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Original Article

Study of pollution in Shatt AL-Arab River using histological alternations and some other biochemical parameters of gill in Nile tilapia (*Oreochromis niloticus*) as water quality biomarkers

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Abstract: This study aims to assess the pollution of Shatt AL-Arab River using histology and some other biochemical parameters of gill in Nile tilapia (*Oreochromis niloticus*) caught in winter 2021 at four locations as water pollution biomarkers. Some water quality parameters were determined in these sites, and the results showed that sites 2 and 3 are polluted at levels above the World Health Organization's guideline. The enzymatic and metabolism activity, histological status, and cytogenetic mutation over time in the gills were assessed. Biotransformation enzymes level showed total cytochrome p450 and Ethoxyresurofin–O– demethylase (EROD) activities are significantly increased in gills of tilapia in site 1, 2 and 3. The antioxidant enzymes activities were recorded significantly high in fish gills of catalase (CAT) and glutathione -S-transferase (GST) in polluted sites of 1, 2, and 3, while superoxide dismutase (SOD) were significantly higher only in site 3 compare with reference site of 4. The metallothionein-like protein (MTLP) was significantly higher in gills at site 3. Also, lipid peroxidation (LOP) and micronucleus analysis showed that sites 2 and 3 samples site the most affected. Gill tissue index was appeared severe alterations levels at sites 2 and 3, followed by site 1 with a relatively lower level of damage, while site 4 (reference one) showed minor or invisible changes in gill tissue. The histological alterations in the gills of Nile tilapia fish at sites 2 and 3 showed atrophy of cellular, hemorrhage, congestions, hyperplasia, and hypertrophy of the filament lamellae epithelium.

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Introduction

Approximately 90% of anthropogenic discharges end up in aquatic systems resulting from industrial, agricultural, and urban activities (Siraj Basha and Usha Rani, 2003). The aquatic environment around industrial units is at high risk of contamination by different pollutants, such as heavy metals (Vinodhini and Narayanan, 2008). The histological alterations may help to detect the most affected target organs and reveal the organism's sensitivity to levels of pollutants (Wester and Canton, 1991). Histological indicators are frequent practices in fish health research that provides information on the sub-lethal and chronic effects of xenobiotics on each biological response (Schwaiger et al., 1997; Van der Oost et al., 2003). They have been used in contaminated ecosystems as proper bio-indicators (Camargo and Martinez, 2007). In a biological system, the toxicant induces changes that are considered a biomarker, providing information about the state of the ecosystem's health (Maria et al., 2009; Monteiro et al., 2010).

The potential of xenobiotics to boost cellular levels of reactive oxygen species (ROS), which can occur either through increased production or an imbalance in antioxidant cell defenses, is widely used to measure their toxicity (Gravato et al., 2006). It may cause DNA changes and oxidation of

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membrane lipids initiating the process of cellular degeneration (Sanchez et al., 2005). Some physiological ways to remove free radicals (ROS) toxicity are cellular defense systems that could be considered bio-indicators of healthy life in the surrounding water body. Defenses against free radicles include scavenger compounds like (CAT) catalase, (GST) glutathione S- transferase, (GSH) glutathione, (SOD) superoxide dismutase, (GPx) glutathione peroxidase, and (GR) glutathione reductase and (MT) Metallothionein, (Storey, 1996) as well as lipid peroxidation (LOP) (Ahamad et al., 2005). This imbalance may also cause DNA damage, which has been recommended as a bioindicator for determining the level of genetic toxicants. The most prominent example of genotoxicity in a test of the micronucleus (Mn) (Rabello-Gay, 1991) is connected with the identification of nuclear changes in fish erythrocytes, representing an important analysis tool (Carrasco et al., 1990).

The gills have important roles because, being the major position of gas exchange and taste (Hughes, 1982), function in the osmoregulation process (Verbost et al., 1994), nitrogen excretion (Sayer and Davenport, 1987), and acid-base equilibrium (Goss *et al.,* 1992). Several studies were performed on the fish gills by microscopical examination that revealed an increase in the tissue damage degrees is correlated to the water quality (Wael et al., 2013). In gills, the morphological alterations represent the adaptation strategies to maintain physiological functions and evaluate chronic or acute exposure to pollutants in the aquatic ecosystem (Morina et al., 2013). Therefore, this work aimed to study the pollution of the Shatt AL-Arab River using histology and some other biochemical parameters of gill in Nile tilapia (*Oreochromis niloticus*) as water quality biomarkers.

