EFFECT OF ULTRAVIOLET-B IRRADIATION ON FATTY ACIDS, AMINO ACIDS, PROTEIN CONTENTS, ENZYME ACTIVITIES AND ULTRASTRUCTURE OF SOME ALGAE

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Abstract

A series of experiments were conducted to determine fatty acids, amino acids, protein contents and enzymes activities of the three algae (*Ulva lactuca, Sargassum hornschuchii* and *Pterocladia capillacea*), which were previously collected in the late of July 2009 from Abu Qir and subjected to UV-B radiation for 20, 40 & 60 minutes daily for five days. These parameters were estimated, when the UV-absorbing compounds contents recorded its maximum after the third day of irradiation of 60 minutes daily for the three algal species. This time was chosen as we expected a suitable response of the algal species to UV-B irradiation. Total saturated, mono unsaturated and polyunsaturated fatty acids of *U. lactuca* and *S. hornschuchii* were increased due to UV-B irradiation, the total fatty acids content in both irradiated algae increased also, while the contents of total saturated, mono and polyunsaturated fatty acids of *Pterocladia capillacea* decreased after exposure to UV-B radiation. The study shows that half of amino acids of *Ulva lactuca* increased after exposure to UV-B radiation for three days, while the other half of amino acids was decreased. *S. hornschuchii* showed the decreasing of all amino acids contents after exposure to UV-B radiation except the two basic amino acids histidine and lysine, and the aliphatic amino acid serine. All amino acids of *P. capillacea* increased after exposure to UV-B radiation for three days except the aliphatic amino acid serine and the aromatic amino acid tyrosine. The total protein content increased in *U. lactuca* and *S. hornschuchii* through out the irradiation experiment, while *P. capillacea* showed notable decreases of protein contents after UV-B irradiation. Exposure to UV-B radiation increased the activity of superoxide dismutase, ascorbate peroxidase and catalase of the three irradiated algal species. The findings also suggest that exposure to UV-B irradiance also affect the ultrastructure of all the irradiated algal species.

Keywords: Algae, ultraviolet, fatty acids, amino acids, proteins, enzyme, ultrastructure

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Introduction

The ozone layer is vital to life on Earth because it is the principal agent that absorbs ultraviolet radiation (UVR) in the Earth's atmosphere. Over the last 50 years, stratospheric ozone has decreased about 5% (**Pyle, 1997**). Depletion of ozone layer is due to anthropogenically released atmospheric pollutants such as chloroflurocarbons (CFC_s), chlorocarbons and organobromides (**Weatherhead & Andersen, 2006**).

Detection of the reduction of stratospheric ozone layer led to the interest in investigating the effects of UV-B radiation on algae since radiation has the ability of reaching algae at any position due to the fact that radiation are capable even of penetrating the water column to significant depth (**Tian & Yu, 2009**).

The damaging effect of UV on algae include growth (**Liu *et al.*, 2007**), morphology (**Wu *et al.*, 2005**), reproduction (**Cordi *et al.*, 2001**), motility (**Häder, 1993**), respiration (**Kashian *et al.*, 2004**), photobleaching of chlorophyll a, reduced photosynthesis (**Rautenberger & Bischof, 2008**), reduction of chlorophyll content (**Zverdanovic *et al.*,2009**), degradation of light harvesting proteins (**Jr, 2005**), inhibition of enzymes of nitrogen metabolism (**Sinha & Häder , 2000**) and other enzyme activities (**Shiu and Lee, 2005**).

However, there are few published papers pertaining to the effects of UVR on the fatty acids composition of microalgae (**Döhler & Biermann, 1994; Goes *et al.*, 1994; Wang & Chai, 1994; Sundbäck *et al.*, 1997; Odmark *et al.*, 1998**) and those that have been published are seemingly contradictory. The extant literature on the subject includes: two studies reporting an overall increase in SFA (saturated fatty acids) and MUFA (monounsaturated fatty acids) and decrease in PUFA (polyunsaturated fatty acids) upon UVR (**Wang & Chai, 1994; Goes *et al.*, 1994**), two papers reporting no significant differences in the fatty acid profile between UVBR treatments and the control (**Sundbäck *et al.*, 1997; Skerratt *et al.*, 1998**), and three reporting an increase in PUFA after UVR exposure (**Döhler & Biermann, 1994; De Lang & Van Donk, 1997; Skerratt *et al.*, 1998**).

Reactive oxygen species (ROS), such as hydroxyl ions, superoxide anions, and peroxyl radicals, are involved in oxidative damage to cell components, regulation of signal transduction and gene expression, and inactivation of receptors and nuclear transcription factors when overproduced (**Imlay & Linn, 1998**). Subsequently it leads to many clinical diseases due to oxidative stress provided by these kinds of free radicals.

The response of superoxide dismutase (SOD), the first line of defense against ROS in plants (**Alscher *et al.*, 2002**), to UV stress depends on the algal

species and the exposure time. SOD activity in the green macro alga *Ulva fasciata* increased by UV radiation (**Shiu and Lee, 2005**) and in the red macroalga *Corallina officinalis* (**Li *et al.*, 2010**). The increase of SOD and catalase (CAT) activities by UV radiation were greater in *Gelidium amansii* than *Pterocladia capillacea*. UV radiation also increased SOD activity in symbiotic dinoflagellate (**Lesser and Shick, 1989**) but long term UV exposure decreased SOD activity in *Chlorella vulgaris* (**Malanga *et al.*, 1997**). Macroalgae (*Monostroma arcticum*, *Acrosiphonia penicilliformis*, *Coccotylus truncates*, *Phycodrys rubens*, *Palmaria palmate* and *Devaleraea ramentacea*) show less SOD induction by UV and it is even depressed in some species (**Aguilera *et al.*, 2002**). The induction of (CAT) by UV radiation was also observed in algae (**Levy *et al.*, 2006**). Because of the low affinity of CAT for H_2O_2 but the high affinity of APX (Ascorbate peroxidase) for H_2O_2, the higher induction of CAT activity accompanied by depression of APX activity & higher H_2O_2 production in *G. Amnsii* seems to indicate that this species faces greater oxidative stress upon exposure to UV radiation than *Pterocladia capillacea* (**Lee and Shui, 2009**).

A number of algae that are simultaneously exposed to visible and UV radiation have evolved mechanisms as accumulation of detoxifying enzymes and antioxidants (Mittler and Tel-Or, 1991) and synthesis of UV-protectants (**Sinha *et al.*, 2001; Oren & Gunde-Cimerman, 2007**).

In radiation exposure experiments, the effects of mild artificial UV conditions on ultrastructure of two red algal species *Palmaria palmata* and *Odonthalia dentata* from the Arctic have been investigated. The transmission electron microscope (TEM) results demonstrated that the photosynthetic apparatus was severely influenced by UV in both species (**Holzinger *et al.*, 2004**). Also the green alga *Dunaliella salina* had many changes in ultrastructures during acclimation to enhanced UV-B radiation (**Tian and Yu, 2009**).

The present study aimed mainly to study the effect of harmful UV-B radiation on some algae in order to investigate the ability of the algal cells to create defense mechanism(s) against this radiation. The intention had been followed the influence of UV-B radiation on amino acids, fatty acids, protein contents and activity of some selected enzymes. The cellular ultrastructure alterations were also monitored by transmission electron microscopy to clarify the response of the investigated species to the exposure of the UV-B radiation.

Materials and Methods

Algal species: *Ulva lactuca*, *Sargassum hornschuchii* and *Pterocladia capillacea* were collected in late July 2009 from Abu Qir in Alexandria. After harvesting, whole algae were extensively washed several times with natural sea water to remove any attached sand and the rhizoidal portions were removed to avoid microbial contamination. Then the algal materials were conveyed to the laboratory in plastic bags filled with sea water.

