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Bioaccumulation of some heavy metals in water and tissues of naturally infected *Oreochromis niloticus* from two polluted sites in Egypt, with reference to related oxidative stress

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ABSTRACT

The present research was planned to assess the harmful effects of heavy metals as well as antioxidant alterations of *Oreochromis niloticus* collected from two polluted fish farms located in Abbassa and Manzala (areas A and B, respectively) in Egypt. They were compared with reference site in central laboratory for aquaculture researchers during the summer season, 2020. A total number of 75 fish collected from all sites was subjected to clinical, postmortem, and bacteriological examination. Water parameters, as well as heavy metals (arsenic, manganese, and nickel) in water, serum, and tissues (muscle, gills, and liver), were evaluated. In addition, antioxidant enzymes (SOD and CAT) and (MDA) were assessed in the serum of *O. niloticus.* Bacterial isolates, *Aeromonas hydrophila*, were identified in fish at both localities, and the recorded mortality rate was about 30% and 5% in areas A and B, respectively. The levels of As in water samples from both polluted areas were higher than the permissible limits. In addition, serum of *O. niloticus* showed a significant decrease in the concentrations of Ni in both polluted areas. In addition, a significant decrease in the mean levels of Ni and As was found in the gills and muscles of *O. niloticus* in both polluted areas. Moreover, a significant decrease in CAT, combined with a significant increase of SOD activities was observed in polluted area A.

INTRODUCTION

Tilapia is the most cultured fish worldwide. Actually, the production of tilapias is considered one of the most important species in the 21st century aquaculture (**Amal & Zamri-Saad, 2011)**. *Oreochromis niloticus* is the most cultured fish, the fastest growing aquaculture, being adapted to a wide range of environmental conditions, tolerant to high stocking density, and is relatively resistant to stress and diseases (**Conroy *et al.*, 2008*)***.**

Water contaminated with heavy metals has become a worldwide problem, especially in aquatic ecosystems; originated from industrial wastes, agriculture drainage and sewage disposal **(Singh *et al.*, 2007)**. Hence, aquatic organisms may face potential hazards that would disturb metabolic pathways of cells; inducing cellular responses attributed to metal property and concentration (**Monteiro *et al.*, 2010)**. Fish is a good bio-indicator, because it concentrates heavy metals at high levels from water. In addition, fish acts as an important indicative factor in freshwater systems to estimate metal pollution and the potential risks of human consumption **(Aktar *et al.*, 2011*)***. Some heavy metals, such as Mn and Ni, are essential to biota at trace levels and recommended as daily dietary supplements **(Alloway, 2013)**. But, if their levels were above the upper permissible limits in aquatic ecosystems, they would be considered contaminants *via* inducing harmful synergistic effects on the living organisms **(CCME, 2008)**. Furthermore, poor water quality (with decreased dissolved oygen and increased NH3) would induce stress factors resulting in increased susceptibility of fish to bacterial diseases. This would lead to a decrease of both fish production and export chances and negatively affects the environment and human health with respect to laborers and consumers **(Mur, 2014)**.

In addition, in the aquatic media, arsenic changes the fish hematological and biochemical parameters that help identifying As pollution in environmental biomonitoring **(Lavanya *et al.*, 2011)**. Moreover, the high concentration of As used in agriculture and industry scales contaminates the soil and water at a localized scale and disturbs many physiological systems such as growth, ion regulation, immune function and enzyme activities **(Pedlar *et al.*, 2002a; Datta *et al.*, 2009)**. Mass mortalities of the Nile tilapia in Egypt in summer are due to poor water quality and high levels of heavy metals accompanied by pathogenic bacterial infection **(Abdel-Razek *et al.*, 2016)**. Nickel has been noted with immunosuppressive effect on teleost fish **(Kubrak *et al.*, 2012)**.Consuming As-contaminated fish leads to opposing health effects on humanbeings because As **(Kar *et al.*, 2011)** induces a series of molecular events involved in oxidative stress, iron homeostasis and lipid metabolism disorder **(Xu *et al.*, 2013)**. Moreover, manganese is a low toxic metal, but has a considerable biological significance and accumulates in fish species **(Ibrahim & Omar, 2013)**.

