Physiological, biochemical and molecular changes in *Nostoc spongiaeforme* Agardh (a freshwater cyanobacterium) and *Phormidium corium* (Agardh) Gomont (a marine cyanobacterium) due to UV-B and high light exposure.

A THESIS SUBMITTED TO GOA UNIVERSITY
FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY
IN BOTANY

By

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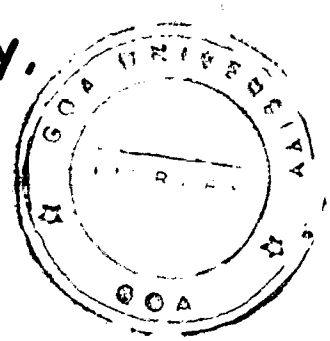

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Rupali R. Bhandari
M Sc. Botany

Under the guidance of

Dr. Prabhat K. Sharma
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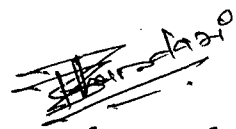
STATEMENT

As required under the university ordinance 0-413, I state that the present thesis entitled "Physiological, biochemical and molecular changes in Nostoc spongiaeforme Agardh (a freshwater cyanobacterium) and Phormidium corium (Agardh) Gomont (a marine cyanobacterium) due to UV-B and high light exposure" is my original contribution and that the same has not been submitted on any previous occasion to the best of my knowledge. The present study is the first comprehensive study of its kind from the area mentioned.

The literature conceiving the problem investigated has been cited. Due acknowledgement have been made wherever facilities have been availed.



Rupali R. Bhandari

Place: Goa University

Date: 16/03/06

CERTIFICATE

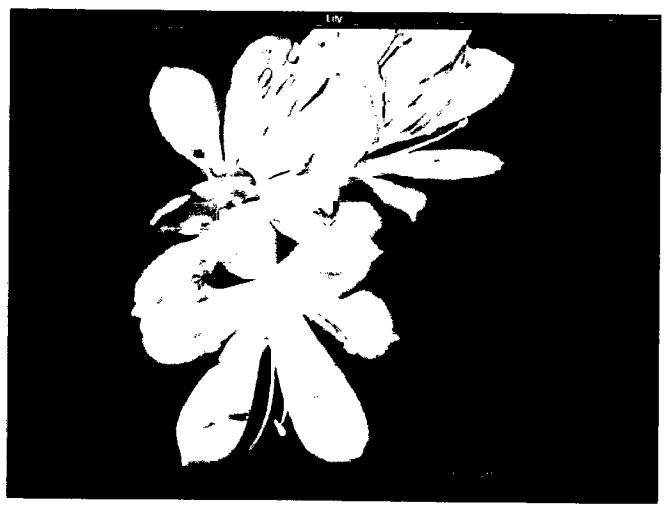
This is to certify that the thesis entitled ""Physiological, biochemical and molecular changes in Nostoc spongiaeforme Agardh (a freshwater cyanobacterium) and Phormidium corium (Agardh) Gomont (a marine cyanobacterium) due to UV-B and high light exposure" submitted by Ms Rupali R. Bhandari for the award of the degree of Doctor of Philosophy in Botany is based on the results of experiments carried out by her under my supervision. The thesis or any part thereof has not previously been submitted for any other degree or diploma.

Guide

Dr. Prabhat Kumar Sharma

Place: Goa University

Date: 16/03/06



DEDICATED TO MY LOVING PARENTS

CONTENTS

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Rupali R. Bhandari

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ABBREVIATIONS

APX	Ascorbate peroxidase
ASC	Ascorbic acid
ATP	Adenosine triphosphate
CAT	Catalase
CFC	Chlorofluorocarbon
CPDs	Cis-syn cyclobutane pyrimidine dimers
Cyt b/f	Cytochrome b_6f complex
D1	Protein containing heterodimeric centre of PS II
DNA	Deoxyribonucleic acid
Fm	Maximum Fluorescence
Fo	Initial Fluorescence
Fv	Variable Fluorescence
LHC I	Light harvesting chl a/b proteins associated with Photosystem I
LHC II	Light harvesting chl a/b proteins associated with Photosystem II
MAAs	Mycosporine like amino acids
MDA	Malondialdehyde
NADP	Nicotiana Adenosine diphosphate
$^1O_2$	Singlet Oxygen
$O_2^{\cdot-}$	Superoxide radicals
PS I	Photosystem I
PS II	Photosystem II
PAR	Photosynthetically active radiation
PFD	Photon flux density
P680	Chl a dimmer which absorb at 680 nm (PS II)
P700	Chl a dimmer which absorb at 700 nm (PS I)

PAM	Pulse Amplitude Modulator
POD	Peroxidase
ROH	Lipid radicals
ROOH	Lipid peroxide radicals
RNA	Ribonucleic acid
SOD	Superoxide dismutase
TBA	Thiobarbuturic acid
TCA	Trichloroacetic acid
UV	Ultraviolet radiation

ABSTRACT

Effect of UV-B radiation (0.8±0.1 mW cm^{-2}), high light (500 µmol $m^{-2}s^{-1}$ PAR) and UV-B radiation supplemented with low intensity PAR (0.8±0.1 mW cm^{-2} with 80 µmol m^{-2} s^{-1}) on *Nostoc spongiaeforme*, a fresh water cyanobacteria and *Phormidium corium*, a marine cyanobacteria with respect to morphology, photosynthesis, photosynthetic pigments, sugar and nitrogen content, lipid peroxidation, lipids profile, fatty acids composition, nucleic acid, UV-B absorbing pigments such as mycosporine like amino acids (MAAs), phenolic compounds, and antioxidant enzymes such as superoxide dismutase and ascorbate peroxidase were studied. Morphological changes were seen due to the treatments in both the species studied. Fv/Fm ratio, which is indicative of photosynthetic efficiency, decreased due to the treatments. The damage to photosynthesis occurred at the level of antenna system as well as at the PS II level. As a result of the UV-B treatment, increase in photosynthetic pigments such as chlorophyll a, phycobilins and β-carotene were observed in *Nostoc* while these pigments decreased in *Phormidium*. High light treatment resulted in photo bleaching of most of the pigments in both species. Sugar and nitrogen content declined due to the treatments, which probably is due to the effect on photosynthesis. UV-B, high light and UV-B supplemented with low light treatment resulted in peroxidation of membrane lipids indicating oxidative damage to lipids and level of unsaturation in the cell membrane. The activity to quench AOS was however, less in *Phormidium* as compared to *Nostoc*. Qualitative as well as quantitative changes in phosphoglyco and neutral lipids were also seen and could probably be due to the damage caused to the cell membrane or damage to enzymes involved in synthesis of lipids. Unsaturation level of fatty acids of both total lipids as well as glycolipids remained unchanged in both the cyanobacteria as a result of UV-B and high light treatment while saturated fatty acids of total and glycolipids declined slightly in *Nostoc* but remained unchanged in *Phormidium*. Also an increase in unsaturation level of total and glycolipids was observed in both the species of the cyanobacteria due to UV-B supplemented with low light. Changes in the lipids under our experimental condition could mean adaptation strategy. There were qualitative as well as quantitative changes in protein band patterns due to the treatments. Degradation of nucleic acid was also

observed due to UV-B, high light and UV-B supplemented with low light treatment in both the species studied. The damaging effect on DNA and RNA could either be due to direct effect of UV-B on nucleic acid or indirectly by AOS generated due to the treatments. Cyanobacteria were able to synthesize mycosporine like amino acids (MAAs) in response to the UV-B treatment, which was further enhanced when UV-B was supplemented with low level visual radiation and the induction may be in order to protect the organism by absorbing UV-B radiation. PAR caused significant decrease in MAAs content, suggesting that MAAs might not be an efficient screening pigment against high light conditions or when exposed to UV-B under high light condition. Activity of antioxidant enzyme such as SOD and APX was also increased probably as a result of oxidative damage observed in the form of lipid peroxidation under our experimental conditions. Increase in MAAs and SOD and APX activity may protect the cyanobacteria against UV-B damage, former by absorbing UV-B radiation while later by metabolizing AOS generated as a result of the UV-B and high light treatment. Damaging effect of UV-B radiation, however, was somewhat attenuated when UV-B treatment was supplemented with low level PAR which could be due to better protection provided by greater amount of MAAs produced under the condition. The better protection to organisms could also be due to the DNA repair mechanism, which is stimulated by low visual radiation, therefore showing relatively less damage to various processes in this study when UV-B treatment is supplemented with low level visual radiation.

CHAPTER -1

INTRODUCTION

INTRODUCTION - CONTENT

INTRODUCTION

The algae are the simplest members of the plant kingdom, and cyanobacteria are the simplest of the algae. In cellular organization, cyanobacteria resemble bacteria by the absence of any membrane bound organelles such as nucleus, chloroplast, mitochondria and the structures that are evident in true photosynthetic algae. Like higher plants, cyanobacteria evolve oxygen during photosynthetic process. They appear to represent a link between bacteria and higher plants. Cyanobacteria were responsible for a major global evolutionary transformation leading to the development of aerobic metabolism and the subsequent rise of higher plants and aerobic animal forms. Cyanobacteria are referred to in literature by various names, such as Cyanophyta, Myxophyta, Cyanochloronta, Cyanobacteria, Blue-green algae, Blue-green bacteria etc. In this work we have used term cyanobacteria (cyan means blue-green) for defining the organism studied.

Cyanobacteria get their name from the combination of green chlorophyll pigment and a unique water-soluble blue pigment, phycocyanin. Phycocyanin is a phycobilins (pigment protein complex), which facilitate harvesting of light for photosynthesis. Cyanobacteria are phylogenetically a primitive group of gram-negative prokaryotes. Their fossils have been identified as over three billion years old, which dates their appearance to the Precambrian era ($2.8–3.5\times10^9$ years). They represent some of the most ancient life forms on earth.

Cyanobacteria have a cosmopolitan distribution ranging from hot springs to Arctic and Antarctic regions and show high variability. They are widely distributed over land and water, often in environments where no other vegetation can exist. They can be

found as greenish slime on the side of damp flowerpot and the wall of house or the trunk of the big tree. They have even been found on the fur of polar bears, to which they impart a greenish tinge. They can grow in full sunlight and in almost complete darkness. Cyanobacteria also form symbiotic relationships with many fungi, forming complex symbiotic organisms known as lichens.

Cyanobacteria are single-celled or colonial. Depending upon the species and environmental conditions, colonies may form filaments, sheets or even hollow balls. Some species grow as a single cell enclosed in a sheath of slime-like material, or mucilage e.g. *Synechococcus, Chroococcus.* The cells of other species aggregate into colonies that are either coiled (*Spirulina*), rod shaped (*Oscillatoria),* rounded or oval (*Nostoc),* or elongated filaments (*Scytonema, Phormidium).* Some filamentous colonies of cyanobacteria show the ability to differentiate into three different cell types namely vegetative cells, akinetes and heterocysts. Vegetative cells are the normal, photosynthetic cells formed under favorable growing conditions. Heterocyst contains the enzyme nitrogenase, which is important for nitrogen fixation. Heterocyst cells have an especially thickened wall that contains an anaerobic environment since they lack thylakoid membrane to carry out photosynthetic light reaction, as nitrification cannot occur in the presence of oxygen. A third type of cell, akinetes are climate resistant spores, which form when environmental conditions become harsh. These akinetes contain large reserves of carbohydrates, and due to their density and lack of gas vesicles, these cells settle to the bottom of the lake. They can tolerate adverse conditions such as the complete drying of a pond or the cold winter temperature, and as a consequence, they serve as seeds for the growth of juvenile filaments when favorable conditions return.

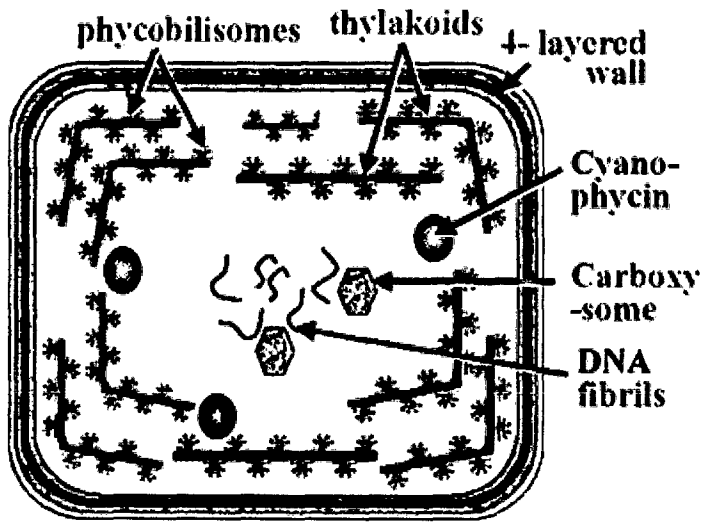


Fig 1.1 A schematic diagram of cyanobacterial cell

Cyanobacterial cell envelope is composed of the outer membrane and plasma membrane separated by a peptidoglycan layer. The cell walls are thick and gelatinous, and lack flagella. They show a variety of movements, such as gliding, rotation, oscillation, jerking and flicking. Sexual reproduction is absent and is able to reproduce through a variety of methods such as binary fission, budding, or fragmentation. Cyanobacteria are rich in chemical diversity. They contain a variety of intracellular compounds such as lipid globules, carboxysomes, polyphosphate and release some of them extracellularly like polysaccharides and peptides. They also accumulate different types of food reserves such as glycogen, cyanophycin etc. that are used as a source of nitrogen, carbon or both.

1.1. CYANOBACTERIA AND THEIR IMPORTANCE:

Studies on cyanobacteria have gained importance especially after the recognition of their role in productivity and ecology and their ability to provide an alternate source of energy, a problem of worldwide interest. These microorganisms are ecologically important in coastal primary production, serving as a food source for herbivores and detritivores, as well as a nursery area for juvenile invertebrates and fishes. They perform over 90% of the total photosynthetic activity.

In addition to being a key player in aquatic productivity, several cyanobacteria are capable of fixing atmospheric nitrogen either as free-living organisms (e.g. *Nostoc, Anaebana, Oscillatoria, Scytonema, Calothrix*) or in symbiosis with many other plants (e.g. *Anaebana* with *Azolla, Nostoc* with *Cycus*). It can convert inert atmospheric nitrogen into an organic form, such as nitrate or ammonia, which is made available for

aquatic eukaryotic phytoplankton as well as higher plants (Kumar et al., 1996). Nitrogen is fixed in structures called heterocysts, which are formed when the cyanobacteria grow in long chains under nitrogen deprivation (Meeks and Elha, 2002). The cell that becomes a heterocyst keeps all oxygen away from the nitrogenase (which is destroyed in the presence of oxygen) and the cell then uses the nitrogenase to make ammonia and passes it on to the other cells in the chain. This function of nitrogen fixing activities is very essential for survival of other form of algae and higher plants. This ability of nitrogen fixation gives them a great advantage over other organisms when there is no usable nitrogen in the environment.

Three kinds of cyanobacteria have been shown to fix nitrogen, the heterocystous filamentous species, non-heterocystous filamentous species and non-heterocystous unicellular species. A heterocystous filamentous species are capable of fixing atmospheric nitrogen under anaerobic conditions. However, several non-heterocystous filamentous cyanobacteria can fix atmospheric nitrogen under microaerophilic conditions in vegetative cells (e.g. *Plectonema boryanum).*

The nitrogen fixing property of cyanobacterial species make major contributions to the world food supply by naturally fertilizing soils and rice fields. The amount of nitrogen being fixed by biological organism on global scale is about 75% or 175 X 10^{12} g per year, or 10^6 metric tons per year and a large part of it is fixed by cyanobacteria (Bezdicek and Kennedy, 1998). Introduction of cyanobacteria to saline and alkaline soils in the state of Uttar Pradesh increased the soil's content of nitrogen and organic matter and also their capacity for holding water and enabled formerly barren soils to grow crops

(Singh, 1961). Similarly cultivation of *Tolypothrix tenuis* in rice field in Japan resulted in a 20% increase of rice crop productivity (Watanabe, 1956). A coating of cyanobacteria on prairie soil binds the particles to the soil to their mucilage coating, which help in maintains a high water content and reduction in soil erosion facilitating better yield (Booth, 1941).

Cyanobacteria are used as a health food due to having high protein content. *Spirulina* is a staple food in parts of Japan, China, Africa and Mexico. It is used as food supplement because of its excellent nutrient composition and digestibility. It has high protein content (60–70%), 20% carbohydrate, 5% lipids, 7% minerals and 6% moisture. *Spirulina* is also a rich source of β-carotene, thiamine and riboflavin and is one of the richest sources of vitamin B_{12}. It is commercially available in the market in the form of powder, granules or flakes and as tablets and capsules. Some strains of *Anabaena* and *Nostoc* are consumed as human food in Chile, Mexico, Peru and Philippines. *Nostoc commune* with high amount of fibre and moderate protein is of potential use as a new dietary fibre source and can play an important physiological and nutritional role in human diet (Jeraci and Vansoest, 1986). Some parts in North America culture and commercially process some cyanobacteria for various food and other products such as vitamins, drug compounds and growth factors. Cyanobacteria e.g. *Microcystin*, *Lyngbya lagerheimii* produce a variety of biologically active natural compounds e.g. *Phormidium tenue*, *Oscillatoria laete-viriane* that have shown potential application in major disease management, such as cancer, asthma, arthritis, diabetes, HIV etc (Skulberg, 2000).

Cyanobacterial/algal lipids have proved to be of considerable commercial value as nutritional supplements in cosmetics, and as substitutes for numerous petroleum products. This is because algae are recognized producers of C_{18} and C_{20} polyunsaturated fatty acids (PUFAs) (Kayama et al., 1989). These acids are essential for nutrition of many animals and humans and are used for the biosynthesis of eicosanoid hormone (Gerwick and Bernart, 1993) and are of interest in biotechnology and more recently in cosmetics (Servel et al., 1994). The lipids found in cyanobacteria provide a concentrated energy source as these glycolipids are composed of both sugar and lipid parts. The lipid part is important in the conversion of β-carotene into vitamin A and serves as a carrier of vitamins A, D, E and K and ionic minerals through the blood cell barrier. Fatty acids found in marine algae has aroused considerable interest among researchers that produce the long chain polyunsaturated fatty acids (LC-PUFAs) docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) since these compounds are now recognized as having a number of important neutraceutical and pharmaceutical application (Shahidi and Wanasundara, 1998; Horrocks and Yeo, 1999).

A variety of fine chemicals such as pigments, vitamins and enzymes with varied applications can be obtained on a commercially viable scale from cyanobacteria. Some cyanobacteria e.g. *Anaebana cylindrica, Anacystis nidulans* are potential source for large-scale production of vitamins of commercial interest such as vitamins of the B-complex group and vitamin E (Borowitzka, 1988). The carotenoids and phycobiliproteins, characteristic of cyanobacteria have high commercial value. They are used as natural food colourants e.g. *Phormidium valderianum*, as food additives to enhance the colour of the flesh of Salmonid fish and to improve the health and fertility of cattle (Emodi, 1978).

Cyanobacteria secrete enzymes that can be exploited commercially, which can be marketed at low cost since relative biomass production of cyanobacteria is much less expensive than bacteria (Elhai and Wolk, 1988). In addition, cyanobacteria are a rich source of several poly-β-hydroxybutyric acid e.g. *Chlorogloea fritschii,* polysaccharides e.g. *Oscillatoria princeps*, lipids e.g. *Arthrospira, Anacystis nidulans*, fatty acids e.g. *Spirulina platensis*, sterols e.g. *Spirulina maxima* etc. with varied properties as flocculants, surfactants and others (Becker, 1994).

Cyanobacteria have also showed immense potential in wastewater and industrial effluent treatment, bioremediation of aquatic and terrestrial habitats, chemical industries, biofertilizers, food, feed and fuel, cosmetics, etc (Fatima, 1999). The use of algae and cyanobacteria in waste treatment is beneficial in different ways since they can bring about oxygenation and mineralization, in addition to serving as food source for aquatic species e.g. *Oscillatoria*, *Aphanocapsa* and a halophilic bacterium *Halobacterium* (Uma and Subramanian, 1990).

Many species of cyanobacteria produce compounds that are toxic to humans and animals e.g. *Microcystin.* Cyanobacterial toxins are the naturally produced poisons stored in the cells of certain species, which is usually released into the water when the cells rupture and die. Although humans ordinarily avoid drinking water that displays a blue-green bloom or scum, they may be affected by toxic strains when they swim or skin recreational water bodies during a bloom. Typical symptoms include redness of the skin and itching around the eyes, sore, red throat, headache, diarrhea, vomiting and nausea. Some are known to attack the liver hepatoxins, or the nervous system neurotoxins, or

irritate the skin. Cyanobacteria e.g. *Microcytin* produce a large number of peptide compounds such as micropeptides, cyanopeptolins, microviridin, circinamide, aeruginosin, with varying bioactivities and potential pharmacological application (Lakshmāna Rao et al., 2002).

Cyanobacteria, being prokaryotic in structure with photosynthesis similar to higher plants form simple model system for the understanding impact of changing environment on photosynthesis and related physiological and biochemical processes. Cyanobacteria also being easy to culture and fast growth cycle are also ideal to study various basic processes such as cellular metabolism, synthesis of macromolecular compounds, cell differentiation and regulation of gene expression.

1.2. CHANGES IN CLIMATIC CONDITIONS AND ITS IMPACT ON PRIMARY PRODUCERS:

The changes in climate, especially increased atmospheric carbon dioxide, temperature and precipitation, associated with changes in tropospheric and stratospheric ozone levels, UV-B radiation, etc. can affect various physiological processes thereby affecting world agricultural production. Increasing consumption of fossil fuels such as oil, gas and coal, in order to satisfy human energy needs, results in large quantities of carbon dioxide being emitted into the atmosphere (Boden et al., 1994). Agricultural and industrial activities also add considerable amounts of methane (CH_4), nitrous oxides (N_2O) and chlorofluorocarbons (CFCs) to the atmosphere. Collectively, all of these gases lead to the enlarged greenhouse effect (Houghton et al., 1990). The heating occurs when gases such as carbon dioxide trap heat escaping from the Earth and radiate it back to the

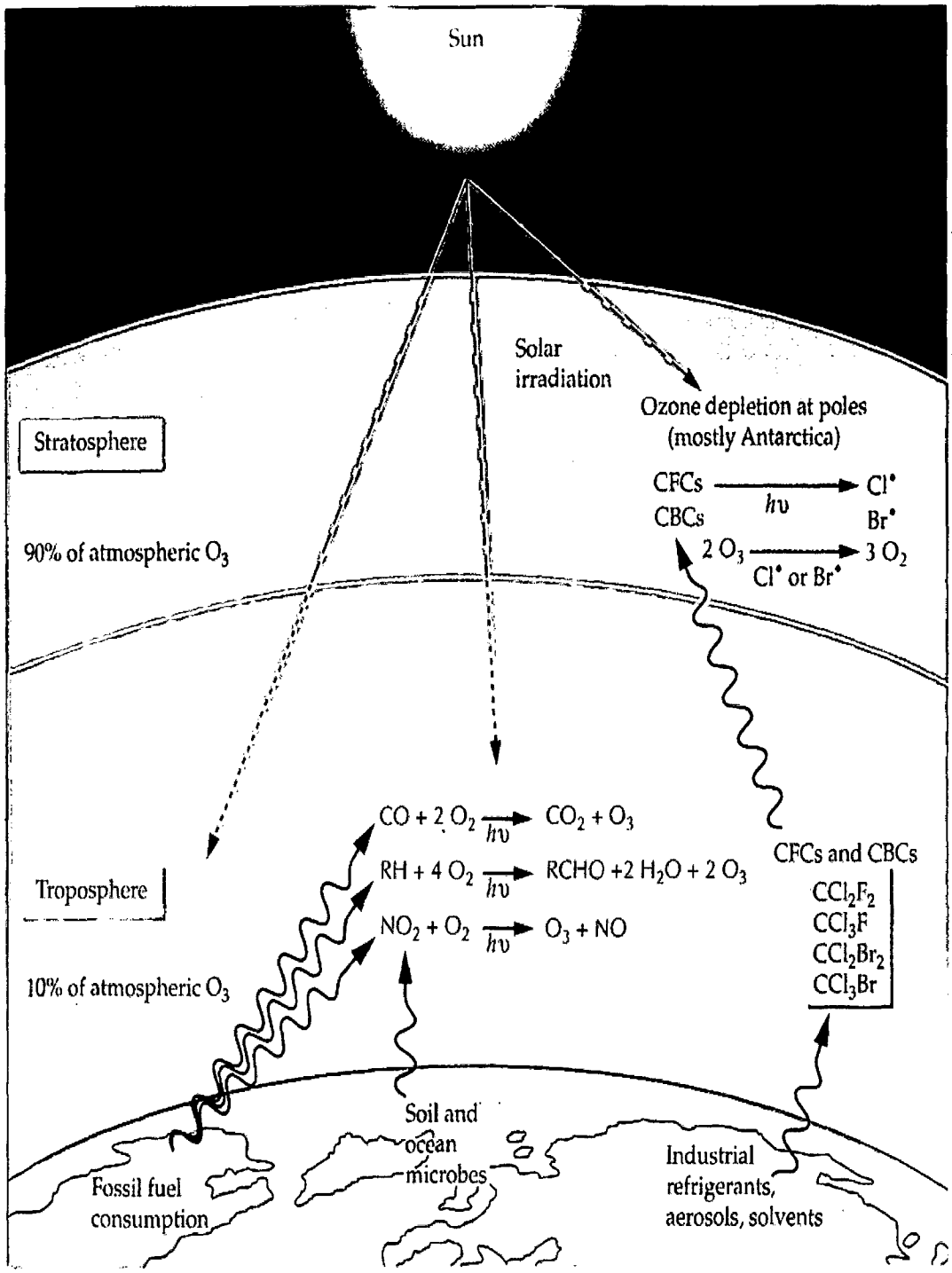


Fig 1.2 Incidence of solar radiation and upward transmission of surface radiated waves showing mechanism of generation of oxygen, carbon dioxide and nitrous oxide which act as green house gases warming the atmosphere and break down of ozone in stratosphere causing increase in the UV-B radiation reaching the earth.

surface because the gases are transparent to sunlight but not to heat and thus act like the glass panel in a greenhouse which is so called greenhouse effect. The greenhouse effect leads to increase in atmospheric carbon dioxide and global warming, with an increase of the global atmospheric temperature. The rise of the atmospheric temperature affects growth of plants and animals and will change the geographical distribution of plant and animal species with unknown effects on the functioning of the ecosystems. Ozone layer depletion is also likely to increase the rate of greenhouse warming, by reducing the effectiveness of the carbon dioxide sink in the oceans as increase in UV-B radiation causes damaging effect to primary producers in the aquatic ecosystem. Sulfur dioxide and nitrogen oxides released during the combustion of fossil fuels combine with water and oxygen in the atmosphere to form the precipitation of sulphuric acid and other acids (acid rain), which affects the quality of natural ecosystems. Singularly and collectively all these changes will have serious consequences on growth and development of primary producers and thereby on consumers and therefore, need to be studied in greater detail.

Ozone depletion and the consequent increases in UV radiations affect terrestrial and aquatic biogeochemical cycles thus altering both sources and sinks of greenhouse and chemically-important trace gases e.g., carbon dioxide (CO_2), carbon monoxide (CO), carbonyl sulfide (COS) and possibly other gases, including ozone. This could lead to a buildup of these gasses in the atmosphere and therefore global warming could also be an indirect effect of ozone depletion; contributing to the melting of the polar ice caps.

1.2.1. ULTRAVIOLET-B RADIATION AND ITS EFFECT:

Atmospheric ozone layer is important to the life on earth, as it is a strong selective absorber of ultraviolet (UV-B) radiation, which acts like a giant sunshade, protecting plants and animals. Ozone (O_3) forms a layer in the stratosphere, 15-40 km above earth surface. Due to the human activities such as production of chlorofluorocarbons, halons, there is a depletion of the ozone layer, resulting in increase in the UV-B radiation on the surface of the earth and to ecologically significant depths in the ocean. The amount of UV-B radiation that penetrates through the ozone layer decreases exponentially with the thickness/density of the layer. The most dramatic loss of ozone has been observed over Antarctica during the austral spring where a 50% reduction in ozone, the widely discussed ozone hole, has been documented. The ozone layer is usually thinnest at the tropics and thickest towards the poles. In the last decades, the quantity and quality of UV radiation reaching the earth's surface changed (Allen, 1994), the increase of UV-B radiation compared to the 1970s ranges from 4 to 130% depending on season and location on earth (Madronich et al., 1998).

Chlorofluorocarbons (CFCs), halons, methyl chloroform, methyl bromide, carbon tetrachloride and several other chemicals are ozone-depleting substances. CFCs are completely artificial substance and used in air conditioning/cooling units, as aerosol spray propellants, and in the cleaning processes of delicate electronic equipment, and are a byproduct of some chemical processes. CFC molecule and halons takes 15 years to go from the ground level up to the upper atmosphere, and it can stay there for about a century. Under the influence of the sun's ultraviolet light, CFC molecules break up and

release chlorine atoms. Free chlorine atoms then react with ozone molecules, taking one oxygen atom to form chlorine monoxide and leaving an ordinary oxygen molecule, destroying up to one hundred thousand ozone molecules.

$$Cl + O_3 \longrightarrow ClO + O_2$$

$$ClO + O \longrightarrow Cl + O_2$$

Because it takes so long for the CFCs and halons to reach the stratosphere, any reduction in their use on earth does not have an immediate effect on the concentration in the stratosphere. Some of the ozone depleting substances is persistent, remaining active in the atmosphere for up to 50 years, therefore it will take considerable time to replenish the depleted ozone, if we even start complete ban of CFC's on earth. According to the global climate model (Shindell et al., 1998), levels of ozone in the stratosphere will continue to fall, despite reduction in emissions of ozone depleting CFCs, with especially severe declines occurring in the years 2010–2019 in the northern hemisphere. Recovery of stratospheric ozone to early 1980s levels is not predicted until roughly 2050.

UV-B is a normal component of sunlight, a significant increase in UV-B radiation above natural levels could be harmful to the environment and human health. UV-B damage depends on the amount of atmospheric ozone layer that can act as a filter, the angle of the sun in the sky, and cloud cover, which shields the surface from some of the ultraviolet radiation.

Ultraviolet radiation is divided into three types, according to wavelength. The shortest and most damaging UV wavelengths, UV-C, which is lethal, (190 - 280 nm), is

absorbed strongly by the oxygen, water vapors and ozone so that negligible amounts reach the earth's surface. UV-B radiation (280-320 nm) is absorbed by ozone and its concentration in the earth's atmosphere is increasing greatly due significant decrease in the stratospheric ozone. Since it is largely UV-B, which is increasing in the atmosphere, it is relevant to study its impact on biological system. There has been considerable study on impact of UV-B on higher plants and crop plants but very little information is available on its impact in aqueous organism. The longest wavelength of ultraviolet radiation (UV-A, 320 - 400 nm) are known to induce both photodamage and photoreactivation processes in living cells (Caldwell et al., 1986). This radiation is relatively unaffected by variations in stratospheric ozone concentrations as it is not absorbed by ozone.

Since plants need sunlight to synthesize their own food by the process of photosynthesis, as a consequence autotrophs are exposed to UV radiation present in the solar energy spectrum. Though UV-B radiation comprise only a small portion of electromagnetic spectrum but has a disproportionately large photo biological effects since the energy content of the UV-B radiation is greater than visual radiation. UV-B radiation is biologically effective and absorbed by various biological compounds such as DNA, pigments, proteins, chromophores, plastoquinol etc that resulted in loss of growth and survival of autotrophs with severe consequences. Some of these components also absorb UV-C (being lethal) and UV-A, but since there population is not increasing as significantly as UV-B in the environment, in this study emphasis is being given in the study of UV-B with reference to two important cyanobacteria.

In aquatic system both incident solar radiation and the depth of penetration into the water column are key factors in assessing the potential for damage to aquatic organism. The penetration of short wavelength solar radiation in an aquatic system strongly depends on the content of dissolved and particulate substances as well as the concentration of phytoplankton. UV rays are not so much reflected from the water surface, as they mostly penetrate it, and may go deep into the water (Kuhn et al., 1999). There is often pronounced variability and seasonal changes in the transparency and it has been shown that UV more than red region of electromagnetic spectrum can penetrate as deep as 40 meters and may lose only 25-30% of its intensity (Menon et al., 2005). Water absorbs strongly in the red and infrared. The net effect of both scattering and absorption of solar radiation effectively enriches the penetrating down welling spectrum in the blue and blue green wavelengths. As irradiance penetrates into the water the scattering process increasingly randomize the angular distribution of light such that the light becomes more diffuse with depth. In nature, the effectiveness of UV-B radiation can be exacerbated by combination of several factors such as increase dissolved/atmospheric carbon dioxide, temperature, mineral nutrients availability, heavy metals and air pollutants etc.

The increased UV-B is detrimental to all forms of life, especially photosynthetic organisms (Jordan, 1996) that cannot avoid UV-B damage because of their requirement for light. The increase in solar UV-B radiation reaching the earth's surface impairs terrestrial and aquatic ecosystems (Häder et al., 1998), affects the health of humans and animals (Longstreth et al., 1998), alters biogeochemical cycles (Zepp et al., 1998) and air quality (Tang et al., 1998) and deteriorates the properties of materials (Andrady et al., 1998). Potential consequences of enhanced level of exposure to UV-B radiation include

changes in species composition, reduced uptake capacity for atmospheric carbon dioxide and loss of biomass, decrease in availability of nitrogen compounds, such as food sources for humans etc.

1.2.1.1. IMPACT ON HIGHER PLANTS:

A high increase in UV-B radiation may disrupt many ecosystems on land. Many agricultural crops including varieties of important food staples have been shown to be adversely affected by the increase in UV-B radiation. UV-B radiation affects the nitrogen fixing organisms by damaging the enzymes by which they can fix atmospheric nitrogen that lead to the reduction in the supply of nitrogen to ecosystems and as a result decrease in rice production (Kumar et al., 1996b).

The effects of increased UV-B radiation are manifold, with marked decreases in yields of agricultural crop plants (Mazza et al., 1999), damage to photosystem II (PS II) and photosystem I (PS I), disturbance in carboxylating enzyme, DNA integrity etc. Ultraviolet-B radiation causes oxidative stress (Mackerness et al., 1998) which in turn affect cell membranes damage (Chow et al., 1992) and ultra structure of various organelles (Brandle et al., 1977) as well as important biochemical pathways was affected, (Jordan et al., 1992). Various developmental processes in plants are also affected leading to inhibition of pollen germination, plants growing more slowly and becoming smaller and more stunted when exposed to UV-B.

1.2.1.2. IMPACT ON AQUATIC PHOTOSYNTHETIC SYSTEM:

Primary producers need solar radiation to synthesize their own food by photosynthesis and thus cannot avoid the damage from the enhanced UV-B radiation and hence resultant oxidative damage (Ehling and Scherer, 1999; Quesada and Vincent, 1997). The penetration of increased amounts of UV-B radiation caused damaging effects on various natural populations of aquatic organisms (Calkins and Thordardottir, 1980), which densely populates the top 2 meters of water. Considering the vital role of phytoplanktons, algae and cyanobacteria as producers and biofertilizers in aquatic ecosystem and crop production, the fluence rate of UV-B radiation impinging on the natural habitat seems to be of major concern.

The works of numerous investigators provides strong evidence that exposure to UV-B radiation decreases primary productivity and causes damage to various forms of organisms in aquatic ecosystems (Cullen and Neale, 1994; Tevini, 1993; Biggs and Joyner, 1994; Karentz et al., 1994; Smith and Cullen, 1995). Karentz et al., (1994) suggests that small organisms because of their size and short generation times are likely to be more susceptible to UV-B stress than larger organisms. UV-B radiation has been reported not only to impair motility and photo-orientation (Donkor and Häder, 1991) but also to affect a number of physiological and biochemical processes such as growth, survival, photosynthesis, pigmentation, total protein profile, DNA, enzyme activity and nitrogen incorporation in cyanobacteria and algae (Sinha et al., 1995; Häder and Worrest, 1991; Wood, 1987). Young developmental stages of algae (zoospores, gametes, zygotes and young germlings) are extremely susceptible to UV radiation stress (Coelho et al.,

2001), there is loss of viability and cellular disintegration in kelp zoospores (Dring et al., 1996). UV-B radiation affects major biochemical constituents by reducing uptake of inorganic nutrients, ammonium, nitrate and phosphorus in phytoplankton, marine diatoms and freshwater flagellates. Thus the reported effects on cell morphology and biochemical constituents could have profound effects on grazers and energy transfer in aquatic food webs (Hessen et al., 1997).

Negative affect of increased UV-B radiation would lead to loss of productivity of phytoplankton and limit the amount of food these organisms create through photosynthesis in aquatic ecosystems on Earth (Smith et al., 1992). Planktons, phytoplanktons as well as zooplankton are highly sensitive to UV-B radiation, as they lack the protective processes that higher forms of plants and animals have. Phytoplankton is the basis of freshwater and marine food chains and phytoplankton losses in aquatic ecosystems caused by ozone depletion reduce the quantity of zooplanktons, fish in the oceans, rivers and lakes and directly affect the global food supply. Solar UV-B radiation has been found to cause damage to early developmental stages of fish, shrimp, crab, amphibians and other animals (Shick et al., 1991). The most severe effects are decreased reproductive capacity and impaired larval development. Fisheries are also affected by increase in UV-B radiation through disruption of the aquatic food chain or the direct killing of eggs and larvae of commercially valuable species.

Human health will also be affected due to enhanced level of UV-B by way of sunburn, snow blindness, eye damage, early ageing of the skin, wrinkle formation and rising rates of skin cancer. It is also known to cause suppression of the immune response

system. A 1% decrease in the ozone layer will cause an estimated 2% increase in UV-B radiation, it is estimated that this will lead to a 4% increase in basal carcinomas and 6% increase in squamous cell carcinomas (Graedel and Crutzen, 1995). High doses of UV light can cause eye damage by temporary clouding of the cornea, called snow-blindness, and chronic doses have been linked to the formation of cataracts. Increased levels of UV-B can have impacts on other aspects of human welfare as well such as disruptions of food supply, deterioration of the quality of the air, water and soils, contribution to the greenhouse effect, and resultant climate change. Industrial pollutants become much more toxic if exposed to UV rays and if these pollutants then get into the waterways it makes the water undrinkable. UV radiation also results in the degradation of plastics and other materials widely used by society. Plastic materials used outdoors will have much shorter lifetimes with small increases of UV radiation.

1.2.2. LIGHT AND ITS EFFECT:

Light is a form of electromagnetic radiation. The electromagnetic spectrum of solar radiation covers a wide range of wavelengths from long waves, the infrared part of the spectrum, the photosynthetic active radiation (PAR 700-400 nm) and the shorter wavelengths including ultraviolet radiation (UV 400-190 nm). Solar radiation is essential for all forms of life on the earth and photochemical reactions are some of the most important processes taking place in our environment.

Light intensity is measured as radiant flux density or irradiance. Since photochemical reactions in photosynthesis depend more on the number of photons incident on a surface rather than on the energy content of these photons, it is more logical

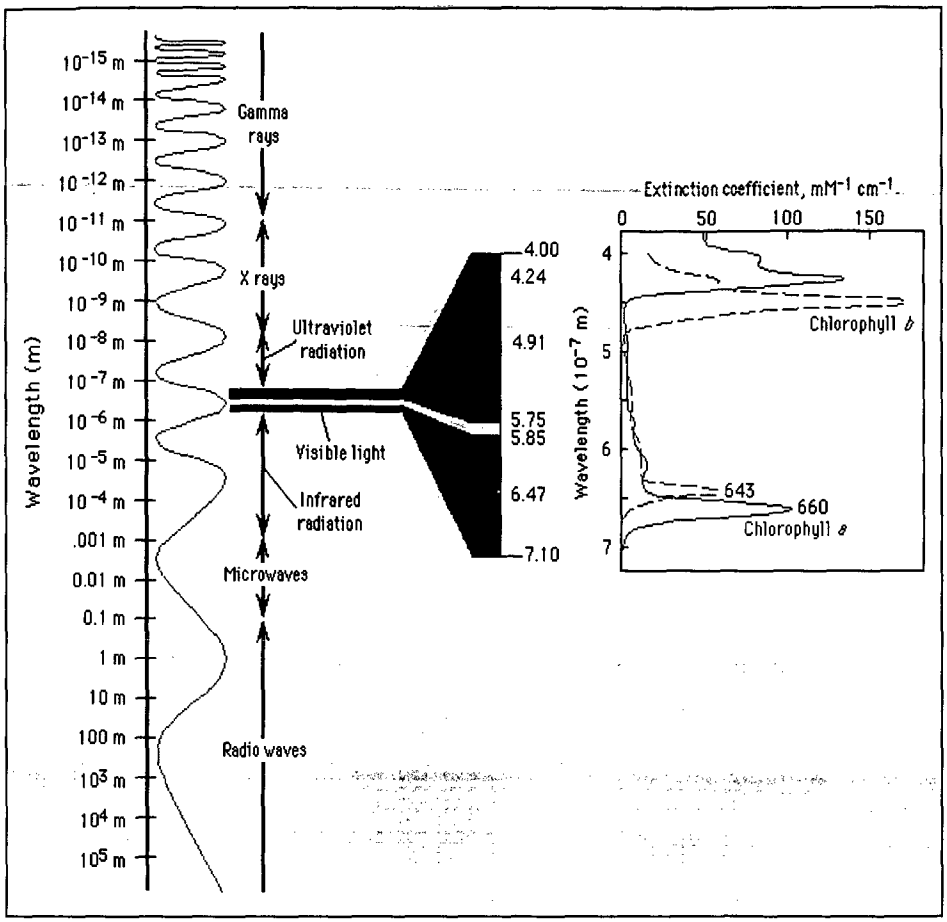


Fig 1.3 Electromagnetic spectrum of solar radiation and absorption of visual radiation by chlorophylls.

to express photosynthetic irradiance in terms of the number of quantum (photons) falling on unit surface in unit time (PFD). The photon flux density in a particular wave region is measured in units of mol m^{-2} s^{-1}.

Light intensity reaching the earth's surface shows much variations being influenced by various factors like atmospheric gases (nitrogen and oxygen), suspended particles (solid particles, dust, smoke) and in waters (clay, silt, plankton). Also water layers, layers of vegetation, topographic factors such as direction and slope of the land surfaces causes marked variations in intensity and daily duration. Light intensity decreases exponentially with depth in the water column with blue green light (480 nm) penetrating the furthest in clear waters (Menon et al., 2005). Light and gravity are employed to guide the organisms to depths of optimal irradiation resulting in typical vertical distribution patterns found in both freshwater and marine ecosystems (Lindholm, 1992; Eggersdorfer and Häder, 1991). Many phytoplanktons are capable of active movements and daily vertical migrations of up to 15 m. Photosynthetic active zone has been reported as deep as 40 m in deep ocean (Menon et al., 2005).

In aquatic ecosystem, solar radiation affects physical, chemical and biological processes such as thermal stratification and the vertical distribution of nutrients, photoautotrophs, herbivore and consumers. Light intensity is known to affect the pattern of macromolecular synthesis from photosynthetically fixed carbon dioxide. Also it is the most significant environmental factor influencing the light harvesting complexes (phycobilisomes) in cyanobacteria.

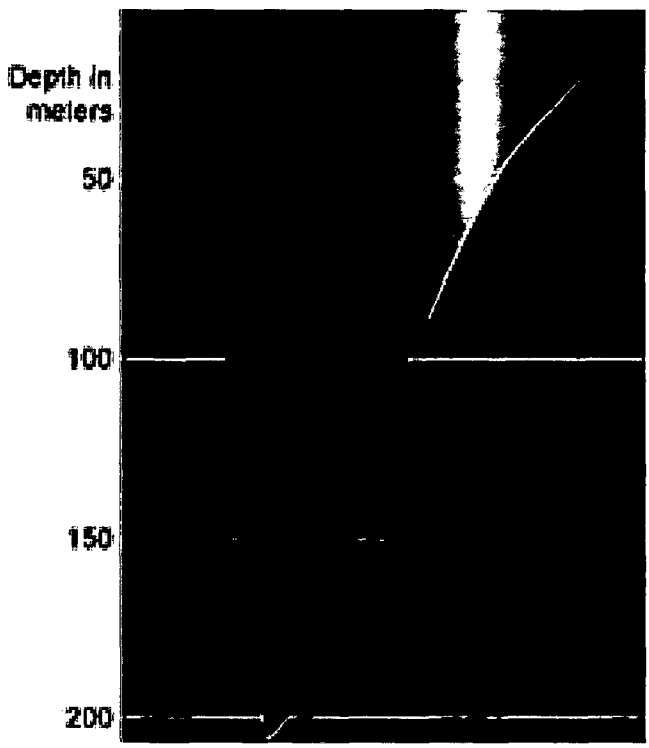


Fig 1.4 Figure showing penetration of light in water

Though sunlight is beneficial as primary producers rely on it for photosynthesis, however, it can also be damaging to photosynthesis, particularly when the planktons are exposed to high light intensities for prolonged periods of time (Xenopoulos et al., 2000). High solar radiation causes damage to the photosynthetic antennae and photobleaching of pigments (Carr and Whitton, 1982; Maegawa et al., 1993; Sagert et al., 1997; Hanelt, 1998). High light exposure results in generation of AOS, which lead to secondary damage through oxidative damage to various biomolecules such as lipids, protein and DNA. The depletion of photosynthetic capacity as a result of over excitation is followed by a decrease in the primary production that is growth rate, increasing pigment photo bleaching and tissue damage in algae from shaded and deep areas after exposure to sunlight (Grobe and Murphy, 1994). Solar radiation also affects the nitrogenase activity and carbon dioxide uptake in cyanobacteria associated with paddy field (Tyagi et al., 1992). At higher light intensities protein synthesis is decreased and the excess carbon is stored as carbohydrate (Konopka and Schnur, 1980). Changes in fatty acid or lipid quality were also observed during light acclimation of algae in addition to quantitative changes in chloroplast lipids (Sewon et al., 1997; Klyachko-Gurvich et al., 1999). Cyanobacteria, like algae and higher plants, have also developed a large number of mechanisms to allow optimal use of absorbed light energy and to avoid oxidative damage induced by excessive excitation (Prasil et al., 1992; Keren and Ohad, 1998).

1.2.3. TEMPERATURE AND ITS EFFECT:

The oceans play a key role with respect to global warming as a sink of carbon dioxide. Reports through various climatic models suggest that due to the doubling of the

carbon dioxide concentration air temperature may rise by 1.5 - 4.5°C accompanied with a 1m rise in sea level by 2080 (IPCC, 1990). Atmospheric levels of carbon dioxide have increased mainly due to human activities such as deforestation as a result of expansion of agriculture and burning of fossil fuels, which leads to the global warming. Primary producers in the oceans assimilate large amounts of atmospheric carbon dioxide thereby acting as sink for environmental carbon dioxide. Climatic changes such as UV-B, temperature etc. significantly reduces photosynthetic activity thus large amounts of carbon dioxide will remain in the atmosphere further exacerbating the global warming phenomenon. A 10% decrease in carbon dioxide uptake by the oceans will leave about the same amount of carbon dioxide in the atmosphere as is produced by fossil fuel burning. The results of all this will be further rise in temperature. Higher temperatures affect primary producers by reducing carbon sink consumers, species composition, expansions of algal populations as well as secondary consumers, trophic dynamics and biogeochemical cycling (Zepp et al., 1998).

1.3. CLIMATIC CHANGES AND ITS IMPACT ON:

1.3.1. GROWTH AND DEVELOPMENT:

The main factors that determine the growth and development of algal populations are light, temperature, pH, nutrient concentration and the presence of organic solutes. The nutritional requirements of the algal culture can easily be met by supply of nutrients, however, environmental factors such as light and temperature, are more difficult to control in outdoor systems. Literature is available describing various techniques and media to be employed in culturing fresh water and marine algal species. All the various

techniques and media employed attempts to do one of the things, either to duplicate natural conditions as nearly as possible or to provide a set of artificial conditions, which are suitable for growth and can produce desired results.

Algae are placed into artificial cultivation conditions for a number of reasons;

(1) To trace the life cycle of a species and to learn the morphological and genetical phases of life cycle.

(2) To study the physiology of algae; to study the mechanism and products of photosynthesis, elaboration of ergastic substance; respiration, nutrition requirements, qualitative and quantitative response to stress factors.

(3) To determine the chemical composition of algae for both scientific and practical purposes.

(4) To study production and productivity rate.

The changes in specific growth rate during the development of culture indicate that the following phases can be distinguished in the growth of a batch culture: (1) lag phase, (2) accelerating phase, (3) logarithmic phase (balanced growth), (4) decelerating phase, (5) stationary phase, (6) death phase. Each growth phase is a reflection of a particular metabolic state of the cell population at any given time.

Algae may be grown in batch cultures or in continuous cultures. A batch culture is initiated by the transfer of a small portion of a culture into a new culture medium resulting in growth and an increase in biomass. Biomass concentration can be measured in many ways, as cell number, dry weight, packed cell volume, or in terms of any

convenient physiological or biochemical components e.g. fv/fm ratio, photosynthetic pigment concentration. Measurements of productivity are based on determination of increase in fresh weight, carbon dioxide incorporation or oxygen production. By experimentation and trial and error, scientists can study the degrees to which media, light, temperature etc. must be varied for the proper growth of different algal groups or even species.

Studies of the effects of solar UV radiation on aquatic primary producers have shown deleterious consequences for growth and survival. Growth and survival of several rice field cyanobacteria have been reported to be severely affected following UV-B irradiation for different durations (Sinha et al., 1998; Häder et al., 1999). High UV radiation resulted in reduced rates of carbon fixation that lower rates of algal growth and changes in algal growth rates can lead to major changes in cellular biochemistry and hence, elemental composition (Kilham et al., 1997). A reduction in primary productivity of phytoplankton was observed in the Antarctic region during the spring due to UV-B and impairment of these processes can affect growth and survival rates (Smith et al., 1992). Growth rate and motility is strongly impaired by solar radiation with or without UV radiation in dinoflagellates (Nielsen et al., 1995). Reports of phytoplankton growth being affected more strongly by UV-A and UV-B radiation in spring than in summer, possibly due to shifts in the dominant species present (Xenopoulos et al., 2002). At high light intensities, the growth rate of cyanobacteria was much lower than that of other algal species (Hoogenhout and Amesz, 1965; Reynolds, 1984).

1.3.2. PHOTOSYNTHESIS

Photosynthesis is the characteristic method of nutrition in the cyanobacteria, which is defined as the synthesis of organic compounds through the assimilation of carbon dioxide using sunlight as an energy source. Carbon dioxide is incorporated into a 5-carbon acceptor, ribulose-1, 5-bisphosphate (RuBP) in an energy requiring reaction catalyzed by the primary carboxylating enzyme, RuBP carboxylase. The product splits into two molecules of a 3-carbon compound, phosphoglyceric acid (PGA), and the reduction of PGA, mediated by the electron carrier NADPH leads to the formation of a series of sugar phosphate intermediates and finally to glucose. During this sequence of metabolic transformations, the acceptor RuBP is generated, ready to accept another carbon dioxide molecule. Cyanobacteria fix carbon dioxide via the Calvin-Benson-Bassham pathway and deposit part of the fixed carbon as glycogen, which serves as a carbon and energy reserve.

$$6H_2O + 6CO_2 \longrightarrow C_6H_{12}O_6 + 6O_2$$

Cyanobacterial photosynthetic apparatus is similar to that of plants except that light is harvested mainly by phycobiliproteins, the water-soluble biliproteins. The light-harvesting complexes of photosystem II of cyanobacteria are the phycobilisomes (PBS), large pigment-protein complexes associated with the cytoplasmic stroma side of the thylakoid membrane. Cyanobacteria have an elaborate and highly organized system of internal membranes, which function in photosynthesis. Chlorophyll *a* and several accessory pigments (phycoerythrin, and phycocyanin) are embedded in these photosynthetic lamellae. It is shown that light absorbed by the phycobiliproteins is used

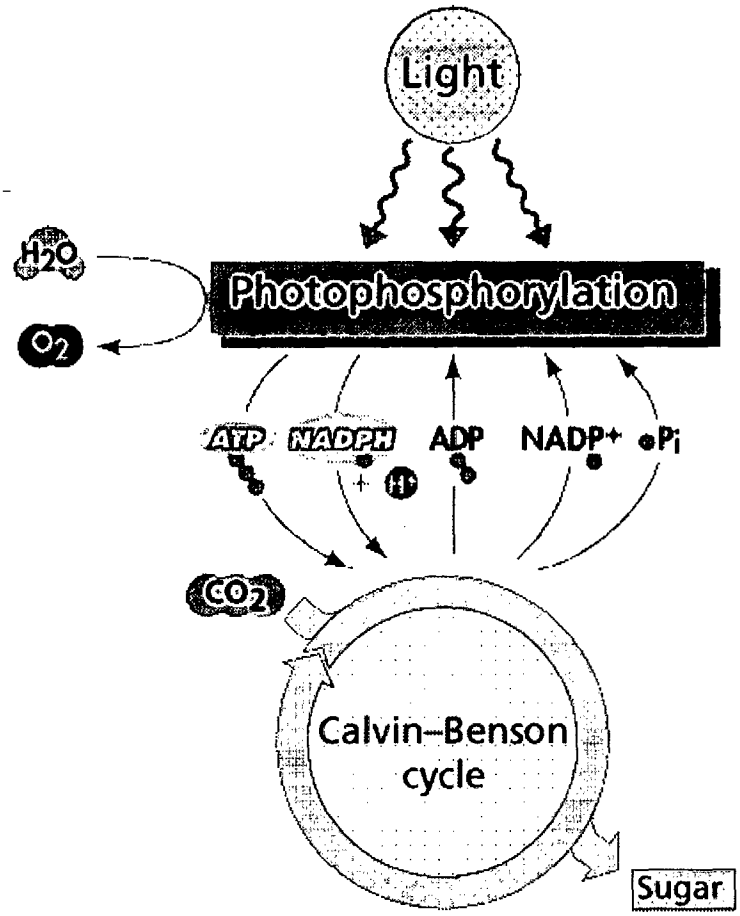


Fig 1.5 The basic steps in photosynthesis

by cyanobacteria as efficiently as light absorbed by chlorophyll. Excitation energy is transmitted from phycobiliprotein pigments to chlorophyll with great efficiency. The concentration of the carboxylating enzyme, RuBP carboxylase is in general high in cyanobacteria, amounting up to 30% of the total cell protein. Cyanobacteria are able to utilize both carbon dioxide and bicarbonate ions as a source of inorganic carbon in photosynthesis. Bicarbonate ions are transported in the light across the plasma membrane and accumulate in the cell to serve as an inorganic carbon pool for photosynthesis. Cyanobacteria use light as their energy source and oxygen as their final electron acceptor when they are going through respiration and making energy.

Light is harvested by pigment protein complexes in the antenna system. Cyanobacterial antenna system is different from the plant system. Cyanobacteria depend on chlorophyll a and specialized water soluble protein complexes (phycobilisomes) to gather light energy (Sidler, 1994). Chlorophyll a is located in membrane bound proteins and phycobilisomes are bound to the outer side of the photosynthetic membrane and act to funnel exciting energy to the photosystem II reaction center. They are composed of phycobiliproteins, protein subunits that contain covalently attached open ring structures known as bilins that are the light absorbing pigments. Primary photochemistry, electron transport, phosphorylation and carbon reduction occur in similar way as they do in chloroplasts of higher plants.

UV-B induced inhibition of photosynthetic activity, decline in carbon fixation, and damage to PS II reaction centre has been demonstrated in a number of marine and freshwater cyanobacteria (Sinha et al., 1997; Babu et al., 1998). The inhibition of

photosynthesis is characterized by photodamage of PS II reaction centers and subsequent proteolysis of the D2 protein (Critchley and Russell, 1994). In addition to the bleaching of the photosynthetic pigments, RUBISCO (ribulose-1,5-bis-phosphate carboxylase/oxygenase) activity was also severely affected by UV-B treatment and reported that at lower UV-B doses the energy transfer to the reaction centre of photosystem II is impaired (Sinha et al., 1996).

Solar UV-B level is reported to depress photosynthesis in microorganisms at Antarctica, resulting in reductions in marine productivity by 5% to 20% (Smith et al., 1992). Phytoplankton organisms are quite sensitive to enhanced UV-B radiation, at the level of photosynthetic apparatus and pigments involved in light reception and in photosynthetic production, carbon and nitrogen metabolism and growth rate (Sinha et al., 1995). Inhibitory effects of UV-B on photosynthesis and chlorophyll fluorescence of several species of marine benthic algae and phytoplankton has been documented (Larkum and Wood, 1993).

Solar radiation of high fluence rate on micro algae (Hanelt, 1992) or phytoplankton (Helbling et al., 1992) causes photoinhibition, which is characterized by a reduction in the quantum yield and in the capacity of photosynthetic oxygen evolution and photosynthetic carbon dioxide fixation. The molecular mechanism of photoinhibition, which is reported to be similar to large extent under visual or UV-B radiation, revealed that the light-induced damage was caused by inactivation of the D1 protein of the PSII complex (Aro et al., 1993; Kanervo et al., 1993; Tyystjärvi et al., 2001). The damaged D1 protein is degraded proteolytically leaving the PSII complex

depleted of the D1 protein. Excess solar radiation causes photoinhibition resulting in decrease oxygen production and affected the ratio of Fv/Fm during periods of bright sunshine in algae (Hanelt et al., 1993; Herrman et al., 1995). Recent studies have demonstrated that photosynthesis was inhibited in many red, brown, and green benthic algae due to excess light conditions.

1.3.3. PHOTOSYNTHETIC PIGMENTS:

Photosynthetic pigments are pigment protein complexes, which reflect certain wavelengths of visible light while absorbing other and this makes them appear colorful. The photosynthetic pigments impart a rainbow of possible colors yellow (carotenoids, xanthophylls), red (phycoerythrin), green (chlorophyll), deep blue (phycocyanin) and blue-green (chlorophyll b). In plants, algae, and cyanobacteria, pigments are the means by which the energy of sunlight is captured to make their own food during photosynthesis. However, since each pigment reacts with only a narrow range of the spectrum, there is usually a need to produce several kinds of pigments, each of a different color, to capture wide spectrum of the solar radiation.

1.3.3.1. CHLOROPHYLL:

Chlorophylls are greenish pigments that contain a porphyrin ring. This is a stable ring shaped molecule around which electrons are free to migrate. Because the electrons move freely, the ring has the potential to gain or lose electrons easily, and thus the potential to provide energized electrons to other molecules. This is the molecule, which makes photosynthesis possible, by passing its energized electrons on to molecules that

will manufacture sugars. Chlorophylls are very effective photoreceptors because they contain a network of alternating single and double bonds, and the orbitals can delocalize stabilizing the structure when molecule is excited. All plants, algae, and cyanobacteria, which photosynthesize, contain chlorophyll *a*. Only chlorophyll species present in cyanobacteria is chlorophyll *a*.

1.3.3.2. CAROTENOIDS:

Carotenoids are C_{40} isoprenoids and tetraterpenes that are located in the plastids of both photosynthetic and non-photosynthetic plant tissues. Carotenoids are usually red, orange, or yellow pigments. These compounds are composed of two small six-carbon rings connected by a chain of carbon atoms. Carotenoids cannot transfer sunlight energy directly to the photosynthetic pathway, but must pass their absorbed energy to chlorophyll and thus are called accessory pigments. There are two classes of carotenoids, the carotenes, which are hydrocarbons and xanthophylls, which are oxygen-containing derivatives. Among the carotenoids, β–carotene appears to be universally present in all cyanobacteria while the presence and abundance of different xanthophylls varies according to species. Myxoxanthin is one of the most abundant xanthophylls in cyanobacteria.

1.3.3.3. PHYCOBILISOMES:

Phycobilisomes (PBS) is mainly composed of the phycobiliproteins, which is water-soluble pigments, and is found in the cytoplasm, or in the stroma of the thylakoid membrane (Liotenberg et al., 1996). They occur only in cyanobacteria and rhodophyta.

Phycobiliproteins are classified on the basis of their color into three large groups, the phycoerythrins (red), allophycocyanin (blue) and the phycocyanins (blue). Absorption maxima for phycoerythrins lie between 490, allophycyanin at 570 nm while absorption maxima for phycocyanins are found between 610 and 665 nm (Nomsawai et al., 1999).

Phycobilisome structure (PBS) is peripheral membrane complexes in cyanobacteria that efficiently harvest light energy and transfer the energy to photosynthetic reaction centers. PBS comprises 30% of the cellular protein of a cyanobacterial cell, representing a large nitrogen reserve. It is organized into two structural domains, the core and rods. Each of these domains contains pigmented and non-pigmented polypeptides. Generally, six rods, each composed of stacks of phycocyanins and phycoerythrins hexamers, radiate from the core, giving PBS a fanlike appearance. All of the phycobiliproteins absorb incident light directly, but in addition, they participate in an energy transfer chain within the phycobilisome in following higher energy to lower energy order phycoerythrin -> phycocyanin -> allophycocyanin ->chlorophyll a.

