A Comparison of Three Types of Compost Materials for the Reduction of Bacterial Pathogens

by

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Abstract

This study compared three compost materials, sand, soil, and stall waste, for their ability to inactivate pathogenic bacteria. For this purpose, a series of mounds were constructed for large-scale testing during the first summer of the project. This was followed by small-scale testing for two winter trials and a second summer trial. In all trials *Escherichia coli* and *Salmonella spp.* were used as indicator organisms, either in unison or individually. Large-scale testing showed complete inhibition of indicator organisms in sand compost mounds. Soil and stall compost mounds showed little to no inhibition of coliforms over the course of both trials. In small-scale winter trials, ANOVA testing showed no significant difference between compost types using turbidity to measure bacterial growth. During the small-scale summer trial, stall compost completely inhibited the Salmonella population by day 9; however, by the end of the trial there was no statistically significant difference between compost types.

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I. INTRODUCTION

Following the occurrence of a natural or human-made disaster of significant magnitude there would be a potential large-scale loss of life, both human and livestock. The carcasses left in the aftermath would need to be secured, stored, and disposed of in a timely manner. Decomposing bodies could potentially shed pathogenic bacteria, viruses, fungi or otherwise hazardous biologic and chemical agents into the environment. In addition, the threat of a widespread pathogenic occurrence, such as the foot-and-mouth disease outbreak in Europe in 2001 (Scudamore and Harris 2002), can limit the methods by which carcasses can be disposed of in a biologically safe manner. Unfortunately, with the suspension of most civil services following a natural disaster it is not always possible to remove corpses for proper preparation and burial.

Several methods of carcass disposal have been utilized following significant natural disasters or disease outbreaks. These include incineration, above and below ground burial, carcass rendering, and composting. All of these methods involve a major investment of time, energy, and resources. The use of incineration and burial has prompted fears of air, water and soil contamination with pathogenically harmful organisms and chemical products (Glanville et al. 2009, Weiping et al. 2009). Rendering has become an unrealistic method due to the decrease in rendering plants in the last quarter century (Schutz and Auvermann 2008). While rendering is considered one of the preferred solutions for the elimination of pathogenically compromised carcasses, it is no longer feasible for usage in large-scale outbreaks of disease (Gwyther et al. 2011). A

better alternative would be the use of natural resources to eliminate carcasses. The use of soil, sand, or other locally available alternatives for composting would require little energy and time investment. The remains of humans or animals could be disposed of with large-scale composting, freeing up resources for other projects and possibly neutralizing biological agents before they can enter into the environment. Short-term composting would breakdown soft tissue but not bones or teeth, thereby allowing remains to be tagged and stored for future identification of human victims.

Composting is the decomposition of organic materials by biological processes in a predominantly aerobic environment (Berge et al. 2009). This process is dependent upon many factors, including available oxygen, moisture, temperature, nutrient accessibility, and pH. In composting, organic material can refer to carcasses from production animals such as swine, horses, chickens, or cattle. The product of composting is called compost, which is a stable mixture that can be used as a soil amendment. The usage of this amendment is regulated by both state and federal guidelines, with various classifications used to identify usage of compost based on days of composting, maximum internal temperature achieved within the compost pile, and number of microorganisms present per gram of compost product (TDEC 2012, U.S. EPA 2012, Walker and Hawkins 2009).

A composting pile is usually composed of a base layer of an absorptive material, such as sawdust, on which a carcass is placed; this layer is meant to absorb liquids draining from the carcass and hinders the passage of microorganisms into the soil below the composting pile. The carcass is then covered by a second layer meant to retain heat and prevent moisture loss; this layer can be made from silage, soil, or many other types of

materials. Composting additives are recommended to maintain the optimal ratio of carbon to nitrogen (30:1) within the pile. The maintenance of this ratio is important for the rapid decomposition of the organic matter (Epstein 1997).

Mounds can vary in set-up while still retaining the basic composition of a generic composting pile. Composting styles include static piles, open and closed windrow piles, and in-vessel composting. Each type varies in the amount of compost produced and the amount of organic matter that can be feasibly broken down, as well as amount of aeration and ability to turn the compost (Hawkins et al. 2006).

Composting piles rely on a complex population of microorganisms to drive the breakdown process; this is both convenient and at the same time presents some potential dangers to humans (Berge et al. 2009). Microorganisms such as fungi, protozoa, and bacteria that either reside within or on the decomposing organism, or contained by the compost material itself, are used in the process of breaking down the organic matter while consuming oxygen and releasing heat, water, and carbon dioxide. Some of the microorganisms that can reside in the mounds during the composting process are considered to be pathogenic to humans, and therefore great care must be taken when composting to ensure complete inactivation of such organisms (Lillywhite et al. 2007). Some of these organisms can escape into the environment through multiple pathways. Agitation caused during turning events or disturbances caused by scavengers are capable of spreading bacteria into the air, as well as directly spreading the bacteria around the local environment. Bacteria are also able to escape the piles during precipitation events during which organisms are flushed from the mounds and into the surrounding

environment. Important pathogenic bacteria that are known to be released during the composting of animals or from the compost pile itself include *Salmonella spp.*, *Escherichia coli, Yersinia spp.*, *Bacillus spp.*, *Streptococcus spp.*, and several others, as well as some fungal species, protozoans, and viruses (Reuter et al. 2011, Bonhotal et al. 2010, Epstein 1997).

The aerobic degradation of complex organic matter into simpler molecules creates an increase in the thermal energy within the compost mound. This process is composed of distinct phases based on the availability of oxygen and the temperatures that are possible under those conditions. Experimentation has shown that external air temperatures are not a major factor in pile temperature or the inactivation of organisms within them (Stanfords et al. 2007). Initial stages of composting are marked by a rapid decrease in the amount of available oxygen within the pile and an increase in core temperature. Temperatures in this initial phase are mesophilic, leading to the proliferation of mesophiles throughout the pile. As the number of microorganisms increases so does the temperature, eventually leading to a thermophilic phase. This phase is considered to begin after temperatures have reached 44°C and is usually reached a few days following the beginning of the composting process. During this phase the amount of thermophiles increases as number of mesophiles decline. The ability to maintain this phase is dependent on moisture and available oxygen. The thermophilic temperatures begin a slow decline, eventually leading to the reestablishment of mesophilic organisms as the dominant bacterial organisms in the compost pile. The mesophiles that begin the regrowth are those that survived the lethal temperatures by living on the periphery of the

pile itself, contaminating organisms originating from outside the compost environment, or spore-forming organisms capable of withstanding the excessive temperatures. Though helpful, the ability to form spores does not necessarily mean that the organism will always survive the thermophilic environment of the pile core. Past research has shown that inactivation of spore formers is possible by reaching sufficient temperature and moisture levels (Reuter et al. 2011). The optimal inactivation temperature of biosoilds for most bacterial organisms in the United States is considered by the U.S. EPA to be those in excess of 55°C. These temperatures must be maintained for at least three days to fulfill inactivation requirements necessary for compost products to be utilized as a soil additive. Specific temperature requirements vary by country, while the guideline temperature tends to 55°C, the composting time fluctuates with some requiring either additional and fewer days (Brinton and Gardner 2000).

Salmonella spp. are Gram-negative, peritrichous, lactose-fermenting, rod-shaped bacteria that produce hydrogen sulfide. Infections caused by these organisms include gastroenteritis, enteric fever, and bacteremia. These infections are considered zoonotic because most of these bacteria are not adapted to humans but are normal microbiota of animal gastroenteric systems (Wales et al. 2010). Salmonella infections can be transferred from both animals to humans and from person to person. Human infections can be traced to contaminated or insufficiently cooked animal food products, including milk, eggs, or dairy products, while some infections have been caused by close contact with reptiles (Pedersen et al. 2009). Salmonella is divided into two species, *S. enterica* and *S. bongori*, with several serotypes within each species. Important serotypes include

S. enterica subspecies enterica serotypes Typhi, Choleraesuis, and Parathyphi. These strains are responsible for the primarily human disease, typhoid fever, which is rare in the U.S. with the majority of cases being travel related (Mahon et al. 2007).

E. coli are Gram-negative, lactose-fermenting, rod-shaped bacteria. *E. coli* is normal microbiota of the gastrointestinal tracts of many animals and humans. Though normal microbiota of many organisms, *E. coli* is an opportunistic pathogen and can cause infections in humans. Infections with *E. coli* can lead to bacteriuria, septicemia, neonatal sepsis, meningitis, and diarrheal syndromes. Some strains of *E. coli* are overtly pathogenic due to the presence of virulence factors, such as toxins, that have been acquired by genetic transfer. *E. coli* can be divided into six major virulence categories, each characterized by specific O and H antigens, virulence factors, and epidemiology, which allow *E. coli* infections to exhibit a wide range of symptoms. These major categories include enterotoxigenic *E. coli*, enteroinvasive *E. coli*, enteropathogenic *E. coli*, enterohemorrhagic *E. coli*, diffusely-adherent *E. coli* and enteroaggregative *E. coli* (Kaspar et al. 2004).

This project sought to compare the ability of three readily available composting materials, sand, soil, and stall scrapings, to serve as effective composting materials for bacterial pathogen reduction, specifically for *Salmonella spp.* and *E. coli*. Evaluation of the effectiveness of the compost materials was accomplished by the analysis of samples taken from compost mounds in field trials, testing the survival of defined amounts of bacteria in small-scale composting experiments, and small-scale laboratory trials using inoculated compost material. It was hypothesized that there would be an observable

difference in the effectiveness between the three types of composting materials in eliminating potential pathogens. The results of these studies will inform and enable local farmers as well as civil authorities to determine the most effective, biosecure, and readily available compost medium for either small-scale or large-scale composting.

II. MATERIALS AND METHODS

LARGE-SCALE COMPOSTING

Construction of piles. Initial compost piles were constructed from one of three types of materials, horse stall scrapings, soil, or sand. Compost piles were constructed in association with the Middle Tennessee State University (MTSU) School of Agribusiness and Agriscience. Horse stall scrapings were obtained from the MTSU Horse Science Center, soil was obtained from MTSU property, and construction-grade sand was obtained from Nashville Ready Mix (Murfreesboro, TN). For these studies both base layers and cover layers were composed of the same material. A base layer of the composting material approximately two feet in height was built on top of a concrete pad. Centrally placed upon each of the bases were full-grown hog carcasses weighing between 200 and 250 lbs. A cover layer of compost material approximately one meter in height was then placed on top of the carcasses. A front loader, provided by the MTSU School of Agribusiness and Agriscience, was used to accomplish the movement of the compost materials and carcasses. Individual composting piles were enclosed by wooden dividers, ensuring separation from the other piles (Figure 1). For the initial summer trial only one carcass per compost material was used.

The second summer composting trial followed a similar pile design except for the use of piglet carcasses, each weighing approximately 30 lb. Due to the size difference in the organic matter being composted, the overall mass of the mounds was divided in half. Two separate mounds were built in each enclosure with each pile containing a piglet carcass. The pig carcasses in both trials were obtained from a commercial meat processing facility.

Figure 1. Completed stall compost mound enclosed within wooden dividers.

Sample collection. Samples were collected from mounds with the use of a soil sample core collector (Forestry Suppliers Inc., Jackson, MS) (Figure 2A). Samples were taken both from both within the mounds (core) and their periphery (base). To clean the corer between collections, the sample collector was placed in a large cylinder containing a 10% Lysol® solution for several minutes and then washed with deionized water (Figure 2B). Samples were collected during the construction of the mounds and the day following construction. Subsequent collections were performed twice a week for the duration of the study period. Samples from each mound were collected in plastic Ziploc® bags and marked as either a core or base sample, along with the date and mound material type. Samples that were not used immediately following collection were stored in a refrigerator at 4°C for future analysis.

Figure 2. Core sampling. (A) One of three compost core samplers used in the first summer trial. (B) Core sampler decontamination with a 10% Lysol® solution.

Precipitation data. Rainfall amounts for the composting periods were collected from the Weather Warehouse data site (http://weather-warehouse.com) for the weather station located at latitude 35.86, longitude -86.34 on Flat Rock Road, Murfreesboro, TN, approximately 4 mi from the composting site.

Salmonella detection. For these studies *Salmonella spp.* were used as indicator organisms to determine the effectiveness of pathogen destruction in the mounds. In order to reduce the amount of resources used to determine the presence of *Salmonella spp*. in the samples, a screening method was used for Salmonella detection (IOS 2002). A 25 g compost sample was homogenized in 225 mL of Buffered Peptone Water (BPW; Oxoid Inc., Lenexa, KS) and then incubated for 18 h at 37°C (Figure 3A). After incubation 300 µL of sample was placed on a Modified Semisolid Rappaport-Vassiliadis agar plate (RVSS; Oxoid Inc.). RVSS plates were then incubated for 24 h at 41.5°C. Plates that showed negative or no growth after the incubation time were incubated for another 24 h. All of the growth and biochemical test media used for the screening of the potential Salmonella colonies were obtained from Becton, Dickinson and Co. (Franklin Lakes, NJ) and all media were prepared according to the manufacturer's instructions. Colonies that demonstrated the characteristic motility of Salmonella on RVSS (Figure 3B) were used to inoculate Xylose Lysine Desoxycholate agar (XLD) and Hektoen Enteric agar (HE) plates. After inoculation XLD and HE plates were incubated for 18 h at 37°C. Colonies that produced hydrogen sulfide were used to streak nutrient agar plates that were then

incubated for 24 h at 37°C. Colonies were selected from nutrient agar plates and used to inoculate triple sugar iron agar slants (TSI), lysine iron agar slants (LIA), and urease broth tubes. All biochemical identification tubes were incubated for 24 h at 37°C. All sample runs were performed with positive and negative controls. *Salmonella enterica* Enteritidis ATCC #13076 (*S.* Enteritidis) was used as the positive control and *Proteus mirabilis* ATCC #25933 was the negative control. The positive Salmonella isolates were stored at -70°C in a 10% glycerol solution for serotyping.

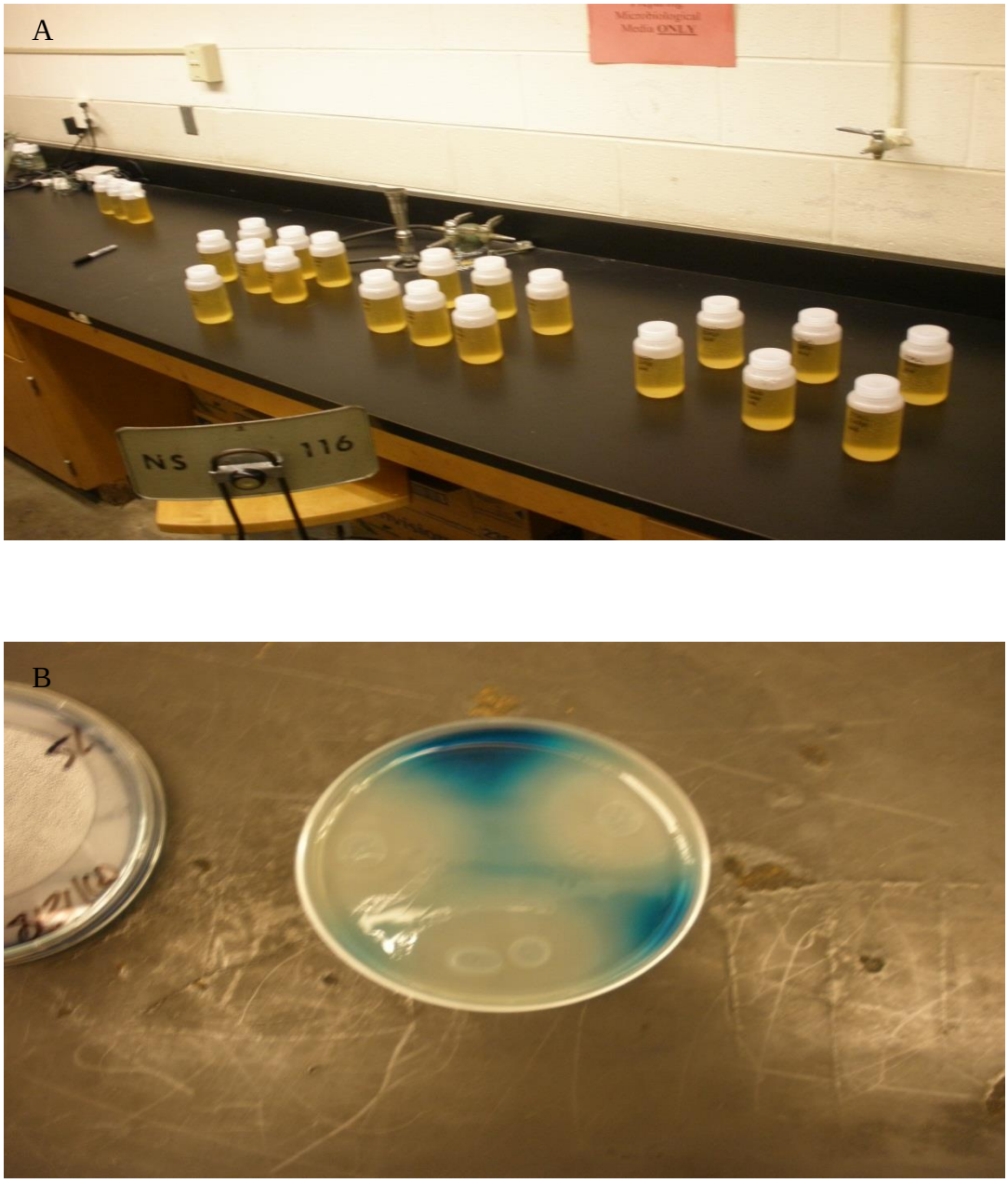

Figure 3. Media for *Salmonella spp.* isolation. (A) Bottles containing BPW prepared for inoculation. (B) RVSS agar showing characteristic spreading growth of *Salmonella spp.*

Salmonella serotyping. Typing of *Salmonella spp.* was performed using the Remel Wellcolex™ Colour Salmonella testing kit (Lenexa, KS). Potential Salmonella isolates were thawed in a water bath at 28°C and then streaked onto nutrient agar plates, which were then incubated for 24 h at 37°C. From each nutrient agar plate, a typical bacterial colony was selected and transferred into 200 µL of 0.85% sterile saline and carefully emulsified. Drops of provided latex reagents were placed on separate circles of reaction testing cards provided with the kit and each latex suspension was mixed with 40 µL of emulsified bacteria. Reaction cards were placed on a Thermo Fisher Scientific 3D rotator (Pittsburgh, PA) for 2 min. After 2 min, cards were observed for background and agglutination colors and then compared to the Wellcolex™ Colour Salmonella reading guide. Positive controls for latex reagent performance were provided in the kit and 0.85% sterile saline was used as a negative control. Positive control reactions were performed by adding one drop of each of the two latex reagents to three separate reaction card circles and then adding one drop of the three positive control types to one circle of each latex reagent type. Negative controls followed the same setup but with saline instead of the provided positive controls. Cards were placed on a Thermo Fisher Scientific 3D rotator for 2 min and then checked for appropriate reactions.

***Escherichia coli* and coliform detection and enumeration.** *E. coli* was also used as an indicator organism for fecal contamination. For the detection and enumeration of *E. coli* in compost samples the Colilert Test kit by IDEXX (Westbrook, ME) was used to assess initial numbers of the bacteria in the field study compost mounds. A 1.0 g sample from each core collection was used for bacterial enumeration by mixing the samples into milk dilution bottles containing 99 mL of deionized water. This represented a 1×10^{-2} dilution of the sample. A 1 mL volume of sample was transferred to another 99 mL bottle for another 1×10^{-2} dilution. Colilert snap packs containing ortho-Nitrophenyl-β-galactoside (ONPG) and 4-methylumbelliferyl-beta-D-glucuronide (MUG) were mixed into each bottle. After mixing, the bottles were incubated at 37°C for 24 h. Following incubation, the bottles were emptied into Colilert quantification trays and heat sealed. The trays were incubated for another 24 h and then were checked for ONPG fermentation as indicated by a yellow color, and the presence of MUG activity as indicated by fluorescence using a 365 nm ultraviolet light (Thermo Fisher Scientific). Coliform enumeration followed the same procedure but only checked for ONPG fermentation. Samples from wells positive for potential *E. coli* were plated onto MAC agar. Lactose positive colonies were transferred to nutrient agar and were confirmed as *E. coli* by testing for 4-methylumbelliferyl β-D-galactopyranoside enzyme (MUG disks; Thermo Fisher Scientific) and the ability to breakdown tryptone into indole with a rapid indole test (Remel). Base sample collections followed the same procedure but were not used for enumeration, only for the recovery of testable *E. coli* isolates. All positive *E. coli* samples were stored at -70°C in a 10% glycerol solution for future virotyping.

***Escherichia coli* virotyping.** *E. coli* isolates recovered from the compost piles were evaluated as potential enterohemorrhagic strains by testing for the presence of the O157 antigen. *E. coli* samples stored for virotyping were thawed and regrown on nutrient agar as described above. Individual colonies from nutrient agar were selected and regrown in TSB in an overnight culture. Enriched broths were tested for presence of O157 antigens by the use of the Biocontrol VIP® for EHEC detection kit. The kit follows the AOAC® Official Method 996.09 for detection of enterohemorrhagic *E. coli* (Biocontrol Systems 2000). The positive control for testing was an O157:H7 isolate, *E. coli* ATCC #43895, which was reconstituted by overnight culture in TSB; the negative control was sterile TSB. After 18 h of incubation each tube was removed from the incubator and allowed to reach room temperature. From each enriched broth tube 100 μL of broth was transferred to a VIP testing unit and then incubated for 10 min at ambient temperature. Following incubation VIP testing units were checked for positive or negative reactions and the results recorded.

SMALL-SCALE COMPOSTING

Construction of compost bins. For comparison of pathogen survivability during colder environmental temperatures, two small-scale composting trials were performed during the time period between January and April 2010. Drainage holes were drilled into the bottom corners of nine 56-qt, translucent, plastic Sterilite® containers with lids (Townsend, MA). Three containers were used for each compost material. A base layer of the composting material was made in the container and a 3-lb pork roast was placed in the center of each tub to serve as the organic material. *Salmonella enterica* Enteritidis ATCC #13076 (*S.* Enteritidis) was grown in TSB for 24 h at 37°C and adjusted to an optical density at 600 nm (OD_{600}) of 0.1 AU using a GeneQuant Pro spectrophotometer (Amersham Biosciences/GE Healthcare, Piscataway, NJ). Twelve closed, heat-transferring PCR tubes (Thermowell, Corning Inc., Corning, NY), each containing 0.5 mL of *S.* Enteritidis at 1 x 10^6 CFU/mL, were inserted into a cut made into each of the pork roasts. Each PCR tube had a loop of dental floss tied off around the cap hinge for easy extraction of the tubes through the compost material. The containers were filled approximately ¾ full with the compost material, taking care that the floss for each tube was visible on top of the compost. The containers were covered with the lids to prevent predators from disturbing the compost.

Twice a week, three tubes were extracted from each of the containers and an OD_{600} for each of the 36 tubes was determined. Optical densities of samples were determined by making a tenfold dilution of the sample in TSB, and measurement with a spectrophotometer as described above. For all optical density measurements, 1.0 mL of

sterile TSB was used as the absorbance control. Compost temperature in each tub was determined with a standard compost thermometer.

A warm weather trial from July to August 2011 was performed using the same experimental design as the winter trial. Nine plastic containers, three replicates containing each type of material, were set up with each containing a 3-lb pork roast. Twelve closed 1.0 mL heat-transferring PCR tubes containing *S.* Enteritidis, optically standardized to 1.0 AU as described above, were inserted into each pork roast. Tubes were removed from the compost twice a week during the course of the study with temperatures being checked by a standard composting thermometer. The OD_{600} of samples was determined by making a tenfold dilution with TSB as above. The number of CFU/g was determined by tenfold serial dilution in TSB followed by spread plating onto MAC agar. For the serial dilutions, 0.1 mL of sample was transferred serially into 0.9 mL tubes containing TSB, and from those dilutions, 0.1 mL of sample from each tube was added to the center of MAC agar and then spread on the agar surface with an alcohol-sterilized glass rod. Plates were incubated at 37°C for 24 h before colonies were counted.

Salmonella enumeration by bacterial optical density and viable population counts at 45ºC and 4ºC. To compare the relationship between optical density and bacterial population counts that were used for the small-scale summer trial, the following procedure was performed. *S.* Enteritidis was grown overnight in TSB and then adjusted to an OD_{600} of 1.0 AU. Volumes of 3 mL from the culture were transferred into six 15

mL glass tubes. Three tubes were placed in an incubator set at 45°C and the other three tubes were placed in a refrigerator set at 4°C. Samples were drawn at 1, 6, 24, and 96 h intervals following initial incubation. Optical densities of samples were determined by making a tenfold dilution with TSB and measurement with a spectrophotometer as described above. For all optical density measurements 1.0 mL of sterile TSB was used as the absorbance control. The number of CFU/g was determined by tenfold serial dilution in TSB followed by spread plating onto MAC agar as described above. Plates were incubated at 37°C for 24 h before colonies were counted.

