

RESEARCH ARTICLE

Distribution of *Cylindrospermopsis raciborskii* (Cyanobacteria) in Sri Lanka

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Abstract: Sri Lanka is a tropical continental island which consists of 103 natural rivers and over ten thousand man-made lakes. Majority of these water resources are known to be contaminated with different types of toxigenic cyanobacteria making water unsuitable for human and animal consumption. *Cylindrospermopsis raciborskii*, a toxin producing tropical cyanobacterium, recently recorded at high abundances in lentic waters of the country. This species is highly adaptive and exhibit different morphotypes: straight, coiled and sigmoid-shaped trichomes under different environmental conditions. Distribution and abundance of the species is mainly depending on the environmental factors and therefore remedies can be proposed to keep the populations under control. This preliminary study was conducted to understand the prevalence of *C. raciborskii*, in Sri Lankan reservoirs. Sixty three reservoirs representing all climatic regions with varying environmental conditions were assessed using standard procedures. The results of the study reviewed that *C. raciborskii* is a dominant cyanobacterium species especially during the dry season in shallow urban reservoirs, terminal reservoirs in large cascades, reservoirs with low flushing rates and deep reservoirs with persistent water stratification. Morphological variations were common in urban and shallow reservoirs that maintain high water retention time. They commonly occur in coiled-shape. However, in eutrophic water bodies they are appeared to support other morphotypes: straight, sigmoid or tricomatous. Further, it was noticed that the cell size of *C. raciborskii* is exceptionally large (width 3.5 μm and length 7 μm) in a high altitudinal wet-zone reservoir that usually maintains temperature range of 10°C - 20°C throughout the year. The results showed that *C. raciborskii* is widely distributed all over the country and the presence of several morphotypes signifies its potential for rapid distribution.

Keywords: Cyanobacteria, cyanotoxin, *Cylindrospermopsis raciborskii*, reservoir, seasons.

INTRODUCTION

Water heritage of Sri Lanka consist of 103 major river basins radiating from the hill country to the lowlands and over ten thousand operational reservoirs (Fernando, 1993). Historical evidence suggests that the origin of many of these reservoirs especially in the dry zone, date back to pre-Christian era during the period the country known to be self sufficient in agriculture (Arumugam, 1969). In contrast, reservoirs constructed during the recent history are used mainly for hydroelectric power generation, irrigation and domestic use, though few reservoirs are used exclusively for drinking. As a result of extensive reservoir construction since ancient time, over 4% of the total 65,000 km² of terrestrial land of Sri Lanka, is now covered with lentic water (Fernando, 1993). Recent studies indicate that these reservoirs are under the threat of pollution which can be directly attributed to human activities (Yatigammana and Cumming, 2017). Over 21 million of human population which distributed unevenly in four major climatic regions of Sri Lanka increase at the rate of 0.8 per year (Census and statistics Sri Lanka, 2016). A larger portion of this population is restricted to urban areas and the others inhabit in remote regions where conventional agricultural practices are common. According to the published reports of the Department of Census and Statistics Sri Lanka and the World Bank indicate, that 42% of the land covered with agricultural fields and experience intense use of agrochemicals. As a result, top soil contains excessive amounts of nutrients, especially Nitrogen and Phosphorus. Although Reservoirs located within the densely populated cities appear to experience eutrophic or hyper-eutrophic conditions, agriculture related cultural eutrophication is now becoming a common problem in less populated areas where

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conventional agriculture is the common livelihood (Yatigammana and Cumming, 2017). These agricultural practices in reservoir catchments and inadequate land use management appear to be the main reasons affecting the reservoir health. Further, global human activities which are known to affect the climate also could have negative impact on water quality in Sri Lanka.

The enrichment and accumulation of nutrients within a water column facilitate invasion and colonization of algae, especially toxigenic species with wider tolerance to various environmental conditions. These toxins are mostly having an allelopathic effect on many native species causing reduction in the diversity of plankton communities (Yatigammana *et al.*, 2011; Jayatissa *et al.*, 2006). Although these toxigenic algal blooms initially appeared in small water bodies located in urban areas, recent studies indicate high abundance of toxigenic cyanobacteria in large reservoirs in remote regions (Perera *et al.*, 2012). *Cylindrospermopsis raciborskii* (Woloszynska, 1912; Seenaya & Subba Raju, 1972) first identified from India as a tropical cyanobacterial species and now it is a globally distributed cyanobacterium which has records from Sri Lanka as early as in 1952 from the Dry Zone reservoirs (Rott, 1983). Now this cyanobacterium has started to colonize in reservoirs in the Highland Wet Zone where the average day temperature remain in 15-20°C (Perera and Yatigammana, 2010). As this species is highly adaptive with wider tolerance to several limnological variables such as pH, temperature and turbidity, they possess the ability to compete with others in the same trophic level. Especially physiological and ecological adaptations and fitness help increase the floatability, allowing them to remain in the photic zone where sunlight is not limited (Padisák, 1997). In addition, high capacity to absorb ammonia and phosphorus shows an additional advantage they possess than the other competing species of the same trophic level (Padisák, 1997). Further, the production of resistant akinetes (resting bodies) helps disperse this species through aquatic birds even into isolated water bodies where usually the invasion of such organisms is limited. Sri Lanka in which the larger portion of land covers with water, provide optimum environmental conditions for such cyanobacteria species. Therefore, rapid dispersal is unavoidable even to small isolated reservoirs that use water solely for drinking.

Unlike other toxigenic cyanobacteria, *C. raciborskii* produce cyanotoxins such as neprotoxin, neurotoxins (carbamate -type alkaloid) and hepatotoxins (cyclicguanidinic alkaloid). These toxins could damage human organs, such as liver, kidney, heart, lungs and even gastric mucosa through the inhibition of protein synthesis (Falconer and Humpage, 2005). Neurotoxins affect sodium channel in nerve cells causing paralysis, hypertension and finally respiratory failure and nephrotoxin damage kidneys (Piyathilaka *et al.*, 2016)

Presence of algal blooms could be controlled using the techniques of biomanipulation, through which filtering of phytoplankton is promoted by aquatic organisms such as *Daphnia* species (Shapiro, 1995). However, application of such techniques to control *C. raciborskii* could also be challenging due to the resistance to such herbivory, a character that could be attributed to toxigenic nature and/or unpalatability.

