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Decontamination of Subway Infrastructure Materials Contaminated with Biological Spores Using Methyl Bromide

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U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

Disclaimer

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Executive Summary

The U.S. Environmental Protection Agency (EPA) is striving to protect human health and the environment from adverse impacts resulting from the intentional release of threat agents. This report provides the results of an assessment to determine the decontamination efficacy of methyl bromide (MB) fumigant in inactivating *Bacillus anthracis* (*B.a.*; causative agent for anthrax) spores on materials typically found in subway system infrastructure. To facilitate future decontaminations employing MB in a subway environment, this investigation focused on finding efficacious conditions when using MB at temperatures that may be encountered in an underground subway system (i.e., temperatures lower than used in previous studies).

This investigation focused on the decontamination of four types of common subway materials (with and without simulated subway grime application): ceramic tile, painted carbon steel, weathered concrete, and granite. Decontamination efficacy tests were conducted with spores of virulent *B.a.* Ames and avirulent *B.a.* Sterne. Decontamination efficacy was quantified in terms of log reduction (LR), based on the difference in the number of bacterial spores recovered from positive control coupons and test coupons. Ten tests were conducted at a target concentration of 212 milligrams per liter (mg/L) MB, target temperatures of 4.5 or 10 degrees Celsius (°C), target relative humidity (RH) of 50% or 75%, and contact times (CT) ranging from 2 to 9 days to assess the effect of these operational parameters on decontamination efficacy.

Summary of Results

As seen in other similar fumigant evaluations[1], the temperature, RH, and CT affect the efficacy of MB against *B.a.* Ames. Table E-1 shows the CT required to achieve ≥6 LR (a decontaminant that achieves an LR value ≥6 is considered effective)[2] on all materials tested for a given set of fumigation conditions (temperature and RH). For example, a CT of 4 days was required to achieve ≥6 LR of *B.a.* Ames on all materials when fumigating at 212 mg/L, 10 °C, and 75% RH.

This study corroborates the importance of RH when fumigating with MB. There were no tests in which ≥6 LR of *B.a.* Ames was achieved on all materials when fumigating at 50% RH. When fumigating at 50% RH, increasing the MB concentration, temperature, or CT generally did not improve decontamination efficacy. In contrast, when fumigating at 75% RH, increasing the temperature and CT improved efficacy. Application of grime to the test materials resulted in longer required CTs to achieve ≥6 LR. Efficacy of MB on *B.a.* Sterne was evaluated against *B.a.* Ames to assess the potential use of *B.a.* Sterne as a suitable surrogate for the virulent strain. Statistical analysis found no significant difference in efficacy for ceramic tile, weathered concrete, and an increased efficacy against *B.a.* Ames on painted carbon steel as compared to *B.a.* Sterne.

Table E-1. CT Required to Achieve ≥6 LR of *B.a.* Ames on all Materials*

Target MB Concentration (mg/L)	Grime Applied to Materials	Target Temperature (° C)	Target RH (%)	Time (days) Required to Achieve ≥6 LR on All Materials	Test Number Reference[a]
				B.a. Ames	
212	No	10	75	4	3
212	Yes	10	75	5	6
212	Yes	4.5	75	7	7

* Materials tested were ceramic tile, painted carbon steel, weathered concrete, and granite.
[a] Detailed data from each test number can be referenced in Table A-1 in Appendix A.

Impact of Study

This research provides information on the efficacy of MB fumigation to decontaminate subway relevant materials that have been contaminated with *B.a.* spores. Such results may be useful in the development of guidance to aid in deployment of MB fumigation after a release of *B.a.* spores within an underground transportation system. These results will provide decision makers with information for effective use of MB at temperatures lower than what has been previously tested, which will facilitate its use in subway systems as well as other applications at cold temperatures.

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Abbreviations/Acronyms

ANOVA	analysis of variance
B.a.	*Bacillus anthracis*
BBRC	Battelle Biomedical Research Center
BSC	biological safety cabinet
CBRN	Chemical, Biological, Radiological, and Nuclear
CMAD	Consequence Management Advisory Division
CFU	colony-forming unit(s)
CI	confidence interval
Cm	centimeter(s)
°C	degrees Celsius
CT	contact time
DNA	deoxyribonucleic acid
EPA	U.S. Environmental Protection Agency
ft^3	cubic feet
HCl	hydrochloric acid
HS	homeland security
HSRP	Homeland Security Research Program
L	liter(s)
LAL	Limulus Amebocyte Lysate (assay)
LED	light emitting diode
LR	log reduction
MB	methyl bromide
Min	minute(s)
Mg	milligram(s)
mL	milliliter(s)
µL	microliter(s)
NHSRC	National Homeland Security Research Center
OLEM	Office of Land and Emergency Management
Oz	ounce(s)
PBST	phosphate-buffered saline + 0.1% Triton X-100
PCR	polymerase chain reaction
ppm	part(s) per million
QA	quality assurance
QC	quality control
QMP	Quality Management Plan
RH	relative humidity
rpm	revolution(s) per minute
SD	standard deviation
SE	standard error
SFW	sterile filtered water
T&E II	Testing and Evaluation II Program
TSA	technical systems audit(s)

1.0 Introduction

The U.S. Environmental Protection Agency (EPA) is helping to protect human health and the environment from adverse impacts resulting from the release of chemical, biological, or radiological agents. With emphasis on decontamination and consequence management, water infrastructure protection, and threat and consequence assessment, the EPA is working to develop tools and information that: help detect the intentional introduction of chemical or biological contaminants into buildings or water systems; contain these contaminants; decontaminate buildings, outdoor environments, or water systems; and facilitate the disposal of material resulting from restoration activities.

In this investigation, the efficacy of methyl bromide (MB) against *Bacillus anthracis* (*B.a.*) Ames spores applied to subway materials (ceramic tile, painted carbon steel, weathered concrete, and granite) was tested. Simulated subway grime was applied to the surface of the materials during Tests 5 through 10 prior to testing to determine impact on the efficacy of MB. Decontamination efficacy was determined based on the log reduction (LR) in viable spores recovered from the inoculated samples (with and without exposure to MB). A decontaminant or fumigant technology is considered effective if a 6 LR or greater is achieved on the materials tested for a given set of fumigation conditions (sporicidal liquid volume, temperature, and relative humidity [RH]).[1] This study builds on previous laboratory research conducted by EPA to assess decontamination efficacy of MB for inactivating *B.a.* spores on various materials and adds data for low temperature as well as grimed material exposures.

Lastly, another objective of this work was to obtain efficacy data for *B.a.* Ames and *B.a.* Sterne, which could be used to assess its suitability as a potential surrogate for *B.a.* Ames when decontaminating with MB. Previous tests[2-4] with *B. atrophaeus* or *B. subtilis* have shown these species to be more resistant to MB compared with *B.a.* Ames. The Ames strain of *B.a.* was chosen for use as a standard since it was the strain identified in the Amerithrax incident in 2001[8].

2.0 Procedures

This section provides an overview of the procedures used for the evaluation of MB fumigant to inactivate *B.a.* Ames on up to four material types. Testing was performed in accordance with the EPA and Battelle Quality Assurance Programs.

2.1 Technology Description

MB (Chemtura, Philadelphia, PA) has been registered by the EPA for soil fumigation (injected into the soil before a crop is planted to effectively sterilize the soil), commodity treatment (used for post-harvest pest control), structural pest control (used to fumigate buildings for termites, and warehouses and food processing facilities for insects and rodents), and quarantine uses (used to treat imported commodities). Although MB has also been demonstrated to be an effective biocide against *B.a.* Ames on building materials and soil[(6)], the focus of this study was to determine effective conditions at lower temperatures, to generate evidence that MB fumigation for *B.a.* Ames may be implemented in the event of a potential release in a subway system. Furthermore, although MB use is being phased out under the Montreal Protocol, MB is still currently and widely used via critical use exemptions as a soil and commodity (quarantine) fumigant[(7)].

2.2 Test Matrix

The test matrix for the MB fumigation is shown in Table 2-1. Each test was performed using four material types inoculated with *B.a.* Ames and *B.a.* Sterne. A subset of tests was conducted using those same four materials, but with simulated subway grime added prior to inoculation of *B.a.* Ames (Tests 5-10). An adaptive management approach was used such that adjustments were made to the fumigation parameters (CT, organism, material grimed or clean, temperature, or relative humidity [RH]) to assess the impact of that parameter and to find efficacious conditions. Tests 1 through 8 and 10 were conducted with all four materials, using *B.a.* Ames. Test 9 was conducted with all four materials, using *B.a.* Sterne to preliminarily assess its use as a comparable surrogate for *B.a.* Ames for future testing.

2.3 Biological Agents

The virulent *B.a.* spores used for this testing were prepared from a qualified stock of the Ames strain at the Battelle Biomedical Research Center (BBRC, Lot B21, West Jefferson, OH). The spore lot was subjected to a stringent characterization and qualification process required by Battelle's standard operating procedure for spore production. Specifically, the spore lot was characterized prior to use by observation of colony morphology, direct microscopic observation of spore morphology, and size and determination of percent refractivity and percent encapsulation. In addition, the number of viable spores was determined by colony count and expressed as colony forming units per milliliter (CFU/mL). Theoretically, once plated onto bacterial growth media, each viable spore germinates and yields one CFU. Variations in the expected colony phenotypes were recorded. Endotoxin concentration of each spore preparation was determined by the Limulus Amebocyte Lysate (LAL) assay to assess whether contamination from gram-negative bacteria occurred during the propagation and purification process of the spores. Genomic deoxyribonucleic acid (DNA) was extracted from the spores and DNA fingerprinting by polymerase chain reaction (PCR) was done to confirm the genotype. This work was performed by Dr. Paul Keim at Northern Arizona University. The virulence of the spore lot was measured at Battelle by challenging guinea pigs intradermally with a dilution series of spore suspensions, and virulence was expressed as the intradermal median lethal dose. In addition, testing was conducted for robustness of the spores via hydrochloric acid (HCl) resistance.

Table 2-1. MB Test Matrix

Test Number	Materials	Organisms	Target Fumigation Parameters			Contact Time (days)
			MeBr Concentration (mg/L)	Temperature (°C)	RH (%)	
1	Ceramic Tile Painted Carbon Steel Weathered Concrete Granite	*Bacillus anthracis* Ames	212	10	75	7
2			212	10	75	2
3			212	10	75	4
4			212	10	75	3
5*			212	10	75	4
6*			212	10	75	5
7*			212	4.5	75	7
8*			212	10	50	7
9*		*Bacillus anthracis* Sterne	212	10	75	5
10*		*Bacillus anthracis* Ames	212	4.5	50	9

*Tests used grimed materials

2.4 Test Materials

Decontamination testing was conducted on ceramic tile, painted carbon steel, weathered concrete, and granite. Information on these materials is presented in Table 2-2, and a picture of each is presented in Figure 2-1. Material coupons were cut to uniform length and width from a larger piece of stock material. Materials were prepared for testing by sterilization via autoclaving at 121 °C for 15 minutes. Autoclaved coupons were sealed in sterilization pouches (Fisher, Pittsburgh, PA) to preserve sterility until the coupons were ready for use. Additionally, when required, simulated grime was prepared by combining 94% Arizona fine dust (Powder Technology Inc., PP2G4A2 find), 2.50% Carbon black (Powder Technology Inc, Raven 410), 0.25% Diesel particulate (NIST, SRM 1650b), 0.13% Motor oil, 0.13% alpha-Pinene 97% (Fisher Scientific, AC16436-0050), 1.00% Lycopodium (Fisher Scientific, S755301), 1.00% Ragweed pollen (Polysciences Inc., 7673), and 1.00% Paper Mulberry pollen (Polysciences Inc., 7670). Application of the prepared grime was achieved by combining 14.0 g of the sterile grime and 300 mL of 95% ethanol into a Binks SV100 spray can. The HPLV sprayer was operated over the test materials in a sweeping motion to achieve the targeted 0.02 g per test material (14.5 cm^2). The simulated grime was applied to ceramic tile, painted carbon steel, weathered concrete, and granite coupons (Figure 2-2).

Table 2-2. Test Materials

Material	Lot, Batch, or ASTM No., or Observation	Manufacturer/ Supplier Name	Approximate Coupon Size, width x length x thickness	Material Preparation
Ceramic Tile	Style Selections White Matte Ceramic Floor Tile Item #: 437485	Lowes Hilliard, OH	1.9 centimeter (cm) x 5.0 cm x 0.8 cm	Autoclave
Painted Carbon Steel	ASTM A1008 Grade CS, Type B Paint: Bond Plex Water based Acrylic	Adept Products, West Jefferson, OH Sherwin Williams, Columbus, OH	1.9 cm x 5.0 cm x 0.1 cm	Autoclave
Weathered Concrete	Military-grade runway concrete; aged 11 years	U.S. Government	1.9 cm x 5.0 cm x 1.0 cm	Autoclave
Granite	Color: Luna Pearl	Konkus Marble, Columbus, OH	1.9 cm x 5.0 cm x 1.0 cm	Autoclave

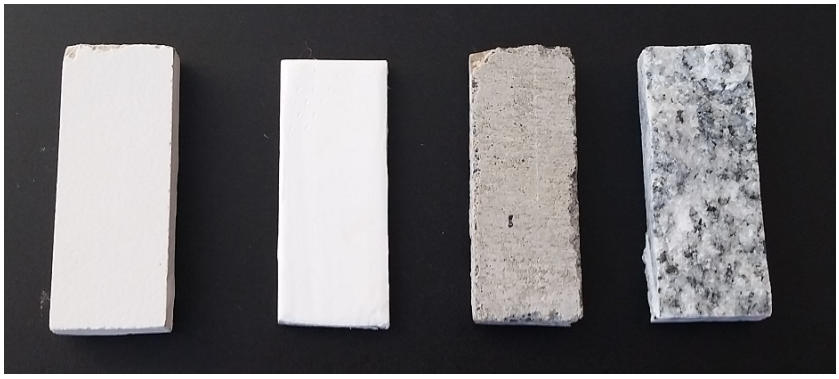

Figure 2-1. Coupons not covered with simulated subway grime. From left to right: ceramic tile, painted carbon steel, weathered concrete, and granite.

