Microcystin-LR contamination in Nile tilapia (*Oreochromis niloticus*) in some water bodies in Sri Lanka

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Received: 04.01.2022 Revised: 26.03.2022 Accepted: 27.04.2022 Online: 15.09.2022

Abstract Microcystin-LR (MC-LR) is a cyanotoxin produced by certain cyanobacteria species. It is toxic to humans and animals. Several studies have demonstrated that cyanotoxins accumulate throughout the food chain, eventually reaching high levels in freshwater fish. The Nile tilapia (*Oreochromis niloticus*) is the most popular freshwater fish in Sri Lanka and in most Asian countries. The current study sought to determine MC-LR concentration in Nile tilapia collected from thirteen freshwater reservoirs; Nallachchiya, Galkulama, Anakattiya, Padaviya, Nachchaduwa and Kalawewa in Anuradhapura District, Parakrama Samudraya, Halmilla, Kaudulla and Ambagaswewa in Polonnaruwa District, Muwapatigewela in Ampara District, and Ulhitiya and Rathkinda in Badulla District. The fish and water samples were collected, transported, and analyzed following standard procedures, and MC-LR was determined using a BEACON ELISA kit. To determine the MC-LR and Tolerable Daily Intake (TDI) levels in fish, samples of the fish skin, flesh, and head were collected and analyzed according to World Health Organization (WHO) guidelines. A comparison was made between the MC-LR levels of fish in different reservoirs and the WHO TDI standards (0.04 $\mu g kg^{-1} day^{-1}$). From the Padaviya, the highest mean concentrations of MC-LR were found in fish skin (3004.25 ± 30 $\mu g\ kg^{-1}$), following head (836.25±18 $\mu g\ kg^{-1}$) and flesh (41.67±8 $\mu g\ kg^{-1}$). The average daily intake of MC-LR in the skin and head of all samples exceeded the WHO's TDI (0.04 $\mu g\ kg^{-1}\ day^{-1}$). According to the findings of this study, consumption of fish heads and skin increases the risk of MC-LR accumulation in the human body by a significant amount.

Keywords: ELISA, irrigation reservoirs, Microcystin-LR, *Oreochromis niloticus*, TDI

INTRODUCTION

In eutrophic waters, cyanobacteria can grow rapidly (Campos and Vasconcelos 2010) and overgrowth results in cyanobacteria blooms, which have become a significant water quality issue in many countries due to the production of cyanotoxins (Blaha et al. 2009). The secondary metabolites, cyclic heptapeptide microcystins (MCs) are the most frequently encountered cyanotoxins. Cyanobacteria of various genera are responsible for the production of MCs (McElhiney and Lawton 2005; Manage 2009; Idroos et al. 2015; Piyathilake et al. 2015). Microcystin-LR (MC-LR) is the most toxic and dominant type of microcystin, with a stable chemical structure (De Figueiredo et al. 2004). It is an intracellular toxin (Aboal and Puig 2005) and is released into the environment when the cyanobacterial cell wall is ruptured under stressful environmental conditions (Idroos and Manage 2014).

Contamination of natural waters by cyanobacterial blooms is a global problem, causing severe water pollution and public health risk to humans and livestock (Park et al. 1998; Oudra et al. 2001). Deaths of pets, birds, and livestock, and infrequently, human deaths due to consumption of MC contaminated surface water or cyanobacterial blooms have been documented (Chorus and Bartram 1999). Many human health effects have been documented from time to time in recent years due to excessive exposure to cyanotoxins (Falconer

et al. 2005). The actual risk posed by cyanotoxins at low concentrations in drinking water, as well as the long-term consequences of exposure to these toxins, are still unresolved (Chorus and Bartram 1999). It is also possible for humans to expose indirectly to cyanotoxins through the consumption of freshwater fish or crops after using cyanotoxin-contaminated water for fish farming or irrigation (Mangaelli et al. 2012). Also, animals, fish, and birds can all be poisoned if there is an excessive amount of toxin-producing cyanobacteria in the environment.

Recent studies have revealed that some of Sri Lanka's water bodies have been contaminated by toxigenic cyanobacteria, which are toxic to humans (Jayatissa et al. 2006; Manage 2009). Manage (2009) discovered approximately 40 species of cyanobacteria belonging to 24 genera in Sri Lanka's drinking, irrigation, and recreational water bodies. According to the many studies, *Microcystis aeruginosa* was found to be the most common toxigenic cyanobacteria in many Sri Lanka's water bodies (Silva and Wijeyaratne 1999; Manage 2009; Magana Arachchi *and Liyanage* 2012).

Several studies have examined microcystin bioaccumulation in common aquatic vertebrates and invertebrates, including fish (Sipia et al. 2001; Magalhaes et al. 2003; Mohomed et al. 2003), mussels (Falconer et al. 1992; Harada 1996; Prepas et al. 1997), clams (Williams et al. 1997). Several studies have been conducted on *Oreochromis niloticus* to understand bioaccumulation and tropic transfer throughout the food chain. The tolerance for MC-LR in *O. niloticus* has been reported to be higher than in other fish species and the detoxification rate within the systems is also higher (Wijerathna and Manage 2018).

O. niloticus is important aquaculture and wild-caught fish species in Sri Lanka's inland fisheries sector. However, because most aquatic animals are exposed to algal blooms regularly in their natural environment, there is an urgent necessity to assess the potential impact of microcystin on edible fish species.

In Sri Lanka, most irrigational reservoirs function as drinking water sources and sources of food fish. The evaluation of risk of MC-LR contamination is therefore important and it is critical to verify the consumable parts of fish and the required TDI to avoid health risks. Thus, the current study was conducted to determine the concentration of MC-LR in edible fish to prevent acute and chronic exposure.