Aeromonas hydrophila is the most pathogenic bacteria, causing motile aeromonas septicemia (MAS) in several fish species worldwide **(Austin & Austin, 2016)**. It is the main cause of mass mortalities among farmed Nile tilapia in Egypt **(Abdel-Latif & Khafaga, 2020)**. Nowadays, farmers who demand freshwater to be reused for crops, argue that drainage water negatively affects the quality of farmed fish owing to the accumulation of pollutants and potential contamination of fish **(FAO, 2014)**. On the other hand, biochemical alterations were documented in a variety of fish intoxicated with heavy metals **(Gioda *et al.*, 2007)**. Specifically, enzyme activities that promote oxidative damage by raising the cellular concentration of reactive oxygen species (ROS) in fish, which can detoxify by induction or inhibition to protect cells and tissues from oxidative

damages as a response to pollutants, consequently, a response of antioxidative defenses is notified **(Monteiro *et al.*, 2010)**. Antioxidant enzymes, such as catalase (CAT), and superoxide dismutase (SOD) are released either through induction or inhibition to protect cells and tissues from oxidative damages as a response to pollutants **(Doherty *et al.*, 2010)**. Nevertheless, an overproduction of ROS cause an increase in the lipid peroxidation (LPO) which is a principal indicator of oxidative damage. Thus, estimation of these indicators is effective biomarkers for observing free radicals, and affecting the cell viability by damaging the cell membrane following the exposure to environmental pollutants **(Nordberg & Arne´r, 2001)**. Hence, the present work was conducted to observe the concentrations of heavy metals (As, Mn, Ni) in water and serum levels, and its bioaccumulation in the muscle, gills, and liver tissues of the Nile tilapia. In addition, this work aimed to estimate antioxidative enzymes (SOD, CAT) and MDA in the serum. The distribution of pollutants in the fish organs and the differences of metal concentrations in sampling locations were examined.

MATERIALS AND METHODS

Study Area

Three selected sites were chosen, including Central laboratory for aquaculture researchers as a reference site, Abbassa private fish farm (an industrial polluted site, where water is supplies from Ismailia Canal), and El Manzala fish farm (an agricultural polluted site, where water is supplied from Hadous drain) (Fig. 1), during summer seasons with heavy recorded mortalities, 2020.

Determination of heavy metals and water quality parameters:

Nine water samples (3 samples from each site) were collected in clean and dark brown-coppered one- litre glass bottles, which had been de-tergered, washed and rinsed with dilute HNO3, double de-ionized distilled water prior to the collection. The container was immersed about 15 cm below water surface for collection in line with standard method **(Gregg, 1989)**. On the sampling day, collected water samples were immediately transported to the laboratory for analysis of water parameters, such as dissolved oxygen (DO), temperature, ammonia toxicity (NH3), pH, and total hardness, in addition to heavy metal analysis (As, Ni, Mn). All were determined using Inductivity Coupled Plasma (iCAP^{TM} 7000 plus Series ICP-OES, Thermo Scientific^{TM}).

Collection of serum and tissue samples of the Nile tilapia

A total number of 75 fish (25 *O. niloticus* at each site) with average body weight of 200±50 g was randomly collected from three selected sites. Blood samples were collected from caudal vein ventral to the anal opening by 23-gauge syringes with acute angle and directly drawn in test tubes without EDTA. Then, blood samples were kept in room temperature until clot formation, centrifuged at 3000 rpm for 10 min for serum

separation, and stored at –20°C until measurement of heavy metals concentrations and antioxidant/oxidant enzymes estimation. Afterwards, fish samples were transported immediately to the laboratory of fish Disease and Management, Faculty of Veterinary Medicine, Mansoura University. Fish was subjected to clinical, postmortem (PM) examination following the methods of **Schaperclaus (1992)**. Following bacterial isolation, three tissues; gills, muscle, and liver were dissected from different areas.