Salmonella enumeration by bacterial optical density and viable population counts at 26ºC. To compare Salmonella growth in tubes inserted into compost to the growth in standardized culture conditions, *S.* Enteritidis was grown overnight in TSB and then adjusted to an OD_{600} of 1.0 AU. The Salmonella was pipetted into 39 1.0 mL-PCR tubes, and all were incubated at 26°C for the course of the study. Three tubes were removed from the incubator at each time point and each was used to make tenfold dilutions in TSB and spread plates on MAC agar as described above. The OD_{600} of each sample was also determined by making a tenfold dilution of each sample with TSB and measurement and measurement in a spectrophotometer.

Survival of *Escherichia coli* in compost material. To test the ability of *E. coli* to survive and replicate in the compost materials, the following procedure was followed. For each compost material, three 500 g portions of each compost type were added to graduated beakers and covered with aluminum foil. Composting materials were sterilized by autoclaving samples for 20 min at 121°C, then allowing samples to cool and sit for 24 h before being autoclaved again. The autoclaved composting material was checked for sterility by plating samples onto MAC agar. *E. coli* was grown in TSB in an overnight culture and then adjusted to an OD_{600} of 1.0 AU. A 1.0 mL volume of *E. coli* was mixed into each material, which were then incubated at 33°C for the course of the study. Twice a week the inoculated compost samples were mixed, and a 1 g portion from each beaker was serially diluted tenfold (1 g / 9 mL) in TSB. Dilutions were plated as described above onto MAC agar and plates were incubated for 24 h at 37°C. Colony counts were performed and the data used to calculate the total number of CFU/g of the composting material. The autoclaved composting samples were checked for sterility by following the same procedure as that of the inoculated samples before use in the experiment.

STATISTICAL ANALYSES

Average colony forming units per gram or mL were calculated for samples with sufficient replicates to derive the individual statistics. Standard deviation was calculated for samples that met the conditions for calculation. Samples with less than two countable replicates were used for an average population based on the countable colonies but not for standard deviation.

Linear regression was utilized for samples from which CFU/mL could not be obtained. Data for these points were estimated using CFU/mL counts and optical densities from both previous and subsequent collections of the same compost type.

For sample sets that met the criteria for comparison, one-way analysis of variance (ANOVA; $p = 0.05$) testing was performed for between group comparisons using Microsoft Excel. Criteria for comparison involved the existence of CFU/g or CFU/mL and optical density data from different compost types of the same trial with corresponding time points. ANOVA comparison testing included both CFU/g or CFU/mL and optical density between data sets.

To determine statistically significant differences between individual data points, t-tests (Microsoft Excel; $p = 0.05$) were used for within group comparisons for sets that showed a significant difference in ANOVA testing results. For samples showing only an increase or decrease following the start of a trial, one-way, directional t-tests were used to determine significance. For samples that showed values both above and below the original inoculum, a two-way, non-directional t-test was used to determine significance.

III. RESULTS

This study was initiated as part of a larger study on the feasibility of different compost materials for large-scale composting of mortalities following natural or anthropogenic disasters. Large amounts of available compost material would differ regionally and for this project, soil, sand and stall scrapings (a mixture of wood chips, hay, and manure) were compared. The goal of this study was to compare the effectiveness of the different compost materials in pathogen reduction and prevention of pathogen contamination of surrounding soil as well as surface and groundwater.

LARGE-SCALE COMPOSTING

First large-scale 2010 summer trial. For the project, swine carcasses were used as the model organisms for the composting. The sampling period for the first set of compost mounds occurred from 5/27/2010 through 6/29/2010 (33 days). Of the three compost mounds, only the stall mound tested positive for detectable levels of *E. coli,* while the sand and soil mounds contained coliforms but no *E. coli*. Over the course of the trial, coliforms from the sand mound went from undetectable levels on the first sampling to 1.0 x 10^4 CFU/g by the second sampling (Figure 4). The increase in coliform levels corresponded with a significant rain event that may have contributed to the movement of bacteria through the sand mound. The sand mound coliform population declined back to undetectable levels for the remainder of the trial following the second collection point two weeks into the sampling.

The soil compost mound coliform population was 1.0×10^4 CFU/g on the first day of sampling, indicating a detectable coliform population was already present in the soil used for the composting. The coliform concentration increased to the maximal detectable level of $>4.35 \times 10^6$ CFU/g by the eighth day of sampling and was maintained at that level until the final sampling 24 days later when the coliform population declined to 1.0×10^4 CFU/g (Figure 4A). The increase in coliform number also correlated with a rainfall event of over 1 inch, which may have facilitated movement of bacteria to the outer areas of the pile.

The stall mound *E. coli* population was determined to be 7.3×10^5 CFU/g on the first day of sampling. Because this material contained manure, this was expected. Following the first day of testing, the *E. coli* population of the stall mound increased to the maximal detectable level of $> 4.35 \times 10^6$ CFU/g and remained at this level for the rest of the trial period (Figure 4B). Because *E. coli* is a coliform and the maximal detectable level of *E coli* was reached, there is no separate plot for the coliforms. The stall material was the only compost mound that did not demonstrate a decrease in the coliform population by the end of the 33-day trial.

Precipitation was recorded as the sum of rainfall in inches occurring on days between sampling events. The largest amount of precipitation recorded coincided with the final sampling period, with a total amount of 2.7 in of rain. All other time points showed a range of precipitation between 0.1-1.53 in of rainfall, with an overall average of 0.9 in of precipitation between samplings (Figures 4A-C).

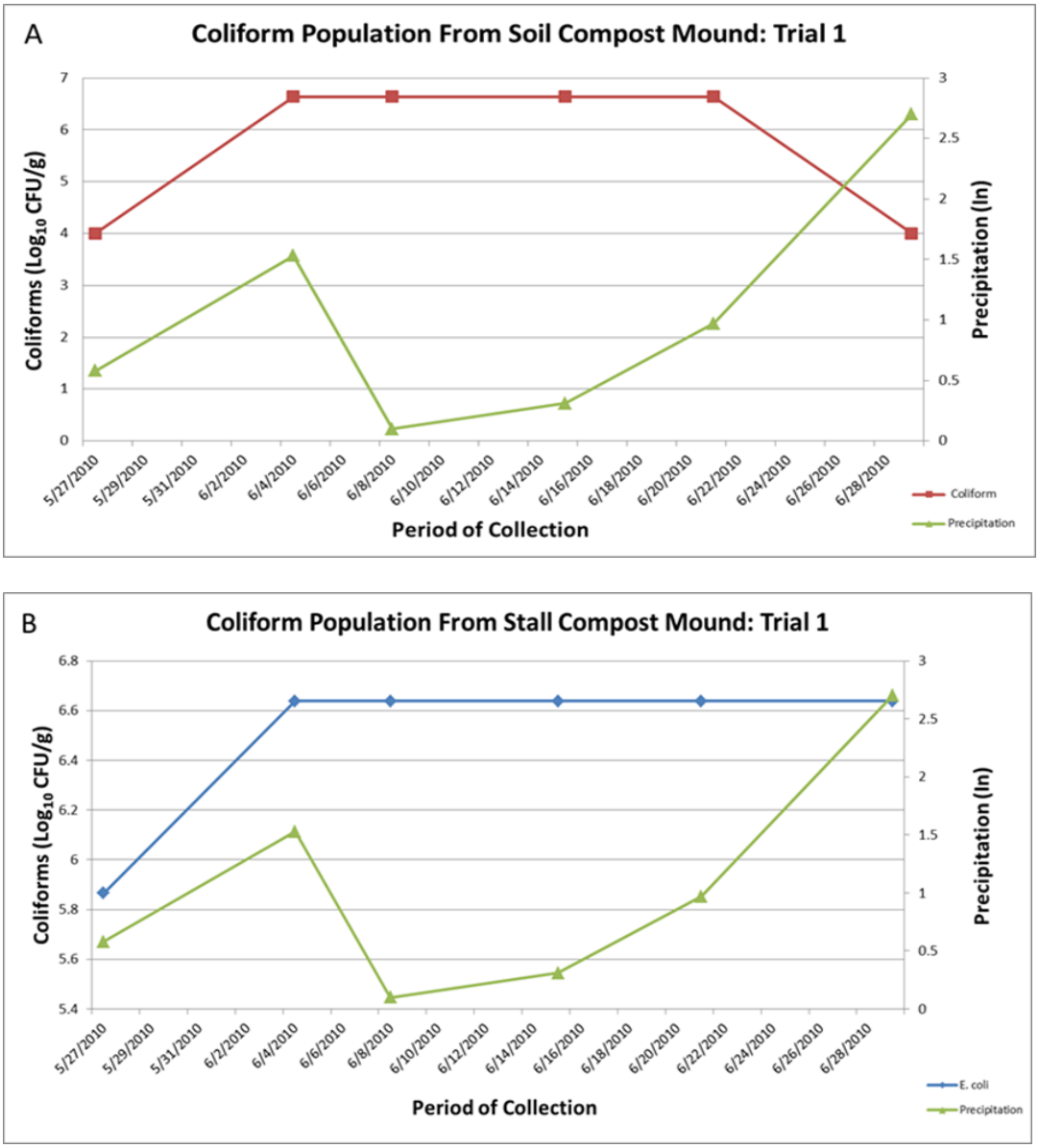

Figure 4. The concentration of bacteria in soil (A), stall (B), and sand (C) compost mounds as measured in CFU/g (left axis). Mounds were sampled at weekly intervals for a period of 33 days, and samples were processed for total coliform and *E. coli* populations. Precipitation was recorded by a weather monitoring station (right axis).

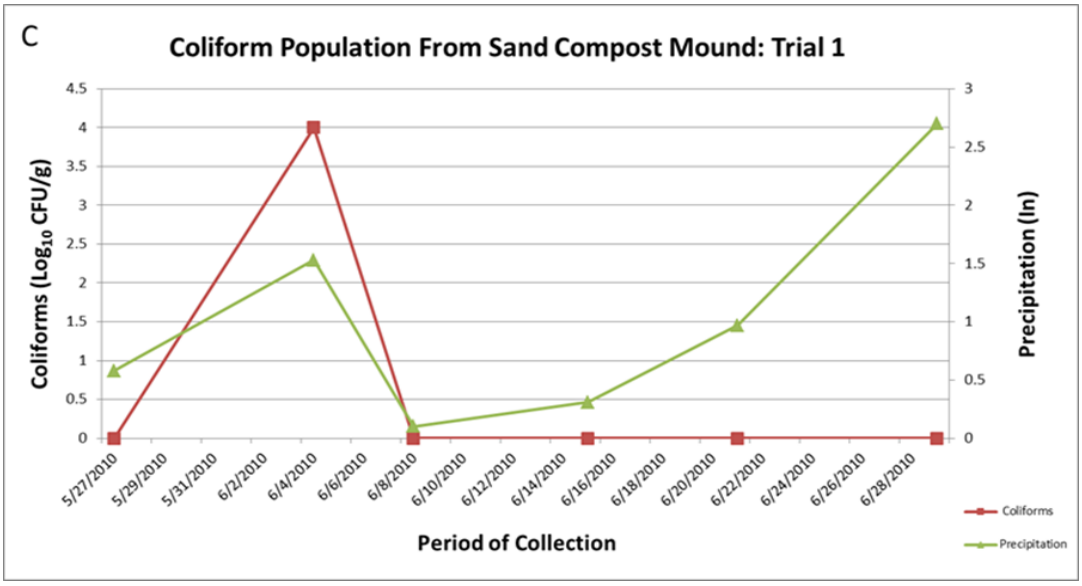

Figure 4 continued. The concentration of bacteria in soil (A), stall (B), and sand (C) compost mounds as measured in CFU/g (left axis). Mounds were sampled at weekly intervals for a period of 33 days, and samples were processed for total coliform and *E. coli* populations. Precipitation was recorded by a weather monitoring station (right axis).

Second large-scale 2010 summer trial. A second field trial using piglet carcasses for composting was performed in order to confirm the effectiveness of the different compost materials and to have a replicate mound of each compost material. The testing period for the second compost mounds occurred between 7/29/2010 and 10/22/2010. The sampling period was extended from 33 to 87 days to determine whether detectable levels of coliforms and *E. coli* would decrease in the compost mounds.

Soil compost coliform populations were at the maximal recordable level of >4.35 x 10^6 CFU/g on the first day of sampling for both mounds signifying that the soil used for

the composting contained coliforms (Figure 5A). The first soil compost mound maintained the maximum detectable level throughout the sampling period, except for one sampling event where the population declined to 1.0×10^4 CFU/g on day 8/7/2010 (day 10). The coliform population of the second soil compost mound maintained a maximum population level for ten of the fifteen sampling points. The coliform population fell to 1.0×10^4 CFU/g on 8/19/2010 (day 21), and reached undetectable levels two days later on 8/21/2010 (day 23). Coliform detection rose again to maximal levels until 9/10/2010 (day 43) where it decreased to undetectable levels. It then rose again to the maximal level until 10/15/2010 (day 80) when it remained undetectable throughout the final sampling on 10/22/2010. The failure to detect the coliforms on days 23 and 43 may have been due to sampling error because maximal levels were obtained on all other sampling days until day 80 of the trial. This second trial was similar to the first in which coliforms were detectable throughout the sampling period in the soil mounds.

In the first field trial, *E. coli* was not detectable in the soil compost mound. For the second trial one mound had no detectable *E. coli,* while in the second mound *E. coli* was detectable only on 8/19/10 (day 21; Figure 5B). This detection followed a significant rainfall event which could have contributed to some movement of the bacteria from the carcass throughout the pile.

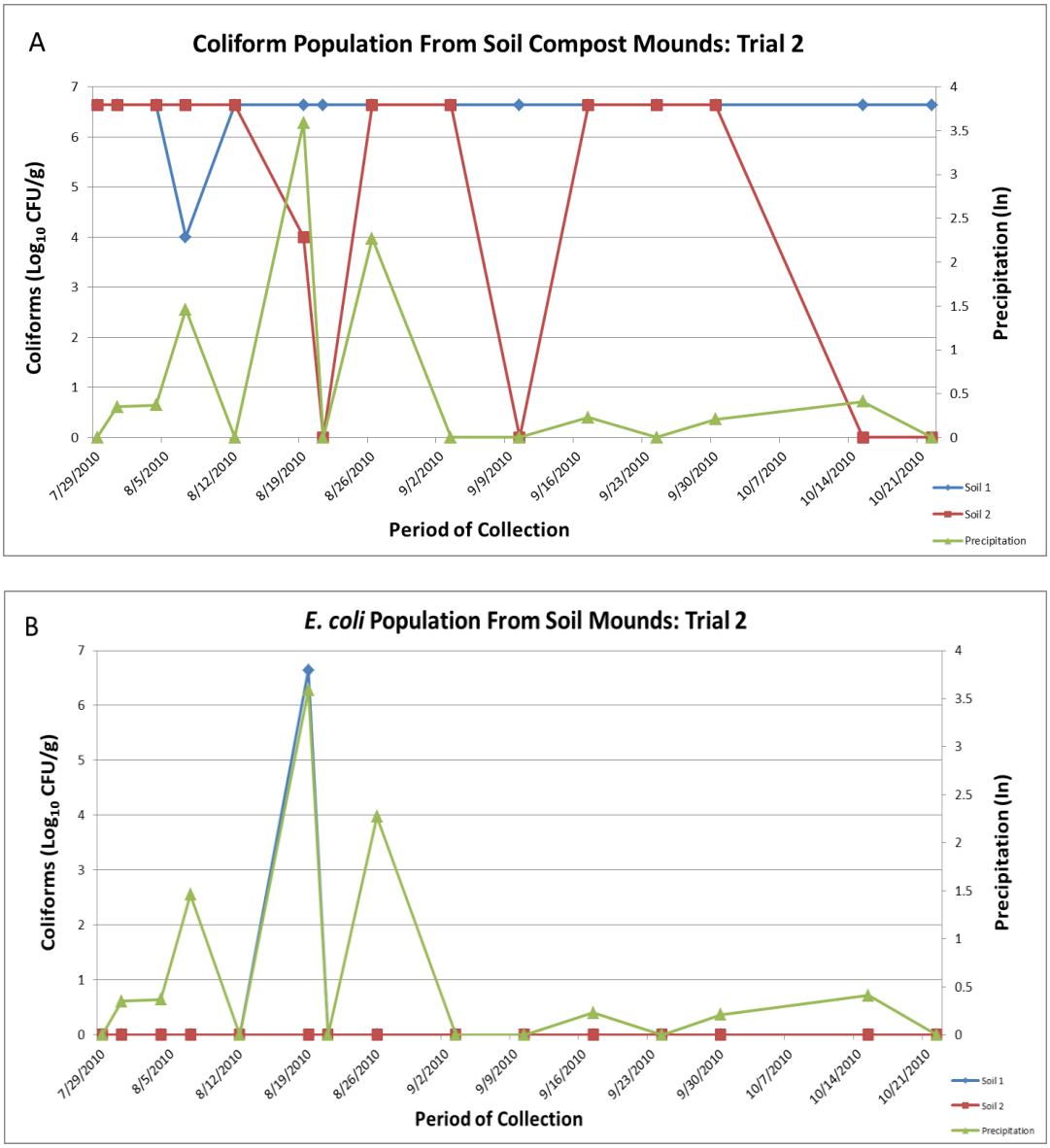

Figure 5. The concentration of coliforms (A) and *E. coli* (B) in soil compost mounds as measured in CFU/g (left axis). Mounds were sampled at biweekly intervals over an 87-day period and samples were processed for total coliform and *E. coli* populations. Each individual mound is represented on the graph. Precipitation was recorded by a weather monitoring station (right axis).

For the second field trial, stall compost piles maintained coliform populations of >4.35 x 10^6 CFU/g except for two sampling collections (Figure 6A). The first stall mound coliform population fell to undetectable levels on two occasions, once on 8/26/2012 (day 29) and again on the final sampling date 10/22/2010 (day 87). These were most likely attributed to sampling error, because the second compost mound maintained the maximum population level detectable by testing over the entire course of the trial period. The *E. coli* population of the first stall compost mound reached detectable levels only once during the trial period. On 9/3/2010, the *E. coli* population of the first stall mound reached the maximal level of > 4.35 x 10^6 CFU/g, otherwise remaining at < 1.0 CFU/g on all other sampling points. The *E. coli* population of the second stall mound reached the maximal detectable level on four occasions, those being on 7/29, 7/31, 8/19, and 8/21/2010. All other collection points of the second stall mound had *E. coli* population levels of < 1.0 CFU/g. Again, these detections followed rainfall events of greater than 1 in.

The material used for the stall mounds was the same material used for trial 1 and had been sitting at the composting site for more than two months before it was used in the second trial. This may have led to a natural decrease in the resident *E. coli* population so that sampling during trial 2 resulted in sporadic recovery of the bacteria. Nonetheless, no *E. coli* were detectable after 9/10/2010 (day 43; Figure 6B).

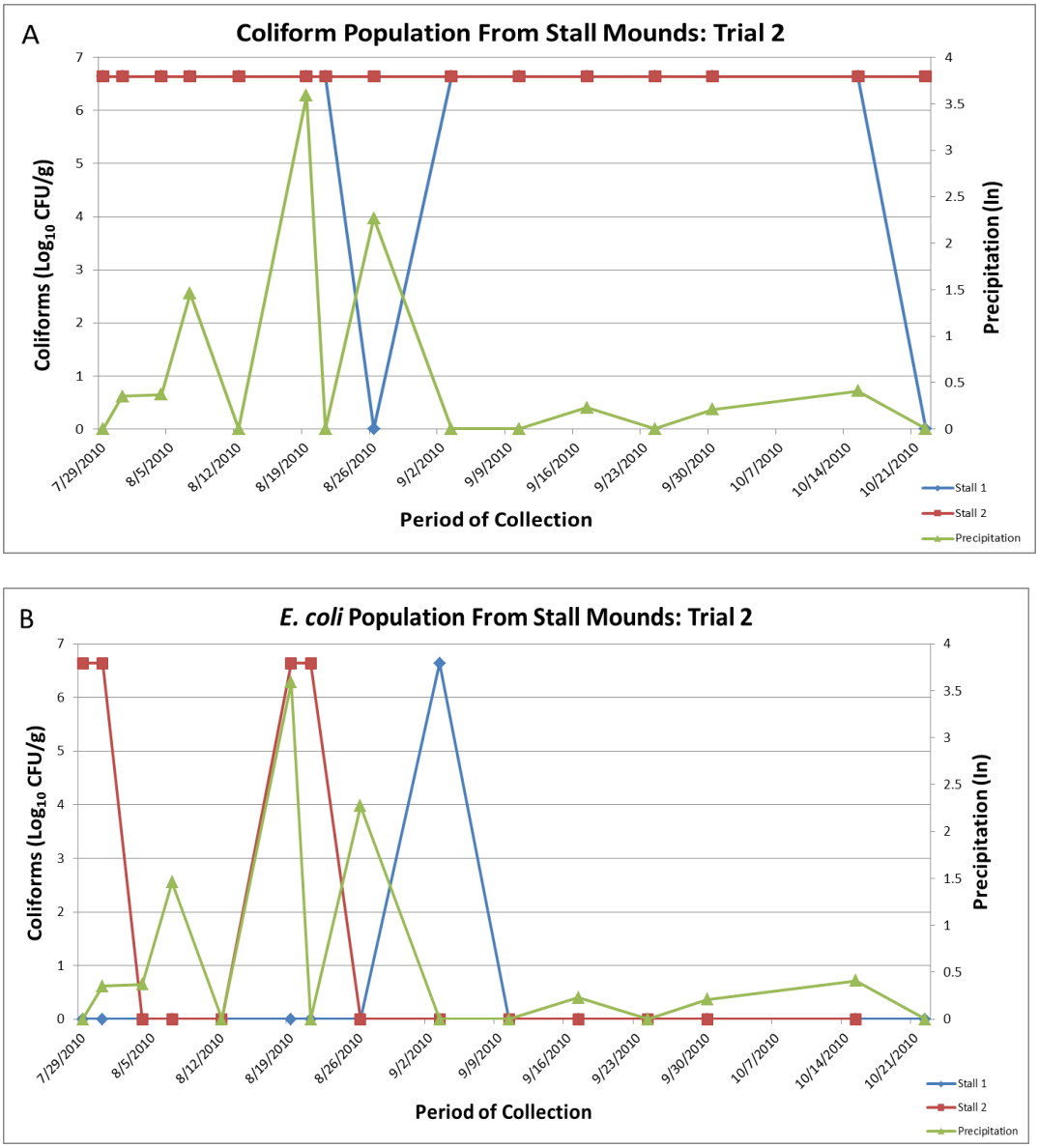

Figure 6. The concentration coliforms (A) and *E. coli* (B) in stall compost mounds as measured in CFU/g (left axis). Mounds were sampled at biweekly intervals over an 87-day period and samples were processed for total coliform and *E. coli* populations. Each individual mound is represented on the graph. Precipitation was recorded by a weather monitoring station (right axis).

For the sand compost mounds, one mound had an *E. coli* population at the maximal detectable level of > 4.35 x 10^6 CFU/g for the first two weeks of the sampling period (Figure 7). One of the sampling dates (day 7) had no detectable *E. coli*; however, this could be attributed to an inconsistency in sampling because levels were again at the maximal level by day 10. By day 21, no *E. coli* or coliforms were detectable in this mound and the second sand compost mound maintained an undetectable level of coliforms and *E. coli* throughout the entire trial period. This pattern of bacterial elimination was similar to the first trial in which the coliforms and *E. coli* were undetectable after day 14.

Precipitation for trial 2 was recorded as amount of rainfall in inches, with each sampling point precipitation amount being the sum of rainfall since the last date of sample collection (Figures 5-7). Over the course of the trial period of 87 days, there was a total of 8.9 inches of rainfall, averaging 0.6 inches per sample collection. The highest amount of rainfall was on 8/19/2010 with 3.59 inches of precipitation. On seven occasions there was no rainfall between collection points. Rainfall events were associated with increases in *E. coli* detection.

