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Elevated Levels of Pathogenic Indicator Bacteria and Antibiotic Resistance Genes after Hurricane Harvey's Flooding in Houston

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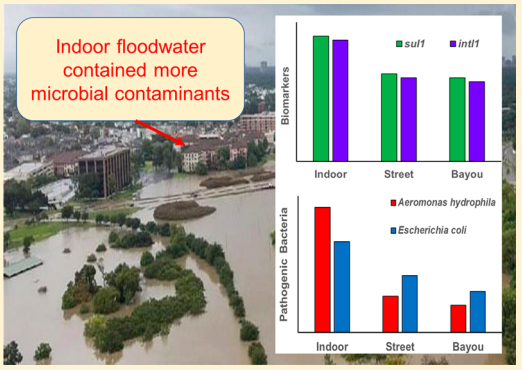
Supporting Information

ABSTRACT: Urban flooding can dramatically affect the local microbial landscape and increase the risk of waterborne infection in flooded areas. Hurricane Harvey, the most destructive hurricane since Katrina in 2005, damaged more than 100000 homes in Houston and flooded numerous wastewater treatment plants. Here we surveyed microbial communities in floodwater inside and outside residences, bayou water, and residual bayou sediment collected immediately postflood. Levels of *Escherichia coli*, a fecal indicator organism, were elevated in bayou water samples as compared to historical levels, as were relative abundances of key indicator genes of anthropogenic sources of antibiotic resistance (*sul*1/16S rRNA and *intI*1/16S rRNA) based on 6 month postflood monitoring. Quantitative polymerase chain reaction measurements showed that gene markers corresponding to putative pathogenic bacteria were more abundant in indoor floodwater than in street floodwater and bayou water. Higher abundances of 16S rRNA and *sul*1 genes were also observed in indoor stagnant waters. Sediments mobilized by floodwater exhibited an increased abundance of putative pathogens postflood in both residential areas and public parks. Overall, this study demonstrates that extreme flooding can increase the level of exposure to pathogens and associated risks.



INTRODUCTION

Hurricane Harvey, a Category 4 hurricane that made landfall in Texas on August 25, 2017,[1] was the most destructive hurricane to strike the U.S. mainland since Hurricane Katrina in 2005.[2] Hurricane Harvey stalled over the Houston area from August 25 to August 29 and dropped >50 in. of rain over the Houston metropolitan area, resulting in extreme flooding of 25−30% of the city.[3] More than 150000 houses were flooded, and many experienced high water for several days.[3] Moreover, Hurricane Harvey led to the widespread discharge of untreated or partially treated sewage due to overloaded and flooded wastewater treatment plants (WWTPs).[4,5]

Urban flooding can significantly affect the microbial landscape and pose potential public health risks.[6−8] Elevated levels of fecal indicator bacteria and microbial pathogens (e.g., *Legionella* spp. and *Aeromonas* spp.) were observed in both floodwater and residual sediments after Hurricanes Katrina and Rita.[9,10] The spread of raw sewage caused by flooded WWTPs may facilitate the dissemination of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) across the flooded areas.[11,12] Additionally, flooded homes may be conducive to the proliferation and survival of pathogens and ARB,[7,13] which is especially important because they may pose a threat to the residents and personnel involved in postflood restoration. In addition to an increased level of contact with contaminated food, water, and surfaces, urban floods can also cause more frequent person-to-person contact due to the massive displacement of population and gathering in shelters.[14] Therefore, flooding is one of the most common events preceding waterborne disease outbreaks in urban areas.[15] An increased number of cases of gastroenteritis has been reported postflood in the United States and United Kingdom, and a positive association between the severity of flooding and the number of gastroenteritis cases has been observed.[16,17]

Flooding is expected to become more frequent due to a combination of global climate change and the expansion of coastal cities.[18] However, few studies have focused on characterizing microbial communities in urban floodwaters, especially with respect to pathogenic bacterial exposures and antibiotic resistance in residential communities. Therefore, there is an urgent need not only to investigate the overall impacts of Hurricane Harvey on public flooded areas in Houston but also to characterize the pathogenic microbial profile and antibiotic resistance in representative samples from flooded residential communities.

