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Phytoplankton community structure in the dry zone reservoirs of Sri Lanka at the declining phase of the tropical monsoon

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Abstract

We studied the phytoplankton community structure and their relationship to the physicochemical properties of water by selecting five major freshwater reservoirs; Bandagiriya, Kattakaduwa, Lunugamvehera, Ridiyagama, and Tissa in the Southern dry zone of Sri Lanka during the declining phase of tropical monsoon rainfall (December 2019 to January 2020). Fifty-seven species, dominated by Cyanophyta (63%), followed by Chlorophyta, Bacillariophyta, and Euglenophyta were identified morphologically and through DNA sequencing. The majority of Cyanophyta were filamentous forms (62%) with approximately 88% being cyanotoxin producing species. The species composition of phytoplankton communities is reservoir specific. *Microcystis* was dominant in Lunugamvehera while the diatom *Melosira* was dominant in Ridiyagama, Kattakaduwa and Tissa. The lowest phytoplankton density and diversity were observed in Bandagiriya mainly due to high turbidity and total suspended solids which interfere with light penetration through the water column. Physicochemical properties of water were significantly different among reservoirs, leading to reservoir specific correlations between phytoplankton density and physicochemical properties of water which might have been driven by the inter-correlative effects of biotic and abiotic factors at the time of sampling. Therefore, the interactive effects might be responsible for the observed variations in phytoplankton community composition. Thus, the present study provides important information on the phytoplankton community structure at the onset of successional episodes in five tropical freshwater reservoirs in relation to their spatial variations in hydrological regimes and physicochemical properties. Such data would provide essential information for planning and implementation of reliable and efficient strategies for monitoring, sampling, forecasting, and managing algal blooms.

Received: 5 March 2022

Accepted: 12 May 2024

Keywords:

Environmental factors;
Freshwater reservoir; Monsoon;
Phytoplankton community;
Species composition

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1. Introduction

Phytoplanktons are one of the most important biotic elements of aquatic ecosystems and are particularly important for maintaining ecological integrity of those systems. Their community structure is a function of environmental factors such as nutrient availability, light intensity, pH, temperature, and grazing pressure

(Furusato et al., 2004; Muhetaer, 2020). Nutrient availability, specifically nitrogen and phosphorous, are the most important elements for their growth and reproduction. Compared to nitrogen, phosphorous critically limits phytoplankton growth in freshwater (Abell et al., 2010 & Xu et al., 2015). In open waters, phytoplankton exhibit a patchy distribution in parallel to the variation in nutrient concentration, degree of stratification, mixing, and wind direction (Huisman &



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Hulot 2005). Further, their community structure is also influenced by biotic factors such as grazing pressure, predation and competitive exclusion (Ting et al., 2006; Rzymiski et al., 2014; Shao et al., 2013).

Sri Lankan aquatic ecosystems provide ideal habitats for excessive growth of phytoplankton due to the presence of favorable conditions such as high light intensity, warm temperatures, and nutrients throughout the year. As a result, algal blooms are frequently formed. During the past few years, there were some observations on algal blooms, fish kills and sharp decline in fish harvest particularly in the dry zone which were linked to the extensive agricultural practices and associated land use change (Manage & Piyasiri 1999; Jayatissa et al., 2006 and). The phytoplankton community structure in the majority of Sri Lankan reservoirs are said to be determined by the combination of monsoon driven rainfall patterns and the variation in concentrations of the major nutrients, nitrogen, and phosphorus (Silva et al., 2013 and Yatigammana et al., 2013). Often, high concentrations of nutrients are accumulated during the wet season. In some reservoirs, site-specific hydrological regimes and physicochemical properties are thought to contribute to location specific succession and development of phytoplankton communities (Silva et al., 2013). The present study was conducted in the months of December 2019 and January 2020 which coincides with the North-east monsoon (NEM) in the Southern dry zone when the annual rainfall exceeds 1750 mm. The rainfall data over the past five years in the Southern dry zone districts showed that it peaked in October-November (second inter-monsoon; SIM) and declined from December to February (NEM) followed by slight increase from March to April (first inter-monsoon; FIM) followed by a decline from May to September until the South-west monsoon (SWM). During the peak rainfall period, the rate of flushing is often high bringing more nutrients to the reservoirs providing favorable conditions for phytoplankton growth during the SIM period (Yatigammana et al., 2013). However, often a considerable amount of phytoplankton biomass is lost from reservoirs due to the spillage of water at SIM resulting in the disappearance of blooms. Therefore, December-January (NEM) is expected to be the period that supports the onset of phytoplankton communities in the Southern dry zone reservoirs. Understanding the phytoplankton community structure at the onset of its community is important for accurate forecasting and timely management of algal blooms.

Algal blooms reduce dissolved oxygen in water, alter odour and taste of drinking water, whilst also reducing the aesthetic and recreational values of these waterbodies. Further, algal blooms negatively affect the

integrity of the entire aquatic ecosystem, physically interrupting water purification and blocking waterways resulting in an array of environmental, ecological and socioeconomic issues and consequences. Some of the algal blooms are considered as harmful algal blooms (HABs) since they produce cyanotoxins with strong cytotoxic, hepatotoxic and neurotoxic potential. These toxins are known to cause serious health complications such as liver and kidney damage, acute pneumonia, acute dermatitis, tumors and sometimes respiratory paralysis, leading to death (Humpage & Cunliffe, 2021). The majority of bloom-forming cyanobacteria such as *Anabaena*, *Aphanizomenon*, *Oscillatoria*, *Microcystis* and *Nodularia* produce cyanotoxins. Cyanotoxin incidence is primarily associated with the presence of HABs (Carmichael, 2008). In Sri Lanka, several outbreaks of cyanobacterial blooms have been reported in freshwater reservoirs such as in the Kandy Lake (Silva, 2003, Kangara et al., 2007), Beira Lake (Karunaratne and Nanayakkara, 2016, Kamaladasa and Jayatunga, 2007) and some major irrigational and drinking water reservoirs in the dry zone of the country which were often associated with large scale fish kills. All such investigated cases revealed that, the algal blooms primarily consisted of cyanobacteria such as *Microcystis* (Silva, 2003, Idroos et al., 2013, Silva, 2007).

Our literature survey revealed that information on the phytoplankton community structure at the onset of favorable conditions for developing algal blooms and its relationship to environmental conditions with special reference to Sri Lankan freshwater reservoirs are lacking. Therefore, the present study was conducted to determine the phytoplankton community structure and its relationship to the physicochemical nature of five selected dry zone reservoirs in the southern part of Sri Lanka, at the declining phase of the tropical monsoon. Since forecasting and managing algal blooms either at regional or national scale have to date, had little success in Sri Lanka, information generated from this study would be useful not only for managing and forecasting of outbreaks of algal blooms, but also to minimize potential human health risks of cyanotoxins.

