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Dynamics of fecal indicator bacteria, bacterial pathogen genes, and organic wastewater contaminants in the Little Calumet River–Portage Burns Waterway, Indiana

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ABSTRACT

Little information exists on the co-occurrence of fecal indicator bacteria (FIB), bacterial pathogens, and organic wastewater-associated chemicals (OWCs) within Great Lakes tributaries. Fifteen watershed sites and one beach site adjacent to the Little Calumet River–Portage Burns Waterway (LCRPBW) on Lake Michigan were tested on four dates for pH, dissolved oxygen, specific conductance, chloride, color, ammonia- and nitrate-nitrogen, soluble phosphorus, sulfate, turbidity, and atrazine; for concentrations of FIB; and for genes indicating the presence of human-pathogenic enterococci (ENT) and of Shiga-toxin producing *Escherichia coli* (EC) from various animal sources. Nineteen samples were also tested for 60 OWCs. Half of the watershed samples met EC recreational water quality standards; none met ENT standards. Human-wastewater-associated OWC detections were correlated with human-influence indicators such as population/km^2, chloride concentrations, and the presence of WWTP effluents, but EC and ENT concentrations were not. Bacterial pathogen genes indicated rural human and several potential animal sources. OWCs of human or ecosystem health concern (musk fragrances AHTN and HHCB, alkylphenols, carbamazepine) and 3 bacterial pathogen genes were detected at the mouth of the LCRPBW, but no such OWCs and only 1 pathogen gene were detected at the beach. The LCRPBW has significant potential to deliver FIB, potential bacterial pathogens, and OWCs of human or ecosystem health concern to the nearshore of Lake Michigan, under conditions enhancing nearshore transport of the river plume. Nearshore mixing of lake and river water, and the lack of relationship between OWCs and FIB or pathogen genes, pose numerous challenges for watershed and nearshore assessment and remediation.

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Introduction

Portions of the nearshore coastal zone of the Great Lakes are influenced substantially by adjacent tributary inputs (Mackey, 2009; Rao and Schwab, 2007), which are in turn controlled by land use and land cover in the tributary watersheds (Howell et al., 2012; Twiss and Marshall, 2012). Great Lakes coastal issues resulting from tributary effects on the nearshore include nutrients and subsequent nuisance algae growth (Higgins et al., 2012), organic wastewater-associated chemicals (OWCs) including pharmaceuticals (Helm et al., 2012), beach closures due to the non-pathogenic fecal indicator bacteria (FIB) such as *Escherichia coli* (EC; IJC, 2012), and the presence of pathogens in Great Lakes tributaries and at beaches (Aslan et al., 2011; Bauer and Alm, 2012; Hamelin et al., 2006; Jenkins et al., 2005; Kinzelman et al., 2008; Wong et al., 2009; Xagoraraki et al., 2007). Nevertheless, there are many gaps in our understanding of the linkage between tributaries and the Great Lakes nearshore, including incomplete knowledge of how land use, and spatial and temporal hydrodynamics, affect tributary influence on the nearshore (Howell et al., 2012; Makarewicz and Howell, 2012; Scopel et al., 2006).

In recent years, enhanced monitoring has provided substantial information regarding FIB in watersheds and at beaches. Monitoring for *E. coli* bacteria in Great Lakes watersheds has been enhanced by the Total Maximum Daily Load (TMDL) Program, mandated by the United States (U.S.) Clean Water Act in 1972. Likewise, monitoring at U.S. Great Lakes beaches for EC has been enhanced by the U.S. BEACH Act of 2000. Concentrations in the water of FIB above designated levels are presumed to indicate fecal contamination, and potential human health risk from pathogens (including bacteria, viruses, and protozoa; USEPA, 2001). However, there is a widely acknowledged variable relationship between FIB and pathogen concentrations (Field and Samadpour, 2007; Savichtcheva et al., 2007).

A variety of modeling approaches have sought to characterize FIB transport at the watershed scale (Benham et al., 2006; Jamieson et al., 2004; USEPA, 2001). Likewise, statistical and mechanistic models of

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E. coli concentrations at Great Lakes beaches have been successfully developed (Francy, 2009; Ge and Frick, 2009; Nevers and Whitman, 2005, 2008; Thupaki et al., 2010), taking advantage of frequent (up to daily) *E. coli* and environmental monitoring at some beaches. Rainfall runoff has been implicated as one major source of FIB at beaches (Kleinheinz et al., 2009; Patz et al., 2008). Where tributaries are adjacent, predictive and mechanistic EC beach models clearly indicate tributary effects on beaches and document the spatial and temporal variability of these effects (Nevers and Whitman, 2005, 2008; Scopel et al., 2006; Thupaki et al., 2010). However, there remain many knowledge gaps regarding the sources, fate, and transport of FIB in watersheds (Jamieson et al., 2004) and at beaches (Boehm et al., 2009).

As of 2006 (USEPA, 2006), 411 km of the combined Little Calumet–Galien River watershed, and 93 km of adjacent Lake Michigan shoreline, were considered impaired by *E. coli* under the TMDL process. The Little Calumet River–Portage Burns Waterway (LCRPBW) influences *E. coli* concentrations at beaches to the west when winds are from the north (onshore direction; Nevers and Whitman, 2005, 2008). The modeled influence of the river on the surrounding nearshore can be visualized at the Great Lakes Coastal Forecasting System site (NOAA, 2012). The Little Calumet and Portage Burns Waterway Final TMDL (Earth Tech Inc., 2004) indicated that permitted point source discharges are generally in compliance with *E. coli* standards, that the major source of *E. coli* is non-point, and that a 90% reduction in non-point source loads will be required to meet water quality standards. However, there remains uncertainty regarding the nature and magnitude of the contribution of various nonpoint *E. coli* sources throughout much of the watershed (Earth Tech Inc., 2004; Northwestern Indiana Regional Planning Commission, 2005). In addition, the significance of the *E. coli* impairments in terms of pathogen occurrence has not been determined, and OWCs have not been studied.

