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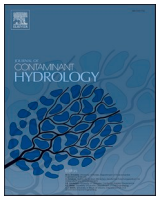

Attachment, re-mobilization, and inactivation of bacteriophage MS2 during bank filtration following simulation of a high virus load and an extreme rain event

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ABSTRACT

Viruses, including human pathogenic viruses, can persist in water. For producing drinking water from surface water via bank filtration, natural attenuation capacities and the fate of viruses during the passage of aquatic sediments are of particular interest. Moreover, the increasing frequency of extreme hydrological events necessitate re-evaluation of the sustainability and efficacy of processes removing viruses. For this purpose, we performed bank sediment filtration experiments using a mesocosm in a technical-scale experimental facility that simulates a field situation under more tightly controlled conditions. We used the bacteriophage MS2 as a surrogate for enteric viruses to study the transport of different viral loads through the bank sediment. Additionally, we simulated a heavy rain event to investigate the re-mobilization of initially attached virus particles. We quantified the abundance of infectious MS2 phages by plaque assay and the total number of MS2 particles by qPCR. Also, we differentiated pore water concentrations by depths of the sediment column and investigated attachment to the sediment matrix at the end of the individual experimental phases. Bank filtration over a vertical distance of 80 cm through sandy sediment revealed a virus removal efficiency of 0.8 $\log_{10}$ for total MS2 particles and 1.7 $\log_{10}$ for infectious MS2 particles, with an initial phage concentration of 1.84×10^8 gene copies mL^{-1}. A low load of infectious MS2 (1.9×10^6 plaque forming units mL^{-1}) resulted in a greater removal efficiency (3.0 $\log_{10}$). The proportion of infectious MS2 phages of the total MS2 particle mass steadily decreased over time, i.e., in the course of individual breakthrough curves and with sediment depth. The simulated pulse of rainwater caused a front of low ionic strength water which resulted in pronounced phage remobilization. The high proportion of infectious MS2 among the detached phages indicated that attachment to the sediment matrix may substantially conserve virus infectivity. Therefore, the re-mobilization of previously attached viruses owing to hydrological extremes should be considered in water quality assessment and monitoring schemes.

1. Introduction

Bank filtration of river and lake water has proven to be of great value for drinking water production by combining treatment and abstraction at low costs (Ahmed and Marhaba, 2017; Massmann et al., 2004; Krauss and Griebler, 2011; Kuehn and Mueller, 2000). The infiltrating surface water is physically, chemically, and microbiologically altered during sediment passage, and this generally improves water quality (Jaramillo, 2011). At some sites, bank filtration plants have been operating for more than 150 years (Kuehn and Mueller, 2000; Nagy-Kovács et al., 2019). For example, in Germany, the country in Europe with most sites of induced bank filtration, 9%–16% of drinking water is produced in this way (Gillefalk et al., 2018). However, if the efficiency of the natural subsurface attenuation processes is insufficient or highly variable, bank filtrate may be contaminated with pathogens, including viruses (Schijven et al., 2002; Tufenkji et al., 2002).

The contamination of raw and drinking water with pathogens is a widespread source of waterborne disease outbreaks (Yang et al., 2012).

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Previous studies found a range of human enteric viruses at densities of 10^2 to 10^3 gc (gene copies) L^{-1} in surface waters (Lodder et al., 2010; Prevost et al., 2015); these were introduced either by wastewater or via surface run-off from agricultural land where wastewater and manure were applied (Fong and Lipp, 2005). The reduction of pathogenic viruses during bank filtration is of particular interest because compared to most pathogenic bacteria, many viruses are more persistent in the aquatic environment. It is not rare for viruses to remain infective for more than 100 days in sediment, freshwater, and sewage (Fong and Lipp, 2005). Moreover, owing to their smaller size, viruses may travel longer distances through the porous subsurface, as underground physical attenuation processes may be less effective than for bacteria (Betancourt et al., 2014; Bition and Harvey, 1992). Viruses have been reported to be transported through subsurface media over distances as far as 1600 m and to a depth of 64 m below the land surface (Bales et al., 1989). For these reasons, a comprehensive understanding of the attenuation efficiency of bank filtration particularly for viruses is important for planning sustainable and safe drinking water production. This requires addressing the impact of dynamic changes in the hydrological cycle on virus transport and retention, including the impact of extended periods of droughts followed by heavy rain events and floods (Eckert et al., 2008).

Climate change is predicted to lead to an increase in extreme hydrological events, including more frequent and extended droughts and heavy rainfall. Understanding the impact of changes in hydrological conditions on the distribution of pathogens during bank filtration is therefore important for public health (Sprenger et al., 2011). Previous studies have already concluded that bank filtrate quality may be compromised by viral contamination induced by extreme precipitation owing both to shorter travel time in the subsurface and to elevated viral contamination in surface water (Rose et al., 2000; Sprenger et al., 2011). Additionally, Drayna et al. (2010) proposed that the re-mobilization (desorption) of formerly attached viruses in sediments can be a relevant process in the contamination of groundwater from infiltrating precipitation. Variation in the ionic strength of the sediment pore water may exert an important influence on virus attachment-detachment and (re-) mobilization (Landry et al., 1979). It is therefore important to understand how heavy rain events causing water with a low ionic strength to percolate through bank sediments affect virus transport.

Although bank filtration has been studied for many years, the focus was chiefly on the fate of a variety of chemical pollutants and pathogenic bacteria. The transport of pathogenic viruses through aquatic sediment has been less well studied, mainly owing to the challenges of virus detection and quantification (Dash et al., 2010; Doussan et al., 1997; Wyn-Jones and Sellwood, 2001). Moreover, studies have so far almost exclusively addressed the infectious viruses (i.e., detection by plaque assay or cell lines) suspended and transported in sediment pore water, while the transport of those which are no longer infectious has scarcely been addressed. However, including them provides information on virus inactivation, virus removal by irreversible attachment to sediment or decay, and virus retardation by attachment and re-mobilization (Loveland et al., 1996). Limiting research to the study of infectious virus particles may further underestimate the number of viruses involved in aggregation. In contrast, exclusively addressing total viruses, as is currently common with molecular tools, misses viral infectivity and inactivation (Hassard et al., 2016). Combining and comparing the fate of both the total virus load and the fraction which remains infectious is therefore the most comprehensive approach for elucidating the mechanisms of virus transport during sediment passage.

The primary objective of the current study was to elucidate the efficacy and contribution of individual natural attenuation processes as viruses travel through aquatic sediment, including irreversible attachment, retardation (attachment and re-mobilization), inactivation, and decay. This included, in particular, quantification of the re-mobilization of formerly attached viruses caused by rainwater infiltration. To achieve the aims of this study, bacteriophage MS2 was used as a surrogate because of its similar size (26 nm) and shape (icosahedral) to enteric viruses (especially enteroviruses), as well as its similar behavioral characteristics (persistence, resistance, no replication in aquatic environments, decay rate in surface water) that determine its fate in the subsurface (Boehm et al., 2019; Harvey and Ryan, 2004; Jofre et al., 2016; Pitol et al., 2018). Two scenarios in the main experiment focused on mimicking the infiltration of heavy viral contamination and a subsequent rainstorm event. Both scenarios are likely to occur frequently in fairly densely settled areas where bank filtrate is obtained from rivers and lakes that carry treated wastewater, e.g., by upstream discharge from sewage treatment plants or by stormwater overflow from mixed sewers. Unlike other studies, we not only looked at the fate of either total or infectious virus particles suspended in sediment pore water but also evaluated their combined dynamics over space and time and included total and infectious MS2 phages attached to the sediment matrix after each treatment phase, i.e., after the contamination event and after the heavy rain event. Moreover, key physical and chemical parameters, i.e., electrical conductivity (EC), dissolved oxygen concentration (DO), pH, redox potential, and temperature, and basic microbial parameters, i.e., adenosine triphosphate concentration (ATP) and total prokaryotic cell counts (TCC), were monitored in sediment pore water throughout the entire experiment.

2. Material and methods

2.1. Experimental facility

The study was conducted using a sediment column embedded in the facility for the simulation of riverbank and slow sand filtration (Fig. S1) at the German Environment Agency's field station in Berlin-Marienfelde. This is fed by water from a storage pond in which groundwater (from which Fe and Mn were removed through microbial precipitation) was exposed to the atmosphere (including natural rainfall and sunlight) so that natural flora and fauna could develop. After a residence time of 2–3 weeks this water entered the slow sand filtration basin, mimicking surface water with a pH of 8 ± 0.2 (29 replicate measurements) and a calculated ionic strength of 13.3 mM. Selected inorganic hydrochemical parameters of the infiltrating water are summarized in Table 1. Mean concentrations of total organic carbon recorded in the months of November/December of preceding years amounted to 2.0 mg L^{-1} (three replicates). A water-saturated sand column, similar to a lysimeter with a diameter of 113 cm (i.e., corresponding to a surface area of 1 m^2) and a height of 130 cm (Fig. 1) was embedded in the slow sand infiltration basin (dimensions 10 × 10 m) and fed with surrounding pond water. The supernatant, i.e., a water column of 10 cm (corresponding to 100 L of water) could be connected and disconnected to the surrounding pond water. The column was packed with 100 cm of natural coarse-grained medium sand (grain size distribution in Table S1) with a porosity of 41% (d_{10} = 0.14 mm, d_{60} = 0.33 mm, uniformity coefficient Cu = 2.36), on top of a supporting layer of 30 cm of gravel. As the ratio of the column diameter (d_{col}) to the effective particle diameter (d_{10}) of the media was

Table 1
Inorganic hydrochemical parameters of the infiltrating pond water in mg L^{-1} and mM (mean values of 14–27 analyses conducted over a period of 7 years).

