

## Seasonal virus removal by alternative onsite wastewater treatment systems

Matthew R. Olson, Richard P. Axler, Randall E. Hicks, Jerald R. Henneck and Barbara J. McCarthy

### ABSTRACT

Viral contamination of public waters is a leading health concern around the world, including in Minnesota where cold climate, abundant onsite systems on poor or thin soils, and abundant surface water resources present a significant risk of wastewater pathogens reaching sensitive water sources. Three alternative onsite treatment systems, a sand filter, peat filter and subsurface-flow constructed wetland (CW) at a field research site were evaluated for seasonal virus removal by seeding each with MS2 bacteriophage. The sand and peat filters and CW removed 2.7, 7.0, and 1.4 log<sub>10</sub> of MS2, respectively, during summer and 1.8 and 6.9 log for the sand and peat filter during winter (CW not seeded). Somatic coliphage reductions for the sand filter, peat filter and CW were 2.9, 3.5, 1.0 log<sub>10</sub> in summer, and 1.5, 2.8, 0.7 log<sub>10</sub> during winter, respectively over a 3 year period. During this period, fecal coliform log<sub>10</sub> reductions were 2.9, 4.6, 2.0 in summer for the sand and peat filters and CW, and 2.0, 4.6, 1.6 in winter. The peat filter was the most effective system for removing MS2, somatic coliphage and fecal coliforms during both winter and summer but all systems removed > 90% of viruses throughout the year.

**Key words** | fecal coliforms, onsite treatment, pathogens, pollution, viruses, wastewater

**Richard P. Axler** (corresponding author)  
**Jerald R. Henneck**  
**Barbara J. McCarthy**  
Natural Resource Research Institute,  
5013 Miller Trunk Highway,  
Duluth, MN 55811,  
USA  
Tel: 218-720-4316/4279  
Fax: 218-720-4328  
E-mail: [raxler@nrri.umn.edu](mailto:raxler@nrri.umn.edu)

**Randall E. Hicks**  
Department of Biology,  
University of Minnesota-Duluth,  
Duluth, MN 55812,  
USA

**Matthew R. Olson**  
Department of Microbiology,  
University of Iowa,  
Iowa city, IA 52242,  
USA

### INTRODUCTION

Viruses are of particular concern to humans due to their highly infectious nature and the low dose required to cause infection (Bosch 1998). Viral pathogens from contaminated water supplies alone are responsible for at least 5–18 million deaths a year, mostly in infants in developing countries (Farthing 1989). Across the United States, about 26 million homes utilize onsite septic systems and nearly half of these are over 30 years old and failing or non-compliant with state regulations (USEPA 2002). Researchers have suggested that septic systems are the most commonly reported cause of groundwater contamination in the US and have indicated that even properly working septic systems remove only 24–83% of enteric viruses (Payment *et al.* 1986; Powelson & Gerba 1994; Higgins *et al.* 2000). These viruses, including polio virus, hepatitis, norwalk virus, rotavirus and others, can easily reach water

sources and become a serious human health concern. This problem is accentuated in communities in northern Minnesota due to lack of centralized wastewater treatment, small lot size, close proximity to water sources, poor or thin soils, and a long, severe winter during which performance may decrease dramatically (Crosby *et al.* 1998; McCarthy *et al.* 1998, 1999; Axler *et al.* 2001; Henneck *et al.* 2001; Pundsack *et al.* 2001; Kadlec *et al.* 2003). In Minnesota, nearly 30% of the population is dependent on onsite treatment systems with more than 500,000 individual onsite treatment systems in use state-wide (MPCA 1996).

Several alternative onsite wastewater treatment systems (more recently called performance-based systems) have been developed which may potentially alleviate these problems. Sand filters, peat filters and constructed wetlands (CWs) in combination with septic tanks, have been shown

to be effective in warm climates at removing solids, pathogens (Gersberg *et al.* 1987; Barrett *et al.* 2001; Hill & Sobsey 2001; Karpiscak *et al.* 1999), biochemical oxygen demand (BOD<sub>5</sub>) and nitrogen and phosphorus (McCarthy *et al.* 1996, 1997, 1998). The efficiency of wastewater treatment has been shown to decrease in cooler climates. McCarthy *et al.* (1997, 1998, 1999) and Axler *et al.* (2001) showed significant decreases in the reduction of nitrogen, phosphorus and BOD<sub>5</sub> in winter relative to summer. Despite the poorer performance, these systems still produced acceptable effluent that was comparable to that produced by secondary treatment in municipal wastewater treatment plants. The winter decrease in treatment is thought to be largely due to a reduction in overall microbial metabolism (Sundaravadivel & Vigneswaran 2001). Although treatment was shown to be acceptable for total suspended solids (TSS) and BOD<sub>5</sub>, much less data exists for the effect of cold temperature on the removal of potential human pathogens, that is, bacteria, parasitic protozoans, helminths and viruses. In fact, the use of these alternative systems in Minnesota was delayed until effective pathogen removal in both winter and summer could be demonstrated (McCarthy *et al.* 1997; Crosby *et al.* 1998; Wallace *et al.* 2001; Kadlec *et al.* 2003).

In northern climates, most of what is known about pathogen removal is based on traditional indicator organisms (i.e. fecal coliforms and *E. coli*). Recently, demonstration projects performed near Duluth, Minnesota, at a cluster CW treatment system at Grand Lake and at replicate residential sized systems at the North East Regional Correction Center (NERCC) research site showed them to be effective in removing fecal coliforms year round, but with reduced efficiency in the winter (McCarthy *et al.* 1998, 1999; Axler *et al.* 2001; Henneck *et al.* 2001; Monson-Geerts *et al.* 2001; Pundsack *et al.* 2001, 2005). Traditional bacterial indicators have come under intense scrutiny as indicator organisms for human pathogens because of their lack of correlation with levels of enteric viruses and their low survivability in aquatic environments (Goyal & Gerba 1983; Stetler 1984; Havelaar *et al.* 1993; Ferguson *et al.* 1996; Calci *et al.* 1998; Leclerc *et al.* 2001). Bacteriophages, viruses that only infect bacteria, have been suggested as a better indicator for evaluating removal of viral pathogens in wastewater treatment systems (Debartolomeis & Cabelli

1991; IAWPRC 1991; Havelaar *et al.* 1993, USEPA 2001). Somatic coliphages (i.e.  $\Phi \times 174$ ) have been proposed for this purpose, but their ability to replicate in the environment makes them a less than ideal candidate for a good indicator of viral contamination. F+ bacteriophages, or male specific bacteriophages, are more resistant to environmental stressors and behave more similarly to the enteric viruses in aquatic environments than traditional indicators of fecal contamination (Havelaar *et al.* 1986; IAWPRC 1991; Meschke & Sobsey 2003). A common male specific phage used as an environmental indicator is the MS2 bacteriophage, which is of similar size, shape and surface potential to many pathogenic viruses (IAWPRC 1991).

Considerably more information exists regarding pollution by onsite systems removal in warm climates than in cold climates. This information gap has hindered acceptance of alternative systems in Minnesota, where the abundance of onsite systems coupled with poor or thin soils, and abundant, sensitive water resources have created a strong need for more effective systems than the traditional septic tank-leach field. The University of Minnesota-St Louis County NERCC research facility in northern Minnesota is a unique site where duplicated, experimental alternative systems have been examined for treatment of wastewater for over 5 years. Removal data for solids, organic matter, nutrients and fecal coliforms has been routinely collected, but little was known about how well these systems removed viruses. Therefore, the objective of the present study was to estimate virus removal for comparison with traditional fecal coliform based estimates by routinely monitoring somatic coliphage densities, and by determining virus removal by inoculating systems with MS2 to determine specific removal rates for this pathogen surrogate.

## MATERIALS AND METHODS

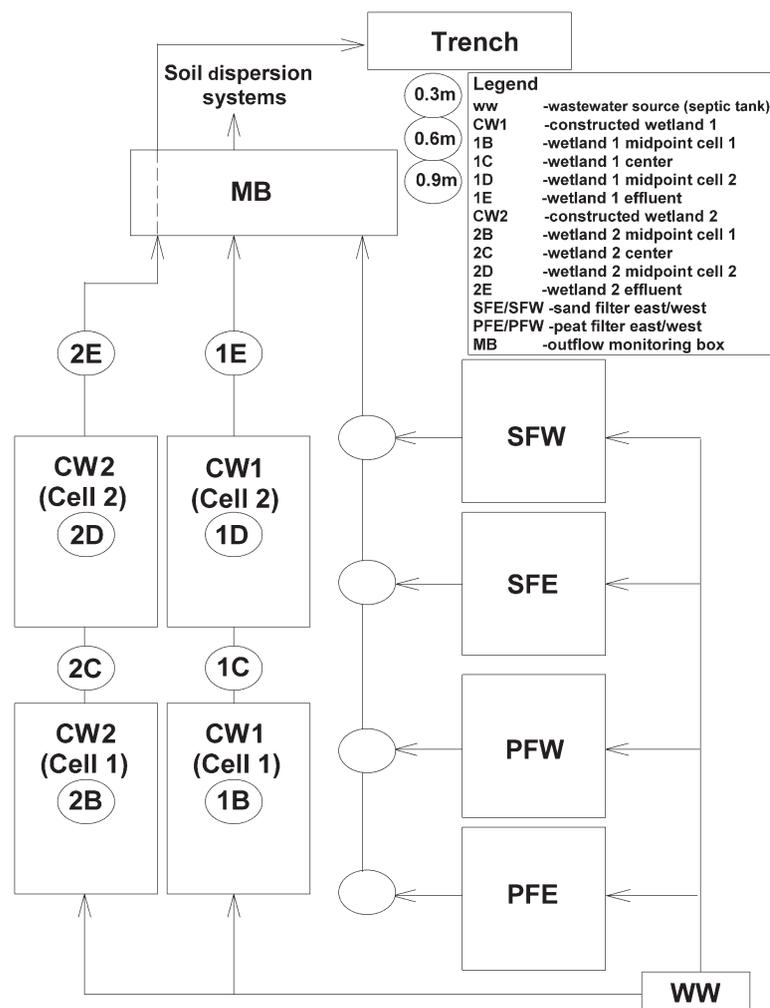
### Site description

Alternative onsite wastewater treatment systems were built between 1995 and 1996 at the Northeast Regional Correction Center (NERCC, ~150 inmates) approximately 18 km north of Duluth, Minnesota, in order to have a large reservoir of continuous wastewater. Each system was designed to simulate a single 2–3 bedroom home using a

