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Transport and survival of bacterial and viral tracers through submerged-flow constructed wetland and sand-filter system

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Abstract

Untreated or improperly treated wastewater has often been cited as the primary contamination source of groundwater. The use of decentralized wastewater treatment systems has applicability around the world since it obviates the need for extensive infrastructure development and expenditures. The use of a submerged flow constructed wetland (CW) and a sand filter to remove bacterial and viral pathogens from wastewater streams was evaluated in this study *Salmonella* sp. and a bacteriophages tracer were used in conjunction with the conservative bromide tracer to understand the fate and transport of these organisms in these treatment systems. Viral breakthrough numbers in the sand filter and CW were similar with a Spearman Rank correlation of 0.8 ($P < 0.05$). In the CW, the virus exhibited almost a 3-log reduction, while in the sand filter, the viruses exhibited a 2-log reduction. The bacterial tracers, however, did not exhibit similar reductions. Low numbers of bacteria and viruses were still detectable in the effluent streams suggesting that disinfection of the effluent is critical. The survival of the tracer bacteria and viruses was as expected dependant on the biotic and abiotic conditions existing within the wastewater. The results suggest that the microbial removal characteristics of decentralized wastewater treatment systems can vary and depend on factors such as adsorption, desorption and inactivation which in turn depend on the design specifics such as filter media characteristics and local climatic conditions.

Keywords: Constructed wetland; Sand filter; Bacteria; Virus; Transport; Survival

1. Introduction

In many countries around the world, a large proportion of the population have no access to wastewater treatment. Centralized wastewater handling and treatment systems are expensive to install and maintain. In the US, as much as 25% of the population rely on single family septic tanks as the only form of domestic wastewater treatment (Neralla et al., 2000; USEPA, 1980). Numerous population centers around the US rely on groundwater as the their primary source of drinking water. According to the latest estimates, over 100 million people use groundwater as their source of drinking water in the US (USEPA, 2000). Pathogen contaminated groundwater has been reported to be responsible for over 50% of all disease outbreaks in the US (USEPA, 2000; Craun and Calderon, 1996). Leaking sewer lines, pathogen infiltration from improperly designed septic tank systems and pathogen infiltration from other sources are ways by which the groundwater can get contaminated (Scandura and Sobsey, 1997). Installation of on-site wastewater handling facilities such as constructed wetlands (CWs) or sand-filtration systems can reduce the potential of groundwater contamination.

Alternate wastewater treatment technologies such as CWs and sand filters have been reported in the literature (Neralla et al., 2000; Hill and Sobsey, 2001; Ayaz and Akça, 2001). CWs and sand filters can be used as low cost alternatives to treat wastewater for multiple single family homes or apartment complexes rather than relying on large centralized systems. Comparatively large treatment plants incorporating these technologies have been built in the US. Though the efficacy of these systems in terms of nutrient removal has been studied quite extensively, their role in reducing microbial pathogen concentrations in the effluent is still limited to a large extent. This is primarily because the fates of microbial

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pathogens are significantly influenced by local climatic and soil conditions. There is a need to understand how CWs and sand filtration units, especially the small scale units, perform under various geographical conditions and pathogen loading rates.

The primary objective of these studies was to understand the fate and transport of a bacterial and a viral tracer in a subsurface flow CW and sand filter unit in an arid geographical location. *Salmonella* spp., was used as the bacterial tracer (to simulate bacterial pathogens) while the male-specific coliphage, MS2 was used as the viral tracer. Bromide, in the form of potassium bromide was used as a conservative chemical tracer. Previous studies have shown that bromide can serve as a conservative non-reactive chemical tracer while bacteriophages serve as a viral tracer for transport studies (Paul et al., 1995; Harvey et al., 1995; Schulze-Makuch et al., 2002). The underlying hypothesis was that the CW and sand filter unit would significantly reduce the number of organisms, thereby demonstrating the applicability of these decentralized sewage treatment options.