The objective of this study was to assess post-Harvey microbial water quality in floodwaters inside and outside of

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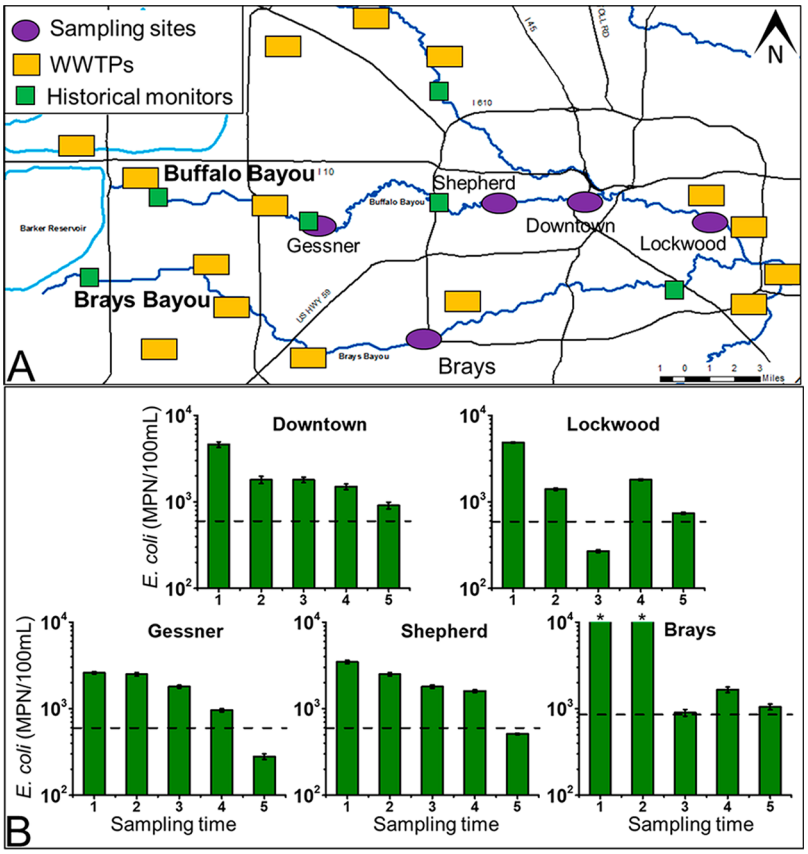

Figure 1. (A) Bayou water sampling sites and (B) impact of flooding on *E. coli* abundance in Houston bayous. The samples were collected on (1) September 1, 2017, (2) September 10, 2017, (3) October 26, 2017, (4) December 11, 2017, and (5) February 16, 2018. Error bars represent ±one standard deviation from the mean for three water samples from the same sites at the same time. The dashed lines represent the 7 year (2006−2013) geometric means of historical *E. coli* values along the bayou. Asterisks indicate the *E. coli* levels are >24000 MPN/100 mL.

residential homes and in nearby flooded bayous, along with characterization of the broader microbial community compositions and comparison to sediments. Bayou water and sediment samples were collected both immediately and several months after the flood event. We tracked the abundance of *Escherichia coli* as a fecal indicator organism in Houston bayou waters for 6 months postflood and compared the results with the historical data provided by the Houston Health Department. We present the first microbial community analysis using 16S rRNA gene amplicon sequencing and quantification of putative pathogenic bacteria in floodwaters and sediments mobilized by flooding during Hurricane Harvey in Houston. We also enumerate the indicators of anthropogenic sources of antibiotic resistance and potential for mobility of multiantibiotic resistance, *sul*1 (sulfonamide resistance) and *intI*1 (a class 1 integrase gene).[12,19] This is the first study to compare the microbial water quality of floodwater collected inside residents' homes to that of street and bayou floodwaters. We also examine the abundance of putative pathogens in residual sediments deposited by floodwaters from residential backyards and public parks over time after the floodwater retreated via quantitative polymerase chain reaction (PCR) measurements.

