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Use of DNA Markers for Investigating Sources of Bacteria in Contaminated Ground Water: Wooster Township, Wayne County, Ohio

By Denise H. Dumouchelle

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Conversion Factors

Multiply	By	To obtain
Length		
inch (in.)	2.54	centimeter (cm)
inch (in.)	25.4	millimeter (mm)
foot (ft)	0.3048	meter (m)
Volume		
gallon (gal)	3.785	liter (L)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:
 $^{\circ}\text{F}=(1.8\times^{\circ}\text{C})+32$

Small sample volumes are reported in milliliters (mL) and microliters (μL). Concentrations of chemical constituents in water are given either in milligrams per liter (mg/L), micrograms per liter ($\mu\text{g/L}$), or nanograms per microliter (ng/ μL).

Bacteria concentrations are given in colony-forming units per 100 milliliters (CFU/100 mL).

Use of DNA Markers for Investigating Sources of Bacteria in Contaminated Ground Water: Wooster Township, Wayne County, Ohio

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Abstract

In 2004, a public-health nuisance was declared by the Wayne County Board of Health in the Scenic Heights Drive-Batdorf Road area of Wooster Township, Wayne County, Ohio, because of concerns about the safety of water from local wells. Repeated sampling had detected the presence of fecal-indicator bacteria and elevated nitrate concentrations. In June 2006, the U.S. Geological Survey (USGS), in cooperation with the Ohio Environmental Protection Agency (Ohio EPA), collected and analyzed samples from some of the affected wells to help investigate the possibility of human-origin bacterial contamination. Water samples from 12 wells and 5 home sewage-treatment systems (HSTS) were collected. Bromide concentrations were determined in samples from the 12 wells. Samples from 5 of the 12 wells were analyzed for wastewater compounds. Total coliform, enterococci and *Escherichia coli* (*E. coli*) bacteria concentrations were determined for samples from 8 of the 12 wells. In addition, two microbial source-tracking tools that employ DNA markers were used on samples from several wells and a composite sample of water from five septic tanks. The DNA markers from the *Enterococcus faecium* species and the order *Bacteroidales* are associated with specific sources, either human or ruminant sources.

Bromide concentrations ranged from 0.04 to 0.18 milligrams per liter (mg/L). No wastewater compounds were detected at concentrations above the reporting limits. Samples from the 12 wells also were collected by Ohio EPA and analyzed for chloride and nitrate. Chloride concentrations ranged from 12.6 to 61.6 mg/L and nitrate concentrations ranged from 2.34 to 11.9 mg/L (as N).

Total coliforms and enterococci were detected in samples from 8 wells, at concentrations from 2 to 200 colony-forming units per 100 milliliters (CFU/100 mL) and 0.5 to 17 CFU/100 mL, respectively. *E. coli* were detected in samples from three of the eight wells, at concentrations of 1 or 2 CFU/100 mL. Tests for the human-specific marker of enterococci, the *esp* gene, were negative in the seven samples tested, including the composite sample of HSTS water. DNA with the general *Bacteroidales* marker was detected in samples from four wells, but the tests for both the human- and ruminant-associated markers were negative. The presence of the PCR (polymerase chain reaction) -detectable DNA for the general fecal *Bacteroidales* marker is indicative of fecal contamination and recently recharged water.

Introduction

In October 2004, the Wayne County Board of Health declared a public-health nuisance in the Scenic Heights Drive-Batdorf Road area of Wooster Township, Ohio (fig. 1) because of concerns about the safety of the local water supply. In January 2006, at the request of Wayne County officials, the Ohio Environmental Protection Agency (Ohio EPA) began an investigation. Repeated sampling of the private water wells in the area found elevated nitrate concentrations and total coliform and *Escherichia coli* (*E.*

coli) bacteria on multiple occasions (Ohio Environmental Protection Agency, 2006). One possible source of contamination is nearby home sewage-treatment systems (HSTS); other possible sources include domestic pets and wildlife (bacteria) and fertilizers (nitrate).