Culturing conditions and UV-irradiation: The whole algae were rinsed and placed in shallow trays with aeration inlet. Water used for culturing was collected from the sampling site. The trays were placed in an environmental cabinet at 30±2°C for 24 hours before. In irradiation experiment, the samples were placed in Petri dishes (15 cm diameter) without covers and exposed directly to UV light. The source of light was UV VL-8.LM lamp supplied by VILBER LOURMAT-France. The UV-B light intensity of the lamp at distance 15 cm was 660 µW cm^{-2} and the supplied light has the wave length 312 nm. Samples were irradiated for 20, 40 & 60 minutes daily for 5 days at distance 15 cm.

Fatty acids determination: It was performed according to **Radwan (1978)**.

Amino acids determination: Amino acid determination was performed according to the method of **Winder and Eggum (1966)**. The system used for the analysis was high performance, amino acid analyzer, (SYKAM Amino acids analyzer, version 6.8).

Protein determination: Total proteins were determined according to **Hartree (1972).**

Enzyme activities estimation: Superoxide oxide dismutase was performed according to **Giannopolitis and Ries (1977)**, ascorbate peroxidase according to **Nakano and Asada (1981)**, while catalase activity was measured as described by **Beers and Sizer (1952).**

Ultrastructure: It was performed according to **Reynolds (1963)** and **Mercer & Birbeck (1966).** Then examined by Philips 400 T electron microscope at 60 – 80 KV.

Statistical analysis: The effect of UV-B radiation on fatty acids, amino acids contents were evaluated by means of a t-test on the parameters estimated before and after exposure to UV-B radiation. The effect of UV-B radiation on these parameters was considered to be statistically significant at a level of $P< 0.05$.

Results

1. Fatty acids contents

Table (1) indicated that all fatty acids of *Ulva lactuca* increased after exposure to UV-B radiation for three days (60 minutes daily) except the saturated fatty acids C12:0 and C15:0, the monounsaturated fatty acid C14:1 and the polyunsaturated fatty acid C18:2, which decreased by 0.162, 0.325, 0.421 and 0.172 µg g^{-1} fresh weight , respectively. Four fatty acids (C20:0, C17:1, C20:5 and C22:2) appeared in the irradiated alga, which were completely absent in the control.

The most affected fatty acids by UV-B irradiation was C14:0, which increased forty times compared to control, also C13:0, C15:1and C22:6, which were increased by nearly four folds their initial concentration of the control. The less affected fatty acids of *U. lactuca* by UV-B irradiation were C8:0, C10:0 and C17:0, which increased by 0.017, 0.022 and 0.077 µg g^{-1} fresh weight), respectively.

The data in Table (1) showed that the total saturated and polyunsaturated fatty acids of *U. lactuca* increased approximately three times after exposure to UV-B radiation, while monounsaturated fatty acids increased nearly two times their initial contents of the control. Total fatty acids content in irradiated *U. lactuca* increased approximately three folds comparing to control.

Results in Table (2) illustrated that all fatty acids of *Sargassum hornschuchii* increased after exposure to UV-B radiation for three days except the three saturated fatty acids (C11:0, C13:0 and C17:0), which decreased by 0.005, 0.476 and 0.002 µg g^{-1} fresh weight, respectively and the monounsaturated fatty acid C22:1 which decreased by 0.024 µg g^{-1} fresh weight. The most increased fatty acid of *S. hornschuchii*, due to UV- B irradiation was the saturated fatty acid C16:0, which increased by 1.941 µg g^{-1} fresh weight. The less increased fatty acids were the saturated fatty acid C23:0, which increased by 0.009 µg g^{-1} fresh weight and the polyunsaturated fatty acid C18:2, which increased by 0.004 µg g^{-1} fresh weight.

It must be mentioned that the two satturated fatty acids, C10:0 and C21:0 appeared in the irradiated alga, which were completely absent in the control. Meanwhile the mono unsaturated fatty acid C18:1 was completely disappeared after exposure to UV-B radiation.

Table (2) showed that the contents of total saturated, mono unsaturated and polyunsaturated fatty acids of *S. hornschuchii* were doubled due to exposure to UV-B irradiation and consequently, the total fatty acids content in irradiated alga

increased also two times compared to the control.

Table (1): Fatty acids content of *Ulva lactuca* before (control) and after (irradiated) exposure to UV-B radiation of 60 minutes daily for three days.

	Fatty acid	Fatty acid content (µg g^{-1} fresh weight)		
		Control	Irradiated	Increase/ decrease
Saturated fatty acids	C6:0	0.054	0.182	0.128
	C8:0	0.016	0.033	0.017
	C10:0	0.008	0.030	0.022
	C12:0	0.196	0.034	-0.162
	C13:0	0.811	2.896	2.085
	C14:0	0.046	1.763	1.717
	C15:0	0.406	0.081	-0.325
	C16:0	0.927	2.227	1.3
	C17:0	0.042	0.119	0.077
	C18:0	0.073	0.194	0.121
	C20:0	-	0.009	0.009
	Total	**2.579**	**7.568**	**4.989**
Mono unsaturated fatty acids	C14:1	0.474	0.053	-0.421
	C15:1	0.480	1.729	1.249
	C16:1	0.232	0.828	0.596
	C17:1	-	0.025	0.025
	C18:1	0.547	0.744	0.197
	C22:1	0.070	0.206	0.136
	Total	**1.803**	**3.585**	**1.782**
Poly unsaturated fatty acids	C18:2	0.262	0.090	-0.172
	C18:3	0.113	0.249	0.136
	C20:3	0.053	0.445	0.392
	C20:5	-	0.038	0.038
	C22:2	-	0.078	0.078
	C22:6	0.544	1.973	1.429
	Total	**0.972**	**2.873**	**1.901**
Total fatty acids		**5.354**	**14.026***	**8.672**

(*) Marked differences are significant at $p \leq 0.05$

Table (2): Fatty acids content of *Sargassum hornschuchii* before (control) and after (irradiated) exposure to UV-B radiation of 60 minutes daily for three days.

	Faty acid	Fatty acid content (µg g^{-1} fresh weight)		
		Control	Irradiated	Increase/ decrease
Saturated fatty acids	C6:0	0.014	0.095	0.081
	C8:0	0.016	0.071	0.055
	C10:0	-	0.015	0.015
	C11:0	0.022	0.017	-0.005
	C12:0	0.090	0.249	0.159
	C13:0	0.497	0.021	-0.476
	C14:0	0.137	0.357	0.22
	C15:0	0.031	0.611	0.58
	C16:0	1.351	3.292	1.941
	C17:0	0.011	0.009	-0.002
	C18:0	0.037	0.135	0.098
	C20:0	0.105	0.344	0.239
	C21:0	-	0.033	0.033
	C23:0	0.020	0.029	0.009
	Total	**2.331**	**5.279**	**2.948**
Mono unsaturated fatty acids	C14:1	0.332	0.704	0.372
	C15:1	0.382	0.719	0.337
	C16:1	0.176	0.308	0.132
	C17:1	0.008	0.035	0.027
	C18:1	0.052	0.144	0.092
	C20:1	0.014	0.044	0.03
	C22:1	0.071	0.047	-0.024
	Total	**0.995**	**2.213**	**1.318**
Poly unsaturated fatty acids	C18:2	0.009	0.013	0.004
	C18:3	0.008	0.064	0.056
	C20:2	0.065	0.145	0.08
	C20:3	0.031	0.154	0.123
	C20:5	0.010	0.022	0.012
	C22:6	0.654	1.124	0.47
	Total	**0.777**	**1.653**	**0.723**
Total fatty acids		**4.103**	**8.828***	**4.725**

INTRODUCTION

The ozone layer is vital to life on Earth because it is th ultraviolet radiation (UVR) in the Earth's atmosphere. Over t Ozone has decreased about 5% (Pyle, 1997). Depletion anthropogenically released atmospheric pollutants such as chlorocarbons and organobromides (Lubin and Jensen, 1995 a concentration of these compounds increases mainly i anthropogenic emissions, thus leading to ozone depletion. depletion is enhanced during long term exposure to very low for winter/spring 95/96 and 96/97. These conditions promot polar stratospheric clouds, containing high concentrations of c to the destruction of O_3-molecules (Müller *et al*., 1997 and Re

The expression "Ozone hole" is used whenever normal drops below 50 %. The concentration of stratospheric ozone units (DU). Ozone is predominantly generated in the low molecular oxygen. As ozone production rate is strongly depe molecular oxygen , as well as solar irradiance, ozone formation oc In the stratosphere, ozone molecules are subject to UVR-medi be degraded due to the reaction within catalytic cycles wi catalysts (Lary, 1997 and Langer 1999).

