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Monitoring of Algal Bloom in Freshwater Lakes, Extraction and Evaluation of Antibacterial Compounds

Shailaja Boorla[1], Akshaya Choppadhandi[2], Shivaleela Kota[3], Gunaswetha Kuraganti[4]

[1-4] *Telangana Social Welfare Residential Degree College (W), Mancherial, Telangana*

*Email: gunaswetha91@gmail.com

ABSTRACT

Cyanobacteria (Blue-green algae) is a photosynthetic bacteria found in both freshwater and marine habitats. Some of the earliest known life forms on Earth are cyanobacteria, which are also thought to be the first species to produce oxygen as a consequence of photosynthesis. They are significant primary producers in aquatic habitats, giving other creatures energy and nutrients. In industrial and agricultural activities including wastewater treatment, bioremediation, and the creation of biofuels, they are also employed. Algal blooms were tracked as part of the Open Filed Collective Project, and each month their diversity is recorded. The freshwater lake near Adilabad and the Mancherial district is being monitored as part of this study by taking water samples, which are then analyzed for physiological features like pH, daytime temperature, the presence of algae in the water, and odor. A spectrophotometer is used to determine the number of algae in the water, and a foldscope and compound microscope are used to view the cells. In order to develop the algae species in tap water, growth promoters like $CaCO_3$ and $NaNO_3$ are added. Following filtering, the algal mat is dried by air. The algal dry mat is pulverized and extracted using a liquid-solid mixture of methanol and ether (7:3). Chromatography is then used to examine the chemicals recovered from the extract using the solvent mixture. The Agar well diffusion method is then used to test for antibacterial activity by the crude extract. By evaluating the Zone of Inhibition against E. coli, S. typhi, and M. luteus, antibacterial activity is measured and findings are documented. The zone of inhibition is measured to be 7mm on average. The suggested framework will assist in keeping track of a water body's biodiversity of species as well as its amount of algal bloom. This will allow for the aquatic ecosystem's health to be monitored, maybe allowing for the discovery of new microbial species.

Key words - Algal Bloom, freshwater lakes, Foldscope, Spectrophotometer, Extraction, Antibacterial activity.

INTRODUCTION

Cyanobacteria also known as blue green algae, are a particular class of bacteria that photosynthesize or create they generate food by changing light energy into chemical energy (Hachicha et al. 2022) They are some of the oldest living things on the planet and were instrumental in creating the oxygen-rich environment that promoted the development of complex life forms. While some cyanobacteria species are advantageous, others can cause toxic algal blooms in aquatic situations (O'Neil et al. 2012). Given their ability to thrive in both challenging and ideal environmental settings, cyanobacteria are found in both terrestrial as well as aquatic ecosystems. These organisms create a wide range of bioactive substances with excellent biocompatibility that may find value in business, agriculture, and medicine. Freshwater-dwelling cyanobacteria produce a wide variety of bioactive substances with medicinal uses such as antimicrobial action (antibacterial, antihelminthic, antifungal, and antiviral chemicals), cytotoxic activity, and anti-inflammatory compounds (Demay et al. 2019; Tiwari and Tiwari,2020; Srinivasan et al. 2021). In light of climatic pressure, oxygen content, and struggle with other microbial strains nearby, antimicrobial synthetic substances are delivered (Şen Karaman et al. 2020).

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The antibacterial synthetic compounds are all the more structurally stable and reliable. Cyclic lipopeptides like cyclosporin A, polyketides like myxoloactone, nonribosomal peptides like microcystins, and various heterocyst glycolipids secreted by heterocyst cyanobacteria are among the antimicrobial bioactive substances made by cyanobacteria (Bionda et al. 2013).

Since cyanobacteria are the ecosystem's principal producers, they feed other creatures, manage the nutrient cycle, produce oxygen, and fix nitrogen. The atmospheric parameters, including as pH, temperature, oxygen concentration, and precipitation content, have a significant impact on the cyanobacteria's ability to flourish in freshwater habitats (Kuraganti et al. 2020). The potential and health of the habitat are dependent on the presence and growth of cyanobacteria under the impact of these conditions (SILVER et al. 2020; Kuraganti et al. 2020).

Our present work is collaborated with Open Field Collective Monitoring Algal Loom Citizen Science project. Open field collective is a communicate driven initiative that is designed to empower individuals and community to become active participants in monitoring and understanding these blooms which can help to mitigate the impacts (https://openfielddata.org/home.php). By providing information to the public on the sources, effects, and solutions to algal blooms, citizens can become better informed and more engaged in protecting their local water resources. In this project Adilabad freshwater Mavala lake has been chosen for the analysis of water body to the environment factors for a year i.e. from November 21 from to November 2022.

