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Integrated use of biomarkers to assess the impact of heavy metal pollution on *Solea aegyptiaca* fish in Lake Qarun

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Abstract

Background: Biomarkers have become a valuable tool in environmental assessment, since they contribute to predicting contaminants in monitoring programmes. This study aimed to investigate the toxicity of heavy metal pollution in Lake Qarun using a multibiomarker approach (morphological, oxidative stress, genotoxicity, stress proteins) in *Solea aegyptiaca* fish. During the winter and summer seasons, water and fish samples were collected from different locations along Lake Qarun; western and northern sectors were away from any source of pollution, while southern and eastern sectors were exposed to effluents discharged from El-Wadi and El-Bats drains, respectively. The environmental quality, as well as the accumulation of metals (Fe, Cu, Zn, Cd, Pb, Ni) in fish gills, liver, and muscles were assessed. Data were integrated using the integrated biomarker response index (IBRv2) for biomarker response interpretation.

Results: Water quality and bioaccumulation of heavy metals revealed a highly significant difference between samples collected from polluted sectors and those collected far from drainage water, and, seasonal differences were detected. Growth indices revealed a significant difference between sites and seasons. Fish from the western sector had the highest total antioxidant capacity in their gills, liver, and muscles, with no seasonal differences detected. However, the maximum value of malondialdehyde, protein carbonyl, 8-hydroxy-2′deoxyguanosine, metallothionein, heat shock protein 70, and DNA strand breaks in gills, liver, and muscles was detected in the polluting sectors. It revealed a significant difference between seasons, with the highest value during the winter season. According to IBRv2 results, the most effective biomarkers in this study were malondialdehyde and 8-hydroxy-2′deoxyguanosine in gills, protein carbonyl and metallothionein in the liver, heat shock protein 70 in gills and liver, and DNA strand break in gills, liver, and muscles.

Conclusions: This multibiomarker approach contributes to distinguishing between locations with varying levels of anthropogenic pollution, identifying the drainage water-exposed sectors as the most stressed and the winter season as the most critical time for *Solea aegyptiaca* owing to spawning. The biomarkers chosen are effective indicators in *Solea aegyptiaca* under stress, indicating the potential for environmental monitoring.

Keywords: Anthropogenic pollution, Oxidative stress, Genotoxicity, Stress proteins, Integrated biomarker response index (IBRv2), Seasonal differences

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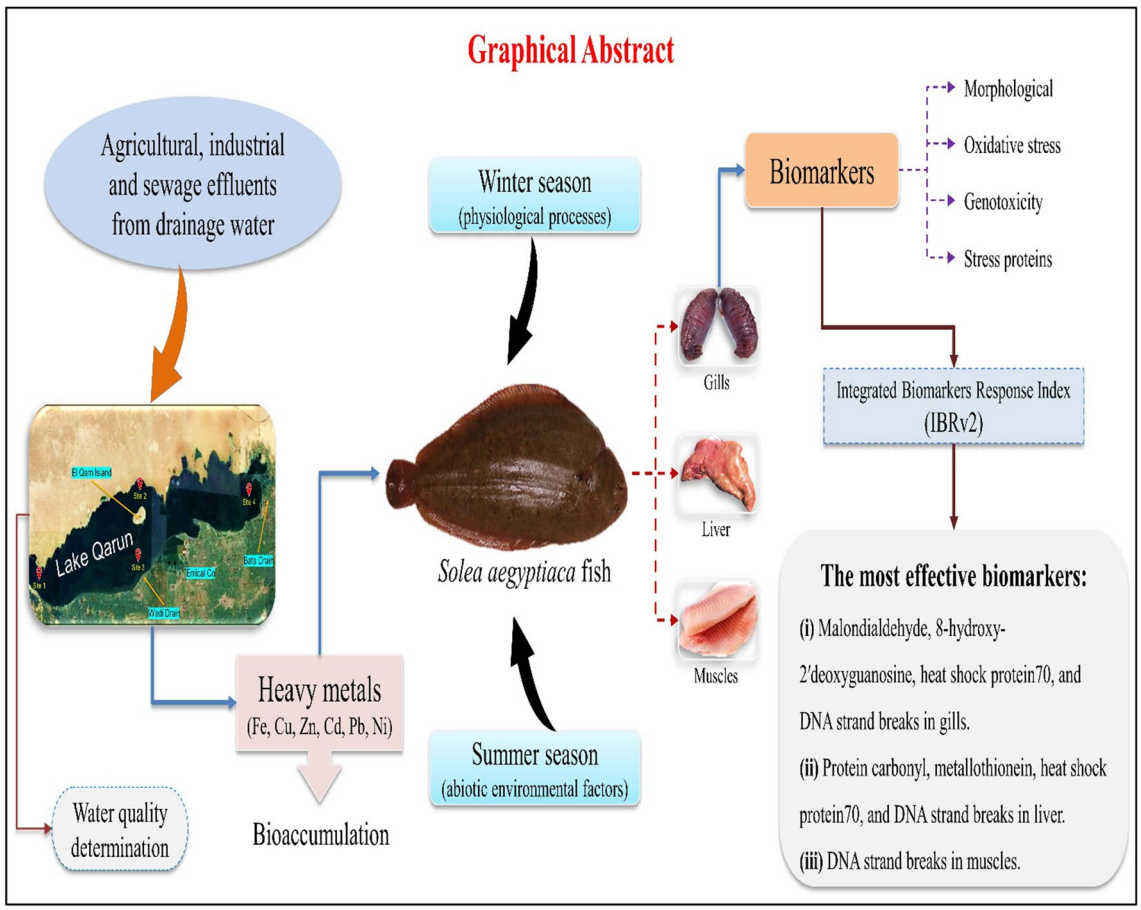
Graphical Abstract



Background

Lake Qarun is a closed saline lake and one of Egypt's most important inland aquatic habitats. It collects agricultural and municipal pollution discharged from the depression of El-Fayoum throughout its two main drains, El-Bats and El-Wadi [1]. The water quality of Lake Qarun had previously been investigated [2, 3]. The increased pollution of water resources in Lake Qarun and the consequent effects on the aquatic environment and human health is an issue of great concern [4]. Fish, generally at the top of the aquatic food chain can bioaccumulate high levels of heavy metals. These metals accumulate in fish organs in diverse ways, posing a significant health risk to humans [5]. Consequently, the issue of hazardous metal contamination in fish has received more attention [6].

Biomarkers are valuable tools for determining how biological systems interact with potential pollutants (chemical, physical or biological) [7] and, therefore, contribute to evaluating the responses of heavy metals in organisms [8]. Metals can lead to oxidative stress in fish by stimulating the generation of reactive oxygen species (ROS), which damages biological components like lipids, proteins and DNA [9, 10]. These oxidative damages can be determined by measuring lipid peroxidation (LPO) [11, 12], protein carbonylation (PC) [12, 13], and DNA base oxidation [14]. ROS can be detoxified by cellular antioxidant defence systems, both enzymatic and non-enzymatic [15] while measuring the total antioxidant capacity (TAC), evaluating the overall efficacy of cellular antioxidants against oxyradicals, and providing a comprehensive assessment of responsiveness to oxidative stress [16]. DNA damage biomarkers can be used to assess the genotoxicity of environmental pollutants and a comet assay is a valuable tool for assessing genotoxicity in fish in the environment [17]. The induction of stress proteins is the only molecular mechanism used by fish to cope with stress [18]. Overexpression of heat shock proteins (HSPs) and metallothioneins (MTs) can be identified in response

to a variety of adverse environmental conditions, including heavy metal exposure [19]. With respect to pollution in general and heavy metals in particular, fish condition factor (K) and hepatosomatic index (HSI) are utilized as biomarkers of health and growth [20].

In environmental monitoring, the indicator organism used is crucial. The sole, genus Solea, is one of the most important and valuable commercial flatfish in Egypt and is well-regarded for human consumption [21]. Thus, the presence of substantial quantities of hazardous metals in these species might severely impact their physiological function and consumers' health [22]. Solea fish are sedentary demersal fish buried in the substrate on muddy bottoms [23, 24]. Eventually, they will be vulnerable to excessive metal concentrations in the sediments. Since they eat predominantly mollusks, invertebrates, small crustaceans, and small fish, which concentrate metals in their tissues, their feeding behaviour may enhance their chances of exposure to increased amounts of these components [24].

The proper application of biological reactions as biomarkers necessitates understanding seasonal fluctuations in biotic and abiotic variables, which have all been proven to impact the basal levels of several biomarkers and their responsiveness to pollution [25]. The combined effects of various natural stressors and pollutants can often be synergistic [26]. Recent studies have revealed that increased summer temperatures have a stronger impact on Solea fish due to increased physiological activity and metal uptake than lower winter temperatures [27, 28]. Biomarker responses demonstrate similar patterns in several fish species, as evidenced by the elevated oxidative stress [29–31], genotoxicity [32–34], and stress proteins [35–37]. There is a paucity of information with respect to *Solea aegyptiaca* inhabiting Lake Qarun, as well as the effect of seasonal variations on these biomarkers. Therefore, this study aimed to determine the toxic impact of heavy metal pollution in Lake Qarun using a suite of biomarkers; morphological, oxidative stress, genotoxicity, and stress proteins in *Solea aegyptiaca* fish to investigate the viability of using the *Solea aegyptiaca* fish as a water pollution indicator at Lake Qarun, El-Fayoum Governate, Egypt. Moreover, to compare the influence of the abiotic environmental factors (temperature, salinity, and dissolved oxygen) during the summer and physiological processes (reproduction) during the winter season on *Solea aegyptiaca* biomarkers. Furthermore, without an integrated strategy to handle issues in linking information and categorizing locations according to contaminant-induced variations in the health condition of organisms, the practical implementation of the biomarker approach in environmental evaluation is restricted [38]. Studies using an integrated biomarker response (IBRv2) index to simplify biomarker response interpretation have never been tested in Egyptian fishes under field or laboratory conditions. Therefore, the biomarker responses of *Solea aegyptiaca* fish were combined into a biomarker response index to assess the extent of contamination along Lake Qarun and to determine the biomarkers that indicate the strongest responses to environmental disturbances.

Materials and methods

Study area

Lake Qarun is Egypt's only enclosed saline lake. It is located in the western desert region of the Fayoum depression, 83 kms southwest of Cairo. The lake is 43 m (140 feet) below sea level and encompasses 243 square kilometres (78 sq mi). It is located between 30°34" and 30°49" east longitudes and 29°25" and 29°34" north latitudes. The lake is 1–8 m deep and holds approximately 1,100,000,000 gallons of water [1]. The lake receives a large amount of untreated agricultural, industrial, sewage, and residential effluents (about 450 million m^3 per year) from El-Fayoum province via two major drains (El-Bats and El-Wadi drains) in addition to several smaller drains that lead to the lake [39]. Figure 1 depicts the chosen locations. Site 1 (30° 31′ 09.6′′E 29° 25′ 42.4′′N): located in the western sector of the lake, it is a relatively unpolluted area with no drainage water. Site 2 (30° 37′ 42"E 29° 28′ 53"N): represents the northern sector of the lake; it lies north of El-Qarn Island, far from drainage water. Site 3 (30° 37′ 43.4′′E 29° 26′ 49.4′′N): it is located at the midpoint of the southern sector near the mouth of the El-Wadi drainage channel. Site 4 (30° 48′ 47.2′′E 29° 31′ 04.1′′N): is at the eastern sector near the mouth of the El-Bats drain.

