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Research Article

Analysis of Total Coliform and Total *Escherichia coli* Levels in the Fox River Watershed

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The Fox River is an important water source for the Elgin, IL community. Fecal contaminants must be removed by water treatment facilities to prevent the spread of infectious diseases and to provide potable water. Water samples from the Fox River watershed on or near Judson University campus were tested using the most probable number (MPN) method to determine coliform counts. Our data from this small independent project demonstrates that tap water is free of fecal contaminants and that the Fox River, its tributary Tyler Creek, and the Volkman Retention Pond often have levels of coliforms exceeding standards for recreational use.

Keywords: Fox River; Tyler Creek; coliform; Escherichia coli

Introduction

In the United States today, access to clean water is generally widespread. City tap water is regulated by municipal treatment plants for fecal (e.g. total coliforms and *E. coli*), inorganic (e.g. nitrate), organic carbon, metal (e.g. lead, mercury, cadmium), and synthetic organic (e.g. polychlorinated biphenyls [PCB], and Dichlorodiphenyltrichloroethane [DDT]) contaminants (City of Elgin, 2016). Coliforms are gram-negative, lactose fermenting bacterial rods that are found in human and other animal intestines; thus, their presence is often used as an indicator of fecal contamination. Coliforms are often enteric bacteria and include such species as *Escherichia coli* and *Klebsiella pneumoniae*. *Enterobacter aerogenes* is also considered a coliform due to its ability to ferment lactose, despite *E. aerogenes* not being an enteric bacterium (Madigan *et al.*, 2003). Coliforms cannot survive outside of the host body indefinitely; however, if recently contaminated water is consumed, *E. coli* can cause gastroenteritis, which can become life-threatening for infants, the immunocompromised, and the elderly (Geldreich, 1972). All three of the above mentioned bacteria can also cause urinary tract infections (UTIs) when bathing or swimming in contaminated water (Madigan *et al.*, 2003).

Knowing that the Elgin section of the Fox River is often above Illinois Pollution Control Board (IPCB) standards for fecal contamination counts (Singh *et al*., 1995), we focused this small study on determining the total coliform and *E. coli* counts for the Fox River watershed encompassing the Judson University campus to determine if this observation still held true at the time this study took place (2010). To be in accordance with IPCB standards, fecal coliform counts in a system used for water supply should not be above 2000 cells/100 mL, and for recreational use should not be above 200 cells/100 mL (Singh *et al*., 1995). The majority of Elgin's potable water originates from the Fox River. Therefore, we employed the MPN method to test water from three sampling sites located on or adjacent to the Judson University campus: Tyler Creek (TC), Volkman Retention Pond (VP), and the Fox River (FR). Tap water from the Volkman Hall dormitory was tested initially to determine if fecal contaminants were screened successfully during the municipal water treatment process. Our data demonstrate that coliforms are absent in tap water but levels in the FR, TC, and VP sometimes exceed IPCB standards for recreational use and approach the water supply coliform limit.

Experimental Procedures

Unless otherwise stated, all media and chemicals were purchased from Remel (Lanexa, KS) and Sigma (St. Louis, MO), respectively.

Description of Sampling Sites

The FR is a 359 km (223 mi)-long tributary of the Illinois River. Its headwaters are ~ 1 mi to the southeast of Colgate, WI and its confluence with the Illinois River is at Ottawa, IL (U.S. Geological Survey, 2012). Sampling was carried out where the FR abuts the Judson University campus (Fig. 1, bottom panel). TC is a minor tributary to the FR. The headwaters of TC lie just to the west-northwest of Elgin in Gilberts, IL and it empties into the FR on the southeast corner of the Judson University campus (Friends of the Fox River, 2016). As Dr. Robert D. Erickson, a founding faculty member of Judson University, relates (July 20, 2017), the VP (Fig. 1, top panel) was originally a marshy depression adjacent to TC at the inception of the university in 1962. As the result of flooding to nearby Volkman Residence Hall in the early 1970s, the marshy area was dredged to form a retention pond emptying into TC via an effluent pipe with a check-valve (Fig. 1, top panel). We visually inspected the effluent pipe and the check-valve is in place and operational. Water samples from TC and VP were collected at sites as indicated on Figure 1.

