Effectiveness of Outdoor Environment Decontamination for Biological Agents

Contamination of outdoor environments could result from intentional or accidental releases of biological materials or human or animal disease outbreaks. Outdoor contamination incidents pose significant challenges in determining the extent of contamination (sampling and analysis), containing the contaminant spread (mitigation), and remediating the areas so that re-occupancy or reuse can occur.

Currently, few methods are well-characterized, efficacious, and readily-available for outdoor decontamination; especially for application over large areas. Further, the range of possible environmental conditions (e.g., temperature, humidity, precipitation, wind) in the outdoor environment is typically much greater than that indoors. Extreme temperatures and the presence of natural, organic-rich materials in outdoor environments are examples that are known to challenge typical (physical and chemical) decontamination processes. For example, low temperatures can cause liquid-based decontaminants to freeze and become ineffective. The presence of organic-rich grime can neutralize the oxidative potential of many chemical-based decontaminants. Understanding the potential challenges to outdoor decontamination and developing effective solutions to overcome those challenges is critical for development of wide-area response capabilities. Two recent research studies have begun to address the knowledge and capability gaps associated with conducting decontamination for biological agents in outdoor environments and challenging settings [1, 2].

The first study aimed to assess the effectiveness of spray-based decontamination methods for inactivating *Bacillus atrophaeus* (surrogate for *B. anthracis*) spores and bacteriophage MS2 (surrogate for foot and mouth disease virus) on neat or heavily soiled concrete and treated plywood) (Figure 1) [2]. Decontamination efficacy was assessed for three different decontamination solutions; pH-amended Bleach (pAB) and Spor-Klenz® Ready-to Use (RTU) were evaluated against *B. atrophaeus* spores, and 2 percent (%) weight/volume (w/v) citric acid in sterilized deionized (DI) water and pAB were evaluated against MS2. Three application methods (handheld sprayer, backpack sprayer, and a chemical sprayer) were utilized to deliver decontaminants to the test surfaces. The evaluation was conducted on two test material surfaces (concrete and treated plywood), with and without agricultural grime. The handheld application method was conducted using a bench-scale test spray apparatus to evaluate the pAB and citric acid spray-based decontamination methods for 18-millimeter (mm) coupons (both grimed and neat) contaminated with MS2. The backpack and the chemical sprayer application methods were conducted on a larger scale (14-inch by 14-inch coupons) to better simulate field operations and were evaluated for both MS2 and *B. atrophaeus*. For all tests, a wetted surface contact time of 30 minutes was administered, followed by a surface rinse with water. The fate of the microorganisms in the runoff generated during the decontamination procedure and in the subsequent rinse step, as well as their potential re-aerosolization in the air, were also investigated.

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Figure 1. Photo of Model Agricultural Grime Application to Concrete Test Coupons.

Decontamination tests with *B. atrophaeus* spores indicated that higher efficacies were achieved on neat materials than on grimed materials, independent of the type of material or application method (Table 1). pAB was found to be more effective than Spor-Klenz® RTU for decontaminating neat concrete materials, while the latter decontaminant was more efficacious for neat plywood materials independent of application method (backpack sprayer versus chemical sprayer). Viable spore levels found in rinsate samples were higher for the backpack sprayer tests than for the chemical sprayer tests, potentially because the chemical sprayer was more effective at physically removing spores before the rinse step. Relatively high re-aerosolization of spores (greater than 1×10^3 colony forming units [CFU] per test) was observed during some tests with both the backpack and chemical sprayers.

Decontamination tests with MS2 indicated that 2% citric acid was not efficacious on concrete and plywood (Tables 2 and 3). However, pAB was found to be efficacious against MS2, with full decontamination on neat or grimed concrete and limited efficacy for neat or grimed plywood. Further, few viable viruses were detected in the runoff from pAB tests, unlike for the 2% citric acid formulation, which had almost complete wash-off (and recovery) of viable viruses from all coupon types. Finally, no viable MS2 re-aerosolization was observed in any of the conducted tests, independent of the type of decontamination solution used. However, it should be noted that the Via-Cell® bio-aerosol cassette sampling method, used in this study, was not validated for MS2 sampling or recovery. A summary of the decontamination results is shown in Tables 1 - 3.