Water sampling

In polyethylene bottles, water samples were obtained from the study sites throughout the winter (January–March) and summer (July–September) seasons of 2019, around 50 cm below the water's surface. Samples were filtered in the field, acidified with 10% nitric acid (HNO3) for preservation, put in an ice bath, and brought to the laboratory.

Fish and tissue sampling

At the same time, a total of 120 fish of *Solea agyptiaca* (30 fish/site) were collected by fishers' nets from the same sites, where the water samples were taken, with an average body weight of 55.38 ± 15.65 and 36.80 ± 11.04 g and an average total length of 19.10 ± 2.50 and 16.30 ± 1.58 cm for winter and summer seasons, respectively, and then transported to the laboratory, National

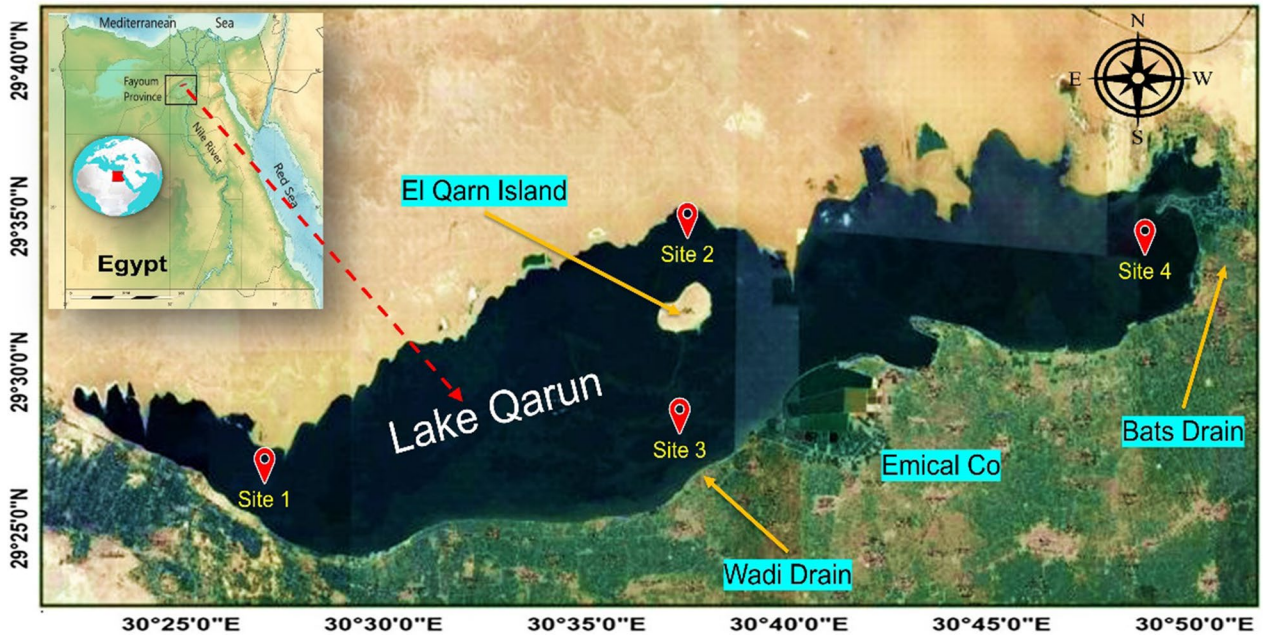


Fig. 1 Lake Qarun map with the studied sites

Institute of Oceanography and Fisheries, Shakshouk Station, El-Fayoum, in aerated, chilled containers containing seawater from the sampling locations.

Prior to decapitation and tissue sampling, fish (20 individuals per site) were assessed (total length, cm) and weighed (total and liver weights, g) to assess growth parameters. Gills, livers, and muscles were sampled, rinsed in a phosphate-buffered saline (PBS) solution (pH 7.4) to eliminate extra blood, and then pooled. Three pooled samples of 9 individuals per site were dried in an oven at 105 °C for 48 h and grounded to a fine powder for metals concentrations. Six pooled samples of 18 individuals per location were immediately frozen in liquid nitrogen and maintained at −80 °C until homogenate preparation for biomarkers analysis. In addition, specimens of gills, liver, and muscles of each group were immediately excised and fixed in normal saline and stored at −80 °C for comet assay analysis.

All applicable international, national, or institutional guidelines for the care and use of animals were followed by the authors.

Water analysis

Field measurements

Water temperature, pH and salinity were monitored in the field using a Hydrolab (model Orion Research Ion Analyzer 399A).

Dissolved oxygen

According to APHA [40], dissolved oxygen (DO) was determined by Wrinkler's iodometric method. A glass stoppered biochemical oxygen demand (BOD) bottle of 300 ml capacities was entirely filled with lake water. The DO in the sample was then fixed by adding a series of reagents (manganous sulphate, alkaline potassium iodide, and conc. H_2SO_4) that formed an acid compound that was then titrated against sodium thiosulphate until the solution became colorless; DO was expressed in mg/l.

Determination of residual heavy metals in Solea aegyptiaca tissues

The dried samples (gills, liver and muscles) were digested in accordance with the procedure outlined by Ghazally [41], in which 1.0 g (dry powder) was digested in (5 ml nitric acid + 5 ml perchloric acid) solution and heated on a hot plate at 80–90 °C until the sample became clear. The solution was cooled, filtered and transferred to a 25 ml volumetric flask, which was then filled with de-ionized water to the desired level. The digests were stored in plastic bottles, and Fe, Zn, Cu, Cd, Pb and Ni concentrations in the gills, liver and muscles were assessed using an Inductively Coupled Plasma Emission Spectrometer (ICP) (ICAP-6300 Duo) according to APHA [42]. The results were expressed in mg/kg dry weight. The limit of detection ranges from 7 µg/l to 100 mg/l for Fe, 2 µg/l

to 100 mg/l for Zn, 6 µg/l to 50 mg/l for Cu, 4 µg/l to 50 mg/l for Cd, 40 µg/l to 100 mg/l for Pb, and 15 µg/l to 50 mg/l for Ni.

The metal pollution index calculation

Metal pollution index (MPI) was utilized to compare the overall metal content of the analyzed fish's gills, liver, and muscles in different year seasons. According to Usero et al. [43], MPI was estimated with the following formula: $MPI = (Fe \times Zn \times Cu \times Cd \times Pb \times Ni)^{1/6}$.

Determination of growth parameters

The condition factor (*K*) and Hepato-somatic index (HSI) were determined using the method of Schreck and Moyle [44] as:

$$K = \text{Weight}\,(g)/\text{Length}^3(\text{cm}) \times 100$$

$$\text{HSI} = \text{Weight of the liver}\,(g)/\text{Total fish weight}\,(g) \times 100$$

Biomarker responses

Oxidative stress parameters

The total antioxidant capacity (TAC) level was estimated spectrophotometrically at 532 nm according to the methodology of Tween 80 oxidation [45]. After the homogenization of the frozen tissues (gills, liver, and muscles) in ice-cold buffer (100 mM Tris-HCl, pH 7.2) in 1:6 ratio (w/v) using Omni International Tissue Master 125 Homogenizer (USA) submerged in an ice water bath, the homogenates were centrifuged at 3000 × g for 15 min at 4 °C. Subsequently, 2 ml of 1% Tween 80 reagent, 0.2 ml of 1 mM $FeSO_4$, and 0.2 ml of 10 mM ascorbic acid were added to 0.1 ml of homogenate supernatant. The sample was replaced by 0.1 ml of dis. water in the blank assay. The solution was boiled in a water bath for 48 h at 37 °C. The mixture was then allowed to cool before adding1 ml of 20% trichloroacetic acid (TCA). 2 ml of 0.25% 2-thiobarbituric acid was mixed with 2 ml of supernatant after centrifugation at 3000 × g for 10 min. Using Spectrophotometer UV–visible double beam-labomed Inc., USA, the absorbance was measured at 532 nm after 15 min of heating in a water bath at 95 °C. The blank was defined as having a 100% absorption. The level of TAC in the sample (%) was quantified regarding the blank absorbance.

Malondialdehyde (MDA) was measured by detecting 2-thiobarbituric acid reactive substrates concentration according to Ohkawa et al. [46], using the Biodiagnostic (Egypt) Kit. The frozen gills, liver, and muscle tissues were homogenized in cold buffer (50 mM potassium phosphate, pH 7.4) in a 1:6 ratio (w/v) with Omni International Tissue Master 125 Homogenizer (USA). After that, the homogenates were centrifuged at 4000 rpm for 15 min. In the assay, 0.2 ml of homogenate supernatant was added to 1.0 ml of chromogen (thiobarbituric acid + detergent + stabilizer) and sealed with a glass bead. In a boiling water bath for 30 min, the solution was heated. The absorbance was measured at 534 nm by UV Visible double beam-labomed Inc. Spectrophotometer (USA). MDA level in the sample was expressed in nmol/g.

Protein carbonyl content (PC) was assessed colorimetrically by dinitrophenylhydrazone (DNPH) assay according to Reznick and Packer [47]. The frozen tissues (gill, liver, and muscles) were homogenized in PBS (pH 7.4) (1:10 w/v) with Omni International Tissue Master 125 Homogenizer (USA). The homogenates were then centrifuged for 15 min at 5000 rpm, and 200 µl of homogenate supernatant was added to 800 µl of 10 mM DNPH in 2.5 M HCl. A sample control was also made by mixing 200 µl of homogenate supernatant with 800 µl of 2.5 M HCl. After incubation at room temperature for 1 h with a 15 min vortexing interval, 1 ml of 20% TCA was added. The solution was then centrifuged at 10,000 × g for 10 min at 4 °C after being incubated on ice for 5 min. After collecting the supernatants, 1 ml of 10% TCA was added before being centrifuged at 10,000 × g for 10 min at 4 °C. The protein pellet was washed three times with 1 ml of 1:1 (v/v) ethanol: ethyl acetate and centrifuged at 10,000 × g for 10 min at 4 °C. The protein pellet was then resuspended in 500 µl of 6 M guanidine hydrochloride and centrifuged at 10,000 × g for 5 min at 4 °C. The supernatant was sampled, and the absorbance was measured at 370 nm with control as a blank by UV Visible double beam-labomed Inc. Spectrophotometer (USA). The protein carbonyl concentration was expressed in nmol/g.

8-hydroxy-2′deoxyguanosine (8-OHdG) concentration was analyzed using an ELISA kit provided by Bioassay Technology Laboratory (China). After the homogenization of the frozen samples (gill, liver, and muscles) in PBS (pH 7.4) (1:10 w/v) with Omni International Tissue Master 125 Homogenizer (USA), the homogenates were centrifuged at 5000 rpm for 15 min. In the assay, samples were added to sample wells with 40 µl of sample, 10 µl of antibody, and 50 µl streptavidin–HRP (enzyme conjugate). After 60 min at 37 °C and five washes with wash buffer, 50 µl substrate solution A and 50 µl substrate solution B were added to the wells and incubated for 10 min at 37 °C. To stop the reaction, 50 µl of stop solution was added to each well, and the blue color was turned yellow. The absorbance was detected at 450 nm using Thermo Scientific Multiskan FC Microplate Photometer (USA). The concentration of 8-OHdG was expressed in ng/g.

