Microbiological Water Quality in Relation to Water-Contact Recreation, Cuyahoga River, Cuyahoga Valley National Park, Ohio, 2000 and 2002

By Rebecca N. Bushon and G.F. Koltun

In collaboration with the National Park Service, Cuyahoga Valley National Park

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Conversion Factors and Abbreviated Water-Quality Units

Multiply	By	To obtain
Length		
inch (in.)	25.4	millimeter (mm)
foot (ft)	0.3048	meter (m)
mile (mi)	1.609	kilometer (km)
Area		
square mile (mi^2)	2.590	square kilometer (km^2)
Volume		
ounce, fluid (fl. oz)	0.02957	liter (L)
gallon (gal)	3.785	liter (L)
cubic inch (in^3)	0.01639	liter (L)
Flow rate		
cubic foot per second (ft^3/s)	0.02832	cubic meter per second (m^3/s)
million gallons per day (Mgal/d)	0.04381	cubic meter per second (m^3/s)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:

$$°F = (1.8 \times °C) + 32$$

Temperature in degrees Fahrenheit (°F) may be converted to degrees Celsius (°C) as follows:

$$°C = (°F - 32) / 1.8$$

Specific conductance is given in microsiemens per centimeter at 25 degrees Celsius (μS/cm at 25°C).

Turbidity is given in Nephelometric Turbidity Units (NTU).

Concentrations of chemical constituents in water are given either in milligrams per liter (mg/L) or micrograms per liter (μg/L).

Concentrations of bacteria in water are given in colonies per 100 milliliters (col/100 mL).

Concentrations of coliphage in water are given in plaques per 100 milliliters (plaques/100 mL).

Concentrations of infectious enteroviruses in water are given in most probable number per 100 liters (MPN/100 L).

Concentrations of Cryptosporidium in water are given in oocysts per 10 liters (oocysts/10 L).

Concentrations of Giardia in water are given in cysts per 10 liters (cysts/10 L).

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Abstract

The microbiological water quality of a 23-mile segment of the Cuyahoga River within the Cuyahoga Valley National Park was examined in this study. This segment of the river receives discharges of contaminated water from stormwater, combined-sewer overflows, and incompletely disinfected wastewater. Frequent exceedances of Ohio microbiological water-quality standards result in a health risk to the public who use the river for water-contact recreation.

Water samples were collected during the recreational season of May through October at four sites on the Cuyahoga River in 2000, at three sites on the river in 2002, and from the effluent of the Akron Water Pollution Control Station (WPCS) both years. The samples were collected over a similar range in streamflow in 2000 and 2002. Samples were analyzed for physical and chemical constituents, as well as the following microbiological indicators and pathogenic organisms: *Escherichia coli (E. coli), Salmonella,* F-specific and somatic coliphage, enterovirus, infectious enterovirus, hepatitis A virus, *Clostridium perfringens (C. perfringens), Cryptosporidium,* and *Giardia*. The relations of the microorganisms to each other and to selected water-quality measures were examined.

All microorganisms analyzed for, except *Cryptosporidium*, were detected at least once at each sampling site. Concentrations of *E. coli* exceeded the Ohio primary-contact recreational standard (298 colonies per 100 milliliters) in approximately 87 percent of the river samples and generally were higher in the river samples than in the effluent samples. *C. perfringens* concentrations were positively and significantly correlated with *E. coli* concentrations in the river samples and generally were higher in the effluent samples than in the river samples.

Several of the river samples that met the Ohio *E. coli* secondary-contact recreational standard (576 colonies per 100 milliliters) had detections of enterovirus, infectious enterovirus, hepatitis A virus, and *Salmonella*, indicating that there are still risks even when the *E. coli* standard is not exceeded. River samples in which the secondary-contact recreational standard for *E. coli* was exceeded showed a higher percentage of the co-occurrence of pathogenic organisms than samples that met the standard. This indicates that in this study area, *E. coli* is a useful indicator of human health risk.

Detections of hepatitis A virus tended to be associated with higher median concentrations of somatic coliphage, F-specific coliphage, and infectious enterovirus. In addition, geometric mean *C. perfringens* concentrations tended to be higher in samples where hepatitis A virus was present than in samples where hepatitis A virus was absent. Hepatitis A virus was not detected in samples collected upstream from the Akron WPCS; all downstream detections had coincident detections in the Akron WPCS effluent, suggesting that Akron WPCS was a principal source of hepatitis A virus at the downstream sites.

Geometric mean concentrations of *E. coli* were calculated on the basis of analytical results from at least five samples collected at each river site during May, July, and September of 2000. In each case, the Ohio geometric-mean primary-contact recreational standard of 126 col/100 mL was exceeded.

E. coli concentrations were significantly correlated with streamflow and increased with streamflow at sites upstream and downstream from the Akron WPCS. This indicates that *E. coli* loads from sources upstream from the Akron WPCS have the potential to appreciably influence the frequency of attainment of recreational water-quality standards at downstream locations.

Introduction

A 23-mile segment of the Cuyahoga River that flows through the Cuyahoga Valley National Park (CVNP) receives discharges of stormwater, combined-sewer overflows, and incompletely disinfected wastewater from urban areas. These discharges can result in health risks to people who use the river for water-contact recreation. *Escherichia coli (E. coli)* frequently is found in the Cuyahoga River at concentrations that exceed recommended maximum levels for water-contact recreation. When present in water, *E. coli* is an indicator of contamination from human and (or) animal feces. *E. coli* concentration has been shown to be a reliable factor in explaining rates of gastrointestinal illness in swimmers exposed to contaminated water (U.S. Environmental Protection Agency, 1986) and is an indicator of the risk of exposure to other pathogenic organisms. Elevated levels of fecal-indicator organisms (such as *E. coli*) are cause for concern in any river but are of particular concern for the Cuyahoga River because of the CVNP and its goal of promoting water-contact recreation.

A considerable amount of information is available on concentrations of *E. coli* and selected other indicator organisms in the Cuyahoga River within the CVNP; however, very little is known about the co-occurrence of waterborne pathogens and how they relate to concentrations of the indicator organisms. Information on the relation of indicator organisms and other environmental factors to the occurrence of waterborne pathogens within the Cuyahoga River is needed for a better understanding of the human-health risk associated with water-contact recreation; such information can aid park managers in formulating recommendations for recreational use of the Cuyahoga River. To meet these information needs, the U.S. Geological Survey (USGS), in collaboration with the National Park Service, conducted an in-depth study to investigate microbiological water quality of the Cuyahoga River within the CVNP.

Purpose and Scope

This report describes the results of a study to (1) characterize the occurrence and distribution of selected microbiological pathogens and indicator organisms in the Cuyahoga River within the CVNP, (2) examine the relations of microorganisms to each other and to selected hydrologic and water-quality measures, and (3) provide general information on the occurrence of waterborne pathogens in relation to *E. coli*-based recreational-use water-quality standards. Data used in this study were collected during the May through October recreational seasons in 2000 and 2002 and consisted of analytical results for water samples collected from four sites in 2000 and three sites in 2002 on the Cuyahoga River within the CVNP and from Akron Water Pollution Control Station (WPCS) effluent. Samples collected during the 2000 recreational season were analyzed for concentrations and (or) the presence/absence of *E. coli, Salmonella*, F-specific coliphage, enterovirus, infec-

tious enterovirus, hepatitis A virus, and selected chemical constituents. Samples collected during the 2002 recreational season were analyzed for concentrations and (or) the presence/absence of *E. coli, Salmonella*, F-specific and somatic coliphage, enterovirus, infectious enterovirus, hepatitis A virus, *Clostridium perfringens (C. perfringens), Cryptosporidium, Giardia,* and selected chemical constituents.

Description of Study Area

The study area consists of a 23-mi segment of the Cuyahoga River, in northeastern Ohio, within the CVNP (fig. 1). The 51.6-mi^2 CVNP, administered by the National Park Service (NPS), was established in 1974 to preserve and protect the natural and recreational values of the Cuyahoga River and adjacent lands (National Park Service, 2003a). According to the National Park Service (2003a), about 3.5 million people visit the park annually; attendance peaks during the May–October recreational season.

The area surrounding the CVNP is predominantly urban and includes two nearby major metropolitan areas—Akron, which is upstream from the study area, and Cleveland, which is downstream from the study area. Most of the undeveloped land near the study area is confined to areas of steep terrain along the Cuyahoga River and tributaries draining into it (Schiefer, 2002). A detailed description of the Cuyahoga River and basin can be found in Myers and others (1998).

The Ohio Environmental Protection Agency (Ohio EPA) has designated the segment of the Cuyahoga River within the CVNP as a warm-water habitat and assigned a recreational-use designation of primary-contact recreation. Primary-contact waters are classified as suitable for full-body contact such as swimming, canoeing, and scuba diving; however, because of poor water quality, these recreation activities are discouraged by the NPS, especially after periods of heavy precipitation (National Park Service, 2003b).

Water quality of the Cuyahoga River within the CVNP is affected during wet weather by discharges of partially treated domestic sewage, intermittent discharges of storm water, and combined-sewer overflows (CSOs). Water quality also is affected by continuous discharge of treated municipal wastewater from the Akron WPCS and other upstream facilities. During the study period, the average daily effluent flow from the Akron WPCS was approximately 68 Mgal/d, and the maximum daily effluent flow was 207 Mgal/d (Donald Calvert, City of Akron, written commun., 2003). Effluent flows less than or equal to 110 Mgal/d receive secondary treatment, whereas wet-weather flows in excess of 110 Mgal/d receive only partial treatment (City of Akron, 2003). Peak effluent flow at the Akron WPCS can reach about 280 Mgal/d during wet weather (City of Akron, 2003).
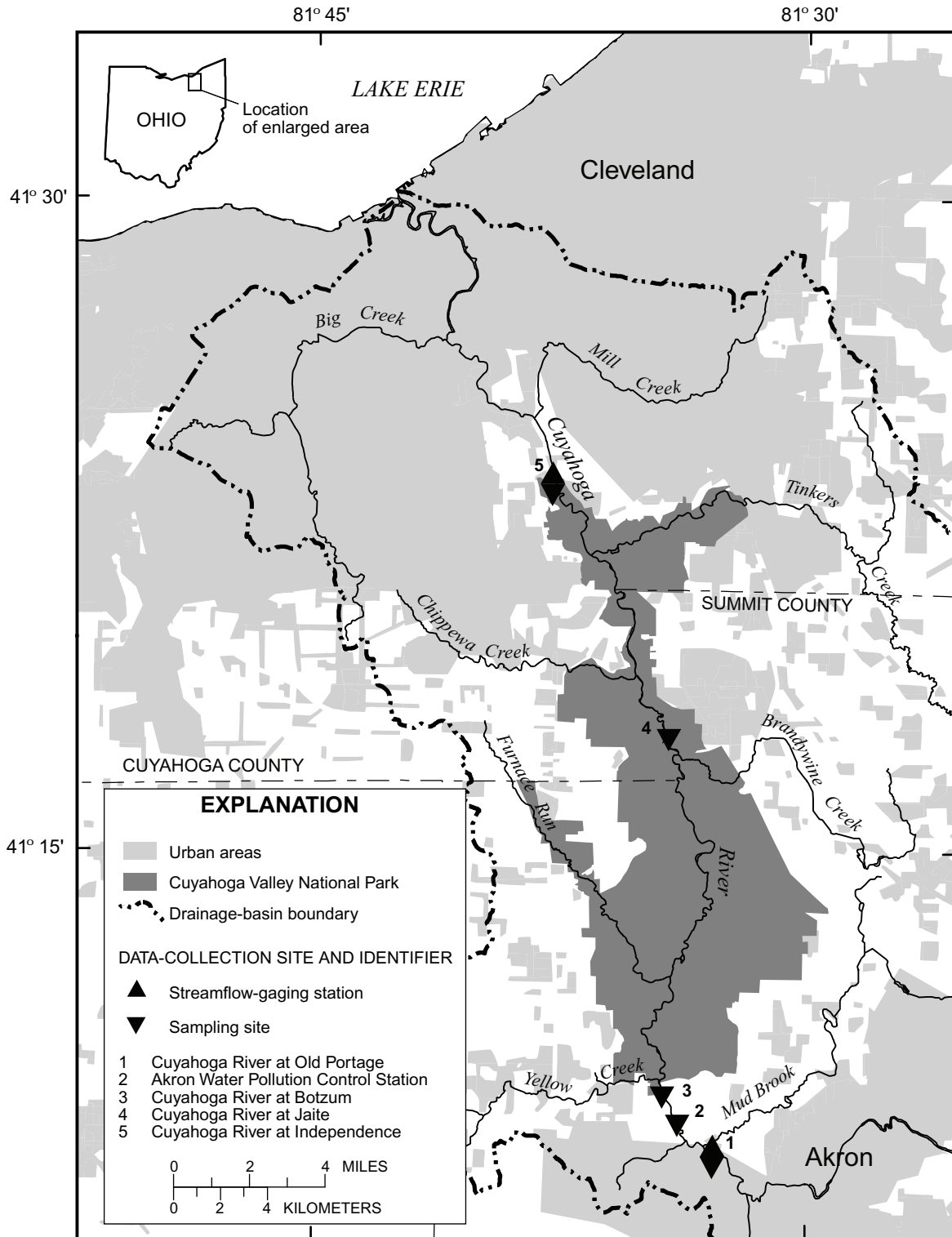
Figure 1.   Location of the study area in northeastern Ohio.

