HHS Public Access

Author manuscript

J Environ Sci Health A Tox Hazard Subst Environ Eng. Author manuscript; available in PMC 2017 October 06.

Published in final edited form as:

J Environ Sci Health A Tox Hazard Subst Environ Eng. 2014 September 19; 49(11): 1236–1243. doi: 10.1080/10934529.2014.910036.

Microbial and chemical contamination during and after flooding in the Ohio River—Kentucky, 2011

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Abstract

Surface water contaminants in Kentucky during and after 2011 flooding were characterized. Surface water samples were collected during flood stage (May 2–4, 2011; n = 15) and after (July 25–26, 2011; n = 8) from four different cities along the Ohio River and were analyzed for the presence of microbial indicators, pathogens, metals, and chemical contaminants. Contaminant concentrations during and after flooding were compared using linear and logistic regression. Surface water samples collected during flooding had higher levels of *E. coli*, enterococci, *Salmonella, Campylobacter, E. coli* O157:H7, adenovirus, arsenic, copper, iron, lead, and zinc compared to surface water samples collected 3-months post-flood ($P < 0.05$). These results suggest that flooding increases microbial and chemical loads in surface water. These findings reinforce commonly recommended guidelines to limit exposure to flood water and to appropriately sanitize contaminated surfaces and drinking wells after contamination by flood water.

Keywords

Natural disaster; emergency response; floods

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Introduction

Floods took more lives and damaged more property than any other type of natural disaster in the United States during the 20th century.[1] In addition to the immediate risk of drowning, floods can flush toxic chemicals and infectious microorganisms from soil and from residential, industrial, agricultural, and waste facilities into storm drains, rivers, and residential areas.[2–4] This places residents and emergency responders at risk of exposure to contaminated water.

From April 12–May 20, 2011, severe storms and tornadoes combined with snowmelt caused flooding along the Ohio River in Kentucky. On May 4, 2011, President Obama declared Kentucky to be a major disaster area and committed over $40 million to aid the clean-up and recovery efforts.[5]

There is limited information available in the published literature describing the type and quantity of contaminants and pathogens found in flood waters. A few studies found increased rates of diarrheal or other illness after heavy rainfall or flooding,[2,6,7] which suggests that increased microbial loads may be present; however, these studies did not investigate which individual pathogens were in the water. Several U.S. studies following Hurricane Katrina measured metal, chemical, and coliform levels in flood water,[8–10] but not pathogens. We only found two published studies that analyzed for a suite of pathogens in flood waters. One of these was conducted in Indonesia,[11] and one in the Netherlands.[12] There is a need to replicate this type of study in different regions of the world to build a more robust knowledge base. Thus, our primary objectives were to characterize microbial and chemical contaminants in surface water during the 2011 Kentucky flood, and to compare these contaminants to surface water samples collected after flood waters receded.

Detecting waterborne pathogens usually requires collecting large volumes of water (~100 L), which can be difficult to collect, transport, and ship. However, a field sampling technique called dead-end hollow-fiber ultrafiltration (DEUF) can filter upwards of 100 L of surface water using a disposable ultrafilter, which is then shipped to the lab instead of water. This has increased the logistical feasibility of testing water sources for multiple bacteria, viruses and parasites.[13] DEUF can recover diverse microbes from large volumes of water (~100 L);[13] however, this was only the second time the method was applied to flood water, which can be highly turbid.[14]

Materials and methods

Flood region

The Ohio River is the largest tributary of the Mississippi River, draining 203,900 square miles. The Ohio River begins in Pittsburgh, Pennsylvania at the confluence of the Allegheny and Monongahela Rivers. From there, the Ohio River flows southwest for approximately 981 miles to Cairo, Illinois, where it joins the Mississippi River. Along the way, it forms much of the northern border of Kentucky. During April–May 2011, the Ohio River flooded along all of northwest Kentucky.