Materials and Methods

Specimens of Nile tilapia with body weight and length of 200±50 g and 17±3 cm, respectively, (n =10, each site) were captured from four different sites in the Shatt AL Arab River, including (1) electric power of the Haratha, (2) electric power of the Najebia, (3) Kandac canal and (4) south part of the river in Basrah, Iraq, using a fishing-net in December 2021 (Fig. 1). The sampling sites were located along the river, having various types of contamination sources (Sites 1, 2, and 3), and a clean site with relatively well preserved, be free of industrial, agricultural, and human domestic effluents was selected as site 4 (Fig. 1).

The physical and chemical properties of the river water were determined using the methods described in (APHA, 1998). In the field, a Water Quality Checker was used to measure temperature, pH, hardness, dissolved oxygen (DO), conductivity (Ec), and biochemical oxygen demand (BOD). The nitrite, PO^4, SO^4, and Chlorides (Cl^-) were determined after filtering the water (Mackereth et al., 1978). The concentration of heavy metals in the column water was determined by AAS Flam of atomic absorption spectrophotometry, using the SRM 3114 NIST (USA) as reference standards for quality control and assurance. In brief, the water samples were fixed with a solution of Nitric acid, filtered through a glass filter (0.45 lm pore diameter), and the heavy metals concentration was determined by a flamer spectrophotometer.

Biochemical analyses: The gill tissue (1 g tissue/15 ml buffer) was homogenized in phosphate buffer (0.1 M, pH 7.4). This homogenate was divided into aliquots for EROD, CAT, SOD, GSH, and GTS activity enzymes. The ethoxyresorufin-o-demethylase (EROD) enzyme level was assayed in fish gills by fluorometric methods (Galgani and Payne, 1991). Glutathione– S–transferase (GST) antioxidant enzyme activity was determined in cytosolic fraction by 1-chloro- 2, 4–dinitrobenzene as a substrate (Habig et al., 1974). Superoxide dismutase (SOD) enzyme level was measured according to McCord and Fridovich (1969). Cytosolic catalase activity (CAT) was measured at 240 nm (Aebi, 1984). The Metallothionein-like protein (MTLP) was determined in fish gills, according to Thompson and Cosson (1984).

Per oxidative and cytogenetic damage evaluation: Ohkawa et al. (1979) procedure with some

Figure 1. Map of Shatt AL-Arab River showing sampling sites.

modifications was used to determine LPO in tissue homogenates. Blood samples were taken from caudal vasculature with a heparinized syringe, the account of erythrocytes micronuclear by preparing a blood smear on a glass slide and stained with Giemsa dye (AL Sabti, 1985).

Histological examinations: The mid-section of the second gill arch from the left side was sampled and fixed in 10% buffered formalin. After that, the samples were decalcified, dehydrated in ethanol in a graded series, cleared in xylene, and embedded in paraffin blocks (Luna, 1968). Sections of 5 µm thickness were cut, and three sections were stained with the standard hematoxylin and eosin (H&E) staining technique.

Histological examinations assessed the distribution of lesions in the analyzed organ and the severity of the alterations. However, depending on the analysis method developed and modified by Tavares et al. (2019), the lesions were distributed in scores (*Sc*) and an important factor (*Fi*). The Sc was distributed as (0 - the absence of lesions or the presence of lesions on up to 10% of the total analyzed tissue), 1 - low-frequency occurrence of lesions ranging from 11-25% for the total analyzed tissue, 2 - moderate frequency lesions occur on 26 to 50% of the total analyzed tissue, 3 - frequently contain apparition of lesions on 51-75% of analyzed tissue, whereas 4 - frequently contain apparition of lesions on 76-100% of analyzed tissue. However, *Fi* indicates how such a change would affect organ function as well as the likelihood of the fish surviving and receiving the following values: (1) lesions that are easily reversible and of minor pathological significance; (2) reversible lesions when the stressor is moderate and has been neutralized histo-pathologically; and (3) lesions that are generally irreversible and have a high histo-pathological prominence. The lesion index was calculated: I Lesion = (*Sc x Fi*), and the lesion organ index is represented by *I LOrgan = I Lesion*.

Statistical analysis: The results were expressed as means±SD (standard deviation) for experimental groups of ten fish. Statistical software was performed in SPSS program. ANOVA was used to determine statistical differences between groups (Zar, 1999). The significance of the results was ascertained at a $P \leq 0.05$.