Previous Studies

The microbiological and chemical water quality and hydrology of the Cuyahoga River and tributaries have been described in previous reports (Childress 1984, 1985; Ohio Environmental Protection Agency, 1994, 1999; Francy and others, 1996; Myers and others, 1998). Concentrations of fecal bacteria that exceed Ohio's bathing-water, primary-contact, and secondary-contact water-quality standards are well documented in the lower 50 mi of the Cuyahoga River (Shindel and others, 1992, 1993; Shindel and others, 1994; Ohio Environmental Protection Agency, 1994, 1999, 2003a). During dry-weather periods investigated in 1992, the river typically met Ohio's geometric-mean primary-contact recreational standard for fecal coliform bacteria (Ohio Environmental Protection Agency, 1994). In an earlier study, Childress (1985) documented elevated concentrations of fecal coliform bacteria in a reconnaissance during summer low-flow conditions in 1982. Fecal coliform concentrations ranged from 38 to 1,900,000 col/100 mL.

The entire reach of the Cuyahoga river was assessed for water-quality impairment by the Ohio EPA as part of their statewide activities in 1991 (Ohio Environmental Protection Agency, 1994) and 1996 (Ohio Environmental Protection Agency, 1999). Improvements observed in microbiological water quality were attributed to improved sewage treatment and disinfection (Ohio Environmental Protection Agency, 1994). Biological and water-quality conditions in the Cuyahoga River reflected minimal change in 1996 (Ohio Environmental Protection Agency, 1999). The Ohio EPA identified the lower 50 mi. of the Cuyahoga River, which includes the study area, as a priority impaired water on the 1998 and 2002 Section 303(d) lists required by the Clean Water Act. The Ohio EPA lists organic enrichment, nutrient enrichment, low instream dissolved oxygen, toxicity, sedimentation, and habitat degradation as the primary causes of impairments and lists point sources, nonpoint sources, and CSOs as sources of impairment (Ohio Environmental Protection Agency, 2003a).

During USGS field studies in 1991–93, the highest concentration of *E. coli* detected in the Cuyahoga River at the upstream boundary of the CVNP (Francy and others, 1993; Myers and others, 1998) was 2,400,000 col/100 mL. That sample concentration exceeded Ohio's single-sample primary-contact recreational standard (298 col/100 mL) by a factor of more than 8,000. The Akron WPCS was identified as the largest source of fecal bacteria to the Cuyahoga River within the CVNP during runoff periods when large volumes of influent result in inadequately disinfected effluent (Myers and others, 1998). The next largest source of fecal bacteria was uncontrolled combined and sanitary sewers that also discharged to the river upstream from the park.

Acknowledgments

Overview of Microbiological Indicators and Pathogens

The presence of pathogenic microorganisms in recreational waters is of great concern. Monitoring the waters for specific pathogens would be ideal but, in practice, is unrealistic because of constraints of time, cost, available methods, laboratory capabilities, and the multitude of pathogens involved in waterborne-disease outbreaks. Instead, indicator organisms traditionally have been used to assess the microbiological quality of water and indicate the potential for pathogens to be present. Fecal-indicator organisms are excreted in large numbers by humans and warmblooded animals; therefore, the concentrations of indicator organisms in fecally contaminated water generally are much higher and more consistent than those of pathogens (Moe, 2002). The numbers of pathogens, which are excreted only by infected individuals, depend on the excretion level and the number of infected individuals in the area. Relative to methods for detecting pathogens, methods to detect indicator organisms are generally much easier and less costly to perform, are easily adaptable by most laboratories, and produce results that are available more quickly. This overview section of the report discusses indicator organisms and pathogens of interest to this study and makes brief mention of analytical approaches for each.

In 1986, the U.S. Environmental Protection Agency (USEPA) recommended the use of *E. coli* as a bacterial indicator organism for monitoring microbiological water quality in freshwater. This recommendation was based on epidemiological studies that showed a strong positive correlation between *E. coli* and the occurrence of swimming-associated gastrointestinal illness (U.S. Environmental Protection Agency, 1986). The Ohio water-quality standards for *E. coli* in recreational waters are listed in table 1 (Ohio Environmental Protection

Table 1.   Ohio water-quality standards for *Escherichia coli* in recreational waters.

[Effective from May 1 through October 15. All values are in colonies per 100 milliliters; na, not applicable. Source: Ohio Environmental Protection Agency, 2003b]

Type of standard	Type of recreational water		
	Bathing waters[a]	Primary contact[b]	Secondary contact[c]
Geometric mean[d]	126	126	na
Single sample[e]	235	298	576

  [a] Bathing waters are suitable for swimming and other full-body-contact exposure where a lifeguard or bathhouse is present.

  [b] Primary-contact waters are suitable for full-body contact, such as swimming, canoeing, and scuba diving.

  [c] Secondary-contact waters are suitable for partial-body contact, such as wading.

  [d] The geometric mean is based on a minimum of five samples in a 30-day period.

  [e] This value cannot be exceeded in more than 10 percent of the samples collected in a 30-day period.

Agency, 2003b). Relative to many viral and protozoan pathogens, *E. coli* has a shorter survival time in water and a greater susceptibility to water-treatment processes. As a consequence, *E. coli* tends to be a poor indicator for viral and protozoan pathogens (Moe, 2002). Other microorganisms, including coliphages and *C. perfringens,* are being investigated as alternative indicators that may better model the survival and disinfection resistance of viruses and protozoa (Moe, 2002).

Coliphages currently are used as indicators of fecal contamination and of the microbiological quality of water. Coliphages are viruses that infect and replicate in coliform bacteria. They are similar to pathogenic human enteric viruses in size and shape, transport characteristics, and resistance to disinfection processes. F-specific and somatic coliphages are two main groups of coliphages used as indicator organisms. They differ in how they infect the coliform bacteria. F-specific coliphage infect the bacteria by attaching to hairlike projections called F pili, and somatic coliphages infect the bacteria by attaching to the outer cell wall. Both groups of coliphage are found in high numbers in sewage and are thought to be reliable indicators of sewage contamination of waters (International Association of Water Pollution Research and Control Study Group on Health Related Microbiology, 1991). Raw sewage typically contains F-specific and somatic coliphage concentrations of about 1,000 plaques per milliliter (Sobsey and others, 1995). A single-agar layer (SAL) direct plating method can be used to enumerate F-specific and somatic coliphage in water samples (Ijzerman and Hagedorn, 1992). Results from this method are available in 24 hours.

C. perfringens has been proposed as an indicator of the presence and density of pathogenic viruses and other stress-resistant microorganisms, such as *Cryptosporidium* and *Giardia* (U.S. Environmental Protection Agency, 1996). *C. perfringens* is a bacterium of fecal origin that is consistently associated with human waste and sewage (Bisson and Cabelli, 1980; Fujioka and Shizumura, 1985). It produces spores that are resistant to disinfection practices and environmental stresses; it is an indicator of present contamination, as well as a conservative tracer of past fecal contamination (U.S. Environmental Protection Agency, 1996). The method for detecting *C. perfringens* is a standard membrane filtration procedure that is similar to that for *E. coli* and produces results in 24 hours. The method requires overnight incubation in an anaerobic environment.

In addition to the organisms discussed previously, samples collected for this study were analyzed for selected pathogens that are of concern in recreational waters. Concentrations of infectious enterovirus, *Cryptosporidium*, and *Giardia* were determined, as was the presence or absence of *Salmonella*, enterovirus, and hepatitis A virus. The microorganisms analyzed for and the methods used in this study are summarized in table 2.

Salmonella continues to be one of the main causes of waterborne illness worldwide (Arvanitidou and others, 1995). Every year in the United States, there are approximately 800,000 to 4 million cases of salmonellosis, which result in

Table 2.   Microorganisms studied and methods used for analyses.

[USGS, U.S. Geological Survey; ODML, Ohio District Microbiological Laboratory; WPCS, Water Pollution Control Station; mL, milliliters; USEPA, U.S. Environmental Protection Agency; APHA, American Public Health Association; ICC-PCR, integrated cell culture-polymerase chain reaction; EHL, Environmental Health Laboratories; MPN, most probable number; L, liters; RT-PCR, reverse transcriptase-polymerase chain reaction]

Microorganism	Method	Analytical laboratory	Volume analyzed	Reporting units	Reference
Escherichia coli	Membrane filtration on mTEC agar	USGS ODML / Akron WPCS	0.1 to 30 mL	Colonies per 100 mL	USEPA, 1985
Salmonella	Enrichment	USGS National Wildlife Health Center	100 mL	Presence or absence per 100 mL	APHA and others, 1998
F-specific and somatic coliphage	Single-agar layer	USGS ODML	100 mL	Plaques per 100 mL	Ijzerman and Hagedorn, 1992
Infectious enterovirus	ICC-PCR	EHL	100 L	MPN per 100 L	Reynolds and others, 1996
Enterovirus	RT-PCR	USGS ODML	100 L	Presence or absence per 100 L	Fout and others, 2003
Hepatitis A virus	RT-PCR	USGS ODML	100 L	Presence or absence per 100 L	Fout and others, 2003
Clostridium perfringens	Membrane filtration on mCP agar	USGS ODML	0.1 to 30 mL	Colonies per 100 mL	USEPA, 1996
Cryptosporidium	USEPA Method 1623	EHL	10 L	Oocysts per 10 L	USEPA, 2001
Giardia	USEPA Method 1623	EHL	10 L	Cysts per 10 L	USEPA, 2001

about 500 deaths (Centers for Disease Control and Prevention, 1999). In Ohio, occurrence of salmonellosis increases slightly during midsummer, with most cases being reported in children under 5 years, adults 20–39 years, and adults over 60 years; however, all ages are at risk (Ohio Department of Health, 1993b). Humans can acquire *Salmonella* from the ingestion of contaminated food or water or by direct contact with infected animals. Symptoms of salmonellosis include acute gastrointestinal illness, characterized by headache, diarrhea, fever, and vomiting. Infection can progress from gastrointestinal illness to septicemia or a focal infection; for example, meningitis (Ohio Department of Health, 1993b). One method for determining the presence or absence of *Salmonella* involves concentration by membrane filtration, selective enrichment in a growth medium, screening for morphological and biochemical characteristics, and serotyping (American Public Health Association and others, 1998, p. 9–22 to 9–25).

More than 100 types of human pathogenic enteric viruses may be present in fecal-contaminated waters (Havelaar and others, 1993). Viruses generally are more persistent in the environment than bacteria and are not removed completely by common treatment processes. The enteric viruses examined in this study were enterovirus and hepatitis A virus. Enteroviruses account for an estimated 10–15 million symptomatic infections in the United States each year (Centers for Disease Control and Prevention, 2000). These viruses can cause a variety of illnesses ranging from gastroenteritis to myocarditis and aseptic meningitis (Melnick, 1990). Enteroviruses in the environment pose a health risk because they are transmitted through the fecal-oral route through contaminated water, and low numbers are able to initiate an infection in humans (Abbaszadegan and others, 1993).

Hepatitis A occurs worldwide with a higher prevalence in developing nations, where sanitary conditions are poor. In the United States, 17,147 cases of hepatitis A were reported to the Centers for Disease Control and Prevention in 1999 (Centers for Disease Control and Prevention, 2003b). Ohio reports approximately 800 cases of the disease each year (Ohio Department of Health, 2000). The hepatitis A virus is transmitted by the fecal-oral route. Symptoms can include

fever, malaise, anorexia, nausea, abdominal discomfort, and jaundice.

Several methods are available for examining water for enteric virus contamination, all with limitations. Two of the most common methods are cell culture and reverse transcriptase-polymerase chain reaction (RT-PCR). The cell culture method provides information on the infectivity of the viruses, but it is expensive and time consuming, requiring several weeks for confirmed positive results. Not all enteric viruses of public-health concern can be detected by this method, and specific strains of viruses in the water samples cannot be determined. Use of this method can be further complicated by the presence of toxic substances in the sample, which could interfere with the analysis. The RT-PCR method is more rapid and less expensive than the cell culture method and allows for detection of the small numbers of viruses usually found in environmental samples. This method can determine specific virus strains and can detect viruses that are unable to grow in cell culture. The disadvantages to the RT-PCR method are the inability to determine the infectivity of the viruses (the viruses do not have to be intact to be detected) and the sensitivity to inhibitory compounds that may be present in the sample and would prevent completion of the analysis.

An integrated cell culture-PCR (ICC-PCR) method can be used to determine concentrations of infectious enteroviruses. The ICC-PCR method combines the cell culture and RT-PCR methods to reduce the effects of toxic compounds on cell culture and inhibitory compounds on PCR. This method increases the sample volume examined and the chance for a more rapid detection of infectious viruses compared to the cell culture method (Reynolds and others, 1996).

Protozoan pathogens are distributed widely in the aquatic environment and have been implicated in several outbreaks of waterborne disease (Lee and others, 2002; Rose and others, 1997). The principal protozoan pathogens that affect the public health acceptability of waters in the United States are *Cryptosporidium* and *Giardia*. Both organisms produce environmentally resistant forms, which facilitate their extended survival in water. These pathogens are transmitted through the fecal-oral route. The infectious dose for both organisms is very small, at approximately 10 organisms.