Several water-quality and microbial source tracking (MST) methods can be used to indicate sewage contamination. Unfortunately, other potential sources can complicate interpretation of the data from many of these methods. Therefore, studies have found that use of multiple methods, including chemical and microbiological indicators, is the best approach for evaluating potential sewage contamination (K. E. Hyer, U.S. Geological Survey, written commun., 2006; Dumouchelle, 2006; Noble and others, 2006). To help identify whether the observed bacterial contamination originated from a human source, the U.S. Geological Survey (USGS), in cooperation with the Ohio EPA, collected and analyzed samples from wells in the Scenic Heights Drive-Batdorf Road area. In particular, the USGS used two MST tools that employ human-associated DNA markers found in bacterial strains of the order *Bacteroidales* and the species *Enterococcus faecium* (Bernhard and Field, 2000; Dick and others, 2005; Scott and others, 2005). Additionally, the presence of the general *Bactroidales* marker was used as another indicator of fecal contamination. The USGS Ohio Water Science Center is testing MST methods for their utility in combination with more traditional tools for contaminant-source identification. The present study is one of several case studies in Ohio.

Purpose and Scope

The purpose of this report is to present data from ground-water samples collected from selected residential wells in the Scenic Heights Drive-Batdorf Road area and to describe the use of DNA markers for investigating the sources of bacteria in contaminated ground water. In June 2006, the USGS collected and analyzed water samples from 12 wells and 5 HSTS. Bromide concentrations were determined at all 12 wells. Wastewater compounds were analyzed in samples from 5 of the 12 wells. Total coliform, enterococci, and *E. coli* bacteria concentrations were determined for samples from 8 of the 12 wells. Analysis of DNA markers was done on water samples from seven wells. A composite sample of HSTS water from 5 of the 12 properties where wells were sampled was analyzed for DNA markers.

Ohio EPA concurrently collected water from the same 12 wells and analyzed the samples for chloride and nitrate concentrations. Data from samples collected and analyzed by the Ohio EPA also are presented.

Description of the Study Area

The study area is in Wooster Township, southwest of the city of Wooster in northeastern Ohio, near the intersection of Batdorf and Tolbert Roads (fig. 1). The land use is rural residential, with most homes on lots ranging from half an acre to more than an acre. The homes are served by residential water wells and leach-line HSTSs. The residential wells in the area are from 70 to 185 ft deep and are completed as open holes in fractured sandstone, siltstone, and shale. The Ohio Department of Health used a downhole camera in July 2003 to investigate a well in the vicinity of WN-36 (fig. 1), and numerous fractures and openings were observed both above and below the water level (Craig Smith, Ohio Environmental Protection Agency, written commun., 2006). In addition, during the sampling for the current study, cascading water was heard in one well (WN-40).

