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RESEARCH ARTICLE

# Occurrence of potentially toxic cyanobacteria *Microcystis aeruginosa* in aquatic ecosystems of central Kerala (south India)

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**Abstract –** *Microcystis aeruginosa* is a potentially toxic bloom-forming freshwater cyanobacterium, usually found in eutrophic water bodies worldwide. The present study reports the occurrence of *Microcystis aeruginosa* and its bloom in freshwater ponds along central Kerala (south India). Monitoring of cyanobacterial blooms was conducted from May 2019 to February 2020 along the aquatic ecosystems of central Kerala and the *M. aeruginosa* blooms were recorded from two freshwater ponds of Kochi. Massive blooms of *M. aeruginosa* was observed during the period prior to summer monsoon (May) with an abundance of $1.17 \times 10^6$ cells $L^{-1}$ (Station 1) and during early summer (February) latter being more thick scum ($2 \times 10^8$ cells $L^{-1}$) with high chlorophyll *a*. Dense aggregates of *M. aeruginosa* scums were more prevalent during the periods characterised by higher Surface Water Temperature (SWT). The nutrient characteristic pattern of the study area showed the abundance of *M. aeruginosa* correlated very well with higher dissolved nitrate (96.7 μmol $L^{-1}$) and phosphate (19.88 μmol $L^{-1}$) concentrations. Thus in the stable freshwater ponds with higher SWT and nutrients were the major factors influencing the growth and abundance of the cyanobacteria *M. aeruginosa.* Toxicological studies conducted revealed that the *Microcystis* bloom was hepatotoxic, inflicting fish mortality.

**Keywords:** CyanoHABs / *Microcystis aeruginosa* / Toxicity / freshwater ecosystems / south India

## 1 Introduction

Cyanobacteria are photosynthetic aquatic or terrestrial prokaryotes that are small, unicellular, often found in colonies and are also known as "blue-green algae" (Shestakov and Karbysheva, 2017). They are the most genetically diverse group and can exist in various habitats like freshwater, marine water and even extreme environment like frozen lakes, salt works and hot springs (Whitton, 1992). Eutrophication has led to the availability of excessive nutrients that leads to algal blooms causing negative impacts on animals and humans. Increased input of nitrogen and phosphorous from various anthropogenic activities into the water bodies substantially favours frequent algal blooms. Frequency and occurrence of harmful cyanobacterial blooms (CyanoHABs) have increased globally in recent years and has been attributed to climate change and eutrophication. Apart from eutrophication, climate change and spread of invasive species has led to variations in aquatic species compositions and alterations in the food web (Paerl and Huisman, 2009; O'Neil *et al.*, 2012; Knoll *et al.*, 2015). One of the characteristic that makes cyanobacteria prevalent during summer blooms when compared to other species of algae is their ability to proliferate at high temperature (Litchman *et al.*, 2010). Temperature increase could minimise vertical mixing, intensifies and prolongs vertical stratification and could make the environment more stable for *Microcystis* (Livingstone, 2003; Paerl and Huisman, 2009).

*M. aeruginosa* is considered as one of the main ecologically detrimental species due to its abundance in freshwater bodies that have varying nutrient loading and its degree of toxicity towards the terrestrial as well the aquatic organisms (Carmichael, 1992; Chorus and Bartram, 1999). Since they are capable of producing cyanotoxin named microcystin they are a major concern to the environment and public health. Microcystin is a hepatotoxin and various cases of human poisoning and mortality of fishes and mammals have been reported (Miller *et al.*, 2010; Tanabe *et al.*, 2018). Microcystin toxins are very stable in nature and are not affected by proteolytic or any hydrolytic attack. They can persist in filter feeders and can finally end up in humans via the food chain (Yu *et al.*, 2009; Chen *et al.*, 2009). These harmful algal blooms could pollute the water bodies with the toxin thereby causing the death of livestock and aquatic animals. *M. aeruginosa* is usually present in freshwater but their

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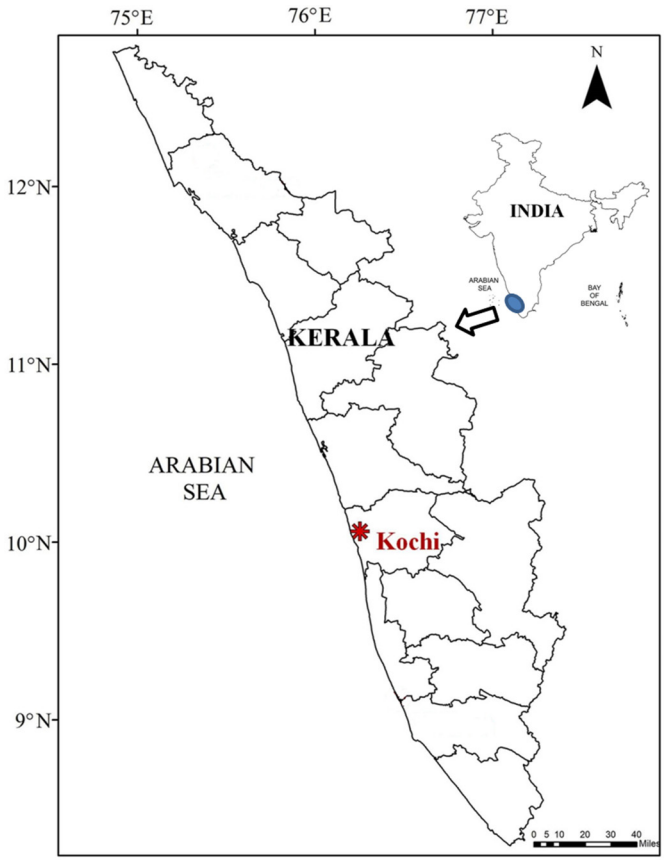


**Fig. 1.** Study area. Red star represents the location in Kochi (Kerala) from where *M. aeruginosa* bloom was recorded.

presence has also been reported in brackish water ecosystems. Their invasion from the freshwater to brackish water bodies has been less investigated (Tanabe *et al.*, 2018). Since the potentially toxic *Microcystis* species are capable of producing microcystin and can have adverse effects on the aquatic ecosystems as well as to humans and other mammals, a detailed study in these aspect stands significant. The present study investigates on the prevalence of *Microcystis* species as well as their development as blooms in the freshwater ecosystems of central Kerala, south India.

