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Application of a microbial source tracking based on bacterial and chemical markers in headwater and coastal catchments

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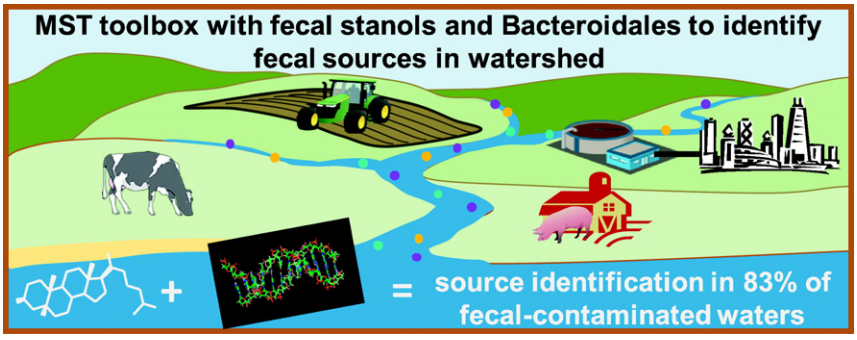
HIGHLIGHTS

- Test of microbial source tracking in contrasting mixed-use catchments.
- Monthly monitoring of specific chemical and microbial markers was performed.
- The toolbox efficiently identified fecal sources in 83% of the samples.
- In nested catchments, the number of fecal sources increases downstream.
- Headwaters and outlets may be integrated for effective management strategies.

GRAPHICAL ABSTRACT



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ABSTRACT

This study identified sources of fecal contamination in three different French headwater and coastal catchments (the Justiçou, Pen an Traon, and La Fresnaye) using a combination of microbial source tracking tools. The tools included bacterial markers (three host-associated Bacteroidales) and chemical markers (six fecal stanols), which were monitored monthly over one or two years in addition to fecal indicator bacteria. 168 of the 240 freshwater and marine water samples had *Escherichia coli* (*E. coli*) or enterococci concentrations higher than "excellent" European water quality threshold. In the three catchments, the results suggested that the fecal contamination appeared to be primarily from an animal origin and particularly from a bovine origin in 52% (Rum2Bac) and 46% (Bstanol) of the samples and to a lesser extent from a porcine origin in 19% (Pig2Bac) and 21% (Pstanol) of the samples. Our results suggested a human fecal contamination in 56% (HF183) and 32% (Hstanol) of the samples. Rainfall also impacted the source identification of microbial contamination. In general, these findings could inform effective implementation of microbial source tracking strategies, specifically that the location of sampling points must include variability at the landscape scale.

1. Introduction

The microbial quality of coastal environments can be affected by fecal pollution from urban and agricultural sources. Fecal contamination

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affects waterways worldwide, leading to the closures or downgrading of shellfish-harvesting areas and bathing areas, and to outbreaks of food poisoning through the consumption of contaminated shellfish or waters (Pommepuy et al., 2005). To prevent these sanitary issues, fecal indicator bacteria; often *E. coli* or enterococci, are used as indicators to determine the level of fecal contamination in water and to classify the water quality as defined by the European regulations (EU 2006/7/EC directive; regulation #854/2004). Different markers have been developed to compliment fecal indicators and allow microbial source tracking toolbox (MST toolbox) (Field and Samadpour, 2007). Specifically, these chemical and microbial markers distinguish fecal pollution sources, i.e. human, bovine, porcine, etc. (Boehm et al., 2003; Rusinol et al., 2016; Sidhu et al., 2013; Tran et al., 2015).

As highlighted by Tran et al. (2015), a suitable MST toolbox needs to be customized to the study area, including characteristics such as anthropogenic activities and land use patterns. In Brittany (northwestern France), the most widespread agricultural activity is intensive livestock, primarily cows and pigs. For this area, it is therefore necessary to distinguish between human, bovine, and porcine sources of fecal contamination. A suitable MST toolbox using microbial and chemical markers has been previously developed for the specific context in Brittany by Gourmelon et al. (2010). This toolbox combines three host-associated real-time PCR Bacteroidales markers designed to identify human (HF183), ruminant (bovine and ovine; Rum2Bac), and porcine (Pig2Bac) fecal contamination (Bernhard and Field, 2000; Mieszkin et al., 2009; Mieszkin et al., 2010; Seurinck et al., 2005) as well as fecal stanols, chemical markers whose distribution can be used to distinguish between human, porcine, and bovine sources of fecal contamination (Derrien et al., 2011; Derrien et al., 2015; Leeming et al., 1996). Real-time PCR markers have been found to offer high sensitivity and specificity, in this French region, ranging from 78% to 100% (Mauffret et al., 2012). A sensitivity of 78%, 98% and 100% was obtained for HF183, Rum2Bac and Pig2Bac, respectively. The lower sensitivity of HF183 was due to the few positive human feces samples whereas all the WWTP effluents tested were found positive. A specificity of 99%, 94%, and 99% was obtained for HF183, Rum2Bac and Pig2Bac, respectively. Furthermore, these markers were found highly sensitive and specific in other countries. The review of 32 studies focusing on the HF183 marker by Ahmed and co-workers in 2016 (Ahmed et al., 2016) show an overall host sensitivity and specificity of 83.1% ($n = 1242$ targeted fecal samples analysed) and 94.6% ($n = 2966$ non targeted fecal samples tested). In addition, Pig2Bac marker was also found highly specific in USA and Israel and Rum2Bac in USA (Raith et al., 2013; Heaney et al., 2015; Ohad et al., 2015).

