Original Article

Adverse Effects of UV-B Radiation on Plants Growing at Schirmacher Oasis, East Antarctica

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ABSTRACT

This study aimed to assess the impacts of ultraviolet-B (UV-B) radiation over a 28-day period on the levels of pigments of *Umbilicaria aprina* and *Bryum argenteum* growing in field. The depletion of stratospheric ozone is most prominent over Antarctica, which receives more UV-B radiation than most other parts of the planet. Although UV-B radiation adversely affects all flora, Antarctic plants are better equipped to survive the damaging effects of UV-B owing to defenses provided by UV-B absorbing compounds and other screening pigments. The UV-B radiations and daily average ozone values were measured by sun photometer and the photosynthetic pigments were analyzed by the standard spectrophotometric methods of exposed and unexposed selected plants. The daily average atmospheric ozone values were recorded from 5 January to 2 February 2008. The maximum daily average for ozone (310.7 Dobson Units (DU)) was recorded on 10 January 2008. On that day, average UV-B spectral irradiances were 0.016, 0.071, and 0.186 W m^{-2} at wavelengths of 305, 312, and 320 nm, respectively. The minimum daily average ozone value (278.6 DU) was recorded on 31 January 2008. On that day, average UV-B spectral irradiances were 0.018, 0.085, and 0.210 W m^{-2} at wavelengths of 305, 312, and 320 nm, respectively. Our results concludes that following prolonged UV-B exposure, total chlorophyll levels decreased gradually in both species, whereas levels of UV-B absorbing compounds, phenolics, and carotenoids gradually increased.

Key words: Carotenoids, phenolics, total chlorophyll, UV-B absorbing compounds, UV-B radiation

INTRODUCTION

The stratospheric ozone layer prevents most UV radiation from penetrating into the earth's surface despite permitting penetration of a limited amount of UV radiation. The first observation of a drastic reduction in total ozone over Halley Bay in Antarctica was reported by Farman *et al.*,[1] and later confirmed by other researchers.[2-4]

Access this article online	
Quick Response Code:	Website: www.toxicologyinternational.com
	DOI: 10.4103/0971-6580.128815

Chlorofluorocarbons (CFCs) and some other air pollutants that diffuse into the ozone layer are responsible for the destruction of the ozone layer, which increases the amount of UV radiation that reaches the earth's surface and adversely affects most living organisms. The potential effects of UV-B radiation on phototrophic organisms may be broadly classified as (a) changes in photosynthesis and growth,[5,6] (b) increased investment in the synthesis and accumulation of UV-B absorbing or screening compounds,[7,8] and (c) DNA damage, repair, and photoreactivation.[9]

Continental Antarctic vegetation is sparse and cryptogamic in nature, with lichens and mosses constituting the dominant flora.[10,11] Lichens, an important component of Antarctic ecosystem, grow in compact turves, mats, and small moss cushions, all of which enable them to collect and retain more

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water. Moss growths are restricted to melted lakes, streams, and other locations where free water is available.[12]

The responses of photosynthetic pigments in plant leaves or thalli to UV-B exposure often differ between studies, with different experiments showing either an increase,[13] no change,[14,15] or a decrease[16-19] in chlorophyll concentrations. Phenolic compounds protect plants from exposure to UV-B, most likely by contributing to the decrease in active oxygen species by acting as antioxidants.[20-22] Here, we report the results of field experiments that examined total levels of chlorophyll, carotenoids, UV-B absorbing compounds, and phenolics in *Umbilicaria aprina* and *Bryum argenteum* with and without UV-B exposure at Schirmacher Oasis in East Antarctica.

MATERIALS AND METHODS

Selection of sites

Experiments involving *U. aprina* were conducted at sites east of the "Maitri" Indian Research Station (70°45.877'S, 011°43.400'E). Experiments involving *B. argenteum* were conducted at sites near the banks of Priyadarshni Lake (70°45.908'S, 011°43.507'E), Schirmacher Oasis. These sites were selected on the basis of availability of plant specimens. The UV filter-frame field experiments with moss (*B. argenteum*) and lichen (*U. aprina*) were conducted for 28 continuous days, beginning on 5 January 2008 and ending on 2 February 2008.

