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Column experiments to study nonlinear removal of bacteriophages by passage through saturated dune sand

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Abstract

In a recent field study on dune recharge, bacteriophages MS2 and PRD1 were found to be removed $3 \log_{10}$ over the first 2.4 m and only $5 \log_{10}$ over the next 27 m. To understand the causes of this nonlinear removal, column experiments were carried out under conditions similar to the field: same recharge water, temperature (5 ± 3 °C) and pore water velocity (1.5 m day^{-1}). Soil samples were taken along a streamline between the recharge canal and the first monitoring well. Bacteriophage ϕ X174 was included for comparison. The high initial removal in the field was found not to be due to heterogeneity of phage suspensions but to soil heterogeneity. Phage removal rates correlated strongly positively with soil organic carbon content, and relatively strongly positively with silt content and the presence of ferric oxyhydroxides. Soil organic carbon content, silt content and the presence of ferric oxyhydroxides were found to decrease exponentially with travel distance. Removal rates of ϕ X174 were found to be 3–10 times higher than those of MS2 and PRD1 due to the lower electrostatic repulsion that the less negatively charged ϕ X174 experiences. It is suggested that the high initial removal in the field is due to the presence of favorable sites for attachment formed by ferric oxyhydroxides that decrease exponentially with travel distance. Similar removal rates may be found at both laboratory and

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field scale. However, due to local variations at field scale detailed knowledge on soil heterogeneity may be needed to enable a reliable prediction of removal.

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1. Introduction

The Netherlands relies for about 14% of its total drinking water production on pre-treated surface water which is artificially recharged in dune areas. Viruses and other pathogenic microorganisms present in surface water are known to be removed during passage in soil. One of the major issues of concern and interest is the efficiency of removal of viruses. Removal rate is defined as the logarithmic reduction in concentration over a certain length, $\Delta \log_{10}C/\Delta x$, or during a certain time, $\Delta \log_{10}C/\Delta t$.

In a recent field study on the effectiveness of dune recharge for virus removal (Schijven et al., 1999), it was shown that bacteriophages MS2 and PRD1 were removed 3 \log_{10} within the first 2.4 m and only 5 \log_{10} over the next 27 m. Several column (Gerba and Lance, 1978; Wang et al., 1981) and field studies (Bales et al., 1995, 1997; DeBorde et al., 1998, 1999; Pieper et al., 1997; Ryan et al., 1999; Schijven et al., 1998, 1999; Schijven and Hassanizadeh, 2000) have also shown that removal often appears to be higher during the first meters of soil passage. This implies that predictions of virus removal at larger distances may be severely overestimated if they are based on removal data from column experiments or from field studies where transport was studied only over short distances. It is therefore important to clarify the cause for the higher initial removal that was observed.

The higher initial removal of virus may be explained by soil and/or virus heterogeneity. Viral heterogeneity may be a cause for the higher initial removal, i.e. the viruses in the suspensions that were used for seeding a column or a field site may have different affinities for attachment sites (Pieper et al., 1997; Schijven et al., 1999). Possibly, viruses that are attached to other colloidal particles behave as particles with different size and density and, therefore, have different single collector efficiencies and also different sticking efficiencies (Schijven and Hassanizadeh, 2000). Viruses that are “stickier” will be removed faster. Transport of MS2 through sand columns has been shown to be greatly enhanced by its attachment to particles of Na-montmorillonite (Jin et al. 2000). The extent of such colloid-facilitated virus transport depends on the type and size of colloids, as well as the extent of virus attachment to the colloids. The extent of colloid-facilitated virus transport under various environmental conditions is unknown (Jin et al., 2000).

Another explanation for higher initial virus removal may be spatial heterogeneity of the soil's adsorption characteristics. In a study on the removal of viruses by deep-well injection (Schijven and Hassanizadeh, 2000), nonlinear removal appeared to have been caused by a change in surface charge of the porous medium. From geochemical mass balances, it was deduced that higher initial removal was caused by preferable attachment of viruses to patches of ferric hydroxides. These were precipitated as a consequence of pyrite oxidation within 8-m distance from the point of injection, but not at larger distances.

The objective of the present study was to investigate the causes of the high initial removal in the dune recharge study (Schijven et al., 1999). To that aim, a series of laboratory experiments was designed and carried out. Soil samples were taken along a streamline between the recharge canal and the first monitoring well in the Castricum field site. Removal rates of the bacteriophages in columns of these soil samples were measured and investigated for a correlation with physicochemical properties of the soil samples.

2. Materials and methods

2.1. Soil samples

Samples of dune sand for filling columns were collected along a flow line from the bottom of the recharge canal to the screen of the first monitoring well W1 at 2.4-m distance. Table 1 lists the locations of the soil samples, denoted A to G. The samples were kept saturated with canal water and transported in stainless steel buckets. Samples of canal water were also collected in 20-l polyvinylchloride containers. The sand and water samples were stored refrigerated (5 ± 3 °C). The soil samples were analyzed for grain size by laser grain size analysis (Konert and Vandenberghe, 1997), and for organic carbon content and Fe-oxalate (Stuyfzand and van der Jagt, 1997). The concentration of Fe-oxalate largely represents amorphous Fe-oxides and -hydroxides.