Phycobiliproteins are relatively large, and contain multiple chromophore prosthetic groups, which are responsible for the fluorescent properties of these proteins, and generally result in fluorochromes of very high efficiency. Both phycocyanin and phycoerythrin fluoresce at a particular wavelength, when they are exposed to strong light, and release it by emitting light of a very narrow range of wavelengths. R-phycoerythrin (R-PE), R symbolizes its red-algal origin, is a bright orange-red coloured protein, with a molecular weight of 250 kDa and containing 34 chromophore prosthetic groups. With

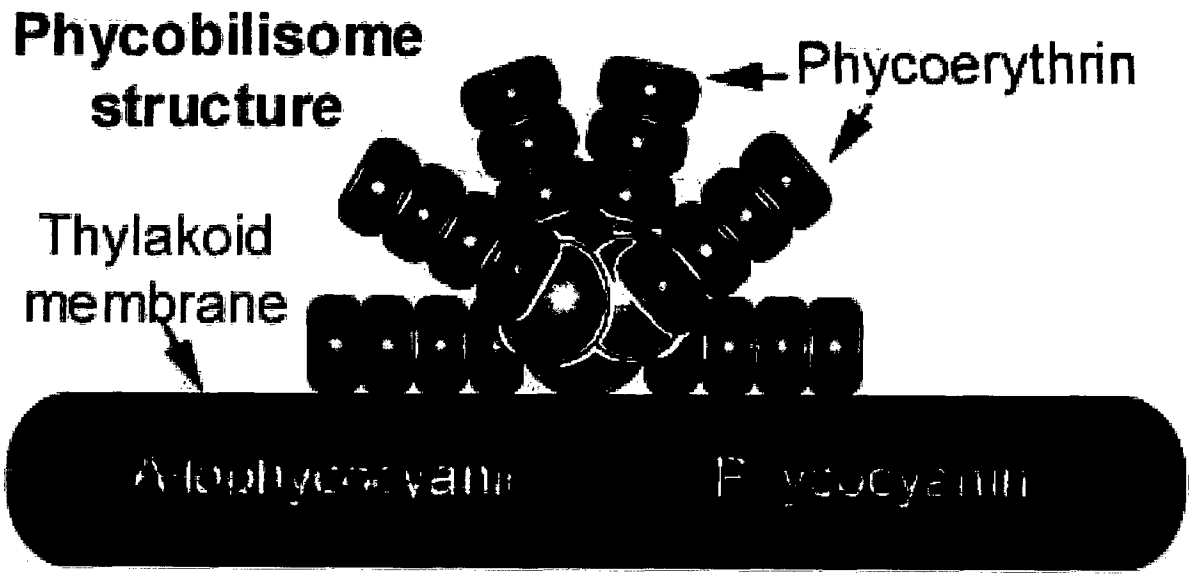


Fig 1.6 Phycobilisome structure

absorption maxima at 492 nm and 565 nm it is excitable by the 488 nm argon-ion laser, and has emission maxima around 578 nm. Allophycocyanin (APC) is a turquoise-blue coloured protein of molecular weight 110 kDa, containing six chromophore prosthetic groups. Absorption maximum is 650 nm, and an emission maximum is 660 nm. APC is excitable by the 633 nm helium-neon laser. The light-absorbing chromophore groups of the phycobiliproteins are constructed from linear or open tetrapyrrole rings, and are structurally related to the bile pigment biliverdin. They are attached to the surrounding protein structure via thioether linkages involving cysteine (cys) residues. Each phycobilisome contains 300-400 bilin chromophores in cyanobacteria, and up to 800 in red algae, and absorb light over much of the visible spectrum. Phycobilins are not only useful to the organisms that use them for soaking up light energy, they have also found use as research tools.

Cyanobacterial pigmentation changes with environmental stress conditions. All type of the photosynthetic pigments such as chlorophyll *a* as well as the accessory light harvesting pigments such as phycocyanin and phycoerythrin are susceptible to UV-B in cyanobacteria (Aráoz and Häder, 1997; Sinha and Häder, 2000), which leads to lower biomass and photosynthesis.

Under mild UV-B an increased synthesis of phycobiliproteins has been observed (Aráoz and Häder, 1997). The fact that phycobiliproteins are capable of intercepting almost 99% of UV-B radiation before it penetrates to the genetic material and that they form a peripheral layer around the sensitive central part containing the DNA, indicating that phycobilins are effective screening pigments against UV-B radiation (Aráoz and

Häder, 1997). The pigmentation of certain cyanobacteria is modified by altering the light quality and shown that the color change of cyanobacterial cells is attributable to a change in the phycoerythrin/phycocyanin ratio (Engelmann, 1992; Borsesch, 1992). This chromatic adaptation enables the cells to make maximum use of the particular wavelength of light available in their environment and is particularly useful in underwater environments (Wyman et al., 1985). Exposure to UV radiation lead to increased production of carotenoids (Goes et al., 1994) as increase in carotenoid production can protect the cells from photoinhibition by quenching the singlet oxygen (Paerl et al., 1983). However, some reports indicate that degradation of algal pigments is not significantly affected by UV-B flux (Maske and Latasa, 1997).

High light on other hand results in increased ROS production (Asada and Takahashi, 1987), which leads to photo oxidation of pigments and proteins (Andersson et al., 1992). High PAR can photooxidise phycobilisomes when cells are shifted from low light to high light. Reports of substantial decrease in allophycocyanin and phycoerythrin content under high light in cyanobacteria such as *Spirulina platensis* is reported (Nomsawai et al., 1999).

1.3.4. SUGARS

Carbohydrates are the universal source of carbon atoms for metabolism, and provide the principal precursors for the biosynthesis of secondary metabolites. In cyanobacteria major carbon and energy reserve compound accumulated is glycogen, which is a polysaccharide consisting of numerous monosaccharide glucoses linked together. Glycogen granules are minute (about 30 x 65 nm in size), ovoid or rod shaped

structures deposited primarily in the cytoplasm between the thylakoids and which is either composed of subunits with a normal glycogen branching pattern, or that the carbohydrate is a novel polyglucan (Shivley, 1988) with a unique branching pattern and tertiary structure. Glycogen is massively accumulated in cyanobacterial cells whenever balanced growth is hampered by a particular nutrient deficiency such as nitrogen (Allen and Smith, 1969) or by sudden increases in energy input (Post, 1987). Growth under sub optimal temperature conditions (Van Eykenlen burg, 1980) or a sudden increase in light energy input may also lead to glycogen accumulation (Ernst and Boger, 1985). Once conditions for balanced growth are reestablished, accumulated glycogen is generally broken down to yield energy and carbon for cell metabolism. It has been suggested that glycogen acts as a dynamic reserve with the dual function of storage product and of buffer substance able to generate the process of carbon supply (Carr, 1966). It has been known for a long time that low molecular mass carbohydrates, such as trehalose, sucrose (Norris et al., 1955) and glucosylglycerol (Kollmann et al., 1979) occur in cyanobacteria. In a few instances, the presence of poly-β-hydroxybutyrate (PHB), a widespread intracellular storage compound typical of prokaryotic organisms has also been reported (Schneegurt et al., 1994).

UV-B exposure to cyanobacteria causes accumulation of larger starch grains (He et al., 1994) and this is suggested to be due to inability of UV-B damaged cells to mobilize starch. High light also affects sugar content. Shifting the *Spirulina* culture from low to high light irradiance showed no induction of storage sugar such as poly-β-hydroxybutyrate was observed (De Philippis et al., 1992). Other stresses such as heat stress is also reported to increase saccharides and pentasaccharide by as many as 10-20

fold and suggested that this might play a role in protection of metabolic enzymes against the stress (Fischer et al., 2006). Elevated temperature is also known to induce higher levels of solutes in a variety of organisms (Hottiger et al., 1987). Cyanobacteria accumulate compatible solutes in response to increased external salinity, with increasing salt level type of sugar molecules changing from sucrose to trehalose to 2-O-(a-D-glucopyranosyl)-glycerol and glycinebetaine accumulating species (Hincha and Hagemann, 2004). Fructose containing oligosaccharides also increased due to salt stress (Salerno et al., 2004). Salt-adapted cells of *Spirulina* strains have a modified biochemical composition; reduced protein and chlorophyll content, and an increased level of carbohydrates (Vonshak, 1997). Accumulation of organic osmolytes such as glucosylglycerol was reported due to changing osmotic environment by various salts and salt concentration in cyanobacterium, *Synechocystis* strain PCC 6803 (Schoor et al., 1999). The data available with respect to salt, temperature and drought stress on sugar in cyanobacteria reported that greater accumulation of sugars in cells due to these abiotic stress conditions (Reed et al., 1986; Reed and Stewart, 1985; Konopka and Schnur, 1980). Very little work has been done with respect to abiotic stress and its impact on sugars in cyanobacteria.

1.3.5. NITROGEN:

Nitrogen is an essential element in all organisms because it is part of amino acids, protein and nucleic acids (DNA and RNA. Nitrogen content of healthy cyanobacteria is usually vary within the range of 4-9% on a dry weight basis depending on the stage of growth and tissue.

At light limiting conditions cyanobacteria accumulate nitrogen in abundant in structured granules, which serve as nitrogen resources, while high light intensity decreases nitrogen content and develops nitrogen chlorosis (Neilson et al., 1971). Stimulation of nitrate utilization by increasing light intensities has been seen in algal species, including the cyanobacteria (Ullrich, 1983).

UV-B radiation leads to reduced uptake of nitrate, ammonium and phosphorus by planktonic algae (Dohler, 1992), followed by reduced availability of nitrogen for the biosynthetic pathway of MAAs synthesis. Enzymes of nitrogen metabolism show differential sensitivity towards UV-B. Inhibition of nitrogenase and glutamine synthetase activity and induction of nitrate reductase activity by UV-B exposure is reported in nitrogen fixing cyanobacterial (Sinha et al., 2001). UV-B also causes reduction in ammonium uptake (Dohler, 1985). The nitrogen assimilation may also be affected due to a decrease in the ATP content reported in algae under UV-B treatment (Vosjan et al., 1990), or due to transport system for inorganic nitrogen in the cell. This is a very important aspect to study as decreased nitrogen assimilation by prokaryotic microorganisms may lead to a nitrogen deficiency for higher plant ecosystems, such as in rice fields and threatening food security.

1.4. OXIDATIVE DAMAGE:

1.4.1. BIOLOGICAL REACTIONS OF OXYGEN RADICALS:

Oxygen is essential for the metabolism of aerobic organisms, however its participation in cellular events results in the appearance of ever present toxic active

oxygen species (AOS). AOS includes a number of compounds such as singlet oxygen ($^1O_2$), superoxide radical (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^\cdot$), which may differ in reactivity, but all of them are more reactive than ground state oxygen. A range of stress factors such as high light, UV radiation, temperature, ozone, desiccation, pollutants etc. are associated with over production of toxic oxygen species and oxidative damage to living organisms including algae (Foyer et al., 1994; Collen and Davison, 1999). These free oxygen radicals are formed as a normal part of the metabolism in photosynthetic organisms but excessive production of AOS can cause damage to lipids, proteins, DNA and other biomolecules.

Superoxide and hydrogen peroxide can react to form hydroxyl radicals ($OH^\cdot$) in the presence of metal ions. Hydroxyl radicals are among the most reactive species able to react indiscriminately to cause lipid peroxidation, the denaturation of proteins and the mutation of DNA. Hydrogen peroxide readily diffuses across membranes and it is therefore not compartmentalized in the cell. The reactions of activated oxygen with biological systems are complex due to the surface properties of membrane, electrical charge, binding properties of macromolecules and compartmentalization of enzymes, substrate and catalysts. Thus, various sites even within a single cell differ in the nature and extent of reactions with oxygen.

UV-B radiation and high light is known to induce significant oxidative stress, lipid peroxidation, DNA breaks, decreased photosynthetic function and chlorohyll bleaching in algae (Halliwell and Gutteridge, 1999, Brown et al., 1994).

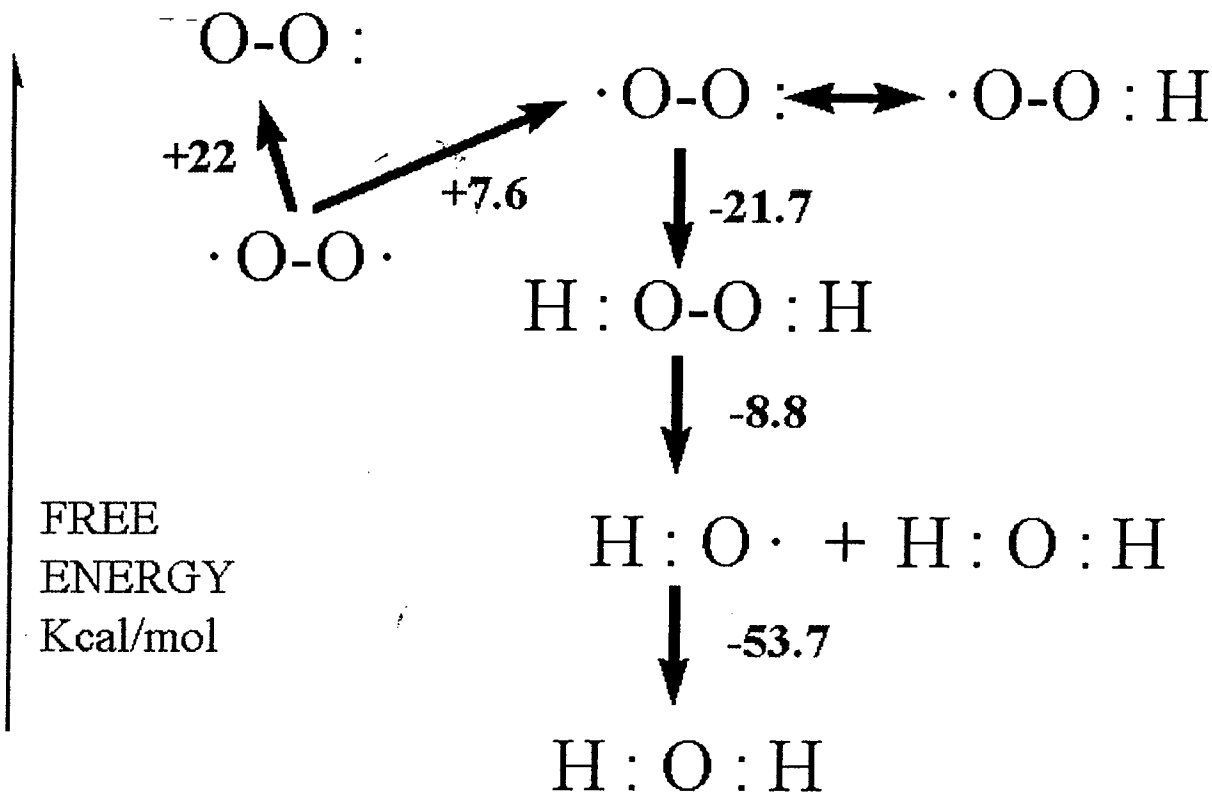


Fig 1.7 Formation of active oxygen species (AOS)

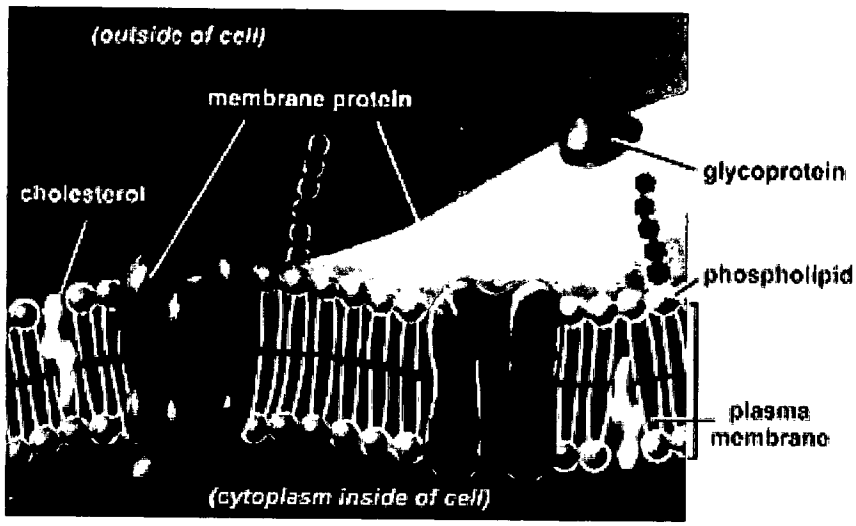
1.4.2. LIPIDS AND FATTY ACIDS:

Lipids are found in the chromatoplasm and produced by processes catalyzed by reactions in the cytosol between the thylakoids. Lipids are integral components of thylakoid membrane and fundamental for the structural and functional integrity of the photosynthetic apparatus. The enzymes for lipid synthesis are translated by ribosomes nearer the chromatoplasm. Cyanobacterial cell membranes are characterized by their unique lipid composition, major components being: glycolipids, such as monogalactosyl diacylglycerol (MGDG), digalactosyl diacylglycerol (DGDG) and sulfoquinovosyl diacylglycerol (SQDG) (Murata, 1998). Glycolipids are polysaccharides formed of sugars linked to lipids and is a part of the cell membrane. Phospholipids are asymmetrical lipid molecules with a hydrophilic head (glycerol and phosphate) and a hydrophobic tail (the non-polar fatty acids) and act as building blocks of cellular membranes. Phosphatidylglycerol (PG) is the only phospholipid in cyanobacterial cell membrane and accounts for approximately 10-20 % of the total membrane lipids. Glycosyldiacylglyceride (GlcDG) is found as a (1%) minor glycerolipid in the outer, inner and thylakoid membranes in cyanobacteria, which is absent in higher plants. The relative level of MGDG in cyanobacterial cell is slightly higher than 50% of the total glycerolipids and relative levels of DGDG, SQDG and PG range from 5% to 25%. The composition of major lipids of the cyanobacteria is similar to that of higher plant chloroplasts (Quinn and Williams, 1983), except a minor component, phosphatidylcholine, in the chloroplasts is replaced by monoglucosyl diacylglycerol in the cyanobacteria.

Fatty acids are one of the main components of lipids and thus of the cytoplasmic membrane which plays a major role in cell function. Fatty acids are hydrocarbon chains that terminate in a carboxyl group, they are termed unsaturated if they contain at least one carbon-carbon double bond and polyunsaturated when they contain multiple double bonds. Unsaturated fatty acids are synthesized from saturated fatty acids by fatty acid desaturases that convert single bonds to double bonds. Polyunsaturated fatty acids have crucial roles in membrane biology and signaling processes in most living organisms.

The composition of fatty acids in cyanobacteria depends on the species and on the environmental conditions. The most abundant fatty acids in cyanobacteria are Palmitic acid (16:0), Palmitoleic acid (16:1), Stearic acid (18:0), Oleic acid (18:1), Linoleic acid (18:2), γ-Linolenic acid (18:3). Cyanobacteria *Synechocystis* spp contains 16:0, 16:1, 18:1, 18:2 and a unique fatty acid, γ-18:3, as major fatty acids (Kenyon, 1972).

The reactions of oxygen free radicals with polyunsaturated lipids have been extensively researched and is defined as lipid peroxidation which is a well-established mechanism of cellular injury in both plants and microorganisms and is commonly used as an indicator of oxidative stress in cells and tissues. It can be caused by a number of active oxygen species. Lipid peroxidation in cells is usually detected and quantified by measuring malondialdehyde (MDA) and other carbonyl by-products of lipid peroxidation, which form a colored complex in the reaction with thiobarbituric acid (TBA) (Pryor and Castle, 1984), or ethane evolution (Kumar and Knowles, 1993), a breakdown product of fatty acid oxidation. Polyunsaturated fatty acid peroxides generate malonaldehyde (MDA) and 4-hydroxyalkenals that have been used as an indicator of



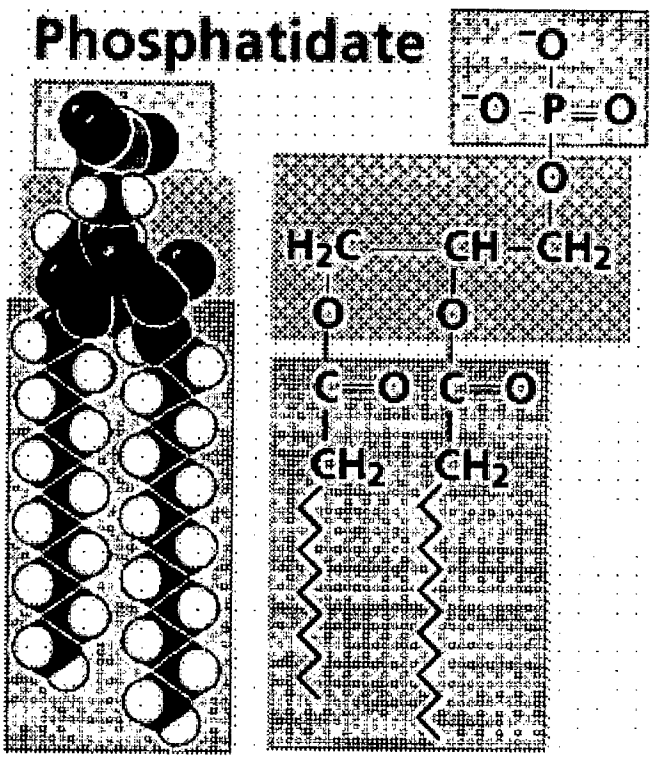


Fig 1.8 Structure of glycolipid and phospholipid

lipid peroxidation (Esterbaurer et al., 1991). Lipid peroxides are unstable and decompose to form a complex series of compounds including reactive carbonyl compounds. Lipid peroxidation is related to increased ion permeability, the loss of membrane fluidity, cross-linking of amino lipids and polypeptides and inactivation of membrane proteins and enzymes (Girotti, 1990). It potentially alters surrounding proteins, nucleic acids and other molecules in addition to the lipid themselves. Thus, damage originating in a lipid membrane compartment could conceivably disseminate to other compartments (Girotti, 1990).

Typically high molecular weight, cross-linked fatty acids and phospholipids accumulate in peroxidized membrane lipid samples. Singlet oxygen can react readily with unsaturated fatty acids producing a complex mixture of hydroperoxides. Oxidation of unsaturated fatty acids by singlet oxygen produces distinctly different products than the hydroxyl radical (Bradley and Minn, 1992). Once formed the lipid hydroperoxides will decompose into a variety of products, some of which can produce oxygen free radicals in the presence of metal catalysts. Mechanism of peroxidation of lipids involves three distinct steps: initiation, propagation and termination. The initiation reaction between an unsaturated fatty acid (linoleate) and the hydroxyl radical involves the abstraction of an H atom from the methylvinyl group on the fatty acid in the case of linoleate, this occurs at carbon-11. The remaining carbon centered radical, forms a resonance structure sharing this unpaired electron among carbons 9 to 13. In the propagation reactions, this resonance structure reacts with triplet oxygen, which is a biradical having two unpaired electrons and therefore reacts readily with other radicals. This reaction forms a peroxy radical. In the case of linoleate, addition occurs at either carbon 9 or 13. The peroxy radical then

abstracts an H atom from a second fatty acid forming a lipid hydroperoxide and leaving another carbon centered free radical that can participate in a second H abstraction. Therefore, once one hydroxyl radical initiates the peroxidation reaction by abstracting a single H atom, it creates a carbon radical product (R) that is capable of reacting with ground state oxygen in a chain reaction. The basis for the hydroxyl radicals extreme reactivity in lipid systems is that at very low concentrations it initiates a chain reaction involving triplet oxygen, the most abundant form of oxygen in the cell. The lipid hydroperoxide (ROOH) is unstable in the presence of Fe or other metal catalysts because ROOH will participate in a Fenton reaction leading to the formation of reactive alkoxy radicals. Among the degradation products of ROOH are aldehydes, such as malondialdehyde and hydrocarbons like ethane and ethylene that are commonly measured end products of lipid peroxidation. The peroxidation reactions are cyclic and continue in membrane lipids and terminated only when the carbon or peroxy radicals cross-link to form conjugated products that are not radicals.

The fatty acids are affected by a number of environmental variables such as light intensity and photoperiod (Brown et al., 1996; Tzovenis et al., 1997), temperature (Thompson et al., 1992; Zhu et al., 1997), salinity (Xu and Beardall, 1996), ozone concentration (Murata, 1998) and the intensity of UV-B irradiation (Skerratt et al., 1998). UV-B radiation is also reported to cause decrease in the total fatty acid content of phytoplankton (Lange and Van Donk, 1997) and the changes in the content of fatty acids were species specific. Polar species, which have a high proportion of unsaturated fatty acids are shown to be susceptible to UV radiation, owing to lipid peroxidation or reduced biosynthesis (Björn, 2002).

High light causes lipid peroxidation to varying effect in various types of algae. It is reported that effect of increasing level of photon fluence on red algae *Gracilaria* did not show any changes in fatty acid composition while green alga *Ulva pertusa* Kjellman showed changes in content of saturated and tetraunsaturated fatty acids (Dawes et al., 1993; Floreto et al., 1993; Floreto and Teshima, 1998).

The changes in fatty acid composition (oxidative or otherwise) due to stresses can also be regarded as an adaptive response (Murata et al., 1984). An increase in unsaturation and decrease in the chain length of esterified fatty acids are known to lower the phase transition temperature of membrane lipids (Chapman et al., 1972). Reports have shown that desaturation of lipids might be involved in the protection against salt stress (Ritter and Yopp, 1993).

1.4.3. PROTEINS:

Proteins are made up of amino acids linked together by peptide bonds that perform a wide variety of cellular functions. It is one of the classes of organic macromolecules that function as structural as well enzymatic and control elements in living systems.

The amino acids in a peptide differ in their susceptibility to oxidative attack, and the various forms of activated oxygen differ in their potential reactivity. Primary, secondary, and tertiary protein structures alter the relative susceptibility of certain amino acids. Sulphur containing amino acids, and thiol groups specifically, are very susceptible sites. Activated oxygen can abstract an H atom from cysteine residues to form a thiyl

radical that will cross-link to a second thiyl radical to form disulphide bridges. Alternatively, oxygen can add to a methionine residue to form methionine sulphoxide derivatives. Reduction of both of these may be accomplished by thioredoxin and thioredoxin reductase (Farr and Kogama, 1991). Many other amino acids undergo specific irreversible modifications when a protein is oxidized. For example, tryptophan is readily cross-linked to form bityrosine products (Davies, 1987). Histidine, lysine, proline, arginine, and serine form carbonyl groups on oxidation (Stadtman, 1986). The oxidative degradation of protein is enhanced in the presence of metal cofactors that are capable of redox cycling, such as Fe, which binds to a divalent cation-binding site on the protein and induce oxidation.

Cellular proteins with aromatic amino acids absorb strongly in the range of UV-B (290 nm-315 nm; Sinha and Häder, 1998), which result in denaturation of proteins. UV-B induced oxidative attack on proteins results in site-specific amino acid modifications, fragmentation of the peptide chain, and aggregation of cross-linked reaction products, altered electrical charge and increased susceptibility to proteolysis resulting in loss in structure and function of proteins, which may lead to changes in cellular membrane permeability, and ultimately death of the organisms (Häder et al., 1998).

1.4.4. NUCLEIC ACIDS:

Cyanobacterial cell is divided internally into two zones, an outer zone, the chromoplasm, in which the pigments or photosynthetic lamellae are localized, and an inner zone in which the nucleic acids and nucleoproteins are localized, the nucleoplasm and contains the extremely folded thin thread of the prokaryotic type of circular

chromosome. Nucleic acids are composed of nucleotides; DNA and RNA. Cyanobacteria have a simpler genetic system than plants and algae that enable them to be easily modified genetically.

Deoxyribonucleic acid (DNA) has been reported to occur in two forms in cyanobacterial cells, as anastamosing fibrils and as elongated rods. DNA fibrils are organized in a complex helical and folded configuration and are distributed uniformly throughout the centroplasm. The DNA is associated with histone like DNA binding proteins and also with RNA

Ribonucleic acid (RNA) is present in the cyanobacterial cell in the form of ribosomes, the protein synthesizing organelles. They are distributed throughout the cell but are more concentrated in the nucleoplasm giving it a slightly yellowish colour. RNA has a sedimentation coefficient of approximately 70s and they dissociate into 50s and 30s subunits. There are three types of RNA; mRNA, tRNA and rRNA, each are involved in protein synthesis.

Activated oxygen and agents that generate oxygen free radicals such as UV and high light, induce numerous lesions in DNA that cause deletions, mutations and other lethal genetic effects. Characterization of this damage to DNA has indicated that both the sugar and the base moieties are susceptible to oxidation, causing base degradation, single strand breakage, and cross-linking to protein (Imlay and Linn, 1986). Degradation of the base produces numerous products, including 8-hydroxyguanine, hydroxymethyl urea, urea, thymine glycol, thymine and adenine ring opened and saturated products. The principle cause of single strand breaks is oxidation of the sugar moiety by the hydroxyl

radical. The short-lived hydroxyl radical then oxidizes an adjacent sugar or base causing breakage of the DNA chain. Cross-linking of DNA to protein is another consequence of hydroxyl radical attack on either DNA or its associated proteins (Oleinick et al., 1986). Treatment with ionizing radiation or other hydroxyl radical generating agents causes covalent linkages such as thymine-cysteine adducts, between DNA and protein. DNA is a weak link in a cells ability to tolerate oxygen free radical attack.

DNA is shown to be one of the key targets for damaging under UV-B radiation in bacteria (Peak et al., 1984), phytoplankton (Buma et al., 1997), macro algae (Pakker et al., 2000a), plants (Quaite et al., 1992), humans and animals (Kripke et al., 1992). UV-B radiation damage to DNA is potentially dangerous to cells, since a single photon hit may have a dramatic or even lethal effect. Several types of DNA damage have been identified that result from free radicals and active oxygen species formed by various photochemical processes. The two major classes of mutagenic DNA lesions induced by UV radiation are cis-syn cyclobutane-pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidone photoproducts (6-4PPs), which are pyrimidine adducts (Thoma, 1999). Both classes of lesions distort the DNA helix. CPDs and 6-4PPs induce a bend or kink of 7-9^{o} and 44^{o}, respectively. The ability of UV radiation to damage a given base is determined by the flexibility of the DNA. Sequences that facilitate bending and unwinding are favorable sites for damage formation, e.g. CPDs form at higher yields in single stranded DNA, at the flexible ends of poly ((dA) (dT)) tracts, but not in their rigid centre (Lyamichev, 1991). Bending of DNA towards the minor groove reduces CPD formation (Pehrson and Cohen, 1992).

UV-B radiation also has direct effect on DNA, which absorbs strongly at 280 nm and may result in the formation of dimeric photoproducts between adjacent pyrimidines and other mutagenic effects (Vincent and Quesada, 1994). In cyanobacteria, UV-B stress caused significantly increased number of DNA strand breaks (He et al., 2002) which was prohibited on addition of exogenous antioxidants such as ascorbic acid and *N*-acetyl cysteine (NAC) suggesting that AOS contributed to the breaks (Reddy et al., 1998). Lower cell viability is also correlated to higher DNA breaks as well as stronger oxidative stress and the damage to DNA and may lead to the potential death of cells (Karentz, 1991).

1.5. PROTECTIVE PROCESSES:

Photoautotrophic cyanobacteria were among the earliest oxygenic photosynthetic organisms that were exposed in the course of their evolution to UV-B fluxes and thus they have developed several strategies for protection against the detrimental effects of UV-B radiation (Ehling and Scherer, 1999). These strategies include stress avoidance by migration, induction of UV absorbing compounds such as MAAs, scytonemin and phenolic compounds, induction of enzymatic and non-enzymatic antioxidants and DNA repair mechanisms.

1.5.1. MIGRATION:

Most cyanobacteria possess several photo responses in order to avoid UV-B or too bright or too dim light intensities in their search for an optimal niche. These include photoaxis, photokinesis and photophobic responses. Solar radiation can penetrate to

significant depths with potential detrimental effects to benthic organisms in the euphotic zone (Smith et al., 1992). Motile algae can avoid harmful radiation by migration to deeper waters (Häder, 1997). Some species of algae and cyanobacteria e.g. *Microcystis*, *Planktothrix Anabaena* and *Aphanizomenon* have the ability to reduce their exposure to solar UV by vertical migration brought about by either active movements or by changing their buoyancy whereby the cells can avoid the surface zone during the hours of excessive irradiance (Vincent and Roy, 1993). In benthic mat communities, UV-induced downward migration of cyanobacteria into deeper strata has been shown (Bebout and Garcia-Pichel, 1995; Quesada and Vincent, 1997; Quesada et al., 1999). In pelagic communities cyanobacteria can avoid UV rays through sinking and floating behaviour from a combination of gas vacuoles and ballast (Reynolds et al., 1987). Organisms which cannot avoid the radiation by migration like sessile macroalgae can protect themselves by absorbing the UV radiation through various compounds.

1.5.2. UV-B ABSORBING COMPOUNDS:

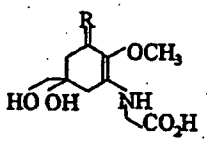
A variety of UV absorbing compounds is found in the aquatic and terrestrial plants. UV screening compounds have evolved to screen UV radiation before it reaches the genetic machinery and other essential biomolecules (Merchant et al., 1991). In addition to functioning as a UV screen, algal mycosporine like amino acids (MAAs) and phenolic compounds serve various other physiological and ecological functions. Terrestrial higher plants have chemically more complex UV screens such as flavonoids. It was reported that the simpler UV absorbing compounds in aquatic plants, such as

MAAs, are as efficient as the more complex flavonoids in terrestrial higher plants (Smith et al., 1992).

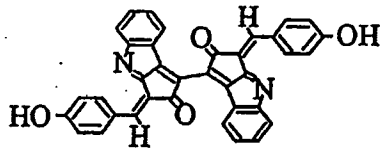
1.5.2.1. MYCOSPORINE LIKE AMINO ACIDS (MAAs):

Mycosporine like amino acids are water soluble substances characterized by a cyclohexenone or cyclohexenimine chromophore conjugated with the nitrogen substituent of an amino acid or its imino alcohol, having absorption maxima ranging from 310-360 nm and an average molecular weight of around 300 kDa (Cockell and Knowland, 1999). Spectroscopic and biochemical analysis of MAAs revealed the presence of shinorine, a bisubstituted MAAs containing both glycine and serine group with an absorption maximum at 334 nm in cyanobacteria. Other MAAs was found to beinduced at 324 nm in the green alga *Prasiola stipitata.* Marine dinoflagellate *Gyrodinium dorsum* showed a set of MAAs such as shinorine, porphyra-334 and palytine having absorption maxima between 310 and 360 nm.

Polychromatic action spectra for the induction of MAAs in *Anabaena* spp and *Nostoc commune* show the induction to be UV-B dependent peaking at 290 nm. The occurrence of high concentration of mycosporine like amino acids in organisms exposed to high levels of solar radiation has been described to provide protection as UV-absorbing pigments (Sinha et al., 1998). Cells with high concentrations of MAAs are approximately 25% more resistant to UV radiation centered at 320 nm than those with no or low concentrations (Garcia-Pichel et al., 1993). Report suggests a circadian induction in the synthesis of MAAs compound by UV-B radiation (Barros et al., 2005).



Basic structure of MAAs



Scytonemin

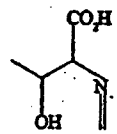
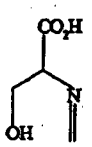
Name	R	λ_{max}	ε	Reference
Mycosporine-glycine	O	310	28100	Ito & Hirata 1977; Dunlap et al. 1986; Gleason 1993
Palythine	NH	320	36200	Takano et al. 1978a; Dunlap et al. 1986; Gleason 1993
Asterina-330		330	43500	Gleason 1993
Palythinol		332	43500	Dunlap et al. 1986; Takano et al. 1978b
Porphyra-334		334	42300	Takano et al. 1979
Shinorine		334	44700	Tsujino et al. 1980; Gleason 1993
Palythene		360	50000	Takano et al. 1978b
Scytonemin	-	386	-	Proteau et al. 1993

Fig 1.9 Structure of mycosporine like amino acids (MAAs), (Sinha and Hader, 2000)

MAAs has been found in blue green, green, red and brown algae from tropical, temperate and Polar Regions. MAAs is even passed down the food chain to consumers where they function as screening pigments. It is interesting to note that in some macroalgae, no MAAs can be found or induced (Groniger et al., 2000). These include deep-water red algae, which, in their natural habitat, never run the risk of exposure to high solar UV radiation (Groniger and Häder, 2000). In other algae like supralitoral red alga *Porphyra*, UV-B-absorbing compounds such, as MAAs cannot be induced. In a third group of macroalgae under normal conditions they have no or low levels of MAAs, which increase upon exposure to UV radiation (Conde et al., 2000).

Screening pigments such as mycosporine like amino acids, scytonemin and carotenoids are incorporated into the cytoplasm or the outer slime sheath (mainly scytonemin) efficiently protect the organisms from solar short-wavelength radiation (Karsten and Garcia-Pichel, 1996). These organisms, which contain MAAs either in their cytoplasm or scytonemin in outer slimy sheath, are more tolerant to UV-B radiation than those that do not contain such covering (Sinha et al., 1995). The MAAs in *Nostoc commune* has been reported to be linked to oligosaccharides in the sheath (Bohm et al., 1995), which was actively excreted and accumulated extracellularly and therefore act as a true screen against solar radiation (Ehling-Schulz et al., 1997). Other wavelength bands such as PAR (400–700 nm) and UV-A (315–400 nm) had only a small effect on MAAs synthesis in *Anabaena and Nostoc* spp. (Sinha et al., 2002). MAAs also plays a vital role as osmotic regulators as well as antifreeze compounds in *Gyrodinium dorsum, Prasiola stipitata* and *Anabaena* spp. (Rozema et al., 2002).

1.5.2.2. SCYTONEMIN:

Scytonemin is a yellow brown, lipid soluble dimeric pigment located in those cyanobacteria, which contain extracellular polysaccharide sheath. It has an *in vivo* absorption maximum at 370 nm, a molecular mass of 544 kDa and a structure based on indolic and phenolic subunits. Scytonemin have been detected in various cyanobacteria e.g. *Chlorogloeopsis, Calothrix* and *Nostoc commune* (Proteau et al., 1993). Scytonemin having absorption maximum at 386 nm as well as in UV-C region of 278 nm, 252 nm and 212 nm may have an advantage as a UV absorbing pigments under the then oxygen-free atmosphere, which also transmitted UV-C.

The role of scytonemin as a sunscreen was demonstrated in the terrestrial cyanobacterium, *Chlorogloeopsis* spp where cyanobacteria accumulate as much as 5% of the cellular dry weight as scytonemin (Garcia-Pichel et al., 1992). Scytonemin content increased in field samples and may reach an even higher specific content (Castenholz, 1997). The correlation between UV protection and scytonemin presence has also been established under solar irradiance in a naturally occurring cyanobacterium, *Calothrix* spp It was shown that high scytonemin content is required for uninhibited photosynthesis under high UV flux (Brenowitz and Castenholz, 1997). Studies indicate the incident UV-A radiation entering the cells may be reduced by around 90% due to the presence of scytonemin in the cyanobacterial sheaths (Garcia-Pichel and Castenholz, 1991). A desiccation tolerant cyanobacterium, *Lyngbya* spp collected from the bark of mango trees in India has been found to contain scytonemin (Sinha et al., 1999).

In addition to scytonemin, report of two unidentified, water-soluble, yellowish and brownish compounds with absorption maximum at 315 nm were recorded only in *Scytonema* spp. The spectral characteristics of these compounds neither matches with MAAs nor with scytonemin and seems to be a novel compound acting as an effective UV screens (Rozema et al., 2002).

1.5.2.3. PHENOLIC COMPOUNDS:

One line of defense in the plants is synthesis of UV-B absorbing non-photosynthetic pigments such as phenolic compounds and flavonoids which are synthesized in the epidermal tissue in order to protect the inner more sensitive sites against UV-B radiation (Graham, 1998). There are evidences that flavonoids reduce damage from UV-B radiation because they act as UV filters, reducing the penetration of potentially damaging UV-B radiation (Sharma et al., 1997). Simpler phenolics occur in lower plants, such as sphagnum acid, and these may have acted as UV filters in the progenitors of vascular plants (Rozema et al., 2002).

Some reports of physodes of brown macroalgae containing phenolic compound have been associated with shielding of UV radiation (Pavia et al., 1997). UV tolerance of some green phytoplankton species was attributed to the cell wall biopolymer phenolic compound sporopollenin (Xiong et al., 1997). In tropical algae, enhanced levels of UV-absorbing compounds were detected in tissues from the canopy compared to tissues from understory locations in turf-forming Rhodophytesm (Beach and Smith, 1996). In phytoplankton, the occurrence and induction of UV screening pigments has also been

Recorded (Wood, 1988). However, there are no reports of phenolic compounds present in cyanobacteria (Rozema et al., 2002)

UV-B exposure in plants induced an increase in a rapid and coordinated expression of genes encoding certain enzymes in the flavonoid biosynthetic pathway (Hahlbrock and Scheel, 1989). Enzymes such as phenylalanine ammonia lyase (PAL), 4-coumarate CoA ligase, chalcone synthetase (CHS) appeared within hours after UV exposure (Strid, 1993) as a result of increased transcription rates (Chappell and Hahlbrock, 1984). These works indicate induction of these enzymes, responsible for shikimic acid pathway, in response to synthesize UV-B absorbing phenolic compounds.

1.5.3. ANTIOXIDANTS:

In order to prevent damage caused by AOS due to various environmental stresses including UV-B and high light, photosynthetic cells have developed several antioxidant enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (POD), catalase (CAT), and glutathione reductase (GR) as well as non-enzymatic antioxidant such as ascorbic acid, carotenoids, tocopherols, glutathione (GSH), hydroquinones, manitol, myoinositol, phenolics, phycocyanin, polyamines and proline to counteract the damaging effects of AOS (Mallick and Mohn, 2000).

1.5.3.1. SUPEROXIDE DISMUTASE:

Superoxide dismutase (SOD) was first isolated by Mann and Keilis, (1938). Superoxide dismutase is a group of metalloisoenzyme and is known to catalyze the

dismutation of superoxide to hydrogen peroxide and oxygen. Superoxide dismutase has a molecular weight of 32.6 KDa. The enzyme contains two atoms of Zn^{2+}, which is necessary for structural stability, and 2 atoms of Cu^{2+} which is necessary for enzymatic activity. Since SOD is present in all aerobic organisms and generates activated oxygen, it has been assumed that SOD has a central role in the defense against oxidative stress (Scandalias, 1993). SOD activity is increased in cells in response to diverse environmental stresses including high light, UV radiation, water logging and drought.

$$2O_2^{\cdot-} + 2H \xrightarrow{SOD} O_2 + H_2O_2$$

There are three distinct types of SOD classified based on the metal cofactor, the copper/zinc (Cu/Zn - SOD), the manganese (Mn-SOD) and the iron (Fe-SOD) isozymes (Bannister et al., 1987). Prokaryotic cells and many eukaryotic algae contain only the Mn-SOD and Fe-SOD isozymes, which are dimers and are believed to be more ancient forms (Scandalias, 1993). All forms of the SOD isozymes are nuclear encoded and are independently regulated according to the degree of oxidative stress experienced in the respective sub cellular compartments. SOD reduces oxidative stress and can play a key role in moderating the ageing process.

1.5.3.2. ASCORBATE PEROXIDASE:

Ascorbate peroxidase is a heme containing protein. Together with glutathione reductase (GR) and dehydroascorbate reductase, it is thought to remove hydrogen peroxide through a mechanism termed Halliwell-Asada pathway. It has been found in

throughout the cytoplasm (Edwards et al., 1990), where it may also cooperate with SOD to remove superoxide radicals. It is a monomeric glycoprotein with the molecular weight of 44 KDa, which contain one molecule of protoheam as prosthetic group and two molecules of Ca^{2+}. Besides dehydroascorbate, ascorbate peroxidase activity also generates monodehydroascorbate. The ascorbate radicals are converted back to ascorbate by a monodehydroascorbate reductase that can use both NADH and NADPH as reductants.

$$H_2O_2 + DH_2 \xrightarrow{\text{Peroxidase}} 2H_2O + D$$

1.5.3.3. CATALASE:

Catalase is a heme containing enzyme that catalyses the dismutation of hydrogen peroxide into water and oxygen. The enzyme is found in all aerobic organisms and is important in the removal of hydrogen peroxide generated in peroxisomes (microbodies) by oxidases involved in β-oxidation of fatty acids, the glyoxylate cycle (photorespiration).

1.5.3.4. EFFECT ON ANTIOXIDANTS:

Increased active oxygen scavenging capacity by increased activities of SOD and APX has been observed in response to UV radiation in algae (Lesser, 1996, Lesser et al., 1996). The increase of SOD activity was detected due to increase in AOS levels and lipid peroxidation (Boldt and Scandalios, 1997; Malanga and Puntarulo, 1995). In cells exposed to UV-B, the amount of mRNA coding for SOD enzymes decreased

significantly whereas the mRNA levels for GR increased (Astrid, 1993). The Zn/Cu-SOD as well as GR activity increased slightly after both UV-B and ozone exposure, while peroxidase activity (POD) was strongly enhanced (Rao et al., 1996). There was no significant change in ascorbate peroxidase activity in isolated chloroplasts of *Chlorella vulgaris* following UV-B stress (Malanga et al., 1997). Increase in the concentration of non-enzymatic antioxidants such as β–carotene and α–tocopherol in *Chlorella vulgaris* was also reported (Malanga and Puntarulo, 1995).

A general induction in activities of ascorbate peroxidase has been reported under high irradiance (Lesser et al., 1990) and temperature stress (Rady et al., 1994). Catalase activity has been reported in the symbiotic photosynthetic prokaryote *Prochloron* spp and its concentration was found to be directly proportional to the irradiance (Lesser et al., 1990). Increased activity of ascorbate peroxidase was also observed in *Nostoc muscorum* when H_2O_2 production was enhanced by photorespiration (Tel-Or et al., 1985). An effective reaction sequence in cyanobacteria for removal of hydroperoxides involves ascorbate peroxidase and recycling of glutathione and ascorbate.

The SOD and catalase activity increased significantly with a decrease in the protein content during the down-shift of temperature in the cyanobacterium, *Synechocystis* spp PCC 6803 (Rady et al., 1994). Bagchi et al., (1991) also reported that catalase activity was not detected in the cyanobacterium, *Anabaena variabilis*. The SOD activity was also shown to be induced under copper stress by the nitrogen fixing cyanobacterium, *Anabaena doliolum* (Mallick and Rai, 1999).

1. 5.4. NON-ENZYMATIC ANTIOXIDANT:

1.5.4.1. ASCORBIC ACID:

L-ascorbic acid (vitamin C) is an important vitamin in the human diet and is abundant in plant tissues. Ascorbate has been shown to have an essential role in several physiological processes in plants, including growth, differentiation and metabolism (Foyer, 1993). Ascorbate can directly scavenge oxygen free radicals with and without enzyme catalysts and can indirectly scavenge them by recycling tocopherol to the reduced form, thereby minimizing the damage caused by oxidative stress.

1.5.4.2. CAROTENOIDS:

In chloroplasts, the carotenoids act as accessory pigments in light harvesting complex, but a more important role is their ability to detoxify various forms of activated oxygen and triplet chlorophyll that are produced as a result of excitation of the photosynthetic complexes by light. The carotenoids can exist in a ground state or in one of two excited states after the absorption of light energy. In terms of its antioxidant properties carotenoids can protect the photosystems in one of four ways,

(a) Reacting with lipid peroxidation products to terminate chain reactions (Burton and Ingold, 1984),

(b) Scavenging singlet oxygen and dissipating energy as heat (Mathis and Kleo, 1973),

(c) Reacting with triplet or excited chlorophyll molecules to prevent formation of singlet oxygen, or

(d) The dissipation of excess excitation energy through the xanthophyll cycle.

1.5.4.3. SOME OTHER IMPORTANT SCAVENGERS:

There are a number of other compounds, which function as antioxidants or scavengers e.g. phycocyanin (found in cyanobacteria and red algae), an accessory light harvesting pigment with open chain tetrapyrroles, which has been found to contain antioxidant properties (Romay et al., 1998). Glutathione (GSH) is a tripeptide (Glu-Cys-Gly) whose antioxidant function is facilitated by the sulphydryl group of cysteine (Rennenberg, 1982). Tocopherol is antioxidants, which scavenge oxygen free radicals, lipid peroxy radicals, and singlet oxygen (Hess, 1993) that include four tocols that have a phytyl chain and analogous tocotrienols that have a geranylgeranyl chain. Proline, manitol, myoinositol etc. have been implicated in the defense against hydroxyl radicals. Myoinositol was found to be more effective than proline in protecting malate dehydrogenase. The antioxidant role of melatonin has been established in members of Dinophyta and Phaeophyta (Balzer and Hardeland, 1996). A compound, usujilene (Nakayama et al., 1999) has been reported to function as a biological antioxidant in marine organisms. UV-B absorbing pigments such as MAAs and flavonoids also act as antioxidants to prevent cellular damage resulting from UV-induced production of active oxygen species (Dunlop and Yamamoto, 1995).

1.5.5. DNA REPAIR MECHANISM:

All microorganisms and plants possess repair processes, which can reverse the damage caused by UV radiation (Sommaruga and Buma, 2000). UV-B induced DNA damage, which shows an increase in incidence of CPDs, which is indicative of potential damage. DNA damage in cells can be repaired by two mechanisms, light-dependent

(photoreactivation) and light-independent mechanisms (dark repair). The mechanism of photoreactivation is well described for DNA as one of the most common UV lesions involves restoration of UV-incurred damage in DNA by photo reactivating enzymes by absorption of light energy monomerize dimers formed in UV irradiated DNA (Karentz, 1994). It has also been shown that action spectra of photorepair of CPD have maximum effectiveness at 400 nm (Park et al., 1995). Photolyases are DNA repair enzymes that use energy of 400-500 nm to energize the direct reversal of pyrimidine dimer bonds. DNA photoreactivating enzymes from different sources vary in their wavelength requirements for most effective catalytic function, but all demand near UV and blue light (Saito and Werbin, 1970; O'Brien and Houghton, 1982; Eker et al., 1990). Light-independent repair systems commonly called as dark repair systems include the nucleotide excision repair, post replication recombination repair and mutagenic or SOS repair. The nucleotide excision repair involves a series of enzymes that recognize and remove pyrimidine dimers and other DNA damage directly from the DNA and resynthesize that segment of DNA (by DNA polymerase) as a complement of the undamaged strand (Karentz, 1994). It is a major mechanism of repair in all types of prokaryotes and eukaryotes, including aquatic organisms (Karentz et al., 1991).

1.5.6. OTHER PROTECTIVE PROCESSES:

As biological methods of UV protection, some microbial communities obtain the benefit of protection from physical substrata that might be present in their habitats. These can include oxidized iron compounds precipitated in the water column that protect benthic organisms (Pierson et al., 1993), overlying rock strata that protect endolithic,

chasmolithic and sublithic microorganisms (Davis, 2000) and salt crystals that protect organisms in evaporitic habitats (Rothschild et al., 1994). Organisms at sufficient depth can also obtain some protection from an overlying liquid water column (Smith and Baker, 1981). The effectiveness of a liquid water column at providing UV protection is greatly increased if organic substances are present (Vincent et al., 1998; Menon et al., 2005).

1.6. WHY TO STUDY IMPACT OF ENVIRONMENTAL FACTORS?

During the course of growth under natural field conditions, microorganisms and plants are exposed to a number of different abiotic stresses such as water stress, temperature stress, salt stress, flooding stress, chemical stress and oxidative stress. The intensity of the abiotic stresses is on rise, implying that various possible solutions for mitigating the damage caused by such stresses must be combined for future increase in photosynthetic productivity. Due to the great impact of changing environment on primary productivity and environment in aquatic as well as terrestrial ecosystem, affecting primary food web, the change in the climatic conditions must be understood and integrated in any future planning.

Research has continued to progress toward understanding the impact of UV on complete ecosystems, both freshwater and marine rather than individual organisms and responses. Excessive visible radiation, non-optimal temperatures, pollutants such as toxic heavy metal ions and changes in salinity can synergistically enhance the inhibitory effects of solar UV, including growth, reproduction, ecosystem structure and food web dynamics. Research confirms that UV-B radiation affects freshwater and marine consumers.

Increases in the level of UV-B radiation are likely to induce changes in community structure since there are great differences in susceptibility of species to UV-induced damage. Species with larger cells, having the ability to accumulate UV screening substances or with more effective repair mechanisms will likely be favored. There is ample evidence that the change in the radiation climate originating from the depletion in stratospheric ozone has significant adverse effects on aquatic organisms. However, the action of defense mechanisms partially mitigates the inhibitory effects of UV-B. Different sensitivity of species and the subsequent changes in community structures may lead to changes in steady state conditions that still sustain a high primary production. Changes in species composition might have consequences propagating within aquatic food webs that are at present difficult to predict.

The algae/plants being the primary producers of oxygen, and stores of carbon dioxide; the loss of algal/plant species due to UV-B radiation will have a tremendous impact on the other species and ecosystems. With the human food supply already strained due to demands of an ever increasing population, changes in weather and climate and loss of soil fertility, even small reductions resulting from UV-B damage to the food supply may be disastrous to large population, especially the poor and indigenous people. Indigenous populations are especially at risk from increases in UV-B radiation since they generally rely on local plants and animals as their food source. Once local food supplies are compromised, they do not have the commercial infrastructures in place to supplement their diet with imported food.

The protective function of UV screening substances has been also verified in both producers and consumers. A number of new compounds absorbing UV-A and UV-B have been identified in cyanobacteria, phytoplankton and macro algae. Consumers such as coral reef organisms, sea urchins and fish can acquire protection by taking up these substances with their food. The synthesis of these substances is induced by UV radiation, and their role as photoprotectants during evolution is recognized. These UV-screening compounds have great potential of commercial application and can be used as sunscreen, colourant in cosmetics and food and giving a bit of abiotic stress can lead to enhance synthesis of such commercially useful molecules.

1.7. OBJECTIVES

Though there is evidence that increased UV-B is harmful to aquatic ecosystems, quantitative estimates are rudimentary at this stage. There is pressing need to increase our efforts to understand the possible short and long-term effects on aquatic ecosystems on a global scale. A major loss in primary biomass productivity may have significant consequences for the intricate food web in aquatic ecosystems and affect food productivity. In order to evaluate the current productivity in the aquatic ecosystem and a possible decrease in the future, under changing climatic condition it is imperative that we understand mechanism of damage and protection in aquatic system under changing environmental conditions.

Since UV-B radiation reaching the earth's surface is increasing and will continue for considerable duration as a result of already emitted CFCs and their long life time as

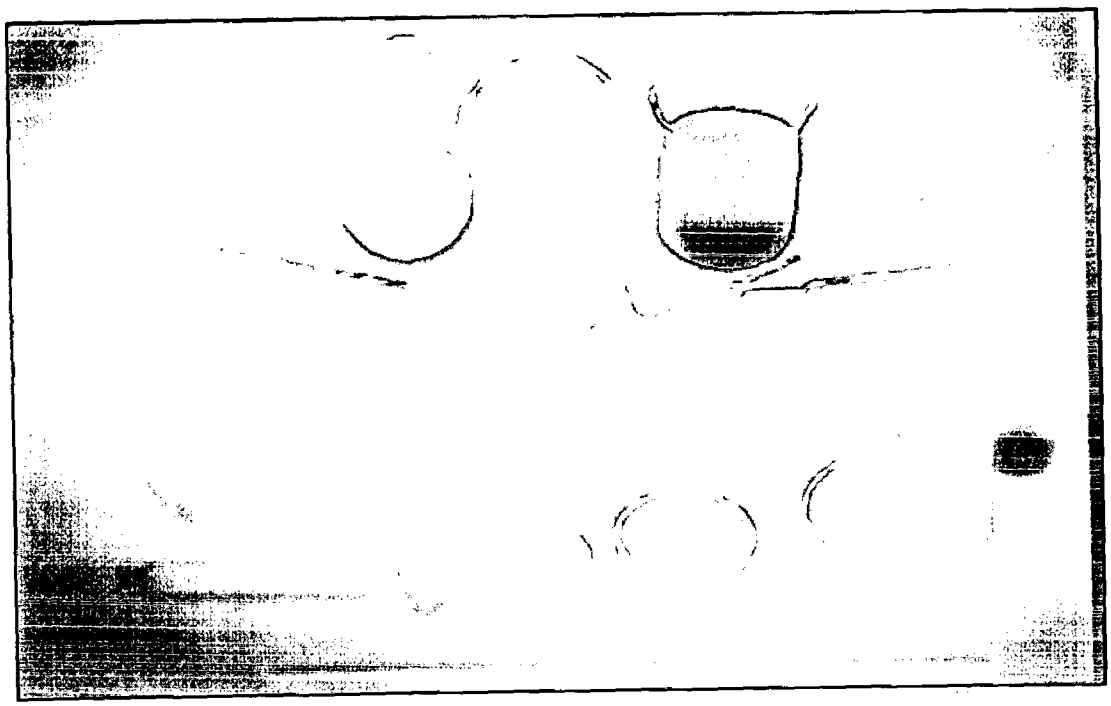
the ozone layer becomes depleted, which in recent years caused increased interest in UV-B induced efforts in the biota. Conducting research in this field is a continuing necessity in order to understand algal/plant response and predict future consequences of a changing climate. The objective of this research work was to study the impact of changing environment; i.e. higher level of UV-B radiation and high light intensity on various physiological, biochemical and molecular aspects in marine and fresh water cyanobacteria, since cyanobacteria are responsible for large part of global photosynthetic productivity and carbon sink which may be affected with changing environmental conditions in the atmosphere. The work has relevance to agriculture and environment and also may result in commercial application. The work is important to understand algal response to changing environmental conditions mainly UV-B, high PAR and predict future consequences.

Following parameters were studied in detail in *Nostoc spongiaeforme* (a freshwater cyanobacterium) and *Phormidium corium* (a marine cyanobacterium) with reference to UV-B radiation, high light (PAR) and UV-B supplemented with low visual light on

- Growth curve
- Morphological changes
- Photosynthetic efficiency (Fv/Fm ratio)
- Changes in photosynthetic pigments (such as chlorophyll, carotenoids, xanthophylls and phycobilisomes)
- Changes in various sugars such as reducing sugars, glycogen and total sugars

- Changes in nitrogen content and total protein profile
- Changes in cell membrane lipids, phospho, glyco and neutral lipids, fatty acid composition, (Estimation, TLC, GC, FTIR, NMR, MS)
- Changes in peroxidation of cell membrane lipids
- Changes in DNA and RNA content.
- Changes in UV-B absorbing compounds such as mycosporine like amino acids (MAAs) and phenolic compounds.
- Changes in antioxidant enzymes such as superoxide dismutase and ascorbate peroxidase.

CHAPTER –2



MATERIALS & METHODS

MATERIALS AND METHODS – CONTENT

2.1. MATERIALS: Cyanobacterial cultures used for the study were *Nostoc spongiaeforme* Agardh, strain GU 1 (freshwater cyanobacteria) and *Phormidium corium* (Agardh) Gomont, strain GU 2 (marine cyanobacteria).

2.1.1. *Nostoc spongiaeforme* Agardh, strain GU 1 belongs to class Cyanophyta, order Nostocales and family Nostocaceae. *Nostoc* is fresh water as well as terrestrial blue green algae. It is found in colonies, which are usually spherical, rarely oval. It has globular, bead-like cells arranged in much-tangled trichomes, many of these enclosed on a copious gelatinous matrix, which is bounded externally by a pellicle-like membrane. The trichomes consist of namely three types of cells, vegetative cells that are bluish green and thin walled, akinetes, and heterocysts, which are colourless or yellowish with thick walls. Reproduction takes place by means of hormogonia, heterocysts and akinetes.

2.1.2. *Phormidium corium* (Agardh) Gomont, strain GU 2 belongs to class Cyanophyta, order Nostocales and family Oscillatoriaceae. *Phormidium* is filamentous, filaments unbranched, parallel, usually in fine, smooth, and layered up to leathery strata. Trichomes isopolar, more or less straight, usually 2-12 μm wide, uniserial, composed of cylindrical up to slightly barrel-shaped cells, constricted or unconstricted at the cross walls. Cell content usually blue-green, thylakoids situated perpendicularly to the cell wall. Heterocytes and akinetes absent, reproduce by means of fragmentation.

2.2. CULTURE CONDITIONS:

2.2.1. CULTURE USED: *Nostoc spongiaeforme* Agardh a freshwater cyanobacterium was provided by Dr. Depak Vyas, Dr. Hari Singh Grover University,

Sagar, Madhya Pradesh. *Phormidium corium* (Agardh) Gomont a marine cyanobacterium was a kind gift from Dr. C. Raghukumar's group at National Institute of Oceanography, Goa who have isolated it from coral *Porites lutea* on the Kavaratti reefs of Lakshadweep Island. The cultures were routinely grown in autoclaved liquid culture medium. The *Nostoc* was grown in BG 11 media (Table 2.1) while *Phormidium* was grown in ASN III media (Table 2.1) according to Rippka et al., (1979).

