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Assessment of some heavy metals in water and fish along Suez Canal with special emphasis to antioxidative enzyme profile

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Abstract
Fish mortalities among cultured Nile tilapia in 4 sectors in the Suez Canal region, Egypt have been recorded. Fishes were transported to the laboratory for clinical examination and samples were taken for histopathological examination. The physical and chemical properties of water quality parameters were evaluated. Additionally, heavy metal levels in water and fish tissues and serum oxidative stress markers such as superoxide dismutase (SOD), Catalase (CAT), glutathione peroxidase (GSH-Px), and glutathione-S-transferase (GST) were assessed. The results showed fish exhibited darkened skin coloration, congested gills with blackening, yellowish discoloration of the liver, and shrunken oocytes of the ovary. Histopathological findings revealed congestion of Branchial blood vessels, hyperplasia of the secondary gill lamellae, and Coagulative necrosis of hepatocytes. The levels of heavy metals in water and tissues were significantly elevated such as Cadmium (Cd), lead (Pb), and Iron (Fe). The oxidative stress markers such as SOD, CAT, GSH-Px, and GST were considerably elevated in the 4-sector regions of the present study. It can be concluded that elevated heavy metals can induce fish mortalities and their elevated levels in fish tissues will cause potential hazardous effects on human consumers.

Keywords: Heavy metals – SOD - CAT - oxidative stress markers – Nile tilapia

Introduction

Nile tilapia, *Oreochromis niloticus* is considered an important species in commercial fisheries around the world, which promptly responds to environmental alterations [1]. Fish is a good bio-indicator because it has the potential to accumulate heavy metals and other organic pollutants [2]. Environmental pollutants, such as metals, pesticides, and other organics, pose serious risks to many aquatic organisms [3]. Heavy metals are major pollutants of aquatic ecosystems due to disposal of industrial effluents in the river of waste material, such as sewage sludge and dredge spoil**.** They are usually toxic at high levels and may accumulate in aquatic organisms as metals are not biodegradable or eliminated from ecosystems**.** Additionally, they may interfere in several metabolic pathways of cells and thereby induce different cellular responses depending on concentration and metal proprieties [4]. Oxidative stress usually occurred through the generation of reactive oxygen species (ROS) such as the superoxide anion, hydroxyl radical (OH), singlet oxygen ($O_{½}$) and hydrogen peroxide (H_2O_2), which may generate DNA alterations and peroxidation of membrane lipids initiating cellular degenerative process [5]. ROS can be attenuated enzymatically and non-enzymatically via various scavenger compounds such as glutathione (GSH) and metallothionein (MT), and antioxidants enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and glutathione S-transferase (GST) [6].

Thus, measurements of these indicators are a valid biomarker for monitoring free radicals, among the most important antioxidant enzymes whose activity changes with pollution [7, 8]. It was reported that both copper (Cu) and zinc (Zn) can play an important and vital role in the cellular metabolism acting co-factors in several important enzymes. However, they can become toxic when elevated concentrations are introduced into the environment [9]. Likewise, nickel (Ni) is considered as an essential element at low concentrations for many organisms; it is toxic at higher concentrations [10].

Histopathological examination is a sensitive tool for determination of the impacts of certain toxicants on fish health and allows for early warning signs of disease and injury in cells, tissues, or organs [11].

Therefore, the study was focused mainly on the assessment of levels of heavy metals in water and fish tissues in 4 sectors of the Suez Canal region. Additionally, evaluation of their effects on fish tissues and oxidative stress markers.

Materials and Methods

1. Sampling area

Sampling area of the present study is illustrated in figure 1, whereas

- Sector 1 (Port Said sector): El-Boughaz, Port Fouad, El-Raswa, and El Qantara,
- Sector 2 (Lake Timsah sector): Northern and Southern entrance of the lake, Infront of Ismailia channel**,**
- Sector 3 (Bitter Lakes sector): El-Defresoir, Fayed, Fanara,
- Sector 4 (Suez Bay sector): Port Tawfiq El-Zeitiya, and Shandora.

