

Enrichments of Metals, Including Methylmercury, in Sewage Spills in South Carolina, USA

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Abstract

Exposure to microbial pathogens is the primary concern of sanitary sewer overflows; however, sewage spills may also be a significant source of toxic metals, including methylmercury (MeHg). Between November 2015 and January 2017, after Hurricane Joaquin, surface water samples were collected routinely from three creeks in Columbia, SC. Routine sampling coincided with six sewage spills. Total mercury (THg) and MeHg (unfiltered and filtered) and 32 other metals (filtered) were measured. Compared with surface water samples, THg (unfiltered and filtered), MeHg (unfiltered), and 19 other metals were significantly higher in sewage spills (all \log_{10} -transformed) (two-tailed *t* test, $p < 0.05$ for all, $n = 38$ –42). Toxic weighting factors were applied to 18 metals, including THg and MeHg, in samples collected directly from sewage spills ($n = 3$ –4) and a wastewater outfall ($n = 5$). On average, sewage was 18.2 and 12.0 times more toxic for THg and MeHg, respectively, and 1.75 times more toxic for all 18 metals, compared to treated effluent from the wastewater outfall. Results suggest sewage spills were a source of inorganic Hg, MeHg, and other metals to the receiving waters and may potentially contribute to water quality impairments.

Core Ideas

- Sewage spills were a significant source of methylmercury (MeHg) to surface waters.
- Sewage spills were a significant source of 19 (of 32) other metals, including copper.
- Sewage was 1.75 times more toxic for 18 metals than treated wastewater.
- Receiving waters are impaired due to copper and fish tissue MeHg.
- Frequent sewage spills may contribute to water quality impairments.

ACROSS THE UNITED STATES, municipalities are serviced by > 20,000 sanitary sewer systems (USEPA, 2004a). Although sanitary sewer systems are less prone to overflows than combined sewer systems, these systems discharge untreated sewage during heavy rains. Sanitary sewage overflows (SSOs) are mainly attributed to excessive inflow and infiltration from storm water and groundwater (USEPA, 2004a). In 2004, the USEPA estimated between 23,000 and 75,000 SSOs occurred annually, releasing between 11 and 38 billion L of untreated wastewater (between 3 and 10 billion gallons) (USEPA, 2004a).

The primary concern of SSOs is exposure to harmful microbial pathogens, including bacteria, viruses, and parasites (USEPA 2004a; McLellan et al., 2007; Donovan et al., 2008; Fong et al., 2010). Sanitary sewage overflows are also a source of toxic metals, including cadmium (Cd), copper (Cu), lead (Pb), mercury (Hg), nickel (Ni), silver, and zinc (Zn) (USEPA 2004a). Treated wastewater contains methylmercury (MeHg) (Bodaly et al., 1998; Balogh and Nollet, 2008; Gbondo-Tugbawa et al., 2010; Mao et al., 2016; Liu et al., 2018), and wastewater effluent also promotes microbial Hg methylation in sediment (Bravo et al., 2011, 2015). Therefore SSOs (untreated sewage) also likely release MeHg directly into surface waters.

Sanitary sewage overflows are a chronic issue in Columbia, SC, due to aging sanitary sewer systems (SCDHEC, 2017d) (Fig. 1). In 2013, 2014, and 2015 (prior to Hurricane Joaquin), there were 174, 149, and 116 sewage spills in Richland County, South Carolina (including Columbia), releasing 5.3, 6.8, and 2.0 million L of sewage, respectively (SCDHEC, 2017d). From 1 to 5 Oct. 2015, Columbia experienced a significant rain event due to Hurricane Joaquin (NWS, 2016). More than 50 cm of rain fell during the 5-d period, which caused unprecedented flooding (NWS, 2016). Multiple wastewater treatment plants bypassed untreated sewage into receiving waters and, simultaneously, scores of SSOs occurred (CRK, 2015).

In November 2015, immediately after Hurricane Joaquin, we began collecting surface water samples from three creeks, which

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Abbreviations: CVAFS, cold vapor atomic fluorescence spectrometry; $\delta^{13}\text{C}_{\text{DOC}}$, $\delta^{13}\text{C}$ of dissolved organic carbon; DOC, dissolved organic carbon; HDPE, high-density polypropylene; *m*, mass; MDL, method detection limits; MeHg, methylmercury; SSO, sanitary sewage overflow; TDN, total dissolved nitrogen; THg, total mercury.

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Fig. 1. Sanitary sewage overflow in Columbia, SC (Location 5E in this study; photo: S. Rothenberg).

were impacted by SSOs before or during the hurricane. We anticipated that surface water metal concentrations would decay as floodwaters receded. Although SSOs are unpredictable and ephemeral, regular monitoring of three creeks coincided with six sewage spills (Table 1). Thus, our main objective was to determine the impacts of sewage spills on metal concentrations in receiving waters, including total mercury (THg), MeHg, and 32 potentially toxic metals. To further assess the impacts of sewage spills, we compared toxic-weighted discharges in effluent from sewage spills with treated wastewater effluent (USEPA, 2004b, 2012).

Materials and Methods

Sampling Approach

Beneficial Uses and Listed Impairments

Surface water samples were collected from Crane Creek, Stoop Creek, and Gills Creek, which feed into the Congaree River (Supplemental Fig. S1). Crane Creek drains into the

Lower Broad River, which is used for Columbia's drinking water supply. Stoop Creek drains into the Lower Saluda River, which is a popular site for contact recreational sports. Segments of the Lower Saluda River and the entire 80-km length of the Congaree River are listed as impaired for Hg, due to elevated concentrations of fish tissue Hg (CRK, 2016). In Gills Creek, there is a fish consumption advisory for Hg (SCDHEC, 2017a, 2017b). The Congaree River is also impaired for Cu (CRK, 2016).

Sewage Spills

Sampling occurred from 18 Nov. 2015 to 3 Jan. 2017 (Table 2). The first group of samples was collected at least once per month from November 2015 to March 2016. During this period, we captured three sewage spills, including a ruptured sewer force main that ran under Stoop Creek. The ruptured pipe was discovered by our team and reported to the South Carolina Department of Health and Environmental Control; repairs were completed within 48 h (Fretwell, 2016). We then targeted and sampled two additional SSOs at Crane Creek (9 Oct. 2016 and 3 Jan. 2017), totaling six sewage spills. Concentrations of THg and MeHg were measured in all six sewage events, other metals and phosphorus (P) were measured in five sewage spills, and total dissolved nitrogen (TDN), dissolved organic carbon (DOC), and $\delta^{13}\text{C}$ of DOC ($\delta^{13}\text{C}_{\text{DOC}}$) were determined in three sewage spills (Table 2). During the entire sampling period, there were 211 sewage spills in Richland County, releasing 25 million L of sewage (SCDHEC, 2017d). Although we only captured six (of 211) sewage spills, the volume released (8.2 million L, Table 1) comprised 33% of the total volume spilled.

Sampling Locations

At Crane Creek (Supplemental Fig. S1b), we sampled directly from two manholes (SSO 1, Location 5D, and SSO 2, Location 5E, Fig. 1). We sampled in a ditch (5B), which funneled sewage from SSO 1 into Crane Creek. Surface water was sampled across the river (5C) and downstream (5A) (~25 and 50 m away from the ditch, respectively). The downstream site (5A) was sampled

Table 1. Sanitary sewage overflows (SSOs) that were sampled for this study, including volume of sewage spilled, precipitation, and mass of total mercury (THg) methylmercury (MeHg), and copper released.

