Final Report
Major Research Project

Studies on the influence of UV-B radiation on cryptogamic flora (Lichen and Moss) of Himalayan region, India

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Annexure No. 1

Objectives of the Project:

1. To measure the UV-B radiation at Himalayan region of India.
2. To study the impact of UV-B radiations on the cryptogamic species, Lichen (*Xanthoria elegans*, *Dermatocarpon miniatum*, *Umblicria indica*) and Moss (*Bryum argenteum*).
3. Quantification of UV-B absorbing compounds and their role in survival mechanism.
4. Quantification of parietina and anthocynin pigment in selected lichens (*X. elegans*, *D. miniatum*, *U. indica*) under UV-B exposed and UV-B unexposed condition.
5. To study the structural adaptations under the harsh environmental conditions of Himalayan region.
6. To study the functional adaptation under the impact of UV-B radiation.

INTRODUCTION

The continuous ozone observations are available from ground-based stations since 1950s for a number of locations, and global observations are available from satellite-based instruments for most of the period since 1979. Ozone is the primary UV-B absorbing component of the atmosphere with approximately 90% of this atmospheric ozone column being in the stratosphere. In 1974 scientists warned that certain industrial chemicals, e.g., chloroflurocabons (CFCs) could migrate to the stratosphere which would deplete upper-atmospheric ozone. Since this was the first observation of a drastic reduction in total ozone over Halley Bay in Antarctica in 1985 (Farman et al., 1985), and later on confirmed by several scientific investigations i.e., Bodeker et al., 2001; McKerzie et al., 2003; NASA, 2006. The minimum ozone levels in the Antarctica decreased steadily throughout the 1990s, and less dramatic decreases have been found above other areas of the world. From 1979-1997, scientists observed losses of ozone have ranged between 5-6% in the winter and spring and about 3% in the summer and fall in the northern mid-latitudes. In 2000 (and again in 2003 and 2006) the depletion reached a record size, extending over more than 27 million sq km, an area greater than that of North America. Recovery of the ozone layer, however, is expected to take 50 to 100 years. UV radiation is known to affect many biological and chemical processes, and is largely detrimental to individual organisms. Increased UV-B radiation is responsible for a wide range of direct and indirect effects on living organisms. In the plants, UV-B appears to affect growth, morphology and secondary metabolism. Reduction in herbaceous plant growth is a common effect of UV-B radiation at midlatitudes and found in a number of species at high latitudes. The other effects of UV-B radiation include disruption of photosynthesis and metabolic processes resulting in reduced growth and eventual cell death by damaging physiologically important molecules such as DNA, protein and lipids. UV-B effects on ecosystem is a complex phenomenon and requires consideration of the ecosystem as a particular climate variability and environmental responses including water, temperature, the timing, season, altitude, extent and duration of ice and snow cover, changes in surface albedo, and level of solar radiation. Therefore, in the proposed project we have carried out measurements of UV-B radiation at higher altitude of Himalaya and the effects of UV-B radiation on the selected cryptogammic plants especially lichen and moss growing over there.

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6. To study the functional adaptation under the impact of UV-B radiation.

Materials and Methods:

I. **Selection of Plant species:** Cryptogamic vegetations are dominant over Himalayan region. On the basis of availability of these plant species some of the lichen and moss species selected for the experiments includes lichens (*X. elegans*, *D. miniatum*, *U. indica*) and moss (*B. argenteum*).

II. **Site Selection:** Three different sites at higher altitudes were selected at Jammu and Kashmir, North Western part of Himalaya. These sites are (i) Jammu about 300m (a.s.l.) altitude, (ii) Udhampur about 800m (a.s.l.) altitude and (iii) the Patnitop about 1800m (a.s.l.) altitude (Figure 1). For the UV-B unexposed conditions UV filter frames were developed.

III. **Standardization of Methodology:** Following parameters selected and standardization were done with the selected plant materials:

1. **Chlorophyll-** Chlorophyll content determined by the methodology of Arnon (1949).
2. **TLC and HPLC-** Plants pigments of exposed and controls will be studied by TLC method of Culberson (1974). Plants pigments will be separated and quantified by HPLC according to methodology of Gilmore and Yamamoto (1991).
3. **Protein-** Protein content of selected plant species analyzed by the method of Lowry et al., (1951).

4. **UV-absorbing compounds-** Methodology of Ruhland and Day (2000) used for the estimation of UV- absorbing compounds.
5. **UV-B measurements-** UV-B irradiances will be measured in term of erythermal dose using UV-Biometer.
6. **Structural adaption-** Morphological adaptation studied by fluorescence microscopy, scanning and transmission electron microscopy.
7. **Functional adaptation-** DNA damage will be studied by the by CPD dimer analysis.

