Biological Contaminant Fate and Transport In an Urban Environment
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Table of Contents

1.0 Introduction .................................................................................................................................. 1
  1.1 Objectives ................................................................................................................................ 3
  1.2 Methods ................................................................................................................................... 4

2.0 Factors Affecting Contaminant Fate and Transport in Urban Areas ........................................... 5
  2.1 Urban Persistence and Migration ............................................................................................ 5
  2.2 Urban Particulate Transport Mechanisms ............................................................................... 5
  2.3 Urban Storm Water Runoff ....................................................................................................... 6
  2.4 Urban Storm Water Modeling .................................................................................................. 7
    2.4.1 Models ............................................................................................................................... 8
    2.4.2 Particle Size Distribution (PSD) ....................................................................................... 8
  2.5 Runoff and Sediment Transport ............................................................................................... 9
  2.6 Overland Pollutant Transport ................................................................................................... 11
    2.6.1 Storm Water Aggregates ................................................................................................. 11
    2.6.2 Particle-Bound Metals ..................................................................................................... 12
    2.6.3 Microbial Aggregation and Transport ............................................................................ 13

3.0 Conclusions and Research Needs ............................................................................................... 15
  3.1 Research Questions .................................................................................................................. 16

4.0 References .................................................................................................................................. 17

5.0 Search Terms .............................................................................................................................. 22

Table of Figures

Figure 1. Scope of report .................................................................................................................... 3
Figure 2. Forces involved with sediment detachment, adapted from Southard, 2006 ..................... 10
Figure 3. Comparison of sediment particle sizes and a Bacillus spore ........................................... 11
## Acronyms and Abbreviations

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ba</td>
<td><em>Bacillus anthracis</em></td>
</tr>
<tr>
<td>Bti</td>
<td><em>Bacillus thuringiensis israelensis</em></td>
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<tr>
<td>Btk</td>
<td><em>Bacillus thuringiensis kurstaki</em></td>
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<tr>
<td>CBR</td>
<td>Chemical, Biological, and/or Radiological</td>
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<td>Cp</td>
<td><em>Clostridium perfringens</em></td>
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<tr>
<td>DHS</td>
<td>Department of Homeland Security</td>
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<td>EPA</td>
<td>U.S. Environmental Protection Agency</td>
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<td>FEMA</td>
<td>Federal Emergency Management Agency</td>
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<td>HSRP</td>
<td>Homeland Security Research Program</td>
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<tr>
<td>Ls</td>
<td><em>Lysinibacillus sphaericus</em></td>
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<tr>
<td>PSD</td>
<td>Particle Size Distribution</td>
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<td>TOC</td>
<td>Total organic carbon</td>
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<td>USDA</td>
<td>U.S. Department of Agriculture</td>
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Executive Summary

This report supports EPA’s mission to address critical needs related to homeland security, which includes decontamination following a chemical, biological, and/or radiological (CBR) attack or release. The release of a biological agent, such as *Bacillus anthracis* (*Ba*) spores, in an urban area could create large areas of complex contamination. Because the many transport pathways in an urban environment create a broad area of study, this report narrowly focuses on the potential for spore transport from urban surfaces during and following precipitation events.

The main process affecting conservative pollutants (pollutants with no chemical formation or loss) in water, like spores, is adsorption onto a solid. In storm water, these aggregates are then transported with the sediment particles in the water. Based upon the literature, most runoff sediment is in the fine particle size range (i.e. <2.5 µm), increasing the likelihood of spore-sediment interaction.

It is possible that many deposited spores will be removed from urban surfaces during the early phase of a precipitation event by what is known as the “First Flush phenomena,” however this needs further study. Many research questions need to be addressed in order to inform site characterization and sampling strategies following an urban release.

As a result of this literature review, the following research questions have been identified that will inform site characterization and sampling strategies following an urban release of *Ba* spores:

- What is the potential for spore entrainment in storm water?
- What is the potential for spores to migrate with fine grain sediments in storm water?
- What is the aggregation rate of spores with fine sediments?
- What is the aggregation rate of spores with fine sediments in rapidly moving water (i.e. with shorter residence times)?
- Does the spore-particle association behavior seen in runoff hold true for overland flow?
- What are the most effective methods to collect particle-bound spores in storm water samples?
- What is the most effective method to collect particle-bound spores directly from active overland flows?
- Do organic and inorganic aggregates containing spores require different decontamination approaches?
- Can precipitation event intensity, volume and duration, be parameterized to model the magnitude of the initial wash off (First Flush) of spores from urban surfaces?
- What are the key parameters needed to predict the extent of spore accumulation and wash off from snow? Are these parameters different for liquid precipitation?
- What is the potential for spore retention in vegetation buffers?
1.0 Introduction

This report supports EPA’s mission to address critical needs related to homeland security, which includes decontamination following a chemical, biological, and/or radiological (CBR) attack. Part of EPA’s Homeland Security Research Program (HSRP) mission is to conduct threat and consequence assessment research and deliver products that improve the ability of EPA to assist decision makers in the preparation for and recovery from public health and environmental emergencies resulting from terrorist threats and incidents. One specific focus area of HSRP research is on decontamination methods and technologies that can be used in the recovery efforts resulting from a CBR contamination incident. In recovering from an incident and decontaminating an area, it is essential to identify and implement appropriate decontamination technologies.

The release of a biological agent, such as Bacillus anthracis (Ba) spores, in an urban area could create large areas of complex contamination including building exteriors, streets and sidewalks, vegetation, and other open spaces (DHS-EPA 2009). The response to a large-scale urban biological release would pose considerable challenges due to these varied and complex surfaces, but perhaps a greater challenge would be how to target environmental sampling to characterize the extent and magnitude of the contamination given the likely dynamic nature of the spatial distribution of the contaminant over time. Understanding contaminant fate and transport in an urban environment is vital in “predicting” contaminant concentrations spatially and temporally, which can inform sampling and mitigation plans. This information can facilitate effective sampling and decontamination technology selection and deployment, to best achieve a reduction in contaminant levels such that the risk to public health is minimized.