Sampling date	Creek and site name	Site ID	Volume of sewage spilled [†]	Precipitation [‡]	THg (unfiltered) [§]	MeHg (unfiltered) [§]	Copper [§]
19 Nov. 2015	Lake Katherine (two SSOs upstream from Gills Creek)	4A	0.14	50	0.0022	0.000023	0.30
31 Dec. 2015	Crane Creek (SSO 1)	5D	4.5	44	0.069	0.0023	15
17 Feb. 2016	Stoop Creek (sewer main rupture)	3C	2.0¶	0	0.018	0.00065	5.2
9 Oct. 2016	Crane Creek (SSO 1)	5D	0.78	74	0.026	0.000074	0.95
9 Oct. 2016	Crane Creek (SSO 2)	5E	0.50	74	0.036	0.00015	0.67
3 Jan. 2017	Crane Creek (SSO 1)	5D	0.25	55	0.0039	0.000032	NA#
Total			8.2	297	0.16	0.0032	22

[†] Volume of sewage (in gallons) reported in SCDHEC (2017d).

[‡] Dates for precipitation were 18–19 Nov. 2015, 30–31 Dec. 2015, 17 Feb. 2016, 7–9 Oct. 2016, and 2–3 Jan. 2017 (USGS, 2018). For 19 Nov. 2015, we used Gauge 021695045 (Richland County), and for all other dates, we used Gauge 02168504 (Lexington County). Two gages were used because there were data gaps for each gauge. Gage 021695045 was 8.7–12 km from the sampling sites, and Gauge 02168504 was 14–32 km from the sampling sites.

[§] Mass of THg (unfiltered), MeHg (unfiltered), and Cu released were calculated by multiplying the volume of sewage spilled (L) by the concentrations given in Supplemental Table S2. Local rivers are impaired for Cu and fish tissue MeHg (see the Materials and Methods).

[¶] The volume of sewage released may have been as high as 19 million L because the spill occurred for at least 1 mo before it was reported (Fretwell, 2016).

[#] NA, not measured and therefore not applicable.

Table 2. Sampling dates, site ID, and parameters measured (A = mercury, B = 32 other metals and phosphorus, and C = carbon and total dissolved nitrogen). Samples collected from six sewage spills are bolded.†

Creek	Location	Location no.	2015					2016					2017			
			18 Nov.	19 Nov.	10 Dec.	31 Dec.	16 Jan.	18 Jan.	22 Jan.	4 Feb.	5 Feb.	17 Feb.	25 Mar.	30 Mar.	9 Oct.	3 Jan.
Crane Creek	Reference sites	5A, 5C			A, B, C		A	A, B	A, B, C	A, B, C		A, B, C	A, B, C	A, B, C	A, B	A
	Ditch	5B			A, B	A, B, C	A	A, B, C	A, B, C	A, C		A, B, C	A, C	A, B		
	SSO# 1	5D			A, B, C									A, B	A	
	SSO 2	5E													A, B	
Stoop Creek	Reference sites	1B, 2A, 2C, 3A, 3B, 3C§	A, B, C		A, B, C		A, B, C				A, B, C	A, B	A, B, C			
	Outfall	2B	A, B, C		A, B, C		A, B, C				A, B		A, B, C			
Gills Creek	Sewer main rupture	3C§											A, B, C	A, B, C	A, B, C	
	Gills Creek	4A	A, B, C	A, B, C	A, B, C		A, B, C				A, B, C		A, B, C		A, B, C	

† Sewage events: 19 Nov. 2015 (SSOs occurred upstream from the Gills sampling site), 31 Dec. 2015 (Crane Creek SSO 1), 17 Feb. 2016 (ruptured sewer main in Stoop Creek), 9 Oct. 2016 (Crane Creek SSOs 1 and 2), and 3 Jan. 2017 (Crane Creek SSO 1).

‡ SSO, sanitary sewage overflow.

§ On 17 Feb. 2016, Site 3C was emitting sewage; however, on 25 and 30 Mar. 2016, Site 3C was repaired, and data were included with other reference sites.

routinely because metal concentrations were comparable between the two sites, and this site was more accessible.

At Stoop Creek (Supplemental Fig. S1c), surface water samples were collected from a wastewater treatment facility outfall (2B). This wastewater treatment plant did not treat the sewage from the SSOs sampled for this study. Surface water samples were also collected from five sites: one upstream from the outfall (1B, ~100 m), two located directly above and below the outfall (2A and 2C, both within 2–3 m of the outfall), and two sites further downstream from the outfall (3A and 3B, ~75 and 90 m downstream from the outfall, respectively). After we discovered the ruptured sewer force main (3C, ~120 m downstream from the outfall), this site was added to our sampling locations.

The Gills Creek sampling site (4A) was located ~1.6 km downstream from Lake Katherine, where SSOs routinely occurred (including on 19 Nov. 2015) (Supplemental Fig. S1d). Unlike Crane Creek and Stoop Creek, we were unable to sample from additional sites due to limited access. Instead, data from 19 November were compared with data collected on the other dates.

In Crane Creek (5A and 5C), Gills Creek (4A), and Stoop Creek (1B, 3A, 3B, and 3C), surface water samples were collected ~1 m from the creek bank.

Sample Collection

Total Mercury and Methylmercury (Unfiltered and Filtered)

Labware was acid cleaned for at least 24 h in 1.2 M hydrochloric acid (HCl), and triple-rinsed with ultrapure H₂O (≥18 MΩ cm⁻¹). Labware was then dried overnight in a bio-safety cabinet (Baker Company) and double bagged. Surface water samples for Hg analyses were collected directly into acid-cleaned 125-mL amber glass bottles with Teflon-lined lids. In the field, two bottles were collected for each surface water sample (for unfiltered and filtered analyses), and bottles were individually double bagged, returned to the laboratory, and processed the same day. At the laboratory, surface water samples were filtered (0.22 μm) into acid-cleaned 125-mL amber bottles. All bottles were preserved with concentrated HCl (0.5% v/v). A 45-mL aliquot from each bottle was poured into a 50-mL acid-cleaned polypropylene vial for MeHg analyses, and vials were individually double bagged and frozen (-80°C). Glass bottles (for THg analyses) were double bagged and stored at 4°C. Methylmercury samples were analyzed within 1 to 2 mo after sample collection, and THg was analyzed within 6 mo.

Other Metals and Phosphorus (Filtered)

A total of 32 other metals were measured, including transition and rare earth metals (i.e., aluminum [Al], barium [Ba], calcium [Ca], Cd, cerium [Ce], cobalt [Co], chromium [Cr], cesium [Cs], Cu, dysprosium [Dy], erbium [Er], europium [Eu], iron [Fe], gallium [Ga], holmium [Ho], lanthanum [La], lutetium [Lu], magnesium [Mg], manganese [Mn], neodymium [Nd], Ni, Pb, praseodymium [Pr], rubidium [Rb], samarium [Sm], strontium [Sr], thallium [Tl], thulium [Tm], uranium [U], vanadium [V], ytterbium [Yb], and Zn); P was also measured. Surface water samples were collected directly into 125-mL polyethylene bottles. Prior to use, bottles were acid washed in 10% nitric acid for at least 24 h, triple rinsed with ultrapure H₂O (≥18 MΩ cm⁻¹), air dried, and double bagged. In the field, samples were individually double bagged and returned to the

laboratory the same day. Approximately 8 mL of each sample was filtered through 0.22- μm hydrophobic syringe filters into acid-cleaned polypropylene vials. Filtered samples were immediately acidified to pH <2 using concentrated nitric acid (0.5 mL), double bagged, and stored at room temperature until analysis (within 2 to 7 d after sample collection).