The purity of both the cultures was maintained by routinely observing the pure cultures under microscope for contaminations. Also contaminations were checked in pure cultures by adding few drops of 5% peptone and observing for the turbidity of the cultures after 24 h.

Cultures were maintained in 100 ml conical flasks filled to 50% of their volume which were plugged with cotton, covered with paper and tied with rubber band and were sterilized by autoclaving at 15 lbs pressure for 20 min. After sterilization and cooling, 5 ml of cyanobacterial healthy stock culture was inoculated in culture media using sterilized pipette and placed on a shaker in culture room at a day/night temperature of 30°C+4 /26 °C ±3 respectively under cool white fluorescent light tubes at 80 μmol m^{-2} s^{-1} with 14h of photoperiod.

2.3. MEASUREMENT OF GROWTH PHASE: To determine appropriate logarithmic phase (balance growth phase) to conduct experiments three different parameters were studied viz: fresh weight, dry weight, chlorophyll estimation and chlorophyll fluorescence. The measurements were continued from 1 to 55 days for *Phormidium corium* and from 1 to 30 days for *Nostoc spongiaeforme* till growth showed a steady state.

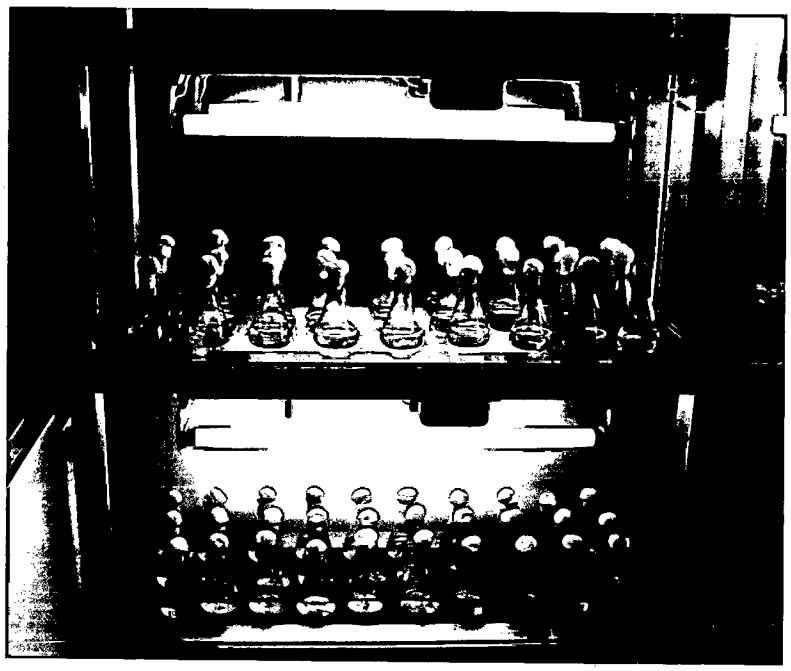
Table 2.1: Composition of standard mineral media for freshwater cyanobacterium *Nostoc spongiaeforme* (BG 11) and marine cyanobacteria *Phormidium corium* (ASN III) according to Rippka et al., (1979).

Ingredient	ASN III (Amount in g/l)	BG 11 (Amount in g/l)
Sodium chloride	25g	-
Magnesium chloride	2.0g	-
Potassium chloride	0.5g	-
Sodium nitrate	0.75g	1.5g
Potassium hydrogen phosphate	0.02g	0.04g
Magnesium sulphate	3.5g	0.075g
Calcium chloride	0.5g	0.036g
Citric acid	0.003g	0.006g
Sodium carbonate	0.02g	0.02g
Ferric ammonium citrate	0.003g	0.006g
EDTA (Di sodium salt)	0.0005g	0.001g
Trace metal mixture (A_5+Co)	1 ml/l	1 ml/l
Distilled water	1000ml	1000ml
pH after autoclaving and cooling	7.5	7.5

Table 2.2: Composition of trace metal mixture (A_5+Co):-

Ingredient	ASN III (g/100 ml)	BG 11 (g/100 ml)
Boric acid	2.86g	2.86g
Manganese chloride	1.81g	1.81g
Zinc sulphate	0.222g	0.222g
Sodium Molybdate	0.390g	0.390g
Copper sulphate	0.079g	0.079g
Cobalt nitrate	0.0494g	0.0494g

A



B

Fig 2.1 Cultures grown in (A) growth chamber and (B) shaker

Logarithmic phase is important to conduct experiment because during this period the growth rate remains constant and biomass concentration changes very little, also the concentration and ratio of the different biochemical components remains constant (Fig 2.2).

2.3.1. FRESH WEIGHT MEASUREMENTS: Cyanobacterial culture (1 ml) of respective growth phase was taken out from well-stirred subcultured conical flask and centrifuged for 10 min at 3000 x g. The excess water in the pellet was drained by gently petting it with blotting paper and fresh weight was taken on a digital balance.

2.3.2. DRY WEIGHT MEASUREMENTS: The pellet of cyanobacterial cells used for fresh weight measurement was dried in an oven at 70°C for 36h and dry weight was measured using digital balance.

2.3.3. CHLOROPHYLL ESTIMATION: 1 ml of cyanobacterial culture was taken out from well stirred sub cultured conical flask and centrifuged for 10 min at 3000 x g. Pellet was drained for excess water using blotting paper. Pellet was weighed and dissolved in 5 ml of methanol and homogenized in glass tissue homogenizer for 5-6 min. The extract was centrifuged for 5 min at 3000 x g and absorbance was recorded using supernatant at 666 nm and 470 nm using spectrophotometer (Schimadzu). The chlorophyll concentration was calculated according to Wellburn and Lichlentheler, (1983).

2.3.4. PHOTOSYNTHESIS MEASUREMENTS: Chlorophyll fluorescence analysis is a useful monitor of photosynthesis, because it is noninvasive, sensitive and scalable over large ranges of times. It has been widely applied to characterize photosynthetic metabolism in cyanobacteria and cyanolichens (Campbell et al., 1998).

Cyanobacterial culture (1 ml) of respective growth phase culture was taken out from well-stirred subcultured conical flask and placed in a cuvette. Photosynthesis measurements were taken using chlorophyll fluorometer (PAM, Walz Germany) according to Sharma et al., (1998a). The top transparent window of the cuvette was fixed with the fibre optic cable carrying modulated and actinic light to the samples in the cuvette and fluorescence from the sample in the cuvette to detector. Cyanobacterial culture was dark adapted for 10 min prior to measurements at room temperature. The dark adapted culture were kept stirred by placing the cuvette on a magnetic stirrer and exposed to a modulated light with an intensity of 3 μmol m^{-2} s^{-1} to measure initial fluorescence (Fo). This was followed by an exposure to a saturating pulse of white light of 4000 μmol m^{-2} s^{-1} to provide the maximum fluorescence (Fm). Variable fluorescence (Fv) was determined by deducting the Fo from Fm (Fv=Fm-Fo) and the Fv/Fm ratio was calculated.

Based on above-mentioned parameters of fresh weight, dry weight, chlorophyll-carotenoid and chlorophyll fluorescence, the most stable growth stage (logarithmic phase) to conduct experiment was 14±1 day for *Nostoc spongiaeforme* and 33±1 day for *Phormidium corium* and all experiments were performed with these particular phase. As far as possible experiments was conducted with 14 and 33 days old culture of *Nostoc* and *Phormidium* respectively.

2.3.5. HARVESTING THE STABLE GROWTH PHASE OF CYANOBACTERIAL CELLS FOR VARIOUS ANALYSIS: Cyanobacterial culture was harvested by centrifuging respective days culture of the 100 ml flask at 8000 x g for 15 min. The pellet was weighed and homogenized in a glass tissue homogenizer for 6 min and redissolved in fresh culture medium with a ratio of 1:5

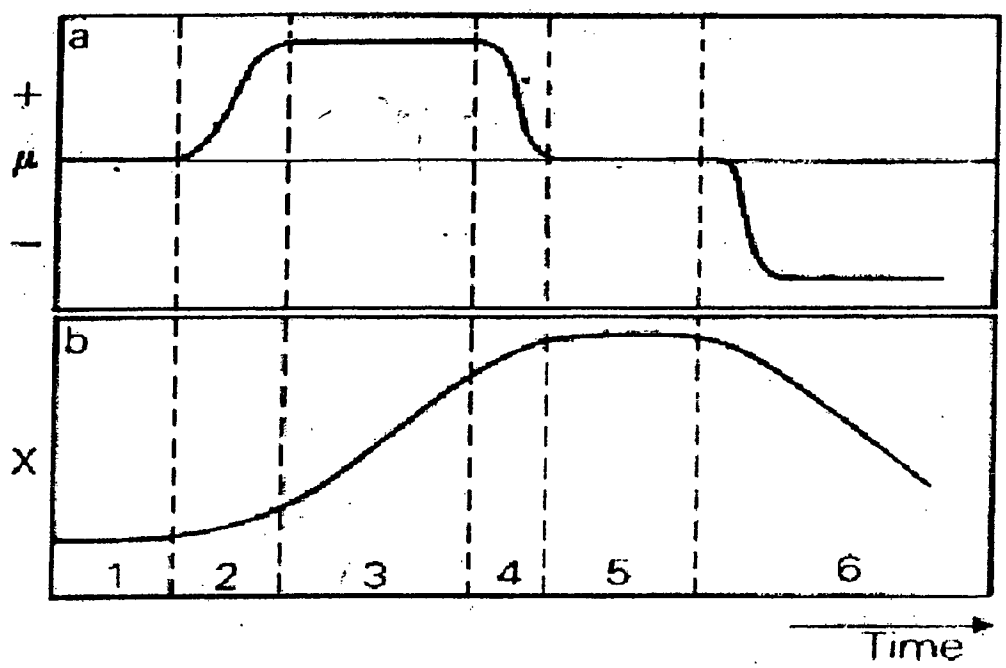


Fig 2.2 Growth curve of alga based on (a) biomass and (b) growth rate

(w/v). This resuspension was used for various analysis presented in this thesis, unless otherwise stated.

2.4. TREATMENT CONDITIONS: The three different treatments viz. UV-B radiation, high light and UV-B supplemented with low visual light at 30°C ± 2°C were given to the cyanobacterial cultures.

2.4.1. EXPOSURE TO UV-B RADIATION: The resuspended culture was transferred to a petri-plate. The UV-B treatment of 0.8±0.1 mW cm^{-2} (Vilbour-Lourmat, France T-6M source with a λ-max at 312 nm) was given in a BOD chamber at 30°C for 0-6 h while keeping the culture continuously stirred in order to prevent the shading effect due to the culture. Spectrum of the UV-B radiation is shown in Fig. 2.3. The culture was stirred using a small magnetic flea (1 mm x 1 cm) at slow speed to avoid mechanical damage to the algal tissue (Fig 2.4a). The UV-B radiation was measured using a UV-B radiometer specific for measuring radiation of 312 nm from the same manufacturer.

2.4.2. EXPOSURE TO UV-B RADIATION SUPPLEMENTED WITH LOW VISUAL LIGHT: The same UV-B treatment (0.8±0.1mW cm^{-2}) was also supplemented with white light of 80 μmol m^{-2} s^{-1} PAR at the culture level using cold light source with fibre optic at 60° angle to the culture (Fig. 2.4b).

2.4.3. EXPOSURE TO HIGH LIGHT: The resuspended culture was exposed to high irradiance of 500 μmol m^{-2} s^{-1} PAR at the culture level at 30°C for 0-6 h in a double walled cuvette circulated with temperature controlled water. The light treatment was given using two slide projectors from opposite sides. The culture was stirred using a small magnetic flea (1 mm x 1 cm) at slow speed to avoid mechanical damage as well as self-shading to the algal tissue. The light irradiance was measured

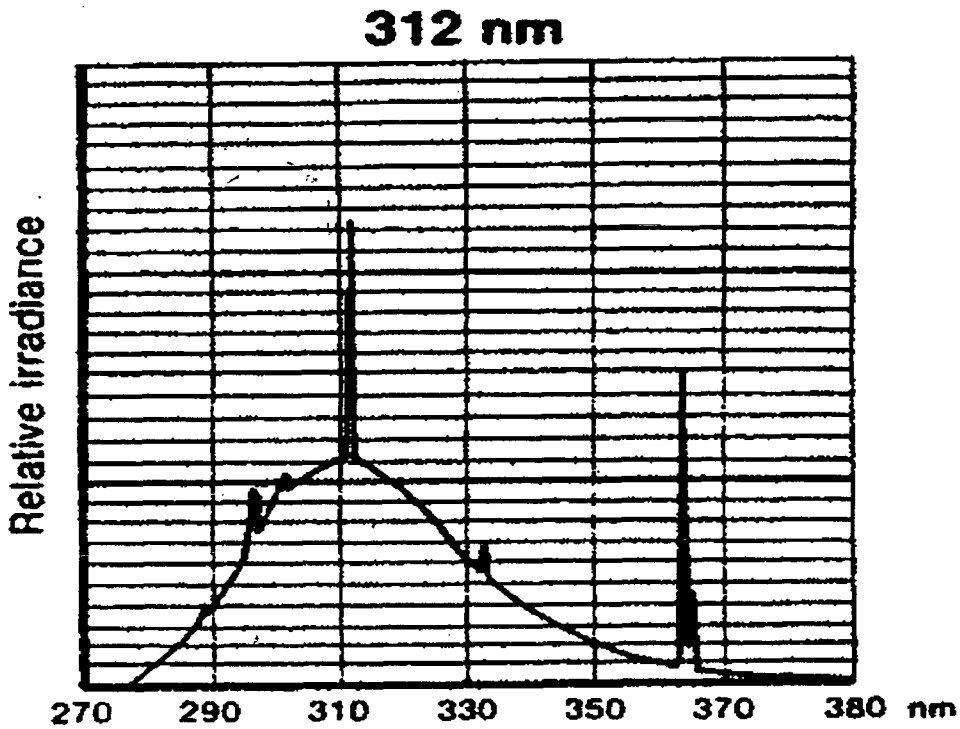
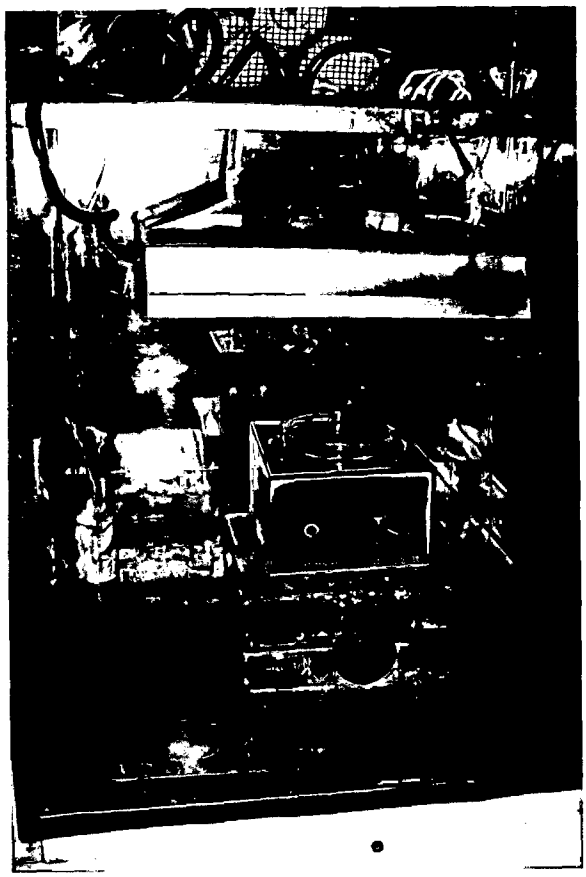


Fig 2.3 Spectral profile of UV-B radiation source

A



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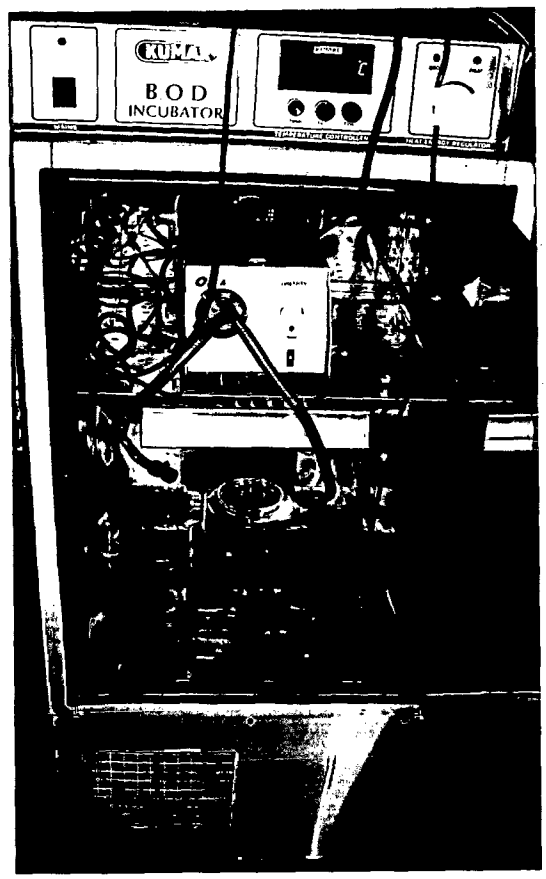

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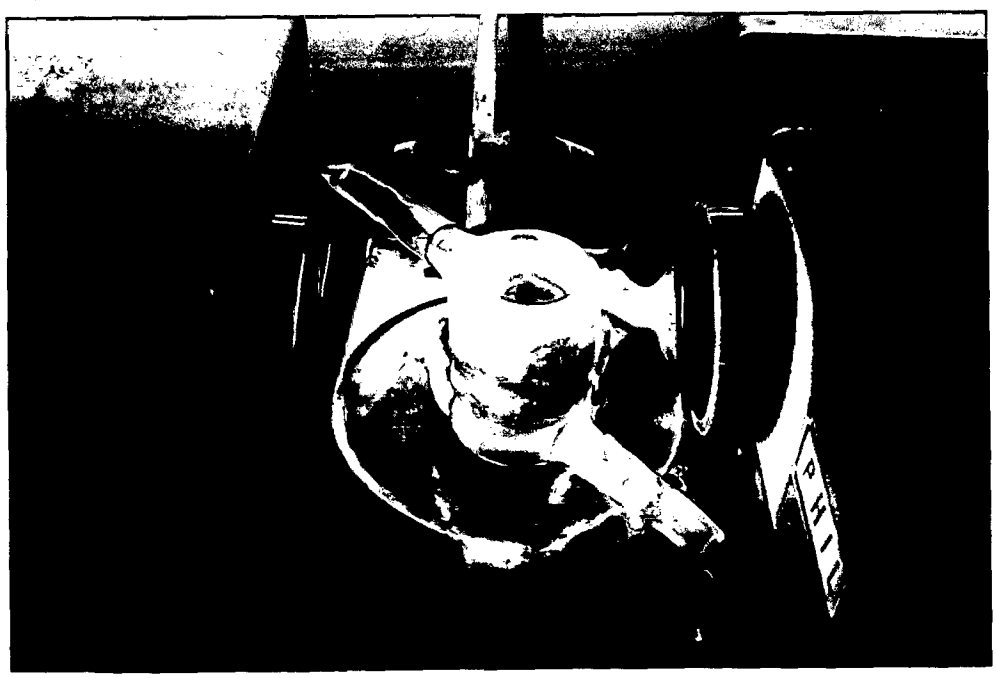


Fig 2.4 Figure showing (A) UV-B, (B) UV-B supplemented with low visual light and (c) high light treatment.

using radiometer (Li-Cor, Model Li-189). The PAR in the cuvette was determined by measuring the PAR value at the beginning of the cuvette and at the end of the water filled cuvette and the difference was divided by two (Fig 2.4c).

2.5. MORPHOLOGICAL CHANGES: Cells were mounted in glycerin on a slide and observed under 40X and 100X (oil emulsion) using Olympus BX41 compound microscope fitted with Olympus DP12 digital camera.

2.6. PHOTOSYNTHESIS MEASUREMENTS: Fv/Fm measurements were taken from resuspended cell culture according to Sharma et al., (1998a) described in the section 2.3.4.

2.7. PHOTOSYNTHETIC PIGMENTS ANALYSIS: Quantitative and qualitative analysis of photosynthetic pigments was carried out using different methods such as high-pressure liquid chromatography (Waters, HPLC), thin layer chromatography (TLC) and UV-Visible spectrophotometer (Schimadzu, UV-2450).

2.7.1. EXTRACTION OF PHOTOSYNTHETIC PIGMENTS: Extraction of photosynthetic pigments was carried out according to method described by Sharma and Hall, (1996). Cells were collected after centrifugation of culture at 6000 x g for 15 min. The supernatant was discarded and pellet of the cells was dried in lyophilizer (Snijders Tilburg, Holland). 0.1g of lyophilized cells were extracted in 1ml of 80% (v/v) methanol containing 0.1% BHT in (10 ml) tissue homogenizer for 5-6 min at 4°C under dim light, followed by centrifugation at 8000 x g for 10 min at 4°C. The supernatant was used for HPLC and TLC analysis of pigments.

2.7.2. HPLC ANALYSIS OF PIGMENTS: The photosynthetic pigments were separated by HPLC with reverse phase column (Waters Spherisorb ODS 25 μm x 4.6 mm x 250 mm) and a detection programme (Waters 2996 phase diode array detector).

The samples were filtered through 0.2 µm pore sized microfilters prior to loading. 10 µl of the pigment sample was injected into the HPLC column. The gradient for separation was 0-100% ethyl acetate in acetonitrile/ water (9:1) over 25 min with flow rate of 1.2 ml/min and the peaks were detected at 445 nm. The quantity of pigments was calculated from peak area value using β-carotene as external standard. Identification of pigments was carried out using retention time against standards and using spectral profile of individual peaks using PDA detector in the range of 400-700 nm.

2.7.3. TLC ANALYSIS OF PIGMENTS: Separation of photosynthetic pigments was carried out using TLC on silica gel plates according to Sankhalkar, (2000). Pigment samples (50 µl) were loaded as discrete spots on the silica plates, 1.5-2 cm from the bottom by means of a syringe. The plates were developed using solvent system, n-hexane:ethyl acetate:triethanolamine (50:38:12). The color spots were identified using their R_f values and spectral profile of individual spot using HPLC.

2.7.4. SPECTROPHOTOMETRIC ANALYSIS OF PHYCOBILISOMES: The resuspended culture (5 ml) was centrifuged at 6000 x g for 15 min at room temperature and dissolved in 5 ml of 20mM sodium acetate buffer with pH 5.5, and cells were broken using sonicator (Bandelin electronic, UW 2200, Germany) at 50% power with 9 cycles for 1 min. Phycobilisomes of sonicated ruptured cells were precipitated with 1% (w/v) streptomycin sulphate for 30 min at 4°C and collected by centrifugation at 6000 x g for 30 min at 4°C. The absorbance of phycocyanin, allophycocyanin and phycoerythrin were measured at 620 nm, 650 nm and 565 nm using UV-Visible spectrophotometer (Schimadzu, UV-2450) and calculated according to Liotenberg et al., (1996).

2.8. SUGAR ANALYSIS:

2.8.1. ESTIMATION OF REDUCING SUGARS: The amount of reducing sugars present in the sample was determined using 2-4, dinitrosalicylic acid (DNS) according to Plummer, (1987). 1 ml of resuspended culture was mixed with 2 ml of distilled water and 1 ml of dinitrosalicylic acid reagent (4% DNS dissolved in alkaline sodium potassium tartarate) and boiled for 5 min in a water bath. The mixture was cooled at room temperature and the extinction was read at 540 nm. The amount of reducing sugars present in the sample was calculated using standard graph with glucose solution.

2.8.2. ESTIMATION OF GLYCOGEN: Glycogen content was determined according to Liotenberg et al., (1996). Resuspended culture (1 ml) was hydrolyzed by boiling for 20 min with 50 µl of 50 % sulphuric acid. To this, 5 ml of O-toluidine reagent was added and samples were boiled for 10 min and cooled on ice. The concentration of glucose released by the acid hydrolysis was calculated from the absorbance at 635 nm using a standard curve established with glucose solution.

2.8.3. ESTIMATION OF TOTAL SUGARS: Total sugar content was determined using anthrone reagent according to method described by Plummer, (1987). Resuspended culture (0.5 ml) was used for estimation of total sugar. 4 ml of anthrone reagent (2% w/v) in concentrated sulphuric acid was added to the cell suspension and incubated at 100°C in a water bath for 10 min. The mixture was cooled and the absorbance was read at 620 nm against reagent blank. In this method carbohydrates are first hydrolyzed into simple sugars using dilute hydrochloric acid. Glucose is hydrated to hydroxymethyl furfural in hot acidic medium and the compound forms with anthrone a green colored product with absorption maximum at 620 nm. Total sugar content was calculated using standard graph with glucose.

2.9. ESTIMATION OF NITROGEN CONTENT: 0.5 g of dry cyanobacterial cells were dissolved in 15 ml of 0.32% potassium permagnete and boric acid with mixed indicator was kept in KEL PLUS nitrogen analyzer. After 6 min of digestion, color will change from pinkish red to greenish blue. This sample was titrated against 0.002N sulphuric acid, thus colour changes from greenish blue to purple. Nitrogen in the sample reacts with sodium hydroxide in the presence of potassium permagnete and forms ammonium borate and nitrogen content was calculated by formula given below according to Tandon, (1987).

Nitrogen = TV X Normality of H_2SO_4 X 0.014/ Weight of sample

2.10. QUANTITATIVE MEASUREMENT OF ACTIVE OXYGEN SPECIES: Production of AOS was determined using epinephrine method (Boveris, 1984). Epinephrine (4.5 mM) was added to the buffer containing 6 mM EDTA in 10 mM sodium carbonate (pH 10.2) with or without the algal tissue. This resulted in Production of AOS, which was determined at 480 nm using double beam spectrophotometer (Shimadzu, UV-2450).

2.11. PEROXIDATION OF CELL MEMBRANE LIPIDS: Lipid peroxidation was determined by the production of thiobarbutaric acid-malonaldehyde (TBA-MDA) adduct formation (2 molecules of TBA reacts with one molecule of MDA) which signify the formation of polyunsaturated fatty acid peroxides in biological systems. Resuspended culture (5 ml) was centrifuged at 5000 x g for 10 min and cell pellet was homogenized in 0.5% TCA in a glass tissue homogenizer. The homogenate was made up to 5 ml and centrifuged at 6000 x g for 15 min. The supernatant was collected and used for measuring the peroxidation of membrane lipids. 1 ml of supernatant was added to the test tube containing 2.5 ml of freshly prepared (0.5%) thiobarbutaric acid (TBA) in (20%) trichloroacetic acid (TCA) and allowed to incubate for 30 min at

90°C in a water bath. After incubation, it was allowed to cool and centrifuged for 3 min at 2000 x g to settle the debris and non-specific precipitation. The optical density was measured at 530-600 nm on UV-Visible spectrophotometer (UV-2450, Schimadzu). The absorbance at 600 nm was subtracted from absorbance at 530 nm in order to correct for the non-specific turbidity. The amount of MDA-formation was quantitated by extinction coefficient of MDA 155 mM^{-1} cm^{-1} according to method described by Sharma et al., (1998b).

2.12. LIPID ANALYSIS:

2.12.1. PURIFICATION OF SOLVENTS: Solvents such as chloroform, methanol, acetone and hexane in the commercial grades contain impurities, which interfere in the lipid analysis. These impurities can be removed by purification of these solvents by washing with concentrated acids and distillation.

2.12.1.1. PURIFICATION OF CHLOROFORM: Chloroform contains 0.25-2% ethanol, which will interfere in the lipid analysis. The ethanol can be removed by washing the chloroform several times with sulphuric acid, distilled water, drying the solvent over anhydrous calcium chloride and storing in dark bottles. 500 ml of chloroform and 25 ml of concentrated sulphuric acid was taken in a separating funnel and shaken vigorously. The lower light yellow color of sulphuric acid was eluted out and the procedure was continued twice or trice until the lower sulphuric acid layer becomes colorless. Enough distilled water was added to solvent, shaken vigorously and this was repeated until the lower chloroform layer becomes clear. The lower chloroform layer was eluted in a conical flask and dried it over 2-5 g of anhydrous calcium chloride overnight to absorb moisture present in the chloroform and this anhydrous chloroform was filtered using filter paper, distilled. Distilled chloroform was stored in dark color bottles at room temperature as light damages chloroform.

2.12.1.2. PURIFICATION OF METHANOL: Commercial grade methanol contains traces of aldehyde and amines, which can be removed by distillation. 5 g of dry magnesium turnings and 30-50 ml of methanol together with 0.5 g of iodine was refluxed until all the iodine disappeared. A vigorous reaction soon starts and when all the magnesium has dissolved, a further 1.5 l of methanol was added and the mixture was refluxed for 1 h (white fungus like dried contamination occurred), after which it is distilled. The first few 10-15 ml of methanol should be discarded but most of the remainder is collected in dark bottles.

2.12.1.3. PURIFICATION OF ACETONE: The major impurities in the commercial grades of acetone are methanol, acetic acid and water, the analytical reagent generally contains less than 0.1% of the organic impurities although the water content may be as high as 1%. In a round bottom flask containing 500 ml of acetone was heated (temperature at which acetone boils) under reflux for about 1-2 h with successive quantities of 0.1 g of potassium permagnete until the violet color of potassium permagnete persists. This solvent was then dried with 25 g of anhydrous potassium carbonate or calcium sulphate to absorb moisture for 2-3 h. Solvent was filtered using filter paper, distilled and stored in brown color bottles.

2.12.1.4. PURIFICATION OF HEXANE: Hexane and most aliphatic hydrocarbons may contain olefins, which can be removed prior to distillation by washing with the concentrated sulphuric acid, followed by 10% sulphuric acid containing potassium permanganate. Most of the aliphatic hydrocarbons may be removed by vigorous shaking of (500 ml) hexane in round separating funnel 2-3 times with 10 ml of concentrated sulphuric acid. The lower layer was eluted out then the solvent was washed 2-3 times with successive portions of 0.05% concentrated solution of potassium permanganate in 10% sulphuric acid by vigorous shaking until the color of

permanganate remains unchanged and lower layer was eluted out. Solvent was then thoroughly washed with 1N sodium carbonate solution 2-3 times, eluted sodium carbonate layer was washed 2-3 times with distilled water, eluted distilled water layer, collected hexane layer in a conical flask and dried over 2-3 g anhydrous calcium chloride overnight, filtered, distilled and stored in brown color bottles.

2.12.2. EXTRACTION OF TOTAL LIPIDS: Total lipids were extracted according to the method described by Turnham and Northcote, (1984). Freshly harvested wet cell pellet (8 g) was boiled in 5 ml of isopropanol for 2 min using spirit lamp to inhibit the lipase activity. This isopropanol solvent was dried using nitrogen gas and cells were homogenized in chloroform: methanol (1:2 v/v) to make the final volume 15 ml and 0.01% BHT was added as antioxidant in the lipid extraction solvent system. Extracted lipids were purified as described by Folch et al., (1957). Lipid extract was centrifuged for 5 min at 2000-3000 x g to get rid of cell debris and to the supernatant, 0.8 ml of double distilled water, 5 ml of chloroform and 5 ml of 0.88% potassium chloride was added in a separating funnel. The mixture was shaken vigorously for 5 min and total lipids were kept for separation for about 30 min. The lower phase of chloroform contains appreciable amounts of extracted lipids. Total lipids were taken into 10 ml screw capped vials fitted with Teflon lining. Again 1 ml of chloroform was added to the separating funnel, shaken and kept for separation for 5 min to remove all total lipids and total lipids were concentrated under the nitrogen gas. The lipid sample was stored in a small volume of chloroform (4-5 ml) at -20°C until further use. The entire extraction and purification process was done in diffused light to protect the lipids from photo oxidation.

2.12.3. GAS CHROMATOGRAPHIC ANALYSIS OF FATTY ACIDS: The fatty acids are easily identified by gas chromatography as methyl esters. The methyl esters

have lower boiling point and would become volatile than fatty acids.

2.12.3.1. ESTERIFICATION OF FATTY ACIDS: Before the fatty acid composition of a lipid can be determined by gas chromatography, it is necessary to prepare the volatile methyl ester derivatives of the fatty acid components.

2.12.3.1.1. Preparation of methanoic-HCL: Methanoic HCL was prepared according to the method described by Christie, (1982). 5 g of ammonium chloride were taken in a conical flask (with side arm), which was connected to another receiver, which was fitted to a side arm conical flask containing 50 ml of methanol. Concentrated sulphuric acid (10 ml) was added to ammonium chloride using buchner funnel and thus reaction liberates hydrochloric acid, which was bubbled in methanol until entire ammonium chloride dissolves with continuous mixing using magnetic stirrer for about 10-15 min. The concentration of hydrochloric acid in methanol was determined by titrating a portion (5ml) of methanoic-HCL against 1N sodium hydroxide (burette solution). 2-3 drops phenolphthalein (1% in 70% ethanol) was used as indicator of end point of neutralization (colorless to pink). Burette reading was noted down and volume of methanol needed for esterification was calculated using formula, $N_1V_1=N_2V_2$.

2.12.3.1.2. Preparation of methyl esters from fatty acid: The fatty acid methyl esters were prepared according to the method described by Christie, (1982). The total lipid sample (1-2 ml) was evaporated to dryness with nitrogen gas after adding known amount (50 µl) of internal standard (1mM heptadeconoic acid) (Allen and Good, 1971) and 5 ml of methanoic-HCl was added to 10 ml of screw capped with teflon lining. The vials were loosely closed with a teflon-lined cap and placed in an oven at 68-70°C for 10 min before the caps were securely tightened. After 2 h in the oven, the vials were cooled at room temperature, 5 ml of distilled water was added (light yellow

color develops), the two phases were mixed by vortexing and methyl esters were extracted with three successive 2 ml portions of distilled hexane, vortexing for 90 seconds. Pasteur pipette were used to separate the hexane extract, which were pooled in another test tube. The hexane solution of methyl esters was treated with 5 ml of saturated solution of sodium bicarbonate and neutralized by shaking (vortexing) for 15 seconds. Then washed with 5 ml of distilled water, vortexed for 15 seconds and upper (hexane) solution was transferred to another test tube and dried it with anhydrous calcium chloride (2-3 crystals), taken in vials and evaporated to dryness in a water bath at 35-40°C with the help of nitrogen gas. The methyl esters were taken in small volume (20 µl) of fresh hexane and vials were capped and stored in a freezer at -20°C until needed for gas chromatography, which should be done as soon as possible to minimize danger of loss of unsaturated esters.

2.12.4. GC ANALYSIS OF FATTY ACIDS: Methyl esters of fatty acids were run on a Nucon gas chromatograph equipped with flame ionization detector and chromatopack data processor. The column (6 mm x 2 mm i.d, stainless steel) was packed with DEGS 10% on 80-100 mesh chromosorb W-HP (Chemlabs, Bangalore). Column temperature was 180°C and injector temperature was 220°C and nitrogen was used as carrier gas (flow rate 30 ml/min). 2 µl of sample was injected to the injector port of gas chromatography using 10 µl fixed-needle syringe. Fatty acid methyl esters peaks were identified by comparing their retention times with methyl esters of pure fatty acid standards and were quantified by using the peak areas of individual fatty acids calculated using the program given by the manufacturers of the instrument. The instrument was programmed to give the Mole % of different fatty acid directly.

(1) The formula for Response Correction Factor (RCF),

RCF=Amount of compound 1/Peak area of 1 X Peak area of reference/Amount of reference

(2) The formula for correction of Peak Area of fatty acid methyl esters for varying detector sensitivity is

CPA = Peak area of the component 1 X RCF

(3) The formula for Conversion of corrected peak area (Wt.% to Moles) is

Mole Corrected peak area = Corrected peak area/Molecular weight of each fatty acid

(4) The formula for Mole Corrected peak area to Mole % fatty acid methyl esters is

Mole % = Mole Corrected peak area/Total of Mole Corrected peak areas of all fatty acid methyl esters X 100

2.12.5. SEPARATION OF GLYCOLIPIDS, PHOSPHOLIPIDS AND NEUTRAL LIPIDS BY COLUMN CHROMATOGRAPHY FOR FATTY ACID ANALYSIS: The total purified lipids were separated into glycolipids, phospholipids and neutral lipid fraction using column chromatography (Kates, 1972) on a column of silicic acid.

2.12.5.1. Preparation of column: In a 10 ml of glass column, glass wool was used to seal it from bottom and rinsed it with chloroform. With the help of stopper cork, the flow of the chloroform from the column was blocked and 1-2 ml of chloroform was added into the column to avoid air bubbles. Slurry of 1-1.5 g of silicic acid was prepared in a 50 ml beaker in about 4-5 ml chloroform so as to get the proper ratio of lipid: silicic acid (1:75). The slurry was poured into the chromatography column using pasture's pipette, stopcock was opened and tube was gently tapped to dislodge air bubbles and this help in settling of the column. Then chloroform was added above the silicic acid and washed the column with 2 column volumes of chloroform. There should always be 1-2 ml of chloroform solvent left above the silicic acid to avoid drying of the column these results in the formation of channels, which will effect the

elution of the solvent. The column should not be expose to light thus always cover it with black paper.

2.12.5.2. Application of sample and elution of column: The total lipids dissolved in a small volume of chloroform was loaded on to the column of silicic acid using pasture's pipette carefully down the sides of the column, allowed solvent to run into column and chloroform was added to ensure quantitative transfer of lipid mixture to the column. Elution of the column at a flow rate of about 1-3 ml/min was carried out with the following solvents. Neutral lipids were eluted with 10 column volumes of chloroform; glycolipids with 40 column volumes of acetone and phospholipids with 10 column volumes of methanol. The total volume of the each of solvents were collected in a test tube and labeled properly and these fractions were concentrated under vacuum under nitrogen gas atmosphere. Dried lipids were immediately dissolved in a known volume of chloroform and stored in vials at -20°C until further use.

2.12.6. QUANTITATIVE ESTIMATION OF GLYCOLIPIDS (TOTAL SUGARS): Total sugars in glycolipids were determined by the phenol-sulfuric acid method according to Kushawa and Kates, (1981). An aliquot of lipid extract (100 µl) was pipetted out in the test tube and evaporated the solvent to dryness under steam of nitrogen, to the residue added 2 ml of distilled water and 1 ml of 5% phenol solution, and mixed gently by vortexing, making sure that the film of lipid at the bottom of tube is undisturbed. To this added 5 ml of concentrated sulphuric acid with vortexing and heated for 5 min in a boiling water bath. The mixture was vortexed briefly and allowed to cool for 30 min. The absorbance of the orange color was read at 490 nm against reagent blank. For calibration, standard containing 1mM D-glucose of different concentration was used. The blank and series of standards starting from

0.01-0.1μM were analyzed simultaneously. The amount of sugars in the unknown sample was read from a calibration curve, prepared at the same time by performing the reaction on known amount of standard sugar solution using D-glucose.

2.12.7. QUANTITATIVE ESTIMATION OF PHOSPHOLIPIDS: Phospholipid was estimated by determining the amount of phosphorus in the lipid sample according to Bartlett method (Christie, 1982). An aliquot of lipid sample (100 μl) was dried using nitrogen gas and 0.4 ml of 70% perchloric acid was added in a test tube and the liquid was digested by gently refluxing for 2 h on sand bath until the solution becomes colorless. The mixture was cooled and 2.4 ml of ammonium molybdate reagent was added, mixed properly, followed by the addition of 2.4 ml of reducing reagent containing sodium bisulphite, sodium sulphite and 1-amino-2-naphthol-4-sulphonic acid. The solutions were mixed thoroughly and were heated on a boiling water bath for 10-15 min for color development. On cooling, the absorbance of the solution was measured at 830 nm. A blank and series of standard samples ranging from 0.01-0.20 μM was analyzed simultaneously. The amount of phosphorus in the unknown sample was read from a calibration curve, prepared at the same time by performing the reaction on known amount of standard phosphate solution using 1mM sodium dihydrogen orthophosphate.

2.12.8. SEPARATION OF TOTAL LIPIDS BY TLC: Separation of total lipids was carried out by thin layer chromatography (TLC) on silica gel H according to Liljenberg and Von Arnold, (1987). Uniform slurry of 100 g of silica gel H was prepared in 100 ml of distilled water and stirred for 1-2 h on a magnetic stirrer. Glass plates was cleaned with acetone and arranged on a table and slurry was pulled at a steady rate across the plate on the table, from left to right using glass rod. The plates were placed in the drying rack, left at room temperature for an hour and dried at

120°C for 2 h. They were then stored in the storage cabinet, but should be heated again at 120°C for 1 h before use. After cooling the plates, samples (100 µl) were applied as discrete spots, 1.5-2 cm from the bottom of the plate, in chloroform by means of a syringe and the plate was then placed in chromatographic chamber containing eluting solvents. In a chromatography chamber, filter papers were placed on both sides to saturate the atmosphere inside with solvent vapors which speeds up the analysis, especially with polar solvents and may occasionally improve the resolution. The plates were run in the chamber up to the top of the plate with solvent system containing chloroform: methanol: glacial acetic acid: water (85:15:10:3.5) for phosphoglycolipids and diethyl ether: water (90:1) for neutral lipids, Plates were then air-dried and spots were visualized as bands with iodine vapors in iodine chamber, identified by their R_f values (Liljenberg and Kates, 1985).

2.13. ANALYSIS OF LIPIDS BY FOURIER TRANSFORM INFRA RED (FTIR) SPECTROPHOTOMETRY: FTIR measurement was done to detect and estimate specific functional and structural groups in lipids. The sample for FTIR was prepared in chloroform according to the method described in section 2. 12. 2. Lipid sample in chloroform was evaporated to dryness using nitrogen gas and immediately dissolved in 1 ml carbon disulphide (CS_2). A drop of the sample is placed on a sodium chloride (NaCl) window and another sodium chloride window was placed on top of the drop, tightened and fixed it to sample holder of the FTIR spectrophotometer (Schimadzu FTIR 8101A with DR-8031 recorder) according to method described by Tilvi et al., (2005). The spectrum was recorded in percent transmittance mode with 4.0 cm^{-1} resolution.

2.14. ANALYSIS OF LIPIDS BY NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROPHOTOMETRY: NMR study was carried out to determine fatty

acid components, particularly to identify, locate double bonds and other functional groups in fatty acids. The sample for NMR spectrum was prepared according to the method described in section 2. 12. 2. Sample in chloroform was evaporated to dryness using dry nitrogen gas and dissolved in deuterated chloroform ($CDCl_3$). Sample was transferred in a NMR tube and was analyzed using 300 MHz, NMR Spectrometer (Bruker, Avance 300) according to method described by Tilvi et al., (2005). Chemical shift for $CDCl_3$ is observed at 7.26 ppm.

2.15. ANALYSIS OF LIPIDS BY MASS SPECTROMETRY (MS): Mass spectroscopy was done for the analysis of different peptides and for the identification of higher molecular weight lipids. Also to locate double bonds in aliphatic chains and to determine the position of the other functional groups in the fatty acid chains. The sample for MS analysis was prepared as described in section 2. 12. 2. The instrument was operated in positive ionization mode. Lipid sample in chloroform was evaporated to dryness using nitrogen gas and dissolved in 1:1 methanol: water with traces of trifluoroacetic acid (TFA). This methanol: water solublized sample was directly infused at a constant flow rate of 10 µl/min into the ion spray source with integrated syringe pump of mass spectrophotometer (Applied Biosystem instrument, Canada, Model QTOF-XL MS/MS equipped with MDS Sciex Analyst software). Full scan data acquisition was performed, scanning from m/z 100 to m/z 2000. MS/MS products were produced by collision-induced dissociation (CID) of selected precursor ions at collision energy between 25-40V and mass analyzed using TOF analyzer of the instrument according to method described by Tilvi et al., (2005).

2. 16. PROTEIN ANALYSIS:

2.16.1. ESTIMATION OF PROTEINS: SDS-PAGE sample loading and antioxidant enzymes activity was based on protein content of the sample. The amount

of protein present in the sample was measured by the method described by Lowry et al., (1951). 1 ml resuspended culture was taken to which 5 ml of solution C containing 100 ml of solution A (2% sodium carbonate in 0.1N sodium hydroxide) and 2 ml of solution B (0.5% copper sulphate in 1% potassium sodium tartarate) was added, mixed well and allowed it to stand for 10 min at room temperature. To this 0.5 ml of Folin-Ciocalteu's reagent (1:1 v/v) was added, mixed well and incubated for 30 min at room temperature in dark. The blue color developed was recorded at 660 nm. Amount of protein present in the sample was calculated using standard graph with bovine serum albumin (BSA).

2.16.2. SDS-PAGE ELECTROPHORESIS: SDS-PAGE electrophoresis was carried out to separate different proteins on the basis of their size and determine the molecular weight of proteins.

2.16.2.1. Sample preparation: Resuspended culture (5 ml) was sonicated for 1 min using sonicator (Bandelin electronic, UW 2200, Germany) with 9 cycles at 50% power followed by centrifugation at 6000 x g for 10 min. Supernatant was discarded and the pellet was washed twice using 0.065M Tris-HCl buffer. To the residue, 100 µl loading buffer containing Tris buffer, SDS, glycine, bromophenol blue, mercaptoethanol was added and thoroughly mixed with toothpick. Sample was incubated at 80°C for 2-3 min in a water bath and immediately transferred to ice. Prior to loading the sample was centrifuged at 4000 x g for 5 min. 10 µl of protein sample was loaded in the wells of stacking gel.

2.16.2.2. Preparation of gel: Electrophoresis of cell membrane protein was performed using a vertical gel system (Hoeffers, gel system) according to the method described by Laemmli, (1970). Separating gel with 10%T and 2.5%C was prepared by mixing the reagents mentioned in Table 2.3 and poured in the chamber between the

glass plates. Water saturated butanol was layered on top of the gel and kept to set for 20-30 min. After polymerization, the water-saturated butanol was decanted from the top of the gel and washed 2-3 times with distilled water. The stacking gel (4%T and 2.5%C) was prepared by mixing the solutions mentioned in Table 2.3, was poured on top of the separating gel. Gel comb was placed and allowed to set for 15-20 min. After polymerization of stacking gel, comb was removed without distorting the shapes of the well and carefully installed the gel in the apparatus.

2.16.2.3. Gel electrophoresis: Tank buffer (0.05M Tris-HCl, 0.192M glycine and 0.1% SDS) was filled in electrophoretic unit and connected the power to check the electrical circuit. The protein sample equivalent to 15 µg was injected into sample well using micro syringe through tank buffer. Similarly, one well was loaded with standard marker proteins in the sample buffer. Electrophoresis was performed using electrophoretic system from Hoefer (PS 500 X T.D.C. power supply) at constant voltage of 50V till the proteins entered the separating gel and then the run was continued at 100V voltage until the bromophenol blue reaches the bottom of the gel. After electrophoresis, the gel from between the plates were removed carefully and immersed in staining solution (1% coomassie brilliant blue in methanol, glacial acetic acid and distilled water) for at least 2 h with uniform shaking on a rocking table. Subsequent to the staining, the gel was transferred to suitable container with at least 200-300 ml of destaining solution (methanol, glacial acetic acid and distilled water) and kept on a rocking table for 30 min, the destainer was changed frequently until the background of the gel is colorless. Analysis of the gel was done using Alpha digiDoc gel documentation system.

Table 2.3: Composition of 10% separating gel and 4% stacking gel.

Stock	Separating gel (10%)	Stacking gel (4%)
Acrylamide solution (30%) Bis acrylamide (0.8%)	3.3 ml	1.5 ml
Tris-HCl (1.875M, pH 8.8)	2.5 ml	---
Tris-HCl (0.6M, pH 6.8)	---	2.5 ml
Water	4 ml	6 ml
SDS (10%)	0.1 ml	0.1 ml
APS (5%)	50 µl	50 µl
TEMED	25 µl	20 µl

2.17. ASSAY OF NUCLEIC ACIDS:

2.17.1. DNA ISOLATION: DNA was isolated using hexadecyltrimethyl ammonium bromide (CTAB) according to Kaufmann et al., (1995). CTAB used in the DNA isolation buffer significantly reduces carbohydrate contamination in the sample. The DNA isolated by this method has a higher molecular weight, maximum purity and high yield. Cells were concentrated by centrifugation at 6000 x g for 10 min and 2 g of cell pellet was washed with 2 ml of 50mM Tris-HCl buffer (pH. 8.0) and resuspended in 5 ml of isolation buffer containing 2% (w/v) CTAB, 1% (w/v) sarkosyl, 20mM EDTA, 1.4M NaCl, 0.1M Tris-HCl, (pH. 8.0) and 1% (v/v) 2-mercaptoethanol and incubated at 60°C for 45 min in a water bath with occasional gentle mixing. The resulting suspension was allowed to cool to room temperature for 5 min and thereafter 5 ml of chloroform:isoamyl alcohol (24:1, v/v) was added and mixed gently by inversion until emulsion was formed. Suspension was centrifuged at 4000 x g for 10 min and supernatant was transferred to sterile centrifuge tubes. DNA from the supernatant was precipitated for 10-15 min in 2 volumes of chilled ethanol and 0.1 volume of 3M sodium acetate (pH 5), pelleted at 4000 x g for 30 min at 4°C and was resuspended in 4 ml of TE buffer (10mM Tris-HCl, 1mM EDTA, pH-8.0) at 37°C followed by addition of 25 µl of RNase A. This was incubated at 37°C for 30 min to hydrolyze the RNAs. To this, 5 ml of Tris buffer saturated with phenol/chloroform was mixed and centrifuged at 6000 x g for 10 min at 4°C. Supernatant was washed twice with 5 ml of chloroform:isoamyl alcohol (24:1, v/v) and centrifuged at 6000 x g for 10 min at 4°C. The supernatant was transferred to fresh test tube and to this 2 ml of sodium acetate (pH 5.2) was mixed and slowly poured the mixture into 10 ml of chilled 100% ethanol. The precipitated DNA was centrifuged at 4000 x g for 5 min at 4°C and briefly rinsed with 70% ethanol, dried

and resuspended in 200 µl of TE buffer which was stored at -20°C until use, in most cases within 24 h. The quality and quantity of DNA was measured using agarose gel electrophoresis as well as spectrophotometer at 260 and 280 nm.

2.17.2. DNA SEPARATION BY AGAROSE GEL ELECTROPHORESIS: Agarose gel electrophoresis is a standard method used to separate, identify and purify DNA and RNA fragments. DNA was separated on agarose gel according to Kaufmann et al., (1995). To 20 µl of DNA, 10 µl of loading buffer containing 50% glycerol, SDS, 1X TAE buffer, 1mM EDTA, 0.25% bromophenol blue was added and mixed by vortexing. Agarose gel (0.8%) was prepared by dissolving 0.8 g of agarose in 100 ml of 1X TAE (0.4M Tris–HCL, 0.5M sodium acetate, 10mM EDTA, pH- 8.0) buffer and boiled in microwave oven followed by addition of 20 µl 10mM ethidium bromide. Gel apparatus was sealed using tape at two open ends, the combs were inserted and agarose was slowly poured into the gel tray, allowed it to harden for 20-30 min at room temperature. The comb and sealing tape was slowly removed from gel tray, placed in the electrophoresis tank with enough 1X TAE buffer, in the tank above the gel. DNA sample after addition of loading buffer was loaded on the agarose gel and allowed it to run at 50-100V for 1 h until the blue dye reaches the end of the gel. The gel was analyzed under UV transilluminator using Alpha digiDoc gel documentation system.

2.17.3. RNA ISOLATION: RNA was isolated using guinidine thiocyanate according to Kaufmann et al., (1995). Guinidine thiocyanate used in the isolation of RNA inhibits the release of RNase from the cell tissue and thus prevent degradation of RNA during the isolation. Cyanobacterial culture cells of balance growth phase were concentrated by centrifugation at 6000 x g for 10 min and 1 g of cell pellet was resuspended in 5 ml of GIT buffer containing 4M guanidine thiocyanate, 25mM

sodium citrate (pH-7.0), 0.5% sarkosyl and 1% (v/v) 2-mercaptoethanol and homogenized using tissue homogenizer for 6 min. To this sample, 1 ml of 2M sodium acetate buffer (pH 4.0), 10 ml of water saturated phenol and 2 ml of chloroform:isoamyl alcohol was added and tubes were vigorously shaken for 20-30 sec. Suspension was centrifuged at 8000 x g for 30 min at 4°C and supernatant was carefully transferred to sterile centrifuge tubes followed by addition of 10 ml of isopropanol, mixed and allowed RNA to be precipitated for 2 h at -20°C. The precipitated RNA was centrifuged at 8000 x g for 30 min at 4°C and RNA pellet was resuspended in 3 ml of GIT buffer and 3 ml of isopropanol, which was mixed and kept at -20°C for overnight. The total RNA was collected after centrifugation at 8000 x g for 30 min at 4°C and pellet was briefly rinsed with 4 ml of 75% ethanol, dried and resuspended in 200 µl of 0.5 % SDS solution which was stored at -20°C in most cases within 24 h. The quality and quantity of RNA was measured using agarose gel electrophoresis as well as spectrophotometer at 260 and 280 nm.

2.17.4. RNA SEPARATION BY AGAROSE GEL ELECTROPHORESIS: RNA molecules are separated according to their sizes using formaldehyde agarose gel electrophoresis according to Davis, (1986). Gel apparatus was cleaned, dried and gel tray was sealed with a tape at two open ends and comb was inserted in it. Formaldehyde agarose gels were prepared by dissolving 1% agarose in sterile distilled water, mixed and boiled in a microwave oven. To the cooled gel mixture, added 10X MOPs buffer, containing 0.2M 3-(N-Morpholino) propanesulfonic acid (MOPS), 0.05M sodium acetate, 0.01M EDTA, pH 7.0, 37% of formaldehyde and 20 µl of ethidium bromide, mixed after each addition. The mixture was slowly poured into the assembled gel tray and allowed it to harden for 20-30 min at room temperature. RNA sample was prepared by addition of denaturing buffer containing 10X MOPs buffer,

37% of formaladehyde, 50% of formamide to the 20 µl of total RNA. This was incubated at 65°C for 15 min and immediately cooled in ice to denature the RNA sample followed by addition of 15 µl of loading buffer (40% glycerol, 10X MOPS buffer, 50% formamide, 1% bromophenol blue) to the sample. After gel polymerized, comb and sealed tapes were carefully removed from the gel. The gel tray was placed in the electrophoresis tank and the tank was filled with 1X MOPs buffer up to the upper edge of the gel. 10 µl of RNA sample was loaded into each well of the gel and electric current was applied and allowed it to run at 50-100V until blue dye band reached at the end of the gel. The gel was analyzed under UV transilluminator using Alpha digiDoc gel documentation system.

2.18. ANALYSIS OF MYCOSPORINE LIKE AMINO ACIDS (MAAs):

2.18.1. EXTRACTION OF MYCOSPORINE LIKE AMINO ACIDS (MAAs):

Extraction and purification of mycosporine like amino acids (MAAs) was carried out according to the method described by Sinha and Häder, (2000). Cyanobacterial culture of stable growth phase was centrifuged and pellet was lypholized-using lyophilizer (Snijders Tilburg, Holland). Lyophilized cells (0.1 g) was homogenized in 5 ml of 20% (v/v) aqueous methanol (HPLC grade) in a tissue homogenizer for 5 min and incubated at 45°C for 3 h. The homogenate was centrifuged at 6000 x g for 15 min. The pellet was reextracted with 20% methanol and supernatant was pooled together and evaporated to dryness using lyophilizer. The dried supernatant was dissolved in 1 ml of 0.2% glacial acetic acid. The sample was filtered through 0.2 µm pore sized microfilters prior to loading to HPLC.

2.18.2. HPLC ANALYSIS OF MYCOSPORINE LIKE AMINO ACIDS (MAAs):

Sample was analyzed using an HPLC (Waters) system with Water-Spherisorb ODS-

C18 reverse phase column (25 µm x 4.6 mm x 250 mm) and a PDA detector (Waters 2996). 10 µl of filtered sample was injected with a micro syringe into HPLC column. The column was irrigated with isocratic mobile phase of 0.2% glacial acetic with a flow rate of 1.0 ml/min and developed for 10 min. The HPLC spectra were obtained at 330 nm with spectral scan wavelength range of 200-700 nm to obtain spectral profile of the isolated peak(s).

2.19. ANALYSIS OF PHENOLIC COMPOUNDS:

2.19.1. EXTRACTION OF PHENOLIC COMPOUNDS: Though most report suggest that cyanobacteria do not contain phenolic compounds however, some derivatives of phenolic compounds were found in algae. Cyanobacterial culture of stable growth phase was centrifuged and pellet was lypholized-using lyophilizer (Snijders Tilburg, Holland). 0.1 g of lyophilized cells was extracted in 5 ml of 80% (v/v) methanolic-HCl in a glasss tissue homogenizer for 5 min at room temperature. This was kept in water bath for 2 h and again homogenized using tissue homogenizer and kept for extraction in dark for 24 h at room temperature. Extracted sample was centrifuged 6000 x g for 10 min and the supernatant was used for the HPLC and spectrophotometric analysis.

2.19.2. HPLC ANALYSIS OF PHENOLIC COMPOUNDS: HPLC analysis of phenolic compounds was done according to Bahorun et al., (2004) using an HPLC (Waters) system with Water-Spherisorb ODS-C18 reverse phase column (25 µm x 4.6 mm x 250 mm) and a PDA detector (Waters 2996). The wavelengths for detection were set to 280 and 360 nm. The sample was filtered through 0.2 µm pore sized microfilters prior to use. After filtration, 20 µl of sample was injected into a column

and eluted using an acetonitrile /water gradient with a flow rate of 0.7ml/min as follows:

Solvent A: Acetonitrile/water 1:9 v/v, pH 2.6

Solvent B: Acetonitrile/water 1:1 v/v, pH 2.6

0-30 min	0-15% B in A
30-50 min	15% B in A
50-60 min	15-25% B in A
60-90 min	15-100% B in A
90-100 min	100-0% B in A

2.19.3. SPECTROPHOTOMETRIC ANALYSIS OF PHENOLIC COMPOUNDS: The sample was diluted using 80% methanolic-HCl and the spectral scan was recorded in the range of 190-700 nm on UV-Visible spectrophotometer (Schimadzu 2450) according to Sharma et al., (1998a).

2.20. ASSAY OF ANTIOXIDANT ASSAY:

2.20.1. ASSAY OF SUPEROXIDE DISMUTASE: Antioxidant enzymes activity was assayed in order to study whether as a result of oxidative damage to the cyanobacteria, antioxidant enzymes are stimulated? Superoxide dismutase was assayed using epinephrine method according to Boveris, (1984). Resuspended culture (10 ml) was centrifuged at 5000 x g for 10 min and cell pellet was extracted in 5 ml of 50 mM sodium dihydrogen phosphate buffer (pH 7.8) using a tissue homogenizer for 3 min. The extract was centrifuged for 10 min at 6000 x g. Supernatant was used for SOD assay. 100 µl of tissue extract was added to 2.6 ml of assay buffer containing 6mM EDTA in 10mM sodium carbonate (pH 10.2) and 300 µl of 4.5mM epinephrine.

Absorbance was recorded at 480 nm on UV-Visual spectrophotometer (Schimadzu 2450) for about 8-10 min. A set of standard with epinephrine but without tissue extract was also assayed separately. Protein content of extract was measured according to Lowry et al (1951) described in section 2. 12. 1.

2.20.2. ASSAY OF ASCORBATE PEROXIDASE: Ascorbate peroxidase activity was assayed according to Sankhalkar and Sharma, (2002). Resuspended culture (10 ml) was centrifuged at 5000 x g for 10 min and cell pellet was extracted in 5 ml of 100mM sodium dihydrogen phosphate buffer (pH 7.5), 1mM EDTA and 5mM ascorbate using glass tissue homogenizer for 3 min. The extract was centrifuged for 15 min at 6000 x g at 4°C. Supernatant was used for peroxidase assay. Activity was assayed in a reaction mixture containing 2.2 ml of potassium hydrogen phosphate (100mM), 400 µl of EDTA (1mM), 400 µl of sodium ascorbate (1.5mM), 400 µl of hydrogen peroxide (10mM) and 200 µl of sample extract. The decrease in absorbance as a result of hydrogen peroxide metabolism was recorded for 3 min at 290 nm using UV-Visible spectrophotometer (Schimadzu, 2450). The amount of protein content of the extract was determined according to Lowry et al., (1951) described in section 2. 12. 1.

CHAPTER –3

RESULTS

RESULTS– CONTENT

RESULTS

3.1. DETERMINATION OF GROWTH PHASE:

Growth phase of both the cyanobacteria was determined by studying biomass, chlorophyll content and chlorophyll fluorescence (Fv/Fm) measurements over a period of 25-50 days.

3.1.1. BASED ON FRESH WEIGHT MEASUREMENT:

Figure 3.1a shows the changes in the fresh weight in *Nostoc* over a period of time. The figure shows almost no lag phase and a continuous increase in the fresh weight up to 18 days of growth, indicating accelerating growth phase. From 18 days to 23 days the fresh weight remained constant, indicating stationary growth rate, and subsequent to 23 days the fresh weight declined indicating death phase. The maximum fresh weight in *Nostoc* ranged from 0.110-0.115 g observed during 20-23 days of growth (Fig 3.1a).

