

PATHOGEN AND PATHOGEN INDICATOR REMOVAL CHARACTERISTICS IN TREATMENT WETLANDS SYSTEMS

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ABSTRACT: This paper reports the performance of two horizontal subsurface flow constructed wetland systems in the removal of pathogen and pathogen indicators under variable loading and operating conditions. The two constructed wetland systems evaluated under this project are located near Lincoln, Nebr. Firethorn is located in a small housing community and Rogers Farm at a rural single-family dwelling. Samples at Firethorn were collected at the wetland cell influent, effluent, and sand filter effluent (system effluent) from June 1996 through April 2000 and at the influent and effluent points from Rogers Farm from June 1999 through April 2000. The main monitoring parameters were fecal coliform at both sites and *Salmonella* spp. (from August 1999 through January 2000) at Firethorn. Firethorn demonstrated effective removal of fecal coliforms with 96.3% removal in the wetland cell and 98% by the wetland system, which included a sand filter. Rogers Farm had an average fecal coliform removal of 99.3%. Although *Salmonella* spp. was not detected at Firethorn, reduction of other bacteria was observed through the wetland system. In general, fecal coliform removal was excellent through the wetland system and no distinct difference in removal efficiency was observed with changes in season and temperature at either facility.

INTRODUCTION

The interest in natural and alternative wastewater treatment systems has resulted in the implementation of various types of constructed wetland (CW) systems. The CW technology is attractive because it offers an effective and potentially low-cost approach to wastewater treatment for small communities and it is ideal for areas in the world that lack funds for conventional mechanical wastewater treatment facilities. Taking advantage of natural wetland removal mechanisms, subsurface flow (SSF) CSs were developed in Germany in the late 1970s. In general, wetlands receive, hold, and recycle nutrients that support macroscopic and microscopic vegetation (Crites and Tchobanoglous 1998; Hammer 1989). Wastewater constituent removal transformation are achieved by a multitude of mechanisms. Some of these mechanisms include sedimentation, filtration, chemical precipitation, adsorption, microbial interactions, and uptake of vegetation (Hammer 1989). SSF wetlands can be likened to a horizontal trickling filter that employs the use of biofilms to treat the wastewater constituents. SSF wetlands support biofilms around the medium and macrophyte roots. Mac-

rophytes provide insulation in the winter, but the amount of nutrient removal from and oxygen transport into the wetland system are somewhat controversial (Brix 1997; Vymazal et al. 1998).

The two systems evaluated in this research project were SSF wetlands located in Lincoln, Nebr. The first system is located in a small housing community and the second at a single-family dwelling. This research focuses on pathogenic-related organisms by monitoring fecal coliform and *Salmonella* spp. and their removal effectiveness by SSF wetland systems. Some pathogen removal mechanisms include filtration, antibiosis, predation, and adsorption. Fecal coliform is a classic indicator organism used to determine the pathogenic characteristic of a water sample. However, it is possible to find some pathogens in the absence of fecal coliforms. Therefore, it may be desirable to test directly for certain pathogens such as *Salmonella* spp.

CWs have been shown to be successful in temperate climates and have shown promise in colder environments by successful implementation in Canada, Scandinavia, Europe, and the northern United States (Maehlum et al. 1995; Vanier 1997; Lionberger 1999; Dahab and Surampalli 2000). However, there may be changes in function in cold climates due to the reduction in the physical, chemical, and biological activity of the system at low temperatures (Maehlum et al. 1995). As their use grows and research continues to show their effectiveness, CWs will undoubtedly become a permanent fixture in small communities and areas where financial constraints are a concern. Besides wastewater treatment, ancillary benefits include their aesthetically pleasing vegetation and ability to attract wildlife, aspects that conventional wastewater facilities or lagoon systems typically lack.

