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Article

Enhanced Release of Cyanotoxins in Freshwater Lakes: Insights on the Causal Mechanisms and Eutrophication Dynamics in the North Bank Plains of Brahmaputra Valley, Assam, India

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ABSTRACT

Cyanotoxin pollution is a radical phenomenon involving depth of water bodies, nutrient enrichment, and dominance of cyanobacterial species. This investigation presents a novel framework for assessing cyanotoxin (MC-LR) risk analysis in the north bank plains of Brahmaputra valley, Assam, Northeast India and cyanotoxin pollution was assessed in seven sub-tropical shallow lakes in Tezpur, Sonitpur, Assam. A positive association was established between cyanotoxin pollution and lake trophic state fluctuations. Microcystin-LR (MC-LR) was quantified through a two-way analytical approach involving ELISA and HPLC methods, and the pathways of eutrophication were systematically evaluated involving CNP dynamics and limnological variables. MC-LR was detected in the seven lakes, 1.12–3.46 ppb (HPLC-method) and 1.143–19.42 ppb (ELISA-method); all lakes contained MC-LR beyond WHO permissible limits (>1 ppb). Trophic State Index (TSI) ranged between 52.05–64.59, eutrophic in four lakes and hypereutrophic in three lakes. CNP enrichment boosted algal density, chlorophyll-a, and hence MC-LR in the lakes ($p < 0.05$; $p < 0.01$). Secchi Disk Depth (SDD) had significant correlation with TSI and MC-LR potentially released by *Microcystis, Dolichospermum,* and *Nostoc* ($p < 0.01$); MC-LR showed substantial associations with CNP and TSI ($p < 0.01$). There are current or past records of MC-LR related epidemiological interventions in the study area. Therefore, this study addresses an urgent necessity to monitor cyanotoxin pollution and address the ecotoxicological concerns of harmful algal blooms (HABs) releasing cyanotoxins Northeast India lakes. Risk analysis and risk mitigation following "state of the art" cyanotoxin guidelines will facilitate systematic remediation of cyanotoxin pollution universally.

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Research Highlights

- Shallow lakes in the sub-tropics are more prone to eutrophication and cyanotoxin pollution.
- Microcystin-LR is a commonly occurring cyanotoxin in water bodies and a potent toxin for living organisms.
- MC-LR depends on harmful algal blooms (HABs), trophic state index (TSI), and bio physicochemical variables of water bodies.
- Ecohydrological monitoring and epidemiological interventions will help in the systematic remediation of cyanotoxin pollution.

1. Introduction

Harmful algal blooms (HABs) and cyanotoxin pollution are emerging threats to the global freshwater and marine ecosystems [1]. Cyanotoxins such as microcystin, cylindrospermopsin, anatoxin-a, and saxitoxin are potent biopollutants that bioaccumulate, go through the food webs, and pose serious health risks to humans and wildlife [2, 3]. Eutrophication frequently promote cyanobacteria dominated HABs, some algae like *Euglena* also produce toxins such as euglenophycin, indicating toxin production is not limited to cyanobacteria [4]. Some of the significant classes of cyanotoxins are—cyclic heptapeptides, alkaloid toxins consisting of a tricyclic guanidine linked to hydroxymethyluracil or structurally similar to acetylcholine and tetrodotoxin, lipopeptide toxins and non-protein amino acids [5]. Cyanotoxin research is predominantly concentrated in the United States, specifically within the Great Lakes, the Gulf of Mexico, and California, although expanding in Europe, with particular emphasis on algal toxins affected freshwater and marine ecosystems in the Netherlands, Germany, Spain, France, and the United Kingdom [6, 7]. Furthermore, the Baltic and Mediterranean Seas are emerging as crucial sites for investigations into cyanobacterial ecology and the management of toxins. In Australia, research efforts are primarily directed towards freshwater ecosystems, with institutions prioritizing the evaluation of cyanotoxins and their associated ecological and public health consequences [8]. Likewise, research is progressing in South America, where Brazil, Argentina, and Chile are actively investigating cyanotoxin-related concerns [9]. In Africa, substantial research is being conducted around Lake Victoria, Lake Chad, and the Nile River, while China, Thailand, Vietnam, Iran, Turkey and India are at the forefront of cyanotoxin research in Asia, concentrating on assessment and management approaches [10, 11]. India experiences a mixed tropical climate with extreme hot and cold regions, and wet humid, dry weather in varied geographical locations; these climatic features are favorable for the growth of HABs and release of cyanotoxin in eutrophic water bodies in freshwater as well as marine ecosystems [12]. Rapid urbanization has increased eutrophication, leading to waterlogging and ecological risks in lakes that are usually overlooked in management plans [3]. Therefore, this study aims to holistically investigate the ecohydrological factors influencing cyanotoxin production, specifically Microcystin-LR (MC-LR) in lakes within the central Brahmaputra Valley. It addresses an urgent need to understand and investigate the ecological and health implications of algal blooms in eutrophic lakes contaminated with algal toxins in Indian lakes. The primary goal is to evaluate MC-LR release from HABs in seven shallow, subtropical lakes and identify the critical ecohydrological drivers linked to trophic state change. This research aims to elucidate lake-specific MC-LR release patterns in lake waters by integrated analyses of biophysicochemical variables, nutrient dynamics, and computation of trophic state indices. By identifying a novel research gap in cyanotoxin (MC-LR) risk analysis in Northeast India, this study pioneers MC-LR characterization in shallow lakes in relation to trophic status and limnological variables. The MC-LR analysis is methodically strengthened through a two-way analysis—Enzyme-Linked Immunosorbent Assay (ELISA) based and confirmatory High Performance Liquid Chromatography (HPLC). The overall outcomes of this paper establish a predictive framework for monitoring ecological risks associated with cyanotoxin pollution and generate robust baseline data on MC-LR occurrence and its ecohydrological drivers, which will be useful for lake pollution management.

