



EFFECT OF UV-B RADIATION ON UV ABSORBING COMPOUNDS AND PIGMENTS OF MOSS AND LICHEN OF SCHIRMACHER OASIS REGION, EAST ANTARCTICA

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Abstract

The survival of Antarctic flora under ozone depletion depends on their ability to acclimate against increasing UV-B radiation by employing photo protective mechanisms either by avoiding or repairing UV-B damage. A fifteen days experiment was designed to study moss (*Bryum argenteum*) and lichen (*Umbilicaria aprina*) under natural UV-B exposure and under UV filter frames at the Maitri region of Schirmacher oasis, East Antarctica. Changes in UV absorbing compounds, phenolics, carotenoids and chlorophyll content were studied for continuous fifteen days and significant changes were observed in the UV exposed plants of *B. argenteum* and *U. aprina*. The change in the UV absorbing compounds was more significant in *B. argenteum* ($P<0.0001$) than *U. aprina* ($P<0.0002$). The change in phenolic contents and total carotenoid content was significant ($P<0.0001$) in both *B. argenteum* and lichen *U. aprina* indicating that the increase in UV absorbing compounds, phenolic contents and total carotenoid content act as a protective mechanism against the deleterious effect of UV-B radiations.

Key words: Carotenoids, chlorophyll, phenolics, UV absorbing compounds, UV filter frame.

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INTRODUCTION

Antarctica is the coldest, driest continent and plants that grow are capable of withstanding severe desiccation. Solar radiation is essential for photosynthesis and growth of plants. Although solar radiation in the visible region drives photosynthesis, absorptance of high levels of visible radiation and radiation at other wavelengths can be damaging. At shorter wavelengths, absorbed UV-B (280-320 nm) radiation can cause lesions to nucleic acid and proteins. Depletion of stratospheric ozone, resulting from anthropogenic activities, atmospheric pollution has led to increased ultraviolet (UV-B) radiation at the Earth's surface, as well as a spectral shift to the more biologically damaging shorter wavelengths (7). The decrease in ozone has been most pronounced and consistent over Antarctica with record levels of austral ozone depletion in the last decade (4, 11, 13, 16). As a consequence, Antarctica now experiences unseasonably high UV-B radiation through much of the spring, caused by the combined effects of the 'ozone hole' and the approach of the natural annual radiation peak, the summer solstice (19). Recovery of the Antarctic ozone hole is currently predicted by 2050, but remains a topic of intense research interest. Lichen and moss are desiccation and freezing tolerant, and able to survive frozen beneath snow during the long polar winter. The emergence from snow and the start of the short growing season currently coincides with peak levels of UV-B radiation due to ozone depletion. Moss may be particularly susceptible to UV-B damage because of their simple structure, with most lacking differentiation and the protective cuticle or epidermal layer of higher plants. Combined with the physiologically stressful effects of repeated freeze/thaw cycles, an intermittent water supply and limiting nutrients, polar bryophytes are likely to be sensitive to the additional stress imposed by elevated UV-B radiation (23, 24). UV-B absorbing pigments are widespread across the plant kingdom, due to

their ability to absorb biologically damaging UV-B radiation while transmitting essential photo synthetically active radiation (6). A meta-analysis of field studies revealed the most striking and consistent response of plants to increased UV-B radiation resulting in increase of UV-B absorbing pigments, on average by 10% (21). In this study we have investigated two Antarctic plants *B. argenteum* and *U. aprina* under natural environmental conditions including the UV-B radiation and under UV-B filter cover with the main objectives to understand the changes in UV absorbing compounds, phenolic content, chlorophyll content and carotenoids under the effect of UV-B radiations. The present paper shows that these plants experienced a quantitative increase of UV absorbing compounds, carotenoid, phenolics which collaborates the positive increase with the increasing UV-B radiation stress.