Cryptosporidiosis and giardiasis occur worldwide. In the United States, 3,785 cases of cryptosporidiosis were reported to the Centers for Disease Control and Prevention in 2001 (Centers for Disease Control and Prevention, 2003a). Symptoms of cryptosporidiosis include diarrhea, stomach cramps, and fever. In Ohio, there is a seasonal peak of giardiasis in late summer, with most cases occurring in children under 5 years and in adults 30–39 years; however, all ages are at risk (Ohio Department of Health, 1993a). Symptoms include chronic diarrhea, cramps, bloating, and weight loss. USEPA Method 1623 is used to detect *Cryptosporidium* and *Giardia* in water by filtration, immunomagnetic separation (IMS), and immunofluorescence assay (FA) microscopy.

Methods of Study

Samples were collected at five sites in 2000: (1) Cuyahoga River upstream from the CVNP at Old Portage (site 1 in fig. 1), (2) effluent from the Akron WPCS (site 2 in fig. 1), (3) Cuyahoga River at the upstream boundary of the CVNP at Botzum (site 3 in fig. 1), (4) Cuyahoga River midway through the CVNP at Jaite (site 4 in fig. 1), and (5) Cuyahoga River at the downstream end of the CVNP at Independence (site 5 in fig. 1). An appreciable amount of streamflow and water-quality data is available for these sites because they were sampled as part of a previous study on the Cuyahoga River (Myers and others, 1998).

Five samples were collected at each of the five sampling sites during May, July, and September of 2000 and were analyzed for *E. coli*. The presence or absence of *Salmonella* was determined in samples collected once in May and twice in July and September at each site. Water samples to determine water-quality characteristics and F-specific coliphage concentrations were collected once in May, July, and September at each site. Because of the high cost of analysis for enterovirus, infectious enterovirus, and hepatitis A virus, samples were collected at only three sites (Old Portage, the Akron WPCS, and Botzum) during the three sampling months. Samples were collected each month for suspended-sediment concentration and chemical water quality. Field measurements of temperature, pH, specific conductance, and dissolved oxygen were made each time samples were collected. Streamflow was obtained from USGS streamflow-gaging stations, where available. Where there were no daily streamflow gages, streamflow was determined from rating curves on the basis of water-level measurements made with a wire-weight gage.

In 2002, samples were collected at four of the five sampling sites; Old Portage, the Akron WPCS, Botzum, and Jaite. (One site from 2000 was dropped becaue of fiscal and logistical constraints.) Water samples were collected during three sampling periods, one in May, June, and July 2002. During each period, samples were collected for determination of the following constituents: *E. coli, Salmonella*, F-specific and somatic coliphage, enterovirus, infectious enterovirus, hepatitis A virus, *C. perfringens, Cryptosporidium, Giardia*, suspended sediment, turbidity, and chemical water quality. Field measurements also were made, and streamflow data were obtained from USGS streamflow-gaging stations where available or determined from rating curves.

Sample Collection

Stream-water samples were collected by USGS personnel by means of the equal-width-increment method described by Edwards and Glysson (1999). Samples for the analysis of *E. coli, Salmonella*, and turbidity were collected in sterile 1-L polypropylene bottles. Samples for the analysis of coliphage and *C. perfringens* were collected in sterile 3-L polypropylene bottles. Stream water to be analyzed for *Cryptosporidium* and *Giardia* was composited into a 10-L sterile cubitainer. Samples for the analysis of suspended sediment and chemical constituents were composited in a churn splitter (Wilde and Radtke, 1999).

Samples from the Akron WPCS effluent were collected in the center of the outfall using the hand-dip method described by Myers and Sylvester (1997). During the recreational season, the effluent from the Akron WPCS is dechlorinated before it is discharged; therefore, further steps to dechlorinate the samples before microbiological analysis were not necessary.

A portable virus-sampling apparatus designed by USGS engineers was used to sample for enteric viruses. The sampler design was based on the protocol described in the USEPA Information Collection Rule (U.S. Environmental Protection Agency, 1996). The apparatus contained a submersible pump, pressure regulator, cartridge housing containing a 1MDS filter (Cuno Inc., Meriden, Conn.), and discharge line. Samples analyzed for enteric viruses were collected by inserting a sterile 1MDS filter into the cartridge housing of the virus sampler and pumping 100 L of water through the filter.

Because of the complexity of the virus-sampling apparatus, stringent disinfection protocols were established. Before sampling and after each use, the sampling equipment was cleaned with a dilute, nonphosphate, laboratory-grade detergent and then sterilized with a 10 percent household bleach (0.5 percent sodium hypochlorite) solution. The sampler was rinsed with a 0.2 percent sodium thiosulfate solution to remove residual chlorine and then was rinsed with sterile deionized water.

Physical Characteristics and Chemical Constituents

Water and air temperature, pH, specific conductance, and dissolved oxygen were measured at each site when samples were collected. Water-quality meters were calibrated daily in the field before use. Streamflow data were obtained from USGS streamflow-gaging stations where available or determined from rating curves. Instantaneous flow from the Akron WPCS (provided by the City of Akron) was based on a standard flume computation.

Turbidity was measured in the field by means of a Hach 2100P portable turbidimeter (Hach Company, Loveland, Colo.). Measurements of each sample were made in duplicate until two consecutive measured values agreed within

5 percent. Turbidimeter calibration was checked daily against turbidity standards before use in the field.

Suspended-sediment samples were sent to the Heidelberg College Water-Quality Laboratory in Tiffin, Ohio, for analysis by means of the filtration method described by Guy (1969).

Nutrient and chloride samples were processed in the field and then sent overnight on ice to the USGS National Water-Quality Laboratory (NWQL) in Lakewood, Colo. Phosphorus samples were collected in polyethylene bottles and acidified with 1 mL of 4.5 N sulfuric acid. Nitrogen and chloride samples were filtered through a 0.45-µm filter and collected in polyethylene bottles. Quality-control samples for nutrients and chloride included one field blank and one replicate for each constituent. No problems were evident from the quality-control results.

Microbiological Methods

All field and laboratory personnel were trained in sample collection and analysis procedures. Quality-assurance and quality-control procedures (Francy and others, 1998; Myers and Sylvester, 1997) were followed for all phases of the project. Unless indicated otherwise, no problems were observed in quality-control samples analyzed for microorganisms described below. The microbiological methods used in this study are summarized in table 2.

Escherichia coli

Water samples were analyzed for concentrations of *E. coli* by membrane filtration using the USEPA-recommended mTEC agar method (U.S. Environmental Protection Agency, 1985). All samples were analyzed in the field within 6 hours of collection to meet specified holding times. A range of volumes was plated to obtain at least one plate with an ideal count of 20 to 80 colonies. Plates were incubated at 35°C for 2 hours (to resuscitate injured or stressed bacteria) then transferred to 44.5°C for 20–22 hours. After incubation, filters were transferred to a pad soaked with a urea-phenol red solution. Colonies that remained yellow after 15 minutes, indicating a negative test for the urease enzyme, were counted as *E. coli*. The results were calculated as described by Myers and Sylvester (1997) and reported as colonies of *E. coli* per 100 milliliters (col/100 mL).

Quality-control samples included a field blank, filter blanks, procedure blanks, and replicates. One field blank was analyzed to assess field contamination of samples and to ensure that equipment cleaning and sterilization techniques were adequate. Filter blanks were analyzed with every water sample to measure the sterility of the equipment and supplies. Procedure blanks were analyzed with every fourth water sample to measure the effectiveness of the analyst's rinsing technique. Seventeen percent of the water samples were analyzed as nested replicates to determine sampling and analytical variability. A nested replicate consists of two water samples collected in separate bottles, each bottle plated in duplicate for concentrations of *E. coli*.

Salmonella

For *Salmonella* analyses, water samples were filtered through 0.45-μm membrane filters (Advantec, MFS, Inc., Pleasanton, Calif.) in the field within 1 hour of sample collection. In 2000, 500 mL of water was filtered; however, in 2002, only 100 mL of water was filtered. Each filter was placed in a bottle of 100 mL chilled Rappaport-Vassiliadis medium (RV) for *Salmonella* screening. The bottles were shipped overnight on ice to the USGS National Wildlife Health Center in Madison, Wis., for analysis. The bottles were incubated at 42°C for 16 to 18 hours. After incubation, an aliquot of the RV enrichment broth was transferred to XLT4 Agar (Difco Laboratories, Detroit, Mich.) and Brilliant Green Agar (Becton Dickinson, Cockeysville, Md.). Both media were incubated at 35–37°C for 18 to 24 hours. If no *Salmonella* isolates were found on the media, another aliquot of the original RV enrichment broth was added to new RV enrichment broth, and the incubation and plating processes were repeated to enhance recovery. All bacterial colonies were screened to identify *Salmonella* spp., and those matching morphological and biochemical characteristics were subcultured on 5 percent sheep blood agar (Becton Dickinson, Cockeysville, Md.). Suspected *Salmonella* isolates were biochemically characterized by either the API-20E or Vitek systems (bioMerieux, St. Louis, Mo.). Isolates yielding a *Salmonella* identification were screened using a polyvalent antisera for *Salmonella* (Becton Dickinson, Cockeysville, Md.) before being serotyped for confirmation at the U.S. Department of Agriculture National Veterinary Services Laboratory (Ames, Iowa).

Quality-control samples for *Salmonella* analyses included one field blank and one matrix spike. A matrix spike was analyzed in order to determine the effect of the matrix on method performance. The matrix spike was prepared by adding a known amount of Salmonella to a water sample in the field.

F-specific and somatic coliphage

Water samples collected for the analysis of F-specific and somatic coliphage were shipped overnight on ice to the USGS Ohio District Microbiology Laboratory (ODML) for analysis.

All coliphage samples were analyzed within the 48-hour holding time. Samples collected in 2000 were analyzed for F-specific coliphage, and samples collected in 2002 were analyzed for F-specific and somatic coliphage. Samples were analyzed using a method described by Ijzerman and Hagedorn (1992), which is based on the induction of the β-galactosidase enzyme found in *E. coli*. Antibiotics are used in this method to prevent interference due to the growth of background bacteria in the sample. Antibiotic-resistant *E. coli* host cultures are used to test for the presence of coliphage. *E. coli* F-amp, which is resistant to the antibiotics ampicillin and streptomycin, was used as a host culture for the detection of F-specific coliphage. *E. coli* CN-13, which is resistant to the antibiotic nalidixic acid, was used as a host culture for somatic coliphage. Sample volumes of 100 mL were mixed with the host culture, antibiotics, molten agar, and chemicals that react with the β-galactosidase enzyme: isopropyl-β-D-thiogalactoside (IPTG) acts as an inducer of β-galactosidase, and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) is a substrate for the enzyme. The mixtures were poured into four 150- x 15-mm sterile petri dishes and incubated for 24 hours at 35°C. If coliphage were present in the samples, the *E. coli* cells were lysed and a stable, dark blue indolyl product was released. Coliphage were easily identified and enumerated by the distinct blue circle within the viral plaque. Results were reported in plaques per 100 milliliters (plaques/100 mL).

Quality-control samples for the analysis of coliphage included positive controls, negative controls, and replicates. A positive control of dilute sewage and a negative control of sterile water were analyzed each day water samples were analyzed to ensure that the method was performing adequately, that the coliphages were correctly identified, and that there was no laboratory contamination. Nine percent of the F-specific coliphage samples and 17 percent of the somatic coliphage samples were analyzed as replicates (one sample plated in duplicate).

Enteric viruses

Cartridge housings containing the 1MDS filters were shipped overnight on ice to the ODML for initial processing. Once received, viruses that were attached to the 1MDS filter via charge interactions were eluted from the 1MDS filter using a beef extract solution at pH 9.5. The eluate was concentrated on a filter using celite at pH 4.0 and then eluted from the filter with sodium phosphate at pH 9.5. The 1MDS filter was left in beef extract solution overnight and reconcentrated the following day. The final concentrate was neutralized and frozen at -70°C to await further processing. An aliquot of each sample was shipped overnight on dry ice to Environmental Health Laboratories (EHL) (South Bend, Ind.) to be analyzed for infectious enteroviruses by the ICC-PCR method, and an aliquot was saved for the ODML to be analyzed for enteric viruses by the RT-PCR method.

Infectious enteroviruses by ICC-PCR

Water samples were analyzed for infectious enteroviruses by EHL by means of a method described by Reynolds and others (1996). For each concentrated sample, a volume of 10 mL was analyzed using 10 cell-culture flasks of Buffalo Green Monkey Kidney (BGMK) monolayer cells. The cells were incubated for 14 days at 36.5°C. Each flask was then treated as an individual sample and was subjected to RNA extraction and then RT-PCR. After RT-PCR, samples were confirmed for enterovirus by oligonucleotide probe hybridization. There was a total of 10 RT-PCR reactions per sample. The MPNV computer program (U.S. Environmental Protection Agency, 2003) was used to compute the most probable number (MPN). The inoculation volume, number of flasks, number of positive RT-PCR reactions, and dilution factors were the parameters for calculation of the MPN, which is reported as MPN/100 L.

Quality-control samples for the analysis of enteroviruses consisted of two equipment blanks, one field blank, and positive and negative controls for both the cell-culture and RT-PCR steps of the method. The equipment blanks were produced in a laboratory and were analyzed to determine potential contamination from the equipment cleaning and sterilization process. The field blank was analyzed to assess field contamination of samples and to ensure that field-equipment cleaning and sterilization techniques were adequate. For the cell-culture step, the positive control consisted of 20–200 plaques of poliovirus inoculated into one flask, and the negative control consisted of 1 mL of sterile sodium phosphate buffer inoculated into one flask. Both were processed the same as water samples. For the RT-PCR step, 1 mL of cell suspension from a positive-control cell-culture flask and viral RNA from polioviruses was used as a positive control, and 1 mL of cell suspension from a negative-control cell-culture flask and 10 µL of sterile buffer was used as a negative control. With the exception of one equipment blank, the quality-control samples for infectious enteroviruses by ICC-PCR were acceptable. A detectable level of infectious enteroviruses was observed in the first equipment blank collected in May 2000. As a result, the water samples collected in May 2000 that did not exceed the concentration of viruses measured in the equipment blank were omitted from the data set.