PATHOGEN BACKGROUND

Previous research of CW systems with emphasis on pathogen issues is somewhat sparse, and direct detection

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of pathogens from wetlands has been attempted, albeit with varying degrees of success. Reviewing the actual relationship between indicator organisms and pathogens can provide some confidence in the use of indicator organisms for pathogen detection. Morinigo et al. (1990) observed the presence of various indicators and their correlation to *Salmonella* detection. They observed that, at high degrees of pollution in marine waters affected by discharges from a polluted river, fecal coliforms and *Clostridium perfringens* correlated most closely with *Salmonella* spp. However, fecal streptococcus showed a close correlation with *Salmonella* spp. when indicator organisms were low. However, *Salmonella* spp. recovery was sometimes high even at low degrees of pollution and the absence of indicator organisms. Similarly, Kenner and Clark (1974) observed that pathogens may be found in the absence of fecal coliforms and that direct detection may prove more useful as pollution indicators than fecal coliforms for recreational, direct use, and potable waters. In a follow-up study, Morinigo et al. (1993) concluded that a total or near absence of *Salmonella* could be predicated by levels of coliphages in waters with pollution of fecal origin. *Salmonella* was used as an alternative to the classically used indicator organisms. This evidence is not considered conclusive for wetland environments, because their research was conducted on freshwater, marine, and submarine outfall areas subjected to sewage discharges. However, it does indicate that other than direct detection of pathogens, indicator organisms still play a key role in predicting water pollution levels. *Salmonella* research [e.g., the marsh wetland pilot project in Arcata, Calif., by Gersberg et al. (1989)] resulted in a reduction of 93–96% in *Salmonella* with residence times of 23–52 h. The study was unique in that spiked samples of *Salmonella* were injected into the wetland cell influent, with hourly effluent samples taken for analysis. Williams et al. (1995) recorded a 97–99% wetland reduction in *Salmonella* when experiments were carried out with primary settled sewage and secondary treated effluent on a gravel-based wetland cell with a residence time of approximately 6 h.

The use of indicator organisms as the only method to determine the pathogen characteristics of some wastewaters is not accurate. However, the methods of indicator organism detection are accomplished fairly easily; hence, their use is widely accepted. When pathogen contamination is suspected, it may be more appropriate to directly enumerate a pathogen such as *Salmonella* to accurately gauge the extent of pathogen contamination in addition to using an indicator organism.

PROJECT OBJECTIVES

The purpose of this research was to observe and document the removal of pathogen and pathogen indicator organisms in mature CW systems that are subjected to variable loading conditions and wide temperature fluctuations. All of the data analyzed will serve to expand the knowledge base concerning CW application in areas with

widely variable climatic conditions. The following research objectives were identified:

- Document the concentration densities and removal efficiency of fecal coliform and *Salmonella* spp. through the wetland cell and sand filter system.
- Explore possible correlation between temperature and seasonal removal of fecal coliform and *Salmonella* spp. through the wetland systems.

METHODOLOGY

The two sites that were investigated in this research project are located on the east outskirts of Lincoln, Nebr. Both sites had experienced failure in their leach field systems because of native soils with high clay content and a high water table (2–4.5 m), causing effluent surface eruptions. These eruptions posed health hazards and adversely affected the aesthetics of the environment by emitting offensive odors (Vanier 1997). CWs were deemed a viable alternative to the existing systems and provided an ideal opportunity for researchers and state regulators to directly observe how these natural systems would perform under harsh Nebraska climatic conditions: one at the small community level and the other at a single-family dwelling.

The Firethorn CW site is comprised of four parallel cells, with each cell measuring 50 × 25 m. The only liner used in the system is a 0.16-m, on-site clay soil liner compacted to 95% of the modified proctor (with a rated percolation of ≤0.318 cm/day) (Vanier 1997). The treatment depth of the wetland is 0.6 m. The gravel bed has a depth of 0.45 m and consists of 15–40 mm diameter gravel with a porosity of approximately 0.4. Each cell is topped off with a 0.15-m layer of 10-mm gravel, which serves as a substrate for vegetative support. The first 10 m of the cells, along with “finger” extensions, were replaced with new gravel (≈25-mm diameter) in November 1999.

Each cell was planted with three vegetation zones, equal in cover area, of common cattails (*Typha latifolia*) and woody cattails (*Typha domingensis*), alkali bulrush (*Scirpus acutus*), and common reeds (*Phragmites communis*). The first third of each cell was planted with cattail sprigs, the middle third with bulrush sprigs, and the final third with reed sprigs.

Wastewater flows by gravity into a series of two buried fiberglass pretreatment tanks that act as dual settling basins and sludge digestion units. The first fiberglass tank has a volume of 37,000 L and the second a volume of 18,000 L. The flow from the pretreatment tanks is split so that each cell receives one-quarter of the flow. The flow is then equally distributed across the width of the cells by perforated, manifold infiltrators (Vanier 1997). The infiltrators are hollow, slotted, and half-cylindrical, with the influent distribution and effluent collection piping being located at the crown level. The design flow rate was calculated at 225 m³/day, with a typical flow of 115–125 m³/day (Vanier 1997).

