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Microbial Assessment of Irrigation Water Used for Production of Fruit and Vegetables in Ontario, Canada

MARINA STEELE,[1] ABDULLAHI MAHDI,[2] AND JOSEPH ODUMERU[1]*

[1]*Laboratory Services Division, University of Guelph, Guelph, Ontario, Canada N1H 8J7; and* [2]*Ontario Ministry of Agriculture and Food, Guelph, Ontario, Canada N1G 4Y2*

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ABSTRACT

Five hundred one irrigation water samples were collected from 27 irrigation water sources on 17 farms in southern Ontario, Canada, over a single irrigation season in 2002. The water samples were tested for the presence of the following bacterial water quality indicators: total coliform bacteria, fecal coliforms, *Escherichia coli*, and fecal streptococci. The median values per 100 ml of these indicators in the irrigation water samples were 3,000, 33, 15, and 1, respectively. Between 70.6 and 98.2% of irrigation water samples contained acceptable levels of fecal coliforms or *E. coli*, according to published irrigation water quality guidelines. Significant correlations ($P < 0.05$) were observed between the concentrations of different bacterial indicators and the degree of recent precipitation and concentrations of total coliforms and fecal streptococci. With the exception of fecal streptococci, which increased in number toward the end of the study, none of the indicators displayed a significant trend over the course of the season, as determined by linear regression analysis of indicator concentrations over time ($P > 0.05$).

There is a growing awareness that fresh or minimally processed fruits and vegetables can harbor disease-causing bacteria, protozoa, viruses, and helminths *(23).* Numerous outbreaks of foodborne illness have been linked to contaminated produce *(8).* Fruits and vegetables may become contaminated with pathogenic microorganisms through contact with soil or improperly composted manure, by irrigation or postharvest washing with contaminated water, or through contact with infected food handlers *(10, 20).* Irrigation water has been directly implicated as a source of pathogenic microorganisms on produce linked to disease outbreaks *(17, 22).* In controlled experimental studies, *Escherichia coli* O157:H7 in irrigation water contaminated lettuce seedlings, and these seedlings remained contaminated even after washing *(19, 21).*

Because irrigation water can be a source of foodborne pathogens, the quality of water routinely used for irrigation of crops is important. The intensity of crop irrigation and the sources of water used within Canada for irrigation vary among provinces *(11).* These water sources include groundwater, surface water, and treated wastewater.

The purpose of this study was to evaluate the microbial quality of water used for irrigation of fruits and vegetables over a single growing season on farms in southern Ontario, Canada, and to compare the results of this study with current Canadian microbial water quality guidelines. Water samples were collected during one growing season from a group of dedicated fruit and vegetable farms in southern Ontario and analyzed for the presence of commonly used bacterial water quality indicators.

* Author for correspondence. Tel: 519-767-6243; Fax: 519-767-6240; E-mail: jodumeru@lsd.uoguelph.ca.

MATERIALS AND METHODS

Selection of irrigation water sources. Twenty-seven irrigation water sources from 17 farms in southern Ontario were sampled. The irrigation water sources were representative of the sources used in southern Ontario. The majority of the sources tested were holding ponds located on farms, and sources of water flowing into these holding ponds included groundwater from wells or springs, water from creeks and lakes, and surface runoff. A canal drawing irrigation water from Lake Simco was also sampled. The farms included in this study were dedicated vegetable farms with little or no livestock production. Characteristics of the irrigation water sources tested and the number of times each source was tested are listed in Table 1. Irrigation water sources located on the same farm were separate water sources but were likely subject to similar environmental contaminants and were thus not completely independent from each other.