Dissolved Organic Carbon and Total Dissolved Nitrogen

Surface water samples for DOC and TDN were filtered in the field through 0.2- μm polyethersulfone syringe filters using acid-washed high-density polypropylene (HDPE) syringes (10% HCl soak overnight, followed by soak in ultrapure H₂O [$\geq 18 \text{ M}\Omega \text{ cm}^{-1}$]). Samples were stored in acid-washed HDPE bottles and kept in the dark until they were returned to the laboratory (<4 h) and stored frozen (-20°C) until analysis.

Laboratory Methods

Total Mercury and Methylmercury

Concentrations of THg were analyzed following EPA Method 1631 (USEPA, 2002). Briefly, samples were digested with bromine monochloride (BrCl) overnight (0.5% v/v). Just before analysis, 30% hydroxylamine hydrochloride (100 μL) was added to neutralize BrCl, and tin(II) chloride (100 μL) was added to convert Hg(II) to volatile Hg(0). Defoaming agent (Spectrum brand Antifoam AF; 2% v/v, 1 mL) was added to analysis vials to reduce foaming. Total mercury was analyzed using cold vapor atomic fluorescence spectrometry (CVAFS) (MERX-T and Model III detector, Brooks Rand Instruments).

Concentrations of MeHg were analyzed following EPA Method 1630 (USEPA, 2001). Briefly, archived samples were distilled in 60-mL acid-washed Teflon vials under N₂. Just before distillation, 200 μL of 1% ammonium pyrrolidine dithiocarbamate was added to each vial. After distillation, MeHg concentrations were analyzed using sodium tetraethyl borate as the derivatizing agent, and gas chromatography–CVAFS (Model III detector, Brooks Rand Instruments). All THg and MeHg concentrations were blank corrected.

Quality assurance/quality control is summarized in Supplemental Table S1. For THg, mean recoveries of aqueous matrix spikes ($n = 15$) and solid-phase standard reference materials ($n = 20$) ranged from of 93 to 105%. For MeHg, recoveries of matrix spikes ($n = 21$) and standard reference materials ($n = 24$) ranged from 69 to 108%. The method detection limits (MDL) were calculated by dividing the lowest point on the calibration curve by the volume of sample analyzed (THg MDL = 0.5 ng L⁻¹, MeHg = 0.01 ng L⁻¹) (USEPA, 2011). Samples below the MDL included 12 filtered THg concentrations (21%), four filtered MeHg concentrations (7.1%), and two unfiltered MeHg concentrations (3.6%); for these observations, half the detection level was imputed for data analyses.

Other Metals and Phosphorus

Thirty-two other metals and P were analyzed using high-resolution inductively coupled plasma mass spectrometry (ThermoFisher ELEMENT II) (Das et al., 2013; Moskalski et al., 2013; Frisby et al., 2016). Briefly, aqueous samples were spiked with In (at 1 $\mu\text{g L}^{-1}$ concentration) as an internal standard to monitor and correct for drift. All samples were blank corrected. Elements with potential isobaric interferences (e.g., Al, Ca, Co, Cr, Cu, Fe, Ga, Mg, Mn,

Ni, P, V, and Zn) were measured in medium resolution (resolving power for mass [m]: $m/\Delta m = 4000$), whereas the rest were measured in low resolution for maximum sensitivity ($m/\Delta m = 300$). Concentrations were calculated against a multi-element standard solution (1 $\mu\text{g L}^{-1}$, High Purity Standards) and a fully digested USGS reference material (Icelandic Basalt, BIR-1) as a rock external standard (Supplemental Table S1). For the latter, the concentrations were compared with reference values reported in the GeoRem database (Jochum et al., 2005, 2016). Recovery for BIR-1 was not determined for P because reference values were not available. For the major cations (Ca, Fe, and Mg), the BIR-1 rock standard was used to calculate concentrations in the samples because the standard was too low to verify concentrations. For 28 metals, the average recovery for BIR-1 using the multi-element solution ranged from 80 to 128% (Supplemental Table S1). The lowest and highest recoveries were for Cs, Cd, and Tl, which also have the highest uncertainty in BIR-1 (i.e., 11, 22, and 33%, respectively) (Jochum et al., 2016). For all 33 elements, the relative SD for field duplicates ($n = 12$) ranged from 1.5 to 24% (Supplemental Table S1).

Dissolved Organic Carbon, Total Dissolved Nitrogen, and $\delta^{13}\text{C}_{\text{DOC}}$

Concentrations of DOC and TDN were analyzed by high-temperature combustion using a Shimadzu total organic carbon and total nitrogen analyzer. Deep seawater reference standards were injected every sixth sample and were within the range of reported values (i.e., DOC = 41–44 $\mu\text{mol L}^{-1}$). Standard deviations of replicate injections were <2% for DOC, <2% for TDN concentrations $\geq 45 \mu\text{M}$ ($n = 18/40$), and <5% for TDN concentrations <45 μM ($n = 22/40$).

Values of $\delta^{13}\text{C}_{\text{DOC}}$ were analyzed by the method of Lang et al. (2012). In brief, 4 mL of sample was transferred to a precombusted (500°C, 5 h) 12-mL borosilicate Exetainer vial (Labco) and acidified to a pH <3 with phosphoric acid (H₃PO₄). One milliliter of sodium persulfate oxidizing solution (100 mL H₂O + 4 g Na₂S₂O₈ + 200 μL H₃PO₄) was added, the vial was sealed, and the samples were flushed with high-purity helium (Grade 5.0, 99.999% He) for 5 min at 100 mL min⁻¹. The samples were heated at 100°C for 1 h to convert organic carbon to carbon dioxide (CO₂). The isotopic signature of the resulting CO₂ was analyzed using a GasBench II preparation device connected to a ConFlo IV interface and a Delta V Plus mass spectrometer (ThermoFisher). Values were determined using standards prepared over a concentration range that bracketed the samples, which had been previously calibrated to International Atomic Energy Agency standards (-12.4 ‰ sucrose, -33.6 ‰ phthalic acid). Values for $\delta^{13}\text{C}_{\text{DOC}}$ are reported vs. Vienna Pee Dee Belemnite (VPDB). The SD of duplicate analyses was 0.05 to 0.2‰, and propagated error based on the external standards was $\pm 0.3\text{‰}$.

Concentrations of THg and MeHg were analyzed at the University of South Carolina Mercury Laboratory (Rothenberg Laboratory). Other metals and P were analyzed at the Center for Elemental Mass Spectrometry at the University of South Carolina. Dissolved organic carbon, TDN, and $\delta^{13}\text{C}_{\text{DOC}}$ were analyzed at the Lang Laboratory at the University of South Carolina.

