

ATTACHMENT A

**JANUARY 10, 2014 LETTER FROM RIVERKEEPER TO
ANGELA LICATA, NYCDEP
REGARDING
NEWTOWN CREEK AERATION SYSTEM**

Local Environmental Pollution Strongly Influences Culturable Bacterial Aerosols at an Urban Aquatic Superfund Site

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S Supporting Information

ABSTRACT: In polluted environments, when microbial aerosols originate locally, species composition of the aerosols should reflect the polluted source. To test the connection between local environmental pollution and microbial aerosols near an urban waterfront, we characterized bacterial aerosols at Newtown Creek (NTC), a public waterway and Superfund site in a densely populated area of New York, NY, USA. Culturable bacterial aerosol fallout rate and surface water bacterial concentrations were at least an order of magnitude greater at NTC than at a neighboring, less polluted waterfront and a nonurban coastal site in Maine. The NTC culturable bacterial aerosol community was significantly different in taxonomic structure from previous urban and coastal aerosol studies, particularly in relative abundances of Actinobacteria and Proteobacteria. Twenty-four percent of the operational taxonomic units in the NTC overall (air + water) bacterial isolate library were most similar to bacterial 16S rRNA gene sequences previously described in terrestrial or aquatic environments contaminated with sewage, hydrocarbons, heavy metals, and other industrial waste. This study is the first to examine the community composition and local deposition of bacterial aerosols from an aquatic Superfund site. The findings have important implications for the use of aeration remediation in polluted aquatic environments and suggest a novel pathway of microbial exposure in densely populated urban communities containing contaminated soil and water.



INTRODUCTION

In 1978, Bovallius and colleagues observed that airborne bacteria concentration was dependent on location, and that urban areas had higher concentrations than nonurban areas.¹ Since then, further work has confirmed these findings² and shown that microbial aerosols are often locally produced both in terrestrial and aquatic environments.^{3,4} Previous studies have established that bacteria, including pathogens, can remain viable after aerosolization from terrestrial and aquatic surfaces and travel a wide range of distances (several meters to 1000s of kilometers) before deposition.^{3,5} This aerial transport mechanism represents a potential public health concern in many urban areas, where terrestrial and aquatic pollution are common, including the release of untreated sewage into local waterbodies.

In aquatic systems, aerosols are primarily formed through the bursting of bubbles introduced to the water column by surface disruption.^{6–8} For example, when sewage is released into the surface waters of estuarine and coastal systems, the fresh water sewage, potentially containing human pathogens, can remain in a density-stratified surface layer, where bubbles release this material into the air.⁹ Once aerosols are formed, onshore winds

transport these particles over land, where they are eventually deposited through gravitational settling, inhalation, or surface interception. In the near-shore environment, particle deposition is dominated by large particles (coarse aerosols) of local aquatic origin.³ The likely high concentration of infective agents in sewage discharges into urban waterways,^{10,11} coupled with the high density of people in the near-shore environment, implies the potential for airborne infection if aerosol formation occurs at the water's edge.¹²

Past studies have found evidence for the transfer of sewage bacteria from water to air at coastal urban sites, including a 12x enrichment of sewage bacteria in coastal aerosols¹³ and increased inhalation contact with fecal coliforms during recreational activity in polluted coastal waters.¹⁴ These concerns are not limited to coastal regions. Researchers have postulated connections between river contamination, air quality, and human health in the Sarno River Basin, Italy,¹⁵

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and on the River Taff, United Kingdom, Wales.¹⁶ Despite these examples of microbial connections between water and air quality, the contribution of bacteria from local waters to near-shore aerosols is poorly characterized, especially in urban areas.

Previous studies of urban microbial aerosols have been conducted in land-locked geographical regions¹⁷ or have not focused on meteorological conditions conducive to microbial contributions from water surfaces.^{18,19} Meanwhile, most waterfront microbial aerosol studies have been conducted in remote areas,^{3,20,21} on a pier in open coastal waters,²² or during a Saharan dust storm.⁵ Moreover, these studies have generally focused on microbial aerosol concentrations and taxonomic identity but have not provided information about the end fate of the aerosols, including their fallout rates and viability upon deposition. Such data are critical because the taxonomic identity of bacterial aerosols is important for source attribution, and demonstrating viability of depositing bacteria is essential for public health and for understanding the role of microbial aerosols in ecological processes.²³

