Title: Simultaneous measurement of genetic fecal indicators in water column and periphyton biofilms in artificial streams

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Abstract: Periphyton is a complex mixture of algae, heterotrophic microbes, fine inorganic sediment, and detritus that are attached to submerged surfaces in most flowing freshwater systems. These communities are known to absorb many pollutants, resulting in improved water quality. However, it remains unknown whether these natural biofilms can sequester genetic fecal indicators from the water column. An indoor mesocosm study was conducted at the U.S. Environmental Protection Agency Experimental Stream Facility to simultaneously measure genetic fecal indicators in the water column and associated periphyton biofilms when subject to wastewater point source loading. The experiment was conducted over 16-weeks and organized into colonization and wastewater loading periods. To simulate point-source wastewater loading, treated sewage effluent from an adjacent facility was pumped directly into mesocosms. Inflow and overflow surface water grabs were paired with the collection of periphyton samples on a weekly basis. Samples were analyzed with five genetic fecal indicator qPCR assays targeting *E. coli* (EC23S857), enterococci (Entero1a), and *Bacteroidales* (GenBac3), as well as human host-associated fecal pollution (HF183 and HumM2). In addition, total suspended solids (water column) and total biotic mass (periphyton) were measured. During sewage loading, genetic indicators were detected at frequencies up to 100% (EC23S857, Entero1a, and GenBac3), 56.3% (HF183), and 37.5% (HumM2) in periphyton confirming sequestration from the water column. Net flux shifts in water column genetic indicator concentrations further supported potential interactions under most test conditions. In addition, positive correlations were observed between periphyton biotic mass and genetic indicators ranging from $r = 0.63$ (Entero1a) to $r = 0.83$ (GenBac3). Overall, findings support the notion that genetic indicators suspended in the water column can be absorbed by periphyton biofilms suggesting that the benthos environment in flowing freshwater systems is an important factor to consider for water quality management.

Keywords: Periphyton, microbial source tracking, qPCR, fecal pollution, water quality managment

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