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Evaluation of the Environmental Oxidative Stress on Apoptotic Related Genes and DNA Damage in *Sparus aurata* and *Dicentrarchus labrax* in Different Areas Along the Mediterranean Sea, Egypt

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ABSTRACT

Marine fish play a crucial role in human diets by providing essential animal protein, particularly where other sources of protein are lacking. However, industrial activities in coastal regions can introduce pollutants that negatively impact the natural environment including aquatic organisms. This study aimed to assess the risks associated with pollution in water, sediments, and fish species—*Sparus aurata* and *Dicentrarchus labrax*—affected by aquaculture, urban rivers, and ports. To achieve this, physicochemical parameters such as pH, oxygen dissolution, chemical oxygen demand (COD), total dissolved solids (TDS), alkalinity, ammonia, nitrite, and nitrate were measured in three locations: Shatby, El-Max, and Anfoushi in Alexandria, Egypt. The research combined these measurements with molecular and biochemical endpoints to evaluate the impact of pollution. The results indicated a significant pollution impact on hepatic antioxidants, apoptotic-related genes, and DNA damage. Bacterial growth density was at its highest at El-Max, followed by Anfoushi and Shatby. Liver enzyme levels of glutathione transferase (GST) and glutathione peroxidase (GPx) were elevated in fish from El-Max and Anfoushi compared to Shatby. Additionally, metal concentrations of Zn, Mn, Cu, and Cd were higher at the El-Max site. Overall, the study highlights that pollution can alter the oxidative state and gene expression in aquatic organisms. The findings underscore the urgent need for stringent regulations to protect aquatic environments and ensure that fish, as a source of animal protein, remain safe for human consumption.

INTRODUCTION

Logistically, coastal zones are significant in terms of economy, ecology, and society **(Martinetto *et al.*, 2020)**. Nonetheless, they frequently experience man-made

stresses like urbanization, population expansion, and industrialization in addition to natural disasters like geological movements, erosion, siltation, and tsunamis. While there is a lot of biodiversity in the Mediterranean Sea, its marine ecosystems are under danger due to an increase in chemical pollution from mining, industry, and agriculture **(Ourgaud *et al.*, 2018; Ghani *et al.*, 2023)**.

The coastal region of Egypt is rich in biodiversity since it contains a wide range of important habitats, and is economically useful. Additionally, it offers ecosystem services that draw in investments and leisure time from people. Alexandria is a city on Egypt's Mediterranean coast that stretches roughly 70 kilometers from Al-Hammam in the west to Abu Qir Bay in the east. Due to the substantial effect of climate change on water bodies and resources, the Egyptian coastal area is a special case that needs to be carefully considered for development and preservation **(Shobier *et al.*, 2011)**.

The coastal area of Alexandria faces issues due to sewage, industrial development, poor waste management, population increase, shipping residues, and agricultural runoff. The fast expansion of the local economy and population could be impacted by changes in the quality of the water **(Ismail, 2018)**. Estimating water quality is crucial for monitoring, but understanding global changes in a range of factors can be difficult. Therefore, assessing water quality using a variety of factors could be the first step in aquatic sustainability management **(Sun *et al.*, 2016)**.

Aquatic ecosystems and human health are at risk due to heavy metal pollution in sources of water, which has become an important worldwide environmental problem. Due to urbanization, climate change, and industry, heavy metal pollution is rising in the aquatic ecosystems. Urban runoff, industrial and municipal wastewater, mining waste, landfill leachates, weathering, and rock abrasion are examples of natural phenomena that can produce pollution. In biological systems, heavy metal ions can bioaccumulate and are hazardous and perhaps cancerous. Even at modest exposure levels, heavy metals can damage the neurological system, kidney, liver, skin, and other bodily systems **(Hama Aziz *et al.*, 2023)**.

Physicochemical parameters, such as heavy metals in the water, fertilizer salts, and commercial fisheries, can be monitored on Alexandria's coastal beaches to offer suggestions for improvement, as well as enhancing both the visitor experience and the standard of living for locals. The Egyptian policy seeks to prevent degradation **(Shakweer *et al.*, 2006; El-Sayed *et al.*, 2022)** and to support decision-makers in managing water availability **(Li & Wu, 2019)**.

The present research attempted to: (i) determine the ecosystem impact factors and contaminants altering the state of Alexandria's coastal beaches; and (ii) estimate the environmental risk and pollution status; these findings are highly relevant in helping to address the issues facing these Egyptian bodies of water. The study also established a database for water quality and regularly maintained it to ensure that the coastline along with waterways has become safe for usage by both visitors and

vacationers. Additionally, the determination of the levels of bacterial and toxic heavy metals in different species of fish collected from Alexandria, Egypt, was also investigated. Evaluation of the contamination levels affecting the antioxidant enzymes; DNA damage and the expression of stress-related genes in two strains of fishes (*Sparus aurata* and *Dicentrarchus labrax*) were correspondingly assessed.

MATERIALS AND METHODS

1. Description of the study area

Samples of fish and water were taken in January 2024 from three locations—Shatby, El-Max, and the Anfoushi area—along Alexandria's Mediterranean Sea shoreline (Fig. 1). The El-Max locale is defined by human and/or industrial activity. On the other hand, Anfoushi and Shatby were chosen as unpolluted areas. Across a coast that stretches 70 kilometers from eastward to westward of the city, Alexandria is known for its many natural beaches and exceptional coves. It is situated within longitudes of 29˚55′E − 30◦04′E and along latitudes of 31˚13′N31˚19′N. This town is situated in Egypt, south of the Mediterranean.

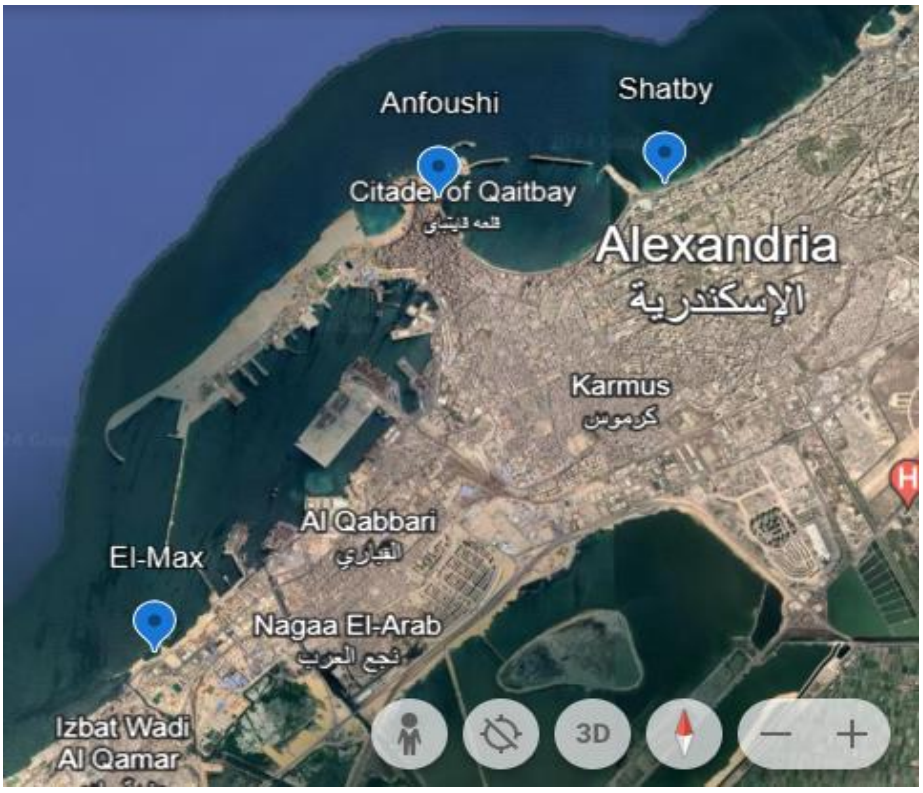


Fig. 1. A map showing the studied areas: (1) Shatby; (2) El-Max; (3) Anfoushi at Alexandria Governorate. The map was made using Google my Maps application.

