

Modeling removal of bacteriophages MS2 and PRD1 by dune recharge at Castricum, Netherlands

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Abstract. Removal of model viruses by dune recharge was studied at a field site in the dune area of Castricum, Netherlands. Recharge water was dosed with bacteriophages MS2 and PRD1 for 11 days at a constant concentration in a 10- by 15-m compartment that was isolated in a recharge basin. Breakthrough was monitored for 120 days at six wells with their screens along a flow line. Concentrations of both phages were reduced about $3 \log_{10}$ within the first 2.4 m and another $5 \log_{10}$ in a linear fashion within the following 27 m. A model accounting for one-site kinetic attachment as well as first-order inactivation was employed to simulate the bacteriophage breakthrough curves. The major removal process was found to be attachment of the bacteriophages. Detachment was very slow. After passage of the pulse of dosed bacteriophages, there was a long tail whose slope corresponds to the inactivation rate coefficient of $0.07\text{--}0.09 \text{ day}^{-1}$ for attached bacteriophages. The end of the rising and the start of the declining limbs of the breakthrough curves could not be simulated completely, probably because of an as yet unknown process.

1. Introduction

In this paper, results of a field study, aimed at investigating virus removal processes during dune recharge, are presented. In the Netherlands, about 14% of the total drinking water production relies on pretreated surface water that is artificially recharged in dune areas. When renovating or designing recharge systems, drinking water companies want to minimize side effects of artificial recharge and keep the land claim for recharge projects within limits [Peters, 1996]. This may conflict with travel time and travel distance that are required for the production of safe drinking water. Drinking water is considered to be safe if certain maximum allowable concentrations of pathogenic microorganisms are not exceeded. Maximum allowable concentrations of pathogens in drinking water can be calculated from a maximum acceptable risk of infection of one per 10,000 persons per year, drinking water consumption, and dose response relations of pathogens [Regli *et al.*, 1991]. For viruses this maximum allowable concentration is 1.8×10^{-7} plaque-forming particles (pfp L^{-1}). This approach has formed the basis for the Extended Surface Water Treatment Rule and is under consideration for the Ground Water Disinfection Rule in the United States [Macler, 1996]. In Netherlands a proposal for drinking water protection policy is being prepared, which leads to similar maximum allowable concentrations. Concentrations of enteroviruses in the source surface water are in the range of $0.02\text{--}10 \text{ pfp L}^{-1}$ but may be consid-

erably higher because of incidental storm water overflow [Schijven *et al.*, 1996]. Prior to recharge in the dune sand the surface water is pretreated by flocculation/sedimentation, rapid sand filtration, and activated carbon filtration thereby reducing virus concentrations by about $1\text{--}2 \log_{10}$ (that is a reduction by a factor of 10–100). Thus a reduction of at least $8 \log_{10}$ may still be required by dune recharge to comply with the maximum allowable concentration of $1.8 \times 10^{-7} \text{ pfp L}^{-1}$.

A field study was carried out at a location for dune recharge at Castricum, Netherlands, in order to show that a reduction of at least $8 \log_{10}$ is possible during the passage of water from the recharge basins to the production wells. This could not be established with the aid of pathogenic viruses because of their very low concentrations. Instead, highly concentrated suspensions of bacteriophages MS2 and PRD1 were added to the recharge water. These bacteriophages impose no health threats, and their enumeration at such high concentrations is not affected by naturally present bacteriophages. MS2 and PRD1 are considered to be good model viruses because they attach less than most pathogenic viruses and are relatively persistent during transport through the subsurface, as evidenced in the literature. Bacteriophages MS2 and PRD1 have relatively low isoelectric points [Bales *et al.*, 1991] and are therefore expected to attach poorly to most soils [Gerba, 1984]. At the field site studied here, the soil mainly consists of calcareous, fine dune sand with an organic carbon content of about 0.1–0.2% and a pH of 7–8 [Stuyfzand, 1993]. Under such conditions, MS2 and PRD1 have been observed to attach poorly [Bales *et al.*, 1989, 1991, 1997; Powelson *et al.*, 1990; Herbold-Pashke *et al.*, 1991; Kinoshita *et al.*, 1993; Jin *et al.*, 1997]. Moreover, to minimize virus inactivation, the study was performed during the winter period. At temperatures less than 7°C the inactivation rate of MS2 in groundwater is very low and is similar to that of PRD1 [Yahya *et al.*, 1993].

The goals of this field study were as follows: (1) to measure reduction in concentrations of bacteriophages MS2 and PRD1 as a function of time and distance; (2) to gain insight into the

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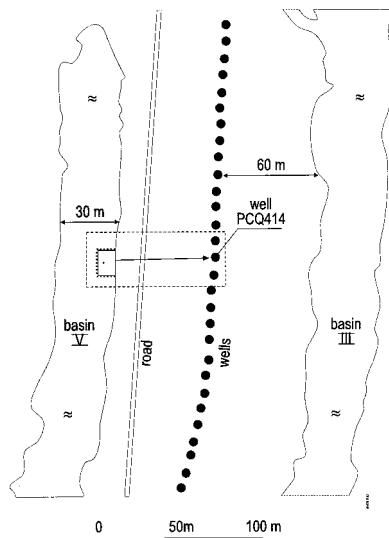


Figure 1. Top view of field site, Castricum, Netherlands. Isolated compartment of 150 m² is situated in basin V, opposite to abstraction well PCQ414.

extent and the manner with which attachment and inactivation contribute to the removal of the bacteriophages in relation to travel time and distance; and (3) to evaluate the applicability of the employed transport model by comparing measured with calculated breakthrough values.

2. Site Description

The Castricum artificial recharge system was constructed in the mid-1950s in old dune reclamation areas near the village of Castricum about 30 km to the west of Amsterdam. The system was last expanded in 1972. Technical renovations were carried out between 1990 and 1993 together with nature conservation and development [Peters *et al.*, 1992]. The system now covers around 150 ha and has a drinking water production capacity of 25×10^6 m³ y⁻¹. Pretreatment of the surface water is carried out near the intake points at the River Rhine and Lake IJssel. The pretreatment consists of coagulation, sedimentation, rapid sand filtration, and partially active carbon filtration. The open water area of the recharge basins is approximately 22 ha. The recharge basins are, on average, 0.8 m deep and have a fairly constant water level of 2.85 m above mean sea level. The water is retrieved using around 600 shallow wells, each approximately

9 m deep, distributed over 12 separate suction lines. The phreatic aquifer is around 10 m thick and consists of fine aeolian dune sands. Hydraulic conductivity is about 12 m d⁻¹ (at a water temperature of 5°C). Effective porosity with respect to flow is approximately 0.35. Below the phreatic layer is about 15 m of silt-containing sand with a lower hydraulic permeability.

