Water quality, Microcystin-LR, and Cylindrospermopsin contamination status of spring and dug well water in CKDu high, low, and non-prevalent areas of Sri Lanka

*H.A.S.N. Abeysiri[1,2] and P. M. Manage[1,2]**

[1]*Centre for Water Quality and Algae Research, Department of Zoology*
[2]*Faculty of Graduate Studies*
[1,2] *University of Sri Jayewardenepura, Sri Lanka*

*Corresponding author: pathmalal@sjp.ac.lk

ORCID ID: https://orcid.org/0000-0003-3876-309X

Received: 04.01.2022 Revised: 24.03.2022 Accepted: 20.04.2022 Online: 15.09.2022

Abstract Cyanotoxins, Microcystin LR (MC-LR) and Cylindrospermopsin (CYN) are hepato and nephro toxins and considered as one of the hypotheses for Chronic Kidney Disease of Unknown Etiology (CKDu) in Sri Lanka. Recent studies have revealed that significant number of dug wells in the North Central and Uva provinces where CKDu is prevalent are contaminated with cyanotoxins MC-LR and CYN. In the present study, cyanotoxin; MC-LR, CYN, and potential toxin-producing cyanobacteria were studied in 330 dug wells and 9 spring water samples collected from CKDu high prevalent areas in several Divisional Secretariats (DS) and Grama Niladhari (GN) divisions of Anuradhapura (Padaviya DS, Medawachchiya DS, Kebithigollewa DS), Polonnaruwa (Medirigiriya DS), Badulla (Girandurukotte GN) and Ampara (Dehiaththakandiya DS) districts, low prevalent areas in Anuradhapura district (Galnewa DS, Rajanganaya DS), non-prevalent areas in Angunakolapelessa DS of the Hambanthota district. General water quality parameters: temperature, pH, conductivity, Dissolved Oxygen (DO), Nitrate ($N\text{-}NO_3^-$), Nitrite ($N\text{-}NO_2^-$), Ammonia ($N\text{-}NH_3$), Total Phosphorus (TP), and Total Hardness (TH) were determined using standard spectrophotometric and titrimetric methods. BACON test kits were used following manufactures instructions to quantify the CYN, and MC-LR concentrations by using the ELISA plate reader. Cyanobacteria density and species composition were determined under light microscopy following the standard algae and cyanobacteria keys. With the exception of Electrical Conductivity, all of the water quality parameters tested remained within the SLSI drinking water quality standard range. *Microcystis* spp. cell densities ranged from 14±3 to 1590±256 cells mL^{-1}, where MC-LR concentration ranging from 0.04±0.01 to 3.89±0.02 µg L^{-1}. At different CYN concentrations (0.04±0.01 to 3.59±0.05 µg L^{-1}), *Cylindrospermopsis* spp. cell densities varied from 15 ±4 to 615±112 cells mL^{-1}. Well water collected from Hambanthota district and spring water was collected Kebithigollewa DS, in Anuradhapura did not record cyanotoxins and cyanobacteria. The factor analysis classified well waters into three clusters of high, low, and non-prevalence areas with presence and absence of cyanobacteria and cyanotoxins. In contrast, springs were classified to a separate cluster. High concentrations of MC-LR and CYN were found in wells where CKDu high prevalent 85% of Padaviya DS and 80% of Medirigiriya DS, respectively. High Nitrate-N (3.01±0.56 mg L^{-1}) and Nitrite-N (0.69±0.09 mg L^{-1}) concentrations were recorded in well water collected from high CKDu prevalence areas. Thus, the results of the present study showed a relationship between cyanobacteria, cyanotoxins and CKDu records in the study area. Accordingly, further comprehensive studies are needed to be carried out to confirm the relationship between cyanotoxins and CKDu in Sri Lanka as cyanotoxin were listed in the WHO report as one of the possible reasons for CKDu.

Keywords: Microcystin-LR, Cylindrospermopsin, dug well, spring, water quality.

INTRODUCTION

The cyanobacteria are a group of over 2000 prokaryotic organisms that are commonly referred to as "blue-green algae" (Mullineaux 2008; Kulasooriya et al. 2011). These organisms have a diverse genetic make-up, which is advantageous. Because they live in a diverse range of habitats across all latitudes, it is possible that their ancestors, who were the planet's first inhabitants, had the ability to adapt to their environment (Kulasooriya et al. 2011). In addition to freshwater, marine, and terrestrial ecosystems, cyanobacteria can be found in hypersaline environments (Chatchawan et al. 2011), hot springs (Hayashi et al. 1994; Wijesekara et al. 2017; Sadeepa et al. 2019), arid deserts (Perera et al. 2018), and freezing environments (Kulasooriya et al. 2011; Perera et al. 2018). It is believed that increased occurrence of cyanobacterial blooms in aquatic systems is due to anthropogenic eutrophication (Paerl and Huisman 2008; Manage et al. 2009). Many cyanobacteria produce highly toxic cyanotoxins, which pose serious health risks to both humans and animals when they are consumed (Lewitus et al. 2012).

The most common cyanotoxins found in the aquatic environment are Microcystins (MCs) and Cylindrospermopsin (CYN) (Sethunga and Manage 2010; Wijewikrama et al. 2019). Over 100 heptapeptides (Lawton et al. 2011; Faassen et al. 2013) make up cyanotoxins. Heptapeptides are highly water-soluble non-volatile compounds (Lambert et al. 1994). Many studies have shown that cyanotoxins have toxic effects on many organs, including the liver (Towner et al. 2002; Manage 2009; the immune system (Lankoff et al. 2004); the nervous system (Lone et al. 2015); the kidney (Torokne et al. 2001; Piyathilaka et al. 2015); Abeysiri et al. 2018a, 2018b). In addition, they are carcinogenic (Newcombe et al. 2002; Lone et al. 2015). Given the toxic nature and environmental stability of MC-LR and CYN, the World Health Organization (WHO) established a provisional guideline limit of 1 μgL^{-1} for MC-LR and 2 μgL^{-1} for CYN in drinking water (WHO 2003).

Human exposure to cyanotoxins is expected because of drinking cyanotoxin-contaminated water (Fleming et al. 2002; Sethunga and Manage 2010; Manage 2019) and eating cyanotoxin-contaminated food such as rice (Chen et al. 2012; Manage 2019), green leafy vegetables (Lefebvre et al. 2013; Wijewickrama et al. 2019). Because cyanotoxins are hydrophilic, humans may concentrate a high amount of MC-LR and CYN through the consumption of contaminated water, crops, and fish harvested from MCs contaminated water (Manage 2019). A number of human health consequences have been reported in recent years as a result of high exposure to cyanotoxins, MCs, and CYN (Falconer et al. 2005). Intoxication of patients with CYN via drinking water sources has also recently been reported in Australia (Falconer et al. 2005). Recent research has shown that MCs can cause lesions in the mitochondria of kidney cells, resulting in kidney injuries (Piyathilaka et al. 2015). Kidney damage by CYN was defined by a decrease in the number of erythrocytes in the glomerulus, an increase in the space surrounding the glomerulus, an increase in the diameter of the tubule lumina, proximal tubule epithelial necrosis, and the presence of proteinaceous material in the distal tubules (Falconer et al. 1999).

Cyanobacteria were found in 75 % of freshwater bodies tested over a two-year period, and MC concentrations were found to be higher than WHO guidelines in more than half of the samples tested in that time (Sethunga and Manage 2010). There have recently been more reports on the number, duration, and intensity of cyanobacteria cell densities in reservoirs in the North Central, Northeast and Uva provinces of Sri Lanka (Sethunga and Manage 2010). A significant relationship was discovered between the number of cyanobacterial cell densities and the amount of toxins present in drinking water (Sethunge and Manage 2010; Hettiarachchi et al. 2014). A provisional guideline limits of 1 $\mu g\ L^{-1}$ for MC-LR and 2 $\mu g\ L^{-1}$ for CYN have been established by the Sri Lanka Standard Institute (SLSI) drinking water standards.

CKDu is common in Sri Lanka's North Central region, where it is thought to have originated. Men between the ages of 30 and 60 who work in agricultural sector are the most affected by the disease (Chandrajith et al. 2011; Jayasekara et al. 2013). A similar disease has been reported in Nicaragua (O'Donnell et al. 2011), El Salvador (Orantes et al. 2014), Costa Rica (Cerdas, 2005), India's Srikakulam District (Machiraju et al. 2009), and Egypt (Kamel and El-Minshawy 2010). Traditional kidney disease risk factors are not associated with this disease, and the pathology is consistent with tubulointerstitial nephritis (Wijethunga 2015). Various hypotheses have been proposed, including exposure to pesticides (Jayasumana et al. 2015), heavy metals, fluoride (Illeperuma et al. 2009; Chandrajith et al. 2011), hard water (Chandrajith et al. 2011)), and heat stress-related dehydration, but no definite aetiological factor has been identified thus far. As a result, implementation of preventive strategies has been slowed down significantly.