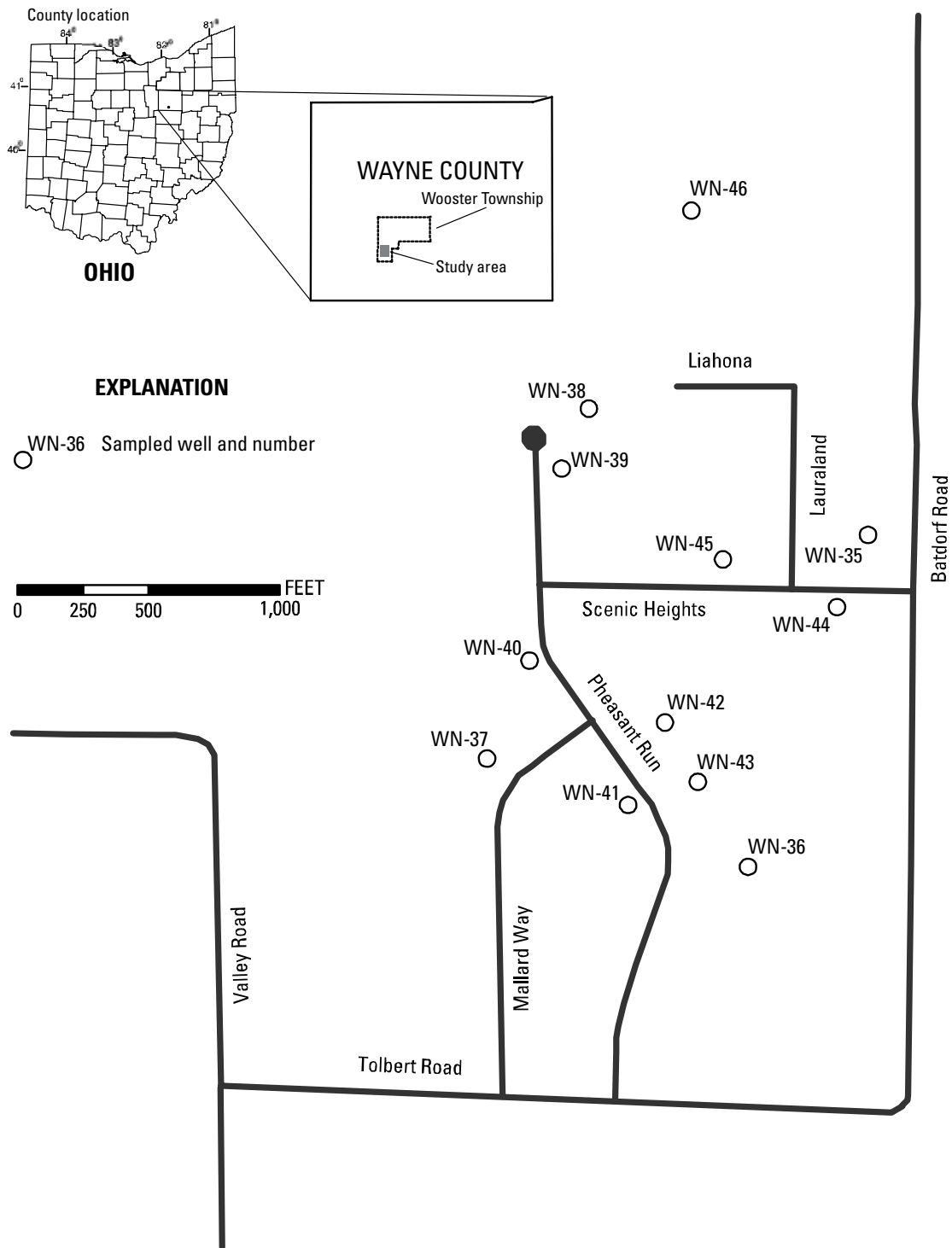


Figure 1. Location of study area and wells sampled in Scenic Heights Drive-Batford Road area, Wayne County, Ohio.

Sampling and Analytical Methods

Selection of wells was based on results of previous sampling by various agencies. Five wells with frequent detections of fecal-indicator bacteria were selected for analysis of wastewater compounds, bacteria, and bromide. Three additional wells were selected for analysis of bacteria and bromide. Another three wells were selected for the analysis of bromide only. A twelfth well, just north of study area, was sampled and analyzed for bromide to obtain background concentration data.

Homeowners of the selected wells gave permission for sampling and identified household spigots from which untreated water could be drawn. All samples were collected from these spigots as supplied by existing plumbing and well pumps. In most cases, an outside spigot was used as the sampling point. Sterile tubing and fittings were connected to the spigot for purging and sample collection. Although the sampled wells were in regular use, all wells were purged before sample collection. During purging, field measurements of pH, specific conductance, dissolved oxygen concentration, and temperature were made. Water samples were collected after the field measurements had stabilized.

The wastewater compounds and bromide analyses were done by the USGS National Water-Quality Laboratory in Denver, Colo. Bromide analyses were done by ion chromatography, with a reporting limit of 0.02 mg/L. The wastewater compounds and detection limits are given in table 1; analytical methods used are documented in Zaugg and others (2002). A field blank sample also was collected and analyzed for wastewater compounds to determine potential contamination from field and transport procedures.

