



Photosynthetic and Biochemical Response of *Ulva lactuca* to Marine Pollution by Polyaromatic Hydrocarbons (PAHs) Collected from Different Regions in Alexandria City, Egypt

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ACTIVITIES of superoxide dismutase (SOD), catalase (CAT) and peroxidase (GR), lipid peroxidation (expressed as MDA), the accumulation of H₂O₂, and photosynthetic activities in *Ulva lactuca* (Ulvales, Chlorophyta) were determined in the present study in order to investigate their response to polyaromatic hydrocarbons (PAHs) at residential and industrial sites in Alexandria, Egypt.

Increasing in PAHs concentrations caused significant ($P < 0.01$) inhibition in the activities of SOD and CAT by 32% each, while, the activity of GR was significantly increased by 1-fold ($P \leq 0.001$). MDA levels were increased with increasing concentrations of PAHs; however, their levels were inversely proportional to activities of antioxidant enzyme (especially CAT). Moreover, photosynthetic rates (P_n), chlorophyll fluorescence (F_v/F_m), and total chlorophyll were significantly reduced ($P < 0.01$) with increasing PAHs by 24, 25, and 19%, respectively. The apparent increase in GR activity could indicate that alga acclimatized to the oxidative stress of PAHs, although oxidation of membrane lipids was not totally prevented, as indicated by stimulated MDA levels.

The results indicated that PAHs could exert oxidative damages, and the harmfulness occurred mainly in samples collected from the industrial site with higher concentrations of PAHs. To the best of our knowledge, this is the first study to investigate the novel and conventional effects of PAHs on the physiological and biochemical traits of alga.

Keywords: Antioxidant enzymes, H₂O₂, MDA, PAHs, Photosynthesis, *Ulva lactuca*.

Introduction

Marine ecosystems are exposed to either biogenic or exogenic petroleum hydrocarbons (Binark et al., 2010; Haynes & Johnson, 2010; El-Sheekh et al., 2016; Qari & Hassan, 2017). Some people in developing countries still dispose of their wastes (domestic, municipal, agricultural, and even industrial) in water bodies (Torres et al., 2010; El Maghraby & Hassan, 2017; Haiba, 2019). These wastes have adverse effects on ecosystems, even when released at low concentrations as they are non-biodegradable

(Torres et al., 2010; Al Meelebi et al., 2014; Qari & Hassan, 2014; Sinaei & Mashinchian, 2014; Haiba & Hassan, 2016; Haiba, 2019; Haiba et al., 2019).

PAHs are ubiquitous in urban areas and distributed widely in marine environments (Qari & Hassan, 2017; Haiba, 2019). They occur naturally (constituents of crude oil) or due to human activities (e.g. petroleum industries, combustion) (Harrison et al., 2016 a; b). They are of special concern because they have cytotoxic properties (mutagenesis and carcinogenesis)

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Received 28/7/ 2020; Accepted 3/2/ 2021

DOI: 10.21608/ejbo.2021.37571.1531

Edited by: Prof. Dr.: Mostafa M. Elsheekh, Faculty of Science, Tanta University, Tanta, Egypt.

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(Hassan & Basahi, 2013; Harrison et al., 2016 a; b; Haiba et al., 2019). In their thorough review, Torres et al. (2010) stated that continuous operative discharges from ships, marine tanker collisions, and refineries discharge high amounts of pollutants in marine ecosystems. PAHs are highly toxic chemicals for marine environments, and they can easily enter the food chain through algae. Moreover, PAHs pose a threat to marine life due to their rapid diffusion and accumulation in algae (Duan et al., 2015).

Algae are promising bioindicators for different types of pollution in aquatic environments. They occupy the first level of the food chain; therefore they ease the transfer of pollutants into higher trophic levels, exacerbating the possibility of harmfulness (Torres et al., 2010; Qari & Hassan, 2017). Conversely, as a food source, algae cannot synthesize PAHs, but they are known to be a good accumulator of PAHs (An-Ping et al., 2018; Geddie & Hall, 2019; Haiba et al., 2019). However, information about the capability of absorption, digestion, and accumulation of PAHs after their uptake from seawater into the algae are scattered and scant, and information in developing countries are even more scarce and fragmentary (Sinaei & Mashinchian, 2014; Haiba & Hassan, 2016; Qari & Hassan, 2017). Exposure of algae to PAHs alter their physiological and biochemical processes, reduces their growth, and even accelerates their death.

