frontiers | Frontiers in Water

TYPE Brief Research Report
PUBLISHED 03 October 2022
DOI 10.3389/frwa.2022.1026768

OPEN ACCESS

EDITED BY
Constantinos V. Chrysikopoulos,
Technical University of Crete, Greece

REVIEWED BY
Petros Kokkinos,
Hellenic Open University, Greece
Vasiliki Syngouna,
University of Patras, Greece

*CORRESPONDENCE
Helena M. Galvão
hgalvao@ualg.pt

SPECIALTY SECTION
This article was submitted to Environmental Water Quality, a section of the journal Frontiers in Water

RECEIVED 24 August 2022
ACCEPTED 05 September 2022
PUBLISHED 03 October 2022

CITATION
Galvão HM, Mendes PJ, Figueiredo A, Santos R and Monteiro S (2022) SARS-CoV-2 contamination potential in environmental and wastewaters in the Algarve region, Southern Portugal. *Front. Water* 4:1026768. doi: 10.3389/frwa.2022.1026768

SARS-CoV-2 contamination potential in environmental and wastewaters in the Algarve region, Southern Portugal

Helena M. Galvão[1]*, Pedro J. Mendes[1], Aida Figueiredo[1], Ricardo Santos[2] and Silvia Monteiro[2]

[1]Centro de Investigação Marinha e Ambiental (CIMA), Universidade do Algarve, Faro, Portugal, [2]Laboratório de Análises, Instituto Superior Técnico (IST), Lisboa, Portugal

The Ria Formosa, Southern Portugal (Algarve) is a mesotidal lagoon with restricted exchange with Atlantic Ocean. Due to arid Mediterranean climate and absence of large freshwater inputs, the lagoon remains hypersaline relative to adjacent ocean ($\geq$37 psu). Generally, fecal coliforms and land-derived bacteria do not survive long in seawater due to UV, osmotic shock, oligotrophy and competition with marine bacteria. However, survival of human pathogenic viruses in natural waters remains relatively unknown. In view of 2020–2021 SARS-CoV-2 pandemic, this preliminary study aimed to estimate potential contamination by wastewater and persistence in marine environment. Five sampling campaigns (45 total samples) were completed in 2020 and 2021 at three stations in the lagoon, as well as inflow and outflow from three wastewater treatment plants (WWTPs). Despite relatively high fecal contamination at lagoon stations, SARS-CoV-2 could not be detected in surface waters using improved methodology. Untreated wastewaters were positive at Faro-Aeroporto 21/10/2020, 14/12/2020, 08/03/2021; Vilamoura 21/10/2020, 14/12/2020; Olhão 14/12/2020. All treated wastewaters were negative, as well as environmental samples. Highest viral titers were observed in Faro-Aeroporto WWTP on 8 March 2021 (1.35×10^5 genomic copies L^{-1}) coinciding with peak SARS-CoV-2 cumulative positive cases in Algarve (2.03×10^4), yet SARS-CoV-2 was not detected in Vilamoura and Olhão raw wastewater then. Thus, the contamination potential of SARS-CoV-2 seemed non-existent in the Ria Formosa during peak pandemic surges in Algarve. However, predicted climate change and its impact on microbial populations remains a challenge to be addressed by both health and tourist authorities.

KEYWORDS

SARS-CoV-2, fecal contamination, wastewater treatment, coastal lagoon, Ria Formosa

Introduction

Coronavirus disease 2019 (Covid-19) is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), an enveloped, single-stranded RNA virus characterized by relatively high infection rate and often asymptomatic infection. The first clinical cases in Portugal were reported on March 2, 2020, with the exponential phase having been reached a couple of weeks later (DGS, 2020).

Wastewater-based epidemiology (WBE) has been applied for several decades to trace pharmaceutical and illicit drug use in urban populations (Choi et al., 2018). The usefulness and potential of wastewater as a surveillance system for pathogens has been demonstrated in the past (Koopmans et al., 2017). It has several advantages, mainly, wastewater analyses represent testing thousands of potentially infected individuals at the same time, with the possibility to identify hotspots of infection prior to syndromic surveillance.