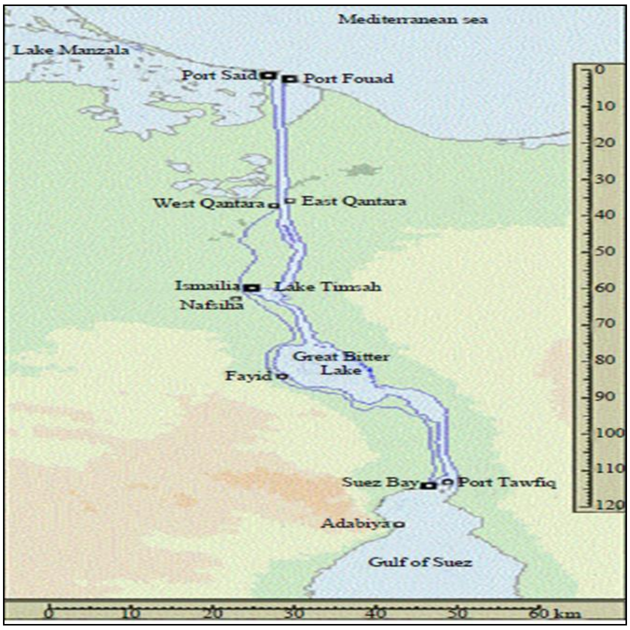

Fig 1: Map of the Suez Canal showing the selected sectors and sampling stations.

2. Data Collection

The sampling schedule for each sector was planned to depend on the collection of different data related to the nature of each sector, suspected sources of pollution, available fish species and water samples.

3. Water samples

Water samples were randomly collected from each station of the selected sectors using dark brown bottles and the column sampler at a depth of half-meter from the pond water surface. The samples were divided into two sets; 1) the first group was used for determination of the physicochemical properties of water quality, 2) the second was acidified by nitric acid and chilled on icebox for transport to the laboratory for heavy metals determination using (the water analysis spectrophotometer that purchased from the project fund).

4. Physico-chemical examination of water samples

The water samples were collected in clean and dark brown coppered glass bottle for measuring of dissolved oxygen, pH, temperature, phosphorus, ammonia toxicity (NH_3), iron, copper, zinc, Nickle, hardness, and salinity these samples are transferred to the laboratory of fish diseases at Veterinary Medicine Mansoura University. The standard method for water quality control

[12], and then the non-ionized ammonia (NH_3) was calculated from total ammonia [13].

5. Clinical and postmortem examination

Collected fish were clinically examined grossly by the naked eye for determining any abnormalities on the external body surface [14]. Gross pathological lesions in gills, abdominal cavity, and internal organs [15].

6. Fish collection and sampling

A total number of 300 fish of Nile tilapia, *Oreochromis niloticus* were randomly collected from different private fish farms (earthen pond) and natural water resources during 2017-2018 from each sector of Suez Canal region.

6. a. Blood samples

The blood samples were collected directly through the insertion of 23-gauge syringes with an acute angle from caudal vein ventral to the anal opening. They are divided into 2 parts; the first part was collected in a test tube free from EDTA and kept in room temperature till clot formation, then they were centrifuged to obtain serum at 3000 rpm for 10 minutes and kept at -20 °C. While the second part was collected utilizing EDTA (10%) to prepare erythrocyte lysates. Serum samples were used for the following determination of Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GSH-Px).

6. b. Samples of tissues

Fishes were dissected after collection of blood samples to obtain gills, brain, liver, kidneys, and muscles which are stocked in 2 parts after washing by normal saline:

1. The first part of muscles and gills were kept also at -20°C and for atomic absorption detection of accumulated metals like Iron, Copper, and Zinc using atomic absorption (Techno sens AA 1.0.2.2.1).
2. The second part of the liver, kidneys, brain, fish skin, muscles, and gills were fully immersed in 20% formalin for histopathological examination and transported to the histopathological department in Veterinary Medicine, Cairo University.

For histopathological techniques, the obtained tissue sections were collected on glass slides, deparaffinized, stained by hematoxylin &eosin stain, for long-term examination, the stained slides are covered using Canada balsam and examined with a light microscope (OLYMPUS CX21), using a reference control tissue and photographed using a digital camera [16].

7. Detection of heavy metals in fish organs and tissues

7. a. Digestion of fish samples

Muscle and gills samples were digested according to the method applied by Agemain *et al.* [17].