Although few studies were done on *C. raciborskii*, the prevalence, auto ecology and environmental preferences of the species is lacking. Since the accessibility to safe drinking water of 21 million people is a primary task in safeguarding the human health and the recent increase of chronic diseases in Sri Lanka which suspect to have originated due to environmental factors, the studies leading to explore the presence of environmental toxins become very essential. Therefore, the present study was designed as an initial step to understand the distribution of the toxigenic species in reservoirs of Sri Lanka.

MATERIALS AND METHODS

Study area

Sixty three reservoirs, varying in size were sampled from three major climatic regions of the country (Figure 1). In order to maximize limnological conditions reservoirs were selected throughout Sri Lanka, cover variety of urban, rural and agricultural regions. These sites range in elevation above mean sea level from 6 to over 1800 m and include reservoirs in: Dry Zone (Mean Annual Rainfall, MARF) = 1 to 1.5 m), the Intermediate Zone (MARF = 1.5 to 2 m) and the Wet Zone (MARF = 2 to 2.5 m) (Figure 1). The catchments of reservoirs sampled include a diversity of vegetation types including: Montane Forests, Sub Montane Forests, Lowland Rain

Forests, Moist Monsoon Forests, Dry Monsoon Forests (National conservation review report, Forest Department, Sri Lanka, 1999).
 Forests, Riverine Dry Forests, Sparse and Open Forest Department, Sri Lanka, 1999).

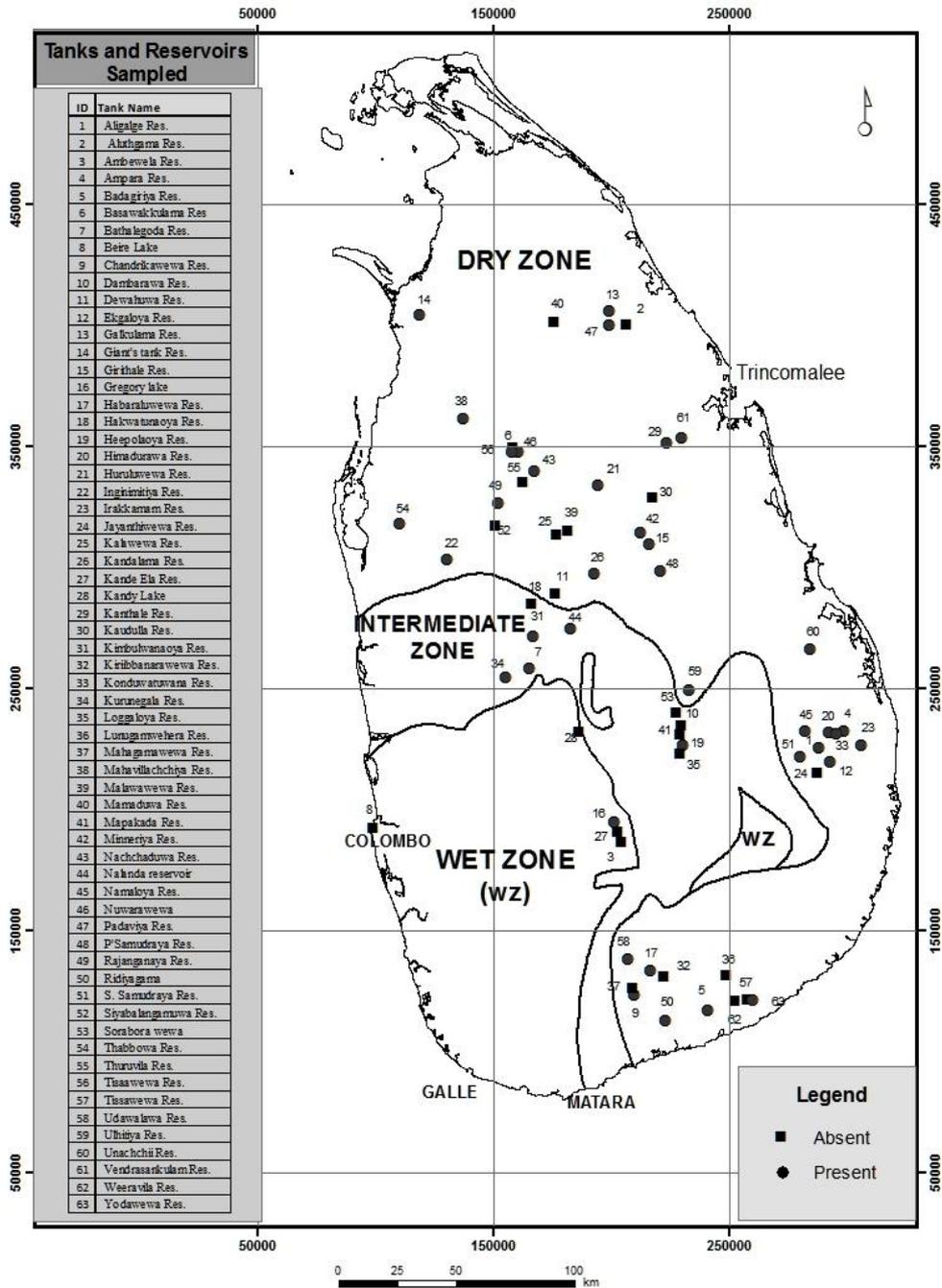


Figure 1: Locations of the 63 study reservoirs in Sri Lanka (closed circle indicate the 38 reservoirs which contain *C. raciboskii*). The country is divided into three main climatic regions: the Dry Zone, the Intermediate Zone, and the Wet Zone.

The geology underlying the study sites is of Precambrian age and is represented by three approximately equally-sized complexes Wannai Complex (WC) the Central Highland Complex (HC) and Vijayan Complex (VC) (Cooray, 1994). The geology of the Dry Zone consists of sites on all of the complexes, whereas the Intermediate Zone occurs on both the WC and HC. The Wet Zones are found on the HC, whereas the northern and southern Dry Zones are found on the WC and VC, respectively. Thus, the study area covers geographically and geologically diverse areas providing grounds for diverse limnological conditions. In addition reservoirs contain diverse morphometric characteristics. Some reservoirs which are located in mountainous regions characterized with funnel shape bathymetry. The reservoirs selected in the Dry Zone were shallow with uneven bottom characteristics whereas reservoirs selected from the Wet Zone known to exhibit permanent bottom anoxia and some have experienced sudden fish kills (Yatigamma and Cumming, 2016). The lowland reservoirs studied consisting of variety of bathymetric characteristics, having single or multiple deep regions while others were shallow throughout the reservoir. Further, the study reservoirs were varied in size ranging from few hectares to over 2,000 hectares.

