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Biomarkers and Ultra Structural Evaluation of Marine Pollution by Polycyclic Aromatic Hydrocarbons

Nehad M. Abd El Moneam[1], Nabila E. Abd El Maguid[1], Amany M. El-Sikaily[2], Marwa G. Zaki[2], Mohamed A. Sheradah[3*]

[1]Biochemistry and Zoology Department, Faculty of Science, Alexandria University, Alexandria, Egypt
[2]Marine Pollution Research Lab., National Institute of Oceanography & Fisheries, Alexandria, Egypt
[3]Marine Biotechnology Lab., National Institute of Oceanography & Fisheries, Alexandria, Egypt
Email: *niof.shreadah@gmail.com

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Abstract

The present study is endeavored to study the oxidative stress and antioxidant response as well as the ultrastructural change of both liver and muscle tissues of *Siganus rivulatus* collected from polluted region (El-Mex Bay) and control region (Marsa Matrouh) to identify the significance of biomarkers. The results revealed that, El-Mex Bay was polluted by different types of chemicals including hydrocarbons comparing with the reference area (Matrouh area). Where**,** the concentration range of total hydrocarbons in sediment samples collected from Matrouh coast was 409.24 - 521.26 ng/g, dry weight, where as this range in sediment samples collected from El-MexBay was 4159.77 - 4589.81 ng/g, dry weight. In this context, the induction of antioxidant systems in fish collected from polluted area in response to oxidative stress should be considered as a clear indication of the presence of pollution and environmental health degradation. Also, The increase in lipid peroxidation was a useful indicator of the pollution load in the present study. The results suggest that chemical pollution is capable of inducing morphological alteration in liver offish collected from polluted area. The present study indicated that ultra-structural changes serveas biomarker of stress in aquatic environment.

Keywords

Biomarkers, Ultra Structural Evaluation, Polycyclic Aromatic Hydrocarbons (PAHs)

1. Introduction

Environmental pollution has increased substantially in the last decades due to a great

number of industrial, agricultural, commercial and domestic waste, effluents and emissions as well as hazardous substances. Aquatic pollution is the predominant form of pollution since the majority of chemical pollutants are entering to sea, rivers, lakes and wetlands [1]-[9]. Marine and coastal ecosystems are characterized by their complexity and their sensitivity to various inorganic and organic pollutants [10]-[18]. Because environmental contaminants can have a broad spectrum of sub lethal effects on organisms, so bioindicators are useful tools for assessing the presence and levels of chemical pollution. Such effects in organisms sensitive to contaminant exposures can be used as early warning signs for the degradation of the environment [19]. Polycyclic aromatic hydrocarbons (PAHs) are a class of organic compounds consisting of two or more fused aromatic rings. They are one of the major categories of pollutants entering the marine environment and finally accumulating in the sediments. Polycyclic aromatic hydrocarbons (PAHs) can accumulate in the tissues of aquatic animals and as such tissue concentrations of chemical pollutants can be of public health concern to both animals and humans [20]-[27]. They are formed by three main processes: diagenesis, petrogenesis, and pyrolysis [28]. Diagenesis and petrogenesis are typically naturally-occurring, where as pyrolysis can be the result of natural or anthropogenic events [29]. Diagenesis occurs in organic matter that has been deposited in soils or sediments [30]. Pollutants accumulated in tissues of fish may catalyze reactions that generate reactive oxygen species (ROS) which may lead to environmental oxidative stress. Indicators of oxidative stress include changes in antioxidant enzyme activity, damaged DNA bases, protein oxidation products, and lipid peroxidation products [31]. Disturbance of living processes at the molecular and subcellular levels of biological organization by xenobiotics can lead to cell injury, resulting in degenerative and neoplastic diseases in target organs. Therefore histopathological biomarkers have been proven to be useful indicators of toxicity in fish organs [32].

The removal of xenobiotics, and even some endogenous substances, from the cell is catalyzed by a number of different enzymes, so called phase I and II enzymes. Phase I enzymes are involved in xenobiotic biotransformation via the introduction of a polar moiety which rendersa lipophilic contaminant to more hydrophilic. The cytochrome P450 (CYP) family is the most well studied in fish, especially CYP1A. CYP1A serves to increase the solubility of hydrophobic molecules through a reduction reaction involving an oxygen molecule [33]. Examples of phase II enzymes commonly used in biomonitoring programs involving fish are glutathione S-transferase (GST) and UDP glucuronyl transferase (UDPGT) [34]. The activity of phase I enzymes can lead to an increase in ROS production or the generation of reactive redox cycling intermediates. Antioxidant enzymes facilitate the removal of these reactive chemical intermediates and resulting ROS molecules. The action ofCYP1A can result in the production of $O_2^{\bullet}$—which in turn can be metabolized by superoxide dismutase (SOD) to H_2O_2. This hydrogen peroxide molecule can then be reduced to H_2O and O_2 by catalase (CAT) [35]. Hydroxyl radicals ($OH^{\bullet}$) can form both H_2O_2 and $O_2^{\bullet}$—via reactions with redox cycling metal ions, for example iron and copper. This highly potent hydroxyl radical can attack both

protein and lipid molecules to form oxidative damage products.

Lipid damage can occur as a result of oxidative stress or a disruption in the balance between prooxidant and antioxidant factors. Reactive oxygen species are known to extract hydrogen atoms from unsaturated bonds thereby altering lipid structure or function [36].

Since fish species, *Siganus rivulatus*, is considered as a sentinel species for environmental monitoring, it was chosen to study the oxidative stress and antioxidant response as well as the ultra-structural change of both liver and muscle tissues of *Siganus rivulatus* collected from polluted region, *i.e.* El-Mex Bay and control region, *i.e.* Marsa Matrouh to identify the significance of biomarkers.

2. Materials and Methods

2.1. Study Area

Matrouh is located in the north-western Mediterranean coast of Egypt 290 km west of Alexandria. It is a protected area far from land-based sources of pollution. On the other hand, El-Mex Bay located west of Alexandria, is a semi-elliptical open basin, extends for about 15km between El-agamy head land in the west and the western harbor to the east and from the coast to a depth line of about 15 km. the bay has a mean depth of about 10 m and surface area of about 19.4 km^2. El-Mex Bay is a highly polluted area, the major types of pollution sources are domestic sewage, industrial waste water, and agricultural run-off, through lake out lets, and river discharged and oil pollution. El-Mex Bay receives mixed agricultural run-off from lake Mariout through El-max pumping station and El-umumdrain, industrial water from chloro-alkali plant, tanneries and slaughterhouse, also, air borne particles from the fumes of adjacent industrial plants including a cement factory [5] [11] [37]-[46]. Petroleum product from Al-Alamien oil field and from Suez Mediterranean pipeline terminal (SUMED) also contaminate the bay [47].