Sampling locations

Water samples were collected in four areas along the northwestern Kentucky border: Carrollton/Gallatin, Louisville, Owensboro, and Paducah. These samples were collected from May 2–4, 2011 (during the flood) and from July 25–26, 2011 (post-flood, after river levels receded). Fifteen samples were collected during flooding (Carrollton/Gallatin, n = 3, Louisville n = 5, Owensboro n = 2, and Paducah n = 5) and 8 samples were collected post-flood (Carrollton/Gallatin, n = 2, Louisville, n = 2, Owensboro, n = 2, and Paducah, n = 2). In Louisville, the Ohio River reaches flood stage at 23 feet; during this flood event, water levels exceeded 50 feet in Louisville at the beginning of May, and receded to normal baseflow levels under 15 feet by the middle of July.[15]

Sample collection

To ensure investigator safety, water samples were collected from accessible locations at the edge of flood pools. Samples taken post-flood were collected as close as possible to the location of a sample collected during the flood to facilitate comparison.

From each location, the following were collected: latitude and longitude; water quality parameters (pH, temperature, turbidity, dissolved oxygen, and conductivity) using a handheld multi-parameter meter (Horiba U-50 Series, Horiba Ltd., Kyoto, Japan); grab samples (i.e., one-time, single point collection) for nitrates, atrazine, and metals; and a DEUF sample for microbes. One exception was in Owensboro, where DEUF samples were not collected during the flood due to logistical challenges. Samplers wore disposable gloves during collection of all samples, and gloves were changed at each location. To implement DEUF, samples used a peristaltic pump with silicone tubing to filter 100 L of water through a REXEED™-25 SX dialysis filter (Asahi Kasei Medical Corporation, Tokyo, Japan) having a pore size of ~30 kDa. To prevent cross-contamination, new tubing was used at each site and the REXEED filters were kept sealed until their use at the collection site.

All grab samples were collected using pre-sterilized bottles that were certified for trace metal analysis. They were collected at a water depth of approximately one foot at each location following US Environmental Protection Agency (US EPA) guidelines for surface water grab sample collection.[16] All grab samples and filters were stored on ice following collection and shipped to the laboratory within 24 h; laboratory analysis began within 24 h of receipt.

Laboratory analysis

Grab samples for nitrates, atrazine, and metals were shipped to the Colorado Department of Public Health and Environment's Laboratory Services Division in Denver, Colorado, and analyzed in accordance with the UE EPA's Standard Methods for drinking water (nitrates: 353.2; atrazine: 525.2; metals (aluminum, arsenic, barium, beryllium, cadmium, calcium carbonate, chromium, copper, iron, lead, magnesium, manganese, molybdenum, nickel, potassium, selenium, silver, sodium, uranium, and zinc): 200.7 and 200.8. Although the metals panel contains a wide suite of metals, we only present metals that have known health- or aesthetic-related implications.

DEUF filters were shipped to the Environmental Microbiology Laboratory at the U.S. Centers for Disease Control and Prevention (CDC), Waterborne Disease Prevention Branch in Atlanta, Georgia. Each ultrafilter was backflushed using two different solutions. The first backflush was performed using 250 mL of backflush solution without sodium polyphosphate (NaPP),[13] (as a precaution out of concern that NaPP could inhibit *Campylobacter* culture), and 100 mL was taken from this aliquot for culture of *Campylobacter*.[17] Each filter was then backflushed a second time using 250 mL of standard backflush solution containing NaPP, Antifoam-Y30 and Tween 80.[13]