Materials and Methods

Collection of water samples

Water samples were collected from 13 reservoirs using a Ruttner sampler to determine the species composition and abundance of cyanobacteria, cyanotoxins (MC-LR). The reservoirs sampled were Nallachchiya, Galkulama, Anakattiya, Padaviya, Nachchaduwa, Kalawewa in Anuradhapura District, Parakrama Samudraya, Halmilla, Kaudulla, Ambagaswewa in Polonnaruwa District, Muwapatigewela in Ampara District, Ulhitiya and Rathkinda reservoirs in Badulla District (Figure 1). Raw water and plankton sampling were performed, in each site at five sampling points in triplicates from 2017 to 2018. There were two sampling occasions during the study period.

Identification and enumeration of cyanobacteria

After natural sedimentation, 100 mL of water samples were fixed with acidified Luogal's solution at a final concentration of 1% (Idroos and Manage 2014). The sedimented plankton sample was concentrated down to 5 mL. Phytoplankton cells from each genus were counted three times in a sedgwick-rafter counting chamber under a light microscope at x400 magnification. Standard taxonomic criteria based on morphology (e.g. cell shape and size, colony shape, mucilage characteristics) were used to identify and confirm the cyanobacteria genera found in the water samples. A sedgwick-rafter counting chamber was used to count the number of cells per millilitre to determine cyanobacteria cell densities.

Collection of Fish Samples

A total of 192 *O. niloticus* specimens of different sizes were purchased from the landings of several fishers in 13 reservoirs studied. Microcystin-LR (MC-LR) was isolated from the fish flesh, head, and skin separately following the standard extraction method (Soares et al. 2004).

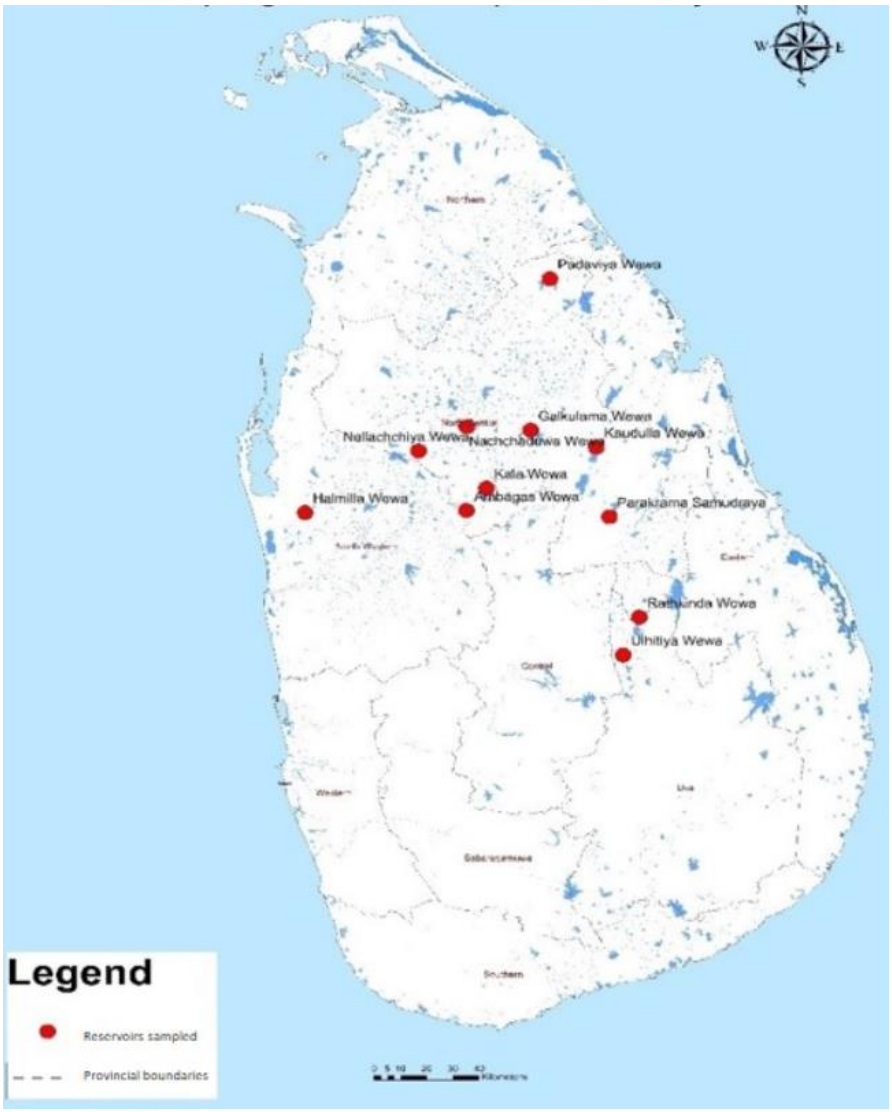


Fig 1. Sampling reservoirs in the present study

Fish sample preparation for detection of MC-LR

Morphometric data of *O. niloticus* specimens (i.e., standard length, width, fork length, and weight) were recorded before processing samples for toxin analysis. The purpose of determining morphometric characteristics of fish was to indicate the nature of fish samples used for the analysis. The fish were washed twice with tap water and once with distilled water. The scales were taken off. Thereafter, the skin was carefully peeled away. The flesh on both sides was then removed. The gills were removed when the skull was separated. The fish flesh, skin, and head were separated, frozen, freeze-dried, and pulverized to extract MC-LR.

Extraction of MC-LR from *O. niloticus* (skin, flesh and head)

All freeze-dried fish samples were treated to MC-LR extraction using methanol–hexane, according to Soares et al. (2004). The samples were ground with 80% methanol (3 mL: 1 g) and shaken at 130 rpm for 12 hours after centrifugation at 6000 rpm for 15 minutes. After collecting the supernatant, an equivalent volume of 100% hexane was added to bind the lipid before passing the mixture through a separating funnel to extract the lipid. The methanol layer was then removed and concentrated, followed by a solid-phase extraction eluting with 80% methanol and rotary evaporated at 50 °C and 115

rpm. In a rotating evaporator, the extracted material was concentrated at 50 °C. After concentration, the dried residue was diluted in 1mL HPLC methanol and filtered through a 0.22 µm Nylon filter (Fisher, UK).