This study aimed to assess the potential for SARS-CoV-2 contamination in environmental and wastewaters in the Algarve region, Southern Portugal. The Ria Formosa lagoon (RF) is an important saltmarsh ecosystem located in the Algarve coast and extends from Faro to Tavira with an area of ca 110 km^2. Besides its ecological importance as a refuge and nursery area for many marine species, it has also economic importance for fisheries, aquaculture and tourism industry. RF is characterized by an arid Mediterranean climate, an absence of significant freshwater inputs, and remains hypersaline relative to open coastal waters throughout the year ($\geq$ 37 psu).

Additionally, as a result of poor management in environmental practices during the installation of the wastewater networks, overflow of wastewater into rainwater collecting systems is common in the cities adjacent to the Ria Formosa (Sul Informação, 2020). This could allow SARS-CoV-2 contamination to bypass water treatment plants and pose a potential contamination risk to the public.

In view of predicted climate change for RF (Rodrigues et al., 2021) and adjacent coastal ocean, increasing temperature and decreased rainfall will probably contribute to intensified hypersaline regime in the lagoon. As a result, it can be surmised that self-depuration processes maybe promoted with respect to fecal bacteria contamination, but consequences to viral pathogens will depend on their type and structure and can only be ascertained with future environmental studies.

Sampling and analytical methods

The sampling program included three Wastewater Treatment Plants (WWTP), namely Vilamoura, Faro Airport and Olhão (see maps in Figure 1) sampled at inflow and outflow on 5 dates 22/09, 21/10, 23/11 & 14/12 in 2020, as well as on 08/03 in 2021. Additionally, surface water from three environmental stations in the RF lagoon, namely Faro Island Bridge, Commercial Dock in Faro, Recreational Dock in Olhão (see maps in Figure 1) were sampled on the same dates.

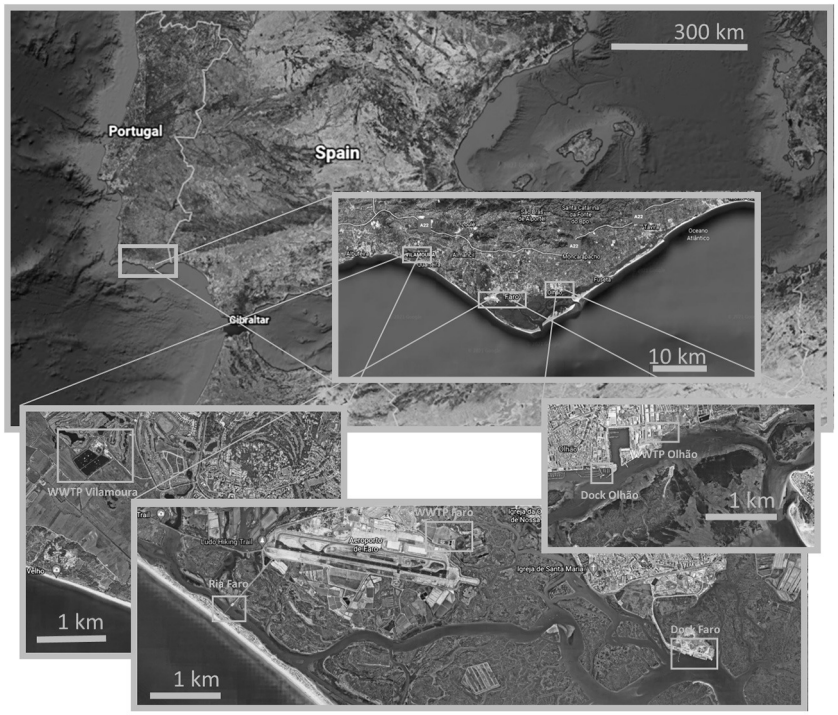

FIGURE 1
Map of the Algarve region, Southern Portugal showing location of 3 sampling stations in the Ria Formosa lagoon (Ria Faro, Dock Faro and Dock Olhão) and 3 Water Treatment Plants (WWTP Vilamoura, WWTP Faro and WWTP Olhão).