The presence of MCs has been identified as another potential environmental nephrotoxin in

CKDu-endemic areas, based on the available data (Zhang et al. 2012; Jayasekara et al. 2013). It has recently been discovered that MCs have the capability of causing lesions in kidney cells, which can result in kidney injuries (Zhang et al. 2012; Piyathilaka et al. 2015). The present study records the presence of MC-LR and CYN producing cyanobacteria and cyanotoxins with reference to some important water quality parameters of well water in CKDu high-prevalent, low-prevalent and non-prevalent areas in Sri Lanka. Therefore, the objective of the study was to determine MC-LR and CYN concentrations and to correlate with water quality parameters according to changes in cell densities of cyanobacteria in randomly selected dug wells in the CKDu high, low, and non-prevalent areas in Sri Lanka.

MATERIALS AND METHODS

Site Selection and Sample Collection

Water and algae samples were collected from randomly selected 330 wells and 9 springs in the districts of Anuradhapura (Padaviya DS, Medawachchiya DS, Kebithigollewa DS, Rajanganaya DS, Galnewa DS), Polonnaruwa (Medirigiriya DS), Badulla (Girandurukotte GN), Ampara (Dehiaththakandiya DS) and Hambanthota (Angunakolapelessa DS) during 2017 – 2018.

Based on patient statistics provided by the Ministry of Health, Medirigiriya, Padaviya, Medawachchiya, Dehiaththakandiya, and Girandurukotte were identified as high CKDu prevalence areas, while Rajanganaya and Galnewa were identified as low CKDu prevalence areas. For the study, Angunakolapellessa in the Hambantota district was chosen as a non-CKDu prevalent area. Secondary data was obtained from divisional secretariats and relevant government officials.

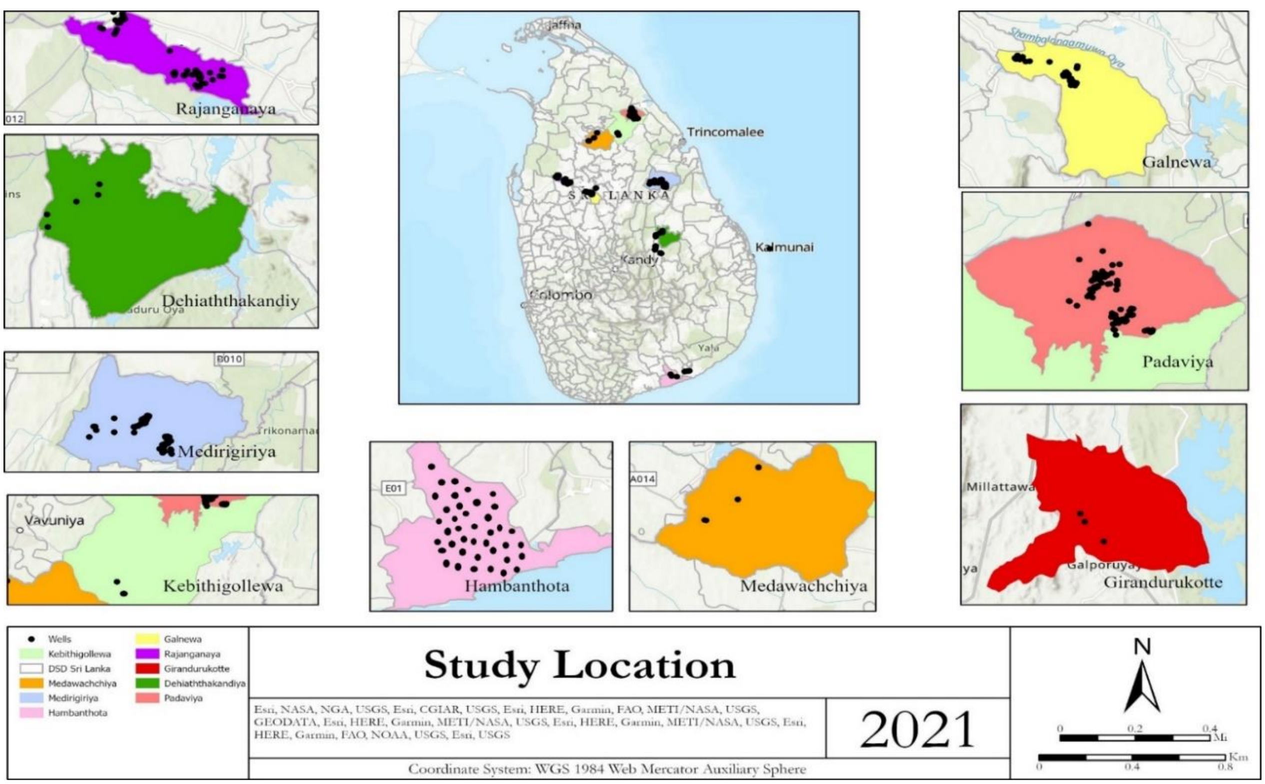

Fig 1. Water sampling locations of the present study

Analyses of water and cyanobacteria

Water temperature (ºC), Dissolved Oxygen (DO), pH, and Electrical Conductivity (EC) were measured on-site using a thermometer, a DO meter (HACH-HQ 40D), a pH meter (HACH-HQ 40D), and a conductivity meter (HACH- Sension EC5). Standard spectrophotometric and titrimetric methods were used to determine Nitrate (N-NO_3^-), Nitrite ($N\text{-}NO_2^-$), Ammonia ($N\text{-}NH_3$), Total Phosphorus (TP), and Total Hardness (TH).

Cyanobacteria genera composition and density were determined by fixing 100mL portions of water with acidified Lugol's solution at a final concentration of 1%. Natural sedimentation was used to concentrate 100mL of fixed sample for cyanobacteria identification. Cyanobacteria cells were counted under a light microscope (×400) using a Sedgwick rafter counting chamber. Standard cyanobacteria identification keys were used to identify cyanobacteria genera (Idroos and Manage 2014).

Quantification of Microcystin-LR and Cylindrospermopsin using ELISA method

Following the manufacturer's instructions, the concentrations of CYN and MC-LR were determined using Beacon ELISA test kits and plate reader (Thermo Scientifica MULTISKAN EX, S/N 3550905398 USA) at 450 nm wavelength (Idroos and Manage 2014).

Data Analysis

Factor Analysis was used to determine the main directions of environmental variables as well as which environmental variables are important in site discrimination (MINITAB 2017).

RESULTS AND DISCUSSION

General water quality parameters in dug well and spring water

CKDu prevalence and non-prevalence areas in the current study were investigated for the quality of dug wells and spring water collected from the various locations.

The most desirable and maximum permissible pH levels in drinking water, according to the Sri Lanka Standard Institute, are 7.0 – 8.5 and 6.5 – 8.5, respectively (SLSI 1983). Findings of the present study show that the pH values of dug well, and spring water remained within Sri Lankan drinking water standards. In Padaviya, Medawachchiya, and Mahiyanganaya, all ground water samples had pH values ranging from 6.45 to 7.14. (Nikagolla et al. 2020). The mean pH values in the Medawachchiya and Galnewa areas were within the allowable range for drinking (Imbulana et al. 2020). The pH values in springs were found to be lower than those recommended by the World Health Organization and the SLSI for drinking water quality (Mahagamage and Manage 2019). Except for one ground well in Padaviya, all ground wells had pH levels within the SLSI's maximum permissible range (Mahagamage and Manage, 2019). The average pH of Girandurukotte's groundwater varied between 6.56 and 7.22 (Kumari et al. 2016). The EC values of well water in CKDu are high and persistent; the areas of Medirigiriya, Padaviya, Medawachchiya, Dehiaththakandiya, and Girandurukotte had EC values ranging from 229 to 1519 µS cm^{-1}, 176 to 1303 µS cm^{-1}, 367 to 903 µS cm^{-1}, 94.3 to 479 µS cm^{-1}, and 77.9 to 340.2 µS cm^{-1} during the study, conductivity values in well water from the Hambanthota district ranged from 234 to 1303 µS cm^{-1}, while conductivity values in spring water collected from the Kebithigollewa area ranged from 77.6 to 646 µS cm^{-1}. Sri Lankan Standards for drinking water (750 µS cm^{-1} – SLSI 1983) were met by the conductivity of spring water collected from Kebithigollewa and well water collected from Girandurukotte GN and Dehiaththakandiya DS and in Kebithigollewa. One well in Medawachchiya, 50 in Medirigiriya, 60 in Padaviya, 37 in Rajanganaya, 31 in Galnewa, and 26 in Hambanthota had an EC greater than 750 µS cm^{-1}, compared to the national average of 450 µS cm^{-1}. The EC values of dug wells in Medirigiriya, Medawachchiya, Padaviya, Rajanganaya, Galnewa, and Agunukolapallassa exceeded the WHO and SLSI recommendations for drinking water approximately by 58.82%, 25%, 57.69%, 75.51%, 67.39 %, and 41.93 %, respectively, in Hambanthota district, following the findings of Chandrajith et al. (2011), it was discovered that there were significant differences in EC in well water samples, having values ranging from 52.5 to 3400 µS cm-1. Due to the mixing of well water with surface water, particularly irrigation water diverted from the wet zone region, the extremely low EC value could be attributed to a variety of factors.

