL-Mahmoudeyia canal on the antioxidant enzymatic activities, GSH content and lipid peroxidation levels (MDA) in *Oreochromis. niloticus* muscles tissues collected from two areas EL-Mahmoudeyia canal as Polluted area and Rosetta branch of river Nile as reference area in summer 2018 and winter 2019 as well as DNA damage was assessed in fish gills(erythrocytes) samples by applying comet assay. EL-Mahmoudeyia canal exposed to excessive of industrial effluents which impact the living organisms especially fish. The herein results showed that higher concentrations of heavy metals (Pb, Zn, Cu, Fe, and Cd) were detected in water and fish samples collected from the polluted area in comparison with the reference area, especially in winter. The accumulation patterns of heavy metals in muscles of *O. niloticus*, were in the following order: Fe > Zn >Pb> Cu and Cd.The antioxidant enzymatic activities of (SOD, CAT, GPx and GST) and the lipid peroxidation biomarker MDA levels in muscles of *O. niloticus* collected from the polluted area were found to be significantly increased compared to that of the reference area. Meanwhile, there was a significant decrease in the GSH content level in muscles of *O. niloticus* collected from the polluted area compared to that of the reference area. A significant elevation in DNA damage frequencies was observed in fish collected from the polluted areas compared with those from the reference area. These noticeable alterations in the selected antioxidant enzymatic activities in muscles of the *O. niloticus* go in parallel with the remarkable elevation in the levels of the detected heavy metals in water from EL Mahmoudeyia canal, as a result of pollution in these areas. This study explored the utility of the DNA damage, the altered antioxidant enzymatic activities, GSH content and MDA level as biomarkers of aquatic pollution.

Keywords : Heavy metals, pollution, oxidative stress, DNA damage, antioxidants enzymes.

Introduction

Pollution levels in aquatic environments have greatly increased recently as a result of intense human activities, which, in some areas have resulted in a substantial impact.(van der Oost et al., 1996).Contamination of the aquatic ecosystem by industrial and agricultural pollutants affects the health of fish, either directly by uptake from the water, or indirectly through their diet of vegetation, invertebrates or smaller fish. Since fish are part of the natural diet of both aquatic mammals and birds, as well as providing an increasingly important protein source for humans, their population and health is of major concern (Kime, 1995).

The pollution problems in the surface water either drainage or fresh water affect the quality of fish in polluted areas. The specific contaminants leading to pollution in water include a wide spectrum of chemicals, pathogens and physical or sensory changes such as elevated temperature and discoloration (Pitt and Burton Jr, 2001).

Heavy metals pollution in aquatic environment has become a worldwide problem during past few decades. This fact is mainly attributed to their persistent stability and toxic effect to aquatic as well as terrestrial creatures(MacFarlane and Burchet, 2000). Among environmental pollutants, metals are of particular concern, due to their potential toxic effect and ability to bioaccumulate in aquatic ecosystems(Censi et al., 2006)Heavy metal concentrations in aquatic ecosystems are monitored by measuring their concentrations in water, sediments and biota. (Namminga, 1976).

Electric power generation also play major role in water pollution. Generally, more than 75% of waste is disposed of in unlined, unmonitored onsite landfills and surface impoundments. Toxic substances in the waste - including arsenic, mercury, chromium, and cadmium can contaminate drinking water supplies and damage vital human organs and the nervous system(Tripathi et al., 2015).The essential use of water in industry is the cooling system (Nriagu and Pacyna, 1988)When water used as a coolant is returned to the natural environment at a higher temperature, the change in temperature impacts organisms by decreasing oxygen supply, and affecting ecosystem composition(El Safty and Siha, 2013).The atmosphere is the major route of Pb entry in natural waters, a fact that has been well documented in the literature(Flegal and Patterson, 1983),(Veron et al., 1987)

Fish are largely being used for the assessment of the aquatic environment quality and can serve as bioindicators of environmental pollution. (Dautremepuits et al., 2004, Lopes et al., 2001). Fish muscle is commonly analyzed to determine contaminant concentrations and to assess the health risks because it is the main part consumed by humans. Fish can be considered as one of the most significant indicators in freshwater systems for the impact of metal pollution (Begum et al., 2005).Tilapia (Oreochromis niloticus) is a fresh water fish that is hardy, prolific, fast growing tropical fish that is farmed mainly in Africa and Asia. Tilapia fish are beneficial to human's beings as they make up a major part of the human diet and provide humans with as much of needed proteins as in meat (Ghorbani and Mirakabad, 2010).

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 (Magdy et al.)

Aquatic ecosystems are not usually able to eliminate heavy metals from waste discharges by their own natural processes. Mercury, cadmium, arsenic, and copper tend to accumulate in bottom sediments, from which they may be released by various processes of remobilisation. They can then, in different forms, move up through biological food chains, eventually to humans in whom they can produce both chronic and acute ailments(Forstner). Metal accumulation causes an increase in highly reactive oxygen species (ROS) such as hydrogen peroxide, superoxide radical, hydroxyl radical which leading to oxidative stress in fish (Dautremepuits et al., 2002).

Oxidative stress is a situation characterized by an imbalance between increased production of oxidant species and/or decreased efficacy of the antioxidant defense system(Gosmaro et al., 2013) leading macromolecule to damage including lipid peroxidation, protein cross linking, DNA damage, changes in growth and function of cells (Ehsaei et al., 2015). Many factors including heavy metals in soil and waste water can affect the DNA genetic material of organisms directly or indirectly and not only to damage the integrity of the DNA structure but also influence its expression and eventually cause genotoxicity to organisms(Yu, 2000).

Comet assay is used as one of the best approaches to study the genotoxic effects of pollutants on fish (Nagarani et al., 2012), as it is used for the estimation of DNA damage to evaluate the genetic risk associated with xenobiotic exposures. The comet assay possesses a number of advantages as compared to other genotoxicity tests. In addition to the capability of this assay to identify DNA damage at the single cell level, other significant advantages include its sensitivity for detecting low levels of DNA damage, the requirement for only small numbers of cells per sample, its ease of application and low cost, and the short time needed to perform the assay. (Tice et al., 2000)

2. MATERIALS AND METHODS

2.1 Study area

EL-Mahmoudeyia Canal, is a 45-mile-long sub-canal from the Nile River which starts at the Nile-port of EL-Mahmoudeyia and goes through Alexandria to the Mediterranean Sea. It was built to supply Alexandria with food and fresh water from the Nile. This study was carried out in EL-Mahmoudeyia province, El-Beheira governorate. Samples were collected from two site areas throughout summer 2018and winter 2019 as shown in Figure 1:

Site 1:

The Rosetta branch of Nile river close to Alatf village which considered as reference area free from the industrial activities and wastes.

