Selective reduction of coliforms in constructed wetlands

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Introduction

Wetland areas around the world have been steadily declining for a number of years due to drainage, development, chemical pollution, sedimentation of contaminants in wetland areas, and other factors. This loss has become an increasing concern because research has shown that both natural and constructed wetlands serve as filters for pollution in the world's water supply (EPA, 2003; Gerba et al., 1999). Fecal coliforms, a group of bacteria found in the intestines and feces of animals, are widely used as indicators of water contamination and water quality. There are many bacteria and viruses that are found in animal waste, including fecal coliforms. Some of the more familiar examples of coliforms include *Escherichia Coli* and *Salmonella enteritis*, which can both be pathogenic to humans. Coliforms may come to inhabit a body of water naturally, through the use of the area as animal habitat, or through pollution containing waste from humans or other animals. Fecal colifoms are useful indicators of pollution because they reveal the potential presence of pathogenic coliforms along with other hazardous bacteria, viruses and chemicals. Both naturally occurring and constructed wetlands have been reported to reduce bacterial concentrations, including fecal coliforms, in the water flowing through them, and wetlands are now being used around the world for water treatment (Antonious and Warner, 2000; Decamp and Warren; 2001, Perkins and Hunter, 2000; Tyrrell et al., 1995).

Natural wetlands are believed to be the most efficient at water filtration, and in efforts to stem the loss of these wetlands, and to acknowledge the vital role wetlands play in maintaining and improving the quality of water, degrading pesticides and agricultural runoff, controlling erosion, and providing habitats for wildlife, the federal government has enacted laws requiring mitigation wetlands be built to replace any wetland area that is drained (United States Code, 1997). It is important for mitigation wetlands to match their natural counterparts as closely as possible if they are to be an adequate substitution. The Olentangy River Wetland Research Park (ORWRP) was constructed for the purpose of research into the design and function of such constructed wetlands. This study was designed to measure the overall bacterial burden of the ORWRP wetlands and explore the effect of the wetlands on fecal coliforms to see how these mitigation wetlands compare to other wetland areas.

Materials and Methods

Sampling

Fifty milliliter samples were collected monthly in sterile centrifuge tubes (Becton Dickinson, Franklin Lakes, NJ). Three samples were collected at each site from the inlet to the outlet of Wetland 1 and 2 starting in October 2000 (Figure 1). Samples were also taken from the Olentangy River above the inlet and from the swale which returns the water to the river after it passes through the wetlands. Samples were not taken in months when the wetlands were frozen or when the water level was too low to collect.

Bacterial Counts

Total bacterial concentration in the samples was determined by standard microbiological plate counting techniques (Koch, 1994) on trypticase soy agar (Difco, Detroit, MI). Coliform concentrations were determined by two methods. One method utilized the multiple tube technique with phenol red lactose broth (Difco) followed by most probable number analysis (Khatiwada, 1999). Positive tubes in this presumptive test were subjected to further testing using the standard protocols for the confirmed test with eosin methylene blue agar (Difco) and completed test of water quality using nutrient agar (Difco) and lactose broth as for the presumptive test (Khatiwada, 1999). Samples collected from the Billabong and all samples collected after the wetlands were drained in the Spring and Summer of 2002, were subjected to membrane filtration (Gerba et al., 1999) through 0.45 μm nitrocellulose membranes (Millipore, Bedford, MA) and grown on M coli Blue 24 medium (Millipore).

Results

Coliform Levels in the Wetlands

Previous studies have shown both natural and constructed surface-flow wetlands to have up to 94% reduction in fecal coliforms (Gerba et al., 1999; Perkins and Hunter, 2000). To determine if the wetland basins at the ORWRP were reducing fecal coliforms, samples were obtained from five sites throughout each wetland. The coliform concentration at each site varied from month to month (Table 1). Despite variation from site to site, in most months, a reduction of coliforms was seen when comparing concentrations at the

