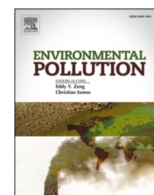
Environmental Pollution 308 (2022) 119594

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol



Binding, recovery, and infectiousness of enveloped and non-enveloped viruses associated with plastic pollution in surface water☆

Vanessa Moresco[a,1], Anna Charatzidou[a], David M. Oliver[a], Manfred Weidmann[b], Sabine Matallana-Surget[a], Richard S. Quilliam[a,*]

[a] Biological and Environmental Sciences, Faculty of Natural Sciences, University of Stirling, Stirling, FK9 4LA, UK
[b] Institute of Microbiology and Virology, Brandenburg Medical School Theodor Fontane, Senftenberg, D-01968, Germany

ARTICLE INFO

Keywords:
Biofilm
Enteric virus
Environmental virology
Microplastics
Plastisphere
Wastewater

ABSTRACT

Microplastics in wastewater and surface water rapidly become colonised by microbial biofilm. Such 'plastisphere' communities are hypothesised to persist longer and be disseminated further in the environment and may act as a vector for human pathogens, particularly as microplastics entering wastewater treatment plants are exposed to high concentrations of pathogenic bacteria. However, the potential for human viral pathogens to become associated with the plastisphere has never before been quantified. Here, we have used rotavirus (RV) SA11 (a non-enveloped enteric virus) and the enveloped bacteriophage Phi6 as model viruses to quantify binding and recovery from biofilm-colonised microplastic pellets in three different water treatments (filtered and non-filtered surface water, and surface water with added nutrients). Viruses associated with biofilm-colonised pellets were more stable compared to those remaining in the water. While infectious particles and genome copies of RV remained stable over the 48 h sampling period, Phi6 stability was highly impacted, with a reduction ranging from 2.18 to 3.94 $\log_{10}$. Virus particles were protected against inactivation factors when associated with the biofilm on microplastic surfaces, and when there was a high concentration of particulate matter in the liquid phase. Although our results suggest that the presence of an envelope may limit virus interaction with the plastisphere, the ability to recover both enveloped and non-enveloped infectious viruses from colonised microplastic pellets highlights an additional potential public health risk of surface waters becoming contaminated with microplastics, and subsequent human exposure to microplastics in the environment.

1. Introduction

The abundance, fate, and impacts of plastic pollution in the environment have been extensively researched in the last two decades (Andrady, 2011; Avio et al., 2017; Cole et al., 2011; Erni-Cassola et al., 2019). Pollution from microplastics (i.e., plastic particles $<$ 5 mm in size) has received particular attention due to their high concentration and potential for widespread dissemination within terrestrial, freshwater, marine, and atmospheric environments (Gao et al., 2020; Leslie et al., 2017; Mohajerani and Karabatak, 2020; Wright et al., 2020). Once in the environment, plastics can become quickly colonised by microorganisms, and studies have started to describe and identify the microbial communities that develop within this so-called 'plastisphere' (Jiang et al., 2018; Wu et al., 2020, 2019; Zettler et al., 2013). Importantly, there are significant differences in microbial diversity within the biofilm on plastic surfaces, compared to the surrounding environment or on naturally occurring substrates (Harrison et al., 2014; Metcalf et al., 2022; Oberbeckmann et al., 2018; Wu et al., 2020). Consequently, the potential risk of human pathogenic bacteria colonising plastics in the environment (particularly in marine contexts) has been well covered in the literature (Bowley et al., 2020; Keswani et al., 2016; Rodrigues et al., 2019).

Locations where high concentrations of microplastics are exposed to high loadings of human pathogens (e.g., the influent and settling tanks of wastewater treatment plants (WWTPs)) provide an ideal environment for biofilm formation on the microplastic surface. The development of specific microbial communities within the biofilm can subsequently evolve throughout the influent-sludge-effluent continuum (Kelly et al.,

☆ This paper has been recommended for acceptance by Christian Sonne.
* Corresponding author.
E-mail address: richard.quilliam@stir.ac.uk (R.S. Quilliam).
[1] Current Address: Division of Biomedical Sciences, School of Medicine, University of California Riverside, 92521, CA, USA.

https://doi.org/10.1016/j.envpol.2022.119594
Received 11 February 2022; Received in revised form 16 May 2022; Accepted 5 June 2022
Available online 6 June 2022
0269-7491/© 2022 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

2021). Despite most WWTPs being highly efficient at removing microplastics from sewage influent (e.g., by up to 99%), and concentrating them in the sludge, treated effluents continue to be one of the main sources of microplastics entering the aquatic environment, and contribute to a global annual input estimated at 10^{15} particles (Kelly et al., 2021; Lares et al., 2018; Pittura et al., 2021; Uddin et al., 2020). Consequently, human pathogens are likely to be released via WWTP effluent into receiving waters as part of the plastisphere community. The interaction of human viruses with this buoyant and persistent substrate could benefit their survival and infectiousness in the environment (Eckert et al., 2018).

Human enteric viruses, such as rotavirus (RV), hepatitis A (HAV) and norovirus (NoV) are shed in high concentrations in the faeces of infected individuals, and are commonly detected in raw sewage, treated effluents, sludge or surface waters that receive treated effluent (Farkas et al., 2018; Iaconelli et al., 2017; Prado et al., 2019; Schlindwein et al., 2010). Some respiratory viruses (which in contrast to the non-enveloped enteric viruses, are covered by a lipid envelope), such as influenza virus and some coronaviruses, can also be found in sewage, although due to their lipid envelope they become inactivated more rapidly (Heijnen and Medema, 2011; Ye et al., 2016). Non-specific electrostatic interactions and hydrophobic forces are considered the main mechanisms involving virus adhesion to abiotic surfaces (Dika et al., 2015). However, there is still a lack of studies evaluating virus interaction with biofilm components, such as extracellular polymeric substances (EPS). Therefore, there is significant scope for human viruses to interact with microplastics in wastewater and surface water, either directly with the surface of the plastic, or by interactions with EPS in the biofilm, which could allow viral capsid proteins, or the envelope, to become incorporated into the plastisphere (Moresco et al., 2021).

The concentration of human pathogenic bacteria colonising microplastics is reduced in treated effluent compared to influent sewage (Kelly et al., 2021); however, in contrast to bacteria, there are different mechanisms and kinetics for the inactivation of human viruses, especially the non-enveloped enteric viruses, which are less susceptible to inactivation processes such as UV irradiation, chlorine or ozone (Wigginton and Kohn, 2012). This increased persistence within WWTPs, could facilitate virus interaction with biofilm components on plastic surfaces (Prado et al., 2019; Simmons and Xagoraraki, 2011). The binding, transfer and persistence of human viruses on biofilm-colonised microplastics has so far never been addressed, although previous studies have recovered infectious and genome copies of enteric viruses and non-enveloped bacteriophages from the biofilm of plastic surfaces immersed in wastewater or drinking water (Helmi et al., 2008; Quignon et al., 1997; Skraber et al., 2009). Specific treatment processes at the WWTP, together with the hydraulic retention time (HRT) and the seasonality of virus outbreaks in the population (Barril et al., 2015) will influence the interaction of human viruses with microplastics. However, the presence of a biofilm on microplastics is likely to be the most significant factor for viruses binding to their surfaces (Moresco et al., 2021). In this study we employed two contrasting virus models: Rotavirus (RV), which is a non-enveloped virus (~80 nm diameter) belonging to the Reoviridae family; this virus is comprised of a triple-layered icosahedral capsid and 11 segments of a double-stranded RNA (dsRNA) genome (Desselberger, 2014). The second virus model was Phi6 (*Pseudomonas* phage Phi6), which is an enveloped bacteriophage (~85 nm diameter), belonging to the family Cystoviridae and contains a segmented dsRNA genome. Phi6 is routinely used as an experimental surrogate for a range of other enveloped viruses (Aquino De Carvalho et al., 2017).