2.2. Bacteriophage suspensions and enumeration

The experiments were carried out with bacteriophages MS2, PRD1 and ϕ X174. MS2 is an icosahedral phage with a diameter of 27 nm and a low isoelectric point (p_1) of 3.5

Table 1
Chemical and physical analysis of soil samples

Soil sample		A	B	C	D	E	F	G
Distance ^a	m	0	0	0	0.35	0.7	1.05	1.4
Depth ^b	m	0–0.05	0.05–0.1	0.1–0.6	0.7–1.0	0.9–1.1	1.2–1.4	1–1.3
L^c	m	0.05	0.1	0.35	1.5	1.9	2.3	2.6
T^c	day	0.035	0.071	0.25	1.1	1.3	1.7	1.8
Grain size ^d	μm	242	220	214	236	237	239	255
Clay ($\leq 2 \mu\text{m}$)	%	0.97	0.69	0.67	1.07	1.01	0.92	1.02
Silt (>2 and $\leq 53 \mu\text{m}$)	%	3.98	1.71	2.08	3.26	2.96	2.38	2.37
Sand ($>53 \mu\text{m}$)	%	95.01	97.56	97.24	95.68	96.00	96.70	96.74
f_{oc}	%	0.88	0.50	0.32	0.50	0.16	0.16	0.27
Fe-oxalate	g kg^{-1} d.w.	2.8	2.1	2.3	2.4	1.8	1.3	1.1
Appearance		black	yellow-brown + shells	yellow-brown + shells	black-grey	yellow-brown	yellow-brown-grey	yellow-grey

^a Distance from bank of recharge canal.

^b Samples A–C relative to bottom of recharge canal, samples D–G relative to groundwater level.

^c Travel distance and time approximately along flow line.

^d Geometric mean.

(Penrod et al., 1996). PRD1 is an icosahedral bacteriophage with a diameter of 62 nm with an inner lipid membrane (Bales et al., 1991; Caldentey et al., 1990). Its p_1 lies between 3 and 4 (Loveland et al., 1996). In order to study adsorption, bacteriophage ϕ X174 was included in the column experiments. Bacteriophage ϕ X174 has a p_1 of about 6.6 and a size of 23 nm (Fujito and Lytle, 1996; Jin et al., 2000). This phage is less negatively charged than MS2 and PRD1. Due to a weaker electrostatic repulsion, bacteriophage ϕ X174 is therefore expected to attach more than MS2 and PRD1.

Highly concentrated suspensions of MS2 and PRD1 were prepared as described in the dune recharge study (Schijven et al., 1999). In fact, the very same batch suspensions used for the field study, which were stored in 1 g/l peptone/5 g/l NaCl at 5 ± 3 °C for over 2 years, were used in the laboratory experiments. Under these conditions, very low inactivation rate coefficients of 0.0019 and 0.0060 day⁻¹ were measured for MS2 and PRD1, respectively (Schijven et al., 1999). Nevertheless, to rule out any role of aging on removal rates of the bacteriophages, in one experiment, freshly prepared, highly concentrated suspensions of MS2 and PRD1 were used for comparison.

A highly concentrated suspension of ϕ X174 (not used in the field study) was also prepared (International Organization for Standardization (ISO), 2000a). Part of each highly concentrated suspension was diluted with 1 g l⁻¹ peptone–saline to a concentration of 10¹⁰–10¹¹ plaque-forming particles (pfp) per liter. These were used as stock suspensions. Prior to each experiment, aliquots from the stock suspensions of all three bacteriophages were diluted 1000-fold in a container with canal water for seeding the columns.

MS2 was assayed using host strain WG49 (Havelaar et al., 1984) in a double agar layer method (ISO, 2000a). PRD1 was assayed according to the same method using *S. typhimurium* LT2 as the host, omitting nalidixic acid in the top agar layer. For ϕ X174 host strain WG5 (ATCC 700078) was used (ISO, 2000b).

2.3. Description of column experiments

Laboratory experiments were designed to closely simulate field conditions. Columns were packed with saturated sand from the field. Water from the recharge canal was used. The experiments were all conducted in a cold room at the same temperature as that of the groundwater during the field study (5 ± 3 °C). The same transport velocity was applied as in the field (1.5 m day⁻¹). Fig. 1 shows a schematic representation of a column; a Plexiglass pipe with an inner diameter of 9 cm and a length of 1.9 m. A stainless steel grid for supporting the sand was placed at the bottom of the pipe. Along the pipe, 14 small stainless steel samplers were placed at 10-cm intervals. These samplers were inserted horizontally towards the center of the column and penetrated the column of sand 2.5 cm. The pipe was filled in small increments with saturated dune sand. During the filling, upward flow of canal water was maintained. At the same time, the pipe was being tapped in order to distribute the sand evenly and to dislodge air bubbles. The pipe was filled up to a length of 1.5 m. Initially, a thin layer of very fine sand particles settled on top of the column. This layer was removed by suction. One to two days later, the flow of water was reversed to downward direction at a rate of 2.4 ml min⁻¹, corresponding to a pore water velocity of about 1.5 m day⁻¹. The sand column was kept saturated at all times. In all experiments, pH of the recharge water as well as that of the column effluent was measured