MATERIAL AND METHODS

Collection and processing of water sample:

Freshwater lake Adilabad Mavala was the source of the water samples. Throughout the course of the year, these samples were taken once every 30 days in clean plastic containers. The samples were then promptly examined by noting the pH, temperature, odour, and colour of the water samples (Kuraganti et al. 2020).

Spectroscopic analysis of lake water:

The water samples were subjected to Calorina spectroscopy chamber, where the transmission light reading are recorded using the red, green and blue paper with the help of RGB colour detector software application (Kuraganti et al. 2020).

Isolation and morphological identification of cyanobacteria:

After being extracted from the water employing Whatman filter paper and being double-cleaned with double-distilled water, the blue-green algal mat that was recovered from the freshwater Mavala lake. Under constant lighting for 20 days, the clean cyanobacterial mat was inoculated in new BG 11 media (Blue Green Algae media). Using a compound microscope, microscopic identification was carried out after 20 days of incubation. *Spirogyra* and *Spirulina* species were used to identify the cyanobacterial strains. Centrifuging at 5000 RPM for 15 minutes was used to extract the cyanobacterium biomass after 20 days of incubation. To eliminate any remaining trace components from the medium, the separated mass was rinsed twice with distilled water. The biomass was then exposed to aseptic shade drying for roughly 48 hours (Kuraganti et al. 2020; Poojari et al, 2014).

Extraction of Bioactive compounds from biomass:

By employing a motor and pistil to crush the shade-dried cyanobacterial biomass, the extraction was completed. Every five minutes, 300ml of the various solvents ethanol, methanol, acetone, and ethyl acetate are combined with 5 grams of the powdered biomass. Thin layer chromatography (TLC) was used to describe the solvent extract, which was then tested for anti-bacterial activity. The solvent extract was stored in the refrigerator for 24 hours (Kuraganti et al. 2020).

Thin Later Chromatography:

On aluminium Silica Gel, TLC was done. 0.25 mm thick plates that have been packed. A small amount of the extract was placed on the TLC plate about 1 cm from the bottom and let to air dry. Methanol, ethyl acetate, and water were combined in a 7:2:1 ratio to provide the mobile phase for the TLC process, which was carried out in a tank. The separated compound dots were seen at 365 nm in both visible and UV light (Kuraganti et al. 2020).

Antibacterial activity:

Gram negative bacteria *E. coli, S. paratyphi*, and gram positive bacteria *M. luteus* were the targets of the antibacterial activity. The agar well diffusion method was used to accomplish the antibacterial activity, and 100µl of suspended was dispersed across the surface of an agar plate using a glass rod to create bacterial lawns that would last for 24 hours. Agar well was drilled using a steel cork borer with a diameter of around 6 mm. Later, 80µl of cyanobacterial crude extract and streptomycin were added to the wells as a positive control. The plates were given a 24-hour incubation period before being checked for the zone of inhibition (Kuraganti et al. 2020). Zone of inhibition is calculated using formula:

Relative percentage of inhibition of test extract = $\frac{\mathbf{100 \times (a-b)}}{\mathbf{c-b}}$

Where,

a. Inhibition zone of test extract
b. Inhibition zone of the solvent
c. Inhibition zone of the standard drug

Table-1. The spectroscopy evaluation throughout the 12 months

Month	Red Value			Blue Value			Green Value		
	Red paper	Blue paper	Green paper	Red paper	Blue paper	Green paper	Red paper	Blue paper	Green paper
November	117	14	96	38	107	126	46	84	77
December	126	28	86	26	54	112	34	28	160
January	79	15	36	53	101	48	89	35	109.2
February	121	23	16	47	133	59	43	59	84
March	98	21	12	32	109	42	45	62	101
April	24	49	14	32	13	37	33	52	83
May	39	42	31	27	45	41	24	40	59
June	156	138	142	143	162	149	139	150	161
August	152	123	121	132	149	118	129	136	135
September	119	83	91	106	111	98	89	102	109
October	124	26	84	26	52	110	34	28	160
November	121	23	16	47	133	59	43	59	84

RESULTS AND DISCUSSION

Analysing the Physiochemical parameters of water sample:

The water samples that were collected on each month for a year, the physiochemical factors like pH, odor, temperature and colour of the water samples along with appearance of cyanobacterial mat were recorded each time. The pH values greatly varied each month as there were dramatic changes in the temperature of the water. The pH was constantly between 6 to 7 in the months of November, 2021 to march 2022.

