



**CPS 2010 RFP
FINAL PROJECT REPORT**

Project Title

Developing and validating practical strategies to improve microbial safety in composting process control and handling practices

Project Period

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Objectives

- 1). Validating the thermal inactivation data collected from outbreak strains in compost using naturally occurring *Escherichia coli* O157:H7 and *Salmonella*.
- 2). Optimizing and validating the finished compost as physical covering and straw as base of freshly formed static compost heaps or windrow compost piles.
- 3). Applying the pathogen growth model to determine the potential of finished composts to support the growth of human pathogens.
- 4). Investigating the growth, survival, and control of foodborne pathogens in the finished compost.

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Abstract

Animal manure-based compost is a valuable organic fertilizer for agricultural applications. Due to the presence of human pathogens in raw animal wastes, proper composting of these wastes and handling of the finished products is critical for ensuring the safety of fresh produce production. This project used a systems approach to address this biological hazard control during composting process and subsequent storage and handling of finished products, developed and validated some practical strategies, which can be used by growers. First, we used naturally occurring *Escherichia coli* O157:H7 and *Salmonella* strains isolated from compost to validate those thermal inactivation data acquired from outbreak strains, which was conducted inside an environmental chamber to mimic early phase of composting process (**Objective I**). The naturally occurring strains of *E. coli* O157:H7 and *Salmonella* survived the thermophilic composting phase better than the corresponding outbreak strains in dairy and poultry composts, respectively. To inactivate pathogens on the compost surface, we applied the finished compost as covering material and the hay as the base of compost heaps to minimize the heat loss (**Objective II**). Our four field trials of static composting heaps in spring and winter revealed that the 20-cm thickness of finished compost (FC) covering resulted in higher compost temperature and rapid inactivation of both *E. coli* O157:H7 and *Salmonella* spp. as compared with the 10-cm FC covering, whereas hay at base had no effect either on the compost heap insulation or the rate of pathogen inactivation. In a commercial scale of composting conducted in Maryland, the use of finished compost as covering (30 cm thickness) significantly increased the number of days $\geq 55^\circ\text{C}$ in windrow piles at all locations and in static piles at top location which resulted in rapid reduction of inoculated pathogens. Further, the rate of bacterial reduction was rapid in windrow piles. Weed seed placed in non-covered piles and static piles were able to germinate after 28 days concurrent with *E. coli* O157:H7 survival in those piles. We also determined the correlation between compost maturity index and the potential of finished composts to support pathogen growth by analyzing 31 finished composts made of different agricultural wastes (**Objective III**). Our results suggested that certain types of compost may have the potential of supporting pathogen growth due to the types and levels of indigenous microorganisms, although all these composts met the microbiological criteria and maturity of finished compost. In the finished compost, pathogen growth and survival can be affected by various factors (**Objective IV**). Three greenhouse trials revealed that the pathogen survived better in the dry compost with larger particle size, and the initial rapid moisture loss in compost may contribute to fast inactivation of pathogens in the finished compost. Application of competitive exclusion microorganisms isolated from the composts successfully reduced up to ca. 2 logs of *E. coli* O157:H7 in compost under greenhouse condition by simulating the storage conditions on farm. These results indicate that compost microflora can be an efficient tool to control foodborne pathogens in the finished compost and reduce the potential for soil and crop contamination. The results from this study will provide the practical methods or practices on compost production and handling to eliminate or reduce pathogen contamination of compost, thereby helping produce industry to grow safe products for human consumption.

Background

Composting represents an excellent method to produce organic fertilizer in an environmental friendly manner by the bioremediation of vegetable waste, animal manure, sludge or sewage waste. In this process, biodegradable solid complex organic matter is transformed into humified and organic fertilizers, which can be used in agriculture with no negative consequences for the environment. The process is complex and influenced by many variables, including environmental factors, composition of the ingredients and indigenous microflora. The typical composting process consists of a series of temperature changes that correlate with a succession of different microbial communities, which utilize complex organic polymers as carbon source such as lignin, hemicelluloses and cellulose also proteins and lipids. Each temperature phase is accompanied by the development of specific microbial communities (mesophiles, thermophiles). The increase in temperature associated with the process is critical for human and animal pathogen reduction.

In general, active composting can achieve 6-log reduction of artificially inoculated pathogens inside the static compost heaps within 3 weeks of composting even without turning the heaps (Singh et al., 2010; Shepherd et al., 2010), data in agreement with several other studies (Jiang et al., 2003;

Lemunier et al., 2005; Lung et al., 2001; Pourrcher et al., 2005). However, reports emerged regarding long-term survival of gram-negative bacteria in bovine feces during composting or in non-aerated manure piles (Inglis et al. 2010; Kudva et al., 1998). The discrepancy in available data could be the results of different experimental settings seasons, raw materials. However, the inconsistency on the length of pathogen surviving the composting process between results from challenged studies and natural setting of composting needs to be verified.

Field studies have shown that pathogenic *E. coli* may have extended survival at the compost surface and on the periphery of the heaps when unturned (Shepherd et al., 2007; Fremaux et al., 2007). For static aerated composting, the use of insulating materials has been recommended to keep compost temperatures up and help reducing odor emissions from a compost pile (CLGA, 2009). Available covers that have been recommended are physical covering such as an insulated pool tarp and a permeable polypropylene or covering with soil (Curtis et al., 2005; Brito et al., 2008; Wu et al., 2009). However, there is insufficient data available on how a physical covering, especially using common farm materials such as finished compost or straw, might affect pathogens at the compost surface for static heap or windrow composting system.

Compost being used for vegetable production needs to pass certain tests to ensure the composting process has been completed and no harmful microorganisms exist. Composting process normally takes several months to a year to complete. During curing phase, the major microbial safety concern is the pathogen regrowth under certain environmental conditions, which has been observed in both biosolid and animal waste-based composts. Although EPA, CLGA, and CCQC have set the microbiological and maturity test guidelines for the finished compost prior to application, these guidelines cannot predict if the compost can support the pathogen growth or not. Although the composting operators or producers rely on the microbiological test results on fecal coliforms, *E. coli*, *Salmonella*, and *E. coli* O157:H7, and maturity or stability tests to determine if the compost can be released to end-users, there are no valid scientific data to link these tests with the potential for pathogen regrowth in the finished compost. Therefore, a practical method is needed to evaluate if the finished compost is conducive to the growth of human pathogens.

Biological control such as competitive exclusion (CE) microorganisms has been used to inhibit foodborne pathogens pre- and post-harvest in agricultural systems (Zhang et al., 2007a,b; Zhao et al., 2006). The finished compost has been identified as possible source of vegetable contamination during preharvest. The finished compost is a complex microcosm with complex microbial interactions. Although rich in nutrients, some hundreds of microbial species that inhabit this ecosystem compete for available nutrients and no doubt produce compounds with antimicrobial properties targeting neighboring microflora. It seems reasonable to isolate the microorganisms with inhibitory properties against foodborne pathogens and then inoculate them back in the finished compost. In spite of the fact that this is a cheap, ecological method to maintain the safety of the finished compost for agricultural applications there is no study related to CE application in compost. Therefore, there is a need to investigate if CE application can be effective for pathogen control in compost products.

Research Methods and Results

Objective 1: Validating the thermal inactivation data collected from outbreak strains in compost using naturally occurring *E. coli* O157:H7 and *Salmonella* serotypes.

Thermal inactivation of outbreak strains (OS) of *E. coli* O157: strains F06M-0923-21 (spinach outbreak strain from California Dept. of Health), F07M-020-1 (Taco John's outbreak strain from California Dept. of Health) and avirulent B6914 (Kindly provided by Dr. Fratamico, Eastern Regional Research Centre, USDA-ARS), and naturally occurring strains (NS): strain F7B-1, 106-4-1, 902-2 (Dr. Elaine Berry, USDA-ARS) were studied by simulating early phase of optimal composting process (2 days of come-up time) in fresh dairy compost. The strains used in the study were induced to resistant to rifampin (100µg/ml). Similarly, OS of *Salmonella* spp, serotypes Typhimurium 8243 (Kindly provided by Dr. Roy Curtis III, Washington University), Enteritidis H2292 (Kindly provided by Dr. Mike Doyle, University of Georgia) and Heidelberg 21380 (Kindly provided by Dr. Hua Zhao, Food Drug and Administration) were compared with NS: serotype Typhimurium 3631 (Dr. David Ingram, Eastern Regional Research Center, USDA-ARS), Kentucky Farm S D0-CMM, and Newport Farm M 12.1bMF (our lab isolates from poultry compost). All the strains were grown in Tryptic soya broth (TSB)

overnight, centrifuge twice at 5,000g and then washed twice with saline. Washed strains were then mixed in equal volume in their respective group to give final inoculums. The three strains mix of each group was then inoculated into the fresh dairy or poultry compost to a ratio of 1:100 (ca. 10^7 cfu/g) for thermal inactivation study for *E. coli* O157:H7 or *Salmonella*, respectively.

Thermal inactivation of *E. coli* O157 in fresh dairy compost. At 50, 55, and 60°C, NS survived for 456 (19 days), 264 (11 days) and 144 h (6 days), respectively, as detected by enrichment (Table 1, Appendix A). Whereas, the duration of survival of OS was shorter with survival duration of 408 (17 days), 216 (9 days), and 96 h (4 days), respectively, at 50, 55, and 60°C in fresh dairy compost. Based on thermal inactivation curve below (Figure 1), the pathogen inactivation rates for both types of strains were about the same, however a few resistant cells of NS survived slightly longer than those OS cells. The decline in the population of both types of strains was very slow with reduction of ca. 0.5, 0.73, and 0.89 logs, respectively, at 50, 55, and 60°C, in NS within 24 h of the come-up time. Similarly, in OS this reduction was 0.78, 0.95, and 1.1 logs, respectively, at 50, 55, and 60°C. Thereafter population decline was quick with ca. 4.28, 5.0, and 5.65 logs reduction, respectively, at 50, 55, and 60°C after 24 h of the temperature of the compost reaching the target temperature. In OS, this decline was ca. 4.21, 5.0, and 5.92 logs, respectively, at 50, 55, and 60°C at same sampling time. *E. coli* O157:H7 strains naturally adapted to compost (NS) survived more than the time-temperature guidelines of EPA for regulating compost safety.

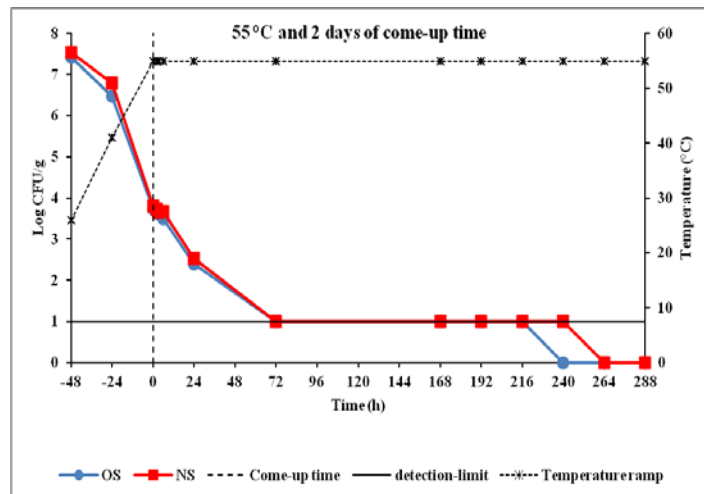


Figure 1. Thermal inactivation of naturally occurring and outbreak strains of *E. coli* O157:H7 in fresh dairy compost at 55°C.

Thermal inactivation of *Salmonella* in fresh poultry compost. Both NS and OS of *Salmonella* were inoculated into fresh poultry compost and exposed to composting temperatures at 50, 55, and 60°C. The survival of NS and OS cultures was >600 h (>25 days) and 552 h (23 days) at 50°C, 360 h (15 days) and 264 h (11 days) at 55°C, and 96 h (4 days) and 24 h (1 day) at 60°C, respectively (Table 2, Appendix A). At 50°C, there was regrowth of pathogen during 24 h before the temperature inside the compost reached the set level. This was evident as there was ca. 0.77 and 1.37 log CFU/g increase in the levels of OS and NS of *Salmonella*, respectively. No regrowth occurred at higher temperatures. When the temperature reached the set level, the decline rates of NS and OS populations were significantly different ($P < 0.05$) at most of the sampling times. Ammonia volatilization was another important factor that contributed to the inactivation of *Salmonella* population in both types of strains (Table 3, Appendix A). In present study NS were apparently better to resist lethal effect of ammonia volatilization than the OS.

In conclusion, **the naturally occurring strains of *E. coli* O157:H7 and *Salmonella* survived the thermophilic composting phase better than the corresponding outbreak strains in dairy and poultry composts, respectively.** In poultry compost, initial levels of ammonia and its volatilization during composting process also contribute to the pathogen inactivation.

Identification of most heat resistant strains: Five colonies each of OS and NS of *E. coli* O157:H7 and *Salmonella* that were picked after heat inactivation at 50, 55 and 60°C were subjected to PCR and PFGE analysis. *E. coli* O157:H7 avirulent strain B6914 was the predominant one in surviving among the

three OS strains used in the study at all three experimental temperatures. Strains F7B-1 and 106-4-1, which were indistinguishable by PFGE, were the predominant surviving strains among NS. Among *Salmonella* serotypes used in the study serotype Heidelberg 21380/ Typhimurium 8243, Enteritidis H2292 and Heidelberg 21380 at 50, 55, and 60°C, respectively, were the dominant surviving serotypes of OS. Among NS, serotypes Typhimurium 3631 was the major surviving serotype at all three temperature used in the study.

Objective 2: Optimizing and validating the finished compost as physical covering and hay as base of freshly formed static compost heaps or windrow compost piles.

Objective 2 consisted of two parts of research. To optimize and validate the depth of finish compost covering and hay as base of the freshly formed compost heaps or piles, passive static compost heaps were evaluated at Clemson University, whereas a commercial scale of composting was conducted at USDA-BARC using a windrow composting system.

At Clemson University: We completed 2 dairy composting trials (Trial #1, 3/21-4/20/2011; Trial #2, 12/8/2011-1/7/2012) and 2 poultry composting trials (Trial #3, 3/13-4/12/2-12; Trial #4, 10/23-11/22/2012) comparing the finished compost as physical covering along with hay as base to insulate the composting heaps under field conditions (all figures are in Appendix B). In each trial, the composting mixture consisted of a sawdust/cow manure mixture, waste feed, old straw and fresh straw at a ratio of ca. 8.5:3:3:1 for dairy composting, and a sawdust, fresh chicken litter plus bedding material, and aged broiler chicken litter at a ratio of ca. 5:3.5:1 for poultry composting. The compost ingredients were mixed thoroughly with the use of a front-end loader, and then equally divided into four heaps {heap #1: 10-cm of hay as base and 10-cm finished compost (FC) (10 cm FC/hay) as physical covering, heap #2: 10-cm of hay as base and 20-cm finished compost as physical covering (20 cm FC/hay), heap #3: no hay base but 20-cm finished compost as physical covering (20 cm FC), heap #4: control without hay base and finished compost covering}(Cont) were constructed.

The newly formed compost heaps were constructed to be ca. 1.2 m in height by ca. 2 m in width, with a conical shape, as previous study performed in our lab demonstrated that these dimensions were suitable for thermophilic composting. The compost heaps were stationed on two concrete staging areas measuring 16 m × 25 m (L × W) enclosed with fencing; two compost heaps were positioned on each staging area. A mixture of three avirulent *E. coli* O157:H7 strains (B6914 stx1⁻/stx2⁻, cv2b7 pGFPuv stx1⁻/stx2⁻, 6980-2 pGFP stx1⁻/stx2⁻) and avirulent *Salmonella* Typhimurium strain 8243 were inoculated into the finished dairy compost at a ratio of 1:100 (v/w) to yield a final bacterial concentration of ca. 10⁹ CFU/g. For poultry composting trials, only avirulent *Salmonella* was inoculated in the same way as described for dairy composting trials. After acclimation in the finished compost for 24 h at room temperature, the inoculated compost was mixed with a portion of the fresh compost mixture at a ratio of 1:100 (w/w) to yield a final population of each pathogen at ca. 10⁷ CFU/g dry weight (DW). Approximately 150 g of the inoculated compost mixture was inserted into Tyvek[®] self-seal pouches (8.89 × 13.33 cm, DuPont, Wilmington DE) to be placed under the compost coverings, whereas ca. 150 g of the sample mixture was placed into polystyrene trays with perforated bottoms to be anchored atop of the uncovered heaps, serving as the experimental controls.

The Tyvek[®] pouches containing compost samples inoculated with avirulent *E. coli* O157:H7 strains and avirulent *S. Typhimurium* strain 8243 were placed into mesh bags, which were secured onto the surface of the compost heaps, and then covered with FC or hay for each heap. Compost samples were obtained at 0, 1, 3, 5, 7, 14, 21, 30 or 60 days after heap construction, and analyzed for the target pathogens, total bacterial counts and *Enterobacteriaceae*. Using an OT-21 temperature and oxygen sensor (Demista Instruments, Arlington Heights, IL), heap temperatures and oxygen contents were monitored and recorded at the center of each heap, and at each sample location (the interface where the surface of the newly formed compost heap met the FC or straw being used as the physical covering) everyday for the first two weeks of composting, and every sampling day thereafter.

Temperature in the composting heaps elevated rapidly in the compost heaps at the interface between the coverings and the surface of the newly-formed compost heap, and at internal locations within each heap. The following is the summary of temperature profiles among treatments for all 4 trials:

Trial #1: For the covered heaps, the temperatures at interface between covering and newly formed compost heaps were above 50°C for more than 2 wks as compared with ca. 22 °C of ambient temperature. Temperatures at the interface followed such order: 20 cm FC = 20 cm FC/hay > 10 cm FC/hay > control, as 20 cm FC/hay = 20 cm FC ≥ 10 cm FC/hay > control for the temperature inside the heaps.

Trial #2: For the covered heaps, the temperatures at interface between covering and newly formed compost heaps were above 50°C for 4~5 days as compared with ca. 20°C of ambient temperature. Temperatures at the interface followed such order: 20 cm FC = 20 cm FC/hay > 10 cm FC/hay > control, as 20 cm FC/hay ≥ 20 cm FC ≥ 10 cm FC/hay > control for the temperature inside the heaps.

Trial #3: For the covered heaps, the temperatures at interface between covering and newly formed compost heaps were above 50°C for 2~13 days of composting as compared with ca. 29°C of ambient temperature. Temperatures at the interface followed such order: 20 cm FC ≥ 20 cm FC/hay > 10 cm FC/hay > control, as 20 cm FC > 20 cm FC/hay > control > 10 cm FC/hay for the temperature inside the heaps.

Trial #4: For the covered heaps, the temperatures at interface between covering and newly formed compost heaps were above 50°C for 11~14 days of composting as compared with ca. 23°C of ambient temperature. Temperatures at the interface followed such order: 20 cm FC ≥ 20 cm FC/hay > 10 cm FC/hay > control, as 20 cm FC/hay ≥ 20 cm FC > 10 cm FC/hay > control for the temperature inside the heaps.

Clearly, the 20-cm thickness of finished compost covering resulted in higher compost temperature at the interface of newly formed compost surface and the compost covering as compared with 10 cm thickness of the finished compost covering. Interestingly, hay at base didn't enhance, and actually reduced the insulation capacity of the finished compost as covering, probably due to the porous nature of the hay, which may let the compost heat evaporate from the base of compost.

Table 1. Survival of avirulent *E. coli* O157:H7 and *S. Typhimurium* during composting under field conditions

Trial	Season	Compost	<i>E. coli</i> O157:H7				<i>Salmonella</i>			
			Cont.	10 cm FC/hay	20 cm FC/hay	20 cm FC	Cont.	10 cm FC/hay	20 cm FC/hay	20 cm FC
1	3/21-4/20/2011	dairy	>30	7*	3	1	>30	7	3	1
2	12/8/2011-2/7/2012	dairy	>60	21	7	14	>60	7	7	7
3	3/13-4/12/2012	poultry	-**	-	-	-	>30	1	3	1
4	10/23-11/22/2012	poultry	-	-	-	-	>30	5	3	3

*Last positive sampling day for the pathogen. ** -, not tested.

Populations of *E. coli* O157:H7 and *Salmonella* were reduced rapidly esp. for composting trials #1, #3, and #4 when the outside temperatures were high and the temperatures at the interface were above 50°C for 1~2 wk. During dairy composting (Trials #1 & #2), the inactivation rates of both pathogens followed the following trend: 20 cm FC (fast) > 20 cm FC/hay > 10 cm FC/hay > Control (slowest). *E. coli* O157:H7 survived slightly longer than *Salmonella* in both trials. Pathogens were eliminated faster in spring than winter months. There was a rapid reduction of *Salmonella* during poultry composting (Trials

#3 & #4). Furthermore, *Salmonella* was inactivated faster in poultry compost than in dairy compost probably due to the bactericidal effect of ammonia on the pathogen.