Enteric viruses by RT-PCR

Water samples were analyzed for enteric viruses by the ODML by means of a method described by Fout and others (2003). Instead of using the multiplex RT-PCR as described in the method (where multiple viruses are analyzed for in a single reaction), each virus was analyzed for in a separate reaction. Viruses in the eluate were concentrated by ultracentrifugation through a sucrose gradient. The concentrates were treated with a solvent mixture that was designed to remove inhibitors, such as humic substances, which can interfere with the activity of the enzymes used in the RT-PCR step. All of the target viruses are RNA viruses; therefore, the enzyme reverse transcriptase (RT) was used to transcribe the RNA into DNA. The DNA was then amplified by means of PCR. All PCR products were confirmed with nucleic acid hybridization. Initially, tests were done for five viruses; however, the results were inconclusive for reovirus, rotavirus, and Norwalk virus, so results for those viruses are not reported.

Quality-control samples for enteric virus analysis by RT-PCR included two equipment blanks, one field blank, and laboratory quality controls for the method. Appendix A contains a list and descriptions of quality-control samples for enteric virus analysis by RT-PCR.

Clostridium perfringens

Water samples to be analyzed for *C. perfringens* were shipped overnight on ice to the ODML. Concentrations of *C. perfringens* were determined by means of membrane filtration on mCP agar with some modifications (U.S. Environmental Protection Agency, 1996). The procedure for the inactivation of vegetative cells as described in the method was not followed for the analysis of these samples. All samples were analyzed within a 48-hour holding time. A range of volumes was plated to obtain at least one plate with an ideal count of 20 to 80 colonies. Plates were incubated in an anaerobic environment for 24 hours at 42°C. All straw-colored colonies that turned dark pink to magenta after exposure to ammonium hydroxide were counted as *C. perfringens*, and results were recorded as col/100 mL.

Quality control for the analysis of *C. perfringens* included positive controls, filter and procedure blanks, and replicates. A positive control of diluted sewage was analyzed each day that water samples were analyzed to ensure that the analytical method was performed adequately and that the *C. perfringens* colonies were correctly identified. Filter blanks were analyzed with each water sample. Procedure blanks were analyzed with every fourth water sample. Seventeen percent of the water samples were analyzed as replicates (one sample plated in duplicate).

Cryptosporidium and *Giardia*

Water samples were shipped overnight on ice to EHL. Filtration of the bulk 10-L samples was initiated within 48 hours of sample collection. Samples were analyzed for concentrations of *Cryptosporidium* oocysts and *Giardia* cysts by means of USEPA Method 1623 (U.S. Environmental Protection Agency, 2001), a performance-based method that requires filtration, immunomagnetic separation of the oocysts/cysts from the material captured, and an immunofluorescence assay for determination of oocyst/cyst concentrations, with confirmation through vital dye staining and differential interference contrast microscopy. The Filta-Max filtration system (IDEXX, Westbrook, Maine) was used in this study. The filter is made of open-cell foam discs that have been compressed to give a nominal porosity of 1 µm. After filtering, the foam is decompressed, enabling the captured oocysts and cysts to be recovered.
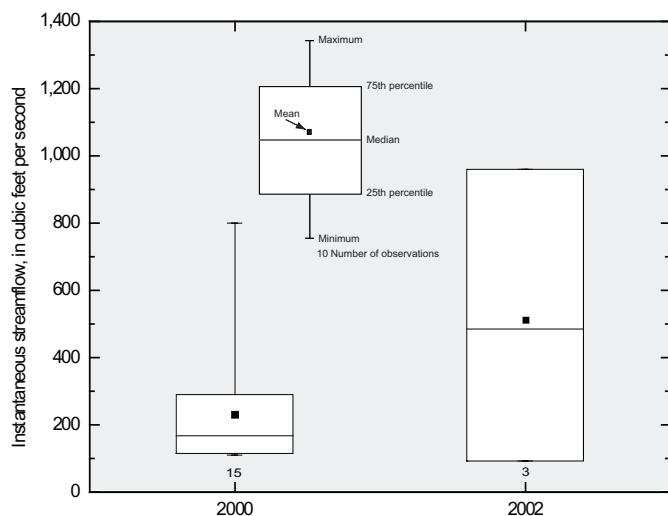
Figure 2.   Distribution of instantaneous streamflows measured in conjunction with samples collected from the Cuyahoga River at Old Portage, by calendar year.

Matrix-spike samples were collected during each sampling trip in 2002 at the Botzum site. The matrix-spike samples consisted of a second 10-L sample that was spiked with a known amount of oocysts and cysts in the laboratory. These samples were analyzed with the regular water sample to determine the effect of the water matrix on the method's recovery.

Characteristics of Streamflows during Sampling Periods

Streamflow was measured by means of standard USGS techniques (Rantz and others, 1982) at the times when nearly all microbiological samples were collected. More than 80 percent of the samples (and streamflow measurements) were obtained during the 2000 recreational season, the remainder being obtained during the 2002 recreational season. To facilitate comparison of streamflow conditions between sampling years, the following discussion focuses on streamflows

measured at the Old Portage site, which has operated as a continuous-record streamflow-gaging station from September 1921 to December 1935 and from October 1939 to the present (November 2003). Instantaneous streamflows measured when microbiological samples were collected from Cuyahoga River sites in 2000 ranged from 111 to 799 ft^3/s; median streamflow was 167 ft^3/s (fig. 2). In comparison, instantaneous stream-flows measured when microbiological samples were collected in 2002 ranged from 92 to 960 ft^3/s; median streamflow was 485 ft^3/s (fig. 2). As a point of reference, the median of daily mean streamflows for the period of record at the Old Portage streamflow-gaging station through water year 2002 is 266 ft^3/s (Shindel and others, 2003). Ten percent of the daily mean streamflows exceeded 1,020 ft^3/s during the same period, and 90 percent of the daily-mean streamflows exceeded 78 ft^3/s (Shindel and others, 2003). Although instantaneous stream-flows are not strictly comparable to daily mean streamflows, these statistics indicate that the samples were not collected at extremes of streamflow. It also is notable that samples were collected in 2000 and 2002 over similar ranges of streamflow.

Analysis of Microbiological Data

Microbiological data used in this study came from analyses of samples collected in 2000 and 2002 from two sources; selected sites on the Cuyahoga River and effluent from the Akron WPCS. Data from the two sources were not combined for analysis except when assessing between-site differences and in selected instances where a combined analysis seemed appropriate. Appendix B lists the microbiological data from samples collected in 2000 and 2002.

The presence of censored observations ("less-than" values) in the data set required special handling. Analytical results for F-specific coliphage, infectious enterovirus, *Giardia*, and *Cryptosporidium* each included some censored values. Of the four microorganisms, only infectious enterovirus and F-specific coliphage had sufficiently small percentages of censored values (7 and 14 percent, respectively) to consider statistical analyses by means of parametric tests. In all applicable parametric tests involving microorganisms with censored values, half of the censoring level was substituted for the censored value.

Because of a lack of general public agreement on methods for handling censored values, data for microorganisms with censored values were also rank transformed. Applicable tests involving infectious enterovirus and F-specific coliphage were done on the both ranks and the base numerical values. Tests involving *Giardia* always were done using the rank-transformed concentrations.

Microbiological data collected for this study were analyzed qualitatively and quantitatively to identify relevant associations between microorganisms and to assess spatial trends in microorganism concentrations. Qualitative analyses were directed at identifying general trends and patterns. Quantitative analyses were directed at identifying correlations between concentrations of organisms and comparing differ-

ences in central values (mean concentration, for example) of one organism as a function of some characteristic of a second organism, as a function of location, or as a function of some other environmental measure.

Correlation analyses were done by computing Spearman's rho. Spearman's rho was chosen over other measures of correlation because it can be used to identify correlations that are not necessarily linear and because it can be used with censored data sets (as long at their ordination can be determined). Comparison tests were done by means of analysis of variance (ANOVA) coupled (where appropriate) with a post hoc Tukey-Kramer multiple-comparison test. The Tukey-Kramer multiple-comparison test is an extension of Tukey's test that facilitates comparisons of classes with unequal sample sizes.

Table 3. Summary statistics for concentrations of *Escherichia coli*, *Clostridium perfringens*, F-specific and somatic coliphage, infectious enterovirus, and *Giardia* at selected sites in the Cuyahoga River and at the Akron Water Pollution Control Station (WPCS), Ohio, 2000 and 2002.

[col/100 mL, colonies per 100 milliliters; mL, milliliters; MPN/100 L, most probable number per 100 liters; L, liters; N, sample size; na, not applicable; nd, not determined]

Site name	Statistic	*Escherichia coli* (col/ 100 mL)	*Clostridium perfringens* (col/ 100 mL)	F-specific coliphage (plaques/ 100 mL)	Somatic coliphage (plaques/ 100 mL)	Infectious enterovirus (MPN/ 100 L)	*Giardia* (cysts/ 10 L)
Old Portage	geometric mean	1,320	115	na	195	na	na
	median	1,350	120	18	130	35	1
	maximum	18,000	190	160	790	190	3
	minimum	64	67	<1	72	<16	<1
	N	18	3	6	3	5	3
Akron WPCS	geometric mean	122	1,140	43	157	112	na
	median	77	1,100	32	170	110	8
	maximum	2,900	1,700	180	290	190	10
	minimum	8	800	12	79	73	<1
	N	19	3	6	3	5	3
Botzum	geometric mean	1,380	353	11	112	na	na
	median	1,250	350	8	63	111	<1
	maximum	12,000	370	63	470	190	16
	minimum	170	340	3	47	8	<1
	N	18	3	6	3	6	3
Jaite	geometric mean	898	227	na	76	106	na
	median	710	270	5	78	109	<1
	maximum	22,000	360	18	170	190	6
	minimum	160	120	<1	33	56	<1
	N	19	3	6	3	3	3
Independence	geometric mean	975	nd	6	nd	nd	nd
	median	620	nd	5	nd	nd	nd
	maximum	25,000	nd	11	nd	nd	nd
	minimum	210	nd	3	nd	nd	nd
	N	15	0	3	0	0	0

The Tukey-Kramer multiple-comparison test is an exact alpha-level test if the sample sizes are the same, and it is slightly conservative for unequal sample sizes (Rafter and others, 2002). An alpha level of 0.05 was used to judge significance of all tests discussed in this report.

The Tukey-Kramer test is based on the assumption that the data are normally distributed, random, and independent and that they have common variance. Quantile-quantile plots and the Shapiro-Wilk test were used to assess normality of the microorganism concentration data. On the basis of these tests, it was determined that logarithmic transformations of concentrations of *E. coli*, F-specific and somatic coliphage, and *C. perfringens* were necessary to meet the normality assumption. No transformation was required for concentrations of infectious enterovirus, and none of the other microorganism data included sufficient percentages of uncensored values to warrant parametric analysis. Bartlett's test (Snedecor and Cochran, 1980) was used to test for departures from the assumption of equal variance (no significant departures were detected) and the data were assumed to be both random and independent based on the sampling design.

General Trends and Patterns

E. coli concentrations in samples collected for this study ranged from 64 to 25,000 col/100 mL in Cuyahoga River samples and from 8 to 2,900 col/100 mL in Akron WPCS effluent samples (table 3). *E. coli* concentrations exceeded 298 col/100 mL (Ohio's single-sample primary-contact recreational standard) 87 percent of the time in Cuyahoga River samples as compared to 26 percent of the time in Akron WPCS effluent samples. Similarly, *E. coli* concentrations exceeded 576 col/100 mL (Ohio's single-sample secondary-contact recreational standard) 64 percent of the time in Cuyahoga River samples as compared to 26 percent of the time in Akron WPCS effluent samples. Both threshold concentrations were exceeded multiple times at all sites. The lower threshold-exceedance rates for the Akron WPCS effluent samples presumably are due to chlorination of the effluent.Those samples that did not exceed the standards generally were associated with the lower range of streamflows for the respective sites. For the range

of effluent flow rates sampled in this study, Myers and others (1998) reported *E. coli* concentrations in the Akron WPCS effluent that were lower than concentrations in Cuyahoga River samples at equivalent flow rates. At effluent flow rates larger than observed in this study, the study by Myers and others (1998) indicated that the Akron WPCS effluent tended to have appreciably higher concentrations of *E. coli* than did river sites at equivalent flow rates. This trend in *E. coli* concentration occurs because capacity of the Akron WPCS to provide full secondary treatment of the effluent stream is exceeded at an effluent flow rate of about 110 Mgal/d.

At least five *E. coli* samples were collected at the Old Portage, Botzum, Jaite, and Independence sites on the Cuyahoga River during May, July, and September of calendar year 2000. Geometric mean *E. coli* concentrations calculated from analytical results from each site and month ranged from 466 to 2,070 col/100 mL (table 4) and in each case exceeded Ohio's geometric-mean primary-contact recreational standard of 126 col/100 mL

Salmonella was detected in 15 of 29 river samples (52 percent) and 3 of 7 effluent samples (43 percent) (Appendix B); all but one of the detections were found in 2000, and multiple detections were noted at each sampling location. *Salmonella* was never detected in combination with hepatitis A virus; however, the majority of their respective detections were in different years.