Genotoxicity estimated by comet assay

The degree of DNA damage was determined by the comet assay (alkaline single-cell gel electrophoresis) as detailed by Singh et al. [48]; 1 ml ice-cold PBS was used to dissolve 0.5 g of crushed samples. After stirring for 5 min, the suspension was filtered. Subsequently, 100 µl of cell suspension was mixed with 600 µl of low melting agarose (0.8% in PBS). 100 µl of this mixture was then placed on pre-coated slides. For 15 min, the coated slides were submerged in lysis buffer [0.045 M Tris-borate-EDTA, pH 8.4, containing 2.5% sodium dodecyl sulphate (SDS)]. The slides were placed in an electrophoresis chamber using the same Tris-borate-EDTA buffer as before but without SDS. The electrophoresis conditions were 2 V/cm for 2 min and 100 mA, and then stained with ethidium bromide 20 µg/ml at 4 °C. The DNA fragment migration patterns of 100 cells for each treatment level were examined using a fluorescence microscope (With excitation filter 420–490 nm [issue 510 nm]). The lengths of the comet's tails were measured from the nucleus to the tail's end, with a 40 × increase to count and measure the comet's size. To visualise DNA damage, ethidium bromide-stained DNA using a 40 × objective on a fluorescent microscope. The Comet 5 image analysis software developed by Kinetic Imaging, Ltd. (Liverpool, UK) linked to a charged-coupled device camera was used to evaluate the quantitative and qualitative extent of DNA damage in the cells by measuring the length of DNA migration and the percentage of migrated DNA. Finally, the program calculates the tail moment. Finally, 50–100 cells were randomly selected and evaluated per sample.

Stress proteins

Metallothionein (MT) was assessed spectrophotometrically using the Ellman's SH according to Viarengo et al. [49] method and a modification of Cataldo et al. [50]. Gills, liver, and muscle tissues were homogenized in three volumes of the homogenization buffer (0.5 M sucrose, 20 mM Tris-HCl buffer, pH 8.6, containing 0.01% β-mercaptoethanol). The homogenates were divided into aliquots (3 ml) and centrifuged at 10.000 × g for 30 min. Afterward, 1 ml of supernatant was mixed with 1 ml of cold (−20 °C) absolute ethanol and 80 µl of chloroform. After centrifugation at 6000 × g for 10 min, three volumes of cold ethanol were added to the resulting supernatant, which was kept at −20 °C for 1 h. The supernatant was centrifuged for 10 min at 6000 × g, and the resulting pellets were washed with ethanol: chloroform: homogenization buffer (87:1:12) before being centrifuged for another 10 min at 6000 × g. The pellets were air-dried before resuspension in 100 µl of 5 mM Tris-HCl, 1 mM EDTA at pH 7, and then added to 420 µl of 0.43 mM 5,5-dithiobis-2-nitrobenzoic acid in a 0.2 M phosphate buffer, pH 8. After 30 min at room temperature, the absorbance was measured at 412 nm with a UV–visible double beam-labomed Inc. spectrophotometer, USA. The glutathione standard curve was used to quantify the amount of MT in the samples, considering that 1 mol of MT comprised 20 mol of cysteine and expressed in µg/g tissue.

Heat shock protein 70 (HSP 70) was quantified using an ELISA kit provided by Bioassay Technology Laboratory (China) and followed the same procedure described for 8-OHdG. The concentration of HSP70 was expressed in ng/g.

The integrated biomarker response index

The results of the biomarkers were incorporated into the integrated biomarker response version 2' (IBRv2) index, described by Beliaeff and Burgeot [51] and updated by Sanchez et al. [52]. In each studied site, the data from each biomarker (Xi) were compared to the reference data (X0), and variance was reduced using a log transformation.: $Yi = \log Xi/X0$. The general mean (µ) and standard deviation (s) of Yi for all sites were computed for each biomarker, and Yi was normalized as $Zi = (Yi—\mu)/s$. The mean of standardized biomarker response (Zi) and mean of reference biomarker data (Z0) were used to establish a biomarker deviation index (A), $Ai = Zi - Z0$ for each biomarker in each site, to draw a basal line centred on 0 and to describe biomarker variation according to this line. The absolute value of A parameters calculated for each biomarker in each site was summed as $IBRv2 = \Sigma \mid A \mid$ to obtain the IBRv2. For a single site, "A" parameters are presented in a star plot to reflect the deviation of each studied biomarker from reference. The area above 0 indicates biomarker activation, while the area below 0 represents biomarker suppression.

Statistical analysis

Two-way analysis of variance (ANOVA) was used to determine the significant difference in season and site, followed by Duncan's multiple range test when the differences were significant. The level of significance in all tests was $P \leq 0.05$. The data were expressed as means ± standard deviation (SD).

Results

Water physicochemical parameters

As detailed in Table 1, water temperature demonstrated the lowest value in the southern sector during winter and the highest value in the northern sector during summer. The highest pH value was found in the eastern sector during summer, while the lowest value was found in the northern sector during winter. Salinity showed the highest value in the northern sector during summer and the lowest value during winter in the southern sector. The

Table 1 Water physicochemical parameters for Lake Qarun during winter and summer seasons, 2019

Seasons	Sites	Water temperature °C	pH	Salinity ‰	DO (mg/l)
Winter	Western sector	18.02±0.08	7.62±0.08	29.51±1.03	10.08±0.20
	Northern sector	18.23 ±0.08	7.52±0.11	30.53±0.98	8.45±0.25
	Southern sector	17.20±0.48	7.91±0.09	26.54±1.44	7.62±0.16
	Eastern sector	17.80±0.10	8.22±0.11	27.30±1.04	5.20±0.10
	Mean	**17.81±0.46**	**7.82±0.30**	**28.47±1.94**	**7.84±1.85**
		B	**B**	**B**	**A**
Summer	Western sector	32.47±0.31	8.39±0.02	34.60±0.80	8.05±0.35
	Northern sector	32.60±0.36	8.31±0.01	35.50±0.56	7.22±0.08
	Southern sector	31.67±0.57	8.56±0.02	32.50±0.80	5.53±0.17
	Eastern sector	31.93±0.61	8.65±0.01	31.25±0.75	4.30±0.20
	Mean	**32.17±0.57**	**8.48±0.14**	**33.46±1.86**	**6.28±1.53**
		A	**A**	**A**	**B**
All over mean	Western sector	**25.24±7.92**	**8.01±0.42**	**32.05±2.91**	**9.06±1.14**
		a	**ab**	**a**	**a**
	Northern sector	**25.42±7.87**	**7.92±0.44**	**33.01±2.82**	**7.84±0.69**
		a	**b**	**a**	**b**
	Southern sector	**24.43±7.94**	**8.23±0.36**	**29.52±3.43**	**6.58±1.15**
		a	**ab**	**a**	**c**
	Eastern sector	**24.87±7.75**	**8.44±0.25**	**29.28±2.31**	**4.75±0.51**
		a	**a**	**a**	**d**
	P.L [160]	**25–35**	**7–8**	**None**	**6–14**

In each column, means values (mean ± SD, $n = 3$) with different letters have a statistically significant difference ($P \leq 0.05$), while means having the same letter in the same column are not significantly different ($P \geq 0.05$). The capital letters indicate the significance between seasons, and small letters indicate significance between sites. *P.L.* Permissible level

highest DO was in the western sector during winter and the lowest in the eastern sector during summer.

Heavy metals bioaccumulation and metal pollution index

The concentrations of Fe, Cu, Zn, Cd, Pb, and Ni in gills, liver, and muscle samples taken from the studied locations during the winter and summer seasons and MPI values are detailed in Tables 2, 3 and 4 for gills, liver, and muscles, respectively. The highest bioaccumulation was recorded in fishes collected from the eastern and southern sectors. The MPI sequence in the investigated *Solea aegyptiaca* tissues was: gills > muscles > liver. Moreover, MPI in gills, liver, and muscles showed the highest value for fishes collected from the eastern sector during summer, while the least MPI was for fishes collected from the western sector during summer for gills and winter for liver and muscles.

Growth parameters

According to the data obtained, the summer season demonstrated the highest K value. In addition, the southern sector had the highest K, while the western sector had the lowest K value. Moreover, results of HSI declared that the highest value was detected in the summer season. With regard to the sampling sites, the highest value of HSI was recorded in the western sector and the lowest value in the eastern sector (Table 5).

Biomarker responses

Oxidative stress parameters

The mean values ± SD of gills, liver and muscle TAC , MDA, PC, and 8-OHdG, are depicted in Table 6. TAC showed an insignificant difference between seasons in terms of gills, liver, and muscles. While the highest TAC was detected in the western sector for gills, liver, and muscles, and the lowest TAC was detected in the eastern sector for gills and southern sector for liver and muscles. MDA demonstrated the highest mean value in gills, liver, and muscles during the winter season. In addition, the obtained data revealed that the eastern sector recorded the highest level in gills, liver, and muscles, and the western sector showed the lowest level in gills, liver, and muscles. Winter season demonstrated the highest PC concentration in gills, liver, and muscles of *Solea aegyptiaca*. Moreover, the southern sector recorded the highest concentration of PC in gills, liver, and muscles, while the western sector detected the lowest concentration in gills, liver, and muscles. The current study revealed an increased concentration of 8-OHdG in gills and muscles during the winter season. Furthermore, the eastern

Table 2 Heavy metal concentrations and MPI in gills during winter and summer seasons, 2019

Seasons	Sites	Fe (mg/kg dry weight)	Cu (mg/kg dry weight)	Zn (mg/kg dry weight)	Cd (mg/kg dry weight)	Pb (mg/kg dry weight)	Ni (mg/kg dry weight)	MPI
Winter	Western sector	136.80±5.60	5.04±1.65	62.59±4.35	0.04±0.03	0.40±0.39	3.05±0.09	3.58
	Northern sector	153.79±4.09	4.77±1.76	52.85±3.00	0.06±0.02	0.54±0.36	3.18±0.25	3.98
	Southern sector	291.71±7.29	10.41±2.51	112.23±2.42	0.26±0.03	1.35±0.30	5.37±0.17	9.29
	Eastern sector	346.33±16.02	10.91±1.99	124.99±5.61	0.35±0.02	1.49±0.18	4.84±0.07	10.30
	Mean	**232.16±93.50**	**7.78±3.46**	**88.16±32.53**	**0.18±0.14**	**0.95±0.57**	**4.11 ± 1.07**	**6.79**
		B	**A**	**B**	**A**	**A**	**A**	
Summer	Western sector	244.92±4.92	2.07±0.32	98.31±4.15	0.04±0.005	0.30±0.03	3.28±0.02	3.54
	Northern sector	277.13±4.43	2.98±0.22	108.82±4.38	0.12±0.004	0.73±0.07	3.88±0.02	5.59
	Southern sector	345.39±4.79	14.47±0.13	134.12±7.76	0.43±0.04	2.12±0.08	6.13±0.04	12.46
	Eastern sector	424.42±3.58	10.93±0.41	141.41±5.42	0.46±0.04	2.46±0.28	6.50±0.47	13.00
	Mean	**322.97±72.06**	**7.62±5.49**	**120.67±19.08**	**0.27±0.19**	**1.40±0.96**	**4.95±1.46**	**8.65**
		A	**A**	**A**	**A**	**A**	**A**	
All over mean	Western sector	**190.86±59.41**	**3.56±1.94**	**80.45±19.93**	**0.04±0.02**	**0.35±0.26**	**3.17±0.14**	
		c	**b**	**b**	**b**	**b**	**b**	
	Northern sector	**215.46±69.66**	**3.88±1.49**	**80.83±30.84**	**0.09±0.03**	**0.64±0.25**	**3.53±0.41**	
		c	**b**	**b**	**b**	**b**	**b**	
	Southern sector	**318.55±29.92**	**12.44±2.73**	**123.18±13.04**	**0.35±0.10**	**1.73±0.46**	**5.75±0.43**	
		b	**a**	**a**	**a**	**a**	**a**	
	Eastern sector	**385.38±44.01**	**10.92±1.28**	**133.20±10.26**	**0.40±0.07**	**1.98±0.57**	**5.67±0.96**	
		a	**a**	**a**	**a**	**a**	**a**	

In each column, means values (mean ± SD, $n = 9$) with different letters have a statistically significant difference ($P \leq 0.05$), while means having the same letter in the same column are not significantly different ($P \geq 0.05$). The capital letters indicate the significance between seasons, and small letters indicate significance between sites

sector recorded the highest 8-OHdG concentration in *Solea aegyptiaca* organs. Meanwhile, the western sector detected the lowest 8-OHdG concentration.