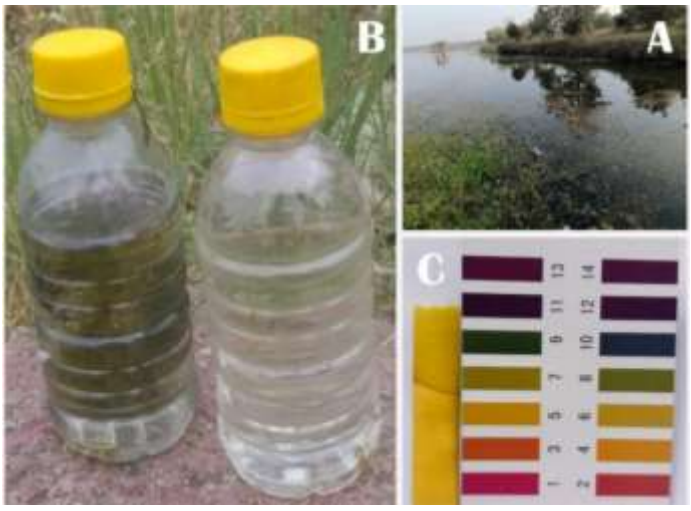

Figure 1: A: Mavala freshwater lake; B: Water sample collected in sterile water bottles; C: pH of the water sample

There are drastic drop of pH in the months of April and May this might because of the high temperature and lowering of water content in the lake that lead to the release of toxins into the surrounding water for eliminating other microbial species in competition for space and nutrients in the lake (Tian et al. 2012), the odor of the water sample was more pungent and strong. Later the pH was observed to be increased to around 7 to 8 in the months of June and July because of the heavy rainfall in Adilabad district. Around same period there was drastic reduction in the appearance of cyanobacterial mat and the odor of the water sample was more likely fresh grass. All the data regarding the factors were updated in the data base of Open Field Collective every month. The sample collection and pH reading is shown in the Figure-1.

Analysis of Spectroscopic values:

As mentioned in the material methos the Calorina spectroscopy chamber was used to measure the transmission light against the red, blue and green paper. The transmission light was measured using the RGB colour detector application where the software is able to capture the red light intensity against red, blue and green paper and same with blue and green light intensity respectively. It was observed that the red and blue light intensities were high in comparison with green light throughout the year. It also significantly showed that the blue green algal mat appearance was moderate from November 2021 to March 2022, where the mat appearance in sample was high in the months of April and May this was correlated with the spectroscopy values.

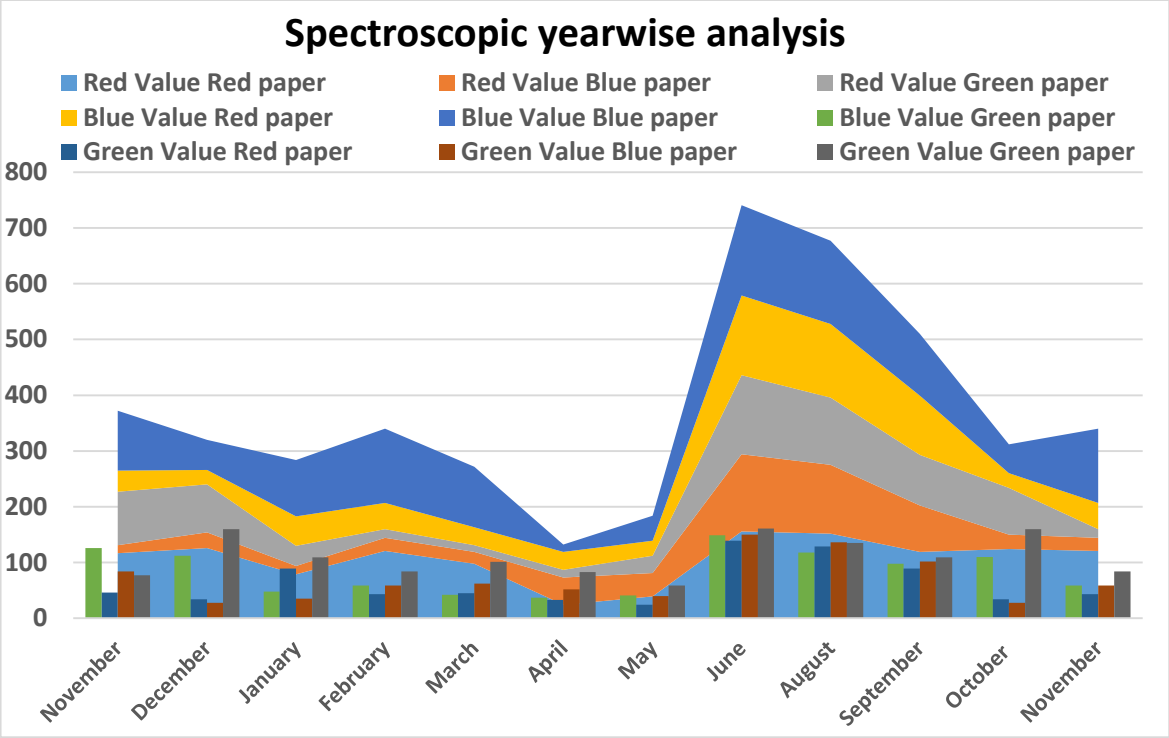

Figure-2. Year-wise spectroscopic analysis

The spectroscopy evaluation throughout the 12 months is tabulate (Table-1) and analysed through Graph in Figure-2.