2. Methods

2.1. Constructed wetland and sand filter treatment systems

The subsurface flow CW and the sand filtration unit (SF) (Figs. 1 and 2) were attached to the septic systems of two separate two-bedroom residences in El Paso, TX. The residences were occupied by single individuals during the course of the study. The CW dimensions were 7.6 m by 3.0 m. It was filled with pea gravel having a grain size of 0.01 m. The gravel depth ranged around 0.3 m with a 1% slope along the long axis of the wetland (Fig. 1). The CW was planted with plants such as Green Taro, Dwarf Umbrella Palm, and Louisiana Iris. The effluent from a septic tank was pumped into the CW and the effluent from the CW flowed into a collection tank before the effluent was discharged via a leach field. The sand filter measured 2.4 m wide and 1.5 m with a sand depth of 0.6 m having an underlying layer (0.2 m) of pea gravel (grain size = 0.01 m). The effluent from a septic tank was pumped into the SF. The sand filtration unit capacity was approximately 2000 l. The study site was located in the arid Chihuahuan desert of the southwestern US. This location has on an average annual temperatures ranging between 9.4 and 25.2 °C.

2.2. Preparation of tracers

Salmonella typhimurium (National Veterinary Service Laboratory, Ames Iowa, Accession #87-26254) resistant to nalidixic acid and novobiocin was utilized as the bacterial tracer. (We had to rely on an antibiotic marked strain to distinguish the introduced strain from the background *Salmonella* spp. that were routinely detected.) A large volume high titer culture was initially prepared. The cells were initially concentrated by centrifugation (10,415*g* for 30 min) and the pellet resuspended in 0.1% peptone solution. The cells were washed using a brief vortexing and the cells were centrifuged again at 10,415*g* for 8 min. The cell pellet was resuspended in 200 ml of 0.1% peptone and the titer determined. The inoculum (200 ml) was stored at 4 °C prior to injection. The MS2 bacteriophage was used as a virus tracer and the host bacterium *E.coli* F_{amp} was used to prepare a high titer lysate (USEPA, 2001). The high titer lysate was filtered (0.2 μm) to remove bacterial cells, the titer determined, and the inoculum (200 ml) stored at 4 °C prior to injection. Potassium bromide (KBr) was used as a conservative chemical tracer at a concentration of 0.3 g/l in distilled water.

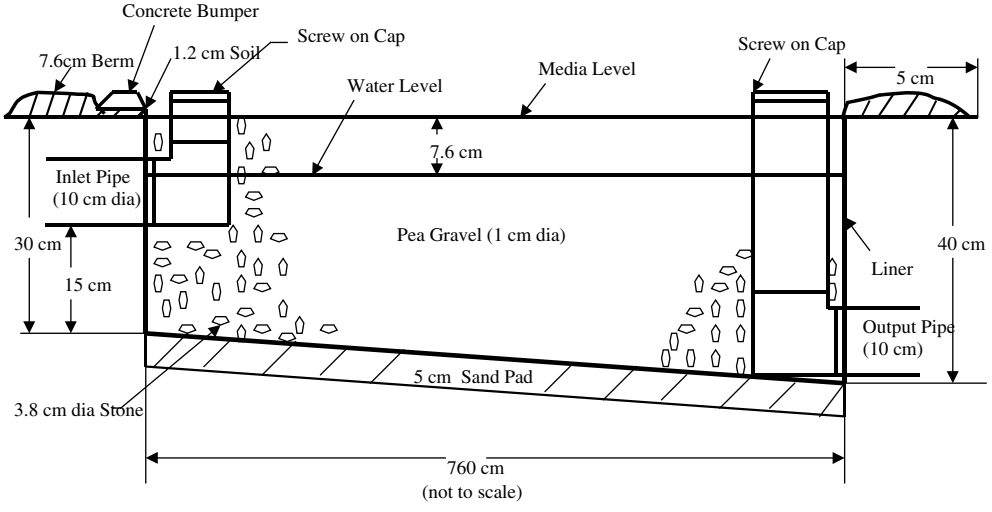

Fig. 1. Schematic representation of the side view of the submerged flow CW (figure not to scale).