There are 16 unsubstituted PAHs identified by Environmental Protection Agency (EPA) as the most aggressive pollutants due to their noxiousness and prevalence in the environment (Harrison et al., 2016 a; Qari & Hassan, 2017).

Egypt is witnessing rapid and tumultuous industrial and demographic growth (Ismail et al., 2014; Hassan et al., 2017; 2018). Alexandria is the second large city in Egypt, and it has about 40% of Egyptian industrial activities (El Maghraby & Hassan, 2017; Haiba, 2019). Improper management of demographic growth as well as rapid expansion and increasing population caused serious environmental problems in Egypt (Hassan, 2017; Haiba, 2019). However, there is a paucity of knowledge about pollution of seawater with PAHs in Egypt, and data about the response of marine life to PAHs is extremely lacking (Geddie & Hall, 2019).

Ulva lactuca is an opportunistic and ubiquitous alga (Guiry & Guiry, 2018). Due to its quick growth, wide prevalence globally, and its high tolerance of a number of environments, it has been widely used as phytoremediators and timely biomonitors and bioindicators of prospective hazards in marine ecosystems (Seoane et al., 2018; Lia et al., 2020).

It is necessary to identify and quantify pollutants and their effects on physiological processes and metabolism in order to diagnosis the situation of the environment. There is a gap in our knowledge on how marine pollution with PAHs affects photosynthetic performance and biochemical traits of macroalga *Ulva lactuca*. Therefore, this work was carried out to further our knowledge about photosynthetic performance and biochemical response of the green macroalga *Ulva lactuca* to marine pollution. To the best of our knowledge, this is the first such investigation to be carried out in Egypt to explain the toxicity mechanisms of PAHs to the alga.

Materials and Methods

Sampling protocol

The thalli of *Ulva lactuca* (Chlorophyta) were collected simultaneously early in the morning from two sites located in Alexandria city (31.2001°N, 29.9187°E), Egypt (Fig. 1), and gently rinsed and transported immediately to the laboratory in a dark icebox. The collected thalli were transferred to the laboratory in boxes filled with seawater, then they were dried in the open air for 24 hours and stored in a liquid nitrogen at -80°C for further analyses (El-Sheekh et al., 2010; Seoane et al., 2018).

One site represents a residential area (site 1, at El Shatby district near downtown), while the other site represents an industrial area (site 2, at the western region of Alexandria City).

Photosynthetic measurements

Healthy and clean thalli of *Ulva lactuca* (about 8-10cm in length) were selected for photosynthetic measurements. Measurements were taken immediately after collection of thalli in the sampling sites, at 12:00 Egyptian local time, using a Portable Photosynthesis System (LI-COR, LI-6400XT Environmental, Biosciences, USA) (Lia et al., 2020).

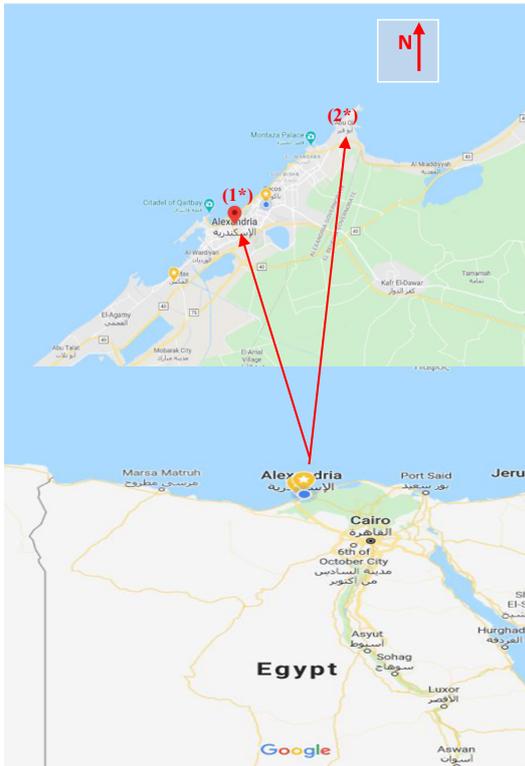


Fig. 1. Location of the study areas in Alexandria city [Site 1 is a residential area, site 2 is an industrial area]

Chlorophyll a fluorescence measurement

Chlorophyll fluorescence was taken simultaneously as photosynthetic rates (at 12:00 PM) using a portable fluorometer (Walz GmbH, Germany). The variable to maximum (F_v/F_m) fluorescence was measured (Beer et al., 2000; Basahi et al., 2016; Shahr et al., 2020).