2.2. Sampling

Sediment samples were collected during March 2009 from ten stations, distributed along El-Mex Bay of Alexandria and, ten stations distributed along Matrouh coast as shown in **Figure 1(a)** & **Figure 1(b)**, respectively. At the same time Fish species (*Siganus rivulatus*), which belong to "*Siganidea*" were collected from El-Mex Bay and also from Matrouh coast. About 100 fish of similar weight (30 - 40 g) were collected from each area. Samples were dissected and their liver as well as, muscles was removed and prepared for electron microscopic studies. While the remaining liver and muscle tissue kept frozen at −20˚C until use. Fish liver samples were weighted to the nearest milligrams and their hepato-somatic index (HIS, liver weight/bodyweight × 100) was calculated according to Jangaard *et al.* [48].

2.3. Methodology

2.3.1. Morphological Study

Morphological features of *Siganus rivulatus* collected from both areas, *i.e.* El-Mex Bay

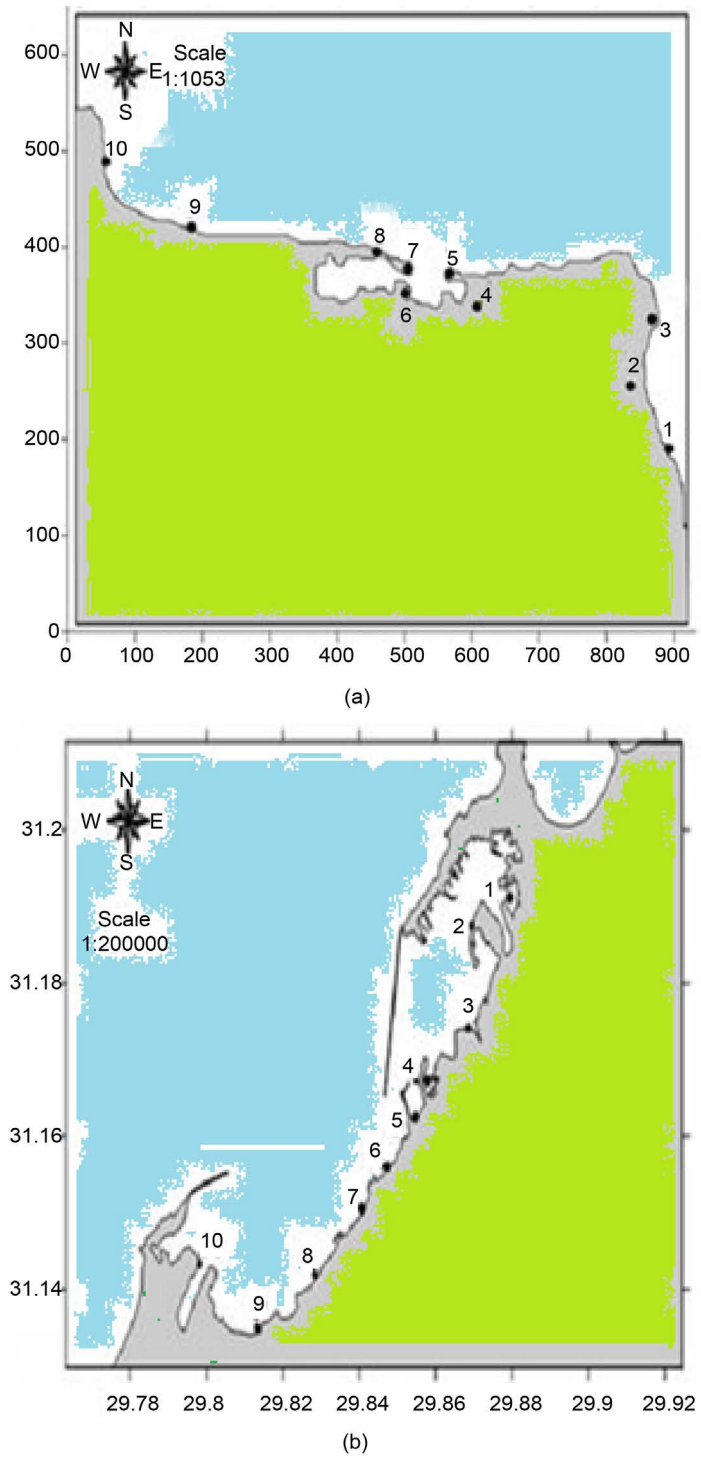

Figure 1. Location of sampling stations in the study area (a) Matrouh area and (b) El-Mex Bey area.

and Matrouh coast were determined. Both weight and length of *Siganus rivulatus* collected from both areas were taken and the hepato-somatic index (HIS = liver weight/body weight × 100) of fish was calculated.

2.3.2. Chemical Analysis

Hydrocarbons have been determined in sediment and fish according to UNEP/IOC/IAEA [49]. The samples were analyzed for aliphatic and aromatic hydrocarbons following these steps extraction, cleaning up and fractionation as well as their instrumental analysis followed by their analytical quality control. To control analytical reliability and assurance recovery efficiency as well as accuracy of the results, six analyses were conducted using hydrocarbon reference materials, HS-5 (sediment) provided by NRC-IMB of Canada and SRM 2974 (freeze-dried mussel tissue) (*Mytilus edulis*) provided by NIST of USA As well as sediment samples of known hydrocarbon levels spiked with a mixture consisting of 2.0 μg from each hydrocarbons were analyzed as above to validate the analytical method used in this study. The laboratory results showed recovery efficiency ranged from 90% - 110% for HS-5, 85% - 97% for SRM-2974 and 94% - 102% for the spiked samples. All solvents were pesticide grade.

2.3.3. Biochemical Analysis

1) Preparation of tissue homogenate

Prior to dissection, liver tissue was perfused with a (phosphate buffer saline solution, pH 7.4 (PBS), containing 0.16 mg/ml heparin to remove any red blood cells. The tissue was homogenized in 5 - 10 ml cold buffer (100 mM potassium phosphate, pH 7.0, containing 2 m MEDTA)/g tissue, and then centrifuge at 10,000 ×g for 15 min. at 4˚C. The supernatant was removed for assay. The biodiagnostic Glutathione *S*-transferase assay kit (CAT. No. GT 25 19) is used to measures total Glutathione *S*-Transferase activity by measuring the conjugation of 1-chloro-2, 4-dinitrobenzene (CDNB) with reduced glutathione. The conjugation is accompanied by an increase in absorbance at 340 nm [50]. Reduced Glutathione (GSH) concentration is determined by Biodiagnostic Gltathione reduced kit (CAT. No. GR 2511). The method based on the reduction of 5, 5' dithiobis (2-nitrobenzoic acid) (DTNB) with glutathione (GSH) to produce a yellow compound, the reduced chromogen directly proportional to GSH concentration and its absorbance can be measured at 405 nm [51]. The determination of lipid peroxidation is based on the reaction of malondialdehyde (break down product of lipid peroxides) with thiobarbituric acid (TBA), the resulting thiobarbituric acid reacting substances (TBARS) concentration, was determined by measuring the absorbance at 535 nm [52].