This volume of filter concentrate was added to the remaining 150 mL from the first backflush and used for additional culture and real-time polymerase chain reaction (PCR) analyses. In preparation for real-time PCR analysis of the water sample, the filter concentrate was further concentrated by PEG precipitation (12% PEG 8000, 0.9 M NaCl, and 1% bovine serum albumin) for 2 h at 4°C.[18] Total coliforms and *E. coli* were analyzed according to Standard Method 9223 using Colilert.[19] Enterococci were enumerated by Enterolert (IDEXX Laboratories). *Salmonella* was detected by membrane filtration using 0.45-μm mixed-cellulose ester filters, followed by broth and agar culture according to Hill and Sobsey,[20] and PCR using the TaqMan assay of Hill et al.[21] *E. coli* O157:H7 was also assayed by culture and real-time PCR.[22]

In addition to the culture of *Campylobacter*, real-time PCR was also performed on the concentrated water sample.[23] Real-time PCR or real-time reverse transcription PCR (RT-PCR) methods were used to detect the following parasites and viruses: *Cryptosporidium, Giardia*, GI and GII noroviruses, enterovirus, adenovirus, and hepatitis A virus.[24–27] In addition to real-time PCR for *Cryptosporidium* and *Giardia*, IMS/FA microscopy (immunomagnetic separation/ immunofluorescence assay) was performed according to US EPA Standard Method 1623 (US EPA 815-R-05-002, 2005) on water samples for which a real-time PCR Ct value below 40 was obtained.[28]

Statistical analysis

Data were analyzed using R version 2.15.1.[29] Values below the limit of detection (LOD) were assigned a value equal to the LOD divided by the square root of two.[30] Most water quality measurements were not normally distributed, so we calculated geometric means and used log-transformed values during statistical comparisons. Most continuous variables were approximately normally distributed after log transformation.

Water quality measurements between flood and post-flood time periods were compared using linear regression for continuous measurements (e.g., pH, total coliform level, etc.) and logistic regression for dichotomous measurements (i.e., presence /absence of *Salmonella, Campylobacter*, and *E. coli* O157:H7). Each water quality variable was tested for statistical significance in a separate linear regression model that accounted for the location (i.e., city) and time period (i.e., flood or post-flood) of collection. $P < 0.05$ was considered statistically significant when comparing water quality variables between flood and post-flood time periods. Because this investigation was hypothesis-generating, analyses were not adjusted for multiple comparisons.

When applicable, contaminant concentrations were compared to the US EPA's maximum contaminant levels (MCLs) and secondary maximum contaminant levels (SMCLs) for drinking water.[31,32] MCLs and SMCLs are the highest level of a contaminant that is allowed in drinking water, and they are enforceable regulations for public drinking water systems. Although they are not applicable to surface water, they provide a general comparison benchmark. When MCLs and SMCLs were not available, levels were compared to the US EPA's maximum allowable instream concentrations of pollutants for human health.[33]

Results and discussion

Water quality parameters

As expected, water temperature was higher in July during post-flood collection (GM = 30.7°C) compared to collection during the flood in May (GM = 15.2°C) ($P < 0.01$) (Table 1). During flooding, surface water was more turbid (GM = 139 NTU vs. 25.5 NTU, $P < 0.01$) and dissolved oxygen was higher (GM = 14.7 mg L^{-1} vs. 9.50 mg L^{-1}, $P < 0.05$). Conductivity was lower during flooding (GM = 360 µS cm^{-1} vs. 170 µS cm^{-}; $P < 0.01$), and pH did not differ.

Chemicals

Nitrates, arsenic, barium, copper, iron, lead, manganese, nickel, and zinc were found in most surface water samples (Table 2). Surface water collected during flooding had higher levels of arsenic, copper, iron, lead, and zinc ($P < 0.01$). All surface water samples exceeded the SMCL for iron and manganese. Two surface water samples collected during the flood exceeded the MCL for lead, and one exceeded the MCL for arsenic.

One surface water sample collected during flooding contained several metals at an order of magnitude higher than any other surface water sample: arsenic (0.015 mg L^{-1}), barium (0.88 mg L^{-1}), copper (0.15 mg L^{-1}), iron (24 mg L^{-1}), lead (0.067 mg L^{-1}), manganese (9.0 mg L^{-1}), nickel (0.051 mg L^{-1}), and zinc (0.65 mg L^{-}).

