

The persistence and removal of enteric pathogens in constructed wetlands

Mohammad R. Karim^{a,*}, Faezeh D. Manshadi^a, Martin M. Karpiscak^b,
Charles P. Gerba^a

^aDepartment of Soil, Water and Environmental Science, University of Arizona, Tucson, AZ 85721, USA

^bOffice of Arid Lands Studies, University of Arizona, Tucson, AZ 85721, USA

Received 6 September 2002; received in revised form 18 August 2003; accepted 17 December 2003

Abstract

Sedimentation is thought to be one of the mechanisms of microbial reduction from wetlands used for wastewater treatment. This study compared the occurrence and survival of enteric indicator microorganisms and pathogens in the water column and sediments of two constructed surface flow wetlands in Arizona. On a volume/wet weight basis the concentration of fecal coliforms and coliphage in the water column and sediment was similar. However, on a volume/dry weight basis the numbers were one to two orders of magnitude higher in the sediment. *Giardia* cyst and *Cryptosporidium* oocyst concentrations were one to three orders of magnitude greater in the sediment compared to the water column. The die-off rates of all the bacteria and coliphage were greater in the water column than the sediment. The die-off rates of fecal coliforms in the water and sediment were $0.256 \log_{10} \text{day}^{-1}$ and $0.151 \log_{10} \text{day}^{-1}$, respectively. The die-off rates of *Salmonella typhimurium* in the water and sediment were $0.345 \log_{10} \text{day}^{-1}$ and $0.312 \log_{10} \text{day}^{-1}$, respectively. The die-off rates of naturally occurring coliphage in water column and sediment were $0.397 \log_{10} \text{day}^{-1}$ and $0.107 \log_{10} \text{day}^{-1}$, respectively, and the die-off rates of and PRD-1 in water and sediment were $0.198 \log_{10} \text{day}^{-1}$ and $0.054 \log_{10} \text{day}^{-1}$, respectively. In contrast *Giardia* die-off in the sediment was greater compared to the water column. The die-off rates of *Giardia* in water and sediment were $0.029 \log_{10} \text{day}^{-1}$ and $0.37 \log_{10} \text{day}^{-1}$, respectively. Coliphage survived the longest of any group of organisms in the sediment and the least in the water column. In contrast *Giardia* survived best in the water column and least in the sediment.

© 2004 Elsevier Ltd. All rights reserved.

Keywords: Constructed wetland; Fecal coliforms; *S. typhimurium*; *Giardia*; *Cryptosporidium*; PRD-1

1. Introduction

Recently, attention has been focused on the ability of wetlands to reduce human pathogens in wastewater. Wetlands have been found to reduce microorganisms with varying but significant degrees of effectiveness. There have been several studies published on microbial

water quality improvement using wetlands [1–8]. Karpiscak et al. [7] reported 57 percent reduction of total coliforms and 62 percent of fecal coliforms from a duckweed-based wetland system. The authors also observed 98 percent reduction of *Giardia*, 87 percent of *Cryptosporidium*, and 38 percent of coliphage by the same system. In a multi-species (bulrush, cattail, black willow, and cottonwood) wetland system, Karpiscak et al. [7] found the reduction of total and fecal coliforms to be 98 and 93 percent, respectively. *Giardia* and *Cryptosporidium* were reduced by an average of 73 and 58 percent, respectively, and enteric viruses by 98 percent.

*Corresponding author. American Water, Quality Control and Research Laboratory, 1115 South Illinois Street, Belleville, IL 62220-3102, USA. Tel.: +1-618-239-0531; fax: +1-618-235-6349.

E-mail address: mkarim@amwater.com (M.R. Karim).

Sedimentation is one of the many processes [2,3,9], which may be involved in the reduction of pathogens in wetlands. Several studies have found that coliforms, fecal coliforms, and *Salmonella* tend to concentrate in sediments of polluted surface waters [10–12]. Gerba and Mcleod [10] observed higher numbers of coliforms and fecal coliforms in marine sediments compared to the overlying water. Hendricks [11] found approximately 90 percent of *Salmonella* isolates in the sediments and showed a higher recovery from sediments than water. Similarly, Van Donsel and Geldreich [12] recovered 100–1000 times more fecal coliforms in river mud than in the overlying water. Enteric bacteria can also survive longer in sediments than the overlying water. *E. coli* has been shown to survive longer in marine sediments than in seawater [10].