Figure 3.1b shows the changes in the fresh weight in *Phormidium* over a period of 50 days. The figure show a somewhat brief lag phase of up to 7 days and from 7-21 days it showed slow acceleration, which increased further during 21-33 days of growth. Subsequent to the 33 days fresh weight remained more or less constant up to 50 days. The maximum fresh weight observed in *Phormidium* was 0.225 g (Fig 3.1b).

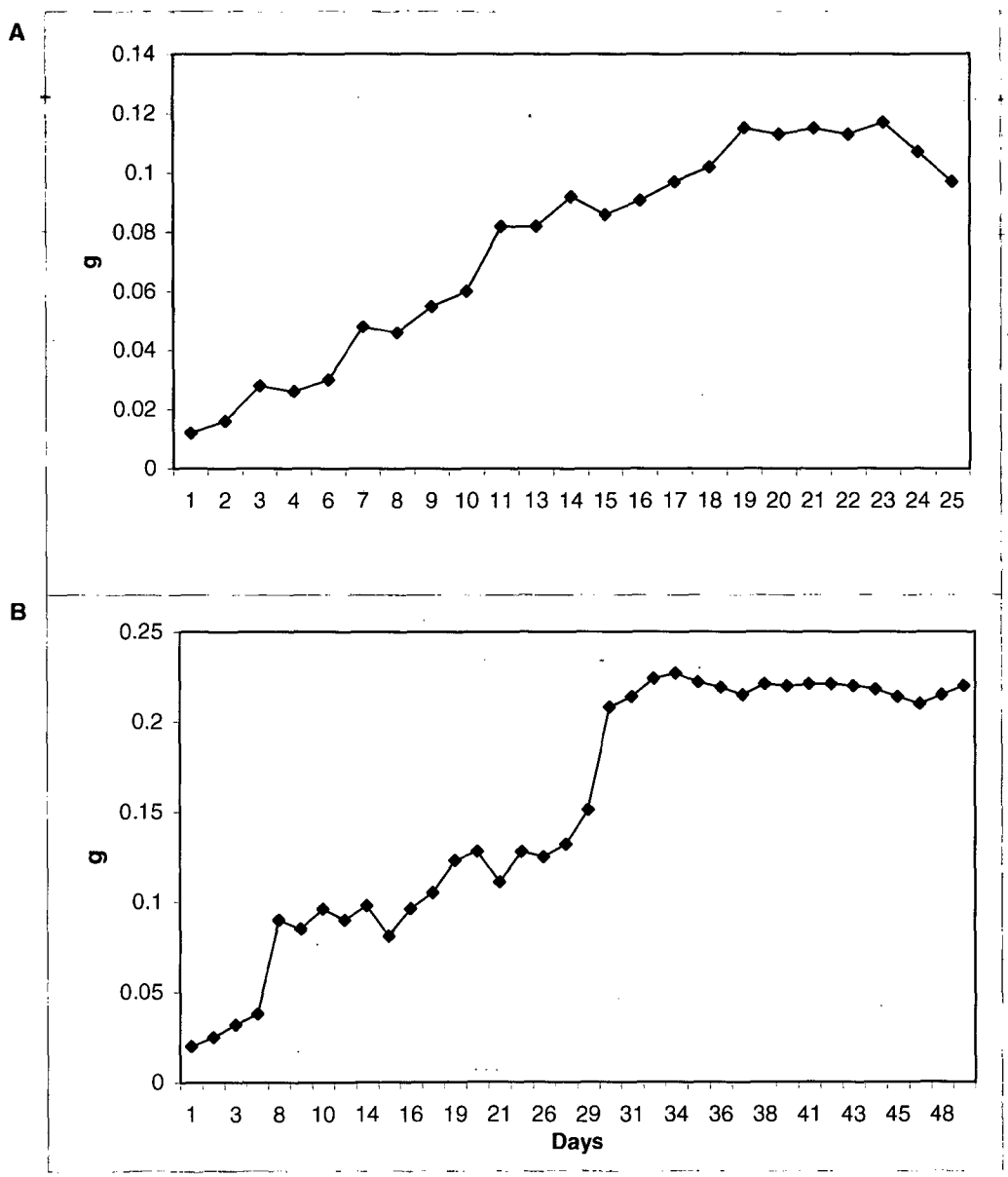


Fig 3.1: Changes in fresh weight over a period of time in (A) *Nostoc spongiaeforme* and (B) *Phormidium corium.*

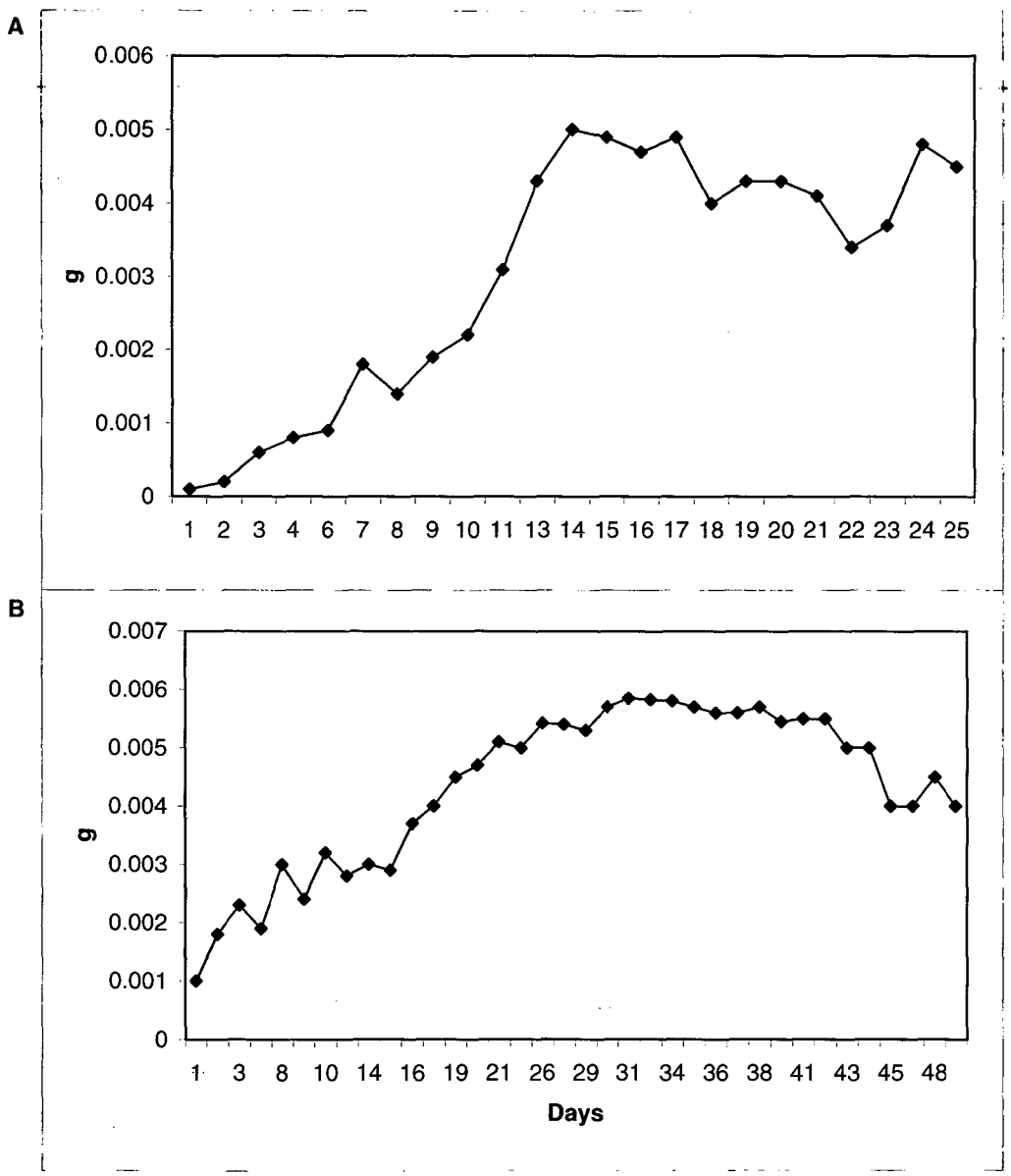


Fig 3.2: Changes in dry weight over a period of time in (A) *Nostoc spongiaeforme* and (B) *Phormidium corium.*

3.1.2. BASED ON DRY WEIGHT MEASUREMENT:

Figure 3.2a shows the changes in the dry weight in *Nostoc* during study of growth. The figure shows a brief lag phase of up to 2 days followed by a slow acceleration phase of up to 8 days. Growth during 9-14 days represent fast acceleration phase followed by constant growth during 14-22 days indicating stationary phase of growth. The maximum dry weight in *Nostoc* was 0.005 g on 14 day of growth (Fig 3.2a).

Dry weight measurements over a period of 50 days were also determined for *Phormidium*. The figure shows no lag phase but a steady continuous increase in dry weight up to 31 days as an acceleration phase. The growth was almost constant thereafter up to 41 days indicating stationary phase, which declined subsequently indicating death phase. The maximum dry weight for *Phormidium* ranged from 0.005-0.006 g on 31-33 day of growth (Fig 3.2b).

3.1.3. BASED ON CHLOROPHYLL MEASUREMENT:

The changes in the chlorophyll content were also calculated over a period of time to determine the various growth phase of *Nostoc*. Figure 3.3a shows the changes in chlorophyll concentration in *Nostoc* over a period of 35 days. A short lag phase of 5 days was observed followed by rapid increase in chlorophyll content up to 12 days indicating acceleration phase. Growth after 12 days showed stable chlorophyll content up to 27-28 days showing balanced growth phase and thereafter it declined indicating death phase (Fig 3.3a).

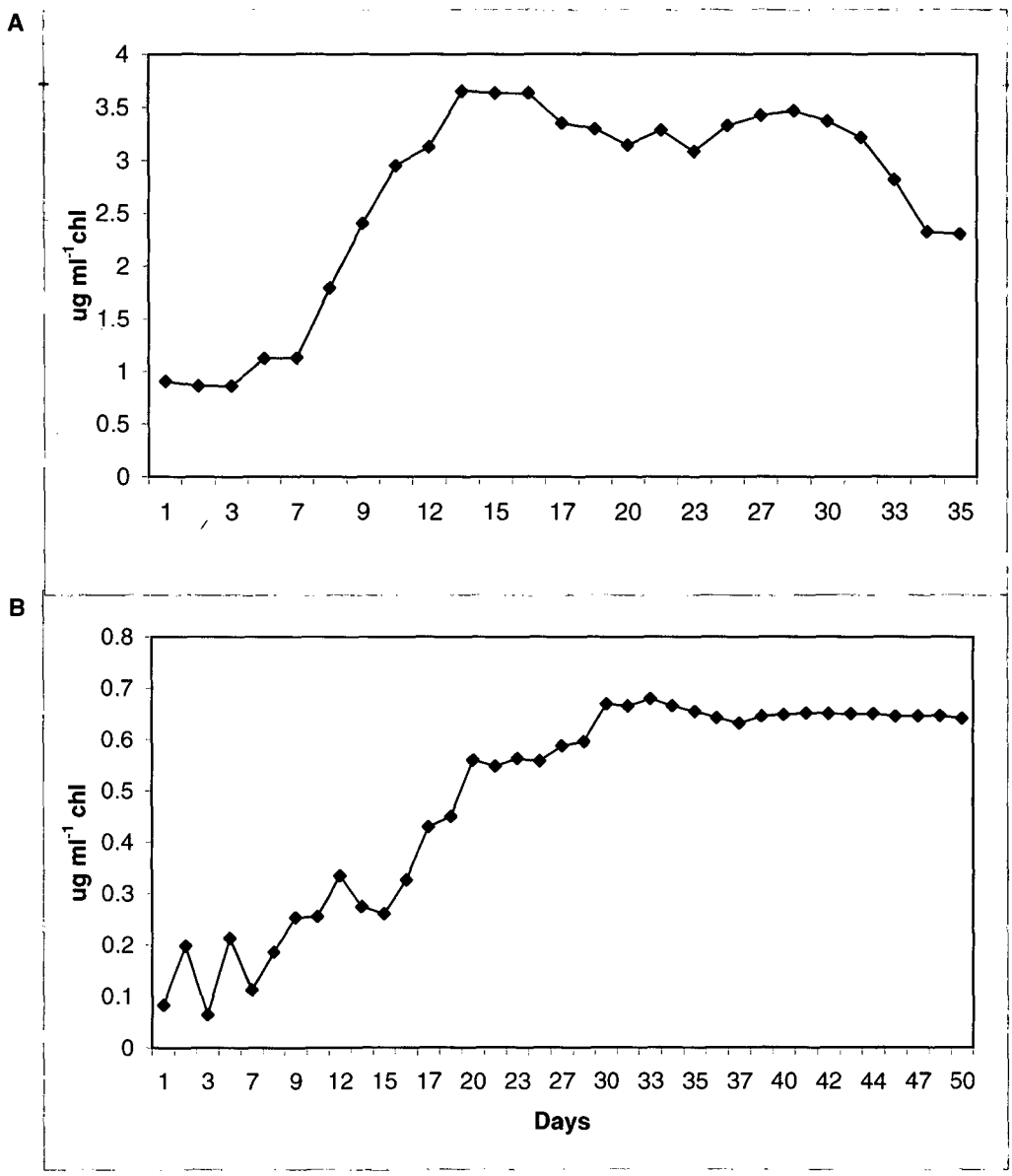


Fig 3.3: Changes in chlorophyll concentration over a period of various days in (A) *Nostoc spongiaeforme* and (B) *Phormidium corium.*

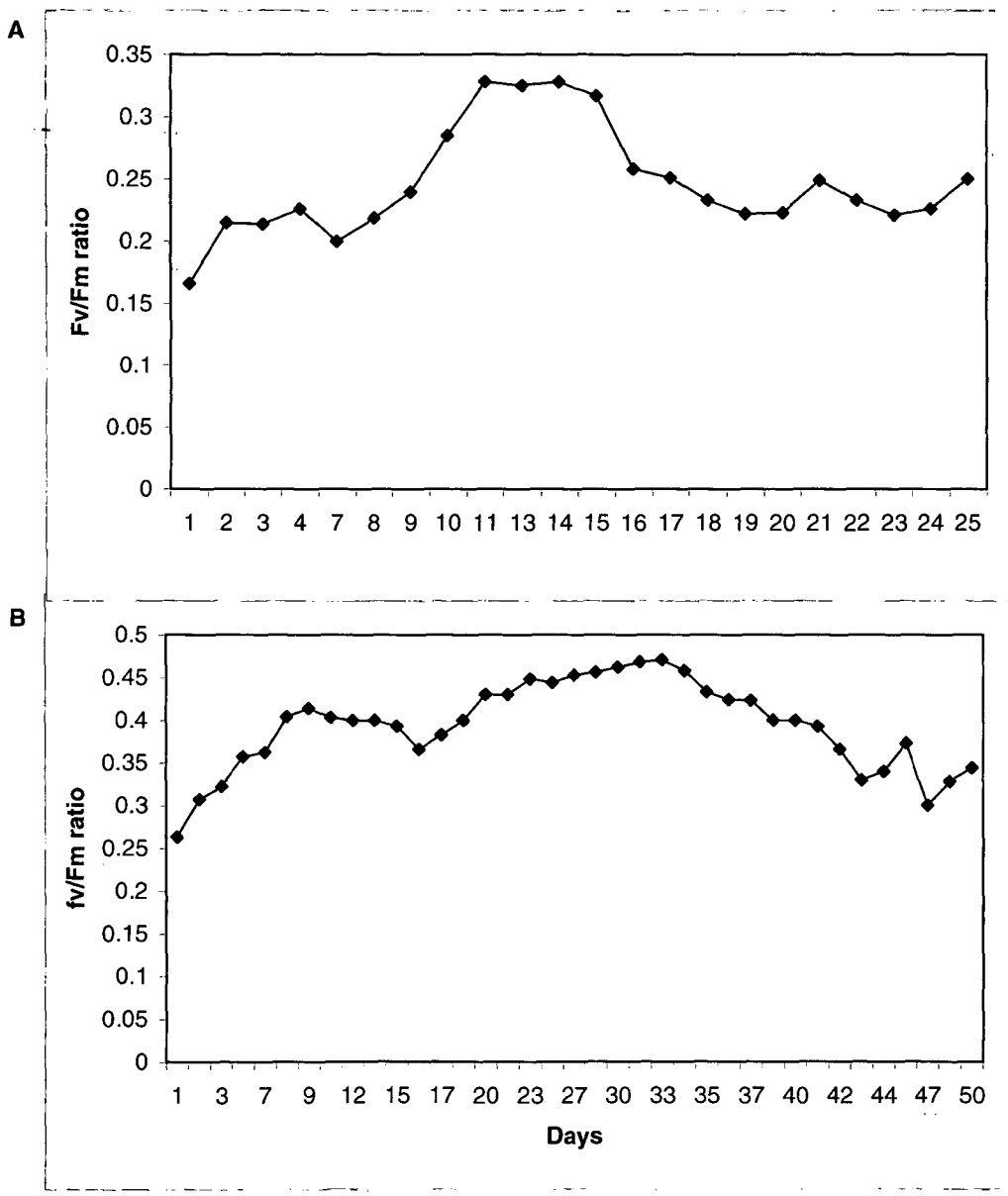


Fig 3.4: Changes in chlorophyll fluorescence (Fv/Fm ratio) over a period of time in (A) *Nostoc spongiaeforme* and (B) *Phormidium corium.*

Figure 3.3b shows the changes in the chlorophyll content in *Phormidium* during development of growth. Figure shows a lag phase of 7 days and acceleration phase during 8-28 days showing continuous increase in the chlorophyll content. Thereafter chlorophyll content remained almost constant up to as late as 50 days of growth indicating stationary phase (Fig 3.3b).

3.1.4. BASED ON CHLOROPHYLL FLUORESCENCE MEASUREMENT:

Fv/Fm ratio during the period of growth was determined in both *Nostoc* and *Phormidium* in order to determine the photosynthetic activity over a period of time in both the cyanobacteria. The figure 3.4a shows changes in the Fv/Fm ratio over a period of 25 days in *Nostoc* culture. The data show a short lag phase of 7 days in the Fv/Fm ratio and thereafter an increase from 7 to 11 days suggesting brief acceleration phase. The Fv/Fm ratio remained constant for short duration from 11 days to 15 days, indicating balanced growth phase and thereafter declined indicating stationary phase. The maximum Fv/Fm ratio in *Nostoc* ranged from 0.325-0.340 during 11-15 days of growth (Fig 3.4a).

Figure 3.4b shows the changes in the Fv/Fm ratio in *Phormidium* during the growth over a period of 50 days. The figure shows no lag phase and continuous increase in the Fv/Fm ratio up to 20 days indicates acceleration growth phase, which increased further during 20-35 days of growth showing balanced growth phase. Subsequent to the 35 days, Fv/Fm ratio remained more or less constant indicating stationary growth phase up to 41 days and further declined indicating death phase. The maximum Fv/Fm ratio in *Phormidium* was 0.450 on 33 day of growth (Fig 3.4b).

Taking these four parameters collectively and the difference in the phases between growth rate and biomass, balance growth phase was determined for *Nostoc* and *Phormidium* to be approximately 14 and 33 days respectively. Therefore, all the experiments have been conducted using 14-15 days old culture of *Nostoc* and 33-34 day old culture of *Phormidium.*

3.2. EFFECT ON MORPHOLOGICAL CHANGES:

The effect of UV-B radiation, high light and UV-B supplemented with low visual light on morphological changes were studied in *Nostoc spongiaeforme* and *Phormidium corium* (Fig. 3.5, 3.6). In *Nostoc spongiaeforme,* UV-B and high light treatment showed breakage of filaments, which resulted in smaller fragments of cyanobacteria. High light treatment showed more breakage of filaments than observed due to UV-B radiation and UV-B supplemented with low light treatment in *Nostoc* (Fig. 3.5).

UV-B and UV-B supplemented with low visual light treatment to *Phormidium* showed degradation of cell wall, which resulted in breakage of cells but the cell wall remained intact with the filaments (Fig. 3.6). There was bluish color accumulation of pigment in the cells of *Phormidium* due to UV-B treatment, which was not seen in case of high light and UV-B supplemented low visual light treatments. High light exposure showed less degradation of cells compared to UV-B and UV-B supplemented low visual light treatment. Also yellowing of the cells was seen due to all the three treatments.

Phormidum showed more damage to the cells due to UV-B and UV-B supplemented with low visual light treatment as compared to *Nostoc.* There was no accumulation of blue colour pigments in *Nostoc* due to UV-B treatment, which was seen

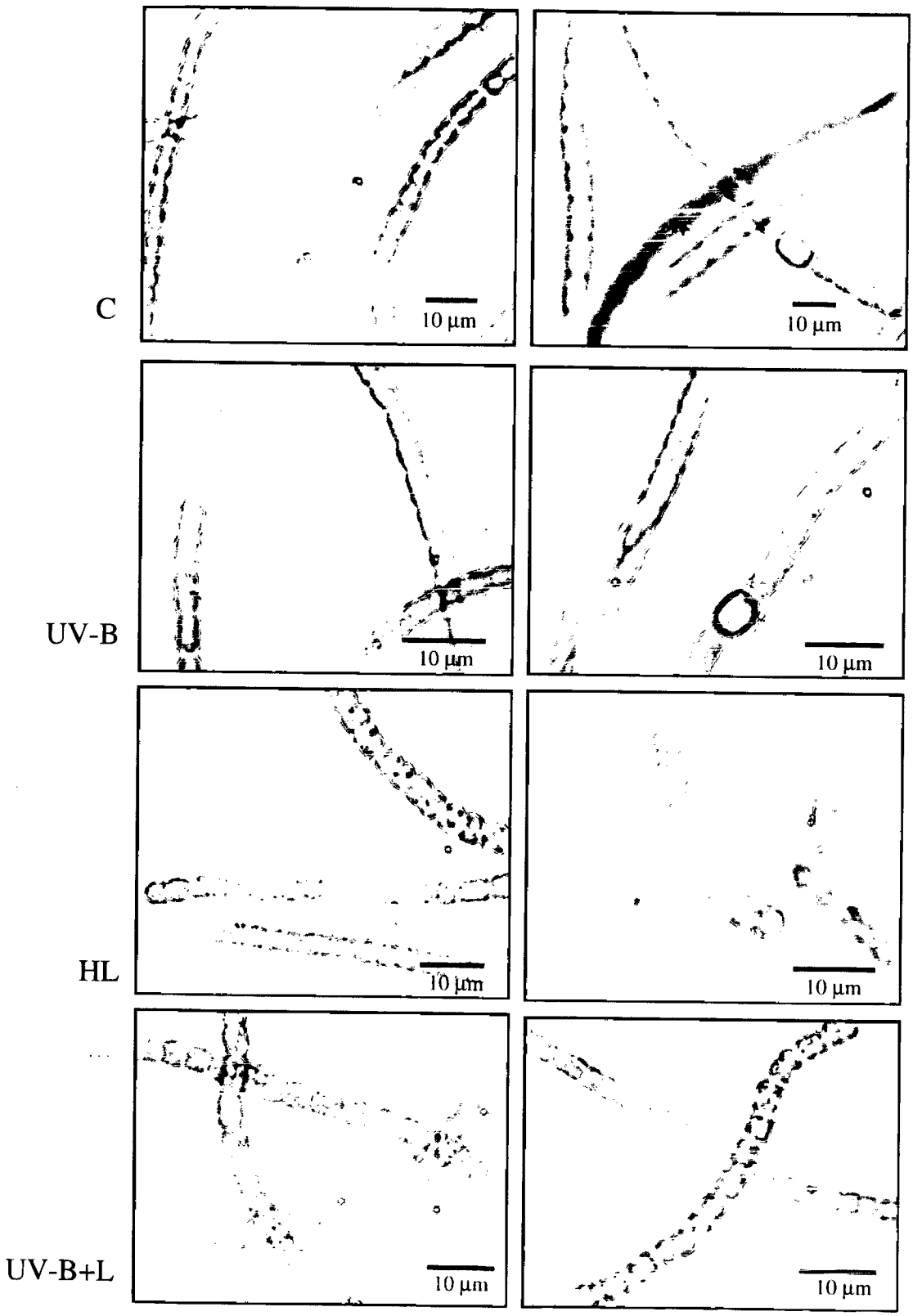


Fig 3.5 Morphological changes due to UV-B, high light and UV-B supplemented with low visual light treatment for 6 h in *Nostoc spongiaeforme* under 100X magnification.

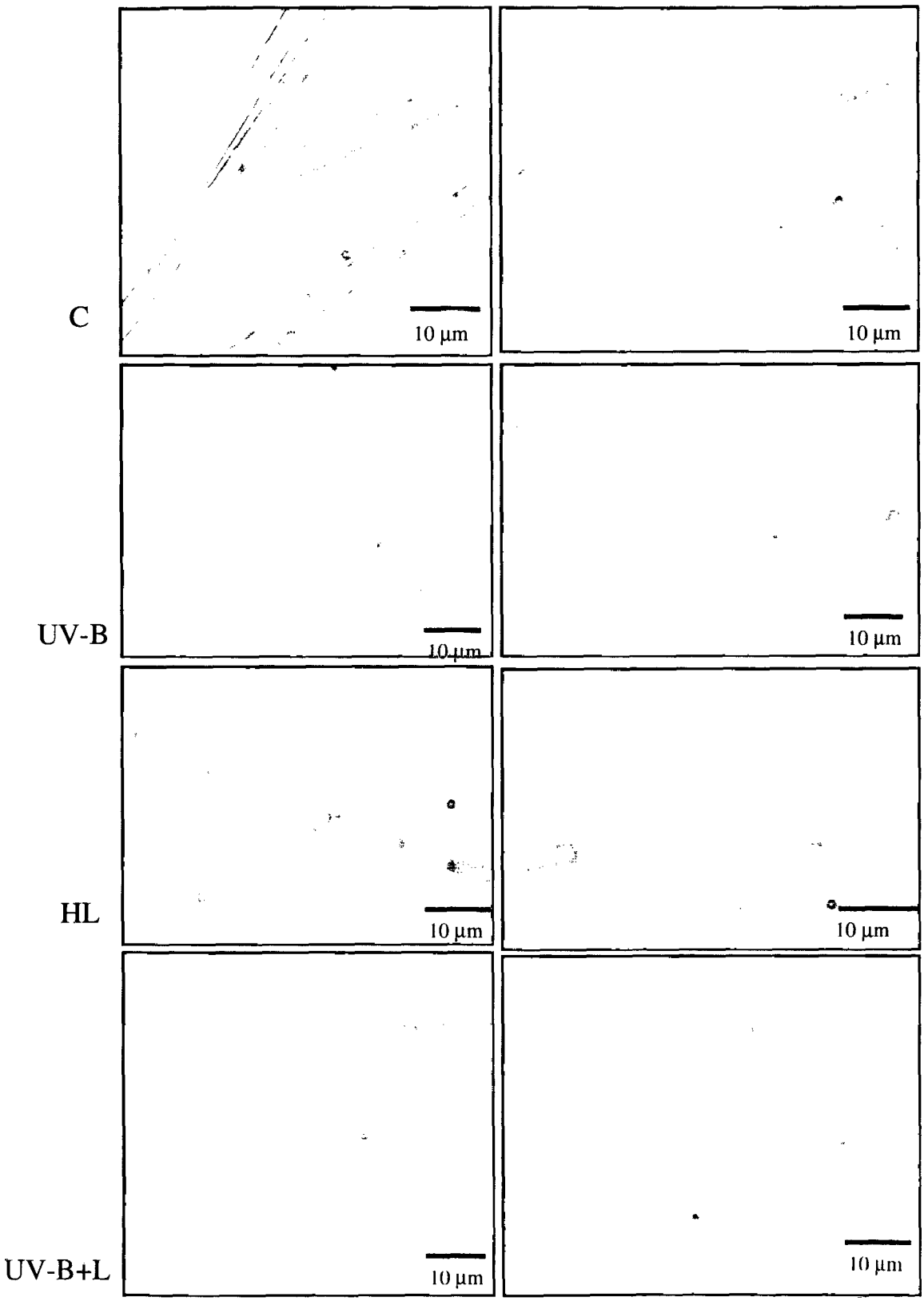


Fig 3.6 Morphological changes due to UV-B, high light and UV-B supplemented with low visual light treatment for 6 h in *Phormidium corium* under 100X magnification.

in case of *Phormidium.* High light treatment in *Nostoc* showed more breakage to the filaments than observed in *Phormidium* for the same treatment.

3.3. EFFECT ON CHLOROPHYLL FLUORESCENCE:

Photosynthetic efficiency of the cyanobacteria was studied by measuring (Fv/Fm ratio) after exposing the culture to UV-B, high light and UV-B supplemented with low visual light (PAR) for 6h duration (Fig. 3.7 a & b).

In *Nostoc spongiaeforme,* 3h of the UV-B treatment caused a rapid decrease in Fv/Fm ratio to an extent of 91% as compared to control. No further decrease in the Fv/Fm ratio was observed up to 5 h of the treatment. Longer treatment resulted in complete inhibition of the photosynthetic efficiency (Fig 3.7a). High light treatment resulted in a decrease of 74% as compared to control after 2h of the treatment. Further increase in the duration of the high light treatment to 3 h did not cause any further decrease in the Fv/Fm ratio and a still longer duration of 4 h of high light treatment resulted in a slight increase of 11 % as compared to the Fv/Fm value observed after 2 h of the treatment. Further increase in the duration of the treatment showed a rapid decrease in the Fv/Fm and 6h of the treatment showed no photosynthetic activity (Fig 3.7a). UV-B treatment supplemented with low PAR showed relatively less decrease in the Fv/Fm ratio than seen in cyanobacteria exposed to UV-B alone or high light. A 2 h treatment of UV-B supplemented with low PAR resulted in 66% decrease in the Fv/Fm ratio as compared to its control and thereafter showed no decrease in the Fv/Fm ratio up to 4 hour of the treatment. A 6 h exposure to the treatment resulted in 90% decrease in the Fv/Fm ratio compared to complete inhibition observed in UV-B and high light treated *Nostoc* (Fig

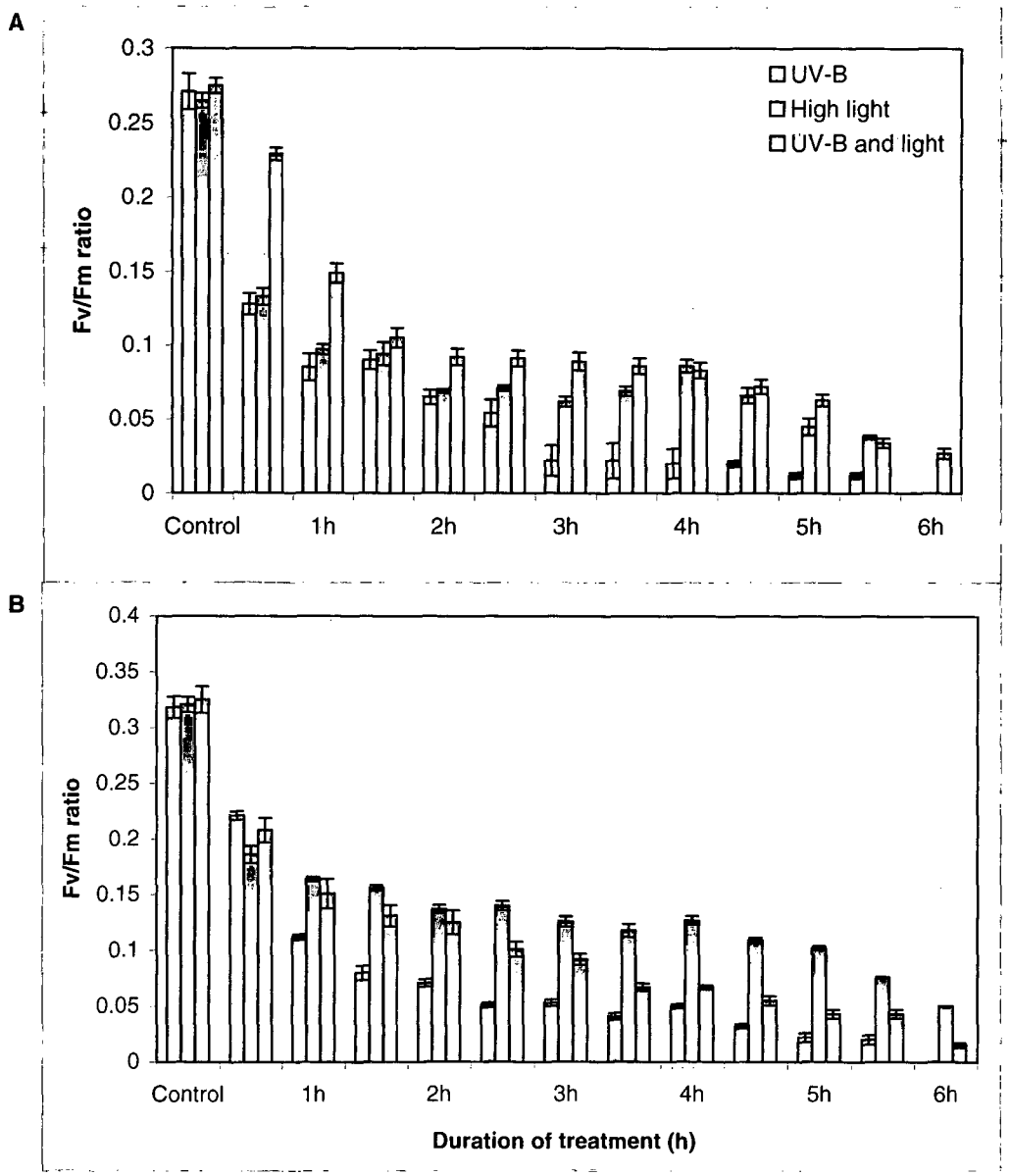


Fig 3.7: Effect of UV-B, high light and UV-B supplemented with low visual light up to 6h on chlorophyll fluorescence (Fv/Fm ratio) in (A) *Nostoc spongiaeforme* and (B) *Phormidium corium.* Each bar represents the mean ±S.D. n=8.

3.7a). The decrease in the Fv/Fm ratio was considerably less when *Nostoc* were treated with UV-B supplemented with low PAR than treated with UV-B alone.

UV-B treatment to *Phormidium corium* showed maximum decrease of 65% with in an hour of the treatment as compared to the control. The Fv/Fm ratio further declined to 83% of control after 3 h of the UV-B treatment and remained more or less constant thereafter till 4 h of the treatment. Subsequent increase in the duration of the treatment beyond 4 h resulted in further decline in the Fv/Fm value. Six hour of UV-B treatment resulted in complete inhibition of the Fv/Fm ratio (Fig. 3.7b). High light treatment resulted in relatively less damage to the Fv/Fm ratio than seen in UV-B treated *Phormidium*. A two hour high light treatment linearly decreased the Fv/Fm value to 59% of the control and subsequent increase in the duration of the high light treatment to 5 h did not decrease the Fv/Fm value considerably and showed a transient leveling of the Fv/Fm ratio. Six hour treatment of the high light, however, decreased the Fv/Fm value to 84% as compared to control (Fig. 3.7b). *Phormidium* treated with UV-B supplemented with low visual light treatment showed almost a linear decrease showing 53%, 71%, and 95% decrease in the Fv/Fm ratio due to 1, 3, and 6h of the treatment respectively (Fig 3.7b). However, similar to the results observed with *Nostoc* (Fig. 3.7a) the decrease in the Fv/Fm ratio as a result of UV-B plus low PAR treatment was significantly less than observed when *Phormidium* was exposed to UV-B alone (Fig. 3.7b).

Figure also indicate that photosynthetic efficiency (Fv/Fm) of *Phormidium* was slightly higher than *Nostoc* under normal growth conditions and UV-B and high light treatment resulted in relatively less damage to photosynthetic efficiency in *Phormidium*

than seen in *Nostoc*. UV-B treatment was more damaging to the photosynthesis than high light as well as UV-B in combination with low PAR in both the species studied.

3.4. EFFECT ON PHOTOSYNTHETIC PIGMENTS:

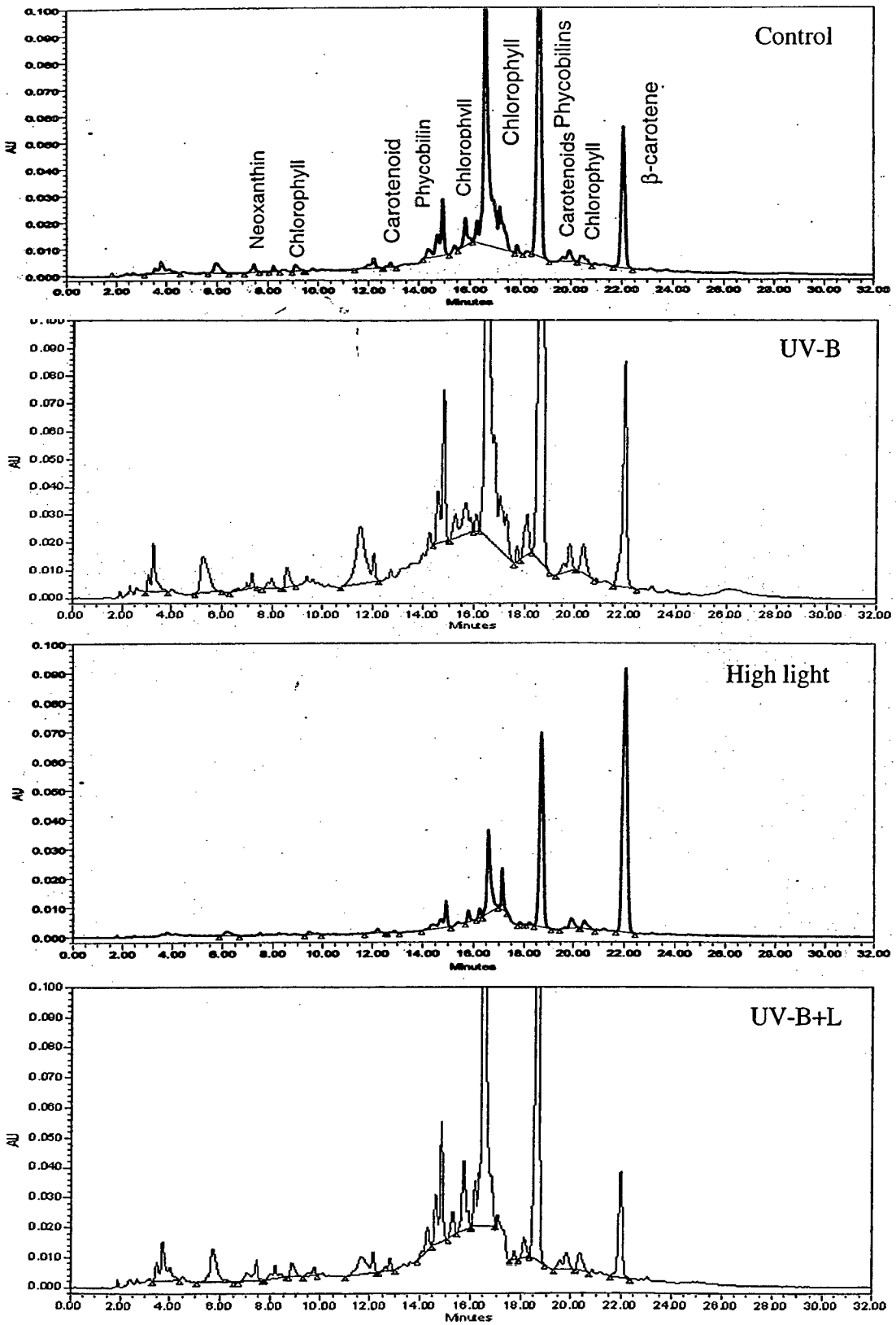
Effect of UV-B, high light and UV-B supplemented with low PAR on photosynthetic pigments such as chlorophyll, carotenoids and phycobillins (phycocyanin, allophycocyanin and phycoerythrin) was also studied in order to relate the changes in the pigments to the photosynthesis. The pigments were studied using TLC and HPLC as well as spectrophotometric measurements.

3.4.1. EFFECT ON PHOTOSYNTHETIC PIGMENTS (HPLC):

HPLC data show significant qualitative and quantitative changes in the pigments in both *Nostoc* as well as in *Phormidium* (Fig. 3.8, 3.9 and 3.10). The different photosynthetic pigments present in *Nostoc spongiaeforme* and *Phormidium corium* were chlorophyll a, phycobilins, carotenoids, including β-carotene.

In *Nostoc spongiaeforme,* UV-B treatment for 6h resulted in considerable increase in all the photosynthetic pigments such as chlorophylls, phycobilins and carotenoids (Fig 3.9). As a result of the 6 h UV-B treatment, chlorophyll a increased by 245%, phycobillins increased by 271% and β-carotene increased by 166% compared to control. Other chlorophyll and carotenoids also increased (Table 3.1). High light treatment, however resulted in marked decline in all pigments except β-carotene compared to control. β-carotene increased to 207% as a result of the high light treatment while chlorophyll a and phycobillins decreased to 82% and 47% respectively compared to

Fig. 3.9 HPLC profile of photosynthetic pigments at 450 nm after 6 h exposure to UV-B, high light and UV-B supplemented with low visual radiation in *Nostoc spongiaeforme*.


Control
Neoxanthin
Chlorophyll
Carotenoid
Phycobilin
Chlorophyll
Chlorophyll
Carotenoids
Phycobilins
Chlorophyll
β-carotene
UV-B
High light
UV-B+L
AU
Minutes

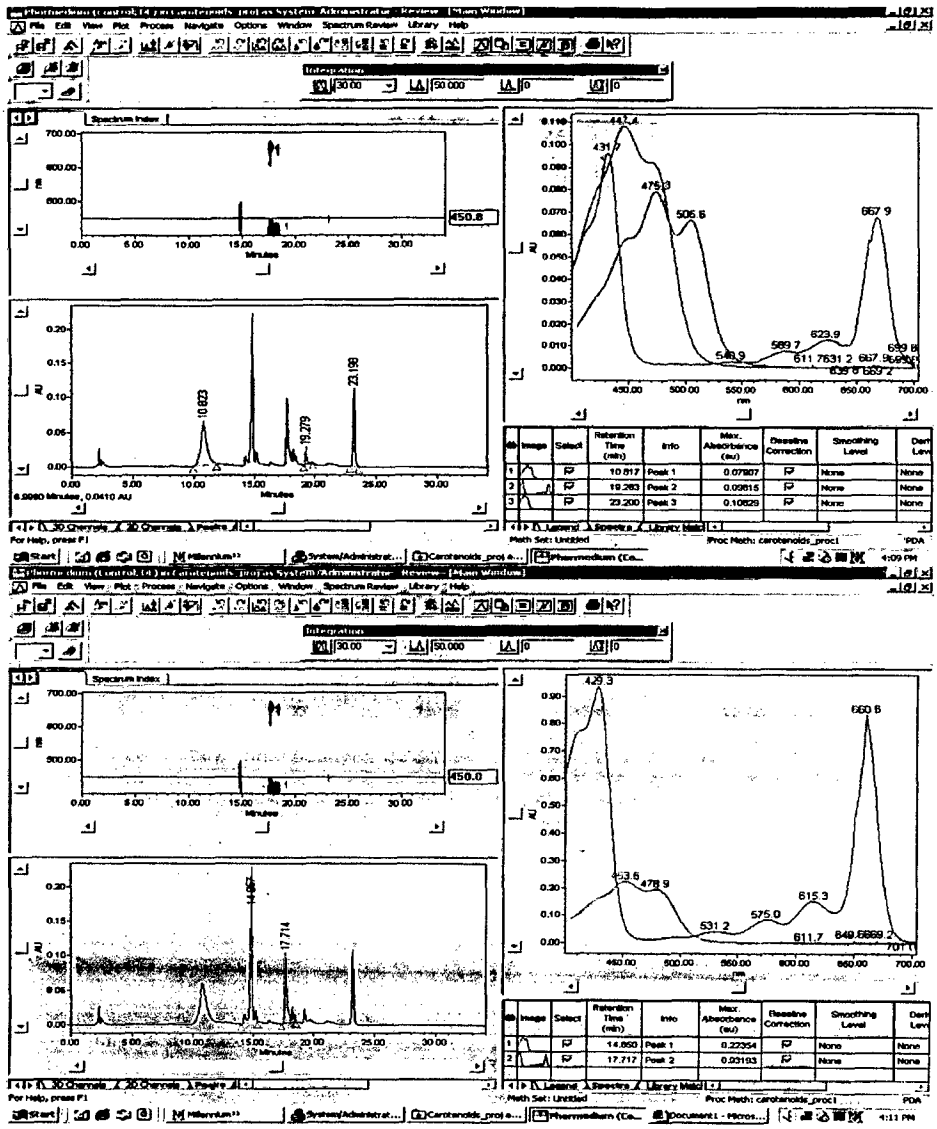


Fig 3.8 HPLC profile of photosynthetic pigments take at 450 nm and absorption spectra of peaks with retention time of 10.817, 14.850, 17.717, 19.283, 23.200 min

Table 3.1 Effect of UV-B and high light for 6 h on photosynthetic pigments ($\mu g\ g^{-1}$ f. w.) in *Nostoc spongiaeforme.*

	Pigments	Control	UV-B	High light	UV-B+L
1	Carotenoids	0.0178	0.2070	0.0051	0.0688
2	Phycobilins	0.0921	0.1845	0.0404	0.1383
3	Chlorophyll	0.0341	0.0981	0.0099	0.0799
4	Chlorophyll a	0.5792	1.4247	0.0993	0.937
5	Phycobilins	0.4501	1.2242	0.2352	0.6013
6	Carotenoids	0.0238	0.0460	0.0156	0.0341
7	Chlorophyll	0.0191	0.0476	0.0120	0.0263
8	β-carotene	0.1801	0.2993	0.3733	0.118

control. All other peaks also declined (Table 3.1). UV-B radiation supplemented with low visual light treatment showed increase in phycobilins(133%), chlorophyll a (161%) while β-carotene content decreased slightly (34%) as compared to control (Fig 3.9, Table 3.1).

Phormidium corium also showed quantitative changes in the photosynthetic pigments. UV-B treatment resulted in over all decrease in all the photosynthetic pigments such as chlorophylls, phycobilins, carotenoids and β-carotene (Fig 3.10). UV-B treatment for 6h showed decrease in chlorophyll a by 34%, phycobillins by 40% and β-carotene decreased by 86% compared to control. Other chlorophyll and carotenoids also decreased due to the treatments (Table 3.2). High light treatment showed even greater decrease in pigments such as chlorophyll a by 50%, phycobilins by 87% and β-carotene content which declined to 63% due to the treatment in *Phormidium* (Fig. 3.10; Table 3.2). UV-B treatment supplemented with low visual light also showed decline in all the pigments but to a lesser extent. As a result of the 6h of UV-B treatment supplemented with low visual light, chlorophyll a decreased by 44%, phycobillins decreased by 47% and β-carotene decreased by 24% compared to control. Other chlorophyll and carotenoids also decreased due to the treatment (Table 3.2).

UV-B treatment to *Nostoc* resulted in quantitative increase in all the pigments while in *Phormidium* the treatment showed decrease in all the pigments. Like wise high light treatment to *Nostoc* resulted in decreased level of chlorophyll a and phycobillins and increase level of carotenoids as compared to its control. While in *Phormidium* high light treatment showed decrease in chlorophyll a phycobillins and β-carotene. UV-B treatment supplemented with low PAR of *Nostoc* resulted in slight increase in chlorophyll a and

Fig. 3.10 HPLC profile of photosynthetic pigments at 450 nm after 6 h exposure to UV-B, high light and UV-B supplemented with low visual radiation in *Phormidium corium.*

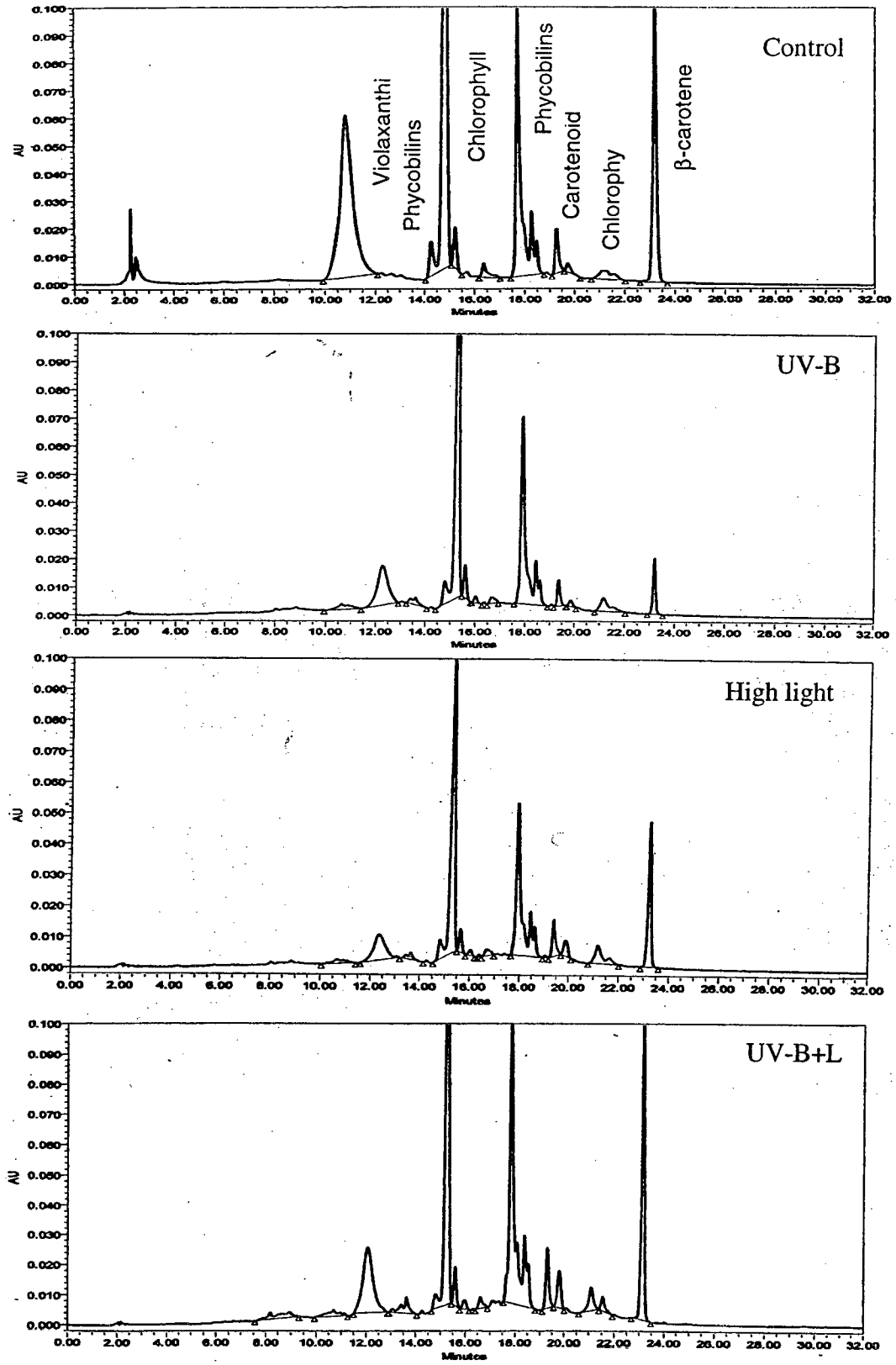

Control
Violaxanthi
Phycobilins
Chlorophyll
Phycobilins
Carotenoid
Chlorophy
β-carotene
UV-B
High light
UV-B+L
AU
Minutes

Table 3.2 Effect of UV-B and high light on photosynthetic pigments (μg g^{-1} f. w.) in *Phormidium corium*.

No.	Pigments	Control	UV-B	High light	UV-B+L
1	Carotenoids	0.719	0.0220	0.0143	0.204
2	Phycobilins	0.060	0.0140	0.0114	0.0336
3	Chlorophyll a	0.478	0.3150	0.2351	0.501
4	Phycobilins	0.898	0.5369	0.109	0.475
5	Carotenoids	0.0600	0.0.0253	0.2605	0.0564
6	Chlorophyll	0.0389	0.0383	0.0447	0.032
7	β-carotene	0.3514	0.0474	0.1299	0.265

phycobillins while decrease in β-carotene while the same treatment in *Phormidium* resulted in slight decrease in all the three major pigments (Fig. 3.9, 3.10 & Table 3.1, 3.2).

3.4.2. EFFECT ON PHOTOSYNTHETIC PIGMENTS (TLC):

The effect of UV-B radiation, high light and UV-B supplemented with visual light on photosynthetic pigments were also studied using TLC in both *Nostoc spongiaeforme* and *Phormidium corium* (Fig. 3.11). In *Nostoc spongiaeforme* and *Phormidium corium*, the photosynthetic pigments observed using TLC were similar to what was observed using HPLC. Main pigments identified were β–carotene, chlorophyll a, carotenoids and phycobilins. The three treatments studied i.e. UV-B, high light and UV-B and low PAR, resulted in only quantitative changes and no qualitative change was observed.

3.4.3. EFFECT ON PHYCOBILINS (SPECTROPHOTOMETRICALLY):

The effect of UV-B, high light and UV-B supplemented with low visual light treatment on phycobilins (phycocyanin, allophycocyanin and phycoerythrin) were studied in both *Nostoc spongiaeforme* and *Phormidium corium* (Fig. 3.12, 3.13).

UV-B treatment to *Nostoc* showed an initial increase in phycocyanin content within 1 h of the treatment by 145% as compared to control followed by a slow linear decrease of 14%, 21%, 39% after 3, 4 and 5h of the treatment respectively as compared to control. However, UV-B treatment of 6h caused a rapid decrease in the phycocyanin content to 90% of control (Fig. 3.12a). Culture of *Nostoc* had much higher allophycocyanin content than phycocyanin and UV-B treatment resulted in greater

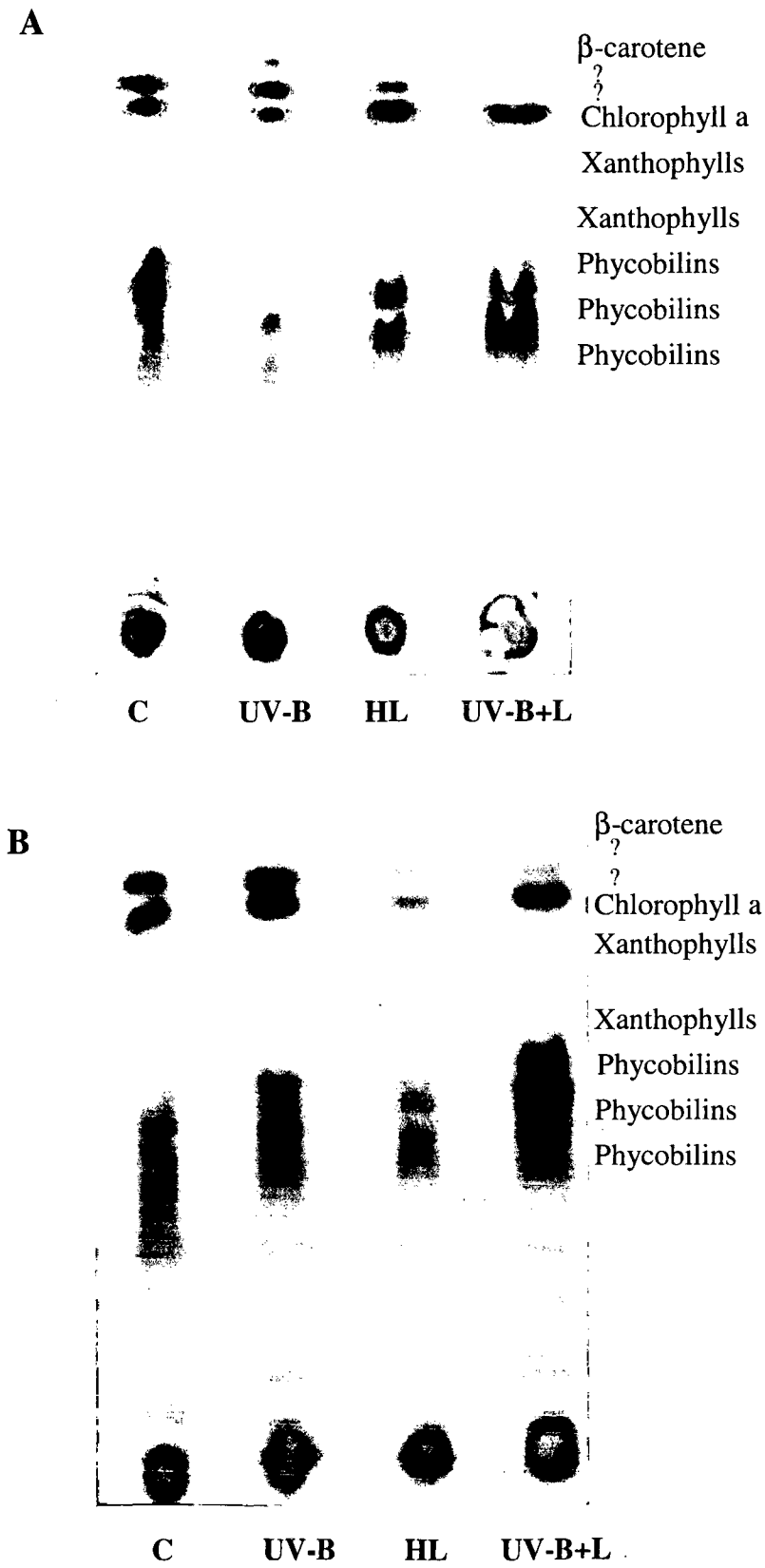
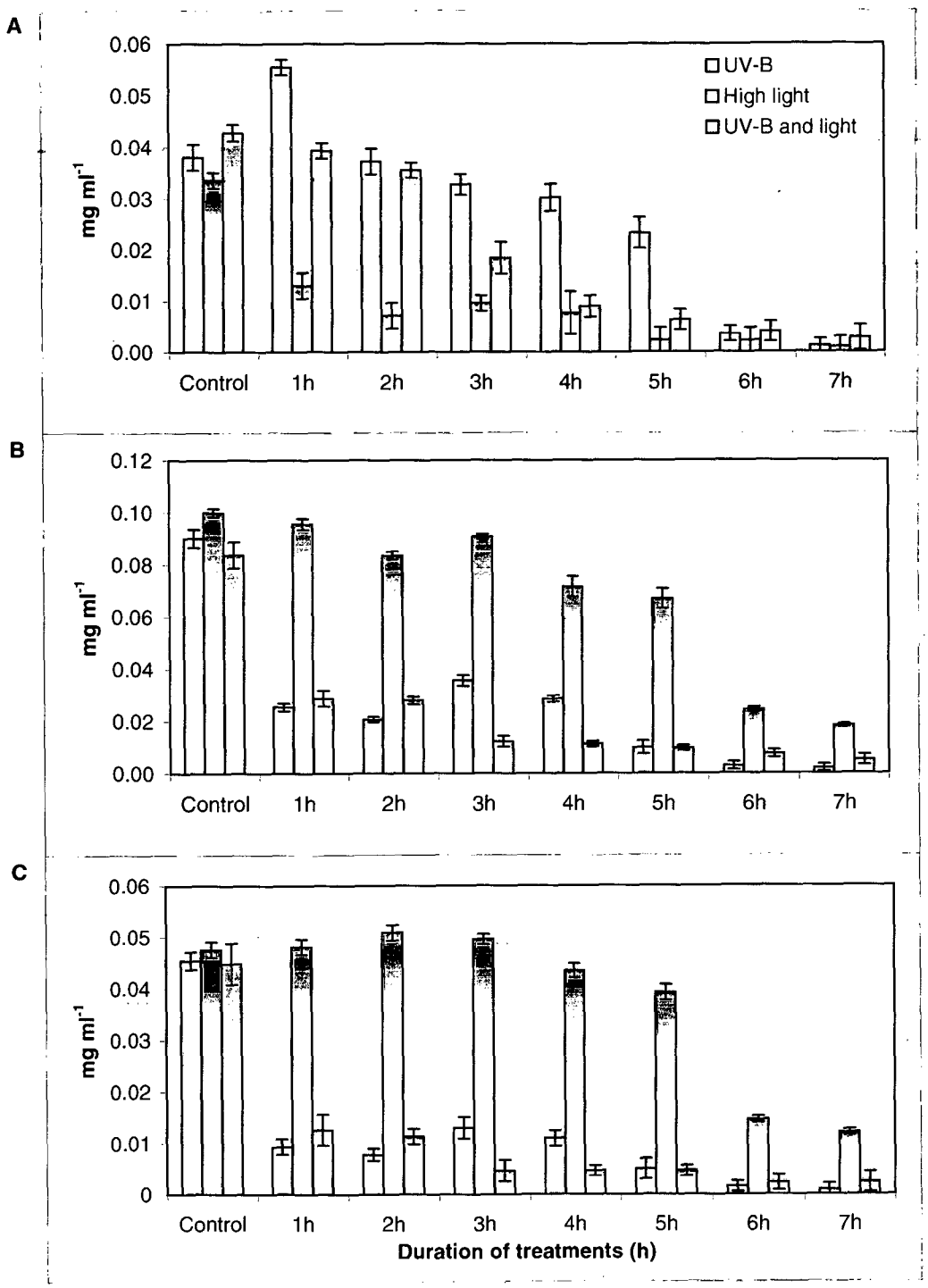


Fig 3.11 TLC profile of photosynthetic pigments after 6 h exposure to UV-B, high light and UV-B supplemented with low visual radiation in (A) *Nostoc spongiaeforme a*nd (B) *Phormidium corium* .

decrease in the allopycocyanin content. UV-B treatment for 1h caused a rapid decrease in allophycocyanin content to an extent of 71% as compared to control. Further increase in the duration of the UV-B treatment up to 4 h resulted in transient increase/leveling off of the allophycocyanin content (Fig. 3.12a). Further increase in the duration of the treatment over and above 4 h resulted in the rapid decrease in the allophycocyanin content showing almost negligible level (97% decline) after 6 h of the UV-B treatment as compared to control. Phycoerythrin content was also higher than phycocyanin content present in *Nostoc* culture. UV-B treatment, however, resulted in much greater decrease in the phycoerythrin content than the other two pigments. An hour of UV-B treatment resulted in 80% decreased in the phycoerythrin content compared to control. However, longer duration of the treatment up to 4 h did not change the percent decrease in the phycoerythrin content significantly. Further increase in the duration of the treatment, however, further decline the phycoerythrin content to a large extent (Fig. 3.12a).