## 2 Materials and methods

As a part of monitoring cyanobacterial blooms, a preliminary survey of various aquatic ecosystems along central Kerala was carried out. Two freshwater ponds at Ernakulam district of Kerala, Station 1 (Lat. 9°58′6.7″N; Long. 76°16′56.8″E) and Station 2 (Lat. 9°57′34″N; Long. 76°17′32″E) with *Microcystis* bloom were selected for monitoring from May 2019 to February 2020 (Figs. 1 and 2).

Quantitative and qualitative analysis of water samples were carried out under a Leica DM 2000 light microscope. Identification of algal species was done with the help of existing literature and standard identification keys (Desikachary, 1959; Rosen and Amand, 2015). Quantitative estimation of algal cells was carried out using Sedgewick- Rafter counting cell. Algal bloom was collected from the ponds by filtering using 63 μm sieve for isolation using BG-11 media and storage for further studies. Isolation was done by single-cell isolation and serial dilution followed by agar plating method (Andersen, 2005). SEM images of *Microcystis* were also captured for detailed taxonomic analysis. Physico-chemical parameters like temperature, salinity, pH were measured with a digital thermometer, refractometer and pH meter respectively. Dissolved oxygen was estimated by Winkler's method (Winkler, 1888). Major nutrients such as nitrate, phosphate and silicate were estimated by following Grasshoff *et al.* (1983). Chlorophyll *a* was determined using 90% acetone extraction method using Hitachi UV-VIS spectrophotometer (Parson *et al.*, 1984). Toxicity studies were conducted by exposing seeds of *Oreochromis mossambicus* to *Microcystis aeruginosa* bloom. Tissue samples like gills, liver and heart of exposed fish were collected, fixed in 10% neutral buffered formalin for 24 hours, dehydrated and paraffin-embedded. Fine sections of tissues were mounted on a clean glass slide and stained with hematoxylin and eosin stains (Sanad *et al.*, 2015; Abdel-Latif and Khashaba, 2017). These sections were viewed under the microscope to study the histological changes due to the toxin.

## 3 Results

The analysis of surface water samples of station 1 and 2 showed the persistent presence of cyanobacteria *M. aeruginosa*. The occasional bloom of *M. aeruginosa* was observed from the study areas as green free-floating scums formed of densely packed colonies and some dried benthic colonies of the species with an unpleasant odour. There was no sign of mortality of any aquatic species from the bloom area.

Massive blooms of *M. aeruginosa* (Figs. 2 and 3) was observed during the period prior to summer monsoon (May) with an abundance of $1.17 \times 10^6$ cells $L^{-1}$ (Station 1) and during the early summer monsoon (February) at station 2, later being more thick scum ($2 \times 10^8$ cells $L^{-1}$). A decline in the cell density was noted at both the stations during monsoon season. In station 2, the coexistence of *M. aeruginosa* with *Anabaena* sp., was observed during the monsoon season which later got dominated by *Microcystis* sp. Light microscopic and SEM images of the species observed from the study area are shown in Figures 3 and 4. Other microalgae present along with the *M. aeruginosa* bloom at station 1 included *Pediastrum* sp., *Scenedesmus* sp., *Chlorella* sp., *Spirulina* sp., *Woronichinia* and diatoms but were found very meagre in cell number.

Seasonal variations in the physicochemical parameters obtained during the monthly monitoring of the study areas are depicted in Figures 5–10. Higher surface water temperature (31 °C) was recorded from both stations prior to monsoon. However, the temperature dropped to an average value of 27.2 °C during the monsoon season. The pH values at station 1 varied between 8.1 and 10.9 with peak value recorded during the end phase of the summer monsoon. At Station 2, the values ranged from 7.4 to 11.5 with higher values during the monsoon season. Dissolved oxygen estimated from station 1 varied between 3.41 ml $L^{-1}$ and 7.56 ml $L^{1}$. Station 2 had dissolved oxygen ranging from 4.31 ml $L^{-1}$ to 11.08 ml $L^{-1}$. A noticeable