2. Sampling and analytical methods

2.1.Water sampling

The water samples were collected from three different locations. Samples were collected in cleaned plastic containers to assess physicochemical values, whereas sterile borosil bottles sealed with brown wrapping were utilized to examine microbiological parameters. All of the samples were gathered between 7.00 a.m. and 9.00 a.m. and safely transported to the laboratory for further examination utilizing standard procedures **(APHA, 2012)**.

2.2. Water sampling for chemical analysis

The samples were collected in sterilized containers and were delivered to the National Research Center for processing. The following parameters were determined *in situ* i) The pH, dissolved oxygen, and total dissolved solids were assessed using the YSI 6000 equipment. ii) Then, in the laboratory, the total alkalinity, chemical oxygen demand, ammonia, nitrite, and nitrate were measured according to **APHA (2012)** standards.

2.3. Fish sampling

A total of 50 samples of the selected commercial fish species *Sparus aurata* and *Dicentrarchus labrax* were collected from three different sites in Alexandria, as shown in Fig. (1). The samples were transported to the laboratory under fully sterile conditions, packed in an ice box, and then examined through both bacteriological and molecular analysis methods.

3. Biological tests

3.1. Bacteriological analysis

The samples were prepared following the guidelines specified in the International Organization for Standardization (ISO) standard 4833-1:2013 **(ISO, 2013)**. Each specimen (water and fish) was decisively shaken then mixed before bacteriological investigation. The samples were diluted in a sterile saline solution. To ensure consistent inoculation, 100µl of each sample, or equivalent dilutions, were plated onto agar. Following that, each plate was incubated at 37°C for 24 hours. After an adequate amount of incubation, each visible colony was counted **(APHA, 2012)**.

4. Enzyme extraction and quantitative measurement

4.1. Preparation of samples

The liver tissues, weighing approximately 250mg each, were first homogenized in order to prepare the samples for measuring the enzyme activities related to oxidative stress. Specifically, the frozen liver tissue samples were thawed and weighed. Then, they were homogenized on ice for 5 minutes at 9,500rpm using a mechanical homogenizer.

The homogenization was conducted in a buffer solution (pH 7.4) containing 100mM KCl and 100mM KH_2PO_4 at a 1:10 tissue to buffer ratio (w/v). After homogenization, the samples were centrifuged at 10,000rpm for 30 minutes at 4°C. This step separated the supernatant, which was then decanted and used for the subsequent measurement of the enzyme activities related to oxidative stress biomarkers **(Kocalar *et al.*, 2023)**.

4.2. Glutathione-S-Transferase (GST) activity

GST activity measured 1-chloro-2, 4-dinitrobenzene compound with reduced glutathione as a precursor. The photographic density was measured at 340nm at 25ºC using a spectrophotometer (Germany). The rise in absorbance was measured for three minutes. The reaction mixture with no fish homogenates was utilized as a blank **(Rudneva *et al.*, 2010)**.

4.3. Glutathione peroxidase (GPx) activity

GPx activity was assessed using a spectrophotometer. The reaction mixture contained 1ml of 50mM monopotassium phosphate buffer, 1mM ethylenediamine tetraacetic acid, 0.075mM hydrogen peroxide, 1mM reduced glutathione, 0.2mM NADPH, 1.6IU/ ml glutathione reductase, and an enzymatic dilution caused a linear reduction of below 0.05 absorbance at 340nm. The decline in absorbance at 340 wavelengths for glutathione peroxidase activity was assessed concerning a blank solution including the enzymes and was handled identically **(Hamed *et al.*, 2004; Rudneva *et al.*, 2010)**.

5. Determination of heavy metals

5.1. Heavy metals in water samples

Water samples (3–4L) underwent filtering via 0.45µm membrane papers. Dissolving metal ions were analyzed with a resin that exchanges cations (chelex100) after preconcentration. The membranes of these filters, which included suspended particle matter (SPM), were cleaned many times before being dried at 65°C for about 48 hours. Each dried membrane paper was put in a Teflon cups, refilled with a 3:2:1 mix of HNO_3, $HClO_4$, and HF (6ml), and burned. The quantities of manganese, copper, zinc, and cadmium were measured using an ICP-OES analysis**(Mohamed *et al.*, 2021)**.

5.2. Heavy metals in sediments samples

Heavy metals found in the surface sediment were digested. Each dried sediment put in a Teflon cup. Three ml of pure HNO_3 was added to the samples by drops. The sample was dried to 80°C before being treated with a 5ml solution of HNO_3/$HClO_4$/HF (3:2:1). The specimen was filtrated as well as repeatedly washed using ionized water. The Mn, Cu, Zn, and Cd concentrations were determined using the ICP-OES technique **(Mohamed *et al.*, 2021)**.

6. Gene expression analysis using quantitative real time-PCR (qRT-PCR) method

6.1. RNA isolation and reverse transcription (RT) reaction

Total RNA was isolated from tissue samples (gills and liver) using the RNeasy Mini Kits and DNaseI (Qiagen). The extracted total RNA was resuspended in DEPC treated water and analyzed photospectrally at 260nm. Aliquots of samples were utilized for rna reverse-transcribing, and then kept at -80°C.

"Fermentas' RevertAidTM Initial Strand cDNA Synthesis Kits" were used to convert Poly(A) RNA from gills and liver samples into cDNA in 20µl. Template RNA (5µg) was mixed with the master mix. Each sample was combined and centrifuged at 1000×g for 30 seconds before being loaded into the preprogrammed thermal cycler. The RT reactions were performed for 10 minutes at 25°C, 1 hour at 42°C, followed by 5 minutes at 99°C for denaturation. The RT preparations tubes were flash-cooled within an ice chamber until being utilized for cDNA amplification by real-time quantitative PCR (qRT-PCR) **(Elateek *et al.*, 2021)**.

