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OPTIMIZATION OF ARTIFICIAL WETLAND DESIGN FOR REMOVAL OF INDICATOR MICROORGANISMS AND PATHOGENIC PROTOZOA

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ABSTRACT

The enhancement of water quality by artificial wetland systems is increasingly being employed throughout the world. Three wetlands were studied in Tucson, AZ to evaluate their individual performance in the removal of indicator bacteria (coliforms), coliphage, and enteric pathogens (*Giardia* and *Cryptosporidium*). A duckweed-covered pond, a multi-species subsurface flow (SSF) and a multi-species surface flow (SF) wetland were studied. Removal of the larger microorganisms, *Giardia* and *Cryptosporidium*, was the greatest in the duckweed pond at 98 and 89 percent, respectively. The lowest removal occurred in the SF wetland, 73 percent for *Giardia* and 58 percent removal for *Cryptosporidium*. In contrast, the greatest removal of coliphage, total and fecal coliforms occurred in the SSF wetland, 95, 99, and 98 percent respectively, whereas the pond had the lowest removals (40, 62, and 61 percent, respectively). Sedimentation may be the primary removal mechanism within the duckweed pond since the removal was related to size, removal of the largest organisms being the greatest. However, the smaller microorganisms were removed more efficiently in the SSF wetland, which may be related to the large surface area available for adsorption and filtration. This study suggests that in order to achieve the highest treatment level of secondary unchlorinated wastewater, a combination of aquatic ponds and subsurface flow wetlands may be necessary.

KEYWORDS

Constructed wetlands; *Cryptosporidium*; fecal coliforms; *Giardia*.

INTRODUCTION

Artificial wetlands are being increasingly used for the tertiary treatment of secondary treated (activated sludge) wastewater. Enhancement of water quality occurs due to the wetland's ability to remove nutrients, various chemical contaminates, and wastewater microorganisms. While there have been numerous studies on the removal of these, there is little information on the fate of viruses and waterborne parasites such as, *Giardia* and *Cryptosporidium* (Kadlec and Knight, 1996).

Depending on the type of wetland system, subsurface flow (SSF), surface flow (SF), or aquatic pond, the fate of these contaminants may depend on individual removal capabilities. For SSF wetlands, plants are not submerged in water but rather the water typically flows horizontally through the gravel substrate allowing a larger surface area for microbial activity and growth. In SF wetlands, the water surface is exposed to the atmosphere and plants are rooted at the bottom of the wetland in some type of substrate (gravel or soil). Contaminant removal/breakdown may be less efficient in SF wetland systems since there is less substrate available for microbial growth. Similarly, aquatic systems or pond systems have very little area for growth of bacteria. In these ponds microbial growth occurs on the shallow roots of floating macrophytes, such as duckweed or on detritus at the bottom of the pond. Different plant species may also enhance water quality within wetland systems. Multi-species plant systems may be able to remove a wider range of wastewater pollutants and endure fluctuating loading rates when compared to monoculture systems, such as reed or bulrush systems (Hammer, 1989).

The purpose of this study was to compare the three primary types of wetlands, a duckweed pond, a multi-species SSF wetland, and a multi-species SF wetland for the removal of indicator bacteria (coliforms), coliphage, and enteric pathogens (*Giardia* and *Cryptosporidium*).

The facility

This research was conducted at the Pima County's Constructed Ecosystems Research Facility (CERF) (Figure 1). The SSF multi-species wetland cells have a maximum depth of 1.4 m and are 61 m long and 8.2 m wide.

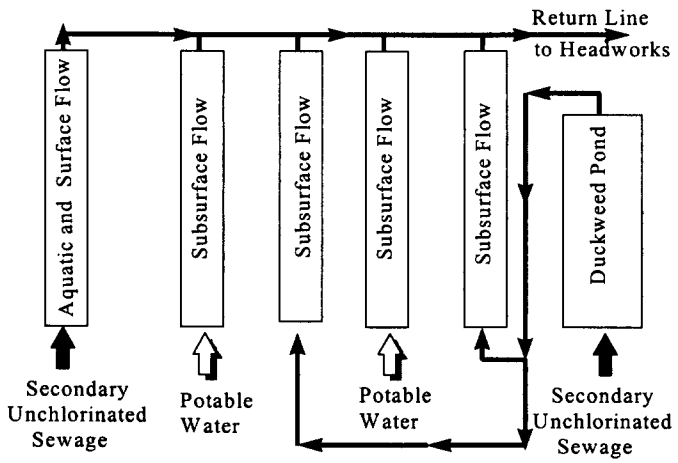

Figure 1. Layout of Constructed Ecosystems Facility and direction of flow of secondary unchlorinated wastewater and potable groundwater.

