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Assessment of Heavy Metal Levels in Water and Their Toxicity in Some Tissues of Nile Tilapia (*Oreochromis niloticus*) in River Nile Basin at Greater Cairo, Egypt

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Abstract: The levels of lead (Pb), copper (Cu), cadmium (Cd), iron (Fe), zinc (Zn) and manganese (Mn) were measured in water samples and in tissues (liver, kidney, gill, spleen and muscle) of Nile tilapia collected from two locations along the River Nile basin at Greater Cairo in Egypt. We investigated the impact of these traces of heavy metals on the physiology, immune responses, genotoxicity and histology of Nile tilapia. The concentrations of heavy metals in water samples from polluted area (El-Tebeen, Area 2) were significantly higher than reference area (El-Zamalek, Area 1) for all investigated metals. The prevalence of each element in various fish tissues was variable between the two sampling areas. The level of plasma total proteins was significantly ($P< 0.001$) elevated in Area 2 compared to Area 1. Lysozyme levels were insignificantly declined in plasma of fish from Area 2 while phagocytosis percentage was markedly reduced ($P< 0.001$) in Area 2 compared to Area 1. DNA damage (comet assay) in blood elevated statistically in tilapia collected from Area 2 compared to Area 1. Additionally, numerous histopathological alterations were observed in Area 2.Thus, innate immune responses of tilapia could be sensitive to environmental contamination.

Key words: Immune Response · Innate Immunity · DNA Damage · Total Protein · Lysozyme Activity · Phagocytosis · Water Pollution

INTRODUCTION

The River Nile is the principal freshwater resource for Egyptians and represents more than 97% of Egyptian water resources [1]. Globally, industrial waste water represents the main source of water pollution. The Uprising increase in modern industries, agriculture urbanization, tourism and human activities are the main sources for chemical pollution to both, aquatic environment and its coexisting ecosystems [2]. Heavy metals are persistent contaminants in the environment causing serious illness in fish, animals and human. Regionally, industrial and agricultural runoffs are considered the primary source of metal poisoning to fish and other aquatic animals in Egypt [3,4].

Fish, the first vertebrate in the evolution tree, exhibits both innate and acquired immune responses. The immune system of fishes on contrary to higher vertebrates is comparatively simple and undifferentiated. The major lympho-myeloid organs of fishes are thymus, head kidney and spleen [5]. The innate immunity is a fundamental defense mechanism of fish [6].The immune system of aquatic organisms, such as fish, is continuously affected by periodic or unexpected changes of their environment. Adverse environmental situations may acutely or chronically stress the health of fish, altering some of their biochemical parameters and suppressing their innate and adaptive immune responses [4,7].

Tilapia (*Oreochromis* (*O.*) spp.) is a teleost fish with a worldwide distribution; therefore it is a good model for assessing the impacts of different environmental pollutants on aquatic ecosystems. A comparative study of five economically important taxa of tilapia showed that Nile tilapia (*O. niloticus*) presents a Godgifted strong immune system that maximizes their capability to tolerate biotic and abiotic types of stress [8,9]. Further, the natural surface feeder omnivorous non predator behavior of Nile

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tilapia might grant them another natural tool that minimizes their possibility of contracting numerous types of pollutants including biological/chemical forms when compared to bottom feeder fishes [4,9]. In fish, chemical pollutants are commonly leading to genotoxicity through their aquatic environments [10].

In rivers, fish are often at the top of the food chain and have the tendency to accumulate heavy metals from water [11]. Therefore, bioaccumulation of metals in fish can be considered as an indicator of metal pollution in aquatic environment [12]. Prolonged exposure to water pollutants even in very low concentrations have been reported to induce morphological, histological and biochemical alterations in fish tissues, which may critically influence the quality and marketability of fishes [3,13]. However, their persistence in the aquatic environment is surely related to the success of their immune system to counteract such impacts [14]. It is also possible that environmental toxicants may increase the susceptibility of aquatic animals to various diseases by interfering with the normal functioning of their immune, reproductive and developmental processes [4].

The present study was carried out to determine the levels of bioaccumulation of some heavy metals [lead (Pb), copper (Cu), cadmium (Cd), iron (Fe), zinc (Zn) and manganese (Mn)] in aquatic environment and in some tissues of Nile tilapia. Another critical goal is to determine the impacts of these heavy metals on the histological normogram of Nile tilapia collected from possibly polluted and non-polluted areas of River Nile basin at the vicinity of Greater Cairo. Ultimately, our main goal was to assess the effects of these heavy metals on the innate immune responses and DNA integrity of collected Nile tilapias.

MATERIALS AND METHODS

Study Areas: Samples were collected from diverse geographical locations along the River Nile basin at the vicinity of two cities of Greater Cairo.