2. Materials and Methods

2.1. Study Area

Seven sub-tropical lakes were selected in Tezpur, Sonitpur district, Assam in the north bank plains of Brahmaputra valley based on their proximity to land use land cover patterns (>1 km apart and earlier records of MC-LR risk analysis in relation to trophic status dynamics. The area experiences an average temperature of 23.95 °C, humidity of 84.2%, and an annual rainfall of 1398 mm, primarily during April–October. Lakes were categorized as residential (2), roadside (3) and agricultural (2), (Figure 1, Table 1).

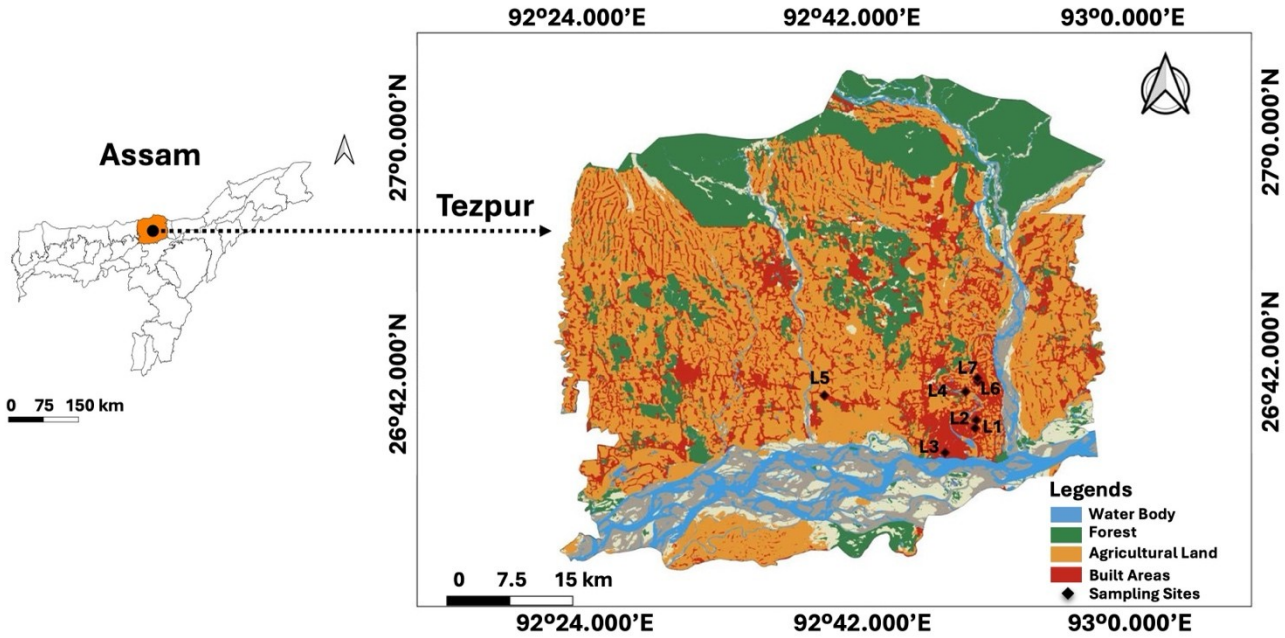

Figure 1. Lake sampling sites that included residential (L1, L2), roadside (L3, L6, L7) and agricultural (L3, L4) localities in Tezpur, India (prepared in QGIS 3.22.1).

Table 1. List of the sampling locations—names, area, geographic location and locality of seven sub-tropical lakes; the lakes were categorized as—agricultural, residential and roadside depending on the locality and land use activities.

Sample Code	Name of the Lakes	Area (m^2)	Geographic Location	Locality	Algal Bloom
L1	Tinilmile Chatai, Dolabari	8213	26°39′26″ N 92°49′44″ E	Residential	Yes, dark-green
L2	Batamari wetland	464,475	26°38′55″ N 92°49′39″ E	Residential	Yes, dark-green
L3	Padam Pukhuri	16,600	26°37′20″ N 92°47′30″ E	Roadside/Township	Yes, dark-green
L4	Porua water body	75,255	26°41′16″ N 92°48′58″ E	Agricultural	No, clear waters
L5	Water body, near Tezpur Medical College	2068	26°41′00″ N 92°38′49″ E	Agricultural field	Yes, black to dark-green
L6	Lake, Tezpur University	4564	26°41′55″ N 92°49′51″ E	Roadside	Yes, dark-green
L7	Niribili Lake, Tezpur University	12,200	26°42′08″ N 92°49′49″ E	Roadside	Yes, dark-green

2.2. Sampling Procedures

Lake waters were via grab sampling at 0–100 cm depth within the euphotic zone, targeting phytoplankton, following APHA guidelines [13]. Samples were collected in triplicate in sterile polyethylene bottles; one set was immediately kept on ice for MC-LR analysis and limnological and phytoplankton assessments.

2.3. Limnological Variables

In situ measurements included pH, electrical conductivity (EC), and dissolved oxygen (DO), using portable meters. Turbidity was measured with a Nephelometer; Total nitrogen (TN) and total phosphorus (TP) were analyzed following APHA guidelines [13]. Total organic carbon (TOC) was estimated via Walkley-Black titration [14]. Fluoride

(F^-) was estimated by the SPADNS colorimetric method (APHA, 4500-F^-D) [13]. Sulphate (SO_4^{2-}) was measured by a turbidimetric method involving $BaCl_2$ and chemical oxygen demand (COD) was estimated through APHA protocols [13].