Microbial indicators and pathogens

During flooding, surface water samples had higher levels of total coliforms (GM = 2.34E + 03 MPN 100 mL^{-1} vs. 420 MPN 100 mL^{-1}, $P < 0.05$), *E. coli* (GM = 285 MPN 100 mL^{-1} vs. 13 MPN 100 mL^{-1}, $P < 0.01$), and enterococci (GM = 335 MPN 100 mL^{-} vs. 30 MPN 100 mL^{-}, $P < 0.01$) compared to post-flood samples (Table 3). A greater proportion of surface water samples collected during flooding contained viable *Salmonella* (100% vs. 38%, $P < 0.01$), adenovirus (77% and 12%, $P < 0.05$), and *Campylobacter* (62% vs. 12%, $P = 0.07$) compared to samples collected post-flood.

Cryptosporidium spp. was detected in similar proportions in surface water samples collected during (85%) and after flooding (62%); these samples were positive for *Cryptosporidium spp.* by real-time PCR but could not be confirmed to contain oocysts by IMS /FA microscopy. Although not statistically significant, two surface water samples collected

during flooding contained viable *E. coli* O157:H7, as compared to zero surface water samples collected post-flood. No samples tested positive for *Giardia*, enterovirus, norovirus GI and GII, or hepatitis A virus.

This study provides rarely collected data that enable us to examine chemical and microbial levels in surface water during and after flooding. Samples were collected at multiple locations along the Ohio River, including both residential and industrial sites. Surface water was heavily contaminated during flooding: *E. coli*, enterococci, and *Salmonella* were identified in every sample we tested. *Campylobacter, Cryptosporidium, E. coli* O157:H7, adenovirus, and several metals were more concentrated during flooding compared to post-flood, suggesting that flooding may temporarily increase the concentration of some microbes and chemicals in surface water. Although the sample size was small and generalizability is limited, this study provides scientific evidence supporting guidelines for limiting exposure to flood water and taking appropriate precautions when cleaning up.

Although this study was not designed to identify the sources of microbial indicators, pathogens, and chemicals in flood water, our results suggest that contaminant levels are likely very heterogeneous and influenced by nearby point sources. Contaminant sources may vary from one house to another or from one city to another, depending on factors such as the strength of the point source, the amount of surface water present to dilute the contaminants, whether the flood water is stagnant or flowing, or the length of time since the point source was submerged. During floods that impact residential areas, items in submerged homes, garages, and vehicles can leak chemicals into the environment. This was observed in one flood water sample collected from a residential neighborhood that contained visible debris in the nearby vicinity (including cars, discarded tires, a battery, and a gasoline can). This sample contained levels of metals that were an order of magnitude higher than the other 14 flood water samples. The other fourteen flood water samples were collected further from residential areas and in locations where no other obvious debris was present.

One previous study assessed chemical levels in New Orleans following Hurricane Katrina in 2005.[9] This study found similar levels of lead and nickel, and lower levels of arsenic, copper, and zinc compared to this New Orleans study. Observed differences are likely due to differences in geography and land usage in the flooded regions. Another study investigated turbidity and microbial loads during heavy rain periods in Germany during 1997–1998 and compared levels to regular samples.[3] Similarly, this paper found that flooding increased turbidity, total coliforms, *E. coli*, fecal *Streptococci*, and *C. perfringens*. A third study assessed the effects of the 2005 flooding in Jakarta, Indonesia and found that flood water contained higher levels of total coliforms, *E. coli*, enterovirus, hepatitis A virus, norovirus G1 and G2 compared to river water.[11]

Although there were higher levels of microbial indicators, pathogens, and metals in flood water, it is the pathogens (i.e., *E. coli* O157:H7, *Salmonella, Campylobacter, Cryptosporidium*, and adenovirus) that pose the greatest public health concern because exposure to them can cause acute illness. For example, the US EPA requires that these organisms not be present in drinking water.[31] Although it is not uncommon to find normal

gastrointestinal microorganisms such as *E. coli* and enterococci in surface water, we found them at much higher concentrations during flooding.