Increase of ultraviolet radiation on the earth's surface c been well documented in recent years not only for polar bu (Smith *et al*. 1992, Madronich, 1993, Frederick *et al*. 1993 and

Ever since the discovery of the Antarctic ozone hole 1985), serious concerns have arisen about the impacts of incr biosphere (Madronich *et al*., 1998 and Bjorn *et al*., 1999). R thinning of the stratospheric ozone layer is becoming more regions of the Northern hemisphere (Jokela *et al*., 1993; Müll 1997). In the Arctic, low light adapted organisms may re alteration in the solar spectrum (Kirst and Wiencke, 1995). I recorded in 30 m depth in Antarctica (Karentz, 1989 and transparent waters UVB can even detected down to 65 m depth

Detection of the reduction of stratospheric ozone layer led the effects of UV-B radiation on algae since radiation has the a position due to the fact that radiation are capable even of pe significant depth (Calkins and Thordardottir, 1982 and Whitehead

The data in Table (3) showed that all concentrations of fatty acids of *Pterocladia capillacea* decreased after exposure to UV-B radiation for three days except C13:0, C15:0, C14:1, C15:1, C16:1, C20:3 and C22:6 where they increased by 2.195, 0.114, 0.165, 0.3, 0.098, 0.002, 0.187 µg g^{-1} fresh weight, respectively.

The most increased fatty acid of *Pterocladia capillacea* due UV-B irradiation was C13:0, where it increased 57 times compared to control, while the less increased fatty acid was C20:3 where it increased by only 0.002 µg g^{-1} fresh weight. The most decreased fatty acids due to UV-B irradiation were C16:0 and C18:1, where they decreased nearly to its half contents compared to control. It must be mentioned that two fatty acids of *P. capillacea* (C20:0 and C20:2) were completely disappeared after exposure to UV-B radiation.

Table (3) showed that the contents of total saturated, mono and polyunsaturated fatty acids of *Pterocladia capillacea* decreased after exposure to UV-B radiation by 1.768, 0.598 and 1.25 µg g^{-1} fresh weight, respectively. UV-B radiation caused the saturated fatty acids content to be lowered by 20.8% than the control. Mono unsaturated and polyunsaturated fatty acids in irradiated samples were found to be less than control by 11.5% and 35.3%, respectively.

It is noteworthy to mention that the increase of fatty acid contents due to UV-B irradiation in both *Ulva lactuca* and *Sargassum hornschuchii* was statistically significant at $P \leq 0.05$, meanwhile the decrease in this content in *Pterocladia capillacea* was statistically unsignificant at $P \leq 0.05$.

2. Amino acids contents

Table (4) illustrated the contents of amino acids groups of *Ulva lactuca* before and after exposure to UV-B radiation for 60 minutes daily for three days. It was noticed that half of amino acids of *Ulva lactuca* increased after exposure to UV-B radiation for three days, while the other half of amino acids was decreased.The most increased amino acid was the aliphatic amino acid alanine, where it increased by 1.243 mg g^{-1} fresh weight and the less increased amino acid was valine, where it increased by 0.210 mg g^{-1} fresh weight. The most decreased amino acid was the basic amino acid arginine, where it decreased by 4.468 mg g^{-1} fresh weight and the less decreased amino acid was the aliphatic amino acid leucine, whichdecreased by 0.013 mg g^{-1} fresh weight. Table (4) showed that the total acidic, aliphatic, aromatic and secondary amino acids groups of *U. lactuca* increased by 0.478, 50.681, 0.563 and 0.276 mg g^{-1} fresh weight, respectively and the most increased amino acid group was the aliphatic amino acid, where it increased nearly three folds their initial concentration of the control, but basic and

sulphur-containing amino acids groups decreased by 6.355 and 0.064 mg g^{-1} fresh weight, respectively. Total amino acids content of *U. lactuca* decreased by 5.07 mg g^{-1} fresh weight after exposure to UV-B radiation for 60 minutes for three days.

Table (3): Fatty acids content of *Pterocladia capillacea* before (control) and after (irradiated) exposure to UV-B radiation of 60 minutes daily for three days.

	Fatty acid	Fatty acid content (µg g^{-1} fresh weight)		
		Control	Irradiated	/Increase decrease
Saturated fatty acids	C6:0	0.180	0.053	-0.127
	C8:0	0.017	0.014	-0.003
	C10:0	0.045	0.007	-0.038
	C11:0	0.032	0.022	-0.01
	C12:0	0.458	0.005	-0.453
	C13:0	0.039	2.234	2.195
	C14:0	0.813	0.356	-0.457
	C15:0	1.130	1.244	0.114
	C16:0	4.898	2.400	-2.490
	C17:0	0.159	0.079	-0.08
	C18:0	0.574	0.204	-0.37
	C20:0	0.144	-	-0.144
	Total	**8.512**	**6.744**	**-1.768**
Mono usaturated fatty acids	C14:1	1.192	1.357	0.165
	C15:1	1.192	1.492	0.3
	C16:1	0.629	0.727	0.098
	C17:1	0.043	0.019	-0.024
	C18:1	2.084	1.002	-1.082
	C22:1	0.079	0.024	-0.055
	Total	**5.219**	**4.621**	**-0.598**
Poly unsaturated fatty acids	C18:2	0.443	0.086	-0.357
	C18:3	0.133	0.022	-0.111
	C20:2	0.125	-	-0.125
	C20:3	0.092	0.094	0.002
	C20:4	1.027	0.373	-0.654
	C20:5	0.357	0.165	-0.192
	C22:6	1.363	1.550	0.187
	Total	**3.540**	**2.290**	**-1.25**
Total fatty acids		**17.271**	**13.655**	**-3.616**

Table (4): Amino acids groups content of *Ulva lactuca* before (control) after (irradiated) exposure to UV-B radiation of 60 minutes daily for three days.

Amino acid		Amino acid content (mg g^{-1} fresh weight)		
		Control	Irradiated	/Increase decrease
Acidic amino acids	Aspartic	13.935	13.887	-0.048
	Glutamic	14.681	15.207	0.526
	Total	**28.616**	**29.094**	**0.478**
Basic amino acids	Arginine	6.539	2.071	-4.468
	Histidine	3.130	1.842	-1.280
	Lysine	1.234	0.635	-0.597
	Total	**10.903**	**4.548**	**-6.355**
Aliphatic amino acids	Threonine	6.635	7.241	0.606
	Serine	16.206	12.941	-3.265
	Glycine	12.922	13.715	0.793
	Alanine	13.834	15.077	1.243
	Valine	9.146	9.356	0.21
	Leucine	10.074	10.061	-0.013
	Isoleucine	6.564	7.020	0.456
	Total	**24.730**	**75.411**	**50.681**
Aromatic amino acids	Tyrosine	0.407	0.070	-0.337
	Phenyl alanine	6.513	7.413	0.9
	Total	**6.920**	**7.483**	**0.563**
Sulphur - containg amino acids	Methionine	1.398	1.334	-0.064
Secondary amino acids	Proline	9.607	9.883	0.276
Total		**132.825**	**127.753**	**-5.072**

Amino acids contents of *Sargassum hornschuchii* were presented in Table (5). It was noticed that all amino acids contents decreased after exposure to UV-B radiation for three days except the two basic amino acids histidine and lysine, and the aliphatic amino acid serine, which increased by 0.257, 0.232 and 0.989 mg g^{-1} fresh weight, respectively. The most increased amino acid of *S. hornschuchii* due to UV-B irradiation was serine, where it increased by 0.989 mg g^{-1} fresh weight and the less increased was lysine where it increased by 0.232 mg g^{-1} fresh weight. The most decreased amino acids by UV-B irradiation was the acidic amino acid glutamic where decreased by 9.34 mg g^{-1} fresh weight and the less decreased one

was the aromatic amino acid tyrosine, where it decreased by 0.187 mg g^{-1} fresh weight.