Bacteriological isolation and identification

Primary isolation was done from previous described tissues according to **Noga (2010)**. Under complete aseptic conditions, loopfuls were taken from internal organs, then inoculated on tryptic soy broth and agar (Oxoid, CM 0129), and incubated at 25°C for 24 hrs. Bacterial isolates were identified according to the methods of **Austin and Austin (2007)**. The biochemical tests used were catalase and oxidase. Final confirmation of bacterial strains was performed using API 20 E®, API 20NE® according to manufacture guide of BioMerieux, Marcy l'Etoile, France **(Nicky, 2013).**

Estimation of heavy metals in tissues of the Nile tilapia

Dissected gills, liver, and muscles were washed by normal saline and kept at -20°C for detection of accumulated heavy metals as As, Mn, and Ni using Buck scientific 210VGP Atomic Absorption Spectrophotometer (5010, Germany).

Estimation of antioxidant enzymes and/or oxidative stress markers in the Nile tilapia serum

Estimation of SOD, CAT, and MDA were determined using Commercial Diagnostic Kits (Biodiagnostic Com., Giza, Egypt) by Atomic Absorption spectrophotometer (5010, Germany). SOD activity was measured calorimetrically in accordance to the method of **Nishikimi *et al.* (1972)**, which depends on the ability of enzyme to prevent the phenazine methosulphate-mediated reduction of nitroblue tetrazolium dye. The CAT activity was estimated in accordance to the method of **Aebi (1984)**, which reacts with a known quantity of H_2O_2. The reaction is blocked after exactly one minute with catalase inhibitor and in the presence of peroxidase (HRP). The remaining H_2O_2 reacts with 3,5 Dichloro -2-hydroxybenazone (AAP) to form a chromophore with a color intensity inversely proportional to the amount of catalase in the original sample**.** In addition, MDA level was measured according to the method of **Ohkawa *et al*. (1979)**. The test depends on MDA which reacts with thiobarbituric acid (TBA) in an acidic medium at temperature of 95^{o}C for 30 min to form thiobarbituric acid reactive product, and the absorbance of the resultant pink product can be measured at 534 nm.

Statistical analysis

The present data were statistically analyzed by SPSS (statistical package version25) *via* the analysis of variance One way "ANOVA" followed by Tukey's multiple

tests as a post-hoc test to compare different parameters between the studied areas. The data values were expressed as means ± standard error of mean (SEM). Significance $^{*}p$ *value*$\leq$ 0.05, $^{**}p$ *value* $\leq$ 0.01,$^{***}p$ *value* $\leq$ 0.001 were also determined. Correlation coefficient (r) was used to fit the relationship between the concentration of heavy metals in water and various tissues of collected fish, while the values of heavy metals in water were compared to the permissible limits **(WHO, 2008)**.

RESULTS

1. Clinical signs and PM examination

Diseased fish from both areas showed an increase in the secreted mucous on the body surface; an exophthalmia of eye and darkness of skin, congestion of the gill lamellae with high mortalities (about 30% and 5% in area A and B, respectively). Congestions and hemorrhagic lesions were detected in some internal organs (Fig. 2).

2. Water quality

As shown in Table (1); the present study revealed that temperature was higher during summer in the water samples collected from all sites; salinity and ammonia levels were also increased in water samples collected from both sites. On the other hand, the DO and the pH levels decreased significantly in water samples collected from both polluted sites compared to the reference site.

3. Water heavy metal concentrations

The current research showed high concentrations of As, Mn, and Ni in water samples collected from both polluted areas (A, B) compared to the reference site. According to the Egyptian standards, the reference site was below the permissible limits in As, Mn, and Ni concentrations. As level exceeded the permissible limits in both polluted areas (A,B). Concentration of heavy metals in water of reference site ranked from: Mn > As = Ni, while heavy metals concentrations in water of area A: As > Mn= Ni. Finally, levels of heavy metals in water of area B: Mn > As > Ni (Table 1).

4. Assessment of heavy metals in serum

A significant decrease was detected in the mean concentrations ($p \leq 0.05$) of Ni in both polluted areas (A and B) compared to reference site. Thus, the concentration of heavy metals in area A and B was ranked as follows: As > Mn>Ni (Table 2).