Despite the elevated levels of metals found in flood water, most metals were within the maximum contaminant levels set by the US EPA.[31] Risk of adverse health effects from exposure to metals at the concentrations we found here is low, particularly if exposure is short-lived and if the water containing the metals is not consumed.[9]

This investigation is subject to several limitations. First, the generalizability is limited. Analyzing water samples for a wide array of pathogens and chemicals is resource intensive, and thus we collected only 15 water samples during flooding and 8 post-flood. Second, contaminant levels were not assessed over time and thus results could be due in part to seasonal variation. Because flooding can be difficult to predict, samples were not collected prior to flooding, and most of the pathogens and chemicals assessed are not measured regularly, so no baseline data were available. Post-flood samples were collected at only one point in time, so no time series analyses could be conducted. Third, sampling locations were selected based on convenience, as it would have been logistically difficult to develop a random sampling frame in the limited time frame available, particularly considering that many locations were inaccessible due to flooded roads.

However, water samples were spaced apart along the sampling area. Also, although samples collected post-flood were collected as close as possible to samples collected during the flood, the specific locations sometimes varied because many areas that were covered in water during flooding were not covered in water post-flood. Finally, with the exception of *Salmonella, Campylobacter* and *E. coli* O157:H7, the viability of most pathogens could not be determined.

Although this study represents only a single flood event and the sample size was limited, the findings reinforce the need to practice commonly recommended guidelines for exposure to flood water. In addition to taking measures to prevent drowning and electrocution, residents and rescue personnel should limit direct contact with flood water. When contact is unavoidable, individuals should wear waterproof boots and gloves and wash any skin that comes into direct contact.[34]

Flood water can also contaminate domestic wells, which provide drinking water to almost 15% of the U.S. population,[35] and which are not protected by the Safe Drinking Water Act. Even when properly constructed and maintained, flood water can seep into a submerged well. Guidelines indicate that any resident with a drinking well that is potentially submerged should clean their well prior to use to prevent waterborne illness. State and local health departments often distribute post-flood well clean-up guidelines. Although the specifics can vary, most guidelines share the same basic principles.[36] These include examining the well for physical damage, cleaning silt and sediment from the well, and then disinfecting the system with a concentrated chlorine solution.

Conclusion

This study provides additional evidence for evaluating flood water exposure risks, and suggests potential future research projects to further characterize flood water quality, including assessing flood water contaminants in different regions of the United States; assessing flood water variability by sampling location (e.g., water depth, distance from pool edge, etc.); and assessing temporal variability of flood water quality during the flood event.

Acknowledgments

We would like to thank the Kentucky Department of Public Health, particularly CDR Margaret Riggs, USPHS, for their time, expertise, and logistical support. We would also like to thank the Louisville Metro Department of Public Health & Wellness, the Three Rivers District Health Department, the Green River District Health Department, and the Purchase District Health Department for their cooperation during this investigation. The use of trade names and names of commercial sources is for identification only and does not imply endorsement by the CDC or the U.S. Department of Health and Human Services. The findings and conclusions in this report are those of the authors and do not necessarily represent those of the CDC.

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Table 1

Water quality parameters in surface water samples following Ohio River flooding, Kentucky, 2011.