Study Area 1: The River Nile basin is at El-Zamalek district, this area can be considered as a referenceor control area due to its distance from any source of industrial/agricultural runoffs). This area located downstream in main River Nile extension at Greater Cairo. It is 37 km south to the industrial zone where no industrial effluent was recognized and thus considered as control area. Geographically, the reference area (Area 1) is located at GPS reading of 30°Z2'20.24"N and 31°13'10.23"E.

Study Area 2: The River Nile basin is at El-Tebeen district, this area located upstream south of River Nile extension at Greater Cairo. This Areais located at core of Cairo industrial zone where cement factory, electric power station, Iron/ steel factory dump their effluents directly to the stream of River Nile. Geographically, Area1 is located at Global Positional System (GPS) reading of 29°41'21.03"N and 31°16'57.23"E.

Water and Fish Sampling: Water samples were collectedmanually in triplicates from each of the studied areas between 10:00 and 12:00 a.m. at a depth of 30 cm below the water surface and stored at 4°C in sterileglass bottles, acidified with concentrated hydrochloric acid (HCl) for preservation. Fifty adult Nile tilapias (*O. niloticus)* ranged from 250±10 g weight were collected during summer season in 2013 from both sampling areas. Fish were collected in closed mesh before transferred to the laboratory in well aerated Styrofoam boxes containing Nile water. Blood samples were withdrawn from the caudal vessels of collected fishes using heparinized syringes [15]. Portion of fresh blood were immediately used for comet assay and phagocytosis while the remaining part was centrifuged to obtain plasma. Collected plasma was kept at -20°C for further analysis to estimate lysozyme, total proteins, albumin and total globulins. Fishes were further dissected using three line incision [15] in order to get spleens, livers, kidneys, gills and muscles. Each tissue sample was divided into two halves. One half was kept frozen at -20°C for assessing heavy metals concentrations and the second half was fixed in 10 % buffered neutral formalin for histological examination.

Heavy Metals Assessment: Trace elements were measured in water and tissue samples using flame atomic absorption spectrophotometer (Thermo Scientific, UK) with double beam and deuterium background corrector according to APHA [16]. Tissue samples were dried, acid-digested and diluted with de-ionized waterto known volume using the dry-ashing procedure proposedby Hseu [17]. Analytical blanks were run in the same way as the samples and concentrationswere determined using standard solutions prepared in the same acid matrix. All reagents used were of analytical grade (Merck, USA). Standards for instrument calibration were prepared on the basis of mono-element certified reference solution inductively coupled plasma standard (Merck). Standard reference material (National Institute of Standards and Technology [NIST], USA) was used to validate analysis and the metal recoveries ranged between 90 and 110%.

Concentrations of all heavy metals in water are expressed as mg/l (ppm) and µg/g wet weight in tissues.

Total Protein Content: Total protein was estimated in plasma according to Burtis *et al.* [18]. Total globulins were calculated by subtracting albumin from total protein.

Phagocytic Activity: Polymorphonuclearleukocytic cells (PMN) were isolated from blood using the method described by Rouse *et al.* [19]. The mixture of PMN and bacteria (*Staphylococcus aureus*) were incubated at 37°C for 2 h with regular stirring and then the mixture was centrifuged at 20000 g for 5 min at 4°C. The supernatants were used to estimate the percentage of bacteria phagocytosed. The mixture of bacteria and PMN were treated with one cycle of freezing and thawing. The percentage of bacteria killed was estimated according to the formula described byWoldehiwet and Rowan [20].

Plasma Lysozyme Activity: Plasma lysozyme activity was measured using the method described by Schultz [21]. Lysozymes diffused through the agarose gel containing a suspension of *Micrococcus lysodeikticus* (Sigma-Aldrich). A clear zone ring of lysis developed in the translucent agarose gel. The wells were swiftly filled with 25 µl aliquots from plasma samples. The plates were then covered tightly and incubated at room temperature on a level surface for 12-18 h. At the end of the incubation period, the clear zone ring diameters were measured to the nearest 0.1 mm.

Comet Assay: DNA damage was determined by alkaline comet assay. Comet assay was performed according to the method described by Tice *et al*. [22]. Ten µl of whole blood was mixed with 75 µl of 0.5% low-melting agarose and spread on pre-coated slides with normal melting agarose (1%). Slides were immersed in cold lysis buffer (2.5 M NaCl, 100 mM EDTA and 10 mM Tris-HCl, pH 10, with freshly added 10% DMSO and 1% Triton X-100) for 24 h at 4°C in darkness. Subsequently, the slides were incubated in fresh alkaline buffer for 20 min (300 mM NaOH and 1 mM EDTA, pH>13) and then electrophoresed for 20 min at 25 V and 300 mA. Neutralization with excess amount of 0.4 M Trizma base (pH 7.5), fixation in 100% cold ethanol and air drying were done. Finally, slides were stained with ethidium bromide (2 µg/ml). The extent of DNA migration for each sample was determined by simultaneous image capture and scoring of 100 cells at 400x magnification using Komet 5 image analysis software developed by Kinetic Imaging, Ltd. (Liverpool, UK).The measured parameters were tail length, the %tail DNA and tail moment.