Chlorophyll content

Chlorophylls of thalli (1g) were extracted using 80% (v/v) cold acetone in a dark-cold room (5°C) and the absorbance readings for Chl *a* and *b* were measured at 645 and 663 nm, respectively (Basahi et al., 2016; Baqasi et al., 2018 a, b).

Protein content

Immediately after return to the laboratory, the protein was extracted from fresh thalli and measured at 595 nm using Shimadzu Spectrophotometer (Bradford, 1976).

Antioxidants bioassay

They were extracted by homogenization of 10g of fresh thalli with 1mL 50mM K_3PO_4 buffer (pH 7.0) on ice. Then, the homogenate was centrifuged at 15,000 rpm for 20min. at 5°C. The

supernatant was used to determine the activities of catalase (CAT), superoxide dismutase (SOD) and glutathione reductase (GR) at 240, 480 and 340nm, respectively. Enzyme activities were measured in five replicates (Hassan, 2006; Basahi et al., 2016; Hassan et al., 2017, 2018; El Dakak & Hassan, 2020).

Determination of H_2O_2 and Lipid peroxidation

H_2O_2 was extracted in 50mM potassium phosphate buffer (pH 6.5) and was centrifuged for 15min. at 18000 rpm at 0°C. Then, 1.5mL 20% H_2SO_4 was added to the supernatant, and absorbance was measured at 410nm (Al Meelebi et al., 2014; Hassan et al., 2017, 2018).

Lipid peroxidation was measured as malondialdehyde (MDA) spectrophotometrically at 535nm (Hassan & Twefik, 2005; Hassan et al., 2017, 2018)

Extraction and analysis of PAHs

1000mL of seawater was collected in acid-washed amber glass from the two locations and they were filtered to remove suspended matter. The 16 EPA PAHs analyzed are; naphthalene (Nap), acenaphthylene (Acy), acenaphthene (Ace), fluorine (Fl), phenalysedanthrene (Ph), anthracene (An), fluoranthene (F), pyrene (Pyr), benzo(a)anthracene (B(a)An), chrysene (Cry), benzo(b)fluoranthene (B(b)F), benzo(k) fluoranthene (B(k)F), benzo(a)pyrene (B(a)Pyr), indeno (1,2,3cd) pyrene (I(c,d) Pyr), dibenzo(a,h) anthracene (da,h)an) (Sinaei & Mashinchian, 2014; Duan et al., 2015; Kafilzadeh et al., 2015).

Alga (*Ulva Lactuca*) was collected in clean polyethylene bags containing water from the same locations and were transferred to the laboratory for analysis. 100 g fresh weight alga was rinsed with dichloromethane (DCM), dried in the open air for 24hrs. and weighed. The dried thalli were ground into a powder and 1000pg μL^{-1} surrogate deuterated internal PAH standards were added to the powdered thalli prior to extraction for quantification (Duan et al., 2015; Mahgoub, 2016). The powdered thalli were extracted in an ultrasonic bath at 20°C for 30min., with 50mL dichloromethane (DCM) and analyzed using GC-MS (Agilent 6890) (Sinaei & Mashinchian, 2014; Duan et al., 2015; Kafilzadeh et al., 2015; Harrison et al., 2016 a, b).

PAHs were classified into LMWPAHs (with 2

to 3-rings) and HMWPAHs (with 4 to 6 rings) to have a better picture of their distribution in water and algal samples. This classification is important to determine the source of PAHs

Quality control and quality assurance (QA/QC)

Blank samples (tubes filled with distilled water as a control) were collected along with field samples to ensure that there was no significant background contamination. They were analyzed, and no PAHs were detected, which ensured the quality of results.

Data analysis

One-way ANOVA was used to determine the effect of sampling sites on the concentrations of PAHs using STATGRAPHS statistical package (Package 4, UK). Data were log-transformed prior to analysis to ensure that they were normally distributed. Light intensity was used as a covariate in analyses of photosynthetic data. Significant difference between means was tested by Tukey's

multiple range test ($P < 0.05$). All the data were expressed as means \pm SD ($n = 5$). Moreover, correlation analyses were performed between all biochemical and physiological traits to determine the relationship between them.

Results and Discussion

Concentrations of PAHs in algal thalli and seawater

Figure 2 shows the concentrations of individual PAHs congener in algal thalli and seawater collected from two different sites around Alexandria city, Egypt.