2. Materials and Methods

2.1 Study area

Five multipurpose reservoirs located in the Southern dry zone of Sri Lanka (Lunugamvehera, Tissa, Bandagiriya, Kattakaduwa, and Ridiyagama) were selected for this study (Fig. 1, Table 1). Water samples from the reservoirs of Lunugamvehera and Tissa were collected in January 2020 and from Bandagiriya, Kattakaduwa and Ridiyagama in December 2019.

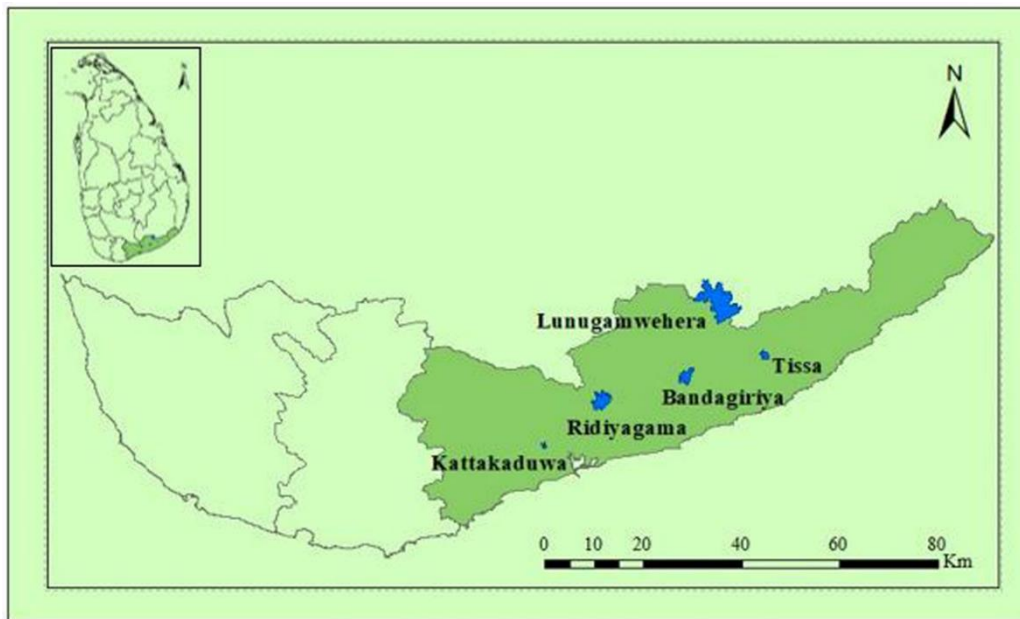


Fig.1 Locations of the five selected dry zone reservoirs

Table 1: Characteristics of five dry zone reservoirs selected for the study.

Reservoir	Location	Major input	Area at full supply level (km ²)	Volume (x 10 ⁶ m ³)
Lunugamvehere	6.385564° N, 81.200852° E	Kirindi Oya	30.32	225.09
Tissa	6.290170 °N, 81.286612° E	Kirindi Oya	2.34	4.32
Bandagiriya	6.247768°N 81.139834°E	Malala Oya	4.5	11.13
Kattakaduwa	6.122894°N 80.875738° E	Chandrika and Muruthawela Wewa	0.80	0.83
Ridiyagama	6.203077°N 80.983253°E	Walawa river	8.8	3.32

2.2 Sampling methods

Water samples were collected on clear sunny days. From each reservoir, sub-surface water samples (~5-10 cm below the surface) were collected from 25-30 sampling sites representing the entire water body. For each sampling point, 2 samples with 3 replicates were collected for the analysis of NO₃⁻-N and PO₄³⁻-P. For the collection of phytoplankton and estimation of chlorophyll-*a*, 50 L of sub-surface water samples were filtered through a 30 µm plankton net (Hydro-Bios, Germany). Filtered samples were collected into vials and brought to the laboratory under cool-dark conditions following the guidelines set out by the United States Environmental Protection Agency (USEPA, 2013).

2.3 Analytical methods

Seven water quality parameters were measured *in-situ* in triplicate using a multi-parameter water quality meter (HI9829-Hanna Instruments, Romania) at the same sites where water samples were collected for laboratory

analyses. The measured parameters were temperature (°C), pH, oxidation-reduction potential (ORP, mV), electrical conductivity (EC, µS/cm), total dissolved solids (TDS, ppm), salinity (psu), dissolved oxygen (ppm) and turbidity (FNU). Water transparency was estimated using the Secchi disk transparency method. The concentrations of NO₃⁻-N (ppm) was determined using a nitrate probe (HI7609829-12 Hanna Instruments, Romania). Prior to use, the probe was calibrated using 10 and 100 ppm KNO₃ calibrating solutions supplied by the manufacturer.

The concentrations of PO₄³⁻-P (ppm) in water samples were determined using the PhosVer3 Phosphate Reagent Powder Pillows (Hatch, USA) having a 0.00 to 2.50 ppm PO₄³⁻ detection range, following manufacture's guidelines. The analytical method was equivalent to the ascorbic acid method of the USEPA protocol 365.2. Absorbance was measured at 881 nm using a UV/visible

spectrophotometer (Genesys 150, Thermo-Scientific, USA). The PO_4^{3-} concentrations were estimated using a calibration curve prepared using a known concentration series of KH_2PO_4 .

Chlorophyll-*a* concentration was determined following the method of Arnon (1949). Briefly, water samples collected were filtered through the 30 μm plankton net in the field and centrifuged at 5000 rpm for 10 minutes. The pellets were resuspended in 10 mL of 80% (v/v) acetone and was kept in dark at 4 °C for 24 h. Extraction of chlorophyll into acetone was facilitated by intermittent shaking during the incubation period. After incubation, the chlorophyll extract was centrifuged at 5000 rpm for 20 minutes. The absorbance of the cleared supernatant was measured at 645 nm and 663 nm using the UV/Visible spectrophotometer (Genesys 150, Thermo-Scientific, USA). The chlorophyll-*a* concentration was calculated following the method described by Arnon (1949).

2.4 Identification and enumeration of phytoplankton

Water samples filtered through a 30 μm plankton net (Hydro-bios, Germany) were used for the identification and enumeration of phytoplankton. Samples were observed using a trinocular bright field microscope equipped with a digital camera (Nikon, Japan). Cyanobacteria, diatoms and green algae were identified up to the genus or species level using morphological characters of colonies and filaments with the aid of standard identification keys (Baker et al., 2012; Nienaber & Steinitz-Kannan, 2018).