MATERIALS AND METHODS

Sample Collection and Water Quality Analysis. Water samples from Houston bayous were collected in triplicate using sterilized 1 L glass bottles (Fisher, Waltham, MA) and coliform sampling bottles (Gamut, Chicago, IL) at five different sites on five separate dates (Figure 1A), ranging from 3 days to 6 months postflood. Indoor and street water samples were collected within 2 weeks postflood from inundated residential areas. Bayou sediment samples deposited by floodwater (Figure S1) were also collected from three public parks and two residential areas 3 and 4 weeks postflood. Deep soil cores (20 cm below the surface) that did not include any visible deposited sediment were collected for comparison. The details of sampling methods, sites, and dates are available in the Supporting Information. All samples were transported back to Rice University on ice within 2 h, and physicochemical parameters [i.e., pH, dissolved oxygen (DO), conductivity, alkalinity, and turbidity] of the water samples were measured immediately. The chemical oxygen demand (COD), ammonia nitrogen (NH_3-N), and total phosphorus (TP) were measured with Hach Methods 8000, 10031, and 10127, respectively. Total *E. coli* was measured by the most probable number (MPN) method.[20] We compared the measured *E. coli* abundances to the 7 year (2006−2013) geometric means of historical *E. coli* values collected from nearby sampling locations along the bayou provided by the Houston Health Department.

DNA Extraction and Microbial Community Analysis. Biomass was concentrated from water samples for DNA extraction by centrifugation (10000*g* for 15 min), and soil/sediment samples were sieved through a 2 mm screen. Microbial DNA was extracted by a FastDNA Spin Kit for soil (MP, Solon, OH) following the manufacturer's instruc-

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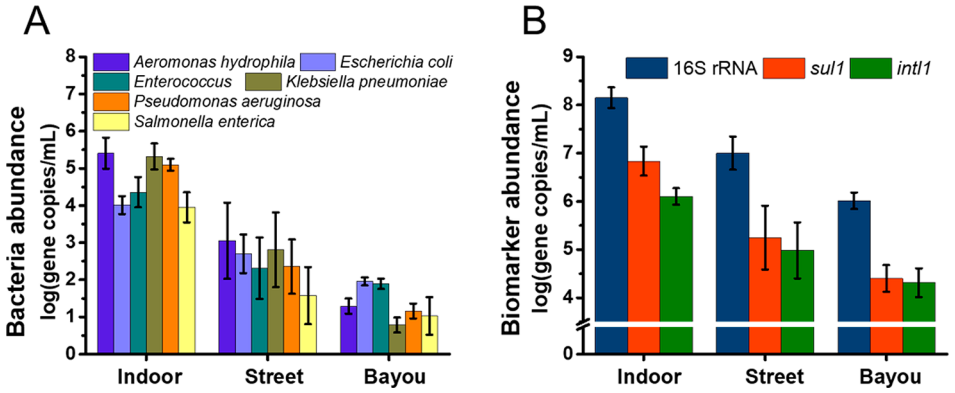

Figure 2. Comparison of gene abundances corresponding to (A) select putative pathogens in community and bayou water samples as determined by qPCR targeting genes *aha1*, *ybbW*, 23S rRNA, *rcsA*, *oprI*, and *hilA* for *A. hydrophila*, *E. coli*, *Enterococcus*, *K. pneumoniae*, *P. aeruginosa*, and *S. enterica*, respectively, and (B) 16S rRNA, *sul*1, and *intI*1 corresponding to total bacteria, resistance to sulfonamides, and class 1 integrons, respectively. Indoor water samples (n = 5) contained significantly higher abundances of putative pathogenic bacteria than street water samples (n = 5) or bayou water samples (n = 5) [t test, Bonferroni-adjusted two-sided $P < 0.0016$ (see Table S4)]. Bayou water samples were taken from Buffalo Bayou and Brays Bayou on September 1, 2017. Error bars represent ±one standard deviation from the mean of five independent samples.

tions. Phage λ DNA was used as an internal standard to calculate the recovery rate of DNA extraction from the concentrated or sieved samples.[21] The bacterial 16S rRNA gene, quantified by Taqman quantitative PCR, was used for total bacteria enumeration.[22] 16S rRNA gene amplicon sequencing was conducted on all samples to profile microbial community composition using methods described previously.[23] Sequences were processed with Mothur following the MiSeq SOP,[24] and operational taxonomic units (OTUs) were defined on the basis of 97% sequence similarity. Principal coordinate analysis of the Brays−Curtis distance matrix was used to compare the microbial communities in indoor water samples, street water samples, and sediment samples, and results were plotted using the phyloseq package in R.[25,26] The sequencing results were also used to screen the microbial communities for OTUs that may have been putative pathogenic bacteria.[27] Additional sequencing details are available in the Supporting Information.