Concentrations of B(k)F were 1.11 (ng L^{-1}) and 2.89 (ng g^{-1}) for seawater and alga thalli samples, respectively; these concentrations are the lowest levels recorded in the present investigation. On the contrary, concentrations of dB(h,h,i)P were 50.22 (ng L^{-1}) and 73.98 (ng g^{-1}) for the same samples, respectively; these levels are the highest concentration recorded in this investigation.

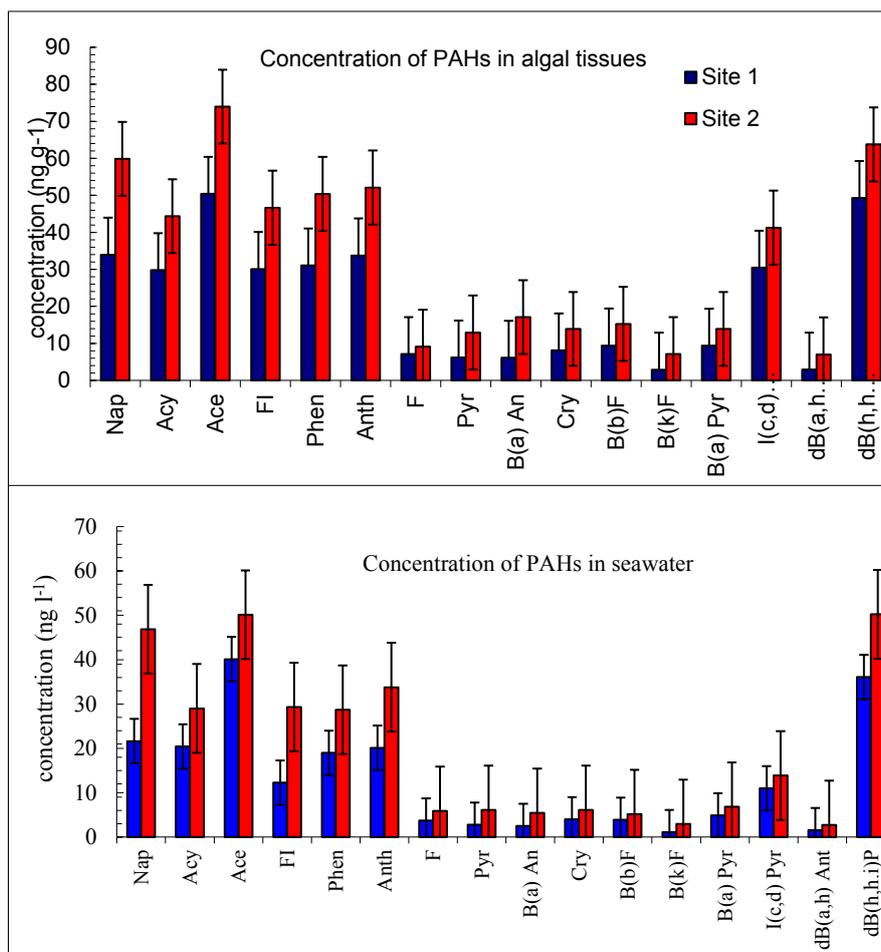


Fig.2. Concentrations of PAHs in algae tissues and seawater ($n = 5 + \text{SE}$).

Total concentrations of PAHs were 341.01 and 528.84 ng g⁻¹ in algal tissues and 205.16 and 224.14 ng L⁻¹ in seawater collected from sites 1 and 2, respectively. Moreover, concentrations LMWPAHs were higher in algal tissues collected from site 2 (327.41 ng g⁻¹) when compared with those collected from algal thalli collected from site 1 (209.12 ng g⁻¹) (Fig. 1). Similarly, LMWPAHs collected from both sites followed the same trend (133.53 and 217.81 for seawater collected from site 1 and 2, respectively). However, HMWPAHs had lower concentrations than LMWPAHs in both algal tissues and seawater. HMWPAHs had lower concentrations (131.89 ng g⁻¹) in thalli collected from site 1 than that collected from site 2 (201.43 ng g⁻¹). Moreover, concentrations of HMWPAHs collected from seawater in site 1 was lower (71.63 ng L⁻¹) than that collected from site 2 (106.33 ng L⁻¹) (Fig. 2).

It was noted that low molecular weight PAHs (LMWPAHs; Nap, Ace, Acy, F, Phe, Anth) had higher concentrations than High molecular weight PAHs (HMWPAHs; Fl, Pyr, BaA, Chry, BaF, BkF, BaP, DahA, IcdP and BghiP) at both sites in algal tissues and seawater.