To test the connection between the local environment and urban microbial aerosols, we characterized fallout rates and taxonomic identity of viable bacteria in aerosols and water surfaces at Newtown Creek (NTC), a public waterway located in a densely populated area along the Hudson River Estuary (HRE) (New York, NY, USA). NTC was recently designated a Superfund site because it receives high volumes of untreated sewage input via a combined sewer system, has been exposed to over 140 years of industrial waste dumping, and ongoing oil seepage occurs from one of the largest underground oil spills in the country (Greenpoint Oil Spill, approximately 1.7×10^6 gallons of underground oil).²⁴ Bacterial concentrations in surface waters at NTC, including sewage indicators, are routinely elevated above concentrations measured in many other parts of the HRE.²⁵ This site houses a city-sponsored aeration project that periodically bubbles the water column to increase dissolved oxygen levels.²⁶ The complex sources of pollution and the potential for enhanced local aerosol production of coarse aerosols, due to aeration, make NTC an ideal location to investigate the connection between local environmental pollution and microbial aerosol production, composition, and deposition.

Our study addressed three hypotheses. First, if local sources, including water surfaces known to have high bacterial concentrations, contributed to bacterial aerosols at NTC, then bacterial fallout at NTC would be significantly greater than at less polluted locations. Second, because of high local levels of industrial and sewage pollution at NTC, bacterial aerosols depositing in NTC should represent a unique bacterial assemblage reflecting the local polluted environment as a source, containing both sewage and hydrocarbon-pollution-associated microbes. Third, aeration remediation would alter the concentration and bacterial composition of aerosols at NTC compared to samples collected when aeration was not occurring. To test these hypotheses, we measured aerosol concentrations and size distributions, bacterial fallout rates, and bacterial community composition of viable isolates from water and aerosols at NTC and compared these results to other less polluted urban and coastal environments. This study is the first to examine the local deposition and community composition of bacterial aerosols from an aquatic Superfund site. The findings have important implications for the use of aeration remediation in polluted aquatic environments and suggest a novel pathway

of microbial exposure in densely populated urban communities containing contaminated soil and water.

METHODS

Study Sites. Bacterial fallout sampling was conducted at two waterfront sites on the HRE in New York, USA. The Superfund site, Newtown Creek (NTC), is a tributary of the HRE located in Brooklyn, NY, USA (40.711731 N, 73.931431 W). Sampling at this site (8 September 2010 – 20 November 2010) was conducted from the Riverkeeper patrol boat, R/V R. Ian Fletcher (www.riverkeeper.org), which was moored to a bulkhead adjacent to an aeration remediation installation. Samples were collected over the course of 5 full sampling days (3 days (7 separate exposure events) with the aerator on, 2 days (4 separate exposure events) with the aerator off). Louis Valentino Pier (LVP) (40.67838 N, 74.01966 W), also on the HRE in Brooklyn, NY, USA, was sampled (6 April 2011 to 8 June 2011) as an urban, but less polluted comparison site. Because LVP is on the open waterfront of NY Harbor, it experiences much greater water flushing and much lower pollutant concentrations than the NTC site.

Meteorological Conditions and Aerosol Concentrations. At both sites sampled for this study, sampling was conducted only under conditions with no wind or low ($\geq 4 \text{ m s}^{-1}$) onshore winds. Wind speed, wind direction, humidity, and temperature data were collected from the Hudson River Environmental Conditions Observing System (www.hrecos.org), the personal weather station network through Weather Underground (www.wunderground.com), and through an onsite Vantage Pro2 Plus Weather Station (Davis Instruments, Hayward, CA) deployed during sampling. At NTC, onshore winds resulted in samplers being downwind of the aeration remediation when it was operating. Humidity and temperature were measured because they are known to affect aerosol particle size and deposition,²⁷ and are thought to play a role in bacterial aerosol viability.²⁸

At both NTC and LVP, a stationary Met One 9012 Ambient Aerosol Particulate Profiler (Met One Instruments, Grants Pass, OR, USA) was used onsite to quantify coarse aerosol particle concentrations during sampling, because the coarse particle mode is where particles containing bacteria of local surface origin would most likely be found.^{3,29} At NTC, the profiler was placed about 2.5 m above water level. At LVP, the profiler was placed at 2.0 m above the pier decking (2.5–5 m above water level, depending on tidal height). One-minute data were recorded in bins of 2, 3, 5, 7, and 10 μm particle diameter (D_p), with a maximum particle cutoff of approximately $D_p = 30 \mu\text{m}$. Aerator effects were tested using Student's *t* test on coarse aerosol concentration data pooled by sample date and by comparison of coarse aerosol particle size distribution curves from each sampling day. Statistical analyses in this study were conducted using R statistical software (R Development Project, 2008).