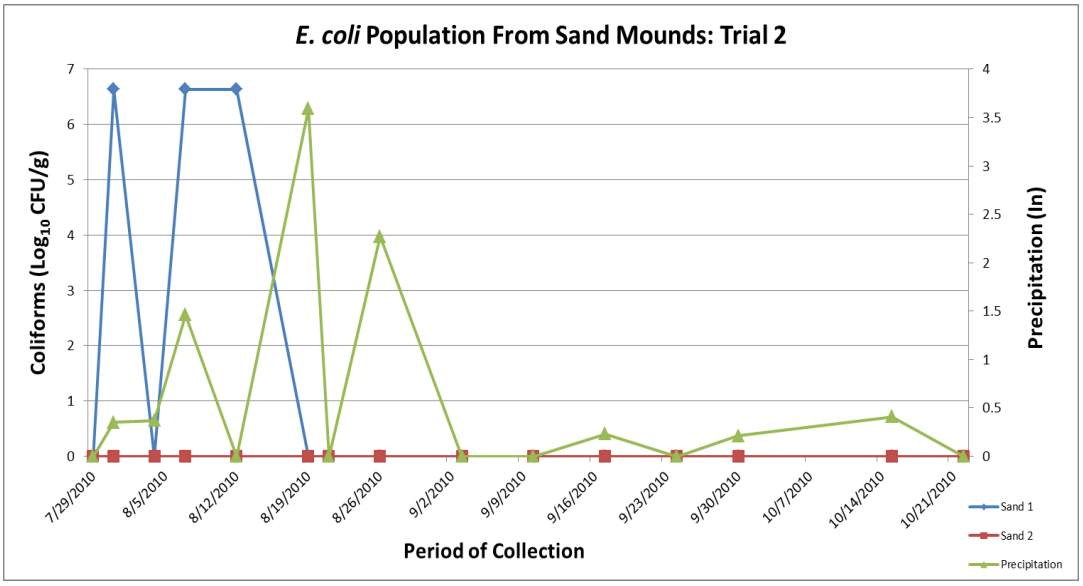

Figure 7. The concentration of *E. coli* in sand compost mounds as measured in CFU/g (left axis). Mounds were sampled at biweekly intervals over an 87-day period and samples were processed for total coliform and *E. coli* populations. Each individual mound is represented on the graph. Precipitation was recorded by a weather monitoring station (right axis).

Survival of *Escherichia coli* in compost materials. To show that *E. coli* growth was not inhibited by the sand or any of the other compost materials, *E. coli* was inoculated into the autoclaved compost media without any animal carcass to provide additional nutrients, and incubated in the laboratory at a constant temperature of 33°C for a 23-day period. The *E. coli* was standardized to an optical density of 1.0 AU at 600 nm, corresponding to a concentration of 2.4×10^6 CFU/g, mixed into the compost sample, and monitored for growth in the compost materials (Figure 8A-C). The data was analyzed with non-directional, two-sample t-tests for soil samples and one-way, paired t-tests for stall and sand samples using Microsoft Excel (Appendices 5.4-5.6). *E. coli* in soil compost showed a slight decline in population after incubation followed by an increase to 5.8×10^6 CFU/g on the second sampling, both collection points showing no significant difference from the initial population concentration ($p = 0.88$, $p = 0.18$) (Figure 8A). The third sampling of the population showed a decline to a low of 7.3×10^4 CFU/g, which is significantly less than the initial population concentration ($p = 0.01$). The final collection showed a rebounding of the population to 4.9×10^5 CFU/g, but was also significantly less than the initial population concentration ($p = 0.01$). Statistical analysis showed that when grown in soil, the *E. coli* population was significantly reduced in comparison to the starting population. This indicates some inhibition of *E. coli* growth in soil compost samples.

The stall compost bacterial population showed a decline over the first three collection points during the testing period, reaching a low of 4.35×10^5 CFU/g (Figure 8B). This decline was found to be significantly less than the population of the original

compost sample ($p = 0.01$). The stall compost bacterial population showed a slight increase in population on the final sampling day, with a final population of 2.2×10^6 CFU/g. This increase in population was not significant in comparison to the original compost sample population ($p = 0.46$). The lack of a significant increase in the *E. coli* population over the course of the trial indicates some level of bacterial inhibition occurring in the stall compost samples.

Samples from the sand compost showed a statistically significant increase in population on the first day of sampling ($p = 0.04$), with a two-log increase to 3.1×10^8 CFU/g (Figure 8C). *E. coli* in the sand compost sample showed a steady decline in population throughout the remainder of the trial. However, the final two collection points showed no statistically significant difference between their population concentrations and that of the initial inoculum ($p = 0.06$, $p = 0.15$). The final population concentration shows that over the course of the study there was no significant change in overall population in the sand compost. While there was a significant difference in the population of the first two collection points ($p = 0.04$, $p = 0.01$), overall there was no significant change when the final collection point is compared to the initial inoculum population.

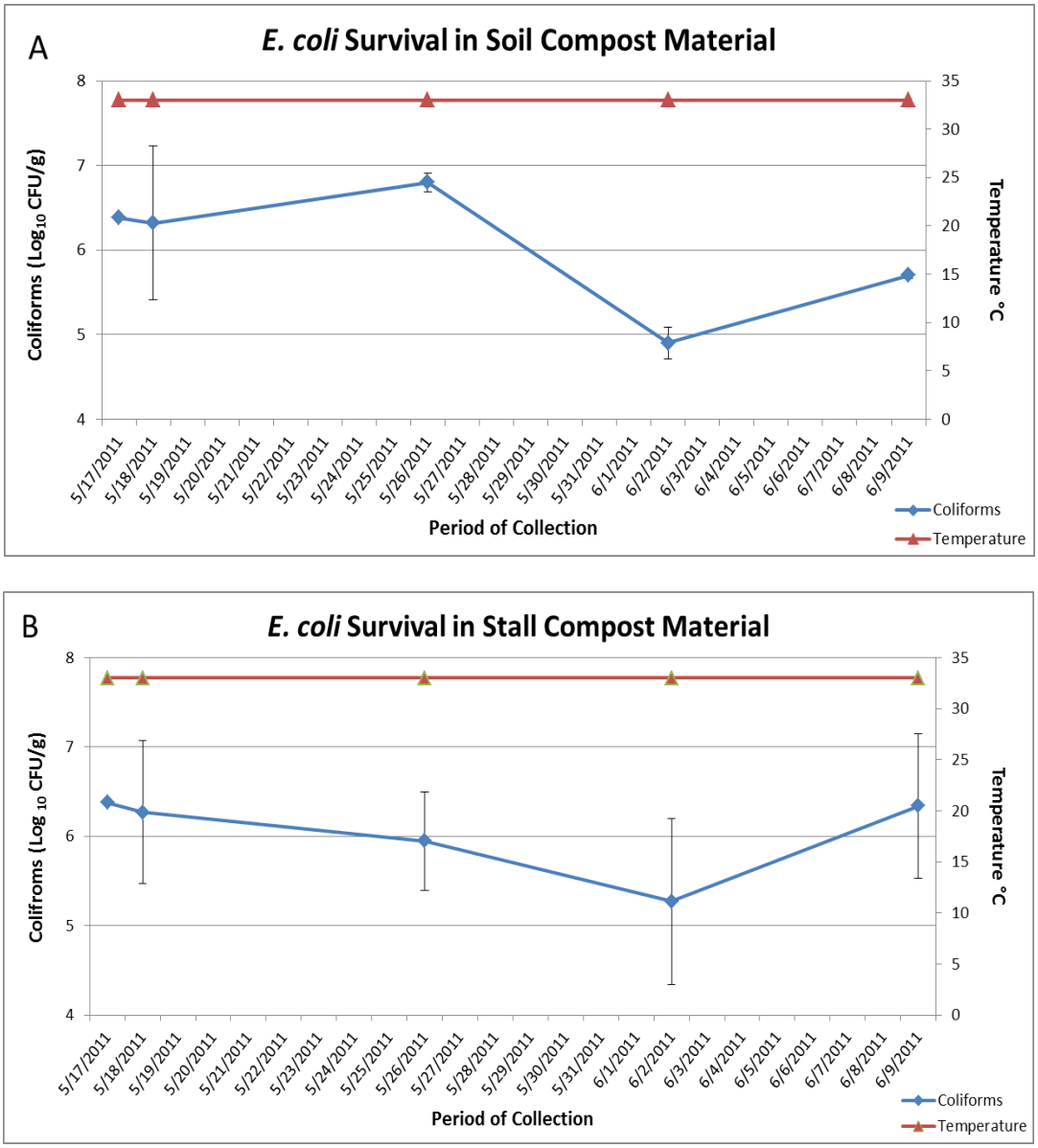

Figure 8. Survival of *E. coli* in the soil (A), stall (B), and sand (C) compost material at 33°C. The compost materials were autoclaved twice and inoculated with 2.4 x 10^6 CFU/g *E. coli*. Three replicates of each compost material were used. Uninoculated controls were also tested for bacterial growth. Bacterial concentration was determined by dilution of compost samples and aerobic bacterial plate counting.

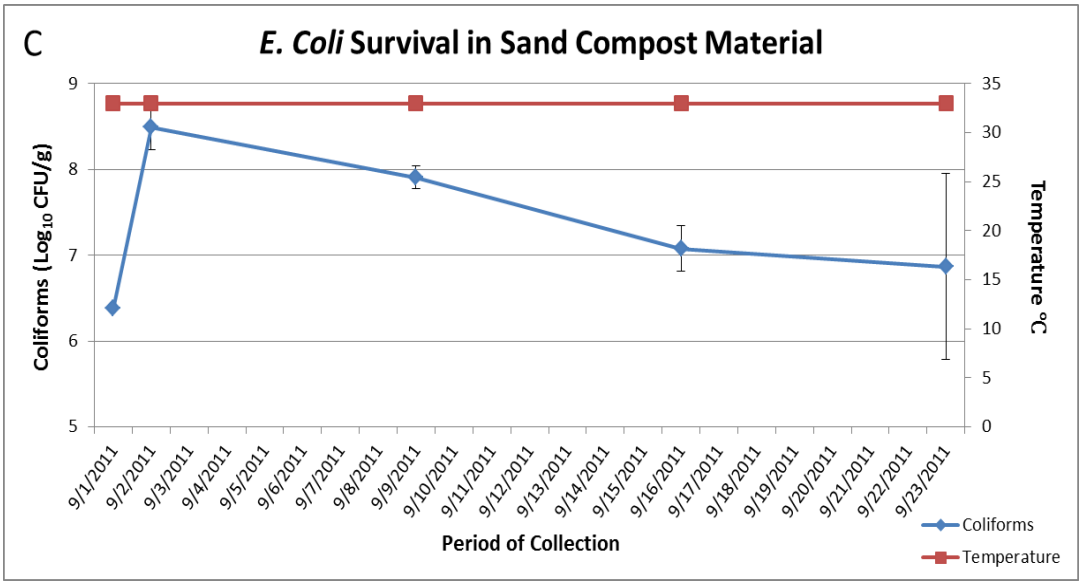

Figure 8 continued. Survival of *E. coli* in the soil (A), stall (B), and sand (C) compost material at 33°C. The compost materials were autoclaved twice and inoculated with 2.4 x 10^6 CFU/g *E. coli*. Three replicates of the compost materials were used. Uninoculated controls were also tested for bacterial growth. Bacterial concentration was determined by dilution of compost samples and aerobic bacterial plate counting.

Identification of *Escherichia coli* and Salmonella isolates from large-scale composting. To determine whether potentially pathogenic strains were surviving in the carcass-containing mounds from the field studies, samples from the mounds were tested for the presence of enterohemorrhagic *E. coli* and *Salmonella spp*. *E. coli* isolates were recovered from positive wells of Colilert quantification trays used to test compost samples. Isolates were confirmed as *E. coli* with additional biochemical testing before testing for the O157 serotype by the VIP® test. Prior to testing, positive and negative controls were used as quality controls. Of four *E. coli* isolates recovered, only one isolate tested positive as an O157 strain (Table 1).

Table 1. Results of *E. coli* VIP® testing for serogroup O157.

Sample Type	Date	Reaction
Stall Base	5/27/2010	Negative
Stall Base 2	7/31/2010	Negative
Sand Base 1	8/7/2010	Positive
Soil Base 1	8/19/2010	Negative

For the large-scale composting trials, samples were also collected for Salmonella testing to determine whether bacteria potentially harbored in the animal body cavity could survive composting and leach through the compost material into the environment. It was not known at the time of burial whether any of the pig carcasses carried

Salmonella. Sample isolates that tested presumptively as *Salmonella spp.* by TSI, LIA, and urease broth were serotyped with the Remel Wellcolex™ Colour Salmonella testing kit. Six of the seven presumptive Salmonella isolates showed the color and clumping pattern distinctive of Group C Salmonella. One isolate tested as negative, showing no distinct color change or clumping pattern. This isolate was retested using the BD BBL Crystal™ system for Gram-negative organisms. Results of that test showed that the isolate from the second stall mound from trial 2 was likely a species of Salmonella that was unreactive with the Wellcolex testing kit.

Table 2. Salmonella serogrouping results on isolates presumptively positive by biochemical testing.

Sample Type	Date	Serotype	BBL Crystal Results
Stall Base 2	8/12/2010	Group C	
Soil Base 1	9/10/2010	Group C	
Stall Core 2	9/10/2010	Negative	Confirmed Salmonella by BBL Crystal
Soil Base 1	9/17/2010	Group C	
Stall Core 1	10/15/2010	Group C	
Stall Core 2	10/22/2010	Group C	
Stall Base 2	10/22/2010	Group C	

SMALL-SCALE COMPOSTING

To further explore the effectiveness of the different composting materials on pathogen reduction, small-scale composting studies were begun to incorporate more replicates and to control the bacterial pathogen used instead of relying on bacteria resident in the compost material or carcass body cavity. Small-scale composting also facilitated the study of environmental temperature on pathogen reduction. Small-scale composting testing included two winter trials and one summer trial. For these trials, Salmonella was used as the test organism. Salmonella represents a human pathogen and has been shown to be more stable in the environment than *E. coli* (Winfield and Groisman 2003).

First small-scale 2011 winter trial. The sampling period for the first winter trial occurred from 1/24/2011 through 3/3/2011. Composting tubs were filled with materials remaining from the large-scale composting trials. PCR tubes were filled with 0.5 mL of cultured *Salmonella enterica* Enteritidis that had been standardized to an optical density 0.1 AU at 600 nm (OD_{600}). The OD_{600} of all standardized Salmonella samples remained measurable over the 39-day study period.

The optical densities of Salmonella samples inserted into pork in soil compost increased significantly beginning on 1/24/2011 (day 4; Figure 9A), reaching a high of 0.77 AU on 2/18/2011 (day 25). The OD_{600} values then began to decline through the end of the trial. All samples except for those drawn on 3/1/2011 (day 36; $p = 0.09$) and 3/3/2011 (day 38; $p = 0.08$), the final two days of the trial, were shown by one-way t-test

analysis to be significantly greater than the OD_{600} of the original inoculum (Appendix 2.3), indicating that bacterial concentration increased significantly over the trial period but then returned to initial concentrations after day 36 of the trial. Increases in the OD_{600} correlated with increases in temperature over the trial period until the last fourteen days when the OD_{600} began to decline. Temperatures of the soil compost ranged from 0°C to 16°C during the trial period with the low of 0°C recorded on 2/11/2011 (day 19) and the high of 16°C on 3/1/2011 (day 36).

The optical densities of Salmonella samples inserted into pork in stall compost (Figure 9B) had a low absorbance reading of 0.33 AU on 1/25/2011 (day 2) and reached a high of 0.71 AU on 2/11/2011 (day 19). The OD_{600} of stall compost Salmonella samples remained between 0.53 AU and 0.42 AU after peaking on day 19, with a final absorbance of 0.44 AU on the final day of the trial. All stall samples except for those drawn on 2/22/2011 (day 30; $p = 0.12$) and 2/25/2011 (day 33; $p = 0.06$) were shown by one-way t-test analysis to be significantly greater than the OD_{600} of the initial inoculum (Appendix 2.2). The Salmonella were able to significantly increase during the trial and remained significantly above the initial inoculum at the end of the trial. Temperatures of the stall compost ranged from 1 to 14°C during the trial period with the low temperature recorded during three collections points (days 4, 11, and 19). The highest temperature was recorded on the 2/22/2011 (day 30). Increases in sample OD_{600} correlated with increases in temperature over the trial period.

The optical densities of Salmonella inserted into pork in sand compost (Figure 9C) had a low absorbance reading of 0.31 AU (day 2) and reached the highest absorbance

value of 0.68 AU on 2/1/2011 (day 8). The Salmonella OD_{600} remained at detectable levels over the remainder of the sampling period with a final value of 0.53 AU on the last day of the trial. One-way t-test analysis of samples show that all samples drawn between 1/25/2011 and 2/16/2011 (days 2 – 23) were significantly greater than the OD_{600} of the initial inoculum, while samples drawn between 2/18/2011 and 3/3/2011 (days 25 – 38) were not significantly different from the OD_{600} of the initial inoculum (Appendix 2.4). Sand compost temperatures ranged from 0°C to 15.6°C during the trial period, with increases in absorbance correlating with increases in temperature.

ANOVA testing of the OD_{600} values showed no significant difference between the three compost types in the first winter trial (Appendix 2.5). This indicates that when compared to each other, the compost type had no significant inhibitory effect on the average optical density of Salmonella populations throughout the course of the trial.

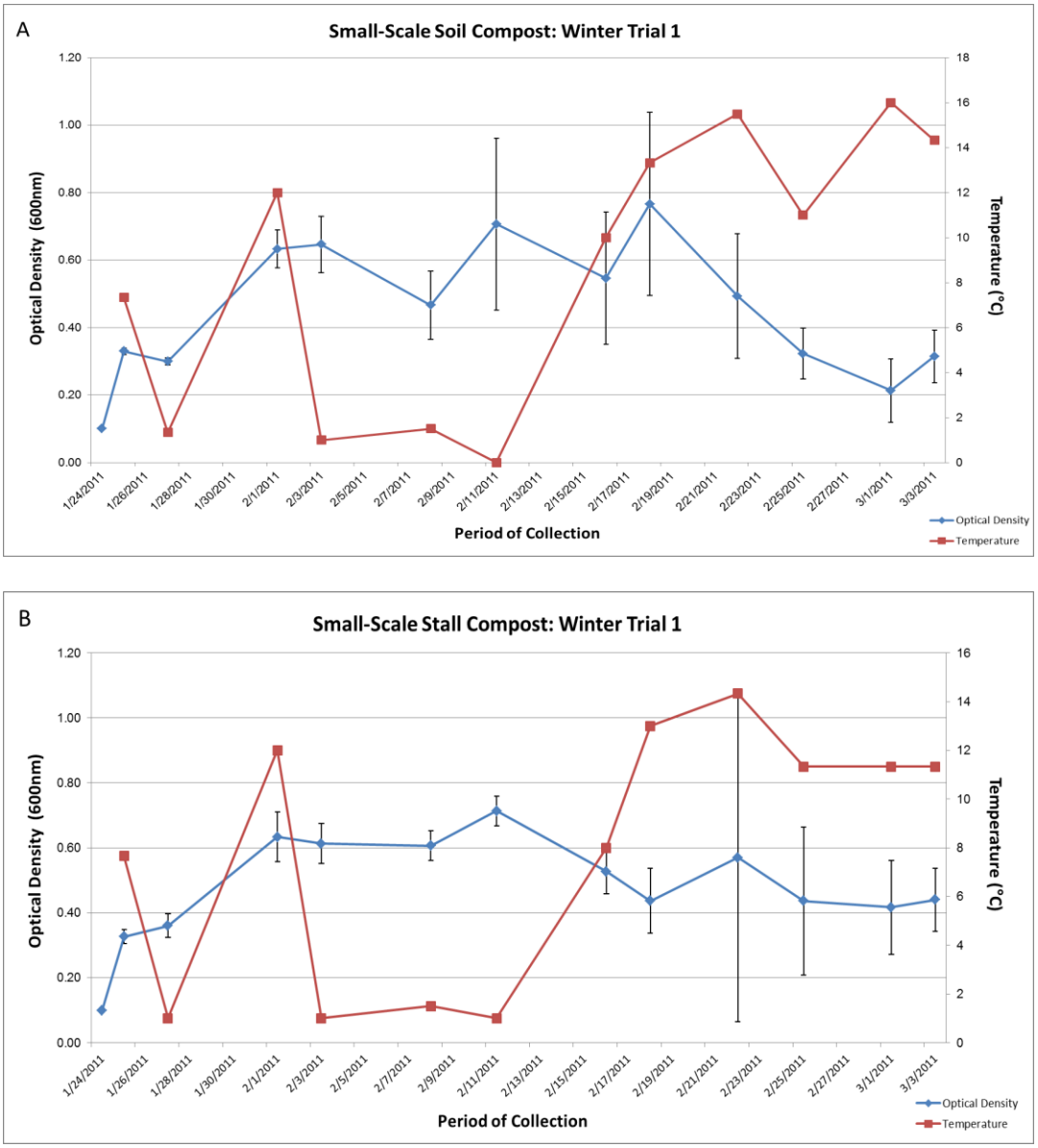

Figure 9. The optical density of small-scale composting Salmonella samples in soil (A), stall (B), and sand (C) compost. Optical density was measured as AU at 600nm (left axis). Tubes of Salmonella in compost tub samples were drawn in biweekly intervals over a 39-day period and samples were measured for optical density. Temperature was recorded by inserting a thermometer into each of the compost tubs (right axis).

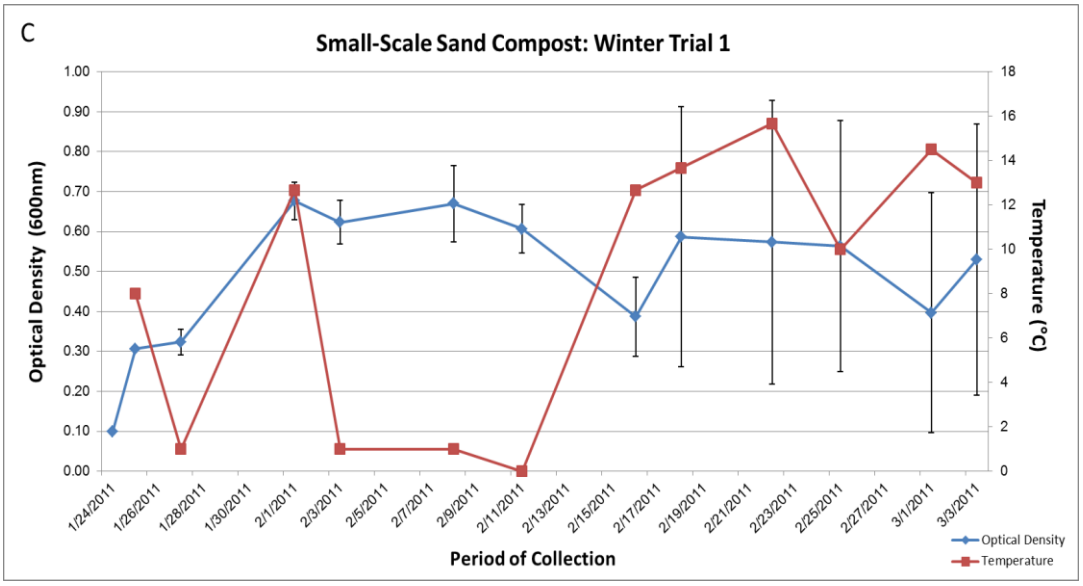

Figure 9 continued. The optical density of small-scale composting Salmonella samples in soil (A), stall (B), and sand (C) compost. Optical density was measured as AU at 600 nm (left axis). Tubes of Salmonella in compost tub samples were drawn in biweekly intervals over a 39-day period and samples were measured for optical density. Temperature was recorded by inserting a thermometer into each of the compost tubs (right axis).

Second small-scale 2011 winter trial. The sampling period for the second winter trial testing occurred from 3/9/2011 through 4/19/2011. A second winter trial was performed for reproducibility of the first trial and to test compost sample performance with warming temperatures. Compost materials from the first small-scale composting trial were reused along with the addition of compost material to make up for volume loss. PCR tubes were again filled with 0.5 mL of cultured Salmonella that had been standardized to an optical density of 0.1 AU at 600 nm. The optical density of all standardized Salmonella samples remained measurable over the 41-day study period.

The optical density of Salmonella in tubes from soil compost (Figure 10A) reached a high absorbance value of 1.54 AU on 4/14/2011 (day 36) and a low of 0.38 AU on the final day of the trial (day 41). One-way t-test analysis showed significant increases in optical density from the initial inoculum for samples drawn on 3/10/2011-3/22/2011 (days 2 through 14; p values ranging from < 0.01 to 0.03), other significant increases occurred on 3/29/2011 and 3/31/2011 (days 21 and 23; $p = 0.01$, $p < 0.01$) and 4/19/2011 (day 41; $p = 0.02$). All other samples differences were not significant when compared to the initial inoculum (Appendix 3.3). Temperatures ranged from 6.7°C to 25°C during the trial period, with the low recorded on the second day of sampling and the high on the 4/14/2011 (day 36). Increases in optical density correlated with increases in temperature during the trial period.