Cyanobacteria that could not be identified using morphological features were confirmed by through DNA analysis. The specific region of the 16S rRNA gene of the cyanobacteria was amplified by the polymerase chain reaction (PCR) and subjected to subsequent DNA sequence analysis of the amplicons. Briefly, axenic cultures of cyanobacteria were established by growing and repeated subculturing in BG11 medium at 26 °C, 12h/12h: day/night. Genomic DNA was extracted from approximately 100 mg of cell pellets following the TRIzol® (Invitrogen, USA) method. The primers used were, CYA106F (5'-CGGACGGGTGAGTAACGCGTG-3') and CYA359F (5'-GGGGAATYTTCCGCAATGGG-3') forward primers and CYA781R(a) (5'-GACTACTGGGGTATCTAATCCCAT-3') and CYA781R(b) (5'-GACTACAGGGGTATCTAATCCCTTT-3') reverse primers. The resulting amplicons were sequenced by Macrogen Inc., South Korea. Sequencing data were analyzed by the Geneious Prime 2021.R.11 software and were identified based on the NCBI/BLAST similarity

search. DNA sequences were submitted to the NCBI GenBank.

Phytoplankton cell density was determined following the method described in Intergovernmental Oceanographic Commission (IOC) Manuals and Guides No.55 (LeGresley & McDermott, 2010) using a Neubauer haemocytometer (Marienfeld, Germany). The relative density (proportion) of each phytoplankton calculated was calculated using the equation by Gusmawati and Deswati (2018) given below.

$$\text{Relative density (proportion)} = \left(\frac{\text{Number of phytoplankton in one species in unit volume}}{\text{Total number of phytoplankton in unit volume}} \right) \times 100\%$$

2.5 Statistical analysis

Data were statistically analyzed using MINITAB version 18. Prior to the statistical analysis, data were checked for normality. Pearson product correlation coefficient (r) was calculated to determine the relationship between water quality parameters and phytoplankton cell density. One-way analysis of variance (one-way ANOVA) followed by the Tukey's HSD test were used to examine significant differences between the reservoirs. The Principal Component Analysis (PCA) was employed to explore the internal structure inter-correlative effects of measured water quality parameters. The statistical significance was defined at $P < 0.05$ for all analyses.

3. Results

3.1 Variation of aquatic environmental factors

The variation of all environmental parameters among the five reservoirs were statistically significant (Table 2). The Tukey's HSD test revealed that the Bandagiriya reservoir differed from the others based on seven parameters, namely, DO, EC, salinity, turbidity, phosphate, nitrogen, and chlorophyll-*a*. The high concentrations of phosphate and nitrogen in the Bandagiriya reservoir had no positive effect on the growth of phytoplankton.

Other than phosphate and nitrogen, EC, TDS and turbidity had relatively higher values in the Bandagiriya reservoir compared to the others. Apart from the Bandagiriya reservoir, the parameters DO, EC, salinity, turbidity, phosphate, nitrogen and chlorophyll-*a* in the other four were categorized into several inter-related groups. Additionally, the Secchi disk transparency and ORP of the Kattakaduwa reservoir were categorized into separate groups and the pH, TDS and temperature were also categorized in to several inter-related groups.

Table 2: The spatial variation of environmental parameters in Lunugamvehera (January 2020), Tissa (January 2020), Bandagiriya (December 2019), Kattakaduwa (December 2019) and Ridiyagama (December 2019) reservoirs. (Values indicate the mean $\pm$ standard deviation).

Parameter	Lunugamvehera	Tissa	Bandagiriya	Kattakaduwa	Ridiyagama
DO (ppm)	8.51 $\pm$ 0.14 ^{b,c}	7.89 $\pm$ 0.52 ^{b,c}	12.49 $\pm$ 0.95 ^a	7.59 $\pm$ 1.66 ^c	9.16 $\pm$ 1.88 ^b
EC (μ S/cm)	319.76 $\pm$ 8.89 ^d	664.00 $\pm$ 28.45 ^{b,c}	1014.56 $\pm$ 13.73 ^a	442.36 $\pm$ 9.12 ^b	375.24 $\pm$ 7.38 ^{c,d}
Salinity (psu)	0.1500 $\pm$ 0.0070 ^d	0.18 $\pm$ 0.09 ^{b,c}	0.49 $\pm$ 0.01 ^a	0.21 $\pm$ 0.01 ^b	0.17 $\pm$ 0.01 ^{c,d}
Turbidity (FNU)	3.94 $\pm$ 0.84 ^d	2.40 $\pm$ 0.31 ^d	83.09 $\pm$ 10.04 ^a	13.76 $\pm$ 2.81 ^c	32.38 $\pm$ 5.45 ^b
Phosphate-P (ppm)	0.0072 $\pm$ 0.0014 ^b	0.0065 $\pm$ 0.0015 ^b	0.0259 $\pm$ 0.0203 ^a	0.0027 $\pm$ 0.0023 ^b	0.0055 $\pm$ 0.0056 ^b
Nitrate-N (ppm)	0.11 $\pm$ 0.01 ^d	0.23 $\pm$ 0.01 ^c	1.10 $\pm$ 0.09 ^a	0.46 $\pm$ 0.08 ^b	0.45 $\pm$ 0.09 ^b
Chlorophyll- <i>a</i> (ppb)	18.85 $\pm$ 4.92 ^a	18.41 $\pm$ 2.61 ^{a,b}	1.68 $\pm$ 0.92 ^c	16.08 $\pm$ 6.02 ^{a,b}	14.44 $\pm$ 4.69 ^b
Secchi disk transparency (m)	1.44 $\pm$ 0.40 ^a	1.57 $\pm$ 0.48 ^a	0.21 $\pm$ 0.02 ^c	0.72 $\pm$ 0.09 ^b	0.35 $\pm$ 0.05 ^c
ORP (mV)	229.23 $\pm$ 12.41 ^c	282.20 $\pm$ 39.76 ^a	214.03 $\pm$ 9.95 ^d	252.59 $\pm$ 12.35 ^b	224.97 $\pm$ 10.70 ^{c,d}
pH	8.52 $\pm$ 0.16 ^b	7.44 $\pm$ 0.89 ^c	8.70 $\pm$ 0.03 ^{a,b}	7.59 $\pm$ 0.37 ^c	8.75 $\pm$ 0.11 ^a
TDS (ppm)	160.03 $\pm$ 0.70 ^c	232.00 $\pm$ 14.07 ^{a,b}	507.19 $\pm$ 6.99 ^a	221.17 $\pm$ 4.85 ^a	187.51 $\pm$ 3.74 ^{b,c}
Temp. (C)	30.62 $\pm$ 0.48 ^b	29.58 $\pm$ 0.61 ^{c,d}	29.75 $\pm$ 0.54 ^c	29.20 $\pm$ 0.35 ^d	31.46 $\pm$ 0.77 ^a

Note: Different letters represent significant differences among environmental parameters between the five reservoirs (Tukey's HSD test: $P < 0.05$).