Results

The physical and chemical parameters of the Shatt AL-Arab River water from the studied sites are presented in Table 1. The chemical and physical profile and contamination status of sites 1, 2, and 3 show a significant polluting load, contain high-level of BOD, nitrite, Chloride (Cl), PO_4, and SO_4, trace metals concentration (Pb, Cu, Cd, Zn, and Cr), a high level of conductivity (Ec) and a low DO. Also, the chemical analysis recorded low concentrations but dangers of Chlorpyrifos insecticide in sites 1, 2, and 3. However, at site 3, the nitrite, heavy metals, and chlorpyrifos insecticide levels exceeded the water

Table 1. Shows the physicochemical profiles of Shatt Al-Arab river water samples collected from Sites 1, 2, 3, and 4.

Parameter	Site 1	Site 2	Site 3	Site 4
Physical and chemical factors				
Temperature (C^0)	17.33±1.22	18.44±1.42	12.56±1.11	10.32±1.01
pH	7.8±0.056	8.01±0.087	8.13±0.045	7.54±0.055
Hardness (mM)	445±44.6	687±63.6	651±105.54	213±30.5
DO (mg O2 /l)	4.62±0.98	4.54±0.97	4.19± 0.88	4.83±0.76
Conductivity (lS cm/1)	4.21±0.003	4.43±0.87	16.54±1.32	2.11±0.00021[a]
BOD (mg/l)	2.15±0.22	2.41±0.34	3.2±0.36	1.5±0.21
Nitrites (mg / l)	0.58±0.055	1.25±0.067	1.99±0.087	0.464±0.099
Chlorides (mg/ l)	550±38.6	801±35.6	822±44.8	312±30.8
PO_4 (mg/l)	0.43±0.02	0.61±0.031	1.02±0.053	0.33±0.012
SO_4 (mg /l)	550±31.7	780±47.8	830±50.3	467±33.1
TDS (mg/l)	2400±87.9	2030±80.00	2105±78.99	1340±68.97
Heavy metals (ug /l)				
Pb	0.143±0.004	0.342±0.010	0.87±0.021	0.054±0.002
Cu	0.057±0.005	0.078±0.003	0.104±0.003	0.055±0.0013
Cd	0.0051±0.00	0.017±0.002	0.031±0.0021	NS
Zn	0.29±0.003	0.33±0.004	0.39±0.002	NS
Cr	0.008±0.001	0.007±0.001	0.014±0.002	NS
Insecticides (ug/l)				
Chlorpyrifos insecticide	NS	NS	0.018± 0.007	NS
Other Organophosphates	NS	NS	NS	NS
Other Organ chlorines	NS	NS	NS	NS