Parameter	Concentration ± standard deviation (mg L^{-1})	Concentration ± standard deviation (mM)
Na^+	50.5 ± 4.6	2.2 ± 0.2
K^+	3.7 ± 0.9	0.09 ± 0.02
Ca^{2+}	123 ± 8	3.1 ± 0.2
Mg^{2+}	17.3 ± 1.4	0.71 ± 0.06
Fe_{tot}	0.018 ± 0.019	0.0003 ± 0.0003
Mn_{tot}	2.2 ± 1.6	0.04 ± 0.03
Cl^-	89 ± 6	2.5 ± 0.2
NO^{3-}	> 0.1	> 0.002
SO_4^{2-}	222 ± 12	2.3 ± 0.2

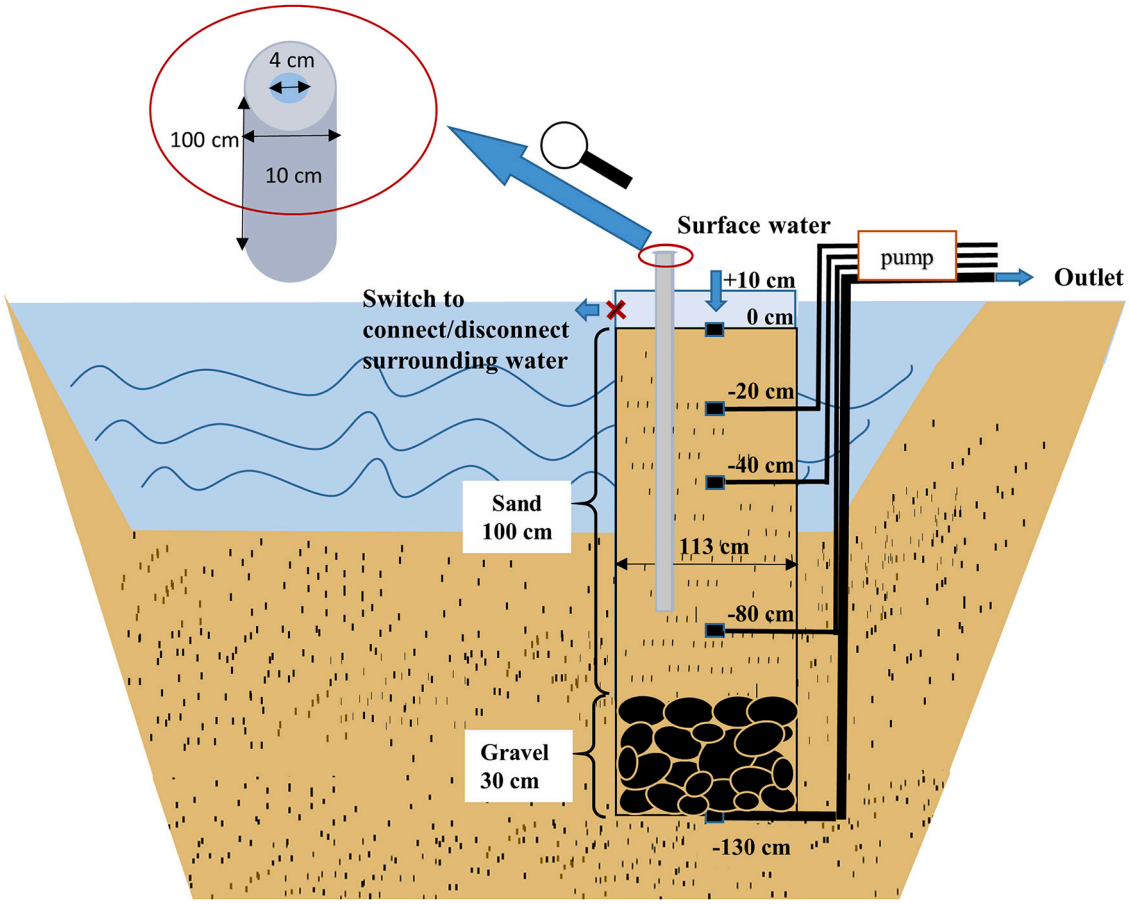

Fig. 1. Schematic drawing of the pond sediment column system.

far greater than 50, a potential wall effect can be neglected (Knappett et al., 2008). Sampling ports for sampling along the flowpath were installed at depths of 20 cm, 40 cm, and 80 cm. The system had been in operation for over 10 years, sufficient for the establishment of a natural bacterial community (Degenkolb et al., 2018).

2.2. Pre-experiments

Pre-experiments included (i) batch tests on the inactivation/decay of MS2 phages in sterile surface water and water-saturated sediment; and (ii) a virus transport experiment in the sediment column with a moderate virus load.

For the batch tests, MS2 was added to filter-sterilized (0.22 μm) Danube River water (pH: 8.19; EC: 401 μS cm^{-1}; DO: 12.76 mg L^{-1}, $n = 3$) or autoclaved water-saturated sand (natural bank sediment; grain size between 63 and 630 μm; porosity of 36%). Duplicate water samples contained an initial total MS2 concentration of 6.47 × 10^8 particles mL^{-1}. Duplicate sediment samples, each water-saturated, consisted of 17 g dry weight sediment with approximately 3.6 mL of pore water and received an initial total MS2 concentration of 1.52 × 10^8 particles mL^{-1}. These samples were incubated at 7 °C in the dark for 19–23 days.

The impact of a moderate virus load on MS2 transport was assessed 2 years before the main experiment, also in November and with the same column system. A pulse of pond water (200*L*) with a moderate virus load, i.e., a final concentration of 1.9 × 10^6 plaque forming units (PFU) mL^{-1} of infectious MS2 (total amount of infectious MS2 of 3.8 × 10^{11} PFU), was supplied with flow rate adjusted to a filter velocity of 1.1 m d^{-1} (corresponding to a pore water velocity of 2.2 m d^{-1}). After the pulse, the column was re-connected to the surrounding pond water just before the pulse solution was completely infiltrated. Water samples were collected before and after the respective treatments at a high temporal and spatial resolution (i.e., the supernatant, 40 cm, 80 cm, and the column outlet at 130 cm depth; Fig. 1). Since this pre-experiment was conducted two years before the main experiment, there was sufficient time for the sediment system to be flushed by MS2-free water.

2.3. Main experimental design

In the first of the two experimental phases, a 100 L pulse of pond water containing a high load of 1.84 × 10^8 gc mL^{-1} MS2 particles, as determined by quantitative real-time polymerase chain reaction (qPCR), and 1.5 × 10^8 PFU mL^{-1} of infectious phage, as determined by the plaque assay, was supplied to the column with a pore water velocity of 2.1 m d^{-1}. Nineteen days after the high virus load pulse, experimental phase 2 was initiated to simulate a heavy rainfall event (corresponding to 37 mm/h) via infiltration of a pulse of 100 L of deionized water. Deionized water was used because its low ionic strength is similar to that of natural rainwater. After each of the pulses, the column was reconnected to the surrounding pond water just before the pulse solution was completely infiltrated. Sampling depths for pore water included the surface (i.e., the supernatant), 20 cm (withdrawal rate: 1.3 L/h), 40 cm (withdrawal rate: 1.4 L/h), 80 cm (withdrawal rate: 1.4 L/h), and the column outlet (at 130 cm depth, withdrawal rate of 33.4 L/h; Fig. 1). From the intermediate ports and at the column outlet water was withdrawn continuously. Samples for infectious MS2, prokaryotic cell counts and microbial activity were collected every hour in the first 7 h and 12 h after the dose of MS2 pulse and the pulse of deionized water, respectively. Subsequently, the sampling intervals increased sequentially with time (see Tables S2 and S3 for details). At the mesocosm outlet in experimental phase 1, water samples for biological monitoring were first

collected 23 h after the supply of MS2 particles. Samples for the analyis of water chemistry were taken once a day during the first 10 days after MS2 dosing and for the entire experimental phase 2 after simulated rainfall. Additional water chemistry samples were taken at 15 and 16 days after MS2 donation.