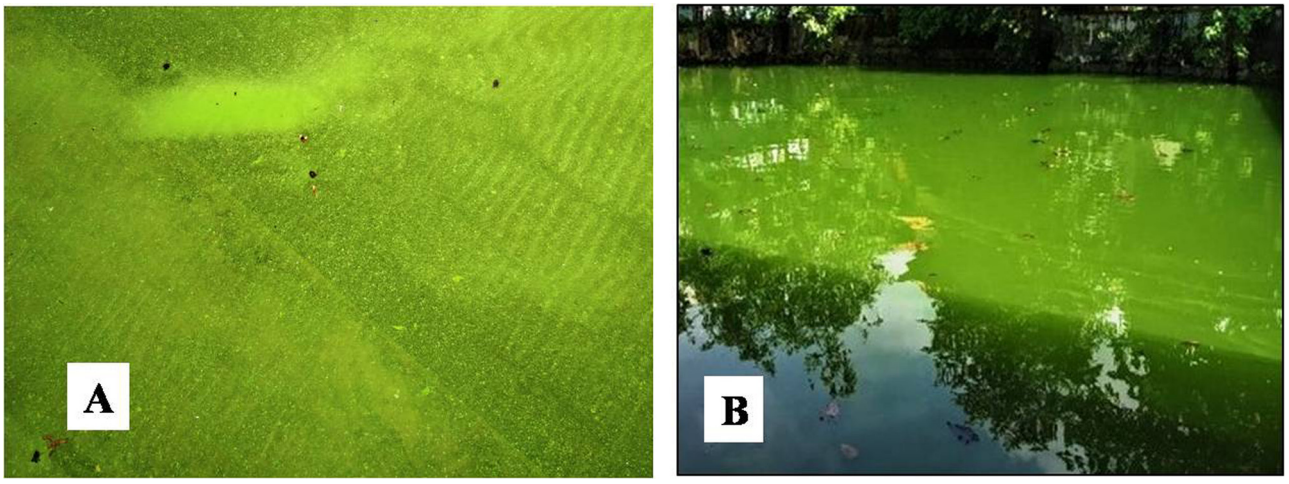


**Fig. 2.** *M. aeruginosa* bloom with green scum floating in the surface. (A) Station-1 (B) Station-2.

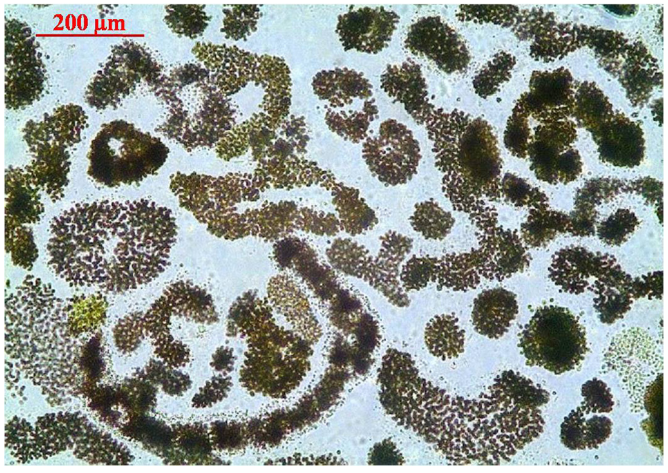


**Fig. 3.** Microphotographs (LM) of *Microcystis aeruginosa* cells from bloom samples.

decrease in dissolved oxygen was observed in both stations during the winter season. The salinity measured from both stations were zero. Chlorophyll *a* estimated was higher at station 1 (82.08 mg $m^{-3}$) prior to summer monsoon when the cell density was at its peak, which gradually decreased during the monsoon period to 20.42 mg $m^{-3}$. At station 2, chlorophyll *a* ranged from 38 mg $m^{-3}$ to 267.13 mg $m^{-3}$ with higher value observed during the monsoon season.

Moderate to high levels of nutrient concentrations were observed during the study period. Nitrate concentrations were high in the surface water (Station 1: 96.78 µmol $L^{-1}$ and Station 2: 43.149 µmol $L^{-1}$) prior to summer monsoon. Both the stations showed higher phosphate concentrations (Station 1: 19.889 µmol $L^{-1}$ and Station 2: 5.388 µmol $L^{-1}$) during the monsoon season. The peak concentration of silicate was observed during the monsoon season at Station 1 (194.105 µmol $L^{-1}$) and post summer monsoon at Station 2 (190.88 µmol $L^{-1}$).

Investigation on the stress response of candidate fish species (*Oreochromis mossambicus*) to exposure of *M. aeruginosa* bloom showed 100% mortality within 15 days of exposure. Tissues of control fishes retained the normal structure whereas moderate to severe histopathological changes were observed in the gills, liver and heart of fishes exposed to *Microcystis* bloom (Figs. 11–13) Structural changes in gills were noticed with the degeneration of primary and secondary lamella. Mild necrosis along with an increase in interlamellar space was also recorded. Liver of the test fish showed complete structural disruption. Vacuolar degeneration was observed in the hepatocytes. Severe necrosis of hepatocytes with inflammatory cells was present in the liver exposed to *Microcystis* bloom. Cardiac muscle cells showed fibrolysis and increased intercellular edema.

## 4 Discussion

*Microcystis* is a common bloom-forming cyanobacterium that is known to produce toxins. *Microcystis* cells are generally of 5–7 µm in diameter with different colony morphology (Baker and Fabbro, 2002). Eutrophic conditions with elevated temperature favour the occurrence of *Microcystis* blooms in aquatic ecosystems (Davis *et al.*, 2009). Various studies regarding diversity and cyanobacterial bloom occurrences has been reported from several countries (Codd *et al.*, 2005), including India (Agrawal *et al.*, 2006). *Microcystis aeruginosa* blooms reported from south west coast of India (Padmakumar *et al.*, 2008), estuaries (Santhosh Kumar *et al.*, 2010; Prasath *et al.*, 2014) and ponds (Muthukumar *et al.*, 2007) in Tamil Nadu, temple ponds of Varanasi city, Uttar Pradesh (Prakash *et al.*, 2009) are few among them from the Indian waters. However, reports regarding the occurrence of microcystin in India and toxicity studies from South Indian water bodies is particularly scarce (Sangolkar *et al.*, 2006; Prakash *et al.*, 2009). Detailed studies on *Microcystis aeruginosa* blooms from south India particularly freshwater ponds along Kerala are fewer.