Bacterial Fallout. Culturable bacterial fallout rate was measured at both sites by exposing triplicate agar plates to ambient aerosols on a platform oriented to onshore winds. This sampling method provided a relative measure of aerosol bacterial abundance, not a total abundance, but has the advantage of confirming viability of enumerated bacteria. For this study, Luria–Bertani (LB) media (Miller, Fisher Scientific) was used to culture both aerosol and surface water bacteria. This medium has been used in previous studies to grow diverse bacterial assemblages from aerosols and water samples in a wide

range of environments, including urban sites.^{21,23} It should be noted that because LB is a nutrient-rich medium, it likely excludes some oligotrophic organisms that are unable to grow in the presence of high nutrient concentrations.

At the NTC site, plates were exposed at 2.5 m above the water surface. At the LVP site plates were exposed at 2.0 m above the pier decking (2.5–5 m above water surface). To prevent overgrowth of bacterial colonies, plates at NTC were exposed for 5–20 min, while at LVP plates were exposed for 20–60 min. After exposure in the field, plates were incubated in the laboratory for 5 days at 25 °C in the dark, after which colony-forming units (CFU) were counted. Bacterial aerosol settling rate ($\text{CFU m}^{-2} \text{ s}^{-1}$) was calculated using the surface area of the exposed Petri dishes (0.0079 m^2) and the duration of exposure. To assess culturability of surface water bacteria at each site, near-shore surface water (<1 m depth) was collected and spread on triplicate LB plates, then incubated and enumerated under the same conditions described for aerosol exposures. Bacterial fallout rates and surface water bacterial concentrations were log-normally distributed, so the geometric mean was used as the best estimate of central tendency. Differences in bacterial fallout rates and culturable surface water bacteria concentrations between sampling sites were tested using Student's *t* test on log-transformed data. The same method was applied to detecting differences in NTC bacterial fallout rates according to aerator operation mode (on/off).

According to a recent review, global estimates of bacterial emissions from aquatic surfaces range from 50–1000 cells m^{-2} .² This range estimate was calculated using a surface water bacterial concentration of 4.2×10^6 cells ml^{-1} , yielding bacterial emission/surface water bacteria concentration ratios of 1.2×10^{-5} – 2.4×10^{-4} . Making the assumption that bacterial emissions = bacterial fallout at steady state, we estimated a bacterial “emissions envelope” by plotting these ratio endmembers and comparing them with bacterial fallout rates and surface bacteria concentrations from NTC, LVP, and coastal Maine.³ While it is unlikely that all bacterial aerosols at any site are strictly aquatic in source, this “emissions envelope” gives perspective on where points would fall within the global estimates of aquatic emissions from water surfaces.

Molecular Analyses of NTC Bacterial Aerosols. After enumeration, NTC LB media plates were stored at 4 °C until colonies were sampled for molecular analysis. Material picked from colonies was suspended in 50 μL of HyClone sterile water (ThermoScientific, Logan, UT, USA), boiled for 5 min to lyse the cells, and frozen at –20 °C until PCR was performed. 16S rRNA genes were amplified from picked colony suspensions using universal bacterial primers 8F (5'-AGRGTTT-GATCCTGGCTCAG-3') and 1492R (5'-CGGCTACCTTGTTACGACTT-3')³⁰ with 35 PCR cycles of 45 s of denaturation at 94 °C, 45 s of annealing at 55 °C, and 1 min elongation at 72 °C.

Single-read sequencing using the 8F primer was performed by SeqWright Laboratories (Houston, TX, USA) and sequences were quality-checked and edited using Geneious software.³¹ High quality sequence longer than 300 base pairs were aligned using the Ribosomal Database Project (RDP)³² and taxonomically classified using RDP's naive Bayesian rRNA classifier at an 80% confidence level.³³ Dominant genera were identified as representing 5% or greater of the entire sequence library. Bacterial aerosols from the NTC site and other published studies^{5,17,20,23,34} were compared for significant

differences in community structure at the phylum level using RDP's Library Compare Tool.³³

Source Analyses of NTC Bacterial Aerosols. To assess dominant source for bacteria in both aerosols and surface waters, a top hits analysis was performed on representative sequences (deposited in GenBank under accession #'s JX853237–JX853447) of operational taxonomic units (OTU's) as assigned by the MOTHUR program³⁵ using the average neighbor algorithm at a 97% similarity cutoff. Representative sequences for each OTU were determined by MOTHUR (i.e., the sequence that was the minimum distance to the other sequences in the OTU), and then blasted against the GenBank database using Geneious' Megablast function. The sequence hit with the highest bit-score was designated the top hit for each sequence, and the reported sequence source was recorded. If more than one top hit was identified, the source for each top hit was recorded, and the source with the majority of hits was used to assign the sequence source category. This breakdown included the categories of “terrestrial”, “aquatic”, and “aerosol”. Top hits were also classified by association with polluted environments, and the type of contaminant and environment was recorded, resulting in the categorization of each OTU as “unpolluted” or “polluted” and pollution type noted as “oil”, “sewage”, “heavy metal”, or “other.”

