

Long term monitoring of host-specific markers for microbial source tracking and catchment management

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Microbial Source Tracking (MST) methods are crucial to identify nonpoint-source pollution by faecal organisms and for effective resource management and remediation. Our objectives were to identify the main correlations and the sentinel host-specific markers among five selected ones helpful to built an efficient water catchment quality management.

In the French catchment of Daoulas (113 km²) with intensive livestock farming (9,500 cows, 60,000 pigs and 434,000 poultry), five host-specific markers were monitored monthly at ten sites over 24 months (20 to date): the human- Hf183, the ruminant- Rum-2-Bac and the pig- Pig-2-Bac specific *Bacteroidales* markers; and animal- (FRNAPHI) and human- (FRNAPHII) specific genogroups of F-specific RNA bacteriophages. Two days rainfall, water temperature, oxygen level, turbidity, pH, concentrations of classical faecal indicators (*Escherichia coli*, enterococci) and of the general AllBac *Bacteroidales* marker were also measured. Correlations between all these variables were determined using the Spearman coefficients ($p < 0.0001$). Variability in the microbiological data was illustrated with a principal component analysis (PCA). Cluster analysis was applied to the data set in order to point out outsider sites.

According to the cluster analysis, the 10 sites were divided into 2 main groups. A first group was composed of 3 sites situated close by the coast, where *E. coli* concentrations were mainly < 2.7 log MPN/100 mL water (87% of the samples, $n=63$). The second group was formed by the four upstream catchment sites where mainly agricultural contaminations were expected and the three sites in the main town of the region where mixed contamination was expected. Higher *E. coli* levels were reported in this second group of sites (Figure 1). Classification using the host-specific *Bacteroidales* markers increased with the *E. coli* levels, and reached 86% with *E. coli* concentrations above 3.3 log MPN / 100 mL. The contamination appeared mainly linked with the ruminants. Human contamination was observed in the Daoulas town. Pig-2-Bac was observed in only 4 samples in the pooled 10 sites and 20 months monitoring. Separately, in water downstream an accidental spillage from a manure tank, Pig-2-Bac levels were close to the limit of quantification pointing out its limitation to trace pig manure spreading contaminations. Finally, FRNAPHI and FRNAPHII were detected in a minority of samples. Classification was given in 34% of the samples when *E. coli* concentrations were above 3.3 log MPN / 100 mL water.

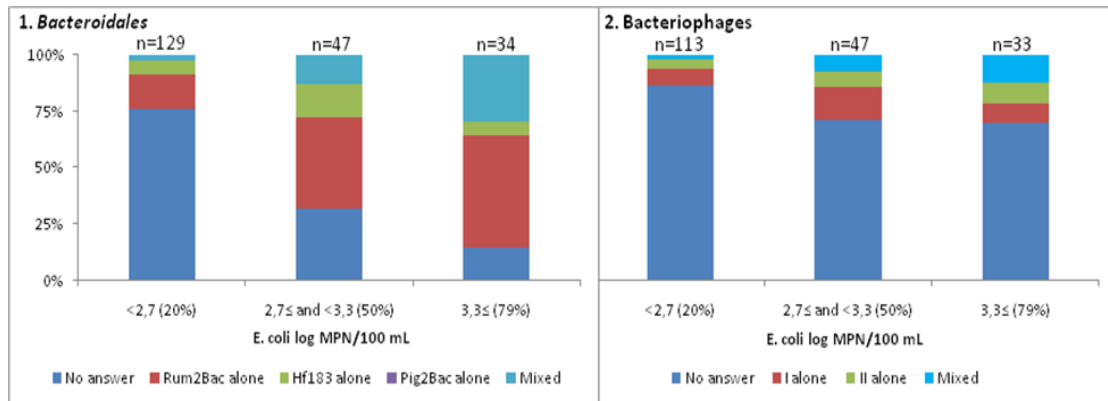


Figure 1: Water sample classification according to the host-specific *Bacteroidale* markers (1) or the FRNAPHI and FRNAPHII (2) at three *E. coli* levels. Total number of samples are indicated at the top of the column. In parenthesis, the percentage of samples collected when the rainfall was ≥ 10 mm is indicated.

In the PCA with the 10 sites and 20 first months data set, enterococci, *E. coli*, AllBac and Rum-2-Bac were positively correlated with the first axis, that explained 33% of the total variability in the microbial parameters (Figure 2). They were also positively correlated with the rainfall and turbidity and anti-correlated with the salinity. Pig-2-Bac, Hf183, FRNAPHI and FRNAPHII were correlated with the second axis (20%) and thus independent with the variables associated with the first axis. Effect of salinity on the microbial variables was probably due to the dilution effect away from the pollution sources on the coast sites, rather than an effect of the salinity on the faecal markers. The correlation between Rum-2-Bac and the rainfall pointed out that ruminant contaminations were likely to be induced by streaming down pasturage soils or bovine manure spreading. On the opposite, rainfall did not affect Hf183 levels indicating that human contamination was likely to be introduced continuously during the year.

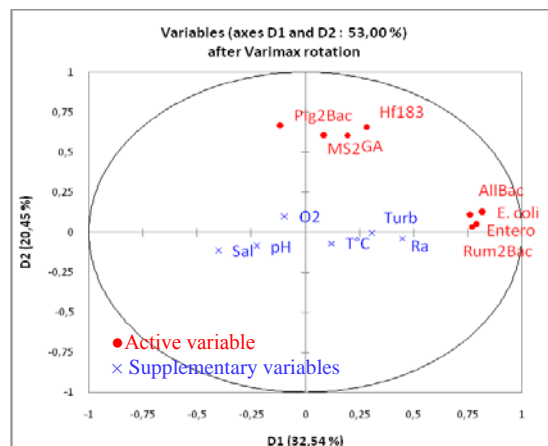


Figure 2: Principal component analysis for the pooled 10 sites and 20 first months data set.

In the present study, Rum2Bac and Hf183 allowed to identify ruminant and human contaminations, respectively. Pig-2-Bac does not seem suitable to trace a contamination by pig manure spreading. The low level of detection of the human or animal genogroups of FRNAPH raise the question of their relevance as MST markers in such a catchment, they should be used with other markers. The present statistical quantitative approach will be compared with a rank qualitative approach. A 24-h sampling study is undergoing to assess variation in these five host-specific markers levels during the day. This research was performed in the framework of the AQUAMANICHE project which was supported by the INTERREG IVA program.