Pathogen Analysis of NYSDOT Road-Killed Deer Carcass Compost Facilities

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Temperature and Pathogen Final Report

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Background

Passively aerated static pile composting is proving to be a good method of managing mortality. However, questions about the hygienic quality of the process and product need to be addressed. “When is the process finished?” and “Where can the finished product be used?”

Evaluation of the effectiveness of static pile composting to inactivate pathogens in road-killed carcasses required identification of the pathogens that might be present and analysis of their sensitivity to inactivation by heating. That, combined with time/temperature data from the compost piles, provided the information needed to assess the hygienic quality of the compost product. A literature review was conducted by CWMI to find data concerning the thermal stability of the pathogens identified. This information and professional opinion helped to determine what pathogens or indicator organisms were tested for in the composts.

The effectiveness of inactivating pathogens through composting is generally assessed by monitoring the reduction in fecal coliform bacteria and *Salmonella* (indicator organisms). It is widely recognized that the sensitivity of different pathogenic organisms to heat varies and questions have been raised about the use of the current indicator organisms. Temperatures achieved in static pile composting suggest an adequate degree and duration of high temperatures to significantly reduce the survival of many pathogenic organisms.

Six static compost piles were established. Three piles were located at New York State Department of Transportation (NYSDOT) facilities across NYS, which served as sites for demonstrations and monitoring. Three piles were located at a controlled Cornell research site where more intensive monitoring could be carried out. Pile design, construction and dimensions were the same for all piles. The piles were comprised of 4 adult deer and 2-3 yards of woodchips. Fecal matter from the deer was put in sentinel bags and placed in the carcasses in the research piles in a manner that enabled them to be recovered for sampling. The bacterial level of the indicator organisms in the fecal matter was analyzed before placement. Data loggers recorded temperatures in three locations within each compost pile. Compost samples and recoverable sentinels were periodically removed and analyzed. The following are results that have been statistically analyzed and interpreted to provide appropriate guidance for road managers.

Executive Summary

Conclusions

Composting of deer carcasses was effective in reducing pathogen levels, decomposing the carcasses and producing a useable end product after 12 months. The composting process used in this project involved enveloping the carcasses of road-killed deer in woodchips and allowing those piles with natural air circulation to sit undisturbed. Temperatures were

measured and samples from the piles were analyzed periodically for pathogens and for compost parameters. While significant pathogen reduction occurred in several months, it took 12 months for all of the measured pathogens to decline to low levels in all of the 6 piles we studied. Samples taken at other sites in NYS that have been composting road-killed deer for over a year also had low pathogen content. We thus suggest a composting duration of 12 months before use. In the interest of being cautious, we also recommend that the end product be used in low public contact settings such as highway rights-of-way.

Project Design

This project investigated the potential of composting as an option for managing road-killed animals. An extensive literature review on hardiness of bacterial pathogens found in wildlife was conducted prior to executing the research (Appendix A). Research and demonstration pilot piles containing deer carcasses enveloped in wood chips were constructed and monitored in several locations in New York State (NYS). Composite samples were taken periodically over the course of a year. Monitoring included temperatures, pathogens and typical compost parameters. More intensive research was also conducted using three replicated piles at the Cornell composting site in Ithaca, NY where sentinel bags containing pathogens were planted within the piles and recovered at intervals throughout the year. Additional samples were taken at other locations in NYS that had been composting road-killed animals for over a year to assess pathogen content and compost parameters at actual composting sites.

Pile Temperature

Even though research and field piles were started at the end of October, and early November, and the temperature of the deer was near 40^{o} F, all piles heated up. Average daily temperatures varied within the piles (depending on location of the probe) and among sites. Temperatures appeared to be higher in the top layer of the pile. All probes in all piles reached 40^{o} C, the recommended temperature for pathogen reduction for Class B use.

Compost Pathogens

Selection of pathogens for monitoring included the typical compost indicators, fecal coliform, *E.coli*, and *Salmonella.* Pathogens of concern in road-killed deer are not limited to this list. Discussions with Cornell Veterinary College faculty and others identified *Clostridium perfringens, Listeria monocytogenes, Campylobacter spp., Yersinia spp., Francisella tularemia*, and *Coxiella burnetii*, as bacterial pathogens. Rabies and Chronic Wasting Disease (CWD) are diseases that might be associated with deer in NYS. In this project we did not have the funding or the laboratory setting necessary to assess prion diseases and the research was done in an area without known CWD. Disease transfer from tick borne diseases, such as rabies, are more important when handling the carcasses than during the composting process and DOT already has safety procedures in place for them. Thermal destruction of some of the bacterial pathogens has been extensively studied in foods. A review of the literature shows that, from least to most hardy (heat-resistant): *Campylobacter jejuni* < *Yersinia enterocolitica* < *E. coli* < *Listeria monocytogenes* and *Salmonella* spp. < *Streptococcus faecalis*. Fecal enterococci, one of the fecal strep groups, have high thermal resistance, and can grow at a wide range of temperatures in the presence of salt and in low pH values. *E. faecium* is not expected to be found in deer, but other

enterococci are. Based on this, enterococci were selected as a good indicator of pathogen destruction in deer compost piles.

Pathogen concentrations were high in the woodchips used in constructing the pilot piles before any deer carcasses were buried. Regardless of the temperatures in the piles, at the pilot sites where pathogen levels (in woodchips only) were high in the beginning, it took 9-12 months to see a significant decrease in pathogen numbers. At Cornell, where the woodchips had lower pathogen numbers, levels remained low through month 9 and then increased significantly at month 12. This was most likely from severe disturbance on the piles just after the month 9 sampling. Piles were deconstructed so that we could find the remaining samples. The piles were left for another 6 months and a set of samples showed that at 18 months, pathogen levels were down to previous levels. No temperature data are available for the Cornell research piles for the last 6 months. In addition, pathogen analyses of compost from piles at several NYSDOT and NYS Thruway facilities that had been established before this research project showed low levels of pathogens.

Sentinel Bags of Deer Intestinal Contents: Deer goo

In order to conduct research on pathogen reduction, sentinel bags containing intestinal matter from deer (deer goo) were placed into deer in the research piles at Cornell. Triplicate bags were withdrawn from each of the three piles at the sampling times.

Fecal coliform and *Escherichia coli* levels in the deer goo in the 3 research piles decreased significantly within 6 weeks of pile establishment (approximately an 8 $\log_{10}$ reduction). Reduction may have occurred earlier, but since the week 3 samples were handled differently in the lab they are not comparable to the other data. A significant decrease of 7 $\log_{10}$ after 6 weeks in the compost pile for fecal coliforms, and 9 $\log_{10}$ reduction for *E. coli* was measured. Fecal streptococci and Enterococci levels took longer to decrease. There was a 3 $\log_{10}$ reduction by week 6 for fecal strep with an additional 3 $\log_{10}$ reduction at week 36. Enterococci levels remained the same until week 36, when there was a 3 $\log_{10}$ reduction.

Mycobacterium avium paratuberculosis (MAP)

While not generally considered an important disease in wild deer, MAP (the bacteria that causes Johne's disease in cattle) was selected as an indicator organism because it is relatively hardy, surviving higher temperatures than fecal coliform, and does not reproduce in the environment. MAP contaminated manure was placed in sentinel bags in the deer carcasses in the research piles. MAP levels decreased significantly to near 0 (from 4.51 $\log_{10}$ cfu/g to 0.19 $\log_{10}$ cfu/g) within 3 weeks, and remained at those levels until week 36. At week 36, several of the samples had values around 2 $\log_{10}$ cfu/g, which would be considered "few" for dairy cattle, posing a slight risk for the spread of Johne's disease to other cattle. One of the samples contained "many" or too numerous to count. It is not clear why these samples had these levels of MAP since this organism is not thought to be able to reproduce outside of a host and previously measured levels were low. This may have occurred because MAP tend to clump and can be hard to find therefore some of the clumps of organism may not have been exposed to high enough temperatures or were not detected in previous sample testing.

Compost Parameters

Samples at all the piles were analyzed for typical compost parameters. The initial samples were taken of wood chips only. As composting progressed, the composite samples taken from different depths in the piles (12", 18" and 24"), included decomposed deer material.

- **Particle size:** The percent of particles in the compost that measured less than 3/8" remained constant over time.
- **Carbon:Nitrogen ratio (C:N):** The C:N ratio started high (71.2) and decreased significantly over time indicating that composting was occurring and that the nitrogen from the carcasses was mixing with the carbon from the wood chips. Linear regression showed a decrease of 4.82/month for the research piles with an r^2 value of 0.808 and 4.18/month with an r^2 of 0.51 for the pilot piles. The C:N ratio after 12 months of composting for this type of pile is approximately the recommended starting value for traditional composting. After one year, there is still sufficient carbon present to start new piles.
- **Total Nitrogen (TN):** Nitrogen levels increased significantly over time in both the pilot and research piles averaging 1.5% in month 12. Percent nitrogen increased by 0.12/month with an r^2 of 0.702 in the research piles, and 0.07/month with an r^2 of 0.337 in the pilot piles.
- **Phosphorus (P):** Phosphorus levels increased significantly over time in both the pilot and research piles, averaging 0.24% in month 12. Percent P increased by 0.01/month with an r^2 of 0.472 in the research piles, and 0.02/month with an r^2 of 0.557 in the pilot piles.
- **Maturity:** Maturity increased significantly by month 12 in both the pilot and research piles averaging 6.9 on a 1:9 scale in month 12. Maturity increased by 0.1/month with an r^2 of 0.235 in the research piles, and 0.2/month with an r^2 of 0.358 in the pilot piles.

Project Design

Pile Construction and Sampling

Six road-killed deer carcass compost piles were set up in 4 different locations. Three pilot piles were set up across New York State to capture differing climatic conditions. The pilot piles were set up at NYSDOT facilities in Watertown, Cortland and Highland, NY, and the research piles were set up at Cornell University in Ithaca, NY. Table 1 shows the location of the sites and the climatic conditions at each obtained from the National Climatic Data Center (http://www.ncdc.noaa.gov/oa/ncdc.html).

Table 1: Pile locations and climatic conditions

			Average Temperature		Average Snowfall (inches)			
Location	Latitude	Longitude	Summer	Winter	December	January	February	March
Watertown	43.98 N	75.91 W	21°C (70°F)	-7°C (20°F)	29	34	23	15
Cortland	42.59 N	76.22 W	21°C (70°F)	-7°C (20°F)	23	23	19	15
Highland	41.72 N	73.96 W	21°C (70°F)	-1°C (30°F)	6	9	9	6
Ithaca	42.15 N	79.25 W	24°C (75°F)	-1°C (30°F)	15	17	15	10

All of the piles were constructed in a similar manner (Figure 1). Approximately 24 inches of "clean" wood chips were laid down, a layer of two deer was placed and two temperature probes were laid in. This layer was covered with approximately 18 inches of chips. Two more deer were laid down and one more probe inserted. Approximately two feet of wood chips were used to cover the piles.

1st layer of deer, temperature probes

Add 2nd layer of deer

Finished pile

Figure 1: Pile building at Highland DOT

The following describes the construction of the pilot piles:

Watertown: arrived 8:09 am 10/31/05

- 8:24 am – chips laid
- 8:30 am –"compost" samples taken (these were samples of the wood chips)
- 8:31 am – two deer laid – data logger temperature probes 1 and 3 under and in between first 2 deer
- 8:35 am - layer of chips on top of first two deer
- 8:38 am – two deer on top of layer – data logger temperature probes 2 and 4 under and in between these
- 8:42 am – pile finished
- 4:30 pm 11/2/05 – temperature probe #4 pulled out of the pile to be ambient temperature

Cortland: arrived 12:25 pm 10/31/05

- 12:30 pm – chips laid
- 12:35 pm – "compost" samples taken (these were samples of the wood chips)
- 12:36 pm – data logger temperature probes 1 and 3 under and in between first layer of deer
- 12:38 pm – 2nd layer of deer
- 12:43 pm - data logger temperature probe 4 under 2nd layer
- Probe 2 ambient

Highland: arrived 11:35 am 11/1/05

- 11:57 am – chips laid
- 12:00 pm – "compost" samples taken (these were samples of the wood chips)
- 12:06 pm – data logger temperature probe 4 under 1st layer deer, probe 3 under 1st layer foot (edge of pile)
- 12:14 pm – data logger temperature probe 2 under 2nd layer of deer, probe 1 ambient
- 12:30 pm – temperature taken with Reotemp thermometer: 100°F

The wood chips used for these piles were wood chips that had been stockpiled by each of these facilities. Three samples of chips from the first layer laid to build the pile were taken at each location and sent to Woods End Laboratories (WEL) for analysis of fecal coliforms, *Escherichia coli*, fecal streptococci, enterococci and *Salmonella spp*. These wood chips started with approximately 4.9 $\log_{10}$ MPN/g solids fecal coliforms and 7.4 $\log_{10}$ MPN/g solids fecal streptococci average for all three sites. The deer in each of these piles were road-killed deer collected either over the weekend (at Watertown, thus the deer were cold) or that morning (at Cortland and Highland) and saved on site until the pile was built. The ambient temperature on Oct 31 and Nov 1, 2005 was approximately 14^{o}C (57^{o}F) at all sites. At the time the piles were built, Hobo U12 Outdoor/Industrial data loggers were set up to record temperatures in the pile every half hour. Three temperature probes from these loggers were placed in the piles for the duration of the study (two probes in the bottom layer of the pile – one underneath one of the deer and the other between the deer, and one probe in the top layer of the pile between the deer). The 4th probe was left outside the pile to record ambient temperature. However, as some probes were left in direct sunlight, temperatures recorded do not necessarily reflect the air temperature. Temperatures recorded by these probes were downloaded via computer connection to the logger and are shown in future graphs.

These 3 piles were truly static piles, that is, they were never turned and were disturbed only to sample. At all the piles, compost was sampled at months 3, 6, 9 and 12. Three samples were taken from each pile. Three holes were dug into the pile at medium height around the perimeter of each of the piles. The first sample was a composite of compost taken from the ceiling of each of the holes at a depth of approximately 12". The second sample was a composite of compost taken from the ceiling of each of the holes at a depth of approximately 18" and the third was taken at approximately 24" into the pile. The results from these pilot piles show what happens to pathogen concentrations over time in road-killed deer static compost piles. In addition, at WEL, the 3 samples from each pile were composited into one sample and tested for the following compost parameters: density, moisture, water holding capacity, pH, organic matter, conductivity, carbon:nitrogen ratio, total nitrogen, phosphorus and Solvita® maturity index.

The research piles (three replicated piles set up at Cornell University in Ithaca, NY) were constructed in the same manner as the pilot piles, except that the deer used in these piles had been collected over a one week period prior to pile construction on 11/8/05 and had been stored at 40^{o}F for that week. In addition, the deer were split open and the spiral colon and blind sac were removed from each of them to create "deer goo" that was placed in bags and used to seed the abdominal cavities of each of the deer being placed in the piles (Figure 2). Temperature probes were placed in each of the 3 research piles as described above. The ambient temperature on Nov 8, 2005 was approximately 6^{o}C (43^{o}F).

Cutting out spiral colon and blind sac

Filling bags and balls with MAP manure

1st layer of deer seeded with balls

Covering 1st layer of deer with chips

Foreground: finished pile, Background: 2nd layer

3 finished research piles

Figure 2: Building the research piles at Cornell University

The wood chips used for these piles came from Tompkins County Department of Transportation. The month 0 compost sample was a single sample of wood chips that were used to build each of the 3 piles. These wood chips started out very clean (2.7 $\log_{10}$ MPN/g solids fecal coliforms, and 5.2 $\log_{10}$ MPN/g solids fecal streptococci average for 3 piles). At months 3, 6, 9 and 12, three samples were taken from each pile as described above for the pilot sites and analyzed for pathogens and compost parameters.

Deer goo was sampled at weeks 0, 3, 6, 9, 17 and 36. At week 0, 5 samples were taken at random from the 50 bags that were prepared for seeding into the deer carcasses. The remaining 45 bags were placed in whiffle balls and 15 were distributed in the 4 carcasses in each pile. At weeks 3, 6, 9, 17 and 36, three whiffle balls with deer goo were pulled from each of the three piles at the research site. Retrieving the whiffle balls required greater disturbance of the piles (digging in to find the balls) than taking compost samples. At week 36 (the week after month 9 compost samples had been taken), we had a hard time finding all of the remaining whiffle balls and used a backhoe in piles 1 and 2 to find them, essentially spreading out and then reforming the piles. Therefore, piles 1 and 2 had been pulled apart prior to taking the month 12 compost samples. The backhoe was not used in pile 3, but the pile was extensively disturbed with a shovel. The backhoe was not cleaned prior to use and it is not known to what materials the bucket had been recently exposed. Due to the disturbance, the research piles were not truly “static” piles.

The entire nylon mesh bags of deer goo were sent to WEL for analysis. At week 0, the 5 samples that were sent for analysis went directly into a plastic Ziploc bag, rather than in the nylon mesh bags being used for seeding. At week 3, the deer goo was removed from the nylon mesh bag at WEL prior to running the analysis procedure. At all other weeks, the deer goo remained in the nylon mesh bag and was put through a stomacher and prepared for analysis.

A second set of whiffle balls containing Johnes (*Mycobacterium avium paratuberculosis* - MAP) contaminated manure in nylon mesh bags were also inserted into the research pile carcasses. Previous work led to the selection of MAP as a relatively hardy indicator bacteria (Appendix A). Samples of MAP contaminated manure were obtained from the Cornell College of Veterinary Medicine. These were sampled at weeks 0, 3, 6, 9, 12 and 36 and sent to the Animal Health Diagnostic Center at Cornell University for Johnes testing. At week 0, 9 samples were taken at random while preparing the 54 bags for seeding into the deer carcasses. At weeks 3, 6, 9, and 12, three whiffle balls with MAP were pulled from each of the 3 research piles. At week 36, only 2 balls were found for MAP analysis in pile 3. Sampling dates were as follows:

Table 2: Sampling dates

Sampling	Cornell Piles	Highland	Watertown	Cortland
Week/Month 0	11/8/05	11/1/05	10/31/05	10/31/05
Week 3 Goo/MAP	11/28/05			
Week 6 Goo/MAP	12/19/05			
Week 9 Goo/MAP	01/09/06			
Month 3 compost	01/30/06	01/17/06	01/16/06	01/17/06
Week 12 MAP only	01/30/06			
Week 17 Goo only	03/13/06			
Month 6 compost	04/17/06	04/24/06	04/24/06	04/24/06
Month 9 compost	07/17/06	07/10/06	07/10/06	07/10/06
Week 36 Goo/MAP	07/24/06			
Month 12 compost	10/09/06	10/16/06	10/16/06	10/16/06
Month 18 compost	04/30/07			

Results

Temperature in the Pilot Piles

The following tables and figures show average daily temperature recorded by the data logger in the pilot piles. Top refers to the probe that was put in the top layer of the pile and bottom 1 and bottom 2 refer to the 2 probes placed in the bottom layer of the pile. The orange, pink and blue curves show the average daily temperature recorded by the data logger probe in the top layer and 2 probes in the bottom layer of each pile, respectively. The green arrows show when compost sampling was done. The red dotted line shows 40^{o}C, which is a recommended temperature for pathogen kill in compost piles to meet United States Environmental Protection Agency (USEPA) Class B standards (i.e. low public contact settings). When there is a break in temperature data in any of the graphs, it indicates a low battery issue with the data logger which resulted in missing data in those time periods.

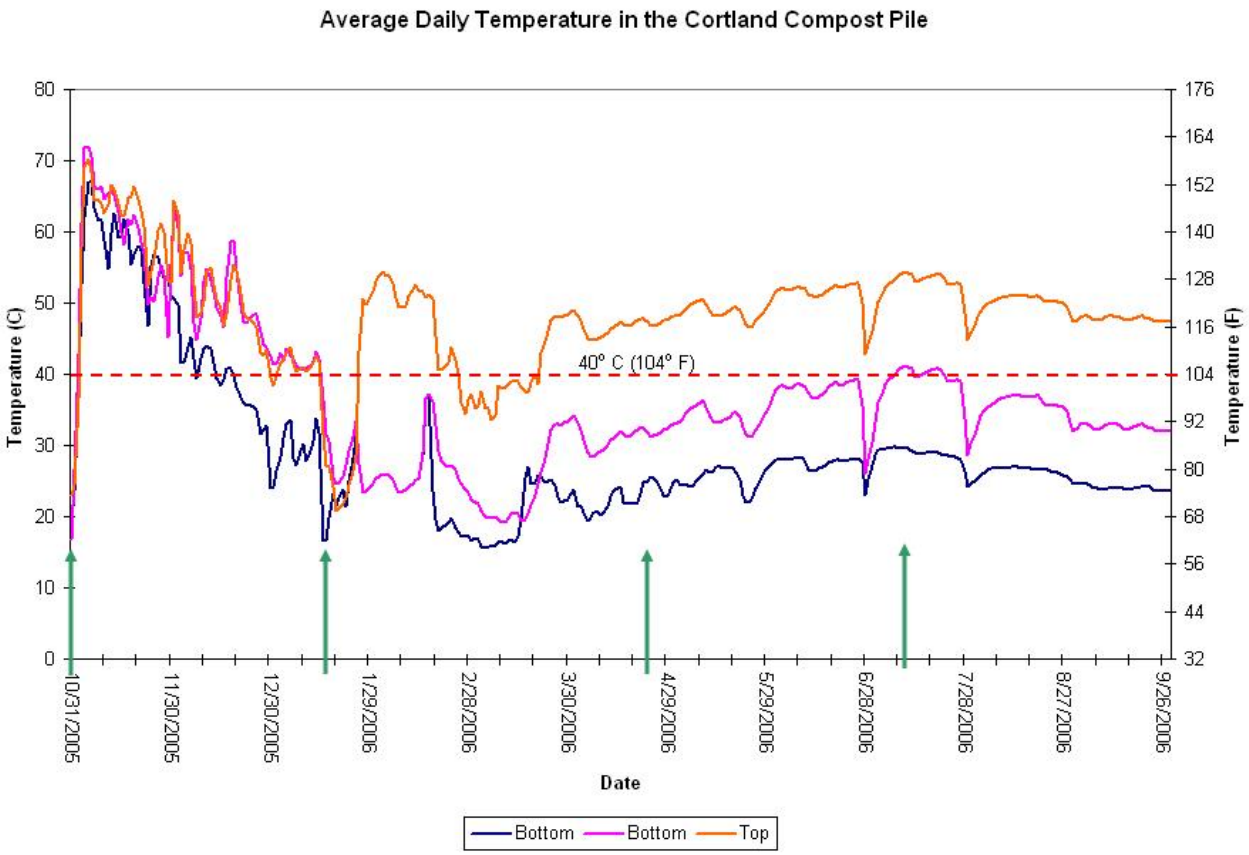


Figure 3: Average daily temperature in the Cortland compost pile over time (green arrows signify sampling dates)

Table 3: Average daily temperature summary statistics in the Cortland pile

	Top		Bottom 1		Bottom 2	
Statistic	°C	°F	°C	°F	°C	°F
Minimum	20.7	69.3	15.6	60.1	16.8	62.2
Mean	48.3	118.9	29.3	84.7	36.7	98.1
Maximum	70.1	158.2	66.9	152.4	72.1	161.8
# of Days missing data	0		18		0	
Total # days recorded	333		315		333	

Table 4: Average daily temperatures greater than 40° C in the Cortland pile

	Top	Bottom 1	Bottom 2
Total # of Days > 40	292	42	87
# of days to reach 40	3	3	2
# Consecutive Days > 40	57	35	74
Date of Consecutive Days	11/3/05 – 12/29/05	11/3/05 – 12/07/05	11/2/05 – 1/14/06

The Cortland pile temperatures were what would be expected in a compost pile. There was an immediate increase in temperature, reaching over 40°C within 2 - 3 days, despite the cold ambient temperature. Temperatures remained over 40°C for 35 – 74 consecutive days.

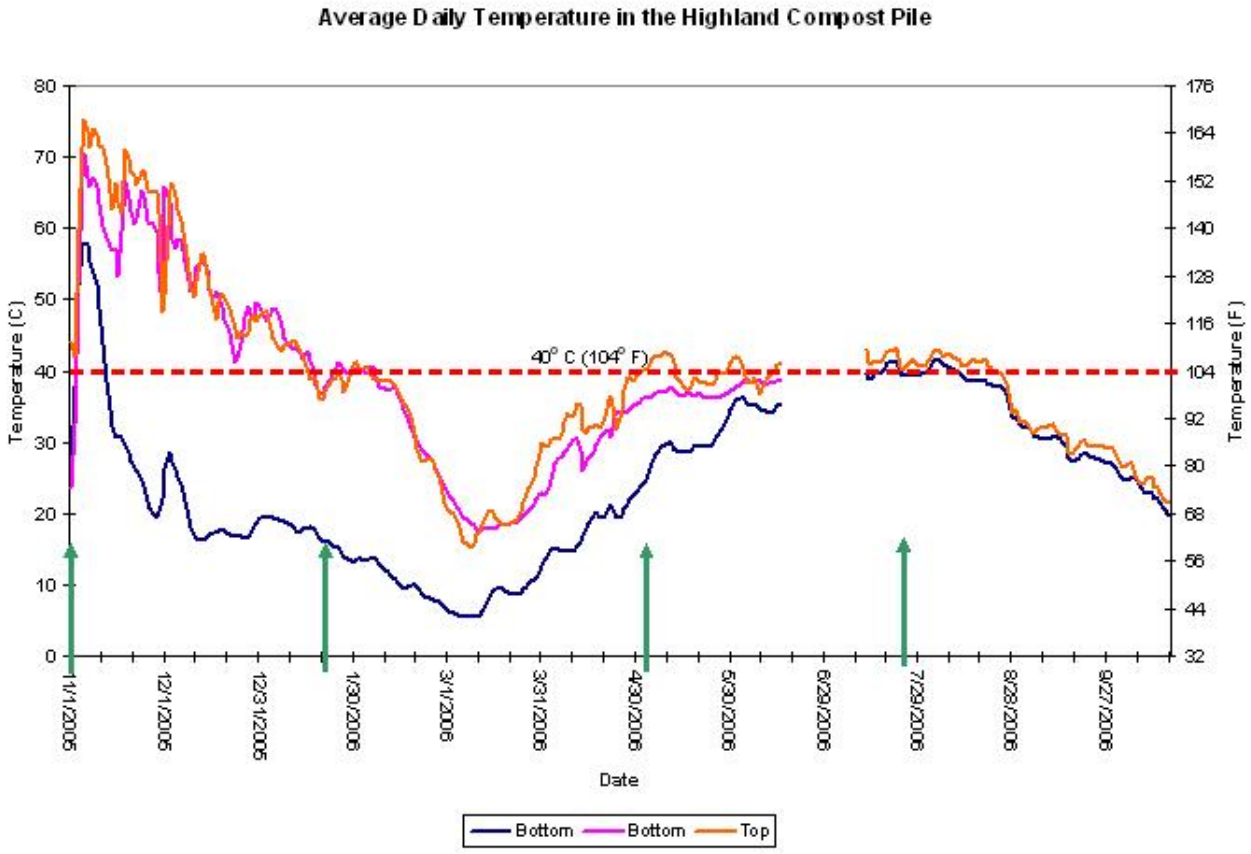


Figure 4: Average daily temperature in the Highland compost pile over time (green arrows signify sampling dates)

Table 5: Average daily temperature summary statistics in the Highland pile

	Top		Bottom 1		Bottom 2	
Statistic	°C	°F	°C	°F	°C	°F
Minimum	15.4	59.7	5.5	41.9	17.6	63.7
Mean	39.0	102.2	24.4	75.9	38.9	102.0
Maximum	75.2	167.4	57.8	136.0	70.5	158.9
# of Days missing data	26		124		26	
Total # days recorded	325		227		325	

Table 6: Average daily temperatures greater than 40° C in the Highland pile

	Top	Bottom 1	Bottom 2
Total # of Days > 40	142	24	87
# of days to reach 40	0	2	2
# Consecutive Days > 40	75	9	76
Date of Consecutive Days	11/1/05 – 1/14/06	11/3/05 – 11/11/05	11/3/05 – 1/17/06

The Highland pile temperatures were also what would be expected in a compost pile. There was an immediate increase in temperature, reaching over 40°C immediately in the top layer and within 2 days in the bottom layers. Temperatures remained over 40°C for 9 – 76 consecutive days.

