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Analysis of cyanotoxins in *Desertifilum* and *Leptolyngbya* from Veeranam Lake: a potential health risk for Chennai, India

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Abstract

Globally, dwindling potable water supplies and toxic waste triggered by human activities and climate change are substantial concerns. Below these occurrences, the suitability of drinking water regulations and their diligent execution are critical issues in maintaining human health. Unfortunately, even in developed countries, many infectious diseases (and other unidentified health effects) are linked to drinking water. These changes impact the harmful growth of Cyanobacteria or blue-green algae. Cyanobacteria-produced toxins can harm humans if they compromise drinking or ingested foods. Toxins like microcystin kill humans and creatures, leading to severe medical conditions. The present research focussed on the isolation, and identification of Cyanobacteria from the Veeranam Lake in the Cuddalore district of Tamil Nadu, India. The (ADDA)-specific ELISA technique is preferred to check the cytotoxicity in identified strains. The IC50 values of *Leptolyngbya* sp. showed 17.72 (g/L), and *Desertifilum* sp. exhibited 19.38 (g/L). Consequently, in (ADDA)-specific Microcystin quantitative assessment, *Desertifilum* sp. had higher toxin concentrations than *Leptolyngbya* sp. after the samples' singlet, duplicate, and triplicate values. Such toxicity evidence indicates that the concerns necessitate monitoring and maximum sustainability, especially when considering the various incipient toxins and the constantly evolving techniques to deal with them.

Keywords Cyanobacterial toxicity, Veeranam Lake, ADDA-ELISA, *Leptolyngbya* sp. and *Desertifilum* sp

Introduction

Surface water is a valuable, vibrant, and constantly replenished global commodity currently in short supply across the platform. Potable surface water is an essential renewable energy source that benefits health, long-term progress, and conservational distinctiveness. Assessing a county's drinking sources includes a thorough evaluation such as geological and hydrogeological approach, as well as close supervision and very well output devices. The

norms in potable get a Maximum Level for every parameter [44].

Freshwater refers to a broad spectrum of surface scenarios all over the globe [1]. To fight contamination, it had become crucial to track drinkable assets' reliability routinely. The physical–chemical parameters of these elements are incredibly beneficial to Cyanobacteria. Only a few Cyanobacteria varieties or strains release potentially dangerous substances to aquatic organisms. These toxins could get into one's system by consuming contaminated fish. Many fish species, including tilapia and carp, can consume cyanobacterial toxins. The pollutants accumulate in the fish's hepatocellular, epidermal cells, muscle development, and guts [22].

Blue–green algae or Cyanobacteria are Gram-negative photosynthesizing prokaryotic cells that can conquer aquatic and terrestrial biotopes [9]. Their dominant focus

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of metabolic activities is photosynthesis [16]. They only need water, CO₂, inorganic compounds, and light to perform tasks. Once perceived through an individual's eyes, microalgae provide both advantageous and adverse characteristics. They tend to serve as plant food or fodder as several variants contain an elevated concentration of macromolecules, critical bioactive molecules, and other vitamin supplements. At the same time, others may also begin producing colorants of intrigue.

They also contribute to pharmaceutically valuable substances, including antibacterial agents [19, 41, 48]. Their substantial expansion can pose a significant hindrance for upriver irrigation systems like water system, amusement and they might furthermore release substances into the water that are irritating or harmful. Problems with water quality induced by high populations of Cyanobacteria are complicated, numerous, and diverse. They can severely affect economic growth and the well-being of humans and animals. Consequently, the negative aspects of five cyanobacteria have piqued the interest of researchers and sparked public concern [16].

Toxins produced by Cyanobacteria

Cyanotoxins are a differing assortment of organic toxicants, either chemically or toxicologically. Microalgae accomplish harmful Cyanobacterial toxins designated as cyanotoxins. Amidst their aquacultural origins, many of the cyanotoxins outlined thus far are more destructive to terrestrial than aquatic species [13, 16]. Cyanotoxins, with their chemical configuration, are grouped into three broad clusters: side-chain proteolytic enzymes (microcystins and nodularin) and toxic metabolites (neurotoxins, hepatotoxin, dermatotoxin). Most scientists are worried about peptidic hepatotoxins and, instead, neurotoxic bioactive constituents or lipopolysaccharides since the latter are not widely distributed, mainly in potable water.

Once microalgal tissue is damaged or implodes; they give off toxins. It could emerge in the environment by itself, well after implementation of *cusO4* to surface water, upon consumption in the ruminants or intestine, or as a consequence of extreme weather. The hepatotoxic benzyl receptors microcystins and nodularin constitute the majority of typically available microalgal toxicants in blooms from mineral and brackish waters globally [15, 35]. The Cyanotoxin cells are often consumed with drinkable water, producing toxicants in the gut lumen [20, 21, 30].