Viruses associated with large particles soon leave a water column and settle into the bottom sediments, while viruses adsorbed on colloidal particles tend to stay suspended in the water for a longer time [13]. Suspended solids-associated viruses that settle out of the water column accumulate in a loose, fluffy layer over the compact bottom sediment. Presence of sediment found to prolong virus survival [14]. Reduction of protozoan parasites (*Giardia* and *Cryptosporidium*) observed in wetlands [1] was thought to be due to the sedimentation of parasites to the bottom of the wetlands. Thus, the bottom sediments of constructed wetlands could potentially serve as a reservoir of human pathogens, which could be released into the water column by storm or manmade events. The purpose of this study was to compare the occurrence and survival of fecal coliforms, coliphages, *Giardia* cyst and *Cryptosporidium* oocyst in the water and sediments from a 10-year-old constructed wetland.

2. Materials and methods

2.1. Study site

Wastewater and sediment samples were collected from the Constructed Ecosystem Research Facility (CERF) located in Pima County, Tucson, AZ. The operation of CERF began in 1989 with the intention of studying the ability of constructed wetlands to be used for treatment of wastewater [7]. CERF receives secondary unchlorinated wastewater from the adjacent Roger Road Municipal Wastewater Treatment Facility. The facility has six raceways lined with 30 mil hyperlon. Water and sediment samples were collected from two of the six raceways. In one of the raceways water hyacinth (*Eichhornia crassipes*) was used as a floating plant. The raceway was 20 m in length, 8.2 m in width, and 0.6 m in depth with an influent flow rate of 58 l min⁻¹. In the second raceway duckweed was used as a floating plant.

The raceway was 65 m in length, 11.9 m in width, and 0.9 m in depth with an influent flow rate of 55 l min⁻¹.

2.2. Study organisms

Salmonella typhimurium (ATCC 23564) was originally received from American-type culture collection. *S. typhimurium* inocula was prepared by centrifuging (1000g for 10 min) late-log phase cultures grown in tryptic soy broth (TSB, Difco, Detroit, MI) at 37°C. Bacterial cells were washed twice with sterile Tris buffered saline (Trisma Base, Sigma Chemical, St. Louis, MO). Cells were then suspended in sterile Tris buffered saline and diluted to a concentration of 2.5 × 10⁶–5 × 10⁶ colony forming units (CFU) ml⁻¹. McFarland standard 0.5 was used as a reference of turbidity to achieve a bacterial concentration of 2.5 × 10⁶–5 × 10⁶ CFU ml⁻¹. Bacteriophage PRD-1 (ATCC 23564B) was grown in *S. typhimurium* (ATCC 23564). Log phase bacteria were used for preparing PRD-1 stocks using the double agar layer (DAL) method [15]. *Giardia muris* cysts (Shiwaji Ramaligam, Beaverton, OH) were produced in infected mice purified by density gradient centrifugation and suspended in deionized water.

2.3. Collection of water and sediment

Wastewater samples (1 l) were collected at the water surface in sterile plastic bottles. Sediment samples were collected with the aid of an Ekman dredge. The samples were kept in an ice-packed cooler and transported to the laboratory at the University of Arizona. The samples for fecal coliforms and coliphages were processed within 4 h of collection. The samples for *Giardia* and *Cryptosporidium* were processed within 24 h of collection. Percent solids in the sediment were determined using the procedure described by Pepper et al. [16].

2.4. Microbial assay of field samples

Fecal coliform bacteria in wastewater samples were determined using the most probable number method [17]. Bacterial numbers in the sediment were determined by diluting 10 g of sediment into 90 ml of sterile deionized water. The sediment was stirred for 10 min and maintained in suspension by continual mixing of the sample before aliquots were taken for assay. EC broth was used for the detection of fecal coliforms. The results were presented as CFU per 100 ml of wastewater or 100 g of sediment.