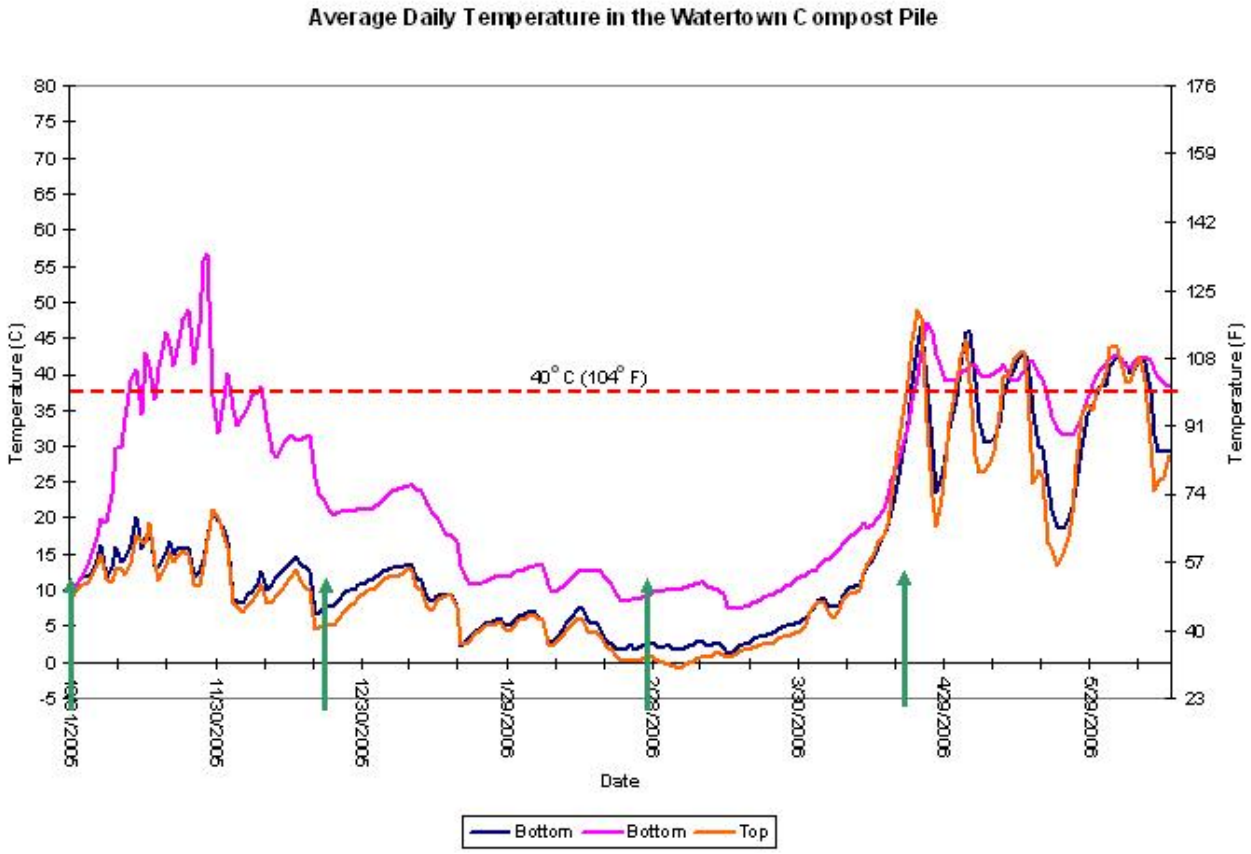


Figure 5: Average daily temperature in the Watertown compost pile over time (green arrows signify sampling dates)

Table 7: Average daily temperature summary statistics in the Watertown pile

	Top		Bottom 1		Bottom 2	
Statistic	^{o}C	^{o}F	^{o}C	^{o}F	^{o}C	^{o}F
Minimum	-0.8	30.6	7.3	45.1	7.5	45.5
Mean	14	57.2	15.2	59.4	24.9	76.8
Maximum	48.8	119.8	46.6	115.9	56.8	134.2
# of Days missing data	0		0		0	
Total # days recorded	227		227		227	

Table 8: Average daily temperatures greater than 40^{o} C in the Watertown pile

	Top	Bottom 1	Bottom 2
Total # of Days > 40	14	17	44
# of days to reach 40	173	174	12
# Consecutive Days > 40	3	2	1
Date of Consecutive Days	4/22/06 – 4/24/06	4/23/06 – 4/24/06	11/13/05

Watertown temperatures did not reflect those of a typical compost pile. Only one of the probes showed an increase in temperature early on, taking 12 days to reach 40^{o}C, while the other two remained below about 20^{o}C for 6 months. This may reflect the fact that the deer were very cold when placed in the pile. In April, all three probes showed an increase in temperature, but the high temperature reached was only around 50^{o}C. The climate in Watertown through the winter includes high winds and little snow. Deer and chips may have been too cold to increase under those conditions. In climates with frequent wind a more dense carbon source may be required.

Combination Temperature/Pathogens in the Deer Goo

Description

The temperatures recorded by the data loggers reflect the temperature in close proximity to the deer where the sentinel bags of deer goo were placed. The following figures show average daily temperature in the research piles on the left-hand y-axis, and the log 10 MPN (most probable number)/g of select pathogens on the right-hand y-axis. The orange, pink and blue curves show the average daily temperature recorded by the data logger probe in the top layer and 2 probes in the bottom layer of each pile, respectively. The solid yellow and turquoise lines in each of the graphs show the mean of the 3 samples from each pile for each parameter, while the yellow square and turquoise diamond show the value for each sample. The green arrows show when sampling was done. This gives a visual trend of the level of pathogens in the deer goo over time as it relates to temperature.

The effectiveness of pathogen inactivation through composting is generally assessed by monitoring the reduction in indicator organisms. *Salmonella* and fecal coliform are the usual indicator organisms. These are the organisms that the USEPA requires for evaluation of the hygienic quality of sewage sludges. One standard for fecal coliform in sewage sludge deemed by USEPA to be Class A, suitable for unrestricted use, is 1000 MPN (3 log 10). For sludges used where

public contact is limited, the Class B standard is 2 million MPN/g (6.3 log 10) for fecal coliform (purple dotted line in the graphs). In addition, the temperature of the compost must remain over 40 ^{o}C (red dotted line in the graphs) for 5 days and above 55^{o}C for 4 hours for Class B. The composts under investigation are not subject to these regulations, but the standards are provided as a benchmark.

Statistical Analysis

Statistical analysis of pathogen levels in the deer goo was done using the S-Plus Statistical package. Differences in pathogen levels at each sampling event were analyzed for the three research piles together using analysis of variance (ANOVA) for multiple comparisons with Tukey corrections. This analysis measures each possible combination of sample dates and compares it to the variation within each of the sample dates. If the between-sample date variation is large and the within-sample date variation is small, a significant difference is concluded. The tables below the figures show the individual values at each pile and the means of 9 samples (3 from each of the 3 replicated piles). Because the week 3 deer goo was analyzed differently (removed from the nylon mesh bags prior to analysis) and the values for week 3 are so variable, it was assumed that in some instances, the bacteria had adhered to the nylon mesh bag and in some instances not, and thus the numbers were biased. Statistical analysis was run both with and without week 3 values.

In addition, regression analysis was run on pathogen levels in the deer goo. Regression analysis differs from the ANOVA analysis in that it examines the relationship between the levels of the pathogens and time. It does not treat each sampling date as a distinct point (as in the ANOVA), but considers the trend over time. As the decrease in fecal coliforms and *E. coli* in deer goo was immediate and dramatic, and then leveled off, non-linear regression was performed using the JMP Statistical Package and the exponential decay equation of:

$y = N_0 * e^{-kx}$, where

- y = the predicted value for the pathogen
- N_0 = the initial value of the pathogen
- e^x = exponential function
- k = constant rate of decay, and
- x = time.

A quantity is said to be subject to exponential decay if it decreases at a rate proportional to its value. Once the rate of decay is determined, then a half-life (the amount of time it will take for ½ of the level to decrease) for that substance can be determined by dividing the natural log of 2 by the rate of decay.

As the decrease in fecal streptococcus and enterococci appeared to be linear over time, linear regressions of those pathogen levels were run using JMP. Linear regression measures whether the change in the pathogen level over time is different from zero. The equation that accompanies the figures is in the form of:

$y = mx + b$, where

- y = the parameter being measured
- m = the slope of the line (i.e. the amount by which the y level changes)
- x = week of composting, and

- b = the y-intercept (i.e. the level of y at time 0).

An r^2 value also accompanies the figures which indicates how well correlated the x variable (in this case week of composting) is with the predicted level of pathogen. That is, it tells how much of the variation in the level of the pathogen is due to the amount of time of composting.

Fecal Coliforms and *Escherichia coli*

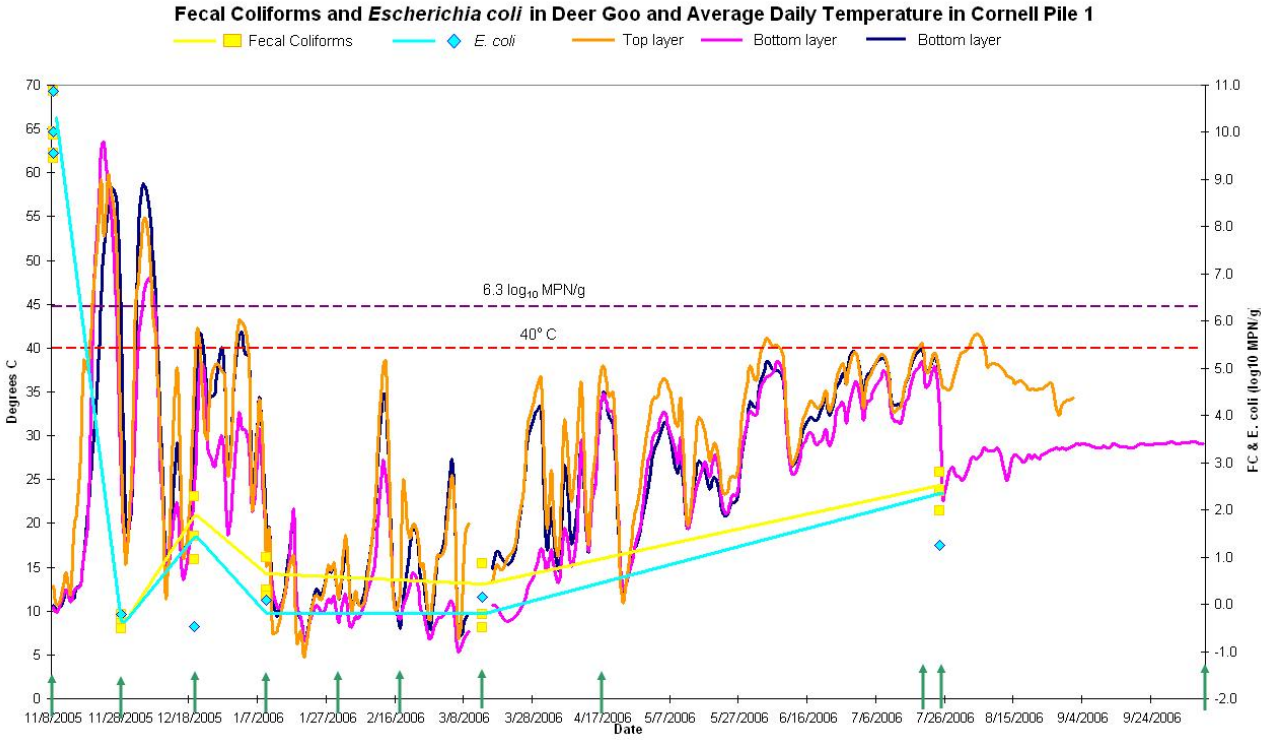


Figure 6: Fecal coliforms and *E. coli* in the deer goo and average daily temperature in pile 1 at Cornell over time (n=5 for week 0, n=3 for all other samplings – samplings indicated by green arrows)

Table 9: Average daily temperature summary statistics in Cornell pile 1

	Top		Bottom 1		Bottom 2	
Statistic	^oC	^oF	^oC	^oF	^oC	^oF
Minimum	4.8	40.6	6.9	44.4	5.5	41.9
Mean	29.0	84.2	26.1	79.0	24.5	76.1
Maximum	59.6	139.3	58.6	137.5	63.5	146.3
# of Days missing data	44		83		6	
Total # days recorded	286		253		330	

Table 10: Average daily temperatures greater than 40° C in Cornell pile 1

	Bottom 1	Bottom 2	Top
Total # of Days > 40	17	13	33
# of days to reach 40	14	12	11
# Consecutive Days > 40	7	7	9
Date of Consecutive Days	11/22/05 – 11/28/05	11/20/05 – 11/26/05	11/19/05 – 11/27/05

Cornell pile 1 took 11 - 14 days to heat up, and remained hot for 7 – 9 days. In addition, there appears to be an increase in temperature after each of the sampling dates (shown by the green arrows) that is likely due to increased air flow resulting from disturbing the pile. There was a large decrease in fecal coliform (Table 15) and *E. coli* (Table 16) levels in the deer goo between the initial sampling when the piles were constructed and the next sampling three weeks later. Levels then remained low throughout the trial.

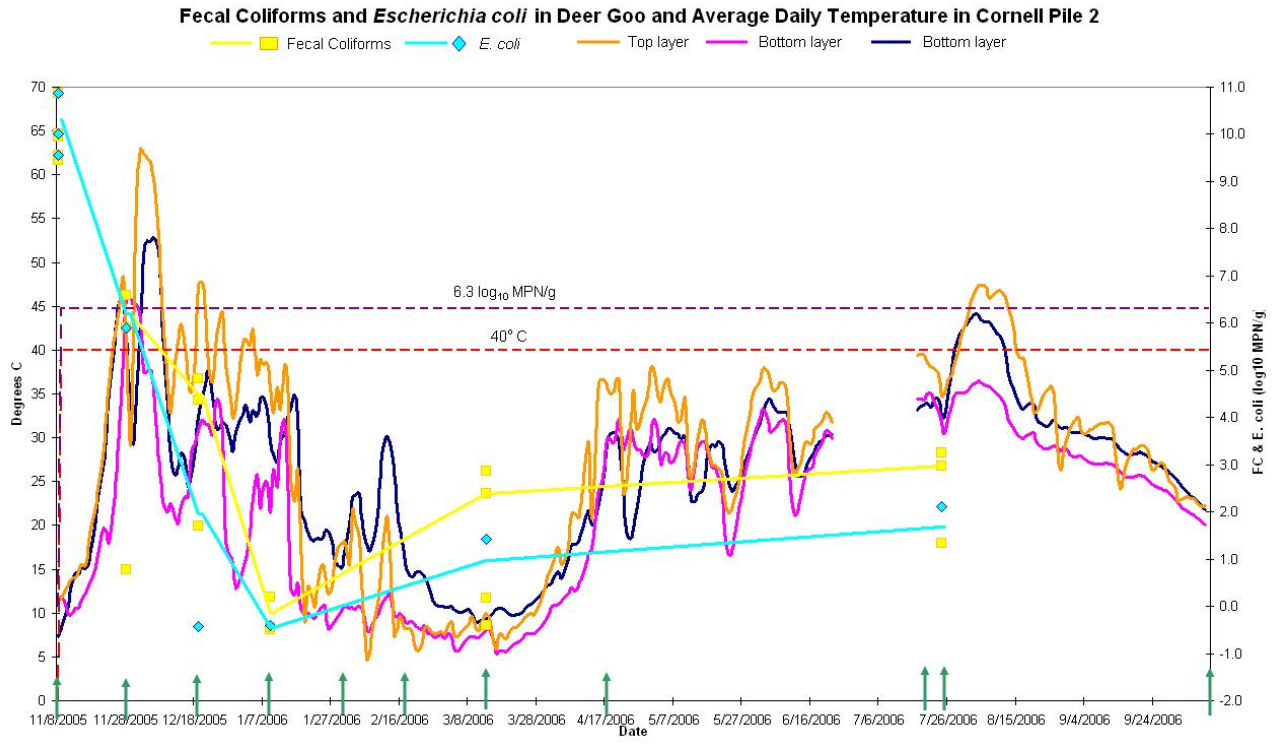


Figure 7: Fecal coliforms and *E. coli* in the deer goo and average daily temperature in pile 2 at Cornell over time (n=5 for week 0, n=3 for all other samplings – samplings indicated by green arrows)

Table 11: Average daily temperature summary statistics in Cornell pile 2

	Top		Bottom 1		Bottom 2	
Statistic	^{o}C	^{o}F	^{o}C	^{o}F	^{o}C	^{o}F
Minimum	4.8	40.6	7.3	45.1	5.3	41.5
Mean	27.8	82.0	26.3	79.3	22.1	71.8
Maximum	62.9	145.2	52.8	127.0	46.3	115.3
# of Days missing data	24		24		24	
Total # days recorded	306		306		306	

Table 12: Average daily temperatures greater than 40^{o} C in Cornell Pile 2

	Bottom 1	Bottom 2	Top
Total # of Days > 40	26	5	45
# of days to reach 40	17	20	16
# Consecutive Days > 40	4	5	15
Date of Consecutive Days	11/25/05 – 11/28/05	11/28/05 – 12/2/05	11/24/05 – 12/9/05

Cornell pile 2 behaved very similar to the Watertown pile, taking 16 - 20 days to reach 40^{o}C and remaining there for 4 - 16 consecutive days. In addition, temperatures appeared to increase after each sampling date. There was a large decrease in fecal coliform (Table 15) and *E. coli* (Table 16) levels in the deer goo between the initial sampling when the piles were constructed and the next sampling three weeks later. Levels then remained low throughout the trial.

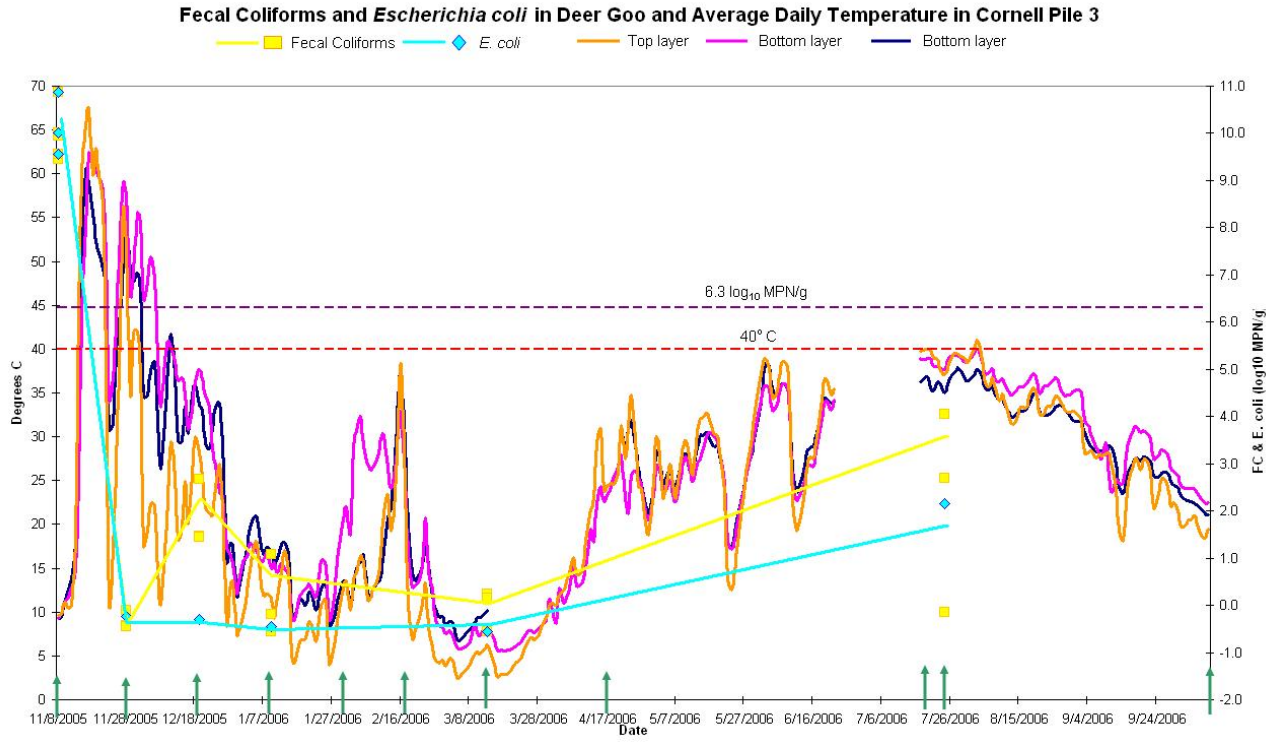


Figure 8: Fecal coliforms and *E. coli* in the deer goo and average daily temperature in pile 3 at Cornell over time (n=5 for week 0, n=3 for all other samplings – samplings indicated by green arrows)

Table 13: Average daily temperature summary statistics in Cornell pile 3

	Top		Bottom 1		Bottom 2	
Statistic	^{o}C	^{o}F	^{o}C	^{o}F	^{o}C	^{o}F
Minimum	2.1	35.8	6.7	44.1	5.6	42.1
Mean	23.0	73.4	26.6	79.9	26.0	78.8
Maximum	67.5	153.5	60.3	140.5	62.3	144.1
# of Days missing data	23		61		23	
Total # days recorded	379		341		379	

Table 14: Average daily temperatures greater than 40^{o} C in Cornell pile 3

	Bottom 1	Bottom 2	Top
Total # of Days > 40	16	22	18
# of days to reach 40	7	7	6
# Consecutive Days > 40	8	8	8
Date of Consecutive Days	11/15/05 – 11/22/05	11/15/05 – 11/22/05	11/14/05 – 11/21/05

Cornell pile 3 took 6 - 7 days to heat up, and it remained hot for 8 consecutive days. In addition, there appears to be an increase in temperature after each of the sampling dates (although not as pronounced as in the other piles). There was a large decrease in fecal coliform (Table 15) and *E. coli* (Table 16) levels in the deer goo between the initial sampling when the piles were constructed and the next sampling three weeks later. Levels then remained low throughout the trial.

Table 15: Fecal coliforms (log_{10} MPN/g solids) in deer goo samples from the 3 research piles over time with and without week 3

	Week 0	Week 3	Week 6	Week 9	Week 17	Week 36
1	10.00	-0.22	1.45	0.30	0.86	1.99
2	9.94	-0.49	0.95	0.18	-0.49	2.81
3	9.45	-0.51	2.28	1.00	-0.22	2.38
4	10.88	5.90	4.38	0.20	2.87	2.97
5	9.56	0.78	4.83	-0.49	0.18	1.34
6		6.59	1.70	-0.44	-0.41	3.26
7		-0.12	1.46	1.08	-0.57	2.70
8		-0.43	2.66	-0.57	0.23	4.04
9		-0.44	1.45	-0.20	0.15	-0.15
Mean	**9.96^{a}**	**1.24^{bcd}**	**2.35^{bc}**	**0.12^{d}**	**0.29^{cd}**	**2.37^{b}**
Mean (excluding week 3)	**9.96^{a}**		**2.35^{b}**	**0.12^{b}**	**0.29^{b}**	**2.37^{b}**

Mean values with differing superscripts are significantly different – $p < 0.05$

Fecal coliforms in the deer goo significantly declined by 8.7 $\log_{10}$ MPN/g by week 3 when it is included, and by 7.6 $\log_{10}$ MPN/g by week 6 whether week 3 is included or not (Table 15). The difference between including and not including week 3 is that after the initial drop, if week 3 is included, there is an increase in fecal coliform levels at week 36, but the levels remain the same over time when it is excluded. Regardless, there is a rapid severe drop in fecal coliform levels. Non-linear regression of the fecal coliform levels in deer goo shows an exponential rate of decay of 0.53. This means that the expected level at any week W is $e^{0.53}$ = 1.7 times the level at week W – 1. In other words, the estimated rate of decay is approximately 1.7 – 1 = 0.7, or approximately 70% per week. The half-life is 1.31 weeks with the week 3 data included. When week 3 data is excluded, the rate of decay is 0.28 ($e^{0.28}$ = 1.3 or 30% decrease per week), with a half-life of 2.45 weeks (Figure 9).

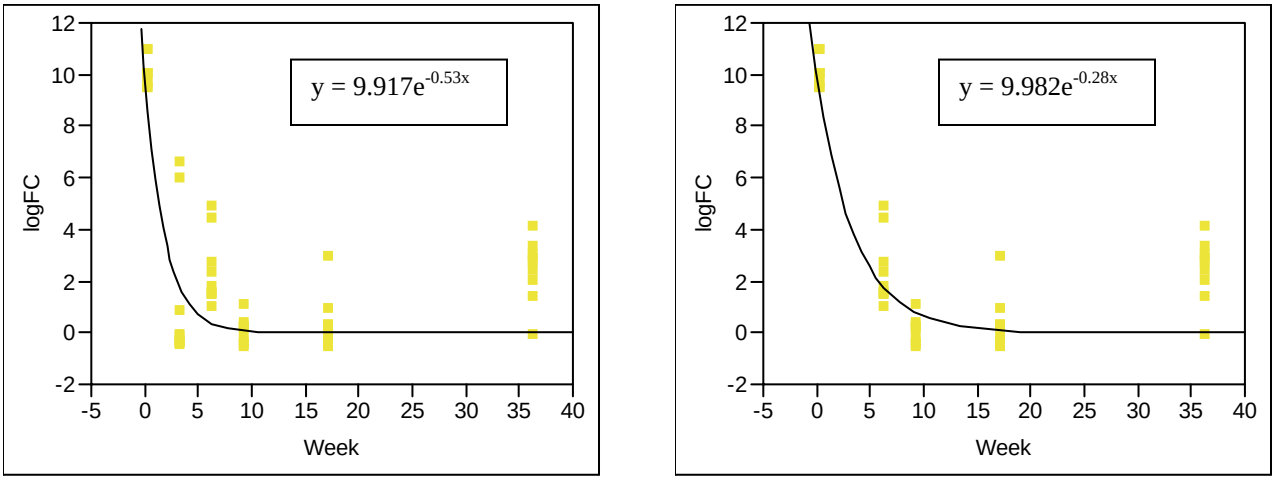


Figure 9: Exponential decay of fecal coliforms in deer goo over time in the research piles
Graph on left is with and graph on right is without week 3 data.

Table 16: *Escherichia coli* (log_{10} MPN/g solids) in deer goo samples from the 3 research piles over time with and without week 3

	Week 0	Week 3	Week 6	Week 9	Week 17	Week 36
1	10.00	-0.22	-0.48	0.08	0.15	1.26
2	9.94	-0.40	-0.60	-0.62	-0.49	2.73
3	9.45	-0.51	1.89	-0.30	-0.60	1.99
4	10.88	5.90	-0.43	-0.40	1.43	2.11
5	9.56	0.08	2.43	-0.49	-0.05	1.18
6		6.59	0.70	-0.44	-0.41	-0.05
7		-0.24	-0.31	-0.46	-0.57	2.15
8		-0.43	-0.40	-0.57	-0.22	0.63
9		-0.44	-0.40	-0.55	-0.52	-0.22
Mean	**9.96^{a}**	**1.15^{b}**	**0.27^{b}**	**-0.42^{b}**	**-0.14^{b}**	**1.31^{b}**
Mean (excluding week 3)	**9.96^{a}**		**0.27^{bc}**	**-0.42^{c}**	**-0.14^{c}**	**1.31^{b}**

Mean values with differing superscripts are significantly different – $p < 0.05$

E. coli values drop significantly and rapidly as well (8.8 log_{10} reduction by week 3 and 9.7 by week 6) (Table 16). In regard to the effect on average concentration of *E. coli*, excluding week 3 samples (due to different handling of samples in the lab as described on page 7), we have the opposite from fecal coliforms. That is, with week 3 included, the levels remain constant over the rest of the trial, but increase at week 36 when week 3 is not included. Non-linear regression of the *E. coli* levels in deer goo shows an exponential rate of decay of 0.72 MPN/g/week, with a half-life of 0.96 weeks with the week 3 data included, and a rate of decay of 0.67 MPN/g/week, with a half life of 1.03 weeks without the week 3 data (Figure 10).