Following a large-scale urban contamination incident, the potential for spores to remain unmitigated, and therefore a potential public health threat, for weeks to months is high. Even if the release is detected immediately, site sampling and characterization will not begin until after the first phase of the response is complete (DHS-EPA 2009, FEMA 2011), a stage during which a characterization strategy is developed (DHS 2012). Following initial site characterization to support law enforcement and public health, further characterization supporting environmental remediation will follow. Environmental sampling will occur to determine the magnitude and extent of contamination, as well as an assessment of the potential for reaerosolization and contamination relocation from both natural and anthropogenic processes (DHS 2012).

Site characterization may involve the sampling and analysis of a complex range of surfaces and matrices, and both the planning and execution of this effort is anticipated to be time consuming. During this time, the spatial distribution of the contamination may evolve and change. According to current guidance, formal written plans need to be in place before sampling can begin. Even once a sampling strategy has been agreed upon and executed, sample analyses, data reduction, and generation of a comprehensive site characterization map will take significant time. During this time, and later while mitigation options are being explored and formally planned based upon initial sampling results, contaminants may migrate to other areas, changing the margins of high concentration zones, potentially contaminating even previously uncontaminated areas. Because “consequence management for wide area disasters can escalate quickly into a substantially unmanageable problem” (DHS 2012), it is critical that the mechanisms behind the relocation of contamination from one area to another in an urban environment be more completely understood.
While guidance on indoor biological sampling and decontamination exists (EPA-CDC 2012), guidance on strategies for outdoor sampling and remediation/mitigation following a release of Ba spores is lacking (Pottage, Goode et al. 2014). Previous reviews of decontamination methods and strategies have all pointed to the difficulty (next to impossible) of decontaminating a large outdoor area (AFRL 1999), and the dearth of tested decontamination strategies for wide-area use (Campbell, Kirvel et al. 2012).

While some authors suggest that many indoor decontamination and sampling strategies would be useful in the outdoor environment (Krauter, Edwards et al. 2011, Campbell, Kirvel et al. 2012) and some sporicidal technologies have demonstrated efficacy on outdoor materials in lab-scale tests (EPA 2010, Calfee, Choi et al. 2011), more research remains to demonstrate how indoor or outdoor strategies can be made applicable to a wide area event. For example, the variety of surface types, potential for contamination relocation, and the potentially limitless spatial distribution of contamination poses both research and operational challenges. For instance, the Census Bureau defines urban areas as densely developed and populated residential and commercial areas having an abundance of impermeable surfaces (76FR 2011), some of these impermeable areas can be as much as 17.5% (Nowak and Greenfield 2012). As such, these areas typically contain concentrated areas of buildings, roads, and sidewalks, as well as smaller areas of vegetation (76FR 2011). Thus, urban areas do not necessarily resemble indoor areas, so it is unclear how indoor area decontamination strategies and techniques will be applicable. Additionally, by definition indoor areas are enclosed whereas outdoor areas are open, which could impact a decontamination strategy as in the outdoors a decontaminant may disperse more quickly than it does indoors, potentially reducing contact times.

Although the first step in the remediation of an outdoor area following the detection of a biological release is to identify the contamination zones (Campbell, Kirvel et al. 2012), tracking the spread of contamination in such an environment could be difficult and may complicate or delay mitigation and remediation efforts. Because no one can predict the location, extent or magnitude of a contamination incident, and all urban areas have unique features, preplanning a sampling and decontamination response for an outdoor incident is nearly impossible. Current response guidance focuses on casualty decontamination (Lake, Divarco et al. 2013), response operational and organizational structures (JP 3-41 2012), and developing mitigation strategies. These mitigation strategies focus on areas of high contamination, followed by areas of lower contamination using unspecified physical methods (chosen following a cost/benefit and risk/benefit analysis), or rely on natural attenuation (Raber, Kirvel et al. 2011).

Outdoor decontamination approaches are not prescriptive, rather they are incident specific, based upon many inputs, and the methods are those which have demonstrated efficacy (DHS-EPA 2009; DHS 2012). Much of the current mitigation response strategy focuses on the identification and characterization of the contaminant, site characterization to determine contaminant distribution, and an assessment of the threat of human exposure (DHS-EPA 2009). As part of the site characterization, contaminant fate and transport can be estimated using mathematical modeling (DHS-EPA 2009), although transport properties will vary based upon spore preparation, potentially making accurate transport estimates problematic (DHS 2012). In spite of this potential limitation, site specific transport estimates will be an important facet in the approach to guiding response planning and optimizing the deployment of resources in order to reduce the overall cost and length required to return to normalcy.
1.1 Objectives

This report analyzes existing scientific knowledge concerning the fate and transport of contaminants in the outdoor urban environment. More specifically, it discusses the major factors that determine contaminant redistribution, and how those factors are used to predict contaminant movement over time when subject to typical environmental and anthropogenic forces. This information may be used to guide sampling and mitigation efforts, and to identify knowledge gaps in need of further scientific study. The scope of this report is limited to the redistribution of contaminants in an urban environment by rain and storm water, as outlined by Figure 1.