Histological Examination: The Nile tilapia tissues (gills, kidney, liver, spleen and muscles) were preserved in 10% buffered formalin, embedded within paraffin, sectioned at 5 µm and stained with Hematoxylin and Eosin (H&E) according to the method described by Prophet *et al.* [23]. The histological alterations of H&E stained tissue sections were microscopically assessed under low / high microscopic powers.

Statistical Analysis: The present data were analyzed by Student's *"t"* Test to compare different parameters between the two studying areas using SPSS statistical package version 20. The data values were expressed as mean ± standard error of mean (SEM). $P< 0.05$ was the accepted significance level. Correlation coefficient (r) was used to fit the relationship between the concentration of heavy metals in water and various tissues of collected fish.

RESULTS

Heavy Metals Assessment: Analysis of water samples collected from the two River Nile sites has indicated that the concentrations of heavy metals in water samples from Area 2 were significantly higher for all investigated metals than Area 1. Briefly, Fe showed the highest mean level in polluted Area (Area 2), followed by Pb, Zn, Mn, Cd and Cu while in the reference area(Area1), the order was Fe followed by Zn then Pb then Mnthen Cd and finally Cu (Table 1).

Results of the assessment of the heavy metal residues in different edible (musculature) and non-edible fish tissues (gill, liver, kidney and spleen) of Nile tilapias collected from both studied areas were tabulated in Table 2. The recorded data indicated the presence of heavy metals traces in all tested fish tissues. Also, the prevalence of each element was variable between the two sampling sites. Gills contaminated with traces of heavy metals with the following order: Fe > Zn > Mn > Cu > Pb > Cd, whereas kidneys Fe > Zn > Pb > Cu > Mn > Cd. Splenic tissue of fishes from polluted area showed marked higher concentrations of Cu and Fe compared to the reference site. However, liver tissues demonstrated no significant changes between the two sampling sites except for Fe. Interestingly, the levels of heavy metals residues in muscle tissues revealed significant higher amounts of Fe, Zn, Cu, Cd and Pb at the polluted area compared to the

Table 1: The concentration of Zn, Mn, Cu, Cd, Pb and Fe (mg/l) in water samples of Nile River surface water collected from areas 1 and 2.

	Water samples	
Metals	Area 1	Area 2
Zn	0.1780±0.0090	0.2680±0.0060*
Mn	0.0039±0.0003	0.0466±0.0010**
Cu	0.0009±0.0001	0.0043±0.0006*
Cd	0.0038±0.0004	0.0150±0.0002**
Pb	0.0081±0.0001	0.3548±0.0080**
Fe	0.2396±0.0026	3.0500±0.1305**

Data are represented as mean± standard error of mean (SEM).
*, **: $P< 0.01$ and $P< 0.001$: significant difference in comparison with the reference standard area at $\alpha= 0.01$and 0.001, respectively.

Table 2: The concentrations of heavy metals in livers, kidneys, spleens, gills and muscles (µg/g wet weight) of *O. niloticus* collected from reference and polluted areas.

	Areas		
Metals	Reference area	Polluted area	%Change
Zn			
Liver	24.03±2.67	20.48±1.91	-14.77
kidney	91.64±1.58	39.58±3.21***	-56.80
Spleen	39.62±8.86	49.85±9.70	+25.82
Gills	8.87±1.54	18.51±0.33***	+108.68
Muscles	0.90±0.22	6.99±0.31***	+676.66
Mn			
Liver	1.38±0.55	1.25±0.49	-9.42
kidney	0.70±0.38	3±0.59**	+328.57
Spleen	0.70.38	3.51±1.20	+401.42
Gills	3.69±0.22	5.61±1.63*	+52.03
Muscles	0.232±0.067	0.151±0.044	-34.91
Cu			
Liver	27.43±8.66	24.03±2.67	-12.39
kidney	8.16±2.12	91.79±2.03***	+1024.87
Spleen	8.42±1.86	31.17±3.43***	+270.19
Gills	1.91±0.21	8.86±1.99**	+363.87
Muscles	0.264±0.023	0.693±0.091**	+162.5
Cd			
Liver	0.07±0.04	0.17±0.06	+142.85
kidney	0.081±0.025	1.07±0.29*	+32.09
Spleen	0.14±.07	1.83±0.85	+1207.14
Gills	0.02±0.003	0.09±0.03	+350
Muscles	0.009±0.002	0.024±0.005*	+2.56
Pb			
Liver	2.25±0.19	4.28±1.01	+90.22
kidney	20.21±7.81	46.52±9.69	+130.18
Spleen	24.37±3.77	59.49±14.61	+144.11
Gills	1.02±0.06	1.66±0.19*	+62.74
Muscles	0.662±0.058	0.833±0.057*	+25.83
Fe			
Liver	24.33±2.25	104.39±15.69**	+329.05
kidney	131.54±20.25	174.35±26.38	+4.18
Spleen	290.31±49.91	496.99±36.18**	+71.192
Gills	49.95±8.6	101.05±11.13**	+102.30
Muscles	5.37±0.5	31.53±4.48***	+487.15