2) Microscopic study

Ultra thin sections (50 nm) were cut using LKB ultra tome with a glass knife, mounted on copper grids. Semi thin sections (1 μm) were also cut from the sample blocks, stained with toluidine blue, and examined under a light microscope. Ultra thin sections were doublestained with freshly prepared uranyl acetate [53] for 20 minutes as well as leadcitrate for 5 minutes, and scoping grids was achieved by using JEOL 100CX transmission electron microscope of the Faculty of Science Alexandria University.

3) Statistical analysis:

This study was statistically analyzed using SPSS (version 10). A difference is considered as significant at $p < 0.05$.

3. Results and Discussion

3.1. Mophological Study

The morphological study showed that the weight and length range for *Siganus rivulatus* collected from each area were relatively similar, but the hepatosomatic index for *siganus rivulatus* collected from El-Mex Bay was 2 fold more than those collected from Matrouh coast (Figure 2). Statistical analysis of data revealed a highly significant difference between the two investigated areas, *i.e.* El-Mex area and Matrouh area at $p < 0.05$.

3.2. Chemical Analysis

The results of the chemical analysis revealed that the concentration range of total hydrocarbons in sediment samples collected from Matrouh coast was 409.24 - 521.26 ng/g, dry weight, whereas the range in sediment samples collected from El-Mex Bay was 4159.77 - 4589.81 ng/g, dry weight revealing the presence of high concentrations of total hydrocarbons in El-Mex Bay (tested area) compared to the reference area (Matrouh area). The statistical analysis of total hydrocarbons in sediment samples revealed a highly significant difference between the two areas at $p < 0.05$. The concentration of total hydrocarbons in sediment collected from El-Mex Bay (tested area) was 9.5 fold more than those of Matrouh coast (control area) as shown in Figure 3. The highest concentration was observed in sediments collected from station 7 (4589.81 ng/g dry weight) followed by stations 9, 1, 5, while the lower concentrations were measured in samples collected from station 6. These would suggested that hydrocarbons accumulated in Mediterranean sea sediments come from nearly human activities and fuel combustion emissions [5] [17] [25] [26] Kim *et al.* [54] demonstrated that the nature of the sediment influences the distribution and concentration of PAHs. Concentrations of PAHs in sediment were affected by chemical composition of the sediment such as organic matter and clay content. Sediments with high organic carbon content was characterized

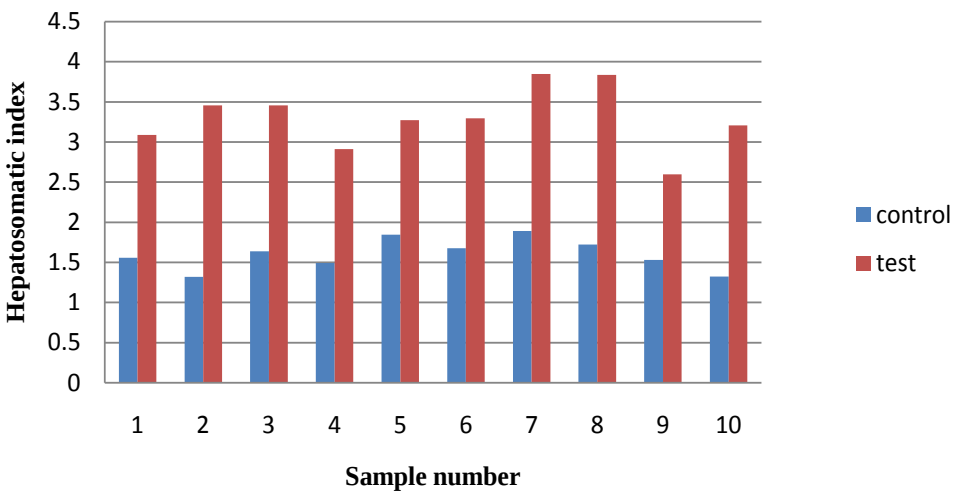

Figure 2. Hepatosomatic index of "*Siganus rivulatus*" collected from El-Mex Bay compared to Matrouh coast.

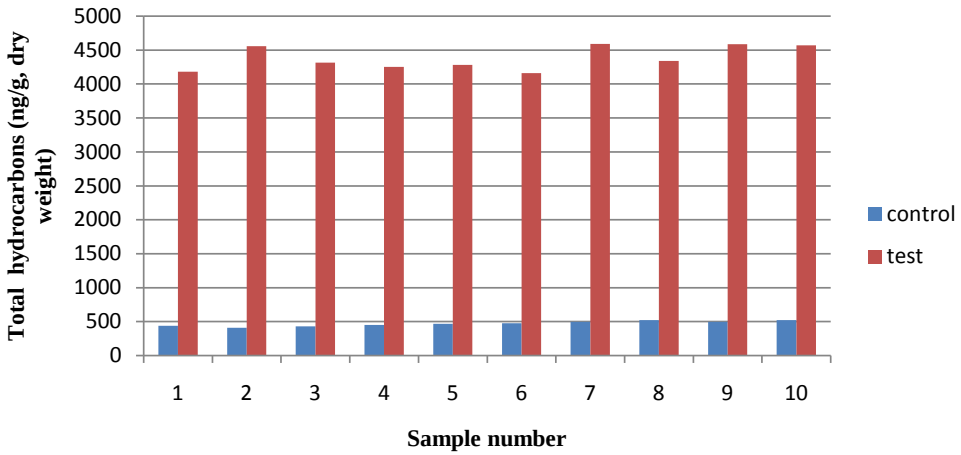

Figure 3. Total hydrocarbons concentration (ng/g, dry weight) of sediment samples Collected from El-Mex Bay compared to Matrouh coast.

by its high values of PAH's [55] [56]. Moreover, Simpson *et al.,* [57] showed that the relationship between total PAHs in sediments and organic carbon was only significant for highly contaminated sites, containing PAHs concentration greater than 2000 ng/g dry weight. In the present study, all sediment samples collected from El-Mex Bay were characterized by its higher total organic carbon (TOC). The importance of sedimentary organic matter on the partitioning of PAHs in sediments has been well documented [58]. Leung *et al.* [19] found that the high partitioning of PAHs to sedimentary organic matter was mainly due to the significant aromatic fraction of the organic matter.