Selected Pathogen and ARG Detection via Quantitative PCR (qPCR). Species-specific functional genes were chosen as biomarkers to verify and quantify fecal indicator bacteria (*E. coli* and *Enterococcus*) and several putative pathogenic bacteria (e.g., *Aeromonas hydrophila*, *Clostridium perfringens*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Pseudomonas aeruginosa*, and *Salmonella enterica*) (Table S2). These bacterial species were targeted because OTUs of the same families were identified in the water and sediment samples on the basis of the results of 16S rRNA gene amplicon sequencing. The extracted genomic DNAs from standard bacterial strains with different bacterial densities were used to establish the standard curve between the threshold cycle (Ct) value and $\log_{10}$(gene copies) for each pathogenic bacterium individually.[28] The sulfonamide ARG *sul*1 and class 1 integrase gene (*intI*1) were quantified as described previously.[29] Detailed information about SYBR Green qPCR on biomarkers, primers, and amplification conditions is included in Table S2.

Statistical Analyses. Abundances of fecal indicator bacteria, putative pathogenic bacteria, and *sul*1 and *intI*1 genes were compared across indoor floodwater (n = 5), street floodwater (n = 5), and bayou water (n = 5) samples. Sediment samples from public parks (n = 15) and those from residential communities (n = 10) and deep soil cores (n = 5) were also compared in terms of fecal indicator bacterium abundance and putative pathogenic bacterium abundance. Analysis of variance and a Student's t test with Bonferonni correction for multiple comparisons were used to determine statistical significance.

RESULTS AND DISCUSSION

Elevated Levels of Fecal Indicators and Markers of Anthropogenic Sources of Antibiotic Resistance in Houston's Bayous Immediately Post-Harvey. Flooding is a well-documented risk factor for environmental safety and human health.[30] To examine the impact of extreme flooding on key indicators of human health risk and the spread of antibiotic resistance immediately postflood, we surveyed the abundance of *E. coli* and the genes *sul1* and *intI1* in water samples collected from Houston's major bayous. Along Buffalo Bayou and Brays Bayou, *E. coli* abundances on September 1 (3 days postflood) were significantly higher than historic *E. coli* abundances (Figure 1B). The immediate post-Harvey *E. coli* levels were also significantly higher than those measured in samples collected over 2 months post-Harvey (Figure 1B). Although immediate preflood *E. coli* values were not obtained, these results strongly indicated that Harvey flooding increased the abundance of fecal bacteria in Buffalo Bayou. Six-month monitoring of Buffalo Bayou also showed that the relative abundances of both the *sul*1 gene (*sul*1/16S rRNA) and the *intI*1 gene (*intI*1/16S rRNA) were highest on September 1 (Figures S2 and S3), consistent with the expectation that flooding would elevate levels of markers of anthropogenic sources of antibiotic resistance. The increased fecal bacterium population and ARG abundance may have resulted from the discharge of untreated or partially treated sewage from overloaded and flooded WWTPs.[5]

Higher Relative Abundance of Pathogen and Antibiotic Resistance Indicators in Indoor than Street or Bayou Water Samples. Fecal indicator bacteria (*E. coli* and *Enterococcus*) and some bacterial families known to contain pathogens (e.g., *Aeromonas*, *Klebsiella*, *Legionella*, and *Salmonella*) were detected in both indoor and outdoor waters (Figure 2). The widespread occurrence of *E. coli* and *Enterococcus* in surface water samples indicates fecal contamination in the flooded areas, which could increase the risks of