6.2. qRT-PCR

Applied Biosystems StepOne™ RT PCR Equipment (Thermo Fisher Scientific) was used to assess cDNA copy counts in the gills and liver tissue. A standard reaction mixture contained 12.5µL of 1× SYBR® Premix Ex TaqTM (Biotech. Co. Ltd.), 0.5µL (0.2µM) of primer (sense and antisense), 6.5µL PCR grade water and 5µL of cDNA templates. The cycling profile was divided into three phases. The initial stage was at 95.0°C for 3min, followed by second stage comprised 40 cycles each with three steps: (a) denaturation at 95.0°C for 15s, (b) primer annealing at 55.0°C for 30s, then (c) extension at 72.0°C for 30s. The third stage included 71 cycles, starting at 60.0°C and rising by around 0.5°C per 10s till 95.0°C. Table (1) displays the sequences of specific primers for genes related to apoptosis in *S. aurata* and *D. labrax*, such as P21, P53, and Caspase-3. The $2^{-\Delta\Delta CT}$ technique was used to calculate the relative quantitation of the target to the reference **(Watanabe *et al.*, 2010; Yang *et al.*, 2017; Refaie *et al.*, 2020; Elateek *et al.*, 2021)**.

Table 1. Primers sequence used for qRT-PCR of *Sparus aurata* and *Dicentrarchus labrax*

Gene	*Primer sequence*	*NCBI (accession no)*
Caspase-3 (*S. aurata*)	F: CACAGCAGTAACGCCACATT R: TCTGCAAGCCTGGATGAAGA	EU722334.1
Caspase-3 (*D. labrax*)	F: GGAACGGAATCTCACGATGC R: TTTCCCTTTTCCGTCCATGC	OQ471908.1
P21 (*S. aurata*)	F: TTGTCTCCCTCAGTCACACC R: GTTCCTTAAGTGCGAGCGAG	NC_044192.1
P21 (*D. labrax*)	F: TGGTACTTTCTCCAGCCCAG R: TTCTCTCGACACACTTCCCC	XM_051380184.1
P53 (*S. aurata*)	F: TACCATGAACAGCAGCTCCA R: GCCTCCTCCTTTTCCTCTGT	XM_030443965.1
P53 (*D. labrax*)	F: TACCTCGCATGTCCAGTCTC R: GCTTACTGGGAACTGGAGGT	XM_051381263.1
GAPDH[1] (*S. aurata*)	F: TCAAGAAGGTCGTCAAGGCT R: GCCGAACTCATTGTCGTACC	XM_030394814.1
GAPDH[1] (*D. labrax*)	F: AAGTATGACTCCACCCACGG R: TCCCTTCAAGTGAGCAGAGG	AJ006883.1

[1]Glyceraldehyde-3-phosphate dehydrogenase

7. Comet assay

Gills and liver samples of *S. aurata* and *D. labrax* taken from various locations in Alexandria were processed through single cell gel electrophoresis (comet test) methods to evaluate the degree of DNA degradation.

The comet technique was carried out according to **Blasiak *et al.* (2004)**, including preparation of reagents, slides, and electrophoresis of microgel slides. The DNA damage analysis program (comet rating, TriTek company, Sumerduck, the VA22742) has 100 cells per animal. Randomly identified nonoverlapping cells were graded on a scale of 0 to 3 based on observed comet tail length migrations and the quantity of DNA in the nucleus **(Collins *et al.*, 1997; Olive *et al.*, 2012)**.

8. Statistical analysis

All gene expressions along with comet assay data were analyzed with the Statistical Analysis Software (1982) General Linear Models (GLM) approach, and the Scheffé-test was used to find any notable group differences. Mean ± SEM is used to express the values. All significant claims were based on a *P*-value of less than 0.05. Plots and heatmaps were produced using the R packages ggplot2 v3.3.5 **(Hadley, 2016)** & pheatmap v1.0.12 (https://github.com/raivokolde/pheatmap).

RESULTS

1. Physicochemical quality

Physical injury and physiological stress are the key reasons for fish sickness and death in aquaculture. Poor water quality has been connected to fish mortality owing to bacterial co-infections **(Nofal & Abdel-Latif, 2017)**. That study collected a significant amount of physicochemical data to identify the important environmental elements in bacterial community formation. Fig. (2) summarizes the key environmental factors for all three locations. pH is a measure of the concentration of ionized hydrogen in a solution. According to the national guidelines, all water samples were in the permissible pH range from 6.5 to 8.5. Nitrite and nitrate levels varied from 0.1 to 0.4 and 2 to 6mg/ L, respectively. National guidelines specify that nitrite and nitrate levels should not exceed 3 and 50mg/ L, correspondingly. The total alkalinity content varied from 69.6 to 446.5mg/ l. Total alkalinity measures water's capacity to resist pH variations. In conclusion, based on physicochemical parameters Shatby's water samples met the quality standards. While in El-Max, there was a drop in pH and dissolved oxygen (DO), accompanied by high concentration in all other parameters.