To determine the source of PAHs, the ratios of phenanthrene/anthracene, fluoranthrene/pyrene and Fluoranthene/Fluoranthene + Pyrene were used [59]-[61]. Where, phe/anth more than 10 indicated to petrogenic input. The ratio of flu/pyr less than 1 was attributed to petrogenic sources and when it is greater than 1 was obviously related to a pyrolytic origin. The present study revealed that, the ratio of phe/anth was less than 10 in both tested and control areas, suggesting that their PAH were pyrolytic-derived. All samples from the control area gave also flu/pyr ratio greater than 1 indicating to a pyrolytic origin of PAHs in this area. On contrast, sediments of El-Mex Bay area gave flu/pyr ratio less than 1 indicating a petrogenic origin of PAHs.

In *Siganus rivulatus* muscles the concentration range of total hydrocarbons collected from Matrouh coast was 104.72 - 219.18 ng/g, dry weight, whereas, this range in *Siganus rivulatus* muscles collected from El-Mex Bay was 2239.52 - 3532.11 ng/g, dry weight. There was a very high significantly difference between the two areas at $p < 0.05$. The concentration of total hydrocarbons in fish muscles collected from El-Mex Bay (tested area) was 16 fold more than those collected from Matrouh coast (control area) as shown in **Figure 4**. An elevation of PAHs in fish samples but it is still less than that found in sediments and higher than that present in the water column due to the increasing metabolism [25]-[27]. PAHs in the fish were mostly dominated by the high molecular weight PAHs (4 - 6 rings). Several studies [62] [63] have shown that fish preferentially bioaccumulate 4, 5 and 6 ringed PAHs rather than 2, 3 ringed PAHs. In

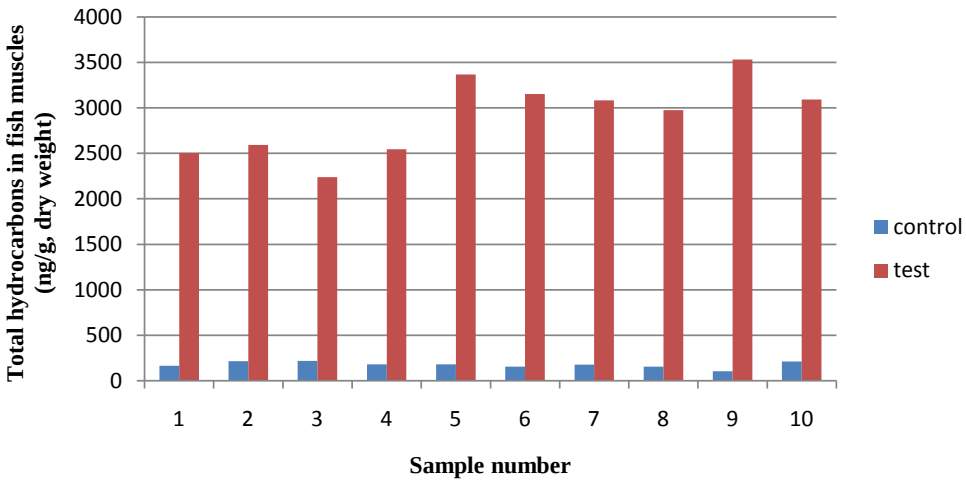

Figure 4. Total hydrocarbons concentration (ng/g, dry weight) of fish muscles collected from El-Mex Bay) compared to Matrouh coast.

the present study, some samples of Matrouh had pr/ph ratio greater than 1 and other samples have undetected phytane this indicated that most PAHs of this control area get from zooplankton, while El-Mex Bay showed that pristine and phytane were present in all samples indicating the petroleum origin of PAHs in this area.

3.3. Biochemical Analysis

Contamination of water with industrial and agricultural pollutants influences the biochemical processes of aquatic organisms [64]. An effective monitoring system using biochemical markers has been established to demonstrate these xenobiotics in the environment [65]. On the other hand, the evaluation of oxidative stress is commonly used in monitoring programs based on measurements of catalyze (CAT), glutathione peroxidase (GPX) and superoxide dismutase (SOD) activities [66]-[68]. The generation of oxyradicals has a connection to the etiology of several human diseases and is probably the same for fish [69]. The statistical analysis of liver and muscle glutathione s-transferase revealed that there was a highly significant increase in liver and muscle glutathione s-transferase activity and glutathione concentration of *Siganus rivulatus* collected from El-Mex Bay than those collected from Matrouh coast at $p < 0.05$. Liver and muscle glutathione s-transferase activity of *Siganus rivulatus* collected from El-Mex Bay increased by 107.21% and 63.41%, respectively, compared to those collected from Matrouh coast as shown in **Figure 5** and **Figure 6**, respectively. Apart from the normal metabolism in a living organism; carcinogens (pesticides, heavy metals), infections (bacterial, parasitical, viral), radiation damage, and environmental stress factors cause an increase in free oxygen radicals and thus cause oxidative stress [70]. Recent findings show also that the pollution toxicity in an aquatic organism may be connected to increased production of reactive oxygen species (ROS) that leads to oxidative stress [71] [72]. In this respect, a number of studies confirmed the successful role of antioxidant enzymes and non-enzymatic antioxidant modulation in identifying environmental stress [73]. In addition,

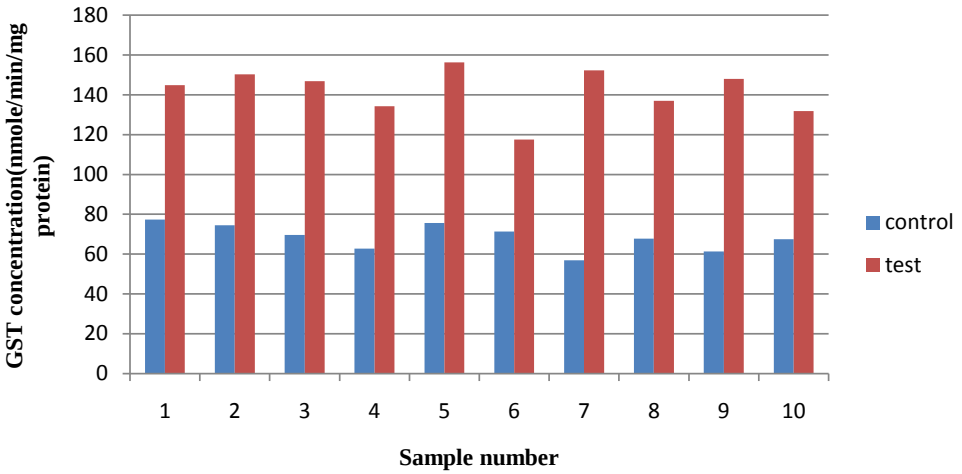

Figure 5. Liver glutathione s-transferase activity of "*Siganus rivulatus*" collected from El-Mex Bay compared to Matrouh coast.