Table 1 Some physico-chemical parameters of dug wells and spring water samples collected from various locations in the North Central, Uva, Eastern, and Southern provinces were determined, as were the mean values and ranges of these parameters. n = number of samples analysed

Province	District	DS Division	Temperature (°C)	pH	Electrical Conductivity (µS cm^{-1})	DO (mg L^{-1})	Nitrate-N (mg L^{-1})	Nitrite-N (mg L^{-1})	Ammonia-N (mg L^{-1})	TP (mg L^{-1})	TH (mg L^{-1})
North Central	Anuradhapura	Medawachchiya (n=4)	29.44 ± 0.80 (28.6 – 30.2)	6.88 ± 0.24 (6.62 – 7.15)	669.33±195 (367 – 903)	4.97 ± 1.15 (3.25 – 6.04)	0.24±0.09 (0.15 – 0.36)	0.69±0.09 (0.56 – 0.81)	<0.01	<0.01	131.40±58.11 (66.1 – 194.2)
		Padaviya (n=104)	28.63 ± 0.98 (26.5 – 30.2)	7.36 ± 0.25 (6.56 – 8.32)	775.79 ±188.30 (176 – 1303)	3.61 ± 1.20 (2.07 – 9.54)	0.02±0.01 (0.01 – 0.42)	0.08±0.01 (0.01 – 1.9)	<0.01	<0.01	64.26±29.81 (5 – 208.11)
		Kebithigollewa (n=9) (Spring)	27.19±0.89 (25.7 – 28.6)	7.42±0.43 (6.67 – 7.98)	347.46±278.58 (77.6 – 646)	4.85±2.09 (1.87 – 8.32)	0.15±0.13 (0.01 – 0.38)	0.56±0.30 (0.01 – 0.79)	<0.01	<0.01	67.30±41.61 (18.12 – 136.14)
		Rajanganaya (n=49)	28.09±0.89 (26.48 – 29.57)	7.39±0.16 (6.8 – 7.59)	853.02±109.31 (637 – 1325)	3.01±0.56 (1.48 – 4.58)	3.01±0.56 (1.48 – 4.58)	<0.01	<0.01	<0.01	66.08±7.14 (27 – 89)
		Galnewa (n=46)	28.11±0.94 (26.7 – 30.2)	7.39 ± 0.24 (6.64 – 7.98)	787.44 ± 194.28 (290 – 967)	3.34 ± 0.79 (1.29 – 5.87)	3.34±0.79 1.29 – 5.87	<0.01	<0.01	<0.01	74.55±17.68 (36 – 89)
	Polonnaruwa	Medirigiriya (n=84)	28.16±1.02 (26.1 – 30.3)	7.32±0.22 (6.27 – 7.86)	798.98±247.29 (229 – 1519)	3.89±1.25 (2.17 – 7.7)	0.22±0.06 (0.01 – 4.09)	0.25±0.08 (0.01 – 0.95)	<0.01	<0.01	64.69±29.78 (19 – 177)
Uva	Badulla	Mahiyanganaya (Girandurukotte GN) (n=12)	29.32±0.72 (28.1 – 30.2)	6.73±0.74 (5.37 – 7.47)	130.68 ± 75.67 (77.9 – 340.2)	5.15±1.27 (2.01 – 6.27)	0.45±0.09 (0.01 – 3.8)	0.59 ± 0.15 (0.29 – 0.84)	<0.01	<0.01	59.61±22.36 (37 – 96)
Eastern	Ampara	Dehiaththakandiya (n=15)	29.07±0.76 (28.1 – 30.2)	7.16 ± 0.41 (6.27 – 7.87)	239.36 ± 132.34 (94.3 – 479)	5.3 ± 1.23 (2.68 – 7.21)	0.2±0.1 (0.01 – 0.36)	0.45 ± 0.23 (0.14 – 0.93)	<0.01	<0.01	72.52±32.98 (28 – 142)
Southern	Hambanthota	Hambanthota (n=62)	27.86±1.03 (25.6 – 30.2)	7.32 ± 0.44 (6.51 – 8.47)	654.30 ± 267.86 (234 – 1303)	3.26± 0.51 (2.14 – 4.26)	<0.01	<0.01	<0.01	<0.01	63.03±15.99 (34 – 96)

According to Paranagama et al. (2013), the physical parameters of pH and conductivity measured did not exceed the Sri Lankan standard for portable water level/SLPWL (SLSI 614, 1983). Ten Padaviya wells and one Medawachchiya well had conductivities greater than 750.0 µS cm^{-1} (Table 1). The EC of ground water in CKDu endemic areas ranged from 134 to 1377 µS cm^{-1}, in two locations of Mahiyanganaya having the lowest and highest average EC of 262 µS cm^{-1} and 1377 µS cm^{-1} respectively. EC of groundwater in Medawachchiya was four times higher than in Mahiyanganaya on average, according to the data published by Nikagolla *et al.* (2020). Groundwater in the Padaviya area had high average EC values (722.6±166.6 µS cm^{-1}), which was unusually high for the area (Edirisinghe et al. 2017). The average EC values of CKDu wells in Dehiaththakandiya are 520.21±57.7 µS cm^{-1}. The average EC values in Girandurukotte groundwater ranged from 131.4 to 556 µS cm^{-1}, with the highest value being 556 µScm^{-1} (Kumari *et al.* 2016).

The TH of well and spring water ranged from 5 to 208.11 mg L^{-1}, which was lower than the SLSI's highest desirable level for drinking water, which was 250 mg L^{-1}. In the Padaviya area, hard water could be found in ten of the wells (Mahagamage and Manage 2019). The Dissolved Oxygen (DO) levels of springs and well water in the study areas remained in the 1.29 to 9.54 mg L^{-1} range throughout the study. Nikagolla et al. (2020) reported that the DO concentration ranged from 1.37 to 7.32 mg L^{-1}, with an average of 3.06 mg L^{-1} in CKDu endemic areas of Sri Lanka. Regarding the presence of nitrogen, land use practices and other anthropogenic activities were reported to be the primary causes of elevated ammonia, nitrate, and nitrite concentrations in reservoir, river, and dug well water has been discovered in recent years in Sri Lanka's Northern, North Central, Northern Eastern, Western, and Central provinces (Sethunga and Manage 2010; Mahagamage and Manage 2014; Imbulana et al. 2020; Nikagolla et al. 2020). In spring water, the mean N-NO_3^- concentrations were 0.15±0.13 mgL^{-1}, whereas the values in dug well water collected from CKDu high prevalent Medirigiriya, Padaviya, Medawachchiya, Girandurukotte, and Dehiaththakandiya were 0.22±0.06 mg L^{-1}, 0.02±0.01 mg L^{-1}, 0.24±0.09 mg L^{-1}, 0.45±0.09 mg L^{-1}, 0.2±0.1 mg L^{-1} and Nitrate concentrations in well waters in Rajanganaya and Galnewa were 3.01±0.56 mg L^{-1} and 3.34±0.79 mg L^{-1}, respectively, while those in Hambanthota were less than 0.01 mg L^{-1}. Springs had an average N-NO_2^- value of 0.56±0.01 mg L^{-1}, while well water had a range of <0.01 to 0.69±0.09 mg L^{-1}, with the mean being 0.56±0.01 mg L^{-1}. The concentration of N-NH_3 in springs and all well water was less than 0.01 mg L^{-1}. Nitrate, nitrite, and ammonia concentrations in drinking water continued to be significantly lower than WHO and SLSI drinking water standards, and total phosphorus (TP) levels were less than 0.01 mg L^{-1} in springs and all well water.