Selection of plant species

Both *U. aprina* and *B. argenteum* grow naturally within the "Maitri" Schirmacher Oasis region, along with other cryptogamic vegetation. *U. aprina*, the most commonly occurring foliose lichen in the Schirmacher Oasis region, is attached to rocks by a central umbilicus. The size of the thallus varies from a few millimeters to about 16 cm in diameter. The *B. argenteum* moss we studied were small to medium sized, with a height ranging from 1 to 25 mm, and were also growing naturally on moist and rocky sites near the melting ice areas of Priyadarshni Lake. Both of the plants selected for field experiments were growing in such a way that they were exposed to light uniformly and we could install UV filter frames on flat surfaces to prevent the perturbation of environmental conditions other than UV-B irradiation.

UV filter frames

The UV filter frames (30.5 cm length × 30.5 cm width × 30.5 cm height) each comprised iron stands covered by a Plexiglas acrylic sheet, to ensure that plants received photosynthetically active radiation, but not UV-B radiation. The acrylic sheet absorbs 98% of total-UV radiation. Each acrylic sheet was 3-mm thick, with a surface area of 30.5 cm^2. The sides of UV filter frames were perforated using a drill bit (37 mm diameter) to create uniform holes to ensure a free flow and exchange of moisture and gases between the environment and the space enclosed by the frames. The holes cover 10% of the total area of the UV filter frames so that moisture and all other environmental parameters besides UV-B irradiation remain identical with and without UV-B exposure. Cubical frames were established over the selected plants at five different sites to control UV-B exposure.

Analyses of pigments

Pigments were analyzed from plants grown without a UV-B filter frame as well as plants covered with UV-B filter frame to prevent exposure to UV-B. Plant samples were harvested at 7-d intervals from 5 January to 2 February 2008, washed with double-distilled water, and blotted dry using Whatman filter paper No. 1 before their fresh weights were recorded. Standard methodologies were used to estimate pigment contents of both species under the two different conditions.

Photosynthetic pigments (total chlorophyll and carotenoids) analyses

Plant samples were homogenized using a pestle and mortar in 80% (v/v) acetone. The ratio of fresh tissue to volume of acetone used for extraction is 10:1 (w/v). Samples were maintained at 4°C throughout the extraction period and centrifuged at a speed corresponding to approximately 25,000*g* for 15 min in a refrigerated centrifuge. The supernatant solutions from centrifuged samples were filtered through Whatman filter paper No. 1 and their absorbances at 663, 645, 480, and 510 nm were determined using a Systronics UV-VIS spectrophotometer-117. The chlorophyll content was calculated from absorbance values at 663 and 645 nm.[23] The carotenoid content was calculated from absorbance values at 480 and 510 nm using a previously described method of Parsons *et al*.[24]

Photoprotective pigments (UV-B absorbing compounds and phenolics) analyses

Levels of UV-B absorbing compounds were assessed as described previously.[25] Plant samples were placed in 25 ml Erlenmeyer flasks containing 5 ml of acidified methanol (MeOH: HCl: H_2O, 90:1:1 (v/v)), and the contents of the flask were heated to 60°C and stirred at that temperature for 10 min before cooling to room temperature over 15 min and filtering through 90 μm filters. Concentrations of soluble UV-B absorbing compounds were estimated by measuring the absorbance of the filtrate at 300 nm using a spectrophotometer.

To assay for levels of phenolics, a 10% (w/v) homogenate was prepared in a methanolic HCl solvent (50% methanol, 0.05% concentrated HCl, approximate pH of 3.5) at room temperature. Extracts were acidified to an approximate pH

of 1.0 by the addition of HCl (1 M). The precipitate was allowed to settle for 15 h in the dark and filtered through Whatman No. 5 filter paper. The absorbance of the supernatant solution was measured at 280 nm as described previously.[26]

Measurement of ozone and UV-B radiation

The UV-B radiation at 305, 312, and 320 nm wasand data on the thickness of the ozone layer were measured at selected sites using a sun photometer (Microtop II 4701, Solar Light) over a 28-day period beginning on 5 January 2008 and ending on 2 February 2008.

Statistical analysis

The statistical analysis was performed by using graph pad Prism 3.0 and five replicates for the experiment were taken. Two-way analysis of variance (ANOVA) was used for pigments of exposed and unexposed experimental plants. The differences between treatments were considered significant at the $P < 0.05$ level.

