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Short Communication

Microcystin-LR Contamination Status and Physico-Chemical Water Quality Parameters of Five Selected Recreational Water Bodies in Sri Lanka

P.C. Piyathilaka[1*] and P.M. Manage[2]

ABSTRACT

Microcystin-LR (MC-LR) is the most toxic and commonly encountered cyanotoxin variant in aquatic systems. MC-LR is a hepatotoxin, but evidences suggest that it might also induce kidney injury. There is an increasing trend in mortality due to chronic kidney disease (CKD) in Sri Lanka where the underlying cause of CKD remains complicated. Cyanobacterial toxins is considered as one of the possible reasons implicated in the aetiology of CKDu in Sri Lanka. A significant source of cyanobacterial toxin exposure is recreational use of contaminated freshwater bodies. Therefore, it was a timely need to assess MC-LR contamination status. Thus, in the present study, freshwater bodies of Beira, Boralesgamuwa, Kandy, Kurunegala and Hot water springs in Kinniya were sampled. Triplicate surface water samples were collected from each water body. Surface water temperature, pH, conductivity and dissolved oxygen (DO) were measured at the site itself and chemical analyses of nitrate, total phosphate and chlorophyll-a were carried out. Analysis of MC-LR contamination was done by Enzyme Link Immuno Sorbent Assay (ELISA). In this study, cyanotoxin MC-LR was not reported from Boralesgamuwa whereas all the other water bodies showed MC-LR contamination. The concentrations of MC-LR from the highest to the lowest were reported respectively from Beira lake (2198 ppb), Kandy lake (103 ppb), Kurunegala lake (75.6 ppb) and the Hot water springs (0.34 ppb). Results of the present study revealed that pH, nitrate, total phosphate and chlorophyll-a contents showed significant positive correlation with the MC-LR concentration of each water body ($p<0.05$) while surface water temperature, DO and conductivity did not show significant correlation to MC-LR contamination.

Keywords: Cyanotoxin, ELISA, Exposure, Microcystin, Recreational, Water quality

INTRODUCTION

People and animals are commonly exposed to cyanobacterial toxins produced by harmful algal blooms (CyanoHABs) through ingesting improperly treated or untreated drinking water contaminated with cyanotoxins. Indirect potential exposure sources include contaminated dietary supplements (Gilroy *et al*., 2000; Hawkins *et al*., 1997) or fish harvested from lakes with current CyanoHABs (Kann, 2008). Cyanotoxins can be categorized into three broad groups according to their chemical structures: cyclic peptides, alkaloids, and lipopolysaccharides (Fromme, 2000).

[1]*Department of Biotechnology, Faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka, Gonawila 60170, Sri Lanka*
[2]*Centre for Water Quality and Algae Research, Department of Zoology, Faculty of Applied Sciences, University of Sri Jayewardenepura, Nugegoda, Sri Lanka*
**mapcpiyathilaka@gmail.com*

The most frequently found cyanobacterial toxins in blooms (MCs) from fresh and brackish waters are cyclic peptide toxins belonging to the microcystin and nodularin families; because these toxins have high molecular weights, chemical stability and water solubility, they are persistent in surface water bodies, which has important inferences for human exposure (Chorus, 2001; Conti *et al*., 2005).

Microcystin-LR (MC-LR) is the most toxic cyanotoxin variant most commonly encountered in contaminated aquatic systems (Jones and Orr, 1994). MC-LR is a potent hepatotoxin, but increasing evidences suggest that it might also induce kidney injury (Piyathilaka *et al*., 2015). There is an increasing trend in mortality and morbidity due to chronic kidney disease (CKD) in Sri Lanka where the underlying cause of CKD remains complicated.

Cyanobacterial toxins is one of the possible causative agents implicated in the aetiology of CKDu in Sri Lanka. A significant source of cyanobacterial toxin exposure is recreational use of contaminated fresh water bodies. Recreational activities in water bodies that experience toxin-producing cyanobacterial blooms can generate aerosolized cyanotoxins, making inhalation a potential route of exposure (Backer *et al*., 2010).

