Resuspension of Bacillus Spores from Operationally Relevant Surfaces

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Abstract

The potential for *Bacillus* spp. spores in outdoor environments to resuspend or reaerosolize following release of biological agent is of concern due to its relative stability and persistence. An improved understanding of reaerosolization from relevant outdoor surfaces is needed to better assess the associated public health risks to secondarily exposed individuals if an event were to occur. The purpose of this study was to assess the potential hazard associated with wind driven reaerosolization off of contaminated surfaces commonly found in urban and military environments, mimicking conditions found outdoors. The surfaces used in this study were vinyl floor tiles, brick, sod, concrete, gravel and chemical agent resistant coating (CARC). In our study, we seeded *Bacillus thuringiensis* var. *kurstaki* dry spores onto these six surfaces in an ambient breeze tunnel (ABT), and then reaerosolized the organisms using a bank of fans which created a wind speed of 3.8 m/s over the seeded surface. Reaerosolized spores were recovered from floor tiles placed every ten feet up to a total distance of 100 feet from the initial point of deposition and were analyzed by culturing. Analysis of variance shows that the settling deposition of reaerosolized Bacillus spores is dependent on the surface that the spores are reaerosolized off of, and the quantity of spores recovered from the ABT floor was inversely correlated with distance traveled from the seeded surface.

Background

Reaerosolization is the reintroduction of previously settled airborne particles back into the atmosphere. Microbial reaerosolization is poorly understood, and the reaerosolization of *Bacillus anthracis* spores in outdoor environments is of particular concern due to its stability and potential for use as a biological weapon. Research has already been conducted that has found that reaerosolization of anthrax spores can occur, although a more complete understanding of this phenomenon is needed in order to improve our ability to estimate exposures to responders and civilians under various scenarios and make accurate assessments of the associated public health risks.

Materials and Methods

Ambient Breeze Tunnel Setup
Testing took place inside of the Edgewood Chemical Biological Center (ECBC) Aerosol Sciences Branch's ABT. The ABT facility features a 196 foot test section, a 14' x 14' entrance, 0-6 mph adjustable wind velocity, and HEPA filtered exhaust.

Six test materials were used: brick, vinyl floor tile, gravel, concrete, sod, and CARC (Figure 1). Each test surface was 10' x 30". A bank of fans was located 5' behind the test surface for reaerosolization. Vinyl floor tiles were used for sample recovery and were placed in three rows every ten feet down the length of the tunnel. Figure 2 shows the layout of the ABT.

Figure 1: Test Surfaces

Materials and Methods (Cont'd)

Deposition Chamber
A deposition chamber was used to seed the test surfaces, shown in Figure 3. Two grams of dry BtK aerosol was generated with sonic nozzles and allowed to settle overnight before reaerosolization.

