

Concentrations of Escherichia coli in Streams in the Kankakee and Lower Wabash River Watersheds in Indiana, June-September 1999

Water-Resources Investigations Report 01-4018

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By Cheryl A. Silcox, Bret A. Robinson, and Timothy C. Willoughby

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Conversion Factors and Abbreviations

Temperature is given in degrees Celsius (°C), which can be converted to degrees Fahrenheit (°F) by use of the following equation:

 ${}^{\circ}F = 1.8({}^{\circ}C) + 32$

Abbreviated water-quality units used in this report: Chemical concentrations and water temperature are given in metric units. Chemical concentration is given in milligrams per liter (mg/L) or micrograms per liter $(\mu g/L)$. Milligrams per liter is a unit expressing the concentration of chemical constituents in solution as weight (milligrams) of solute per unit volume (liter) of water. One thousand micrograms per liter is equivalent to one milligram per liter. For concentrations less than 7,000 mg/L, the numerical value is the same as for concentrations in parts per million. Concentrations of bacteria are given in colonies per 100 milliliters (col/100 mL).

Specific conductance of water is expressed in microsiemens per centimeter at 25 degrees Celsius (μ S/cm). This unit is equivalent to micromhos per centimeter at 25 degrees Celsius (µmho/cm), formerly used by the U.S. Geological Survey.

Volumes of water-quality samples are given in liters (L) and milliliters (mL).

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Abstract

Water samples collected from 58 surface-water sites in the Kankakee and Lower Wabash River Watersheds from June through September 1999 were analyzed for concentrations of *Escherichia coli* bacteria. Each site was sampled five times in a 30-day period. Twentynine sites were sampled during June and July, and 29 different sites were sampled during August and September. A five-sample geometric mean of concentrations was computed for each site. Concentrations of *Escherichia coli (E. coli)* in 126 of the 289 samples exceeded the State of Indiana single-sample standard of 235 colonies per 100 milliliters for waters used for recreation. Concentrations in samples from 38 of the 58 sites exceeded the State of Indiana standard for a five-sample geometric mean of 125 colonies per 100 milliliters for waters used for recreation.

Ten of the 58 sites were at or near U.S. Geological Survey streamflow-gaging stations. Based on records from the streamflowgaging stations, 18 percent of the samples collected at these sites were collected at streamflows above the median daily discharge for each station.

E. coli concentrations and turbidity measurements collected during 1999 were analyzed in concert with similar concentration and turbidity data collected in 1998 at streams within the Upper Wabash River Watershed in Indiana to investigate the relation between concentrations of bacteria and turbidity. The analysis indicated a statistically significant correlation between concentrations of *E. coli* and turbidity. If the turbidity was greater than 83 nephelometric turbidity units, the *E. coli* concentration always exceeded the singlesample standard. If, however, the turbidity was less than 83 nephelometric turbidity units, concentrations of *E. coli* were not always below the single-sample standard.

Introduction

The presence of *E. coli* in water is direct evidence of the presence of fecal contamination from warm-blooded animals and indicates the possible presence of pathogens (Myers and Sylvester, 1997). *E. coli* is one of the two preferred indicator bacteria used by the U.S. Environmental Protection Agency (USEPA) to determine the suitability of surface waters for recreational use. The water-quality standards for *E. coli* in recreational waters in Indiana require the concentration of *E. coli* to be less than the single-sample standard of 235 colonies per 100 mL (milliliters) and less than the geometric mean of 125 colonies per 100 mL computed from five samples collected within a 30-day period (Oddi, 1995).

The Indiana Department of Environmental Management (IDEM) is responsible for monitoring watersheds in Indiana and reporting the quality of the State's waters to Congress through the State's Report to Congress on Water Quality, 305B Report. As part of this statewide watershed assessment program, IDEM entered into a cooperative agreement with the U.S. Geological Survey (USGS) to measure concentrations of the bacteria *Escherichia coli* (*E. coli*) in the Upper Wabash River Watershed in 1998 (Silcox and others, 2000). Continuing the program in 1999, the USGS measured concentrations of *E. coli* at 58 surface-water sites in the Kankakee and Lower Wabash River Watersheds from June through September.

Purpose and Scope

This report documents the concentrations of *E. coli* measured in samples from selected streams in the Kankakee and Lower Wabash River Watersheds from June through September 1999. The report also discusses the relation between concentrations of *E. coli* and streamflow at sites where streamflow records were available and examines the relation between concentrations of *E. coli* and turbidity. Quality-assurance data for the *E. coli* samples are presented. Field measurements of water temperature, pH, dissolved oxygen, specific conductance, and turbidity collected at the same time as the samples analyzed for *E. coli* also are presented.

