Changes of the Specific Infectivity of Tracer Phages during Transport in Porous Media

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Abstract

Phages (i.e. viruses infecting bacteria) are considered to be good indicators and tracers for fecal pollution, hydraulic flow or colloidal transport in the subsurface. They are typically quantified as total virus particles (VLP) or plaque forming units (PFU) of infectious phages. As transport may lead to phage deactivation, VLP quantification can overestimate the number of infectious phages. In contrast, PFU counts may underestimate the transport of total virus particles. Using PFU and tunable resistive pulse sensing-based counting for active and total phages, resp., we quantified the effect of transport through laboratory percolation columns on the specific infectivity (SI). The SI is defined by the ratio of total VLP to PFU and is a measure for the minimum particle numbers needed to create a single infection. Transport of three marine tracer phages and the coli-phage (T4) was described by colloidal filtration theory. We found that apparent collision efficiencies of active and total counts differed. Depending on the phage properties (e.g. morphology or hydrophobicity) passage through a porous medium led to either an increasing or decreasing SI of effluent phages. Our data hence suggest that both phage mass recovery and the SI should be considered in quantitative phage tracer experiments.

One sentence brief. Transport through a porous medium dynamically changes the specific infectivity of tracer phages as quantified by particle-to-PFU ratios.

KEYWORDS: Marine phage, particle-to-PFU ratio, specific infectivity, tracer, transport, qNano.

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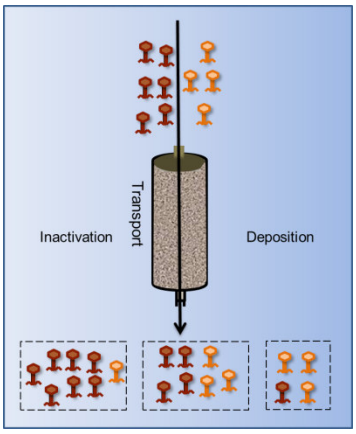

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INTRODUCTION

Bacteriophages (phages) are viruses that infect bacteria. They play an important role in ecological-,[1,2] and health-related environmental research.[3,4] Phages are considered to be good indicators for the transport of pathogenic viruses in surface and groundwater.[5,6] Although rarely applied marine phages hold promise as tracers for hydraulic flow and colloidal transport.[7,8,9] Marine phages are virtually absent in the terrestrial ecosystem, nonpathogenic and quantifiable with highest sensitivity. It is possible to apply as many as 10^{15} phages (~1 g) in tracer experiments and to detect <5 phages per mL of recovered water via specific interactions with host bacteria in active count enumeration.[10] Generally, phage transport is quantified by either total counting of virus-like particles (VLP) or by assessment of their active counts in a plaque forming unit (PFU) assay.[11,12]

Several studies have analyzed transport-induced virus removal due to deposition and inactivation.[13,14] Using PFU quantification such research assessed the effect of eluent properties (e.g. beef extract, sewage, rainwater and others)[15,16] on the release of attached viruses/phages from adsorbent surfaces.[3,17,18] However, only few studies so far have jointly analyzed total (e.g. using radioactive[17,19,20,21] or fluorescent-dyed[22] viruses) and active phages . None of them emphasized the specific infectivity (SI) as a reflection of the probability of suspended VLP to initiate an infection.[23] The SI can be calculated based on total counts and PFU,[23] and be defined as the minimum particle numbers needed to create a single infection. A value of 1 indicates that each virus particle is infective. The SI is thus a measure for the quality of the titer of viruses[24] and can be used to assess phage dynamics in natural viral lysates. In viral lysates noninfectious particles are often found as a

likely result of genome modification (i.e. mutation or damage), failures in the infection cycle[25] or inactivation.

The exclusive use of VLPs counts or quantitative molecular biological techniques (e.g. Bettarel et al,[26]) may lead to overestimations of the infectivity of virus suspensions. By contrast, as inactivated phages are not any longer detectable by PFU, results of PFU-based tracer tests (e.g. for hydraulic water flows or of the quantification of colloidal transport) may be distorted. Virus inactivation thereby is one of the most important factors controlling virus fate and transport in the subsurface. The impact of many environmental factors on virus inactivation has been tested. This included the ionic strength, variations of pH and temperature,[27,28] the clay mineral structures,[29,30] the presence of liquid-air-interface,[31] types of soil[32] and sand, or the nature and initial concentration of the phages used.[33] No study however has analyzed the effects of phage properties (i.e, morphology and hydrophobicity) and transport on SI and associated errors.

In the frame of the Collaborative Research Centre AquaDiva[34] (http://www.aquadiva.uni-jena.de/) we here utilized three marine and one coli tracer phage (T4) that belong to two different virus families (*Siphoviridae* and *Myoviridae*) and vary in their physicochemical surface properties. Active and total counts were quantified by PFU and tunable resistive pulse sensing (TRPS), respectively. The TRPS technology uses the coulter principle on the nanoscale and was chosen due to its relative sensitivity, since it measures particle-by-particle, the possibility to adjust the size range of the particles counted and its relatively low price. TRPS and PFU counts allowed us to investigate the effect of transport on the SI of phages in laboratory percolation columns while simultaneously describing phage deposition and transport by colloidal filtration theory approaches.

