

MICROBIAL SOURCE TRACKING OF RIVER WATER USING HOST-SPECIFIC BACTEROIDES AND FAECALIBACTERIUM ENDPOINT-PCR ASSAYS

G.K. Kapukotuwa^{1*}, C.L. Abayasekara², K.C. Weerakoon³ and R.S. Rajakaruna⁴

¹Postgraduate Institute of Science, University of Peradeniya, Peradeniya, Sri Lanka

²Department of Botany, Faculty of Science, University of Peradeniya, Peradeniya, Sri Lanka

³Department of Zoology, The Open University of Sri Lanka, Nugegoda, Sri Lanka

⁴Department of Zoology, Faculty of Science, University of Peradeniya, Peradeniya, Sri Lanka

*kapukotuwag@gmail.com

Microbial source tracking is helpful in the effective management of water pollution. The objective of this study was to identify faecal pollution sources spatially and temporally along the Rawan-Oya tributary of the Mahaweli River using host-specific *Bacteroides* and *Faecalibacterium* markers. Water samples were collected aseptically over 19 months from June 2020 to March 2022 from nine sites representing pristine, agricultural, rural, semi-urban and urban areas along the river. Water samples (n = 171) were filtered through 0.45 µm sterile membrane filters. DNA was extracted from faecal samples of humans, cattle and dogs to test the specificity of markers and from isolates on membrane filters using the gDNA Tissue Miniprep System. The Bac32 assay was performed for general *Bacteroides spp*, while human and cattle faecal contamination was tracked using *Bacteroides* HF183 and CF193 markers, and dog faecal contamination was tracked using *Faecalibacterium* ED-1 marker. All the samples were positive for the universal *Bacteroides* marker, confirming faecal contamination of water collected from all the sites. Every site along the stream was positive for human-faecal contamination except for the pristine area. Urban and semi-urban sites had high levels of human-faecal contamination, which could be attributed to the presence of toilets and wastewater outlets in the vicinity. The recreational sites had human-faecal contamination, while agricultural areas with paddy fields, cattle farms and urban sites with meat shops in the vicinity of the stream had cattle faeces contamination. Urban and semi-urban areas had high dog faecal contamination. Both human-specific ($\chi^2 = 29.18$; $p < 0.0001$) and dog-specific ($\chi^2 = 4.23$; $p = 0.039$) faecal contaminations were significantly higher during the wet season compared to the dry season. This study represents a significant and pioneering step towards using PCR tools to identify faecal pollution sources in the Rawan-Oya tributary, showing diverse faecal contamination patterns along the waterway.

Financial assistance from the National Science Foundation of Sri Lanka (Grant No. NSF/SCH/2019/04) is acknowledged.

Keywords: Faecal contamination, Host-specific genetic markers, Microbial source tracking, PCR, River water