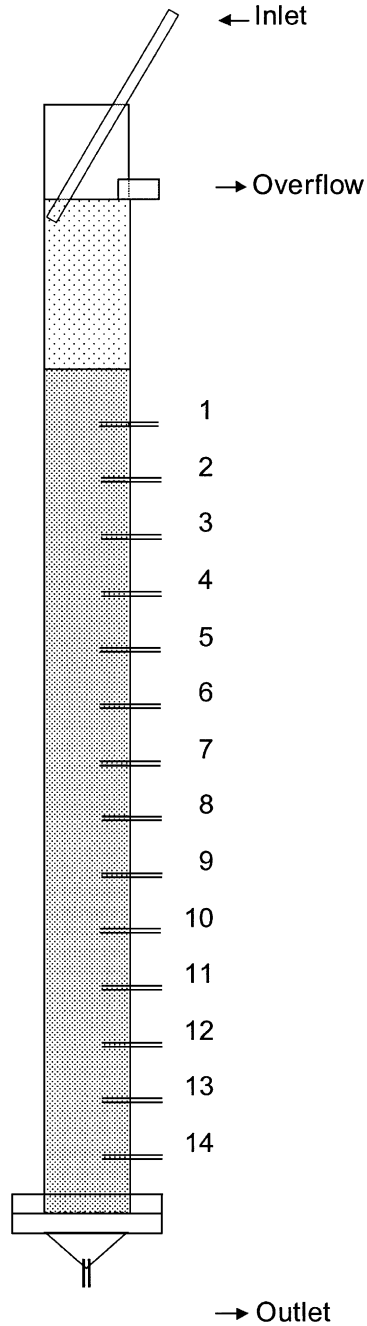


Fig. 1. Schematic representation of a column, filled with sand to a length of 1.5 m. Inner column diameter is 9 cm. Numbers 1 to 14 represent stainless steel samplers, each 10 cm apart.

to be 7.5–8.0. The electric conductivity of the canal water was $800 \mu\text{S cm}^{-1}$. At the inlet, canal water was fed and microorganisms were seeded. The overflow kept the level of water in the column constant and led excess of water to the feeding tank. The pump that was connected to the outlet determined the flow rate of water through the column and led the effluent to a disposal tank.

Five different columns were prepared, each of them filled with different portions of soil samples A to G described in Table 1. These columns, soil samples and their corresponding lengths (figures inside parentheses, measured from the top of the column) are as follows:

- Column I containing soils A (0–0.2 m), B (0.2–0.4 m) and C (0.4–1.5 m);
- Column II containing soils D (0–0.5 m), E (0.5–1.0 m) and F (1.0–1.5 m);
- Columns III containing soil G (0–1.5 m), taken near the first monitoring well;
- Columns IV and V containing soil G* (0–1.5 m), that was collected at a later date at the same location as soil G.

Note that columns I and II each contained three different soil samples emplaced in series in separate sections. The idea was to measure removal in three different samples of soil within one experiment.

In order to investigate the linearity of removal, soil columns I–V were fed continuously for 4 days with a suspension of MS2, PRD1 and ϕX174 . In column III, this was continued for 17 days in order to investigate blocking effects. From the second day of seeding, i.e. after steady state was established, 1.2-ml samples were taken every day from all sampling points at a flow rate of 0.2 ml min^{-1} and three decimal dilutions were assayed. To measure inactivation of bacteriophages in the aqueous phase, samples from the column influent and effluent were taken every few days for 1–3 weeks and eight replicates were analyzed.

2.4. Conceptual model

Under steady state conditions, the relative contributions of inactivation and adsorption to the removal of viruses by soil passage can be compared easily. A steady state situation occurs when input of virus is continuous and may be seen as a worst-case situation. In the field study (Schijven et al., 1999), it was observed that detachment of MS2 and PRD1 from the soils grains was much slower than attachment. Therefore, we may neglect detachment. This also implies disregarding inactivation of attached viruses. Also, dispersivity in the columns is very small, and is therefore neglected. With these assumptions, under steady state conditions, the removal rate, r [$\log_{10} \text{ day}^{-1}$], is described by the following simple equation (Schijven and Hassanizadeh, 2000):

$$r = \frac{-\log_{10}\left(\frac{C}{C_0}\right)}{t} = \frac{k_{\text{att}} + \mu_1}{2.3} \quad (1)$$

where t is the travel time defined by $t=x/v$, x is the distance [m] and v is the average interstitial water velocity [m day^{-1}]. C is the concentration of free phages [pfu m^{-3}], C_0

is the concentration at $x=0$, $\log(C/C_0)$ is a measure of virus removal, k_{att} is the attachment rate coefficient [day^{-1}] and μ_1 is the inactivation rate coefficient of free phages [day^{-1}].

2.5. Analysis of inactivation rates and removal rates

The inactivation of free bacteriophages is assumed to be first order. As mentioned above, the inactivation rate coefficient of the bacteriophages in water was estimated from the decrease in concentrations with time in samples taken from both column influent and effluent in each experiment. First, inactivation data from different column experiments were compared in order to test whether these data could be pooled. Next, effects of passage through columns on inactivation of free phages were studied by comparing inactivation rates in column influent and effluent. Removal of the bacteriophages with time or distance is also expected to be first order (see Eq. (1)), provided blocking does not occur. The removal capacities of various soil samples as a function of travel time and distance were determined and compared among soil samples.