Table (5): Amino acids groups content of *Sargassum hornschuchii* before (control) and after (irradiated) exposure to UV-B radiation of 60 minutes daily for three days.

Amino acid		Amino acid content (mg g^{-1} fresh weight)		
		Control	Irradiated	/Increase decrease
Acidic amino acids	Aspartic	9.764	7.899	-1.865
	Glutamic	16.178	6.838	-9.34
	Total	**25.942**	**14.737**	**-11.205**
Basic amino acids	Arginine	2.651	2.130	-0.521
	Histidine	0.576	0.833	0.257
	Lysine	1.039	1.271	0.232
	Total	**4.266**	**4.234**	**-0.032**
Aliphatic amino acids	Threonine	3.436	2.113	-1.323
	Serine	4.327	5.316	0.989
	Glycine	5.351	4.907	-0.444
	Alanine	5.025	3.540	-1.485
	Valine	3.860	3.535	-0.325
	Leucine	5.596	4.321	-1.275
	Isoleucine	4.071	3.507	-0.564
	Total	**31.666**	**27.239**	**-4.427**
Aromatic amino acids	Tyrosine	0.236	0.049	-0.187
	Phenyl alanine	3.124	2.607	-0.517
	Total	**3.360**	**2.656**	**-0.704**
Sulphur –containig amino acids	Methionine	1.024	0.457	-0.567
Secondry amino acids	Proline	5.476	4.115	-1.361
Total		**71.734**	**53.438**	**-18.296**

Table (5) represent the contents of amino acids groups of *S. hornschuchii* before and after exposure to UV-B radiation for 60 minutes daily for three days. These results showed that all total acidic, basic, aliphatic, aromatic, secondary and sulphur-containing amino acids decreased after exposure to UV-B radiation by 11.205, 0.032, 4.424, 0.704, 1.361 and 0.567 mg g^{-1} fresh weight, respectively. All amino acids of *Pterocladia capillacea* increased after exposure to UV-B

radiation for three days except the aliphatic amino acid serine and the aromatic amino acid tyrosine, where they decreased by 13.887 and 0.02 mg g^{-1} fresh weight, respectively (Table 6).

Table (6): Amino acids groups content of *Pterocladia capillacea* before (control) and after (irradiated) exposure to UV-B radiation of 60 minutes for three days.

Amino acid		Amino acid content (mg/g fresh weight)		
		Control	Irradiated	/Increase decrease
Acidic amino acids	Aspartic	15.399	22.867	7.468
	Glutamic	17.202	28.460	11.258
	Total	**32.601**	**51.327**	**18.726**
Basic amino acids	Arginine	8.298	10.198	1.900
	Histidine	1.268	2.262	0.976
	Lysine	1.699	2.040	0.341
	Total	**11.265**	**14.500**	**3.235**
Aliphatic amino acids	Threonine	8.717	11.071	2.354
	Serine	20.945	7.058	-13.889
	Glycine	15.670	18.471	2.801
	Alanine	13.761	15.368	1.607
	Valine	12.153	15.790	3.637
	Leucine	13.444	16.671	3.227
	Isoleucine	11.682	25.332	13.650
	Total	**96.372**	**109.761**	**13.389**
Aromatic amino acids	Tyrosine	0.050	0.030	-0.02
	Phenyl alanine	8.190	10.669	2.479
	Total	**8.240**	**10.699**	**2.459**
Sulphur -containing amino acids	Methionine	2.344	2.643	0.299
Secondary amino acids	Proline	11.735	15.220	3.485
Total		**162.557**	**204.150***	**41.593**

The highest increase in amino acids content of *P. capillacea* due to UV-B irradiation was the aliphatic amino acid isoleucine, where it increased by 13.65 mg g^{-1} fresh weight, while the less increased one was the sulphur-containing amino acid methionine where it increased only by 0.299 mg g^{-1} fresh weight. The most decreased amino acid due to UV-B irradiation was the aliphatic amino acid serine

(13.887 mg g^{-1} fresh weight) and the less decreased amino acid was tyrosine (0.02 mg g^{-1} fresh weight).

All the total acidic, basic, aliphatic, aromatic, secondary and sulphur-containing amino acids groups increased by UV-B irradiation by 18.726, 3.235, 13.389, 2.459, 3.485 and 0.299 mg g^{-1} fresh weight, respectively. The most increased content was noticed in the acidic amino acids group where it increased by 57.4% compared to control. Total amino acids content of *P. capillacea* increased by 41.593 mg g^{-1} fresh weight (25.6%) after exposure to UV-B radiation (Table 6).

It was found from the statistical analysis that there are no significant differences at $P \leq 0.05$ between the mean value of the total amino acids contents of the both *U. lactuca* and *S. hornschuchii* before and after treatments by UV-B radiation, while this mean values was statistically significant in the case of *P. capillacea* at $P \leq 0.05$ (Appendix 16).

3. Protein contents:

Protein contents were determined only in the samples of the three algae (*Ulva lactuca, Sargassum hornschuchii* and *Pterocladia capillacea*), which contained maximum UV-absorbing compounds. Table (7) indicated that the total protein content increased in *U. lactuca* through out the irradiation experiment. This increase was notably large after the first dose of irradiation, where its value was 11.20 mg g^{-1} fresh weight and then the increment was gradually quite little by increasing the irradiation dose, where its values were 11.83, 12.41, 12.43 & 12.64 mg g^{-1} fresh weight, respectively.

4. Enzymes activities:

Enzymes activities of the three algal species (*Ulva lactuca, Sargassum hornschuchii* and *Pterocladia capillacea*) were estimated after irradiation by UV-B for three days for 60 minutes. Table (8) showed that the activity of ascorbate peroxidase (APO) increased in all the three algae (*U. lactuca, S. hornschuchii* and *P. capillacea*) as a result of UV-B irradiation for three days by nearly 101.4, 25.2 and 43.5%, respectively. It was noticed that superoxide dismutase activity (SOD) in *U. lactuca* increased due to UV-B irradiation by approximately 51.2% compared to control. SOD activity in S. *hornschuchii* increased also by approximately 19.7%, while the highest increase (126.3%) of this activity was recorded by *P. capillacea* (Table 9). Catalase activity (CAT) was remarkably

increased after UV-B irradiation (Table 10) in all the three algal species for three days. These increases were approximately 32.3, 56.4 and 35.9% for *U. lactuca*, *S. hornschuchii* and *P. capillacea*, respectively.

Table (7): Total protein content of *Ulva lactuca, Sargassum hornschuchii* and *Pterocladia capillacea* before (control) and after (irradiated) exposure to UV-B radiation of 60 minutes daily for five days.

Species	Content of protein (mg g^{-1} fresh weight).					
	Control	1st day	2nd day	3rd day	4th day	5th day
Ulva	10.46	11.20	11.83	12.41	12.43	12.64
Sargassum	14.72	16.35	17.67	18.00	18.19	18.20
Pterocladia	24.72	22.72	21.46	21.04	20.72	20.71

Table (8): Ascorbate peroxidase activity of *Ulva lactuca, Sargassum hornschuchii* and *Pterocladia capillacea* before (control) and after (irradiated) exposure to UV-B radiation of 60 minutes daily for three days.

Species	Enzyme activity (µmol H_2O_2 $minutes^{-1}$ g^{-1} fresh weight)			
	Control	Treated	Increase/ decrease	%Increase/ decrease
Ulva lactuca	1.488	2.997	1.509	101.4
Sargassum hornschuchii	0.329	0.412	0.083	25.2
Pterocladia capillacea	0.411	0.590	0.179	43.5

Table (9): Superoxide dismutase activity of *Ulva lactuca, Sargassum hornschuchii* and *Pterocladia capillacea* before (control) and after (irradiated) exposure to UV-B radiation of 60 minutes daily for three days.