High light treatment to *Nostoc,* however, had somewhat opposite effect than seen under UV-B treatment. The high light treatment for 5 h in general had very little effect on allophycocyanin and phycoerythrin and greater effect on phycocyanin. One hour of the high light treatment decreased the phycocyanin content by 61% as compared to control, which was slowly declined to 94 after 6 h of the treatment (Fig. 3.12b). Allophycocyanin content, however, showed only 9% decrease after 3 h of the high light treatment and further increase in the duration of the treatment to 5 h resulted in only 31% decrease in the allophycocyanin content, but declined to 78% after 6 h of the treatment (Fig. 3.12b). Phycoerythrin content showed a slight increase of 1-7% in *Nostoc* within first 3 h of the high light treatment, which declined to only 17% after 5 h of the treatment and 6 h of the high light treatment decreased the phycoerythrin content by 69% as compared to control.

Fig. 3.12 Effect of UV-B, high light and UV-B supplemented with low visual light up to 6 h on phycobilins in *Nostoc spongiaeforme*. A-Phycocyanin, B-Allophycocyanin, C- phycoerythrin. Each bar represents the mean ±S.D. n=5.

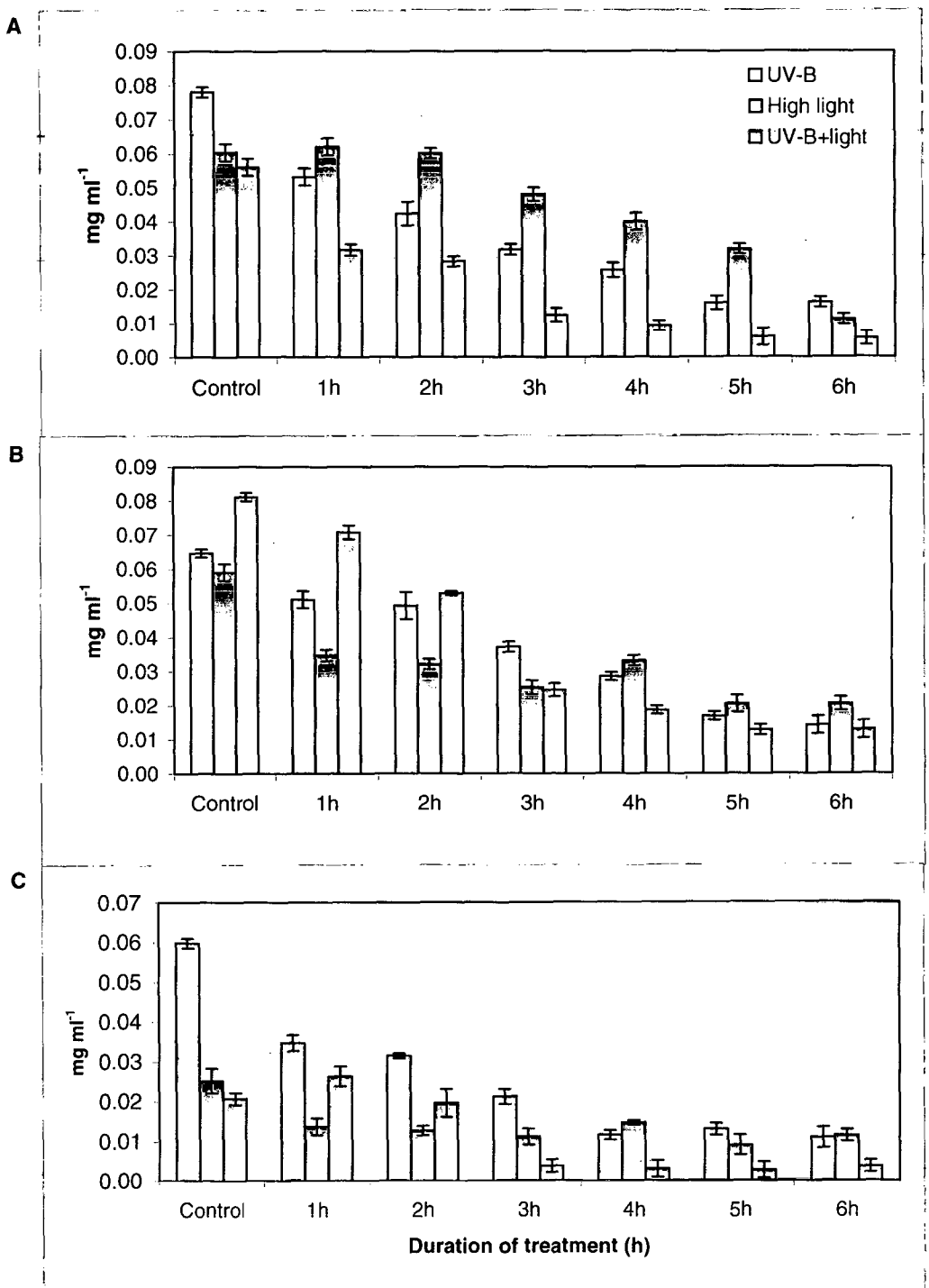

A
B
C
mg ml-1
UV-B
High light
UV-B and light
Control
1h
2h
3h
4h
5h
6h
7h
Duration of treatments (h)

UV-B treatment in combination with low level PAR also showed decreases in phycocyanin, allophycocyanin and phycoerythrin content, however, decrease in the phycocyanin content, during the early stage of the treatment (up to 2h), was relatively less (Fig. 3.12c). Up to two hour of the UV-B + low PAR treatment resulted in 17% decrease in the phycocyanin content in *Nostoc* as compared to control, which rapidly declined to 57% after 3 h and 90% after 6 of the treatment. Decline in the allophycocyanin content within first two hours was, however, considerable (66%) as compared to control, which further decreased to 91% after 6h of the same treatment. Phycoerythrin also declined to a greater extent (72%) on exposure to the UV-B+ low PAR treatment within 1h, which declined to 95% after 6 h duration of the treatment (Fig. 3.12c).

UV-B treatment to *Phormidium corium* also resulted in a linear decline in all the three pigments, phycocyanin, allophycocyanin and phycoerythrin. Decrease in the allophycocyanin was comparatively less than seen in phycocyanin and phycoerythrin as a result of the UV-B treatment. Phycocyanin content declined linearly to 59% after 3 h of the UV-B treatment, which was further, declined to 79% after 6 of the treatment as compared to control. However, allophycocyanin declined to relatively lesser extent of 42% after 3 h and 78% after 6 of the UV-B treatment as compared to its control. Phycoerythrin declined even more to 64% after 3 h and 82% after 6 of the treatment as compared to its control (Fig. 3.13a).

High light treatment to *Phormidium* resulted in two-phase effect for short duration (up to 2-3h) and longer duration (up to 6h). It was observed that short duration treatment resulted in greater declined to allophycocyanin and less declined in phycocyanin content

Fig. 3.13 Effect of UV-B, high light and UV-B supplemented with low visual light up to 6 h on phycobilins in *Phormidium corium*. A-Phycocyanin, B-Allophycocyanin, C- phycoerythrin. Each bar represents the mean ±S.D. n=5.


A
B
C
mg ml-1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
UV-B
High light
UV-B+light
Control
1h
2h
3h
4h
5h
6h
Duration of treatment (h)

while longer duration of the treatment resulted in reversing of the process and showed more decline in phycocyanin and less decline in allophycocyanin. Two hour of the high light treatment resulted in almost no decrease in the phycocyanin content and declined to 47% after 5 h of the high light treatment as compared to control. Six hour of the high light treatment, however, decreased the phycocyanin content to 81%. Allophycocyanin declined to 41% within 1h of the treatment as compared to control and thereafter remained more or less constant up to 4h of the high light treatment. Six hour of the high light treatment resulted in a decline of 65% as compared to control (Fig. 3.13b). Similarly phycoerythrin also showed a rapid decline of 45% on the onset of the high light treatment as compared to control and thereafter remained more or less constant up to h of the treatment. Even six hour of the treatment resulted in only 55% decrease in the phycoerythrin content compared to 81% observed in phycocyanin and 65% in allophycocyanin for the same duration of the treatment (Fig. 3.13b).

UV-B radiation supplemented with low level of light showed a linear decline in all the three pigments. However decline in the allophycocyanin up to 2 h of the treatment was almost negligible (Fig. 3.13c).

When pigment content of *Nostoc* and *Phormidium* were compared it was observed that phycocyanin and phycoerythrin content were higher in *Phormidium* than seen in *Nostoc* while allophycocyanin content was higher in *Nostoc* than seen in *Phormidium.* The UV-B treatment resulted in relatively less decrease in all the phycobilins content in *Phormidium* than seen in *Nostoc.* Also decrease of all the three phycobilins, phycocyanin, allophycocyanin and phycoerythrin was more or less same in *Phormidium* as a result of the UV-B treatment while in *Nostoc,* allophycocyanin and phycoerythrin decreased more

than the phycocyanin. High light also showed diversity of changes in the phycobillins in both *Nostoc* as well as *Phormidium.* Phycocyanin decreased to a lesser extent in *Phormidium* than in *Nostoc* while allophycocyanin decreased more in *Phormidium* than seen in *Nostoc* as a result of the high light treatment (Fig. 3.12 & 3.13).

3.5. EFFECT ON REDUCING SUGARS, GLYCOGEN CONTENT AND TOTAL SUGARS:

The effect of UV-B, high light and UV-B supplemented with low visual treatment on reducing sugar, glycogen and total sugar content was carried out to study the effect of these factors on carbohydrate metabolism and relate it to photosynthesis.

3.5.1. EFFECT ON REDUCING SUGARS:

The effect of UV-B, high light and UV-B in combination with low PAR showed two distinct type of effect on reducing sugar in both *Nostoc* and *Phormidium*: one rapid and another slower. UV-B and UV-B+ low PAR resulted in a rapid decrease in reducing sugar content in both *Nostoc* and *Phormidium* within 2 h of the treatment and thereafter the decrease in the reducing sugar content was comparatively much less even after 6 h of the respective treatment. Similarly high light caused a rapid decrease in the reducing sugar content within one hour of the treatment and thereafter the decrease in the reducing sugar content was slight even after 6 h of the treatment in *Nostoc* and *Phormidium* (Fig. 3.14 a & b).

The UV-B treatment to *Nostoc spongiaeforme* for 2 h caused a decrease of 44% in reducing sugar content as compared to control, which further declined to 55% after 4 h of

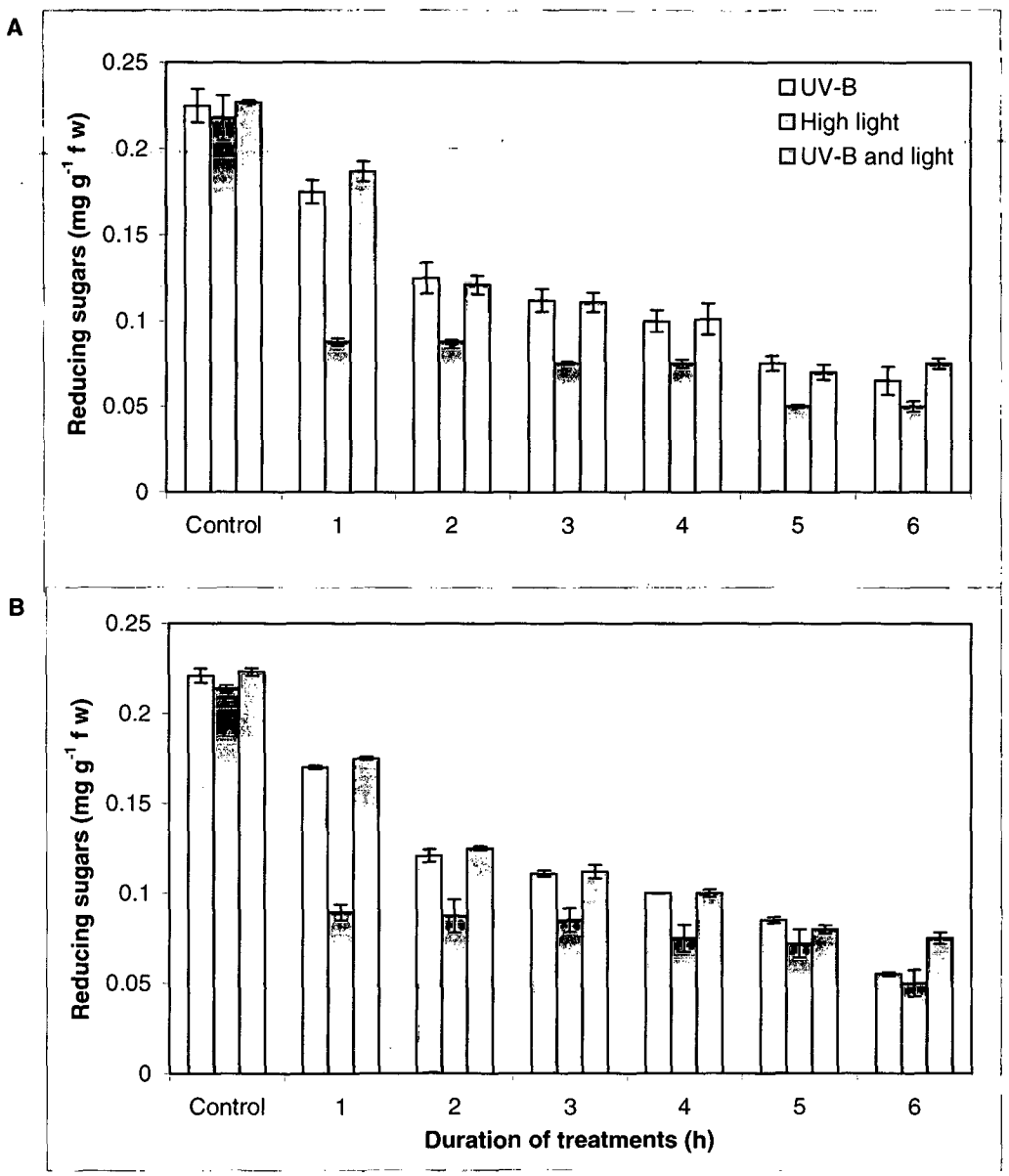


Fig 3.14: Effect of UV-B, high light and UV-B supplemented with low visual light up to 6 h duration on reducing sugars in (A) *Nostoc spongiaeforme* and (B) *Phormidium corium*. Each bar represents the mean ±S.D. n=5.

the treatment and 71% after 6 h of the UV-B treatment. High light treatment to *Nostoc* for 1 h decreased the reducing sugar content to 60% which declined only slightly even after 4 h of the treatment (66% decrease) and 6 h of the treatment resulted in a decrease of 77% as compared to control (Fig. 3.14a). UV-B + low PAR treatment resulted in less decrease in the reducing sugar content as compared to seen under UV-B or high light treatment. Two hour of the UV-B + low PAR treatment resulted in 46% decrease in the reducing sugar content which declined to 55% and 67% after 4 and 6 h of the treatment respectively (Fig. 3.14a).

More or less same results were observed with *Phormidium*. Reducing sugar decreased rapidly to 45% within 2 h of the UV-B treatment as compared to control and 75% after 6 h of the treatment. High light caused more rapid decrease in the reducing sugar content than seen under UV-B treatment. 1 h of high light treatment resulted in 58% decrease in the reducing sugar content as compared to control, which declined to 77% after 6 h of the treatment (Fig. 3.14b). UV-B + low PAR also caused rapid decline of 44% in reducing sugar within 2 h of the treatment as compared to control which was declined to 66% after 6 of the treatment (Fig. 3.14b).

3.5.2. EFFECT ON GLYCOGEN:

Glycogen content was also affected as a result of the treatment studied in both *Nostoc* and *Phormidium*. Distinct two phase decrease of glycogen content was not observed in *Nostoc*. All the three treatment (UV-B, high light and UV-B+low PAR) resulted in a linear decrease in glycogen content in *Nostoc* with respect to the duration of the respective treatment and the glycogen content declined to 60%, 53% and 65% as a result of 6 h of the UV-B, high light and UV-B +PAR treatment respectively (Fig. 3.15a).

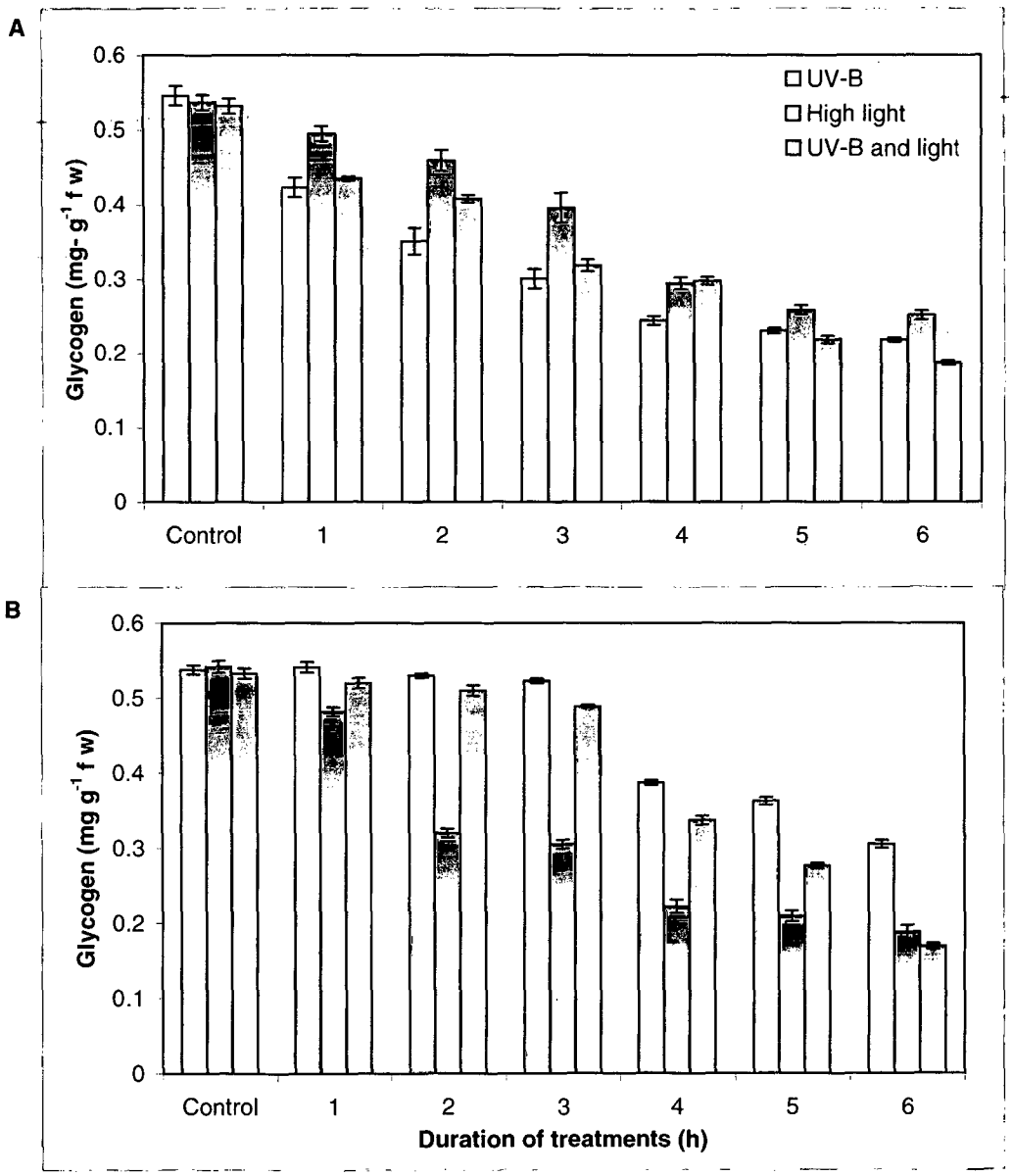


Fig 3.15: Effect of UV-B, high light and UV-B supplemented with low visual light up to 6 h on glycogen in (A) *Nostoc spongiaeforme* and (B) *Phormidium corium*. Each bar represents the mean ±S.D. n=6.

However, *Phormidium* showed two distinct phases of decrease in glycogen content. UV-B treatment for 3 h decreased the glycogen content by only 3% compared to control followed by a rapid decrease of 43% after 6h of the treatment. Similar results were observed when *Phormidium* was exposed to UV-B treatment supplemented with low visual light. Three hour of the UV-B + low PAR treatment showed a decrease of only 8% as compared to control, thereafter the glycogen content declined more rapidly and a decrease of 68% in glycogen content was observed after 6 h of the treatment (Fig. 3.15b). High light, however, resulted in a faster decrease in glycogen content on the onset of treatment. Glycogen content declined to 41% as compared to control with in 2 h of high light treatment, which remained more or less constant even after 3 h of the treatment but there after declined linearly to 65% after 6 h of the treatment (Fig. 3.15b).

3.5.3. EFFECT ON TOTAL SUGARS:

UV-B treatment of *Nostoc spongiaeforme* resulted in an initial decrease of 36% in total sugar content within 1h following which it showed transient stability in decline up to 2 h of the treatment (Fig. 3.16a). Further increase in the duration of the treatment resulted in a linear decrease in the total sugar content to 84% after 6 h of the treatment as compared to control. High light also resulted in a decline in the total sugar content and showed a slight transient leveling of the decrease in sugar content during 3-4 h of the treatment following which a slow decline was observed. A 6 h of the high light treatment decreased the total sugar content by 66% as compared to control (Fig. 3.16a). UV-B in combination with low level PAR initially did not show any decrease in the total sugar content up to 3 h of the treatment but declined slightly thereafter. A 6 h of the UV-B +

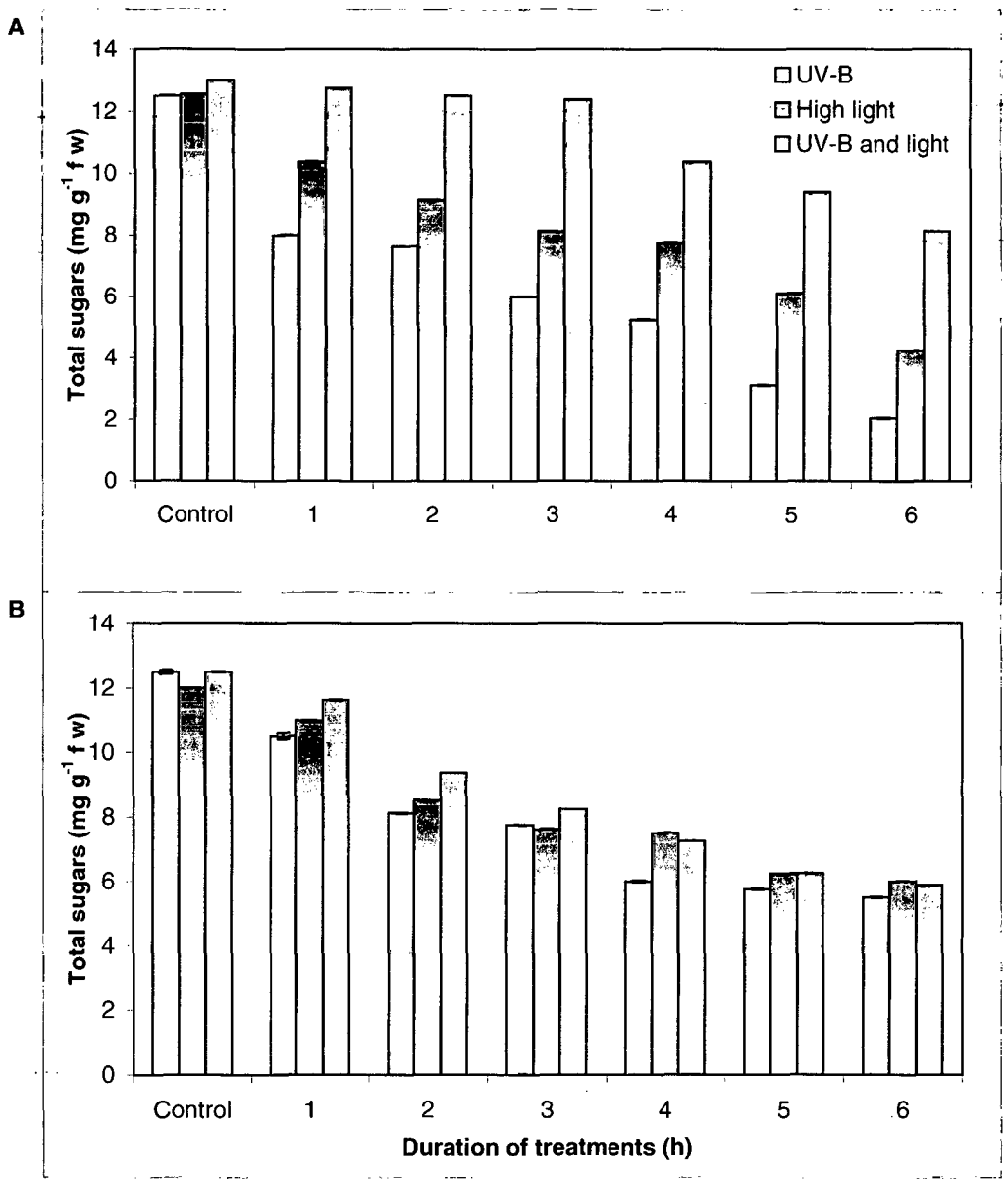


Fig 3.16: Effect of UV-B, high light and UV-B supplemented with low visual light up to 6 h on total sugar in (A) *Nostoc spongiaeforme* and (B) *Phormidium corium*. Each bar represents the mean ±S.D. n=5.

low PAR treatment resulted in only 37% decrease in the total sugar content as compared to 84% decrease seen during UV-B treatment of the same duration.

Phormidium showed more or less same extent of decrease in the total sugar content as a result of the UV-B, high light and UV-B + PAR treatment. However, a slight transient leveling of the decrease in total sugar content was observed during 2-3 h of UV-B treatment and 3-4 h during high light treatment (Fig. 3.16b). UV-B + low PAR showed relatively less decrease in the total sugar content in *Phormidium* than seen during UV-B treatment alone (Fig. 3.16b).

3.6. EFFECT ON NITROGEN CONTENT:

The effect of UV-B radiation, high light and UV-B supplemented with low visual light on nitrogen content in *Nostoc spongiaeforme* and *Phormidium corium* shows that all the three treatment caused decrease in the nitrogen content as compared to control and extent of decrease in the nitrogen content in both the species was more or less same (Fig. 3.17). UV-B treatment for 6h decreased the nitrogen content by 38% in *Nostoc* and 30% in *Phormidium*. Similarly high light treatment and UV-B + low PAR treatment resulted in more or less same level of decrease in the nitrogen content in both the cyanobacteria.

3.7. EFFECT ON ACTIVE OXYGEN SPECIES:

We compared the ability of AOS quenching of culture of *Phormidium* with *Nostoc spongiaeforme* maintained under growth conditions. It was seen that normally grown tissue homogenate of *Phormidium corium* could quench the AOS by only 15% as compared to 32% seen in *Nostoc* as compared to control (epinephrine without any tissue

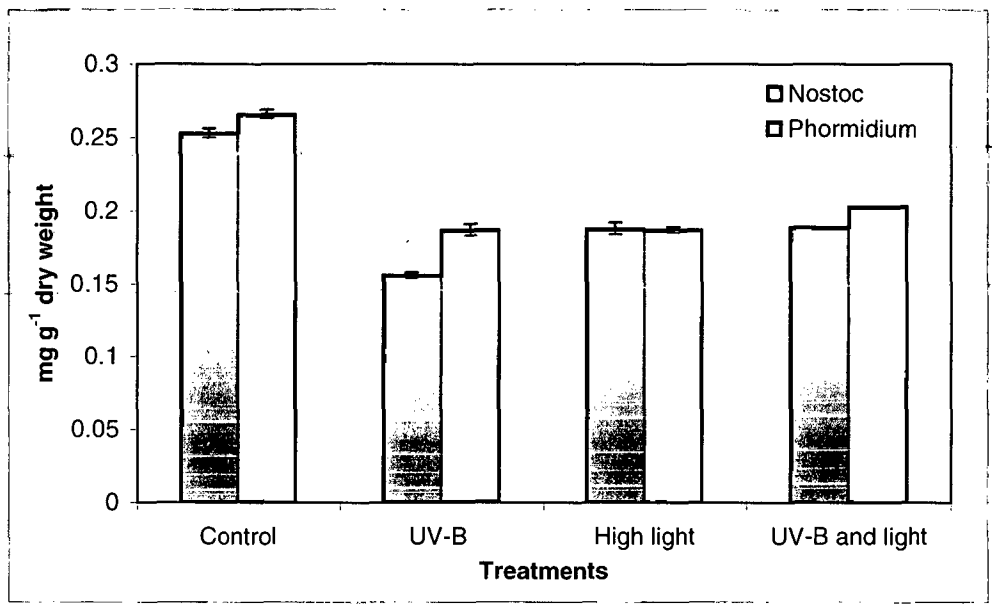


Fig 3.17: Effect of UV-B, high light and UV-B supplemented with low visual light up to 6 h on nitrogen content in *Nostoc spongiaeforme* and *Phormidium corium*. Each bar represents the mean ±S.D. n=3.

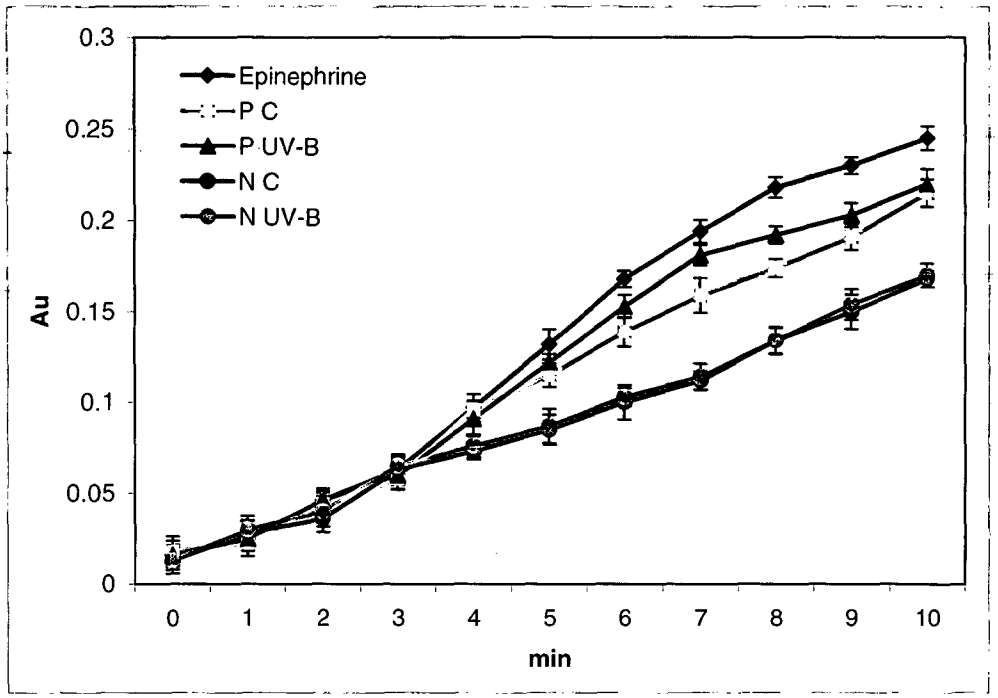


Fig 3.18: Generation of active oxygen species (AOS) in *Nostoc spongiaeform* e and *Phormidium corium* after 6h of UV-B treatment. Blue colour shows generation of AOS in the presence of epinephrine and without any tissue homogenate, yellow and green colour show generation of AOS in the presence of *Nostoc* control and UV-B treated culture respectively, purple and orange colour show generation of AOS in the presence of *Phormidium* control and UV-B treated for 6 h culture respectively. Data represents the mean ±S.D. n=6.

homogenate; Fig. 3.18). UV-B treatment for 6h resulted in slightly decreasing in the quenching of AOS in *Nostoc* and *Phormidium.*

3.8. EFFECT ON PEROXIDATION OF LIPIDS:

The effect of UV-B radiation, high light and UV-B supplemented with low visual radiation on MDA formation, which is indicative of the level of peroxidation of cell membrane lipids, was also studied in *Nostoc spongiaeforme* and *Phormidium corium* (Fig. 3.19 a & b).

UV-B radiation showed rapid increase (135%) in the lipid peroxidation, seen as MDA formation, within 2h of the treatment as compared to control. Further increase in the duration of treatment to 4 h slowed down the peroxidation to a considerable extent showing only 27% further increase as compared to 2 h of the treatment. Further increase in the duration of the treatment to 6h rapidly increased the extent of peroxidation of lipids to 270% as compared to control in *Nostoc spongiaeforme* (Fig. 3.19a). High light treatment of *Nostoc* showed only a 40% increase in lipid peroxidation as a result of 6 h of the treatment as compared to control (Fig. 3.19a). UV-B supplemented with low visual light for 1, 2 and 3h showed an increase of 16%, 26% and 41% respectively in MDA formation in *Nostoc,* however, increase in the duration of the treatment to 6h showed an increase of 135% in the MDA formation as compared to the control (Fig. 3.19a).

The level of peroxidation even in the control culture of *Phormidium* was considerably higher than observed in *Nostoc.* However, treatment to UV-B, high light and UV-B + low PAR further increased the level of lipid peroxidation albeit to a lesser extent than seen in *Nostoc.* Three hour of UV-B treatment to *Phormidium corium* showed

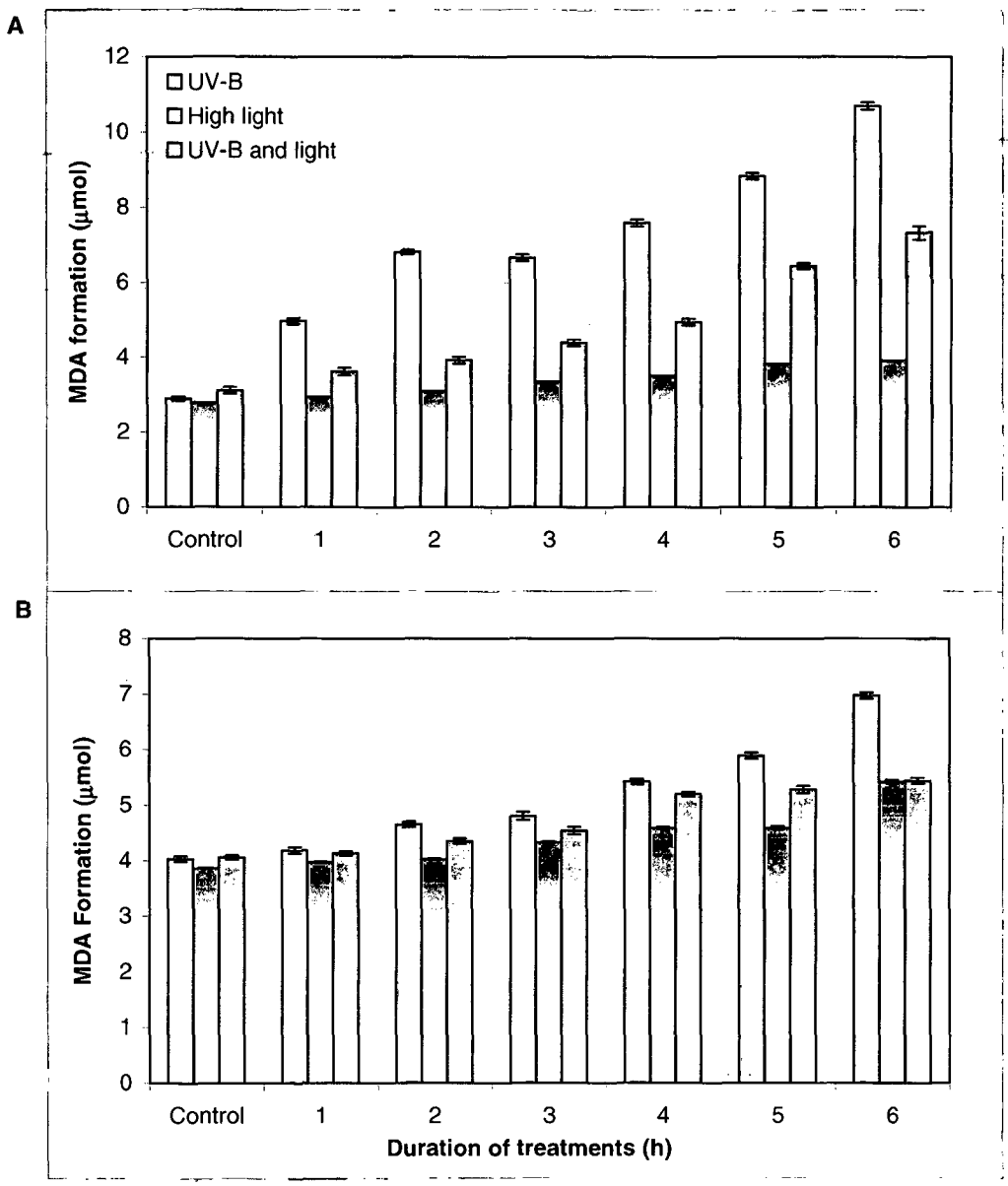


Fig 3.19: Effect of UV-B, high light and UV-B supplemented with low visual light up to 6 h duration on lipid peroxidation in cell membrane of (A) *Nostoc spongiaeforme* and (B) *Phormidium corium.* Each bar represents the mean ±S.D. n=7.

only 19% increase in MDA formation which was further increased to 73% after 6 h of the UV-B treatment as compared to control, while the same treatment in *Nostoc* resulted in a increase of 270% in lipid peroxidation. Similarly high light treatment to *Phormidium* also showed a slow increase of only 40% after 6h of the treatment as compared to control. UV-B + low PAR treatment resulted in lesser extent of peroxidation than seen in UV-B treated *Phormidium*. A six h UV-B + low PAR treatment resulted in 34% increase in the peroxidation level compared to 73% peroxidation level seen in the UV-B treatment alone (Fig. 3.19b).

UV-B treatment showed more MDA formation compared to high light and UV-B supplemented with low light in both *Nostoc* and *Phormidium*. The extent of peroxidation due to UV-B treatment was far greater in *Nostoc* compared to *Phormidium*. High light treatment showed more or less same level of increase in the peroxidation in *Nostoc* and *Phormidium*, however, basal level of peroxidation in *Phormidium* was far greater than seen in *Nostoc*. UV-B supplemented with low light showed relatively less peroxidation than seen due to UV-B alone in both the species.

3.9. EFFECT ON LIPIDS:

3.9.1. EFFECT ON PHOSPHOGLYCOLIPIDS:

Phosphoglycolipids separation was carried out by thin layer chromatography in *Nostoc spongiaeforme* and *Phormidium corium* (Fig. 3.20a & b). Identification of these spots was done according to Rf values compared with standards. There were quantitative changes in phospho-glycolipids due to UV-B, high light and UV-B supplemented with low visual light treatment in *Nostoc spongiaeforme* and *Phormidium corium*. In *Nostoc*

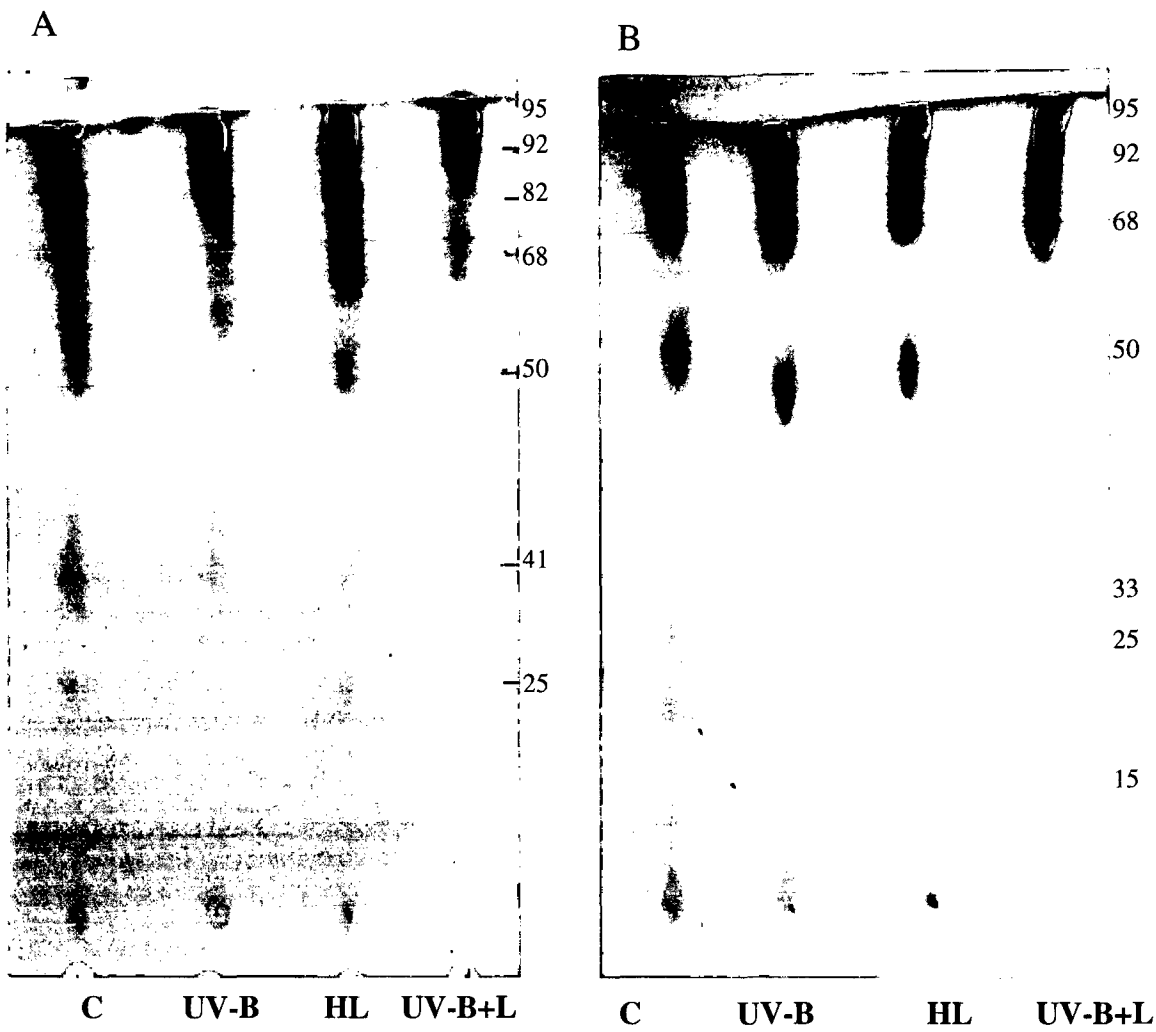


Fig 3.20 TLC profile of phosphoglycolipids in (A) *Nostoc spongiaeforme* and (B) *Phormidium corium* after exposure to 6 h of UV-B, high light and UV-B supplemented with low visual light treatment. Tentative nature of phosphoglycolipids is shown in table 3.3.

Table 3.3 Rf value and possible nature of lipids seen in TLC profile.

Sr. No.	Phosphoglycolipids		Neutral lipids	
	Rf x100	**Components**	**Rf x 100**	**Components**
1	15	Sulpholipid	10	Monoglyceride
2	25	Digalactosyldiglyceride	15	Monoglyceride
3	33	Digalactosyldiglyceride	20	Monoglyceride
4	41	Phosphotidylglycerol	38	Diglyceride
5	50	Phosphatidylethanolamine	70	Diglyceride
6	68	Sulfoquinosylglycerol	85	Fatty acid
7	82	Monogactosyldiglyceride	89	Triglyceride
8	92	Monogactosyldiglyceride	93	Esters
9	95	Pigments	98	Pigments

spongiaeforme, 5 and in *Phormidium corium,* 7 different phospho-glycolipids spots were observed. Tentative nature of these phospho-glycolipid is given in the table 3.3. Due to the UV-B treatment alone and UV-B supplemented with low PAR there was slight decline in phosphotidylglycerol components (Rf 41) in *Nostoc* (Fig. 3.20a). Sulfoquinosylglycerol (Rf 68) declined as a result of the UV-B in *Phormidium.* Monogalactosyldiglyceride (Rf 82 and 92) showed slight decrease in *Nostoc* due to UV-B and UV-B supplemented with low PAR but increased as a result of the high light treatment. Monogalactosyl diglyceride (Rf 82) increased in *Phormidium* as a result of UV-B and high light but decreased due to the UV-B supplemented with low PAR as compared to control (Fig. 3.20b).

3.9.2. EFFECT ON NEUTRAL LIPIDS:

Neutral lipids separation was carried out by thin layer chromatography in *Nostoc spongiaeforme* and *Phormidium corium* (Fig. 3.21a & b). Identification of these spots was done according to Rf values compared with standards, which is shown in table 3.2. There were no changes in neutral lipids as a result of UV-B, high light and UV-B supplemented with low visual light in *Nostoc spongiaeforme* while *Phormidium corium* showed qualitative change in neutral lipids due to the treatments. In *Nostoc spongiaeforme,* 5 different neutral lipids spots were observed which remained unchanged due to the treatments (Fig. 3.21a). *Phormidium corium* showed 6 different neutral lipid spots, which changed slightly due to the treatments (Fig. 3.21b).

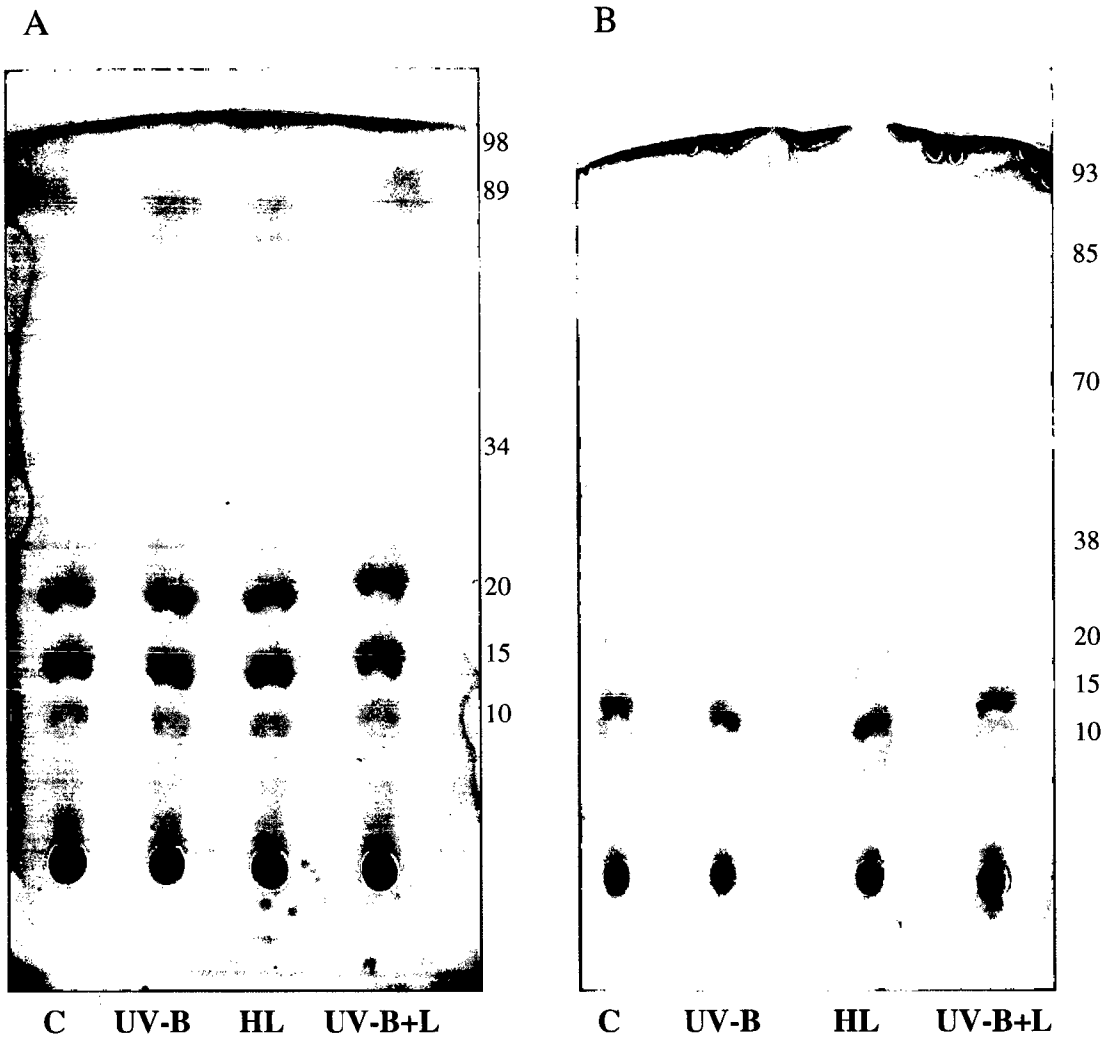


Fig 3.21 TLC profile of neutral lipids in (A) *Nostoc spongiaeforme* and (B) *Phormidium corium* after exposure to 6 h of UV-B, high light and UV-B supplemented with low visual light treatment. Tentative nature of neutral lipids is given in table 3.3.

3.9.3. EFFECT ON QUANTITATIVE ESTIMATION OF GLYCOLIPIDS AND PHOSPHOLIPIDS:

The effect of UV-B, high light and UV-B supplemented with low visual radiation on glycolipid in *Nostoc spongiaeforme* and *Phormidium corium* was studied (Fig. 3.22a & b). UV-B treatment resulted in greater decrease in glycolipids than high light or UV-B + low PAR. Six hour of the UV-B treatment to *Nostoc* resulted in 63% decline in glycolipids content as compared to 36% and 20% declined observed under high light and UV-B + PAR treatment respectively for the same duration (Fig. 3.22a). *Phormidium* also showed similar trend of changes in glycolipid content as a result of the three treatments studied. A 6 h UV-B treatment to *Phormidium* resulted in 42% decrease in the glycolipid content and high light and UV-B + low PAR decreased the glycolipid content by 18 and 15% respectively as compared to control. The extent of decreased in glycolipid content was relatively less in *Phormidium* than seen in *Nostoc* (Fig. 3.22a).

Phospholipids also declined as a result of the treatments in both *Nostoc* and *Phormidium* (Fig. 3.22b). UV-B treatment for 6 h resulted in a decrease of 53 and 64 % in *Nostoc* and *Phormidium* respectively as compared to their control. High light treatment for 6 h caused a decrease in phospholipids content by 60 and 65% in *Nostoc* and *Phormidium* respectively as compared to their respective controls. UV-B + low PAR treatment for 6 h showed a decline of 56% in phospholipid content in *Nostoc* and a decline of 51% in *Phormidium* compared to their control (Fig. 3.22b).

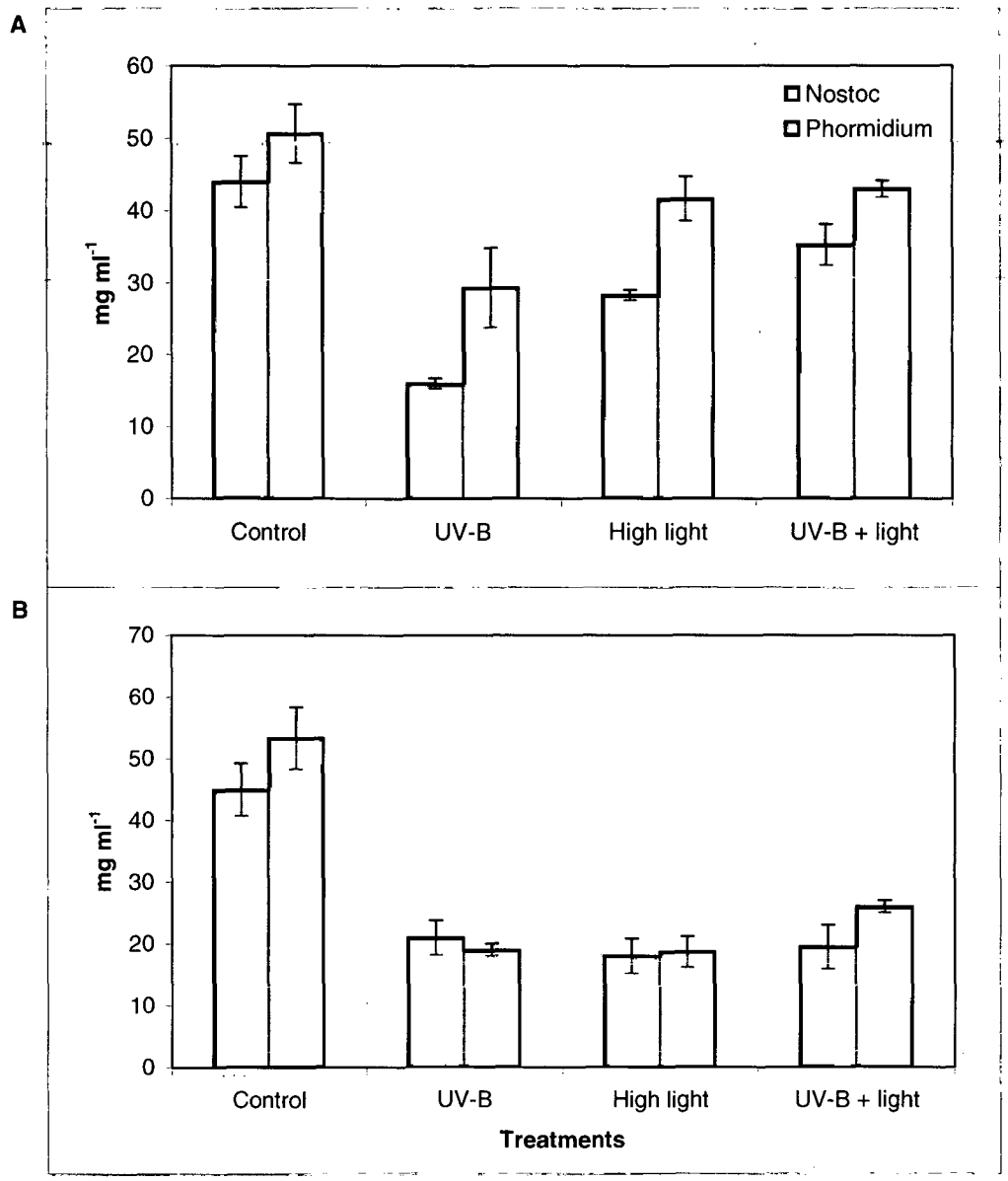


Fig 3.22: Effect of UV-B, high light and UV-B supplemented with low visual light for 6 h on (A) glycolipids and (B) phospholipid content in *Nostoc spongiaeforme* and *Phormidium corium*. Each bar represents the mean ±S.D. n=5.

3.9.4. FATTY ACID ANALYSIS:

The fatty acids composition of the *Nostoc spongiaeforme* and *Phormidium corium* was carried out using gas chromatography (GC). GC profile of the fatty acid composition of *Nostoc spongiaeforme* and *Phormidium corium* is shown in figure 3.23 & 3.24 respectively. The fatty acid groups present in both the species are Lauric acid C_{12}, Myristic acid C_{14}, Palmitic acid C_{16}, Stearic acid C_{18} Oleic acid $C_{18:1}$, Linoleic acid $C_{18:2}$ and Linolenic acid $C_{18:3}$ (Fig. 3.23; Table 3.4). Extent of variation in the fatty acid content of the total lipids fraction of *Nostoc* and *Phormidi*um grown under growth conditions (control) was negligible seen (Table 3.4 and 3.5). However, fatty acid content of the glycolipid fraction of *Nostoc* grown under control conditions was considerable higher than the fatty acid content of the glycolipid fraction of control grown *Phormidium.*

3.9.4.1. FATTY ACID ANALYSIS OF *NOSTOC*:

Fatty acid analysis of glycolipid and total lipid fraction of *Nostoc* grown under growth conditions showed greater content of saturated fatty acid such as Lauric acid, Myristic acid, Stearic acid in glycolipid fraction than seen in the total lipid component. Lauric acid was 56% higher in glycolipid fraction than seen in total lipid fraction. Myristic acid was 13% higher while Stearic acid was 169% higher in glycolipid fraction as compared to total lipids fraction of *Nostoc*. Quantity of palmatic acid was more or less same in both glycolipids as well as total lipids fractions. Unsaturated fatty acids such as Oleic acid, Linoleic acid and Linolenic acid was considerably higher in glycolipids component than in the total lipid. Oleic acid, Linoleic acid and Linolenic acid content were 158%, 161% and 81% greater respectively in glycolipid fraction than in total lipid fraction (Fig. 3.23; Table 3.4).

Fig. 3.23 GC profile of total fatty acids after 6 h exposure to UV-B, high light and UV-B supplemented with low visual radiation in *Nostoc spongiaeforme.*

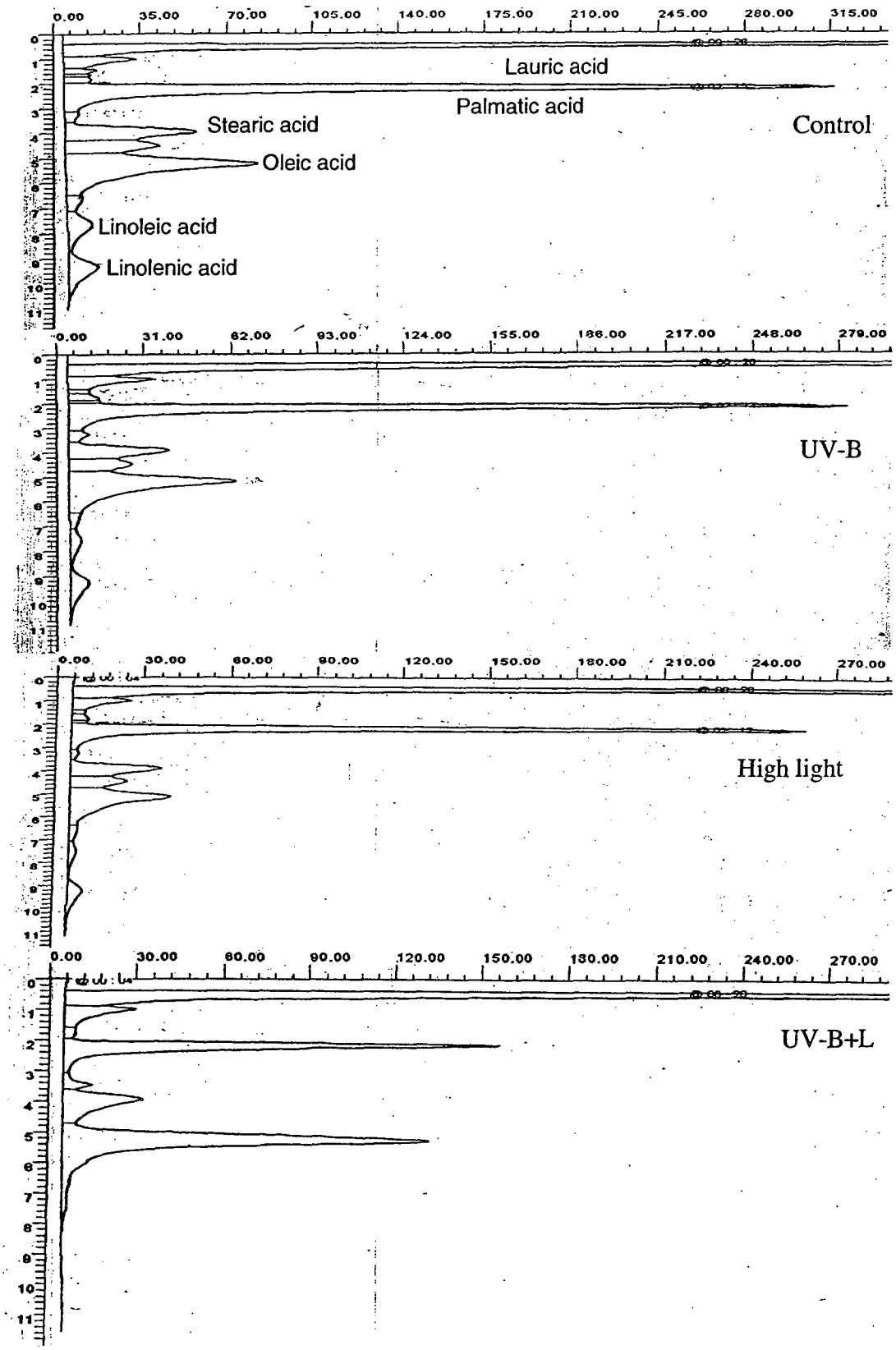

0.00 35.00 70.00 105.00 140.00 175.00 210.00 245.00 280.00 315.00
Lauric acid
Palmatic acid
Stearic acid
Oleic acid
Linoleic acid
Linolenic acid
Control
0.00 31.00 62.00 93.00 124.00 155.00 186.00 217.00 248.00 279.00
UV-B
0.00 30.00 60.00 90.00 120.00 150.00 180.00 210.00 240.00 270.00
High light
0.00 30.00 60.00 90.00 120.00 150.00 180.00 210.00 240.00 270.00
UV-B+L

Table 3.4 Effect of UV-B, high light and UV-B supplemented with low visual light for 6 hours on fatty acid composition of total and glycolipid in *Nostoc spongiaeforme* (mole %).