Determination of MCs

The test was performed according to the manufacturer's instructions using a Beacon Microcystin plate kit (Catalogue number: 20-0068). To make 1x wash solution, 100x wash solution was diluted. To make 500 mL of wash solution, 5 mL of 100x concentrate was combined with 495 mL of de-ionized water. Depending on the number of samples, the required number of assay wells were removed and placed on a holder to prepare a test plate. Each control, calibrator, and test sample well-received 50 µL of HRP enzyme conjugate. Then, 50 µL of calibrators, control and test samples were added to each well. A total of 50 µL of antibody solution was supplied to each well. The test plate was then covered with a parafilm strip and incubated for 30 minutes in a plate rotator with continuous mixing. The contents were removed after the incubation period, and the plate was cleaned five times with 1x wash solution. To remove surplus wash solution, the plate was tapped on a paper towel. After adding 100 µL of a substrate to each well, the plate was incubated for 30 minutes before being covered with parafilm. Finally, 100 µL of stop solution was added to each well, and the wells' extinction levels were evaluated using a ELISA plate reader (Multiskan EX, Thermo Scientific) at 450 nm wavelength.

Evaluation of human health risk

Human health risk via consumption of MC-LR contaminated fish could be indicated as the amount of MC-LR in µg per kilogram of body weight and in a 60kg adult per day. The calculations were done using the following equation.

$$\text{Human health risk } (\mu g\, kg^{-1}) = \frac{\text{MC} - \text{LR intake } (\mu g\, day^{-1} person^{-1})}{\text{Body weight of an adult (60 kg)consumption}}$$

Statistical Analysis

The mean and standard deviation (SD) were used to represent the data. MINITAB (version 17) statistical software was used for all statistical analyses (MINITAB, State College, PA, USA). Principal Component Analysis (PCA) was carried out for physico-chemical and MC-LR concentrations in environmental samples (i.e., water as well as skin, head and flesh of fish) for thirteen sampling reservoirs. In order to determine whether there was a statistically significant difference in the physio-chemical and MC-LR concentration in water and fish flesh, skin and head samples obtained, a one-way analysis of variance (ANOVA) was performed on the data ($p < 0.05$).

RESULTS

Cyanobacteria

The possible toxin-producing cyanobacteria recorded in each of the 13 water bodies and cell density of each taxon are given in Table 1. All 13 reservoirs support drinking water supply, irrigation and fish production.

Table 1 Cell densities ± SD of potential toxin-producing cyanobacteria genera in 13 reservoirs

Reservoir	Taxon	Cell density (cells mL^{-1})
Nallachchiya	*Microcystis* sp.	92,000±368
	Cylindrospermopsis sp.	4,500±145
	Anabaena sp.	9,234±230
	Oscillatoria sp.	1,245±80
	Total	**106,979±903**
Galkulama	*Microcystis* sp.	12,492±250
	Cylindrospermopsis sp.	232±25
	Anabaena sp.	ND
	Oscillatoria sp.	ND
	Total	**12,724±275**
Anakattiya	*Microcystis* sp.	10,647±190
	Cylindrospermopsis sp.	327±30
	Anabaena sp.	ND
	Oscillatoria sp.	ND
	Total	**10,974±220**
Padaviya	*Microcystis* sp.	111,496±410
	Cylindrospermopsis sp.	76,347±250
	Anabaena sp.	9,538±210
	Oscillatoria sp.	3,594±120
	Total	**200,975±990**
Nachchaduwa	*Microcystis* sp.	74,000±169
	Cylindrospermopsis sp.	5,534±128
	Anabaena sp.	10,435±218
	Oscillatoria sp.	1,378±95
	Total	**91,347±610**
Kalawewa	*Microcystis* sp.	17,150±259
	Cylindrospermopsis sp.	14,650±239
	Anabaena sp.	7,398±134
	Oscillatoria sp.	1,102±24
	Total	**40,300±656**
Parakrama Samudraya	*Microcystis* sp.	7,348±158
	Cylindrospermopsis sp.	2,500±137
	Anabaena sp.	1,234±59
	Oscillatoria sp.	1,223±65
	Total	**12,305±419**
Halmillewa	*Microcystis* sp.	54,000±158
	Cylindrospermopsis sp.	11,476±210
	Anabaena sp.	20,000±210
	Oscillatoria sp.	2,357±110

	Total	**87,833±688**
Kaudulla	*Microcystis* sp.	41,170±126
	Cylindrospermopsis sp.	11,331±358
	Anabaena sp.	288±41
	Oscillatoria sp.	ND
	Total	**52,789±525**
Ambagaswewa	*Microcystis* sp.	51,256±140
	Cylindrospermopsis sp.	21,113±190
	Anabaena sp.	296±38
	Oscillatoria sp.	1,256±98
	Total	**73,921±466**
Muwapatigewelawewa	*Microcystis* sp.	14,223±94
	Cylindrospermopsis sp.	3,804±110
	Anabaena sp.	2,345±132
	Oscillatoria sp.	1,510±85
	Total	**21,882±421**
Ulhitiya	*Microcystis* sp.	21,870±75
	Cylindrospermopsis sp.	11,741±20
	Anabaena sp.	ND
	Oscillatoria sp.	ND
	Total	**33,611±95**
Rathkinda	*Microcystis* sp.	35,289±119
	Cylindrospermopsis sp.	6,331±113
	Anabaena sp.	ND
	Oscillatoria sp.	ND
	Total	**41,620±232**

ND-Not Detected

Some toxin-producing cyanobacteria among the phytoplankton; *Microcystis* sp., *Cylindrospermopsis* sp., *Anabaena* sp. and *Oscillatoria* sp. were found in various densities in all water bodies. *Microcystis* sp., the most detected potential toxic cyanobacteria, is responsible for MCs production in all water bodies studied. In addition, *Cylindrospermopsis* sp. was found in all the water bodies studied. In most reservoir phytoplankton samples, *Microcystis* spp. was dominant, while *Cylindrospermopsis* spp. was codominant. Except in Galkulama, Anakattiya, Ulhitiya, and Rathkinda, the most common toxic cyanobacteria found was *Anabaena* sp., which is known to produce MCs, CYNs, anatoxins, and saxitoxins and *Oscillatori*a sp., which is known to produce anatoxins, was found in Nallachchiya. The maximum density of cyanobacteria was found in the Padaviya (111,496±410 cells mL^{-1}), where the lowest was in Parakrama Samudraya (7,348±158 cells mL^{-1}) (Table 1).

