Water Research 119 (2017) 1–11

Contents lists available at ScienceDirect

Water Research

journal homepage: www.elsevier.com/locate/watres

Effect of freshwater sediment characteristics on the persistence of fecal indicator bacteria and genetic markers within a Southern California watershed

Amity G. Zimmer-Faust [a, 1], Vanessa Thulsiraj [a, 2], Catalina Marambio-Jones [a], Yiping Cao [b], John F. Griffith [b], Patricia A. Holden [c], Jennifer A. Jay [a, *]

[a] Department of Civil and Environmental Engineering, University of California at Los Angeles, Los Angeles, CA 90095, United States
[b] Southern California Coastal Water Research Project, 3535 Harbor Blvd Ste 110, Costa Mesa, CA 92626, United States
[c] Earth Research Institute and Bren School of Environmental Science & Management, University of California, Santa Barbara, CA 93106, United States

ARTICLE INFO

Article history:
Received 1 November 2016
Received in revised form
22 March 2017
Accepted 9 April 2017
Available online 18 April 2017

Keywords:
Fecal indicator bacteria
Genetic markers
Microbial source tracking
Decay rate
Freshwater sediment

ABSTRACT

In this study, the aging of culturable FIB and DNA representing genetic markers for *Enterococcus* spp. (ENT1A), general *Bacteroides* (GB3), and human-associated *Bacteroides* (HF183) in freshwater sediments was evaluated. Freshwater sediment was collected from four different sites within the upper and lower reach of the Topanga Creek Watershed and two additional comparator sites within the Santa Monica Bay, for a total of six sites. Untreated (ambient) and oven-dried (reduced microbiota) sediment was inoculated with 5% sewage and artificial freshwater. Microcosms were held for a 21-day period and sampled on day 0, 1, 3, 5, 7, 12, and 21. There were substantial differences in decay among the sediments tested, and decay rates were related to sediment characteristics. In the ambient sediments, smaller particle size and higher levels of organic matter and nutrients (nitrogen and phosphorus) were associated with increased persistence of the GB3 marker and culturable *Escherichia coli* (cEC) and enterococci (cENT). The HF183 marker exhibited decay rates of −0.50 to −0.96 day^{-1}, which was 2–5 times faster in certain ambient sediments than decay of culturable FIB and the ENT1A and GB3 markers. The ENT1A and GB3 markers decayed at rates of between −0.07 and −0.28 and −0.10 to −0.44 day^{-1}, and cEC and cENT decayed at rates of between −0.22 and −0.81 and −0.03 and −0.40 day^{-1}, respectively. In the oven-dried sediments, increased persistence of all indicators and potential for limited growth of culturable FIB and the GB3 and ENT1A markers was observed. A simplified two-box model using the HF183 marker and cENT decay rates generated from the microcosm experiments was applied to two reaches within the Topanga Canyon watershed in order to provide context for the variability in decay rates observed. The model predicted lower ambient concentrations of enterococci in sediment in the upper (90 MPN g^{-1}) versus lower Topanga watershed (530 MPN g^{-1}) and low ambient levels of the HF183 marker (below the LLOQ) in sediments in both lower and upper watersheds. It is important to consider the variability in the persistence of genetic markers and FIB when evaluating indicators of fecal contamination in sediments, even within one watershed.

1. Introduction

Decay of bacteria and fecal markers in sediments is particularly understudied even though sediments, soils, and beach sands have been implicated as potential reservoirs in a variety of climates and environments (Alm et al., 2014; Craig et al., 2004; Garzio-Hadzick et al., 2010; Haller et al., 2009; Lee et al., 2006). Sediments can promote persistence of fecal organisms both by providing protection from ultraviolet radiation, temperature fluctuations, and

* Corresponding author.
E-mail address: jjay@seas.ucla.edu (J.A. Jay).
[1] Present address: U.S. Environmental Protection Agency, Office and Research and Development, National Health and Environmental Effects Research Laboratory, 2111 Marine Science Drive, Newport, OR 97365, United States.
[2] Present address: Department of Biological Sciences, Mount Saint Mary's University Los Angeles, Los Angeles, CA 90049, United States.

http://dx.doi.org/10.1016/j.watres.2017.04.028
0043-1354/

predation by microorganisms in the overlying water column (Davies et al., 1995; Korajkic et al., 2013; Wanjugi and Harwood, 2013; Wheeler Alm et al., 2003) and by supplying critical nutrients (Craig et al., 2004; Labelle et al., 1980; Mika et al., 2009; Wanjugi et al., 2016). Under favorable sediment conditions, fecal indicator bacteria (FIB) exhibit extended survival and even regrowth, which can lead to false positive indications of recent fecal pollution in water quality monitoring when organisms colonize sediment and levels no longer correspond with the presence of fecal pollution and pathogens (Byappanahalli and Fujioka, 1998; Byappanahalli et al., 2012; Davies and Bavor, 2000; Garzio-Hadzick et al., 2010; Lee et al., 2006).