Site 2:

EL-Mahmoudeyia canalas polluted area where the EL-Mahmoudeyia electric power station and a textile mill dump directly their effluents to the

stream of the canal. The distance between the two   studied areas is about 2 Km.

Fig. 1: The two sites of the study area at El Mahmodia(El Behaira, Egypt)

2.2 Sample collection

Water and fish (Nile tilapia, Oreochromis niloticus) samples were collected from the previously mentioned areas during the first season (summer, July 2018) and the second season (winter, February2019). Fish samples were caught by fisherman's net, the collected fish were with an average body weight (120 ± 10 gm), and an average body length (16±4 cm). After dissection of fish, muscle tissues were separated for estimation of heavy metals, antioxidant enzymes activity, thiobarbituric acid-reactive substances (MDA)concentration and DNA damage by comet assay. Water samples were collected in clean bottles from a 2-meter depth of the studied areas.

2.3 Chemicals

The assay kits used for biochemical estimation of lipid peroxide (Malondialdehyde, MDA), Reduced glutathione concentration (GSH) , catalase (CAT), glutathione-s transferase (GST), superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities were purchased from Biodiagnostic Co., Egypt. All other chemicals and reagents were of analytical grade and were commercially available from local scientific distributors in Egypt.

2.4. Chemical analysis

The concentration of heavy metals (Pb, Zn, Cu, Fe, and Cd) in the samples were determined after digestion by using Atomic Absorption Spectrophotometer (Perkin Eelmer E. Analyst, 2000, USA).according to the method described by(Vitošević et al., 2007). Results in water were expressed in (mg/L) and in fish muscles in mg/kg dry wt.

2.5. Antioxidants and lipid peroxidation biomarkers

Tissue homogenates were prepared from muscle samples in 10 volumes of 0.1 M Tris-EDTA buffer

(pH7.4), centrifuged at $1,000 \times g$ at 4°C for 30 min. Aliquots of the supernatant were utilized for the following spectrophotometric assessments.

2.5.1. Superoxide Oxide Dismutase activity

(SOD) was determined spectrophotometrically at 560 nm according to the method of Nishikimi et al. (1972). The method based on the ability of SOD enzyme to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium dye. Briefly, 0.05 mL sample was mixed with 1.0 mL buffer (pH 8.5), 0.1 mL nitroblue tetrazolium (NBT) and 0.1 mL NADH. The reaction was initiated by adding 0.01 mL phenazine methosulphate (PMs) and then increased in absorbance was read at 560 nm for 5 min. SOD activity was expressed as U/gm tissue.

2.5.2.The enzymatic activity of Catalase (CAT)

was measured according to the method described by (Aebi, 1984). The CAT reacts with a known quantity of H_2O_2, and the reaction is stopped after 1 min with a CAT inhibitor. In the presence of peroxidase, the remaining H_2O_2 reacts with 3,5-dichloro-2-hydroxybenzene sulfonic acid and 4-aminophenazone to form a chromophore, with a color intensity inversely proportional to the amount of CAT in the sample. The absorbance was measured at 510 nm.

2.5.3. The enzymatic activity of Glutathione peroxidase(GPx)

in tissue was estimated colorimetrically using kits from Bio-diagnostic Company. The assay is an indirect measure of the activity of GPx. The GSSG that is produced upon the reduction of organic peroxide by GPx is recycled to its reduced state by GR. The oxidation of NADPH to NADP+ is accompanied by a decrease in absorbance at 340 nm (A340),

providing a spectrophotometric means for monitoring GPx enzymatic activity. To assay GPx, an aliquot of tissue homogenate was added to a solution containing GSH, GR, and NADPH. The enzyme reaction was initiated by adding the substrate, tert-butyl hydroperoxide, and the A340 was recorded. The rate of the decrease in A340 is directly proportional to the GPx activity in the tested sample(Paglia and Valentine, 1967)

2.5.4.Glutathione-S-Transferase (GST) enzymatic activity

in tissue homogenates were assayed spectrophotometrically according to the method of (Habig et al., 1974). By measuring the conjugation of 1- chloro- 2,4- dinitrobenzene (CDNB) with reduced glutathione. The conjugation is accompanied by an increase in absorbance at 340 nm. The rate of increase is directly proportional to the GST activity in the sample.

2.5.5.The reduced glutathione (GSH)

level was assayed using a method based on the reductive cleavage of 5, 5′-dithiobis (2-nitrobenzoic acid) (DTNB) by a sulfhydryl (-SH) group to yield a yellow color. The reduced chromogen (absorbance measured at 412 nm) is directly proportional to the GSH concentration.(Beutler et al., 1963)

2.5.6. lipid peroxidation (LPO):

lipid peroxidation (LPO) was determined by a colorimetric method of (Kei, 1978) according to the details given in the kit's instructions. A thiobarbituric acid reactive substance (TBARS) was used for the estimation of LPO and expressed in terms of malondialdehyde (MDA) content. For this purpose, 0.5 mL of trichloroacetic acid (10%) solution was added into 0.5 mL sample in test tube. The mixture was centrifuged at 600×g for 10

min and 0.2 mL of the supernatant was transferred into a new test tube containing 1.0 mL of TBA (25 mmol /L) solutions and boiling for 30 min. The solution was then cooled and a pink color chromogen for samples and standard were read at 534 nm using spectrophotometer. The MDA values were expressed as nmol of MDA/gm tissue.

2.6. DNA damage using the comet assay

Isolated gills cells of fish from both areas were subjected to the modified single-cell gel electrophoresis or comet assay(Fairbairn et al., 1995). Before running the comet assay, cell viability for erythrocytes and gill cells was determined using the trypan blue exclusion method. To obtain the cells, a small piece of the gills was washed with an excess of ice-cold Hank's balanced salt solution (HBSS) and minced quickly into approximately 1 mm^3 pieces while immersed in HBSS, with a pair of stainless steel scissors. After several washings with cold phosphate-buffered saline, the minced tissues were dispersed into single cells using a pipette(Lai and Singh, 1995). In brief, the protocol for electrophoresis involved embedding of the isolated cells in agarose gel on microscopic slides and lysing them with detergent at high salt concentrations overnight (in the cold). The cells were treated with alkali for 20 min to denature the DNA and electrophoresis under alkaline conditions (30 min) at 300 mA, 25 V. The slides were stained with ethidium bromide and examined using a fluorescence microscopy (Zeiss, axiostar plus USA)with a green filter at × 40 magnifications. For each experimental condition, about 100 cells per a fish were examined to determine the percentage of cells with DNA damage that

appear like comets. The non-overlapping cells were randomly selected and were visually assigned a score on an arbitrary scale of 0–3 (i.e., class 0 = no detectable DNA damage and no tail; class 1 = tail with a length less than the diameter of the nucleus; class 2 = tail with length between 1× and 2× the nuclear diameter; and class 3 = tail longer than 2× the diameter of the nucleus) based on perceived comet tail length migration and relative proportion of DNA in the nucleus(Collins et al., 1997, Kobayashi, 1995). A total damage score for each slide was derived by multiplying the number of cells assigned to each class of damage by the numeric value of the class and summing up the values. Slides were analyzed by one observer to minimize the scoring variability.