Water quality and MC-LR contamination

The mean values of water quality parameters and total MC-LR concentration in thirteen freshwater reservoirs are summarized in Table 2.

Table 2 Mean values of water quality parameters and MC-LR concentration (mean±SD) in 13 reservoirs

Reservoir	Temperature (°C)	pH	DO (mg/L)	EC (µS/cm)	N-NO3 (mg/L)	N-NO2 (mg/L)	N-NH_3 (mg/L)	TP (mg/L)	MC-LR Concentration (ppb)
Nallachchiya	30.10±0.09	8.20±0.01	8.10±0.09	172.20±1.45	0.18±0.05	0.52±0.01	0.21±0.03	0.73±0.07	5.30±0.04
Galkulama	29.00±0.07	8.00±0.06	8.10±0.05	674.00±2.13	0.31±0.03	0.56±0.06	0.29±0.03	0.14±0.08	ND
Anakattiya	31.80±1.02	8.30±0.03	9.90±0.04	340.40±1.09	0.19±0.01	0.71±0.04	0.16±0.01	0.21±0.05	ND
Padaviya	28.30±0.03	6.70±0.01	8.50±0.01	290.30±1.67	0.32±0.01	0.64±0.04	0.27±0.02	1.79±0.12	5.98±0.03
Nachchaduwa	28.30±0.03	8.30±0.04	7.80±0.03	719.00±2.47	0.27±0.02	0.47±0.02	0.12±0.01	1.90±0.21	ND
Kalawewa	29.10±0.04	8.10±0.04	7.30±0.02	179.10±0.09	0.29±0.01	0.45±0.03	0.15±0.02	0.81±0.19	ND
Parakrama Samudraya	27.60±0.01	7.20±0.07	7.20±0.05	97.00±0.07	0.29±0.01	0.95±0.04	0.24±0.01	0.63±0.13	3.20±0.02
Halmilla	28.20±1.23	8.40±0.05	8.50±0.03	478.50±1.47	0.28±0.02	0.55±0.05	0.17±0.02	1.45±0.21	2.80±0.01
Kaudulla	29.10±0.06	8.50±0.04	8.10±0.06	440.10±1.38	0.24±0.01	0.54±0.03	0.15±0.01	1.36±0.21	3.10±0.01
Ambagaswewa	28.40±0.05	8.50±0.03	7.80±0.07	462.00±1.25	0.35±0.04	0.44±0.04	0.16±0.02	1.30±0.21	4.20±0.03
Muwapatigewela	30.30±0.03	7.70±0.03	8.70±0.06	88.50±0.06	0.34±0.02	0.72±0.01	0.18±0.02	1.74±0.19	ND
Ulhitiya	27.90±0.02	6.50±0.02	6.70±0.03	93.10±0.09	0.24±0.04	0.54±0.05	0.21±0.01	1.23±0.17	3.10±0.03
Rathkinda	28.50±0.02	6.10±0.01	7.10±0.03	90.70±0.08	0.26±0.02	1.16±0.01	0.25±0.01	0.71±0.10	2.30±0.02

DO-Dissolved Oxygen, EC–Electrical Conductivity, N-NO_3^--Nitrate nitrogen, N-NO_2^--Nitrite nitrogen, N-NH_3-Ammonia nitrogen, TP–Total Phosphorous, MC-LR–Microcystin-LR, ND-Not Detected

All the water bodies had temperatures ranging from 27.6 - 31.8 ^{o}C, with the maximum temperature being recorded in Anakattiya (31.80±1.02 ^{o}C) and the lowest being reported in Parakrama Samudraya (27.60±0.0 ^{o}C). The highest and lowest pH values were found in Kaudulla (6.1) and Rathkinda (8.5). DO levels ranged from 7.1 to 9.9 mg L^{-1}, with the lowest in Rathkinda (7.10±0.03 mg L^{-1}) and the maximum value of 9.90±0.04 mg L^{-1} in Anakattiya. Electrical Conductivity (EC) values ranged from 93.10±0.09 µS cm^{-1} in Ulhitiya to 719.00±2.47µS cm^{-1} in Nachchaduwa. Ambagaswewa had the highest N-NO_3^- concentration (0.35±0.04 mg L^{-1}), whereas Nallachchiya had the lowest (0.18±0.05 mg L^{-1}). In all the water bodies examined in this study, N-NO_2^- and N-NH_4^+ levels were over the detection thresholds. Total phosphorus levels ranged from 0.14±0.08 mg L^{-1} in Galkulama to 0.73±0.07 mg L^{-1} in Nallachchiya.

Microcystin-LR, a cyanotoxin, was found in great concentration in Nallachchiya (5.30±0.04 µg L^{-1}), Padaviya (5.98±0.03 µg L^{-1}), Parakrama Samudraya (3.20±0.02 µg L^{-1}), Halmilla (2.80±0.01 µg L^{-1}), Kaudulla (3.10±0.01 µgL^{-1}), Ambagaswewa (4.20±0.03 µg L^{-1}), whereas Rathkinda (2.30±0.02 µg L^{-1}) had the lowest. In Galkulama, Anakatttiya, Nachchaduwa, Kalawewa, and Muwapatigewela, MC-LR was not detected.