RESULTS

Effects of Local Pollution on Bacterial Fallout Rates and Community Composition. During bacterial fallout sampling at NTC, onshore winds were low ($2.9 \pm 0.4 \text{ m s}^{-1}$ mean velocity) (Supporting Information Table 1). RH was lower and temperature was higher at the NTC site as compared to the sampling conditions at the LVP comparison site. Despite this, the geometric mean of bacterial fallout rate on LB plates at NTC ($2.00 \pm 0.50 \text{ CFU m}^{-2} \text{ s}^{-1}$) was significantly higher ($p < 0.01$) than those measured at the comparison site (LVP, $0.26 \pm 0.05 \text{ CFU m}^{-2} \text{ s}^{-1}$) or at a high-humidity nonurban coastal site (coastal Maine, USA, $0.09 \pm 0.05 \text{ CFU m}^{-2} \text{ s}^{-1}$)³ under similar low onshore wind conditions.

PCR amplification and sequencing of NTC bacterial aerosol and surface water isolates resulted in a sequence library of 530 isolates after quality control: 239 bacterial aerosol sequences and 291 surface bacteria sequences. This isolate library included diverse bacteria from 4 phyla: Bacteroidetes, Firmicutes, Actinobacteria, and Proteobacteria (Figure 1). Other urban and coastal aerosol libraries using both culture and culture-independent techniques had similar phylum representation, but the NTC bacterial aerosols were significantly different ($p < 0.01$) in community structure from all libraries, particularly in terms of representation in the Proteobacteria and Actinobacteria (Figure 1). This was in contrast to the fact that, with the exception of the Fahlgren et al.²⁰ clone library, there were no significant differences detected ($p > 0.35$) between the other libraries in terms of Actinobacteria representation. Within the Actinobacteria, NTC bacterial aerosols contained significantly ($p < 0.01$) more *Microbacterium* than all other comparison libraries.

The genera *Microbacterium*, *Pseudomonas*, *Shewanella*, and *Bacillus* dominated the total sequence library at NTC (Table 1). *Microbacterium*, *Pseudomonas*, and *Shewanella* were present in both aerosols and surface waters, but *Microbacterium* were more commonly found in bacterial aerosols, and *Pseudomonas* and *Shewanella* were more commonly found in surface waters

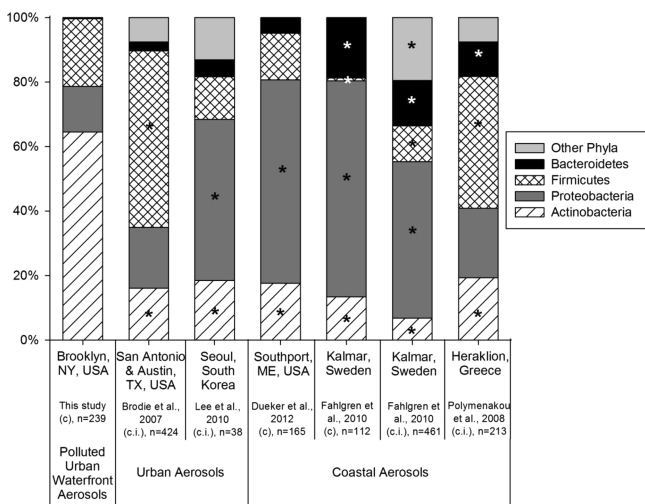


Figure 1. Comparison of Newtown Creek (NTC, Brooklyn, NY, USA) bacterial aerosol phyla with other published urban and coastal aerosol libraries. Studies were either culture-based (c.) or culture-independent (c.i.), and number of sequences used in the analysis noted (*n* =). An asterisk (*) indicates significant differences in phylum-level community structure from this study at *p* < 0.01.