MATERIALS AND METHODS

Study site and experimental set-up

The Schirmacher Oasis region of east Antarctica consists (Latitude: 70 Deg 45'01.65»South, Longitude: 11 Deg 43'01.45» East) of a series of low-lying peninsulas and islands, which become partially ice-free during the summer melt period. Screening treatments of UV-B radiation by UV-B filter frames were established near the Priyadarshini Lake and around Indian Antarctic Station, Maitri. These sites were chosen because the selected plant species grow naturally and have greater exposure to both sunlight and wind.

A metal frame box was designed fitted with a 12" X 12" UV filter sheet (280 to 380 nm) used to screen the UV-B radiation. These UV filter frames placed over *B. argenteum* and *U. aprina* under natural conditions other than UV-B radiation, served as UV unexposed condition for continuous study of the plant specimen over fifteen days of duration. A simultaneous study without UV filter frame

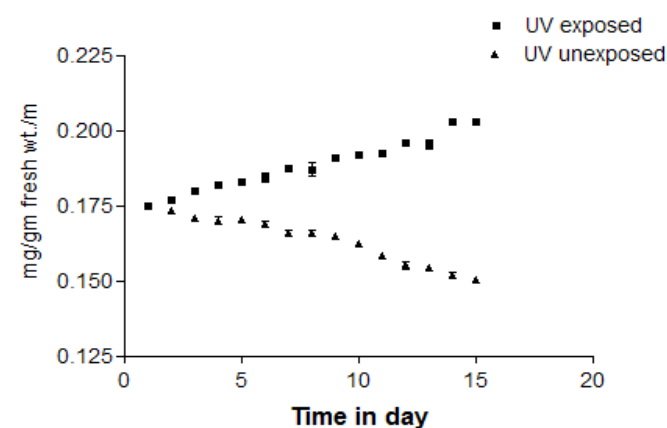
Table 1. Concentration of chlorophyll a and chlorophyll b under UV exposed conditions at Maitri, East Antarctica.

Plant	Chl. a			Chl. b		
	mg/gm fresh wt (Mean value \pm S.D)			mg/gm fresh wt (Mean value \pm S.D)		
	Day 1	Day 7	Day 15	Day 1	Day 7	Day 15
<i>B. argenteum</i>	0.137 \pm 0.002	0.132 \pm 0.003	0.126 \pm 0.001	0.060 \pm 0.128	0.053 \pm 0.064	0.046 \pm 0.045
<i>U. aprina</i>	0.142 \pm 0.003	0.138 \pm 0.002	0.132 \pm 0.003	0.040 \pm 0.041	0.033 \pm 0.066	0.020 \pm 0.060

Table 2. Concentration of chlorophyll a and chlorophyll b under UV Filter frame conditions at Maitri, East Antarctica.

Plant	Chl. a			Chl. b		
	mg/gm fresh wt (Mean value \pm S.D)			mg/gm fresh wt (Mean value \pm S.D)		
	Day 1	Day 7	Day 15	Day 1	Day 7	Day 15
<i>B. argenteum</i>	0.140 \pm 0.003	0.146 \pm 0.001	0.151 \pm 0.002	0.060 \pm 0.146	0.066 \pm 0.144	0.071 \pm 0.001
<i>U. aprina</i>	0.141 \pm 0.002	0.149 \pm 0.001	0.150 \pm 0.002	0.040 \pm 0.002	0.046 \pm 0.002	0.058 \pm 0.003

serving as UV exposed condition was carried over the plants from 2 January 2010 to 17 January 2010. Samples were randomly collected in triplicate daily from the nearby three sites of Indian research station “Maitri,” situated at Schirmacher Oasis, East Antarctica from both the specified conditions and were analysed for the changes in UV absorbing compounds, phenolic contents, chlorophyll content and carotenoids.

**Figure 1.** Change in UV absorbing compounds under UV exposed and UV unexposed conditions in *B. argenteum*. $P < 0.0001$.