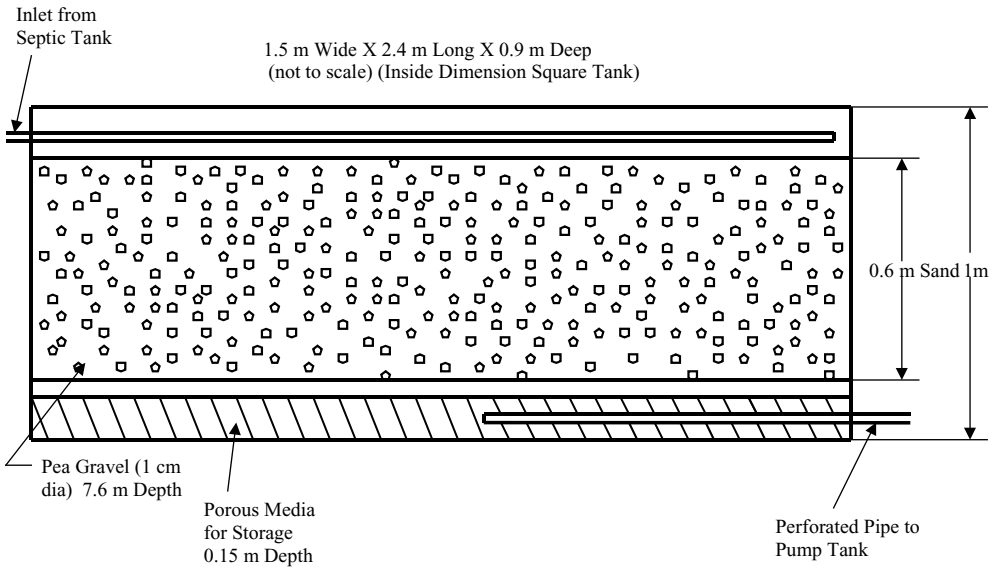

Fig. 2. Schematic representation of the cross sectional length view of the sand-filter system (figure not to scale).

2.3. Transport studies

Prior to the transport studies, wastewater samples were collected from different locations of the CW and SF and analyzed for the presence of nalidixic acid and novobiocin resistant bacteria on BGA and for background levels of male-specific phage. This was done to ensure that no potentially interfering background levels of these organisms were present. A total of 1.0×10^{13} PFU of MS2 virus and 6.4×10^9 CFU of *S. typhimurium* along with 5 gallons of KBr (final concentration = 0.3 g/l) was added to the toilet bowl of the residence to which the sand filter was attached. A total of 1.1×10^{11} PFU of phage, 6.4×10^9 CFU of *Salmonella* sp. and 5 gallons of KBr (final concentration = 0.3 g/l) solution were added into the toilet bowl at the residence where the CW was installed. Wastewater samples were collected every 24 h at the pump tank outlet and the CW outlet. *Salmonella* sp. was enumerated by appropriate dilutions using 0.1% peptone and using BGA plates containing 25 $\mu g\,ml^{-1}$ of nalidixic acid and novobiocin. Characteristic colonies (red–pink, opaque colored colonies) after a 24-h incubation at 35 °C were enumerated as *Salmonella*. MS2 bacteriophages were enumerated using the double agar layer method and *E. coli* F_{amp} as the host (USEPA, 2001). Plaques were enumerated after the plates were incubated at 37 °C for 24 h. Bromide concentration was quantitated using ion chromatography.

2.4. Survival of Salmonella sp. and MS2 phage

The survival of the bacterium and phage under temperature conditions within the CW and SF was studied during the summer and winter. Wastewater samples were collected from the septic tanks that were connected to CW and SF and aliquoted (25 ml) into multiple 50 ml polypropylene conical tubes. Each of the tubes was separately inoculated with the MS2 phage (7.9×10^9 PFU/ml) and *Salmonella* (4.0×10^9 CFU/ml). The 50 ml conical tubes were placed at random throughout the CW and SF. During the winter study, the initial levels of *Salmonella* and MS2 phage were 3.9×10^7 CFU/ml and 1×10^9 PFU/ml respectively. During both the summer and winter studies, triplicate sample tubes, covered in foil, were placed at ambient room temperature in the laboratory as controls. The study was conducted over a total duration of 4 weeks. At weekly intervals, three replicate tubes were collected from the CW, three replicate tubes from the SF, and three replicate tubes from the laboratory controls were assayed for *S. typhimurium* and MS2.

2.5. Data and statistical analysis

The tracer transport data were represented as moving average concentrations over the duration of the study to understand the tracer transport patterns. However, the actual concentrations of *S. typhimurium*, MS2 phage, and Bromide were used for calculating the Spearman Rank correlation. For the survival studies, linear regression was used to compare the survival rates for *S. typhimurium* and MS2 phage. SigmaPlot was used for graphical representation while the statistical analyses were performed with SPSS 10.0 for Windows (SPSS Inc., Chicago, IL).