2.4. Biological Variables

Secchi disk depth (SDD) was measured with a Secchi disk [15]. Phytoplankton was sampled using a plankton net, preserved with Lugol's solution, and identified microscopically at 10× and 40× magnification (OLYMPUS BX43) following standard taxonomic keys [16]. Chlorophyll-a was extracted with acetone, quantified spectrophotometrically at 663 and 645 nm, and algal density was calculated based on absorbance at 684 nm using the following equations [17]:

$$\text{Chlorophyll a } (\text{mg L}^{-1}) = \text{O.D. } (663) \times 12.7 - \text{O.D. } (645) \times 2.69 \quad (1)$$

Algal density was enumerated through thr following equation [18]:

$$\text{Cell density } (\text{cells mL}^{-1}) = e\{[\ln(\text{absorbance\_}684) + 16.439]/1.0219\} \quad (2)$$

Moreover, the trophic state index (TSI) was calculated using the following equations [19]:

$$\text{TSI (SD)} = 60 - 14.41 \text{ Ln (SD)} \quad (3)$$

$$\text{TSI (chl-a)} = 9.81 \text{ Ln (chl-a)} + 30.6 \quad (4)$$

$$\text{TSI (TP)} = 14.42 \text{ Ln (TP)} + 4.15 \quad (5)$$

$$\text{CTSI} = \text{TSI (SD)} + \text{TSI(chl-a)} + \text{TSI (TP)}/3 \quad (6)$$

The range of Carlson TSI is approximately 0 to 100 (<30: oligotrophy; 30–40: moderately mesotrophic; 40–50: mesotrophic and less productive; 50–60: eutrophic; and >60: hypereutrophic) [19].

2.5. Cyanotoxin Quantification

Microcystin-LR (MC-LR) was measured using Cayman's ELISA kit (Item No. 502000), with detection limits of 0.0205–8 ng/mL in ELISA reader (Thermo Scientific Multiskan Sky Multiplate Spectrophotometer) and HPLC (Waters Corporation, USA) following standard protocols [20].

2.6. Statistical Analysis

The limnological data was parametrically tested and verified to obtain meaningful interpretation. Normalization of data was performed through log transformation (except pH), and the z-score of the data was computed using the following equation [21]:

$$Z_n = (X_n - \mu/\text{SD}) \quad (7)$$

where $Z_n = z$-score of the nth variable, X_n is the nth variable, and μ is the mean of the nth variable in this investigation. Descriptive statistics, Pearson's correlation, and regression and Principal Component Analysis (PCA) were analysed with normalized data in IBM SPSS 20 software.

3. Results

3.1. Limnological Variables and Nutrient Dynamics

The lake waters showed slightly acidic pH (6.32 ± 0.03 in L2, residential lake) to marginally alkaline pH (7.21 ± 0.02 in L5, agricultural lake), all values falling within the range compatible for supporting diverse aquatic lives (Table 2). The water temperature ranged between 25.37 °C ± 0.07 and 26.70 °C ± 0.09 and the DO levels varied from 4.36 ± 0.04 mg L^{-1} to 7.02 ± 0.04 mg L^{-1} across all lakes (Table 2). The COD was recorded as 12.20 ± 0.09 mg L^{-1} (L4) to 60.97 ± 0.10 mg L^{-1} (L2) and the EC measurements varied from 23.70 ± 0.06 µS/cm (L5) to 51.49 ± 0.11 µS/cm (L2) (Table 2). SDD measurements across the lakes ranged from a maximum of 2.34 ± 0.01 m (L4), indicating relatively clear water, to a minimum of 0.83 ± 0.01 m (L2), reflecting non-transparent water, while turbidity levels were high in L7 (22.72 ± 0.13 NTU) and low in (10.15 ± 0.05 NTU) in L1 (Table 2). Cl^- concentrations varied from 8.55 ± 0.39 mg L^{-1} (L4) to 80.94 ± 0.13 mg L^{-1} (L7), sourcing from natural and water treatment activities. Sulphate ranged between 2.20 ± 0.06 mg L^{-1} (L1) to 5.29 ± 0.08 mg L^{-1} (L2), arising from natural sources and causing non-algal turbidity in the lake waters. F^- was lowest in L7 (3.68 ± 0.09 mg L^{-1}) and highest in L2 (5.44 ± 0.03 mg L^{-1}) indicating anthropogenic influences (Table 2).

TOC averaged around 0.03 mg L^{-1} (0.02 ± 0.005 to 0.05 ± 0.005 mg L^{-1}) suggesting abundant organic matter derived from algal biomass, sediments, and terrestrial runoff. TN was recorded between 3.26 ± 0.05 mg L^{-1} (L4) to 13.58 ± 0.14 mg L^{-1} (L2), reflecting substantial nitrogen input from domestic sewage, agricultural runoff, and land-uses. TP levels ranged from 1.37 ± 0.05 mg L^{-1} to as high as 3.27 ± 0.03 mg L^{-1} (in L2) (Table 2). Nearly all sampling sites showed elevated phosphorus amounts, a recommended concentration level of 0.05 mg L^{-1} for inland water lakes by USEPA [22].

Table 2. A comprehensive analytical report on the bio-physicochemical variables in the seven lakes.

Lakes	Temp (°C)	SDD (m)	pH	EC (µS/cm)	DO (mg L^{-1})	Chloride (mg L^{-1})	TN (mg L^{-1})	Turbidity (NTU)	TP (mg L^{-1})	Sulphate (mg L^{-1})	TOC (mg L^{-1})	COD (mg L^{-1})	Fluoride (mg L^{-1})
L1	25.4 ± 0.05	2.08 ± 0.01	6.80 ± 0.03	37.58 ± 0.16	6.65 ± 0.03	35.50 ± 0.67	4.64 ± 0.09	10.15 ± 0.05	1.37 ± 0.05	2.20 ± 0.06	0.027 ± 0.010	17.23 ± 0.05	5.28 ± 0.04
L2	26.3 ± 0.07	0.83 ± 0.01	6.32 ± 0.03	51.49 ± 0.11	4.36 ± 0.04	49.70 ± 0.67	13.58 ± 0.14	22.72 ± 0.13	3.27 ± 0.03	5.59 ± 0.08	0.050 ± 0.005	60.97 ± 0.10	5.44 ± 0.03
L3	26.1 ± 0.07	1.49 ± 0.01	6.94 ± 0.03	45.53 ± 0.17	5.74 ± 0.04	30.29 ± 0.39	4.87 ± 0.11	12.34 ± 0.03	1.41 ± 0.03	3.84 ± 0.04	0.020 ± 0.005	17.20 ± 0.05	4.50 ± 0.09
L4	26.5 ± 0.09	2.34 ± 0.01	7.06 ± 0.02	38.31 ± 0.08	7.02 ± 0.04	15.15 ± 0.39	3.26 ± 0.05	10.56 ± 0.03	1.45 ± 0.03	3.47 ± 0.04	0.020 ± 0.005	12.20 ± 0.09	5.43 ± 0.04
L5	25.9 ± 0.07	1.74 ± 0.01	7.21 ± 0.02	23.70 ± 0.06	4.86 ± 0.05	79.05 ± 1.68	5.56 ± 0.15	17.45 ± 0.04	2.56 ± 0.04	4.12 ± 0.03	0.027 ± 0.010	20.50 ± 0.12	4.24 ± 0.04
L6	25.2 ± 0.07	1.26 ± 0.02	6.47 ± 0.04	42.13 ± 0.10	5.53 ± 0.12	78.31 ± 0.10	7.14 ± 0.11	19.76 ± 0.04	2.16 ± 0.04	3.63 ± 0.10	0.037 ± 0.005	32.63 ± 0.12	3.97 ± 0.06
L7	25.1 ± 0.19	1.03 ± 0.01	6.57 ± 0.03	36.28 ± 0.10	5.40 ± 0.05	80.94 ± 0.13	6.46 ± 0.08	20.82 ± 0.05	2.35 ± 0.09	5.10 ± 0.07	0.033 ± 0.007	45.47 ± 0.15	3.68 ± 0.09

