Reduction of fecal coliform levels in two created wetlands at the Olentangy River Wetland Research Park

Terry D. Hinds, Jr., Rachel R. Brown, and Eugene H. Burns, Jr.*

Department of Natural Sciences, Shawnee State University, Portsmouth OH 45662

Introduction

The presence of fecal coliform bacteria in aquatic environments is often used as an indicator of contamination with fecal material and other possible pollutants (Tyrrell et al., 1995; Hernandez et al., 1997; Gearheart, 1999; McMath et al., 1999; Perkins and Hunter, 2000). Fecal coliform pollution may occur in ambient water as a result of the overflow of domestic sewage or non-point sources of human or animal waste (McMath et al., 1999). River water may be contaminated with fecal material of man or other animals. At the time of contamination, pathogens may be introduced into the water (Ricca and Cooney, 1999; Tyrrell et al., 1995). Over 140 different virus types and many different bacteria are excreted in animal and human feces and urine, including *Escherichia coli*, *Salmonella enteritis*, hepatitis A and viral gastroenteritis that are pathogenic to humans (Hernandez et al., 1997; Newman et al., 2000; Ricca and Cooney, 1999; Tyrrell et al., 1995). Thus, the presence of fecal contamination is an indicator that a potential health risk exists for individuals exposed to this water (Newman et al., 2000; Ricca and Cooney, 1999; Tyrrell et al., 1995).

Increasingly, constructed wetlands are being used for lowering pollutant levels in contaminated water and wastewater, including treatment for urban and agricultural storm water runoff that may contain chemical and other pollutants (Carleton et al., 2001; Gerba et al., 1999; Khatiwada and Polprasert, 1999; Newman et al., 2000; Ostroumov, 1998; Perkins and Hunter, 2000; Shutes, 2001). Studies have shown that wetlands improve water quality by reducing nutrients, chemical contaminants, and pathogenic microbes (Gerba et al., 1999; Ostroumov, 1998; Lau and Chu, 2000). Previous studies at the Olentangy River Wetland Research Park (ORWRP) have shown decreasing levels in nitrate+nitrite, soluble-reactive phosphorus, and total phosphorus as the water passes through the wetlands (Mitsch et al., 2000).

The purpose of this study was to examine fecal coliform levels in two constructed wetlands at ORWRP and to determine the ability of these basins to reduce coliforms. Samples were collected from locations throughout both wetlands, the Olentangy River, and the swale from October 2000 to June 2001 during months when the wetlands were not frozen. Samples were subjected to the multiple tube fermentation technique using most probable number analysis (Hernandez et al., 1997; Khatiwada and Polprasert, 1999) to determine the concentration of fecal coliforms in each sample. The data from five months of sampling show that the two constructed wetlands are reducing fecal coliform levels as water flows through them.

*Corresponding author
Eugene H. Burns, Jr., Dept. of Natural Sciences,
Shawnee State University,
940 Second Street, Portsmouth, Ohio 45662
email: eburns@shawnee.edu
phone: (740) 351-3685

Materials and Methods

Sample Collection

Samples of 100 ml were collected from locations in wetlands 1 and 2 (Figure 1), the Olentangy River, and the entrance to the swale, using sterile 50 ml conical tubes (Becton Dickinson, Franklin Lakes, NJ). Samples were taken monthly in October 2000, March 2001, April 2001, May 2001, and June 2001. During December 2000, January 2001, and February 2001 the wetlands were frozen and samples were unable to be collected.