Table 3. A list of phytoplankton species and their relative density (proportions) (%) identified in four reservoirs in the Southern dry zone of Sri Lanka. The Bandagiriya reservoir was excluded from the list due to the very low phytoplankton count.

Generic name	Relative density (%)			
	Lunugamvehera	Ridiyagama	Tissa	Kattakaduwa
Cyanophyta				
1. <i>Anabaenopsis</i> *	-	3	-	-
2. <i>Aphanocapsa</i> *	-	2	-	-
3. <i>Aphanizomenon</i> *	-	11	-	-
4. <i>Chroococcus</i>	-	6	2	-
5. <i>Cylindrospermopsis</i> *	-	8	-	-
6. <i>Dolichospermum</i> *	-	-	-	-
7. <i>Geitlerinema</i> *	2	-	-	-
8. <i>Gloeocapsa</i>	3	5	-	-
9. <i>Leptolyngbya</i> *	5	3	-	9
10. <i>Microcystis</i> *	48	4	6	-
11. <i>Oscillatoria</i> *	5	3	2	9
12. <i>Phormidium</i> *	5	3	-	8
13. <i>Planktolyngbya</i> *	-	6	-	-
14. <i>Pseudanabaena</i> *	5	4	2	7
15. <i>Synechocystis</i> *	-	1	-	-
16. <i>Synechococcus</i> *	-	5	-	-
Chlorophyta				
17. <i>Ankistrodesmus</i>	-	2	2	-
18. <i>Pandorina</i>	3	3	-	-

19. <i>Pediastrum</i>	-	4	8	-
Euglenozoa	-	-	-	-
20. <i>Euglena</i>	-	-	5	-
21. <i>Phacus</i>	-	-	2	
Bacillariophyta				
22. <i>Aulacoseira</i>	4	7		23
23. <i>Cymbella</i>	1	-		-
24. <i>Fragilaria</i>	3	-	7	-
25. <i>Melosira</i>	11	16	50	44
26. <i>Navicula</i>	3	-		-
27. <i>Nitzschia</i>	2	4	6	-
28. <i>Pleurosigma</i>	-	-	5	-
29. <i>Rhizosolenia</i>		-	3	

Note. * = Toxin-producing cyanobacteria

3.2 Phytoplankton community composition

The highest number of cyanobacteria belonging to 13 and 10 genera were identified from Lunugamvehera and Ridiyagama reservoirs respectively. *Microcystis* was dominant in the Lunugamvehera reservoir with an average density of 1.83×10^7 cell/mL (Table 3, Fig.2-a). Among the filamentous species, *Geitlerinema* was found only in the Lunugamvehera reservoir (Fig.2-l). *Geitlerinema* is considered as a rare species in Sri Lanka (Hossain et al., 2020) and was recorded for the first time through this study in Southern dry zone freshwater bodies. Although *Geitlerinema* was less dominant ($\leq 2\%$ relative density), it was seen to dominate in cultures grown from reservoir water samples in the BG11 medium (Fig.2-l). In addition, several other filamentous types were also grown in cultures. Their identities as confirmed by DNA sequencing were *Leptolyngbya* (MW288942, MW288946), *Phormidium* (MW288941, MW288943) and *Pseudanabaena* (MW288940, MW288940) (Fig. 3). The identity of *Geitlerinema* was also confirmed by DNA sequencing (MW288944).

A prominent thin surface film of greenish bloom was present along the shoreline of the Ridiyagama reservoir on the day of sampling while such visible blooms were absent in other four reservoirs (Fig. 3). A total of 57 phytoplankton species belonging to 4 phyla and 29 genera were identified from the Ridiyagama reservoir. Of these 35 species were cyanobacteria which represented approximately 63% of the total phytoplankton species identified in this study (Table 3). In the Ridiyagama reservoir, the most abundant phytoplankton species was a diatom, *Melosira* with an average density of 26.58 filaments/mL (Fig.2b). Among the cyanobacteria observed in the same reservoir, *Aphanizomenon* was the dominant species with a density of 8.58 filaments/mL. The filaments of *Aphanizomenon* were frequently observed as individual trichomes and less frequently as bundles comprising 2-4 trichomes

(Fig.2-f). The other cyanobacteria species *Anabaenopsis* (Fig. 2-g) and *Cylindrospermopsis* (Fig.2-j) had moderate densities of 4.95 and 3.79 filaments/mL, respectively. Among the five reservoirs studied, *Cylindrospermopsis* was only found in Ridiyagama. In addition, *Microcystis* (6.64 cells/mL), *Chroococcus* (9.96 cells/mL), *Leptolyngbya* (4.98 filaments/mL) and *Pseudanabaena* (6.64 filaments/mL) were less observed in some sampling sites.

Melosira was the dominant species in the Tissa reservoir with a density of 27.96 filaments/mL (Fig.2c). In addition, *Microcystis*, *Aphanizomenon* and *Oscillatoria* cyanobacterial genera were identified. The Bandagiriya reservoir had the lowest phytoplankton diversity and density observed in this study. This observation was further supported by the low chlorophyll-*a* content as compared to the other four reservoirs (Table 2). We could only observe a single filament of *Anabaenopsis* in the collected water samples. However, when water samples from the same reservoir were cultured in BG11 medium, we were able to recover four additional filamentous cyanobacteria species; *Leptolyngbya*, *Pseudanabaena*, *Oscillatoria* and *Phormidium*. In the Kattakaduwa reservoir, *Melosira* was the dominant species in all sampling sites with an average density of 39.18 filaments/mL (Fig.2-d). Among the cyanobacteria, only filamentous species including *Aphanizomenon*, *Oscillatoria*, *Leptolyngbya*, *Pseudanabaena*, and *Phormidium* were identified.

According to our overall observations, cyanobacteria dominated-phytoplankton community was found only in Lunugamvehera, whereas *Melosira*, a diatom dominated the community in the Kattakaduwa, Ridiyagama and Tissa reservoirs. Compared to solitary cells and colonies, filamentous cyanobacteria were generally more abundant in all reservoirs.