Coliphage was detected using the double agar layer (DAL) method [15]. *Escherichia coli*, strain ATCC 15597 (American Type Culture Collection, Rockville, MD) was used as the host bacterium. Coliphage titers in the sediment was determined by adding 10 g of sediment to

90 ml of 3 percent beef extract (Becton Dickinson Microbiology Systems, Cockeysville, MD). The sediment was then mixed for 30 min and serial dilutions were made and assayed. Selective mEndo agar (Difco) was used as the top agar to reduce the growth of the other species of bacteria that could make plaque visualization difficult. All sample dilutions were assayed in duplicate. Plaques were enumerated after 18 h and results were expressed as plaque forming units (PFU) per 100 ml of wastewater or 100 g of sediment.

2.5. Efficiency of coliphage recovery from sediment

In order to determine recovery efficiency of coliphage from sediment, 1 ml of 10^6 PFU ml⁻¹ MS-2 stock was mixed with 10 ml of deionized water and 10 g of sediment was then added and stirred for 30 min. The mixture was then centrifuged at $1000 \times g$ for 30 min. The supernatant was aspirated off and assayed. A control (without sediment) was done at the same time and the bacteriophage absorption to sediment was determined by comparing the titer with the control. The pellet was eluted with 90 ml of 3 percent beef extract (Becton Dickinson Microbiology Systems, Cockeysville, MD) and recovery efficiency determined.

2.6. Detection of *Giardia* and *Cryptosporidium* in water and sediment

Both *Giardia* and *Cryptosporidium* were determined simultaneously using an immunofluorescent method similar to the protocol described in the Information Collection Rule laboratory Manual [18] with the exceptions that wastewater samples were directly centrifuged for concentrating the parasite rather than filtration and for clarification by flotation, not more than 1 ml of packed pellet was floated per centrifuge tube. A 20 ml aliquot of each sediment sample was processed for *Giardia* cysts and *Cryptosporidium* oocysts. The weight of the samples was recorded and the dry matter content was determined as described by Pepper et al. [16]. Sediment samples were mixed with 30 ml of sterile deionized water. After homogenization, the samples were passed through a series of two sieves of gradually finer mesh (opening 2 mm, No. 10, Fisher Scientific Company, USA; 300 μ m, No. 50, Gilson Company, Inc. Worthington, OH) to remove fibrous and other large materials. The samples were then transferred to 750 ml plastic centrifuge bottles and concentrated by centrifugation at 1050g for 10 min (CS-6 centrifuge, Beckman, Palo Alto, CA). The supernatant was aspirated off without disrupting the pellet. The cyst and oocyst containing pellet was then washed twice in deionized water. Elution solution was added to achieve a final volume of 20 ml. The sample was then processed as described for the water sample. For

recovery efficiency studies, 20 ml of sediment was mixed with 1 ml of 10^5 cysts of *Giardia*. A 1 ml aliquot was removed and used as the control and the remaining 20 ml were processed as described previously. The control was diluted with Tris buffered saline and counted.

2.7. Survival studies

Wastewater and sediment samples were collected from the duckweed pond. Sediment of 100 g (wet weight) was mixed with 400 ml of pond water in 11 sterile plastic bottles. Wastewater without adding any sediment was used as a control. The bottles were kept in the dark at room temperature (22.5°C). After various times, 0.3 ml samples were withdrawn from the bottles and 1:10 serial dilutions were made in Tris buffered saline before assaying for fecal coliforms on mFC agar (Difco Laboratories, Detroit, MI) by the spread plate method. The plates were incubated at 44.5°C for 24 h. Indigenous coliphage was enumerated using the double layer method described by Adams [15].

2.8. *S. typhimurium* and PRD-1

S. typhimurium was added to achieve a concentration of 10^5 CFU ml⁻¹ in 500 ml of wastewater with or without 20 percent sediment. The bottles were kept in the dark at room temperature (22.5°C). After various times, 0.3 ml samples were withdrawn from the bottles and *S. typhimurium* enumerated by plating on Hektoen enteric agar (Difco) and incubating at 37°C for 24–48 h. PRD-1 was added at a concentration of 10^6 PFU ml⁻¹ in 500 ml wastewater with or without 20 percent sediment and samples collected at various intervals for assay.