EXPERIMENTAL PROCEDURES

Material and Methods

Propagation of phages and their hosts: Three lytic marine phages and a common coli-phage used in our earlier work[9] were used (Table 1). Marine phages PSA-HS2 and PSA-HM1 and their hosts strains were kindly provided by Dr. B. M. Duhaime (University of Michigan, USA). Phage vB_PspS-H40/1[35] together with its host strain was obtained from Dr. J. Zopfi (University of Basel, Switzerland). The well-characterized T4 coliphage[36,37] and its host *E. coli* (Migula 1895) were purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ, Germany). All phages were propagated using the double agar-layer technique and purified as in a previous study.[9] Diluted 2216E medium (50 %) was used to grow marine bacterial strains at room temperature.[38]

Enumeration of phages: Active phage counts PFU (mL^{-1}) were obtained using a modified spotting plaque assay technique as detailed earlier.[23] The plates of the phage-host pairs were incubated overnight at room temperature (37°C for *E. coli*). Total phage counts (Phage particles mL^{-1}) were determined by tunable resistive pulse sensing (TRPS) using an Izon qNano Gold (Izon Science Europe Ltd., Magdalen Centre, The Oxford Science Park, Oxford, UK) particle counter and the Izon Control Suite Software V3.2. This instrument has been used before to quantify and characterize (e.g. particle size) viruses and nanoparticles.[39,40,41] For the measurements samples are passed through a nanopore (Izon Science) induced by varying trans-membrane voltage and pressure.[39] The samples were analyzed in 100 mM sterile phosphate buffer (PB; 0.87 g L^{-1} K_2HPO_4, 0.68 g L^{-1} KH_2PO_4; pH 7). The phage suspension (40 µL) was added to the upper chamber and subsequent the nanopore stretch and the ionic current were recorded. The numbers of phage particles were calculated and calibrated using 1.2×10^{13} particles mL^{-1} calibration particles (size of 110 nm) of allowing for a minimum of 500 particles per event. Izon Control Suite Software V3.2 was used to calculate the size of phage particles.

Column deposition experiments: Percolation experiments using 100 mM PB (ionic strength, $I \approx 0.12$ M) were conducted as presented in previous work.[9] Briefly, glass columns (diameter: 1 cm, length:

10 cm) confined at the bottom by a glass frit (pore size: 100 – 160 µm) were used. Columns were packed wet with a d50 median particle size of 0.31 mm commercial quartz sand (Euroquarz-group; porosity of ≈ 0.4 (estimated gravimetrically)). The sand gave no detectable background signal in TRPS-based counting of phages in the column effluents as verified in controls in the absence of phages. Then >8 pore volumes (PV) of sterilized 100 mM PB were allowed to pass to equilibrate the columns. In order to account for the sensitivity of the particle counter and to minimize the phage inactivation rate by applying low concentrations[33] phages suspension of 10^8-10^{10} PFU mL^{-1} (in 100 mM PB) were applied. About 8 PV of phage suspensions were pumped from the top to the bottom of the columns using a peristaltic pump at a hydraulic flow rate of $Q = 0.7 \times 10^{-4}$ m s^{-1} (19.8 mL h^{-1}). During the experiments the phage concentrations in the influent and effluent were quantified at regular intervals using both enumeration techniques. Data represent averages and standard deviations of triplicate analyses of samples obtained from either two (phage vB_PspS-H40/1 and T4 coliphage) or three (phages PSA-HM1 and PSA-HS2) independent column experiments.

Colloidal stability of phage suspensions: The colloidal stability of the phage suspensions (10^8-10^{10} PFU mL^{-1} in 5 mL PB (100 mM)) was determined in triplicate by quantifying active and total phage particles of unstirred suspensions at t = 0 h and t = 2 h. Colloidal stability experiments were performed in glass vials (30 mL) to best possibly avoid phage inactivation.[42] In order to assess the effect of sand on stability and infectivity of phage suspensions, identical experiments were performed in presence of 10 g of sand (described above).

Calculations

Collision efficiency : The collision efficiency α_t was calculated by the colloid filtration theory (eq. 1)[43] as the ratio of the experimental single-collector removal efficiency to the predicted single-collector contact efficiency.

(1)

α_t is the apparent relative affinity of a phage for the packing material and α_0 the hypothetical collision efficiency at t = 0. The collision efficiency of total phages (and the apparent collision efficiency of active phages (were calculated from experimental phage breakthrough curves.[44] C_t is the effluent phage concentration, C_0 the influent phage concentration, ε the porosity of the packed bed, a_s the radius of the sand particles (mean diameter 0.29 mm), L the length of the column, and η_{trans} the predicted single-collector contact efficiency.[44]

Mass recovery (***M***): The mass recovery of active (M_a, eq. 2) and total (M_{tot}, eq. 3) phage was determined by the %-ratio of total phages in the effluent and the influent as quantified either by PFU or total VLP enumeration (eqs. 2 & 3) with $C_{t,a,effluent}$, $C_{t,a,influent}$ and $C_{t,tot,effluent}$, $C_{t,tot,influent}$ being the effluent and influent phage concentrations assessed by PFU and total VLP enumeration, resp..

(2)

(3)

Specific Infectivity (SI): The specific infectivity of the phages at a given time t () was calculated as the particle-to-PFU ratio,[9] i.e. by the ratio of the total phage $C_{t,tot}$ (VLP mL^{-1}) and the active (i.e. infectious) phage concentrations $C_{t,a}$ (PFU mL^{-1}) in a sample (eq. 4).

(4)

The relative change of the SI () was determined (Fig. 2) as the ratio of the SI in the effluent () and the influent () at a given time *t* (eq.5).

(5)

The time averaged SI of the inflow () and effluent () were calculated by eqs. 6 & 7 with Q being the hydraulic flow rate (mL h^{-1}) in the column.

(6)

(7)

RESULTS

Phage Transport in Saturated Percolation Columns. The transport experiments were conducted using sand-packed columns under continuous, saturated flow conditions. Three marine phages (PSA-HM1, vB_PspSH40/1, PSA-HS2) and one coli phage (T4) of differing morphology, size and physicochemical surface properties were used (Table 1). All phages exhibited a high colloidal stability (96-112%) during the observation period (2 h) (Table 1).