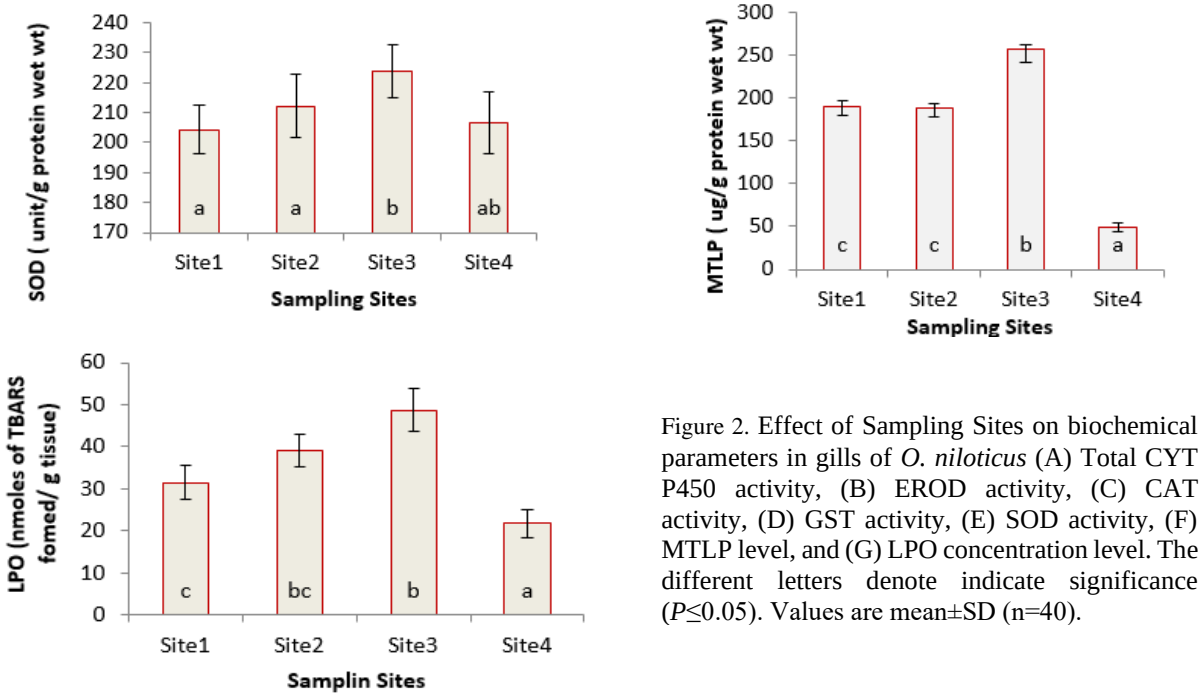

Figure 2. Effect of Sampling Sites on biochemical parameters in gills of *O. niloticus* (A) Total CYT P450 activity, (B) EROD activity, (C) CAT activity, (D) GST activity, (E) SOD activity, (F) MTLP level, and (G) LPO concentration level. The different letters denote indicate significance ($P \leq 0.05$). Values are mean±SD (n=40).

quality guidelines for freshwater life protection established by national and international legislation. Our results acquired after assessing the contamination indices provided supplementary evidence of the low water quality at sites 1, 2, and 3, where the fish were captured, compared with site 4.

Biotransformation enzymes as biomarkers: The fish gills of sites 1, 2, and 3 showed an increase in their biotransformation enzyme activities compared to site 4, which indicates a point of reference.

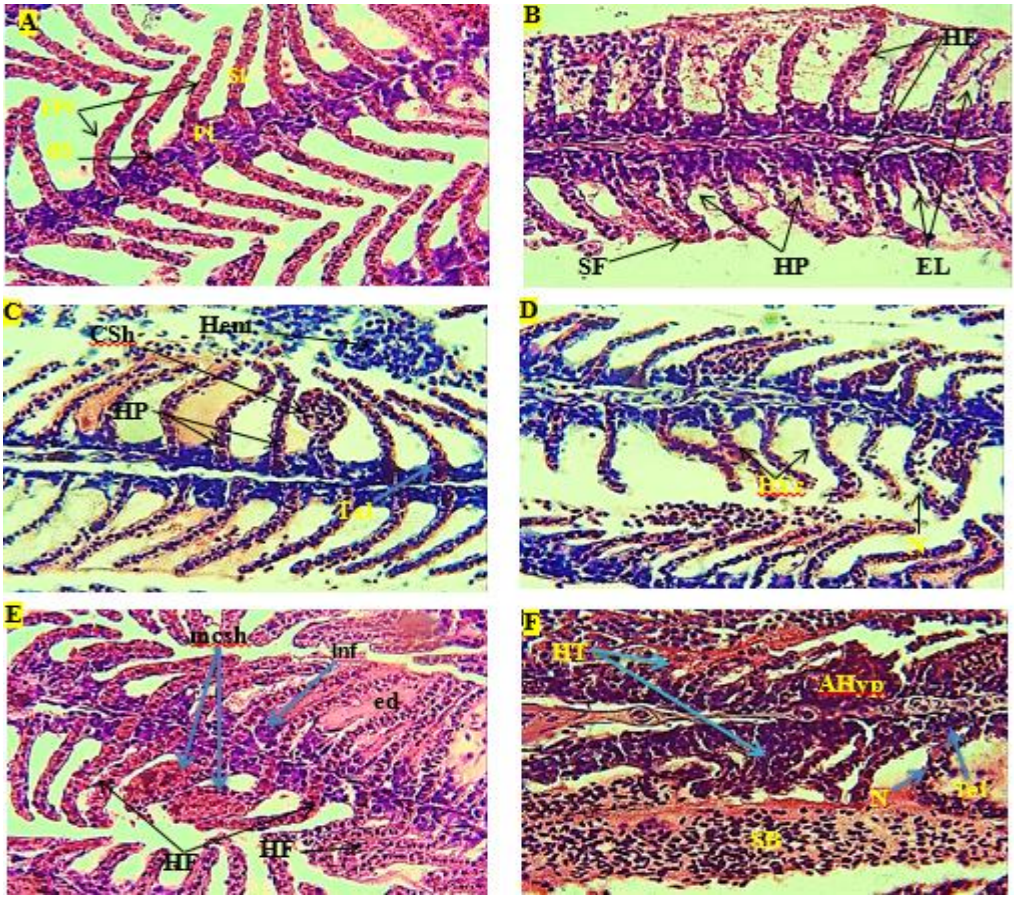

Figure 3. Microphotographs of the blood smear of *O. niloticus* fish, note Micronucleus mutation (arrows) in Sites 1, 2, and 3 (A, B, and C). Giemsa Stain (1000X).