2.7. Statistical analysis:

The results were expressed as Mean±SE and statistical significance was evaluated by one way ANOVA using SPSS (version 20.0) program. Values were considered statistically significant when $p < 0.05$.

3. Results

The mean heavy metals concentrations in water from both reference and polluted area are presented in table (1). The concentration of Pb, Zn, Cu, Fe and Cd in water from the selected areas ranged between (0.009-0.035), (0.178- 0.281), (0.00096-0.0034), (0.233-1.308) and (0.0032-0.0128) mg/L in winter, and ranged between (0.0086-0.28), (0.177-0.272), (0.00086-0.003), (0.235-1.263) and (0.0029-0.0110) mg/L in summer respectively. EL-Mahmoudeyia canal (polluted area) had the highest levels of heavy

metals while, the reference area exhibited the lowest values during period of sampling. The results demonstrated that, Fe had the highest concentration (0.235-1.263 mg/l) among the tested metals, while Cd exhibited the lowest one (0.0029-0.0110 mg/l) during the study period. Also, the concentrations of Pb, Zn, Cu, Fe and Cd in water were elevated in winter compared to summer at two studied areas.

Table (1): The concentration of heavy metals (mg/l) in water samples collected from both reference and polluted area

Heavy Metal	Metal concentration (mg/l)			
	Summer		Winter	
	Reference area	Polluted area	Reference area	Polluted area
Pb	0.0086±0.0005	0.028±0.0009*	0.009±0.0002	0.035±0.001*
Zn	0.177±0.006	0.272±0.005*	0.178±0.006	0.281±0.006*
Cu	0.00086±0.00003	0.003±0.00005*	0.00096±0.00005	0.0034±0.00007*
Fe	0.235±0.003	1.263±0.015*	0.233±0.004	1.308±0.020*
Cd	0.0029±0.0002	0.0110±0.0004*	0.0032±0.0.0001	0.0128±0.0003*

Values are expressed as mean ± S.E
*: Significant difference in comparison with the reference area ($P < 0.05$)

All data of heavy metals (Pb, Zn, Cu, Fe and Cd) accumulations in muscles of *Oreochromis niloticus* collected from the Rosetta River Nile (Reference area) and EL-Mahmoudeyia canal (polluted area) (mg/kg dry wt.) during summer and winterrecorded in table 2.The values of iron concentrations in the muscles of *Oreochromis niloticus* showed a highly significant increase ($p < 0.05$) at polluted area during winter and summer seasons in comparison with non-polluted area.

Lead concentrations in the muscles of *Oreochromis niloticus* samples showed a significant increase ($P < 0.05$) at polluted area during summer and winter in comparison with non-polluted area. Lead concentrations in muscles reached the value of 0.163 ± 0.005 mg/kg dry wt. in summer to 0.809 ± 0.011 mg/kg dry wt. in winter season.

Zinc concentrations in the muscles of *Oreochromis niloticus* samples had a highly significant increase at ($p < 0.05$) in polluted area during summer and winter seasons in comparison with non-polluted area. Zinc concentrations in muscles reached the value of 2.974 ± 0.012 mg/kg dry wt. in summer to 3.530 ± 0.047 mg/kg dry wt. in winter.

Copper concentrations in the muscles of *Oreochromis niloticus* samples had a highly significant increase ($p < 0.05$) at polluted area during summer and winter seasons in comparison with non-polluted area. Copper concentrations in muscles reached the quantity of $0.086.207 \pm 0.0085$ mg/kg dry wt. in winter to 0.080 ± 0.0008 mg/kg dry wt. in summer.

The iron concentrations in muscles reached the value of 3.939 ± 0.038 mg/kg dry wt. in winter to 3.632 ± 0.085 mg/kg dry wt. in summer, the iron

concentrations in muscles of *Oreochromis niloticus* showed highly significant increase (p < 0.05) at the polluted area during the two seasons summer and winter in comparison with non-polluted area.

The cadmium concentrations in muscles reached the value of 0.0255 ± 0.0005 mg/kg dry wt. in

winter to 0.0224 ± 0.0008 mg/kg dry wt. in summer, the cadmium concentrations in muscles of *Oreochromis niloticus* showed a significant increase (p < 0.05) at the polluted area during the two seasons summer and winter in comparison with non-polluted area.

Table (2): The concentration of heavy metals (mg/kg) in muscle samples collected from both reference and polluted area

Heavy Metal	Metal concentration (mg/kg)			
	Summer		Winter	
	Reference area	Polluted area	Reference area	Polluted area
Pb	0.163±0.005	0.691± 0.010*	0.238±0.541	0.809±0.011*
Zn	0.760±0.012	2.974±0.039*	0.860±0.017	3.530±0.047*
Cu	0.0276±0.0009	0.0808±0.0008*	0.0329±0.0014	0.0865±0.0085*
Fe	0.968±0.007	3.632±0.085*	1.144±0.031	3.939±0.038*
Cd	0.0101±0.0006	0.0224±0.0008*	0.0127±0.0004	0.0255±0.0005*

Values are expressed as mean ± S.E ($N = 10$)
*: Significant difference in comparison with the reference area ($P < 0.05$)

Biochemical parameters

The SOD activity in muscles of *Oreochromis niloticus* increased significantly ($P \leq 0.05$) at polluted area during the two seasons in comparison with the reference area (Fig.2). The increase in winter and summer was 27.8% and 25% respectively.
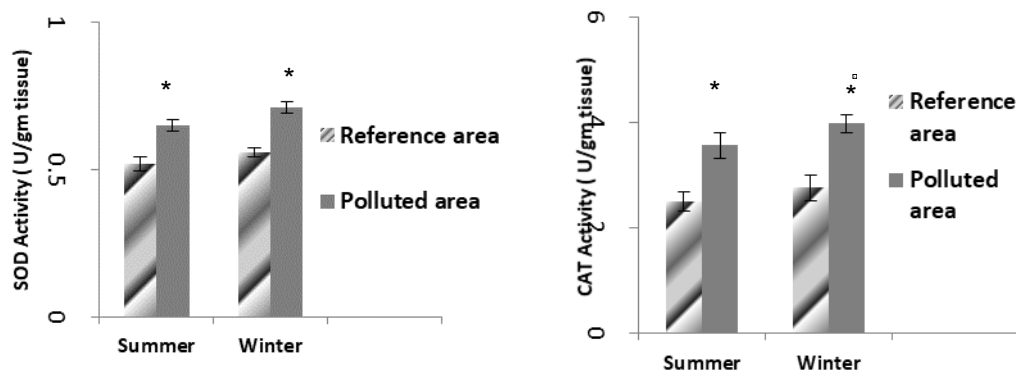
Figure(2): SOD and CAT activities (U/gm wet tissue) in white muscles of *O. niloticus* collected from both areas (Mean±S.E).