From the analysis, the bloom of freshwater cyanobacteria was observed in two ponds which were dominated by 90–95% *Microcystis aeruginosa.* Green free-floating scums were present on the surface of the water which was due to the formation of colonies by *Microcystis aeruginosa*. *Microcystis*

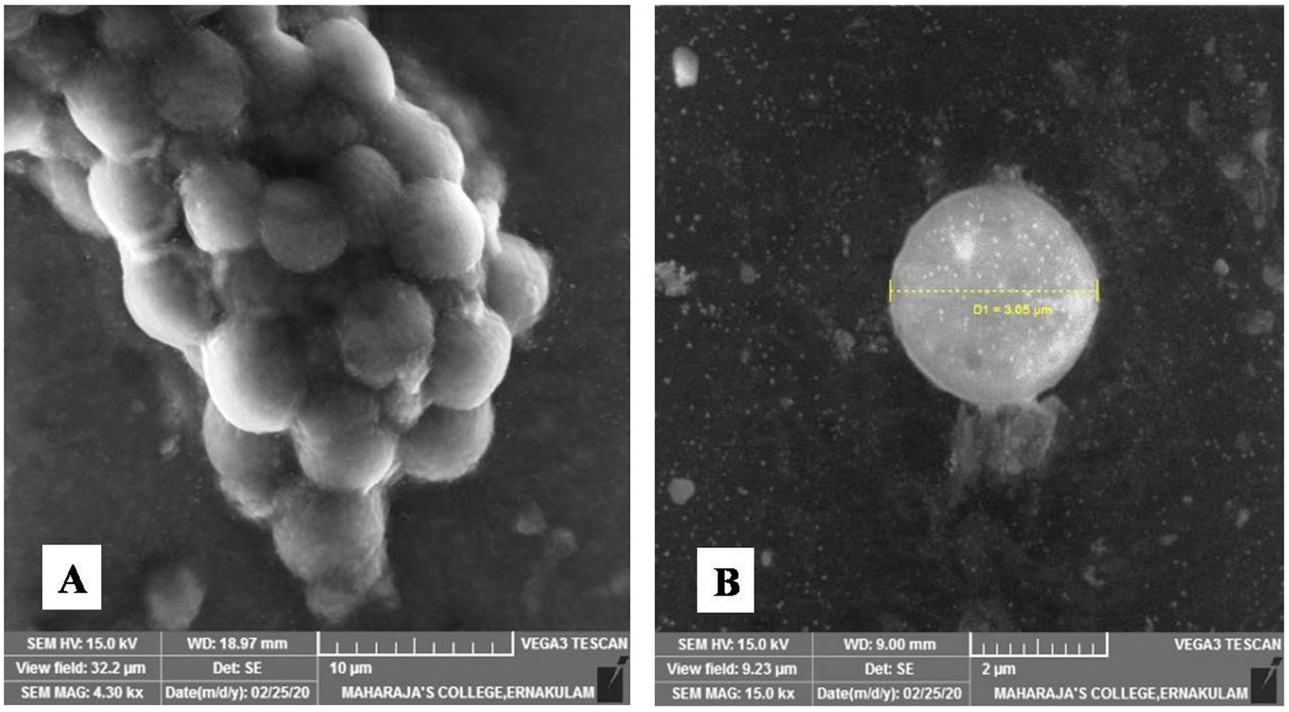


**Fig. 4.** SEM images of (A) *M. aeruginosa* colony (B) *M. aeruginosa* single.

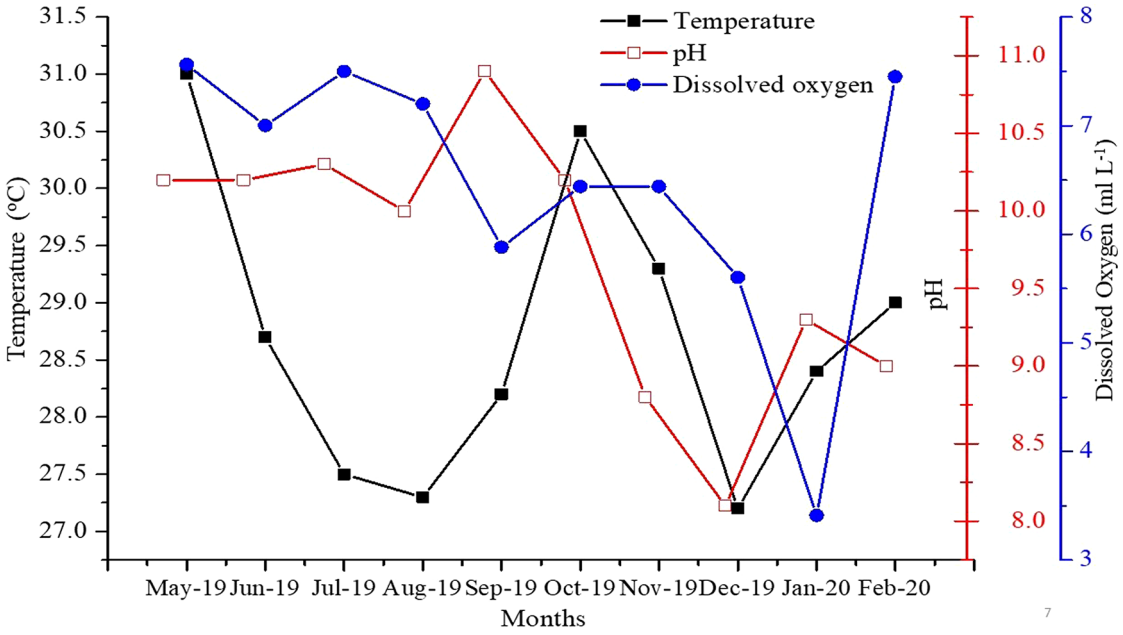


**Fig. 5.** Variations in temperature, pH and dissolved oxygen in Station-1 during the study period.

strains that contain gas vesicles can change their cell density and corresponding buoyancy which enables them to form blooms at different depths in the water column (Reynolds, 2006). Like the other buoyant cyanobacteria, *Microcystis* also prefers calm water columns, in which they will be able to adjust their buoyancy and thus regulate their vertical position (Reynolds and Walsby, 1975; Chorus and Bartram, 1999; Huisman *et al.*, 2004).