common source of septic tank effluent and similar daily flows so that different systems could be directly compared. As of 2001, 28 alternative onsite systems have been evaluated at the NERCC site as part of a collaborative study with local and state agencies and private businesses coordinated by the Natural Resource Research Institute (NRRI) at the University of Minnesota-Duluth. The systems examined in the present study were duplicated and included subsurface flow (SSF) constructed wetlands (CW), peat filters and intermittent sand filters (Figure 1). All systems were designed to achieve a performance level typical of secondary treatment of  $25 \text{ mg TSS l}^{-1}$  and  $30 \text{ mg BOD}_5 \text{ l}^{-1}$ , and disinfection to a recreational bathing standard of 200 fecal coliform bacteria per 100 ml. Two trenches were also

present that received peat filter and constructed wetland effluent. They were ponded with 2–5 cm of wastewater and sampled at 0.3 m (1'), 0.6 m (2'), and 0.9 m (3') depth in the soil using pan lysimeters (details in McCarthy *et al.* 1997). Each system was semi-continuously dosed with septic tank effluent (hourly for the CW and every 4–6 hours for the peat and sand filters) at a daily rate of approximately  $1,000 \text{ l day}^{-1}$ . Average septic tank effluent constituents of concern were  $191 \text{ mg BOD l}^{-1}$ ,  $1.4 \times 10^5 \text{ CFUs } 100 \text{ ml}^{-1}$  fecal coliforms,  $12 \text{ mg TP l}^{-1}$ , and  $55 \text{ mg TN l}^{-1}$  during the experiments reported here.

Each lined, constructed wetland (duplicates CW1 and CW2) was filled with gravel to a depth of 0.45 m and consisted of two cells in a series each  $37 \text{ m}^2$  in surface area with a



**Figure 1** | Schematic of Northeast Regional Corrections Center (NERCC) experimental site located 18 km north of Duluth, Minnesota. The systems located at this site were intended as a demonstration comparing year round, long-term performance of various alternative onsite wastewater treatment systems including replicated constructed wetlands, sand filters, peat filters and traditional drain field trenches. Circles within the figure represent sampling points.

nominal retention time of 13 days and areal loading rate of 2.6 g BOD per m<sup>2</sup> day<sup>-1</sup>. The first cell of each wetland was planted with cattail species, *Typha latifolia* and *Typha angustifolia*, and was designed to achieve a 6.5 day hydraulic retention time (HRT) and achieve the level of secondary treatment based on best available design information (details in Axler *et al.* 1996, 2001; Kadlec & Knight 1996; McCarthy *et al.* 1997). The second cells of each constructed wetland were designed to enhance nutrient removal by doubling the HRT of the wetlands to 13 days to improve nitrogen removal (see Axler *et al.* 2001). These cells were planted with greenhouse raised soft-stem bullrush, *Scirpus taebertmontani*, also intended to aid in N-removal. In addition, limestone gravel was used as second cell substrate to potentially improve phosphate removal. A shallow standard treatment trench constructed in a loamy sand soil received constructed wetland and peat filter effluent as described above.

Each sand filter was approximately 30 m<sup>2</sup> in surface area and 1.2 m deep with an areal loading rate of 6.4 g BOD per m<sup>2</sup> day<sup>-1</sup>; peat filters were 28.7 m<sup>2</sup> in area, 1.4 m deep and had an areal loading rate of 6.7 g BOD per m<sup>2</sup> day<sup>-1</sup> (design and construction details in McCarthy *et al.* 1997 and Monson-Geerts *et al.* 2001). Both filters were vertically pressure-dosed and retention times were typically on the order of hours or less. Inflow rates measured by water meters, and effluent rates measured by tipper buckets at effluent ports, were recorded daily during virtually all sampling periods. The peat filters and constructed wetlands began receiving septic tank effluent (STE) in November 1995 and the sand filters in May 1996. The wetlands were planted in 1996 and were fully vegetated by late 1997. All systems were operated and maintained nearly continuously from 1996 to 2001. Further description, images and results for these and other NERCC alternative systems (currently referred to as performance-based systems) may be found at <http://septics.coafes.umn.edu/research/index.html>.

### MS2 propagation

An overnight culture of *E. coli* HS(pFamp)R, a pilus forming, F<sup>+</sup> bacteria, was prepared in 50 ml of nutrient broth supplemented with 1 ml each of 0.22 µm filter sterilized supplemental solutions (30 g l<sup>-1</sup> calcium chloride, 100 g l<sup>-1</sup> glucose, 15 g l<sup>-1</sup> magnesium sulfate) and incubated at 37°C.

Stock cultures of MS2 (ATCC 15597-B1) were prepared by inoculating 100 ml of *E. coli* Famp culture in nutrient broth with 1 ml of MS2 and incubating at 37°C for 18–24 hours. This mixture was then centrifuged at 4,500 rpm for 20 minutes to pellet any bacterial cells in the broth and the supernatant, containing the MS2 phage, was decanted into a sterile flask stored in the dark at 4°C until used.

### MS2 dosing

MS2, in nutrient broth, was seeded into a sand filter, a peat filter and a constructed wetland system during a morning dose of wastewater. The sand and peat filters were seeded once with the MS2 virus due to their relatively short retention times, and the CW was seeded with equal portions daily at similar times for 5 consecutive days. Summer was defined by systems having effluent temperatures greater than 13°C and winter was defined as having average effluent temperatures below 9°C which corresponded to the period when above-ground vegetation was absent in the CW beds. Snow and/or ice persist to early May in most years in northern Minnesota. The sand filter was inoculated on 7 April 2001 (winter conditions) with 9.2 × 10<sup>11</sup> plaque forming units (PFU) of MS2 and on 25 June 2001 (summer conditions) with 2.5 × 10<sup>11</sup> PFU. The peat filter was inoculated with 2.4 × 10<sup>11</sup> PFU of MS2 on 7 May 2001 (winter) and with 5.8 × 10<sup>11</sup> PFU on 6 June 2001 (summer). The CW was inoculated for five consecutive days from 1 August to 5 August 2001 (summer) with a total of 4.0 × 10<sup>12</sup> PFU approximately evenly distributed. These inocula were added during the routine dosing cycle of ~40–240 litres of wastewater, depending on the system. Viral doses were increased in the summer for all systems to compensate for expected increases in treatment performance.

### Sample collection and analysis

Influent and constructed wetland effluent samples were collected manually in sterile 100 ml polypropylene bottles; sand and peat filter samples were collected by an automated sampler. Constructed wetland effluent and centre samples (2E and 2C in Figure 1) were collected from a single collection port, whereas mid-cell samples (2B and 2D) were collected by compositing water pumped from sampling pipes at two points

across the wetland, each at three different depths. After collection, all samples were transported to the laboratory on ice. Viral assays for the sand and peat filter samples were initiated as soon as possible after collection, typically less than 2 hours. However, samples from days 1 to 7 in the CW experiment were frozen at  $-80^{\circ}\text{C}$  after collection for several days because of a temporary problem with the SAL technique (see below). Subsequently, a set of storage experiments comparing freshly analysed to frozen samples yielded a correction factor of 1.78 for these dates to correct for storage losses (details in Olson 2004). Effluent samples were collected from each system until MS2 levels decreased to less than one standard deviation of background concentrations or, for the peat filter, three consecutive samples below detection.

The MS2 titre of all stocks and wastewater samples were determined using the single agar layer technique (DeBorde *et al.* 1998; USEPA 2001). In brief, samples were serially diluted (if needed) in 1X phosphate buffered saline (PBS) and 10 ml of the diluted sample along with 250  $\mu\text{l}$  of an 18 hour culture of *E. coli* HS(pFamp)R were added to a 150 mm petri dish. Double strength tryptone agar supplemented with  $\text{MgSO}_4$  ( $15\text{ g l}^{-1}$ ), glucose ( $100\text{ g l}^{-1}$ ),  $\text{CaCl}$  ( $30\text{ g l}^{-1}$ ) and ampicillin/streptomycin ( $15\text{ }\mu\text{g ml}^{-1}$  each) were then added to each plate and allowed to solidify. Viral plaques on each plate were counted and recorded the following day (18–24 hours later). Fecal coliforms and somatic coliphage determinations followed *Standard Methods* (1998).