Exposure of humans to cyanotoxins

During its history, water sources have also been connected to acute gastroenteritis, waterborne diseases, intestinal parasites, and typhoid resulting in a great deal

of social distress and mortality. The aspects of health damage inflicted by cyanobacterial toxins were also investigated judicially, and accomplish epidemiological evidence—in particular on disclosure (percentage of microbes, form, and amount of cyanotoxins) seem to be relatively uncommon [28]. The epidemiologic studies indication for sentient injury induced concerning cyanobacterial toxins. It must, therefore, be calibrated against the substantiation for those other reasons, with microbial, pathogens or cryptosporidiosis illnesses initially to be probed [16].

Predominantly inherited Cyanobacterial toxins affect health via daily intake (consumption) and actual interaction (scuba diving, laundry) with industrial wastewater [26, 34]. *Leptolyngbya* is among the most prevalent (and taxonomically most difficult) Cyanobacterial genera, with multiple different strains and based on usage (species) that inhabit soil, decaying organic matter, and meta-phyton in a broad range of freshwater and saltwater (ocean) surroundings [2, 4]. Toxicants found in organic matter excerpts of the *Desertifilum* sp. (Strain SP-D) and *Nostoc* sp. (Strain SP-45) are cyclic depsipeptides, in addition to *cyanopeptolines*, *microcystilides*, *oscilopeptins*, *anabenoptylides*, *eruginopeptins*, *microcystins*, and others [11].

A case study on Cyanobacterial toxins

In surface water, Cyanobacterial isolates can produce multiple toxins at once. In general, researchers identified multiple MCs toxin from a discrete Microalgal strain. Though several microalgal isolates create various MCs simultaneously, just one or two are usually dominant in either strain [45]. Qualitative alteration in MCs is present in *Nostoc*, *Anabaena*, and *Microcystis* strains. Scientists observed many identical MCs toxin in Planktonic *Anabaena* sp., *Leptolyngbya* sp., *Microcystis* sp., and *Oscillatoria* sp. [46, 47]. A few *Microcystis*, in addition to *Oscillatoria*, and *Nodularia* exhibit a single substantial toxic substance.

Francis of Adelaide stated in his report that the first specific instance of farm animal death rates in Australia was in 1878. [26]. Since then, there have been numerous reports of animal poisoning caused by phytoplankton blooms all around the community [10, 12, 14, 17, 25, 27, 28, 32, 36–38, 40, 43]. *Desertifilum* sp. and *Nostoc* sp. strains proved particularly poisonous in the secluded isolates test, based on the *Daphnia magna* experiment (complete death observed 48 h after testing the animals). Such variants had the maximum suppressive activities on HeLa tumour cells proliferating [7].

The scheduling and length of the *Microcystis* bloom period have become entirely dictated by the country's weather factors; in tropical regions, widespread microalgae incidents are prevalent in early summer and early

fall, which may last 2–4 months. The bloom period could initiate prior to then last longer in regions with much more Coastal or subarctic environments [31, 50]. Toxicogenic (toxic chemical able to produce) and non-toxicogenic isolates of Cyanobacteria reconcile, once developed in the research laboratories, distinct cultures are, therefore, identified as yielding so much toxic compounds over others [8].

Depiction of Cyanotoxins and its biochemical interactions

Secondary metabolic compounds of blue–green cyanotoxins are hazardous chemicals that substantially impact organic sciences [6]. The stomach, intestinal disruption, gastrointestinal troubles, and unusual reactions to allergens are among them. Cyanotoxins end up causing toxicosis that incorporates hepatocytes, neurotoxicity, and respiratory issues.

Cyanobacteria that are filamentous or colonial generate a wide variety of toxicants. *Anabaena*, *Microcystis*, *Nodularia*, *Aphanizomenon*, *Planktothrix*, *Cylindrospermopsis*, and *Lyngbya* constitute the most common toxin-producing microalgae-based species found in water sources [5, 49]. Hepatotoxins include MCs and NOD, neurotoxins typically involve anatoxins and SXTs; paralytic shellfish toxins include *Aphanizomenon*; cytotoxins entail CYN; and dermatotoxins comprise LPS [23, 42].

Biopolymers are something that hepatotoxins are. Hypovolemic shock and more emphasis on serum in

the liver cause hepatotoxins to fissure the hepatic cell framework. In the filamentous form of Cyanobacteria: *Nodularia spumigena*, residues of the NOD gene are involved. NOD toxin comprises five amino acid residues. New species of the taxon of freshwater microalgal species produce microcystins with five amino acid residues [29].

General configuration of Microcystin (MCs)

Microcystin (MCs) seem to be nonlinear heptapeptide toxicants encompassing seven peptide-linked amino acids obtained by *Planktothrix*, *Microcystis*, *Anabaena*, and more than nine *Hapalosiphon*. Nodularin are nonlinear pentapeptides provided by the microbes *Nodularin* sp. and *Microcystis* sp. [13].