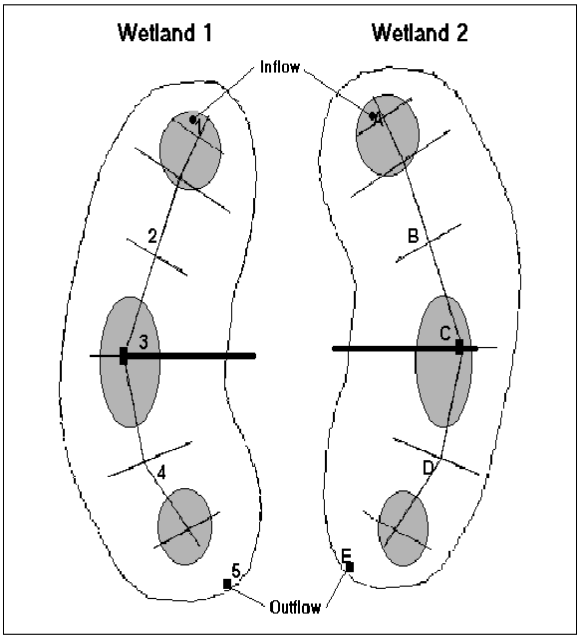

Figure 1. Sample locations in wetlands

Testing for fecal coliforms

Samples were subjected to presumptive, confirmed, and completed tests in the multiple tube technique as described in *Standard Methods for the Examination of Water and Wastewater* (APHA, 1998). Briefly, phenol red lactose fermentation broth tubes containing an inverted Durham tube for gas collection were inoculated with each water sample, for the presumptive test. Five tubes of 1X lactose broth (phenol red broth base [Difco, Detroit, MI] with 5g lactose/ L) were inoculated with 0.1 ml of each sample. Five tubes of 1X lactose broth were inoculated with 1.0 ml of each sample, and five tubes of 2X lactose broth (10 g lactose/ L) were inoculated with 10 ml of each sample. Tubes were incubated for 48 hours at 37°C. Results were recorded as positive for lactose fermentation and production of gas. In the confirmed test, each positive tube from the presumptive test was used to inoculate an eosin methylene blue agar plate (Difco) and incubated for 24 hours at 37°C. Positive colonies produce a dark center or green metallic sheen. Positive colonies from the confirmed test were then subjected to the completed test. The positive colonies were inoculated into 1X lactose fermentation broth and streaked on nutrient agar plates (Difco). Fermentation tubes and plates were incubated for 24 hours at 37°C. Tubes were recorded as positive if they showed lactose fermentation and gas production. Colonies on nutrient agar were subjected to Gram stains and recorded as positive if they showed Gram negative rods (Khatiwada and Polprasert, 1999). Positive results at the end of the completed test were used for most probable number analysis to determine the fecal coliform concentration per 100 ml in each wetland sample (Khatiwada and Polprasert, 1999; Hernandez et al., 1997).

Results

To determine the effects of water flow through the wetlands on fecal coliform concentration, samples were collected each month from October 2000 to June 2001, except during months when the wetlands were frozen. The sampling pattern was designed to distribute samples across each wetland and provide adequate space for reduction of fecal coliforms between each water sample (see Figure 1). Sampling in different locations within each wetland provided data to analyze the reduction of fecal coliform levels throughout each wetland. In addition, one sample was collected in the Olentangy River and the swale. Fecal coliform levels recorded each month at the inlet and outlet of wetlands 1 and 2, the river, and the swale are shown in Table 1.

The maximum coliform concentration in the river was

Table 1: Reduction of fecal coliforms in each wetland during five months sampled.

Month	Inlet[1]	Outlet[2]	Number Reduced[3]	%Reduction[4]
October 2000				
Wetland 1	22	2	20	90.9
Wetland 2	33	9	24	72.7
River to Swale[5]	50	2	48	96.0
March 2001				
Wetland 1	27	15	12	44.4
Wetland 2	23	9	14	60.9
River to Swale	33	13	20	60.6
April 2001				
Wetland 1	49	7	42	85.7
Wetland 2	30	16	14	46.7
River to Swale	47	5	42	89.4
May 2001				
Wetland 1	37	4	33	89.2
Wetland 2	44	6	38	86.4
River to Swale	37	18	19	51.3
June 2001				
Wetland 1	40	4	36	90.0
Wetland 2	33	2	31	93.9
River to Swale	60	4	56	93.3

[1] Fecal coliforms / 100ml in samples collected at the inlet to the wetland or in the river or swale.
[2] Fecal coliforms/ 100ml in samples collected at the outlet of each wetland or in the river or swale.
[3] Difference in fecal coliform concentration between inlet and outlet.
[4] Percent reduction in fecal coliform concentration from inlet to outlet calculated as (number reduced/ inlet concentration) x 100.
[5] Fecal coliform levels for the river sample are reported as inlet values and fecal coliform levels at the entrance to the swale are reported as outlet values.

60 coliforms/100ml, in June 2001, whereas, the maximum coliform concentration found in either wetland basin was 49 coliforms/100ml, found in wetland 1 in April 2001. With the exception of wetland 1 in June 2001 and wetland 2 in May 2001 the data consistently show a slight reduction in coliforms between the river sample and the sample taken at the inlet to the wetland basin.

Table 1 shows that coliform concentration varied from month to month, but always decreased from inlet to outlet of the wetland. Wetland 1 showed fecal coliform reduction between 44% and 91% with an average reduction of 80% ± 18%. Wetland 2 reduced fecal coliform levels an average of 72 ± 17% with monthly reduction varying between 47% and 94%.

Figure 2 shows average fecal coliform concentration in each sample site in wetland 1 and wetland 2 over the five month sampling period. These data show a steady decrease in fecal coliform concentration as water moves away from the inlet through the wetland. The difference in average fecal coliform reduction between the two wetlands is not statistically significant (t-test, p= 0.2801). These data demonstrate that both wetlands are reducing fecal coliform concentration in a similar manner.