Description of the Study Area

Samples were collected in the Kankakee River and Lower Wabash River Watersheds (fig. 1). Within the Kankakee River Watershed, samples were collected from the Indiana-Illinois state line upstream to sites east and north of Plymouth, Ind. The Kankakee River Watershed drains 1,920 mi² (Hoggatt, 1975) in Indiana. Major tributaries to the Kankakee River from which samples were collected include the Little Kankakee River, Pine Creek, Singleton Ditch, the Yellow River, and the Iroquois River. Within the Iroquois River Watershed, which discharges to the Kankakee River in Illinois, samples were collected from 5 mi east of the Indiana-Illinois state line upstream to sites east of Rensselaer, Ind. This watershed drains 661 mi2 in Indiana.

The remaining samples were collected from the Lower Wabash River Watershed, which, for the purposes of this study, extends from Lafayette, Ind., to that point at which the Wabash River discharges to the Ohio River in southwestern Indiana. Major tributaries to the lower Wabash River from which samples were collected include Big Pine Creek, Coal Creek, Sugar Creek, and Big Raccoon Creek. At its mouth, the Wabash River drains 23.921 mi^2 in Indiana.

The study area falls within three distinct physiographic areas (Schneider, 1966) and can be divided roughly into thirds. The northern third of the study area, including the Kankakee River and the Iroquois River Watersheds, falls within the Northern Moraine and Lake Region. This region includes broad moraines composed of glacial till and expansive valleys underlain by thick and topographically subdued sand deposits.

The middle third of the study area, from southern White and Benton Counties in the north to northern Vigo County in the south, falls within the Tipton Till Plain physiographic unit. This physiographic unit is nearly flat in most areas and shows appreciable relief only where river valleys, like the Wabash, have dissected the till plain.

The southern third of the study area, from Terre Haute to the mouth of the Wabash River, is entirely within the Wabash Lowland physiographic unit. In this area, the Wabash River Valley is broad and underlain by thick glacial-outwash deposits. Uplands are described as undulating to rolling and typically stand 100 to 150 ft above the adjacent valley floors (Schneider, 1966).

Study Methods

Selection of the sampling sites, the procedures used to collect the samples to meet a 6-hour sample-holding time limit, and the methods used to measure field parameters are described. Methods

used to collect and process the *E. coli* samples and the equation used to calculate the geometric mean are discussed. Statistical methods used to evaluate the relation between concentrations of *E. coli* and turbidity also are outlined.

Selection of Sampling Sites

Sampling sites initially were selected by IDEM personnel. Responses from a 1987 poll of local health officials, conservation officers, and sheriff's departments regarding known areas of stream recreational uses provided a core list of potential sampling sites. Additional sites were added to improve spatial coverage or to position sites at existing USGS streamflow-gaging stations. Site locations were verified on topographic maps and field verified by USGS personnel prior to sample collection. Where sampling conditions were unsafe or where site characteristics interfered with the ability to collect a sample, the site was relocated as close to the initial site as possible. Changes to sampling-site locations were agreed upon by USGS and IDEM personnel.

The 58 selected sampling sites were divided into two groups so that all sites could be sampled five times at equally spaced intervals within a 30-day period. Figure 2 shows the locations of the sampling sites. Table 1 lists the Group 1 sites (sites 1 through 25, 30, 33, 41, and 42) that were sampled during June and July 1999. Most of the Group 1 sites were in the Kankakee River Watershed. Table 2 lists the Group 2 sites (sites 26 through 29, 31, 32, 34 through 40, and 43 through 58) that were sampled during August and September 1999. All of the Group 2 sites were in the Lower Wabash River Watershed.

Field Measurements

At each sampling site, a multi-parameter water-quality probe was used to make field measurements of water temperature, dissolved oxygen, pH, and specific conductance at several locations across the width of the stream. The probes used

to measure dissolved oxygen, pH, and specific conductance were calibrated daily.

Field determinations of turbidity were made by collecting samples of stream water in polyethylene bottles and analyzing the samples with a portable turbidimeter. The measuring range of the turbidimeter was checked daily with reference standards. Water temperature, dissolved-oxygen concentration, pH, specific conductance, and turbidity were measured at the same locations in the stream where the samples were collected for analysis of *E. coli*.

Collection of Samples

Water samples were collected during the recreational season in Indiana, defined as April through October. The samples were collected by two-person field crews in order to expedite the sampling process and to meet the mandated 6-hour sample-holding time limit prior to processing the samples. Duties at the sampling sites included

- measuring and recording field parameters,
- measuring and recording water-surfaceelevation data by using a measuring tape lowered to the surface of the stream from the top of the bridge or
- documenting the stage levels of streams at sites where streamflow-gaging stations were present or nearby, and
- collecting the water samples for analysis of *E. coli*.