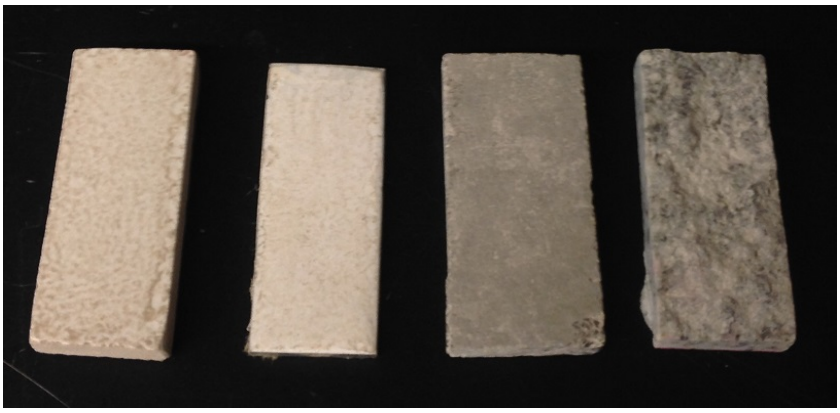

Figure 2-2. Coupons coated with simulated subway grime. From left to right: ceramic tile, painted carbon steel, weathered concrete, and granite.

2.5 Preparation of Coupons

Test and positive control coupons were placed on a flat surface within a Class II biological safety cabinet (BSC) and inoculated with approximately 1×10^8 CFU of viable *B.a.* Ames or *B.a.*

Sterne spores per coupon. A 100 microliter (µL) aliquot of a stock suspension of approximately 1×10^9 CFU/mL was dispensed using a micropipette applied as 10 µL droplets across the coupon surface (see Figure 2-3). This approach provided a more uniform distribution of spores across the coupon surface than would be obtained through a single drop of the suspension. After inoculation, the coupons were transferred to a Class III BSC and left undisturbed overnight to dry under ambient conditions, approximately 22°C and 40% RH.

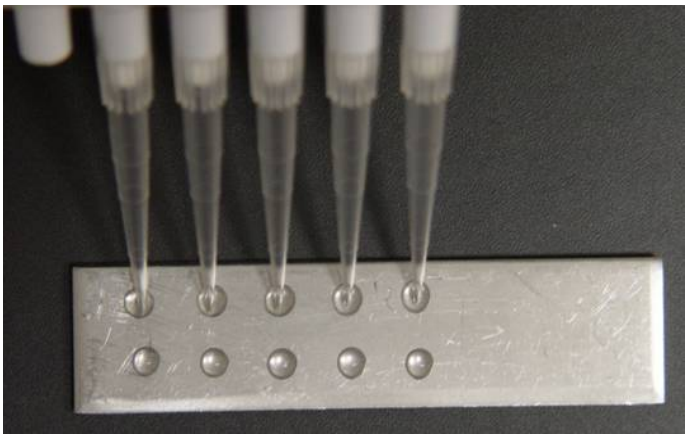

Figure 2-3. Liquid inoculation of coupon using a micropipette.

The number and type of replicate coupons used for each combination of material, decontaminant, concentration, and environmental conditions were:

- five test coupons (inoculated with *B.a.* and exposed to decontaminant)
- five positive controls (inoculated with *B.a.* but not exposed to decontaminant)
- one laboratory blank (not inoculated and not exposed to the decontaminant)
- one procedural blank (not inoculated and exposed to the decontaminant)

On the day following spore inoculation, coupons intended for decontamination (including blanks) were transferred into the test chamber and exposed to the MB fumigant using the apparatus and application conditions specified in Section 3.0 of this report. Control coupons were added to the control chamber as described in Section 3.0.

2.6 Coupon Extraction and Biological Agent Quantification

For sample extraction, test coupons, positive controls, and blanks were placed in 50 mL polypropylene conical tubes containing 10 mL of sterile phosphate buffered saline + 0.1% Triton X-100 (PBST). The vials were capped, placed on their sides and agitated on an orbital shaker for 15 minutes (min) at approximately 200 revolutions per minute (rpm) at room temperature.

Residual viable spores were determined using a dilution plating approach. Following extraction, the extract was removed and a series of 10-fold dilutions was prepared in sterile filtered water (SFW). An aliquot (0.1 mL) of either the undiluted extract and/or each serial dilution were plated onto tryptic soy agar in triplicate and were incubated for 18-24 hours at 37 ± 2 ºC. Colonies were counted manually and CFU/mL were determined by multiplying the average number of colonies per plate by the reciprocal of the dilution. Dilution data representing the greatest number of individually definable colonies were expressed as arithmetic mean ± standard deviation (SD) of the numbers of CFU observed. Laboratory blanks controlled for sterility, and procedural blanks

controlled for viable spores inadvertently introduced to test coupons. The target acceptance criterion was that extracts of laboratory or procedural blanks were to contain no CFU.

After each decontamination test, the BSC III and the MB test and control chambers were thoroughly cleaned (using separate steps involving bleach, ethanol, water, then drying).

2.7 Decontamination Efficacy

The mean percent spore recovery from each coupon was calculated using results from positive control coupons (inoculated, not decontaminated), by means of the following equation:

$$\text{Mean \% Recovery} = [\text{Mean } CFU_{pc}/CFU_{spike}] \times 100 \quad (1)$$

where Mean CFU_{pc} is the mean number of CFU recovered from five replicate positive control coupons of a single material, and CFU_{spike} is the number of CFU spiked onto each of those coupons. The value of CFU_{spike} is known from enumeration of the stock spore suspension. One aliquot of the stock suspension is plated and enumerated on each day of testing to confirm CFU_{spike} concentration. Spore recovery was calculated for *B.a.* Ames or Sterne on each coupon, and the results are included in Section 5 and Appendix A.

The efficacy of MB was assessed by determining the number of viable organisms remaining on each test coupon after decontamination. Those numbers were compared to the number of viable organisms extracted from the positive control coupons.

The number of viable spores of *B.a.* Ames or Sterne in extracts of test and positive control coupons was determined to calculate efficacy of the decontaminant. Efficacy is defined as the extent (as $\log_{10}$ reduction or LR) to which viable spores extracted from test coupons after decontamination were less numerous than the viable spores extracted from positive control coupons. The logarithm of the CFU abundance from each coupon extract was determined, and the mean of those logarithm values was then determined for each set of control and associated test coupons, respectively. Efficacy of a decontaminant for a test organism/test condition on the i^{th} coupon material was calculated as the difference between those mean log values, i.e.:

$$Efficacy\,(LR) = \overline{(\log_{10} CFUc_{ij})} - \overline{(\log_{10} CFUt_{ij})} \quad (2)$$

where $\log_{10} CFUc_{ij}$ refers to the j individual logarithm values obtained from the positive control coupons, $\log_{10} CFUt_{ij}$ refers to the j individual logarithm values obtained from the corresponding test coupons, and the overbar designates a mean value. In tests conducted under this plan, there were five positive controls and five corresponding test coupons (i.e., $j = 5$) for each test. A decontaminant that achieves a 6 LR or greater is considered effective.[2]

In the case where no viable spores were found in any of the five test coupon extracts after decontamination, a CFU abundance of 1 was assigned, resulting in a $\log_{10}$ CFU of zero for that material. This situation occurred when the decontaminant was highly effective, and no viable spores were found on the decontaminated test coupons. In such cases, the final efficacy on that material was reported as greater than or equal to (≥) the value calculated by Equation 2.

The variances (i.e., the square of the SD) of the $\log_{10} CFUc_{ij}$ and $\log_{10} CFUt_{ij}$ values were also calculated for both the control and test coupons (i.e., S^2c_{ij} and S^2t_{ij}), and were used to calculate the pooled standard error (SE) for the efficacy value calculated in Equation 2, as follows:

$$SE = \sqrt{\frac{S^2 c_{ij}}{5} + \frac{S^2 t_{ij}}{5}} \quad (3)$$

where the number 5 again represents the number *j* of coupons in both the control and test data sets. Each efficacy result is reported as a LR value with an associated 95% confidence interval (CI), calculated as follows:

$$95\%\ \text{CI} = \textit{Efficacy (LR)} \pm (1.96 \times \text{SE}) \quad (4)$$

The significance of differences in efficacy across different test conditions was assessed based on the 95% CI of each efficacy result.

2.8 Statistical Analysis

The mean and 95% CIs on the percent recovery for the control coupons were calculated by strain, material, and simulated subway grime coating. For *B.a.* Ames and each material, an analysis of variance (ANOVA) model with main effect for simulated subway grime was fit to percent recovery. An ANOVA model with main effect for material was fit to the percent recovery data for control coupons with and without the simulated subway grime for *B.a* Ames. Finally, an ANOVA model with main effects for *B.a.* strain and material and the two-factor interaction effect was fit to the percent recovery data, comparing Test 9 to the combined data from Tests 5, 6, 7, 8, and 10. For any effects of factors with more than two levels found to be statistically significant, Tukey comparisons were used to identify which levels of the effect are different; for effects of factors with two levels found to be statistically significant, least squares means were calculated.

The mean and 95% CIs on the reduction of the logarithm (base 10) *B.a* for the decontaminated coupons were calculated by strain, material, simulated subway grim coating, temperature, relative humidity and CT. For *B.a* Ames, an ANOVA model with main effects for material, simulated subway grime coating, temperature, RH, and CT was fit to the reduction of logarithm (base 10) spores. For any effects of factors with more than two levels found to be statistically significant, Tukey comparisons were used to identify which levels of the effect are different; for effects of factors with two levels found to be statistically significant, least squares means were calculated.

An ANOVA model with main effects for *B.a* strain and material and the two-factor interaction was fit to the reduction of logarithm (base 10) spores for Tests 6 and 9. For any effects of factors with more than two levels found to be statistically significant, Tukey comparisons were used to identify which levels of the effect are different; for effects of factors with two levels found to be statistically significant, least squares means were calculated.

All statistical analyses were performed using Statistical Analysis Software (SAS; version 9.4, Cary, NC). All results are reported at the 0.05 level of significance.

2.9 Surface Damage

The physical effect of MB on the materials was also qualitatively monitored during the evaluation. This approach provided a gross visual assessment of whether the decontaminants altered the appearance of the test materials. The procedural blank (coupon that is decontaminated, but has no spores applied) was visually compared to a laboratory blank coupon (a coupon not exposed to the decontaminant and that has no spores applied). Obvious visible damage might include structural damage, surface degradation, discoloration, or other aesthetic impacts.

3.0 Fumigation Description and Procedures

Methyl bromide is a colorless and odorless volatile gas. Chloropicrin (Sigma Aldrich, St. Louis, MO) was added to the MB source gas (0.5% chloropicrin, 99.5% MB) as a warning irritant (lacrimator) for the safety of laboratory staff. The gas mixture was used at full strength and injected into the test chamber at the indicated target concentrations.

Figure 3-1 shows a schematic drawing of the MB test chamber and containment system. Decontamination testing was conducted inside an approximately 38 liter (L) stainless steel chamber. The chamber was insulated to prevent condensation on the inside chamber walls. As a means of secondary containment and laboratory personnel safety, this test chamber was housed inside a custom acrylic compact glove box (Plas Labs, Inc., Lansing, MI) that was hard-ducted to the facility exhaust system.

Temperature was controlled using a heated/cooled water bath, and RH was elevated using a Nafion tube pervaporation system (controlled using a water bath). Temperature and RH in the test chamber were measured using an HMT368 temperature and humidity probe (Vaisala, Inc., Woburn, MA). Temperature, RH, and MB concentration were controlled with a CNI-822 controller (Omega Engineering, Stamford, CT) and the data were recorded every minute during the contact time (CT) using the associated iLOG software.

The MB concentration in the test chamber was measured continuously during the contact period using a Fumiscope™ Version 5.0 (Key Chemical and Equipment Company, Clearwater, FL). MB was added to the chamber, as necessary, to maintain the 212 mg/L within ±10% as was show to be efficacious previously[2-4, 8] . The Fumiscope meter was calibrated by the manufacturer for MB, displaying the concentration on a digital light-emitting diode (LED) display in ounces (oz) of MB per 1000 cubic feet (ft^3). 1 oz per 1000 ft^3 is approximately 257 parts per million (ppm) at 25 °C and is approximately 1 mg/L (independent of temperature). The Fumiscope meter included an air pump that pulled a gas sample from the test chamber through the thermal conductivity meter at a controlled rate and exhausted the gas back into the test chamber. Moisture was removed from the gas sample using a small paper filter before it was measured in the Fumiscope to eliminate interference from water.