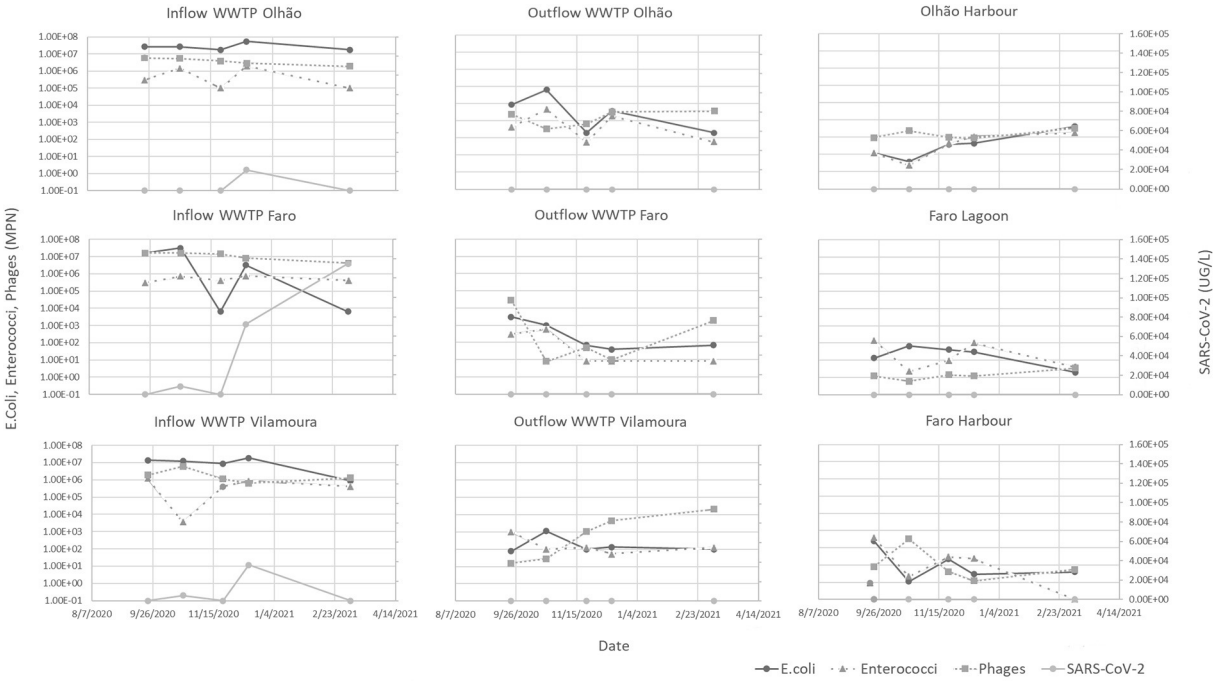

FIGURE 2
Escheria coli, Enterococci (MPN per 100 mL), somatic coliphage abundances (Plaque Forming Units per 100 mL) and SARS-CoV-2 genomic copies (µg. L^{-1}) on 5 sampling dates (22/09, 21/10, 23/11 and 14/12 in 2020; 08/03 in 2021) at 3 Ria Formosa stations (Faro lagoon, Faro harbor/dock and Olhão harbor/dock) and 3 WWTP (inflow and outflow) at Vilamoura, Faro airport and Olhão.

The environmental stations were selected due to both proximity to WWTPs and sampling logistics (eg. readily accessible). Faro commercial dock although further away from WWTPs was selected since it is more exposed to ocean influence. As an initial risk evaluation for human health, sampling dates were not planned to describe seasonality, but instead were designed to detect presence or absence of SARS-CoV-2 in both natural and wastewaters during the Covid-19 pandemic surge in the Algarve.