Bacterial analyses were done at the USGS Ohio Water Microbiology Laboratory in Columbus, Ohio. Samples were analyzed within 26 hours of collection for total coliforms and *E. coli* by use of the MI membrane-filtration method (U.S. Environmental Protection Agency, 2002a) and for enterococci using the mEI membrane-filtration method (U.S. Environmental Protection Agency, 2002b). Because of the need to collect enterococci colonies for DNA analysis, up to 1 L of sample water was filtered, which is more than the standard 100 mL filtered for the method. The MST methods that can detect the presence of genetic markers within the order *Bacteroidales* and the species *Enterococcus faecium* were used to try to determine whether fecal contamination included a human source. Water samples were tested for the presence of a general fecal, human-associated, and ruminant-associated marker in the order *Bacteroidales*, as described in Dick and others (2005), according to the protocol originally described by Bernhard and Field (2000). Water samples were tested for the presence of *Enterococcus faecium* human-specific *esp* marker (enterococci marker) according to the protocol originally described by Scott and others (2005). About 500 mL of wastewater was collected from 5 septic tanks and combined into a single sample that also was analyzed for bacterial DNA markers.

Procedures for the microbiology quality-assurance/quality-control (QA/QC) laboratory practices are described in Francy and others (2005). In the laboratory, filter blanks were included for at least every third *E. coli* sample (and every sample for *Bacteroidales* or enterococci markers) to document that filtration equipment and buffered water were not contaminated.

Control DNA for human contamination sources (Delaware, Ohio, wastewater influent sample) and ruminant contaminant sources (Delaware, Ohio, cattle feces) were similarly processed for the presence of the *Bacteroidales* and enterococci markers. The presence of the general *Bacteroidales* marker (Bac32) was used as evidence that the polymerase chain reaction (PCR) was successful; in the absence of a positive response for Bac32, a matrix spike was included. For the enterococci marker, matrix spikes were included for each environmental sample, including the composite HSTS sample, to identify any possible matrix inhibition. PCR reagent blanks were included to test for contamination of PCR reagents with amplifiable target DNA.

Table 1. Wastewater-method compound names, USGS National Water-Quality Laboratory reporting limits, and possible compound uses (modified from Zaugg and others, 2002).

[Laboratory reporting limits are in micrograms per liter; F, fungicide; H, herbicide; I, insecticide; GUP, general-use pesticide; FR, flame retardant; WW, wastewater; Manuf, manufacturing; %, percent; >, greater than; CP, combustion product; UV, ultraviolet]

Compound name	Laboratory reporting limit	Possible compound uses or sources
1,4-Dichlorobenzene	0.5	Moth repellent, fumigant, deodorant
17beta-Estradiol	5	Estrogen replacement therapy, estrogen metabolite
1-Methylnaphthalene	0.5	2-5% of gasoline, diesel fuel, or crude oil
2,6-Dimethylnaphthalene	0.5	Present in diesel/kerosene (trace in gasoline)
2-Methylnaphthalene	0.5	2-5% of gasoline, diesel fuel, or crude oil
3beta-Coprostanol	2	Carnivore fecal indicator
3-Methyl-1H-indole (skatol)	1	Fragrance, stench in feces and coal tar
3-tert-Butyl-4-hydroxyanisole (BHA)	5	Antioxidant, general preservative
4-Cumylphenol	1	Nonionic detergent metabolite
4-n-Octylphenol	1	Nonionic detergent metabolite
4-tert-Octylphenol	1	Nonionic detergent metabolite
5-Methyl-1H-benzotriazole	2	Antioxidant in antifreeze and deicers
Acetophenone	0.5	Fragrance in detergent and tobacco, flavor in beverages
Acetyl-hexamethyl-tetrahydro-naphthalene (AHTN)	0.5	Musk fragrance (widespread usage) persistent in ground water
Anthracene	0.5	Wood preservative, component of tar, diesel, or crude oil, CP
Anthraquinone	0.5	Manuf dye/textiles, seed treatment, bird repellent
Benzo[a]pyrene	0.5	Regulated PAH, used in cancer research, CP
Benzophenone	0.5	Fixative for perfumes and soaps
beta-Sitosterol	2	Plant sterol
beta-Stigmastanol	2	Plant sterol
Bisphenol A	1	Manuf polycarbonate resins, antioxidant, FR
Bromacil	0.5	H (GUP), >80% noncrop usage on grass/brush
Bromoform	0.5	WW ozonation byproduct, military/explosives
Caffeine	0.5	Beverages, diuretic, very mobile/biodegradable
Camphor	0.5	Flavor, odorant, ointments
Carbaryl	1	I, crop and garden uses, low persistence
Carbazole	0.5	I, Manuf dyes, explosives, and lubricants
Chlorpyrifos	0.5	I, domestic pest and termite control (domestic use restricted as of 2001)
Cholesterol	2	Often a fecal indicator, also a plant sterol
Cotinine	1	Primary nicotine metabolite
Diazinon	0.5	I, > 40% nonagricultural usage, ants, flies
Dichlorvos	1	I, pet collars, flies, also a degradate of naled or trichlofon
d-Limonene	0.5	F, antimicrobial, antiviral, fragrance in aerosols
Equilenin	5	Hormone replacement therapy drug
Estrone	5	Biogenic hormone