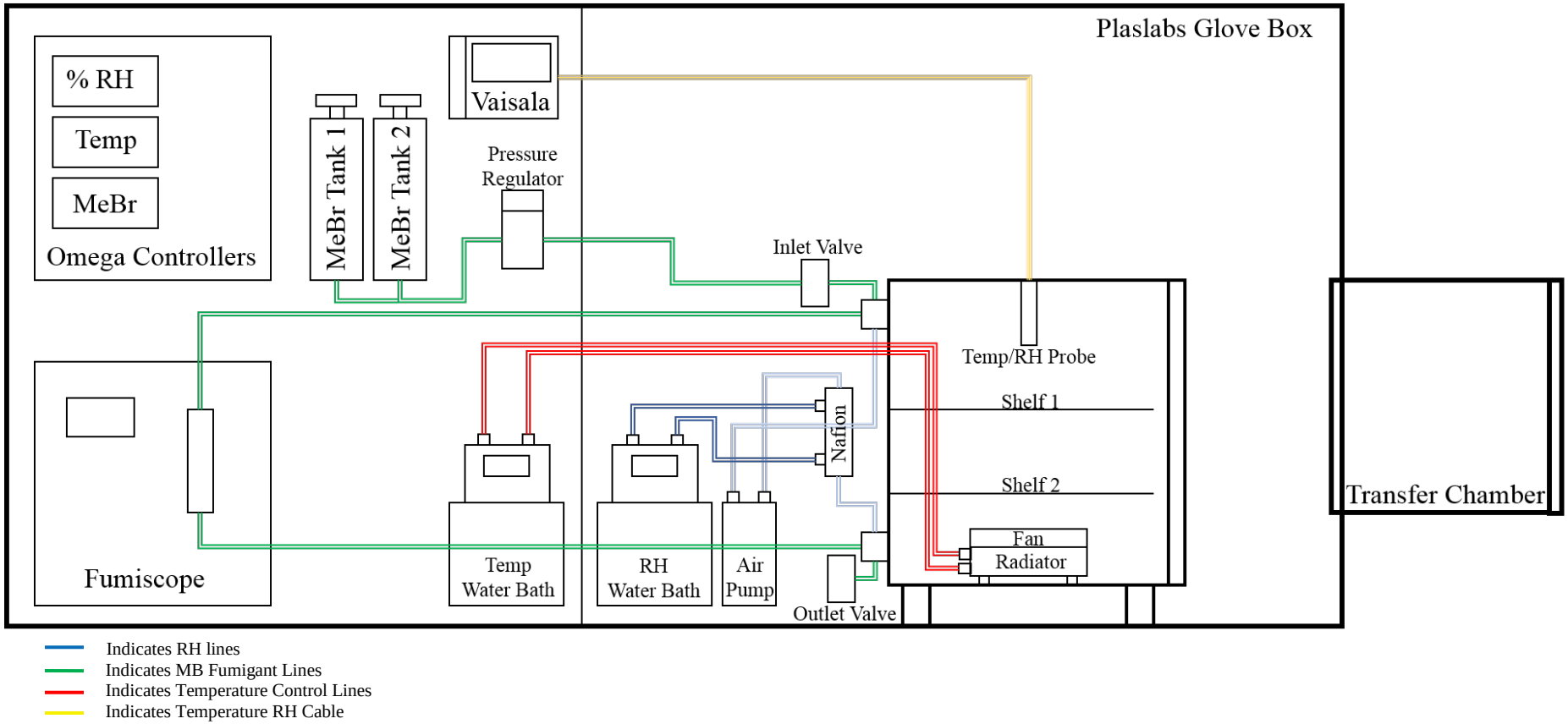

Figure 3-1. Schematic of MB decontamination test chamber housed inside custom compact glove box.

A 9-L Lock & Lock® airtight container (Lock & Lock, Farmers Branch, TX) served as the positive control chamber. Fixed humidity point salts[9] were added as a slurry to a separate container placed in the bottom of the positive control chamber. Sodium chloride was used to control the RH at 75% and sodium bromide was used to the control the RH at 50%. The control chamber was placed in an incubator (Thermo Scientific, Waltham, MA) for all tests and set to the appropriate temperature (i.e., 10 °C). The temperature and RH of the positive control chamber were measured and the data logged once every minute using a HOBO® data logger model U12-11 (Onset Computer Corporation, Cape Cod, MA).

As in previous studies with MB[1], multiple coupons of each material were inoculated with the biological agent and placed on a wire rack inside the test chamber. Blank (i.e., uninoculated) and positive control (i.e., inoculated but not decontaminated) samples were also prepared for each material and were utilized with data from the test samples (inoculated and decontaminated) to determine decontamination efficacy.

Ten MB tests were conducted at a concentration of 212 mg/L (Table 2-1). Target CTs ranged from 2 to 9 days, target temperature from 4.5 or 10 °C and RH from 50 or 75%. During each test run, inoculated test samples were placed inside the MB test chamber, and the chamber was sealed. The chamber was allowed sufficient time to equilibrate to the target temperature and RH prior to start of the run. Once the temperature and RH were stable, MB was slowly injected into the chamber until the target concentration was reached. The test chamber remained sealed until the end of the required CT. At this time, the MB was turned off and the seal of the test chamber broken by removing the lid. The test chamber and BSC III were allowed to off-gas until the MB levels in the chamber reach 0.00 mg/L, which occurred within minutes of lid removal. At this time, the samples were removed and processed as stated in Section 2.6.

4.0 Quality Assurance/Quality Control

Quality Assurance (QA) and quality control (QC) procedures were performed in accordance with the Testing and Evaluation (T&E) II program *Quality Management Plan* (QMP).. The QA/QC procedures and results are summarized below.

4.1 Equipment Calibration

All equipment (e.g., pipettes, incubators, biological safety cabinets) and monitoring devices (e.g., thermometer, hygrometer) used at the time of evaluation were verified as being certified, calibrated, or validated.

4.2 QC Results

QC efforts conducted during decontaminant testing included positive control samples (inoculated, not decontaminated), procedural blanks (not inoculated, decontaminated), laboratory blank (not inoculated, not decontaminated), and inoculation control samples (analysis of the stock spore suspension).

All positive control results were within the target recovery range of ≥5 to ≤120% of the inoculated spores, and all procedural and laboratory blanks met the criterion of no observed CFU for both organisms.

Inoculation control samples were taken from the spore suspension on the day of testing and serially diluted, nutrient plated, and counted to establish the spore density used to inoculate the samples. The spore density levels met the QA target criterion of 1×10^9 CFU/mL (±1 log) for all tests.

4.2.1. Operational Parameters

The temperature, RH, and MB concentration during each test were controlled using Omega controllers, as described in Section 3.0. These controllers were set to the target conditions and allowed temperatures, RH, or MB to be adjusted or injected as needed to stay within target ranges of ±2 °C, ±20% RH and ±10% MB. Readings were taken once every minute for the duration of the CT. The actual operational parameters for each test are shown in Table 4-1 and reported as the average value ± SD.

Table 4-1. Actual Fumigation Conditions for MB Tests.

Test Number	MB Concentration (mg/L)		Temperature (°C)		RH (%)		CT (days)
	Target	Actual*	Target	Actual*	Target	Actual*	
1†	212	214.1 ± 14.3	10	10.3 ± 1.6	75	72.3 ± 4.1	7
2	212	216.0 ± 3.21	10	9.5 ± 0.6	75	77.9 ± 2.4	2
3	212	220.8 ± 8.6	10	9.5 ± 1.4	75	80.9 ± 5.1	4
4	212	230.5 ± 15.7	10	10.4 ± 0.8	75	78.7 ± 4.2	3
5	212	212.8 ± 3.4	10	10.0 ± 0.3	75	82.3 ± 5.5	4
6	212	215.7 ± 4.1	10	10.2 ± 0.9	75	81.6 ± 4.0	5
7	212	221.8 ± 11.7	4.5	3.1 ± 0.8	75	79.9 ± 4.5	7
8	212	214.4 ± 3.2	10	9.6 ± 0.1	50	52.4 ± 6.4	7
9	212	215.4 ± 4.3	10	9.6 ± 0.0	75	77.1 ± 0.5	5
10	212	217.1 ± 6.9	4.5	4.5 ± 0.1	50	48.9 ± 4.8	9

* Data reported as average ± SD.

† Parameters deviated from target during Test 1 which is outlined in Section 4.4

4.3 Audits

4.3.1 Performance Evaluation Audit

Performance evaluation audits were conducted to assess the quality of the results obtained during these experiments. Table 4-2 summarizes the performance evaluation audits that were performed.

No performance evaluation audits were performed to confirm the concentration and purity of *B.a.* Ames or *B.a.* Sterne spores because quantitative standards do not exist for these organisms. The control coupons and blanks support the spore measurements.

Table 4-2. Performance Evaluation Audits.

Measurement	Audit Procedure	Allowable Tolerance	Actual Tolerance
Volume of liquid from micropipettes	Gravimetric evaluation	± 10%	± 0.15% to 2.5%
Time	Compared to independent clock	± 2 seconds/hour	0 seconds/hour
Temperature	Compared to independent calibrated thermometer	± 2 °C	All differences were ≤0.3 °C
Relative Humidity	Compare to independent calibrated hygrometer	± 10%	All differences were ≤1.8%

4.3.2 Technical Systems Audit

Observations and findings from the technical systems audit (TSA) were documented and submitted to the laboratory staff lead for response. TSAs were conducted on August 8, August 9, and August 11, 2016 to ensure that the tests were being conducted in accordance with the EPA and Battelle quality assurance programs. As part of the audit, test procedures and data acquisition and handling procedures were reviewed. None of the findings of the TSA required corrective action.

4.3.3 Data Quality Audit

At least 10% of the data acquired during the evaluation were audited. A QA auditor traced the data from the initial acquisition, through reduction and statistical analysis, to final reporting to ensure the integrity of the reported results. All calculations performed on the data undergoing the audit were checked.

4.4 Quality Assurance Project Plan Deviations

Section 4.2.1 Operation Parameters states "MB concentration has an allowable test measurement tolerance of ±10%." Test #1 started on July 21, 2016 with target parameters of 212 mg/L, 10 °C, 75% RH, and a 7-day CT. Due to the target temperature of 10°C, the water circulating through the radiator was set to 4 °C. This low temperature combined with the high level of desired RH (75%) resulted in large amounts of condensation within the radiator inside the test chamber. This buildup of moisture in turn caused the temperature to rise in the test chamber. To mitigate this, the test chamber had to be opened approximately every 8 to 10 hours to remove the condensation from the radiator. As the door to the testing chamber was opened, the MB concentration (mg/L) in the chamber would briefly drop below the allowable test measurement tolerance. To mitigate any issues in further testing, the circulating water bath was adjusted to stay above the dew point of the chamber which minimized condensation inside the test chamber, which reduced the need to periodically open the door to the chamber.

4.5 QA/QC Reporting

Each assessment and audit were documented in accordance with EPA and Battelle quality assurance programs. For these tests, findings were noted (none significant) in the data quality audit, but no follow up corrective action was necessary. The findings were mostly minor data transcription errors requiring some recalculation of efficacy results, but none were gross errors in recording. Copies of the assessment reports were distributed to the EPA and laboratory staff. QA/QC procedures were performed in accordance with the EPA and Battelle quality assurance programs.

4.6 Data Review

Records and data generated in the evaluation received a QC/technical review before they were utilized in calculating or evaluating results and prior to incorporation in reports.

5.0 Summary of Results and Discussion

The decontamination efficacy of MB against virulent *B.a.* Ames was evaluated at a target concentration of 212 mg/L, target temperatures of 4.5 or 10 °C, target RH of 50 or 75%, and CTs ranging from 2 to 9 days for a total of nine tests. Table 5-1 shows the CT required to achieve ≥6 LR on all material types tested (ceramic tile, painted carbon steel, weathered concrete, and granite) with or without grime added, and at all target operational parameters. Actual operational parameters, as measured, were well within acceptable ranges for all tests except for Test 1 which is outlined in Section 5.4. The detailed decontamination efficacy results are found in Appendix A.

Table 5-1. CT Required to Achieve ≥6 LR of B. anthracis on all Materials*

Target MB Concentration (mg/L)	Grimed	Target Temperature (° C)	Target RH (%)	Time (days) Required to Achieve ≥6 LR on All Materials	Test Number Reference[a]
				B.a. Ames	
212	No	10	75	4	3
212	Yes	10	75	5	6
212	Yes	4.5	75	7	7

* Materials tested were ceramic tile, painted carbon steel, weathered concrete and granite.
[a] Detailed data from each test number can be referenced in Table A-1 in Appendix A.

5.1 Effects of Test Materials on MB efficacy for *B.a.* Ames

The LR results by material, for each test, are shown in the bar graphs in Figure 5-1. Differences in efficacy between two materials are significant if the 95% CIs of the two efficacy results do not overlap. In general, ceramic tile and weathered concrete were most difficult to decontaminate (exhibited lower efficacy than painted carbon steel or granite) when testing with Ames. Further details and statistical analysis on the decontamination efficacy results are found in Appendices A and B.