Morphometric data of environmental fish samples

The morphometric data of *O. niloticus* samples from 13 reservoirs are summarized in Table 3. The largest body length was recorded from Padaviya and the heaviest fish were from Parakrama Samudraya.

Table 3 Morphometric data (mean±SD) of *O. niloticus* samples from 13 reservoirs

Reservoir	No. of fish sampled	Mean Standard Length (cm)	Mean Fork Length (cm)	Mean Total Length (cm)	Mean Weight (g)
Nallachchiya	15	16.20±1.34	17.50±1.69	22.80±2.34	200.00±17.19
Galkulama	14	17.00±1.67	17.90±1.72	22.00±2.31	199.50±17.12
Anakattiya	13	18.00±1.85	18.70±1.87	22.60±2.42	231.12±18.36
Padaviya	18	19.90±1.92	20.50±2.12	26.00±2.67	173.50±15.12
Nachchaduwa	15	18.20±1.89	18.50±1.83	23.80±2.57	200.00±17.23
Kalawewa	16	16.00±1.25	16.50±1.25	21.60±2.14	156.60±13.57
Parakrama Samudraya	14	16.90±1.92	17.50±2.12	23.00±2.67	288.40±23.24
Halmilla	12	16.50±1.28	17.00±1.57	21.40±2.11	194.30±16.49
Kaudulla	15	17.20±1.68	17.50±1.62	20.40±2.14	200.00±17.12
Ambagaswewa	17	19.10±1.87	19.50±2.14	24.00±2.47	210.80±17.13
Muwapatigewela	15	19.00±1.85	20.00±2.17	24.00±2.41	180.90±16.14
Ulhitiya	14	16.80±1.31	17.20±1.59	22.00±2.48	206.80±17.17
Rathkinda	14	16.90±1.35	17.10±1.61	18.70±1.59	176.40±15.96

MC-LR concentrations in the fish head, skin and flesh

Concentrations of MC-LR in the fish head, skin and flesh in thirteen reservoirs are given in Figure 2. *O. niloticus* in Padaviya had high mean MC-LR concentrations, with 3004.25±30 µg kg^{-1} in the skin, 41.67±8 µg kg^{-1} in the flesh, and 836.25±18 µg kg^{-1} in the head. The presence of MC-LR in fish samples collected from Galkulama, Anakattiya, Nachchaduwa, Kalawewa (fish skin and head), and Muwapatigewela (fish head) indicates that the fish samples had previously been exposed to the MC-LR.

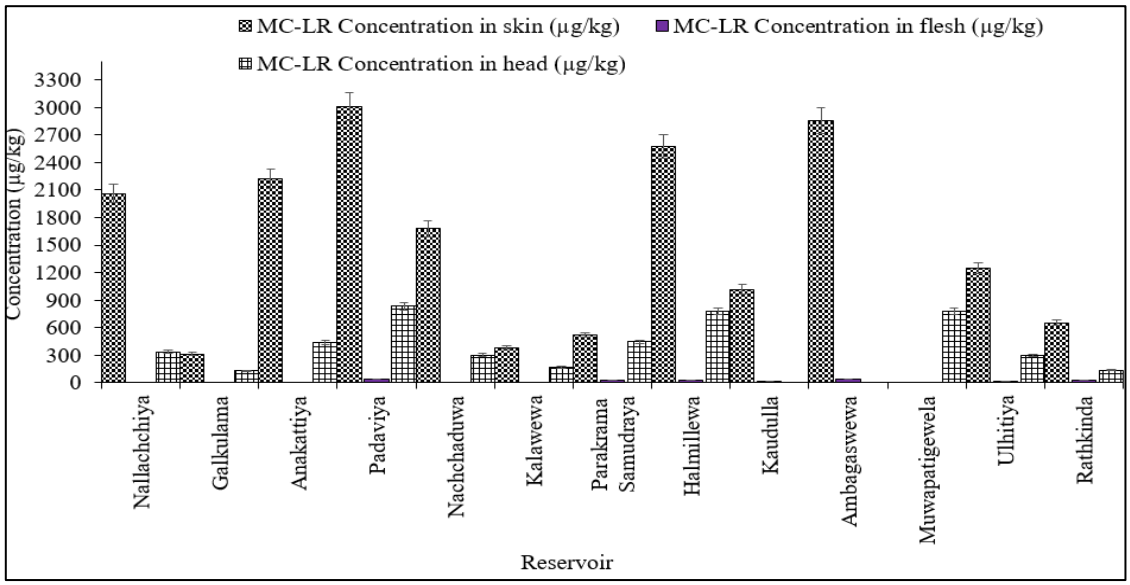


Fig 2. MC-LR concentrations in head, skin and flesh of *O. niloticus* collected from different water bodies