Interestingly, the data show differences in fecal coliform reduction between summer (June 2001) and winter (March 2001) months. The samples taken in March 2001 were only reduced in fecal coliform levels by 44% in wetland 1 and 61% in wetland 2. Samples taken in June 2001 were reduced 90% in wetland 1 and 94% in wetland 2. The fecal coliform reduction in wetland 1 in March 2001 was 51%

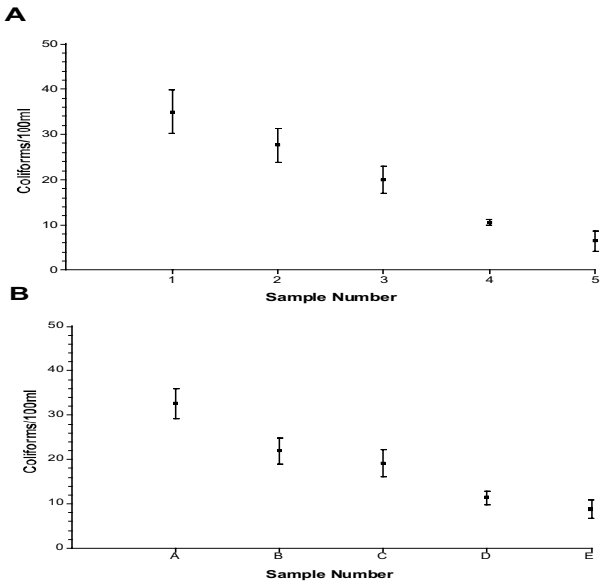

Figure 2. Fecal coliform levels in the wetlands. A) mean fecal coliforms/ 100 ml ± standard deviation in each sample site in wetland 1 for the five months sampled. B) mean fecal coliforms/ 100 ml ± standard deviation in each sample site in wetland 2 for the five months sampled.

less than in June 2001. Wetland 2 reduced fecal coliform concentration 35% less in March 2001 than in June 2001.

Discussion

Fecal coliforms have long been used as indicators of pollution in water (Gearheart, 1999; Hernandez et al., 1997; McMath et al., 1999; Perkins and Hunter, 2000; Tyrrell et al., 1995) due to the potential for introduction of pathogens and other pollutants along with these bacteria (Ricca and Cooney, 1999; Tyrrell et al., 1995). This study examined the concentration of fecal coliforms in two created wetlands at the Olentangy River Wetlands Research Park. During the months sampled, both wetlands consistently showed fecal coliform reduction ranging from 44 to 96% with average reductions of 80 ± 18% and 72% ± 17% for wetland 1 and wetland 2, respectively. The data did not show a significant difference in fecal coliform reduction between the two wetlands. The level of reduction in fecal coliforms observed is similar to that reported by Perkins and Hunter (Perkins and Hunter, 2000) and others (Lau and Chu, 2000; Newmann et al., 2000; Perkins and Hunter, 2000) in constructed wetlands in other areas. Similar reductions in fecal coliforms have also been reported in natural wetland areas (Lau and Chu, 2000).

Reduction in fecal coliforms by wetlands may involve several factors including amount of plant coverage, hydraulic retention time, and settling of microorganisms (Perkins and Hunter, 2000; Khatiwada and Polprasert, 1999; Shutes, 2001). Seasonal changes in any of these factors may account for the variability in reduction from month to month. Interestingly, this study showed substantial differences in fecal coliform reduction potential observed in winter months as compared to summer months. In fact, fecal coliform reduction was 1.5 to two times greater in June as compared to March. Further studies will be needed to determine the cause of these differences but Newman et al. (2000) and others (Khatiwada and Polprasert, 1999) have suggested that differences in reduction potential between winter and summer months may be due to differences in plant coverage, temperature, and retention time.

Increased plant coverage in summer months may lead to greater filtration through plant material providing more entrapment of bacteria and more time for sedimentation. Gerba et al. (1999) suggested that microorganism removal as water flows through constructed wetlands may be primarily due to sedimentation. Preliminary studies of total bacterial burden in the wetlands suggest that sedimentation of bacteria is occurring in the ORWRP (data not shown). Further studies will be necessary to confirm these preliminary observations and to relate bacterial sedimentation to coliform reduction.