Isolation and Identification of Cyanobacteria:

The cyanobacteria mal collected from the freshwater lake is washed twice with double distilled water and inoculated in BG_{11} broth media also called as Blue Green algal media which is specific media used for algae. The BG_{11} broth media contains chemical entities that are identical to the freshwater composition with dissolvement of varies salts and minerals that enhance the cyanobacteria growth. The cyanobacteria were incubated for 20days at 35 ^{0}C under continuous emission of white fluorescent light maintained for 16x 8 L/D cycles. Constant light can give photosynthesis a reliable source of energy, enabling algae to continuously generate and assemble biomass (Peter et al. 2022). However, it's crucial to remember that too much light exposure can also be bad for algae because it can cause photoinhibition and injury to the photosynthetic machinery (Ramanna et al. 2017). In order to maximise productivity and prevent stress on the algae, thorough monitoring and management of light exposure are required. This is because the ideal light conditions for algae growth rely on a range of parameters. The Microscopic pictures of the biomass was taken under compound microscope under 40X lenses and identified as *Spirogyra* and *Spirulina* species. The microscopic images are shown in Figure-3.

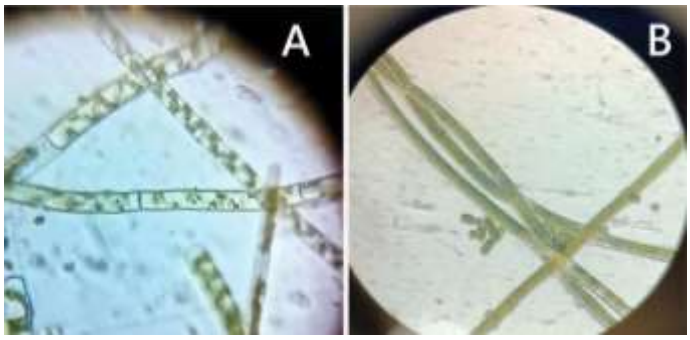

Figure-3. Microscopic images of cyanobacterial cultures isolated from Mavala freshwater lake , Adilabad, A: Spirogyra Sp; B: Spirulina Sp

Figure 4: Plates exhibiting Zone of Inhibition against bacterial cultures

Extraction and Characterization of Bioactive compounds:

The extraction is carried out my mixing 300ml of solvents like ethanol, methanol, acetone and, ethyl acetate to the shade dried pulverized cyanobacterial biomass powder simultaneously for every 5min of interval. The biomass is the mixture of two cultures that is *spirogyra* and *spirulina* sp. The extraction mixture is kept at 4^0C for 24 hours and later filtered. The filtered extract was subjected to characterize the bioactive compounds by separating them using TLC. The mobile mixture was chose based upon the solvent polarity since the most cyanobacterial antimicrobial compounds are lipid in nature and lipids are soluble in the polar mixture of ethanol, acetone and ethyl acetate (Kuraganti et al. 2020). After the completion of TLC the separated compounds were observed under visible and UV light and the RF values have been calculated according to below formula.

$$\text{Retention Factor} = \frac{Distance\ travelled\ by\ solute}{\text{Distance travelled by the solvent}}$$

Antibacterial Activity:

The bioactivity of cyanobacterial solvent extract (ethanol, methanol, acetone and ethyl acetate) was tested for antimicrobial activity by Agar well diffusion method. Solvent extract was tested for biological activity on Gram negative and Gram-positive bacteria (*Escherichia coli*, *Salmonella paratyphi* and, *Micrococcus luteus*) streptomycin is used as positive test compound. After 24hours of incubation the zone of inhibition was measured **(Fig 4)**, inhibition zone was considerably seen against *E.coli* and *M.luteus* with 5mm and 7mm respectively, whereas the inhibition zone was 4mm against *S.paratyphi*. the antibacterial activities of the cyanobacteria could be attributed to the type and amount of free bioactive compounds which have a role in the overall defence against the gram positive and gram negative bacteria (Kuraganti et al. 2020).

CONCLUSION

The investigation unequivocally demonstrated that algae have an impact on the variety of other species in the lake and its surroundings. The algal concentration id mostly effected by physiochemical factors of the lake. The isolated algae have strong antimicrobial properties against both gram positive and gram negative bacteria. The bioactive compounds produced by the cyanobacteria have biomedical and pharmaceutical applications.

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Conflicts of Interest

Authors declare that there is no conflict of interests regarding the publication of this paper.

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