![Figure 1. Scope of report.](image)

Formulating mitigation strategies to a biological release in an urban setting requires a thorough understanding of how such contaminants spread through this unique environment. Sampling and mitigation plans should account for contaminant fate and transport in order to be comprehensive. In the absence of existing fate and transport data for Ba spores in the days, weeks and months post-release in a large urban area, the purpose of this report is to formulate hypotheses regarding the fate and transport of Ba spores in an outdoor urban environment based upon the reported behavior of similarly sized particles and other contaminants, and to identify research gaps that can more precisely address these questions.
1.2 Methods

Information about contaminant fate and transport in an urban environment for this literature review was considered from unclassified peer reviewed journal articles, conference proceedings and textbooks found by searching citation databases including the EPA Desktop Library, PubMed, and Google Scholar. Additionally, published technical and guidance reports, and books were included. Articles referenced include original research and literature reviews. Reports referenced include research, current state and local response guidance documents, and government or military response guidance documents. Books referenced were published within the last five years or are the most recent edition of an established source. Search terms are included in Section 5 of this report. The search was limited to articles published in the English language, but there was no restriction on geographic location.

This report was generated using references (secondary data) that could not be evaluated for accuracy, precision, representativeness, completeness, or comparability and therefore no assurance can be made that the data extracted from these publications meet the U.S. Environmental Protection Agency (EPA) quality assurance requirements. However, the sources of secondary data were limited to peer-reviewed documents.
2.0 Factors Affecting Contaminant Fate and Transport in Urban Areas

2.1 Urban Persistence and Migration

Field studies examining the fate and transport (via air, water and surface mechanisms) of Ba spores following an urban release are not publically available. For this reason, preplanning sampling and mitigation efforts following such an event is difficult because the quality of the preplanning is hampered by a lack of data. There are data in the literature on the transport of some radionuclides following nuclear release incidents (Chernobyl) that can be used to inform potential urban fate and transport, but this would only be applicable to an atmospheric release (because such nuclear accidents often result in emission of contaminants high into the atmosphere), not to a low/street level release. One long-term field study on urban persistence of Bacillus thuringiensis kurstaki (Btk) spores, a Ba surrogate, over a 4-year period showed persistence in soil throughout the study. The surrogate remained detectable to a lesser extent on surfaces (up to 48 weeks), vegetation (grass up to six weeks and leaves up to 24 weeks), and in water (up to 48 weeks), but these results were not consistent across all sampling sites. The authors concluded that in urban environments, some Bacillus species will remain detectable for many years (Van Cuyk, Deshpande et al. 2011).

Another study tracked spore transport in an urban area following the release of less than 50 g B. amyloliquefaciens (Group VI Bacillus species) in the 0-10 µm diameter range mixed with other pesticides as dry powder via a blower from a truck on a road. Seventy two hours after release, the surrogate was not detected at the release site, but was detected by air samplers outdoors about 2 miles away, which authors attribute to reaerosolization, which is particle detachment from a surface following settling. Surface samples at this remote location indicate a surface loading of $10^2$/m² CFU/wipe, while surface samples collected at the release site were negative. Air samplers also detected the surrogate in underground subway stations near the release site and several miles away, although the transport mechanism was not reported (Garza, Van Cuyk et al. 2014).

2.2 Urban Particulate Transport Mechanisms

Particle transport by any mechanism is primarily a function of particle diameter (Hinds 1999), but many other variables can impact the fate and transport of particles in an urban environment. Potentially confounding our understanding of particle transport in an urban environment are some of the unique environmental features including buildings, numerous impervious surfaces, thermal convection from paved surfaces, the presence of street canyons (advection from canyons), and microenvironments, to name a few.

Following a release and initial deposition, other factors potentially impacting transport and confounding consequence management include surface water runoff to drainage areas/ponds; the formation of concentrated spore pools; weathering of spore (or other) materials; resuspension (causing redistribution) from road and foot traffic, wind, and sampling (Van Cuyk, Veal et al. 2011), causing a potentially ever-changing contamination zone. Spores may also be transported on clothing during sampling. In a large scale study tracking the movement of Btk following spraying of a slurry in an urban area, the authors
found contamination in sampler personnel vehicles and hotel rooms, and evidence of reaerosolization in the field throughout the study (Van Cuyk, Veal et al. 2011).

Because the many transport pathways create a broad area of study, this report narrowly focuses on the potential for spore transport from above ground urban surfaces (Figure 1) during and following precipitation events. This transport mechanism is likely to be a major factor in spore relocation in an urban environment, and covering all potential mechanisms would not be possible in the time frame of this study. Other transport mechanisms not addressed in this report will be covered in future studies.

2.3 Urban Storm Water Runoff

Urban areas are characterized by a high degree of impervious surfaces that decrease water infiltration, increase peak flows, and the total volume of water runoff. These impervious areas increase runoff velocities and decrease travel time, transporting runoff more rapidly (USDA 1986). Some factors impacting runoff travel time include surface roughness, channel and slope flow, and flow over plane surfaces (USDA 1986). One study showed that runoff volumes could be about 14.5% greater from urban areas as compared to forested areas (Corbett, Wahl et al. 1997). The highest sediment loads in storm water are typically seen when discontinuous impervious areas comprise about 35% of the watershed (Corbett, Wahl et al. 1997). The impact of rain intensity on runoff is important for slow moving storms, however sensitivity of runoff to storm patterns decrease at high storm speeds (De Lima and Singh 2002).

It is possible that many deposited spores will be removed from urban surfaces with other pollutants during the early phase of a precipitation event when high pollutant concentrations are seen in runoff waters, a phenomenon known as “first flush” (Sansalone and Buchberger 1997; Zoppou 2001). First flush is related to both the buildup and wash off of pollutants (Zoppou 2001) and applies to both overland and sewer processes (Zoppou 2001). First flush has been attributed to increased rain intensity at the start of a storm (Zoppou 2001) and correlated to overall storm intensity (Sansalone, Koran et al. 1998). During high intensity precipitation events, short residence times are seen between rainfall runoff and “dry deposited” particulate matter (Sansalone and Buchberger 1997; Sansalone, Koran et al. 1998), resulting in particle instability and a lack of equilibrium between particle aggregation and disaggregation processes (Blazier 2003).