Field sampling and laboratory analyses

Preliminary assessment to investigate the presence of *C. raciborskii* was done using a plankton net (pore size 10 μ m) for all selected reservoirs (63 in total) during dry and wet seasons. Taxonomic description of Baker (1991) was used to identify *C. raciborskii*. After preliminary screening, 38 reservoirs that contain *C. raciborskii* were selected for detailed study (Figure 1). The physico-chemical and biological analyses were done according to the standard procedures using the samples obtained at three months interval for a period of over one year from October, 2010 to January, 2012. The average relative abundance data were used to understand the distribution of *C. raciborskii* in relation to environmental conditions.

Collection of limnological data

Water samples were obtained from thirty eight reservoirs at each visit, in a 1-L acid-washed, triple-rinsed Nalgene bottle. Samples were taken at 0.3 m below from the air-water interface from

the deepest area or middle of the each reservoir to analyze alkalinity, nutrients, dissolved oxygen (DO) and preserved immediately according to the American Public Health Association (APHA, 2005) standards. For the analysis of chlorophyll *a*, 500 ml of water obtained >1m below the air water interface of the same area of each reservoir using a depth sampler and was filtered through Whatman glass microfiber filters (pore size 0.45 μ m). The filters were then folded and inserted into test tubes, which was wrapped in aluminum foils. The samples were kept in ice box while transporting to the environmental research laboratory at the National Institute of Fundamental Studies (NIFS) Kandy and then preserved in a freezer until analysis. Temperature, salinity, specific conductance and conductivity measurements were also obtained at the site itself. The average values of each measured variable of each season were used for the statistical analysis. In total, 13 physico-chemical variables were measured, including nutrients [total phosphorus (TP), dissolved phosphorus (DP), nitrite nitrogen, nitrate nitrogen, ammonia], conductivity, alkalinity, sulphate (SO₄), temperature, dissolved oxygen, turbidity and chlorophyll *a* and pH.

Collection of biological data

At least four sites from each reservoir were selected for maximum representation of the reservoirs. Samples were preserved onsite by adding 2 drops of acid Lugol's solution and stored under 4°C until analysis. The samples collected at each sampling site was filtered through a 10- μ m mesh and then the material retained on the mesh was backwashed into glass vials, and diluted to a final volume of 5 ml. One to two drops of acid Lugol's solution were added to each vial for further preservation. Following agitation of the samples, 100 μ l of this solution was placed directly onto a microscope slide. Identification and enumeration of *C. raciborskii* was performed at \times 400 magnification using a Olympus research microscope (CX 31) equipped with phase contrast optics. Photomicrographs were taken using a Olympus digital camera (CCD resolution 600 pixels). At least 500 cells, filaments and clumps (only for *Microcystis* sp.) were counted to understand the dominance of the species. The relative abundance of each species was calculated.

Data Screening and analysis

The normality of all environmental variables from the 38 reservoirs were assessed and transformed, if appropriate, using the computer program Canoco 5 (v. 5) for windows. Principle Component Analysis (PCA) was used to find the main directions of environmental variables and which environmental variable is important in discriminating of sites.

Cluster analysis was performed to understand the variations of study areas based on the environmental data using the computer program Minitab (v. 16) in both dry and wet seasons. The correlation between the environmental variables was assessed by Pearson correlation analysis using the computer program SYSTAT.

RESULTS

The 38 reservoirs that had *C. raciborskii* showed variation in their limnological characteristics. The average physical and chemical characteristics of the study reservoirs during wet and dry seasons are summarized in Table 1. The temperature of the surface waters varied from ~ 25.0 °C to 32.0°C, during the wet season and ~ 20.1 °C to 32.3°C during the dry season (Table 1). However the variation of median values (29.7- 31.1) and the average values (29.37-30.92) were mostly similar in both dry and wet seasons showing a normal distribution. Higher turbidity values were encountered in the dry season in Padaviya reservoir having 66.2 NTU. Conversely median and mean values were similar in both

seasons. As expected, the pH values were neutral in the wet season and slightly alkaline during the dry season (median and mean). Extreme conductivity values were recorded in reservoirs located closer to the arid Zone (Badagiriya, Giant's tank, Inginimitiya, Mahawilachchiya, Thabbowa, Yodawewa) in both wet and dry seasons. In addition, terminal reservoirs in main cascades (e.g. Nachchaduwa, Nuwarawewa, Rajanganaya) also recorded high conductivity values in both seasons. TP, DP, NH₃, Nitrate, and Nitrite values showed that the reservoirs are eutrophic or hypereutrophic except Chandrikawewa, Habaraluwewa, Inginimitiya, Kanthale and Udawalawa in which TP values are less than 30 µg/L and therefore fall under mesoeutrophic conditions. Dissolved oxygen levels were in the normal range (6.9 -6.5) in majority of the reservoirs.

The results of the Pearson correlation clearly shows a strong relations with: 1) concentration of alkalinity and conductivity, 2) nutrients and primary production (Chl. a), 3) nutrients and turbidity, 4) Sulphate and alkalinity, especially during the Dry season (Table 2 and Table 3). For example, Sulphate and alkalinity strongly correlate with conductivity while TP, DP NH₃, nitrite and nitrate correlate with Chl. a. In addition these variables correlate with the turbidity as well. However, the correlation between the primary production, nutrients, TP and turbidity were apparent during the dry season indicating the effect of the concentration of nutrients especially in algal growth.

Table 1: Minimum, maximum, mean and median of Environmental data of 38 study reservoirs during the wet and dry season (October 2010 – January 2012) Abbreviations: TP = Total Phosphorus; DP = Dissolved Phosphorus; CHLA = Chlorophyll-*a*.