The optical density of Salmonella stall compost samples (Figure 10B) showed an initial increase, reaching a high of 1.32 AU on the final day of the trial, which may have been the result of a trend of increasing compost temperatures from 10°C up to 25°C

towards the end of the trial period. One-way t-test analysis showed that samples drawn on 3/10/11 through 3/29/11 (days 2 through 21; p values ranging from $p < 0.01$ to $p = 0.02$) were significantly greater than the OD_{600} of the initial inoculum. This was followed by a decline in optical density with the lowest OD_{600} value recorded on 4/12/2011 (day 34; $p = 0.02$). All other sample differences were not statistically significant when compared to the initial inoculum OD_{600} (Appendix 3.2). Temperatures ranged from 7°C to 25.7°C, the lowest temperature was recorded on 3/31/2011 (day 23) and the highest on 4/14/2011 (day 36). Over the trial period increases in OD_{600} correlated with increases in compost temperature.

The optical density of Salmonella sand compost samples (Figure 10C) ranged from 0.36 to 1.22 AU over the course of the trial. The lowest value was recorded on 4/5/2011 (day 28) and the highest on the 4/14/2011 (day 36). One-way t-test analysis showed that all the samples except for those drawn on 3/24/2011 (day 16; $p = 0.12$) and 3/31/2011 (day 23; $p = 0.08$) were significantly higher when compared to the initial inoculum (Appendix 3.4). Temperatures ranged from 6°C to 30°C, with the lowest temperature recorded on day 22 of sampling and the highest on the 4/14/2011 (day 36).

ANOVA analysis of the three compost types over the course of the trial showed no statistical difference among the optical density results (Appendix 3.5). This indicates that compost type had no significant inhibitory effect on the average optical density of Salmonella populations inserted into pork samples during the winter trial.

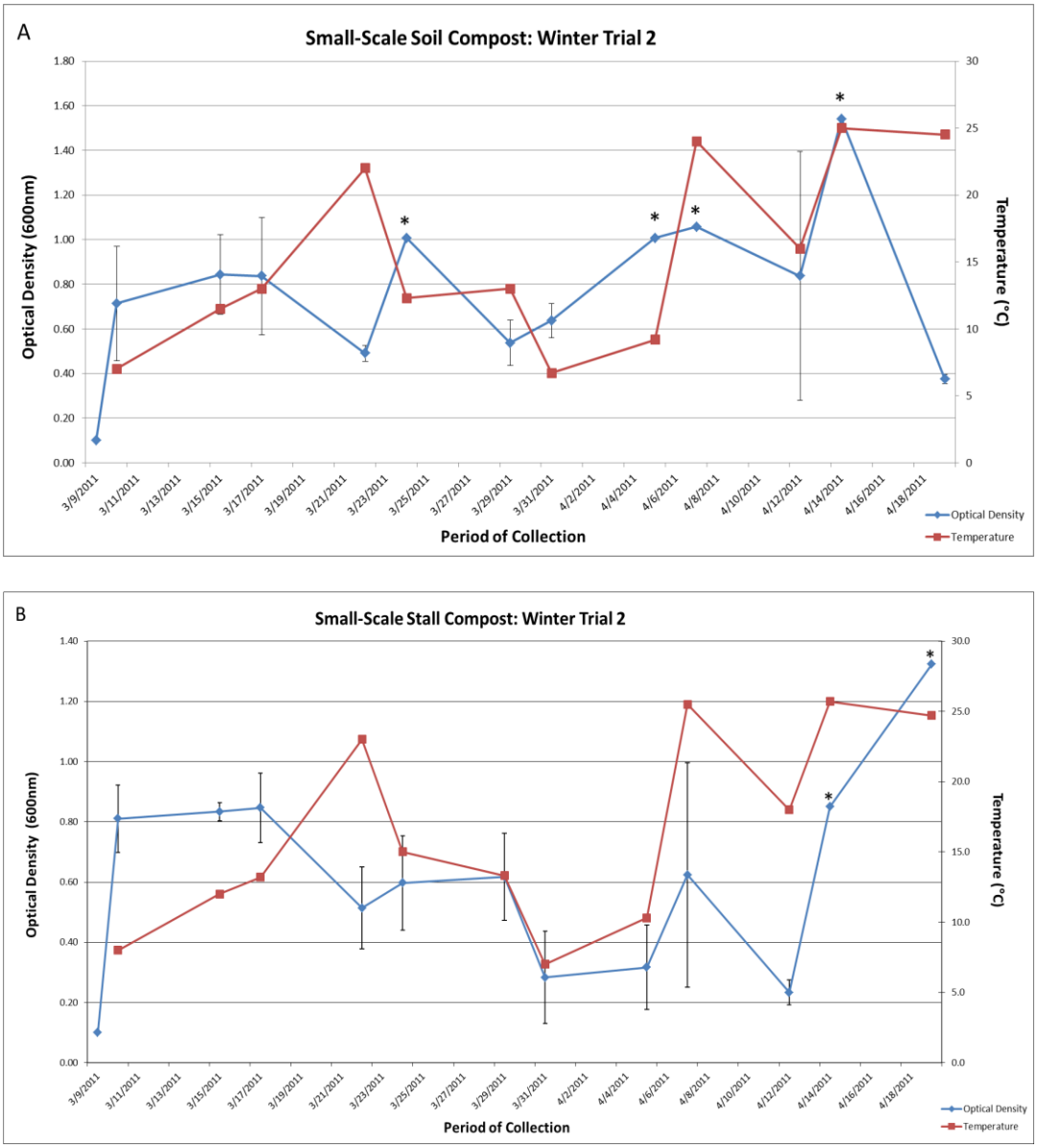

Figure 10. The optical density of small-scale composting Salmonella samples in soil (A), stall (B), and sand (C) compost. Optical density was measured as AU at 600nm (left axis). Tubes of Salmonella in compost tub samples were drawn in biweekly intervals over a 41-day period and samples were measured for optical density. Temperature was recorded by inserting a thermometer into each of the compost tubs (right axis).

* Standard deviation not shown.

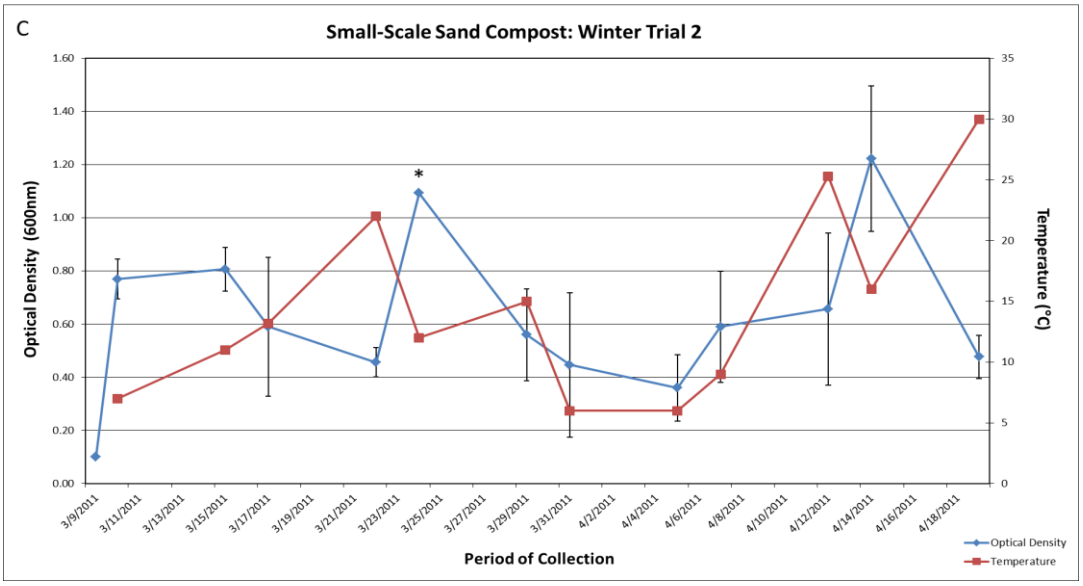

Figure 10 continued. The optical density of small-scale composting Salmonella samples in soil (A), stall (B), and sand (C) compost. Optical density was measured as AU at 600nm (left axis). Tubes of Salmonella in compost tub samples were drawn in biweekly intervals over a 41-day period and samples were measured for optical density. Temperature was recorded by inserting a thermometer into each of the compost tubs (right axis).

* Standard deviation not shown.

Small-scale 2011 summer trial. A small-scale summer trial was performed in order to test the effectiveness of the different compost materials in warm weather and also for comparison to the winter trials. The testing period for the summer trial occurred between 7/6/2011 and 8/8/2011 (34 days). For the summer trial two parameters were changed. The winter trials began with Salmonella cultures at an OD_{600} of 0.1 AU. Because higher summer temperatures were expected to reduce the Salmonella population more quickly, the summer trial began with an OD_{600} of 1.0 AU to more accurately detect a decrease in the bacteria. Additionally, Salmonella viable population counts were determined in addition to the optical density measurements to more effectively determine whether the viability of the population was decreasing.

Composting tubs were filled with materials remaining from the large-scale composting trials. PCR tubes were filled with 1.0 mL of cultured Salmonella that had been standardized to 1.0 AU at 600 nm corresponding to approximately 7.4×10^8 CFU/mL. The optical density of all standardized Salmonella samples remained measurable over the 33-day study period. Due to resource availability, sample population numbers for the sample collections between 7/21/2011 and 8/4/2011 were determined by regression analysis using sample optical density (Appendices 4.3 – 4.6).

The OD_{600} values for Salmonella soil compost samples throughout the trial were all significantly greater than the original inoculum (p values ranged from <0.1 to 0.02; Appendix 4.15). In contrast, population counts reached a high of 6.9×10^{11} CFU/mL on the second day of the trial and then declined to a low of 1.12×10^8 CFU/mL on 7/12/2011 (day 7, Figure 12B), which was a statistically significant decrease ($p < 0.01$, Appendix

4.8). Population counts on day 9 were also statistically significantly less ($p = 0.01$; Appendix4.8). This decline corresponds with the compost reaching the maximum temperature on day 7. Population counts returned to the initial baseline population on day 17, there was no significant change in concentration for the remainder of the trial (Figure 11B, Appendix 4.8). This corresponds with a relatively unchanging compost temperature. Soil compost temperatures ranged from 35°C to 44°C. Sample counts for specimens drawn between 7/21/2011 and 8/4/2011 were determined by regression analysis using OD_{600} values for comparison to previously established population values (Appendices 4.3 and 4.4).

For the Salmonella in soil compost, both population counts and optical density increased initially followed by a statistically significant decrease after a temperature increase. The population counts decreased to values equivalent to those of the initial inoculum, while the absorbance values remained significantly greater than the initial OD_{600} value.

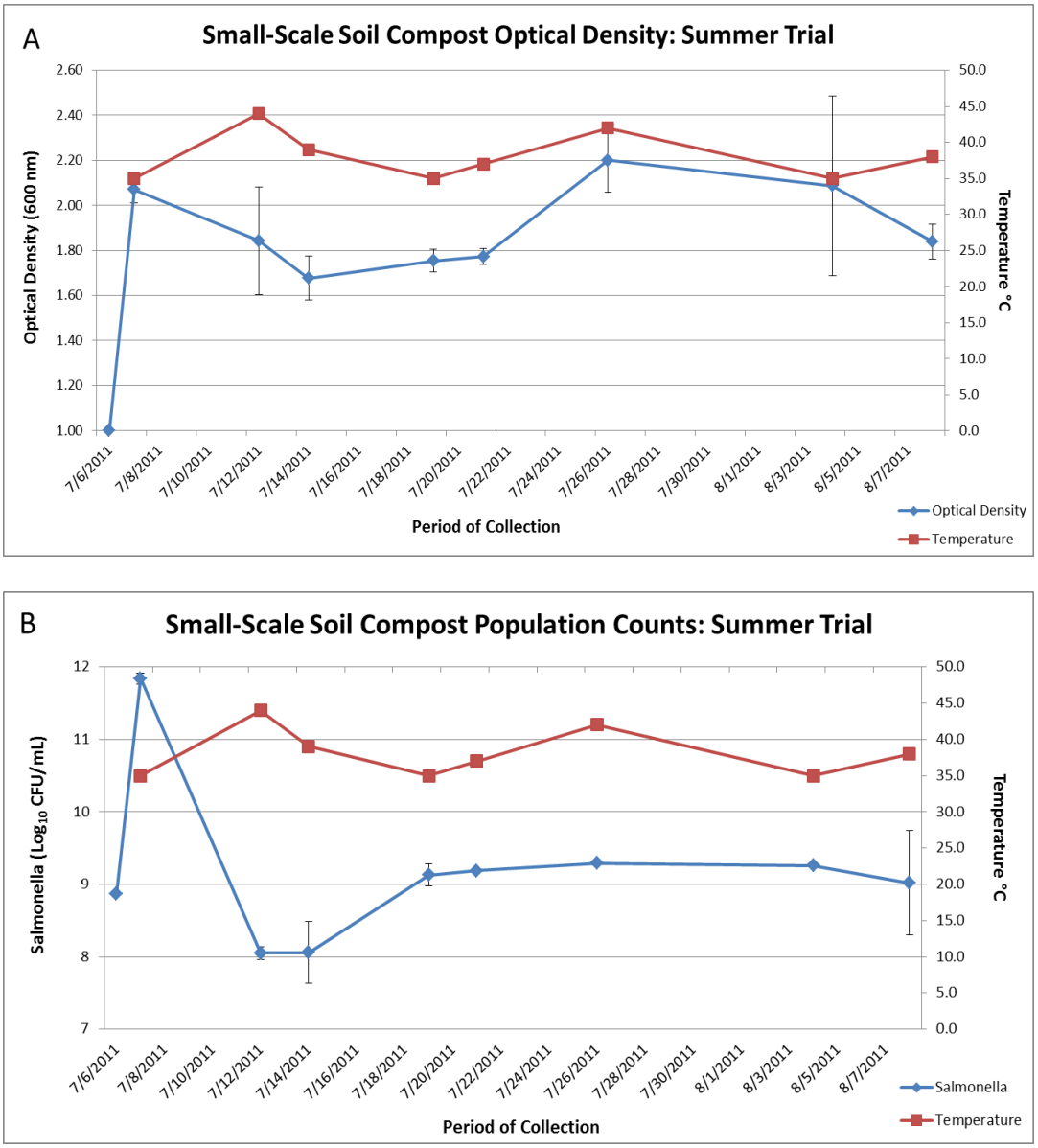

Figure 11. Salmonella optical density (A), measured as AU at 600 nm (left axis), and population counts (B), measured as CFU/mL (left axis), from small-scale soil composting. Population count values on 7/21/2011, 7/26/2011, and 8/4/2011 represent colony number estimates based on regression analysis. Temperature was recorded by inserting a thermometer into each of the compost tubs (right axis).

The optical density of Salmonella samples in stall compost (Figure 12A) reached their peak on the second day of the trial with 2.05 AU and declined to a low of 1.68 AU on 7/26/2011 (day 21). The optical densities of all samples during the trial were statistically significantly increased when compared to the original inoculum (*p* values ranged from < 0.01 to 0.04; Appendix 4.14). The stall compost Salmonella population (Figure 12B) also reached a high concentration on the second day of the trial of 5.13 x 10^{11} CFU/mL. Unlike the optical density, the population underwent a significant decline ($p < 0.01$; Appendix 4.14) to undetectable levels by the collection point on 7/14/2011 (day 9) and remained undetectable during the remainder of the trial. Stall compost had the highest recordable temperature of all the compost materials for the summer trial with 48°C on 7/12/2011 (day 7). This time point also corresponds with the elimination of detectable Salmonella by population counts in two of the replicate samples (Appendix 4.1). Stall compost temperature declined to 35°C by 7/19/2011 (day 14) and remained within the range of 35°C to 41°C for the remainder of the trial. Thus for the Salmonella in stall compost, both population counts and optical density saw a significant increase initially, but only the population count had a significant decrease following a temperature increase.

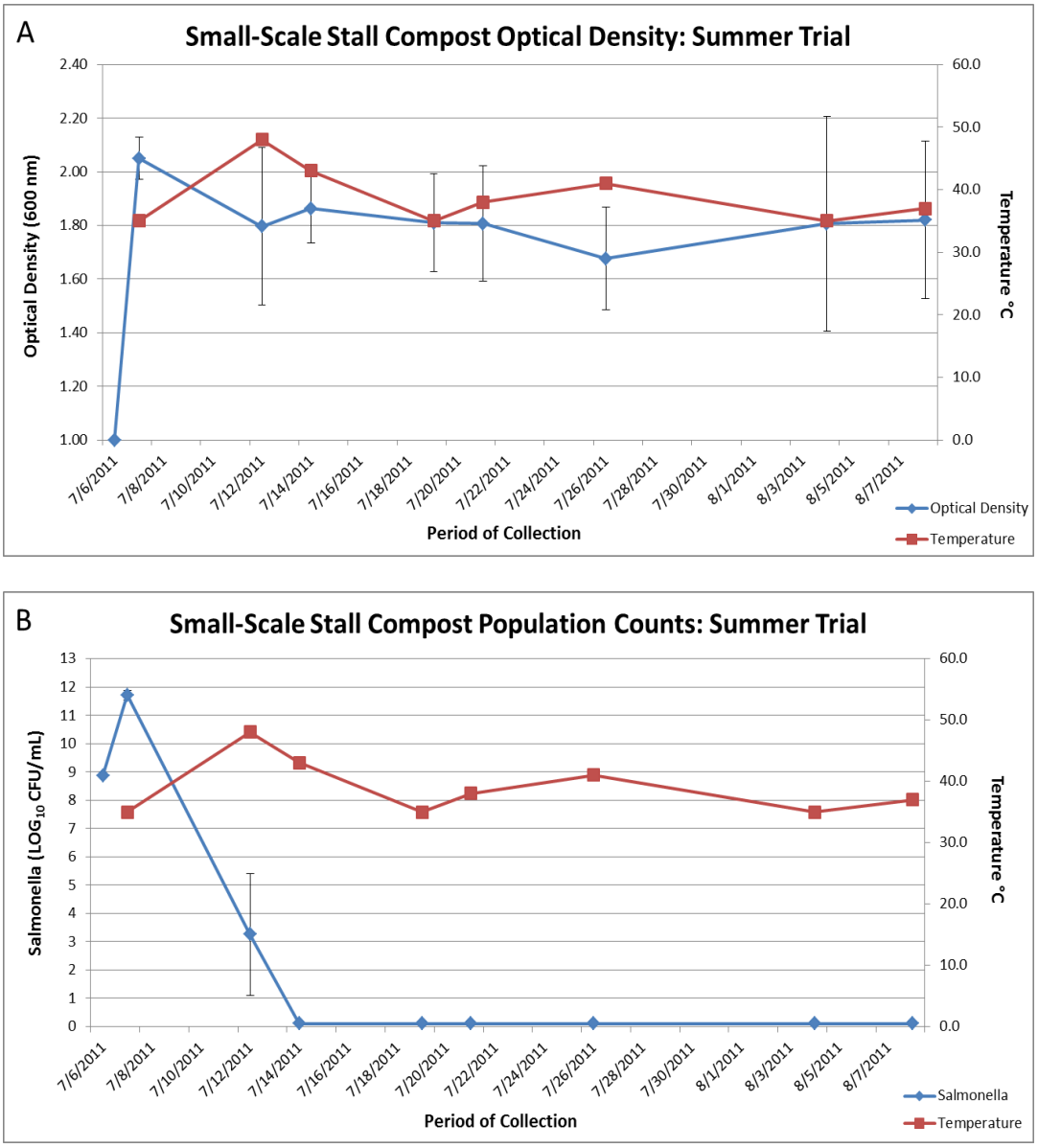

Figure 12. Salmonella optical density (A), measured as AU at 600 nm (left axis), and population counts (B), measured as CFU/mL (left axis), from small-scale stall composting. Population count values on 7/21/2011, 7/26/2011, and 8/4/2011 represent colony number estimates based on regression analysis. Temperature was recorded by inserting a thermometer into each of the compost tubs (right axis).

The optical density of Salmonella in the sand compost sample reached a peak of 1.88 AU on the second day of the trial and then declined to a low of 1.58 AU on 7/19/2011 (day 14; Figure 13A). This was followed by a period of statistically unchanging values (*p* values ranged from <0.01 to 0.02; Appendix 4.16). The final collection point of the trial was the only one that was not statistically significantly less when compared to the original inoculum OD_{600} ($p = 0.07$) (Figure 13A; Appendix 4.16). Population counts of Salmonella from sand compost also reached a high of 5.23×10^{11} CFU/mL on the second day of the trial ($p = 0.04$; figure 14B). Salmonella population counts declined to or below baseline population by 7/14/2012 (day 9, Figure 14B), this decline was statistically significant ($p < 0.01$; Appendix 4.9). This decrease followed the compost reaching its maximum trial temperature of 43°C. The sample population did decline significantly on the final collection day to 1.45×10^7 ($p < 0.01$; Appendix 4.9), which was below the original population number; this also corresponded with an increase in temperature; though this is speculative due to three preceding population values being calculated by regression analysis. Sand compost temperatures fell within a range of 33°C and 43°C , with the highest temperature being recorded on 7/12/2011 (day 7). Sample counts for specimens drawn between 7/21/2011 and 8/4/2011 were determined by regression analysis using OD_{600} values for comparison to previously established population values (Appendix 4.5 and 4.6). As with Salmonella cultured in stall compost, both population counts and optical density of Salmonella cultured in sand increased

initially, and was then followed by a statistically significant decrease in viable population counts after a temperature increase.

ANOVA analysis of population counts between the three types of compost showed no statistical difference between compost materials over the course of the trial (Appendix 4.13). The viable population counts from 7/14/2011 and 7/19/2011 did show a statistical difference when compared to the initial inoculum population (Appendix 4.13). These statistical differences correspond with the large decline in stall sample population to undetectable levels. This shows a significant difference in the performance of stall compost in reducing numbers of viable Salmonella when compared to the other materials at those time points. ANOVA analysis of optical density measurements showed no statistically significant difference in OD_{600} values over the course of the trial when compared to the initial inoculum (Appendix 4.19). One statistical difference between these compost materials was seen on 7/26/2011. This time point corresponded with a statistically significant increase in optical density for the soil compost samples ($p < 0.01$; Appendix 4.15).

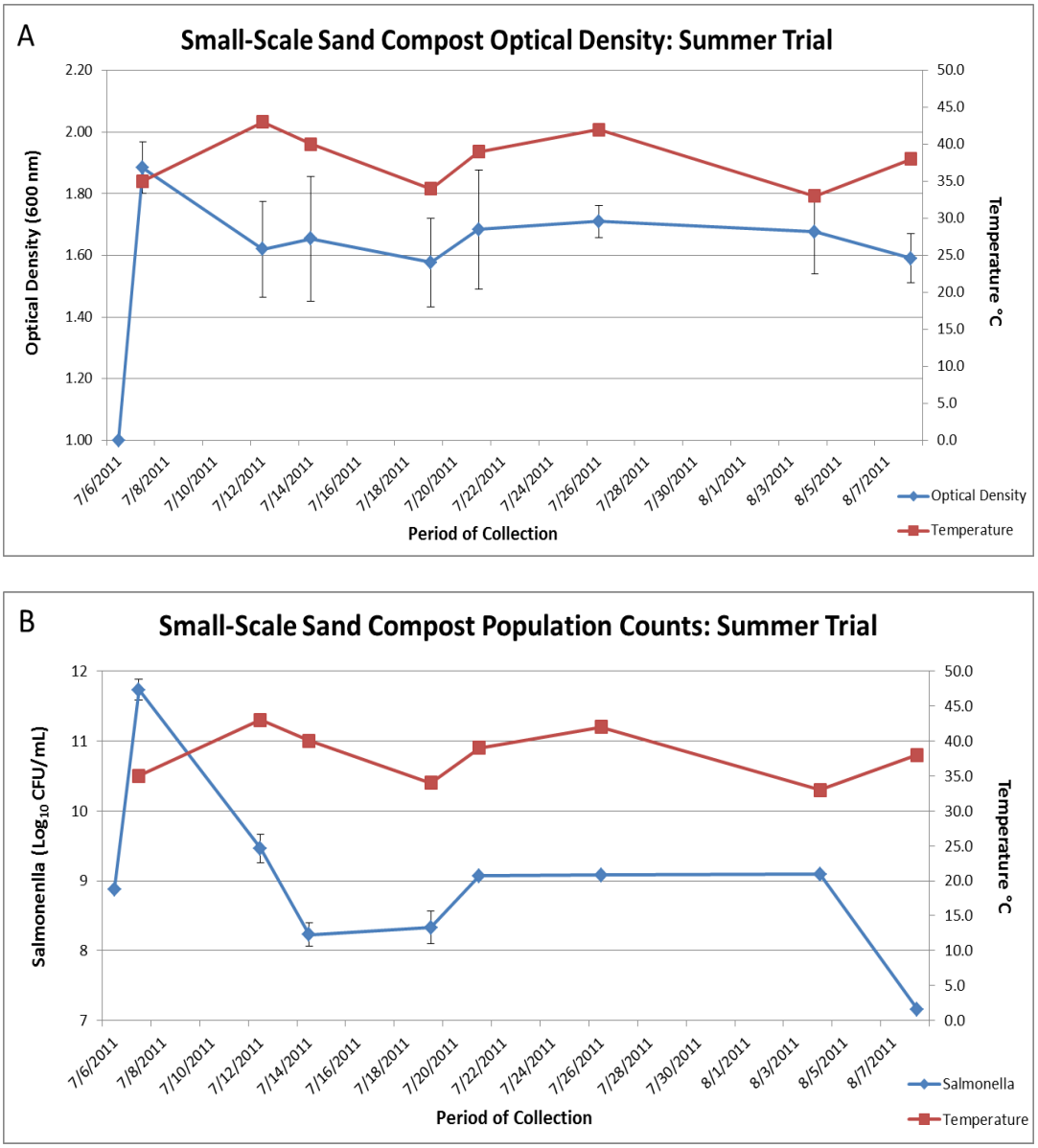

Figure 13. Salmonella optical density (A), measured as AU at 600 nm (left axis), and population counts (B), measured as CFU/mL (left axis), of small-scale sand composting. Population count values on 7/21/2011, 7/26/2011, and 8/4/2011 represent colony number estimates based on regression analysis. Temperature was recorded by inserting a thermometer in each of the compost tubs (right axis).