The overarching aim of this study was to evaluate virus binding and recovery from biofilm-colonised microplastics and the role of this interaction as a factor contributing to virus stability and dissemination through the wider environment. As far as we are aware, this is the first study that has evaluated virus interactions with microplastics, by comparing enveloped and non-enveloped infectious virus stability.

2. Material and methods

2.1. Virus preparation

Simian rotavirus (SA11) was cultivated in MA104 (Monkey African Green kidney epithelial cells) obtained from the European Collection of Authenticated Cell Cultures using 1X Minimum Essential Media (MEM) supplemented with 10% Foetal Bovine Serum (FBS) (Gibco – Thermo Fisher Scientific, UK), 100 U/mL of penicillin G, 100 μg/mL of streptomycin, 0.025 μg/mL of amphotericin (PSA), and 1% Non-Essential Amino Acids (NEAA) (Gibco – Thermo Fisher Scientific), maintained at 35 ± 2 °C and 5% CO_2. RV SA11 stocks were propagated according to Moresco et al. (2015) and aliquots were maintained at −80 °C until used in the experiments.

Phi6 (Pseudomonas phage Phi6) and the host bacteria *Pseudomonas syringae* van Hall 1902 were both obtained from DSMZ (German Collection of Microorganisms and cell cultures GmbH). *P. syringae* was cultivated in TSB (Tryptic Soy Broth; Sigma-Aldrich) and incubated at 25 ± 2 °C. Phi6 stocks were obtained by infecting *P. syringae* in the exponential growth phase using the liquid lysate method, followed by incubation at 25 °C for a period up to 5h. The lysate was centrifuged at 4000g, for 10 min at 4 °C and filtered through a 0.22 μm pore size membrane filter (Sartorius Stedim Biotech., Gottingen, Germany) to remove bacteria cell debris. Stock aliquots were maintained at 4 °C for a period up to three weeks before being used in virus binding experiments.

2.2. Biofilm colonisation of microplastic

2.2.1. Freshwater samples and biofilm production

Surface freshwater from a eutrophic lake on the campus of the University of Stirling, Scotland (56°08′50.2″ N 3°55′25.2″ W) was used as a natural inoculant to produce biofilm on microplastics. Background conductivity, pH, and turbidity characteristics of the water were measured (Hanna Instruments, Bedfordshire, UK) (Table 1).

Polyethylene (PE) microplastics pellets (2 mm) (Goodfellow Cambridge Ltd, Huntingdon, UK) were used as the plastic surfaces for biofilm colonisation and subsequent virus binding experiments. Spherical stainless steel cages (tea infusers, Arktek, Amazon, UK) diameter 4.5 cm, containing 2 g of PE microplastic pellets (approx. 150 pellets), were first autoclaved and added to sterile 2.5 L glass flasks (six cages per flask) containing 1 L of either: 1) filtered lake water (FW) (filtered to pass through 0.45 μm); 2) lake water (LW) without filtration and containing the natural microbial community; or 3) lake water containing LB broth (LWLB) at a final concentration of 10 g/L; this nutrient source was used to increase microbial growth.

Three replicate flasks were set-up for each water type (i.e., nine mesocosmsin total) and incubated in a horizontal shaker (100 rpm) at room temperature (±22 °C) for up to 14 days. At day 7, three cages from each flask were removed to quantify the biofilm formation on the microplastic pellets and for subsequent virus binding experiments. Additionally, at day 7, 250 mL of the water in each flask was removed and replaced with fresh FW, LW or LWLB water.

2.2.2. Biofilm quantification

Biofilm colonising the microplastic pellets was evaluated using an

Table 1
Physicochemical parameters of lake water treatments used as matrix for biofilm development on microplastic pellets.

Water samples	pH	Turbidity (NTU)	Conductivity (μS/cm)
FW	7.9	1.17	320
LW	7.8	5.31	308
LWLB	7.8	8.72	469

FW: filtered lake water; LW: lake water; LWLB: lake water with LB broth.
NTU: Nephelometric Turbidity unit.

Table 2
Log_{10} and percentage (%) reduction of infectious RV and Phi6 recovered from mesocosm water.

Water treatment	Log_{10} reduction (%)	
	RV	Phi6
FW	1.19 (93.6)	>4
LW	1.05 (91.2)	>4
LWLB	0.08 (23)	>4

adapted crystal violet method (Lobelle and Cunliffe, 2011). After either seven or 14 days of incubation, samples of 10 and 20 microplastic pellets were collected from each replicate cage with sterile forceps, placed in a sterile 12 well-plate, and rinsed twice with sterile distilled water (dH_2O) to remove weakly attached bacteria. The plates were dried at room temperature in a biological safety cabinet for 30 min and stained with a solution of 1% crystal violet (Sigma-Aldrich) in water. Excess stain was removed, and the microplastic pellets rinsed again with sterile dH_2O and left to dry before adding 200 μL of 90% ethanol to produce the decolouring solution. Immediately after, the decolouring solution was added to a sterile 96 well-plate with an appropriate blank (i.e., 90% ethanol) and absorbance at 595 nm measured in a spectrophotometer (TECAN Infinite M200, Switzerland). The absorbance of the solubilised crystal violet stain (A595) was considered proportional to the amount of biofilm colonising the microplastic pellets.

2.3. Virus binding and recovery from biofilm colonised microplastics

To evaluate virus adhesion and recovery from the biofilm, colonised microplastics were collected after seven days of incubation, rinsed with sterile dH_2O and added to a new sterile flask containing 100 mL of fresh lake water and 1 mL of each virus stock (RV and Phi6) at a concentration from 10^7 to 10^9 PFU/mL (which is within the concentration range of enteric viruses commonly found in wastewater samples). One mL of water from each flask was collected at time zero (T0) to quantify the exact number of virus particles added. At 3, 24 and 48 h, samples of both 10 and 20 microplastic pellets from each replicate cage in each flask were removed, together with 1 mL of the water. The microplastic pellets were collected using sterile forceps and added to a 12 well-plate, rinsed with sterile dH_2O to remove weakly attached viruses and left dry at room temperature for 30 min. The pellets were then transferred to 1.5 mL polypropylene tubes with 1 mL of TGBE elution buffer (Tris-Glycine Beef Extract) at pH 9.5. Tubes were vortexed for 1 min to allow detachment of viruses, and the eluate used to quantify virus concentration by plaque assay (for RV and Phi6) and by RT-qPCR (for RV).

2.3.1. RV and Phi6 plaque assay

The plaque assay methodology was used to detect infectious viruses recovered from both the biofilm and the water for each treatment. RV plaque assays were performed as described by Moresco et al. (2015): briefly, a confluent MA104 cell monolayer, seeded in six-well plates (Greiner, Austria) at the final concentration of 2 x 10^5 cells/mL was inoculated with 400 μL of ten-fold dilutions of the virus stock (positive control) or the samples (either water or biofilm eluate), preactivated with 10 μg/mL of trypsin (Sigma-Aldrich) for 30 min at 37 °C and incubated for 1h at 35 ± 2 °C to allow virus adsorption. Samples were then removed, and the cell monolayer overlaid with 1.5% of Bacto Agar (Dibco-BD) diluted in 1X DMEM high glucose (4.5 mg/L) (Gibco) without FBS and supplemented as described above but with the addition of 2 mol/L of L-glutamine, 2 mol/L of sodium pyruvate (Sigma), 100 μg/mL of DEAE-Dextran (Sigma), and 10 μg/mL of trypsin. Cells were incubated at 37 °C under 5% CO_2 for 4–5 days and subsequently stained with 0.4% crystal violet. Macroscopic plaques were counted to establish viral titres and expressed as plaque-forming units per mL (PFU/mL).