Assuming both inactivation and removal are first order, the logarithms of the measured concentrations must decline linearly with time or distance. Assuming normally distributed errors, a log likelihood function, L , which includes parameters a (slope), b (intercept) and s (standard error) can be formulated for each set of n measured concentrations C_i at time t_i from an experiment (Hogg and Craig, 1995):

$$L(a, b, s) = 2 \sum_{i=1}^n \left[\ln(s\sqrt{2\pi}) + \frac{[\ln C_i - (at_i + b)]^2}{2s^2} \right] \quad (2)$$

where i is the i -th of n observations. In the case of inactivation experiments, parameter a equals inactivation rate coefficient μ_1 , and in the case of removal experiments, it is equal to removal rate r . Values for parameters a , b and s for different sets of data or combinations of these sets of data were obtained by maximizing this log likelihood function (equivalent to least squares solution) using numerical optimization in Mathematica 4.0.0 (Wolfram Research, 1999).

The question was whether the slopes a for different sets of data were different or not. This can be done by means of likelihood ratio tests (Cox and Hinkley, 1974). The linear model can be applied to all separate data sets as well as pooled data sets. For pooled data sets, a common value of slope a was evaluated using the following likelihood function:

$$L(a, b_1, b_2, \dots, b_m, s) = 2 \sum_{j=1}^m \sum_{i=1}^{n_j} \left[\ln(s\sqrt{2\pi}) + \frac{[\ln C_{ij} - (at_{ij} + b_j)]^2}{2s^2} \right] \quad (3)$$

for m data sets with n_j observations.

The sum of the log likelihoods of the separate data sets (Eq. (2)) models is compared to that of the pooled data (Eq. (3)). The difference is interpreted as a χ^2 deviate with number of degrees of freedom equal to the difference in the number of parameters in the pooled data set ($2+m$) and the total number of parameters of all separate data sets ($3m$) (Teunis et al., 1996). If the log likelihood of the pooled data is found to be significantly higher than

that of the sum of the log likelihoods of the separate data sets, then significant differences exist between the data sets. If not, they may be pooled.

In the case of columns I and II, both with three layers of soil ($0 - x_1, x_1 - x_2, x_2 - x_3$ at end of column), a linear model with slope a_1 was compared with a model defining three slopes a_1, a_2, a_3 , each corresponding to one layer of soil, thus incorporating spatial heterogeneity into the regression model:

$$f(t) = \left\{ \begin{array}{ll} a_1 t + b & \text{if } 0 \leq t \leq \frac{x_1}{v} \\ a_2(t - t_1) + a_1 t_1 + b & \text{if } \frac{x_1}{v} \leq t \leq \frac{x_2}{v} \\ a_3(t - t_2) + a_2(t_2 - t_1) + a_1 t_1 + b & \text{if } \frac{x_2}{v} \leq t \leq \frac{x_3}{v} \end{array} \right\} \quad (4)$$

3. Results

3.1. Soil analysis

Results of chemical and physical analysis of soil samples are given in Table 1. Grain size distribution was similar for all sand samples, but clay and silt contents were lower in soils B and C. The soil organic carbon content was higher in samples A, B and D. The values of silt and Fe-oxalate are highest in soil A. Fe-oxalate concentrations, the organic carbon content and silt content appeared to decrease with travel distance. Fig. 2 shows the logarithms of the Fe-oxalate concentrations, the organic carbon content and silt content with travel distance at field scale. The decrease as a function of travel distance was quantified by means of regression analysis. Parameter values from linear regression analysis are listed in Table 2. There appears to be a significant first order decrease with travel distance. In the case of silt content, soils B and C were omitted from regression

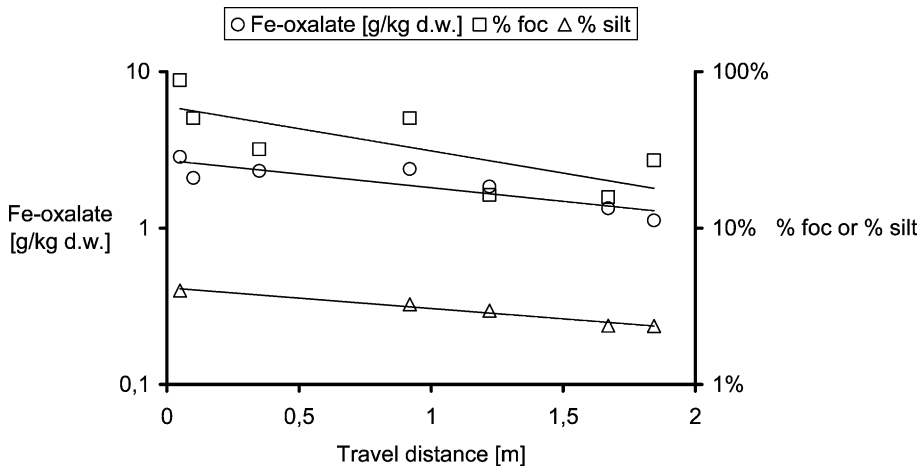


Fig. 2. Change in concentration of Fe-oxalate and contents of f_{oc} and silt as a function of travel distance.