Comparison of Salmonella enumeration by bacterial optical density and viable population counts at 45°C and 4°C. Because the summer small-scale compost trials indicated viable Salmonella by optical density readings but not by population counts, especially for Salmonella in stall compost, a laboratory trial was performed to determine if a correlation existed between the optical density of a sample and the viable number of organisms in the same sample while the population was in decline. For this study, Salmonella samples were standardized at 1.0 AU at 600 nm, which corresponded to 7.4 x 10^8 CFU/mL. Samples were incubated at either 4°C or 45°C for four days with samples collected at 1, 6, 24, and 96 h time points for population enumeration and optical density determination following previously described methods.

Salmonella samples incubated at 45°C reached a statistically significantly increased viable population count of 1.13 x 10^9 CFU/mL by the 1 h time point ($p = 0.04$; Figure 14A). The population then declined significantly to 1.26 x 10^5 CFU/mL ($p < 0.01$) by the final 96 h time point (Appendix 7.2). Conversely, sample optical density showed a steady increase over the course of the trial, beginning at the standardized 1.0 AU and ending at statistically significantly higher OD_{600} of 2.3 AU ($p < 0.01$; Appendix 7.4) at 96 h. The data indicate that the optical density of a sample does not correlate with population counts when the population is declining at 45°C (r = -0.49; Appendix 7.11).

Salmonella samples incubated at 4°C reached a viable population count of 2.54 x 10^9 CFU/mL by the 1 h time point (Figure 14B). For the rest of the trial period the Salmonella population remained steady with no statistically significant changes when compared to the 1 h time point (Appendix 7.7), ending with a population of 1.86 x 10^9

CFU/mL. Optical densities of samples increased significantly over the course of the trial, reaching 2.08 AU at the final time point ($p < 0.01$; Appendix 7.8). Optical density increases were statistically significant (p values ranged from < 0.01 to 0.03; Appendices 7.8 and 7.9) when compared to one another except for the increase from 24 h to the 96 h time points ($p = 0.38$; Appendix 7.9). Optical density and population showed some correlation ($r = 0.71$; Appendix 7.13) in that both exhibited an immediate increase followed overall by non-significant changes throughout the remainder of the trial, suggesting that at 4°C the optical density is reflective of the viable population count.

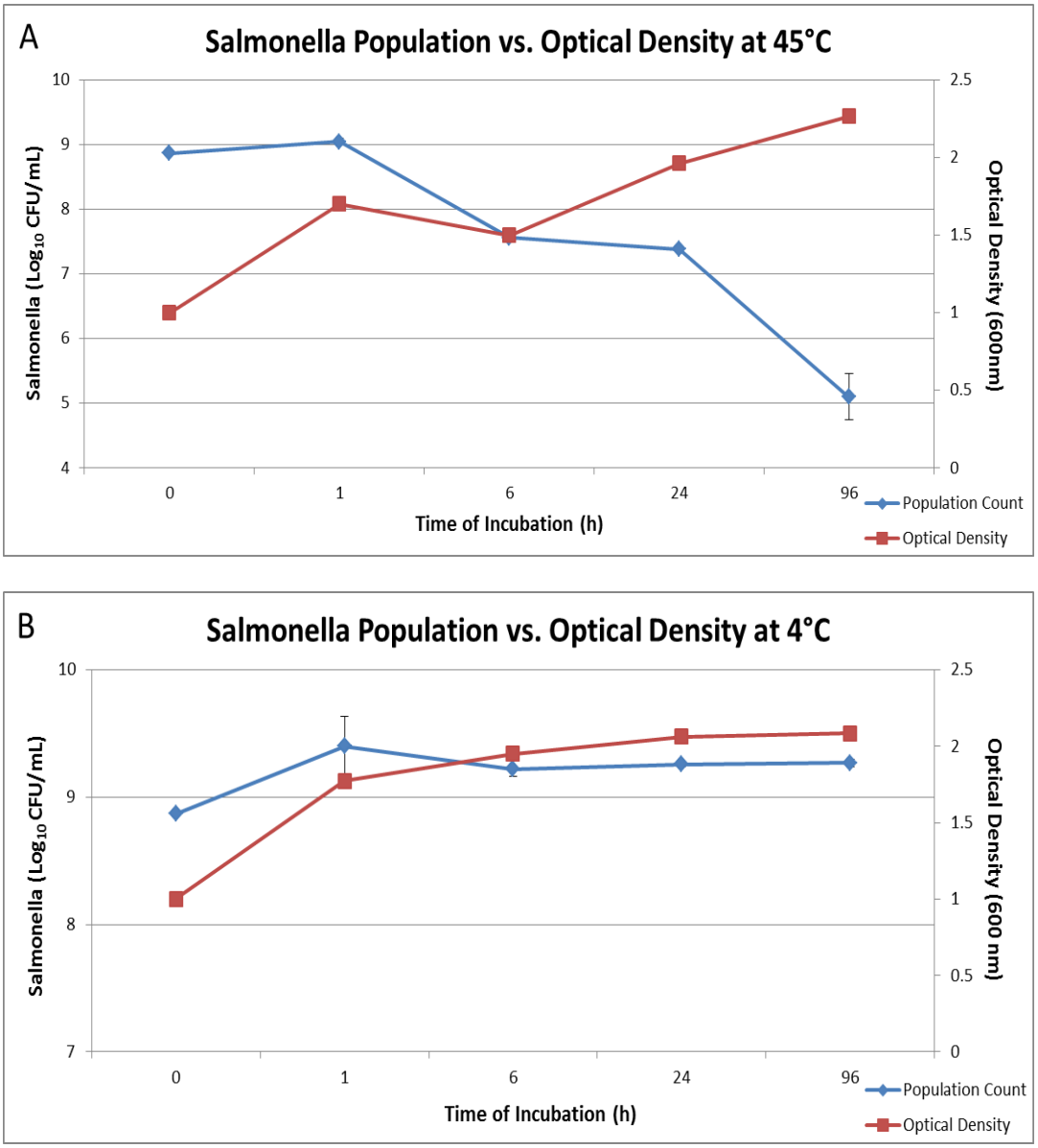

Figure 14. Salmonella population counts versus sample optical density at 45°C (A) and 4°C (B). Viable populations of samples were measured as CFU/mL (left axis). Optical density was measured as AU at 600nm (right axis). Samples were collected over a four day period and enumerated by population counting and optical density measurement. Temperatures were determined by incubator/refrigerator display and thermometers.

Comparison of Salmonella enumeration by bacterial optical density and viable population counts at 26°C. The 4°C and 45°C comparison was performed short term to compare population counts and optical density at the temperature extremes of the winter and summer trials. To determine how population counts and absorbance of Salmonella compare at room temperature over an extended period as for the small-scale composting trials, a 30-day room temperature laboratory trial was performed. Salmonella samples were grown and prepared following the same steps used for the summer trial samples. PCR tubes were filled with 1.0 mL of standardized (1.0 AU at 600 nm) Salmonella culture and then incubated at 26°C. Samples collected over the trial period were used to determine both optical density and population counts. Temperatures were recorded during each sampling point with the use of a thermometer in the incubator.

Sample optical density peaked at 2.38 AU on day 2 of the trial followed by a gradual decline to 1.11 AU on day 28. There was a statistically significant difference between the high (1/5/2012; day 2) and low (1/31/2012; day 28) OD_{600} values ($p < 0.01$; Appendix 6.3). However, the final time point was not statistically significant ($p = 0.08$; Appendix 6.2) when compared to the original inoculum. The viable population followed a similar trend to that of the bacterial density, a rapid increase, followed by a gradual decline over the rest of the trial period (Figure 15A). Room temperature Salmonella samples showed a significant increase in growth during the first two sampling periods on 1/5/2012 (day 2; $p = 0.03$) and 1/7/2012 (day 4; $p = 0.01$) (Figure 15B; Appendix 6.2). After reaching peak growth on day 4, the sample population significantly declined throughout the rest of the trial period in comparison to the initial inoculum, eventually

declining to 2.63 x 10^8 CFU/mL ($p < 0.01$; Appendix 6.2). Optical density showed a strong correlation with population counts in the room temperature trial (r = 0.87; Appendix 6.5), suggesting that at standard incubation conditions both population counts and optical density are indicative of bacterial survival.

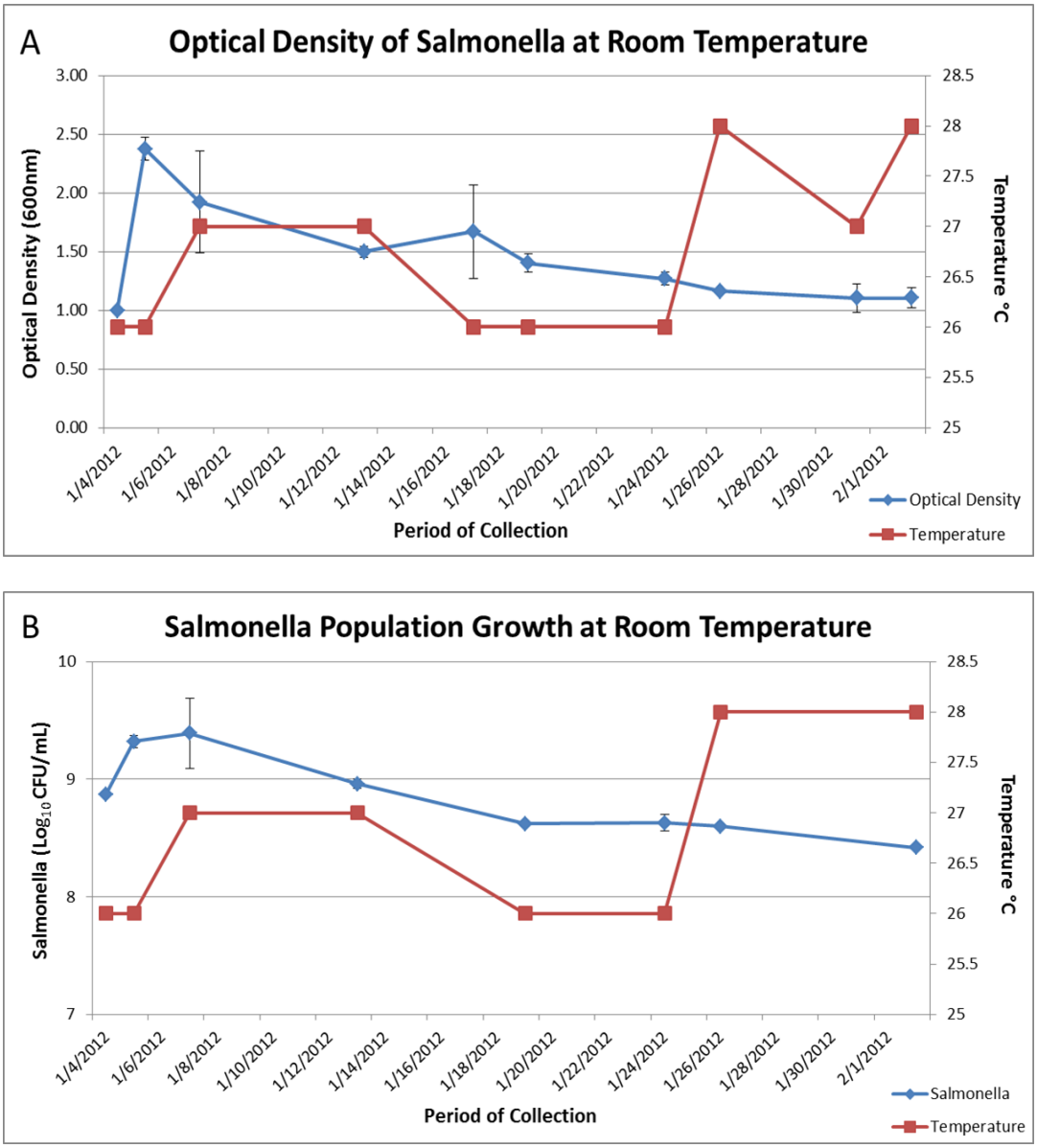

Figure 15. Salmonella population growth at room temperature over a 30 day period. (A) Sample optical density was measured as AU at 600 nm (left axis). (B) Population counts of samples were measured as CFU/mL (left axis). Temperatures were determined by a thermometer in the incubator (right axis).

IV. Discussion

The aim of this project was to compare the pathogen-reducing qualities of three different composting materials. The expected outcome of these studies was recommendations about the pathogen-reducing effectiveness of commonly used composting materials that would be used by both farmers and civil authorities in making decisions for small-scale and large-scale composting. Composting materials used in the project, sand, soil, and stall waste, were chosen based on availability in the surrounding region, with all three materials being readily available. The organisms used as model pathogens, coliforms, *Escherichia coli*, and *Salmonella spp.*, were chosen based on previous composting research and their potential existence within the composting carcasses (De Clercq et al. 2007, Pourcher et al. 2005, De´portes et al. 1998). The use of adult hog and piglet carcasses as composting matter was chosen due to needing large carcasses and their relative availability. For the small-scale composting trials, pork butt was used as composting matter to maintain consistency with the organic material used for the large-scale trials and also due to the ease of attainability.

LARGE-SCALE COMPOSTING

Large-scale composting was used in the first set of trials to replicate a real-world scenario in which local materials would be used to build composting mounds. These in turn would then be used to compost carcasses following a natural disaster. The mounds were constructed on a field site on a concrete pad with wooden barriers separating the compost mounds. Several other investigators observing carcass composting also used

these same large-scale trials for their observations. Due to the failure of the temperature logging devices set up by other investigators in the mounds, the only measurable parameter available affecting detection of bacteria from the mounds was precipitation recorded by a proximal weather station.

For these large-scale studies, coliform and *E. coli* numbers were monitored from core samples retrieved from the mounds. The soil mound in the first field trial already had a measurable coliform population at the start of the collection period, indicating the presence of potential pathogens. During composting, the soil compost mound had an increase in measurable coliforms to the high end of the measurable range between the second and fifth collection points (days 8 and 26), before declining back to the original collection point population on day 30. No *E. coli* was detectable in the soil mound in the first trial. The second large-scale field trial had two mounds for each compost material. In this second trial, soil mounds showed an overall lack of pathogen inhibition, with most collection points having coliform populations above the measurable high end point, although one of the two mounds decreased to undetectable levels by day 80 of the 87-day trial. *E. coli* was only detected in one of the soil mounds from trial 2 at one collection point and this coincided with a significant rainfall event.

Stall compost did not inhibit the bacterial coliform populations in either of the trials. In both trials, stall compost started with high levels of coliforms present, but this would be expected due to the nature of the material which is a combination of hay, wood chips, and horse manure. Coliform numbers at all but two collection points were above the measurable range, with the few that were not attributed to sampling error and

inconsistency of the bacterial populations in the mounds. *E. coli* was also detected in the stall mounds. In the first trial with the stall compost, *E. coli* had the same detection levels as the coliform population, but in the second trials *E. coli* was detected at collection points following rainfall events during the first six weeks of the second trial. No *E. coli* was detected during the final six weeks. Stall compost mounds also had the most occurrences of recoverable Salmonella, with all five isolates from the mounds confirmed as Group C.

In both large-scale trials, the sand compost mounds showed the complete elimination of recoverable *E. coli* and coliforms within 2 to 3 weeks. Unlike the other composting material mounds, sand compost mounds did not show an increase in bacterial populations following precipitation events, with bacterial populations remaining undetectable for a prolonged period. This could be due to insufficient precipitation to cause resurgence in the *E. coli* population (Desmarais et al. 2002), because a lack of moisture in a sand environment is known to cause a decline in *E. coli* numbers (Mika et al. 2009). However, only trial 2 had less than 0.5 in of rainfall in the last 8 weeks of the trial, while trial 1 had rainfall during the final 10 days of the trial. Additionally, although the sand was most effective at pathogen reduction in these large-scale trials, it is unlikely that the sand did a proper job of actually composting the swine carcasses in the mounds. Follow up surveys of the first mounds in the months following the initial trial period revealed that the carcasses in the sand mounds had not significantly decomposed during the trial.

By the rules that govern solid waste disposal in Tennessee, in order for compost samples to be considered disinfected, they must have less than or equal to 1×10^3 fecal coliform MPN/g of volatile suspended solids (TDEC 2012). In addition, the solids must have been maintained at 55ºC for three consecutive days during the composting period. By this definition of adequate disinfection, only the sand compost would be acceptable as a soil amendment, although no temperature data is available for the actual composting temperature to confirm temperatures of 55ºC. At the beginning of both trials, the concentration of the coliforms in the soil compost material was already higher than the acceptable coliform amount. Although coliform populations decreased in trial 1 and in one of the mounds from trial 2, the coliforms in the second soil mound from trial 2 did not decrease. It is unclear whether the pig carcass contributed any of the coliforms to the amounts detected in the mounds; nonetheless, by TDEC guidelines, this soil would be unacceptable for spreading.

Throughout both trials stall compost samples maintained coliform levels that were $> 4.35 \times 10^6$ CFU/g and therefore could not be safely used for any purpose following the composting process without additional composting time. Current static horse manure composting methods recommend periods of 3-8 months for compost maturity (Guthrie 2012, Hamilton 2010). It was not determined in these studies whether a period of composting longer than 87 days would have decreased coliform levels in the stall compost mounds.

To determine whether these compost materials were able to support the growth of *E. coli*, laboratory studies were performed with the three compost materials. Triplicate

samples of the compost materials were sterilized by two rounds of autoclaving and then inoculated with *E. coli*. The inoculated compost was incubated at 32-33ºC for 25 days and samples were removed weekly for colony counts. No significant increase in *E. coli* occurred in the sand or stall compost material, and a significant decrease of *E. coli* was observed in the soil compost material. These results indicate that neither the sand or stall material was inhibitory to bacterial viability nor were they lacking in nutrients to maintain a bacterial population. There was a decrease in *E. coli* inoculated into soil. This could be attributed to either a lack of sufficient nutrients or inhibitory compounds in the soil. Nonetheless, in the large-scale field trials, soil was not inhibitory to coliforms.

In addition to sampling large-scale compost mounds for total coliforms and *E. coli*, samples that tested positive for *E. coli* were reserved for *E. coli* O157 testing. Additionally, samples removed from the compost mounds were tested for Salmonella. Salmonella are found in the gastrointestinal tracts of animals, and are considered to be human pathogens. The passage of these bacteria from the animal through the compost presents a risk for both human and animal health. Several types of pathogenic *E. coli* exist; however, in the U.S., the strain that causes the most illnesses in humans is *E. coli* O157:H7 (CDC 2011). This strain is also found in animals, primarily ruminants such as cattle and sheep, but it has also been found in pigs (Chapman et al. 1997). As with Salmonella, the passage of these *E. coli* from the carcass into the environment represents a health risk, thus compost materials and the temperatures achieved during composting need to ensure pathogen inactivation.

For these studies, several Salmonella and *E. coli* isolates were collected from the large-scale compost mounds. Virotyping of potential *E. coli* isolates from the large-scale compost mounds using the Biocontrol VIP® for EHEC detection kit showed the recovery of the O157:H7 strain from one of the sand mounds of the second large-scale trial. *E. coli* O157:H7 is an enterohemorrhagic strain of *E. coli* known to cause hemorrhagic diarrhea, colitis, and hemolytic uremic syndrome (Mahon et al. 2007). The *E. coli* O157:H7 strain is known to cause infections mostly among the very young and the elderly (Reiss et al. 2006). Spread of *E. coli* O157:H7 is normally associated with contaminated agricultural products such as undercooked meats, apple cider, and unpasteurized dairy products (Mahon et al. 2007). This is especially important to note since this study was interested in potential movement of pathogens from the mounds into the surrounding environment including soil and groundwater. It is unknown how much of the *E. coli* O157:H7 stain was present in the mounds, as the strain was not specifically enumerated. Also, the isolation of *E. coli* from mounds sampled was partially dependent on the production of β -glucuronidase which is produced by 92% of *E. coli* strains but not by *E. coli* O157:H7 (Mahon et al. 2007). However, even if the *E. coli* O157:H7 strain was only present in low amounts, the infectious dose is much lower than for other *E. coli* strains and would still represent a health risk (Vernozy-Rozand et al. 2002).

The recovery of *E. coli* O157:H7 from the sand mounds occurred within the first two weeks of the second trial. The first weeks of an in-use composting mound are considered the most active, with most bacterial decline occurring during this period (Epstein 1997). In these studies, *E. coli* was reduced to undetectable levels following the

initial two-week period in sand mounds, indicating that the possible spread of the O157:H7 strain would have to occur within the first two weeks of composting. *E. coli* population counts in the sand mounds showed increases in population following precipitation events during the first two weeks of composting of the second summer large-scale trial, suggesting that movement of surviving pathogens from compost into surrounding soil and water environments is a possibility.

Salmonella was recovered predominantly from the stall compost mounds; this is most likely due to the fecal matter in the compost material. Detectable Salmonella occurred only twice in other compost types, indicating either trace amounts present in the compost or possible environmental contamination from birds or scavenging animals. All but one of the isolates of recoverable Salmonella typed by Remel Wellcolex™ Colour Salmonella testing kit was serogrouped as belonging to Group C. The remaining isolate was unreactive to the typing antisera and was later confirmed as Salmonella by the BD BBL Crystal™ testing system. Group C by the Kauffman–White classification system includes *S. enterica* subspecies enterica serotype Newport, *S. enterica* subspecies enterica serotype Montivideo, *S. enterica* subspecies enterica serotype Paratyphi C, *S. enterica* subspecies enterica serotype Choleraesuis, and many others (Grimont and Weill 2007). Typing of Salmonella only went as far as the serogrouping and therefore serotypes for the isolates are unknown. As with *E. coli,* the presence of Salmonella in samples would indicate pathogen survival and the ability to escape the mounds into the surrounding environment, including soil, ground and surface water. This could lead to the contamination of food crops in the surrounding area. This is potentially a problem due to

Salmonella-related illnesses being caused by contaminated food products (Forbes et al. 2007). Also, different serotypes of Salmonella are capable of causing a wide range of infective states such as gastroenteritis, bacteremia, and Typhoid fever (Forbes et al. 2007). Salmonella serogroup C has been associated with human Salmonellosis outbreaks (Sheth et al. 2011, Cook et al. 1998).

SMALL-SCALE COMPOSTING

Because the large-scale composting field trials resulted in the detection of pathogenic bacteria and composting with sand showed greater pathogen reduction compared to the soil and stall composting, small-scale static composting trials were begun with replicates for statistical comparisons and temperature monitoring. These small-scale trials used cuts of pork as the organic compost material and composting was performed in large, plastic containers. For these studies, vials of Salmonella were inserted into the pork and removed for assessment of survival throughout the trial period.

The first small-scale trial was begun on January 24, 2011 and continued through March 3, 2011 and was designated as the first winter trial. A second winter trial was begun on March 9, 2011 and extended to April 19, 2011. Winter trials used optical density readings to evaluate bacterial cell survival and growth. The use of optical density as an estimate of population is a standard practice for procedures such as determining Minimum Inhibitory Concentrations for antibiotic testing and other research applications (Lianou and Koutsoumanis 2011, CLSI 2007, Jean-Christophe et al. 1999). Also,

laboratory studies of Salmonella growth at low temperatures show a positive correlation between optical density and bacterial population (Figure 14B, Appendix 7.13).

During both winter trials, Salmonella samples from all compost types showed significant increases in optical density at some point during the trial periods. This would indicate failure of the compost materials to inhibit Salmonella growth. Declines in optical density, especially those later in the testing period, may not have been due to temperatures changes but instead due to a lack of available nutrients. Salmonella survival in the compost is a possibility due to winter trial temperatures never increasing above 30ºC, far from the optimal inactivation temperature of 55ºC. Also, further laboratory studies with Salmonella at room temperature showed a strong positive correlation between bacterial optical density and population counts at this temperature (Figures 16A-B, Appendix 6.5). Following the end of the winter trials, a more direct method of viable population estimation was needed due to expected increases in temperatures. For the summer small-scale trial, population counts were included to provide more information about the number of viable organisms present and also for comparison to turbidity readings.