Phi6 plaque assays were performed by the double agar layer methodology. Firstly, the bacterial host, *P. syringae* was cultivated in TSB and incubated at 25 ± 2 °C overnight (16h). Petri dishes containing TSB with 1.5% w/v of Bacto Agar (BD) as the bottom layer were overlaid with a mixture of semi-solid TSB (0.6% w/v Bacto Agar), 1 mL of sample (i.e., water or biofilm eluate) diluted in phage buffer (40 mM Na_2HPO_4, 20 mM KH_2PO_4, 80 mM NaCl, 0.1M $CaCl_2$, 0.1M $MgSO_4$) pH 7.5, and 3 mL of *P. syringae* suspension. After solidification, plates were incubated for 25 ± 2 °C for a period up to 18h. The visualisation of clear spots (plaques) on the developed lawn of *P. syringae* on the top agar layer indicated the presence of infectious Phi6 which were counted and calculated as PFU/mL.

2.3.2. RNA extraction and RT-qPCR for RV detection

RV RNA was extracted from the water and biofilm eluate samples with a QIAmp Viral RNA Mini Kit (Qiagen), following the manufacturer's instructions. RV genome copies were quantified in a LightCycler® 480 instrument (Roche) employing a one-step Quantitect Probe RT-PCR kit (Qiagen), with 0.5 μm of NSP3F (5'–ACCTCTWCACRTRACCCTCTATGAG-3')and NSP3R (5′-GGTCACATAACGCCCCTATAGC - 3′) primers that amplify a region of the RV non-structural protein NSP3, and 0.2 μm of the NSP3 (5′-6FAM-AGTTAAAAGCTAACACTGTCAAA–BBQ-3′) hydrolysis probe (TIB MOLBIOL, Berlin, Germany) (Zeng et al., 2008). RT-qPCR reactions were performed in duplicate with cycling conditions consisting of an initial step at 50 °C for 30 min (reverse transcription step) followed by 45 cycles of 94 °C 1 min, 60 °C 1 min, and 72 °C 1 min. In each run, positive controls consisted of an RV standard curve (constructed using 10-fold dilutions of the linearised synthetic plasmid pMA-RQ (AmpR) (Invitrogen, Thermo Fisher) containing an 85 bp sequence of the NSP3 gene) obtained via the same RT-qPCR reaction and cycling conditions as described above. Non-template controls (NTC) were used as negative controls during each run. The values of genome copies of RV were expressed as genome copies per mL (gc/mL).

2.4. Data analyses

The reduction of viral load was established using the values of viral titres determined at 48 h (Nt) compared to the initial concentration of virus immediately after inoculation T0 (for the water samples) or 3 h (for colonised microplastics pellets) (N0), using equation [$\log_{10}(Nt/N0)$]. An analysis of covariance (ANCOVA) was used to determine whether water composition (FW, LW and LWLB), time, and plastic concentration (either 10 or 20 pellets) had a significant effect on virus detection from biofilm samples, by either PFU or RT-qPCR. An analysis of variance (one-way ANOVA) was performed to compare the values of virus recovery in the water of the different treatments, and time points, followed by a Tukey post-hoc test when necessary. Statistical analyses were performed using R software version 1.4 1103. Differences at the $p < 0.05$ level (95% confidence interval) were considered statistically significant.

3. Results

3.1. Biofilm quantification on microplastics in freshwater samples

Biofilm quantification on the surfaces of microplastic pellets in samples containing 20 pellets were similar in all treatments after seven days of incubation and did not significantly change ($p > 0.05$) following a further seven days incubation (Fig. 1). However, in samples that contained only 10 pellets, samples from filtered lake water (FW10) and lake water (LW10) had significantly lower values of absorbance ($p < 0.001$) than all other samples. The same was not observed for pellets incubated in unfiltered lake water supplemented with LB broth, which had similar absorbance values regardless of whether the sample contained 10 or 20 plastic pellets.

3.2. Binding and recovery of RV and Phi6 from colonised microplastics

The recovery of RV from colonised microplastic pellets after an

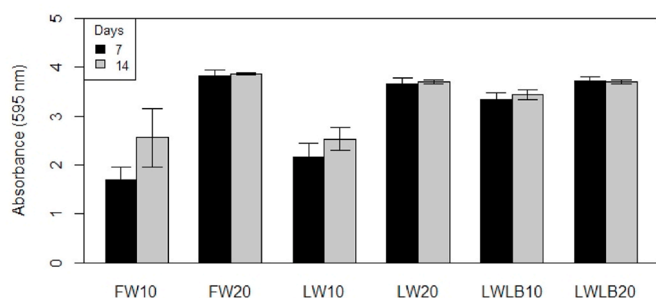


Fig. 1. Absorbance values (A595) as a proxy for microbial biofilm formation on microplastic pellets after 7 or 14 days incubation (mean of three replicates ± standard deviation). FW10 and FW20 (filtered lake water, 10 or 20 microplastics); LW10 and LW20 (lake water, 10 or 20 microplastics); LWLB10 and LWLB20 (lake water and LB broth, 10 or 20 microplastics).

incubation time of 3, 24 and 48 h was evaluated by plaque assay (i.e., presence of infectious virus particles) and RT-qPCR (genome copies) (Fig. 2a and b, respectively). Infectious RV particle recovery at the initial time point (3 h), ranged from 1.23 x 10^3 to 1.16 × 10^4 PFU/mL for samples containing 10 pellets and from 2.45 x 10^3 to 1.88 × 10^4 PFU/mL for samples containing 20 pellets (Fig. 2a). For the FW and LW samples these values remained stable (10^3 PFU/mL on average), regardless of the period of incubation (i.e., 24 or 48 h), or whether there were 10 or 20 pellets in the sample. In contrast, the values of infectious RV particles recovered from the LWLB10 and LWLB20 treatments increased by 0.91 and 0.23 $\log_{10}$ respectively at 24h of incubation, followed by subsequent decay at the end of the 48h of incubation (Table 3). Overall, the recovery of RV was more significantly affected by the number of plastic pellets in the sample (ANCOVA, $p = 0.003$) than the incubation time ($p = 0.02$), or the water treatment ($p = 0.04$). When RV recovery from the pellets was evaluated by RT-qPCR, the average RV genome detected at the end of 3 h was 10^6 gc/mL with a higher average gc:PFU ratio of 3 x 10^2 $\log_{10}$,

Table 3
Log_{10} and percentage (%) reduction of infectious RV and Phi6 recovered from biofilm-colonised microplastic pellets.

	Microplastics no.	Log_{10} reduction (%)	
		RV	Phi6
FW	10	0.60 (74)	3.75 (99.98)
	20	0.36 (56)	3.94 (99.98)
LW	10	0.30 (50)	2.18 (99.35)
	20	0.58 (74)	2.84 (99.85)
LWLB	10	0.03 (6)	3.94 (99.98)
	20	0.07 (16)	3.72 (99.98)