7. b. Heavy metals estimation

The detection and estimation of these metals collected from fish farms were carried out by using the Atomic Absorption model (Techno sens AA 1.0.2.2.1) spectrometer with hydride system Thermo made in the UK (Solar Atomic Absorption spectrophotometer). Detection of heavy metals in samples made by Atomic Absorption Spectrometry (AAS) Determination of Heavy metals Fe, Zn, Cu, Pb, Hg, Ni and Cd concentrations which as expressed by µg / g dry weight using water analysis spectrophotometer that is purchased from the project fund. The concentrations of heavy metals were expressed as mg/l for water and µg/g dry weight of the fish organ.

8. Detection of oxidative stress compounds

By using a spectrophotometer (SPEKOL11 CARL ZEISS JENA) (Germany) by colorimetric method for the detection of oxidative stress compounds in the blood, serum, and tissues by using a commercially available chemical kit (Biodiagnostic company, Cairo, Egypt). Spectrophotometer (Spekoll 11, Germany). The activities of SOD [18], CAT [19], GSH-Px [20], and GST [21].

Results and discussion

Water pollution is one of the principal environmental and public health problems facing Egypt and the Middle East region [22].

1. Clinical findings (signs and PM lesions)

Clinical signs of collected and examined fish were retarded growth, loss of activity, a dark discoloration of the skin. Gills showed congested filaments, sometimes dark in color and the gill chamber filled with turbid mucous. The gonads of *O. niloticus* are clearly suffering from deformation from their ideal shapes and shrunk of the oocyte diameter.

Fig 2: Mass mortalities among cultured Nile tilapia in sector 1, Port said area.

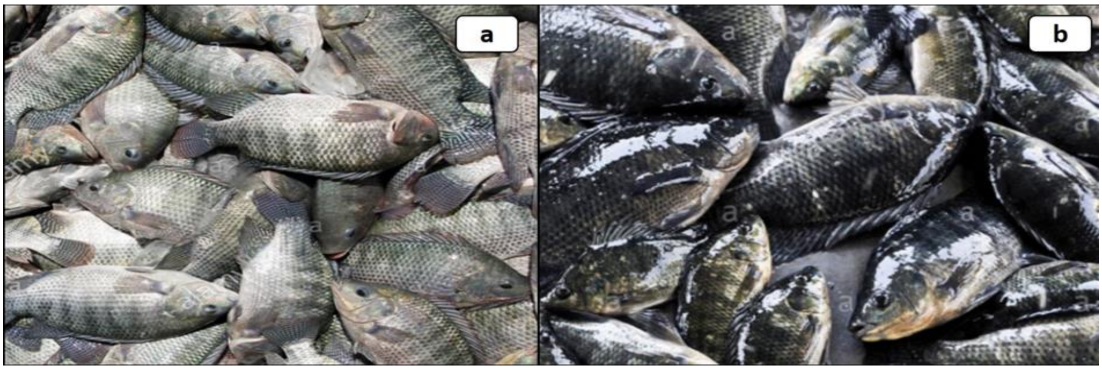

Fig 3: Nile tilapia exhibited normal skin color (Photo a), and dark discoloration of fish post-harvesting (Photo b).

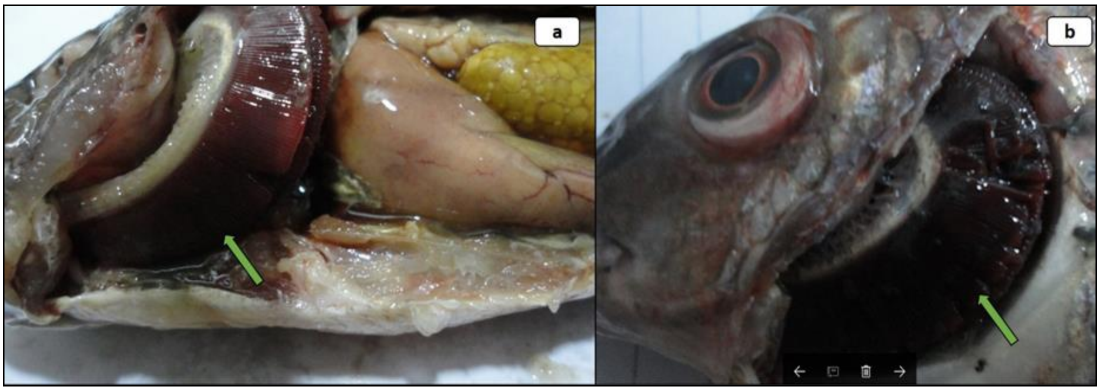

Fig 4: PM lesions of Nile tilapia showed dark discoloration of the gills (arrow) (Photo a, b) with yellowish discoloration of the liver with shrunken oocytes in gonads (Photo a).