Basin V was selected as the test site because its flow pattern was known and could be kept constant throughout the test period (Figure 1). The pumping rate was 330 m³ h⁻¹ from a series of 55 wells, each 10 m apart. Within basin V a 150-m² compartment was constructed using PVC sheeting along wooden poles. The water level in the compartment was kept the same as that of the basin. The distance from this compartment to the extraction well PCQ414 was 63 m with a water travel time of approximately 40 days. Along a line almost perpendicular to the bank of basin V, six monitoring wells (W1 to W6) were installed at distances of 2.4, 3.8, 6.4, 10, 17, and 30 m. The well screens were 0.25 m long and were positioned at different depths so as to be along a calculated flow line. A schematic representation of the groundwater situation is shown in Figure 2.

3. Experimental and Modeling Methods

3.1. Soil Analysis

Soil samples were taken very near to the screens of the monitoring wells. Also, soil was collected from the first few centimeters of the bottom of the compartment and from the subsurface between the compartment and the first monitoring well. The samples were analyzed for grain size by laser grain size analysis [Konert and Vandenberghe, 1997] and for organic carbon content, cation exchange capacity, metal ions (Si, Ti, Al, Fe, Mg, Mn, Ca, K, Na, and P), and Al-, Fe-, and Mn-oxalate as described by Stuyfzand and van der Jagt [1997].

3.2. Salt Tracing

Prior to the dosage of the water with bacteriophages, sodium chloride was added to the compartment as a conservative salt tracer to estimate interstitial flow velocity and medium dispersivity. The chloride concentration of the water in the compartment was increased in one step from 150 mg L⁻¹ to 750 mg L⁻¹ and kept at this level for exactly 7 days. During 35 days, electrical conductivity (EC) was measured at 10-min intervals in the six monitoring wells by means of fixed EC sensors with a data logger. Water was continuously pumped from the wells

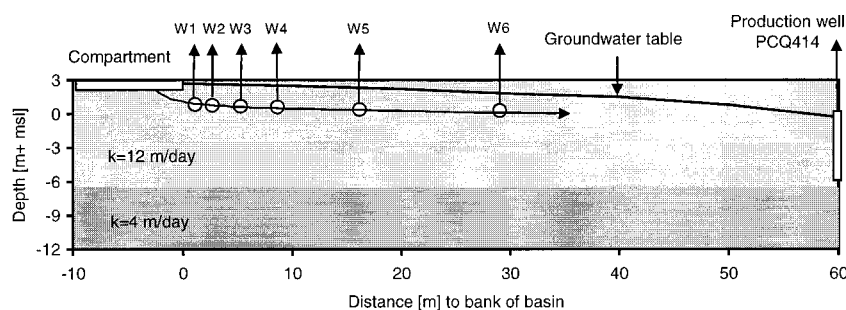


Figure 2. Schematic cross section of field site with compartment and monitoring wells W1 to W6. Curved arrow indicates flow path; k is the hydraulic conductivity (in meters day⁻¹); depth is given in meters above sea level (msl).

at a rate of 4 L h^{-1} to prevent distortion of the measurements by stagnant water in the borehole pipes above the screens.

3.3. Bacteriophage Preparation and Dosage

Suspensions of 2 L with 3×10^{14} pfp of MS2 and 2 L with 3×10^{13} pfp of PRD1 were prepared as described by the *International Organization for Standardization (ISO)* [1995] using hosts *E. coli* HfrH (WG21) for MS2 and *S. typhimurium* LT2 for PRD1. Phage suspensions were prediluted, distributed in bottles, and kept at $5^\circ \pm 3^\circ\text{C}$. In preparation for the dosage in the compartment, 2.5×10^{13} pfp of MS2 and 2.5×10^{12} pfp of PRD1 were added to 200 L of cold water from the recharge basin in a 1000-L PVC container. Then, uniform dilution was obtained by filling the container up to 1000 L with basin water. No stirring was applied to avoid enhancing inactivation of the phages. Dosage of the water with phages started by emptying bottles with 2.2×10^{13} pfp of MS2 and 2.2×10^{12} pfp of PRD1 at the center of the compartment. This was done in order to raise the concentrations of MS2 and PRD1 in the compartment immediately to about 10^8 and 10^7 pfp L^{-1} , respectively. The concentrations were kept constant by pumping phages from the 1000-L container at a rate of 30 L h^{-1} into the compartment. When a container was empty, it was replaced by another freshly prepared 1000-L container with the same concentrations of MS2 and PRD1 as before. The water in the compartment was continuously circulated with a pump to promote mixing and to achieve a uniform concentration. Dosage of the phages was carried out for a period of exactly 11 days.

3.4. Bacteriophage Sampling

To avoid cross contamination, all activities related to the dosage were kept strictly separated from sampling and were performed by different crew. Samples of 100 mL were taken from two central locations in the compartment every day at 9:00 A.M. Temperature and pH of the water in the compartment were also measured at the same time.

At the surface the monitoring wells were covered with polyester covers. Under these covers, water was constantly pumped up at a rate of 4 L h^{-1} . This water was led through PVC pipes to a place downstream of the recharge compartment to avoid its contamination. A heating cable was placed in the covers and in the PVC pipes to avoid freezing of water. Samples were also taken at 4 L/h by disconnecting the tubing to fill a bottle. The volume of water samples varied depending on the expected concentration. The scheme for taking samples from the monitoring wells was based on the assumption that the phages traveled equally as fast as the salt tracer. Preliminary calculations were made to predict the breakthrough curves at the six monitoring wells. Starting 2 days before the expected breakthrough time at a given well, samples were taken every 6 hours so as to capture the rising limb of the breakthrough curve. After breakthrough was observed, samples were taken every 24 hours. Nine days later, before a decrease in phage concentration was anticipated, sampling frequency was increased to every 6 hours for about 2 days so as to capture the declining limb of the breakthrough curve. Then, samples were taken every 24 hours and later in the experiment once or twice a week. At wells W1 to W4, sample size was 250 mL as long as concentrations were above 10 pfp per 100 mL, then samples of 10 L were taken. At well W5, only 10-L samples were taken during breakthrough. At well W6, three samples of 1000 L were taken every 48 hours during maximum expected breakthrough. To that aim, a PVC container of 1000 L was filled at a rate of 1000

L d^{-1} . These samples of 1000 L were concentrated in the field by the filtration method as described by *van Olphen et al.* [1984]. If more than one well needed to be sampled at the same time, the well with the lowest breakthrough concentration, usually farthest away from the compartment, was sampled first. Temperature, and pH were immediately measured in an additional (small) sample. All samples were collected in separate cooling boxes and processed for phage enumeration within 18 hours.