Table 1. Dominant Genera in the Newtown Creek (NTC, Brooklyn, NY, USA) Isolate Library^a

	total library (<i>n</i> = 530)	surface water library (<i>n</i> = 291)	aerosols library (<i>n</i> = 239)
<i>Microbacterium</i>	18.5%	2.1%	38.7%
<i>Pseudomonas</i>	13.6%	22.3%	2.9%
<i>Shewanella</i>	8.9%	15.8%	0.4%
<i>Bacillus</i>	6.2%		13.9%
<i>Vibrio</i>	3.8%	6.8%	
<i>Acinetobacter</i>	3.2%	5.1%	0.8%
<i>Arthrobacter</i>	2.5%	0.3%	5.0%

^aPercentages represent the portion of *n* sequences represented by each genera in the Total Library (Surface Water + Aerosols), the Surface Water Library, and the Aerosols Library. Bold and italic percentages signify genera considered dominant in that library.

(Table 1). *Bacillus* was only detected in bacterial aerosols, and *Vibrio* was only detected in surface waters. Bacteria classified as gram-positive dominated bacterial aerosols (>75%) and gram-negative bacteria dominated surface waters (>90%). Genera known to include human pathogens were present both in surface waters and bacterial aerosols at this site (Supporting Information Table 2). These genera comprised large portions of both libraries, representing 44% of all surface water isolates and 74% of all aerosol isolates.

OTU analysis of the combined surface water and aerosol isolate sequences yielded 212 OTU's, 93 in the surface water library and 124 in the aerosol library. Five OTUs (representing 8% of the total sequence library) were shared between surface waters and aerosols. The shared OTUs represented 10% of the surface water isolates and 5% of the aerosol isolates, and were classified as *Shewanella* sp., *Pseudomonas* sp. (2 OTUs), an unclassified Pseudomonadacea, and *Microbacterium* sp. The source analysis of representative sequences for each OTU revealed that bacteria previously detected in terrestrial environments made up 83% of total aerosol OTUs and 42% of the total surface water OTUs. Also, 15% of the total aerosol OTUs were most similar to sequences previously detected in aquatic

environments. As hypothesized, 50 OTUs, representing 28% of the total sequence library (26% of surface water isolates, 23% of aerosol isolates), were most similar to organisms found in environments contaminated by heavy metals, hydrocarbons, sewage, and other industrial waste (Figure 2).

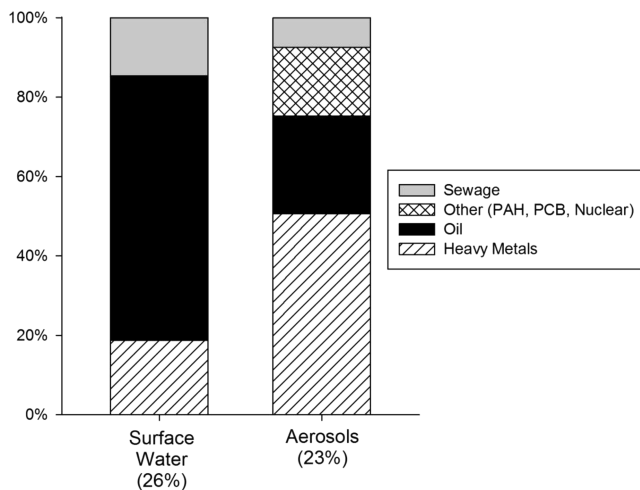


Figure 2. Pollution source analysis of OTU's from Newtown Creek (NTC, Brooklyn, NY, USA) aerosols and surface waters. % of OTU's with top hits from polluted environments from each library noted. Other = PCB, PAH, nuclear and/or unspecified polluted environments, sewage = organisms isolated from sewage, heavy metals = organisms isolated from heavy metal-contaminated soils and water, oil = organisms isolated from oil-contaminated soils and water.

Effects of Aeration Remediation on Bacterial Fallout Rates and Community Composition.

As hypothesized, mean NTC coarse aerosol concentrations pooled by day increased significantly (*p* < 0.05) when the aerator was on ($4.52 (\pm 0.9) \times 10^5 \text{ m}^{-3}$) as opposed to when the aerator was off ($1.86 (\pm 0.76) \times 10^5 \text{ m}^{-3}$). Comparison of size distribution curves for each sample day confirmed the increased presence of large coarse aerosol particles ($D_p \geq 6 \mu\text{m}$) when the aerator was operating (Figure 3). The difference in bacterial fallout rates