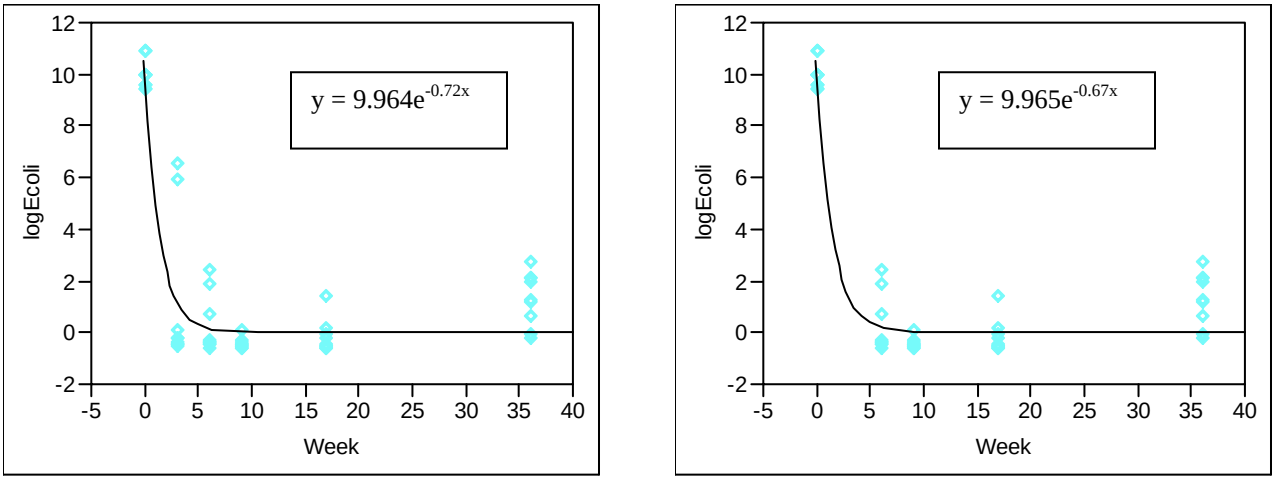


Figure 10: Exponential decay of *E. coli* in deer goo over time in the research piles
Graph on left is with and graph on right is without week 3 data.

Fecal Streptococci and Enterococci

There is no benchmark value for enterococci below which compost would be considered "safe", but the purple dotted line representing 6.3 $\log_{10}$ MPN/g as well as the red dotted line representing 40^{o} C has been put in the graphs for reference. Figures 11, 12 and 13, show fecal streptococcus and enterococci levels in deer goo, as well as pile temperatures in Cornell piles 1, 2 and 3 respectively.

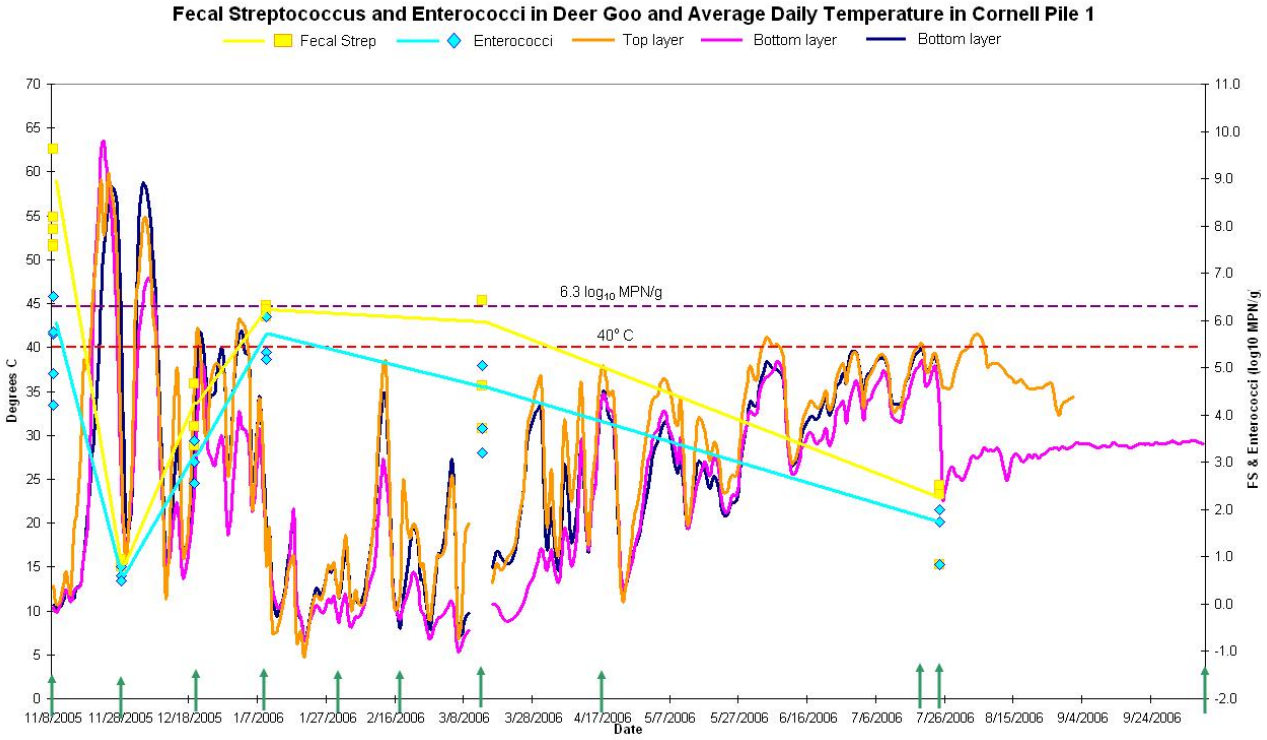


Figure 11: Fecal streptococcus and enterococci in the deer goo and average daily temperature in pile 1 at Cornell over time
(n=5 for week 0, n=3 for all other samplings)

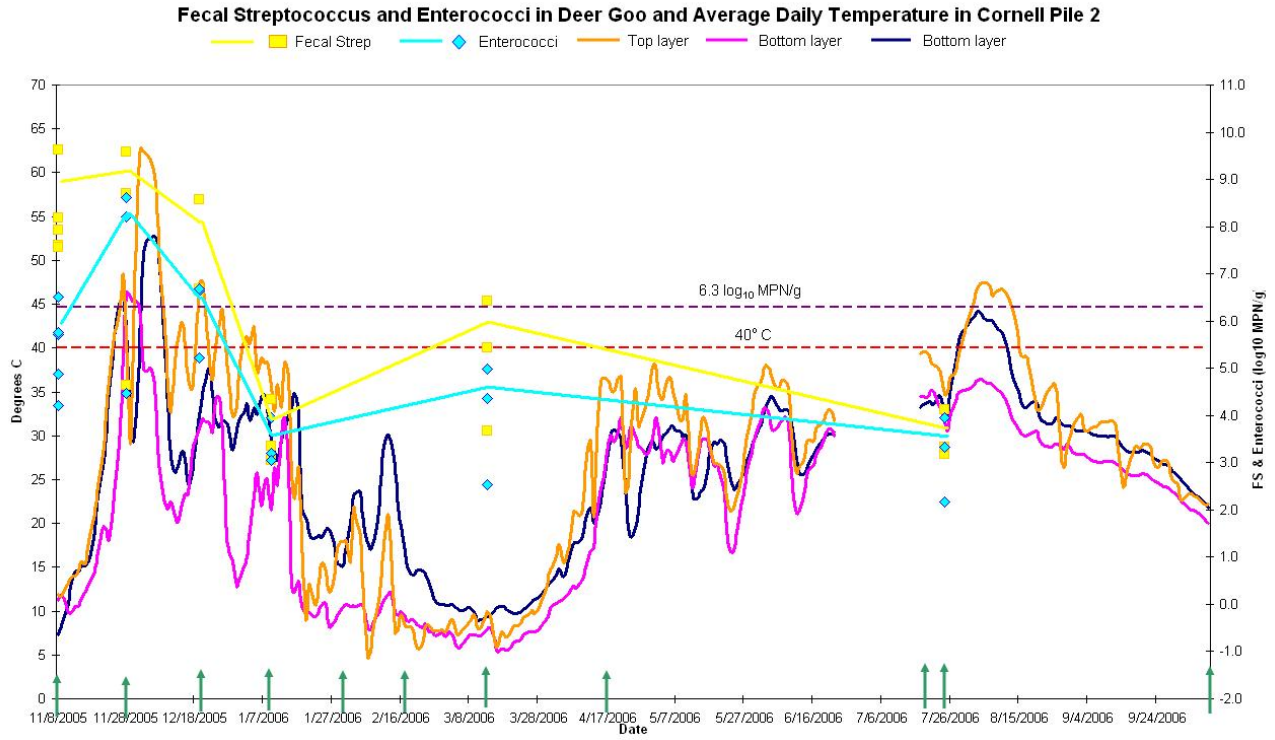


Figure 12: Fecal streptococcus and enterococci in the deer goo and average daily temperature in pile 2 at Cornell over time
(n=5 for week 0, n=3 for all other samplings)

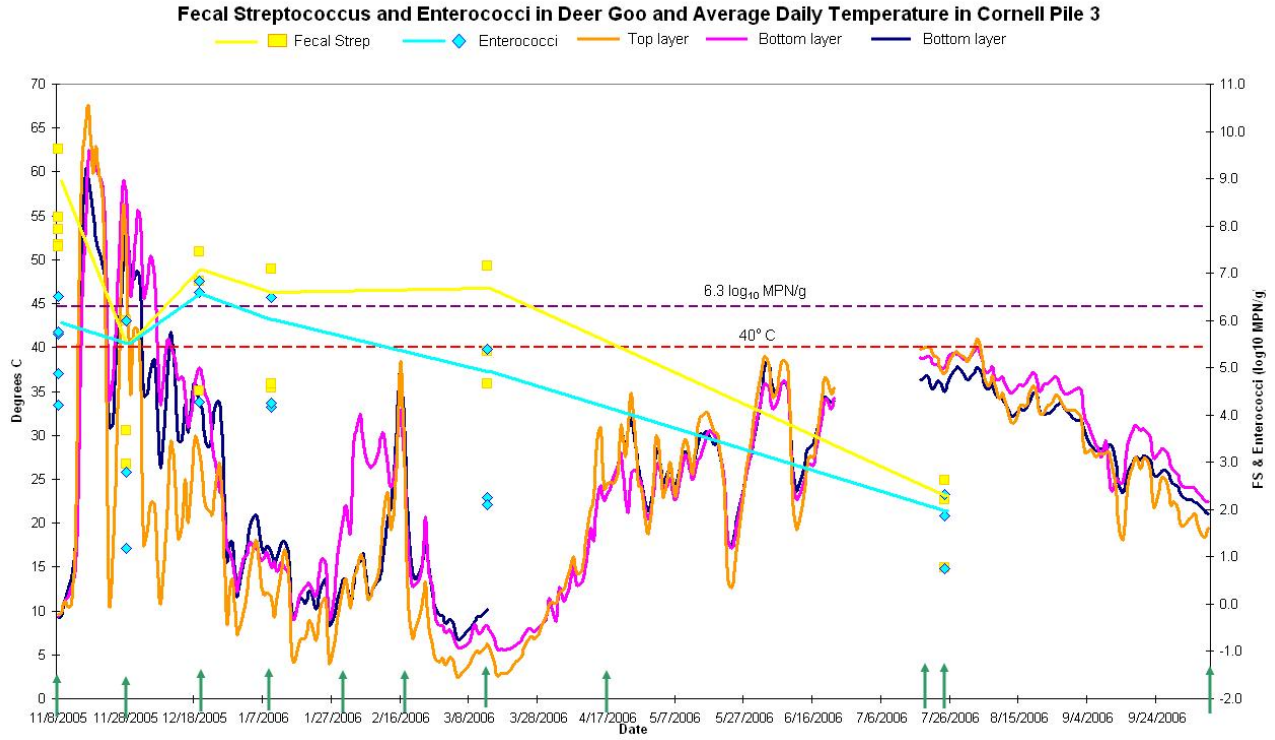


Figure 13: Fecal streptococcus and enterococci in the deer goo and average daily temperature in pile 3 at Cornell over time
(n=5 for week 0, n=3 for all other samplings)

Table 17: Fecal streptococci (log_{10} MPN/g solids) in deer goo samples from the 3 research piles over time with and without week 3

	Week 0	Week 3	Week 6	Week 9	Week 17	Week 36
1	9.63	0.79	4.66	6.32	4.62	0.83
2	6.61	0.95	3.34	6.23	3.72	2.34
3	6.92	0.79	3.75	6.08	6.43	2.51
4	6.57	9.59	6.64	3.34	5.43	4.11
5	8.18	4.64	8.57	3.04	3.66	3.18
6		8.70	6.67	4.34	6.41	3.32
7		3.67	4.51	7.08	4.65	2.62
8		6.00	6.84	4.58	5.34	0.78
9		2.97	7.45	4.66	7.15	2.20
Mean	**8.18^{a}**	**4.23^{b}**	**5.83^{ab}**	**5.08^{ab}**	**5.27^{ab}**	**2.43^{c}**
Mean (excluding week 3)	**8.18^{a}**		**5.83^{b}**	**5.08^{b}**	**5.27^{b}**	**2.43^{c}**

Mean values with differing superscripts are significantly different – $p < 0.05$

Fecal streptococcus in the deer goo decreased significantly by week 3 then decreased significantly again at the end (Table 17). Linear regression of fecal streptococcus levels in the deer goo shows a significant decrease of 0.124 MPN/g/week when the week 3 data was included and a decrease of 0.146 MPN/g/week when it was not included (due to different sample handling) (Figure 14). The correlation is better without week 3 (0.651) versus with (0.411).

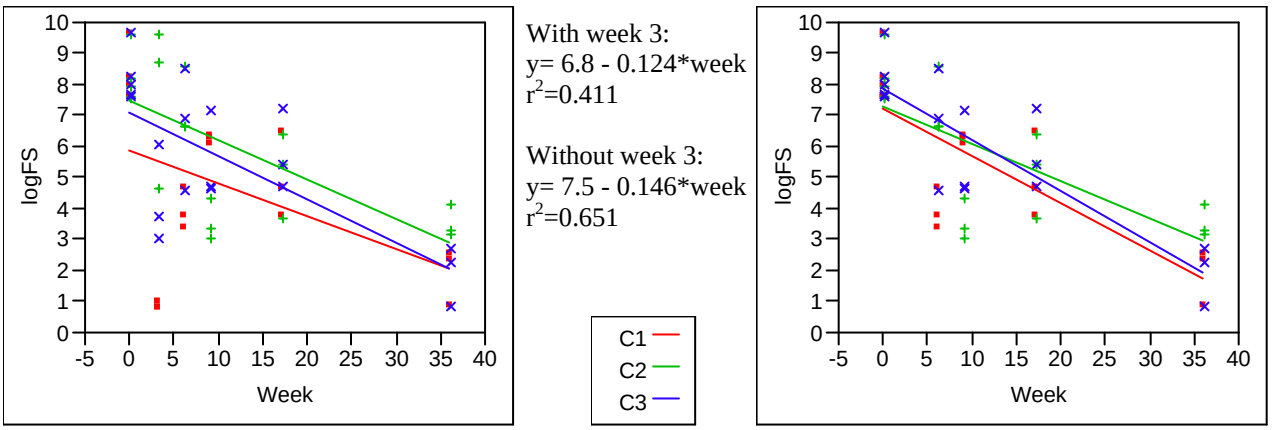


Figure 14: Linear regression of fecal streptococcus over time in the deer goo
Graph on left is with and graph on right is without week 3 data.

Table 18: Enterococci (log_{10} MPN/g solids) in deer goo samples from the 3 research piles over time with and without week 3

	Week 0	Week 3	Week 6	Week 9	Week 17	Week 36
1	6.51	0.79	2.56	5.32	3.20	0.83
2	5.72	0.60	3.00	5.18	3.72	2.00
3	4.20	0.49	3.46	6.08	5.04	1.73
4	5.76	8.61	5.23	3.20	4.36	3.95
5	4.88	4.46	6.63	3.04	2.53	2.18
6		8.20	6.67	3.96	4.99	3.32
7		2.80	4.28	6.48	2.26	2.32
8		6.00	6.84	4.18	2.11	0.74
9		1.18	6.60	4.26	5.40	1.86
Mean	**5.42^{a}**	**3.68^{ab}**	**5.03^{a}**	**4.63^{ab}**	**3.74^{ab}**	**2.10^{b}**
Mean (excluding week 3)	**5.42^{a}**		**5.03^{a}**	**4.63^{a}**	**3.74^{ab}**	**2.10^{b}**

Mean values with differing superscripts are significantly different – $p < 0.05$

Enterococci in the deer goo decreased significantly by week 36 (Table 18). Linear regression of the deer goo data shows a significant decrease of enterococci of 0.079 MPN/g/week when the week 3 data was included, and a decrease of 0.091 MPN/g/week when it was not included (due to different sample handling) (Figure 15). The correlation is better without week 3 (0.529) versus with (0.360).

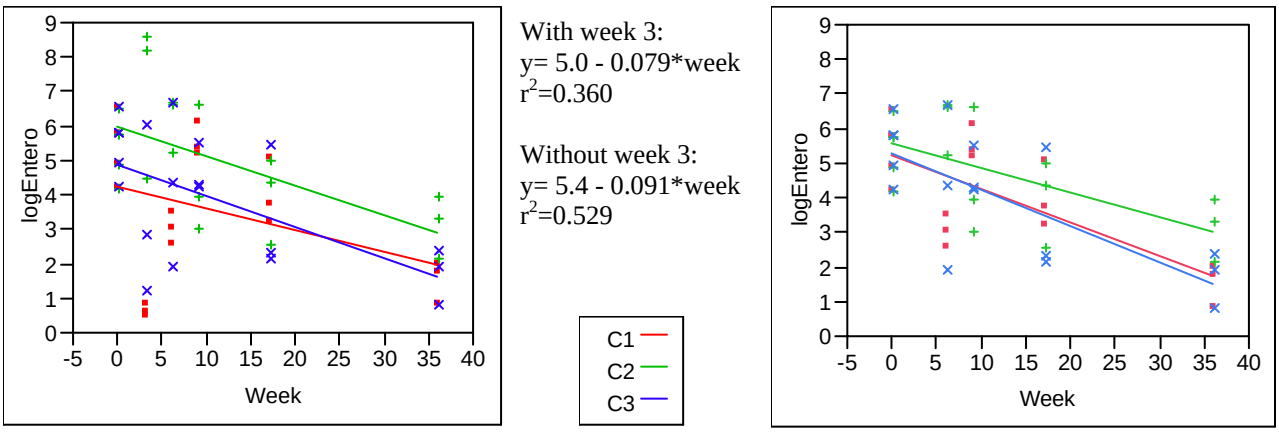


Figure 15: Linear regression of enterococci over time in the deer goo
Graph on left is with and graph on right is without week 3 data.

Pathogens in the Compost

Description

Statistical analysis of pathogen levels in the composts, deer goo and MAP was done using the S-Plus Statistical package. Differences in pathogen levels at each sampling were analyzed for the three pilot piles together, and the three research piles together using analysis of variance (ANOVA) for multiple comparisons with Tukey corrections. Linear regressions of pathogen levels over time were run for the three pilot piles together, as well as the three research piles together using the JMP Statistical package

Fecal Coliforms and *Escherichia coli* – Pilot Piles

The graphs for the pilot piles (Figure 16 for fecal coliforms and Figure 17 for *E. coli*) show each of the sites separately, while the tables show the analysis of all piles together. Individual samples are denoted by the yellow square, green diamond and turquoise triangle for Cortland, Highland and Watertown respectively, and the corresponding colored line shows the average of the three samples at each site. There was severe flooding in the Southern Tier in the last week of June 2006 and the Cortland pile (which sits next to a ditch) was surrounded by water during that time (just before the month 9 sampling on July 10th) which could explain the increase in fecal coliforms and *E. coli* at that site at that time. Therefore, statistical analysis on fecal coliforms and *E. coli* was also run without the Cortland month 9 samples and is shown in Tables 19 and 20.

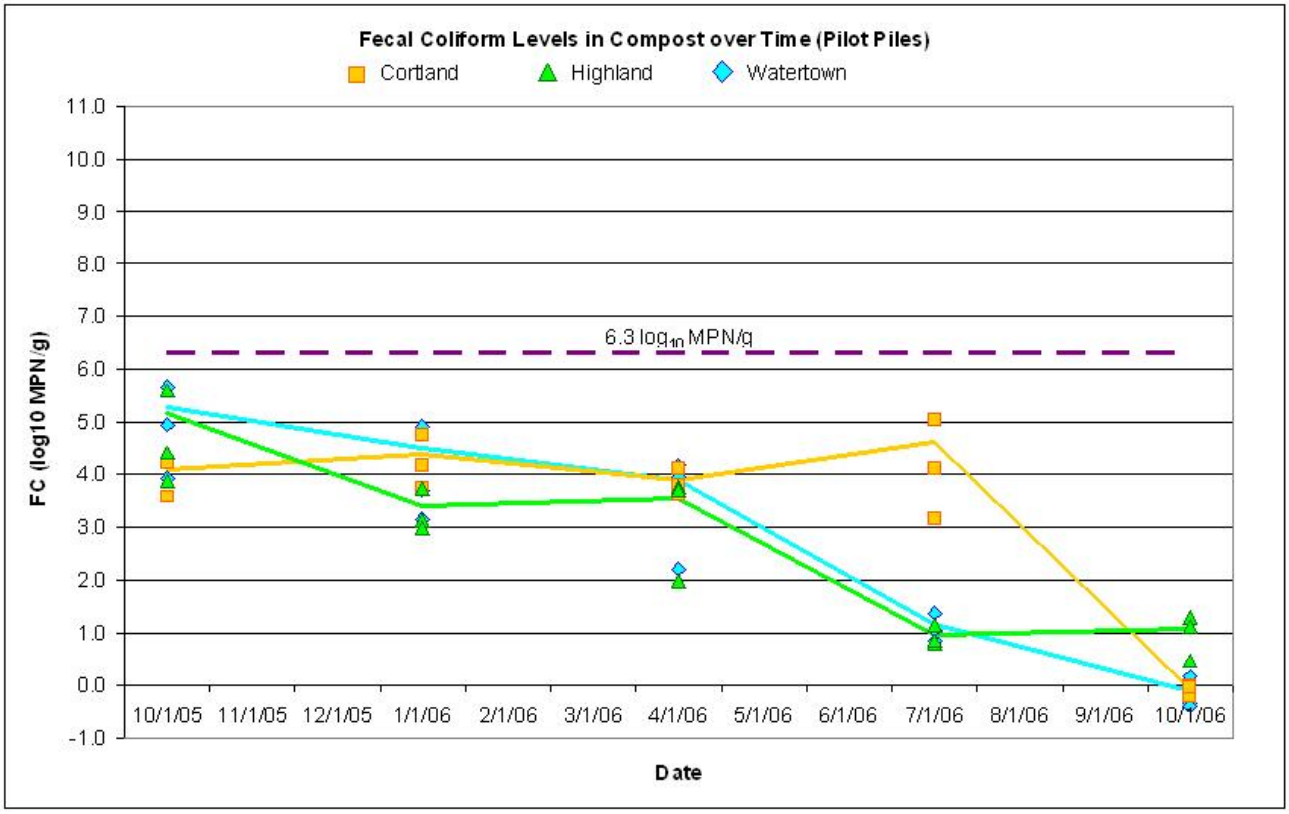


Figure 16: Fecal coliform levels in compost over time at the pilot sites
n=3 at each site

Fecal coliform levels in the compost were below the benchmark of 6.3 $\log_{10}$ MPN/g at all sites in the woodchips prior to composting (Figure 16). Fecal coliform levels were reduced significantly by month 9 (2.5 $\log_{10}$ reduction with the Cortland month 9 data [severe flooding may have caused an increase in fecal coliforms in the Cortland pile], and 3.5 $\log_{10}$ reduction without it), and then reduced significantly again by month 12 (Table 19).

Table 19: Fecal coliforms (log_{10} MPN/g solids) in compost (pilot piles) over time

	Month 0	Month 3	Month 6	Month 9	Month 12
Cortland	4.20	4.15	3.63	3.15	-0.22
Cortland	4.20	3.72	3.81	4.11	-0.03
Cortland	3.59	4.73	4.11	5.04	-0.06
Highland	3.88	3.11	3.76	0.78	0.46
Highland	4.40	2.97	1.98	1.15	1.28
Highland	5.60	3.74	3.70	0.85	1.11
Watertown	4.94	3.15	3.96	0.85	-0.36
Watertown	5.67	3.72	4.18	1.36	-0.39
Watertown	3.92	4.92	2.20	1.04	0.15
Mean	**4.49[a]**	**3.80[a]**	**3.48[a]**	**2.04[b]**	**0.22[c]**
Mean (excluding Cortland 9)	**4.49[a]**	**3.80[ab]**	**3.48[b]**	**1.00[c]**	**0.22[c]**

Mean values with differing superscripts are significantly different – $p < 0.05$

Linear regression of the pilot pile data for fecal coliforms showed a decrease of 0.082 log_{10} MPN/g /week with the Cortland month 9 data included, and a decrease of 0.088 log_{10}/week without it. In both cases, the r^2 value is high (0.734 and 0.825 respectively), indicating a reduction of fecal coliform numbers over time with composting (Figure 17).

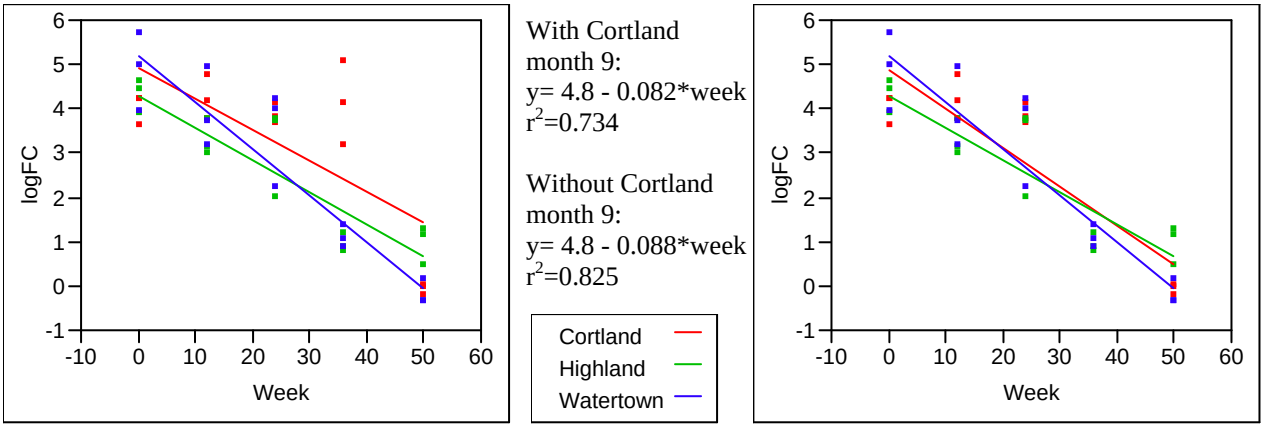


Figure 17: Linear regression of fecal coliforms over time in the pilot piles
Graph on left is with and graph on right is without month 9 Cortland data.