Sediment samples were collected after the high virus load pulse (experimental phase 1) and the simulated rain event (experimental phase 2) using a double-wall sediment corer (high-density polyethylene, 10 cm outer diameter, 4 cm inner diameter, and 100 cm height), which was pushed into the sediment to a depth of 70 cm at the end of each experimental phase (day 15 and day 23 after the MS2 pulse). The inner core was withdrawn carrying the sediment, whereas the outer tube remained in place for the remainder of the experiment to maintain undisturbed hydrological conditions. The cored sediment samples were divided into portions of 5 cm depth and transferred into falcon tubes (50 mL) for the subsequent quantification of total and infectious MS2 particles. All experiments were conducted at the end of the calendar year (November/December).

2.4. Cultivation and quantification of MS2

Bacteriophage MS2 (DSMZ 13767) and MS2 host strain *Escherichia coli* (DSMZ 5695) were obtained from the DSMZ (German Collection of Microorganisms and Cell Cultures GmbH). Isolation and purification of MS2 phages after propagation with a co-incubation of *E. coli* 5695 were performed following the protocol described in Feichtmayer (2019) to produce a high-density phage suspension in buffer solution.

For the pre-experiment with the moderate virus load, infectious phages were quantified with the plaque assay in accordance with DIN EN ISO 10705–1:2001. For the pre-experiment batches and the main experiments, this was adapted to follow the double-agar overlay method of Kropinski et al. (2009). In brief, a solid agar base (10 mL of 1.5% agar, *w/v*) was overlaid with a mixture of soft agar (4 mL of 0.75% agar, w/v) and 1 mL of a well-mixed solution consisting of the (diluted) sample and host bacteria in the logarithmic growth phase (1:1, *v*/v). Serial dilutions of the mixture were incubated at 37 °C overnight and PFUs were counted in duplicate. The titer of infectious phages in the sediment samples was estimated after the elution of 0.5 mL of sediment with 1 mL of deionized water and vigorous shaking on a vortex mixer (3 times, each for 1 min, with 30 s between each mixing step). The limit of detection (LOD) for the plaque assays was 0.5 PFU mL^{-1}.

Triplicate quantification of the total number of phages was performed using qPCR analysis of phage RNA transcribed into cDNA. Before phage RNA extraction, all water samples were concentrated to a volume of 1 mL using Amicon® Ultra-15 Centrifugal Filter Units (Merck). Phage RNA was then extracted using the AllPrep PowerViral DNA/RNA Kit (Qiagen) and cDNA synthesis was performed using the Maxima First Strand cDNA Synthesis Kit (ThermoFisher). The isolation of phage RNA and the reverse transcription was conducted following the manufacturer's instruction. The qPCR targeted a 315 bp fragment using the MS2-specific primer sequences MS2-2717Fw (5′-CTG-GGC-AAT-AGT-CAA-A-3′) and MS2-3031Rv (5′-CGT-GGA-TCT-GAC-ATA-C-3′),and using the Brilliant III UltraFast SYBR Green QPCR Master Mix (Agilent). Then, 2 μL of cDNA were quantified in triplicate; each 25 μL reaction contained 12.5 μL of qPCR master mix, 0.375 μL of reference dye (1:500 v/v dilution in RNase-free water) and 0.5 μL of each primer (10 μM, Eurofins). The qPCR analyses were performed in a Stratagene MX3000P qPCR cycler (Agilent, $R^2 > 0.99$) and a Light Cycler 480 SW 1.5 (Roche, $R^2 > 0.98$) following a protocol modified from Dreier et al. (2005). Initial denaturation (95 °C, 10 min) was followed by 40 cycles of denaturation (95 °C, 15 s), annealing (50 °C, 30 s) and elongation (72 °C, 30 s). Afterwards, the melting curve was recorded between 55 °C and 95 °C to verify the specificity of the amplification products. Owing to the different sample volumes available for concentration, there was no single LOD; instead, the LOD range was determined as 5.8×10^2–5.6×10^3 gc mL^{-1}.

2.5. ATP and TCC analyses

To study the relationship between virus reduction and the amount and activity of autochthonous microorganisms, microbial activity and prokaryotic cell counts were monitored in both water and sediment samples throughout the experiment. The microbial activity in water and sediment samples was estimated using ATP concentration measurements using the BacTiter-Glo Microbial Cell Viability Assay kit (Promega) following the protocol described in Hammes et al. (2010), but with minor modifications. Water samples and the BacTiter-Glo reagent, prepared in accordance with the manufacturer's instructions, were prewarmed separately at 38 °C for at least 2 min before 1 mL of sample was mixed with 50 μL of reagent. The mixture was incubated at 38 °C for 1 min and luminescence measured on a GloMax 20/20 Luminometer (Promega). Concentrations were determined by comparison with external ATP standards dissolved in ATP-free water (Fisher Scientific) using ATP-free water as blank. To correct for the contribution of extracellular ATP in the samples the measurements were performed on an untreated sample fraction representing the total ATP concentration as well as a fraction of the same sample that was filtered (0.1 μm syringe filter, Merck Millipore) to remove microbial cells. The concentration of intracellular ATP was then calculated by subtracting the extracellular concentration from the total. All measurements were conducted in triplicate.

The total ATP in sediment samples was determined as follows: 50 μL of 0.1 μm filtered Milli-Q water was added to 200 μL of homogenized sediment. Next, 100 μL preheated (38 °C) BacTiter-Glo reagent was added and the sample was mixed using a Thermomixer (1400 rpm) at 38 °C for 2.5 min. Subsequently, 900 μL of 0.1 μm filtered Milli-Q water was added and the sample was briefly mixed using a vortex mixer and centrifuged. Finally, triplicate measurements of the supernatant were performed using a similar protocol to that for the water samples (see above). The extracellular ATP in sediment samples was quantified using a similar setup, but without the addition of 100 μL preheated (38 °C) BacTiter-Glo reagent to lyse the cells.

TCC was quantified using flow cytometry (FC 500 CYTOMICS, Beckman Coulter) as described in Fillinger et al. (2019) for pore water samples and as detailed in Bayer et al. (2016) for sediment samples.

2.6. Physicochemical conditions

Pore water temperature and pH were measured on-site using a temperature data logger (Voltcraft) and microprocessor pH meter, respectively. EC, redox potential, and DO were measured on-site using field sensors (WTW).

2.7. Data analysis

The recovery of total and infectious MS2 during experimental phase 1 and phase 2 was calculated to a depth of 80 cm. The deeper zone of the mesocosm including the gravel layer was not included, thus avoiding inconsistency due to its large sediment heterogeneity and the risk of having missed the MS2 peak concentration at the mesocosm's outlet. For samples with a total MS2 concentrations below the detection limit, the infectious MS2 concentration was used instead for calculation. The number of total and infectious sediment-attached MS2 was calculated as the product of the total or infectious MS2 concentration detected at various depths of the sediment cores and the volume of the corresponding column layer. The phage particle concentrations collected at depths between 65 cm and 70 cm were assumed to be representative of the depth interval of 65–80 cm.

To test the relationships between different biological and physicochemical variables, a Spearman rank correlation analysis was performed with the Hmisc package in R (Harrll et al., 2018) and applied to all available variables collected for pore water and sediments encompassing data from both experimental phases (Tables S4 and S5).

Student's *t*-test ("t.test" in R) and the Wilcoxon test ("wilcox.test" in R) were used to analyze the significance of difference between samples using t with a significance level of 0.05.

3. Results and discussion

3.1. Pre-experiments

3.1.1. Inactivation and decay of MS2 in batch incubations

Following the MS2 inoculum of 6.5 × 10^8 gc mL^{-1} in sterilized surface water for 23 days in batch experiments (Fig. 2) showed the concentration of total MS2 particles, as measured by qPCR, to remain constant, i.e., without any significant reduction (*Wilcoxon test*, $n = 12$, $P = 0.5$; Fig. 2). This indicates that no MS2 decay occurred during incubation. However, the fraction of infectious MS2 showed some decline at 19 days after inoculation, i.e. from 5.6 × 10^8 to 9.8 × 10^7 PFU mL^{-1}, accounting for 15% of the total MS2 and corresponding to a 0.8-log reduction of the initial infectious MS2 concentration. This decrease in infectious MS2 during incubation is assumed to be due to inactivation and implies an inactivation rate of MS2 in the sterilized surface water at 7 °C was 0.045 $\log_{10}$ day^{-1} (Fig. 2), similar to the inactivation rate of MS2 (0.043 $\log_{10}$ d^{-1}) determined at 4 °C in raw groundwater by Ogorzaly et al. (2010).

Similar to the experiments only in sterilized water, no reduction in total MS2 was observed after inoculation into water-saturated sediments (*Wilcoxon test*, $n = 12$, $P = 1$). The initial concentration of attached total MS2 in the sterile water-saturated sediment mixture was 1.5 × 10^8 gc mL^{-1}. Infectious MS2 attached to the sediment with an initial concentration of 8.9 × 10^7 PFU mL^{-1} declined at an inactivation rate of 0.035 $\log_{10}$ d^{-1} (Fig. 2). The assumed inactivation rate of attached MS2 phages was lower than that of the suspended MS2, indicating that although attachment is an important process for attenuating MS2 during the subsurface passage, phage activity is preserved. Prolonged survival of the virus by attachment to sediment was also observed by Melnick et al. (1980) and Schijven and Hassanizadeh (2000).

