Viral Persistence in Landfill Leachate

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Background

- Municipal Solid Waste (MSW landfills) dominate US solid waste disposal.
- Outbreaks of diseases involving CDC Category A Select Agents (e.g., Ebola) produce significantly more medical waste than normal activities
- Biological incidents (e.g., natural outbreaks, terrorist/intentional events, unintentional release) may result in infectious viruses inoculated into US Landfills via the disposal of building materials, non-patient waste, diapers, and other porous waste.
- Vegetative bacteria and spores vary in persistence (weeks to years). Limited information on viral persistence in landfills.

Background

Human and animal waste containing viruses enter into our landfills daily.

- Aerosol-generation, manual sorting and other practices expose workers.
- Viruses, including highly pathogenic avian influenzae (HPAI), persist on glass and galvanized metal surfaces for >13 days depending on environment.[1]
- We still have limited understanding of viral persistence in landfill leachate.

1: Wood et al. ES&T 44(19):7515-7520 (2010)

Past Work

- Previous EPA tests 2015-2016 Testing (Data presented at 2016 Meeting)
  - Persistence in landfill leachate from three landfills in Ohio
  - 3 viral surrogates (RNA viruses)
    - TGEV (Transmissible gastroenteritis virus), ssRNA enveloped mammalian virus
    - MS2, ssRNA non-enveloped bacteriophage
    - Phi6, dsRNA enveloped bacteriophage
  - 2016 data identified that bacteriophages MS2 and Phi6 persists for months (55-113 days) at 12^{o}C and only a few days (0.2-3 days) at 37^{o}C.
  - TGEV persists for between 5-17 days at 12^{o}C
  - Persistence in leachate is variable. Choice of test agents is critical for accurate risk estimate and prediction.

Study Scope

Can infectious viruses pose a threat to environmental and human health once introduced into a landfill?

Current disposal practices of building materials, animal carcasses, non-patient waste, diapers, etc. can result in infectious viral agents being inoculated into US landfills. Residual agents in materials from natural outbreaks, terrorist or intentional events, etc. may result in the dissemination of live biological threat agents.

Approach

- Leachate collected and characterized from three landfills
- Survival of three viral agents in leachate determined under controlled conditions to assess the risk of persistence
- Enveloped and non-enveloped ssRNA and dsRNA viruses used to represent emerging infectious disease (EID) agents of concern

Parameter	Description
RNA Viruses	Zika virus (enveloped ssRNA) Phi 6 (enveloped bacteriophage dsRNA) MS2 (non-enveloped bacteriophage ssRNA)
Landfill Leachate	Three; each from different landfill facilities
Incubation Temperature	12˚C for ZIKV / 37˚C for Phi6 12˚C and 37˚C for MS2
Time Points	ZIKV: 0, 6, 24, 48 and 96 hours Phi6: 0, 2, 4, 8, 24, 30, 56, and 96 hours MS2: 0, 4, 8, 24, 30, 56, 96 and 168 hours (37°C) 0, 3, 7, 14, 21, 28, 42 days (12°C)

Study Landfills

Characteristic	Landfill A	Landfill B	Landfill C
Waste Acceptance Rate	In 2014, accepted approximately 3,200 tons per day	3,500 to 5,000 tons per day, Approximately 1,000,000 tons of waste received in 2014	Average 1,400 tons/day
Footprint	100 acres permitted to accept waste	283 acres permitted to accept waste	168 acres permitted to accept waste
Year Opened	1997	1995	1995
Expected Closure Date	2023 or 2024 (could extend by 25 years via expansion)	2030 to 2045	Information not provided
Gas collection system	Yes	Yes (~190 gas collection wells/points)	No

Leachate Characterization

Chemical and biological characterization in 2015 and 2017

Analyte	Landfill A		Landfill B		Landfill C	
	2015	2017	2015	2017	2015	2017
Metals (milligrams per liter [mg/L])						
Calcium	11.6	45	200	536	312	83.1
Iron	6.36	5.60	17.4	120	31.5	7.87
Magnesium	130	245	84.3	79.5	297	175
Manganese	0.0468	0.0951	0.152	3.78	2.26	0.246
Potassium	468	367	260	151	937	747
Sodium	1,880	1,870	1,500	549	2,360	2,090
Zinc	0.140	0.0699	0.0199	0.259	0.0711	0.255
Anions (mg/L)						
Chloride	2,070	2,280	1,980	533	2,810	2,530
Nitrate-N	4.00	6.40	3.08	1346	<1.00	<0.500
Sulfate	3.19	5.40	10.1	168	33.0	58.6
Total Alkalinity as $CaCO_3$ (mg/L)						
Total Alkalinity	6,100	5,120	2,600	2,020	8,040	8,450
Ammonia as Nitrogen (mg/L)						
Ammonia	1,050	828	386	298	1,370	1,550
Oxygen Demand (mg/L)						
COD	1,500	1,880	2,470	3,270	9,060	1,290
BOD	187	421	2,020	2,070	2,350	198
pH (Standard Units), Oxidation Reduction Potential (millivolts) and Temperature (degrees Celsius)						
pH (field)[1]	7.88	ND	7.14	ND	7.36	ND
pH (lab)[2]	7.76	7.95	7.06	6.57	7.55	7.95
ORP (field)[1]	47.4	ND	-60.7	ND	-96.8	ND
Temperature (field)[1]	21.8	ND	25.0	ND	20.0	ND
Total Dissolved Solids, Total Organic Carbon, Total Suspended Solids (all in mg/L)						
TDS	6,680	8,440	5,980	5,000	13,500	9,420
TOC	448	632	796	1,330	2,960	500
TSS	12.3	69.0	82.0	122	72.0	26.4
Visual Observations						
Color	yellow	dark gray/black	brown	light gray	dark brown	golden brown
Particulates	N/A	N/A	Present	N/A	present	N/A
Microbial Enumeration via Standard Plate Count (CFU/mL)						
Bacterial load[3]	3×10^6	6.07×10^7	9×10^5	1.35×10^7	8×10^5	2.69×10^6
Fungal Load[3]	3×10^2	1.64×10^4	8×10^4	7.60×10^6	9×10^3	5.73×10^4