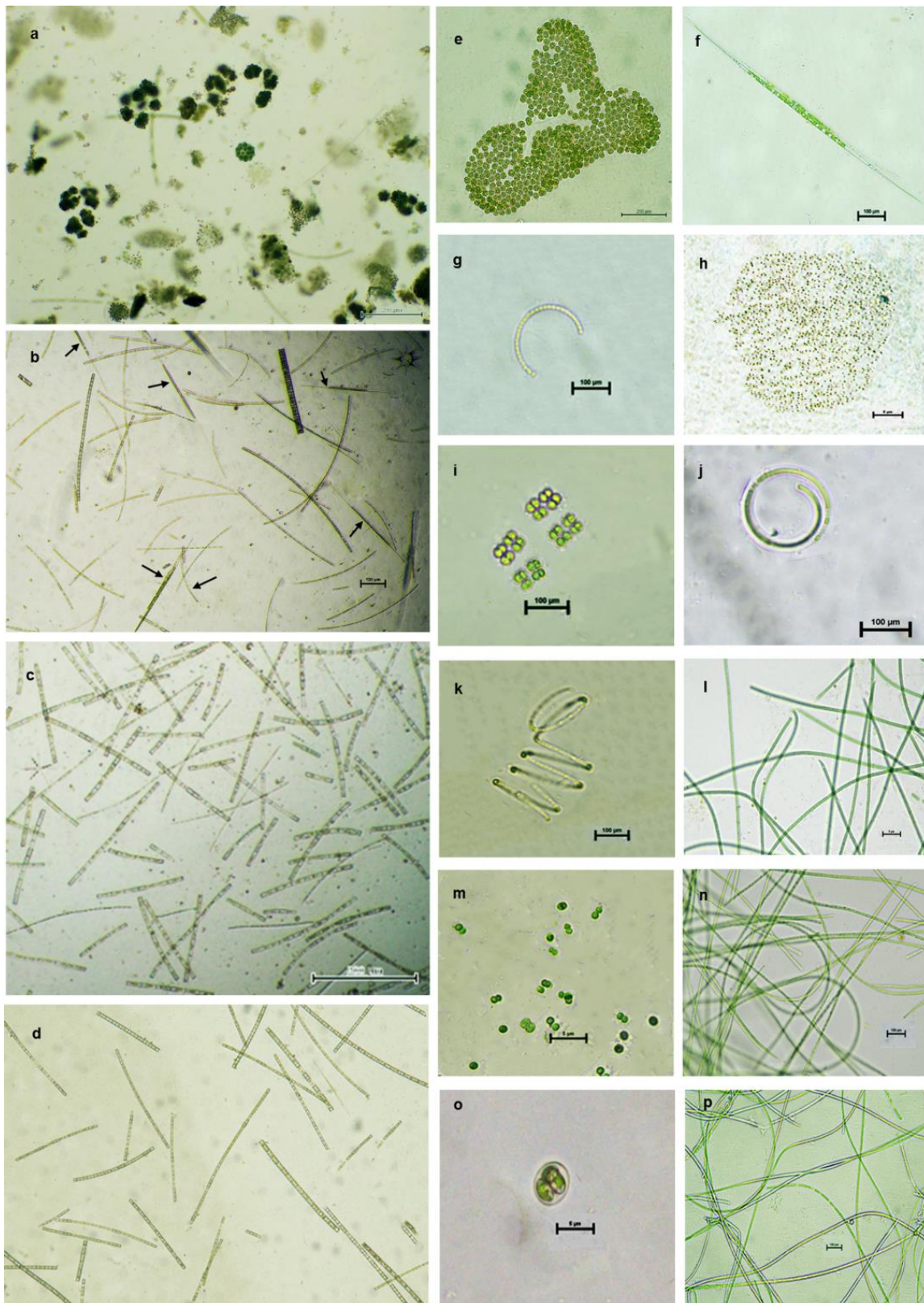


Fig.2 Dominant phytoplankton communities and members of Cyanophyta and Bacillariophyta identified from Lunugamvehera, Ridiyagama, Tissa and Kattakaduwa reservoirs. a: colonies of *Microcystis* dominant in Lunugamvehera, b: Filaments of *Aphanizomenon* (arrow) and *Melosira* dominant in Ridiyagama, c and d: Filaments of *Melosira* dominant in Tissa and Kattakaduwa reservoirs respectively, e: *Microcystis*, f: *Aphanizomenon*, g: *Anabaenopsis*, h: *Aphanocapsa*, i: *Chroococcus*, j: *Cylindrospermopsis*, k: *Planktolyngbya*, l: *Geitlerinema*, m: *Synechocystis*, n: *Oscillatoria*, o: *Gloeocapsa* and p: *Phormidium*



Fig.3 Algal blooms on the shoreline of the Ridiyagama reservoir observed in December 2019

3.3 Relationship between phytoplankton community and environmental factors

There were significant positive correlations between the phosphate concentration and phytoplankton density in the Lunugamvehera and Ridiyagama reservoirs (Table 4). This was further confirmed by the correlation between phosphate and chlorophyll-*a* concentrations observed in both reservoirs ($P < 0.05$, and $P < 0.001$, respectively). Further, in the Lunugamvehera reservoir, phosphate levels correlated positively ($P < 0.001$) with average cell density of *Microcystis* (dominant genus) while similar trends were observed in Ridiyagama for the two dominant species, *Melosira* and *Aphanizomenon* although the associations were not significant. All correlations observed between cell/filament density and nitrate levels were negative, and none of the reservoirs exhibited with no significant trends. The results of the Pearson correlation analysis further revealed that average phytoplankton density or the density of dominant species in Tissa and Kattakaduwa reservoirs were not significantly correlated with any environmental factors.

Interestingly, both nitrate and phosphate concentrations were high in the Bandagiriya reservoir compared to the other reservoirs but were not positively correlated with the phytoplankton cell densities (data not shown). Turbidity and TDS of the Bandagiriya reservoir were significantly higher than that of other reservoirs while the lowest Secchi disk transparency was also detected in this reservoir (Table 2). We observed a noticeable population of zooplankton in the Bandagiriya reservoir which comprised species belonging to the three genera, *Trichocerca*, *Pseudodiaptomus* and *Heliodiaptomus* which were equally dominant in all sampling sites and

had an average density of between 5 – 17.5 individuals/mL.

According to the principal component analysis, EC, TDS and salinity in the majority of the reservoirs were categorized into a single group (Fig. 5). Strong and statistically significant ($P > 0.05$) positive correlations ($r > 0.7$) between these parameters were obtained for all reservoirs except Lunugamvehera (Fig. 5-a). Although the correlations observed for Lunugamvehera, were positive, none of those relationships were significant ($P > 0.05$). The TDS appeared to have a negative influence on the dominant phytoplankton community in Lunugamvehera, Ridiyagama and Tissa reservoirs.