3. Results

3.1. Transport studies

3.1.1. Constructed wetland pump tank

All three tracers were detected in the pump tank soon after their addition into the toilet bowl and remained constant for up to 5 days (Fig. 3A). There was, however, a significant reduction in concentration of the tracers compared to the injection concentration. There was about a 2-log difference in the maximum phage concentration as compared to *Salmonella*. After 5 days, all three tracers showed a decline in concentration. *Salmonella* remained relatively constant throughout the 30-day study other than for a moderate decline between day 7 and 17. The numbers averaged between 1 and 10 CFU/ml. The phages were also detected within 10 min of the injection. MS2 phage levels, however, decreased by 3-log orders of magnitude from approximately day 8 until day 22. The levels of surviving phages were lower than that of *Salmonella* towards the end of the study. The chemical tracer (bromide) also showed a decrease which mimicked that of the phage. Even at the end of 30 days, there were detectable levels of phages, *Salmonella* and bromide.

3.1.2. Constructed wetland effluent

The CW effluent showed a similar pattern as compared to the pump tank effluent in terms of the microbial tracers (Fig. 3B). While the levels of phages and *Salmonella* showed a decreasing trend over the 30-day period, the bromide tracer concentrations increased between day 5 and 10. Bromide was detectable even at the end of 30 days. There was a greater decline in phage numbers than that of the bacterial pathogen, which remained relatively constant even at the end of 30 days.

3.1.3. Sand filter pump tank

The sand filtration pump tank served as the primary reservoir for the tracers spiked into the sand-filter system. All three tracers remained at relatively constant levels for the first 7 days of the study (Fig. 4A). Subsequently, the phage concentration declined rapidly in contrast to the *Salmonella* concentrations which increased to slightly above 10^1 CFU/ml. By the end of the study, *Salmonella* numbers remained constant at around 10^1 CFU/ml. Even at the end of 30 days, all three tracers were detectable.

3.1.4. Sand-filter effluent

Salmonella was not detected in the sand-filter effluent until around day 18. This is in contrast to the phage and bromide tracers which were detectable even on day 1 (Fig. 4B). Even though the bacterial tracer was not detectable as early as the phage and bromide tracers, bacterial numbers in the effluent actually increased and remained constant between 1 and 100 CFU/ml in the sand-filter effluent. Bromide concentration decreased steadily over the course of 30 days after an initial increase. Phage numbers declined almost 2-log units over 30 days.

3.2. Salmonella survival in constructed wetland and sand filter

The survival of *Salmonella* sp. in the CW during summer and winter months is shown in Fig. 5A and B. During the summer month, the survival in the field mimicked its survival under laboratory conditions. In the winter months, however, the survival patterns of the cells maintained under laboratory conditions and the field conditions were significantly different ($P < 0.05$).