3.5. Inactivation Rate of Free Bacteriophages in Water

The field study was performed in the wintertime when water temperature is low and thus inactivation is minimal. Nevertheless, in contrast to column studies, inactivation is not negligible because of longer timescales and larger distances. To measure inactivation of the phages in the water phase during the experiment, both field and laboratory experiments were carried out. In the field a sample of 2 L was taken from well W1 at a rate of 4 L h^{-1} on the third day of maximum breakthrough. This sample was distributed over 20 bottles of 100 mL, which were kept in a dark plastic bag hanging in the basin outside the compartment. Once a day, a bottle was taken from this bag for phage counting. In the laboratory, 2 L of three different waters (peptone saline, water from the recharge basin, and water from well W1 after breakthrough when phage concentrations were less than 1 pfp L^{-1}) were dosed with MS2 and PRD1 at about the same concentration as the maximum breakthrough concentration at well W1. These suspensions were also distributed over 20 bottles of 100 mL, stored in the dark at $5^\circ \pm 3^\circ\text{C}$, and analyzed regularly for a period of about 1 month. In this manner, inactivation rates under four different conditions were determined: under field conditions in well water and under laboratory conditions in peptone saline, compartment water, and well water.

3.6. Bacteriophage Enumeration

All samples were split in half. MS2 was assayed as described by the *ISO* [1995] using host strain WG49 [*Havelaar et al.*, 1984]. PRD1 was assayed according to the *ISO* [1995] using *S. typhimurium* LT2 as the host, omitting nalidixic acid in the top agar layer. If concentrations in the 100-mL samples were expected to be higher than 10^4 pfp L^{-1} , 1 mL of the appropriate dilutions was applied to 9-cm petri dishes with tryptone yeast glucose agar (TYGA). Samples of 100 mL with expected concentrations in the range of 10 – 10^4 pfp L^{-1} were divided in 20 portions of 5 mL and applied separately to 15-cm petri dishes with TYGA. Samples of 10 L were concentrated by ultrafiltration as described by *van Olphen et al.* [1984] to about 20 mL. Samples of 1000 L were filtrated with Milligard 1.2- μm cartridge filters (Millipore) in the field. These filters were eluted with beef extract at pH 9 and concentrated further by ultrafiltration to a final volume of about 20 mL. All concentrated samples were applied in aliquots of about 5 mL to 15-cm petri dishes with TYGA.

3.7. Elution Experiments

A soil sample of about 5 kg was taken from a location next to the screen of W1 at day 45 of the field experiment. This was done in order to measure the amount of attached bacteriophages that are still viable and to investigate detachment as a function of pH. The sample was kept saturated by an excess of water from the same location. Five 6-cm columns were made with soil from this sample in glass cylinders with a diameter of

Table 1. Chemical and Physical Analysis of Soil Samples

	A	B	C	D	E	W1	W4	W6
Distance, m	0	2.4	2.4	10	30
Depth, m	0–0.05	0.1–0.15	0.3–1.5	1–1.5	2–2.3	2–2.3	3.2–3.3	6–6.5
Grain size,* μm	235	240	240	203	209	209	209	203
Clay ($\leq 2 \mu\text{m}$), %	0.55	0.49	0.49	0.70	0.50	0.85	0.77	0.86
Silt (> 2 and $\leq 53 \mu\text{m}$), %	1.19	2.55	0.98	1.52	1.07	3.42	2.14	4.28
Sand ($> 53 \mu\text{m}$), %	98.26	97.26	98.53	97.78	98.43	95.70	96.87	94.84
f_{oc} , %	0.37	0.25	0.15	2.7	0.088	0.10	0.20	0.079
CEC, meq kg^{-1}	16	13	8.6	102	6.5	14	15	14
Al-oxalate, g kg^{-1} (dw)	0.20	0.18	0.23	0.23	0.19	0.32	0.27	0.41
Fe-oxalate, g kg^{-1} (dw)	1.0	1.1	1.1	0.56	0.73	1.2	1.1	1.7
Mn-oxalate, g kg^{-1} (dw)	0.026	0.022	0.012	0.021	0.0047	0.019	0.020	0.020

Sample A is taken from bottom of the compartment. Samples B–E are taken between bottom of the compartment and W1. Abbreviation dw is dry weight. Ellipses indicate that these soil samples were taken from the bottom of the compartment of approximately 1 m distance from the bank of the compartment.

*Grain size is geometric mean.

9 cm. At $5^\circ \pm 3^\circ\text{C}$, bacteriophages were eluted from a column with 400 mL of water from the recharge basin (no phages added) and from columns using the same water but with pH adjusted to 8, 9, and 10. One column was eluted with 600 mL of beef extract at pH 9. The pore water velocity was adjusted to 1.5 m d^{-1} to approximate field conditions. Fractions of 11 mL were analyzed for pH and for MS2 and PRD1 concentrations.

3.8. Conceptual Model

The major transport processes included in the mathematical model of our field experiments are advection, hydrodynamic dispersion, attachment, detachment, and inactivation. The water flow at the field site has a predominantly one-dimensional direction: from the recharge basin toward the line of wells. The temporal changes in flow velocity are negligible. Therefore it was assumed that the water flow is steady state and one-dimensional. This allowed the use of analytical solutions. In one dimension, dispersion is equal to the dispersivity times flow velocity: $\alpha_L v$.

Attachment of MS2 and PRD1 has been shown to be reversible and kinetically limited [Bales et al., 1991, 1993, 1997; Kinoshita et al., 1993]. In columns with sandy soil [Bales et al., 1989] and silica beads [Bales et al., 1991], dispersion of both MS2 and PRD1 and the salt tracer have been shown to be similar. In field studies where preferential flow was absent, PRD1 was transported at about the same rate as a conservative salt tracer [Bales et al., 1995; Pieper et al., 1997]. Therefore phage transport may be simulated using a one-site kinetic model [Bales et al., 1991; McCaulou et al., 1994; Toride et al., 1995]. Inactivation is modeled as first-order decay with different rate coefficients for free and attached phages. The governing equations read as follows:

$$\frac{\partial C}{\partial t} = \alpha_L v \frac{\partial^2 C}{\partial x^2} - v \frac{\partial C}{\partial x} - k_{att}C - \mu_f C + k_{det} \frac{\rho_B}{\theta} S \quad (1)$$

$$\frac{\rho_B}{\theta} \frac{\partial S}{\partial t} = k_{att}C - k_{det} \frac{\rho_B}{\theta} S - \mu_s \frac{\rho_B}{\theta} S \quad (2)$$

Subject to boundary conditions $C = C_0$ at $x = 0$ and $\partial C / \partial x = 0$ at $x = \infty$. Here C is the concentration of free phages (pfp m^{-3}); S is the concentration of attached phages (pfp kg^{-1}); t is the time (days); x is the distance (in meters); α_L is the dispersivity (in meters); v is the average interstitial water velocity (m

d^{-1}); ρ_B is the bulk density (kg m^{-3}); θ is the porosity (–); k_{att} and k_{det} are the attachment and detachment rate coefficients (day^{-1}) respectively; μ_f and μ_s are the inactivation rate coefficients of the free and attached phages (day^{-1}) respectively.