	Average		Maximum		Minimum		Median	
	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet
Temperature (°C)	30.9	29.3	36.8	32.0	20.1	25.0	31.2	29.5
Turbidity (NTU)	12.5	11.8	66.2	45.6	1.4	1.8	7.9	8.1
pH	8.3	7.7	9.2	9.0	7.2	7.0	8.2	7.6
Conductivity (µS cm ⁻¹)	263.2	238.1	928.0	637.0	46.0	38.0	198.0	200.0
Alkalinity (mg L ⁻¹)	121.9	105.7	402.7	241.0	30.5	25.6	112.9	103.1
Nitrite (µg L ⁻¹)	21.2	35.3	59.5	132.3	6.3	13.1	20.0	23.3
Nitrate (mg L ⁻¹)	0.7	0.2	23.1	1.3	0.0	0.0	0.1	0.1
NH ₃ (mg L ⁻¹)	57.1	67.3	145.1	220.4	11.9	19.3	49.4	57.5
TP (µg L ⁻¹)	57.0	55.4	230.0	134.5	15.8	18.3	40.8	48.6
DP (µg L ⁻¹)	18.1	19.1	421.8	114.6	0.5	4.2	6.5	15.6
SO ₄ ²⁻ (mg L ⁻¹)	3.4	4.0	21.1	20.2	0.4	1.5	2.2	3.1
DO (mg L ⁻¹)	6.9	6.5	9.5	9.0	5.1	3.4	6.8	6.5
CHLA (mg L ⁻¹)	29.4	22.0	148.5	63.0	0.5	1.1	18.2	18.2

In the study, cluster analysis was performed to understand the variations of study reservoirs based on the environmental data. Results of the study revealed that the reservoirs are having more similarities during the wet season than in the dry season (Figure 2a and Figure 2b). During the wet season, majority of them indicated over 90% of similarity and all the reservoirs showed 80% similarity. In contrast, during the dry season all the reservoirs indicated ~50% of similarity. In addition, three clusters were clearly visible and they show ~ 85% of similarity. It is clear that the water quality characteristics of the dry season help separate the reservoirs in different cascades or geological regions. For example Aligalge, Ekgaloya, Himadurawa, Senanayake samudraya are all Gal Oya Basin (GOB) reservoirs are closely located in the eastern part of the country and belong to the same cascade are clustered together especially during the dry season (Figure 2b). Tissa wewa located in the southern region of the country are different from other reservoirs shows only 50 % of similarity.

The results of the principle component analysis also explain that all the measured environmental variables during the dry season have strong correlation with the 1st axis, the main component which helps discriminate the sites

(Figure 3a &3b). The dissolved phosphorus is strongly associated with the second most important component. However during the wet season the most important environmental variables appear to be conductivity, alkalinity and sulphate. Further, nutrient and associated variables appear to be contributed more on the 1st component (Figure 4a & 4b). The measured environmental variables help to separate the reservoirs sampled during the wet season than in the dry season (Figure 3a &3b; Figure 4a &4b).

C. raciborskii was recorded in 38 out of the 63 reservoirs in the study (Figure 1). The dominance of *C.raciborskii* appears to be favored by environmental factors prevail in urban reservoirs such as Gregory, Nuwarawewa and Padaviya. In addition, the isolated and terminal reservoirs also appear to experience high abundance of *C. raciborskii* during both wet and dry seasons.

Further, the morphology of this species was varied and included straight, coiled and sigmoid- shaped trichomes. Populations were recorded as mixtures of all three morphotypes (Table 4 and Figure 5). The relative abundance of the cyanobacterium during the wet and dry seasons is given in Figure 6. During the dry season, the organisms tend to be more abundant than in the wet season.

Table 2: Pearson correlation matrix on transformed environmental variables with Bonferroni adjusted probabilities for 13 measured environmental variables during the dry season in the 38 reservoirs. * Indicates significant correlation at $p < 0.05$.

	Temperature	Turbidity	pH	Conductivity	Alkalinity	Nitrite	Nitrate	Ammonia	TP	DP	Sulphate	DO	CHLA
Temperature	1												
Turbidity	0.035	1											
pH	-0.293	0.325	1										
Conductivity	-0.137	0.045	0.464	1									
Alkalinity	-0.104	-0.073	0.098	0.538*	1								
Nitrite	-0.128	0.383	0.101	0.191	-0.012	1							
Nitrate	0.18	-0.117	-0.39	-0.164	-0.21	-0.021	1						
Ammonia	-0.394	0.342	0.145	0.101	-0.04	0.414	0.182	1					
TP	-0.148	0.872*	0.391	-0.07	-0.176	0.347	-0.153	0.309	1				
DP	0.017	-0.045	0.01	0.051	-0.06	0.055	-0.045	-0.043	-0.037	1			
Sulphate	-0.059	0.264	0.33	0.631*	0.396	0.13	-0.126	0.139	0.147	-0.038	1		
DO	0.038	0.13	0.179	0.217	-0.012	0.106	0.33	0.172	0.131	-0.026	-0.08	1	
CHLA	-0.246	0.678*	0.481	-0.096	-0.156	0.178	-0.089	0.279	0.865*	0.044	0.115	0.24	1

Table 3: Pearson correlation matrix on transformed environmental variables with Bonferroni adjusted probabilities for 13 measured environmental variables during the wet season in the 38 reservoirs. * Indicates significant correlation at $p < 0.05$.

	Temperature	Turbidity	pH	Conductivity	Alkalinity	Nitrite	Nitrate	Ammonia	TP	DP	Sulphate	DO	CHLA
Temperature	1												
Turbidity	0.46	1											
pH	-0.225	0.099	1										
Conductivity	-0.18	-0.082	0.347	1									
Alkalinity	-0.228	-0.254	0.305	0.885*	1								
Nitrite	0.273	0.006	-0.103	-0.216	-0.201	1							
Nitrate	-0.275	0.243	0.466	-0.229	-0.334	0.058	1						
Ammonia	0.51	0.634	0.007	-0.066	-0.096	0.058	0.047	1					
TP	0.149	0.205	-0.156	-0.088	-0.196	-0.021	0.109	0.134	1				
DP	0.458	0.376	-0.234	-0.255	-0.232	0.115	0.06	0.515	0.159	1			
Sulphate	0.019	0.137	0.321	0.61	0.604*	-0.158	-0.117	0.417	0.054	-0.092	1		
DO	-0.008	0.194	0.154	0.001	0.042	-0.048	0.25	-0.046	-0.189	0.004	-0.084	1	
CHLA	0.18	0.497	0.102	-0.298	-0.397	-0.231	0.399	0.536*	0.403	0.44	-0.05	0.112	1