In conclusion, all compost heaps covered with the finished compost resulted in high temperatures at the interface between the compost coverings and the surface of the newly-formed compost heaps, which is correlated with rates of pathogen reduction during dairy composting process (Trial #1 & 2) and poultry composting trial #4. However, during poultry composting process of Trial #3, the rates of *Salmonella* inactivation with these treatments didn't correlate well with the compost temperature profile probably due to rapid die-off of the pathogen in poultry compost and unusual high ambient temperature during the time of that trial.

At USDA-ARS: This portion of the study was conducted at the on-farm compost facility at USDA-BARC (Beltsville, MD) from September to December of 2012 (all figures and tables are in Appendix B). Conical-shaped static compost piles (5 cu. yd. each, 6 ft dia. base) were constructed on top of a 15cm layer of straw. Feedstock consisted of dairy solids (from a solids/liquids separation process), calf manure and straw bedding, excess (unconsumed) feed, and old hay (85:5:5:5). Three static piles were covered with 30 cm finished mature compost (SFC), and three additional static piles which served as controls were uncovered (SN). Two windrow piles each 30 m long and comprising 50.5 cu. m (66 cu yd.), without a straw base, were constructed using the same feedstock mixture as used for the static piles. The four pile-cover treatment depths used for the insulating layer of finished mature compost placed on top of the feedstock mass were: 0 cm (control-WN), 10, 15, and 30 cm (WFC). Two windrows (8 ft wide at the base x 90 ft long x 5 ft high) were divided into two sections of 45 ft lengths, and each part was subdivided into 15 ft each to obtain three replicates (Figure 1). Windrow piles were turned on day 14, and thereafter twice during the whole trial.

Three strains of bacteria were used in the study: 1) a nonpathogenic strain of *E. coli* O157:H12 from a local watershed and spontaneously resistant to nalidixic acid, 2) an avirulent green fluorescent protein expressing *E. coli* O157:H7 (strain B6914) from the EMFSL stock culture and 3) *Salmonella enterica* (strain LT2) rifampicin resistant were streaked onto Tryptic Soy agar (TSA), incubated 24 h at 37°C, and a colony of each strain was streaked onto sorbitol MacConkey Agar supplemented with 0.05 mg/l of cefixime, 2.5 mg/l of potassium tellurite, and 50 mg/l of nalidixic acid (CTSMAC-NA) for *E. coli* and on Xylose Lysine Tergitol4 agar supplemented with 80 mg/l of rifampicin (XLT-R) for *Salmonella*. A single colony of each strain (*E. coli* O157:H7 B6914 and *Salmonella enterica* LT2) was transferred into 9 ml of sterile fecal slurry and incubated for 24 h at 37°C. Then, 2.5 ml of each strain was inoculated in 1.25 L slurry and incubated at 37°C for 24h. Inoculated slurries with B6914, and LT2 yielded concentrations of 8.39 and 7.73 log CFU/ml, respectively.

Microbial sample preparation: Inoculated slurry of *E. coli* O157:H7 and *Salmonella* were concentrated and equal volumes of each strain were mixed to form a cocktail. Feedstock consisting of separated dairy solids was inoculated with a cocktail and mixed thoroughly. Portions of 30 g of inoculated feedstock were placed on a piece of organza fabric and sealed with a plastic cable tie to form a sample sachet. The sachets were placed in PVC bio-sentry tubes (7.5 cm dia, 60 cm long) in three different locations (East side, top middle, West side) of all static compost piles and in windrow piles that were covered with 30 cm finished compost and uncovered windrow piles. Side locations were approximately 0.5 m from the base of the pile and at the inner interface of the compost cover layer and the new feedstock. The top was placed at least 0.3 m below the interface between the compost cover layer and new feedstock. As a consequence of the rapid die-off of inoculated *E. coli* O157:H7 and *Salmonella* strains, *E. coli* O157:H12 strain was prepared and inoculated into dairy solids. The latter was placed into sachets which were loaded into the biosentry tubes as described above and placed into 7-day old compost piles.

Physico-chemical sample preparation and measurements: Approximately 50 g of uninoculated feedstock (the same used for microbial analysis) was placed on the organza fabric and securely tied closed using a plastic cable; these samples were for pH, moisture content, and total carbon (C) and nitrogen (N) determination. Samples were placed in bio-sentry tubes along with the inoculated sample sachets in two

locations (West and Top) of static and windrow piles. The pH and electrical conductivity measurements were performed according to standard methods using 5 g sample (TMECC, 2000). Moisture content of samples was analyzed according to Standard Methods (APHA, 1998). Total carbon and total nitrogen were analyzed using an elemental analyzer.

Seed preparation and test germination percentage: Temperature tolerance of seeds from 20 different weed species and tomato (Table 1) was determined to facilitate selection of weed species to be used in field-scale compost pile studies. Tolerance to 55°C was used as the screening test incubation temperature because it represents the minimum value that must be sustained for three consecutive days with in-vessel or static aerated pile composting of sewage sludge, or for 14 days for windrow composting, in order to meet pathogen reduction standards (US EPA, 1993). The initial screening tests for weed seed germination involved preparation of 21 separate aliquots of 20-seeds each per weed species for exposure testing in triplicate at 55°C for 0, 3, 6, 24, 48, 72, 96 hr. The testing methods used in the screening study and subsequent samples from compost piles were adapted from those described by Dahlquist et al. (2007).

Seed preparation and germination after exposure in windrow and static compost piles

Three species of weed seeds, *Echinochloa crus-galli* (barnyard grass), *Rumex obtusifolius* (bitter dock), *Setaria viridis* (green bristle grass), and *Lycopersicon esculentum* cultivar *Roma* (tomato seed, control) were selected for the experiment, based on results from preliminary studies of tolerance to high temperatures by seeds of 20 species of weeds (Table 1) and tomato. Small bags of 50 undamaged seeds of each type of weed and 20 seeds of tomato were made using organza fabric and tied with cable tie. Each of 4 small bags of seed was placed into another organza bag of (3' x 4'). The large seed bags were placed into bio-sentry tubes along with the inoculated samples for exposure to compost conditions with sampling over time and germinability analysis. The samples were analyzed at day 0, 28, 56 and 84. Germination test was processed following the weed seed test germination protocol (Millner, 2012).

Compost sampling scheduling and temperature measurements

A total of 54 perforated bio-sentry tubes covered on both edges with fiberglass were prepared for the study. Each bio sentry tube has 24 holes staggered equidistantly around the length of the tube to allow gas exchange between the compost material and the samples inside the tube. Each bio-sentry tube was labeled and contained a temperature data logger (LogTag datalogger), seven sample bags for microbial analysis, six for physical analysis, three for an external laboratory, and three bags for seed germination. Bio-sentry tubes were placed at approximately 10, 10, and 30 cm depths beneath the surface at three locations, east, west, and top, with a numerated flag on the pile surface indicating tube location. On days 1, 3, 7, 14, 21, 28, 56, and 84, tubes were pulled from their pile locations with aid of a ~2 m rope tied around the tube on day 0. Tubes were reinserted into their pile location immediately after sample removal.

Microbiological analysis: *Salmonella* LT2, *E. coli* O157:H7 B6914, and *E. coli* O157: H12 as surrogate pathogenic organisms and total coliforms, fecal coliforms, and *E. coli* were monitored throughout the composting period. Sampling methods outlined in this study were adapted from the US Testing Methods for the Examination of Composting and Compost (TMECC) with a slight modification. Compost samples (n=252) were transferred aseptically and individually into a sterile filter bag, massaged for 2 min in 10x diluted with 0.1% sterile buffer peptone water and then sonicated for 5 min using a Sonicator. The diluted suspensions were plated on XLT4-Rif agar (enumeration of LT2, black colonies), CTSMac-Na agar (enumeration of B6914 and H12, light pink with dark center), and Mac plates for total coliforms (bright pink or red colonies) following incubation for 24 h at 35°C. The Mac supplemented with 4-methylumbelliferone- β -D-Glucuronide (MUG) was incubated at 44.5 °C for enumeration of fecal coliforms (colorless, pink to red colonies) and *E. coli* (red colonies with blue fluorescence under the ultraviolet light (~365nm). Besides direct plating, the three-tube, three-dilution most-probable number (MPN) technique was used for enumeration of *Salmonella*, *E. coli* O157, total coliforms, fecal coliforms, and *E. coli* following the TMECC with a slight modification. For total coliforms, 1 ml of each dilution

was transferred into each of three tubes containing 9 ml of lauryl tryptose broth (LTB; Acumedia) and an inverted gas tubes (6 x 50 mm; Kimble and Chase, USA) and then incubated at 35°C for 18-24h. Identification of coliforms was confirmed by the presence of gas in the tube in each dilution set and most probable number (MPN) was calculated. For *E. coli* O157, positive tubes of LTB were streaked accordingly on CTSMac-Na and incubated at 35°C for 18-24h for MPN. For *Salmonella* MPN, 1 ml of each dilution was transferred into each of three tubes containing 9 ml of BPW. The tubes were then incubated for 24 h at 35 °C for primary enrichment and 1ml of primary enriched suspension into 9 ml of tetrathionate broth (Acumedia) for 24 h at 35°C. After 24h, tubes were mixed, and streaked onto XLT4-Rif and incubated again at 35° C for 24h.

Statistical analysis: Bacterial populations obtained as MPN were transformed to log CFU per gram. The counts were analyzed with a three-way ANOVA using the PROC MIXED procedure of SAS version 9.3 (SAS institute, NC). The effects of compost pile type, FC cover, sample location, sampling days, and their interactions were compared. Similarly, the pH and moisture contents were analyzed using the PROC Mixed procedure. The level of statistical significance was set at $p < 0.05$.

Survival of enteric pathogens in compost piles: Initial *Salmonella* populations in static and windrow compost piles were 6.46 log CFU/g. The *Salmonella* were reduced significantly within 24 h in windrow piles irrespective of sampling location (top-internal or east/west-interface) and in static piles sampled from the top location (Figures 2-5). The populations of *Salmonella* were reduced below detection limit within 1 and 3 days in WFC and WN piles, respectively. However, the *Salmonella* persisted for longer duration in static piles. This pathogen was undetectable after 7 and 28 days in SFC and SN piles when sampled from internal (top) and west interface locations, respectively. *Salmonella* were recovered after 84 days in SN pile sampled from east interface. Use of 30 cm FC as cover reduced *Salmonella* at a faster rate in both, static and windrow compost piles.

The initial *E. coli* O157:H7 populations (7.41 log CFU/g) in static and windrow piles were reduced significantly within 24 h in all internal samples (top location). *E. coli* O157:H7 were undetectable after 1 day in WFC and WN piles in samples collected from interface and top locations, respectively (Figures 2-5). After 3 days, *E. coli* O157:H7 could not be detected in WFC and WN piles at every location. The effect of sample location was evident in static piles as *E. coli* O157:H7 populations were detected after 84 days in interface locations from SN and SFC piles. Nevertheless, *E. coli* O157:H7 were reduced below detectable levels in SN and SFC pile samples collected from top location.

E. coli O157:H12 strain, an environmental strain isolated from local watershed, was placed one week later in compost piles to compare its survival during the composting process. The strain persisted for longer durations than *E. coli* O157:H7 (Figure 6). The H12 strain was undetectable after 7 and 28 days in WFC and SFC piles, respectively, sampled at top location. However, it was recovered from both side locations in WFC and SFC piles after 84 days. In SN and WN compost piles, *E. coli* O157:H12 persisted for more than 84 days irrespective of sampling location.

Survival of coliforms and E. coli in compost: Initial populations of coliforms, fecal coliforms and *E. coli* were 8.73, 7.4 and 5.63 log CFU/g (Table 8-10). Coliforms were reduced significantly after 3 days in all samples of windrow piles. Coliforms persisted in all compost samples in the range of 2.26-3.04, irrespective of sample location, compost pile or cover. Fecal coliforms were reduced with composting process, the rate of reduction was rapid in those samples collected at top location. Fecal coliforms were undetectable after 3 days in WFC piles at all three locations, but recovered at day 14 followed by reduction to undetectable level on 28-84 days. Fecal coliforms were detected in all static piles at a level below 3 log CFU/g. The populations of generic *E. coli* reduced significantly by 3 days in all compost samples except in SN pile of west locations. Further, this bacterium was undetectable by 3 days in all samples from windrow piles irrespective of cover or sample location. Similarly, *E. coli* were reduced below detectable level within 3 days in SN and SFC pile samples from top locations.

Moisture, pH, and C:N ratio of compost samples: The pH of the compost reduced gradually during 84 days; however, the reduction was not significant (Table 2). The effect of pile, use of cover, or sample location did not have any effect on compost pH. The initial moisture content of compost (69%) was reduced significantly by day 3 in some instances (Table 3). There was no specific pattern in moisture loss, as precipitation also influenced moisture content of compost. The C:N ratio was 30% at the beginning of the compost which was reduced at day 84 to ~16%, indicating carbon loss during composting process (Table 4). There were no significant differences in C:N ratio due to type of compost pile or cover or sample location.

Compost Pile temperatures and weed seed germination: Temperature increased rapidly in static and windrow piles particularly at top locations (Figures 2-5). Overall, the temperature increase in piles with FC cover was significantly greater compared to corresponding static and windrow piles without FC cover. Total number of days the exposures exceeded $\geq 55^{\circ}\text{C}$ were 40, 33, 27, and 10 at top locations of WFC, WN, SFC, and SN piles, respectively (Table 5). The location of sampling significantly influenced temperature variations. Temperature above $\geq 55^{\circ}\text{C}$ was < 3 days in east and west (side) locations of SN, SFC, and WN piles. The temperature was $\geq 55^{\circ}\text{C}$ for at least 5 days of the first week at top locations of static and windrow piles. In contrast, at one week later with the *E. coli* O157:H12 study, days above 55°C were few (Table 6). The germination rate for tomato, green foxtail, broadleaf dock and barnyard grass was 95, 100, 100, and 50%, respectively, at the beginning of the composting process (Table 7). None of these seeds germinated in windrow piles after 28 days except east location of WN piles, where 6% of barnyard grass seeds were germinated. Most seeds were germinated in static piles after 28 days especially sampled from east and west locations. Nevertheless, seeds failed to germinate in static piles sampled from top location.

Objective 3: Applying the pathogen growth model to determine the potential of finished composts to support the growth of human pathogens.

In Objective 3 samples collected from different composting sites or sources were evaluated for their physicochemical and microbiological properties as well as their potential associated with pathogen regrowth. A total of 31 finished compost samples (12 dairy, 6 poultry, 2 horse and 4 other manure-based, and 7 plant-based compost) were collected from SC, PA, FL, and CA. The physico-chemical properties such as pH, a_w , C:N ratio, organic matter, Solvita CO_2 and NH_3 test, and microbiological properties including mesophiles, thermophiles, mesophilic actinomycetes, thermophilic actinomycetes, fecal coliforms, fungi, *E. coli* O157:H7 and *Salmonella* were tested (Table 1, Appendix C). The growth potential of ca. 3 log CFU/g of 3-strain mixture of *E. coli* O157:H7 or *Salmonella* spp. was determined in these samples (Table 1, Appendix C). The populations of both pathogens increased ca. 0.1 ~ 0.8 and 0.2 ~ 0.6 log CFU/g for *E. coli* O157:H7 and *Salmonella*, respectively, within 3 days at 22°C in 8 compost samples, respectively. In general, composts supporting pathogen growth contained lower number of indigenous actinomycetes and fungi. These results suggested that **certain types of compost may have the potential of pathogen growth due to the types and levels of indigenous microorganisms, although all these composts met the microbiological criteria and maturity of finished compost.**

The background microbial community in compost samples was analyzed using denaturing gradient gel electrophoresis (DGGE). Total microbial DNA was extracted from 22 out of 31 compost samples using the PowerMax Soil DNA Isolation Kit (Mo-Bio). Total DNA extracted from soil samples was PCR-amplified for 16 s rRNA genes (U968F and L1401R primers). PCR amplicons were not obtained for samples #23 and #29 after several attempts. Amplified PCR products were then analyzed by DGGE using a BioRad D code system. Briefly, PCR products were loaded onto a 7% polyacrylamide gel with a 30 to 60% denaturant gradient in 0.5 X TAE buffer. Electrophoresis was performed for 16 h at 50 V, and the bands of soil samples on the gel were stained with ethidium bromide. Ethidium bromide stained bands of soil samples evidence that each sample has its own microbial community. The 31 finished compost samples were made from different raw materials. The distinguishable band pattern in

each sample is due to presence of numerous microorganisms in the finished compost product. But, when compared the band pattern of samples with the same raw materials, some common bands can be seen in DGGE profiles (plant based #22, #24 and dairy compost #6, #12).

The community level physiological profiles (CLPP) were assayed with the Biolog Ecoplate method for all compost samples except #11, #21 and #31. Compost samples were diluted in 0.85% saline to reach a bacterial concentration of approximately 10^4 CFU/ml then wells consisting of 31 carbon sources and 3 water controls were inoculated. Plates were incubated for 7 days at room temperature and the color development was monitored by optical density at 590 nm every 24 h. The analysis was performed on data collected at 120 h since there was no color development after that time point. The principal component analysis (PCA) was performed with XLSTAT and the Cluster analysis with SPSS Statistics 21 for Windows. Different compost samples were clustered into four groups (Fig.1, Appendix C), the cutoff between groups was established where the clustering distances were equal or below five. Thus it becomes clear that the metabolic activities of the composts were different, therefore the result supports the previous finding that the microbial communities inhabiting these compost samples were different. **The compost samples (n=8) that supported pathogens growth were present in all clusters except one, indicating that multiple conditions in addition to the type of microbiota may be responsible for promoting regrowth.**

To further investigate if pathogen regrowth is translated in any changes in metabolic profiling, we carried out PCA on the Biolog data from these samples along with other two compost samples (#1-Philadelphia dairy compost and #4-Chicken litter compost) negative for pathogen growth. In this case the metabolic response is designed to complement the DGGE analysis; moreover the color development for the same carbon source from different compost samples can be produced by non-related microflora. PCA is a method aimed at reducing the amount of data when there is a correlation present, and then the relationship between samples can be visualized by plotting individual principal components against each other. The PC (PC1/PC2) that presented variance from the data of single carbon source readings was taken and their accumulative variance contributions were revealed as 54.91% for *Salmonella* regrowth samples and 65.58% for *E. coli* O157:H7 samples. The PC1 and PC2 were used to generate statistical analysis reflecting the metabolic capacities of microbial communities in the process of composting. Therefore, the scoring coefficients of the 31 carbon sources in terms of the two principal components were used to group the compost samples (Figure 2, Appendix C). For *Salmonella* regrowth, the largest group is represented by the positive plant-based composts and the positive hen/chicken compost, suggesting a similarity in their community-level metabolic profile (Figure 2A). Samples #1 and #4 are each unique in their community metabolic profile, and both did not support the pathogen growth. These samples are dairy- and poultry based, respectively. Interestingly the dairy-based compost samples that supported regrowth are also grouped together. A similar outcome can be described for compost samples that allowed *E. coli* O157:H7 regrowth (Figure 2B): the plant-based composts are similar in their community metabolism whereas composts #1 and #4 have unique distinctive patterns. **These results confirmed that one of the conditions that allow the pathogen regrowth is the type of microflora in the finished compost.**

Objective 4: Investigating the growth, survival, and control of foodborne pathogens in the finished compost.

In Objective 4, the effects of environmental conditions typically found in agriculture settings (temperature, air relative humidity, sun-radiation intensity) were investigated with respect to survival of *E. coli* O157:H7 in finished compost as affected by particle size. In addition, we isolated competitive exclusion microflora (CE) from various compost samples (n= 30), which we applied as an antimicrobial treatment to inhibit *E. coli* O157:H7 in finished compost. To simulate field conditions these experiments were performed in greenhouse conditions. The followings are detailed outcomes from this research

Pathogen survival in compost particles with different sizes: Both avirulent, ampicillin-resistant and green fluorescent protein (GFP)-labeled *E. coli* O157:H7 strain B6914 and avirulent *Salmonella* Typhimurium strain 8243 were used for this study. *S. Typhimurium* strain was induced to be resistant to 100 µg of rifampin ml using the gradient plate method (Rice et al., 2005). A mixture of these avirulent strains was inoculated into the finished composts with initial moisture content of 20, 30 and 40%. Then, the finished compost samples were sieved into three portions with particle sizes of >1000, 500-1000 and <500 µm, and stored in greenhouse for 30 days. At selected intervals, compost samples were tested for pathogen population. Three greenhouse trials were performed during Nov.-Dec., 2011 (late fall trial), Jan.-Feb. 2012 (winter trial) and July-Aug 2012 (summer trial). For each season, the second trial was initiated one week after the beginning of the first trial.

