Jomard Publishing

Advances in Biology & Earth Sciences., V.1, N.1, 2016, pp.126-152

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

Nadia H. Noaman[1], Faiza M.A. Akl[2], Magda A. Shafik[2], Mohamed S.M. Abdel-Kareem[1], Wafaa M. Menesi[2]

[1]Department of Botany, Faculty of Science, Alexandria University, Egypt
[2]Department of Biological and Geological Sciences, Faculty of Education, Alexandria University, Egypt
e-mail: myabdelkareem@yahoo.com, msabdelkareem@gmail.com

Abstract. A series of experiments were conducted to determine fatty acids, amino acids, protein contents and enzymes activities of three algae (*Ulva lactuca*, *Sargassum hornschuchii* and *Pterocladia capillacea*) subjected to UV-B radiation. These parameters were estimated, when the UV-absorbing compounds contents recorded its maximum after the third day of irradiation of 60 minutes daily. Total saturated, mono unsaturated and polyunsaturated fatty acids of *U. lactuca* and *S. hornschuchii* were increased due to UV-B irradiation, while that of *Pterocladia capillacea* decreased. The study shows that amino acids of the three studied algal species varied greately due to UV-B rirradiation. The total protein content showed also different responses 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 suggests that exposure to UV-B irradiance also affect the ultrastructure of all the irradiated algal specis.

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

1. 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), changing of protein profile (Abdel-Kareem, 1999), 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 (e.g. Wang & Chai, 1994 and Odmark *et al.,* 1998) and those that have been published are seemingly contradictory. The extant literature on the subject includes: some studies reporting an overall increase in SFA (saturated fatty acids) and MUFA (monounsaturated fatty acids) and decrease in PUFA (polyunsaturated fatty acids) upon UVR (e.g. Wang & Chai, 1994), two papers reported no significant differences in the fatty acid profile between UVBR treatments and the control (Skerratt *et al.,* 1998), and other reported an increase in UFA after UVR exposure (Gupta *et al.,* 2008).

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 defence 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 *Gelidiumamansii* 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 (*Monostromaarcticum, Acrosiphoniapenicilliformis, Coccotylus truncates, Phycodrysrubens, Palmaria palmate* and *Devaleraearamentacea*) 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 speciesfaces 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 (Oren and Gunde-Cimerman, 2007).

In radiation exposure experiments, the effects of mild artificial UV conditions on ultrastructure of two red algal species *Palmariapalmata* and *Odonthaliadentata* 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 *Dunaliellasalina* had many changes in ultrastructures during acclimation to enhanced UV-B radiation (Tian and Yu, 2009).

The present study aimed to study the defense mechanism(s) created by some algal cells against the effect of harmful UV-B radiation. For this purpose the influence of UV-B radiation on amino acids, fatty acids, protein contents enzyme activities and the ultrastructure of the selected algae has been studied.

2. 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. 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: Five days irradiated samples were used for total proteins determination 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 paired samples t-test on the parameters estimated before and after exposure to UV-B radiation using SPSS version 10.0. Differences were considered significant at $P \leq 0.05$. The effect of UV-B radiation on these parameters was considered to be statistically significant at a level of $P < 0.05$.

3. 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.

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 $p \leq 0.05$

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.

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	0.759	0.337
	C16:1	0.176	0.308	0.132
	C17:1	0.008	0.035	0.027
	C18:1	0.052	-	0.052
	C20:1	0.014	0.044	0.03
	C22:1	0.071	0.047	-0.024
	Total	**0.995**	**2.213**	**1.218**
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	1.254	0.6
	Total	**0.777**	**1.652**	**0.223**
Total fatty acids		**4.103**	**8.828***	**4.725**

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

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 saturated 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. Consequently, the total fatty acids content in irradiated alga increased also two times compared to the control.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.

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**

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 dropped 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, which decreased 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. 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.

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 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**

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).

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).

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

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		**162.56**	**204.150***	**41.593**

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 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.

The protein contents of irradiated *S. hornschuchii* increased gradually through out the irradiation experiment. This increase was large after the first and second doses of irradiation, where their values were 16.35 & 17.67 mg g^{-1} fresh weight, respectively. The increments were gradually quite little by increasing the irradiation dose, where their values were 18.00, 18.19 & 18.20 mg g^{-1} fresh weight, respectively. *P. capillacea* showed notable decreases of protein contents after the first and second doses, where their values were 22.72 & 21.46 mg g^{-1} fresh weight, respectively and also a gradual quite little decrease by increasing the following three irradiation doses, where their values were 21.04, 20.72 & 20.71 mg g^{-1} fresh weight.