Toxic Weighting Factors

Chemicals differ in their toxicity; therefore, the USEPA developed toxic weighting factors for 1064 chemicals, which were based on chronic aquatic life criteria and human health criteria (USEPA

2004b, 2012). Toxic-weighted discharges are used to rank the relative toxicity of wastewater effluent between different sources (USEPA, 2004b, 2012). Chemical toxicity changes once the waste stream is discharged into surface water; therefore, toxic weighting factors are not applicable to sewage mixed with surface water (USEPA, 2012). In the present study, toxic weighting factors were applied to effluent directly collected from sewage spills (Locations 5D and 5E), and from the wastewater treatment plant outfall (i.e., treated effluent) (Location 2B). This analysis was not applied to other sampling sites or other sewage spills due to mixing between surface water and sewage effluent. Of the 34 metals measured for this study, toxic weighting factors were published for 18 metals (Table 3). Metal concentrations were converted to grams per liter

and then multiplied by the appropriate toxic weighting factor, yielding the toxic weighted gram equivalents per liter (USEPA, 2012). Toxicities for all 18 metals were summed, and the proportional contribution of each metal was determined. Toxic weighting factors were applied to filtered concentrations for 16 metals, whereas the unfiltered concentrations were used for THg and MeHg. The water quality criterion for MeHg is based on fish tissue MeHg, which is strongly positively correlated with unfiltered surface water MeHg (USEPA, 2010).

Data Analysis

Histograms were used to examine the distribution of each variable, and right-skewed variables were \log_{10} -transformed.

Table 3. Toxic weighting factors for unfiltered total mercury (THg) and methylmercury (MeHg) concentrations, and filtered metal concentrations (USEPA, 2004b). Toxic-weighted concentrations in effluent are compared between sewage spills and the wastewater outfall.

Metal†	CAS‡	Toxic weighting factor	Avg. \pm 1 SD toxicity		Ratio of avg. sewage/outfall
			in sewage ($n = 3-4$)§	in outfall ($n = 5$)	
g equivalent $L^{-1} \times 10^{-8}$					
THg (unfiltered)	7439976	110	375 \pm 295	20.6 \pm 7.45	18.2*
MeHg (unfiltered)	22967926	23,493.7	610 \pm 453	50.7 \pm 56.4	12.0*
Al	7429905	0.06	511 \pm 94.0	153 \pm 168	3.33*
Ba	7440393	0.00199076	2.85 \pm 1.02	2.57 \pm 1.80	1.11
Ca	7440702	0.000028	20.1 \pm 14.3	38.3 \pm 14.5	0.525
Cd	7440439	22.8	26.3 \pm 31.6	16.9 \pm 4.41	1.55
Ce	7440451	NA¶			
Co	7440484	0.11	8.34 \pm 4.08	6.26 \pm 3.68	1.33
Cr	7440473	0.07	2.68 \pm 0.725	0.943 \pm 0.328	2.84**
Cs	7440462	NA			
Cu	7440508	0.623	123 \pm 74.3	93.9 \pm 66.5	1.30
Dy	NA	NA			
Er	NA	NA			
Eu	NA	NA			
Fe	7439896	0.0056	237 \pm 166	56.7 \pm 89.2	4.18
Ga	NA	NA			
Ho	NA	NA			
La	7439910	NA			
Lu	NA	NA			
Mg	7439954	0.00086553	133 \pm 61.9	182 \pm 52.7	0.728
Mn	7439965	0.103	900 \pm 344	1000 \pm 1780	0.898
Nd	NA	NA			
Ni	7440020	0.1	12.7 \pm 4.63	7.05 \pm 1.85	1.80*
P	7723140	NA			
Pb	7439921	2.24	76.3 \pm 28.6	19.1 \pm 4.30	4.00**
Pr	NA	NA			
Rb	7440177	NA			
Sm	7440199	NA			
Sr	7440246	0.000022167	0.0764 \pm 0.0193	0.103 \pm 0.0322	0.743
Tl	7440280	2.85	2.14 \pm 1.09	1.47 \pm 0.536	1.46
Tm	NA	NA			
U	7440611	NA			
V	7440622	0.28	28.5 \pm 3.62	11.4 \pm 5.86	2.49**
Yb	NA	NA			
Zn	7440666	0.04	58.0 \pm 38.2	123 \pm 62.6	0.472
Sum of all 18 metals			3130 \pm 259	1790 \pm 232	1.75

*** Significant at the 0.05 and 0.01 probability levels, respectively. Significance was determined by two-tailed *t*-test.

† Al, aluminum; Ba, barium; Ca, calcium; Cd, cadmium; Ce, cerium; Co, cobalt; Cr, chromium; Cs, cesium; Cu, copper; Dy, dysprosium; Er, erbium; Eu, europium; Fe, iron; Ga, gallium; Ho, holmium; La, lanthanum; Lu, lutetium; MeHg, methylmercury; Mg, magnesium; Mn, manganese; Nd, neodymium; Ni, nickel; P, phosphorous; Pb, lead; Pr, praseodymium; Rb, rubidium; Sm, samarium; Sr, strontium; THg, total mercury; Tl, thallium; Tm, thulium; U, uranium; V, vanadium; Yb, ytterbium; Zn, zinc.

‡ CAS, Chemical Abstracts Service.

§ THg and MeHg: $n = 4$; all other metals: $n = 3$.

¶ NA, not applicable.

Metal concentrations were compared using parametric tests, including Student's two-tailed *t* test and one-way ANOVA. For ANOVA, multiple comparisons were estimated using the Sidak test, and *p* values for pairwise comparisons were reported in the text. For comparisons within each creek, we considered metal concentrations elevated if sewage concentrations were ≥ 1.5 times higher than average surface water concentrations within each creek. Bivariate associations were investigated using Pearson's correlation. For all statistical tests, an α level of 0.05 was chosen as a guide for significance. Data were analyzed using Stata 9.2 (StataCorp, 2005) and the R platform 3.3.2 (R Core Team, 2013).

Results

Total Mercury and Methylmercury Concentrations in Sewage Spills vs. Surface Water

Compared with surface water samples ($n = 36$), concentrations of \log_{10} THg (unfiltered and filtered) and \log_{10} MeHg (unfiltered) were significantly higher in sewage spills ($n = 6$) (two-tailed *t* test, $p < 0.02$ for all, $n = 42$; Fig. 2). The percentage of MeHg (of THg, unfiltered and filtered) was significantly lower in sewage spills compared to surface water samples (when \log_{10} -transformed), while the percentage of particulate-bound MeHg was significantly higher in sewage spills compared to surface water samples (two-tailed *t* test, $p \leq 0.01$ for all, $n = 42$; Fig. 2).

Considering each sewage spill separately, unfiltered THg concentrations were elevated in all six sewage spills, while unfiltered MeHg concentrations were higher in three (of six) sewage spills (i.e., one spill in each creek; Supplemental Tables S2–S3). Specifically, average unfiltered THg concentrations were 1.9 to 8.8 times higher than average values for creek-specific surface water samples. Filtered THg and unfiltered MeHg concentrations were 1.6 to 1.8 times higher and 1.7 to 3.8 times higher,

respectively, in three (of six) sewage spills (one in each creek), compared to creek-specific surface water samples. Filtered MeHg was 1.7 times higher in just one of four SSOs in Crane Creek than average values for Crane Creek surface water samples.

