International Journal of Scientific Research in Biological Sciences
Vol.9, Issue.6, pp.01-16, December (2022)

Research Paper

E-ISSN: 2347-7520

The study of the Relationships of *Microcystis aeruginosa* to the Geographically Diverse Distribution

Kailash Prasad Jaiswal[1*], Ragini Gothalwal[2], A.S. Yadav[3]

[1,2]Department of Biotechnology, Barkatullah University, Bhopal-462026 (M.P.), India
[3]Department of Higher Education, Govt. of M.P., India

**Corresponding Author: kailashjaiswal6@gmail.com*

Available online at: www.isroset.org | DOI: https://doi.org/10.26438/ijsrbs/v9i6.116

Received: 17/Oct/2022, Accepted: 19/Nov/2022, Online: 31/Dec/2022

***Abstract*—**Cyanobacteria blooms occurred worldwide, including in India *Microcystis aeruginosa* is a cyanotoxins producing species that occurs in aquatic habitats such as lakes and rivers and varies with different geographical locations. Microcystin (Hepatotoxin) causes serious health hazards in humans, animals, and aquatic living organisms. The ability of microcystin to inhibit the growth of cancer cell lines may lead to the discovery of effective anticancer drugs, pharmacological and toxicological significance of the cyanobacterial genera *Microcystis.* The influence of nutritional, physicochemical, and environmental factors had effects on the release of microcystin (Hepatotoxin) in *Microcystis aeruginosa* in different aquatic environments of central India. In this study, we investigated the distribution of potentially-toxic *Microcystis aeruginosa* for environmental factors that influence the abundance of *Microcystis aeruginosa* cell density in lakes, dams, and rivers of different aquatic regions (Bhopal, Ujjain, Tikamgarh, Gwalior, and Rewa) of central India. Microcystis aeruginosa populations were found in 97.5% of the sites, with the number of cells peaking in the rainy season (7.3 x 106 cells L^{-1}), followed by similar trends in the winter season (6.1 x 106 cells L^{-1}), and a significant decrease in the summer (4.2 x 106 cells L^{-1}). Seasonal significant differences were observed in the rainy ($p>0.07$), winter ($p>0.05$) and summer ($p>0.02$) seasons in *Microcystis aruginosa*. Thus, a significant difference in microcystin-producing Microcystis aeruginosa cells and Chl-concentrations throughout the year, correlating with these various biological and physicochemical parameters, confirms the importance of local environmental factors like phosphorus, nitrate, DO concentration, and pH, as well as regional conditions like distance from built areas and nature reserve parameters, in influencing the geographical distribution of toxigenic Microcystis. Furthermore, the results show that microcystin identified the pharmacological importance of the cyanobacterial genera *Microcystis aeruginosa*.

***Keywords*—**Diversity, Toxigenic, Microcystis, anticancer, Secondary Metabolite

I. INTRODUCTION

Toxic *Microcystis aeruginosa* blooms are a growing threat to freshwater bodies worldwide. *Microcystis aeruginosa* has been reported in more than 106 countries, including Australia, China, Europe, Pakistan, Bangladesh, Israel, Japan, Latin America, North America, South Africa, Thailand, and Sri Lanka. Microcystis is the most common species of bloom-forming cyanobacteria known to produce cyanobacterial hepatotoxins known as Microcystins, which have cytotoxic and genotoxic effects in animal cells, induce oxidative stress in animal cells, and are thought to be one of the main mechanisms of microcystin toxicity [1]. It is well documented that Microcystis forms large colonial phenotype in eutrophic reservoirs and is very common in Indian aquatic environments. Poisoning and serious chronic effects in humans, such as cancer, have been reported, but many studies have reported a microcystin toxin with anticancer potential in human cell lines, resulting in promising control of human adenocarcinomas [2]. *Microcystis* is the most common genus of bloom-forming freshwater cyanobacteria and exists as single cells with a size of 30 to 40 μm and with varying colonies ranging from a few to thousands of cells under natural conditions [3]. More than 50 *Microcystis* morpho species have been recognized according to variations in colony form, mucilage structure, cell diameter, cell arrangement within a colony, ratio of the pigments phycocyanin and phycoerythrin, and details of the seasonal life cycle and their morphological characteristics [4]. Under suitable environmental conditions such as light intensity, total sunlight duration, nutrient availability, water pH, increased precipitation events, and water column stability, they rapidly multiply in surface water, causing *Microcystis* blooms [5].

Parameters like light intensity, pH, temperature, nutrients like nitrogen (N) and/or phosphorus (P), and trace metals could play a critical role in microcystin toxin production in *Microcystis* blooms in aquatic environments [6]. Urban water develops under the strong influence of anthropogenic factors and, hence, significantly differs in the main physical and physicochemical properties from that of natural zonal fresh water, which is responsive to the

anthropogenic transformation of its properties, which influences *Microcystis aeruginosa* abundance [7]. The influence of meteorological parameters in a changing climate can lead to predictable consequences on light and temperature, which have been shown to influence the growth rates of potentially toxic *Microcystis* sp. in different geographical areas [8] and toxin biosynthesis by Terray *et al.,* [9].

Bagchi (2005) have reported in the region of western Asia included India incidence of cyanotoxins producing cyanobacterial *Microcystis sp., Arthrospira sp,. Pharmidum sp., Oscillatoria sp.,* in nutrients nitrogen (N) and/or phosphorus (P), rich in aquatics of different geographical regions of, Eastern and south and India Central, Codd *et al.,* [10], also many studies reported *Microcystis sp.* Blooms increasing threats occupy in rich nutrients nitrogen (N) and/or phosphorus (P) in lake, fresh water, resorvirors widely used in drinking water and daily needed in central India[11]. Therefore, the central India was selected for identification of cyanotoxins producing cyanobacteria and determination of cyanotoxins in different geographical areas such as Bhopal, Ujjain, Rewa, Gwalior and Tikamgarh region of central India, study of biodiversity of occurrence of *Microcystis aeruginosa* in different aquatic habitats and bioactive metabolites microcystin toxins synthesis in different physicochemical environmental and nutritional condition of different aquatics and also metrological study on the abundance of *Microcystis aeruginosa* in different aquatic habitats and in different geographical areas of central India.

II. MATERIALS AND METHOD

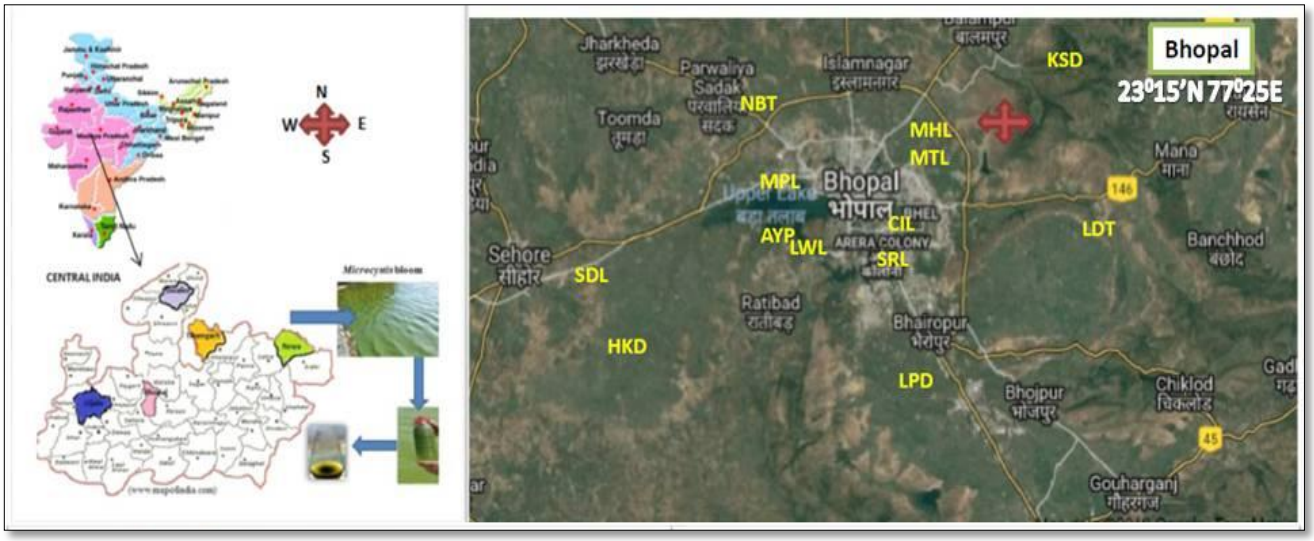
Study sites and sample collection

The cyanobacterial bloom sample of natural lakes, artificial lakes, dam and River were included for studies from different aquatics of different geographical location of central India. Clean glass bottles were used to collect 1L water to brought to the laboratory, during March-2018 to Feb-2019. Out of 113 water sample 25 samples were taken from natural lake of Bhopal sampling station, 21 samples were taken from natural lakes of Ujjain sampling station, 10 samples were taken from natural lakes of Gwalior sampling station, 19 samples were taken from natural lakes of Tikamgarh sampling station, and 38 samples were taken from natural lakes, rivers and Dam of Rewa sampling station of central India.

All latitude and longitude records for each lake were verified by checking visually the information provided in Figure 1. Study site code: Lower lake (LWL), Siddki Husain lake (SDL), Sahpura lake (SPL), Laharpur dam (LPD), Hathaikheda dam (HKD), Kaliasote dam (KSD), Ayodhya nagar pond (AYP), Neelbad tank (NBT), Ladiya Talab (LDT), Motia lake (MTL), Munshi Husain lake (MHL), Chachai Dam (CCD), Chachai Dam (CCD), Chachai Dam (CCD), Chachai Dam (CCD), Chachai Dam (CCD), Chachai Dam (CCD), Chachai Dam (CCD), Chachai Dam (CCD), Chachai Dam (CCD), Chachai Dam (CCD), Chachai Dam (CCD), Chachai Dam (CCD), Chachai Dam (CCD), Chachai Dam (CCD), Chachai Dam (CCD).

Media preparation and Culture of cyanobacteria

The different liquid media BG-11, BG-12 and Hughes were prepared as Rippka *et al.,* [12], the components of media were weigh using weighing balance (Axis India) then components transfer in the 500 ml measuring cylinder to measure the required volume of media makeup by adding distilled water, the dissolved components pour in the 1 Litre beaker and pH-7.9 of media adjust by using digital pH meter (µpH system 361), then sterilized at 121°C for 30 min. For culture of collected samples the 1ml sample were inoculated in BG-11 medium, and maintained at 26 ± 2^0C under illumination with cool light fluorescence tube (intensity approximately 10-50 W/m^2), maintained under photoautotrophic growth conditions with slight modification of procedure developed by Ripkka *et al.,* [13]. The composition of modified Hughes medium, pH-7.5, Hughes *et al.,* [16]; modified by Allen [17] and the composition of BG-11 medium, pH-7.9, [12] ,the composition of BG-12 medium, pH-8.0, [14].



