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First identification of the microcystins – producing cyanobacterium *Microcystis flos-aquae* and *Microcystis wesenbergi* in Valankulam lake, Coimbatore district, Tamil Nadu, India

Primera identificación de las cianobacterias productoras de microcistinas Microcystis flos-aquae y Microcystis wesenbergi en el lago Valankulam, distrito de Coimbatore, Tamil Nadu, India

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ABSTRACT

Microcystis was major toxin-producing cyanobacterial species, may highly found in eutrophication water bodies. The cyanobacterial toxins are increase the aquatic stress, and may leads to nutrients load in aquatic ecosystems. In the present study, *Microcystis flos-aquae,* and *Microcystis wesenbergi* was recorded for the first in the valankulam lake, at Coimbatore district, Tamil Nadu, India. In addition, some hydrographical characteristics viz., Water Temperature, P^H, Salinity, DO, TDS, EC, Phosphate, Total alkalinity, and Nitrate was also analysed during the study period from September-2021 to May-2022.

Keywords: First record, Cyanobacteria, *Microcystis*, hydrographical profile, Valankulam lake

RESUMEN

Microcystis fue una de las principales especies de cianobacterias productoras de toxinas, y es posible que se encuentre en gran medida en cuerpos de agua en eutrofización. Las cianotoxinas incrementan el estrés acuático, pudiendo conducir a la carga de nutrientes en los ecosistemas acuáticos. En el presente estudio, Microcystis flos-aquae y Microcystis wesenbergi se registraron por primera vez en el lago valankulam, en el distrito de Coimbatore, Tamil Nadu, India. Además, también se analizaron algunas características hidrográficas,

a saber, temperatura del agua, PH, salinidad, OD, TDS, EC, fosfato, alcalinidad total y nitrato durante el período de estudio de septiembre de 2021 a mayo de 2022.

INTRODUCTION

Algal species are ranging from unicellular to multicellular in form and they are very unique because they can carry out the photosynthetic activity with help of chlorophyll pigments. Moreover, they found in all habitats of environmental ecosystems and especially found in the surface of all aquatic ecosystems (Dittami et al., 2017; Ramirez-Llodra et al., 2010). The aquatic ecosystems can be classified into types, due to dissolved salt contents in their environment and namely freshwater and marine water ecosystems. The freshwater ecosystems are essential for all human beings and their associated animals. A freshwater ecosystem includes streams, lakes, ponds, wetlands, rivers and etc., (Gopal, 2020). The algae communities are mainly classified by their presents in the environment namely, phytoplankton, benthos, epiphytic, epipelic, endozoic and epilithic. However, they are very crucial aquatic primary producers and help in the purification, but also they act as bio-indicators of water quality (Stancheva and Sheath, 2016). In the past few decades, scientists have estimated that a total of 50,000 algal species are around the world but currently, 30,000 species were only identified. Nowadays algae species are widely used for further purposes such as industrial, food technology, biotechnology, and bioremediation field to degrade the contaminants which present in the environmental ecosystems, but also widely used for food additives, and food preservative process. Among the algal communities, Phytoplankton species are plays a crucial role in energy transformation to higher tropic levels of aquatic ecosystems (Hallegraeff, 2010; Weitere et al., 2018). The microalgae species are mainly classified into four major groups such as, Cyanophyceae, Chlorophyceae, Bacillariophyceae, and Euglenophyceae.

Cyanophyceae is the major group of freshwater phytoplankton species, can produce toxic substances by releasing blooms into aquatic ecosystems, and these toxic substances are produced by 40 cyanobacterial species which includes 20 genera like viz., *Microcystis*, *Anabaena, Aphanizomenon*, *Nodularia*, *Nostoc*, *Cylindrospermopsis, Chroococcus*, *Planktothrix*, and etc (Sood et al. 2015; Gaysina et al. 2019). The cyanobacterial blooms predominately found during the summer and autumn seasons, which accumulates at the surface of all water bodies. Freshwater bodies are mainly used for drinking, or irrigation purposes, if the high level of cyanobacterial blooms occurs in freshwater bodies, may cause severe health effects to human beings, livestock, and wildlife animals due to continuous releasing of toxic substances into the surface of water bodies (Codd et al., 2019; Carmichael, 2001). The cyanobacterial communities can adapt to any environmental conditions, because of their cellular mechanisms. The cyanobacterial species are greatly influenced by the environmental factors like, temperature, light, and heavy nutrients load in aquatic ecosystems may affect the cyanobacterial density. Toxin-producing cyanobacterial communities mainly found in freshwater bodies due to favourable environmental conditions and the consumption of contaminated water may cause liver failure which leads to death. Among the cyanobacterial toxins (nodularins, microcystins, cylindrosperomopsin, anatoxin, and saxitoxins), particularly microcystins produced by *Microcystis, Anabaena,* and *Planktothrix*