2.9. *Giardia muris*

Giardia muris cysts were placed in 11 plastic bottles containing wastewater or wastewater plus sediment (20 percent). Cysts were added to a concentration of 10^6 cysts per ml to 200 ml of water or water plus sediment sample. A 30 ml aliquot was taken from each sample at 1-, 2-, 3-, and 5-day intervals and *Giardia* cysts were concentrated for the determination of viability by excystation. Sediment samples were mixed with 20 ml of sterile deionized water. After homogenization, the samples were passed through a series of two sieves of gradually finer mesh (opening 2 mm, No. 10, Fisher Scientific Company, USA; 300 μ m, No. 50, Gilson Company, Inc. Worthington, OH) to remove fibrous and other large materials. Samples were then collected into 50 ml sterile polypropylene tubes and centrifuged at 1050g for 10 min. The supernatant was aspirated off and the cyst suspension was washed twice in deionized water. After the second wash, the pellet was resuspended in

approximately 15 ml of deionized water and carefully layered over 15 ml of 1 M sucrose contained in 50 ml plastic centrifuge tubes. The tubes were then centrifuged at 600g for 10 min. The interface layer containing the cysts was aspirated into a collection flask using a pipette. This cyst containing solution was then washed an additional two times as described above. After the second wash, the supernatant was aspirated off down to 0.2 ml above the pellet. Following that, 5 ml of prewarmed (37°C) reducing solution (1 percent glutathione, 1 percent L-cysteine-HCL and Hank's balanced salt solution, Sigma, St. Louis, MO) and 5 ml of prewarmed (37°C) 0.1 M sodium bicarbonate solution (Fisher Scientific Co., Fairlawn, NJ) were added to the pellet and incubated at 37°C for 30 min. The cysts were pelleted again and resuspended in 10 ml of chilled (4°C) trypsin-tyrode's (0.5 percent trypsin and tyrode's solution) solution and then centrifuged at 1050g for 10 min. The supernatant was then aspirated off to no less than 0.2 ml above the pellet. A 0.3 ml of prewarmed (37°C) trypsin-tyrode's solution was added to the pellet and mixed by low speed vortexing. Then 10- μ l samples were removed from each tube and placed in a hemocytometer (Baxter Health Care Corp, McGraw Park, IL). Samples were examined at 400 \times magnification with a phase-contrast microscope (BH-2, Olympus, Japan). Cysts that were seeded into the wastewater (control) were also processed using the same method. All experiments were done in duplicate.

2.10. Data analysis

Linear regression analyses were used to calculate die-off rates (\log_{10} reduction per day) by the following equation: $\log_{10} N_t/N_0 = -mx + b$, where $\log_{10} N_t/N_0$ is the ratio of the \log_{10} value at time t (measured in days) to the initial \log_{10} value ($\log N_0$), x is the time in days, b is the intercept value, and m is the slope.

3. Results

The number of fecal coliforms in wetlands containing duckweed and water hyacinth is shown in Table 1. On a wet weight basis of sediment the number of fecal coliforms in the sediment of the duck weed pond was slightly lower than the water column. However, on a dry weight basis of the sediment the number is greater in the sediment than in the water column. The number of fecal coliforms in the sediment of the hyacinth pond was slightly greater than in the water column. The number was 1–2 orders of magnitude greater on a dry weight basis of sediment. The number of coliphage in the wetland containing duckweed and water hyacinth is presented in Table 2. The number of coliphage in the water column and sediment was a similar order of magnitude on a volume/wet weight basis. However, on a dry weight basis the number of coliphage was 1–2 orders of magnitude greater in both wetlands. The concentration of *Giardia* in the sediment was 2–3 orders of magnitudes greater in both wetlands (Table 3). Similar to *Giardia*, larger numbers of *Cryptosporidium* were found in the sediment than the overlying water. Generally, from 1 to 3 orders of magnitude greater *Cryptosporidium* numbers were found in the sediment than in the water column (Table 3).

The mean recovery efficiency of fecal coliforms, coliphage, *Giardia* and *Cryptosporidium* were 98.6, 34, 29 and 95 percent, respectively. The rate of die-off for all the microorganisms studied are presented in Table 4. The die-off of both fecal coliforms and *S. typhimurium* was lower in the presence of sediments. The mean reduction of fecal coliforms after 14 days in water and sediment was 3.78 \log_{10} and 2.15 \log_{10} , respectively. The die-off rates of fecal coliforms in water and sediment were 0.256 \log_{10} day⁻¹ and 0.151 \log_{10} day⁻¹, respectively. The reduction of *S. typhimurium* in water and sediment were 4.16 \log_{10} and 3.39 \log_{10} , respectively.