### Data analysis

Percentage and  $\log_{10}$  removal efficiencies were calculated for each onsite treatment system by estimating the total amount of MS2 virus retained by each system. These calculations assumed that there was no viral replication once the virus was released into the treatment systems, as has been shown by numerous studies of wastewater within constructed wetlands (e.g. Gersberg *et al.* 1987; IAWPRC 1991).

$$\% \text{ Reduction} = [100 - \{(\sum \text{effluent MS2} / \sum \text{influent MS2}) \times 100\}] \quad (1)$$

$$\text{Log}_{10} \text{ Reduction} = \log_{10}(\sum \text{influent MS2} / \sum \text{effluent MS2}) \quad (2)$$

Minimum detection limit of MS2 in this study was 1 viral plaque within a sample of a given volume and determined as:

$$\text{MS2 detection limit} = (1/\text{millilitres of sample plated}) \quad (3)$$

$Q_{10}$  values were calculated to summarize the relative temperature sensitivity of each system. A  $Q_{10}$  value of 1.0 signifies that the system is non-temperature sensitive, while a higher  $Q_{10}$  denotes increased temperature sensitivity.

$$Q_{10} = (k_2/k_1)^{10/(T_2-T_1)} \quad (4)$$

where,  $k_1$  is the rate of viral removal at temperature  $T_1$  and  $k_2$  is the rate of removal at temperature  $T_2$ .

MS2 removal rates in each system were determined by plotting the natural log of the fractional decrease in MS2 concentration,  $1n(C_t/C_o)$ , as a function of time ( $t$ ). A linear regression was fit to this data using SPSS v10.1 for Windows, the slope of this regression line indicating the virus removal rate.  $T_{90}$  values, the time (in days) required to remove 90% of the virus, were calculated based on removal rates for each system.

### Statistical analysis

Removal of fecal coliforms and somatic coliphages were summarized for winter (October–May) and summer (June–September) through years 1996–2001 as the geometric means of  $\log_{10}$  reductions during these periods. Student  $t$ -tests were performed to determine significant differences between winter and summer indicator removal using SPSS v10.1 for Windows.

Error was estimated for MS2 experiments based on propagation of errors as described in Bevington & Robinson (1992). In brief, error was propagated for MS2 virus plaque count variations and daily flow readings, the products of which were summed throughout all sampling dates. This summation was divided by the total number of samples from each seeding experiment and was then converted to  $\log_{10}$  form to estimate the overall error.

## RESULTS

### MS2 spike recovery experiments

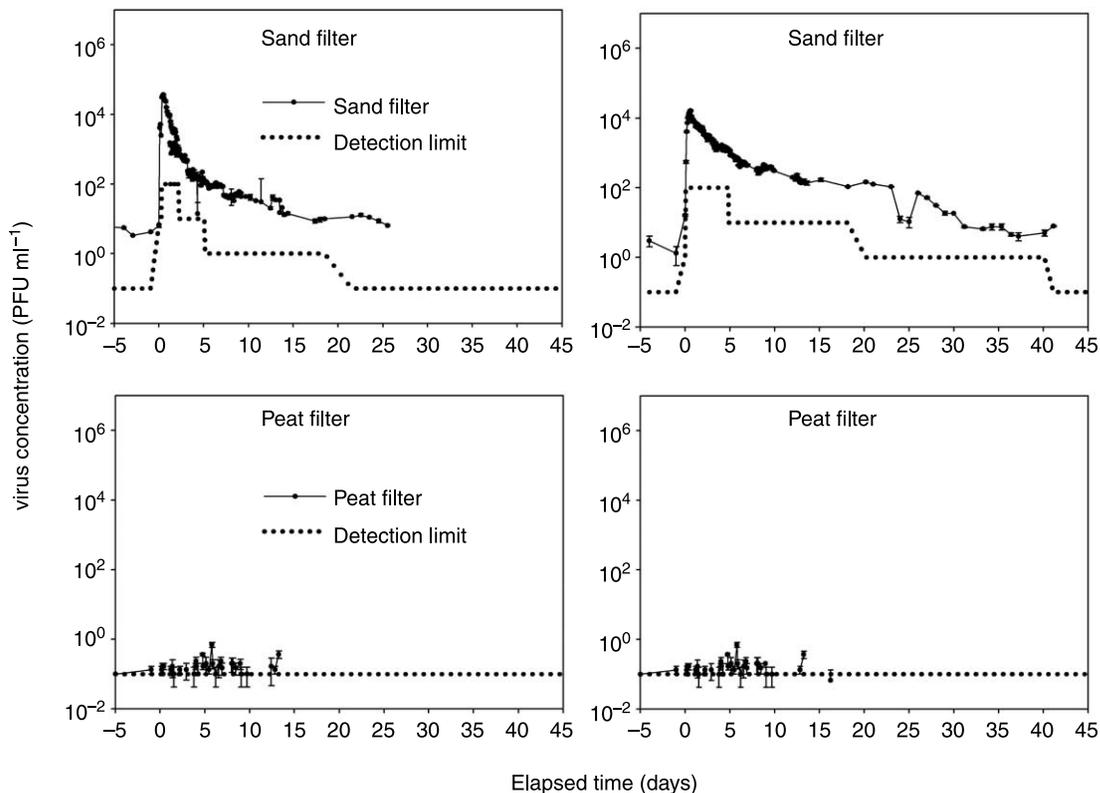
In summer 2001, the peat filter showed the highest removal efficiency of the seeded MS2 virus, removing 99.99999% (7.0 log) and taking less than a day to reach 90% removal (Figure 2, Table 1). Removal was virtually identical at 99.99998% (6.9 log) at winter water temperatures. The sand filter showed the second highest viral removal of the systems tested, removing 99.8% (2.7 log) in summer and taking 23 days for 90% removal. Winter removal efficiency was 98.7% (1.8 log) and 38 days for 90% removal. The constructed wetland demonstrated the poorest viral removal of the three systems, with a summer efficiency of 96.4% (1.4 log) by the midpoint of the first cell to ~96% at the point of discharge after the second cell, and taking 93 days for 90% viral removal (Figure 3). The sand filter had a  $Q_{10}$  of 2.6 indicating strong temperature sensitivity while the peat filter was so efficient at removing virus that the effluent in winter was near the

detection limit and so the calculated  $Q_{10}$  of 1.1 indicated that its performance was essentially temperature insensitive. A  $Q_{10}$  for viral removal could not be calculated for the constructed wetland because of a lack of winter data.

The standard trench receiving CW effluent provided an additional 87.9% (0.91 log) removal after 0.3 m of soil, 88.4% (0.93 log) reduction after 0.6 m, and 98.5% (1.9 log) after the state-mandated depth of 0.9 m of unsaturated soil (Figure 4, Table 1). At the 0.6 m depth, the trenches reached 90% viral removal after ~110 days, and an additional 0.3 m (0.9 m total) of soil decreased the  $T_{90}$  by nearly 20 days (Table 1).

### Fecal coliform and somatic coliphage removal

Overall, fecal coliforms were removed more efficiently than somatic coliphages by each system. The peat filter removed 4.6 log fecal coliforms and 3.5 log somatic coliphage during summer and 4.6 log and 2.8 log in winter, respectively. The sand filter reduced fecal coliforms by 2.9 log in summer and



**Figure 2** | Concentration of MS2 virus in sand and peat filter effluents after seeding in the summer and winter of 2001. In summer, both sand and peat filters were seeded with MS2 on day 0 and monitored until MS2 reached background levels. Error bars represent standard error of three MS2 determinations.

**Table 1** | Winter and summer MS2 virus removal experiments based on seeding individual treatment systems. Winter was defined as average system effluent temperatures below 9°C. Summer was defined as having average system effluent temperatures above 13°C. The trench inflow was entirely constructed wetland (CW) effluent. CW+ trench values are for total removal by the combined systems. The wetland was not seeded with MS2 in winter due to logistical difficulties. T<sub>90</sub> represents the time (in days) required for each system to remove 90% of seeded virus. ND = not determined

System	Average inflow (g day <sup>-1</sup> )		Outflow temp (°C)		Inflow MS2 (PFU × 10 <sup>10</sup> )		Outflow MS2 (PFU × 10 <sup>10</sup> )		Reduction (%)		Log <sub>10</sub> reduction (±sd)		T <sub>90</sub>		Removal rate (h <sup>-1</sup> ) × 10 <sup>-3</sup>	
	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
Sand filter	344	241	8.7	16.6	24.8	91.7	4.01	0.206	98.4	99.8	1.8 (0.6)	2.7 (0.7)	58	23	8.50	10.7
Peat filter	221	233	8.9	16.1	24.2	58.0	0.00011	0.0000098	99.999995	99.999998	6.9 (0.2)	7.0 (0.4)	<1	<1	1,690	1,620
CW	297	298	4.5	13.6	ND	403	ND	14.5	ND	96.4	ND	1.4 (0.9)	ND	93	ND	7.1
Trench 0.3 m	ND	208	ND	12.5	ND	14.5	ND	1.76	ND	87.9	ND	0.9 (0.8)	ND	110	ND	5.7
0.6 m	ND	208	ND	11.9	ND	14.5	ND	1.69	ND	88.4	ND	0.9 (0.9)	ND	109	ND	5.6
0.9 m	ND	208	ND	12.5	ND	14.5	ND	0.232	ND	98.5	ND	1.9 (0.9)	ND	92	ND	5.9
CW+ trench	297	298	4.5	12.5	ND	40.3	ND	0.232	ND	99.94	ND	3.3 (0.9)	ND	89	ND	ND