The steps are the significant indication of microcystin: X and Z seem to be varying L-amino acid, D-Ala is D-Alanine, D-Glu is D glutamic acid (glutamate), MeAsp3 is D-erythro—methyl aspartic acid, Mdha is N-methyl dehydroalanine. Adda is 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-4,6-dienoic acid (Fig. 1). Adda specific amino acid, is required to express biological activity [20, 21]. Contaminants after the dependent variables L-amino acids found at positions X and Z. There are reportedly more than 60 microcystin variations currently classified and vary throughout their systemic toxicity. The much more familiar MC is microcystin-LR (MC-LR), with leucine (L) and arginine (R).

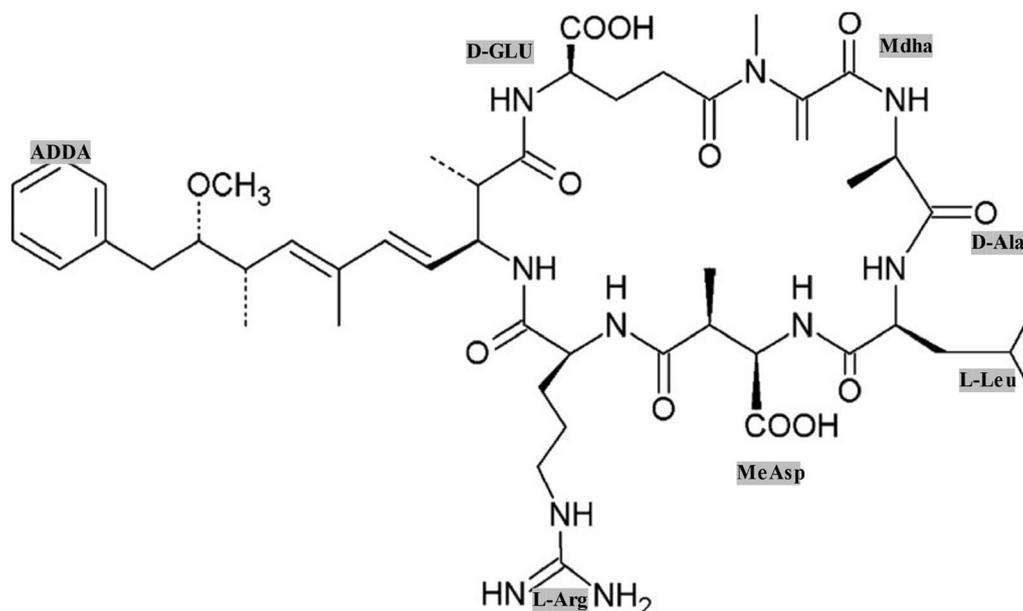


Fig. 1 Chemical structure of microcystin-LR (MC-LR). *D-Ala* D-Alanine; *L-Leu* L-Leucine (Lamino acid), *MeAsp* D-erythro-β-methylaspartic acid, *L-Arg* L-Arginine (L-amino acid), *Adda* 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-4,6-dienoic acid, *D-Glu* D-glutamate, *Mdha* Nmethyldehydroalanine [33]

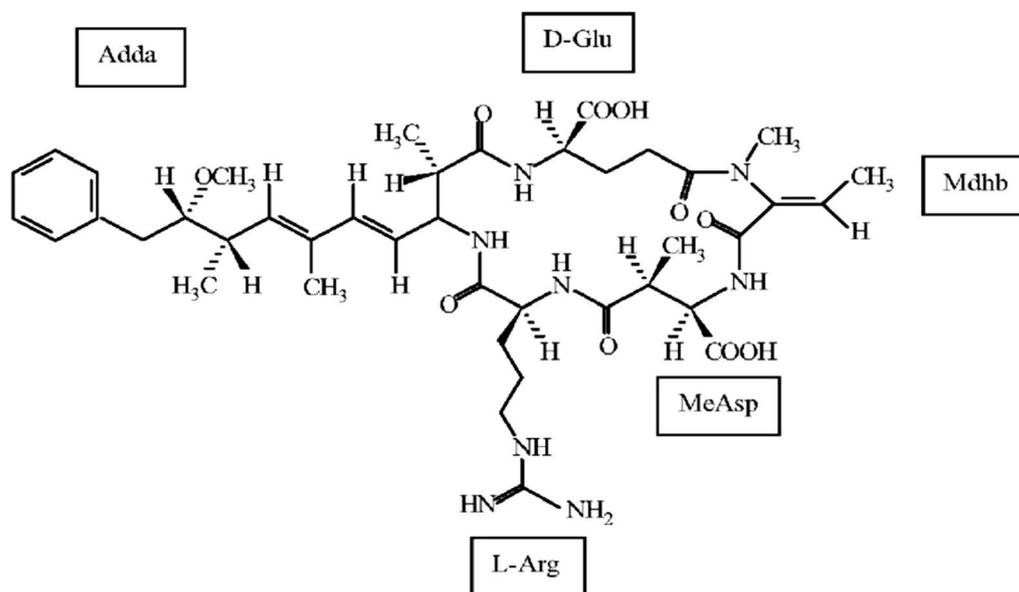


Fig. 2 Chemical structure of nodularin. *MeAsp* D-erythro-β-methylaspartic acid, *L-Arg* L-Arginine (L-amino acid), *Adda* 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-4,6-dienoic acid, *D-Glu* D-glutamate, *Mdha* N-methyldehydroalanine [33]