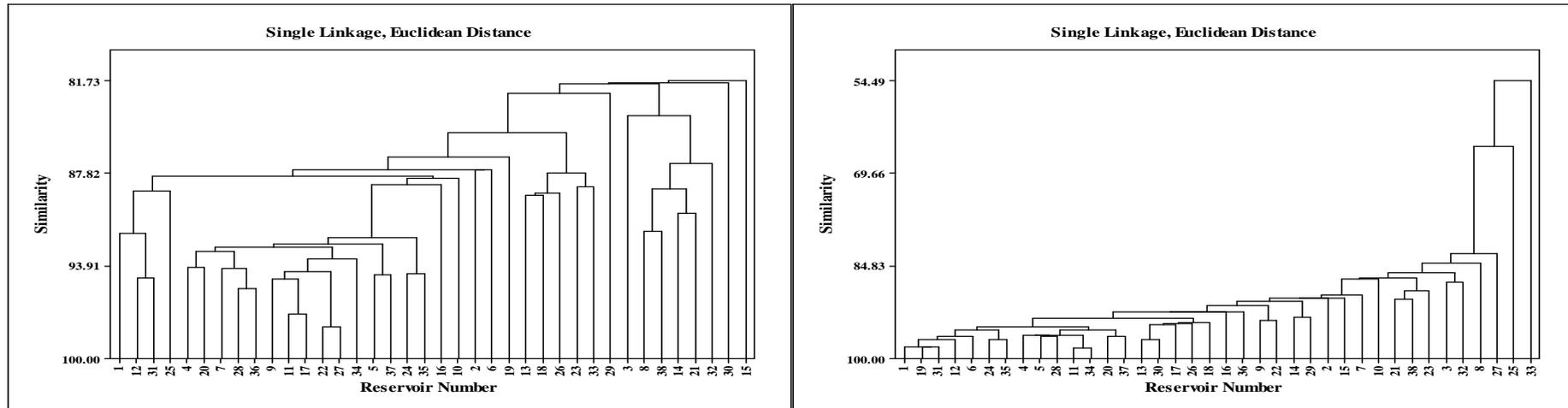


Figure 2: Dendrogram showing the similarity of study sites based on measured environmental variables during wet season (a) and during dry season (b).

Reservoir List:

1.Ampara Tank, 2.Badagiriya, 3.Bathalegoda wewa, 4. Chandrika wewa, 5. Ekgaloya Tank, 6. Galkulama, 7. Giants Tank, 8.Girithale, 9.Gregary lake, 10.Habaralu wewa, 11.Heepolaoya, 12. Himadurawa Tank, 13. Hurulu wewa, 14. Inginimitiya, 15. Irakkammam Tank, 16. Kandalama, 17. Kanthale wewa, 18. Kimbulawana oya, 19. Konduwatuwana, 20. Kurunegala wewa, 21. Mahavillachchiya, 22.Minneriya, 23.Nachchaduwa, 24.Nalanda, 25.Namal Oya, 26.Nuwarawewa, 27. P'samudraya, 28.Padaviya wewa, 29.Rajanganaya, 30.Ridiyagama wewa, 31.Senayanake S., 32.Thabbowa, 33.Tissa wewa, 34.Udawalawa, 35. Ulhitiya, 36.Unachchiya tank, 37. Vendrasan Kulam, 38. Yoda wewa.

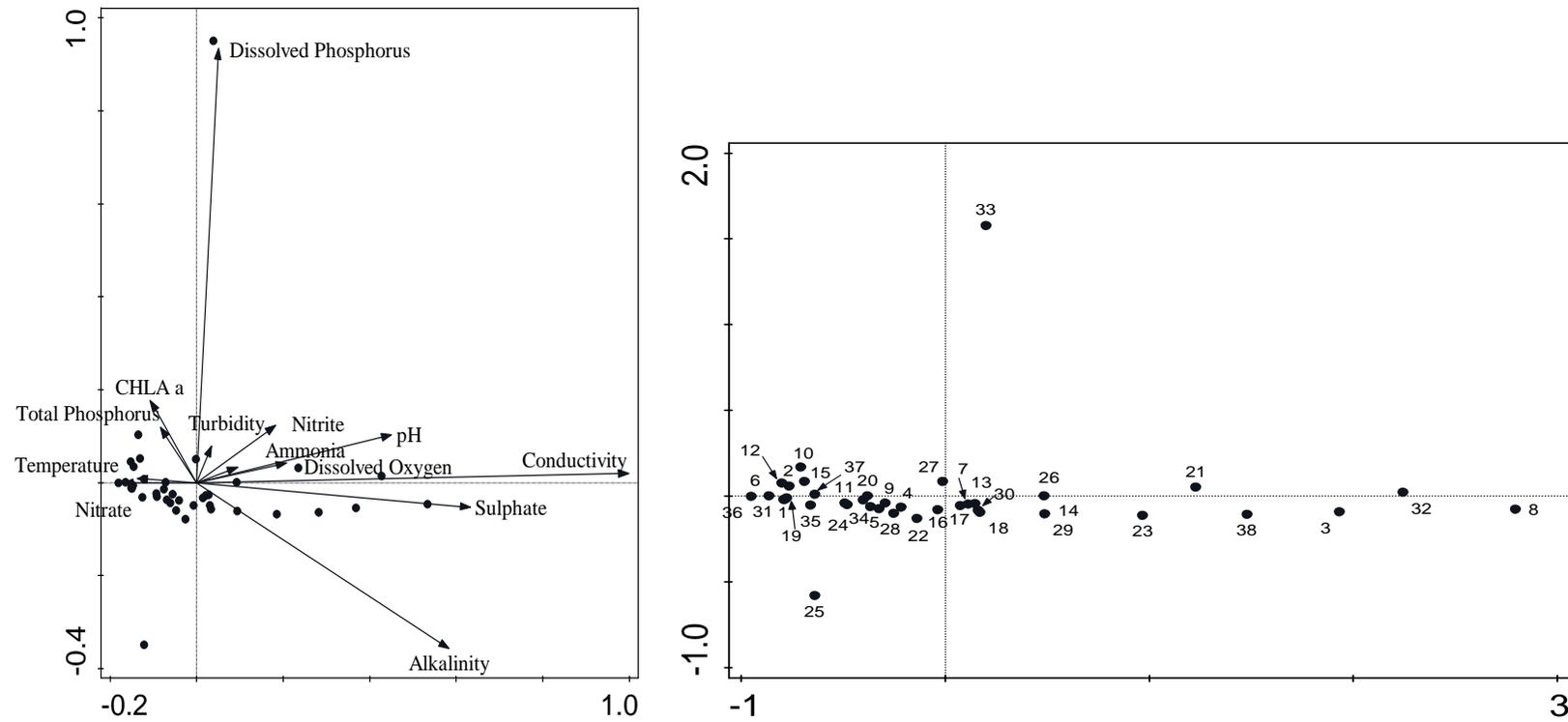


Figure 3: Principal Components Analysis (PCA) ordination diagram of the 38 study sites (list given under the Figure 2.) and associated environmental variables during the dry season (a=distribution of environmental variable; b=distribution of sites).