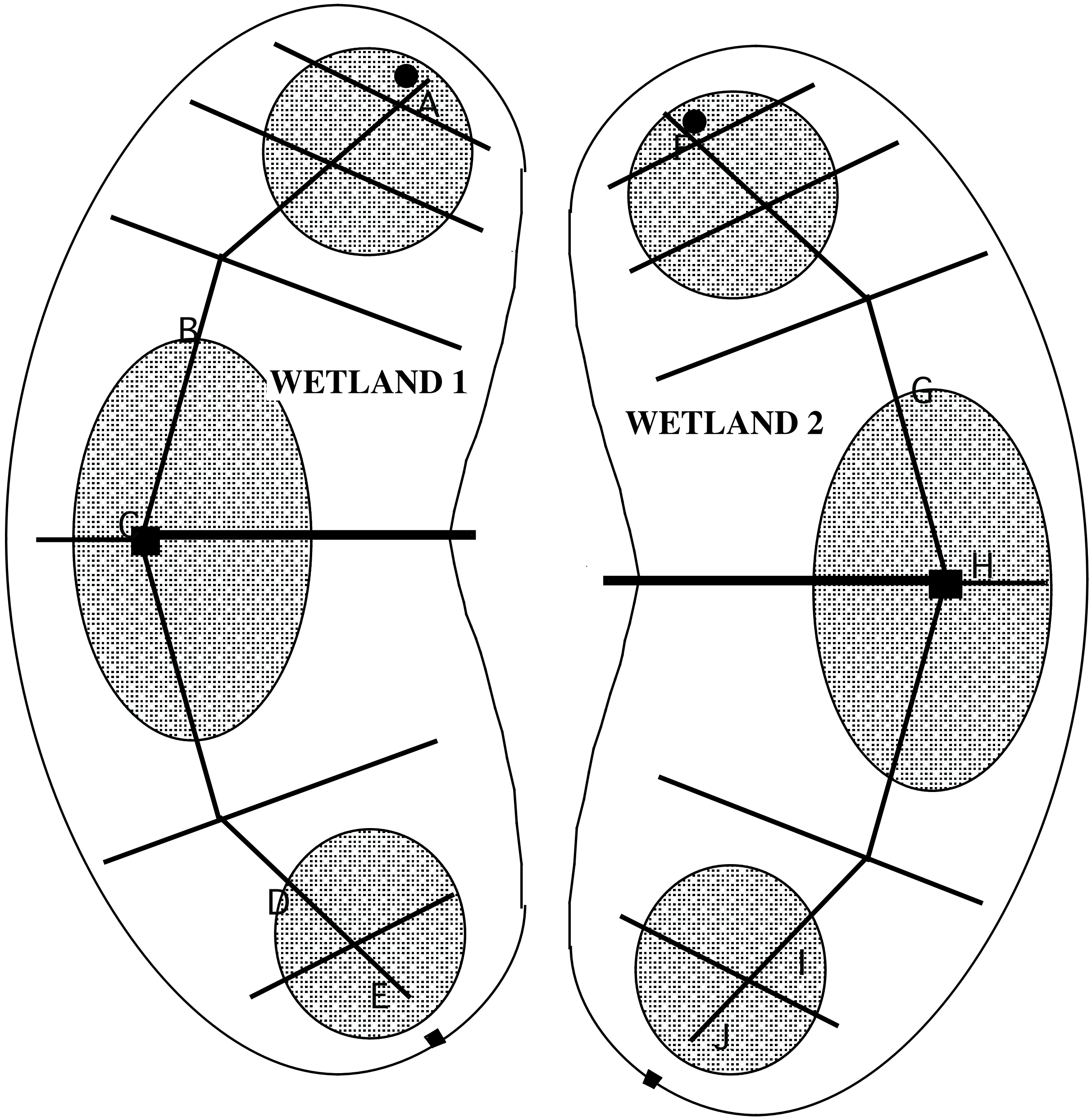

Figure 1. Sampling locations in the two experimental wetland basins.

inlet and the outlet (Tables 2 & 3) or comparing coliform concentrations between the river and the swale (Table 4). During the period before the wetlands were drained, fecal coliform levels decreased an average of 80.04 ± 17.9% in Wetland 1 and 72.12 ± 17.0% in Wetland 2. This reduction was significant as shown by student t-test (p = 0.0019 and 0.0005, respectively).

In the Spring and Summer of 2002, the continuous flow pumps supplying the wetland basins were turned off to allow recovery and new growth of plants which had been devastated by muskrat activity. Although the pumps were shut off suddenly, water remained in the wetlands for a period of time during which evaporation and outflow reduced its levels. During this period of wetland drainage, bacteria, including coliforms, were being concentrated into increasingly smaller puddles of water. Samples taken after pumping resumed showed substantially different levels of coliforms than those taken before the drainage. In general, coliform concentrations in months after the drainage were higher than those in the period before the drainage (Tables 2 & 3). In most months, a reduction was seen when comparing the inlet to the outlet. However, this reduction was not significant.