Similar concentrations of N-NO_3^-, N-NO_2^-, and N-NH_3 were found in reservoirs (Sethunga and Manage 2010), and springs, dug wells in the Anuradhapura district (Mahagamage and Manage 2019). Nitrate concentrations were below the detection limit in all groundwater samples from Mahiyanganaya and the spring sample (Nikagolla et al. 2020). Phosphate concentrations in groundwater can rise because of agricultural activities. According to Paranagama (2013), Phosphorus levels in well water ranged from 61.1x10^{-6} to 80.25x10^{-6} mg L^{-1}, depending on the source. Organic phosphorous levels in well water ranged from 0.1 to 0.39 mg L^{-1}, according to Dhanapala et al. 2015).

Except for one sample from Medawachchiya, Padaviya, and Mahiyanganaya, phosphate levels in well water samples were below the detection limit (Nikagolla et al. 2020). The ammonium concentrations measured in well water collected from the Anuradhapura district were low and negligible, indicating that the water was not contaminated (Imbulana et al. 2020). Cyanobacteria are thought to have higher optimum temperatures for growth than other phytoplankton groups (Thomas *et al*. 2015). The optimal temperatures for the two species (*Microcystis aeruginosa* and *Cylindrospermopsis raciborskii*) were 28ºC and 37ºC (Xu et al. 2010). Our findings support the preference mean temperature for cyanobacterial growth of 28.09±0.89ºC, 28.11±0.94ºC in well water collected from CKDu low prevalent Rajanganaya and Galnewa, respectively, and 28.16±1.02 – 29.44±0.80ºC in well water collected from CKDu high prevalent areas. Spring water had a mean temperature of 27.19±0.89ºC and control area water had a temperature of 27.86±1.03ºC.

Table 2 MC-LR, CYN contamination levels and cyanobacteria (*Microcystis* spp. and *Cylindrospermopsis* spp.) cell densities in some dug wells in North Central, Uva, Eastern and Southern provinces. n = number of samples analysed.

CKDu prevalence	District	DS Division	*Microcystis* spp. cell density, cells mL^{-1}		Total MC-LR, $\mu g\ L^{-1}$ (by ELISA)		*Cylindrospermopsis* spp. cell density, cells mL^{-1}		Total CYN, $\mu g\ L^{-1}$ (by ELISA)	
			Mean ±SD	Range	Mean±SD	Range	Mean ±SD	Range	Mean ±SD	Range
High prevalent	Anuradhapura	Medawachchiya (n=4)	141±4	143 - 144	0.50±0.38	0.12 – 2.05	40±6	31 - 47	1.06±0.59	0.15 – 2.94
		Padaviya (n=104)	155±128	120 - 1456	0.71±0.09	1.00 – 3.30	42±6	25 - 57	1.28±0.17	1.00 – 7.60
		Kebithigollewa (n=9) (Springs)	NR	NR	NR	NR	NR	NR	NR	NR
	Polonnaruwa	Medirigiriya (n=84)	45±5	30 - 56	1.55±0.22	0.67 – 4.40	27±5	16 - 42	2.35±0.24	0.75 – 5.30
	Badulla	Mahiyanganaya (Girandurukotte GN) (n=12)	32±3	25 - 37	NR	NR	71±4	64 - 78	0.31±0.10	0.04 – 0.45
	Ampara	Dehiaththakandiya (n=15)	27±5	21 - 38	NR	NR	21±4	15 - 26	1.35±1.14	0.17 – 3.59
Low prevalent	Anuradhapura	Rajanganaya (n=49)	26±6	13 - 32	1.52±0.33	1.00 – 2.20	26±4	21 - 34	1.02±0.10	1.00 – 2.20
		Galnewa (n=46)	46±10	34 – 59	1.60±0.36	1.00 –2.20	36±9	25 – 48	1.45±0.09	1.00 –1.90
Control	Hambanthota	Hambanthota (n=62)	NR	NR	NR	NR	NR	NR	NR	NR

Several studies have found that nitrogen (N), phosphorus (P), or the availability of both influence phytoplankton growth and biomass (Elmgren and Larsson 2001; Cloern 2001; Bledsoe et al. 2004)). In terms of controlling algal production and bloom formation, it is widely assumed that nitrogen is the most important limiting nutrient in marine systems, whereas phosphorus is the most important limiting nutrient in freshwater systems (Nixon 1995). Because of the leaching of nitrogenous fertilizers from soils, nitrite, nitrate, and ammonia can be released into the environment (Burakham et al. 2004). Generally, nitrate levels in groundwater are low, but they can rise as a result of leaching or runoff from agricultural lands, as well as human and animal wastes (Magagamage and Manage 2019). Groundwater nitrate levels were higher in areas with high and low CKDu prevalence than in areas where the disease was not prevalent. Kebithigollewa spring water, on the other hand, had a higher nitrate concentration than wells in CKDu high prevalence areas in Padaviya, that the springs were surrounded by forest.

Information about the cell density of *Microcys*tis spp. and *Cylindrospermopsis* spp., and the concentrations of MC-LR and CYN found in well water samples is given in Table 2. The concentrations of CYN and MC-LR in dug well waters ranged from 0.31±0.10 to 2.35±0.24 µg L^{-1} and 0.50±0.38 to 1.60±0.36 µg L^{-1}, respectively. The CYN and MC-LR standard concentrations for drinking water quality were exceeded by 33% and 37%, respectively, in the dug wells. Mean cell density (±SD) of *Cylindrospermopsis* spp. in Padaviya was 42±6 cells mL^{-1} (range: 25-57 cells mL^{-1}), while mean CYN concentration (±SD) was 1.28±0.17 µg L^{-1} (range 1.00 – 7.60 µg L^{-1}). *Microcystis* spp. cell density (±SD) was found to be 155±128 cells mL^{-1} (range 120 – 1456 cells mL^{-1}), whereas MC-LR concentration (±SD) was 0.71±0.09 µg L^{-1} (range 1.00 - 3.30 µg L^{-1}). These values of CYN and MC-LR exceeded 29% and 25% respectively in wells above the WHO recommended concentrations. Medirigiriya area in Polonnaruwa district, the MC-LR and CYN concentrations were 1.55±0.22 µg L^{-1} (range 0.67 – 4.40 µg L^{-1}) and 2.35±0.24 µg L^{-1} (range 0.75 – 5.30 µg L^{-1}) respectively. This indicated that MC-LR and CYN concentrations of wells were 23 % and 27 %, respectively, above the WHO and SLSI drinking water quality standards. *Cylindrospermopsis* spp. was the dominant cyanobacteria with 21±4 cellsmL^{-1} (range: 15 – 26), CYN 1.35±1.14 µgL^{-1} (range: 0.17 – 3.59) in Dehiathtakandiya and 71±4 cells mL^{-1} (range 64 – 78 cells mL^{-1}), CYN 0.31±0.10 µg L^{-1} (range: 0.04 – 0.45 µg L^{-1}) Girandurukotte being the only cyanotoxin detected in well waters in these areas. Thirty-three percent of dug wells had levels of CYN that exceeded WHO and SLSI standards. MC-LR and CYN concentrations in CKDu low prevalent Rajanganaya and Galnewa were 1.52±0.33 µg L^{-1} (range 1.00 – 2.20 µg L^{-1}), 1.60±0.36 µg L^{-1} (range 1.00 – 2.20 µg L^{-1}), and 1.02±0.10 µg L^{-1} (range 1.00 – 2.20 µg L^{-1}), 1.45±0.09 µg L^{-1} (range 1.00 – 1.90 µg L^{-1}), respectively. Cell densities of *Microcystis* spp. 46±10 cells mL^{-1} (range 34–59 cells mL^{-1}) and *Cylindrospermopsis* spp. 36±9 cells mL^{-1} (range 25–48 cells mL^{-1}) were higher in Galnewa than in Rajanganaya, indicating that Galnewa is a more productive site. All dug wells in Rajanganaya and Galnewa areas exceeded the WHO's recommended MC-LR concentration for drinking water. According to the findings 4% of wells in the Rajanganaya area and 14% of wells in the Gallnewa area exceeded the WHO's recommended CYN concentration for drinking water. All 62 dug well water samples collected from the CKDu non-prevalence Hambanthota area, as well as the 9 spring water samples collected from the CKDu high prevalence area in Anuradhapura district, were found to be free of MC-LR and CYN. According to Madhushankha et al. (2016), cyanobacteria with the potential to produce MC and CYN were found in well waters collected from CKDu prevalent areas in Girandurukotte.