First flush has been defined as the normalized cumulative mass load $m(t)$ divided by the normalized cumulative runoff volume $v(t)$ over a given time interval:

$$\frac{\int_0^t c(t)q(t)dt}{M} > \frac{\int_0^t q(t)dt}{V}$$

where $c(t)$ is the incremental (i.e. at a specific time point) concentration and $q(t)$ is the incremental flow rate, $M$ is the constituent mass and $V$ is the total runoff volume. (Sansalone, Hird et al. 2005).

Some research has found that the first flush is likely limited to small quantities of fine particles (Svensson 1987). Other research has shown that about 40 percent of particles were discharged in the first 20 percent of runoff volume (Li, Lau et al. 2005) with increased concentrations of settleable particles and microbes at the beginning of a storm (Krometis, Characklis et al. 2007). In one study, high
intensity precipitation events associated with high runoff volumes were found to exhibit sustained flushes discharging about 80 percent of the particle number density (PND) in the first 60 percent of the runoff volume, while low intensity events resulted in a somewhat restricted flush, where 80 percent of the PND was not flushed until the storm had nearly concluded (Cristina and Sansalone 2003). This is consistent with previous work demonstrating weak first flush behavior for low flow precipitation events, although particles between 2-8 µm rapidly washed off during each rain event studied (Sansalone, Koran et al. 1998).

Highway construction has been shown to result in up to a six-fold increase in total suspended solids during first flush in storm water from sites during construction, increasing the percent particles in the clay fraction (<75 µm) from 76% to 96% in the drainage ditch adjacent to the site (Cleveland and Fashokun 2006). Other variables impacting particle transport across roadways include both the runoff rate and duration, and the traffic intensity, and that particles in the 2-8 µm range can be quickly washed off of pavement during the intense rain events (Sansalone, Koran et al. 1998). Another study found that only events with an average volumetric flow rate of about 1 Lm⁻¹ of drainage width exhibited a rapid removal of all particulate matter (Cristina and Sansalone 2003). Another found that while first flush may occur on sloped, small watersheds, site-specific data should be obtained before it is assumed that the “entire runoff volume” does not require treatment (Sansalone and Cristina 2004).

The results of these studies imply that many deposited spores may be removed from urban surfaces with other pollutants during the early phase of a precipitation event. However the magnitude of the removal may be dependent upon precipitation event intensity and duration. Detailed information about storm events, including volume and intensity, in the days and weeks following an urban release may aid sampling and mitigation planning and response activities. Additionally, laboratory studies should be conducted to identify the key parameters needed to predict the extent of spore wash off from urban surfaces during precipitation events.

### 2.4 Urban Storm Water Modeling

Urban storm water management is generally focused on water flow management to protect public health, property, and to decrease pollution loads in rivers and streams. The hydrological regime of a catchment area drives water quality (Merritt, Letcher et al. 2003), and, as a result, storm water models tend to focus on water volumes and the spatial/temporal distribution of precipitation (Zoppou 2001). Storm water quality models generally focus on sediment load and concentration (Obropta and Kardos 2007), however these models contain a lot of uncertainty in parameter values and model outcomes stemming from a lack of understanding about the interactions between contaminants and sediments (Merritt, Letcher et al. 2003). Buildup and wash off models are also frequently used to simulate overland runoff quality. However, more research is needed to decrease model uncertainties (Obropta and Kardos 2007) by enhancing our understanding of the physicochemical processes governing how specific pollutant particles adsorb to sediment particles (Zoppou 2001). Current storm water models tend to predict water quantity better than quality (Obropta and Kardos 2007; Merritt, Letcher et al. 2003), and uncertainty in water quality predictions is greater than for water quantity predictions (Merritt, Letcher et al. 2003). Since water quantity models are better understood, they may be better predictors of spore relocation than current water quality models.
2.4.1 Models

While there are numerous urban storm water models available in the literature, as outlined by Zoppou et al (2001), most are deterministic-distributed models that focus on water volume and the spatial-temporal distribution of precipitation (Zoppou 2001). For these models, precipitation volumes are considered to be uniformly distributed (Zoppou 2001). The two fundamental elements of urban storm water modeling are rainfall/runoff modeling, including the not well understood buildup and wash off of pollutants from impervious surfaces (Zoppou 2001; Chen et al, 2006), and transport modeling of flows and pollutants through sewer systems (Zoppou 2001).

The buildup equation presented by Zoppou et al (2001) is:

\[
\frac{dP_B}{dt} = I - k_B P_B(t)
\]

where \(P_B(t)\) is the mass of surface pollutant buildup at time \(t\), \(I\) is mass of pollutant accumulation between storms and \(k_B\) is the pollutant buildup coefficient (Zoppou 2001).

The wash off equation presented by Zoppou et al (2001) assumes the wash off rate is proportional to the surface concentration of pollutant and is:

\[
\frac{dP_W}{dt} = -k_W r P_W(t)
\]

where \(P_W(t)\) is the pollutant mass at time \(t\), \(k_W\) is the coefficient of pollutant removal, and \(r\) is the runoff flow rate (Zoppou 2001). For both equations, the coefficients are empirically derived (Zoppou 2001).

Both equations are similar to those presented by Chen et al, 2006, and are representative of those typically found in the literature (Zoppou 2001; Chen and Adams 2006). The models could be evaluated experimentally in laboratory tests using spores, the results of which could help inform sampling and other consequence management decisions.

Current models that rely on storm water quantity are not as good at predicting storm water quality (Obropta and Kardos 2007). Due to the complexity of the many required input parameters needed to accurately model storm water quality, most models do not produce reliable predictions (Obropta and Kardos 2007), although the magnitude of the uncertainty has not been quantified. One significant parameter not typically included in storm water quality models is the particle size distribution (PSD) (Obropta and Kardos 2007).