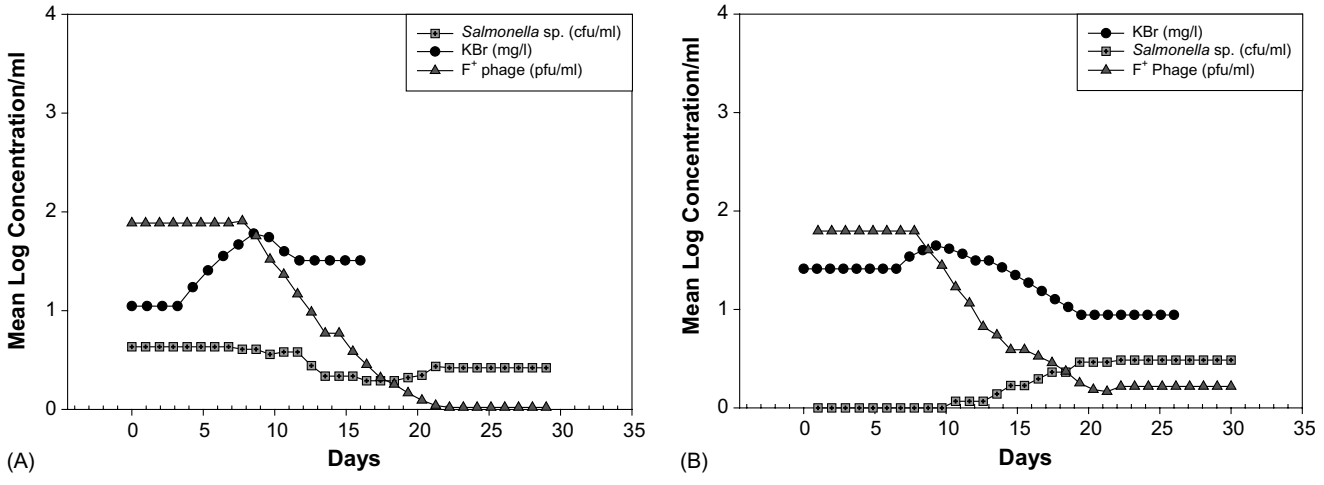

Fig. 3. Concentration (geometric mean) of microbial tracers and bromide: (A) in the pump tank prior to entering the CW and (B) in the CW effluent.

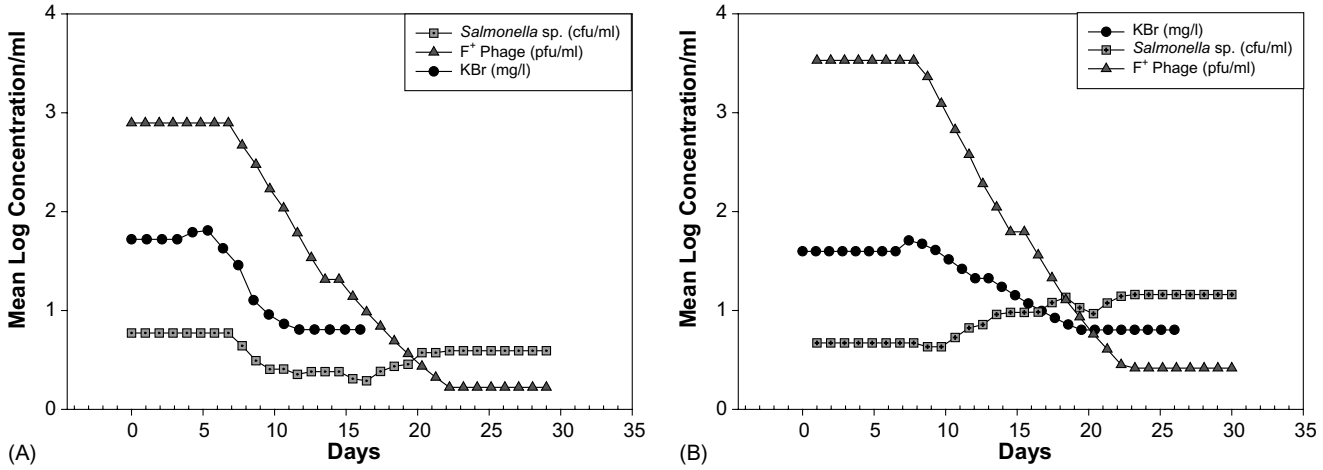

Fig. 4. Concentration (geometric mean) of microbial tracers and bromide: (A) in the pump tank prior to entering the sand filter and (B) in the sand-filter effluent.

