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Screening of microcystin and microcystin-producing genes in Labugama and Kalatuwawa reservoirs in Sri Lanka

I. U. De Silva and P. M. Manage*

Centre for Water quality and Algae Research, Department of Zoology,
University of Sri Jayewardenepura, Sri Lanka
*pathmalal@sjp.ac.lk

Microcystins (MCs) are the most widely studied carcinogenic cyanobacterial hepato- and neurotoxins. The MCs are synthesized by a non-ribosomal pathway through a multifunctional enzyme complex known as microcystin synthetase (mcy) encoded by the mcy gene clusters. The present study was aimed to screen MCs and MC-producing genes in Labugama and Kalatuwawa reservoirs which satisfies 60% of drinking water requirement for Colombo District. In the study, plankton and water samples were collected from the two reservoirs prior to water treatment process. The sampling was performed using a boat on the first week of each month from August to October in 2017 where three sampling locations were selected to collect water at each sampling time. Horizontal plankton samples were collected at 10 cm depth in each location using a 55 µm plankton net while the boat was moving. Water temperature and pH were measured on site using digital meters. Total inorganic nitrogen ($N\text{-}NO_3^-$, $N\text{-}NO_2^-$, $N\text{-}NH_3$) and total phosphorous were determined using standard spectrophotometric methods, and cyanobacteria were identified under a light microscope using standard algae and cyanobacteria identification keys. *Microcystis* spp. were isolated and monocultures were prepared on cyanospecific BG 11 media from which the genomic DNA was extracted for the screening of MC-producing gene cluster in water samples using the Polymerase Chain Reaction (PCR). MCs were analysed by High Performance Liquid Chromatography (HPLC) and Enzyme Linked Immunosorbent Assay (ELISA) respectively. Temperature of water samples ranged between 27.8 and 28 ^oC while pH fluctuated in a range between 7.2 and 7.5. Total inorganic nitrogen was recorded from 0.02 to 0.03 mg/L and total phosphorous fluctuated from 0.01 to 0.02 mg/L during the sampling period. *Microcystis* spp. was identified as dominant cyanobacteria in cell density range between 176 and 226 cells/mL for both reservoirs. Amazingly, MCs were not present at detectable levels following the HPLC method (detection limit 0.5 mg/L) and ELISA method (detection limit 0.1 µg/L). Also, the PCR amplification showed the absence of mcy cluster genes E, A and B in the water samples. Thus, the results of the study revealed that both Labugama and Kalatuwawa reservoirs have non toxic strains of cyanobacterium *Microcystis* spp. The results of the present study were supported HPLC, ELISA analysis and molecular analysis.

Keywords: ELISA, genomic DNA, HPLC, mcy gene clusters microcystins, microcystin synthetase, PCR