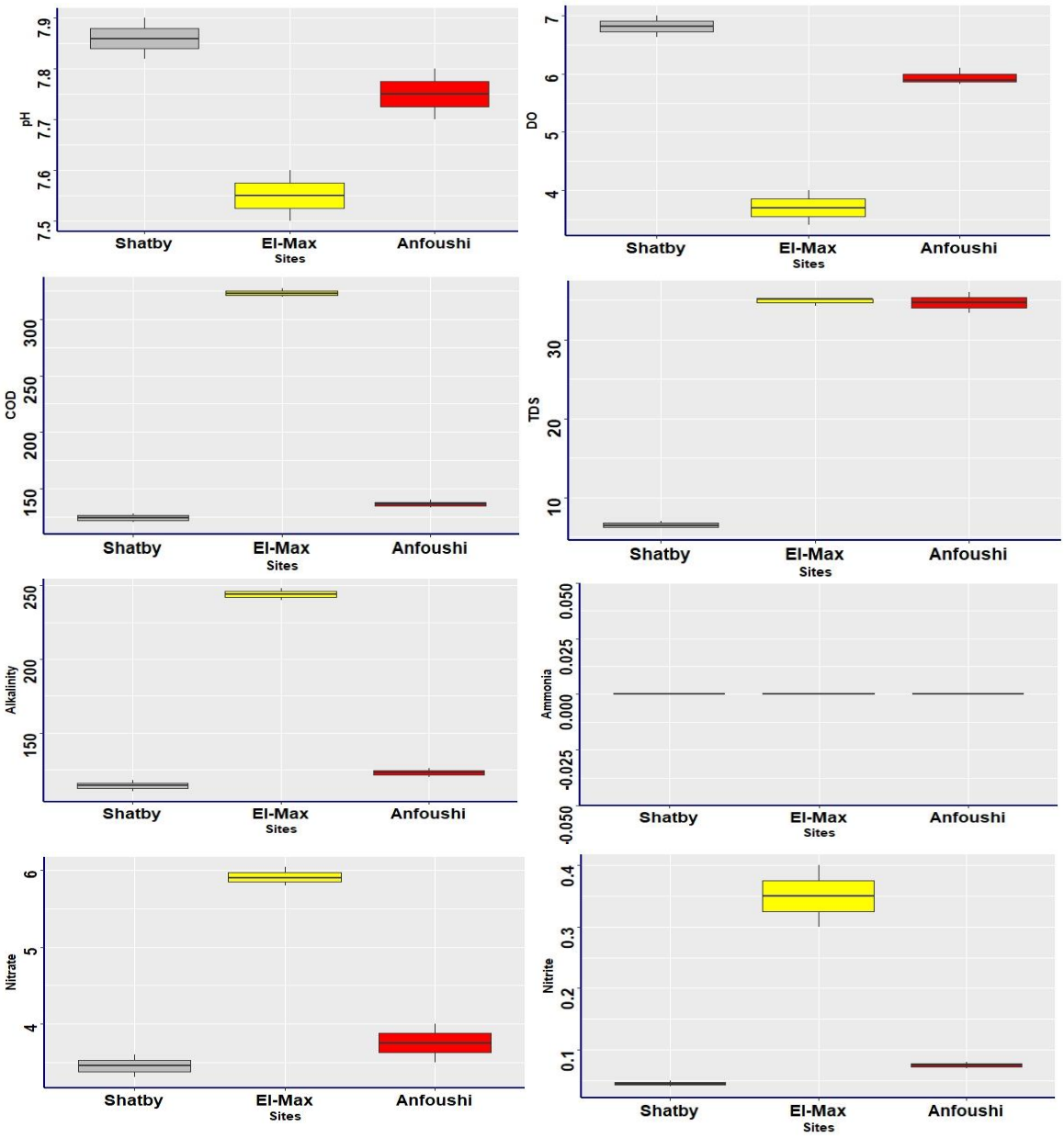


Fig. 2. Box plots for physiochemical parameters. Each box plot includes water samples collected from each site. The boxplots' bottom and upper margins correspond to the first and third quartiles, while the whiskers extend to the highest or lowest value at 1.5 times the interquartile, and the black bars across the box reflect median values

2. Bacterial quality

Fish infected with bacterial infections exhibit synergistic interactions, resulting in severe sickness and much-increased mortality rates **(Xu *et al.*, 2012)**. Bacterial isolates were counted to distinguish location, and the relationship between the bacterial isolates was evaluated based on the different sites. A heat map of all bacterial isolates shows relative growth on a scale ranging from positive (red) to negative (light blue). Each column presented distinct samples, whereas each row presented a fingerprint of responses for a specific strain. The columns are organized by strain similarity, whereas the rows are organized by response similarity. In the right panel, black lines represent each sample that is present. Fig. (3) illustrates the heat-map analysis of the bacterial isolates. However, this approach was effectively used to cluster pathogenic bacteria. Heatmap and hierarchical clustering of different bacterial isolates according to their phenotypic profile showed differences between isolates. The heat map's red and blue colors indicate the presence and absence of bacteria, respectively. The red color indicates the highest level of contamination, whereas the light blue indicates the absence of microorganisms. In Fig. (3), the red color scale represents a major percentage (highest abundance) of the isolated bacteria, whereas the blue one represents a minor percentage (lowest abundance). Some bacteria in the Shatby region differ markedly from those in the El-Max region; for example, *Aeromonas* was red in El-Max samples but yellow in Shatby samples. The heatmap's left side shows a color depiction of the different origins (hatchling in blue and hatchery in red), serotypes, and hatcheries. Wald's approach was used in conjunction with a binary distance matrix to produce hierarchical clustering.

3. Heavy metals

The principal component analysis biplot for heavy metal concentrations (Fig. 4a) demonstrated the link between heavy metal concentrations and various sites. Fig. (4a) represents the mean concentrations of heavy metals in water and sediments samples. The first dimension explained 87.1% of the total variation observed. Zn, Mn, and Cu showed a favorable correlation with the El-Max location. Cd contents in water samples and sediments were below the detectable limit.