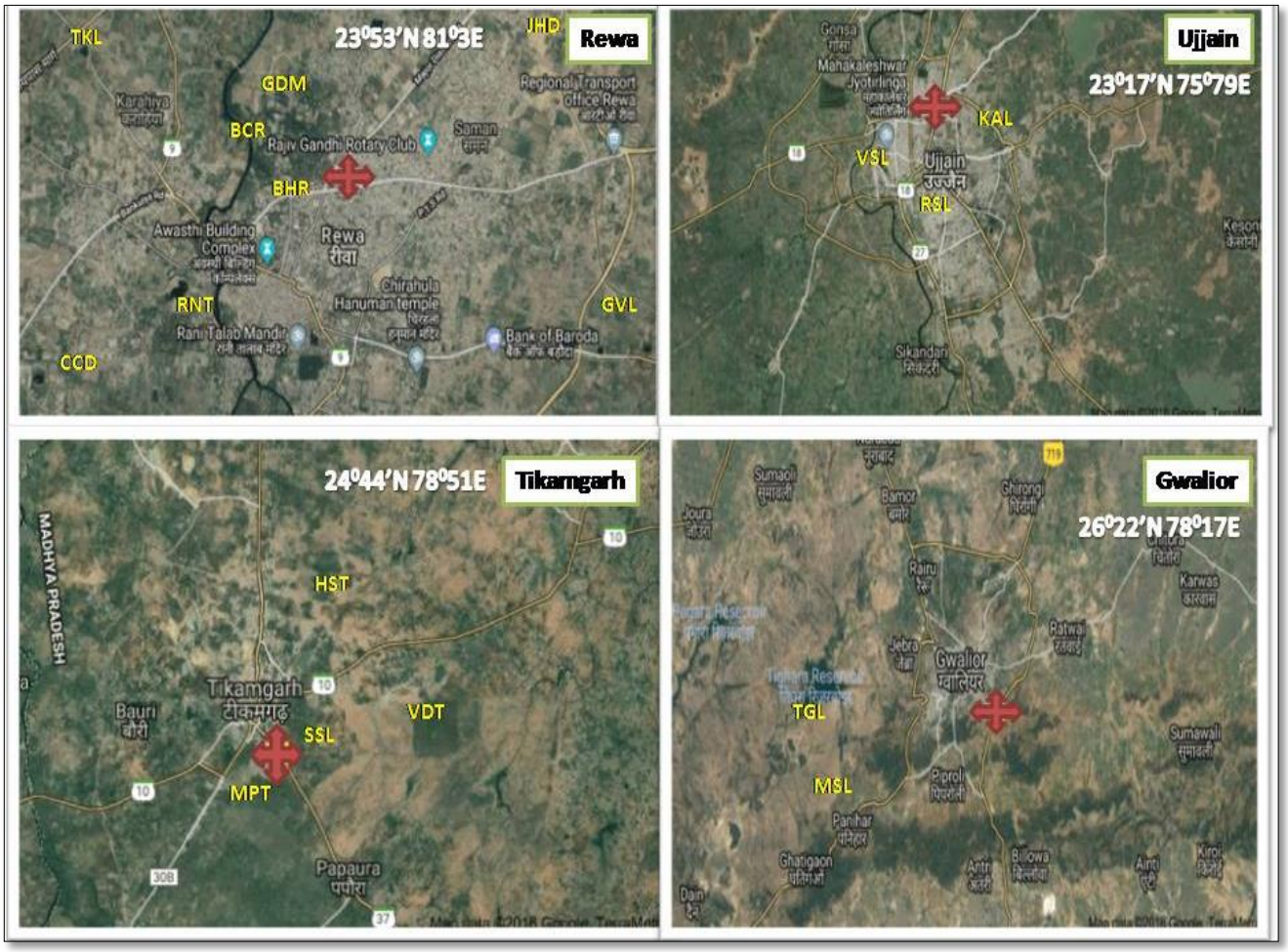


Figure 1: Map of sampling station geographical position and locations of *Microcystis* bloom forming study sites of different aquatic habitats of central India, [46].

Isolation, Identification and Purification of *Microcystis aeruginosa*

The each cyanobacterial sample was observed under the light microscope (Leica analytical system) using oil immersion under 100X magnifications, the cyanobacterial genera were identified taxonomically on the basis of morphological characteristics, cell shape, and surface areas, following the monograph [15]. For removal of bacterial and fungal contamination from *Microcystis aeruginosa* isolates culture, the 10 day old 2 ml cultures of each isolates were centrifuge (eppendrof pvt. Ltd.) at 6000 rpm for 10 min at 4^0C, after centrifugation the supernatant were discarded and pellet dissolved in sterilized distilled water and vortexed it. The 0.1ml inoculums was cultured in solid BG-12 medium contained Petri dishes, the plates were placed under illumination at 26±2°C. For purification of *Microcystis aeruginosa* isolates culture using pour plate technique and streaking method, where 0.8% agar used in BG-11 medium and sub cultured till pure cyanobacterial colony visible under light microscope [12]. Then checked the bacterial and fungal contamination in the *Microcystis* culture in BG-11 solid media was used under dark/light incubation, the 10 days old culture was used for growth on nutrients agar media (NAM) and potato dextrose agar (PDA) at 37^0C and 28^0C, respectively for 24 hrs.

Optimization of different nutrients media

The different nutrient media were prepared as modified Hughes medium, BG-11 medium and BG-12 medium . The pure *Microcystis aeruginosa* isolates culture were grown in liquid medium BG-11 and modified Hughes and BG-12 in 250 ml of conical flask with 100 ml containing liquid media. All other components and environmental conditions of the prepared media (BG-11 and modified Hughes and BG-12 liquid media) For 8 days, all isolates were placed at 26 20C under illumination with a cool light fluorescence tube (intensity approximately 10-50 w/m2), the flasks were shaken by hand twice a day, and photoautotrophic growth conditions were maintained [12]. The optical densities of isolates were measured at 680 nm according to Cummings, (2015).

Measurement of Chlorophyll-*a*

Chlorophyll-*a* was Measured by the method described by the [18], 4 ml of culture centrifuge at 5000rpm for 10 min at 4^0C, after centrifugation the pellet were suspended and gently mixed with methanol and placed for 30 min incubation, then centrifuge at 5000rpm for 10 min at 4^0C cell debris removed and Chlorophyll-a content dissolved in methanol and measured by spectrophotometer.

The quantitative estimation of Chlorophyll-*a*.

Chl-*a* (mg ml^{-1}) = 13.42 X OD_{665} X 4 ml (volume of methanol) 1ml (vol. of sample)

Evaluation of *Microcystis aeruginosa* growth using different growth parameters:

The growth rate (μ) day^{-1} was calculated based on OD measured at 680 nm following the equation

$$\mu = \frac{\ln (X_2 - X_1)}{\mu t (t_2 - t_1)}$$

Where, X_1 and X_2 are the optical densities at times t_1 and t_2 , growth rate (μ) occurring at any time during the incubation (Sorokin, 1975).

The doubling time **(G)** was calculated as: G = ln2/ μ, where ln2=0.693 and μ =growth rate.

Subsequently, the nutrients optimization for growth of *Microcystis aeruginosa* in BG-11 and Hughes medium results found that out of 30 only 10 isolate were highly potent. The 5ml culture of *Microcystis aeruginosa* isolates were taken at exponential growth phase and harvested using centrifuge at 6000 rpm for 5min. and inoculated in liquid medium BG-12 at pH-7.9 in 250 ml of conical flask in triplicates and placed at 26 $\pm 2^0$C under illumination with cool light fluorescence tube (intensity approximately **(**50 KW/cm^2) maintained under photoautotrophic growth conditions up to 9^{th} day.The optical density of each *Microcystis aeruginosa* isolates culture was measured at 680 nm at 48 hrs of interval ,the quantitative estimation of Chlorophyll-*a*.was performed. Extraction of protein from *Microcystis aeruginosa* isolates culture. The concentration of protein in the solution was estimated from a calibration curve prepared by BSA as a known standard, the protein was measured according to the Lowry *et al.,* [19].

Optimization of physiological conditions

Experimental condition for pH effects

Buffer used to adjust pH of BG-11 culture medium for experiments, sodium phosphate buffer (0.01M) pH-6.0, 7.0, borax buffer(0.01M) pH-9.0,HEPES buffer (0.05M) pH-8.0. Borate (10 mM) pH-11 (Dawson and Elliot, 1989). The *Microcystis aeruginosa* isolates were harvested by using centrifugation (6000 rpm for 10 min) and *Microcystis aeruginosa* isolates was inoculated in pH-6.0 and 7.0, 8.0, 9.0 and 11.0 of BG-11 media at 28±1^0C under florescent light (50 W/cm^2) for 9 days according to [20].

Experimental condition for light exposures

The isolates were concentrated by centrifugation (6000 rpm for 10 minutes), pellet washed with distilled water, and inoculated in a 150 ml conical flask containing BG-11 medium. *Microcystis aeruginosa* were grown at different light intensity levels (A-50.08 W/cm^2, B-12.30 W/cm^2, C-5.32 W/cm^2).. Light was supplied from cool-white fluorescent tubes (BAJAJ, 6500^0K 36W 5M8) and different intensities were maintained by white paper (shade sheet), [20].

Experimental condition for temperature exposures

The experiments were performed with *Microcystis aeruginosa* isolates for experiments *Microcystis aeruginosa* isolates culture were concentrated by using centrifugation (eppendrof centrifuge 5415R) at 6000 rpm for 10 min. and the cultures were grown in liquid BG-11. The temperatures were applied 18°C ("low temperature"), 26°C ("control"), 30°C and 36°C, 40°C ("high temperature"). The isolates culture was placed in shaker (20 rpm) (ORBITECH, Scigenics Biotech) for each temperature and placed under cool light fluorescent tubes, [21].

Optimization of nutrients concentration

Experimental condition for growth experiment *Microcystis aeruginosa* isolates were cultured in BG-11 medium. Ferric ammonium citrate. $NaNO_3$, K_2HPO_4, were used to regulate the concentrations of NO_3^-, PO_4^{3-} and NH_4^+ in BG-11 media. Before each experiment, all *Microcystis aeruginosa* isolates e were adapted to a medium without N or P for 48 hrs. In each experiment, 150-ml conical flasks experiments were performed in triplicate at pH 8.0, temperature of 28±1°C, light intensity of (50 W/cm^2) on 16: 8 h light-dark cycle. The effects of nutrients were tested at different concentration of nutrients, different concentrations of NH_4^+ (10, 30, 50, 100 μM), NO_3^-, (5, 10, 20, 50 mM) and PO_4^- (0.1, 0.3, 1.0, 5 mM) according to [22].

Morphological analysis of *Microcystis* and Physicochemical and meteorological

Colonial *Microcystis* sp. were collected 30 samples from the five different sampling station of central India, as *Microcystis* blooms occur frequently at this site. The sampling, which was conducted seasonally from march-2018to Feb.-2019 and the collected samples were maintained at 20°C.

Cells were counted using optical microscope (Leica Pvt. Ltd.) at 100X magnification for distribution of cells (Schoen, 1988 and Guillard, 1978). The measurement was average number of cells per 1mm square, so the centre large square is usually counted and to obtain the total number of cells in this large square, the number of cells in each of the 25 medium squares were counted, recorded then added. Usually the procedure is repeated twice more to give a total of 6 counts. To obtain the cell density, calculate the average cell count and multiply by the conversion factor (for Neubauer = $x10^4$).

With the help of a thermometer, the physical characteristics of a water sample from various sampling sites in central India were analysed physicochemically (76MM, Immersio, Zeal, England) pH metre was used to measure the pH of the water, and the concentration of dissolved nutrients, including free carbon dioxide (free CO2), alkalinity, chloride, dissolved oxygen (DO), nitrate, conductivity, and total dissolved solids, was determined (TDS).The central Indian cities of Bhopal, Ujjain, Rewa, Tikamgarh, and Gwalior were among those whose meteorological conditions were studied.

Principal component analysis of samples
Multivariate factor analysis was performed on the physico-chemical and biological variables average data one year study period of study sites using principal component analysis (PCA) by help of SYSTAT software.

Statistical analysis
All statistical analyses were performed with SYSTAT software package-5.0. Two way ANOVA for significance level, PCA analysis.

III. RESULTS AND DISCUSSION

Out of total of 113 samples collected only 30 *Microcystis aeruginosa* were isolated from different aquatic habitats of different geographical location of central India, the identification of cyanotoxins producing cyanobacteria, study of biodiversity of occurrence of *Microcystis aeruginosa* and bioactive metabolites microcystin toxins synthesis in different environmental, nutritional condition and genes involved in microcystin toxins regulation and synthesis, understanding their toxic effects on animal cell line models with a view to exploit naturally occurring cyanotoxins effects and implications, pharmacological action of aggregation process under influence of crude extracts of *Microcystis aeruginosa*. The results obtained from the present investigation are presented here under.

Isolation and identification cyanobacteria
The present study was conduct to enumerate cyanobacteria from different aquatic habitats of different geographical location of central India. The 30 isolates of *Microcystis aeruginosa* were taxonomically identified during microscopy examination of samples. The existence of intracellular structures, the gas vesicles provides buoyancy to cells, in culture also all cells ovoid and spherical in shape were observed, the variation of *Microcystis aeruginosa* colonies 40-90 µm were observed.

In the field of science, for many years, researchers and scientists in different parts of India have conducted microscopic detection and enumeration of cyanobacteria species in freshwater bodies containing visual algal blooms. Water samples found to contain potentially toxin-producing species of *Microcystis aeruginosa* above a cell density threshold of 20,000 cells L^{-1} indicate a scarcity of information describing the occurrence of toxic Microcystis aeruginosa blooms in various aquatic habitats of India, as well as the toxins typically encountered. The Central India region Bhopal, Ujjain, Gwalior, Tikamgarh and Rewa, have different subtropical climates, rainfall, day light and temperature, due to these reasons effects on the morphological and physiological characteristics growth of *Microcystis aeruginosa*. Similar observation were also proposed *Microcystis* morphospecies [23] and colonies size variation (90- 100 µm) geographical distribution and climate conditions of aquatic habitats[24],[25].