3.2. Chlorophyll-a (Chl-a) and Algal Density and Carslon TSI

Chl-a concentrations ranged from very low (0.04 $\pm$ 0.007 µg L^{-1}) in L4 (roadside lake) to a high level (0.13 $\pm$ 0.003 µg L^{-1}) in hypereutrophic L2 (residential lake), reflecting variations in phytoplankton biomass (Table 3). Algal density, measured via cell counts, ranged from approximately 42109.87 $\pm$ 939.362 cells mL^{-1} in the least productive lakes to over 80735.467 $\pm$ 272.674 cells mL^{-1} in the mesotrophic L2 (Table 3). The TSI values ranged from 52.04 in L4, indicating eutrophic conditions, to 60.87 in L2, classifying some lakes as hypereutrophic (Table 3, Figure 2).

3.3. Comparative Detection and Quantification of MC-LR in Lake Waters

MC-LR across the lakes ranged from 1.08 ppb in L7 to 19.42 ppb in L2 in ELISA based method and 1.12 ppb (L2) to 3.46 ppb (L5) in HPLC method (Figure 3). These elevated levels, particularly in L2, L5 and all other lakes, significantly exceeded the World Health Organization (WHO) permissible limit for freshwater (1.0 ppb) [23]. The detection range of MC-LR was similar between HPLC and ELISA ($p = 0.45$); however, site-wise MC-LR estimates were markedly different, underscoring the need to determine the most suitable method for MC-LR studies.

Table 3. Biological variables influencing TSI fluctuation and MC-LR release in the seven lakes.

Samples	Chlorophyll-a (µg L^{-1})	Algal density (Cells mL^{-1})	Microcystin-LR (ELISA) (ppb)	Microcystin-LR (HPLC) (ppb)	Trophic State Index	Trophic State
L1	0.07 $\pm$ 0.007	51287.61 $\pm$ 472.23	1.35	1.73	54.19	Eutrophic
L2	0.13 $\pm$ 0.003	80735.47 $\pm$ 272.67	19.42	1.12	64.59	Hypereutrophic
L3	0.10 $\pm$ 0.004	53892.22 $\pm$ 1033.32	1.29	2.54	56.87	Eutrophic
L4	0.04 $\pm$ 0.003	42109.87 $\pm$ 939.36	1.14	1.18	52.05	Eutrophic
L5	0.12 $\pm$ 0.007	72420.34 $\pm$ 223.88	1.37	3.46	59.67	Eutrophic
L6	0.11 $\pm$ 0.051	60934.88 $\pm$ 275.16	1.24	–	60.16	Hypereutrophic
L7	0.09 $\pm$ 0.029	66431.02 $\pm$ 1062.83	1.08	–	60.87	Hypereutrophic

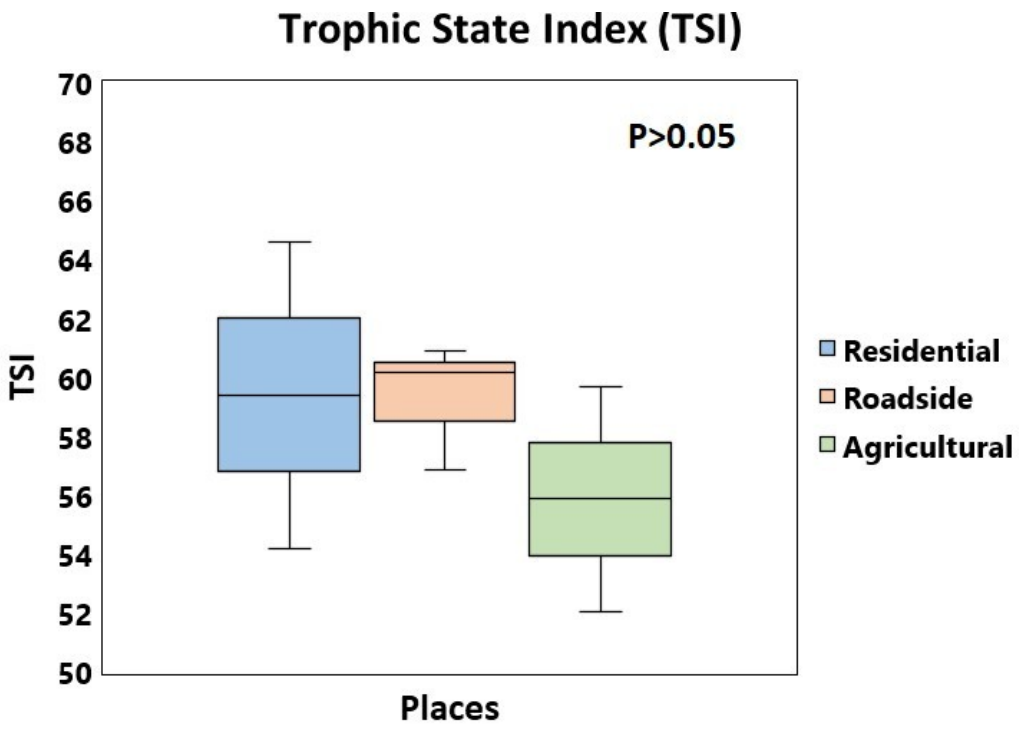

Figure 2. TSI of the lakes categorized as—residential (M = 35.50), roadside (M = 37.59) (M = median) and agricultural (M = 27.64); M = median.