Regarding the clinical examination of *O. niloticus*, it was found that, due to pollution, there was a loss of appetite, reduce growth, increased opercular movements, and discoloration of the skin. The gill chamber was congested and filled with mucus, congestion of the lamellae, suffocation and finally death. These findings were like the results obtained by Svobodová [23]. The gill surface tends to be alkaline, soluble ferrous iron can be oxidized to insoluble ferric compounds which then cover the gill lamellae and inhibit respiration [24].
The heavy metals like Cu and Zn, individually or in combination with other heavy metals, may exert a strong inhibitory effect on the cell division [25]. There were heavy mortalities recorded among the examined fish. It was found that due to numerous environmental stressors affect the liver and cause metabolic disturbances and structural damage, possibly leading to death [26].
Regarding the PM examination, there were pale discoloration and necrosis of the liver due to damage to liver cells, blood-filled abdominal cavity, and darkened and congested gills. These findings were agreed with the results of Abd El-Gawad [27].

2. Histopathological findings

Fig 5: Gills of Nile tilapia showing intense hemorrhage in lamellar blood vessels (arrow) (HE, 100x) (Photo a), and proliferation, adhesions of secondary lamellae and severe congestion in lamellar capillaries (arrow) (HE, 100x) (Photo b).

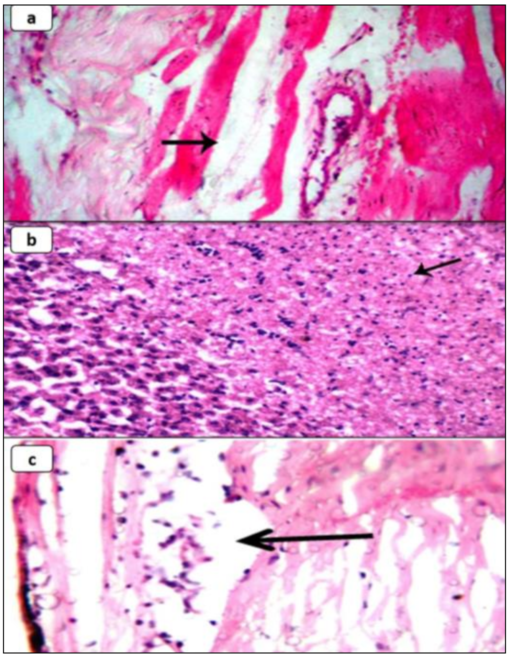

Fig 6: Skin of Nile tilapia showing edema and inflammatory cells infiltration in hypodermis (H&E stain) (x400) (Photo a), liver displayed massive coagulative necrosis of hepatocytes (arrow) (HE, 100x) (Photo b), and muscles displayed dilatation of blood capillaries in interstitial tissue with edema separating muscle fibers (arrow) (HE, 400x) (Photo c).

Histopathological changes in animals' tissues are reliable and direct indicators of environment stressors [52]. It is also the easiest method for assessing both short- and long-term toxic effects [53]. The liver is the most organs associated with the detoxification and biotransformation process is the liver, and due to its function, position and blood supply [54].

In the present study, our results revealed that there was a massive number of inflammatory cells infiltration in between the proliferated hyperplastic pancreatic cells at the portal areas and severs congestion of the portal vein while the controlled sample showing normal histopathological stricture which was agreed with Figueiredo-Fernandes *et al*. [55] who reported that the adverse effects of heavy metals were vacuolar hydropic, degeneration of cytoplasm in hepatocytes, which were finally necrotic and infiltrated with inflammatory cells that is due to exposure of Nile tilapia to sublethal levels of Cu has been shown to cause histopathological alterations in liver [56]. Also, the cellular degeneration in the liver may be also due to oxygen deficiency as a result of gill degeneration and/or to the vascular dilation and intravascular hemolysis observed in the blood vessels with subsequent stasis of blood [57].