Comet assay

DNA damages of *Solea aegyptiaca* fish collected from the studied sites during the winter and summer seasons are displayed in Table 7. Concerning DNA damage in gills of *Solea aegyptiaca* presented as tail DNA (%), tail length (μm), and tail moment, the present study demonstrated that the highest value was recorded during the winter season. The eastern sector also showed the maximum gills DNA damage. Regarding liver DNA damage, the highest value of tail DNA (%), tail length (μm), and the tail moment was observed during winter, whereas the maximum damage in *Solea aegyptiaca* liver was recorded in the eastern sector. In addition, muscular DNA recorded the highest value during winter, and the eastern sector showed maximum DNA damage. (Fig. 2).

Stress proteins

The results (mean values ± S.D) of MT and HSP70 in the gill, liver, and muscles of *Solea aegyptiaca* are presented in Table 8. MT revealed the highest concentration in gills, liver, and muscles during winter. Furthermore, the results showed the maximum MT concentration in the eastern sector for gills and muscles and the southern sector for the liver. On the contrary, the minimum concentration in gills, liver, and muscles was detected in the western sector. HSP70 is declared the highest HSP70 level in gills, liver, and muscles during winter. Regarding the studied sites, the highest HSP70 level was in the eastern sector for gills, liver, and muscles, while the lowest level was in the western sector.

The integrated biomarker response index

The winter season showed higher IBRv2 scores, where *Solea aegyptiaca* were more stressed than the summer season. Furthermore, the findings revealed a potential distinction between the sites in terms of pollution levels along the lake. The eastern sector had the highest IBRv-2value, followed by the southern sector, which had the most polluted sites in direct contact with sewage and agricultural contaminants from El-Bats and El-Wadi drainage channels. In contrast, the western sector presented the lowest IBRv2. With regard to each site individually, the results showed that increased gills MDA, liver PC, gills and liver HSP70, liver MT, gills 8-OHdG and gills, liver and muscles DNA strand breaks were the most discriminant factors for the eastern sector,

Table 3 Heavy metal concentrations and MPI in liver during winter and summer seasons, 2019

Seasons	Sites	Fe (mg/kg dry weight)	Cu (mg/kg dry weight)	Zn (mg/kg dry weight)	Cd (mg/kg dry weight)	Pb (mg/kg dry weight)	Ni (mg/kg dry weight)	MPI
Winter	Western sector	353.73±5.40	23.44±3.00	30.99±10.77	0.13±0.02	0.28±0.11	3.01±0.18	5.52
	Northern sector	341.90±7.50	25.65±5.44	45.16±9.86	0.16±0.02	0.47±0.15	3.86±0.17	6.97
	Southern sector	618.05±11.81	45.62±5.10	72.93±9.92	0.27±0.05	0.85±0.05	4.94±0.17	11.52
	Eastern sector	504.13±18.40	58.65±4.78	94.86±9.96	0.38±0.04	1.06±0.27	6.08±0.45	13.79
	Mean	**454.45±119.57**	**38.34±15.73**	**60.98±27.21**	**0.24±0.11**	**0.66±0.35**	**4.47±1.23**	**9.45**
		A	**B**	**A**	**A**	**A**	**A**	
Summer	Western sector	348.84±4.04	49.97±0.63	56.98±4.49	0.17±0.01	0.31±0.07	2.93±0.33	7.32
	Northern sector	359.03±5.13	54.20±0.70	62.88±11.40	0.23±0.04	0.65±0.15	3.46±0.26	9.27
	Southern sector	496.13±12.33	80.18±0.38	84.69±4.19	0.43±0.03	1.26±0.08	5.39±0.21	14.64
	Eastern sector	672.92±14.63	67.82±0.61	104.23±8.33	0.58±0.01	1.41±0.12	5.91±0. 21	16.86
	Mean	**469.23±137.30**	**63.04±12.43**	**77.20±20.62**	**0.35±0.17**	**0.91±0.48**	**4.42±1.33**	**12.02**
		A	**A**	**A**	**A**	**A**	**A**	
All over mean	Western sector	**351.29±5.04**	**36.71±14.66**	**43.99±16.04**	**0.15±0.02**	**0.30±0.08**	**2.97±0.24**	
		b	**b**	**c**	**c**	**c**	**d**	
	Northern sector	**350.47±11.00**	**39.93±16.02**	**54.02±13.60**	**0.20±0.05**	**0.56±0.17**	**3.66±0.29**	
		b	**b**	**c**	**c**	**b**	**c**	
	Southern sector	**557.09±67.65**	**62.90±19.20**	**78.81±9.37**	**0.35±0.09**	**1.05±0.23**	**5.17±0.30**	
		a	**a**	**b**	**b**	**a**	**b**	
	Eastern sector	**588.53±93.64**	**63.24±5.88**	**99.55±9.68**	**0.48±0.11**	**1.23±0.27**	**6.00±0.32**	
		a	**a**	**a**	**a**	**a**	**a**	

In each column, means values (mean ± SD, $n = 9$) with different letters have a statistically significant difference ($P \leq 0.05$), while means having the same letter in the same column are not significantly different ($P \geq 0.05$). The capital letters indicate the significance between seasons, and small letters indicate significance between sites

while variations in MDA level in gills and PC and MT concentrations in liver, were the most crucial responses that explain the IBRv2 score in the southern sector. The western and northern sectors, on the contrary, demonstrated minimal changes and inhibition in the biomarkers analyzed. Moreover, considering IBRv2 for each organ (gills, liver, and muscles) of *Solea aegyptiaca* inhabiting Lake Qarun, regardless of sites and seasons, it revealed different results, with the liver having the highest value, followed by the gills and muscles IBRv2 showing suppression (Fig. 3).

Discussion

Lake Qarun water quality

Water temperatures are a critical control parameter in the aquatic system, influencing the aquatic environment's physical, chemical, and biological transformation [53]. The water temperature of Lake Qarun showed the expected seasonal pattern. The increase in temperature during the summer season corresponds to what Al-Afify et al. [4] had recorded and results from low water levels and solar heating, with summer days being longer than winter days [54]. The pH of natural water impacts biological and chemical reactions, as well as metal ion solubility and natural aquatic life [53]. During the summer, the relatively higher pH values in the lake's eastern and southern sectors may be due to an increase in photosynthetic activity, lowering CO_2 levels in the water. Low concentrations of DO may also play a role [55]. These findings are consistent with those obtained Al-Afify et al. [4]. Water salinity is an essential parameter for fast evaluation of water quality, Vicente-Martorell et al. [56] stated that salinity decreases fish intake and accumulation of metals and contaminants and has an inverse relationship with the water level. In the current study, salinity demonstrated a significant decrease in the eastern and southern sectors due to the diluting impact caused by drainage water from the El-Bats and El-Wadi drains, as recorded by Abd Ellah [57]. DO is critical for aquatic organisms' survival and wellness. Fish and other aquatic species rely on the DO concentration of a water body for breathing. The distribution of DO is influenced by the solubility of various inorganic nutrients [58]. In the present study, DO demonstrated low levels in the eastern and southern sectors, which may be due to dissolved oxygen depletion for oxidation of large amounts of organic materials from sewage and agricultural wastes discharged to the lake directly from El-Bats and El-Wadi drains at these areas, this observation agrees with that obtained by Tayel et al. [59]. Furthermore, the increased rate of oxygen uptake

Table 4 Heavy metal concentrations and MPI in muscles during winter and summer seasons, 2019

Seasons	Sites	Fe (mg/kg dry weight)	Cu (mg/kg dry weight)	Zn (mg/kg dry weight)	Cd (mg/kg dry weight)	Pb (mg/kg dry weight)	Ni (mg/kg dry weight)	MPI
Winter	Western sector	33.69±1.05	3.25±1.34	20.21±1.84	0.07±0.02	0.24±0.11	0.77±0.12	1.75
	Northern sector	47.25±3.05	5.23±0.84	24.12±3.30	0.09±0.02	0.56±0.04	1.19±0.01	2.66
	Southern sector	136.67±6.65	10.72±1.26	68.44±4.16	0.16±0.03	0.80±0.11	2.81±0.26	5.75
	Eastern sector	111.21±1.59	8.52±0.58	72.88±3.05	0.21±0.03	0.85±0.15	3.19±0.21	5.83
	Mean	**82.21±44.99**	**6.93±3.15**	**46.41±25.56**	**0.13±0.06**	**0.61±0.27**	**2.00±1.08**	**4.01**
		A	**A**	**A**	**A**	**A**	**A**	
Summer	Western sector	46.27±5.57	2.62±0.53	43.41±1.57	0.09±0.02	0.11±0.06	1.25±0.14	2.01
	Northern sector	54.80±5.40	2.79±0.54	48.62±1.55	0.12±0.01	0.30±0.02	1.47±0.12	2.71
	Southern sector	119.85±5.45	11.22±0.65	67.82±2.30	0.19±0.01	1.00±0.17	3.03±0.13	6.12
	Eastern sector	151.88±5.22	8.10±0.55	99.29±3.60	0.30±0.01	1.09±0.21	3.43±0.13	7.18
	Mean	**93.20±46.44**	**6.18±3.84**	**64.79±22.96**	**0.17±0.08**	**0.62±0.46**	**2.29±1.00**	**4.51**
		A	**A**	**A**	**A**	**A**	**A**	
All over mean	Western sector	39.98±7.77	2.93±0.97	31.81±12.80	0.08±0.02	0.18±0.11	1.01±0.29	
		b	c	c	c	c	d	
	Northern sector	51.03±5.70	4.01±1.47	36.37±13.62	0.10±0.02	0.43±0.14	1.33±0.17	
		b	c	c	c	b	c	
	Southern sector	128.26±10.70	10.97±0.94	68.13±3.02	0.18±0.03	0.90±0.17	2.92±0.22	
		a	b	b	b	a	b	
	Eastern sector	131.55±22.54	8.31±0.55	86.09±14.77	0.25±0.05	0.97±0.21	3.31±0.20	
		a	a	a	a	a	a	
	P.L	**5**	**5**	**40**	**0.5**	**2**	**0.5–0.6**	
	WHO							

In each column, means values (mean ± SD, $n = 9$) with different letters have a statistically significant difference ($P \leq 0.05$), while means having the same letter in the same column are not significantly different ($P \geq 0.05$). The capital letters indicate the significance between seasons, and small letters indicate significance between sites. *P.L.* Permissible level. [161] for (Fe, Cu, Zn, Pb, and Cd) & [162] for (Ni)

through a breakdown of organic matter by microorganisms, augmented by the rise in water temperature, is the leading cause of DO deficit in the summer [54, 60]. Long-term exposure to low DO levels (< 5 mg/l) increases an organism's susceptibility to other environmental stresses [61] and has dire implications for fish and other aquatic animals' survival, as reduced DO activates physiological regulatory mechanisms responsible for maintaining the oxygen gradient between water and tissues, which is necessary for the aerobic metabolic pathways to function [62].