The cell densities in both stations were higher prior to monsoon with comparatively high surface water temperature (~31 °C). The temperature may well influence not just bloom dynamics, but also influence the preferred production of the toxic fraction of any given cyanobacterial population (Davis *et al.*, 2009; Dziallas and Grossart, 2011). *Microcystis* species is found to predominate during summer season favoured with high surface water temperatures and thereby conducive for bloom formation (Van der Westhuizen and Eloff, 1985; Imai *et al.*, 2009). During peak monsoon season with lower surface water temperature (27 °C) *Microcystis* cells were observed in fewer cell densities but in healthy cell conditions. Intense

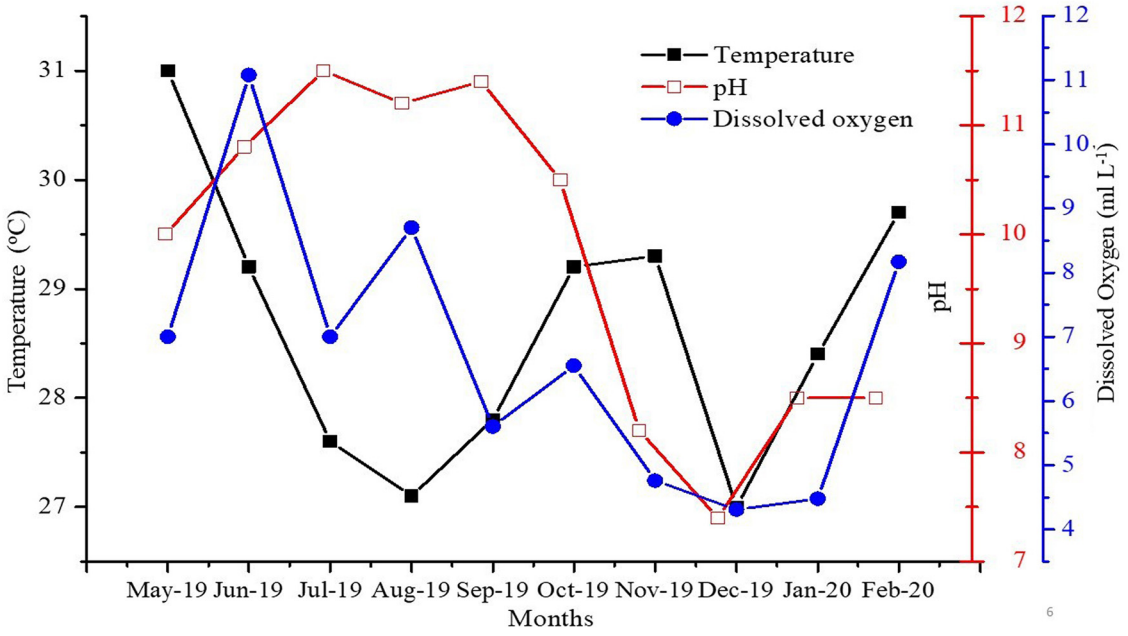


**Fig. 6.** Variations in temperature, pH and dissolved oxygen in Station-2 during the study period.

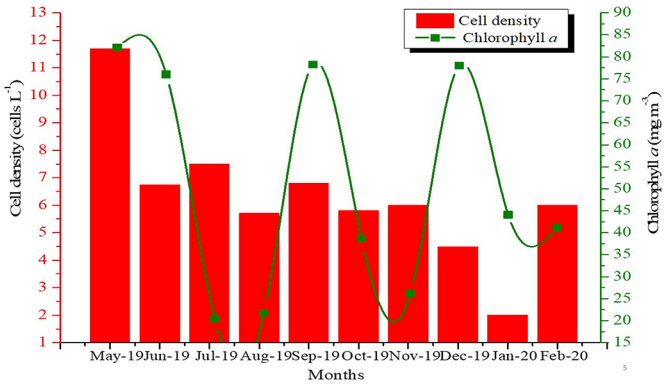


**Fig. 7.** Variations in *M. aeruginosa* cell density ($\times 10^5$ Cells $L^{-1}$) and chlorophyll *a* in station-1 during the study period.

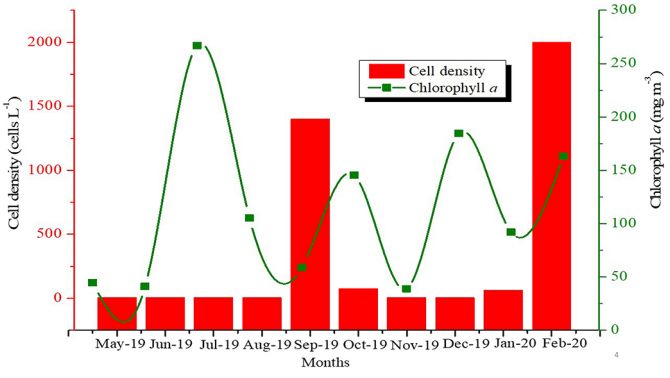


**Fig. 8.** Variations in *M. aeruginosa* cell density ($\times 10^5$ Cells $L^{-1}$) and chlorophyll *a* in station-2 during the study period.