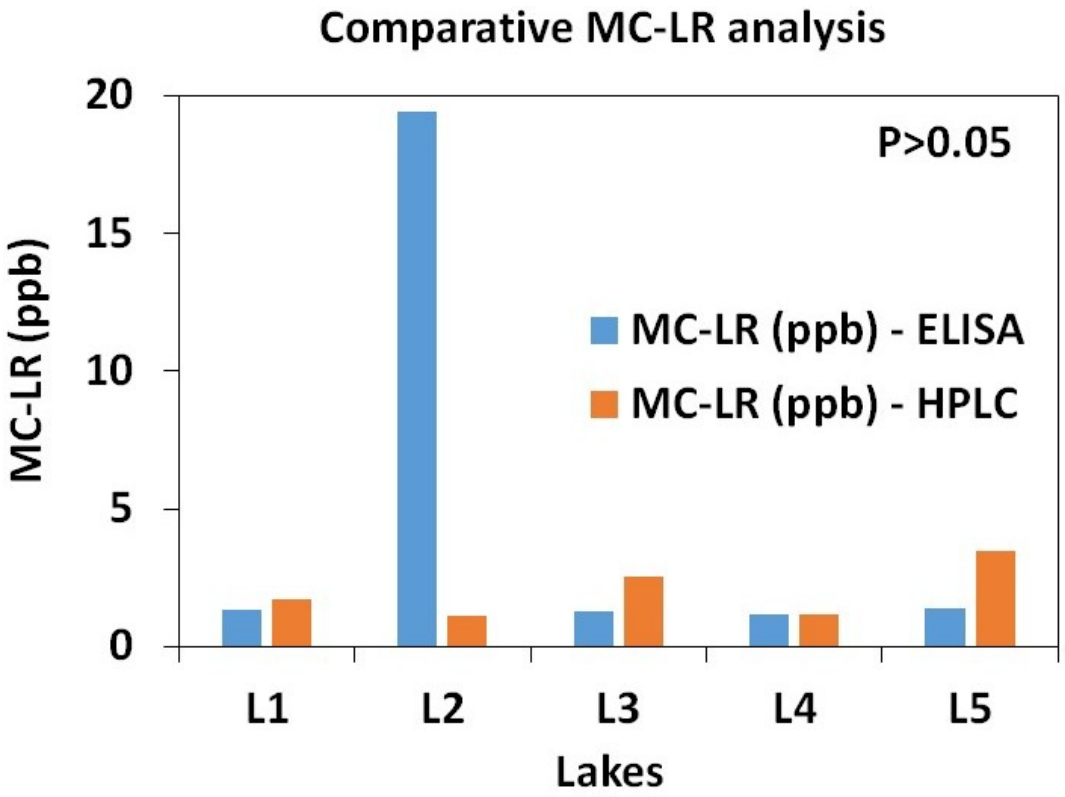

Figure 3. A comparative MC-LR analysis through HPLC and ELISA methods; quantification were similar except L2.

4. Discussions

4.1. The Role of Critical Ecohydrological Drivers in Trophic State Change

The limnological variables, nutrient, MC-LR and trophic state of the seven lakes in Tezpur, Assam varied notably in land use, with agricultural, residential, and roadside categories, reflecting different sources and intensities of anthropogenic runoff. The lake diversity provided a broader understanding of how local human activities affects water quality and trophic dynamics. Spatial differences across lakes highlighted the influence of nearby land use, with roadside and residential lakes generally exhibiting higher nutrient loads, turbidity, and algal richness, whereas some lakes maintained better water clarity and lower nutrient concentrations. These baseline data are fundamental for identifying pollution hotspots, understanding nutrient fluxes, and establishing a framework for assessing future ecological changes and management needs in the study area.

Slightly acidic conditions in lakes like L2, L6, and L7 suggest active organic decay processes and cyanobacterial overgrowth, which produce organic acids and influence overall chemical balances (Table 2). The acidic pH also influences toxin stability, nutrient availability, and microbial activity. The temperature data, ranging from 25.37 °C $\pm$ 0.07 to 26.70 °C $\pm$ 0.09, indicated warm water conditions typical of subtropical lakes, conducive to cyanobacteria proliferation, especially *Microcystis* sp., which thrive in warmer temperatures. Higher temperatures correlate with increased metabolic rates and growth of algae, accelerating eutrophication processes and temperature-driven changes in biogeochemical cycles affect lake chemistry and biological activity, contributing to bloom dynamics. The average DO values indicated active growth of algal biomass in the lake waters [24]. Such hypoxic or near anoxic conditions are critical indicators of water quality, threaten aquatic fauna, lead to fish kills, and may reduce biodiversity. An inverse relationship between DO and Chl-a underscores the impact of algal blooms i.e., excessive phytoplankton production results in increased organic matter when these algae decay, depleting oxygen (DO-Chl-a: $r = -0.97^{**}$ at $p < 0.01$) (Tables 3 and 4). In regions like Tezpur, where geogenic factors contribute to ion levels, EC variability can serve as an indirect indicator of pollution sources. High EC values may also influence nutrient availability and algal growth, especially in nutrient-enriched waters subjected to continuous runoff from settlements or agricultural land along with natural causes [22]. Also, high turbidity levels observed in L7 (22.72 $\pm$ 0.13NTU), are symptomatic of dense algal blooms, mainly cyanobacteria like *Microcystis*, which produce significant biomass and extracellular organic matter. Elevated turbidity causes light attenuation, thereby shortening the euphotic zone where maximum photosynthesis occurs. An inverse relationship

