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# POLLUTION SOURCE IDENTIFICATION, TRACKING AND SANITARY SURVEY IN ITALIAN BEACHES

*Carducci A.[1], Verani M.[1], Federigi I.[1], Iannelli R.[2]*

*[1] Laboratory of Hygiene and Environmental Virology, Department of Biology, University of Pisa, Italy (E-mail: annalaura.carducci@unipi.it); [2] Department of Engineering for Energy, Systems, Territory and Constructions, University of Pisa, Italy*

## Aims

Italy has about 5.500 Km of monitored coastline, that are almost fully suitable for bathing according to ongoing European Regulation. Besides the significance for public health, recreational water quality is an important indicator of tourism development, since beach advisories and closures due to lack of compliance with bathing suitability standards have a negative impact on economy of coastal areas. The study area is a notorious tourist destination in the north-west of Tuscany, combining beautiful landscape and popular beaches (**figure 1**). Short-term pollution posed the problem of possible classification as "scarce" of these area, owing to the fecal contamination caused by drainage ditches. Our goal was to understand the impact of polluted streams on seawater contamination and the role of meteorological conditions on freshwater and seawater bacterial indicator levels. To this aim, the monitoring results from 2012 to 2015 bathing seasons were analyzed and, only for 2015, fecal-oral pathogens and viral indicators were searched at ditch mouths, using cultural and biomolecular techniques.

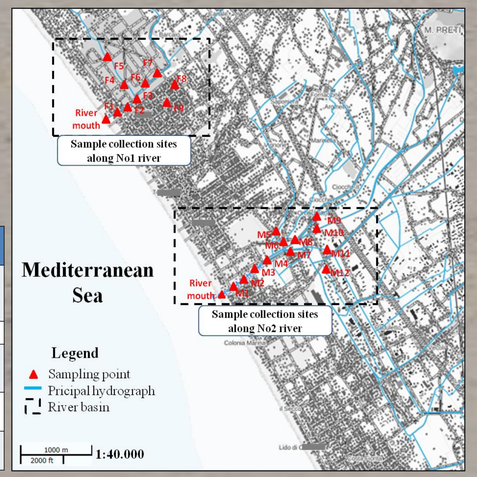
**Figure 1. Aerial picture of shoreline.**

## Materials and Methods

During summer seasons from 2012 to 2015, data on fecal bacteria contamination were collected: weekly samples from a series of sampling points along the rivers and monthly samples from the sea at the river mouths (**figure 2**). Rainfall amount was also recorded for the sampling dates. During 2015 bathing season, the environmental monitoring was focused on terminal tract of the rivers, and samples were analyzed for bacterial (intestinal enterococci, *Escherichia coli*) and viral (human adenovirus, F-specific coliphage) fecal-indicator microorganisms and fecal-oral pathogens (*Salmonella* spp., norovirus, enterovirus, hepatitis A virus), following analytical methods described in table below. Statistical analyses of data was carried out using parametric approach.

| Microbial parameter | Analytical methods |
|---|---|
| *Escherichia coli*, intestinal enterococci | Standardized microplates method (ISO 9308-3 for *E.coli* and ISO 7899-1 for IE) |
| *Salmonella* spp. | EPA method for drinking water (US EPA, 2006) |
| Human Adenovirus | Ultrafiltration, biomolecular test (DNA extraction, qPCR) and infectivity assay on A549 cells (MPN) |
| Coliphage | EPA method 1602 for ground water (US EPA, 2001) |
| Enteric RNA viruses | Ultrafiltration, biomolecular test (RNA extraction, *two step* RT-qPCR ) |

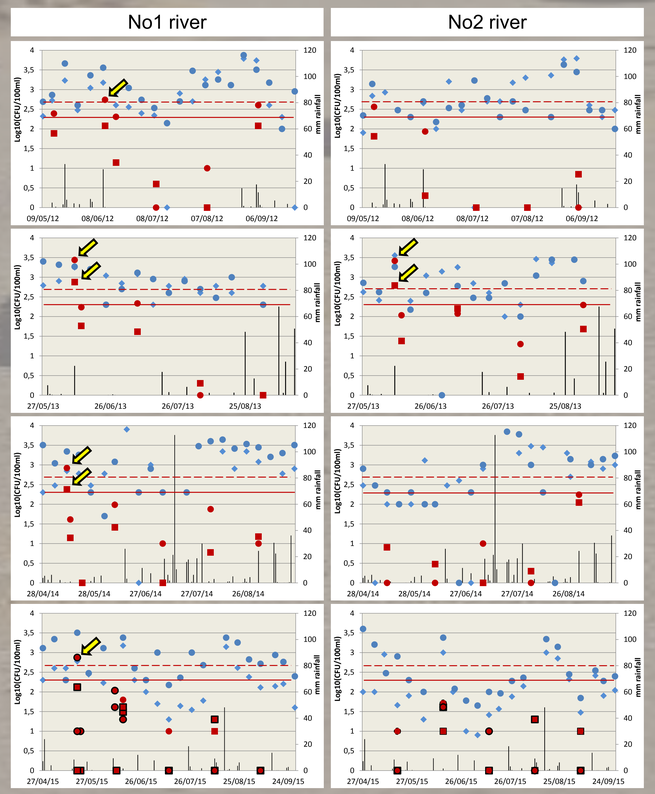
**Figure 2. Location of the study area indicating the sampling sites for each river.**