Species	Enzyme activity (unit gm^{-1} fresh weight)			
	Control	Irradiated	Increase/ decrease	%Increase/ decrease
Ulva lactuca	0.43	0.65	0.220	51.2
Sargassum hornschuchii	0.66	0.79	0.130	19.7
Pterocladia capillacea	0.38	0.86	0.480	126.3

Table (10): Catalase activity of *Ulva lactuca, Sargassum hornschuchii* and *Pterocladia capillacea* before (control) and after (irradiated) exposure to UV-B radiation of 60 minutes daily for three days.

Species	Enzyme activity (µmol H_2O_2 $minutes^{-1}$ g^{-1} fresh weight)			
	Control	Treated	Increase/ decrease	%Increase/ decrease
Ulva lactuca	26.894	35.576	8.682	32.3
Sargassum hornschuchii	11.647	18.211	6564	56.4
Pterocladia capillacea	13.552	18.423	4.871	35.9

5. Ultrastructure of the algal species:

The electron micrograph of *U. lactuca* before UV-B irradiation showed arrangement of the cell components and clear cell wall (CW) (Plate 1). When the cells were exposed to 60 minutes daily doses of UV-B radiation for five days, chloroplast showed dissipation and irregularity in shape and a small vacuole appeared (V). On the other hand, the cell wall and the pyrenoids appeared unaffected (Plate 2). The electron micrograph of untreated *S. hornschuchii* showed the clear arrangement of thylakoid membranes, clear nucleus with clear nuclear envelope and clear cell wall (Plate 3). The irradiated cells showed some disorganization of cell components, malformation of the cell, wrinkled cell wall and appearance of some vacuoles. The nucleus is not affected (Plate 4). Plate (5) showed untreated cell of *P. Capillacea* with typical chloroplasts that have clear arrangement of thylakoids, well organized nucleus and clear cell wall. Meanwhile, irradiated cell (Plate 6) showed partially damaged cell wall, disturbance of the cell inclusions including chloroplasts and appearance of vacuoles.

Discussion

Algae have an important role as food for fish and crustaceans and their nutritional value is mainly related to the content of essential fatty acids. Two studies reporting an overall increase in saturated fatty acid and monounsaturated fatty acids and decrease in polyunsaturated fatty acids of algae (**Wang & Chai, 1994; Goes *et al.*, 1994**), one study reporting an increase in saturated fatty acid, monounsaturated & polyunsaturated fatty acids of algae (**Noaman, 2007**), two papers reporting no significant differences in the fatty acid profiles between UV radiation treatments and the control (**Sundbäck *et al.* 1997; Skerratt *et al.* 1998**), and three reporting an increase in polyunsaturated fatty acids after UV radiation exposure (**Dohler & Biermann, 1994; De lang & van Donk, 1997; Skerratt *et al.* 1998**).

The composition of fatty acids of *Spirulina platensis* in response to UV-B radiation was found to have 23.5% saturated fatty acid (SFA), 76.4% monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA). In contrast to its UV-B untreated counterpart, SFA was 46.6% and MUFA and PUFA were 53.3%, which suggested that UV radiation reduces saturated fatty acid and increased unsaturated fatty acids in *S. platensis* (**Gupta *et al.*, 2008**). It is also observed that gamma linolenic acid was an important component of the total content of PUFAs of UV treated alga.

Liang *et al.* (2006) concluded that the effect of UV radiation on algal fatty acid compositions depends on algal species, the nitrogen concentration and time of UV radiation exposure. This was true for our results, since the total saturated, mono- and polyunsaturated fatty acid contents of *U. lactuca* and *S. hornschuchii* (Tables 1 & 2) increased significantly due to UV-B irradiation, while these of *P. capillacea* were unsignificantly decreased (Table 3). Several studies showed that UV radiation resulted in an increase of (PUFA) and reduction of SFA. For example, **Liang *et al.* (2006)** who showed that UV radiation resulted in an increase of PUFA and reduction of SFA both in the marine diatoms *Phaeodactylum tricornutum* and *Chaetoceros mulleri*, which agrees with the findings of **De Lang and Van Donk (1997)** for *Cryptomonas pyrenoidifera* and **Skerrat *et al.* (1998)** for *Phaeocystis antaractica*.

In the same topic, **Meireles *et al.* (2003)** recorded that UV irradiation increased the two fatty acids eicosapentaenoic and docosahexaenoic (n-3 fatty acids) by the alga *Pavlova lutheri*. Meanwhile **Döhler & Biermann (1994)** reported that UV increased long-chained FA (C-18 and C-20) of *Ditylum brightweelwii*, while there was a reduction of short-chained FA (C-14, C-16).

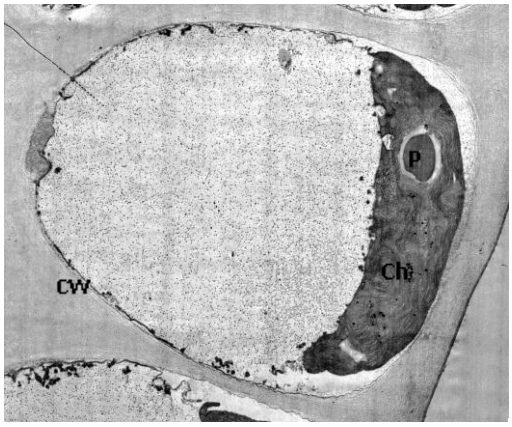

Plate (1): The electron micrograph of *Ulva lactuca* before UV-B irradiation showing chloroplasts (Ch), pyrenoid (P), nucleus (N) and cell wall (CW) (2.5 ×10^3).

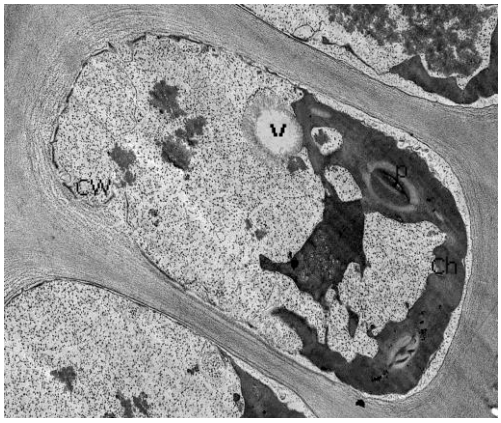

Plate (2): The electron micrograph of *Ulva lactuca* irradiated by UV-B of 60 minutes daily for five days showing dissipation of chloroplast (Ch) and appearance of a vacuole (V) and lamellated cell wall (CW) (2.5 ×10^3).

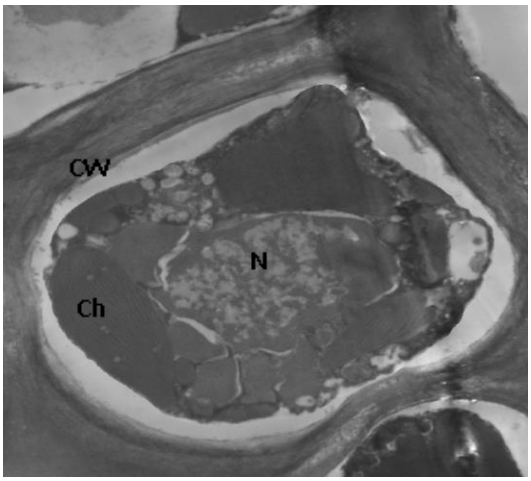

Plate (3): The electron micrograph of *Sargassum hornschuchii* before UV-B irradiation showing the typical chloroplast (Ch), cell wall (CW) with clear arrangement of thylakoids (7.5×10^3).