General configuration of Nodularin

The cyclic pentapeptide, nodularin, contains amino acids like those found in MCs, namely D-erythro-β-methyl aspartic acid, L-Arginine, Adda, D-glutamic acid and N-methyl dehydrobutyryne (Fig. 2). Nodularin is structurally like MCs and exerts similar toxicities.

Experimental methods for detecting Cyanotoxins

Various expedited screen tests and experimental methods are available to distinguish and recognize Cyanobacteria

cells and cyanotoxins in water. The complexity of these methodological approaches and the details they provide may vary significantly [18]. The procedures are as follows:

- Enzyme-Linked Immunosorbent Assays (ELISA)
- Protein Phosphatase Inhibition Assay (PPIA)
- Reversed-phase High Performance Liquid Chromatographic methods (HPLC) combined with Mass

Table 1 BG11 media preparation [3]

Stock solution	Elements	50 mL (50x) Concentration	1000 mL (1L) 1 mL from 50x
1	NaNO ₃	75.0 g	1 mL
2	K ₂ HPO ₄	2.0 g	1 mL
3	MgSO ₄ · 7H ₂ O	3.75 g	1 mL
4	CaCl ₂ · 2H ₂ O	1.80 g	1 mL
5	Citric acid · 1H ₂ O	0.30 g	1 mL
6	Ammonium ferric citrate green	0.30 g	1 mL
7	Na ₂ EDTA	0.05 g	1 mL
8	Na ₂ CO ₃	1.00 g	1 mL
9	Trace elements (1 mL from trace elements from 50x)		
	H ₃ BO ₃	0.143 g	
	MnCl ₂ · 4H ₂ O	0.0905	
	ZnSO ₄ · 7H ₂ O	0.011	
	CuSO ₄ · 5H ₂ O	0.004	
	CO(NO ₃) ₂ · 6H ₂ O	0.0025 g	
	NaMoO ₄ · 2H ₂ O or MoO ₄ · (85%)	0.0195	