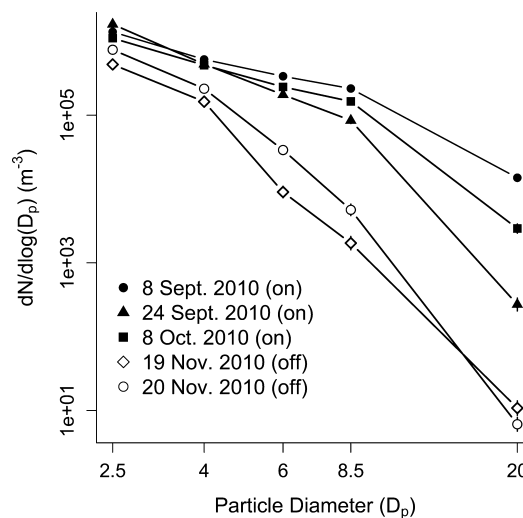


Figure 3. NTC coarse aerosol particle size distribution by sample date, with aerator status (on/off) noted. Vertical lines denote standard error for each point, if error bars are larger than plot point character.

with aerator operation was not as pronounced, however. The geometric mean of bacterial fallout rates was higher when the aerator was on ($2.12 \pm 0.71 \text{ CFU m}^{-2} \text{ s}^{-1}$) than when the aerator was off ($1.72 \pm 0.57 \text{ CFU m}^{-2} \text{ s}^{-1}$), but the difference was not statistically significant ($p = 0.6802$). The geometric mean of culturable surface bacterial concentrations, however, was almost an order of magnitude lower when the aerator was on ($4.1 (\pm 0.3) \times 10^3 \text{ CFU ml}^{-1}$) than when the aerator was off ($2.43 (\pm 0.09) \times 10^4 \text{ CFU ml}^{-1}$) ($p < 0.01$). With the aerator off, plotting NTC bacterial fallout rate versus surface bacterial concentrations was well within the Burrow's et al.² "emissions envelope", but when the aerator was on the point was well above the envelope (Figure 4).

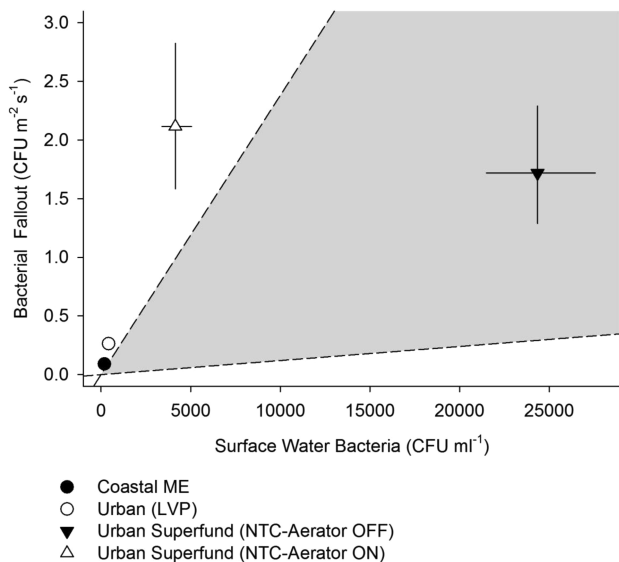


Figure 4. Geometric mean of bacterial fallout rates ($\text{CFU m}^{-2} \text{ s}^{-1}$) plotted against geometric mean of culturable surface water bacteria (CFU ml^{-1}) for NTC (Brooklyn, NY, USA) and Louis Valentino Pier (LVP, Brooklyn, NY, USA) waterfront sites. Data from sampling in coastal Maine (ME) (Dueker et al.³) included for non-urban coastal reference. Horizontal and vertical bars represent geometric standard error. If error bars are not visible, they are smaller than the plot point character. Dashed lines and shaded region represent the range of global bacterial emissions estimates from surface waters as calculated by Burrows et al.²

Molecular analysis of bacterial aerosols and culturable surface water bacteria revealed differences in bacterial taxonomy with aerator operation. There were significantly more ($p < 0.05$) *Microbacteriaceae* (particularly *Microbacterium*) in surface waters when the aerator was on compared to when the aerator was off. The same was true for aerosols, with significantly more *Microbacterium* ($p < 0.05$) present when the aerator was on than when the aerator was off. Sewage OTUs were present in surface waters both when the aerator was on and off, but were only found in aerosols when the aerator was on.