*: Significant difference in comparison with the reference area ($P < 0.05$)

The CAT activity in muscles of *Oreochromis niloticus* increased significantly (P ≤ 0.05) at polluted area during the two seasons in comparison with the reference area (Fig.2). The increase in winter and summer was 43.8% and 42.7% respectively.

The GPx activity in muscles of *Oreochromis niloticus* increased significantly (P ≤ 0.05) at polluted area during the two seasons in comparison with the reference area (Fig.3). The increase in winter and summer was 47.2% and 32.2% respectively.

The GST activity in muscles of *Oreochromis niloticus* increased significantly (P ≤ 0.05) at polluted area during the two seasons in comparison with the reference area (Fig.3). The increase in winter and summer was 54% and 45% respectively.
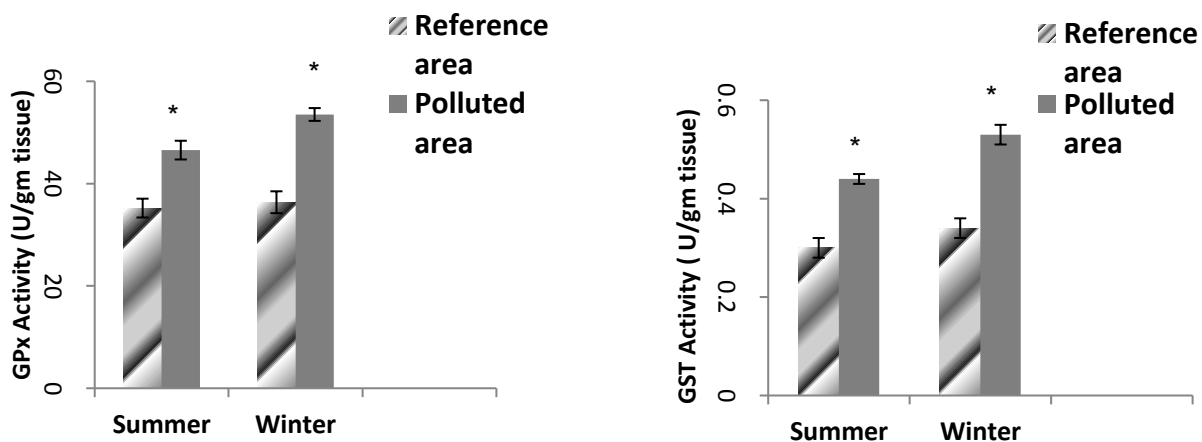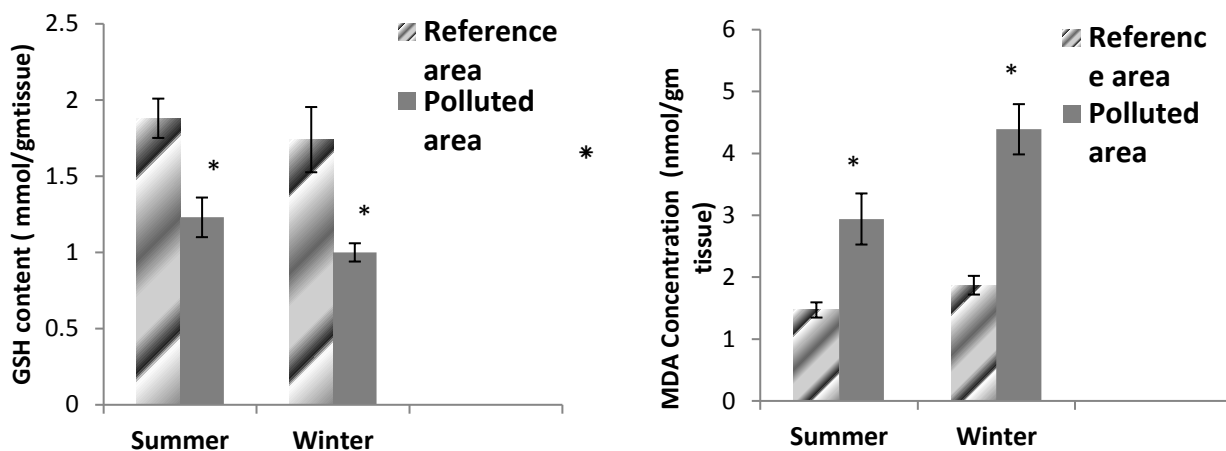

Figure (3): GPx and GST activities (U/gm wet tissue) in white muscles of *O. niloticus* collected from both areas (Mean±S.E).

*, Significant difference in comparison with the reference area ($P < 0.05$)

The GSH concentration in muscles of Oreochromis niloticus decreased significantly (P ≤0.05) at the polluted area during the two seasons in comparison with the reference area (Fig.4). The decrease in winter and summer was 42.4% and 34.6% respectively.

The MDA concentration in white muscles of *Oreochromis niloticus*increased significantly (P ≤0.05) at the polluted area during the two seasons in comparison with the reference area (Fig.4). The increase in winter was 2.3 fold, and 2.0fold in summer.

Figure(4): GSH content (mmol/gm wet tissue) and MDA concentration (nmol/gm wet tissue) in muscles of *O. niloticus* collected from both areas (Mean±S.E).

*,: Significant difference in comparison with the reference area ($P < 0.05$)

Comet assay

The results obtained in the current study using the comet assay(Table 3) revealed that during both seasons, the average scores for DNA damage in O. niloticus from the polluted area (EL-Mahmoudyia canal) were statistically significant (p<0.05) when compared with that of   reference area (Rosetta branch of Nile River). There was a predominance of comets in Classes 2 and 3, with medium and high DNA damage in cells from fish collected from the polluted area (Fig.5). The results revealed that blood cells from fish collected from reference area showed lower comet score than that collected from the polluted area (Fig.6).