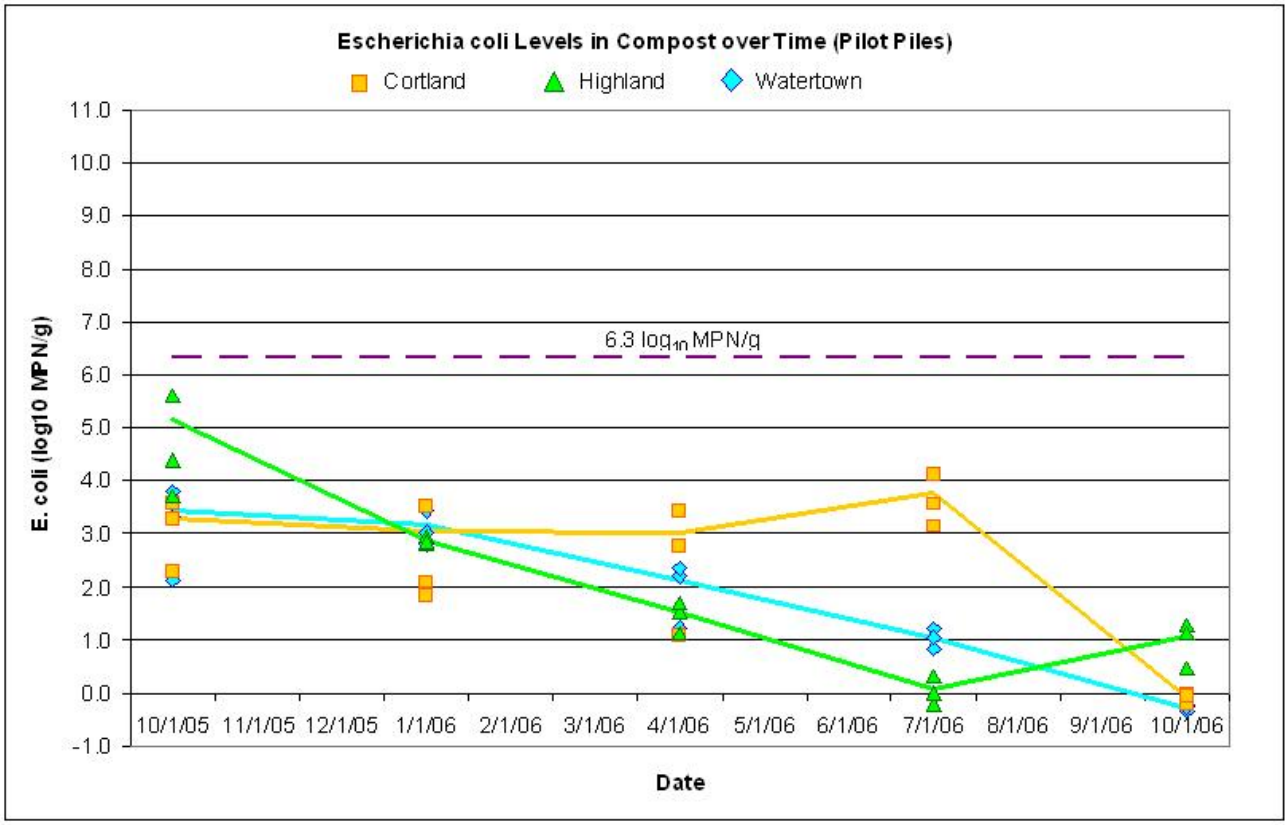


Figure 18: *Escherichia coli* levels in compost over time at the pilot sites.
n=3 at each site

Escherichia coli levels in the compost were below the benchmark of 6.3 $\log_{10}$ MPN/g at all sites in the woodchips prior to composting (Figure 17). Although *E. coli* levels rose again at month 9 in the Cortland pile, they were still well below 6.3 og_{10} MPN/g. For all pilot piles together, *E. coli* levels were reduced significantly by month 6 (1.5 $\log_{10}$ reduction), and then reduced significantly again by month 12 (2 $\log_{10}$ reduction, Table 20).

Table 20: *Escherichia coli* (log_{10} MPN/g solids) in compost (pilot piles) over time

	Month 0	Month 3	Month 6	Month 9	Month 12
Cortland	2.28	1.83	1.08	3.15	-0.22
Cortland	3.58	2.08	3.43	4.11	-0.03
Cortland	3.28	3.51	2.77	3.57	-0.06
Highland	3.72	2.94	1.11	0.30	0.46
Highland	4.40	2.83	1.54	-0.22	1.28
Highland	5.60	2.84	1.70	0.00	1.11
Watertown	3.32	2.79	2.20	0.85	-0.36
Watertown	3.80	3.43	2.34	1.20	-0.24
Watertown	2.11	3.04	1.23	1.04	-0.27
Mean	**3.57^{a}**	**2.81^{ab}**	**1.94^{b}**	**1.56^{b}**	**0.19^{c}**
Mean (excluding Cortland 9)	**3.57^{a}**	**2.81^{ab}**	**1.94^{b}**	**0.53^{c}**	**0.19^{c}**

Mean values with differing superscripts are significantly different – $p < 0.05$

Linear regression of the pilot pile data showed a decrease of 0.065 log_{10} MPN/g /week with the Cortland month 9 data included, and a decrease of 0.071 log_{10} MPN/g/week without it. In both cases, the r^2 value is high (0.636 and 0.759 respectively), indicating a reduction of *E. coli* numbers over time with composting (Figure 16).

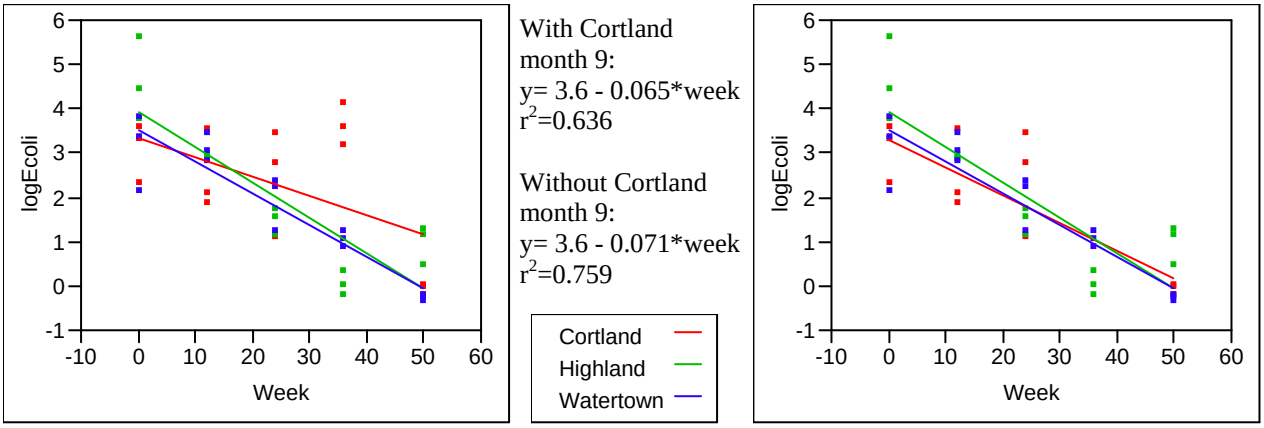


Figure 19: Linear regression of *Escherichia coli* over time in the pilot piles
Graph on left is with and graph on right is without month 9 Cortland data.

Fecal Coliforms and *Escherichia coli* – Research Piles

The graphs for the research piles (Figures 20 and 22) show each of the sites separately, while the tables show the analysis of all piles together. Individual samples are denoted by the yellow square, green diamond and turquoise triangle for Pile 1, Pile 2 and Pile 3 respectively, and the corresponding colored line shows the average of the three samples at each site. The tables show the results of statistical analysis of pathogen levels over time for all three piles combined. Because the research site piles were disturbed at month 12 more than they would be in a normal road-killed compost pile possibly

causing contamination of the pile (as described on page 7), statistical analysis was run with and without the month 12 values.

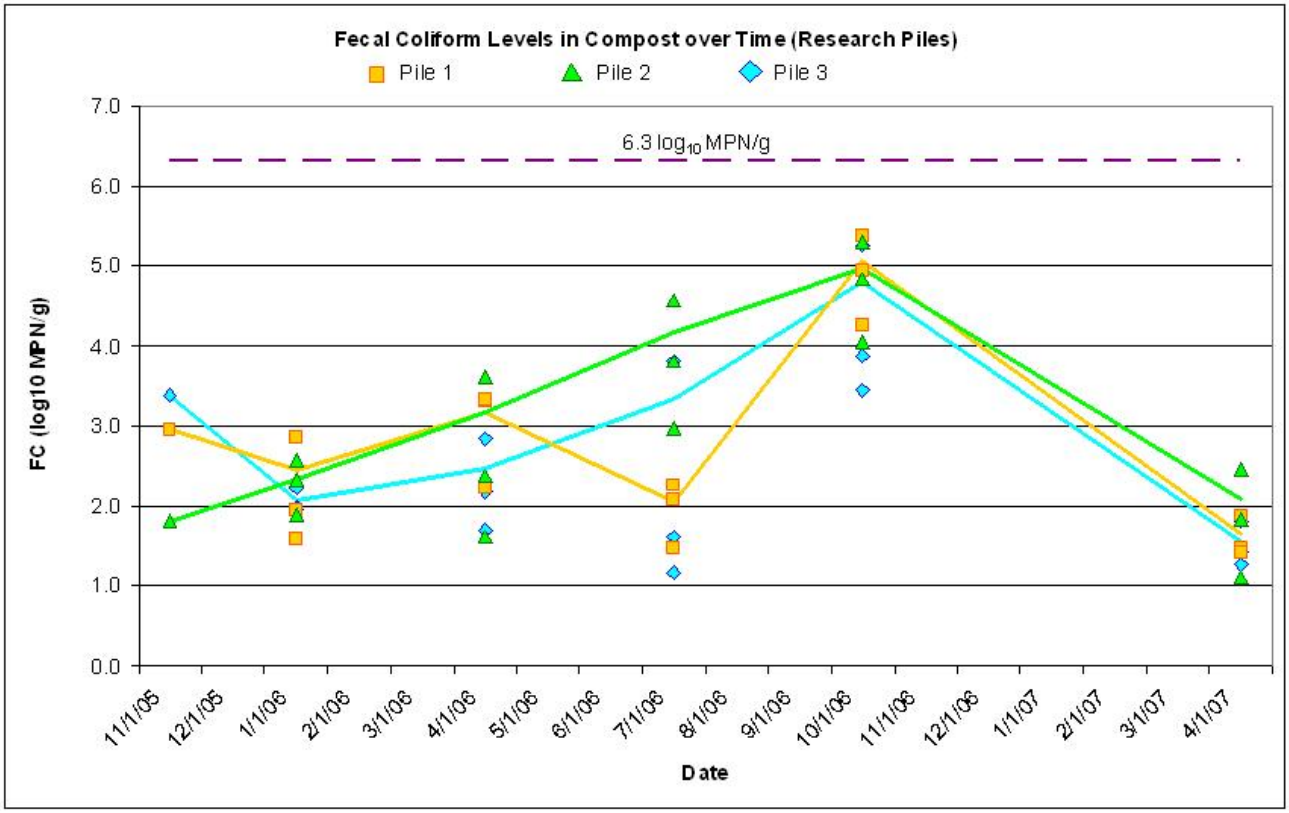


Figure 20: Fecal coliform levels in compost over time at the research site
n=3 for each pile

Fecal coliform levels were already below the benchmark figure of 6.3 $\log_{10}$, and remained below that level throughout the trial. However, at month 12, fecal coliform levels increased significantly by 2 $\log_{10}$. Additional sampling at month 18 showed a 3 $\log_{10}$ decrease from month 12, but no significant decrease from the earlier months. When month 12 data is excluded due to the large amount of disturbance that took place at week 36, fecal coliform levels in the compost remain the same throughout the study (Figure 20 and Table 21).

Table 21: Fecal coliforms (log_{10} MPN/g solids) in compost (research piles) over time

	Month 0	Month 3	Month 6	Month 9	Month 12	Month 18
Cornell 1-1	2.95	1.60	3.30	1.47	5.38	1.89
Cornell 1-2		1.94	3.34	2.08	4.26	1.49
Cornell 1-3		2.87	2.23	2.26	4.94	1.42
Cornell 2-1	1.82	2.57	3.62	2.96	4.04	1.84
Cornell 2-2		1.90	1.62	3.81	4.84	1.11
Cornell 2-3		2.32	2.38	4.57	5.30	2.46
Cornell 3-1	3.38	2.00	2.18	1.62	3.88	1.28
Cornell 3-2		1.97	1.71	1.18	5.26	1.82
Cornell 3-3		2.23	2.85	3.81	3.45	1.43
Mean	**2.72[a]**	**2.15[a]**	**2.58[a]**	**2.75[a]**	**4.59[b]**	**1.64[a]**
Mean (excluding month 12)	**2.72[a]**	**2.15[a]**	**2.58[a]**	**2.75[a]**		**1.64[a]**

Mean values with differing superscripts are significantly different – $p < 0.05$

Linear regression of the research pile data showed that fecal coliform levels remained constant over time both with and without the month 12 data (Figure 21). This is different from the pilot piles, where there was a significant decrease in fecal coliform levels over time, because those piles started with high coliform counts in the initial woodchips. In contrast, coliform counts in the Cornell research piles began and remained low.

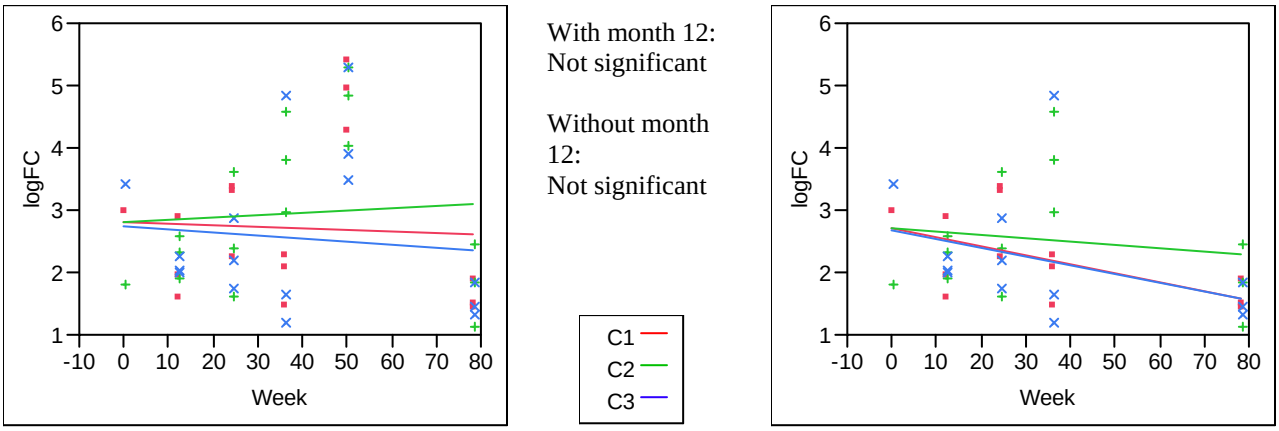


Figure 21: Linear regression of fecal coliforms over time in the research piles
Graph on left is with and graph on right is without month 12 data.

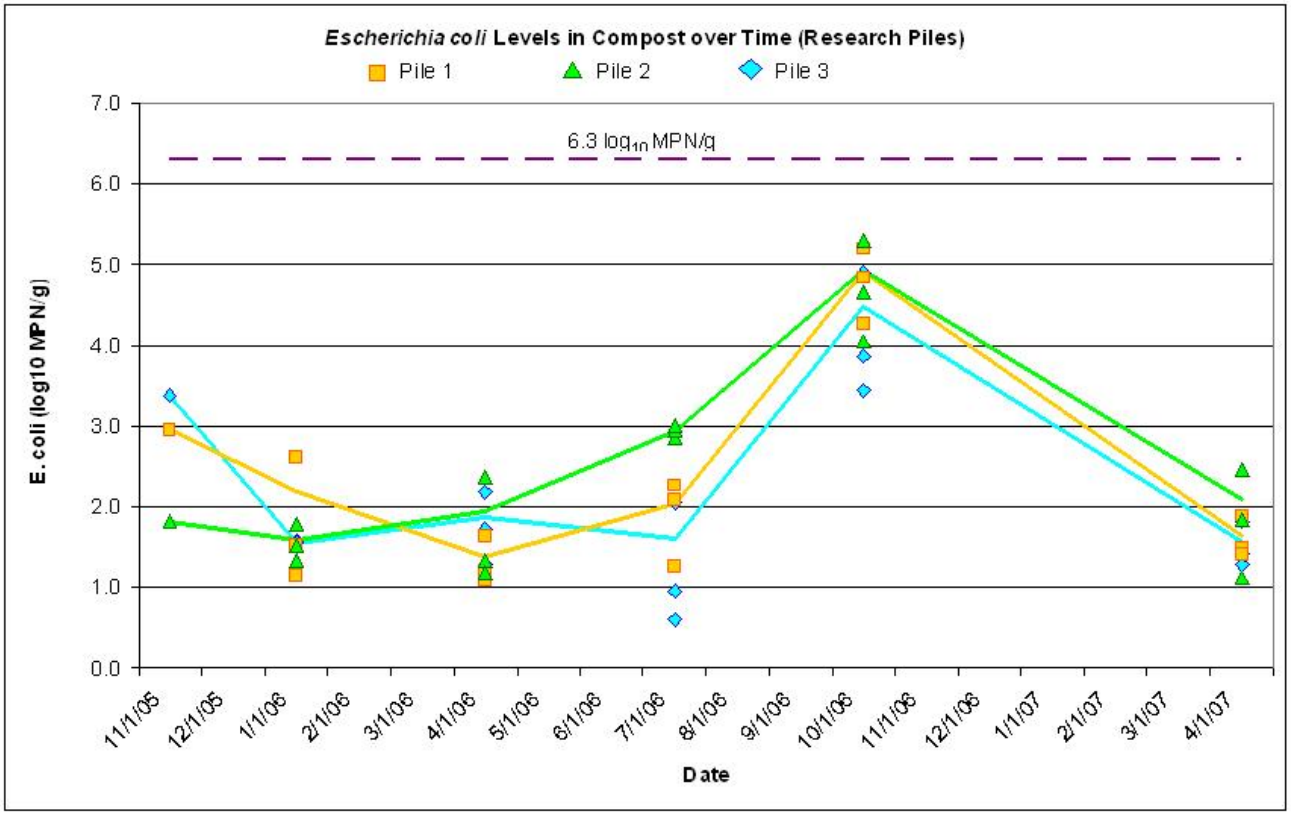


Figure 22: *Escherichia coli* levels in compost over time at the research site
n=3 for each pile

E. coli levels in the research piles remained the same over time until month 12 when they increased by 2 $\log_{10}$. Additional sampling at month 18 showed a 3 $\log_{10}$ decrease from month 12, but no significant decrease from the earlier months (Figure 22 and Table 22). When month 12 data is excluded due to the large amount of disturbance that took place at week 36, the only difference is that month 6 *E. coli* levels are significantly lower than month 0 levels.

Table 22: *Escherichia coli* (log_{10} MPN/g solids) in compost (research piles) over time

	Month 0	Month 3	Month 6	Month 9	Month 12	Month 18
Cornell 1-1	2.95	1.15	1.20	1.26	5.20	1.89
Cornell 1-2		1.53	1.63	2.08	4.26	1.49
Cornell 1-3		2.60	1.08	2.26	4.83	1.42
Cornell 2-1	1.82	1.79	2.36	2.85	4.04	1.84
Cornell 2-2		1.52	1.32	2.94	4.65	1.11
Cornell 2-3		2.32	1.18	3.00	5.30	2.46
Cornell 3-1	3.38	1.54	2.18	0.95	3.88	1.28
Cornell 3-2		1.57	1.30	0.60	4.90	1.82
Cornell 3-3		1.56	1.72	2.04	3.45	1.43
Mean	**2.72^{a}**	**1.62^{a}**	**1.55^{a}**	**2.00^{a}**	**4.50^{b}**	**1.63^{a}**
Mean (excluding month 12)	**2.72^{a}**	**1.62^{ab}**	**1.55^{b}**	**2.00^{ab}**		**1.63^{ab}**

Mean values with differing superscripts are significantly different – $p < 0.05$

Linear regression of the research pile data showed that *E. coli* levels remained constant over time both with and without the month 12 data (Figure 23). Again, this is different from the pilot piles, where there was a significant decrease in fecal coliform levels over time, because those piles started with high coliform counts in the initial woodchips. In contrast, coliform counts in the Cornell research piles began and remained low.

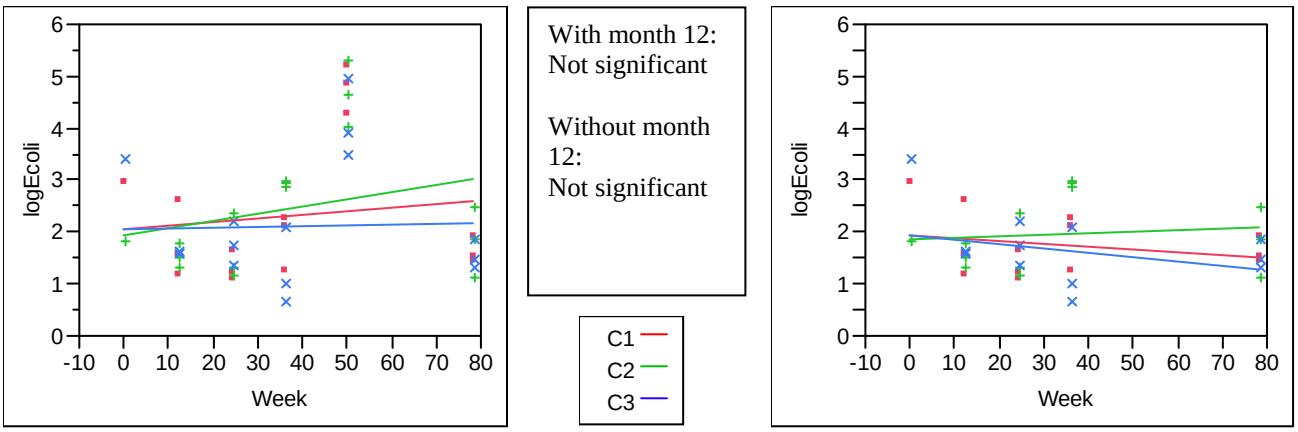


Figure 23: Linear regression of *E. coli* over time in the research piles
Graph on left is with and graph on right is without month 12 data.

Fecal Coliforms and *Escherichia coli* in Other Piles

Samples of compost were taken from three additional road-killed compost piles in New York State (Angola and LeRoy NYS Thruway Authority sites and Salamanca, NYS DOT site) that had been composting road-kill for over a year. Triplicate samples were taken from piles at each site that were considered finished and were being used on highway

rights-of-way. They were analyzed for pathogen content. Table 23 shows the levels of fecal coliforms and *E. coli* found in the samples. The level of fecal coliforms and *E. coli* in these samples was essentially zero indicating good pathogen kill in the finished compost.

Table 23: Fecal coliforms and *Escherichia coli* (log_{10} MPN/g solids) in finished road-killed compost at Angola, LeRoy and Salamanca

	Fecal Coliforms	*Escherichia coli*
Angola 1	< 0.34	< 0.34
Angola 2	0.28	0.04
Angola 3	< 0.37	< 0.37
LeRoy 1	< 0.27	< 0.27
LeRoy 2	< 0.28	< 0.28
LeRoy 3	< 0.28	< 0.28
Salamanca 1	0.11	0.11
Salamanca 2	< 0.24	< 0.24
Salamanca 3	< 0.30	< 0.30

Fecal Streptococci and Enterococci – Pilot Piles

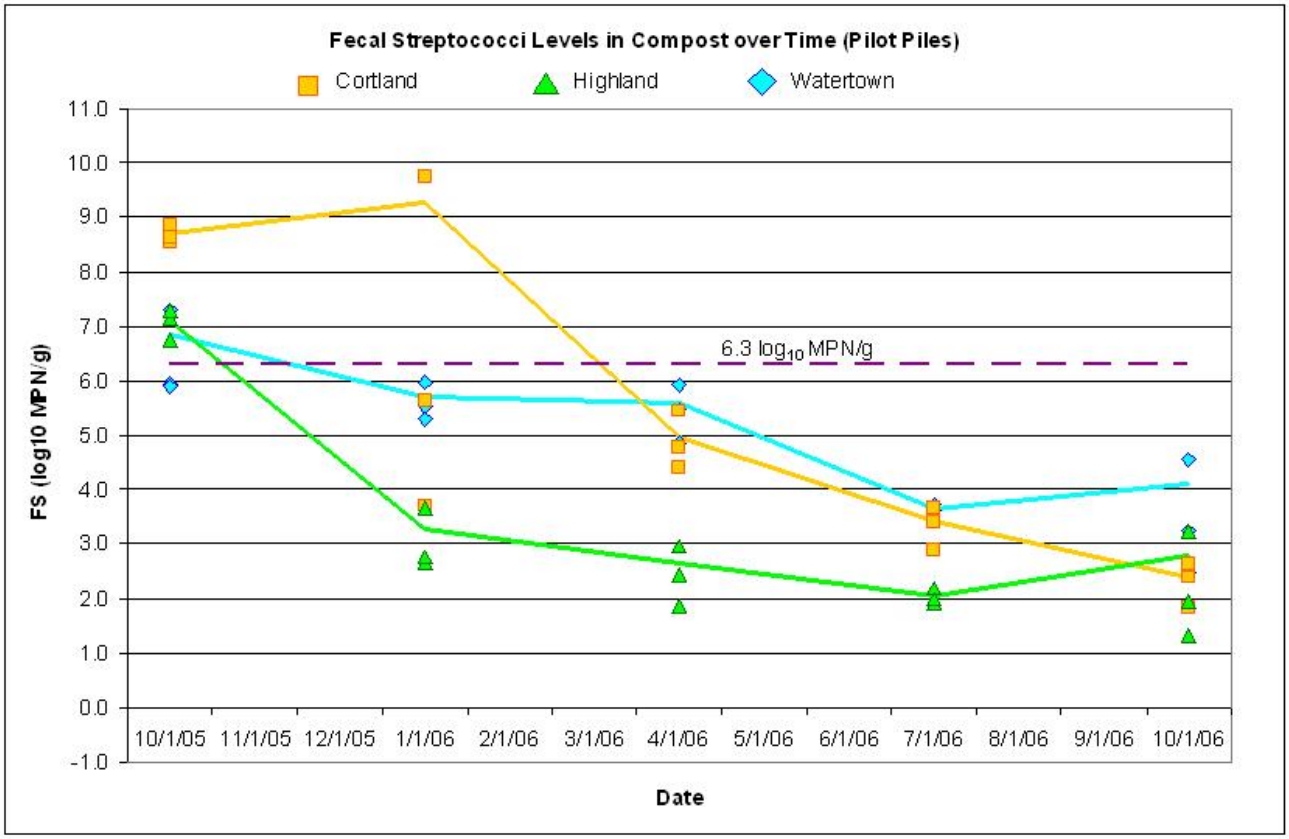


Figure 24: Fecal streptococcus levels in compost over time at the pilot sites
n=3 at each site

Fecal streptococcus decreased by approximately 3 $\log_{10}$ after 6 months in the compost pile at Cortland and an additional 3 $\log_{10}$ by 12 months. At Highland, it decreased by 3 $\log_{10}$ after 3 months in the compost pile and an additional 1 $\log_{10}$ by 12 months. It took longer at the Watertown pile to see any decrease in fecal streptococcus. There was a 3 $\log_{10}$ reduction for fecal strep at month 9 with no additional decrease at month 12 in Watertown (Figure 24).This may have resulted from the lower temperatures in that pile. For all pilot piles together, fecal streptococcus levels were reduced significantly by month 3 (2.3 $\log_{10}$ reduction), and then reduced significantly an additional 2 $\log_{10}$ by month 9 (Table 24).

Table 24: Fecal streptococci ($\log_{10}$ MPN/g solids) in compost (pilot piles) over time

	Month 0	Month 3	Month 6	Month 9	Month 12
Cortland	8.54	3.70	5.45	2.89	2.63
Cortland	8.86	5.61	4.78	3.41	2.38
Cortland	8.62	9.76	4.40	3.64	1.84
Highland	6.74	2.65	2.96	1.92	1.93
Highland	7.15	2.77	1.85	2.18	3.23
Highland	7.30	3.66	2.45	1.99	1.32
Watertown	7.28	5.52	5.48	3.60	3.23
Watertown	5.95	5.30	4.85	3.62	2.48
Watertown	5.90	5.98	5.92	3.73	4.56
Mean	**7.37^{a}**	**5.00^{b}**	**4.24^{bc}**	**3.00^{c}**	**2.62^{c}**

Mean values with differing superscripts are significantly different – $p < 0.05$

Linear regression of the pilot pile fecal coliform data showed a decrease of 0.094 $\log_{10}$ MPN/g /week with an r^2 value of 0.756 (Figure 25).

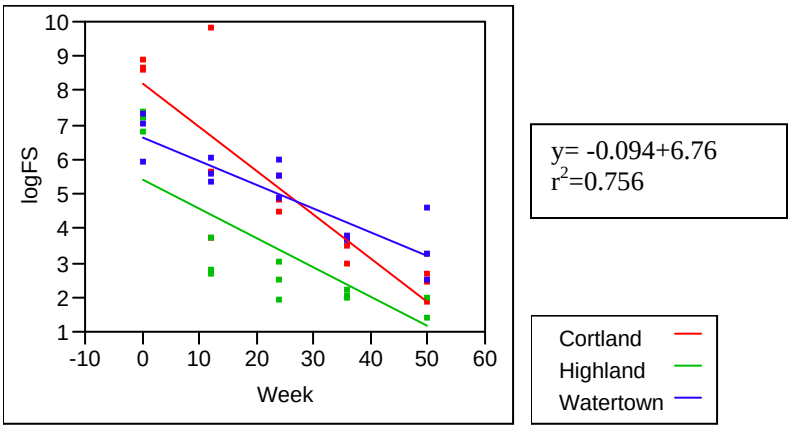


Figure 25: Linear regression of fecal streptococcus over time in the pilot piles.