There are over 2,300 known serotypes of *Salmonella*. In Ohio, the *Salmonella* serotypes Typhimurium and Enteritidis account for more than half of all human isolates (Ohio Department of Health, 1993b). *Salmonella* serotypes Braenderup, Hartford, Java (also called paratyphi B variant java), Manhattan, Miami, Muenster, Newport, Oranienburg, and Senftenberg were identified in samples collected in 2000. Braenderup, Java, and Newport were the most commonly found *Salmonella* serotypes. *Salmonella* Arizona was identified in the one detection (at Jaite) in 2002.

F-specific coliphage samples were collected in 2000 and 2002; however, somatic coliphage, *C. perfringens*, *Cryptosporidium*, and *Giardia* samples were collected in 2002 only. No samples were collected from the Independence site in 2002. Concentrations of somatic coliphage and F-specific coliphage

Table 4. Geometric mean *Escherichia coli* concentrations for samples collected during May, July, and September 2000 at selected sites on the Cuyahoga River, Ohio.

[col/100 mL; colonies per 100 milliliters]

| Month | Geometric mean *Escherichia coli* concentration (top number), in col/100 mL, and number of observations on which it was based (bottom number), at indicated site | | | |
	Old Portage	Botzum	Jaite	Independence
May	1,880	1,020	697	961
	5	5	6	5
July	2,060	1,960	1,270	466
	5	5	5	5
September	841	1,510	1,070	2,070
	5	5	5	5

ranged from 79 to 290 plaques/100 mL and 12 to 180 plaques/100 mL, respectively, in the Akron WPCS effluent samples (table 3). Concentrations of somatic coliphage ranged from 33 to 790 plaques/100 mL in the nine river samples collected in 2002. F-specific coliphage concentrations ranged from <1 to 160 plaques/100 mL in 21 river samples collected in 2000 and 2002 and were less than detection in two (about 10 percent) of the river samples (table 3).

Hepatitis A virus was detected only in samples collected in 2002. Of 21 samples analyzed for hepatitis A virus, 6 tested positive (contained hepatitis A virus), with 3 of the 6 samples coming from the Akron WPCS effluent (Appendix B). The Centers for Disease Control and Prevention (2003b) states that hepatitis A virus is killed by adequate chlorination of water; however, hepatitis A virus was found in half of the Akron WPCS effluent samples analyzed. The Akron WPCS effluent is chlorinated (with a contact time of approximately 30 minutes) and treated with sodium bisulfite (to reduce chlorine residual) just prior to discharge (City of Akron, 2003).

Hepatitis A virus was not detected in any of the six samples from the Old Portage site (upstream from the Akron WPCS). Hepatitis A virus was detected in samples from Akron WPCS, Botzum, and Jaite collected May 21–22, 2002; in samples from the Akron WPCS and Botzum collected July 15–16, 2002; and in a sample from the WPCS only on June 17, 2002. In no case was hepatitis A virus detected in samples from a site other than the Akron WPCS without a coincident detection of hepatitis A virus in the Akron WPCS sample. The fact that hepatitis A virus was never detected upstream from the Akron WPCS, coupled with the fact that all downstream detections of hepatitis A virus had coincident detections of hepatitis A virus in the Akron WPCS effluent, suggests that the Akron WPCS was a principal source of hepatitis A virus at the downstream sites.

Enterovirus (as determined by the RT-PCR method) was detected when tested for in most samples (19 out of 21) and in all samples from the Akron WPCS and Botzum site (Appendix B). Infectious enterovirus concentrations (as determined by the ICC-PCR method) ranged from <16 to 190 MPN per 100 L in

river samples and from 73 to 190 MPN per 100 L in samples from the Akron WPCS effluent (table 3).

Concentrations of *C. perfringens* in samples collected from river and effluent sources exceeded 100 col/100 mL with only one exception (at Old Portage). Concentrations of *C. perfringens* in samples collected from the Cuyahoga River ranged from 67 to 370 col/100 mL, whereas concentrations in samples from the Akron WPCS effluent ranged from 800 to 1,700 col/100 mL (table 3). The mean concentration of *C. perfringens* in Akron WPCS effluent samples (1,200 col/100 mL) was more than 4 times the mean concentration in river samples (243 col/100 mL). This result is not unexpected because *C. perfringens* is present in large numbers in human and animals wastes, and its spores are resistant to disinfection (Francy and others, 2000).

Cryptosporidium concentrations greater than or equal to 1 oocyst/10 L were not found in any sample. *Giardia* concentrations were greater than 1 cyst/10 L in four of the nine (44 percent) Cuyahoga River samples and two of the three (67 percent) Akron WPCS effluent samples analyzed for *Giardia* and *Cryptosporidium*. The highest measured *Giardia* concentration (15.8 cysts/10 L) was from a sample collected at the Botzum site. The second and third highest concentrations (9.5 and 8.2 cysts/10 L, respectively) were from samples collected from the Akron WPCS effluent. *Giardia* was detected at least once at each site sampled in 2002.

Relation of *Escherichia coli* Water-Quality Standards to Presence of Selected Infectious Microorganisms

Ohio's water-quality standards for *E. coli* vary as a function of designated recreational use (table 1). Ohio has established standards for three categories of recreational uses: bathing waters, primary contact, and secondary contact. Bathing-water standards are applicable to designated swimming areas and therefore are the most stringent of the three. In contrast, primary-contact standards are applicable to waters that are not designated as swimming areas but are capable of

Table 5. Numbers and percentages of time microorganisms were present in Cuyahoga River samples when the indicated threshold *Escherichia coli* concentrations were not exceeded, Ohio, 2000 and 2002.

[Total number of river samples analyzed for *Escherichia coli* = 70; NA, not applicable; col/100 mL, colonies per 100 milliliters]

Ohio contact recreation standard	Threshold *Escherichia coli* concentration (col/100 mL)	Number of river samples not exceeding threshold	Number of current analyses for enterovirus, infectious enterovirus, and hepatitis A virus	Number of concurrent analyses for *Salmonella*	Number of times (percentage of time) indicated organism was present		
					Enterovirus and infectious enterovirus	Hepatitis A virus	*Salmonella*
Bathing	235	5	0	0	NA (NA)	NA (NA)	NA (NA)
Primary	298	9	1	1	1 (100%)	0 (0%)	1 (100%)
Secondary	576	25	7	10	5 (71%)	1 (14%)	3 (30%)

Table 6. Numbers and percentages of time microorganisms were present in Cuyahoga River samples when the indicated threshold *Escherichia coli* concentrations were exceeded, Ohio, 2000 and 2002.

[Total number of river samples analyzed for *Escherichia col*i = 70; col/100 mL, colonies per 100 milliliters]

Ohio contact recreation standard	Threshold *Eschericha coli* concentration (col/100 mL)	Number of river samples exceeding threshold	Number of current analyses for enterovirus, infectious enterovirus, and hepatitis A virus	Number of concurrent analyses for *Salmonella*	Number of times (percentage of time) indicated organism was present		
					Enterovirus and infectious enterovirus	Hepatitis A virus	*Salmonella*
Bathing	235	65	15	29	13[a] (87%) 11[b] (73%)	3 (20%)	15 (52%)
Primary	298	61	14	28	12[a] (86%) 10[b] (71%)	3 (21%)	14 (50%)
Secondary	576	45	8	19	8[a] (100%) 6[b] (75%)	2 (25%)	12 (63%)

[a]Value is for enterovirus.

[b]Value is for infectious enterovirus.

supporting full-body-contact recreation (such as swimming or canoeing). Secondary-contact standards are applicable to waters that can support only partial-body-contact recreation (such as wading).

The *E. coli* water-quality standards were based in part on results of a USEPA study in which rates of swimming-associated gastrointestinal illness were found to be related to concentrations of *E. coli* in the water (Dufour, 1984). An equation developed by Dufour (1984) relating illness rate to *E. coli* concentration shows that exposure to waters that meet *E. coli* water-quality standards is not necessarily without risk. For example, bathing waters with a geometric-mean *E. coli* concentration of 126 col/100 mL (based on a minimum of five samples in a 30-day period) have an associated risk of 8 gastrointestinal illnesses per 1,000 bathers (according to Dufour's equation). Given that some gastrointestinal illnesses can be expected even when *E. coli* standards are not exceeded, an analysis was done to examine the frequency of occurrence of pathogenic organisms when selected recreational standards were not exceeded.

As listed in table 5, only 5 out of the 70 river samples analyzed had *E. coli* concentrations less than 235 col/100 mL (Ohio's single-sample bathing-water recreational standard). Unfortunately, no other bacterial or viral analyses were done for those samples.

One of nine river samples with *E. coli* concentrations less than 298 col/100 mL was analyzed for other bacteria and viruses. Both infectious enterovirus and *Salmonella* were detected in the sample; however, hepatitis A was not detected (table 5).

Seven of the 25 river samples with *E. coli* concentrations less than 576 col/100 mL were analyzed for enterovirus, infectious enterovirus, and hepatitis A virus. Of those seven samples, five (71 percent) were found to contain enterovirus and

infectious enterovirus at concentrations above detection, and one (14 percent) was found to contain hepatitis A virus. Ten of the 25 river samples with *E. coli* concentrations less than 576 col/100 mL were analyzed for *Salmonella*, which was found in three (30 percent) of the samples (table 5). By comparison, *E. coli* concentrations were greater than 576 col/100 mL in 45 river samples (table 6). Eight of those 45 samples were analyzed for enterovirus, infectious enterovirus, and hepatitis A virus with rates of detection of 100 percent, 75 percent, and 25 percent, respectively. Nineteen of the 45 samples with *E. coli* concentrations greater than the single-sample secondary-contact recreational standard were analyzed for *Salmonella*, which was detected in approximately 63 percent of those samples. In all cases, percentages of pathogenic-organism detections were larger in samples where the single-sample secondary-contact recreational standard for *E. coli* was exceeded than in samples where the standard was not exceeded. A comparable analysis cannot be made with respect to the single-sample primary-contact recreational standard because only one sample that did not exceed that standard was analyzed for other pathogenic organisms.

Associations Between Bacteria, Viruses, and Protozoa

Much information has been published on the relation of *E. coli* concentrations to fecal coliform concentrations in the Cuyahoga River and other selected rivers in Ohio (Francy and others, 1993; Myers and others, 1998); however, little information is available about the relation between concentrations of *E. coli* and other microorganisms or between combinations of other microorganisms.

Table 7.   Correlation between microorganism concentrations in Cuyahoga River samples and combined river and Akron Water Pollution Control Station (WPCS) effluent samples, Ohio, 2000 and 2002.

[Shaded cells designate river samples only, unshaded cells designate combined river and effluent samples]

	Spearman's rho (top number), p value (middle number), and number of observations (bottom number) for indicated microorganism pairs				
	Escherichia coli	**F-specific coliphage**	**Somatic coliphage**	**Infectious enterovirus**	*Clostridium perfringens*
Escherichia coli		0.09 0.652 27	0.37 0.240 12	-0.04 0.661 19	0.21 0.505 12
F-specific coliphage	0.33 0.145 21		0.63 0.027 12	0.17 0.490 19	0.13 0.687 12
Somatic coliphage	0.37 0.332 9	0.54 0.130 9		-0.35 0.264 12	0.11 0.737 12
Infectious enterovirus	-0.25 0.383 14	-0.22 0.445 14	-0.55 0.125 9		-0.01 0.966 12
Clostridium perfringens	0.68 0.045 9	0.06 0.881 9	-0.11 0.781 9	0.03 0.949 9	

A matrix of correlation information for samples collected for this study is shown in table 7. The shaded cells in table 7 list correlation information for concentration data for samples collected from Cuyahoga River sites only. The unshaded cells show correlation information for concentration data for samples collected from river sites combined with concentration data for samples collected from the Akron WPCS effluent (hereafter referred to as the "combined data set").

Absolute values of correlation coefficients were greater than 0.5 for only three microorganism pairs (table 7) and only two of those pairs had coefficients that were statistically significant. *C. perfringens* concentrations were positively (rho = 0.68) and significantly correlated with concentrations of *E. coli* in river samples. The correlation between these two microorganisms was also positive in the combined data set; however, the correlation was weaker (rho = 0.21) and not statistically significant. The poorer correlation in the combined data set is attributed to the markedly different characteristics of *C. perfringens* and *E. coli* concentrations in the effluent as compared to river samples.

Somatic coliphage concentrations and F-specific coliphage concentrations were positively and significantly correlated (rho = 0.63) in the combined data set. The correlation between this same pair of microorganisms was also positive (rho = 0.54) in samples collected from river sites only; however, the number of observations was smaller and the correlation was not statistically significant (p = 0.13).

Infectious enterovirus concentration was negatively correlated with somatic coliphage concentrations in river samples (rho = -0.55) and in the combined data set (rho = -0.35). The magnitudes and signs of these correlation coefficients, however, were influenced strongly by one observation, and neither correlation was statistically significant. As a matter of note, Gantzer and others (1998) found a significant positive correlation between the concentration of somatic coliphage and the presence of infectious enterovirus in treated wastewater.