The wetland cell effluent collects in a dosing tank and

then flows into a two-cell (each 15 × 18 m) sand filter with a depth of 1.1 m. The sand filter is comprised of a 0.76-m layer of 1–2 mm clean masonry sand topped by a 0.34-m layer of 6-mm washed pea gravel (Vanier 1997). The sand filter effluent is then collected in a concrete wet well and periodically pumped to a drainage ditch.

The Rogers Farm site consists of one cell measuring 14 × 4 m. The liner used in the system is PVC with a thickness of 1.0 mm. The depth of the wetland is 0.75 m; however, the treatment media depth is 0.6 m, leaving the wetland recessed approximately 0.15 m below grade (Glunz 1997). To prevent infiltration of runoff, a 0.76-m berm was constructed around the perimeter of the wetland cell. The treatment media consists of pea-sized gravel varying in diameter, with the majority from 4.76 to 9.53 mm. A 0.10-m layer of fine gravel (2–4.76 mm diameter) was mixed with the top 15 cm of pea gravel, which raised the treatment media by 5 cm (Glunz 1997). The cell is planted with two vegetation zones, equal in area, of cattails (*Typha* spp.) and common reeds (*Phragmites* spp.). The influent half of the cell was planted with cattail sprigs and the effluent half with reed sprigs.

Wastewater flows from the house to a 4,700-L concrete septic tank. Wastewater flows by gravity through a V-notch weir and enters the wetland via a slotted, manifold infiltrator that equally distributes the wastewater across the width of the cell. After passing through the wetland, the wastewater exits and flows through a V-notch weir to its final destination in a total containment pond. The design flow rate was calculated at 2.27 m³/day, with an average actual flow rate of 1.51 m³/day. However, the flow is intermittent because of the fluctuation in the usage rate throughout the day.

Wastewater samples were collected at the Firethorn CW from June 1996 through April 2000 on a biweekly basis. Data were collected for *Salmonella* spp. analysis from August 1999 through January 2000. Although sand filter data are included, the main focus of the study was the removal of pathogen and pathogen indicator organisms in the wetland. In addition, nine samples of influent and effluent wastewater were collected for fecal coliform analysis at the Rogers Farm CW between June 1999 and April 2000. At Firethorn, the sampling sites were the CW influent, CW cell effluent, and sand filter effluent (system effluent). Rogers Farm sampling was done at the CW influent and effluent points. Note that, in addition to pathogens and pathogen indicators, the wetlands systems were monitored using an extensive list of parameters including soluble and total BOD₅ and COD, solids, nitrogens, phosphorous, pH, and temperature (Vanier 1997; Vanier and Dahab 1997, 2001; Stone 1998; Lionberger 1999; Dahab and Surampalli 2000; Jillson 2000). Firethorn data from June 1996 through February 1997 and from April 1997 through mid-October 1998 were gathered by Vanier (1997) and Lionberger (1999), respectively, Stone (1998) initiated the collection of water quality data at the Rogers Farm site.

All of the analyses in the research followed the proce-

dures, as outlined in *Standard methods* [American Public Health Association (APHA) 1998] with the exception of *Salmonella* spp. enumeration, which was accomplished using the U.S. EPA–approved method developed by Kenner and Clark (1974). *Salmonella* spp. detection was accomplished by the use of serial dilutions using an enrichment broth, followed by isolation plating with two types of agar: Xylose Lysine Desoxycholate (XLD) agar and Brilliant Green (BG) agar media. In addition, direct plating of wastewater samples on the two types of agar was done to aid in finding appropriate serial dilutions (Jillson 2000).

RESULTS AND DISCUSSION

Fig. 1 shows the temperature profile of the Firethorn CW influent and the effluent. The largest differences between the influent and effluent temperatures occurred during the summer (April–September) and winter (October–March) seasons. Vanier (1997) observed that the wetland exerted a slight warming of the influent temperatures in the summer and cooling of influent temperatures in the winter. However, as the CW system matured, this temperature difference appeared to be less extreme. The average temperatures were 14.5° and 13.5°C for the influent and effluent, respectively. The influent experienced a high of 26.2°C and a low of 1.5°C. The high and low temperatures for the effluent were 28.9° and 0.3°C, respectively. No ice formation was observed inside the sampling wells, wet well, weir boxes, and sump pit during the course of sampling at either wetland site.