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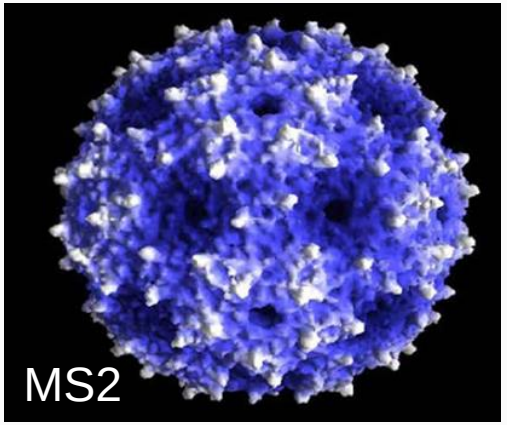
Red – decrease from 2015
Green – increase from 2015

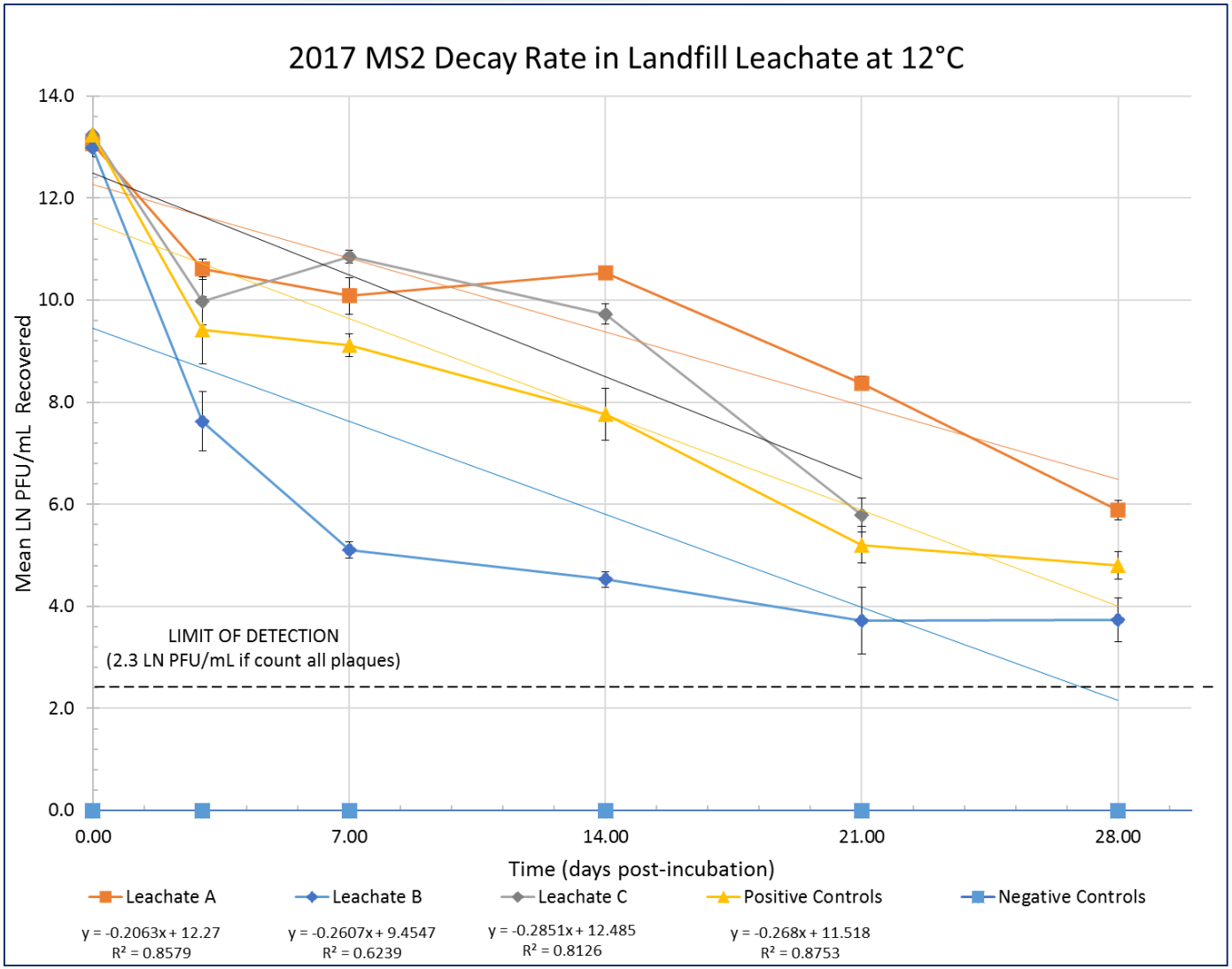
Viral Persistence (MS2, ssRNA, non-enveloped)