In Europe, shellfish-harvesting areas and bathing areas are classified according to the fecal indicator bacteria level in shellfish (*E. coli*) and water (*E. coli* and enterococci; EU 2006/7/EC directive; regulation #854/2004). With regards to bathing areas, fecal indicator bacteria are used to monitor and classify bathing waters as insufficient, good, or excellent (threshold values in Table S1). Furthermore, a water bathing profile has to be established in all the European bathing areas, identifying the potential sources of fecal contamination (2006/7/EC directive).

There is therefore a need for tools to efficiently identify sources of fecal contamination in waters that do not comply with European water quality guidelines.

In this study, we used a MST toolbox based on bacterial and chemical markers at the catchment scale to study seasonal variations in term of intensity and sources of fecal contamination in three contrasting catchments in Brittany, France. We hypothesized that the use of a MST toolbox at a monthly frequency, in concert with precipitation data, should allow the determination of the main seasonal fecal contamination sources depending on the catchment land use and land cover. These results could help local stakeholders to improve land use management by decreasing microbial contamination threats.

2. Material and methods

2.1. Catchment characteristics and water sampling strategy

2.1.1. Justiçou, Pen an Traon and La Fresnaye catchments

The Justiçou catchment (catchment J; 2064 inhabitants; 9 km long; 5.6 km^2) is located in the upstream part of the Elorn catchment (Fig. 1, S1). The catchment of the Elorn estuary (Brittany, France) covers 385 km^2 and is located to the north-east of the Bay of Brest. A detailed description of this catchment can be found in Mieszkin et al. (2013). Fecal bacteria sources in the catchment J include one wastewater treatment plant located at Plouneventer (WWTP; 1298 inhabitants-equivalents, extended aeration activated sludge process, located just upstream of the J1 sampling point) pig, cattle, and poultry breeding (animal and human densities are shown in Fig. S1), manure spreading, and livestock grazing area. Five sampling points (J1 to J5) were selected in order to include the impact of the WWTP on a small stream with pressure from intensive agriculture (Table S2, Fig. 1).

The Pen an Traon catchment (catchment P; 629 inhabitants) is a coastal catchment, which covers 3.7 km^2 and includes a swimming area (Pen an Traon beach, P5) (Figs. 1, S2). Catchment P is divided into two areas that drain two streams flowing into the Pen an Traon beach: (i) the Poul ar Vilin stream (1.2 km; 1.2 km^2; P1 and P2) flows along a rural area mainly dominated by pastures and livestock, and (ii) the Pouldu stream (1.3 km; 1 km^2; P3 and P4) flows along two small urban areas without a municipal WWTP and including pastures (Fig. S2). In a recent swimming-water profile study (Patris and Perenne, 2011), these two streams were identified as potential contributors (4% of the *E. coli* fluxes in the Elorn estuary) to fecal contamination inputs. The high *E. coli* concentration and their proximity to the swimming beach increase risk of contamination. Five sampling points were monitored upstream and downstream from the Poul ar Vilin (P1 and P2) and Pouldu streams (P3 and P4) and at the Pen an Traon beach (P5, Table S2). These two headwater catchments were chosen to evaluate point and non-point fecal contamination sources.

The La Fresnaye catchment (catchment F; 121 km^2; 6923 inhabitants) is located in the northeastern part of Brittany (Figs. 1, S3). Catchment F is divided into four sub-catchments: Fremur (river length = 50 km, area = 77.4 km^2, F1), le Rat (river length = 10.5 km, area = 19.2 km^2, F2), le Clos (river length = 8.7 km, area = 13.3 km^2, F3), and Kermiton (river length = 2.3 km, area = 6.3 km^2, F4). The catchment includes 12 towns with seven WWTPs: two activated sludge WWTPs, three lagoon-based WWTPs, two reed bed filter WWTPs, and one using rotating biological contactors (dimensioned for 250 to 16,000 inhabitants-equivalents). Catchment F has intensive livestock production (Fig. S3). Sampling points were monitored at the outlet of each sub-catchment (F1: Fremur; F2: Le Rat; F3: Le Clos; F4: Kermiton) and in the seawater (F5), which was always sampled at high tide, downstream of the outlet of the Kermiton stream (F4).

2.1.2. Water sampling strategy

Monthly monitoring was performed over a one-year period in the catchments J and P (from August 23rd, 2010 to July 17th, 2011) and over a two-year period in the catchment F (from February 25th, 2013 to January 5th, 2015). Water samples were collected from the surface (top 10-cm) using sterilized bottles and were immediately placed in an ice chest, where they were kept until analysis. All samples were filtered in the laboratories within one day of sampling.

2.2. Water sample analysis

2.2.1. Enumeration of fecal indicator bacteria

Escherichia coli and enterococci were counted using microplate methods (EN ISO 9308-3 (Anonymous, 1999a) and EN ISO 7899-1 (Anonymous, 1999b)), respectively, with a detection limit of the 15 most probable number (MPN) per 100 mL of water sample.