3.1.2. Transport of a moderate MS2 pulse through the sediment column

In the pre-experiment that lasted for 50 h, we observed a significant decrease in the peak concentrations of the infectious virus breakthrough curves with depth, from 1.9 × 10^6 PFU mL^{-1} at the surface to 2.1 × 10^3 PFU mL^{-1} at a depth of 80 cm (Fig. 3). After 80 cm of sediment passage, the attenuation or removal of infectious MS2 amounted to approximately 3.0 $\log_{10}$. No significant difference in the concentration of infectious MS2 was detected at the depth of 80 cm and the outlet at the depth of 130 cm (*t*-test, $t = 0.97$, $df = 9.7$, $P = 0.35$).

3.2. Main experiments

3.2.1. Dynamics of physico-chemical water quality

During the entire course of the main experiment, the pond water infiltrating into the bank sediment differed significantly in temperature (*Wilcoxon test*, $n = 34$, $P < 0.05$), DO concentration (*t-test*, $t = -3.6$, $df = 31$, $P < 0.01$) and pH (*t-test*, $t = -4.0$, $df = 24.6$, $P < 0.001$) from the water collected at the outlet after 130 cm of sediment passage. Compared to the surface water temperature ($mean_{surface}$ = 5.9 °C ± 2.4 °C) water temperature at the column outflow was higher and more constant ($mean_{outlet}$ = 7.8 °C ± 2.0 °C; Fig. 4). This difference was partially due to the transition from autumn to winter and the delayed response of the sediment temperature to the decrease in air temperature during the course of the experiment. The mean DO concentration decreased from 13.9 ± 1.8 mg L^{-1} ($mean_{surface}$) to 11.5 ± 2.2 mg L^{-1} ($mean_{outlet}$) during the passage of pore water through the column, which was likely due to higher oxygen solubility at lower temperature and microbial processes, e.g., aerobic respiration (Diem et al., 2013). A peak value of 17.2 mg L^{-1} DO in the infiltrating surface water on day 9 corresponded to a sudden drop in temperature (Fig. 4). The pH decreased from a mean value at the surface of 8.1 ± 0.1 to a mean value at the outlet of 7.8 ± 0.3 (Fig. 4). Heterotrophic biological processes result in DO consumption and oxidation of organic matter with the production of CO_2 and/or organic acids which generally reduces pH (Kuehn and Mueller, 2000). During sampling, the mean redox potential at the surface (350 ± 71 mV) was not significantly different from that at the column outlet (347 ± 67 mV; *Wilcoxon test*, $n = 32$, $P > 0.5$). As redox

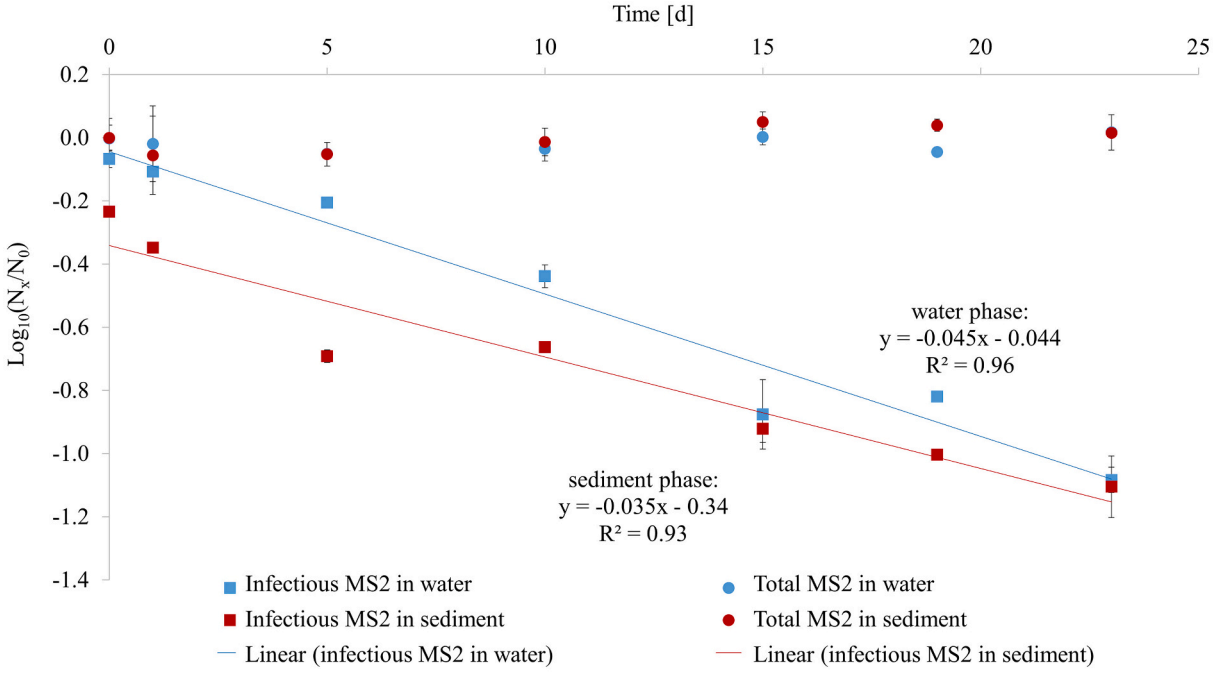

Fig. 2. The variation of total and infectious bacteriophage MS2 in sterilized surface water or sterilized water-saturated sediment (the abundance of total MS2 at time 0 in water or in sediment was recorded as N_0 and the abundance of total or infectious MS2 detected at the corresponding time point in water or in sediment was defined as N_x).

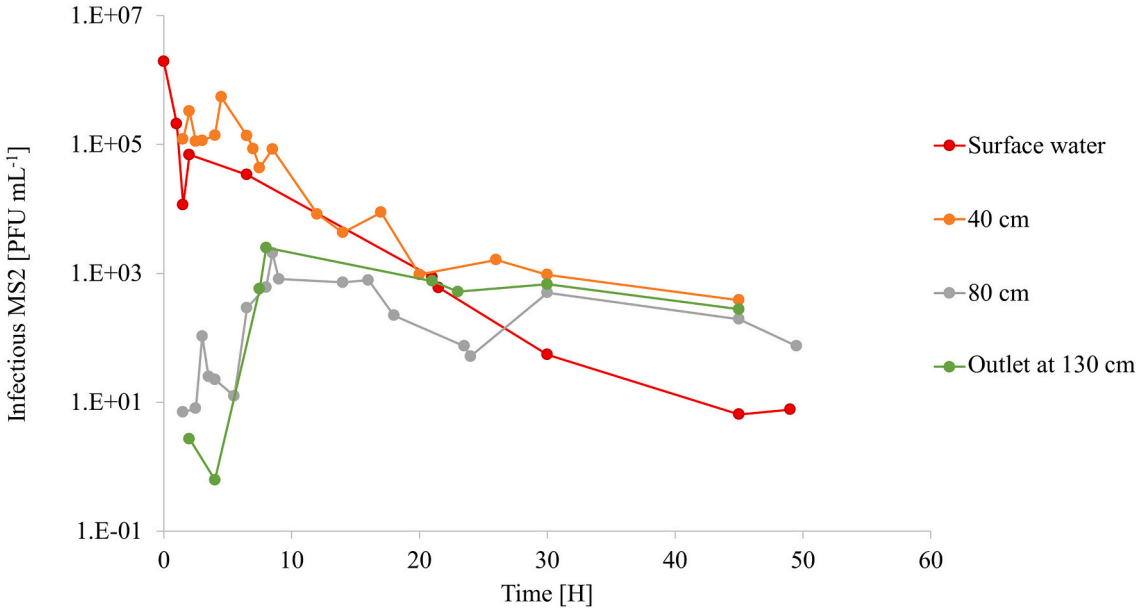

Fig. 3. Breakthrough of infectious MS2 at different depths of the column, as determined from the transport pre-experiment.

conditions remained oxic throughout the entire experiment effects on the survival of the virus by a redox shift are likely to have been negligible (Feichtmayer et al., 2017). EC remained constant during the experiment ($mean_{surface} = 878 \pm 223$ µS cm^{-1}, $mean_{outlet} = 879 \pm 192$ µS cm^{-1}) and was independent of sediment depth, with one exception, namely the supply of deionized water at the beginning of phase 2 which caused a temporary shift throughout the sediment column. The lowest EC caused by deionized water was 15.1 µS cm^{-1} at the surface; a higher value of 135.8 µS cm^{-1} was detected at the outlet. The simulated heavy rainfall, as intended, led to a reduction in the ionic strength of the sediment pore water. Overall, the differences in DO and pH indicated the activity of biological processes during bank filtration (Kuehn and Mueller, 2000; Diem et al., 2013).