Evaluation of growth in different nutrient media
Cyanobacteria are photosynthetic prokaryotes, which can produce a wide range of bioactive compounds with different properties; including a variety of toxic compounds, also known as cyanotoxins. The growth of all *Microcystis aeruginosa* isolates from different aquatics of central India were chosen to evaluate its growth by O.D. measure and chl-a estimation, and its toxin production categorized under different nutritional regimes. The growth of *Microcystis aeruginosa* were studied in BG-11 nutrient media for 9 days, during exponential phase on the day 5-7 higher growth rate (µ) (0.8 ml^{-day}) and Chl-a (0.12 mg^{-ml}) content were observed in all *Microcystis aeruginosa* isolates (MTL, MHL, SPL, CIL, LWL, SDL, SRL, LPD, HKD, KSD, AYP, NBT, RSL, VSL and SSL), however in (VDT, HST, TKL, MPT, MSL, LDT, BCR, BHR, GDM, CCD, GVL, RNT and JHD during exponential phase higher growth rate (µ) (0.5 ml^{-day}) and chl-a (0.12 mg^{-ml}) content were observed which is shown in Figure 2.

The growth of *Microcystis aeruginosa* were studied in Hughes nutrient media for 9 days, during exponential phase higher growth rate (0.8 ml^{-day}) and chl-a (0.18 mg^{-ml}) content were observed in isolates (MTL, MHL, SPL, CIL, LWL, SDL, SRL, LPD, HKD, KSD, AYP, NBT, RSL, VSL and SSL), however, VDT, HST, TKL, MPT, MSL, LDT, BCR, BHR, GDM, CCD, GVL, RNT and JHD during exponential phase on the day 5-7 day lower growth rate (µ) (0.5 ml^{-day}) and chl-a (0.14 mg^{-ml}) content were observed, which is shown in Fig. 3.

Subsequently, the growth optimization of *Microcystis aeruginosa* in BG-11 and Hughes media were performed. The experimental results found that the out of 30 isolates only 10 isolates growth were significant which were selected for growth optimization of *Microcystis aeruginosa* were studied in BG-12 nutrient media for 9 days. The growth characteristics of isolates in BG-12 nutrients medium, the isolates (MTL, MPL, HST, GVL, LWL, TKL, RNT, SRL, HKD, MSL), these isolates grow faster has higher growth rate and long exponential phase from day 4^{th} to 7^{th} growth rate (0.8 mg^{-ml}), chl-a (0.18 mg^{-ml}) and protein (1.36 mg^{-l} d^{-1}), the results of growth profile of isolates in BG-12 nutrients medium shown in (Fig.4), showed pronounced differences between the exponential and stationary phase.

The *Microcystis aeruginosa* isolates of different aquatic habitats showed variation in growth rate, exponential and stationary phase, some of isolates have a greater specific growth rate, chl-*a* and proteins content as compared to other isolates. Because the *Microcystis aeruginosa* isolates collected from different aquatic habitats and geographical location therefore the growth pattern was different in laboratory condition. Many researchers reported *Microcystis aeruginosa* from different aquatic growth rate and exponential phase times were different in different due to different geographical location of water source. Similarly the collected *Microcystis aeruginosa* from different aquatic habitats shown growth rate, chl-a and exponential phase time were vary in different aquatic source, growth rates of *Microcystis aeruginosa* were varying due to different light intensities, nutrient

concentrations environmental factors water temperature factor determining the dominance of the *Microcystis sp.* due to sample were collected from different geographical location[26,27].

The experimental results showed that the growth rate, protein and chl-a content in *Microcystis aeruginosa* isolates were significant, cellular protein content increased from 0.6 to 0.9 mg^{ml-1} during exponential phase. Protein is the main component of *Microcystis* cell architecture and is closely related to the life activities of the cells [28], thus, high proportions of protein at high specific growth rates favor the maintenance of *Microcystis aeruginosa* [29,30]. Chlorophyll-a is a measure of the amount of *Microcystis aeruginosa* growing in a water body which can be used to classify the trophic condition of a water body [31]. As per guideline World Health Organization, the chlorophyll-a is the indicator of microcystin concentration, the similar levels for microcystin for cyanobacteria abundance and chl *–a* proposed ranges low (< 10 μg^{-L}), moderate (between 10 and 50 μg^{-L}, high (between 50 and 5000 μg^{-L}), and very high risk (>5000 μg^{-L}) are useful tools to overestimate actual risk [7]. Parameters to express microcystin production, such as protein content, chlorophyll-a and comparison of intracellular microcystin production are very difficult to indicate protein and chlorophyll-a dependent cellular processes, which are expected to change under different tropic condition [32].

The experimental results showed that the variation in growth rate and chl-a content in *Microcystis aeruginosa* isolates in different culture media Hughes, BG-11 and BG-12, showed that the BG-11 and BG-12 nutrient medium were well supported the growth of *Microcystis aeruginosa* isolates of highly significant at ($P<0.05$) . The Hughes medium also supported growth, although the growth rate were very slow. The growth indicated that the *Microcystis* displayed exponential growth and fast adaptation to the BG-11, BG-12 nutrient media condition compared to Hughes media. The results reinforces the nutrients are essential for the growth of *Microcystis aeruginosa*. The growth of *Microcystis aeruginosa* and production of pigments depends on composition of medium and its pH, light intensity exposures, in the experiment composition of nutrients medium and pH were different therefore, in BG-11 and BG-12 media, higher growth rate and concentration of chl-*a* were recorded in all isolates where in Hughes nutrient media moderate growth rate, chl-*a* content were noticed. Similarly, Cifuentes et al., reported highest nitrogen content which resulted in high accumulation of chl-*a* in *Microcystis aeruginosa*, which is due to the well-known effect of limitation of nutrient as an inductive factor for chl-*a* content in *Microcystis aeruginosa*. Hughes media contain low nitrogen source, therefore the lower production of chl-*a*, Chl-*a* is the essential photosynthetic pigment in cyanobacteria, because only chl-*a* can utilize the absorbed light energy for the synthesis of chemical energy ATP. According to Sedmak and Elersek [33] *Microcystis aeruginosa* possessed higher photosynthetic activity after cells became aggregated, which supported the view that the unicellular *Microcystis aeruginosa*, this physiological characteristic might facilitate *Microcystis* maintaining predominance in natural water [34],[35]. The culture media used in this work have different nutrient compositions, in particular regarding to metals, vitamins and iron. These elements is essential to several metabolic processes for cyanobacteria such as chlorophyll-a synthesis, respiration, photosynthesis and nitrogen fixation [36]. The nutrients such as nitrogen, phosphorus and ammonium influence growth of *Microcystis aeruginosa* isolates. The growth of isolates more in BG-11 and BG-12 medium than Hughes media because of appropriate Ka^{+}, Na^{+} ions required in the cytoplasm of *Microcystis aeruginosa* for optimum growth and nucleic acid synthesis, BG-11 and BG-12 medium consists higher concentration of Na^{+} and K^{+} where Hughes medium has moderate concentration of Na^{+} and K^{+} source [37]**.**

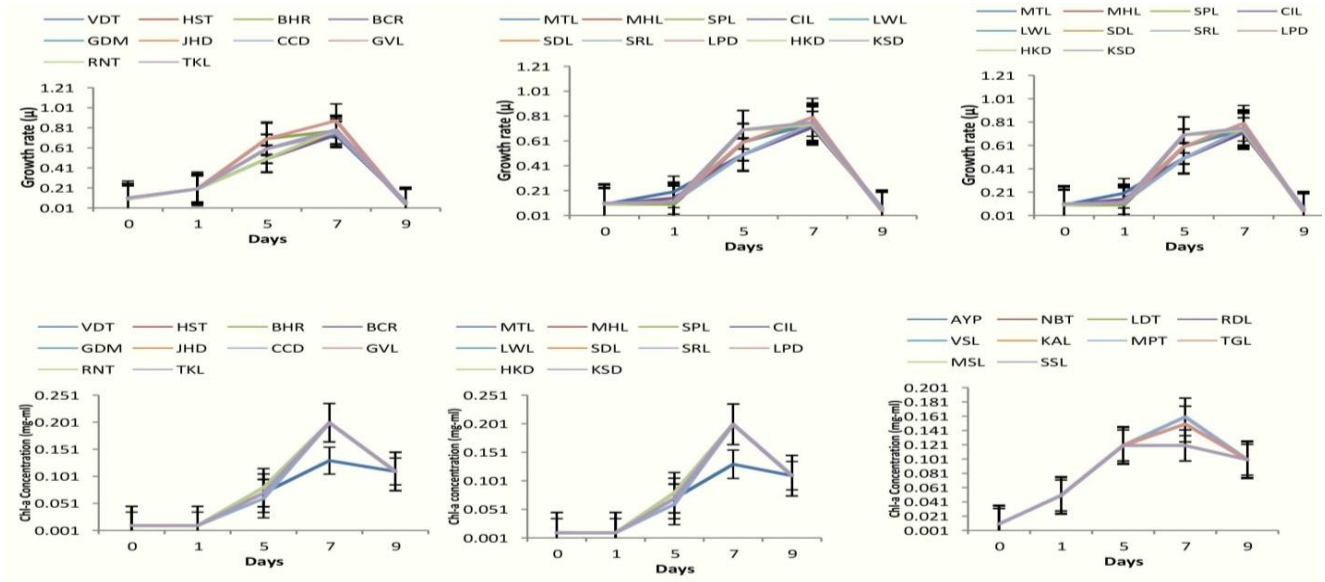


Figure 2. Growth profile of *Microcystis aeruginosa* isolates during growth in BG-11 nutrients media, A-Growth rate and B- Chl-a concentration.

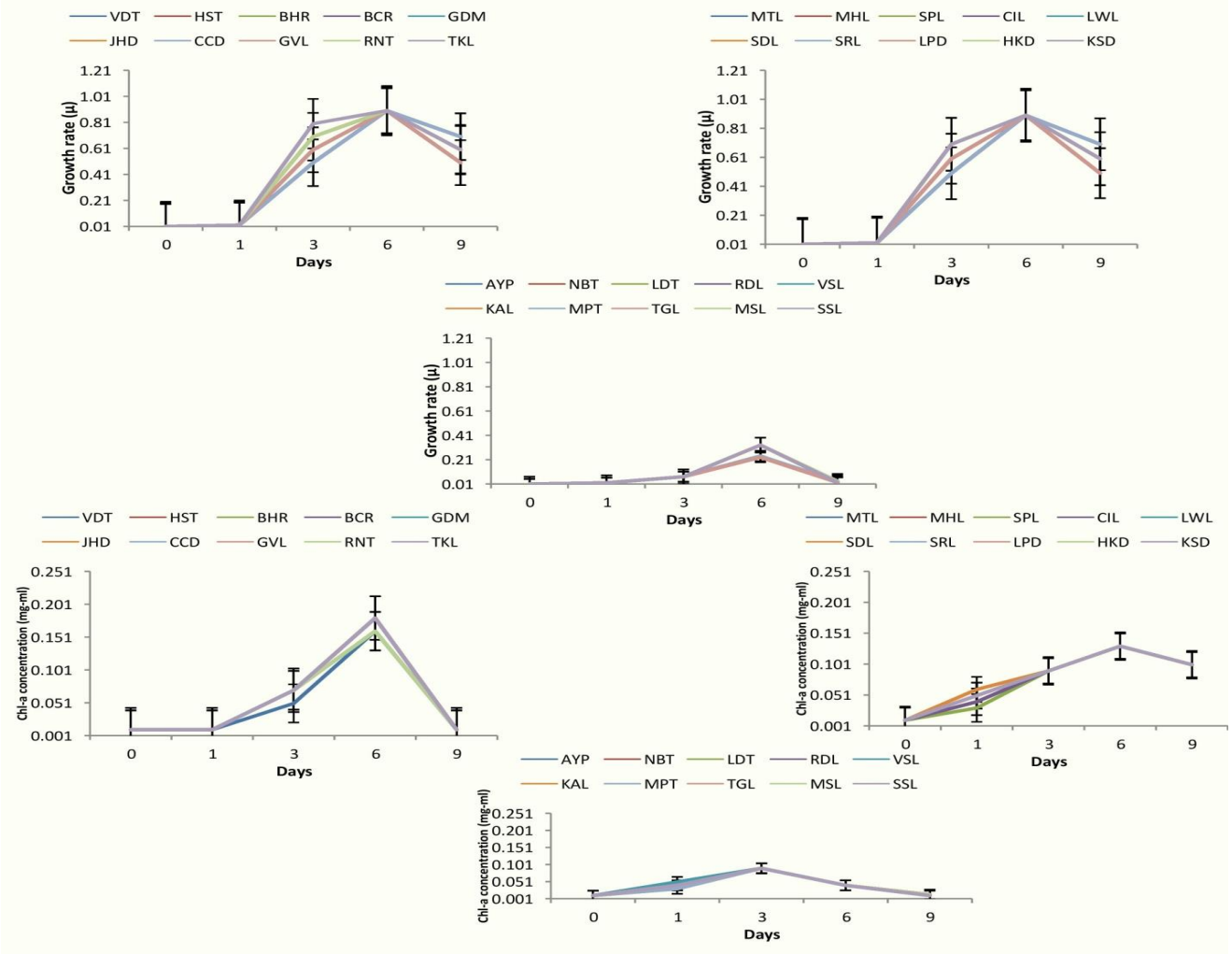


Figure 3. Growth profile of *Microcystis aeruginosa* isolates during growth in Hughes nutrients media, A-Growth rate and B- Chl-a concentration.