Table 1. Decontamination Results for Large Coupon (Lab-Scale) Tests with *Bacillus atrophaeus*

Test	Decontamination Application Method	Material Type	Decontamination Liquid	Coupon Condition	Positive Controls (CFU)		Test Coupons		LR (CFU)	
					Average	STD	Average	STD	Average	STD
1	Backpack sprayer	Concrete	pAB	Neat	1.63E+07	1.67E+06	ND	-	7.3	0.02
2				Grimed	1.02E+06	1.77E+05	1.24E+03	8.78E+02	3.0	0.36
3	Backpack sprayer	Treated plywood	pAB	Neat	2.92E+06	1.08E+06	1.99E+02	3.65E+01	6.6	0.90
4				Grimed	6.46E+05[1]	3.01E+05	6.36E+02	5.99E+02	3.3	0.64
5	Backpack sprayer	Concrete	Spor-Klenz® RTU	Neat	7.21E+06	3.72E+06	2.67E+02	2.03E+02	4.6	0.62
6				Grimed	1.24E+04	1.51E+03	1.01E+02	9.22E+01	2.4	0.66
7	Backpack sprayer	Treated plywood	Spor-Klenz® RTU	Neat	1.59E+07	7.09E+06	ND	-	7.4	0.01
8				Grimed	1.27E+06	5.26E+05	1.88E+03	2.20E+03	3.1	0.53
9	Chemical sprayer	Concrete	pAB	Neat	2.01E+06	1.46E+06	ND	ND	6.4	0.01
10				Grimed	1.66E+05[1,2]	1.44E+05	4.65E+02	4.03E+02	3.5	0.52
11	Chemical sprayer	Treated plywood	pAB	Neat	6.73E+06	2.72E+06	1.27E+00	9.33E-01	6.8	0.27
12				Grimed	4.29E+05[1]	2.05E+05	1.96E+02	3.40E+02	3.9	0.79
13	Chemical sprayer	Concrete	Spor-Klenz® RTU	Neat	4.94E+04[1]	2.39E+04	5.10E+02	3.33E+02	2.5	1.31
14				Grimed	1.51E+06	2.80E+05	3.60E+01	3.78E+01	4.8	0.43
15	Chemical sprayer	Treated plywood	Spor-Klenz® RTU	Neat	9.58E+06	3.09E+05	ND	-	7.1	0.14
16				Grimed	Samples were exposed to exccess heat during heat shock process					

CFU – colony forming unit; LR – log reduction; STD – standard deviation
[1]Positive control recoveries below 6 logs, prevent achievement of 6 LR
[2]Some replicates were too contaminated to enumerate.

Table 2. Decontamination Results for Small Coupon (Bench-Scale) Tests with MS2

Decon Agent	Material	Positive Control PFU		Test Coupon PFU		Surface Decontamination Efficacy (LR)	
		Average	STD	Average	STD	Average	Cumulative STD
pAB	Neat concrete	6.77E+06	2.68E+06	ND	-	7.1	0.12
	Grimed concrete	2.99E+07	2.59E+07	2.83E+05	6.34E+05	6.4	1.3
	Neat plywood	1.37E+08	7.97E+07	4.54E+05	1.46E+05	2.4	0.19
	Grimed plywood	4.91E+07	7.36E+07	8.57E+05	9.86E+05	3.7	1.7
2% Citric acid	Neat concrete	3.68E+07	1.24E+07	1.39E+07	7.93E+06	0.46	0.15
	Grimed concrete	6.17E+07	1.03E+08	4.99E+06	4.21E+06	1.1	1.1
	Neat plywood	6.21E+07	1.12E+07	3.52E+04	3.83E+04	3.5	0.25
	Grimed plywood	6.35E+07	8.05E+07	7.88E+07	6.96E+07	0.08	0.56