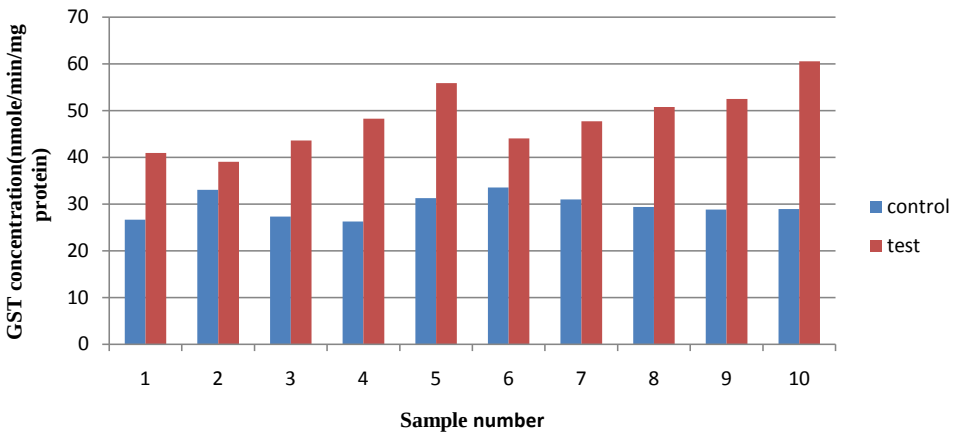

Figure 6. Muscle glutathione s-transferase activity of "*Siganus rivulatus*" collected from El-Mex Bay compared to Matrouh coast.

lipid peroxidation estimation in particular has also been found to have a high predictive importance as a biomarker of exposure [74]-[76]. Therefore, this study aimed to detect the oxidation stress response in liver and muscle of *Siganus rivulatus* collected from El-Mex Bay (tested area) and Matrouh (controlarea) to identify the significance of oxidative biomarkers as an early indicator of the health of marine ecosystem. Antioxidant enzymes were included in this study because of their inducibility under conditions of oxidative stress and their potential role in adaptation to pollutant-induced stress. An important liver biomarkers used for environmental pollution are glutathione S-transferase (GST) enzyme and glutathione in its reduced form (GSH) [77]. The presence of organic pollutants in fish muscle and their relation to specific biomarkers were also determined by Siroka *et al.,* [78]. Where, the GST is an important intracellular enzyme of the second stage of xenobiotic metabolism. Its main function is to catalyze the conjugation of glutathione and electrophilic substances of exogenous origin that might be one of etiological factors of carcinogenesis and development of degenerative diseases [79]. It

is used as a biochemical marker of aquatic environmental contamination with exogenous substances [80].

The liver and muscle tripeptide glutathione concentration of *Siganus rivulatus* collected from El-Mex Bay was increased by 73.63% and 77.54%, respectively compared to its corresponding value collected from Matrouh coast (**Figure 7** and **Figure 8**, respectively). The liver and muscle lipid peroxidation concentration for "*Siganus rivulatus*" collected from El-Mex Bay increased by 255% and 176%, respectively, compared to those collected from Matrouh coast (**Figure 9** and **Figure 10**, respectively). Moreover, measurements of lipid peroxidation which has been described as a biomarker for effect of pollution in several studies was also a useful indicator for the pollution load [71]. An elevation of MDA registered the oxidative stress due to the presence of contamination [79]. This study indicated the existence of a significant elevation in liver and muscle lipid peroxidation of "*Siganus rivulatus*" collected from El-Mex Bay compared to that

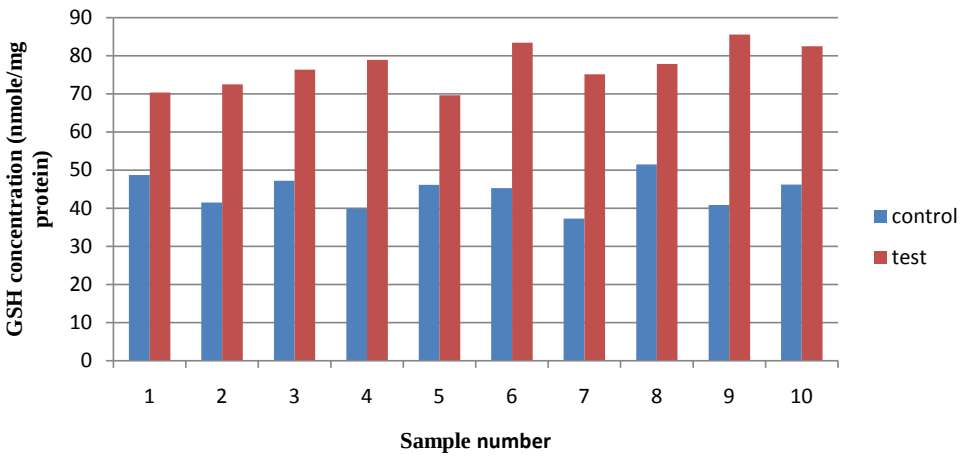

Figure 7. Liver glutathione reduced concentration of "*Siganus rivulatus*" collected from El-Mex Bay compared to Matrouh coast.

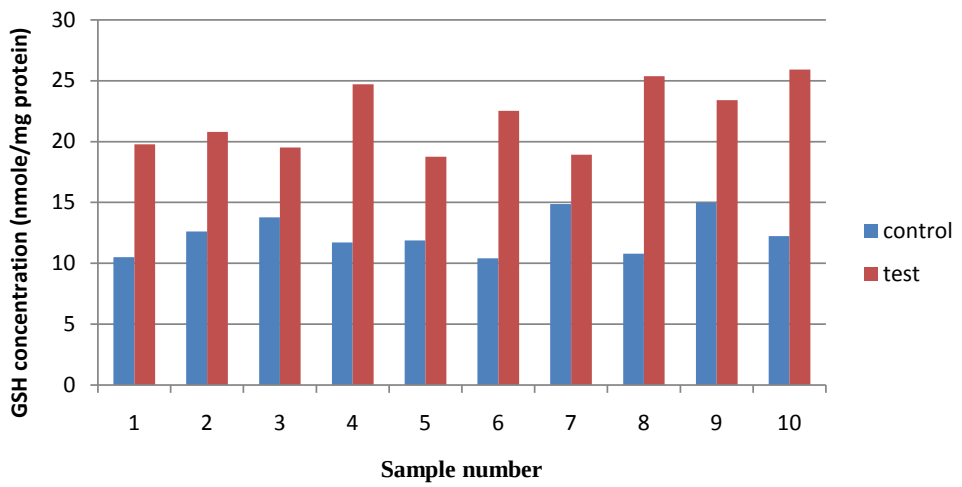

Figure 8. Muscle glutathione reduced concentration of "*Siganus rivulatus*" collected from El-Mex Bay compared to Matrouh coast.