DISCUSSION

This study demonstrated substantial influences of local environmental pollution on bacterial aerosols at NTC, a Superfund waterway. As predicted, bacterial fallout rates were significantly elevated at NTC when compared to a neighboring waterfront site and coastal Maine. Also, the bacterial aerosol community composition at NTC was unique when compared

to other coastal and urban aerosol studies, reflecting heavy sewage and industrial pollution in the local terrestrial and aquatic environment. Furthermore, aeration remediation of this waterway resulted in increased aerosol production from surface waters, strengthening the connection between water and air quality at this Superfund site.

While the surface waters of NTC were previously known to sustain elevated surface water bacterial counts,²⁵ the bacterial aerosols data presented here are the first confirmation that the air at this Superfund site also supported high bacterial loads. Differences in meteorological conditions between NTC and the comparison sites would not explain the difference in fallout rates. Wind speeds were comparably low and onshore during all sampling reported in this study. In addition, RH was much lower at NTC than at both the LVP comparison site and the Maine coastal site.³ Low RH is known to result in smaller aerosol particle size³⁶ and is thought to decrease bacterial aerosol viability.²⁸ Low RH would therefore decrease the NTC fallout rate relative to other sites. A more likely explanation is that the elevated fallout at NTC is related to the elevated surface water bacterial concentrations at the site. Supporting this explanation, Hultin et al.³⁷ found a linear relationship between culturable bacterial concentrations in coastal surface waters and culturable bacterial aerosols produced from simulated wave action in controlled experimental conditions. Similarly, Bradley et al.³⁸ found a positive correlation between aerosolized seawater coliforms and increased aerosol creation through recreational activities in polluted coastal waters. More generally, when the aerator was off, the ratio of bacterial aerosols to surface water bacterial concentrations at NTC fit well within the "emissions envelope" developed using Burrows et al.² estimates for bacterial emissions from surface waters (Figure 4).

Given the complex urban setting of the NTC site, bacterial aerosols most likely derive from both terrestrial and aquatic surfaces, but the overlap in community composition of surface water and aerosol bacteria indicate that surface waters were a major contributor to aerosols. NTC water and air isolate libraries shared dominant genera and OTUs, particularly *Microbacterium* (soil bacteria) and *Pseudomonas* (aquatic bacteria). While terrestrial sources did appear to dominate bacterial aerosols (83% of aerosol OTU's), they also dominated surface waters at NTC (42% of surface water OTU's). This means that the reported percentage of aquatic-associated bacteria in bacterial aerosols (15%) can be considered a lower limit estimate of the water–air transfer of bacteria occurring at this site.

As predicted, pollution at this site appears to have produced a unique bacterial aerosol community at NTC, as demonstrated by the significant phylum-level differences between the NTC aerosols library and other published urban and nonurban aerosol libraries (Figure 1).^{5,17,20,23,34} While some of these differences may be a function of media selection, Fahlgren et al.²⁰ and Bowers et al.¹⁸ observed that culture-based techniques do generally succeed in sampling dominant bacterial types in bacterial aerosols (both urban and nonurban). The significant dominance of Actinobacteria (particularly the soil-associated *Microbacterium*) at NTC may stem from the long-term ecological impact of NTC's heavy pollution loading. Actinobacteria are known to process hydrocarbons and heavy metals in both terrestrial and aquatic environments,^{39–41} and have been dominant in past culture-based studies of sewage-polluted coastal waters.⁴² Furthermore, 23% of NTC bacterial aerosols

were close matches with bacteria previously associated with heavy metal, hydrocarbon, and sewage contamination (Figure 2).

Although association with polluted environments does not mean that a bacterium is necessarily a public health concern, it does suggest that the impact of polluting water and soil is not restricted to these surfaces and instead also impacts the types of microbes found in local air masses. In addition, the presence of sewage-associated bacteria in surface waters indicates high likelihood of pathogen presence in both the water and air. The potential for polluted public waterways to incubate or act as a reservoir for pathogens that can then be emitted to the air has important public health implications, particularly in crowded urban environments. NTC surface waters are known to frequently contain high concentrations of *Enterococcus* sp.,²⁵ which are used as indicators of the presence of sewage and associated pathogens known to cause gastrointestinal illnesses.⁴³ Our sampling further confirmed the presence of genera known to contain pathogenic bacteria in NTC surface waters (Supporting Information Table 2), including *Aeromonas*, *Enterobacter*, *Enterococcus*, *Francisella*, and *Vibrio*. Furthermore, *Acinetobacter*, *Microbacterium*, and *Psychrobacter*, genera often implicated in opportunistic infections, were detected in viable state both in surface waters and aerosols. Bacterial aerosols also harbored genera containing pathogens thought to be aquatic, such as *Massilia*⁴⁴ and *Roseomonas*.⁴⁵

When compared to the neighboring reference site (LVP), surface waters at NTC harbored extraordinarily high culturable bacterial concentrations. At NTC, 90% of these culturable surface water bacteria were gram-negative, which can pose a further public health concern in terms of increasing potential for aerosolization of bacterial products, including endotoxins and other lipid antigens.⁴⁶ Negative health consequences have been documented for human exposure to aerosolized endotoxins in sewage treatment facilities^{47–51} but have not yet been explored in urban public waterways with high sewage inputs and potential aerosol production from surface waters through mechanisms including recreational activities, commercial boat traffic, and aeration remediation.