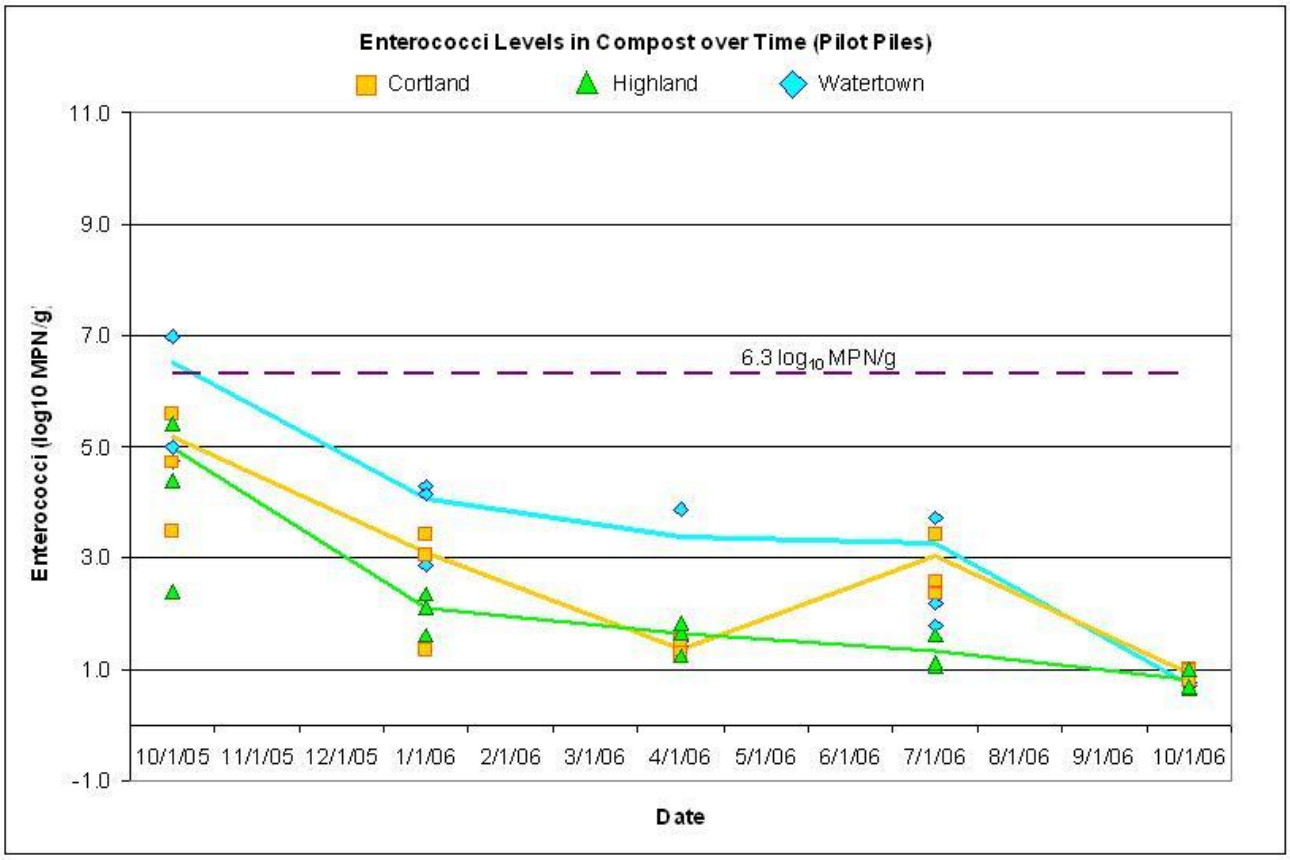


Figure 26: Enterococci levels in compost over time at the pilot sites
n=3 at each site

Enterococci levels in the compost at the pilot sites followed the same pattern as fecal streptococcus. In Cortland and Highland, the levels had decreased by approximately 3 $\log_{10}$ after 6 months of composting, while the Watertown levels took longer to decrease (Figure 26). All piles together show a significant decrease of 2 $\log_{10}$ by month 3 and an additional 2 $\log_{10}$ after 12 months (Table 25).

Table 25: Enterococci (log_{10} MPN/g solids) in compost (pilot piles) over time

	Month 0	Month 3	Month 6	Month 9	Month 12
Cortland	5.59	1.34	1.20	2.36	0.78
Cortland	4.72	3.43	1.34	3.41	1.00
Cortland	3.48	3.04	1.51	2.57	0.95
Highland	4.40	1.60	1.65	1.04	0.67
Highland	5.40	2.34	1.23	1.61	1.00
Highland	2.41	2.11	1.83	1.11	0.70
Watertown	6.97	2.87	1.48	2.18	0.70
Watertown	4.74	4.30	1.40	1.79	0.76
Watertown	5.00	4.15	3.86	3.73	0.70
Mean	**4.75**^{a}	**2.80**^{b}	**1.72**^{bc}	**2.20**^{bc}	**0.81**^{c}

Mean values with differing superscripts are significantly different – $p < 0.05$

Linear regression of the pilot pile data showed a decrease of 0.068 log_{10} MPN/g /week with an r^2 value of 0.666 (Figure 27).

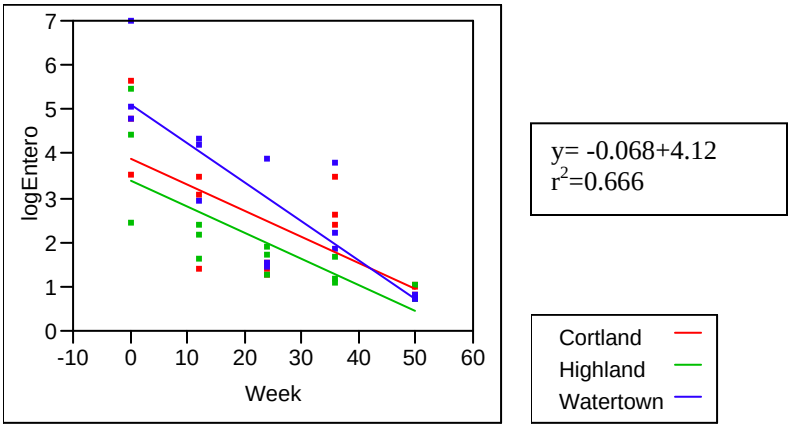


Figure 27: Linear regression of enterococci over time in the pilot piles.

Fecal Streptococci and Enterococci – Research Piles

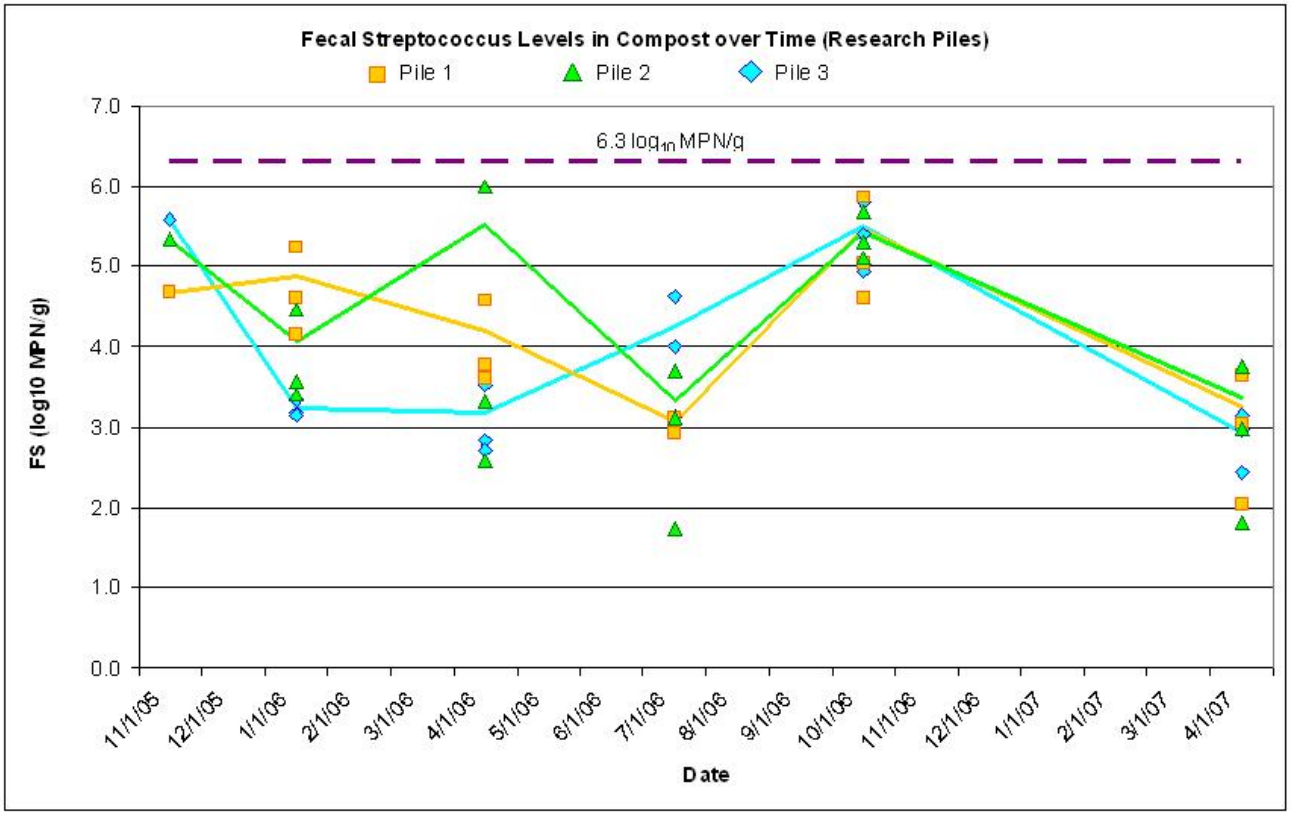


Figure 28: Fecal streptococcus levels in compost over time at the research site
n=3 for each pile

Fecal streptococcus in the samples from the initial wood chips was lower by approximately 2 $\log_{10}$ than levels in the pilot piles. These levels in the research piles decreased by 2 $\log_{10}$ by month 6, remained there through month 9 then increased back to original levels at month 12, although all levels were below 6.3 $\log_{10}$ MPN/g. Levels measured at month 18 are similar to those at month 12 in the pilot piles. If the month 12 data is not included, there is a significant reduction in fecal strep levels by month 9 that remains low in month 18 (Table 26).

Table 26: Fecal streptococci (log_{10} MPN/g solids) in compost (research piles) over time

	Month 0	Month 3	Month 6	Month 9	Month 12	Month 18
Cornell 1-1	4.67	4.15	4.58	3.11	5.04	3.04
Cornell 1-2		5.23	3.60	3.11	4.61	3.63
Cornell 1-3		4.61	3.77	2.93	5.86	2.04
Cornell 2-1	5.34	3.57	2.59	1.73	5.11	2.99
Cornell 2-2		3.41	5.99	3.11	5.30	1.81
Cornell 2-3		4.46	3.32	3.70	5.69	3.76
Cornell 3-1	5.58	3.18	2.84	3.13	5.40	2.96
Cornell 3-2		3.34	3.52	4.0	5.80	3.15
Cornell 3-3		3.15	2.72	4.62	4.94	2.43
Mean	**5.20^{ab}**	**3.90^{bc}**	**3.66^{c}**	**3.27^{c}**	**5.31^{a}**	**2.87^{c}**
Mean (excluding month 12)	**5.20^{a}**	**3.90^{ab}**	**3.66^{ab}**	**3.27^{b}**		**2.87^{b}**

Mean values with differing superscripts are significantly different – $p < 0.05$

Linear regression of the research pile data was not significant when month 12 data were included, but there was a significant decrease of 0.02 MPN/g/week without month 12 data, although the r^2 value was low (Figure 29).

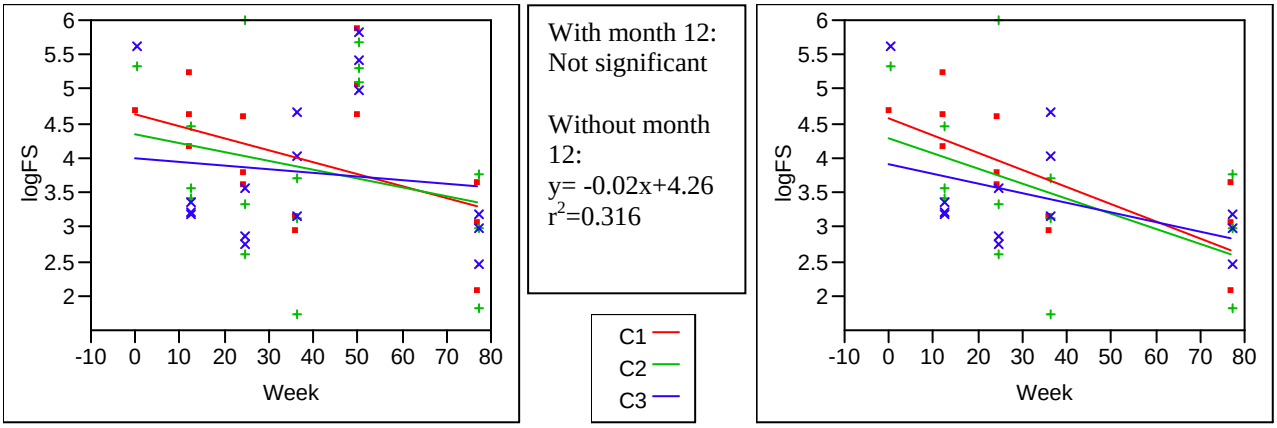


Figure 29: Linear regression of fecal streptococcus over time in the research piles
Graph on left is with and graph on right is without month 12 data.

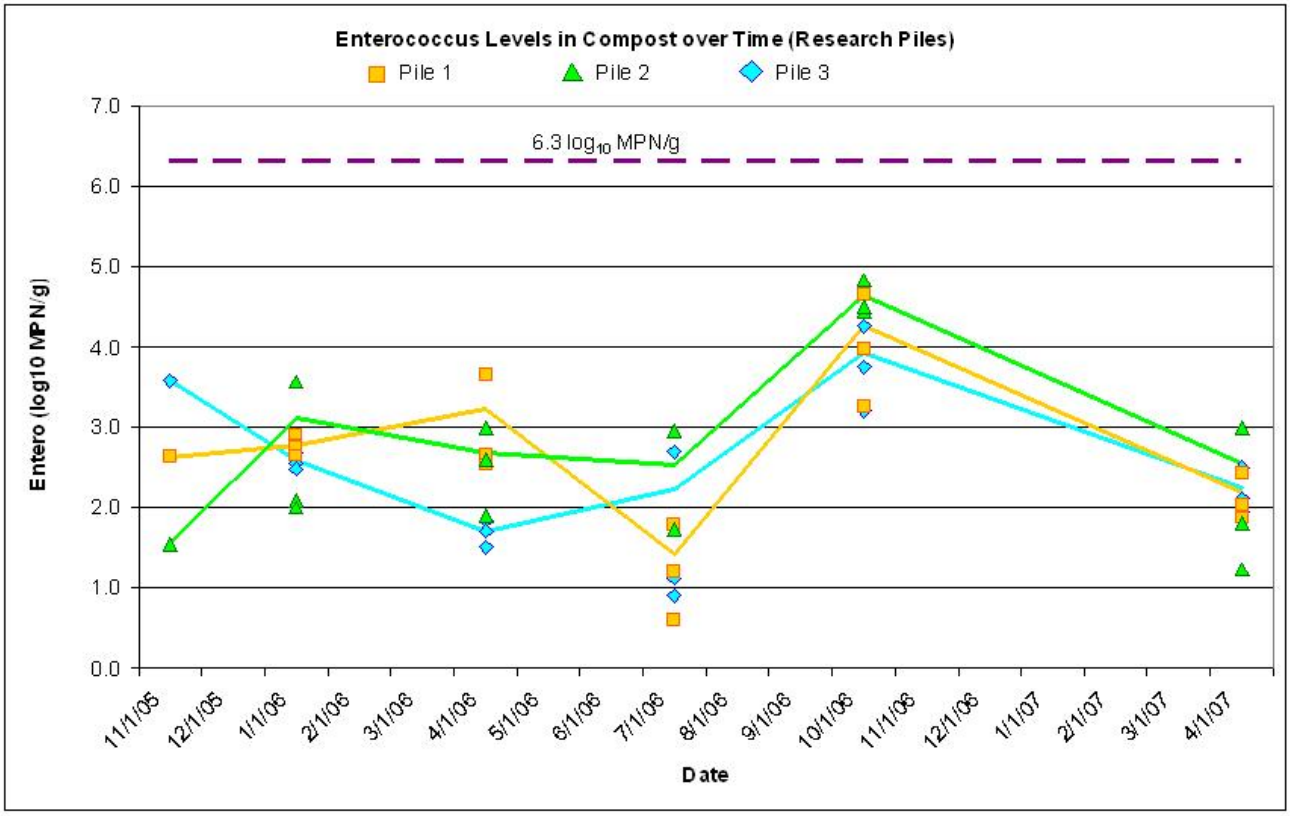


Figure 30: Enterococci levels in compost at the research site
n=3 for each pile

Enterococcus levels remained fairly constant over time with the exception of month 12 levels (Figure 30). Enterococcus in the samples from the initial wood chips was lower by approximately 2 $\log_{10}$ than levels in the pilot piles. These levels in the research piles decreased by 1 $\log_{10}$ by month 9, then increased to above original levels at month 12. Levels measured at month 18 are similar to those at month 12 in the pilot piles and back to original levels in the research piles. If the month 12 data is not included, there is a significant reduction in enterococci levels by month 9 that remains low in month 18 (Table 27).

Table 27: Enterococci (log_{10} MPN/g solids) in compost (research piles) over time

	Month 0	Month 3	Month 6	Month 9	Month 12	Month 18
Cornell 1-1	2.63	2.75	3.64	1.20	3.98	1.89
Cornell 1-2		2.64	2.53	1.79	3.26	2.43
Cornell 1-3		2.90	2.67	0.60	4.64	2.18
Cornell 2-1	1.54	3.57	2.60	1.73	4.43	2.99
Cornell 2-2		2.08	2.99	2.96	4.51	1.81
Cornell 2-3		2.0	1.91	1.72	4.83	1.23
Cornell 3-1	3.58	2.54	1.84	0.90	3.20	1.94
Cornell 3-2		2.68	1.52	1.11	4.26	2.49
Cornell 3-3		2.48	1.72	2.69	3.75	2.11
Mean	**2.59^{ab}**	**2.63^{a}**	**2.38^{ab}**	**1.64^{b}**	**4.10^{c}**	**2.10^{ab}**
Mean (excluding month 12)	**2.59^{ab}**	**2.63^{a}**	**2.38^{ab}**	**1.64^{b}**		**2.10^{ab}**

Mean values with differing superscripts are significantly different – $p < 0.05$

Linear regression of the research pile data showed that enterococci levels remained constant over time both with and without the month 12 data (Figure 31).

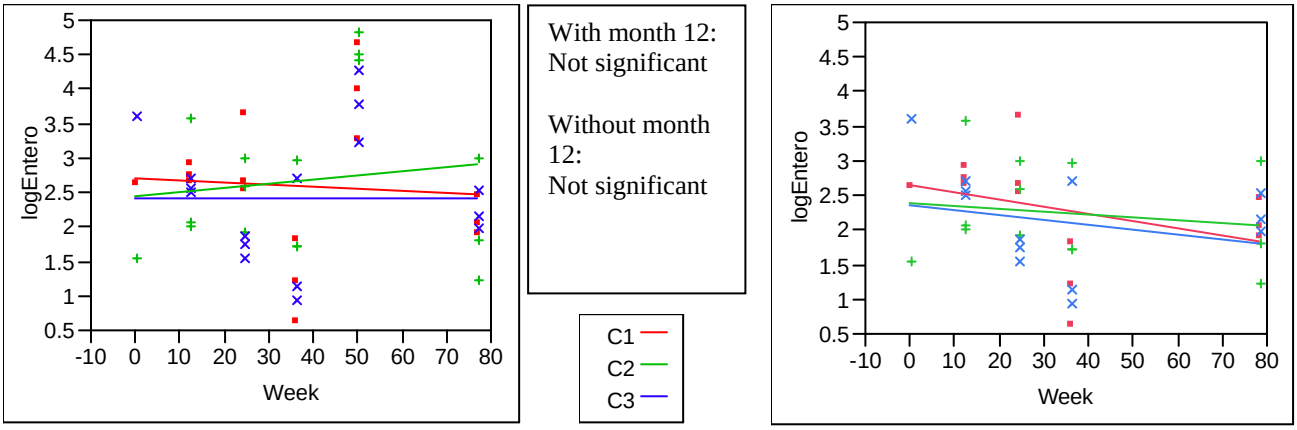


Figure 31: Linear regression of enterococci over time in the research piles
Graph on left is with and graph on right is without month 12 data.

Fecal Streptococci and Enterococci in Other Piles

Samples of compost were taken from three additional road-killed compost piles in New York State (Angola and LeRoy NYS Thruway Authority sites and Salamanca, NYS DOT site) that had been composting road-kill for over a year. Triplicate samples were taken from piles at each site that were considered finished and were being used on highway rights-of-way. They were analyzed for pathogen content. Table 28 shows the levels of fecal streptococcus and

enterococci found in the samples. The level of fecal streptococcus and enterococci in these samples was essentially zero indicating good pathogen kill in the finished compost.

Table 28: Fecal streptococcus and enterococci (log_{10} MPN/g solids) in finished road-killed compost at Angola, LeRoy and Salamanca

	Fecal Streptococcus	Enterococcus
Angola 1	2.76	< 0.66
Angola 2	1.72	< 0.65
Angola 3	< 0.63	< 0.63
LeRoy 1	0.78	< 0.73
LeRoy 2	1.36	< 0.72
LeRoy 3	< 0.70	< 0.70
Salamanca 1	0.11	0.11
Salamanca 2	< - 0.24	< -0.24
Salamanca 3	< - 0.30	< -0.30

Mycobacterium avium paratuberculosis (MAP)

Sampling for MAP occurred at weeks 0, 3, 6, 9, 12 and 36 in the Cornell research piles as described previously (page 8). Analysis was done at the Animal Health Diagnostic Center at the New York State College of Veterinary Medicine at Cornell University. When the number of colonies found on a plate is greater than 300 colonies (which equates to 3.35 log 10 colony forming units/gram wet weight), it is reported as "Too Numerous To Count (TNTC)". At week 0, we expected all of the samples to have greater than 300 colonies, and thus had the lab run the appropriate dilutions to be able to enumerate the colonies. In the remaining weeks, we did not ask for that enumeration. One of the samples at week 36 came back as TNTC. As it takes 3 months to grow the colonies for counting, we were not able to have them go back and run the appropriate dilutions to get an actual value. Therefore, the statistics for MAP were run using the value of 300 colonies or 3.35 log_{10} cfu/g for that particular sample.

MAP levels decreased significantly to near 0; (from 4.51 log_{10} cfu/g to 0.19 log_{10} cfu/g) within 3 weeks, and remained at those levels until week 36. At week 36, several of the samples had values of around 2 log_{10} cfu/g, (actual counts of 1 – 30 colonies) which would be considered "few" for dairy cattle, posing a slight risk for the spread of Johne's disease to other cattle. One of the samples contained "many" or too numerous to count. It is not clear why these samples had these levels of MAP since this organism is not thought to be able to reproduce outside of a host, and thus it is puzzling how an increase could occur. One possible explanation may be that because MAP tends to clump, they were either not detected in the laboratory on previous sample testing or the aliquots placed in the sentinel bags were not uniformly contaminated. It is also possible that some early change in the pile environment renders the MAP organisms difficult to grow in culture, yet not completely inactivated, and that later pile dynamics allow them to recover to the point of easier culturing capability.

Figure 32 shows the values for MAP over time in each of the research piles. The purple diamonds show individual values and the line shows the average of all 9 samples (three from each of the 3 research piles) at each date. Table 29 shows the results of the statistical analysis (ANOVA for multiple comparisons with Tukey corrections).

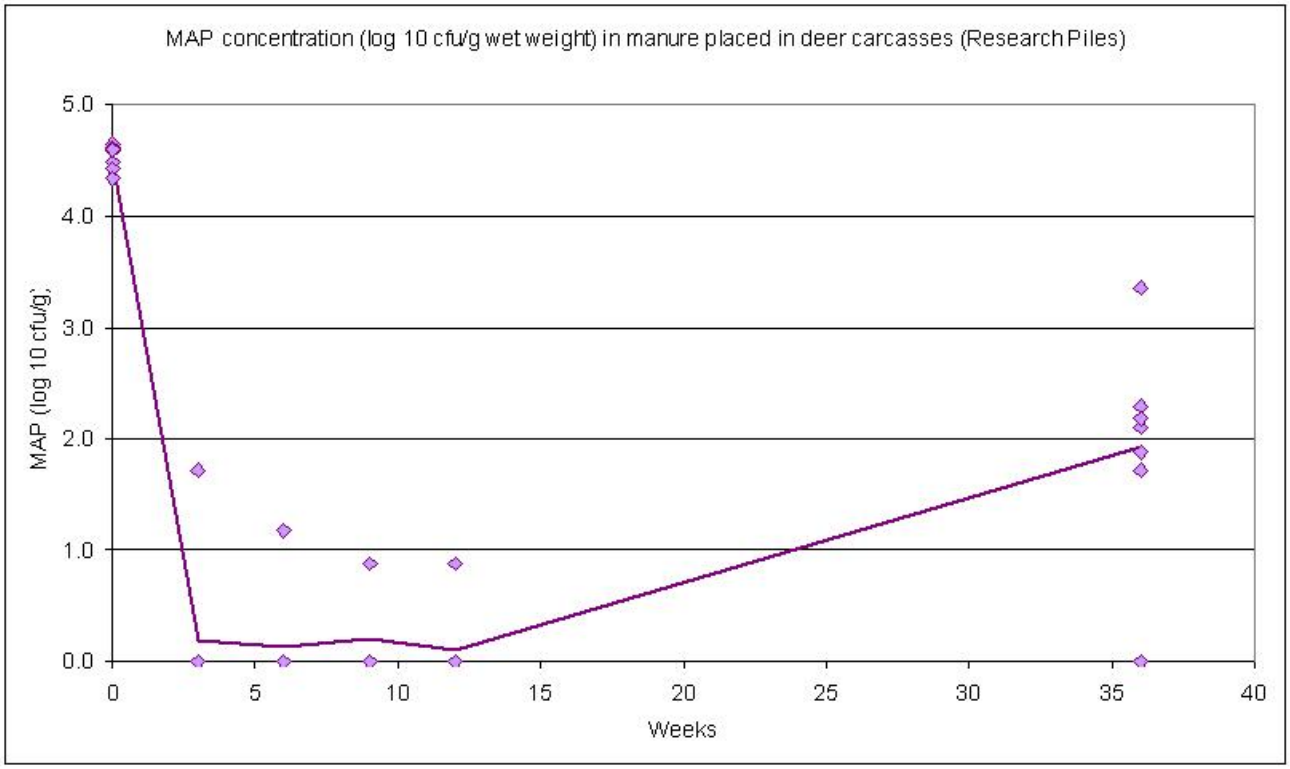


Figure 32: *Mycobacterium avium paratuberculosis* in manure in the compost piles at Cornell over time
(n=8 for week 36 and n=9 for all other samplings)

Table 29: MAP (log_{10} cfu/g wet weight) in manure over time

	Week 0	Week 3	Week 6	Week 9	Week 12	Week 36
1	4.58	0.00	0.00	0.00	0.00	2.11
2	4.58	0.00	0.00	0.00	0.00	1.88
3	4.65	0.00	0.00	0.88	0.88	No sample
4	4.35	1.72	0.00	0.00	0.00	2.29
5	4.49	0.00	1.18	0.00	0.00	1.72
6	4.42	0.00	0.00	0.88	0.00	0.00
7	4.62	0.00	0.00	0.00	0.00	1.88
8	4.34	0.00	0.00	0.00	0.00	3.35
9	4.60	0.00	0.00	0.00	0.00	2.18
Mean	**4.51^{a}**	**0.19^{b}**	**0.13^{b}**	**0.19^{b}**	**0.10^{b}**	**1.92^{c}**

Mean values with differing superscripts are significantly different – $p < 0.05$

MAP values drop significantly and immediately and thus exponential decay was calculated. Non-linear regression of the MAP levels in manure seeded in the research piles shows an exponential rate of decay of 1.03 per week, with a half-life of 0.67 weeks (Figure 33).