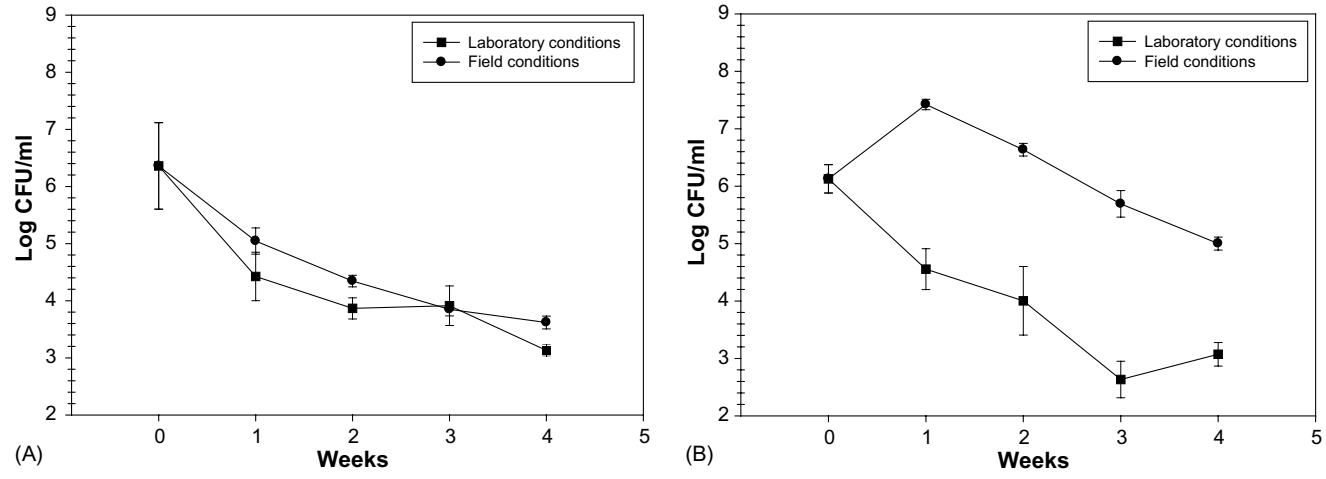

Fig. 5. Survival of *Salmonella* sp. in the CW and laboratory conditions during: (A) summer months and (B) winter months.

Salmonella exhibited a 3-log decrease under laboratory conditions, while the bacterium showed only a 1-log decline under field conditions. During both the summer and winter months, viable *Salmonella* cells were present in the field samples even after 4 weeks. The survival of *Salmonella* in the sand filter is shown in Fig. 6A and B. There was an approximate 4-log decline within 1 week in both the laboratory and field conditions. Thereafter, the bacterial pathogen remained at approximately the same level in the field while in the laboratory conditions the decline was more pronounced. In the warmer summer month, both the laboratory and field incubated samples showed similar inactivation patterns of over 5-log units. The populations of viable *Salmonella* cells averaged over 10^2 CFU/ml even after 4 weeks.

3.3. Phage survival in constructed wetland and sand filter

Phage survival in the CW water was different in the summer and winter months (Fig. 7A and B). There was a rapid decline in phage numbers during the winter months in the microcosms maintained under both laboratory and field conditions. In the summer months, however the phage populations remained relatively stable over the course of 4 weeks. There was a 3-log inactivation of phages under laboratory conditions compared to almost a 2.5-log decline in the field. During winter, however, the phages in the field exhibited an 8-log decline within 3 weeks. The decline was faster for the viruses incubated under the field conditions as compared to the laboratory controls. There were no detectable

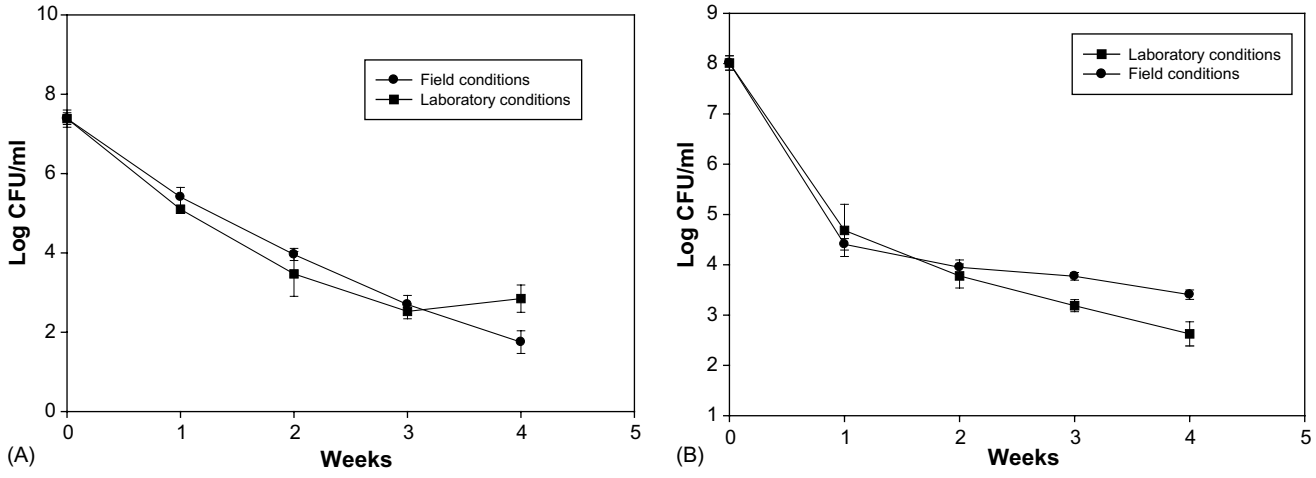

Fig. 6. Survival of *Salmonella* sp. in the sand filter and laboratory conditions during: (A) summer months and (B) winter months.