5. Antioxidant and/or oxidant enzymes in the serum of *O. niloticus*

The results of the antioxidant activity levels in serum shown in Fig. (3) revealed that a significant decrease in serum CAT activity ($p \leq 0.05$) was observed in area A compared to the reference site. Moreover, a significant increase was noted in the mean serum of SOD activity ($p \leq 0.05$) in area A compared to the reference site.

6. Determination of heavy metals in tissues of *O. niloticus*

Considering As presented in Table (3), a significant decrease was determined in the mean levels of As ($p \leq 0.01$) in the liver of the *O. niloticus* in both areas (A, B) compared to the reference site. However, As levels in gills were significantly decreased

($p \leq 0.05$) in area A more than in area B ($p \leq 0.01$) compared to the reference site. In addition, muscle As levels were significantly decreased ($p \leq 0.001$) in both areas (A, B). Moreover, the mean levels of Ni in gills were significantly decreased in area A and B ($p \leq 0.01$; $p \leq 0.001$) when compared to the reference site. Besides, levels of Ni in muscle were significantly decreased in area A and B ($p \leq 0.05$; $p \leq 0.01$) in comparison to reference site.

7. Correlation between heavy metal levels in water and tissue samples

The Pearson coefficient indicates that, the concentrations of different traces of heavy metals in various tissues of fish caught from polluted areas are greatly dependent on the concentrations of those elements in the raw water. The relationship between heavy metal concentrations in fish organs and external water environment was variable between positive and negative correlations. As demonstrated in Table (4), in polluted site, concentration of Mn in water exhibited positive correlation with those in all tissues of *O. niloticus* (liver and muscles) and lower positive correlation in gills. In addition, levels of As in water showed a strong negative correlation with As in liver and muscles and significant correlation in gills, despite the strong correlation in muscles. Ultimately, concentrations of Ni in water exhibited a strong negative correlation with its concentrations in gills and muscles more than in the liver.

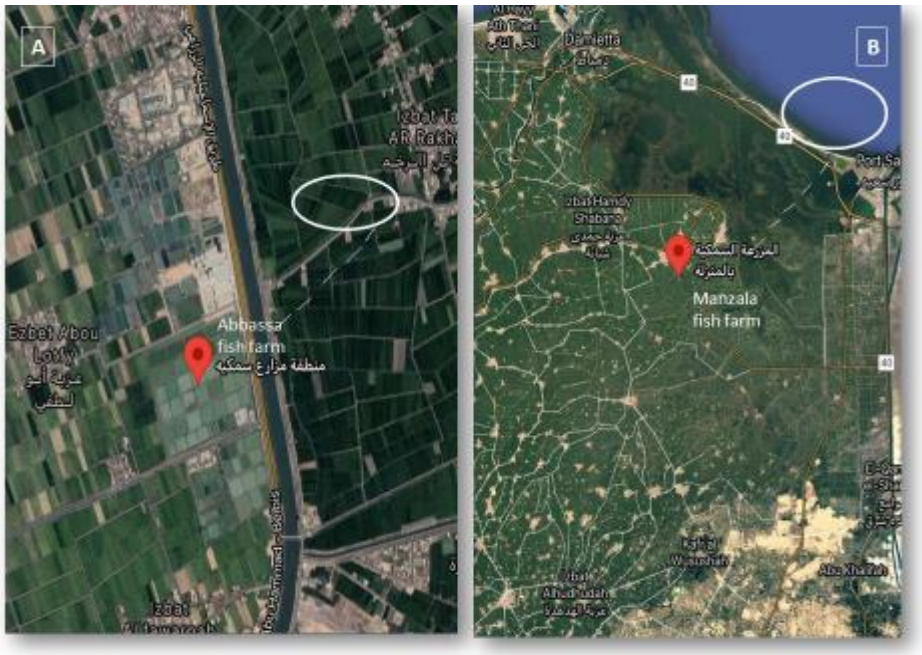

Fig. 1. A map from the Google earth showing the polluted areas (Abbassa private fish farm (area A) in Abbassa, Abou-Hammad, Sharkeia governorate, Egypt and Manzala fish farm two 5.2-ha earthen ponds Manzala, Dakahlia governorate, Egypt (area B).