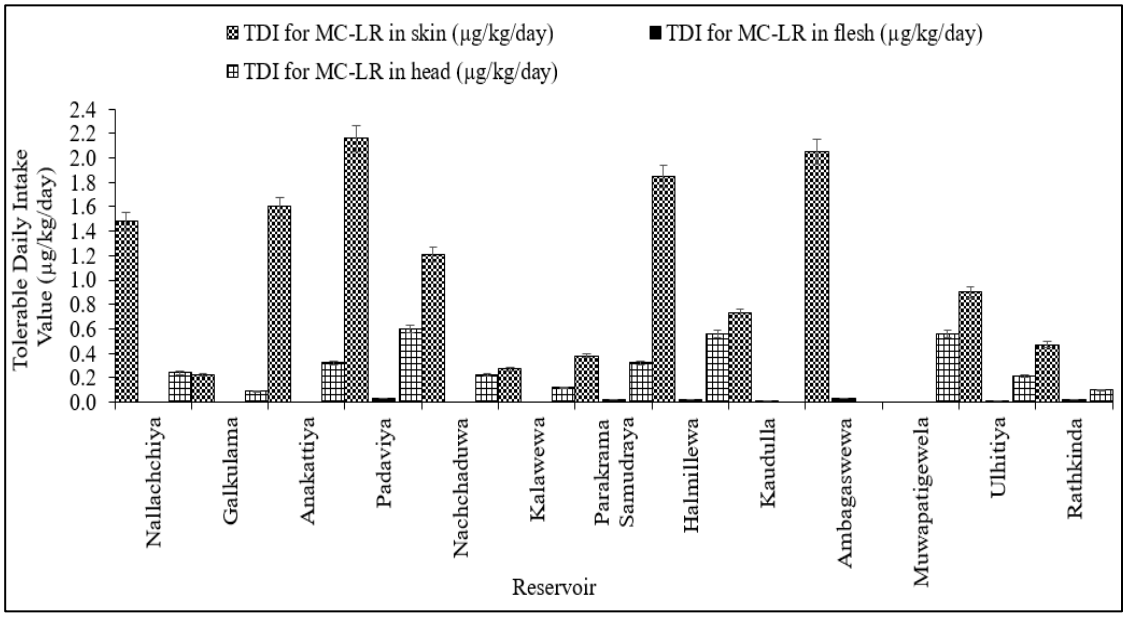


Fig 3. The Tolerable Daily Intake Values (TDI) of MC-LR in human who consume *O. niloticus*. WHO standard (0.04 µgkg^{-1}day^{-1})

Health Risk

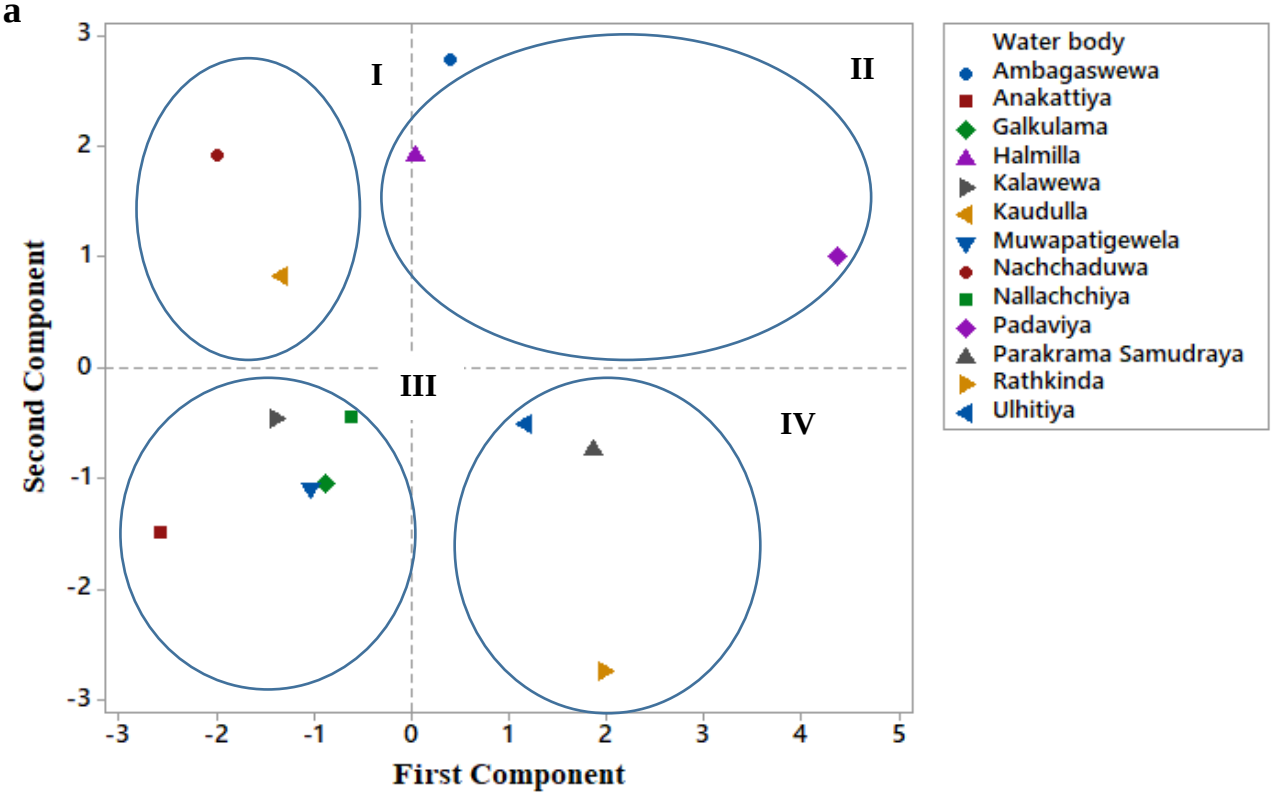
Figure 3 shows tolerable dietary intake (TDI) to MC-LR from fish heads, skin, and flesh of *O. niloticus* taken from thirteen reservoirs. According to WHO, 0.04 µg kg^{-1} day^{-1} is acceptable amount of MC-LR for human consumption. The TDI in fish skin (2.16 ± 0.02 µg kg^{-1} day^{-1}) and head (0.60±0.01 µg kg^{-1} day^{-1}) in Padaviya were much higher than the WHO recommended value. However, the TDI value in fish flesh in Padaviya was 0.03 ± 0.01 µg kg^{-1} day^{-1}, which was lower than the WHO's recommended value. In Nallachchiya, the TDI of MC-LR in fish skin and the head was 1.48±0.01 µg kg^{-1} day^{-1} and 0.24 ± 0.01 µg kg^{-1} day^{-1}, respectively, whereas in Galkulama, they were 0.22±0.01 µg kg^{-1} day^{-1} in skin and 0.09 µg kg^{-1} day^{-1} in head. In Anakattiya, the TDI values in fish skin and the head were 1.60±0.01 µg

kg^{-1} day^{-1} and 0.32±0.01 µg kg^{-1} day^{-1}, respectively. These values were higher than the WHO-recommended TDI. In Parakrama Samudraya, the TDI of MC-LR in fish skin and the head was 0.38±0.01 µg kg^{-1} day^{-1} and 0.32±0.01 µg kg^{-1} day^{-1}, respectively. In Kaudulla, the TDI of MC-LR in fish skin was 0.73±0.01 µg kg^{-1} day^{-1}. These values were higher than the WHO-recommended TDI. The TDI value in fish flesh in Parakrama Samudraya, on the other hand, was 0.02 µg kg^{-1} day^{-1}, which was below the WHO standard. In Halmilla, Nachchaduwa, Kalawewa, Ulhitiya, and Rathkinda, TDI values of fish skin and head were higher than WHO TDI values.