ANOVAs on data for the Cuyahoga River sites indicated that neither geometric-mean somatic coliphage concentration nor geometric-mean F-specific coliphage concentration differed significantly as a function of the presence/absence of enterovirus, infectious enterovirus, or hepatitis A virus. Similarly, an ANOVA on data for the Cuyahoga River sites indicated that mean infectious enterovirus concentrations did not differ significantly as a function of the presence/absence of hepatitis A virus. In spite of the lack of significant differences, there were observable tendencies for the presence of hepatitis A virus to be associated with higher median concentrations of somatic coliphage, F-specific coliphage, and infectious enterovirus (fig. 3). When considering the significance of these results, it is important that the reader be aware that they are based on a small sample: only 9 observations for somatic coliphage, 15 observations for F-specific coliphage, and 14 observations for infectious enterovirus.

Hepatitis A virus was not detected in samples when infectious enterovirus concentrations were less than 80 MPN/100 L. There were instances, however, when infectious enterovirus was detected (in some cases at concentrations greater than 80 MPN/100 L) and hepatitis A virus was not detected.
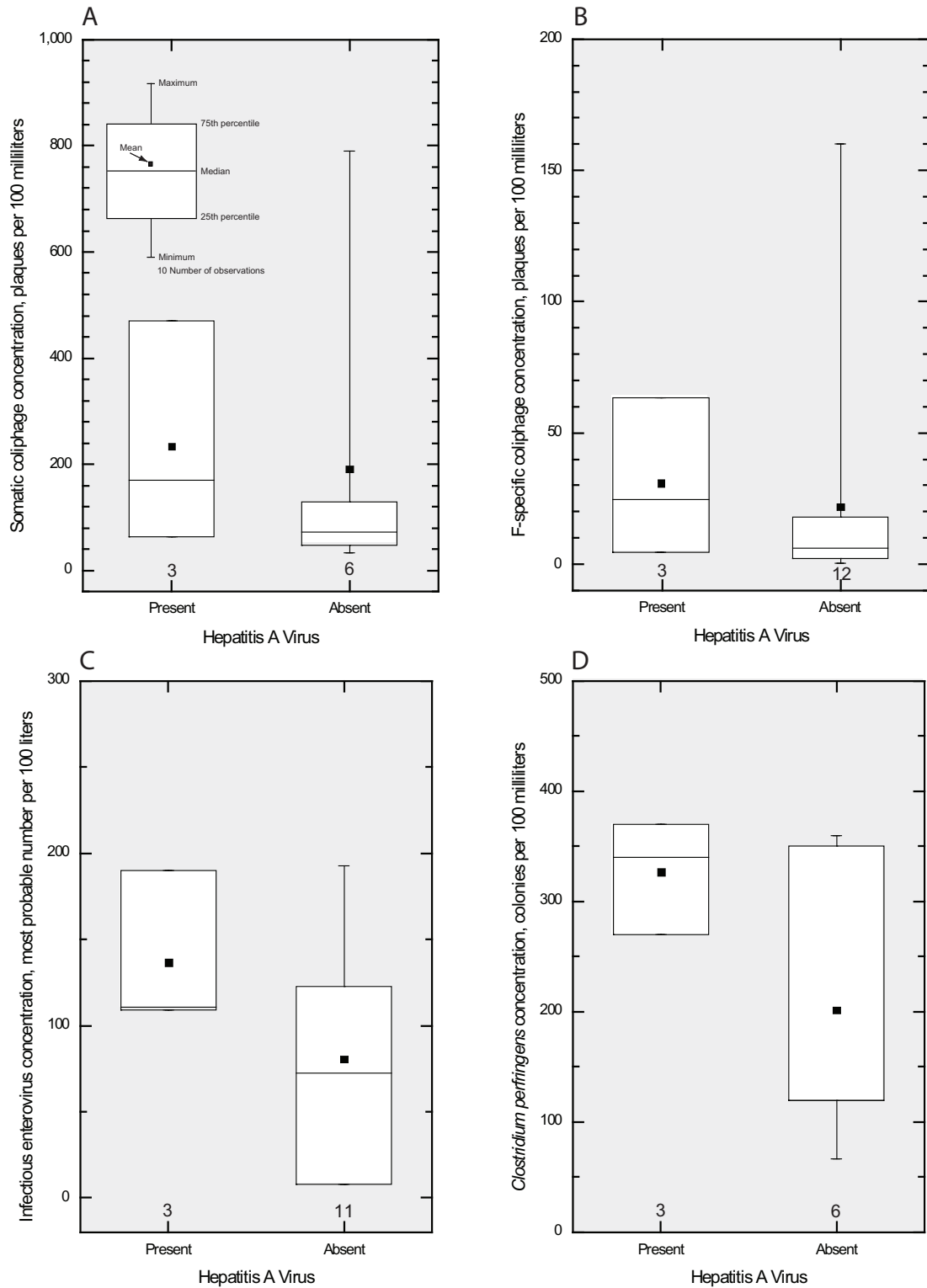
Figure 3. Distribution of (A) somatic coliphage, (B) F-specific coliphage, (C) infectious enterovius, and (D) *Clostridium perfringens* concentrations as a function of the presence of hepatitis A virus in Cuyahoga River samples, Ohio, 2000 and 2002.
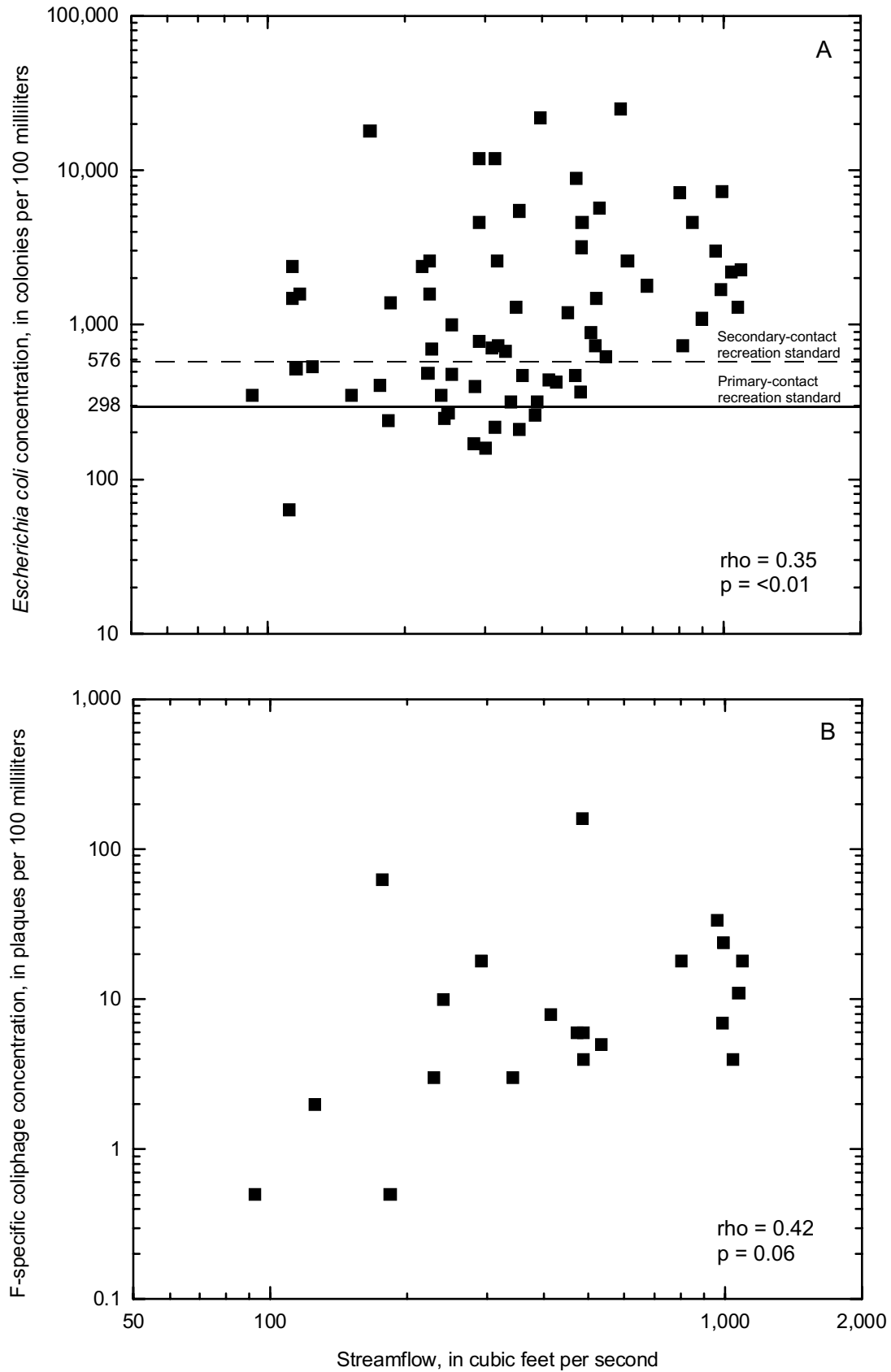
Figure 4.   Relation between streamflow and concentrations of (A) *Escherichia coli* and (B) F-specific coliphage in Cuyahoga River samples, Ohio, 2000 and 2002.

An ANOVA followed by a Tukey-Kramer test on the combined data set indicated that geometric-mean *C. perfringens* concentration was significantly higher in samples where hepatitis A virus was present (detected) than in samples where hepatitis A virus was absent (not detected). Figure 3 (D) shows that a similar tendency was present for data from the Cuyahoga River sites alone; however, the difference in geo-metric-mean concentration was not statistically significant.

Relation of Streamflow and Water-Quality Measures to Bacteria, Viruses, and Protozoa

Scatterplots and correlation analyses were used to assess the relation of streamflow to concentrations of *E. coli*, F-specific coliphage, somatic coliphage, infectious enterovirus, *C. perfringens*, and *Giardia* at the Cuyahoga River sites. *E. coli* had a weak (rho = 0.35) but significant positive cor-relation with streamflow. F-specific coliphage concentration also was positively correlated (rho = 0.42) with streamflow; however, the p value for this correlation (p = 0.06) was slightly larger than alpha, and so the relation was not statistically sig-nificant. The strength of these relations are illustrated further in figure 4 which shows scatterplots of *E. coli* and F-specific coliphage concentrations against streamflow. No other micro-organisms exhibited a notable trend with streamflow.

E. coli concentration tended to increase with increas-ing streamflow at Cuyahoga River sites both upstream and downstream from the Akron WPCS, an indication that *E. coli* loading is an increasing function of rainfall /runoff processes and that *E. coli* sources upstream from the Akron WPCS may have appreciable influ-ence on concentrations in the river down-stream from the Akron WPCS. These results are consistent with the findings of Myers and others (1998).

A variety of water-quality charac-teristics, measured in selected samples, may be related to observed concentrations of *E. coli*. Because many water-quality characteristics are highly correlated with streamflow, apparent correlations between these measures and *E. coli* concentra-tions may be due solely to the influence of streamflow. As a consequence, it is necessary to account for the influence of streamflow on concentrations of *E. coli* before assessing its correlation with water-quality characteristics. This was done by regressing logarithms of *E. coli* concentration on logarithms of stream-flow and using the residuals (the differ-ences between the observed and predicted values of *E. coli*) for further analyses. The residuals represent the distribution

of log-transformed *E. coli* concentrations with the variability attributable to streamflow removed.

Residuals from the regression of logarithms of *E. coli* concentration on logarithms of streamflow (for Cuyahoga River sites) were analyzed for correlation with the water-qual-ity characteristics. Fluoride, pH, specific conductance, and total phosphorus were the only water-quality characteristics with significant correlation to the residuals. Site-by-site investigations of these correlations suggest that the observed correlation resulted from the association of these parameters with spatial trends in concentration as opposed to within-site associations of the parameters with *E. coli* concentrations.

To better illustrate how some water-quality parameters can exhibit spatial trends, figure 5 shows boxplots of pH measured in Cuyahoga River samples and in Akron WPCS effluent samples. An ANOVA followed by a Tukey-Kramer test indicates that mean pH in the Akron WPCS effluent samples was statistically lower than in samples from any of the river sites. Further, the mean pH in samples from Botzum, the site immediately downstream from the Akron WPCS, was statistically lower than in samples from the Old Portage and Jaite sites.