**Table (3): Visual score of DNA damage in fish samples.**

Season	Area	No. of cells		Class**				DNA damaged cells% (Mean±SEM)
		Analyzed*	Comets	0	1	2	3	
Summer	Reference	300	41	259	24	11	6	13.67±1.53
	Polluted	300	68	232	33	24	11	22.72±1.15*
Winter	Reference	300	53	247	31	14	7	17.67±1.52
	Polluted	300	70	230	27	29	14	23.33±1.50*

Values are expressed as mean ± S.E
*: Significant difference in comparison with the reference area ($P < 0.05$)
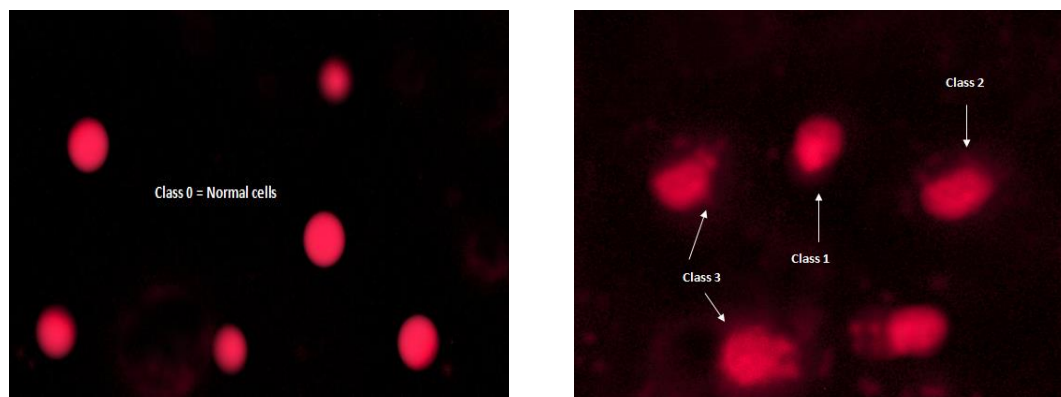
Figure 5: Visual score of normal DNA (class 0) and comet (class 1, class 2) using comet assay in fish cells collected from Rosetta branch on Nile River
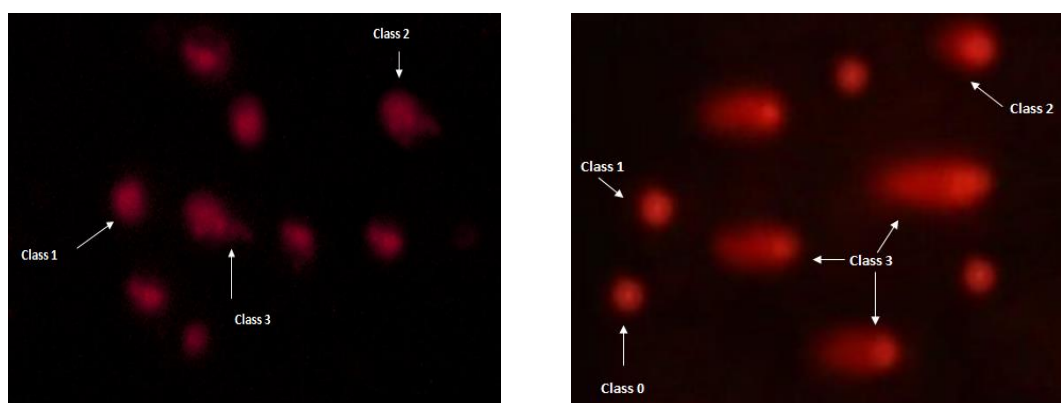
Figure6: Visual score of comet (class1, class 2, class 3) using comet assay in fish cells

collected from EL-Mahmoudeyia canal

Discussion

The present results showed that the mean concentration of heavy metals in water tended to be higher in the polluted area; this may be due to the impact of pollution sources in this area, as the industrial effluents that discharged directly into the sampling area (EL-Mahmoudeyia canal), as sewage, domestic wastes.

The present study revealed that the concentrations of the estimated heavy metals in water samples were in the following order: Fe > Zn > Pb > Cu and Cd. Furthermore, the heavy metal concentrations in water samples demonstrated seasonal variations as it was higher in winter than in summer. This in agreement with the results obtained by(Magdy et al.),(El-Bouraie et al., 2010) and (Islam et al., 2015a)whom reported that, heavy metals concentration showed seasonal variations and increased in winter. The perceived decrease in heavy metals concentration in the present work during summer may be due to phytoplankton growth that absorb large quantity of heavy metals from water and heavy metals are more likely to be attached by organic matter and clays, which have negatively charged surface functional groups that bind heavy metal that are positively charged and settled down in sediments. (Islam et al., 2015b)

Preceding studies presented that the bioaccumulation of heavy metals does not only depend on the structure of the organ, but also on the interaction between metals and the target organs(Mersch et al., 1993). It was reported that fish could accumulate trace metals and act as indicators of pollution(El-Naggar et al., 2009).The present results showed that the concentration of estimated heavy metals in muscles tissues were always higher than in water, this was confirmed by other previous studies (Chale, 2002, Abumourad et al., 2014, Riani, 2015) may be due to the variety of occurrences on fish body and other aquatic biota, including the regular diffusion, bio-magnifications, and bioconcentration (Boisson et al., 2003).The present study showed that there was a significant increase in the mean concentration of the heavy metals (Pb, Zn, Cu, Fe, and Cd) in the muscles of fish which collected from the polluted area in comparison with that of the reference area as shown in Table (2).The order of accumulation of such heavy metals was Fe > Zn>pb>cu>cd.

The highest accumulation of (Fe) in fish muscles may be due to the increase of total dissolved Fe in Nile water and consequently increases the free metal Fe concentration and thereby lead to an increase in metal uptake by different organs (Tayel et al., 2008)

Cd and Pb are toxic at low concentrations, non-biodegradable non-essential heavy metals and have no role in biological processes in living organisms. Thus, even in low concentration, they could be harmful to fish. The current data showed that pattern of Cd and Pb accumulation in muscles were in agreement with (Rashed, 2001)who indicated the lowest concentration of Pb and cd in muscles of O. niloticus from Nile River at Assiut region.

The present results agreed with previous studies which found that Cu showed lower accumulation in fish muscles which may be due to rapid deposition of Cu in muscles at early exposure, but liver represented the terminal Cu storage area at prolonged exposure (Tsai et al., 2013).

In the present study the Zn concentration in muscles of O. niloticus collected from polluted area (Tables 2) showed an elevation in winter. These results are in agreement with those obtained by(Magdy et al.)

The present study demonstrated that the antioxidant enzymatic activities of SOD and CAT(Fig.1),GPx and GST(Fig.2) and oxidative stress biomarker (MDA)(Fig.3) in muscles of O. niloticus collected from EL-Mahmoudyia canal (polluted area), were increased significantly when compared with that collected from Rosetta branch of Nile River (Reference area). The high lightened increase in the antioxidant enzymatic activities

demonstrated the adaptive responses of fish to the oxidative damage caused by the generated reactive oxygen species from exposure to water pollutants (dos Santos Carvalho et al., 2012).

The current study revealed that the antioxidant enzymatic activities of (SOD, CAT, GPx and GST) as well as oxidative stress biomarker (MDA), were increased in winter in comparison to that in summer, in agreement with (Magdy et al.). This explained that, more free radical are produced at lower temperature, which results in oxidative damage in tissues exposed to pollutants and increased antioxidant enzymes expression to protect the exposed tissues against the oxidative damage of free radicals.