Most Probable Number (MPN) Method

The MPN method was used to measure total coliform and *E. coli* densities (cells/100 mL) in water samples as described in Leboffe and Pierce (2006). Briefly, 100 mL samples were collected in sterile bottles from TC, VP, and FR. Time of day, date, air temperature (°C), water temperature (°C), location, weather/site conditions, and precipitation in the previous 48 hrs were recorded for each sample (Table 1). Screw-cap glass test tubes (13 x 150 mm) were filled with 9 mL distilled water (dH_2O), and autoclaved 15 min at 121°C and 15 PSI (STM-E, Market Forge, Everett, MA.). Serial dilutions in sterile dH_2O (10^0, 10^{-1}, and 10^{-2}) of collected water samples were used to inoculate media.

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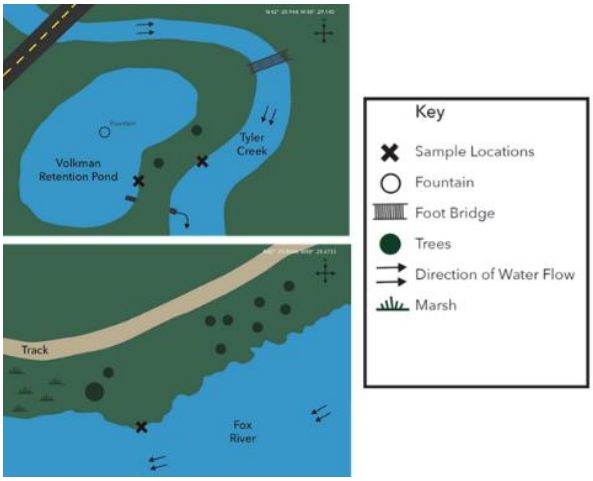

Figure 1. Water sampling sites. *Top panel*, location of VP and TC sampling sites. *Bottom panel*, location of FR sampling site. Map coordinates for each site are located in the upper right hand corner of each image. See key for legend to symbols.

Fifteen Lauryl Tryptose Broth tubes (LTB; 2% w/v Tryptose, 0.5% w/v Lactose, 16 mM K_2HPO_4, 20 mM KH_2PO_4, 86 mM NaCl, 0.01% w/v Sodium Lauryl Sulfate [SLS] in dH_2O; 10 mL were dispensed into 13 x 150 mm tubes having an inverted Durham tube [6 x 50 mm] and sterilized as above) were divided into three groups of five broths. Each tube in a group was aseptically inoculated with 1 mL of the appropriate dilution, mixed well, and incubated 48 hrs at 35 ± 2ºC. LTB tubes that display gas production, as indicated by a bubble in the Durham tube, or turbidity with no gas production were recorded as (+).

Brilliant Green Lactose Bile Broth tubes (BGLB; 1% w/v Peptone, 1% w/v Lactose, 2% w/v Oxgall [bile salts], 0.00133% w/v Brilliant Green dye in dH_2O; 10 mL were dispensed and sterilized as for LTB) were aseptically inoculated with (+) LTB using an inoculating loop, mixed well, and incubated 48 hrs at 35 ± 2ºC. Concurrently, *E. coli* Broth tubes (EC; 2% w/v Tryptose, 0.5% w/v Lactose, 0.15% w/v Oxgall, 23 mM K_2HPO_4, 11mM KH_2PO_4, 86 mM NaCl in dH_2O; 10 mL were dispensed and sterilized as for LTB) were aseptically inoculated with (+) LTB using an inoculating loop, mixed well, and incubated 48 hrs in a water bath at exactly 45.5ºC. BGLB and EC broth tubes positive for gas production, but not turbidity alone, were recorded as (+).