For our model computations a modified version of the computer code CXFIT [Toride et al., 1995] has been used. This code is based on analytical solutions of equilibrium and non-equilibrium transport models, including governing equations (1) and (2).

4. Evaluation of Results and Parameter Estimation

4.1. Soil Analysis and Water Chemistry

Results of chemical and physical analysis of soil samples are given in Table 1. The samples taken near wells W1, W4, and W6 show no differences in composition. However, the samples that were taken between the compartment and well W1 show that the organic carbon content is higher near the compartment (0.4%) and then decreases to 0.1–0.2% toward W1. The very high organic carbon content (2.7%) at 1–1.5 m (sample D) is most probably only a local feature. Related to the organic carbon content is the cation exchange capacity, which, indeed, shows a similar spatial pattern. The geometric mean grain size of samples A–C is higher in combination with a lower percentage of clay and silt than the other samples. The values of the metal ions and metal oxalates show some variability with distance but show no significant trends.

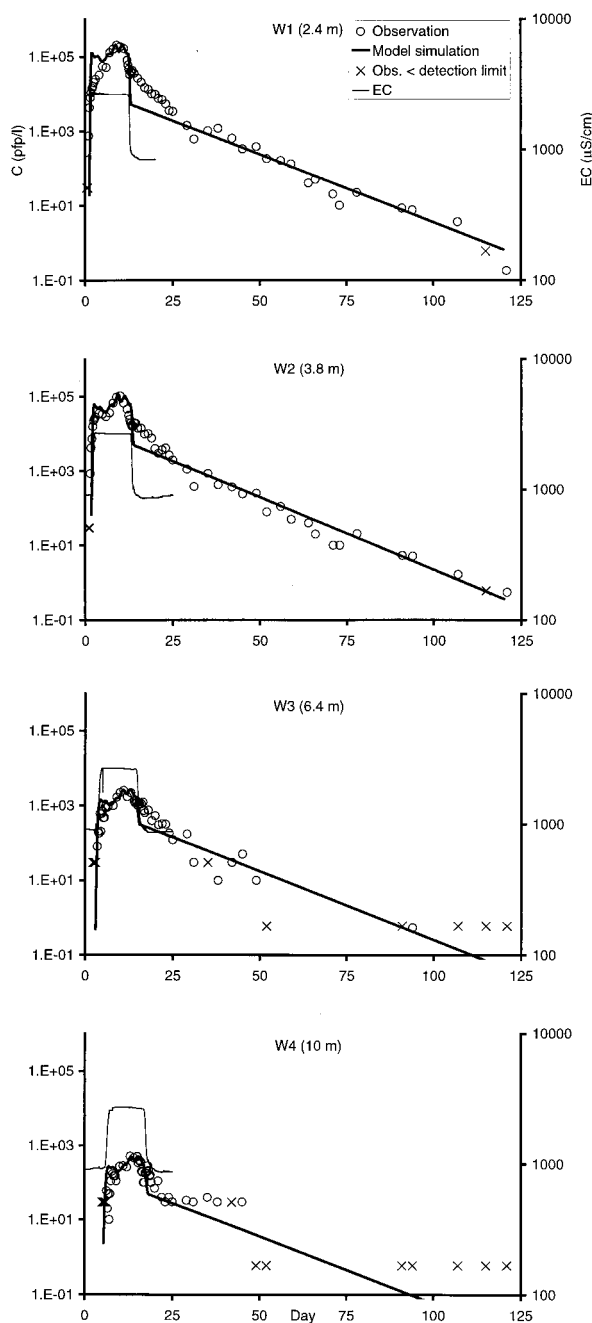
In Table 2 the chemistry of the water in the recharge basin and at the production well are given (data from drinking water company). EC is twice as high as in the water of the contaminated zone reported by Pieper et al. [1997], because concentrations of chlorine, bicarbonate, and bivalent cations are much higher. During soil passage, dissolved oxygen concentrations decrease, whereas the dissolved iron concentrations increase, because of oxidation of iron sulphides [Stuyfzand, 1993].

4.2. Description of the Breakthrough Curves of the Salt Tracer and the Phages

The concentration of MS2 measured in the recharge compartment was on the average 1.1×10^8 pfp L^{-1} and varied between 5.4×10^8 and 2.1×10^8 pfp L^{-1} . The concentration of PRD1 was on the average 1.0×10^7 pfp L^{-1} and varied

Table 2. Chemistry of Recharge and Production Well Water During Field Experiment

	Recharge Water		Production Well Water	
	Average	Standard Deviation	Average	Standard Deviation
Electric conductivity, $\mu\text{S cm}^{-1}$	822	57	914	11
Dissolved oxygen, mg L^{-1}	9.7	1.9	1.1	0.38
Ca^{2+} , μM	2200	220	2300	52
Mg^{2+} , μM	520	38	630	19
Fe (dissolved), μM	1.9	1.7	8.8	3.2
HCO_3^- , μM	2400	220	2800	41
Cl^- , μM	4000	37	4500	220

**Figure 3.** Breakthrough curves of sodium chloride (measured as electrical conductivity (EC) and MS2 at wells W1 to W4. Detection limit depends on sample volume.

between 5.4×10^6 and 2.4×10^7 pfu L^{-1} . This may be ascribed to variations in power supply to the dosage pump but not to mixing problems in the compartment. This can be deduced from the fact that EC measurements showed complete mixing of the salt in the compartment within 30 min. The temperature of the recharge water increased gradually from 2° to 7°C during the first 52 days, and that of the monitoring wells increased from 3° to 9°C during the first 93 days. Temperature of the recharge water fluctuated $\pm 1^\circ\text{C}$ during the day. The pH of the water varied between 7.3 and 8.3.

At wells W1 to W4, complete breakthrough was monitored, but at W5 and W6 only maximum breakthrough concentrations were measured (68 and 2.2 pfu L^{-1} at W5 and 0.83 and 0.060 pfu L^{-1} at W6 of MS2 and PRD1, respectively). The breakthrough curves of phages and salt tracer for wells W1 to W4 are shown in Figures 3 and 4 with the salt breakthrough curve projected over a period of 11 days for the sake of comparison. Maximum EC levels were about $2700 \mu\text{S cm}^{-1}$ at all wells. This implies that there was no dilution. As expected, breakthrough times of MS2 and PRD1 at wells W1 to W4 were the same as those of the salt tracer.

4.3. Parameter Estimation and Sensitivity Analysis

Model parameters that needed to be evaluated were ν , α_L , k_{att} , k_{det} , μ_I , and μ_S . The quantities ν and α_L were found from fitting the salt breakthrough curves using CXTFIT with the values of k_{att} , k_{det} , μ_I , and μ_S set to zero. The resulting values are shown in Table 3. The flow velocity variations were relatively small between wells W1 and W5. The values for dispersivity (α_L) were very low, confirming that the soil was rather homogeneous.