Each of the SSF wetlands was planted with cattail (*Typha domingenisis*), bulrush (*Scirpus olneyi*), black willow (*Salix nigra*), and cottonwood (*Populus fremontii*). The SSF wetland studied received unchlorinated secondary treated wastewater that was previously treated by passage through the duckweed covered pond. Two raceways received only chlorine disinfected potable water and serve as controls. The detention time of the SSF wetland was approximately 4 days. The aquatic pond, planted with the floating macrophyte duckweed (*Lemna gibba*), received secondary unchlorinated wastewater. This pond measured 65 m in length, 11.9 m in width, and 0.9 m in depth, with a detention time of approximately 6 days. The SF wetland measured 61 m in length, 8.2 m in width, and 0.6 m in depth, with an approximate detention time of 4 days. Similar to the SSF wetland, the SF wetland was planted with cattail (*Typha domingensis*), bulrush (*Scirpus olneyi*), giant reed (*Arundo donax*), black willow (*Salix nigra*), and cottonwood (*Populus fremontii*). A small aquatic system containing water hyacinth (*Eichhornia crassipes*) was placed inline just prior to the gravel-based SF multi-species wetlands (Figure 2).

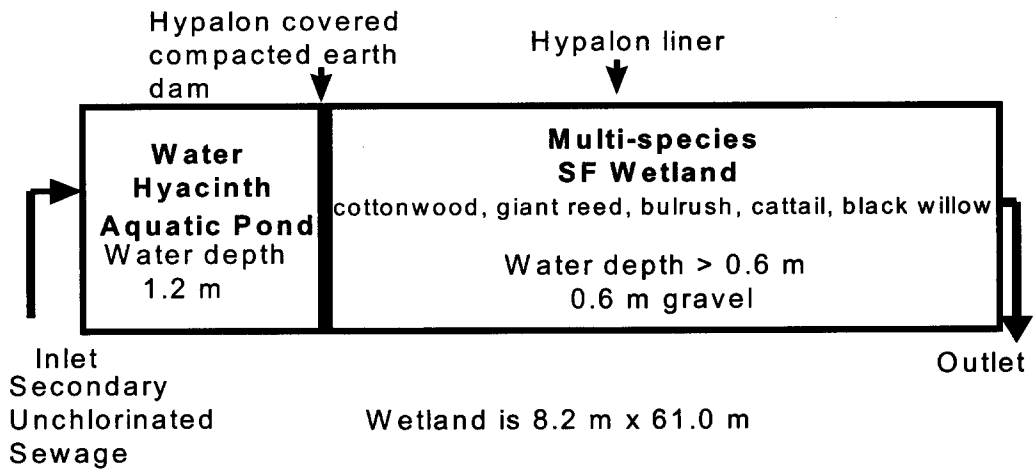

Figure 2. Diagram of the SF multi-species wetland located at CERF

METHODS

Samples from the influent and effluent flows for each wetland were collected over a 34-month period (pond sampled from July 1994 to December 1995; SSF wetland from February 1995 to August 1996; and SF wetland from July 1994 to December 1994). Volumes collected for protozoan parasites were 1-400 L, coliform bacteria 0.05-1 L, and coliphage 50 mL. Water samples and filters collected for analysis were placed in separate sealable bags and transported to the laboratory at The University of Arizona in ice-packed coolers. A portion of the water samples was used for the determination of turbidity and pH for both the influent and effluent. Water temperature was determined during sampling for both the influent and effluent of each wetland.

Total and fecal coliforms were detected using the membrane filtration technique as described in *Standard Methods for the Examination of Water and Wastewater* (1995). Detection of coliphages was achieved by the double-layer method using *Escherichia coli*, strain ATTC 15597, as described by Adams, (1959). *Giardia* and *Cryptosporidium* were detected simultaneously in the samples by a modification of the indirect immunofluorescence method as described in *Standard Methods for the Examination of Water and Wastewater* (1995).

RESULTS

The inflow and water exiting each wetland was sampled from 15-20 times during the course of the study. The average percent reductions of the microorganisms studied are presented in Figures 3 and 4. Individual wetland ranges are presented in Tables 1 to 3.

Aquatic pond

The ranges in the concentration of the organisms studied in the aquatic pond during the sampling period are presented in Table 1. In the aquatic pond average densities in the influent were 15.6 *Giardia* cysts/L, 1.58 *Cryptosporidium* oocysts/ L, 4.24 x 10^6 colony forming units (CFU)/100 mL for total coliforms, 1.77 x 10^6 CFU/100 mL for fecal coliforms, and 1.2 x 10^3 plaque forming units (PFU)/mL for coliphage. The effluent contained 0.35 *Giardia* cysts/L, 0.17 *Cryptosporidium* oocysts/L, 1.65 x 10^6 CFU/100 mL and 5.97 x 10^5 CFU/100 mL for total and fecal coliforms, respectively. The concentration of coliphage averaged 7.4 x 10^2 PFU/mL. Average reductions observed were 98 and 89 percent for *Giardia* cysts and *Cryptosporidium* oocysts, respectively. Fecal coliforms were reduced by an average of 61 percent and total coliforms by 62 percent. An average reduction of 40 percent was observed for coliphage (Fig. 4). Hence, the removal of

microorganisms was related to size, the larger organisms had the highest removal rates followed by the smaller organisms (Figure 3) (Falabi, 1996). A positive correlation between the removal of *Giardia* and *Cryptosporidium* and influent turbidity was determined ($p = 0.10$). No other correlations were observed.