The MPN for total coliform and *E. coli* was determined using (+) BGLB broth tubes and EC broth tubes, respectively, as follows: $\frac{MPN}{100}mL = \frac{100P}{\sqrt{VnVa}}$. Where P = total number of positive results (BGLB or EC), V_n = combined volume (Dilution [D] x 1.0 mL x # negative tubes) of original sample in LTB tubes that produced negative results in BGLB or EC, and V_a = combined volume (D x 1.0 mL x # tubes) of original sample in all LTB tubes inoculated.

Eosin Methylene Blue test

2.75% w/v Eosin Methylene Blue (EMB) agar was resuspended in dH_2O with heat while stirring, autoclaved as above, and aseptically poured into sterile 15 x 100 mm polystyrene petri dishes (VWR, West Chester, PA). EMB plates were inoculated using the quadrant streak technique with a loop-full of (+) BGLB and (+) EC broth. Inverted plates were incubated 24 - 48 hrs at 35 ± 2ºC.

Nutrient Agar

Nutrient Agar (NA; 0.8% w/v Nutrient Broth, and 1.5% w/v Agar-Agar [Difco, Detroit, MI]) in dH_2O was dissolved using heat with stirring. Seven mL of NA was pipetted into 13 x 150 mm screw-cap glass test tubes and autoclaved as above. Tubes were then placed at an angle to cool, creating a slant. NA slants were aseptically inoculated with a loop from (+) EC and BGLB broth tubes. Inoculated slants were incubated 24 - 48 hrs at 35 ± 2ºC.

Gram Stain

Gram Stain reagent recipes and methodology are from Leboffe and Pierce (2006). Glass slides were cleaned and labeled. A drop of dH_2O was placed on the slide, bacteria from EMB or NA was aseptically transferred and mixed with the drop of dH_2O to prepare a smear, allowed to air-dry, and heat-fixed. Bacterial smears were flooded with Gram Crystal Violet (Modified Hucker's) for 1′, rinsed with tap water, and exposed to Gram Iodine for 1′. Slides were rinsed with tap water and 95% ethanol was added drop-wise until the effluent was light blue. Slides were rinsed with tap water and the smears counterstained with Gram Safranin for 45″, rinsed with tap water, blotted dry with bibulous paper and examined under oil immersion microscopy at x1000 magnification

Table 1. Sampling information collected for TC, VP, and FR over the course of three collections. 100 mL samples were taken at each site. Precipitation (Precip.) is the amount of rainfall in the previous 48 hrs. "Conditions" are subjective and objective observations made by J.R.

Collection Number	Sample Site	Date	Time	Air Temp. (ºC)	Water Temp. (ºC)	Conditions	Precip. (cm)
1	TC	10.20.10	9:35 am	5.5	9.0	Cloudy	0.0
	VP	10.20.10	9:40 am	5.5	11.0	Cloudy	0.0
	FR	10.20.10	10:40 am	5.5	10.0	Sun/wind	0.0
2	TC	10.27.10	6:12 pm	11.0	12.0	Very windy	0.54
	VP	10.27.10	6:14 pm	11.0	12.6	Very windy	0.54
	FR	10.27.10	6:03 pm	11.0	11.5	Very windy, Canadian geese on site	0.54
3	TC	11.05.10	5:01 pm	5.0	7.0	Sun/wind	0.03
	VP	11.05.10	5:10 pm	5.0	7.0	Sun/wind	0.03
	FR	11.05.10	3:15 pm	5.5	10.0	Sun/wind, water extremely murky	0.03