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 lactuca	10.46	11.20	11.83	12.41	12.43	12.64
Sargassum hornschuchii	14.72	16.35	17.67	18.00	18.19	18.20
Pterocladia capillacea	24.72	22.72	21.46	21.04	20.72	20.71

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.

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

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

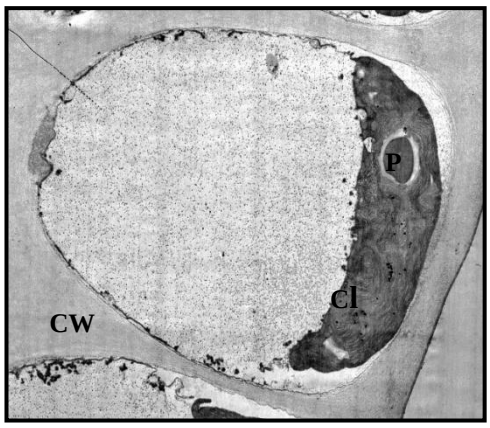

Plate (1): The electron micrograph of *Ulva lactuca* before UV-B irradiation showing chloroplasts (Ch), pyrenoid (P) and cell wall (CW) (2.5×10^3).

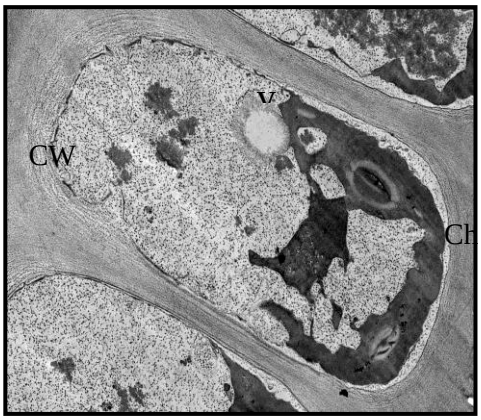

Plate (2): The electron micrograph of *Ulva lactuca* irradiated by UV-B of 60 minutes daily for five days showing dissipation of chloroplast (Ch) and appearance of a vacuole (V) and lamellated cell wall (CW) (2.5×10^3)

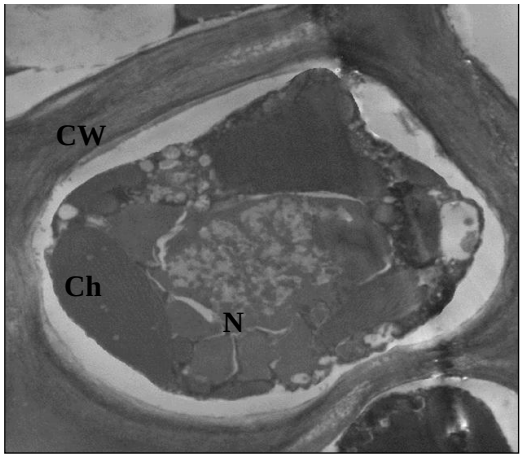

Plate (3): The electron micrograph of *Sargassum hornschuchii* before UV-B irradiation showing the typical chloroplast (Ch), cell wall (CW) with clear arrangement of thylakoids (7.5×10^3).

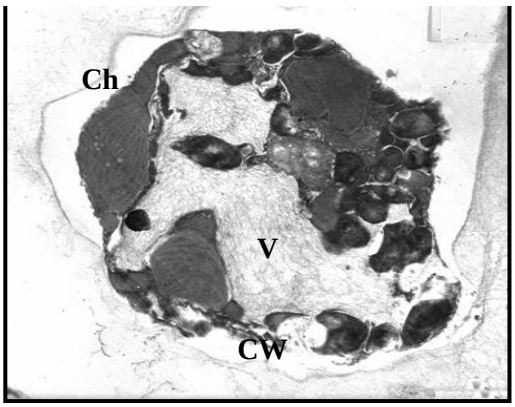

Plate (4): The electron micrograph of *Sargassum hornschuchii* irradiated by UV-B irradiation of 60 minutes daily for five days showing dramatic disorganization of cell components of the alga, irregularity of cell wall and appearance of vacuole (V) (7.5×10^3).