The variation in the precedent antioxidants enzymatic activities and oxidative stress biomarker MDA during winter and summer may be due to decreased ROS elimination systems at lower temperature (Lushchak, 2011) , also fish at lower temperatures have higher polyunsaturated fatty acids content in their membrane lipids to maintain its function which increase the risk for lipid peroxidation and oxidative damage affecting its membrane integrity(Guderley and St-Pierre, 2002).

The pronounced elevation of the antioxidants enzymatic activity of SOD, CAT, GPx and GST in the current study was in agreement with the results presented in gold fish, Carassius auratus(Aliko et al., 2018), in O. niloticus (Magdy et al 2017).
The highlighted elevation of the enzymatic activities of SOD, CAT, GST and GPx in the muscles of O.niloticus collected from the polluted area could be due to the high accumulation level of the estimated heavy metals (Pb, Zn Cu, Fe and Cd) in fish muscles that generate free radicals which disturb the pro-oxidants/antioxidants balance creating a situation of oxidative stress in muscles tissues which in response to that situation increase the expression of precedent antioxidants enzymes as a type of defense mechanism against the free radicals produced by the increased level of heavy metals accumulation in the fish muscles.

Our results are in agreement with the findings of Farombi et al (Farombi et al., 2007b)

GSH is well known as a metal protective molecule that has the ability to change the tendency to metal (Killa and Rabenstein, 1989). GSH is taken into consideration as a first line of protection against metals via chelating and detoxifying them, scavenging and detoxification of oxyradicals via reactions catalysed by GPx (Sies, 1999). The current results showed a significant decrease in the GSH content in fish muscles collected from the polluted area in comparison with that of fish collected from the reference area, this could be linked to the increased GST activity which uses GSH for converting xenobiotics into more hydrophilic compounds. Other factors such as GST may induce the consumption of GSH (Eroglu et al., 2015). Some studies reported the GSH reduction with a significant increase in GST activity, which is in accordance with the present data(Zhang et al., 2004) , others reported that GSH levels decrease in response to oxidative stress after initial exposure to toxicants and can also increase as a compensatory action (Guyonnet et al., 1999, Tan et al., 1998)

Malondialdehyde (MDA) is widely used as an indicator of lipid peroxidation and oxidative stress in cells and tissues(Esterbauer et al., 1991).The herein results revealed that there was a significant increase in MDA level in muscles collected from the polluted area when compared with that of the reference area, the increased level of MDA in the polluted area may be resulted from the high levels of ROS produced by the accumulated heavy metals in fish (Dautremepuits et al., 2002).Same results were recorded in C. gariepinus collected from heavily polluted river nearby major industries in Nigeria(Farombi et al., 2007a).

It has been reported that there is an association between DNA damage in aquatic animals and aquatic environment pollution (Klobučar et al., 2010, Fatima et al., 2014). Fish is the best accessible vertebrate model to estimate potential

risks, due to their capability to metabolize and accumulate contaminants in their bodies(Diekmann et al., 2004), furthermore, fish blood erythrocytes are the most suitable for DNA damage analyses as it displays the complete health status of the organism so fish blood cells have attained specific attention as their erythrocytes are nucleated and, therefore, suitable for acquiring nucleoids for single cell gel electrophoresis(Costa et al., 2011).The herein results showed that there is a significant increase in the average scores for DNA damage in O. niloticus from the polluted area when compared with that of from the reference area in agreement with(Badr et al., 2014) who had found that the DNA strand breaks increased statistically in tilapia collected from polluted area compared to fish collected from reference area. During the present study significantly higher percentages of DNA damage in O. niloticus indicate its higher susceptibility to metals. In addition, metal accumulation causes an elevation in production of ROS such as hydrogen peroxide, superoxide radical, hydroxyl radical which results in oxidative stress in fish(Dautremepuits et al., 2002), and hence more MDA is produced that capable of attacking biomolecules such as protein and DNA producing a variety of adducts (Adeyeye et al., 1996) such as that with DNA bases which considered as mutagenic (Becker et al., 2008)

Conclusion

Continuous discharge of industrial effluents without treatment into fresh canals can cause physiological changes in fish as Nile tilapia and hence the human health. Fish biomarkers as genotoxic damage in peripheral blood erythrocytes and oxidative stress in Nile tilapia are necessary to assess the impact of xenobiotic compounds (i.e. heavy metals) on fish. Moreover, the treatment of all kinds of waste waters, sewage and agricultural wastes is recommended before discharge into the aquatic systems.

References

ABUMOURAD, I., ABBAS, W., AUTHMAN, M. & GIRGIS, S. 2014. Environmenta l impact of heavy metal pollution on metallothionein expression in Nile Tilapia. *Res J Pharm Biol Chem Sci,* 5**,** 998-1005.

ADEYEYE, E. I., AKINYUGHA, N. J., FESOBI, M. E. & TENABE, V. O. 1996. Determination of some metals in Clarias gariepinus (Cuvier and Vallenciennes), Cyprinus carpio (L.) and Oreochromis niloticus (L.) fishes in a polyculture fresh water pond and their environments. *Aquaculture,* 147**,** 205-214.

AEBI, H. 1984. [13] Catalase in vitro. *Methods in enzymology.* Elsevier.

ALIKO, V., QIRJO, M., SULA, E., MORINA, V. & FAGGIO, C. 2018. Antioxidant defense system, immune response and erythron profile modulation in gold fish, Carassius auratus, after acute manganese treatment. *Fish Shellfish Immunol,* 76**,** 101-109.

BADR, A. M., MAHANA, N. A. & EISSA, A. 2014. Assessment of Heavy Metal Levels in Water and Their Toxicity in Some Tissues of Nile Tilapia (Oreochromisniloticus) in River Nile Basin at Greater Cairo, Egypt. *Global Veterinaria,* 13**,** 432-443.

BECKER, J., FULLNER, K., SEELING, U., FORNALCZYK, G. & KUHN, A. 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. *Analytical and bioanalytical chemistry,* 390**,** 571-578.

BEGUM, A., AMIN, M. N., KANECO, S. & OHTA, K. 2005. Selected elemental composition of the muscle tissue of three species of fish, Tilapia nilotica, Cirrhina mrigala and Clarius batrachus, from the fresh water Dhanmondi Lake in Bangladesh. *Food chemistry,* 93**,** 439-443.

BEUTLER, E., DURON, O. & KELLY, M. 1963. Colorimetric method for determination of

glutathione reduced. *J. Lab. Clin. Med,* 61**,** 882.

BOISSON, F., COTRET, O., TEYSSIE, J.-L., EL-BARADE₁, M. & FOWLER, S. 2003. Relative importance of dissolved and food pathways for lead contamination in shrimp. *Marine Pollution Bulletin,* 46**,** 1549-1557.