FIB are used as proxies for the myriad of pathogens present in fecal matter, and their presence has been linked to adverse health effects (Cabelli et al., 1982; Kay et al., 1994; USEPA, 2010). However, FIB do not provide information regarding the source of fecal contamination (Field and Samadpour, 2007). Quantification of source-associated DNA markers using quantitative polymerase chain reaction (qPCR) methods have greatly advanced microbial source tracking (MST) efforts. Molecular MST methods enable successful source tracking by allowing for same-day water quality monitoring results and information regarding the source of the fecal contamination (Boehm et al., 2013; Griffith et al., 2013, 2009; Noble et al., 2006). Although there has been limited research evaluating the decay rates of source tracking markers along with culturable FIB in water (e.g. Bae and Wuertz, 2015; Mattioli et al., 2017), marker decay has not yet been fully investigated in sediments. FIB are typically measured by culture-based technique, whereas DNA-based markers measure DNA from both culturable, viable but not culturable (VNBC), and dead cells. Further, decay of fecal microorganisms is dependent on both the bacterium itself and on physical (e.g. organic matter content, temperature, salinity) (Haller et al., 2009; Shelton et al., 2014; Thelaus et al., 2009) and biotic factors (predation and competition) (Kinnaman et al., 2012; Korajkic et al., 2013; Wanjugi and Harwood, 2013). Ideally, indicators should provide a warning that fecal contamination by human-derived sewage has recently occurred, and their presence should correlate with the presence of human pathogens.

Studies so far evaluating genetic marker decay in sediments are limited and have focused on evaluating the effect of moisture content on decay of genetic markers in freshwater sand (Eichmiller et al., 2014) and in marine beach sand (Yamahara et al., 2012), decay in freshwater sediment under aerobic and anaerobic conditions (Kim and Wuertz, 2015), decay of the LA35 poultry litter marker in fresh and marine sediments and waters (Nayak et al., 2015), and decay of a single genetic marker: *Catellicoccus marimmalium* in wetted and unwetted beach sand (Brown and Boehm, 2015). With the exception of the Nayak et al. (2015) study, these efforts consider decay in one sediment type only; the relative aging of genetic markers and FIB may vary among sites due to differences in particle size, nutrient concentrations, and ambient microbial communities which has not yet been addressed.

In this study, laboratory microcosm experiments were used to investigate differences in the relative aging of FIB and genetic markers in six different freshwater sediments. In addition, the effect of ambient microbiota on decay was evaluated by oven-drying four of the six sediments and comparing decay rates between the ambient and oven-dried sediments. The main objectives were to: 1) examine the variability in genetic marker and FIB decay rates with respect to varied freshwater sediment characteristics, 2) investigate the role of the ambient microbiota on persistence of genetic markers and FIB in freshwater sediments, and 3) assess the variability in sediment decay rates within one watershed (Topanga Creek Watershed). In addition, a simplified two-box model was applied, using HF183 marker and cENT decay rates generated from the microcosm experiments, to better contextualize the variability in decay rates observed within the Topanga Canyon watershed.

2. Methods

2.1. Sediment microcosm experiments

2.1.1. Sediment collection and characterization

Freshwater sediments were collected from six sites: a total of four sites were selected within the Topanga Canyon Watershed (S1–S4), two within the upper reach of Topanga Canyon and two within the lower reach of the Topanga Canyon; and, for comparison, two additional sites: Ballona Creek freshwater marsh (C1) and Medea Creek in Malibu Canyon (C2). In a previous study, chronically high levels of fecal contamination entering the upper Topanga Creek watershed were observed (Riedel et al., 2015). Inputs include runoff from the town of Topanga and Topanga Canyon Boulevard, which parallels the creek. The upper reach (4500–4650 m) is pool-dominated and larger substrate, such as cobbles, boulder, and bedrock are more frequent, while the lower reach of Topanga Canyon is dominated by riffles and runs (25 and 75%) and a higher percentage of smaller substrates, such as fines and gravel (Dagit et al., 2014). Site coordinates, map of sites selected, and site descriptions can be found in the Supplemental Information (SI).

Approximately the top 5 cm sediment was collected from at least three locations within a 5 m × 5 m transect at each site using sterile sediment cores. Sediments were held at 4 °C in the dark for approximately 48 h before microcosm experiments. Particle size, organic matter content, and nutrient, and chlorophyll *a* levels were measured in each sediment type; for detailed methods see the SI.

2.1.2. Microcosm setup

Microcosms were constructed in 2 L Pyrex beakers with a 2:1 sediment: water ratio by volume using homogenized sediment and artificial fresh water ((AFW) distilled water with 0.3 mM $MgCl_2$, 0.6 mM $CaCl_2$, and 1.4 mM $NaHCO_3$). Two types of sediment treatments were conducted: ambient (untreated) and reduced microbiota (oven-dried).

Sediment collected from selected sites (S2, S3, S4, and C2) was oven-dried at 180 °C for 48 h to reduce the impact of the ambient microbial community and predators (Korajkic et al., 2013; Wanjugi and Harwood, 2013). Reduction of the ambient microbial community was assured by running an Enterolert and Coliert IDEXX on sediment resuspension and by streaking the sediment resuspension onto Tryptic Soy Agar; bacterial levels after oven-drying were below the LOD on all media. Oven-dried sediment moisture content was adjusted with AFW to match moisture content of ambient sediment prior to seeding of sewage inoculum.

Sewage influent was collected from the Orange County Sanitation District in Fountain Valley, CA and held for 24 h prior to sediment inoculation. On Day 0 of the microcosm experiments, ambient and oven-dried sediment from each site was mixed with sewage (5% primary influent) for two hours in separate 26 L Sterilite containers. After two hours, 400 mL seeded sediment was distributed into each of three replicate, 2 L Pyrex beakers. 200 mL AFW was then added to each beaker. A preliminary microcosm experiment showed no difference in cENT decay in freshwater sediment collected from Site S2 when ambient site water or filter-sterilized site water was used instead of AFW (see SI for details, Fig. S2).

Microcosm experiments were conducted in a Precision Environmental Chamber set at 20 °C on a 12-h light/dark cycle. Each beaker had an airstone to ensure mixed and oxygenated conditions (Whisper 100 Aquarium Air Pump, Tetra, Blacksburg, VA). Sediment was inspected visually to ensure that it was not suspended by air movement.