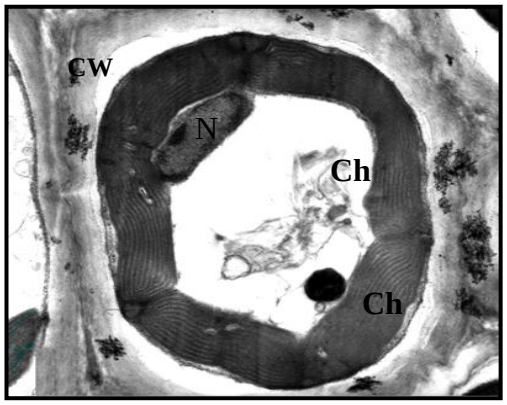

Plate (5): The electron micrograph of *Pterocladia capillacea* before UV-B irradiation showing the clear arrangement of thylakoids inside chloroplast (Ch), cell wall (CW) and nucleus (N).

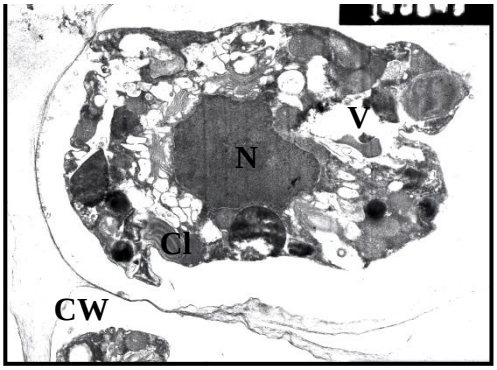

Plate (6): The electron micrograph of *Pterocladia capillacea* irradiated by UV-B irradiation of 60 minutes daily for five days showing disturbance of the cell inclusions, appearance of vacuoles (V), irregularity of cell wall (CW) and chloroplast structure (Ch) is less clear with disorganization of thylakoids.

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

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 obvious arrangement of thylakoid membranes, obvious nucleus with visional nuclear envelope and cell wall (Plate 3). The irradiated cells showed some disorganization of cell components, malformation of the cell, wrinkled cell wall and appearance of some vacuols. The nucleus is not affected (Plate 4). Plate (5) showed untreated cell of *P. Capillacea* with typical chloroplasts that have obvious arrangement of thylakoids, well organized nucleus and visional cell wall. Meanwhile, irradiated cell (Plate 6) showed partially damaged cell wall, disturbance of the cell inclusions including chloroplasts and appearance of vacuoles.

6. 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. Some studies reporting an overall increase in saturated fatty acid and monounsaturated fatty acids and decrease in polyunsaturated fatty acids of algae (e.g. Wang & Chai, 1994), while others reported an increase in saturated fatty acid, monounsaturated & polyunsaturated fatty acids of algae (e. g. Noaman, 2007). Meanwhile, few papers reported no significant differences in the fatty acid profiles between UV radiation treatments and the control (e.g. 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 slight 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 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 there was a reduction of short-chained FA (C-14, C-16). UV increases fatty acids of *Chaetoceros simplex* (Boutry *et al.,* 1976) and *Pavalvo lutheri* (Meireles *et al.,* 2003). 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). They 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.

Noaman (2007) 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. *Ulva. 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 (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 increase 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 and 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 authers 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 and 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 and 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 and 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 and 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 discussion was 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.

References

1. Abdel-Kareem M.S.M., Influence of Ultraviolet Radiation on Growth, Photosythetic Pigments and Protein Profile of *Dunaliella salina* Teod. (Chlorophyceae), Egypt. J. Bot., Vol.39, No.1, pp.1999, pp.77-95.
2. Aguilera J., Dummemuth A., Karsten U., Chriek R., Wiencke C., Enzymatic defenses against photooxidative stress induced by ultraviolet radiation in Arctic marine marcrolage, Polar Boil., 25, 2002, pp.432-441.
3. Alia P., Mohanty P., Matysik J., Effect of proline on the production of singlet oxygen, Biomed. Life Sci., 21, 1995, pp.195-200.
4. Alscher R.G., Erturk N., Health L.S., Role of superoxide dismutases (SODs) in controlling oxidative stress in plants, J. Exp. Bot., 53, 2002, pp.1331-1341.
5. Asada K., The water-water cycle in chloroplasts: scavenging of active oxygen and dissipation of excess photons, Annu. Rev. Plant Physiol., 50, 1999, pp.601-639.
6. Asada K., Takahashi M., Production and scavenging of active oxygen in photosynthesis, In: Kyle DJ, Osmond CB, Arntzen CJ (Eds) Photoinhibition,