Table 2. Micronucleus (%) in *O. niloticus* RBC from various sites of Shatt Al-Arab River.

Cytogenetic mutation	Site1	Site2	Site3	Site4
Micronucleus (RBC) (%)	7.88 ± 0.51^{d}	10.51 ± 2.11^{c}	18.8 ± 3.32^{b}	2.61 ± 0.87^{a}

Values are expressed as mean ±SD (n = 40), the significant differences were obtained ($P\leq0.05$) when compared to control group values.

Table 3. Distribution of the detected histopathological damage in the gills of the *O. niloticus* collected from different sites along the whole course of the river Shatt AL-Arab River.

Fish Species	pathological signs	Site 1	Site 2	Site 3	Site 4
O. niloticus	Hypertrophy of the epithelium	-	+	+++	-
	Hyperplasia of the epithelium	++	++	+++	-
	Hyperplasia of the Mucous cell	-	-	+	+
	Hyperplasia of the Chloride cell	-	+	++	-
	Hypertrophy of the Chloride cell	-	+	+	-
	Secondary lamellae Fusion	++	++	+++	+
	Edema	-	+	+++	
	Lifting of the epithelium	+	+	+++	-
	Necrosis	-	+	++	-
	Hyperemia	+	+	++	-
	Hemorrhage	-	+	++	-
	Telangiectasia	-	+	+	-
	inflammatory infiltration	-	+	++	-

(–) no histological alterations or mild histopathological alterations; (+) moderate histological alterations; (++) severe histological alterations; and (+++) very severe histological alterations in the gill surface architecture.