Water quality parameters, MC-LR content in water and MC-LR content in fish skin, head and flesh

PCA was used to investigate the underlying patterns of the overall MC-LR content in water and environmental parameters, and to find out the key elements that drove toxin formation in a natural setting. Eigenvalues greater than 1.0 were considered for PCA scoring, and four scores were chosen. The first principal component (PC1) accounts for 30.9 % of total data variance, whereas the second component (PC2) accounts for 20.8 %. On the PC1 axis, four clusters may be seen in the score plot of PC2 vs PC1. The samples in groups I, II, III, and IV have distinct water quality metrics. The two groups were separated along PC1 by a strong association between N-NO_2^- and N-NH_3, which also influenced the separation of the two groups. pH related to Electrical Conductivity (EC), DO correlate with temperature, and N-NO_3^-, Total Phosphorous (TP), MC-LR in fish skin, head, and flesh correlated with MC-LR in water as seen in the PCA scores plot. The concentration of MC-LR in water was found to have a significant relationship with the fish skin (p = 0.036). Our findings also revealed a statistically significant relationship between total MC-LR in water and DO (p = 0.036).

a



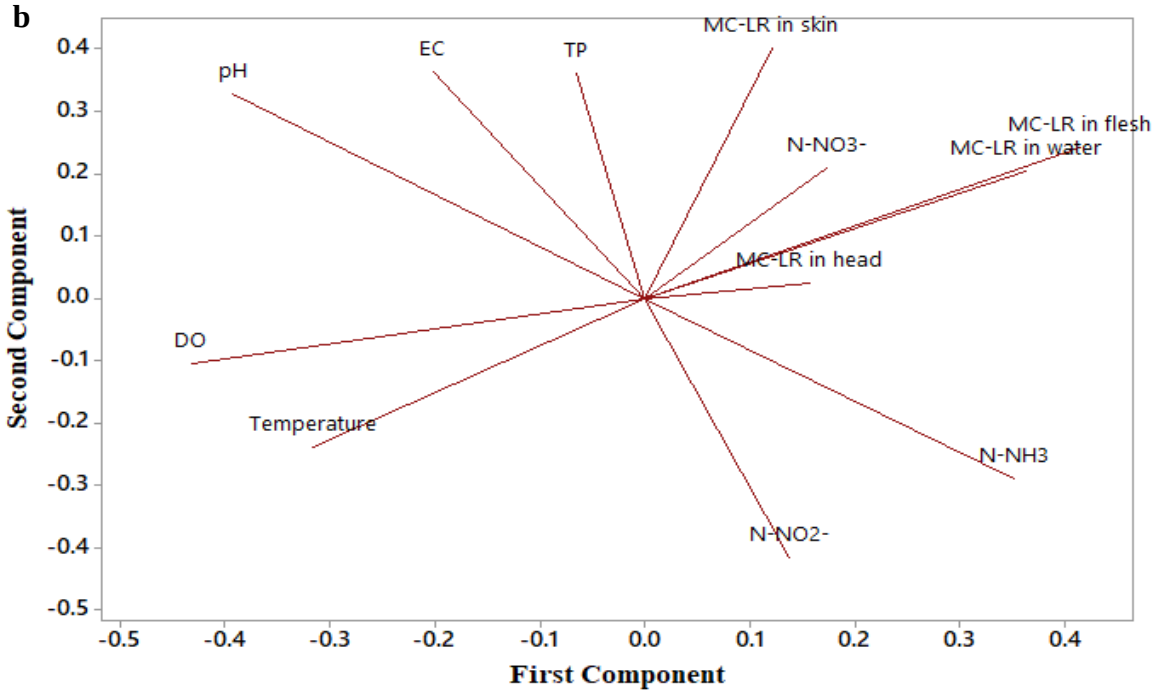


Fig 4. (a) Score plot (b) Loading plot) PCA results for water quality metrics and MC-LR concentrations in water, fish skin, fish head, and fish flesh

DISCUSSION

Temperature, phosphorus, nitrogen, and pH are known to influence cyanobacteria growth (Chen et al. 2009). According to the findings of the present study, there were significant linear relationships between DO and MC-LR content in water. The dimensions of the measured variables were reduced to their representative primary components using PCA based on a correlation matrix (PC). N-NO_2^- and N-NH_3 were found to account for approximately 30.9% in PC1, while 20.8% in PC2 comprised N-NO_3^-, Total Phosphorous (TP), MC-LR in fish tissue (flesh and skin), and MC-LR in water.