## Results

In the study period, the limits of European Bathing Directive (500 CFU/100 ml for *E. coli* and 200 CFU/100 ml for enterococci) were exceeded only in the first half of each bathing season (from April to June), six times at the No1 river and two at the No2 river mouths (**figure 3**). This can be related to a time-dependent dilution effect of the sea on river waters, that is statistically significant for No1 river (t test, $P < 0.05$), ranging from a minimum of 0.39 dilution log in June to a maximum of 2.30 dilution log in September. Comparing the average concentrations of indicators between dry and wet days separately for each river and year, a statistically significant difference was observed at the river mouths, while for sea waters data were too scarce for statistical analysis. Considering the total study period, the differences of microbial concentrations between dry and wet days were statistically significant (t test, $P < 0.05$) in the majority of the sampling points along the rivers (**figure 4**). During the last year of monitoring, besides bacteria indicators, human adenovirus and coliphages were found, but not RNA pathogenic viruses (**figure 5**).

**Figure 3. River and seawater contamination related to rainfall from 2012 to 2015 bathing seasons. Amount of rainfall are black histograms (▮). Samples collected at river mouth are blu symbols (*E. coli* ● ; IE ◆ ) and samples collected from the sea are red symbols (*E. coli* ● ; IE ◆ ). Only for 2015 bathing season additional sampling points were introduced (*E. coli* ● ; IE ◆ ). Continued and dotted lines indicate bathing suitability limits for IE and *E. coli*, respectively. Arrows indicate values over limits.**




$Log_{10}$ average adenovirus DNA concentration and infectivity were respectively 7.2 ± 0.6 GC/10L and 1.8 ± 0.4 MPN/10L in No1 river, 6.2 ± 1.8 GC/10L e 2.2 ± 0.7 MPN/10L in No2 river. Concerning coliphages, 1.6 ± 0.6 PFU/1L and 1.2 ± 0.6 PFU/1L were detected in No1 and No2 river, respectively. No significant correlations were found between these parameters and bacterial indicators (Pearson, $p > 0.05$), the only statistically significant correlation was observed between *E. coli* and enterococci.

**Figure 4. Average mean and standard deviation of fecal contamination for each sampling point along the rivers, in wet (■) and dry (□) days. Data from 2012 to 2015 bathing seasons are taken into account. Asteriscs indicate the statistical significativity: (*) significant, P< 0.05; (**) very significant, P < 0.01; (***) extremely significant, P < 0.001.**

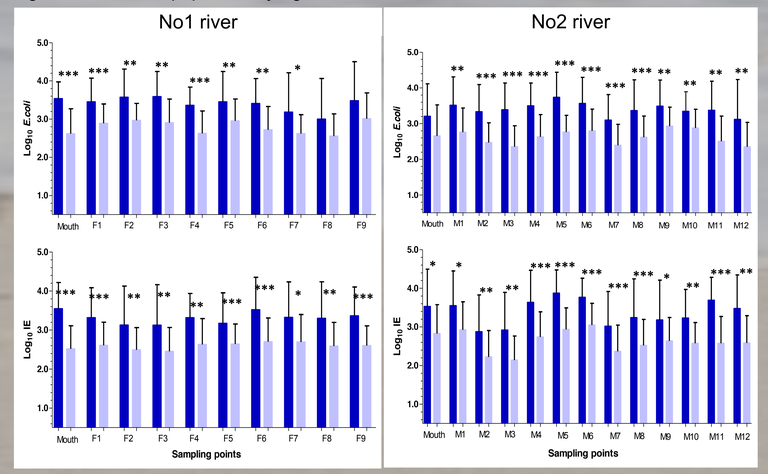
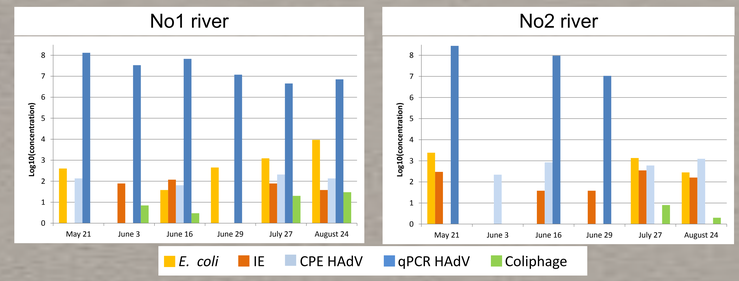



**Figure 5. Traditional and new indicators of fecal contamination during 2015 monitoring campaign (HAdV, adenovirus; CPE, Cytopatic Effect; qPCR, quantitative Polimerase Chain Reaction).**




## Conclusion

In the studied area, this is the first environmental survey on the recreational water sources of pollution. The collected data allowed us to better explain the pollution dynamics along watershed and the effect of this microbial contamination on beach water quality. In particular, they confirmed the high impact of rainfall events. Nevertheless, the absence of this effect in some points suggests the presence of constant pollution sources (i.e. abusive discharges). The monitoring campaign during the 2015 bathing season confirmed the abundant presence of adenovirus, but without any correlation with the other indicators. This analytical survey on the sources of pollution of recreational waters could be used to create a large monitoring data set for developing predictive models of microbial contamination in relation with climatic conditions and possible sanification interventions.