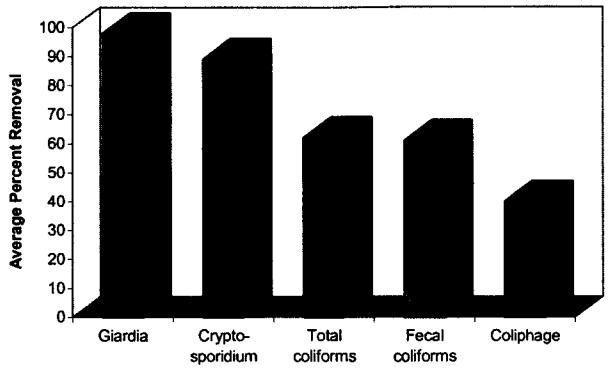

Figure 3. Average percent removal of microorganisms by a duckweed pond.

Table 1. Concentration of microorganisms observed in the aquatic duckweed system

Water Source	*Total Coliforms (CFU/100mL)*	*Fecal Coliforms (CFU/100mL)*	*Giardia (cysts/L)*	*Cryptosporidium (oocysts/L)*	*Coliphage (PFU/mL)*
Influent Range	1.5×10^5- 8.8×10^6	1.3×10^5-5.3×10^6	4-33	0-3	650-2.0×10^3
Effluent Range	8.5×10^4-3.8×10^6	6.5×10^4-1.2×10^6	0-1	0-1	69-1.5×10^3

Surface flow wetland

The concentrations of microorganisms studied in the surface flow are presented in Table 2. Average concentrations of total and fecal coliforms in the influent were 1.0×10^7 CFU/100 mL and 7.1×10^6 CFU/100 mL, respectively. The protozoans, *Giardia* cysts and *Cryptosporidium* oocysts, averaged 55.3 cysts/L and 12.6 oocysts/L in the influent, respectively. Average concentrations in the effluent were 8.3×10^4 CFU/100 mL for total coliforms, 4.0×10^4 CFU/100 mL for fecal coliforms, 16.1/L for *Giardia* cysts, and 4.9/L for *Cryptosporidium* oocysts. Unlike the other two wetlands studied, data on the occurrence of coliphage was not obtained. Total coliforms were reduced by an average of 98 percent, fecal coliforms by an average of 93 percent, *Giardia* cysts by an average of 73 percent, and *Cryptosporidium* oocysts by an average of 58 percent (Fig. 4) (Karpiscak *et al.*, 1995).

Table 2. Concentration of microorganisms observed in the multi-species surface flow wetland

Water Source	*Total Coliforms (CFU/100 mL)*	*Fecal Coliforms (CFU/100 mL)*	*Giardia (cysts/L)*	*Cryptosporidium (oocysts/L)*
Influent Range	1.8×10^5-2.2×10^7	4.5×10^4-4.0×10^7	22-161	6-24
Effluent Range	6.2×10^3-3.7×10^5	1.4×10^3-8.9×10^4	<1-64	<1-12

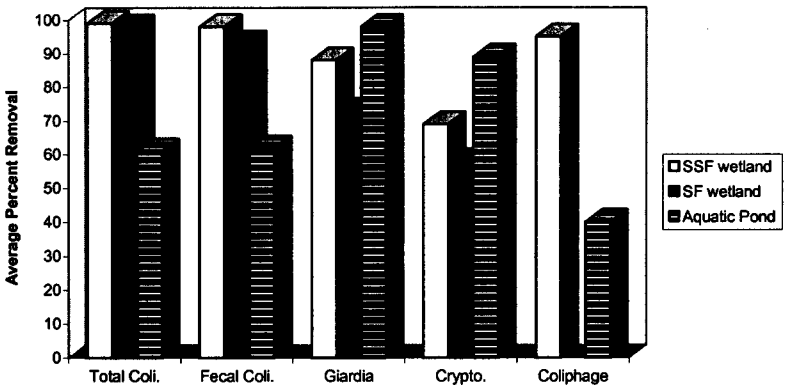

Figure 4. Comparison of average percent removals of microorganisms for an aquatic pond, surface flow, and subsurface flow wetland systems.