LR – log reduction; PFU – plaque forming unit; STD – standard deviation

Table 3. Decontamination Results for Large Coupon (Lab-Scale) Tests with MS2

Decon Agent	Material	Positive Coupon (PFU)		Test Coupon (PFU)		Surface Decontamination Efficacy (LR)	
		Average	STD	Average	STD	Average	Cumulative STD
pAB	Neat Concrete	2.46E+04	6.61E+03	ND	-	4.7	0.06
	Grimed Concrete	1.54E+06	2.65E+05	ND	-	6.2	0.04
	Neat Plywood	3.64E+06	-	9.78E+01	4.44E+01	4.8	0.33
	Grimed Plywood	4.70E+06	4.71E+04	ND	-	7.0	0.00
2% Citric Acid	Neat Concrete	6.20E+03	6.74E+03	2.89E+03	1.98E+03	0.20	0.36
	Grimed Concrete	8.36E+05	3.26E+05	1.15E+02	1.06E+02	4.3	0.35

LR – log reduction; PFU – plaque forming unit; STD – standard deviation

The second study sought to determine the efficacy of spray-applied bleach decontamination formulations, specifically formulated to remain liquid at low temperatures (i.e., below the freezing point for water) [1]. These non-freezing bleach formulations (NFB) could be beneficial when conducting remediation activities during cold weather conditions. The materials utilized during testing were glass and concrete, surface types common to building exteriors in outdoor environments.

The tests were conducted in an environmental test chamber (ETC) so that temperature conditions ranging from -25 °C to 25 °C could be precisely achieved (Figure 2). An automated spray system, completely contained within the environmental chamber was developed. The use of this setup allowed easy control of test parameters (i.e., spray duration, spray pressure, volume of spray, temperature and relative humidity), and allowed a more realistic challenge to the decontamination method as all components (spray nozzles, spray reservoir, hoses, etc.) were located inside the chamber and at the test temperature.

Eight non-freezing bleach-based formulations were prepared from recipes provided by the EPA-Environment and Climate Change Canada (ECCC) working group. Each solution was evaluated for its ability to inactivate *Bacillus atrophaeus* spores on building material (concrete and glass) surfaces. The solutions contained de-icing agents that depressed the freezing point of the solutions below the target test temperatures so that the solutions could be spray-applied. In addition to the NFB solutions, traditional pH-amended bleach (pAB) solution was included in the evaluations (when temperatures permitted) as a reference decontamination agent.