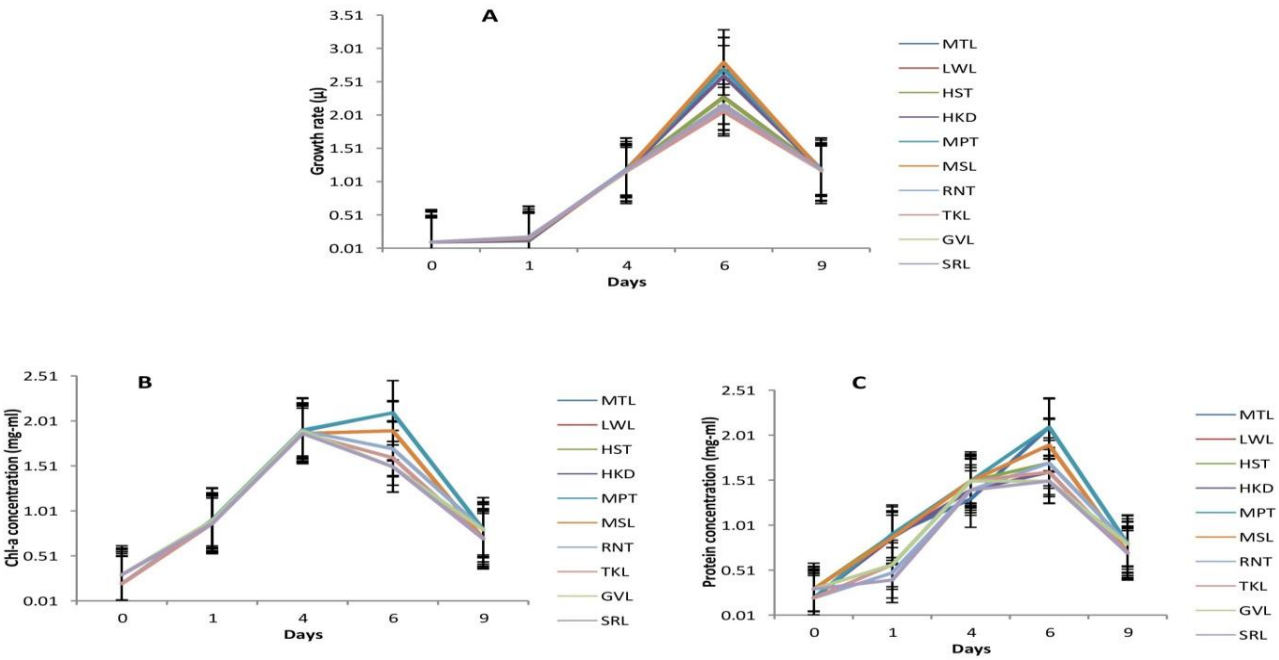


Figure 4. Growth profile of *Microcystis aeruginosa* during growth in BG-12 nutrients media, A-growth rate, and B- Chl-a concentration and C- protein concentration.

Growth characteristics of Microcystis aeruginosa under different physiological conditions

A common cyanobacterium called Microcystis aeruginosa can harm freshwater ecosystems both ecologically and economically when it is in large quantities. However, physiological studies reported physiological variation among different strains of Microcystis in determining growth, and potent of bloom formation. Because of this, research in the lab on the development characteristics of Microcystis aeruginosa under various physiological conditions revealed a wide range of physiological reactions to modification oftemperature, light, salinity, and macronutrients.

Growth of *Microcystis aeruginosa* under different pH

Growth of *Microcystis aeruginosa* isolates at different pH values (pH 6, 7, 8, 9 and 11) were studied different medium, when isolates at different pH, the isolates MTL, MPL, HST, GVL, LWL, TKL, RNT, SRL, HKD, MSL have showed optimum growth and maximum growth was evident at high pH-8.0 , of observed growth rate (μ) = 3.0 d^{-1} shown in Fig. 5. The growth characteristics all of isolates statistical analysis results showed that the growth of all *Microcystis aeruginosa* isolates highly significant ANOVA, P<0.005) at pH-8, while it was not significant at pH- 7.0, 9.0 and 11.0.

Due to the collecting of Microcystis samples from various aquatic habitats in central India, the growth of the isolates was impeded at low and high pH levels. Chiu, Kao, and Wang et al. [38] revealed similar findings on the growth rate fluctuation in different pH conditions in laboratory settings as well as in aquatic environments. According to the experimental findings, the BG-12 medium's pH-8 growth rate and chlorophyll-a concentration significantly differed from each other. According to similar studies, Microcystis aeruginosa grows best at a pH- 8.0 under lab conditions, according to Wangwibulkit et al. [47]. According to these findings, Microcystis aeruginosa growth is inhibited in an acidic environment [39].

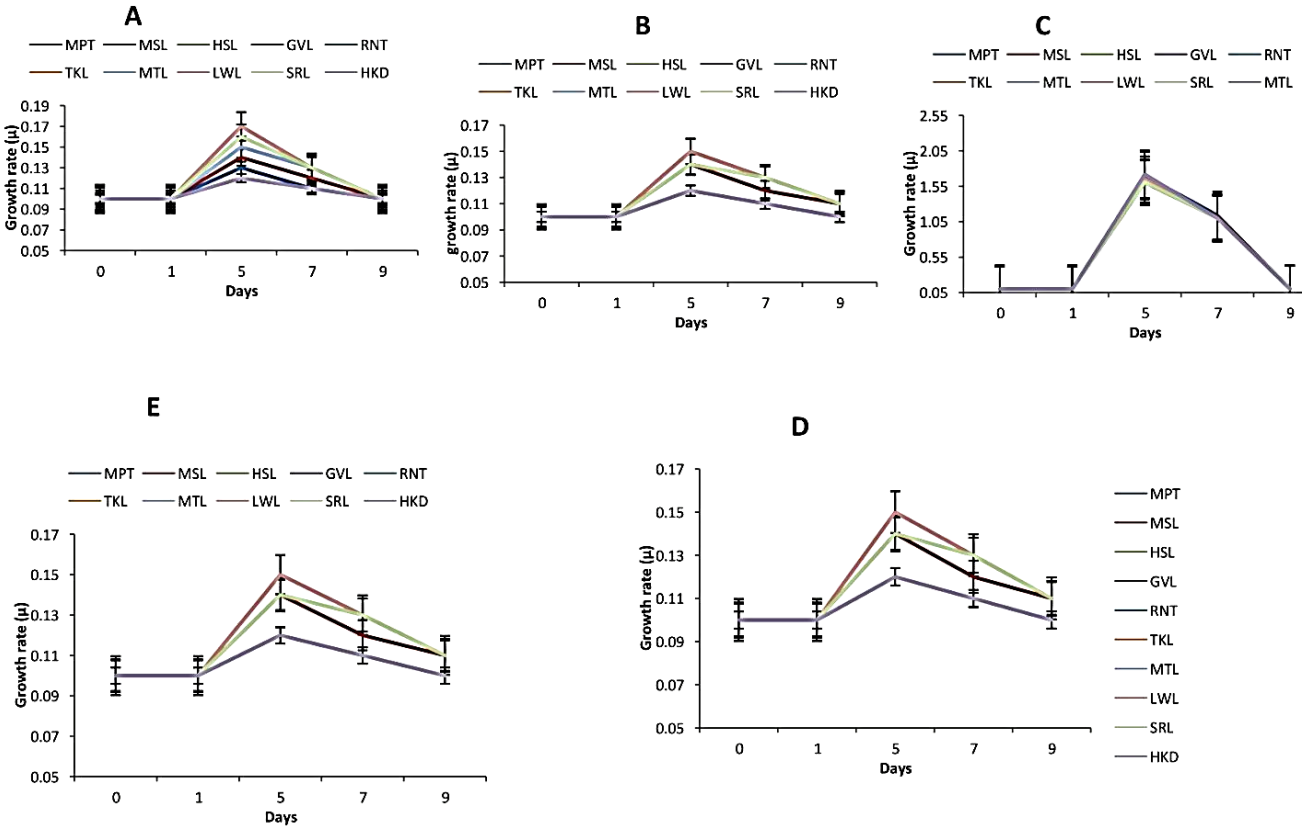


Figure 5: Growth profile of *Microcystis aeruginosa* isolates on 5th day of incubation under the influence of different pH: A-6,B-7,C-8,D-9 and E-11.

Growth under different light intensity

Growth of isolates MTL, MPL, HST, GVL, LWL, TKL, RNT, SRL, HKD and MSL were studied under different light intensity conditions, in all isolates where cumulative growth rate (μ) = 3.8 d^{-1} at 50 W/cm^2 light intensity occurred and long exponential growth phase was found on 5th - 7th day. The optimum growth rate (μ) = 3.4 d^{-1} at (12 W/cm^2) light intensity was shown in Fig. 6, at low light intensity (5.38 W/cm^2) growth was not significant.

The experimental findings unambiguously show that Microcystis aeruginosa grows and behaves differently depending on the amount of light it receives. According to the studies, the dominance of the Microcystis aeruginosa strains in Australian freshwater systems with variable light intensity is demonstrated by their capacity to survive in a range of light intensities, from low to high. When photosynthesizing cells are exposed to light of any intensity, two distinct phenomena occur simultaneously,

namely the photodamage (light-induced inactivation of PSII) and the repair of photodamaged PSII. Light is necessary for photosynthesis, but excessive light energy is harmful to the photosynthetic machinery [40]. The extent of photoinhibition of PSII depends on the balance between the rate of photodamage and the rate of repair. The correlations between *Microcystis aeruginosa* cells density and cellular pigment chl-*a* content under 50-60 W/cm^2 light intensities were shown by Mehnert *et al.*,[41]. The higher *Microcystis aeruginosa* growth rate could be due to a maximum chl-*a* concentration due to higher light (50-80 W/cm^2) conditions, which may a significant factor controlling *Microcystis aeruginosa* growth and toxin production[40].

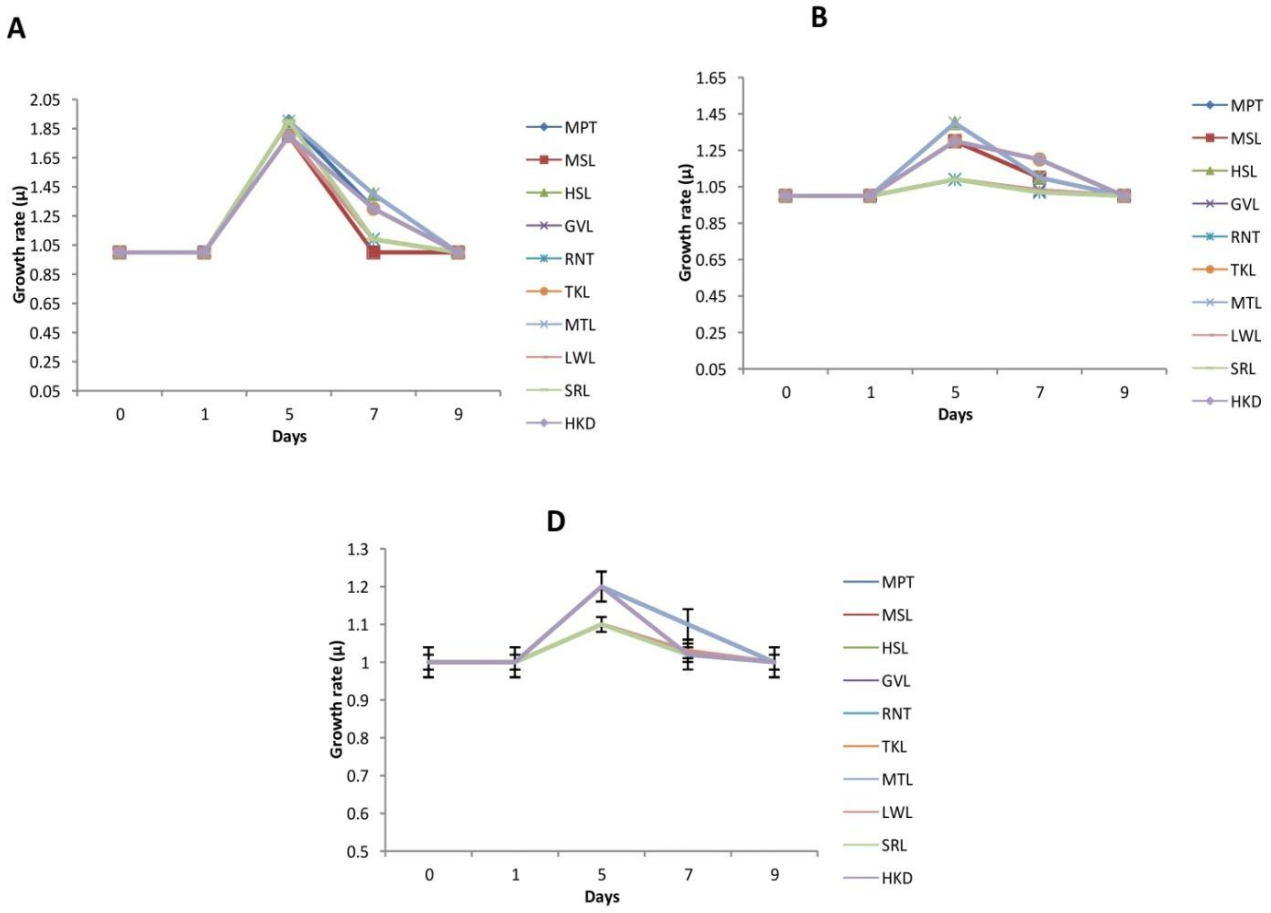


Figure 6: Growth profile of *Microcystis aeruginosa* isolates on 5th day of incubation under different light intensity A-50.38 W/cm^2, B-10.75 W/cm^2,C- 7.02 W/cm^2.