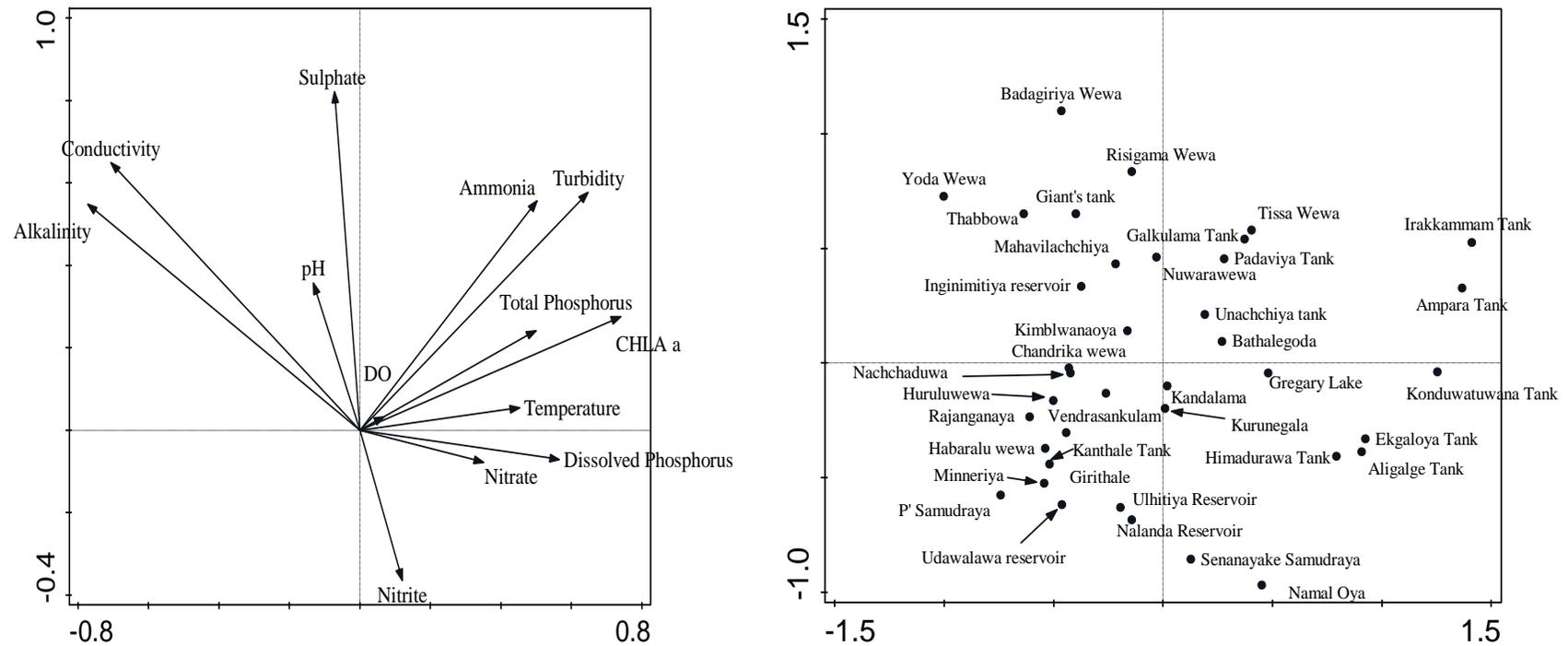


Figure 4: Principal Components Analysis (PCA) ordination diagram of the 38 study sites and associated environmental variables during the wet season (a=distribution of environmental variable; b=distribution of sites).

Table 4 : Main Characteristics of the three observed morphological forms of *C. raciborskii*.

	Straight	Coiled	Sigmoid
Cell width (µm)	2.5 – 3.5	2.5 – 3.2	1.5 – 1.9
Cell length (µm)	4.7 – 6.2	5.9 – 6.9	6 - 7
Trichome	Straight, rarely pointed	Coiled in a circular manner	Spirally or irregularly twisted
Heterocytes	Drop like, formed at one or both ends	Drop like, formed at one end or both ends	Drop like, formed at one end or both ends

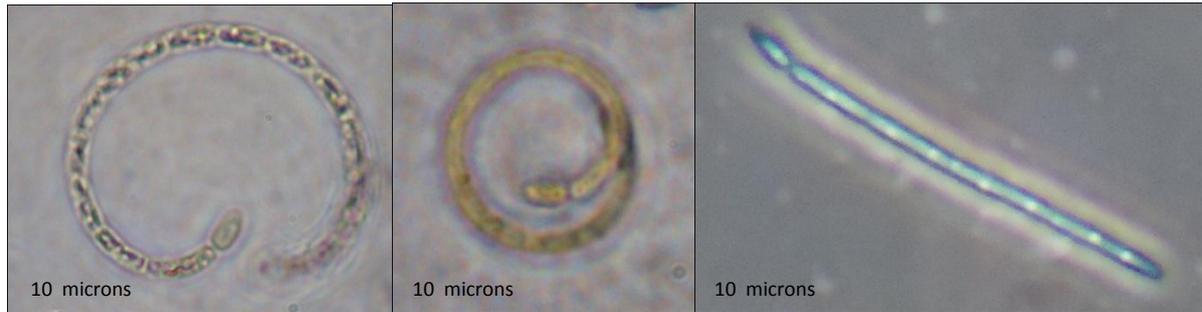


Figure 5: Photomicrographs showing morphological variations of *C. raciborskii* from Sri Lankan reservoirs.