The inactivation rates of free phages were determined directly from laboratory measurements as described in section 4.4. The remaining parameters, k_{att} , k_{det} and μ_S , can, in principle, be determined with the aid of CXTFIT in inverse simulation mode. However, our computations and sensitivity analysis have shown that CXTFIT is not suitable for fitting the phages breakthrough curves. The fitted breakthrough curve obtained with CXTFIT in inverse mode is shown in Figure 5a (semilog scale) and Figure 5b (linear scale). The corresponding values for parameters k_{att} , k_{det} and μ_S are given in column for curve A of Table 4. The fitted breakthrough curve deviates significantly from the observed breakthrough curve both at high concentrations (curve A in Figure 5b) and at the tail section (curve A in Figure 5a). This is due to the fitting procedure used in CXTFIT, where model parameters are estimated by minimizing the sum of squared residuals. Because the tail concentrations are orders of magnitude smaller than

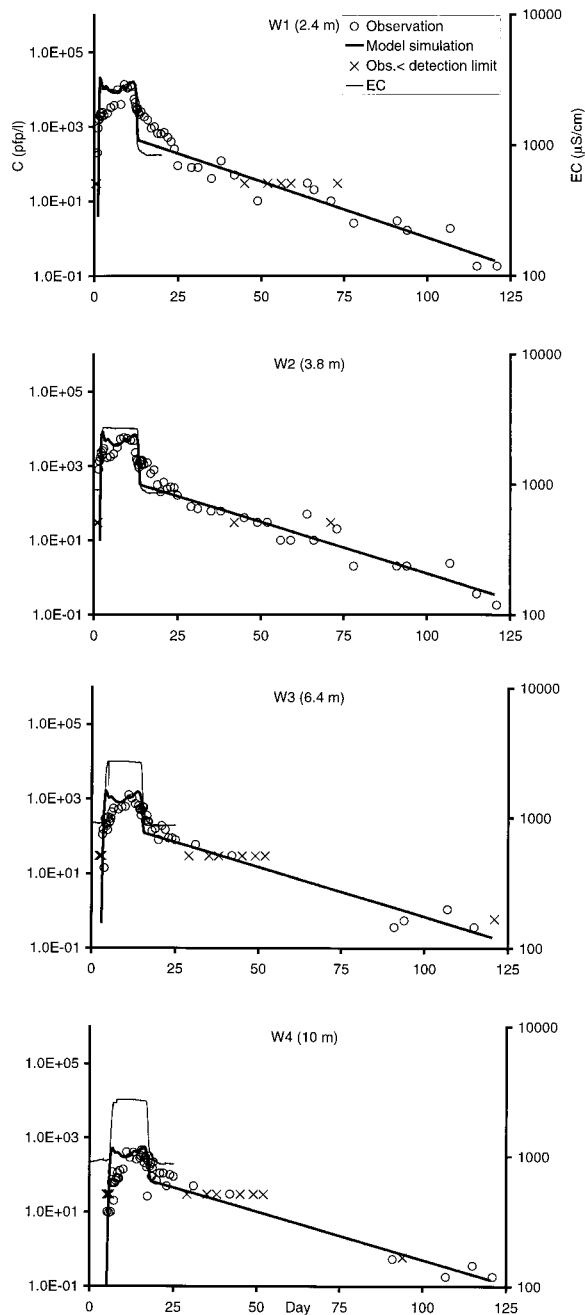


Figure 4. Breakthrough curves of sodium chloride (measured as electrical conductivity (EC) and PRD1 at wells W1 to W4. Detection limit depends on sample volume.

the maximum breakthrough concentration, their weight in the fitting procedure will be negligible. This procedure does not account properly for the processes that affect the tail section. Therefore a different approach was chosen to estimate the parameter values of k_{att} , k_{det} , and μ_S . This is described in detail in sections 4.5–4.7.

In order to show the effects of various parameters on the shape of the breakthrough curve and to aid our parameter estimation strategy, the following limited sensitivity analysis was carried out. Starting with the set of parameter values suggested by CXTFIT (column for curve A in Table 4), k_{att} was decreased from 4.6 to 4.0, and the other parameter values were

kept constant (column for curve B of Table 4). This resulted in curve B of Figures 5a and 5b having higher maximum concentrations than observed. Next the values of μ_S and k_{det} were varied over a wide range. We found that the tail slope is mainly determined by the value of μ_S , whereas its intercept is mainly affected by the value of k_{det} . Some typical values are given in Table 4. Keeping k_{det} constant, variation of μ_S results in the change of the tail slope with little effect on the intercept (compare curves A, B, and C or D and E). However, variation of k_{det} at a constant μ_S changes the intercept significantly but has a small effect on the slope (compare curves B and D or C and E). Curve E comes closest to the observed breakthrough curve. In all cases, k_{det} is much smaller than k_{att} . In none of the simulations was it possible to fit the rising and declining limbs of the observed breakthrough curve completely.

One of the major assumptions in our model is that the transport is one-dimensional. This is justified by the fact that the flow is almost everywhere horizontal and the dispersion is very small. The influence of dispersion on the calculated breakthrough curves has been investigated by varying the value of dispersivity from 0.008 to 0.024 m. The corresponding values of k_{att} that were needed to obtain the same breakthrough curves were found to be 4.0 and 4.1, respectively. This shows that variability of α_L does not significantly affect the estimation of k_{att} . The effect of lateral dispersion is expected to be even smaller as transversal dispersivity is about 10 times less than longitudinal dispersivity. Therefore a one-dimensional modeling approach is valid.

4.4. Inactivation Rates of Free Bacteriophages

Results of the inactivation experiments for free phages are shown in Figure 6. It is evident that the inactivation rate is first order under all conditions. MS2 was stable in peptone/saline. Rate coefficients calculated from these curves are given in Table 5. Inactivation of MS2 in the field test was less than in the laboratory test in water from either the compartment or the well; this could be due to temperature effects. PRD1 was very stable under all conditions in the laboratory, which is consistent with the reported stability of this phage [Yahya *et al.*, 1993]. Surprisingly, however, PRD1 was found to inactivate at a rate of 0.12 day^{-1} in the field test, 4 times faster than MS2 (0.030 day^{-1}). Both phages were enumerated from exactly the same bottles; thus, conditions were exactly the same for both phages. Naturally, for the simulation of the breakthrough curves of MS2 and PRD1 the values of μ_i obtained from the field inactivation experiment were used. These values are well within the range of values reported by others. The inactivation rate coefficient of MS2 in groundwater at 4°C has been reported to be $0.028\text{--}0.15 \text{ day}^{-1}$ by Yates *et al.* [1985] and 0.041 day^{-1} by Powelson *et al.* [1990]. Also, according to Yahya *et al.* [1993] the inactivation rate coefficients of both MS2 and PRD1 at 7°C are $0\text{--}0.092 \text{ day}^{-1}$. It should be noted that values of inactivation rate coefficients presented in this paper (in units per day) are a factor of 2.3 higher than found in many other works. This is because inactivation rate coefficients are often expressed in units of $\ln 10 \text{ day}^{-1}$ [see, e.g., Yates *et al.*, 1985]; this unit is equivalent to the value per day divided by 2.3 ($\ln 10$).