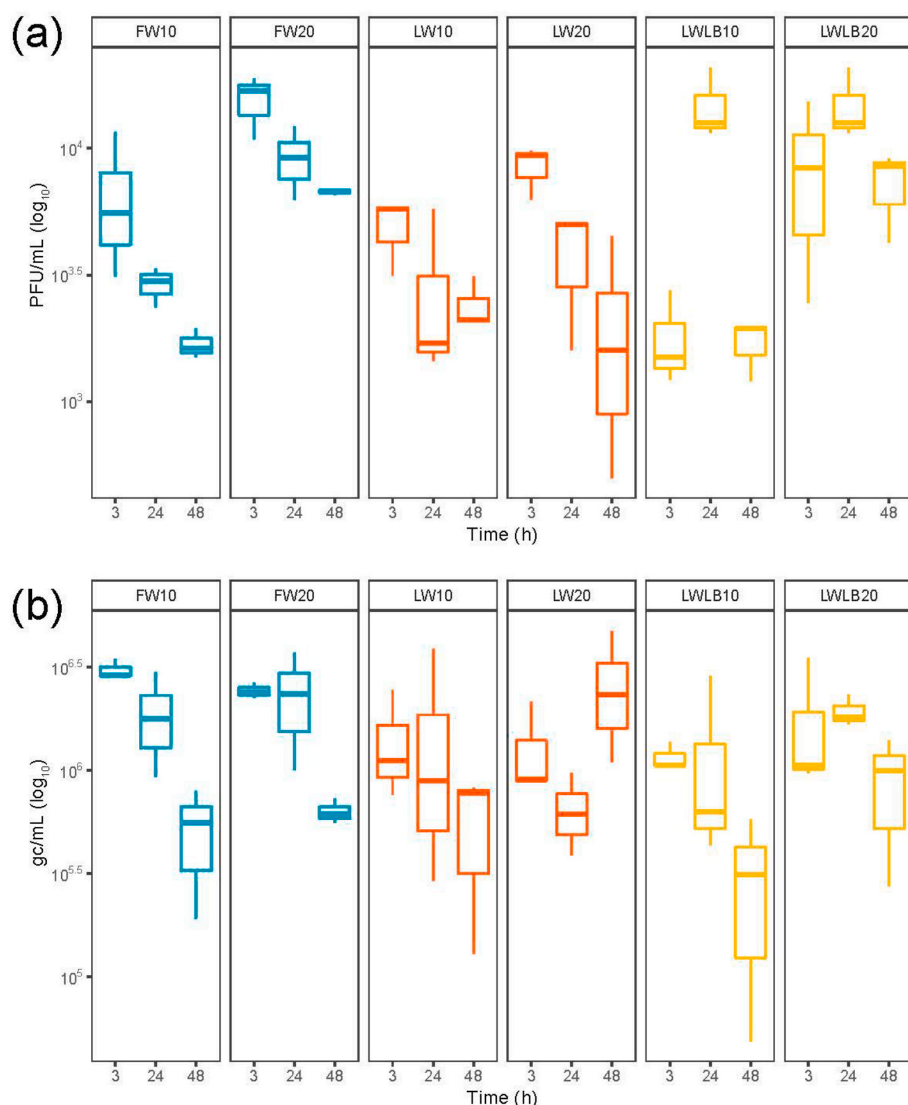


Fig. 2. RV recovery from biofilm-colonised microplastics (using either 10 or 20 microplastic pellets) at 3, 24 and 48 h of contact time. (a) infectious RV analysed by plaque assay (PFU/mL) and (b) RT-qPCR (gc/mL). Box and whisker plot of $\log_{10}$ RV detection. Horizontal lines represent the median, and the top and bottom of the box represents the 75th and 25th percentiles (n = 3). Top and bottom of the whisker represents the highest and lowest values.
FW10 and FW20 (filtered lake water, 10 or 20 microplastics); LW10 and LW20 (lake water, 10 or 20 microplastics); LWLB10 and LWLB20 (lake water and LB broth, 10 or 20 microplastics).

regardless of the concentration of microplastic pellets or water treatment ($p > 0.05$) (Fig. 2b). The number of RV genome copies remained fairly stable, nevertheless the small reduction by 48 h was significant ($p < 0.001$).

Recovery of infectious Phi6 particles from the microplastic pellets at the first time point (3 h) ranged from 10^4 PFU/mL for the samples FW10 and LW10 (which both had lower biofilm concentrations – Fig. 1), to 10^5 PFU/mL for the remaining treatments (Fig. 3). Although Phi6 inactivation varied from 0.3 to 1.24 $\log_{10}$ within the first 24 h period, virus recovery and stability was strongly reduced at 48 h (Table 3), with final detection values of 10^1 PFU/mL regardless of the water treatment or concentration of microplastic pellets. The duration of incubation was the main factor influencing virus inactivation (ANCOVA, $p < 0.001$), whereas water treatment and the number of plastic pellets in the sample were not significant factors for the recovery of Phi6.

3.3. RV and Phi6 recovery and stability in water

The initial RV concentration detected by plaque assay and RT-qPCR respectively, was on average 3×10^5 PFU/mL (Fig. 4a) and 4.5 x 10^6 gc/mL (Fig. 4b) with an average detection gc:PFU ratio of 2 x 10^1 $\log_{10}$. In general, at the end of 48 h of incubation, there was a higher $\log_{10}$ reduction of infectious RV virus particles compared to the copies of the RV virus genome (Table 2), except for the LWLB treatments. At 3 h of incubation, there was a $\log_{10}$ reduction of 0.44 and 0.23 in the water treatments FW and LW respectively, while no reduction was observed in the LWLB treatment. At 24 h, there was a 1.19 $\log_{10}$ reduction in the water of the FW treatment, which remained constant at 48 h, representing a 93% reduction of infectious RV particles compared to T0 (Table 2). There was a more gradual $\log_{10}$ decay of infectious RV over 24 h and 48 h (0.60 and 1.05 respectively) in the water of the LW treatment, equivalent to a 91% reduction relative to T0. The lowest reduction of infectious viral particles occurred in the water of the LWLB treatment, with a 23% (0.36 $\log_{10}$) reduction at 24 h, followed by an increase of 0.45 $\log_{10}$ infectious virus particles at 48 h. Water sample treatment did not impact on the number of genome copies detected ($p > 0.05$), but time did decrease the number of RV genome copies ($p < 0.001$), mainly due to the period between T0 and T3 h (Fig. 4b). At 48 h, $\log_{10}$ reduction values ranged from 0.52 to 0.64, which equated to 73, 75 and 82%, for the FW, LW and LWLB treatments respectively.

The initial Phi6 concentration at T0 in the different water treatments was on average 7.7×10^6 PFU/mL for all water samples (Fig. 5). In the water of the FW and LW treatments, there was on average a 1 $\log_{10}$ reduction after 3 h of incubation, followed by another 1 $\log_{10}$ at 24 h, or 98 and 99% virus reduction respectively, with no detection of viable Phi6 by 48 h (Table 2). In contrast, Phi6 in the water of the LWLB treatment underwent an initial inactivation of 2.7 $\log_{10}$ at 3 h, which was maintained at 24 h followed by more than a 4 $\log_{10}$ (99.99%) reduction at 48 h. No differences ($p > 0.05$) in Phi6 reduction values were evident in the LWLB water quantified at 0, 3 and 24 h; however, by 48 h the inactivation of Phi6 was significantly increased ($p < 0.001$).

4. Discussion

4.1. Biofilm colonisation of microplastic pellets

Biofilm formed on microplastic pellets in all three water treatments (regardless of incubation time, or plastic concentration), although the presence of a nutrient source favoured the more rapid formation of biofilm on plastic surfaces (Simcox et al., 2019). Microbial substrate competition, surface saturation, the potential synthesis of antimicrobial compounds (e. g., antibiotics), and the expression of *quorum sensing* molecules are all factors associated with the establishment of biofilms (López et al., 2010; Skraber et al., 2009). However, the occurrence of secondary biofilm on the pellets during the development of successional bacterial colonisation during the extra seven days of incubation, probably masked any differences in the dynamics of biofilm colonisation between the three water treatments (Harrison et al., 2014). This was likely due to the nature of the closed-system mesocosms, and the controlled incubation conditions employed in this study.

4.2. Infectious virus recovery and stability

During the duration of these experiments (3–48 h), it was possible to recover both enveloped and non-enveloped model viruses from microplastic pellets. Although virus stability declined with time, virus inactivation was lower when they were recovered from biofilm-colonised pellets compared to viruses recovered from the water phase. However, inactivation of the enveloped Phi6 viruses was always relatively higher than the non-enveloped RV, highlighting the importance of the envelope as a driver for persistence in the environment (Casanova and Weaver, 2015).