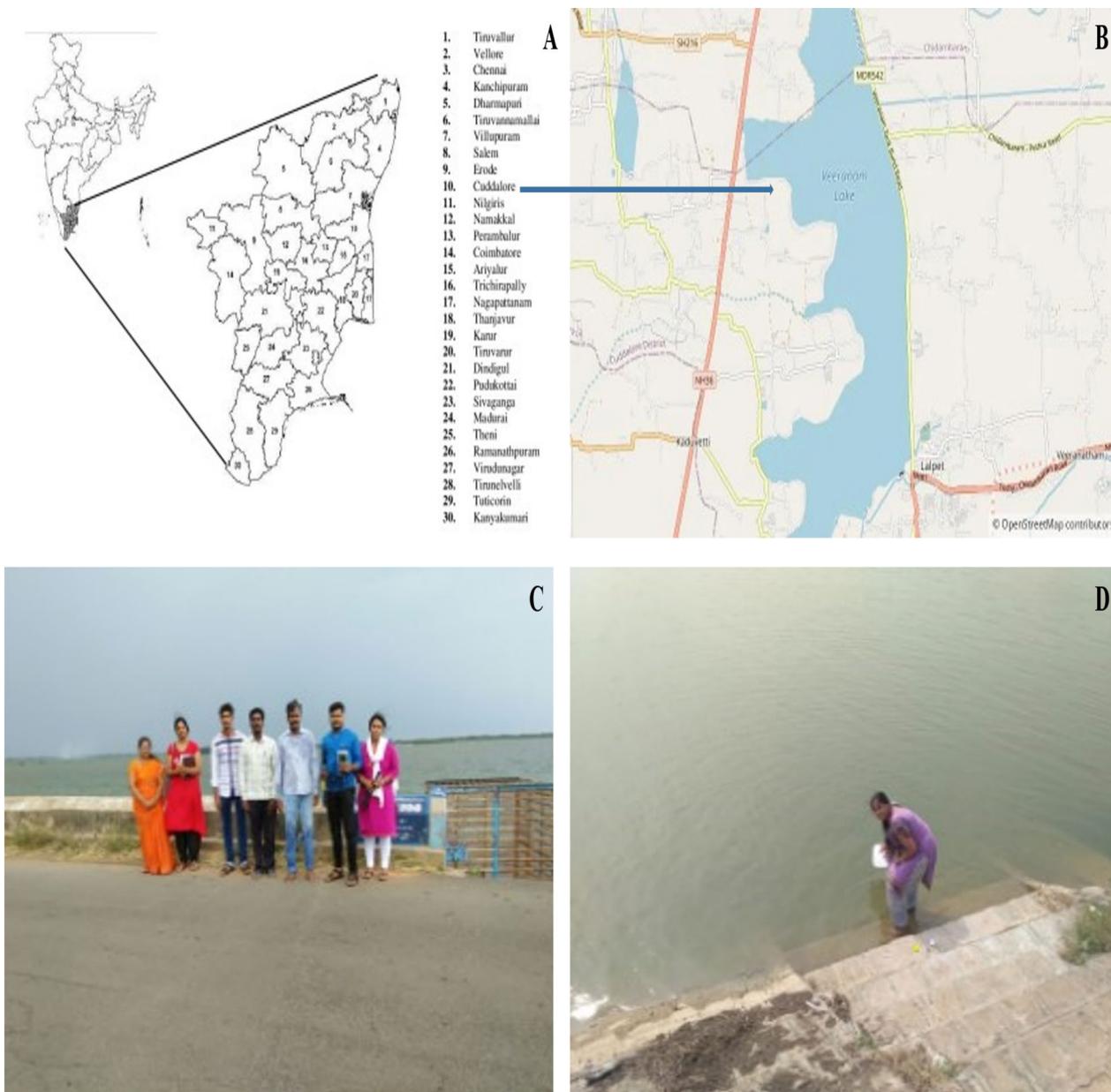


Fig. 3 A and B depict the map view of Veeranam Lake. C and D depict the seasonal collection of water samples for Cyanobacterial isolation

Spectrometric (MS, MS/MS) or Ultraviolet/Photodiode Array Detectors (UV/PDA).

- Liquid Chromatography/Mass Spectrometry (LC/MS)
- Conventional Polymerase Chain Reaction (PCR), quantitative
- real-time PCR (qPCR) and microarrays/DNA chips.

The current study aimed to isolate Cyanobacterial isolates from Veeranam Lake in Cuddalore District, Tamil

Nadu, India. The cytotoxicity quantification study was conducted on two axenic cultures.

Materials and methods

Study area

The Lake has positioned fourteen kilometres south-southwest (SSW) of Chidambaram and in every critical district of Tamil Nadu in Cuddalore. It is a 9.9-mile (16 km) long wetland in Tamil Nadu. Water aggregates in the body of water from the river Kollidam well to the river Vadavaru. Besides that, the Veeranam Lake testing

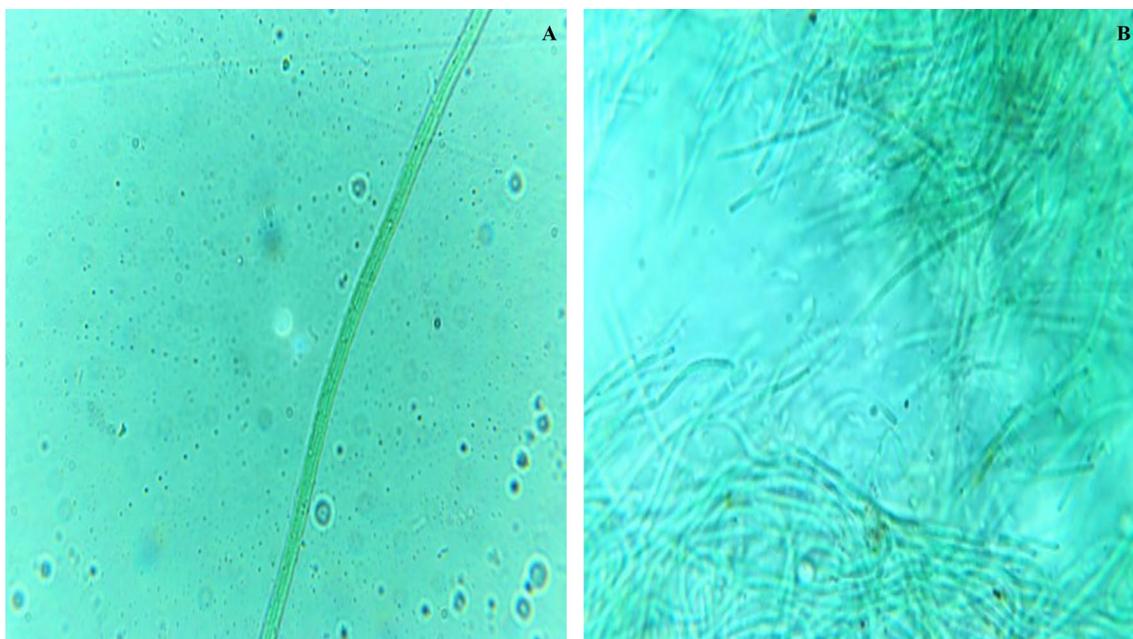


Fig. 4 **A** Showing the image of *Desertifilum* sp and **B** showing *Leptolyngbya* sp

site secures groundwater all the possible way from either the bottom elevation of Anicut, upon mettur dam, as well as the river basin of Cauvery "Bhavani and Amaravati."