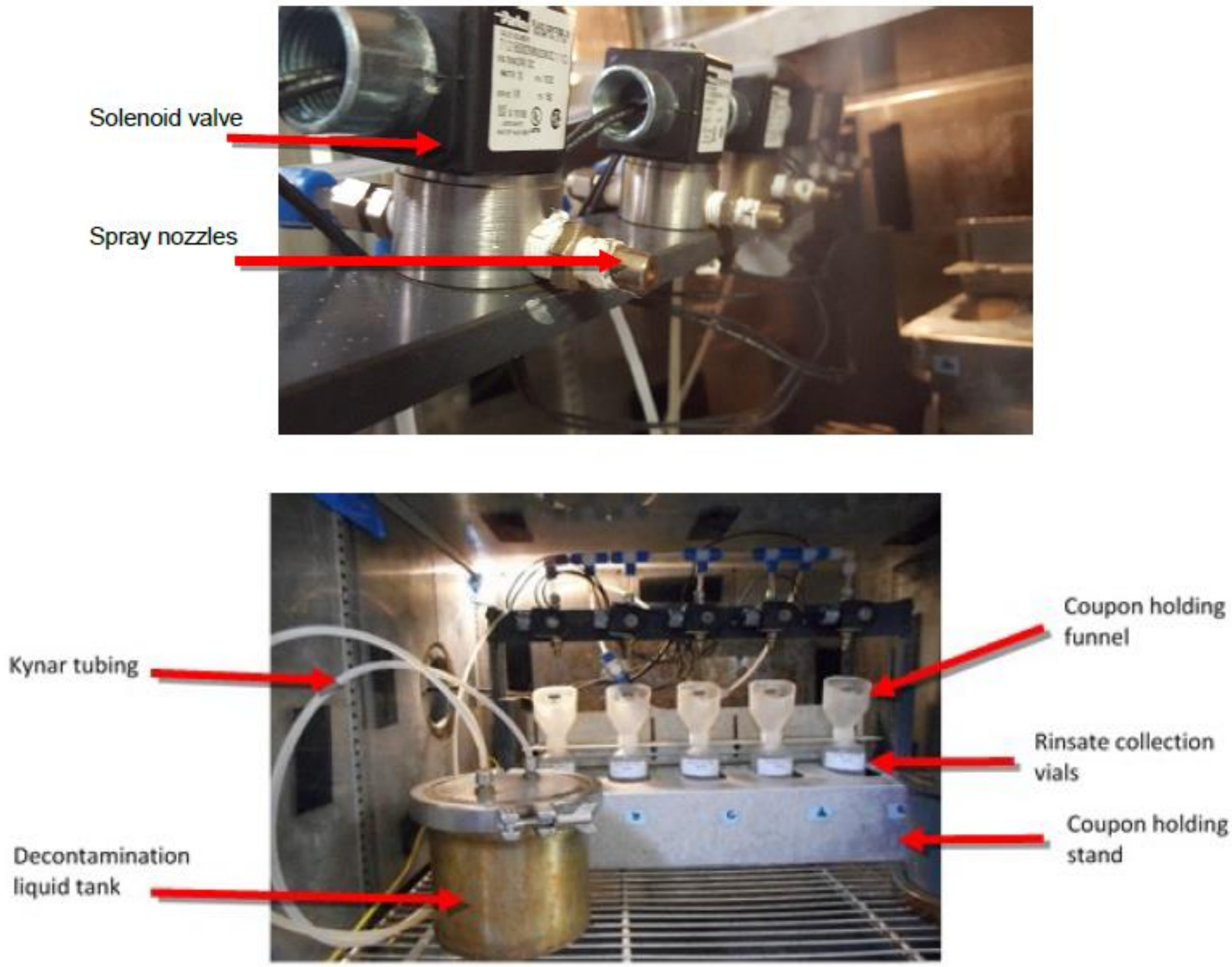
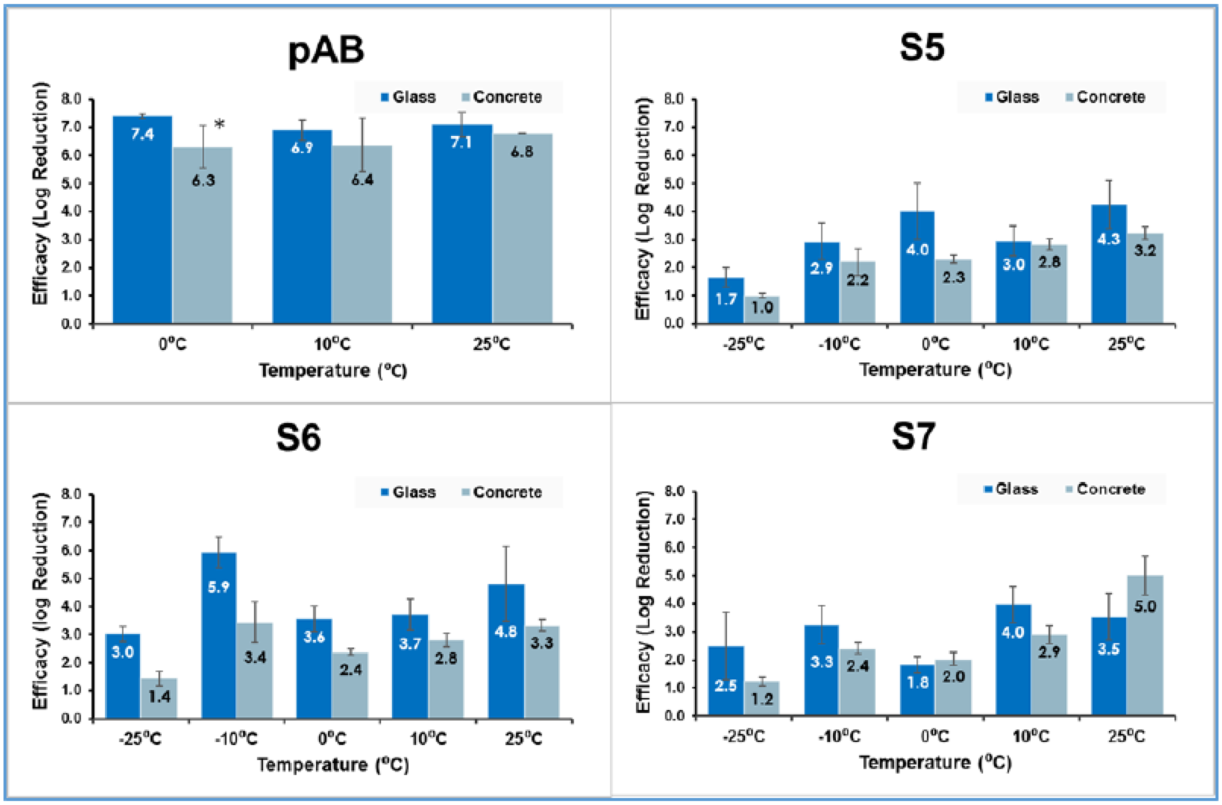