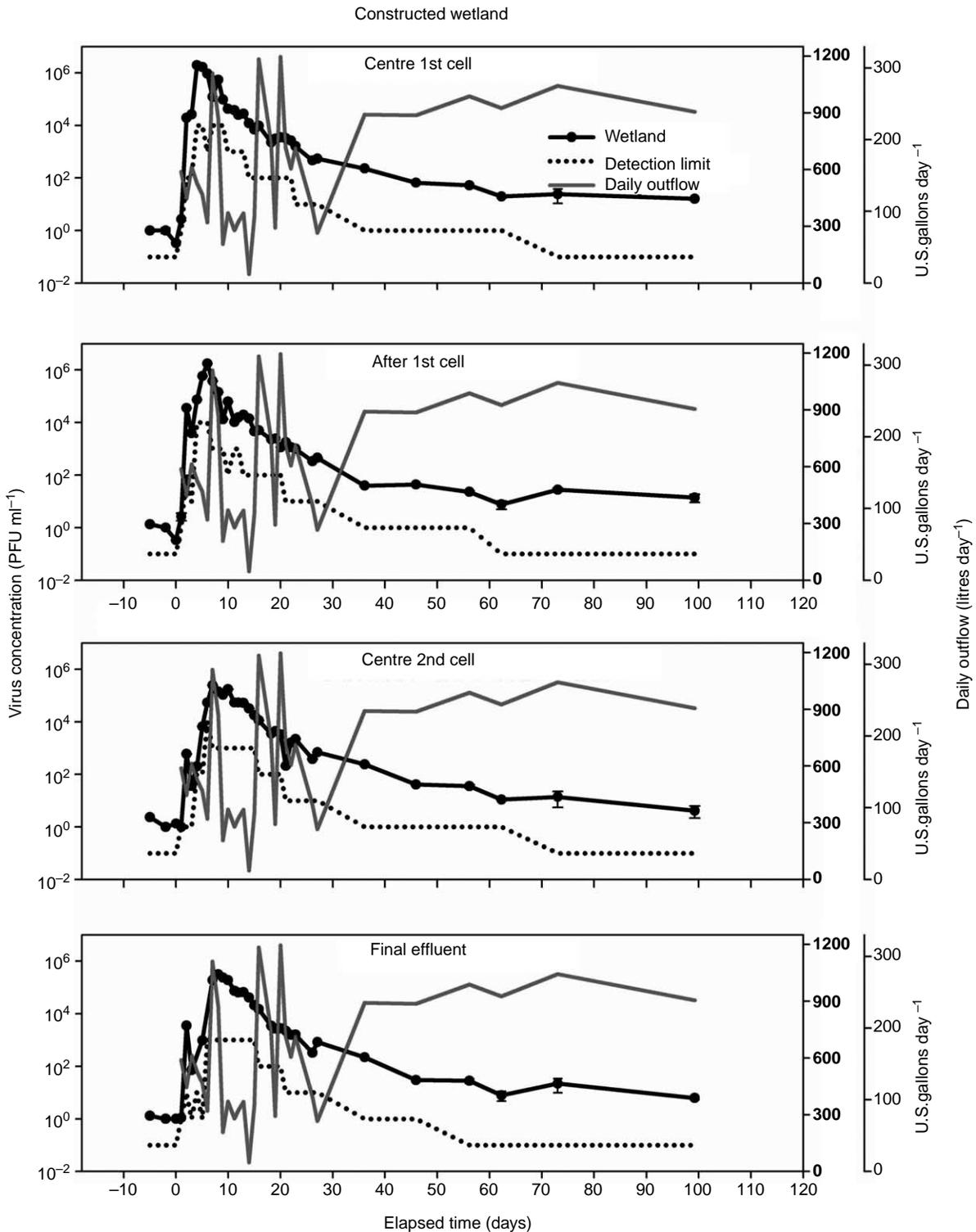
2.0 log in winter and somatic coliphages by 2.6 log in summer and 1.4 log in winter. The constructed wetland reduced fecal coliforms by 2.0 log in summer and 1.6 log in winter and somatic coliphages by 1.0 log in summer and 0.7 log in the winter (Table 2).

## DISCUSSION

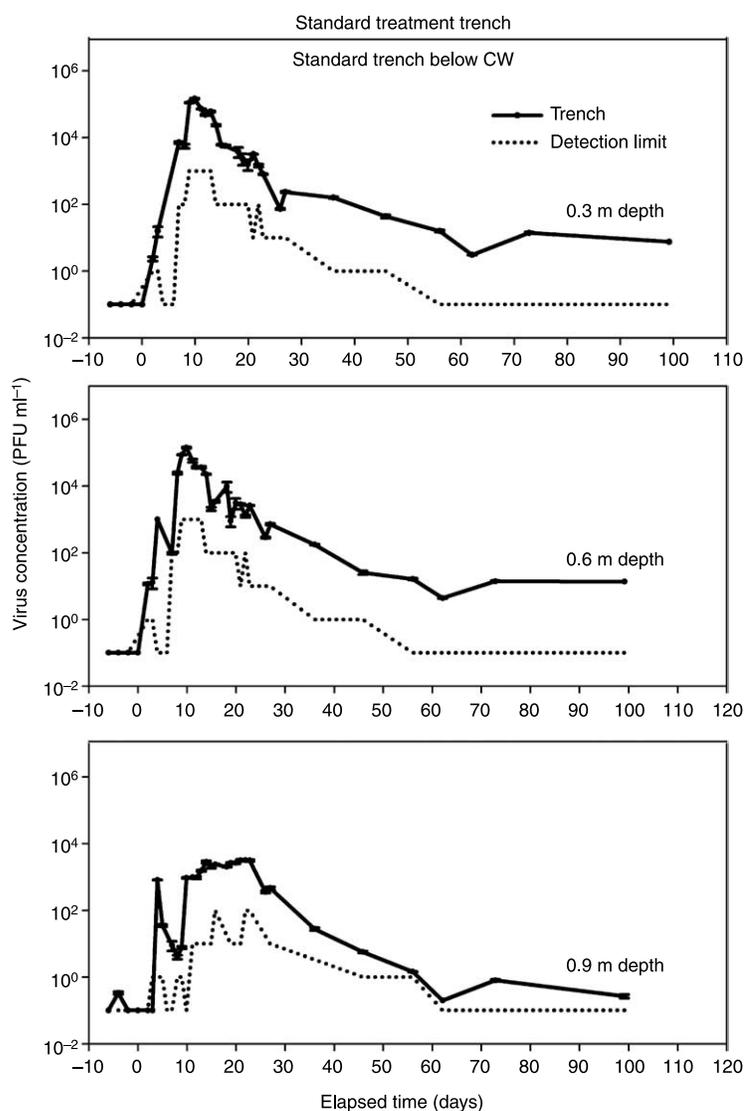
### Overall performance

The NERCC systems efficiently removed bacteria and viruses to varying degrees compared with similar systems around the world (Table 3). The sand filter removed 2.4–3.8 log fecal coliforms and 1.5–3.4 log virus (including MS2 and somatic phages) compared with literature medians of 3.3 log fecal ( $n = 16$ ) and 2.5 log virus removal ( $n = 5$ ). The peat filter removed 4.5–4.8 log fecal coliforms and 3.3–7.0 log virus compared with literature median values of 2.4 log removal of fecal coliforms ( $n = 27$ ), but no data was found for viruses. The constructed wetlands removed 1.6–3.0 log fecal coliforms and 0.8–1.4 log viruses compared with literature medians of 1.7 log fecals ( $n = 52$ ) and 1.9 log virus ( $n = 22$ ). Sand filter performance fell within the range of previous literature values. The constructed wetland performed somewhat better than the wetlands described in the literature for fecal coliforms in summer, but worse for viral removal in both summer and winter. Peat filter performance was outstanding for both fecal coliforms and viruses and was consistently better than the other systems with removal rates for the MS2 virus nearly 800 times that of the sand filter or CW.

Pundsack *et al.* (2001) also reported higher performance by the same peat filter for fecal coliforms and seeded *Salmonella choleraesuis* (serotype typhimurium) compared with the sand filters and constructed wetlands. Acidity may play an important role in the peat filter's ability to remove pathogens. The average pH of the peat filter, sand filter and constructed wetland during this study were  $4.7 \pm 0.6$ ,  $6.7 \pm 0.2$  and  $7.1 \pm 0.1$ , respectively (mean  $\pm$  S.D.). Virus structure and pH are largely responsible for viral adsorption to particles in wastewater and soil (Schijven & Hassanizhadeh 2000). The viral protein coat, or capsid, and the amino acid sequence of the capsid can give a virus a net positive or negative charge (Gerba *et al.* 1975; Hurst



**Figure 3** | Concentrations of MS2 virus at the centre of cell 1, after cell 1, the centre of cell 2, and effluent of CW2 after seeding 5 consecutive days with MS2 from 2001 1–5 August (summer temperatures). Error bars represent the standard error of three MS2 concentration determinations.