Extraction of pigments

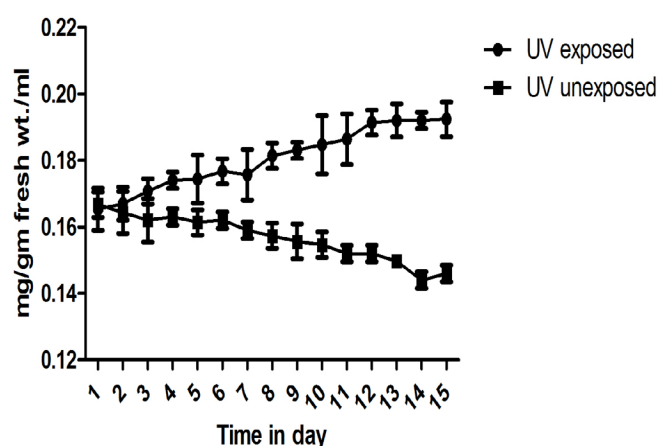
For chlorophyll content estimation, 100 mg of the plant sample was crushed in 5ml of 80% acetone solution (80ml acetone finally make up to 100 ml with distilled water) at 4°C and spin in centrifuge at 10,000 rpm for 10 minutes. Supernatant was taken and optical density (O.D) was measured at 663nm, 645nm, 510nm for chlorophyll a, chlorophyll b and carotenoids respectively (1).

For UV absorbing compounds 5 ml of acidified methanol (MeOH : HCl: H₂O 90 : 1 : 1) was taken and 100 mg of plant sample was heated at 60°C and stirred for 10 minutes in 25 ml flask. It was cooled at room temperature for 15 minutes and filtered through Whatman paper no. 5 and absorbance was recorded at 300 nm (17).

For phenolic content study the 100 mg of plant sample was homogenised (10% w/v) in acidified methanol (50% methanol 0.05 % HCl, pH 3.5). Homogenate allowed to settle for 15 h in dark at 0-4°C and filtered through Whatman paper no. 5 and absorbance recorded at 280 nm (15).

Statistical analyses

The statistical analyses of the data was performed by using graph pad Prism version 3.0. For the chlorophyll and pigment data of the UV exposed and UV unexposed experiment, two way ANOVA was used. Treatment effects were considered significant at the $P < 0.05$ level.

**Figure 2.** Change in phenolic contents under UV exposed and UV unexposed conditions in *B. argenteum*. $P < 0.0001$.

RESULTS

The change in the UV absorbing compounds, phenolics and carotenoids was significant in *B. argenteum* and *U. aprina*. The change in the UV absorbing compounds was more significant in *B. argenteum* ($P < 0.0001$) than lichen *U. aprina* ($P < 0.0002$). UV absorbing compounds in *B. argenteum* gradually increased from initial concentration

of 0.175 mg/gm fresh wt. /ml at day 1st to 0.205 mg/gm fresh wt. /ml on day 15th under UV exposed condition and decreased to 0.151 mg/gm fresh wt. /ml on day 15th under UV unexposed condition. In *U. aprina* an increase in UV absorbing compounds were recorded from initial concentration of 0.176 mg/gm fresh wt. /ml at day 1st to 0.203 mg/gm fresh wt. /ml on day 15th under UV exposed condition and decreased to 0.145 mg/gm fresh wt. /ml on day 15th under UV unexposed condition.

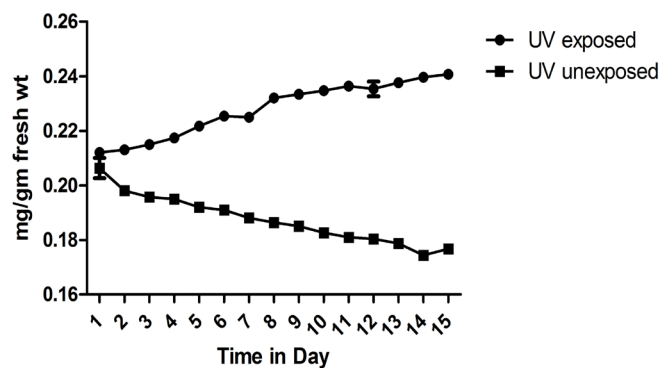


Figure 3. Change in total carotenoids content under UV exposed and UV unexposed conditions in *B. argenteum*. $P < 0.0001$.