Data are represented as Mean ± standard error of mean (SEM).
*, **, ***: significant difference in comparison with the reference area at $\alpha= 0.05$, 0.01, 0.001 and 0.0001, respectively.

Table 3: Relationship between the concentrations of metals (Zn, Cu, Mn, Cd, Pb and Fe) in water (mg/l) and in some tissues (µg/g wet weight) of *O. niloticus* collected from polluted area.

	Heavy metals in water (mg/l)					
	Zn	Cu	Mn	Cd	Pb	Fe
Liver	+0.902	+0.264	+0.995	-0.153	-0.772	+0.010
Kidney	+0.837	+0.999	+0.975	+0.028	-0.182	-0.699
Spleen	+0.995	+0.085	+0.993	+0.388	+0.998	+0.530
Gills	+0.747	+0.850	+0.863	+0.406	+0.939	-0.968
Muscles	+0.998	-0.941	+0.989	+0.912	+0.373	+0.368

Pearson correlation coefficient (r)

reference area with a decreasing order of Fe > Zn > Pb > Cu > Mn > Cd at both sites. The splenic tissue revealed the highest percentage of change for Cd, Pb and Mn while muscle tissues recorded the highest percentage of change for Zn and Fe and kidney tissues for Cu.

Correlation Between Heave Metals Levels in Water and Tissue Samples: The Pearson Coefficient has indicated that the concentrations of different traces of heavy metals in various tissues of fish caught from polluted area were greatly dependent on the concentrations of these elements in the raw water (Table 3). The relationship between heavy metals concentrations in fish organs and external water environment were variable between positive and negative correlations. As demonstrated in Table 3, in polluted site, levels of Zn and Mn in water exhibited positive correlation with those in all tissues of *O. niloticus*. In addition, Cu in water showed a strong positive correlation with Cu in kidney and gills, despite the strong negative correlation in muscles. Ultimately, concentrations of Cd in water exhibited a strong positive correlation with its concentrations in muscles, while Pb showed a strong positive correlation with that in spleen and gill tissue.

Total Proteins: The level of plasma total proteins was significantly ($P< 0.001$) elevated in the polluted industrial area compared to the reference area as shown in Fig. 1. The albumin mean level was not significantly different between the two sites. Further, the mean level (3.29±0.1) of total globulins was significantly reduced ($P< 0.001$) in polluted area compared to reference area (7.15±0.06).

Plasma Lysozyme Activity: Analysis has indicated that lysozyme levels were insignificantly declined in plasma of fishes from polluted area compared to that of reference area. The mean level (90.09±3.66) of lysozyme in Area 1 was not significantly different from that of fish in Area 2 (96.522±2.21) (Fig. 2A).

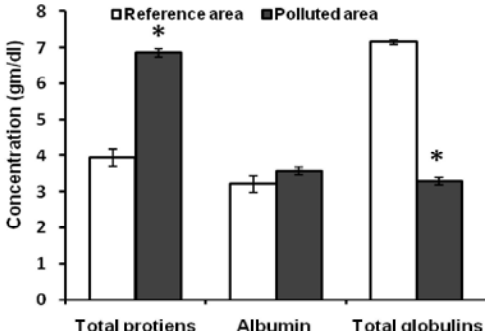

Fig. 1: Estimation of total protiens: Concentrations of total protiens, albumin and total globulins in plasma of *O. niloticus* collected from reference and polluted areas. *: significant difference in comparison to reference area at $\alpha = 0.0001$.