Figure 3: Deposition Chamber

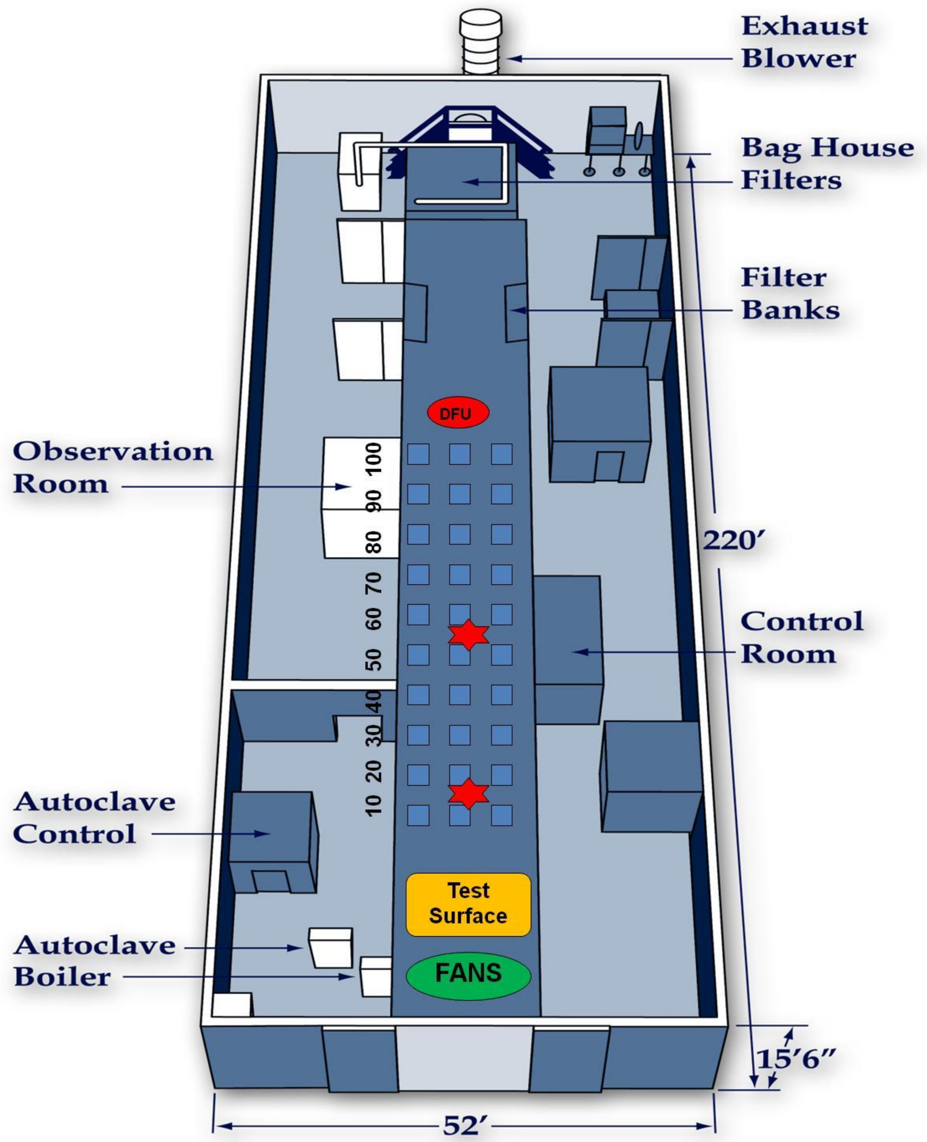

Figure 2: ABT Setup

Materials and Methods (Cont'd)

Reaerosolization
Fans were used to reaerosolize material from the test surface. The fans produced a wind speed of 3.8 m/s measured using a hot wire anemometer just over the center line of the test surface. After reaerosolization, the ABT was closed and particles were allowed to settle overnight before sample collection took place.

Sample Collection
Vinyl floor tiles were sampled using sterile non-cotton swipes wetted in phosphate buffered saline solution with 0.1% triton X-100 (PBST). Swiping technique followed CDC surface sampling procedures for *Bacillus anthracis* spores from smooth, non-porous surfaces. During sampling the ABT fans were turned to the lowest speed and samples were taken starting from the 100 foot mark and working forward to minimize reaerosolization from walking through the ABT during sample collection.

Sample Analysis
Samples were extracted in 20 ml of 50% PBST 50% 2-propanol and plated onto TSA plates using an Advanced Instruments Autoplate® Spiral Plating System. Plates were incubated overnight at 30° C and counted using an Advanced Instruments Qcount® Colony Counter.

Data

From the colony counts we were able to determine the colony forming units (CFU)/tile. Analysis confirmed that BtK spores were relatively evenly distributed across the width of the ABT. We were able to use this information to approximate the total deposition onto the floor of the ABT in the sampling area which allowed us to perform a qualitative comparison of reaerosolization from each of the test materials. Figure 4 shows the total CFU of BtK deposited on the ABT floor.

A One-way ANOVA test of the CFU/sq.ft. versus test surface is shown below

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One-way ANOVA: logA versus Substrate

Source      DF      SS     MS     F      P
Substrate    5  17.032  3.406  7.63  0.000
Error      173  77.196  0.446
Total      178  94.228