At the time of sampling, the characteristics of stage change, water clarity, and weather conditions also were recorded on the field forms.

Water samples for *E. coli* determinations were collected in 300 mL (milliliter) glass bottles with glass stoppers. Prior to use, the bottles were washed with detergent, rinsed three times with tap water and three times with deionized water, and sterilized by autoclaving. To ensure optimum growth conditions for *E. coli*, two solutions were added to each sample bottle before the bottle was sterilized. To counter the effects of residual chlorine or other halogens used in water-disinfection pro-

Figure 2. Location of *Escherichia coli* sampling sites in the Kankakee and

Table 1. Sites in the Kankakee and Lower Wabash River Watersheds in Indiana at which water samples were collected during June and July 1999 (Group 1 sites) for analysis of Escherichia coli

[USGS, U.S. Geological Survey; SR, State Road; CR, County Road; US, U.S. Highway; E, N, and S, denote the geographic directions of east, north, and south]

6 Concentrations of Escherichia coli, Kankakee and Lower Wabash River Watersheds, Indiana, June–September 1999

Table 2. Sites in the Lower Wabash River Watershed in Indiana at which water samples were collected during August and September 1999 (Group 2 sites) for analysis of Escherichia coli

[USGS, U.S. Geological Survey; SR, State Road; CR, County Road; US, U.S. Highway; I-64, Interstate 64; E, W, and N, denote the geographic directions of east, west, and north]

cesses, 0.3 mL of a 10-percent solution of sodium thiosulfate was added to the bottles. Residual chlorine and other halogen compounds act as bacterial-growth inhibitors; their effects need to be reduced so that *E. coli* can fully recover on the growth medium and produce accurate counts (Bordner and Winter, 1978; American Public Health Association and others, 1992). In addition, 0.9 mL of a 15-percent solution of ethylenediaminetetraacetic acid (EDTA) was added to neutralize the effects of trace-element concentrations greater than 10 µg/L (micrograms per liter). EDTA, a chelating agent, binds particularly with copper and zinc, making the metals neutral so that they do not adversely affect bacterial growth (Britton and Greeson, 1989, p. 5–6; Bordner and Winter, 1978; American Public Health Association and others, 1992).

In the field, a weighted hand-line sampler that held the sample bottle was lowered beneath the surface of the water. Some samples were collected by immersing the bottles by hand when the stream was too shallow for the hand-line sampler. At each site, the sample was a composite of water from one to six well-mixed areas of flow, depending on the width of the stream. The samples were kept on ice until processed. Duplicate samples were collected concurrently with the environmental samples at selected sites.

Processing of Samples

Equipment used to process the samples was washed with detergent prior to field work, rinsed three times with hot tap water and three times with deionized water, and then sterilized with an 8-watt ultraviolet (UV) lamp having a wavelength of 254 nanometers for a minimum of 15 minutes. Processing equipment included a multi-port manifold filter stand, stainless-steel filter holders, vacuum pumps, sterile disposable pipets, and glass graduated cylinders. After the samples were processed, aluminum-block incubators were used to provide optimum conditions for bacterial growth.

The USGS Ohio District Microbiological Laboratory prepared and provided fresh

membrane-filter Thermotolerant (mTec) agar on which the *E. coli* were grown*.* The fresh mTec agar was poured into petri dishes in the USGS Indiana District laboratory for use in the field. Refrigeration units were used to keep the mTec agar chilled before being used. The 2-week holding time for mTec agar, once it was prepared, was monitored in the laboratory and field. Urea/phenol red reagent was obtained from the USGS Quality of Water Services Unit (QWSU), prepared in the field, and used to confirm the presence of *E. coli* colonies.

The samples collected for analysis of *E. coli* were processed either in hotel rooms or in the USGS Indiana District laboratory. Surfaces on which the samples were processed were cleaned with isopropyl alcohol before the first sample was processed, between samples, and after the last sample was processed each day. Analysts washed their hands with bactericidal soap before processing the first sample, between samples, and after processing the last sample.

Five to eight different sample volumes, including one to three different dilutions, were filtered for each site because the extent of possible *E. coli* contamination was unknown at each site. A range of small to large sample volumes and dilutions was used for processing. This was done to obtain at least one sample volume capable of producing one or more filter plates with sufficient colony growth to obtain an ideal *E. coli* colony count of 20 to 80 colonies per filter plate (Myers and Sylvester, 1997). Stream conditions at the time of each sampling and previous colony counts for each site obtained after the first week of sampling guided the analysts in determining the quantity and types of sample volumes to process to obtain one or more filter plates in an ideal range. Sample dilutions were made by adding 11 mL of sample water to 99 mL of sterile dilution water for a 1:10 ratio and 1 mL of sample water to another 99 mL of sterile dilution water for a 1:100 ratio.