Total Mercury and Methylmercury Concentrations in Crane Creek vs. Other Creeks

Crane Creek was most heavily impacted by SSOs, compared with the other two creeks. In 2013, 2014, and 2015 (prior to Hurricane Joaquin), of the total amount of sewage spilled in Richland County, sewage overflows from SSO 1 (Location 5D) accounted for 10, 39, and 51%, respectively, whereas sewage overflows from SSO 2 (Location 5E) accounted for 4.3, 15, and 8.3%, respectively (SCDHEC, 2017d).

Compared to sewage spills ($n = 6$), \log_{10} MeHg (filtered) and \log_{10} percentage of MeHg (of THg) (unfiltered and filtered) were significantly higher in the Crane Creek ditch ($n = 9$, Location 5B), which funneled sewage from SSO 1 into the creek (two-tailed *t* test, $p < 0.01$ for all, $n = 15$). Inputs of THg and MeHg from the ditch impacted Crane Creek, as follows. Surface water THg (unfiltered) and MeHg (unfiltered) concentrations were significantly higher in Crane Creek compared to surface water samples in the other two creeks (when \log_{10} -transformed; ANOVA, $p < 0.05$ for all, $n = 36$). The highest MeHg concentration was measured on 10 Dec. 2015 in the ditch (Supplemental Tables S2–S3). No SSOs occurred on that day; however, SSOs occurred three times in November (2 November, 10 November, and 19 November), releasing 3.7 million L of untreated sewage (SCDHEC, 2017d). Results suggested frequent sewage spills at Crane Creek contributed to elevated THg and MeHg concentrations throughout the creek, including the ditch, across the creek, and 50 m downstream from the site of the sewage spills, compared with surface water samples in the other creeks.

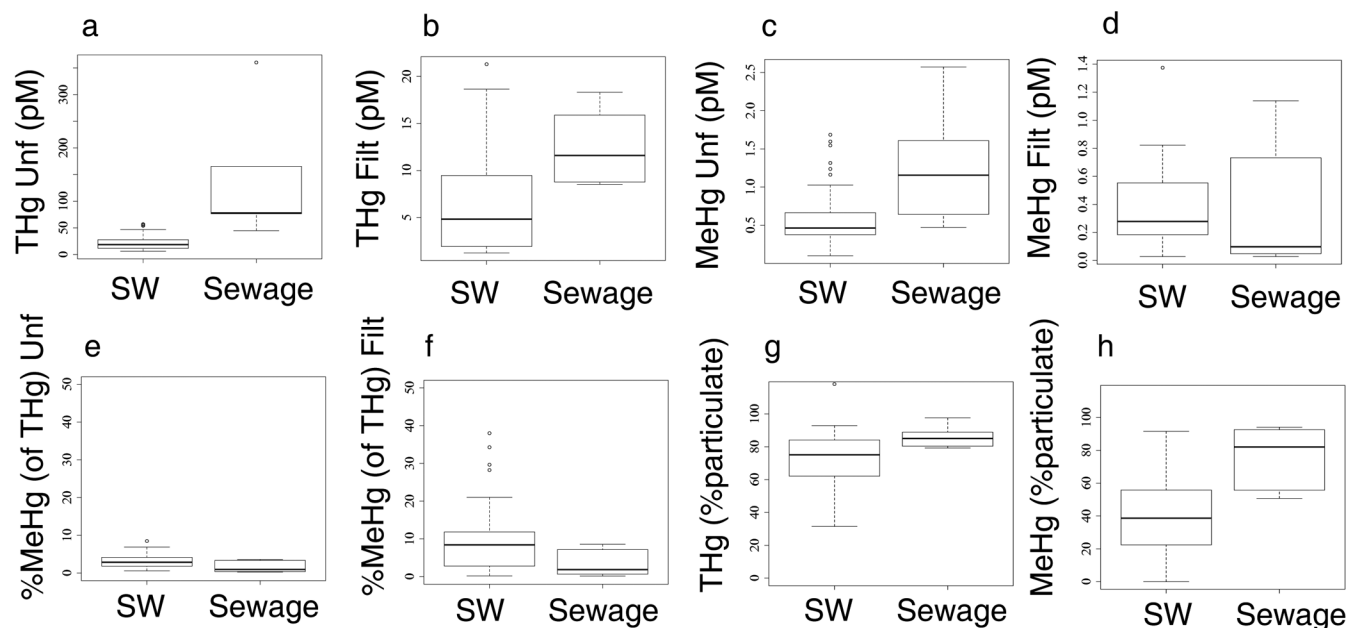


Fig. 2. Boxplots comparing total mercury (THg) and methylmercury (MeHg) concentrations in surface water (SW) ($n = 36$) and in sewage spills ($n = 6$), including (a) THg unfiltered (Unf)***, (b) THg filtered (Filt)*, (c) MeHg (Unf)*, (d) MeHg (Filt), (e) percentage MeHg (of THg) (Unf)***, (f) percentage MeHg (of THg) (Filt)*, (g) THg (% particulate), and (h) MeHg (% particulate)***. Boxplots show raw data; *p* values are for two-tailed *t* tests using \log_{10} -transformed data for Panels a–f and raw data for Panels g–h (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Percentage (%) particulate = $100(\text{unfiltered} - \text{filtered})/\text{unfiltered}$. For Panels a–d, divide pM by five to obtain ng L^{-1} .

Sewage and Other Metals

Sewage spills released other metals (aside from Hg) (Supplemental Tables S2–S3, Supplemental Fig. S2–S3). Compared with surface water sampling sites ($n = 33$), 19 other metals were significantly higher in sewage spills ($n = 5$), including Al, Cr, Cs, Cu, Ga, U, V, and all 12 rare earth elements (Ce, Dy, Er, Eu, Ho, La, Lu, Nd, Pr, Sm, Tm, and Yb) when all were \log_{10} -transformed (two-tailed t test, $p < 0.05$ for all, $n = 38$).

In Crane Creek SSOs ($n = 3$), concentrations of 11 to 31 metals (of 32 metals) were 1.5 to 8.9 times higher compared to average values for surface water samples (Supplemental Tables S2–S3). Metals most enriched in sewage were Cs (8.9 times higher), Zn (6.5 times higher), and V (3.2 times higher). Metals enriched in all three sewage spills included Co, Cs, Ga, Ni, V, and Zn and the light rare earth elements (Ce, La, Nd, Pr, and Sm) (Supplemental Tables S2–S3). In Gills Creek downstream from SSOs ($n = 1$), 21 metals were 1.5 to 3.8 times higher than the Gills Creek average (Supplemental Tables S2–S3). The most enriched elements were Al (3.6 times higher) and the light to middle rare earth elements (La, Ce, Pr, Nd, Sm, and Dy), which were 3.4 to 3.8 times higher than the Gills Creek average. At Stoop Creek, the ruptured force main had three metals with elevated concentrations compared with surface water samples, including Cu (1.8 times higher), Ga (2.1 times higher), and Mn (2.8 times higher). Both Mn and Ga were also 1.8 and 1.5 times higher, respectively, in the upstream wastewater treatment plant outfall compared to reference sites (Supplemental Tables S2–S3). Therefore, higher concentrations of these two metals measured downstream near the ruptured pipe possibly reflected inputs from the outfall.