CENSI, P., SPOTO, S., SAIANO, F., SPROVIERI, M., MAZZOLA, S., NARDONE, G., DI GERONIMO, S., PUNTURO, R. & OTTONELLO, D. 2006. Heavy metals in coastal water systems. A case study from the northwestern Gulf of Thailand. *Chemosphere,* 64**,** 1167-1176.

CHALE, F. 2002. Trace metal concentrations in water, sediments and fish tissue from Lake Tanganyika. *Science of the total environment,* 299**,** 115-121.

COLLINS, A., DUŠINSKá, M., FRANKLIN, M., SOMOROVSKá, M., PETROVSKá, H., DUTHIE, S., FILLION, L., PANAYIOTIDIS, M., RAŠLOVá, K. & VAUGHAN, N. 1997. Comet assay in human biomonitoring studies: reliability, validation, and applications. *Environmental and molecular mutagenesis,* 30**,** 139-146.

COSTA, P. M., NEUPARTH, T. S., CAEIRO, S., LOBO, J., MARTINS, M., FERREIRA, A. M., CAETANO, M., VALE, C., DELVALLS, T. A. & COSTA, M. H. 2011. Assessment of the genotoxic potential of contaminated estuarine sediments in fish peripheral blood: laboratory versus in situ studies. *Environmental Research,* 111**,** 25-36.

DAUTREMEPUITS, C., BETOULLE, S. & VERNET, G. 2002. Antioxidant response modulated by copper in healthy or parasitized carp (Cyprinus carpio L.) by Ptychobothrium sp.(Cestoda). *Biochimica et Biophysica Acta (BBA)-General Subjects,* 1573**,** 4-8.

DAUTREMEPUITS, C., PARIS-PALACIOS, S., BETOULLE, S. & VERNET, G. 2004. Modulation in hepatic and head kidney parameters of carp (Cyprinus carpio L.) induced by copper and chitosan. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology,* 137**,** 325-333.

DIEKMANN, M., WALDMANN, P., SCHNURSTEIN, A., GRUMMT, T., BRAUNBECK, T. & NAGEL, R. 2004. On the relevance of genotoxicity for fish populations II: genotoxic effects in zebrafish (Danio rerio) exposed to 4-nitroquinoline-1-oxide in a complete life-cycle test. *Aquatic toxicology,* 68**,** 27-37.

DOS SANTOS CARVALHO, C., BERNUSSO, V. A., DE ARAúJO, H. S. S., ESPíNDOLA, E. L. G. & FERNANDES, M. N. 2012. Biomarker responses as indication of contaminant effects in Oreochromis niloticus. *Chemosphere,* 89**,** 60-69.

EHSAEI, M., KHAJAVI, M., ARJMAND, M. H., ABUEE, M. A., GHAYOUR-MOBARHAN, M. & ALAMDARI, D. H. 2015. Prooxidant–antioxidant balance in patients with traumatic brain injury. *Acta Neurologica Belgica,* 115**,** 69-73.

EL-BOURAIE, M., EL-BARBARY, A., YEHIA, M. & MOTAWEA, E. 2010. Heavy metal concentrations in surface river water and bed sediments at Nile Delta in Egypt. *Suo,* 61**,** 1-12.

EL-NAGGAR, A. M., MAHMOUD, S. A. & TAYEL, S. I. 2009. Bioaccumulation of some heavy metals and histopathological alterations in liver of Oreochromis niloticus in relation to water quality at different localities along the River Nile, Egypt. *World Journal of Fish and Marine Sciences,* 1**,** 105-114.

EL SAFTY, A. & SIHA, M. 2013. ENVIRONMENTAL AND HEALTH IMPACT OF COAL USE FOR ENERGY

PRODUCTION. *Egyptian Journal of Occupational Medicine, 37*, 181-194.

EROGLU, A., DOGAN, Z., KANAK, E., ATLI, G. & CANLI, M. 2015. Effects of heavy metals (Cd, Cu, Cr, Pb, Zn) on fish glutathione metabolism. *Environmental Science and Pollution Research, 22*, 3229-3237.

ESTERBAUER, H., SCHAUR, R. J. & ZOLLNER, H. 1991. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free radical Biology and medicine, 11*, 81-128.

FAIRBAIRN, D. W., OLIVE, P. L. & O'NEILL, K. L. 1995. The comet assay: a comprehensive review. *Mutation Research/Reviews in Genetic Toxicology, 339*, 37-59.

FAROMBI, E., ADELOWO, O. & AJIMOKO, Y. 2007a. Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African cat fish (Clarias gariepinus) from Nigeria Ogun River. *International Journal of Environmental Research and Public Health, 4*, 158-165.

FAROMBI, E. O., ADELOWO, O. A. & AJIMOKO, Y. R. 2007b. Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African cat fish (Clarias gariepinus) from Nigeria Ogun River. *Int J Environ Res Public Health, 4*, 158-65.

FATIMA, M., USMANI, N., HOSSAIN, M. M., SIDDIQUI, M. F., ZAFEER, M. F., FIRDAUS, F. & AHMAD, S. 2014. Assessment of genotoxic induction and deterioration of fish quality in commercial species due to heavy-metal exposure in an urban reservoir. *Archives of environmental contamination and toxicology, 67*, 203-213.

FLEGAL, A. & PATTERSON, C. 1983. Vertical concentration profiles of lead in the Central Pacific at 15 N and 20 S. *Earth and Planetary Science Letters, 64*, 19-32.

FORSTNER, U. GTW Wittman. 1983. Metal Pollution In The Aquatic Environment. Springer-Verlag, Berlin.

GHORBANI, M. & MIRAKABAD, H. Z. 2010. Factors influencing on trout production in Khorasan Razavi Province. *Trends Agric. Econ, 3*, 19-27.

GOSMARO, F., BAGNATI, M., BERTO, S., BELLOMO, G. & PRENESTI, E. 2013. Measurement of total antioxidant capacity of human plasma: Setting and validation of the CUPRAC–BCS method on routine apparatus ADVIA 2400. *Talanta, 115*, 526-532.

GUDERLEY, H. & ST-PIERRE, J. 2002. Going with the flow or life in the fast lane: contrasting mitochondrial responses to thermal change. *Journal of Experimental Biology, 205*, 2237-2249.

GUYONNET, D., SIESS, M.-H., LE BON, A.-M. & SUSCHETET, M. 1999. Modulation of phase II enzymes by organosulfur compounds from allium vegetables in rat tissues. *Toxicology and applied pharmacology, 154*, 50-58.

HABIG, W. H., PABST, M. J. & JAKOBY, W. B. 1974. Glutathione S-transferases the first enzymatic step in mercapturic acid formation. *Journal of biological Chemistry, 249*, 7130-7139.

ISLAM, M. S., AHMED, M. K., HABIBULLAH-AL-MAMUN, M. & MASUNAGA, S. 2015a. Assessment of trace metals in fish species of urban rivers in Bangladesh and health implications. *Environmental toxicology and pharmacology, 39*, 347-357.