Total Bacteria in the Wetlands

In addition to coliform concentrations, total bacterial levels were determined for each sample collected from the wetlands. As with coliform levels, total bacterial concentrations varied from month to month and from site to site. In some months, total bacterial concentration actually increased from inlet to outlet (Tables 5 & 6) and from the river to swale (Table 7). Over the period studied, neither Wetland 1 or Wetland 2 showed a significant reduction in total bacterial concentration from inlet to outlet (p = 0.1726 and 0.4203, respectively). Total bacterial levels after drainage were comparable to those before drainage. After drainage, total bacterial concentration was still not significantly reduced from inlet to outlet (p = 0.1083 and 0.4135).

Discussion

As water flowed across the wetlands, variation was seen in the concentration of both coliforms and total bacteria. At some sampling sites, bacterial levels and/or coliform levels were actually higher than at the inlet. This increase may be attributable to many factors including sedimentation of

Table 1. Average coliforms at each site in the wetlands prior to drainage.

	Wetland 1		Wetland 2
Site	Cfu's/mL	Site	Cfu's/mL
A	0.35±0.11	F	0.33±0.08
B	0.28±0.08	G	0.23±0.07
C	0.20±0.07	H	0.17±0.03
D	0.11±0.02	I	0.10±0.04
E	0.06±0.05	J	0.08±0.05

Table 2: Wetland 1 coliform concentrations (cfu's/mL)

Month I	Inlet	Outlet	% Reduction
October 2000	0.22	0.02	91%
March 2001	0.27	0.15	45%
April 2001	0.49	0.07	89%
May 2001	0.37	0.04	89%
June 2001	0.40	0.04	90%
After Drainage			
May 2002	0.88	1.90	--
September 2002	0.48	0.07	85%
October 2002	1.92	0.81	58%
November 2002	0.30	0.34	--

Table 3: Wetland 2 Coliform Concentrations (cfu's/mL)

Month	Inlet	Outlet	% Reduction
October 2000	0.33	0.09	73%
March 2001	0.23	0.09	61%
April 2001	0.30	0.16	47%
May 2001	0.44	0.06	86%
June 2001	0.33	0.02	94%
After Drainage			
May 2002	0.31	1.92	--
September 2002	4.74	7.47	--
October 2002	1.29	1.43	--
November 2002	0.04	0.17	--

Table 4: River and Swale Coliform Concentrations (cfu's/mL)

Month	River	Swale	% Difference
October 2000	0.50	0.02	-96%
March 2001	0.33	0.13	-60%
April 2001	0.47	0.05	-89%
May 2001	0.37	0.18	-51%
June 2001	0.60	0.04	-93%
After Drainage			
September 2002	48.00	0.73	16%
October 2002	19.30	8.10	-90%
November 2002	3.00	3.40	-8%

Table 5: Wetland 1 Total Bacterial Concentration (cfu's/mL)

Month	Inlet	Outlet	% Difference
October 2000	8,593	4,666	-46%
April 2001	6,347	1,493	-75%
May 2001	3,347	853	-38%
June 2001	21,280	13,226	23%
July 2001	6,320	8,160	-73%
August 2001	24,373	6,720	-77%
After Drainage			
September 2002	7,520	160	-98%
October 2002	2,400	1,920	-20%
November 2002	4,240	400	-91%

Table 6: Wetland 2 Total Bacterial Concentration (cfu's/mL)

Month	Inlet	Outlet	% Difference
October 2000	480	1,840	74%
April 2001	2,067	4,170	-42%
May 2001	1,373	800	65%
June 2001	5,867	16,747	-51%
July 2001	28,640	14,187	-65%
August 2001	5,480	906	50%
After Drainage			
September 2002	26,560	11,600	-56%
October 2002	10,800	6,520	-40%
November 2002	800	3,040	74%

Table 7: River and Swale Total Bacterial Concentration (cfu's/mL)

Month	River	Swale	% Difference
October 2000	4,653	2,273	-51%
April 2001	6,720	3,440	-49%
May 2001	8,827	427	-95%
June 2001	11,786	13,440	14%
July 2001	7,360	9,760	33%
August 2001	3,440	1,787	-48%
After Drainage			
September 2002	3,040	2,440	-20%
October 2002	5,200	6,520	25%
November 2002	320	600	88%

the bacteria in certain areas, variation in plant coverage offering different degrees of filtration, or variation in the wildlife population (Gerba et al., 1999; Pollard et al., 1995). Wetland 2 tended to show more areas with high bacterial levels than Wetland 1. This may be due to the increased usage of Wetland 2 by ducks, geese, muskrats and other wildlife. These animals were found less frequently in Wetland 1 due to its proximity to the parking lot and observation tower as well as the high number of tour groups which enter Wetland 1. Further studies are needed to correlate the spikes in bacterial concentration with the areas of muskrat hutches or other indicators of wildlife.