Carbon (Dissolved Organic Carbon and $\delta^{13}\text{C}_{\text{DOC}}$) and Nutrients (Phosphorus and Total Dissolved Nitrogen)

Concentrations of P were analyzed in five (of six) sewage spills, whereas TDN and DOC were analyzed in three (of six) sewage spills (Supplemental Table S4). When data were combined from all sites, P, TDN, and DOC (all \log_{10} -transformed) were strongly positively correlated (Pearson's $\rho = 0.51$ – 0.91 , $p < 0.001$, $n = 39$ – 40). Concentrations of P, TDN, and DOC (all \log_{10} -transformed) were significantly higher in sewage spills compared to other surface water samples in all three creeks (two-tailed t test; $p < 0.002$; $n = 38$, 30, and 31, respectively; Supplemental Fig. S4).

Associations between DOC and metals were investigated in surface water samples with and without the three sewage samples ($n = 31$ and 28, respectively; all variables were \log_{10} -transformed). Including sewage spills, THg (unfiltered and filtered) and MeHg (unfiltered) had significant, positive relationships with DOC (Pearson's $\rho = 0.45$ – 0.69 , $p \leq 0.01$ for all). Additionally, 11 of 12 rare earth elements (excluding Eu), as well as six other metals (Al, Cu, Cr, Fe, Ga, and V), were positively correlated with DOC (Pearson's $\rho = 0.37$ – 0.53 , $p < 0.05$ for all). Excluding sewage spills, the correlation with DOC was attenuated for THg (filtered), six (of 11) rare earth elements, and five (of six) other metals. However, MeHg (filtered) was significantly correlated with DOC when sewage spills were excluded (Pearson's $\rho = 0.58$, $p < 0.01$).

Differences in $\delta^{13}\text{C}_{\text{DOC}}$ provide insight into the origin of C sources (Wayland and Hobson, 2001; Finlay and Kendall, 2007). The $\delta^{13}\text{C}_{\text{DOC}}$ values were significantly more positive in sewage

spills ($n = 3$) compared to surface water samples ($n = 26$) (two-tailed t test, $p = 0.01$, $n = 29$). The surface water samples in Crane Creek and Stoop Creek, as well as all samples in Gills Creek, had $\delta^{13}\text{C}_{\text{DOC}}$ values that were more typical for terrestrial freshwater systems (mean = -27‰) (Kendall et al., 2001). More positive $\delta^{13}\text{C}_{\text{DOC}}$ values in sewage samples likely reflected the more ^{13}C -enriched human diet, and/or microbial mineralization of organic matter (Cravotta, 1997).

Comparison of Toxicity in Effluent from Sewage Spills and in Treated Wastewater

Toxic weighting factors were applied to 18 metals to compare the relative toxicity of effluent from sewage spills ($n = 3$ – 4) with treated effluent from the wastewater treatment plant outfall ($n = 5$) (Table 3). On average, sewage was 18.2 and 12.0 times more toxic for THg and MeHg, respectively, and 1.75 times more toxic for all 18 metals, compared to treated effluent from the wastewater outfall. The toxicities of THg, MeHg, Al, Cr, Ni, Pb, and V were significantly higher in sewage compared to the outfall (two-tailed t test, $p < 0.05$ for all, $n = 8$ – 9), while toxicity due to all metals was higher, but not statistically significant (two-tailed t test, $p = 0.11$, $n = 8$) (Table 3). The average proportional contribution of each metal was determined (Supplemental Fig. S5). Compared with effluent from the outfall, the largest average increases in sewage toxicity were due to MeHg (+15%) and THg (+13%), while the largest average decreases were due to Mg (–12%) and Zn (–10%).

Discussion

Enrichment of Methylmercury and Other Metals in Sewage

Wastewater sewage treatment is designed to remove at least 90% of organic matter, before treated wastewater is discharged to water bodies (USEPA, 2004a). Metals often bind to organic matter, including Hg, which is one of the most strongly complexed metals (Evans, 1989). Therefore, it was not surprising that concentrations of THg, MeHg, the percentage of particulate-bound MeHg, and 19 other metals were significantly higher in sewage spills compared to surface water samples. Concentrations of DOC and $\delta^{13}\text{C}_{\text{DOC}}$ were also significantly higher in sewage compared to surface water samples. Most of the metals that were significantly higher in sewage compared to surface water were also strongly positively correlated with DOC (the exceptions were Cs, Eu, Fe, and Ga). Conversely, Mn is one of the least complexed metals by organic matter (Evans, 1989). Concentrations of \log_{10} Mn did not differ between sewage and surface water samples, and Mn toxicity did not differ between sewage and the outfall (see above).

Methylmercury in Sewage

Unfiltered MeHg concentrations were significantly higher in sewage spills compared to surface water samples. Methylmercury may be introduced into sewer systems with other wastes or wastewaters. Although most Hg emitted by the human body is inorganic Hg(II), MeHg can also be excreted (Rand et al., 2016; Rothenberg et al., 2016); therefore, it is possible that MeHg was sourced from municipal waste and transported through the sewage system complexed with DOC. Alternatively,

higher MeHg concentrations may reflect in situ Hg methylation within the sewer system. In the environment, Hg(II) methylation typically occurs in anoxic zones by anaerobic microorganisms, including sulfate-reducing bacteria (Gilmour et al., 2013). Similar dark, anoxic conditions may be found within sewer collection systems, which contain microbes within anaerobic biofilms on pipe walls, including sulfate-reducing bacteria (Nielsen and Hvitved-Jacobsen, 1988).

Previous studies reported higher MeHg concentrations in untreated sewage (influent), including Winnipeg, Canada (average = 2.2 ng L⁻¹; Bodaly et al., 1998), St. Paul, MN, USA (average = 3.3 ng L⁻¹; Balogh and Nollet, 2008), Syracuse, NY, USA (average = 5.05 ng L⁻¹; Gbondo-Tugbawa et al., 2010), Henan Province, China (average = 7.5 ng L⁻¹; Mao et al., 2016), and 24 provinces in China (average = 6.5 ng L⁻¹; Liu et al., 2018). Lower average MeHg concentrations in sewage in the present study (unfiltered MeHg = 0.25 ng L⁻¹, *n* = 6) may reflect dilution due to inflow and infiltration of groundwater (in Crane Creek) or mixing between sewage and surface water (in Gills Creek and Stoop Creek), or sewage MeHg concentrations were lower in this population.

Rare Earth Elements as Potential Tracers for Wastewater

Rare earth elements are a proxy for continental crust-derived material (in the form of eroded or dissolved rock and fine suspended sediment) (Moskalski et al., 2013). We sampled from sewage spills in each creek; however, the causes for sewage spills differed. The SSOs sampled at Crane Creek and Gills Creek were mainly due to excessive infiltration of groundwater and storm water, whereas in Stoop Creek, the sewage spill was due to a ruptured force main running under the creek. All three pipes transported municipal waste. The light rare earth elements (i.e., La, Ce, Pr, Nd, and Sm) had elevated concentrations in Crane Creek sewage spills, including SSO 1 on 31 Dec. 2015 and both SSO 1 and SSO 2 on 9 Oct. 2016. Similarly, in Gills Creek (downstream from two SSOs), the same five rare earth elements had higher concentrations on 19 Nov. 2015. In Stoop Creek, rare earth elements were not elevated in the ruptured pipe (on 17 Feb. 2016).