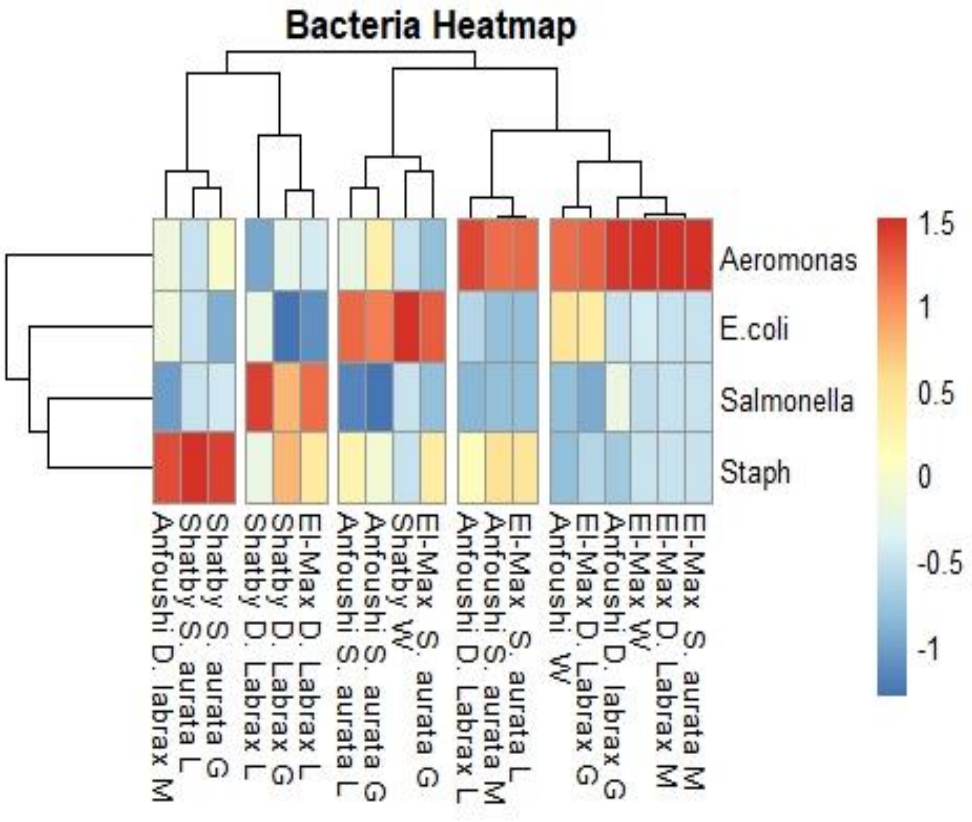


Fig. 3. Heatmap with hierarchical clustering of bacterial isolates

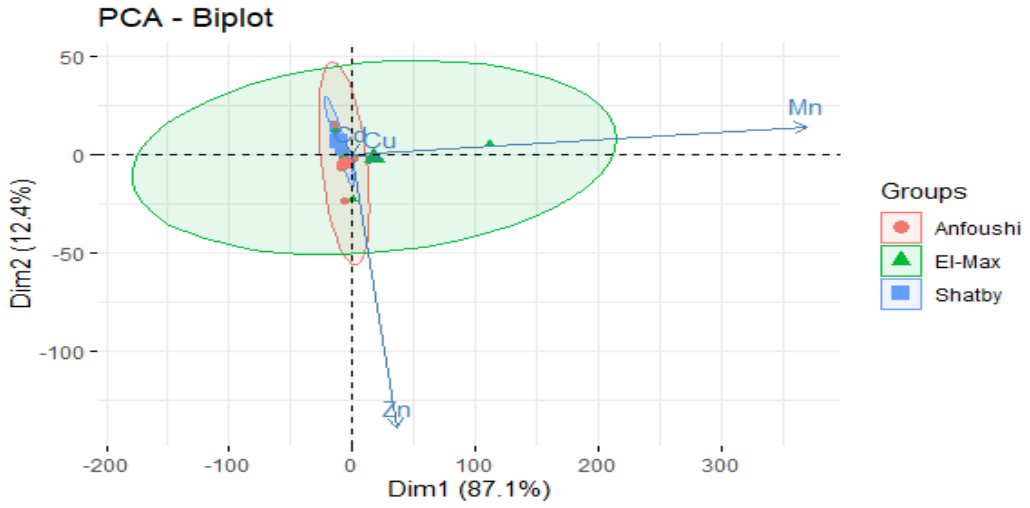


Fig. 4a. Plot of principal component analysis (PCA) of heavy metal concentration (blue squares: Shatby; green triangles: El-Max and red dots: Anfoushi). Ellipses are used to illustrate the groups' confidence intervals. Biplots have been overlapped

with PCA. Small angles between arrows imply positive, strong associations between biomarkers

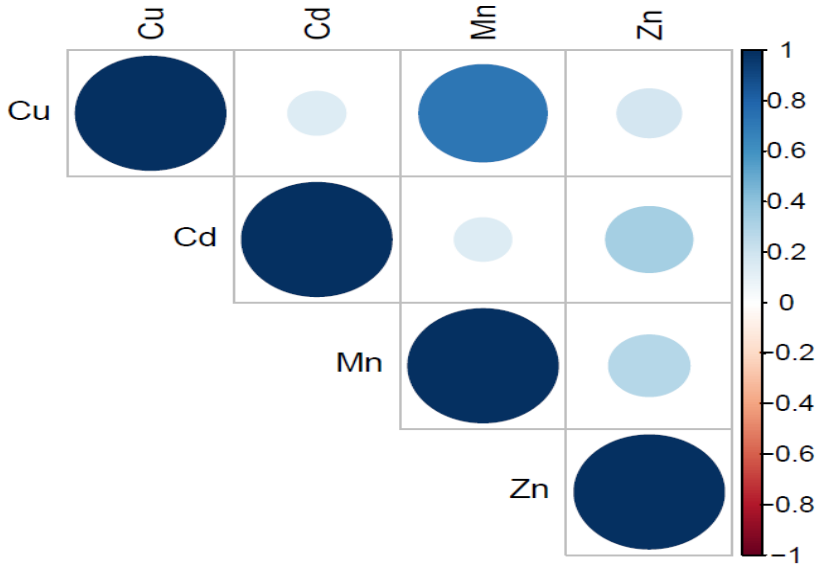


Fig. 4b. Correlation circle from principal component analysis (PCA) showing three main groupings

4. Biochemical enzymes

Regarding liver tissues, GST and GPx activity differed significantly between sites. The average levels of GST were substantially greater in the El-Max site with respect to the Shatby control (Fig. 5a). Additionally, GPx activity levels from the El-Max and Anfoushi sites significantly differed from the control site (Fig. 5b). The current study found a considerable rise in antioxidant enzyme activity (GST and GPx) the liver of El-Max fish compared with control fish. Lipid peroxidation is an adverse oxidative stress response on cellular and tissue constituents. Oxidative stress is described as an imbalance in oxidant/antioxidant responses **(Almeida *et al.*, 2009)**. The infection causes an imbalance between the oxidative and an antioxidant defense mechanisms by decreasing antioxidant activity and preventing the ability to neutralize the effect of ROS produced **(Abou-Okada *et al.*, 2023)**.

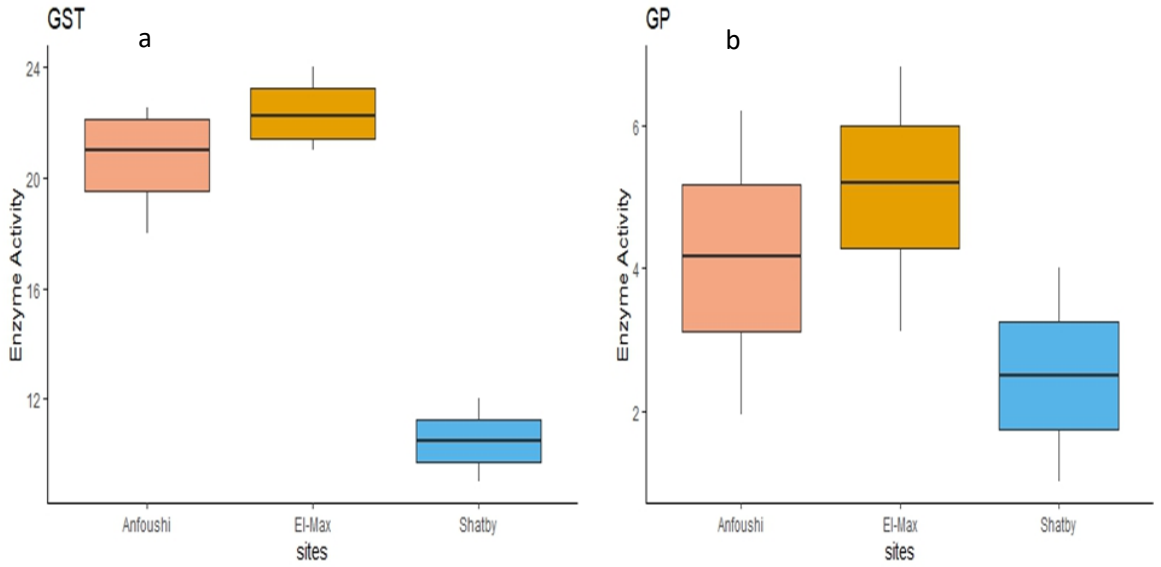


Fig. 5. Boxplot of the biomarker activities in fish tissues: a) Glutathione S transferase (GST) and b) Glutathione peroxidase. All fish activities were determined in each of the three locations. The central line represents the median value, the cross in the center of the box represents the mean value, and the box's borders represent the 25th and 75th percentiles, respectively