This study also showed a decrease in fecal coliforms as water traveled from the river through the pumping system into the wetlands, although this is unlikely due to sedimentation. With the exception of wetland 1 in June 2001 and wetland 2 in May 2001, the data show a 10% to

56% decrease in fecal coliform concentration between the river and the wetland inlet. These data suggest that bacteria may be accumulating in the pipes and pumps. Other researchers have noted differences in nitrate+nitrites and phosphorus between river samples and samples taken at the inlet to the wetlands (Mitsch, 2000). These differences in chemical concentrations may be related to bacterial metabolic processes in the piping machinery. Biofilm formation in pipes is a common occurrence (Batista et al., 2000; Jenkinson and Lappin-Scott, 2001; MacDonald et al., 2000) that may eventually lead to pipe damage (Batista et al., 2000; MacDonald et al., 2000). Further research will be necessary to determine the presence and extent of biofilms in the ORWRP pump and piping system.

Because this study only measured fecal coliform levels for five months, observations are continuing. However, these preliminary data suggest that biofilm may be forming in the pumping machinery, and the wetlands at ORWRP may be reducing fecal coliforms in a similar manner as observed at other constructed wetlands and in natural wetland areas.

Acknowledgments

The authors thank Dr. Robert Deal, Dr. Bill Mitsch, Judy Gardner, and Hui Suk Jones. This research was supported by the Department of Natural Sciences, Shawnee State University and a grant from the Ohio Board of Regents Incentive for Performance Fund Research Challenge Program.

References

APHA. 1998. Standard Methods for the Examination of Water and Wastewater, 20th ed.. American Public Health Association, Washington, DC.

Batista, J. F., R. F. C. Pereira, J. M. Lopes, M. F. M. Carvalho, M. J. Feio, and M. A. M. Reis. 2000. In situ corrosion control in industrial water systems. Biodegradation 11: 441-448.

Carleton, J. N., T. J. Grizzard, A. N. Godrej, and H. E. Post. 2001. Factors affecting the performance of stormwater treatment wetlands. Water Research 35: 1552-1562.

Gearheart, R. A. 1999. The use of free surface constructed wetland as an alternative process treatment train to meet unrestricted water reclamation standards. Water. Science Technology 40: 375-382.

Gerba, C. P., J. A. Thurston, J. A. Falabi, P. M. Watt, and M. M. Karpiscak. 1999. Optimization of artificial wetland design for removal of indicator microorganisms and pathogenic protozoa. Water Science Technology 40: 363-368.

Hernandez, F., R. Monge, C. Jimenez, L. Taylor. 1997. Rotavirus and Hepatitis A virus in market lettuce (*Latuca sativa*) in Costa Rica. International Journal of Food Microbiology 37: 221-223.

Jenkinson, H. F. and H. M. Lappin-Scott. 2001. Biofilms adhere to stay. TRENDS in Microbiology 9: 9-10.

Khatiwada, N. R. and C. Polprasert. 1999. Kinetics of fecal coliform removal in constructed wetlands. Water Science Technology 40: 109-116.

Lau, S. S. S. and L. M. Chu. 2000. Nutrient and faecal contamination and retention in wetland enclosures (gei wais) in the Mai Po Marshes, Hong Kong. Hydrobiologia 431: 81-92.

MacDonald, R., M. Santa, and V. S. Brozel. 2000. The response of bacterial biofilm community in a stimulated industrial cooling water system to treatment with an anionic dispersant. Journal of Applied Microbiology 89: 225-235.

McMath, S. M., C. Sumpter, D. M. Holt, A. Delanoue, and A. H. L. Chamberlain. 1999. The fate of environmental coliforms in a model water distribution system. Letters in Applied Microbiology. 28: 93-97.

Mitsch, W. J., V. Bouchard, L. Zhang, and M. Hunter. 2000. Biogeochemical and nutrient removal patterns of created riparian wetlands: Sixth-year results. In: Mitsch, W. J. and V. Bouchard (ed.), Olentangy River Wetland Research Park at The Ohio State University, Annual Report 2000. Columbus, OH, pp. 83-90.

Newman, J. M., J. C. Clausen, and J. A. Neafsey. 2000. Seasonal performance of a wetland constructed to process dairy milkhouse wastewater in Connecticut. Ecological Engineering. 14: 181-198.

Ostroumov, S. A. 1998. Biological Filtering and Ecological Machinery for Self-Purification and Bioremediation in Aquatic Ecosystems: Towards a Holistic View. Biology Forum. 91: 221-232.

Perkins, J., C. Hunter. 2000. Removal of enteric bacteria in a surface flow constructed wetland in Yorkshire, England. Water Research 34: 1941-1947.

Ricca, D. M. and J. J. Cooney. 1999. Coliphages and Indicator Bacteria in Boston Harbor, Massachusetts. John Wiley & Sons, Inc. 404-408.

Shutes, R.B.E. 2001. Artificial wetlands and water quality improvement. Environment International. 26: 441-447.

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 Research 29: 2483-2490.