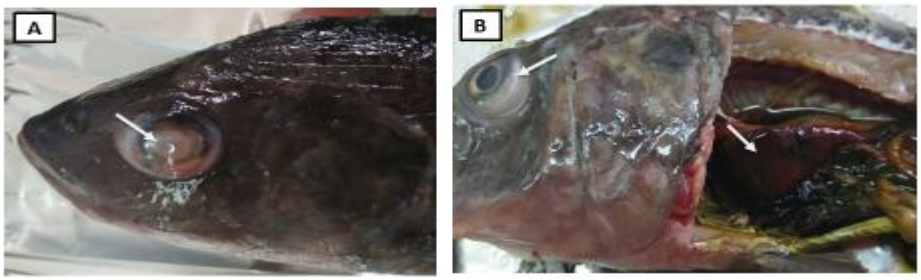

Fig. 2. Clinical signs (A) of *O. niloticus* fish showing exophthalmia (White arrow), excessive mucous secretion and hemorrhage spots at the head and postmortem lesions (B) of diseased *O. niloticus* fish, showing exophthalmia (white arrow) and congestion of liver (white arrow), enlargement of intestine and gall bladder.

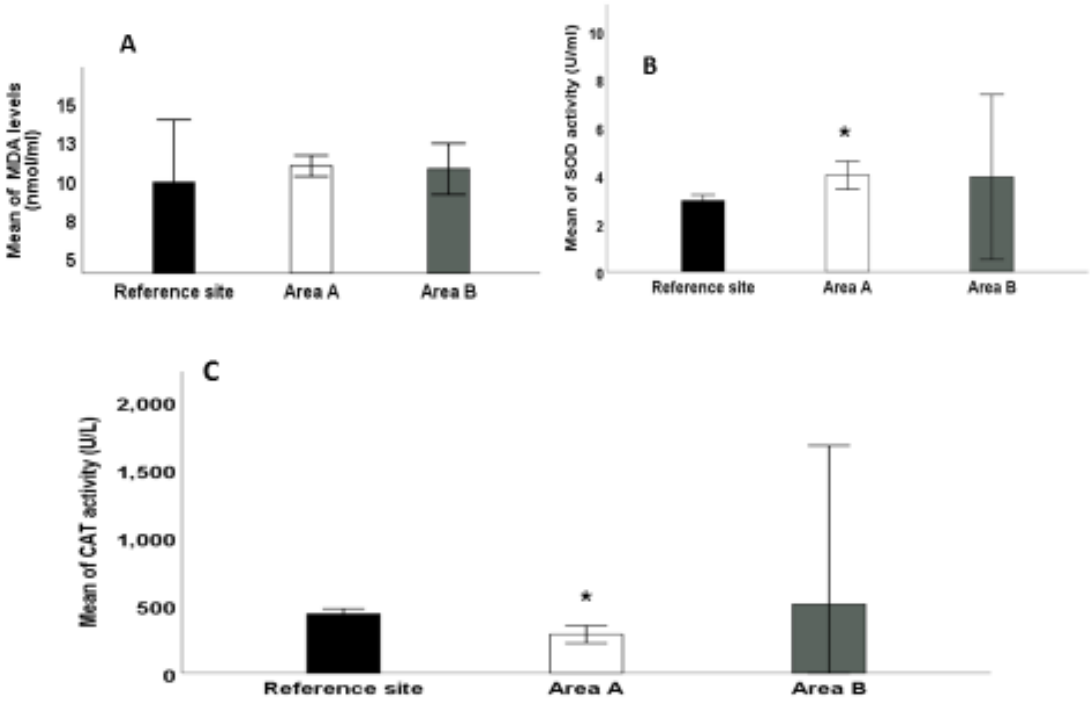

Fig. 3. The mean levels of **MDA** (nmol/ml) (A), **SOD** activity (U/ml) (B), **CAT** activity (U/L) (C) in the serum of *Oreochromis niloticus* from two different polluted sites (area A, B), compared to the reference site. Statistical significance ($^{*}p \leq 0.05$).

Table 1. Physico-chemical parameters of water quality and concentration of heavy metals (As, Mn, Ni) in water samples (mg/l) collected from reference site, polluted area A and polluted area B.