Table 1. Wastewater method compound names, USGS National Water-Quality Laboratory reporting limits, and possible compound uses.— Continued

Compound name	Laboratory reporting limit	Possible compound uses or sources
Ethynyl estradiol	5	Oral contraceptive
Fluoranthene	0.5	Component of coal tar and asphalt (only traces in gasoline or diesel fuel), CP
Hexahydrohexamethyl-cyclopentabenzopyran (HHCB)	0.5	Musk fragrance (widespread usage) persistent in ground water
Indole	0.5	Pesticide inert ingredient, fragrance in coffee
Isoborneol	0.5	Fragrance in perfumery, in disinfectants
Isophorone	0.5	Solvent for lacquer, plastic, oil, silicon, resin
Isopropylbenzene (cumene)	0.5	Manuf phenol/acetone, fuels and paint thinner
Isoquinoline	0.5	Flavors and fragrances
Menthol	0.5	Cigarettes, cough drops, liniment, mouthwash
Metalaxyl	0.5	H, F (GUP), mildew, blight, pathogens, golf/turf
Methyl salicylate	0.5	Liniment, food, beverage, UV-absorbing lotion
Metolachlor	0.5	H (GUP), indicator of agricultural drainage
N,N-diethyl- <i>meta</i> -toluamide (Deet)	0.5	I, urban uses, mosquito repellent
Naphthalene	0.5	Fumigant, moth repellent, major component (about 10%) of gasoline
Nonylphenol, diethoxy- (total, NPEO2)	5	Nonionic detergent metabolite
Octylphenol, diethoxy- (OPEO2)	1	Nonionic detergent metabolite
Octylphenol, monoethoxy- (OPEO1)	1	Nonionic detergent metabolite
<i>para</i> -Cresol	1	Wood preservative
<i>para</i> -Nonylphenol (total)	5	Nonionic detergent metabolite
Pentachlorophenol	2	H, F, wood preservative, termite control
Phenanthrene	0.5	Manuf explosives, component of tar, diesel fuel, or crude oil, CP
Phenol	0.5	Disinfectant, manuf several products, leachate
Prometon	0.5	H (noncrop only), applied prior to blacktop
Pyrene	0.5	Component of coal tar and asphalt (only traces in gasoline or diesel fuel), CP
Tetrachloroethylene	0.5	Solvent, degreaser, veterinary anthelmintic
Tri(2-chloroethyl) phosphate	0.5	Plasticizer, flame retardant
Tri(dichloroisopropyl) phosphate	0.5	Flame retardant
Tributyl phosphate	0.5	Antifoaming agent, flame retardant
Triclosan	1	Disinfectant, antimicrobial (concern for acquired microbial resistance)
Triethyl citrate (ethyl citrate)	0.5	Cosmetics, pharmaceuticals
Triphenyl phosphate	0.5	Plasticizer, resin, wax, finish, roofing paper, FR
Tri(2-butoxyethyl) phosphate	0.5	Flame retardant

Ground-Water-Quality Data

Data on the wells and the onsite measurements (pH, specific conductance, temperature, and dissolved oxygen (DO)) are listed in table 2. The pH, specific conductance, and temperature values are typical for ground water from bedrock in northeastern Ohio (Eberts and others, 1990; Jagucki and Darner, 2001); however, the DO concentrations are unusually high for ground water. Although the use of the residential well pumps in the sample collection could introduce some oxygen into the water, the DO concentrations are still unusually high. In general, DO concentrations in ground water are expected to be less than 0.1 mg/L because buried organic matter and oxidizable minerals deplete available oxygen quickly; however, ground water in recharge areas can have relatively high DO concentrations, similar to concentrations in surface water (Hem, 1989; Freeze and Cherry, 1979). The DO concentrations measured in the wells in this study are consistent with recently recharged water.