Potentially toxic blooms of cyanobacteria occur in inland waters which are mostly reservoirs used as supplies of drinking water or for recreation in Sri Lanka (Hettiarachchi *et al*., 2014). Problems associated with filter clogging in water purification plants as well as bad odors and tastes in drinking water have been detected; however, toxicity has scarcely been evaluated (Conti *et al*., 2005). According to the guidelines for recreational water bodies, if the occurrence of cyanobacteria is found at a site, those using the site must be warned about and received information on the cyanobacteria. The guidelines advise microcystin analysis only as an option for narrowing down the health risk assessment, recommending closure at total microcystin concentrations above 100 µg/L (ppb). Further, it advises to include analysis of total phosphorus concentration as a measure of a water body's carrying capacity for the mass development of cyanobacteria (Manage *et al*., 2009).

The concentration of chlorophyll a (Chl a) at which the guidelines specify warning notices be issued is 40 µg/L (if cyanobacteria are dominant in the phytoplankton) while a concentration of 150 µg/L means temporary closure of a site. According to the guidelines, the critical threshold concentration of total phosphorus (TP) for mass development of cyanobacteria is 40 µg/L (Frank, 2002).

Therefore, it was a timely need to assess the MC-LR contamination status of major recreational water bodies in Sri Lanka. Thus, Beira, Boralesgamuwa, Kandy, and Kurunegala lakes and Hot water springs in Kinniya were sampled to analyse MCs contamination status.

MATERIALS AND METHODS

Beira, Boralesgamuwa, Kandy and Kurunegala lakes and Hot water springs in Kinniya were selected to conduct the study taking into consideration their extensive use in recreational activities. Locations of the reservoirs in Sri Lanka are indicated in Figure 1. Triplicate surface water samples were collected at a depth of 10 cm from the surface of each reservoir into sterile polypropylene bottles from at least three sample locations in each water body. Plankton samples were collected using 55 µm mesh size plankton net by filtering 100 L of surface water.

Physico-Chemical water quality Parameters such as surface water temperature, pH, conductivity and dissolved oxygen (DO) of water samples were measured at the site itself. Collected water samples were subjected to analysis of nitrate, total phosphate and chlorophyll-a in the laboratory. Analysis of MC-LR was done by Enzyme Link Immuno Sorbent Assay (ELISA).

With regards to chemical analysis, water samples were subjected to nitrate, total phosphate and chlorophyll-a analysis. Total nitrate concentration (mg/L) and total phosphate concentration (mg/L) were measured according to standard spectrophotometric methods described previously (APHA, 1995) where chlorophyll-a concentration was measured within 24 h according to a standard spectrophotometric method described bymArvola (1981). Quantitative analyses of MCs were done by Enzyme Link Immuno Sorbent Assay (ELISA).

Freeze-thawed water samples were used for ELISA analysis to quantify the MC-LR. Lyophilization of available cyanobacterial cells were achieved by three subsequent freeze-thaw cycles. Then the sample was filtered through 0.22 µm Nylon syringe filters. Assay was done according to the manufacturer's instructions.

The average optical density value (AOD) of each calibrator, control and sample was calculated. Percentage of corrected optical density (% Bo) values were calculated using the following equation,

% Bo = [(AOD calibrator, control or sample) / (AOD negative control)] x 100

% Bo values were graphed on the Y axis against its Microcystin concentration on the X axis and the calibration curve was created by drawing the best fitted line through the calibrator points. Microcystin concentration in the samples was calculated with the use of corresponding AOD value of each sample in respective calibration curve.

RESULTS

Table 1 shows the mean physico-chemical water quality parameters of the recreational water bodies subjected to the present study. Temperature of the reservoirs varied between 25.2 (Kandy lake) to 39.2 ºC (Hot water springs), and lowest recorded pH was 5.46 in Beira lake and the highest was in Boralesgamuwa (7.31).