Topics in Photosynthesis 9, Elsevier Sci. Publish., Amsterdam, 1987, pp.89-109.
7. Beers R.F., Sizer I.W., A spectrphotometric method for measuring the breakdown of hydrogen peroxide by catalase, J. Biol. Chem., 195, 1952, pp.133-140.
8. Bhandari, R.,Sharma, P.K., Effect of UV-B on photosynthesis, membrane lipids and MAAS in marine Cyanobacterium *Phormidium corium* (Agardh) Gomont. Indian. J. Exp. Biol., 44, 2006, pp.330-335.
9. Bischof K., Effects of enhanced UV-radiation on photosynthesis of Arctic/cold-temperate macroalgae, Ber. Polarforsch, 357, 2000.
10. Bischof K., Hanelt D., Wiencke C., Effects of ultraviolet radiation on photosynthesis and related enzyme reactions of marine macroalgae, Palnta, 211, 2000, pp.555-562.
11. Boutry J.L., Barbier M., Ricard M., The marine diatom *Chaetoceros simplex* calcitrans Paulsen and its environment, Effects of light and ultraviolet irradiation on the biosynthesis of fatty acids, CR. Acad. Sci. D., 282, 1976, pp.239-242.
12. Bowler, C., Van Montagu, M., Inzé, D., Superoxide dismutase and stress tolerance. Annu. Rev. Plant Physiol., 43, 1992, pp.83-116.
13. Buma A.G.J., Boelen P., Jeffrey W.A., UVR-induced DNA damage in aquatic organisms, In: Helbling, E. W., Zagarese, H. (Eds.). Comprehensive series in photochemistry and photobiology 1, UVeffects in aquatic Organisms and ecosystems, Roy. Soc. Chem., Cambridge, UK, 2003, pp.291-327.
14. Buma A.G.J., van Oijen T., van De Poll W., Veldhuis M.J.W., Gieskes W.W.C., The sensitivity of *Emiliania Huxleyi* (Prymnesiophyceae) to ultraviolet-B- radiation, J. Phycol., 36, 2000, pp.296-303.
15. Buma A.G.J., Zemmelink H.J., Sjollema K., Gieskes W.W.C., UVB radiation modifies protein and photosynthetic pigment content, volume and ultrastructure of marine diatoms, Mar. Ecol.-Prog. Ser., 142, 1996, pp.47–54.
16. Campos J.L., Figueras, X., Pinol, M.T., Boronat, A., Tiburcio A.F., Carotenoid and conjugated polyamine levels as indicators of ultraviolet-C induced stress in *Arabidopsis thaliana,* Photochem. Photobiol., 53, 1991, pp.689-692.
17. Chaturvedi R., Shyam R., Degradation and de novo synthesis of D1 protein and psbA transcript levels in *Chlamydomonas reinhardtii* during UV-B inactivation of photosynthesis and its reactivation, J. Biosci., Vol.25, No.1, 2000, pp.65-71.
18. Collén J., Davison I.R., Reactive oxygen production and damage in intertidal *Fucus* spp. (Phaeophyceae), J. Phycol., 32, 1999a, pp.54-61.
19. Collén J., Davison I.R., Reactive oxygen metabolism in intertidal Fucus spp. (Phaeophyceae), J. Phycol., 35, 1999b, pp.62-69.
20. Cordi B., Donkin M.E., Peloquin J., Price D.N., Depledge M.H., The influence of UV-B radiation on the reproductive cells of the intertidal macroalga, *Enteromorpha intestinalis,* Aqua. Toxicol., 56, 2001, pp.1-11.