between turbidity, SDD and nutrients specifies that increased nutrient load impairs water transparency and affects phytoplankton photosynthesis cum oxygen production (Tables 3 and 4) [25]. As algal biomass (measured via Chl-a) increases, water becomes more turbid, further diminishing SDD, which may create a feedback loop favouring bloom persistence. The relationship between turbidity and DO was also significant (Table 4). L2, with prominent turbidity of 22.72 $\pm$ 0.13NTU, exhibited the lowest DO levels ($\sim 4.36 \pm 0.04$ mg L^{-1}), suggesting that dense algal populations and their subsequent decay consume oxygen rapidly. As SDD decreases, the oxygen levels also tend to decline ($r = 0.80^{*}$, $p < 0.05$) (Table 4), corroborating that eutrophic, turbid lakes are prone to hypoxia, affecting aquatic life.

Chlorides, fluoride, and sulphate are some of the predominant anionic species in the lake waters, these anionic species were sourced from the natural surroundings, parent shale, and human-based activities such as agricultural runoff and wastewater release in the study areas [22]; however, F^{-} may originate from residential and urban effluents in the investigated lakes. L2 is the most polluted lake and vulnerable to high nutrient enrichment and hence rapid algal growth that will cause eutrophication immediately. Sulphate^{-} might have reduced transparency i.e., non-algal turbidity and pH (6.32 $\pm$ 0.03), but it did not cause severe acidification of L2; however, in this investigation, the anthropocentric releases may cause a cumulative surge of these anions in the lakes.

TOC levels (0.02 $\pm$ 0.005 to 0.05 $\pm$ 0.005 mg L^{-1}) indicated eutrophication since organic load stimulates microbial activity and further oxygen depletion. High concentrations of TN in 13.58 $\pm$ 0.14 mg L^{-1} in L2 and other residential and roadside lakes reflect substantial nitrogen input from domestic sewage, agricultural runoff, and land-uses near the lakes (Table 2). These sources introduce ammonia, nitrate, and nitrite compounds, which act as nutrients causing algal growth, particularly cyanobacteria. Elevated TP levels (1.37 $\pm$ 0.05 mg L^{-1} to 3.27 $\pm$ 0.03 mg L^{-1}) mostly results from domestic sewage, fertilizer runoff, and soil erosion. Phosphorus (P) is often the limiting nutrient in freshwater systems, excess P catalyzes algal proliferation, especially of bloom-forming cyanobacteria [26]. The positive correlations between TOC, TN, TP, Chl-a, and algae density suggest nutrient enrichment directly drives eutrophication and HAB events (Table 4). Furthermore, the relationship of nutrients with decreasing SDD and DO indicates that nutrient loading accelerates organic decay and oxygen consumption, creating hypoxic conditions (Table 4).

Chl-a concentrations (0.04 $\pm$ 0.007 to 0.13 $\pm$ 0.003 µg L^{-1}) in mesotrophic L2 (residential lake), indicated proliferation algal blooms, especially HABs (Table 3, Figure 4). Chl-a is a primary indicator of trophic status, higher levels signify abundant algae, often resulting from nutrient over-enrichment (Tables 3 and 4). *Microcystis*, *Dolichospermum*, and *Nostoc* species dominated these high-density blooms, suggesting scenario for HAB development and associated toxin production (Figure 4) [27]. The high algal densities contribute to increased Chl-a and cyanotoxin release, which further impact water transparency, oxygen balance, and ecosystem stability (Table 4). The positive correlations between Chl-a and nutrients underscore the eutrophication process, where nutrient surplus drives rapid algal growth. The dense blooms containing cyanobacteria (*Microcystis*, *Dolichospermum*, and *Nostoc*) may have produce MC-LR, which pose health risks to humans, livestock, and aquatic fauna, entailing targeted management strategies to control nutrient inputs and prevent HAB formation (Figure 4) [28, 29].

Table 4. Details of correlation analysis among the bio-physicochemical variables in the seven sub-tropical lakes.

	Temperature	SDD	pH	DO	TN	Turbidity	TP	TOC	Chl-a	Algal Density	MC-LR
SDD	0.22										
pH	0.61	0.81 *									
DO	−0.36	0.80 *	0.42								
TN	0.15	−0.88 **	−0.63	−0.86 *							
Turbidity	0.33	−0.77 *	−0.49	−0.91 **	0.93 **						
TP	−0.12	−0.83 *	−0.85 *	−0.73	0.83 *	0.83 *					
TOC	−0.17	−0.96 **	−0.84 *	−0.78 *	0.91 **	0.84 *	0.93 **				
Chl-a	0.37	−080 *	−0.46	−0.97 **	0.88 **	0.92 **	0.79 *	0.83 *			
Algal Density	−0.12	−0.63	−0.57	−0.67	0.48	0.64	0.71	0.64	0.61		
MC-LR	0.22	−0.87 *	−0.55	−0.93 **	0.89 **	0.83 *	0.75	0.84 *	0.95 **	0.50	
TSI	0.09	−0.93 **	−0.66	−0.92 **	0.91 **	0.84 *	0.81 *	0.90 **	0.93 **	0.57	0.98 **