The average values of temperature in greenhouse were 21.3, 21.7 and 28.4°C for late fall, winter and summer trials, respectively, while the average values of relative humidity (RH) in greenhouse were 41.3, 36.0 and 64.4% for late fall, winter and summer trials, respectively. Fig.1 (Appendix D) presented the average temperature, RH and light intensity inside the greenhouse for a typical day. The moisture contents in compost samples dropped rapidly to under 10% within 5 days of storage followed by gradual decline till 30 days in all treatments (Table 1, Appendix D). For compost with moisture contents of 20 and 30%, the average *Salmonella* reductions in compost with particle sizes of >1000, 500-1000 and <500 µm were 2.15, 2.27 and 2.47 log CFU g⁻¹ within 5 days of storage in summer, respectively, as compared with 1.60, 2.03 and 2.26 log CFU g⁻¹ in late fall, and 2.61, 3.33 and 3.67 log CFU g⁻¹ in winter, respectively (Table 1, Appendix D). For samples with initial moisture content of 40%, the *Salmonella* reductions in compost with particle sizes of >1000 and <1000 µm were 2.14 and 3.17 log CFU g⁻¹ within 5 days in summer, respectively, as compared with 3.17 and 3.16 log CFU g⁻¹ in late fall, and 2.93 and 3.36 log CFU g⁻¹ in winter, respectively. For compost with moisture contents of 20 and 30%, the average *E. coli* O157 reductions in compost with particle sizes of >1000, 500-1000 and <500 µm were 1.98, 2.30 and 2.54 log CFU g⁻¹ within 5 days of storage in summer, respectively, as compared with 1.70, 2.56 and 2.90 log CFU g⁻¹ in winter, respectively. For samples with initial moisture content of 40%, the *E. coli* O157 reductions in compost with particle sizes of >1000 and <1000 µm were 2.08 and 2.48 log CFU g⁻¹ within 5 days in summer, respectively, as compared with 2.20 and 2.84 log CFU g⁻¹ in winter, respectively. Table 2 (Appendix D) presented data on pathogen reduction for the entire experiment (30 days). **Our results revealed that the pathogens survived better in the dry compost with larger particle size, and the initial rapid moisture loss in compost may contribute to fast inactivation of pathogens in the finished compost.**

To further understand the survival strategies of foodborne pathogens during composting storage, we have attempted to develop a microscope technique to visualize the association of bacterial cells with compost components. First, the morphological and surface characteristics of compost particles with different particle sizes were analyzed by a surface profiler to determine the diameter, area and surface roughness. The results showed the sample particle sizes are in good agreement with the sieve pore sizes which were 500 and 1000 µm used in above experiments (Table 3, Appendix D). Due to the autofluorescence emitted from compost matrix, green fluorescence signal from GFP-labeled *E. coli* O157:H7 could not be differentiated by epifluorescence microscope. Our next approach was to use immunofluorescence (IF) technique to enhance the GFP signal. IF results showed that GFP in *E. coli* O157:H7 and autofluorescence of compost can be differentiated both visually and quantitatively according to the signal intensity, esp. with quantum dot conjugates (Qdots) (Figure 2, Appendix D).

Selection of CE compost microflora with inhibitory activity against E. coli O157:H7.

The microorganisms isolated from compost through a growth chamber method were tested for antimicrobial activity against *E. coli* O157:H7 6914. A derivative Rifampicin resistant of the strain has been previously generated in our laboratory and it was used for bacterial enumeration to differentiate from the CE strain or the compost microflora. Testing included (i) an initial screening by an agar layer method (ii) strains were individually tested in liquid broth (TSB without glucose) in the presence of the target microorganism and (iii) finally tested in compost in laboratory conditions. In laboratory conditions

compost-isolated cultures tested individually in broth, achieved from 0.7 to 3.9 log CFU/ml reduction of *E. coli* O157:H7 B6914 (Table 4, Appendix D). Temperature did not play a role in pathogen reduction (incubation versus room temperature), however when inoculated in compost, pathogen reduction was reduced probably because of the limited diffusion environment and the lack of growth of CE cultures (Tables 5&6, Appendix D). Genomic DNA was isolated from CE strains that showed promising antimicrobial activity, and 16S rRNA gene was amplified with universal primers 8F and E1406R. The PCR amplicons were sequenced at Clemson University Genomic Institute. The obtained sequences were compared to the 16S rRNA genes available in the database and the closest genus/species were determined using sequence match analysis in the ribosomal project database (RDP) (Table 4, Appendix D)

To confirm antimicrobial properties of the selected CE strains in greenhouse settings two experimental approaches were used for the finished dairy compost with adjusted 20, 30, and 40% moisture levels. First, to simulate pathogen contamination after active composting (Table 5, Appendix D), ten bacterial strains selected for their competitive exclusion properties (Table 4, Appendix D) were grown in TSB without glucose until early stationary phase, then centrifuged and washed twice with 0.85 % saline solution. Compost was inoculated 10^8 - 10^9 CFU/g with the cocktail of CE cultures and after 24 h with a cocktail (10^5 CFU/g) of three *E. coli* O157:H7 avirulent and rifampicin resistant strains (*E. coli* O157:H7 B6914, *E. coli* O157:H7 MD46 and *E. coli* O157:H7 MD47).

The second approach simulated the survival of the pathogen during composting and persistence in the compost and agricultural environment (Table 6, Appendix D). Heat-shocked cocktail of *E. coli* O157:H7 was inoculated in compost, and then 24 h later samples were inoculated with the CE cultures cocktail. Samples consisted of (i) compost inoculated only with *E. coli* O157:H7, (ii) compost inoculated with CE cocktail, (iii) compost inoculated with both *E. coli* O157:H7 and CE cocktail and (iv) uninoculated compost.

For both approaches, two independent experiments were performed in triplicate. Sterile cups containing compost samples were arranged in large plastic containers and data loggers for temperature and relative humidity were placed inside. Containers had recipients with saturated KCl solution and were closed every evening and opened in the morning. The moisture levels of the samples were adjusted every evening based on the weight loss. Adjustment in the morning was not necessary since there was little moisture loss due to the overnight storage in high relative humidity. Therefore, samples were subjected to high humidity and lower temperatures overnight and high temperatures and decreased humidity during the day. Treatments were sampled every 4 days and analyzed for moisture content and bacterial enumeration. Experiments were performed in Summer, Fall and Winter trials and data was statistically analyzed by multifactor analysis of variance (ANOVA) using SAS. The comparisons between the means were performed $P < 0.05$ significance test.

Overall, the season or the compost moisture levels did not influence overall pathogen survival (the controls) in the compost samples. On the other hand the compost inoculated with CE, *E. coli* O157:H7 survived longer over time in the colder seasons (Fall and Winter) than the summer months, possibly due to lower temperatures in the greenhouse. *E. coli* O157:H7 inoculated in composts with 20% MC survived better at the presence of CE than the compost with high moisture levels (30 and 40%) regardless of the inoculation method (Table 2, below). Pathogen reduction for non-heat adapted cells and heat-adapted cells by CE treatment ranged from 0.06 to 2.14, and 0.02 to 1.54 logs, respectively, for all trials.

The physiological state of the pathogen seemed to be important in pathogen survival in the compost environment. Depending on the season, the non heat-adapted cells were inactivated at a faster rate than heat-adapted cells. Although the pathogen declined in CE treated samples in all cases as compared with the controls, significant inactivation of non-heat adapted *E. coli* O157:H7 (Approach #1) occurred after only 2 days of storage in the greenhouse in compost samples with higher moisture content (40 and 30 %) during the Fall and Winter trials. The 20 %MC treatments were different than controls at 16 days of storage for the same conditions (Table 2, below). The heat-adapted cells (Approach #2) showed resistance to inhibitory action by CE since significant differences between treatments and controls were present after 12 days for 40 and 30% MC compost and 16 days of storage for compost with

20% MC in the Fall trial. Similar outcome resulted from the Winter trial: in compost with 40% MC heat-adapted cells CE treatments were different than controls at day 8 of greenhouse storage, in composts with 30 and 20% MC dropped to significant levels by day 12 and 16, respectively. As for summer trial, there was no significant difference between the treatment and the controls in the first 4 days of greenhouse incubation for both heat-adapted and non-adapted cells. Most of the treatments dropped to significant levels after 8 days of storage in the greenhouse.

Seasons influenced the pathogen inactivation. The non-adapted cells were more susceptible to CE inhibitory action in the colder season Fall and Winter than the Summer, whereas there was no significant influence of the trial season in the susceptibility to CE action of heat-adapted cells. The temperature in the greenhouse varied greatly between the 3 tested seasons (in the Summer trial occasionally temperatures over 50 °C were recorded at the sample level whereas in the Fall and Winter the temperature did not exceed 38°C).

Table 2. *E. coli* O157:H7 reduction in the finished dairy compost as affected by CE cultures

Season	Moisture (%)	Time (d) required to have difference (p<0.05) in pathogen reduction between treatment and control	
		Approach #1	Approach #2
Summer	40	8	8
	30	8	8
	20	8	12
Fall	40	2	12
	30	2	12
	20	16	16
Winter	40	2	8
	30	2	12
	20	16	16

In conclusion, the application of competitive exclusion microorganisms can effectively inactivate those *E. coli* O157:H7 cells due to cross-contamination within 2 days in the finished compost with at least 30% moisture during colder seasons (winter and fall). As for those heat-adapted *E. coli* O157:H7 cells surviving the thermophilic composting process, the antimicrobial activities from CE became significant only after 12 days, suggesting the cross-resistance of those heat-adapted *E. coli* O157:H7 population.

Outcomes and Accomplishments

Objective 1: Validating the thermal inactivation data collected from outbreak strains in compost using naturally occurring *E. coli* O157:H7 and *Salmonella* serotypes.

Thermal inactivation of naturally occurring *E. coli* O157:H7 and *Salmonella* serotypes was compared with that of outbreak strains, which was conducted inside an environmental chamber to mimic early phase of composting process. At 50, 55, and 60°C, NS of *E. coli* O157:H7 survived in fresh dairy compost for 19, 11 and 6 days, respectively, as detected by enrichment, whereas, the duration of survival of OS was shorter with survival duration of 17, 9, and 4 days, respectively. The survival of NS and OS cultures of *Salmonella* was >25 and 23 days at 50°C, 15 and 11 days at 55°C, and 4 and 1 day at 60°C, respectively. Overall the naturally occurring strains of *E. coli* O157:H7 and *Salmonella* survived the thermophilic composting phase better than the corresponding outbreak strains in dairy and poultry composts,

respectively. In poultry compost, initial levels of ammonia and its volatilization during composting process also contribute to the pathogen inactivation.

Objective 2. Optimizing and validating the finished compost as physical covering and straw as base of freshly formed static compost heaps or windrow compost piles.

Four field trials of static composting validated that the 20-cm thickness of finished compost covering resulted in higher compost temperature and rapid inactivation of both *E. coli* O157:H7 and *Salmonella* spp. as compared with the 10-cm FC covering, whereas hay at base of the compost heaps didn't show much impact. Commercial scale windrow composting results showed that the use of finished compost as a cover significantly increased the number of days above 55°C in static piles at the top location and in windrow piles at all three locations (top, and both sides). Reduction of target pathogens, *E. coli* O157:H7 and *Salmonella*, was associated with the temperature and time of exposure in the compost piles. Both pathogens were reduced rapidly in FC covering compared to that in non-covered control piles validating the use of the 30 cm layer of finished compost in the composting process. Also the rate of reduction was substantially more rapid in the windrow than in the static piles. Fecal coliforms were < 3 log CFU/g (California LGMA levels) after 84 days. Weed seed germination studies revealed germination of weed seeds at day 28 in static piles from east and west (side) locations and non-covered piles. In addition, *E. coli* O157:H7 were recovered from these locations after 28 days indicating strong association between weed seed temperature tolerance, especially broadleaf dock, and *E. coli* O157:H7 survival.

Objective 3: Applying the pathogen growth model to determine the potential of finished composts to support the growth of human pathogens.

A total of 31 finished compost samples of agricultural wastes were analyzed for the physico-chemical properties and microbiological properties. Eight of those composts supported the growth of either 3-strain mixture of *E. coli* O157:H7 or *Salmonella* spp. with growth potential as ca. 0.1 ~ 0.8 and 0.2 ~ 0.6 log CFU/g within 3 days at 22°C, respectively, although all these composts met the microbiological criteria and maturity of the finished compost. In general, composts supporting pathogen growth contained lower number of indigenous actinomycetes and fungi. The clusterings in the Biolog output of 28 analyzed composts were not associated with their origin (dairy-, poultry-, or plant-) suggesting that these compost samples share some common background microflora. The analysis of community-level metabolic profile indicates that the type of microbiota in the compost may be responsible for promoting pathogen regrowth.

Objective 4: Investigating the growth, survival, and control of foodborne pathogens in the finished compost.

Pathogen survival in the finished compost as affected by compost particle size, moisture and at the presence of competitive exclusion microorganisms was evaluated under greenhouse conditions. The population reduction of both *E. coli* O157:H7 and *Salmonella* in compost with particle sizes of >1000, 500-1000 and <500 µm was faster in small particle size than the larger ones, which was correlated with compost dehydration rates for all seasons. The application of competitive exclusion microorganisms effectively inactivated those *E. coli* O157:H7 cells due to cross-contamination within 2 days in the finished compost with at least 30% moisture during colder seasons (winter and fall). As for those heat-adapted *E. coli* O157:H7 cells surviving the thermophilic composting process, the antimicrobial activities from CE became significant only after 12 days, suggesting the cross-resistance of those heat-adapted *E. coli* O157:H7 population. These results indicate that some indigenous compost microflora can be an efficient tool to control foodborne pathogens in finished compost and reduce the potential for soil and crop contamination, however, factors such as the physiological state of the bacteria, the environmental conditions and compost moisture levels should be considered. The advantage of using the finished compost native microflora is that (i) these bacteria are adapted to the compost environment thus ensuring

their survival; (ii) the method provides microbiological protection to the compost after CE application and (iii) avoid major changes of compost physicochemical and microbiological properties.

Summary of Findings and Recommendations

Obj. 1: The naturally occurring strains of *E. coli* O157:H7 and *Salmonella* survived the thermophilic composting phase better than the corresponding outbreak strains in dairy and poultry composts, respectively. Therefore, the time/temperature combination data reported for these 2 pathogens using outbreak strains should be considered as the minimum and need to be verified for specific composting process in order to reduce the risk of pathogen survival during composting. Due to the prolonged survival of a few resistant NS cells in compost, it is recommended to validate the complete killing of the pathogens in the finished compost by using sensitive detection methods coupled with enrichment step.

Obj. 2. The finished compost as cover can increase the temperature at the interface of freshly constructed compost surface and the finished compost cover, while hay at the base of the composting heaps showed little impact on the composting temperature. Due to the presence of ammonia, *Salmonella* was inactivated rapidly even when the depth of finished compost only 10 cm thickness. The effectiveness of finished compost as a covering material on pathogen reduction was also verified in static and windrow composting systems at a commercial scale. Additionally, there is a strong association between inactivation of weed seed germination and *E. coli* O157:H7 growth, further studies to validate weed seed germination as an indicator of pathogen reduction in finished compost would be helpful. Based on the results of this study, it is recommended to cover the composting heaps or piles with about 10~30 inches of the finished compost, esp. during winter months when the ambient temperature is low, and use less finished compost for covering poultry compost heaps.

Obj. 3: By analyzing some representative agricultural wastes-based composts, we have found certain types of compost may have the potential for supporting pathogen growth due to the types and levels of indigenous microorganisms, although all these composts met the microbiological criteria and maturity of finished compost. Further studies are needed to identify those key microbial species responsible for pathogen control in the compost. Therefore, just relying on microbiological test results on fecal coliforms, *E. coli*, *Salmonella*, and *E. coli* O157:H7, and maturity or stability tests may not be sufficient to predict if pathogen growth can occur in the finished compost.

Obj. 4: Our results revealed that compost with larger particle size supports pathogen survival more than the compost with small particle size, and the initial rapid moisture loss in compost contributes to fast inactivation of pathogens in the finished compost. By applying competitive exclusion microorganisms to the finished compost with at least 30% moisture, up to 99% population of those *E. coli* O157:H7 cells due to cross-contamination can be effectively inactivated within 2 days during colder seasons (winter and fall). As for those heat-adapted *E. coli* O157:H7 cells surviving the thermophilic composting process, longer treatment with CE cultures is needed, suggesting the cross-resistance of those heat-adapted population. Additionally, both compost moisture and season of application may affect the efficacy of this biological control method as well. Based on the results of this study, we'd recommend covering the fresh compost surface with the finished compost or other physical barrier to reduce the aerosolization of compost particles. Produce field in very close proximity to the composting site should be checked periodically for possible pathogen transmission from the fresh compost heaps. To avoid the pathogen growth in the finished compost due to cross contamination, a cocktail mixture of CE can be applied a few days prior to the use of the finished compost, preferably in the colder seasons.

APPENDICES

Publications and Presentations (required)

Publication list:

Kim, J., S. Heringa, J. Diao, and X. Jiang. 2011. Fate of *Escherichia coli* O157:H7 and *Salmonella* spp. in animal manure-based composts during storage. Abs. 111th Gen. Mtg. Am. Soc. Microbiol. New Orleans, LA, May 22-24.

Jiang, X.P. 2011. Developing and validating practical strategies to improve microbial safety in composting process control handling practices. The 2nd Produce Research Symposium, Center for Produce Safety, Omni Orlando Resort at Champions Gate, FL, 6/28/2011.

Singh, R., M. Shepherd, X. Liu, J. Kim, J. Diao, C. Ionita, and X. Jiang. 2012. Developing and validating practical strategies to improve microbial safety in composting process control handling practices. Western Food Safety Summit, Hartnell College, May 10 & 11, 2012.

Diao, J., Z. Chen, and X. Jiang. 2013. Influence of Compost Particle Size on Pathogen Survival Under Greenhouse Condition. Abs. 100th Annu. Mtg. Intern. Assoc. Food Prot., Charlotte, NC, July 28-31.

Ionita, C., J. Kim, and X. Jiang. 2013. Survival of *Escherichia coli* O157:H7 in finished compost in the presence of competitive exclusion bacteria. Clemson University, Life Sciences Facility Dedication - Feb. 8, 2013.

Copies of Publication:

Fate of *Escherichia coli* O157:H7 and *Salmonella* spp. in animal manure-based composts during storage (ASM, 2011)

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ABSTRACT

Background: Treating chicken litter with heat prior to land application has been recommended due to the concern of *Salmonella*. However, there was difference in processing methods and no validation studies of these heat treatments for *Salmonella* have been reported. Therefore, the purpose of this study was to evaluate thermal inactivation of *Salmonella* spp. in chicken litter under different environmental conditions.

Methods: A mixture of three *Salmonella* serotypes was inoculated into fresh and aged chicken litter with different moisture levels (30, 40, and 50%) at the final concentration of ca. 7 log CFU/g. Samples were exposed to 75, 80, or 85°C up to 5 h inside a controlled oven/ incubator, and analyzed for *Salmonella* levels at selected intervals.

Results: Original chicken litter samples were absent of *Salmonella* spp. by enrichment and the population of background microorganisms was ca. 6.9 and 8.4 log CFU/g for fresh and aged chicken litter, respectively. Moisture content of chicken litter samples was reduced below 10% within 60 - 120 min upon exposure to above temperatures, with longer times in samples with initial high moisture content. The D-values of *Salmonella* in fresh chicken litter with 30% of moisture were 14.4, 13.3, and 9.0 min at 75, 80, and 85°C, respectively. For 40% moisture samples, D-values were 13.4, 11.3, and 6.4 min at above temperatures, respectively, as compared with 11.5, 11.2, and 6.3 min for the samples with 50% of moisture. On the other hand, linear inactivation of *Salmonella* was not observed in aged chicken litter. *Salmonella* survived in the range of 60 to over 300 min depending on temperatures and initial moisture

contents. Analysis of nutrients and metal contents revealed a significant difference between fresh and aged chicken litter samples.

Conclusions: Our results revealed that a 7-log reductions of *Salmonella* can be achieved by exposing fresh chicken litter for 80.5~100.8, 78.4~93.1, and 44.1~63 min at 75, 80, and 85°C, respectively, depending on initial moisture contents. However, aged chicken litter requires more heat treatment.

Developing and validating practical strategies to improve microbial safety in composting process control handling practices (The 2nd Produce Research Symposium, Center for Produce Safety, Omni Orlando Resort at Champions Gate, FL, 6/28/2011)
Jiang, X.P. Clemson University, SC, 29634

Summary

Animal manure-based compost is a valuable organic fertilizer for agricultural applications. Due to the presence of human pathogens in raw animal wastes, proper composting of these wastes and handling of finished products is critical for ensuring the safety of fresh produce production. In this study, we validated the thermal inactivation data collected from outbreak strains of *Escherichia coli* O157:H7 in compost using naturally occurring strains. The thermal inactivation data revealed similar inactivation rates for these two types of strains with naturally occurring strains persisting slight longer. To increase the insulation capacity of compost heaps, the finished compost as a physical covering and hay at the base of heaps were compared under field condition. Apparently the 20-cm thickness of finished compost covering resulted in higher compost temperature and rapid inactivation of both *E. coli* O157:H7 and *Salmonella* spp., whereas hay at the base didn't make a difference. Furthermore, we determined the correlation between compost maturity index and the potential of finished composts to support growth of human pathogens. Our analysis revealed that 2 out of 10 finished composts with low population of fungi and fecal coliforms supported the growth of above pathogens even though all samples were considered as mature compost.