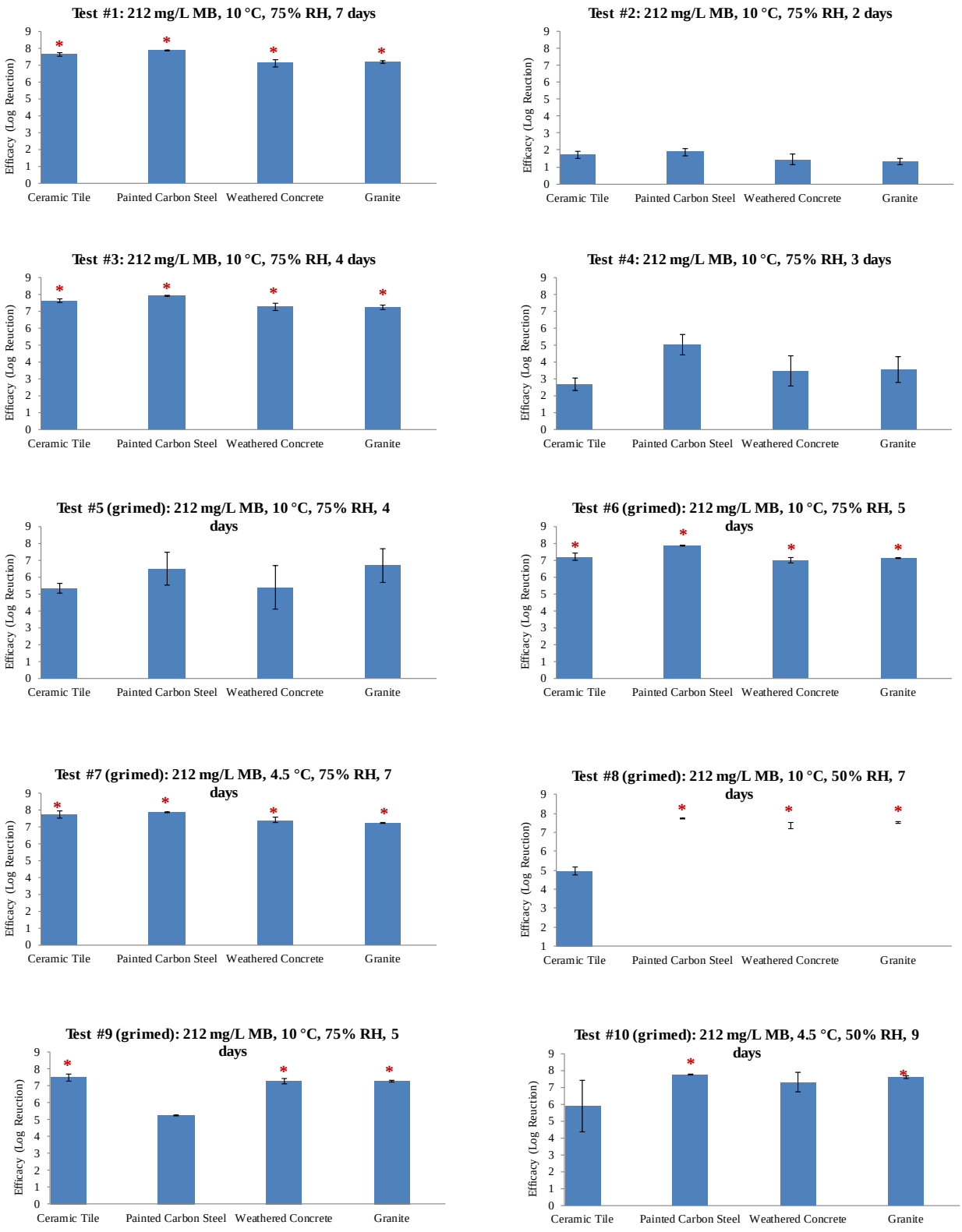

Figure 5-1. Summary of MB efficacy results, by material, for *B. anthracis* Ames and *B. anthracis* Sterne[a]. Results shown are average LR ± 95% CI.

*** Complete inactivation achieved**

[a] Test 9 was tested with *Bacillus anthracis* Sterne, Test 6 is the comparative test with B.a. Ames

5.2 Effect of Temperature on Efficacy of MB against *B. anthracis* Ames

The decontamination efficacy of MB against virulent *B.a.* Ames was evaluated at target temperatures of 4.5 or 10 °C. These temperatures were tested at various combinations of RH and CTs with and without grime added to the test materials. Due to complexity of test matrix, a direct comparison of temperature was not achievable.

In general, increasing temperature either increased decontamination efficacy or had no significant impact on efficacy. Test 10 (212 mg/L, 4.5 °C, 50% RH, 9 Day CT), achieved complete inactivation on painted carbon steel and granite but only 5.89 LR was achieved on ceramic tile and 7.30 LR on weathered concrete. For Test 8, all parameters but temperature (10 °C) were the same and the CT decreased to 7 days. Complete inactivation was achieved on painted carbon steel, weathered concrete, and granite but only 4.95 LR was achieved on ceramic tile (Table 5-2). Thus, it can be concluded that increasing temperature at 50% RH will have minimal effect on efficacy. Additional analyses of the effect of temperature, including LR data for each specific material, are included in Appendix A.

Table 5-2. Average Difference in Efficacy between Test 10 (4.5°C) and Test 8 (10°C)

Material Type	Test 10				Test 8				Average Difference in Efficacy
	212 mg/L	4.5 °C	50%	9 Days	212 mg/L	10 °C	50%	7 Days	
Ceramic Tile	5.89				4.95				-0.24
Painted Carbon Steel	≥7.73				≥7.73				
Weathered Concrete	7.30				≥7.34				
Granite	≥7.58				≥7.52				

5.3 Effect of Relative Humidity on Efficacy of MB against *B. anthracis* Ames

The decontamination efficacy of MB against *B.a.* Ames was evaluated at a target RH of 50 and 75%. The actual %RH conditions for each test are shown in Table 4-1. These RH levels were tested at various temperatures and CTs with and without grime added to the test materials. The comparisons are shown in Figures 5-2 and 5-3. Detailed tabulated results to assess the effect of RH are summarized Appendix A.

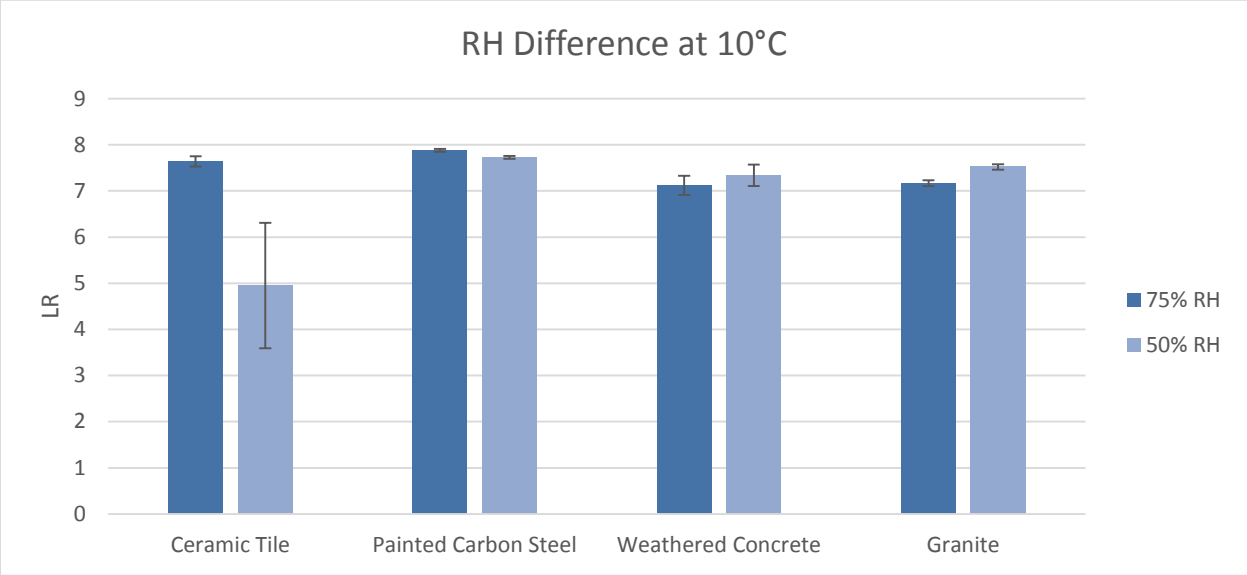

Figure 5-2. Effect of relative humidity at 10°C on MB decontamination efficacy against *B. anthracis* Ames. Results shown as average log reduction ± CI.

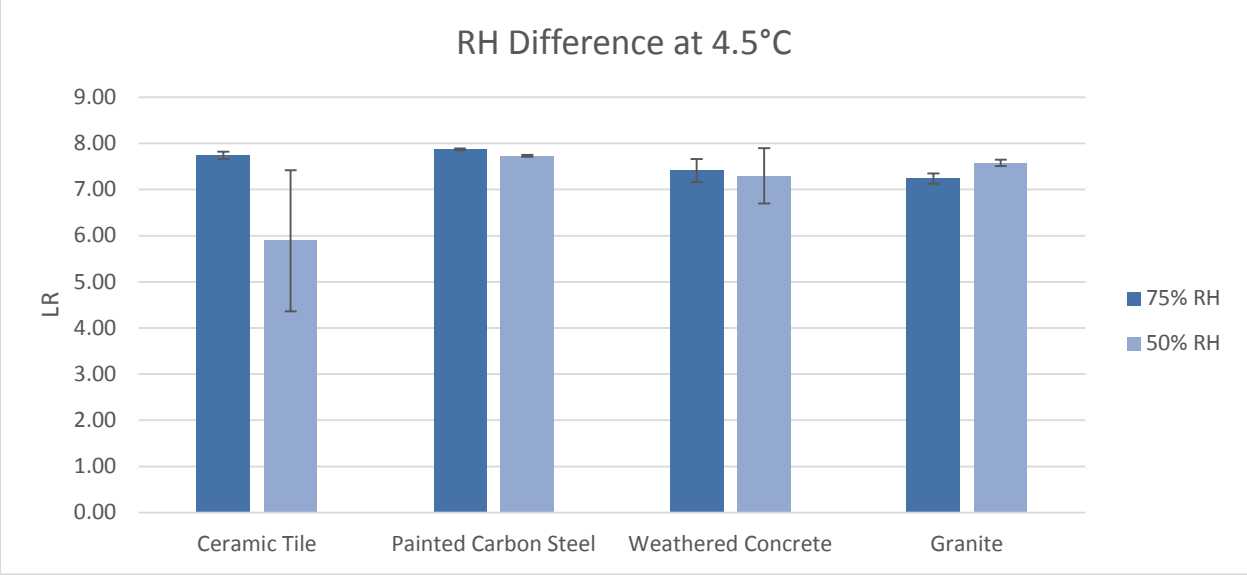

Figure 5-3. Effect of relative humidity at 4.5°C on MB decontamination efficacy against *B. anthracis* Ames. Results shown as average log reduction ± CI.

Complete inactivation was achieved in Test 1 on all materials tested. For Test 8, the RH was lowered to 50% and simulated subway grime was added to each coupon materials prior to inoculation. In this test, complete inactivation was achieved on all coupons except ceramic tile (4.95 LR), while this may suggest that 75 %RH promoted greater decontamination efficacy for

ceramic tile compare to 50 %RH, this cannot be confirmed due to the application of simulated subway grime for test 8.

5.4 Effect of CT on Efficacy of MB against *B. anthracis* Ames

The effect of increasing the CT on the efficacy against *B.a.* Ames was also assessed. The CTs tested ranged from 2 to 9 days; four non-grimed test conditions (Tests 1-4) and two grimed test conditions (Tests 5 and 6) could be compared to assess the effect of increasing CT. These comparisons are summarized in Figures 5-4 and 5-5 and presented in full detail in Appendix A.

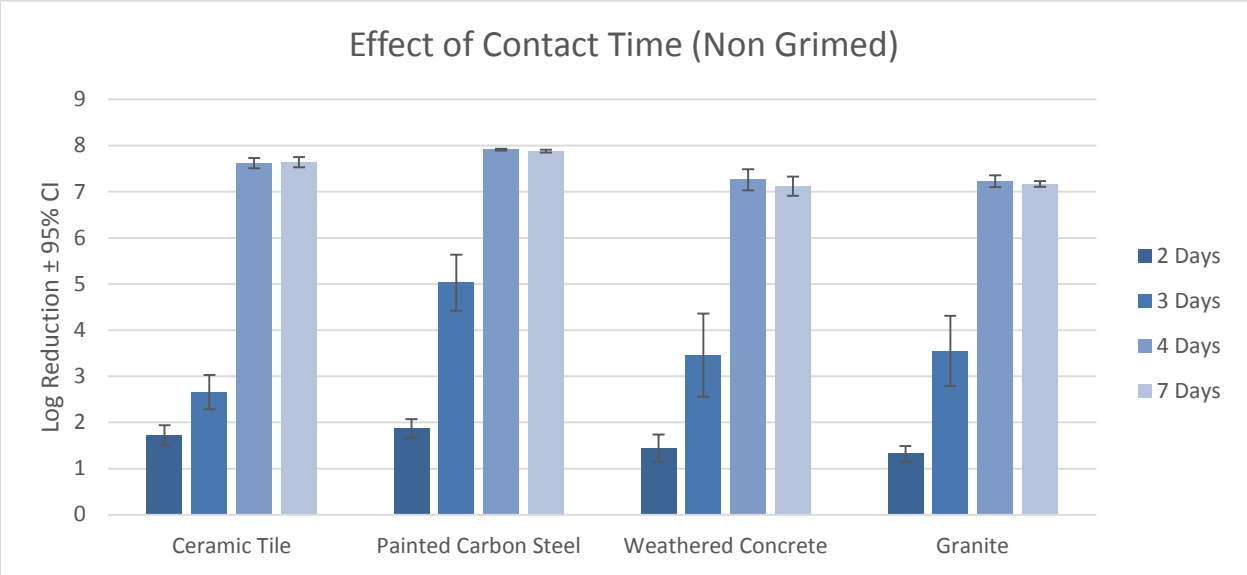

Figure 5-4. Summary of the effect of CT on average MB decontamination efficacy against *B. anthracis Ames* (Non Grimed Coupons).

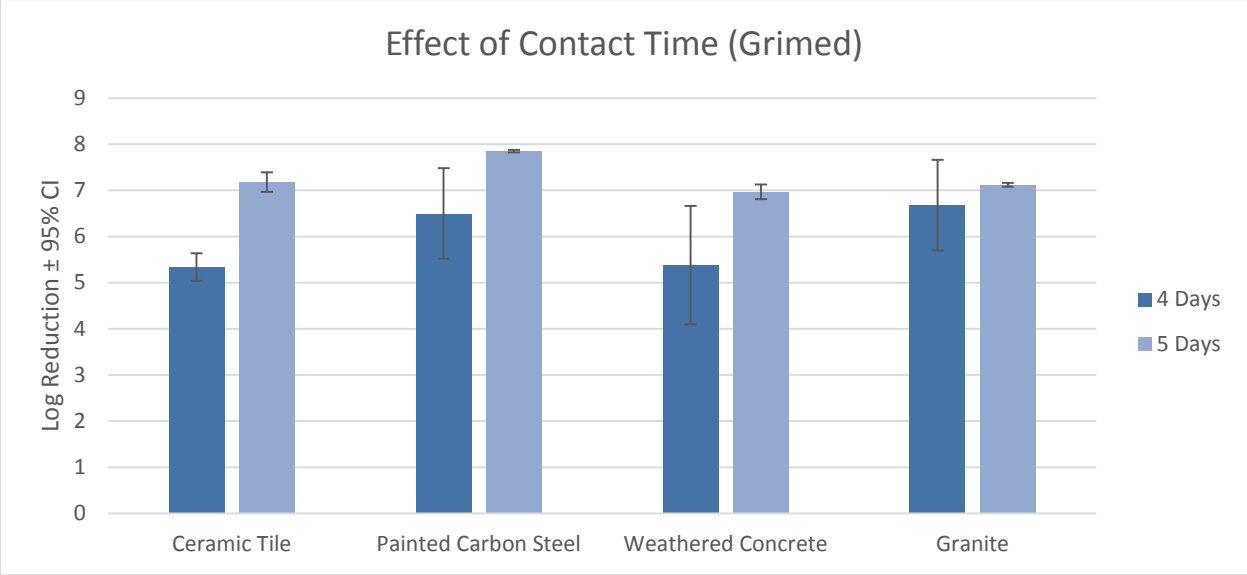

Figure 5-5. Summary of the effect of CT on average MB decontamination efficacy against *B. anthracis Ames* (Grimed Coupons).