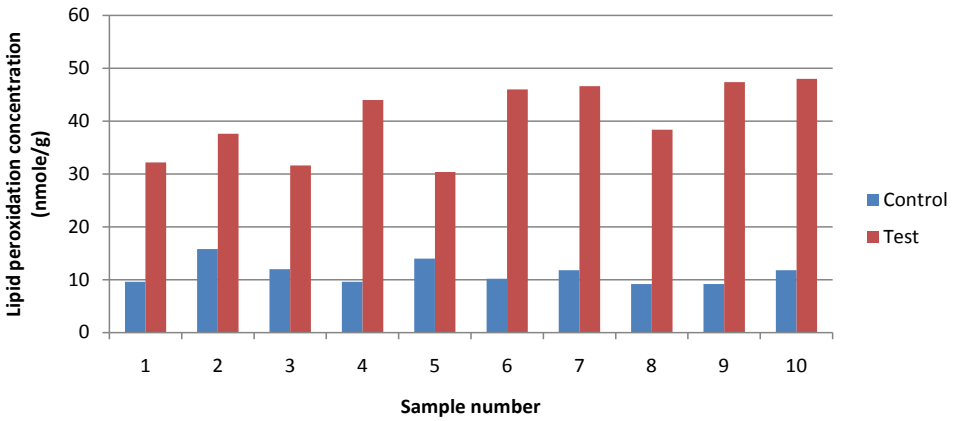

Figure 9. Liver lipid peroxidation concentration (nmole/g) of "*Siganus rivulatus*" collected from El-Mex Bay compared to Matrouh coast.

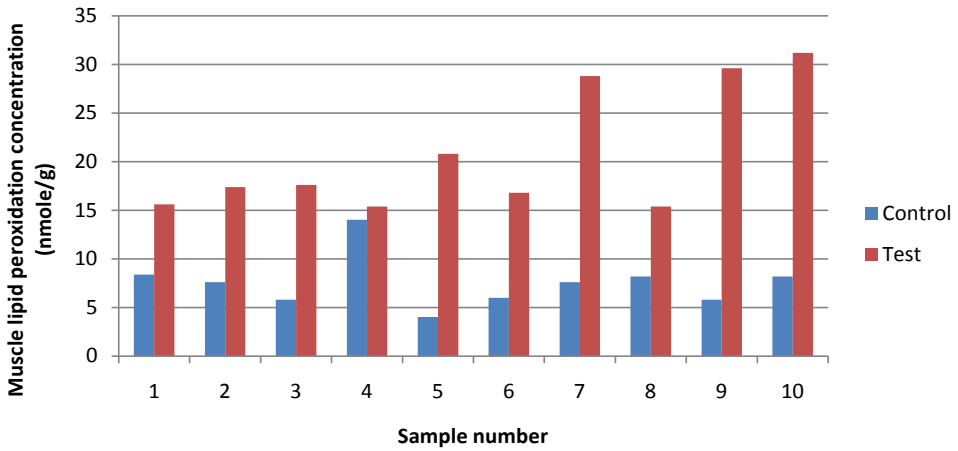

Figure 10. Muscle lipid peroxidation concentration (nmole/g) of "*Siganus rivulatus*" collected from El-Mex Bay compared to Matrouh coast.

collected from Matrouhcoast (Control area) as shown in **Figure 9** and **Figure 10**. The apparent increase in lipid peroxidation may be attributed to the accumulation of the chemical pollutant in the organs under investigation indicating the existence of significant pollutant concentration in the fish liver and muscle of this area. Chemical pollutant catalyzes the formation of ROS capable to damage tissues such as DNA, proteins and lipids. [81]. The results of this study were in agreement with the work of Farombi *et al.*, [82] who found that the level of MDA activity in the liver of Cat fish (*Clarias gariepinus*) collected from Ogun River (polluted area) was significantly elevated in all fish organs compared to the control fishes collected from the Agodi fish farm, the percentage increase in liver, kidney, gills and heart lipid peroxidation were 177%, 102%, 168% and 71% respectively compared to control.

3.4. Microscopic Observations

3.4.1. Microscopic Observation of Hepatocytes

Ultrastructurally, it was noticed that hepatocytes of liver of fish "*S. rivulatus*" collected

from El-Mex Bay, showed hepatic alternations. The present study pointed out nuclear alternations in "*Siganus rivulatus*" hepatocytes collected from a polluted area (El-Mex Bay) using transmission electron microscopy, including shrunk nuclei, centric nucleoli, decreased heterochromatin and irregular nuclear envelopes (Figure 11). Ultrathin preparations (electron microscope) of liver of "*S. rivulatus*" collected from El-Mex Bay showed also indistinct membrane of mitochondria with some of which exhibited partial loss of cristea, other exhibited swelling. One of the main alterations in liver cells of "*S. rivulatus*" collected from El-Mex Bay in the present study was the increase in size and number of lipid droplets (Figure 11). An increase, on the other hand, in the number of lysosomes, peroxisome proliferations and extensive vacuolization were observed in "*Siganus rivulatus*" from El-Mex Bay. Moreover, accumulation of vacuoles resulted in the displacement of nuclei to the cell margin with pyknosis of the nuclei and increased number of melanomacrophage centers in hepatocytes of "*S. rivulatus*" from El-Mex Bay was observed (Figure 12). Most of such ultra-structural changes included fragmentation of rough endoplasmic reticulum. Irregular nuclear outline and induction of glycogenosomes and phospholipids are of nonspecific nature.