Samples were shaken vigorously before each sample dilution volume was withdrawn to ensure uniform distribution of the bacteria throughout the sample. Sterile, disposable 1-mL and 10-mL glass pipets were used to measure and deliver concentrated sample volumes to dilution bottles and to

measure and deliver dilution volumes to the interior of the funnel filter assembly. For sample dilution volumes less than 10 mL, about 20 mL of sterile saline buffer solution was poured into the funnel before pipetting the sample dilution to evenly distribute the bacteria on the filter. A sterile graduated cylinder was used to transfer sample dilution volumes greater than 10 mL. A three-port manifold with funnels or a single-use stainless-steel-filter system was used to support a 0.45-µm (micron) filter designed to facilitate colony capture, incubation, and quantification. The water was pulled through the filter either by a vacuum pump set not to exceed 5 lb/in^2 or by a hand vacuum assembly.

After filtering each of the sample dilution volumes, 20 to 30 mL of sterile saline buffer solution were used to flush the sides of the funnel to ensure that any bacteria present on the funnel walls were rinsed on to the filter. The graduated cylinders used to measure and deliver sample dilution volumes to the funnel also were rinsed with sterile saline buffer solution, and the rinsate was processed through the filter.

Petri dishes containing the mTec agar used to encourage growth of *E. coli* colonies on the prepared filters were labeled prior to processing the sample. Undiluted (environmental) samples and sample dilutions were filtered from smallest to largest. The filters then were placed in petri dishes with the mTec agar and placed inverted in a preheated incubator set at 35.0°C for 1.75 to 2 hours, removed, and then placed in a preheated incubator set at 44.5°C for 22 to 24 hours. After the second incubation period was completed, the filter was transferred to a filter pad saturated with urea/phenol red reagent. After 15 to 20 minutes at room temperature, the yellow to yellow-brown *E. coli* colonies were counted. If the filter plate had a colony count in the ideal range, verification of the count was made either by the second crew member or by rotating the filter 90 degrees and recounting the colonies. Concentrations of *E*. *coli* were calculated according to the methods described by (Myers and Sylvester, 1997, p. 31–33-FIB) and recorded on the field sheet. If more than one dilution were within the ideal colony count, the concentration of *E. coli* was computed as the sum of the colony counts for

each sample volume multiplied by 100 and divided by the sum of the sample volumes. For example, if 24 colonies were counted for a sample volume of 3 ml and 60 colonies were counted for a sample volume of 10 ml, the concentration of *E. coli* is calculated as follows:

 $Col/100$ mL = $(24 + 60)$ x $100/(3 + 10) = 646$ (1)

where:

- $Col/100$ mL = colonies per 100 milliliters;
	- $24 + 60$ are the colony counts on two different filter plates;
		- $3 + 10$ are the sample volumes filtered for each plate.

Reporting whole numbers as two significant figures for results greater than or equal to 10 resulted in a reporting value of 650 colonies per 100 mL. The same calculation was used if *E. coli* colonies were present but none of the dilutions had concentrations of *E. coli* within the ideal colony count. In these cases, all dilutions having colonies present were used in the calculation and reported as an estimate (denoted with a "K"). If no *E. coli* colonies were present, a value of one colony was assigned to the largest sample volume filtered and the same calculation method was used. These calculations, using one colony for the largest sample volume filtered, were reported as the calculated number preceded by a less than symbol. Colony counts were recorded on field sheets labeled for each site. Concentrations of *E. coli* were reported in whole numbers for results less than 10, and results greater than or equal to 10 were reported in two significant figures (Myers and Sylvester, 1997, p. 30-FIB). After counting the number of colonies, petri dishes were filled with chlorine bleach, placed in sealed plastic bags, and discarded.

Geometric Mean

The five-sample geometric mean was calculated, using equation 2.

$$
GM = 5\sqrt{S_1 \cdot S_2 \cdot S_3 \cdot S_4 \cdot S_5}
$$
 (2)

where:

GM is the geometric mean, and

 Si is the concentration of *E. coli* measured in each of the five samples.

One field form was lost before the *E. coli* concentration could be entered into the data base for site 16. The sample was collected on June 30, 1999. To compute the five-sample geometric mean for this site, the value of one colony per 100 mL was substituted for the lost *E. coli* concentration. If the five-sample geometric mean exceeded the standard after the substitution was made, which it did, any value selected for substitution would have produced a five-sample geometric mean that exceeded the standard.