Infectious and total phage particles in effluent samples were quantified by PFU and TRPS counts at given time points of the breakthrough curves. Additionally, TRPS allowed us to assess the average size of the VLP in measured samples. Inspection of breakthrough curves revealed differences between the mass recoveries of total vs. active phage particles of three of the phages (Figs. 1 & S1). Only phage vB_PspSH40/1 showed a similar mass recovery of active and total phages ($M_{a\text{-}p} \approx M_{tot\text{-}p} \approx$ 30%; cf. Table 1 and eq. 2&3). Mass recoveries of the infectious forms ($M_{a\text{-}p}$ = 6-50%) of the other phages were clearly reduced relative to $M_{tot\text{-}p}$ = 47-107% of the total phages. The phages T4 ($M_{tot\text{-}p}$ = 63%) and PSA-HM1 ($M_{tot\text{-}p}$ = 107%) reached high transport efficiencies whereas phages vB_PspSH40/1 ($M_{a\text{-}p}$ = 30%) and PSA-HS2 ($M_{tot\text{-}p}$ = 56%) were generally more retained despite of efficient transport of phagePSA-HS2 ($C/C_0 \approx 1$) during initial breakthrough (Fig. S1).

The differences of the breakthrough of active and total phage particles were also reflected by the apparent collision efficiencies (Table 1) of active (and total phages (for both the initial collision efficiency (and once quasi steady state was reached (. For phages T4 and PSA-HM1 the was three- to fivefold increased relative to , while of phages vB_PspSH40/1 and PSA-HS2 the was and about threefold smaller than , respectively. TRPS measurements revealed a decreased particle sizes of both PSA-HM1 and T4 phages eluting at later pore volumes (Fig. S3).

Changes of the Specific Infectivity. The SI was calculated as the particle-to-PFU ratio in samples of the phage suspensions (eq. 4). The SI of the influent ($SI_{influent}$) depended on the phage type (Table 1) and varied from 1.7 (most infectious, PSA-HM1) via 4.2 (PSA_HS2) and 5.3 (T4) to 10 (least infectious; vB-PspSH40/1). This means that about 60, 24, 19, and 10% of the total VLP of the influents of PSA-HM1, PSA-HS2, T4 and vB-PspSH40/1 were infectious (Fig. S2).No changes of the colloidal stability and the SI of the phages were observed in static batch experiments in the presence of sand.(Table 1, Fig. S4). Transport in the sand-filled columns however changed the SI of the phages as was best observed when quasi steady state effluent concentrations of the breakthrough curves were reached (Fig. 1): While the $SI_{effluent}$ of phage vB-PspSH40/1 remained unchanged, the $SI_{effluent}$ of the phages PSA-HM1 and T4 increased 2.5- and 9-fold (i.e. 2.5 -9 fold more viruses were needed to initiate a single PFU in the effluent as compared to the influent).

By contrast, in the effluent of phage PSA-HS2 only 2.9 phages were needed to initiate the infection (cf. Tab.1) as compares to 4.2 in the influent. This points either at a similar transport-induced decrease of total and active phages (vB-PspSH40/1) or a selective enrichment of active (PSA-HS2) or inactivated (PSA-HM1, T4) phages (Fig. S1) as also reflected by the mass recovery of total and active phages (Table 1). Figure 2 shows that the relative changes of the SI () may vary significantly during a transport experiment. Interestingly, ≈1 at the front of the breakthrough were observed likely pointing at straining effects[45] and poor deactivation of phages eluting at the front of the breakthrough.

DISCUSSION

In this study we analyzed the effect of phage properties and transport on the specific infectivity of phages. Thereby, we evaluated the usefulness of PFU-based phage enumeration approach as a proxy for the quantification of total phage particle transported. . For this purpose, we studied the commonly described coli-phage T4 and three marine phages that had been reported to be a good

tracers for hydrological flow and colloidal transport.[46,47,9]. To our knowledge this is the first study that simultaneously enumerates active and total phages during transport through porous media. We found that passage through a porous medium changed the ratios between active and total phage particle to extents that depended on the individual phages (Fig. 1). This was reflected by distinct apparent collision efficiencies of active and total phages (Table 1). Observed phage mass recovery based on PFU counts was in agreement with previous studies[37,9] and revealed lower mass recovery of phage T4 than PSA-HM1 and PSA-HS2. Using total counts, by contrast, we found high mass recoveries of colloidal phage particles after passage through a porous medium. The SI depended on the phages tested and always was >1 (cf. Table 1) and, hence, higher than for many of the phages reported.[25] Phage-specific differences of the SI are in accordance with earlier studies, showing that the variations depended on virus type and quantification method.[48, 49,23]

Effect of transport on the Specific Infectivity. No effects of the suspension buffer on the colloidal stability and the SI of the phages were observed (Fig. S5). The relative changes of the SI of phages during transport hence seemed to be driven by both phage deposition and inactivation (Fig. S2). Known reasons for phage inactivation are the break off of the phage tail or a damage of the capsids of phages.[50,51,52] This may either lead to changes of the phage particle size, to alterations of the physico-chemical surface properties and, as a consequence, to differing deposition and transport properties of active and inactive phages. Smaller phages are generally better transported than bigger phages,[53,9] while more hydrophobic phages are thought to exhibit a higher (i.e. the phages are more retained during transport) than more hydrophilic phages.[54,55] If > consistently lower PFU than total phage counts were detected in the effluent (cf. phages T4 and PSA-HM1 in Fig. 2). This either proposes that the retention of active phages was higher than the retention of total phage particles and/or that transport led to significant deactivation of the phages. PFU-based quantification will in such cases will underestimate the transport of total phage particles. In case of ≈ (cf. phage vB_PspS-H40/1) the inactivation and deposition rates of phage particles are similar and particle transport is

suitably reflected by PFU enumeration. Finally, if ,< selective enrichment of PFU in the effluent is seen (cf. phage PSA-HS2) and PFU-based tracer experiments can overestimate transport of total phage particles.