Results

To determine coliform presence, the MPN method was utilized (see Experimental Procedures). Water samples from control municipal tap water were negative for bacterial growth and fermentation of lactose (data not shown). All environmental water samples (FR, TC, VP) were positive for fermentation of lactose (gas production; Fig. 2). LTB is selective for the coliform group due to the inclusion of lactose and SLS. LTB inhibits the growth of gram-negative cocci and gram-positive rods and cocci. However, because it does not completely inhibit the growth of all noncoliforms, it is used to presumptively identify the presence or absence of coliforms. Since coliforms ferment lactose, which produces gas, we knew that perceiving trapped gas in the inverted Durham tube in the media would indicate the presence of coliforms (+). Absence of gas is negative if the broth is clear. Since we are analyzing an environmental sample, coliforms may only be a very small percentage of the bacteria present in the sample. Therefore, the occurrence of turbidity (bacterial growth) in LTB, but no gas production, does not rule out the presence of coliforms and is also marked as a (+). LTB did not select against all noncoliforms; therefore, there were, in aggregate, more (+) LTB tubes than the other two media types (Fig. 2). LTB tubes determined to be (+) were used to inoculate BGLB and EC

broth. BGLB broth containing lactose and 2% bile inhibits growth of noncoliforms and is utilized to confirm the presence of coliforms (gram-negative rods). EC broth, which includes lactose and a small amount of bile salts, is selective for *E. coli* when incubated at 45.5ºC (Leboffe and Pierce, 2006).

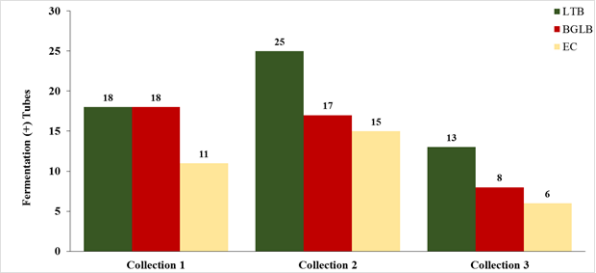

Figure 2. Total number of tubes positive (+) for lactose fermentation (gas production). LTB showed the greatest number of positive tubes due to the inability to select against all noncoliforms. EC broth produced the least amount of (+) tubes due to its increased selectivity for *E. coli* over other coliforms that could not survive incubation at 45.5ºC.

Bacterial growth was evident if the media showed fine turbidity, flocculence, pellicle, and/or sediment formation. As expected, more selective media resulted in fewer broth tubes exhibiting growth. Analysis of growth patterns between the least selective media, LTB, and the most selective media, EC, reveals over twice as many growth-positive LTB tubes compared to EC tubes (Fig. 3). Additionally, as expected, this correlation was also observed for lactose fermentation with LTB samples exhibiting the most positive samples, then BGLB, and finally the most selective media, EC (Fig. 2). The data from collection 2 indicate a positive correlation between the number of EC tubes exhibiting turbidity and fermentation with rainfall occurring in the previous 48 hrs (Table 1). The increase in the number of EC tubes promoting the growth of *E. coli* after rainfall is consistent with run-off containing fecal matter from farms, suburban lawns, and parking lots entering the watershed upstream of the Judson University campus.

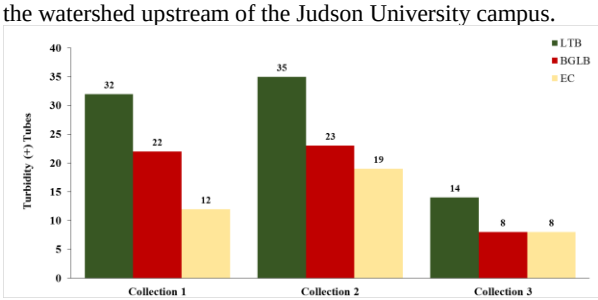

Figure 3. Total number of tubes positive (+) for bacterial growth (turbidity). Tubes were considered positive for bacterial growth if the media showed fine turbidity, pellicle formation, and/or sediment formation. LTB (least selective) fostered the most bacterial growth over the course of the three collections, while EC (most selective) had the least number of tubes positive for growth.