Developing and validating practical strategies to improve microbial safety in composting process control handling practices (Western Food Safety Summit, Hartnell College, May 10 & 11, 2012)
Singh, R., M. Shepherd, X. Liu, J. Kim, J. Diao, C. Ionita, and X. Jiang. Clemson University, SC, 29634

ABSTRACT

Animal manure-based compost is a valuable organic fertilizer for agricultural applications. Due to the presence of human pathogens in raw animal wastes, proper composting of these wastes and handling of the finished products is critical for ensuring the safety of fresh produce production. In this study, we validated the thermal inactivation data collected from outbreak strains of *Escherichia coli* O157:H7 and *Salmonella* in fresh dairy compost using naturally occurring strains. The thermal inactivation data revealed similar inactivation rates for these two types of strains with naturally occurring strains persisting slight longer. To increase the insulation capacity of compost heaps, the finished compost as a physical covering and hay at the base of heaps were compared under field condition. Two field trials revealed that the 20-cm thickness of finished compost covering resulted in higher compost temperature and rapid inactivation of above pathogens. To better understand the microbiological safety of the finished compost, the correlation between compost maturity index and the potential of finished composts to support pathogen growth was studied. Our analysis revealed that 2 out of 10 finished composts with low population of fungi and fecal coliforms supported the growth of above pathogens even though all samples were considered as mature compost. Additionally, some indigenous microorganisms isolated from various compost products possess strong antimicrobial activities against both pathogens. Our results demonstrated that the microbiological safety of animal manure-based compost can be ensured by

understanding various factors critical for pathogen growth and survival during composting and subsequent handling of finished compost.

Influence of compost particle size on pathogen survival under greenhouse condition (IAFP, 2013)

Diao, J., Z. Chen, and X. Jiang. Clemson University, SC, 29634

ABSTRACT

Introduction: Animal waste directly applied to agricultural field is one of the possible contamination sources of fresh produce. Although pathogens can be eliminated by proper composting process, pathogens are able to survive, recolonize and regrow on compost heap surfaces. Furthermore, bioaerosols can be generated on compost surfaces with different particle sizes which can carry pathogens, travel via air and contaminate fresh produce nearby.

Purpose: This study was to investigate the survival of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium in compost with different particle sizes as affected by initial moisture content and seasonality under greenhouse condition.

Methods: The mixture of avirulent *S. Typhimurium* and *E. coli* O157:H7 were inoculated into the finished compost with initial moisture content of 20%, 30% and 40%. Then, the finished compost samples were sieved into three portions with particle size of >1000, 500-1000 and <500 μm , and stored in greenhouse for 30 days. At selected intervals, compost samples were tested for pathogen population.

Results: The moisture contents in compost samples dropped to under 10% within 5 days of storage followed by gradual decline till 30 days in all treatments. For moisture contents of 20 and 30%, the average *Salmonella* reductions in compost with particle sizes of >1000, 500-1000 and <500 μm were 2.15, 2.27 and 2.47 log CFU g^{-1} within 5 days of storage in summer, respectively, as compared with 1.60, 2.03 and 2.26 log CFU g^{-1} in late fall, and 2.61, 3.33 and 3.67 log CFU g^{-1} for winter, respectively. *E. coli* O157: H7 reduction in compost with particle sizes followed the same trend as *Salmonella*.

Significance: Our results revealed that compost with larger particle size supports pathogen survival more than the compost with small particle size, and the initial rapid moisture loss in compost contributes to fast inactivation of pathogens in the finished compost.

Survival of *Escherichia coli* O157:H7 in finished compost in the presence of competitive exclusion bacteria (Life Sciences Facility Dedication - Feb. 8, 2013)

Ionita, C., J. Kim, and X. Jiang. Clemson University, SC, 29634

ABSTRACT

Composting is an environmental friendly method for converting livestock and agricultural waste into organic fertilizer. During composting the high temperature achieved in the thermophilic phase is critical for pathogen inactivation. However, extended survival of pathogens in compost has been reported despite high temperature. This study evaluated the effectiveness of selected competitive exclusion microorganisms (CE) isolated from compost for *Escherichia coli* O157:H7 reduction in compost with 20-, 30- and 40 % moisture levels. A cocktail of three avirulent *E. coli* O 157:H7 strains was inoculated onto dairy waste-based compost (10^5 CFU/g) along with CE strains (10^8 CFU/g). Two inoculation approaches were used: (i) heat-adapted *E. coli* O157:H7 to simulate cells that survived composting and (ii) inoculation in compost to simulate environmental recontamination. Samples were stored in a greenhouse and monitored for a month. Results indicate that heat-adaption and higher moisture content afforded *E. coli* O157:H7 survival in compost. In CE treatments *E. coli* O157:H7 population significantly decreased compared with the controls ($P \leq 0.05$) by day 2 and 4 in heat-adapted cultures for 40- and 30% moisture contents, respectively. Our findings will identify scenarios to effectively eliminate *E. coli* O157:H7 in compost.

Budget Summary (required)

The fund provided by CPS was adequate for us to carry out the project. We overspent in Supplies categories due to the expanded research on microscopic imaging of bacterial interaction with compost particles (Objective 4), and replacement of a broken freezer and a balance. Since there was a gap between the departure of previous postdoc and the hiring of the current one, the fund from Salaries and Fringes category was used to cover the shortfall of Supplies categories. A request for re-budget was submitted to our Sponsored Program Office. The breakdown of the grant funds spent by category is:

Graduate Salaries - \$66,368.03

Hourly Salaries - \$61,105.93

Fringe Benefits - \$22,001.88

Subcontract - \$54,680.00

Supplies - \$67,384.03

Travel - \$10,716.13

Tables and Figures (optional)

Suggestions to CPS (optional)

We enjoyed the close contact with CPS, and all those activities such as attending research symposiums and making industry contacts, which helps us to refine our research approaches in order to develop the effective solutions for produce industry.

Attachment for SCB10055

Developing and validating practical strategies to improve microbial safety in composting process control and handling practices.

Appendix A

Table 1. Thermal inactivation of naturally adapted and outbreak strains of *E. coli* O157:H7 in fresh dairy compost

Temperature	Treatment	Population (log CFU/g) at heating times (h) ¹														
		(-) 48 h	(-) 24 h	0	3	6	24	96	144	168	216	264	288	408	456	504
50	OS ²	7.36±0.14	6.58±0.04	4.31±0.41	NS ³	4.07±0.07	3.15±0.07	NS	NS	(+) ⁴	(+)	(+) ⁵	(+)	(+)	(-)	(-)
	NS ⁶	7.43±0.04	6.93±0.05	4.35±0.07	NS	4.19±0.04	3.15±0.16	NS	NS	(+)	(+)	(+)	(+)	(+)	(+)	(-)
55	OS	7.42±0.06	6.47±0.13	3.73±0.12	3.62±0.20	3.5±0.08	2.41±0.56	NS	NS	(+)	(+)	(-)	(-)			
	NS	7.53±0.06	6.8±0.06	3.8±0.11	3.65±0.12	3.67±0.09	2.53±0.29	NS	NS	(+)	(+)	(+)	(-)			
60	OS	7.26±0.07	6.16±0.13	3.53±0.09	3.6±0.13	3.38±0.22	1.35±0.39	(+)	(-)	(-)						
	NS	7.34±0.08	6.45±0.07	3.72±0.21	3.62±0.34	3.57±0.14	1.69±0.74	(+)	(+)	(-)						

¹ Values are mean ± Std Deviation

² OS, Outbreak strains

³ NS, not sampled

⁴ Positive by enrichment

⁵ Negative by enrichment

⁶ NS, Naturally adapted strains

Table 2. Thermal inactivation of naturally adapted and outbreak strains of *Salmonella* in fresh poultry compost

Temperature	Treatment	Population (log CFU/g) with different heating times (h) ¹																				
		(-) 48h	(-) 24h	0	1	2	3	4	6	24	48	72	96	120	168	264	312	360	408	456	552	600
50 °C	OS ²	7.03 ±0.06	7.80 ±0.15	6.41 ±0.08	ND	6.25 ±0.08	ND	6.22 ±0.06	6.22 ±0.05	5.48 ±0.20	ND	ND	ND	4.88 ±0.33	4.83 ±0.27	3.85 ±0.09		1.41 ±0.45		1.18 ±0.30	(+) ³	(-) ⁴
	NS ⁵	6.90 ±0.17	8.27 ±0.17	7.44 ±0.10	ND	7.31 ±0.03	ND	7.27 ±0.04	7.27 ±0.04	6.77 ±0.15	ND	ND	ND	5.88 ±0.39	5.67 ±0.16	4.75 ±0.15		3.34 ±0.66		2.52 ±0.63	(+)	(+)
55 °C	OS ²	7.03 ±0.08	4.23 ±0.90	3.15 ±0.51	ND	2.95 ±0.57	ND	2.75 ±0.64	2.44 ±0.52	1.83 ±0.59	ND	1.58 ±0.63	ND	(+) ³	(+)	(+)	(-) ⁴	(-)	(-)	(-)	(-)	(-)
	NS ⁵	7.00 ±0.10	5.26 ±1.16	4.54 ±0.72	ND	4.4 ±0.74	ND	4.33 ±0.79	4.27 ±0.80	4.17 ±0.75	ND	3.89 ±1.16	ND	2.55 ±1.66	(+)	2.07 ±0.42	1.12 ±0.35	(+)	(-)	(-)	(-)	(-)
60 °C	OS ²	7.09 ±0.10	3.9 ±1.34	1.86 ±0.93	1.78 ±0.85	1.72 ±0.77	1.37 ±0.41	1.22 ±0.38	(+) ³	(+)	(-) ⁴	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
	NS ⁵	7.06 ±0.09	5.45 ±0.75	3.73 ±1.04	3.37 ±0.76	3.81 ±0.42	3.54 ±0.57	2.69 ±0.45	2.56 ±0.32	2.1 ±0.38	(+)	(+)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)

¹ Values are mean ± Std Deviation

² OS, Outbreak strains

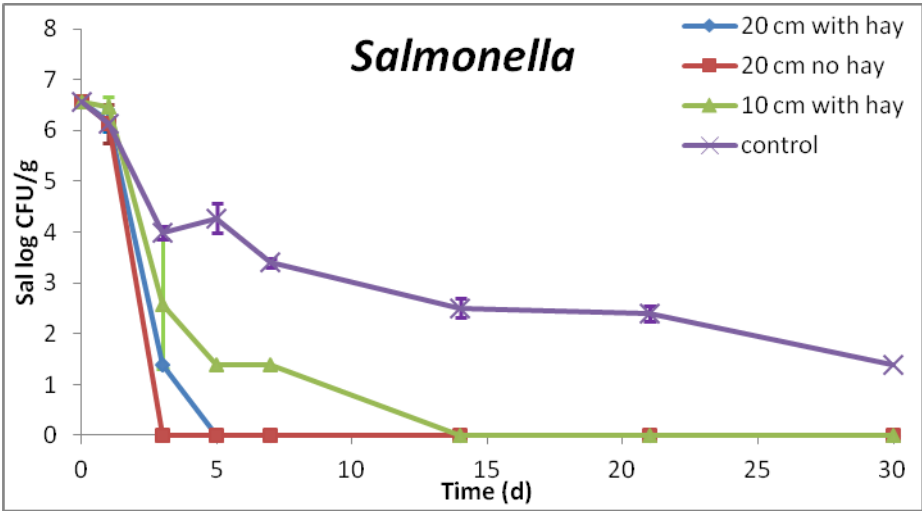
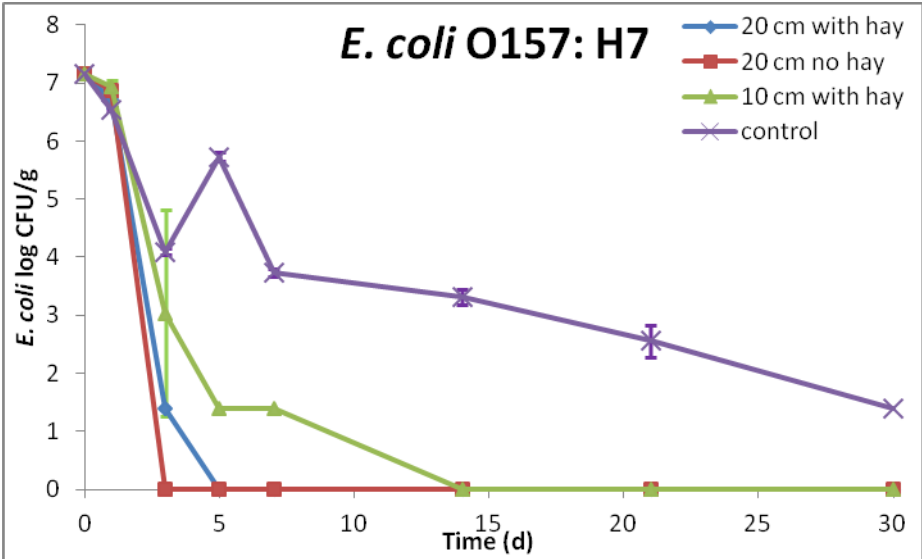
³ Positive by enrichment

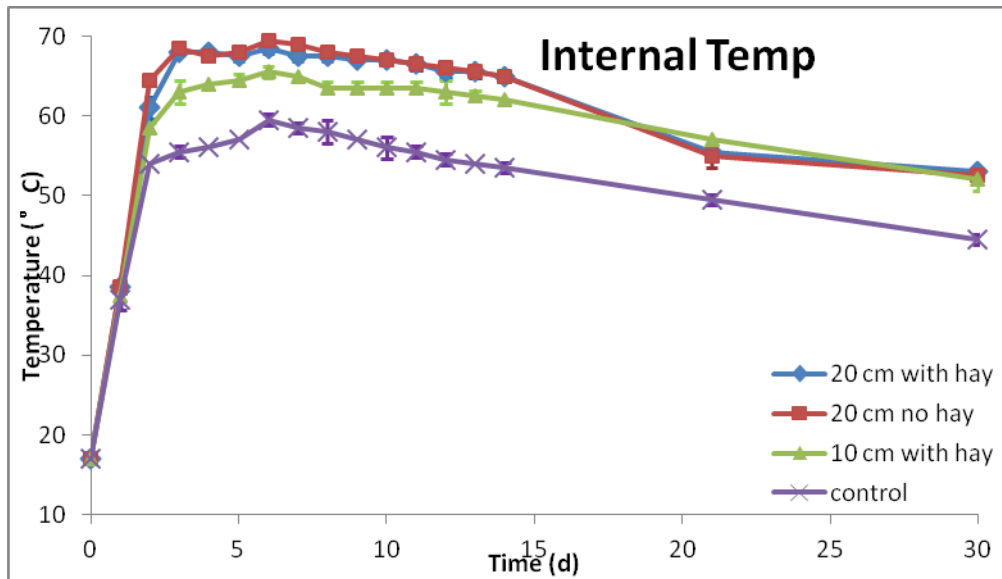
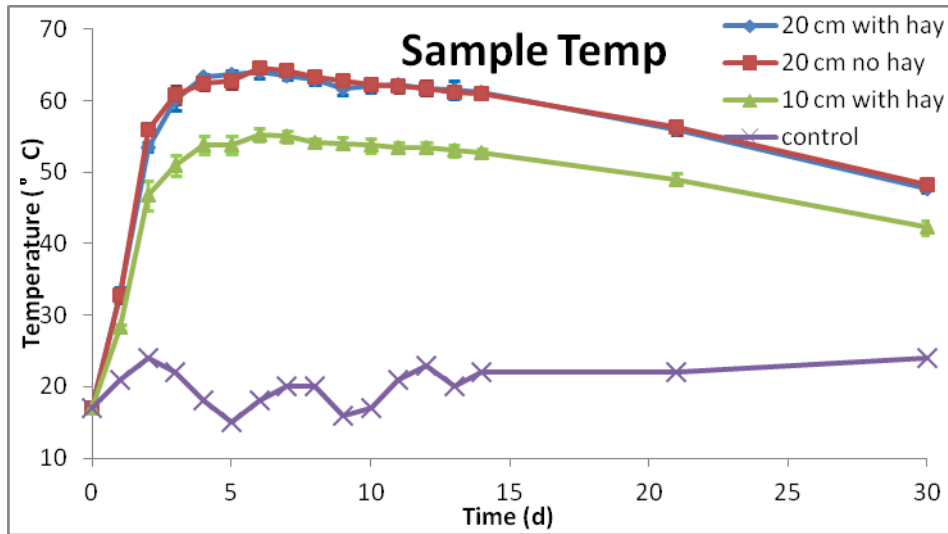
⁴ Negative by enrichment

⁵ NS, Naturally adapted strains

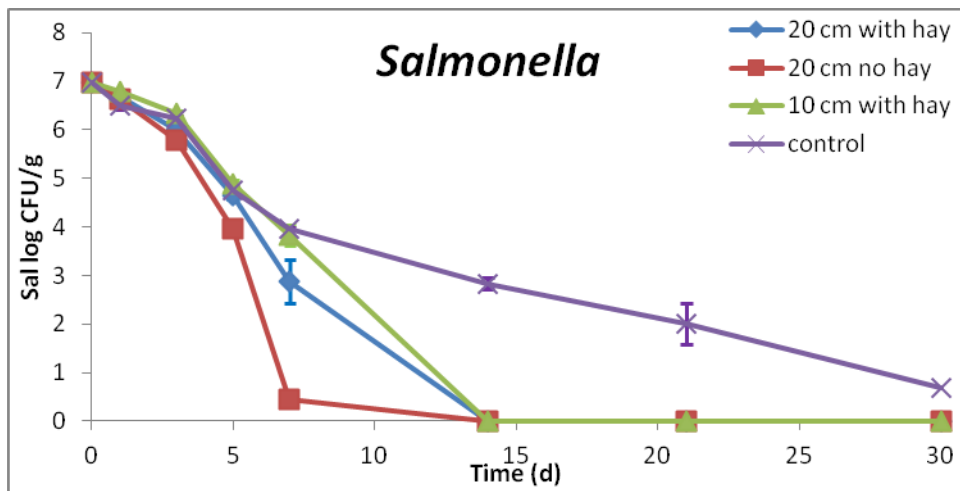
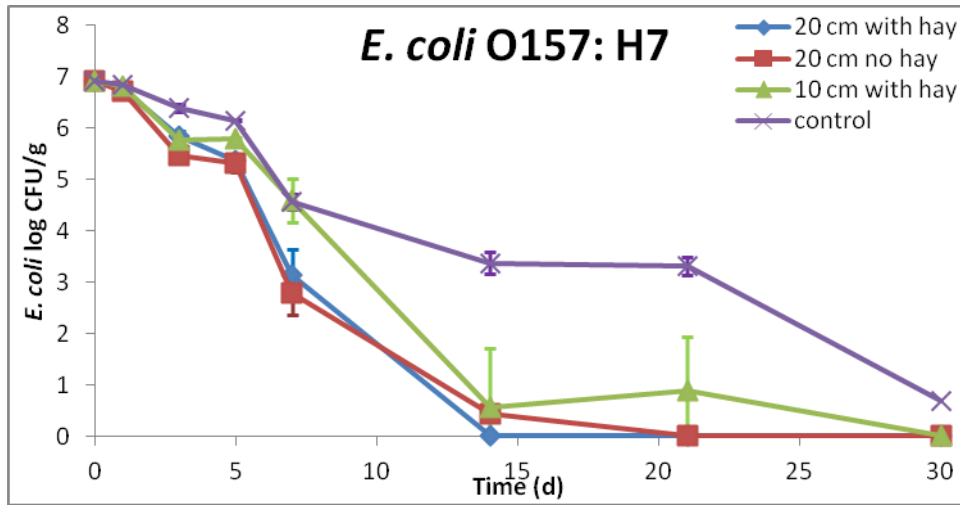
Appendix B