Our overall objective was to explore how the use of newer potential monitoring tools might influence monitoring or restoration strategies within the LCRPBW and the adjacent nearshore. In this study we evaluated the concentrations of FIB and OWCs, and occurrence of bacterial pathogen genes, plus several field-measured water quality parameters, on four dates under wet and dry conditions. Sites ranged from upper portions of the watershed to the mouth of the river, and included an adjacent beach. Selected bacterial pathogen genes were included to relate FIB to potential human health risk, and to infer source in some cases. Atrazine and OWCs were included again to infer source and because atrazine and some OWCs are of human and environmental health concern (Graymore et al., 2001; Uslu et al., 2012). Our objectives were to 1) identify factors and sources that may influence the detection of FIB, bacterial pathogen genes, and OWCs within the watershed and 2) evaluate the potential for the river to deliver bacterial pathogens and OWCs to the nearshore.

Materials and methods

Setting and site delineation

Three major subwatersheds were identified for the purposes of this study (Fig. 1; Table 1): the East Branch of the Little Calumet River including Coffee Creek (hereafter East Branch); Salt Creek; and Deep River, the West Branch of the Little Calumet River, and the portion of the Portage Burns Waterway upstream from the confluence with the East Branch (hereafter West Branch). Sampling point buffers (100 m) were generated using ArcMap 9.3.1 software (Environmental Systems Research Institute, ESRI, 2006). Total upstream drainage areas for stream sampling points were delineated using 8-digit Hydrologic Unit Code (HUC) boundaries (U.S. Geological Survey; USGS); 14-digit sub-watershed boundaries (Michigan Department of Environmental Quality); and 1:100,000 scale stream catchment boundaries (USEPA). Watershed delineations were overlaid with the USGS 1:24,000 2001 National Land Cover Data set and the area and percentage of land-cover classes within each buffer and drainage area was calculated (Table 1). Land cover for Ogden Dunes Beach, Lake Michigan, was obtained from the delineated catchment immediately surrounding the beach. Population/km^2 for the upstream drainage areas for all sample points was derived from U.S. Census Tracts, Tele Atlas North America Inc. (ESRI, 2006).

Sampling

All sites with the exception of Salt Creek near McCool, IN (November 16 only), were sampled on four dates: August 24, September 7 and 14, and November 16, 2005. On August 24, no rain had fallen for 4 days prior to sampling when 21.4 mm was recorded; on September 7, no rain had fallen for 17 days; 5.84 mm of rain fell on September 14; and on November 16, 13.05 mm had fallen in the previous 24 h, and 6.35 mm fell on that date. For convenience of discussion, these conditions are hereafter referred to as DRY (Sept. 7), RAIN1 (Sept. 14), RAIN2 (Aug. 24), and RAIN3 (Nov. 16), with rain codes reflecting the relative influence of likely rainfall runoff during the sampling. However, for the USGS hydrologic gaging station Little Calumet River at Porter, IN (04094000) stream discharge on all sampling dates was below the 25th percentile of historical flow conditions, reflecting overall dry conditions in 2005.

Water physical and chemical analysis

Water pH, temperature, dissolved oxygen (DO), and specific conductance (SC) were measured using a multi-parameter probe (Hach Co., Loveland, CO). Field tests on grab samples included test strips or DREL/2000 colorimeter (HACH Co.) for chloride (Cl), ammonia-N (NH_4-N), nitrate-N (NO_3-N), soluble phosphorus as P (PO_4-P), and sulfate (SO_4); color wheel for color (COL; Hach Co.); and DREL/2000 colorimeter (HACH Co.) for turbidity (TURB). Atrazine (ATZ) was tested in the field by enzyme-linked immunosorbent assay triazine test (RapPID Assay Atrazine; Strategic Diagnostics Inc., Newark, DE) and reported as detected if the concentration in water exceeded the stated limit of quantitation (0.1 μg/L) for the assay.

Organic wastewater chemicals were tested on water samples that were filtered (450 °C baked 0.7 μm nominal pore-size glass fiber filters) into 450 °C-baked amber glass bottles in the field. Sampling personnel avoided the use of pharmaceuticals and personal care products on the day of sampling. Target compounds were extracted with disposable, polypropylene solid phase extraction cartridges that contain a polystyrene-divinylbenzene phase. Concentrated extracts were analyzed by capillary-column gas chromatography/mass spectrometry (GC/MS), and compounds were identified using authentic standards (Zaugg et al., 2002). The analysis targeted 60 organic chemicals previously detected in a wide range of human wastewater effluents including an array of fire-retardants, musk fragrances, detergent constituents, solvents, and other chemicals widely used in cleaning agents, as well as selected pesticides (Glassmeyer et al., 2005; Zaugg et al., 2002; see Supplemental information). Nineteen samples were collected from 11 watershed sites for the analyses of wastewater chemicals: 4 samples on September 7, 6 on September 14, and 9 on November 16. In addition, one replicate sample and one field blank were collected on November 16.

Bacteria enumeration, DNA extraction, and polymerase chain reaction (PCR)

Methods followed those reported in Haack et al. (2009). Briefly, 100 mL grab water samples were analyzed using standard membrane-filtration methods for enumeration of EC and ENT. Growth on replicate filters from 100 mL of water was frozen at −70 °C until DNA extraction and analysis. PCR was conducted using a PerkinElmer GeneAmp PCR