Surface attachment/detachment dynamics of viruses from the biofilm in the treatment with the added nutrient source, in addition to natural virus inactivation under the experimental conditions, are likely responsible for fluctuations in the concentrations of infectious RV

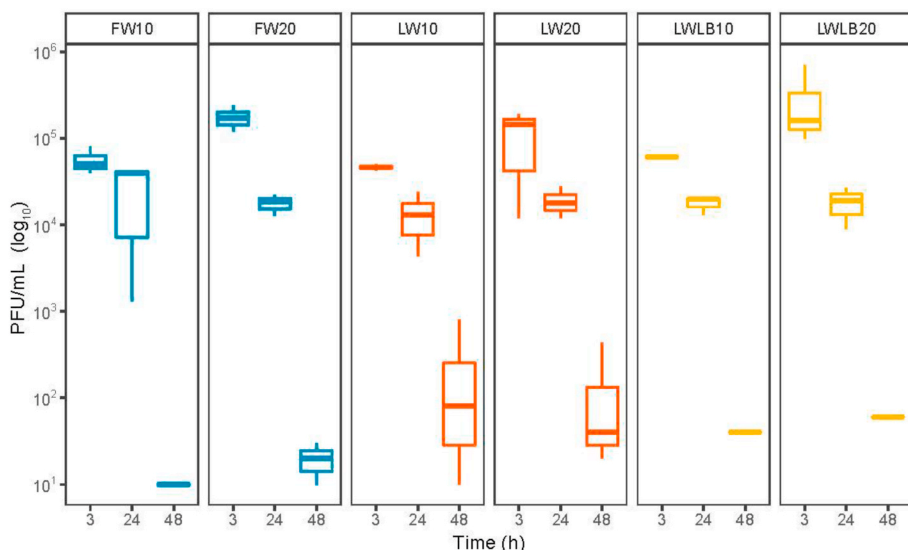


Fig. 3. Phi6 recovery from biofilm-colonised microplastics (using either 10 or 20 microplastic pellets) at 3, 24 and 48 h of contact time evaluated by plaque assay (PFU/mL). Box and whisker plot of $\log_{10}$ Phi6 detection. Horizontal lines represent the median, and the top and bottom of the box represents the 75th and 25th percentiles ($n = 3$). Top and bottom of the whisker represents the highest and lowest values. FW10 and FW20 (filtered lake water, 10 or 20 microplastics); LW10 and LW20 (lake water, 10 or 20 microplastics); LWLB10 and LWLB20 (lake water and LB broth, 10 or 20 microplastics).

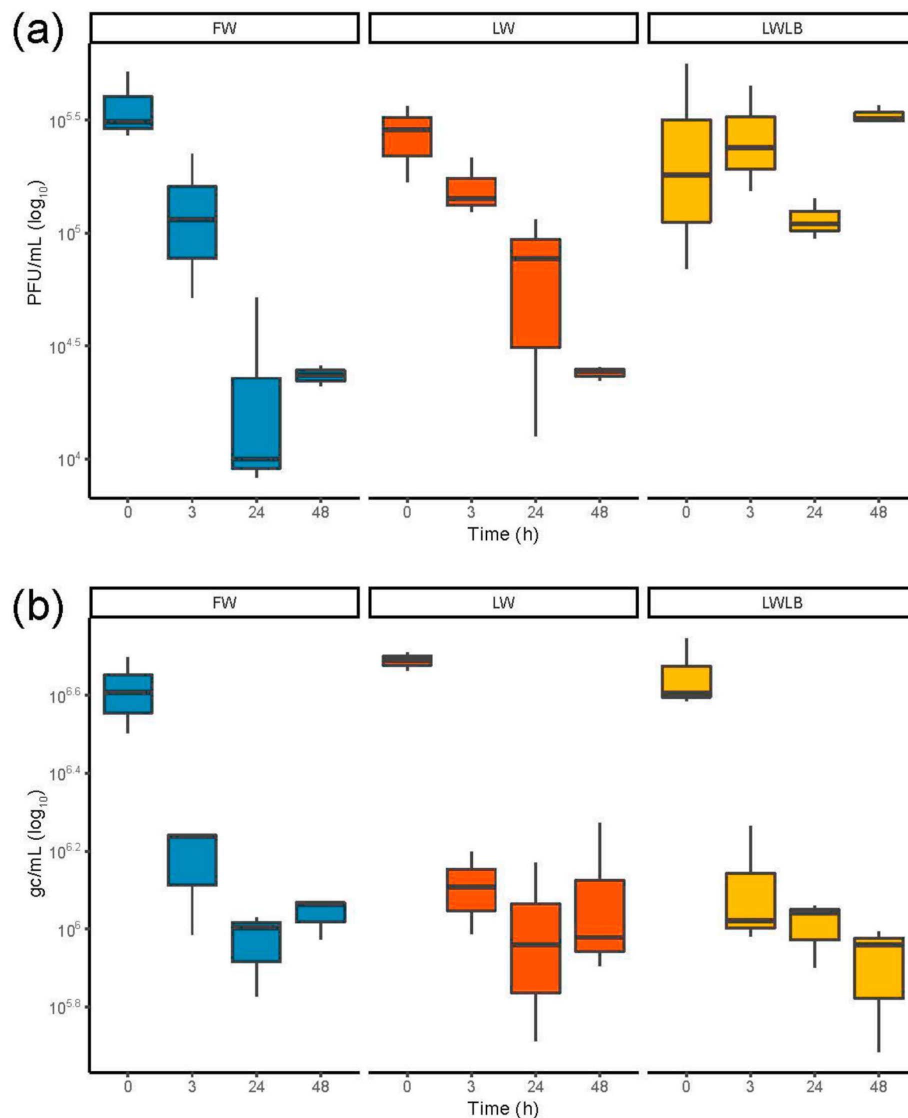


Fig. 4. RV recovery from mesocosm water at time zero (after spiking), and 3, 24 and 48 h. (a) infectious RV analysed by plaque assay (PFU/mL) and, (b) RT-qPCR (gc/mL). Box and whisker plot of log_{10} RV detection. Horizontal lines represent the median, and the top and bottom of the box represents the 75th and 25th percentiles ($n = 3$). Top and bottom of the whisker represents the highest and lowest values. FW, filtered lake water; LW, lake water; LWLB, lake water and LB broth.

particles recovered from either the water or the biofilm-colonised microplastic (Helmi et al., 2008). In addition to the nutrient input, the higher ionic strength provided by the LB broth would have affected the biofilm depth, structure and microbial composition, in turn facilitating increased virus interaction with the biofilm surrounding the microplastic (Langlet et al., 2008). This increased level of protection afforded by the biofilm was likely responsible for the reduced rate of inactivation of the RV particles associated with the surfaces of microplastic pellets.

The formation of viral aggregates, between viral particles or between viruses and other organic/inorganic particles varies according to virus characteristics, e.g., isoelectric point (IEP) or pH of the media, but can provide a level of protection against inactivation factors (Gerba and Betancourt, 2017). Particulate matter in the lake water, and the increased turbidity resulting from the addition of LB broth, may have protected RV particles and limited their inactivation as they became associated with the colonised microplastic pellets. However, in the treatments with no extra nutrient source, and thus lower biofilm colonisation of the microplastic pellets, there was a gradual decay in infectious RV particles that was likely due to differences in biofilm structure, coverage and thickness and the subsequent higher rate of detachment of RV particles.

Detection of the RV genome in both the water and the biofilm on the microplastic pellets remained stable throughout the analysed period, which is consistent with other studies evaluating enteric virus genome presence and stability either in environmental (water and sediments) or biofilm samples (Espinosa et al., 2008; Gassilloud et al., 2003; Gassilloud and Gantzer, 2005; Helmi et al., 2008). Importantly, the detection of viral genome copies by molecular methods such as quantitative PCR, does not indicate the presence of infectious virus particles (and any subsequent human health risk) (Hamza et al., 2011). On average, the number of genome copies detected was 1–3 log_{10} higher than the PFU values, which is in accordance with other studies comparing RV detection in environmental samples (Fongaro et al., 2012; Moresco et al., 2016). However, the composition and concentration of bacterial biofilm components and the elution buffer composition (TGBE) are known to be able to inhibit PCR amplification (Skraber et al., 2009), and therefore the genome copies detected may be an underrepresentation of the true number.