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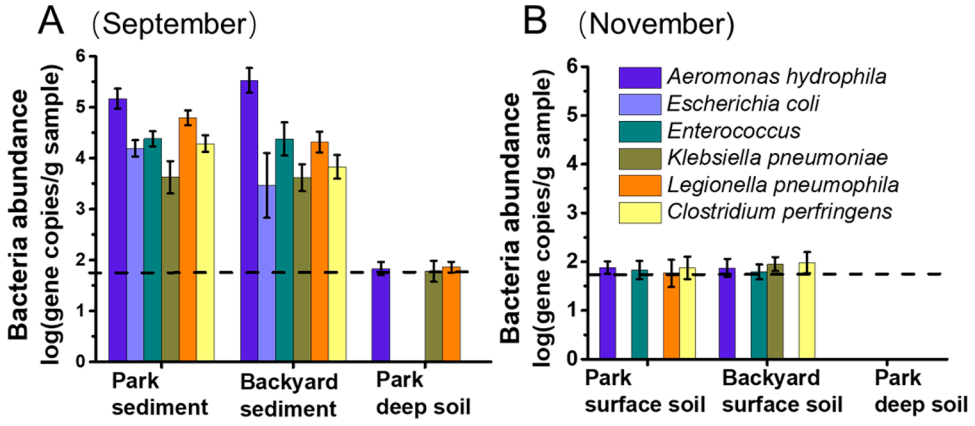

Figure 3. Gene abundances corresponding to targeted pathogenic bacteria quantified in sediments and surface soil by qPCR targeting the same genes indicated in Figure 2. Sediment samples were taken on September 20 and 28 (A) from three parks (n = 15) and two residential communities (n = 10), while surface soil (0−5 cm) samples were taken on November 16 (B) at the same location (Table S1). Deep soil cores (15−20 cm) taken from park 1 (n = 5) were used for comparison. Dashed lines indicate limits of quantification (50 gene copies/g) for qPCR-based bacterial enumeration. Error bars represent ±one standard deviation from the mean of independent samples.

eye and skin infection as well as gastroenteritis.[31,32] OTUs in the Aeromonadaceae family, which includes pathogenic bacteria that have previously been identified as a significant cause of infections associated with natural disasters (hurricanes, flooding, and earthquakes),[33] were among the most dominant taxonomic groups known to contain pathogens in all Houston floodwater samples.

We collected floodwater samples from inside residential homes, from outside homes (street water), and from nearby bayous to compare the microbial water quality across water samples. Of the indoor water samples that were collected (from September 4 to 8), four were collected from homes that were closed off for more than 1 week and contained primarily stagnant water, while the others were collected from open homes with floodwater flowing continuously throughout the first floor of the home. Principal coordinate analysis of the bacterial community16S rRNA gene amplicon sequences revealed that the open house floodwater samples clustered together, while samples collected from closed houses with stagnant water clustered separately (Figure S4). This indicates that when a home was closed off and water was allowed to stagnate, it developed a distinct microbial community.

To assess whether stagnation enabled the growth of pathogenic bacteria, we used qPCR for sensitive and specific quantification of gene markers corresponding to a suite of fecal indicators and human pathogens. The abundances of fecal indicators and human pathogens were significantly higher in stagnant indoor waters than in street waters, bayou waters, and open indoor waters (Figure 2A, Figure S5, and Table S4). Additionally, the abundance of *E. coli* had a relatively strong positive correlation with the other pathogenic bacteria such as *A. hydrophila* (R^2 = 0.81), *K. pneumoniae* (R^2 = 0.83), and *P. aeruginosa* (R^2 = 0.79). The levels of *E. coli* were as high as 1.0 × 10^4 gene copies/mL in stagnant indoor floodwaters. While we recognize that qPCR measurements capture both viable and nonviable bacteria, it is notable that such measurements are still substantially higher than the EPA standard for safe water for direct contact (126 colony-forming units/mL).[34] The levels of *K. pneumoniae*, a bacterium that is commonly reported to exhibit multidrug resistance,[35] and *P. aeruginosa*, a resilient opportunistic pathogen notorious for biofilm formation,[36] were 2.0 × 10^5 and 1.3 × 10^5 gene copies/mL, respectively. Consistent with the levels of pathogens detected, the *sul*1 and *intI*1 genes in closed indoor floodwaters were more abundant than in the street waters or bayou waters (Figure 2B). The absolute concentrations of *sul*1 and *intI*1 genes were around 250 and 60 times greater in closed indoor floodwaters than in bayou waters, respectively. These results indicate that indoor floodwater exposures, particularly in homes containing stagnant water, may pose significant microbial exposure risks. Precautions should be taken to avoid direct contact with stagnant floodwater during re-entry to flooded homes and postflood restoration.