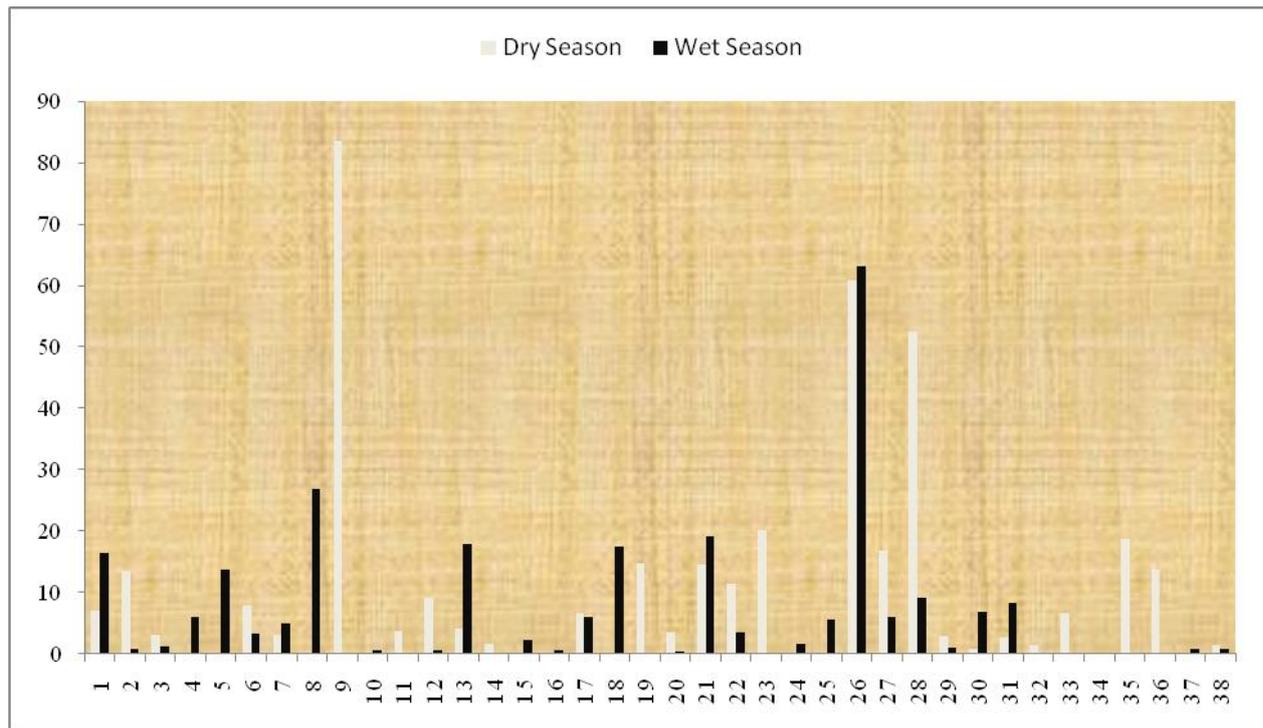


Figure 6: Comparison of the relative abundance of *C. raciborskii* during wet season and the dry season in 38 reservoirs (list of reservoirs are given under Figure 2) .

DISCUSSION

Cylindrospermopsis raciborskii is known to be highly adaptable, extremely small cyanobacterium distributed over 45 countries throughout the world (Codd *et al.*, 2005). They usually occur at 1-3 m below the air-water interface and therefore rarely form blooms (Sethunga and Manage 2010; Saker and Griffith, 2001). They do not produce volatile organic compounds, geosmin or 2-methylisoborneol (MIB), which usually produce by other bloom-forming cyanobacteria (Chiswell *et al.*, 1997). Therefore, farm and wild animals are unknowingly affected by the cylindrospermopsin, which is secreted by *C. raciborskii*. This organism could not be detected by foul tastes or odors caused by volatile organic compounds, or scums in drinking water (Hawkins *et al.*, 1997; Saker and Eaglesham, 1999).

There have been several cases of cyanotoxin poisoning in human by toxigenic cyanobacteria. For example, 76 patients were killed in a dialysis clinic in 1996, in Brazil. Later they found that the water used to treat the patients was contaminated with cyanotoxin (MC-LR) contaminated water (Carmichael *et al.*, 2001). In addition, the Palm Island Mystery Disease was also caused by the toxins produced by cyanobacteria. (Hawkins *et al.*, 1985). The disease was recorded after the authorities have used copper sulphate to control algae in drinking water supply. According to the scientists, the outbreak has resulted from the cell lysis of the cyanobacterium, which occurs after they die out following the cyanotoxin release into the water (Manage *et al.*, 2009, Manage *et al.*, 2010). In Sri Lanka too, epidemics related to algal toxins were reported in the recent history (e.g. Yatigammana *et al.*, 2011), though it was caused by a dinoflagellate, *Peridinium aciculiferum*. Piyathilaka *et al.* (2015) recorded that Microcystin-LR-induced cytotoxicity and apoptosis in human embryonic kidney and human kidney adenocarcinoma cell lines.

As the records on toxic algae are increasing in Sri Lankan reservoirs (Sethunga and Manage 2010), it has become important to understand the distribution of the cyanotoxin producing cyanobacteria in Sri Lanka, in where especially the surface waters are used for drinking purposes. There are frequent studies on

the distribution of toxigenic cyanobacteria, mainly due to the common toxin, Microcystin, produced by a variety of cyanobacterial species (Sethunga and Manage 2010, Idroos *et al.*, 2017). The lack of information on *C. raciborskii* suggests that studies leading to understand the prevalence of toxigenic cyanobacteria done in the past may have failed to see the small cyanobacterial species including *C. raciborskii*, especially due to sampling errors including; 1) plankton samples are usually taken from the first 1m of the water column, 2) *C. raciborskii* cannot be detected from the blooming, odor or foul taste, 3) pore sizes of the plankton nets might not have been small enough (e.g. 20 µm). However, the present study was able to detect and identify the organism as the sampling was carried out with the aim of identifying the specific organism. Accordingly, the study confirmed that the cyanobacterium residing in >60 % of the sampling reservoirs, demonstrating its high prevalence throughout the country. According to the findings of the study, among the different morphotypes, straight morphotype was the most common in Sri Lankan reservoirs, a condition which is also noticed by Saker and Griffiths (2001) from a study on domestic water supply in Australia. This situation could arise when they are derived from akinetes or structures produced to overcome unfavorable environmental conditions. However, St Amand (2002) found that straight morphotypes produce more toxins than coiled morphotypes, which gives us a warning signal for the safety and quality of drinking water in Sri Lanka. Fluctuations of temperature and increase of phosphorus concentration in water promote akinete production, a condition common in Sri Lankan reservoirs. However, the morphotype could also be varied with the age of the bloom and later produce fast growing coiled morphotypes (Saker and Eaglesham, 1999). Variation of morphotypes could also be attributed to genetic variability as well (Hawkins *et al.*, 1997; Neilan *et al.*, 2003). Although many different types of morphotypes were detected, they were not quantified to assess the severity of the problem, which is a limitation of the study.