Table 1 is a summary of fecal coliform removal data for the summer and winter periods at Firethorn and the mean for the study. Fig. 2 shows the fecal coliform influent, effluent, and sand filter effluent concentration at Firethorn. The sand filter generally increased the overall removal efficiency; however, the majority of the removal occurred in the wetland system.

Summer removal efficiency of fecal coliform at Firethorn was found to be 97% for the initial start-up period from June 1996 through September 1996, and 94, 98.2, and 97.6% for subsequent summer periods; winter removal rates were 97.7, 98.9, 90.9, and 98% for each 6-month period. The average fecal coliform removal for the entire study was quite high at 96.3%, with the sand filter raising the removal rate to 98%.

Fecal coliform removals expressed as log reductions at the Firethorn wetlands systems are shown in Fig. 3. As seen, fecal coliform removal in the entire wetlands systems (including the sand filter) ranged from 1.6 to nearly 5. The average log removal during the entire study was found to be 2.0 and 2.8 for the wetland effluent and the system effluent, respectively. Fig. 3 clearly illustrates the value of the sand filter system in polishing the constructed wetlands effluent.

Figs. 2 and 3 chronologically depict the fecal coliform removal characteristics at the Firethorn wetland facility. Analysis of the data presented in these figures shows that

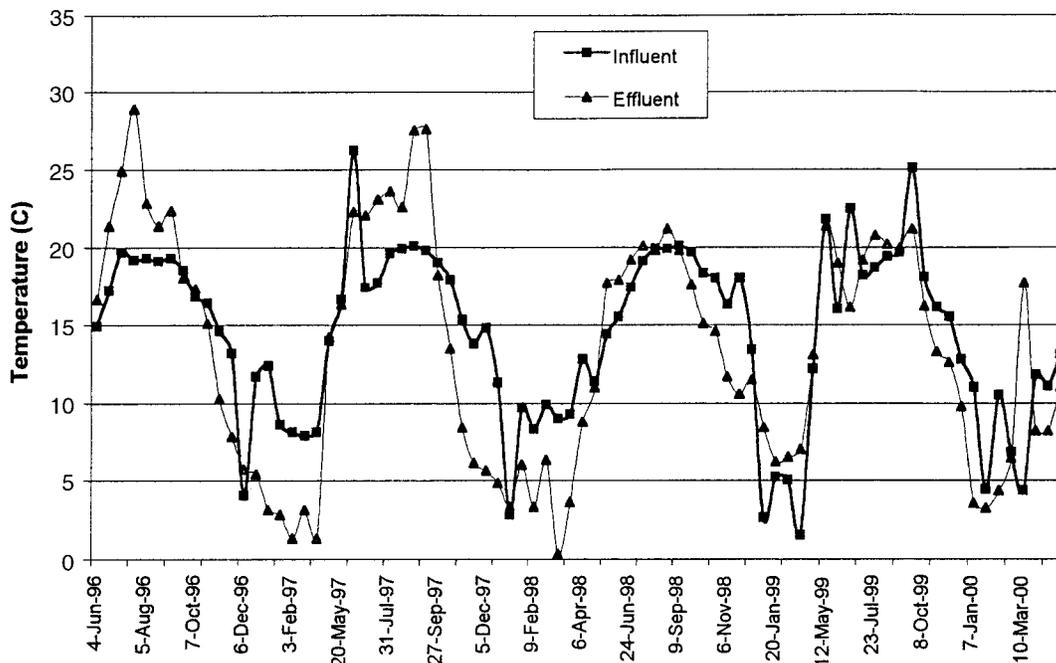


FIG. 1. Firethorn Wastewater Temperature Profile

TABLE 1. Summary of Fecal Coliform Removal versus Temperature for Firethorn

Time period	Temperature (C)	Wetland removal efficiency (%)	Wetland system removal efficiency (%)	Wetland log removal	Sand filter log removal	Wetlands system log removal
June 1996 to September 1996	20.5	97.0	86.7	1.74	-0.51	1.23
Standard deviation	2.9	4.1	22.3	1.75	-0.39	—