Effect of phage properties on the Specific Infectivity. Several mechanisms control the SI of phages during transport in porous media. IIn case of *Myoviridae* phages (i.e. phages T4 or PSA-HM1) inactivation may be the main driver for our observations. Their (nonflexible) contractile tail make *Myoviridae* phages more sensitive to inactivation than Siphoviridae (i.e. phages PSA-HS2 or vB_PspS-H40/1) with flexible tails. This was reflected by the decrease in size of *Myoviridae* phages tested whereas no change was recorded for *Siphoviridae* phages (Fig. S3). This is also in accordance with observations that phages of the *Siphoviridae* family are generally highly resistant to adverse conditions.[56,52] The physical stress resulting from phage transport in conjunction with strong sand-phage interactions (as was confirmed by the extended Derjaguin−Landau−Verwey−Overbeek (XDLVO) calculations in a previous study)[9] may break off the nonflexible tails and lead to phage inactivation.[50] The higher transport-induced inactivation of phage T4 than of phage PSA-HM1 could be attributed to its higher hydrophobic affinity to sand than of PSA-HM1. Inactivation is further supported by the size of the particles recorded by the TRPS revealing clearly decreasing sizes for both *Myoviridae* yet not for the *Siphoviridae* (Fig. S3). Additional support for deactivation of phages by transport-induced processes comes from sand batch experiments under static conditions that showed no significant change of the SI of all phages tested (Fig. S4 and Table S1). This finding is in agreement with earlier studies that illustrated the role of solid matrices in preventing viruses from inactivation.[57,33,19] The decreasing relative (eq. 5, Fig. 2) of phage PSA-HS2, however, may only be explained by preferential attachment of inactivated phages during transport (cf. Fig. S2).

Implications for phage transport studies. Due to the limited sensitivity of the TRPS method and its inability to discriminate phages from other particles of similar size, PFU enumeration approaches

may remain the method of choice in field tracer phage experiments. PFU enumeration thereby appeals through its high specificity of phage-host interactions and its extremely high sensitivity of one or two phages mL^{-1} of recovered water.[46] Our approach however allows for easy comparison and calibration of tracer phages for their SI and transport properties and, hence, for better selection and tailored use of phages as tracers at given environmental conditions. At present, our data do not allow for the quantitative discrimination of inactivation and deposition rates of active and inactivated phages. They though show that transport through porous media changes the SI of certain phages and leads either to a relative enrichment or depletion of infectious phages. Hence, phage properties and SI should be considered in quantitative tracer experiments. Transport effects on the SI may also be relevant in health-related phage indicator and tracer studies or during transport of pathogenic viruses: active viruses may get selectively enriched during transport in porous media, resulting in decreased specific infectivity and increased risk to potential receptors. Finally, transport-induced selective enrichment of infectious viruses may further be used to concentrate titers or to produce therapeutic viruses at high concentrations.

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SUPPORTING INFORMATION AVAILABLE

Supporting Information is available and contains four figures and one table.

Figure Captions

Figure 1: Breakthrough curves of total (particles mL^{-1}; empty circles) and active (PFU mL^{-1}; filled circles) counts of three marine tracer phages (PSA-HM1, vB_PspS-H40/1, PSA-HS2) and a commonly used coli model phage (T4) of sand filled percolation columns. Dashed and long-short dashed lines reflect total and active phages in the inflow. Please note that the scales of the y-axes differ.

Figure 2: Time-dependent relative change of the specific infectivity (cf. eqs. 4&5) of three marine tracer phages and a commonly used non-marine model phage during transport in a sand filled column. Increasing $SI_{rel,t}$ refers to a transport-induced phage deactivation while decreasing $SI_{rel,t}$ point at a relative enrichment of active phages.

Table 1. Overview of selected phage characteristics, specific infectivity (eq. 4) of the influent ($SI_{influent}$, eq.6), specific infectivity of the effluent of a complete transport experiment ($SI_{effluent}$, eq. 6) and at quasi steady state effluent concentrations ($SI_{effluent\text{-}p}$) , the apparent collision efficiencies (eq. 1) of total (α_{tot}) and active (α_a) phages, the apparent mass recovery (eq. 2&) of active (M_a, $M_{a\text{-}p}$) and total (M_{tot}, $M_{tot\ p}$) phages of a complete transport experiment and at quasi plateau as well as the colloidal stability of phages in suspension. The last column refers to the calculated apparent relative loss due to adhesion.

Phage name (Family name)	**Host name**	**Zeta potential** ζ [a] (mV)	**Water contact angle** Θ_w [a] (degree)	**Size** [a] (Head/tail) (nm)	**Specific infectivity of influent** $SI_{influent}$ (VLP/PFU)	**Specific infectivity of effluent** [a] $SI_{effluent\text{-}p}$ [b] ($SI_{effluent}$) (VLP/PFU)	**Apparent collision efficiency of active phages** $\alpha_{a\text{-}p}$ [c] ($\alpha_{0,a}$) (×10^{-2})	**Apparent collision efficiency of total phages** $\alpha_{p,tot}$ [c] ($\alpha_{0,tot}$) (×10^{-2})	**Mass recovery of active phages** $M_{a\text{-}p}$ [d] (M_a) (%)	**Mass recovery of total phages** $M_{tot\text{-}p}$ [d] (M_{tot}) (%)	**Colloidal stability of active phages** [d] (total phages) [e] (%)
PSA-HM1 (*Myoviridae*)	*Pseudo-alteromonas H71*	- 18 ± 1	40 ± 5	173 (60/113)	1.7	4.2 (3.7)	16 ± 4 (12)	5 ± 2 (4)	50 (37)	107 (80)	96 ± 7 (106 ± 11)
T4 (*Myoviridae*)	*E. coli (Migula 1895)*	-13 ± 1	95 ± 5	203 (90/113)	5.3	50 (50)	64 ± 5 (67)	11 ± 9 (18)	6 (7)	63 (63)	101 ± 0 (107 ± 5)
vB_PspSH40/1 (*Siphoviridae*)	*Pseudo-alteromonas H40*	-11 ± 3	53 ± 3	111 (42/69)	10	11 (9.1)	15 ± 4 (19)	19 ± 7 (27)	30 (34)	30 (28)	96 ± 17 (99 ± 6)
PSA-HS2 (*Siphoviridae*)	*Pseudo-alteromonas H13-15*	-13 ± 4	40 ± 5	210 (60/150)	4.2	2.9 (2.9)	4 ± 4 (4)	16 ± 7 (23)	89 (75)	56 (47)	112 ± 8 (109 ± 14)