Considering the abundance of the species in reservoirs located in different climatic regions with different morphological and physicochemical conditions, it was not clear about the exact environmental condition that supports the species distribution. However, the

highest relative abundance of 60% during the dry and 63% during the wet season was recorded from Nuwarawewa, a thermally stratified urban reservoir located in the dry zone of Sri Lanka. The average temperature of the reservoir during the dry season was 31°C and 29 °C in the wet season. According to Briand *et al.*, (2004) the temperature is the most important environmental factor that determines the population dynamics of *C. raciborskii*. Briand *et al.* (2004) and Shafik *et al.*, (2001) explained that the optimum temperature for the growth of *C. raciborskii* is 30°C although 'sub-optimum' temperatures range from 25-35°C. In addition, the species prefer stratified waters in tropics and shallow waters in temperate environments. According to Yatigammana and Cumming (2016), most of the reservoirs in Sri Lanka are chemically and thermally stratified, where the condition rarely breaks at high temperatures. However, large fluctuations of temperature negatively affect the population size of *C. raciborskii*. Padisák (1997) records a large population of this cyanobacterium in extremely warm years and few in cool years. Hill (1970) records the same situation having a high population in one year and could not find any until two years in a lake in Minnesota. Therefore, it is apparent that the organism becomes vulnerable in extreme temperatures. This may be the reason why high altitudinal (>6000ft MASL) lake (Gregory Lake) had extreme abundance of *C. raciborskii* in the year 2011, during the wet season and none during the dry season (Perera *et al.*, 2012). Briand *et al.*, (2002) explained that *C. raciborskii* is highly tolerant to turbidity. It is clear that the cyanobacterium *C. raciborskii* was abundant (RA 52%) in Padaviya reservoir in the dry season, during which the turbidity level was very high (~66.2 NTU). Therefore, it is apparent that they are shade tolerant. In contrast, high light intensity also promotes akinete production. According to Moore *et al.*, (2006), a four-fold increase in light intensity (25–100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) resulted in approximately 14-fold increase in akinete concentration. However, optimal irradiance had been reported as 80 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Shafik *et al.*, 2001). As Sri Lanka is located in the tropical belt, the irradiance should be optimum throughout the year. This may be the reason for having more straight morphotypes of *C. raciborskii* in Sri Lankan waters. Due to its high affinity and storage capacity of phosphorus, *C. raciborskii* shows the ability to grow in low levels of

phosphorus (Branco *et al.*, 1994; Briand *et al.*, 2002; Presing *et al.*, 1996) and may give *C. raciborskii* a competitive advantage. However, in high phosphorous levels, *C. raciborskii* has a negative correlation with dissolved TP. Nevertheless, the theory is contradictory with our findings where the high abundance of *C. raciborskii* was detected in hypereutrophic waters. When consider the past studies in Sri Lanka where the generally accepted phenomenon is that nitrogen-limited aquatic environments promote the growth of cyanobacteria when phosphorous is not limited (Silva and Samaradivakara, 2005) which is comparable with the findings of the present study. It is known that most cyanotoxin producing and bloom forming cyanobacteria needs high concentrations of phosphorous for their mass growth and it has been noticed that in lowland reservoirs in Sri Lanka, phosphorous is known to be a limiting factor (Jayatissa *et al.*, 2006, Sethunga and Manage 2010). The condition helps interpret our findings of low relative abundance of *C. raciborskii* in majority of our study reservoirs where TP is limited. Similarly, our results clearly indicate that the reservoirs where *C. raciborskii* was recorded had relatively low nitrate concentrations and the reservoirs that had TP at high concentrations the organism was highly abundant.

Similar to many other cyanobacteria, *C. raciborskii* have heterocysts that allow them to utilize atmospheric nitrogen through a process known as nitrogen fixation. Typically, this adaptation allows cyanobacteria to survive in low nitrogen systems. However, studies have found that *C. raciborskii* have few heterocysts even under low nitrogen content. Some other studies suggest that the organism prefer ammonia as the nitrogen source (Briand *et al.*, 2002; Bouvey *et al.*, 2000; Presing *et al.*, 1996). High relative abundance of the species recorded in our study was not always affiliated with the ammonia or other measured nitrogen sources. All the study reservoirs are of pH neutral or maintain high alkalinity which is preferred by *C. raciborskii* and known to be a common character of all cyanobacteria. The most preferred pH of *C. raciborskii* was found to be 8.0-8.7 (Padisák 1997). Although Saker and Griffiths (2001) found that the *C. raciborskii* present during the dry season when the water level is low, Branco and Senna (1994) found that they appear with the rain when the nutrients in water get diluted.

Primary health concerns surrounding *C. raciborskii* relate to the production of Cylindrospermopsin. Human exposure to this cyanotoxin via drinking water and water based recreational activities.. A health alert level for *C. raciborskii* of 15000 cells per ml has been set at which to begin monitoring for the presence of Cylindrospermopsin in source waters. The lack of scum formation, variation in colour of the water body, rapid germination of large number of cells, highly variable morphology, relative toxicity and persistence of this species year round in many areas, continues to make this species a primary focus to water managers (Mcgregor and Fabbro, 2000). A number of hypotheses have been suggested to explain the successful spread and invasive behavior of *C. raciborskii* (Komáerk, 2002). Some authors have proposed that high physiological tolerance to light and temperature (Padisák, 1997) coupled with higher temperatures promoted by climate warming may explain the expansion of this species to new regions (Briand *et al.*, 2004). Finally, the present study help scientists to study areas that need to be concentrated to safeguard surface water in Sri Lanka that serve a significant resource of Sri Lanka's economy.

CONCLUSION

Our study clearly indicates that *C. raciborskii* is now widely spread in Sri Lankan reservoirs. The most abundant morphotype is the straight morphotype that has high potential for toxin production. Although Sri Lanka is a small island, the climatic variation that the reservoirs experience, provides variable environmental conditions in surface freshwater lentic systems. Among the measured environmental factors, temperature and nutrients are the most important environmental factors that determine the distribution of the cyanobacterium *C. raciborskii*. The presence of several morphotypes signifies the potential for rapid distribution of *C. raciborskii* in other reservoirs of Sri Lanka.

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