Statistical Analysis

A Wilcoxon signed-rank test (Helsel and Hirsch, 1992, p.142) was used to determine if there were statistically significant differences between the environmental samples and the concurrent duplicates. The Wilcoxon signed-rank test measured whether one group of data produced larger observations than the second group and made no assumptions regarding how the data were distributed (Helsel and Hirsch, 1992, p.118).

A Kendall's Tau test (Helsel and Hirsch, 1992, p. 212) for significant correlation was used to determine if there was a statistically significant correlation between the concentration of *E. coli* and turbidity. For this report, a five-percent level of significance (a=0.05) was identified as the criterion for the statistical correlation. The p-value is derived from the data and measures the believability of the null hypothesis (no correlation exists between concentrations of *E. coli* and turbidity). The smaller the p-value, the more likely there is a correlation between concentrations of *E. coli* and turbidity and the stronger the evidence for rejection of the null hypothesis.

Quality-Assurance and Quality-Control Procedures

Quality-assurance and quality-control procedures were followed for collection and processing of the samples. These procedures include frequent checking and calibration of equipment as well as collection of additional samples for quality control.

Analysis of the quality-control samples provides quantitative information not only about the potential for sample contamination during collection and processing but also about the variability of sampling.

The pH buffers and specific-conductance solutions used to calibrate the multi-parameter probe were quality assured by the QWSU. The multi-parameter water-quality measuring meter was calibrated daily for pH, specific conductance, and dissolved oxygen before any field measurements were made. The measuring range of the portable turbidimeter used to measure turbidity in the surface-water samples was checked daily with reference standards. If parameters measured in the field were not stable or if they did not have reasonable values, the meters were recalibrated at the site and field parameters were remeasured.

E. coli fresh substrate media kits were quality assured by the USGS Ohio District Microbiological Laboratory. Membrane filters, sterile saline buffer solution, premeasured sterile dilution water, petri dishes, and petri dishes with pads also were quality assured by the QWSU. The incubators were checked weekly with an American Society for Testing and Materials (ASTM) certified thermometer to assure that temperature ranges shown on the internal thermometer in the incubator were accurate to ±0.5°C. The incubators were inspected daily to assure they were operating properly.

Quality-control samples consisted of 289 filter blanks, 71 process blanks, 19 field blanks, and 39 duplicate samples. Results of the qualityassurance and *E. coli* determinations are presented in tables 3 and 4 at the back of this report. Blanks and duplicate samples are discussed separately.

Filter Blanks

Filter blanks were processed before every set of samples to determine if the equipment used to process the samples was clean and the saline buffer solution used to rinse sample-processing equipment was not contaminated. Filter blanks were processed for all 289 samples collected and were acquired by passing 100 ml of the sterile saline buffer solution

through the filter prior to processing any dilutions of the environmental samples. While passing the saline buffer solution through the filter, every attempt was made to have the saline buffer solution come in contact with every surface that the environmental sample might touch. One filter blank for site 58 had eight observable *E. coli* colonies. The presence of the colonies on the filter blank indicates that the sample-processing glassware had been contaminated, the saline buffer solution used for the sample set was contaminated, or the analyst made a procedural error. The results for site 58 are not greatly affected, however, because the *E. coli* concentration for that sample was very low and below the ideal colony-count range, resulting in the data being flagged as estimated.

Process Blanks

Process blanks consisted of 100 mL of saline buffer solution filtered through the same equipment used to process samples, ensuring the equipment rinses that followed the filtering of each sample were adequate. Process blanks were filtered for one sample daily. Sixteen of the 71 process blanks contained observable *E. coli* colonies. The maximum concentration of *E. coli* measured in the process blanks was seven colonies per 100 mL. All of the process blanks that had observable *E. coli* colonies contained less than 1 percent of the concentration of *E. coli* measured in the environmental samples; therefore, the sample results were not affected and nothing was done to modify the data.

Field Blanks

Nineteen field blanks were filtered during the length of the study on randomly selected days determined by the analysts. Field blanks consisted of 250 mL of sterile saline buffer solution that was poured into a sample-collection bottle. The field blanks were kept chilled and remained with the samples collected at all sites for that day. The field blanks were processed by passing 100 mL of the blank solution through the filter. None of the 19 field blanks had observable *E. coli* colonies, indicating that there was no contamination resulting

from transporting the samples and that there was adequate sterilization of the sample-collection bottles.

Duplicate Samples

Duplicate samples were collected concurrently with the environmental samples at selected sites. The concurrent duplicates were processed in the same manner as the environmental samples and were used to evaluate the natural variability in the samples.