Sample collection

Water samples were drawn from various sites in Veeranam Lake from August 2018 to March 2019 using scientific procedures and sequestered in pre-cleaned small screwcap vials for Cyanobacterial isolation.

Blue green algal media (bg11) for the isolation of cyanobacteria

Cultured from solid and liquid media, the samples are infurated at 25 °C under luminance. The test results are then dispersed to a homogenizer tube and centrifuged for 30 min. The centrifuged test results were diluted with distilled water to minimize the number of pathogens. As for the dilution method, the sample size was examined using a microscope to prove its appropriateness [52]. Table 1 shows BG 11 media composition preferred in this study.

Biomass production

For further analysis, biomass production of axenic culture is a must. The colonies were isolated from groups and subcultures. The single isolated colonies were

identified morphologically through a Light microscope and took the image from Scope Image 9.0 camera X5N.

The quantitative analysis of toxins include microcystin and nodularin in water samples using ADDA-ELISA

The best way to analyze the water purity is the enzyme-based assay. The experiment works well for detecting toxins found in microalgae. The enzyme-based approach presumably enhances the simultaneous check on quality and quantity in recognizing Microcystin and Nodularin. The ADDA-specific ELISA kit, an indirect competitor, utilizes diagnostics MCs and NOD toxicants without congeners. Once imbued, MC Toxin and an MC-protein comparator encased on a microplate tray contend for the kit's anti-Microcystin/Nodularin Ab receptor. After thoroughly cleansing the micro-titre plate, added the 2nd Ab-HRP identifier. A colour change is a signal emitted after quite a second cleansing step and the addition of the substrate solution. The yellow to blue coloration intensity is proportional to the number of Microcystin in the test sample. After a retention time, the colour reaction ceases; access the colouring in an ELISA reader. Interpolation is employed to figure out the concentrations of the extracts with the calibration curves erected in every cycle.

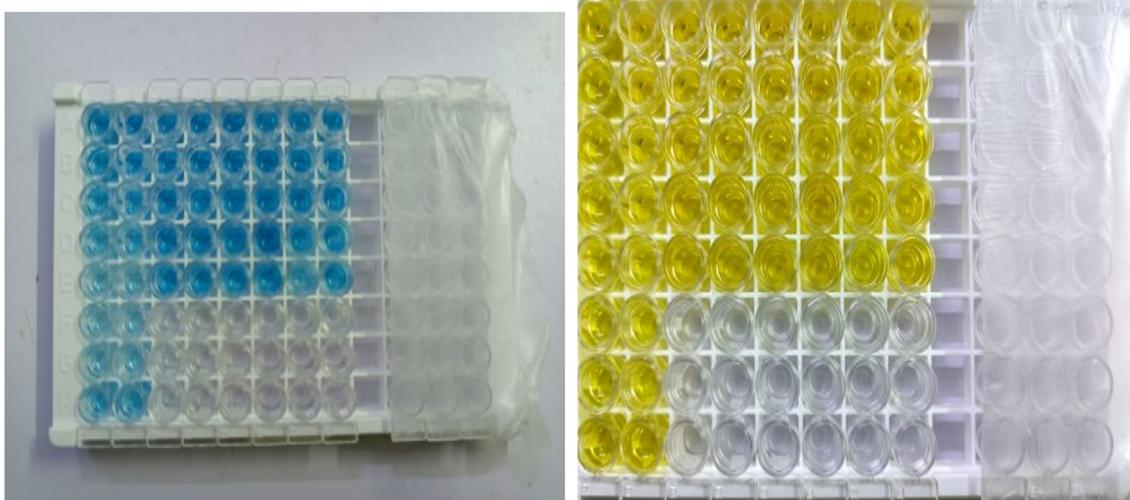


Fig. 5 ADDA specific ELISA kit used for toxicity study and the color change from blue to yellow represents the presence of toxins in both the samples

Collection of samples and handling methods

The extracts are obtained in glass or PETG material (Polyethylene Terephthalate Glycol) boxes and reviewed for 24 h. Several materials initiate or storage containers can cause Microcystin intake, which causes erroneous (falsely low) results. Extracted tests or samples of water are required to be cleaned with a solution of sodium thiosulfate as quickly as they can (referring to the significant scientific bulletin). Refrigerate the samples for longer than 5 days. The samples ought to remain preserved icy for at least 5 days.