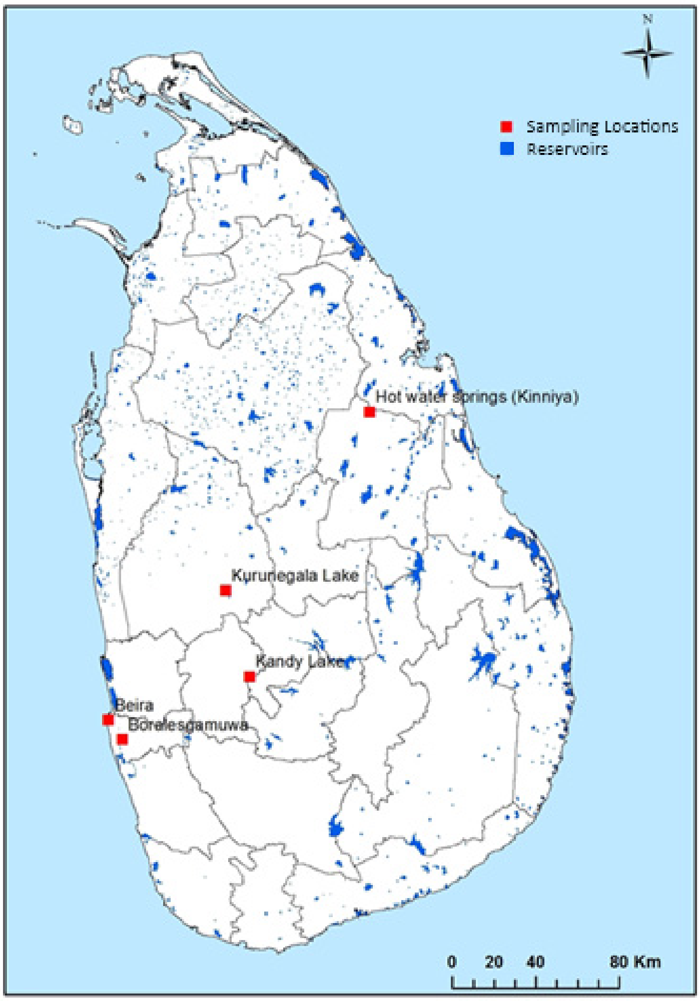

Figure 1. Sampling locations of selected reservoirs and hot water springs for the present study

Table 1. Physico-chemical water quality results of five recreational water bodies

Water body	Temperature (˚C)	pH	DO (mg/L)	Conductivity (µS/cm)	Total NO_3^- (mg/L)	Total PO_4^{3-} (mg/L)	Chlorophyll-a (mg/L)	MC-LR (ppb)
Beira lake	28.6±0.12	5.46±0.01	6.97±0.02	389.2±2.31	1.56±0.01	2.65±0.01	25.61±0.20	2198.00±0.14
Boralesgamuwa lake	28.7±0.19	7.31±0.05	7.27±0.03	298.1±1.26	0.00±0.01	1.03±0.01	26.33±0.01	0.00±0.01
Kandy lake	25.2±0.10	7.14±0.10	8.11±0.05	209.3±0.98	0.66±0.01	1.15±0.01	22.65±0.21	103.00±0.23
Kurunegala lake	29.6±0.25	7.04±0.23	6.58±0.02	145.7±1.52	0.08±0.01	0.88±0.01	10.64±0.17	75.60±0.21
Hot water springs	39.2±0.21	6.45±0.16	4.34±0.02	215.3±1.20	0.00±0.01	0.31±0.01	10.36±0.02	0.34±0.03

Values indicated with ± SD

DO and conductivity values ranged between 4.34 mg/L to 8.11 mg/L and 145.7 µS/cm to 389.2 µS/cm respectively. The highest total nitrate was detected in Beria lake (1.56 mg/L) where Kandy lake (0.66 mg/L) and Kurunegala lake (0.08mg/L) followed in the descending order. In Boralesgamuwa lake and Hot water springs, the total nitrate concentration remained below 0.01mg/L.