Although aerosol creation through aeration remediation of a public waterway has not before been studied, aerosol creation has been documented (as transfers of both chemical and bacterial content from air to water) in waste treatment plants using aeration technology.^{52–54} As hypothesized, coarse aerosol concentrations were significantly increased at NTC when the aerator was operating versus when it was off, and large aerosol particles in particular ($D_p \geq 6 \mu\text{m}$) were more concentrated when the aerator was operating. Given that large aerosol particles have short residence times, and therefore are local in origin, this increase in large particles with aeration is strong evidence of aerator-produced surface water aerosol emissions.

Despite the clear signal of coarse aerosol production through aeration remediation, NTC bacterial fallout rates did not increase during aeration. This may be related to the concomitant decrease in surface water bacterial concentrations during aeration. Taking those concentrations into account, bacterial fallout rates during aeration actually exceeded what would be expected by Burrows et al.'s² emissions estimates (Figure 4). In addition, the bacterial composition of the aerosols changed when the aerator was on, providing further evidence of aerator-facilitated bacterial aerosol production from surface waters. For instance, sewage-associated bacteria were only detected in bacterial aerosols when the aerator was on.

Also, *Microbacterium* significantly increased in relative abundance in both surface waters and aerosols when the aerator was on. Changing aerosol bacterial community composition and increased coarse aerosol concentrations with aeration operation indicate that aeration remediation strengthens the connection between water quality and air quality at this public Superfund waterway and has the potential for unintended and unexplored health impacts on surrounding urban populations.

■ ASSOCIATED CONTENT

📄 Supporting Information

Supplemental tables and figures referred to in the text. This information is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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Supporting Information

Local environmental pollution strongly influences culturable bacterial aerosols at an urban aquatic Superfund site

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Supporting Information Summary: 1 page, 2 tables

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Supplemental Table 1. Mean meteorological conditions during bacterial fallout sampling at Newtown Creek (NTC, Brooklyn, NY, USA) and the comparison site, Louis Valentino Pier (LVP, Brooklyn, NY, USA).

	NTC	LVP
Exposure Days	5	5
Exposure Events	11	16
Temperature (°C)	20.7 ± 3.7	18.3 ± 1.9
Relative Humidity (%)	45.1 ± 6.8	77.1 ± 5.4
Wind Speed (m s⁻¹)	2.9 ± 0.4	1.7 ± 0.5
Coarse Aerosols (m⁻³ air)	4.1 (± 0.9) x 10 ⁵	4.1 (± 1.1) x 10 ⁵

Supplemental Table 2. Genera known to contain human pathogens detected in water and air at Newtown Creek (NTC, Brooklyn, NY, USA). Genera assigned using Ribosomal Database Project (RDP), 80% confidence threshold.

	Aerosols	Surface Water
<i>Acinetobacter</i>	x	x
<i>Aerococcus</i>	x	
<i>Aeromonas</i>		x
<i>Bacillus</i>	x	
<i>Brevundimonas</i>	x	
<i>Citrobacter</i>		x
<i>Comamonas</i>		x
<i>Enterobacter</i>		x
<i>Enterococcus</i>		x
<i>Exiguobacterium</i>	x	
<i>Francisella</i>		x
<i>Kocuria</i>	x	
<i>Massilia</i>	x	
<i>Microbacterium</i>	x	x
<i>Micrococcus</i>	x	
<i>Nocardiopsis</i>	x	
<i>Paenibacillus</i>	x	
<i>Paracoccus</i>	x	
<i>Pseudomonas</i>	x	x
<i>Psychrobacter</i>	x	x
<i>Rhodococcus</i>	x	
<i>Roseomonas</i>	x	
<i>Sphingobacterium</i>	x	
<i>Sphingomonas</i>	x	
<i>Staphylococcus</i>	x	
<i>Streptomyces</i>	x	
<i>Vibrio</i>		x