The gills are more exposed to contaminated water and metals can penetrate through their thin epithelial cells [58], also the gills perform various vital functions (respiration, osmoregulation, and excretion) and have a large surface area in contact with the external environment**.** In the present study, the result of histopathological alteration of gills of *O. niloticus* fish due to pollution was congestion with edema in the lamellae of the filaments of intoxicated samples while controlled one was showing normal histological structure of the filament with branching lamella**e** that result was agreed with Figueiredo-Fernandes *et al*. [55] who reported that exposure of Nile tilapia to sublethal levels of Cu has been shown to cause histopathological alterations in gills (edema and vasodilation of the lamellar vascular axis). Additionally, Rosety-Rodríguez *et al*. [59] who suggested that when fishes suffer a more severe type of stress an inflammatory response could be occurred as lamellar aneurysms and blood congestion with dilation of marginal channels together with leukocyte infiltration all these results may be due to due to increase of ammonia, heavy metals, pH change and oxygen depletion [60, 61]. In the present study, the result of histopathological alteration of skin and muscles of *O. niloticus* fish due to pollution was edema with inflammatory cells infiltration in hypodermal tissue and dilatation of blood capillaries in interstitial tissue with edema separating muscle fibers that results were agreed with Tayel *et al*. [60] who reported that periphery inflammatory cells with some aggregate of glomeruli like under its connective tissue with infiltration of inflammatory cells, subcutaneous adipose tissue partly with fibrosis. A muscle with vacuolation, small blood vessels were in between, these alterations in skin and muscles may be attributed to inorganic fertilizers**,** ammonia [61], heavy metals [62] and changes in water quality [63].

3. Physico-chemical properties of water and heavy metals levels

Table 1: Average of some physical characters and heavy metals concentrations (μg/l) in water samples collected during 2017-2018 from different sectors of the Suez Canal region.

Parameters	Sector 1	Sector 2	Sector 3	Sector 4
Temperature °C	23	24.5	28	29
pH value	7.2	8.4	8.7	8.9
Salinity %	14	28.50	32	42
Heavy metals				
Cadmium (Cd)	2.00	0.09	0.08	0.40
Nickle (Ni)	1.20	1.70	2.20	4.10
Lead (Pb)	2.90	3.40	2.60	3.70
Iron (Fe)	32.40	42.50	71.10	55.90
Zinc (Zn)	17.80	2.20	12.10	20.4
Copper (Cu)	6.30	0.40	2.00	0.60

4. Levels of heavy metals in fish tissues (muscles and gills)

Table 2: Heavy metals concentration (µg /g) of Nile tilapia collected from Suez Canal Corridor during 2017-2018.

Heavy metals	Muscles	Gills
Fe	0.5 – 0.7	10.8 – 11.9
Zn	0.3– 0.4	1.9 – 2.5
Cu	0.4 – 0.5	2.1-1.8
Pb	0.2-0.1	1.3-o.9
Hg	0.0	0.0
Ni	0.0	0.0
Cd	0.0	0.0

5. Levels of oxidative stress markers

The highest values of Fe were reported in gills, and muscle when compared with the permissible limits. These results were agreed with Van Rensburg [28] who reported that the highest bio concentration of Fe in fish tissues was found in the liver and gonads. To best of our knowledge, it was found that the gills, liver, and kidney are commonly the primary target organs for many pollutants [29].

The most important antioxidant defense systems include antioxidant enzymes such as SOD, CAT, GSH-Px, and GST [30]. It was found that SOD converts superoxides (O_2-) generated in peroxisomes and mitochondria to hydrogen peroxide [31]. The SOD can catalyze the breakdown of the superoxide anion (O_2^-) to water and hydrogen peroxide (H_2O_2) that were further detoxified by the CAT and SOD enzyme system which gives the initial defense in combating the oxygen toxicity [32].

In addition, CAT plays an important role in the cell redox equilibrium [33], where removes the hydrogen peroxide by converting it to water and oxygen [31].