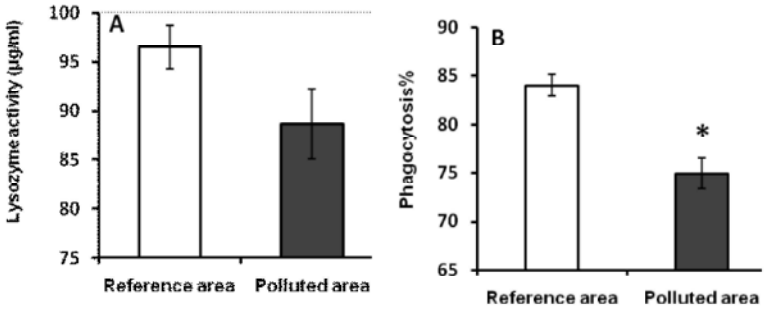

Fig. 2: Lysozyme activity and percentage of phagocytosis: (A) lysozyme activity in plasma of O. *niloticus* collected from reference and polluted areas. (B) percentage of phagocytosis of blood cells of *O.niloticus* collected from reference and polluted areas. *: significant difference compared to reference site at $\alpha = 0.0001$.

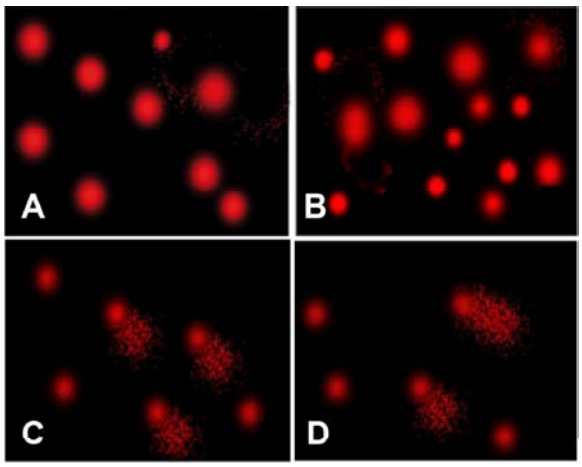

Fig. 3: Comet assay showing DNA distribution: (A& B) photomicrographs showed DNA damage of blood cells of Nile tilapia collected from reference area (Area 1). (C, D) Photomicrographs showed DNA damage of blood cells of Nile Tilapia collected from polluted area (Area 2).

Phagocytosis: Ingestion of bacteria is a method for evaluating the effect of heavy metals residues on phagocytes. The results of ingestion of bacteria by leukocytes showed a significant (75.0±1.236) reduction ($P< 0.001$) in fish collected from polluted area compared to reference area (84.0±1.64) (Fig. 2B).

Comet Assay: The Photomicrographs of DNA damage were presented in Fig. 3. The relative amounts of DNA strand breaks were higher in the polluted area (Area 2) than the reference area (Area 1) as shown in Fig. 4. The comet assay revealed a significant ($P< 0.01$) increase of DNA tail lengths (5.71±0.35) in blood cells of *O. niloticus*

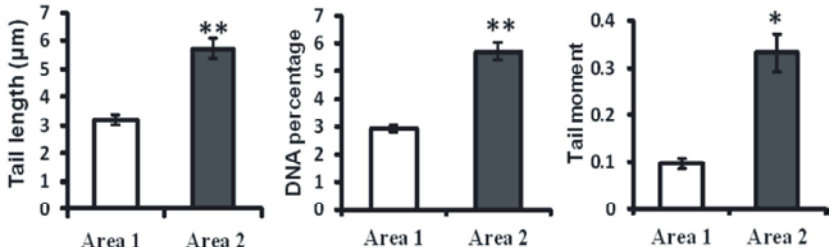

Fig. 4: Tail length, DNA percentage and Tail moment of comet parameters of blood cells of *O. niloticus* collected from reference (Area 1) and polluted (Area 2) areas. The data are represented as Mean ±Standard Error of Mean (SEM); *, **: significant difference in comparison to reference area at α=0.01 and 0.0001, respectively.