Seawater had significantly ($P \leq 0.01$) lower concentrations of PAHs than thalli (Fig. 2). The observed differences in PAHs could be attributed to the presence of lipids in algal tissues (Qari & Hassan, 2017).

Table 1 shows the ratios phenanthrene to anthracene (Phen/Anth), fluoranthene to pyrene (F/Pyr), and LMWPAHs/HMWPAHs, which are indicators of PAHs's sources. The ratio of Phen/An and F/Pyr are commonly used to identify pyrolytic origin of PAHs (An-Ping et al., 2018).

Low Phen/Anth ratios (<10) indicate combustion processes. For the F/Pyr ratios, values >1 have been used to indicate pyrolytic origins, and values <1 are attributed to petrogenic sources (Haiba, 2019). The ratios of LMW/HMW were higher than 1.0 at both sites in water samples and thalli in the present study, which indicated the prevalence of LMWPAHs, suggesting petroleum-related compounds are the main sources (Qari & Hassan, 2017; Haiba, 2019). Moreover, due to the bioavailability of LMWPAHs in the water, they have been accumulated in algal tissues.

Table 1 showed that the ratio of F/Pyr ratios in algae tissue and in seawater were 1.15 and 1.35 in site 1 and 0.71 and 0.96 in site 2, respectively, which could indicate that the source could be pyrogenic (oil pollution). Similarly, the Phen/Anth ratios proved a pyrogenic origin as they were 0.92 and 0.94 in site 1 and 0.97 and 0.85 in site 2 for the same samples, respectively. Moreover, the transport of aerosols with their PAH in atmospheric is another pyrogenic source. Due to the presence of many industrial and anthropogenic activities (e.g. petroleum, cement, fertilizers) nearby site 2, it is suggested that the source could be pyrolytic. Mahgoub (2016) stated that water bodies can become contaminated with PAHs from runoff in urban areas, waste water from industries and petroleum spills.

An F/Pyr ratio >1 indicates pyrolytic origin (i.e. occurrence of the combustion-derived material). The LMW/HMW and F/Pyr ratios in the present investigation clearly indicated that PAHs have pyrolytic origin, and this is in agreement with previous results in Saudi Arabia (Qari & Hassan, 2017).

TABLE 1. The ratios of concentrations of sum of the lower molecular weight PAHs versus sum of the higher molecular weight PAHs and the ratios of concentrations of sum of the Fluoranthene versus pyrene; Phenanthrene versus anthracene

Ratio	Sample	Site 1	Site 2
LMW/HMW	Algal tissue	1.76	2.99
	seawater	1.44	1.57
F/Pyr	Algal tissue	1.15	0.71
	seawater	1.35	0.96
Phen/Anth	Algal tissue	0.92	0.97
	seawater	0.94	0.85

LMW= Lower molecular weight PAHs, HMW= Higher molecular weight PAHs.

F/Pyr = Fluoranthene versus pyrene, Phen/Anth = Phenanthrene versus anthracene

Response of chlorophyll content, chlorophyll fluorescence and photosynthesis

Table 2 shows photosynthetic efficiency and chlorophyll contents of alga collected from both sites. Photosynthetic rates (P_n), chlorophyll fluorescence (F_v/F_m) Chl *a*, Chl *a/b*, and total chlorophyll were reduced by 24, 25, 24, 18 and 19% in alga collected from site 2 when compared with that collected from site 1, respectively. However, Chl *b* showed insignificant response ($P > 0.05$).

TABLE 2. Effects of different locations on photosynthetic pigments, photosynthetic rates (P_n) and chlorophyll fluorescence (F_v/F_m) of *Ulva lactuca*

Parameter	Site 1	Site 2
Chl <i>a</i> (mg g ⁻¹ fresh wt)	1192.8 ^b ± 123.35	908.7 ^a ± 67.22
Chl <i>b</i> (mg g ⁻¹ fresh wt)	423.7 ± 33.98	393.9 ± 45.45
Chl <i>a/b</i>	2.82 ^b	2.31 ^a
Total Chl. (mg g ⁻¹ fresh wt)	1616.5 ^b ± 157.87	1302.6 ^a ± 129.23
F_v/F_m	0.721 ^b ± 0.056	0.548 ^a ± 0.058
P_n ($\mu\text{molO}_2 \text{ min}^{-1} \text{ s}^{-1}$)	15.03 ^b ± 2.17	11.26 ^a ± 1.52

- n= 5 ± SD.