Bromide concentrations from 12 ground-water samples ranged from 0.04 to 0.18 mg/L (table 3). Samples were collected concurrently by Ohio EPA and analyzed by their laboratory for chloride and nitrate¹ concentrations (table 3). Chloride concentrations ranged from 12.6 to 61.6 mg/L. Nitrate concentrations ranged from 2.34 to 11.9 mg/L (as N). Water samples from three of the wells had nitrate concentrations that exceeded the Maximum Contaminant Level of 10 mg/L (U.S. Environmental Protection Agency, 2004). Samples from 10 of the wells had nitrate concentrations greater than 3.1 mg/L; in Ohio, this may indicate effects of human activity (Baker and others, 1989). High nitrate concentrations are not common in ground water because aquifers are normally low in oxygen, forcing soil bacteria to strip oxygen from nitrate. These nitrate concentrations are consistent with the high DO concentrations described earlier.

Samples from eight wells were collected and analyzed for bacteria concentrations (table 4). Enterococci bacteria were found in samples from every well, although the concentrations in samples from three wells were less than 0.5 CFU/100 mL (volumes filtered were greater than 100 mL). Total coliforms were found in samples from every well at concentrations from 2 to 200 CFU/100 mL. *E. coli* were found in samples from three wells at concentrations greater than 1 CFU/100 mL.

Concentrations of enterococci from wells WN-37, WN-39, WN-40, WN-42, and WN-43 did not allow for the collection of more than 100 colonies for the DNA-marker analysis. This is significant because Scott and others (2005) reported consistent detection of the *esp* gene in human-derived enterococci when at least 100 colonies were cultivated. Regardless, the colonies were tested for the presence of the *esp* gene. The presence of this gene indicates fecal contamination from a human source; the absence of the gene indicates either that fecal contamination is from nonhuman sources, or that the enterococci concentrations from human sources were below the detection limit, or that the marker was not present in the fecal source. The *esp* gene was not found in any of the samples, including the composite sample from five septic tanks (table 4). In another study, the *esp* gene was reported to be present in 8 of 10 septic-system samples that had enterococci concentrations of 58 ± 24 CFU/100 mL (Scott and others, 2005), so the failure to detect the marker in the composite sample with 51,000 CFU/100 mL is a strong indication that the gene would not have been detected in the ground-water samples even if the enterococci concentrations were greater. Therefore, the fecal contamination in the wells is likely from either human sources that did not have the marker or from nonhuman sources.

¹ Actual analysis was for nitrite plus nitrate; however, “nitrate” is used in this report for simplicity because the proportion of nitrite to nitrate is negligible in most ground water.

Table 2. Well data and water-quality field data for ground-water samples from wells in the Scenic Heights Drive-Batdorf Road area of Wayne County, Ohio, June 2006

[μ S/cm, microsiemens per centimeter at 25 degrees Celsius; deg. C, degrees Celsius; mg/L, milligrams per liter; U, unknown; <, less than; --, no data]

Site name	Date sampled	Approximate depth of well/length of casing (feet)	Depth to water, below ground surface (feet)	pH (standard units)	Specific conductance (μ S/cm)	Temperature, water (deg. C)	Dissolved oxygen (mg/L)
WN-35	6-21-2006	150*/U	102	7.0	485	13.0	9.45
WN-36	6-21-2006	118 / 70	59.7**	6.9	430	12.2	1.26
WN-37	6-19-2006	80*/U	29.4	6.3	417	13.1	8.56
WN-38	6-21-2006	140 / 116	94.8	6.5	298	11.3	6.77
WN-39	6-20-2006	140*/U	82.4	6.3	273	13.0	7.65
WN-40	6-19-2006	116 / 52	51.6	6.6	465	13.8	6.51
WN-41	6-20-2006	135 / 79	55.0	7.0	466	13.5	2.25
WN-42	6-20-2006	140*/U	65.8	6.5	361	13.2	6.73
WN-43	6-20-2006	100 / 79	63.0	6.5	355	12.6	4.04
WN-44	6-21-2006	140*/U	104.3	7.0	554	12.8	4.96
WN-45	6-19-2006	150 / 120	98.0	6.8	422	12.9	4.59
WN-46	6-19-2006	--	--	7.0	618	12.7	7.15

* Reported by the Ohio Environmental Protection Agency.