communities may cause chronic effects and promote tumour growth substances in human beings (Yang et al., 2010). Moreover, microcystins are heptapeptide hepatotoxins in nature, and they contain both the D and L-amino acids plus N-methyldehyoalanine with unique β-amino acid side group, 3-amino-9- methoxy-2-6, 8-trimethyl -10-ophenyldeca-4, 6-dienoic acid, and they comprise of largest and most structurally diverse group among the toxin- producing cyanobacterial species. Around the world, more than 90 microcystins strains have been identified by varying their degree of hydroxylation, methylation, epimerization, and their peptide sequence (Welker and Von Döhren, 2006). However, the world health organization has underlined the importance of monitoring microcystins concentration in drinking water and has recommended 1mg /L.

Coimbatore city is the Manchester of southern India, and this city is well known for its cottage industries, dying, and automobile factories. In Coimbatore city, only seven wetland tanks are majorly contribute to the habitat for aquatic biodiversity. In the past few years, the rapid growth human population, urbanization, human exploitation, anthropogenic activities, and global warming also affect the aquatic biodiversity. In the present study, *Microcystis flos-aquae* and *Microcystis wesenbergi* was recorded for the first time in the valankulam lake, at Coimbatore city, Tamil Nadu, India.

MATERIALS AND METHODS

Microcystis flos-aquae and *Microcystis wesenbergi* was collected from valankulam lake, at the sampling stations by using Towing-Henson's standard plankton net (mouth diameter 0.35 nm) made up of nylon bolting cloth (mesh size 50 µm) at the water surface and about 10 min at a uniform speed of the boat (10 Km/h) during early hours between 5 AM to 7 AM. The quantitative analysis of plankton samples 100 L of water was filtered through the plankton net and immediately the plankton biomass was transferred to specimen bottles containing 5% of neutralized formalin and subjected to microscopic analysis. The sample is poured into graduated centrifuge tubes of 10 to 30 mL capacity and revolved in an electric centrifuge for 10-20 min at different rates of revolutions (1500–2000 rpm). After which the supernatant water was removed. The species were separated under a light microscope by using a fine needle and brush. Individual species of plankton were mounted on microscopic slides on a drop of 20% glycerine. The plankton species were identified by referring to the standard manuals, textbooks, and monographs (Anand, 1989; Agarker et al., 1994; Adoni et al., 1985). The taxonomic identification was done under the compound light microscope at a magnification of 40 X to 100 X and they were photomicrographed by using, Inverted Biological Microscope (Model Number INVERSO 3000 (TC-100) CETI) attached to the camera (Model IS 300). Furthermore, morphological features of the collected specimens were described for further studies. In addition, surface water samples were collected by plastic containers to check the on-field hydrographical parameters viz., water temperature, P^H, salinity, DO, TDS, EC, Alkalinity, Nitrate, and Phosphorus by using “µP Based Water and Soil Analysis Kit” (Model 1160).

RESULTS

Microcystis flos-aquae and *Microcystis wesenbergi* description: *Microcystis* are colonial species. Colonies tend to float near the surface in nutrient-rich freshwater bodies and other low-salinity water bodies, and cells have no individual mucilaginous envelopes, but the colony is encased in fine colourless mucus. They can produce toxins (microcystins) with dense blooms can be dangerous to aquatic ecosystems and cell sizes are 2 to 3 um. (Table 1 and Figure 1).

Table 1. Morphologically identified of *Microcystis flos-aquae and Microcystis wesenbergi* species

Group	Family	Genus	Species
Cyanophyceae	Microcystaceae	*Microcystis*	*Microcystis flos-aquae* (Wittrock) Kirchnner, 1898 *Microcystis wesenbergi* Komarek in Kondrateva 1968

Hydrographical characteristics of lake water: The hydrographical characteristics of lake water temperature range between 29.88±0.49 to 24.26±0.96 ^{o}C and P^{H} range between 7.06 ± 0.25 to 8.93 ± 0.40 respectively. The salinity, DO, TDS, and EC was ranged between 107.92±0.06 to 132.48±0.20 (ppt), 6.21±0.29 to 8.31 ± 0.50 (mg/l^{-1}), and 109.21±24.06 to 209.03± 0.24 (µS cm^{-1}) were recorded during the study period. Phosphorus, Nitrate and total alkalinity was range between 33.14±0.09 to 35.75±1.27 (mg/l^{-1}), 22.42±1.27 to 24.83 ± 1.69 (mg/l^{-1}), and 120.80 ± 7.04 to 134.36 ± 8.86 (mg/l^{-1}). Above all the parameter was found higher in summer season expect of DO, and TDS (Table 2).