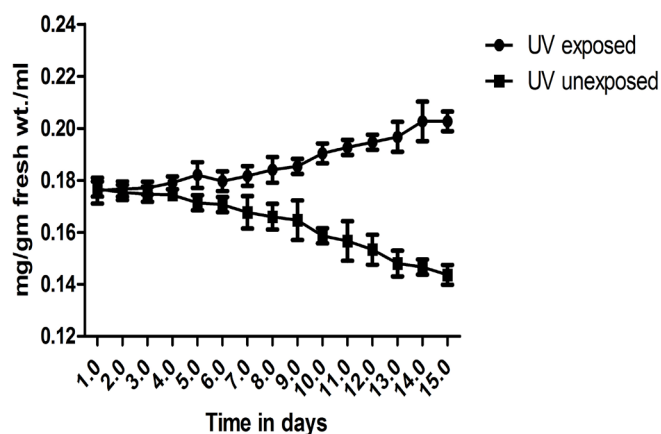


Figure 4. Change in UV absorbing compounds under UV exposed and UV unexposed conditions in *U. aprina*. $P < 0.0002$.

The change in phenolic contents and total carotenoid content was significant $P < 0.0001$ in both *B. argenteum* and *U. aprina*. Phenolic contents in *B. argenteum* increased from initial concentration of 0.168 mg/gm fresh wt. /ml at day 1st to 0.194 mg/gm fresh wt. /ml on day 15th under UV exposed condition and decreased to 0.147 mg/gm fresh wt. /ml on day 15th under UV unexposed condition. In *U. aprina*, phenolic contents increased from initial concentration of 0.142 mg/gm fresh wt. /ml at day 1st to 0.172 mg/gm fresh wt. /ml on day 15th under UV exposed condition and decreased to 0.114 mg/gm fresh wt. /ml on day 15th under UV unexposed condition. Change in total carotenoid in *B. argenteum* was from initial concentration of 0.210 mg/gm fresh wt. /ml at day 1st to 0.242 mg/gm fresh wt. /ml on day 15th under UV exposed condition and decreased to 0.177 mg/gm fresh wt. /ml on day 15th under UV unexposed condition. In *U. aprina* total carotenoids increased from initial concentration of 0.135 mg/gm fresh wt. /ml at day 1st to 0.165 mg/gm fresh wt. /ml on day 15th under UV exposed condition and decreased to 0.135 mg/gm fresh wt. /ml on day 15th under UV filter condition.

The change in the chlorophyll were not considerable in

both plants which clearly indicates that the increase in UV absorbing compounds, phenolic contents and total carotenoid content act as a protective mechanism against the deleterious effect of UV-B radiations. The change in chlorophyll a and chlorophyll b is summarized in table 1 and 2. Results are represented in Fig. 1 to 6.

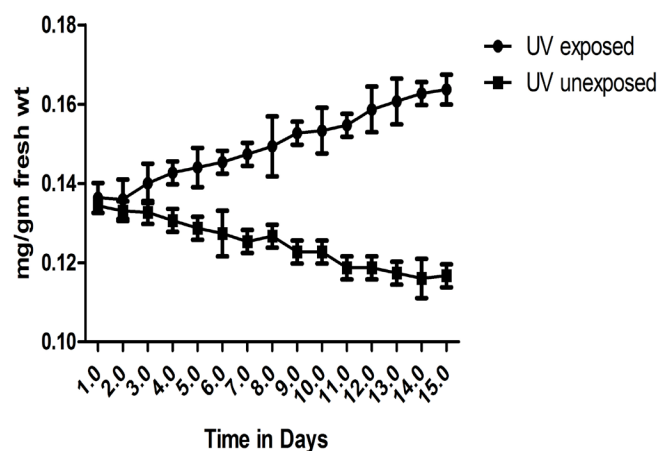


Figure 5. Change in phenolic contents under UV exposed and UV unexposed conditions in *U. aprina*. $P < 0.0001$.