A significant increase in LR was observed with extended CT for both grimed and non-grimed test materials. In Test 2 (212 mg/L, 10 °C, 75% RH, 2 Day CT) complete inactivation was not achieved on any materials tested and achieved an average of 1.59 LR across materials. Using those same test parameters but extending CT to 4 days resulted in complete inactivation on all non-grimed test materials. Similarly, using grimed test materials, increasing CT from 4 days to 5 resulted in complete inactivation on all test materials.

5.5 Effect of Grime

The addition of a simulated subway grime to each of the four test materials was evaluated in Tests 5-10. Comparing Tests 3 and 5 (212 mg/L, 10 °C, 75% RH, 4 Day CT), test materials that did not have grime applied resulted in complete inactivation for all test materials, but when grime was applied significant reductions in efficacy for all materials but granite were observed. These comparisons are summarized in Figures 5-6 and presented in full detail in Appendix B.

While the primary focus of this study was to determine log reduction values, it is worth noting that statistical analysis showed the addition of grime increased the recovery of spores from weathered concrete and granite as shown in Appendix B, page B-3 conclusions.

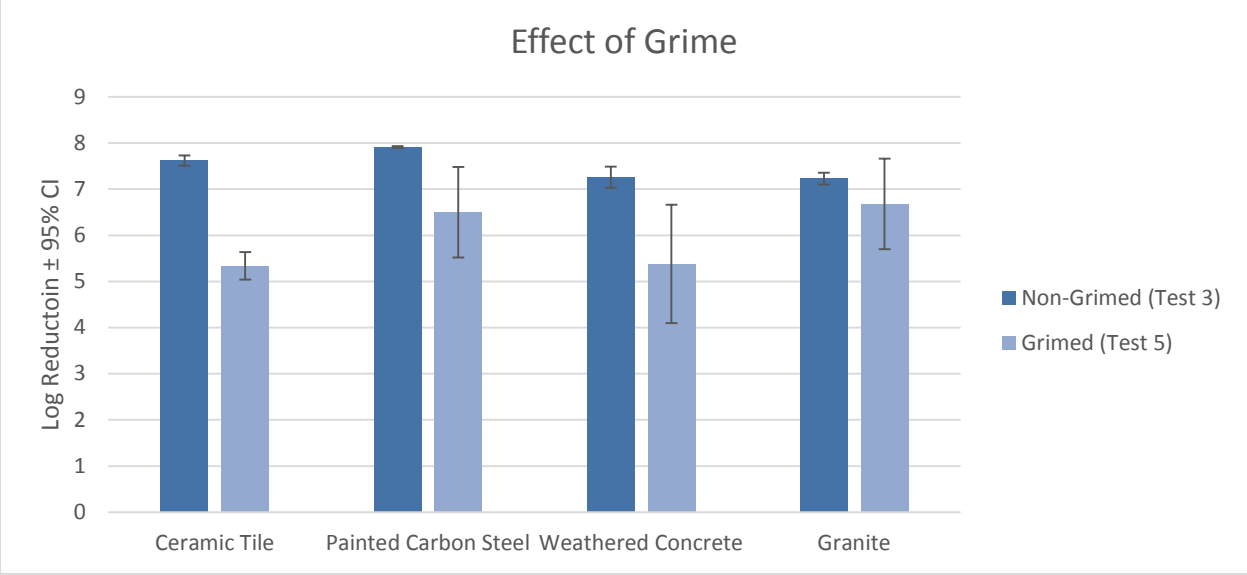

Figure 5-6. Summary of the effect of grime on average MB decontamination efficacy against *B. anthracis Ames*.

5.6 Comparison of *B.a.* Ames vs Sterne

Efficacy of MB on *B.a.* Sterne was evaluated against *B.a.* Ames to assess the potential use of *B.a.* Sterne as a suitable surrogate for the virulent strain. Comparing Test 6, which used *B.a.* Ames (212 mg/L, 10 °C, 75% RH, 5 Day CT) and Test 9 which had identical testing parameters but used *B.a.* Sterne, statistical analysis found no significant difference in efficacy for ceramic tile, weathered concrete, and granite. MB was more efficacious against *B.a.* Ames on Painted Carbon Steel than *B.a.* Sterne, resulting in ≥7.85 and 5.25 LR, respectively. These comparisons are summarized in Figure 5-7 and presented in full detail in Appendix B.

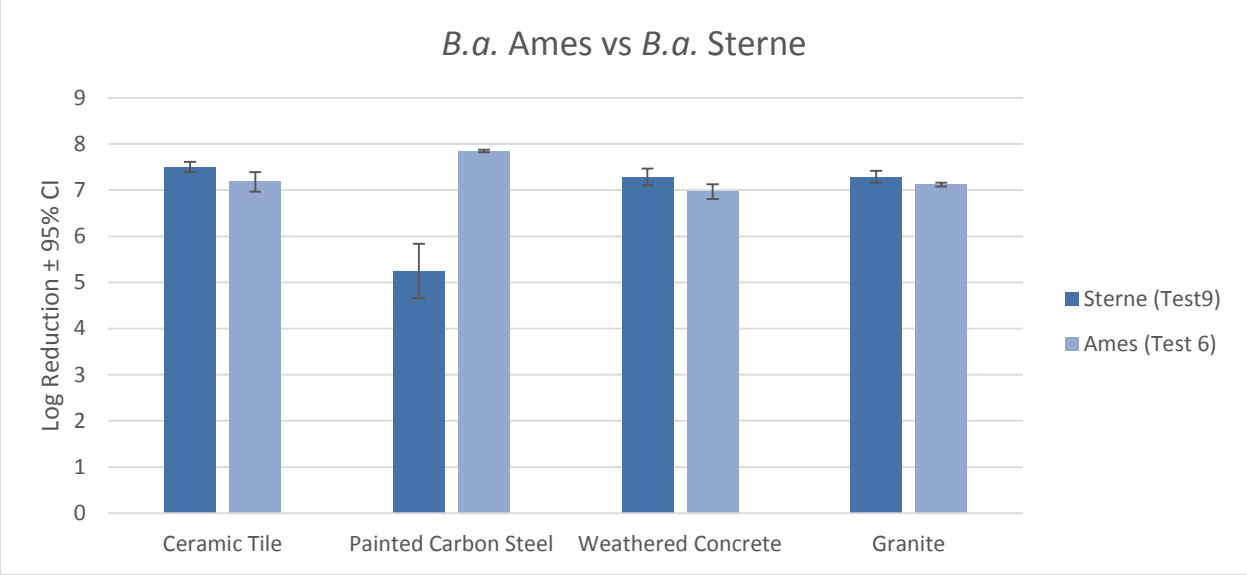

Figure 5-7. MB decontamination efficacy against *B. anthracis* Ames and Sterne (Grimed Coupons).

5.7 Surface Damage to Materials

At the end of each decontamination test, the procedural blanks were visually compared to the laboratory blanks, and test coupons were visually compared to positive controls, to assess any impact MB may have had on each material type. Based on the visual appearance of the decontaminated coupons, there were no apparent changes in the color, reflectivity, or roughness of the six material surfaces after being exposed to MB. Note that chloropicrin is often added to MB as a warning agent and chloropicrin has the potential to cause oxidation to some surfaces[(9)].

5.8 Summary and Conclusion

This investigation focused on decontamination efficacy when fumigating with MB at temperatures (low temperatures) and examining the effect of RH. Eliminating or reducing the need to humidify and/or heat an area of interest would facilitate MB fumigation when used to decontaminate a subway contaminated with *B.a.* spores.

This study highlights the roles of CT, RH, temperature, and application of simulated subway grime when fumigating with MB. There was a clear impact of time as evidenced by the increase in LR of *B.a.* Ames with the increase of time (2, 3, 4 & 7 days) resulting in ≥6 LR at 4 and 7 days. There were no tests (only two tests conducted at 50% RH) in which ≥6 LR of *B.a.* Ames was achieved on all materials when fumigating at 50% RH. Application of grime to the test materials in the case of weathered concrete, painted steel, and granite decrease decontamination efficacy. It also increased the time to achieve complete inactivation on all test materials from 4 to 5 days. Lastly when comparing *B.a.* Ames to *B.a.* Sterne, three of the test materials resulted in similar reductions while *B.a.* Sterne was more resistant on Painted Carbon Steel. This was only conducted for a single test but shows that *B.a.* Sterne may be a suitable surrogate for the virulent Ames strain. Additional testing would need to be performed to confirm this result.

Impact of Study

This work provides information on the operational parameters of MB fumigation that are required to achieve efficacy when tested at temperatures and on materials that would be typical of a subway underground transit system that has been contaminated with *B.a* Ames and Sterne spores. Such results may be useful in the development of guidance to aid in deployment of MB fumigation after a wide-area release of *B.a* Ames spores in a subway environment.

6.0 References

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10. Compatibility of Material and Electronic Equipment with Methyl Bromide and Chlorine Dioxide Fumigation, EPA Technology Evaluation Report. October 2012. EPA/600/R-12/664. (https://cfpub.epa.gov/si/si_public_file_download.cfm?p_download_id=508138)

Appendix A
Detailed Test Results

Efficacy Results

The detailed decontamination efficacy results for methyl bromide against *B.a.* Ames on four material types (ceramic tile, painted carbon steel, weathered concrete and granite) are shown in Table A-1. Zero CFU were observed on all laboratory and procedural blanks.

Table A-1. Inactivation of *B. anthracis* Ames Spores using Methyl Bromide[a]

Test Number	Target Parameters		Material	Inoculum (CFU/coupon)	Mean Recovered *B.a.* Ames (CFU/coupon)		Decontamination Efficacy ± CI[d]
	Concentration (mg/L) / CT (days)	Temp (°C) / RH (%)			Positive Control[b]	Test Coupon[c]	
1	212/7	10/75	Ceramic Tile	7.97×10^7	$4.50 \pm 1.47 \times 10^7$	0.00 ± 0.00	≥7.64 ± 0.11
			Painted Carbon Steel		$7.55 \pm 0.54 \times 10^7$	0.00 ± 0.00	≥7.88 ± 0.03
			Weathered Concrete		$1.48 \pm 0.90 \times 10^7$	0.00 ± 0.00	≥7.12 ± 0.21
			Granite		$1.50 \pm 2.46 \times 10^6$	0.00 ± 0.00	≥7.17 ± 0.06
2	212/2	10/75	Ceramic Tile	8.23×10^7	$3.60 \pm 0.78 \times 10^7$	$7.56 \pm 3.49 \times 10^5$	1.72 ± 0.22
			Painted Carbon Steel		$5.63 \pm 0.80 \times 10^7$	$8.22 \pm 3.32 \times 10^5$	1.87 ± 0.20
			Weathered Concrete		$1.24 \pm 0.39 \times 10^7$	$5.29 \pm 3.23 \times 10^5$	1.44 ± 0.30
			Granite		$1.20 \pm 0.26 \times 10^7$	$6.01 \pm 2.44 \times 10^5$	1.32 ± 0.17
3	212/4	10/75	Ceramic Tile	8.93×10^7	$4.32 \pm 1.30 \times 10^7$	0.00 ± 0.00	≥7.62 ± 0.11
			Painted Carbon Steel		$8.08 \pm 0.34 \times 10^7$	0.00 ± 0.00	≥7.91 ± 0.02
			Weathered Concrete		$2.17 \pm 1.70 \times 10^7$	0.00 ± 0.00	≥7.26 ± 0.23
			Granite		$1.79 \pm 0.67 \times 10^7$	0.00 ± 0.00	≥7.23 ± 0.13
4	212/3	10/75	Ceramic Tile	8.13×10^7	$2.65 \pm 0.58 \times 10^7$	$8.99 \pm 11.3 \times 10^4$	2.66 ± 0.37
			Painted Carbon Steel		$6.00 \pm 0.89 \times 10^7$	$9.26 \pm 5.72 \times 10^2$	5.03 ± 0.61
			Weathered Concrete		$1.35 \pm 0.76 \times 10^7$	$1.86 \pm 2.40 \times 10^4$	3.46 ± 0.90
			Granite		$8.05 \pm 2.23 \times 10^6$	$7.75 \pm 1.31 \times 10^3$	3.55 ± 0.76
5[e]	212/4	10/75	Ceramic Tile	7.77×10^7	$5.87 \pm 0.58 \times 10^7$	$3.20 \pm 1.46 \times 10^2$	5.34 ± 0.30
			Painted Carbon Steel		$5.19 \pm 0.84 \times 10^7$	$6.69 \pm 6.60 \times 10^1$	6.50 ± 0.98
			Weathered Concrete		$3.08 \pm 2.00 \times 10^7$	$1.05 \pm 1.41 \times 10^3$	5.38 ± 1.28
			Granite		$3.14 \pm 0.47 \times 10^7$	$4.73 \pm 7.27 \times 10^1$	6.68 ± 0.98
6[e]	212/5	10/75	Ceramic Tile	7.70×10^7	$1.70 \pm 0.96 \times 10^7$	0.00 ± 0.00	≥7.18 ± 0.21
			Painted Carbon Steel		$7.07 \pm 0.53 \times 10^7$	0.00 ± 0.00	≥7.85 ± 0.03
			Weathered Concrete		$1.01 \pm 0.43 \times 10^7$	0.00 ± 0.00	≥6.97 ± 0.16
			Granite		$1.33 \pm 0.14 \times 10^7$	0.00 ± 0.00	≥7.12 ± 0.04
7[e]	212/7	4.5/75	Ceramic Tile	7.63×10^7	$5.57 \pm 1.26 \times 10^7$	0.00 ± 0.00	≥7.74 ± 0.08
			Painted Carbon Steel		$7.34 \pm 0.43 \times 10^7$	0.00 ± 0.00	≥7.87 ± 0.02
			Weathered Concrete		$2.99 \pm 1.62 \times 10^7$	0.00 ± 0.00	≥7.41 ± 0.25
			Granite		$1.80 \pm 0.60 \times 10^7$	0.00 ± 0.00	≥7.24 ± 0.11
8[e]	212/7	10/50	Ceramic Tile	9.13×10^7	$3.95 \pm 0.55 \times 10^7$	$2.67 \pm 3.03 \times 10^3$	4.95 ± 1.36
			Painted Carbon Steel		$5.43 \pm 0.41 \times 10^7$	0.00 ± 0.00	≥7.73 ± 0.03
			Weathered Concrete		$2.52 \pm 1.27 \times 10^7$	0.00 ± 0.00	≥7.34 ± 0.23
			Granite		$3.33 \pm 0.48 \times 10^7$	0.00 ± 0.00	≥7.52 ± 0.06
10[e]	212/9	4.5/50	Ceramic Tile	9.60×10^7	$5.93 \pm 1.17 \times 10^7$	$1.20 \pm 1.86 \times 10^3$	5.89 ± 1.53
			Painted Carbon Steel		$5.38 \pm 0.34 \times 10^7$	0.00 ± 0.00	≥7.73 ± 0.02
			Weathered Concrete		$4.12 \pm 0.98 \times 10^7$	$7.46 \pm 14.4 \times 10^1$	7.30 ± 0.60
			Granite		$3.87 \pm 0.74 \times 10^7$	0.00 ± 0.00	≥7.58 ± 0.07