** Depth to water is below the top of casing.

Table 3. Results of bromide, chloride, and nitrate analyses for ground-water samples from wells in the Scenic Heights Drive-Batdorf Road area of Wayne County, Ohio, June 2006.

[Samples for chloride and nitrate analyses were collected and analyzed by the Ohio Environmental Protection Agency; mg/L, milligrams per liter]

Site name	Bromide, dissolved (mg/L)	Chloride, dissolved (mg/L)	Nitrite + nitrate, (mg/L as N)
WN-35	0.19	43.6	11.9
WN-36	.07	39.7	2.34
WN-37	.14	59.2	9.87
WN-38	.09	13.0	10.9
WN-39	.16	12.6	11.3
WN-40	.18	61.6	9.15
WN-41	.06	37.1	2.42
WN-42	.12	37.2	8.66
WN-43	.08	46.8	5.88
WN-44	.05	28.0	6.50
WN-45	.05	44.5	5.08
WN-46	.04	53.2	6.38

Table 4. Results of analyses of bacteria concentrations and bacterial DNA in ground-water samples from wells in the Scenic Heights Drive-Batdorf Road area of Wayne County, Ohio, June 2006.

[CFU/100 mL, colony-forming units per 100 milliliters; <, less than; E, results based on estimated colony count; k, results based on colony count outside of the ideal range, potentially reducing accuracy of estimates; g, present at concentrations less than 0.5 colonies per 100 milliliters; --, not determined; Pos., positive for detection of bacterial DNA marker; Neg., negative for detection of bacterial DNA marker]

Site name	Enterococci ^a (CFU/100mL)	Total Coliform ^a (CFU/100ml)	<i>E. coli</i> ^a (CFU/100mL)	<i>Enterococcus</i> <i>esp</i> marker ^b present	<i>Bacteroidales</i> ^c markers present		
					Fecal	Human	Ruminant
WN-36	g ^d	14 E, k	< 1	--	Neg.	--	--
WN-37	17 E, k	200 E, k	1 E, k	Neg.	Pos.	Neg.	Neg.
WN-39	2 E, k	28 E	2 E, k	Neg.	Neg.	--	--
WN-39 replicate	2 E, k	9 E, k	< 1	Neg.	Neg.	--	--
WN-40	2 E, k	32 E	1 E, k	Neg.	Pos.	Neg.	Neg.
WN-41	g ^d	2 E, k	< 1	--	--	--	--
WN-42	2 E, k	26 E	< 1	Neg.	Pos.	Neg.	Neg.
WN-43	2 E, k	16 E, k	< 1	Neg.	Pos.	Neg.	Neg.
WN-45	g ^d	4 E, k	< 1	--	Neg.	--	--
Septic composite	51,000 E	--	--	Neg.	--	Pos.	Neg.

^a Samples were processed within 26 hours of collection.

^b The *esp* marker is on a gene in the *Enterococcus faecium* DNA that is specific to bacteria from a human source. Presence of the marker indicates fecal contamination of human origin but does not indicate humans as a dominant source. Absence of the marker indicates either no human-origin fecal contamination or concentrations less than the minimum detection limit.

^c The presence of the general fecal *Bacteroidales* marker indicates fecal contamination. The presence of the human-associated or ruminant-associated marker indicates human or ruminant sources contributed to the fecal contamination. The absence of a marker indicates either no associated host-source for the fecal contamination or that the concentrations were less than the minimum detection limit.

^d Concentrations less than 1 CFU/100 mL were determined by filtering up to 1 liter of water.

Samples from seven wells were analyzed for the detection of the general fecal Bacteroidales marker; four were positive (table 4). DNA samples from these four wells were then tested for the host-specific, human- and ruminant-associated markers; all tests for host-specific markers were negative. The presence of the general fecal marker indicates fecal contamination. Although the presence of the human- or ruminant-associated marker indicates human or ruminant sources contributed to the contamination, the absence of the marker may indicate one or more of the following situations: (1) there was no fecal contamination from the host-associated sources, (2) the amount of contamination from the host-associated sources was below the detection limit of the method, or (3) the marker was not present in the fecal sources or was not present at expected concentrations.

