

Effect of Ultraviolet (UV)-B Radiation on Morphology and Photosynthetic Activity of *Spirogyra* sp.



Davendra Singh Malik and Prachi Rathi

Abstract *Spirogyra* sp. is phytoplankton species belonging to *chlorophyceae* group and also use as a bioindicator. They are very sensitive to overexposure from solar ultraviolet (UV) radiation. In this study, *Spirogyra* sp. was collected from freshwater and then cultured in the Pringsheims Medium. The aim of the present study was to find out the impact of ultraviolet (UV-B) radiation on morphology and photosynthetic activity of *Spirogyra* sp. For this different experimental setup were divided on the basis of time duration. Exposure duration was 2 h/day for six groups except control. Reading of artificial UV-B was recorded at 5th, 10th and 15th day respectively and one is kept as control (covered with acrylic sheet to avoid any light penetration). After 15th days of artificial UV-B exposure it is observed that chlorophyll content decreased and carotenoid increased. Maximum effect and fragmentation were observed after 15th day of artificial UV-B exposure. The study provides basic scientific data about the impacts of UV-B radiations on aquatic ecosystem.

Keywords *Spirogyra* species · UV-B radiation · Chlorophyll · Carotenoid

1 Introduction

Phytoplankton is an autotrophic individual which found in upper most layer (up to 200 m) of the surface water body. In freshwater, various phytoplankton species found which are belonging to different phytoplankton groups. The most common is green algae which belong to *Chlorophyceae* group and are among the oldest group living on the earth's surface [5]. Algae are spread in soils, swamps, wetlands, and freshwater resources such as river and lakes. In green algae, *Spirogyra* sp. well known freshwater bioindicator species and also known for its pharmacological activities as an anti-hypertension resulted from its isolated gallic acid [8]. In modern era, one of the serious environmental problems on earth has been experiencing due to increase

D. S. Malik · P. Rathi (✉)

Department of Zoology and Environmental Science, Gurukul Kangri (Deemed to be University), Haridwar, Uttarakhand 249404, India

e-mail: rathi.pr94@gmail.com

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in ultraviolet (UV) radiation. The ozone layer protects us from UV radiation, but due to the higher pollution the amount of green house gases such as CFCs, CCS and organic bromides is increase and they are responsible for damage the ozone layer [6]. The solar radiation penetrates in it up to some depth and provides them an optical energy for the process of photosynthesis and production of food [10]. Phytoplankton organisms are preferred to move into the photic zone due to their energetic requirements for solar radiation and on the other hand they are affected by excessive solar radiation. The increased amount of UV radiation which penetrates the euphotic zone has affected the phytoplankton productivity. These autotrophic organisms live in an environment where factors such as light availability, uptake of nutrients, sinking and grazing pressure affects the growth and distribution. Since algae are simple, small sized organisms do not contain protective layers as the skin, so the effect of these rays will be fast and direct [9]. The effects of short ultraviolet rays in these organisms are shown by their diminished ability to adjust their position in the water column. So, keeping this view, we investigate the impacts of UV-B radiation on photosynthetic pigments in *Spirogyra* sp. on different time exposure.