3.4.2. Microscopic Observation of Muscle Fiber

Electron microscopic examination of the muscle fibers of "*S. rivulatus*" collected from the control area showed their composition of sarcoplasm and longitudinal arrays of myofibrils. The center of each dark (A-band or actin) was occupied by a pale area

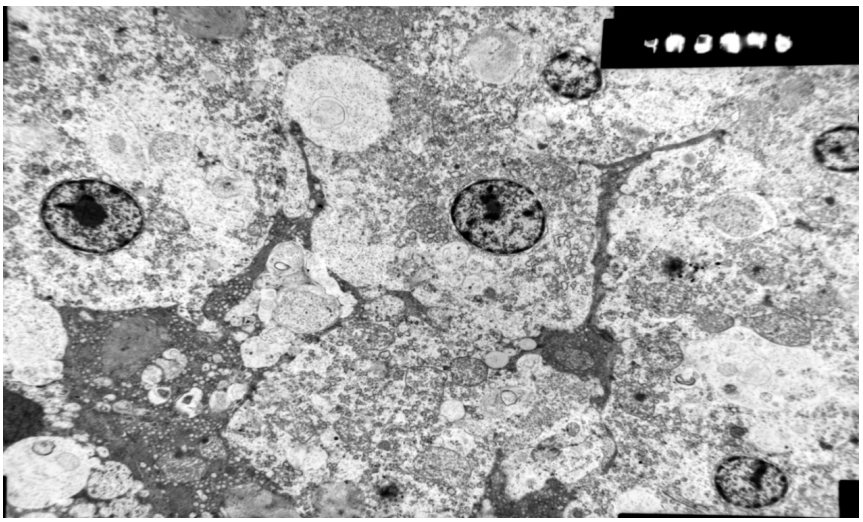

Figure 11. Electron micrograph. Liver section of "*Siganus rivulatus*" collected from Matrouh coast (control area). Showing, mononucleated hepatocytes interrupted by electron dense sinusoidal space (S), spherical central nucleus (N), with regular nuclear envelope (Ne), some nuclei showed centric large nucleoli (thin arrow), some nuclei revealed eccentric nucleoli (thick arrow), the hepatocyte nucleus possessed mostly regular nuclear envelope, small amount of condensed heterochromatin attached with the nucleolus (Nu), rough endoplasmic reticulum (RER), spherical and avoid mitochondria (M) with short tubular cristae, small number of lipid droplets (L) [Glutaraldehyde fixed-OsO_4 post fixed-uranyl acetate-lead citrate stained preparation ×6000].

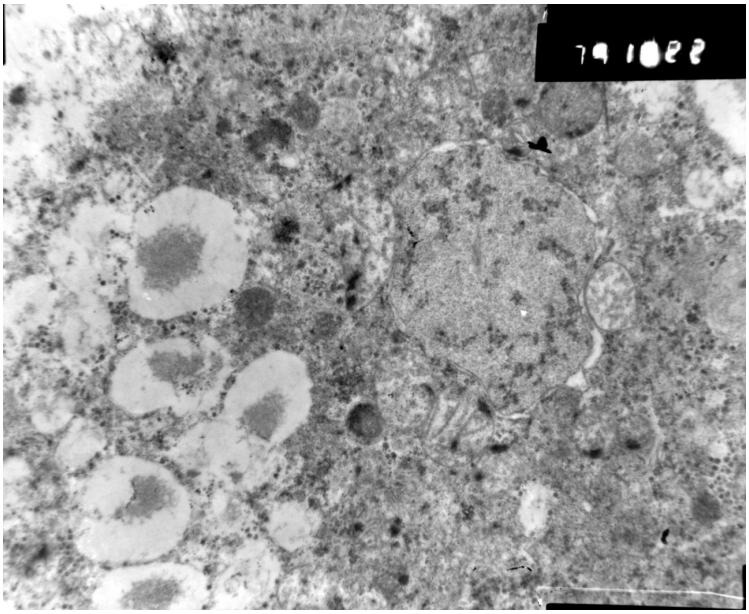

Figure 12. Electron micrograph, liver section of "*Siganus rivulatus*" collected from El-Mex Bay (tested area). Showing, altered nucleus (N) with irregular nuclear envelope (Ne), most chromatin aggregate peripherally, large number of microbodies (Mi), numerous primary lysosomes (Ly), perixosomes (P), large empty vacuoles (V) with flocculent materials and myline [Glutaraldehyde fixed-OsO_4 post fixed-uranyl acetate-lead citrate stained preparation ×10,500].

known as H-zone (Hensen's zone) which is bisected by an additional thin striation known as M-line (Mittelsheibe). A thin dark line, known as a Z-line (Zwischenscheibe) bisected the I-band which was relatively thick and distinguished by its density (**Figure 13**). Measurements based on electron microscopic preparations showed that there were insignificant differences in sacromere length, A-band, I-band and H zone in muscle fibres of "*Siganus rivulatus*" collected from control and test area at $p < 0.05$. Electron microscopic examination of the muscle of "*Siganus rivulatus*" collected from control area showed that the sarcoplasm is differentiated into peripheral sarcoplasm occupied a thin region and the nearest myofibrils, interfibrillar sarcoplasm filled the spaces between myofibrils and perinuclear sarcoplasm which is found at the pole of the nucleus. Moreover, the sarcoplasm contains non-myofibillar components including nuclei, mitochondria and sarcoplasmic reticulum, and cellular inclusions such as glycogen particles (**Figure 13**).

The nuclei of the "*Siganus rivulatus*" muscle collected from control area appeared just beneath the sarcolemma. They were oval or spindle shaped in appearance with predominant heterochromatin. Adjacent to the inner nuclear membrane, the heterochromatin appeared as dense aggregates surrounded by a regular nuclear envelope. The envelope appeared perforated by nuclear pores (**Figure 13**).

Electron microscopic preparations of the "*Siganus rivulatus*" muscle collected from tested area showed also that muscle fibers possessed highly altered nuclei, with large

size nucleoli and irregular as well as disrupted nuclear envelope, with dilated nuclear pores (Figure 14). The analysis of the present study data using student t-test revealed that there is a highly significant decrease in the nuclear length in muscle cells of fish

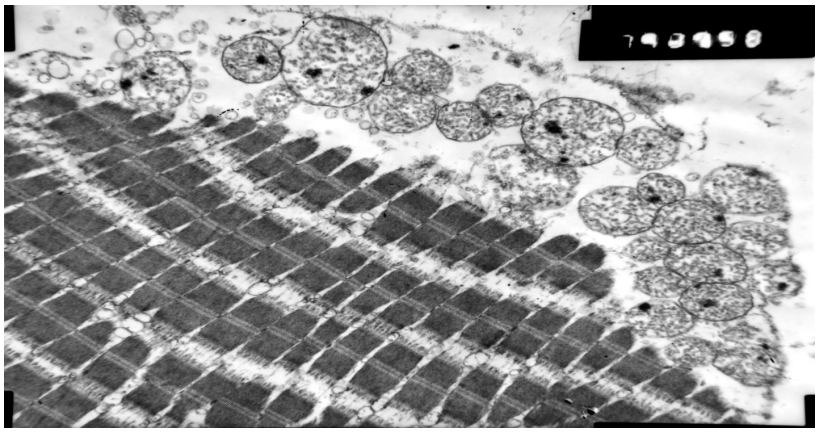

Figure 13. Electron micrograph. Longitudinal section of muscle of fish "*Siganus rivulatus*" collected from Matrouh coast (control area). Showing, regular striations, part of sarcolemma (Sl), numerous large size mitochondria (M) with distinct membrane, light matrix dense cristae. Arrow pointed at mitochondrial dense granules, sarcoplasmic reticulum (Sr). Note also, obvious isotropic band (I), Z-line (Z), anisotropic band (A), less distinct H-zone (H) [Glutaraldehyde fixed-OsO_4 post fixed-uranyl acetate-lead citrate stained preparation ×10,500].