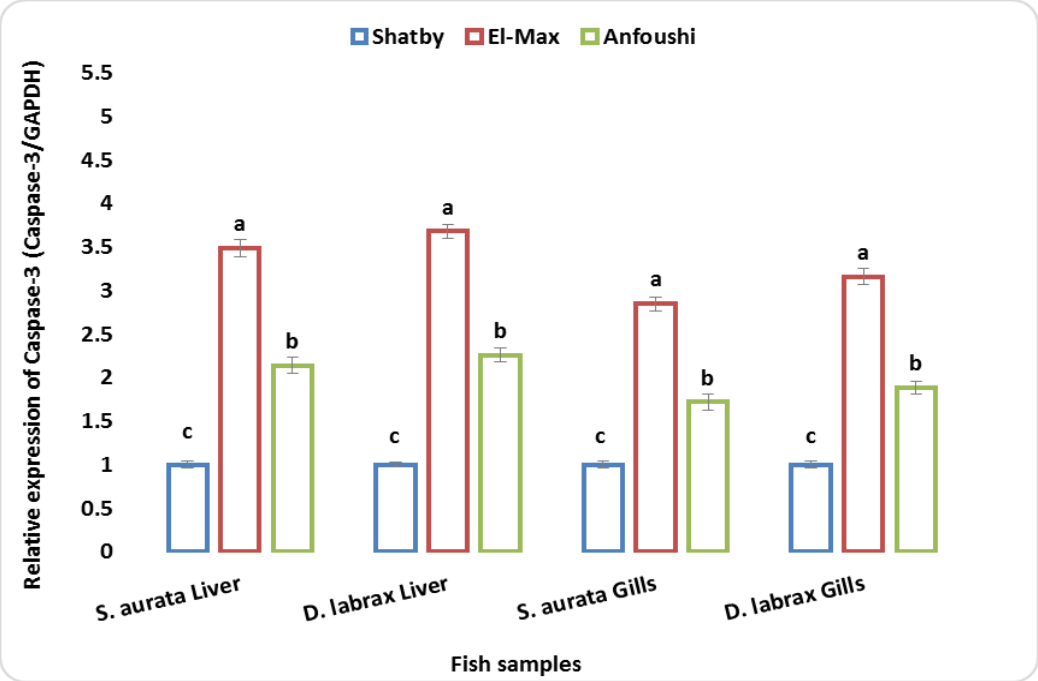
5. Expression of apoptosis genes in *S. aurata* and *D. labrax*

Expression of Caspase-3, p21 and p53 genes in the liver and gills of *S. aurata* and *D. labrax* collected from Shatby, El-Max and Anfoushi in Alexandria, Egypt, are summarized in Figs. (6- 8).

The results demonstrated that the expression levels of the Caspase-3 gene in the liver's tissues of *S. aurata* and *D. labrax* were relatively higher than those in gills tissues (Fig. 6). Moreover, expression levels of Caspase-3 gene within tissues samples of *S. aurata* or *D. labrax* collected from El-Max location were significantly increased (P<0.01) in comparison with those from Shatby and Anfoushi locations (Fig. 6). Additionally, expression levels of Caspase-3 gene within tissues samples of *S. aurata* or *D. labrax* collected from Shatby location reached the lowest levels compared to El-Max and Anfoushi locations (Fig. 6).

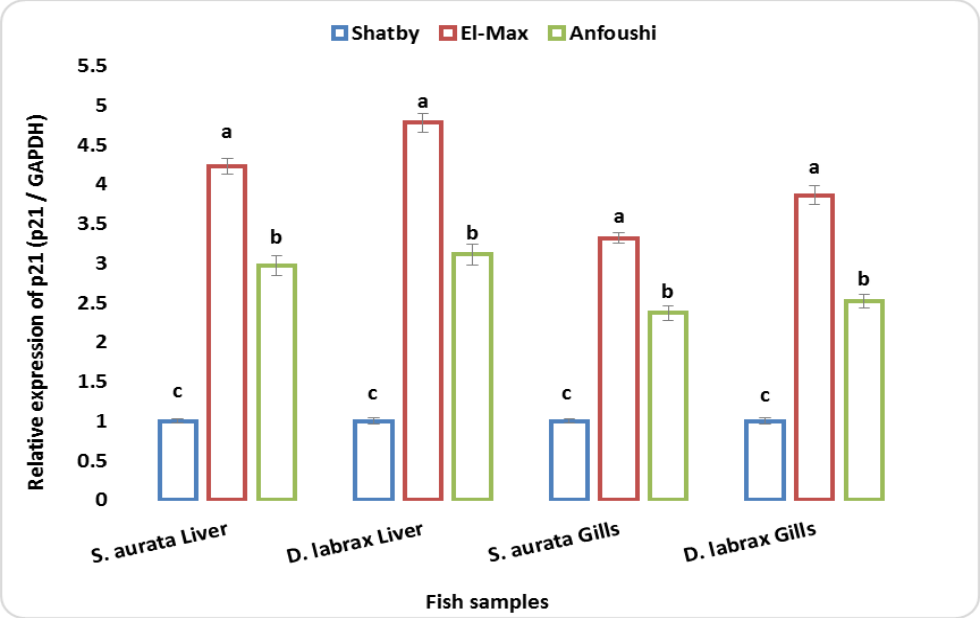
In the same trend, p21 gene exhibited expression values similar to Caspase-3 gene in *S. aurata* and *D. labrax*. Expression levels of p21 gene within gills tissues of *S. aurata* and *D. labrax* were relatively lower than those in liver tissues (Fig. 7). Furhtermore, expression levels of p21 gene within tissues samples of *S. aurata* or *D. labrax* collected from El-Max location were considerably up-regulated (P<0.01) as compared to those from Shatby and Anfoushi locations (Fig. 7). Moreover, expression levels of p21 gene within tissues samples of *S. aurata* or *D. labrax* collected from Shatby location exhibited

lowest values in comparison to El-Max and Anfoushi locations (Fig. 7). The p53 gene displayed expression levels in *S. aurata* and *D. labrax* not like the previous genes. The expression levels of p53 gene in liver tissues of *S. aurata* and *D. labrax* were relatively near to those in gills tissues (Fig. 8). Also, the expression levels of p53 gene in tissues samples of *S. aurata* or *D. labrax* collected from El-Max location were over-expressed like those in Anfoushi location without significant differences (Fig. 8). In contrast, the expression levels of p53 gene in tissues samples of *S. aurata* or *D. labrax* collected from El-Max and Anfoushi locations increased significantly (P<0.05) compared with those in Shatby location (Fig. 8).



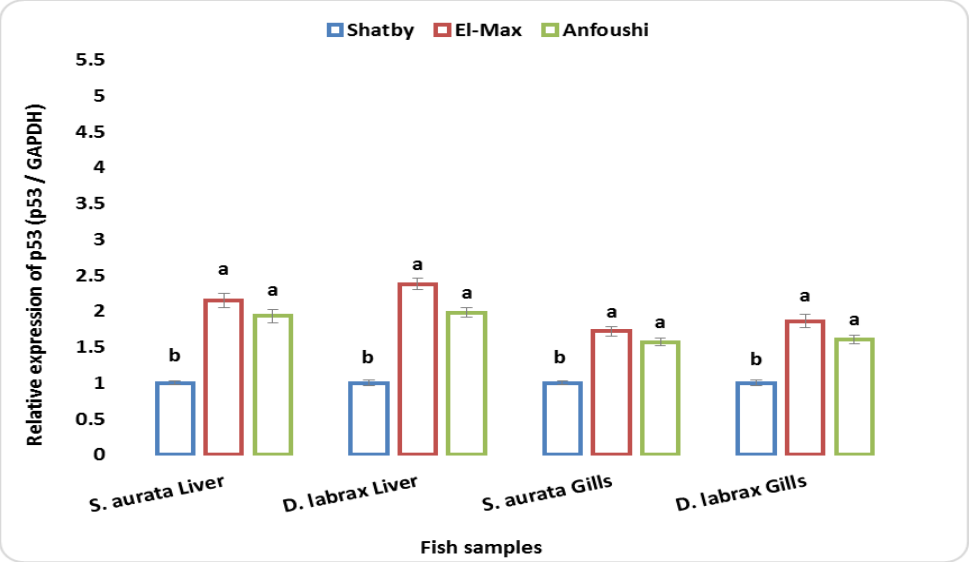
Fish sample	Shatby	El-Max	Anfoushi
	Mean± SEM	Mean± SEM	Mean± SEM
***S. aurata* liver**	1.0±0.04^{c}	3.49±0.10^{a}	2.14±0.09^{b}
***D. labrax* liver**	1.0±0.03^{c}	3.68±0.08^{a}	2.26±0.08^{b}
***S. aurata* gills**	1.0±0.04^{c}	2.85±0.07^{a}	1.72±0.09^{b}
***D. labrax* gills**	1.0±0.04^{c}	3.16±0.09^{a}	1.88±0.07^{b}