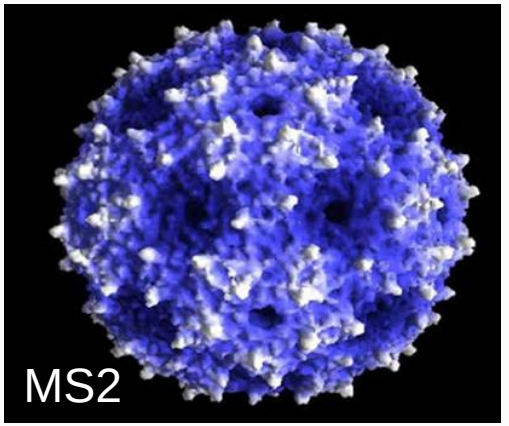
Matrix	12°C[a]			37°C[c]		
	Slope	D-Value (days)	Persistence[b] (days)	Slope	D-Value (days)	Persistence[b] (days)
Leachate A	-0.206	4.85	48.3	-0.253	0.16	1.78
Leachate B	-0.261	3.84	27.4	-0.203	0.21	2.21
Leachate C	-0.285	3.51	35.7	-0.231	0.18	2.03
PBS	-0.268	3.73	34.4	-0.014	3.0	31.31

[a]D-values and Persistence calculated from all positive values: leachate A, B, and positive samples analyzed from timepoints T=0, 6 hours, and 3, 7, 14, 21 and 28 days post incubation; leachate C samples analyzed from timepoints T=0, 6 hours, 3, 7, 14 and 21 days post-incubation.
[b]Calculated time in days at which measured linear decay rate intersects with assay limit of detection.
[c]D-values and Persistence calculated from all positive values: all samples analyzed from timepoints T=0, 4, 8, 24 and 30 hours post incubation.



Viral Persistence (MS2, ssRNA, non-enveloped)





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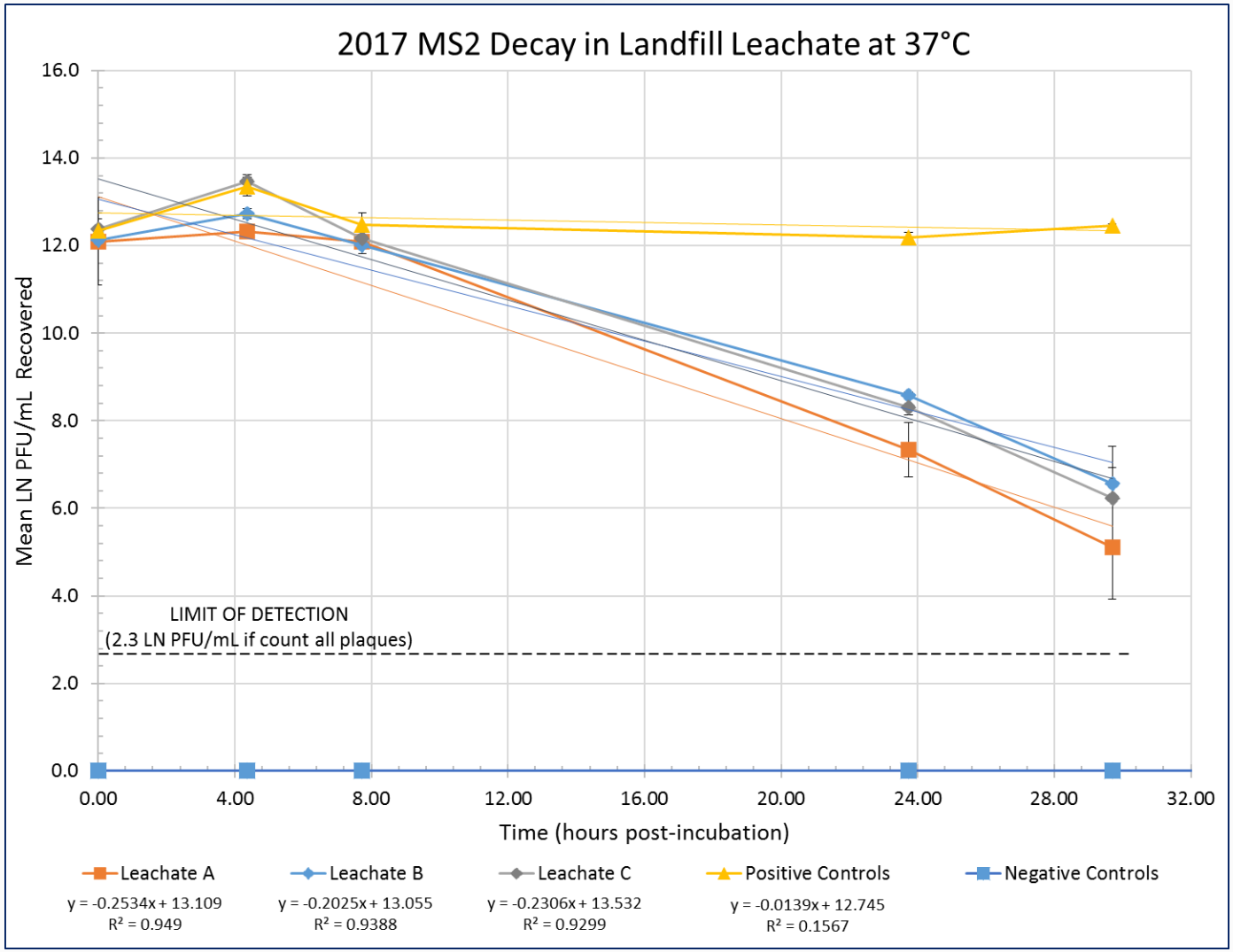