2.4.2 Particle Size Distribution (PSD)

Sediment particle size is important because smaller sediment particles adsorb a higher percent of pollutants per unit mass (Obropta and Kardos 2007). Several studies have shown that urban runoff contains a large fraction of fine particles having a high surface to volume ratio, increasing adsorption of pollutants into particle pores and on surfaces (Characklis and Wiesner 1997; Sansalone, Koran et al. 1998; Roger, Montrejaud-Vignoles et al. 1998). Given the size (~1 µm) and density (~1 g/cm³) of a single \(Ba\) spore, it seems likely they will behave similarly to other pollutants by adhering to clay or silt and being transported along with these particles in runoff.
Knowledge of PSD in runoff is key to understanding the fate and transport of particulate contaminants and how those contaminants partition in the different size fractions (Kim and Sansalone 2008). Wide particle size ranges are seen in runoff during different precipitation events depending upon the event intensity and duration as well as spatial variations during the same event (Kim and Sansalone 2008). One study showed greater than 90 percent of the sediment collected from highway runoff to be less than 10 µm (Li, Lau et al. 2006), the mass of which made up less than 10% of total mass collected (Li, Lau et al. 2005). A one year study of runoff from an urban French highway with about 30,000 vehicles per day showed that ninety percent of the solid matter by weight was less than 100 µm, and of those less than 50 µm, fifty six percent were clays (Roger, Montrejaud-Vignoles et al. 1998). Another study found about fifty v/v percent particles with diameter less than 15.2 µm (Andral, Roger et al. 1999) in highway runoff. A fourth study reported 25-80% on mass basis of fine particulate matter in urban runoff was less than 75µm (suspended settleable fraction) (Kim and Sansalone 2008). Collectively, it has been found that much of the road particulate matter can be attributed to tire abrasion of the road surface (Sansalone and Tribouillard 1999). Those abraded road particles which are less than 10 µm can remain entrained in storm water and thus are very difficult to remove (Sansalone and Tribouillard 1999).

Given that PSD varies both spatially and with event characteristics (Kim and Sansalone 2008), having an understanding of PSD for a particular event and location may prove difficult. Additionally, there is currently no available research in the literature that identifies a correlation between pathogenic organisms and PSD (DeGroot and Weiss 2008). This gap, however, may be due to sampling issues, since it is difficult to accurately sample particles and associated contamination in storm water because they are stratified in the water (buoyant, suspended, settled or settling) (DeGroot and Weiss 2008). Another study reported particle aggregation in samples occurring in less than six hours, with the fine particle concentration decreasing and an increase of larger particles in stored samples (Li, Lau et al. 2005).

Based upon the literature, most runoff sediment is in the fine particle size range, increasing the likelihood of spore-sediment interaction. This suggests contaminants entrained in storm water, including spores, will likely migrate where fine grain sediments go. For this reason, these areas may be ideal locations for sampling and mitigation activities following a release. This hypothesis should be tested in laboratory studies for confirmation.

### 2.5 Runoff and Sediment Transport

Surface runoff, or overland flow, occurs when precipitation volumes surpass infiltration and depression storage capacities (Merritt, Letcher et al. 2003). During precipitation events, sediments are suspended by two types of processes: rainfall splash detachment, and entrainment via overland flow shear stress (Svensson 1987; Merritt, Letcher et al. 2003). When shear stress is greater than the cohesive strength, sediment detachment occurs (Merritt, Letcher et al. 2003). (Figure 2 provides an overview of the forces related to sediment detachment.) The sediment transport rate is governed by flow conditions and particle properties, with smaller particles “always” transported, and the water transport capacity dependent upon the particle size distribution (Svensson 1987).
Figure 2. Forces involved with sediment detachment, adapted from Southard, 2006 (Southard 2006). In Figure 2, $F_F$ is the fluid force, $F_C$ is the contact force, $F_L$ is the lift force, $F_D$ is the drag force, $W$ is the weight of the particle, $\tau$ is the viscous sheer stress, and $+$ and $-$ are the high and low pressures, respectively.

In surface runoff, contaminants and sediments are dislodged and transported in four phases: suspended solids or sediment; dissolved or fine particulate; near-bed layer; and bed-load layer (Obropta and Kardos 2007). Sediment transport generally occurs in the suspended load or bed load, depending on the properties of the sediment particle and the storm water. The suspended sediment load is primarily silt and sand moving through the water column, remaining suspended when the upwards velocity is approximately equal to the settling velocity (Hickin 2009). When the upwards velocity is less than the settling velocity, the particle will move as bed load by rolling, sliding, and saltating (briefly carried by the fluid before settling). If the upwards velocity is greater than the settling velocity, the particle will be transported near the surface in the wash load (Hickin 2009).

*Bacillus anthracis* spores have a diameter of approximately 1 µm (Carrera, Zandomeni et al. 2007), clay particles are < 2 µm; silt ranges from 2 – 50 µm; and sand is in the range of 50 – 2000 µm (USDA 2015). Figure 3 provides a visual comparison of these particle sizes for perspective. On a mass basis, the surface area of clay particles is typically several orders of magnitude higher than silt particles, and almost six orders of magnitude more than coarse sand particles (USDA 2015). Sediments with high surface to mass ratios adsorb and transport particulate and other substances in water systems (Lick 2008). Conservative substances can be adsorbed to sediments and transported with these solids by advection, the dominant pollutant transport mechanism in runoff (Zoppou 2001). Fine silt is very easily suspended, which results in more evenly distributed silt in the water column than coarser materials that tend to settle out faster (Hickin 2009). The wash load is the part of suspended sediment in the clay range (i.e. < 2 µm), which is finer than silt and remains suspended in the water without the force of turbulence; it is kept uniformly distributed in suspension via Brownian motion (Hickin 2009). It is possible that individual *Ba* spores may adsorb to silt and clay particles forming aggregates, as suggested by some literature discussed below, but this is not known.
2.6 Overland Pollutant Transport