Figure 3 displays the differences between concentrations of *E. coli* measured in the environmental samples and duplicate samples and the natural log percent difference between the two. The median natural log percent difference between the environmental samples and the duplicate samples was 8 percent. No statistically significant differences between the environmental samples and the concurrent duplicate samples were determined at the 5-percent significance level. The significance level obtained by the data, or the pvalue (Helsel and Hirsch, 1992, p.112), was 0.623.

Concentrations of Escherichia coli

The Indiana environmental rules establish the bacteriological quality standard for waters for recreational uses (Oddi, 1995). These rules are used to evaluate waters for full-body-contact recreational uses, to establish wastewater-treatment requirements, and to establish effluent limits during the recreational season. The standard states:

> E. coli bacteria, using membrane filter (MF) count, shall not exceed one hundred twenty-five (125) per one hundred (100) milliliters as a geometric mean based on not less than five (5) samples equally spaced over a thirty (30) day period nor exceed two hundred thirty-five (235) per one hundred (100) milliliters in any one (1) sample in a thirty (30) day period.

Table 3, at the back of the report, lists field measurements and concentrations of *E. coli* for

Figure 3. Concentrations of Escherichia coli measured in the environmental samples and duplicate samples and the natural log percent difference. Samples were collected in the Kankakee and Lower Wabash River Watersheds in Indiana, June–September 1999.

all 58 sites. The five-sample geometric mean is shown below the last sample-collection date for each site. Figure 4 shows the range in concentrations of *E. coli* for the five samples collected at each site, and figure 5 shows the five-sample geometric-mean concentration of *E. coli* determined for each site.

Concentrations of *E. coli* at all 58 sites ranged from less than 1 to greater than 8,000 colonies per 100 mL, with concentrations in 126 of the 289 samples processed exceeding the single-sample standard in samples from 40 sites. The five-sample geometric mean of concentrations of *E. coli* for all sites ranged from 4 to 1,400 colonies per 100 mL. The concentrations of *E. coli* in samples from 38 sites exceeded the five-sample geometric-mean standard.

Concentrations of *E. coli* were examined relative to their location at sampling sites along several stream reaches in the Kankakee and Lower Wabash River Watersheds (figs. 6 and 7). Stream reaches included the Kankakee, Yellow, and Iroquois Rivers in the Kankakee River Watershed and the Big Pine, Sugar, Big Raccoon, and Coal Creeks and the Wabash River in the Wabash River Watershed. Fifty-three of 85 samples from 17 sites on the Kankakee, Yellow, and Iroquois Rivers had concentrations of *E. coli* that exceeded the single-sample standard. Concentrations of *E. coli* exceeded the standard for the five-sample geometric mean at all sites (fig. 6).

Twenty of 35 samples from all seven sites (1, 5, and 7–11) on the Kankakee River had concentrations of *E. coli* that exceeded the single-sample standard. Concentrations of *E. coli* in samples from the Kankakee River ranged from 32 to 2,100 colonies per 100 mL. The five-sample geometric means ranged from 150 to 370 colonies per 100 mL.

Twenty-one of 29 samples from all six sites (14–19) on the Yellow River had concentrations of *E. coli* that exceeded the single-sample standard. Concentrations of *E. coli* in samples from the Yellow River ranged from 73 to 5,100 colonies per 100 mL. The five-sample geometric means ranged from 190 to 1,400 colonies per 100 mL.

Twelve of 20 samples from four sites (20, 21, 23, and 25) on the Iroquois River exceeded the single-sample standard. Concentrations of *E. coli* in samples from the Iroquois River ranged from less than 5 to 3,600 colonies per 100 mL. The fivesample geometric means ranged from 160 to 1,100 colonies per 100 mL.

Eight sites having only one or two sampling locations on a particular stream are shown as "other sites in the Kankakee River Watershed" (fig. 6). Thirty-one of 40 samples collected from these eight sites had concentrations of *E. coli* that exceeded the single-sample standard, and all sites exceeded the five-sample geometric mean. Concentrations of *E. coli* in these samples ranged from an estimated 65 to greater than 8,000 colonies per 100 mL. The five-sample geometric means ranged from 370 to 770 colonies per 100 mL.

Twenty-six of 55 samples from 11 sites on Big Pine, Sugar, Big Raccoon, and Coal Creeks and the Wabash River had concentrations that exceeded the single-sample standard, and seven of the sites exceeded the standard for the fivesample geometric mean (fig. 7). Five of 10 samples from two sites (26 and 28) on Big Pine Creek had concentrations of *E. coli* that exceeded the singlesample standard, and site 26 exceeded the standard for the five-sample geometric mean. All 10 samples from two sites on Big Pine Creek (27 and 29) had concentrations that did not exceed the single-sample standard; however, the five-sample geometric-mean standard was exceeded at site 27. Concentrations of *E. coli* in samples from Big Pine Creek ranged from 17 to 700 colonies per 100 mL. The five-sample geometric means ranged from 29 to 400 colonies per 100 mL.