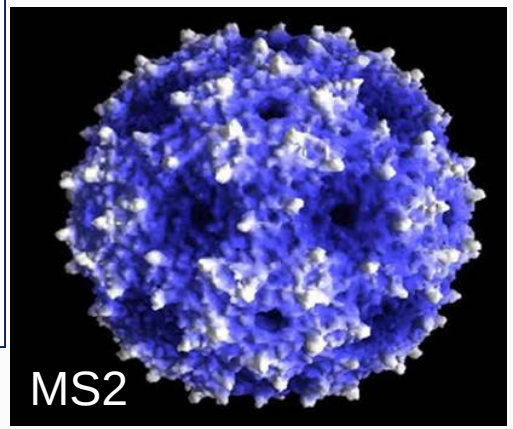

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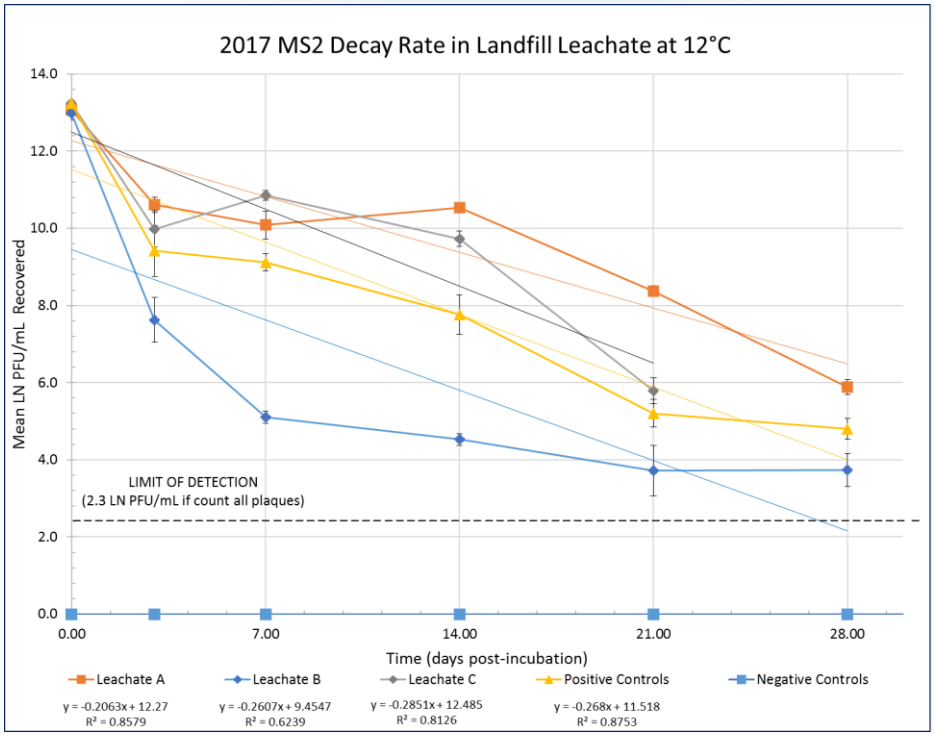
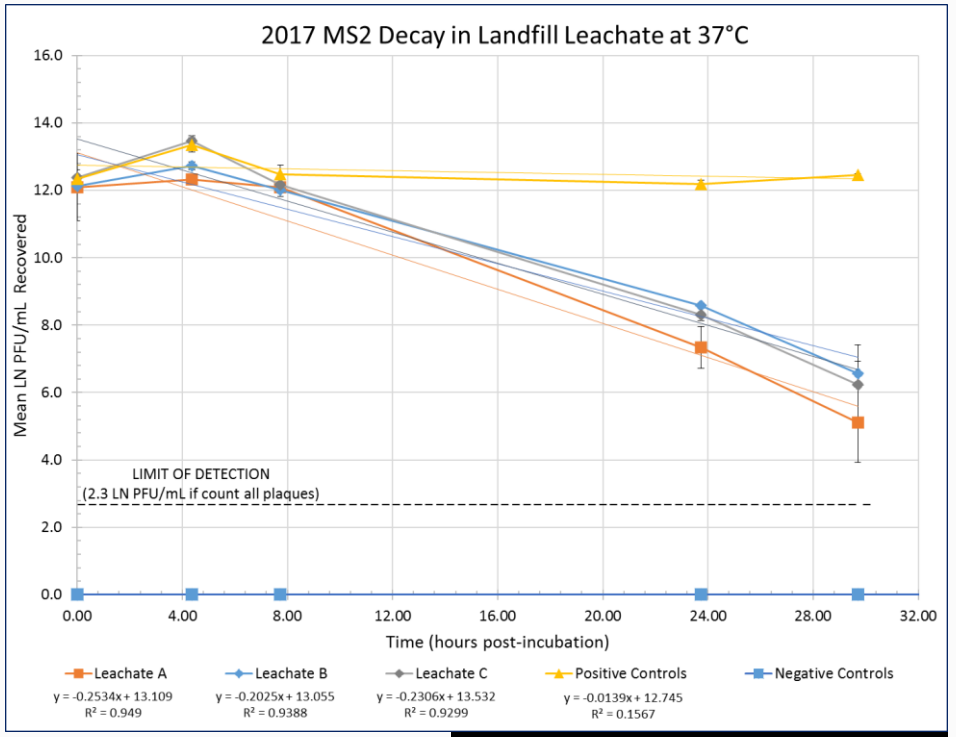
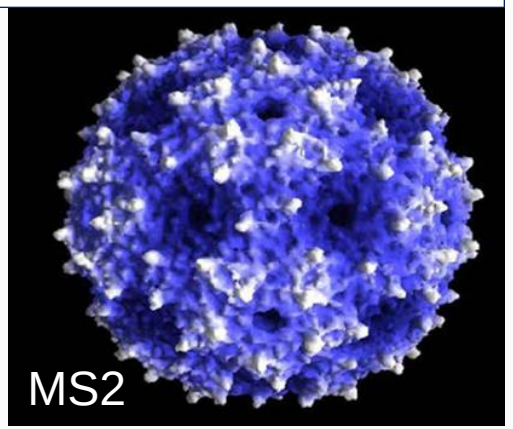