**Figure 4** | Concentrations of bacteriophage MS2 in standard trench field lysimeters at 0.3, 0.6, 0.9 m (1', 2' and 3') beginning 1 August 2001. The treatment trench received CW effluent that had been seeded with MS2 as in Figure 3. Error bars represent standard error of three MS2 determinations. Dotted lines represent the detection limit of the SAL technique.

*et al.* 1980). Most enteric viruses tend to have a weak negative charge at a pH above 5 and a positive charge below pH 5 (Schijven & Hassanizhadeh 2000). When pH falls into an acidic range, viruses such as MS2 and the enteric viruses have a greater positive charge, increasing their affinity to negatively charged soil or substrate particles and increasing repulsion from like charged particles. Goyal & Gerba (1979) demonstrated this effect by measuring a >3 log decrease in MS2 adsorption due to an increase in pH from 4.5 to 8.2. In other laboratory

studies, Bales *et al.* (1993) observed an 87% (0.9 log) decrease in MS2 sticking efficiency using silica beads and a pH shift from 5.0 to 7.0. Penrod *et al.* (1996) also noted a similar reduction (75%; 0.6 log) in MS2 sticking efficiency using a quartz medium after a pH shift from 3.5 to 5.0. These data are consistent with the data from the NERCC systems, where in the peat filter effluent (pH 4.7) the MS2 virus was probably more likely to adsorb to peat granules at a lower pH than to the sand and gravel substrates found in the sand filter and CW at higher pHs.

**Table 2** | Log<sub>10</sub> removal of indigenous fecal coliform bacteria and somatic coliphages. Winter samples were collected from October through May 1999/2000, 2000/2001 and 2001/2002. Summer samples were collected between June and September 1999, 2000 and 2001. Standard deviations ( $\pm$ ) are noted in parentheses and *n* is the number of samples. \* represents significant increased indicator removal in summer compared with winter at  $p < 0.05$  as determined by the student *t*-test

Treatment system	Fecal coliforms Log <sub>10</sub> removal		Somatic coliphage Log <sub>10</sub> removal	
	Summer	Winter	Summer	Winter
Constructed wetland	2.0 (1.3) <i>n</i> = 13	1.6 (1.0) <i>n</i> = 24	1.0 (0.7) <i>n</i> = 11	0.8 (0.3) <i>n</i> = 20
Sand filter	2.9 (1.2) <i>n</i> = 11	2.0 (1.3) <i>n</i> = 28	2.6*(1.3) <i>n</i> = 16	1.5 (1.2) <i>n</i> = 29
Peat filter	4.6 (0.3) <i>n</i> = 12	4.6 (0.4) <i>n</i> = 28	3.4*(0.6) <i>n</i> = 17	2.8 (1.1) <i>n</i> = 26

### Winter vs. summer performances

In general, few differences were seen in the removal of indicator organisms between winter and summer. In fact, the average difference between the water temperature in the summer and winter experiments was only 6.6°C (15.4°C  $\pm$  1.6°C versus 8.8°C  $\pm$  0.14°C; mean  $\pm$  S.D.). These systems also showed relatively little variation in removal rates due to increased temperature. In the same systems, Pundsack *et al.* (2001) reported a 1.0–6.2 log increase in seeded *Salmonella choleraesuis* (serotype *typhimurium*) removal during summer experiments. Similar alternative systems, reported in the literature, demonstrated a 0.1 to 0.7 log increase in fecal coliform removal during warmer temperatures, compared with a 0 to 1.2 log increase by the NERCC systems (Table 3). Temperature is usually considered the most important factor in determining virus survival in aquatic environments (Hurst *et al.* 1980; Yates *et al.* 1985; Blanc & Nasser 1996; Schijven & Hassanizhadeh 2000). Higher temperatures may damage viral capsids, increase microbial metabolism (which can increase predation), and increase the production of harmful metabolic byproducts (Nasser & Oman 1999). Several laboratory scale studies have shown the effects of temperature on virus survivability. Blanc & Nasser (1996) reported much higher rates of inactivation for MS2 and Hepatitis A at 23°C compared with that at 10°C in soil saturated with wastewater. Nasser & Oman (1999) showed negligible loss of poliovirus and Hepatitis A between 4°C and 10°C in soils saturated with PBS and groundwater whereas both viruses were quickly inactivated at 20°C and 30°C. Although these studies demonstrate increased viral decay with temperature,

the most significant effect occurred above 20°C and it should be noted that no NERCC systems averaged over 20°C during the winter or summer.

Q<sub>10</sub> values based on MS2 removal were used to characterize the relative temperature sensitivity of each system. The peat filter showed little temperature sensitivity with a Q<sub>10</sub> value of 1.1 (a value of 1.0 indicating no temperature effect) with both winter and summer experiments reaching >90% virus removal in less than 1 day. The sand filter had a Q<sub>10</sub> of 2.7 indicating that a 10°C increase in temperature would increase virus removal rates 2.7 fold. In winter, the sand filter required 16 additional days to reduce MS2 levels by 90%. A Q<sub>10</sub> could not be calculated for the constructed wetland because it was not seeded with MS2 in winter. These data may be interpreted to suggest that MS2 removal by the peat filter is relatively temperature insensitive in the conditions tested because removal is accomplished largely by physical filtration and/or pH dependent adsorption. However, viral removal was so complete, even in winter, that any temperature effect was probably masked by the method detection limit.

### Effects of substrate saturation on virus removal

Substrate saturation levels can also have an effect on virus survivability in wastewater treatment systems. The constructed wetland was designed to be saturated, operating as a plug flow reactor, although it was loaded intermittently with septic tank effluent (hourly doses). The constructed wetland had the lowest virus removal of all systems tested and commonly experienced anoxia and negative redox

**Table 3** | Summary of literature estimates for removal of microbial pathogens as indicated by fecal coliforms, somatic coliphages and the F + bacteriophage MS2 in alternative (performance based) onsite wastewater treatment systems. This review focuses on residential or larger systems unless noted. All data reported as log<sub>10</sub> removal (see methods). ‘-’ represents no data available and \* refers to notes. Numbers in parentheses refer to years of data collected. Data separated by ‘/’ indicates winter/summer reduction values. SSF and SF refer to subsurface flow and surface flow constructed wetlands (CWs), respectively. Median reduction values include NERCC data

System	Bacteria		Viruses		Notes	Ref
	Fecal coliforms	Salmonella	Somatic phage	MS2 spike		
NERCC						
Sand filter	2.7/3.9 (6)	1.4/7.6	1.7/3.5 (4)	1.8/2.7	MS2 and Salmonella spikes	This, 37
Peat filter	4.6/4.5 (6)	5.6/8.9	3.4/3.5 (4)	6.9/7.0	”	”
CW	1.7/2.8 (6)	1.3/2.4	0.8/1.4 (4)	1.4	MS2 summer only for virus.	”
Other systems	Fecal coliforms		Somatic phage	MS2/F +	Notes	Ref
<b>Sand filters</b>						
Recirculating	0.9/1.1 (3)		-	-	Mean- 2 research, 2 residential	1
Single pass	-		-	2.7	1.5 m deep, reported in citation	2
Lateral flow	>6 (1)		-	2.5 (1)	Mean for 3 pilot scale models	3
Recirculating	2.2 (2-6)		-	-	Mean for 3 systems	4
Single pass	3.2-3.8 (1)		-	-	-	5
Single pass	5.9-6.2 (1)		-	-	Range for 9 gravity and pressure	6
Intermittent	1.6-3.0 (<1)		-	0.9-2.8	Range for 12 systems dosed	7
					0.04-0.16 m d <sup>-1</sup>	
<b>Median</b>	<b>3.3</b>		<b>2.6</b>	<b>2.5</b>		
<b>Peat filters</b>						
NERCC PuraFlow	1.5-3.3/2.3-4.0 (1)		-	-	Recirculating and 1-pass filters	8
Pressure	1.4/1.5 (3.5)		-	-	Lake Washington, MN	8
Peat/sand	4.8 (8)		-	-	Campground system (200	9
PuraFlow	3.4-6.2* (1)		-	-	*Additional UV treatment	5
PuraFlow	1.6 (1)		-	-	Mean for 10 systems	10
Sphagnum	>4 (~2)		-	-	Mean for 3 systems	11
Sphagnum	>5 (<1)		-	-	Mean for 2 systems + 2 soils	12
Peat biofilters	1.5-2.1 (1-3)		-	-	Average for 3 Quebec filters	13, 14
Lab scale columns	2.5-3.0 (<1)		-	-	1.2 m long, 3-100 mm diameter	15, 16
<b>Median</b>	<b>2.5</b>		<b>3.5</b>	<b>7.0</b>		

Table 3 | (continued)