21. Döhler G., Effects of UV-B radiation on the marine diatoms *Lauderia annulata* and *Thalassiorsira rotula* grown in different salinities, Mar. Biol., 83, 1984, pp.247-253.
22. Döhler G., Impact of UV-B radiation on uptake of ^{15}N-ammonia and ^{15}N-nitrate by phytoplankton of the Wadden Sea. Mar. Biol., 112, 1992, pp.485-489.
23. Döhler G., Effect of UV-B radiation on utilization of inorganic nitrogen by Antarctic microalgae, Photochem, Photobiol., 66, 1997, pp.831-836.
24. Döhler G., Drebes G., Lohmann M., Effect of UV-A and UV-B radiation on pigments, free amino acids and adenylate content of Dunaliella tertiolecta Butcher (Chlorophyta).J. Photochem. Photobiol., B: Biol., 40, 1997, pp.126-131.
25. Drake J.W., The molecular basic of mutation,. Holden-Day, San Francisco, 1970, pp.161-176.
26. Duval B., Shetty K., Thomas W.H., Phenolic compounds and antioxidant properties in the snow alga *Chlamydomonas nivalis* after exposure to UV light. J. Appl. Phycol., 11, 2000, pp.559-566.
27. Ehling S.M., Scherer S., UV protection in cyanobacteria, Advance in photosynthesis and respiration, Vol.1, Dordrecht: Springer, 1999, pp.119-138.
28. Foyer C.H., Lelandais M., Kunert K.J., Photooxidative stress in plants. Physiol. Plant., 92, 1994, pp.696-717.
29. Gantt E., Supramolecular membrane organization, In The Molecular Biology of Cyanobacteria, Dordrecht, Kluwer Academic, 1994, pp.119-138.
30. Garrard L.A., Brandle J.R., Effects of UV radiation on component processes of photosynthesis. In: Climatic Impact Assessment Program (CIAP), Monograph 5: D. S. Nachtwey, M. M. Caldwell & R. H. Biggs, Ed, pp. 4-20-4-32, US. Department of Transportation, Washington, D.C. 20590, 1975.
31. Giannopolitis C.N., Ries S.K., Superoxide dismutases, I. Occurrence in higher plants, Plant Physiol., 59, 1977, pp.309-314.
32. Golecki J.R., Drews G., Supramolecular organization and composition of membranes. In: Carrand, N. G. and Whitton, B.A., (Eds) The biology of Cyanobacteria, Oxford: Blackwell Scientific Publications Ltd., 1982, pp.125-141.
33. Gupta R., Bhadauriya P., Singh V.C., Singh P.B., Impact of UV-B radiation on thylakoid membrane and fatty acid profile of *Spirulina platensis*, Curr. Microbiol., 56, 2008, pp.156-161.
34. Häder D.P., Risk for enhanced solar ultraviolet radiation for aquatic ecosystem, Prog. Phycol. Res., 9, 1993, pp.1-45.
35. Hall J.M., Parrish C.C., Thompson R.J., Eicosapentaenoic acid regulates scallop (*Placopecten magellanicus*) membrane fluidity in response to cold, Biol. Bull., 202, 2002, pp.201-203.
36. Halliwell B., Gutteridge J.M.C., Free radicals in biology and medicine, Oxford: Calendon Press, 1989.
37. Hartree E.F., A modification of Lowry method that gives a linear photometric response, Analyt. Biochem., 48, 1972, pp.422-425.

38. He Y.Y., Klisch M., Hadder D.P., Adaptation of cyanobacteria to UV-B stress correlated with oxidative stress and oxidative damage, J. Photochem. Photobiol., 76, 2002, pp.188-196.
39. Holzinger A., Lütz C., Algae and UV irradiation: Effects on ultrastructure and related metabolic functions, Micron, 37, 2006, pp.190-207.
40. Holzinger A., Karesten U., Lütz C., Wiencke C., Ultrastructure and photosynthesis in the supralittoral green macroalga *Prasiola crispa* (Lightfoot) Kützing from Spitsbergen (Norway) under UV exposure, Phycologia, Vol.45, No.2, 2006, pp.190-207.
41. Holzinger A., Lütz C., Karesten U., Wiencke C., The effect of ultraviolet radiation on ultrastructure and photosynthesis in the red macroalgae *Palmaria palmate* and *Odonthalia dentate* from Arctic waters, Plantbiol., Vol.6, No.5, 2004, pp.568-577.
42. Imlay J.A., Linn S., DNA damage and oxygen radical toxicity, Science, 240, 1998, pp.1302-1309.
43. Jansen M.A.K., Noort R.E., Lagrimini M.Y.A., Phenol-oxidizing peroxidases contribute protection of plants from ultraviolet radiation, Plant Physiol., 26, 2001, pp.1012-1023.
44. Jiménez C., Berl T., Rivard C.J., Edelstein C.L., Capasso J.M., Phosphorylation of MAP kinase-like proteins mediate the response of the halotolerant alga *Dunaliella viridis* to hypertonic shock. Biochim, Biophys. Acta., Vol.1644, No.1, 2004, pp.61-9.
45. Jobson R.W., Qiu Y.L., Amino acid compositional shifts during streptophyte transitions to terrestrial habitats, J. Mol. Evol., 72, 2011, pp.204-214.
46. Jordan B.R., The effects of ultraviolet-B radiation on plants: a molecular perspective, Adv. Bot. Res., 22, 1996, pp.97-103.
47. Jr O.N., Light related photosynthetic characteristics of freshwater Rhodophytes, Aqua. Botany, 82, 2005, pp.193-209.
48. Karentz D., Bothwell M.L., Coffin R.B. Hanson A, Herndl G.J., Kilham S.S., Lesser M.P., Lindell M., Moeller R.E., Morris D.P., Neale P.J., Sanders R.W., Weiler C.S., Wetzel R.G., Impact of UV-B radiation on pelagic fresh water ecosystem: Report of working group on bacteria and phytoplankton, Arch. Hydrobiol. Beih., 43, 1994, pp.31-69.
49. Kashian D.R., Prusha B.A., Clemenrs W.H., Influence of total organic carbon and UV-B radiation on zinc toxicity and bioaccumulation in aquatic communities, Environ. Sci. Technol., 38, 2004, pp.6371-6376.
50. Kobayashi Y., Change of hydroperoxy fatty acids formed by ultraviolet radiation of bovine retinas-determination of chemiluminescence assay, Nippon Ganka Gakkai Zassi, 102, 1998, pp.15-21.
51. Korbee N., Figueroa F.I., Aguilera J., Effect of light quality on the accumulation of photosynthetic pigments, proteins and mycosporine-like amino acids in the red *Porphyra leucosticte* (Bangiales, Rhodophyta), J. Photochem. Photobiol., B, 80, 2005, pp.71-78.
52. Kovacik J., Klejdus B., Backor M., Physiological responses of *Scenedesmus quadricauda* (Chlorophyceae) to UV-A and UV-C light, Photochem, Photobiol., 86, 2010, pp.612-616.