[a] Data taken from [9]; [b] SI (eq. 6 & 7) in the overall effluent ($SI_{effluent}$) and at quasi steady state effluent concentrations of active and total and phages ($SI_{effluent\text{-}p}$). [c] Average and standard deviations (n≥8) of the apparent initial collision efficiency of active ($a_{0,a}$) and total ($a_{0,tot}$) phages: apparent collision efficiency at the quasi steady state effluent concentrations of active ($a_{a\text{-}p}$) and total and phages ($a_{tot\text{-}p}$) (cf. eq. 1). [d] Average and standard deviations (n≥8) of the mass recovery of active and total phages (cf. eq. 2 & 3) of the total breakthrough curves (M_a, M_{tot}) and at quasi steady state effluent concentrations ($M_{a\text{-}p.}$, $M_{tot\text{-}p.}$). [e] Colloidal stability determined in batch experiment after 2 h.

References

(1) Williamson, K. E.; Harris, J. V.; Green, J. C.; Rahman, F.; Chambers, R. M. Stormwater runoff drives viral community composition changes in inland freshwaters. *Front. Microbiol.* **2014**, *5*, 105.

(2) Rehmann, L. L. C.; Welty, C.; Harvey, R. W. Stochastic analysis of virus transport in aquifers. *Water Resour. Res.* **1999**, *35* (7), 1987–2006.

(3) Morales, I.; Atoyan, J.; Amador, J.; Boving, T. Transport of Pathogen Surrogates in Soil Treatment Units: Numerical Modeling. *Water* **2014**, *6* (4), 818–838.

(4) Masciopinto, C.; La Mantia, R.; Chrysikopoulos, C. V. Fate and transport of pathogens in a fractured aquifer in the Salento area, Italy. *Water Resour. Res.* **2008**, *44* (1), W01404.

(5) Redman, J. A.; Grant, S. B.; Olson, T. M.; Hardy, M. E.; Estes, M. K. Filtration of recombinant Norwalk virus particles and bacteriophage MS2 in quartz sand: Importance of electrostatic interactions. *Environ. Sci. Technol.* **1997**, *31* (12), 3378–3383.

(6) Keswick, B. H.; Wang, D.-S.; Gerba, C. P. The Use of Microorganisms as Ground-Water Tracers: A Review. *Ground Water* **1982**, *20* (2), 142–149.

(7) Flynn, R. M.; Schnegg, P.-A.; Costa, R.; Mallen, G.; Zwahlen, F. Identification of zones of preferential groundwater tracer transport using a mobile downhole fluorometer. *Hydrogeol. J.* **2005**, *13* (2), 366–377.

(8) Flynn, R. M.; Mallèn, G.; Engel, M.; Ahmed, A.; Rossi, P. Characterizing Aquifer Heterogeneity Using Bacterial and Bacteriophage Tracers. *J. Environ. Qual.* **2015**, *44* (5), 1448.

(9) Ghanem, N.; Kiesel, B.; Kallies, R.; Harms, H.; Chatzinotas, A.; Wick, L. Y. Marine Phages As Tracers: Effects of Size, Morphology, and Physico–Chemical Surface Properties on Transport in a Porous Medium. *Environ. Sci. Technol.* **2016**, *50* (23), 12816–12824.

(10) Rossi, P. Advances in biological tracer techniques for hydrology and hydrogeology using bacteriophages, Université de Neuchâtel, 1994.

(11) Wielen, P. W. J. J. van der; Senden, W. J. M. K.; Medema, G. Removal of Bacteriophages MS2 and ΦX174 during Transport in a Sandy Anoxic Aquifer. *Environ. Sci. Technol.* **2008**, *42* (12), 4589–4594.

(12) Nasser, A. M.; Oman, S. D. Quantitative assessment of the inactivation of pathogenic and indicator viruses in natural water sources. *Water Res.* **1999**, *33* (7), 1748–1752.

(13) Gerba, C. P.; Lance, J. C. Poliovirus removal from primary and secondary sewage effluent by soil filtration. *Appl. Environ. Microbiol.* **1978**, *36* (2), 247–251.

(14) O'Luanaigh, N. D.; Gill, L. W.; Misstear, B. D. R.; Johnston, P. M. The attenuation of microorganisms in on-site wastewater effluent discharged into highly permeable subsoils. *J. Contam. Hydrol.* **2012**, *142–143*, 126–139.

(15) Landry, E. F.; Vaughn, J. M.; Thomas, M. Z.; Beckwith, C. A. Adsorption of Enteroviruses to Soil Cores and Their Subsequent Elution by Artificial Rainwater. *Appl. Environ. Microbiol.* **1979**, *38* (4), 680–687.

(16) Hurst, C. J.; Gerba, C. P. Development of a quantitative method for the detection of enteroviruses in soil. *Appl. Environ. Microbiol.* **1979**, *37* (3), 626–632.

(17) McConnell, L. K.; Sims, R. C.; Barnett, B. B. Reovirus removal and inactivation by slow-rate sand filtration. *Appl. Environ. Microbiol.* **1984**, *48* (4), 818–825.

(18) LaBelle, R. L.; Gerba, C. P. Influence of pH, salinity, and organic matter on the adsorption of enteric viruses to estuarine sediment. *Appl. Environ. Microbiol.* **1979**, *38* (1), 93–101.

