

## Coliphages as indicators of fecal contamination in wastewater treatment (SCA.Re.S. Project)

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### Introduction

Bacteriophages are fecal indicators of viral contamination (**Figure 1**), because they are more similar to human pathogenic viruses than the traditional fecal indicator bacteria, in terms of log-reduction during wastewater treatment and persistence in the aquatic environment. The aim of this study was to evaluate the presence of bacteriophages and enteric viruses: Enterovirus, Adenovirus, Norovirus, Hepatitis A and E, Rotavirus and Pepper mild mottle virus (PMMoV), in the context of SCA.Re.S. (Evaluation of sanitary risk related to the discharge of wastewater to the ground) project

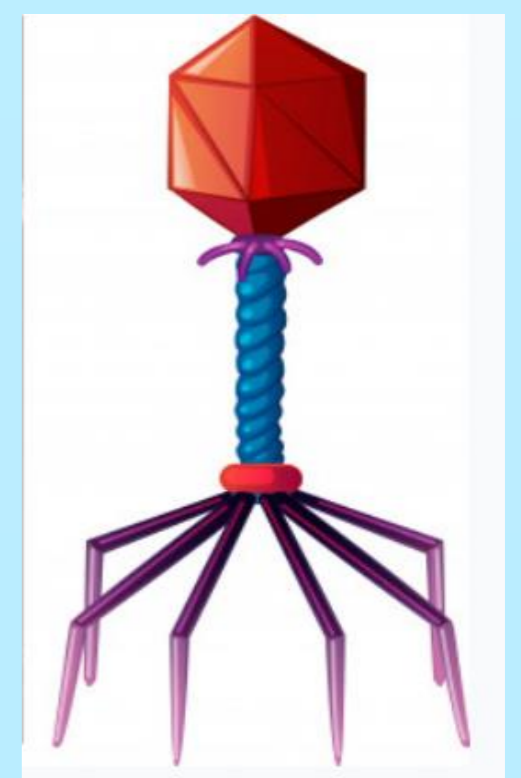


Figure 1: Bacteriophage

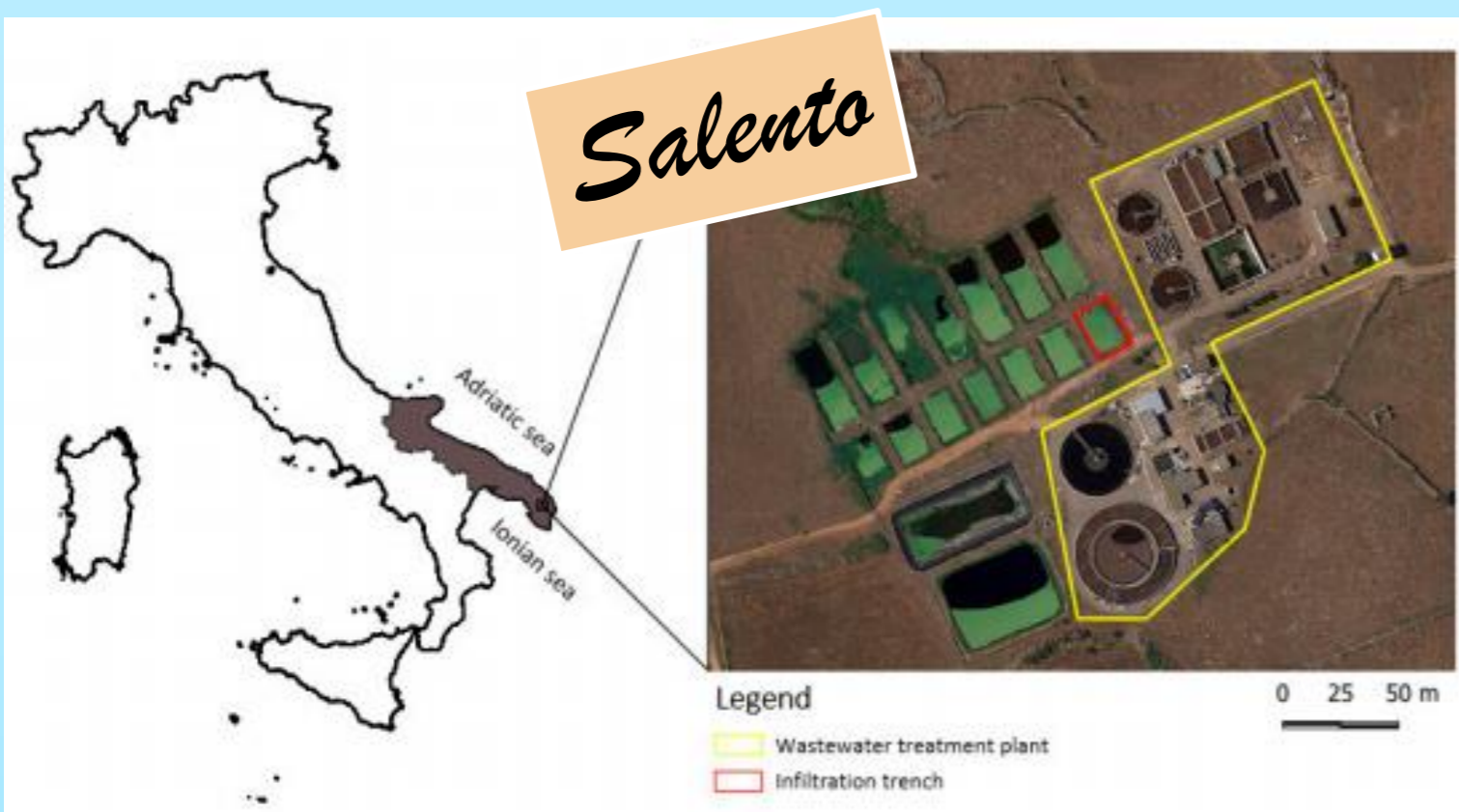


Figure 2: Wastewater treatment plant located in the karst-fractured area in Salento peninsula, Apulia, Italy.