4. Discussion

4.1 Relationship between abiotic aquatic environmental factors and phytoplankton community

Several abiotic and biotic factors affect community structure of phytoplankton in aquatic ecosystems. The present study found significant variations in several abiotic environmental factors among the five reservoirs. The Bandagiriya reservoir had distinct environmental conditions compared to other four reservoirs. The high phosphate and nitrogen concentrations in the Bandagiriya reservoir seemed to have no positive impact on the growth and reproduction of phytoplankton because, phytoplankton density and chlorophyll-*a* concentration in the reservoir were fairly low (ten-fold lower than other four reservoirs). The nitrogen:phosphorus (N:P) ratio of the five reservoirs were in the range of 15 to 81. For Bandagiriya, the ratio was 42 suggesting that it is unlikely that the N:P ratio contributed to the low growth of the phytoplankton community in this reservoir. In contrast to our results, many previous research have found that phosphates and nitrates are the major nutrients that determine phytoplankton growth and reproduction in waterbodies (Niamien-Ebrottie J.E., 2015; Harris et al., 2016; Yatigammana & Perera, 2017; Shan et al., 2019). Hence, there must be other factors that determine phytoplankton growth in Bandagiriya. We think that turbidity and TDS which were high in Bandagiriya might have interfered with light penetration through the water column and consequently limited phytoplankton growth. TDS also appeared to have a negative influence on the dominant phytoplankton community in Lunugamvehera, Ridiyagama and Tissa reservoirs. The Secchi disk transparency was used as a proxy for light penetration. Light penetration is mostly dependent on the total suspended solids and organic detritus that increase turbidity in water (Cai et al., 1996).

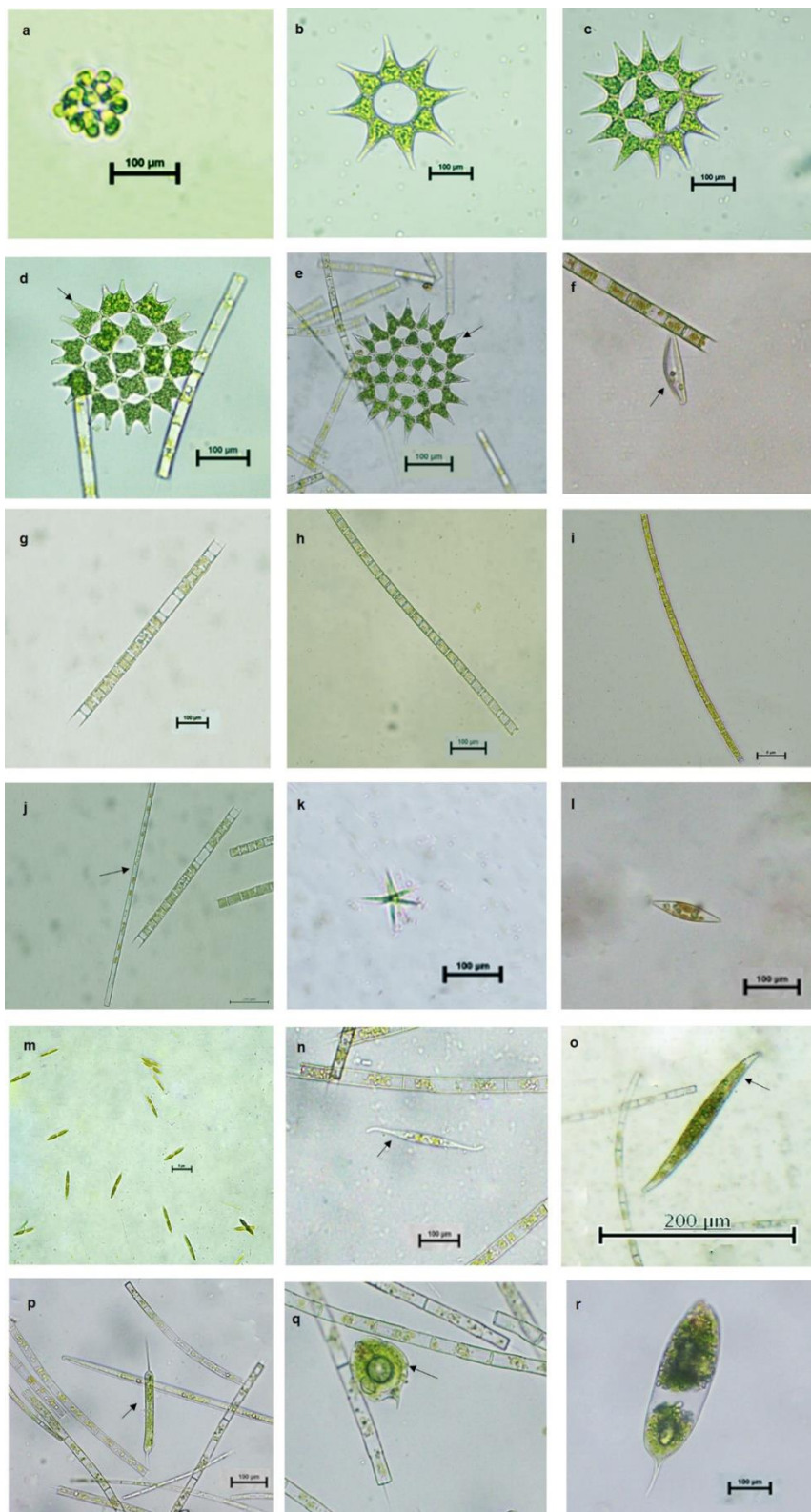
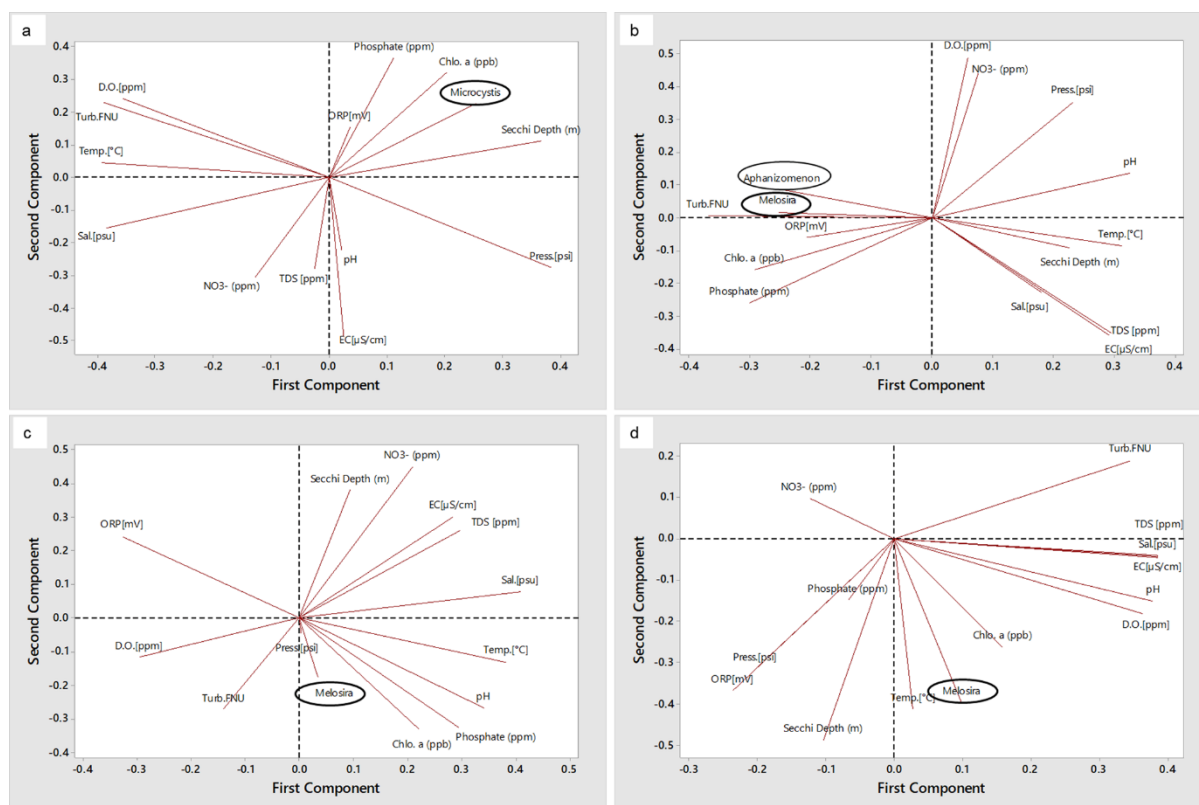