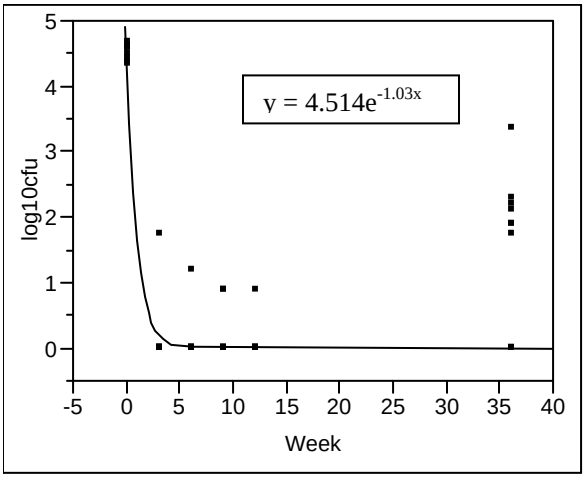


Figure 33: Exponential decay of *Mycobacterium avium paratuberculosis* in manure over time in the research piles.

Compost Quality Parameters

The compost sampled at months 3, 6, 9 and 12 was analyzed at WEL for compost quality parameters. When the samples arrived at the laboratory, they were screened to less than 3/8" (10mm). This portion of the sample was then ground and analyzed for compost quality parameters. The following graphs and tables show the combined analysis from all piles together for particle size, carbon:nitrogen ratio (C:N), total nitrogen (TN), phosphorus (P) and Solvita® maturity. Differences in compost parameters were analyzed for all piles together, using analysis of variance (ANOVA) for multiple comparisons with Tukey corrections. Linear regression analysis was run using the JMP statistical package for pilot and Cornell piles separately. Linear regression differs from the ANOVA analysis in that it examines the relationship between the levels of the compost parameters and time. It does not treat each sampling date as a distinct point (as in the ANOVA), but considers the trend over time and measures whether the change in the parameter over time is different from zero. Therefore, even if the ANOVA shows a difference between individual points, the linear regression may show no change over time. Results of linear regression are shown if they are significant (i.e. the change over time was significantly different from zero) and correlation values (r^2) are given. The closer the r^2 value is to one, the better the correlation between the parameter and time.

Particle Size

Particle size was measured by sieving the compost (Figure 34). The percent of particles in the compost that measured less than 3/8" over time remained the same (Table 30), and linear regression analysis was not significant.

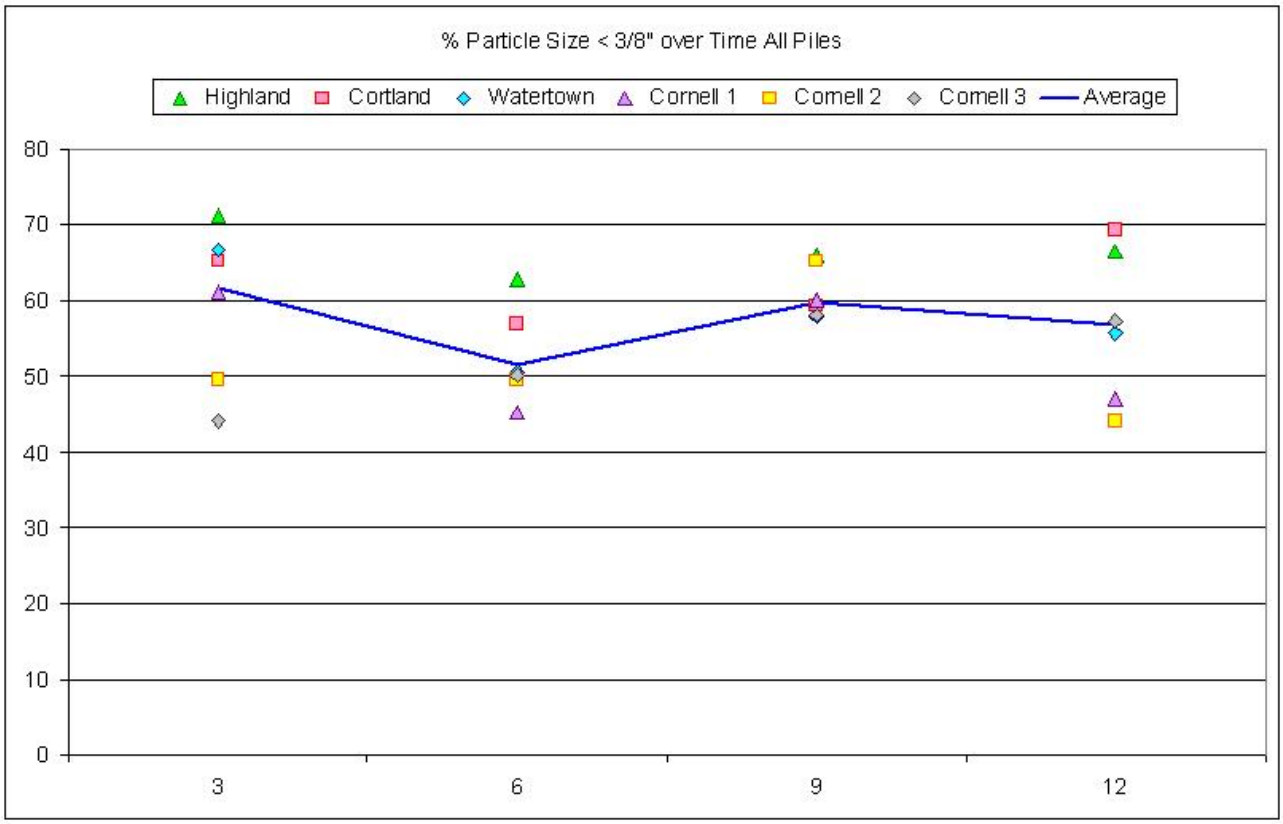


Figure 34: Percent particle size of less than 3/8" in the compost piles over time
(n=6 – one at each site/pile)

Table 30: Percent particle size of less than 3/8" in compost piles over time

	Month 3	Month 6	Month 9	Month 12
Cortland	65.2	57.0	59.3	69.4
Highland	71.3	62.8	65.9	66.5
Watertown	66.7	50.6	58.0	55.8
Cornell 1	61.2	45.3	60.1	47.0
Cornell 2	49.7	49.7	65.3	44.1
Cornell 3	55.5	44.2	50.2	58.1
Mean	**61.6[a]**	**51.6[a]**	**59.8[a]**	**56.8[a]**

Mean values with differing superscripts are significantly different – $p < 0.05$

C:N Ratio

The C:N ratio decreased significantly over time indicating that composting was occurring and that the nitrogen from the carcasses was mixing with the carbon from the wood chips (Figure 35). As composting progressed, the C:N ratio decreased until month 9 when it was at levels considered appropriate for initiating a compost process (Table 31). The final material is thus not a "finished compost" and should be reused as a carbon source for other piles, or continue through the curing process. Linear regression showed a decrease of 4.82/month for the Cornell piles with an r^2 value of 0.808 and 4.29/month with an r^2 of 0.49 for the pilot piles (Figure 36).

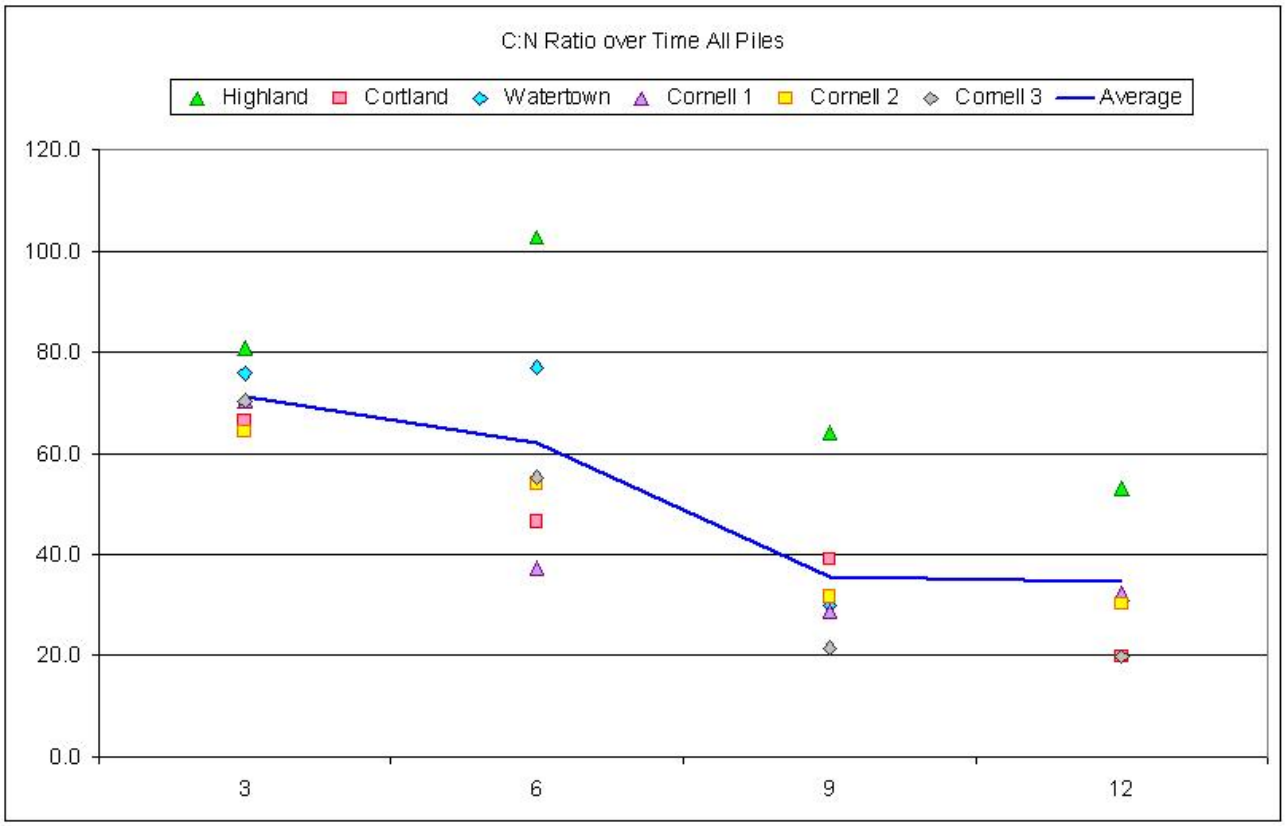
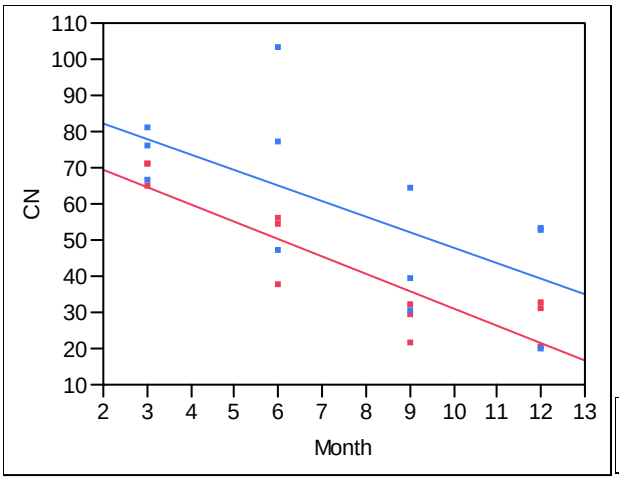


Figure 35: Carbon:Nitrogen ratio in the compost piles over time
n=6 – one at each site/pile

Table 31: Carbon:Nitrogen Ratio in compost piles over time

	Month 3	Month 6	Month 9	Month 12
Cortland	66.1	46.5	39.0	19.7
Highland	80.6	102.6	64.0	52.9
Watertown	75.7	76.8	30.0	52.2
Cornell 1	70.4	37.4	28.7	32.0
Cornell 2	64.2	54.1	31.6	30.3
Cornell 3	70.4	55.3	21.3	19.9
Mean	**71.2[a]**	**62.1[a]**	**35.8[b]**	**34.5[b]**

Mean values with differing superscripts are significantly different – $p < 0.05$



Cornell piles:	y = -4.82x + 79.1 p-value < 0.001	r^2 = 0.808
Pilot piles:	y = -4.29x + 91.0 p-value 0.0028	r^2 = 0.409

Figure 36: Linear regression of C:N ratio over time in the Cornell piles (red line) and the pilot piles (blue line)

Total Nitrogen

Nitrogen levels increased significantly over time in compost samples in both the pilot and research piles. The average value of 1.5 % at month 12 is within the range typical for composts. Linear regression showed that percent nitrogen increased by 0.12/month with an r^2 of 0.702 in the Cornell piles but was not significant in the pilot piles.

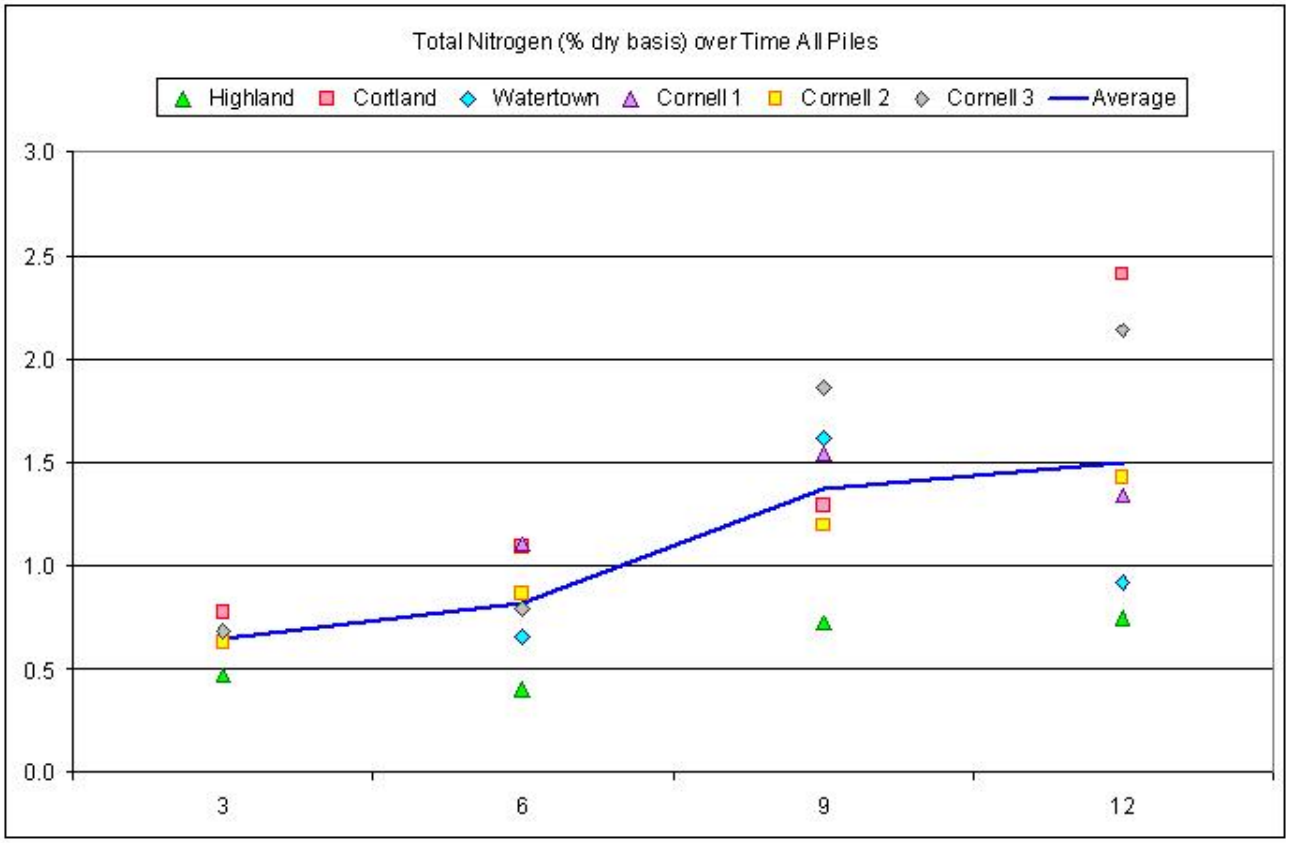
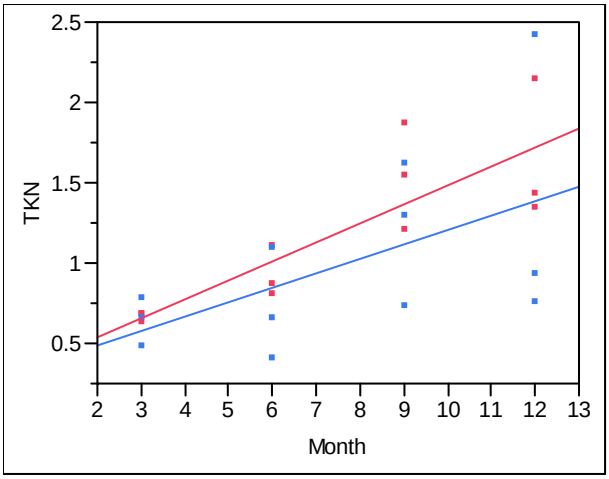


Figure 37: Total nitrogen (% dry basis) in the compost piles over time
(n=6 – one at each site/pile)

Table 32: Total nitrogen (% dry basis) in compost piles over time

	Month 3	Month 6	Month 9	Month 12
Cortland	0.77	1.09	1.29	2.41
Highland	0.47	0.40	0.73	0.75
Watertown	0.66	0.66	1.61	0.92
Cornell 1	0.68	1.11	1.54	1.34
Cornell 2	0.63	0.86	1.20	1.43
Cornell 3	0.68	0.80	1.87	2.14
Mean	**0.65^{a}**	**0.81^{ab}**	**1.37^{bc}**	**1.50^{c}**

Mean values with differing superscripts are significantly different – $p < 0.05$



Cornell piles: $y = 0.117x + 0.305$ $r^2 = 0.702$
p-value 0.0007

Pilot piles: not significant

Figure 38: Linear regression of total nitrogen over time in the Cornell piles (red line) and the pilot piles (blue line)

Phosphorus (P)

Phosphorus increased in the composts over the project period, although there was no statistical difference until month 12. The average value of 0.24 % at month 12 is within the range typical for composts. Linear regression showed that percent P increased by 0.01/month with an r^2 of 0.472 in the Cornell piles, and 0.02/month with an r^2 of 0.550 in the pilot piles.

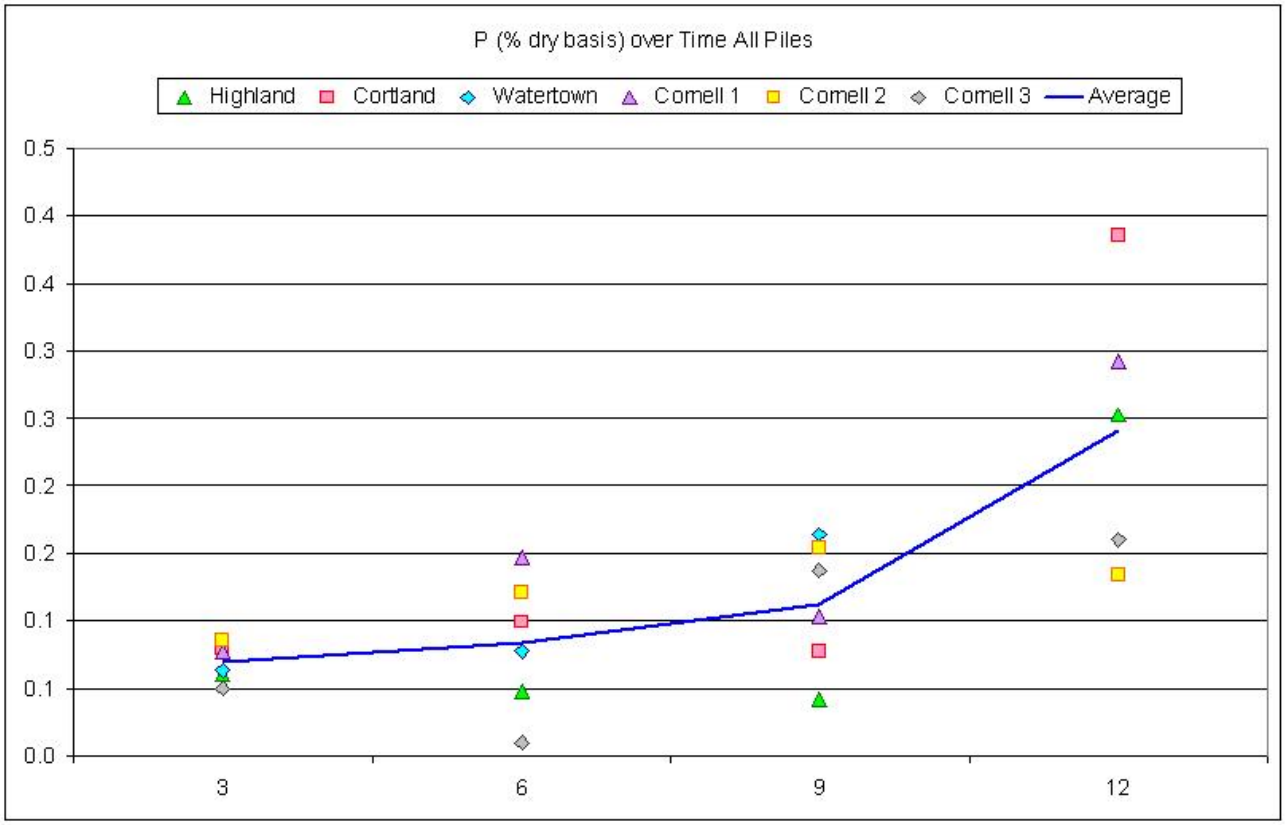
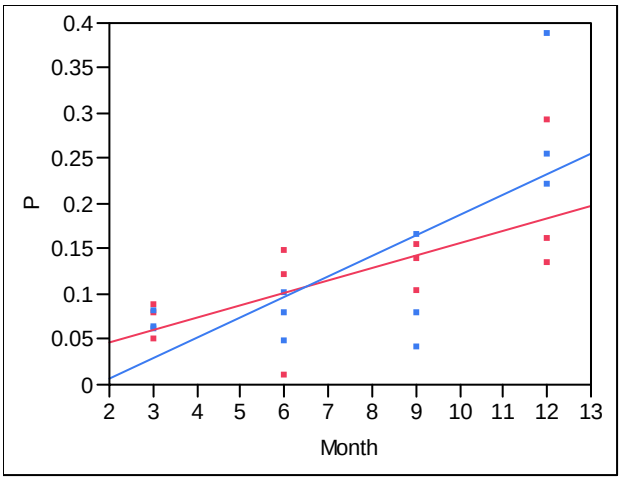


Figure 39: Total phosphorus (% dry basis) in the compost piles over time
(n=6 – one at each site/pile)

Table 33: Total phosphorus (% dry basis) in compost piles over time

	Month 3	Month 6	Month 9	Month 12
Cortland	0.079	0.099	0.077	0.386
Highland	0.060	0.047	0.041	0.253
Watertown	0.063	0.077	0.164	0.220
Cornell 1	0.077	0.147	0.103	0.292
Cornell 2	0.086	0.121	0.154	0.134
Cornell 3	0.050	0.009	0.137	0.160
Mean	**0.069[a]**	**0.083[a]**	**0.113[a]**	**0.241[b]**

Mean values with differing superscripts are significantly different – $p < 0.05$



Research piles:	y = 0.014x + 0.020 p-value 0.0136	r^2 = 0.472
Pilot piles:	y = 0.023x – 0.039 p-value 0.0053	r^2 = 0.550

Figure 40: Linear regression of phosphorus over time in the Cornell piles (red line) and the pilot piles (blue line)

Maturity

Maturity was calculated using the Solvita® maturity test. This procedure measures the evolution of carbon-dioxide (CO_2) and emission of ammonia (NH_3), two important factors for determining stability and maturity. The results are recorded as a maturity index ranging from 1 (“raw” compost) to 8 (“finished” compost). Solvita 6 and above is commonly recognized as suitable maturity for official uses. The maturity of the compost was generally low until month 12. Maturity increased significantly over time in both the pilot and Cornell piles averaging 6.9 in month 12. Linear regression showed that the change per month in maturity was not significant in the Cornell piles, but was in the pilot piles (0.3/month with an r^2 of 0.395).

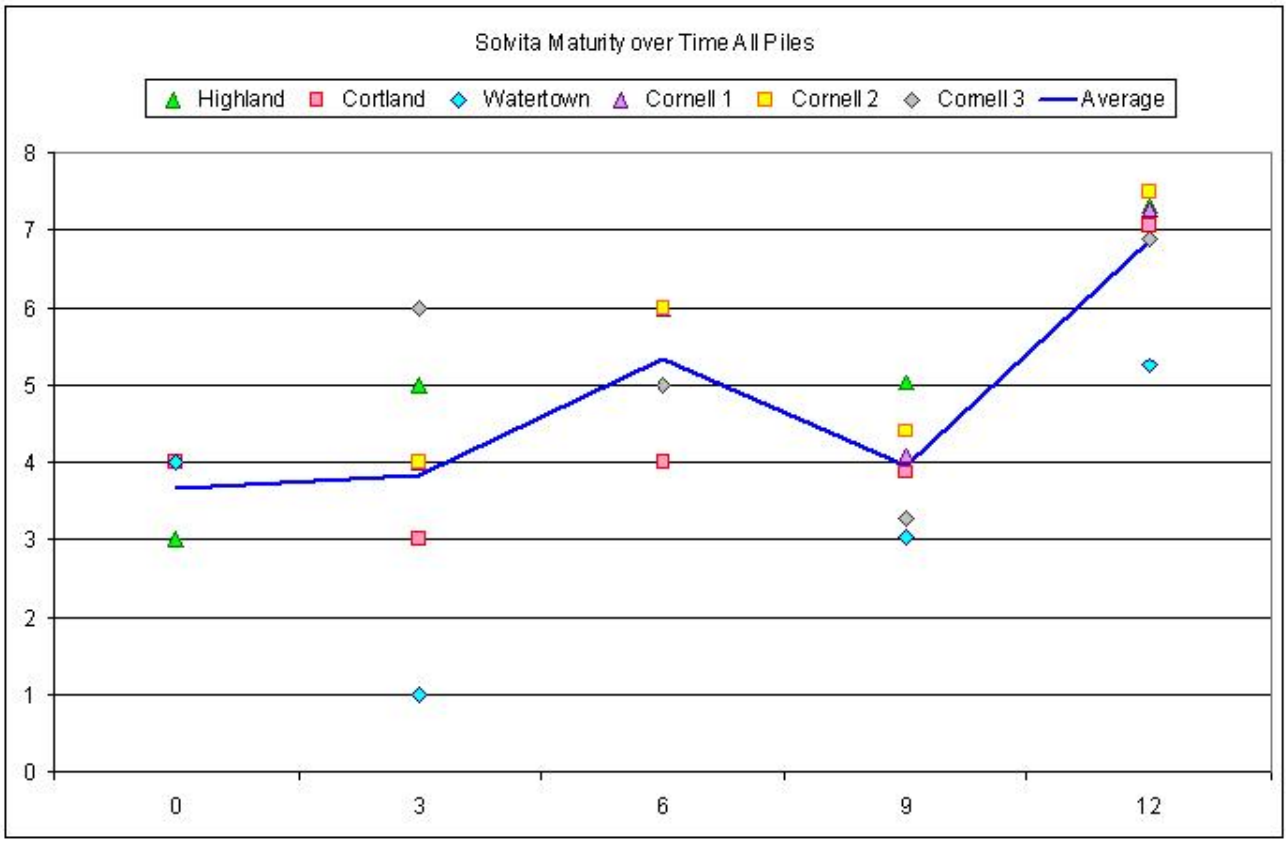
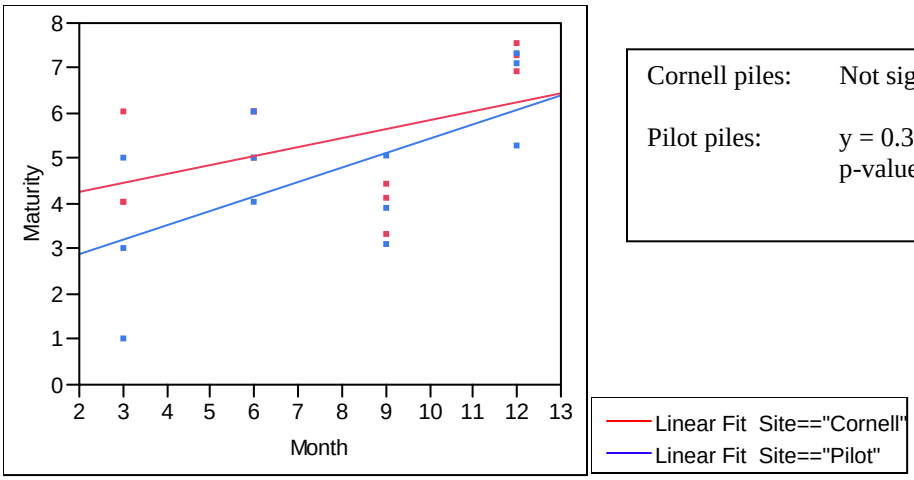


Figure 41: Solvita® maturity of the compost piles over time
(n=6 – one at each site/pile)

Table 34: Solvita® maturity of the compost piles over time

	Month 3	Month 6	Month 9	Month 12
Cortland	3.0	4.0	3.9	7.1
Highland	5.0	6.0	5.0	7.3
Watertown	1.0	5.0	3.1	5.3
Cornell 1	4.0	6.0	4.1	7.3
Cornell 2	4.0	6.0	4.4	7.5
Cornell 3	6.0	5.0	3.3	6.9
Mean	**3.8[a]**	**5.3[ab]**	**4.0[a]**	**6.9[b]**

Mean values with differing superscripts are significantly different – $p < 0.05$



Cornell piles:	Not significant	
Pilot piles:	y = 0.320x + 2.23 p-value 0.0287	r^2 = 0.395

Figure 42: Linear regression of maturity over time in the Cornell piles (red line) and the pilot piles (blue line)

Conclusions

Composting of deer carcasses was effective in reducing pathogen levels, decomposing the carcasses and producing a useable end product after 12 months. In general, temperatures in the piles, regardless of the cold ambient temperature, reached over 40^{o}C within 2 weeks of building the piles, allowing for the decomposition process of the carcasses to start. Pathogen levels were reduced to near zero levels in all of the piles and within the deer carcasses and thus can be used for highway department projects.