	Reference site	Polluted Area A	Polluted Area B	Range Guide line according to WHO 2008
pH	7.9	7.2	7.4	6.5-8.5 mg/l
Salinity	0.1 g/L	0.2 g/l	0.9 g/l	0-5 g/l
Dissolved Oxygen	5	٣	3.5	>4mg/l
Temperature	31^{o}c	34^{o}c	32^{o}c	35^{o}c
NH3	Zero	0.01	over range	0.02 mg/l
Hardness	240	158	460	500mg/l
As	N.D	0.04	0.03	0.01mg/l
Mn	0.0003	0.01	0.1	0.5mg/l
Ni	N.D	0.01	0.01	0.1 mg/l

Table 2. Concentration of heavy metals in serum of *O. niloticus* fish.

Metals	Reference site	Polluted Area A	Polluted Area B
As	4.38±0.82	3.1 ±0.56	2.5± 0.26
Mn	0.09±0.04	0.7 ±0.24	0.3±0.07
Ni	1.3 ± 0.37	0.54±0.09*	0.3±0.1*

Data are represented as mean ± SEM (standard error of the mean).
Indicate a significant variation between 2 polluted areas compared to reference site by One way "ANOVA" at: **p value* ≤0.05

Table 3. The concentration of heavy metals in liver, gills, and muscle (mg/g wet weight) of *O. niloticus* collected from reference and polluted areas.

Metals	Reference site	Polluted Area A	Polluted Area B
As			
Liver	9.2±1.01	2.75±0.89**	2.6±0.7**
Gills	8.83±1.21	5.48±0.76*	3.79±0.51**
Muscle	8.4±0.43	4.34±0.24***	3.2±0.43***
Mn			
Liver	0.24±0.03	0.22±0.08	0.45±0.16
Gills	0.44±0.05	0.92±0.16،	0.74±0.17
Muscle	0.1±0.02،	0.69 ±0.28،	0.53±0.24،
Ni			
Liver	1.48±0.59	1.1±0.4	0.73±0.25
Gills	3.03±0.16	1.03±0.26**	0.78±0.21***
Muscle	1.82±0.3	0.78±0.24*،	0.52± 0.13**

Data are represented as mean ± SEM (standard error of the mean).
Indicate a significant variation between 2 polluted areas compared to reference site by One way "ANOVA" at: *p *value* ≤0.05, **p *value* ≤0.01, ***p *value* ≤0.001.

Table 4. Relationship between the concentration of heavy metals (As, Mn and Ni) in water (mg/l) and in some tissues (mg/g wet weight) of *O. niloticus* collected from polluted areas.

	Heavy metals in water (mg/l)		
	As	**Mn**	**Ni**
Liver	**-0.944**	**0 .963**	**-0.670**
Gills	**-0.997 ***	**0.488**	**-0.888**
Muscles	**-0.995**	**0 .938**	**-0.992**

Pearson correlation coefficient (r)

DISCUSSION

Water pollution is the greatest environmental and public health problem facing aquaculture in Egypt **(Anwar, 2003)**. *Oreochromis niloticus* is an important commercial fish in the world, which promptly responds to environmental alterations **(Vijayan *et al.*, 1996)**. Regarding clinical observations in affected *O. niloticus* in selected polluted areas, the previous clinical findings agree with those of **Svobodová (1993)**. Concerning the internal hemorrhagic lesions in the liver and kidney, results concur with those of **Abd El-Gawad (1999)**. In addition, many environmental stressors affected the liver, causing metabolic disorders and structural destruction, and possibly lead to death **(Brusle & Anadon, 1996)**. Consequently, bacterial pathogens, such as *Aeromonas* spp. have been

associated with lethal impacts in the farmed tilapia **(Marcel *et al.*, 2013).** In this study, *Aeromonas hydrophila* was isolated from two polluted areas. It was reported that, *A. hydrophila* can induce severe economic losses of cultured fish, especially when correlated with poor water quality as un-ionized NH_3 **(Abdel-Latif & Khafaga, 2020).** Similar results were determined on investigating heavy mortalities of the farmed Nile tilapia during the summer season following an *A. hydrophila* infection with relevant poor water parameters **(El-Son *et al.*, 2019**). This poses the strong relationship between environmental pollution and the stress response in fish, thus facilitating infectious diseases to be pathogenic microbes **(Velkova-Jordanoska *et al.*, 2008).**