Studies are reported on MCs pollution of drinking water sources (Song et al. 1998). Fish is exposed to MC through ingestion of toxic cyanobacteria or MCs contaminated food, and to a lesser extent, dissolved MCs (Cazenave et al. (2005)). In the present study, it was found that the Nallachchiya (5.30±0.04 µg L^{-1}), Padavaiya (5.98±0.03 µg L^{-1}), Parakrama Samudraya (3.20±0.02 µg L^{-1}), Halmillawa (2.80±0.01 µg L^{-1}), Kaudulawa (3.10 ±0.01 µg L^{-1}), Ambagaswewa (4.20±0.03 µg L^{-1}), Ulhitiya (3.10±0.03 µg L^{-1}), and Rathkinda (2.30±0.02 µg L^{-1}) reservoirs, total MC-LR concentrations were substantially higher than the WHO (1998) recommended safe drinking water threshold of 1 µg L^{-1}. In Galkulama (12, 492±250 cellsmL^{-1}), Anakattiya (10,647±190 cellsmL^{-1}), Nachchaduwa (74,000±169 cells mL^{-1}), Kalawewa (17,150±259 cells mL^{-1}), and Muwapatigewela (14,223±94 cells mL^{-1}), high *Microcystis* spp. cell densities were detected without detecting MC-LR which may be due to presence of non-toxic form of cyanobacteria. Thus, it should be emphasized, that the digestibility of toxic cyanobacteria differs by species.

It is crucial to know the toxic contamination status of the fish before they are consumed. MC-LR contamination has been recorded in vertebrates and invertebrates (Liang et al. 2007; Nyakairu et al. 2010; Carneiro et al. 2015). Cichlids, particularly *O. niloticus*, have been implicated as bioindicators in some contexts around the world. Toxic tolerance in aquatic organisms is more established than in mammals. Because MC-LR is a potent hepatotoxin, researchers have focused their efforts on understanding how it works (Manage 2009).

The prevalence of cyanobacteria blooms in Sri Lanka has been investigated, and contaminated water sources have been reported by Jayatissa et al. (2006). Because MC-LR is also found in edible parts of *O. niloticus* in the reservoirs studied, its impact on

human health cannot be neglected. According to the findings of this study, the accumulation of MC-LR in the skin and head is greater than in the flesh. As such, for preventing potential health issues, it is recommended that people should avoid consumption of edible parts of fish contaminated with MC-LR, i.e., skin and head.

MC-LR concentrations in fish tissues are dependent on the type of food consumed, the duration of time exposed to the toxin, and the rates used among different species (Dyble et al. 2011). On the other hand, the accumulation of MC-LR has devastating consequences specific to each species. According to previous studies, the livers of omnivorous fish are more severely damaged by cyanobacterial blooms than the livers of carnivorous fish (Qiu et al. 2007). Ame et al. (2010) reported a significant correlation between MC content in *Odontesthes bonariensis* liver and MC concentrations in water, but not between those in fish muscle. Magalhaes et al. (2001) found a positive relationship between cellular MC and muscle MC concentration in *Tilapia rendalli*. As shown in another Sri Lankan study, there was accumulation of MC-LR in cultured and natural populations of *O. niloticus* in edible parts (Wijerathna and Manage 2018).

As found in this study, concentrations of MC-LR in the head and flesh were lower than in the skin (skin > head > flesh). In the flesh samples of *O. niloticus* obtained from Nallachchiya, Galkulama, Anakattiya, Nachchaduwa, Kalawewa, and Muwapatigewela, no MC-LR was found. MC concentrations in the intestinal walls of fish are substantially higher than in other organs (Zhang et al. 2009), implying that the intestinal wall may prevent MC accumulating in other body tissues. The concentrations of MC-LR in the heads and skin of the fish differed greatly between reservoirs. During the study, that the highest cell density of *Microcystis* spp. was found to be in Padaviya was 111,496±410 cells mL^{-1}, having the highest MC-LR concentration (5.98±0.03 µg L^{-1}). The greatest MC-LR concentrations [head (836.25±18 µg kg^{-1}), skin (3,004.25±30 µg kg^{-1}), and flesh (41.67±8 µg kg^{-1})] were also found in *O. niloticus* collected from Padaviya, where the calculated TDI value was higher than the WHO's recommended TDI (0.04 µg kg^{-1} day^{-1}). The Nallachchiya registered the second-highest cell density of *Microcystis* spp. (92,000±368 cells mL^{-1}), with a mean MC-LR concentration of 5.30±0.04 µg L^{-1}. In this reservoir, MC-LR levels found in fish skin (2,061.02±0.01 µg kg^{-1}) and fish head (336.02±0.01 µg kg^{-1}) were also higher. Their TDI levels were higher than the WHO TDI value.

Also as shown by the results of PCA (Figure 4b), there is a close association of MC-LR in water and those in fish skin. This suggests that environmental factors favoring growth of cyanobacteria play a role in accumulation of MC-LR in fish. Contaminant levels of MC-LR in fish may change over time due to seasonal variation of water quality where they inhabit and the life stages of fish having differences in susceptibility, especially associated with ontogenetic changes of dietary patterns. As a result, determining the basic determinants of changes of accumulation of MCs in fish may be challenging.

Human exposure to the MC-LR via consuming contaminated fish, poses health problems for the consumer (Peng et al. 2010). During the study period, the mean TDI of skin and head via *O. niloticus* consumption in all reservoirs studied was greater than the WHO-recommended TDI value for a 60 kg adult who consumes an average 300 g of fish per day. The results of the present study revealed that high concentration of toxin accumulations in the fish skin and head of fish harvested from several reservoirs.

It is also important to note that FAO food safety limit for MC-LR was set for healthy adult humans; children and the elderly are more vulnerable to the adverse health effects of MC-LR (Magalhaes et al. 2001).

CONCLUSION

The current research is the first to investigate cyanotoxin (MC-LR) in reservoirs of Sri Lanka and in edible parts of *O. niloticus* inhabiting those reservoirs. The contamination levels varied widely over the geographical range. In some reservoirs, concentrations of MC-LR in the fish skin and head were higher than in flesh. It is therefore suggested that consumers avoid eating head and skin parts of *O. niloticus* from reservoirs where MC-LR levels are high.

ACKNOWLEDGMENTS

This research was funded by National Research Council, Sri Lanka (Grant No. NRC-16-078), University Research grant (Grant No. ASP/01/RE/SCI/ 2019/29) and Algae and Water Quality Research Centre, University of Sri Jayewardenepura.

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