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

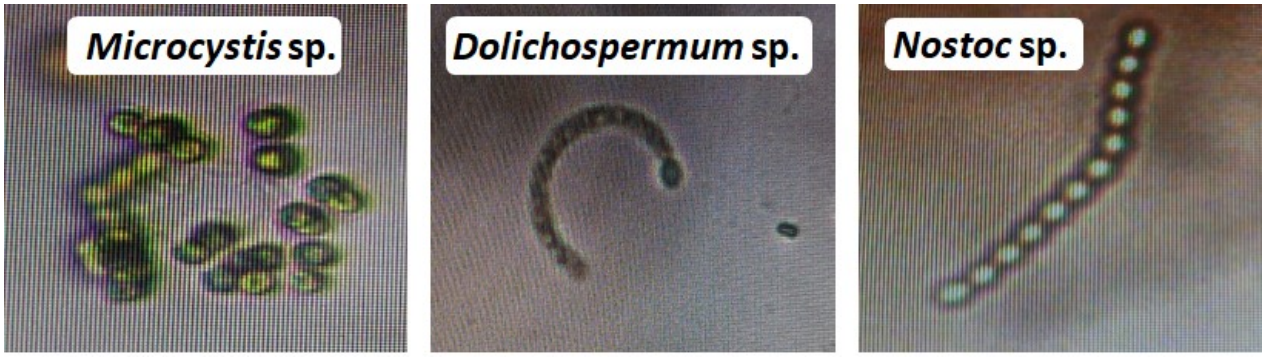

Figure 4. Microscopic images of algae (scale 10 µm): blue–green algae (cyanobacteria)—*Microcystis* sp., *Dolichospermum* sp. and *Nostoc* sp. recorded in L2 following standard taxonomic keys [16] (these cyanobacteria were recorded in all lakes).

4.2. Carlson Trophic State Index (TSI) and as an Indicator of Lake Productivity

The strong correlation between TSI and some key parameters (SDD, water transparency, Chl-a, algal density, TP, and nutrient loads) indicates that nutrient enrichment directly drives trophic state escalation to eutrophic conditions and high productivity (Tables 3 and 4). Elevated TSI values coincided with decreased SDD and increased Chl-a, which collectively reduce light penetration and promote bloom conditions and release algal toxins (Table 3). The strong correlation was observed between TSI and MC-LR, this emphasizes the importance of regular trophic monitoring as an integrative tool for early detection of eutrophication and cyanotoxin-related health risks, enabling timely management interventions to reduce nutrient loads and protect lake health (Table 4).

However, the Carlson TSI model has certain limitations that may not fully predict the original state of water systems, and there is a risk of misinterpreting the exact origins of input parameters, especially non-algal turbidity in lake waters [30]. The empirical relationships among SDD, Chl-a, and TP do not capture the climatic variations and other relevant limnological variables (DO, water color and pH) [31]. Yet, modified TSI models rely on transient TN limitation in trophic-state indexing, which significantly contributes to planktonic Chl-a in waters [32]. An important finding shows that TSI model based on SDD, Chl-a, TP, and TN accounts a log-based score of 0–100 that may lead to misinterpretation because an increment of 10 units corresponds to a doubling of algal biomass or a reduction to half in relation to trophic state; this challenging factor often leads to misestimating the trophic state [33]. Careful interpretation of data and validation of results through other physicochemical and climatic indicators will help in validating the TSI based trophic status classifications.

4.3. Risk Analysis of MC-LR in Freshwater Lakes

The observed MC-LR concentrations, ranging from 1.08 ppb to 19.42 ppb, significantly exceed the WHO permissible limit of 1.0 ppb/µg L^{-1} for freshwater systems [23]. The higher MC-LR concentrations were detected mostly in lakes with high TSI and algal biomass, reinforcing the strong link between eutrophication and toxin production. *Microcystis*, *Dolichospermum*, and *Nostoc* species, known to produce MC-LR, dominated these blooms, particularly in shallow, nutrient-enriched lakes with extensive phytoplankton proliferation (Figure 4) [34, 35]. Temperature plays a vital role in microcystin biosynthesis, with optimal production occurring between 20–25 °C; observed lake temperatures (∼25.88 °C) align well with this window (Tables 2 and 3). The strong association between MC-LR and nutrients underscores the importance of nutrient management in controlling toxin levels (Table 4). This finding suggests that cyanobacterial dominance and their toxigenic potential can be predicted from nutrient parameters and TSI, providing a pathway for targeted management strategies.

The dual approach for MC-LR detection and quantification revealed that HPLC offers consistent recovery that makes it relevant for confirmatory assessment for a wide range of environmental pollutants; while ELISA based assessment are suitable for biological assays. The striking differences in the results indicate scope of limitations in sample preparation and method validation in both the analytical approaches. Earlier reports also show that there are chances of cross reactivity and matrix effects that can lead to overestimation of the target compound, this could be the reason of higher MC-LR quantification in L2 i.e., 19.42 ppb as compared to HPLC quantification i.e., 1.12 ppb in in L2 [36]. Overall, it was observed that ELISA may be used for

early screening and detection of cyanotoxins, and HPLC may be used for confirmatory analysis to avoid technical and methodological uncertainties.

The US Environmental Protection Agency (USEPA) recommends a microcystin limit of 0.3 µg L^{-1} for children (i.e., feeding babies to nursery children) and 1.6 µg L^{-1} in drinking water for adults (i.e., including those above pre-school age) [37]. WHO advises a low probability effect advisory for microcystin in recreational water of up to two µg L^{-1} [38]. The Oregon Health Authority (OHA) has set the human tolerable daily intake (TDI) of microcystin at 0.05 µg kg^{-1} per day; the tolerable limit for drinking water is 1.0 µg L^{-1} and for recreational water is 10 µg L^{-1} for acute or short-term exposure to microcystin [39]. Health Canada (HC) advocates a limit of 1.5 µg L^{-1} for MC-LR in drinking water in Canada; overall, HC follows a guideline of 0.3 to 1.6 µg L^{-1} of microcystin for drinking water and ≤ 20 µg L^{-1} for recreational purposes that brings in contact with microcystin [39]. The existing guidelines appear ambiguous because the database of microcystin concentrations worldwide is incomplete, and an uncertainty factor of 1000 may reflect a lapse in current records on MC-LR (WHO, 2022). In this investigation, the higher concentration range indicates that HAB (cyanobacteria) proliferation in the study area is harmful to human health, and there is always a plausible risk associated with acute or chronic exposure to MC-LR. Management implications include establishing routine cyanotoxin surveillance, implementing nutrient control strategies to limit bloom formation, and developing policies aligned with international guidelines. The current evidence underscores the need for localized epidemiological studies to assess potential health impacts and to formulate risk mitigation protocols.