(19) Moore, R. S.; Taylor, D. H.; Sturman, L. S.; Reddy, M. M.; Fuhs, G. W. Poliovirus Adsorption by 34 Minerals and Soils. *Appl. Environ. Microbiol.* **1981**, *42* (6), 963–975.

(20) Murray, J. P.; Laband, S. J. Degradation of poliovirus by adsorption on inorganic surfaces. *Appl. Environ. Microbiol.* **1979**, *37* (3), 480–486.
(21) Ryan, J. N.; Harvey, R. W.; Metge, D.; Elimelech, M.; Navigato, T.; Pieper, A. P. Field and Laboratory Investigations of Inactivation of Viruses (PRD1 and MS2) Attached to Iron Oxide-Coated Quartz Sand. *Environ. Sci. Technol.* **2002**, *36* (11), 2403–2413.
(22) Asraf-Snir, M.; Gitis, V. Tracer studies with fluorescent-dyed microorganisms—A new method for determination of residence time in chlorination reactors. *Chem. Eng. J.* **2011**, *166* (2), 579–585.
(23) Alfson, K. J.; Avena, L. E.; Beadles, M. W.; Staples, H.; Nunneley, J. W.; Ticer, A.; Dick, E. J.; Owston, M. A.; Reed, C.; Patterson, J. L.; et al. Particle-to-PFU Ratio of Ebola Virus Influences Disease Course and Survival in Cynomolgus Macaques. *J. Virol.* **2015**, *89* (13), 6773–6781.
(24) Carpenter, J. E.; Henderson, E. P.; Grose, C. Enumeration of an Extremely High Particle-to-PFU Ratio for Varicella-Zoster Virus. *J. Virol.* **2009**, *83* (13), 6917–6921.
(25) Flint, S. J.; Enquist, L. W.; Racaniello, V. R.; Skalka, and A. M. *Principles of Virology, 3rd Edition, Volume I: Molecular Biology*; ASM Press, 2009.
(26) Bettarel, Y.; Sime-Ngando, T.; Amblard, C.; Laveran, H. A Comparison of Methods for Counting Viruses in Aquatic Systems. *Appl. Environ. Microbiol.* **2000**, *66* (6), 2283–2289.
(27) Rossi, P.; Aragno, M. Analysis of bacteriophage inactivation and its attenuation by adsorption onto colloidal particles by batch agitation techniques. *Can. J. Microbiol.* **1999**, *45* (1), 9–17.
(28) Feng, Y. Y.; Ong, S. L.; Hu, J. Y.; Tan, X. L.; Ng, W. J. Effects of pH and temperature on the survival of coliphages MS2 and Q? *J. Ind. Microbiol. Biotechnol.* **2003**, *30* (9), 549–552.
(29) Carlson, G. F.; Woodard, F. E.; Wentworth, D. F.; Sproul, O. J. Virus Inactivation on Clay Particles in Natural Waters. *J. Water Pollut. Control Fed.* **1968**, *40* (2), R89–R106.
(30) Babich, H.; Stotzky, G. Reductions in inactivation rates of bacteriophages by clay minerals in lake water. *Water Res.* **1980**, *14* (2), 185–187.
(31) Trouwborst, T.; Kuyper, S.; De Jong, J. C.; Plantinga, A. D. Inactivation of some bacterial and animal viruses by exposure to liquid-air interfaces. *J. Gen. Virol.* **1974**, *24* (1), 155–165.
(32) Yeager, J. G.; O'Brien, R. T. Enterovirus inactivation in soil. *Appl. Environ. Microbiol.* **1979**, *38* (4), 694–701.
(33) Chrysikopoulos, C. V.; Aravantinou, A. F. Virus inactivation in the presence of quartz sand under static and dynamic batch conditions at different temperatures. *J. Hazard. Mater.* **2012**, *233–234*, 148–157.
(34) Küsel, K.; Totsche, K. U.; Trumbore, S. E.; Lehmann, R.; Steinhäuser, C.; Herrmann, M. How Deep Can Surface Signals Be Traced in the Critical Zone? Merging Biodiversity with Biogeochemistry Research in a Central German Muschelkalk Landscape. *Front. Earth Sci.* **2016**, *4*, 32.
(35) Kallies, R.; Kiesel, B.; Schmidt, M.; Kacza, J.; Ghanem, N.; Narr, A.; Zopfi, J.; Hackermüller, J.; Harms, H.; Wick, L. Y.; et al. Complete genome sequence of Pseudoalteromonas phage vB_PS-H40/1 (formerly H40/1) that infects Pseudoalteromonas sp. strain H40 and is used as biological tracer in hydrological transport studies.
(36) Hijnen, W. A. M.; Brouwer-Hanzens, A. J.; Charles, K. J.; Medema, G. J. Transport of MS2 Phage, *Escherichia coli, Clostridium perfringens* , *Cryptosporidium parvum,* and *Giardia intestinalis* in a Gravel and a Sandy Soil. *Environ. Sci. Technol.* **2005**, *39* (20), 7860–7868.
(37) Aronino, R.; Dlugy, C.; Arkhangelsky, E.; Shandalov, S.; Oron, G.; Brenner, A.; Gitis, V. Removal of viruses from surface water and secondary effluents by sand filtration. *Water Res.* **2009**, *43* (1), 87–96.
(38) Oppenheimer, C.; Zobell, C. The Growth and Viability of 63 Species of Marine Bacteria as Influenced. *J. Mar. Res.* **1952**, *11* (1), 10–18.
(39) Garza-Licudine, E.; Deo, D.; Yu, S.; Uz-Zaman, A.; Dunbar, W. B. Portable nanoparticle quantization using a resizable nanopore instrument-The IZON qNano™. In *Engineering in*

Medicine and Biology Society (EMBC), 2010 Annual International Conference of the IEEE; IEEE, 2010; pp 5736–5739.