In the present study, there were increased activities of CAT, GSH-Px, GST and SOD and these findings were parallel to the results obtained by Lushchak [34] who reported that of the levels of these enzymes in carps from industrially polluted site as compared to the reference site due to the presence of higher peroxide concentrations [35]. GSH-Px catalyzes the reduction of both hydrogen peroxide and lipid peroxides, thus preventing the formation of free radicals formed by peroxide decomposition [36]. In our study, the results indicate that there was increased activity of GSH-Px, which agreed with Hamed *et al*. [37] who reported that increase of GSH-Px activity in *O. niloticus* and *Clarias lazera* collected from industrially polluted sites when compared to control groups.

Because GSH-Px depletion promotes the generation of ROS and oxidative stress which affecting the functional and structural integrity of cell and organelle membranes [38].

Low DO levels in water interfere with the fish population, causing death and abnormalities in the offspring. Disturb the balance oxygen supply/demand influencing oxygen levels in tissues, which interfere with antioxidant defenses [39]. In this study, the results were shown a decreased in DO in all sectors when compared to the range of guidelines [40], and this finding was agreed with Vidal *et al*. [41] who reported that hypoxia increased the activities of CAT and GSH-Px. Lushchak and Bagnyukova [42] reported that hypoxia increased the activities of SOD and CAT in the liver of common carp, *Cyprinus carpio*. It was demonstrated that the fish under low-oxygen conditions (hypoxia, anoxia) has increased their antioxidant capacity to enhance their ability to quench ROS production upon return to normal oxygen concentrations [43].

The increase in temperature stimulates all metabolic processes in accord with known thermodynamic principles; it enhances oxygen consumption and, therefore, may increase ROS production as side products of intensified metabolism resulting in oxidative stress in fish [44]. Temperature and pH affect the catalytic efficiency and binding capacity of enzymes [45]. The present results were agreed with Osman *et al*. [46] who reported that the High levels of heavy metals during spring and summer could be attributed to the changes associated with higher water temperatures, which can cause higher activity and ventilation rates in fish where in this study there are high levels of heavy metals especially zinc, copper and iron.

Ammonia is a toxic metabolite and excess ammonia is known to trigger the operation of detoxification or utilization systems, chiefly by way for the formation of less toxic nitrogenous substances [47]. The excessive presence of NH_3 alters cellular metabolism, resulting in decreased cellular concentrations of ATP [48]. In this study, our results revealed that there was increased in all levels of total ammonia, toxic ammonia (NH_3) and non-ionized ammonia (NH_4) in all sectors, and this finding was similar to that of Hegazi *et al*. [49] who reported that chronic ammonia exposure significantly increased SOD activity which was approved in our results where there are increased in SOD activity in liver, blood, and serum. Additionally, the present results were agreed with Sinha *et al*. [50] & Nofal and Abdel-Latif [51] who reported that exposure fish to high environmental ammonia (HEA) resulted in induced production of H_2O_2, increased activity of CAT, increased lipid peroxide content (MDA), increased levels of GSH-Px in the liver and no change of GST activity in the liver of goldfish and carp that is due to ammonia exposure can lead to oxidative stress in fish species.

Table 3: Levels of SOD, CAT, GSH-Px, and GST in the serum of Nile tilapia collected from Suez Canal Corridor during 2017-2018.

Oxidative stress markers	Sector 1	Sector 2	Sector 3	Sector 4
SOD(unit/mg protein)	839.708±46.95	656.77±81.24	707.35±22.42	488.19±44.56
CAT (µmol H2O2 consumed / min / mg protein)	15.77±0.54	16.29 ± 0.36	17.45±0.06	16.09±0.24
GSH-Px (µmol GSH consumed / min / mg protein)	9.50±3.06	7.20 ± 0.40	6.65±0.79	9.65±0.79
GST (µmol CDNB conjugates / min / mg protein)	0.0307 ± 0.0018	0.0482 ± 0.0063	0.0122±0.0043	0.0898±0.0242

Conclusions

Generally, it can be concluded that levels of the studied heavy metals (Pb, Cd, Cu, Zn, Ni, Hg, and Fe) in water and fish tissues that farmed in the Suez Canal corridor are significantly elevated which may seriously cause public health hazards of human consumers. These heavy metals are directly enhanced by negative human-based activities. The obtained results showed that metals accumulation in fish varied between gills and muscles and usually associated with oxidative stress responses and DNA damage of the affected fish. Using different techniques for rapid diagnosis of water pollution as histopathological alterations of fish tissues and antioxidant enzymes can be considered as promising tools.

Acknowledgments

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