Table 2. Hydrographical characteristics of lake water

Parameters	Monsoon (2021)	Post Monsoon (2022)	Summer (2022)
Water temperature (°C)	24.26 ± 0.96^{a}	26.99 ± 0.42^{b}	29.88 ± 0.49^{c}
pH	7.06 ± 0.25^{a}	7.20 ± 0.35^{b}	8.93 ± 0.40^{c}
Salinity (ppt)	107.92 ± 0.06^{a}	109.15 ± 0.22^{b}	132.48 ± 0.20^{c}
DO (mg/l^{-1})	8.31 ± 0.50^{c}	7.79 ± 0.14^{b}	6.21 ± 0.29^{a}
TDS (mg/l^{-1})	109.21 ± 24.06^{b}	108.28 ± 15.20^{ab}	104.34 ± 24.06^{a}
EC (µS cm^{-1})	193.42 ± 0.16^{a}	207.03 ± 0.24^{b}	209.35 ± 0.29^{c}
Phosphorus (mg/l^{-1})	35.41 ± 0.28^{a}	33.14 ± 0.09^{b}	35.75 ± 1.27^{bc}
Nitrate (mg/l^{-1})	22.42 ± 1.27^{a}	23.50 ± 1.80^{ab}	24.83 ± 1.69^{bc}
Total alkalinity (mg/l^{-1})	120.80 ± 7.04^{a}	128.23 ± 9.10^{b}	134.36 ± 8.86^{c}

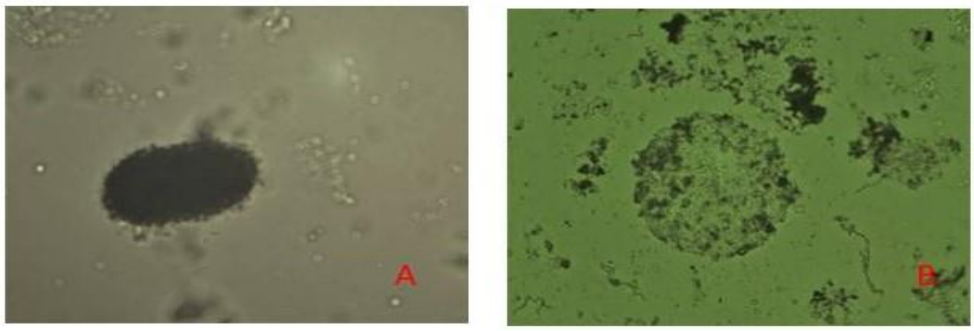

Figure 1. Species of Cyanophyceae (400x) recorded in the Valankulam lake A) *Microcystis flos-aquae,* B) *Microcystis wesenbergi*

DISCUSSION

In the present study, *Microcystis flos-aquae* and *Microcystis wesenbergi* was recorded for the first time in the Valankulam lake, at Coimbatore city, Tamil Nadu. The cyanobacterial species are much more diverse and proliferate in temperate environments, and they produce motile filaments (Hormogonia), which helps in the travel away from the main cyanobacterial biomass to bud and form new colonies (Muruga, 2015; Adams et al., 2012). They can fix the atmospheric nitrogen in aquatic ecosystems, where specialized cells are responsible for the nitrogen fixation, which are referred to as heterocysts. The absorbed atmospheric nitrogen converts into ammonia, by heterocysts. Heterocysts are made up of a thick polysaccharide layer, which isolates from the cells thereby activating the enzymatic nitrogenase complex and they transfer to the partner with key factor relationships (Oliver et al., 2012; Kulasooriya, 2011). The growth of cyanobacterial species decided by the availability nitrogen and phosphorus are considered as an important nutrient which abundantly found in highly saline water bodies. The toxic-producing cyanobacterial species are mainly occurs in eutrophication water bodies, due to the heavy load of organic materials.