Fig. 6. The expression alterations of *Caspase-3* gene in liver and gills of *S. aurata* and *D. labrax* collected from different locations (Shatby, El-Max and Anfoushi) in Alexandria, Egypt. Data are presented as mean ± SEM. $^{a,b,c:}$ Mean values in tissues with unlike superscript letters showed significant differences (P<0.05)



Fish sample	Shatby	El-Max	Anfoushi
	Mean± SEM	Mean± SEM	Mean± SEM
***S. aurata* liver**	1.0±0.03^{c}	4.23±0.10^{a}	2.97±0.12^{b}
***D. labrax* liver**	1.0±0.04^{c}	4.78±0.12^{a}	3.11±0.13^{b}
***S. aurata* gills**	1.0±0.03^{c}	3.32±0.07^{a}	2.37±0.09^{b}
***D. labrax* gills**	1.0±0.03^{c}	3.86±0.11^{a}	2.52±0.08^{b}

Fig. 7. The expression alterations of *p21* gene in liver and gills of *S. aurata* and *D. labrax* collected from different locations (Shatby, El-Max and Anfoushi) in Alexandria, Egypt. Data are presented as mean ± SEM. $^{a,b,c:}$ Mean values in tissues with unlike superscript letters showed significant differences (P<0.05)



Fish sample	Shatby	El-Max	Anfoushi
	Mean ±SEM	Mean ±SEM	Mean ±SEM
***S. aurata* liver**	1.0±0.03^{c}	2.15±0.10^{a}	1.93±0.09^{a}
***D. labrax* liver**	1.0±0.04^{c}	2.38±0.08^{a}	1.98±0.07^{a}
***S. aurata* gills**	1.0±0.03^{c}	1.72±0.07^{a}	1.57±0.05^{a}
***D. labrax* gills**	1.0±0.04^{c}	1.86±0.09^{a}	1.61±0.06^{a}

Fig. 8. The expression alterations of *p53* gene in liver and gills of *S. aurata* and *D. labrax* collected from different locations (Shatby, El-Max and Anfoushi) in Alexandria, Egypt. Data are presented as mean ± SEM. $^{a,b,c:}$ Mean values in tissues with unlike superscript letters showed significant differences (P<0.05)

6. DNA damage in fish tissues

The DNA damage in the gills and liver tissues of *S. aurata* and *D. labrax* collected from different locations in Alexandria is shown in Table (2) and Fig. (9). The results displayed that DNA damage was increased in liver tissues of *S. aurata* and *D. labrax* more than gills tissues (Table 2 & Fig. 9). Moreover, fish collected from El-Max area showed significantly high levels of DNA damage in liver and gills compared with Shatby (P<0.01) and Anfoushi (P<0.05) areas. Furthermore, DNA damage of class three (tails longer than 2× nucleus diameter) was increased in liver and gills of *S. aurata* and *D. labrax* collected from El-Max area compared with those recorded in Shatby and Anfoushi areas.

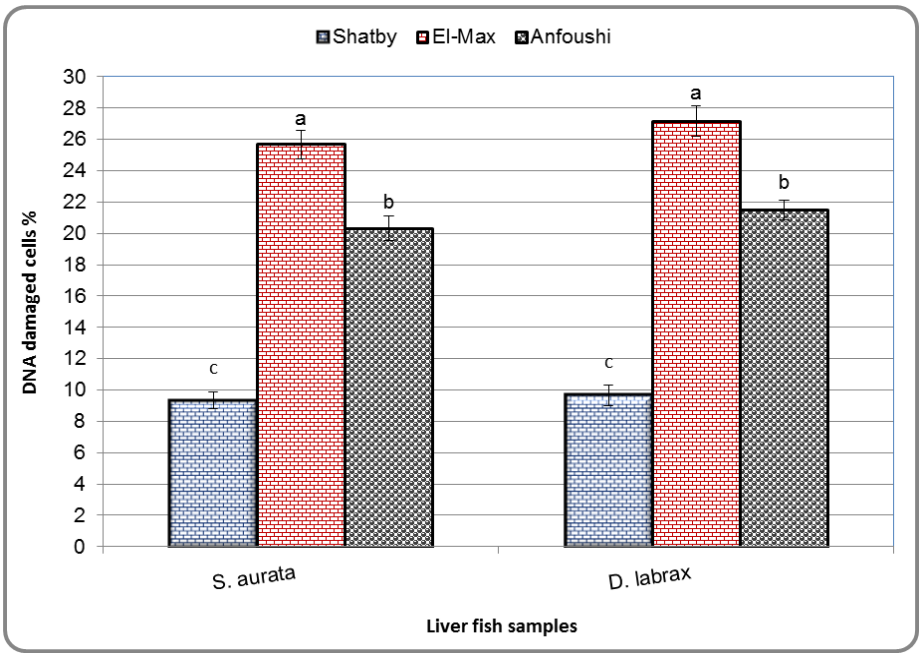
Additionally, DNA damage in liver and gills of *S. aurata* and *D. labrax* collected from Anfoushi area was significantly increased (P<0.05) compared to Shatby area.

Table 2. Visual score of DNA damage in gills and liver tissues of *S. aurata* and *D. labrax* collected from different areas in Alexandria

Treatment	No of samples	No. of cells		Class**				DNA damaged cells % (Mean± SEM)
		Analyzed*	Comets	0	1	2	3	
Shatby area								
***S. aurata* liver**	6	600	56	544	45	8	3	9.33±0.54[a]
***D. labrax* liver**	6	600	58	542	43	11	4	9.67±0.67[a]
***S. aurata* gills**	6	600	47	553	37	10	0	7.83±0.42[a]
***D. labrax* gills**	6	600	51	549	39	12	0	8.50±0.61[a]
El-Max area								
***S. aurata* liver**	6	600	154	446	46	57	51	25.67±0.92[a]
***D. labrax* liver**	6	600	163	437	43	64	56	27.17±0.95[a]
***S. aurata* gills**	6	600	141	459	54	38	49	23.50±0.76[a]
***D. labrax* gills**	6	600	149	451	51	48	50	24.83±0.80[a]
Anfoushi area								
***S. aurata* liver**	6	600	122	478	40	39	43	20.33±0.80[a]
***D. labrax* liver**	6	600	129	471	39	44	46	21.50±0.62[a]
***S. aurata* gills**	6	600	113	487	45	31	37	18.83±0.70[a]
***D. labrax* gills**	6	600	117	483	43	33	41	19.50±0.76[a]

*: Number of cells examined per a group, **: Class 0= no tail; 1= tail length < diameter of nucleus; 2= tail length between 1X and 2X the diameter of nucleus; and 3= tail length > 2X the diameter of nucleus. Data are presented as mean ± SD. [a:] Mean values within tissue with like superscript letters were insignificantly different (P>0.05).