53. Kramer G.F., Norman H.A., Krizek D.T., Mirecki, R.M., Influence of UV-B radiation on polyamines, lipid peroxidation and membrane lipids of cucumber, Phytochemistry, 30, 1991, pp.2101-2108.
54. Kumar,A., Tyagi M.B., Sinha N., Tyagi R., Jha P.N., Sinha R.P., Häder D.P., Role of white light in reversing UV-B mediated effects in the N_2-fixing cyanobacterium *Anabaena* BT2, J. Photochem. Photobiol., 71, 2003, pp.35-42.
55. Kwok D., Shetty K., Effects of proline and proline analogs on total phenolic and rosmarinic acid levels in shoot clones of thyme (*Thymus vulgaris* L), J. Food Biochem., 22, 1997, pp.37-51.
56. Lee T.M., Shiu C.T., Implication of mycosporine-like aminutesa acid and antioxidant defenses in UV-B radiation tolerance for the algae species *Pterocladia capillacea* and *Gelidium amansii*, Mar. Environ. Res., 67, 2009, pp.8-16.
57. Lesser M.P., Shick J.M., Effects of irradiance and ultraviolet radiation on photoadaptation in the zooxanthellae of *Alptasia pallida*: primary production, photoinhibition and enzymic defenses against oxygen toxicity, Mar. Biol., 102, 1989, pp.243-255.
58. Levy O., Achituv Y., Yacobi Y.Z., Dubinsky Z., Stambler N., Diel 'tunning' of coral metabolism: physiological responses to light cues, J. Exp. Biol., 209, 2006, pp.273-283.
59. Li L., Zhao J., Tang X., Ultraviolet radiation induced oxidative stress and response of antioxidant system in an intertidal macroalga Corallina officinalis L. J. Environ. Sci., 22, 2010, pp.716-722.
60. Liang Y., Beardall J., Heraud P., Effects of nitrogen source and UV radiation on the growth, chlorophyll fluorescence and fatty acid composition of *phaeodactylum tricornutum* and *chaetoceros muelleri* (Bacillariophyceae), J. Photochem. Photobiol, B: Biol., 82, 2006, pp.161-172.
61. Liu X. J., Duan S.S., Li A.F., Overcompensation effect of Pavlova viridis under ultraviolet (UV) stress, Ying Yong Sheng Tai Xue Bao, Vol.18, No.1, 2007, pp.169-173.
62. Lütz C., Seiditz H.K., Meindl U., Physiological and structural changes in the chloroplast of the green alga *Micrasterias denticulata* induced by UV-B simulation, Plant Ecol., 128, 1997, pp.54-64.
63. Malanga G., Calmanovici G., Puntarulo S., Oxidative damage to chloroplasts from *Chlorella vulgaris* exposed to ultraviolet-B radiation, Physiol. Plant., 101, 1997, pp.455-462.
64. Martin D.W., Mayes P.A., Rodwell V.W., Amino acids and peptides in: Harpers review of Biochemistry, 20th Edn., Lange Medical Publications, Los Altos California, 1985, pp.21-31.
65. Meindl U., Lütz C., Effects of UV irradiation on cell development and ultrastructure of the green alga *Micrasterias*, J. Photochem. Photobiol., 36, 1996, pp.285–292.
66. Meireles L.A., Guedes, A.C., Malcata F.X., Increased of yields of eicosapentaenoic and docosahexaenoic acids by the microalga *Pavalvo*