3.2.2. *Experimental phase 1 – virus contamination*

3.2.2.1. Fate of MS2 during transport through the bank sediment. The peak concentrations of total MS2 in the breakthrough curves decreased with increasing depth and distance to the surface (Fig. 5), i.e., from 1.84 × 10^8 gc mL^{-1} in the surface water to 3.2 × 10^7 gc mL^{-1} at 80 cm. Thus, the peak of total virus concentration was reduced by 0.8 log units through 80 cm of sediment passage. A total amount of 1.75 × 10^{13} MS2 particles was estimated to be transported through the 80 cm sediment column (Table 2), i.e., at the end of experimental phase 1, only 5% of the total MS2 particles remained in the column. At 219 h after the inoculation with MS2, 10^3 total MS2 particles mL^{-1} were detected in the surface water, corresponding to a log decrease rate of 0.0185 h^{-1}. We attribute these remaining MS2 particles to kinetic phage attachment to and detachment from the top sediment, taking into account the mixing and non-uniform infiltration of the pond water (Frohnert et al., 2014; Schijven and Hassanizadeh, 2000).

As with the concentration of total MS2 phage particles, the peak concentration of infectious MS2 in pore water decreased with increasing distance from the surface, i.e., from 1.5 × 10^8 PFU mL^{-1} in the surface water to 2.9 × 10^6 PFU mL^{-1} measured at depth of 80 cm (Fig. 5) corresponding to a removal of approximately 1.7 log units. With a reduction in peak concentration of 0.8 log units for total MS2 particles and 1.7 log units for infectious MS2 particles, the overall reduction of virus in our main experiment was in the range of that reported in other studies using MS2 as a model agent, i.e., a 0.1–1.9 log unit reduction over a filtration distance of 0.4–1.5 m as reviewed by Bauer et al. (2011). With the moderate virus load applied in the pre-experiment, which was run under the same conditions as the main experiment, a 3.0 log unit removal of infectious MS2 in pore water was found, exceeding the removal in the main experiment.

The lower removal efficiency in the main experiment can be explained by a conceptual model for colloid transport derived from column studies and micromodel observations, as suggested by Bradford et al. (2009). At high initial input concentrations, a lower relative mass transfer to the solid phase occurs because weakly associated colloids are knocked off the solids' surface owing to more frequent collisions. Moreover, Bradford et al. (2009) found that only a small fraction of the porous media play an active role in colloid retention, the reason being that colloid retention is highest near grain–grain contact where low-velocity areas form. Furthermore, another possible explanation is the enhanced aggregation caused by the higher virus concentration, leading to less interaction of the individual viruses with the grain surfaces. Indeed, all virus particles within one aggregate must be inactivated before this aggregate group will not appear as active in the plaque assay (Schijven and Hassanizadeh, 2000). The high virus load we applied reflects a worst-case scenario with a high share of sewage in river flow, as raw sewage is reported to contain between 10^9 and 10^{12} infectious coliphages per L (Lodder and de Roda Husman, 2005). Applying a high virus load also enabled the reliable detection of total and infectious MS2 particles over time and space.

19 days after the major MS2 pulse 0.1% of MS2 particles that were transported through the 80 cm sediment column were still infective. Compared to the infectious MS2 fraction of the sterilized water in batch experiments, a much higher proportion of infectious MS2 particles lost their infectivity during the sediment passage in the main experiment. We assumed that the higher fraction of inactivated MS2 phages in the column pore water relative to the sterile batch tests was mainly attributable to the interplay of pore water flow and attachment, as well as to autochthonous microorganisms and their activity. For example, Hurst et al. (1980) and Schijven et al. (2002) found that the presence of aerobic microorganisms, especially sediment-attached microorganisms, could affect virus activity and may promote virus decay. Yates et al. (1990) provided an early review of the mechanisms through which microorganisms may cause the inactivation of viruses. For example, certain bacteria release proteolytic enzymes that destroy the protein coating of virus particles (Cliver and Herrmann, 1972). Deng et al. (2014) indicated that the number of infectious MS2 phages may be

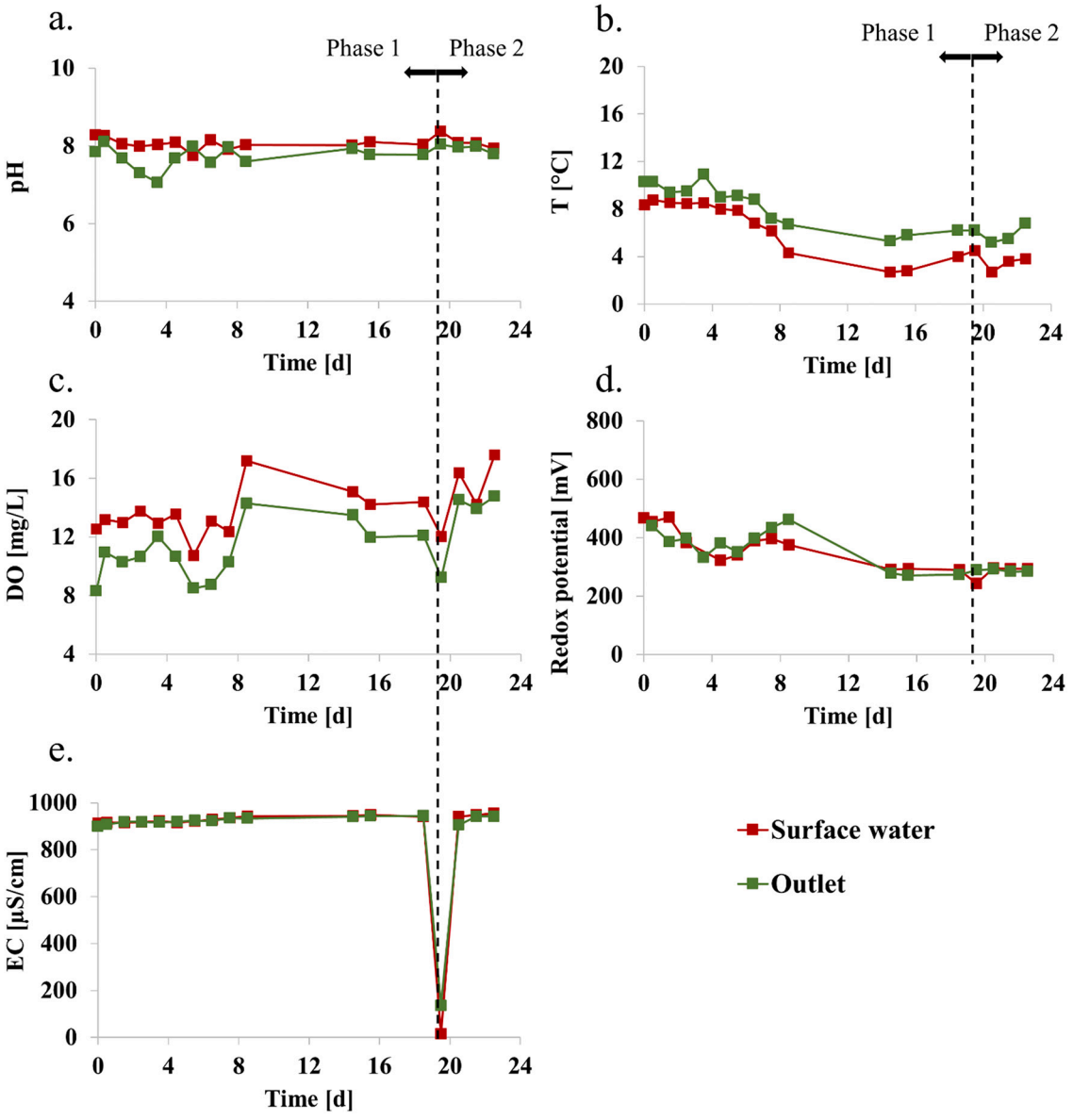

Fig. 4. Physico-chemical conditions in the surface water and column outlet over the course of the main virus transport experiment. T: temperature; DO: dissolved oxygen; EC: electrical conductivity.

reduced by grazing protozoa. More recently, Feichtmayer et al. (2017) provided a comprehensive review of the antagonistic microbial processes inactivating and/or eliminating viruses in aquatic ecosystems.

Importantly, the average percentage of infectious MS2 in pore water decreased with increasing distance from the point of infiltration (Fig. 5). Moreover, the proportion of infectious MS2, 0.4%–79%, also changed over time in the breakthrough water. The highest proportion of infectious MS2 particles was found near or at the peak of the individual breakthrough curves, independently of sediment depth (i.e., 79% at 0 cm, 59% at 20 cm, 47% at 40 cm, and 30% at 80 cm).

3.2.2.2. Fate of MS2 attached to the sediment in phase 1 core samples. The concentration of sediment-attached total MS2 particles was distributed rather evenly with depth in the sediment column at the end of phase 1 (i.e., 15 days after the inoculation with MS2) (Fig. 6). Starting with a total concentration of attached MS2 phages of 1.5×10^5 gc mL^{-1} in the uppermost sediment layer, the concentration increased to 4.8×10^5 gc mL^{-1} to a depth of 15 cm and then remained constant, independently of further sediment depth. This pattern indicated that the top sediment layer experienced significant detachment owing to the ongoing infiltration of MS2-free surface water whereas sediments below 15 cm experienced a MS2 saturation with a balance between the attachment and detachment of MS2 particles.