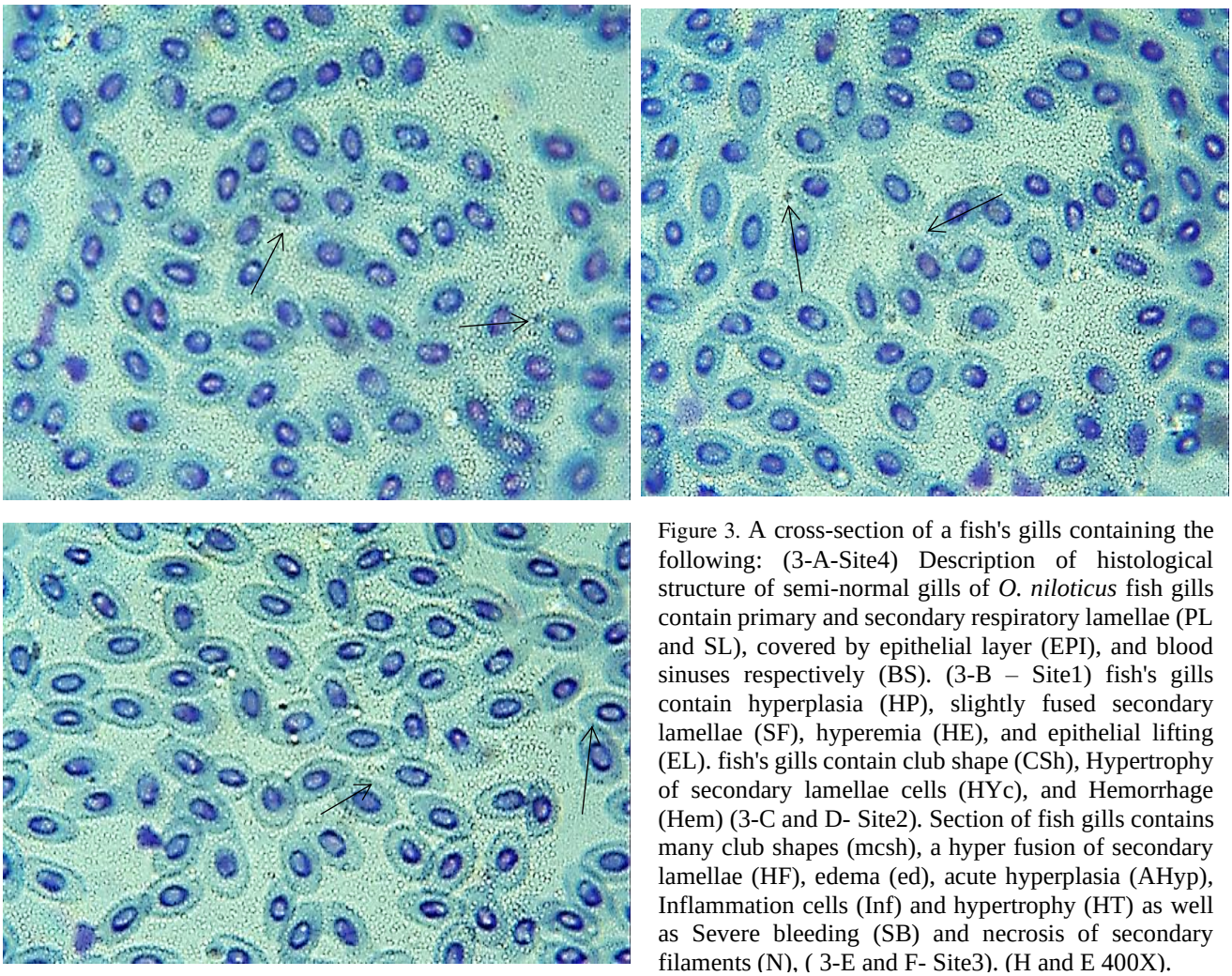

Figure 3. A cross-section of a fish's gills containing the following: (3-A-Site4) Description of histological structure of semi-normal gills of *O. niloticus* fish gills contain primary and secondary respiratory lamellae (PL and SL), covered by epithelial layer (EPI), and blood sinuses respectively (BS). (3-B – Site1) fish's gills contain hyperplasia (HP), slightly fused secondary lamellae (SF), hyperemia (HE), and epithelial lifting (EL). fish's gills contain club shape (CSh), Hypertrophy of secondary lamellae cells (HYc), and Hemorrhage (Hem) (3-C and D- Site2). Section of fish gills contains many club shapes (mcsh), a hyper fusion of secondary lamellae (HF), edema (ed), acute hyperplasia (AHyp), Inflammation cells (Inf) and hypertrophy (HT) as well as Severe bleeding (SB) and necrosis of secondary filaments (N), (3-E and F- Site3). (H and E 400X).

Table 4. Gill alteration index (I Lesion %) of *O. niloticus* from sites selected on the Shatt Al-Arab River. Fi important factor.

Histopathological signs	Gill Lesion Index (I *Lesion%*)							
	Site 1		Site 2		Site 3		Site 4	
	Fi	Sc	Fi	Sc	Fi	Sc	Fi	Sc
Epithelial hypertrophy	1	15.6	2	28.9	3	70.9	0	2.22
Epithelial hyperplasia	1	11.7	2	25.5	4	86.5	1	3.11
Mucous cell hyperplasia	1	11.8	2	25.8	3	75.4	0	0.00
Chloride cell hyperplasia	1	13.8	1	12.8	3	66.8	0	0.00
Chloride cell hypertrophy	1	11.1	1	13.9	3	60.7	0	0.00
Fusion of secondary lamellae	1	14.6	1	12.7	4	90.8	1	5.35
Edema	0	7.42	1	11.9	3	55.9	0	0.00
Epithelial lifting	1	11.7	1	14.9	3	74.9	0	0.00
Necrosis	0	6.11	1	13.8	4	80.6	0	0.00
Hyperemia	1	12.9	2	28.9	3	67.7	1	0.00
Hemorrhage	1	11.9	2	26.7	4	80.3	0	0.00
Telangiectasia	1	11.3	1	15.8	3	65.4	0	0.00
inflammatory infiltration	0	7.71	1	12.1	3	60.7	0	0.00

However, in sites 1, 2, and 3 the t-Cyp450 and EROD showed a significantly higher level ($P \leq 0.05$) than site 4. However, the EROD activity was more noticeable and affected by the low water quality compared to the t-Cyp450 enzyme (Figs. 4, 5).

Antioxidant enzymes as biomarkers: The fish gills

of sites 1 2, and 3 showed an increase in their antioxidant enzyme activities compared to site 4. However, in sites 2 and 3, the CAT showed a significant increase compared to sites 1 and 4. The increase in GST and SOD activities was significant only in site 3, compared with other regions. This reveals that low water quality in sites 2 and 3 had a greater impact on CAT than GST and SOD enzymes (Figs. 1, 2, 3).