Statement on Implementation

Implementation of this practice has been occurring throughout the state as a result of the research performed. Numerous workshops were held to train DOT and other highway personnel, as well as NYS Thruway Authority personnel on the proper procedures for composting road-killed animals. In addition, a 12 page fact sheet, a poster, a road kill compost sign and an 8-minute DVD on composting road kill is available through Cornell Waste Management Institute, as well as on our website at http://cwmi.css.cornell.edu/tirc.htm. This project has generated interest from other states as well, and we continue to field inquiries into composting road killed animals.

Appendix A: Prevalence and Persistence of Pathogens in New York State Road-Kill Disposed of Through Composting: A Literature Review

Executive Summary

Composting is being investigated by New York State Department of Transportation (NYSDOT) as a tool for managing road-killed animals in New York State, particularly white-tailed deer. As part of a project to evaluate the effectiveness of static pile composting to inactivate pathogens in road-killed carcasses, the Cornell Waste Management Institute conducted a literature review and consulted with experts to identify the pathogens that might be present and to assess their sensitivity to inactivation by heating.

The literature reviewed on prevalence, suggest that the pathogens expected to be found in white-tailed deer and other wildlife are as follows:

- *Salmonella* – very little to none in deer
- *E. coli* and fecal coliforms – conflicting reports on *E. coli* O157:H7, but other fecal coliforms are present and can be a source of human infection
- *Clostridium* – present, especially in the gut and multiply when the animal dies
- *Listeria* – present, but deer are probably not an important source
- *Campylobacter* – very little to none
- *Yersinia* – wild ruminants may be important carriers
- Tularemia (*Francisella tularensis*) and other tick-borne diseases (as well as rabies) are carried by wildlife, but are more important in the handling of the carcasses rather than the composting process
- *Coxiella* – not prevalent
- CWD – present, hard to manage, it is not known how it would be affected by composting
- *Leptospira* – conflicting reports
- *Cryptosporidia* and *Giardia* – also conflicting reports, but more likely in younger animals
- *Mycobacteria* – present, but probably in less than 5% of the population

The hardiness of these pathogens is summarized in the following table:

	Hardiness Rating		
Pathogen	1	2	3
Salmonella spp.	*		
E. coli and *E. coli* O157:H7	*		
Campylobacter spp.	*		
Yersinia spp.	*		
Listeria spp.	*		
Leptospira spp		*	
Streptococcus (enterococci)		*	
Clostridium perfringens			*
Mycobacterium			*

A rating of 3 indicates that there is sufficient data to suggest that an organism is capable of surviving when exposed to various stressors, while a rating of 1 would indicate that the organism would not be expected to survive when exposed to stressors (Smith, et al 2005).

Extensive literature review on inactivation of these pathogens suggests that the temperatures reached in static pile composting of road-killed white-tailed deer will be sufficiently high enough to inactivate the pathogens of importance:

- *Salmonella* – unlikely to survive in compost where temperatures exceed 50^{o}C over a period of several days to two weeks. In ground meat, a 1 $\log_{10}$ reduction in bacterial numbers can be obtained after 46 minutes at 55^{o}C, 0.8 minutes at 60^{o}C, and 0.1 minutes at 70^{o}C.
- *E. coli* and fecal coliforms – unlikely to survive in compost where temperatures exceed 50^{o}C over a period of several days to two weeks. In ground meat, a 1 $\log_{10}$ reduction in *E. coli* bacterial numbers can be obtained after 33 minutes at 55^{o}C, 1.2 minutes at 59^{o}C, 0.5 minutes at 60^{o}C, and 0.1 minutes at 70^{o}C.
- *Clostridium* – few studies have been done in compost. In ground meat, a 1 $\log_{10}$ reduction in bacterial numbers can be obtained after 5.2 or 16.9 minutes at 59^{o}C and 41.7 minutes at 70^{o}C.
- *Listeria* – in compost, the rising temperature had little effect on eliminating *Listeria*, but when exposed to the athermic factors (such as alkalinisation of compost to pH 8.8) of composting, it was eliminated. In ground meat, a 1 $\log_{10}$ reduction in bacterial numbers can be obtained after 47 minutes at 55^{o}C and 1.1 minutes at 60^{o}C.
- *Campylobacter* – holding manure at 25^{o}C for 90 days will decrease bacterial numbers to concentrations below detection. In lamb meat, a 1 $\log_{10}$ reduction in bacterial numbers can be obtained after 1.2 minutes at 55^{o}C and 0.3 minutes at 60^{o}C.
- *Yersinia* – holding manure at 25^{o}C for 90 days will decrease bacterial numbers to concentrations below detection. In milk, a 1 $\log_{10}$ reduction in bacterial numbers can be obtained after 0.5 minutes at 60^{o}C.
- *Francisella tularensis* – survived less than 10 minutes in liver and cured ham at 56 and 57^{o}C, respectively.
- *Coxiella* – pasteurization times and temperatures (between 63 and 80^{o}C) needed to kill this organism.

- CWD – extremely high heat needed to inactivate the prion responsible for CWD, will most likely not be affected by composting.
- *Leptospira* – hardiness level two organism. Should be inactivated at the same temperatures as *Streptococcus* spp. In ground beef, a 1 $\log_{10}$ reduction in *Streptococcus faecalis* bacterial numbers can be obtained after 12 minutes at 60°C.
- *Cryptosporidium* and *Giardia* – holding manure at 25°C for 90 days will decrease protozoal numbers to concentrations below detection.
- *Mycobacteria* – hardiness level three organism. Depending on the species, mycobacterium may grow at a wide temperature range from 10 to 65°C, though the optimum growth range for most species is between 29 and 45°C.

In summary, the relative hardiness of the pathogens expected to be found in road-killed deer and other wildlife is *Campylobacter jejuni* < *Yersinia enterocolitica* < *Escherichia coli* < *Listeria monocytogenes* and *Salmonella* spp. < *Streptococcus faecalis* (based on D-values in food from various studies – E&A Environmental Consultants, 2001; Ahmed, et al 1995; Craven and Blankenship, 1983; Lihono, et al 2003; and Price and Tom, 2005).

One of the goals of the literature review was to identify the organisms to be monitored in the field-component of this project. Pilot piles comprised of four deer carcasses embedded in wood chips were established at three NYSDOT facilities around New York State and three replicated research piles were established at Cornell University in Ithaca, NY. At each site, samples of compost were tested periodically and at the research site, additional testing includes bags of deer intestinal contents that were placed inside the deer carcasses and retrieved at intervals.

Based on the literature review and consultation, the following pathogens were selected for analysis in both intestinal content bags and compost:

- % solids
- fecal coliform (MPN/g solids)
- *E. coli* (MPN/g solids)
- *Salmonella* spp. (MPN/4 g solids)
- fecal streptococci (MPN/g solids)
- enterococci (MPN/g solids)
- *Mycobacterium Avian paratuberculosis* (MAP) – in intestinal content bags only

Prevalence of Pathogens in Road-Killed White-Tailed Deer and Other Wildlife

Evaluation of the effectiveness of static pile composting to inactivate pathogens in road-killed carcasses first requires identification of the pathogens that might be present. A preliminary meeting held with the faculty at the Cornell School of Veterinary Medicine[1] identified *Clostridium perfringens, Salmonela spp., Escherichia coli*, fecal or total coliform, *Listeria monocytogenes, Campylobacter spp., Yersinia spp., Tularemia, Coxiella burnetii*, rabies and chronic

[1] Edward Dubovi, Patrick McDonough, S.J. Shin, Sue Stehman, Belinda Thompson, and Susan Wade.

wasting disease (CWD) as pathogens/diseases of potential concern in road-killed animals in New York State. Additional communications with experts[2] in wildlife diseases also identified leptospirosis, *Cryptosporidium*, and *Mycobacterium*. A review of the literature for the prevalence of these pathogens in white-tailed deer and other wildlife follows.

Salmonella and *E. coli* are the two most commonly studied bacteria. Mammalian intestines are full of fecal coliform, mostly *E. coli*, but there is particular concern of the pathogenic strain 0157. Most of the literature agrees that *Salmonella* spp. and the *E. coli* O157:H7 are absent from deer. In a study on samples collected from wild red deer, roe deer, moose and reindeer in Norway, *Salmonella* spp. and the potentially human pathogenic verocytotoxic *Escherichia coli* were not isolated (Lillehaug, et al 2005). Weber and Weidt (1986) report 73 roe deer fecal samples were negative for *Salmonella*. No *Salmonella* or *E. coli* O157:H7 were isolated from 450 samples from wild boar, red deer and roe deer in Poland (Koronkiewicz, et al 2004). Henderson and Hemmingsen (1983) report no *Salmonella* spp. were found from 3810 fecal samples of roe deer. They state that the apparent inability of deer to act as carriers [of *Salmonella*] may be because they lack a gall bladder, a site where *Salmonella* spp. can colonize. However, none of these studies were done on white-tailed deer. In one study involving white-tailed deer, Branham, et al (2005) collected samples from deer and other livestock in Texas, in which *Salmonella* spp. were found in the highest quantities in white-tailed deer (7.69%), followed by sheep (7.32%).

In the same study (Branham, et al 2005), *E. coli* O157 was found only in cattle, sheep and water, and only in September (sampling was September through December) most likely due to the well-documented seasonal shedding pattern of these bacteria. In a study on free ranging white-tailed deer in southeastern United States, no *E. coli* O157:H7 were detected in 310 fresh fecal samples collected from the ground. However, when sampled directly from the deer, it was isolated from the feces, but not the meat, of three of 469 (0.64%) deer, but when returning to the same site the following year, it was not found at all in 140 deer (Fisher, et al 2001). The low overall prevalence of *E. coli* O157:H7 and the identification of only one site with positive deer suggest that wild deer are not a major reservoir of *E. coli* O157:H7 in the southeastern United States. Dunn, et al (2004), agree that deer are not a major reservoir, showing only 0.3% prevalence in hunter-harvested deer and 1.8% in captive herds.

Renter, et al (2001) states that even if the presence of *E. coli* O157:H7 in the feces of free-ranging deer is infrequent, any water or food sources contaminated by deer feces should be considered potentially infectious. Their study cultured *E. coli* O157:H7 from 4 (0.25%) of 1,608 hunter-killed white-tailed deer fecal samples in Nebraska. Renter states that the presence of *E. coli* O157:H7 in the feces of free-ranging deer has implications not only for hunters, consumers of venison, and others in contact with deer or deer feces, but also for the development of strategies aimed at reducing and/or controlling this pathogen in water sources and domestic livestock. Others agree although the incidence of prevalence is small; five of 630 (0.79%) over a seven-year period (Rice, et al 2003), five of 212 (2.4%) of white-tailed deer in Kansas sharing pasture with cattle (Sargeant, et al 1999). Pagano, et al 1985, found no *Salmonella* in fecal samples of wild ruminants and marmots in Italy, but did find antibiotic resistant *E. coli* suggesting they may be important carriers.

[2] Cornell College of Veterinary Medicine: Elizabeth Buckles; NYS Department of Health Services: David Dziewulski; NYS Department of Environmental Conservation: Terry Laibach, Sally Rowland, Alan Woodard; USDA: Richard Chipman, Larry Clark, Pat Millner; EPA: Fran Kremer, Jim Smith; Woods End Research Laboratory: Pam Storms, Will Brinton; Cornell University Department of Natural Resources: Paul Curtis, Gary Goff; Cornell School of Industrial and Labor Relations, Worker Health and Safety Program: Nellie Brown.

According to Rice, et al 1995, it is possible that wild animals sharing the same habitat as cattle may also be colonized by *E. coli* O157 and that interspecies transmission may occur. Deer and cattle fecal samples in an area used by both were collected and cultured for *E. coli* O157. Of the 108 deer samples, two were positive and five of the 191 cattle samples collected were positive. The seven isolates were compared and found to be identical. One case of *E. coli* poisoning from eating venison from white-tailed deer was found in Connecticut. It was found in three packages of meat that had been frozen for 25 days. It was assumed the deer acquired it from cows grazing on dairy farms in Vermont. The authors state that deer are most likely to carry O157 during the time of greatest human exposure, the fall hunting season (Rabatsky-Her, et al 2002). Nine of 11 persons in three households reported symptoms including diarrhea, abdominal cramps, and nausea, from consuming the same venison jerky. *E. coli* O157:H7 was isolated from leftover jerky and one piece of uncooked venison from the same carcass. It was also recovered from the band saw used to cut the deer, and from remnants of the rotting deer skin. *E. coli* O157:H7 was isolated from three (9%) of 32 fecal pellets collected in the surrounding forest one month later, but none was isolated from samples obtained seven months later.

The prevalence of *Clostridium perfringens*, *Listeria*, *Campylobacter* and *Yersinia* in wildlife have all been studied. *Clostridium perfringens* types A and C are found in human and animal feces, soil, green and decaying plant material, sewage, and water (Atwill, 2005). In a study of the causes of morbidity and mortality in farmed white-tailed deer (Haigh, et al 2005), it was found that the following bacterial pathogens were implicated in cases of enteritis and diarrhea in the deer: *E. coli* (n=4), *Clostridium perfringens* (n=2), *Salmonella spp*. (n=2), and *Yersinia spp*. (n=1). Clostridia that survive at low temperatures were isolated from hides, feces and tonsils of deer slaughter stock, making handling of these animals important in slaughter plants to control the spread of *Clostridium* contamination which can cause spoilage of vacuum-packed meats (Broda, et al 2002). *Clostridium botulinum* may be found in the intestinal tract and perhaps other organs of healthy animals. It does no harm there, but, if the animals die, it may multiply and produce toxins in their carcasses.

In a study on 450 animals in Poland, it was confirmed that wild boars, red deer and roe deer were carriers of *Listeria* and *Campylobacter*, but *Yersinia* was isolated only from the feces of wild boar (2.4%) and red deer (18.2%) (Koronkiewicz, et al 2004). In two studies on wildlife, *Listeria monocytogenes* was isolated from deer (7-11%), foxes (14%), badgers (30%), hares (27%) and birds (17%) (Schonberg and Gerigk, 1991). In a study by Weber and Weidt (1986), where the feces of 196 hare and 73 roe deer were studied, *Campylobacter spp*. were isolated from feces from nine hares, but none were isolated from the roe deer. From samples collected from wild red deer, roe deer, moose and reindeer in Norway, *Campylobacter jejuni* was found in one roe deer sample only (Lillehaug, et al 2005). No *Campylobacter* were found in fecal samples from 60 wild red deer, 13 wild roe deer, seven wild chamois, 41 wild alpine marmot and soils mixed with deer feces in Italy, but *Yersinia* was found suggesting wild ruminants may be important carriers of *Yersinia* (Pagano, et al 1985).

This statement is backed up by other studies. In 1984, Henderson obtained fecal samples from farmed and feral deer in New Zealand and isolated 176 strains of *Y. enterocolitica* from 922 samples (isolation rate of 19.1%). Henderson states that this rate exceeds rates described from most other species indicating deer as a major reservoir of *Y. enterocolitica*. In a study done by Shayegani, et al 1983, approximately 1,426 wild animals in New York (most with traumatic injuries) were submitted to the Wildlife Pathology Unit and specimens were collected for identification of pathogens. 133 (9.3%) tested positive for *Yersinia* spp. Distribution across the state positive for *Yersinia spp*. was even, with a range of 4.7 to 12.8% of samples being positive. *Y. enterocolitica* was isolated from the following: 19 of 213

(8.9%) raccoon, 27 of 145 (18.6%) white-tailed deer, nine of 76 (11.8%) gray fox, eight of 59 (13.6%) red fox, and 18 of 196 (10.9%) other mammals.

Tularemia is a very rare disease that is caused by the bacteria *Francisella tularensis*. It is often referred to as rabbit fever or deerfly fever. Humans most commonly contract this disease by handling or eating undercooked wild animal meat that has been infected by disease carrying ticks. Ticks are the most important group of ectoparasites of wild mammals. Soft ticks (family Argaside) feed rapidly, so are rarely collected on their hosts. Hard ticks (Ixodid ticks) require longer blood meals on their hosts. *Ixodes scapularis* (black-legged tick) is found on white-tailed deer in the eastern United States and is responsible for the spread of Lyme disease, human babesiosis, human granulocytic ehrlichiosis, tularemia and spotted fever group Rickettsia. All stages of *Ixodes* are highly prone to desiccation, and microhabitat contributes greatly to off-host survival (Allan, 2001). The *Ehrlichiae* (small, gram-negative bacteria that primarily invade leucocytes) are grouped according to the type of blood cell most commonly infected. HGE agent (human granulocytic ehrlichiosis) infects granulocytes (the leukocytes involved in the immune defense against pathogens, parasites and allergens), and *Ehrlichia chaffeensis* (the agent involved in human monocytic ehrlichiosis) infects monocytes (leukocytes that develop into macrophages within tissues to ingest bacteria, dead cells, and other debris). Although white-tailed deer harbor a variant strain of the agent of human granulocytic ehrlichiosis not associated with human infection, they are not a reservoir for strains that cause the human disease (Massung, et al 2005). White-tailed deer are, however, natural reservoirs of *E. chaffeensis* and a major host of the Lone Star Tick – the causative agent of human monocytotropic ehrlichiosis (Yabsley, et al 2003).

Coxiella burnetii, the etiologic (the cause or origin of a disease or disorder) agent of Q fever, is a worldwide zoonotic pathogen. Evidence of antibody to *C. burnetii* was reported among various wild-animal species, including coyotes, foxes, rodents, skunks, raccoons, rabbits, deer, and birds. (McQuiston and Childs, 2002). Their literature review suggests that *C. burnetii* is enzootic (a disease that is constantly present in an animal, but usually only affects a small number of animals at any one time) among ruminants and wild animals throughout much of the United States and that there is widespread human exposure to this pathogen. However, sheep and goats appear to be a more important risk for human infection in the United States than cattle or wild animals. A study in Nova Scotia concluded that there is extensive infection of the hare population by *Coxiella burnetti*, with lesser degrees of infection of the moose, raccoon, and deer population (Marrie, et al 1993).

Chronic Wasting Disease (CWD), a fatal brain disease of North American deer and elk, has recently emerged as an important wildlife management issue (Samuel, et al 2002). Despite the lack of evidence that CWD affects humans or livestock, a significant concern has been the perceived risk to humans and livestock. Unfortunately in dealing with CWD, many important biological facts are still unknown and further research will be required to answer these questions.

Destruction of PrP CWD (the prion responsible for CWD) is difficult, and there are few treatments documented to be completely effective; however, high-temperature incineration and alkaline digestion are two such treatments for disposal of CWD-positive carcasses (Fischer, et al 2003). CWD can be transmitted to susceptible animals indirectly, from environments contaminated by excreta or decomposed carcasses (Miller, et al 2004). Under experimental conditions, mule deer (*Odocoileus hemionus*) became infected in two of three paddocks containing naturally infected deer, in two of three paddocks where infected deer carcasses had decomposed in situ ~1.8 years earlier, and in one of

three paddocks where infected deer had last resided 2.2 years earlier. Indirect transmission and environmental persistence of infectious prions will complicate efforts to control CWD and perhaps other animal prion diseases.

In New York State, several CWD-infected deer were found in 2005 in the vicinity of Oneida-Herkimer counties. This area has been designated a containment area and no road-killed deer will be taken from this area or included in composting programs.

There are conflicting reports among the researchers as to whether or not deer are primary carriers of *Leptospira spp*. A total of 403 deer blood specimens (both sexes, ages six months to eight years) were examined for *Brucella abortus* and *Leptospira pomona* in 1958. The results indicated an incidence of 0.25% brucellosis and 1.73% leptospirosis among the deer herds of the southeastern United States (Shotts, et al 1958). Reilly, et al 1962, detected antibodies to antigens of one or more of 10 leptospiral serotypes tested in serum of 23 (22.8%) of 103 deer at the Seneca Ordnance Depot, Seneca County, New York. Roth, et al 1964, reports that *Leptospirosis pomona* was isolated in white-tailed deer in IL, MN, WI and LA. Deer can be carriers of leptospires. In 1979, Fleming and Nusbaum found two of 36 (5.5%) samples from white-tailed deer that have no contact with domestic animals were positive for *L. icterohemorrhagiae*, one of 36 (2.8%) were positive for *L. Pomona* and one of 36 (2.8%) were positive for *Toxoplasma*. None were positive for *Brucella*. According to the authors, this survey indicated that deer are not a primary reservoir of *Leptospira* sp., *Brucella* sp. or *Toxoplasma*. A 1986 paper by Ingebrigtsen, et al indicated that tests for antibodies to the etiologic agents of leptospirosis on 628 white-tailed deer produced positive results of only 3%.

White-tailed deer shed *Giardia* sp. cysts and *Cryptosporidium* sp. oocysts in the environment and must be considered potential sources of contamination. However, the incidence decreases in animals greater than six months of age (Rickard, et al 1999). In a study by Perz and LeBlancq 2001, *Cryptosporidium parvum* was found in 22 of 111 wildlife fecal samples collected over a two-year period in lower New York State. They came from 10 of 91 white-tailed deer, three of five chipmunk, one of five raccoon and six of six muskrat. This study provided evidence of *C. parvum* transmission cycles involving deer and other mammalian hosts in lower New York State, affirming the potential role of wildlife species as sources of *Cryptosporidium* in the catchments of public water supplies. In a study in the North Saskatchewan River Basin in Alberta, Canada, *Giardia duodenalis* and *C. parvum*-like oocysts were detected very rarely in wildlife scat samples (66 positive out of 2011 for *Giardia* and 19 out of 2011 for *C. parvum*). The majority of samples containing *Giardia* came from muskrat (78.26%) and beaver (8.68%). *Cryptosporidium* was detected in one deer sample, representing prevalence of 0.15%, and in eight beaver samples, representing a prevalence of 2.4%. However, collection techniques (rectally for the aquatic mammals and on the ground, exposed to elements, for the others) could have caused bias (Heitman, et al 2002). Trout, et al 2003 and 2004 indicate that research suggests deer could be a potential source of infectious cysts of both *C. parvum* and *G. duodenalis* for humans and cattle. The findings of a study that took fecal specimens from 520 dairy calves and 22 coyotes, 82 white-tailed deer and 25 beaver in eastern United States suggest that deer, beaver and cattle could be potential sources of infectious *Giardia* cysts for humans and other animals (Santin-Duran, et al 2004).

Tuberculosis is primarily a respiratory disease and transmission of infection within and between species is mainly by the airborne route. *Mycobacterium bovis,* the cause of bovine-type tuberculosis, has an exceptionally wide host range. Susceptible species include cattle, humans, non-human primates, goats, cats, dogs, pigs, buffalo, badgers, possums, deer and bison (O'Reilly and Daborn, 1995). *Mycobacterium* is a genus of non-sporing, non-motile Gram-positive bacteria.

Of the obligate pathogens, the most important include the mammalian tubercle bacilli which include *M. bovis* and *M. tuberculosis*. The latter is primarily a human disease, while the former is one of animals, and zoonotic (Clifton-Hadley, 2001). Survival of *M. bovis* outside its host is dependent on ambient environmental conditions: maximum survival occurs in cold, damp conditions, while exposure to direct sunlight under dry conditions lessens its survival. Estimates of distribution of bovine TB in free-ranging white-tailed deer suggest prevalence of less than 5%. The risk to human health is greater for those in close contact with live deer or handling infected carcasses (Clifton-Hadley, 2001).

Conclusion

The literature reviewed here suggests that the pathogens expected to be found in white-tailed deer and other wildlife are as follows:

- *Salmonella* – very little to none
- *E. coli* and fecal coliforms – conflicting reports on *E. coli* O157:H7, but other coliform are present and can be a source of human infection
- *Clostridium* – present, especially in the gut and multiply when the animal dies
- *Listeria* – present, but probably not an important source
- *Campylobacter* – very little to none
- *Yersinia* – wild ruminants may be important carriers
- Tularemia and other tick-borne diseases (as well as rabies) are carried by wildlife, but are more important in the handling of the carcasses rather than the composting process
- *Coxiella* – not prevalent
- CWD – present, hard to manage, will most likely not be affected by composting
- *Leptospira* – conflicting reports
- *Cryptosporidia* and *Giardia* – also conflicting reports, but more likely in younger animals
- *Mycobacteria* – present, but probably in less than 5% of the population

Hardiness and Temperature Sensitivity of Pathogens

The effectiveness of inactivating pathogens through composting is generally assessed by monitoring the reduction in indicator organisms. *Salmonella* and fecal coliform are the usual indicator organisms. These are the organisms that the USEPA requires for evaluation of the hygienic quality of sewage sludges. It is widely recognized that the sensitivity of different pathogenic organisms to heat varies significantly and questions have been raised about the use of the current indicator organisms. Evaluation of the effectiveness of static pile composting to inactivate pathogens in road-killed carcasses requires identification of the pathogens that might be present and analysis of their sensitivity to inactivation by heating. That, combined with time/temperature data from the compost piles, will provide the information needed to assess the hygienic quality of the compost product.

Most of the research done on pathogens in compost has examined *Salmonella*, *E. coli*, and total fecal coliforms. In addition, the compost examined is, most generally, made from manure and farm waste or municipal solid waste. This is because these organisms are responsible for many human gastrointestinal illnesses, and fecal matter is where they are found. According to Deportes, et al 1988, fecal coliforms and fecal streptococci are good candidates for assessing municipal solid waste compost hygienization. Table 1 shows survival and/or inactivation times in compost or manure. Other than in the Shuval, Jiang, and Droffner studies, both *Salmonella* and *E. coli* were either inactivated or undetectable within 24 hours at temperatures greater than 50^{o}C.