- Means not followed by the same letter are significantly different from each other at $P \leq 0.05$

In the present study, PAHs could damage the biosynthesis of chlorophyll in *U. lactuca*, especially in site 2. Moreover, The reduction of chlorophyll *a/b* in site 2 suggesting that PAHs converted some of Chl *a* into Chl *b* by oxidation of the methyl group on the ring II, this is in agreement with the results of Cheng et al. (2016), who found that some of Chl *a* was converted to Chl *b* due to Cd stress in *Chlorella vulgaris*. Similar results were found in cyanobacteria (El-Sheekh et al., 2010).

The reduction in P_n and F_v/F_m in site 2 compared with control site 1, is probably due to inhibition in chlorophyll *a* biosynthesis as a compensatory mechanism to maintain the availability of carbon compounds for antioxidant compounds synthesis (Tammam al., 2011; Samanta et al., 2019). Moreover, higher growth in site 1 was significantly ($R^2 = 0.582$, $P < 0.01$) correlated with chlorophyll *a* content and photosynthetic capacity in *U. lactuca*

(data not shown). Torres et al. (2010) stated that among the cellular defenses against ROS, photosynthetic pigments (mainly chlorophylls) can scavenge free radicals and quench electronically excited-state molecules such as H_2O_2 , which have been known to be mutagenic and have the ability to cause severe damages to DNA molecules (Binark et al., 2020).

Antioxidant defense responses

Protein content and activities of CAT and SOD were reduced by about 32% each in thalli collected from site 2 when compared with that collected from site 1 (Table 3). On the contrary, the activity of GR was increased by about 1-fold.

TABLE 3. Antioxidant activities of *U. lactuca* at the two selected sites

Parameter	Site 1	Site 2
Total Protein (mg g ⁻¹ fresh wt)	7.62 ^b ± 1.22	4.39 ^a ± 1.77
CAT (Unit CAT mg ⁻¹ protein)	2.98 ^b ± 0.79	1.99 ^a ± 0.56
SOD (Unit SOD mg ⁻¹ protein)	237.4 ^b ± 33.56	157.18 ^a ± 22.78
GR (Unit GR mg ⁻¹ protein)	26.78 ^a ± 5.34	59.11 ^b ± 11.82

- Different letter in each row indicate significant differences ($P < 0.05$) according to Tukey's test ($P < 0.05$)

Antioxidant enzymes such as CAT, SOD, and GR are the importance in maintaining the redox state within the cell in order to protect the cell against peroxidation damage (Cheng et al., 2016; Hassan et al., 2017, 2018).

CAT activity increased at site 1 (2.98 Unit CAT mg⁻¹ protein) where PAHs concentrations were lower than that in site 2, which has higher levels of PAHs and thalli collected from that site had relatively lower CAT activity (1.99 Unit CAT mg⁻¹ protein). Cat could reduce H_2O_2 toxicity by catalyzing its conversion into H_2O (Torres et al., 2010). The massive production of reactive oxygen species (ROS) could reduce activity of CAT, therefore causing oxidative stress.

SOD plays an important protective mechanism by controlling free radical concentration, and it is considered as the first line of defense against ROS in living cells. The high activity of SOD activity

in site 1, where PAHs are relatively low, proposes the enhanced dismutation of toxic ROS (e.g. superoxide radical " $O_2^{\cdot-}$ ") to yield a less toxic ROS e.g. H_2O_2 which can be demolished further by associated increased GR activity (Samanta et al., 2019). Nevertheless, the lowered activity of SOD under High PAHs levels is most likely related to the excessive buildup of H_2O_2 (Binark et al., 2010).

The drastic and significant ($P < 0.01$) increase in the activity of GR (more than 1-fold) in the present study indicates that GR is mainly involved in oxidative defense. This is in agreement with previous results with salt and heavy metal stresses (Manoj et al., 2010; Tammam et al., 2011; Samanta et al., 2019). This increase in GR activity indicated higher scavenging of ROS (Manoj et al., 2010; Basahi et al., 2016). Enhanced GR activity helps in lowering ROS production via the reduction of NADPH/NADP⁺ ratio. The lower ration of NADPH/NADP⁺ makes NADP⁺ readily available to accept electrons; thereby, less electrons will flow to O_2 (Manoj et al., 2010; Tammam et al., 2011). Enhanced levels of antioxidants are crucial for withstanding photooxidative stress triggered by reduced energy utilizing capacity, resulting from PAHs toxicity (Torres et al., 2010). GR is known to reduce the oxidized form of glutathione (GSH) that consists of the corresponding disulfide (GSSG) back to two molecules of GSH using NADPH as a cofactor (Ogawa, 2005).