Number of observations	8	8	8	8	8	8
October 1996 to March 1997	6.0	97.7	99.8	1.88	1.30	3.18
Standard deviation	5.5	2.3	0.34	1.83	1.06	—
Number of observations	11	11	11	11	11	11
April 1997 to September 1997	18.6	94.0	96.1	1.44	0.16	1.60
Standard deviation	5.5	9.4	8.8	1.00	0.31	—
Number of observations	11	11	11	11	11	11
October 1997 to March 1998	6.3	98.9	99.9	2.29	1.15	3.44
Standard deviation	5.7	1.0	0.10	2.50	1.12	—
Number of observations	12	12	12	12	12	12
April 1998 to September 1998	17.2	98.2	99.8	2.20	0.88	3.08
Standard deviation	3.9	2.4	0.49	2.27	0.87	—
Number of observations	12	12	12	12	12	12
October 1998 to March 1999	9.6	90.9	98.3	1.23	0.92	2.15
Standard deviation	4.9	7.4	2.7	1.10	1.17	—
Number of observations	10	10	10	10	10	10
April 1999 to September 1999	19.5	97.6	99.7	2.25	0.49	2.75
Standard deviation	2.9	5.0	0.34	2.20	0.89	—
Number of observations	10	10	10	10	10	10
October 1999 to March 2000	9.1	98.0	98.9	2.01	0.23	2.23
Standard deviation	3.6	2.3	1.1	2.22	0.02	—
Number of observations	11	11	11	11	11	11
Study mean	13.0	96.3	98.0	2.0	0.8	2.8
Standard deviation	7.2	5.7	7.2	0.8	0.9	—
Number of observations	85	85	85	85	85	85

Note: Wetlands system includes wetland and sand filter.

the average summer removal percentage was >95%, with the winter average >99%. If the initial start-up period from June through October 1996 is excluded, the removal rate for the summer increases to 98.5%.

Fig. 4 shows the linear regression of the removal per-

centages versus temperature. As shown, the regression line indicates that there was no clear trend of fecal coliform removal efficiency with seasonal changes in temperature (i.e., coefficient of determination, $R^2 = 0.01$).

Nebraska Standards for Water Quality, under the Ne-

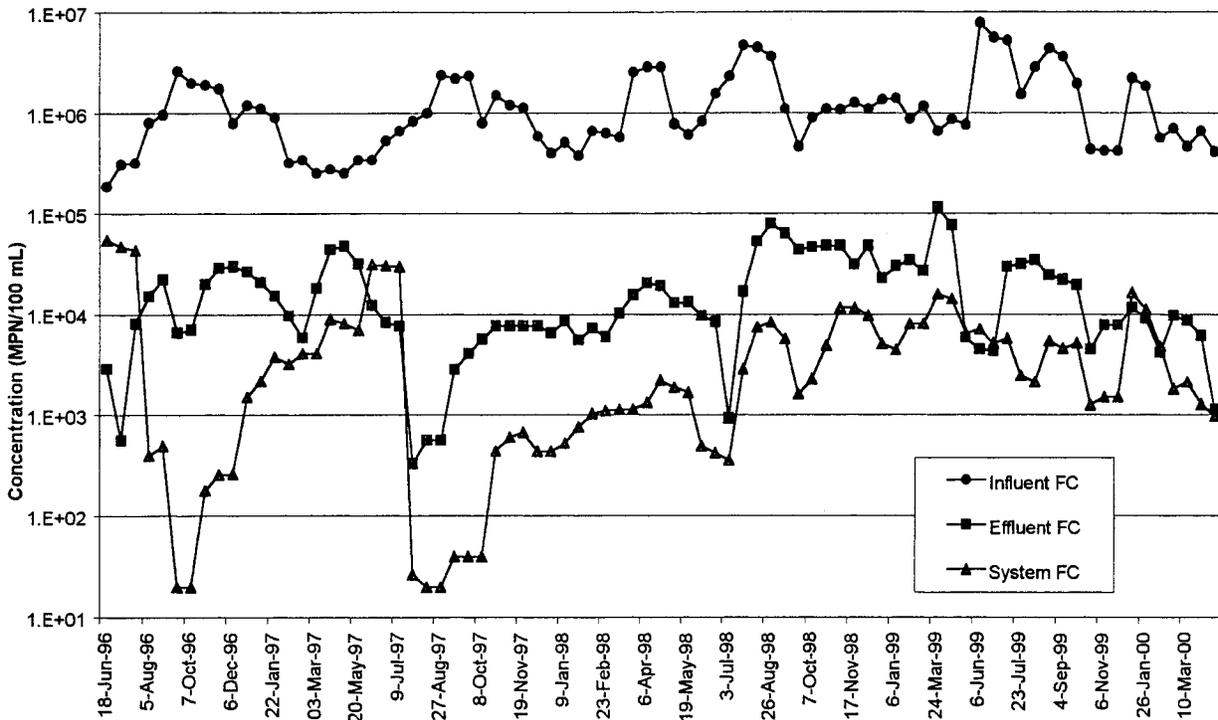


FIG. 2. Fecal Coliform (FC) Concentration at Firethorn (Three-Point Moving Average)