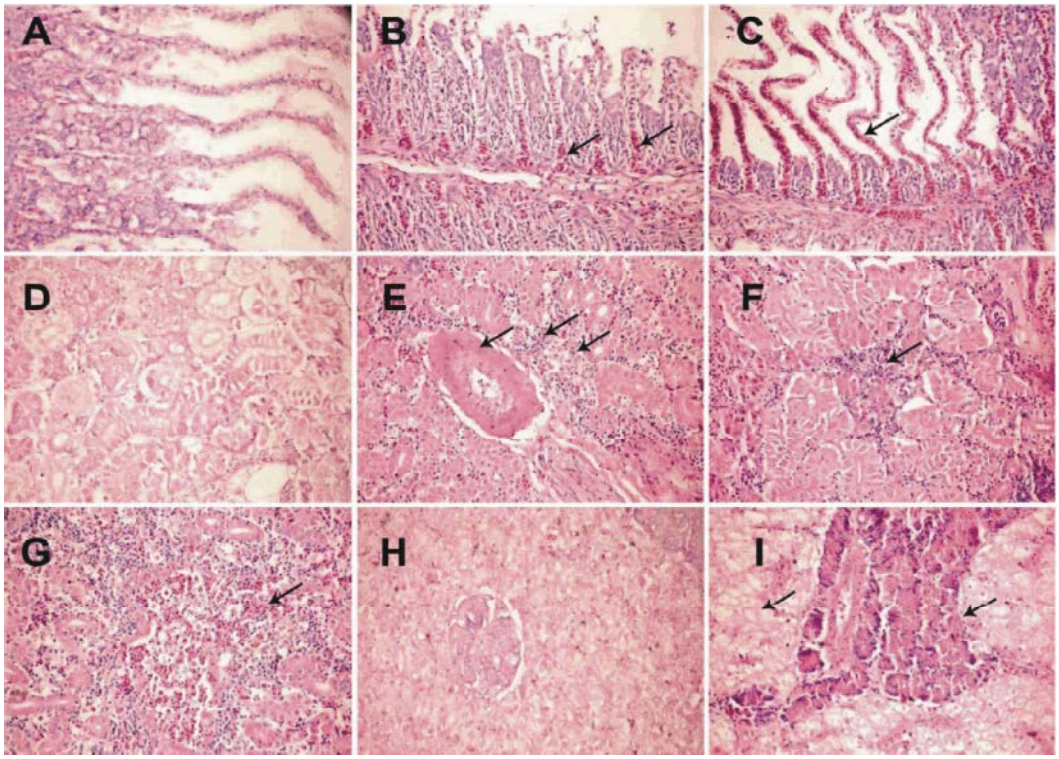

Fig. 5: Histological appearance of different tissues in *O. niloticus*. Photomicrographs of the gill tissue; (A) normal histological structure (B) necrosis (C) lamellar congestion. Photomicrographs of the kidney tissue; (D) no histological changes (E) showed marked interstitial nephritis and hyalinosis in the wall of renal blood vessels (F) focal tubular necrosis associated with leucocytic cells infiltration. (G) Photomicrograph of kidney showing massive inflammatory cells and free RBCs. Photomicrographs of liver tissue; (H) normal architecture (I) necrosis of hepatopancreas and vacuolization of hepatocytes. All tissues stained with hematoxylin and eosin (x400). A, D and H photomicrographs represented reference area, while other photomicrographs represented polluted area.

collected from polluted area compared to that of reference area (3.21±0.17). The percentage of DNA also elevated significantly ($P< 0.01$) in polluted area (5.71±0.32) compared to the reference area (2.95±0.14). *O. niloticus* from polluted area in Nile River had blood cells with higher significant ($P< 0.0001$) DNA tail moments (0.332±0.039) than referenced area (0.098±0.011).

Histological Findings: The histological examination of different sampled organs of collected Nile tilapias has revealed the presence of numerous histopathologicalalterations among examined muscles, livers, gills and kidney tissues (Figs. 5, 6). The gills from the reference area had normal structure as shown in Fig. 5A, while gills from the polluted area have presented

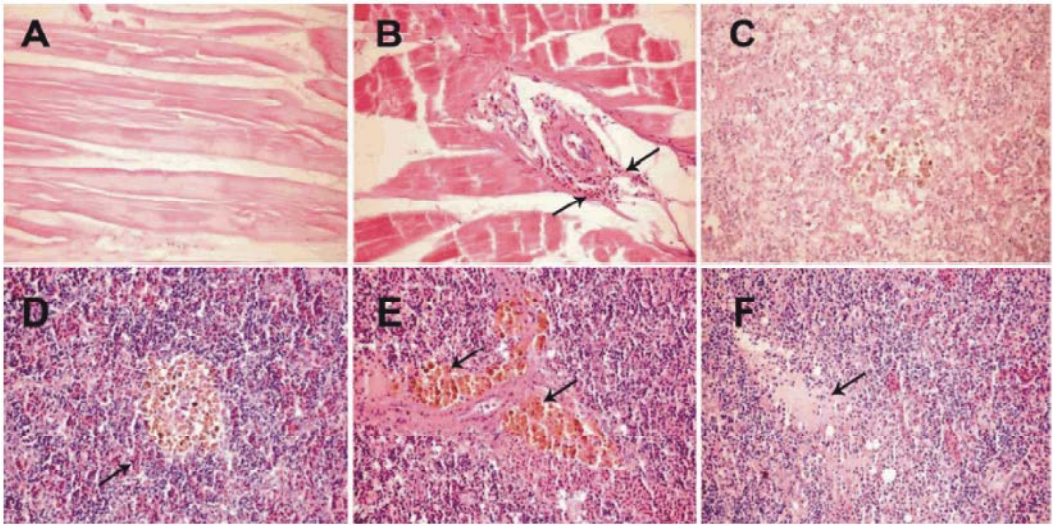

Fig. 6: Histological appearance of different tissues in *O. niloticus*. Photomicrographs of muscle tissues; (A) no histopathological changes (B) perivascular oedema and hemorrhage Photomicrographs of spleen tissues; (C) normal structure (D) marked congestion associated with diffuse infiltration with inflammatory cells (E) hyperactivity of melanomacrographe centers around blood vessels; (F) focal area of necrosis. All tissues stained with hematoxylin and eosin (x400). A and C photomicrgraphs represented reference area, while the others represented polluted area.