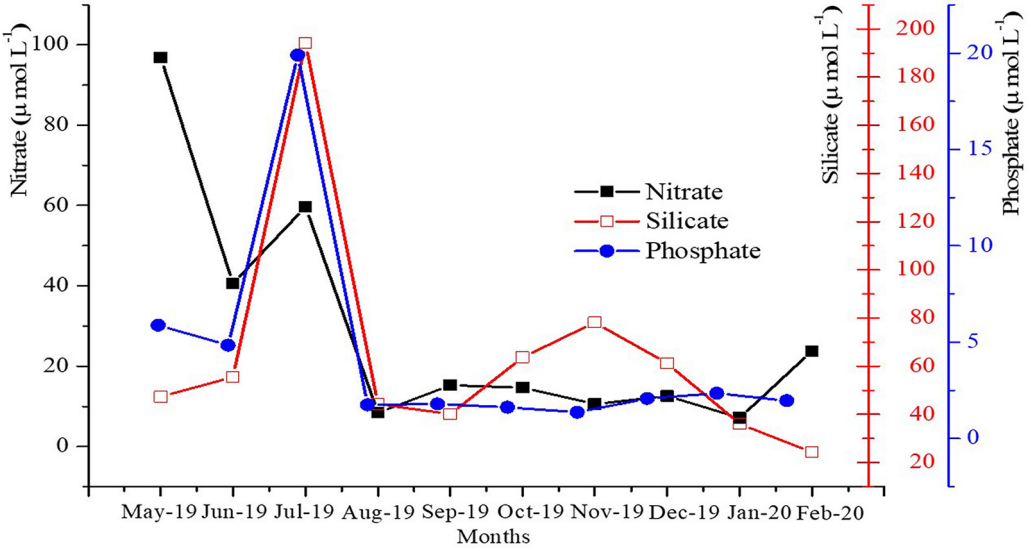


**Fig. 9.** Variations in nutrient concentrations in Station-1 during the study period.

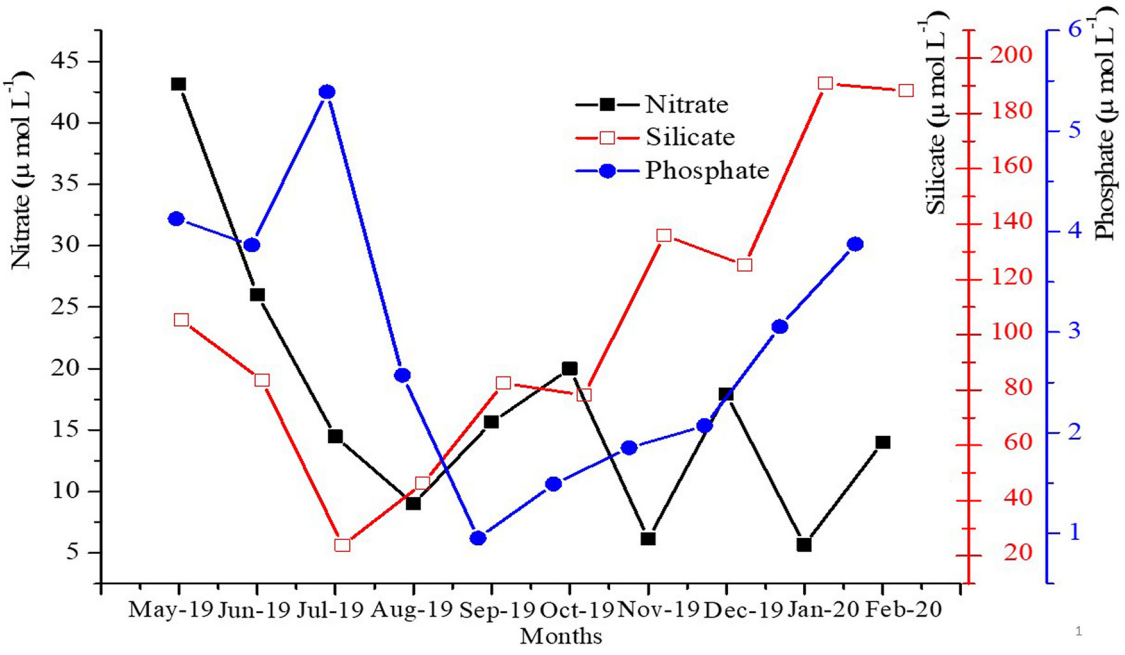


**Fig. 10.** Variations in nutrient concentrations in Station-2 during the study period.

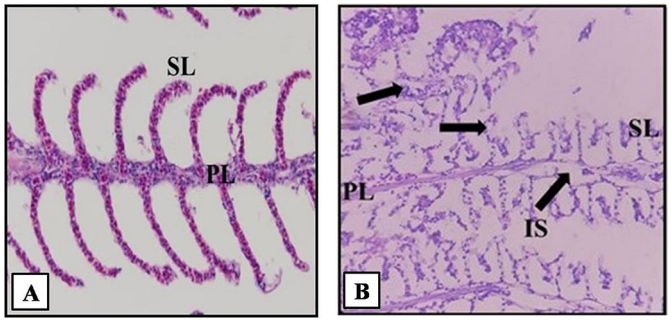


**Fig. 11.** (A) Control gills-normal structure with well-arranged primary lamella (PL) and secondary lamella (SL). (B) Test gills-degeneration of the primary and secondary lamella (arrow heads), increase in inter lamellar space (IS).