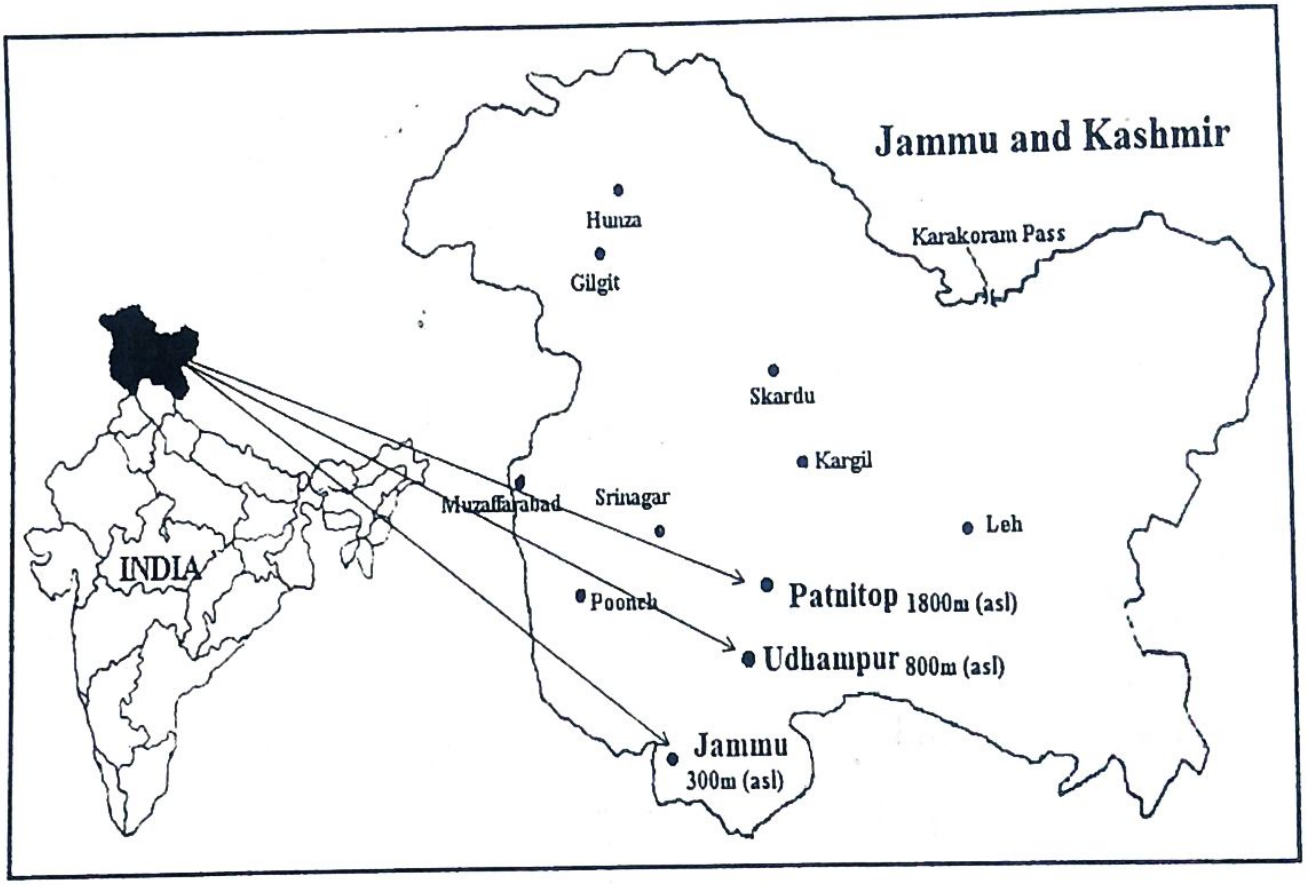

Figure 1: Showing selected sampling sites in Jammu and Kashmir.

Results and Discussion:

UV-B radiation UV-B radiation (280 to 320 nm) were measured for the continuous 120 h at the selected sites and the average UV-B irradiances was 3.426 MED/h. The maximum average UV-B irradiance (4.38 MED/ h) was recorded at 72 h whereas minimum was 1.72 MED/ h at 120 h. It was observed that the UV-B irradiance values were increasing from morning to noon and then decreased till evening.

There was decrease ($p<0.02$) in total chlorophyll of UV-B exposed plants of *B. argenteum* at 96 h as compared to UV-B unexposed plants and at that time the UV-B irradiance was 3.67 MED/h. In UV-B exposed plants of *X. elegans*, the decrease ($p<0.05$) in total chlorophyll was found at 120 h as compared to UV-B unexposed plants and at that time the UV-B irradiance was 1.72 MED/h. The increase ($p<0.02$) in carotenoids of UV-B exposed plants of *B. argenteum* and *X. elegans* was recorded at 120 h as compared to UV-B unexposed plants and at that time the UV-B irradiance was 1.72 MED/h.