	All Locations		Carrollton		Louisville		Owensboro		Paducah	
	May (n = 15)	**July (n = 8)**	**May (n = 3)**	**July (n = 2)**	**May (n = 5)**	**July (n = 2)**	**May (n = 2)**	**July (n = 2)**	**May (n = 5)**	**July (n = 2)**
pH (SU)										
Minimum	5.05	4.49	5.36	7.58	6.63	7.71	7.35	4.79	5.05	4.49
Maximum	8.72	7.89	6.51	7.72	7.49	7.89	7.47	5.30	8.72	4.50
GM	6.80	6.06	5.85	7.65	6.86	7.80	7.41	5.04	7.14	4.49
Temperature (°C) **										
Minimum	11.8	28.1	16.1	28.7	11.8	30.9	16.1	28.1	13.0	33.1
Maximum	20.0	33.2	17.1	29.3	15.8	31.9	17.4	30.8	20.0	33.2
GM	15.2	30.7	16.6	29.0	12.9	31.4	16.7	29.4	16.2	33.1
Turbidity (NTU) **										
Minimum	47	16	158	19	92	16	76	19	47	31
Maximum	728	54	728	45	238	16	227	26	173	54
GM	139	25.5	336	29.2	142	16.0	131	22.2	81.7	40.9
Dissolved Oxygen (mg L^{-1}) **										
Minimum	8.51	5.55	21.4	5.55	11.2	9.30	10.1	7.33	8.51	10.1
Maximum	44.4	14.3	44.4	8.50	26.1	14.3	10.8	11.6	18.0	12.3
GM	14.7	9.50	27.6	6.87	14.7	11.5	10.5	9.22	11.6	11.1
Conductivity (µS cm^{-1}) **										
Minimum	68	154	247	429	119	453	111	429	68	154
Maximum	435	473	328	460	435	473	114	442	155	231
GM	170	360	278	444	263	463	112	435	97	189

** *P* value < 0.01 for linear regression comparing geometric mean levels between May and July, controlling for city;

GM = Geometric mean.

Table 2

Chemical analysis of surface water samples following Ohio River flooding, Kentucky, 2011.

Chemical	All Locations		Carrollton		Louisville		Owensboro		Paducah	
	May (n = 15)	July (n = 8)	May (n = 3)	July (n = 2)	May (n = 5)	July (n = 2)	May (n = 2)	July (n = 2)	May (n = 5)	July (n = 2)
Nitrates (mg L^{-1})										
% above LOD (0.02 mg L^{-1})	93	100	100	100	80	100	100	100	100	100
% above MCL (10 mg L^{-1})[a]	0	0	0	0	0	0	0	0	0	0
GM (mg L^{-1})	0.29	0.48	0.37	0.91	0.25	0.87	0.64	0.33	0.21	0.20
Arsenic (mg L^{-1}) **										
% above LOD (0.001 mg L^{-1})	100	100	100	100	100	100	100	100	100	100
% above MCL (0.010 mg L^{-1})[a]	7	0	0	0	0	0	0	0	20	0
GM	0.004	0.002	0.003	0.001	0.004	0.001	0.005	0.003	0.005	0.003
Barium (mg L^{-1})										
% above LOD (0.005 mg L^{-1})	100	100	100	100	100	100	100	100	100	100
% above MCL (2 mg L^{-1})[a]	0	0	0	0	0	0	0	0	0	0
GM	0.103	0.103	0.063	0.099	0.103	0.105	0.078	0.120	0.153	0.089
Copper (mg L^{-1}) **										
% above LOD (0.005 mg L^{-1})	100	62	100	100	100	100	100	0	100	50
% above MCL (1.3 mg L^{-1})[a]	0	0	0	0	0	0	0	0	0	0
GM	0.018	0.005	0.013	0.009	0.018	0.005	0.018	0.004	0.021	0.005
Iron (mg L^{-1}) **										
% above LOD (0.01 mg L^{-1})	100	100	100	100	100	100	100	100	100	100
% above SMCL (0.3 mg L^{-1})[b]	100	100	100	100	100	100	100	100	100	100
GM	6.66	1.05	7.29	0.93	6.48	0.73	7.97	1.20	6.04	1.51
Lead (mg L^{-1}) **										
% above LOD (0.001 mg L^{-1})	100	25	100	0	100	0	100	0	100	100
% above MCL (0.015 mg L^{-1})[a]	13	0	0	0	20	0	0	0	20	0
GM	0.005	<LOD	0.003	<LOD	0.007	<LOD	0.005	<LOD	0.006	0.001