Table 1: Survival and/or Inactivation Times of Pathogens in Compost or Manure

Pathogen	Temp (^{o}C)	Time	Source	Comment
S. enteriditis in compost	45	2 days	Lung, et al, 2001	Undetectable
E. coli in compost	45	3 days	Lung, et al, 2001	Undetectable
E. coli in pig manure	50	24 hours	Turner, 2002	Inactivation
E. coli in cattle manure	50	14 days	Jiang, et al, 2003	None detected
E. coli in pig manure	55	2 hours	Turner, 2002	Inactivation
Fecal enterococci in manure	55	2.1 hours	Lund, et al, 1996	4 log reduction
Salmonella in compost	55	80 days	Shuval, et al, 1991	None detected
Fecal coliforms in compost	55	<120 days	Shuval, et al, 1991	5 log reduction
Fecal strep in compost	55	<120 days	Shuval, et al, 1991	4 log reduction
Salmonella in sewage sludge	60	25 min	Mitscherlich and Marth, 1984	Survival time
E. coli in manure compost	60	24 hours	Hess, et al, 2004	Undetectable
Total coliforms in compost	60	24 hours	Hess, et al, 2004	Undetectable
S. typhimurium in food compost	60	9 days	Droffner and Brinton, 1995	Survival time
E. coli in food compost	65	9 days	Droffner and Brinton, 1995	Survival time
M. tuberculosis in biosolids	70	20 min	E&A Environ Consult, 2001	Destruction

In the Shuval study, *Salmonella* was reduced to very low levels in the first few days of composting. *S. enteriditis* and *E. coli* were undetectable in compost after two and three days, respectively, at 45^{o}C (Lung, et al 2001). These data show that *Salmonella* and *E. coli* are unlikely to survive in compost where temperatures exceed 50^{o}C over a period of several days to two weeks. They also show that fecal coliforms and streptococcus may be more resistant to temperature in compost than either *Salmonella* or *E. coli*.

Additional pathogens have been studied to a smaller extent in manure and manure-based composts. Based on actual data plus some data extrapolated from cattle manure environments, holding manure at 25^{o}C for 90 days will decrease pathogens [*Escherichia coli* O157:H7, *Salmonella*, *Campylobacter*, *Yersinia*, *Cryptosporidium*, and *Giardia*] to concentrations below detection (Guan and Holley, 2003). In a study of pathogen survival during mesophilic anaerobic digestion of animal waste, it was found that *Yersinia enterocolitica* was the least resistant of pathogens studied, followed by *Listeria monocytogenes, Salmonella typhimurium*, *E. coli* and lastly, *Campylobacter jejuni* (Kearney, et al 1993).

Except for *Listeria*, the rising temperature (in short time composting of poultry manure) was sufficient to eliminate the vegetative forms of pathogens investigated (*Salmonella typhimurium, S. pullorum, E. coli, Proteus vulgaris, Pasteurella hamolytica, Past. multocida,* haemolytic *Micrococci*, haemolytic *Streptococci*, and *Listeria monocytogenes Type I*) after exposure to an average 22-hour composting process. *Listeria* was eliminated when exposed to the athermic factors (such as alkalinisation of compost to pH 8.8) of composting. *Clostridium perfringens* was not affected by either temperature or athermic factors (Platz, 1977).

Pathogens of concern in road-killed deer are not necessarily just *E. coli, Salmonella* and fecal coliforms. A meeting with Cornell Veterinary College faculty identified, in addition, *Clostridium perfringens, Listeria, Campylobacter, Yersinia, Francisella tularensis, Coxiella*, rabies and CWD as pathogens/diseases that might be associated with deer in New York State. Thermal destruction of these pathogens has been extensively studied in foods. Table 2 gives D-values (minutes needed to get a 1 $\log_{10}$ reduction in bacterial numbers) of these organisms in various foods. It appears from this table that *Campylobacter jejuni* is less resistant to heat than *Yersinia enterocolitica, which* is less resistant than *E. coli,* which is less resistant than *Listeria monocytogenes* and *Salmonella* spp., which are less resistant than either *Streptococcus faecalis* or *Clostridium botulinum. Escherichia coli* is less resistant than *Clostridium perfringens*, but as *C. perfringens* data was not conducted at the same temperature as the others, it is hard to say where it might fit in the whole list. In other words, from least to most hardy: *Campylobacter jejuni* < *Yersinia enterocolitica* < *E. coli* < *Listeria monocytogenes* and *Salmonella* spp. < *Streptococcus faecalis*.

Table 2: D-values of pathogens in various foods

Pathogen	Temp (^{o}C)	Time (min)	Source
Campylobacter jejuni in lamb meat	55	1.2	Price and Tom, 2005
Escherichia coli in turkey	55	8.0	Ahmed, et al, 1995
Escherichia coli in pork sausage	55	8.5	Ahmed, et al, 1995
Escherichia coli in chicken	55	9.3	Ahmed, et al, 1995
Escherichia coli in ground pork	55	33.4	Murphy, et al, 2004
Salmonella in ground pork	55	45.9	Murphy, et al, 2004
Listeria monocytogenes in ground pork	55	47.2	Murphy, et al, 2004
Escherichia coli O157:H7 in ground beef	59	1.2	Doyle and Schoeni, 1984
Clostridium perfringens in ground beef	59	5.2	Roy, et al, 1981
Clostridium perfringens in ground beef	59	16.9	Price and Tom, 2005
Campylobacter jejuni in lamb meat	60	0.3	Price and Tom, 2005
Yersinia enterocolitica in milk	60	0.5	E&A Environ Consultants, 2001
Escherichia coli O157:H7 in ground beef	60	0.5	Ahmed, et al, 1995
Escherichia coli in pork sausage	60	0.5	Ahmed, et al, 1995
Escherichia coli in chicken	60	0.5	Ahmed, et al, 1995
Escherichia coli in turkey	60	0.6	Ahmed, et al, 1995
Salmonella in ground beef	60	0.8	Craven and Blankenship, 1983
Listeria monocytogenes in pork slurry	60	1.1	Lihono, et al, 2003
Salmonella in egg, pH 8.0	60	1.5	Price and Tom, 2005
L. monocytogenes in blue crabmeat	60	1.9	Price and Tom, 2005
L. monocytogenes in lobster	60	2.4	Price and Tom, 2005
L. monocytogenes in mussels	60	5.5	Price and Tom, 2005
Salmonella in egg, pH 5.5	60	9.5	Price and Tom, 2005
Salmonella in pea soup	60	10.0	Price and Tom, 2005
Streptococcus faecalis in fish cakes	60	11.3	Mitscherlich and Marth, 1984
Streptococcus faecalis in tuna pie	60	11.3	Mitscherlich and Marth, 1984
Streptococcus faecalis in chicken a la king	60	12.2	Mitscherlich and Marth, 1984
Streptococcus faecalis in ground beef	60	12.2	Mitscherlich and Marth, 1984
Streptococcus faecalis in fish sticks	60	15.7	Mitscherlich and Marth, 1984
Escherichia coli in ground pork	70	0.1	Murphy, et al, 2004
Salmonella in ground pork	70	0.1	Murphy, et al, 2004
Clostridium botulinum in ground turkey	70	41.7	Juneja, et al, 1995

The pathogens of concern in road-killed deer that do not appear in Table 2 (*Francisella tularensis*, and *Coxiella burnetti*) have been studied. *Francisella tularensis* survived less than 10 minutes in liver and cured ham at 56 and 57^{o}C, respectively. *Coxiella burnetti* is one of the organisms that have been used as an indicator of successful pasteurization of

milk. The extent of the pasteurization treatment required is determined by the heat resistance of the most heat-resistant enzyme or microorganism in the food (University of Guelph, 2005) indicating the need for pasteurization times and temperatures (between 63 and 80^{o}C) to kill this organism. Most of the pathogens found in road-killed deer appear to be fairly easily killed by temperatures above 60^{o}C, so in order to assess the effectiveness of destruction of pathogens due to composting, it was decided that indicator organisms should be used.

In food studies, indicator organisms have been generally *Escherichia coli*, *Salmonella*, *Aeromonas*, *Listeria* and *Yersinia* species (Simpson, et al 1994). But, as seen above, these are not necessarily the most heat resistant. *Streptococcus faecium* is a thermoduric enterococcus microorganism that is most likely to survive the mild pasteurization heat treatment given to some foods, and to withstand the presence of salt and nitrite at normal usage levels (Simpson, et al 1994). Lund, et al 1996, state that data indicate that fecal enterococci measurements give a good indication of inactivation of enterovirus and other more heat sensitive viruses, especially under thermophilic conditions. Due to the high thermal resistance, ability to grow at a wide range of temperatures in the presence of salt and in low pH values, *Enterococcus faecium* has been frequently considered as a reference microorganism for thermal treatments to be applied in pasteurized meals or "sous vide" (a method of cooking that is intended to maintain the integrity of the ingredients by cooking it for many hours at relatively low temperatures – 60^{o}C) type foods (Martinez, et al 2003). *E. faecium* is not expected to be found in deer, but other enterococci are. Based on this, enterococci appear to be a good indicator of pathogen destruction in deer compost piles.

Smith, et al 2005 describe the heartiness of bacteria as the relative ability of the organism to survive environmental stress and/or treatment processes. A rating of three indicates that there is sufficient data to suggest that the organism is capable of surviving when exposed to various stressors, while a rating of one would indicate that the organism would not be expected to survive when exposed to stressors. The range is one to three. In Smith's book, there is a table that rates the heartiness of different organisms. *Salmonella*, *E. coli* and *E. coli* O157:H7, as well as *Campylobacter* spp, *Yersinia* spp., and *Listeria* spp. all are a heartiness rating of one. *Leptospira* spp. and *Streptococcus* (enterococci) are a two and *Clostridium perfringens* and *Mycobacterium* are a three. Data on numbers of pathogens in each category were deemed necessary as an indicator that all pathogens of concern in road-killed deer could be destroyed during composting. From heartiness level one, fecal coliforms, *E. coli* and *Salmonella* were chosen, and fecal strep and enterococci from level two. *Mycobacterium avium* subspecies *Paratuberculosis* (MAP) was chosen as the level three bacteria.

Clostridium perfringens is not only found in the intestinal tracts of animals, but is also found as part of the micro flora of soil (Smith, 1975). As they are ubiquitous, they are likely to be everywhere. Woods End Research Lab has done *Clostridium* testing and has found it declines rapidly in composting (personal communication). It also sporulates at high temperatures and when exposed to air. It was thus deemed not appropriate for use as an indicator.

Of the three mycobacterium used to set pasteurization standards, *M. bovis* is the most sensitive to heat, followed by *M. avium* and then *M. paratuberculosis* (Sung and Collins, 1998). Depending on the species, mycobacterium may grow at a wide temperature range from 10 to 65^{o}C, though the optimum growth range for most species is between 28 and 45^{o}C (Kusnetsov, et al 2003).

Seeding with lab-reared organisms was the original intention of this study; however, as the literature review progressed and resulted in the selection of study organisms different from those in the original proposal, the methods also evolved. It is doubtful if the behavior of seeded bacteria, viruses and parasites mimics that of naturally occurring

organisms (Strauch, 1987). Therefore, since the level one and level two organisms can all be found in the intestinal tract of deer, it was decided to use actual intestinal contents of the road-killed deer for seeding into recoverable bags placed in the compost piles. These organisms will also be studied in the compost itself. Due to the fact that we have a relationship with Veterinary School faculty in the Johne's laboratory, we also have access to manure testing positive for *Mycobacterium avium* subspecies *Paratuberculosis* (MAP). Therefore, this was chosen as the level three bacteria with which to seed carcasses. MAP testing will not be done on the compost though, as it is not found in white-tailed deer. It is being used as an indicator organism.

References Cited

Ahmed, N.M., D.E. Conner and D.L. Huffman. (1995) Heat-resistance of *Escherichia coli* O157:H7 in meat and poultry as affected by product composition. Journal of Food Science. 60(3):606-610.

Allan, Sandra A. Ticks (Class Arachnida: Order Acarina) Part I Chap. 4 In: Parasitic Diseases of Wild Animals 2nd Edition. Eds. William M. Samuel, Margo J. Pybus and A. Alan Kocan. Iowa State University Press, Ames, Iowa. 2001. P. 72-106.

Atwill, Edward R. (2005) Microbial pathogens excreted by livestock and potentially transmitted to humans through water. Retrieved 8/17/05 from University of California Veterinary School Web site http://www.vetmed.ucdavis.edu/vetext/INF-EC_Microb.html

Branham, L.A., M.A. Carr, C.B. Scott, and T.R. Callaway. (2005) *E. coli* O157 and *Salmonella* spp. in white-tailed deer and livestock. Current Issues in Intestinal Microbiology 6:25-29.

Broda, D.M., R.G. Bell, J.A. Boerema, and D.R. Musgrave. (2002) The abattoir source of culturable psychrophilic *Clostridium* spp. causing 'blown pack' spoilage of vacuum-packed chilled venison. Journal of Applied Microbiology. 93:817-824.

Clifton-Hadley, R.S., C.M. Sauter-Louis, I.W. Lugton, R. Jackson, P.A. Durr, and J.W. Wilesmith. Mycobacterial Diseases. Part II Chap. 21 In: Infectious Diseases of Wild Animals 3rd Edition. Eds. Elizabeth S. Williams and Ian K. Barker. Iowa State University Press, Ames, Iowa. 2001. P. 340-371.

Craven, S.E. and L.C. Blankenship. (1983) Increased heat resistance of Salmonellae in beef with added soy proteins. Journal of Food Protection. 46(5):380-384.

Deportes, I., J.L. Benoit-Guyod, D. Zmirou and M.C. Bouvier. (1998) Microbial Disinfection Capacity of Municipal Solid Waste (MSW) Composting. Journal of Applied Microbiology. 85:238-246.

Doyle, M.P. and J.L. Schoeni. (1984) Survival and Growth Characteristics of *Escherichia coli* Associated with Hemorrhagic Colitis. Applied and Environmental Microbiology. 48(4):855-856.

Droffner, M.L. and W.F. Britton. (1995) Survival of E. coli and Salmonella populations in aerobic Thermophilic Composts as Measured with DNA Gene Probes. Zbl Hyg., 197:232-239.

Dunn, J.R., J.E. Keen, D. Moreland, and R.A. Thompson. (2004) Prevalence of *Escherichia coli* O157:H7 in white-tailed deer from Louisiana. Journal of Wildlife Diseases. 40(2):361-365.

E&A Environmental Consultants. (2001) Research Concerning Human Pathogens and Environmental Issues Related to Composting of Non-Green Feedstocks. Prepared for: Oregon Department of Environmental Quality.

Fischer, J.R., T. Zhao, M.P. Doyle, M.R. Goldberg, C.A. Brown, C.T. Sewell, D.M. Kavanaugh, and C.D. Bauman. (2001) Experimental Field Studies of *Escherichia coli* O157:H7 in White-Tailed Deer. Applied and Environmental Microbiology. 67, 3:1218-1224.

Fischer, J.R., L.H. Creekmore, R.L. Marchingon, S.J. Riley, S.M. Schmitt, E.S. Williams. (2003) External Review of Chronic Wasting Disease Management in Wisconsin. Wisconsin Department of Natural Resources.

Fleming, W.J., and S.R. Nusbaum. (1979) A survey of deer on the Seneca Army Depot for evidence of leptospirosis, brucellosis and toxoplasmosis. New York Fish and Game Journal. 26(2):198.

Guan, Tat Yee and Richard A. Holley. (2003) Pathogen survival in swine manure environments and transmission of human enteric illness - a review. Journal of Environmental Quality 32:383-392.

Haigh, J., J. Berezowski, and M.R. Woodbury. (2005) A cross-sectional study of the causes of morbidity and mortality in farmed white-tailed deer. Canadian Veterinary Journal. 46:507-512.

Heitman, T.L., L.M. Frederick, J.R. Viste, M.J. Guselle, U.M. Morgan, R.C., Thompson and M.E. Olson. (2002) Prevalence of *Giardia* and *Cryptosporidium* and characterization of *Cryptosporidium* spp. isolated from wildlife, human, and agricultural sources in the North Saskatchewan River Basin in Alberta, Canada. Canadian Journal of Microbiology. 48, 6:530-541.

Henderson, T.G. and P. Hemmingsen. (1983) Faecal survey of deer for *Yersinia pseudotuberculosis* and *Salmonella* sp. New Zealand Veterinary Journal. 31:225-226.

Henderson, T.G. (1984) The isolation of *Yersinia* sp. from feral and farmed deer faeces. New Zealand Veterinary Journal. 32(6):88-90.

Hess, Thomas F., Inga Grdzelishvili, Haiqing Sheng and Carolyn J. Hovde. (2004) Heat Inactivation of E. coli During Manure Composting. Compost Science and Utilization 12(4):314-322.

Ingebrigtsen, D.K., J.R. Ludwig, and A.W. McClurkin. (1986). Occurrence of antibodies to the etiologic agents of infectious bovine rhinotracheitis, parainfluenza 3, leptospirosis, and brucellosis in white-tailed deer in Minnesota. Journal of Wildlife Diseases. 22(1):83-86.

Jiang, X., J. Morgan and M.P. Doyle. (2003) Fate of Escherichia coli O157:H7 during Composting of Bovine Manure in a Laboratory-Scale Bioreactor. Journal of food protection. 66(1):25-30.

Juneja, V.K., B.S. Marmer, J.G. Phillips, and A.J. Miller. (1995) Influence of the intrinsic properties of food on thermal inactivation of spores of nonproteolytic *Clostridium botulinum*: Development of a predictive model. Journal of Food Safety. 15(4):349-364.

Kearney, T.E., M.J. Larkin, J.P. Frost and P.N. Levett. (1993) Survival of pathogenic bacteria during mesophilic anaerobic digestion of animal waste. Journal of Applied Bacteriology. 73:215-219.

Keene, W.E., E. Sazie, J. Kok, D.H. Rice, D.D. Hancock, V.K. Balan, T. Zhao, and M.P. Doyle. (1997) An outbreak of *Escherichia coli* O157:H7 infections traced to jerky made from deer meat. Journal of the American Medical Association. 277(15):1229-1231.

Koronkiewicz, A. E. Daczkowska-Kozon, K. Markiewicz, A. Wojciechowska, E. Zmuda and W. Dabrowski. (2004) Game animals as carriers of enteric pathogens. Folia Universitatis Agriculturae Stetinensis, Scientia Alimentaria. 3:79-84.

Kusnetsov, J., E. Torvinen, O. Perola, T. Nousiainen and M.L. Katila. (2003) Colonization of hospital water systems by legionellae, mycobacteria and other heterotrophic bacteria potentially hazardous to risk group patients. Acta Pathologica, Microbiologica et Immunologica Scandinavica. 111:546-556.

Lillehaug, A., B. Bergsj, J. Schau, T. Bruheim, T. Vikren, K. Handeland. (2005) *Campylobacter* spp. *Salmonella* spp., verocytotoxic *Escherichia coli*, and antibiotic resistance in indicator organisms in wild cervids. Acta Veterinaria Scandinavica. 46(1/2):23-32.

Lund, B., V.F. Hensen, P. Have, and B. Ahring. (1996) Inactivation of virus during anaerobic digestion of manure in laboratory scale biogas reactors. Antonie Van Leeuwenhoek. 69(1):25-31.

Lung, A.J., C. M. Lin, J.M. Kim, M.R. Marshall, R. Nordstedt, N.P. Thompson and C.I. Wei. (2001) Destruction of Escherichia coli O157:H7 and Salmonella Enteritidis in Cow Manure Composting. Journal of Food Protection 64(9):1309-1314.

Marrie, T.J., J. Embil, and L. Yates. (1993) Seroepidemiology of *Coxiella burnetii* among wildlife in Nova Scotia. American Journal of Tropical Medicine and Hygiene. 49(5):613-615.

Martinez, S., M. Lopez, and A. Bernardo. (2003) Thermal inactivation of Enterococcus faecium: effect of growth temperature and physiological state of microbial cells. Letters in Applied Microbiology. 37:475-481.

Massung, R.F., J.W. Courtney, S.L. Hiratzka, V.E. Pitzer, G. Smith, and R.L. Dryden. (2005) *Anaplasma phagocytophilum* in White-tailed deer. Emerging Infectious Diseases. 11(10):1604-1606.

McQuiston, Jennifer H.; Childs, James E. (2002) Q Fever in Humans and Animals in the United States. Vector Borne and Zoonotic Diseases. 2(3):179-191.

Miller, M.W., E.S. Williams, N.T. Hobbs, L.L. Wolfe. (2004) Environmental Sources of Prion Transmission in Mule Deer. Emerging Infectious Diseases [serial on the Internet]. Available from: http://www.cdc.gov/ncidod/EID/vol10no6/04-0010.htm

Mitscherlich, E. and E.H. Marth. (1984) Microbial Survival in the Environment: Bacteria and Rickettsiae Important in Human and Animal Health. Springer-Verlag, Berlin.

Murphy, R.Y., B.L. Beard, E.M. Martin, L.K. Duncan and J.A. Marcy. (2004) Food Microbiology and Safety (FMS) - Comparative Study of Thermal Inactivation of Escherichia coli O157:H7, Salmonella, and Listeria monocytogenes in Ground Pork Journal of Food Science : an official publication of the Institute of Food Technologists. 69(4): FMS97.

O'Reilly, L.M., and C.J. Daborn. (1995) The epidemiology of *Mycobacterium bovis* infection in animals and man: a review. Tubercle and Lung Disease. 76 (Supplement 1), 1-46.

Pagano, A., G. Nardi, C. Bonaccorso, V. Falbo, C. Passi, V. Sanguinetti, and A. Mantovani. (1985) Faecal bacteria of wild ruminants and the alpine marmot. Veterinary Research Communications. 9:227-232.

Perz, J.F. and S.M. LeBlancq. (2001) *Cryptosporidium parvum* infection involving novel genotypes in wildlife from lower New York state. Applied and Environmental Microbiology. 67, 3:1154-1162.

Platz, S. (1977) Survival of Pathogenic Bacteria During Composting of Poultry Manure. Zentralblatt fur Verterinarmedizin 24B(1):25-34

Price, R. J. and P.D. Tom. Compendium of Fish and Fishery Product Processes, Hazards and Controls. National Seafood HACCP Alliance for Training and Education. Retrieved 9/12/05 from the University of California Seafood Information Network Web site. http://seafood.ucdavis.edu/haccp/compendium/compend.htm

Rabatsky-Her, T., D. Dingman, R. Marcus, R. Howard, A. Kinney, and P. Mshar. (2002) Deer meat as the source for a sporadic case of *Escherichia coli* O157:H7 infection, Connecticut. Emerging Infectious Diseases. 8(5):525-527.

Reilly, J.R., T.F. Muraschi, and D.J. Dean. (1962) Leptospirosis in the white-tailed deer, *Odocoileus virginianus*. The Cornell Veterinarian. 52:94-98.

Renter, D.G., J.M. Sargeant, S.E. Hygnstorm, J.D. Hoffman, and J.R. Gilliespie. (2001) *Escherichia coli* O157:H7 in free-ranging deer in Nebraska. Journal of Wildlife Diseases. 37(4):755-760.

Rice, D.H., D.D. Hancock, and T.E. Besser. (1995) Verotoxigenic *E. coli* O157 colonisation of wild deer and range cattle. Veterinary Record. 237:524.

Rice, D.H., D.D. Hancock, and T.E. Besser. (2003) Faecal culture of wild animals for *Escherichia coli* O157:H7. The Veterinary Record. 152:82-83.

Rickard, L.G., C. Siefker, C.R. Boyle, E.J. Gentz. (1999) The prevalence of *Cryptosporidium* and *Giardia* spp. in fecal samples from free-ranging white-tailed deer (*Odocoileus virginianus*) in the southeastern United States. Journal of Veterinary Diagnostic Investigation. 11:65-72.

Roth, E.E., W.V. Adams, G.E. Sanford, K. Newman, M. Moore, and B. Greer. (1964) Isolation of *Leptospira pomona* from white-tailed deer in Louisiana. American Journal of Veterinary Research. 25(104):259-261.

Roy, R.J., F.F. Busta, and D.R. Thompson. (1981) Thermal inactivation of *Clostridium perfringens* after growth at several constant and linearly rising temperatures. Journal of Food Science. 46:1586-1591.

Samuel, M.D., D.O. Joly, M.A. Wild, S.D. Wright, D.L. Otis, R.W. Werge, and M.W. Miller. (2002) Surveillance Strategies For Detecting Chronic Wasting Disease In Free-Ranging Deer and Elk. Results of a CWD Surveillance Workshop Madison, Wisconsin December 10-12, 2002. Available at http://www.nwhc.usgs.gov/publications/fact_sheets/pdfs/cwd/CWD_Surveillance_Strategies.pdf

Santin-Duran, M., R. Fayer, and J.M. Trout. (2004) Epidemiology of zoonotic protozoa in dairy cattle and wildlife in eastern United States. Journal of Eukaryotic Microbiology. 51(2) Supplement:12A.

Sargeant, J.M., D.J. Hafer, J.R. Gillespie, R.D. Oberst, S.J. Flood. (1999) Prevalence of *Escherichia coli* O157:H7 in White-Tailed Deer Sharing Rangeland With Cattle. Journal of the American Veterinary Medical Association. 215(6):792-794.

Schonberg, A. and K. Gerigk. (1991) *Listeria* in effluents from the food-processing industry. Revue Scientifique et Technique. Office International des Epizooties. 10(3):733-748.

Shayegani, M. W. Stone, I. Deforge, T. Root, L. Parsons and P.S. Maupin. (1986) *Yersinia enterocolitica* and related species isolated from wildlife in New York state. Applied and Environmental Microbiology. 52, (3):420-424.

Shotts, E.B., W.E. Greer, and F.A. Hayes. (1958) A preliminary survey of the incidence of Brucellosis and Leptospirosis among white-tailed deer (*Odocoileus virginianus*) of the Southeast. Journal of the American Veterinary Medical Association. 133(7):359-361.

Shuval, H., R. Jocide, M. Consiglio, G. Spagiarri, and C. Spigoni. (1991) Control of enteric microorganisms by aerobic thermophilic co-composting of waster water sludge and agro-industry wastes. Water Science and Technology 24:401-405.

Simpson, M.V., J.P. Smith, H.S. Ramaswamy, B.K. Simpson and S. Ghazala. (1994) Thermal resistance of Streptococcus faecium as influenced by pH and salt. Food Research International 27:349-353.

Smith, Jr., J.E., P.D. Millner, W. Jakubowski, N. Goldstein, and R. Rynk, eds. (2005) Contemporary Perspectives on Infectious Disease Agents in Sewage Sludge and Manure. The JG Press.

Smith, L.D. (1975) Chapter 7:*Clostridium perfringens* In: The Pathogenic Anaerobic Bacteria. Charles C. Thomas Press, Springfield, IL. p.115-176.

Strauch, D. (1987) Microbiological specifications of disinfected compost. In: Compost: Production, Quality and Use. Edited by M. DeBertoldi, M.P. Ferranti, P. L'Hermite and F. Zucconi. Elsevier Applied Science, London and NY. Pages 210-229.

Sung, N., and M.T. Collins. (1998) Thermal Tolerance of Mycobacterium paratuberculosis. Applied and Environmental Microbiology, 64(3):999-1005.

Trout, J.M., M. Santin, and R. Fayer. 2003. Identification of Assemblage A *Giardia* in White-Tailed Deer. Journal of Parasitology. 89, 6:1254-1255.

Trout, J.M., M. Santin and R. Fayer. Identifying and Quantifying Sources of Parasitic Organisms. (2004) In: Workshop Report Pathogens in the Environment, Edited by: William Hargrove, Director Kansas Center for Agriculture Resources and the Environment. Kansas City, MO, Feb 23-25, 2004, pp 19-22.

Turner, C. (2002) The thermal inactivation of *E. coli* in straw and pig manure. Bioresource Technology 84:57-61.

University of Guelph. Thermal Destruction of Microorganisms. Retrieved Aug 24, 2005 from the University of Guelph Dairy Science and Technology Education Series website. http://www.foodsci.uoguelph.ca/dairyedu/TDT.html

Weber, A. and H. Weidt. (1986) Occurrence of salmonellae and thermophilic *Campylobacter* in faeces samples from captured field hares and roe deer. Praktisch Tierarzt. 67(12):1092-1094

Yabsley, M.J., V.G. Dugan, D.E. Stallknecht, S.E. Little, J.M. Lockhart, J.E. Dawson, and W.R. Davidson. (2003) Evaluation of a Prototype *Ehrlichia chaffeensis* surveillance system using white-tailed deer (*Odocoileus virginianus*) as natural sentinels. Vector-Borne and Zoonotic Diseases. 3(4):195-207.