Runoff is available for overland flow, depression storage (low points that store precipitation), and infiltration in to the soil (Zoppou 2001). Because most urban surfaces are impervious, most models do not account for subsurface water in the unsaturated zone (Zoppou 2001). Conservative pollutants, like spores, are inert and not chemically changed during transport (Zoppou 2001). The main process affecting conservative pollutant transport is adsorption/adhesion onto a solid (typically a sediment) (Zoppou 2001). These aggregates are then transported by advection (diffusion not believed to be significant) with the sediment particles in the water (Zoppou 2001). For optimum model accuracy, both adsorption and advection should be considered, however most models lack these parameters (Zoppou 2001). Adding these parameters to existing storm water models would allow the prediction of spore transport in a city of interest. The magnitude of pollutant adsorption is a function of pollution concentration, surface area of the sediment (Sansalone, Koran et al. 1998), and temperature (Zoppou 2001). It is possible that microorganisms attached to high surface area sediments may be protected from disinfectants, making them less vulnerable to decontamination (Zoppou 2001).

2.6.1 Storm Water Aggregates

Much of the literature on storm water aggregates pertains to holding ponds and tanks and are not specific to overland flow, however it is not unreasonable to relate these data to overland flow. Although the residence time of the particles in storm water moving across impervious surfaces is much different than that of pooling water, many of the same mechanisms apply.

Storm water aggregates are usually comprised of inorganics, microbes and biota (plant matter). As the particle aggregates increase in size, their porosity also increases, causing the aggregate density to decrease to near the density of water (Droppo 2001). Additionally, the organic content of particles plays a significant role for the aggregate, contributing to particle density, settling velocity and pollutant binding tendencies (DeGroot and Weiss 2008). A range of street sediment particle densities reported in

Figure 3. Comparison of sediment particle sizes and a Bacillus spore.
the literature are from 2.2 – 2.8 g/m³ (Zanders 2005). In storm water, microbes may remain as singlets or become aggregates of microbes and particles, potentially becoming more transportable (Characklis, Dilts et al. 2005).

Three transport processes that foster particle contact and increase the likelihood of aggregation in storm water are Brownian motion, fluid shear, and differential settling (Lick 2008). Brownian motion, or thermal diffusion, is applicable to particles less than 0.1 µm (Lick 2008) and in the context of this report, is significant in terms of collisions between very fine clay particles with single B.a. spores (about 1 µm). Increased collisions could increase the probability of agglomeration with other particles. Fluid shear is a collision mechanism applicable to particles between 0.1 and 50 µm (Lick 2008), and would apply to collisions between a 1 µm spore and clay and silt particles. Differential settling, which is due to gravity, is applicable to particles greater than 50 µm (Lick 2008), like sand.

2.6.2 Particle-Bound Metals

While particulate metals are likely not a good surrogate for spores, this review would be incomplete without at least considering what is widely known in the literature about the fate of these particles in an urban setting. Metals have shown to be the most prevalent sediment-bound contaminant in roadway runoff (Sansalone, Buchberger et al. 1996) and the particle-bound metals may be an indicator of the fate of spores in urban runoff. However due to density and surface chemistry differences, correlations between metal particulate and spore transport should be viewed critically.

Particle-bound metals from roadways appear to persist in the environment for some time. One study demonstrated that lead from vehicle exhaust remains in the top 15 cm of soil, bound to organic matter until it is mechanically redistributed or becomes incorporated in surface runoff (Turer, Maynard et al. 2001). The authors estimate that 40% of deposited lead from leaded fuel exhaust still remains in the soil, bound to organic matter (Turer, Maynard et al. 2001). Other studies have found that surface runoff of particle bound metals is a function of rainfall intensity (Sansalone, Buchberger et al. 1996; Sansalone and Buchberger 1997; De Lima and Singh 2002). Additionally, snow wash-off contained more particle-bound metals and solid particulate than the rainfall runoff, which was attributed to longer pollutant accumulation times and the high surface area of the snow banks (De Lima and Singh 2002). Further studies showed that some metals, Zn and Cu, sorbed to rainfall solids more than in snow, but lead had a higher sorption rate onto solids for first snow (Sansalone and Buchberger 1997).

Particle-bound metals are mostly found in the less than 125 µm range, and are not easily caught by roadside vegetation buffers (Zanders 2005). Vegetation buffers are not good at retaining particles less than 60 µm and poor for 6-30 µm particles (Zanders 2005). And, although their weight was only about ten percent of the total suspended solids load, another study found particles less than 100 µm accounted for greater than fifty percent of lead, zinc, and copper pollutants (Furumai, Balmer et al. 2002). For zinc and copper, the amount of metals sorbed to solids was higher in runoff samples as compared to road dust, but was about equal for lead (Furumai, Balmer et al. 2002).

While density and surface chemistry differences between spores and particulate metals preclude any direct comparisons, some of the results outlined above may be useful in identifying laboratory studies on spore fate in urban environments. For example, studies on the impact of snow on spore to solids sorption rates, accumulation and wash off would be useful to inform response efforts in a winter release.
Additionally, studies determining if spores are retained by vegetation buffers would enhance our understanding of spore transport from road surfaces.