Other systems	Fecal coliforms	Somatic phage	MS2/F +	Notes	Ref
<b>Constructed wetlands</b>					
SSF	2.0/2.5 (4)	1.5 (2)	–	Grand Lake CWs, Duluth, MN	17
SSF	2.3/3.0 (3)	–	–	Lake Washington, MN	18
SSF/SF	1.7 (~2)	1.3 (~2)	1.9 (<1)*	MS2 and PRD1	19, 20, 21
SSF	0.4–4.0 (<1)	0.8–2.4 (<1)	1.3–2.8 (<1)	Range for 8 CWs	22
SSF	0.7–2.3	1.9–2.1 (<1)	2.7 (<1)	Range for 3 systems, seeded F and attenuated polio virus	23, 24
3 cell SSF	0.9 (7)	–	–	Mean for 21 systems	25
SSF	1.9 (<1)	2.5 (<1)	1.9 (<1)*	*Indigenous enteric viruses	26
SSF	1.1–2.4 (1)	–	–	Range of 5 systems at flows from 14.7 to 69.2 m day <sup>-1</sup>	27
SSF	0.2–2.1 (<1)	–	–	Range for 3 systems	28
SSF	6.0 (3)	–	–	Hospital waste, 45 beds	29
SSF	0.4, 0.5, 0.6 (<1)	–	–	None, <i>Phragmites</i> and <i>Typha</i>	30
SSF Reed Beds	0.9–2.7 (<1)	–	–	Mean for 4 systems	31
SSF Mesocosm	1–3.7 (2)	1–2.8 (2)	–	1.5 × 1.0 m, 400 l mesocosms	32
SF	0.9 (2)	–	–	Mean for 12 systems	33
SF	–	–	1.4/1.9 (2)	1 and 3 phase CWs at flows 2.0–3.2 m <sup>3</sup> h <sup>-1</sup>	34
SF	1.7 (3)	2 (3)	1.7 (3)	*Salmonella removal	35
Peat/Cypress	1.2 (<1)	2.7 (<1)	6.6* (<1)	CW channels flooded with STE	36
				*Indigenous enteric viruses	
<b>Median</b>	<b>1.7</b>	<b>2.0</b>	<b>1.9</b>		

References: 1. Christopherson *et al.* 2001. 2. Van Cuyk & Sigriest 2001. 3. Check *et al.* 1994. 4. Piluk & Peters 1995. 5. Loomis *et al.* 2001. 6. Ausland *et al.* 2002. 7. Emerick *et al.* 1997. 8. Monson-Geerts *et al.* 2001. 9. Nicholas & Boelter 1982. 10. White *et al.* 1995. 11. Daigle 1993. 12. Brooks *et al.* 1984. 13. Talbot *et al.* 1996. 14. Jowett & McMaster 1995. 15. Rana & Viraraghavan 1987. 16. Coleman & Gaudet 1994. 17. Axler *et al.* 2001. 18. Henneck *et al.* 2001. 19. Gerba *et al.* 1999. 20. Gerba *et al.* 1999. 21. Vidales *et al.* 2003. 22. Barrett *et al.* 2001. 23. Gersberg *et al.* 1987. 24. Reed *et al.* 1995. 25. Steer *et al.* 2002. 26. Quinonez *et al.* 2001. 27. Tunner *et al.* 1998. 28. Conte *et al.* 2001. 29. Shrestha *et al.* 2000. 30. Kaseva *et al.* 2000. 31. Davison *et al.* 2001. 32. Hench *et al.* 2003. 33. Gearhardt *et al.* 1989. 34. Chendorian *et al.* 1998. 35. Hill & Sobsey 2001. 36. Scheuerman *et al.* 1989. 37. Pundsack *et al.* 2001.

potentials within the wetland (Axler *et al.* 2001; Axler unpublished data). The peat filter and sand filter were loaded intermittently with septic tank effluent and had relatively shorter retention times (1–6 hours; McCarthy *et al.* 1997, 1998) but remained unsaturated most of the time. The lower level of virus reduction by the CW could be due to a decrease in viral attachment or inactivation in saturated media. Viruses have been shown to travel longer distances in saturated soils than unsaturated soils. Lance & Gerba (1984) showed that polio virus traveled 40 cm in an unsaturated sand column, compared with 160 cm in saturated conditions. Viruses are removed from unsaturated soil not only by adsorptive processes but also by inactivation (Powelson & Gerba 1994). This inactivation is likely to occur at the air water interface (AWI) where viruses are vulnerable to attack by chemical disinfectants, free oxygen radicals or other environmental stressors (Wan *et al.* 1994).

Unfortunately, substrate saturation can cause other problems in cold climate onsite treatment systems. Freezing of the entire system or inflow/outflow ports is most likely to occur during periods of low flow in saturated systems. Such periods are more likely to happen in northern Minnesota seasonal resorts or cabins where water usage is minimal in the winter. At the NERCC facility, freezing and icing have posed greater problems for the constructed wetlands than the other systems because of their larger size, shallow depths and saturated conditions (McCarthy *et al.* 1998; Axler *et al.* 2001; Henneck *et al.* 2001). Winters with early and abundant snowfall, even though much colder, produced no freezing problems in any of the systems whereas winters characterized by reduced snowfall (1999–2000 and 2000–2001) necessitated additional insulation (also see Wallace *et al.* 2001; Kadlec 2001).

### Traditional indicators vs. MS2

In this study removal of seeded MS2 virus was compared with the removal of indigenous fecal coliforms and somatic coliphages. MS2 is regarded as a worst case indicator of viral contamination due to its low adsorption to soils and its general hardiness in aquatic systems compared with traditional bacterial indicators (Van Cuyk & Sigriest 2001). The seeded MS2 removal was similar to fecal coliform and somatic coliphage removal in all systems tested. MS2

reduction values may be inflated because each system was artificially seeded with the virus, which was necessary to establish a removal efficiency representative of a number of system “flushings”. Gersberg *et al.* (1987) noted that removal efficiencies of seeded MS2 and attenuated polio virus were often higher than removal of indigenous MS2 and polio viruses. Results from this study also suggested that indigenous somatic coliphages were a more conservative indicator for virus removal than fecal coliform bacteria. However, the levels of somatic coliphages determined in the present study were quite variable and often below detection. Somatic phage and fecal coliform densities for all the NERCC systems were not very well correlated during the period 1998–2001 (Olson 2004). Neither the influent nor CW effluent showed a significant association between fecal coliforms and somatic phages and values from the peat filter were too low for such an analysis. The two indicators were significantly ( $P < 0.01$ ) but not very strongly correlated ( $r^2 = 0.68$ ) for the SF effluent. Somatic coliphages are also known to have a much greater potential to replicate in the environment relative to MS2 (IAWPRC 1991).

### SUMMARY AND CONCLUSIONS

Very little information has been collected regarding cold climate performance of alternative onsite wastewater treatment systems with regard to virus removal. Alternative onsite treatment systems were evaluated in winter and summer periods for removal of the MS2 virus, somatic coliphages and fecal coliforms. All systems provided >95% removal of seeded or indigenous viruses in summer and typically >90% removal in winter. An additional 0.6 m of loamy sand soil from a conventional trench dispersal system would improve these values to 99.5% and 98%, respectively. The peat filter demonstrated the greatest removal by far of all indicators in summer and winter, followed by the sand filter and then the constructed wetland. The sand filter and the CW performed better in the summer than the winter, while the peat filter appeared to be virtually temperature insensitive. Virus removal was generally poorer than fecal coliform removal for the SF and CW. Somatic coliphage removal was a more conservative indicator of pathogen removal than fecal coliforms but this data set was more

variable and influent levels were often near detection limits. The peat filter typically removed all viral and bacterial indicators to the limit of detection. A literature review of viral and bacterial removal by similar systems to those evaluated in this study indicated a wide range of performance and that the NERCC SF and CW were generally near the median performance. The peat filter outperformed all literature systems for fecal coliform bacteria but no comparative viral data was found.