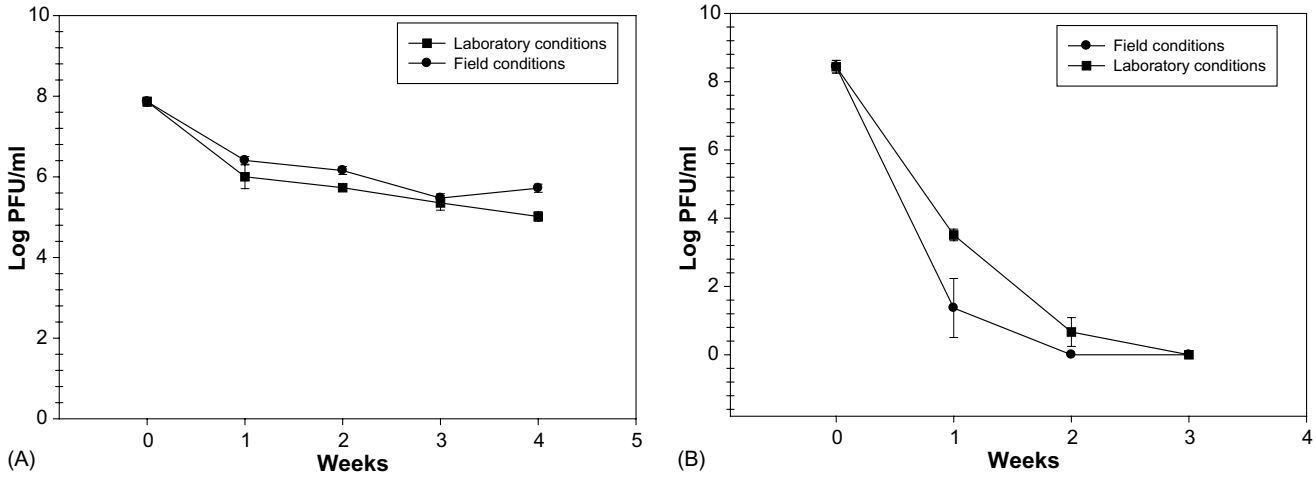

Fig. 7. Survival of MS2 bacteriophage in the CW and laboratory conditions during: (A) summer months and (B) winter months.

phages at the end of 4 weeks in both the field and laboratory incubated samples. The phages exhibited a 4-log decline in numbers within the sand filter during the summer (Fig. 8) At the end of 4 weeks, greater than 10^3 PFU/ml of phages were still viable in the field samples.

4. Discussion

Spearman rank statistics were used to analyze the chemical and biological tracers in the sand filter and the CW. Bromide transport in the CW and SF had a strong correlation (0.9) ($P < 0.05$) suggesting that the behavior of the chemical tracer was similar in both the treatment systems.

Phage transport patterns through the CW and SF were also similar yielding a correlation of 0.8 ($P < 0.05$).

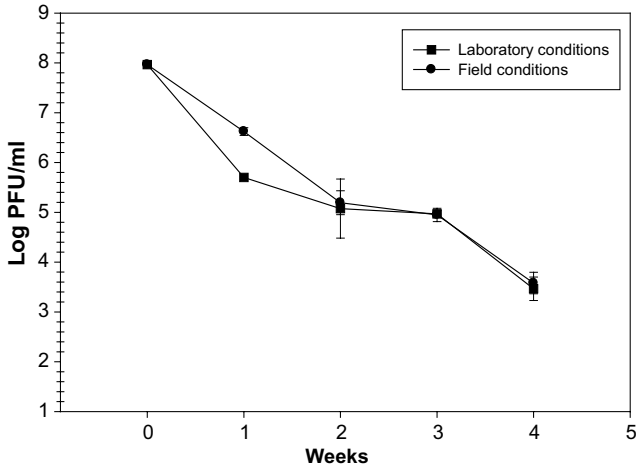

Fig. 8. Survival of MS2 bacteriophage in the sand filter and laboratory conditions during summer months.