The significant increase ($p<0.02$) in UV-B absorbing compounds and phenolics of *X. elegans* were recorded at 120 h under the UV-B exposed conditions and the values of UV-B irradiance was 1.72 MED/h. In *B. argenteum*, significant increase ($p<0.02$) in UV-B absorbing compounds and phenolics ($p<0.05$) were found at 120h under UV-B exposed condition and the UV-B irradiance was 1.72 MED/h. However, increase in the phenolics in *B. argenteum* is more or less same at 72, 96 and 120 h. The UV-B absorbing compounds and phenolics of both plants were positively associated with UV-B radiation exposure. In both the UVB unexposed plants, there were no significant changes in UV-B absorbing compounds and phenolics during the course of the study.

The experimental evidences suggest that the ultraviolet radiation reaching on earth surface varies with altitude, atmospheric conditions and types of instrument used for UV-B radiation measurement. We have found that UV-B absorbing compounds and phenolics in the exposed plants were increasing under the influence of UV-B radiation. The UV-B absorbing compounds and phenolics are produced by the plants under the influence of UV-B radiation and thereby provides protection against the UV-B radiation. Therefore, in *B. argenteum* and *X. elegans* UV-B absorbing and phenolic might be responsible for providing protection against UV-B radiation.

Our study demonstrates about the changes in pigments of two cryptogamic plants under UV-B exposed conditions. *B. argenteum* and *X. elegans* were naturally exposed to UV-B radiation at study sites with a maximum average UV-B irradiance of 4.38 MED/ h and minimum average UV-B irradiance of 1.72 MED/ h during the study period. In both the UV-B exposed plants, UV-B absorbing compounds and phenolics were increasing during the study period. These findings suggest that the UV-B radiation induces synthesis of UV-B absorbing compounds and phenolics, therefore, these plants are able to deal with negative effects of UV-B radiation.

Due to delay in the appointment of project fellow and continuous disturbances at selected sites of Jammu, subsequent field studies could not be performed, further delay in release of grant the project fellow resigned and left the project.

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Annexure No. 3

Achievements from the Project: Majority of the studies on the effects of UV-B radiation have been carried out in the laboratory, and very few studies have been performed in the field conditions. The cryptogamic vegetations are an important flora at the high altitude of Himalaya. With changing altitude environmental conditions vary, the environmental factors such as temperature, moisture, solar radiations (UV-B radiation) and nutrient levels influences the plant pigments, growth and development. The UV-B radiation measurement is an important parameter in the studies carried out. Determination of natural UV-B exposure level and its effects on selected plants species is important to know the survival of these species at high altitudes. The study of photosynthetic and photoprotective pigments is important in naturally UV-B exposed plants. The chlorophyll, carotenoids, UV-absorbing compounds and phenolics are the important plant pigments and were analyzed under UV-B exposed and UV-B unexposed conditions. The morphological study of selected plants was important to know the structural adaptations of flora under the harsh environmental condition of Himalayan region. The mechanism how plants are able to survive under the impact of UV-radiation is important to study the functional adaptation of selected plants. The data of UV-B radiation measurements is helpful in comparing levels of UV-B radiation at other important locations on the earth such as Antarctic and Arctic regions.

Summary of the Findings: The Himalayan region is well known for its rich plant diversity, including lichens. The account of lichens and mosses from the study area are available but their distribution pattern at different altitudinal gradient were not known. Any change in distribution and richness indicates changes in environmental conditions and effects of anthropogenic activities. The distribution of lichens and mosses in the study area clearly distinguishes the different sites by varied species richness. The number and richness of both the plant groups showed a decreasing trend towards the higher elevations.

The experimental evidences suggest that the ultraviolet radiation reaching on earth surface varies with altitude, atmospheric conditions. UV-B radiation (280 to 320 nm) were measured for the continuous 120h at the selected sites and the average UV-B irradiances was 3.426 MED/h. The maximum average UV-B irradiance (4.38 MED/ h) was recorded at 72 h whereas minimum was 1.72 MED/ h at 120 h. It was observed that the UV-B irradiance values were increasing from morning to noon and then decreased till evening.

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The diversity of lichens and mosses in Himalayan region can be useful as biosensors, helping to assess the overall health of an ecosystem. Lichens and mosses being the most sensitive plant groups

to microclimatic changes indicate such anthropogenic activities distinctly. Our study demonstrates about the changes in pigments of two cryptogamic plants under UV-B exposed conditions at high altitude of study area. *B. argenteum and X. elegans* were naturally exposed to UV-B radiation at study sites with a maximum average UV-B irradiance of 4.38 MED/ h and minimum average UV-B irradiance of 1.72 MED/ h during the study period. In both the UV-B exposed plants, UV-B absorbing compounds and phenolics were increasing during the study period. These findings suggest that the UV-B radiation induces synthesis of UV-B absorbing compounds and phenolics, therefore, these plants are able to deal with negative effects of UV-B radiation.

Annexure No. 5

Contribution to the Society:

The studies carried out in the project indicates that the UV-B irradiations during the clear sky day periods were measured high and the selected plants have the capability of protection against the high UV irradiation, by the induction of UV protecting pigments.

As the study area is having great human pressure therefore, a regional conservation strategy is needed for effective conservation of lichens and mosses in such fragile areas.