IPCB standards for total coliform and total *E. coli* for recreational water usage (200 cells/100 mL) were exceeded in FR in collection 2, TC in collection 1, and VP in collection 1 and 2 (Fig. 4). Consistent with our data from Figure 2, total coliform and total *E. coli* levels were measurably higher within 48 hrs of rainfall (collection 2) for VP and FR. These data are consistent with increased coliforms in the FR watershed due to run-off from upstream sources contaminated with fecal material. Since the measurable amount of precipitation was only 0.54 cm (Table 1), sewage back-up from overloaded drains on the Judson University campus is highly unlikely.

IPCB total coliform level standards for water supply (2000 cells/100 mL) were not exceeded at sampling times during the course of this project, but were closely approached (1960 cells/100 mL) by the FR sample in collection 2 following a 0.54 cm rainfall (Fig. 4, top panel). Total *E. coli* levels closely mirrored that of total coliform levels (Fig.4, bottom panel). IPCB total coliform level standards for recreational water usage were exceeded in TC and VP in collection 1 and by VP and FR in collection 2. On average, the FR displayed the highest total coliform and *E. coli* counts of the three bodies of water tested over the course of this study. All three sampling sites revealed low levels of total coliforms and *E. coli* in collection 3 (Fig. 4). The low levels of coliforms, relative to the other two collections, may be due to the ~2 – 4ºC drop in water temperature, thereby inhibiting bacterial viability (Table 1; see also Fig. 2 and 3).

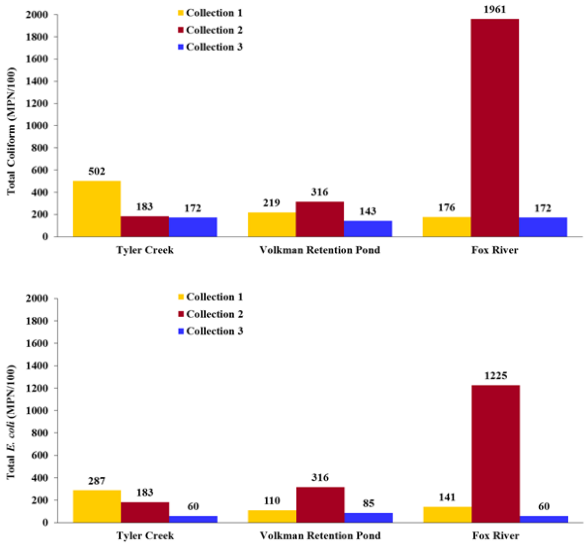

Figure 4. Total coliform and total *E. coli* levels for the Fox River Watershed. *Top panel*, total coliform levels (MPN/100 mL) at each site over three collections. *Bottom panel*, total *E. coli* levels (MPN/100 mL) at each site over three collections.

To give a presumptive determination of the major coliforms present in fermentation (+) BGLB and EC broth tubes, EMB plates were streaked using the quadrant technique and analyzed for growth and color of colonies. EMB permits differentiation between lactose fermenters and nonfermenters as well as the colon bacillus, *E. coli*. In addition, EMB medium partially inhibits the growth of gram-positive organisms. Luxuriant growth of *E. coli* will result in colonies with a distinct metallic-green sheen due to the large amount of acid produced leading to precipitation of dye on the colony surface. However, well-isolated *E. coli* colonies can be either metallic-green or pink with black centers. *E. aerogenes* present as pink-

mucoid colonies and *K. pneumoniae* produces pink-mucoid colonies with purple centers (Cappuccino and Sherman, 2008; Leboffe and Pierce, 2006). EMB plates inoculated from EC fermentation (+) samples exhibited pink colonies with black centers, with a minority of colonies being either pink-mucoid or pink-mucoid with purple centers. In contrast, EMB plates inoculated from BGLB fermentation (+) tubes exhibited a majority of pink-mucoid or pink-mucoid with purple center colonies with *E. coli* colonies being scant (data not shown). No non-coliform colonies (beige) were detected from the BGLB and EC broths tested. These data confirm that EC broth preferentially selected for *E. coli* and that we had at least three different species of enteric bacteria growing in BGLB.