2 Materials and Methods

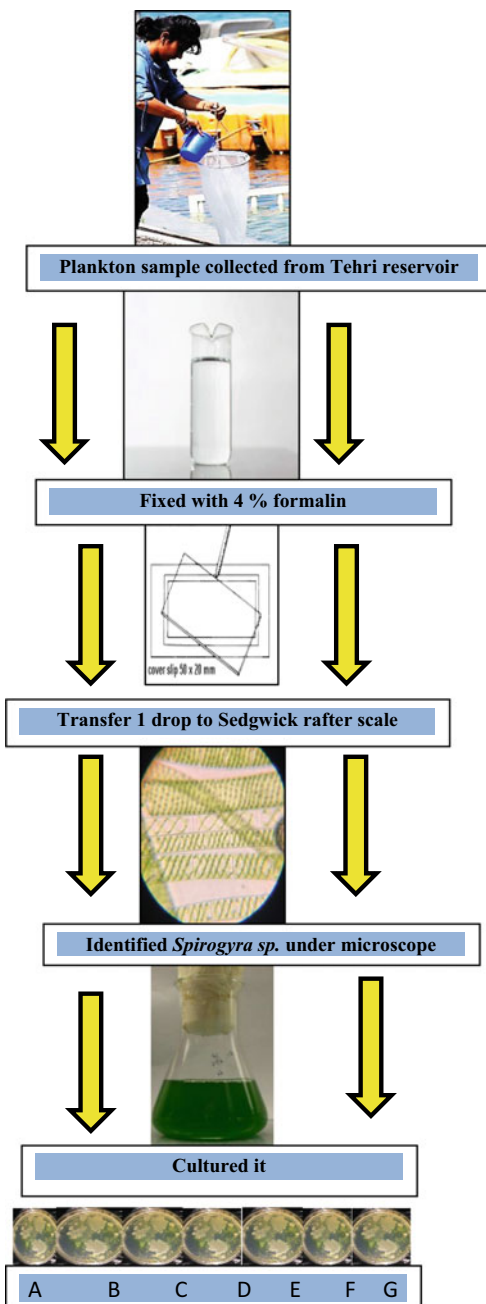
2.1 Identification and Culturing of *Spirogyra* sp.

Methodology and culturing of *Spirogyra* sp. were shown in Fig. 1. One drop of collected plankton sample was putted on Sedgwick rafter scale (because Sedgewick rafter holds a much higher volume, and is good for large species and useful for the abundance study of mixed samples). Identifying under a light microscope (Optika B-150 series) with camera attached in to it. Identified *spirogyra* sp. was isolated and then grown in a Pringsheims medium, for multiple growths of selected species. The equal number of tested organisms was then transmitted into seven different sterilised petri dish by using magnifying glass (Carson triview 5x, 10x, 15 × folding loupe with built in case tv-15).

2.2 Experiment Set up for Laboratory Experiment

Seven wooden piece frames were taken of same dimension (45 cm long and 22 cm wide). Each has one circular open space (diameter = 18 cm) and fitted them on seven different and the test organisms may not pass through the net and remains at the surface as shown in a Fig. 2. Seven aquariums were taken for the experimental set up and divided them into seven groups. Group A (control), group B (expose to natural solar radiation for 5 days), Group C (expose to natural solar radiation for 10 days), Group D (expose to natural solar radiation for 15 days), Group E (UV-B exposure for

Fig. 1 Methodology of collection and culture of *Spirogyra* sp.

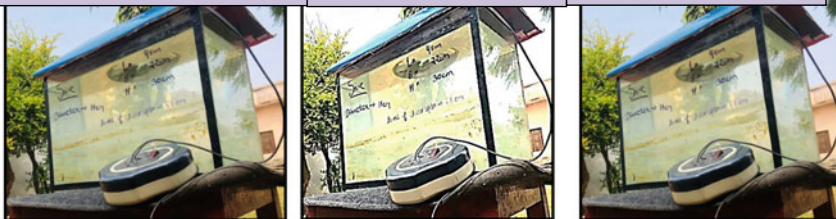




Group A (Control)



| | | |
|---|--|--|
| Group B (Natural solar exposure for 5 days) | Group C (Natural solar exposure for 10 days) | Group D (Natural solar exposure for 15 days) |
|---|--|--|



| | | |
|---|--|--|
| Group E (Artificial UV-B exposure for 5 days) | Group F (Artificial UV-B exposure for 10 days) | Group G (Artificial UV-B exposure for 15 days) |
|---|--|--|

Fig. 2 Laboratory experiment set up

5 days) Group F (UV-B exposure for 10 days) Group G (UV B exposure for 15 days). Petri plates containing the material in 10 ml of culture solution were exposed to a distance of 15 cm from the source for equal irradiation of tested organisms. Group A was covered by acrylic sheet and kept in a laboratory. Group B, C and D were kept outside the lab for natural solar exposure and group E, F, G were kept in a lab to provide artificial UV-B through UV-B Philips lamp of intensity 0.638 at 312 nm. The exposure was given for two hour/day. Natural solar radiation was measured by coleparmer radiometer (WW-09811-56) daily.