The distribution of infectious MS2 particles followed a slightly different trend, with a stable concentration in the top 45 cm and a moderate decrease in concentration and proportion between 50 cm and 70 cm. The percentage of infectious MS2 phages was thus high at the top (15% at 5 cm), decreased with depth, and became lowest at the bottom of the column (0.8% at 70 cm). Both the attached and suspended infectious MS2 decreased with depth, suggesting MS2 experienced more inactivation than decay during the sediment passage. This result also suggests that the retention of infectious phage particles is higher than that for the total MS2 particles because inactivation reduces particle size and alters the physico-chemical characteristics of the phage surface, facilitating transport (Ghanem et al., 2018).

3.2.2.3. Microbial biomass and activity in phase 1. The abundance of prokaryotic cells and microbial activity in the pore water at individual

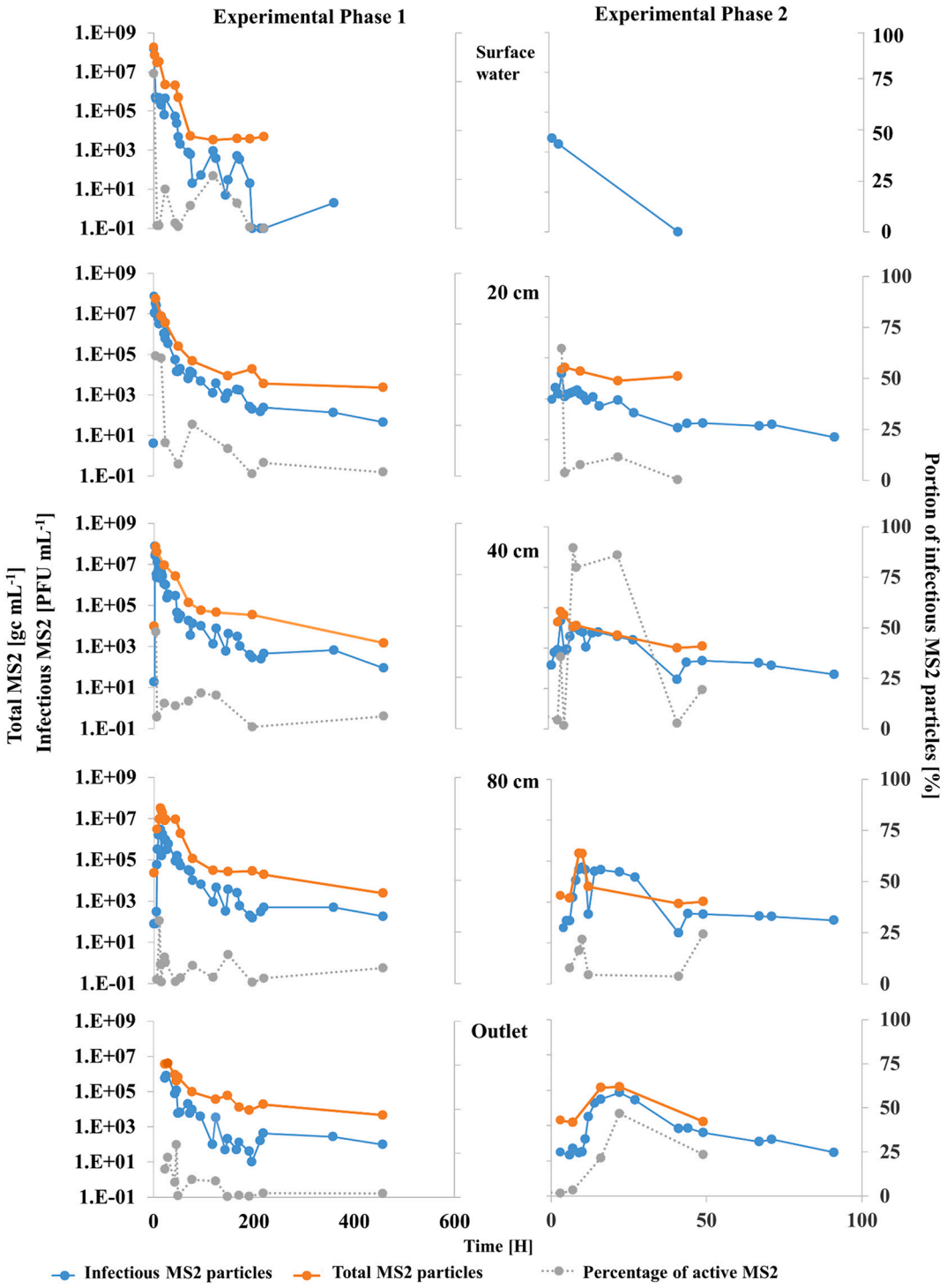

Fig. 5. Breakthrough of total and infectious MS2 particles at various sediment depths in pore water during experimental phases 1 and 2.

sediment depths remained stable during phase 1 (Fig. 7). This indicates that the inoculation of surface water with MS2 did not significantly alter other microbial patterns in sediment pore water. As expected, the concentration of prokaryotic cells in the pore water decreased with sediment depth, from 8.12×10^5 cells mL^{-1} in the surface water to 5.39×10^4 cells mL^{-1} at 100 cm. A similar pattern was observed for pore water microbial activity (Fig. 7). Prokaryotic cell counts and microbial activity of the attached microbial communities from core sampling were also highest in the top-most sediments (Fig. 8). Decreasing prokaryotic cell numbers with increasing sediment depth were consistent with previous studies (as reviewed by Hassard et al., 2016), while the stable microbial activity throughout the column below 10 cm the presence of a well-established depth-independent active bacterial community (Degenkolb et al., 2018). The activity of indigenous microbes is assumed to have a negative impact on the survival of allochthonous viruses and bacteria, particularly those that do not have a host in the environment, such as human pathogenic viruses (Sobsey et al., 1980; Feichtmayer et al., 2017).

Table 2
Estimate of total and infectious MS2 during the main experiment down to a column depth of 80 cm (Phase 1: dose of MS2; Phase 2: pulse of deionized water).

Experimental phase	Samples	Total MS2 (gc)	Distribution of inoculum	Proportion of infectious to total MS2
	Inoculum	1.84×10^{13}	100%	79%
Phase 1	Sediment-attached	3.3×10^{11}	1.8%	4%
	Flow through 80 cm	1.75×10^{13}	95%	6.7%
	Retained–MS2	3.3×10^{11}	1.8%	4%
Phase 2	Sediment-attached	4.2×10^{11}	2.4%	2.9%
	Flow through 80 cm	3.3×10^{10}	0.2%	69%

3.2.3. *Experimental phase 2 – Phage re-mobilization through a simulated heavy rainfall event*

As described in Section 2.2, the simulated heavy rainfall event was initiated 19 days after the supply of a high load of MS2. The infiltration of 100 L of deionized water was equivalent to a recharge of precipitation of 37 mm/h, which represents an extreme rainfall for the Berlin area. The pulse of deionized water caused a significant decrease in EC, i.e., a reactive front of low ionic strength water migrating down through the sediment column (Fig. 4). After 1 day, the EC values were fully recovered, not only in the surface water but also at the outlet of the column.

3.2.3.1. Re-mobilization of attached MS2 phages. Although the number of total MS2 particles had dropped below or close to the detection limit in sediment pore water immediately before the simulated rainfall event, the pulse of deionized water caused a new moving front of MS2 phages re-mobilized from the sediment migrating through the sediment column. In contrast to experimental phase 1, the peak concentrations of total MS2 particles in pore water increased with depth, reaching concentrations as high as 10^5 gc mL^{-1} (Fig. 5).

The fraction of infectious MS2 phages followed a distribution pattern similar to that of the total MS2 phages (Fig. 5). The average percentage of infectious MS2 in pore water samples at different depths was in the range of 13%–40% and hence significantly higher than in the first experimental phase (where the range was 7.6%–21%). Notably, in experimental phase 2, 3.3×10^{10} MS2 particles in total were transported through the 80 cm sediment column after the application of deionized water, and a significant portion (on average 69%) were still infectious (Table 2). This suggested that attachment somehow conserves infectivity of phage particles better than transport in suspension.

Similar findings were obtained in the batch experiments (Fig. 2) and in earlier studies (Yates et al., 1987; Melnick et al., 1980; Schijven and Hassanizadeh, 2000). Gerba (1984) proposed that the mechanisms protecting viruses attached to sediment might include shielding from proteolytic enzymes or other substances that inactivate and degrade viruses. The attachment of virus particles to the soil and sediment surface is mediated by a combination of electrostatic and hydrophobic interactions (Gerba, 1984). A decrease in pore water salt concentration is known to increase the surface potential of the attached viruses owing to the expansion of the electrostatic double layer surrounding the virus particles and soil particle surfaces, causing virus detachment (Quanrud et al., 2003). A rapid shift to low ionic strength conditions has a strong desorption effect on phages and other particles from sediment surfaces (Yates et al., 1987; Gerba, 1999). Deionized water, as shown in this experiment, can cause a quantitative re-mobilization of previously immobilized virus and thus a second pulse of contamination (Guber et al., 2006; Landry et al., 1979). Although the reactive front of the low ionic strength water pulse initiated the desorption of part of the attached phages, the phage peak in the column outflow was broader. We assumed that some virus particles underwent re-attachment further down the sediment column, as observed by Wellings et al. (1975).