Assessment of Between-Site Differences in Microbiological Characteristics

Between-site differences in central values of concentra-tions of microorganisms were assessed by means of a combi-
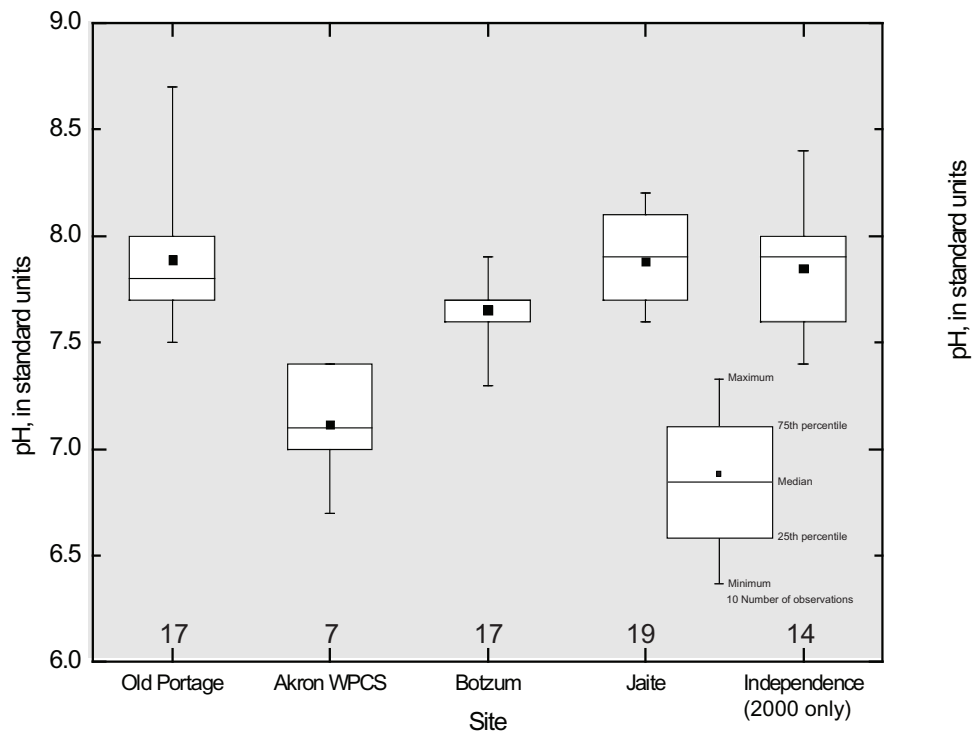
Figure 5.   Distribution of pH as a function of site on the Cuyahoga River, including the Akron Water Pollution Control Station (WPCS), Ohio, 2000 and 2002.
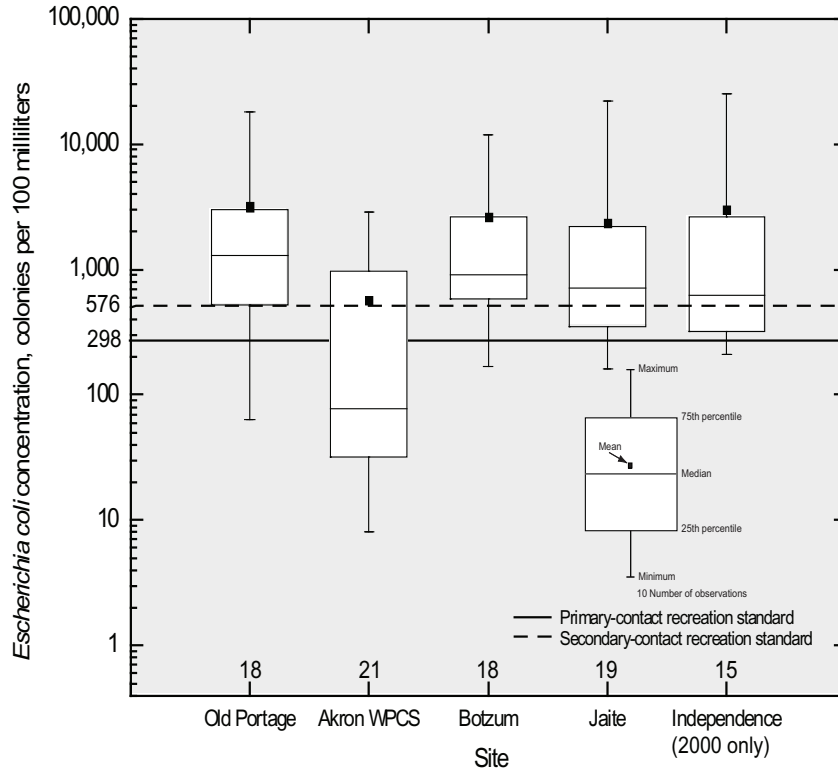
Figure 6.   Distribution of *Escherichia coli* concentrations as a function of site on the Cuyahoga River, including the Akron Water Pollution Control Station (WPCS), Ohio, 2000 and 2002.
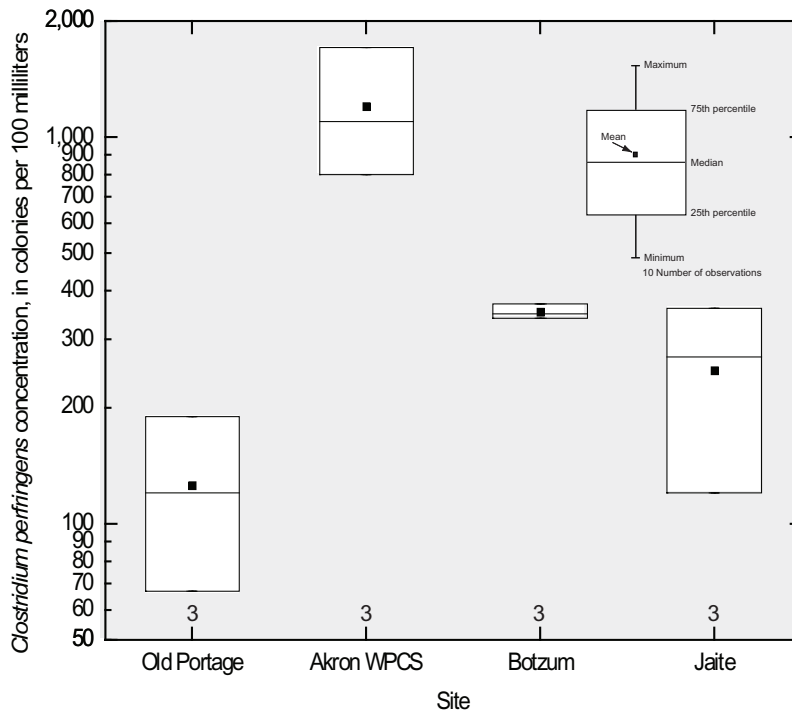
Figure 7.   Distribution of *Clostridium perfringens* concentrations as a function of site on the Cuyahoga River, including the Akron Water Pollution Control Station (WPCS), Ohio, 2000 and 2002.

nation of ANOVA and (where appropriate) the Tukey-Kramer test. Two basic types of assessments were done.

The first type of assessment focused on identifying differences in concentrations between samples collected from all combinations of the Cuyahoga River sites and the Akron WPCS site. Based on an ANOVA coupled with the Tukey-Kramer test, geometric-mean *E. coli* and *C. perfringens* concentrations were found to be statistically lower and higher, respectively, in the Akron WPCS effluent than in water samples from the Cuyahoga River sites. These relations are evident in figures 6 and 7. A comparable analysis on ranks of F-specific coliphage concentration indicated that the mean rank in samples from the Akron WPCS was statistically higher than in samples from Jaite; significant differences in ranks of F-specific coliphage concentration were not found between other sites. ANOVAs on the logarithms of somatic coliphage concentrations, logarithms and ranks of infectious enterovirus concentrations, and on ranks of *Giardia* concentrations did not show any significant between-site differences for these microorganisms.

The second type of assessment was done to determine whether significant differences existed between central values of concentration or rank considering only those samples from Cuyahoga River sites immediately upstream (Old Portage) and downstream (Botzum) from the Akron WPCS. Because the minimum significant difference between mean concentrations determined in the Tukey-Kramer test is a function of the weighted average variance (Sokal and Rohlf, 1981), it was felt that inclusion of the Akron WPCS results (as was done in the first type of assessment) potentially could increase or decrease the weighted average variance in a way that could obscure differences between central values observed for the river sites. Based on ANOVAs and a post hoc Tukey-Kramer test, *C. perfringens* was the only microorganism to exhibit significant between-site differences (in this case for geometric-mean values). Elevated concentrations of *C. perfringens* in Akron WPCS effluent (fig. 6) discharged to the Cuyahoga River likely explain why geometric-mean concentrations at Botzum were significantly higher than at Old Portage.

Summary and Conclusions

Discharges of stormwater, combined-sewer overflows, and incompletely disinfected wastewater to the Cuyahoga River cause frequent exceedances of the bacteriological water-quality standards for body-contact recreation. People who come in contact with the contaminated water are at risk for gastrointestinal and (or) other illnesses. Elevated levels of fecal-indicator organisms would be cause for concern in any river but are of particular concern for the Cuyahoga River because of the CVNP and its goal of promoting water-contact recreation. In response to this concern, the U.S. Geological Survey, in collaboration with the National Park Service, con-

ducted an in-depth study to investigate microbiological water quality of the Cuyahoga River within the CVNP.

Water samples were collected from the Cuyahoga River and from the effluent of the Akron Water Pollution Control Station (WPCS) in 2000 and 2002. Four river sites were sampled in 2000, but only three river sites were sampled in 2002. The samples were collected over a similar range in streamflow in 2000 and 2002. In 2000, water samples were analyzed for *E. coli*, *Salmonella*, F-specific coliphage, enterovirus, infectious enterovirus, hepatitis A virus, and physical and chemical characteristics. In 2002, water samples were analyzed for the same constituents as in 2000, as well as for somatic coliphage, *C. perfringens, Cryptosporidium,* and *Giardia*.

With the exception of *Cryptosporidium*, all microorganisms analyzed for were detected one or more times at each location sampled. *E. coli* concentrations in river samples exceeded the single-sample primary-contact recreational standard in approximately 87 percent of the samples and tended to be higher than in the effluent samples (presumably because of chlorination of the effluent). In contrast, concentrations of *C. perfringens*, a spore-forming bacterium present in large numbers in human and animal waste, tended to be higher in effluent samples than in river samples.

Detections of *Salmonella* were, with one exception, limited to samples collected in 2000; however, detections of hepatitis A virus were limited exclusively to samples collected in 2002. It is unknown why the detection of hepatitis A virus was limited to one year; however, the reduction in detections of *Salmonella* in 2002 may be due to analysis of smaller sample volumes.

Hepatitis A virus was not detected in samples collected upstream from the Akron WPCS. All downstream detections of hepatitis A virus were coincident with detections in the Akron WPCS effluent. The pattern of detections and the presence of hepatitis A virus in the Akron WPCS effluent suggests that the Akron WPCS was a principal source of hepatitis A virus at the downstream sites.

Geometric-mean concentrations of *E. coli* were calculated on the basis of analytical results from at least five samples collected at each river site during May, July, and September 2000. In each case, the geometric-mean primary-contact recreational standard of 126 col/100 mL was exceeded.

Microbiological data for river samples in which *E. coli* concentrations were less than the single-sample secondary-contact recreational standard (576 col/100 mL) were examined to assess the co-occurrence of other microorganisms. Seven of the 25 river samples with *E. coli* concentrations less than 576 col/100 mL were analyzed for enterovirus, infectious enterovirus, and hepatitis A virus. Of those seven samples, five (71 percent) contained enterovirus and infectious enterovirus at concentrations above detection, and one contained hepatitis A virus. Ten of the 25 river samples with *E. coli* concentrations less than 576 col/100 mL were analyzed for *Salmonella*, which was detected in three (30 percent) of the samples. An analysis of the co-occurence of pathogenic microorganisms in samples in which the single-sample secondary-contact recreational

standard was exceeded consistently showed higher percentages of co-occurrence than in samples in which the single-sample secondary-contact recreational standard was not exceeded.

C. perfringens concentrations were positively and significantly correlated with concentrations of *E. coli* in river samples. The correlation between these two microorganisms was also positive in samples collected from Cuyahoga River sites combined with samples from the Akron WPCS effluent (the combined data set); however, the correlation was weaker and not statistically significant.

Somatic coliphage concentrations and F-specific coliphage concentrations were positively and significantly correlated in the combined data set. The correlation between this same microorganism pair also was positive in samples collected from river sites alone; however, the number of observations was smaller and the correlation was not statistically significant.

ANOVA results indicated that geometric-mean concentrations of somatic coliphage and F-specific coliphage in river samples did not differ significantly as a function of the presence/absence of enterovirus, infectious enterovirus, or hepatitis A virus. Similarly, mean infectious enterovirus concentrations did not differ significantly as a function of the presence/absence of hepatitis A virus. In spite of the lack of statistically significant differences, there was an observable tendency for detections of hepatitis A virus to be associated with higher median concentrations of somatic coliphage, F-specific coliphage, and infectious enterovirus. There also appears to be a relation between *C. perfringens* and hepatitis A virus wherein geometric mean *C. perfringen*s concentrations were significantly higher in combined dataset samples where hepatitis A virus was present than in samples where hepatitis A virus was absent. A similar tendency was present for data from the Cuyahoga River sites alone; however, the difference in geometric-mean concentration was not statistically significant.

E. coli concentrations exhibited a weak but significant positive correlation with streamflow. The tendency for *E. coli* concentration to increase with streamflow was observed for Cuyahoga River sites upstream and downstream from the Akron WPCS, indicating that *E. coli* loading is an increasing function of rainfall /runoff processes and that *E. coli* sources upstream from the Akron WPCS may have appreciable influence on concentrations in the river downstream from the Akron WPCS.

F-specific coliphage concentration also was positively correlated with streamflow; however, the p value for that correlation was slightly larger than alpha, so the relation was not statistically significant. Correlations between streamflow and concentrations (or ranks of concentrations) of other microorganisms were not significant, nor were there significant site-specific correlations between physical or chemical water-quality measures and concentrations of microorganisms.

ANOVA and the Tukey-Kramer test were used to assess between-site differences in central values of concentrations (or ranks of concentrations) of microorganisms. *E. coli* and

C. perfringens concentrations were statistically lower and higher, respectively, in the Akron WPCS effluent than in water samples from the other Cuyahoga River sites. The mean rank of F-specific coliphage concentrations in samples from the Akron WPCS was statistically higher than in samples from the Jaite site; however, no significant differences in ranks of F-specific coliphage concentration were found between other sites. No significant between-site differences in central values of concentrations (or ranks of concentrations) were detected for other microorganisms.