RESULTS

UV-B radiation and ozone levels

Over the 28-day period when ozone levels were measured (5 January–2 February 2008), the average ozone level over the "Maitri" Schirmacher Oasis region was 295.4 DU [Figure 1a]. The maximum daily average ozone value, recorded on 10 January 2008, was 310.7 DU. On the same day, UV-B spectral irradiances of 0.016, 0.071, and 0.186 W m^{-2} were recorded at wavelengths of 305, 312, and 320 nm, respectively. The minimum daily average ozone value, recorded on 31 January 2008, was 278.6 DU. On the same day, UV-B spectral irradiances of 0.018, 0.085, and 0.210 were recorded at wavelengths of 305, 312, and 320 nm, respectively.

Throughout the study period, values for the daily average UV-B radiation above the selected sites were 0.016, 0.071, and 0.186 W m^{-2} at wavelengths of 305, 312, and 320 nm, respectively [Figure 1b-d]. Of all of the daily average measurements of UV-B levels made, the highest were recorded on 5 January 2008, with values of 0.025, 0.10, and 0.25 W m^{-2} at wavelengths of 305, 312, and 320 nm, respectively. At that time, average ozone values were 308.6 DU. The minimum daily average UV-B values, recorded on 12 January 2008, were 0.004, 0.028, and 0.091 W m^{-2} at wavelengths of 305, 312, and 320 nm, respectively. At that time, average ozone values were 291.1 DU.

Photosynthetic pigments (total chlorophyll and carotenoids)

For both species, total chlorophyll concentrations decreased gradually throughout the period of exposure to UV-B. In contrast, carotenoid concentrations of both species increased gradually over the same period under the similar conditions. A significant decrease in total chlorophyll ($P < 0.02$) was observed for both species after 28 days of exposure to UV-B [Figure 2], although slight decreases measured after 7, 14, and 21 days of exposure were not statistically significant. In both species, a significant increase in carotenoid concentrations ($P < 0.001$) was found after 28 days of exposure to UV-B, although increases measured after 7, 14, and 21 days of exposure were not statistically significant [Figure 3]. There were no significant changes in total levels of chlorophyll and carotenoids for either species when plants were not exposed to UV-B.

Photoprotective pigments (UV-B absorbing compounds and phenolics)

In both species, levels of UV-B absorbing compounds and phenolics both increased gradually following exposure to UV-B. The increase in UV-B absorbing compounds of both species was significant ($P < 0.05$)

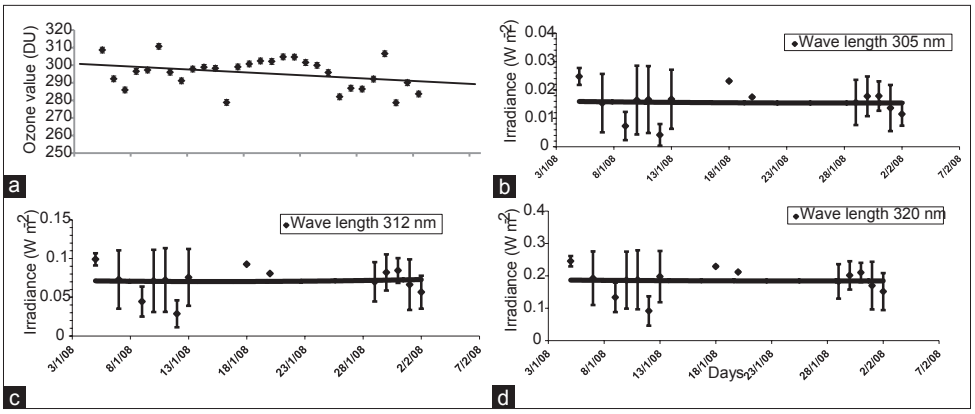

Figure 1: Fluctuations in environmental parameters in the "Maitri" Schirmacher Oasis region over the experimental period. (a) Average atmospheric ozone concentrations. (b) Changes in daily average spectral irradiance at 305 nm. (c) Changes in daily average spectral irradiance at 312 nm. (d) Changes in daily average spectral irradiance at 320 nm

after 28 days of continuous UV-B exposure [Figure 4]. In *U. aprina*, a significant increase ($P < 0.02$) in phenolics was recorded on day 21 under UV-B-exposed conditions [Figure 5]. However, in *B. argenteum*, a significant increase in phenolics ($P < 0.001$) was only evident at the end of the experiment (after 28 days of UV-B exposure) [Figure 5]. No significant changes in UV-B absorbing compounds and phenolics were observed without exposure to UV-B.