Table 1
Occurrence of fecal coliforms in the water column and sediment of the duckweed and hyacinth pond

| Date | MPN 100 ml ⁻¹ of water | MPN 100 g ⁻¹ of sediment (wet weight) | % Solids | MPN 100 g ⁻¹ of sediment (dry weight basis) |
|----------------------|-----------------------------------|--------------------------------------------------|----------|--------------------------------------------------------|
| <i>Duckweed pond</i> | | | | |
| 12/26/98 | 5.0×10^4 | 5×10^3 | 5.0 | 1.0×10^5 |
| 12/27/98 | 1.7×10^4 | 8×10^3 | 13.5 | 5.9×10^4 |
| 12/31/98 | 1.6×10^4 | 3×10^3 | 5.0 | 6.0×10^4 |
| Mean | 2.7×10^4 | 5.3×10^3 | 7.6 | 7.3×10^4 |
| <i>Hyacinth pond</i> | | | | |
| 12/26/98 | 2.4×10^4 | 1.1×10^4 | 3.5 | 3.1×10^5 |
| 12/27/98 | 1.3×10^4 | 5.0×10^4 | 3.0 | 1.6×10^6 |
| 12/31/98 | 1.7×10^3 | 8.0×10^3 | 5.0 | 1.6×10^5 |
| Mean | 1.2×10^4 | 2.3×10^4 | 3.8 | 6.9×10^5 |

Table 2
Occurrence of coliphage in the water column and sediment of the duckweed and hyacinth pond

| Date | PFU 100 ml ⁻¹ of water | PFU 100 g ⁻¹ of sediment (wet weight) | % Solids | PFU 100 g ⁻¹ of sediment (dry weight basis) |
|----------------------|-----------------------------------|--------------------------------------------------|----------|--------------------------------------------------------|
| <i>Duckweed pond</i> | | | | |
| 12/26/98 | 5.9×10^4 | 1.5×10^4 | 5.0 | 3.0×10^6 |
| 12/27/98 | 1.7×10^4 | 7.5×10^3 | 13.5 | 5.5×10^4 |
| 12/29/98 | 2.4×10^4 | 4.4×10^4 | 7.0 | 6.2×10^5 |
| Mean | 3.3×10^4 | 1.4×10^4 | 8.3 | 1.2×10^6 |
| <i>Hyacinth pond</i> | | | | |
| 12/24/98 | 3.5×10^3 | 3.5×10^3 | 3.5 | 1.0×10^5 |
| 12/27/98 | 5.1×10^3 | 1.3×10^4 | 3.0 | 4.3×10^5 |
| 12/29/98 | 1.7×10^3 | 9.0×10^3 | 6.0 | 1.5×10^5 |
| Mean | 3.4×10^3 | 8.5×10^3 | 4.1 | 2.2×10^5 |

Table 3
Occurrence of *Giardia* cysts and *Cryptosporidium* oocysts in the water column and sediment of the duckweed and hyacinth pond

| Date | % Solid in the sediment | <i>Giardia</i> | | | <i>Cryptosporidium</i> | | |
|----------------------|-------------------------|------------------------------|--------------------------|----------------------------------------|------------------------------|--------------------------|----------------------------------------|
| | | Water column l ⁻¹ | Sediment l ⁻¹ | Sediment kg ⁻¹ (dry weight) | Water column l ⁻¹ | Sediment l ⁻¹ | Sediment kg ⁻¹ (dry weight) |
| <i>Duckweed pond</i> | | | | | | | |
| 11/23/98 | 4.0 | 2.8×10^1 | 2.6×10^4 | 6.9×10^5 | 14.5 | $<2.1 \times 10^3$ | $<5.8 \times 10^4$ |
| 12/01/98 | 3.7 | 1.7×10^2 | 1.6×10^4 | 4.5×10^5 | 29.4 | 2.3×10^3 | 6.5×10^4 |
| 12/04/98 | 4.0 | 1.8×10^2 | 2.1×10^5 | 5.3×10^6 | <23.25 | $<2.3 \times 10^3$ | $<6.1 \times 10^4$ |
| Mean | 3.9 | 1.3×10^2 | 8.4×10^4 | 2.1×10^6 | 21.9 | 2.3×10^3 | 6.5×10^4 |
| <i>Hyacinth pond</i> | | | | | | | |
| 11/30/98 | 3.5 | 95.2 | 1.1×10^4 | 3.0×10^5 | 47.6 | 4.5×10^3 | 1.2×10^5 |
| 12/01/98 | 4.0 | <5.4 | 9.5×10^3 | 2.4×10^5 | <5.43 | 2.3×10^3 | 6.1×10^4 |
| 12/04/98 | 4.5 | <55.5 | 2.3×10^4 | 5.2×10^5 | <55.5 | $<2.5 \times 10^3$ | 5.8×10^4 |
| Mean | 4.0 | 95.2 | 1.4×10^4 | 3.5×10^5 | 47.6 | 3.4×10^3 | 4.0×10^4 |