The highest total phosphate was detected in Kandy lake (2.15 mg/L), then Boralesgamuwa lake (2.03 mg/L), Beria lake (1.65 mg/L), Kurunegala lake (0.88 mg/L) and Hot water springs (0.31 mg/L) respectively. The highest chlorophyll-a concentration was recorded in Boralesgamuwa lake (26.33 mg/L) followed by Beira lake (25.61 mg/L) and Kandy lake (22.65 mg/L) respectively. In the other water bodies, chlorophyll- a concentration ranged between 10.64 mg/L to 10.36 mg/L. The cyanotoxin MC-LR was not reported from Boralesgamuwa whereas all the other water bodies showed MC-LR contamination. The concentrations of MC-LR from the highest to the lowest were detected in Beira lake, Kandy lake, Kurunegala lake and the Hot water springs respectively (Table 1).

Statistical analysis of water quality data of the five recreational water bodies tested revealed that among the water quality parameters, pH, total nitrate, total phosphate and chlorophyll-a concentrations showed significant positive correlation to the MC-LR concentration of each water body ($p<0.05$, Pearson correlation analysis). In contrast, temperature, DO content and conductivity did not show a positive correlation to the MC-LR concentration ($p>0.05$, Pearson correlation analysis).

DISCUSSION

Cyanobacteria produce toxins which are hazardous to human populations; and the humans can be exposed to such toxins through recreational activities (Frank, 2002). The production of cyanobacterial hepatotoxins (microcystins) and their presence in drinking and recreational waters represent a growing danger to human and animal health (Wood *et al.*, 2006). Risk management deals with the probability that a certain exposure to toxins will lead to specific health outcomes (Backer *et al.*, 2010; Conti *et al.*, 2005). Genera of cyanobacteria known to possess cyanotoxins producing strains are *Microcystis, Planktothrix, Anabaena, and Aphanizomenon*. The toxins include hepatotoxic peptides (microcystins), cytotoxic and neurotoxic alkaloids (cylindrospermopsin, anatoxins, and saxitoxins) and lipopolysaccharides (LPS), which have been known to cause allergic reactions, poisonings, and through hemodialysis, even deaths (Frank, 2002).

Therefore, the present study was carried out to examine the MC-LR contamination status of five recreational water bodies in Sri Lanka along with some selected physico-chemical water quality parameters. According to the findings, the MC-LR contamination of Beira, Kurunegala and Kandy lakes were well above the maximum level suggested by WHO (WHO, 2003) for recreational purposes (2–10 ppb) and, in addition, they were above the higher limit proposed for recreational water by Germany (100 ppb) (Chorus *et al.*, 2000). However, Boralesgamuwa lake and Hot water springs were having very low contamination levels with respect to recommended levels given for MC-LR in recreational waters.

Profusely, the literature highlights that elevated levels of physico-chemical water quality parameters such as total nitrate, total phosphate and chlorophyll-a concentrations are direct limiting factors of cyanobacterial abundance (Arvola, 1981; Chorus *et al.*, 2000; Pathmalal, and Piyasiri, 1995). If a recreational site is already been contaminated with toxin producing cyanobacteria, the aim of measures to minimize is not to close sites, but rather to restore water quality with desirable levels. In most water bodies, this can be achieved by keeping total phosphorus concentrations below 0.01 mg/L. Cyanobacterial densities rarely reach hazardous levels even in water bodies containing 0.02–0.03 mg/L total phosphorus (Chorus *et al.*, 2000). Among the water quality parameters tested, pH, total nitrate, total phosphate and chlorophyll-a concentrations showed positive correlation with MC-LR contamination status of each recreational water body.

CONCLUSION

The high microcystin content detected in the recreational water bodies tested represents a serious risk for users' health. The Alert Level framework proposed by WHO should be adapted for the other water bodies with moderately high concentrations of microcystin levels, though the contamination levels are still below the threshold proposed by WHO. Protective measures should be promoted to avoid possible extreme effects of microcystin on humans and animals due to chronic and/or acute recreational exposures.

Furthermore, physico-chemical water qualities such as pH, total nitrate, total phosphate and chlorophyll-a contents which act as factors with positive correlation to microcystin level revealed by the present study should be managed in low levels to avoid reaching non-desirable levels of MC-LR contaminations in the recreational water bodies studied.

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