### 2.6.3 Microbial Aggregation and Transport

When microbes are part of an aggregate, they attach to dense inorganic particles, and are likely to settle more quickly and survive longer than single microbes, while most planktonic organisms and those attached to organic particles will remain suspended and transportable (Characklis, Dilts et al. 2005). A study investigating the persistence of *B. sphaericus* spores in a pond following mosquito treatment saw rapid spore settling into the mud, with larger clumps settling within two hours of treatment while fine particles remained suspended; the spores remained detectable in the mud layer for the duration of the 21 day study (Davidson, Urbina et al. 1984). In another study, the fate of *Bacillus thuringiensis israelensis* (*Bti*) in deionized water naturally seeded with soil dust comprised of 21 percent clay, 59 percent sand and 20 percent silt and less than 1% organic matter was examined (Ohana, Margalit et al. 1987). The spores quickly adsorbed to soil particles, with 99.8 percent settling into the mud fraction within 45 minutes (Ohana, Margalit et al. 1987).

The results of these studies suggest that single spore contaminants may quickly become part of aggregates in urban settings, although research needs to be conducted to determine the aggregation rate of spores and sediments. Some studies have shown that high surface area suspended sediments with attached/adsorbed microorganisms may be more difficult to disinfect as there may be areas where the microbes do not come into contact with the disinfectant (Berman, Rice et al. 1988; Zoppou 2001). This could be an important consideration for mitigation strategies following a release. There is a research need to determine if organic and inorganic aggregates containing virulent spores are more difficult to decontaminate, and if so, what steps can be taken to mitigate this issue following a large-scale urban release.

Not much is known about microbes and particle association in storm water transport (Krometis, Dillaha et al. 2009), although a few studies have looked at *Clostridium perfringens* (*Cp*) spores in urban storm water. They have shown positive partitioning behavior-affinity to associate with settleable particles (clay, silicates) in urban storm water at rate of greater than 50 percent (Krometis, Characklis et al. 2007; Characklis, Dilts et al. 2005; Cizek, Characklis et al. 2008) and can persist in sediments for years (Mueller-Spitz, Stewart et al. 2010). Similar physically to *Ba*, *Cp* spores are in the size range of 0.8 – 1µm (Novak, Juneja et al. 2003), have an exosporium (Hoeniger, Stuart et al. 1968; Henriques and Moran 2007), and a density of about 1.2-1.3 g/cm³ (Characklis, Dilts et al. 2005).

While the number of settleable particles in storm water increases after a storm water event (Characklis, Dilts et al. 2005), for one study, the highest concentration of both particle bound and free *Cp* spores were seen during the early storm stages (Cizek, Characklis et al. 2008), and for another study partitioning behavior did not change over the course of the storm (Krometis, Characklis et al. 2007), although the average settleable fraction of *Cp* and total suspended solids decreased as the storm continued (Krometis, Characklis et al. 2007). No significant relationship was seen between microbial associations with settling particles and particle concentration, total organic carbon (TOC) or temperature (Cizek, Characklis et al. 2008), or PSD, total suspended solids, and TOC (Characklis, Dilts et al. 2005).

In another study, the authors looked at the long-term persistence of *Bti* and *Lysinibacillus sphaericus* (*Ls*) after direct application to urban catchment basins (for mosquito control). Two days post treatment,
most of the applied spores were found in the bottom sludge where they remained at consistent high concentrations for the 275 day study (Guidi, Lehner et al. 2013). This is consistent with other studies that show high removal by sedimentation for \( Cp \) in suburban detention ponds (Krometis, Characklis et al. 2007) but does raise questions about spore persistence in sludge and how partitioning may effect fate and transport and the duration a microbe remains a public health threat (Characklis, Dilts et al. 2005).

While these studies suggest spores will be removed from storm water via their association with fine particles, research needs to be performed to determine if this holds on urban surfaces during overland transport. Given the potential short residence times between spores and storm water sediments as they travel rapidly across surfaces, different partitioning behavior may result.
3.0 Conclusions and Research Needs

The fate of spores following a large-scale urban release is unknown. It is possible that the highest risk to public health is the primary aerosol during initial release (CDC 2013). While the literature contains some information about the fate of other contaminants, including clay, silts, microbes, and particle-bound metals, many questions remain unanswered that need to be addressed in order to inform site characterization and sampling strategies following such a release. For longer term mitigation strategies, further research is needed to gain a better understanding of the consequences of spores released in the urban environment. These consequences include spore transport in air and water over time and the fate of spores following the application of decontaminants (NSTC 2013).

Based upon the available literature reviewed for this report, there are many research questions to be addressed regarding the fate and transport of spores in urban runoff following a large scale urban release. Research suggests most runoff sediment is in the fine particle size range, increasing the likelihood of spore-sediment interaction. This suggests the potential for spore entrainment in storm water, where they will likely migrate with fine grain sediments. These areas may be unique in each urban environment, based upon the catchment features, however these areas may be ideal locations for sampling and mitigation activities following a release. This hypothesis should be tested in laboratory studies for confirmation.

The literature also suggests that single spores entrained in storm water may quickly become part of aggregates, changing their susceptibility to decontaminants. The aggregation rate of spores with sediments and the resulting impacts on sampling, detection, and decontamination efforts, all of which are important considerations for mitigation strategies following a release, need to be studied. Data are needed to determine if organic and inorganic aggregates containing spores are more difficult to decontaminate, and whether steps can be taken to mitigate this issue following a large-scale urban release. Sampling studies should also be performed to determine the most appropriate and effective methods to collect particle-bound contaminants in storm water samples as there are currently no standardized or approved methods for the collection of these samples (Grant, Rekhi et al. 2003). Developing methods to collect samples directly from overland flows may better inform wash off model development.

Given that the magnitude of initial spore removal from urban surfaces may be dependent upon precipitation event intensity, volume and duration, research identifying any correlation between these parameters and the strength of the initial wash off are needed. Studies of the dominant parameters in first flush phenomena will help inform a response to prepare effective mitigation approaches following the first rain event after wide area release. In addition, parameterized studies will help predict the extent of spore accumulation and wash off from snow and the potential for spore retention in vegetation buffers.