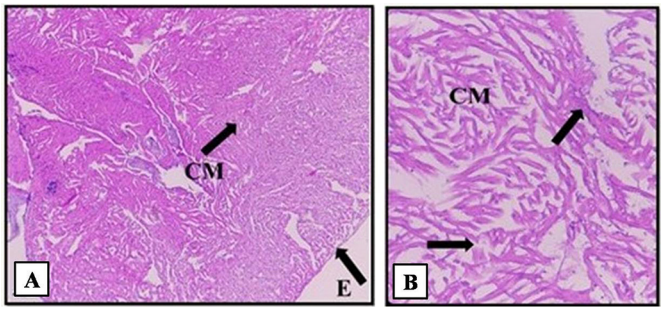


**Fig. 12.** (A) Control heart-normal structure with no histological changes (CM – Cardiac muscle, E – epicardium). (B) Test heart-Fibrillation of cardiac muscle cells and increased intercellular edema (arrows heads).

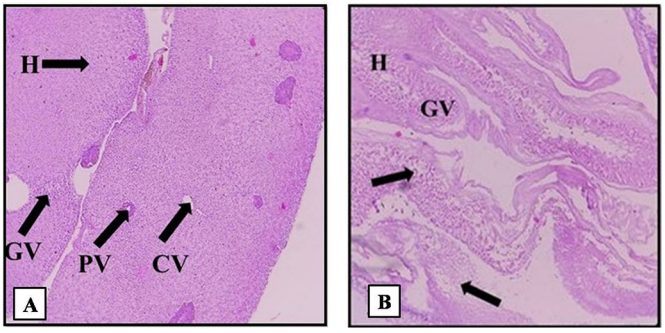


**Fig. 13.** (A) Normal liver-normal architecture with no histological abnormalities was observed in liver of control fish. Normal hepatocytes can be seen (GV – Glycogen vacuole, H – Hepatocytes, CV – Central vein, PV – Portal vein). (B) Test liver – Complete structural disruption, severe necrosis of hepatocytes (arrow heads) with inflammatory cells were observed.

summer monsoon had reduced the surface water temperature and the dilution with rainwater might have caused the decline in cell density.

*Microcystis* showed coexistence of with Cyanobacteria *Anabaena* sp. at Station 2. A similar coexistence was reported in Finnish freshwater lakes (Ekebom *et al.*, 1992; Sivonen *et al.*, 1989). Coexistence of *Microcystis* with *Anabaena* observed in the study during the monsoon period was later dominated by *Microcystis*. The shift of dominance from *Nostoc* and *Anabaena* to *Microcystis* has been noted in an early *Microcystis*-dominated summer bloom in Harsha lake (Lu *et al.*, 2019). This shift was explained by the nitrogen fixation process and the subsequent production of nitrogen along with the phosphate scavenging activity that favoured the succession

of *Microcystis*. Chia *et al.* (2018) investigated the allelopathic interactions between *Microcystis* and *Anabaena sp.,* based on nutrient levels.

During the bloom of *Microcystis* ambient water pH was more on an alkaline range (Station 1: 10.2 and Station 2: 8.5). Neutral to alkaline pH conditions were found to be most favourable for the *Microcystis* growth and also favours their succession over the other phytoplankton (Yang *et al.*, 2018).

Chlorophyll *a* concentration was at peak with higher cell density in both the stations prior to monsoon and was found to decrease with the decline in cell density. The maximum concentration of chlorophyll *a* observed at station 1 was 82.08 mg $m^{-3}$. Chlorophyll *a* data showed a positive correlation with the *Microcystis* cell density at station 1. While at station 2, a noticeable increase in chlorophyll *a* concentration (267.13 mg $m^{-3}$) was observed during monsoon season when the cell density was at moderate range. This might be due to the coexistence of *Microcystis* with *Anabaena* which was found during the monsoon period, where both the microalgae might have contributed to the chlorophyll *a* concentration. Microcystis bloom with chlorophyll *a* varying between 13.3 and 216 μg $L^{-1}$ was reported by Albay *et al.* (2005). Almanza *et al.* (2016) have reported chlorophyll *a* value of 163.3 μg $L^{-1}$ from Lo Galindo urban lake during their study. Yuan *et al.* (2014) in their analysis suggested that total nitrogen and chlorophyll *a* was found to have the strongest association with microcystin. World Health Organization have proposed related advisory levels for cyanobacteria abundance and chlorophyll *a*. The chlorophyll *a* concentrations $<10$ μg $L^{-1}$ have low, values between 10 and 50 μg $L^{-1}$ have moderate, values between 50 and 5000 μg $L^{-1}$ have high and values $>5000$ μg $L^{-1}$ have a very high risk (Chorus and Batram, 1999).

Dissolved oxygen present in water can depict the water quality during algal blooms. Increase in dissolved oxygen could be due to active photosynthesis and reaeration along with sufficient sunlight. Oxygen depletion is rendered by the decomposition of algae creating an anoxic condition leading to the dysfunctioning of the whole aquatic ecosystem (Huang and Chen, 2013; Garnier *et al.*, 1999a, 1999b). Dissolved oxygen in the present study ranged from 3.41 mg $L^{-1}$ to 11.08 mg $L^{-1}$. Increased dissolved oxygen was observed at both stations during intense *Microcystis* bloom owing to the active growth of algal cells which slightly declined subsequently during the post-bloom period as a result of degeneration stage. Lowest values were observed during the month of January 2020 at both stations when the cell density declined. Hence, the result implies that variations in the dissolved oxygen observed during the study were as a result of algal bloom and eutrophication (Huang and Chen, 2013).