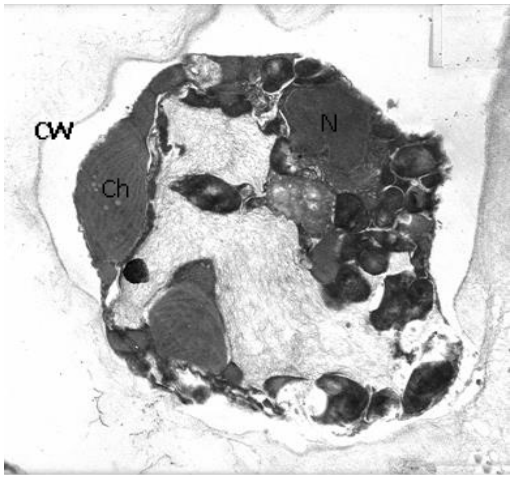

Plate (4): The electron micrograph of *Sargassum hornschuchii* irradiated by UV-B irradiation of 60 minutes daily for five days showing dramatic disorganization of cell components of the alga, irregularity of cell wall and appearance of vacuol (V) (7.5×10^3).

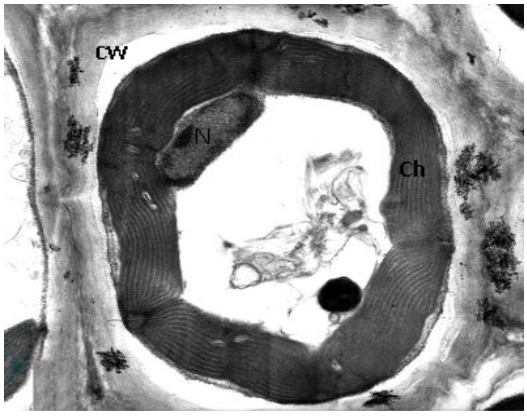

Plate (5): The electron micrograph of *Pterocladia capillacea* before UV-B irradiation showing the clear arrangement of thylakoids inside chloroplast (Ch), cell wall (CW) and nucleus (N).

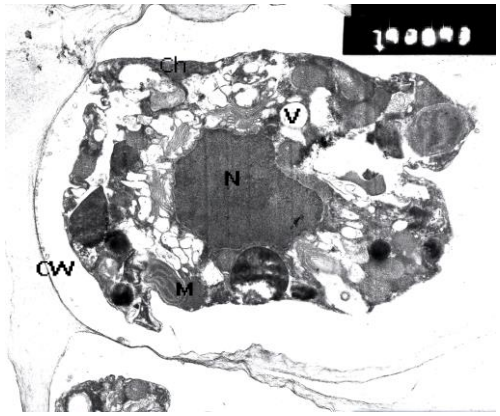

Plate (6): The electron micrograph of *Pterocladia capillacea* irradiated by UV-B irradiation of 60 minutes daily for five days showing disturbance of the cell inclusions, appearance of vacuoles (V), irregularity of cell wall (CW) and chloroplast structure (Ch) is less clear with disorganization of thylakoids.

UV increases fatty acids of *Chaetoceros simplex* (**Boutry *et al.*, 1976**) and *Pavalvo lutheri* (**Meireles *et al.*, 2003**). Also Kobayashi (1998) found an increase in fatty acids of 18 carbon atoms by UV irradiation but that of 20-22 carbon atoms was not affected by the exposure time.

The increase of fatty acid contents of both *U. lactuca* and *S. hornschuchii* may be interpreted as a physiological response to adapt the alga to UV-B irradiation stress. Since PUFAs are known in regulating membrane fluidity (**Hall *et al.*, 2002**) and physiological processes under stress (**Golecki and Drews, 1982**). At the same time membrane lipid unsaturation increases tolerance of Cyanophyta to UV radiation (**Ehling & Scherer, 1999**). SFAs and MUFAs provide the energy required for rebuilding of the photosynthetic apparatus and PUFAs are essential for chlorophyll membrane development (**Skerratt *et al.*, 1998**). Meanwhile, the decrease of fatty acids content of *P. capillacea* may be due to splitting of fatty acids as a result of UV-B irradiation (Kobayashi, 1998) and/or Lipid peroxidation (**He *et al.*, 2002**). The previous data are in complete agreement with the results of **Goes *et al.*, (1994)** who showed that the formation of PUFA in the green alga *Tetraselmis* sp. was suppressed by UV, the results of **Skerratt *et al.* (1998)** who reported that PUFA decreased in *Chaetoceros simplex* under UV radiation and

Noaman (2007) who found that the drop in C18:3 in *Synechococcus leopoliensis* by its exposure to UV for 5 minutes was followed by the increase of that fatty acid of 18 carbon atoms with increasing the exposure time, while polyunsaturated fatty acid of 22 carbon atoms decreased by increasing the exposure time.

In contrast to **Bhandari and Sharma (2006)** who found that fatty acid profile of *Phormidium corium* did not show any qualitative changes due to exposure to UV-B irradiation, *U. lactuca* showed appearance of four new fatty acids (SFA C20:0, MUFA C17:1 and PUFAs C20:5 & C22:2), while *S. hornschuchii* showed appearance of two fatty acids (SFAs C10:0 & C21:0) and disappearance of one fatty acid (MUFA C18:0). At the same time two fatty acids (SFA C20:0 & PUFA C20:2) disappeared from the fatty acid profile of *P. capillacea*. Similar disappearance was reported by **Noaman (2007)** for *Synechococcus leopoliensis*, while UV radiation causes induction of PUFA C20:20 in the marine diatom *Chaetoceros simplex* (**Boutry *et al*., 1976**). At the same time, UV effects on cell components (e.g. lipids, fatty acids, proteins, amino acids) and metabolic processes have been studied by several scientists (**Karentz *et al*., 1994**). Meanwhile, UV irradiation may change the contents of proteins and amino acids of marine algae (**Korbee *et al*., 2005**).

In the obtained literatures, we noticed no general or specific trend for the effect of UV-B radiation on the concentration of individual amino acids. Meanwhile, **Döhler (1984)** reported that the effect of UV-B radiation on concentration of amino acids was species-dependent. For example: Some amino acids in *Synechococcus leopoliensis* as lysine and arginine decreased by the exposure to UV irradiation, while aspartic increased (**Noaman, 2007**). The same author showed that cysteine, alanine and valine completely disappeared from mutants M_1, M_2 and M_3. Exposure of *Scendesmus quadricauda* to UV-A caused five (including proline) of 17 detected amino acids to increase, while only aspartic acid and histidine increased in UV-C treatment (**Kovacik *et al*., 2010**). UV-A and UV-B irradiance resulted in an increase of main amino acid biosynthesis and an enhancement of the main free amino acids (**Döhler *et al*., 1997**), results are discussed in relation to the UV-effects on photosynthetic pigments and the key enzymes of the carbon and nitrogen metabolism. This conclusion was noticed in our results, where some amino acids decreased and others increased due to UV-B irradiation in the three studied species (Tables 4-6).

The aromatic amino acid phenyl alanine, which can absorb UV-B radiation (**Martin *et al*., 1985**) was found to increased in *U. lactuca* and *P. capillacea*

(Tables 4 &6). Meanwhile, the decrease of this amino acid in *S. hornschuchii* (Table 5) may be due to the formation of phenyl alanine ammonia-lyase in response to UV radiation (**Campos *et al*., 1991**).

UV radiation accumulates proline that can protect plant cell against UV radiation induced peroxidative processes (**Sarkar *et al*., 2011**). This was true for *U. lactuca* and *P. capillacea* in which proline increased due to UV-B irradiation (Tables 4 & 6). Since proline is one of the important solutes, which accumulate in many organisms by exposure to environmental stresses, it is likely that proline accumulation is related to the protection of these organisms against singlet oxygen production during stress conditions (**Alia *et al*., 1995**). At the same time, proline accumulation may be critical for stimulating the pentose phosphate pathway in order to provide key precursors for the phenylpropanoid pathway (**Kwok & Shetty, 1997**). With the same respect, a three-fold increase in proline occurred in *Chlamydomonas nivalis* by exposure to UV (**Duval *et al*., 2000**) was accounted by stimulation of UV to the biochemical pathways related to proline metabolism.