Heavy metal bioaccumulation and metal pollution index in *Solea aegyptiaca*

Metal contamination in aquatic systems has received special attention due to its toxicity, persistence/bioaccumulation in the environment, and ecological risks [63]. The increased concentrations of all the studied heavy metals in the gills, liver, and muscles of *Solea aegyptiaca* gathered from the eastern and southern sectors of the lake were inconsistent with our recent results of chemical analysis of Lake Qarun water [64]. This finding indicates that the eastern and southern parts are the most polluted sides of the lake due to the large amount of agricultural and industrial effluents discharged directly to the lake at these sites from El-Bats and El-Wadi drains. Furthermore, these findings are compatible with those of Hussein et al. [65]. The MPI of the total combination of metals revealed that metal concentrations in gills, liver, and muscles varied seasonally, with a greater MPI for fish taken in the summer. These results are supported by Ibrahim and Omar [66] and Mohamed et al. [67].

Status and condition of *Solea aegyptiaca*

K and HSI are morphological markers that can provide information on a fish's overall health status [68]. Although physiological activity (growth, reproduction, and secretion) may influence these values under certain environmental circumstances, they may act as a first screening biomarker to detect effects [69]. The significant increase in the *K* values in fish collected from the eastern and southern sectors could be explained by the fact that untreated sewage and agricultural wastewater discharged directly from El-Bats and El-Wadi drainage canals in these lake areas is high in nutrients contributing to a high *K* value, which aligns with Fonseca et al. [70]. While

Table 5 Growth parameters of *Solea aegyptiaca* during winter and summer seasons, 2019

Seasons	Sites	K	HSI
Winter	Western sector	0.60±0.06	1.43±0.36
	Northern sector	0.64±0.06	1.35±0.30
	Southern sector	0.79±0.10	0.92±0.20
	Eastern sector	0.77±0.12	0.81±0.22
	Mean	**0.70±0.12**	**1.13±0.38**
		B	**B**
Summer	Western sector	0.74±0.13	1.94±0.78
	Northern sector	0.73±0.11	1.55±0.41
	Southern sector	0.91±0.19	1.38±0.43
	Eastern sector	0.87±0.14	1.34±0.29
	Mean	**0.81±0.16**	**1.55±0.56**
		A	**A**
All over mean	Western sector	**0.67±0.12**	**1.68±0.65**
		b	**a**
	Northern sector	**0.68±0.10**	**1.45±0.37**
		b	**b**
	Southern sector	**0.85±0.16**	**1.15±0.41**
		a	**c**
	Eastern sector	**0.82±0.14**	**1.07±0.37**
		a	**c**

In each column, means values (mean ± SD, $n = 20$) with different letters have a statistically significant difference ($P \leq 0.05$), while means having the same letter in the same column are not significantly different ($P \geq 0.05$). The capital letters indicate the significance between seasons, and small letters indicate significance between sites

lowest values of *K* were found in the winter season at which spawning of *Solea aegyptiaca* occurs, as indicated by Ahmed et al. [71] and Kalifa et al. [72]. Similar patterns of *K* value decline have been observed in various fish species and have become an indicator of spawning processes [73, 74]. The significant decrease in HSI, which is another parameter of fish growth, in fish collected from the eastern and southern sectors of the lake is consistent with that of Zaghloul et al. [75], who attributed it to depletion of hepatic glycogen accompanied by hyperglycemia, indicating the fish's requirement for energy to withstand the heavy metal contamination stress. Bakhoum et al. [76] reported that the lowest values of hepatosomatic indices were recorded only during the peak spawning period. This finding is in agreement with our finding, where the lowest values of HSI were found in the winter season at which spawning of *Solea aegyptiaca* occurs.

Oxidative stress responses

Oxidative stress is an inherent element of aerobic life, resulting from the imbalance between ROS formation and antioxidant defences in biological systems [77]. The production of radicals such as ROS, which can induce oxidative stress, is one of the most well-studied pathways by which HM can cause pathophysiological processes. These active species can further induce oxidative damages to biomolecules (such as DNA, proteins, and lipids), impairing their functional properties, resulting in changes in the normal activities of cells, tissues, organs, and eventually organisms, as manifested by disease symptoms and other pathological conditions [10, 78]. However, the antioxidant defence mechanism is triggered as a radical scavenging system to counteract the threat of reactive species [79]. An antioxidant is defined as any substance that, when present in low concentrations, inhibits or prevents oxidative damage to essential molecules [80].

Individual antioxidant responses were combined with the measurement of TAC [81]. The considerable decline in TAC in gills, liver, and muscles of fish caught from Lake Qarun's eastern and southern regions, exposed to heavy metal pollution from El-Bats, and El-Wadi drainage channels at these sites, is similar to those of Benedetti et al. [82] showed a negative response of TAC in the liver of *Anguilla Anguilla* under field conditions. In addition, Mehrpak et al. [83] and Banaee et al. [84] reported antioxidant depletion following cadmium exposure in common carp (*Cyprinus carpio*) liver and muscles, respectively. Moreover, Drąg-Kozak et al. [85] found that the total antioxidant capacity level in the hepatopancreas was significantly decreased in Cd-treated Prussian carp (*Carassius gibelio*) fish. The present study demonstrated no seasonal variations in gills, liver, and muscles of Lake Qarun, s *Solea aegyptiaca* fish which could be because, according to the study of Gorbi et al. [16] in the European Eel *Anguilla Anguilla* liver, the seasonal rise in natural oxidative pressure is balanced by fluctuations in individual antioxidants, without changing the organisms' total redox status.

Peroxidation of lipids is a sign of oxidative damage in cells and tissues resulting from a disturbance in the balance between prooxidant and antioxidant agents [11, 86]. The most common method for monitoring LPO is measuring the end-product, MDA [87]. The significant increase in MDA levels in gills, liver, and muscles of *Solea aegyptiaca* fish gathered from the eastern and southern sectors of Lake Qarun, exposed to heavy metals from drainage channels at these sites, is a clear oxidative stress indication. These observations are similar to that provided by da Silva Montes et al. [86], Nunes et al. [88], and Sifi and Soltani [89], who attributed the observed rise in MDA to heavy metals accumulating in several tissues of polluted fish. Heavy metal metabolism frequently leads to ROS production, which is known to extract hydrogen atoms from unsaturated bonds, changing lipid structure and function. The extraction of hydrogen atoms from a

Table 6 Oxidative stress biomarkers of *Solea aegyptiaca* organs during winter and summer seasons, 2019

Seasons	Sites	TAC (%)			MDA(nmol/ g tissue)			PC (nmol/ g tissue)			8-OHdG (ng/ g tissue)		
		Gills	Liver	Muscles	Gills	Liver	Muscles	Gills	Liver	Muscles	Gills	Liver	Muscles
Winter	Western sector	65.26±1.85	72.12±3.00	85.29±3.48	37.35±5.17	27.31±5.53	11.13±3.32	50.35±9.49	100.98±6.54	32.51±9.27	22.22±3.52	27.13±2.39	17.07±2.57
	Northern sector	59.86±3.05	66.74±2.86	80.74±3.13	67.40±19.28	45.08±8.67	13.60±2.62	68.73±10.87	136.16±7.81	40.35±8.46	31.62±1.89	31.55±2.32	24.70±2.75
	Southern sector	53.61±2.42	56.58±2.99	71.40±3.21	135.54±57.01	109.44±17.07	24.89±2.88	180.04±12.21	249.59±8.29	77.27±8.98	44.35±2.07	42.78±2.49	35.47±3.54
	Eastern sector	49.52±2.21	59.92±1.77	73.06±3.22	163.46±43.11	152.75±7.34	27.59±2.55	142.97±17.33	233.69±4.93	70.85±7.22	56.02±2.48	54.24±4.48	40.41±3.09
	Mean	**57.06±6.53 A**	**63.84±6.65 A**	**77.62±6.53 A**	**100.94±62.27 A**	**83.65±52.28 A**	**19.31±7.86 A**	**110.52±55.48 A**	**180.11±64.72 A**	**55.24±21.12 A**	**38.55±13.28 A**	**38.92±11.12 A**	**29.41±9.73 A**
Summer	Western sector	61.32 ± 2.93	76.69 ± 3.87	89.07 ± 3.91	25.88 ± 7.73	16.34 ± 1.80	2.64 ± 0.70	23.29 ± 8.62	52.68 ± 9.43	12.26±5.04	14.88±1.58	21.32±3.17	12.39±2.42
	Northern sector	62.42 ± 1.91	71.01 ± 2.72	83.76 ± 5.02	36.73 ± 8.52	29.45 ± 3.45	5.59 ± 0.72	33.52 ± 7.08	67.47 ± 8.68	21.39±4.90	19.14±2.43	26.63±2.75	17.12±2.20
	Southern sector	54.28 ± 2.41	59.11 ± 4.22	73.70 ± 6.07	89.72 ± 14.05	66.79 ± 5.35	9.59 ± 1.68	86.42 ± 7.03	115.24±10.45	33.88±6.74	37.70±2.34	36.26±2.24	25.96±3.50
	Eastern sector	51.36 ± 1.39	63.54 ± 3.77	76.86 ± 5.06	106.23 ± 11.45	96.02 ± 6.65	11.92 ± 2.46	74.77 ± 7.17	105.71±12.54	30.06±6.36	45.28±2.33	47.10±2.55	32.24±2.49
	Mean	**57.35 ± 5.19 A**	**67.59 ± 7.72 A**	**80.85 ± 7.73 A**	**64.64 ± 36.20 B**	**52.15 ± 32.34 B**	**7.44 ± 3.93 B**	**54.50 ± 28.12 B**	**85.27±28.22 B**	**24.40±10.10 B**	**29.25±13.05 B**	**32.83±10.35 A**	**21.93±8.25 B**
All over mean	Western sector	**63.29 ± 3.12 a**	**74.41 ± 4.08 a**	**87.18 ± 4.04 a**	**31.62 ± 8.67 b**	**21.83 ± 6.95 c**	**6.88 ± 4.99 b**	**36.82 ± 16.56 b**	**76.83 ± 26.38 b**	**22.38 ± 12.74 b**	**18.55 ± 4.63 d**	**24.22 ± 4.04 d**	**14.73 ± 3.41 d**
	Northern sector	**61.14 ± 2.77 b**	**68.88 ± 3.47 b**	**82.25 ± 4.29 b**	**52.06 ± 21.41 b**	**37.26 ± 10.30 c**	**9.61 ± 4.58 b**	**51.12 ± 20.36 b**	**101.81 ± 36.72 b**	**30.87 ± 11.89 b**	**25.38 ± 6.84 c**	**29.09 ± 3.53 c**	**20.91 ± 4.62 c**
	Southern sector	**53.94 ± 2.33 c**	**57.84 ± 3.73 d**	**72.55 ± 4.78 c**	**112.63 ± 46.26 a**	**88.11 ± 25.33 b**	**17.24 ± 8.30 a**	**133.23 ± 49.81 a**	**182.41 ± 70.74 a**	**55.57 ± 23.89 a**	**41.02 ± 4.06 b**	**39.52 ± 4.09 b**	**30.71 ± 5.10 b**
	Eastern sector	**50.44 ± 2.00 d**	**61.73 ± 3.39 c**	**74.96 ± 4.50 c**	**134.85 ± 42.40 a**	**124.38 ± 30.37 a**	**19.76 ± 8.53 a**	**108.87 ± 37.80 a**	**169.70 ± 67.45 a**	**50.45 ± 22.27 a**	**50.65 ± 6.06 a**	**50.67 ± 5.10 a**	**36.32 ± 5.04 a**