lamellar deformations with prominent abnormal arrangement, congestion and necrosis of gills' lamellae (Fig. 5B-C). Microscopical examination of kidney sections of tilapias collected from the reference area revealed the presence of normal renal corpuscles, glomerular tufts and renal tubules (Fig. 5D). In polluted area, histopathological examination of sampled tilapias' kidney tissues revealed an interstitial nephritis and hyalinosisof the renal blood vessels' wall. The tubular system exhibited focal necrosis which further replaced by massive inflammatory cells and free RBCs (Fig. 5 E,F,G) together with interstitial nephritis in some renal portions. Hepatic tissue of fishes collected from polluted areahave presented vacuolar degeneration with hepato-pancreatic necrosis (Fig 5H) compared to Norma liver section reference area (Fig. 5I). Muscle tissues of tilapias collected from Area 2 showed perivascular edema and hemorrhagic myositis (Fig. 6B) compared to normal arrangement of muscle bundles in case of those fishes from reference area (Fig. 6A). Splenic tissues of reference area fishes have demonstrated normal organization of splenic compartments (Fig. 6C). However, the splenic tissues of Area 2 showed activation of melanomacrophage centersaround blood vessels (Fig. 6D). Splenic tissues were congested, necrotic together with diffuse infiltration of inflammatory cells (Fig. 6E,F).

DISCUSSION

Alterations in immunological functions of an aquatic organism can be used as immunological indicators for evaluating the direct effects of exposure to any pollutant [24]. The external barriers separating the fish from its environment, i.e., the epithelia of skin, gills and alimentary canal, is the first line of defense. These epithelia work as mechanical barriers to invading pathogens, but they also contain chemical (antibodies, lysozyme, etc.) and cellular (immune cells) defenses. In fact, a wide variety of chemicals has been reported to impact immune parameters of teleost fishes [25,26].

According to Egyptian Chemical Standards, the maximum permissible limits of heavy metal in surface water are 5 mg for Zn, 1 mg/l for Cu, 0.01 mg/l for Cd, 0.05 mg/l for Pb and 1 mg/l for Fe [27]. Comparable U.S. Environmental Protection Agency(US-UPA) standards are 5 mg for Zn, 0.5 mg/l for Mn, 1 mg/l for Cu, 0.01 mg/l for Cd, 0.005 mg/l for Pb and 0.3 mg/l for Fe [28]. In the current study, the water samples from Area 2 were polluted with Pb and Fe which exceeded the permissible limitsaccording to US-EPA and ESC. In contrast, levels of Zn, Cu, Mn and Cd from both sites are within the permissible limits according to US-EPA [28], ESC [27] and WHO [29] standards. This pattern of heavy metal traces

in water of predictably polluted area was consistent with the levels of heavy metals in water samples from the river Tributaries in Egypt [30]. Cadmium and Pb concentrations were comparable with Gomaa *et al.* [31] from Greater Cairo and with Monroy *et al*. [32] in Lake Titicaca. However, lower concentrations in raw water samples from Greater Cairo in Egypt were reported by Mohamed and Osman [33]. In polluted area, there are industrial activities that can introduce Cd and Pb to River Nile water. In addition, discharge of various treated and untreated liquid wastes to the water can introduce large quantities of heavy metals to the River Nile [34].

Interestingly, the fish collected from the industrial zone showed a significant increase in the total proteins. This result was in full agreement with previous study of Salah El-Deen *et al.* [35]. The increase in serum total protein level in fish may be attributed to several biological conditions as damage of liver, kidney and gills [36]. Also, elevation in serum protein level was reported by Ghazalyand Said [37] in case of *O. niloticus* exposed to sub-lethal concentration of Cu (1.14 mg/l) for 96 h. Herein, the increase of total proteins is associated with the reduction of total globulins. It is reported that Cd treatment inhibited the antibody levels in cunners (*Tautogolabrus adspersus*) [38].