The lack of fractionation between these elements and the lack of a single enriched rare earth element suggested the source water for the Crane Creek and Gills Creek sewage spills originated from a common background, consisting of crustal origin (as eroded rock or dust). Other studies have used rare earth elements, particularly gadolinium, as tracers of urban wastewater inputs (Verplanck et al., 2005). Here, we found enrichments of the light rare earth elements in sewage spills, which we attributed to excessive inflow and infiltration. This information may be useful in future studies distinguishing the causes of sewage spills; however, more research is needed to verify these results.

Implications for Water Quality

All three creeks sampled for this study drain into the Congaree River (Supplemental Fig. S1), which is impaired for Hg and Cu (CRK, 2016). For Hg, emissions from coal-fired power utilities and incineration of municipal waste are considered the most significant sources to local watersheds (SCDHEC, 2017c). Atmospheric Hg is mainly Hg(0), which has an atmospheric residence time of up to 2 yr (Schroeder and Munthe, 1998). After Hg(0) is oxidized to Hg(II) and deposited to the earth's

surface, inorganic Hg(II) may be converted to MeHg by anaerobic microorganisms and biomagnified in fish tissue, or Hg(II) may be photoreduced to Hg(0) and volatilized (Schroeder and Munthe, 1998; Gilmour et al., 2013). Our results indicate that SSOs release MeHg directly into surface waters. Although the mass of MeHg released is likely lower than the mass of Hg released from power plants, it is of the form that is most toxic and readily biomagnified in fish tissue. The contributions of sewage to surface water impairments should be evaluated when establishing total maximum daily loads for Hg and Cu in the Congaree River. Given the volume of sewage released annually by SSOs in the United States (11–38 billion L; USEPA, 2004a), it is possible other US water bodies are similarly impacted with elevated concentrations of MeHg and other metals, which should be further investigated.

There are some limitations of the study that are worth noting. First, we did not sample during warmer months. However, sewage MeHg concentrations are higher in the summer compared to the winter due to increased microbial activity (Gbondo-Tugbawa et al., 2010), and therefore our results potentially underestimated the importance of SSOs to surface water MeHg. Second, we began sampling after Hurricane Joaquin, and we do not have pre-Hurricane data. Sanitary sewage overflows are a chronic issue in Richland County (including Columbia) (SCDHEC, 2017d), and the collection of baseline data before Hurricane Joaquin (i.e., without SSOs) was probably not possible within these creeks. Lastly, we were unable to calculate loads because we did not have flow data for all three creeks; however, these measurements will be included in future studies.

Supplemental Material

Supplemental Material includes Fig. S1 (maps), Table S1 (quality assurance/quality control), Tables S2 to S4 (concentration data), Fig. S2 to S4 (boxplots), and Fig. S5 (toxicity pie charts).

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References

- Balogh, S.J., and Y.H. Nollet. 2008. Methylmercury input to the Mississippi River from a large metropolitan wastewater treatment plant. *Sci. Total Environ.* 406:145–153. doi:10.1016/j.scitotenv.2008.07.039
- Bodaly, R.A., J.W.M. Rudd, and R.J. Flett. 1998. Effect of urban sewage treatment on total and methyl mercury concentrations in effluents. *Biogeochemistry* 40:279–291. doi:10.1023/A:1005922202681
- Bravo, A.G., S. Bouchet, D. Amouroux, J. Pote, and J. Dominik. 2011. Distribution of mercury and organic matter in particle-size classes in sediments contaminated by a waste water treatment plant: Vidy Bay, Lake Geneva, Switzerland. *J. Environ. Monit.* 13:974–982. doi:10.1039/c0em00534g
- Bravo, A.G., S. Bouchet, S. Guedron, D. Amouroux, J. Dominik, and J. Zopf. 2015. High methylmercury production under ferruginous conditions in sediments impacted by sewage treatment plant discharges. *Water Res.* 80:245–255. doi:10.1016/j.watres.2015.04.039