3.2.3.2. Phages remaining attached to sediment after simulated heavy rain. A significant number of total MS2 particles (around 10^{11} gc) remained attached to the sediment after both phases of the experiment (Table 2). After the artificial rain event, the abundance of sediment-attached total MS2 particles was moderately lower at the top of the sediment column than in experimental phase 1, down to a depth of 20 cm, but below that it remained constant and similar to the numbers found at the end of experimental phase 1 (Figs. 6 and 8). Indeed, the

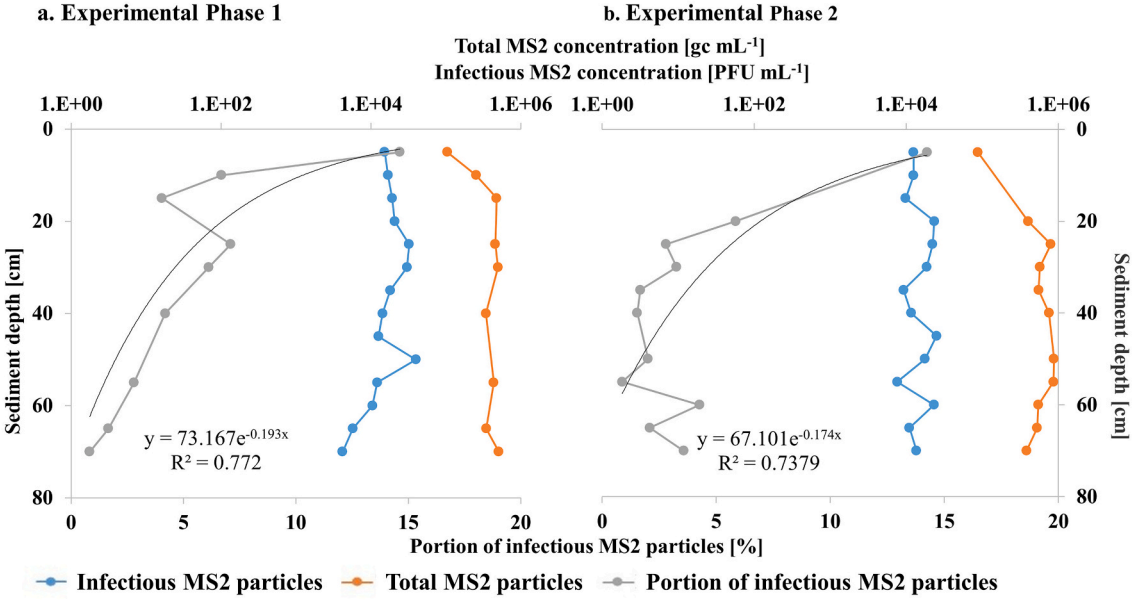

Fig. 6. Distribution of total and infectious sediment-attached MS2 particles with depth (a: sediment samples taken from core samples after 15 days, at the end of experimental phase 1; b: sediment samples taken from core samples 23 days after the application of deionized water, at the end of experimental phase 2; the grey solid line models the proportion of infectious MS2 collected from the sediment core against the sediment depth).

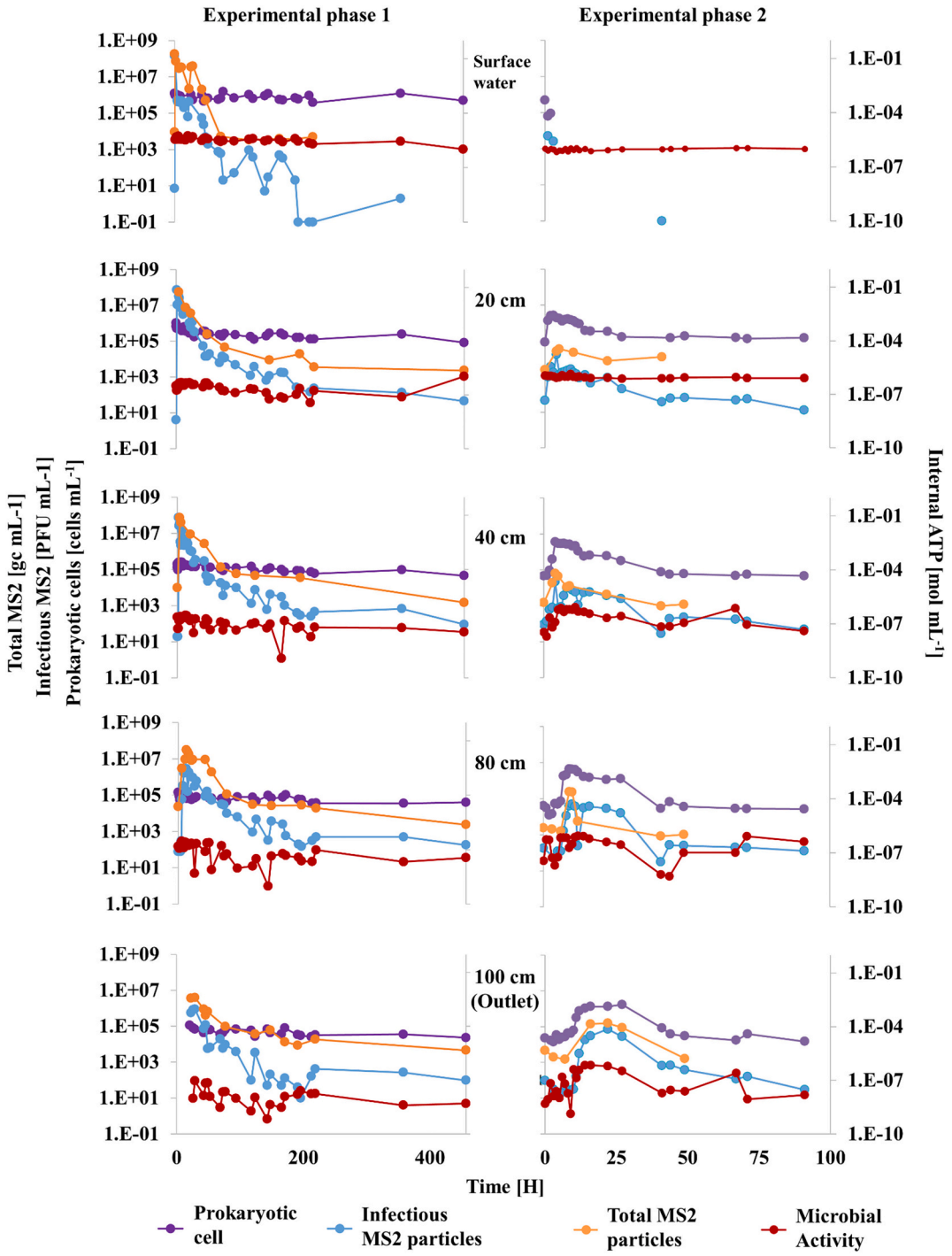

Fig. 7. Spatio-temporal distribution of bacteriophage MS2 (total and infectious), microbial activity, and prokaryotic cell concentration in sediment pore water of the column for both experimental phases (virus pulse and simulated rain event) and in various depths of pore water.

number of sediment-bound infectious MS2 phages after the simulated rainfall event was not significantly different from that in experimental phase 1 (*t-test, t = 0.80, df = 26, P = 0.43*). Infectious MS2 particles attached to the sediment matrix were distributed more evenly with depth than total phage particles, ranging from 7.6×10^3 to 2.5×10^4 PFU mL^{-1}. Modelling the proportion of infectious MS2 collected from the sediment core against the sediment depth showed that the decrease in the proportion of infectious MS2 phages with depth was less steep after the artificial rainfall than in experimental phase 1 (Fig. 6), indicating that infectious MS2 particles were preferentially re-mobilized.