Small-scale testing subjected bacterial populations in the study to the internal temperatures of the compost materials without having to interact with the other internal pressures of the composting environment (Epstein 1997). During the small-scale summer trial, the most effective compost material was the stall compost, which reduced Salmonella population counts to undetectable levels by the ninth day of the trial. This could be due to a larger degree of heat being generated by aerobic respiration by naturally

occurring bacteria in the stall compost. While recorded temperatures only reached a high of 48ºC, it is possible that stall compost may have reached the inactivation temperature of 55ºC on one or several of the non-sampling trial days.

During the summer trial, optical density was used for comparison between compost material types and for use in filling in population data that was unavailable due to equipment issues. When comparing the summer trial compost materials, both the stall and sand compost types showed a similar relationship between optical density and bacterial population, with optical density showing no significant change over the trial period while bacterial population counts decreased significantly to zero. Salmonella in the soil compost instead showed a significant increase in optical density, but not in population counts. This may have been due to an initial significant increase in cell number reflected by an increase in both optical density and colony counts on day 2, but a decrease in viability, although not total cells, on subsequent days. These results led to laboratory studies to determine the effect of temperature on the correlation of turbidity (optical density) and bacterial populations in the samples. The laboratory study showed that a negative correlation existed between Salmonella optical density and viable population counts when the temperature was high enough to inactivate the bacteria (Figure 14A, Appendix 7.11). Salmonella has been reported to enter the viable but non-culturable (VBNC) state when exposed to environmental stresses including temperature, nutrients, biocides, and ultraviolet radiation (Zeng et al. 2013, Passerat et al. 2009, Saroj et al. 2009). If colony counts are utilized to determine populations of bacteria from

compost, it is possible that viable organisms could still exist in the compost as significant differences occur between optical density measurements and viable population counts.

Comparison of compost types in small-scale trials shows the importance of temperature in compost material effectiveness. In the winter trials, significant reduction of Salmonella did not occur in any of the compost materials for the 39-day test period. This suggests that at least for the first five weeks of winter composting, viable organisms are present that can contaminate surrounding soils and water sources. For the summer trial, optical density showed cell numbers throughout the trial period despite temperatures of the compost materials reaching 45-48ºC. Viable population counts showed decreases in viable cells to zero for stall compost material; however, this does not discount the existence of VBNC bacteria. Moreover, although the Salmonella tubes from the stall compost did not contain any recoverable organisms by the end of the trial, recorded temperature data did not show that the compost reached the necessary 55ºC limit required by TDEC. Also, these trials only measured the Salmonella bacteria in the tube that was inserted into the compost materials and not the populations existing in the materials themselves. Therefore it is unknown how the natural bacterial populations of the compost materials were affected during the trial periods.

ANOVA analyses of winter trial data showed no significant differences between materials insofar as effectiveness in reducing Salmonella inserted into pork samples in the compost. ANOVA analyses of small scale-scale summer trial data showed a significant difference in compost material bacterial populations on two collection dates, both on 7/14/2011 and 7/19/2011. These dates correspond with the decline of the stall

compost bacterial population to undetectable levels. For these studies, temperature was a critical factor for pathogen reduction, with winter trials showing little decrease in Salmonella at lower temperatures between 0-29ºC. Similarly, others have reported the survival of *E. coli* and Salmonella strains at temperatures above 55ºC (Singh et al. 2011, Hutchinson et al. 2005). Thus, an ideal temperature for inactivation in a specific compost material is difficult to define and time-temperature criteria set by TDEC and the U.S. EPA of 55ºC for 3 days may not be sufficient to reduce pathogens. In addition to temperature, factors such as moisture, aeration, carbon/nitrogen content, and populations of bacteria in compost material may all be important in pathogen reduction (Singh et al. 2011, Ceustermans et al. 2007, Davis et al. 1992). Further studies will be needed to assess composting conditions, in addition to temperature, necessary for pathogen reduction in a specific compost material. However, it would be advisable that those using compost as soil amendments first have samples assessed for bacterial numbers at county, cooperative, or agricultural extension offices before spreading or selling compost from carcasses.

Part of this project was the feasibility of using readily available materials for composting in the event of animal or human mass fatalities in a natural disaster. With all composting, there is the risk of pathogenic microorganisms reaching the environment before sufficient heat is generated to destroy pathogens. With a large number of fatalities, the risk that pathogen transport to the environment is even greater. Many extension offices or states recommend or require an impervious material or surface for composting to prevent or reduce runoff events (Gamroth 2009, Morse 2009). For large

mortality events, areas with controlled drainage such as parking lots would be necessary. Nonetheless, composting remains an inexpensive alternative for animal mortalities on farms and more studies are needed to ensure the biosecurity of this process for the protection of natural resources surrounding composting sites as well as human health.

The studies presented here showed sand to be most effective at reducing bacterial numbers during large-scale summer trials, although decomposition was reduced. There was no advantage using soil or stall material for bacterial reduction. However, small-scale composting trials during winter and summer months showed no statistically significant differences between the three compost materials in reducing bacterial numbers by the end of the trials.

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APPENDICES

Appendix 1: 2010 Large-scale Summer Trials

Appendix 1.1: First large-scale 2010 summer trial data

Date	Sample	E. Coli CFU/g	Coliform CFU/ g	Precipitation (In)
5/27/2010	Stall Origin	2.03E+05	0.00	0.58
	Soil Origin	0.00	0.00	
	Sand Origin	0.00	0.00	
5/27/2010	Stall	7.33E+05	0.00	0.58
	Soil	0.00	1.00E+04	
	Sand	0.00	0.00	
6/4/2010	Stall	> 4.35E+6	0.00	1.53
	Soil	0.00	> 4.35E+6	
	Sand	0.00	1.00E+04	
6/8/2010	Stall	> 4.35E+6	0.00	0.10
	Soil	0.00	> 4.35E+6	
	Sand	0.00	0.00	
6/15/2010	Stall	> 4.35E+6	0.00	0.31
	Soil	0.00	> 4.35E+6	
	Sand	0.00	0.00	
6/21/2010	Stall	0.00	> 4.35E+6	0.97
	Soil	0.00	> 4.35E+6	
	Sand	0.00	0.00	
6/29/2010	Sand	0.00	0.00	2.70
	Soil	0.00	1.00E+04	
	Stall	0.00	> 4.35E+6	

Appendix 1.2: Second large-scale 2010 summer trial data

Date	Sample	E. Coli CFU/g	Coliform CFU/g	Precipitation (In)
7/29/2010	Stall 1	0.00	0.00	0.00
	Stall 2	> 4.35E+6	0.00	
	Soil 1	0.00	> 4.35E+6	
	Soil 2	0.00	> 4.35E+6	
	Sand 1	0.00	0.00	
	Sand 2	0.00	0.00	
7/31/2010	Stall 1	0.00	> 4.35E+6	0.35
	Stall 2	> 4.35E+6	0.00	
	Soil 1	0.00	> 4.35E+6	
	Soil 2	0.00	> 4.35E+6	
	Sand 1	> 4.35E+6	0.00	
	Sand 2	0.00	0.00	
8/4/2010	Stall 1	0.00	> 4.35E+6	0.37
	Stall 2	0.00	> 4.35E+6	
	Soil 1	0.00	> 4.35E+6	
	Soil 2	0.00	> 4.35E+6	
	Sand 1	0.00	0.00	
	Sand 2	0.00	0.00	
8/7/2010	Stall 1	0.00	> 4.35E+6	1.46
	Stall 2	0.00	> 4.35E+6	
	Soil 1	0.00	1.00E+04	
	Soil 2	0.00	> 4.35E+6	
	Sand 1	> 4.35E+6	0.00	
	Sand 2	0.00	0.00	
8/12/2010	Stall 1	0.00	> 4.35E+6	0.00
	Stall 2	0.00	> 4.35E+6	
	Soil 1	0.00	> 4.35E+6	
	Soil 2	0.00	> 4.35E+6	
	Sand 1	> 4.35E+6	0.00	
	Sand 2	0.00	0.00	

Date	Sample	E. Coli CFU/g	Coliform CFU/g	Precipitation (In)
8/19/2010	Stall 1	0.00	> 4.35E+6	3.59
	Stall 2	> 4.35E+6	0.00	
	Soil 1	> 4.35E+6	0.00	
	Soil 2	0.00	1.00E+04	
	Sand 1	0.00	0.00	
	Sand 2	0.00	0.00	
8/21/2010	Stall 1	0.00	0.00	0.00
	Stall 2	> 4.35E+6	0.00	
	Soil 1	0.00	0.00	
	Soil 2	0.00	0.00	
	Sand 1	0.00	0.00	
	Sand 2	0.00	0.00	
8/26/2010	Stall 1	0.00	0.00	2.27
	Stall 2	0.00	> 4.35E+6	
	Soil 1	0.00	> 4.35E+6	
	Soil 2	0.00	> 4.35E+6	
	Sand 1	0.00	0.00	
	Sand 2	0.00	0.00	
9/3/2010	Stall 1	> 4.35E+6	0.00	0.00
	Stall 2	0.00	> 4.35E+6	
	Soil 1	0.00	> 4.35E+6	
	Soil 2	0.00	> 4.35E+6	
	Sand 1	0.00	0.00	
	Sand 2	0.00	0.00	
9/10/2010	Stall 1	0.00	> 4.35E+6	0.00
	Stall 2	0.00	> 4.35E+6	
	Soil 1	0.00	> 4.35E+6	
	Soil 2	0.00	0.00	
	Sand 1	0.00	0.00	
	Sand 2	0.00	0.00	

Date	Sample	E. Coli CFU/g	Coliform CFU/g	Precipitation (In)
9/17/2010	Stall 1	0.00	> 4.35E+6	0.23
	Stall 2	0.00	> 4.35E+6	
	Soil 1	0.00	> 4.35E+6	
	Soil 2	0.00	> 4.35E+6	
	Sand 1	0.00	0.00	
	Sand 2	0.00	0.00	
9/24/2010	Stall 1	0.00	> 4.35E+6	0.00
	Stall 2	0.00	> 4.35E+6	
	Soil 1	0.00	> 4.35E+6	
	Soil 2	0.00	> 4.35E+6	
	Sand 1	0.00	0.00	
	Sand 2	0.00	0.00	
9/30/2010	Stall 1	0.00	> 4.35E+6	0.21
	Stall 2	0.00	> 4.35E+6	
	Soil 1	0.00	> 4.35E+6	
	Soil 2	0.00	> 4.35E+6	
	Sand 1	0.00	0.00	
	Sand 2	0.00	0.00	
10/15/2010	Stall 1	0.00	> 4.35E+6	0.41
	Stall 2	0.00	> 4.35E+6	
	Soil 1	0.00	> 4.35E+6	
	Soil 2	0.00	0.00	
	Sand 1	0.00	0.00	
	Sand 2	0.00	0.00	
10/22/2010	Stall 1	0.00	0.00	0.00
	Stall 2	0.00	> 4.35E+6	
	Soil 1	0.00	> 4.35E+6	
	Soil 2	0.00	0.00	
	Sand 1	0.00	0.00	
	Sand 2	0.00	0.00	

Appendix 2: First Small-scale Winter Trial

Appendix 2.1: First small-scale 2011 winter trial data

Date	Sample	OD_{600}	Avg	Std Dev	Temperature (°C)	Avg (°C)
1/24/2011	Stall 1	0.10	0.10	0.00	ND*	ND
	Stall 2	0.10				
	Stall 3	0.10				
	Soil 1	0.10	0.10	0.00	ND	ND
	Soil 2	0.10				
	Soil 3	0.10				
	Sand 1	0.10	0.10	0.00	ND	ND
	Sand 2	0.10				
	Sand 3	0.10				
1/25/2011	Stall 1	0.35	0.33	0.02	8.0	7.7
	Stall 2	0.32			8.0	
	Stall 3	0.31			7.0	
	Soil 1	0.33	0.33	0.01	7.0	7.3
	Soil 2	0.32			8.0	
	Soil 3	0.34			7.0	
	Sand1	0.31	0.31	0.01	8.0	8.0
	Sand 2	0.31			8.0	
	Sand 3	0.30			8.0	
1/27/2011	Stall 1	0.40	0.36	0.04	1.0	1.0
	Stall 2	0.33			1.0	
	Stall 3	0.35			1.0	
	Soil 1	0.30	0.30	0.01	1.0	1.3
	Soil 2	0.29			1.0	
	Soil 3	0.31			2.0	
	Sand 1	0.30	0.32	0.03	1.0	1.0
	Sand 2	0.36			1.0	
	Sand 3	0.31			1.0	

*ND – Not determined

Date	Sample	OD_{600}	Avg	Std Dev	Temperature (°C)	Avg (°C)
2/1/2011	Stall 1	0.55	0.63	0.08	12.0	12.0
	Stall 2	0.70			12.0	
	Stall 3	0.65			12.0	
	Soil 1	0.68	0.63	0.06	12.0	12.0
	Soil 2	0.57			12.0	
	Soil 3	0.65			12.0	
	Sand 1	0.64	0.68	0.05	13.0	12.7
	Sand 2	0.73			12.5	
	Sand 3	0.66			12.5	
2/3/2011	Stall 1	0.68	0.61	0.06	1.0	1.0
	Stall 2	0.60			1.0	
	Stall 3	0.56			1.0	
	Soil 1	0.55	0.65	0.08	1.0	1.0
	Soil 2	0.69			1.0	
	Soil 3	0.70			1.0	
	Sand 1	0.57	0.62	0.06	1.0	1.0
	Sand 2	0.62			1.0	
	Sand 3	0.68			1.0	
2/8/2011	Stall 1	0.65	0.61	0.05	2.0	1.5
	Stall 2	0.56			1.5	
	Stall 3	0.61			1.0	
	Soil 1	0.35	0.47	0.10	1.0	1.5
	Soil 2	0.53			1.5	
	Soil 3	0.52			2.0	
	Sand 1	0.72	0.67	0.10	1.0	1.0
	Sand 2	0.73			1.0	
	Sand 3	0.56			1.0	

Date	Sample	OD_{600}	Avg	Std Dev	Temperature (°C)	Avg (°C)
2/11/2011	Stall 1	0.74	0.71	0.21	1.0	1.0
	Stall 2	0.49			1.0	
	Stall 3	0.91			1.0	
	Soil 1	1.00	0.71	0.25	0.0	0.0
	Soil 2	0.58			0.0	
	Soil 3	0.54			0.0	
	Sand 1	0.66	0.61	0.06	0.0	0.0
	Sand 2	0.62			0.0	
	Sand 3	0.54			0.0	
2/16/2011	Stall 1	0.45	0.53	0.07	8.0	8.0
	Stall 2	0.55			8.0	
	Stall 3	0.58			8.0	
	Soil 1	0.36	0.55	0.20	10.0	10.7
	Soil 2	0.75			10.0	
	Soil 3	0.53			12.0	
	Sand1	0.50	0.39	0.10	15.0	12.7
	Sand 2	0.32			11.0	
	Sand 3	0.34			12.0	
2/18/2011	Stall 1	0.55	0.44	0.10	13.0	13.0
	Stall 2	0.40			13.0	
	Stall 3	0.36			13.0	
	Soil 1	1.08	0.77	0.27	13.0	13.3
	Soil 2	0.59			13.0	
	Soil 3	0.63			14.0	
	Sand 1	0.96	0.59	0.33	13.0	13.7
	Sand 2	0.44			14.0	
	Sand 3	0.36			14.0	

Date	Sample	OD_{600}	Avg	Std Dev	Temperature (°C)	Avg (°C)
2/22/2011	Stall 1	1.15	0.57	0.51	14.0	14.3
	Stall 2	0.33			15.0	
	Stall 3	0.23			14.0	
	Soil 1	0.31	0.49	0.19	15.5	15.5
	Soil 2	0.68			14.0	
	Soil 3	0.49			17.0	
	Sand 1	0.55	0.57	0.36	16.0	15.7
	Sand 2	0.94			15.0	
	Sand 3	0.23			16.0	
2/25/2011	Stall 1	0.69	0.44	0.23	12.0	11.3
	Stall 2	0.25			11.0	
	Stall 3	0.37			11.0	
	Soil 1	0.29	0.32	0.08	11.0	11.0
	Soil 2	0.27			11.0	
	Soil 3	0.41			11.0	
	Sand1	0.74	0.56	0.31	10.0	10.0
	Sand 2	0.75			10.0	
	Sand 3	0.20			10.0	
3/1/2011	Stall 1	0.42	0.42	0.15	11.0	11.3
	Stall 2	0.27			12.0	
	Stall 3	0.56			11.0	
	Soil 1	0.14	0.21	0.09	16.0	16.0
	Soil 2	0.18			14.0	
	Soil 3	0.32			18.0	
	Sand 1	0.74	0.40	0.30	14.0	14.5
	Sand 2	0.27			14.5	
	Sand 3	0.18			15.0	

Date	Sample	OD_{600}	Avg	Std Dev	Temperature (°C)	Avg (°C)
3/3/2011	Stall 1	0.37	0.44	0.10	11.0	11.3
	Stall 2	0.55			12.0	
	Stall 3	0.40			11.0	
	Soil 1	0.26	0.32	0.08	13.0	14.3
	Soil 2	0.37			13.0	
	Soil 3	ND*			17.0	
	Sand 1	ND	0.53	0.34	14.0	13.0
	Sand 2	0.77			12.0	
	Sand 3	0.29			13.0	

*ND – Not determined

Appendix 2.2: First winter trial stall sample OD_{600} t-tests

1 way, paired t-test comparisons for stall samples to the original inoculum						
Sample	**Mean**	**r^2**	**t Stat**	**t Critical**	**p value**	**p < 0.05**
1/25/2011	0.33	4.33E-04	-18.86	2.92	<0.01	Yes
1/27/2011	0.36	1.30E-03	-12.49	2.92	<0.01	Yes
2/1/2011	0.63	5.83E-03	-12.09	2.92	<0.01	Yes
2/3/2011	0.61	3.73E-03	-14.55	2.92	<0.01	Yes
2/8/2011	0.61	2.03E-03	-19.46	2.92	<0.01	Yes
2/11/2011	0.71	4.46E-02	-5.03	2.92	0.02	Yes
2/16/2011	0.53	4.63E-03	-10.86	2.92	<0.01	Yes
2/18/2011	0.44	1.00E-02	-5.82	2.92	0.01	Yes
2/22/2011	0.57	2.55E-01	-1.61	2.92	0.12	No
2/25/2011	0.44	5.17E-02	-2.56	2.92	0.06	No
3/1/2011	0.42	2.10E-02	-3.78	2.92	0.03	Yes
3/3/2011	0.44	9.30E-03	-6.11	2.92	0.01	Yes

Appendix 2.3: First winter trial soil sample OD_{600} t-tests

1 way, paired t-test comparisons for soil samples to the original inoculum						
Sample	**Mean**	**r^2**	**t Stat**	**t Critical**	**p value**	**p < 0.05**
1/25/2011	0.33	1.00E-04	-39.84	2.92	<0.01	Yes
1/27/2011	0.30	1.00E-04	-34.64	2.92	<0.01	Yes
2/1/2011	0.63	3.23E-03	-16.25	2.92	<0.01	Yes
2/3/2011	0.65	7.03E-03	-11.29	2.92	<0.01	Yes
2/8/2011	0.47	1.02E-02	-6.28	2.92	0.01	Yes
2/11/2011	0.71	6.49E-02	-4.12	2.92	0.03	Yes
2/16/2011	0.55	3.82E-02	-3.96	2.92	0.03	Yes
2/18/2011	0.77	7.40E-02	-4.24	2.92	0.03	Yes
2/22/2011	0.49	3.42E-02	-3.68	2.92	0.03	Yes
2/25/2011	0.32	5.73E-03	-5.11	2.92	0.02	Yes
3/1/2011	0.21	8.93E-03	-2.08	2.92	0.09	No
3/3/2011	0.32	6.05E-03	-3.91	6.31	0.08	No

Appendix 2.4: First winter trial sand sample OD_{600} t-tests

1 way, paired t-test comparisons for sand samples to the original inoculum						
Sample	**Mean**	**r^2**	**t Stat**	**t Critical**	***p* value**	***p* < 0.05**
1/25/2011	0.31	3.33E-05	-62.00	2.92	<0.01	Yes
1/27/2011	0.32	1.03E-03	-12.03	2.92	<0.01	Yes
2/1/2011	0.68	2.23E-03	-21.14	2.92	<0.01	Yes
2/3/2011	0.62	3.03E-03	-16.46	2.92	<0.01	Yes
2/8/2011	0.67	9.10E-03	-10.35	2.92	<0.01	Yes
2/11/2011	0.61	3.73E-03	-14.36	2.92	<0.01	Yes
2/16/2011	0.39	9.73E-03	-5.03	2.92	<0.01	Yes
2/18/2011	0.59	1.06E-01	-2.59	2.92	0.06	No
2/22/2011	0.56	1.28E-01	-2.21	2.92	0.08	No
2/25/2011	0.56	9.90E-02	-2.55	2.92	0.06	No
3/1/2011	0.40	9.04E-02	-1.71	2.92	0.11	No
3/3/2011	0.53	1.15E-01	-1.79	6.31	0.16	No

Appendix 2.5: First winter trial ANOVA test results

ANOVA test results of first winter trial OD_{600} values										
Date	**f**	***p***	**f Critical**	**MSB SS**	**MSB MS**	**DF**	**MSE SS**	**MSE MS**	**DF**	***p* < *0.05***
1/25/2011	2.53	0.16	5.14	0.0010	0.0005	2	0.0011	0.0002	6	No
1/27/2011	3.38	0.10	5.14	0.0055	0.0027	2	0.0049	0.0008	6	No
2/1/2011	0.50	0.63	5.14	0.0038	0.0019	2	0.0226	0.0038	6	No
2/3/2011	0.19	0.83	5.14	0.0018	0.0009	2	0.0276	0.0046	6	No
2/8/2011	4.56	0.06	5.14	0.0650	0.0325	2	0.0427	0.0071	6	No
2/11/2011	0.28	0.76	5.14	0.0214	0.0107	2	0.2266	0.0378	6	No
2/16/2011	1.30	0.34	5.14	0.0456	0.0228	2	0.1052	0.0175	6	No
2/18/2011	1.29	0.34	5.14	0.1638	0.0819	2	0.3804	0.0634	6	No
2/22/2011	0.04	0.96	5.14	0.0122	0.0061	2	0.8316	0.1386	6	No
2/25/2011	0.83	0.48	5.14	0.0865	0.0432	2	0.3130	0.0522	6	No
3/1/2011	0.94	0.44	5.14	0.0754	0.0377	2	0.2408	0.0401	6	No
3/3/2011	0.67	0.56	6.94	0.0468	0.0234	2	0.1399	0.0350	4	No

Appendix 3: Second Small-scale Winter Trial

Appendix 3.1: Second small-scale 2011 winter trial data

Date	Sample	OD_{600}	Avg	Std Dev	Temperature (°C)	Avg (°C)
3/9/2011	Stall 1	0.10	0.10	0.00	ND*	ND
	Stall 2	0.10				
	Stall 3	0.10				
	Soil 1	0.10	0.10	0.00	ND	ND
	Soil 2	0.10				
	Soil 3	0.10				
	Sand 1	0.10	0.10	0.00	ND	ND
	Sand 2	0.10				
	Sand 3	0.10				
3/10/2011	Stall 1	0.69	0.81	0.11	8.0	8.0
	Stall 2	0.91			8.0	
	Stall 3	0.83			8.0	
	Soil 1	0.90	0.71	0.26	8.0	7.3
	Soil 2	0.42			7.0	
	Soil 3	0.82			7.0	
	Sand 1	0.70	0.77	0.08	7.0	7.0
	Sand 2	0.76			7.0	
	Sand 3	0.85			7.0	
3/15/2011	Stall 1	0.80	0.83	0.03	12.0	12.0
	Stall 2	0.84			12.0	
	Stall 3	0.86			12.0	
	Soil 1	0.74	0.84	0.18	11.5	11.5
	Soil 2	0.74			11.5	
	Soil 3	1.05			11.5	
	Sand 1	0.75	0.81	0.08	11.0	11.0
	Sand 2	0.77			11.0	
	Sand 3	0.90			11.0	

