Proceedings of the

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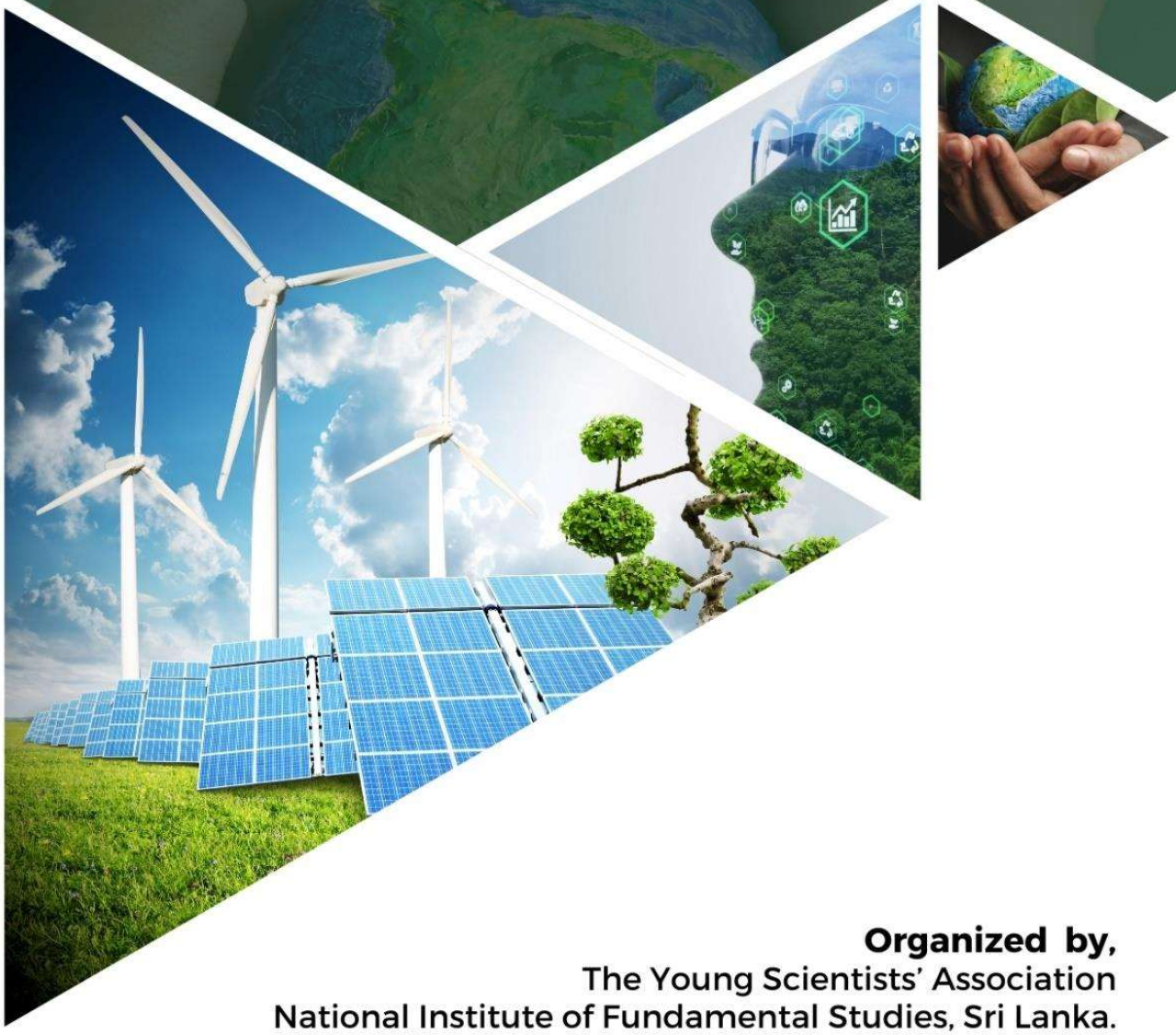

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PCR-based identification and HPLC profiling of microcystin production in selected cyanobacterial genera

H.M.S.A.T. Gunathilaka[1], W.R.P. Wijesinghe [2], D.N. Magana-Arachchi[1*]

[1]*Molecular Microbiology and Human Diseases Programme, National Institute ofFundamental Studies, Kandy, Sri Lanka*
[2]*Department of Botany, Faculty of Science, University of Peradeniya, Peradeniya, Sri Lanka*

[*]*dhammika.ma@nifs.ac.lk*

Cyanotoxins, especially microcystins (MC), pose serious health risks to humans and animals. Understanding their production mechanisms is essential for controlling their spread in freshwater systems. The study aimed to analyze the MC production in five freshwater cyanobacterial genera using high-performance liquid chromatography (HPLC) and conducted gene screening related to MC production via polymerase chain reaction (PCR). These strains were sourced from the culture collection of the Molecular Microbiology and Human Diseases Project (MM&HD) at the National Institute for Fundamental Studies; *Microcystis aeruginosa* (EF051239), *Fischerella* sp. A22 (OR727806), *Nostoc* sp. A13 (OR727808), *Pseudanabaena* sp. CMP3 (ON870364), and *Leptolyngbya* sp. CMP4 (ON870366) was grown in BG11 medium under controlled conditions, with a photon flux of 25 µmol m^{-2} s^{-1} and a photoperiod of 14 light:10 hours darkness. MC was extracted with 1 mL of 80% methanol, evaporated to dryness, and reconstituted in 1 mL of methanol. HPLC quantified the microcystin content in the cyanobacterial pellet extracts using a reference standard (SIGMA ALDRICH 33578). Genomic DNA was extracted from pure cultures using a modified cetyltrimethylammonium bromide method, and conventional PCR was performed to detect selected "*mcy*" genes (A, B, E, H, and T). The results indicated that *Microcystis aeruginosa* (0.982 ± 0.57 mg/L) and *Fischerella* sp. (0.8519 ± 0.14 mg/L) exhibited the highest toxin concentrations. There is a significant positive correlation ($p < 0.05$, $r = 0.9066$) between the presence of the selected "*mcy*" genes and the cyanotoxin production in cyanobacterial cells. None of the samples tested positive for the "*mcy*" T gene, but all species showed positive amplification for the "*mcy*" A and "*mcy*" H genes. *Pseudanabaena* and *Leptolyngbya* species isolated from the Mahapelessa hot springs only carried the "*mcy*" A and "*mcy*" H genes. However, toxin production in these organisms can vary due to different micro-environmental and molecular factors.

Keywords: *Cyanobacteria, cyanotoxin, high-performance liquidchromatography, microcystin, "mcy" gene cluster*