***Microcystis aeruginosa* growth under different temperature**

The growth of isolates MTL, MPL, HST, GVL, LWL, TKL, RNT, SRL, HKD and MSL were studied under different temperature and growth characteristics of isolates were observed from 5th to 7th day growth rate (μ) 3.0 d^{-1} growth was maximum and long exponential phase was observed 28^{0}C and lower growth occurred at18^{0}C . At high temperature (36^{0}C) the growth rate (μ) was 3.0 d^{-1} and chl-a was 0.031 mg^{-ml} d^{-1} was found at 40^{0}C Fig. 7. The growth characteristics showed the growth rate of isolates at temperature 28 ^{0}C was significant (ANOVA, P<0.005), where as 18^{0}C, 36^{0}C and 40^{0}C temperature were non significant towards growth rate.

The growth rate and chl-*a* content in isolates gradually declined (stationary phase) until the end of the experiment due depletion of nutrients in culture media. The results presented in this study clearly indicate differences in growth and physiological characteristics of *Microcystis aeruginosa* in relation to temperature and the growth rate of *Microcystis aeruginosa* isolates were strongly affected by different temperature conditions because sample were collected from different geographical locations. *Microcystis aeruginosa* may have a maximum growth at 28 °C due to adaptation of isolates to optimum temperature found in different habitats. The significant differences were found in the growth of low and high temperature which were according to Imai *et al.*, [26]. In freshwater ecosystems, phytoplankton generally shifts from diatoms to green algae to cyanobacteria accompanied by seasonal temperatures increase from 10 to 30 °C [28,38]. The data results revealed that *Microcystis aeruginosa* outcompetes as temperature increased. Thus, the rise climate condition may accelerate the phytoplankton succession and *Microcystis aeruginosa* bloom formation [38].

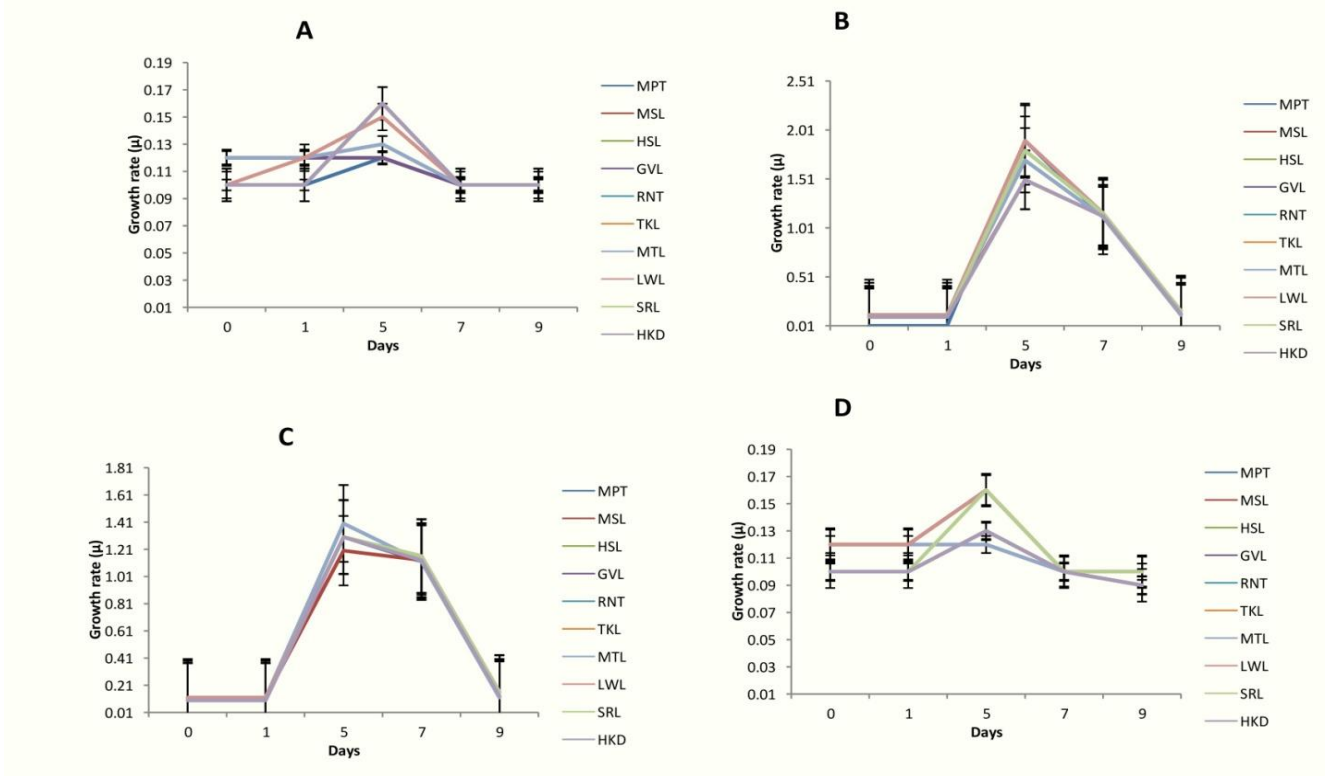


Figure 7: Growth profile of *Microcystis aeruginosa* isolates on 5th day of incubation in different temperatures A-18 ^{0}C, B-28 ^{0}C,C-36 ^{0}C, and D-40 ^{0}C.

Growth characteristics of *Microcystis aeruginosa* in presence of different nutrients

Cyanobacteria blooms are typically associated with eutrophic and poorly flushed waters. Nutrient concentrations and water quality influence on the growth rates of toxic and non-toxic strains of *Microcystis*. Different nutrient N, P and NH_4^+-limited conditions effects on the cell morphology, cell number and life history.

Impacts of NO_3^-

Growth of isolates were studied under different concentration of NO_3^- **, t**he maximum growth rate (μ) 3.0 d^{-1}, found at 20 mM NO_3^- during exponential growth phase on day 5th to 7th , the optimum growth rate (μ) 3.0 d^{-1} of isolates (MSL, GVL and RNT) were occurred at 10mM (0.8 mg^{-L}) NO_3^- and growth of isolates were significant at ($p < 0.01$), different NO_3^- concentration growth rate in all isolates growth profile of isolates shown in Fig.-8.

Impacts of PO_4^{3-}

The growth of MTL, MPL, HST, GVL, LWL, TKL, RNT, SRL, HKD and MSL were studied under different concentration of PO_4^{3-} **,** the growth (μ) 3.0 d^{-1} , were occurred at 0.3 mM PO_4^{3-} and isolates (MSL, GVL and RNT) growth rate (μ) 3.0 d^{-1} occurred at the PO_4^{3-} concentration 0.05 mg^{-L} (1.5 μM PO_4^{3-}). *Microcystis aeruginosa* required 0.8 mg^{-L} (10-30 μM PO_4^{3-}) for a high growth rate which is shown fig-8 in the different PO_4^{3-} concentration.

Impacts of NH_4^+

The maximum growth rate (μ) 3.0 d^{-1} and Chl-*a* 0.71 μl) was found at 30μM (7.8 mg^{-L}) NH_4^+ and significant at (Chl-*a* $p < 0.01$) and where MSL, GVL and RNT, maximum growth rate (2.7 d^{-1}) were observed at 10μM NH_4^+ (Fig.-10).

In agreement with previous studies, our experimental results showed that the growth of Microcystis aeruginosa increased as the NO3- and NH4+ concentrations increased[43,44].The current findings also demonstrated that initially NH4+promoted slightly faster cell growth, but that both substances behaved differently in terms of Microcystis aeruginosa growth. Microcystis aeruginosa can only use NO3 -after enzymatic reduction to NO2- and NH4 + , and these enzymatic reactions consume cellular energy and consequently have an impact on cell growth. [42].

The results of the effect of the PO_4^{3-} concentration are in agreement with previous studies [43], which reported that a minimum of 0.05 mg L^{-1} (1.5 μM PO_4^{3-}) is necessary for *Microcystis aeruginosa* growth, and 0.3-0.8 mg L^{-1} (10-30 μM PO_4^{3-}) is needed for a high growth rate. Baldia *et al.,* [48] reported that the growth rate of *Microcystis aeruginosa* increased with nitrate concentrations up to of 620 μM (8.7 mg L^{-1}) and with phoaphate concentrations up to 7 μM (0.22 mg L^{-1}). It was also indicated that maximum growth of *Microcystis aeruginosa* occurred at a relatively high nitrate concentration, but at a relatively low phosphate concentration [42].

As a result, Microcystis aeruginosa that uses NH4+ before NO3- [45] may experience NO3- uptake inhibition. Similarly, our results showed that *Microcystis aeruginosa* had a lower growth rate at a nitrate concentration of 500 µM, indicating that decreased NO_3^- absorption in the presence of NH_4^+ seemed to hinder the growth of *Microcystis aeruginosa*. However, according to Kim et al. [42], the inhibition of NO3- uptake by NH4+ and the preference for NH4+ uptake differ depending on environmental conditions and species. We observed a similar effect for NH_4^+ concentrations below 250 µM, suggesting that NH_4^+ might inhibit NO_3^- uptake at concentrations above 250 µM. However, this effect only occurred during the initial growth phase, and cell densities at 4 days were similar for different nitrate concentrations. Therefore, results suggest that the nitrate concentration has a significant role in the growth of *Microcystis aeruginosa* [42].

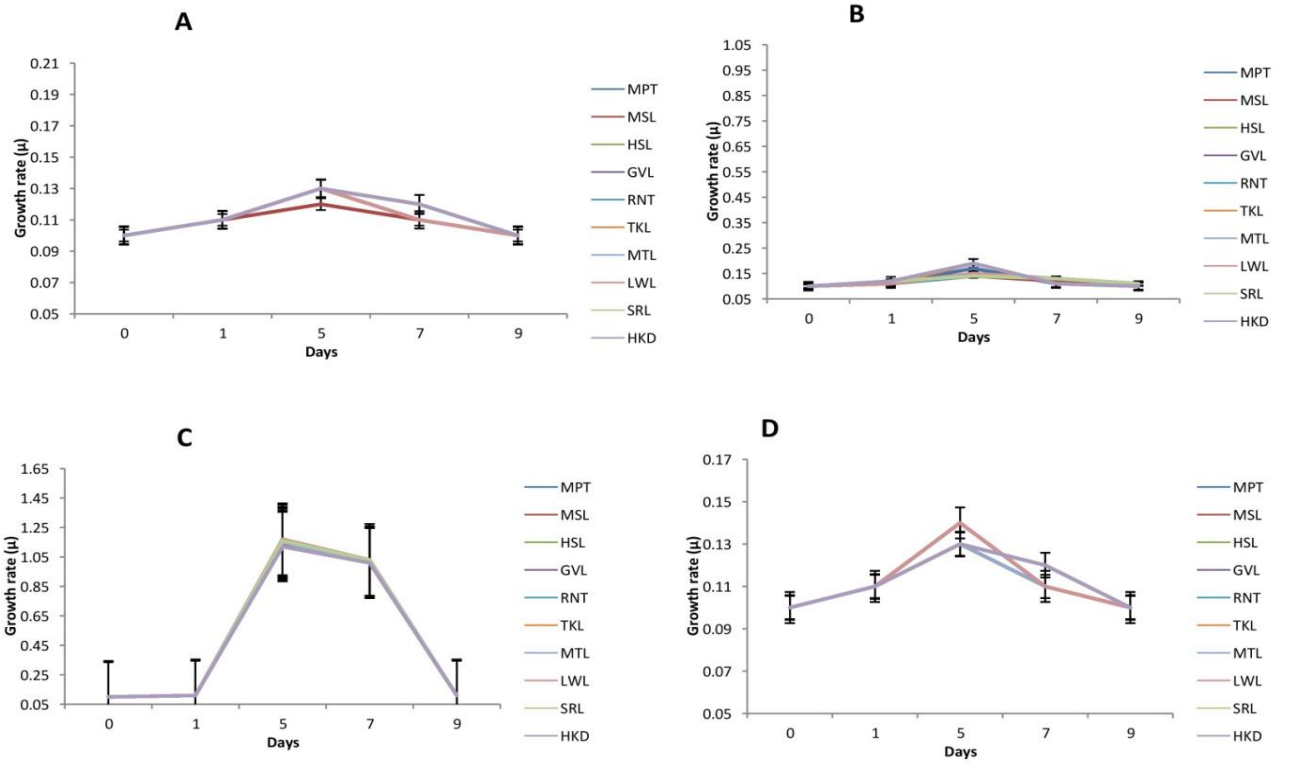


Figure 8: Growth profile of *Microcystis aeruginosa* isolates at different NO_3^- concentration A-50, B-100,C-250,D-500 µM after 5 day of incubation.