*ND – Not determined

Date	Sample	OD_{600}	Avg	Std Dev	Temperature (°C)	Avg (°C)
3/17/2011	Stall 1	0.85	0.85	0.12	13.5	13.2
	Stall 2	0.96			13.0	
	Stall 3	0.73			13.0	
	Soil 1	0.60	0.84	0.26	13.0	13.0
	Soil 2	1.12			13.0	
	Soil 3	0.79			13.0	
	Sand 1	0.66	0.79	0.12	13.0	13.2
	Sand 2	0.90			13.5	
	Sand 3	0.81			13.0	
3/22/2011	Stall 1	0.43	0.51	0.14	23.0	23.0
	Stall 2	0.44			24.0	
	Stall 3	0.67			22.0	
	Soil 1	0.53	0.49	0.03	21.0	22.0
	Soil 2	0.47			21.0	
	Soil 3	0.47			24.0	
	Sand 1	0.43	0.46	0.06	22.0	22.0
	Sand 2	0.42			22.0	
	Sand 3	0.52			22.0	
3/24/2011	Stall 1	0.72	0.60	0.16	15.0	15.0
	Stall 2	0.42			16.0	
	Stall 3	0.65			14.0	
	Soil 1	0.43	1.01	0.92	12.0	12.3
	Soil 2	2.07			12.0	
	Soil 3	0.52			13.0	
	Sand 1	0.56	1.09	1.03	12.0	12.0
	Sand 2	2.28			12.0	
	Sand 3	0.44			12.0	

Date	Sample	OD_{600}	Avg	Std Dev	Temperature (°C)	Avg (°C)
3/29/2011	Stall 1	0.69	0.62	0.14	14.0	13.3
	Stall 2	0.45			13.0	
	Stall 3	0.71			13.0	
	Soil 1	0.42	0.54	0.10	14.0	14.0
	Soil 2	0.61			13.0	
	Soil 3	0.58			15.0	
	Sand 1	0.76	0.56	0.17	15.0	15.0
	Sand 2	0.47			15.0	
	Sand 3	0.45			15.0	
3/31/2011	Stall 1	0.46	0.28	0.15	7.0	7.0
	Stall 2	0.21			7.0	
	Stall 3	0.18			7.0	
	Soil 1	0.62	0.64	0.08	7.0	6.7
	Soil 2	0.72			6.0	
	Soil 3	0.57			7.0	
	Sand 1	0.28	0.45	0.27	6.0	6.0
	Sand 2	0.30			6.0	
	Sand 3	0.76			6.0	
4/5/2011	Stall 1	0.18	0.32	0.14	10.0	10.3
	Stall 2	0.31			10.0	
	Stall 3	0.46			11.0	
	Soil 1	0.68	1.01	0.64	9.5	9.2
	Soil 2	1.74			9.0	
	Soil 3	0.60			9.0	
	Sand 1	0.22	0.36	0.12	9.0	9.0
	Sand 2	0.46			9.0	
	Sand 3	0.40			9.0	

Date	Sample	OD_{600}	Avg	Std Dev	Temperature (°C)	Avg (°C)
4/7/2011	Stall 1	0.36	0.62	0.37	26.0	25.5
	Stall 2	1.05			25.5	
	Stall 3	0.46			25.0	
	Soil 1	0.30	1.06	0.72	23.0	24.0
	Soil 2	1.13			24.0	
	Soil 3	1.74			25.0	
	Sand 1	0.35	0.59	0.21	25.0	25.3
	Sand 2	0.69			25.0	
	Sand 3	0.73			26.0	
4/12/2011	Stall 1	0.28	0.23	0.04	18.0	18.0
	Stall 2	0.20			18.0	
	Stall 3	0.22			18.0	
	Soil 1	0.25	0.84	0.56	16.0	16.0
	Soil 2	1.36			16.0	
	Soil 3	0.90			16.0	
	Sand 1	0.59	0.66	0.29	16.0	16.0
	Sand 2	0.41			16.0	
	Sand 3	0.97			16.0	
4/14/2011	Stall 1	0.44	0.85	0.70	26.0	25.7
	Stall 2	0.45			26.0	
	Stall 3	1.66			25.0	
	Soil 1	0.81	1.54	1.03	25.0	25.0
	Soil 2	ND*			25.0	
	Soil 3	2.27			25.0	
	Sand 1	1.06	1.22	0.27	33.0	30.0
	Sand 2	1.07			30.0	
	Sand 3	1.54			27.0	

*ND – Not determined

Date	Sample	OD_{600}	Avg	Std Dev	Temperature (°C)	Avg (°C)
4/19/2011	Stall 1	0.46	1.32	0.75	25.0	24.7
	Stall 2	1.73			24.0	
	Stall 3	1.78			25.0	
	Soil 1	0.36	0.38	0.02	24.0	24.5
	Soil 2	ND*			ND	
	Soil 3	0.39			25.0	
	Sand 1	0.42	0.48	0.08	25.0	25.0
	Sand 2	0.44			25.0	
	Sand 3	0.57			25.0	

*ND – Not determined

Appendix 3.2: Second winter trial stall sample OD_{600} t-tests

1 way, paired t-test comparisons for stall samples to the original inoculum						
Sample	**Mean**	**r^2**	**t Stat**	**t Critical**	**p value**	**p < 0.05**
3/10/2011	0.81	0.01	-11.04	2.92	<0.01	Yes
3/15/2011	0.83	0.01	-41.58	2.92	<0.01	Yes
3/17/2011	0.85	0.01	-11.24	2.92	<0.01	Yes
3/22/2011	0.51	0.02	-5.27	2.92	0.02	Yes
3/24/2011	0.60	0.02	-5.48	2.92	0.02	Yes
3/29/2011	0.62	0.02	-6.19	2.92	0.01	Yes
3/31/2011	0.28	0.02	-2.07	2.92	0.09	No
4/5/2011	0.32	0.02	-2.68	2.92	0.06	No
4/7/2011	0.62	0.14	-2.43	2.92	0.07	No
4/12/2011	0.23	0.00	-5.55	2.92	0.02	Yes
4/14/2011	0.85	0.49	-1.85	2.92	0.10	No
4/19/2011	1.32	0.56	-2.83	2.92	0.05	No

Appendix 3.3: Second winter trial soil sample OD_{600} t-tests

1 way, paired t-test comparisons for soil samples to the original inoculum						
Sample	**Mean**	**r^2**	**t Stat**	**t Critical**	**p value**	**p < 0.05**
3/10/2011	0.71	0.07	-4.13	2.92	0.03	Yes
3/15/2011	0.84	0.03	-7.19	2.92	0.01	Yes
3/17/2011	0.84	0.07	-4.85	2.92	0.02	Yes
3/22/2011	0.49	0.01	-19.5	2.92	<0.01	Yes
3/24/2011	1.01	0.85	-1.70	2.92	0.12	No
3/29/2011	0.54	0.01	-7.40	2.92	0.01	Yes
3/31/2011	0.64	0.01	-12.17	2.92	<0.01	Yes
4/5/2011	1.01	0.40	-2.47	2.92	0.07	No
4/7/2011	1.06	0.52	-2.29	2.92	0.07	No
4/12/2011	0.84	0.31	-2.29	2.92	0.07	No
4/14/2011	1.54	1.07	-1.97	6.31	0.15	No
4/19/2011	0.38	4.50E-04	-18.33	6.31	0.02	Yes

Appendix 3.4: Second winter sand sample OD_{600} t-tests

1 way, paired t-test comparisons for sand samples to the original inoculum						
Sample	**Mean**	**r^2**	**t Stat**	**t Critical**	**p value**	**p < 0.05**
3/10/2011	0.77	0.01	-15.37	2.92	<0.01	Yes
3/15/2011	0.81	0.01	-15.03	2.92	<0.01	Yes
3/17/2011	0.59	0.07	-3.24	2.92	0.04	Yes
3/22/2011	0.46	0.03	-11.22	2.92	<0.01	Yes
3/24/2011	1.09	1.06	-1.67	2.92	0.12	No
3/29/2011	0.56	0.03	-4.59	2.92	0.02	Yes
3/31/2011	0.45	0.07	-2.21	2.92	0.08	No
4/5/2011	0.36	0.02	-3.61	2.92	0.03	Yes
4/7/2011	0.59	0.04	-4.06	2.92	0.03	Yes
4/12/2011	0.66	0.08	-3.37	2.92	0.04	Yes
4/14/2011	1.22	0.08	-7.09	2.92	0.01	Yes
4/19/2011	0.48	0.01	-8.01	2.92	0.01	Yes

Appendix 3.5: Second winter trial ANOVA test results

ANOVA test results of second winter trial OD_{600} values										
Date	**f**	***p***	**f Critical**	**MSB SS**	**MSB MS**	**D F**	**MSE SS**	**MSE SS**	**D F**	***p* < 0.05**
3/10/2011	0.25	0.79	5.14	0.0142	0.0071	2	0.1684	0.0281	6	No
3/15/2011	0.08	0.92	5.14	0.0022	0.0011	2	0.0792	0.0132	6	No
3/17/2011	1.26	0.35	5.14	0.1268	0.0634	2	0.3023	0.0504	6	No
3/22/2011	0.32	0.74	5.14	0.0049	0.0024	2	0.0453	0.0076	6	No
3/24/2011	0.32	0.73	5.14	0.4222	0.2111	2	3.8688	0.6448	6	No
3/29/2011	0.25	0.79	5.14	0.0102	0.0051	2	0.1229	0.0205	6	No
3/31/2011	2.72	0.14	5.14	0.1876	0.0938	2	0.2064	0.0344	6	No
4/5/2011	3.05	0.12	5.14	0.8962	0.4481	2	0.8803	0.1467	6	No
4/7/2011	0.87	0.47	5.14	0.4067	0.2033	2	1.4101	0.2350	6	No
4/12/2011	2.19	0.19	5.14	0.5756	0.2878	2	0.7890	0.1315	6	No
4/14/2011	0.67	0.56	5.79	0.5891	0.2945	2	2.2000	0.4401	5	No
4/19/2011	3.28	0.12	5.79	1.4887	0.7444	2	1.1330	0.2266	5	No

Appendix 4: Small-scale 2011 Summer Trial

Appendix 4.1: Small-scale 2011 summer trial population data

Date	Sample	CFU/mL	Avg	Std Dev	Temperature (℃)	AVG (℃)
7/6/2011	Stall 1	7.40E+08	7.40E+08	0.00	ND*	ND
	Stall 2	7.40E+08				
	Stall 3	7.40E+08				
	Soil 1	7.40E+08	7.40E+08	0.00	ND	ND
	Soil 2	7.40E+08				
	Soil 3	7.40E+08				
	Sand 1	7.40E+08	7.40E+08	0.00	ND	ND
	Sand 2	7.40E+08				
	Sand 3	7.40E+08				
7/7/2011	Stall 1	7.30E+11	5.1E+11	2.00E+11	35.0	34.7
	Stall 2	4.60E+11			35.0	
	Stall 3	3.40E+11			34.0	
	Soil 1	5.70E+11	6.90E+11	1.15E+11	34.0	34.7
	Soil 2	8.00E+11			35.0	
	Soil 3	7.00E+11			35.0	
	Sand 1	4.30E+11	5.23E+11	1.88E+11	34.0	35.0
	Sand 2	4.00E+11			35.0	
	Sand 3	7.40E+11			36.0	
7/12/2011	Stall 1	0.00	1.80E+03	3.06E+03	49.0	47.7
	Stall 2	5.30E+03			46.0	
	Stall 3	0.00			48.0	
	Soil 1	1.00E+08	1.12E+08	2.43E+07	43.0	43.7
	Soil 2	1.40E+08			44.0	
	Soil 3	9.60E+07			44.0	
	Sand 1	4.50E+09	2.87E+09	1.42E+09	44.0	43.3
	Sand 2	2.20E+09			43.0	
	Sand 3	1.90E+09			43.0	

*ND – Not determined

Date	Sample	CFU/mL	Avg	Std Dev	Temperature (°C)	AVG (°C)
7/14/2011	Stall 1	0.00	0.00	0.00	45.0	43.0
	Stall 2	0.00			43.0	
	Stall 3	0.00			41.0	
	Soil 1	1.10E+08	1.17E+08	9.02E+07	39.0	39.3
	Soil 2	3.00E+07			40.0	
	Soil 3	2.10E+08			39.0	
	Sand 1	2.50E+08	1.70E+08	7.00E+07	41.0	40.3
	Sand 2	1.40E+08			40.0	
	Sand 3	1.20E+08			40.0	
7/19/2011	Stall 1	0.00	0.00		35.0	34.7
	Stall 2	0.00			35.0	
	Stall 3	0.00			34.0	
	Soil 1	3.00E+08	4.27E+08	1.48E+08	34.0	35.3
	Soil 2	5.90E+08			35.0	
	Soil 3	3.90E+08			37.0	
	Sand 1	3.50E+08	2.17E+08	1.19E+08	34.0	34.3
	Sand 2	1.80E+08			35.0	
	Sand 3	1.20E+08			34.0	
7/21/2011	Stall 1	ND*	0.00		40.0	38.3
	Stall 2	ND			39.0	
	Stall 3	ND			36.0	
	Soil 1	ND	1.58E+09	Regression	35.0	37.0
	Soil 2	ND			37.0	
	Soil 3	ND			39.0	
	Sand 1	ND	1.17E+09	Regression	38.0	38.7
	Sand 2	ND			38.0	
	Sand 3	ND			40.0	

*ND – Not determined

Date	Sample	CFU/mL	Avg	Std Dev	Temperature (℃)	AVG (℃)
7/26/2011	Stall 1	ND*	0.00		42.0	40.7
	Stall 2	ND			41.0	
	Stall 3	ND			39.0	
	Soil 1	ND	1.93E+09	Regression	41.0	42.3
	Soil 2	ND			42.0	
	Soil 3	ND			44.0	
	Sand 1	ND	1.19E+09	Regression	43.0	41.7
	Sand 2	ND			40.0	
	Sand 3	ND			42.0	
8/4/2011	Stall 1	ND	0.00		36.0	35.0
	Stall 2	ND			36.0	
	Stall 3	ND			33.0	
	Soil 1	ND	1.83E+09	Regression	34.0	34.7
	Soil 2	ND			35.0	
	Soil 3	ND			35.0	
	Sand 1	ND	1.22E+09	Regression	32.0	33.0
	Sand 2	ND			33.0	
	Sand 3	ND			34.0	
8/8/2011	Stall 1	0.00	0.00	0.00	38.0	37.0
	Stall 2	0.00			38.0	
	Stall 3	0.00			35.0	
	Soil 1	1.70E+09	1.06E+09	8.58E+08	35.0	38.0
	Soil 2	1.40E+09			40.0	
	Soil 3	8.70E+07			39.0	
	Sand 1	1.50E+07	1.45E+07	7.07E+05	37.0	38.0
	Sand 2	/			/	
	Sand 3	1.40E+07			39.0	

*ND – Not determined

Appendix 4.2: Small-scale 2011 summer trial OD_{600} data

Date	Sample	OD_{600}	Avg	Std Dev	Temperature (℃)	Avg (℃)
7/6/2011	Stall 1	0.10	0.10	0.00	ND*	ND
	Stall 2	0.10				
	Stall 3	0.10				
	Soil 1	0.10	0.10	0.00	ND	ND
	Soil 2	0.10				
	Soil 3	0.10				
	Sand 1	0.10	0.10	0.00	ND	ND
	Sand 2	0.10				
	Sand 3	0.10				
7/7/2011	Stall 1	2.00	2.05	0.08	35.0	34.7
	Stall 2	2.01			35.0	
	Stall 3	2.14			34.0	
	Soil 1	2.03	2.07	0.06	34.0	34.7
	Soil 2	2.04			35.0	
	Soil 3	2.14			35.0	
	Sand 1	1.79	1.88	0.08	34.0	35.0
	Sand 2	1.91			35.0	
	Sand 3	1.95			36.0	
7/12/2011	Stall 1	1.47	1.80	0.29	49.0	47.7
	Stall 2	1.88			46.0	
	Stall 3	2.04			48.0	
	Soil 1	1.95	1.84	0.24	43.0	43.7
	Soil 2	1.57			44.0	
	Soil 3	2.01			44.0	
	Sand 1	1.61	1.62	0.16	44.0	43.3
	Sand 2	1.47			43.0	
	Sand 3	1.78			43.0	

*ND – Not determined

Date	Sample	OD_{600}	Avg	Std Dev	Temperature (℃)	Avg (℃)
7/14/2011	Stall 1	1.87	1.86	0.13	45.0	43.0
	Stall 2	1.99			43.0	
	Stall 3	1.73			41.0	
	Soil 1	1.62	1.68	0.10	39.0	39.3
	Soil 2	1.62			40.0	
	Soil 3	1.79			39.0	
	Sand 1	1.71	1.65	0.20	41.0	40.3
	Sand 2	1.43			40.0	
	Sand 3	1.82			40.0	
7/19/2011	Stall 1	1.61	1.81	0.18	35.0	34.7
	Stall 2	1.85			35.0	
	Stall 3	1.97			34.0	
	Soil 1	1.70	1.75	0.05	34.0	35.3
	Soil 2	1.76			35.0	
	Soil 3	1.80			37.0	
	Sand 1	1.66	1.58	0.14	34.0	34.3
	Sand 2	1.41			35.0	
	Sand 3	1.66			34.0	
7/21/2011	Stall 1	1.60	1.81	0.22	40.0	38.3
	Stall 2	1.79			39.0	
	Stall 3	2.03			36.0	
	Soil 1	1.81	1.77	0.04	35.0	37.0
	Soil 2	1.74			37.0	
	Soil 3	1.77			39.0	
	Sand 1	1.46	1.68	0.19	38.0	38.7
	Sand 2	1.81			38.0	
	Sand 3	1.78			40.0	

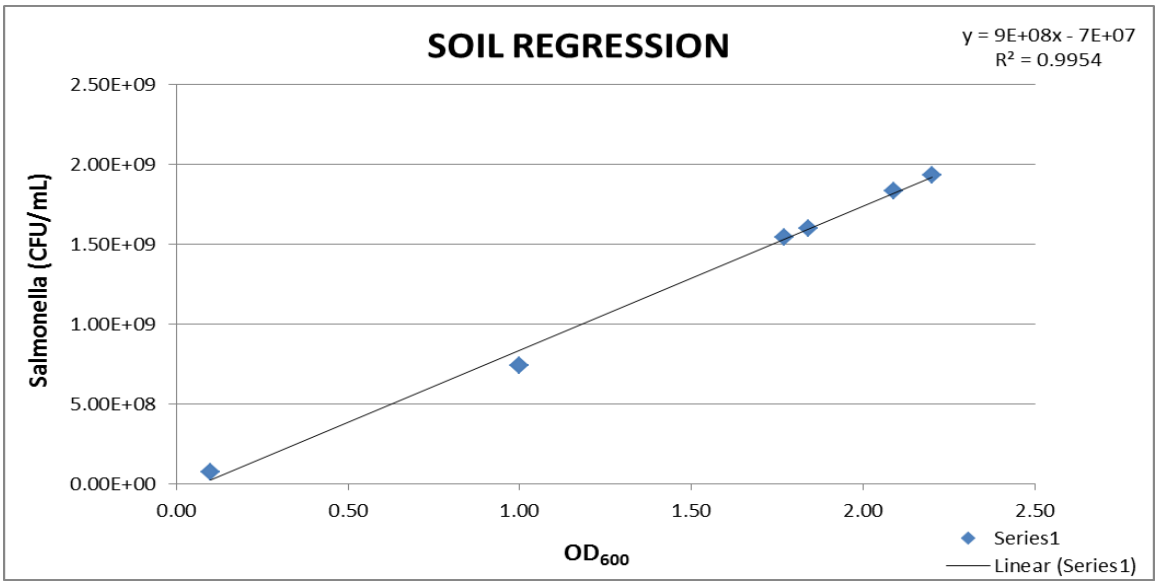
Date	Sample	OD_{600}	Avg	Std Dev	Temperature (℃)	Avg (℃)
7/26/2011	Stall 1	1.46	1.68	0.19	42.0	40.7
	Stall 2	1.82			41.0	
	Stall 3	1.75			39.0	
	Soil 1	2.22	2.20	0.14	41.0	42.3
	Soil 2	2.33			42.0	
	Soil 3	2.05			44.0	
	Sand 1	1.68	1.71	0.05	43.0	41.7
	Sand 2	1.77			40.0	
	Sand 3	1.68			42.0	
8/4/2011	Stall 1	1.35	1.81	0.40	36.0	35.0
	Stall 2	1.97			36.0	
	Stall 3	2.10			33.0	
	Soil 1	2.49	2.09	0.40	34.0	34.7
	Soil 2	1.69			35.0	
	Soil 3	2.08			35.0	
	Sand 1	1.63	1.68	0.14	32.0	33.0
	Sand 2	1.83			33.0	
	Sand 3	1.57			34.0	
8/8/2011	Stall 1	1.50	1.82	0.29	38.0	37.0
	Stall 2	2.08			38.0	
	Stall 3	1.88			35.0	
	Soil 1	1.79	1.84	0.08	35.0	38.0
	Soil 2	1.93			40.0	
	Soil 3	1.80			39.0	
	Sand 1	1.72	1.59	0.18	37.0	38.0
	Sand 2	ND*				
	Sand 3	1.46			39.0	

*ND – Not determined

Appendix 4.3: Summer soil sample regression results

Summer soil compost population data as determined by regression analysis						
Soil Compost	**Point 1**	**Point 2**	**Point 3**	**Point 4**	**Point 5**	**Point 6**
OD_{600}	0.1	1	1.77	1.84	2.09	2.2
Population	7.40E+07	7.40E+08	1.54E+09	1.60E+09	1.83E+09	1.93E+09

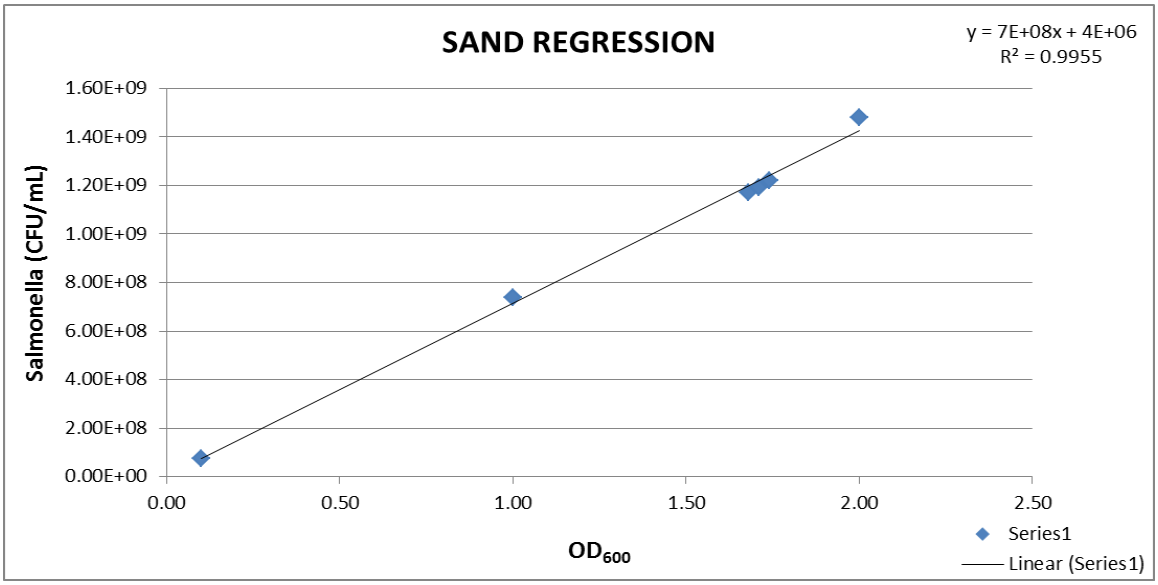
Appendix 4.4: Summer soil sample regression chart



Appendix 4.5: Summer sand sample regression results

Summer sand compost population data as determined by regression analysis						
Sand Compost	**Point 1**	**Point 2**	**Point 3**	**Point 4**	**Point 5**	**Point 6**
OD_{600}	0.1	1	1.68	1.71	1.74	2
Population	7.40E+07	7.40E+08	1.17E+09	1.19E+09	1.22E+09	1.48E+09

Appendix 4.6: Summer sand sample regression chart



Appendix 4.7: 2011 summer stall population t-tests

2 way, paired t-test comparisons for stall samples to the original inoculum						
Date	**Mean**	**r^2**	**t Stat**	**t Critical**	***p* value**	***p* < 0.05**
7/7/2011	5.10E+11	3.99E+22	-4.42	4.30	0.05	No
7/12/2011	1.77E+03	9.36E+06	4.19E+05	4.30	<0.01	Yes
7/14/2011	0.00	0.00	6.55E+04	2.78	<0.01	Yes
7/19/2011	0.00	0.00	6.55E+04	2.78	<0.01	Yes
8/8/2011	0.00	0.00	6.55E+04	2.78	<0.01	Yes

Appendix 4.8: 2011 summer soil population t-tests

2 way, paired t-test comparisons for soil samples to the original inoculum						
Date	**Mean**	**r^2**	**t Stat**	**t Critical**	***p* value**	***p* < 0.05**
7/7/2011	6.90E+11	1.33E+22	-10.35	4.30	0.01	Yes
7/12/2011	1.12E+08	5.92E+14	44.71	4.30	<0.01	Yes
7/14/2011	1.17E+08	8.13E+15	11.97	4.30	0.01	Yes
7/19/2011	4.27E+00	2.20E+16	3.66	4.30	0.07	No
8/8/2011	1.06E+09	7.36E+17	-0.65	4.30	0.58	No