This method accomplishes preanalytical treatments as effectively as an appropriate cell membrane lysing approach to keep the substances' toxic effects. (Freeze and thaw). Overall, the sonication within cell lysing can decrease toxins amounts, leading to falsely minimal outcomes of samples. For additional details on the lysis

of cells, consult the corresponding sample set-up scientific bulletin. Glass fibre adjustments are best to use than employing non-glass fibre filters due to erroneous results. Test results must be lysed before to separation process and toxin intensity to mitigate the separation of cell-bound MC toxins.

Methodology for working in kit

Filled 50 µl of the control, reagent blank, LCRC, five standard stocks, or tests into the test strip wells depending on the operational strategy. Accessed duplicate and triplicate values. With the help of a multi-channel pipette, added 50 µl of the antibody solution in each well. Wrapped the wells with parafilm/ tape and mixed the mixture for thirty seconds by progressing the strip holder in a circular movement on the workbench. Ensured not

Table 2 Assay for microcystin-ADDA-ELISA

Microcystin-ADDA ELISA								
Sample	Concentration (µg/L)	OD at 450 nm			Mean	%B/B0	Microcystin (µg/L)	%B/B0
		Singlet	Duplicate	Triplicate				
Standard	0	1.745	1.762	1.798	1.768333333	100	0	100
	0.15	1.64	1.639	1.633	1.637333333	92.5918944	0.15	92.5918944
	0.4	1.494	1.364	1.377	1.411666667	79.8303487	0.4	79.8303487
	1	1.044	1.027	1.062	1.044333333	59.0574929	1	59.0574929
	2	0.681	0.649	0.687	0.672333333	38.0207352	2	38.0207352
	5	0.59	0.56	0.61	0.586666667	33.1762488	5	33.1762488
Sample	Concentration (µg/L)	Singlet	Duplicate	Triplicate	Mean	%B/B0	Microcystin (µg/L)	%B/B0
<i>Leptolyngbya</i>	100	0.092	0.996	1.101	0.096333	5.44769086	17.72	
<i>Desertifilum</i>	100	0.736	0.069	0.072	0.067667	3.8265787	19.38	
		Singlet	Duplicate	Triplicate	Mean			
Positive control	0.75 (ppb)	1.163	1.146	1.152	1.153666667	65.2403393		

to leak the entirety. Incubated the contents at optimum temperature for 90 min.

Removed the cover and squeezed out the solutions from the wells into the sink. Stripes are washed three times using the diluted wash buffer. For every washing procedure, we exploited a minimum of 250 µl of 1X wash buffer. Blotted the inverted plate using paper towels after subsequent wash. Using a pipette, added 100 µl of an enzyme conjugate solution in every single well. Again, wrapped the wells with masking tape and mixed the substances for 30 s by evolving the strip in a circular movement on the tabletop. The process was carried out without leaking the components and incubated at optimum temperature for 30 min.

After removing the wrapping, the entirety of the wells are discarded into a faucet, and blot dried the inverted plate with paper towels. Using the diluted wash buffer, the strips are washed three times. For each cleaning step of the well, we utilized a minimum of 250 µl of 1X wash

buffer. After each wash step, blotted using a stack of paper towels. Added 100 µl of substrate which is a colour solution in each well at a same time. The wells are protected with parafilm or tape and mixed the entire contents for 30 s. Circular movement given for strip holder on tabletop without any leak. Incubated the strips for 20-min at 37°C. And kept away from direct sunlight. In the strips, added 50 L of stop solution as prescribed in the substrate solution. Within 15 min, we have obtained the results by incorporating the stop solution. Using an ELISA photometer reader, the results were observed at 450 nm O.D.

Estimation

Calculated the mean absorption spectrum elements on every standard for detailed calculation. To estimate the %B/B0 with each assessment, divided the mean absorbance value by the Zero Standard (Standard "0") mean absorption spectrum. Established a guideline slope on the diagram by predicting the percentage B/B0 on every characteristic against the comparable toxic chemical density on the lateral logarithmic (x) axis.

Results and discussion

Study area

The Veeranam Lake, located in the Cuddalore district of Tamil Nadu, India (Fig. 3A–D), where water gets transported to major urban cities, including Chennai, is first examined in this research. Samples were collected at

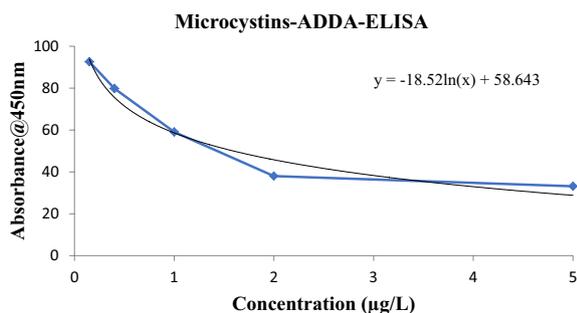


Fig. 6 Standard curve showing the ELISA absorbance @450 nm again concentration

three intervals during various seasons from six distinct places from August 2018 to March 2019.