Moderate to increased levels of nutrients observed in both the stations could have greatly contributed to the formation of *Microcystis* bloom throughout the study period. An increased concentration of phosphorous (P) is considered as an important factor responsible for promoting eutrophication and cyanobacterial blooms. High phosphorous concentrations in lakes could lead to high biomasses of phytoplankton community that mainly consists of cyanobacteria. Nitrogen availability could influence the production of microcystin, in *Microcystis* thereby impacting the bloom toxicity (Davis *et al.*, 2010; Gobler *et al.*, 2016; Chaffin *et al.*, 2018). The effects of phosphorous, nitrogen and ammonia on the growth of *Microcystis* were previously studied (Kim *et al.*, 2017). Phosphorus can stimulate the growth of *Microcystis* by supplying nutrient element and has complex interactions with other “environmental factors". They play major roles in the growth of *Microcystis* (Jin *et al.*, 2005). Silicate concentrations were also found to be higher during post summer monsoon at station2 and monsoon at station1. Higher silicate concentrations have been reported during *Microcystis* bloom from backwaters of the southeast coast of India (Prasath *et al.*, 2014). Nitrate concentration was high prior to monsoon whereas a peak concentration of phosphate was observed during monsoon season. The ponds were mainly used for domestic use like washing clothes and bathing. Use of soaps and detergents which could likely increase the phosphate content might have favoured the development of *Microcystis* bloom in these freshwater ecosystems. Fertilizer runoff could elevate the nitrate content in water. Since the water is stagnant and high temperature along with higher nutrient conditions together builds a favourable environment for the proliferation of *M. aeruginosa* (Dhanya *et al.*, 2012; Somek *et al.*, 2008; Levy, 2017). Even though eutrophication and climate change are the key drivers of algal blooms, their combined influence on microcystin concentration is less investigated. Lürling *et al.* (2017) hypothesized that warming could promote the cyanobacterial blooms and eutrophication could enhance the biomass and microcystin concentration. This represents the vulnerability of eutrophic waters to future climate changes that might provoke more cyanobacterial nuisance. Increased incidents of eutrophication caused by the use of fertilizers, detergents, due to sewage discharge and other effluents from chemical industries containing nitrate and phosphate have been inducing algal blooms in the aquatic ecosystem.

Reports on the occurrence of toxic *M. aeruginosa* bloom from Indian waters are less. Since the species is known to produce hepatotoxin called microcystin, it can cause a potential threat to humans and the environment. *Microcystis* species is of both economic and ecological importance. They produce hepatotoxin that affects the health of humans as well as other livestock’s. Besides, they pollute the water bodies making it unsuitable for use.

Bloom associated toxicity studies are of significant scientific value especially for providing an overall understanding of the occurrence of cyanotoxin to enhance risk management and risk assessment strategies (Metcalf and Codd, 2014). During the toxicity study, 100% mortality was observed in the fish exposed to *Microcystis* bloom within 15 days of exposure. The most affected organ was liver which has been previously reported as the target organ of Microcystin (Hermansky and Stoh’s, 1991; Tencalla *et al.*, 1994). Histological changes were also observed in gills and heart. The important histological findings included structural alterations, necrosis and vacuolization. Concomitant histological alterations were reported elsewhere in connection with *Microcystis* blooms (Atencio *et al.*, 2008; Preeti *et al.*, 2016; Abdel-Latif & Khashba, 2017; Sanad *et al.*, 2015; Ahmed *et al.*, 2017).

*M. aeruginosa* blooms are well studied and documented in the temperate and subtropical aquatic ecosystems. However such studies particularly toxicity examinations of *Microcystis* blooms are least attempted from Indian aquatic systems (Sangolkar *et al.*, 2006). Hence the study addresses the

occurrence of cyanobacteria *Microcystis*, their bloom formation and possible toxicological effects from the freshwater lentic ecosystem. Variations in the hydrobiological conditions of the systems during bloom and non-bloom conditions are addressed.

## 5 Conclusion

*M. aeruginosa* bloom along two selected freshwater ponds of central Kerala was monitored with the changes in the water quality, bloom characteristics and toxic effects. The species is known to produce potent toxin microcystin that has an adverse effect on various aquatic organisms. Huge scum formation and depletion of dissolved oxygen following the bloom occurs in the study sites. *M. aeruginosa* was observed in both the study sites throughout the period however they developed to bloom during post-monsoon and pre-monsoon period with decreased cell densities during monsoon. High surface water temperature with stable water column and alkaline water pH was observed to be conducive for *M. aeruginosa* blooms. Coexistence of *M. aeruginosa* with *Anabaena* sp. was observed during the non-bloom conditions. Exposure of the *M. aeruginosa* bloom to fish (*Oreochromis mossambicus*) observed high mortality at ~15 days. Histological examination of the fish revealed that the liver was most effected followed by gills and heart indicating the hepatotoxicity of microcystin. Therefore, regular monitoring of the freshwater ecosystem is necessary to address the occurrence of freshwater toxic algal bloom and their ecological impacts. This can help in the development of eco-friendly control and mitigation techniques.

Variations in physicochemical parameters like temperature, pH and nutrient concentration might be the reason for the emergence of *M. aeruginosa* bloom. These blooms could affect the water quality as well as a threat to the health of humans, pets and aquatic organisms. Therefore, regular monitoring of the freshwater ecosystem is necessary to preserve and maintain the aquatic systems. Due to the impacts and toxicity of blooms, the development of eco-friendly control and mitigation techniques to reduce the occurrence of blooms is necessary. An awareness regarding the harmful algal blooms and its effect should be created among the people. Further studies should be carried out to determine the extent of toxicity.

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