Treatment	Fatty acid composition (mole %)						
	Lauric acid 12:0	Myristic acid 14:0	Palmatic acid 16:0	Stearic acid 18:0	Oleic acid 18:1	Linoleic acid 18:2	Linolenic acid 18:3
Total lipids							
Control	11.4±0.6	18.2±0.4	15.4±0.6	9.5±0.4	3.4±0.6	20.7±0.6	3.5±0.3
UV-B (6h)	11.2±0.4	17.7±0.5	14.9±0.7	9.2±0.6	3.4±0.5	20.7±0.4	3.5±0.5
High light (6h)	10.3±0.5	17.6±0.3	14.8±0.3	9.1±0.7	3.4±0.4	20.7±0.3	3.5±0.4
UV-B+L (6h)	12.7±0.5	19.8±0.3	16.6±0.3	10.2±0.7	3.6±0.4	22.4±0.3	3.8±0.4
Glycolipids							
Control	17.9±0.4	20.6±0.5	15.1±0.3	25.6±0.5	8.8±0.4	37.7±0.6	9.1±0.7
UV-B (6h)	12.3±0.6	14.2±0.4	10.4±0.4	17.9±0.4	8.8±0.3	37.7±0.4	9.1±0.4
High light (6h)	16.7±0.3	19.4±0.6	14.1±0.4	25.3±0.3	8.8±0.6	37.7±0. 5	9.1±0.4
UV-B+L (6h)	18.1±0.3	21.6±0.6	16.1±0.4	26.6±0.3	9.8±0.6	38.4±0. 5	10.1±0.4

UV-B treatment resulted in greater decrease in the saturated fatty acid such as Lauric acid, Myristic acid, Palmatic acid, Stearic acid of glycolipids components in *Nostoc*. Unsaturated fatty acids of glycolipids fraction remained unchanged due to the UV-B treatment in *Nostoc spongiaeforme* (Fig. 3.23). High light treatment also resulted in slight decrease in saturated fatty acids of glycolipids while unsaturated fatty acids showed no appreciable change due to the treatment in *Nostoc spongiaeforme.* There was increase in content of both saturated as well as unsaturated fatty acids of glycolipids fraction in case of UV-B supplemented with low visual light treatment compared to control in *Nostoc spongiaeforme* (Table 3.4).

UV-B radiation and high light treatment resulted in decrease in saturated fatty acids content such as Lauric acid, Myristic acid, Palmatic acid, Stearic acid in total lipid fraction of *Nostoc spongiaeforme.* Unsaturation level of fatty acids such as Oleic acid, Linoleic acid and Linolenic acid of total lipids remained unchanged in *Nostoc* due to the UV-B as well as high light treatment. UV-B supplemented with low visual light treatment resulted in slight increase in the content of both saturated as well as unsaturated fatty acids of total lipids as compared to control in *Nostoc spongiaeforme* (Fig. 3.23; Table 3.4).

3.9.4.2. FATTY ACID ANALYSIS OF *PHORMIDIUM*:

The fatty acid analysis of glycolipid fraction and total lipid fraction of *Phormidium* grown under growth conditions showed low content of saturated fatty acid such as Myristic acid and Palmatic acid in glycolipid fraction than seen in the total lipid component. Myristic acid content decreased to 23% while Palmatic acid decreased to 33% in glycolipid fraction as compared to total lipids fraction. Lauric acid content was

Fig. 3.24 GC profile of total fatty acids after 6 h exposure to UV-B, high light and UV-B supplemented with low visual radiation in *Phormidium corium*.

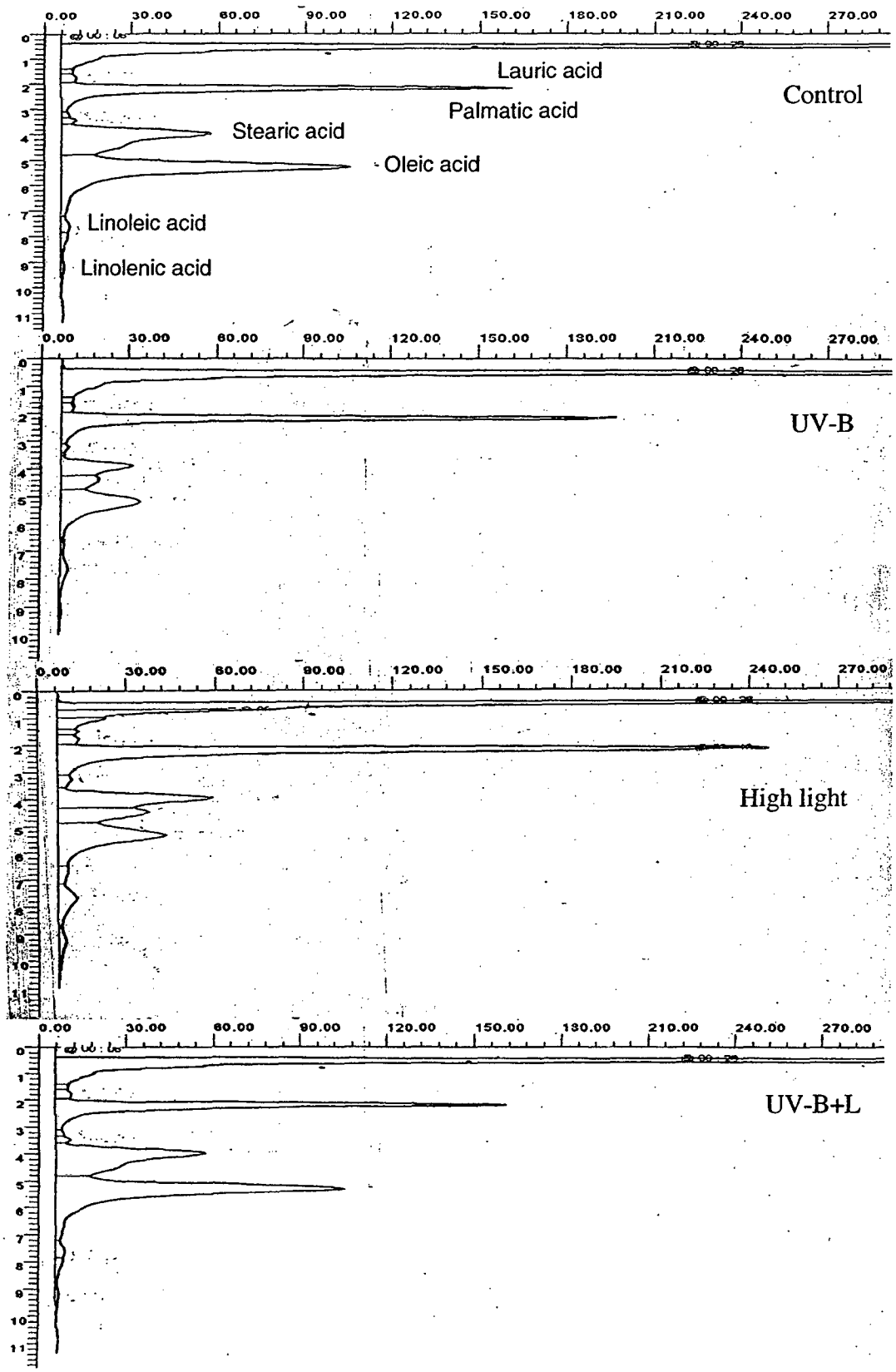

0.00
30.00
60.00
90.00
120.00
150.00
180.00
210.00
240.00
270.00
Lauric acid
Palmatic acid
Stearic acid
Oleic acid
Linoleic acid
Linolenic acid
Control
UV-B
High light
UV-B+L

Table 3.5 Effect of UV-B, high light and UV-B supplemented with low visual light for 6 hours on fatty acid composition of total and glycolipid in *Phormidium corium* (mole %).

Treatment	Fatty acid composition (mole %)						
	Lauric acid 12:0	Myristic acid 14:0	Palmatic acid 16:0	Stearic acid 18:0	Oleic acid 18:1	Linoleic acid 18:2	Linolenic acid 18:3
Total lipids							
Control	11.3±0.6	17.6±0.4	14.8±0.6	9.4±0.4	3.2±0.6	19.9±0.6	3.3±0.3
UV-B (6h)	11.3±0.4	17.6±0.5	14.8±0.7	9.1±0.6	3.2±0.5	19.9±0.4	3.3±0.5
High light (6h)	11.2±0.5	17.4±0.3	14.7±0.3	9.0±0.7	3.2±0.4	19.9±0.3	3.3±0.4
UV-B+L (6h)	11.4±0.7	17.7±0.7	14.9±0.5	9.4±0.5	3.6±0.5	21.3±0.3	4.3±0.2
Glycolipids							
Control	11.6±0.4	13.4±0.5	9.8±0.3	16.6±0.5	5.7±0.4	35.0±0.6	5.9±0.7
UV-B (6h)	11.4±0.6	13.4±0.4	9.8±0.4	16.6±0.4	5.7±0.3	35.0±0.4	5.9±0.4
High light (6h)	11.6±0.3	13.3±0.6	9.8±0.4	16.6±0.3	5.7±0.6	35.0±0. 5	5.9±0.4
UV-B+L (6h)	13.1±0.5	15.1±0.3	11.2±0.3	18.7±0.4	7.80±0.5	39.75±0.7	8.95±0.3

more or less same in both glycolipid fraction as well as total lipid fraction. Quantity of Stearic acid was (77%) higher in glycolipids fraction than seen in total lipids components. Unsaturated fatty acids such as Oleic acid, Linoleic acid and Linolenic acid were considerably higher (75-76%) in glycolipids fraction than in the total lipid components (Fig. 3.24; Table 3.5).

The effect of UV-B radiation, high light and both UV-B and light treatment on fatty acid composition of total and glycolipids fraction of *Phormidium corium* is shown in figure 3.24 and Table 3.5. Saturated and unsaturated fatty acids of total lipid components as well as glycolipid fraction showed no appreciable change due to UV-B and high light treatment in *Phormidium*. UV-B supplemented with low visual light treatment also did not show any appreciable changes in the saturated fatty acid contents of total lipids fraction, however, slight increase (up to 14%) in the various saturated fatty acids of glycolipids components was observed due to the treatment. UV-B + low PAR treatment resulted in a slight increase in the unsaturated fatty acids Linoleic acid (9%) and Linolenic acid (29%) while Oleic acid content remained more or less same as compared to control in total lipids fraction. In glycolipids fraction, however, Oleic acid increased to 35% while Linoleic acid and Linolenic acid increased by 13% and 51% respectively as compared to control due to the UV-B + low PAR treatment (Table 3.5).

3.10. EFFECT ON LIPIDS (FTIR):

FTIR spectra of *Nostoc spongiaeforme* showed absorption bands at 3425, 2900, 2825, 2365, 1700, 1625, 1550, 1480, 1200, 1180, 770 cm^{-1} which indicate the presence of both saturated and unsaturated fatty acids (Fig. 3.25, Table 3.6). Quantitative changes

were observed as a result of UV-B, high light and UV-B supplemented with light treatment. UV-B treatment resulted in significant decrease in the unsaturation level of fatty acids as seen by the decrease in the amplitude of 1550 cm^{-1} band as compared to control. UV-B + low PAR seem to completely inhibit unsaturation of lipid molecule while high light treatment caused only slight change in the unsaturation level. Triple bond formation (band at 2365 cm^{-1}) also decreased as a result of the UV-B and UV-B + low PAR treatment, while it did not show any change under high light condition. Spectral band at 1700 cm-1, indicative of C=O group declined under high light condition but remain almost unchanged under UV-B and UV-B + low PAR treatment. Slight changes in C-H stretch band, seen as peaks at 2825 and 2900 cm^{-1}, was also observed due to all the three treatments studied, but more so under high light condition. Hydroxyl group observed at absorption band 770 cm^{-1} decreased in all the three treatment but OH group observed at 3425 cm^{-1} increased due to the three treatments studied (Fig. 3.25, Table 3.6).

FTIR spectra of *Phormidium corium* also showed similar absorption bands at 3425, 2900, 2825, 2365, 1700, 1625, 1550, 1480, 1180 cm^{-1} (Fig. 3.26, Table 3.7). Quantitative changes observed in *Phormidium* as a result of the UV-B, high light and UV-B + low PAR treatment were relatively less than seen in *Nostoc*. Spectral band at 1550 cm^{-1} indicative of unsaturation decreased only slightly as a result of the treatments compared to control. However, triple bond (band at 2365 cm^{-1}) declined considerably as a result of the high light and UV-B + low PAR treatment but UV-B alone did not result in any change in the triple bond as compared to control. Spectral band at 1700 cm^{-1} indicative of C=O bond and 2825/2900 indicative of C-H stretch did not change as a result of the treatments. Hydroxyl group detected at 3425 cm^{-1} increased as a result of the treatments (Fig. 3.26).

Fig. 3.25 FTIR spectra of total lipids extracted from UV-B, high light and UV-B supplemented with low visual light treatment in *Nostoc spongiaeforme* culture for 6 h. The spectrum was taken using carbon disulphide (CS_2) as solvent.

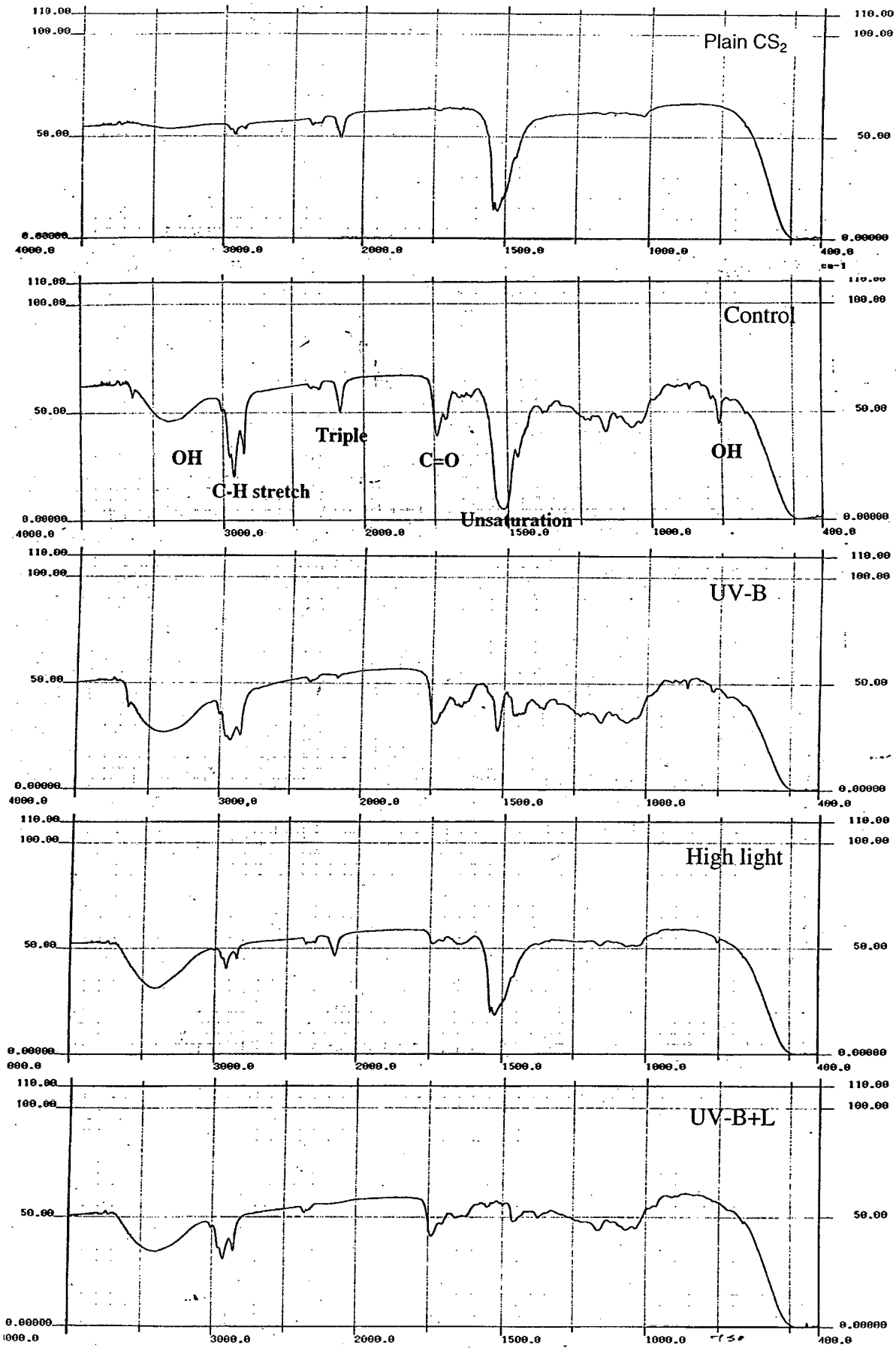

Plain CS_2
Control
OH
C-H stretch
Triple
C=O
Unsaturation
OH
UV-B
High light
UV-B+L
4000.0
3000.0
2000.0
1500.0
1000.0
400.0
cm-1

Table 3.6 FTIR profile of lipids indicating qualitative as well as quantitative changes of lipids in *Nostoc spongiaeforne* due to UV-B, high light and UV-B supplemented with low visual light. + indicates presence of spot and – indicates absence of spot.

Peaks	Components	Control	UV-B	High light	UV-B + light
3425	Hydroxyl group	+++	++++	++++	++++
2900	C-H stretch	++++	+++	++	+++
2825	C-H stretch	+++	+++	+	++
2365	Triple bond	+++	+	+++	+
1700	Carbonyl group	+++	+++	+	++
1625	-	+	+++	++	++
1550	Unstaurated group	+++++	++	+++	+
1480	-	++	++++	-	+
1200	C-O esters	++	++	+	++
1180	-	+++	+	+	-
770	Hydroxyl group	++	-	-	-

Fig. 3.26 FTIR spectra of total lipids extracted from UV-B, high light and UV-B supplemented with low visual light treatment in *Phormidium corium* culture for 6 h. The spectrum was taken using carbon disulphide (CS_2) as solvent.

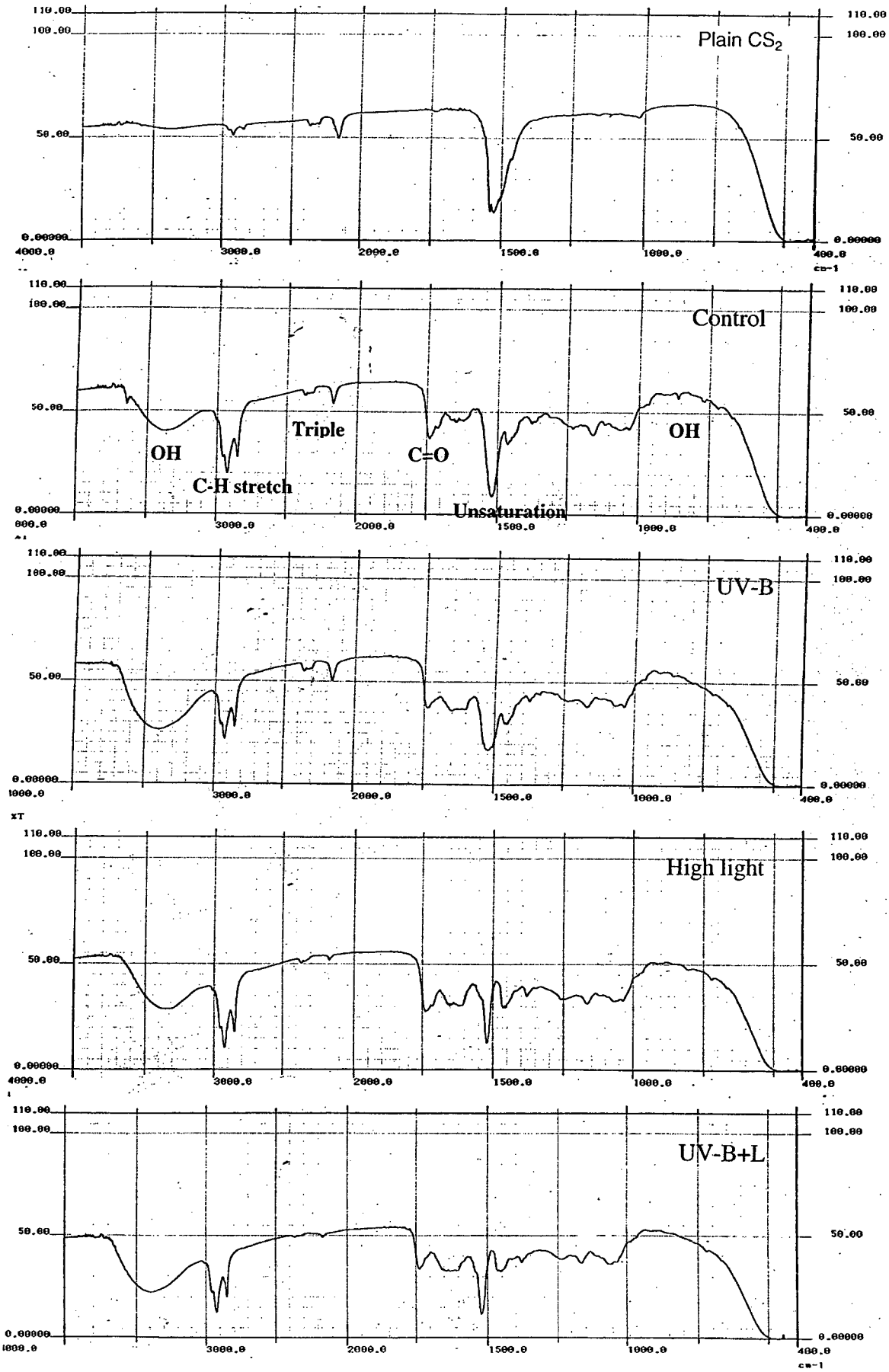

Plain CS_2
Control
OH
C-H stretch
Triple
C=O
Unsaturation
OH
UV-B
High light
UV-B+L
110.00
100.00
50.00
0.00000
4000.0
3000.0
2000.0
1500.0
1000.0
400.0
cm-1
%T

Table 3.7 FTIR profile of lipids indicating qualitative as well as quantitative changes of lipids in *Phormidium corium* due to UV-B, high light and UV-B supplemented with low visual light. + indicates presence of spot and – indicates absence of spot.

Peaks	Components	Control	UV-B	High light	UV-B + light
3425	Hydroxyl group	+++	+++++	++++	+++++
2900	C-H stretch	++++	+++	++++	++++
2825	C-H stretch	++	++	++	++
2365	Triple bond	+++	+++	+	+
1700	Carbonyl group	++++	+++	+++	+++
1625	-	++	+++	++++	+++++
1550	Unstaurated group	+++++	++++	+++	+++
1480	-	++	++	++	++
1350	-	+++	+++	+++	+++
1180	Hydroxyl group	++	++	++	++

Hydroxyl group seen at 770 cm^{-1}in *Nostoc* was not observed in *Phormidium*. Similarly changes in C=O and C-H stretch was observed in *Nostoc* as a result of the treatments but was not seen with *Phormidium*.

3.11. EFFECT ON LIPIDS (NMR):

Nuclear magnetic resonance spectra (NMR) showed peaks at δ0.85, δ1.25, δ1.43, δ1.59, δ2.17 which are indicative of aliphatic fatty acid chain and δ3.5 and δ5.3 which are indicative of unsaturation level in *Nostoc spongiaeforme* (Fig. 3.27, Table 3.8). No qualitative changes were observed in the peak profile except for the peak at δ2.17 which was observed in control *Nostoc* culture and decreased significantly as a result of the UV-B treatment but was not seen under high light or UV-B + low PAR treatment. Slight increase in the peak at δ5.3 which indicate unsaturation was increased due to UV-B, high light and UV-B + low PAR treatment.

NMR spectra of *Phormidium corium* showed peaks at δ0.85, δ1.25, δ1.31, δ1.43, δ2.27, which are indicative of aliphatic fatty acid chain, another peak at δ4.2 indicative of O-CH/N-CH group and δ5.3 which is indicative of unsaturation level (Fig. 3.28; Table 3.9). Greater quantitative change in the aliphatic fatty acid chain was seen as a result of UV-B supplemented with light treatment as compared to control while UV-B and high light caused only slight change. The peak at δ4.2 indicative of O-CH/N-CH group, which was seen in control, disappeared due to the three treatments. Spectral peak at δ5.3 which indicate unsaturation remained more or less same as a result of UV-B and high light treatment while decreased due to UV-B + PAR treatment. Also there was appearance of peaks at δ5.0 (unsaturation) and δ6.9 due to UV-B supplemented with low

Fig. 3.27 NMR spectra of UV-B, high light and UV-B supplemented with low visual light treatment for 6 h on lipids in *Nostoc spongiaeforme*. The spectrum was taken using $CDCl_3$ as solvent.

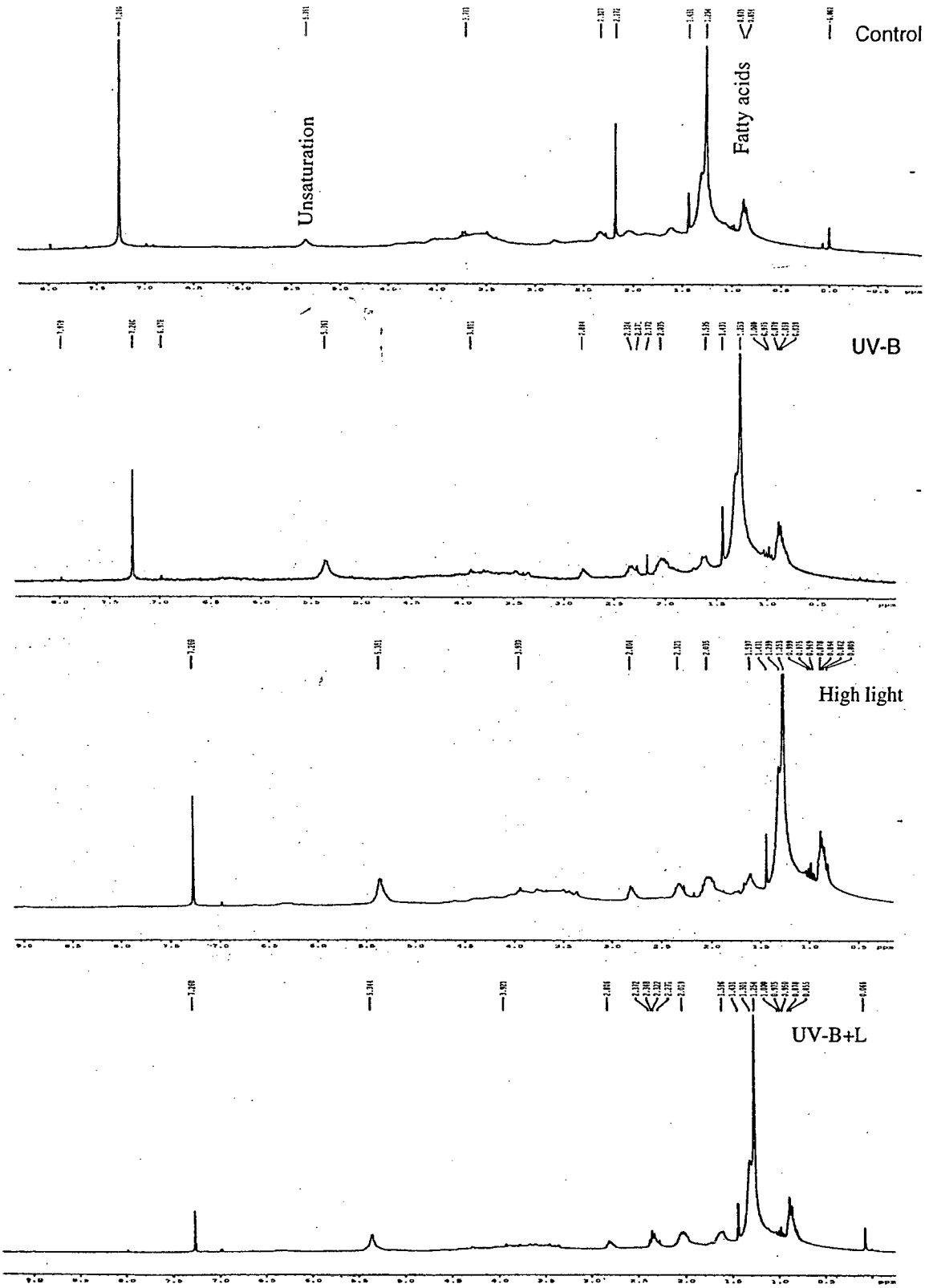

Control
Unsaturation
Fatty acids
UV-B
High light
UV-B+L

Table 3.8 NMR profile of lipids indicating qualitative as well as quantitative changes of lipids in *Nostoc spongiaeforne* due to UV-B, high light and UV-B supplemented with low visual light. + indicates presence of spot and – indicates absence of spot.

Peaks	Components	Control	UV-B	High light	UV-B + light
0.85	Fatty acid chain	++	+++	+++	++
1.25	Fatty acid chain	++++	+++++	+++++	++++
1.43	Fatty acid chain	++	++++	+++	++
1.59	Fatty acid chain	+	++	++	+
2.035	Fatty acid chain	+	+++	++	++
2.17	Fatty acid chain	++++++	+	-	-
2.327	-	++	+++	++	++
3.9	O-CH, N-CH group	+	+	+	-
5.3	Unsaturation	+	+++	++++	+++

Fig. 3.28 NMR spectra of UV-B, high light and UV-B supplemented with low visual light treatment on lipids in *Phormidium corium.* The spectrum was taken using $CDCl_3$ as solvent.

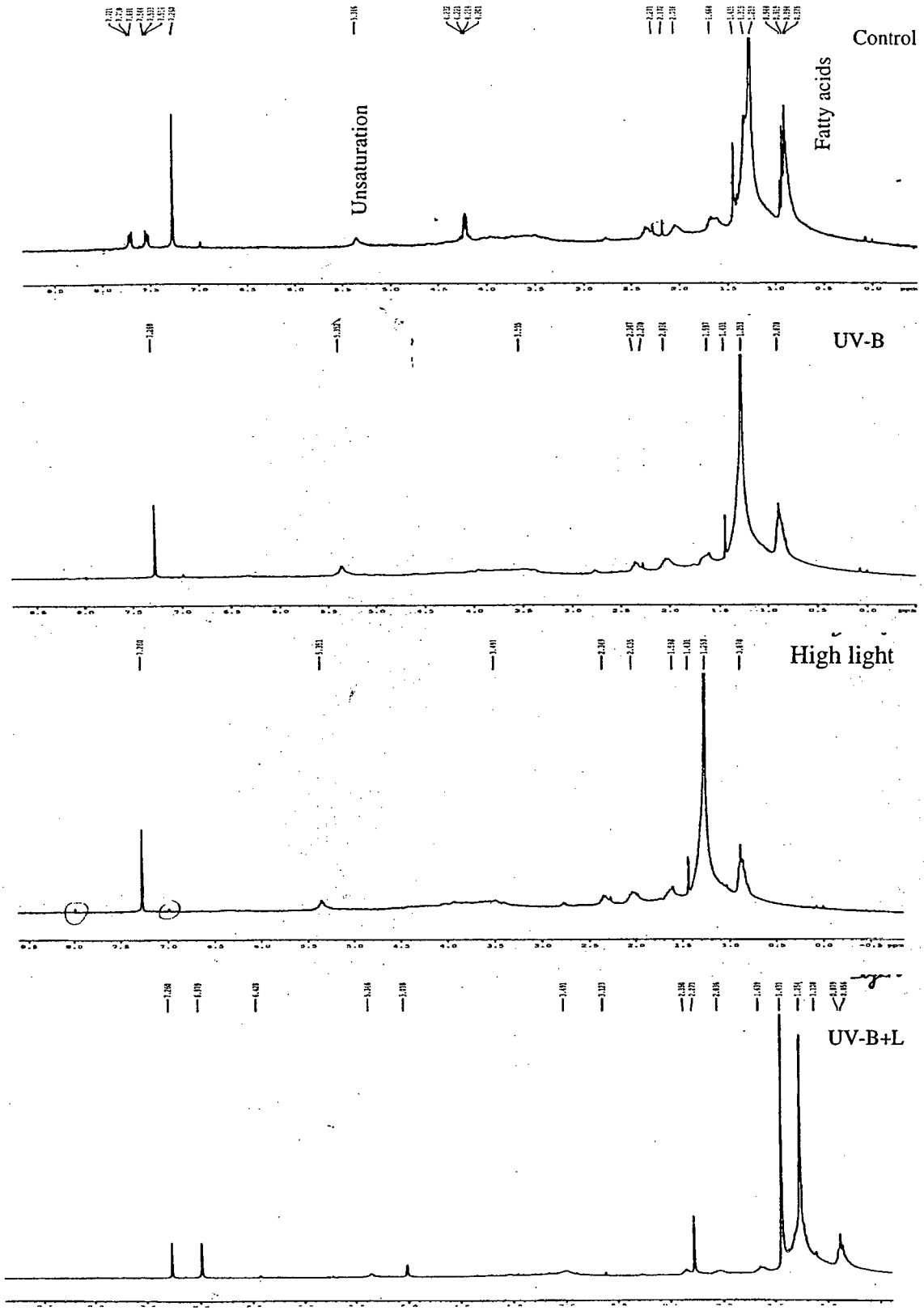

Control
Unsaturation
Fatty acids
UV-B
High light
UV-B+L

Table 3.9 NMR profile of lipids indicating qualitative as well as quantitative changes of lipids in *Phormidium corium* due to UV-B, high light and UV-B supplemented with low visual light. + indicates presence of spot and – indicates absence of spot.

Peaks	Components	Control	UV-B	High light	UV-B + light
0.85	Fatty acid chain	+++++	+++	+++	++
1.25	Fatty acid chain	+++++	+++++	+++++	+++
1.31	Fatty acid chain	++++	-	-	-
1.43	Fatty acid chain	++++	+++	+++	++++++
1.59	Fatty acid chain	-	++	++	-
2.17	-	++	++	-	-
4.2	O-CH, N-CH group	++	-	-	-
5.0	Unsaturation	-	-	-	+
5.3	Unsaturation	+++	+++	+++	-
6.9	-	-	-	-	+++
7.0	Peptides	-	-	+	-
8.03	Peptides	-	-	+	-

light treatment. High light treatment showed appearance of peaks at δ7.0 and δ8.03 in the downfield region of NMR indicating the presence of peptide moieties.

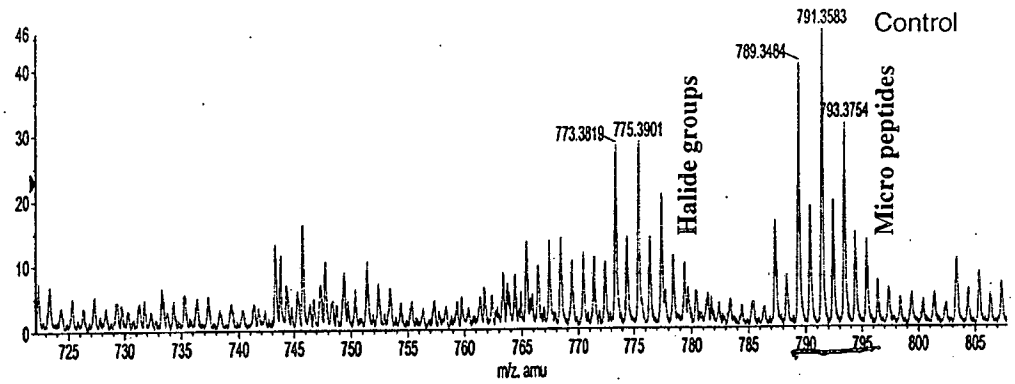
The peak at δ4.2 indicative of O-CH/N-CH group was seen in case of *Phormidium,* which was not seen in *Nostoc.* Also appearance of peaks at δ7.0 and δ8.03, indicating the presence of peptide moieties, was also observed in *Phormidium* as a result of high light treatment but not seen in *Nostoc.*

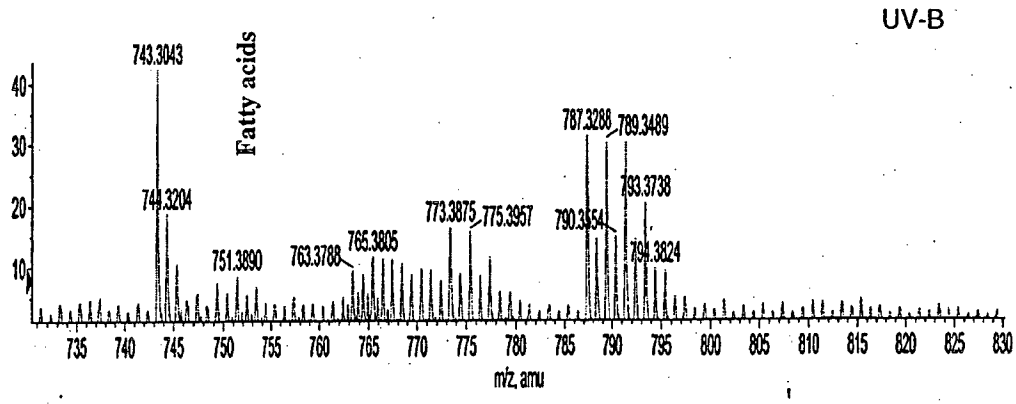
3.12. EFFECT ON LIPIDS (MS):

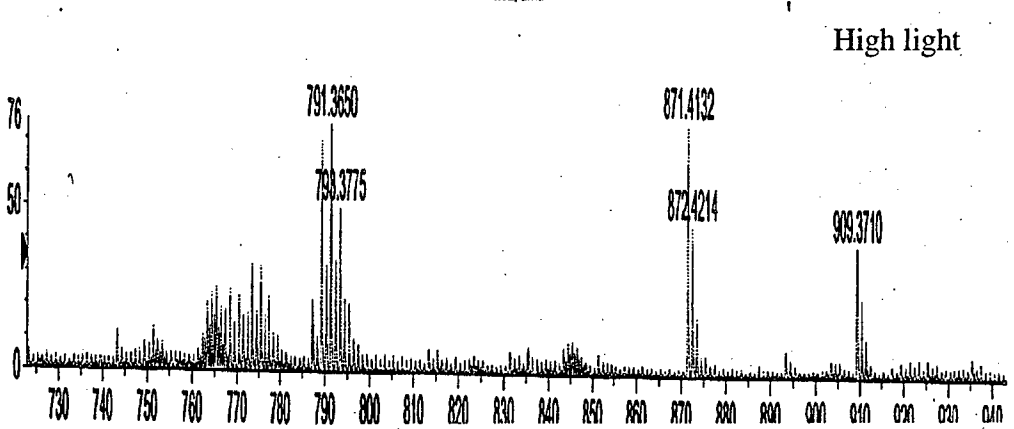
The ESI-MS spectra of *Nostoc spongiaeforme* showed molecular $[M+H]^+$ ions at m/z 773, 775, 789, 791, 793 amu (Fig. 3.29; Table 3.10). Spectra showed the presence of small peptides and halides group in addition of fatty acid molecules. UV-B treatment resulted in observation of additional ions at m/z 743, 744, 751, 763, 765, 787, 790, 794 amu and also loss of ions at m/z 791 amu. High light and UV-B supplemented with low light treatment resulted in observation of other additional ions at m/z 871, 873, 909 amu as well as loss of ions at m/z 773, 775, 789 amu.

The ESI-MS spectra of *Phormidium corium* showed molecular $[M+H]^+$ ions peaks at m/z 743, 744, 747, 749, 750, 751, 765, 766, 771 amu (Fig. 3.30; Table 3.11). UV-B treatment resulted in appearance of additional molecular ions at m/z 787 amu. High light treatment showed more or less same ions but the intensity of ions was less compared to control.

Fig. 3.29 MS spectra of UV-B, high light and UV-B supplemented with low visual light treatment on lipids in *Nostoc spongiaeforme*. The spectrum was taken using 1:1 methanol: water with traces of trifluoroacetic acid (TFA).


Control
791.3583
789.3484
793.3754
773.3819
775.3901
Halide groups
Micro peptides
m/z, amu


UV-B
743.3043
Fatty acids
744.3204
751.3890
763.3788
765.3805
773.3875
775.3957
787.3288
789.3489
790.3554
793.3738
794.3824
m/z, amu


High light
791.3650
798.3775
871.4132
872.4214
909.3710

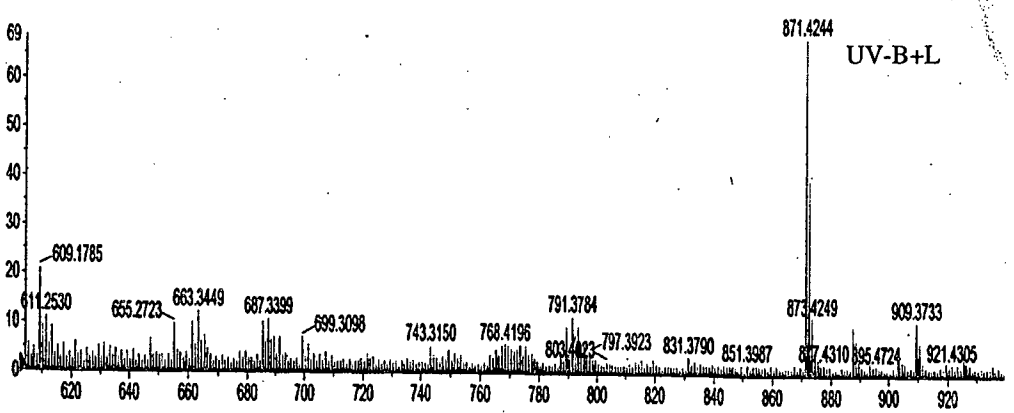
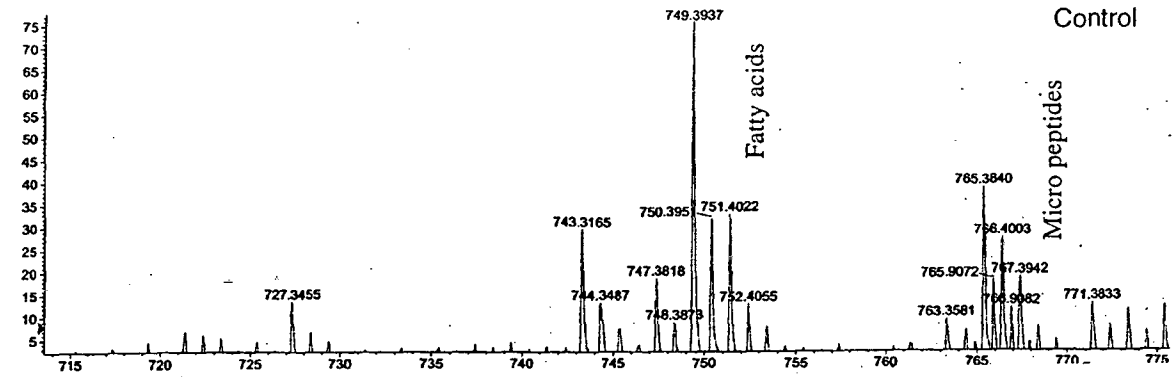
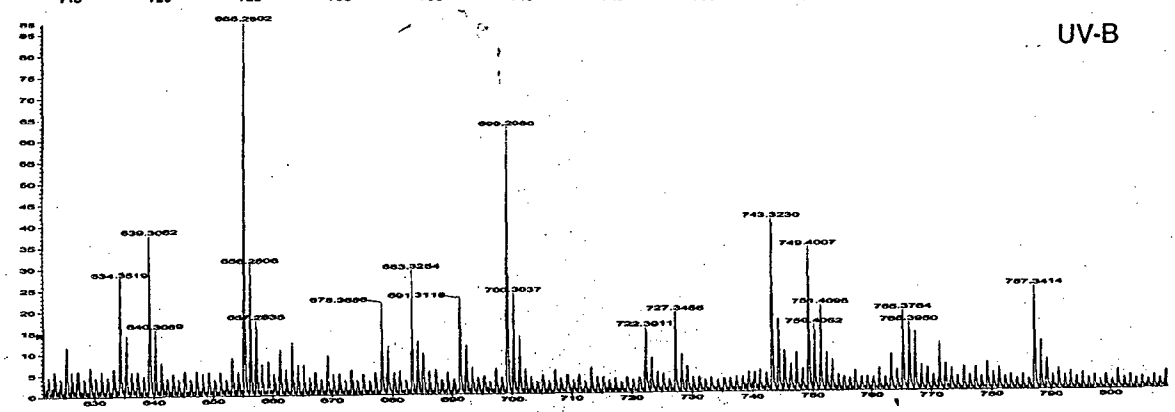

871.4244
UV-B+L
609.1785
611.2530
655.2723
663.3449
687.3399
699.3098
743.3150
768.4196
791.3784
797.3923
831.3790
851.3987
873.4249
895.4724
909.3733
921.4305

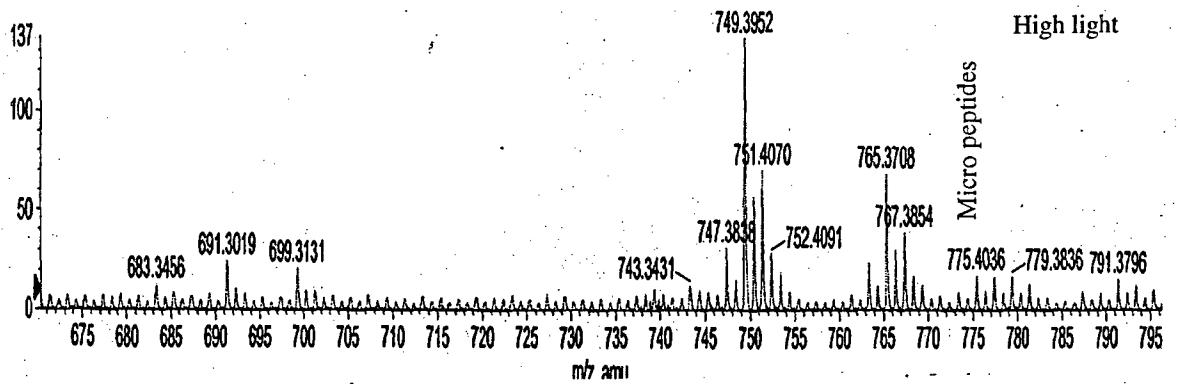
Table 3.10 MS profile of lipids indicating qualitative as well as quantitative changes of lipids in *Nostoc spongiaeforme* due to UV-B, high light and UV-B supplemented with low visual light. + indicates presence of spot and – indicates absence of spot.

Peaks	Components	Control	UV-B	High light	UV-B + light
743	Fatty acid chain	-	+	-	+
744	Fatty acid chain	-	+	-	-
751	Fatty acid chain	-	+	-	-
763	Halides	-	+	-	-
765	Halides	-	+	-	-
773	Peptides	+	+	-	-
775	Peptides	+	+	-	-
777	Peptides	+	+	-	-
781	Peptides	-	-	-	-
787	Peptides	+	+	-	-
789	Peptides	+	+	-	-
791	Peptides	+	-	+	-
790	Peptides	-	+	-	-
793	Peptides	+	+	+	-
795	Peptides	-	+	-	-
871	-	-	-	+	+
873	-	-	-	+	+
909	-	-	-	+	+
921	-	-	-	-	+

Fig. 3.30 MS spectra of UV-B, high light and UV-B supplemented with low visual light treatment on lipids in *Phormidium corium*. The spectrum was taken using 1:1 methanol: water with traces of trifluoroacetic acid (TFA).


Control
Fatty acids
Micro peptides
749.3937
743.3165
750.395
751.4022
747.3818
727.3455
744.3487
748.3873
752.4055
765.3840
766.4003
765.9072
767.3942
763.3581
771.3833


UV-B


High light
Micro peptides
749.3952
751.4070
765.3708
747.3838
767.3854
752.4091
691.3019
699.3131
683.3456
743.3431
775.4036
779.3836
791.3796
m/z amu

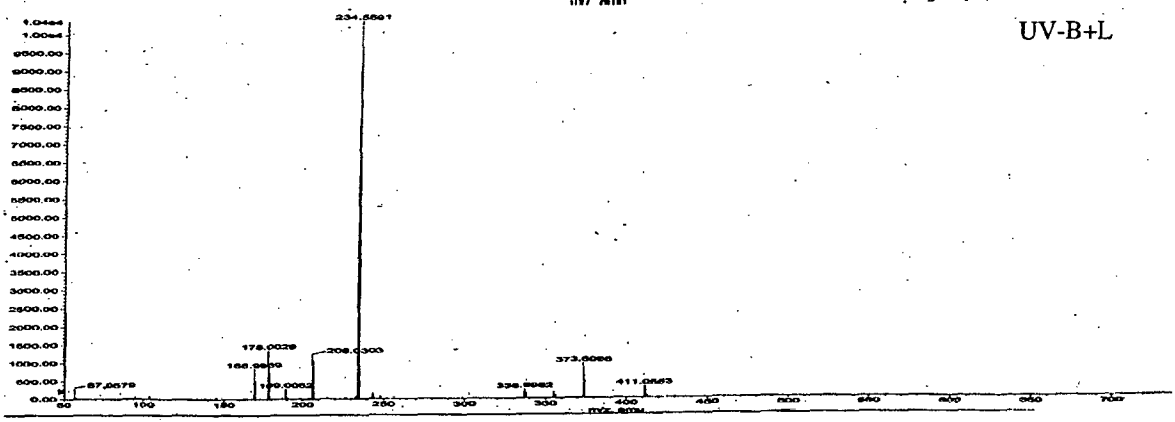

UV-B+L
234.5561
178.0029
208.0300
373.0088
411.0563

Table 3.11 MS profile of lipids indicating qualitative as well as quantitative changes of lipids in *Phormidium corium* due to UV-B, high light and UV-B supplemented with low visual light. + indicates presence of spot and – indicates absence of spot.

Peaks	Components	Control	UV-B	High light	UV-B + light
743	Fatty acid chain	+	+	+	-
744	Fatty acid chain	+	-	-	-
747	Fatty acid chain	+	-	+	-
749	Fatty acid chain	+	+	+	-
750	Fatty acid chain	+	+	-	-
751	Fatty acid chain	+	+	+	-
765	Peptides	+	+	+	-
766	Peptides	+	+	+	-
787	Peptides	-	+	-	-

3.13. EFFECT ON CELL MEMBRANE PROTEINS:

UV-B radiation, high light and UV-B supplemented with low visual light treatment showed changes in the SDS-PAGE profile of cell membrane in both the organisms (Fig. 3.31 a & b). UV-B treatment to *Nostoc* did not cause any significant changes in the protein profile of the cell membrane as compared to control. High light treatment, however, quantitatively increased 26 kDa proteins. UV-B supplemented with low visual radiation resulted in quantitative as well as qualitative changes in the protein profile of *Nostoc*. It was seen that protein of 26 and 28 kDa increased along with de novo induction of protein of low molecular weight in the region of 14-17 kDa and some even below that weight (Fig. 3.31a).

Phormidium also showed changes in the protein profile as a result of the treatment studied. It was seen that UV-B treatment caused slight induction of 28 kDa and significant decrease in the 26 kDa protein as compared to control (Fig. 3.31b). However, high light showed opposite effect i.e. it caused decrease of 28 kDa protein while 26 kDa proteins increased significantly. UV-B supplemented with low visual radiation showed similar changes as seen during the high light treatment (Fig. 3.31b).

3.14. EFFECT ON NUCLEIC ACID:

3.14.1. EFFECT ON DNA:

The effect of UV-B, high light and UV-B supplemented with low visual light treatment on DNA was studied in both species (Fig. 3.32a & b). Degradation of DNA in *Nostoc spongiaeforme* and *Phormidium corium* was observed as a result of UV-B, high light and UV-B supplemented with light treatment, however, *Nostoc* showed greater

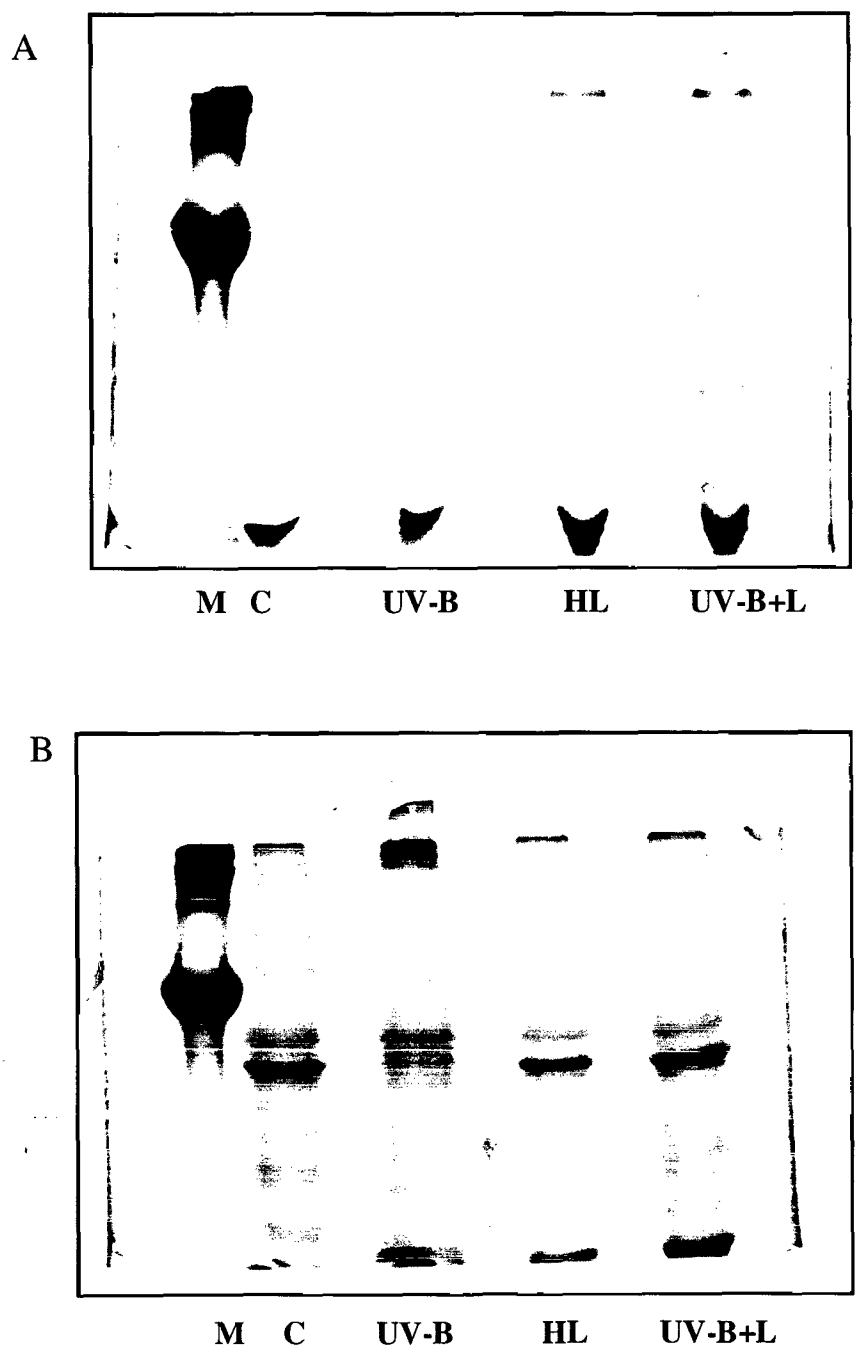


Fig 3.31 SDS-PAGE protein profiles of cell membrane in (A) *Nostoc spongiaeforme* and (B) *Phormidium corium* due to 6 h of UV-B, high light and UV-B supplemented with low visual light. M represents standard protein mixture.

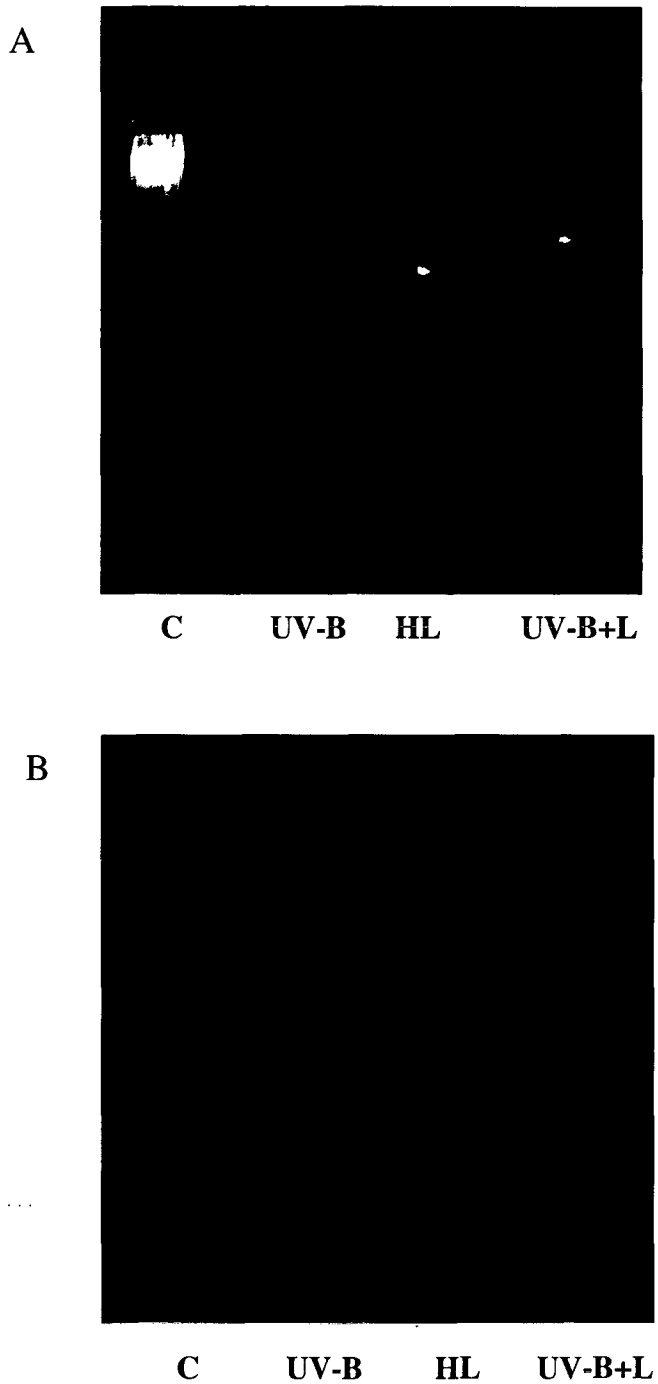


Fig 3. 32 Agarose gel showing DNA due to UV-B, high light and UV-B supplemented with low visual light for 6 h in (A) *Nostoc spongiaeforme* and (B) *Phormidium corium.*

degradation of DNA (Fig. 3.32a) as compared to *Phormidium* (Fig. 3.32b). It was also seen that UV-B was more damaging to DNA than high light, which caused great deal streaking in the DNA. UV-B supplemented with low visual light also showed relatively higher damage than seen in high light treated culture (Fig. 3.32).

3.14.2. EFFECT ON RNA:

Three RNA bands, two rRNA and sheared mRNA, were seen in control sample of *Nostoc spongiaeforme* and *Phormidium corium* (Fig. 3.33 a & b). UV-B, high light and UV-B supplemented with low visual light showed denaturation of RNA bands.