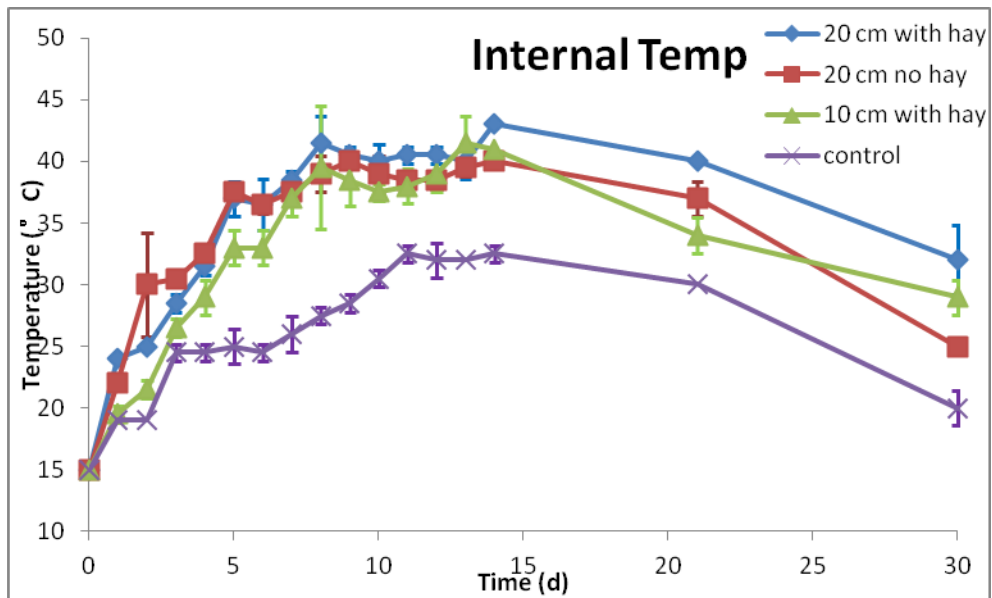
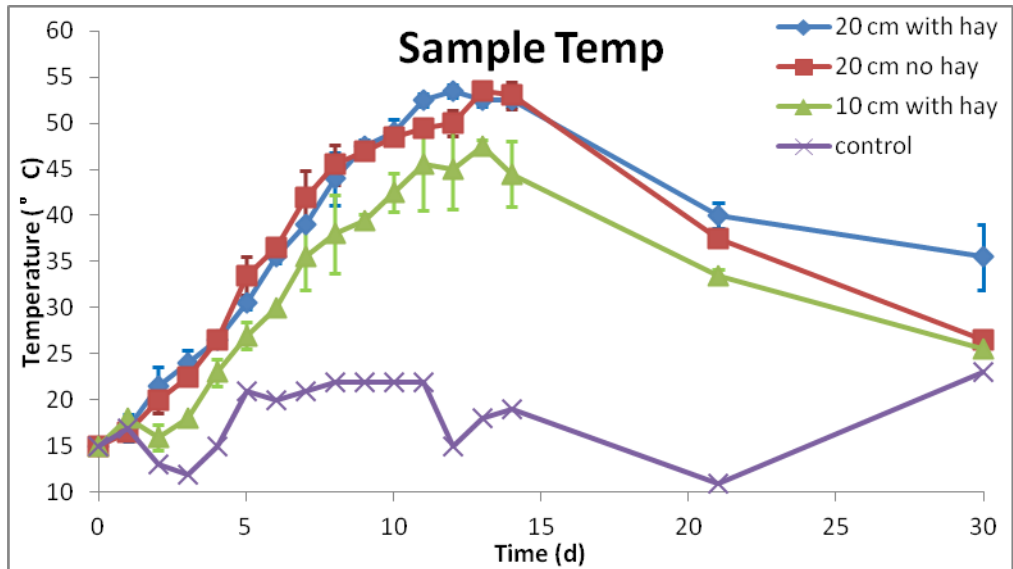
Trial #1



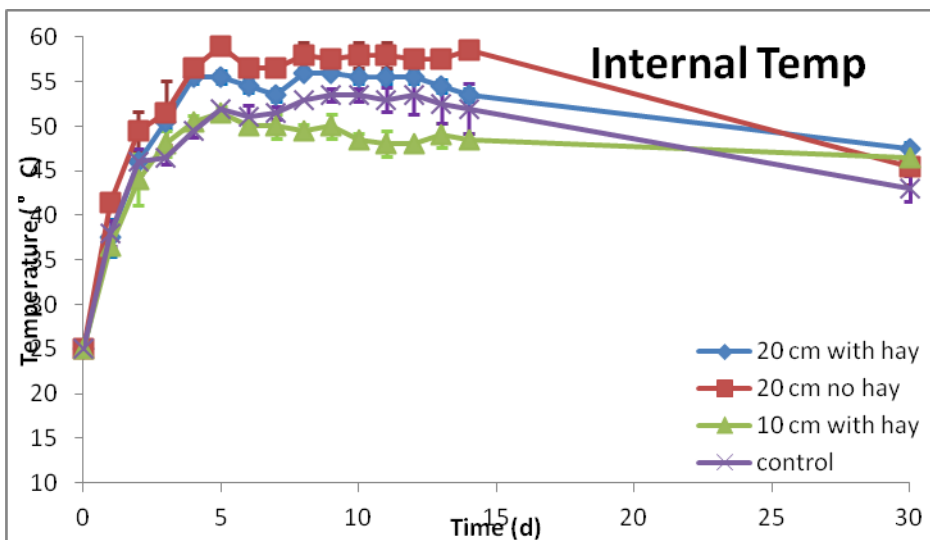
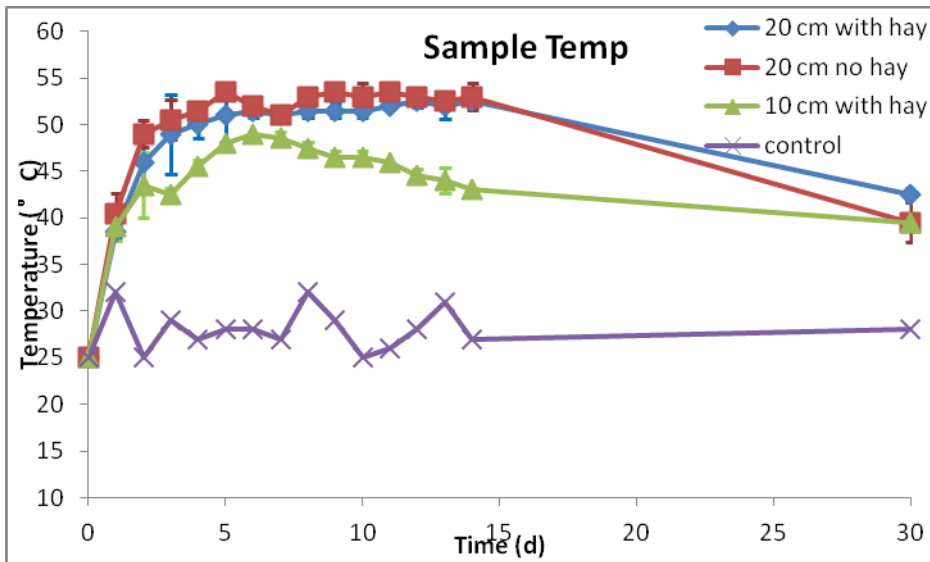
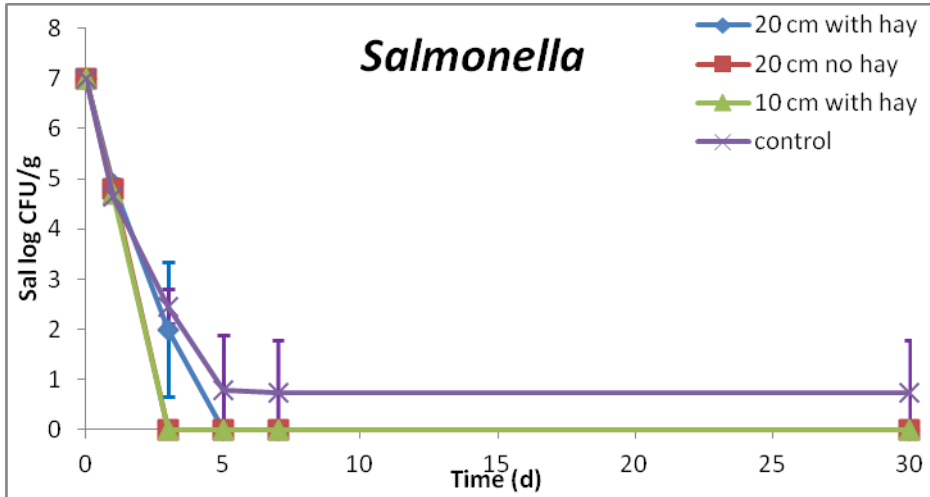


Trial #2





Trial #3



Trial #4

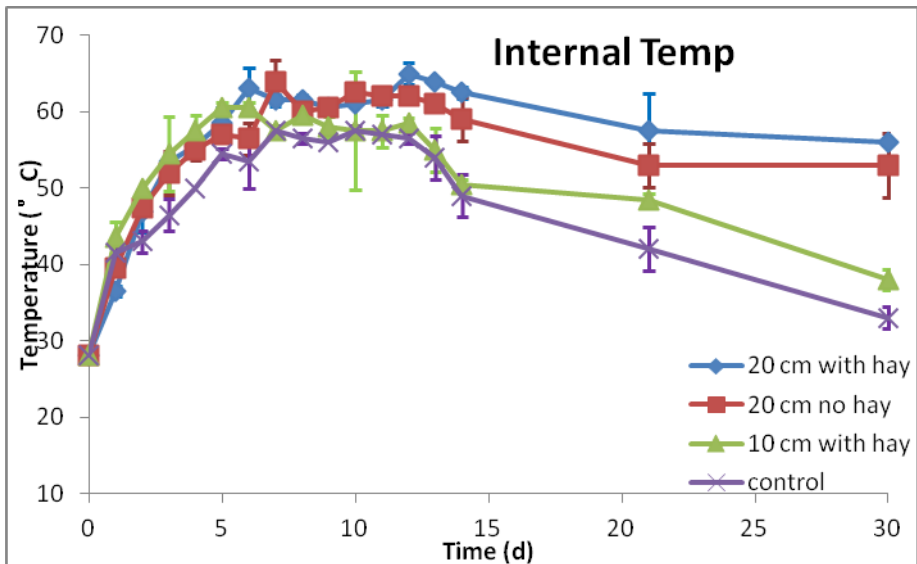
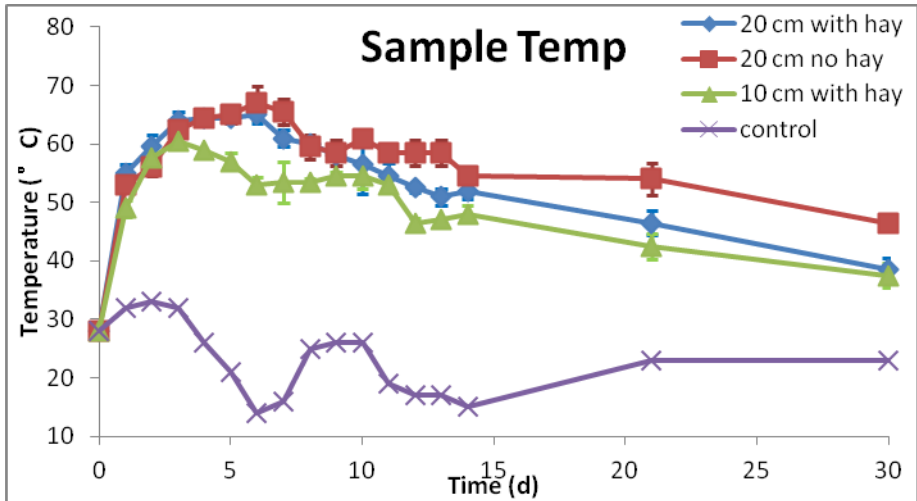
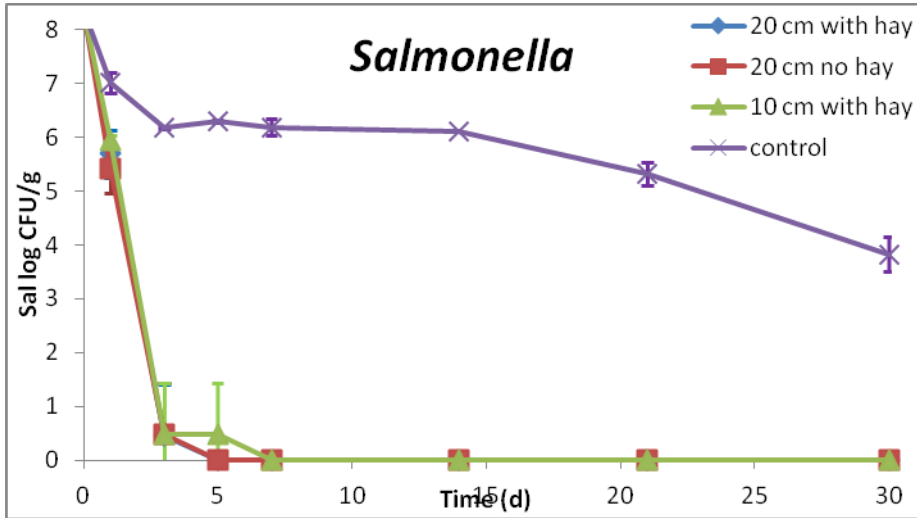


Figure 1: Construction of Static and Windrow piles at BARC, Beltsville, MD



Static pile - base



Static pile



Static piles



Static pile with 30 cm finished compost as cover



Windrow pile with no cover



Windrow pile with finished compost as cover

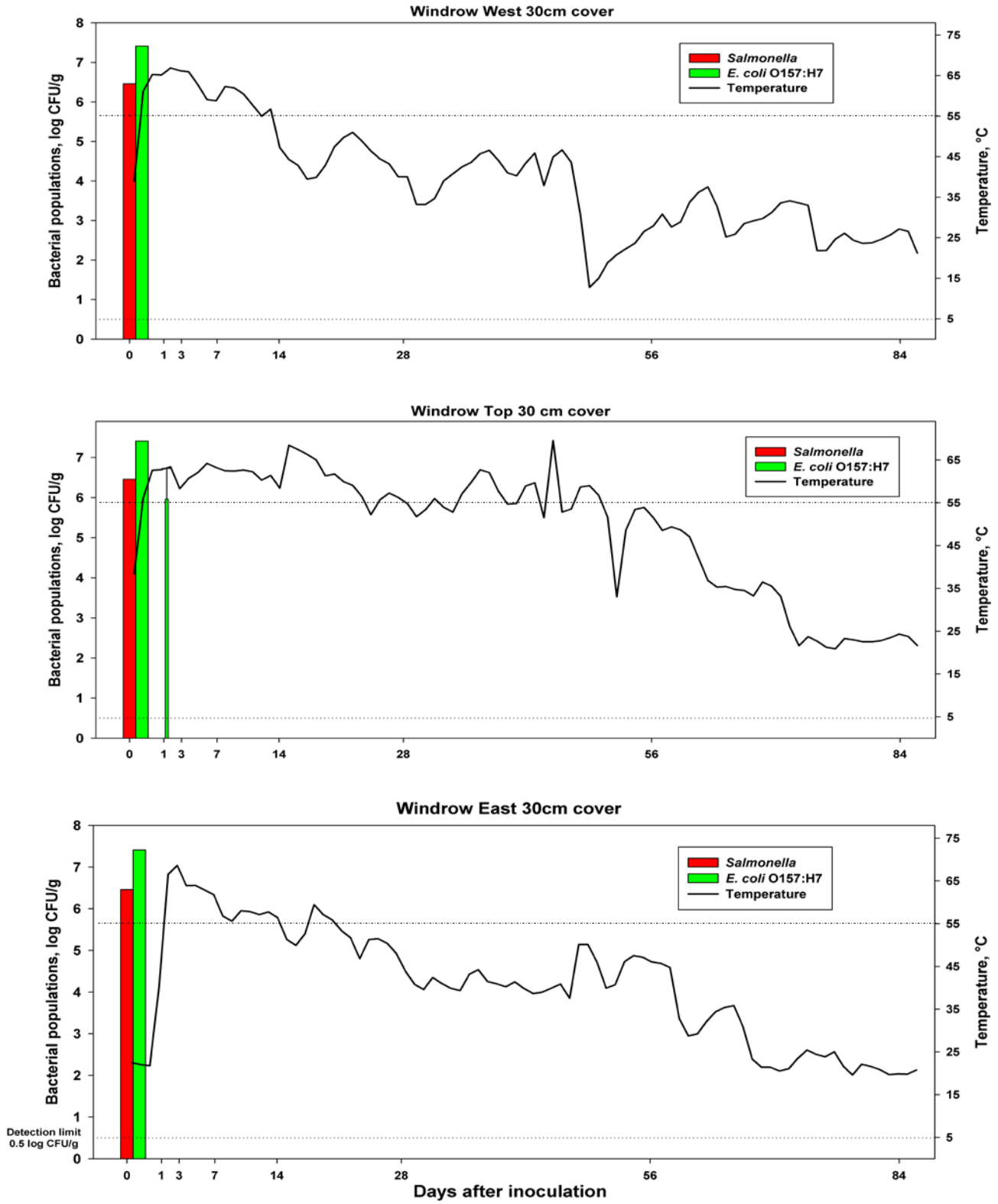


Figure 2. Survival of target pathogens and location-specific temperature profiles during composting process (windrow - 30 cm finished compost as cover).

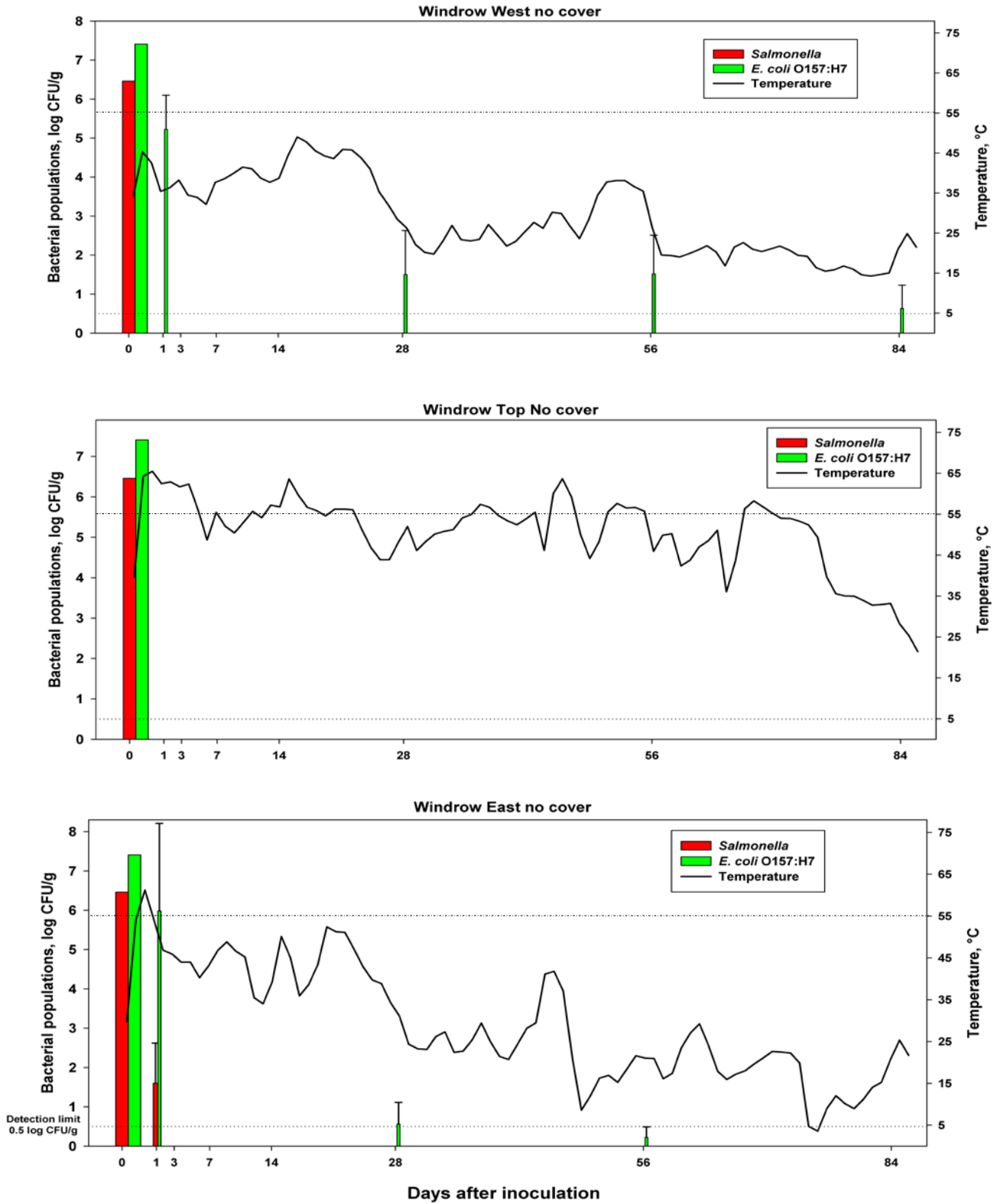


Figure 3. Survival of target pathogens and location-specific temperature profiles during composting process (windrow - no finished compost as cover).