In each column, means values (mean ± SD, $n = 18$) with different letters have a statistically significant difference ($P \leq 0.05$), while means having the same letter in the same column are not significantly different ($P \geq 0.05$). The capital letters indicate the significance between seasons, and small letters indicate significance between sites

Table 7 DNA damage of *Solea aegyptiaca* organs during winter and summer seasons, 2019

Seasons	Sites	Gills DNA strand breaks			Liver DNA strand breaks			Muscles DNA strand breaks		
		Tail DNA (%)	Tail Length (µm)	Tail moment	Tail DNA (%)	Tail Length (µm)	Tail moment	Tail DNA (%)	Tail Length (µm)	Tail moment
Winter	Western sector	2.25 ± 0.04	2.33 ± 0.06	5.24 ± 0.04	2.47 ± 0.04	2.40 ± 0.03	5.90 ± 0.03	2.29 ± 0.04	2.20 ± 0.15	5.59 ± 0.03
	Northern sector	2.42 ± 0.05	2.37 ± 0.04	5.74 ± 0.04	2.51 ± 0.07	2.44 ± 0.05	6.12 ± 0.09	2.31 ± 0.05	2.45 ± 0.03	5.66 ± 0.05
	Southern sector	3.12 ± 0.09	3.25 ± 0.07	9.99 ± 0.76	2.71 ± 0.32	2.82 ± 0.03	7.64 ± 0.61	2.50 ± 0.26	2.82 ± 0.21	6.60 ± 0.70
	Eastern sector	3.68 ± 0.09	3.58 ± 0.05	13.17 ± 0.04	3.58 ± 0.09	3.53 ± 0.05	12.60 ± 0.89	3.64 ± 0.07	3.72 ± 0.04	13.52 ± 0.58
	Mean	**2.87 ± 0.59**	**2.88 ± 0.56**	**8.54 ± 3.34**	**2.82 ± 0.49**	**2.80 ± 0.46**	**8.07 ± 2.81**	**2.69 ± 0.58**	**2.80 ± 0.60**	**7.84 ± 3.40**
		A	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**
Summer	Western sector	2.04 ± 0.14	2.05 ± 0.04	4.06 ± 0.05	1.35 ± 0.03	1.23 ± 0.30	1.66 ± 0.06	1.37 ± 0.03	1.49 ± 0.03	2.07 ± 0.60
	Northern sector	2.37 ± 0.03	2.33 ± 0.03	5.69 ± 0.04	1.31 ± 0.03	1.21 ± 0.31	1.59 ± 0.03	1.58 ± 0.04	1.67 ± 0.03	2.64 ± 0.59
	Southern sector	2.44 ± 0.02	2.34 ± 0.02	5.81 ± 0.03	2.88 ± 0.04	2.59 ± 0.03	7.46 ± 0.05	2.49 ± 0.04	2.31 ± 0.04	5.75 ± 0.04
	Eastern sector	2.69 ± 0.05	2.43 ± 0.06	6.29 ± 0.05	3.01 ± 0.08	2.95 ± 0.03	8.90 ± 0.07	2.52 ± 0.05	2.49 ± 0.04	6.27 ± 0.05
	Mean	**2.39 ± 0.25**	**2.29 ± 0.15**	**5.46 ± 0.86**	**2.14 ± 0.83**	**2.00 ± 0.83**	**4.90 ± 3.39**	**2.00 ± 0.53**	**2.00 ± 0.43**	**4.18 ± 1.93**
		B	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**
All over mean	Western sector	**2.15 ± 0.15**	**2.19 ± 0.16**	**4.65 ± 0.62**	**1.91 ± 0.59**	**1.82 ± 0.64**	**3.78 ± 2.21**	**1.83 ± 0.48**	**1.85 ± 0.39**	**3.83 ± 1.88**
		c	**b**	**c**	**c**	**c**	**c**	**c**	**c**	**c**
	Northern sector	**2.40 ± 0.05**	**2.35 ± 0.04**	**5.72 ± 0.05**	**1.91 ± 0.63**	**1.82 ± 0.67**	**3.86 ± 2.37**	**1.95 ± 0.38**	**2.06 ± 0.41**	**4.15 ± 1.63**
		c	**b**	**c**	**c**	**c**	**c**	**c**	**c**	**c**
	Southern sector	**2.78 ± 0.36**	**2.80 ± 0.48**	**7.90 ± 2.24**	**2.80 ± 0.24**	**2.71 ± 0.12**	**7.55 ± 0.42**	**2.50 ± 0.18**	**2.57 ± 0.30**	**6.18 ± 0.63**
		b	**a**	**b**	**b**	**b**	**b**	**b**	**b**	**b**
	Eastern sector	**3.19 ± 0.52**	**3.01 ± 0.60**	**9.73 ± 3.59**	**3.29 ± 0.31**	**3.24 ± 0.30**	**10.75 ± 2.03**	**3.08 ± 0.59**	**3.11 ± 0.64**	**9.89 ± 3.81**
		a	**a**	**a**	**a**	**a**	**a**	**a**	**a**	**a**

In each column, means values (mean ± SD) with different letters have a statistically significant difference ($P \leq 0.05$), while means having the same letter in the same column are not significantly different ($P \geq 0.05$). The capital letters indicate the significance between seasons, and small letters indicate significance between sites

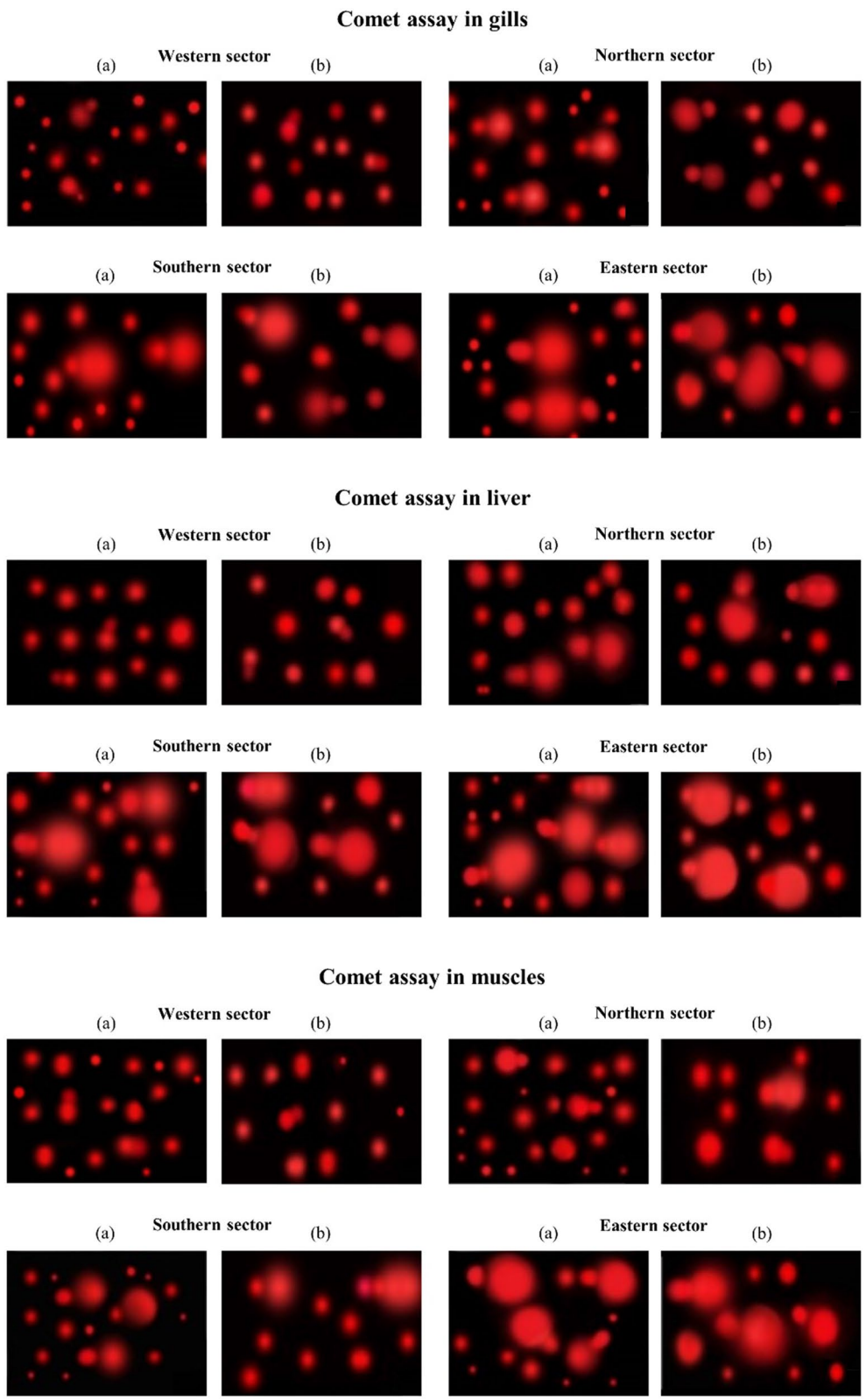


Fig. 2 Representative results of comet assay in gills, liver, and muscles of *Solea aegyptiaca* fish collected from different sites along Lake Qarun during winter **a** and summer **b** seasons, 2019. [The more the damage, the larger the DNA tail area ((%)) or the longer the DNA tail length]

Table 8 Stress proteins biomarkers of *Solea aegyptiaca* organs during winter and summer seasons, 2019