S = 0.6680   R-Sq = 18.07%   R-Sq(adj) = 15.71%

                                Individual 95% CIs For Mean Based on
                                Pooled StDev
Level      N    Mean   StDev  --------+---------+---------+---------+-
Brick     30  5.2629  0.4721                        (-----*-----)
CARC      30  4.8018  0.9568              (-----*-----)
Concrete  29  4.8093  0.6564              (-----*-----)
Gravel    30  5.0703  0.4048                    (-----*-----)
Sod       30  4.3090  0.3584  (-----*-----)
Tile      30  5.1012  0.9029                     (-----*-----)
                              --------+---------+---------+---------+-
                                    4.40      4.80      5.20      5.60
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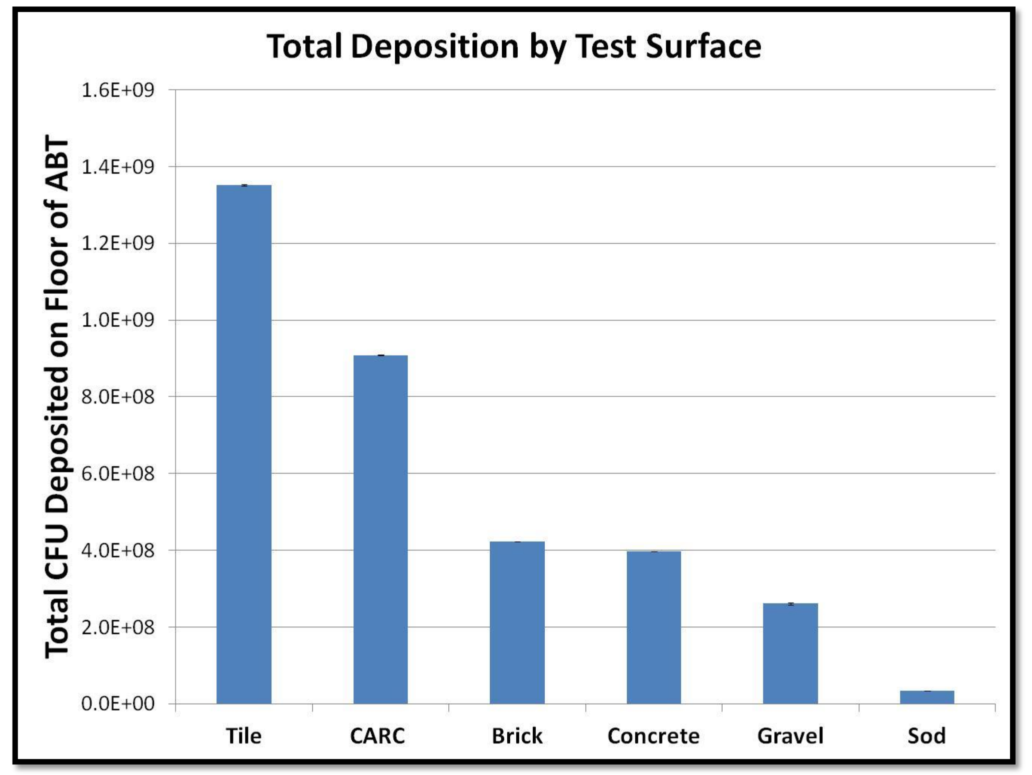

Figure 4: Total deposition of BtK onto the ABT floor by surface type

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Data (Cont'd)