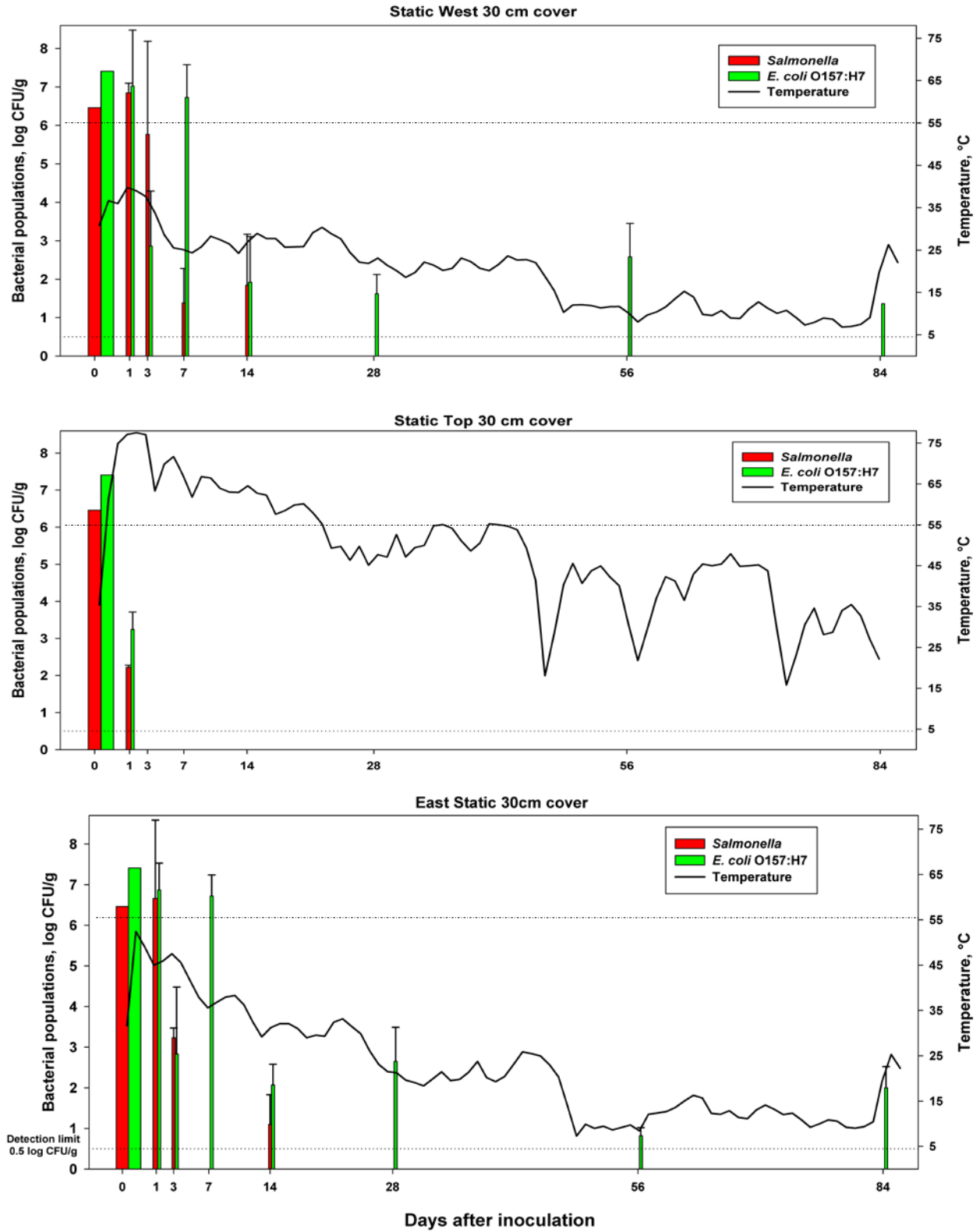


Figure 4. Survival of target pathogens and location-specific temperature profiles during composting process (static - 30 cm finished compost as cover).

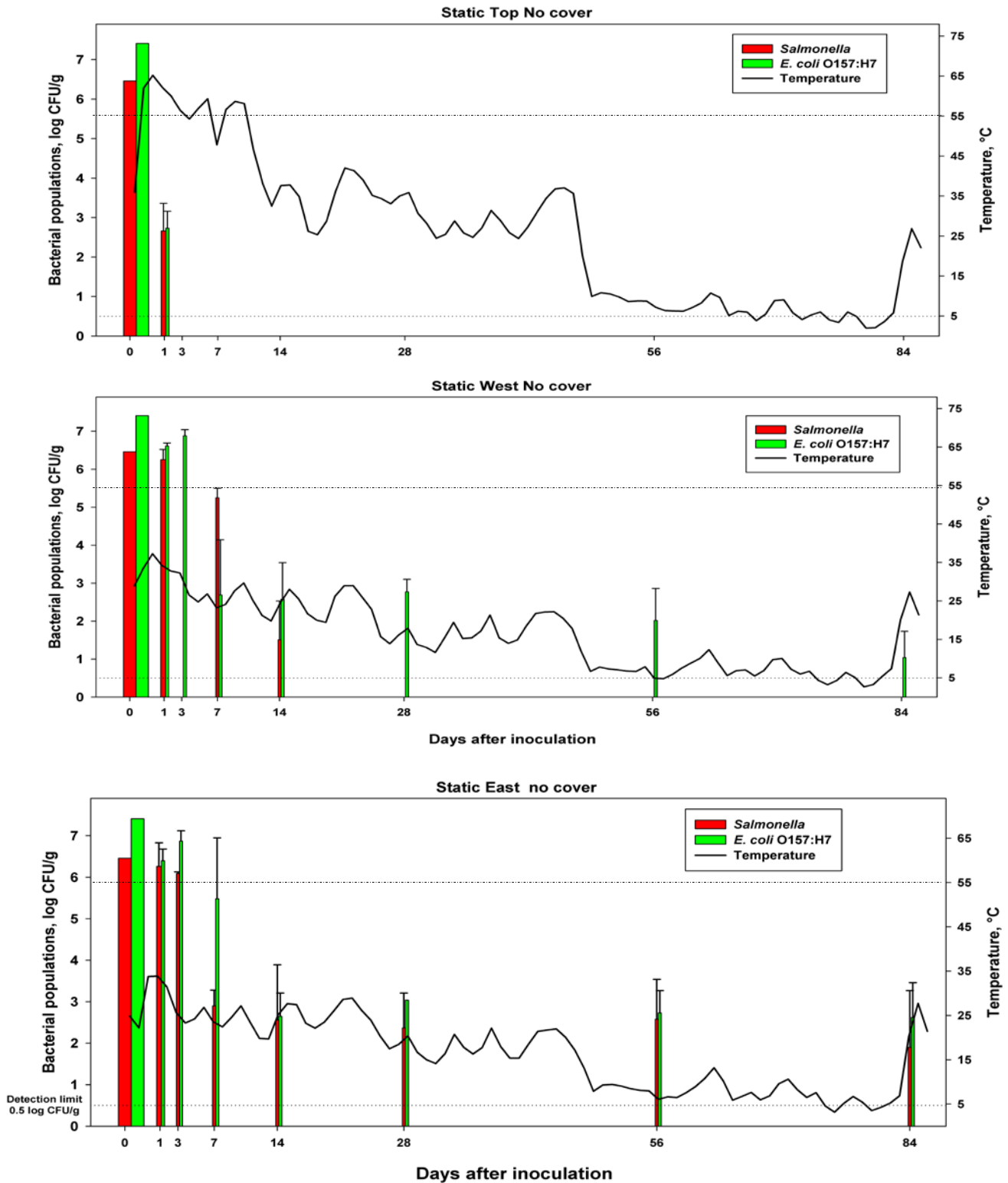


Figure 5. Survival of target pathogens and location-specific temperature profiles during composting process (static - 30 cm finished compost as cover).

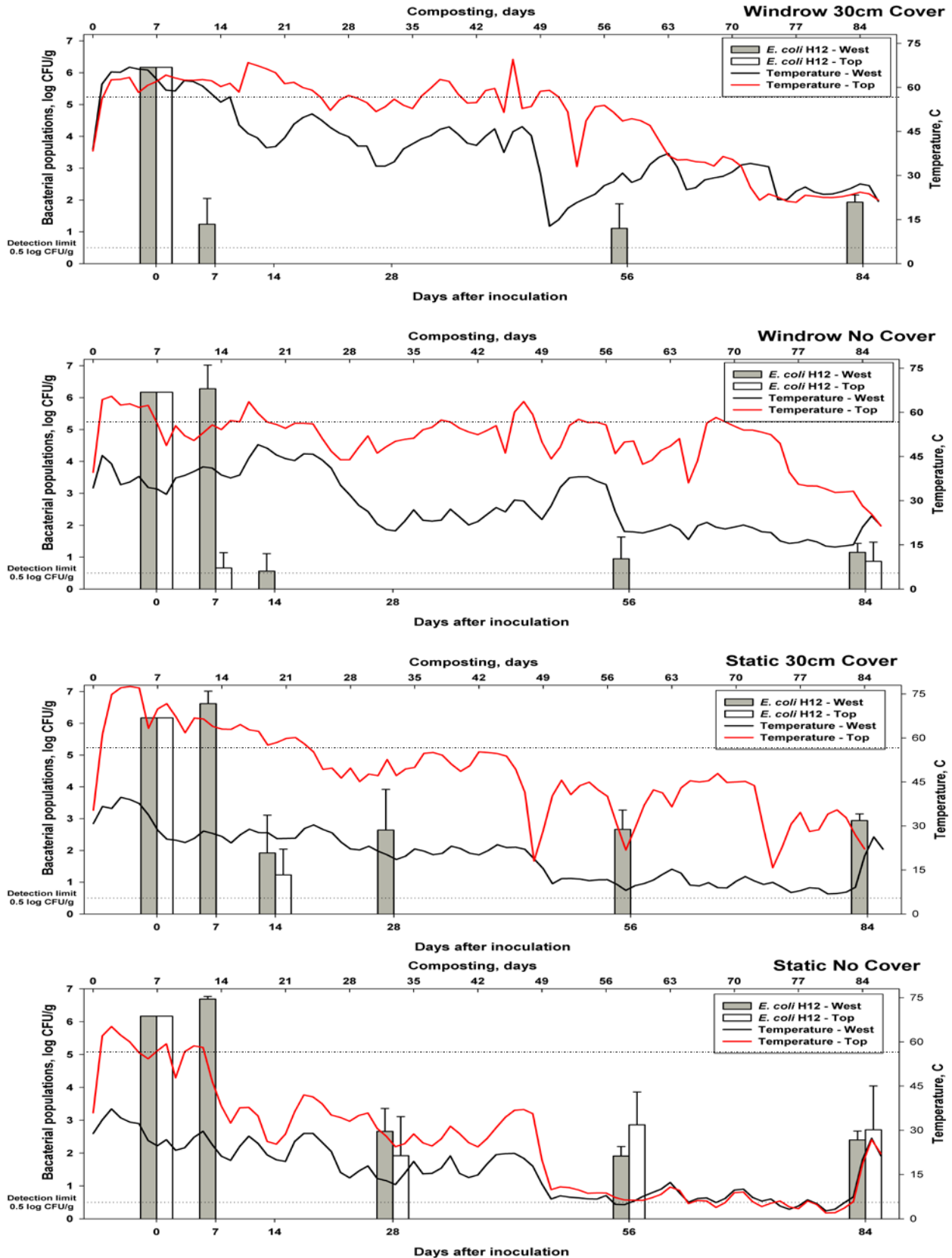


Figure 6. Survival of *E. coli* O157:H12 and location-specific temperature profiles during composting process (**E. coli* O157:H12 was inoculated in 7 day old compost).

Table 1. Mean percentage weed seed germination after timed exposure to moist 55C conditions†

Genus species	Common Name	0 hr	3 hr	6 hr	24 hr
<i>Amaranthus albus</i>	Tumble pigweed	100%	0%	0%	0%
<i>Amaranthus retroflexus</i>	Redroot pigweed	100%	0%	0%	0%
<i>Avena fatua</i>	Wild oats	83%	17%	7%	0%
<i>Chenopodium album</i>	Common lambsquarters	39%	14%	4%	0%
<i>Cirsium arvense</i>	Canada thistle	24%	0%	0%	0%
<i>Echinochloa crus-galli</i>	Barnyardgrass	100%	100%	100%	0%
<i>Eragrostis ciliaris</i>	Stinkgrass	2%	9%	2%	0%
<i>Geranium carolinianum</i>	Carolina geranium	1%	0%	0%	0%
<i>Ipomoea hederacea</i>	Ivy leaf morningglory	97%	93%	86%	0%
<i>Polygonum convolvulus</i>	Wild buckwheat	1%	1%	0%	0%
<i>Polygonum pennsylvanicum</i>	Pennsylvania smartweed	0%	0%	0%	0%
<i>Polygonum persicaria</i>	Ladysthumb	7%	0%	0%	0%
<i>Portulaca oleraceae</i>	Common purslane	69%	18%	18%	0%
<i>Rumex crispus</i>	Curly dock	100%	86%	19%	0%
<i>Rumex obtusifolius</i>	Broadleaf dock	100%	91%	39%	0%
<i>Sesbania exaltata</i>	Hemp sesbania	85%	75%	17%	7%
<i>Setaria viridis</i>	Green foxtail	10%	17%	4%	0%
<i>Solanum nigrum</i>	Black nightshade	0%	0%	0%	0%
<i>Sorghum halpense</i>	Johnsongrass	7%	11%	7%	0%
<i>Thlaspi arvense</i>	Field pennycress	17%	0%	0%	0%
<i>Control –Lycopersicon esculentum cv. Roma</i>	Tomato ‘Roma’	100%	95%	87%	0%

† Mean of three replicates of 20 seeds each per exposure time; see text for details

Table 2: Changes in pH values during the composting

Compost Pile	Cover (cm)	Sample location	Days				
			0	3	28	56	84
Static	0	Top	8.58 ± 0.08	8.68 ± 0.11	8.22 ± 0.15	8.02 ± 0.17	7.28 ± 0.06
	0	West	8.58 ± 0.08	8.82 ± 0.05	8.15 ± 0.41	7.69 ± 0.24	7.39 ± 0.22
	30	Top	8.58 ± 0.08	8.36 ± 0.06	8.11 ± 0.09	7.90 ± 0.16	7.39 ± 0.10
	30	West	8.58 ± 0.08	8.67 ± 0.21	8.27 ± 0.06	7.54 ± 0.04	7.42 ± 0.71
Windrow	0	Top	8.58 ± 0.08	8.71 ± 0.16	8.26 ± 0.13	7.96 ± 0.10	7.45 ± 0.07
	0	West	8.58 ± 0.08	8.63 ± 0.22	8.42 ± 0.38	8.05 ± 0.27	7.56 ± 0.04
	10	Top	8.58 ± 0.08	8.66 ± 0.02	8.28 ± 0.05	8.03 ± 0.01	7.38 ± 0.16
	10	West	8.58 ± 0.08	8.59 ± 0.15	8.17 ± 0.17	7.93 ± 0.13	7.53 ± 0.17
	15	Top	8.58 ± 0.08	8.53 ± 0.05	8.12 ± 0.09	7.87 ± 0.07	7.46 ± 0.07
	15	West	8.58 ± 0.08	8.53 ± 0.06	8.18 ± 0.07	7.88 ± 0.04	7.38 ± 0.06
	30	Top	8.58 ± 0.08	8.60 ± 0.14	8.34 ± 0.02	7.76 ± 0.09	7.57 ± 0.13
	30	West	8.58 ± 0.08	8.60 ± 0.15	8.31 ± 0.06	7.68 ± 0.04	7.48 ± 0.05

Table 4: Change in Carbon:Nitrogen ratio during the composting process

Compost Pile	Cover (cm)	Sample location	Day	
			0	84
Static	0	Top	30.22 ± 1.27	14.96 ± 1.03
	0	West	30.22 ± 1.27	15.27 ± 1.18
	30	Top	30.22 ± 1.27	16.1 ± 0.17
	30	West	30.22 ± 1.27	16.61 ± 0.91
Windrow	0	Top	30.22 ± 1.27	17.29 ± 0.35
	0	West	30.22 ± 1.27	17.27 ± 1.13
	10	Top	30.22 ± 1.27	16.73 ± 0.58
	10	West	30.22 ± 1.27	16.48 ± 1.19
	15	Top	30.22 ± 1.27	16.32 ± 1.27
	15	West	30.22 ± 1.27	16.75 ± 0.94
	30	Top	30.22 ± 1.27	15.29 ± 0.76
	30	West	30.22 ± 1.27	14.95 ± 1

Table 5. Time-temperature exposures for sample locations in static and windrow piles with and without compost cover layers.

Compost Pile	Cover (cm)	Sample location	Max temp (°C)	No. of days \geq 55°C	No. of days \geq 50°C
Static	0	Top	65.2	10	11
	0	West	37.2	0	0
	0	East	33.9	0	0
	30	Top	77.5	27	32
	30	West	39.8	0	0
	30	East	52.4	0	1
Windrow	0	Top	65.5	33	55
	0	West	49.0	0	0
	0	East	61.2	2	7
	30	Top	69.6	40	55
	30	West	66.8	15	16
	30	East	68.6	16	25

Table 6. Time-temperature exposures for sample locations in static and windrow piles with and without compost cover layers for the- *E. coli* O157:H12 study

Compost Pile	Cover (cm)	Sample location	Max temp (°C)	No. of days \geq 55°C	No. of days \geq 50°C
Static	0	Top	59.3	5	5
	0	West	29.7	0	0
	30	Top	71.7	22	28
	30	West	30.4	0	0
Windrow	0	Top	63.7	27	49
	0	West	49.0	0	0
	30	Top	69.6	34	49
	30	West	62.7	9	10

Table 3: Moisture content of compost

Compost Pile	Cover (cm)	Sample location	Days ^x						
			0	3	7	14	28	56	84
Static	0	Top	69.1 ± 0.9a	69.7 ± 4.1a	52.3 ± 10.72 a	33.0 ± 2.8def	15.4 ± 5.0d	16.1 ± 1.1de	20.5 ± 6.5bc
	0	West	69.1 ± 0.9a	59.4 ± 2.0abc	60.7 ± 2.57a	43.5 ± 2.8bcd	36.5 ± 11.8abc	30.6 ± 12.8cde	24.2 ± 11.9bc
	30	Top	69.1 ± 0.9a	66.9 ± 2.3ab	58.7 ± 10.5a	41.2 ± 18.8cde	20.9 ± 6.0cd	17.5 ± 12.4cde	22.56 ± 14.5bc
	30	West	69.1 ± 0.9a	67.0 ± 0.9ab	65.6 ± 2.87a	58.8 ± 9.5abc	53.5 ± 3.6a	45.7 ± 8.1ab	25.6 ± 8.1bc
Windrow	0	Top	69.1 ± 0.9a	63.8 ± 6.5abc	60.7 ± 2.6a	61.6 ± 12.9ab	17.9 ± 11.2d	35.2 ± 14.1bc	25.3 ± 4.6bc
	0	West	69.1 ± 0.9a	65.1 ± 2.4ab	51.1 ± 22.8a	35.8 ± 7.7de	36.5 ± 30.6abc	33.1 ± 26.4bcd	17.3 ± 4.6c
	10	Top	69.1 ± 0.9a	51.1 ± 27.3bc	62.5 ± 3.1a	58.2 ± 16.5abc	26.1 ± 18.5bcd	53.8 ± 4.9a	23.4 ± 15.2bc
	10	West	69.1 ± 0.9a	45.7 ± 17.6c	20.7 ± 5.5b	15.5 ± 5.3f	17.0 ± 5.2d	50.3 ± 4.2ab	19.8 ± 12.6c
	15	Top	69.1 ± 0.9a	68.9 ± 3.8ab	61.1 ± 7.2a	38.4 ± 15.9de	42.6 ± 7.9ab	28.1 ± 4.9cde	30.4 ± 11.4bc
	15	West	69.1 ± 0.9a	62.6 ± 8.0abc	60.4 ± 4.1a	24.4 ± 18.7ef	24.3 ± 4.9bcd	12.90 ± 1.7e	24.0 ± 19.8bc
	30	Top	69.1 ± 0.9a	68.9 ± 4.5ab	64.9 ± 4.9a	61.4 ± 1.6ab	41.6 ± 33.2ab	51.4 ± 22.9ab	38.3 ± 7.2b
	30	West	69.1 ± 0.9a	71.2 ± 4.5a	64.8 ± 5.3a	40.8 ± 12.0cde	29.5 ± 9.5abcd	17.9 ± 7.5cde	61.1 ± 20.2a

^x Values with different letters within the same column are statistically different (P < 0.05)

Table 7: Weed Seed germination rate (%) during composting process

	Location	Cover	Day 0				Day 28				Day 60	Day 90
			Green foxtail	Broadleaf dock	Barnyard grass	Tomato	Green foxtail	Broad leaf dock	Barnyard grass	all seeds	all seeds	
Static	Top	0	95	100	100	50	0	0	0	0	0	0
	West	0	95	100	100	50	60	15	42	0	0	0
	East	0	95	100	100	50	98	82	57	0	0	0
	Top	30	95	100	100	50	0	0	0	0	0	0
	West	30	95	100	100	50	33	0	3	21	0	0
	East	30	95	100	100	50	2	36	57	25	0	0
Windrow	Top	0	95	100	100	50	0	0	0	0	0	0
	West	0	95	100	100	50	0	0	6	6	0	0
	East	0	95	100	100	50	0	0	0	0	0	0
	Top	10	95	100	100	50	0	0	0	0	0	0
	West	10	95	100	100	50	0	0	0	0	0	0
	East	10	95	100	100	50	0	0	0	0	0	0
	Top	15	95	100	100	50	0	0	0	0	0	0
	West	15	95	100	100	50	0	0	0	0	0	0
	East	15	95	100	100	50	0	0	0	0	0	0
	Top	30	95	100	100	50	0	0	0	0	0	0
	West	30	95	100	100	50	0	0	0	0	0	0
	East	30	95	100	100	50	0	0	0	0	0	0

Table 8. Changes in coliform populations (log CFU/g) during composting process

Compost Pile	Cover (cm)	Sample Location	Days							
			0	1	3	7	14	28	56	84
Static	0	East	8.73	6.26	6.96	6.81	2.65	3.04	3.04	2.9
Static	0	Top	8.73	6.34	5.78	2.86	0	0.27	2.28	3.04
Static	0	West	8.73	6.36	6.95	6.84	2.66	2.77	3.04	2.9
Static	30	East	8.73	7.09	7.91	6.79	2.65	2.65	2.88	2.94
Static	30	Top	8.73	6.54	0.66	2.86	1.03	0	0	2.57
Static	30	West	8.73	7.13	6.85	6.79	2.94	2.14	3.04	2.16
Windrow	0	East	8.73	6.06	0	3.04	2.66	1.24	2.88	2.62
Windrow	0	Top	8.73	1.97	0	3.04	1.97	0.56	2.63	2.72
Windrow	0	West	8.73	6.02	0.91	3.04	2.87	1.51	3.04	2.73
Windrow	30	East	8.73	1.17	0	3.04	3.04	1.06	0	2.89
Windrow	30	Top	8.73	6.74	0	2.87	1.62	0	1.17	2.56
Windrow	30	West	8.73	2.56	0	3.04	2.86	0.91	2.57	2.6

Table 9. Changes in generic *E. coli* populations (log CFU/g) during composting process

Compost Pile	Cover (cm)	Sample Location	Days							
			0	1	3	7	14	28	56	84
Static	0	East	5.63	5.42	4.14	3.66	0.06	1.27	0.88	0.5
Static	0	Top	5.63	1.09	0	0	0	0	0	0
Static	0	West	5.63	5.39	5.87	0	0	0	0	0
Static	30	East	5.63	5.39	0	3.12	0	0	0	0
Static	30	Top	5.63	3	0	0	0	0	0	0
Static	30	West	5.63	4.55	2.59	2.95	0	0	0	0

Windrow	0	East	5.63	3.43	0	0	0	0	2.18	0
Windrow	0	Top	5.63	1.65	0	0	0	0	0	0
Windrow	0	West	5.63	0	0	0	0	0	0	0
Windrow	30	East	5.63	0.63	0	0	0	0	0	0
Windrow	30	Top	5.63	0	0	0	0	0	0	0
Windrow	30	West	5.63	1.44	0	0	0	0	0	0

Table 10. Changes in fecal coliform populations (log CFU/g) during composting process

Compost Pile	Cover (cm)	Sample Location	Days							
			0	1	3	7	14	28	56	84
Static	0	East	7.4	7	6.82	5.99	2.67	2.94	3.04	2.64
Static	0	Top	7.4	5.16	0	0	0	0	0	2.87
Static	0	West	7.4	7	6.37	5.8	2.05	2.49	2.23	2.71
Static	30	East	7.4	7.05	2.57	6.07	0.79	2.65	2.64	2.71
Static	30	Top	7.4	6.29	0.66	0.56	0	0	0	2.56
Static	30	West	7.4	6.86	2.59	6.09	1.34	1.94	2.26	1.97
Windrow	0	East	7.4	5.37	0	2.61	2.56	0	2.18	0
Windrow	0	Top	7.4	5.12	0.27	2.66	0.66	0	2.26	0
Windrow	0	West	7.4	5.85	0.56	2.58	2.56	1.41	2.72	2.57
Windrow	30	East	7.22	0	0	0	0.91	0	0	0
Windrow	30	Top	7.4	4.31	0	0	1.62	0	0	0
Windrow	30	West	7.4	1.45	0	0	1.75	0	0	0

Appendix C

Table1. Summary of the compost samples investigated in this study.