Table 2
Regression analysis of soil properties (on log-scale) as a function of travel distance (see Fig. 2)

	Slope	p (%)	Intercept	p (%)	R^2 (%)
$\ln(\text{Fe-oxalate})$	– 0.40	0.81	1.0	0.023	78
$\ln(f_{oc})$	– 0.65	4.7	– 0.51	12	58
$\ln(\text{silt})$	– 0.30	0.21	– 3.2	0.00042	97

analysis. Possibly, the silt content may vary irregularly, or silt and clay were partly lost when taking samples B and C from the bottom of the recharge canal.

3.2. Inactivation of free bacteriophages

Table 3 summarizes the measured values of the inactivation coefficient μ_1 for the phages. Likelihood-ratio tests showed that there were no significant differences between the inactivation rates in column influents and effluents and a single value for the inactivation rate coefficient for each bacteriophage could be estimated. The value of μ_1 for PRD1 is now found to be lower than in the field study. The value found in the present study is more realistic, because PRD1 is expected to be more stable than MS2 (Yahya et al., 1993). It appears that ϕX174 is the most stable one.

3.3. Removal rates

Fig. 3 shows the removal of bacteriophages with travel distance in columns I, II and III containing soils A–G 2 days after seeding. Within each soil sample, removal appears to proceed linearly. This was also observed on days 3 and 4 after seeding. Table 4 summarizes the removal rates that were found by fitting the log likelihood function (Eqs. (2) and (3)). No significant change in removal rate within 4 days was found for MS2 in any of the columns and for ϕX174 in column III. However, in other cases, significantly different removal rates between days were found. In columns I and II, both of which contain three layers of different soil samples, removal rates were found to be significantly different among the different soils for all bacteriophages. It appears that removal rate is highest in soil A. This is most apparent for ϕX174 because removal of this bacteriophage is higher than that of MS2 and PRD1. In soil E, removal of phages appeared to be very low; even an increase in concentrations with travel time was found. Samples from soil E in column II appeared to contain silt particles, which have phages attached. Bacteriophages attached to small particles can be still be enumerated (Formentin et al., 1997). These data points were therefore excluded from subsequent analyses.

Table 3
Inactivation rate coefficient μ_1 [day^{-1}]

	N^a	μ_1	95% CI
MS2	38	0.082	0.068–0.096
PRD1	30	0.044	0.038–0.049
ϕX174	30	0.012	0.0072–0.016

^a Number of observations.