A gradual transfer of Phi6 particles from the aqueous phase to the biofilm-colonised microplastic pellets, occurred between 3 and 24 h of incubation. Despite higher inactivation values compared to RV, the concentration of recovered Phi6 from the colonised pellets suggests that

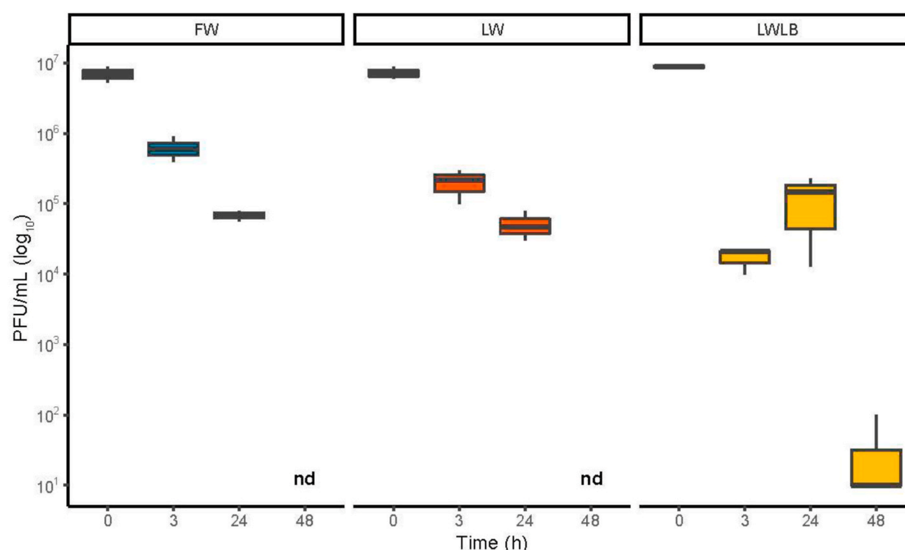


Fig. 5. Phi6 recovery from mesocosm water at time zero (after spiking), and 3, 24 and 48 h analysed by plaque assay (PFU/mL). Box and whisker plot of log_{10} Phi6 detection. Horizontal lines represent the median, and the top and bottom of the box represent the 75th and 25th percentiles ($n = 3$). Top and bottom of the whisker represents the highest and lowest values. FW, filtered lake water; LW, lake water; LWLB, lake water and LB broth. nd: non-detected.

associating with the biofilm can increase virus stability and persistence compared to its relative survival in the water phase, where with exception of the treatments with added nutrient broth, no Phi6 viruses were detected at the end of 48 h. The persistence of Phi6 phage, which is commonly used as a model for studying the stability of enveloped viruses, is influenced by the physio-chemical composition and temperature of the water, and the activity of autochthonous microbial communities (Aquino De Carvalho et al., 2017). Thus, the natural composition of the lake water used in each treatment may have played a role in virus particle aggregation and the apparent Phi6 inactivation in the water phase, with increased inactivation most evident at 48 h. The detection limit of the Phi6 plaque assay of 6×10^1 PFU/mL (data not shown) may have had an impact on the non-detection values for the FW and LW treatments at 48 h of incubation; however, viable Phi6 particles were recovered from the colonised microplastic pellets suggesting that most of the virus particles were associated with the biofilm and not in the water phase.

Characteristics of different viruses can influence their interaction with biofilm; however, the similarity between the two virus models used in this study, e.g., size (~80 nm diameter), genome composition (segmented double stranded RNA) and an isoelectric point of 6.44 and 6.94 for RV SA11 and Phi6 respectively (Kozlowski, 2017) suggests that the presence or absence of an envelope plays an important role for viruses binding to biofilm-colonised plastics. The interaction of non-enveloped viruses, with bacterial cell wall components, such as peptidoglycans and lipopolysaccharides, can increase virion infectivity and thermostability, e.g., as seen with several genogroups of norovirus and coxsackievirus (Almand et al., 2017; Robinson et al., 2014; Waldman et al., 2017). Conversely, the infectivity of some important enveloped human viral pathogens (e.g., HCoV (229E) and MERS-CoV), can be substantially reduced when interacting with specific peptidoglycans from the cell wall of *Bacillus subtilis* (Johnson et al., 2019). Although the exact mechanisms of how viruses interact with the biofilm of colonised plastics in natural environmental conditions are not yet known, it is likely that enveloped and non-enveloped viruses will behave differently in terms of their persistence and subsequent dissemination.

Among the different plastic particles entering a WWTP, microplastic pellets show lower retention in the sludge (Bayo et al., 2020). The combination of high microplastic abundance in wastewater, the intrinsic characteristics of plastics, such as buoyancy and hydrophobicity, together with a high loading of human viral pathogens into wastewater treatment processing provides significant scope for viruses to become associated with the surfaces of microplastics, and increase their potential environmental dissemination once discharged to receiving waters. The recovery of infectious viruses from colonised microplastic pellets, therefore, highlights the potential public health risk of surface waters becoming contaminated with microplastics, and subsequent human exposure to microplastics in the environment.

5. Conclusion

The presence and survival of human viruses associated with biofilm colonising the surface of microplastics could play an important role in virus transfer. Although virus stability in the plastisphere is influenced by different environmental factors and viral structural characteristics, the recovery of both virus models used in this study emphasizes the potential for plastic pollution to act as a novel pathway for viral dissemination and persistence in the environment.

Author statement

VM, **RSQ**: Conceptualization; **VM**, **AC**: Data curation; **VM**: Formal Analysis; **VM**: Writing-Original draft preparation; **RSQ**: Supervision; **VM**, **RSQ**, **DMO**, **MW**, **SM-S**: Writing-reviewing, and editing; **RSQ**: Funding Acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was funded by the UKRI Natural Environment Research Council (NERC) as part of the project, “Microbial hitch-hikers of marine plastics: the survival, persistence & ecology of microbial communities in the ‘Plastisphere’” [grant number NE/S005196/1], and the NERC-GCRF SPACES project [grant number NE/V005847/1] both awarded to RQ. SMS was supported by UKRI NERC as part as the NRF-NERC-SEAP-‘Understanding the impact of plastic pollution on marine ecosystems in Southeast Asia (South East Asia Plastics (SEAP))’. Sources, impacts and

solutions for plastics in Southeast Asia coastal environment (NRF Award No. NRF-SEAP-2020-0001, NERC Award No. NE/V009621/1). We thank Dr Gerhard Dobler (German Centre for Infection Research, München, Germany) for donating the Simian rotavirus (SA11).

References

Almand, E.A., Moore, M.D., Outlaw, J., Jaykus, L.A., 2017. Human norovirus binding to select bacteria representative of the human gut microbiota. PLoS One 12, 1–13. https://doi.org/10.1371/journal.pone.0173124.

Andrady, A.L., 2011. Microplastics in the marine environment. Mar. Pollut. Bull. 62, 1596–1605. https://doi.org/10.1016/j.marpolbul.2011.05.030.

Aquino De Carvalho, N., Stachler, E.N., Cimabue, N., Bibby, K., 2017. Evaluation of Phi6 persistence and suitability as an enveloped virus surrogate. Environ. Sci. Technol. 51, 8692–8700. https://doi.org/10.1021/acs.est.7b01296.

Avio, C.G., Gorbi, S., Regoli, F., 2017. Plastics and microplastics in the oceans: from emerging pollutants to emerged threat. Mar. Environ. Res. 128, 2–11. https://doi.org/10.1016/j.marenvres.2016.05.012.

Barril, P.a., Fumian, T.M., Prez, V.E., Gil, P.I., Martínez, L.C., Giordano, M.O., Masachessi, G., Isa, M.B., Ferreyra, L.J., Ré, V.E., Miagostovich, M., Pavan, J.V., Nates, S.V., 2015. Rotavirus seasonality in urban sewage from Argentina: effect of meteorological variables on the viral load and the genetic diversity. Environ. Res. 138, 409–415. https://doi.org/10.1016/j.envres.2015.03.004.