Between-site differences in central values of concentrations (or ranks of concentrations) also were assessed on the basis of samples collected at the Old Portage and Botzum sites only. That assessment was aimed at identifying differences that likely can be attributed to the Akron WPCS, which discharges its effluent to the Cuyahoga River between the two sites. *C. perfringens* was the only microorganism that exhibited significant between-site differences, probably because of the elevated concentrations of *C. perfringens* in the Akron WPCS effluent.

Results from this study demonstrate that pathogenic microorganisms can be present in the Cuyahoga River even when *E. coli* concentrations do not exceed the single-sample primary- or secondary-contact recreational standards. However, the fact that the percentage of detections of pathogenic organisms consistently was greater in samples in which the single-sample secondary-contact recreation standard was exceeded demonstrates that *E. coli* is a useful indicator of human health risk in the study area.

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Appendix A

Appendix A.   Quality-control samples for the detection of enterovirus and hepatitis A virus by means of the reverse transcriptase-polymerase chain reaction (RT-PCR) method.

[PCR, polymerase chain reaction]

Sample type	Description	Purpose
Negative-process control	Sterile water analyzed with each batch of five samples, beginning at the inhibitor removal step.	To determine whether cross-contamination of samples occurs during the inhibitor removal, RT-PCR, and hybridization steps.
Seeded negative-process control	Positive-control viruses that are added to a duplicate negative-process control with each batch of five samples before the RT- PCR step.	To verify the proper completion of the RT-PCR reaction.
Matrix spike	Positive-control viruses added to duplicate environmental samples before the RT-PCR step.	To indicate whether inhibitors are present in the environmental samples.
PCR positive control	Positive-control viruses added to sterile water before the RT-PCR step (also serves as the hybridization positive control).	To verify the proper completion of the RT-PCR and hybridization steps.
PCR negative control	Sterile water analyzed at the RT-PCR step.	To determine whether cross-contamination of samples occurs during the RT-PCR step.
Hybridization negative control	Sterile water analyzed at the hybridization step.	To determine whether cross-contamination of samples occurs during the hybridization step.

Appendix B

Appendix B. Streamflow and microbiological data for Cuyahoga River samples and Akron Water Pollution Control Station (WPCS) effluent samples collected in 2000 and 2002.

[ft³/s, cubic feet per second; col/100 mL, colonies per 100 milliliters; plaques/100 mL, plaques per 100 milliliters; MPN/100 L, most probable number per 100 liters; oocysts/10 L, oocysts per 10 liters; cysts/10 L, cysts per 10 liters;--, no data; **bold**, results based on colony count outside the ideal range of 20-80 colonies per plate; <, less than]

Date	Stream-flow (ft³/s)	Escherichia coli (col/100 mL)	Salmonella (presence/absence)	Salmonella serotype	F-specific coliphage (plaques/100 mL)	Somatic coliphage (plaques/100 mL)	Infectious enterovirus (MPN/100 L)	Enterovirus (presence/absence)	Hepatitis A virus (presence/absence)	Clostridium perfringens (col/100 mL)	Cryptosporidium (oocysts/10 L)	Giardia (cysts/10 L)
							Old Portage					
05/03/00	290	780	--	--	--	--	--	--	--	--	--	--
05/10/00	290	**12,000**	--	--	--	--	--	--	--	--	--	--
05/11/00	253	1,000	--	--	--	--	--	--	--	--	--	--
05/17/00	152	350	--	--	--	--	--	--	--	--	--	--
05/23/00	799	7,200	present	S. java	18	--	33[a]	present	absent	--	--	--
07/06/00	185	1,400	--	--	--	--	--	--	--	--	--	--
07/12/00	113	2,400	absent	--	--	--	--	--	--	--	--	--
07/18/00	290	4,600	present	S. braenderup	18	--	82	present	absent	--	--	--
07/25/00	117	1,600	--	--	--	--	--	--	--	--	--	--
07/26/00	113	1,500	--	--	--	--	--	--	--	--	--	--
09/05/00	115	520	--	--	--	--	--	--	--	--	--	--
09/06/00	111	**64**	--	--	--	--	--	--	--	--	--	--
09/11/00	167	18,000	present	S. hartford	--	--	--	--	--	--	--	--
09/19/00	125	540	absent	--	2	--	<16	present	absent	--	--	--
09/26/00	350	1,300	--	--	--	--	--	--	--	--	--	--
05/21/02	960	3,000	absent	--	34	790	35	present	absent	190	<1	<1
06/17/02	485	370	absent	--	160	130	<16	absent	absent	67	<1	3
07/15/02	92	350	absent	--	<1	72	190	present	absent	120	<1	1
							Akron WPCS					
05/23/00	143	2,500	--	--	--	--	--	--	--	--	--	--
05/23/00	143	2,400	present	S. miami	180	--	33[a]	present	absent	--	--	--
05/24/00	112	110	--	--	--	--	--	--	--	--	--	--
06/01/00	118	240	--	--	--	--	--	--	--	--	--	--

Appendix B. Streamflow and microbiological data for Cuyahoga River samples and Akron Water Pollution Control Station (WPCS) effluent samples collected in 2000 and 2002.—Continued

[ft³/s, cubic feet per second; col/100 mL, colonies per 100 milliliters; plaques/100 mL, plaques per 100 milliliters; MPN/100 L, most probable number per 100 liters; oocysts/10 L, oocysts per 10 liters; cysts/10 L, cysts per 10 liters;--, no data; **bold**, results based on colony count outside the ideal range of 20-80 colonies per plate; <, less than]

Date	Stream-flow (ft³/s)	Escherichia coli (col/100 mL)	Salmonella (presence/ absence)	Salmonella serotype	F-specific coliphage (plaques/100 mL)	Somatic coliphage (plaques/100 mL)	Infectious enterovirus (MPN/100 L)	Enterovirus (presence/ absence)	Hepatitis A virus (presence/ absence)	Clostridium perfringens (col/100 mL)	Cryptospo-ridium (oocysts/10 L)	Giardia (cysts/10 L)
06/05/00	196	30	--	--	--	--	--	--	--	--	--	--
06/08/00	114	77	--	--	--	--	--	--	--	--	--	--
06/12/00	192	32	--	--	--	--	--	--	--	--	--	--
07/11/00	102	2,200	present	*S. braenderup*	--	--	--	--	--	--	--	--
07/18/00	109	2,900	present	*S. manhattan*	28	--	190	present	absent	--	--	--
09/19/00	86	30	--	--	--	--	--	--	--	--	--	--
09/19/00	86	110	absent	--	110	--	73	present	absent	--	--	--
09/26/00	93	38	--	--	--	--	--	--	--	--	--	--
09/28/00	89	32	--	--	--	--	--	--	--	--	--	--
09/29/00	87	8	--	--	--	--	--	--	--	--	--	--
10/02/00	90	14	--	--	--	--	--	--	--	--	--	--
10/03/00	88	18	--	--	--	--	--	--	--	--	--	--
10/16/00	86	38	--	--	--	--	--	--	--	--	--	--
10/23/00	84	55	--	--	--	--	--	--	--	--	--	--
05/21/02	119	210	absent	--	29	79	81	present	present	1,700	<1	<1
06/17/02	108	970	absent	--	34	290	110	present	present	800	<1	10
07/15/02	84	1,300	absent	--	12	170	150	present	present	1,100	<1	8
Botzum												
05/03/00	--	580	--	--	--	--	--	--	--	--	--	--
05/10/00	474	**8,900**	--	--	--	--	--	--	--	--	--	--
05/17/00	283	**170**	--	--	--	--	--	--	--	--	--	--
05/24/00	987	1,700	present	*S. muenster*	7	--	120	present	absent	--	--	--
05/25/00	811	730	--	--	--	--	--	--	--	--	--	--
07/06/00	317	2,600	--	--	--	--	--	--	--	--	--	--
07/12/00	217	2,400	absent	--	--	--	--	--	--	--	--	--
07/18/00	486	3,200	present	*S. braenderup*	6	--	73	present	absent	--	--	--
07/19/00	511	900	--	--	--	--	--	--	--	--	--	--
07/26/00	226	1,600	--	--	--	--	--	--	--	--	--	--
09/05/00	224	490	--	--	--	--	--	--	--	--	--	--

Appendix B.   Streamflow and microbiological data for Cuyahoga River samples and Akron Water Pollution Control Station (WPCS) effluent samples collected in 2000 and 2002.—Continued

[ft³/s, cubic feet per second; col/100 mL, colonies per 100 milliliters; plaques/100 mL, plaques per 100 milliliters; MPN/100 L, most probable number per 100 liters; oocysts/10 L, oocysts per 10 liters; cysts/10 L, cysts per 10 liters;--, no data; **bold**, results based on colony count outside the ideal range of 20-80 colonies per plate; <, less than]

Date	Stream-flow (ft³/s)	Escherichia coli (col/100 mL)	Salmonella (presence/absence)	Salmonella serotype	F-specific coliphage (plaques/100 mL)	Somatic coliphage (plaques/100 mL)	Infectious enterovirus (MPN/100 L)	Enterovirus (presence/absence)	Hepatitis A virus (presence/absence)	Clostridium perfringens (col/100 mL)	Cryptosporidium (oocysts/10 L)	Giardia (cysts/10 L)
09/11/00	314	12,000	--	--	--	--	--	--	--	--	--	--
09/12/00	226	2,600	present	S. java	--	--	--	--	--	--	--	--
09/19/00	228	700	present	S. oranien-burg	3	--	<16	present	absent	--	--	--
09/26/00	521	730	--	--	--	--	--	--	--	--	--	--
05/22/02	992	7,300	absent	--	24	63	110	present	present	370	<1	<1
06/18/02	413	440	absent	--	8	47	110	present	absent	350	<1	16
07/16/02	176	410	absent	--	63	470	190	present	present	340	<1	<1
Jaite												
05/03/00	--	900	--	--	--	--	--	--	--	--	--	--
05/11/00	429	430	--	--	--	--	--	--	--	--	--	--
05/17/00	300	**160**	--	--	--	--	--	--	--	--	--	--
05/18/00	320	730	--	--	--	--	--	--	--	--	--	--
05/24/00	1,090	2,300	absent	--	18	--	--	--	--	--	--	--
05/25/00	896	1,100	--	--	--	--	--	--	--	--	--	--
07/06/00	309	710	--	--	--	--	--	--	--	--	--	--
07/07/00	331	680	--	--	--	--	--	--	--	--	--	--
07/11/00	356	5,500	present	S. seftenberg	--	--	--	--	--	--	--	--
07/17/00	488	4,600	present	S. newport	4	--	--	--	--	--	--	--
07/25/00	248	270	--	--	--	--	--	--	--	--	--	--
09/06/00	243	**250**	--	--	--	--	--	--	--	--	--	--
09/11/00	396	22,000	present	S. newport	--	--	--	--	--	--	--	--
09/18/00	240	**350**	present	unknown	10	--	--	--	--	--	--	--
09/20/00	253	480	--	--	--	--	--	--	--	--	--	--
09/26/00	524	1,500	--	--	--	--	--	--	--	--	--	--
05/22/02	1,040	2,200	absent	--	4	170	110	present	present	270	<1	<1
06/18/02	472	470	absent	--	6	78	56	absent	absent	360	<1	6
07/16/02	183	240	present	S. arizona	<1	33	190	present	absent	120	<1	<1

Appendix B. Streamflow and microbiological data for Cuyahoga River samples and Akron Water Pollution Control Station (WPCS) effluent samples collected in 2000 and 2002.—Continued

[ft³/s, cubic feet per second; col/100 mL, colonies per 100 milliliters; plaques/100 mL, plaques per 100 milliliters; MPN/100 L, most probable number per 100 liters; oocysts/10 L, oocysts per 10 liters; cysts/10 L, cysts per 10 liters;--, no data; **bold**, results based on colony count outside the ideal range of 20–80 colonies per plate; <, less than]

Date	Stream-flow (ft³/s)	Escherichia coli (col/100 mL)	Salmonella (presence/absence)	Salmonella serotype	F-specific coliphage (plaques/100 mL)	Somatic coliphage (plaques/100 mL)	Infectious enterovirus (MPN/100 L)	Enterovirus (presence/absence)	Hepatitis A virus (presence/absence)	Clostridium perfringens (col/100 mL)	Cryptosporidium (oocysts/10 L)	Giardia (cysts/10 L)
						Independence						
05/03/00	676	1,800	--	--	--	--	--	--	--	--	--	--
05/04/00	550	620	--	--	--	--	--	--	--	--	--	--
05/11/00	454	1,200	--	--	--	--	--	--	--	--	--	--
05/18/00	361	470	--	--	--	--	--	--	--	--	--	--
05/24/00	1,070	1,300	present	S. java	11	--	--	--	--	--	--	--
07/07/00	385	260	--	--	--	--	--	--	--	--	--	--
07/11/00	390	320	present	S. newport	--	--	--	--	--	--	--	--
07/12/00	356	210	--	--	--	--	--	--	--	--	--	--
07/17/00	532	5,700	absent	--	5	--	--	--	--	--	--	--
07/25/00	315	220	--	--	--	--	--	--	--	--	--	--
09/06/00	284	400	--	--	--	--	--	--	--	--	--	--
09/11/00	592	25,000	present	multiple	--	--	--	--	--	--	--	--
09/18/00	340	**320**	absent	--	3	--	--	--	--	--	--	--
09/25/00	854	4,600	--	--	--	--	--	--	--	--	--	--
09/26/00	614	2,600	--	--	--	--	--	--	--	--	--	--

[a]Results were omitted from dataset because concentrations did not exceed levels measured in the equipment blank.