- Cravotta, C.A. 1997. Use of stable isotopes of carbon, nitrogen, and sulfur to identify sources of nitrogen in surface waters in the Lower Susquehanna river basin, Pennsylvania. Water Supply Paper 2497. USGS. <https://pubs.usgs.gov/wsp/wsp2497/pdf/wsp2497.pdf> (accessed 19 Apr. 2018).
- CRK. 2015. The flood: What happened and what happens next. Congaree Riverkeeper. <http://www.congareeriverkeeper.org/river-watch/flood-what-happened-and-what-happens-next-by-congaree-riverkeeper> (accessed 19 Apr. 2018).
- CRK. 2016. 2016 list of impaired waters. Congaree Riverkeeper. <http://congareeriverkeeper.org/river-watch/2016-list-impaired-waters-by-congaree-riverkeeper> (accessed 19 Apr. 2018).
- Das, R., M. Bizimis, and A.M. Wilson. 2013. Tracing mercury seawater vs. atmospheric inputs in a pristine SE USA salt marsh system: Mercury isotope evidence. *Chem. Geol.* 336:50–61. doi:10.1016/j.chemgeo.2012.04.035
- Donovan, E., K. Unice, J.D. Roberts, M. Harris, and B. Finley. 2008. Risk of gastrointestinal disease associated with exposure to pathogens in the water of the Lower Passaic River. *Appl. Environ. Microbiol.* 74:994–1003. doi:10.1128/AEM.00601-07
- Evans, L.J. 1989. Chemistry of metal retention by soils. *Environ. Sci. Technol.* 23:1046–1056. doi:10.1021/es00067a001
- Finlay, J.C., and C. Kendall. 2007. Stable isotope tracing of temporal and spatial variability in organic matter sources to freshwater ecosystems. In: R. Michener and K. Lajtha, editors, *Stable isotopes in ecology and environmental science*. 2nd ed. Blackwell Publ., Oxford, UK., doi:10.1002/9780470691854.ch10.
- Fong, T.T., M.S. Phanikumar, I. Xagorarakis, and J.B. Rose. 2010. Quantitative detection of human adenoviruses in wastewater and combined sewage overflows influencing a Michigan river. *Appl. Environ. Microbiol.* 76:715–723. doi:10.1128/AEM.01316-09
- Fretwell, S. 2016. Sewage leaks into Saluda River tributary. The State, 19 February. <http://www.thestate.com/news/local/article61424152.html> (accessed 19 Apr. 2018).
- Frisby, C.M., M. Bizimis, and S. Mallick. 2016. Seawater-derived rare earth element addition to abyssal peridotites during serpentinization. *Lithos* 248–251:432–454. doi:10.1016/j.lithos.2016.01.025
- Gbondo-Tugbawa, S.S., J.A. McAlear, C.T. Driscoll, and C.W. Sharpe. 2010. Total and methyl mercury transformations and mass loadings within a wastewater treatment plant and the impact of the effluent discharge to an alkaline hypertrophic lake. *Water Res.* 44:2863–2875. doi:10.1016/j.watres.2010.01.028
- Gilmour, C.C., M. Podar, A.L. Bullock, A.M. Graham, S.D. Brown, A.C. Somenhally, et al. 2013. Mercury methylation by novel microorganisms from new environments. *Environ. Sci. Technol.* 47:11810–11820. doi:10.1021/es403075t
- Jochum, K.P., U. Nohl, K. Herwig, E. Lammel, B. Stoll, and A.W. Hofmann. 2005. GeoRem: A new geochemical database for reference materials and isotopic standards. *Geostand. Geoanal. Res.* 29:333–338. doi:10.1111/j.1751-908X.2005.tb00904.x
- Jochum, K.P., U. Weis, B. Schwager, B. Stoll, S.A. Wilson, G.H. Haug, et al. 2016. Reference values following ISO guidelines for frequently requested rock reference materials. *Geostand. Geoanal. Res.* 40:333–350. doi:10.1111/j.1751-908X.2015.00392.x
- Kendall, C., S.R. Silva, and V.J. Kelly. 2001. Carbon and nitrogen isotopic compositions of particulate organic matter in four large river systems across the United States. *Hydrol. Processes* 15:1301–1346. doi:10.1002/hyp.216
- Lang, S.Q., S.M. Bernasconi, and G.L. Frueh-Green. 2012. Stable isotope analysis of organic carbon in small ($\mu\text{g C}$) samples and dissolved organic matter using a GasBench preparation device. *Rapid Commun. Mass Spectrom.* 26:9–16. doi:10.1002/rem.5287
- Liu, M., P. Du, C. Yu, Y. He, H. Zhang, X. Sun, et al. 2018. Increases of total mercury and methylmercury releases from municipal sewage into environment in China and implications. *Environ. Sci. Technol.* 52:124–134. doi:10.1021/acs.est.7b05217
- Mao, Y., L. Cheng, B. Ma, and Y. Cai. 2016. The fate of mercury in municipal wastewater treatment plants in China: Significance and implications for environmental cycling. *J. Hazard. Mater.* 306:1–7. doi:10.1016/j.jhazmat.2015.11.058
- McLellan, S.L., E.J. Hollis, M.M. Depas, M. Van Dyke, J. Harris, and C. Scopel. 2007. Distribution and fate of *Escherichia coli* in Lake Michigan following contamination with urban stormwater and combined sewer overflows. *J. Great Lakes Res.* 33:566–580. doi:10.3394/0380-1330(2007)33[566:DA FOEC]2.0.CO;2
- Moskalski, S.M.R., R. Torres, M. Bizimis, M. Goni, B. Bergamaschi, and J. Fleck. 2013. Low-tide rainfall effects on metal content of suspended sediment in the Sacramento-San Joaquin Delta. *Cont. Shelf Res.* 56:39–55. doi:10.1016/j.csr.2013.02.001
- Nielsen, P.H., and T. Hvitved-Jacobsen. 1988. Effect of sulfate and organic matter on the hydrogen sulfide formation in biofilms of filled sanitary sewers. *J. Water Pollut. Control Fed.* 60:627–634.
- NWS. 2016. The historic South Carolina flood of October 1–5, 2015. Natl. Weather Serv. https://www.weather.gov/media/publications/assessments/SCFlooding_072216_Signed_Final.pdf (accessed 19 Apr. 2018).
- R Core Team. 2013. R: A language and environment for statistical computing. R Found. Stat. Comput., Vienna, Austria.
- Rand, M.D., D. Vorobjikina, E. van Wijngaarden, B.P. Jackson, T. Scrimale, G. Zareba, et al. 2016. Methods for individualized determination of methylmercury elimination rate and de-methylation status in humans following fish consumption. *Toxicol. Sci.* 149:385–395. doi:10.1093/toxsci/kfv241
- Rothenberg, S.E., S. Keiser, N. Ajami, M.C. Wong, J. Gesell, J.F. Petrosino, and A. Johs. 2016. The role of gut microbiota in fetal methylmercury exposure: Insights from a pilot study. *Toxicol. Lett.* 242:60–67. doi:10.1016/j.toxlet.2015.11.022
- SCDHEC. 2017a. Approved TMDLs by watershed area. South Carolina Dep. Health Environ. Control. <http://www.scdhec.gov/HomeAndEnvironment/Water/ImpairedWaters/WatershedAreas/> (accessed 11 Jan. 2018).
- SCDHEC. 2017b. Gills Creek. South Carolina Dep. Health Environ. Control. <http://www.scdhec.gov/HomeAndEnvironment/Docs/03050110-02.pdf> (accessed 19 Apr. 2018).
- SCDHEC. 2017c. Mercury in the environment. South Carolina Dep. Health Environ. Control. <http://www.scdhec.gov/HomeAndEnvironment/Mercury/MercuryintheEnvironment/> (accessed 19 Apr. 2018).
- SCDHEC. 2017d. Sewer sanitary overflows. South Carolina Dep. Health Environ. Control. <http://www.scdhec.gov/apps/environment/SSO/> (accessed 19 Apr. 2018).
- Schroeder, W., and J. Munthe. 1998. Atmospheric mercury: An overview. *Atmos. Environ.* 32:809–822. doi:10.1016/S1352-2310(97)00293-8
- StataCorp. 2005. Stata statistical software: Release 9. StataCorp, College Station, TX.
- USEPA. 2001. Method 1630: Methyl mercury in water by distillation, aqueous ethylation, purge and trap, and CVAFS. EPA-821-R-01-020. USEPA, Office of Water, Washington, DC.
- USEPA. 2002. Method 1631, Revision E: Mercury in water by oxidation, purge, and trap and cold vapor atomic fluorescence spectrometry. EPA-821-R-02-019. USEPA, Office of Water, Washington, DC.
- USEPA. 2004a. Report to Congress on impacts and control of combined sewer overflows and sanitary sewer overflows, EPA 833-R-04-001. USEPA, Office of Water, Washington, DC.
- USEPA. 2004b. Technical support document for the 2004 effluent guidelines program plan. EPA-821-R-04-014. USEPA, Office of Water, Washington, DC.
- USEPA. 2010. Guidance for implementing the January 2001 methylmercury water quality criterion. EPA-823-R-10-001. USEPA, Office of Water, Washington, DC.
- USEPA. 2011. 40 code of federal regulation, Appendix B to Part 136: Definition and procedure for the determination of the method detection limit-revision. USEPA. <https://www.gpo.gov/fdsys/pkg/CFR-2011-title40-vol23/pdf/CFR-2011-title40-vol23-part136-appB.pdf> (accessed 19 Apr. 2018).
- USEPA. 2012. Toxic weighting factors methodology. EPA-820-R-12-005. USEPA, Office of Water, Washington, DC.
- USGS. 2018. National Water Information System: Web interface. USGS. <http://waterdata.usgs.gov> (accessed 19 Apr. 2019).
- Verplanck, P.L., H.E. Taylor, D.K. Nordstrom, and L.B. Barber. 2005. Aqueous stability of gadolinium in surface waters receiving sewage treatment plant effluent, Boulder Creek, Colorado. *Environ. Sci. Technol.* 39:6923–6929. doi:10.1021/es048456u
- Wayland, M., and K.A. Hobson. 2001. Stable carbon, nitrogen, and sulfur isotope ratios in riparian food webs on rivers receiving sewage and pulp-mill effluents. *Can. J. Zool.* 79:5–15. doi:10.1139/z00-169