Seasons	Sites	MT (µg / g tissue)			HSP70 (ng/ g tissue)		
		Gills	Liver	Muscles	Gills	Liver	Muscles
Winter	Western sector	131.06 ± 1.89	255.53 ± 5.21	105.61 ± 3.83	1.70 ± 0.29	1.76 ± 0.24	1.62 ± 0.20
	Northern sector	133.01 ± 1.55	260.07 ± 2.88	106.73 ± 1.49	1.87 ± 0.20	1.91 ± 0.23	1.84 ± 0.23
	Southern sector	140.22 ± 1.32	335.15 ± 0.91	111.85 ± 1.53	2.78 ± 0.27	2.81 ± 0.27	2.39 ± 0.22
	Eastern sector	142.37 ± 2.27	329.54 ± 1.46	113.17 ± 2.88	3.09 ± 0.38	3.19 ± 0.30	2.71 ± 0.24
	Mean	**136.66 ± 5.12**	**295.07 ± 38.27**	**109.34 ± 4.11**	**2.36 ± 0.66**	**2.42 ± 0.66**	**2.14 ± 0.49**
		A	**A**	**A**	**A**	**A**	**A**
Summer	Western sector	120.50 ± 2.67	242.56 ± 5.59	97.88 ± 3.83	1.48 ± 0.22	1.46 ± 0.16	1.13 ± 0.21
	Northern sector	122.10 ± 3.20	249.39 ± 4.24	100.75 ± 3.27	1.61 ± 0.21	1.64 ± 0.16	1.30 ± 0.21
	Southern sector	129.98 ± 4.17	280.35 ± 4.62	104.85 ± 3.91	2.00 ± 0.18	2.23 ± 0.24	1.81 ± 0.21
	Eastern sector	133.72 ± 2.01	271.69 ± 5.14	106.14 ± 3.06	2.38 ± 0.24	2.51 ± 0.18	2.13 ± 0.40
	Mean	**126.58 ± 6.30**	**261.00 ± 16.51**	**102.41 ± 4.71**	**1.87 ± 0.41**	**1.96 ± 0.47**	**1.59 ± 0.48**
		B	**B**	**B**	**B**	**B**	**B**
All over mean	Western sector	**125.78 ± 5.94**	**249.04 ± 8.51**	**101.74 ± 5.44**	**1.59 ± 0.27**	**1.61 ± 0.25**	**1.38 ± 0.32**
		b	**b**	**b**	**c**	**c**	**c**
	Northern sector	**127.55 ± 6.18**	**254.73 ± 6.56**	**103.74 ± 3.95**	**1.74 ± 0.24**	**1.77 ± 0.24**	**1.57 ± 0.35**
		b	**b**	**b**	**c**	**c**	**c**
	Southern sector	**135.10 ± 6.10**	**307.75 ± 28.80**	**108.35 ± 4.62**	**2.39 ± 0.46**	**2.52 ± 0.39**	**2.10 ± 0.36**
		a	**a**	**a**	**b**	**b**	**b**
	Eastern sector	**138.05 ± 4.95**	**300.62 ± 30.42**	**109.65 ± 4.64**	**2.73 ± 0.48**	**2.85 ± 0.43**	**2.42 ± 0.44**
		a	**a**	**a**	**a**	**a**	**a**

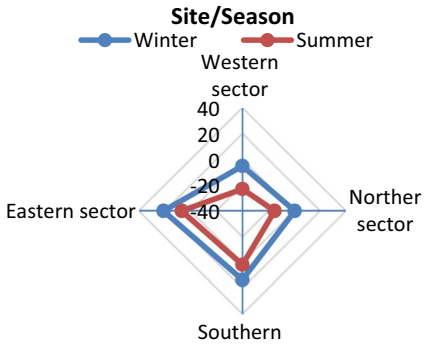
In each column, means values (mean ± SD, $n = 18$) with different letters have a statistically significant difference ($P \leq 0.05$), while means having the same letter in the same column are not significantly different ($P \geq 0.05$). The capital letters indicate the significance between seasons, and small letters indicate significance between sites

methylene group is easier in polyunsaturated fatty acids due to the near closeness of the unsaturated bonds, which allows for the easier abstraction of hydrogen atoms. The fluidity of the membrane, and thus the organelle's function and cell health, may be impaired as a result [90]. Therefore, the observed increased MDA level indicated lipid molecule vulnerability to ROS and the magnitude of oxidative damages imposed on these molecules.

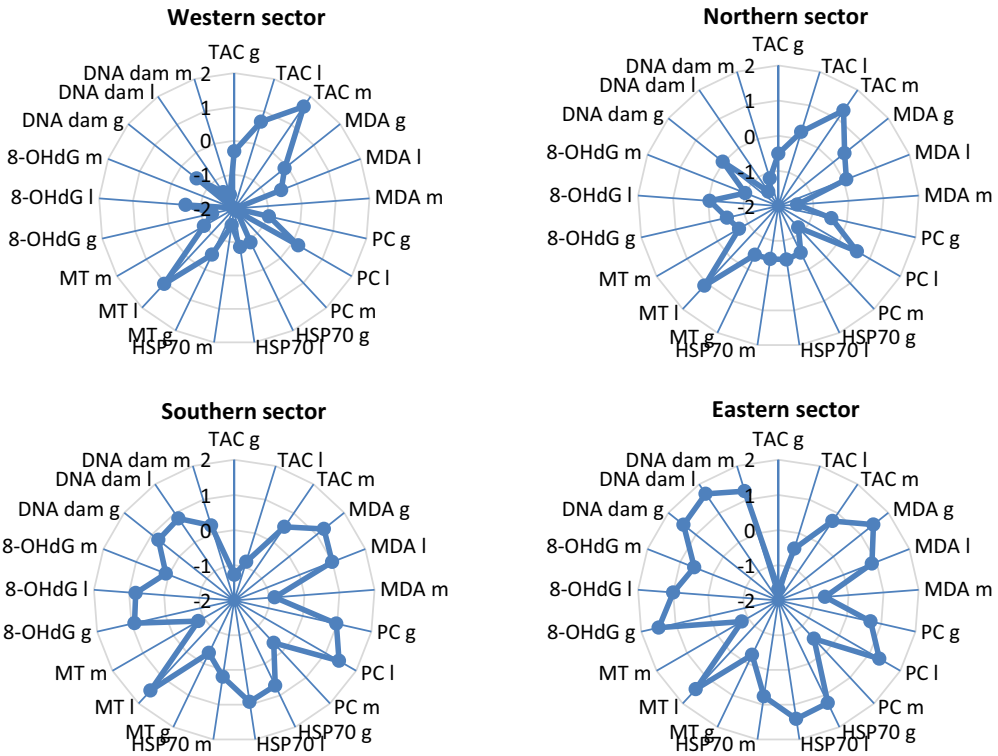
In addition, previous evidence has indicated that acidic or alkaline pH can trigger a prooxidant in different fish organs [91, 92]. The higher LPO and lower TAC in the gills, liver, and muscles of *Solea aegyptiaca* exposed to alkaline pH at the eastern and southern sectors in this study were expected and demonstrated that the function of these organs was affected, and fish were not able to neutralise ROS. Similarly, in tambaqui (*Colossoma macropomum*), Nile tilapia (*Oreochromis niloticus*), Amazon catfish (*Pseudoplatystoma reticulatum*), and pacu (*Piaractus mesopotamicus*), alkaline pH present more deleterious effects than acidic pH [93–95]. However, the deterioration of water quality in extreme drought conditions during the dry season (summer season) causes oxidative stress and enhanced peroxidation of lipids as fish are subjected to hypoxia under these conditions [96]. The present results showed the highest gills, liver, and muscle MDA levels during the winter season. Elevated MDA levels during the spawning period were also shown by Soldatov et al. [97] in bivalve *Mytilus galloprovincialis* (L.) and Morozov et al. [98] in common bream *Abramis brama* (L.). Therefore, spawning could be a physiological state in which aquatic organisms are naturally exposed to oxidative stress [97]. Moreover, cold aquatic habitats had higher DO levels (in harmony with our previous results) and higher intracellular lipid contents [99]. Increased lipid concentrations and improved oxygen solubility facilitate oxygen transport in fish tissues, as does higher lipid unsaturation, which stimulates LPO chain reactions [100].

Another critical parameter by which to evaluate oxidative stress damage is PC formation. PCs are an irreversible post-translational alteration that occurs early in the oxidative stress process [101]. Proteins are targets for free radicals, and protein oxidation is described as a covalent modification of proteins caused by ROS or reaction with by-products of oxidative stress, resulting in the change of specific amino acid residues and the formation of PC derivatives [102]. It is a valuable biomarker for the exposure of aquatic organisms to environmental pollutants

a) IBRv2 for all 4 sites during winter and summer seasons



b) Biomarkers star plots for each site



c) IBRv2 for *Solea aegyptiaca* organs

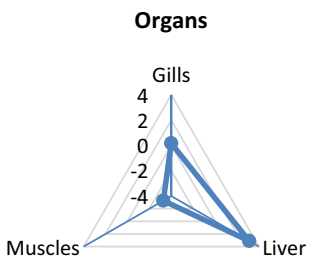


Fig. 3 Integrated biomarker response index (IBRv2**)** depending on the following biomarkers: TAC, MDA, PC, HSP70, MT, 8-OHdG, and DNA damage (DNA dam) in different organs (g = gills, l = liver, m = muscles) of *Solea aegyptiaca* collected from Lake Qarun during winter and summer seasons, 2019

[103], and metal-catalyzed protein oxidation is an important process by which carbonyls are incorporated into proteins [104]. The significant increase in PC level in gills, liver, and muscles of *Solea aegyptiaca* from the eastern and southern sectors indicates that heavy metal pollution at these locations had induced oxidative stress in fish. Previous studies have shown similar observations that exposure to metals induces PC in the gill, liver, brain, kidney, and muscle of red sea bream [105], gill, liver, kidney, and brain of *Channa striatus* and *Heteropneustes fossilis* [106] and gill, liver, and muscle of *Notopterus notopterus* [107]. In the metal-catalyzed oxidation reactions, an electron donor system is needed to catalyze the reduction of O_2 to H_2O_2 and Fe(III) to Fe(II). Superoxide anions can react directly with Fe(III) to produce the reduced form of the metal. The Fe(II) ion then binds to the metal-binding site on the protein, followed by a reaction with H_2O_2. This reaction generates •OH, OH-•, and Fe(III). The active oxygen species react locally with the amino acid side chains at the metal-binding site leading to metal oxidation and protein carbonylation [108]. The significant increase in protein damage (oxidation) during winter season, spawning period of *Solea aegyptiaca*, as indicated by elevated PC content in gills, liver, and muscle tissues was stated by Husmann et al. [109] found an induction in PC levels in some Mollusk species, such as *Laternula ellipti*ca contaminated with Copper and Iron during the reproductive period. This finding could be due to the release of sexual products during spawning, which causes tissue damage and oxidation [110]. In addition, the reduced environmental temperature may raise DO levels in seawater and, consequently, in ectothermic animals' internal fluids, thus increasing the danger of ROS generation [111]. Moreover, during cold acclimation, all tissues are expected to increase oxygen concentration and mitochondrial ROS generation and decrease protein turnover [112].