4.4. Correlation and Principal Component Analysis of the Lake Limnological and Biological Variables and TSI and MC-LR

Pearson correlation analysis revealed convincing links between the limnological parameters pH, temperature, DO, turbidity, Chl-a, algal density, nutrients (TP, TN, TOC), TSI and MC-LR. As anticipated, a strong correlation exists among MC-LR occurrence and the key limnological variables, nutrients and trophic state underscores the integrated role of bloom intensity, nutrient enrichment and eutrophic conditions in cyanotoxin release in the lakes (Table 4, Figures 4 and 5). The rotated PCA plots clearly exhibited two major gradients that govern the lake water quality and MC-LR occurrence. PC1 (74.19% variance) shows an eutrophication gradient linked to MC-LR releases, limnological variables, nutrient dynamics and trophic state (i.e., Chl-a, turbidity, algal density, MC-LR, TSI and TN, TOC and TP), while DO and evidently decreases with increase in turbidity, Chl-a, algal density, TSI and nutrients surge (Table 4, Figure 5). PC2 (16.27% variance) presents a secondary physicochemical gradient, controlled by pH and temperature, and specifying their potential role in moderating TSI, nutrient dynamics and MC-LR release. The statistical interpretations the variables were interdependent and tend to transform simultaneously as a response to shifting in the climate regime, human interventions, and unspecified environmental flows.

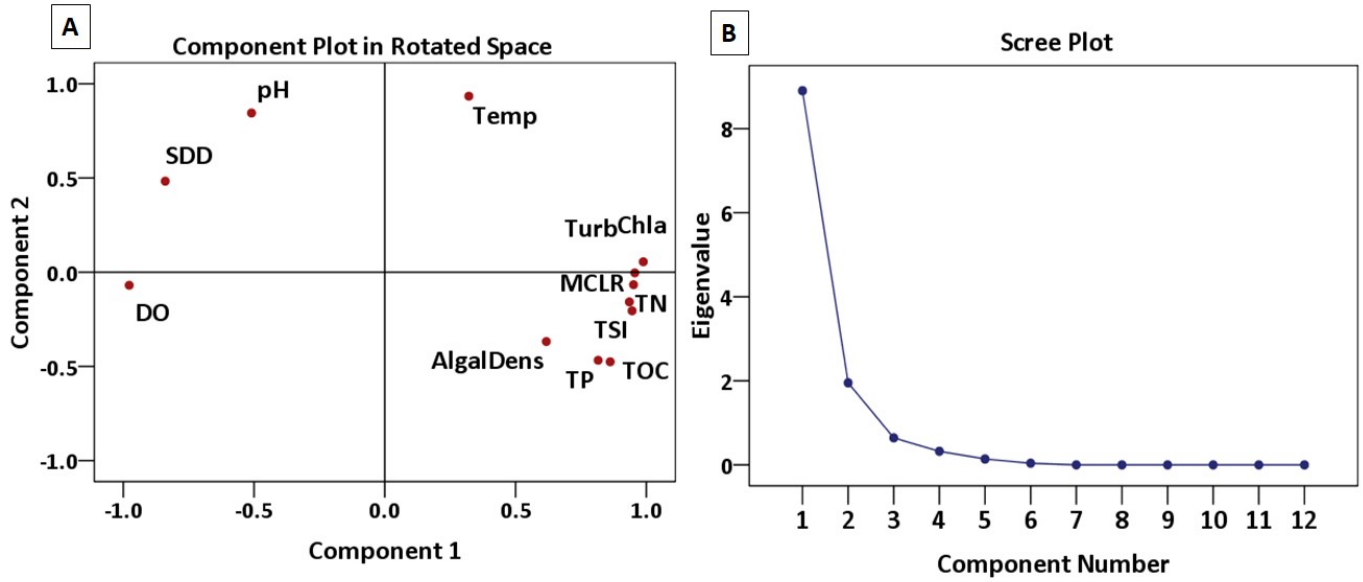

Figure 5. (**A**) Rotated Principal Component PC plots showing visual association between limnological variables and biological variables under similar environmental influence; (**B**) Scree plot showing prominent 3 PCs for the limnological and biological variables (PC1 = 74.19%, PC2 = 16.27%, PC3 = 5.36% and remaining PCs = upto 4.19% variance).

5. Conclusions

This investigation highlights the vulnerability of shallow lake eutrophication and cyanotoxin (MC-LR) contamination. MC-LR was detected and quantified across all sites in the north bank plains of Brahmaputra valley, Tezpur, Assam. The lakes were eutrophic to hypereutrophic exhibiting high MC-LR levels i.e., 1.08 ppb to 19.42 ppb (ELISA method) and 1.12 ppb to 3.46 ppb (HPLC method), and exceeded the WHO permissible limit for freshwater (1.0 ppb). Nutrient enrichment, as indicated by elevated TOC, TP, and TN levels in a few lakes, demonstrated a strong correlation with MC-LR and TSI. The investigation was exploratory in nature and showed the interdependency of the limnological variables, nutrients, MC-LR and trophic status; MC-LR strongly correlated with Chl-a, turbidity and CNP while exhibiting an inverse relationship with DO and SDD indicating the key role of limnological and biological variables in promoting algal blooms and release of algal toxins. *Microcystis* sp., *Dolichopermum* sp., and *Nostoc* sp., may have potentially released MC-LR alongside increasing algal blooms and lake water turbidity. The outcomes of this investigation emphasize a tenacious need for routine cyanotoxin monitoring, nutrient management, and sustainable remediation technique. Since there is limited epidemiological data in in the north bank plains of Brahmaputra valley, this study advocates for immediate intervention to mitigate cyanotoxin risks with implications for global water management strategies.

Author Contributions

N.G.: Conceptualization, review, supervision, writing; G.P.: Validation, supervision, review of original draft; P.B.: Grammar and language check and preparation of maps; B.P.: Investigation, field work, data analysis, writing.

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Conflicts of Interest

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.

Use of AI and AI-Assisted Technologies

The authors have checked the grammar and language in an AI tool.

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