Three of 10 samples from two sites (41 and 42) on Sugar Creek had concentrations of *E. coli* that exceeded the single-sample standard. Both of these sites exceeded the standard for the fivesample geometric mean. Samples from sites 43 through 45 on Sugar Creek did not exceed either standard. Concentrations of *E. coli* in samples from Sugar Creek ranged from 7 to 1,500 colonies per 100 mL, and the five-sample geometric means ranged from 29 to 280 colonies per 100 mL.

Figure 4. Ranges in concentrations of Escherichia coli for sampling sites in the Kankakee

Figure 5. Five-sample geometric mean Escherichia coli concentration determined for sampling sites

 \triangle E. coli concentration based upon non-ideal colony counts

Figure 6. Concentrations of *Escherichia coli* and five-sample geometric means for Group 1 sites in the Kankakee River Watershed in Indiana, June–July 1999.

Figure 6. Concentrations of Escherichia coli and five-sample geometric means for Group 1 sites in the

EXPLANATION

Five-sample geometric mean E. coli water-quality standard for full-body contact Single-sample E. coli water-quality standard for full-body contact Five-sample geometric mean E. coli concentration \ast Single-sample E. coli concentration $^{+}$

E. coli concentration based upon non-ideal colony counts \triangle

Figure 7. Concentration of *Escherichia coli* and five-sample geometric means for Group 1 and Group 2 sites in the Lower Wabash River Watershed in Indiana, June–September 1999.

Figure 7. Concentration of Escherichia coli and five-sample geometric means for Group 1 and Group 2 sites in the Lower Wabash River Watershed in Indiana, June–September 1999—Continued.

Seven of 15 samples from three sites (49–51) on Big Raccoon Creek had concentrations of *E. coli* that exceeded the single-sample standard, and site 49 exceeded the standard for the five-sample geometric mean. Concentrations of *E. coli* in samples from Big Raccoon Creek ranged from 13 to 970 colonies per 100 mL. The five-sample geometric means ranged from 79 to 490 colonies per 100 mL. All 10 samples from two sites on Big Raccoon Creek (52 and 53) had concentrations that did not exceed the single-sample standard or the five-sample geometric-mean standard.

Ten of 15 samples from three sites (34, 35, and 37) on Coal Creek had concentrations that exceeded the single-sample standard. All of these sites had concentrations that exceeded the five-sample geometric-mean standard. Concentrations of *E. coli* in samples from Coal Creek ranged from 87 to 800 colonies per 100 mL. The five-sample geometric means ranged from 130 to 470 colonies per 100 mL.

Concentrations for three samples from site 30 on the Wabash River exceeded the single-sample standard. The five-sample geometric mean for site 30 also exceeded the five-sample standard. Site 30 was the only site on the Wabash River to exceed either the single-sample or the five-sample geometric-mean standards. Concentrations of *E. coli* in samples from the Wabash River sites ranged from less than 1 to 8,000 colonies per 100 mL. The five-sample geometric means ranged from 4 to 380 colonies per 100 mL.

Two sites having only one sampling location on a particular stream are shown as "other sites in the Lower Wabash River Watershed" on figure 7. Seven of 10 samples from these two sites had concentrations of *E. coli* that exceeded the single-sample standard. One of the two sites had concentrations of *E. coli* that exceeded the fivesample geometric mean.

Relation between Concentrations of Escherichia coli and Streamflow

The relation of concentration of *E. coli* to stream discharge was examined for selected sites in the Kankakee and Lower Wabash River Watershed

(figs. 8 and 9). Streamflow conditions varied during the 5-week sample-collection periods, June–July and August–September. This study was dependent more on collecting samples at the prescribed sample-collection times (five samples collected at evenly spaced intervals within a 30-day period) rather than at particular streamflow conditions. Stream discharges presented in this report were taken from data collected at USGS streamflowgaging stations where stage-discharge relations have been developed. Ten of the 58 sites were at or near streamflow-gaging stations. Based on records of streamflow from these stations (Stewart and others, 2000), 18 percent of the samples collected at these sites were collected at discharges above the long-term daily mean discharge. Six of the Group 1 sites were at or near streamflow-gaging stations and 30 percent of the samples were collected at discharges above the long-term median daily mean discharge. Four of the Group 2 sites were at or near streamflow-gaging stations and none of the samples were collected at discharges above the long-term median daily mean discharge reported by Stewart and others (2000).