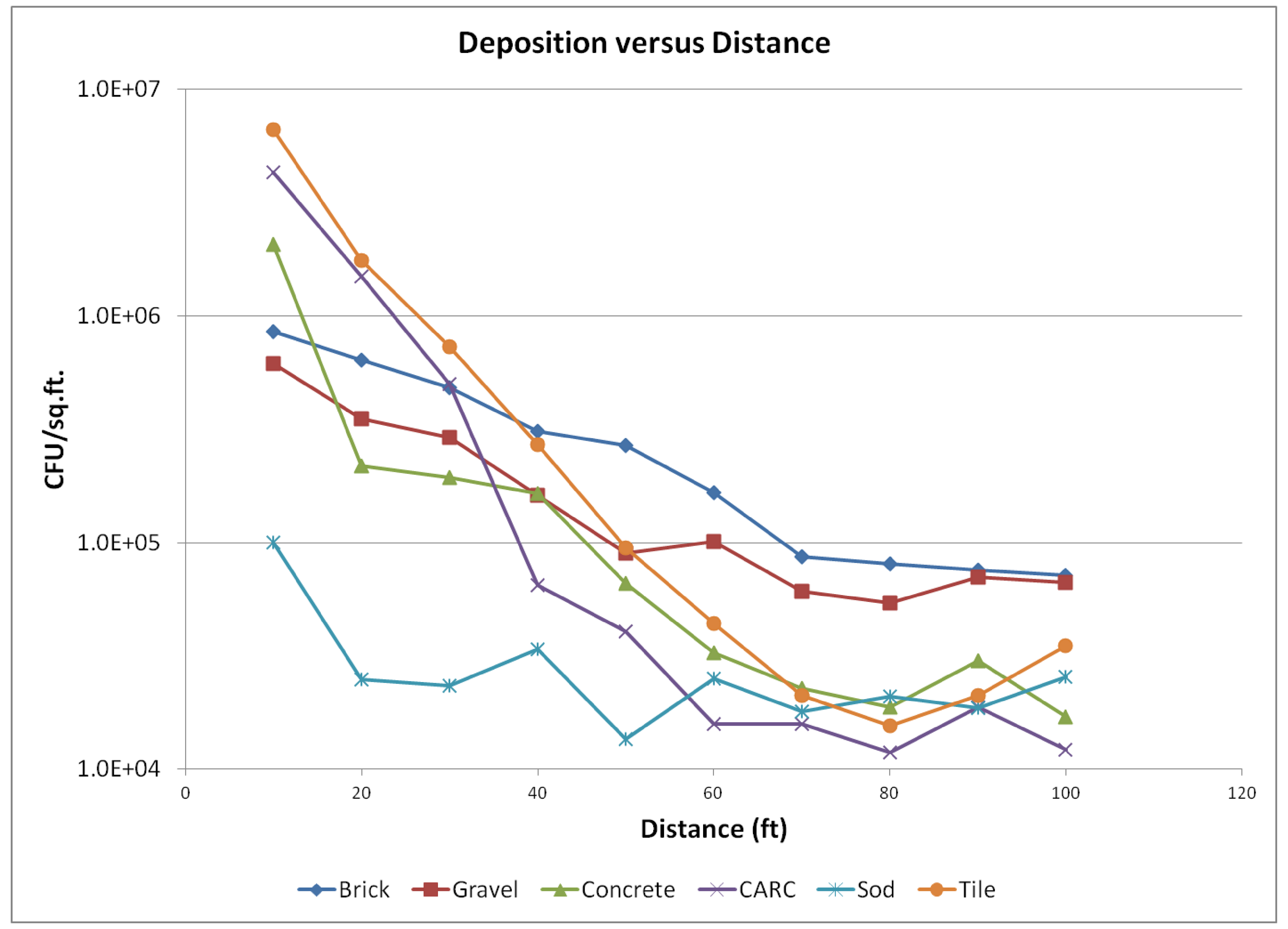

Figure 5: Deposition in CFU/sq. versus distance in feet.

Conclusions

This study unambiguously indicates that dry powder *Bacillus thuringiensis* Var. *kurstaki* spores seeded onto surfaces are reaerosolized by wind at a speed of 3.8 m/s under ambient conditions. Analysis of variance shows that both distance and test surface influence passive deposition of BtK spores onto tiles placed on the floor of the ABT, both having p-values of 0.00. Tests were conducted in the ABT from May to July under ambient uncontrolled conditions. It is understood that various environmental factors, such as humidity, temperature, and environmental particulates such as pollens and dusts, can affect reaerosolization and passive deposition. Temperature and humidity were recorded for these experiments for reference, but further experimentation is needed to quantitatively determine how environmental conditions affect the reaerosolization hazard. The general trend of the data shows that the deposition of spores on the ABT floor was inversely correlated with distance travelled from the seeded surfaces. It is hypothesized that the deposition values rise from approximately 80 to 100 feet because of turbulent air flow in the tunnel. During the tests the fans at the back of the ABT were not turned on, so as particles reached the back of the ABT they would be forced back forward.

This is preliminary qualitative work on the topic of outdoor reaerosolization. Uniform deposition and reaerosolization from multiple surfaces aids in a comparative analysis of associated hazards. Reaerosolization in an outdoor environment is a very complex event. Factors such as dissemination method, meteorological conditions, and the nature of the agent could affect the reaerosolization hazard. Additional work to obtain a more quantitative understanding of the reaerosolization off of surfaces is needed.