Table 4
The die-off rates of fecal coliforms, *S. typhimurium*, coliphage, PRD-1 and *Giardia* in the water and sediment of the artificial wetland

| Microorganisms | Water | | | Sediment | | |
|-----------------------|-----------------------------------------------------------|----------------|---------|-----------------------------------------------------------|----------------|---------|
| | Log reduction/ (day log ₁₀ day ⁻¹) | R ² | p-value | Log reduction/ (day log ₁₀ day ⁻¹) | R ² | p-value |
| Fecal coliform | -0.256 | 0.899 | 0.0001 | -0.151 | 0.986 | 0.0001 |
| <i>S. typhimurium</i> | -0.345 | 0.769 | 0.0019 | -0.312 | 0.916 | 0.0001 |
| Coliphage | -0.397 | 0.983 | 0.0001 | -0.107 | 0.867 | 0.0008 |
| PRD-1 | -0.198 | 0.865 | 0.0008 | -0.054 | 0.930 | 0.0001 |
| <i>Giardia</i> | -0.029 | 0.490 | 0.187 | -0.370 | 0.861 | 0.022 |

Coliphages were reduced by $3.16 \log_{10}$ in water in 10 days compared to $1.26 \log_{10}$ in sediment. The inactivation rate of both coliphage and PRD-1 in sediment was lower compared to water (Table 4). The inactivation rate

of coliphage in water and sediment was $0.397 \log_{10} \text{ day}^{-1}$ and $0.107 \log_{10} \text{ day}^{-1}$, respectively. The inactivation rate for PRD-1 in water and sediment was $0.198 \log_{10} \text{ day}^{-1}$ and $0.054 \log_{10} \text{ day}^{-1}$, respectively. The results suggest

that sediment prolonged the survival of both bacteria and phage in the bottom of the wetlands constructed for the purpose of wastewater treatment. However, *Giardia* survival in the sediment was found to be opposite of what was observed in the case of bacteria and phages. *Giardia* reduction in the water and sediment in 5 days was $0.03 \log_{10}$ and $1.81 \log_{10}$, respectively. The die-off rate of *Giardia* in sediment was greater than the die-off rate in water. The die-off rate in water and sediment was $0.029 \log_{10} \text{ day}^{-1}$ and $0.37 \log_{10} \text{ day}^{-1}$, respectively, suggesting that the wetland sediment environment is unfavorable for the survival of *Giardia*.

4. Discussion

Several processes may be involved in the reduction of microorganisms by wetlands. Sedimentation is thought to be one of the mechanisms in the reduction process. In this study we examined the relative occurrence and survival of fecal indicator microorganisms and pathogens in the water column and sediments of two constructed wetlands.

On a volume/wet weight basis the number of fecal coliforms and coliphage in the water column and sediment were not significantly different. However, on a dry weight basis of the sediment, the numbers in the sediment were generally higher by 1–2 orders of magnitude. Several studies have found that coliforms, fecal coliforms, and *Salmonella* tend to concentrate in sediments of natural waters [10,11,19,20]. All of these experiments were conducted in water that contained no plants. In contrast, in the constructed wetlands used in this study, the water was covered with either duckweed or water hyacinth to which the microorganisms could attach. Filtration and attachment of microbes in the plant root surfaces is thought to be one of the processes of microbial reduction in wetlands [3,21]. It is plausible that the microbial attachment to the root surface reduces the settling of bacteria and viruses in constructed wetlands. This could explain why we did not observe a large difference in numbers of fecal coliforms and coliphage in the water column and sediments. Moreover, most of the previous studies were conducted in seawater and estuarine sediments. The high salt concentration and relatively clear seawater compared to wastewater might cause a difference in the settling of bacteria and viruses and thus their accumulation in sediment. In contrast, *Giardia* and *Cryptosporidium* concentrations were 2–3 orders of magnitude greater in sediment compared to the water column. *Giardia* and *Cryptosporidium* are much larger compared to bacteria and viruses, which may have resulted in a greater degree of settling. Karpiscak et al. [7] studied the reduction of indicator microorganisms and parasites from a multi-species subsurface flow wetland and the duckweed pond