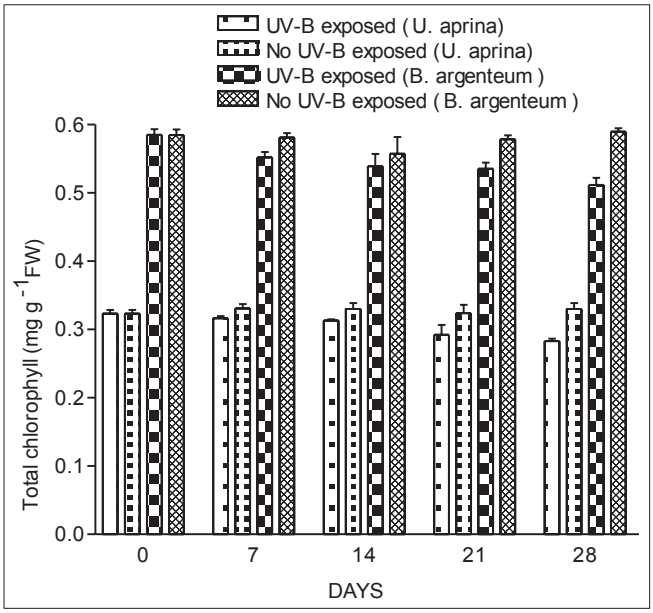

Figure 2: Concentrations of total chlorophyll in U. aprina and B. argenteum with and without exposure to UV-B at Schirmacher Oasis in East Antarctica

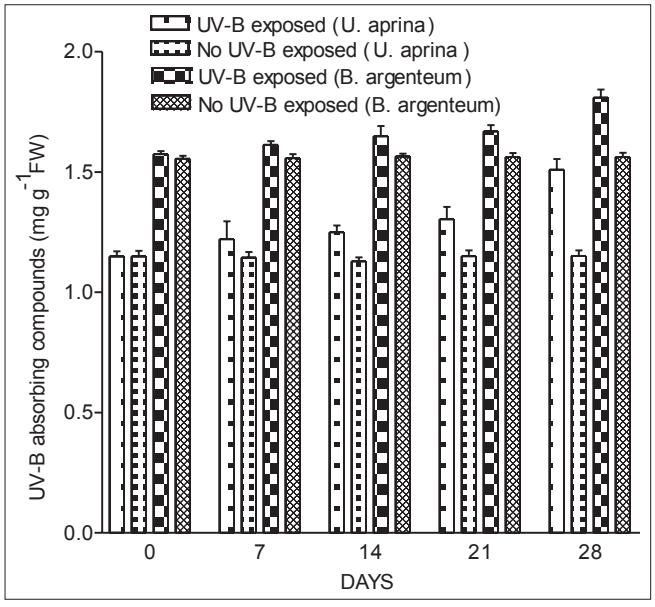

Figure 4: Concentrations of UV-B absorbing compounds in U. aprina and B. argenteum with and without exposure to UV-B at Schirmacher Oasis in East Antarctica

DISCUSSION

Our study demonstrated that changes in UV-B radiation altered the pigmentation of *B. argenteum* and *U. aprina*. It shows that the total chlorophyll concentration of both plants decreases gradually following exposure to UV-B for a 28-day period. Conversely, carotenoid concentrations in *B. argenteum* and *U. aprina* increased gradually over the same time and under identical conditions. Robinson *et al*.,[18] reported lower concentrations of chlorophyll in *Grimmia antarctici* under near-ambient UV radiation and correspondingly high relative concentration of carotenoids under reduced UV radiation at Windmill Islands of east Antarctica. For *Sanionia uncinata* and *Cephaloziella varians*, carotenoid contents increased under naturally elevated UV-B radiation, although the total chlorophyll

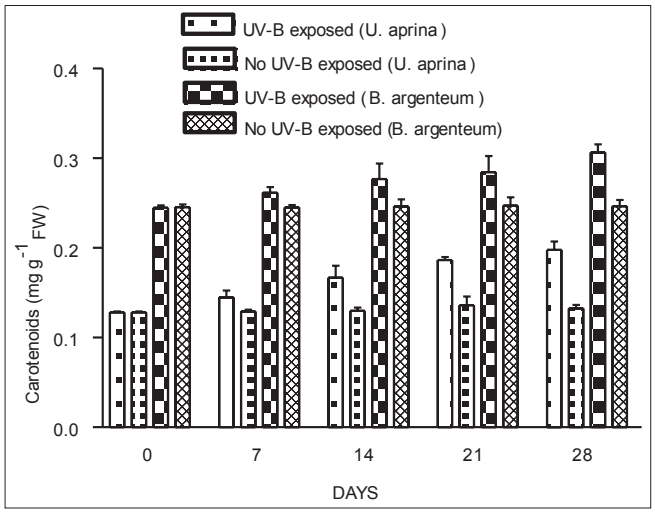

Figure 3: Concentrations of carotenoids in U. aprina and B. argenteum with and without exposure to UV-B at Schirmacher Oasis in East Antarctica