Appendix 4.9: 2011 summer sand population t-tests

2 way, paired t-test comparisons for sand samples to the original inoculum						
Date	**Mean**	**r^2**	**t Stat**	**t Critical**	***p* value**	***p* < 0.05**
7/7/2011	5.23E+11	3.54E+22	-4.81	4.3	0.04	Yes
7/12/2011	2.87E+09	2.02E+18	-2.59	4.3	0.12	No
7/14/2011	1.70E+08	4.90E+15	14.1	4.3	<0.01	Yes
7/19/2011	2.17E+08	1.42E+16	7.6	4.3	0.02	Yes
8/8/2011	1.45E+07	5.00E+11	1451	12.71	<0.01	Yes

Appendix 4.10: 2011 summer selected stall population t-tests

1 way, paired t-test comparisons for selected stall samples				
Dates	**t Stat**	**t Critical**	***p* value**	***p* < 0.05**
7/7/2011 and 7/12/2011	4.42	2.92	0.02	Yes
7/7/2011 and 7/14/2011	4.42	2.92	0.02	Yes

Appendix 4.11: 2011 summer selected soil population t-tests

1 way, paired t-test comparisons for selected soil samples				
Dates	**t Stat**	**t Critical**	***p* value**	***p* < 0.05**
7/7/2011 and 7/12/2011	10.36	2.92	<0.01	Yes

Appendix 4.12: 2011 summer selected sand population t-tests

1 way, paired t-test comparisons for selected sand samples				
Dates	**t Stat**	**t Critical**	***p* value**	***p* < 0.05**
7/7/2011 and 7/14/2011	4.81	2.92	0.02	Yes

Appendix 4.13: 2011 summer population ANOVA test results

ANOVA test results for summer trial population values										
Date	**F**	***p***	**f Critical**	**MSB SS**	**MSB MS**	**D F**	**MSE SS**	**MSE MS**	**D F**	***p* < 0.05**
7/7/2011	1.05	0.41	5.14	1.21E +23	6.03E +22	2	3.44E +23	5.73E +22	6	No
7/12/2011	2.98	0.13	5.14	9.27E +18	4.63E +18	2	9.30E +18	1.55E +18	6	No
7/14/2011	5.22	0.04	5.14	4.54E +16	2.27E +16	2	2.61E +16	4.34E +15	6	Yes
7/19/2011	11.30	0.01	5.14	2.73E +17	1.37E +17	2	7.25E +16	1.21E +16	6	Yes
8/8/2011	3.56	0.12	5.79	2.09E +18	1.04E +18	2	1.47E +18	2.94E +17	5	No

Appendix 4.14: 2011 summer stall sample OD_{600} t-tests

1 way, paired t-test comparisons for stall samples to the original inoculum						
Date	**Mean**	**r^2**	**t Stat**	**t Critical**	***p* value**	***p* < 0.05**
7/7/2011	2.05	0.01	-23.29	2.92	<0.01	Yes
7/12/2011	1.80	0.09	-4.69	2.92	0.02	Yes
7/14/2011	1.86	0.02	-11.49	2.92	<0.01	Yes
7/19/2011	1.81	0.03	-7.65	2.92	<0.01	Yes
7/21/2011	1.81	0.05	-6.48	2.92	0.01	Yes
7/26/2011	1.68	0.04	-6.14	2.92	0.01	Yes
8/4/2011	1.81	0.16	-3.49	2.92	0.04	Yes
8/8/2011	1.82	0.09	-4.82	2.92	0.02	Yes

Appendix 4.15: 2011 summer soil sample OD_{600} t-tests

1 way, paired t-test comparisons for soil samples to the original inoculum						
Date	**Mean**	**r^2**	**t Stat**	**t Critical**	***p* value**	***p* < 0.05**
7/7/2011	2.07	3.70E-03	-30.47	2.92	<0.01	Yes
7/12/2011	1.84	0.06	-6.12	2.92	0.01	Yes
7/14/2011	1.68	0.01	-11.94	2.92	<0.01	Yes
7/19/2011	1.75	2.53E-03	-25.92	2.92	<0.01	Yes
7/21/2011	1.77	1.23E-03	-38.14	2.92	<0.01	Yes
7/26/2011	2.20	0.02	-14.73	2.92	<0.01	Yes
8/4/2011	2.09	0.16	4.70	2.92	0.02	Yes
8/8/2011	1.84	0.01	-18.63	2.92	<0.01	Yes

Appendix 4.16: 2011 summer sand sample OD_{600} t-tests

1 way, paired t-test comparisons for sand samples to the original inoculum						
Date	**Mean**	**r^2**	**t Stat**	**t Critical**	***p* value**	***p* < 0.05**
7/7/2011	1.88	0.01	-18.37	2.92	<0.01	Yes
7/12/2011	1.62	0.02	-6.92	2.92	0.01	Yes
7/14/2011	1.65	0.04	-5.63	2.92	0.02	Yes
7/19/2011	1.58	0.02	-6.92	2.92	0.01	Yes
7/21/2011	1.68	0.04	-6.1	2.92	0.01	Yes
7/26/2011	1.71	2.70E-03	-23.67	2.92	<0.01	Yes
8/4/2011	1.68	0.02	-8.61	2.92	0.01	Yes
8/8/2011	1.59	0.03	-4.54	6.31	0.07	No

Appendix 4.17: 2011 summer selected stall OD_{600} t-tests

1 way, paired t-test comparisons for selected stall samples				
Dates	**t Stat**	**t Critical**	***p* value**	***p* < 0.05**
7/7/2011 and 7/14/2011	1.83	2.35	0.08	No
7/26/2011 and 8/4/2011	-0.51	2.35	0.32	No
7/26/2011 and 8/8/2011	-0.71	2.35	0.27	No

Appendix 4.18: 2011 summer selected soil OD_{600} t-tests

1 way, paired t-test comparisons for selected soil samples				
Dates	**t Stat**	**t Critical**	***p* value**	***p* < 0.05**
7/7/2011 and 7/12/2011	1.59	2.92	0.13	No
7/7/2011 and 7/14/2011	5.9	2.35	<0.01	Yes

Appendix 4.19: 2011 summer ANOVA test results

ANOVA test results for summer trial OD_{600} values										
Date	**f**	***p***	**f Critical**	**MSB SS**	**MSB MS**	**D F**	**MSE SS**	**MSE MS**	**D F**	***p* < 0.05**
7/7/2011	5.65	0.04	5.14	0.063	0.032	2	0.033	0.006	6	Yes
7/12/2011	0.75	0.51	5.14	0.083	0.042	2	0.335	0.056	6	No
7/14/2011	1.78	0.25	5.14	0.079	0.040	2	0.134	0.022	6	No
7/19/2011	2.34	0.18	5.14	0.089	0.044	2	0.114	0.019	6	No
7/21/2011	0.43	0.67	5.14	0.024	0.012	2	0.171	0.028	6	No
7/26/2011	13.09	0.01	5.14	0.515	0.258	2	0.118	0.020	6	Yes
8/4/2011	1.16	0.37	5.14	0.263	0.132	2	0.678	0.113	6	No
8/8/2011	0.99	0.43	5.79	0.087	0.044	2	0.220	0.044	5	No

Appendix 5: 33℃ Laboratory Trial

Appendix 5.1: Stall population data

Date	Sample	CFU/g	Avg	Std Dev	Avg (℃)
5/17/2011	Stall 1	2.40E+06	2.40E+06	0.00E+00	ND*
	Stall 2	2.40E+06			
	Stall 3	2.40E+06			
5/18/2011	Stall 1	1.20E+05	1.88E+06	2.55E+06	33
	Stall 2	4.80E+06			
	Stall 3	7.10E+05			
	Control 1	0.00	0.00	0.00	33
	Control 2	0.00			
	Control 3	0.00			
5/26/2011	Stall 1	2.40E+05	9.00E+05	1.13E+06	33
	Stall 2	2.60E+05			
	Stall 3	2.20E+06			
	Control 1	0.00	0.00	0.00	33
	Control 2	0.00			
	Control 3	0.00			
6/2/2011	Stall 1	7.40E+05	4.35E+05	3.75E+05	33
	Stall 2	1.60E+04			
	Stall 3	5.50E+05			
	Control 1	0.00	0.00	0.00	33
	Control 2	0.00			
	Control 3	0.00			
6/9/2011	Stall 1	5.70E+06	2.20E+06	3.05E+06	33
	Stall 2	1.40E+05			
	Stall 3	7.50E+05			
	Control 1	0.00	0.00	0.00	33
	Control 2	0.00			
	Control 3	0.00			

*ND – Not determined

Appendix 5.2: Soil population data

Date	Sample	CFU/g	Avg	Std Dev	Avg (℃)
5/17/2011	Soil 1	2.40E+06	2.40E+06	0.00	ND*
	Soil 2	2.40E+06			
	Soil 3	2.40E+06			
5/18/2011	Soil 1	5.90E+06	2.08E+06	3.31E+06	33
	Soil 2	2.30E+05			
	Soil 3	1.20E+05			
	Control 1	0.00	0.00	0.00	33
	Control 2	0.00			
	Control 3	0.00			
5/26/2011	Soil 1	4.80E+06	5.80E+06	1.41E+06	33
	Soil 2	6.80E+06			
	Soil 3	ND			
	Control 1	0.00	0.00	0.00	33
	Control 2	0.00			
	Control 3	0.00			
6/2/2011	Soil 1	5.10E+04	7.30E+04	3.11E+04	33
	Soil 2	ND			
	Soil 3	9.50E+04			
	Control 1	0.00	0.00	0.00	33
	Control 2	0.00			
	Control 3	0.00			
6/9/2011	Soil 1	5.20E+05	4.90E+05	4.24E+04	33
	Soil 2	4.60E+05			
	Soil 3	ND			
	Control 1	0.00	0.00	0.00	33
	Control 2	0.00			
	Control 3	0.00			

*ND – Not determined

Appendix 5.3: Sand population data

Date	Sample	CFU/g	Avg	Std Dev	Avg (℃)
9/1/2011	Sand 1	2.40E+06	2.40E+06	0.00E+00	ND*
	Sand 2	2.40E+06			
	Sand 3	2.40E+06			
9/2/2011	Sand 1	1.40E+08	3.13E+08	1.53E+08	33
	Sand 2	3.70E+08			
	Sand 3	4.30E+08			
	Control 1	0.00E+00	0.00E+00	0.00E+00	33
	Control 2	0.00E+00			
	Control 3	0.00E+00			
9/9/2011	Sand 1	5.70E+07	8.07E+07	2.18E+07	33
	Sand 2	8.50E+07			
	Sand 3	1.00E+08			
	Control 1	0.00E+00	0.00E+00	0.00E+00	33
	Control 2	0.00E+00			
	Control 3	0.00E+00			
9/16/2011	Sand 1	1.80E+07	1.21E+07	6.34E+06	33
	Sand 2	1.30E+07			
	Sand 3	5.40E+06			
	Control 1	0.00E+00	0.00E+00	0.00E+00	33
	Control 2	0.00E+00			
	Control 3	0.00E+00			
9/23/2011	Sand 1	1.20E+07	7.38E+06	6.34E+06	33
	Sand 2	1.00E+07			
	Sand 3	1.50E+05			
	Control 1	0.00E+00	0.00E+00	0.00E+00	33
	Control 2	0.00E+00			
	Control 3	0.00E+00			

*ND – Not determined

Appendix 5.4: Stall sample t-tests

1 way, paired t-test comparisons for stall samples to original inoculum						
Date	**Mean**	**r^2**	**t Stat**	**t Critical**	***p* value**	***p* < 0.05**
5/18/2011	1.88E+06	6.50E+12	0.36	2.92	0.38	No
5/26/2011	9.00E+05	1.27E+12	2.31	2.92	0.07	No
6/2/2011	4.35E+05	1.41E+11	9.07	2.92	0.01	Yes
6/9/2011	2.20E+06	9.30E+06	0.12	2.92	0.46	No

Appendix 5.5: Soil sample t-tests

2 way, paired t-test comparisons for soil samples to original inoculum						
Date	**Mean**	**r^2**	**t Stat**	**t Critical**	***p* value**	***p* < 0.05**
5/18/2011	2.08E+06	1.09E+13	0.17	4.30	0.88	No
5/26/2011	5.80E+06	2.00E+12	-3.4	12.71	0.18	No
6/2/2011	7.30E+04	9.68E+08	105.77	12.71	0.01	Yes
6/9/2011	4.90E+05	1.80E+09	63.67	12.71	0.01	Yes

Appendix 5.6: Sand sample t-tests

1 way, paired t-test comparisons for sand samples to original inoculum						
Date	**Mean**	**r^2**	**t Stat**	**t Critical**	**p value**	**p < 0.05**
9/2/2011	3.13E+08	2.34E+16	-3.52	2.92	0.04	Yes
9/9/2011	8.07E+07	4.76E+14	-6.21	2.92	0.01	Yes
9/16/2011	1.21E+07	4.03E+13	-2.66	2.92	0.06	No
9/23/2011	7.38E+06	4.02E+13	-1.36	2.92	0.15	No

Appendix 5.7: 33℃ trial ANOVA test results

ANOVA test results for 33℃ trial										
Date	**f**	***p***	**f Critical**	**MSB SS**	**MSB MS**	**D F**	**MSE SS**	**MSE MS**	**DF**	***p* < 0.05**
Point 1	12.40	0.01	5.14	1.93E +17	9.69E +16	2	4.69E +16	7.82E +15	6	Yes
Point 2	29.75	< 0.01	5.79	1.14E +16	5.69E +15	2	9.57E +14	1.91E +14	5	Yes
Point 3	8.14	0.03	5.79	2.63E +14	1.32E +14	2	8.08E +13	1.62E +13	5	Yes
Point 4	1.72	0.27	5.79	6.81E +13	3.40E +13	2	9.91E +13	1.98E +13	5	No

Appendix 6: Room Temperature Laboratory Trial

Appendix 6.1: Room temperature laboratory trial data

Date	Sample	OD600	AVG	Std Dev	CFU/mL	Avg CFU/mL	Std Dev2
1/4/2012	1	1.00	1.00	0.00	7.40E+08	7.40E+08	0.00
	2	1.00			7.40E+08		
	3	1.00			7.40E+08		
1/5/2012	1	2.36	2.38	0.10	2.20E+09	2.07E+09	4.16E+08
	2	2.48			1.60E+09		
	3	2.29			2.40E+09		
1/7/2012	1	1.70	1.92	0.43	2.50E+09	2.43E+09	3.06E+08
	2	2.42			2.10E+09		
	3	1.64			2.70E+09		
1/13/2012	1	1.45	1.50	0.05	1.10E+09	9.20E+08	1.61E+08
	2	1.54			8.70E+08		
	3	1.51			7.90E+08		
1/17/2012	1	1.38	1.67	0.40	ND*	ND	ND
	2	1.95			ND		
	3				ND		
1/19/2012	1	1.36	1.40	0.08	4.60E+08	4.17E+08	3.79E+07
	2	1.49			4.00E+08		
	3	1.36			3.90E+08		
1/24/2012	1	1.24	1.27	0.05	3.50E+08	4.23E+08	1.27E+08
	2	1.24			3.50E+08		
	3	1.33			5.70E+08		

*ND – Not determined

Date	Sample	OD600	AVG	Std Dev	CFU/mL	Avg CFU/mL	Std Dev2
1/26/2012	1	1.17	1.16	0.02	ND*	4.00E+08	ND
	2	1.14			ND		
	3	1.18			4.00E+08		
1/31/2012	1	1.02	1.10	0.12	ND	ND	ND
	2	1.19			ND		
	3	ND			ND		
2/2/2012	1	1.20	1.11	0.08	2.30E+08	2.63E+08	4.93E+07
	2	1.04			2.40E+08		
	3	1.08			3.20E+08		

*ND - Not determined

Appendix 6.2: Room temperature OD_{600} t-tests

1 way, paired t-test comparisons for room temperature samples to the original inoculum						
Date	**Mean**	**r^2**	**t Stat**	**t Critical**	***p* value**	***p* < 0.05**
1/5/2012	2.38	0.01	-24.81	2.92	<0.01	Yes
1/7/2012	1.92	0.19	-3.67	2.92	0.03	Yes
1/13/2012	1.50	2.10E-03	-18.90	2.92	<0.01	Yes
1/17/2012	1.67	0.16	-2.33	2.92	0.13	No
1/19/2012	1.40	0.01	-9.31	2.92	0.01	Yes
1/24/2012	1.27	2.70E-03	-9.00	2.92	0.01	Yes
1/26/2012	1.16	4.33E-04	-13.59	2.92	<0.01	Yes
1/31/2012	1.11	0.01	-1.24	2.92	0.22	No
2/2/2012	1.11	0.01	-2.22	2.92	0.08	No

Appendix 6.3: Room temperature population t-tests

2 way, paired t-test comparisons for room temperature samples to the original inoculum						
Date	**Mean**	**r^2**	**t Stat**	**t Critical**	***p* value**	***p* < 0.05**
1/5/2012	2.07E+09	1.73E+17	-5.52	4.30	0.03	Yes
1/7/2012	2.43E+09	9.33E+16	-9.60	4.30	0.01	Yes
1/13/2012	9.20E+08	2.59E+16	-1.94	4.30	0.19	No
1/19/2012	4.17E+08	1.43E+15	14.79	4.30	<0.01	Yes
1/24/2012	4.23E+08	1.61E+16	4.32	4.30	0.05	No
2/2/2012	2.63E+08	2.43E+15	16.74	4.30	<0.01	Yes

Appendix 6.4: Selected OD_{600} t-tests for room temperature samples

1 way, paired t-test for OD_{600} for selected samples incubated at room temperature				
Sample	**t Stat**	**t Critical**	***p* value**	***p* < 0.05**
1/5/2012 - 1/31/2012	12.53	2.92	< 0.01	Yes

Appendix 6.5: Room temperature OD_{600} and population data

OD_{600} and population data for Salmonella incubated at room temperature								
OD_{600}	1.00	1.11	1.16	1.27	1.40	1.50	1.92	2.38
CFU/mL	7.40E +08	2.63E +08	4.00E +08	4.23E +08	4.17E +08	9.20E +08	2.43E +09	2.07E +09

Appendix 6.6: Room temperature OD_{600} and population correlation

OD_{600} and population correlation for samples incubated at room temperature		
	OD_{600}	**CFU/mL**
OD_{600}	1.00	
CFU/mL	0.86	1.00

Appendix 7: 45℃ and 4℃ Laboratory Trials

Appendix 7.1: 45℃ laboratory trial data

Date	Sample	OD_{600}	AVG	CFU/mL	AVG CFU/mL
5/9/2012	1	1.00	1.00	7.40E+08	7.40E+08
0 h	2	1.00		7.40E+08	
	3	1.00		7.40E+08	
5/9/2012	1	1.74	1.70	1.29E+09	1.13E+09
1 h	2	1.70		1.05E+09	
	3	1.66		1.05E+09	
5/9/2012	1	1.52	1.50	3.70E+07	3.67E+07
6 h	2	1.52		3.10E+07	
	3	1.46		4.20E+07	
5/10/2012	1	1.88	1.96	2.28E+07	2.39E+07
24 h	2	1.92		2.33E+07	
	3	2.09		2.55E+07	
5/12/2012	1	2.30	2.27	4.10E+04	1.26E+05
96 h	2	2.32		1.48E+05	
	3	2.18		1.89E+05	

Appendix 7.2: 45℃ population t-tests

2 way, paired t-test comparisons for Salmonella population incubated at 45℃						
Sample	**Mean**	**r^2**	**t Stat**	**t Critical**	***p* value**	***p* < 0.05**
1 h	1.13E+09	1.92E+16	-4.88	4.30	0.04	Yes
6 h	3.67E+07	3.03E+13	221.19	4.30	<0.01	Yes
24 h	2.39E+07	2.06E+12	863.51	4.30	<0.01	Yes
96 h	1.26E+05	5.84E+09	16770.62	4.30	<0.01	Yes

Appendix 7.3: 45℃ selected population t-tests

1 way, paired t-test comparison for selected population samples incubated at 45℃ samples				
Sample	**t Stat**	**t Critical**	***p* value**	***p* < 0.05**
1h - 96 h	14.12	2.92	<0.01	Yes

Appendix 7.4: 45℃ OD_{600} t-tests

1 way, paired t-test comparisons for Salmonella OD_{600} incubated at 45℃						
Sample	**Mean**	**r^2**	**t Stat**	**t Critical**	***p* value**	***p* < 0.05**
1 h	1.70	1.60E-03	-30.31	2.92	<0.01	Yes
6 h	1.50	1.20E-03	-25.00	2.92	<0.01	Yes
24 h	1.96	1.24E-02	-14.96	2.92	<0.01	Yes
96 h	2.27	5.73E-03	-28.97	2.92	<0.01	Yes

Appendix 7.5: 4℃ laboratory trial data

Date	Sample	OD_{600}	AVG	CFU/mL	AVG CFU/mL
5/9/2012	1	1.00	1.00	7.40E+08	7.40E+08
0 h	2	1.00		7.40E+08	
	3	1.00		7.40E+08	
5/9/2012	1	1.78	1.77	2.24E+09	2.54E+09
1 h	2	1.68		1.41E+09	
	3	1.86		3.97E+09	
5/9/2012	1	1.92	1.95	1.78E+09	1.66E+09
6 h	2	1.92		1.77E+09	
	3	2.01		1.42E+09	
5/10/2012	1	2.03	2.06	1.88E+09	1.85E+09
24 h	2	2.10		1.75E+09	
	3	2.06		1.92E+09	
5/12/2012	1	2.17	2.08	2.01E+09	1.86E+09
96 h	2	2.10		1.82E+09	
	3	1.98		1.74E+09	

Appendix 7.6: 4℃ population t-tests

1 way, paired t-test comparisons for Salmonella population incubated at 4℃						
Sample	**Mean**	**r^2**	**t Stat**	**t Critical**	***p* value**	***p* < 0.05**
1 h	2.54E+09	1.71E+18	-2.39	2.92	0.07	No
6 h	1.66E+09	4.20E+16	-7.74	2.92	0.01	Yes
24 h	1.85E+09	7.90E+15	-21.63	2.92	<0.01	Yes
96 h	1.86E+09	1.92E+16	-13.95	2.92	<0.01	Yes

Appendix 7.7: 4℃ selected population t-tests

1 way, paired t-test comparisons for selected population samples incubated at 4℃				
Sample	**t Stat**	**t Critical**	***p* value**	***p* < 0.05**
1h and 6h	1.16	2.92	0.18	No
1h and 24h	0.91	2.92	0.23	No
1h and 96h	0.90	2.92	0.23	No

Appendix 7.8: 4℃ OD_{600} t-tests

1 way, paired t-test comparisons for Salmonella OD_{600} incubated at 4℃						
Sample	**Mean**	**r^2**	**t Stat**	**t Critical**	***p* value**	***p* < 0.05**
1 h	1.77	8.13E-03	-14.85	2.92	<0.01	Yes
6 h	1.95	2.70E-03	-31.7	2.92	<0.01	Yes
24 h	2.06	1.23E-03	-52.44	2.92	<0.01	Yes
96 h	2.08	9.23E-03	-19.53	2.92	<0.01	Yes

Appendix 7.9: 4℃ selected OD_{600} t-tests

1 way, paired t-test comparisons for selected OD_{600} samples incubated at 4℃				
Sample	**t Stat**	**t Critical**	**p value**	**p < 0.05**
1h and 6h	-2.94	2.35	0.03	Yes
6h and 24h	-3.13	2.13	0.02	Yes
24h and 96h	-0.34	2.35	0.38	No

Appendix 7.10: 45℃ trial OD_{600} and population data

OD_{600} and population data for Salmonella incubated at 45℃					
OD_{600}	1.00	1.70	1.50	1.96	2.27
CFU/mL	7.40E+08	1.13E+09	3.67E+07	2.39E+07	1.26E+05

Appendix 7.11: 45℃ trial OD_{600} and population correlation

OD_{600} and population correlation for samples incubated at 45℃		
	OD_{600}	**CFU/mL**
OD_{600}	1.00	
CFU/mL	-0.49	1.00

Appendix 7.12: 4℃ trial OD_{600} and population data

OD_{600} and population data for Salmonella incubated at 4℃					
OD_{600}	1.00	1.77	1.95	2.06	2.08
CFU/mL	7.40E+08	2.54E+09	1.66E+09	1.85E+09	1.86E+09

Appendix 7.13: 4℃ trial OD_{600} and population correlation

OD_{600} and population correlation for samples incubated at 4℃		
	OD_{600}	**CFU/mL**
OD_{600}	1.00	
CFU/mL	0.71	1.00