used in this study. Reduction of *Giardia* and *Cryptosporidium* from the multispecies wetlands was 73 and 58 percent, whereas the reduction from the duckweed pond was 98 and 89 percent, respectively. The greater numbers of *Giardia* and *Cryptosporidium* in sediments suggest that sedimentation may be the primary reduction mechanism of *Giardia* and *Cryptosporidium* observed in wetlands.

The die-off of fecal coliforms and *S. typhimurium* was less in the sediment compared to the water column. A similar result was reported by Gerba and McLeod [10] in marine sediments. The authors reported that *E. coli* survived longer when sediment was present than in the seawater alone. The authors also reported that *E. coli* could grow in the presence of sediment. The longer survival of fecal coliforms in the wetland sediment could be due to the greater content of organic matter present in the sediment. Coliphage and seeded PRD-1 also survived for longer periods of time in the presence of sediments compared to the control. Previous studies in the estuarine environment found that virus survival was prolonged in the presence of marine sediments [14,22]. Laboratory studies demonstrated that sediment was capable of protecting poliovirus type 1 from the inactivating effects of microorganisms, heat, salt and temperature [22,23]. The observed lower inactivation rates of coliphage and PRD-1 in sediment compared to the water column in the wetland sediment could be due to the protective effect of sediment on phages. Future studies are needed to examine the survival of human viruses in the sediments of constructed wetlands.

The survival of *Giardia* was adversely affected in the presence of sediment. The high concentration of *Giardia* cysts and *Cryptosporidium* oocysts found in the sediment were analyzed microscopically only for presence/absence with no measurements for viability or infectivity. It appears that at least *Giardia* does not survive long periods of time in the bottom sediments of artificial wetlands. To our knowledge no published literature exists on the survival of *Giardia* in sediments. A few survival studies suggest that temperature plays an important role in the survival of *Giardia* [24,25]. Biological antagonism is thought to be a mechanism of *Cryptosporidium* oocyst survival in natural waters [26]. A plausible explanation of greater reduction of *Giardia* in the sediment could be due to biological antagonism or the presence of organic substances, which enhanced die-off of the protozoan parasites. Future studies are necessary to study the effect of sediments on the survival of *Cryptosporidium*.

5. Conclusion

This study demonstrated that sediments of artificial wetlands, built for the purpose of wastewater treatment,

accumulate significant concentrations of pathogens, indicating that sedimentation is one of the mechanisms involved in the reduction of pathogens from artificial wetlands. Sedimentation of pathogens in vegetated wetlands appears to be size related with higher sedimentation of larger particles. Wetland sediments prolong the survival of bacteria and virus but not parasites.