4.5. Inactivation Rates of Attached Bacteriophages

As pointed out earlier, the slope of the tail of the breakthrough curves is mainly determined by the value of μ_S . Here an approximate solution for the tail is obtained, and then measured breakthrough tails are used to evaluate μ_S . When a

Table 3. Estimated Values of Model Parameters for Six Wells

	W1	W2	W3	W4	W5	W6
NaCl						
x , m	2.4	3.8	6.4	10.2	17.1	30.1
v , m d ⁻¹	1.41	1.56	1.59	1.57	1.52	1.19
α_L ,* m	0.008	0.012	0.017	0.017	0.0096	0.08
MS2						
μ_s , day ⁻¹	0.085	0.092	0.092			
k_{att} , day ⁻¹	4.1	3.2	2.8	2.0	1.3	0.8
η^{\ddagger}	0.61	0.60	0.56	0.57	0.58	0.69
α^{\ddagger}	0.0014	0.0010	0.00092	0.00065	0.00043	0.00027
k_{det} , day ⁻¹	0.00087	0.0016	0.0026	0.0018	0.00052	0.0030
PRD1						
μ_s , day ⁻¹	0.071	0.067	0.067	0.067		
k_{att} , day ⁻¹	4.0	3.1	2.2	1.5	1.3	0.7
η	0.34	0.32	0.31	0.32	0.32	0.38
α	0.0024	0.0018	0.0013	0.00086	0.00075	0.00043
k_{det} , day ⁻¹	0.00077	0.0011	0.0018	0.0025	0.0021	0.0034

*Variable α_L is dispersivity.†Variable η is single collector efficiency.‡Variable α is sticking efficiency.

pulse of dosed phages has passed a position x , the attachment starts to decrease. Assuming that dispersion is negligible and rewriting (1) and (2) in a Lagrangian reference frame (i.e., following the water motion), we obtain

$$\frac{\partial C}{\partial t} = -(k_{att} + \mu_l)C + k_{det} \frac{\rho_B}{\theta} S \quad (3)$$

$$\frac{\rho_B}{\theta} \frac{\partial S}{\partial t} = -(k_{det} + \mu_s) \frac{\rho_B}{\theta} S + k_{att} C \quad (4)$$

Elimination of S between these two equations yields

$$\frac{\partial^2 C}{\partial t^2} + \lambda_1 \frac{\partial C}{\partial t} + \lambda_2 C = 0 \quad (5)$$

where

$$\lambda_1 = k_{att} + \mu_l + k_{det} + \mu_s \text{ and } \lambda_2 = k_{att}\mu_s + k_{det}\mu_l + \mu_l\mu_s$$

The solution of (5) is

$$C = C_0 \exp(-\xi t) \quad (6)$$

with

$$\xi = \frac{1}{2} \lambda_1 \left[1 - \sqrt{1 - \frac{4\lambda_2}{\lambda_1^2}} \right] \quad (7)$$

Taylor series expansion of the term inside the brackets and neglecting second and higher-order terms yields

$$\begin{aligned} \xi &= \frac{1}{2} \lambda_1 \left[1 - \left(1 - 2 \frac{\lambda_2}{\lambda_1^2} + \frac{1}{2} \left(\frac{\lambda_2}{\lambda_1^2} \right)^2 + \dots \right) \right] \\ &\equiv \frac{\lambda_2}{\lambda_1} = \left(\mu_s + \frac{k_{det}\mu_l + \mu_l\mu_s}{k_{att}} \right) \left(1 + \frac{\mu_l + k_{det} + \mu_s}{k_{att}} \right)^{-1} \end{aligned} \quad (8)$$

Now, because k_{att} is orders of magnitude bigger than the other rate coefficients, ξ may be approximated with μ_s so that the solution (6) becomes

$$C = C_0 e^{-\mu_s t} \quad (9)$$

Equation (9) shows that the slope of the tail of the breakthrough curve in a semilog graph is mainly determined by μ_s .

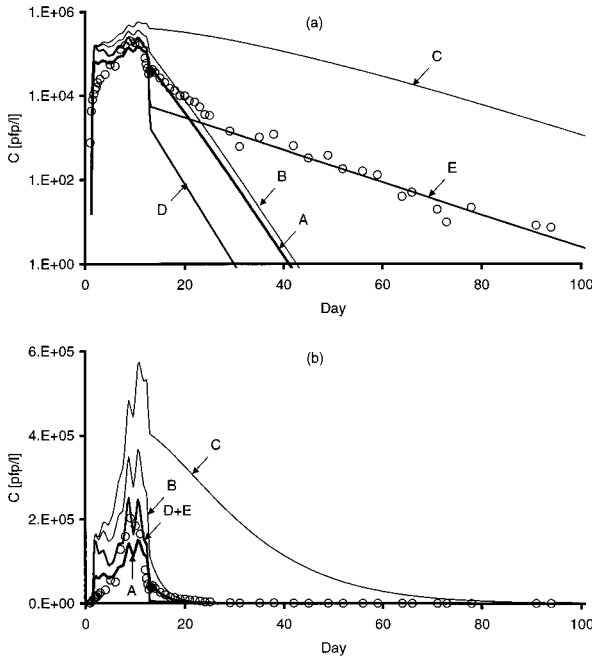


Figure 5. Breakthrough curves of MS2 at well W1 with concentrations on (a) a logarithmic scale and (b) a linear scale. Curve A is a fitted curve using CXTFIT in inverse mode. Curves B, C, D, and E are simulated curves. For corresponding parameter values see Table 4.

Table 4. Sensitivity Analysis: Parameter Values Used to Calculate the Breakthrough Curves A–E in Figures 5a and 5b

	A	B	C	D	E
k_{att}	4.6	4.0	4.0	4.0	4.0
k_{det}	0.041	0.041	0.041	0.0008	0.0008
μ_s	0.44	0.44	0.09	0.44	0.09

Parameter values are given per day.