The spatial distribution patterns of MC-LR contamination (Figure 2) and CYN contaminations (Figure 3) are shown in ground well water from Padaviya DS, Medirigiriya DS, Rajanganaya DS, and Galnewa DS.

Padaviya DS division in Anuradhapura district, 34% and 33% of MC-LR and CYN concentrations in well water were recorded ranged between 1.0–3.3 µg L^{-1}, 1.0–7.6 µg L^{-1} from Parakramapura and Bogaswewa area, respectively (Figures 2a and 3a). In Medirigiya DS division of Polonnaruwa district, MC-LR and CYN concentrations in well water ranged between 0.67–4.40 $\mu g\ L^{-1}$ and 0.75–5.30 $\mu g\ L^{-1}$ in Ambagaswewa and Thalakolawewa, respectively (Figures 2b and 3b). In Rajanganaya DS, 14% of MC-LR contamination in well water ranged from 1.0 to 2.2 $\mu g\ L^{-1}$ (Figure 2c) and in Galnewa DS, 4% ranged from 1.0 to 2.2 $\mu g\ L^{-1}$ (Figure 2d), while 60.5% and 29% of CYN contaminations in well water ranged from 0.17 to 3.59 $\mu g\ L^{-1}$ (Figure 3e) and from 0.04 to 0.45 $\mu g\ L^{-1}$ in Dehiaththakandiya DS and Girandurukotte GN (Figure 3f).

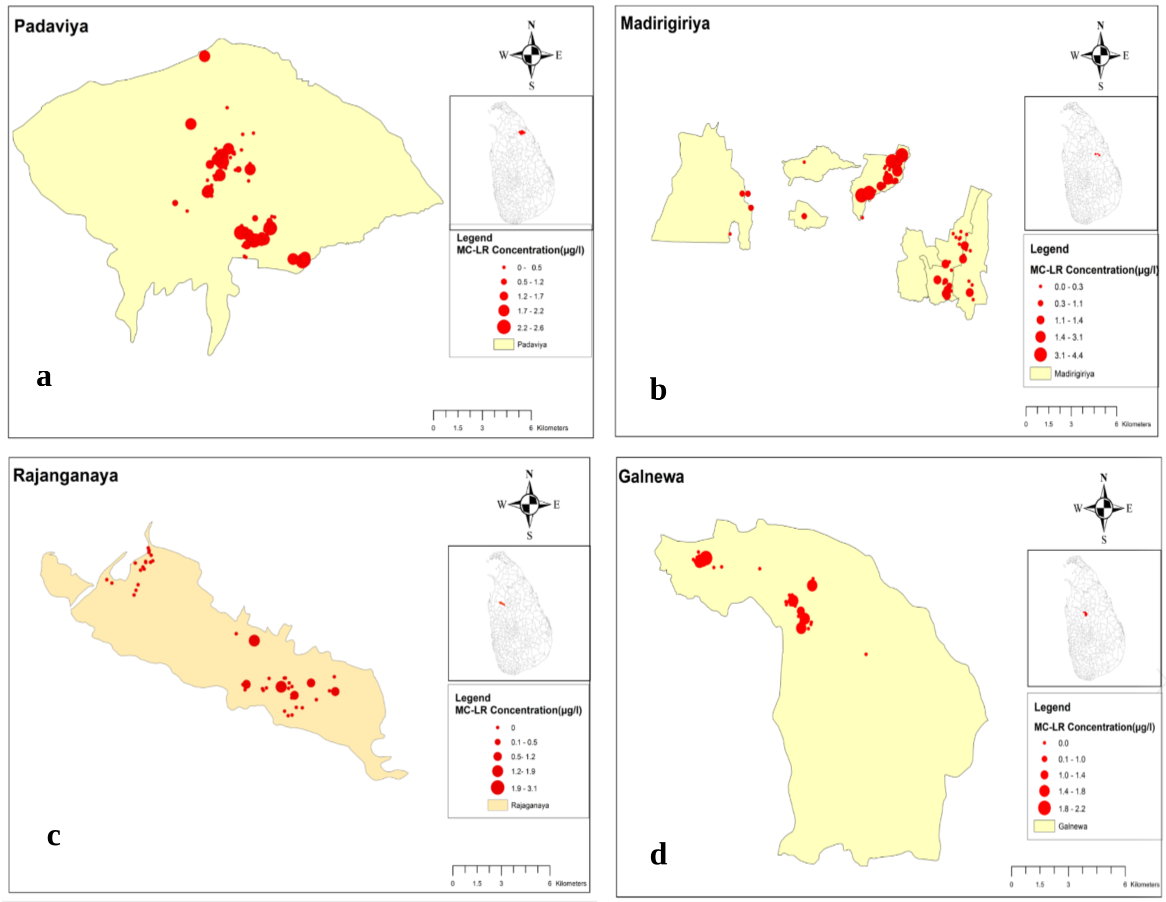

Fig 2. Spatial distribution pattern of MC-LR contamination in dug well water of (a) Padaviya DS (b) Medirigiriya DS (c) Rajanganaya DS (d) Galnewa DS

Factor analysis is a statistical method for describing variability in observed, correlated variables in terms of a smaller number of unobserved variables known as factors. Its goal is to identify the 'common factor' that the correlated variables are measuring (Hiyassat et al. 2016). A factor analysis was performed to determine the relationship between wells, cyanobacteria cell densities, cyanotoxin concentrations, and the number of CKDu patients. The analysis revealed that 95% of the CKDu high-prevalent, low-prevalent, and non-prevalent areas were classified into three clusters. Furthermore, springs were grouped into a single cluster (Figures 4a and 4b). As a result, CYN concentration, *Cylindrospermopsis* spp. cell density, MC-LR concentration, *Microcystis* spp. cell density, Nitrate-N, Nitrite-N, Ammonia-N, DO, temperature, electrical conductivity, pH, TH and TP were correlated with well water collected from CKDu high prevalence areas (Figures 4a and 4b). Pearson Correlation revealed a strong significant correlation between CYN concentration (p=0.000), MC-LR concentration (p=0.000), *Cylindrospermopsis* spp. cell density (p=0.000), and *Microcystis* spp. cell density (p=0.000) and the number of CKDu patients in high CKDu prevalence areas.