Subsurface flow wetland

The ranges of microorganisms in the subsurface flow wetland detected during the sampling period are presented in Table 3. Total and fecal coliforms averaged 2.8 x 10^6 CFU/100 mL and 1.2 x 10^6 CFU/100 mL in the influent and 2.2 x 10^4 CFU/100 mL and 1.7 x 10^4 CFU/100 mL in the effluent, respectively. *Giardia* cysts averaged 62/100 L in the influent and 1.0/100 L in the effluent while *Cryptosporidium* oocysts averaged 45.1/100 L in the influent and 7.4/100 L in the effluent. Coliphage averaged 4.3 x 10^2 PFU/mL in the influent and 8.1 PFU/mL in the effluent. The percent reductions averaged 99% for total coliforms, 98% for fecal coliforms, 88% for *Giardia* cysts, 69% for *Cryptosporidium* oocysts, and 95% for coliphage (Figure 4).

Table 3. Microorganism ranges observed in the subsurface flow wetland system

Water Source	*Total Coliforms (CFU/100 mL)*	*Fecal Coliforms (CFU/100 mL)*	*Giardia (cysts/L)*	*Cryptosporidium (oocysts/L)*	*Coliphage (PFU/mL)*
Influent Range	1.3 x 10^5-9.2 x 10^6	1.0 x 10^4-3.2 x 10^6	<1.0-330	0.9-110	40-2.5 x 10^3
Effluent Range	6.0 x 10^2-2.2 x 10^5	2.6 x 10^2-2.6 x 10^5	<0.5-2.9	<1.0-50	1.0-43

DISCUSSION

In all wetland systems studied, the concentration of total coliforms, fecal coliforms, *Giardia*, *Cryptosporidium*, and coliphages decreased from the influent to the effluent. The lowest removal of the smaller sized organisms, total and fecal coliforms and coliphage, occurred in the aquatic pond. The removal of microorganisms seemed to be related by size. The largest microorganisms, *Giardia* (8-12 μm) and *Cryptosporidium* (2-6 μm), were reduced at the highest rates, 98 and 89 percent, whereas total and fecal coliforms (1.1-1.5 μm) were removed at 62 and 61 percent, followed by coliphage (0.045-0.065 μm), at 40 percent. Moreover, the longer detention time of the pond, 6 days, did not allow more efficient removal of bacteria and coliphage over the shorter 4-day detention time of the other two wetlands studied. The primary mechanism of removal within the pond system is likely to be sedimentation, although other factors such as natural die-off, adsorption to detritus and duckweed roots may also be involved to some degree.

The SSF and SF systems were the most efficient in the removal of total coliforms, fecal coliforms, and coliphage. The SSF wetland had the highest removal of all the organisms, total coliforms averaged 99 percent, fecal coliforms by 98 percent, and coliphage by 95 percent whereas the SF wetland had average removal rates of 98 and 93 percent, respectively (coliphage removal not determined). Removal of coliphage was greater in the SSF than the pond. In both SF and SSF wetlands, increased physical contact of pathogens

with plant roots and wetland substrate may be the primary mechanisms of pathogen removal. SSF wetlands are filled with gravel media and rooted plants, unlike SF wetlands which have the plants rooted only in the bottom of the wetland where the gravel media occurs.

The increased filtration and physical contact of the wastewater in SSF wetlands may be the cause of increased reduction of both bacteria and viruses.

The removal of coliforms and fecal coliforms in the multi-species SSF and SF systems in this study are similar to monoculture systems previously reported (Gearheart *et al.*, 1989; Gersberg *et al.*, 1987, 1989; Hendrey *et al.*, 1979).

No correlations were observed between the removal of total coliforms, fecal coliforms, coliphage, *Giardia*, or *Cryptosporidium* and turbidity, temperature, or pH in either the SF or SSF wetlands. There was also no relationship between the removal of total coliforms, fecal coliforms, or coliphage with temperature, pH, and turbidity in the aquatic pond. However, a positive correlation (p = 0.10) between both *Giardia* and *Cryptosporidium* and the influent turbidity of the aquatic pond was observed.

CONCLUSIONS

The removal of enteric microorganisms in the duckweed pond was related to size with the larger organisms being removed more effectively. Thus, settling was likely the primary mechanism of microorganism removal. The greater removal of viruses and bacteria in the SSF wetland was probably related to the large surface area for adsorption and filtration. This suggests that for efficient removal of pathogenic microorganisms the combined use of both an aquatic pond (for the most efficient removal of parasites) and a SSF wetland (for the most effective removal of bacteria and viruses) may be required. The removal may be increased further with longer retention times, permitting increased contact between wastewater, plant roots, and wetland media in both SSF and SF wetlands, and allowing increased settling in aquatic ponds.

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