The main amino acids in Antarctic microalgae changed in response to UV exposure, alanine, asparagine and glutamate increased after UV-B irradiation. The same results recorded in *U. lactuca* and *P. capillacea*, where alanine and glutamic acid increased after UV-B irradiation (Tables 4 & 6). The marked increase of alanine after exposure of *U. lactuca* and *P. capillacea* to UV-B might due to an enhancement of the alanine aminotransferase activity (**Döhler *et al*., 1997**).

Alanine decreased in *S. hornschuchii*, a result, which occurred at *Phaeocystis pouchetii* by its exposure to UV-B radiation, which was discussed by the damaging effect on the uptake of inorganic nitrogen and nitrogen metabolism (Döhler, 1992). Meanwhile, results found with UV sources regarding glutamine and glutamate indicate a different influence on the glutamine synthetase/ glutamate synthase (GS/GOGAT) system (**Döhler *et al*., 1997**).

Aspartic acid was reduced in all tested diatoms, a drastic reduction in glutamic acid could be observed in *L. annulata* samples (**Döhler, 1984**) which was discussed in relation to the impact of UV-B upon carbon and nitrogen metabolism. These data were in agreement with our results, where aspartic acid decreased in *U. lactuca* & *S. hornschuchii*. **Döhler (1997)** recorded that glutamine, serine and glycine decreased in antarctic microalgae by exposure to UV-B radiation. This was true for *S. hornschuchii*, where glutamic and glycine decreased due to UV-B irradiation (Table 5). The ^{15}N-incorporation into the amino acids was reduced as a result of UV-B exposure of phytoplankton and ice algae. Results are discussed with reference to an inhibitory effect on the enzymes

of both carbon and nitrogen metabolism as well as adaptation strategies (**Döhler, 1997**).

UV-B radiation is readily absorbed by nucleic acid and protein chromatophores, and their participation in plant responses to UV radiation has been documented (**Buma *et al*., 2003; Jobson & Qiu, 2011**). The involvement of these components in biological responses to UV radiation would indicate that protein synthesis and enzyme activities could be affected if biological systems were exposed to UV-B radiation (**Garrard and Brandle, 1975**). In addition, ultraviolet light can dimerize thymidine bases and cause lesions in DNA (**Drake, 1970**).

In the present study, the exposure of *U. lactuca* and *S. hornschuchii* to UV-B radiation caused the increase of protein content compared to the control as shown in Table 7. Exposure of *Scenedesmus quadricauda* to UV-A and UV-C caused increase in soluble proteins (**Kovacik *et al*., 2010**) and *Dunaliella viridis* was found by **Jiménez *et al*. (2004)** to have the ability to adapt to a variety of environmental stresses including nitrogen starvation, osmotic or thermal shocks and UV irradiation by formation of proteins (50, 45 and 43 KDa) and increase its contents. **Tominaga *et al.* (2010)** found the accumulation of heat shock protein 70 (HSP70) in the alga *Ulva pertusa* by exposure to high temperature & suggested that this protein play a particularly important roles in adaptation to the stress conditions. UV-B exposure of higher plants leaves induces the synthesis of special polypeptids like stress-proteins (**Santos *et al*., 2004**).

Kovacik *et al*. (2010) exposed axenic cultures of *Scenedesmus quadricauda* to UV-A (366 nm) and UV-C (254 nm) light over 1 h. Both wavelengths stimulated increase in soluble proteins. Primary photosynthetic carboxylating enzymes and soluble proteins in leaves of C3 and C4 crop plants were greatly affected by UV-B radiation (**Vu *et al*., 1982**). Evidences suggest that polyamine accumulation may serve as indicator of UV radiation stress (**Kramer *et al*., 1991**).

On the other hand, the protein content of *P. capillacea* decreased due to UV-B irradiation Table 7. This result was in agreement with those of some authors such as **Noaman (2007)** who noticed that the total proteins of *Synechococcus leopoliensis* exposed to UV decreased by increasing the exposure time. UV exposure for 24h caused the reduction of the protein content of *Dunaliella bardawil* (**Salguero *et al.* 2005**) and **Bischof *et al.* (2000)** noticed that UV radiation resulted in loss of protein of some marine macroalgae. The same results obtained also by **Bischof *et al*. (2000)** who recorded that exposure to UV resulted

in loss of total protein only in the deepwater species *Laminaria solidungula* and *Phycodrys rubens*. The different sensitivities to UV exposure of the species tested reflect their zonation pattern in the field.

Damage and degradation of protein by UV is proved in algae (**Xue *et al*., 2005**). **Kumar *et al*. (2003)** proved the inhibition of nitrogenase enzyme by UV which may be the cause for inhibition of protein synthesis in *P. capillacea* or the damage may be due to the ability of protein to absorb UV which was proved by **Ziska and Teramura (1992)**. **Chaturvedi and Shyam (2000)** proved that the degradation of protein of *Chlamydomonas reinhardtii* by its exposure to UV-B and **Prasad *et al*. (1998)** showed the inhibition of contents of protein by exposure *Chlorella vulgaris* to UV-B stress.

Formation of reactive oxygen species (ROS) in response to environmental stresses such as UV-B radiation is a common feature in plants (**Rao *et al*., 1996**). In general, oxidative stress results from the disruption of cellular homeostasis of ROS production from the excitation of O_2 to form singlet ($O_{1/2}$) and the transfer of 1, 2 or 3 electrons to O_2 to form superoxide (O_2^-), hydrogen peroxide (H_2O_2) and the hydroxyle radical (HO^-), respectively (**Halliwell and Gutteridge, 1989**). The generation of ROS leads to oxidative destruction of the cell components through oxidative damage of membrane lipids, nucleic acid and protein (**Imlay & Linn, 1998**). To counteract the toxicity of ROS, defense systems that scavenge cellular ROS have been developed in plants to cope with oxidative stress via the non-enzymatic and enzymatic systems (**Noctor and Foyer, 1998; Asada, 1999**). Antioxidants including water-soluble ascorbate (AsA) and water-insoluble ά-tocopherol and carotenoids have been considered to be the nonenzymatic agants for scavenging ROS (**Noctor & Foyer, 1998; Smirnoff & Wheeler, 2000; Munné-Bosch & Alegre, 2002**). In the enzymatic ROS-scavenging pathways, superoxide dismutase (SOD) converts O_2^- to H_2O_2 and then ascorbate peroxidase (APX) and glutathione reductase (GR) in the ascorbate-glutathione cycle (AGC) are responsible for H_2O_2 removal (**Asada, 1999**). Catalase (CAT) (**Willekens *et al*., 1997**) and peroxidase (POX) (**Asada and Takahashi, 1987**) are also involved in H_2O_2 removal.

In the three irradiated species, *U. lactuca*, *S. hornschuchii* & *P. capillacea*, the activity of superoxide dismutase (Table 9), a prominent biomarker of defense against oxidative stress (**Bowler *et al*., 1992**) increases with UV-B irradiation as a direct consequence. Fortunately antioxidant systems in plant and algae can scavenge ROS, including the antioxidant molecules such as carotenoids, ascorbate and reduced glutathione and antioxidant enzymes such as SOD, CAT, APX as

well as several other enzymes involved in the ascorbate-glutathione cycle, which is considered to be an efficient ROS detoxifying system in chloroplasts (**Jordan, 1996; Niyogi, 1999; Foyer *et al*., 1994**). This was true for our results of APX (Table 8) and CAT (Table 10) in the three irradiated species, where the two enzymes of the antioxidant systems obviously increased due to UV-B radiation. It was demonstrated by **Jansen *et al*., (2001)** that peroxidases are able to contribute to the protection of PSII in plants from UV radiation stress (as a result of their oxygen radical scavenging activity through removing H_2O_2).