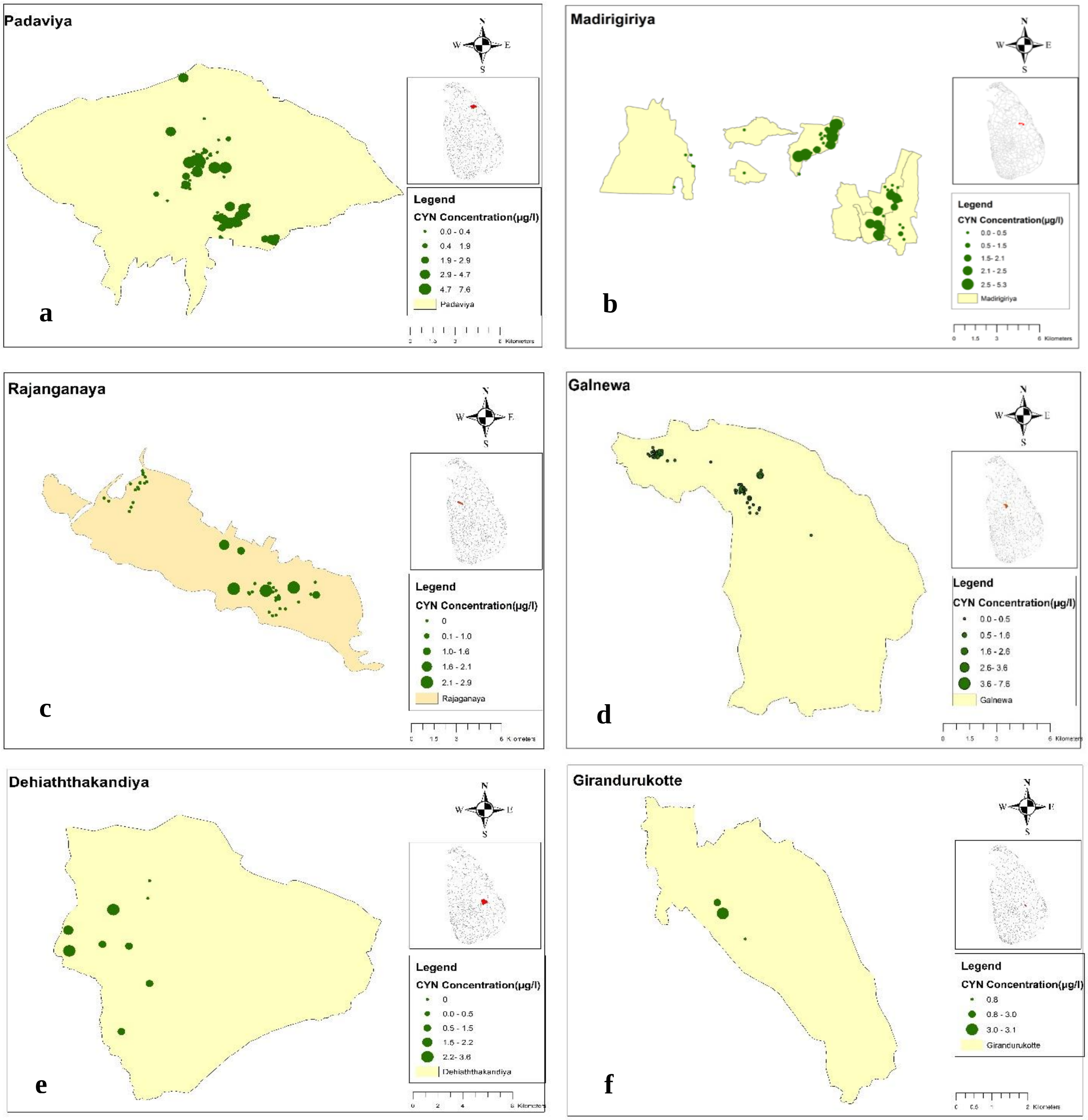

Fig 3. Spatial distribution pattern of CYN contamination in dug well water in (a) Padaviya DS (b) Medirigiriya DS (c) Rajanganaya DS (d) Galnewa DS (e) Dehiaththkandiya DS
(f) Girandurukotte GN

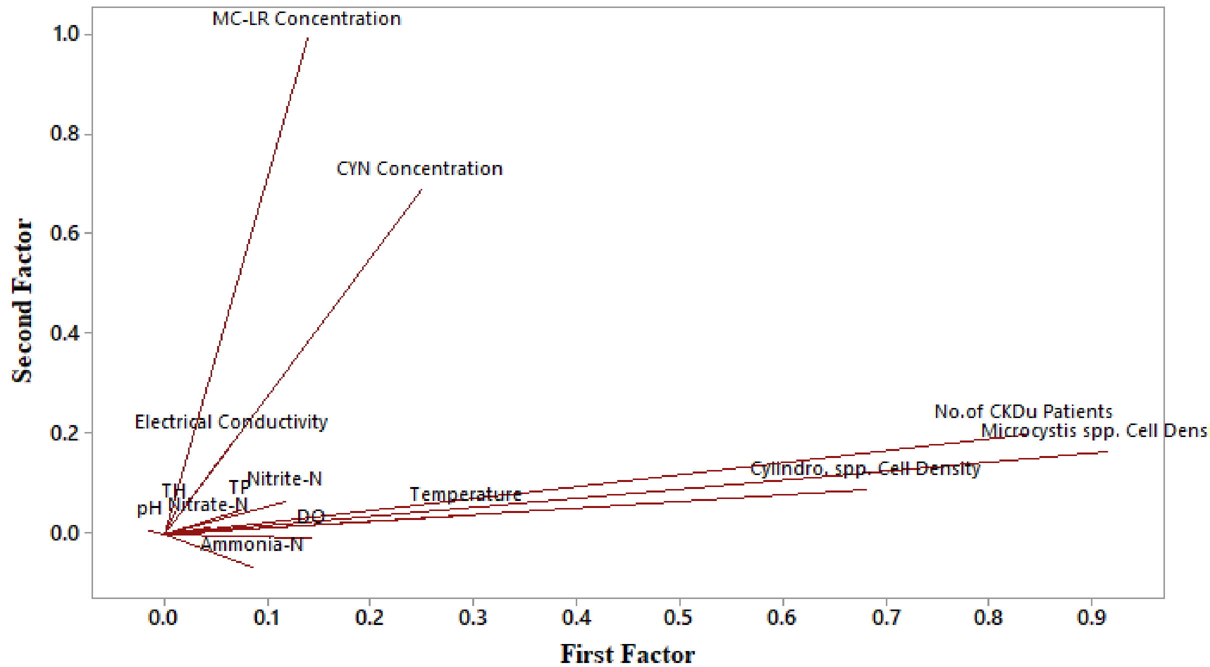

Fig 4a. Loading plot derived from factor analysis; source: own study

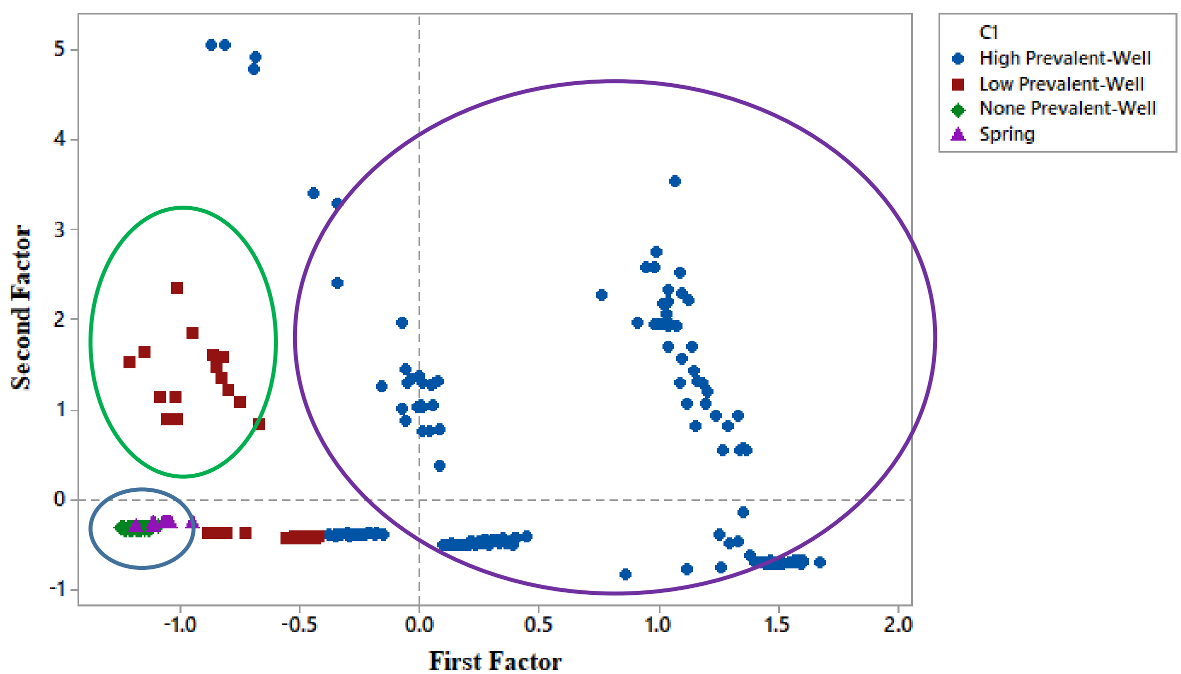

Fig 4b. Score plot derived from factor analysis; source: own study

CONCLUSIONS

In CKDu high prevalence areas, the WHO and SLSI desirable CYN and MC-LR contamination values were exceeded respectively by 27.65% and 29.26% of groundwater wells. Rural societies in the districts of Anuradhapura, Polonnaruwa, Ampara, and Badulla face serious problems due to a lack of safe drinking water, according to the study's final findings. It was also found that spring water in these areas is safe to drink. Findings of the present study revealed a link between cyanobacteria, cyanotoxins, and CKDu patients in CKDu-endemic areas. In Sri Lanka, however, more comprehensive studies are needed to confirm the relationship between MC-LR, CYN, and CKDu. This is particularly due to the reason that prevalence of CKDu has been relatively a recent incidence in some geographical areas of the country, where most reservoir favouring growth of cyanobacteria have been in existence over centuries.

REFERENCES

Abeysiri, H.A.S.N., K. Wanigasuriya & P. M. Manage 2018a. Cyanobacterial cyanotoxins and water quality in well water at CKDU endemic Girandurukotte and Dehiaththakandiya, Sri Lanka, pp.155, International Conference on Multidisciplinary Approaches Proceedings 5, ICMA, University of Sri Jayewardenepura, Sri Lanka.

Abeysiri, H.A.S.N., K. Wanigasuriya & P. M. Manage 2018b. Detection of Cylindrospermopsin from well water in CKDu endemic Padaviya, Medawachciya and Kebithigollewa area in Anuradhapura District, Sri Lanka, pp.32, International Symposium on Water Quality and Human Health: Challenges Ahead Proceedings 5, University of Peradeniya, Sri Lanka.