2.3 Determination of Total Amount of Chlorophyll and Carotene Pigments

The effect of natural solar and artificial UV-B radiation on chlorophyll content, carotenoid and fragmentation were investigated in seven different groups. After exposure, samples were treated with acetone for the pigment extraction i.e., for chlorophyll a, b and total carotenoid. Samples were filtered and crushed with 90% acetone in mortar so that the pigment comes out of the cell. The obtained solution vortexed and then centrifuged at around 2000 rpm for 5 min after centrifugation, supernatant was separately collected and pigments (chlorophyll 'a', Chlorophyll 'b' and total carotenoid) was estimated spectrophotometrically using UV-VIS spectrophotometer. The chlorophyll concentration and the concentration of carotenoid were determined using equations of [12, 13].

3 Result and Discussion

Solar UV-B radiation reaching the earth surface exerted significant effect on microalgae [4]. Photosynthesis is potentially the main target of UV radiation due to multiplicity of possible effects [7]. Daily natural solar radiation variation during the study period was shown in Fig. 3. Effect of natural solar radiation and artificial UV B on photosynthetic pigments of *Spirogyra* sp. was shown in Table 1.

Natural solar radiation in Haridwar was measured in range from 0.512 to 0.955 mW/cm² (Fig. 3). Minimum natural solar radiation was measured on 9th day during the experimental study it was due to the cloudy weather on that day.

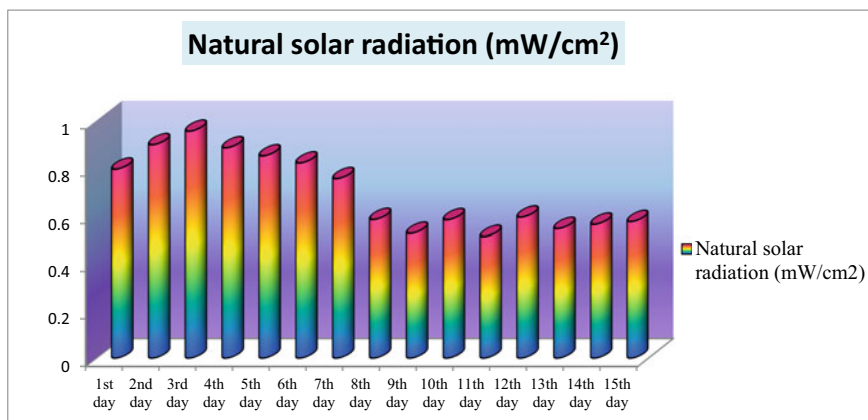


Fig. 3 Natural solar UV radiation variation of fifteen days in Haridwar

Table 1 Effect of solar and artificial ultraviolet radiation on *spirogyra* sp. in laboratory

| Parameter/experiment setup for 15 days | Control (Group A) | Natural solar UV | | | Artificial UV-B exposure | | |
|--|-------------------|------------------------------------|-------------------------------------|-------------------------------------|------------------------------------|-------------------------------------|-------------------------------------|
| | | After 5 days of exposure (Group B) | After 10 days of exposure (Group C) | After 15 days of exposure (Group D) | After 5 days of exposure (Group E) | After 10 days of exposure (Group F) | After 15 days of exposure (Group G) |
| Chlorophyll 'a' (mg/l) | 10.46 ± 0.08 | 9.51 ± 0.35 | 9.45 ± 0.38 | 9.38 ± 0.32 | 6.42 ± 0.31 | 5.79 ± 0.28 | 4.63 ± 0.53 |
| Chlorophyll 'b' (mg/l) | 3.95 ± 0.79 | 3.82 ± 0.88 | 3.76 ± 0.28 | 3.65 ± 0.23 | 3.05 ± 0.65 | 2.66 ± 0.49 | 2.28 ± 0.45 |
| Carotenoid (mg/g) | 3.99 ± 0.17 | 4.31 ± 0.25 | 4.35 ± 0.18 | 4.42 ± 0.21 | 5.26 ± 0.21 | 6.33 ± 0.17 | 7.19 ± 0.09 |
| Fragmentation | — | — | — | — | + | + | ++ |