The over-production of ROS and the induction of oxidative stress by UV-B radiation have been observed in microalgae as *Chlorella vulgaris* (**Malanga *et al*., 1997**), cyanobacteria (**He *et al*., 2002**) and diatom (**Rijstenbil, 2002**). An increase in the activities of ROS scavenging enzymes was observed in algae exposed to oxidative stress (**Rijstenbil, 2002**). It is known that antioxidant defense mechanism against ROS is pivotal for algal survival under stressful conditions, higher antioxidant contents and antioxidant enzyme activities are associated with higher stress tolerance in algae (**Collén & Davison, 1999 a,b**). UV-B also increased APX and GR activities in *Pterocladia capillacea* but decreased them in *Gelidium amansii* .UV-B also increased SOD and CAT activities but to a higher degree in *G. amansii*. So, *G. amansii* suffered greater oxidative stress from UV-B radiation. *P.capillacea* can effectively reduce UV-B sensitivity by increasing sunscreen ability and antioxidant defense capacity (**Lee and Shiu, 2009**).

Additionally, the imbalance between light phase and Calvin cycle probably due to the decreased activity of ribulose-1,5-biphosphate carboxylase/ oxygenase (Rubisco) by UV irradiation (**Bischof, 2000**) promoted the formation of superoxide radical at the level of ferredoxin at photosystem I (PS I). The direct effect of UV-B on respiration pathway might contribute to the increased ROS formation. It is well-known that the overproduction of ROS in living organisms including photoautotrophs under stress conditions is potentially toxic which may attack biomolecules such as lipid, protein, DNA and some small molecules and results in oxidative damage, even the death of the organisms (**Halliwell and Gutteridge, 1989**).

SOD activity was increased in the marine macroalga *U. fasciata* by UV radiation (followed by a decrease at higher UV doses), which also increased the activities of CAT, POX, APX and GR. The induction of antioxidant enzyme activities for detoxifying reactive oxygen species (**Shiu & Lee, 2005**), which serves as the defense system against oxidative stress occurring in *U. fasciata* upon

exposure to UV radiation. The excretion of H_2O_2 as well as the availability of antioxidants and the activation of SOD, CAT, guaicol POX and reactive oxygen scavenging enzymes in the ascorbate-glutathione cycle serve as the defence system against oxidative stress occurring in *U. fasciata* upon exposure to UV-B. UV-B disrupts the balance between the production and removal of H_2O_2 and subsequently accumulated H_2O_2 initiates the signaling responses leading to the induction of enzymatic antioxidant defense systems to overcome ROS production in *U .fasciata* (**Shiu and Lee, 2005**).

Despite the fact that a large number of publications, especially during the last 20 years, is devoted to UV research in different algal systems, these mostly neglected to study possible influences on cell ultrastructure or the use of different transmission/scanning electron microscopy (TEM/SEM) methods to address structural changes in cellular components. **Holzinger & Lütz (2006)** postulated that UV-B effects on ultrastructure of algal cells can be found in one article in the book edited by **Rozema *et al*. (1997)** and a single communication by **Lütz *et al*. (1997).** The latter group used freshwater green algae also in another study on ultrastructure changes and physiological adaptations under different stimulated UV regimes (**Meindle and Lütz, 1996**). More studies, presented as single communications, report on ultrastructure and UV-effects in marine diatoms (**Buma *et al*., 1996**), Haptophyta (**Buma *et al*., 2000**), marine red algae (**Poppe *et al*., 2002, 2003; Holzinger *et al*., 2004**) and marine green algae (**Holzinger *et al*., 2006**).

Exposure of the three species to extra doses of UV radiation caused the cells to show dissipation of the chloroplasts and irregularity in shapes (Plates 2, 4 & 6). *U. lactuca* showed disrupted chloroplast structure with severe damage in the thylakoid membranes when the alga irradiated for five days, 60 minutes daily. The same observation was also noticed, with different degrees, in chloroplasts of both *S. hornschuchii* and *P. capillacea*.

Holzinger *et al.* (2004) studied the effect of UV radiation on the ultrastructure of two red algae *Plamaria palmate* and *Odonthalia dentate.* Their TEM results demonstrated that the photosynthetic apparatus was severely influenced by UV, because thylakoid membranes appeared wrinkled, lumen dilatations occurred, and the outer membranes were altered. This dissipation of chloroplast was in full agreement with our results.

Poppe *et al.* (2002) reported destruction in chloroplasts, by exposure of the alga *Palmaria decipiens* to UV for 8 hours. UV irradiation of *Palmaria palmata* for 6 hours caused damage in the outer chloroplast envelope and lumen dilatation

(**Holzinger *et al.*, 2004**). Meanwhile irradiation for 24 hours caused severe damage with irregular lumen of the thylakoids. As the same time severe damage in the thylakoid membranes occurred after 24 hours exposure of the red alga *Odonthaila dentata* to UV irradiation (**Holzinger *et al.*, 2004**).

Under UV-B stress, the thylakoid membrane of *Spirulina platensis* becomes distorted (**Gupta *et al*., 2008**). It must be mentioned that the thylakoid membrane is the site for both photosynthesis and respiration (**Gantt, 1994**). Structural disturbance to membranes is likely to result in a reduced photosynthetic activity, e. g. due to dilation of the thylakoid membranes and rupture of the chloroplast double membrane (**Strid *et al*., 1994**).

The chloroplast envelope and the thylakoids membranes of the red macroalga, *Phycodrys austrogeogica* were damaged and the phycobilisomes were detached from the thylakoids after 12 hours UV irradiance (**Poppe *et al.*, 2003**). The same study showed that in the red alga *Palmaria decipiens*, UV irradiation for 4 hours lead to changes in ultrastructure of chloroplasts, with dilated thylakoids as compared to the control cell, while 6 and 8 hours irradiation caused disrupted thylakoids and the formation of inside-out translucent thylakoids vesicles, which also shown in irradiated *S. hornschuchii* as shown in plate 7B. Changes to the ultrastructure of chloroplast due to UV treatment and a vesiculation of the thylakoids was observed. Drastic changes in the arrangement of thylakoids membranes were found and a large number of small plasma vesicles accumulated at the plasma membrane as a consequence of UV irradiation (**Holzinger *et al.*, 2004**).

The effect of ultraviolet (UV) radiation on the ultrastructure of four red algae, the endemic Antarctic *Palmaria decipiens* and *Phycodrys austrogeorgica*, the Arctic-cold temperate *Palmaria palmate* and the cosmopolitan *Bangia atropurpurea* was studied. All four species showed a formation of 'insideout' vesicles from the chloroplast thylakoids upon exposure to artificial UV-radiation. In *P. decipiens*, most vesicles were developed after 8 h and in *P. palmate* after 48 h of UV exposure. In *B. atropurpurea*, vesiculation of thylakoids was observed after 72 h of UV irradiation. In *Ph. Austrogeorgica*, the chloroplast envelope and thylakoid membranes were damaged and the phycobilisomes became detached from the thylakoids after 12 h of UV exposure (**Poppe *et al.*, 2003**).

Buma *et al*. (1996) proved that the irradiated diatoms *Cyclotella sp, Nitzschia colsterium* and *Thalassiosira nordenskioldii* by UV radiation showed that vacuolization had taken place, the initial large vacuole was found to be

fragmented into small vacuoles and the nuclear envelope as well as membranes appeared to be unaffected by UV irradiation in the three diatoms. The same results were showed by **Holzinger *et al.* (2004)**, where no changes in nuclei or Golgi bodies in *Palmaria decipiens* treated by UV irradiation, and the nuclear membrane of *Palmria palmate* appeared normal, which demonstrates that membranes are not generally destroyed but selectively targested and altered by UV treatment.

The above results were in consistence with the ultrastructure of the investigated species, where, as shown in plates (2, 4 & 6) the nucleus of *U. lactuca* was damaged and showed wrinkled nuclear envelope due to UV-B irradiation. On the other hand, the nucleus in *S. hornschuchii* and *P. capillacea* is nearly unaffected. At the same time some vacuoles were appeared in the three investigated species due to UV-B irradiation, where *U. lactuca* contained a small vacuole, meanwhile *S. hornschuchii* and *P. capillacea* showed appearance of some vacuols. UV-B irradiation also affected the cell wall of the three species, since *U. lactuca* showed lamellated cell wall, while *S. hornschuchii* and *P. capillacea* showed irregularity of their walls.

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