Fecal bacteria of the order Bacteroidales are anaerobes and are expected to survive for only a relatively short time in oxygenated waters. A study by Kreader (1998) found that in river water at 4°C, PCR-detectable DNA from Bacteroidales was detectable for at least 2 weeks; at 14° C, the DNA was detectable for only 4 to 5 days. Given the temperatures in the sampled water in this study (11-13°C), the presence of detectable DNA from Bacteroidales in samples from four wells —WN37, WN-40, WN-42, and WN-43 — is indicative of recently recharged water.

Water from five wells — WN-37, WN-39, WN-40, WN-42 and WN-43 — was sampled and analyzed for wastewater compounds (table 1). Metolachlor, an herbicide and general-use pesticide and an indicator of agricultural drainage (table 1), was detected in all five samples but at concentrations below the laboratory reporting limit of 0.5 µg/L (data on file at the USGS Ohio Water Science Center). The estimated concentrations of metolachlor ranged from 0.0102 to 0.1160 µg/L. The only other compound detected was caffeine, in the sample from WN-40; the concentration was estimated at 0.120 µg/L, below the reporting limit of 0.5 µg/L.

Summary

In 2004, a public-health nuisance was declared by the Wayne County Board of Health in the Scenic Heights Drive-Batdorf Road area of Wooster Township, Wayne County, Ohio, because of concerns about the safety of water from local wells. Repeated sampling of the private water wells had found elevated nitrate concentrations and the fecal-indicators total coliform and *Escherichia coli* (*E. coli*). To help identify whether the bacterial contamination was from a human source, the U.S. Geological Survey (USGS), in cooperation with the Ohio Environmental Protection Agency (Ohio EPA), collected and analyzed samples from some of the affected residential water wells. The wells in the area are completed as open holes in fractured bedrock. In June 2006, the USGS collected water samples from 12 wells and 5 septic tanks. Bromide concentrations were analyzed in samples from all 12 wells. Samples from 5 of the 12 wells were analyzed for wastewater compounds. Total coliform, enterococci, and *E. coli* bacteria concentrations were determined in samples from 8 of the 12 wells. In addition, two microbial source tracking (MST) tools that employ DNA markers were tested on several samples from wells and a composite sample of water from 5 septic tanks. The DNA markers from the *Enterococcus faecium* species and the order Bacteroidales are associated with specific sources, either human or ruminant. The general Bacteroidales marker is also an indicator of fecal contamination from warmblooded animals.

Onsite measurements of pH, temperature, specific conductance, and dissolved oxygen were made during sampling. The unexpectedly high concentrations of dissolved oxygen, ranging from 1.26 to 9.45 mg/L, are indicative of recently recharged waters. Bromide concentrations in samples from 12 wells ranged from 0.04 to 0.18 mg/L. No wastewater compounds were detected at concentrations above the reporting limits in five wells. Metolachlor was detected in the five samples but at concentrations below the laboratory reporting limit of 0.5 µg/L. Caffeine was detected in one sample but also at a concentration below the reporting limit of 0.5 µg/L. Samples from the 12 wells were collected by Ohio EPA and analyzed for chloride and nitrate; chloride concentrations ranged from 12.6 to 61.6 mg/L and nitrate concentrations ranged from 2.34 to 11.9 mg/L.

Total coliforms were detected concentrations from 2 to 200 CFU/100 mL in eight wells. Similarly, enterococci bacteria were detected in all eight wells sampled, ranging in concentration from 0.5 to 17 CFU/100 mL. *E. coli* were detected in samples from only three of the eight wells, at concentrations of 1 or 2 CFU/100 mL. The test for the human-specific marker, the *esp* gene, of enterococci was negative in seven samples tested, including the composite sample of HSTS water. The general fecal Bacteroidales marker was detected in samples from four wells, but the tests for both the human- and ruminant-associated markers were negative. The presence of the PCR-detectable DNA for the general fecal Bacteroidales marker is indicative of fecal contamination and recently recharged water.

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