lutheri following random mutagenesis, Biotechnol. Bioeng., 81, 2003, pp.50-55.
67. Mercer E.H., Birbeck M.S.C., Electron Microscopy: A Handbook for Biologists. Oxford, Blackwell .2nd edition, 1966, pp.85.
68. Mittler R., Tel-Or E., Oxidative stress responses in the unicellular cyanobacterium *Synechococcus* PCC7942. Free Rad. Res. Commun., 12, 1991, pp.845-850.
69. Munné-Bosch S., Alegre L., The function of tocopherols and tocotrienols in plants, Crit. Rev. Plant Sci., 21, 2002, pp.31-57.
70. Nakano Y., Asada K., Hydrogen peroxide is scavenged by ascorbate-specific peroxidases in spinach chloroplasts, Plant Cell Physiol., 22, 1981, pp.867-880.
71. Niyogi K.N., Photoprotection revisited:genetic and molecular approaches, Annu. Rev. Plant Physiol. Plant Mol. Biol., 50, 1999, pp.333-359.
72. Noaman N.H., Ultraviolet-B irradiation alters Amino acids, proteins, fatty acids contents and enzyme activites of *Synechococcus leopoliensis*, Int. J. Bot., Vol.3, No.1, 2007, pp.109-113.
73. Noctor G., Foyer C.H., Ascorbate and glutathione: Keeping active oxygen under control, Annu. Rev. Plant Physiol. Plant Mol. Biol., 49, 1998, pp.249-279.
74. Odmark S., Wulff A., Wangberg, S.A., Nilsson C., Sundback K., Effects of UV-B radiation in a microbenthic community of a marine shallow-water sandy sediment, Mar. Boil., 132, 1998, pp.335-345.
75. Oren A., Gunde-Cimerman N., Mycosporine and mycosporine-like Amino acids: UV protectants or multipurpose secondary metabolites?. FEMS Microbiol. Lett., 269, 2007, pp.1-10.
76. Poppe F., Hanelt D., Wiencke C., Changes in ultrastructure, photosynthetic activity and pigments in the Antarctic red alga *Palmaria decipiens* during acclimation to UV radiation, Bot. Mar., 45, 2002, pp.253-261.
77. Poppe F., Schmidt R.A.M., Hanelt D., Wiencke C., Effects of UV radiation on the ultrastructure of several red algae, Phycol. Res., 51, 2003, pp.11-19.
78. Prasad V., Kumar A., Kumar H.D., Effects of UV-B on certain metabolic processes of the green alga *Chlorella vulgaris*, International J. Environ. Studies, 55, 1998, pp.129-140.
79. Pyle J.A., Global ozone depletion observation and theory. in: Lumbsden, P.J (Ed). Plants and UV-B responses to environmental change, Cambridge university press, Cambridge, 1997, pp.3-12.
80. Radwan S.S., Coupling of two dimensional thinlayer chromatography with GC for the quantitative analysis of lipid classes and their constituented fatty acids, J. Chromatograph Sci., 16, 1978, pp.538-542.
81. Rao M.V., Paliyath C., Ormord D.P., Ultraviolet-B-induced and ozone-induced biochemical changes in antioxidant enzymes of *Arabidopsis thaliana*. Plant Physiol., 110, 1996, pp.125-136.
82. Rautenberger R., Bischof K., UV-susceptibility of photosynthesis of adult sporophytes of four brown Antarctic macroalgae (Phaeophyceae), Rep. Pol. Mar. Res., 2008, pp.263-269.