Sample collection. Duplicate samples were collected 1 to 18 times from each irrigation source between 28 May and 8 October 2002, for a total of 501 water samples. Each duplicate sample was analyzed separately. Water was collected with a sampling pole as close to the water intake valve of the irrigation pump as possible to obtain water that was representative of what was being applied to crops. Samples were collected in sterile 500-ml high-density polyethylene collection bottles and transported on ice in the dark to the Laboratory Services Division of the University of Guelph for water quality testing. Water temperature was measured immediately after collection using a thermometer. If testing could not be performed on the day of collection, the water samples were stored at 4°C until testing was done. Testing was always initiated within 24 h of the time of sample collection in the field. The temperature of the irrigation water samples and evidence of precipitation in the environment surrounding the irrigation water source at the time of sample collection were recorded. Evidence of precipitation was described by the following numeric code: 0,

TABLE 1. *Characteristics of irrigation water sources sampled in study*

Source[a]	No. of samples	Description
1	30	Spring-fed pond
2	34	Creek-fed pond
3	30	Well-fed pond
4A[b]	22	Holding pond for source 4B
4B	8	Spring-fed pond
5A	34	Surface runoff-fed pond
5B	36	Spring-fed pond
6	10	Surface runoff-fed pond
7A	16	Spring-fed pond
7B	16	Spring-fed pond
8	24	Well-fed pond
9A	4	Spring, Lake Erie–fed pond
9B	4	Lake-fed pond
10A	6	Surface runoff-fed pond
10B	4	Surface runoff-fed pond
11A	24	Creek-fed pond
11B	24	Surface runoff-fed pond
12	27	Canal from Lake Simco
13A	20	Surface runoff-fed pond
13B	22	Creek-fed pond
13C	22	Surface runoff-fed pond
14	28	Spring-fed pond
15A	24	Creek-fed pond
15B	26	Creek-fed pond
16	2	Creek
17A	2	Surface runoff-fed pond
17B	2	Surface runoff-fed pond

[a] Numbers refer to farms. Letters refer to sources within a farm.
[b] This pond was lined with man-made materials.

no evidence of recent precipitation; 1, evidence of current or recent light rainfall; 2, evidence of current or recent moderate rainfall; 3, evidence of recent or current heavy rainfall.

Microbiological testing of water samples. Shortly before testing, each water sample was resuspended by shaking to evenly disperse the bacteria throughout the water in the container, and the bacteria were enumerated by filtering the water through sterile 0.45-μm-pore-size membrane filters (QA Life Sciences, Santiago, Calif.) and plating the filters on selective media. The membrane filtration method published by the U.S. Environmental Protection Agency *(6)* was used to determine both total coliforms (TC) and *E. coli* (EC) per 100 ml of water. This method utilizes 4-methylumbelliferyl-β-D-galactopyranoside indoxyl-β-D-glucuronide agar plates incubated at 35°C for 22 to 24 h. Fecal coliforms (FC) per 100 ml were enumerated using mFC agar plates incubated at 44.5°C for 18 to 22 h as previously described *(2)*. Fecal streptococci (FS) were enumerated using oxolinic acid–esculin–azide agar plates *(7)* incubated for 48 h at 42°C. Results were expressed as numbers of indicator bacteria per 100 ml.

Statistical analysis of results. From the 501 samples tested in this study, 494 TC counts, 446 FC counts, 501 EC counts, and 501 FS counts were made. Seven TC counts and 55 FC counts were excluded from the analysis because the results were difficult to interpret accurately. The seven TC counts were invalidated because the plates contained high moisture levels that prevented an accurate count of colonies. The 55 FC counts were invalidated because high levels of nonfecal coliform bacteria on these plates interfered with accurate FC counts. Twenty-six TC counts (5.3%), 29 FC counts (6.5%), and 26 FS counts (5.2%) were beyond the detection limits of the assays used. Because these detection limits were significantly higher than the median values obtained from these counts, the detection limits were used instead of numerical values for these results in statistical tests that required continuous numerical data. The data were organized into categories representing the proportion of samples with counts that fell above or below different bacterial indicator cutoff concentrations published in water quality guidelines. The cutoffs evaluated were 1,000 TC, 100 FC, 200 FC, 1,000 FC, 77 EC, 200 EC, and 20 FS per 100 ml of water.

Descriptive statistics (median, quartiles, mean, and standard deviation) characterizing each of the microbial water quality indicators were determined using the summary statistics function of Statistical Analysis Software (SAS) system for Windows, release 8.01 (SAS Institute Inc., Cary, N.C.). Pearson correlation coefficients, determined using the SAS system, were used to describe the relationships between the different microbial water quality indicator results and between indicator results and the water temperature at the time of sample collection or evidence of precipitation at the time of sample collection.

