

## Abstract

# Coliphages as Indicators of Fecal Contamination in Wastewater Treatment (SCA.Re.S. Project) <sup>†</sup>

Osvalda De Giglio <sup>1,\*</sup>, Ileana Federigi <sup>2</sup>, Marco Verani <sup>2</sup>, Giusy Diella <sup>1</sup>, Francesco Triggiano <sup>1</sup>, Carla Calia <sup>1</sup>, Francesca Apollonio <sup>1</sup>, Giusy Bonanno Ferraro <sup>3</sup>, Pamela Mancini <sup>3</sup>, Carolina Veneri <sup>3</sup>, Giuseppina La Rosa <sup>3</sup>, Annalaura Carducci <sup>2</sup> and Maria Teresa Montagna <sup>1</sup>

<sup>1</sup> Regional Reference Laboratory of Clinical and Environmental Surveillance of Legionellosis, Department of Biomedical Science and Human Oncology, University of Bari Aldo Moro, Piazza G. Cesare 11, 70124 Bari, Italy; giusy.diella@uniba.it (G.D.); francesco.triggiano@uniba.it (F.T.); carla.calia@uniba.it (C.C.); francesca.apo@libero.it (F.A.); mariateresa.montagna@uniba.it (M.T.M.)

<sup>2</sup> Laboratory of Hygiene and Environmental Virology, Department of Biology, University of Pisa, Via S. Zeno 35/39, 56127 Pisa, Italy; ileana.federigi@biologia.unipi.it (I.F.); marco.verani@unipi.it (M.V.); annalaura.carducci@unipi.it (A.C.)

<sup>3</sup> Department of Environment and Health, Istituto Superiore di Sanità, 00161 Rome, Italy; giusy.bonannoferraro@iss.it (G.B.F.); pamelamancini@iss.it (P.M.); carolina.veneri@guest.iss.it (C.V.); giuseppina.larosa@iss.it (G.L.R.)

\* Correspondence: osvalda.degiglio@uniba.it

<sup>†</sup> Presented at the 3rd International Electronic Conference on Environmental Research and Public Health—Public Health Issues in the Context of the COVID-19 Pandemic, 11–25 January 2021; Available online: <https://ecerph-3.sciforum.net/>.



**Citation:** De Giglio, O.; Federigi, I.; Verani, M.; Diella, G.; Triggiano, F.; Calia, C.; Apollonio, F.; Bonanno Ferraro, G.; Mancini, P.; Veneri, C.; et al. Coliphages as Indicators of Fecal Contamination in Wastewater Treatment (SCA.Re.S. Project). *Med. Sci. Forum* **2021**, *4*, 47. <https://doi.org/10.3390/ECERPH-3-08997>

Academic Editor: Jon Øyvind Odland

Published: 11 January 2021

**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Copyright:** © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

**Abstract:** Bacteriophages are fecal indicators of viral contamination, 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. The investigation focused on a wastewater treatment plant, located in an area fractured by karst in the Salento peninsula (Apulia, Italy). 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 the plaque assay method on appropriate host bacteria (*E. coli* Famp), and expressed as plaque-forming units, PFU/100 mL. A nested RT-PCR assay was used for the detection of enteric viruses. The median values of coliphages were 590 PFU/100 mL in treated wastewater and 1000 PFU/100 mL in infiltration trench, while all samples from the monitoring well were under the detection limit. All samples were positive for at least one viral pathogen. PMMoV was detected only in the monitoring well. 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, until complete removal in the monitoring well. Different soil properties are likely 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.

**Keywords:** coliphages; virus; wastewater

**Author Contributions:** Conceptualization, O.D.G., M.T.M., A.C.; Methodology, M.T.M., O.D.G., I.F., M.V., A.C., G.L.R.; Software and formal analysis, I.F., M.V.; Validation, M.T.M., O.D.G., G.L.R., A.C.;

Bacteriological and virological investigation, F.T., F.A., C.C., G.D., G.L.R., P.M., G.B.F., C.V., I.F., M.V.; Writing—original draft preparation, I.F., O.D.G., M.T.M., A.C., M.V.; Writing—review and editing, M.T.M., O.D.G., I.F., A.C., M.V., G.L.R.; Funding acquisition, M.T.M. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by the Apulia Regional Government, Bari, Italy (DGR 1346 del 04/08/2021 Not applicable Not applicable All data generated or analyzed during this study are included in this published article The authors declare no conflict of interest.

**Conflicts of Interest:** The authors declare no conflict of interest.