Bayo, J., Olmos, S., López-Castellanos, J., 2020. Microplastics in an urban wastewater treatment plant: the influence of physicochemical parameters and environmental factors. Chemosphere 238. https://doi.org/10.1016/j.chemosphere.2019.124593.

Bowley, J., Baker-Austin, C., Porter, A., Hartnell, R., Lewis, C., 2020. Oceanic hitchhikers – assessing pathogen risks from marine microplastic. Trends Microbiol. 29, 1–10. https://doi.org/10.1016/j.tim.2020.06.011.

Casanova, L.M., Weaver, S.R., 2015. Inactivation of an enveloped surrogate virus in human sewage. Environ. Sci. Technol. Lett. 2, 76–78. https://doi.org/10.1021/acs.estlett.5b00029.

Cole, M., Lindeque, P., Halsband, C., Galloway, T.S., 2011. Microplastics as contaminants in the marine environment: a review. Mar. Pollut. Bull. 62, 2588–2597. https://doi.org/10.1016/j.marpolbul.2011.09.025.

Desselberger, U., 2014. Rotaviruses. Virus Res. 75–96. https://doi.org/10.1016/j.virusres.2014.06.016, 190C.

Dika, C., Duval, J.F.L., Francius, G., Perrin, A., Gantzer, C., 2015. Isoelectric point is an inadequate descriptor of MS2, Phi X 174 and PRD1 phages adhesion on abiotic surfaces. J. Colloid Interface Sci. 446, 327–334. https://doi.org/10.1016/j.jcis.2014.08.055.

Eckert, E.M., Di Cesare, A., Kettner, M.T., Arias-Andres, M., Fontaneto, D., Grossart, H.P., Corno, G., 2018. Microplastics increase impact of treated wastewater on freshwater microbial community. Environ. Pollut. 234, 495–502. https://doi.org/10.1016/j.envpol.2017.11.070.

Erni-Cassola, G., Zadjelovic, V., Gibson, M.I., Christie-Oleza, J.A., 2019. Distribution of plastic polymer types in the marine environment; A meta-analysis. J. Hazard Mater. 369, 691–698. https://doi.org/10.1016/j.jhazmat.2019.02.067.

Espinosa, A.C., Mazari-Hiriart, M., Espinosa, R., Maruri-Avidal, L., Méndez, E., Arias, C.F., 2008. Infectivity and genome persistence of rotavirus and astrovirus in groundwater and surface water. Water Res. 42, 2618–2628. https://doi.org/10.1016/j.watres.2008.01.018.

Farkas, K., Cooper, D.M., McDonald, J.E., Malham, S.K., de Rougemont, A., Jones, D.L., 2018. Seasonal and spatial dynamics of enteric viruses in wastewater and in riverine and estuarine receiving waters. Sci. Total Environ. 634, 1174–1183. https://doi.org/10.1016/j.scitotenv.2018.04.038.

Fongaro, G., Nascimento, M. a, Viancelli, A., Tonetta, D., Petrucio, M.M., Barardi, C.R.M., 2012. Surveillance of human viral contamination and physicochemical profiles in a surface water lagoon. Water Sci. Technol. 66, 2682–2687. https://doi.org/10.2166/wst.2012.504.

Gao, D., Li, X., Liu, H., 2020. Source, occurrence, migration and potential environmental risk of microplastics in sewage sludge and during sludge amendment to soil. Sci. Total Environ. 742, 140355 https://doi.org/10.1016/j.scitotenv.2020.140355.

Gassilloud, B., Gantzer, C., 2005. Adhesion-aggregation and inactivation of Poliovirus 1 in groundwater stored in a hydrophobic container. Appl. Environ. Microbiol. 71, 912–920. https://doi.org/10.1128/AEM.71.2.912-920.2005.

Gassilloud, B., Schwartzbrod, L., Gantzer, C., 2003. Presence of viral genomes in mineral water: a sufficient condition to assume infectious risk? Appl. Environ. Microbiol. 69, 3965–3969. https://doi.org/10.1128/AEM.69.7.3965-3969.2003.

Gerba, C.P., Betancourt, W.Q., 2017. Viral aggregation: impact on virus behavior in the environment. Environ. Sci. Technol. 51, 7318–7325. https://doi.org/10.1021/acs.est.6b05835.

Hamza, I.A., Jurzik, L., Überla, K., Wilhelm, M., 2011. Methods to detect infectious human enteric viruses in environmental water samples. Int. J. Hyg Environ. Health 214, 424–436. https://doi.org/10.1016/j.ijheh.2011.07.014.

Harrison, J.P., Schratzberger, M., Sapp, M., Osborn, A.M., 2014. Rapid bacterial colonization of low-density polyethylene microplastics in coastal sediment microcosms. BMC Microbiol. 14, 1–15.

Heijnen, L., Medema, G., 2011. Surveillance of influenza A and the pandemic influenza A (H1N1) 2009 in sewage and surface water in The Netherlands. J. Water Health 9, 434–442. https://doi.org/10.2166/wh.2011.019.

Helmi, K., Skraber, S., Gantzer, C., Willame, R., Hoffmann, L., Cauchie, H.M., 2008. Interactions of Cryptosporidium parvum, Giardia lamblia, vaccinal poliovirus type 1, and bacteriophages φX174 and MS2 with a drinking water biofilm and a wastewater biofilm. Appl. Environ. Microbiol. 74, 2079–2088. https://doi.org/10.1128/AEM.02495-07.

Iaconelli, M., Muscillo, M., Della Libera, S., Fratini, M., Meucci, L., De Ceglia, M., Giacosa, D., La Rosa, G., 2017. One-year surveillance of human enteric viruses in raw and treated wastewaters, downstream river waters, and drinking waters. Food Environ. Virol. 9, 79–88. https://doi.org/10.1007/s12560-016-9263-3.

Jiang, P., Zhao, S., Zhu, L., Li, D., 2018. Microplastic-associated bacterial assemblages in the intertidal zone of the Yangtze Estuary. Sci. Total Environ. 624, 48–54. https://doi.org/10.1016/j.scitotenv.2017.12.105.

Johnson, B.A., Hage, A., Kalveram, B., Mears, M., Plante, J.A., Rodriguez, S.E., Ding, Z., Luo, X., Bente, D., Bradrick, S.S., Freiberg, A.N., Popov, V., Rajsbaum, R., Rossi, S., Russell, W.K., Menachery, V.D., 2019. Peptidoglycan-associated cyclic lipopeptide disrupts viral infectivity. J. Virol. 93, 1–15. https://doi.org/10.1128/jvi.01282-19.

Kelly, J.J., London, M.G., McCormick, A.R., Rojas, M., Scott, J.W., Hoellein, T.J., 2021. Wastewater treatment alters microbial colonization of microplastics. PLoS One 16, 1–19. https://doi.org/10.1371/journal.pone.0244443.

Keswani, A., Oliver, D.M., Gutierrez, T., Quilliam, R.S., 2016. Microbial hitchhikers on marine plastic debris: human exposure risks at bathing waters and beach environments. Mar. Environ. Res. 118, 10–19. https://doi.org/10.1016/j.marenvres.2016.04.006.

Kozlowski, L.P., 2017. Proteome-pI: proteome isoelectric point database. Nucleic Acids Res. 45, D1112–D1116. https://doi.org/10.1093/nar/gkw978.

Langlet, J., Gaboriaud, F., Duval, J.F.L., Gantzer, C., 2008. Aggregation and surface properties of F-specific RNA phages: implication for membrane filtration processes. Water Res. 42, 2769–2777. https://doi.org/10.1016/j.watres.2008.02.007.

Lares, M., Ncibi, M.C., Sillanpää, Markus, Sillanpää, Mika, 2018. Occurrence, identification and removal of microplastic particles and fibers in conventional activated sludge process and advanced MBR technology. Water Res. 133, 236–246. https://doi.org/10.1016/j.watres.2018.01.049.