Linear regression analysis was used to determine whether there were trends evident in microbial indicator concentrations in water samples collected during the experiment. Linear regression analysis was performed using the regression function of Microsoft Excel 97 SR-2 (i) (Microsoft Corporation, Redmond, Wash.).

RESULTS AND DISCUSSION

In many microbiological surveys, the presence of pathogenic microorganisms has been demonstrated on fruits and vegetables *(8, 9)*, and numerous disease outbreaks have been linked to contaminated fruits and vegetables *(8, 9, 16, 18)*. These outbreaks emphasize the impact that contaminated produce can have on human health. The risk of disease transmission is increased when fruits and vegetables are consumed raw. Fruits and vegetables may become contaminated with pathogenic microorganisms by contact with soil or improperly composted manure, by irrigation with contaminated water, by postharvest washing with contaminated water, or by contact with infected food handlers *(10)*. In experimental studies on contamination of lettuce with *E. coli* O157:H7, irrigation water effectively transmitted *E. coli* to lettuce plants, which emphasizes the importance of using good quality irrigation water for ready-to-eat crops *(19, 21)*. Sources of irrigation water include groundwater, surface water, and human wastewater. Groundwater is located in aquifers beneath the earth's surface. Surface water includes various freshwater sources, such as ponds, lakes, rivers, and creeks. Wastewater refers to human sewage, which is commonly used for irrigation in countries where water is limited.

Pathogenic microorganisms in irrigation water are closely associated with fecal contamination. Fecal contamination is commonly detected by the use of water quality indicators. In this study, four types of microbial quality indicator bacteria were examined: total coliforms, fecal coliforms, *E. coli*, and fecal streptococci (enterococci). Total coliforms are a heterogeneous group of bacteria that are used to evaluate the general hygiene level of water but are not directly related to fecal contamination. Fecal coliforms

TABLE 2. *Descriptive statistics for microbial water quality indicator results, water temperature at time of collection, and evidence of precipitation at time of collection for each water sample*

Statistic	TC/100 ml	FC/100 ml	EC/100 ml	FS/100 ml	Water temperature (°C)	Precipitation[a]
n	494	446	501	501	456	493
Range	29–50,000	0–7,000	0–4,500	0–156	12–31	0–3
25% quantile	2,325	7	3	0	18	0
50% quantile (median)	3,000	33	15	1	22	0
75% quantile	8,500	100	46	9	24	1
Mean	8,559	162	105	14	21	0.6
Standard deviation	12,730	545	395	29	4	1.0

[a] Precipitation was scored on a scale of 0 (none) to 3 (recent or current heavy rain).

are a subset of coliform bacteria that are used to estimate the concentration of *E. coli* present in water samples. *E. coli* is a thermotolerant coliform bacterium that is closely associated with fecal contamination of water. Although *E. coli* is now considered a better indicator of fecal contamination than fecal coliforms *(15),* this bacterium is listed in most water quality guidelines and was included in this study for this reason. Fecal streptococci (enterococci) were included because these bacteria survive longer in nature than do other bacterial fecal indicators *(1)* and because a direct relationship between fecal streptococci concentrations in beach waters and incidence of gastroenteritis in swimmers has been reported *(12).*

Several guidelines have been published that include acceptable concentrations of these indicator bacteria for irrigation water in Canada. The *Canadian Water Quality Guidelines for the Protection of Agricultural Water Uses* published by the Canadian Council of Ministers of the Environment (CCME) *(5)* are widely accepted, although province-specific guidelines are also available *(1, 3–5, 23).* The CCME guidelines and the provincial guidelines from Saskatchewan and Alberta are the most restrictive, calling for 1,000 or fewer total coliforms per 100 ml and 100 or fewer fecal coliforms (*E. coli*) per 100 ml of irrigation water, whereas provincial guidelines of Manitoba and British Columbia allow from 200 to 1,000 fecal coliforms per 100 ml in water used for irrigation. Several of these guidelines recommend testing multiple water samples over a period of time to obtain a better representation of water quality.