Differences in bacterial and coliform concentrations between the wetlands at ORWRP may also be influenced by the flow rate, as in other wetland areas (Gerba et al., 1999). Flow rate has been shown to influence bacterial concentration in surface flow wetlands (Gerba et al., 1999; Perkins and Hunter, 2000). Interestingly, during the Spring and Summer of 2002, the flow rate for the wetland basins at ORWRP decreased to zero due to the stopping of the pumps for a period long enough to allow the wetlands to dry. The data indicate that the wetlands are not functioning the same with respect to bacteria as they were before the drainage. Coliform reduction was no longer significant in either wetland. The variation in coliform filtering ability between the months before the drainage and the months after the drainage may be due to excessive concentration of coliforms in the small puddles which developed as the wetlands were drying. The data suggest that it may take some time for the wetlands to recover from this event. Natural wetlands experience similar situations during the cyclical periods of drought of flooding, yet studies indicate that natural wetlands still efficiently filter coliforms (Lau and Chu, 2000).

While preliminary, due to the low number of months sampled, the results of this study also suggest that the wetlands at the ORWRP may be selectively reducing coliform concentrations while not significantly affecting total bacterial concentrations. A number of factors contribute to reduction of coliforms by natural and constructed wetlands. Among these are sedimentation, filtration by wetland plants, solar radiation, flow rate and temperature (Khaitwada, 1999; Pollard et al., 1995). One would expect these factors should contribute not only to coliform reduction, but also to a reduction in total bacterial concentration. Further experimentation is clearly needed in order to determine why total bacterial concentration is not being reduced significantly in the ORWRP. One hypothesis is that certain wetland plants may be producing antimicrobial chemicals that may affect coliforms differently from many other bacteria. Studies are underway to test this hypothesis.

References

Antonious, G. F., and R. C. Warner. 2000. Constructed Wetlands for Domestic Wastewater Treatment: Survey and Performace in Kentucky. J. KY. Acad. Sci. 61: 23-29.

Decamp, O., and A. Warren. 2001. Abundance, biomass and viability of bacteria in wastewaters: impact of treatment in horizontal subsurface flow constructed wetlands. Water Res. 35: 3496-501.

Environmental Protection Agency. Jan. 17 2003, posting date. Wetlands Status and Trends. Environmental Protection Agency. [Online.]

Gerba, C. P., J. A. Thurston, J. A. Falabi, P. M. Watt, and M. M. Karpiscak. 1999. Opimization Of Artificial Wetland Design For Removal Of Indicator Microorganisms and Pathogenic Protozoa. Water Sci. Technol. 40: 363-368.

Khatiwada, N. R. 1999. Kinetics of fecal coliform removal in constructed wetlands. Water Sci. Technol. 40:109-116.

Koch, A. L. 1994. Methods for General and Molecular Bacteriology. American Society for Microbiology, Washington, D.C.

Lau, S. S., and L. M. Chu. 2000. Nutrient and faecal contamination and retention in wetland enclosures (gei wais) in the Mai Po Marches, Hong Kong. Hydrobiologia 431: 81-90.

Perkins, J., and C. Hunter. 2000. Removal of Enteric Bacteria in a Surface Flow Constructed Wetland in Yorkshire, England. Water Res. 34: 1941-1947.

Pollard, P.C., J. A. Flood, and N. J. Ashbolt. 1995. The Direct Measurement of Bacterial Growth in Biofilms of Emergent Plants (Schoenoplectus) of an Artificial Wetland. Water Sci. Technol. 32: 251-256.

Tyrrell, S. A., S. R. Rippey, and W. D. Watkins. 1995. Inactivation of bacterial and viral indicators in secondary sewage effluents, using chlorine and ozone. Water Res. 29: 2483-2490.

United States Code (1997). Clean Water Act, 33 U. S. C. 1344.