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Chemical	All Locations		Carrollton		Louisville		Owensboro		Paducah	
	May (n = 15)	July (n = 8)	May (n = 3)	July (n = 2)	May (n = 5)	July (n = 2)	May (n = 2)	July (n = 2)	May (n = 5)	July (n = 2)
Manganese (mg L^{-1})										
% above LOD (0.002 mg L^{-1})	100	100	100	100	100	100	100	100	100	100
% above SMCL (0.05)[b]	100	100	100	100	100	100	100	100	100	100
GM	0.374	0.190	0.714	0.109	0.312	0.081	0.145	0.346	0.446	0.425
Nickel (mg L^{-1})										
% above LOD (0.005 mg L^{-1})	80	88	67	100	100	100	100	100	60	50
% above MCL (0.61 mg L^{-1})[a]	0	0	0	0	0	0	0	0	0	0
GM	0.007	0.003	0.004	0.004	0.009	0.003	0.010	0.003	0.006	0.003
Zinc (mg $/^{-1}L$) **										
% above LOD (0.01 mg L^{-1})	100	100	100	100	100	100	100	100	100	100
% above SMCL (5.0 mg L^{-1})[b]	0	0	0	0	0	0	0	0	0	0
GM	0.065	0.021	0.046	0.030	0.067	0.024	0.053	0.020	0.083	0.014

** *P* value < 0.01 for linear regression comparing geometric mean levels between May and July, controlling for city

[a] MCL = Maximum Contaminant Limit per US EPA regulations.

[b] SMCL = Secondary Maximum Contaminant Limit per US EPA regulations.

GM = Geometric mean.

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Table 3

Microbial indicators and pathogens in surface water following Ohio River flooding, Kentucky, 2011

Indicator/ Pathogen	All Locations		Carrollton		Louisville		Owensboro		Paducah	
	May (n = 13)	July (n = 8)	May (n = 3)	July (n = 2)	May (n = 5)	July (n = 2)	May (n = 0)[a]	July (n = 2)	May (n = 5)	July (n = 2)
Total Coliforms *										
% above LOD	100	100	100	100	100	100	N/A	100	100	100
GM (MPN)	2.34E+03	420	1.99E+03	1.99E+03	2.77E+03	1.07E+03	N/A	121	2.17E+03	121
E. coli **										
% above LOD	100	100	100	100	100	100	N/A	100	100	100
GM (MPN)	285	13	554	3.33	402	23.6	N/A	8.74	135	43.4
Enterococci **										
% above LOD	100	100	100	100	100	100	N/A	100	100	100
GM (MPN)	335	30	519	24.7	439	22.3	N/A	27.0	197	54.9
Salmonella **										
% above LOD	100	38	100	0	100	50	N/A	50	100	50
Cryptosporidium										
% above LOD	85	62	67	100	100	50	N/A	50	80	50
Adenovirus *										
% above LOD	77	12	67	0	80	50	N/A	0	80	0
Campylobacter *										
% above LOD	62	12	100	0	80	0	N/A	0	20	0
E. coli O157:H7										
% above LOD	15	0	67	0	0	0	N/A	0	0	0

GM = Geometric mean; MPN = Most probable number per 100 mL.

* *P* value < 0.05,

** *P* value < 0.01 for linear (total coliforms, *E. coli*, and *Enterococci*) and logistic (*Salmonella, Campylobacter*, and *E. coli* O157:H7) regression comparing May to July, controlling for city.

[a] Due to logistical constraints, we were unable to collect filter samples from Owensboro in May.