3.2.3.3. Effects of rain on the distribution of bacteria and microbial activity. The artificial heavy rainfall not only mobilized sediment-attached MS2 particles but also caused prokaryotic cells to detach (Fig. 7). This result supported the findings of earlier studies, which reported that the contamination of groundwater wells was promoted by the detachment of bacteria from surrounding sediments in periods of heavy rainfall (Bition and Harvey, 1992; Zyman and Sorber, 1988). Interestingly, in our study, no apparent changes were observed in microbial activity down to a depth of 40 cm, indicating that cells with no or low activity were predominantly dislodged from the sediment following rainfall. However, at deeper zones of the sediment column, the microbial

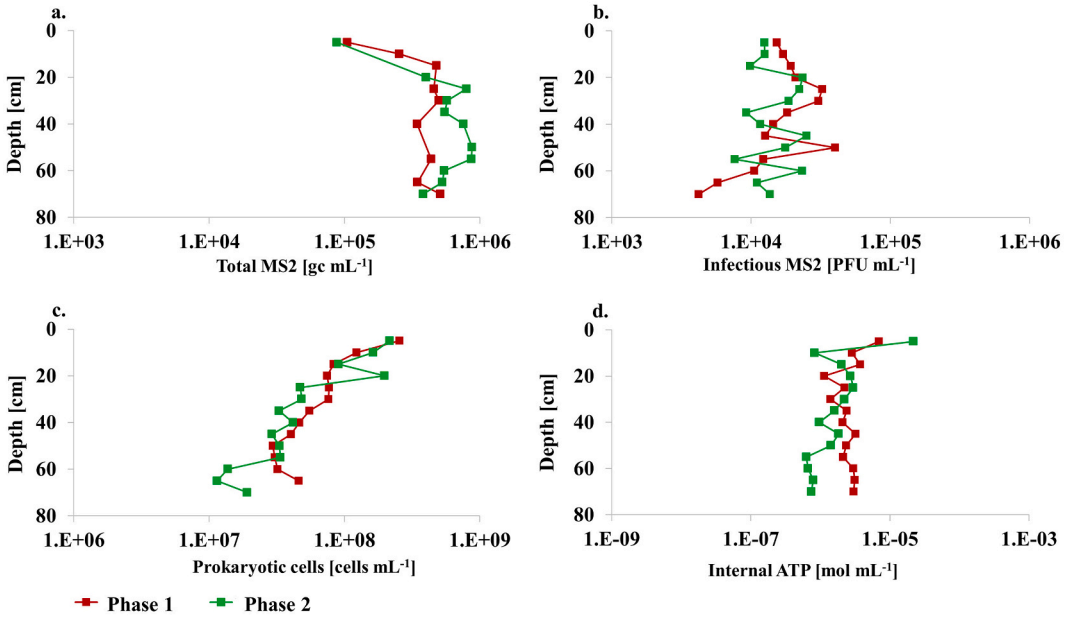

Fig. 8. Vertical distribution of sediment-attached bacteriophage MS2 (total: a and infectious: b), prokaryotic cell concentration (c), and microbial activity (d) in the enclosure at the end of experimental phase 1 (virus pulse) and phase 2 (simulated rain event).

activity appeared to follow the dynamics of the bacterial numbers (Fig. 7). The peak concentration of prokaryotic cells in the sediment pore water following the simulated rainfall was more than 10 times higher than the pore water concentrations detected in experimental phase 1. However, at 91 h after the simulated rainfall, lower concentrations of prokaryotic cells were detected in pore water compared with phase 1, but the overall pattern was similar: fewer prokaryotic cells were present further down the column.

As in experimental phase 1, the sediment-attached prokaryotic cell concentration was highest at the top of the column (5 cm sediment depth) and 10 times lower at a depth of 70 cm. After the rainfall, although the numbers of sediment-attached microorganisms were lower mainly in the top sediments, the overall abundance of bacteria that remained attached to the sediment was lower in experimental phase 2 than in experimental phase 1. Although a breakthrough of prokaryotic cells migrating through the sediment column was observed following the simulated rain event, the total number of sediment-attached cells did not change significantly (*Wilcoxon test*, $n = 28$, $P = 0.38$), as shown by the cell count, which was two orders of magnitude lower in pore water than attached to the sediment matrix. Similar patterns were found for microbial activity (Fig. 8). The simulated rainfall significantly increased the activity of the sediment-attached microbial consortia in the top sediment. Apart from this exception, most of the sediment-bound microbial activity was lower in phase 2, with a significant difference between phase 1 and phase 2 revealed (*Wilcoxon test*, $n = 28$, $P = 0.003$).

3.3. A comprehensive perspective on the main experiment

Although it may be assumed that the virus concentrations applied in the experiment rarely occur in natural environments, in face of the rather small sample volumes available and the LOD of the methods applied for the quantification of total and infectious MS2, the seeding of high phage numbers allowed the spatio-temporal resolution of several processes involved in the retardation and attenuation of virus in bank sediment infiltration, including attachment, detachment, and inactivation. Our study revealed that high peak concentration of viruses in recharge water can lead to exceedance of the retention capacity of bank sediments, presumably caused by the displacing interactions between attached MS2 and suspended MS2 in the water phase. In our main experiment, only a minor fraction of the supplied virus particles was retarded by attachment, and for the peak concentrations of total MS2 and infectious MS2 phage particles the reductions achieved amounted only to of 0.8 and 1.7 log units, respectively, for a sediment passage of 80 cm. In contrast, for the lower initial MS2 concentration in the supply water, the sediment removal efficiency was much higher, amounting to 3.0 $\log_{10}$. If we only consider infectious virus particles to be a health hazard, our results are more promising. We showed that with travel distance through the sediment and water residence time, the proportion of non-infectious virus particles steadily increased. After a sediment passage of 80 cm, 1.2 $\log_{10}$ of the total MS2 particles were inactivated and could no longer be detected by the plaque assay. Indeed, compared with MS2, other viruses, particularly many human pathogenic viruses, tend to be attached more efficiently, especially within the first centimeters of sediment passage (Schijven and Hassanizadeh, 2000). Thus, the breakthrough of viruses of health concern is likely to be lower than for the bacteriophage MS2.

The attachment of a virus particle does not imply that it will be inactivated and decay. Our data showed that re-mobilized virus particles were characterized by a higher proportion of infectious particles (0.3%–89.4%) than that found in the suspended virus fraction during the initial contamination, i.e., experimental phase 1 (Fig. 5). The inactivation of viruses during attachment is reported to be virus-specific (Frohnert et al., 2014). Yates et al. (1987) reviewed further factors that could affect the transport and inactivation of viruses during soil and sediment passage, including temperature, pore water pH, sediment organic matter content, autochthonous microbial activity, and ionic strength. While earlier studies commonly agree that viruses remain infective and intact for longer at low temperatures (Yates et al., 1987; Feng et al., 2003) and inactivation rates increase with an increase in temperature (John and Rose, 2005; Krauss and Griebler, 2011), during our experiment, temperature showed a positive correlation to both infectious MS2 and total MS2 phage concentrations in pore water (Table S4). However, this correlation is caused by a decrease of virus particles over time and a simultaneous seasonal decrease of ambient temperature in the sediment mesocosm. Moreover, water temperatures detected during our experiment remained $<10\,^{\circ}C$, a temperature range characterized by a very low inactivation rate of MS2 (Schijven and Hassanizadeh, 2000). EC was negatively correlated with infectious MS2 phage concentrations. This supports the view that compared with total MS2, the desorption of infectious MS2 is more sensitive to ionic strength. For the sediment

samples tested, prokaryotic cell abundance and microbial activity were significantly negatively correlated with the concentration of total MS2 particles rather than the concentration of infectious MS2 particles (Table S5). This indicates an antagonistic relationship between the autochthonous microbial communities and the allochthonous phages. Microbial mediated antagonistic processes that lead to a reduction of allochthonous viruses and microbes have been reported (Feichtmayer et al., 2017). A further possibility is competition between total viruses and autochthonous bacteria for attachment sites in the aquifer medium.

The simulated recharge of rainwater clearly showed that a significant proportion of the initially attached viruses was re-mobilized, resulting in a second pulse of virus-contaminated pore water moving through the bank sediment and thus demonstrating that extreme hydrological events indeed have the potential to cause a temporary impact on water quality. As the low EC of the simulated rainwater exceeded 130 cm of sediment passage (Fig. 4), the re-mobilization of attached viruses can be expected to have an impact over a distance of several meters, depending on the time and intensity of the rain event, water flow velocity, and pore-water ionic strength (Quanrud et al., 2003).

4. Conclusions

Attachment, inactivation, and decay are important processes in the attenuation of viruses, as exemplified with the bacteriophage MS2, during bank sediment passage. Even when challenged with an extreme virus concentration of 10^8 particles mL^{-1}, at the upper end of loads to be expected in rivers, bank filtration can be efficient in reducing a viral contamination by 1–2 log units after only a short transport distance of less than one meter. At lower concentrations of viruses infiltrating bank sediments and a lower water flow velocity (i.e., high water residence time), and/or higher temperatures, the natural attenuation efficiency is likely to be much higher, in the range of 3–4 log units. Equally important, only a small fraction of virus particles remained infectious after the sediment passage, indicating that virus inactivation was a quantitatively important process in bank filtration. Extreme hydrological events, such as heavy rainfall, will cause the re-mobilization of previously attached virus particles and autochthonous microorganisms from the sediment matrix. As such, rain and flood events may pose a hazard for drinking water production, and they require particular attention in risk assessment and management.

Author statement

He Wang: Sample and data analysis, visualization, writing of the MS. Judith Kaletta: Field work, sample analysis. Sigrid Kaschuba: Field work, sample analysis. Sondra Klitzke: Field work, sample analysis, writing of the MS. Ingrid Chorus: Writing of the MS. Christian Griebler: Conceptualization, supervision, writing of the MS.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jconhyd.2022.103960.

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