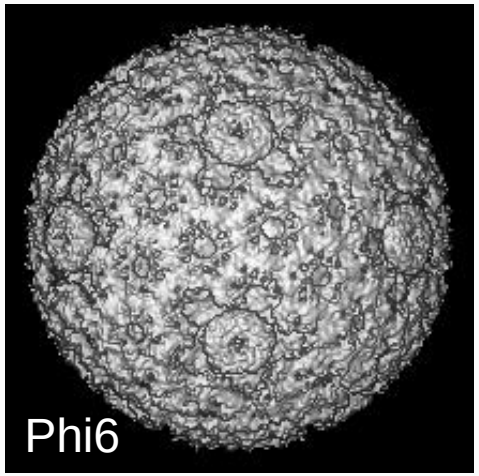
Viral Persistence (Phi6, dsRNA, enveloped)

Matrix	Slope	D-Value (days / hours)	Persistence[b] (days / hours)
Leachate A	-1.1356	0.04 / 0.96	0.46 / 11.2
Leachate B	NR[c]	NR[c]	NR[c]
Leachate C	-1.2573	0.03 / 0.72	0.43 / 10.4
PBS	-0.0694	0.60 / 14.41	7.54 / 180.9

D-values and Persistence calculated from all positive values: all samples analyzed from timepoints T=0, 2.3, 4.3, and 8.3 hours post incubation.

[b] Calculated time in days at which measured linear decay rate intersects with assay limit of detection.

[c] NR = Phi6 not recoverable from leachate B, no persistence test performed.