Low oxygen concentration in water causing balance disturbance of oxygen levels in tissues interferes with antioxidant defenses **(Oliveira *et al.*, 2010)**. Moreover, excess NH3 modifies cellular metabolism, and thus, reduces the cellular ATP concentrations **(Costa *et al.*, 2008)**. In this study, higher NH3 and lower DO levels were detected in both polluted areas. The current results are correspondent with those of **Nofal *et al.* (2019)**, who reported a decrease in DO and an increase in NH3 in Manzala ponds rearing the Nile tilapia in summer season. Besides, the rise in temperature stimulates all metabolic processes *via* oxygen consumption, thus, increasing ROS production with relevant oxidative stress in fish **(Bagnyukova *et al.*, 2007)**. Increased water temperature in the present study is in agreement with the finding of **Osman *et al.* (2010)**, who related the high concentration of heavy metals during summer to the increase in water temperature, causing high metabolic and respiratory activities in fish.

Agricultural drainage water, used illegally in farms, contains high concentrations of heavy metals, which in turn, contaminate fish tissues with toxins, pathogenic micro-organisms, and several chemical substances (**Authman *et al.*, 2013)**. Consequently, fish are the main aquatic food that may accumulate large amounts of metals from water **(Mansour & Sidky, 2002)**. Therefore, concentration of heavy metals in different organs is used as an indicator of metal pollution in the aquatic system **(Qadir & Malik, 2011)**. Concernrs have been directed to heavy metals due to their toxicity and ability to bio-accumulate in aquatic ecosystems **(Mohammadi *et al.*, 2011)**. Those metals are classified as potentially toxic (As), probably essential (Ni) and essential (Mn) **(Biswas *et al.*, 2011)**. These metals produce toxic effects when the metal intake elevates the permissible limits excessively **(Tuzen, 2003)**.

As is considered one of the most alarming chemical in the environment **(ATSDR, 2002)**, exhibiting heavy toxicity even at very low concentrations **(Olmedo *et al.*, 2013)**. Exposure of fish to different concentrations of arsenic affects the phagocytic ability of macrophages and helps in the diffusion of bacterial pathogens into distant host tissues **(ATSDR**, **2003)**. As accumulation affects several physiological systems in fish as immune function and enzyme activity **(Datta *et al.*, 2009)**, and so waterborne As significantly accumulated in the gills and liver, causing toxicity to fish (**Tsai & Liao, 2006)**. Moreover, Mn is low toxic metal, but has a considerable biological significance

and accumulates in fish species **(Ibrahim & Omar, 2013)**. In addition, Ni is a potential hazard to the environment and has immunosuppressive effect in teleost fish (**Atchison *et al.*, 1987; Kubrak *et al.*, 2012).**

It was observed that higher As concentration was detected in water, however, it showed a significant decrease in the serum and tissues. While, the higher levels were in gills and the lowest concentration was in the liver (Gills > Muscles > Liver). Similar results showed higher concentration of As in the water of southern Thailand **(Jankong *et al.*, 2007)**. Dissimilar results reported that no evidence of As accumulation from water in either rainbow or brown trout **(Robinson *et al.* 1995)**. Other studies observed higher accumulation of As in liver and gills **(Bears *et al.*, 2006; Hamdi *et al.*, 2009)**. Previous reports indicated that the accumulation of As is higher in marine fish than in freshwater fish due to lower concentrations of As in freshwater environments (**Gaim *et al.* 2015).** Lower levels of Mn and Ni in water in the present study could be related to their low solubility as previously reported in the study of **Pandey *et al.* (1995)**. Similar results reported that Mn and Ni concentrations in water were under the permissible limits and recorded higher concentrations of Mn during summer at El-Kossia and Ni at Mankabad in Assiut governorate around the River Nile **(Ibrahim & Omar, 2013)**.