Bledsoe, E.L., E.J. Phlips, C.E. Jett & K.A. Donnelly 2004. The relationships among phytoplankton biomass, nutrient loading and hydrodynamics in an inner-shelf estuary. Ophelia 58(1): 29-47. https://doi.org/10.1080/00785236.2004.10410211

Burakham, R., M. Oshima, K. Grudpan & S. Motomizu 2004. Simple flow-injection system for the simultaneous determination of nitrite and nitrate in water samples. Talanta 64(5): 1259-1265. https://doi.org/10.1016/j.talanta.2004.03.059

Cerdas, M. 2005. Chronic kidney disease in Costa Rica. Kidney International 68: S31-S33. https://doi.org/10.1111/j.1523-1755.2005.09705.x

Chandrajith, R., S. Nanayakkara, K. Itai, T.N.C. Aturaliya, C.B. Dissanayake, T. Abeysekera, K. Harada, T. Watanabe & A. Koizumi 2011. Chronic kidney diseases of uncertain etiology (CKDu) in Sri Lanka: geographic distribution and environmental implications. Environmental Geochemistry and Health 33(3): 267-278. https://doi.org/10.1007/s10653-010-9339-1

Chatchawan, T., Y. Peerapornpisal & J. Komárek 2011. Diversity of cyanobacteria in man-made solar saltern, Petchaburi Province, Thailand–a pilot study. Fottea 11(1): 203-214.

Chen, Y., J. Xu, Y. Li, & X. Han 2011. Decline of sperm quality and testicular function in male mice during chronic low-dose exposure to microcystin-LR. Reproductive Toxicology 31(4): 551-557. https://doi.org/10.1016/j.reprotox.2011.02.006

Chen, P., L. Zhu, S. Fang, C. Wang & G. Shan 2012. Photocatalytic degradation efficiency and mechanism of microcystin-RR by mesoporous Bi2WO6 under near ultraviolet light. Environmental Science and Technology 46(4): 2345-2351. https://doi.org/10.1021/es2036338

Cloern, J.E. 2001. Our evolving conceptual model of the coastal eutrophication problem. Marine Ecology Progress Series 210: 223-253. doi:10.3354/meps210223

Dhanapala, D.M.K.M., H.B. Asanthi & M.H.J.P. Gunarathne 2015. Exposure analysis of drinking and dietary contaminants in a selected population, Padaviya, Anuradhapura. International Journal of Multidisciplinary Studies (IJMS) 2(1): 43-50. http://dr.lib.sjp.ac.lk/handle/123456789/3333

Edirisinghe, E.A.N.V., H. Manthrithilake, H.M.T.G.A. Pitawala, H.A. Dharmagunawardhane & R.L. Wijayawardane 2018. Geochemical and isotopic evidences from groundwater and surface water for understanding of natural contamination in chronic kidney disease of unknown etiology (CKDu) endemic zones in Sri Lanka. Isotopes in Environmental and Health Studies 54(3): 244-261. https://doi.org/10.1080/10256016.2017.1377704

Elmgren, R and Larsson, U. 2001. Nitrogen and the Baltic Sea: managing nitrogen in relation to phosphorus. The Scientific World Journal 1: 371-377. https://doi.org/10.1100/tsw.2001.291

Faassen, E.J. & M. Lürling 2013. Occurrence of the microcystins MC-LW and MC-LF in Dutch surface waters and their contribution to total microcystin toxicity. Marine Drugs 11(7): 2643-2654. https://doi.org/10.3390/md11072 643

Falconer, I.R. 1999. An overview of problems caused by toxic blue–green algae (cyanobacteria) in drinking and recreational water. Environmental Toxicology 14(1): 5-12. https://doi.org/10.1002/(SICI)1522-7278(1999 02)14:1<5::AID-TOX3>3.0.CO;2-0

Falconer, I.R. & A.R. Humpage 2005. Health risk assessment of cyanobacterial (blue-green algal) toxins in drinking water. International Journal of Environmental Research and Public Health 2(1): 43-50. https://doi.org/10.3390/ijerph2005 010043

Fleming, L.E., C. Rivero, J. Burns, C. Williams, J.A. Bean, K.A. Shea & J. Stinn 2002. Blue green algal (cyanobacterial) toxins, surface drinking water, and liver cancer in Florida. Harmful Algae 1 (2): 157–168. https://doi.org/ 10.1016/S1568-9883(02)00026-4

Hayashi, N.R., Y. Peerapornpisal, H. Nishihara, M. Ishii, Y. Igarashi & T. Kodama 1994. Isolation and cultivation of thermophilic cyanobacteria from hot springs of northern Thailand. Journal of Fermentation and Bioengineering 78(2): 179-181. https://doi.org/10.1016/0922-33 8X(94)90260-7

Hettiarachchi, I.U. & P. M. Manage 2014. Cyanobacterial cell density & intracellular Microcystin-LR levels in drinking/ irrigation reservoirs in Anuradhapura, Sri Lanka, pp. 42, Environment and Natural Resources International Conference Proceedings 1. ENRIC, Thailand.

Hiyassat, M.A., M.A. Hiyari & G.J. Sweis 2016. Factors affecting construction labour productivity: a case study of Jordan. International Journal of Construction Management 16(2): 138-149. https://doi.org/ 10.1080/15623599.2016.1142266

Idroos, S.F. & P.M. Manage 2014. Seasonal occurrence of Microcystin-LR with respect to physico-chemical aspects of Beira lake water. International Journal of Multidisciplinary Studies, 1 (2).

Illeperuma, O.A., H.A. Dharmagunawardhane & K.P.R.P. Herarh 2009. Dissolution of aluminium from substandard utensils under high fluoride stress: A possible risk factors for chronic renal failures in the north-central province. Journal of the National Science Foundation of Sri Lanka 37: 219-222.

Imbulana, S., K. Oguma & S. Takizawa 2020. Evaluation of groundwater quality and reverse osmosis water treatment plants in the endemic areas of Chronic Kidney Disease of Unknown Etiology (CKDu) in Sri Lanka. Science of the Total Environment 745: 140716. https://doi .org/10.1016/j.scitotenv.2020.140716

Jayasekara, J.M., D.M. Dissanayake, S.B. Adhikari & P. Bandara 2013. Geographical distribution of chronic kidney disease of unknown origin in North Central Region of Sri Lanka. Ceylon Medical Journal 58(1): 6-10.

Jayasumana, C., S. Gunatilake & P. Senanayake 2014. Glyphosate, hard water and nephrotoxic metals: are they the culprits behind the epidemic of chronic kidney disease of unknown etiology in Sri Lanka. International Journal of Environmental Research and Public Health 11(2): 2125-2147. https://doi.org/10.3390 /ijerph110202125

Kamel, E.G. & E.L. Minshawy. 2010. Environmental factors incriminated in the development of end stage renal disease in El-Minia Governorate, Upper Egypt. Nephro-Urology Monthly 2(3): 431-437.

Kulasooriya, S.A. 2011. Cyanobacteria: pioneers of planet earth. Ceylon Journal of Science (Biological Science) 40(2): 71-88.

Kumari, M.K.N., R.M.C.P. Rathnayake, K.M.A. Kendaragama, M.H.J.P. Gunarathna & K.G.S. Nirmanee 2016. Drinking water quality in chronic kidney disease of unknown Aetiology (CKDu) prevalent and non-prevalent areas in Giradurukotte, Sri Lanka. International Journal of Advance Agriculture Environment Engineering 3(1): 57-60. http://dx.doi.org/ 10.15242/IJAAEE.ER0116026

Lambert, T.W., C.F.B. Holmes & S.E. Hrudey 1994. Microcystin class of toxins: health effects and safety of drinking water supplies. Environmental Reviews 2(2): 167-186. https://doi.org/10.1139/a94-011

Lankoff, A., W.W.Carmichael, K.A. Grasman, & M. Yuan 2004. The uptake kinetics and immunotoxic effects of microcystin-LR in human and chicken peripheral blood lymphocytes in vitro. Toxicology 204(1): 23-40. https://doi.org/10.1016/j.tox.2004.05.016

Lawton, L.A., A. Welgamage, P.M. Manage & C. Edwards 2011. Novel bacterial strains for the removal of microcystins from drinking water. Water Science and Technology 63(6): 1137-1142. https://doi.org/10.2166/wst.2011.352

Lefebvre, B.R. 2013. The accumulation of the cyanobacterial toxin, microcystin, in cherry tomato (*Solanum lycopersicum*) and bush bean (*Phaseolus vulgaris*) plants. *Center for Freshwater Biology*. 1, University of New Hampshire, Durham, USA

Lewitus, A.J., R.A. Horner, D.A. Caron, E. Garcia-Mendoza, B.M. Hickey, M. Hunter, D.D. Huppert, R.M. Kudela, G.W. Langlois, J.L. Largier & E.J. Lessard 2012. Harmful algal blooms along the North American west coast region: History, trends, causes, and impacts. Harmful Algae 19:133-159. https://doi.org /10.1016/j.hal.2012.06.009

Lone, Y., R.K. Koiri & M. Bhide 2015. An overview of the toxic effect of potential human carcinogen Microcystin-LR on testis. Toxicology Reports 2: 289-296. https://doi.org/ 10.1016/ j.toxrep.2015.01.008

Machiraju, R.S., K. Yaradi, S. Gowrishankar, K.L. Edwards, S. Attaluri, F. Miller, A.P. Grollman, & K.G. Dickman 2009. Epidemiology of Udhanam endemic nephropathy. Journal of American Society of Nephrology 20: 643A.