[a] Data are expressed as the mean (± SD) of the logs of the number of spores (CFU) observed on five individual samples, and decontamination efficacy (log reduction).
[b] Positive Controls = samples inoculated, not decontaminated.
[c] Test Coupons = samples inoculated, decontaminated.
[d] CI = confidence interval (± 1.96 × SE).
[e] Test 5-10 had materials applied with simulated subway grime

Table A-2. Inactivation of *B. anthracis* Sterne Spores using Methyl Bromide[a]

Test Number	Target Parameters		Material	Inoculum (CFU/coupon)	Mean Recovered *B.a. Sterne* (CFU/coupon)		Decontamination Efficacy ± CI[d]
	Concentration (mg/L) / CT (days)	Temp (°C) / RH (%)			Positive Control[b]	Test Coupon[c]	
9[e]	212/5	10/75	Ceramic Tile	6.10×10^7	$3.25 \pm 0.80 \times 10^7$	0.00 ± 0.00	$\geq 7.50 \pm 0.11$
			Painted Carbon Steel		$5.71 \pm 1.00 \times 10^7$	$5.99 \pm 5.25 \times 10^2$	5.25 ± 0.59
			Weathered Concrete		$2.10 \pm 0.94 \times 10^7$	0.00 ± 0.00	$\geq 7.29 \pm 0.18$
			Granite		$2.07 \pm 0.76 \times 10^7$	0.00 ± 0.00	$\geq 7.29 \pm 0.13$

[a] Data are expressed as the mean (± SD) of the logs of the number of spores (CFU) observed on five individual samples, and decontamination efficacy (log reduction).
[b] Positive Controls = samples inoculated, not decontaminated.
[c] Test Samples = samples inoculated, decontaminated.
[d] CI = confidence interval (± 1.96 × SE).
[e] Test 9 had materials applied with simulated subway grime

Effect of Relative Humidity on Efficacy of MB against *B. anthracis* Ames

The decontamination efficacy of MB against *B.a.* Ames was evaluated at target relative humidity of 50 or 75%. The actual %RH conditions for each test are shown in Section 4.2. These RH levels were tested at various temperatures, and CTs and results are summarized in Table A-5 below and discussed in Section 6.4. The comparisons are made for two test conditions which share the same fumigation parameters except RH and grime application.

Table A-5. Effect of Increasing Relative Humidity at High Temperatures on *B. anthracis* Ames*

Material Type	Test 1[a]			Test 8[ab]			Average Increase in Efficacy
	10 °C	75%	7 days	10 °C	50%	7 days	
Ceramic Tile	≥7.64			4.95			-0.57
Painted Carbon Steel	≥7.88			≥7.73			
Weathered Concrete	≥7.12			≥7.34			
Granite	≥7.17			≥7.52			

* Data are expressed as decontamination efficacy (log reduction).
[a] Parameters of each test listed in order of MB concentration (mg/L), temperature (°C), %RH, and CT (days).
[b] Grime applied to test materials

Table A-6. Effect of Increasing Relative Humidity at Low Temperatures on *B. anthracis* Ames*

Material Type	Test 7[ab]				Test 10[ab]				Average Increase in Efficacy
	212 mg/L	4.5 °C	75%	7 days	212 mg/L	4.5 °C	50%	9 days	
Ceramic Tile	≥7.74				5.89				-0.70
Painted Carbon Steel	≥7.87				≥7.73				
Weathered Concrete	≥7.41				7.30				
Granite	≥7.24				≥7.58				

* Data are expressed as decontamination efficacy (log reduction).
[a] Parameters of each test listed in order of MB concentration (mg/L), temperature (°C), %RH, and CT (days).
[b] Grime applied to test materials

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Effects of CT on Efficacy of MB against *B. anthracis* Ames

The effect of increasing the CTs to MB at low and high %RH on the efficacy against *B.a.* Ames was assessed by comparing Tests 1-4 for non-grimed test materials and Tests 5 and 6 for grimed materials. The CTs tested ranged from 2 to 7 days and actual CTs did not deviate from these targets except for Test 1 which is described in Section 5.4. The results are summarized in Table A-6. The comparisons are made for two test conditions that share the same fumigation parameters except CT.

Table A-7. Effect of Increasing CT at High Relative Humidity on *B. anthracis* Ames* with no Grime.

Material Type	Test 2[a]				Test 4[a]				Average Increase in Efficacy
	212 mg/L	10 °C	75%	2 days	212 mg/L	10 °C	75%	3 days	
Ceramic Tile	1.72				2.66				2.09
Painted Carbon Steel	1.87				5.03				
Weathered Concrete	1.44				3.46				
Granite	1.32				3.55				
Material Type	**Test 4[a]**				**Test 3[a]**				**Average Increase in Efficacy**
	212 mg/L	10 °C	75%	3 days	212 mg/L	10 °C	75%	4 days	
Ceramic Tile	2.66				≥7.62				6.62
Painted Carbon Steel	5.03				≥7.91				
Weathered Concrete	3.46				≥7.26				
Granite	3.55				≥7.23				
Material Type	**Test 3[a]**				**Test 1[a]**				**Average Increase in Efficacy**
	212 mg/L	10 °C	75%	4 days	212 mg/L	10 °C	75%	7 days	
Ceramic Tile	≥7.62				≥7.64				-0.05
Painted Carbon Steel	≥7.91				≥7.88				
Weathered Concrete	≥7.26				≥7.12				
Granite	≥7.23				≥7.17				

* Data are expressed as decontamination efficacy (log reduction).

[a] Parameters of each test listed in order of MB concentration (mg/L), temperature (°C), %RH, and CT (days).

Table A-8. Effect of Increasing CT at High Relative Humidity on *B. anthracis* Ames* with Grime.

Material Type	Test 5[a]				Test 6[a]				Average Increase in Efficacy
	212 mg/L	10 °C	75%	4 days	212 mg/L	10 °C	75%	5 days	
Ceramic Tile	5.34				≥7.18				1.31
Painted Carbon Steel	6.50				≥7.85				
Weathered Concrete	5.38				≥6.97				
Granite	6.68				≥7.12				

* Data are expressed as decontamination efficacy (log reduction).
[a] Parameters of each test listed in order of MB concentration (mg/L), temperature (°C), %RH, and CT (days).

Appendix B
Detailed Statistical Analysis

Results

Table B-1 contains the mean percent recoveries for each strain, material, and simulated subway grime coating, with 95 percent confidence intervals. Percent recoveries for each *B. anthracis* Ames control coupon are plotted in Figure B-1. Percent recoveries comparing *B. anthracis* strain for tests 5 through 10 are plotted in Figure B-2.

Table B-2, B-Table B-3, B-Table B-4, and Table B-6 present the ANOVA summary tables for testing the effect of simulated subway grime for each material. Percent recovery was significantly different with and without simulated subway grime for weathered concrete ($p = 0.0071$) and granite ($p < 0.0001$), with simulated subway grime resulting in greater percent recovery for both materials (Table B-5 and Table B-7).

ANOVA summary tables for testing the effect of material without and with simulated subway grime are presented in Table B-8 and Table B-10. Percent recovery was significantly different among the materials both without and with simulated subway grime. Table B-9 and Table B-11 present the Tukey comparisons among the differences, indicating only granite and weathered concrete were not significantly different with respect to percent recovery on the control coupons.

Table B-12 presents the ANOVA summary table for testing whether there is a difference in control coupon recovery between the two strains and materials. There was no statistically significant difference in percent recovery between the two strains ($p = 0.2108$), but there was a statistically significant difference among the materials ($p < 0.0001$). The Tukey comparisons for materials are presented in Table B-14. All materials were significantly different from one another except for granite and weathered concrete. Painted carbon steel had a significantly greater percent recovery than all other materials. Ceramic tile had a significantly greater percent recovery than granite and weathered concrete.

Estimates with exact 95 percent confidence intervals for the reduction in log (base-10) *B. anthracis* spores are presented in Table B-15.

The main effects ANOVA model fitted to the *B. anthracis* Ames strain log-reduction data summary table is presented in Table B-16. All main effects were statistically significant. The Tukey comparisons for material are presented in Table B-17 and for CT are presented in Table B-21; least squares means for simulated subway grime, temperature, and relative humidity are presented in Table B-18, Table B-19, and Table B-20, respectively. From Table B-17, the log-reduction for ceramic tile was significantly less than the other materials, and no other materials were significantly different from each other. Log-reduction was statistically greater when there was no simulated subway grime (Table B-18); log-reduction was statistically greater at 4.5 degrees (Table B-19) and 50% relative humidity (Table B-20). From Table B-21, a CT of 4 days was not statistically different from CTs of 7 and 9 days, while all other pairs of CTs were statistically different with respect to reduction in log (base 10) *B. anthracis* Ames spores. Generally, longer times had greater log-reduction except for 7 and 9 days which had significantly less log-reduction than 5 days and 9 days had significantly less log-reduction than 7 days.

Table B-22 presents the ANOVA summary table for testing whether there was a difference in decontaminated coupon reduction between the two *B. anthracis* strains and materials. This model only included data from Tests 6 and 9 because they had the same combination of subway grime, temperature, relative humidity and CT. There was a statistically significant interaction between

strain and material ($p < 0.001$), indicating that the difference in reduction between the two strains of log (base 10) *B. anthracis* depends on the material. Table B-25 presents the Tukey comparisons among the material combinations for Ames strain, and shows the model did not estimate any difference in log-reduction between the materials for Ames strain. Table B- presents the Tukey comparisons among the material combinations for Sterne strain, and shows the that reduction of log (base 10) *B. anthracis* Sterne spores on painted carbon steel was statistically less than that for all other materials. Table B-16 presents the Tukey comparisons between the strains for each material, and shows that there was a significantly greater reduction for Ames strain than Sterne on painted carbon steel. Results are consistent with the data that show all combinations of material and strain had a complete kill except for Sterne strain on painted carbon steel.

Conclusions

Analysis of the percent recovery showed statistically significant differences in percent recovery with and without simulated subway grime for weathered concrete and granite. For both materials, the percent recovery was greater for coupons with simulated subway grime. In addition, painted carbon steel had a significantly greater percent recovery than all other materials while ceramic tile had a significantly greater percent recovery than granite and weathered concrete. It can also be concluded that *B. anthracis* Ames and *B. anthracis* Sterne were not significantly different with respect to the percent recovery.

Reduction in log (base-10) *B. anthracis* Ames spores was statistically different among the different materials, simulated subway grime, temperatures, relative humidity, and CTs. The least percent reduction in spores was seen for ceramic tile. Simulated subway grime resulted in lower reduction in spores. Greater reduction in spores was observed for temperature equal to 4.5 degrees and relative humidity equal to 50%. Finally, CT of 5 days resulted in the greatest reduction in *B. anthracis* Ames spores.

B. anthracis strains were differentially reduced depending on the material. A lower log (base 10) reduction in *B. anthracis* Sterne on painted carbon steel was observed compared to *B. anthracis* Ames on painted carbon steel and compared to Sterne on other materials.

Table B-1. Mean Percent Recovery for Control Coupons for Each Strain, Material, and Simulated Subway Grime Combination with 95 Percent Confidence Intervals.