Samples were transported refrigerated within 24 h of collection to the Laboratório de Análises, Instituto Superior Técnico (IST) in Lisbon. Fecal contamination included routine analyses for *Escherichia coli* and intestinal *Enterocci* using Idexx water analyses methods (Colilert-18 and Enteroalert), somatic coliphages by plaque assay (ISO 10705-2:2000) and additionally SARS-CoV-2 by concentration and RT-PCR (Philo et al., 2020; Monteiro et al., 2022a).

Results

Results are summarized in Figure 2. Notwithstanding relatively high fecal contamination, determined by indicators such as *E. coli, Enteroccoci* and somatic coliphages, SARS-CoV-2 could not be detected in surface waters at the three environmental stations in Ria Formosa lagoon on all 5 sampling dates. Due to limited database, in-depth and/or non-parametric statistical analyses were not performed. Nevertheless, in environmental waters, there was no correlation between *E. coli* and *Enterocci* (Pearson $r > 0.7$, $p > 0.1$) or between *E. coli* and coliphages (Pearson $r < 0.5$, $p > 0.05$). Whilst in untreated wastewaters, *E. Coli* and *Enterocci* were weakly correlated ($r = 0.6$, $p < 000.1$) but no correlation was observed between fecal bacteria and coliphages.

SARS-CoV-2 was detected in untreated sewage (inflow) on several dates, namely Faro-Aeroporto WWTP 21/10/2020, 14/12/2020, 08/03/2021; Vilamoura WWTP 21/10/2020, 14/12/2020; and Olhão WWTP 14/12/2020. However, treated wastewater (outflow) remained negative. Highest viral titers were observed at Faro-Aeroporto WTP untreated wastewater on 8 March 2021 (1.35 x 10^5 genomic units L^{-1}) coinciding with peak SARS-CoV-2 cumulative positive cases in the Algarve (DGS, 2021).

Discussion

In this study, raw and treated wastewater and marine waters were tested for the presence of fecal indicator bacteria (*E. coli* and *Enterococci*), somatic coliphages, and SARS-CoV-2. Although fecal contamination was detected in all sampling points, as determined by the three indicators tested in this study,

SARS-CoV-2 was undetected in treated wastewater and marine waters, showing that wastewater treatments could remove SARS-CoV-2 before the release into environmental waters, therefore diminishing the potential risk for human health.

Fecal coliforms and other allochthonous land-derived bacteria do not survive long in seawater due to abiotic and biotic challenges such as UV radiation, osmotic shock, oligotrophic regime, grazing by marine protists and competition with autochthonous marine bacteria (see review in Rozen and Belkin, 2001). On the other hand, survival of viruses in seawater is less studied but point to environmental factors such as wet vs dry season, as well as organic matter concentration regulating decay rates and loss of infectivity of human adenoviruses, enteroviruses and coliphages (Noble and Fuhrman, 2001; Jiang et al., 2007). Moreover, the relationship between somatic coliphages and human viruses remains poorly understood, although correlation between fecal indicator bacteria and coliphages seems to be established (Verani et al., 2018).

Not enough data was available in this study to ascertain significant correlations between variables. However, absence of correlations between fecal indicators could also be the result of differential survivability of the tested microorganisms in marine waters. RF is characterized to be oligotrophic with respect to inorganic nutrients and hypersaline relative to adjacent ocean ($\geq$ 37 psu) but has relatively low penetration of solar radiation (see Domingues et al., 2017). Furthermore, marine bacteria abundance is generally high (Galvão Helena et al., 2019). Altogether, these biotic and abiotic factors are expected to contribute to relatively rapid decay of fecal bacteria and perhaps some types of human viruses (eg. poliomyelitis virus).

It can be postulated that survival (or persistence of infectious viral particles) of SARS-CoV-2 in marine waters, as detected by RT-qPCR, is improbable due to its structure and presence of an outer membrane. A few studies have already demonstrated that SARS-CoV-2 is unlikely to be infectious at the WWTP level (Westhaus et al., 2021; Monteiro et al., 2022b). In fact, human pathogenic viruses detected so far in seawater have been found to be non-enveloped viruses and are thus less susceptible to osmotic shock in full strength seawater.