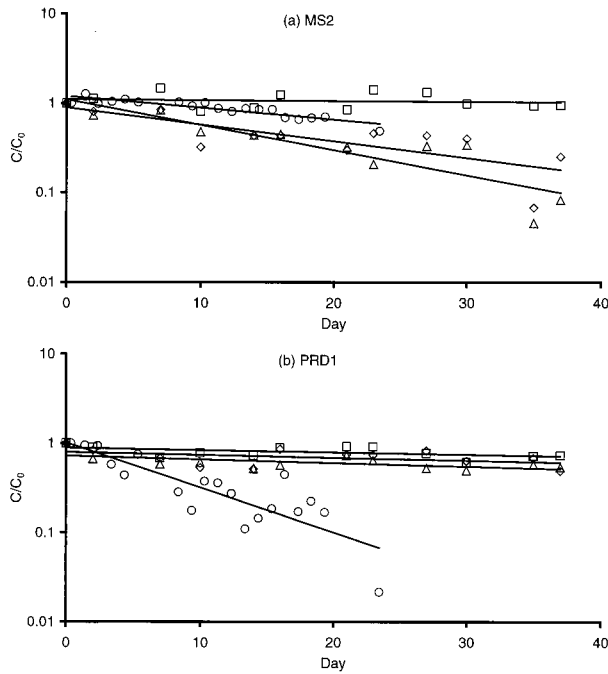


Figure 6. Inactivation of (a) MS2 and (b) PRD1 at the laboratory in peptone/saline (squares), in compartment water (diamonds), in W1 well water (triangles), and at the field site in W1 well water (circles). Lines are the corresponding linear regression lines.

On the basis of the values of μ_s from the breakthrough curves were estimated, and the results are given in Table 3.

At wells W1 to W3, μ_s for MS2 varied only slightly from 0.085 to 0.092 day⁻¹. The value of 0.092 day⁻¹ was also employed in simulation of breakthrough at W4. Likewise, μ_s for PRD1 at wells W1 to W5 was fairly constant: 0.067–0.071 day⁻¹. It is evident that the inactivation rate of attached MS2 was higher than that of free MS2. The same can be said for attached PRD1 compared to free PRD1 in the laboratory test but not compared to PRD1 in the field test. An increase in inactivation rate of MS2 and PRD1, by a factor of 2 to 3, due to attachment to soil has also been reported by *Blanc and Nasser* [1996].

4.6. Attachment Rate Coefficients

For the evaluation of k_{att} an approximate solution of (1) was developed for the duration of the experiment where maximum breakthrough concentrations (C_{max}) were observed. In this period a steady state situation was assumed to have been reached. Neglecting detachment, (1) then reads as follows:

$$\alpha_L v \frac{\partial^2 C_{max}}{\partial x^2} - v \frac{\partial C_{max}}{\partial x} - (k_{att} + \mu_l) C_{max} = 0 \quad (10)$$

The solution of this equation is

$$C_{max} = C_0 \exp(-\psi x)$$

where

$$\psi = \frac{\left[\sqrt{1 + \frac{4\alpha_L}{v} (k_{att} + \mu_l)} \right] - 1}{2\alpha_L} \quad (11)$$

Here C_0 is the maximum concentration of dosed bacteriophages. This solution can be rearranged to

$$k_{att} = v \frac{\left[1 - 2\alpha_L \frac{2.3}{x} \log_{10} \left(\frac{C_{max}}{C_0} \right) \right]^2 - 1}{4\alpha_L} - \mu_l \quad (12)$$

With μ_l already determined from inactivation experiments, (12) can be used to estimate the value of k_{att} . The results are reported in Table 3. Apparently, k_{att} decreases with distance from about 4 to 0.8 day⁻¹ for both phages.

Attachment rate coefficient k_{att} is known to depend on microscale flow and diffusion characteristics as well as surface properties of viruses and soil grains. It is possible to exclude the effects of flow and diffusion by expressing the attachment rate of viruses in terms of sticking efficiency α , defined by [*Yao et al.*, 1971; *Bales et al.*, 1991, 1993; *McCaulou et al.*, 1994; *Penrod et al.*, 1996]:

$$\alpha = \frac{2}{3} \frac{d_g}{(1 - \theta)} \frac{k_{att}}{v} \frac{1}{\eta} \quad (13)$$

Here d_g is the average diameter of the grains (in meters). MS2 and PRD1 are small in size (26 and 62 nm, respectively), and their transport in the immediate vicinity of the soil grains is dominated by Brownian diffusion [*Penrod et al.*, 1996]. Thus the single collector efficiency η was calculated as given by *Penrod et al.* [1996].

Values for α are also given in Table 3. Similar to k_{att} , α decreases with distance from 0.0014 to 0.00027 for MS2 and from 0.0024 to 0.00043 for PRD1. These very low values of α reflect unfavorable conditions for attachment, mainly because of the relatively high pH of 7.3–8.3.

4.7. Detachment Rate Coefficients

Given the values of k_{att} , μ_l and μ_s as described above, the value of k_{det} was adjusted so as to obtain a best fit to the measured breakthrough curve. These values are given in Table 3. It is apparent that, compared to attachment, detachment is very slow.

4.8. Removal Versus Distance

Figure 7 shows reduction of the maximum concentrations of both phages versus distance. Removal of both phages appeared to be very similar. It can be seen that reduction is not linear with distance. However, linear regression analysis of the reduction data with distance from wells W1 to W6 revealed high correlation coefficients of 93% and 97% for MS2 and PRD1, respectively. This suggests that reduction was linear with distance between the monitoring wells and that the first 3.1 and 2.6 log₁₀ reduction in concentrations of MS2 and PRD1, re-

Table 5. Inactivation Rate Coefficient μ_l for MS2 and PRD1

Location	Temperature	Water	MS2	PRD1
Laboratory	5° ± 3°C	peptone/saline	0.0019	0.0060
		recharge water	0.044	0.0074
		water from W1	0.064	0.0094
Field site	2°–5°C	water from W1	0.030	0.12

Values are given per day.

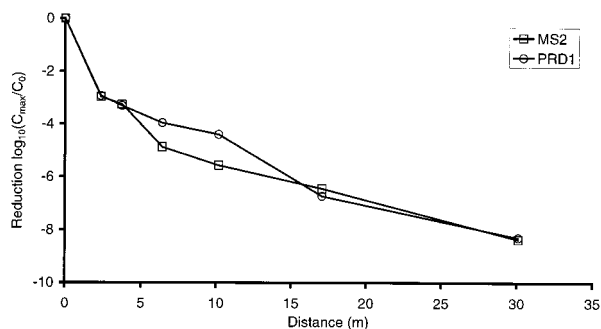


Figure 7. Reduction ($\log_{10}(C_{\max}/C_0)$) of MS2 and PRD1 versus distance (in meters).

spectively, occurred somewhere before the first well at a higher attachment rate.

4.9. Elution Experiments

The results of the elution experiments described in section 3.7 are shown in Table 6. Total numbers of bacteriophages eluted at different pH are given. With increasing pH, more phages were eluted. This effect seems stronger for PRD1 than for MS2. Nevertheless, the amount of phages that detached is much smaller than predicted by the model. According to the simulation with CXTFIT [Toride *et al.*, 1995] the number of phages that should have been eluted by beef extract is about 35,000 for MS2 and 4900 for PRD1. These values are 80 times higher for MS2 and 270 times higher for PRD1 than actually were eluted with beef extract. Assuming that elution with beef extract at pH 9 released all attached phages, it seems that phages were removed already at a much higher attachment rate prior to well W1. This is in agreement with the high removal rate between the compartment and W1.