Algal communities are mainly depends on water temperature and the availability of nutrients in aquatic ecosystems. Moreover, the maintenance of water temperature, P^H, salinity, and other hydrographical characteristics is very important for all aquatic organisms (Mirzaei et al., 2017). In the present study, water temperature was increased during the summer season and decreased during in the monsoon season. If any significant elevation in water temperature, may affects the physiological process, rate of photosynthetic activities, and base of food chain in aquatic environment. However, the water temperature has positively support for the aquatic organisms in valankulam lake. P^H is the measure of hydrogen ions in the water. Water has hydrogen ions and hydroxyl ions. When there are equal numbers of both, the water is neutral. If hydrogen ions increase, water becomes an acidic or hydroxyl ions increase, water becomes an alkaline. In the present study, P^H value indicates that lake water is slightly alkaline during the summer season, due to the interactions of limestone, the sediment of cyanobacterial blooms, and other anthropogenic activities. The salinity is the measurement of the dissolved salts concentration of water and, EC to determine the level of impurities in water and also used to measures the ability of water to conduct the electricity. In the present study, the salinity and EC was higher in summer and lower in monsoon seasons. If high level of salinity and EC was found in water bodies, may affects the dissolved oxygen levels. However, the salinity and EC was positively supports in the growth of aquatic organisms. The dissolved oxygen and total dissolved suspended solids are vital sources for all living organisms in aquatic ecosystems. In the present findings, DO and TDS are higher in monsoon season, and lower summer season. The availability of DO levels in aquatic ecosystems may vary daily or seasonally due to the consumption of oxygen by aquatic organisms (Garvey et al., 2007). TDS are essential for all the living organisms, and which helps to inbuilt the metabolic activities. Moreover, the high level TDS alters the taste of water and it makes it salty or metallic, and also it increase the water temperature in aquatic environment. However, in the present findings TDS was positively supports for the growth of aquatic organisms in the valankulam lake. The nitrate and phosphorus are the good indicators of eutrophication in the lentic and lotic water bodies. During the study period, phosphorus and nitrate was increased during the

summer season and lower in the monsoon season. The phosphorus is an important driver for the dominance of *Microcystis* species, when dissolved inorganic phosphorus concentrations are below detectable limits, and have higher phosphate uptake, and affinity when compared to other cyanobacterial species (Aubriot and Bonilla, 2018). The *Microcystis* regulates their uptake of inorganic phosphorus, therefore termed as phosphorus opportunist. However, phosphorus concentration was below detectable levels during the monsoon season, may elevated from the base of aquatic sediments and it decreases the abundance of nitrogen fixing cyanobacterial species, but they were no changes among the *Microcystis* species. Furthermore, *Microcystis* could also utilize the organic sources therefore it expands their flexibility in phosphorus uptake (O'Neil et al., 2012; Burford and Davis, 2011). The dissolved inorganic nitrogen plays important role in the growth of blooms producing cyanobacterial species, and due to the reduction of nitrate and nitrogen di-oxide into ammonia, can proliferate in the preferred sources of dissolved inorganic nitrogen in aquatic ecosystems. However, Cyanobacterial species can adapt in low temperatures, which are also found in -20 ^{o}C like Atlantic and Antarctic regions, and permanently found in warm water bodies (Clarke, 2003; Pienitz et al., 2004). The results from the study revealed, hydrographical characteristics of lake water show that high levels of nutrients loaded in valankulam lake, due to various anthropogenic activities like the release of sewage sludge, industrial effluents, dumping of municipal waste, and other man made activities which directly or indirectly affects the aquatic biota.

Cyanobacterial blooms are usually comprised of toxic and non-toxic strains, and it is almost impossible to distinguish to identify the toxic or non-toxic strains, because of their same identical appearances under the light microscope. However, *Microcystis flos-aquae* and *Microcystis wesenbergi* is the major toxin producing species among all the cyanobacterial communities, which are mainly found in the summer and end of autumn seasons (Mendez-Jimenez, 2020). Moreover, the aquatic ecosystems suffer due to an abundance of cyanobacterial blooms, may cause deterioration in the water surface, water discolouration and unpleasant odour also impacts on the hydrographical properties of lake water. All cyanobacterial toxic compounds may cause severe health effects in human begins, but particularly microcystins are very dangerous from a chronic point of view. Recently, microcystins substance was observed in the kidney cell line regions, may affect cell viability and they decayed after 24 hours by the exposure of toxins (Liu et al., 2018; Pavagadhi and Balasubramanian, 2013). Furthermore, *Microcystis* stimulates the growth of heterotrophic bacteria, and produces specific secondary metabolites, which much more difficult to remove (*Microcystis*) from the water bodies. Chlorination is a generally effective method of removing dissolved toxic substance at sufficient concentrations. The carbon activated powder is most effective to remove cyano-toxic substances from drinking water, but it considered as most highly expensive treatment. The *Microcystis* compounds in surface scums are extremely stable and may persist for weeks after being released from their cells (Dyble et al., 2008). Therefore, WHO recommended continuous monitoring of *Microcystis* in freshwater water bodies are essential one in throughout the world.

As conclusion, Coimbatore is one of the metropolitan cities in India, and due to the increase in human population, over exploitation, industrialization, and urbanization may cause various impacts on aquatic

ecosystems. *Microcystis* is the major toxin producing cyanobacterial species, may cause severe health effects in human begins and also in wild animals. The *Microcystis* majorly found in freshwater bodies, and may affect the liver cells in human begins. Therefore, WHO underlined the importance of freshwater bodies, and compulsory to maintain by distilling processes. In the present study, *Microsytis flos-aquae,* and *Microcystis wesenbergi* was recorded for the first in the Valankulam lake, at Coimbatore district, Tamil Nadu.

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