References

- [1] Falabi JA, Gerba CP, Karpiscak MM. *Giardia* and *Cryptosporidium* removal from waste-water by a duckweed (*Lemna gibba* L.) covered pond. *Lett Appl Microbiol* 2002;34:384–7.
- [2] Green MB, Griffin P, Seabridge JK, Dhoibie D. Removal of bacteria in subsurface flow wetlands. *Water Sci Technol* 1997;35:109–16.
- [3] Gersberg RM, Lyon SR, Brenner R, Elkins BV. Survival of bacteria and viruses in municipal wastewaters applied to artificial wetlands. In: Reddy KR, Smith WH, editors. *Aquatic plants for water treatment and resource recovery*. Orlando, FL: Magnolia Publishing Inc; 1987.
- [4] Gersberg RM, Lyon SR, Brenner R, Elkins BV. Integrated wastewater treatment using artificial wetlands; A gravel marsh case study. In: Hammer DA, editor. *Constructed wetlands for wastewater treatment; municipal, industrial, and agricultural*. Chelsea, MI: Lewis Publishers Inc; 1989. p. 1–2.
- [5] Gearheart RA, Klopp F, Allen G. Constructed free surface wetlands to treat and receive wastewater: Pilot project to full scale. In: Hammer DA, editor. *Constructed wetlands for wastewater treatment; municipal, industrial, and agricultural*. Chelsea, MI: Lewis Publishers, Inc; 1989.
- [6] John C. Treatment of agro-industrial wastes using water hyacinth. *Water Sci Technol* 1984;17:781–90.
- [7] Karpiscak MM, Gerba CP, Watt PM, Foster KE, Falabi JA. Multi-species plant systems for wastewater quality improvements and habitat enhancement. *Water Sci Technol* 1996;33:231–6.
- [8] Mandi L, Houhoum B, Asmama S, Schwartzbrod J. Wastewater treatment by reed beds an experimental approach. *Water Res* 1996;30:2009–16.
- [9] Decamp O, Warren A. Bacteriophage in cistes isolated from constructed wetlands (reed beds) used for wastewater treatment. *Water Res* 1998;32:1989–96.
- [10] Gerba CP, Mcleod J. Effect of sediments on the survival of *E. coli* in marine waters. *Appl Environ Microbiol* 1976; 32:114–20.
- [11] Hendricks CW. Increased recovery rate of *Salmonellae* from stream bottom sediment verses surface waters. *Appl Microbiol* 1971;21:379–80.
- [12] Van Donsel DJ, Geldreich EE. Relationships of *Salmonella* to fecal coliforms in bottom sediments. *Water Res* 1971;5:1079–87.
- [13] Rao C, Bitton G. Methods of recovering viruses from aquatic sediments. In: Rao VC, Melnick JL, editors. *Human viruses in sediments, sludges, and soils*. Boca Raton, FL: CRC Press; 1987.
- [14] Smith EM, Gerba CP, Melnick JL. Role of sediments in the persistence of enteroviruses in the estuarine environment. *Appl Environ Microbiol* 1978;35:685–9.
- [15] Adams MH. *Bacteriophages*. New York: Interscience Publisher, Inc; 1959.
- [16] Pepper IL, Gerba CP, Brendecke JW. *Environmental microbiology. A laboratory manual*. San Diego, CA: Academic Press; 1995.
- [17] American Public Health Association (APHA). *Standard methods for the examination of water and wastewater*, 19th ed. Washington, DC: American Public Health Association (APHA); 1995.
- [18] USEPA, 1996. *ICR microbial laboratory manual*. EPA/600/R-95/178.
- [19] Matson EA, Hornor SG, Buck JD. Pollution indicators and other microorganisms in river water. *J Water Pollut Contr Fed* 1978; 13–9.
- [20] Erkenbrecher Jr. CW. Sediment bacterial indicators in an urban shellfishing subestuary of the lower chesapeake Bay. *Appl Environ Microbiol* 1981;42:484–92.
- [21] Gerba CP, Thurston JA, Falabi JA, Watt PM, Karpiscak MM. Optimization of artificial wetland design for the removal of indicator microorganisms and pathogenic protozoa. *Water Sci Technol* 1999;40:363–8.
- [22] Labelle R, Gerba CP. Investigation into the protective effect of estuarine sediment on virus survival. *Water Res* 1982;16:469–78.
- [23] Liew PF, Gerba CP. Thermostabilization of enteroviruses by estuarine sediment. *Appl Environ Microbiol* 1980; 40:305–8.
- [24] Deregner DL, Cole L, Schupp DG, Erlandsen SL. Viability of *Giardia* cysts suspended in lake, river and tap water. *Appl Environ Microbiol* 1989;55:1223–9.
- [25] Johnson DC, Enriquez CE, Pepper IL, Daris TI, Gerba CP, Rose JB. Survival of *Giardia*, *Cryptosporidium*, poliovirus, and *Salmonella* in marine waters. *Water Sci Technol* 1997;35:261–8.
- [26] Chauret C, Bagwell B, Meybodi S, Nolan K, Chen P, Springthorpe S, Sattar S. Effect of microbial antagonism on the survival of *Cryptosporidium parvum* oocysts in fresh waters. IAQW 19th Biennial International Conference, Vancouver, Canada, 1998.