Morphology of bacteria grown on EMB was examined by Gram staining. NA slants were individually inoculated with the three different types of colonies for use as stock cultures. All colonies displayed echinulate (dense and opaque with a spiny edge) growth. Analysis of bacteria under oil immersion at x1000 revealed that colonies isolated from EMB plates inoculated with EC were gram-negative rods of varying lengths, most likely *E. coli* with a smattering of other gram-negative coliforms. For the most part, colonies from BGLB source tubes were gram-negative rods. However, there were a few gram-positive cocci scattered throughout the preparation, indicating that selection for gram-negative rods is not absolute in BGLB media and that EMB, as expected, does not completely inhibit the growth of gram-positive cocci (data not shown; Cappuccino and Sherman, 2008; Leboffe and Pierce, 2006). Further isolation, with concomitant biochemical tests, would be required to definitively determine the major bacterial species present in the BGLB and EC (+) samples and was beyond the scope of this study.

Discussion

Our findings demonstrate that coliforms were present in every water system tested in the Fox River watershed running through or adjacent to the Judson University campus. Coliform levels were inconsistent from collection to collection, most likely due to variations in the quantity of run-off, dependent on rainfall, water temperature, and overall water quality (e.g. nitrate levels, pH; not tested). The sharp spike in total coliform and total *E. coli* counts from the FR collection 2 exemplifies this process. Not only can rainfall wash fecal matter into the FR watershed, but significant rainfall upstream of Judson University could lead to septic and other waste systems to release fecal matter into the watershed. The FR was highly turbid (data not shown) when water sampling occurred during collection 2 indicating an increase in volume and flow rate. During collection 2, the VP did not exhibit the same increase in coliform levels, most likely because the pond is relatively self-contained with little water entering the system other than from run-off from surrounding land. Though VP is connected to TC by an effluent pipe (Fig. 1), a check-valve prevents water flow from TC to VP mitigating transfer of coliforms. Nonetheless, we were surprised that TC did not have a similar spike in coliforms during collection 2 as the FR, since TC carries water from adjacent neighborhoods and, further upstream, farms. However, with the decrease of farm land and concurrent urbanization adjacent to Elgin, IL and the flow-rate of TC (it is not an ephemeral waterway), it is likely that coliforms are rapidly washed into the Fox River.

IPCB standards for coliform levels appropriate for recreational use (200 cells/100 mL) were exceeded at least once for each body of water during a collection period. During the fall and winter months, when water recreation is less prevalent, this is not much cause for concern; but, based on the observed correlation between temperature and coliform levels, we expect coliform counts to increase during the warmer summer months when water recreation is at its peak. The IPCB standard for water supply (2000 cells/mL) was never exceeded; however, one of Elgin's main water sources, the Fox River, closely approached the limit at 1960 cells/100 mL in collection two, within 48 hrs of rainfall. These data suggest that water enthusiasts, when practicable, would do well to avoid ingesting, or having contact with, water from these sources to avoid acute gastroenteritis or UTIs (Singh *et al.*, 1995).

In conclusion, our findings on total coliform and *E. coli* levels in the Fox River watershed, encompassing the Judson University campus, are in agreement with the IPCB study (Singh *et al.*, 1995) that coliform counts for recreational water use are often exceeded. As noted above, water run-off after significant amounts of precipitation increases coliform levels. The contamination of the Fox River watershed surrounding the Judson University campus with coliforms will only be exacerbated by increased run-off due to urbanization and replacement of farmland, which absorbs water, by parking lots serving commercial properties.

Acknowledgments

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