Presence of high concentrations of antioxidants in chloroplasts enhances the oxidative injury leading to excessive flow of electrons in their microenvironment, therefore tolerance of algae to PAHs toxicity depends mainly on defense mechanism that alleviates oxidative injury (Torres et al., 2010).

Hydrogen peroxide and Lipid peroxidation

Table 4 shows the variation in levels of H_2O_2 and MDA collected from thalli of *Ulva* from the two sites. There were significant ($P < 0.05$) increase in the content of H_2O_2 and MDA levels by 6- and 2-fold, respectively, in thalli collected from an industrial site (site 2) when compared with those collected from the residential site (site 1). These significant increases indicate that PAHs have a redox potential which helps in the stimulation of ROS that stimulates the production of MDA and H_2O_2 with the apparent oxidative damage to membranes. There are

similar results in the literature due to pollution with trace elements but not with PAHs. This is the first study indicating that PAHs stimulates the production of H_2O_2 and lipid peroxidation (represented by production of MDA). Moreover, these increases were significantly correlated with the inhibition in CAT activity ($R^2 = -0.671$ and -0.683 for MDA and H_2O_2 , respectively (Table 4).

TABLE 4. Levels of hydrogen peroxide and lipid peroxidation (as MDA) in thalli of *Ulva* collected from different sites

Parameter	Site 1	Site 2
H_2O_2 (mg g ⁻¹ fw)	3.52 ^b ±1.22	24.31 ^a ±5.29
MDA (nmol g ⁻¹ f wt)	298.98 ^b ±12.11	947.19 ^a ± 56.37

- Different letter in each row indicate significant differences ($P < 0.05$) according to Tukey's test ($P < 0.05$)

Algae as other living organisms, produce different reactive oxygen species (ROS) due to oxidative metabolism (e.g. H_2O_2), which could be injurious to at high concentrations (Torres et al., 2010). Production of these ROS are slow under normal conditions, however, pollution (including PAHs) stimulates their production (Binark et al., 2010; Torres et al., 2010). This could be the case in the present study, especially H_2O_2 production was higher in site 2 (which has high levels of PAHs) than in site 1 (which had lower levels of PAHs).

Correlation analysis between all traits

Proper and early environmental risk assessments must establish functional relationships between abiotic and biotic levels of pollution (Torres et al., 2010). In the present study, correlation coefficients varied between physiological and biochemical parameters (Table 5). There was a significant positive correlation between photosynthetic rates and chlorophyll contents, which suggests that pigment levels directly influenced physiological and biochemical processes and eventually the growth of *Ulva lactuca*. Recently, Binark et al. (2020) found similar results on marine algae contaminated with oil pollution. Additionally, correlation analysis showed that chlorophyll *a* positively correlated ($0.05 > P < 0.01$) with photosynthetic rates (P_n),

photosynthetic efficiency (F_v/F_m), CAT, and SOD, photosynthesis, but not GR ($P > 0.05$). Total PAHs were negatively correlated with all traits except GR. This negative correlation indicates the release of reactive oxygen species (ROS) that cause oxidative damage of cells. Similar results were found with *Ulva australis* (Samanta et al., 2019).

Generally, PAHs inhibited, especially at higher concentrations in an industrial site (site 2), net photosynthetic rates, chlorophyll fluorescence, chlorophyll content, activities of CAT and SOD. On the other hand, MDA contents and activity of GR were enhanced. These responses might be ascribed to excess ROS, such as H_2O_2 , which were induced by PAHs.

Pathak et al. (2018) reported that algae could be used as green biosorbents for the removal of PAHs and their biodegradation as their cellular constituents act as binding sites for removal of pollutants, and share enzymatic systems.

In summary, this work effects of pollution with PAHs was determined for first time on physiological and biochemical traits of *U. lactuca*; concentrations of PAHs changed with location and they were responsible in the apparent variation in photosynthetic activity and

antioxidant response of alga.

Conclusion

In conclusion, novel and conventional stress responses of alga to PAHs, an organic environmental pollutant, have been identified for the first time. These findings support the importance of using *Ulva lactuca* as a bioindicator of seawater pollution.