5. Discussion and Conclusions

Removal of model viruses by dune recharge was studied at a field site in the dune area of Castricum, Netherlands. After dosage of the recharge water with bacteriophages MS2 and PRD1 their breakthrough was followed at different monitoring wells along a flow line. A one-site kinetic model was employed to estimate parameter values for attachment, detachment, and inactivation rate coefficients. Comparison of calculated and measured breakthrough curves shows that maximum breakthrough concentrations and the tail sections are modeled satisfactorily. Noticeable discrepancies are found at the end of the

rising limbs and the onset of the declining limbs. This effect, however, becomes less and less pronounced as we get farther away from the recharge basin. This indicates the following: (1) There is an unknown removal/release process, which is not being modeled here. (2) This process seems to be present mainly in the section before W1. (3) The extra attachment process seems to be reversible, as we can find a higher measured concentration at the declining limb than calculated.

At this stage we can only speculate on the nature of this extra process. One explanation may be that the attachment rate coefficient is proportional to the concentration of free phages and the detachment rate coefficient is proportional to the concentration of attached phages. Another possibility is that because of heterogeneities of the soil or within the phage population, attachment and detachment rate coefficients are much higher in the section before W1 (see below).

This field study has shown that at least 8 \log_{10} reduction of phage concentration can be achieved within 30 m of passage in sandy dunes for both MS2 and PRD1. This corresponds to a travel time of about 25 days. Within the first 2.4 m, reduction was about 3 \log_{10} , and an additional 5 \log_{10} was removed within 30 m. Thus the reduction in concentrations of MS2 and PRD1 was not linear with distance. The removal of the bacteriophages before reaching W1 is much higher than thereafter. After W1 the reduction increased linearly with distance whereby the maximum breakthrough concentrations were mainly determined by attachment. The following observations indicate the existence of an extra removal process before well W1:

1. Linear regression of the reduction between wells W1 and W6 shows a correlation coefficient of 93% and 97% with distance for MS2 and PRD1, respectively. Deviations from the regression line probably reflect subsurface heterogeneities and measurement errors.

2. The calculated amount of attached phages was found to be 100 times higher than the amount that could actually be eluted by beef extract from a soil sample taken near the screen of W1. This suggests that the bacteriophages were removed before reaching W1 by processes unaccounted for in the model.

3. Soil analysis showed a higher organic carbon content in the soil samples from the first 5 cm and 10–15 cm, as well as in soil from 1–1.5 m. Bales *et al.* [1993] showed that with increasing small amounts of hydrophobic organic material bonded to silica beads, the attachment rate coefficient of MS2 increased. The initial higher removal of the bacteriophages may thus be explained by hydrophobic interactions with organic matter.

Table 6. Elution of MS2 and PRD1 From Soil Columns at Different pH

pH			MS2-Phages, pfp		PRD1-Phages, pfp	
Influent	Effluent	Column Dry Weight,* g	Eluted	Simulated [†] (CXTFIT)	Eluted	Simulated [‡]
7.5	7.5–7.9	576	129	33,000	0	4,600
8	7.7–8.0	572	117	33,000	0	4,600
9	8.1–8.5	615	191	36,000	2	4,900
10	7.8–8.9	572	339	33,000	30	4,600
9+ beef extract	8.2–8.7	607	430	35,000	18	4,900

*Weight is measured after 24 hours at 105°C.

[†]MS2 measured C equals 330 pfp L⁻¹; simulated C equals 358 pfp L⁻¹; simulated S equals 58 pfp g⁻¹.

[‡]PRD1 measured C equals 33 pfp L⁻¹; simulated C equals 48 pfp L⁻¹; simulated S equals 8 pfp g⁻¹.

Yet another possibility that could explain the initially greater removal of the bacteriophages may be variations in the surface properties of the phage population. Phages that are stickier are removed faster. This possibility has been posed before by Pieper *et al.* [1997], who also showed initial greater removal of PRD1.

One may contemplate that the existence of fine-grained sediments at the bottom of the compartment may be the cause of more early attachment. This possibility can be ruled out. It was shown that the average grain size of the soil was larger in the samples from the first 5 and 10–15 cm than in soil samples taken at the monitoring wells.

Although attachment was found to be the major process in removing the phages, the corresponding very low estimated values of the sticking efficiencies show that conditions for attachment were highly unfavorable. This is consistent with the concept that in sandy soils with relatively high *pH*, electrostatic repulsion is important [Bales *et al.*, 1991, 1993].

However, it must be mentioned that we had expected higher values for phages sticking efficiencies for two reasons. First, in our case the concentrations of oxalate-extractable iron were found to be 3–10 times higher than in the sewage-contaminated soil reported by Pieper *et al.* [1997]. Furthermore, their soil contained about 1% bonded organic matter, which is considerably higher than in our case. This would imply that in our case, there is a greater amount of available iron-oxide coatings, which promote attachment. Second, concentrations of bivalent cations were rather high in our case (Table 2). Bales *et al.* [1991] showed that Ca^{2+} -concentrations of 1–100 μM at *pH* 7 are already sufficient to promote attachment of viruses. However, in our case, *pH* was still the major factor in suppressing the attachment rate of the bacteriophages. According to Loveland *et al.* [1996], electrostatic forces between PRD1 and quartz sand or ferric oxyhydroxide are repulsive at all separation distances at a *pH* above 7.3. The *pH* values in our field study were found to be 7.3–8.3, and these are clearly higher than the *pH* values in the study by Pieper *et al.* [1997], which were reported to lie between 6 and 6.7.

The removal rates of MS2 and PRD1 were almost equal. The very low sticking efficiencies found in this study reflect relatively conservative behavior of both MS2 and PRD1 and suggest they are suitable indicators for virus transport. In other words, these low sticking efficiencies indicate that the soil conditions are unfavorable to attachment of other viruses too.

Attachment was shown to be reversible, with a very small detachment rate. The elution experiment at different *pH* values and with beef extract confirm the finding of Bales *et al.* [1993] that large chemical perturbations are needed to enhance detachment, at least for MS2. In the present field study, *pH* varied from 7.3 to 8.3. The elution experiment indicated that within this range release of MS2 and PRD1 was not enhanced dramatically. This is in agreement with the observations of the field study.

This study has increased our understanding of the removal processes for bacteriophages MS2 and PRD1 at field scale. A number of important properties, in particular, attachment characteristics, have been quantified. Reasonably good simulation results have been obtained with a simple one-dimensional analytical model. However, a number of important questions have remained unanswered. The role of heterogeneities in medium properties and possible variation of attachment and detachment coefficients with concentrations

have to be investigated. These will be subjects of future numerical modeling and laboratory experiments.

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