The difference in the starting concentrations of the phages in the SF can be attributed to the higher inoculum levels of phages that were introduced into the system. It is interesting, however, that within 20 days, both the CW and SF had only trace levels of phages suggesting that in both these systems, a 3-log reduction can be expected by attenuation processes. Greater than 99% removal of MS2 bacteriophage was observed in sand-based lysimeters (Van Cuyk et al., 2001). Chendorain et al. (1998) observed a 97% reduction in MS2 bacteriophage numbers in single celled and multi-celled surface water CWs. Adsorption and inactivation can be considered to be the primary factors controlling virus attenuation within submerged flow systems and sand-filter systems. The concept of critical pH has recently been proposed as a key factor controlling viral adsorption onto sediments (Schulze-Makuch et al., 2003). It is also evident that virus particles can be expected to be present in the effluent (possibly due to desorption) until the numbers of viable phage particles decrease below the detection limit. Such prolonged low-level detection has been previously reported (Dowd and Pillai, 1997; Dowd et al., 1998). Meschke and Sobsey (1998) have shown using Norwalk virus, poliovirus, and MS2 bacteriophages that viruses can exhibit different adsorption characteristics with different soil textures. In this study, within the sand filter there was a very weak negative, but statistically significant, correlation (−0.4) ($P < 0.05$) between *Salmonella* and phage. It is evident that phages are removed in greater numbers than the bacterial pathogen.

The gradual increase in the bacterial concentration in the SF could be due to increased desorption as the study progressed. A similar increase, though appearing later in the study, was also noted in the CW. It must be emphasized that neither the CW nor the sand filter totally eliminated the bacterial or the viral tracer. While the bacterial reductions in the wetland and the sand filter were negligible, there was a marked reduction of the viral tracer. There was almost a 3-log reduction of viruses in the wetland as compared to more than a 3-log reduction in the sand filter. Other studies have reported on the reduction of microbial tracers under wetland conditions (Gersberg et al., 1987; Neralla et al., 2000; Hill and Sobsey, 2001). The detection of low numbers in the effluent indicates that both bacteria and virus particles can migrate through the CW and SF. Studies conducted at seven onsite CWs in Alabama and North Carolina suggest that microbial removal efficiencies can vary significantly (Barrett et al., 2001). Effluent disinfection may therefore be required to provide an additional barrier against potential environmental contamination. Bromide and phage transport in the SF exhibited a weak correlation of 0.5 ($P < 0.05$). The differences in migration patterns between the bromide and the virus tracers have been reported previously (Bales et al., 1995; Schijven, 2001). This supports the findings that bromide transport patterns cannot be used to model microbial tracers especially since viruses are reactive with their surrounding matrices. Iqbal and Krothe (1996) have reported on the greater mobility of conservative tracers such as Br^- and Cl^- compared to reactive tracers.

The survival of the target organisms was dependant primarily on the factors in the septic effluent and the ambient temperature. Since the organisms were not in contact with the gravel/sand material, adsorption was not a factor in these studies. The reduced survival or persistence of phages in contrast to the bacterium in these septic tanks agrees with previous results. We have previously shown that in the arid southwest regions of Texas, the high cation content of the water is detrimental to phage survival (Dowd and Pillai, 1997). Studies have shown that wastewater associated bacteria could be harbored directly on the root surfaces of plants within the CWs (Vymazal et al., 2001a,b). The decline in bacterial numbers could be attributed to biotic and abiotic factors. Davies and Bavor (2000) have reported that bacterial numbers tend to decrease more rapidly in CWs than in ponds and that bacterial predation can be responsible for the decline.

5. Conclusions

Overall, these results suggest that submerged flow CWs and sand-filter systems are effective at reducing the viral concentration of waste effluent streams. However, the reduction of bacterial concentrations (when *Salmonella* sp. was used a tracer) was not significant. This result indicates that disinfection of effluent from decentralized waste treatment systems is critical to prevent environmental contamination. Factors such as adsorption, desorption and inactivation do play a role in reducing microbial concentrations in the effluent, which suggests that performance of these systems will depend on the specifics of the design, filter media and local climatic conditions.

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