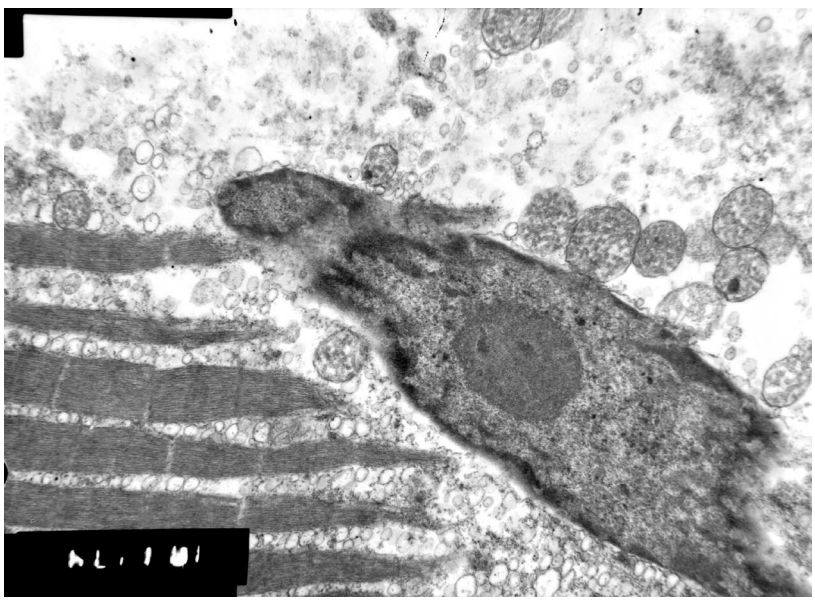

Figure 14. Electron micrograph. Longitudinal section of muscle of "*Siganus rivulatus*" collected from El-Mex Bay (tested area). Showing, highly altered nucleus (N), with altered large size nucleolus (Nu), with irregular disrupted nuclear envelope (Ne), with dilated nuclear pores (Np), small size mitochondria (M) with dense matrix, sarcoplasmic reticulum (Sr), increase lipid droplets (L), lysis in the cytoplasm [Glutaraldehyde fixed-OsO_4 postfixed-uranyl acetate-lead citrate stained preparation ×15,000].

caught from the test area comparing to its corresponding length in control area. However, there was insignificant difference in the nuclei width between the two area at $p < 0.05$.

Ultrastructural examination showed that the mitochondria of "*Siganus rivulatus*" muscle collected from the control area were oval in shape. They were clearly visible in the sarcoplasm adjacent to the nuclei and were also seen between the myofibrils. On the other hand, it was noticed that in "*Siganus rivulatus*" muscle collected from the test area, mitochondria appeared small in size with dense matrix. In these preparations the sub-sacrolemma mitochondria appeared disrupted and lysis in the cytoplasm (**Figure 14**). Data analysis using student t-test demonstrated that there is a highly significant decrease in the mitochondria length and width in the test area, comparing to that of control area at $p < 0.05$. In this context, the histopathological study confirmed the biochemical result where, the hepatocytes of liver of fish "*S. rivulatus*" collected from El-Mex Bay, showed hepatic alterations. It is suggested that this is due to increase concentration of different pollutants in El-Mex Bay [83]. This study also pointed out nuclear alterations in *S. rivulatus* hepatocytes collected from a polluted area using transmission electron microscope, including shrunk nuclei, centric nucleoli, decreased heterochromatin and irregular nuclear envelopes. It showed also mitochondrial indistinct membrane, some of which exhibited partial loss of cristea, other exhibited swelling. The mitochondrial degeneration may account for the impaired oxidative capability of hepatocytes in fish collected from the polluted location. According to the obtained biochemical results, the chemical pollutants induce lipid peroxidation and decrease the functional lipid content of tissues which can easily disturb the cellular metabolism and affect the ultra-structure. Thus, mitochondrial membranes damage affects their features; permeability, and selectivity. In consequence, they cannot regulate physiological processes and damaged mitochondria do not produce enough ATP, resulting in impair enzymatic detoxification within the exposed tissues [84]. These results are in agreement with results reported by Abdel-Moneim and Abdel-Mohsen in 2010, who found that catfish (Clariidae) hepatocytes collected from the polluted area showed accumulation of heterochromatin, enlarged nucleoli, and an extremely folded nuclear envelope. Tripathi and Shukla [85] Showed the mitochondrial degeneration may account for the impaired oxidative capability of hepatocytes in fish collected from the polluted area. Indeed, marked ultrastructural changes including, the presence of swollen mitochondria with loss of functional cristae due to exposed to methyl parathion. Electron microscopic examination of the *S. rivulatus* collected from test area showed also that muscle fibers (**Figure 14**) possessed highly altered nuclei with large size nucleoli, with irregular and disrupted nuclear envelop and mitochondria appeared small in size with dense matrix revealing that the histopathological biomarker of toxicity in fish organs is a useful indicator of environmental pollution.

4. Conclusions

The present study showed that El-Mex Bay area was polluted by different types of

chemicals including hydrocarbons comparing with the reference area (Matrouh area). It was proven that the use of selected biochemical markers and chemical analysis has been a suitable to monitor the level of contamination of aquatic environment. The present study suggests that oxidative stress biomarkers, especially estimation of antioxidant systems in fish could provide a useful indicator of pollution of marine ecosystem. The induction of antioxidant systems in fish collected from polluted area in response to oxidative stress should be considered as a clear indication of the presence of pollution and environmental health degradation. The increase in lipid peroxidation revealed as a useful indicator of the pollution load in the present study. These results suggest also that chemical pollution is capable of inducing morphological alteration in liver of fish collected from polluted area. The present study indicated that ultrastructural changes serve as biomarker of stress in aquatic environment. When evaluating aquatic environmental pollution, it should always take into account a combination of several biochemical markers in any final assessment together of course with the results of chemical monitoring because only a combination of all this information will give the most objective picture of the status of the environmental monitoring.

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