Conclusions

This preliminary study indicated that all three WWTP effectively removed SARS-CoV-2 despite moderately high concentrations in untreated urban waters. It also confirmed that surveillance of SARS-CoV-2 in wastewater in the Algarve region remains an efficient WBE tool to detect Covid-19 illness at a population level in agreement with results from other national wastewater monitoring (Monteiro et al., 2022a). Further monitoring of wastewaters (but not environmental waters) for SARS-CoV-2 is being conducted by Águas do Algarve, S.A., a state-owned company managing regional WTPs and WWTPs.

Future perspectives

How the persistence of human viral pathogens will be affected by climate change in adjacent coastal ocean (IPCC, 2014) and in the Ria Formosa (Rodrigues et al., 2021) is difficult to evaluate. In the latter study, predicted changes in abiotic variables are antagonistic and reveal a complex scenario. However, tangled inter-actions of physico-chemical variables with microbial populations is beyond the scope of any current numerical model considering that most antagonistic effects and feedback loops are still unknown or poorly understood.

Following the European Blue Flag campaign and EU Bathing Water Directives (ec.europa.eu/environment/water/water-bathing), all recreational waters in Portugal are routinely monitored for bacterial contamination and other water quality variables by the Agência Portuguesa do Ambiente (apambiente.pt/agua/aguas-balneares), but not for viral pathogens. Besides representing a risk to humans, microbial pathogens may also be transmitted to marine mammals. For instance, the importance of Algarve coastal waters as an ecosystem for common dolphins are increasingly being recognized with respect to foraging, feeding and nursery behavior (Castro et al., 2022).

Hence, in view of future pandemics, it is urgently needed that health authorities monitor the contamination potential of viral pathogens in the marine environment, particularly in highly developed tourist regions such as the Algarve. Future environmental research should include besides routine monitoring, *in situ* survival studies of both bacterial and viral pathogens in different coastal, transition and inner waters, to help with quantitative risk assessment and improvement of molecular biology techniques to infer viral infectivity.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

HG wrote paper. PM analyzed data and prepared figures. AF collected samples and performed fecal contamination analyses. SM and RS performed SARS analyses and supervised AF. All authors contributed to the article and approved the submitted version.

Funding

This study was partially funded by Centro de Investigação Marinha e Ambiental (Ref. CIMA, UIDB/00350/2020CIMABASE), Universidade do Algarve, Faro, Portugal.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

Castro, J., Faustino, C., Cid, A., Quirin, A., Matos, F., Rosa, R., et al. (2022). Common dolphin (Delphinus delphis) fission–fusion dynamics in the south coast of Portugal. *Behav. Ecol. Sociobiol.* 76:128. doi: 10.1007/s00265-022-03235-0

Choi, P. M., Tscharke, B. J., Donner, E., O'Brien, J. W., Grant, S. C., Kaserzon, S. L., et al. (2018). Wastewater-based epidemiology biomarkers: past, present and future. *Trends Anal. Chem.* 105, 453–469. doi: 10.1016/j.trac.2018.06.004

DGS. (2020). *Novo Coronavirus - Covid-19: relatório da situação. Direção Geral de Saúde*. Lisbon, Portugal. Available online at: https://covid19.minsaude.pt/relatorio-de-situacao/ (accessed August 24, 2022).

DGS. (2021). *Novo Coronavirus - Covid-19: relatório da situação. Direção Geral de Saúde*. Lisbon, Portugal. Available online at: https://d.minsaude.pt/relatorio-de-situacao/

Domingues, R. B., Guerra, C. C., Barbosa, A. B., and Galvão, H. M. (2017). Will nutrient and light limitation prevent eutrophication in an anthropogenically-impacted coastal lagoon? *Cont. Shelf Res.* 141, 11–25. doi: 10.1016/j.csr.2017.05.003

Galvão Helena, M., Mendes Pedro A., Caetano Sandra, M., Icely John, D., and Newton, Alice (2019). Role of microbes in the Ria Formosa lagoon. In: *RIA FORMOSA - Challenges of a Coastal Lagoon in a Changing Environment*. Faro: Universidade do Algarve. p. 67–81.