Madhushankha, L., M.A. Dhammika, P. Charitha, A. Tilak & G. Lishantha 2016. Cyanobacteria and cyanotoxins in well waters of the Girandurukotte, CKDu endemic area in Sri Lanka: do they drink safe water? Journal of Ecotechnology Research 18(1), pp.17-21. https://doi.org/10.11190/jer.18.17

Mahagamage, M.G.Y.L. & P.M. Manage 2014. Water quality index (CCME-WQI) based assessment study of water quality in Kelani River basin, Sri Lanka, pp.199-204, Environment and Natural Resources International Conference Proceedings 1, ENRIC, Mahidol University, Thailand.

Mahagamage, M.G.Y.L. & P.M. Manage 2019. Water quality and microbial contamination status of Madawachchiya, Padaviya and Kebithigollewa areas in Anuradhapura District, Sri Lanka. Journal of Water and Land Development 42(1): 1-11. 10.2478/jwld-2019-0039

Manage, P.M., 2019. Cyanotoxins: A hidden cause of Chronic Kidney Disease of unknown etiology (CKDu) in Sri Lanka - A review. Sri Lanka Journal of Aquatic Sciences, 24(1): 1–23. http://doi.org/10.4038/sljas.v24i1.7562

Manage, P.M., C. Edwards, B.K. Singh & L.A. Lawton 2009. Isolation and identification of novel microcystin-degrading bacteria. Applied and Environmental Microbiology 75(21): 6924-6928. https://doi.org/10.1128/AEM.01928-09

Mullineaux, C.W., V. Mariscal, A. Nenninger, H. Khanum, A. Herrero, E. Flores & D.G. Adams 2008. Mechanism of intercellular molecular exchange in heterocyst-forming cyanobacteria. The EMBO Journal 27(9): 1299-1308. https://doi.org/10.1038/emboj.2008.66

Newcombe, G. & B. Nicholson 2002. Treatment options for the saxitoxin class of cyanotoxins. Water Science and Technology: Water Supply 2(5-6): 271-275. https://doi.org/10.2166/ws. 2002.0179

Nikagolla, C., K.T. Meredith, L.A. Dawes, R.B. Banati & G.J. Millar 2020. Using water quality and isotope studies to inform research in chronic kidney disease of unknown aetiology endemic areas in Sri Lanka. Science of the Total Environment, 745: 140896. https://doi.org /10.1016/j.scitotenv.2020.140896

Nixon, S.W. 1995. Coastal marine eutrophication: a definition, social causes, and future concerns. Ophelia 41(1): 199-219. https://doi.org/10.10 80/00785236.1995.10422044

O'Donnell, J.K., M. Tobey, D.E. Weiner, L.A. Stevens, S. Johnson, P. Stringham, B. Cohen & D.R. Brooks 2011. Prevalence of and risk factors for chronic kidney disease in rural Nicaragua. Nephrology Dialysis Transplantation 26(9): 2798-2805.

Orantes, C.M., R. Herrera, M. Almaguer, E.G. Brizuela, L. Núñez, N.P. Alvarado, E. Jackeline Fuentes, H.D. Bayarre, J.C. Amaya, D.J. Calero & X.F. Vela 2014. Epidemiology of chronic kidney disease in adults of Salvadoran agricultural communities. MEDICC Review 16: 23-30.

Paerl, H.W. & J. Huisman 2008. Blooms like it hot.

Science 320(5872): 57-58.

Paranagama, P.A. 2013. Potential link between ground water hardness, arsenic content and prevalence of CKDu, pp. 1-8, Symposium on chronic kidney disease of uncertain origin (CKDu): A scientific basis for future action Proceedings, University of Peradeniya, Sri Lanka.

Perera, I., S.R. Subashchandrabose, K. Venkateswarlu, R. Naidu & M. Megharaj 2018. Consortia of cyanobacteria/microalgae and bacteria in desert soils: an underexplored microbiota. Applied Microbiology and Biotechnology 102(17): 7351-7363.

Piyathilaka, M.A.P.C., M.M. Pathmalal, K.H. Tennekoon, B.G.D.N.K., De Silva, S.R. Samarakoon & S. Chanthirika 2015. Microcystin-LR-induced cytotoxicity and apoptosis in human embryonic kidney and human kidney adenocarcinoma cell lines. Microbiology 161(4): 819-828. https://doi.org/10.1099/mic.0.000046

Sadeepa H.D.D., K.A. Sirisena & P.M. Manage 2019. Isolation and characterization of industrially important enzyme producing bacteria from hot springs of Sri Lanka, International Conference of Multidisciplinary Approaches Proceedings, ICMA, University of Sri Jayewardenepura, Sri Lanka.

Sethunge S. & P.M. Manage 2010. Nuisance algae in water supply projects in Sri Lanka, Sustainable Built Environment Conference Proceedings, ICSBE, Kandy, Sri Lanka.

SLS 714 (Sri Lanka Standards Institution) 2013. Specification for Potable Water, Colombo: SLSI. SLS 614: 2013 UDC 663.6.

Thomas, M.K. & E. Litchman 2016. Effects of temperature and nitrogen availability on the growth of invasive and native cyanobacteria. Hydrobiologia 763(1): 357-369. DOI: 10.1007/s10750-015-2390-2

Torokne, A., A. Palovics & M. Bankine 2001. Allergenic (sensitization, skin and eye irritation) effects of freshwater cyanobacteria—experimental evidence. Environmental Toxicology 16(6): 512-516. https://doi.org/10.1002/tox.10011

Towner, R.A., S.A. Sturgeon & K.E. Hore 2002. Assessment of in vivo oxidative lipid metabolism following acute microcystin-LR-induced hepatotoxicity in rats. Free Radical Research 36(1): 63-71. https://doi.org/10.1080/10715760210171

Wijesekara W.A.M.A. & P.M. Manage 2017. In vitro screening of antibacterial and phytochemical properties of hot water spring cyanobacterium *Lynbya* sp., pp. 38, NARA Scientific Sessions Conference Proceedings, Colombo.

Wijewickrama, M.M. & PM. Manage 2019. Accumulation of microcystin-LR in grains of two rice varieties (*Oryza sativa* L.) and a leafy vegetable, *Ipomoea aquatica*. Toxins 11(8): 432. https://doi.org/10.3390/toxins11080432

Wijetunge S., N.V. Ratnatunga, T.D. Abeysekera, A.W. Wazil, M. Selvarajah 2015. Endemic chronic kidney disease of unknown etiology in Sri Lanka: Correlation of pathology with clinical stages. Indian Journal of Nephrology 25(5): 274-280. DOI: 10.4103/0971-4065.145095

World Health Organization 2016. Cyanobacterial Toxins: Microcystin-LR in Drinking-Water, 2nd ed.; World Health Organization: Geneva, Switzerland, 2003; Available online:http://www.who.int/water_sanitation_health/dwq/chemicals/cyanobactoxis.pdf (accessed on 21 January 2016).

Xu, H., H.W. Paerl, B. Qin, G. Zhu & G. Gaoa 2010. Nitrogen and phosphorus inputs control phytoplankton growth in eutrophic Lake Taihu, China. Limnology and Oceanography 55(1): 420-432. http://doi.org/10.4319/lo.2010.55.1.0420

Zhang, H., Q. Huang, Z. Ke, L. Yang, X. Wang & Z. Yu 2012. Degradation of Microcystin-LR in water by glow discharge plasma oxidation at the gas–solution interface and its safety evaluation. Water Research 46(19): 6554-6562. https://doi.org/10.1016/j.watres.2012.09.041