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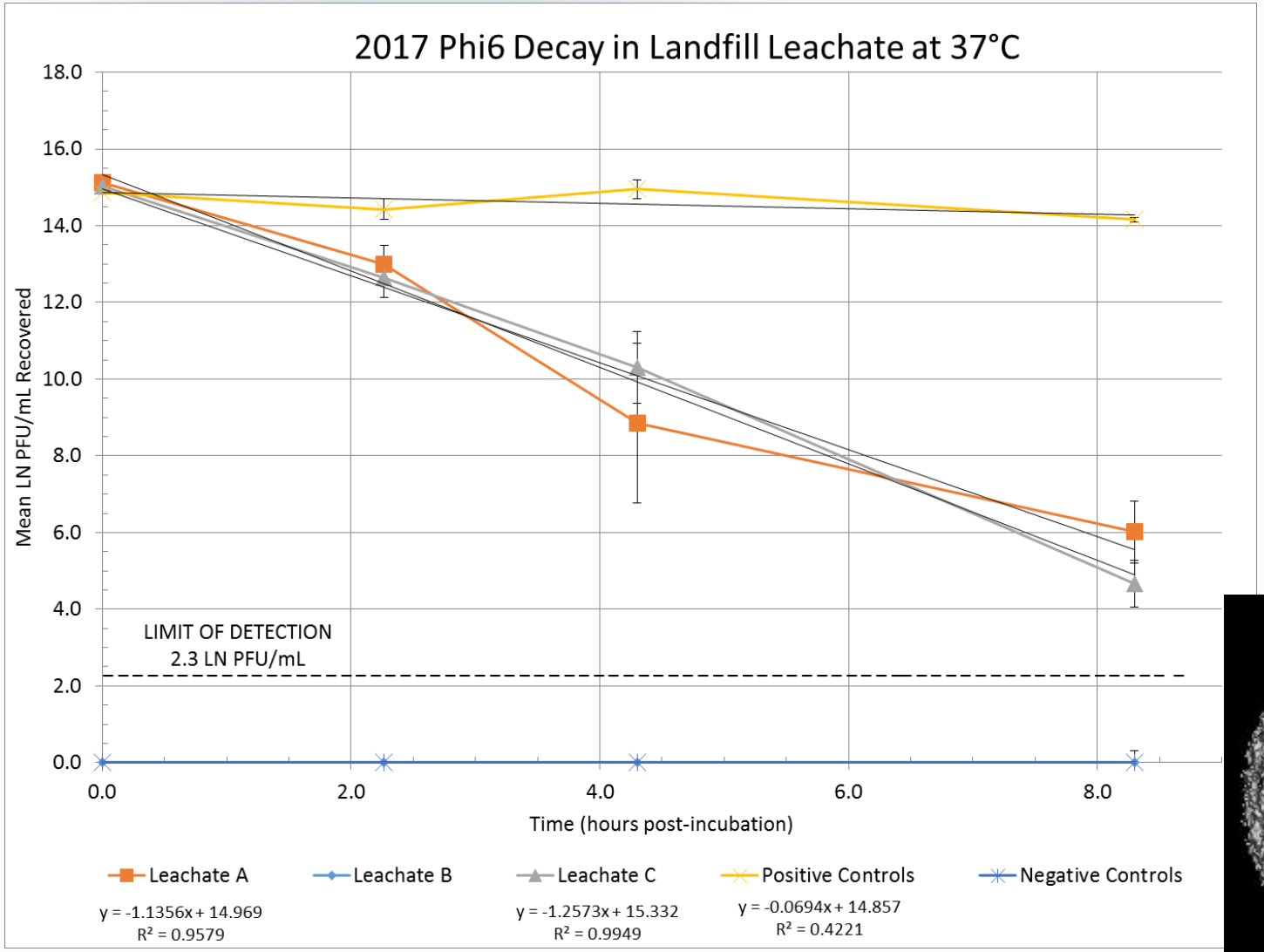
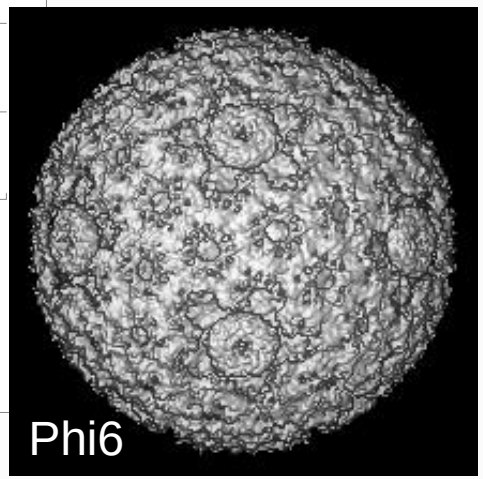
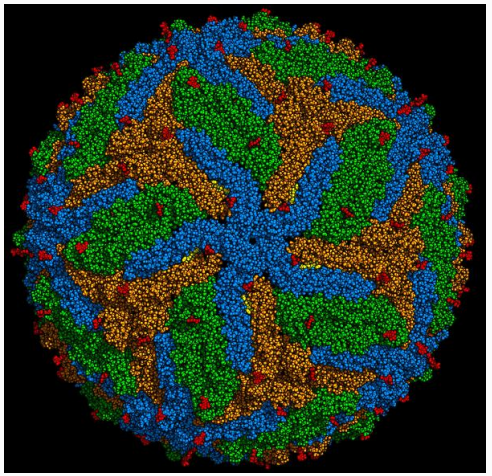




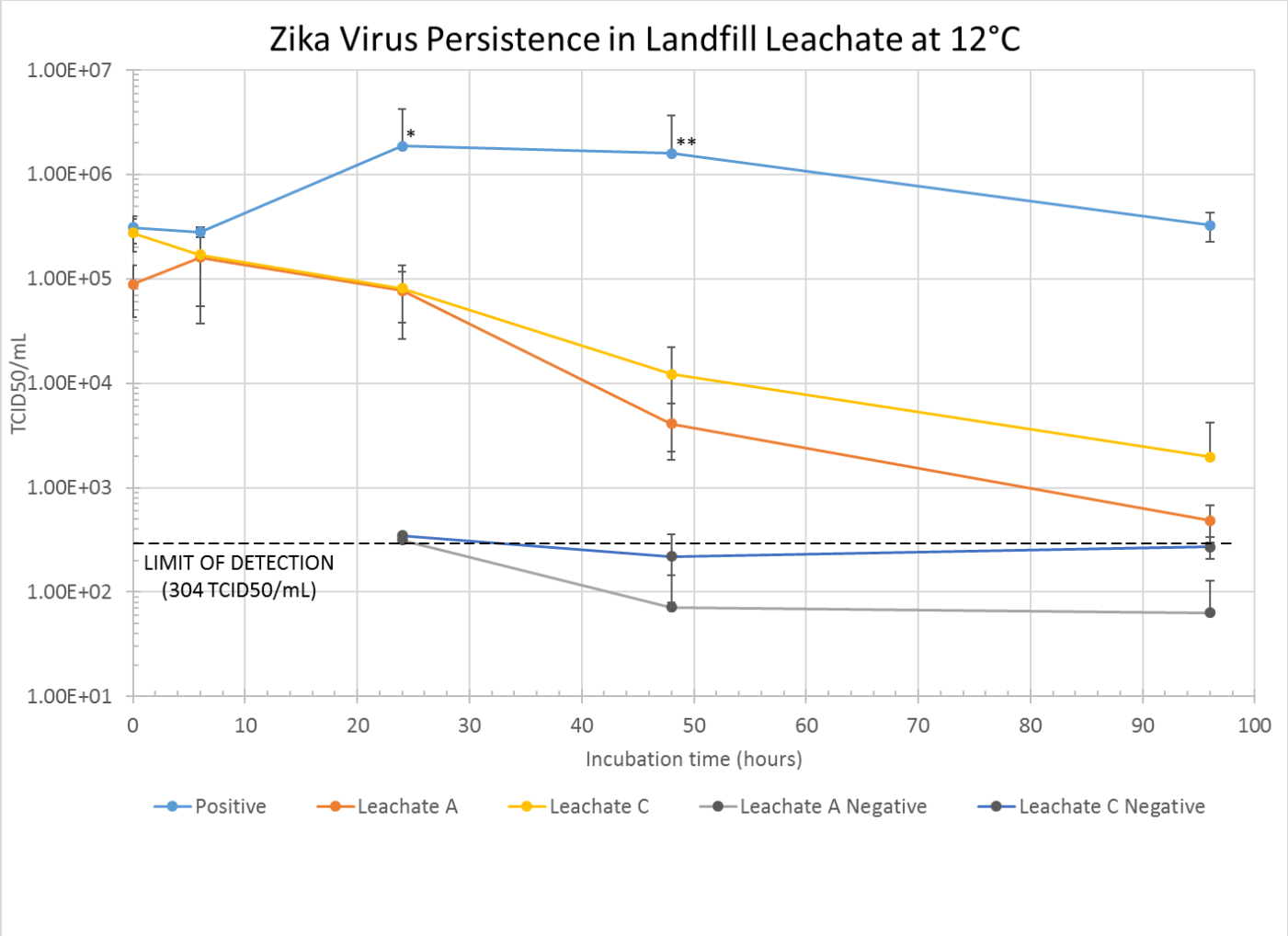
Viral Persistence (ZIKV, ssRNA, enveloped)