Metallothionein protein (MLTP): One of the goals of this study was to evaluate the relationship between low water quality on the gill quantitative expression of metallothionein as a defense response against heavy metals pollution, which shows significantly greater values ($P \leq 0.05$) in sites 1, 2, and 3 compared to site 4. However, site 3 recorded the highest value.

Lipid peroxidation: Sits 1, 2, and 3 show an increase in LPO of fish gills compared to site 4; however, site 3 recorded the highest LPO ($P \leq 0.05$) than other locations. Sites 1 and 3 also showed a significant increase in fish gills LPO ($P \leq 0.05$) than site 4.

Histological structure of gills: The micronucleus–RBC mutation is shown in Table 2. Our results confirmed that micronucleus–RBC mutation was higher in all sites compared with reference site 4 and site 3 had the highest value (Figs. 1, 2, 3). The fish from sites 1, 2, and 3 revealed several histological changes in the gills (Table 1). The gills in the reference region had normal structure (Fig. 3A). The individuals from site 1 had a low prevalence of lesions. An increase in the histological lesion of fish gills was recorded in site 2, and the acute histological lesion in site 3. The most relevant histological changes were hyperplasia, which is accompanied by lamellar fusion and rupture of the epithelial layer (Fig. 3B). The damage was more pronounced in site 2 due to the vacuole formation and necrosis in the interfilament region (Fig. 3C). Vasodilation, slight necrosis and epithelial lifting in site 2 (Fig. 3D) and hypertrophy, spiked secondary lamellae, and end filaments in the shape of a club in site 3 (Fig. 3E) were main observed histopathological alternations. In some cases, in site 3, the damage is exacerbated by the complete loss of gills structure at several points and prominent hemorrhage and necrosis of secondary lamellae (Fig. 3F).

The significant changes in the I Lesion % index occurred in fish gills coinciding with the low water quality in sites 1, 2, and 3 compared with the reference i.e. site 4. However, site 3 recorded significant alterations in primary and secondary filaments represented by hypertrophy, hyperplasia, and the fusion of secondary lamellae, edema, epithelial lifting, necrosis, hyperemia, hemorrhage, telangiectasia, and inflammatory infiltration (Table 3).

Discussion

In the present article, *O. niloticus* was chosen as a bio-model because its fish is a pollution-resistant species ideal for a biomarker of water pollution. Generally, the quality of physical and chemical factors provides vital data regarding water health. Sites 2 and 3 had poor water quality containing higher concentrations of hardness, DO, Ec, BOD, nitrate, chloride PO_4, SO_3, and TDS; also recorded higher concentrations of trace metals and the Chlorpyrifos insecticide. Most physical and chemical standards in site 3 do not contain the limits permitted by the World Health Organization (WHO) for freshwater quality (Al-Asadi, 2014). However, the high concentrations of pollutants mentioned above were due to Hartha and Najebbia power plants and human activities, which discharge organic, inorganic compounds, heavy metals, ions, and insecticides into theriver. Most of the physical and chemical compounds in the Shatt AL Arab River were not within the limits of acceptability according to WHO and Iraq standards (Al-Hejuje, 2015). Therefore, the fish inhabiting the Shatt al-Arab river were exposed to many mixtures and complex pollutants, whether chemical compounds or physical factors and our findings showed the eco-toxicological impact of multiple contaminants on *O. niloticus*.

Oliveira et al. (2010a) mention that low levels of DO interfere with the fish population, causing

abnormalities in breeding and death. Disturbing the balance of oxygen supply/demand influences oxygen levels in tissues, interfering with antioxidant defenses. Generally, the catalytic efficiency and binding capacity of enzymes were affected by pH and temperature. PH alteration may also affect the bioavailability of severely contaminated with special reference to metals (Carvalho et al., 2004). Because biological and chemical processes cannot degrade trace metals, they tend to accumulate in aquatic sediments and soils with water; therefore, they have the potential to enter the food chain, posing a health risk to aquatic animals such as fish (Castiglione et al., 2009; Eagderi et al., 2022). Contamination substances such as heavy metals, pesticides, and other chemical compounds may immediately affect aquatic organisms by generating free oxygen radicals (ROS), resulting in primary degeneration and genotoxicity (Halliwell and Gutteridge, 1985).