Morphological identification of cyanobacteria under light microscope (scope image 9.0 camera X5n) and sem image
Veeranam Lake constituted well over ten Cyanobacteria that were engaged in toxic effects. Nevertheless, this research selected only two organisms for comparison because of the challenges in raising selected species in lab conditions, particularly during the Covid pandemic. In many instances, misidentifications of isolates in culturing make applying phylogenetic tasks premised on cultures to field populations difficult. Figure 4 shows two morphologically recognized samples designated as DD for *Desertifilum* and PM for *Leptolyngbya*.

Assay for microcystin-ADDA-ELISA

The ADDA-specific ELISA assay test depicts the quantification of toxic elements, including both samples. Figure 5 depicts a change in colour from blue to yellow, suggesting the presence of a toxic substance in samples. Table 2 shows the average score of the *Desertifilum* and *Leptolyngbya* sp. test results. *Desertifilum* has a higher toxic intensity than the *Leptolyngbya* test sample. Figure 6 depicts the absorption intensity calibration curves at 450 nm.

For the bioassay, we opted an ADDA-specific Enzyme-linked immunosorbent kit for this research. The IC₅₀ values of *Leptolyngbya* and *Desertifilum* demonstrated Microcystin (g/L) concentration levels of 17.72 (g/L) and 19.38 (g/L), respectively. As a result, in ADDA detailed Microcystin sensing, *Desertifilum* sp. displayed greater toxin densities than the *Leptolyngbya* test upon the specimen's singlet, duplicate, and triplicate virtues. Thus, the change in colour from blue to yellow symbolizes the existence of toxicants among both isolates.

Conclusion

The World Health Organization (WHO) has also postulated a recommended value for MC-LR, the most prevalent MC version, in potable water of 1.0 g/L [51]. Therefore, choosing instant, and naive tests are must for routine analysis. ELISAs for MCs that use polyclonal or specific antibodies are incredibly specialized, 47 responsive, and rapid methodologies of detecting MCs and nodularin [39]. This same ELISA test in this research relies on composition and reacts to toxins enclosing the ADDA methyl group. As a result, it helps to detect the large percentage of the renowned poisonous penta- and hepta-peptide toxic chemical congeners [24]

Enzyme-linked immunosorbent assays are utilized skilfully by many scientists to diagnose Microcystin, equating to sequence genetics. In this study, we have

used Microcystin (Adda-specific) Enzyme-linked immunosorbent assay kit that satisfactorily identified the toxic substance in the test samples. Although the package is rigorous and precise to Microcystin and nodularin dependant toxins, comparing the outcomes with specific other analytical techniques, such as HPLC or LC-MS, is beneficial. The Extract concentration of the *Leptolyngbya* sp. and *Desertifilum* sp. study in the existing research suggests the Microcystin (g/L) intensity of *Leptolyngbya*: 17.72 (g/L) and *Desertifilum*. 19.38 (g/L). Besides that, well after the specimens' singlet, duplicate, and triplicate scores in ADDA-specific Microcystin treatment, DD had quite a greater density of toxins than the LE/PM specimen.

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Author contributions

MR: Writing—original draft, Supervision. SE: Software, Conceptualization, Methodology, Visualization. AU: Validation, Writing—review and editing, Investigation.

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Availability of data and materials

Data publicly available in a repository: *Desertifilum tharense* UAM-C 16S ribosomal RNA gene, partial sequence was deposited into the National Library of Medicine (NCBI: National Centre for Biotechnology Information) under accession number OK422864 and are available at the following URL: [https://www.ncbi.nlm.nih.gov/nucleotide/2108473155?log\\$=activity](https://www.ncbi.nlm.nih.gov/nucleotide/2108473155?log$=activity). *Leptolyngbya* sp. lkmLPT16 16S ribosomal RNA gene, partial sequence was deposited into the National Library of Medicine (NCBI: National Centre for Biotechnology Information) under accession number: ON383825 URL: [https://www.ncbi.nlm.nih.gov/nucleotide/2232459489?log\\$=activity](https://www.ncbi.nlm.nih.gov/nucleotide/2232459489?log$=activity). The experimental data and the simulation results that support the findings of this study are available in Current Botany Journal with the identifier Current Botany 2020, 11: 65–74. <https://doi.org/10.25081/cb.2020.v11.6059>. <https://updatepublishing.com/journal/index.php/cb>. Data available with the paper or supplementary information: All data supporting the findings of this study are available within the paper and its Supplementary Information. Lake water ecosystem variables indicating ecosystem functions that support the findings of this study are included within this paper and its Supplementary Information files. Lake water analysis for pollution check that supports for this research article mentioned in: <https://doi.org/10.1016/j.matpr.2021.11.354>.

Declarations

Consent to participate

ADDA-Specific ELISA KIT has been purchased from RE: 546531192013 EURO-FINS AMAR IMMUNODIAGNOSTIC ROD// Abrax. Eurofins India, Bangalore.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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