Fig.4 Members of Chlorophyta (a-e), Bacillariophyta (f-p) and Eluglenophyta (q-r) identified from Lunugamvehera, Tissa, Kattakadu and Ridiyagama reservoirs. a: *Pandorina*, b and c: *Pediastrum simplex*, d: *Pediastrum duplex*, e: *Pediastrum biwae*, f: *Cymbella*, g: *Melosira herzogii*, h: *Melosira ambigua*, i: *Aulacoseira granulate*, j: *Fragilaria* (synonym: *Synedra*), k: *Ankistrodesmus*, l: *Navicula*, m: *Nitzschia*, n: *Nitzschia reversa*, o: *Pleurosigma*, p: *Rhizosolenia*, q: *Phacus* and r: *Euglena*.

Table 4. Correlation coefficients (r) (Pearson correlation analysis) between the average cell/filament density of the dominant species of phytoplankton and critical environmental factors.

	Lunugamvehera	Ridiyagama	Tissa	Kattakaduwa
	<i>Microcystis</i>	<i>Aphanizomenon</i>	<i>Melosira</i>	<i>Melosira</i>
Phosphate	0.851 (P = 0.002)*	0.241 (P = 0.199)	0.514 (P = 0.004)*	0.101 (P = 0.812)
Chl-a	0.837 (P = 0.045)*	0.517 (P = 0.003)*	0.577 (P = 0.001)*	0.554 (P = 0.154)
Nitrate	-0.16 (P = 0.658)	-0.158 (P = 0.403)	-0.163 (P = 0.391)	-0.114 (P = 0.778)
Turbidity	-0.049 (P = 0.894)	0.258 (P = 0.169)	0.303 (P = 0.103)	0.03 (P = 0.994)
TDS	-0.167 (P = 0.645)	-0.39 (P = 0.033)*	-0.303 (P = 0.104)	-0.004 (P = 0.992)
Secchi disk transparency	0.164 (P = 0.65)	-0.034 (P = 0.857)	-0.12 (P = 0.527)	-0.105 (P = 0.804)
pH	0.091 (P = 0.803)	-0.293 (P = 0.117)	-0.36 (P = 0.05)*	0.061 (P = 0.886)
				<i>Melosira</i>
				0.184 (P = 0.368)
				0.503 (P = 0.009)*
				-0.108 (P = 0.598)
				0.053 (P = 0.796)
				0.219 (P = 0.282)
				0.424 (P = 0.031)
				0.31 (P = 0.123)

* = statistically significant (P < 0.05)

**Fig.5** Loading plots of the Principal Component Analyses of the four reservoirs showing the variability between aquatic environmental factors and the dominant phytoplankton community (circled) in, a: Lunugamvehera, b: Ridiyagama, c: Tissa and d: Kattakaduwa reservoirs

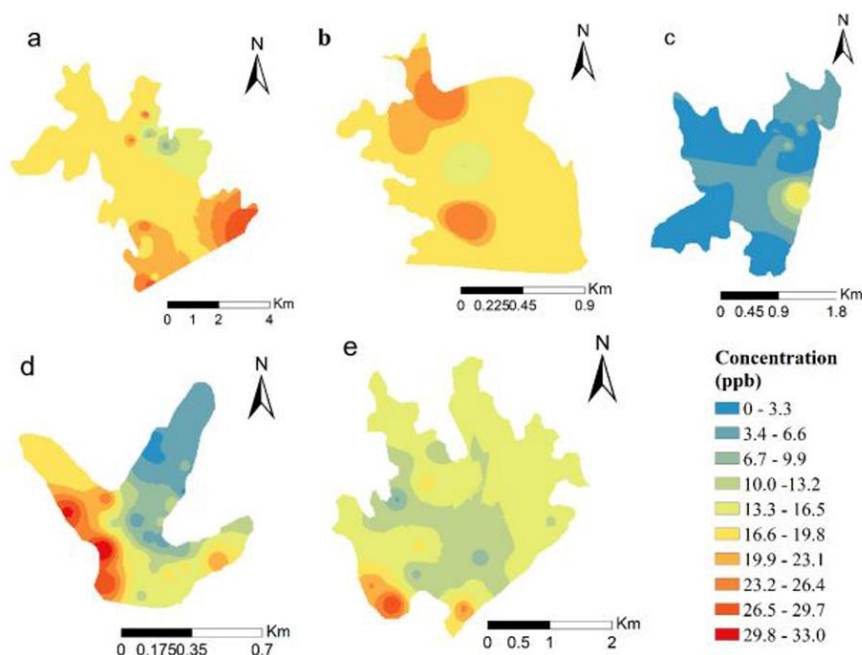


Fig.6 Spatial distribution of chlorophyll-*a* in a: Lunugamvehera, b: Tissa, c: Bandagiriya, d: Kattakaduwa and e: Ridiyagama reservoir.

Therefore, the effect of turbidity and TDS on the floating phytoplankton community is apparent. Further, grazing pressure exerted by the high population density of zooplankton may also in part, be responsible for the low phytoplankton community in the Bandagiriya reservoir.

All the observed zooplankton in Bandagiriya are comparatively larger zooplankton species, and their grazing on phytoplankton may have contributed to the reduction of the phytoplankton population (Vanni, 1987). Further, Bergquist et al., (1985) observed that *Diaptomus oregonensis* causes a decline in phytoplankton with greatest axial linear dimensions. However, this interaction is complex and it is decided by a combination of factors such as nutrient levels and temperature (Gusha, 2019). Silva et al., (2007) however argues that phytoplankton density and species dominance are not regulated by grazing pressure, but might result from water release from the euphotic zone.