Finally, given the likely short residence times between spores and storm water sediments in overland flow during a precipitation event, partitioning behavior needs to be studied to confirm that particle association behavior seen in runoff holds true for overland flow. Since many current studies focus on runoff captured in holding ponds, it is important to determine the aggregation behavior of spores only briefly exposed to sediments that are then left behind on surfaces following a precipitation event. While many studies suggest that spores and other contaminants are ultimately removed from storm water
through their association with fine particles, research needs to be performed to determine if this holds true on urban surfaces during overland transport and what happens to the spores left behind on surfaces following a precipitation event in order to inform sampling and mitigation planning following such a release. For example, having an understanding of whether the spores have attached to other sediments and are no longer singlets that could potentially be inhaled would be important to know for response planning.

3.1 Research Questions

As a result of this literature review, the following research questions emerge that are relevant to site characterization, decontamination, and sampling strategies following an urban release of *Ba* spores:

- What is the potential for spore entrainment in storm water?
- What is the potential for spores to migrate with fine grain sediments in storm water?
- What is the aggregation rate of spores with fine sediments?
- What is the aggregation rate of spores with fine sediments in rapidly moving water (i.e. with shorter residence times)?
- Does the spore-particle the association behavior seen in runoff hold true for overland flow?
- What are the most effective methods to collect particle-bound spores in storm water samples?
- What is the most effective method to collect particle-bound spores directly from active overland flows?
- Do organic and inorganic aggregates containing spores require a different decontamination approaches?
- Can precipitation event intensity, volume, and duration, be parameterized to model the magnitude of the initial wash off (First Flush) of spores from urban surfaces?
- What are the key parameters needed to predict the extent of spore accumulation and wash off from snow? Are these parameters different for precipitation?
- What is the potential for spore retention in vegetation buffers?
- What mitigation measures can be taken in the early stages of an incident (before it rains) that would enhance the containment of spores from a wide area release once it does rain?
4.0 References


5.0 Search Terms

allintitle: street dust runoff OR flushing OR migration OR transport OR urban spread OR migration OR containment "bacillus" "outdoor"

Migration transformation metals street dusts urban runoff

allintitle: street runoff OR flushing OR migration OR transport

allintitle: street dust resuspension

allintitle: road resuspension

allintitle: fate and transport particle

allintitle: fate and transport urban

allintitle: fate and transport particulate

allintitle: urban bacillus

allintitle: urban release

urban aerosol spore washout


allintitle: anthracis field OR transport OR resuspension OR fate OR spore OR properties OR water OR soil OR wind OR rain OR UV OR solar -genome -genetic -plasmid -virulence -vaccine -molecular -mice -lethal -pcr -macrophage -genomes -infection -toxin

allintitle: aerosol generation

allintitle: aerosol generation rain OR soil OR urban OR raindrop OR wind

Particle, metals, and water quality in runoff from large urban watershed

a non-equilibrium relationship between particle aggregation and disaggregation

A Study of The 2001 Anthrax Terror Attacks and the History of Biological Warfare

Aggregation rate of time-particle size resolved

Aggregation rate of time-particle size resolved runoff

Aggregation rate of time-particle size resolved tss

allintitle: bacillus fate OR transport

allintitle: bacillus metals

allintitle: cytology author:hoeniger
Assessing microbial pollution of rural surface waters: a review of current watershed scale modeling approaches

B. thuringiensis water
B. thuringiensis water fate
bacillus metals
bacillus sediment
bacillus sediment urban
bacillus sphaericus spore μm
bacillus spore surface charge
c perfringens spore μm
C. perfringens stormwater
C. perfringens urban stormwater
C. perfringens urban stormwater sediment
Clostridium perfringens adhesion
Clostridium perfringens bacillus spores
Clostridium perfringens spore adhesion
dlvo theory
dlvo theory bacillus
dlvo theory bacillus wastewater
first flush
first flush shear stress
floccular sediment transport
floccular sediment transport urban
genome sequence
hydrodynamic forces generated on a spherical sediment particle during entrainment
hydrodynamic forces sediment particle
hydrodynamic forces sediment particle sheet flow
hydrodynamic forces sediment particle urban
hydrograph
Initial adhesion of Bacillus subtilis on soil minerals as related to their surface properties
Langmuir adsorption model
metals gully pot
microbes Assessing microbial pollution of rural surface waters: a review of current watershed scale modeling approaches
microbial fate and transport
microbial fate and transport clostridium OR spores
microbial fate transport clostridium
microbial partitioning
Microbial partitioning stormwater
Microbial partitioning stormwater perfringens
Microbial partitioning to settleable particles in stormwater
motion of body in fluid
overland flow
particle size suspended author: slattery
perfringens "spore density" g/cm3
perfringens author: Yolton
perfringens spore density g/cm3
perfringens spore morphology
perfringens spore surface
perfringins author: Yolton
pollution follows stormwater
pollution follows water
rous number sediment
rous number sediment urban
sediment transport
Sediment transport mechanics
sediment transport urban
sediment transport urban runoff
shear stress "first flush"
shear stress "first flush" urban
shear stress "shields parameter" clay urban
shear stress shields parameter clay
shields parameter clay
STRUCTURE ASSEMBLY function spore surface layers
The Shields Diagram
The Shields Diagram urban
urban critical shear stress for cohesive sediment transport
urban critical shear stress sediment transport
urban critical shear stress sediment transport (road, pavement)
urban critical shear stress sediment transport road OR pavement
urban hydrograph
urban overland flow
urban particle fate transport
urban particle transport
urban runoff metals distribution
urban sediment transport
urban sediment transport forces
urban sediment transport forces impervious
wide area release
ZETA BACILLUS