3.15. EFFECT ON UV-B ABSORBING COMPOUNDS:

3.15.1. EFFECT ON MYCOSPORINE LIKE AMINO ACIDS (MAAs):

HPLC analysis of 20% methanol extract showed mycosporine like amino acids (MAAs) in *Nostoc spongiaeforme* and *Phormidium corium.* The HPLC analysis of *Nostoc* showed two peaks having lambda max at 224 and 323 nm whereas *Phormidium* showed a single peak with lambda max at 224 nm (Fig. 3.34; 3.35).

Exposure of *Nostoc* to UV-B radiation for 6h increased the MAAs with λ max at 324 nm by 108% as compared to control while the peak 2 with λ max at 224 nm disappeared as result of the treatment. UV-B in combination with low level PAR increased the MAA content by 376% as compared to control. High light treatment for the

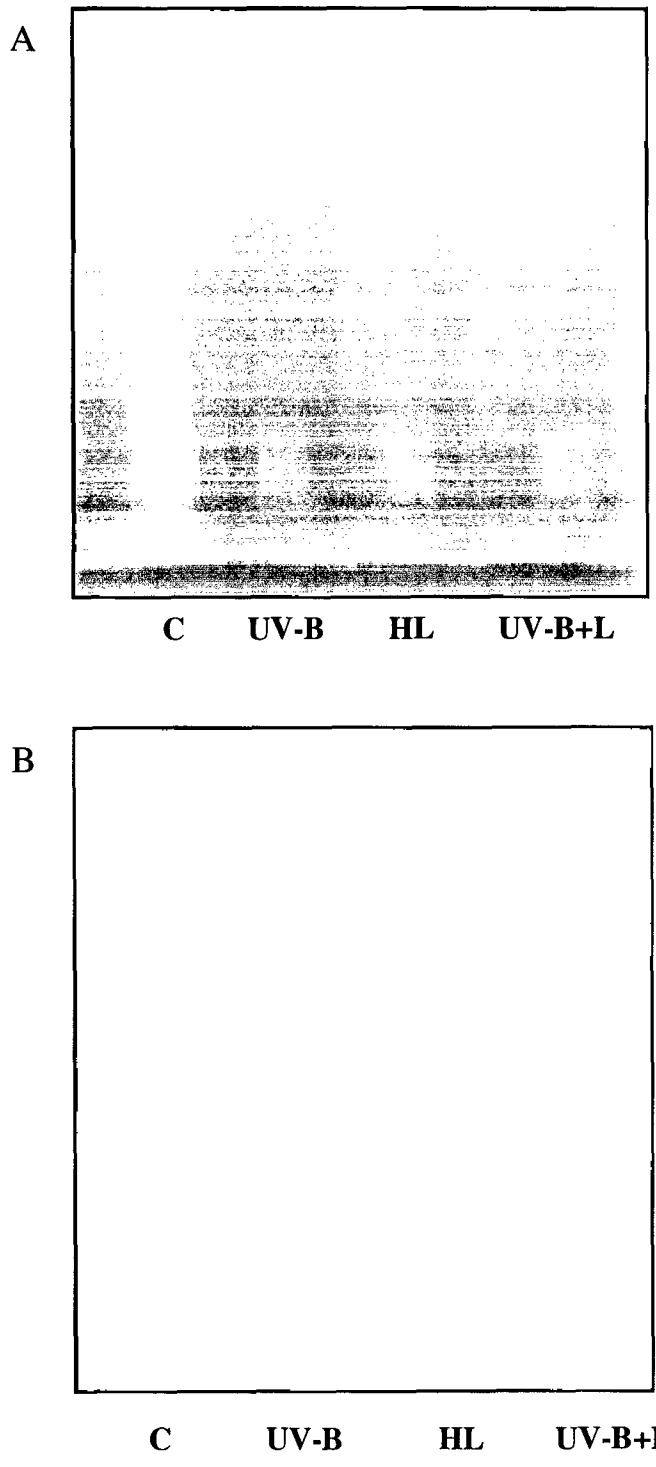


Fig 3. 33 Agarose gel showing RNA due to UV-B, high light and UV-B supplemented with low visual light for 6 h in (A) *Nostoc spongiaeforme* and (B) *Phormidium corium*.

Fig. 3.34 HPLC profile of mycosporine like amino acids (MAAs) extracted from *Nostoc spongiaeforme* after exposure to UV-B, high light and UV-B supplemented with low visual light treatment for 6 h.

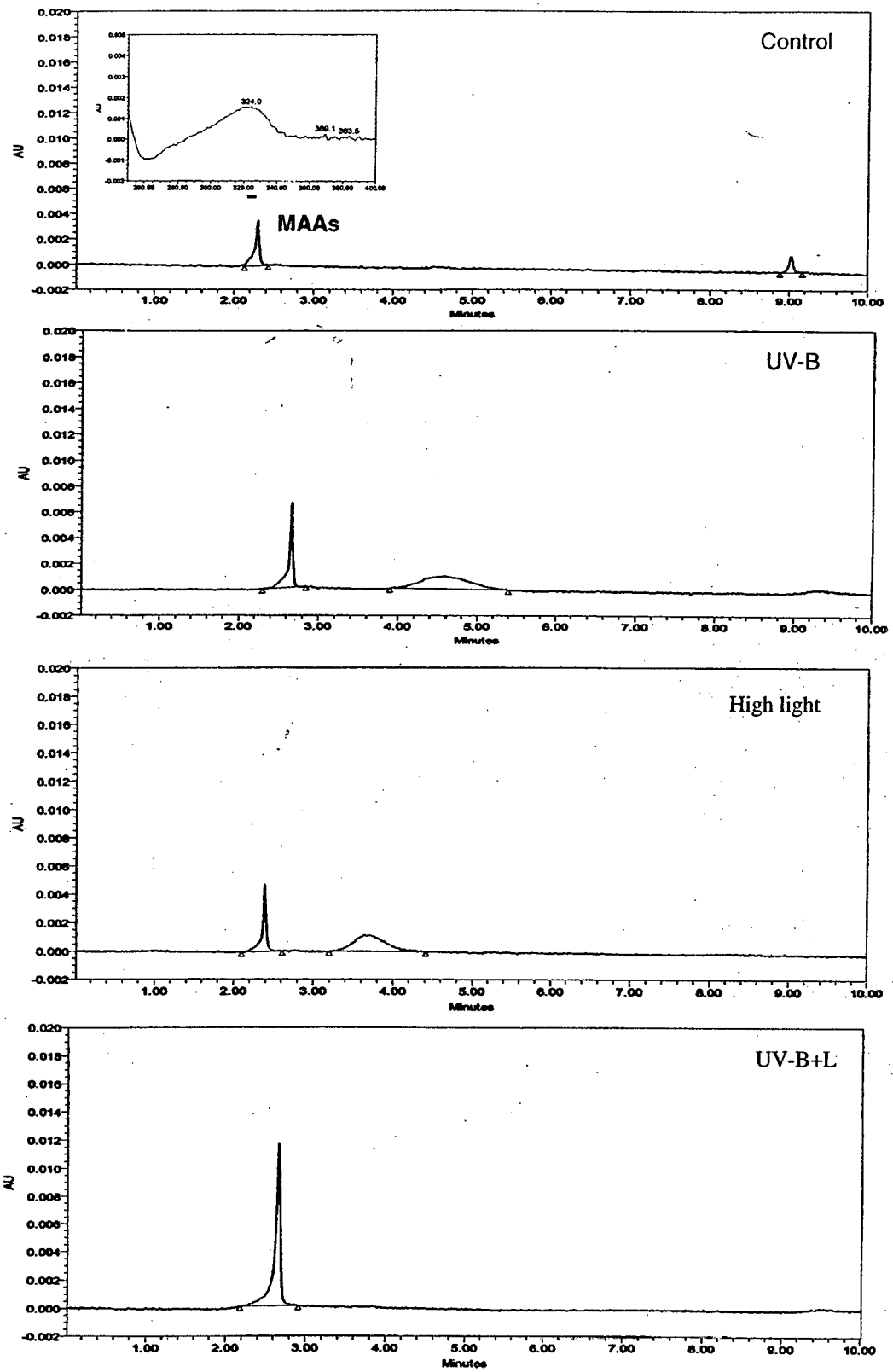
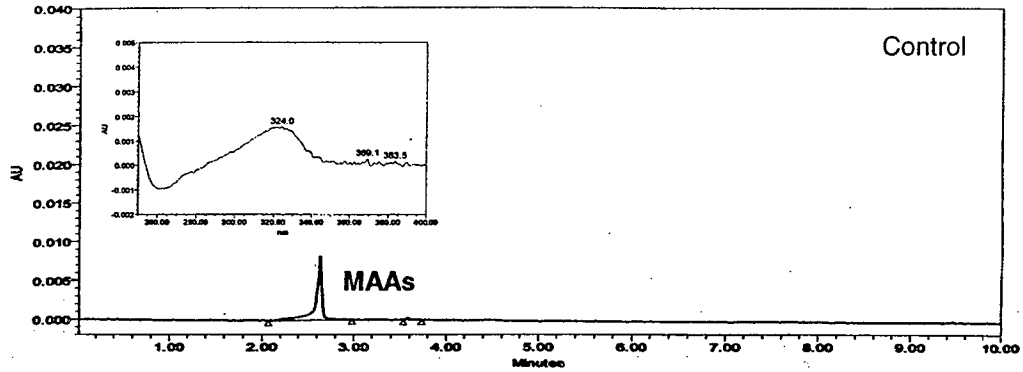
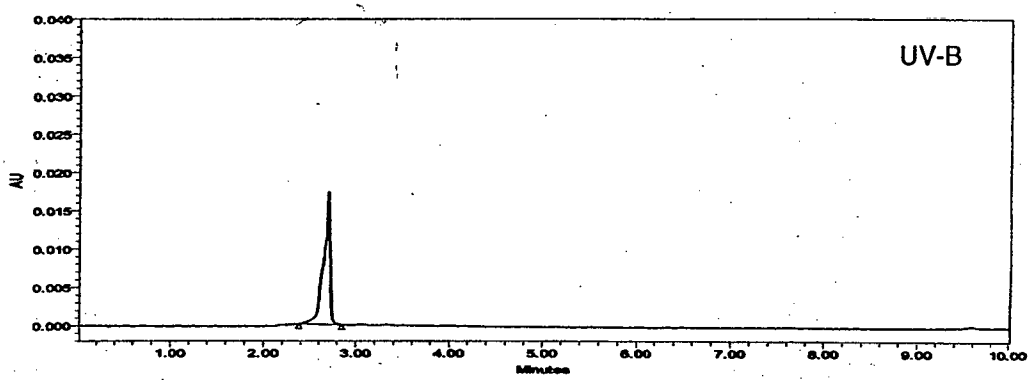
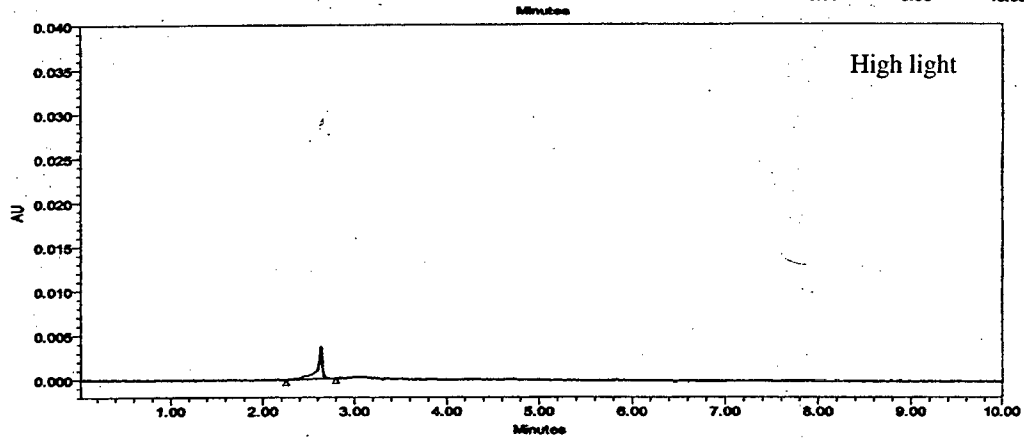
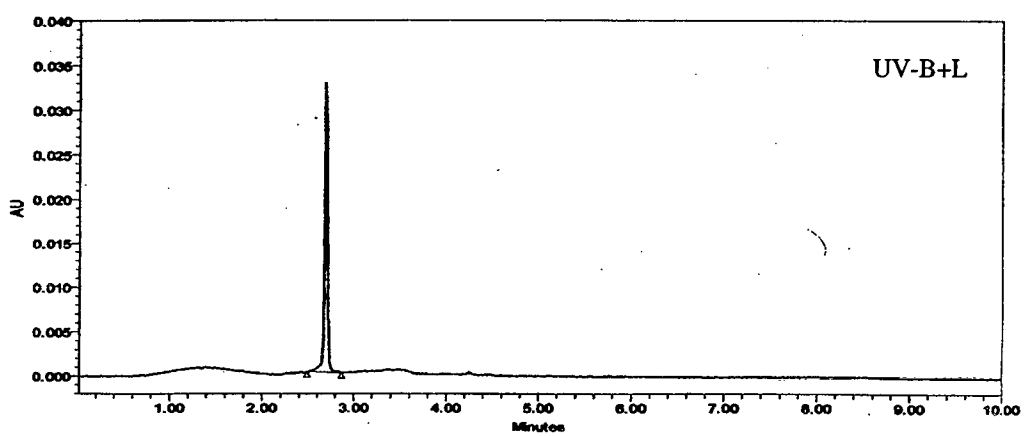

Control
MAAs
324.0
UV-B
High light
UV-B+L
AU
Minutes

Fig. 3.35 HPLC profile of mycosporine like amino acids (MAAs) extracted from *Phormidium corium* after exposure to UV-B, high light and UV-B supplemented with low visual light treatment for 6 h.


Control
AU
MAAs
Minutes


UV-B
AU
Minutes


High light
AU
Minutes


UV-B+L
AU
Minutes

same duration, however, resulted in a decrease of 40% in the MAAs as compared to control in *Nostoc spongiaeforme* (Fig. 3.34).

Similar results were obtained with *Phormidium* as a result of the treatments studied (Fig. 3.35). A 6 h UV-B radiation to *Phormidium* resulted in an increase of 80% in the amount of MAAs than seen in control. In *Phormidium corium,* UV-B treatment in combination with low visual radiation showed even greater induction (230%) of MAAs. Increase in the MAAs as a result of UV-B + low PAR treatment was considerably higher in *Nostoc spongiaeforme* (376%) than seen in *Phormidium corium* (230%). High light treatment for 6 h to *Phormidium* also resulted in a decrease of 40 % in MAAs content (Fig. 3.35).

3.15.2. EFFECT ON PHENOLIC COMPOUNDS:

HPLC chromatogram for phenolic compounds of *Nostoc spongiaeforme* and *Phormidium corium* showed qualitative as well as quantitative increase in peaks after exposure to 6h of UV-B radiation (Fig. 3.36, 3.37, 3.38). Identification of these peaks was based on spectral profile. The spectra of all these peaks showed approximately two to three peaks in the UV region. All the components showed a peak at 210 nm, between 250-260 nm and a slight peak at 330 nm region indicating UV absorbing compounds.

There were 4 peaks observed in *Nostoc* with retention time of and 4.221, 4.4487, 5.721 and 7.177 in control, which were increased due to 6h of UV-B treatment (Fig. 3.37). Also two additional peaks were observed as a result of the UV-B treatment at retention time of 5.616 and 19.486 in *Nostoc.* In *Phormidium,* the numbers of peaks

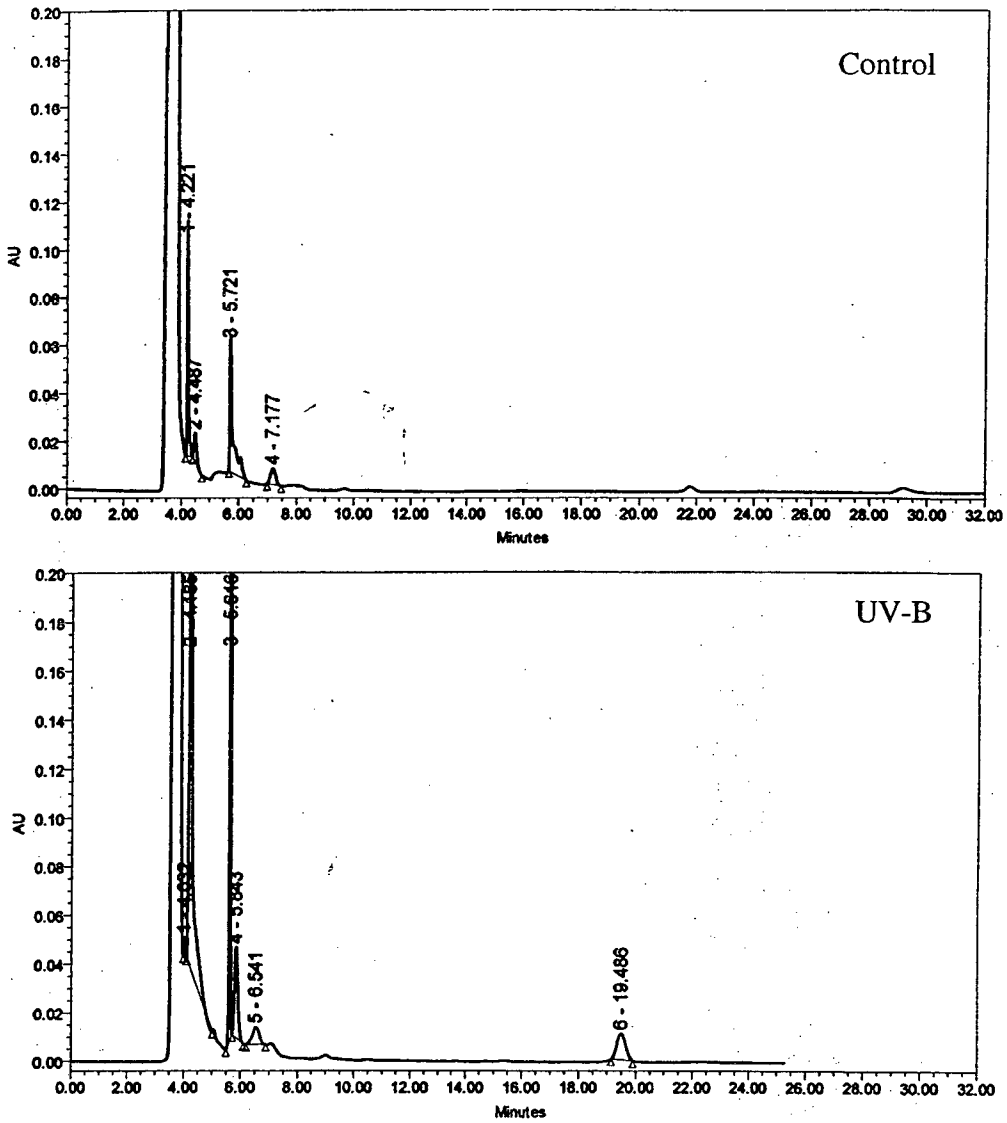


Fig 3.37: HPLC profile of phenolic compounds extracted from *Nostoc spongiaeforme* after exposure to UV-B, high light and UV-B supplemented with low visual light treatment for 6 h.

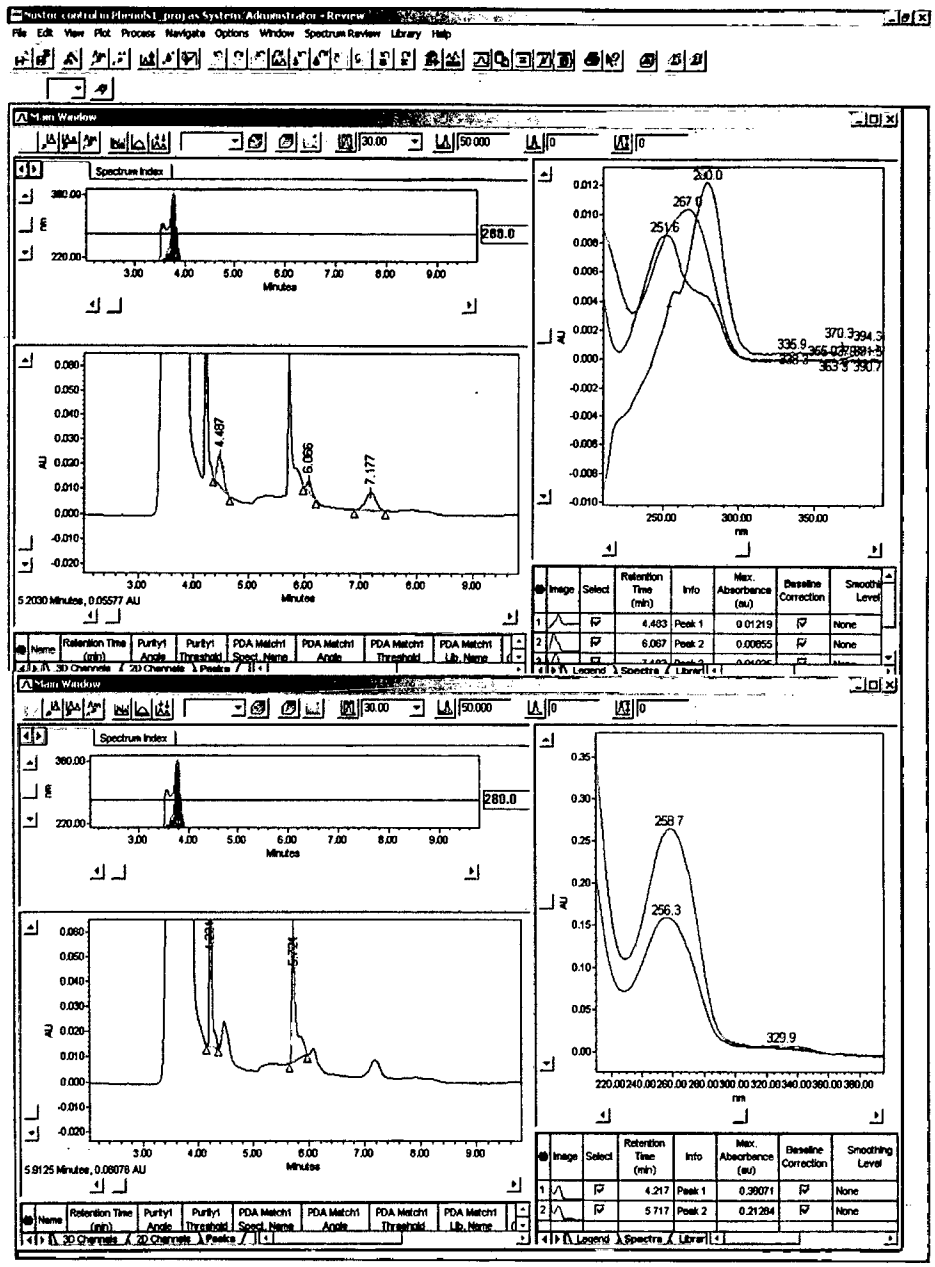


Fig 3.36 HPLC profile of phenolic compounds taken at 280 nm and absorption spectra of peaks with retention time of 4.217, 4. 448, 5.721, 7.177, 6.067 min.

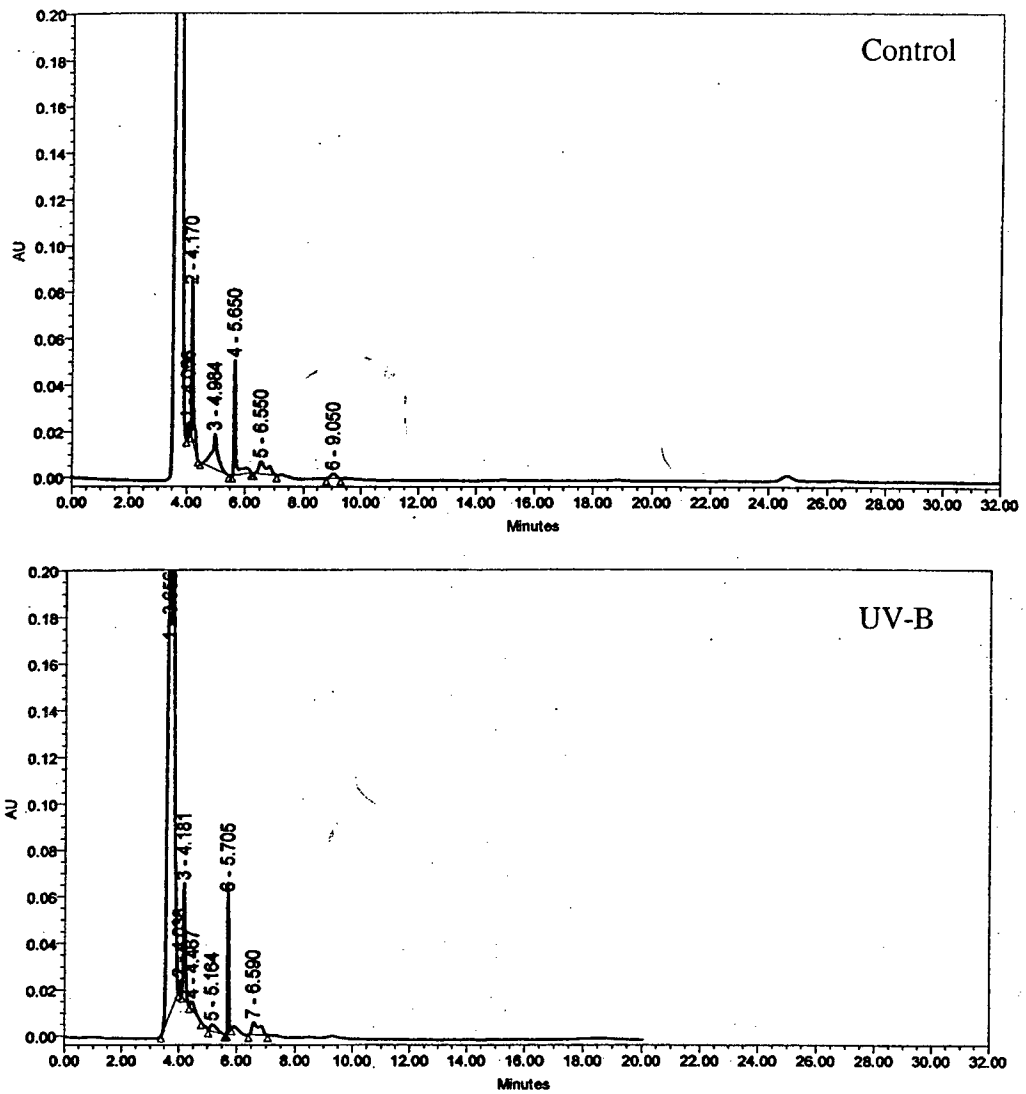


Fig 3.38: HPLC profile of phenolic compounds extracted from *Phormidium corium* after exposure to UV-B, high light and UV-B supplemented with low visual light treatment for 6 h.

observed were 6 with retention time at 4.056, 4.170, 4.984, 5.650, 6.550, 9.050, which mainly resulted quantitatively due to the UV-B treatment (Fig. 3.38).

Spectrophotometric analysis for phenolic compounds of *Nostoc spongiaeforme* and *Phormidium corium* showed quantitative increase in peaks after exposure to 6h of UV-B and UV-B supplemented with low visual light (Fig. 3.39, 3.40; Table 3.12). It was observed that absorption scan of *Nostoc spongiaeforme* showed 3 peaks each in the UV and visual region. Peaks in the UV region were at 210, 318 and 325 nm. The peaks observed in the visual region were 380 nm, 408 nm and 655 nm. Peaks at 210, 332 and 318 nm increased as a result of UV-B treatment. A 6h of UV-B treatment resulted in over all decrease in the compounds absorbing in the visual region. Also UV-B treatment resulted in the formation of additional peak at 280 nm, which was not seen in control. High light treatment resulted in decrease in the compounds absorbing in the UV region as well as visual region. UV-B supplemented with low visual light showed 5 distinct peaks, which resulted in slight increase in peaks at 210 nm, 348 nm and 408 nm in *Nostoc* (Fig. 3.39; Table 3.12a).

In *Phormidium corium,* the absorption scan showed 5 distinct peaks out of which 3 were in UV-B region and 2 peaks seen in visual region. There was increase in peaks at 210 nm, 353 nm and 651 nm due to 6h of UV-B treatment. High light treatment showed in 4 distinct peaks at 210 nm, 353 nm and 651 nm, which remained unchanged. UV-B supplemented with low visual light treatment showed 5 peaks at 210 nm, 353 nm, 603 nm and 651 nm. UV-B treatment resulted in slight increase in peak at 210 nm, 353 nm and 651 nm (Fig. 3.40; Table 3.12b).

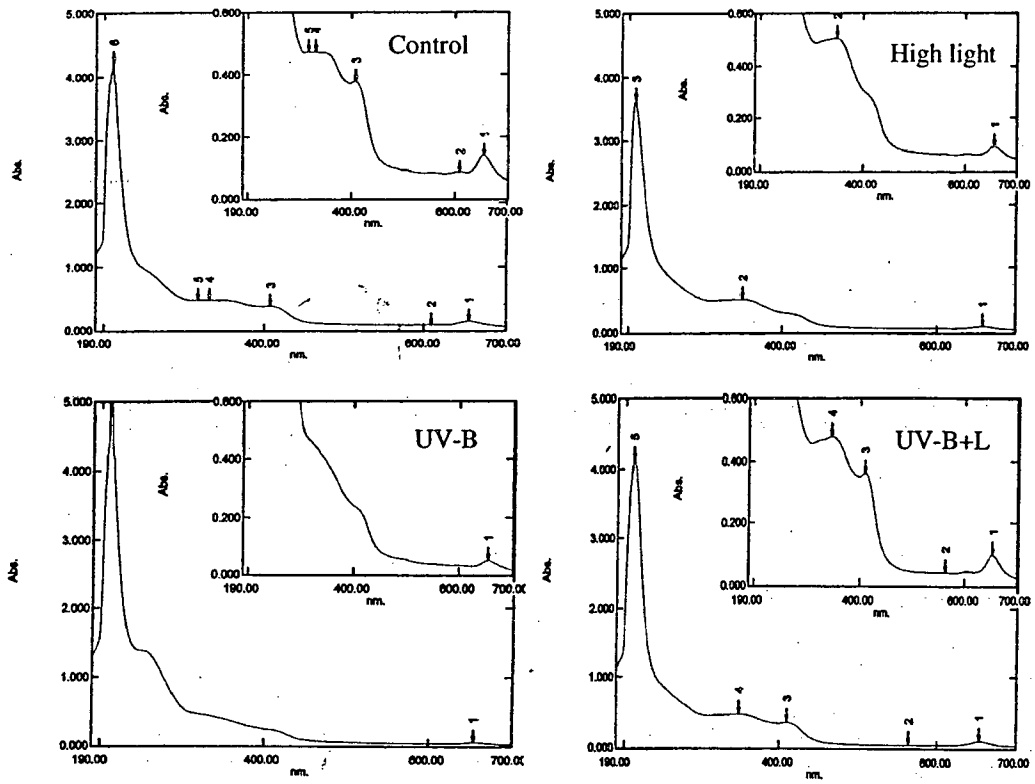


Fig 3.39: Spectral scan of phenolic compounds extracted from *Nostoc spongiaeforme* treated with UV-B, high light and UV-B supplemented with low visual light treatment for 6 h. Inset shows the spectral profile from 400 - 700 nm region in different absorbance range to highlight the changes in phenolic compounds in visual range.

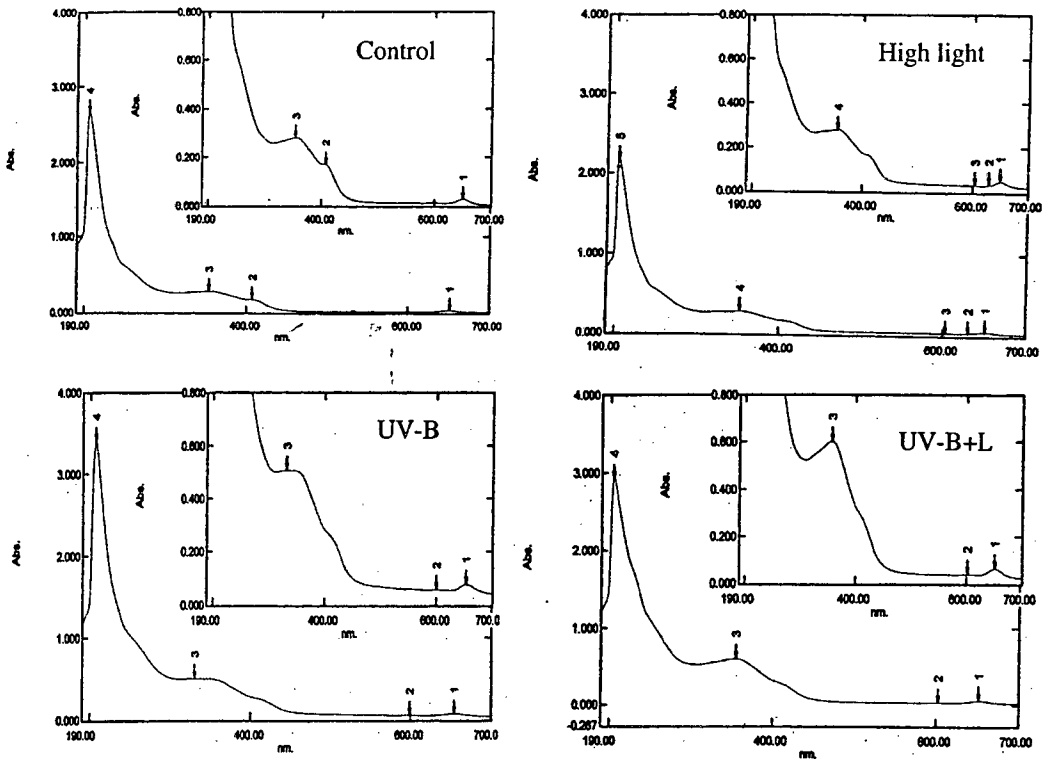


Fig 3.40: Spectral scan of phenolic compounds extracted from *Phormidium corium* treated with UV-B, high light and UV-B supplemented with low visual light treatment for 6 h. Inset shows the spectral profile from 400 -700 nm region in different absorbance range to highlight the changes in phenolic compounds in visual range.

Table No. 3.12 Spectral analysis indicating qualitative as well as quantitative changes of phenolic compounds in (A) *Nostoc spongiaeforne* and (B) *Phormidium corium* due to UV-B, high light and UV-B supplemented with low visual light. + indicates presence of spot and – indicates absence of spot.

A

Peaks (nm)	Control	UV-B	High light	UV-B + light	Components
651	+	++	++	+++	Anthocyanin
631	+	-	+	++	?
602	++	+++	+++	+++	?
353	++	-	+	++	?
331	+	+	++	++	Flavonol
205	++	++++	+	++	Caumarin

B

Peaks (nm)	Control	UV-B	High light	UV-B + light	Components
655	+	++	++	+++	Anthocyanin
609	+	-	+	++	?
563	++	+++	+++	+++	?
408	++	-	+	++	?
348	+	+	++	++	?
332	++	++++	+	++	Flavonol
318	+	+++	++	+++	?
210	+	++++	++	++++	Caumarin

3.16. EFFECT ON ANTIOXIDANT ENZYMES:

3.16.1. EFFECT ON SUPEROXIDE DISMUTASE:

An increase in the activity of superoxide dismutase was observed as a result of UV-B, high light and UV-B supplemented with low visual light treatment in both *Nostoc spongiaeforme* and *Phormidium corium* (Fig. 3.41 a & b). UV-B treatment to *Nostoc spongiaeforme* for 1 h and 3 h resulted in 22% and 30% increase in the SOD activity as compared to control, which was declined to 19% after 6 h of the same treatment (Fig. 3.41a). In *Nostoc spongiaeforme,* high light treatment on the other hand resulted in continuous increase in the SOD activity over a period of 6 h, however, actual activity was relatively less than observed under UV-B treatment. A one h of the high light treatment resulted in an increase of 19% in the SOD activity as compared to control followed by an increase of and 26% after 3 h and 28% after 6 h of the treatment. UV-B supplemented with visual radiation also resulted more or less same pattern of increase in the SOD activity in *Nostoc* as seen under the UV-B treatment but the level of increase was comparatively less than observed for the UV-B alone treatment (Fig. 3.41a).

In *Phormidium corium,* activity of superoxide dismutase was increased slightly but continuously by 16%, 17% and 21% as a result of the UV-B treatment for 1, 3 and 6 h respectively than seen in the control (Fig. 3.41b). High light treatment also resulted in a continuous increase in the SOD activity in *Phormidium.* Six hour of high light treatment resulted in 50% increase in the activity of SOD as compared to their control in *Phormidium corium* (Fig. 3.41b). SOD activity was relatively higher in *Phormidium* than seen in *Nostoc.* UV-B supplemented with visual radiation showed very little increase in the activity of the SOD in *Phormidium.* A six hour UV-B+PAR treatment resulted in 3 %

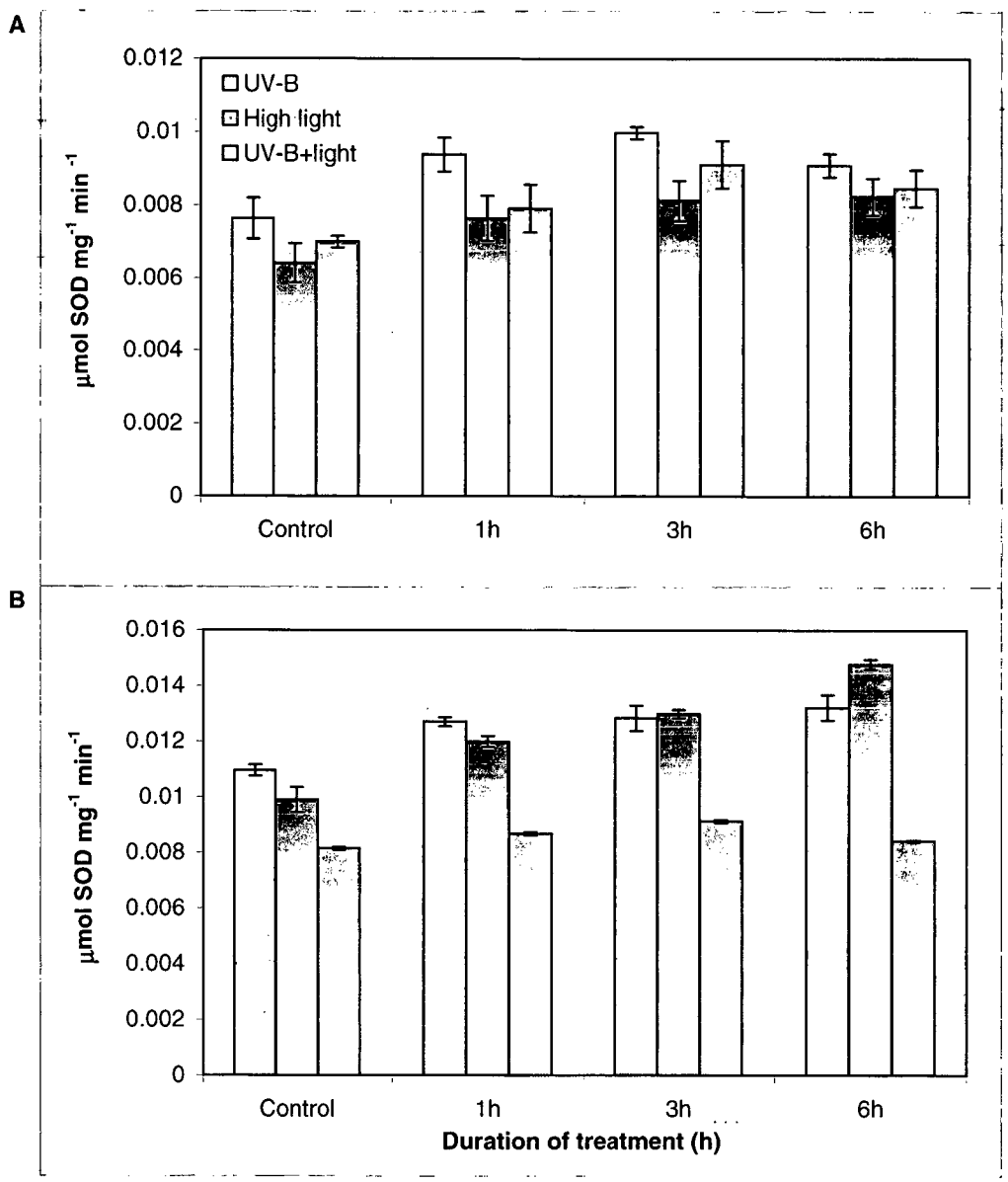


Fig 3.41: Effect of UV-B, high light and UV-B supplemented with low visual light treatment for 6 h duration on superoxide dismutase (SOD) activity in (A) *Nostoc spongiaeforme* and (B) *Phormidium corium.* Each bar represents the mean ±S.D. n=5.

increase in the activity as compared to 21% observed under UV-B alone and 50% seen under high light treatment. Results show that UV-B treatment showed more increase in SOD activity in *Nostoc* while high light treatment resulted in greater activity of SOD in *Phormidium* (Fig. 3.41b)..

3.16.2. EFFECT ON ASCORBATE PEROXIDASE:

An increase in the activity of ascorbate peroxidase was observed as a result of UV-B, high light and UV-B supplemented with low visual light treatment in both *Nostoc spongiaeforme* and *Phormidium corium* (Fig. 3.42a & b). *Nostoc* showed a linear increase in the APX activity as a result of UV-B alone and UV-B + PAR treatment while high light treatment showed an initial increase in the APX up to 3 h of the treatment which declined on further exposure to high light. UV-B treatment of 1 and 3 h resulted in an increase of 9% and 32% in the APX activity in *Nostoc spongiaeforme,* which further increased to 46% as a result of 6 h of the treatment (Fig. 3.42a). High light treatment resulted in an increase of 47% after 3 h of the treatment in *Nostoc spongiaeforme,* however, further increase in the duration of the treatment decreased the APX activity to only 28% as compared to control. UV-B supplemented with low visual light increase the APX activity linearly to 85% as a result of 6 h of the treatment (Fig. 3.42a).

Similarly in case of *Phormidium corium,* APX activity increased to 22%, and 58% due to 1 and 3h of UV-B treatment and further increase in duration of treatment for 6h resulted in further increase of 70% of APX activity as compared to control (Fig. 3.42b). Similarly high light treatment also resulted in a linear increase in the APX activity. High light treatment up to 6 h resulted in an increase in the activity of ascorbate peroxidase by 87% in *Phormidium corium* (Fig. 3.42b). Likewise UV-B + low PAR also

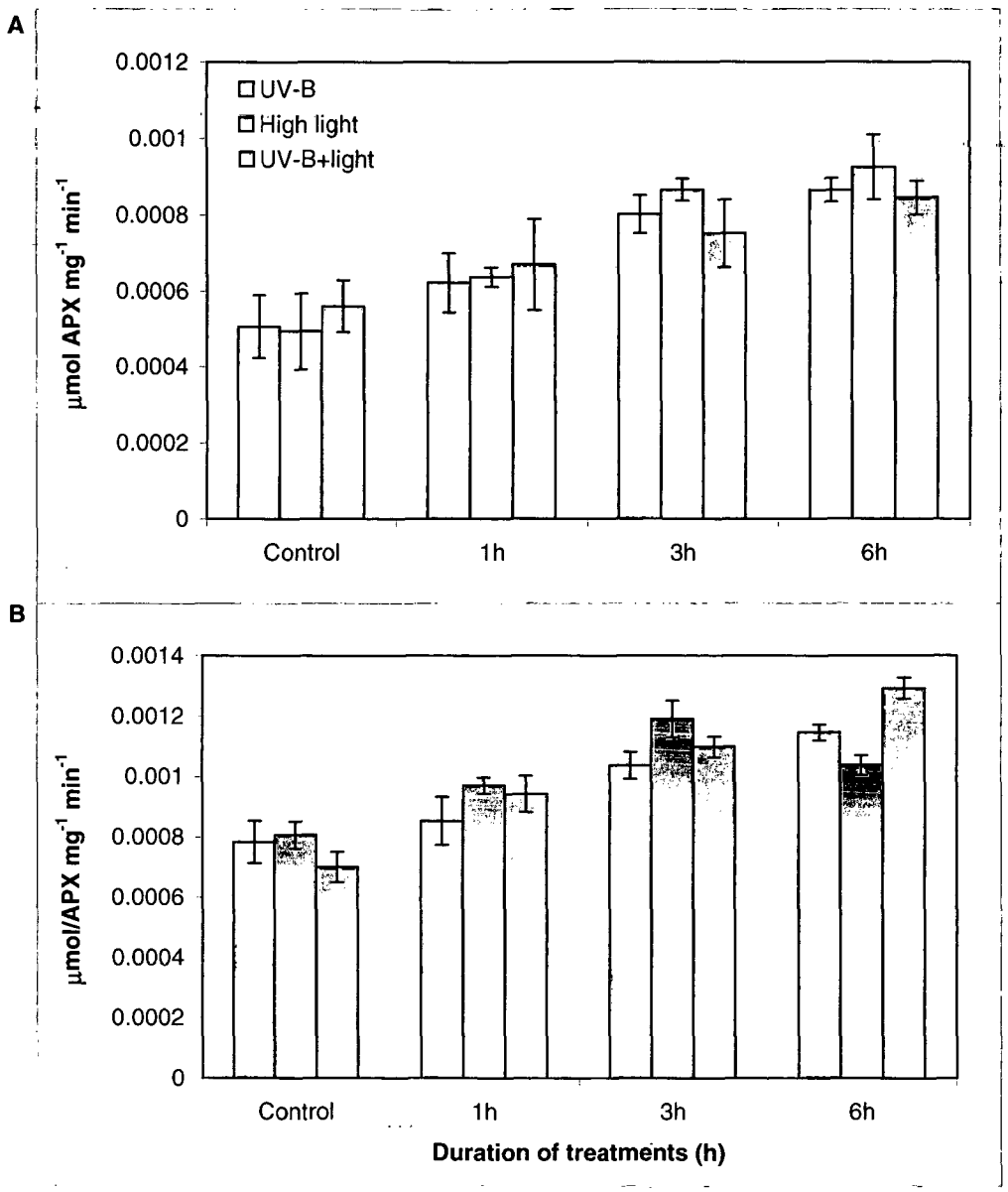


Fig 3.42: Effect of UV-B, high light and UV-B supplemented with low visual light treatment for 6 h duration on ascorbate peroxidase (APX) activity in (A) *Nostoc spongiaeforme* and (B) *Phormidium corium* . Each bar represents the mean ±S.D. n=5.

resulted in a linear increase in the APX activity. Exposure to 6h of UV-B supplemented with low visual light resulted in increase of 50% in the APX activity in *Phormidium.*

Though difference in the extent of increase in APX activity as a result of UV-B, high light or UV-B + low PAR was only slight in both *Nostoc* and *Phormidium*, the actual level of APX activity in the control culture was significantly less in *Phormidium* than observed in *Nostoc* (Fig. 3.42).

CHAPTER – 4

DISCUSSION

DISCUSSION – CONTENT

DISCUSSION

To determine the appropriate growth phase, we used four different physiological parameters such as fresh weight, dry weight, chlorophyll estimation and chlorophyll fluorescence and depending upon these parameters, we observed that in *Nostoc spongiaeforme,* 14th day and in *Phormidium corium,* 33rd day is logarithmic phase which is defined as a stage where most of biochemical components show stability. Balanced growth phase (logarithmic phase) i.e. 14th day for *Nostoc spongiaeforme* and 33rd for *Phormidium corium* were used for setting all the experiments conducted in this work as well as subculture using fresh culture medium by batch culture (Fig. 3.1-3.4). We have used logarithmic phase for our experimental work because during this period the growth rate remains constant and biomass concentration changes very little, also the concentration and ratio of the different biochemical components remains constant.

4.1. MORPHOLOGY:

Results indicate physical damage to both the cyanobacteria as UV-B, high PAR and UV-B radiation supplemented with low light resulted in bleaching and breakage of the filaments of both species of cyanobacteria (Fig. 3.5 & 3.6). High light intensity have resulted in photobleaching of pigments and thus affected the growth of alga. The bleaching of the cyanobacteria may be a result of generation of active oxygen species (AOS) as seen in this study (Fig. 3.18). The effects of UV-B and high light on morphological changes correlate with reductions in the photosynthetic efficiency of both the cyanobacteria studied.

UV-B radiation is known to exert negative influence on a variety of aspects in algae (Häder and Figueroa, 1997; Häder, 2001). UV-B radiation affects different biological processes such as photosynthesis, nitrogen metabolism, growth rate, motility and orientation of phytoplankton (Häder and Häder, 1991). Quesada and Vincent, (1997) have shown that impairment of various metabolic processes and subsequent killing of cyanobacteria at higher UV-B doses was believed to be due to irreversible damage to membrane components, photosynthetic pigments, DNA, nitrogen transport systems, the reaction center of PS II and Rubisco. Sinha et al., (2000) have suggested that the cellular constituents absorbing radiation between 280-320 nm destroyed by UV-B radiation, which may further affect the cellular membrane permeability and protein damage resulting in the death of the cell. Karentz et al., (1991) also reported that cell survival strongly depends on the efficiency of photo reactivation or excision repair and thus loss in survival of these species may have been due to irreversible damage to the DNA by UV-B radiation.

Malanga and Puntarulo, (1995) reported that UV-B can affect algal development and induce significant alterations in algal morphology as UV-B radiation causes damage to DNA, which is potentially dangerous to cells. Salih et al., (1998) observed that elevated temperature caused significant morphological damage to the chloroplasts in the symbiotic dinoflagellates with severity of degradation increasing with the degree of irradiance (dark < low light < high light treatments). They also reported numbers of lipid globules within degraded chloroplasts with the concomitant massive reduction and vacuolation of chloroplasts and suggested role of oxygen species in chloroplast membranes. Studies on symbiotic dinoflagellates from naturally bleached or bleaching

corals had disrupted cellular components, and showed increased vacuolation, shrinkage of chloroplasts and bloating of cells (Hayes and Bush, 1990; Szmant and Gassman, 1990; Brown et al., 1995; Glynn et al., 1985).

4.2. PHOTOSYNTHESIS:

Fv/Fm ratio, which is an indicator of photosynthetic efficiency, decreased due to UV-B, high light and UV-B supplemented with low visual light (Fig. 3.7). Such a decrease in the Fv/Fm ratio was due to decrease in the Fo, indicative of decrease in the excitation energy reaching the photosynthetic reaction centre II probably due to loss of pigments in the light harvesting complex II, as well as Fm indicating damage to the PS II reaction centre itself under both UV-B and PAR treatment.

Mechanism of damage to photosynthesis under UV-B and high light is reported to be more or less same (Nishiyama et al., 2001 and Campbell et al., 1998). A decrease in photosynthetic activity during UV-B or high light exposure may be a result of direct damage to key components within the D1 protein of photosystem II (Vass, 1997), loss of photosynthetic pigments (Dohler et al., 1995), as well as due to a reduced expression of genes involved in photosynthesis (Jordan et al., 1992; Mackerness et al., 1999). Strid et al., (1990) showed that total chlorophyll content, the content of PS II, ATP hydrolysis and Rubisco activity decreased when plants were exposed to UV-B radiation. Bjorkman, (1987) and Demming and Bjorkman, (1987) found that the decrease in Fv/Fm ratio caused by photoinhibitory irradiation was linearly related to the decrease in the optimal quantum yield of photosynthesis.

Gomez et al., (1998) observed decreased yield of variable chlorophyll fluorescence with increasing sunlight in green alga *Dasycladus vermicularis*. Campbell et al., (1998) reported that UV-B stress significantly depressed productivity in aquatic habitats, largely because UV-B inhibits several steps of photosynthesis, including the photooxidation of water catalyzed by photosystem II. Water splitting site and the reaction center of PS II may also be damaged by ultraviolet radiation (Bhattacharya et al., 1987). The degradation of D1 protein, the key protein in PS II reaction center induced by UV-B radiation may also contribute to the decrease in photosynthesis (Jordan, 1996). Schofield et al., (1995) and Nilawati et al., (1997) observed that the most studied effect of UV on primary producers was the inhibition of photosynthesis due to photo system damage and bleaching of photo pigments. Impairment of photosynthetic electron transport also occurs, which in particular affects PS II activity (Campbell et al., 1998). Bjorn (2002) reported that UV-B resulted in inhibition of growth in Antarctic cyanobacteria, while photosynthesis was not affected. In photosynthetic organisms, UV-B radiation exerts direct effects on photosynthetic light reactions and carbon reduction (Teramura and Ziska, 1996). It has been suggested that the specific target of UV-B on photosynthesis of chromophytes could be the PS II reaction centre and the light-harvesting antenna (Lesser et al., 1996; Cullen and Neale, 1994), but carbon dioxide fixing enzyme, ribulose bisphosphate carboxylase-oxygenase was more sensitive than PS II in the dinoflagellate *Prorocentrum micans* (Lesser, 1996), indicating effect of UV-B on both light reaction as well as dark reaction enzymes. However, we have not studied the effect of UV-B on CO_2 fixing enzyme in this study.

Our results with Fv/Fm ratio show a non-linear pattern of damage showing transient stability/increase in the Fv/Fm ratio after 2-3 h of the treatment in both the cyanobacteria studied, which on longer duration of treatment resulted in rapid decline in the photosynthesis. These results indicate transient adaptation after initial onset of the stress, which is overcome by longer duration of the stress. Similar results were observed by Flores-Moya et al., (1999) and Häder et al., (2003) who observed that photosynthetic response of the brown alga *Dictyota dichotoma* and red alga *Corallina officinalis* was inhibited by solar radiation conditions at midday and recovers during the afternoon which suggest that UV-B may be involved both in the impairment and the recovery of photosynthesis. This diurnal pattern is believed to be mainly due to dynamic photoinhibition (Henley et al., 1992). Karentz et al., (1994) reported that the photosynthetic activity was affected due to natural radiation as shallow water algae were more resistant than algae from deeper waters, indicating that alga was able to acclimate to changes in irradiance by synthesizing UV-B absorbing compounds (MAAs).

The decrease in the Fv/Fm ratio under high light conditions may be a result of photoinhibition, which is known to affect quantum yield of photosynthesis and the PS II reaction centre (Vass, 1997). Krause, (1988) reported that in cyanobacteria, photoinhibition was mainly due to the photoinduced damage to the photosynthetic machinery. It is known that both oxidizing as well reducing conditions in the photosynthetic electron transport are source of generation of AOS, which may lead to bleaching of surrounding pigments and peroxidation of lipid membranes (Sharma, 2002). UV effects on photosynthesis occurred only in conjunction with PAR exposure (Bischof et al., 2002), indicating that UV effects are induced primarily in the presence of oxygen.

UV exposure increased the formation of active oxygen species (Lesser et al., 1996) and the damage to photosynthesis by UV radiation is caused by the generation of free radicals (Hideg and Vass, 1996). These radicals accumulate in the thylakoids and are responsible for peroxidation reactions, which were also observed in this study. Peroxidation destroy various components of the photosynthesis apparatus (Malanga et al., 1997), among which are the D1 (largely under PAR) and D2 (more so under UV-B) proteins of PS II, which are highly susceptible to peroxidative conditions (Greenberg et al., 1989). Apart from damaging photosynthetic components, active oxygen species also have been implicated as a messenger for signaling down regulation of several photosynthetic genes (Mackerness et al., 1996). Nishiyama et al., (2001) observed increased concentration of intracellular AOS caused photodamage to PS II mainly by inhibiting the repair of damage to PS II by inhibiting the synthesis of D1 protein. Campbell et al., (1998) also showed generation of AOS under UV-B treatment in *Synechococcus* spp PCC 7942, which down regulated psbA genes encoding PS II D1 protein.

Herrmann et al., (1995) demonstrated that the extent of photoinhibition depends on the fluence rate and the duration of exposure as well as on the spectral distribution of solar radiation. Franklin et al., (1992) demonstrated that short exposure to solar irradiation of high fluence rates resulted in a rapid decrease in Fv/Fm and Fv, corresponding to an increase in Fo, which is rapidly reversible under dim light. Hanelt et al., (1992) found an increase in Fo in several red algae after photoinhibitory light treatment and a recovery of photosynthesis in most of *Dunaliella dichotoma* algae. However, in this study with cyanobacteria we observed decrease in both Fo as well as Fm. Algae growing in transparent waters show a higher photoinhibition than algae

growing in turbid waters (Hanelt et al., 1993). Decrease in Fv/Fm ratio and changes in the pigments due to light in green alga *Spongiochloris*, *Chlorella* and *Scenedesmus* was also reported by Koblizek et al., (1999); Masojidek et al., (1999). Reduction in productivity, impaired development and reproduction for phytoplankton as well as for macro algae under UV-B light has also been reported (Häder et al., 2003; Huovinen et al., 2000). A large number of studies investigating the effects of UV-B radiation on seaweeds were reported, showing that UV-B causes deleterious effects such as depression in growth and photosynthesis, photodestruction of pigments (Franklin and Forster, 1997; Häder and Figueroa, 1997). Richter et al., (1999) reported that ultraviolet radiation induced opening of the membrane bound calcium channels in the cyanobacterium, *Anabaena* spp.

4.3. PHOTOSYNTHETIC PIGMENTS:

An increase in photosynthetic pigments, mainly chlorophyll, carotenoids and phycobillins in *Nostoc spongiaeforme* under UV-B treatment, in this study (HPLC profile) would imply that their synthesis is induced, directly or indirectly by UV-B radiation (Fig. 3.9). The increase in photosynthetic pigments mainly carotenoids in response to UV-B treatment in our study might be in response to provide protection against the UV-B damage probably as quenchers of AOS and excitation energy. Photosynthetic pigments especially β-carotene play a dual role in photosynthesis, as accessory pigments to transfer excitation energy to chlorophyll a and as protectant against damaging light as quencher of active oxygen species. Changes in carotenoids and phycobillins may also regulate wavelengths of light absorbed (chromatic adaptation) to protect it against the UV-B damage.

High light treatment to *Nostoc* though increased the β-carotene, which may again indicate its role as an antioxidant under high light stress conditions (Fig 3.9). However, interesting results of decrease in chlorophyll a during high light treatment (which was not seen during UV-B or UV-B supplemented with low PAR) may suggest a process by which light absorption could be limited to protect itself against over energization.

Our data showed contrasting behavior in *Nostoc* and *Phormidium* with reference to pigment. While chlorophyll a, carotenoids and phycobiliprotein content increased in *Nostoc* as a result of the UV-B treatment. In *Phormidium,* all these pigments declined (Fig. 3.10). The decrease in photosynthetic pigments due to UV-B treatment in *Phormidium corium* could be due to more generation of active oxygen species (Fig 3.18) as *Phormidium* had less level of MAAs induced in response to UV-B treatment compared to seen in *Nostoc* (Fig. 3.35), which could also affect pigment content as pigments are highly sensitive to oxidation and peroxidation reactions (Sharma, 2002). The chromopheric compounds involved in photosynthesis such as chlorophyll, phycobiliproteins and quinones absorb UV-B radiation and photosensitize the generation of AOS.

Jahnke, (1999) have shown accumulation of β-carotene in *Dunaliella baradawil* in response to UV exposure, which they correlated with protection against UV damage as β-carotene is an efficient quencher of oxygen radicals. Goes et al., (1994) reported that UV radiation induces a rapid increase in intracellular carotenoids in the unicellular marine prasinophyte *Tetraselmis.* They also reported decline in the chlorophyll a under UV-B exposure, which was not the case in our study with *Nostoc.*

Detres et al., (2001) observed that significant reductions in total chlorophyll and carotenoids were found in *Thalassia testudinum* exposed to UV radiation. Also Dawson and Dennison, (1996) reported that increments in UV fluxes induced significant decreases in chlorophyll in five species of sea grasses from Australia. UV-B was found to reduce the amounts of photosynthetic pigments and cause photoinhibition in the red alga *Porphyra leucosticta* (Figueroa et al., 1997; Strid and Porra, 1992). Strid et al., (1994) observed that photosynthetic pigments can be bleached by UV-B and the structure of the light harvesting complexes was affected resulting in impaired photosynthesis.

Häder and Worrest, (1991) observed that UV-B radiation can photooxidize and thereby bleach all types of photosynthetic pigments. Bischof et al., (2000); Han et al., (2003) also reported that there was loss of photosynthetic pigments due to UV-B treatment in macroalgae. Götz et al., (1999) have reported that there was degradation of phycobilins, the inactivation of photosynthetic oxygen evolution, and the activity of photosystem II in the cyanobacterium, *Synechococcus* PCC 7942 that was exposed to UV-B radiation. Dohler et al., (1995) also observed that UV-B caused a decrease in chlorophyll a concentration, affected chlorophyll a periodicity, damaged phycobilisome proteins, and caused a decrease in photosynthetic efficiency in *Synechococcus* spp. Gerber and Häder, (1995) have shown that UV-B reduces the content of photosynthetic pigments in phytoplankton and leads to lower photosynthetic rates.

High light treatment also resulted in decrease in chlorophyll a, which was greater than seen in UV-B treated culture, and β-carotene, which was lesser than seen in UV-B treated culture, indicating that there was greater over energization under high light

treatments, therefore, greater decrease in chlorophyll a and the greater over energization resulted in more AOS formation, thus the higher increase in β-carotene, as compared to UV-B treated culture.