IPCC. (2014): Climate change 2014: synthesis report. In: Core Writing Team, Pachauri R.K.,and Meyer, L.A., editors. *Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Geneva: IPCC. p. 151.

Jiang, S. C., Chu, W., and He, J.-W. (2007). Seasonal detection of human viruses and coliphage in Newport Bay, California. *Appl. Environ. Microbiol.* 73, 6468–6474. doi: 10.1128/AEM.01370-07

Koopmans, J. S., Henry, C. J., Park, J. H., Eisenberg, M. C., Ionides, E. L., and Eisenberg, J. N. (2017). Dynamics affecting the risk of silent circulation when oral polio vaccination is stopped. *Epidemics* 20, 21–36. doi: 10.1016/j.epidem.2017.02.013

Monteiro, S., Rente, D., Cunha, M., Gomes, M., Marques, T., Lourenço, A., et al. (2022b). A wastewater-based epidemiology tool for COVID-19 surveillance in Portugal. *Sci. Total Environ.* 804, 150264. doi: 10.1016/j.scitotenv.2021.150264

Monteiro, S., Rente, D., Cunha, M. V., Marques, T., Cardoso, E., Vilaça, J., et al. (2022a). Discrimination and surveillance of infectious severe acute respiratory syndrome Coronavirus 2 in wastewater using cell culture and RT-qPCR. *Sci. Total Environ.* 815, 152914. doi: 10.1016/j.scitotenv.2022.152914

Noble, R. T., and Fuhrman, J. A. (2001). Enteroviruses detected by reverse transcriptase polymerase chain reaction from the coastal waters of Santa Monica Bay, California: low correlation to bacterial indicator levels. In: Porter, J.W., editor. *The Ecology and Etiology of Newly Emerging Marine Diseases. Developments in Hydrobiology*. Dordrecht: Springer. p. 159.

Philo, S., Keim, E., Swanstrom, R., Ong, A., Burnor, E., Kossik, A., et al. (2020). A comparison of SARS-CoV-2 wastewater concentration methods for environmental surveillance. *Sci. Total Environ.* 760, 144215. doi: 10.1016/j.scitotenv.2020.144215

Rodrigues, M., Rosa, A., Cravo, A., Jacob, J., and Fortunato, A. (2021). Effects of climate change and anthropogenic pressures in the water quality of a coastal lagoon (Ria Formosa, Portugal). *Sci. Total Environ.* 780, 146311. doi: 10.1016/j.scitotenv.2021.146311

Rozen, Y., and Belkin, S. (2001). Survival of enteric bacteria in seawater. *FEMS Microbiol. Rev.* 25 513–529. doi: 10.1111/j.1574-6976.2001.tb00589.x

Sul Informação. (2020). *Olhão: Descargas de esgotos na ria continuam sem solução, mas não é por falta de aviso. Sulinformação.pt*. Available online at: https://www.sulinformacao.pt/2020/07/trashed-4/ (accessed September 4, 2022).

Verani, M., Federigi, I., Donzelli, G., Cioni, L., and Carducci, A. (2018). Human adenoviruses as waterborne index pathogens and their use for quantitative microbial risk assessment. *Sci. Total Environ.* 651, 1469–1475. doi: 10.1016/j.scitotenv.2018.09.295

Westhaus, S., Weber, F.-A., Sciwy, S., Linnemann, V., Brinkmann, M., Widera, M., et al. (2021). Detection of SARS-CoV-2 in raw and treated wastewater in Germany - suitability for COVID-19 surveillance and potential transmission risks. *Sci. Total. Environ.* 751, 141750. doi: 10.1016/j.scitotenv.2020.141750