Converse results recorded that the highest levels of Mn and Ni were depicted in the surface water of the river Ganga **(Kar *et al.*, 2008)**. The current results detected that the highest concentration of Mn were in the gills, while the lowest was in the liver (Gills > Muscles > Liver). The same results reported the highest levels of Mn in the gills of *Tilapia Zilli* and *Clarias anguillaris* at Afikpo, Nigeria **(Nwani *et al.*, 2010)**. Dissimilar results reported that the highest value of Mn in *O. niloticus* liver was found during summer in Lake Burullus, Egypt **(Aly *et al.*, 2020)**. Moreover, a significant decrease was identified in the mean levels of Ni in the serum in both polluted areas. The Ni recorded the highest levels in the gills while the lowest levels were in the muscle (Gills > Liver> Muscles). Similar results observed gills and liver containing higher concentrations of Mn, Ni in *Clarias gariepinus* and *Labeo umbratus* **(Coetzee *et al.*, 2002)**, whereas *Cyprinus carpio* fish from the Avşar Dam Lake showed the highest concentrations of Mn and Ni in the liver followed by the gills **(Öztürk *et al.*, 2009)**. Thus, even low concentration of heavy metals in water may result in high concentration in fish flesh; which matches with the results of **Kock and Hofer (1998).**

The results showed that strong negative correlation of As were detected in muscles, while dissimilar results reported positive correlation of As content in the muscles of channel catfish; *Ictalurus punctatus,* and green sunfish; *Lepomis cyanellus* **(Maher *et al.*, 1999; Neva´rez *et al.*, 2011)**. Moreover, a positive strong correlation with the levels of Mn was observed in water and in all tissues of *O. niloticus.* This finding coincides with that of **Badr *et al.* (2014)** who reported that, in polluted site levels of Mn in water exhibited positive correlation with those in all tissues of *O. niloticus*.

Antioxidant enzymes such as SOD and CAT are considered the early defense system in opposing oxygen toxicity **(Sheriff *et al.*, 2014)**. In the present work, a significant increase was observed in the serum SOD activity combined with significant decrease in activity of CAT in polluted area A. Similar results detected higher trend of SOD and lower trend of CAT activity in *O. niloticus* in the polluted area of the Manzala farm **(Nofal *et al.*, 2019)**. This may be attributed to chronic exposure to ammonia which induces antioxidant enzymes. In addition, hypoxia could increase the antioxidant capacity, and thus, enhances their ability to reduce ROS production upon returning to normal oxygen concentrations (**Vidal *et al.*, 2002)**. These results are in accordance with those of **Huang *et al.* (2007)**. On the other hand, a decrease of SOD activity and increase of CAT enzyme were notified in the blood of *Barbus m. petenyi* Heck from Lake Ohrid in response to environmental pollution **(Velkova-Jordanoska *et al.*, 2008)**. Remarkably, MDA is the main marker of oxidative damage and its concentration gives direct evidence of the toxic process caused by free radicals **(Sieja & Talerczyk, 2004)**. Additionally, significant changes were recorded in the SOD activity and higher levels of LPO were detected in the blood of three cichlid species, *Oreochromis niloticus*, *Tilapia rendalli*, and *Geophagus brasiliensis* from river polluted by industrial effluents **(Ruas *et al*., 2008)**. On the contrary, no difference was depicted in the MDA levels in the liver of *Silurus glanis* fish in the Kizilirmak River, Kirikkale, Turkey **(Avci *et al.*, 2005**).

CONCLUSION

Alteration of water quality parameters with subsequent heavy metals bioaccumulation caused changes in the antioxidant defenses. Oxidative stress markers and antioxidative enzymes were assessed in the *O. niloticus* at polluted areas: Abbassa and Manzala farms. The current results revealed that, even low concentration of heavy metals in water may result in high concentration of metals in fish tissues. Such studies could be useful in fish farming and aquaculture production. Thus, it was concluded that the altered activities of SOD and CAT could be useful biomarkers of water pollution. In addition, this research sheds a considerable light on the bacterial infection of the Nile tilapia associated with heavy metals pollution and water quality, which are potentially pathogenic to humans.

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