Our study revealed that the biotransformation enzymes, total CYP 450, and EROD enzymes are good indicators for organic and inorganic pollutants in the water. The EROD enzyme is considered the best biological marker for water quality. Van Der Oost et al. (2003) pointed out that total CYP 450 and EROD activities appeared in many fish species and seemed to be sensitive biomarkers for the pollution of aquatic systems. In freshwater fish, the cytochrome P450 has been studied as a biomarker indicating environmental contaminations due to agricultural sewage or industrial wastes. Therefore, it is used for water pollution at low levels, or the pollutants are no longer dissolved in water but persist in the living organism, such as residues of biocidal agents (Machala et al., 1997). However, in fish, the class of CYP P450 isozymes and EROD are in charge of the biotransformation of a wide range of chemical compounds such as PAHs, PCBs, and dioxins, etc.. EROD enzyme is the most sensitive catalytic probe for determining fish's CYT P450 system's inductive response (Goksøyr and Forlin, 1992; Whyte et al., 2000). The total CYP 450 is responsible for a wide range of chemical material biotransformation whose catalytic activity (Siroka and Drastichova 2004). EROD is a highly sensitive indicator of contaminant uptake in fish (Arellano-Aguilar et al., 2009).

The results of the current study confirmed that the antioxidant enzymes (CAT and GST) are proper indicators for water quality monitoring in the studied sites 1, 2, and 3, while the SOD was less sensitive to water pollutants. GST, CAT, and SOD are the primary line of defense compared to oxygen toxicity induced by the different contamination compounds such as heavy metals, insecticides, and organic and inorganic compounds. GST, CAT, and SOD are metalloenzymes that play an important role in ROS defense by converting superoxide anions to hydrogen peroxide, which is detoxified by both CAT and GPX activities (Olsen et al., 2001). An increase in CAT enzyme activity indicates the presence of a higher peroxide level, for the increased activity of this enzyme at sites 1, 2, and 3 indicated that oxidant defenses were insufficient to prevent the formation of LPO. Hence, the removal strategy of free radicals by CAT activity is a functional response to oxidative stress (Monteiro et al., 2009). GST enzyme significantly prevents oxidative damage by conjugating GSH to lipid peroxide breakdown products (Fernandes et al., 2007). GST induction indicates the presence of a variety of chemical pollutants (Ahmad et al., 2005). Monteiro et al., (2010) explain that GST enzyme induction occurs in various tissues at various times after exposure to inducers.

Our results showed that metallothionein levels were significantly higher in fish gills collected from the Shatt AL-Arab River (sites 3) compared to the reference sites, i.e. site 4 and sites 1 and 2. Metallothionein proteins (MT) have a physiological role in protecting cells from the poisonous effects of free oxygen radicals, which must be considered before elevated MT levels can be interpreted as a marker of metal exposure in aquatic organisms (Oliveira et al., 2010b). Our study found higher LPO levels in the fish gills, indicating oxidative stress in sites 2 and 3 compared to reference site 4. However, LPO compounds are typically formed due to

complex free radical reactions in cell membranes that produce lipid hydroperoxides that decompose double bonds of unsaturated fatty acids and degrade membrane lipids. After being absorbed, pollutants have the potential to react with endogenous substances, creating a state of biological harm that may destroy the life quality (Cerqueira and Fernandes, 2002). The results confirmed that LPO is an excellent biomarker for sensing lower water quality.

Our results showed a wide range of gill alteration index (I Lesion %) in sites of 1, 2, and 3 compared to the reference site 4. Our results agreed with Santos et al. (2014), who found several histological changes, such as hyperplasia and hypertrophy of epithelium lamellae, lifting of respiratory epithelium, edema of epithelial cells, and clubbing of secondary filaments. The fish gills account for the top 50% of the surface area of a body; therefore, understanding the level of tissue damage produced can help determine the toxicity of the environment, making fish highly suitable for assessing the health of aquatic systems (Ogundiran et al., 2009).

Conclusions

Our study confirmed that the enzyme activities, micronucleus mutation of RBC, and histological alterations of the gill of *O. niloticus* could be used as a biomarker to monitor the water quality of the aquatic ecosystem and its effects on organisms that live in it. Our results contribute to the knowledge of the linkage between biological responses and environmental variations and the importance of these biomarkers in environmental monitoring. Further, it is proved that *O. niloticus* could be used in further environmental pollution studies in freshwater ecosystems.

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