Strain	Material	Simulated Subway Grime	N	Mean Percent Recovery (95% Confidence Interval)
B. anthracis Ames	Ceramic Tile	No	20	45.28 (38.26,52.29)
B. anthracis Ames	Ceramic Tile	Yes	25	55.12 (45.60,64.63)
B. anthracis Ames	Granite	No	20	15.82 (13.09,18.56)
B. anthracis Ames	Granite	Yes	25	31.60 (27.01,36.19)
B. anthracis Ames	Painted Carbon Steel	No	20	81.87 (75.51,88.24)
B. anthracis Ames	Painted Carbon Steel	Yes	25	74.08 (66.59,81.57)
B. anthracis Ames	Weathered Concrete	No	20	18.65 (13.13,24.17)
B. anthracis Ames	Weathered Concrete	Yes	25	32.47 (24.57,40.38)
B. anthracis Sterne	Ceramic Tile	Yes	5	53.25 (36.92,69.57)
B. anthracis Sterne	Granite	Yes	5	33.93 (18.48,49.39)
B. anthracis Sterne	Painted Carbon Steel	Yes	5	93.64 (73.32,100.0)[a]
B. anthracis Sterne	Weathered Concrete	Yes	5	34.48 (15.36,53.61)

[a] Confidence limits less than 0 or greater than 100 truncated to 0 or 100 to reflect valid range of percent recovery values.

Table B-2. Percent Recovery ANOVA Summary Table Testing the Effect for Simulated Subway Grime on Ceramic Tile Control Coupons.

Source	Degrees of Freedom	Sum of Squares	F Statistic	P-value
Grime	1	1075.88	2.72	0.1064
Residual Error	43	17007.56		

Table B-3. Percent Recovery ANOVA Summary Table Testing the Effect for Simulated Subway Grime on Painted Carbon Steel Control Coupons.

Source	Degrees of Freedom	Sum of Squares	F Statistic	P-value
Grime	1	674.69	2.54	0.1182
Residual Error	43	11412.63		

Table B-4. Percent Recovery ANOVA Summary Table Testing the Effect for Simulated Subway Grime on Weathered Concrete Control Coupons.

Source	Degrees of Freedom	Sum of Squares	F Statistic	P-value
Grime	1	2123.58	7.98	0.0071*
Residual Error	43	11437.93		

* Effect is statistically significant at $\alpha = 0.05$ level.

Table B-5. Percent Recovery Least Squares Means for Simulated Subway Grime Conditions on Weathered Concrete Control Coupons.

Simulated Subway Grime	Mean	Standard Deviation
No	18.65	3.65
Yes	32.47	3.26

Table B-6. Percent Recovery ANOVA Summary Table Testing the Effect for Simulated Subway Grime on Granite Control Coupons.

Source	Degrees of Freedom	Sum of Squares	F Statistic	P-value
Grime	1	2766.32	32.87	<0.001*
Residual Error	43	3618.36		

* Effect is statistically significant at $\alpha = 0.05$ level.

Table B-7. Percent Recovery Least Squares Means for Simulated Subway Grime Conditions on Granite Control Coupons.

Simulated Subway Grime	Mean	Standard Deviation
No	15.82	2.05
Yes	31.60	1.83

Table B-8. Percent Recovery ANOVA Summary Table Testing the Effect for Material Without Simulated Subway Grime Applied Control Coupons.

Source	Degrees of Freedom	Sum of Squares	F Statistic	P-value
Material	3	56415.41	129.09	<0.001*
Residual Error	76	11070.90		

* Effect is statistically significant at $\alpha = 0.05$ level.

Table B-9. Percent Recovery Tukey Comparisons# for Materials Without Simulated Subway Grime Applied Control Coupons.

	Granite	Painted Carbon Steel	Weathered Concrete
Ceramic Tile	29.45*	-36.6*	26.63*
Granite		-66.05*	-2.82
Painted Carbon Steel			63.22*

* Differences are statistically significantly at $\alpha = 0.05$ level.

Positive values indicate the row level results in greater percent recovery than the column level; negative values indicate the column level results in greater percent recovery.

Table B-10. Percent Recovery ANOVA Summary Table Testing the Effect for Material with Simulated Subway Grime Applied Control Coupons.

Source	Degrees of Freedom	Sum of Squares	F Statistic	P-value
Material	3	31007.48	30.62	<0.001*
Residual Error	96	32405.58		

* Effect is statistically significant at α = 0.05 level.

Table B-11. Percent Recovery Tukey Comparisons[#] for Materials with Simulated Subway Grime Applied Control Coupons.

	Granite	Painted Carbon Steel	Weathered Concrete
Ceramic Tile	23.51*	-18.96*	22.64*
Granite		-42.48*	-0.87
Painted Carbon Steel			41.61*

* Differences are statistically significantly at α = 0.05 level.

Positive values indicate the row level results in greater percent recovery than the column level; negative values indicate the column level results in greater percent recovery.

Table B-12. Percent Recovery ANOVA Summary Table Testing the Effect for Strain and Material for Control Coupons with Simulated Subway Grime.

Source	Degrees of Freedom	Sum of Squares	F Statistic	P-value
B. anthracis Strain	1	505.49	1.58	0.2108
Material	3	28820.79	30.11	<0.001*
B. anthracis Strain*Material Interaction	3	1142.58	1.19	0.3155
Residual Error	112	35736.33		

* Effect is statistically significant at α = 0.05 level.

Table B-13. Percent Recovery ANOVA Summary Table Testing the Main Effects for Strain and Material for Control Coupons with Simulated Subway Grime.

Source	Degrees of Freedom	Sum of Squares	F Statistic	P-value
B. anthracis Strain	1	505.49	1.58	0.2118
Material	3	41641.43	43.28	<0.001*
Residual Error	115	36878.90		

* Effect is statistically significant at α = 0.05 level.

Table B-14. Percent Recovery Tukey Comparisons[#] for Material for Control Coupons.

	Granite	Painted Carbon Steel	Weathered Concrete
Ceramic Tile	22.81*	-22.54*	22*
Granite		-45.35*	-0.82
Painted Carbon Steel			44.53*

* Differences are statistically significantly at $\alpha = 0.05$ level.

Positive values indicate the row level results in greater percent recovery than the column level; negative values indicate the column level results in greater percent recovery.

Table B-15. Mean Log (Base 10) Reduction[*] for Decontaminated Coupons for Each Decontamination Scenario with 95 Percent Confidence Intervals.

Strain	Material	Simulated Subway Grime	Temperature	Relative Humidity	CT	N	Number of Samples that were Complete Kill	Mean Log10 Reduction (95% Confidence Interval
B. anthracis Ames	Ceramic Tile	No	10	75	2	5	0	2.08 (1.79, 2.38)
B. anthracis Ames	Ceramic Tile	No	10	75	3	5	0	3.15 (2.64, 3.67)
B. anthracis Ames	Ceramic Tile	No	10	75	4	5	5	7.95 (--, --)
B. anthracis Ames	Ceramic Tile	No	10	75	7	5	5	7.90 (--, --)
B. anthracis Ames	Ceramic Tile	Yes	4.5	50	9	5	2	6.11 (3.95, 8.28)
B. anthracis Ames	Ceramic Tile	Yes	4.5	75	7	5	5	7.88 (--, --)
B. anthracis Ames	Ceramic Tile	Yes	10	50	7	5	1	5.32 (3.39, 7.25)
B. anthracis Ames	Ceramic Tile	Yes	10	75	4	5	1	5.46 (5.04, 5.88)
B. anthracis Ames	Ceramic Tile	Yes	10	75	5	5	5	7.89 (--, --)
B. anthracis Ames	Granite	No	10	75	2	5	0	2.16 (1.96, 2.37)
B. anthracis Ames	Granite	No	10	75	3	5	0	4.57 (3.51, 5.63)
B. anthracis Ames	Granite	No	10	75	4	5	5	7.95 (--, --)
B. anthracis Ames	Granite	No	10	75	7	5	5	7.90 (--, --)
B. anthracis Ames	Granite	Yes	4.5	50	9	5	5	7.98 (--, --)
B. anthracis Ames	Granite	Yes	4.5	75	7	5	5	7.88 (--, --)
B. anthracis Ames	Granite	Yes	10	50	7	5	5	7.96 (--, --)
B. anthracis Ames	Granite	Yes	10	75	4	5	4	7.08 (5.69, 8.47)
B. anthracis Ames	Granite	Yes	10	75	5	5	5	7.89 (--, --)
B. anthracis Ames	Painted Carbon Steel	No	10	75	2	5	0	2.04 (1.77, 2.31)
B. anthracis Ames	Painted Carbon Steel	No	10	75	3	5	1	5.17 (4.31, 6.03)
B. anthracis Ames	Painted Carbon Steel	No	10	75	4	5	5	7.95 (--, --)

Table B-15. Mean Log (Base 10) Reduction for Decontaminated Coupons for Each Decontamination Scenario with 95 Percent Confidence Intervals. (Continued)

Strain	Material	Simulated Subway Grime	Temperature	Relative Humidity	CT	N	Number of Samples that were Complete Kill	Mean Log10 Reduction (95% Confidence Interval
B. anthracis Ames	Painted Carbon Steel	No	10	75	7	5	5	7.90 (--, --)
B. anthracis Ames	Painted Carbon Steel	Yes	4.5	50	9	5	5	7.98 (--, --)
B. anthracis Ames	Painted Carbon Steel	Yes	4.5	75	7	5	5	7.88 (--, --)
B. anthracis Ames	Painted Carbon Steel	Yes	10	50	7	5	5	7.96 (--, --)
B. anthracis Ames	Painted Carbon Steel	Yes	10	75	4	5	3	6.68 (5.29, 8.06)
B. anthracis Ames	Painted Carbon Steel	Yes	10	75	5	5	5	7.89 (--, --)

-- Confidence interval could not be calculated because all sample were a complete kill.

Table B-15. Mean Log (Base 10) Reduction for Decontaminated Coupons for Each Decontamination Scenario with 95 Percent Confidence Intervals. (Continued)

Strain	Material	Simulated Subway Grime	Temperature	Relative Humidity	CT	N	Number of Samples that were Complete Kill	Mean Log10 Reduction (95% Confidence Interval
B. anthracis Ames	Weathered Concrete	No	10	75	2	5	0	2.27 (1.88, 2.66)
B. anthracis Ames	Weathered Concrete	No	10	75	3	5	0	4.30 (3.06, 5.54)
B. anthracis Ames	Weathered Concrete	No	10	75	4	5	5	7.95 (--, --)
B. anthracis Ames	Weathered Concrete	No	10	75	7	5	5	7.90 (--, --)
B. anthracis Ames	Weathered Concrete	Yes	4.5	50	9	5	5	7.68 (6.83, 8.52)
B. anthracis Ames	Weathered Concrete	Yes	4.5	75	7	5	5	7.88 (--, --)
B. anthracis Ames	Weathered Concrete	Yes	10	50	7	5	5	7.96 (--, --)
B. anthracis Ames	Weathered Concrete	Yes	10	75	4	5	3	5.86 (4.08, 7.64)
B. anthracis Ames	Weathered Concrete	Yes	10	75	5	5	5	7.89 (--, --)
B. anthracis Sterne	Ceramic Tile	Yes	10	75	5	5	5	7.79 (--, --)
B. anthracis Sterne	Granite	Yes	10	75	5	5	5	7.79 (--, --)
B. anthracis Sterne	Painted Carbon Steel	Yes	10	75	5	5	1	5.28 (4.46, 6.11)
B. anthracis Sterne	Weathered Concrete	Yes	10	75	5	5	5	7.79 (--, --)

-- Confidence interval could not be calculated because all sample were a complete kill.

Table B-16. Log-reduction ANOVA Summary Table for Testing Main Effects of Material, Simulated Subway Grime, Temperature, Relative Humidity, and CT for Decontaminated Coupons.

Source	Degrees of Freedom	Sum of Squares	F Statistic	P-value
Material	3	22.05	14.02	<0.001*
Coat	1	28.30	53.97	<0.001*
Temperature	1	13.83	26.38	<0.001*
RH	1	5.84	11.14	0.001*
CT	5	518.62	197.85	<0.001*
Residual Error	168	88.08		

* Effect is statistically significant at $\alpha = 0.05$ level.

Table B-17. Log-reduction Tukey Comparisons[#] for Materials for Decontaminated Coupons.

	Granite	Painted Carbon Steel	Weathered Concrete
Ceramic Tile	-0.85*	-0.86*	-0.66*
Granite		-0.01	0.19
Painted Carbon Steel			0.2

* Differences are statistically significantly at $\alpha = 0.05$ level.

Positive values indicate the row level results in greater log-reduction than the column level; negative values indicate the column level results in greater log-reduction.

Table B-18. Log-reduction Least Squares Means for Simulated Subway Grime for Decontaminated Coupons.

Simulated Subway Grime	Mean	Standard Deviation
No	7.74	0.28
Yes	6.06	0.13

Table B-19. Log-reduction Least Squares Means for Temperature for Decontaminated Coupons.

Temperature	Mean	Standard Deviation
10	6.07	0.09
4.5	7.73	0.33

Table B-20. Log-reduction Least Squares Means for Relative Humidity for Decontaminated Coupons.

Relative Humidity	Mean	Standard Deviation
50	7.44	0.33
75	6.36	0.09

Table B-21. Log-reduction Tukey Comparisons[#] for CT for Decontaminated Coupons.

Time (days)	3	4	5	7	9
2	-2.16*	-5.81*	-7.43*	-5.76*	-4.24*
3		-3.65*	-5.27*	-3.6*	-2.08*
4			-1.62*	0.05	1.57
5				1.67*	3.19*
7					1.52*

* Differences are statistically significantly at $\alpha = 0.05$ level.

Positive values indicate the row level results in greater log-reduction than the column level; negative values indicate the column level results in greater log-reduction.

Table B-22. Log-reduction ANOVA Summary Table Testing the Effect for *B. anthracis* Strain and Material for Decontaminated Coupons.