### Materials and Methods

The investigation focused on a wastewater treatment plant located in an area fractured by karst in the Salento peninsula (Apulia, Italy) (**Figure 2**). In autumn (September–November) 2019, water samples were monthly collected from three sites (treated wastewater, infiltration trench and monitoring well). The somatic coliphages were analyzed by standardized culture-based methods, according to BS EN ISO 10705-2:2001. Coliphage density was enumerated using plaque assay method on appropriate host bacteria (*E. coli* Famp) and expressed as plaque forming units PFU/100mL. Nested RT-PCR assay was used for detection of enteric viruses.

### Results

The median values of coliphages were 590 PFU/100mL in treated wastewater, 1000 PFU/100mL in infiltration trench while all samples from monitoring well were under the detection limit (<25 PFU/100ml) (**Figure 3**). All samples were positive for at least one viral pathogen. PMMoV was detected only in monitoring well.

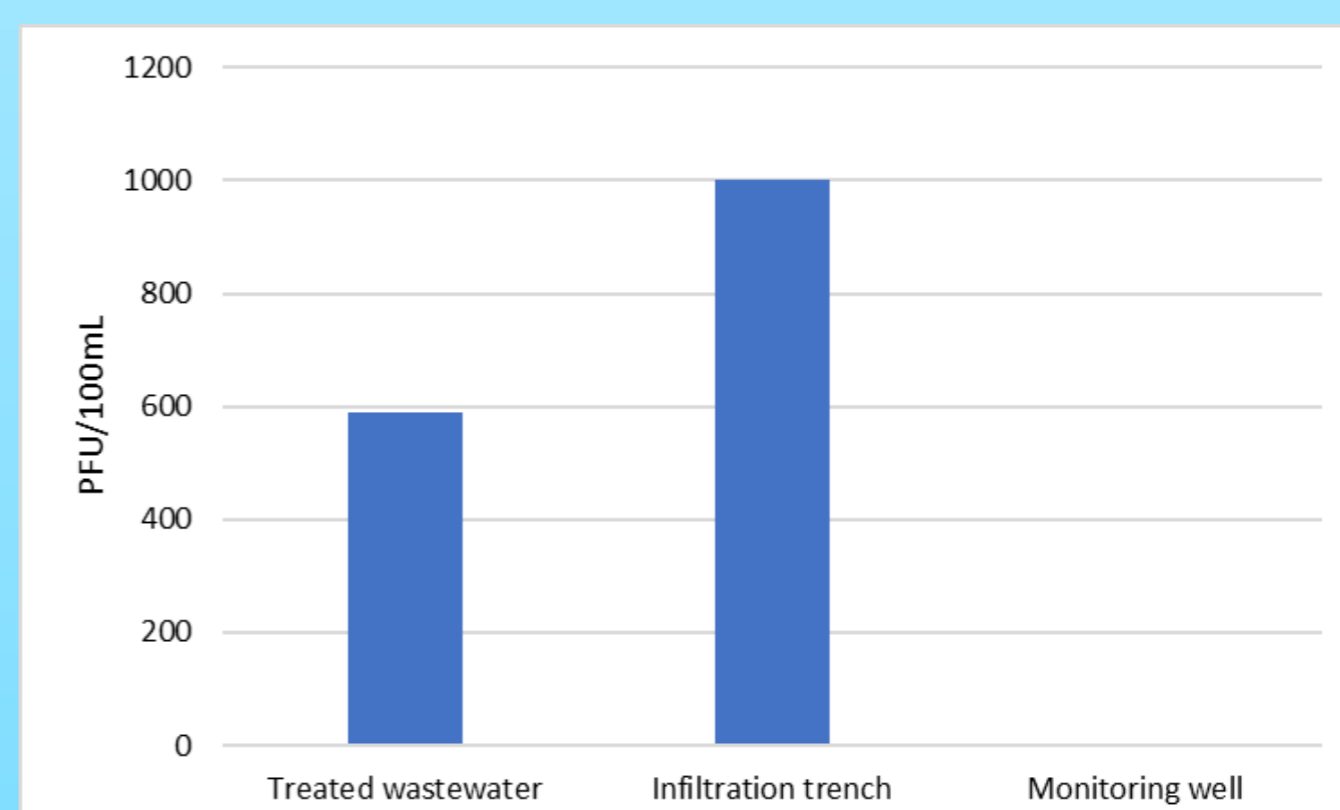


Figure 3: Median values of coliphages in treated wastewater, infiltration trench and monitoring well.

### Conclusions

The results confirmed the role of coliphages as indicators of viral contamination. Overall, we observed a gradual reduction in the concentration/occurrence of coliphages and viruses across the karst-fissured soil (**Figure 4**), until the complete removal in the monitoring well. Different soil properties are probably involved in this phenomenon such as straining, soil pores, microorganism size, and adsorption onto soil particle. Moreover, we can also hypothesize natural degradation over time, phototoxicity or ingestion by multicellular organisms in the soil and/or monitoring well water.



Figure 4: Karst-fissured soil.