(40) Gutiérrez-Granados, S.; Cervera, L.; Segura, M. de las M.; Wölfel, J.; Gòdia, F. Optimized production of HIV-1 virus-like particles by transient transfection in CAP-T cells. *Appl. Microbiol. Biotechnol.* **2016**, *100* (9), 3935–3947.

(41) Arjmandi, N.; Van Roy, W.; Lagae, L. Measuring Mass of Nanoparticles and Viruses in Liquids with Nanometer-Scale Pores. *Anal. Chem.* **2014**, *86* (10), 4637–4641.

(42) Thompson, S. S.; Flury, M.; Yates, M. V.; Jury, W. A. Role of the air-water-solid interface in bacteriophage sorption experiments. *Appl. Environ. Microbiol.* **1998**, *64* (1), 304–309.

(43) Syngouna, V. I.; Chrysikopoulos, C. V. Transport of biocolloids in water saturated columns packed with sand: Effect of grain size and pore water velocity. *J. Contam. Hydrol.* **2011**, *126* (3–4), 301–314.

(44) Tufenkji, N.; Elimelech, M. Correlation Equation for Predicting Single-Collector Efficiency in Physicochemical Filtration in Saturated Porous Media. *Environ. Sci. Technol.* **2004**, *38* (2), 529–536.

(45) Sasidharan, S.; Torkzaban, S.; Bradford, S. A.; Kookana, R.; Page, D.; Cook, P. G. Transport and retention of bacteria and viruses in biochar-amended sand. *Sci. Total Environ.* **2016**, *548–549*, 100–109.

(46) Rossi, P.; Dorfliger, N.; Kennedy, K.; Muller, I.; Aragno, M. Bacteriophages as surface and ground water tracers. *Hydrol. Earth Syst. Sci.* **1998**, *2* (1), 101–110.

(47) Flynn, R.; Hunkeler, D.; Guerin, C.; Burn, C.; Rossi, P.; Aragno, M. Geochemical influences on H40/1 bacteriophage inactivation in glaciofluvial sands. *Environ. Geol.* **2004**, *45* (4), 504–517.

(48) Frey, T. K. Molecular Biology of Rubella Virus. In *Advances in Virus Research*; Karl Maramorosch, F. A. M. and A. J. S., Ed.; Academic Press, 1994; Vol. 44, pp 69–160.

(49) Coffey, L. L.; Vignuzzi, M. Host Alternation of Chikungunya Virus Increases Fitness while Restricting Population Diversity and Adaptability to Novel Selective Pressures. *J. Virol.* **2011**, *85* (2), 1025–1035.

(50) Harvey, R. W.; Ryan, J. N. Use of PRD1 bacteriophage in groundwater viral transport, inactivation, and attachment studies. *FEMS Microbiol. Ecol.* **2004**, *49* (1), 3–16.

(51) Yeager, J. G.; O'Brien, R. T. Structural changes associated with poliovirus inactivation in soil. *Appl. Environ. Microbiol.* **1979**, *38* (4), 702–709.

(52) Jończyk, E.; Kłak, M.; Międzybrodzki, R.; Górski, A. The influence of external factors on bacteriophages—review. *Folia Microbiol. (Praha)* **2011**, *56* (3), 191–200.

(53) Dowd, S. E.; Pillai, S. D.; Wang, S.; Corapcioglu, M. Y. Delineating the specific influence of virus isoelectric point and size on virus adsorption and transport through sandy soils. *Appl. Environ. Microbiol.* **1998**, *64* (2), 405–410.

(54) Schijven, J. F.; Hassanizadeh, S. M. Removal of Viruses by Soil Passage: Overview of Modeling, Processes, and Parameters. *Crit. Rev. Environ. Sci. Technol.* **2000**, *30* (1), 49–127.

(55) Attinti, R.; Wei, J.; Kniel, K.; Sims, J. T.; Jin, Y. Virus' (MS2, φX174, and Aichi) Attachment on Sand Measured by Atomic Force Microscopy and Their Transport through Sand Columns. *Environ. Sci. Technol.* **2010**, *44* (7), 2426–2432.

(56) Lasobras, J.; Muniesa, M.; Frías, J.; Lucena, F.; Jofre, J. Relationship between the morphology of bacteriophages and their persistence in the environment. *Water Sci. Technol.* **1997**, *35* (11), 129–132.

(57) Pecson, B. M.; Decrey, L.; Kohn, T. Photoinactivation of virus on iron-oxide coated sand: Enhancing inactivation in sunlit waters. *Water Res.* **2012**, *46* (6), 1763–1770.