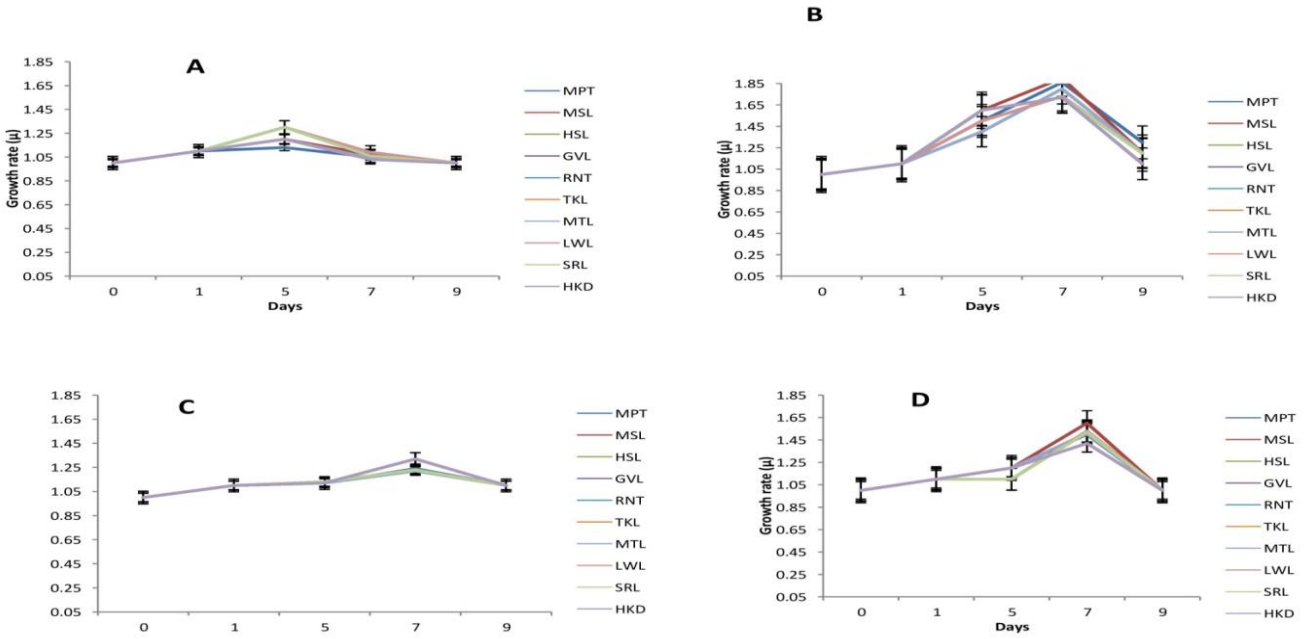


Figure 9: Growth profile *Microcystis aeruginosa* isolates at different PO_4^{3-} concentration A-1.0, B-5.0,C-10.0,D-50.0 µM after 5 day of incubation.

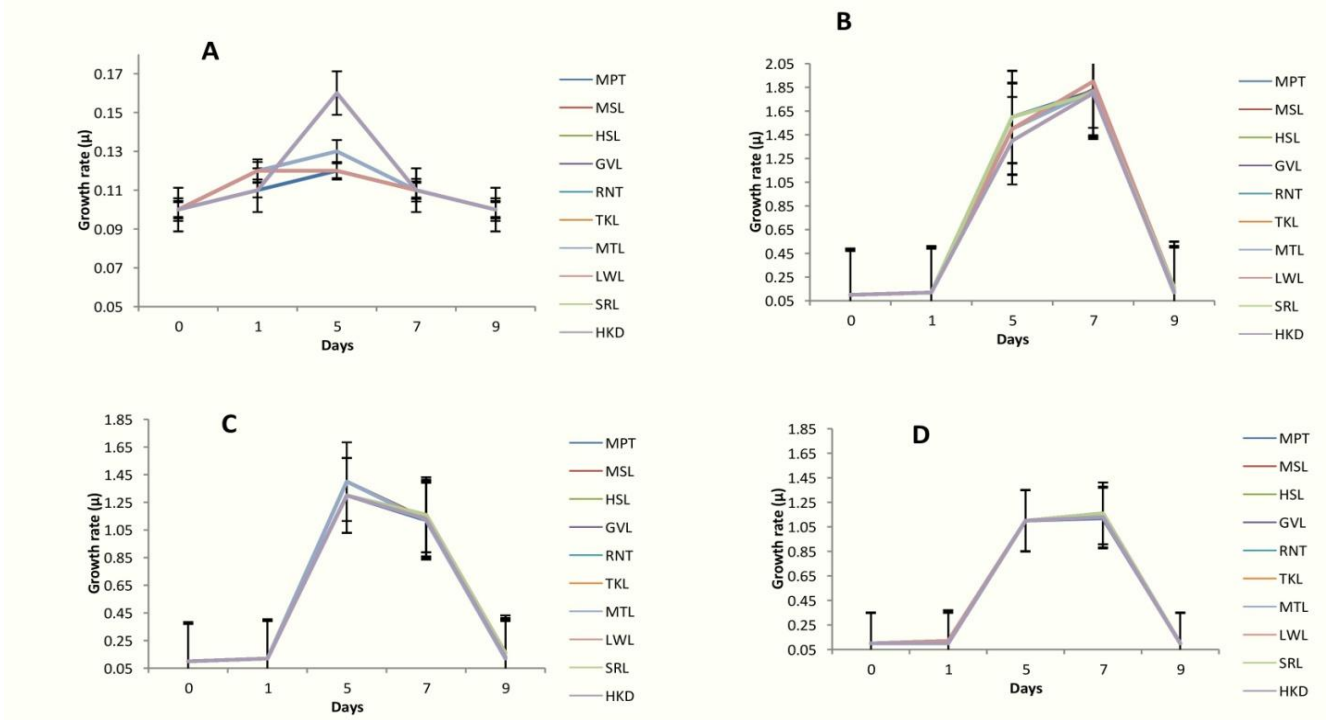


Figure 10: Growth profile of *Microcystis aeruginosa* isolates at different NH_4^+ concentration A-50 µM, B-100 µM,C-250 µM,D-500 µM after 5 day of incubation.

Enumeration of physicochemical meteorological data and *Microcystis* occurrence:

During the microscopic examination of sample, the *Microcystis aeruginosa* were present large number of cells during all seasons over period of one years. The indices chl-*a* were used to characterize trends in the *Microcystis aeruginosa* cells abundance in different aquatic habitats. The water analysis from different sampling site (MTL, LWL, SRL, HKD, HST, TKL, MPT, MSL, GVL and RNT) from different geographical location of central India, the identification results found the concentration of *Microcystis* cells, varies in different sample contained an average of 12 - 2.4X10^6 cells of *Microcystis*, and recorded chl-a content. *Microcystis aeruginosa* populations were commonly identified in 97.5% and the number of cells reached a highest peak in rainy season 7.3×10^6 cells $^{L-1}$, similar trends in winter season 6.1×10^6 cells $^{-L}$, and significant decrease was during summer season 4.2×10^6 cells $^{-L}$ in the studied sites Table-2. Seasonal significant difference was observed in rainy ($p>0.05$), winter ($p>0.05$) and summer ($p>0.05$) season in *Microcystis aruginosa*. The seasonally chl-*a* pigment concentration variation in sampling sites as highest mean chl-*a* value (42.67 µg^{-L}) was reached in rainy similarly (42.67 µg^{-L}) in winter and the lowest chl-*a* mean value (22.92 µg^{-L}) was reached in summer, Misson *et al.*, and Agha *et al.*,[49] reported that the variation of *Microcystis aeruginosa* density as well as chl-*a* content differ significantly in all sampling sites may be due to physical factors and biological condition.

Physicochemical and meteorological study

The abiotic factors (pH values, phosphorus, and nitrite concentrations), which had direct effect on *Microcystis aeruginosa* cells abundance. The pH value of water samples ranges from 6.5 to 9.3 in the study sites generally neutral to slightly alkaline. Although pH has direct effect on aquatic ecosystem, it shows close relationship with chemical constituents of all sampling water. The alkalinity of water is the important parameter indicating the tropic status and biodiversity pattern of an aquatic ecosystem. Seasonal variation of total alkalinity was recorded 120 mg^{-L} maximum value during summer season and minimum 60, 89 mg^{-L} in rain and winter season respectively. Total alkalinity ranges from 70.0 to 442.0 (mg^{-L}) in the sampling sites (MTL, LWL, SRL, HKD, HST, TKL, MPT, MSL, GVL and RNT). The study site receives sewage water and drainage from 28 sewage-filled nallahs, suffers from pollution due to drainage from sewage-filled nallahs, lack of fresh water source, its water is not suitable for drinking [50]. The in this lake water bodies reported presence of *Microcystis aeruginosa*, due to different geographical location of study sites pH values varies which directly effect on *Microcystis aeruginosa* cells abundance [51,52]. Alkalinity level in sampling sites found to be higher than the permissible limit (WHO: 120 mg^{-L}) in many sampling sites, evidenced the highest value in sampling sites. Similarlily In monsoon months, heavy rains caused higher turbidity and so photosynthetic rate is also decreased because reduced intensity of light causing decrease in pH value during monsoon [53]. The water temperatures effects the growth of *Microcystis aeruginosa* bloom during study. It was observed that the water temperature varies seasonally in selected sampling sites, the minimum water temperature was recorded in winter season at sampling sites (MTL, LWL, SRL, HKD, HST, TKL, MPT, MSL, GVL and RNT), where as maximum value of 39.1 ^{0}C was

registered in summer season. Similarlily the increasing in water temperature particularly in summer can be attributed to overall increasing trend in atmospheric temperature in addition to exothermic chemical process of the human activities prevail all along the lakes. The seasonal presence of free carbon dioxide was recorded which is the maximum as to be 5.8 mg^{L-1} at sampling sites in summer season, whereas the minimum free carbon dioxide 1.6 mg^{L-1} which was observed in monsoon season, the natural aquatic habitats resources, beautiful fauna and flora including many rivers, lakes, pond, dams, pools, tanks and waterfalls, water bodies reported presence of *Microcystis aeruginosa*, due to different geographical location of study sites water temperature varies which directly effect on *Microcystis aeruginosa* cells abundance [54]. The metabolic rate and reproductive activities of aquatic life are greatly influenced by water temperature, which varies with season, elevation, geographical location, and climatic conditions. Seasonal changes for pH, turbidity, TDS [53], and alkalinity were also considerably different. Nitrate is a naturally occurring type of nitrogen that is present in the soil and water. An excessive amount of it results in the production of an algal bloom, which causes eutrophication [55].

The electrical conductivity of water followed a seasonal pattern in different aquatic. During monsoon the minimum value 190.3 µS/cm was observed at sampling sites, where as the maximum 390 µS/cm was noted at sampling sites (MTL, LWL, SRL, HKD, HST, TKL, MPT, MSL, GVL and RNT) in summer season, water bodies reported presence of *Microcystis aeruginosa*, due to different geographical location of study sites electrical conductivity of water values varies which directly effect on *Microcystis aeruginosa* cells abundance. The higher conductivity may be due to the evaporation of water in summer season similar result was noted by Reddy and Solanki and Acharya [55]. Similarlily reported by Upadhyay *et al.*, [53]. The oxygen is one of the most important constituents needed for the metabolic process of plants and animals. In the present investigation minimum 6.1 mg^{-L} and maximum 12.2-16.2 mg^{-L} was recorded in samples during summer, rainy and winter season in all study sites (MTL, LWL, SRL, HKD, HST, TKL, MPT, MSL, GVL and RNT) respectively, water bodies reported presence of *Microcystis aeruginosa*, due to different geographical location of study sites. The dissolved oxygen in water varies which is directly related to *Microcystis aeruginosa* cell abundance. Similarlily the oxygen concentration showed decreasing trend from summer onwards, low oxygen during the peak summer may be attributed to the temperature rise and stratification which leads to the increased bacterial consumption of oxygen[56].