ID #	Type of compost	pH	MC(%)	aw	C:N ratio	OM	S	FC	M	T	MA	TA	Fungi	<i>E. coli</i> O157:H7	<i>Salmonella</i>
1	Dairy	6.75	62.18	0.98	13.8	55.99	A/C	5.46	9.33	7.91	8.04	6.10	7.42	NG	NG
		±	±	±	±	±		±	±	±	±	±	±		
		0.06	0.02	0.003	0.27	0.33		0.24	0.13	0.21	0.07	0.14	0.14		
2	Dairy	8.72	37.42	0.99	17.25	17.78	F	0.00	6.91	6.08	5.64	5.56	2.80	NG	0.37 ± 0.09
		±	±	±	±	±		±	±	±	±	±	±		
		0.24	0.31	0.002	1.18	3.99		0.00	0.06	0.39	0.02	0.20	0.35		
3	Poultry	9.21	45.34	0.98	10.12	20.56	f	0.00	7.27	6.52	6.91	6.83	3.58	NG	0.35 ± 0.30
		±	±	±	±	±		±	±	±	±	±	±		
		0.2	0.21	0.013	0.13	2.19		0.00	0.18	0.12	0.02	0.05	0.13		
4	Poultry	6.97	41.68	0.95	14.93	58.67	F	2.27	8.33	6.52	5.71	4.69	6.56	NG	NG
		±	±	±	±	±		±	±	±	±	±	±		
		0.08	0.45	0.013	0.34	0.93		0.27	0.12	0.10	0.15	0.15	0.05		
5	Dairy	8.16	57.26	0.99	13.44	35.65	C	4.08	7.68	2.82	7.64	6.37	5.40	NG	NG
		±	±	±	±	±		±	±	±	±	±	±		
		0.06	2.24	0.001	0.44	1.34		0.10	0.05	0.08	0.08	0.78	0.09		
6	Dairy	8.28	65.36	0.95	12.44	48.02	A/C	2.94	8.48	8.01	8.08	7.15	5.36	NG	NG
		±	±	±	±	±		±	±	±	±	±	±		
		0.18	2.04	0.001	0.18	0.11		0.10	0.04	0.05	0.08	0.09	0.14		
7	Goat	9.48	58.76	0.98	10.46	43.65	C	5.15	6.96	6.95	9.00	6.78	5.89	NG	NG
		±	±	±	±	±		±	±	±	±	±	±		
		0.11	0.69	0.005	0.09	1.06		0.09	0.05	0.11	0.14	0.06	0.19		
8	Poultry	8.62	56.94	0.96	12.61	49.80	A/C	4.20	9.33	7.03	9.40	7.00	7.05	NG	NG
		±	±	±	±	±		±	±	±	±	±0.11	±		
		0.05	0.20	0.009	8.05	2.55		0.48	0.12	0.12	0.05		0.06		
9	Horse	8.79	37.69	0.98	18.74	48.85	C	2.40	7.72	6.38	7.91	6.16	6.01	NG	NG
		±	±	±	±	±		±	±	±	±	±	±		
		0.13	2.03	0.004	0.22	3.04		0.03	0.06	0.08	0.03	0.07	0.22		

10 Goat	8.12	50.58	0.97	12.76	24.60	F	3.71	7.73	6.88	7.71	6.73	5.61	NG	NG
	±	±	±	±	±		±	±	±	±	±	±		
	0.09	0.91	0.004	0.26	1.31		0.07	0.06	0.09	0.09	0.04	0.15		
11 Alpaca compost, straw	7.82	33.54	0.95	11.56	27.25	F	5.17	7.87	7.10	8.04	7.47	5.32	0.29 ±	NG
	±	±	±	±	±		±	±	±	±	±	±	0.13	
	0.09	1.89	0.003	0.18	3.89		0.05	0.03	0.04	0.01	0.09	0.13		
12 Dairy	9.18	26.52	0.94	14.54	27.15	F	1.67	9.01	6.82	8.90	7.43	6.36	NG	NG
	±	±	±	±	±		±	±	±	±	±	±		
	0.16	0.16	0.021	0.37	0.21		0.37	0.03	0.05	0.02	0.07	0.09		
13 Dairy	8.54	44.49	0.96	19.38	35.40	C	2.11	8.50	7.34	8.87	7.02	5.11	NG	NG
	±	±	±	±	±		±	±	±	±	±	±		
	0.18	0.84	0.011	0.23	1.56		0.06	0.13	0.08	0.10	0.09	0.17		
14 Dairy	8.48	64.28	0.93	33.12	46.20	F	2.72	7.13	6.87	6.80	6.84	4.94	NG	NG
	±	±	±	±	±		±	±	±	±	±	±		
	0.21	0.27	0.015	0.22	0.57		0.20	0.10	0.16	0.04	0.05	0.23		
15 Dairy	8.18	29.82	0.91	13.8	47.00	A	4.55	8.96	6.03	8.77	6.53	6.71	0.76 ±	0.57 ± 0.73
	±	±	±	±	±		±	±	±	±	±	±	0.09	
	0.08	2.50	0.020	0.03	1.27		0.37	0.03	0.28	0.04	0.08	0.07		
16 Poultry	7.82	46.19	0.98	88.30	64.50	F	1.69	7.47	5.26	7.01	5.73	6.77	NG	NG
	±	±	±	±	±		±	±	±	±	±	±		
	0.11	0.00	0.004	4.07	1.70		0.49	0.08	0.26	0.09	0.23	0.04		
17 Dairy	9.32	24.28	0.97	38.02	16.75	C	1.65	6.84	5.32	6.81	5.18	3.72	NG	NG
	±	±	±	±	±		±	±	±	±	±	±		
	0.00	0.00	0.001	1.44	2.76		0.15	0.10	0.04	0.01	0.25	0.24		
18 Dairy	9.08	35.95	0.96	20.33	24.70	C	0.00	6.54	5.91	6.57	5.63	0.00	0.14 ±	NG
	±	±	±	±	±		±	±	±	±	±	±	0.08	
	0.04	1.73	0.002	2.29	1.84		0.00	0.04	0.12	0.06	0.12	0.00		
19 Dairy	9.26	38.14	0.97	19.42	26.15	C	0.00	6.69	6.15	6.60	5.87	0.00	NG	NG
	±	±	±	±	±		±	±	±	±	±	±		
	0.28	0.89	0.004	0.52	3.46		0.00	0.08	0.12	0.08	0.17	0.00		
20 Horse	8.24	55.11	0.96	16.16	46.90	F	0.00	6.91	6.70	6.71	6.35	0.00	NG	NG
	±	±	±	±	±		±	±	±	±	±	±		
	0.05	0.56	0.001	0.64	0.00		0.00	0.05	0.04	0.01	0.07	0.00		

21	Plant based	8.78	63.40	0.98	29.76	56.00	C	3.16	8.55	6.80	7.78	6.65	5.89	NG	NG
		±	±	±	±	±		±	±	±	±	±	±		
		0.05	0.40	0.006	1.85	0.57		0.16	0.08	0.06	0.13	0.34	0.17		
22	Plant based	9.16	47.97	0.98	25.98	44.65	F	1.83	7.94	6.90	7.70	6.75	3.58	0.58 ±	0.18 ± 0.02
		±	±	±	±	±		±	±	±	±	±	±	0.11	
		0.11	0.90	0.000	0.71	0.92		0.38	0.04	0.07	0.09	0.05	0.00		
23	Plant based	9.29	38.61	0.97	24.12	40.25	C	2.14	9.05	6.36	8.33	6.60	5.52	0.26 ±	0.45 ± 0.06
		±	±	±	±	±		±	±	±	±	±	±	0.01	
		0.13	1.55	0.001	0.49	0.92		0.14	0.11	0.03	0.12	0.17	0.15		
24	Plant based	9.26	53.35	0.98	27.48	49.75	C	0.00	7.39	6.59	7.37	6.75	4.32	0.75 ±	0.31 ± 0.04
		±	±	±	±	±		±	±	±	±	±	±	0.04	
		0.03	0.37	0.002	0.98	2.19		0.00	0.05	0.07	0.08	0.11	0.18		
25	Dairy	9.38	63.96	0.94	12.54	48.15	C	2.68	7.54	6.65	7.54	7.13	4.32	NG	NG
		±	±	±	±	±		±	±	±	±	±	±		
		0.02	0.27	0.004	0.73	0.78		0.20	0.10	0.07	0.18	0.18	0.43		
26	Leaf based	9.41	59.58	0.96	20.60	29.65	C	2.99	7.34	6.55	7.12	6.17	4.76	NG	NG
		±	±	±	±	±		±	±	±	±	±	±		
		0.06	4.32	0.004	0.29	1.34		0.10	0.10	0.08	0.17	0.15	0.13		
27	Crab	9.20	42.91	0.95	14.68	15.55	F	1.78	7.93	6.24	8.61	7.16	4.11	NG	NG
		±	±	±	±	±		±	±	±	±	±	±		
		0.11	0.18	0.014	0.64	3.75		0.09	0.11	0.11	0.20	0.36	0.11		
28	Poultry	8.15	20.76	0.84	10.22	28.90	F	2.91	7.41	5.68	7.51	5.90	4.11	NG	NG
		±	±	±	±	±		±	±	±	±	±	±		
		0.04	0.00	0.005	0.15	0.28		0.07	0.11	0.20	0.38	0.22	0.11		
29	Poultry	7.67	30.12	0.88	11.19	50.25	F	2.21	7.68	6.88	8.01	6.91	4.79	NG	NG
		±	±	±	±	±		±	±	±	±	±	±		
		0.01	0.00	0.024	0.07	2.47		0.92	0.10	0.15	0.29	0.15	0.10		
30	Green waste, mushroom mulch	9.73	36.39	0.94	14.42	48.65	A	0.00	8.83	7.43	8.55	6.88	5.93	NG	NG
		±	±	±	±	±		±	±	±	±	±	±		
		0.01	0.00	0.012	0.21	5.02		0.00	0.06	0.04	0.10	0.12	0.07		
31	Food waste,	6.68	20.09	0.82	16.69	56.25	F	0.00	5.21	5.71	5.31	5.37	5.26	NG	NG
		±	±	±	±	±		±	±	±	±	±	±		

green waste	0.16	0.00	0.000	0.04	5.44	0.00	0.35	0.12	0.21	0.14	0.31
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A number of 31 compost samples of different origin were determined pH, moisture content (MC%) water activity (a_w), C:N ratio, organic matter (OM), Solvita test (S), fecal coliform (FC), total mesophiles (M), total thermophiles (T), meso-actinomycetes (MA), thermo-actinomycetes (TA) and fungi. Concomitantly these samples were inoculated with a cocktail of *E. coli* O157:H7 and *Salmonella* respectively and monitored for growth/no growth (NG). Values indicate averages of triplicates and their standard deviations.

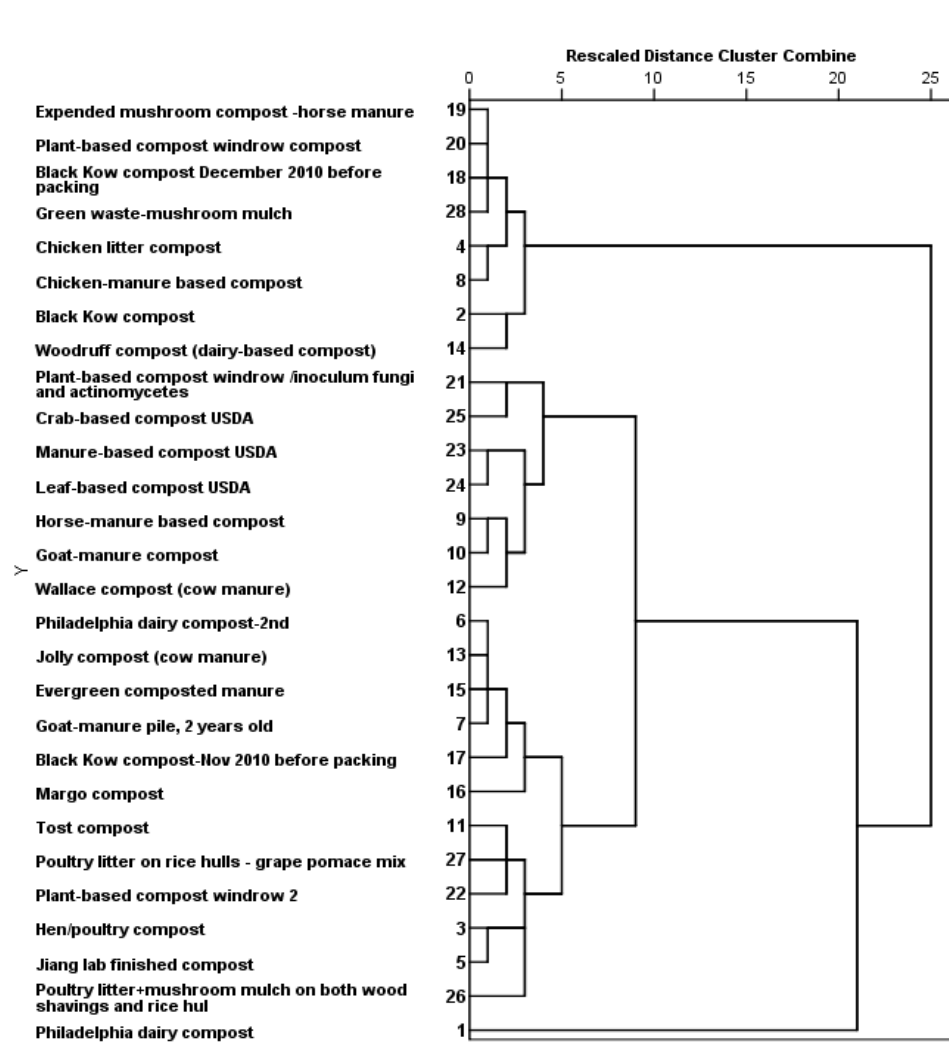


Figure 1. Cluster analysis on metabolic activity of microbial communities associated with the analyzed compost samples.

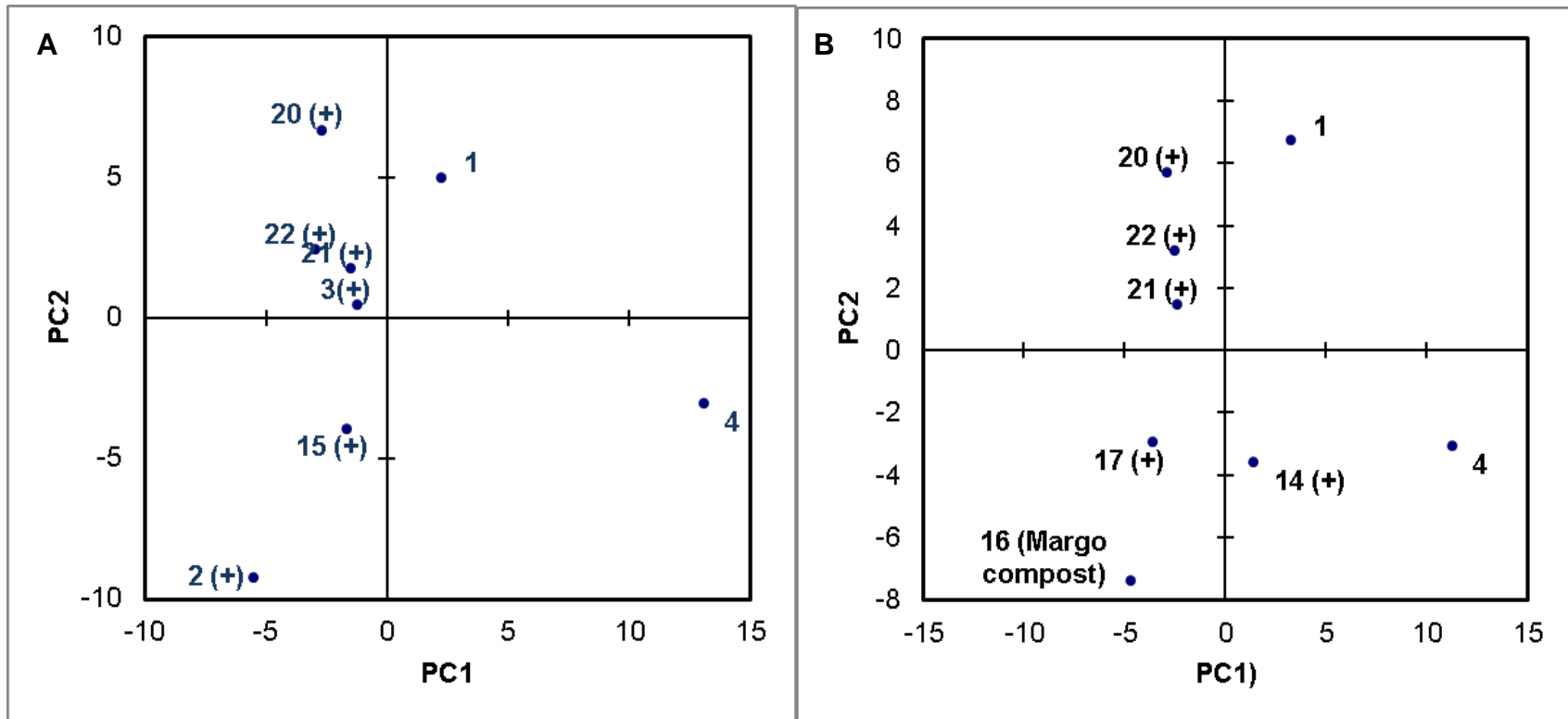


Figure 2. Principal component analysis on microbial metabolism for elected samples. **(A)** Analysis of finished compost samples that allowed *Salmonella* spp. regrowth {2(+)-Black Kow compost, 3(+)-hen/poultry compost, 15(+)-Woodruff compost, 20(+)-Plant-based

compost windrow plus inoculum, 21 (+)Plant-based compost windrow, 22(+) Plant-based compost static pile} and for comparison purposes {1-Philadelphia dairy compost and 4-Chicken litter compost}. **(B)** Analysis of finished compost samples that allowed *E. coli* O157:H regrowth {14(+) Woodruff compost, 17(+)Black Kow compost before packing, 20(+)Plant-based compost windrow plus inoculum, 21 (+)Plant-based compost windrow, 22(+)Plant-based compost static pile} and for comparison purposes {1-Philadelphia dairy compost and 4-Chicken litter compost}.