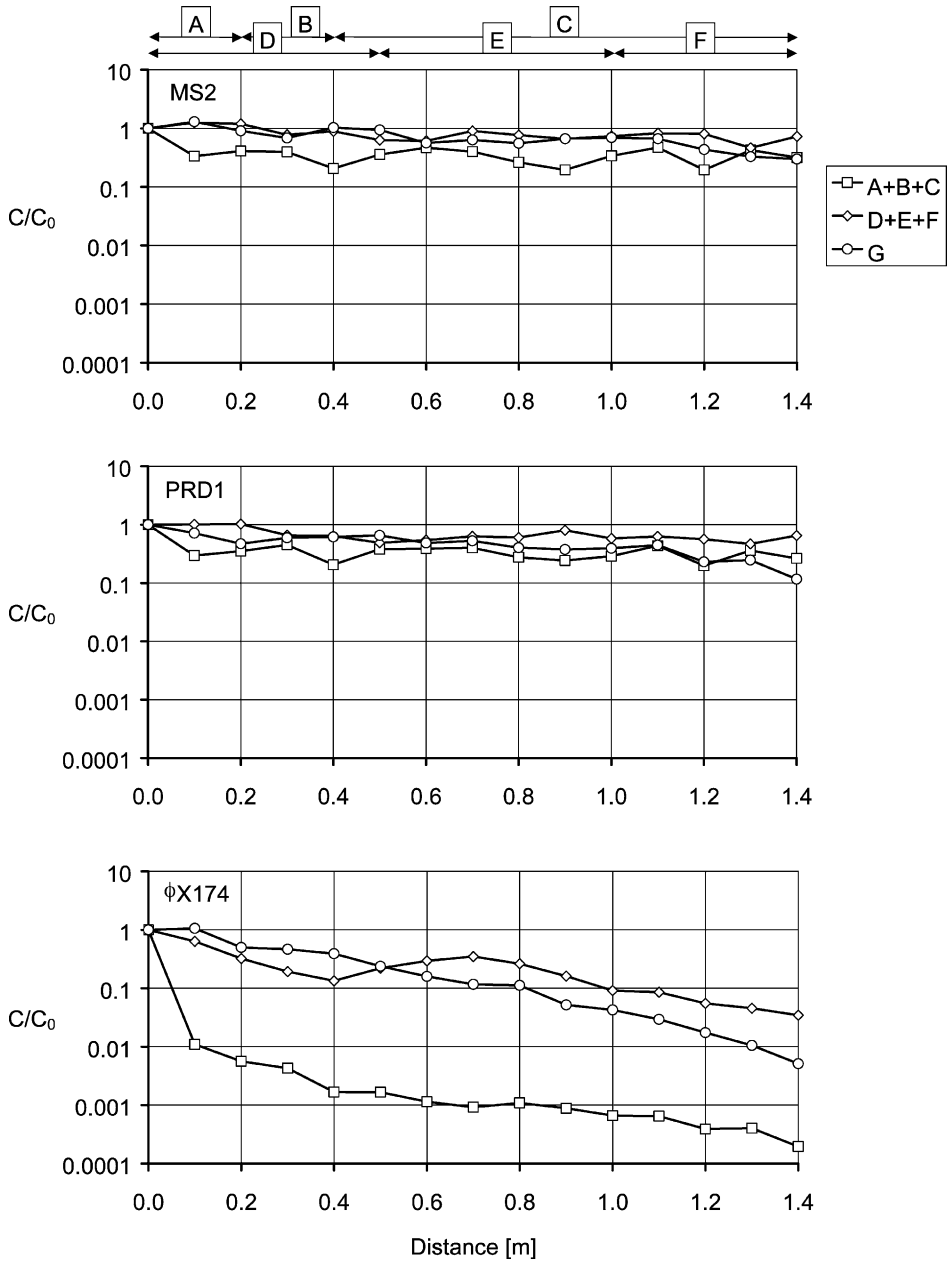


Fig. 3. Removal of bacteriophages with distance after 2 days of seeding in columns I (A+B+C), II (D+E+F) and III (G).

Table 4
Removal rates r (\log_{10} day $^{-1}$) in soils A to G (columns I to III)

Column Day	I			II			III
	A	B	C	D	E	F	G
<i>MS2</i>							
2	2.7	0.45	0.021	0.75	0.12	0.36	0.54 (0.35–0.73)
3	2.2	0.65	0.18	0.44	–0.063	0.12	0.59 (0.38–0.80)
4	1.1	1.9	0.24	0.43	0.17	0.39	
Pooled data	2.0	1.0	0.13	0.54	0.039	0.21	0.57 (0.44–0.70)
<i>PRD1</i>							
2	3.0	–0.11	0.10	1.0	–0.27	0.32	0.70 (0.46–0.93)
3	2.7	1.7	0.39	1.1	–0.34	–0.20	0.61 (0.37–0.85)
4	1.6	1.9	0.20	0.91	–0.19	1.2	
Pooled data	2.4	1.2	0.23	1.0	–0.26	0.45	0.66 (0.50–0.81)
<i>ϕX174</i>							
2	17	1.9	1.4	1.6	0.52	2.6	2.5 (2.3–2.7)
3	13	4.1	1.2	1.2	–0.037	2.5	2.1 (1.7–2.4)
4	14	3.3	1.6	1.6	0.22	1.8	
Pooled data	15	3.0	1.4	1.5	0.23	2.3	2.3 (2.0–2.5)

95% confidence intervals are given inside brackets for removal rates in column III with soil G.

Table 5 gives the correlation coefficients of soil properties and removal rates. The removal rates of all three bacteriophages are very highly positively correlated with the soil organic carbon content. This means that either organic carbon provides attachment sites for the bacteriophages, or that organic carbon and bacteriophages attach to the same type of sites, probably ferric oxyhydroxides of which there are more present in soils A and D. Removal rates of the bacteriophages also show a relatively high and positive correlation with Fe-oxalate and the silt-content, but a low correlation with clay content. Removal rates of the bacteriophages are very highly correlated, indicating interaction of the bacteriophages with the same type of sites. Fe-oxalate was found to be positively correlated with the silt content and highly positively with the organic carbon content.

Seeding of bacteriophages in column III containing soil G was continued for a period of 17 days to investigate the possibility of blocking attachment sites with bacteriophages

Table 5
Correlation coefficients of soil properties and removal rates r (\log_{10} day $^{-1}$)

	% Sand	% Silt	% clay	% f_{oc}	Fe-oxalate	r	
						MS2	PRD1
% Silt	–1.00						
% Clay	–0.74	0.68					
% f_{oc}	–0.67	0.71	0.14				
Fe-oxalate	–0.48	0.53	–0.16	0.83			
r MS2	–0.61	0.64	0.18	0.92	0.57		
r PRD1	–0.72	0.74	0.29	0.95	0.62	0.98	
r ϕ X174	–0.70	0.74	0.19	0.85	0.56	0.92	0.92