Deoxyribonucleic acid (DNA) nucleotides are common targets for oxidative damage because of their significant mutagenic and oxidative capacity [113]. Guanine is the most prone to oxidation of all nucleic base pairs [114]. When a free hydroxyl group oxidizes guanine, it becomes 8-OHdG, a common form of DNA distortion caused by free radicals [114, 115]. The levels of 8-OHdG in aquatic species have been studied and are thought to be a biomarker of oxidative stress caused by pollution [116]. The significant induction in 8-OHdG level in gills, liver, and muscles of *Solea aegyptiaca* collected from eastern and southern sectors of Lake Qarun revealed that exposure to heavy metals from the non-treated sewage and agricultural wastewater resulted in significant oxidative damage to DNA in *Solea aegyptiaca* fish, indicating that the lake water was genotoxic. This finding is confirmed by the study of Sun et al. [14] in zebrafish liver and Jebali et al. [117] in gills of clams, which found an increase in 8-OHdG levels in fish inhabiting ecosystems subjected to anthropogenic disturbances from wastewater effluents. In addition, according to our findings, Alak et al. [118] and Topal et al. [119] reported that the activity of 8-OHdG may be increased in the liver and brain tissues of rainbow trout as a result of toxic chemicals under laboratory conditions. The significantly increased levels of DNA oxidation in *Solea aegyptiaca* gills, liver, and muscles during winter (spawning period) are in line with the study of Nagasaka et al. [120] observed increased expression of 8-OHdG in ayu brain DNA during the spawning period, implying that DNA damages caused by oxidative stress and ROS had induced during reproduction.

Genotoxicity

Genotoxic agents can impair the DNA integrity, and the extent of DNA strand breaks can be utilized as a sensitive genotoxicity indicator and thus, as a biomarker of exposure. Single-cell gel electrophoresis, or comet assay, is a simple, adaptable, quick, sensitive, and widely used way of assessing DNA damages at the cellular level [121]. In this study, the increased DNA damage in *Solea aegyptiaca* collected from the eastern sector exposed to the El-Bats drainage channel may be related to the high detected levels of metals and the excessive metal bioaccumulation in fish tissues at this site. Heavy metals have been found to create adducts with DNA via intercalating or covalently interacting with the molecule. These metals generate ROS, inducing oxidative stress and single and double-strand breakage [122]. These results are confirmed by previous studies on Lake Qarun that reported increased DNA damage on *Solea Vulgaris* and *Tilapia Zilli* liver [123] and *Tilapia Zilli* and *Mugil cephalus* liver, gills, and muscle [124], which was related to the level of heavy metal pollution in the studied sites. The increased DNA damage during *Solea aegyptiaca* spawning (winter season) could be attributed to an increase in ROS generation during the reproductive cycle [125]. These free radicals can indirectly harm DNA through LPO [126] as MDA, an LPO product, forms adducts with DNA, causing DNA damage [127]. This finding is inconsistent with our results of increasing LPO during the spawning season of *Solea aegyptiaca*.

Stress proteins

In fish, sub-lethal exposure to heavy metals triggers a number of cellular defence mechanisms. The principal defence against heavy metals is the heavy metal-binding proteins gene expression in impacted cells. Heavy metal stress stimulates the production of several stress proteins, which reduce the harmful effects of heavy metals [128].

These stress proteins, such as HSPs and MTs, function as molecular chaperones [129] and have been proposed as a biomarker, because they are specific and provide a quick inductive signal of heavy metal contamination before the deleterious effects of heavy metals [130].

MT is a cysteine-rich low-molecular-weight cytosolic protein that binds heavy metals [129] and plays a crucial role in regulating metal homeostasis within cells, preventing metal toxicity, and guarding against oxidative damage within cell sequesters these metals, thus acting as a detoxification protein [131]. The detoxication occurs via metal-mediated activation of the gene transcription that expresses MTs, consequently increasing its synthesis and removing metals that bind to them. Considering the absorption of metals by aquatic organisms and the induction of MTs due to the increase in these elements, MTs are significant biomarkers in aquatic environments [132]. This importance is supported by our results of the significant increase in gills, liver, and muscles MT expression in the heavy metal contaminated sectors, which is in agreement with the study of Khodadoust, and Ahmad [133], who suggested that there was a connection between some heavy metal contamination and induction of MT in gill, liver, and muscle of Java medaka fish (*Oryzias javanicus*). In addition, Carvalho et al. [134] reported that MT levels were substantially induced in the liver and gill of the tilapia from Monjolinho River exposed to metal pollution from industrial and urban waste effluents as compared to their references. Therefore, it can be speculated that the induction of MTs is the most common way of metal detoxification in fish [135] and can be susceptible to a combination of metals (Fe, Cu, Zn, Cd, Pb, Ni), each in low concentration. Although MTs are commonly related to exposure to metals, their values can be affected by both endogenous and exogenous variables such as pH, salinity [136], reproduction, the existence of glucocorticoids, specific stress situations, or cold temperatures [137, 138]. The significant increase in MT levels in gills, liver, and muscles of *Solea aegyptiaca* fish during the spawning period (winter season) suggests that the seasonality of the reproductive cycle influences MT levels in *Solea aegyptiaca*. Similarly, Gorbi et al. [16] observed that the levels of hepatic MT revealed a double rise in October when the spawning of *Mugil cephalus*. Changes in MTs due to reproduction have also been identified as crucial in monitoring programmes for European flounders [139].

In addition to MT, HSP70 has been found to play a protective role in cells exposed to stress [140, 141]. It prevents non-native proteins from aggregating and promotes their refolding and transportation to cellular organelles [142]. The significant increase in HSP70 levels in gills, liver, and muscles of *Solea aegyptiaca* fish obtained from the heavy metal polluted sites was observed by many investigators who reported increased expression of HSP70 due to metal exposure in different fish organs/species such as *Chanos chanos* gills, liver and muscle [143], *Tanichthys albonubes* liver [144], *Oreochromis niloticus* muscle [145] and *Cirrhinus cirrhosis* liver [146]. In response to metal pollution stress, heat shock factors (HSFs) that are typically bind HSPs in the cytosol are dissociated from HSPs. Once liberated, HSFs are phosphorylated and form trimers. The trimerized HSF then enters the nucleus and binds to heat shock elements (HSEs) in the promoter region of the HSP70 gene, initiating the expression of HSP70 [147]. As a result, the significant induction of HSP70 in the organs of the *Solea aegyptiaca* fish verifies stress resistance and appears to be closely linked to metal pollution management through a cytoprotective role.

Contrary to the hypotheses, the present study showed that gills, liver, and muscles HSP70 levels increased significantly in *Solea aegyptiaca* during winter (spawning period). This finding may be related to the enhanced ROS production during the reproductive cycle [125], which can trigger the HSP induction [148]. In addition, during spawning, feeding activity may diminish or completely cease [149], as confirmed by our previous results of the decrease in growth indices of *Solea aegyptiaca* during winter, which in turn induced HSP70 expression as reported by Antonopoulou et al. [150] who observed increased HSP70 levels in the 3-week hunger. Furthermore, hormonal processes linked to reproduction may also elicit physiological changes before water temperatures rise to summer levels, leading to HSP70 activation [151]. Similarly, Bildik et al. [140] and Feidantsis et al. [152] illustrated that cold acclimatisation had enhanced HSP70 expression in a variety of fish tissues. Moreover, a direct impact of low temperature on protein stability is likely related to the heat shock response, because cold stress, like heat stress, could destabilize hydrophobic interactions, leading to damaged or misfolded proteins, and seems to stimulate chaperone or regulatory protein mechanisms [153]. Overall, although the stimulation of MT and HSP70 expression did not result in death, it compromised the ability of *Solea aegyptiaca* gills, liver, and muscles to handle xenobiotics and spawning-related stress through the expression of stress proteins.

The integrated biomarker response index

Several authors have recently proposed adopting approaches that combine numerous biomarker responses into a single value, thereby explaining the data and simplifying large-scale biomarker arrangement in environmental monitoring [154, 155]. Beliaeff–Burgeot's IBR is a valuable tool for describing the health state of organisms

exposed to stressful situations in a simple and comprehensive manner by presenting a graphical overview and an integrated numerical value of the various biomarkers [156]. The present work used a suite of biomarkers including TAC, MDA, PC, 8-OHdG, DNA dam, MT, and HSP-70 in gills, liver, and muscles to calculate IBRv2. Here, IBRv2 revealed that the winter season was more stressful for *Solea aegyptiaca* (high IBRv2 value), possibly due to stress during spawning. Similar results were recorded by Bodin et al**.** [157] and Leinio¨ and Lehtonen [158].

In addition, the IBRv2 star plots are consistent with the variations in the pollution levels at each site and the extent of anthropogenic impact in these ecosystems, since the highest value of IBRv2 was detected in eastern and southern sectors of Lake Qarun, exposed to heavy metals from El-Bats and El-Wadi drainage channels at these sites. Indeed, the different forms of the star plots of the examined locations revealed a distinct contamination pattern, supporting the chemical analysis of those sites. This finding demonstrates biomarker integration's effectiveness for environmental assessment as previously reported [155, 159]. Several biomarkers in the current study showed a response triggered or inhibited depending on the investigated location. The spatial arrangement of these biomarkers in the star plot provided a more precise visualisation of which biomarkers were the most sensitive in this kind of evaluation.

Consequently, MDA in gills, PC in the liver, HSP70 in gills and liver, MT in the liver, 8-OHdG in gills, and the comet assay, which measures DNA damage in gills, liver, and muscles were the biomarkers that turned out to be the most effective in this study. Moreover, IBRv2 could distinguish organs of *Solea aegyptiaca* depending on the responses of biomarkers. The liver was the most affected organ (higher IBRv2 value), followed by the gills, while muscles IBRv2 showed inhibition. This finding may be related to the level of heavy metal accumulation confirmed by the metal pollution index of these organs.

Conclusions

The use of an integrated approach based on fish biomarkers is a precise and efficient technique for assessing Lake Qarun's environmental state. Heavy metals had an impact on the contaminated sites (eastern and southern sectors) in concentrations enough to influence the health of *Solea aegyptiaca* fish. Moreover, the obtained results suggest that the examined responses are related to seasonality, and the most common finding in the current study was that *Solea aegyptiaca* showed the most significant fluctuations in the winter when spawning occurs. Therefore, *Solea aegyptiaca* fish has proven to be an appropriate sentinel species for assessing environmental pollutants using a set of biomarkers. Overall, IBRv2 analysis was able to compare seasons, sites, and organs based on integrating biomarkers responses, demonstrating the efficacy of this biomonitoring method.

Abbreviations

IBRv2: Integrated biomarker response index version 2; ROS: Reactive oxygen species; LPO: Lipid peroxidation; PC: Protein carbonylation; TAC: Total antioxidant capacity; HSPs: Heat shock proteins; MTs: Metallothioneins; K: Condition factor; HIS: Hepatosomatic index; DO: Dissolved oxygen; BOD: Biochemical oxygen demand; PBS: Phosphate buffered saline; ICP: Inductively coupled plasma emission spectrometer; MPI: Metal pollution index; TCA: Trichloroacetic acid; MDA: Malondialdehyde; DNPH: Dinitrophenylhydrazone; 8-OHdG: 8-Hydroxy-2′deoxyguanosine; SDS: Sodium dodecyl sulphate; HSP 70: Heat shock protein 70; HSFs: Heat shock factors; HSEs: Heat shock elements; ANOVA: Analysis of variance; SD: Standard deviation; P.L.: Permissible level; NS: Non-significant; DNA dam: DNA damage; G: Gills; L: Liver; M: Muscles; DNA: Deoxyribonucleic acid.

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Author contributions

AE contributed to the study design, the sampling and processing of samples, analysis, evaluation and interpretation the data and wrote the manuscript. ME, HG, EH and SH supervised the study, contributed to the study design, the interpretation of the results and support writing the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The data used and analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

All applicable international, national, or institutional guidelines for the care and use of animals were followed by the authors.

Consent for publication

All the authors read and approved this paper.

Competing interests

The authors declare that they have no competing interests.

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