The effects of UV-B photosynthetic pigments chlorophyll content showed decreasing trend with increasing duration/time period of natural solar radiation and artificial UV-B radiation. The chlorophyll content decreased in all exposure either it is natural solar radiation or artificial UV-B exposure. It was found that highest chlorophyll content (10.46 ± 0.08) was found in Group A (control) and lowest (4.63 ± 0.53) in Group G (15 days of UV-B exposure). Chlorophyll 'b' pigment was found maximum (3.95 ± 0.79) in Group A (control) and minimum (2.28 ± 0.45) in Group G (15 days of UV-B exposure).

Carotenoids are the accessory pigments in plants that absorb light energy for use in photosynthesis. Highest carotenoid content (7.19 ± 0.09) was found in a group G (after 15 days of UV-B exposure) while lowest carotenoid content (3.99 ± 0.17) was found in group A (control). They also protect chlorophyll from photo damage [1]. The increase in the amount carotenoids when exposing algae to UV-B is due to its important role in protecting chlorophyll. Carotenoids cover the chlorophyll and protect it from the negative impact of these rays. Carotenoid content increases as the stress or duration of solar radiation on phytoplankton increases [14].

Fragmentation of filaments, shrinkage, deformation and degeneration of chloroplasts were frequently noticed in all treatments of UV-B radiation but not in an exposure of natural solar radiation and in control. The degree of the above effects was found to be linearly increased with increase in the time period to which the algae was exposed. The responses of *Spirogyra* sp. are depending on duration/time period of UV-B radiation exposure and protective mechanism of the organisms being exposed. *Spirogyra* sp. was exposed to UV radiation (artificial and solar) for 5, 10 and 15 days. There is no fragmentation in *Spirogyra* sp. cell wall was found in control and natural solar but on UV-B exposure low fragmentation was found on 5th day and the fragmentation increases as the number of days increases. Highest fragmentation was found after 15 days. Morphological changes upon UVR exposure may be due to the release and accumulation of reactive oxygen species that oxidizes the lipids of sheath or cell membrane in the presence of UV radiation [11]. Deformation in *Spirogyra* sp. first observed in chloroplast and then breakage of filaments (after 15 days treatment). It showed deformed chloroplast and lack of usual spiral arrangement in the UV exposed *Spirogyra* sp. filaments. Besides, UVR also induced photo orientation in *Spirogyra* sp. it shows migration to its lit area. This behaviour may be considered as a tolerant strategy against UV-B radiation [2, 3, 15].

4 Conclusion

In the present study it can concluded that artificial UV-B possess potential impact on morphology and physiology of *Spirogyra* sp. when exposed for 5th, 10th and 15th days. Morphological changes include deformation in *Spirogyra* sp. was observed in chloroplast along with the breakage of filaments (after 15 days treatment). It showed deformed chloroplast and lack of usual spiral arrangement in the UV exposed *Spirogyra* filaments. Besides, UV radiation also induced photo orientation in *Spirogyra*

sp. it shows migration to its little area. This behaviour may be considered as a tolerant strategy against UV-B radiation. The fragmentation in the selected filamentous algae was corresponding to its exposure to UV radiation. No fragmentation was observed in Group A, Group B, Group C and Group D but fragmentation increased as we move from Group E to Group G due to the harmful UV-B radiation effect on *Spirogyra* sp. cell wall it may be due to the release and accumulation of reactive oxygen species that oxidizes the lipids of sheath or cell membrane.

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