ISLAM, M. S., AHMED, M. K., RAKNUZZAMAN, M., HABIBULLAH-AL-MAMUN, M. & MASUNAGA, S. 2015b. Metal speciation in sediment and their bioaccumulation in fish species of three urban rivers in Bangladesh. *Archives*

of environmental contamination and toxicology, 68**,** 92-106.

KEI, S. 1978. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. *Clinica chimica acta,* 90**,** 37-43.

KILLA, H. & RABENSTEIN, D. L. 1989. Separation and detection of bis (alkylthio) selenides of penicillamine and glutathione by liquid chromatography with electrochemical detection. *Journal of chromatography,* 465**,** 359-368.

KIME, D. E. 1995. The effects of pollution on reproduction in fish. *Reviews in Fish Biology and Fisheries,* 5**,** 52-95.

KLOBUČAR, G. I., ŠTAMBUK, A., PAVLICA, M., PERIĆ, M. S., HACKENBERGER, B. K. & HYLLAND, K. 2010. Genotoxicity monitoring of freshwater environments using caged carp (Cyprinus carpio). *Ecotoxicology,* 19**,** 77.

KOBAYASHI, H. 1995. A comparison between manual microscopic analysis and computerized image analysis in the single cell gel electrophoresis. *MMS Commun.,* 3**,** 103-115.

LAI, H. & SINGH, N. P. 1995. Acute low-intensity microwave exposure increases DNA single-strand breaks in rat brain cells. *Bioelectromagnetics,* 16**,** 207-210.

LOPES, P. A., PINHEIRO, T., SANTOS, M. C., DA LUZ MATHIAS, M., COLLARES-PEREIRA, M. J. & VIEGAS-CRESPO, A. M. 2001. Response of antioxidant enzymes in freshwater fish populations (Leuciscus alburnoides complex) to inorganic pollutants exposure. *Science of the total environment,* 280**,** 153-163.

LUSHCHAK, V. I. 2011. Environmentally induced oxidative stress in aquatic animals. *Aquatic toxicology,* 101**,** 13-30.

MACFARLANE, G. & BURCHET, M. 2000. Cellular distribution of Cu, Pb, and Zn in the Grey Mangrove, Avicemnia marina (Forsk.), Vierh Aquatic Botanic 68: 45-59.

MAGDY, T., NAHED, S. G., NASR, A. & SALLY, S. M. Antioxidant Defense System Alternations in Fish as a Bio-Indicator of Environmental Pollution.

MERSCH, J., DUBOST, N. & PIHAN, J. Year. Comparison of several inert and biological substrata to assess the trace metal contamination in the reservoir of the nuclear power plant in Cattenom, France. *In:* Annales de limnologie. Toulouse, 1993. 325-337.

NAGARANI, N., DEVI, V. J. & KUMARAGURU, A. 2012. Identification of DNA damage in marine fish Therapon jarbua by comet assay technique. *Journal of environmental biology,* 33**,** 699.

NAMMINGA, H. E. Year. Effects of high discharge and an oil refinery cleanup operation on heavy metals in water and sediments in Skeleton Creek. *In:* Proceedings of the Oklahoma Academy of Science, 1976. 133-138.

NRIAGU, J. O. & PACYNA, J. M. 1988. Quantitative assessment of worldwide contamination of air, water and soils by trace metals. *nature,* 333**,** 134.

PAGLIA, D. E. & VALENTINE, W. N. 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *The Journal of laboratory and clinical medicine,* 70**,** 158-169.

PITT, R. & BURTON JR, G. A. 2001. *Stormwater effects handbook: A toolbox for watershed managers, scientists, and engineers*, CRC Press.

RASHED, M. 2001. Cadmium and lead levels in fish (Tilapia nilotica) tissues as biological indicator for lake water pollution. *Environmental monitoring and assessment,* 68**,** 75-89.

RIANI, E. 2015. The Effect of Heavy Metals on Tissue Damage in Different Organs of Goldfish Cultivated in Floating Fish Net in

Cirata Reservoir, Indonesia Marine Science.

SIES, H. 1999. Glutathione and its role in cellular functions. *Free radical Biology and medicine,* 27**,** 916-921.

TAN, S., SAGARA, Y., LIU, Y., MAHER, P. & SCHUBERT, D. 1998. The regulation of reactive oxygen species production during programmed cell death. *The Journal of cell biology,* 141**,** 1423-1432.

TAYEL, S., YACOUB, A. & MAHMOUD, S. 2008. Histopathological and haematological responses to freshwater pollution in the Nile catfish Clarias gariepinus. *Journal of Egyptian Academic Society for Environmental Development,* 9**,** 43-60.

TICE, R. R., AGURELL, E., ANDERSON, D., BURLINSON, B., HARTMANN, A., KOBAYASHI, H., MIYAMAE, Y., ROJAS, E., RYU, J. C. & SASAKI, Y. 2000. Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. *Environmental and molecular mutagenesis,* 35**,** 206-221.

TRIPATHI, L., MISHRA, A., DUBEY, A. & TRIPATHI, C. 2015. Water pollution through energy sector. *International Journal of Technology Enhancements and Emerging Engineering Research,* 3**,** 2347-4289.

TSAI, J.-W., JU, Y.-R., HUANG, Y.-H., DENG, Y.-S., CHEN, W.-Y., WU, C.-C. & LIAO, C.-M. 2013. Toxicokinetics of tilapia following high exposure to waterborne and dietary copper and implications for coping mechanisms. *Environmental Science and Pollution Research,* 20**,** 3771-3780.

VAN DER OOST, R., GOKSøYR, A., CELANDER, M., HEIDA, H. & VERMEULEN, N. P. 1996. Biomonitoring of aquatic pollution with feral eel (Anguilla anguilla) II. Biomarkers: pollution-induced biochemical responses. *Aquatic toxicology,* 36**,** 189-222.

VERON, A., LAMBERT, C. E., ISLEY, A., LINET, P. & GROUSSET, F. 1987. Evidence of recent lead pollution in deep north-east Atlantic sediments. *nature,* 326**,** 278.

VITOŠEVIĆ, B., SAMARDŽIĆ, S., ANTONIJEVIĆ, V. & JAKOVLJEVIĆ, V. 2007. Heavy metals in some imported food products and their potential toxic implications. *Medicus,* 8**,** 62-66.

YU, R. 2000. Cadmium and DNA damage, oncogene expression as well as cell apoptosis. *Overseas Medicine Hygienics Fascicule,* 27**,** 359-363.

ZHANG, J., WANG, X., GUO, H., WU, J. & XUE, Y. 2004. Effects of water-soluble fractions of diesel oil on the antioxidant defenses of the goldfish, Carassius auratus. *Ecotoxicology and Environmental Safety,* 58**,** 110-116.