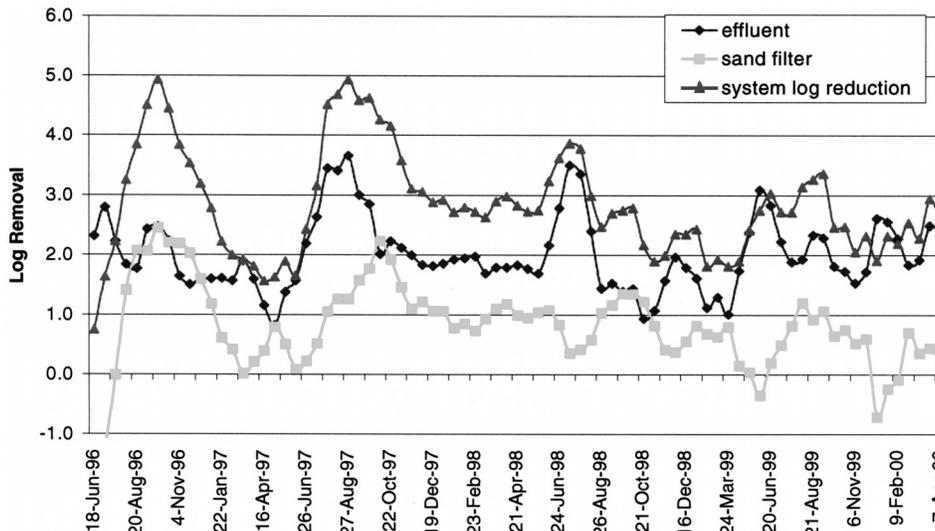


FIG. 3. Fecal Coliform Log Reductions at Firethorn (Three-Point Moving Average)

Nebraska Department of Environmental Quality Title 117 regulations, stipulate that bacteria of the fecal coliform group shall neither exceed a geometric mean of 200/100 mL nor equal or exceed 400/100 mL in >10% of the samples during the period of May 1 through September 20 in wastewater effluents discharged to primary contact (recreational) streams. This criterion is based on a minimum of five samples within a 30-day period and applies to wastewater discharged to surface water. Currently, the fecal coliform effluent at Firethorn does not meet this criterion. However, Firethorn discharges into the Steven's Creek watershed, which is currently not classified as a

surface water used for primary contact recreational activities. Therefore, Firethorn is not subjected to the fecal coliform discharge criteria at this time. Fecal coliform regulations are reviewed every 3 years, with the next review scheduled for 2002. Based on recommendations by the Nebraska Department of Environmental Quality, it is possible that the segment of the Steven's Creek watershed of concern here could be reclassified, requiring compliance with the regulation.

Fig. 5 shows the fecal coliform influent and effluent concentrations at the Rogers Farm CW. Removal was consistently >98%, with an average of 99.3%. The average

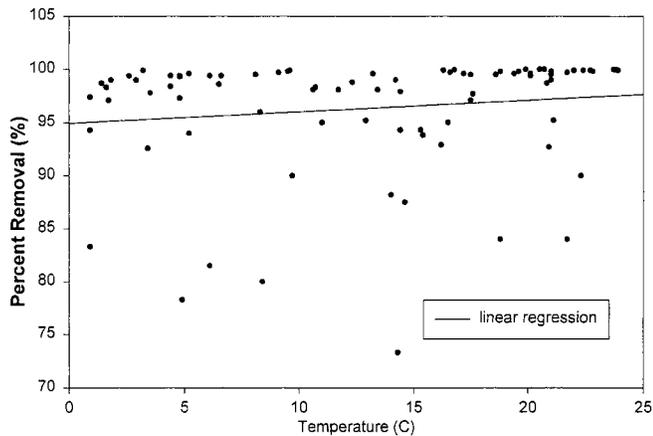


FIG. 4. Firethorn Fecal Coliform Removal versus Temperature

log removal was 2.8, as was that of the Firethorn system effluent. Overall, effluent concentrations were lower at Rogers Farm than Firethorn. This may be due to the longer design detention time of 6 days at Rogers Farm, compared to 5 days at Firethorn. Only one sample from the Rogers Farm had an effluent fecal coliform concentration <200/100 mL.