Discrepancies in guidelines among jurisdictions reflect a lack of understanding of the actual risk of disease transmission from foodborne pathogens present in irrigation water. For a foodborne pathogen in irrigation water to cause disease in humans, the pathogen must survive in the water and/or soil, be transferred effectively to an edible portion of the plant, survive on the plant until harvest, adhere to the plant during postharvest washing, survive food-processing steps, and be consumed by a susceptible human. Foods that are consumed with minimal washing or processing, such as salad crops and strawberries, represent a higher risk of disease transmission from pathogens present on their surfaces at harvest. Many guidelines, such as British Columbia's *Water Quality Criteria for Microbiological Indicators (1),* set lower cutoffs for irrigation of crops consumed raw than for irrigation of other crops.

Descriptive statistics for microbial indicators, water temperature, and evidence of precipitation at the time of collection for all water samples tested in this study are summarized in Table 2. The counts of total coliforms, fecal coliforms, and *E. coli* were quite variable, displaying wide ranges and high standard deviations, whereas fecal streptococci counts were less variable. All of the microbial indicator summaries revealed positive skewness, indicating that these results had asymmetric distributions with an asymmetric tail tending toward more positive values. This skewness of distribution suggests that the median is a better measure of typical results than the mean and that multiple water samples should be tested from each irrigation water source over a time to obtain a more accurate representation of water quality.

Pearson correlation coefficients describing the relationships between microbial indicators, water temperature, and evidence of precipitation at the time of sampling are summarized in Table 3. Positive coefficients were obtained for the relationships between the presence of each of the different microbial indicators (0.1368 to 0.7145) and for the relationships between the presence of either total coliforms (0.1759) or fecal streptococci (0.2386) and evidence of recent precipitation. All of these associations were significant ($P < 0.0001$). The positive Pearson correlation coefficient (0.7146) between counts of *E. coli* and those of fecal coliforms in this study suggested that either fecal coliforms or *E. coli* could be used to measure fecal contamination. Because fecal coliform counts enumerate bacteria other than *E. coli,* cutoffs for *E. coli* should be somewhat lower than those for fecal coliforms in water quality guidelines *(1).* The associations observed between evidence of precipitation and counts of total coliforms or fecal streptococci suggested that rainfall increased the concentrations of these indicator bacteria in water, but other factors such as water temperature or environmental conditions may have influenced these results. There was a significant negative Pearson correlation coefficient (-0.2021, $P < 0.0001$) between evidence of precipitation and the water temperature of the samples at the time of collection.

The proportions of irrigation water samples deemed acceptable by different water quality guidelines are summarized in Figure 1. Between 70.6 and 96.4% of irrigation water samples were acceptable for fecal coliform bacteria, and between 78.0 and 98.2% of irrigation water samples

TABLE 3. *Pearson correlation coefficients between microbial indicator, sample water temperature, and evidence of precipitation at time of sample collection*[a]

	TC/100 ml (n = 494)	FC/100 ml (n = 446)	EC/100 ml (n = 501)	FS/100 ml (n = 501)	Water temperature (n = 456)	Precipitation (n = 493)
TC/100 ml	1.0000					
FC/100 ml	0.21438 (<0.0001)	1.0000				
EC/100 ml	0.28996 (<0.0001)	0.71458 (<0.0001)	1.0000			
FS/100 ml	0.26518 (<0.0001)	0.13679 (0.0038)	0.20133 (<0.0001)	1.0000		
Water temperature	0.02561 (0.5863)	0.01014 (0.8388)	−0.01169 (0.8033)	−0.12364 (0.0082)	1.0000	
Precipitation	0.17593 <0.0001	−0.04541 90.3431	0.04867 0.2808	0.23860 <0.0001	−0.20208 <0.0001	1.0000

[a] *P*-values are given in parentheses.