Source	Degrees of Freedom	Sum of Squares	F Statistic	P-value
B. anthracis Strain	1	5.28	95.24	<0.001*
Material	3	11.73	70.56	<0.001*
B. anthracis Strain*Material Interaction	3	11.73	70.56	<0.001*
Residual Error	32	1.77		

* Effect is statistically significant at α = 0.05 level.

Table B-23. Log-reduction Tukey Comparisons[#] for Material for *B. anthracis* Ames Decontaminated Coupons.

Material	Granite	Painted Carbon Steel	Weathered Concrete
Ceramic Tile	0.00	0.00	0.00
Granite		0.00	0.00
Painted Carbon Steel			0.00

* Differences are statistically significantly at α = 0.05 level.

Positive values indicate the row level results in greater log-reduction than the column level; negative values indicate the column level results in greater log-reduction.

Table B-24. Log-reduction Tukey Comparisons[#] for Material for *B. anthracis* Sterne Decontaminated Coupons.

Material	Granite	Painted Carbon Steel	Weathered Concrete
Ceramic Tile	0.00	2.50*	0.00
Granite		2.50*	0
Painted Carbon Steel			-2.50*

Positive values indicate the row level results in greater log-reduction than the column level; negative values indicate the column level results in greater log-reduction.

*Differences are statistically significantly at α = 0.05 level.

Table B-25. Log-reduction Tukey Comparisons for *B. anthracis* Strain by Material for Decontaminated Coupons.

Material	Difference[#]
Ceramic Tile	0.10
Granite	0.10
Painted Carbon Steel	2.60*
Weathered Concrete	0.10

Positive values indicate *B. anthracis* Ames results in greater log-reduction than *B. anthracis* Sterne; negative values indicate *B. anthracis* Sterne results in greater log-reduction.

* Difference is statistically significantly at α = 0.05 level.

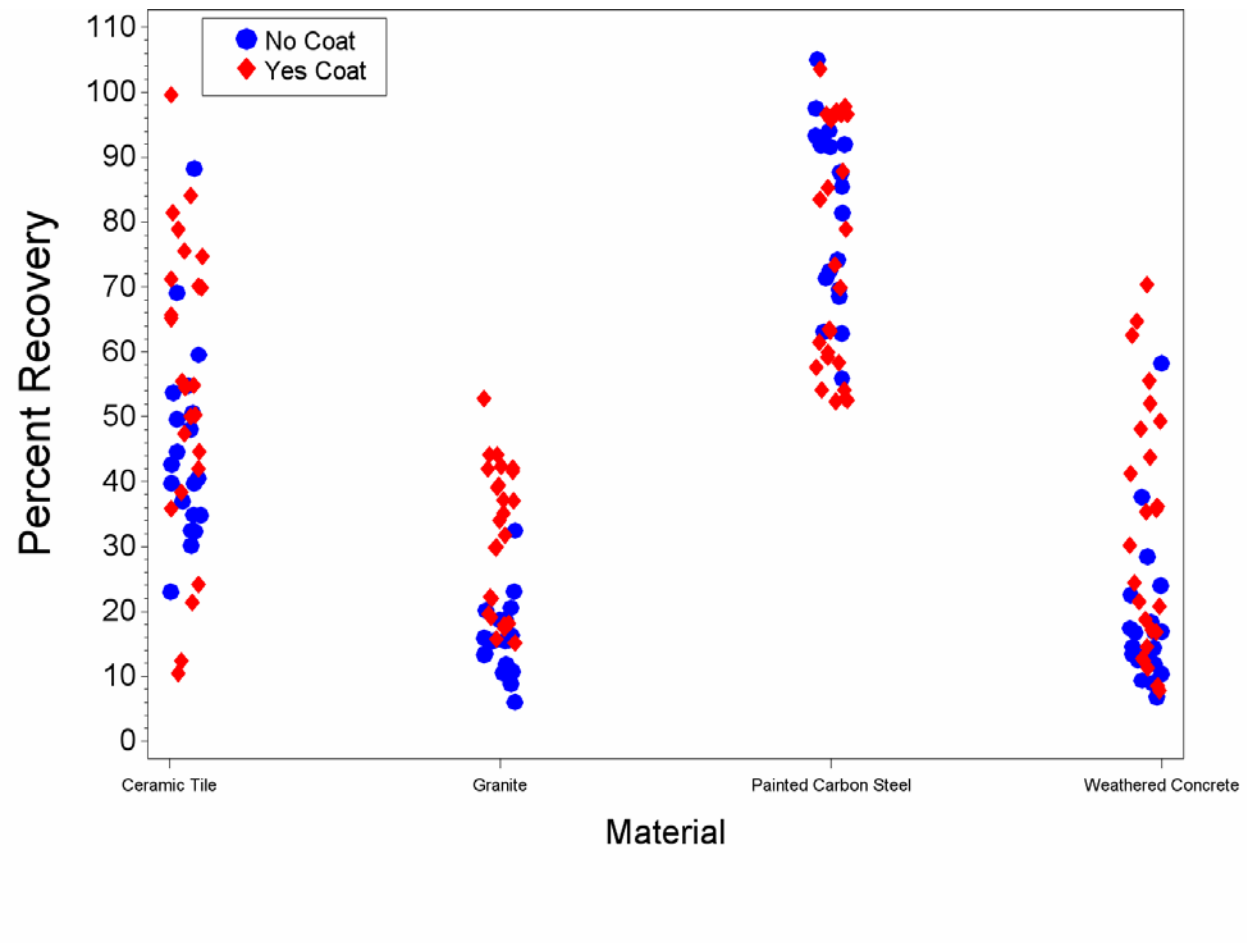

Figure B-1. Plot of Control Coupon Percent Recovery of Inoculum by Material and Simulated Subway Grime for B. anthracis Ames. Note That Percent Recovery Values Greater than 200% Are Not Included in the Plot.

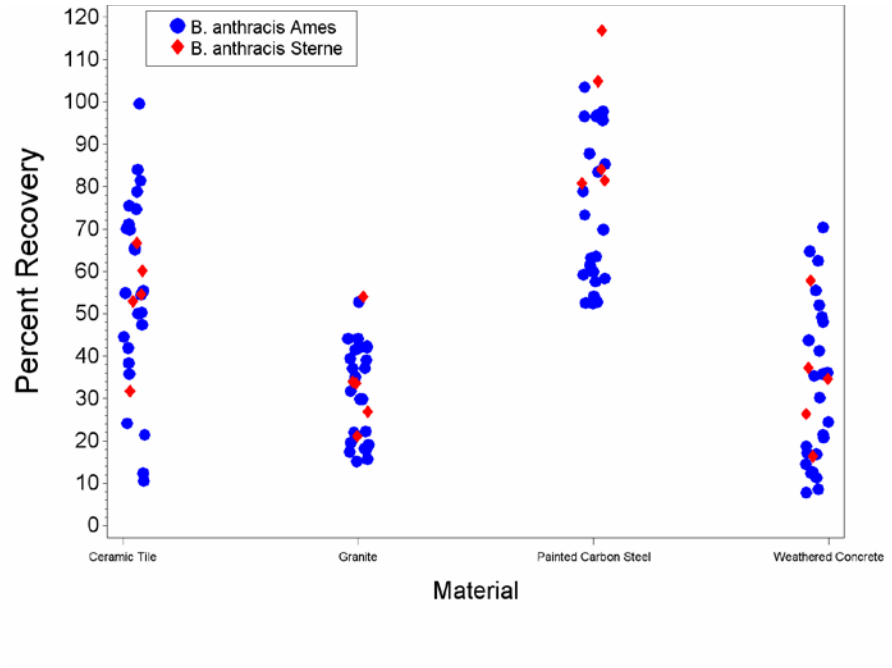

Figure B-2. Plot of Control Coupon Percent Recovery of Inoculum by Material and Strain. Note That Percent Recovery Values Greater than 200% Are Not Included in the Plot.

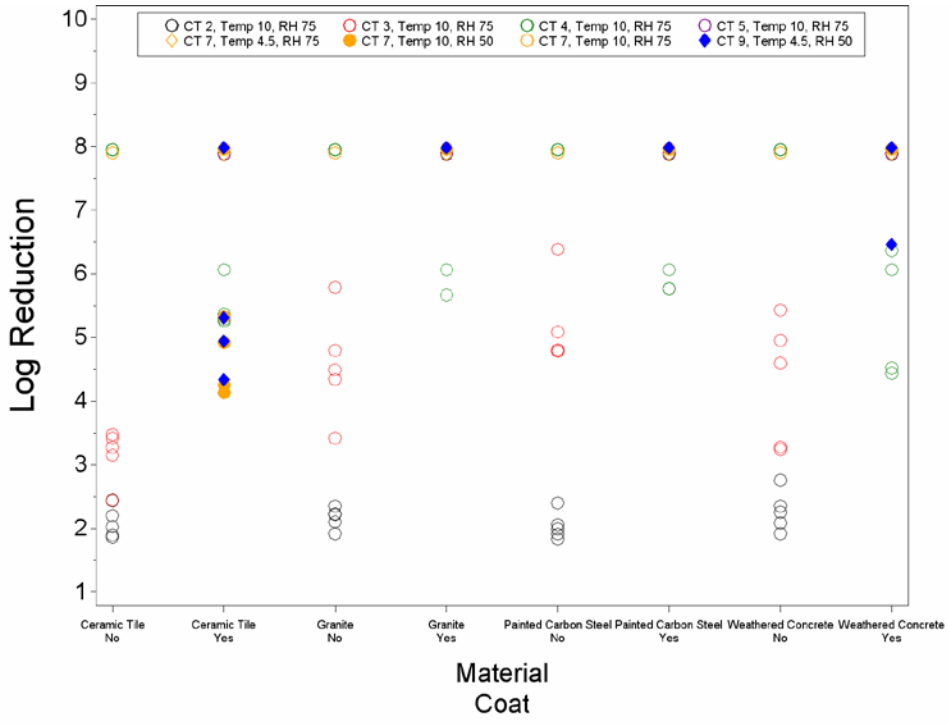

Figure B-3. Plot of Decontaminated Coupon Log (Base 10) Reduction of Inoculum by Material, Simulated Subway Grime, Temperature, Relative Humidity, and CT.

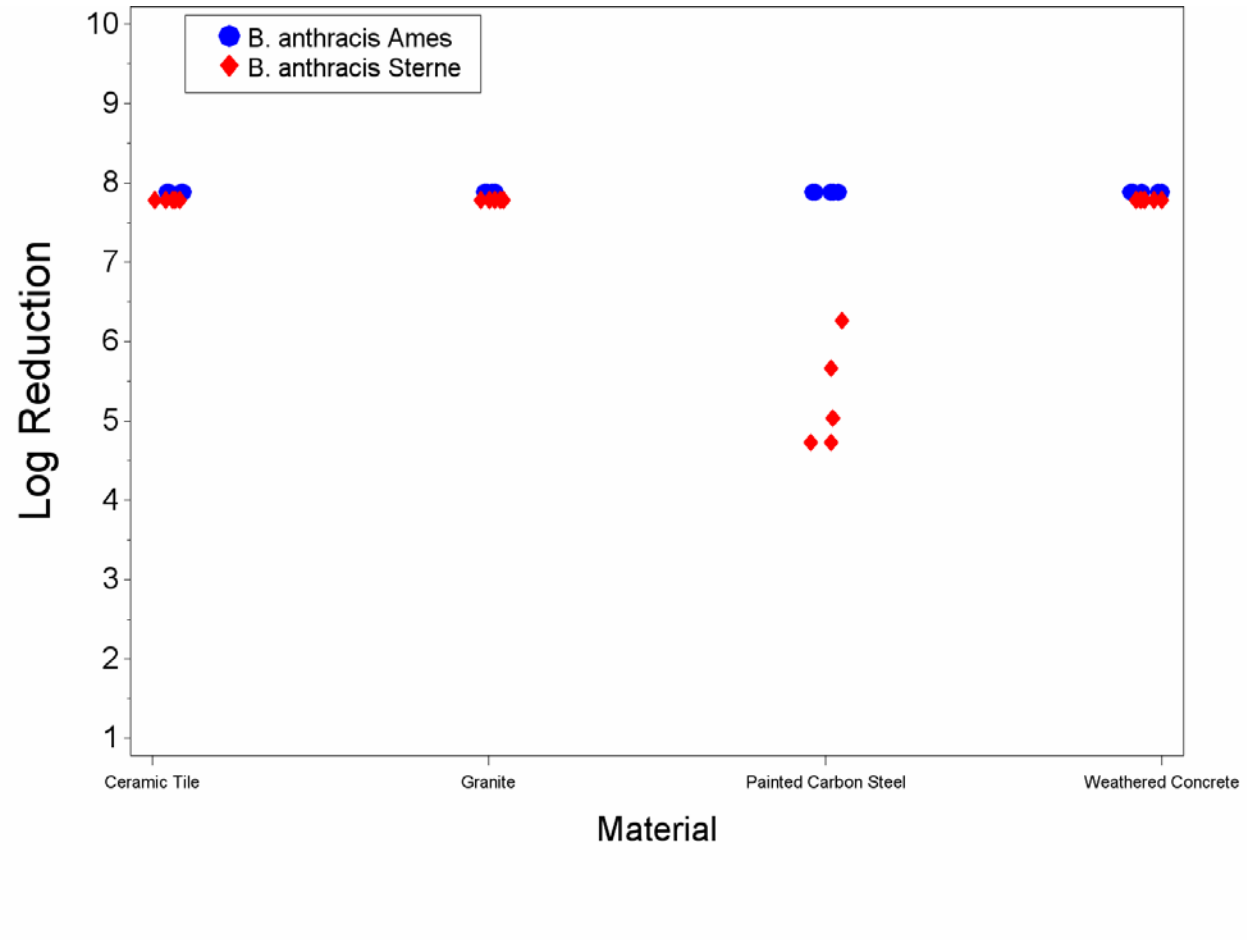

Figure B-4. Plot of Decontaminated Coupon Log (Base 10) Reduction of Inoculum by Material and Strain.

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