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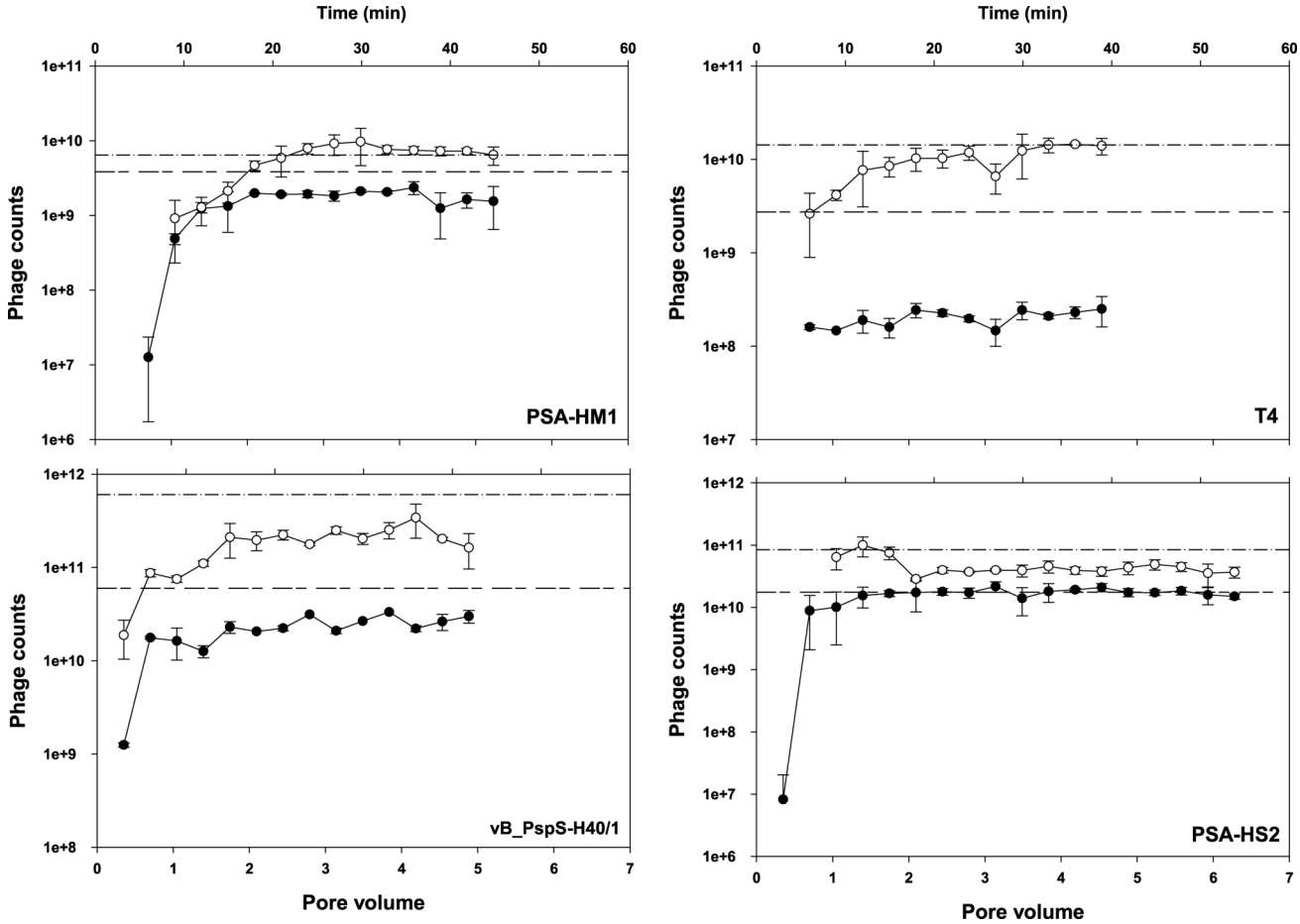

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454 **Figure 1**

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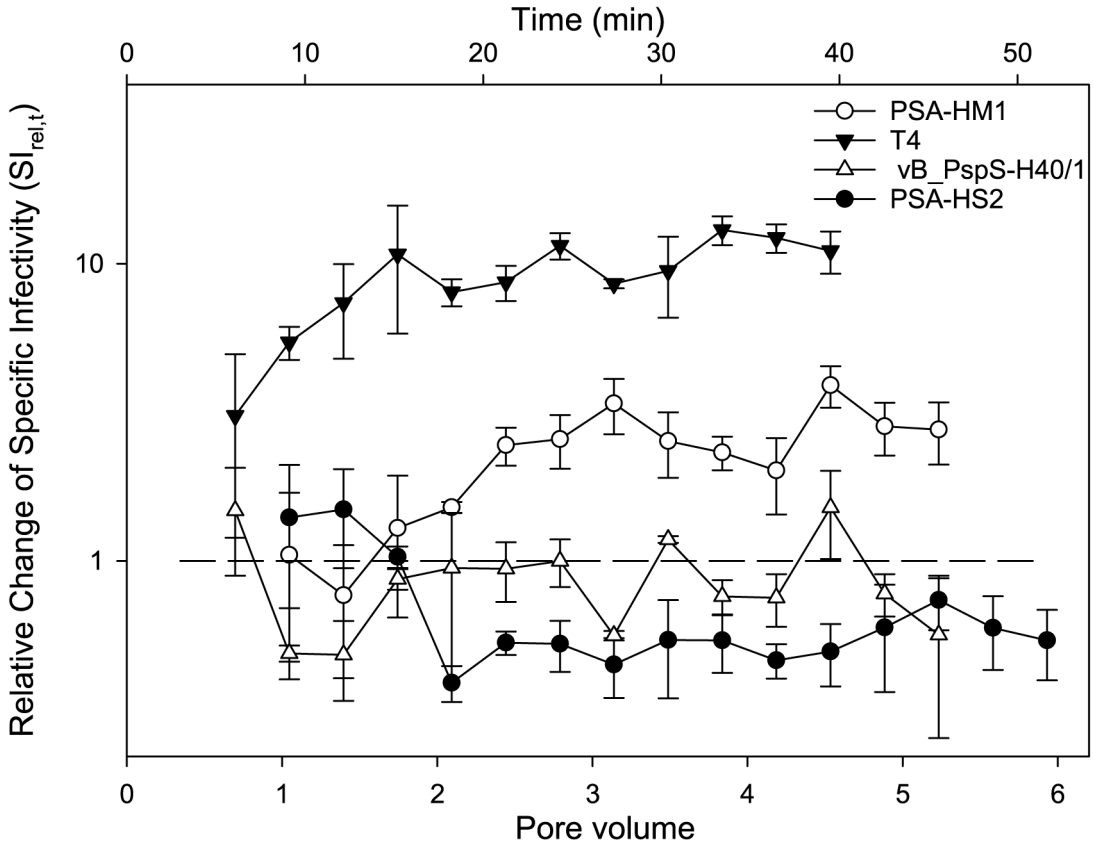

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458 **Figure 2**

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