Leslie, H.A., Brandsma, S.H., van Velzen, M.J.M., Vethaak, A.D., 2017. Microplastics en route: field measurements in the Dutch river delta and Amsterdam canals, wastewater treatment plants, North Sea sediments and biota. Environ. Int. 101, 133–142. https://doi.org/10.1016/j.envint.2017.01.018.

Lobelle, D., Cunliffe, M., 2011. Early microbial biofilm formation on marine plastic debris. Mar. Pollut. Bull. 62, 197–200. https://doi.org/10.1016/j.marpolbul.2010.10.013.

López, D., Vlamakis, H., Kolter, R., 2010. Biofilms. Cold Spring Harbor Perspect. Biol. 2, 1–12.

Metcalf, R., Oliver, D.M., Moresco, V., Quilliam, R.S., 2022. Quantifying the importance of plastic pollution for the dissemination of human pathogens: the challenges of choosing an appropriate 'control' material. Sci. Total Environ. 810, 152292 https://doi.org/10.1016/j.scitotenv.2021.152292.

Mohajerani, A., Karabatak, B., 2020. Microplastics and pollutants in biosolids have contaminated agricultural soils: an analytical study and a proposal to cease the use of biosolids in farmlands and utilise them in sustainable bricks. Waste Manag. 107, 252–265. https://doi.org/10.1016/j.wasman.2020.04.021.

Moresco, V., Damazo, N.A., Barardi, C.R.M., 2016. Thermal and temporal stability on the enteric viruses infectivity in surface freshwater. Water Sci. Technol. Water Supply 16, 620–627. https://doi.org/10.2166/ws.2015.171.

Moresco, V., Damazo, N.A., Barardi, C.R.M., 2015. Rotavirus vaccine stability in the aquatic environment. J. Appl. Microbiol. 120, 321–328. https://doi.org/10.1111/jam.13021.

Moresco, V., Oliver, D.M., Weidmann, M., Matallana-Surget, S., Quilliam, R.S., 2021. Survival of human enteric and respiratory viruses on plastics in soil, freshwater, and marine environments. Environ. Res. 199, 111367 https://doi.org/10.1016/j.envres.2021.111367.

Oberbeckmann, S., Kreikemeyer, B., Labrenz, M., 2018. Environmental factors support the formation of specific bacterial assemblages on microplastics. Front. Microbiol. 8, 1–12. https://doi.org/10.3389/fmicb.2017.02709.

Pittura, L., Foglia, A., Akyol, Ç., Cipolletta, G., Benedetti, M., Regoli, F., Eusebi, A.L., Sabbatini, S., Tseng, L.Y., Katsou, E., Gorbi, S., Fatone, F., 2021. Microplastics in real wastewater treatment schemes: comparative assessment and relevant inhibition effects on anaerobic processes. Chemosphere 262. https://doi.org/10.1016/j.chemosphere.2020.128415.

Prado, T., de Castro Bruni, A., Barbosa, M.R.F., Garcia, S.C., de Jesus Melo, A.M., Sato, M.I.Z., 2019. Performance of wastewater reclamation systems in enteric virus removal. Sci. Total Environ. 678, 33–42. https://doi.org/10.1016/j.scitotenv.2019.04.435.

Quignon, F., Sardin, M., Kiene, L., Schwartzbrod, L., 1997. Poliovirus-1 inactivation and interaction with biofilm: a pilot-scale study. Appl. Environ. Microbiol. 63, 978–982. https://doi.org/10.1128/aem.63.3.978-982.1997.

Robinson, C.M., Jesudhasan, P.R., Pfeiffer, J.K., 2014. Bacterial lipopolysaccharide binding enhances virion stability and promotes environmental fitness of an enteric virus. Cell Host Microbe 15, 36–46. https://doi.org/10.1016/j.chom.2013.12.004.

Rodrigues, A., Oliver, D.M., McCarron, A., Quilliam, R.S., 2019. Colonisation of plastic pellets (nurdles) by E. coli at public bathing beaches. Mar. Pollut. Bull. 139, 376–380. https://doi.org/10.1016/j.marpolbul.2019.01.011.

Schlindwein, A.D., Rigotto, C., Simões, C.M.O., Barardi, C.R.M., 2010. Detection of enteric viruses in sewage sludge and treated wastewater effluent. Water Sci. Technol. 61, 537–544. https://doi.org/10.2166/wst.2010.845.

Simcox, L.J., Pereira, R.P.A., Wellington, E.M.H., Macpherson, J.V., 2019. Boron doped diamond as a low biofouling material in aquatic environments: assessment of Pseudomonas aeruginosa biofilm formation. ACS Appl. Mater. Interfaces 11, 25024–25033. https://doi.org/10.1021/acsami.9b07245.

Simmons, F.J., Xagoraraki, I., 2011. Release of infectious human enteric viruses by full-scale wastewater utilities. Water Res. 45, 3590–3598. https://doi.org/10.1016/j.watres.2011.04.001.

Skraber, S., Ogorzaly, L., Helmi, K., Maul, A., Hoffmann, L., Cauchie, H.-M., Gantzer, C., 2009. Occurrence and persistence of enteroviruses, noroviruses and F-specific RNA phages in natural wastewater biofilms. Water Res. 43, 4780–4789. https://doi.org/10.1016/j.watres.2009.05.020.

Uddin, S., Fowler, S.W., Behbehani, M., 2020. An assessment of microplastic inputs into the aquatic environment from wastewater streams. Mar. Pollut. Bull. 160, 111538 https://doi.org/10.1016/j.marpolbul.2020.111538.

Waldman, P., Meseguer, A., Lucas, F., Moulin, L., Wurtzer, S., 2017. Interaction of human enteric viruses with microbial compounds: implication for virus persistence and disinfection treatments. Environ. Sci. Technol. 51, 13633–13640. https://doi.org/10.1021/acs.est.7b03875.

Wigginton, K.R., Kohn, T., 2012. Virus disinfection mechanisms: the role of virus composition, structure, and function. Curr. Opin. Virol. 2, 84–89. https://doi.org/10.1016/j.coviro.2011.11.003.

Wright, S.L., Ulke, J., Font, A., Chan, K.L.A., Kelly, F.J., 2020. Atmospheric microplastic deposition in an urban environment and an evaluation of transport. Environ. Int. 136, 105411 https://doi.org/10.1016/j.envint.2019.105411.

Wu, N., Zhang, Y., Zhao, Z., He, J., Li, W., Li, J., Xu, W., Ma, Y., Niu, Z., 2020. Colonization characteristics of bacterial communities on microplastics compared with ambient environments (water and sediment) in Haihe Estuary. Sci. Total Environ. 708, 134876 https://doi.org/10.1016/j.scitotenv.2019.134876.

Wu, X., Pan, J., Li, M., Li, Y., Bartlam, M., Wang, Y., 2019. Selective enrichment of bacterial pathogens by microplastic biofilm. Water Res. 165, 114979 https://doi.org/10.1016/j.watres.2019.114979.

Ye, Y., Ellenberg, R.M., Graham, K.E., Wigginton, K.R., 2016. Survivability, partitioning, and recovery of enveloped viruses in untreated municipal wastewater. Environ. Sci. Technol. 50, 5077–5085. https://doi.org/10.1021/acs.est.6b00876.

Zeng, S.Q., Halkosalo, a, Salminen, M., Szakal, E.D., Puustinen, L., Vesikari, T., 2008. One-step quantitative RT-PCR for the detection of rotavirus in acute gastroenteritis. J. Virol. Methods 153, 238–240. https://doi.org/10.1016/j.jviromet.2008.08.004.

Zettler, E.R., Mincer, T.J., Amaral-Zettler, L.A., 2013. Life in the “plastisphere”: microbial communities on plastic marine debris. Environ. Sci. Technol. 47, 7137–7146. https://doi.org/10.1021/es401288x.