Figure 2. Photographs of the Spray Apparatus Inside the Temperature-Controlled Environmental Test Chamber.

Figure 3 summarizes the surface decontamination efficacy results for pAB and three of the most efficacious NFB formulations. As the figure shows, pAB achieved a surface LR greater than 6 at temperatures greater than 0 °C on both materials tested. None of the NFB formulations were as effective as pAB. As the testing temperature was lowered, decontamination efficacy also tended to decrease. However, at temperatures greater than 0 ºC, no test solutions were as effective as pAB, and none were observed to achieve a 6 LR (Figure 3). Decontamination efficacy data for the pAB solution were gathered only to 0 °C because the freezing point of pAB was determined to be -8 °C. Despite the NFB solutions demonstrating lower decontamination efficacies compared to pAB, these solutions currently are the only NFB decontaminants evaluated against *Bacillus* spores. At conditions below -8 °C, these solutions may be useful in reducing surface-bound spore concentrations during remediation efforts. The results from this project provide an important baseline that further work can build upon to develop and characterize new decontamination options under environmentally-challenging conditions such as freezing temperatures.



*Denotes Full Surface Decontamination Based on Detection Limit

Figure 3. Surface Decontamination Efficacy (Log Reduction) for pH-Amended Bleach and three non-freezing Decontamination Solutions (S5, S6, and S7).

In summary, many factors can influence decontamination efficacy in outdoor environments. Currently, our grasp of decontamination capabilities is lacking for outdoor areas. Further work is needed to determine impacts of weather (rain, wind, snow, humidity, extreme temperatures) and surface/matrix types on our ability to select viable options for remediation. Also, application of decontamination methods over large areas with readily-available devices, chemicals, supplies, and workers will be challenging and should be addressed prior to an incident. While the current two studies have begun to address questions regarding outdoor decontamination, many more need to be answered in order to develop robust and comprehensive remediation strategies for large, outdoor areas.

Contact Information

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Disclaimer

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1. Calfee, W., L. Mickelsen, S. Serre, R. Rupert, AND M. Nalipinski. Evaluation of Spray-Based, Low-Tech Decontamination Methods under Operationally Challenging Environments: Cold Temperatures. U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-17/211, 2017.

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