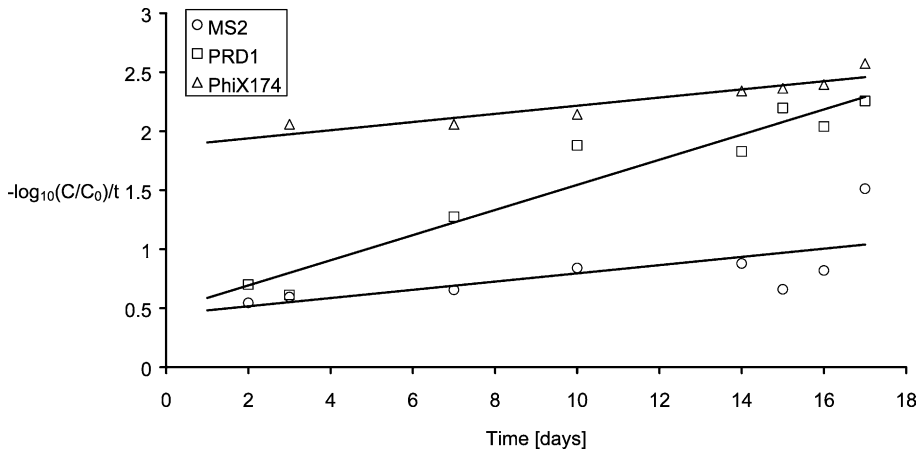


Fig. 4. Removal rates of bacteriophages as a function of time during continuous seeding (see also Table 6). Slopes of regression lines: 0.035 (MS2 and ϕ X174) and 0.11 (PRD1); R^2 : 45% (MS2), 93% (PRD1) and 87% (ϕ X174); p : 6.8% (MS2), 0.010% (PRD1) and 0.24% (ϕ X174).

upon prolonged seeding. In the event of blocking, the grain surfaces become progressively occluded, resulting in a decrease in attachment rate. Removal rates are deduced from linear regression analysis (Fig. 4). Apparently, blocking did not occur. On the contrary, the removal rates of all phages increased significantly with time. This increase was the same for MS2 and ϕ X74 but three times higher for PRD1. With column IV containing soil G*, this experiment was repeated, but now seeding was performed for the first 3 days and again 30 days later. In the intermediate period, the column was not seeded with bacteriophages, only recharge water was fed. In this experiment, removal rates did not change significantly. Therefore, the observed increase in removal rates in column III appears to be due to the continuous seeding of bacteriophages.

Table 6 shows the removal rates that were observed in column IV, containing soil G*, after seeding bacteriophages for 2 days. Table 6 also shows the removal rates measured in column V containing soil G*, where freshly prepared suspensions of MS2 and PRD1 were used. Removal of bacteriophages from the freshly prepared suspensions appeared to be linear as well at approximately the same removal rate as the 2-year-old suspensions. This means that the initially higher removal of MS2 and PRD1 that was observed in the field was not resulting from faster attachment of sub-populations of phage that are only present in freshly prepared suspensions.

Table 6

Removal rate r ($\log_{10} \text{ day}^{-1}$) of 2-year-old suspension of MS2 and PRD1 in column IV with soil G* and of fresh suspensions of MS2 and PRD1 in column V with soil G*

	IV	V
MS2	0.27 (0.077–0.46)	0.56 (0.26–0.87)
PRD1	0.72 (0.43–1.0)	0.53 (0.19–0.70)
ϕ X174	1.2 (0.90–1.5)	1.4 (0.81–1.7)

95% confidence intervals are given between brackets.

Table 7

Removal and removal rates of MS2 and PRD1 at field scale (Schijven et al., 1999)

Well	Travel distance [m]	Travel time [day]	Removal ($-\log_{10}(C/C_0)$)		Removal rate from well to well ($-\log_{10}(C/C_0)/t; \text{day}^{-1}$)	
			MS2	PRD1	MS2	PRD1
	0	0	0	0		
W1	2.4	1.7	3.0	2.9	1.8	1.8
W2	3.8	2.4	3.3	3.3	0.40	0.53
W3	6.4	4.0	4.9	4.0	1.0	0.40
W4	10	6.5	5.6	4.4	0.30	0.18
W5	17	11	6.5	6.7	0.18	0.49
W6	30	25	8.4	8.3	0.14	0.11

For columns III, IV and V with only one type of soil (G or G*), 95% confidence intervals were calculated (Tables 3 and 6) to give an idea of the uncertainty of the observed removal rates. For MS2 and PRD1, the 5% and 95% limits differ by a factor of 1.6–2.3 in column III (Table 4). For $\phi X174$, this ratio is only 1.2–1.4. In column IV (Table 7), the 5% and 95% limits for MS2 and PRD1 differed with factors 2 and 6. The confidence interval of the removal rate of $\phi X174$ is much smaller because the removal rate is much higher than that of MS2 and PRD1.

4. Discussion and conclusions

Two possible causes have been considered for the nonlinear removal of bacteriophages in our earlier field study: heterogeneity in the phage population and heterogeneity in soil properties. Removal of the bacteriophages within each soil sample increased linearly with travel time. We may therefore conclude that the nonlinear removal that was observed in the field study was not due to heterogeneity of the bacteriophages. In the field study, freshly prepared suspensions of MS2 and PRD1 were used. In the present study, these suspensions were used again after 2 years of storage. One might be concerned about the possibility of significant changes in these suspensions due to aging. For example, subpopulations of bacteriophages in the suspensions may exist that differ in inactivation rate. They may also differ in attachment properties, e.g. numbers of stickier phages may have decreased more than those of less stickier ones. To investigate this issue, removal of MS2 and PRD1 from freshly prepared and 2-year-old suspensions was compared. Removal appeared to elapse in a linear fashion at similar rates for both types of suspensions. Moreover, we did not observe blocking in our 17-day continuous seeding experiment. Apparently, an excess of attachment sites was available and thus there was no competition for attachment sites. This was confirmed in an additional experiment where we measured breakthrough curves when seeding for 1 day with a concentration 10 times higher than in our other experiments. No difference in removal rates were found. Therefore, we may also safely state that inactivated phages in the 2-year-old suspension did not noticeably affect removal of intact bacteriophages.