A



B

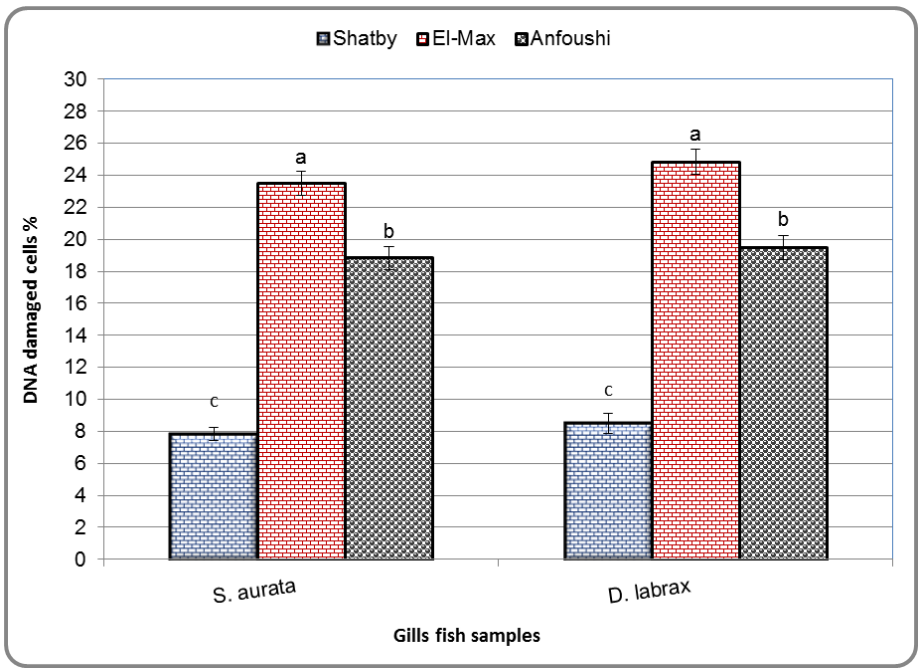


Fig. 9. Rate of DNA damage in liver (A) and gills (B) tissues of *S. aurata* and *D. labrax* collected from different areas in Alexandria. The data are provided as mean ± SEM. [a, b,c:] Mean values in tissues with unlike superscript letters showed significant differences (P<0.05)

DISCUSSION

In the last 15 years, coinciding with the introduction of the European Union's (EU) Water Framework Directive (WFD) **(Cini, 2002)**, the focus has been on achieving

and maintaining "Good Chemical Status" in EU surface waters. While chemical tests provide valuable insights into water quality, they may not fully capture ecological changes such as variations in aquatic plant life or flow regimes, which could affect the overall environmental status **(Karr & Chu, 2000)**.

Stressors significantly impact aquatic ecosystems, leading to drastic declines in biodiversity **(Sala *et al.*, 2000)**. Physicochemical characteristics are key to understanding spatiotemporal fluctuations in aquatic species. Meteorological and hydrographical studies are essential for assessing productivity and fertility in ecosystems **(Rajasegar, 2003)**. Alexandria's Mediterranean coastline, with its high maritime and recreational boating traffic, including marinas and harbors **(Shreadah *et al.*, 2013)**, faces significant environmental pressures. The study revealed that while Shatby and Anfoushi met quality standards, El-Max experienced a drop in pH and dissolved oxygen (DO) with high concentrations of other pollutants. This is consistent with the finding of **Salem *et al.* (2021)**, who reported similar issues in El-Max, attributed to rainfall and the impact of wastewater from household, agricultural, and industrial sources **(Shreadah *et al.*, 2016)**.

Bacteriophage analysis showed high levels of total coliform and various bacterial strains in El-Max compared to Shatby and Anfoushi. **Divizia *et al.* (1997)** also found *Salmonella* in El-Max, with high levels of total coliform and fecal streptococci, indicating human fecal contamination. **Amer *et al.* (2015)** noted high bacterial pollution levels and potential new bacterial strains in El-Max sediments, which may be linked to high hydrocarbon pollution levels.

Heavy metal pollution is a serious issue in Alexandria. The study found elevated levels of Zn, Mn, and Cu in El-Max compared to Shatby and Anfoushi. **Ghani *et al.* (2013)** reported high levels of various metals in El-Max, attributed to pollution from Lake Mariout and the surrounding areas **(CMCS, 2023)**.

Pollution stress leads to oxidative metabolism, producing reactive oxygen species (ROS) that cause oxidative stress and potential lipid peroxidation, damaging cellular membranes **(Kelly *et al.*, 1998; Sweetman *et al.*, 2010)**. Antioxidants like SOD, GPx, GST, and CAT are critical for defending against pollution **(Liu *et al.*, 2010)**. In this study, higher GPx and GST levels in *Sparus aurata* and Dicentrarchus labrax from El-Max reflect the impact of pollution stress.

Pollutants, including pesticides, can induce apoptosis or programmed cell death **(Ojha & Gupta, 2017; Yang *et al.*, 2017)**. Caspase-3 is a key apoptotic marker, and higher levels of this gene were observed in fish from El-Max compared to Shatby and Anfoushi.

The p53 protein regulates the cell cycle, apoptosis, and DNA repair. Exposure to genotoxic substances increases p53 protein levels **(Park *et al.*, 2006)**. The current study found up-regulated p21 and p53 gene expression in fish from El-Max, indicating higher exposure to environmental pollutants **(Brzuzan *et al.*, 2006; Ruiz *et al.*, 2012)**.

DNA damage, assessed using the comet assay, is a significant indicator of genotoxic impact. Fish from El-Max exhibited significantly higher DNA damage in liver

and gills compared to Shatby and Anfoushi, highlighting the genotoxic effects of environmental pollutants **(Delunardo *et al.*, 2013; Goswami *et al.*, 2014)**.

CONCLUSION

The current study gives significant information on the properties of surface seawater of three points along the Egyptian Mediterranean coastline of Alexandria City. The current findings show that the pollutants in the El-Max area had an impact on aquatic organisms by causing an increase in the antioxidant enzyme activity, altering the expression of apoptotic- related genes and elevating the DNA damage in gills as well as the liver of *Sparus aurata* and *Dicentrarchus labrax*. Consequently, this study allows us to predict and evaluate how marine fish respond to changes in environmental circumstances and pollutants.

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