The summer sample locations showed a mean value of 8.1 mg-L for the nitrate concentration. According to the findings of the current investigation, N2 was found in the water at high temperatures during the rainy season at the lowest quantity of 2.2 mg L-1. The WHO recommends a 50 mg/L nitrate level for drinking water. All of the water samples' nitrate readings are below acceptable limits. Chloride levels were high in the summer and low in the monsoon season, suggesting that the evaporation of the water body and higher temperatures may be to blame for the increase in chloride content. Chloride is a crucial criterion for the detection of contamination in water bodies. detected chlorides 496.0 mg-L on a rainy [56].

The turbidity of the water samples ranged from 0.0 to 5.0 NTU-L, while the acceptable range for turbidity values for drinking water is between 10 NTU and 20 NTU (WHO,2004). Water turbidity varied from 4 NTU, which is the lowest, to 7 NTU, which is the highest, at sampling sites, where 12 NTU was measured in the summer. Higher turbidity has an indirect impact on life because it allows cyanobacterial blooms to use more light for photosynthesis, which lowers the rate of primary productivity [53]. According to WHO regulations, the maximum amount of total dissolved solids (TDS) in drinking water is 500 mg/L, but the amount measured was 1296.0 mg/L. A high TDS concentration in fresh water can alter the taste and reduce microbial activity[55].

The meteorological data highest rainfall was observed at study sites during the rainy season (22.15 mm), while summer rainfall was not as noticeable (0.17 mm). According to statistical analysis, rainfall was positively correlated with the density of Microcystis aeruginosa cells. In the rainy and winter seasons, there was a positive correlation between the amount of light and the density of Microcystis aeruginosa cells. The results of the study show that the concentration of Microcystis aeruginosa cells was correlated with nutrient-related variables, such as chlorophyll-a, population dynamics of Microcystis aeruginosa, nutrient levels, rainfall (positive correlations), and irradiance (negative correlation) in the summer, the variation of meteorological data in study sites due to different geographical location of study sites, which directly affect on Microcystis aerugi Major blooms are likely to happen after the rainy season [55,52]. Although this study was conducted in 30 water bodies of central Indian aquatic habitats, the results reinforce similar studies in other environments globally and support the present knowledge of potentially-toxic *Microcystis aeruginosa* distribution in tropical environments [57].

Influence of environmental and physiochemical factors on *Microcystis aeruginosa*

The principal component analysis (PCA) of study sites of different sampling station Bhopal, Ujjain, Tikamgarh, Gwalior and Rewa, included the biological variables are microcystin, chl-a and environmental variables are conductivity, total dissolved solid, total alakality, water temperature, dissolved oxygen, turbidity, rain fall, light irradiance, Phosphate, nitrate, pH, CO_2, chloride, the PCA results of the different geographical location of study sites of central India are following.

The first component of the analysis (inertia value = 31.0%) between variables related to rain and nutrients on the one

hand, and variables related to water temperature and light on the other, was determined using the PCA findings of the study sites LWL, SRL, and LDT. Conductivity and pH were in opposition on the second PCA component (inertia value = 18%), with pH on one side of the axis and temperature on the other. The PCA's second component was primarily related with the other side concentrations of Microcystis and chl-a, but in the other direction, suggesting that alakality and water temperature interact negatively. This placement along the second axis of the PCA further implies that microcystin concentration is adversely correlated with nutritional factors and water quality.

Results from the PCA analysis of the research site MPT (Marajapura Tal) showed that there was a significant inertia (inertia value = 35.0%) between variables related to rain and nutrients on the one hand, and variables related to water temperature and light on the other. Conductivity and DO, pH were in opposition on the second PCA component (inertia value = 10%), with conductivity on one side of the axis and DO, pH on the other. This shows that the concentration of Microcystis, chl-a, and nutrient loading in the MPT sample were primarily linked with the second PCA component, but in the opposite direction, which suggests adverse interactions with water temperature and alkalinity. This placement along the second axis of the PCA further shows that microcystin concentration isOverall the PCA results indicated that the biological and environmental variable observed in study sites MTL, LWL, SRL, HKD, MPT, MSL, HST, RNT, GVL and TKL, the nutrients and temperature variation was much higher which was link between nutrients which directly influence the microcystin production study sites MPT, GVL and TKL. As a result, the findings indicate a significant risk to humans and animals using recreational waters, which may increase in the future due to predicted changes in climatic change, specifically temperature and rainfall patterns [52]. The physicochemical parameters influence *Microcystis* dominance in high nutrient load conditions, and the elevated turbidities that probably occurred following the heavy rainfall in winter coincident with lower temperatures could have contributed to the microcystin production in study site MPT, RNT and TKL. The effects of climatic changes on Microcystis concentration variation in aquatic habitats as a result of extreme rain events and environmental conditions may have implications for the dominance of potentially toxic Microcystis blooms in the study sites. Similar findings revealed that the influence of rain fall and light irradiance was highly significant for the growth of Microcystis aeruginosa and microcystin production at moderate light conditions and low temperatures [57]. Torres *et al.*, reported on the effect of light on the abundance of *Microcystis aeruginosa* during winter, which might favour the growth of freshwater ecosystems . The data obtained from this study would fit with the well-established notion that *Microcystis aeruginosa* growth rates and thus bloom occurrence are more widespread during periods when light intensities and water temperatures are higher [58] and agree with the common occurrence of *Microcystis aeruginosa* blooms in late summer. Other previous studies have concluded that the summer months are dominated by *Microcystis aeruginosa* succeeding in late summer, autumn, and early winter. Temperature may well influence not just bloom dynamics, but also the preferred production of the toxic fraction of any given cyanobacterial population [22]. While there may be evidence for such a relationship in Microcystis sp., there is contradictory evidence in aquatic organisms showing an inverse relationship between temperature and toxin production [57].

IV. CONCLUSION

In order to improve our understanding of the factors influencing the distribution and abundance of microorganisms in nature, our discoveries underscore the necessity for improved linkage between sequence and environmental data. Predicting when and where aquatic microorganisms, including Microcystis, will live and bloom is essential in order to facilitate the design of affordable monitoring systems and management strategies to reduce human and environmental exposure as the Earth's climate, population, water-, and land-use rapidly change. The different nutrients media BG-11, BG-12 and Hughes were used for optimization of growth of *Microcystis aeruginosa* experimental results found that the BG-12 liquid culture medium was selected as the best culture medium suitable for the culturing of *Microcystis aeruginosa* isolates MPT, MSL, HST, GVL, RNT, and TKL, because of the media differ in nutrients concentration, presence or absence of vitamins and micronutrients, BG-11 and BG-12 medium consists highest concentration of Na^+ and K^+ but Hughes medium there is moderate concentration of Na^+ and K^+ source therefore, growth of isolates more in BG-11 and BG-12 medium than Hughes media, appropriate Ka^+, Na+ ions required in the cytoplasm of *Microcystis aeruginosa* for optimum growth, high Na+ and ammonia for nucleic acid synthesis. Physicochemical study results found that toxigenic *Microcystis aeruginosa* more abundance in higher nutrients (Nitrate and Phosphorus) load and physiological factors pH, light and temperature variation in study sites from different geographical location of central India. Microcystin toxin concentrations in all study sites comparatively not higher than guideline recommended by the World Health Organization i.e. $1\mu g^{-L}$. However, because backpacking and camping are common in the study areas from different geographical location of central India, lakes possibly could be used as drinking-water sources. The release of microcystin bioactive compound during stress condition of *Microcystis aeruginosa*, the microcystin identified in laboratory condition in different media and natural condition in different geographical distribution suggesting that microcystin identified application of pharmacological significance may be emerging as anticancer therapeutics.

REFERENCES

[1] Zhang, M.; Duan, H.; Shi, X.; Yu, Y.; Kong, F.,.Contributions of meteorology to the phenology of cyanobacterial blooms: Implications for future climate change. *Water Research*, Vol. **46**, pp.**442–452**, **2012**.

[2] Monks N.R., Liu Shuqian and Moscow, Jeffrey A.,. Microcystins as Agents for Treatment of Cancer. *Pharmaceutical Sciences Faculty Patents*.. Vol.**35**, pp.**211-219, 2015.**

[3] Xiao, M., Willis, A., Burford, M. A. & Li, M.,. Review: a meta-analysis comparing cell-division and cell-adhesion in *Microcystis* colony formation. *Harmful Algae* Vol**. 67,** pp**.85–91, 2017**.

[4] Qianqian SUN,Wei ZHU, Ming LI, Xiao TAN,. Morphological changes of *Microcystis aeruginosa* colonies in culture, *Journal of limnology*, Vol.**10,** pp**.1-20, 2015**.

[5] Paerl, H. W., Gardner, W. S., McCarthy, M. J., Peierls, B. L. & Wilhelm, S. W.. Algal blooms: noteworthy nitrogen. *Science*. Vol.**32**, **346:175 ,2014.**

[6] Srivastava Ankita, So-Ra Ko, Chi-Yong Ahn, Hee-Mock Oh, Ravi Alok Kumar and Asthana Ravi Kumar,. Microcystin Biosynthesis and *mcyA* Expression in Geographically Distinct *Microcystis* Strains under Different Nitrogen, Phosphorus, and Boron Regimes. *BioMed Research International*, **2016**. doi.org/10.1155/2016/5985987.

[7] Kumari Simmi and Choudhary Shikha ,. Study Of Various Physico-Chemical Parameters On Growth Behavior On Different Algal Isolates From Shastra Districts, *World Journal Of Pharmacy And Pharmaceutical Sciences*, Vol.**7, 10,** pp**.1002-1012, 2018, ISSN 2278 – 4357.**

[8] Taranu, Z.E.; Zurawell, R.W.; Pick, F.; Gregory-Eaves, I.,. Predicting cyanobacterial dynamics in the face of global change: the importance of scale and environmental context. *Global Change Biology*, Vol.**18,** pp**.3477–3490, 2012**.

[9] Terray, L.; Boé, J..Quantifying 21st-century France climate change and related uncertainties.*Geoscience*, Vol.**345,** pp**.136–149, 2013.**

[10] Codd G.A., Azevedo S.M.F.O., Bagchi S.N., Burch M.D., Carmichael W.W., Harding W.R., Kaya K. and Utkilen H.C.,. CYANONET: A Global Network for Cyanobacterial Bloom and Toxin Risk Management *Initial Situation Assessment and Recommendations*, IHP-VI Technical Document in Hydrology. **2005**, No.76 UNESCO Working Series SC-2005/WS/55.

[11] Mukhopadhyay S, Naskar N,. Int. J. Innov. *Res. and Rev*. Vol. **3,** pp.**107–113, 2015.**

[12] Rippka, R., Deruelles, J., Waterbury, J., Herdman, M., Stanier, R.,. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *Journal of General Microbiology*. Vol.**111**, pp.**1–61, 1979**.

[13] Rippka R.,.Isolation and purification of cyanobacteria. *Method Enzymol*. Vol.**167,** pp**.3-27, 1989**.