Fig. 6 represents the fecal coliform removal percentage in relation to changes in temperature at the Rogers Farm system. The linear regression analysis shows no clear relationship between temperature and removal efficiency at this facility. This is similar to the findings at Firethorn.

In general, the exact mechanisms by which constituents are removed in wetland systems are not completely understood. Sand filters of the type used at the Firethorn facility typically rely on a bacterial biofilm that coats the sand medium. Research by Weber-Shirk and Dick (1997) found that one primary method of *E. coli* removal was by bacterial predation. They suggested that bacterial predators are even more important in removal than the sticky biofilm, especially on particles >2 μ m. It is also known that predation by nematodes, protozoans or cladocera, lytic bacteria, and bacteriophage attacks reduce fecal coliforms and pathogens (Hammer 1989; Ottova et al. 1997). In addition, Williams et al. (1995) found a strong corre-

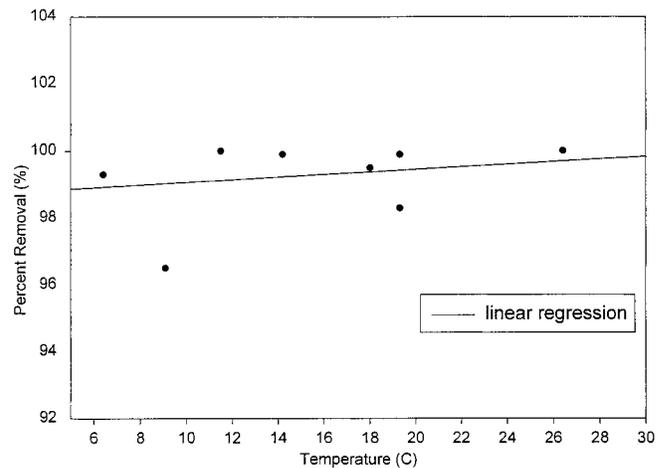


FIG. 6. Rogers Farm Fecal Coliform Removal versus Temperature

lation between BOD and fecal coliform reduction during treatment with gravel-based hydroponics. Because adsorption is the primary removal mechanism of BOD, the association between BOD and fecal coliform suggests that adsorption may likely be a primary mechanism of pathogen removal (Gray 1989). Therefore, fecal coliform removal appears to be the result of a complex combination of mechanisms. Based on these data, if fecal coliforms are temperature sensitive, the temperature ranges experienced in the Firethorn and Rogers Farm CWs did not appear to have enhanced or hampered the removal of fecal coliform.

Testing for *Salmonella* spp. was conducted at the Firethorn CW from August 1999 through January 2000. However, *Salmonella* was not successfully detected in the wetlands samples. The analysis methods were checked repeatedly using controls obtained from the Department of Food Science and Technology, University of Nebraska-Lincoln.

Samples were considered positive for *Salmonella* if XLD plate cultures contained black-centered red colonies or if BG plate cultures contained pinkish-white, opaque colonies with a brilliant red background. Although cultures with the indicated black-centered colonies for *Salmonella* were not specifically recovered, yellow colonies

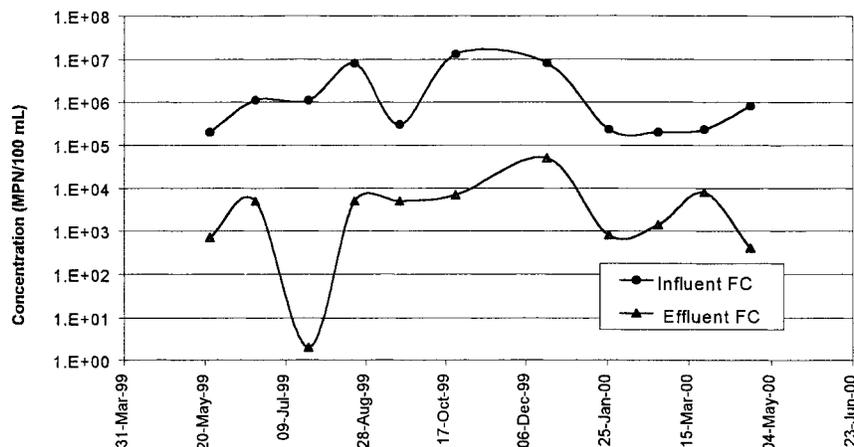


FIG. 5. Rogers Farm Fecal Coliform (FC) Concentration

were isolated by the XLD agar. Some of these colonies could have been *Salmonella* spp., but with hundreds of subtypes *Salmonella* was difficult to isolate (D. L. Peters, various personal communications, 2000). Other bacteria that form yellow colonies on XLD agar are *Citrobacter*, *Klebsiella*, *Proteus*, *Enterobacter*, *Shigella*, or *Coliform* (Dynamac Corp. 1993). Reduction of these yellow colonies was observed through the length of the wetland system.