Analysis of figures 8 and 9 indicates that although concentrations of *E. coli* can exceed the single-sample standard during low stream discharge, the standard is always exceeded at discharges greater than the median daily mean.

Relation between Concentrations of Escherichia coli and Turbidity

To determine if there was a relation between concentrations of *E. coli* and turbidity, data for *E. coli* and turbidity from samples collected from 1998 (Silcox and others, 2000) and the study in 1999 were combined to provide a larger statistical base. Sixty-two percent of the samples were collected at discharges above the long-term median daily mean discharge during 1998, compared to 18 percent during 1999.

Figure 8. Stream discharge and concentrations of Escherichia coli at selected Group 1 sites in the Kankakee River Watershed in Indiana, June–July 1999.

22 Concentrations of Escherichia coli, Kankakee and Lower Wabash River Watersheds, Indiana, June–September 1999

Site 12: Singleton Ditch at Schneider

Figure 8. Stream discharge and concentrations of Escherichia coli at selected Group 1 sites in the Kankakee

Figure 8. Stream discharge and concentrations of Escherichia coli at selected Group 1 sites in the Kankakee River Watershed in Indiana, June–July 1999—Continued.

24 Concentrations of Escherichia coli, Kankakee and Lower Wabash River Watersheds, Indiana, June–September 1999

Figure 9. Stream discharge and concentrations of Escherichia coli at selected Group 2 sites in the Lower

Relation between Concentrations of Escherichia coli and Turbidity 25

DISCHARGE, IN CUBIC FEET PER SECOND

26 Concentrations of Escherichia coli, Kankakee and Lower Wabash River Watersheds, Indiana, June–September 1999

Figure 10 displays scatter plots of concentration of *E. coli* versus turbidity for 1998, 1999, and for 1998 and 1999 combined. Turbidity and *E. coli* data were examined for the 2-year period because they were collected in the same way in both years. Data collected at these sites represented watersheds with different land uses and runoff rates and were sampled at different times.

A locally weighted scatterplot smoothing technique (LOWESS) was used to generate the line in the plot for the 1998 and 1999 combined data. The LOWESS method depicts the relation between turbidity and *E. coli* and accommodates outlying data (Helsel and Hirsch, 1992, p. 48). A statistically significant correlation (p<0.001) was determined between concentrations of *E. coli* and turbidity for the 2-year data composite. If the turbidity measured during sample collection was greater than 83 NTU (nephelometric turbidity units), the sample always had concentrations of *E. coli* above the singlesample standard, indicating that runoff is a major factor affecting *E. coli* concentrations. If, however, the measured turbidity was less than 83 NTU, the concentrations of *E. coli* were not always below the single-sample standard, indicating other environmental or anthropogenic factors besides turbidity are influencing the concentrations of *E. coli*.

Summary

Water samples collected from 58 stream sites in the Kankakee and Lower Wabash River Watersheds from June through September 1999 were analyzed for concentrations of *E. coli*. Samples were collected at 29 sites during June and July, and at 29 different sites during August and September. A five-sample geometric mean was computed for each site. The five-sample geometric-mean concentrations ranged from 4 to 1,400 colonies per 100 mL, and concentrations for 38 sites exceeded the five-sample geometric-mean standard of 125 colonies per 100 mL. Of the 289 individual samples processed, 126 exceeded the single-sample standard of 235 colonies per 100 mL. Concentrations of *E. coli* ranged from less than 1 to greater than 8,000 colonies per 100 mL during the study.

Ten of the 58 sites were at or near USGS streamflow-gaging stations. Based on records from these stations, 18 percent of the samples collected at these sites were collected at streamflows above the median daily mean discharge.

E. coli concentration data and turbidity measurements collected in 1998 and 1999 showed a statistically significant correlation. The concentration of *E. coli* always exceeded the single-sample standard when the turbidity exceeded 83 NTU; however, when the measured turbidity was less than 83 NTU, concentrations of *E. coli* were not always below the single-sample standard.

Figure 10. Turbidity and concentrations of Escherichia coli measured in samples collected at sites in the Kankakee and the Upper and Lower Wabash River Watersheds in Indiana, June–September 1998 (wet year) and 1999 (dry year).

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Supplemental Data

(Tables 3 and 4)

Table 3. Water-quality data for sampling sites in the Kankakee and Lower Wabash River Watersheds, June–September 1999

(geometric mean for site 16 computed by substituting a value of one colony per 100 milliliters for missing data, as explained on p. 10 of text)

^aFive-sample geometric mean was calculated by use of one or more samples having non-ideal colony counts.

[Time is in military notation; -- , no data; K, non-ideal colony count; < , less than; > , greater than]