Higher COD, NH_3-N, and TP Co-Occurred with More Severe Indoor Microbiological Contamination. We observed higher concentrations of nutrients in closed indoor floodwaters than in street water and bayou water samples (Table S3). Statistical analysis of 15 indoor and outdoor floodwater samples showed that the log-normal abundance of COD, NH_3-N, and TP exhibited strong positive correlations with observed *E. coli* abundances [in log(copies/mL)], with R^2 values of 0.87, 0.71, and 0.84, respectively. These associations could be due to the proliferation of most indoor bacteria, including pathogenic and antibiotic resistant strains, under high-nutrient conditions. Another possible explanation is that the nutrients and bacteria in the street water were diluted, while the floodwater inside homes was significantly less diluted. Further research is needed to understand the causes of severe indoor microbiological contamination (growth of endogenous bacteria vs inoculation from floodwater).

Pathogen Gene Markers Were More Abundant in Surface Sediments Than in Deep Soil Cores. Significant amounts of bayou sediment were deposited in parks bordering the bayous as a result of the flooding (Figure S1),[37] and these sediments may pose microbial risks after floodwater retreat if they harbor pathogens. Principal coordinate analysis revealed that the sediment samples clustered together and separated from the floodwater samples, indicating distinct microbial community compositions (Figure S4). qPCR enumeration showed that the most readily detected pathogen markers (e.g., *aha1* for *A. hydrophila*, *rcsA* for *K. pneumoniae*, *cpa* for *C. perfringens*, and *mip* for *L. pneumophila*) were similar between public parks and residential communities but different from those in floodwaters (Figures 2A and 3A). *C. perfringens*, one

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of the most common causes of food poisoning,[38] and *L. pneumophila*, the primary cause of Legionnaires' disease,[39] were among the most abundant pathogens in the sediment samples. *A. hydrophila* and *K. pneumoniae*, which were frequently detected in the floodwater samples, were also widely distributed in the sediments. All tested pathogen levels were at least 1 order of magnitude higher in the sediment samples than in deep soil cores (Figure 3A). In addition, as shown in Figure 3B, the levels of the targeted pathogen markers declined around or below quantification limits (50 copies/g) in the surface soil (0−5 cm) samples taken at the same sites on November 16, 2017 (80 days postflood). These results suggested that flood-mobilized sediments contain elevated levels of pathogens and may pose a public health risk if people come in contact with the sediments immediately postflood via recreation or cleanup efforts. More time-resolved samples should be gathered in the future to estimate pathogen die-off time, investigate sources of the detected pathogens, and study the factors that control pathogen propagation in soils and sediments postflood to inform remediation and recommendations for public park use.

Overall, the epic flooding caused by Harvey temporarily shifted the local microbial landscape, increasing the levels of gene markers for pathogenic bacteria, multiantibiotic resistance, and its extent of dissemination in the flooded areas. Stagnant indoor floodwater contained relatively high nutrient concentrations and likely provided a niche for the proliferation of potentially pathogenic bacteria. Sediments mobilized by floodwater were potential sources of infection in the 3 weeks after floodwaters retreated, and the pathogen profiles found in sediments were distinct from those of the floodwaters and bayou waters. Our results demonstrate that the elevated abundance of microbial contamination in stagnant indoor floodwaters and sediments increases the potential level of exposure of residents and relief workers in the aftermath of extreme floods.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.estlett.8b00329.

Methods of soil and sediment sampling, 16S rRNA gene amplicon sequencing and analysis, and quantitation and verification of selected biomarkers; figures of additional *E. coli* abundance, relative abundance of *sul*1 and *intI*1 genes, and principal coordinate analysis of microbial communities; and tables of locations and dates of water, soil, and sediment samples, primers and probes for qPCR analysis, and physical chemical parameters of floodwater samples (PDF)

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Notes

The authors declare no competing financial interest.

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