The removal rates of all three bacteriophages are very highly positively correlated with the organic carbon content of the soil, and to a lesser extent with Fe-oxalate and the silt content. In addition, the soil organic carbon content, silt content and the presence of ferric oxyhydroxides were found to decrease exponentially with travel distance. We therefore conclude that the high initial removal that was found in the field was due to heterogeneity of the soil's adsorption characteristics.

Removal rates of MS2 and PRD1 were found to be lower than that of ϕ X174. A similar difference in adsorption was also found between MS2 and ϕ X174 in Ottawa sand at pH 7.5 by Jin et al. (1997, 2000) and between MS2 and Poliovirus I by Bales et al. (1993). At pH 7.5–8.0, the dune sand is predominantly negatively charged and, thus, conditions are unfavorable for attachment to negatively charged viruses. Therefore, one may argue that the virus–grain interaction is the rate-limiting step for attachment and not the transport (e.g., by diffusion) to attachment sites (Ryan and Elimelech, 1996). Thus, the lower removal rates of MS2 and PRD1 can be explained by greater electrostatic repulsion that they experience compared to the less negatively charged ϕ X174. Since electrostatic interaction appears to determine attachment, this suggests that attachment to ferric oxyhydroxides, which form favorable (positively charged) sites, is the major removal process. This is supported by the same findings at the deep well injection study (Schijven et al., 2000). Organic matter attaches also to the same sites of ferric oxyhydroxides (Pieper et al., 1997), hence the high correlation that was found between phages removal rates and the soil organic carbon content.

The removal rates of the bacteriophages are also highly positively correlated, indicating that the bacteriophages interact with the same type of attachment sites. This implies that if the removal rate of, say, MS2 is measured both at laboratory and field scale, but that of another virus, say ϕ X174, is only measured at laboratory scale, one can estimate removal of that virus at field scale based on its correlation with MS2. The finding that MS2 and PRD1 are removed less than ϕ X174 supports the conclusion that MS2 and PRD1 can be considered as relatively conservative tracers for virus transport in saturated sandy soils at pH 6–8 and with a low organic carbon content (Bales et al., 1989; Herbold-Paschke et al., 1991; Jin et al., 1997; Kinoshita et al., 1993; Powelson et al., 1990; Schijven et al., 1999).

Although removal rates were found to be higher in soil samples A and B, this does not fully explain the high removal that was observed during the field study (Schijven et al., 1999), since soils A and B account for only the first 5–10 cm of soil passage in the field. The removal within soil A suggests a nonlinear behavior, because the removal rate appears to be higher in the first than in the next 10 cm. The removal rates of the bacteriophages were found to vary more than a factor 10 among various soils.

For comparison between removal rates from the column experiments and the field study, details of removal and removal rates from the field study are given in Table 7. Concentrations of MS2 and PRD1 decreased about 3 \log_{10} within the first 2.4 m at a rate of 1.8 \log_{10} day⁻¹. Thereafter, another 3.5–3.8 \log_{10} was removed over a distance of 15 m at a rate of 0.36–0.40 \log_{10} day⁻¹. Finally, over the next 13 m, an additional 1.6–1.9 \log_{10} was removed at a rate of 0.11–0.14 \log_{10} day⁻¹. The removal rates that were found for MS2 and PRD1 between the first and second monitoring well in the field study are similar to the ones found in columns III, IV and V with soil G and G*, taken near the first observation well. The removal rates of MS2 and PRD1 in soil A were a bit higher than the

initial removal rates found in the field. Overall, one can say that the removal rates of MS2 and PRD1 observed in the column experiments with soils taken between the recharge canal and the first monitoring well vary over a very similar range ($0.13\text{--}2.4 \log_{10} \text{day}^{-1}$) as the removal rates observed in the field study over a distance of 30 m ($0.11\text{--}1.8 \log_{10} \text{day}^{-1}$). However, considerable variation in removal rates was also found within each experiment over the days of observation in the columns with soils A–F. This variation is probably due to uncertainties in the measurements. According to the 95% confidence intervals that were calculated for the removal rates of bacteriophages in columns III, IV and V with 1.5 m of soils G and G*, predictions of removal may vary by a factor 2 to 3. Nevertheless, the main source for uncertainty in predicting removal at field scale is heterogeneity of the soil properties. Therefore, detailed knowledge of soil heterogeneity is needed in order to be able to predict removal at field scale.

During 17 days of continuous seeding of bacteriophages in column III with soil G, removal rates appeared to increase linearly with time. This increase was on average 0.035day^{-1} for both MS2 and ϕX174 , and 0.11day^{-1} for PRD1. The cause for this increase is not clear. It cannot be explained by an increase of inactivation of free bacteriophages. For example, in the case of MS2 or PRD1, a 100-fold increase of the inactivation rate would be needed to account for the observed increase, which is unlikely. In the field study (Schijven et al., 1999), only a small increase in inactivation of MS2 and PRD1 was observed in water samples from the first monitoring well compared to those from the recharge water. However, an increase by a factor of approximately 2.5 for MS2, 5 for PRD1 and 1.4 for ϕX174 of k_{att} would account for the observed increases in removal rate.

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