Phosphate concentration appears to be the most important factor that determined the phytoplankton density in Lunugamvehera and Ridiyagama reservoirs. Since chlorophyll-*a* is the primary photosynthetic pigment in green algae and cyanobacteria, their abundance is indicated by the chlorophyll-*a* concentration. This feature has already been used as a proxy of cyanobacterial biomass in the literature (Lambou et al., 1983; Kasprzak et al., 2008; Lu et al., 2013). Therefore, according to this study, phosphates can be considered as one of the major driving forces of

phytoplankton growth, and consequently the increase in the chlorophyll-*a* contents. In addition, phosphate appeared to determine the dominant phytoplankton communities of *Microcystis*, and *Melosira* and *Aphanizomenon*, in Lunugamvehera and Ridiyagama, respectively.

The observed negative correlation between nitrate and chlorophyll-*a* in the studied reservoirs are not in agreement with findings of previous studies in which nitrate was identified as one of the major limiting factors that determine phytoplankton biomass (Cunha & Calijuri 2011; Trommer et al., 2020). Our data further revealed the absence of an association between a given environmental parameter and the total phytoplankton density or the density of the dominant species in both Tissa and Kattakaduwa reservoirs. This indicates that preferences may be affected by a combination of parameters (Sharma & Singh 2018). Although, temperature is considered as a limiting factor for phytoplankton growth in temperate regions (Hansson, 1996; Grover et al., 2006; Mesquita et al., 2020), this may not be the case of tropical countries.

In general, phytoplankton exhibit a patchy distribution in open waters and their distribution patterns are highly dynamic being affected by changes in wind-driven currents and the mobility of phytoplankton (Cyr, 2017). Spatial distribution of chlorophyll-*a* in all studied reservoirs are supported by this argument (Fig. 6). Maps showed comparatively higher chlorophyll-*a* levels in

isolated corners of reservoirs compared to locations located centrally, suggesting the effect of wind currents and nutrient input.

4.2 Phytoplankton community composition and implications for human and ecosystem health

The dominant phytoplankton community in multipurpose freshwater reservoirs determines the socioeconomic value of a particular reservoir. According to the present study, the dominant phytoplankton community observed in Ridiyagama, Tissa and Kattakaduwa reservoirs was *Melosira*. There is no documented evidence that suggests the potential of *Melosira* develop into blooms in Sri Lanka. However, such *Melosira* blooms have been reported in other countries (Miyajima, 1994; Katz et al., 2015). Nonetheless, phytoplankton communities dominated by cyanobacteria cause detrimental impacts on the ecosystem integrity, impacts recreation and imposes health risks to fish and humans due to the production of cyanotoxins, particularly when there are blooms (Smith & Schindler, 2009). Therefore, early detection of cyanobacterial blooms as well as environment factors conducive for bloom formation are important. Of the total cyanobacteria identified in this study, 88% species are known to produce cyanotoxins (Cheung et al., 2013; Rastogi et al., 2015).

At least one species of bloom forming cyanobacteria was identified from all five reservoirs. *Microcystis*, which is the most prevalent bloom forming cyanobacterial genus in tropical Asia (Mowe et al., 2015) was identified as the dominant species in Lunugamvehera and it was also present in Ridiyagama and Tissa reservoirs indicating the high potential for toxic bloom formation during the successional development of the plankton community. Microcystin produced by *Microcystis* are mainly hepatotoxic and neurotoxic to fish and humans (Niamien-Ebrottie et al., 2015).

In the Ridiyagama reservoir, other than *Microcystis*, several other cyanobacterial genera are known to have toxic bloom forming potential and these include *Anabaenopsis*, *Aphanizomenon*, *Cylindrospermopsis*, *Leptolyngbya* and *Pseudanabaena* (Ballot et al., 2005; Zhang, 2020; Humbert et al., 2010; Niamien-Ebrottie et al., 2015). Since *Aphanizomenon* was found to be dominant in sites where the bloom was visible, *Aphanizomenon* may be responsible for the bloom observed in the Ridiyagama reservoir. In Kattakaduwa and Lunugamvehera reservoirs, the potential toxic bloom forming cyanobacterial community mostly overlaps with that of Ridiyagama with the presence of *Aphanizomenon*, *Leptolyngbya*, *Oscillatoria*, *Pseudanabaena*, and *Phormidium* whereas *Oscillatoria* was also present in Tissa reservoir. *Oscillatoria* is known to produce anatoxin-a and microcystins

(Niamien-Ebrottie, 2015). Except *Microcystis* and *Cylindrospermopsis*, outbreaks of cyanobacterial blooms associated with other cyanobacterial genera identified from the studied reservoirs have not been recorded so far from freshwater bodies in Sri Lanka (Kulasooriya, 2017). However, the likelihood of an outbreak of these genera into toxic blooms cannot be ignored. As apparent from this study, cyanobacterial dominance or bloom formation in a waterbody is complex and may be driven by synergistic environmental factors (Hyenstrand, 1998; Dokulil & Teubner, 2000; Lu et al., 2013). Therefore, continuous monitoring of physicochemical properties and strict reduction in phosphate input are essential for the management of bloom forming phytoplankton in waterbodies in Southern Sri Lanka.

Conclusion

Physicochemical properties of five reservoirs were significantly different to one another leading to reservoir specific correlations between phytoplankton density and physicochemical properties. *Microcystis* was dominant in Lunugamvehera while *Melosira* was dominant in Ridiyagama, Kattakaduwa and Tissa. Of the cyanobacterial communities, 88% are known to produce cyanotoxins and majority of them are reported to form harmful cyanobacterial blooms in Sri Lanka and elsewhere. In the Lunugamvehera and Ridiyagama reservoirs, phosphate appears to be the most important abiotic factor influencing phytoplankton density, whereas in the Tissa and Kattakaduwa reservoirs, phytoplankton density did not correlate with any of the physicochemical parameters. We hypothesize that their prevalence may have been determined by combined effects of several parameters. In Bandagiriya, the high turbidity and TDS appear to be the reasons for having very low phytoplankton density. In conclusion, our data showed that the phytoplankton community composition and species dominance in the studied reservoirs were site specific and their dynamics may have been driven by specific environmental factors such as phosphate level, turbidity, TDS and their inter-correlative effects. Thus, this study provides important information on the phytoplankton community structure at their early stage in the development of a succession in tropical freshwater reservoirs in relation to their location-specific hydrological regimes and physicochemical properties. These successions may lead to the formation of algal blooms when the conditions become more favorable. Therefore, our data would support future decision making for monitoring, forecasting and managing of algal blooms.

5. Acknowledgments

This research was supported by the Accelerating Higher Education Expansion and Development (AHEAD) Operation of the Ministry of Higher Education, Sri

Lanka, funded by the World Bank under the grant number AHEAD/RA3/DOR/RUH/SCI/CYANO.

6. Conflict of Interests

The authors declare there is no conflict of interest.

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