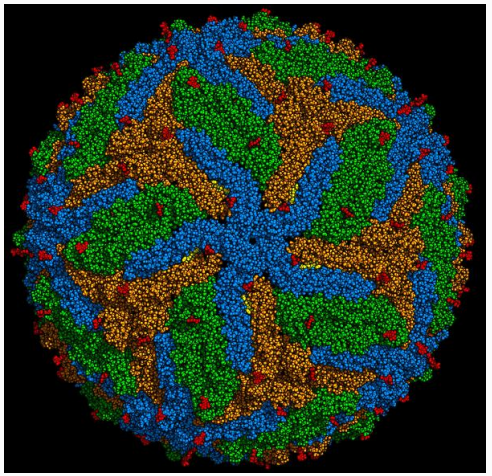
Matrix	Slope	Measured D-Value (hours)	Measured D-Value (days)	Persistence[a] (hours)	Persistence[a] (days)
Leachate A	-0.0611	16.4	0.68	100.3	4.18
Leachate B	ND[b]	ND	ND	ND	ND
Leachate C	-0.0551	18.2	0.76	121.1	5.04
DMEM Medium (Positive Control)[c]	0.0009	N/A	N/A	N/A	N/A

[a] Calculated time at which measured linear decay rate intersects with assay limit of detection.
[b] ND = Not Done. Leachate B had a toxic effect on Zika virus, thus persistence testing was not performed.
[c] Zika virus in positive control did not appreciably degrade over testing period. Persistence and D-values were not calculated due to the positive slope.



Viral Persistence (ZIKV, ssRNA, enveloped)