Decrease observed in almost all the photosynthetic pigments during high light stress in both species in this study may be due to photo bleaching of pigments as excess light causes photooxidation of antenna pigment more rapidly (Fig. 3.9 & Fig. 3.10). High light treatment showed decreased photosynthetic pigments so as to provide protection to organisms against over energization by dissipating the excess light energy in the pigment bed and preventing over excitation of reaction centre due to the absorption of light and thus extent of photoinhibition can be decreased.

Quantitative increase in photosynthetic pigments in *Nostoc* due to the treatments (Fig. 3.9) did not correlate with decreased photosynthetic efficiency measured as Fv/Fm ratio, which declined as a result of the treatments (Fig. 3.7). This indicate that decline in Fv/Fm ratio may not be so much dependent on the pigment content only but may also be due to the oxidative damage to lipids and protein resulting in structural and functional alteration of membrane lipids and protein which is also substantiated as oxidative damage to lipids (Fig. 3.19) and proteins in thylakoid membrane (Fig. 3.31) was also observed in this study. However, comparison of the carotenoids content with the photosynthetic or growth performance under UV suggests incomplete functioning of the pigments, since there was a significant decline in photosynthesis and sugar content despite the marked increase in carotenoid content.

Others have also reported similar changes in pigment profile as a result of UV-B and high light treatment. Campos et al., (1991) suggested that UV-B and UV-C radiation increases the levels of 3-hydroxy-3-methylglutaryl COA reductase mRNA, inducing additional synthesis of carotenoids for protection of chlorophyll against UV damage. In cyanobacteria, soluble carotenoid proteins or carotenoids in the cell wall (Jürgens and Weckesser, 1985) could act as a filter for UV-B radiation. The protective mechanism of carotenoids has been found to prevent the damaging oxidation of pigments and proteins by UV radiation (Telfer and Barber, 1995).

Cyanobacteria also have the ability to vary their phycobiliprotein composition (phycocyanin/phycoerythrin ratio), which allows regulation of the balance of wavelengths of light absorbed, a phenomenon known as chromatic adaptation to protect it against the UV-B damage (Tandeau de Marsac, 1977). Wyman et al., (1985) have demonstrated that phycoerythrin plays an important role in attenuating photodamage.

Our data also showed changes in phycobilins content in order to change their ratio vis a vis each other. These changes in the ratio of various phycobilins differently under UV-B and high light treatment may indicate a role for phycobilins in absorbing quantity and quality of light under our experimental conditions. Our data show change of phycobilins in favour of phycocyanin over other phycobilins under UV-B treatment, and changes in favour of allophycocyanin under high light treatment.

Aráoz and Häder, (1997) have reported phycoerythrins being more resistant to photobleaching than phycocyanin and allophycocyanin. They also reported induction of phycoerythrin under UV-B stress, which can form a peripheral layer around the DNA,

and intercepting more than 98% of UV-B radiation before it penetrates to the genetic material. Liotenberg et al., (1996) observed that the changes in cell pigmentation are reminiscent of the phenomenon of complementary chromatic adaptation in which green and red wavelengths promote the synthesis of phycoerythrin and phycocyanin.

Our data also showed increase in xanthophylls under UV-B treatment in *Nostoc* which may indicate a role of xanthophylls in protection against oxidation either by quenching oxygen radicals or by dissipating excess energy, may be by a process similar to xanthophylls cycle, though xanthophylls cycle is not present in the cyanobacteria. Sandman et al., (1998) had observed that UV-B caused enhanced production of the photosynthetic pigments zeaxanthin and phycouroblin, and caused a decrease in photosynthetic efficiency in *Synechococcus* spp. Similarly increase in the xanthophylls in our study may limit the photosynthetic efficiency thereby limiting the damage against over excitation. Bischof et al., (2002) showed UV radiation exposure resulted in higher concentration of lutein and zeaxanthin as it strongly contribute to the protection of plants against photooxidative damage by dissipating excess light energy (Niyogi et al., 1997; Sharma and Hall, 1996). Middleton and Teramura, (1993) observed that UV-B radiation increased photosynthetic pigment content, along with UV-B-absorbing compounds, since both carotenoids and flavonoids may be involved in photoprotection against UV-B stress.

The contrasting results on pigment bleaching in *Nostoc* and *Phormidium* may be due to difference in their tolerance and sensitivity level as a result of adaptation to changing environment due to recovery and protective processes as both the organisms suffered different extent of oxidative damage (Fig. 3.19). The difference in the extent of

peroxidation was largely due to difference in induction of antioxidant enzymes and MAAs in the two species studied. Our results indicate that *Nostoc* showed slightly less damage compared to *Phormidium*, which was probably due to greater increase in activity of antioxidant enzymes and more induction of MAAs in *Nostoc* than *Phormidium* as a result of the three treatments studied.

Quantitative decrease in the phycobilisome content due to UV-B, high light and combination of UV-B and light treatment in both *Nostoc spongiaeforme* and *Phormidium corium* may also be due to bleaching caused to accessory light harvesting pigments (Fig. 3.12, 3.13). In *Phormidium* short term treatment i.e. up to 2-3 hours resulted in lesser oxidation to pigments than seen in *Nostoc* but during longer duration of the treatment *Nostoc* showed lesser damage/oxidation to the pigments than seen in *Phormidium.* These results indicate greater induction of protective processes such as antioxidant enzymes, MAAs etc. in *Nostoc* thus better protection against damage due to UV-B/high light.

Pandey et al., (1997) reported that exposure to UV-B radiation in *Synechococcus* PCC7942, resulted in decrease in phycobilins with a concurrent loss of energy transfer from phycobilisomes to the photosystem (Banerjee and Hader, 1996; Sinha et al., 1998). Lao and Glazer, (1996) reported that UV light resulted in photodestruction of cyanobacterial phycobilisomes of *Anabaena* spp PCC 7120 suggesting that damage to individual phycobilisomes was amplified by the resulting dissociation of the macromolecular complex. The initial photo damage in the phycobilisomes protein was the sum of the direct damage to the aromatic acids in the protein moiety and the direct damage to the bilins. Nedunchezian et al., (1996) suggested that the decrease in

photosynthetic activity was primarily due to the loss of energy transfer from phycobilisomes to chlorophyll, which in turn induced the dissociation of phycobilisomes. The PS II protein is extremely susceptible to protease activity and cleavage of the anchor releases an intact phycobilisomes from the membrane (Hiller et al., 1983). Thus, the UV-B induced photodamage of the anchor protein will preferentially expose the phycobilisomes to possible dissociation and the resultant degradation of other phycobiliproteins.

Sinha et al., (1997) observed fluorescence emission spectra of phycobiliproteins after UV-B irradiation which first shows an increase followed by a shift towards shorter wavelength and finally a decrease in fluorescence, indicating impaired energy transfer from the accessory pigments to the photosynthetic reaction centers and subsequent bleaching of the pigments. Fujita et al., (1989) suggested that smaller size of rod under high light allows the light harvesting system to escape from an excess photon flux and the larger size under low light intensity provides an efficient trap for photons. Schwarz and Grossman, (1998) suggested that higher level of phycocyanin is related to susceptibility of organisms to high light. MacDonald et al., (2003) also reported that high levels of UV-B induces a decrease in the photosynthetic activity of cyanobacteria due in part to the loss of energy transfer from phycobilisomes to chlorophyll in the reaction centres of PS II (Pandey et al., 1997).

Han et al., (2003) reported significant percent decrease in chlorophyll a and phycocyanin after exposure to intense PAR in *Anabaena* spp and they suggested that it might be an adaptation strategy, as indicated by an increase in cell production. Neidhardt

et al., (1998) observed that high light resulted in lower pigment content, a highly truncated chlorophyll antenna size and accumulation of photo damaged PS II centers in the chloroplast thylakoids in *Dunaliella salina* cells. They also suggested possibility of maximizing photosynthetic productivity and light utilization by minimizing the light harvesting chlorophyll antenna size thereby indicating that if light absorption can be reduced by pigments (chl/phycobilins) at antenna level than extent of photoinhibition can be decreased. Enlargement of chlorophyll antenna size as well as chlorophyll a on shifting of *Dunaliella salina* cells from high light to low light has been reported by Masuda et al., (2002) suggesting role for these pigments in protection against photoinhibition probably by limiting the over load of excitation energy. Nomsawai et al., (1999) observed disappearance of large size phycocyanin transcript and 33 kDa linker polypeptides of phycobilisomes when cells of *Spirulina platensis* were shifted from low light to high light. Schwarz and Grossman, (1998) reported that *Synechococcus* nbIR mutant cells unable to properly modulate the phycobilisome level, die rapidly due to its inability to degrade its light-harvesting complex when exposed to high light. The nbIR mutant cells which also had more phycobilisomes than wild type are unable to properly modulate phycobilisome level during exposure to high light which compromise their survival. Oxidation of pigments under high light conditions may also due to the generation of AOS. All these work suggest that changes in the accessory light harvesting pigments such as phycobilisomes in order to limit absorption of excess light may prevent photodamage against excess high light by reducing the antenna size as well as light absorption. Häder et al., (2003) suggested that low photosynthetic pigment content of the cells also led to decrease in the level of other parameters, a result of decreased carbon and

nitrogen assimilation. De Chazal and Geoffrey, (1994) observed that the brown *Nostoc* was resistant to photobleaching under conditions of high continuous light intensity and elevated temperature under which conditions green *Nostoc* spp photobleached.

4.4. SUGARS:

Decline in reducing and stored sugar content observed due to UV-B, high light and combination of UV-B and light treatment in both *Nostoc spongiaeforme* and *Phormidium corium* (Fig. 3.14, 3.15, 3.16) may be a result of damage caused to photosynthesis by the treatments. UV-B may affect sugar by lowering of rate of photosynthesis. Phytoplankton organisms are quite sensitive to enhanced UV-B radiation, suffering changes at the level of photosynthetic apparatus and pigments involved in light reception and in photosynthetic production, carbon and nitrogen metabolism and growth rate. Our data with chlorophyll fluorescence and lipid analysis suggests that processes such as photosynthesis and membrane structure got affected due to the treatments, which may affect assimilation of carbon dioxide. Fv/Fm indicating lowering the photosynthesis and changes in lipid profile needing greater maintenance cost could result in decrease in reducing and total sugar. Greater decrease observed in the reducing sugar than seen in total sugar (Fig. 3.14) further indicating primary effect on the primary synthesis processes such as photosynthesis.

Babu et al., (1998) have reported that UV-B exposure resulted in decrease in total starch content in *Nostoc, Anaebaena* and *Scytonema* and the inhibition of specific growth rate was in accordance with the inhibition of photosynthesis. Our results also support these observations. He et al., (1994) showed larger starch grains accumulated after a

single exposure to UV-B in algae, which could be due to inability of UV-B damaged cells to mobilize starch. Strid et al., (1994) showed that the immobilization could be related to either membrane damage resulting in alterations in permeability or to destruction of enzymes. On the other hand work by De Philippis et al., (1992) have shown increase in glycogen content in *Spirulina maxima* from shifting the culture from low light to high light for duration of 200 h. Though there are a few study on impact of UV-B and high light on sugar content in higher plants, in depth studies are lacking on the effect of these factor on carbohydrate status in algae. Yue et al., (1998) observed that supplemental levels of UV-B induced a decrease of soluble carbohydrates in leaves of *Triticum aestivum* and decreased sucrose and starch contents in leaves of the moss *Polytrichum commune* (Barsig et al., 1998). A decrease in non-structural carbohydrates on exposure to high UV-B irradiation was recorded in cotton by Zhao et al., (2003). According to Garrard et al., (1977), UV-B sensitive phanerogams are characterized by reduced amounts of sucrose. Barsig and Malz, (2000) reported that there was no impact of enhanced UV-B on starch grains and sucrose content but resulted decrease in glucose content in sugar maize leaves. It has been reported that mulberry plants irradiated with UV from lamps suffered less herbivory by silkworms (*Bombyx mori*) and the lower consumption was attributed to lower sucrose content of the foliage (Yazawa et al., 1992). These observations in general are similar to our observations of decline in sugar content with algae suggesting similar mode of effect under UV-B/high light treatment.

There are reports, which also show increased accumulation of sugars under UV-B conditions in different plants species. Britz and Adamse, (1994); Santos et al., (1993) reported that UV-B induced an accumulation of starch in cucumber leaves, which

suggested that high starch content could be related to its function as a carbohydrate storage pool. Rozema et al., (1997); Day (2001) showed that plants increase their content of carbohydrate rich secondary metabolites under elevated UV-B. Musil et al., (1999) observed that non-structural carbohydrate concentrations were significantly increased in leaves of four species viz. *L. laureolum, P. pubescens* and *Protea burchellii* under enhanced UV-B. Santos et al., (1993); He et al., (1994) have reported that increase in starch content in UV-B-irradiated leaves of pea, corn and a desert annual plant, which they attributed to mitochondrial damage resulting from decreased respiratory consumption of substrate favoring starch accumulation. They also indicated increased accumulation of starch was not due to photosynthetic stimulation in the absence of any UV-B effect on phosphoenolpyruvate or Rubisco activity. Hilal et al., (2004) reported that exposure to UV-B radiation led to strong increase in the fructose content, which is related to higher requirement of erythrose-4P as a substrate for the synthesis of lignin and phenolics. Glycogen reserves displayed a dynamic pattern of accumulation and disappearance during cell growth, which varied only slightly with nitrogen source (Liotenberg et al., 1996). Schneegurt et al., (1994) observed that poly-β-hydroxybutyrate (PHB) a widespread intracellular storage compound typical of prokaryotic organism, was accumulated under stress conditions in *Cyanothece* spp strain ATCC. Chimphango et al., (2003) reported that elevated UV-B exposure had no effect on soluble sugars, starch and nitrogen fixed in the three species of tropical grain legumes studied. Lindroth et al., (2000) reported that high UV-B exposure showed increase in nitrogen concentration and decreased carbohydrate content and suggested that increase in sugar content may be related to adaptation for synthesizing other secondary carbohydrate molecules such as

phenolic compounds, lignin, tannins etc. Data discussed here indicate that sugar changes vary from species to species depending on kind of adaptation mechanism followed by the organism.

4.5. NITROGEN:

Decrease in nitrogen content observed under UV-B, high light and UV-B supplemented with low light treatments in *Nostoc spongiaeforme* and *Phormidium corium* (Fig. 3.17), may be due to greater degradation of nitrogenase or decrease in the enzyme activity itself or inhibition of ATP synthesis. Prokaryotic microorganisms responsible for nitrogen fixation are also susceptible to UV-B, which could result in changes in the biogeochemical cycling of nitrogen, potentially leading to a drastic nitrogen deficiency for higher plant ecosystems, such as rice paddies and detrimental effects on plant growth. Decrease in photosynthesis will also have negative effect on assimilation of nitrogen. Zhang et al., (1994) observed inactivation of nitrogenase might possibly be due to the inhibition of ATP synthesis by UV-B as well. Kumar et al., (1996b) have also observed decrease in nitrogenase activity within 35-55 minutes of UV-B treatment depending upon the species and this inactivation is due to the inhibition of ATP synthesis by UV-B. Tyagi et al., (2003) reported that exposure of UV-B radiation for 20 min completely inactivated nitrogenase activity while in *vivo* nitrate reductase activity was not completely lost even after 80 min of UV-B exposure. Kumar et al., (2003) also showed that UV-B exposure for 30 min resulted in complete loss of nitrogenase activity in the nitrogen fixing cyanobacterium, *Anabaena* BT2 which suggests that the activity of nitrogenase did not recover upon transfer of UV-B exposed

cells to fluorescent light, suggesting that the inhibition may be due to specific inactivation of the enzyme. Protein peroxidation (though not studied here) may also cause an overall decrease in nitrogen content due to formation of carbonyl from amino group and loss of NH_3 by production of aldehyde. Solheim et al., (2002) showed that UV-B radiation affected the biological nitrogen fixation in arctic cryptogam species by 50% over a period of 3-4 years.

Sinha et al., (1996) have also shown that UV-B induced inactivation of the nitrogen fixing enzyme nitrogenase in many cyanobacteria. They observed that differentiation of vegetative cells into heterocysts was severely affected by UV-B irradiation in a number of rice field cyanobacteria. The C: N ratio was altered following UV-B irradiation, which in turn affected the spacing pattern of heterocysts in filaments. In addition, major heterocyst polypeptides of around 26, 54 and 55 kDa have been shown to be decreased in concentration due to UV-B irradiation, suggesting that the multilayered thick wall of heterocysts may be disrupted resulting in the inactivation of the nitrogen fixing enzyme nitrogenase by the penetrating oxygen (Sinha et al., 1996). In our study we also reported disruption of cell as well as decrease in the number of hetrocyst as a result of the treatments.

Hessen et al., (1997) reported reduced uptake rates of nitrate, ammonium and phosphorus due to UV exposure. Ronner et al., (1983) found that phytoplankton of Scotia Sea, Antarctica showed a preferential uptake of ammonium in comparison to nitrate. Lehtimaki et al., (1997) reported intracellular nodularin concentrations increased with high temperature, high irradiance, and high phosphate concentration and decreased with

low and high salinities and high inorganic nitrogen concentrations in *N. spumigena* and *A. flos-aquae* indicating changes in the membrane behaviour with respect to ion uptake.

4.6. ACTIVE OXYGEN SPECIES:

In this study we observed negligible ability of quenching of active oxygen species existed in *Phormidium* (Fig. 3.18) compared to *Nostoc*. This is substantiated by increased oxidative nature of damage, observed as lipid peroxidation in *Phormidium*. This may also suggest one of the reason why corals, largely associated with *Phormidium,* are getting bleached. Lesser, (1997) had proposed generation of reduced oxygen intermediates within both the algal symbionts and host, resulting in oxidative stress which causes decrease in photosynthesis and subsequent bleaching. In this study, UV-B exposure also resulted in production of AOS measured using epinephrine method (Fig. 3. 17), which may also be responsible for decreased photosynthetic efficiency through photooxidative effect.

Increased formation of AOS species by stress factors such as high light, UV radiation, temperature etc. is a common process in living organisms including algae. The formation of these compounds is a normal part of the metabolism, excessive production can cause damage to DNA, proteins and lipids (Yue et al., 1998). Our results are also in agreement with other studies, which indicated increased production of oxygen radicals indicating oxidative damage to cyanobacterial species due to the stress conditions. During photosynthesis, oxygen is produced by photosystem II, which increases the internal oxygen concentrations and potentially augments the chances for active oxygen species

(AOS) formation especially under stress conditions (Halliwell, 1987). The rich presence of photosynthetic pigments and redox components such as chlorophylls, phycobiliproteins and quinones which exhibit absorption in the UV range (Franklin and Forster, 1997) photosensitize the formation of AOS including superoxide radical, hydroxyl radical, hydrogen peroxide and singlet oxygen by electron or energy transfer to oxygen, and tend to exert oxidative damage, inducing lipid peroxidation, DNA damage, inhibition of photosynthesis and bleaching of photosynthetic pigments such as chlorophylls and phycobilins (An et al., 2000; Vega and Pizarro, 2000; Landry et al., 1995). Jordan, (1996); Niyogi, (1999) reported that under UV-B stress, the inhibition in electron transport chain due to the degradation of the D1 protein in photosystem II might promote the energy transfer from triplet chlorophylls to oxygen to form singlet oxygen by UV irradiation (Bischof et al., 2000) and promoted the formation of superoxide radical at the level of ferredoxin at photosystem I (PS I).

He and Häder, (2002) observed that UV-B radiation increased AOS levels causing lipid peroxidation and DNA strand breaks as well as decreased photosynthetic performance and survival. They suggested that UV-B irradiation promotes the formation of AOS significantly and exerts oxidative stress to the cyanobacterium, *Anabaena* spp. Niyogi, (1999) reported that in the presence of light, photosensitizer mediated production of singlet oxygen especially under stress conditions results in oxidative stress to photosynthetic organisms. They showed the respiratory reduction of oxygen in the dark to water leads to the stepwise formation of AOS such as superoxide, hydrogen peroxide and hydroxyl radical. UV-B-induced photosensitization of photosynthetic pigments such as chlorophylls, phycobiliproteins generates singlet oxygen as well as other AOS.

Additionally, the direct damage to the key enzymes involved in photosynthesis and respiration pathway may also promote AOS formation (Jordan, 1996; Bischof et al., 2000; Halliwell and Gutteridge, 1999). In green alga, *Chlamydomonas reinhardtii* under excess light, changes in Rubisco and D1 protein were found to be closely related to AOS increase and changes of the redox status regulated by the glutathione pool (Irihimovitch and Shapira, 2000). Foyer et al., (1994) reported that AOS generated under stress conditions including UV-B stress, especially hydroxyl radical ($OH^{\cdot}$) and singlet oxygen ($^1O_2$), react with sugars, purines and pyrimidines thus, causing DNA strand breakage is in cells subjected to oxidative stress.

These generations of AOS results in oxidative damage and lipids along with proteins are some of the oxidative targets attacked by the elevated AOS, peroxidation of lipids was observed in this study (Fig. 3.19). More peroxidation of cell membrane lipids in *Phormidium* indicates two things: greater generation of AOS under stress conditions and second, limited ability to remove/quench AOS by the *Phormidium* as compared to *Nostoc* (Fig. 3.19). Lipid peroxidation occurs especially at sites where polyunsaturated fatty acids (PUFA) occur in high concentrations. Ultraviolet radiation has been shown to be very effective in inducing lipid oxidation of biological membranes (Kochevar, 1990), polyunsaturated fatty acids (Yamashoji et al., 1979) and phospholipid liposomes (Pelle et al., 1990). Several studies have reported that UV-B and high light are known to cause oxidation of membrane lipids (Kappus, 1985; Malanga et al., 1997; Girotti, 1990; He et al., 2002; Strid et al., 1994). The treatment in our study similarly may have resulted changes in the oxidation level of membrane affecting various biochemical processes. These observations are also substantiated by our study with fatty acid profile, which

showed slight changes in unsaturation/saturation ratio of fatty acids. He and Häder, (2002) observed induction of AOS under *in vivo* condition in cyanobacterium, *Anabaena* sp and suggested that impaired photochemical reactions of PSII can enhanced the production of AOS in algae. He et al., (2002) observed that when cyanobacteria were subjected to UV-B irradiation, they exhibited increased oxidative stress, oxidative damage, decreased photosynthetic efficiency and inhibited growth, indicating role of active oxygen species (AOS) in the oxidative damage of the cyanobacterium, *Anabaena* spp. Salmon et al., (1990) have reported that UV-B can promote the formation of lipid oxidation products, which can destroy the lipid soluble antioxidants as well. Foyer et al., (1994) have observed that the signaling role of oxidative stress originally caused by UV-B stress may mediate the protective modulation that finally protects the organisms from continuous and lethal damage and reverses the negative effect after a certain period of response progression. In our study, however, no such reversal of oxidative damage under UV-B exposure was seen but UV-B treatment supplemented with low visual radiation resulted in slight decrease in the photosynthetic damage (Fig. 3.7) as well as low peroxidation of membrane lipid (Fig. 3.19) as compared to the UV-B treatment alone.

4.7. LIPIDS AND FATTY ACIDS:

Plants change their membrane lipid composition in response to the alteration of major environmental factors. Environmental conditions can lead to significant changes in the lipid compositions of micro algae (Shifrin and Chisholm, 1981) and can have even more dramatic effect on fatty acid distributions (Dunstan et al., 1993). The proportions of

the lipids can vary significantly in response to changes in light intensity, salinity and most importantly nutrient levels.

Quantitative decrease in phospho and glycolipids observed with UV-B treatment in this study may again be due to oxidative damage caused to cell membrane as seen in this study (Fig. 3.20, 3.21) and also reported by others (Vigh et al., 1993). But the observed changes in lipids could also be due to enzymes involved in lipid synthesis (Nishida and Murata, 1996), however, we have not studied enzymes involved in the lipid biosynthesis. These changes in lipids composition and content may be related to adaptation and survival of the organism under the changing environmental conditions through maintaining membrane integrity and physiological functions by changing composition and content of its various constituents. Murphy, (1983) observed that the UV-B affects the integrity of the membrane thereby causing a decrease in the lipid content and membrane transport system. UV-B treatment was also reported to decrease lipid content in *Odontella weissflogii* due to degradation of complex lipid (Skerratt et al., 1998). Predieri et al., (1995) reported that UV-B irradiation decreased monogalactosyldiacylglycerol (MGDG) content, being the major lipid component of the chloroplast, but did not affect digalactosyldiacylglycerol (DGDG). In the presence of oxygen, UV-B radiation produces large reduction in lipids, thus affecting membrane systems (Murphy, 1983; Kramer et al., 1991).

Klyachko-Gurvich et al., (1999) reported that (n-3) desaturation of fatty acids in MGDG is an adaptive response of algal cells to alterations in light conditions and may indicate a high degree of control of the ionic permeability and structure of the cell

membranes. Also this may be considered as an adaptive response of the algal cell to the growth conditions requiring the rearrangement of the structure of thylakoids to provide more efficient photosynthesis. It has been shown that the polar lipids can associate firmly with chlorophyll and may help to orient the chlorophyll-protein complexes within the membrane (Harwood and Russell, 1984). Light dependent alteration in the content of polar lipids and proportion of fatty acids in them may influence lipid interplay with proteins of the membranes thus affecting various membrane associated physiological and biochemical processes (Klyachko-Gurvich et al., 1999).

Norman and Thompson, (1985), Xu, (1994) have reported decrease in phospho and glycolipids, changes in fatty acid composition and changes in the unsaturation level in response to environmental variations such as high light, temperature and salinity has been reported in cyanobacterium, *Spirulina platensis.* Funteu et al., (1997) have shown decrease in the level of sulfoquinovosyl diacylglycerol and phosphatidylglycerol due to light exposure in *Spirulina platensis.* Nishida and Murata, (1996) attributed the survival of algae under higher irradiance conditions to membrane fluidity as a result of higher level of fatty acid unsaturation. Tedesco and Duerr, (1989) also shown that high light slightly increased total lipids and percent composition of polyunsaturated fatty acids such as γ–linolenic acid in *Spirulina platensis.* Khotimchenko and Yakovleva, (2004) reported variations in lipid composition of the marine green alga *Ulva fenestrata* grown under different irradiance conditions which suggest that changes in lipid composition can be considered as a mechanism of adaptation and survival of thalli subjected to variations in solar irradiance. Adams et al., (1987) and Ohno et al., (1979) reported that phospholipid content was affected after increase in salt concentration, which may control the ionic

permeability of the organisms in stress conditions.

Our results showed that UV-B and high light exposure resulted in slight decrease in saturated fatty acid content of total lipids and glycolipids (Table 3.4, 3.5), thus changing the ratio of unsaturation to saturation level of fatty acid in favour of unsaturation, which is required for adaptation. The unsaturation level of fatty acids increased after treatment with both UV-B and light in combination while it remained unchanged after treatment with UV-B alone and high light. Slight decrease observed in saturated fatty acids in our study would change the ratio of fatty acids in membrane toward the unsaturation level leading to increased membrane fluidity and adaptation to stress conditions. However, others have shown changes in fatty acid composition with reference to visual light.

Dawes et al., (1993) observed that light intensity influenced primarily the content of saturated and tetraunsaturated fatty acids in *Ulva pertusa*. Walsh et al., (1997) reported that the changes in the fatty acid composition due to high level of light intensity may occur as a cellular response to reduce the susceptibility of the cyanobacterial membranes to photo oxidation. Cohen et al., (1987) observed no change in fatty acid distribution with light intensity in *Spirulina minor* while Tedesco and Duerr, (1989) shown that high light slightly increased total lipids and percent composition of unsaturated fatty acid γ-linolenic acid in *Spirulina platensis*. Transformation of the cyanobacterium, *Synechococcus* spp. PCC 7942 with the *desA* gene for a D12 desaturase have been reported to increase the unsaturation of membrane lipids and thereby enhance the tolerance of cyanobacterium to high light (Gombos et al., 1997).

Increase in the unsaturation level of fatty acids due to UV-B supplemented with low light is probably due to increase in the activity of desaturase level as reported by Funtue et al., (1997) in *Spirulina platensis* under high temperature. The fluidity of membrane lipids is directly correlated with the level of unsaturated fatty acids in biological membranes. The increase in unsaturation of fatty acids in membrane lipids may help in providing protection to cyanobacteria against UV-B radiation. Gombos et al., (1997) have reported that increase in the unsaturation of membrane lipids enhanced the tolerance of cyanobacterium to abiotic stress. Samala et al., (1998) observed the degree of fatty acid unsaturation under abiotic stress conditions, increase in the ratio of unsaturation to saturation of fatty acids under low temperature. However, changes in fatty acid composition in response to alteration of stress conditions still remain a less studied process.

4.8. PROTEINS:

Changes in protein profile due to UV-B and high light exposure indicate that cellular proteins are also one of the targets of UV-B and high light (Fig. 3.31). UV-B affects the protein largely due to the presence of aromatic amino acids that absorbs strongly in the range of 290-314 nm, which could lead to denaturation of proteins (Vass, 1997). Ultraviolet radiation is known to damage proteins and enzymes, especially those rich in aromatic amino acids such as tryptophan, tyrosine, phenylalanine and histidine, all of which show strong absorption in the UV range from 270-290 nm. UV-B can induce the photo oxidation of tryptophyan residues into the photoproduct N-formyl kyneurenine which acts as a photosensitizer allowing the formation of oxygen radicals leading to

protein structural changes, intra and intermolecular cross links, proteolysis, enzyme inactivation. Therefore, observed decrease in our study could also be due to the formation of active oxygen species under our experimental conditions. High light is known to causes generation of AOS, which may also lead to oxidation of protein.

Sinha and Häder, (1998) reported that as a result of UV-B, proteins may undergo a number of modifications including photo degradation, increased aqueous solubility of membrane proteins, and fragmentation of the peptide chain, leading to inactivation of proteins (enzymes) and disruption of their structural entity. Dohler et al., (1995) observed that another adaptation to environmentally enhanced levels of UV irradiance might be the synthesis of heat shock proteins and a special 43 kDa protein under UV stress. However, we have not studied these aspects.

4.9. DNA AND RNA:

Damage seen to DNA and RNA under UV-B treatment (Fig. 3.32) may be a result of the formation of dimmers between adjacent pyrimidine bases, cis-syn cyclobutane dimmers and pyrimidine (6-4) pyrimidine photoproducts. Such DNA lesions interfere with DNA transcription and replication and can lead to misreading of the genetic code causing mutation and death. It is known that UV-B radiation is readily absorbed by DNA and RNA that alter the DNA configuration which involve dimerization of adjacent pyrimidine bases, which results in cyclobutane-pyrimidine dimmers (CPDs) and, less frequently, 6-4 pyrimidine pyrimidone dimers (6-4PPDs), and mutagenic lesions in many cyanobacteria and therefore result in cytotoxicity which disrupt metabolism and cell division.

The observed damage to DNA and RNA in our study may also be due to the formation of active oxygen species, especially in the case of high light, as it does not cause formation of thimidine dimmer, indicating oxidative damage in response to stress conditions. It can be suggested that UV-B can cause damage to DNA and RNA directly (through formation of thimidine dimmers and CPD) as well as indirectly through AOS, while high light may cause oxidative damage only through AOS formation.

UV induced effects on algal physiology, and damage of molecular targets such as DNA, RNA and proteins have been documented by Iwanzik et al., (1983); Buma et al., (1997); Davidson et al., (1996). There is increasing evidence that AOS are also involved in the DNA damage (Mackerness et al., 1998; Foyer et al., 1994). These damages to DNA may have an effect on cell division, organism's mortality, marine community structure and consequently aquatic productivity (Hader and Figueroa, 1997; Karentz, 1991).

Baumstark-Khan et al., (2000) reported that the secondary breaks induced by the excision repair of photochemically formed CPD or 6-4 photoproducts may cause the number of AOS mediated primary breaks induced by UV-B irradiation. Danno and Horio, (1987) also reported the generation of superoxide anions or secondarily generated hydroxyl radicals which can induce DNA damage following UV-B irradiation in *Nostoc* spp. Huovinen et al., (2000); Wiencke et al., (2000) reported that DNA damage is known to result in the loss of spore viability and the delay in spore germination and development in macroalgae which is correlated to higher DNA breaks as well as stronger oxidative stress. They also suggested that the damage to DNA may lead to the potential death of

cells directly in addition to damage to lipids and proteins (Dring et al., 1996; Swanson and Druehl, 2000; Makarov and Voskoboinikov, 2001; Banãres et al., 2002).

4.10. PROTECTIVE PROCESSES:

Organisms have developed number of protective mechanisms by which they protect or reduce the damaging impact of UV and high light treatment. These include the synthesis of UV absorbing or screening compounds such as mycosporine-like amino acids, phenolic compounds (Karsten et al., 1998), the production of enzymatic and non-enzymatic antioxidative mechanism responsible for scavenging of active oxygen species (Sharma et al. 1998b), and repair of UV-induced damage of DNA by photoreactivation and excision repair (Häder, 2001).

4.10.1. MYCOSPORINE LIKE AMINO ACIDS (MAAs):

Our study showed that *Nostoc spongiaeforme* and *Phormidium corium* are able to synthesize mycosporine like amino acids (MAAs) in order to provide protection against UV-B radiation (Fig. 3.34, 3.35). In our study we observed far higher level of MAAs in cyanobacteria exposed to UV-B supplemented with low visual light than observed when exposed to only UV-B radiation. This may be correlated with slight attenuation of UV-B impact when UV-B radiation is supplemented with low-level visual radiation.

Quesada et al., (1999) and Cockell and Knowland, (1999) have observed the protection against UV-B stress in some cyanobacteria has been attributed to the synthesis of UV-B absorbing MAAs and scytonemin and has been suggested to provide protection

as a UV absorbing sunscreen (Garcia-Pichel et al., 1993; Sinha et al., 1999). Several authors have reported synthesis of increased MAAs due to UV-B exposure. Matsunga et al., (1993); Bandaranayake, (1998); Conde et al., (2000) in a marine cyanobacterium, *Oscillatoria* spp,; Portwich and Garcia-Pichel, (2000) in a cyanobacterium, *Chlorogloeopsis* PCC 6912; Vernet and Whitehead, (1996) in a dinoflagellate, *Lingulodinium* and Karsten et al., (1998) in a red alga, *Chondrus crispus.*

Besides providing protection as a UV-absorbing/screening compound it is possible that MAAs play more than one role in the cellular metabolism of all or some organisms (Castenholz, 1997). Oren, (1997) has reported accumulation of MAAs as a result of osmotic stress, which may suggest a role for MAAs in organic osmoticum. However, there may be physiological limitations to the accumulation of MAAS osmotically active compounds within the cell. Portwich and Garcia-Pichel, (1999) have also suggested that UV-B and osmotic stress regulate the synthesis of MAAs in the cyanobacterium, *Chlorogloeopsis* spp. Dunlap and Yamamoto, (1995) observed that some MAAs has mild antioxidant properties. Dunlap et al., (1998) reported that mycosporine glycine functions as a biological antioxidant in marine organisms while the imino mycosporine like amino acids such as shinorine, porphyra-334, palythine, asterina-330 and palythinol were oxidatively robust.

Several experiments on the induction of MAAs in macro algae have shown two kinetics for the induction, one responding only to UV radiation and another responding to UV radiation and PAR (Karsten et al., 1998). In *Anabaena* spp, a maximum induction of the MAA synthesis was found in the short wavelength UV-B (Sinha et al., 2002) and for

Gyrodinium dorsum a maximum at 310 nm was found (Klisch and Häder, 2002). The concentration of UV absorbing substances such as MAAs can be correlated with various factors such as irradiance (Post and Larkum, 1993; Sivalingam and Nisizawa, 1990). However, in our study we did not observed induction of MAAs as a result of high light treatment rather a significant decline in the MAAs in both the cyanobacterial spp. was seen. In nature cyanobacteria are also exposed to high light and combination of both UV-B and high light might compromise the protective ability of MAAs under UV-B only conditions. Rozema et al., (2002) observed that visible (400-700 nm) and long wavelength UV-A (315-400 nm) showed only a slight effect in the induction of mycosporine-like amino acids (MAAs) but was effectively induced by UV-B in the marine dinoflagellate, *Gyrodinium dorsum*, the green algal species, *Prasiola stipitata* and in the cyanobacterium, *Anabaena* spp. But when UV-B was supplemented with low level PAR it caused far greater increased in the MAAs content, indicating that high level of visual radiation may be inhibitory/degrading for the MAAs.

The UV absorbing substance found in *Prasiola stipitata* shows a similar behaviour as MAAs and was reported earlier as an unknown MAAs (Hoyer et al., 2001). Lesser, (1996); Neale et al., (1998); Karsten et al., (1998); Franklin et al., (2001) demonstrated that MAAs accumulation coincides with a reduction of UV effects on photosynthesis in phytoplankton and macro algae. Garcia-Pichel et al., (1993) showed that for various cyanobacteria, high MAA concentrations are correlated with increased, albeit not complete, resistance to UV photodamage. These authors estimated sunscreen factors due to the presence of MAAs of up to 0.3, i.e. 30% of harmful UV quanta were absorbed before hitting cytoplasmic molecular targets. In a similar study with Antarctic

phytoplankton, Riegger and Robinson, (1997) calculated sunscreen factors up to 0.5 for diatom cells and up to 0.72 for *Phaeocystis antarctica* colonies. Our results indicated decrease in the MAAs induction due to the high light.

Reef–building corals also contain UV-absorbing compounds capable of blocking potentially damaging UV radiation as a first line of defense. *Phormidium* was isolated from coral reefs and these coral reefs are also showing bleaching, it was appropriate to correlate the content of MAAs with the bleaching observed. Our study showed that *Phormidium corium* was also able to synthesize mycosporine like amino acids (MAAs) having absorbance in UV region, in response to UV-B radiation. Kawaguti, (1944) speculated that some coral pigments offered protection against strong sunlight. Many reef animals can produce natural sunscreens (MAAs) to protect themselves against UV but MAA concentrations are possibly a response to the amount of UV to which they are subjected. The amount of MAAs in a coral's tissues helps to determine how much UV, it can withstand without bleaching (Gleason, 1993). Lesser and Farrell, (2004) observed that both photosynthetic pigments and MAAs are depressed after experimental exposure to high solar radiation and thermal stress to common Caribbean coral, *Montastraea faveolata*. Our results show that when a cyanobacterium was exposed to UV-B radiation there was considerable increase in the MAA content, however, high light resulted in considerable decline in the MAA content (Fig. 3.35). This antagonistic effect of UV-B and high light on MAA may probably explain greater damage to *Phormidium* under *in vivo* conditions even when having ability to produce higher MAA under UV-B conditions.

4.10.2. PHENOLIC COMPOUNDS:

The role of phenolic compounds as defense mechanisms in various terrestrial plants is well studied while there are no reports on the occurrence of phenolic compounds in cyanobacteria. But in our study we observed the certain compounds showing absorbance in the UV-B region, generally shown by phenols or its derivatives (Fig 3.37, 3.38). Increase in the phenolic compounds in this study may be one of the protective mechanisms of cyanobacteria against stress conditions, as phenolic compounds are able to absorb radiation in the UV range. Van de Staaij et al., (1995) observed that phenolic acids absorb highly in the UV spectral region and suggested a role in protection from UV-B radiation.

There are few reports on the presence of phenolic compounds derivatives in different algae. McMillan et al., (1980) reported that the presence of sulphated flavones and sulphated phenolic acids in sea grasses. Dawson and Dennison, (1996) observed that sea grasses rely on the production of UV absorbing pigments for protection against harmful effects of UV radiation. Abal et al., (1994) reported flavonoids in marine phanerograms, which absorb at slightly longer wavelength than in other marine plants with absorbance peaks located between 330 and 345 nm.

In plants, however, induction of phenolic compounds in response to UV-B is well documented. It has been reported that secondary metabolites such as phenolic compounds and flavonoids accumulate in the epidermis and shield the underlying tissues against harmful UV-B radiation (Sharma et al., 1997, Sharma et al., 1998a). Bornman and Vogelmann, (1991) suggested that it is the cellular location of UV-B-screening

phenylpropanoid pigments, rather than the total amounts present in leaves, that is of importance in UV-B protection. Also, increased production of various phenylpropanoids in response to UV-B irradiation has been widely reported (Rozema et al., 1997) and associated with inter and intraspecific differences in UV-B sensitivity (Day et al., 1994).

It is known that the derivatives of the phenylpropanoid pathway include flavonoids, hydroxycinnamic acid derivatives and related phenolics, which absorb UV-B radiation. Negative correlations have consistently been observed between leaf phenylpropanoid concentrations and UV-B transmittance in many taxa (Day, 1993), with different UV-B absorption spectra apparent in whole-leaf extracts of woody and herbaceous forms (Day et al., 1994). The UV-B absorption, strong antioxidant and energy dissipating properties of phenylpropanoids assist in limiting damage to the photosynthetic apparatus (Tevini et al., 1991) and to DNA (Stapleton and Walbot, 1994).

Solar ultraviolet-B radiation is known to stimulate the enzymes PAL (Phenylalanine Ammonium Lyase) and CHS (Chalcone Synthase) and other branch-point enzymes of the phenyl propanoid pathway. PAL catalyses the transformation of phenylalanine to *trans*-cinnamic acid, which leads to the formation of complex phenolic compounds such as flavonoids, tannins and lignin. Expression study of APL, 4CL and CHS under UV-B condition is under progress.

In addition to the absorption of UV-B radiation, these polyphenolic compounds also serve as an antioxidant, specially the anthocyanin (Sarma, 1996). Many other functions such as in allelopathy, signal transduction, plant hormones and chemical defense against microorganisms, herbivory and structural rigidity have been reported.

4.10.3 ANTIOXIDANTS:

There are number of defense mechanisms against active oxygen species (AOS) which primarily divided into two major groups enzymatic and non-enzymatic antioxidant systems. UV-B and high light treatment resulted in higher activity of superoxide dismutase and ascorbate peroxidase, which metabolizes active oxygen radicals and free radicals generated during the treatment as seen by oxidative damage to membrane lipids, and thus may provide some protection against photodamage (Fig. 3.41, 3.42). However, induction of SOD and APX was considerably less in these two cyanobactetria than observed in other organisms, which may explain damage to photosynthesis and membrane under our treatment conditions. This limitation could be genetic as after a slight induction of SOD under short duration of the UV-B and high light treatment, no further increase in the enzyme activity was observed with longer duration of the treatment indicating limitation of SOD in quenching ROS. However, APX showed continuous but slight increase in its activity due to the treatments. These results also indicate limitation in quenching of superoxide radicals while hydroxyl radicals and hydrogen peroxide may be quenched more efficiently. Ascorbate, a non-enzymatic antioxidant system, however, remained constant as a result of the UV-B treatment even after 6 h.

Our results support various studies, which indicate increase in the SOD and APX activity in response to stress conditions. It has been reported that UV-B and high light exposure resulted in enhanced superoxide dismutase and ascorbate peroxidase activity to protect it from UV-B/high light induced oxidative damage in cyanobacteria, algae and

higher plants (Lesser et al., 1996; Hazzard et al., 1997; Aguilera et al., 2002; Shirkey et al., 2000; Danno and Horio, 1987). Mittler and Tel-Or (1991) observed that high activity of APX and catalase in cells of the unicellular cyanobacterium, *Synechococcus* PCC 7942 could effectively remove low concentrations of peroxides. Mackerness et al., (1998); Boldt and Scandalios, (1997) also showed that increased accumulation of antioxidant enzymes in *Anabaena* spp to continuous moderate UV-B treatment reversed the oxidative stress and oxidative damage.

Barros et al., (2003); Lornmann et al., (2004) also reported greater increase in SOD which prevented peroxidation of lipid under higher AOS conditions but no increase in CAT and APX activity indicating a particular antioxidant enzyme may be specific to a particular algae. Herbert et al., (1992) reported that a mutant strain of *Synechococcus* spp strain PCC 7942 lacking FeSOD activity was shown to be much more sensitive to AOS.

SOD activity has been shown to be induced under photooxidative conditions due to high light in bloom forming cyanobacterium, *Microsystis aeruginosa* (Canini et al., 2001) and plants (Jansen et al., 1989). Yoshimura et al., (2000); Sen Gupta et al., (1993) also reported that under high light conditions, the steady state transcript level of cytosolic ascorbate peroxidase (APX) was found to increased in Spinach. Our results with antioxidant enzymes indicate that slight increase in the SOD activity in *Nostoc* and greater increase of SOD in *Phormidium* due to high light treatment and this was correlated to greater decline in the photosynthetic efficiency (Fv/Fm ratio) in *Nostoc* compared to *Phormidium*. Varkonyi et al., (2000) observed a membrane associated SOD to protect membrane molecules from oxidative damage in an environment of high oxygen

concentration in filamentous cyanobacteria. Li et al., (2002) also reported MnSOD in cyanobacteria associated with membranes and suggested that membrane associated MnSOD is possibly related to the function of protecting thylakoid membranes from superoxide anion damage.

There are also reports of no activity of antioxidant enzymes due to stress conditions. Araoz and Häder, (1999) observed that in *Nostoc* spp, there was no change in peroxidase (POD) activity due to solar radiation as well as artificial UV-B radiation and increased activity of POD in *Nostoc muscorum* after exposure to 2h of solar UV-B radiation that decreased after prolonged exposure time. They suggested that observed differences between both cyanobacteria species in activity of POD are important to assess the defense capacity of both the organisms to cope with oxidative damage and also observed the induction of new antioxidant isoenzymes together with the increased activity and the number of isoforms in *Nostoc* spp compared to *Nostoc muscorum,* therefore, indicating better protection to *Nostoc* spp from UV-B induced oxidative damage. Miyake et al., (1993) reported that the scavenging systems for hydrogen peroxide (H_2O_2) involve POD and APX enzymes as well as non-enzymatic scavengers such as ascorbic acid and α–tocopherol, which requires the presence of ascorbate to metabolize H_2O_2 into H_2O. Foyer et al., (1994) reported that ascorbate is regenerated by the photosynthetic electron transport chain or by the ascorbate glutathione cycle. In our study we also observed that APX activity was greater than SOD activity and ascorbate content remained almost unchanged as a result of the UV-B treatment. Though even a moderate increase in SOD and APX activity had been shown to improve tolerance to oxidative stress (Araoz and Hader, 1999; Mackerness et al., 1998), in plants as much as

400% increase in SOD and APX has been reported which also failed to provide complete protection against the oxidative damage (Sankhalkar and Sharma, 2002). In this study we have observed increase only up to 20-40% which may not be sufficient to provide a good protection against the higher doses of UV-B/high light but might be effective against moderate level of such stresses. Low induction of SOD and APX under stress condition may indicate shade adapted nature of the organisms.

With specific problem of bleaching of coral associated with *Phormidium*, we have observed that induction of the SOD and APX was considerably less and was found to be for short duration. Longer duration of the treatment further declined the activity of SOD and APX activity. This may explain greater damage to growth and oxidative damage in the species under longer duration of the UV-B and high light treatment. Ascorbic acid content, a non-enzymatic antioxidant, was also found to be less in *Phormidium* (1.2mg g^{-1} F.W.) and UV-B and light treatment slightly decreased the ascorbate content (data not shown). It seems that inability of *Phormidium corium* to quench AOS species on account of low inherent level of antioxidant enzymes and ascorbic acid and limited induction of antioxidant enzymes on imposing of the stress resulted in greater formation of AOS that led to oxidative damage and subsequent bleaching and death of the coral reefs. Downs et al., (2002) observed strong positive correlations between accumulation of oxidative damage products and bleaching in corals. They also observed that coral bleaching is tightly coupled to the antioxidant and cellular stress and that coral bleaching (algal loss) may be a final strategy to defend corals from oxidative stress.

Our study shows the bleaching of coral reefs may be due to the oxidative damage caused to symbiotic algae as a result of generation of AOS under high UV-B and PAR conditions due to inefficient enzymatic (SOD and APX) and non- enzymatic (ascorbic acid) antioxidant system. The formation of AOS resulted in repelling of corals from symbiont algae as a protection to itself against AOS loading which subsequently may result in starving and death of the corals as autotrophic algae are the source of providing food to corals.

4.10.4. DNA REPAIR:

Cyclobutane dimers, the well-known DNA lesions induced by UV-B also possess enzyme-catalyzed repair in the presence of visible and UV-A light. It has been reported that DNA damage occurs when cyclobutane-type pyrimidine dimers are formed by cross-linking of strands, these dimers can subsequently be monomerized, a process known as photo reactivation or photo repair, by the blue/UV-A-activated enzyme photolyase This repair of UV-B induced thymine dimers has been reported to occur within a few hours in visible light in marine phytoplankton (Buma et al., 1996; Gieskes and Buma, 1997). However, we have not studied the DNA repair mechanism.

Sancar, (1996) reported that CPDs can be photorepaired by a specific enzyme (photolyase) in the presence of and using the energy of UV-A or visible light at permissive temperatures. Ehling-Schulz and Scherer, (1999) showed repair mechanisms such as photo reactivation as well as light dependent DNA repair mechanism, which may also helps in overcoming deleterious effects of UV-B radiation. The enzyme-catalyzed reversal of CPDs in situ has been found in a wide range of terrestrial and aquatic plants

and reported to be saturated at relatively low photon level (Hidema et al., 1997). Both these processes, protection as well as repair are seems to be stimulated by low visual radiation, therefore, showing relatively less damage to photosynthesis and related processes in this study when UV-B treatment is supplemented with low level visual radiation. Similar results on photodamage under UV-B supplemented with low visual radiation were observed in this study. It was also seen that UV-B supplemented with low visual light also resulted in much higher level of MAAs accumulation again suggesting mitigating effect against UV-B damage.

Rozema et al., (2002) observed that DNA repair by photolyase may effectively remove incidence of CPDs formation in *Cladonia arbuscula*. They also studied temperature dependence of the formation and photorepair of the two most frequently UV-B-induced DNA lesions, CPDs and (6–4) photoproducts.

The distinct photolyase enzymes that specifically reverse CPDs and 6-4PPDs have been identified in higher plants (Batschauer, 1993; Nakajima et al., 1998). These enzymes recognize UV-induced lesions and reverse dimerization by absorbing light between 350 and 450 nm (Pang and Hays, 1991). Same kind of mechanism has also seen in the unicellular alga, *Chlorella pyrenoidosa* (Hsu et al., 1998). Apart from light-dependent photolyases, various lights independent, versatile repair mechanisms such as nucleotide excision repair and recombinational repair have been detected in plants (Liu et al., 2000). These responses are not specific for UV-induced damage and involve multiple enzymatic steps that remove and replace damaged DNA fragments. Kinetics of DNA repair are fast when light is adequate for photolyase activity, whereas DNA repair rates in

darkness are considerably lower (Pang and Hays, 1991, Quaite et al., 1992, Takeuchi et al., 1996). Both light-dependent and independent DNA repair have been observed in red macro algae *Rhodymenia pseudopalmata* and *Palmaria palmata* (Pakker et al., 2000b), indicating that macroalgae posses the same DNA repair mechanism as higher plants.

The presence of peptides seen in both species in this study (Fig 3.29, 3.30) will be an important aspect to further study, as peptides are known to have microtoxicity. Cyanobacteria produce a large number of peptide compounds e.g. micropeptins, cyanopeptolins, microviridin, circinamide, aeruginosin, with varying bioactive and potential pharmacological application (Okino et al., 1992). They also produce numerous secondary metabolites such as macrolides, alkaloids, sulphur compounds, cytotoxins, fungicides, which show characteristic biological activities including cytotoxic, immunosuppressive, antifungal, antiviral, cardioactive and enzyme inhibitory activity (Namikoshi and Rinchart, 1996).

CONCLUSION:

UV-B, high light and UV-B supplemented with low light exposure caused damaging effect on various parameters such as morphology, photosynthesis, photosynthetic pigments, sugar content, nitrogen, phosphoglycolipids, peroxidation of cell membrane lipids, degradation of the cell membrane proteins, DNA and RNA etc in both *Nostoc spongiaeforme* and *Phormidium corium.* The observed effect may be consequences of generation of active oxygen species as a result of the treatments. The resultant oxidative damage leads to the decreased survival of the test organisms. Changes in some of the parameters such as photosynthesis, peroxidation etc. may be a consequence of damage while changes in other parameters such as carotenoids, phycobilins, lipids fatty acids may be a result of adaptation process and still further changes in some other parameters such as MAAs, SOD, APX and phenolic compounds etc. may be inorder to provuide protection against the harmful effect of UV-B and high light.

We have observed some of the protective and adaptive processes of cyanobacteria due to UV-B, high light and UV-B supplemented with low light, such as synthesis of UV-B absorbing compounds like mycosporine like amino acids (MAAs). Work also shows that in response to oxidative damage, activity of antioxidant enzymes such as SOD and APX increased to provide protection against the damage. Role of fatty acid composition of membrane in photoinhibition in protection of the organism is also suggested.

Damaging effect of UV-B radiation was attenuated to some extent when UV-B treatment was supplemented with low level PAR, the survival of cyanobacteria was slightly better and damage to photosynthesis, sugar and lipid peroxidation was also attenuated to some extent. This may be due to repair mechanisms such as photo reactivation as well as light dependent DNA repair mechanism which helps in overcoming deleterious effects of UV-B radiation. Better survival of alga and lesser damage to photosynthesis, sugar and lipid peroxidation under UV-B treatment supplemented with low-level visual radiation may also be due to increased synthesis of UV-B absorbing compounds such as mycosporine like amino acids (MAAs), quenchers such as β-carotene and increase level of antioxidant enzymes such as SOD and APX formation.

FUTURE PLAN OF WORK:

1. **Microtoxins:**

   Cyanobacteria produce a large number of micropeptides, which are known to have microtoxicity. These microtoxins are known to have commercial applications. The characterization and isolation of microtoxins will be studied in detail.

2. **Assay of nitrogenase activity:**

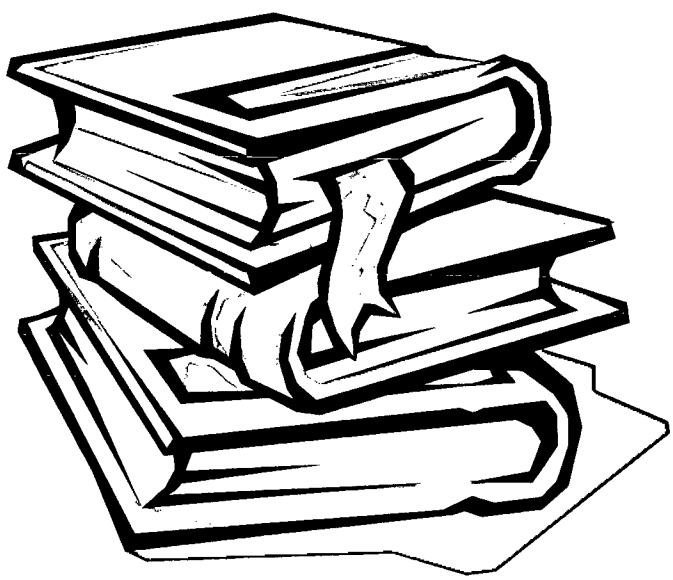
   The ability of cyanobacteria to fix nitrogen using enzyme nitrogenase will be studied.

2. **Assay of enzyme involved in lipid synthesis:**

   Enzymes such as desaturase, saturase which is involved in lipid synthesis will be studied in detail.

3. **Gene expression studies:**

   Gene expression studies for three enzymes such as phenylalanine (PAL), 4-coumarate (4-CO), chalchone synthetase (CHS) which are involved in the synthesis of UV-B absorbing pigments such as phenolic compounds will be studied.



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High Light Induced Changes on Photosynthesis, Pigments, Sugars, Lipids and Antioxidant Enzymes in Freshwater (*Nostoc spongiaeforme*) and Marine (*Phormidium corium*) Cyanobacteria.

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ABSTRACT

Effects of high light (500 μmol m^{-2} s^{-1} PAR) on cyanobacteria *Nostoc spongiaeforme* Agardh, a fresh water alga and *Phormidium corium* Agardh (Gomont), a marine alga with respect to photosynthesis, pigments, sugar content, lipid peroxidation, fatty acids composition, antioxidant enzymes activity and DNA was studied. It was seen that Fv/Fm ratio, indicative of photosynthetic efficiency, decreased due to the light treatment. The damage to photosynthesis occurred at the level of both antenna system as well as at the PS II level. Photo bleaching of photosynthetic pigments was also observed. High light treatment also resulted in decline in sugar content, probably due to the effect on photosynthesis. Peroxidation of membrane lipids, indicating oxidative damage to lipids and level of unsaturation in the cell membrane, was also observed. Activity of antioxidant enzyme superoxide dismutase (SOD) and ascorbate peroxidase (APX) was increased, probably as a result of oxidative damage observed in the form of lipid peroxidation. Quantitative decrease in phospho and glycolipids was also observed. Unsaturation level of fatty acid composition of both total lipids as well as glycolipids remains unchanged in both the species, however, saturated fatty acid declined slightly changing the ratio in favor of unsaturated fatty acids. Degradation of DNA was also observed in both the species studied. Results showed transient plateau during 2-4h of the high light treatment in Fv/Fm ratio, phycobilisome pigments, sugars and antioxidant enzymes after initial decrease within 1h of the treatment, which may indicate period of partial adaptation to high light as a combined efficiency of protective processes operational in the two species, which subsequently failed upon longer exposure of 4-6 h.

Key words: chlorophyll fluorescence, cyanobacteria, high light, lipid peroxidation, pigments, phosphoglycolipids, photosynthesis, sugars

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—UV-B induced changes on photosynthesis, membrane lipids and MAAs in marine cyanobacterium, *Phormidium corium* (Agardh) Gomont

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UV-B radiation (0.8±0.1 mW cm^{-2}) and UV-B radiation supplemented with low intensity PAR (~80 μ mol m^{-2} s^{-1}) affected photosynthesis at the level of antenna system as well as PS II reaction centre (F_o and F_m declined) in *Phormidium corium* (Agardh) Gomont. UV-B radiation resulted in decline in sugar content, peroxidation of membrane lipids as well as quantitative and qualitative changes in phosphoglycolipids and neutral lipids. Fatty acid profile did not show any qualitative changes due to the treatment, however, UV-B supplemented with low PAR resulted in slightly higher level of unsaturation. *P. corium* synthesized MAAs in response to UV-B. Quantity of MAAs increased when UV-B treatment was supplemented with low level PAR.

Keywords: Chlorophyll fluorescence, Cyanobacteria, Lipid peroxidation, Mycosporine like amino acids, Phosphoglycolipids, Sugars, UV-B radiation.
IPC Code: Int. Cl.[7]

Depletion of the stratospheric ozone layer has resulted in an increasing incidence of solar UV-B radiations (280-320 nm) at the Earth's surface[1]. Since cyanobacteria use sunlight for photosynthesis, they are also exposed to UV-B radiation. UV-B is a small component of the solar radiation reaching the earth's atmosphere and is known to penetrate to different levels in fresh water as well as marine environments[2]. The energy level of UV-B photon is high which makes it photochemically active form of radiation and it is largely absorbed by DNA, protein, chromophores, pigments, plastoquinone and plastoquinol. This lead to wide ranging effects, including alteration in the structure of proteins, DNA and depression of key physiological processes[3]. A number of physiological and biochemical processes in cyanobacteria such as growth, pigmentation, photosynthesis, enzymes of nitrogen and carbon metabolism have been reported to be susceptible to UV-B[4-5]. In this study we investigated the effect of UV-B and UV-B radiation supplemented with low intensity PAR on photosynthesis, lipids, sugars content and mycosporine like amino acids (MAAs) in a cyanobacterium, *Phormidium corium* (Agardh) Gomont, isolated from coral reefs of Lakshadweep Island, India.

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Materials and Methods

Phormidium corium was obtained from Dr C Raghukumar, National Institute of Oceanography, Goa. It has been isolated from corals of Lakshadweep Island, India. The cultures were grown in ASN III medium (Rippka *et al*[6]) and maintained at 30°±2°C under fluorescent light at 80 μmol m^{-2} s^{-1} PAR with 12h light/12h dark cycle. All experiments were carried out during active growth phase inoculating 30 days old culture of the organism.

Exposure to UV-B radiation—UV-B exposure of 0.8±0.1 mW cm^{-2} was given to the cells while stirring in a BOD chamber for 0-6 h. UV-B radiation was measured using a UV-B radiometer (Radiometer model VLX-312 and light source model T-6M with a λ-max at 312 nm were from Vilbour-Lourmat, France). For certain experiments, white light of 80 μmol m^{-2} s^{-1} PAR was supplemented using light source with fibre optic at 60° angle to the culture during UV-B treatment.

Photosynthesis measurement—Photosynthesis was measured using chlorophyll fluorometer (PAM, Walz, Germany) according to Sharma *et al*[7]. Culture was dark adapted for 10 min prior to measurement. The dark-adapted culture was exposed to a modulated light at 4 μmol m^{-2} s^{-1} to measure initial fluorescence (F_o) followed by exposure to a saturating pulse of white light of 4000 μmol m^{-2} s^{-1} to provide the maximum fluorescence (F_m). Variable fluorescence