The results indicated that PAHs could provoke oxidative damages in the alga, which occurred mainly in samples exposed to higher concentrations of these compounds. Moreover, results highlighted the importance of algae as bioaccumulator of pollutants such as PAHs and could be used for phytoremediation for sustainable development.

Acknowledgments: We would like to thank Prof Iqbal Ismail and Mr. Jaykumr, CEES, King Abdulaziz University, Jeddah, for their help in the extraction and analysis of PAHs. We are indebted to Prof A.F. Khalafa, Alexandria University, and Prof M. El-Sheekh, Tanta University, for their invaluable comments and guidance. Last but not least, we would like to thank late lawyer M.S. El Maghraby who inspired this work.

TABLE 5. Pearson correlation coefficients (n= 24) between biochemical and physiological traits in *Ulva lactuca*

	Total PAHs	Chl <i>a</i>	Chl <i>b</i>	P _n	F _v /F _m	Protein	CAT	SOD	GR	MDA	H ₂ O ₂
Total PAHs	-	-0.721	-0.112	-0.846	-0.784	-0.549	-0.621	-0.573	0.503	0.784	0.902
Chl <i>a</i>		-	0.308	0.671	0.739	0.479	0.589	0.704	0.226	-0.811	-0.593
Chl <i>b</i>			-	0.289	0.302	0.331	0.281	0.116	0.174	-0.511	-0.287
P _n				-	0.937	0.527	0.403	0.454	0.281	-0.704	-0.683
F _v /F _m					-	0.718	0.627	0.578	0.092	-0.773	-0.648
Protein						-	0.564	0.538	0.702	-0.572	-0.556
CAT							-	0.657	-0.543	-0.671	-0.683
SOD								-	-0.652	-0.618	-0.594
GR									-	0.718	0.664
MDA										-	0.649
H ₂ O ₂											-

- Bold figures are significant at $0.01 \leq P \leq 0.05$

Conflict of interests: The authors declare no conflict of interest.

Authors contribution: Dr Dhalia El Maghraby constituted by about 85% (She suggested the idea, collected samples, wrote the first draft, helped in analysis and helped in the final revision). Prof Ibrahim Hassan contributed by 15% (He revised the manuscript, helped in analysis and final revision and was responsible for communication with the Journal)

Ethical approval: Not applicable.

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إستجابة العمليات الكيموحيوية والبناء الضوئي للتلوث البحري بالمركبات الأروماتية عديدة الحلقات في طحلب خس البحر (الأولفا) المجمع من مناطق مختلفة بمدينة الأسكندرية- مصر

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تناولت هذه الدراسة تقدير النشاط الإنزيمي لسوبر أكسيد الديسميوتيز والكاتاليز والبير أوكسيداز والجلوتاثيون ريداكناز وكذلك تركيزات المالونألدهيد وفوق أكسيد الهيدروجين في عينات من طحلب الأولفا التي تم تجميعها من شواطئ مختلفة بالأسكندرية لدراسة تأثير المركبات الأروماتية عديدة الحلقات .

أدت الزيادة في تركيز المركبات الأروماتية عديدة الحلقات لإنخفاض ملحوظ ومعنوي في نشاط إنزيمي الماتاليز وسوبر أكسيد ديسميوتاز ب 32 % لكل منهما بينما تضاعف نشاط إنزيم الجلوتاثيون ريداكناز. وتزايد كذلك تركيز المالونألدهيد مع زيادة تركيز المركبات العضوية بينما انخفض نشاط إنزيم الكاتاليز مع زيادة تركيز تلك المواد.

كما تسببت الزيادة في تركيز المركبات الأروماتية عديدة الحلقات لإنخفاض الوميض الكلوروفيللي (24%) والمحتوى الكلوروفيللي (25%) مما أدى إلى إنخفاض في عملية البناء الضوئي (19%).

تشير الزيادة الملحوظة في نشاط إنزيم الجلوتاثيون ريدكناز إلى أن الطحلب قد يكون تأقلم وتكيف مع الإجهاد التأكسدي للمركبات العضوية الأروماتية عديدة الحلقات بالرغم من عدم الأكسدة الكلية لدهون الغشاء الخلوي.

بينت الدراسة أن المركبات العضوية عديدة الحلقات سببت تدمير تأكسدي وخاصة في العينات التي تم تجميعها من مناطق صناعية ذات التركيزات المرتفعة من تلك المركبات. وعلى حد علمنا- هذه هي الدراسة الأولى من نوعها لدراسة تأثير تلك المركبات العضوية على الخصائص الفسيولوجية والحيوية في الطحالب.