The BG agar exhibited yellow-green colonies surrounded by a yellow-green zone. *Escherichia*, *Klebsiella*, and *Proteus* were reported to exhibit these types of colonies (Dynamac Corp. 1993). Reduction of these colonies was observed through the wetland. It was more difficult to isolate bacterial colonies on the BG agar because of the lack of a distinct colony color and the poor growth of some *Salmonella* species because of the brilliant green dye content. Therefore, the BG agar also proved inconclusive.

At first, recovery of *Salmonella* spp. seemed practical because these organisms are widespread in nature and occur in humans and nearly all known animals (Gray 1989). Of the 1,800 subtypes or serotypes reported, some are largely specific to a single host such as the typhoid organism, yet many others are not host specific (Gray 1989). The most common origins of *Salmonella* spp. are in large animal reservoirs in carriers (e.g., chickens, turkeys, pigs, cows, and other domestic and wild animals). Nontyphoidal *Salmonella* spp. are reported to have the ability to survive in meats and animal products that are not thoroughly cooked. Therefore animal products are the main vehicles of transmission in humans. The *Salmonella* spp. that cause typhoid fever and other enteric fevers lack an animal reservoir and are generally transmitted by person-to-person contact or from various water sources (e.g., bathing waters and well waters).

The diversity and density of *Salmonella* spp. depend on the nature of the wastewater, which is determined by the general health of the community. *Salmonella* spp. are susceptible to removal by various methods. The majority of pathogen content is reported to be generally concentrated in sludge, which may offer a food source for survival (Ponugoti et al. 1997). *Salmonella* spp. can survive in both aerobic and anaerobic environments, but prolonged anaerobic exposure can be detrimental (D. L. Peters, various personal communications, 2000). In activated sludge, *Salmonella* spp. are reduced by 80–90%, mainly by predation due to amoebae, ciliate protozoans, and rotifers (Gray 1989). However, the ciliate protozoans and rotifers feed only on suspended bacteria and not on the flocculated bacteria, whereas amoebae feed on both (Gray 1989). Thus, Firethorn does not appear to provide a suitable environment where *Salmonella* spp. are likely to be found unless an outbreak occurs among the resident population.

CONCLUSIONS

Based on the analyses of data collected at the two SSF wetlands facilities during this study and presented above, the following conclusion can be drawn:

- Both CW systems demonstrated effective removal of fecal coliforms. The average fecal coliform removal in wetland cells at Firethorn was 96.8%, with the sand filter raising the removal percentage to an average of 98%. The average log removal was 2.0 in the wetlands cell alone and 2.8 in the wetland system including the sand filter. The average fecal coliform removal in the Rogers Farm wetland was 99.3%, which corresponded to a 2.8 log reduction.
- Recovery of *Salmonella* spp. from the wetlands systems was not conclusive. Isolation of other bacteria colonies was observed along with a reduction of these colonies through the length of the wetland. *Salmonella* spp. contamination is indicative of the health and lifestyles of the population; therefore, *Salmonella* spp. were extremely difficult to detect, especially in a small community.
- From a seasonal perspective, analyses of data collected from June 1995 through April 2000 at Firethorn indicated that the summer periods resulted in fecal coliform reductions of 97, 94, 98.2, and 97.6% for the summer seasons of 1996–1999, respectively. The corresponding winter removals were 97.7, 98.9, 90.9, and 98% for each winter period, respectively. The average removal for all summer periods was 96.7%, and the average winter removal was 99%. If the original start-up period is excluded, summer removal increases to 98.5%. No clear difference in removal efficiency occurred with a change in temperature at Firethorn. Rogers Farm data consisted of nine sampling occasions from June 1999 to April 2000, with no clear correlation between removal efficiency and temperature changes being found. This result mirrored the findings at Firethorn.

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