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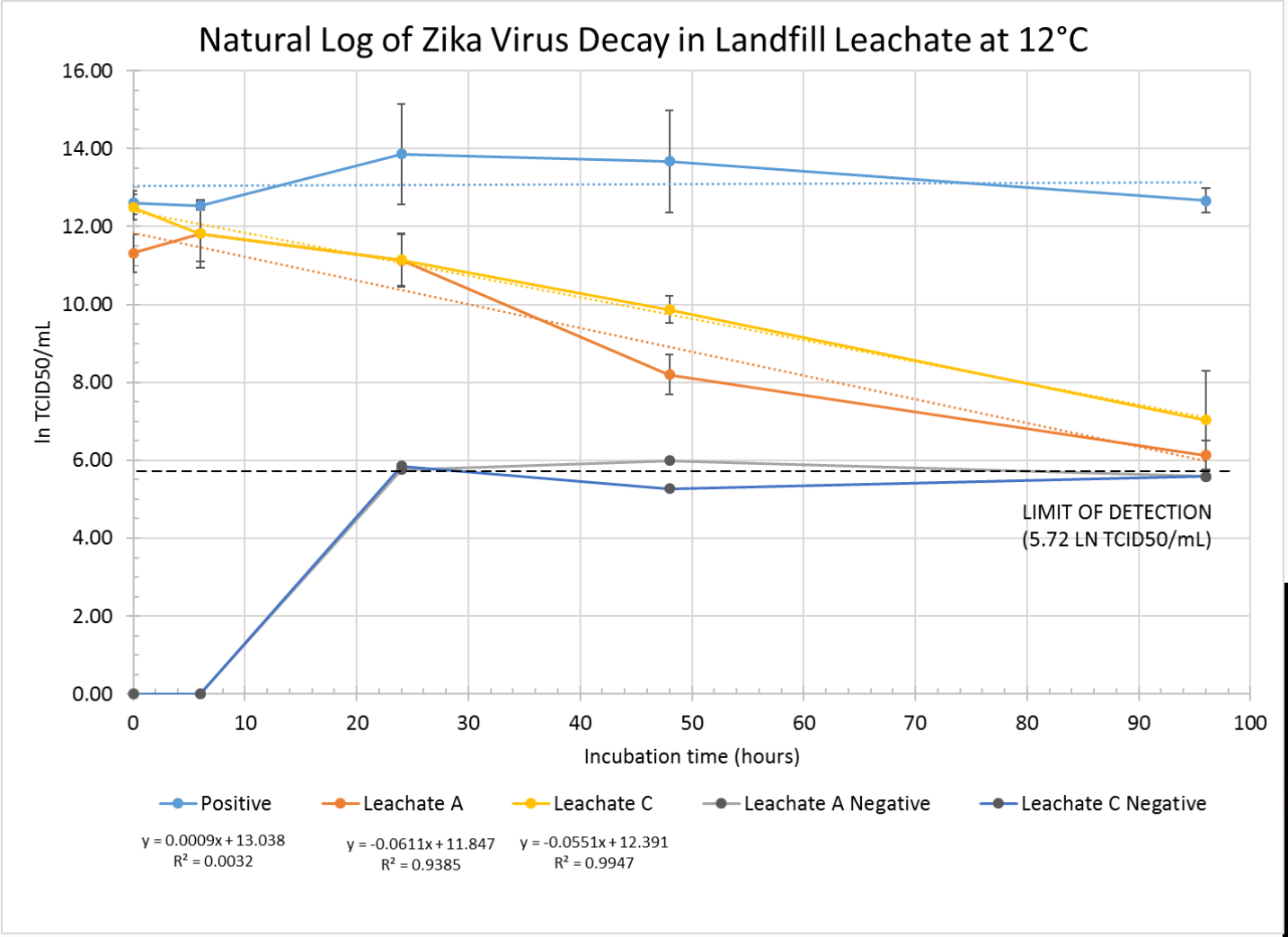

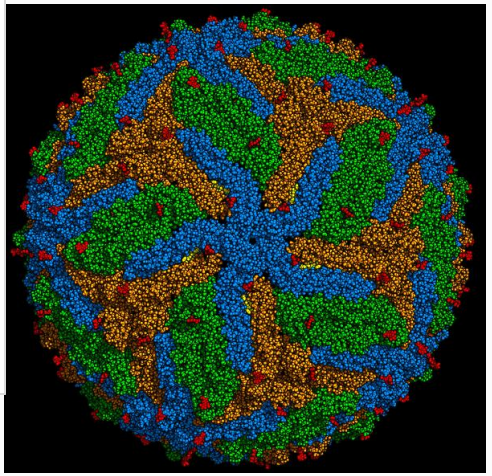

Summary Findings

Virus	Temperature Test Condition	Calculated Persistence (Days)[a] *Time Until No Longer Detected*			
		Leachate A	Leachate B	Leachate C	Control Matrix[b]
Zika (enveloped RNA virus)	12°C	4.18	ND[c]	5.04	NR[d]
MS2 Bacteriophage (non-enveloped phage)	12°C	48.3	27.4	35.7	34.4
	37°C	1.78	2.21	2.03	31.31
Phi6 Bacteriophage (enveloped phage)	37°C	0.46	NRec[e]	0.43	7.54

[a]Calculated time (days) when measured linear decay rate intersects with assay limit of detection.
[b]ZIKV in sterile incomplete Dulbecco's Modified Essential Medium (DMEM) medium; bacteriophage in sterile phosphate buffered saline.
[c]ND= Sample unrecoverable from leachate during testing, persistence testing not performed.
[d]NR = No decay, or minimal, observed within incubation period tested
[e]NRec = Sample unrecoverable from leachate during persistence testing.

Summary Findings

Virus	Temperature Test Condition	D-value (Days/Hours)			
		Leachate A	Leachate B	Leachate C	Control Matrix[a]
Zika Virus (enveloped RNA virus)	12°C	0.68 / 16.4	ND[b]	0.76 / 18.2	NR[c]
MS2 Bacteriophage (non-enveloped phage)	12°C	4.85 / 116.4	3.84 / 92.2	3.51 / 84.2	3.73 / 89.5
	37°C	0.16 / 3.95	0.21 / 5	0.18 / 4.3	3.0 / 72
Phi6 Bacteriophage (enveloped phage)	37°C	0.04 / 0.96	NRec[e]	0.03 / 0.72	0.60 / 14.4

[a]ZIKV in sterile incomplete DMEM; bacteriophage in sterile phosphate buffered saline (PBS).
[b]No decay observed within incubation period tested.
[c]ND= Sample unrecoverable from leachate during testing, persistence testing not performed.
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Conclusions and Implications

- Viral Persistence at 12°C is on the order of days to months, suggesting that viruses may persist in landfill leachate for extended times.
- Viral persistence and D-values are reduced at moderately elevated temperatures.
- Leachate composition likely dramatically effects viral persistence; data suggests this may be in part due to leachate chemical constituents.
- Further analysis is needed to 1) gain insight into characteristics that affect viral decay rates, and 2) generate actionable data for use in waste management.
- Viral persistence in landfill leachates varies from days to months. Choice of test agents is critical as data suggest that viral structure and/or structural characteristics are key for viral persistence.

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