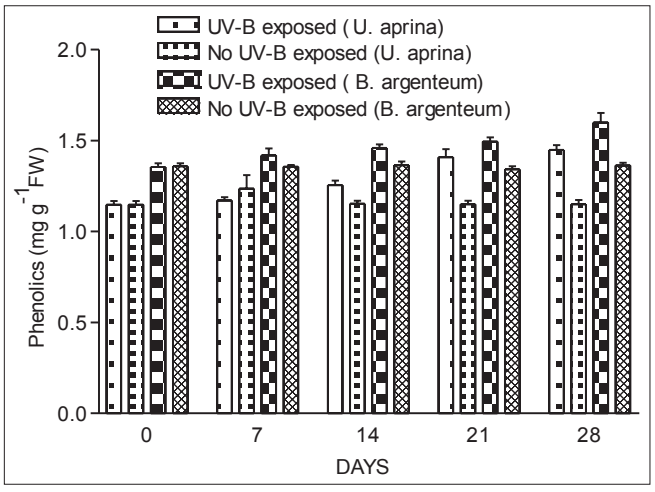

Figure 5: Concentrations of phenolics in U. aprina and B. argenteum with and without exposure to UV-B at Schirmacher Oasis in East Antarctica

concentration was not affected by ozone depletion over a 4-6 week *in situ* study.[27] Nevertheless, many workers have reported no effect of UV-B radiation on the chlorophyll concentration of plants.[28-31] For instance, a study similar to ours, involving the lichens *Lobaria pulmonaria* and *Xanthoria aureola* (also known as *Xanthoria ectaneoides*), documented no significant reduction in either chlorophyll a or chlorophyll b at different UV-B levels under laboratory conditions.[32] Similarly, exposure of the lichen *Turgidosculum complicatulum* to various combinations of UV radiation had no significant effect on levels of chlorophyll, carotenoids, and UV-absorbing compounds or photosystem II efficiency.[33]

Throughout the plant kingdom, UV-B absorbing pigments play essential roles in the absorption of biologically damaging UV-B radiation, whereas transmitting essential photosynthetically active radiation.[7] For example, concentrations of UV-B screening pigments in *C. varians* and *S. uncinata* were positively correlated with daily doses of UV-B radiation at Rothera Point over a 4-6 week *in situ* study.[27] De la Rosa *et al*.,[34] reported that induction of several phenolics takes place in silver birch (*Betula pendula*) by UV-B radiation and their concentration was dependent on UV-B daily time-integrated irradiance. Analysis of the response to UV-B radiation over a season (November 1999–March 2000) revealed higher concentrations of UV-B absorbing compounds in the two cosmopolitan moss species *Bryum pseudotriquetrum* and *Ceratodon purpureus* but lower concentrations of UV-B absorbing compounds in *Schistidium antarctici*. Another study, conducted on Svalbard, observed no changes in UV-B absorbing compounds in high Arctic tundra plant in response to UV-B radiation.[35] Several other studies on the effects of UV on plants suggested that lichens contain a variety of polyphenolic compounds, including usnic acid, parietin, and melanin, with strong capacities to absorb UV radiation and that might contribute to photoprotection mechanisms in lichen.[36-39] Our results also shows that UV-B absorbing compounds and phenolics in *B. argenteum* and *U. aprina* increases gradually on the exposure of UV-B and protects the plants from the damaging effects of UV-B radiation.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the financial support from the National Centre for Antarctic and Ocean Research (NCAOR, Goa), Ministry of Earth Sciences, New Delhi, India.

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How to cite this article: Singh J, Singh RP. Adverse effects of UV-B radiation on plants growing at schirmacher oasis, East Antarctica. Toxicol Int 2014;21:101-6.

Source of Support: Nil. **Conflict of Interest:** None declared.