83. Reynolds E.S., The use of lead citrate at high pH as an electron-opaque stain in electron microscopy, J. Cell Biol., 17, 1963, pp.208-213.
84. Rozema J., Gieskes W.W.C., van de Geijn S.C., Nolan C., de Boois H. (Eds.), UV-B and Biosphere, Kluwer Academic Publishers, Dordrecht, 1997.
85. Salguero A., Leon R., Mariotti A., de la Morena B., Vega J.M., Vilchez C., UV-A mediated induction of carotenoid accumulation in *Dunaliella bardawil* with retention of cell viability, Appl. Microbiol. Biotechnol., Vol.66, No.5, 2005, pp.506-511.
86. Santos I., Fidalgo F., Almeida J.M., Salema R., Biochemical and ultrastructural changes in leaves of potato plants grown under-supplementary UV-B radiation, Plant Sci., Vol.167, No.4, 2004, pp.925-935.
87. Sarkar D., Bhowmik P.C., Kwon Y.N., Shetty K., The role of praline-associated pentose phosphate pathway in cool-season turfgrasses after UV-B exposure. Environ, Exp. Bot., 70, 2011, pp.251-258.
88. Shiu C.T., Lee T.M., Ultraviolet-B- induced oxidative stress and response of the ascorbate-glutathione cycle in a marine macroalga Ulva fasciata, J. Exp. Bot., 56, 2005, pp.2851-2865.
89. Sinha R.P., Häder D.P., Effects of UV-B radiation on cyanobacteria. Recent Res. Dev. Photochem. Photobiol., 4, 2000, pp.239-246.
90. Skerratt J.H., Davidson A.D., Nichols P.D., McMeekin T.A., Effect of UV-B on lipid content of three Antarctic marine phytoplankton, Phytochemistry, 49, 1998, pp.999-1007.
91. Smirnoff N., Wheeler G.L., Ascorbic acid in plants: biosynthesis and function, Critical Rev. Plant Sci., 19, 2000, pp.267-290.
92. Strid A., Chow W.S., Anderson J.M., UV-B damage and protection at the molecular level in plants, Photosynth. Res., 39, 1994, pp.475-489.
93. Tian J., Yu, J., Changes in ultrastructure and responses of antioxidant systems of algae (*Dunaliella salina*) during acclimation to enhanced ultraviolet-B radiation. Photochem, Photobiol., 97, 2009, pp.152-160.
94. Tominaga H., Coury D.A., Amano H., Kakinuma M., Isolation and characterization of a cDNA encoding a heat shock protein 70 from a sterile mutant of *Ulva pertusa* (Ulvales, Chlorophyta). Ecotoxicology, 19, 2010, pp.577-588.
95. Vu C.V., Allen L.H., Garrard L.A., Effect of supplemental UV-B radiation on primary photosynthetic carboxylating enzymes and soluble proteins in leaves of C_3 and C_4 crop plants. Physiol. Plant, 55, 1982, pp.11-16.
96. Wang K.S., Chai T.J., Reduction in omega-3 fatty acids by UV-B irradiation in microalgae, J. Apple. Phycol., 6, 1994, pp.415-421.
97. Weatherhead, E.C., Andersen S.B., The search of signs of recovery of the ozone layer. Nature, 441, 2006, pp.39-45.
98. Willekens H., Chamnogopol S., Davey M., Schraudner M., Langebartels C., Catalase is a sink for H_2O_2 and is indispensable for stress defense in C3 plants, EMBO J., 16, 1997, pp.4806-4816.
99. Winder K., Eggum O.B., Protein hydrolysis. Adescription method used at the department of animal physiology in Copenhagen, Acta. Agric., 16, 115, 1966.

100. Wu H., Gao K., Villafance V.J.E., Watanabe T., Effects of solar UV radiation on morphology and photosynthesis of filamentous cyanobacterium *Anthrospira plantensis*, Appl. Environ. Microbiol., 71, 2005, pp.5004-5013.
101. Xue L., Zhang Y., Zang T., An L., Wang X., Effects of enhanced ultraviolet-B radiation on algae and cyanobacteria, Crit. Rev. Microbiol., 17, 2005, pp.79-89.
102. Ziska L.H., Teramura A.H., CO_2 enhancement of growth and photosynthesis in Rice (*Oryza sativa*): Modification by increased ultraviolet-B radiation, Plant Physiol., 99, 1992, pp.473-481.
103. Zverdanovic J., Cvetic T., Jovanovic S.V., Markovic D., Chlorophyll bleaching by UV-irradiation in vitro and in situ: Absorption and fluorescence studies, Rad. Phys. Chem., 78, 2009, pp.25-32.