Lysozyme is a major component of the fish innate immune system involved in inflammatory processes [39] acting against parasitic, bacterial and viral infections [40]. In the present study, the lysozyme activity was inagreement with Zelikoff *et al.* [41] who have concluded that lysozyme activity was not affected in serum of rainbow trout exposed to similar types of pollution. In other studies, lysozyme levels were reduced in the kidney of carp [42]. It is possible that heavy metals had a limited effect on lysozyme activity in the Nile Tilapia.

The principal cells involved in phagocytosis process are tissue macrophages, neutrophils and monocytes in blood [6]. The main functions of the phagocytic cells are to phagocytose tissue debris and microorganisms, to mount immune response regulating mediators and to bridge innate and adaptive immune responses. In the current work, there was a marked reduction in phagocytosis percentage in polluted area. Our results contradicts other studies documenting that zebra fish Cu exposure suppressed phagocytosis in European sea bass [43] and *Daniorerio* [44]. Similar results were also demonstrated in experimental fish [45].

It has been implied that there is an association between DNA damage in aquatic animals and contamination of aquatic environment [46,47]. In the present assay, we found that the DNA strand breaks increased statistically in tilapia collected from polluted area compared to fish collected from reference area. Likewise, comet fish exposed subchronically and chronically to effluents from a Swine industry associated with greater DNA damage [48]. Increased number of DNA strand breaks correlated with immunotoxicity in dolphins [49]. Further, Erythrocytes genotoxicity of *O. niloticus* exposed to polluted river water samples was most recently confirmed in Fuzinatto *et al*. [50] study.

In the literature, heavy metal concentrations in the tissue of freshwater fish vary considerably among different studies. The accumulation patterns of contaminants in fish depend on both uptake and elimination rates [51]. In the current study, the low accumulation of Cu in gills may be due to development of some defensive mechanism such as excessive mucous secretion and clogging of gills which runs parallel with similar explanation of Eissa *et al.* [4] who described similar mechanisms of gills of Nile tilapias and catfish that have survived a colossal environmental crisis of Mariotteya water body in 2009. Additionally, Cu can combine with other contaminants such as ammonia, mercury and Zn to produce an additive toxic effect on fish. The present results agreed with previous studies which found that Cu and Zn showed higher concentrations in liver than flesh muscles of different fish species from Egypt and different places of the world [31, 52]. Recently, it has been pointed to Cu rapid deposition in muscles at early exposure, but liver acted as a terminal Cu storage area at prolonged exposure [53]. Our data revealed that accumulation pattern of the trace heavy metals in fish liver from contaminated area were in comparable with liver of *O. niloticus* obtained from industrial area of Shoubra El-Khema [54].

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 obtained data illustrated that pattern of Cd and Pb accumulation in different tissues were in agreement with Rashid [55] who indicated that the highest concentration of Pb in fish were kidney and liver followed by bone and muscles of *O. niloticus* from Nile River at Assiut region. Our results showedthat the Mn is highly accumulated in gills than other organs of fish collected from both areas. These results are in accordance with Osman and Kloas [56] who demonstrated that Mn was highly concentrated in the gills of fish from early six sites along the whole River Nile. Fe is an abundant and important element, unsurpassed by any other heavy

metals in the earth's crust [54]. The increase of Fe accumulation in fish in all examined tissues was higher than the other metals possibly due to the increase of total dissolved Fe in Nile water and consequently increases the free metal Fe concentration and there by lead to an increase in metal uptake by different organs [57]. Further, the presence of the regional Iron and steel factory in El-Tebeen and its chronic dumping of factory effluents into River Nile area could be another staggering cause of such elevation of Fe levels in water as well as fish tissues. Interestingly, muscles were found to accumulate the lowest levels of heavy metal residues for all investigated metals in fish muscles from the studied both sites. Such tiny concentrations of heavy metals might have reached tissues through circulation [56].

Histological examination of liver showed increased vaccuolation with congestion of blood vessels and necrosis of hepatocytes which may be attributed to the cumulative effect of heavy metals and the increase of their concentrations in the hepatic tissue. These results agreed with several previous studies [58]. In addition, the pathological liver steatosis was reported by Kranz and Peters [59], accompanied by additional features such as necrosis, lymphocyte infiltration, deposition of ceroids and aggregations of macrophages. The current histological study of the gills of *O. niloticus* in polluted area showed several histological alterations, including glamellar deformations, congestion and necrosis compared to reference area. It is possible that the damage of the gills could be a direct result of the heavy metals [60]. It is previously indicated to the presence of infiltrations of inflammatory cells in secondary cells [61]. Mucinous metaplasia of lamellar epithelial lining is considered as adaptive mechanism against heavy metal toxicity.

CONCLUSION

The results presented in this work suggested the possible immunotoxic and genotoxic effects of heavy metals on Nile tilapia. These data implied into the importance of immunological assays as environmental monitoring especially in industrial effluents.

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