[14] Nakagawa, M., Taicaniura, Y. And Yagi,. Isolation and characterization ofthe slime froma cyanobacterium Microcystisaeruginosa K-3A. Agricultural and Biological Chemistry Vol.**51,** pp**.329-337, 1987.**

[15] Desikachary,T.V., (1959). In. Premnath (Eds.) Cyanophyta.Indian council of Agriculture Research, New Delhi India.: **3,1-302, 1959.**

[16] Hughes, E. O., Gorham, P. R., and Zehnder, A.. Toxicity of a unialgal culture of *Microcystis aeruginosa*. *Can. J. Microbiol*. Vol.**4,** pp**.225–36, 1958.**

[17] Allen, M. M., and Stanier, R. Y.. Growth and division of some unicellular blue-green algae. *J. Gen. Microbiol*. Vol.**51,** pp**.199–202, 1968.**

[18] Mackinney Gordon , Absorption Of Light By Chlorophyll Solutions, Journal Of Biological Chemistry, ,Vol.**140,** pp.**315-322, 1941.**

[19] Lowry Oh, Rosebrough Nj, Farr Al, Randall Rj. Protein Measurement With The Folin Phenol Reagent. J Biol Chem. ; Vol.**1,** pp**.265-75, 1931. PMID: 14907713.**

[20] Wynne, D. and Rhee, G.-Y.,. Effects of light intensity and quality on the relative N and P requirement (the optimum N: P (ratio) of marine planktonic algae. *Journal of Plankton Research*. Vol.**8,** pp**.91-103, 1986.**

[21] Van der Westhuizen, A.J. and Eloff, J.N.,. Effect of temperature and light on the toxicity and growth of the blue-green alga *Microcystis aeruginosa* (UV-006). *Planta*. Vol.**163,** pp**.55-59, 1985.**

[22] Liu, X., Lu, X. & Chen, Y.. The effects of temperature and nutrient ratios on *Microcystis* bloom in Lake Taihu, China: an 11-year investigation. *Harmful Algae*.Vol.**10,** pp**.337-343, 2011.**

[23] Desikachary,T.V.,. In. Premnath (Eds.) Cyanophyta.Indian council of Agriculture Research,New Delhi India.: vol **3,** pp**.1-302, 1959.**

[24] Yang Jingwen, Tang Hengxing , Zhang Xingxing, Yang Zhou,. High temperature and pH favor Microcystis aeruginosa to outcompete Scenedesmus obliquus. *Environmental Science and Pollution Research*,Vol.**25,5,** pp**.1-9, 2017.**

[25] Li, D.; Yu, Y.; Yang, Z.; Kong, F.; Zhang, T.; Tang, S.**.** The dynamics of toxic and nontoxic microcystis during bloom in the large shallow lake, Lake Taihu, china. *Environmental Monitoring and Assessment* ., Vol.**186,** pp**.3053–3062, 2014.**

[26] Imai, H., Kwang-Hyeon, C., Kusaba,M., Nakano, S.-I.,. Temperature-dependent dominance of Microcystis (Cyanophyceae) species: M. aeruginosa and M. wesenbergii. *Journal of Plankton Research* vol. **31,2,** pp**.171–178, 2009.**

[27] Shen Hong, Yuan Niu†, Ping Xie, Min Tao And Xi Yang,. Morphological and physiological changes in Microcystis aeruginosa as a result of interactions with heterotrophic bacteria. *Freshwater Biology*. Vol.**56,** pp**.1065–1080, 2011.**

[28] Reynolds CS.. Growth and replication of phytoplankton. In: The ecology of phytoplankton. New York (NY): *Cambridge University Press.* pp.**179–180, 2006.**

[29] Singh Satya Shila, Kikku Kunui, Minj Robin Anigo, Singh Prashant.. Diversity and distribution pattern analysis of cyanobacteria isolated from paddy fields of Chhattisgarh, India. Journal of Asia-Pacific Biodiversity, Vol.**7,4,** pp**.462-470, 2014.**

[30] Li Ming, Philip Nti Nkrumah & Man Xiao,. Biochemical composition of *Microcystis aeruginosa* related to specific growth rate: insight into the effects of abiotic factors. *Inland Waters*, Vol.**4:4,** pp**.357-362, 2014. ISSN: 2044-2041.**

[31] Kotak B, Lam A, Prepas E, Hrudey S:,. Role of chemical and physical variables in regulating microcystin-LR concentration in phytoplankton of eutrophic lakes. *Canadian Journal of Fisheries and Aquatic Sciences*. Vol.**57,8, 2000** : 10.1139/f00-091 1584-1593 10.1139/f00-091.

[32] Haider Alghanmi, Haifaa Muttar,. Effect of Physicochemical Factors on Cyanobacteria Biodiversity in Some Agricultural Soil of Al - Diwaniyah City during Spring Period, *Journal of Global Pharma Technology*, **vol 12,9,pp43-52, 2017.**

[33] Sedmak B. & Elersˇek T.,. Microcystins induce morphological and physiological changes in selected representative phytoplanktons. *Microbial Ecology, vol.* **50, pp298–305, 2005.**

[34] Yeung Anna C. Y.,'Agostino Paul M. D, Poljak Anne, McDonald James, W. Bligh Mark, T. David Waite,and Neilan Brett A.,.Physiological and Proteomic Responses of Continuous Cultures of *Microcystis aeruginosa* PCC 7806 to Changes in Iron Bioavailability and Growth Rate. Appllied Environmental Microbiology. Vol.**1; 82, 19: pp5918–5929, 2016.**

[35] Xiao Man, Colin Ming Liand S. Reynolds,. Colony formation in the cyanobacterium *Microcystis. Biological Review*. **93, pp. 1399 – 1420, 2018**.doi: 10.1111/brv.

[36] Alexoval R, Fujii M, Birch D, Cheng J, Waite TD, Ferrari BC, Neilan BA .,. Iron uptake and toxin synthesis in the bloom-forming *Microcystis aeruginosa* under iron limitation. *Environ Microbiology.* Vol. **13,4, pp1064–1077, 2011.**

[37] Bortoli Stella, Oliveira-Silva Diogo, Krüger Thomas Dörr Felipe A. , ColepicoloPio, Volmer Dietrich A. , Pinto Ernani,. Growth and microcystin production of a Brazilian *Microcystis aeruginosa* strain (LTPNA 02) under different nutrient

conditions. Revista Brasileira de Farmacognosia., vol. **24, pp.4, 2014.**

[38] Wang Xin, Chunbo Hao , Feng Zhang , Chuanping Feng , Yingnan Yang,. Inhibition of the growth of two blue-green algae species (*Microsystis aruginosa* and *Anabaena spiroides*) by acidification treatments using carbon dioxide. *Bioresource Technology* vol. **102,pp.5742–5748, 2011.**

[39] Yang Jingwen, Tang Hengxing , Zhang Xingxing, Yang Zhou,. High temperature and pH favor Microcystis aeruginosa to outcompete Scenedesmus obliquus. *Environmental Science and Pollution Research*, vol **25,5,pp.1-9, 2017.**

[40] Pierangelini, M.; Stojkovic, S.; Orr, P.T.; Beardall, J.,. Photosynthetic characteristics of two Cylindrospermopsis raciborskii stains differing in their toxicity. *Journal of Phycology*, vol. 50,pp 292–302, 2014.

[41] Pan J, Guo B. Effects of Light Intensity on the Growth, Photosynthetic Characteristics, and Flavonoid Content of Epimedium pseudowushanense B.L.Guo. Molecules. Vol **4;21(11):1475, 2016**. doi: 10.3390/molecules21111475. PMID: 27827937; PMCID: PMC6273461.

[42] Kim Hocheol, Bok Yeon Jo and Kim Han Soon.. Effect of different concentrations and ratios of ammonium, nitrate, and phosphate on growth of the blue-green alga (cyanobacterium) *Microcystis aeruginosa* isolated from the Nakdong River, Korea. *Algae*. Vol.**32,4 :pp 275-284, 2017**.

[43] Lee, O. H. & Cho, K. J.. Nitrogen and phosphorus uptake and growth kinetics of *Microcystis aeruginosa* cultured under chemostats. *Korean Journal of Limnology*. Vol 39:pp119-130, 2006.

[44] Chen Qin, Christiansen Guntram, Li Deng, and Rainer Kurmayer.. Emergence of nontoxic mutants as revealed by single filament analysis in bloom-forming cyanobacteria of the genus Planktothrix, *BMC Microbiology*. Vol 3,pp16:23, 2016.

[45] Liu, X., Lu, X. & Chen, Y.. The effects of temperature and nutrient ratios on *Microcystis* bloom in Lake Taihu, China: an 11-year investigation. *Harmful Algae*.vol. **10:pp337-343, 2011.**

[46] Jaiswal Kailash, Gothalwal R, A S Yadav,2018. The influence of environment in the distribution of toxinogenic Microcystis aeruginosa in aquatic habitats. Conference: 59th Annual Conference of Association of Microbiologists of India (**AMI-2018**). DOI: 10.13140/RG.2.2.26848.12807

[47] Wangwibulkit, S.; Limsuwan, C. and Chuchird, N.,. Effect of salinity and pH on the growth of Blue-green algae, Oscillatoria sp. and Microcystis sp., isolated from pacific white shrimp (Litopenaeus vannamei) ponds. Kasetsart univ. F*isheries Research*, vol. **32,1,pp 1-9, 2008.**

[48] Baldia, S. F., Evangelista, A. D., Aralar, E. V. & Santiago, A. E. Nitrogen and phosphorus utilization in the cyanobacterium *Microcystis aeruginosa* isolated from Laguna de Bay, Philippines. *Journal of Applied Phycology* vol. 19:pp607-613, . 2007.

[49] Zhou Y, Zhang X, Li X, Jia P, Dai R. Evaluation of changes in Microcystis aeruginosa growth and microcystin production by urea via transcriptomic surveys. Sci Total Environ. Vol. **10;655:pp181-187, 2019.** doi: 10.1016/j.scitotenv.2018.11.100. Epub 2018 Nov 8. PMID: 30469064.

[50] Pani Subrata, Dubey Amit and Khan M.R.,. Decadal Variation in Microflora and Fauna in Water Bodies of Bhopal, Madhya Pradesh, *Current World Environment*, vol l. **9,1, 137-144, 2014.**

[51] Pathak Hemant,. Assessment Of Eutrophic Status Of Water Resources Of Madhya Pradesh, India: A Review, *International Journal of Innovative Research & Growth*, vol.**1,1,pp.1-5, 2015**. ISSN: 2455-1848.

[52] Acharya Ruchi, Saify Tayyab, Sharma Bhawna and Gautam Rajani,. Relationship Analysis between Phytoplankton Diversity and Water Quality of Lower Lake of Bhopal. *Asian Journal of Water, Environment and Pollution, vol.***12, 4, pp.83–86, 2015.**

[53] Upadhyay S.K., , Assessment of Lake Water Quality by Using Palmer and Trophic State Index- a Case Study of Upper Lake, Bhopal, India, *International Research Journal of Environment Sciences*,. Vol. **2,5, pp.1-8, 2013.**

[54] Mishra Neeta and Pandey Devendra N.,. Phytoplankton Diversity of Atarital Dam At Mauganj, Rewa Madhya Pradesh, India. *International Journal of Applied and Universal Research*,vol. **4,3,pp6-11, 2017**. E-ISSN No: 2395-0269.

[55] Mahapatra kalyani,MKS kushwa,.Study the seasonal variation and correlation analysis of various parameters of tekanpur lake, *International Journal of Multidisciplinary research and development*,vol. **2,6,pp.201-205, 2015.**

[56] Yan YW, Dai RH, Liu Y, Gao JY, Wu XH ,. Comparative effects of inorganic and organic nitrogen on the growth and microcystin production of *Microcystis aeruginosa*. *World Journal of Microbiology Biotechnology*., vol.**31,5,pp.763–772, 2015.**

[57] Santhanam P. Balaji Prasath B., Nandakumar R., and Jayalakshmi T.,. Detection of Cyanotoxins of Cyanobacterial (*Microcystis aeruginosa*) Strain Using Microtox® Bioluminescence Bioassay .*Basic and Applied Phytoplankton Biology*, **2019**. https://doi.org/10.1007/978-981-10-7938-2_10.

[58] Metcalf, J.S.; Codd, G.A.. A Review of Current Knowledge, Cyanobacterial Toxins (Cyanotoxins) in Water; Foundation for Water Research: Marlow, UK, . vol **47, 2014.**

AUTHORS PROFILE

Dr. K.P. Jaiswal worked as Faculty of Biotechnology, Barkatullah University, Bhopal, M.P., India, assistant professor in the Department of Biotechnology and Bioinformatics at the Maulana Azad National Institute of Technology (MANIT), Bhopal, India. Bachelor of Science in Biotechnology from Punjab Technical University (I.K. Gujral Punjab Technical University), Jalandhar, Punjab, India, in 2009; master of Science in Animal Biotechnology from the National Dairy Research Institute, Karnal, India, in 2012; and Ph.D. in Biotechnology from Barkatullah University, Bhopal, India, in 2020; Bioinformatics Professional, TAUBER Bioinformatics Research Centre, Israel, in 2019 and Bioinformatics Carrier Professional, CERTC, New Delhi, India in 2020, Life member in society and academia, life member of Indian Science Congress Association (ISCA) in New Biology (Biotechnology) also member of Global Initiative of Academic Network (GIAN),Ministry of Education, MHRD, New Delhi, Government of India, Life Member, Life Science (Biotechnology), of Akhil Vigyan Bharti, India.

.

Dr. AS Yadav

Prof. Life Science , OSD In Mp Higher Education, Govt. Of M.P.

Dr. R. Gothalwal

Prof. Head Department of Biotechnology and Coordinator-SUB-DIC Bioinformatics Centre

Barkatullah University, Bhopal, M.P.