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## REFERENCES

- Ausland, G., Stevik, T. K., Hansen, J. F., Kohler, J. C. & Jenssen, P. D. 2002 Intermittent filtration of wastewater: removal of fecal coliforms and fecal streptococci. *Wat. Res.* **36**, 3507–3516.
- Axler, R. P., Henneck, J., Bridgham, S., Tikkanen, C., Nordman, D., Bamford, A. & McDonald, M. E. 1996 Constructed wetlands in northern Minnesota for treatment of aquaculture wastes. In *Symposium Proceedings for Constructed Wetlands in Cold Climates: Design, Operation, Performance*, 4–5 June 1996, Niagara-on-the-Lake, Ontario, Canada.
- Axler, R., Henneck, J. & McCarthy, B. 2001 Residential subsurface flow treatment wetlands in northern Minnesota. *Wat. Sci. Technol.* **44**(11–12), 345–352.
- Bales, R. C., Li, S., Maguire, K. M., Yahya, M. T. & Gerba, C. P. 1993 MS2 and poliovirus transport in porous media: Hydrophobic effects and chemical perturbations. *Wat. Resour. Res.* **29**, 957–963.
- Barrett, E. C., Sobsey, M. D., House, C. H. & White, K. D. 2001 Microbial indicator removal in on-site constructed wetlands for wastewater treatment in the Southeastern US. *Wat. Sci. Technol.* **44**(11–12), 177–182.
- Bevington, P. R. & Robinson, D. K. 1992 *Data Reduction and Data Analysis for the Physical Sciences*. McGraw-Hill, New York.
- Blanc, R. & Nasser, O. 1996 Effect of effluent quality and temperature on the persistence of viruses in soil. *Wat. Sci. Technol.* **33**(10–11), 237–242.
- Bosch, A. 1998 Human enteric viruses in the water environment: A minireview. *Int. Microbiol.* **1**, 191–196.
- Brooks, J. L., Rock, C. A. & Struchtemeyer, R. A. 1984 Use of peat for onsite wastewater treatment II. Field studies. *J. Environ. Qual.* **13**(4), 524–530.
- Calci, K., Burkhardt, W., Watkins, W. & Rippey, S. 1998 Occurrence of male-specific bacteriophages in feral and domestic animal wastes, human feces, and human-associated wastewaters. *Appl. Environ. Microbiol.* **64**(12), 5027–5029.
- Check, C. G., Waller, D. H., Lee, S. A., Pask, D. A. & Mooers, J. D. 1994 The lateral flow sand filter system for septic tank effluent treatment. *Wat. Environ. Res.* **66**, 919–928.
- Chendorian, M., Yates, M. & Villegas, F. 1998 The fate and transport of viruses through surface water constructed wetlands. *J. Environ. Qual.* **27**, 1451–1458.
- Christopherson, S. H., Anderson, J. L. & Gustafson, D. M. 2001 Evaluation of recirculating sand filters in Minnesota. In: *Proceedings of the 9th National Symposium on Individual and Small Community Sewage Systems*. American Society of Agricultural Engineers (ASAE), St Joseph, Michigan, pp. 201–214.
- Coleman, R. N. & Gaudet, I. D. 1994 Septic tank effluent treatment using laboratory-scale peat filters. *Wat. Pollut. Res. J. Can.* **29**(1), 19–37.
- Conte, G., Matinuzzi, N., Giovannelli, L., Pucci, B. & Masi, F. 2001 Constructed wetlands for wastewater treatment in central Italy. *Wat. Sci. Technol.* **44**(11–12), 339–343.
- Crosby, J. B., McCarthy, B., Gilbertson, C. & Axler, R. 1998 A regulatory perspective on impediments and solutions to the use of performance standards for on-site wastewater treatment. In *Onsite Wastewater Treatment, Proceedings of the 8th International Symposium on Individual and Small Community Sewage Systems*. American Society of Agricultural Engineers (ASAE), St Joseph, Michigan, pp. 259–267.
- Daigle, J. 1993 Peat moss for wastewater treatment. *Natural*

- Resources and Energy, Mineral Resources Division, New Brunswick, Canada. Open file report 93-3.
- Davison, L., Headley, T. & Edmonds, M. 2001 On-site wastewater treatment by reed bed in the moist subtropics. *Wat. Sci. Technol.* **44**(11–12), 353–360.
- Debartolomeis, J. & Cabelli, V. J. 1991 Evaluation of an *Escherichia coli* host strain for enumeration of F male-specific bacteriophages. *Appl. Environ. Microbiol.* **57**(5), 1301–1305.
- DeBorde, D. C., Woessner, W., Lauerman, B. & Ball, P. 1998 Virus occurrence and transport in a school septic system and unconfined aquifer. *Ground Water* **36**(4), 825–834.
- Emerick, R. W., Test, R. M., Tchobanoglous, G. & Darby, J. 1997 Shallow intermittent sand filtration: Microorganism removal. *The Small Flows J.* **3**(1), 12–21.
- Farthing, M. G. 1989 *Viruses and the Gut.* Smith, Kline & French, Welwyn Garden City, UK.
- Ferguson, C., Coote, B., Ashbolt, N. & Stevenson, I. 1996 Relationships between indicators, pathogens and water quality in an estuarine system. *Wat. Res.* **30**, 2045–2054.
- Gearhardt, R. A., Klopp, F. & Allen, G. 1989 Constructed free surface wetlands to treat and receive wastewater: Pilot project to full scale. In *Constructed Wetlands for Wastewater Treatment* (ed. D. A. Hammer). Lewis Publishers, Chelsea, Michigan, USA, pp. 121–138.
- Gerba, C. P., Wallis, C. & Melnick, J. L. 1975 Fate of wastewater bacteria and viruses in soil. *J. Irrig. Drain. Div., Am. Soc. Civ. Eng.* **101**, 157–174.
- Gerba, C. P., Thurston, J. A., Falabi, J. A., Watt, P. M. & Karpisack, M. M. 1999 Optimization of artificial wetland design for removal of indicator microorganisms and pathogenic protozoa. *Wat. Sci. Technol.* **40**(4–5), 363–368.
- Gersberg, R. M., Lyon, S. R., Brenner, R. & Elkins, B. 1987 Fate of viruses in artificial wetlands. *Appl. Environ. Microbiol.* **53**(4), 731–736.
- Goyal, S. M. & Gerba, C. P. 1979 Comparative adsorption of human enteroviruses, simian rotavirus, and selected bacteriophages to soils. *Appl. Environ. Microbiol.* **38**, 241–247.
- Goyal, S. M. & Gerba, C. P. 1983 Viradel method for detection of rotavirus from seawater. *J. Virol. Meth.* **7**(5–6), 279–285.
- Havelaar, A. H., Furse, K. & Hogeboom, W. M. 1986 Bacteriophages and indicator bacteria in human and animal faeces. *J. Appl. Bacteriol.* **3**, 255–262.
- Havelaar, A. H., van Olphen, M. & Drost, Y. C. 1993 F-specific RNA bacteriophages are adequate model organisms for enteric viruses in fresh water. *Appl. Environ. Microbiol.* **59**(9), 2956–2962.
- Hench, K. R., Bissonnette, K. G., Sexstone, A. J., Coleman, J. G., Garbutt, K. & Skousen, J. G. 2003 Fate of physical, chemical, and microbial contaminants in domestic wastewater following treatment by small constructed wetlands. *Wat. Res.* **37**, 921–927.
- Henneck, J., Axler, R., McCarthy, B., Monson-Geerts, S. D., Christopherson, S., Anderson, J. & Crosby, J. 2001 Onsite treatment of septic tank effluent in Minnesota using SSF constructed wetlands: performance, costs, and maintenance. In *Proceedings of the 9th National Symposium on Individual and Small Community Sewage Systems*. American Society of Agricultural Engineers (ASAE), St Joseph, Michigan, pp. 650–662.
- Higgins, J., Heufelder, G. & Foss, S. 2000 Removal efficiency of standard septic tank and leach trench septic systems for MS2 coliphage. *Small Flows Q.* **1**(2), 26–28.
- Hill, V. R. & Sobsey, M. D. 2001 Removal of Salmonella and microbial indicators in constructed wetlands treating swine wastewater. *Wat. Sci. Technol.* **44**(11–12), 215–222.
- Hurst, C. J., Gerba, C. P. & Cech, I. 1980 Effects of environmental variables and soil characteristics on virus survival in soil. *Appl. Environ. Microbiol.* **40**(6), 1067–1079.
- IAWPRC Study Group on Health Related Water Microbiology 1991 Bacteriophages as model viruses in water quality control. *Wat. Res.* **5**, 529–545.
- Jowett, C. E. & McMaster, M. L. 1995 Onsite wastewater treatment using unsaturated absorbent biofilters. *J. Environ. Qual.* **24**, 86–95.
- Kadlec, R. H. 2001 Thermal environments of subsurface treatment wetlands. *Wat. Sci. Technol.* **44**(11–12), 251–258.
- Kadlec, R. & Knight, R. 1996 *Treatment Wetlands*. Lewis Publishing, Boca Raton, Florida.
- Kadlec, R. H., Axler, R., McCarthy, B. & Henneck, J. 2003 Subsurface treatment wetlands in the cold climate of Minnesota. In *Constructed Wetlands for Wastewater Treatment in Cold Climates* (eds. U. Mander & P. D. Jenssen). WIT Press, Northampton, Massachusetts, pp. 19–52.
- Karpisack, M. M., Freitas, R. J., Gerba, C. P., Sanchez, L. R. & Shamir, E. 1999 Management of dairy waste in the sonoran desert using constructed wetland technology. *Wat. Sci. Technol.* **40**(3), 57–65.
- Kaseva, M. E., Mbwette, T. S., Katima, J. H. & Mashauri, D. A. 2000 Performance of a sub-surface horizontal flow constructed wetland in polishing upward flow anaerobic sludge blanket pre-treated domestic wastewater under tropical conditions: A case study in Dar Es Salaam, Tanzania. In *Wetland Systems for Water Pollution Control* (eds. K. Reddy & R. Kadlec). Lake Buena Vista, Florida. International Water Association, London, UK, 7th International Conference on Wetlands for Water Pollution Control, 11–16 November 2000, pp. 1051–1060.
- Lance, J. C. & Gerba, C. P. 1984 Virus movement in soil during saturated and unsaturated flow. *Appl. Environ. Microbiol.* **47**(2), 335–337.
- Leclerc, H., Mossel, D., Edberg, S. & Struijk, C. 2001 Advances in the bacteriology of the coliform group: their suitability as markers of microbial water safety. *Ann. Rev. Microbiol.* **55**, 201–234.
- Loomis, G. W., Dow, D. B., Stolt, M. H., Green, L. T. & Gold, A. J. 2001 Evaluation of innovative onsite wastewater treatment systems in the Green Hill Pond Watershed, Rhode Island: A NODP II project update. In *Proceedings of the 9th National Symposium on Individual and Small Community Sewage*