were acceptable for *E. coli.* These values include 70.6 and 83.43% of samples that were acceptable according to CCME guidelines for fecal coliforms and *E. coli,* respectively. For fecal streptococci, 81.0% of samples were acceptable at 20 bacteria per 100 ml. In contrast, only 8.10% of irrigation water samples were deemed acceptable by the CCME criterion of 1,000 total coliforms per 100 ml *(5).* The high proportion of samples exceeding the CCME total coliform cutoff may have been due in part to climate conditions during the study. Higher than average temperatures and lower levels of precipitation were observed on the farms in the study during this summer, reducing water levels in many of the irrigation water sources (climate data for 2002 is summarized on Environment Canada's web site: http://www.msc-smc.ec.gc.ca/ccrm/bulletin/annual02/national_e.cfm). These low water levels resulted in high levels of sediment from the bottom of irrigation water storage ponds; the sediment was collected along with the water samples and may have increased the concentration of total coliforms in the water samples. The warm temperatures also may have promoted reproduction of coliform bacteria, which unlike fecal coliforms and *E. coli* will reproduce in the environment *(13).* Although total coliform counts provide a measure of water quality, they are not necessarily indicative of fecal contamination *(14).*

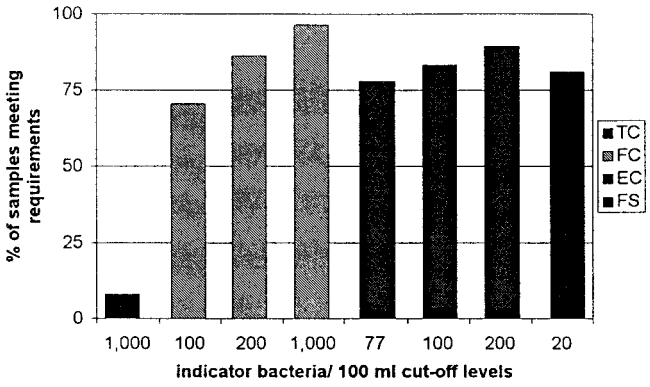

FIGURE 1. *Proportions of irrigation water samples that were deemed acceptable by different water quality cutoffs.*

Of the 27 irrigation water sources sampled, 20 sources were tested at least five times over at least 30 days, fulfilling the criteria for British Columbia's water quality guidelines. Sample means from these 20 irrigation water sources were evaluated in comparison with these standards. For irrigation of crops eaten raw, 12 (60%), 11 (55%), and 14 (70%) of the 20 irrigation water sources contained water of acceptable quality according to fecal coliform counts, *E. coli* counts, and fecal streptococci counts, respectively. For irrigation of areas open to the public or to livestock grazing, 19 (95%) and 20 (100%) of the 20 irrigation water sources contained water of acceptable quality according to *E. coli* counts and fecal streptococci counts, respectively. For general irrigation, all 20 irrigation water sources contained water of acceptable quality.

Linear regression analysis of plots of indicator bacteria concentrations versus day of the study was used to determine whether there was a strong positive linear trend of bacterial indicator concentrations over the course of the study. Only fecal streptococci concentrations displayed a positive trend, with higher counts obtained in the fall. The regression line for this plot was $y = 0.2388x - 5.1103$, where x is the day of the study on which the water sample was collected and y is the FS/100 ml concentration in the water sample. The regression lines for total coliform, fecal coliform, and *E. coli* concentrations versus day of study were $y = 4.0718x + 8232.6$, $y = -0.6127x + 209.24$, and $y = -0.5061x + 145.54$, respectively. Higher fecal streptococci counts might be attributed to surface runoff resulting from higher levels of precipitation and/or to the application of manure fertilizer to the farms in the study or to neighboring farms at this time of the year. A negative trend for water temperature versus day of the study ($y = -0.057x + 26.177$) and a positive trend in history of precipitation versus day of the study ($y = 0.0104x - 0.2047$) were also observed in the fall months at the end of the study.

The microbial quality of the majority of irrigation water samples from the fruit and vegetable farms tested was generally acceptable for fecal coliforms, *E. coli,* and fecal streptococci, but many samples exceeded the recommended

levels for total coliform bacteria. A high proportion of these irrigation water samples (45%) were not acceptable for irrigation of crops consumed raw according to British Columbia's *Water Quality Criteria for Microbial Indicators.* These results suggest that water used to irrigate crops consumed without cooking should be tested regularly to ensure that it is of acceptable microbial quality and that this water should be treated if the water quality is poor. Close correlations were observed between the different microbial water quality indicators, suggesting that one indicator, such as *E. coli*, might be sufficient for testing water quality. Variation in the concentrations of fecal indicators indicated that testing of multiple samples of irrigation water over the course of the season is advisable.

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