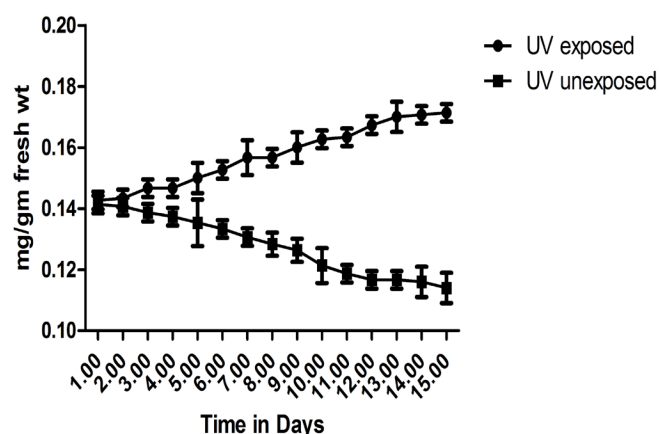


Figure 6. Change in total carotenoids content under UV exposed and UV unexposed conditions in *U. aprina*. $P < 0.0001$.

DISCUSSION

In present study we observed that the chlorophyll content experienced insignificant changes under UV exposed and UV unexposed conditions. Changes in chlorophyll have been observed in some species but are not a consistent response to natural variations in UV-B exposure, although they have previously been observed in Arctic bryophytes in response to enhanced UV-B radiation (21, 8, 5). No change in chlorophyll concentration was observed as a result of seasonal changes in UV-B radiation in either the South American *Sphagnum magellanicum* (22) or two Antarctic bryophytes studied by Newsham *et al.* (14).

Carotenoid synthesis is known to be induced by exposure to UV-B radiation (6). Carotenoids are considered to act as protective pigments which form a cover around the plants photo system to protect it from any damage under stress conditions. The data from the present study indicate that carotenoid concentration positively associated with increases in UV-B radiation arising from ozone depletion, corroborating the data of Xiong and Day (25),

who showed increased concentrations of these pigments in foliage of *Colobanthus quitensis* and *Deschampsia antarctica* plants exposed to near-ambient solar UV-B, compared with plants exposed to reduced UV-B. Similarly, increased carotenoid concentrations have been found in foliage of the bryophytes *Cephaloziella exiliflora* and *Sanionia uncinata* at Rothera Point during periods of ozone depletion.

UV-B absorbing pigments are widespread across the plant kingdom, due to their ability to absorb biologically damaging UV-B radiation while transmitting essential photo synthetically active radiation (20). The concentration of UV-B absorbing compounds increased significantly when studied under UV exposed condition as compared to those studied under UV unexposed condition. The increase in the UV-B absorbing compounds is positively associated with the UV-B exposure. Antarctic field experiments have shown increased concentrations of UV-B screening pigments in foliage of the pearlwort *C. quitensis* and the grass *D. antarctica* exposed for four months to near-ambient solar UV-B radiation under plastic screens on the western Antarctic Peninsula, compared with plants exposed to reduced UV-B radiation (18). The widespread accumulation of UV-B screening pigments in plant tissues in response to UV-B radiation owes at least in part to flavonoid synthesis, caused by the induction of genes encoding chalcone synthase, a key enzyme in the flavonoid biosynthesis pathway (10).

Some of the indirect effects relate to physiological and ecological functions of various protective absorbing (poly) phenolics, whose production is induced by UV-B, in addition to other environmental factors. These form part of the phenyl propanoid pathway (2, 20, 3). In our study the change in the phenolic content was significantly increasing in *B. argenteum* and *U. aprina* which clearly indicates that under the effect of UV-B the production of phenolics is induced. Solar UV-B radiation is known to stimulate the synthesis of the enzymes phenyl alanine ammonia lyase (PAL) and chalcone synthase (CHS) and other branch-point enzymes of the phenylpropanoid pathway (9). PAL catalyzes the transformation of phenylalanine into trans-cinnamic acid, which is a central intermediate in the formation of complex phenolic compounds such as flavonoids, condensed tannins and lignin (12). These UV-B filters prevent UV-B damage to the DNA and other targets in the plants, in addition to photo-reactivation and dark repair processes.

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Other articles in this theme issue include references (26-53).

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