- Systems. American Society for Agricultural Engineers (ASAE), St Joseph, Michigan, pp. 506–515.
- McCarthy, B., Axler, R., Crosby, J., Kadlec, R., Anderson, J., Monson Geerts, S., Gustafson, D., Hage, D., Henneck, J., Nordman, D., Otis, R., Sabel, G. & Weidman, P. 1996 Development of alternative on-site treatment systems for wastewater treatment: A demonstration project for Northern Minnesota. *Symposium Proceedings for Constructed Wetlands in Cold Climates: Design, Operation and Performance*, 4–5 June 1996 Niagara-on-the-Lake, Ontario, Canada.
- McCarthy, B., Axler, R., Monson-Geerts, S., Henneck, J., Crosby, J., Norman, D., Weidman, P. & Hagen, T. 1997 Development of alternative on-site treatment systems for wastewater treatment: A demonstration project for Northern Minnesota. NRRI Technical Report, NRRI/TR-97/10, Natural Resources Research Institute, University of Minnesota, Duluth, Minnesota.
- McCarthy, B., Axler, R., Monson Geerts, S., Henneck, J., Crosby, J., Nordman, D. & Weidman, P. 1998 Performance of alternative treatment systems in Northern Minnesota. In *Proceedings of the 8th International Symposium on Individual and Small Community Sewage Systems*, Orlando, Florida, March 1998. American Society for Agricultural Engineers (ASAE), St Joseph, Michigan, pp. 446–457.
- McCarthy, B., Axler, R., Monson Geerts, S., Henneck, J., Crosby, J. & Weidman, P. 1999 Cold-weather operation and performance of alternative treatment systems in Northern Minnesota. In: *Proceedings of the National On-Site Wastewater Recycling Association (NOWRA), 8th Annual Conference*, Jekyll Island, Georgia, November 1999.
- Meshcke, J. S. & Sobsey, M. D. 2003 Comparative reduction of Norwalk virus, poliovirus type 1, F+ RNA coliphage MS2 and *Escherichia coli* in miniature soil columns. *Wat. Sci. Technol.* **47**(3), 85–90.
- Monson-Geerts, S. D., McCarthy, B., Axler, R., Henneck, J., Christopherson, S., Crosby, J. & Guite, M. 2001 Performance of peat filters in the treatment of domestic wastewater in Minnesota. In *Proceedings of the 9th National Symposium on Individual and Small Community Sewage Systems*. American Society of Agricultural Engineers (ASAE), St Joseph, Michigan, pp. 295–304.
- MPCA 1996 Individual sewage treatment systems standards: Chapter 7080 MN Rules. Minnesota Pollution Control Agency, Water Quality Division, St Paul, Minnesota.
- Nasser, A. & Oman, S. 1999 Quantitative assessment of the inactivation of pathogenic and indicator viruses in natural water sources. *Wat Res.* **33**(7), 1748–1752.
- Nichols, B. D. & Boelter, D. 1982 Treatment of secondary effluent with a peat sand filter bed. *J. Environ. Qual.* **11**(1), 86–92.
- Olson, M. R. 2004 *Seasonal virus removal by alternative onsite wastewater treatment systems*. MS thesis (Biology). University of Minnesota, Duluth.
- Payment, P., Fortin, S. & Trudel, M. 1986 Elimination of human enteric viruses during conventional waste water treatment by activated sludge. *Can. J. Microbiol.* **32**(12), 922–925.
- Penrod, S. L., Olson, T. M. & Grant, S. B. 1996 Deposition kinetics of two viruses in packed beds of quartz granular media. *Langmuir* **12**, 5576–5587.
- Piluk, R. & Peters, E. 1995 Recirculating sand filters: A better nitrogen removing alternative to conventional septic systems. *Watershed Prot. Tech.* **2**(1), 2–4.
- Powelson, D. K. & Gerba, C. P. 1994 Viral removal from sewage effluents during saturated and unsaturated flow through soil columns. *Wat. Res.* **28**, 2175–2181.
- Pundsack, J., Axler, R., Hicks, R., Henneck, J., Nordman, D. & McCarthy, B. 2001 Seasonal pathogen removal by alternative on-site wastewater treatment systems. *Wat. Environ. Res.* **73**(2), 204–212.
- Pundsack, J. R., Hicks, R. E. & Axler, R. P. 2005 Effect of alternative on-site wastewater treatment on the culturability and viability of *Salmonella choleraesuis*. *J. Wat. Health* **3**(1), 1–14.
- Quinonez-Diaz, M., Karpisack, M. M., Ellman, E. D. & Gerba, C. P. 2001 Removal of pathogenic and indicator microorganisms by a constructed wetland receiving untreated domestic wastewater. *J. Environ. Sci. Health* **A36**(7), 1311–1320.
- Rana, S. & Viraraghavan, T. 1987 Use of peat in septic tank effluent treatment: Column studies. *Wat. Pollut. Res. J. Can.* **22**(3), 491–504.
- Reed, S. C., Crites, R. W. & Middlebrooks, E. J. 1995 *Natural Systems for Waste Management and Treatment*, 2nd edn. McGraw-Hill, New York, NY.
- Scheuerman, P. R., Bitton, G. & Farrah, S. R. 1989 Fate of microbial indicators and viruses in a forested wetland. In *Constructed Wetlands for Wastewater Treatment* (ed. D. A. Hammer). Lewis Publishers, Chelsea, Michigan, pp. 657–663.
- Schijven, J. F. & Hassanizadeh, S. M. 2000 Removal of viruses by soil passage: Overview of modeling, processes, and parameters. *Critical Rev. Environ. Sci. Technol.* **30**(1), 49–127.
- Shrestha, R. R., Haberl, R., Laber, J., Manandhar, R. & Mader, J. 2000 Application of constructed wetlands for wastewater treatment in Nepal. In *Wetland Systems for Water Pollution Control* (ed. K. Reddy & R. Kadlec). Lake Buena Vista, Florida. International Water Association, London, UK, 7th International Conference on Wetlands for Water Pollution Control, 11–16 November 2000, pp. 991–1001.
- Standard Methods for the Examination of Water and Wastewater* 1998 19th edn. American Public Health Association American Water Works Association/Water Environment federation, Washington, DC.
- Steer, D., Fraser, L., Boddy, J. & Seibert, B. 2002 Efficiency of small constructed wetlands for subsurface treatment of single-family domestic effluent. *Ecol. Engng.* **18**, 429–440.
- Stetler, R. E. 1984 Coliphages as indicators of enteric viruses. *Appl. Environ. Microbiol.* **48**(3), 668–670.
- Sundaravadivel, M. & Vigneswaran, S. 2001 Constructed wetlands for wastewater treatment. *Critical Rev. Environ. Sci. Technol.* **31**(4), 351–409.

- Talbot, P., Belanger, G., Pelletier, M., Laliberte, G. & Arcind, Y. 1996 Development of a biofilter using an organic medium for onsite wastewater treatment. *Wat. Sci. Technol.* **34**(3–4), 435–446.
- Tunner, C. C., Subius, J. P. & Upsdell, M. P. 1998 Relationships between loading rates and pollutant removal during maturation of gravel bed constructed wetlands. *J. Environ. Qual.* **27**, 448–458.
- USEPA Environmental Protection Agency 2001 Method 1602: Male-specific (F+) and somatic coliphage in water by single agar layer (SAL) procedure. EPA 821-R-01-029, Washington, DC.
- USEPA Environmental Protection Agency 2002 Onsite wastewater treatment systems manual. Office of Water and Research and Development, EPA 625-R-00-008, Washington, DC.
- Van Cuyk, S. & Siegrist 2001 Pathogen fate in wastewater soil absorption systems as affected by effluent quality and soil clogging genesis. In *Proceedings of the 9th National Symposium on Individual and Small Community Sewage Systems*. American Society for Agricultural Engineers (ASAE), St Joseph, Michigan, pp. 125–136.
- Vidales, J. A., Gerba, C. P. & Karpiscak, M. M. 2003 Virus removal from wastewater in a multispecies subsurface-flow constructed wetland. *Wat. Environ. Res.* **75**(3), 238–245.
- Wallace, S., Parkin, G. & Cross, C. 2001 Cold climate wetlands: Design and performance. *Wat. Sci. Technol.* **44**(11–12), 259–265.
- Wan, J. M., Wilson, J. L. & Kieft, T. L. 1994 Influence of the gas-water interface on transport of microorganisms through unsaturated porous media. *J. Appl. Environ. Microbiol.* **60**, 509–516.
- White, K. D., Robertson, S. C. & O'Driscoll, J. P. 1995 Evaluation of peat biofilters. *Environ. Health*, 11–18 November 1995.
- Yates, M. V., Gerba, C. P. & Kelley, L. M. 1985 Virus persistence in ground water. *Appl. Environ. Microbiol.* **31**(4), 778–781.