Appendix D

Fig.1 Greenhouse temperature, relative humidity and light intensity in a typical day

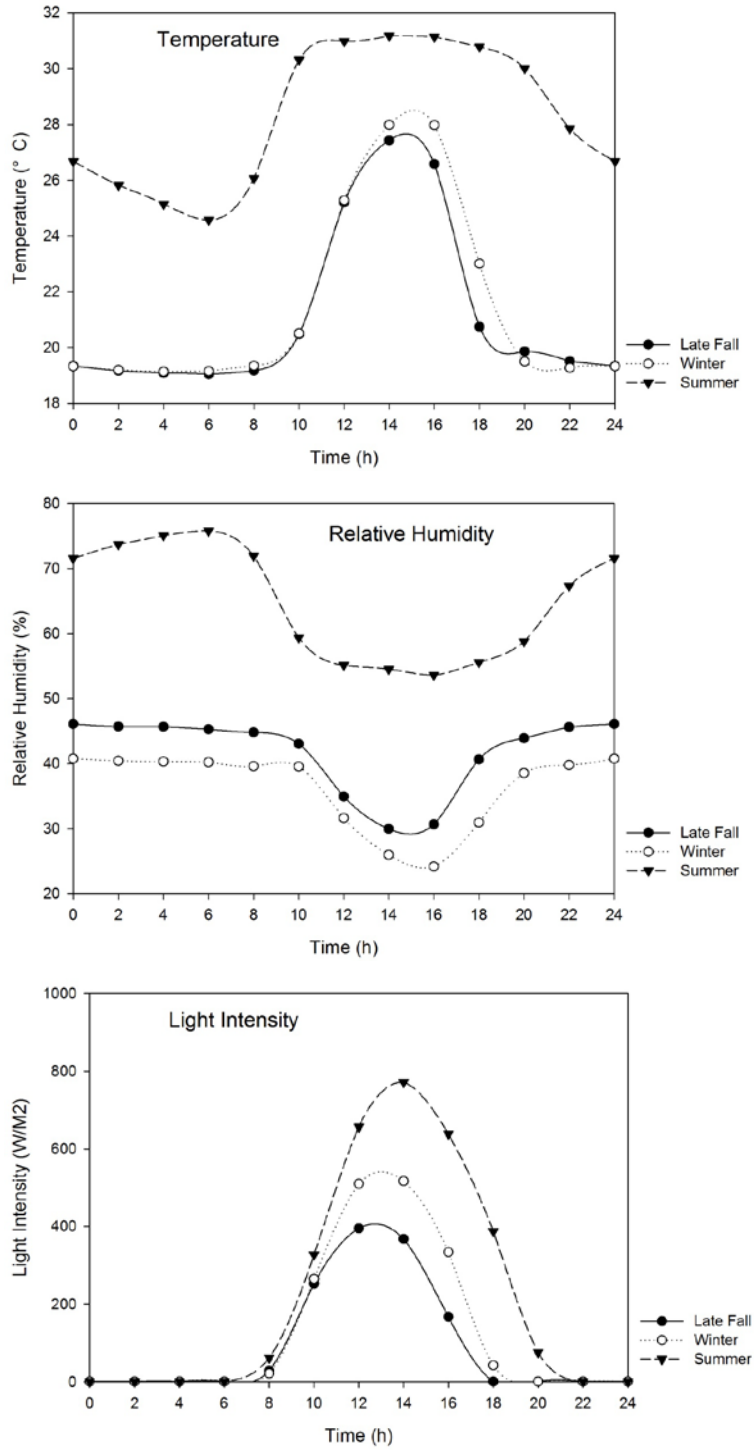


Table 1. Pathogen reduction and dehydration rate of compost samples as affected by particle sizes, initial moisture and seasonality

Trial	Initial MC	Particle size	log reduction in first 5 days		Dehydration rate in 5 (3) days (%/d) ***		
			<i>Salmonella</i>	<i>E. coli</i> O157			
Late fall	40%	>1000 µm	3.19±0.22a*	NT**	6.68****		
		<1000 µm	3.16±0.19a	NT	7.39		
		30%	>1000 µm	1.80±0.09bc	NT	4.02	
	20%	500-1000	2.35±0.34ab	NT	5.26		
		<500 µm	2.74±0.23a	NT	5.50		
		>1000 µm	1.39±0.12c	NT	2.43		
		500-1000	1.70±0.02bc	NT	3.14		
		<500 µm	1.78±0.17bc	NT	3.45		
		40%	>1000 µm	2.93±0.37ab	2.20±0.15b	6.44 (10.25)	
Winter	40%	<1000 µm	3.36±0.06ab	2.84±0.21ab	6.81 (10.78)		
		30%	>1000 µm	2.33±0.22b	1.28±0.05b\c	4.48 (7.60)	
		500-1000	3.23±0.33ab	2.05±0.09bc	4.99 (8.04)		
	20%	<500 µm	3.68±0.09a	2.68±0.09ab	4.90 (7.93)		
		>1000 µm	2.88±0.21ab	2.11±0.14b	2.49 (3.64)		
		500-1000	3.42±0.85ab	3.07±0.24a	3.03 (4.75)		
		<500 µm	3.66±1.19a	3.11±0.28a	3.07 (5.12)		
		Summer	40%	>1000 µm	2.14±0.07a	2.08±0.08a	3.10
				<1000 µm	3.17±0.02a	2.48±0.03a	6.16
30%	>1000 µm			2.35±1.20a	2.14±0.44a	1.69	
20%	500-1000		2.61±1.13a	2.40±0.51a	3.82		
	<500 µm		2.84±0.62a	2.54±0.38a	4.70		
	>1000 µm		1.95±1.18a	1.82±0.75a	-0.23		
	500-1000		1.93±1.12a	2.19±0.51a	1.51		
	<500 µm		2.10±1.08a	2.53±0.46a	2.62		

*Data are expressed as means±SD of two trials. Means with different letters in the same column within each season are significantly different (P < 0.05) according to the LSD test.

**NT, not tested.

***In winter trial only, the dehydration rate is significantly different between day 3 and 5.

****Dehydration rate is expressed as moisture content reduction/days of storage.

Table 2. Pathogen reduction in 3 trials as affected by MC and particle size of compost

Trial	Initial MC**	Particle size	log reduction in 30 days	
			<i>S. Typhimurium</i>	<i>E. coli</i> O157
Late fall	40%	>1000 µm	3.92±0.70ab*	NT***
		<1000 µm	4.57±0.98a	NT
	30%	>1000 µm	2.66±0.00cde	NT
		500-1000 µm	3.17±0.29bcd	NT
		<500 µm	3.47±0.06abc	NT
	20%	>1000 µm	1.80±0.32e	NT
		500-1000 µm	2.18±0.51de	NT
		<500 µm	2.34±0.10de	NT
	Winter	40%	>1000 µm	4.70±0.98ab
<1000 µm			4.88±0.57ab	3.55±0.93ab
30%		>1000 µm	2.98±0.32c	1.95±0.03c
		500-1000 µm	4.50±1.03bc	2.68±0.49bc
		<500 µm	5.04±0.92ab	3.69±0.47ab
20%		>1000 µm	3.90±0.16bc	2.15±0.24bc
		500-1000 µm	5.23±0.49ab	3.09±0.29a
		<500 µm	6.10±0.10a	3.19±0.12a
Summer		40%	>1000 µm	3.55±0.57a
	<1000 µm		4.07±0.42a	3.72±0.33a
	30%	>1000 µm	3.08±1.29a	3.04±0.61a
		500-1000 µm	3.27±1.42a	3.00±0.68a
		<500 µm	3.55±0.99a	3.27±0.58a
	20%	>1000 µm	2.69±1.10a	2.59±1.20a
		500-1000 µm	2.65±0.88a	3.11±1.11a
		<500 µm	2.76±0.78a	3.52±0.99a

*Data are expressed as means±SD of two trials. Means with different letters in the same column within each season are significantly different ($P < 0.05$) according to the LSD test.

**Initial moisture content, which is not adjusted during the storage.

***NT, not tested.

Table 3. The 3D Optical Profile analysis of Compost Samples with Different Particle sizes

Sample size	Sample No.	Ra (μm)*	Area (μm^2)	Diameter (μm)**	volume (μm^3)
>1000 μm	1	142.80	1.35×10^6	1965	7.35×10^8
	2	77.79	1.27×10^6	2022	5.98×10^8
	3	117.38	9.60×10^5	2123	4.24×10^8
	ave	112.66 ± 32.76	$1.19 \times 10^6 \pm 2.04 \times 10^5$	2037 ± 80	$5.85 \times 10^8 \pm 1.56 \times 10^8$
500-1000 μm	1	63.99	2.21×10^5	853	4.33×10^7
	2	72.12	3.03×10^5	864	7.09×10^7
	3	93.73	3.49×10^5	934	1.15×10^8
	ave	76.61 ± 15.37	$2.91 \times 10^5 \pm 6.48 \times 10^4$	884 ± 44	$7.63 \times 10^7 \pm 3.60 \times 10^7$
<500 μm	1	19.44	3.20×10^4	267	2.26×10^6
	2	14.70	8.00×10^3	122	4.60×10^5
	3	45.20	7.10×10^4	382	1.04×10^7
	ave	26.45 ± 16.41	$3.70 \times 10^4 \pm 3.18 \times 10^4$	257 ± 130	$4.37 \times 10^6 \pm 5.29 \times 10^6$

*Ra, represents the surface roughness. It is the arithmetic average of the absolute values of height difference.

**Diameter is the average measurement of particle length.

Fig 2. Background subtracted images of GFP-labeled *E. coli* O157: H7 in compost sample detected by Qdots.

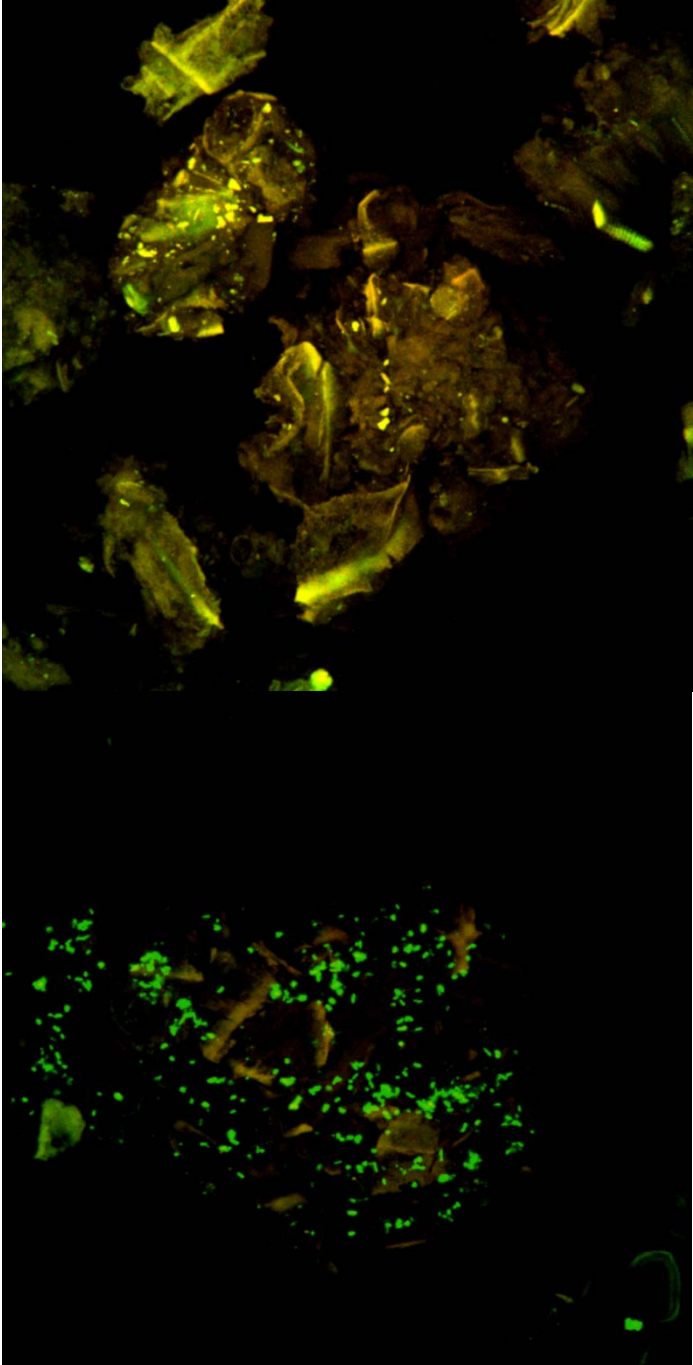


Table 4. Strain identification based on 16S rRNA used as CE against *E. coli* O157:H7 in this study and their inhibitory activity in laboratory conditions

Isolate identification based on 16S rRNA	<i>E. coli</i> O157:H7 log reduction	
	Liquid (TSB)	Compost (stored in laboratory conditions)
unclassified <i>Comamonadaceae</i>	2.45±0.15	2.14 ±0.25
unclassified <i>Enterobacteriaceae</i>	1.63±0.47	1.06 ±0.33
genus <i>Comamonas</i>	1.38 ±0.26	1.44 ±0.28
genus <i>Kluyvera</i>	3.92 ±0.38	3.72 ±0.41
genus <i>Raoultella</i>	1.89 ±0.19	1.77 ±0.32
uncultured bacterium <i>Citrobacter</i> spp.	1.13 ±0.29	1.15 ±0.47
genus <i>Enterobacter</i>		
uncultured bacterium	1.75 ±0.34	1.44 ±0.35
<i>Comamonas testosteroni</i> CNB-2	1.75 ±0.17	0.89 ±0.05
family <i>Nannocystaceae</i> uncultured proteobacterium	1.97 ±0.28	2.36 ±0.44
genus <i>Enterobacter</i>		
uncultured bacterium	2.15 ±0.51	1.93 ±0.27

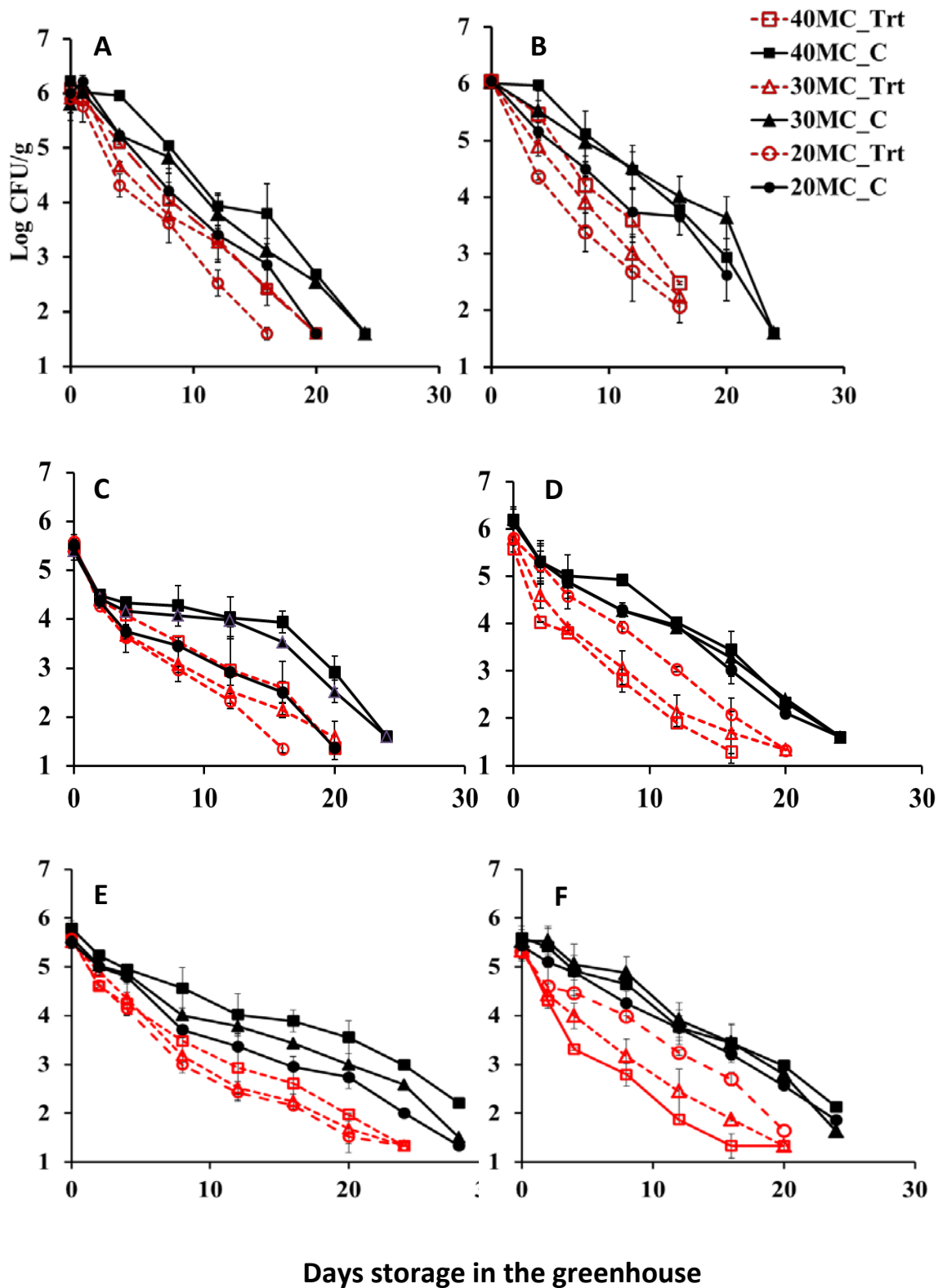


Figure 3. Inhibition of *E. coli* O157:H7 in the presence of CE in the greenhouse conditions. (A) Summer trial heat-adapted cells; (B) Summer trial non-adapted cells; (C) Fall trial heat-adapted; (D) Fall trial non-adapted; (E) Winter trial heat-adapted and (F) Winter trial non-adapted cells.

Table 5. Inhibition of *E. coli* O157: H7 cocktail in the presence of CE compost microflora (Inoculation method simulated a possible recontamination event- Approach #1)

Season	MC (%)	Time 0		48h		96 h	
		Trt	Control	Trt	Control	Trt	Control
Summer	40	6.04 ± 0.07 (Aa*)	6.00 ± 0.11 (Aa*)	ND	ND	5.46 ± 0.23 (Aa*)	5.96 ± 0.09 (Aa*)
	30	6.03 ± 0.08 (Aa*)	6.03 ± 0.15 (Aa*)	ND	ND	4.89 ± 0.16 (Aa*)	5.52 ± 0.48 (Aa*)
	20	6.05 ± 0.03 (Aa*)	6.04 ± 0.10 (Aa*)	ND	ND	4.35 ± 0.05 (Aa*)	5.15 ± 0.43 (Aa*)
Fall	40	5.45 ± 0.05 (Aa*)	5.59 ± 0.02 (Aa*)	4.32 ± 0.07 (Ba*)	5.41 ± 0.07 (Aa*)	3.31 ± 0.07 (Bb*)	4.81 ± 0.07 (Ab*)
	30	5.34 ± 0.09 (Aa*)	5.53 ± 0.05 (Aa*)	4.43 ± 0.07 (Ba*)	5.52 ± 0.07 (Aa*)	3.79 ± 0.07 (Bb*)	5.05 ± 0.07 (Aa*)
	20	5.37 ± 0.44 (Aa*)	5.43 ± 0.28 (Aa*)	4.30 ± 0.07 (Aa*)	5.08 ± 0.33 (Aa*)	4.36 ± 0.12 (Aa*)	4.99 ± 0.22 (Aa*)
Winter	40	5.45 ± 0.24 (Aa*)	5.59 ± 0.55 (Aa*)	4.30 ± 0.44 (Ba*)	5.41 ± 0.24 (Aa*)	3.31 ± 0.05 (Bb*)	4.91 ± 0.57 (Ab*)
	30	5.34 ± 0.32 (Aa*)	5.53 ± 0.45 (Aa*)	4.41 ± 0.44 (Ba*)	5.61 ± 0.11 (Aa*)	3.81 ± 0.41 (Bb*-**)	5.06 ± 0.14 (Aa*)
	20	5.30 ± 0.14 (Aa*)	5.43 ± 0.64 (Aa*)	4.60 ± 0.44 (Aa*)	5.00 ± 0.52 (Aa*)	4.47 ± 0.36 (Aa*)	4.89 ± 0.12 (Aa*)

Samples consisted of composts with established moisture content (MC) levels. Means of treatment and controls are compared, within each combination numbers followed by different capital letters are significantly different ($P < 0.05$), different small letters indicate significant differences between seasons for the same conditions (treatment, MC) and star symbol indicate comparison between moisture levels. For the data points at 48 h factors season and moisture levels included for analysis only Fall and Winter trials.

Table 6. Inhibition of *E. coli* O157: H7 cocktail in the presence of CE compost microflora (Inoculation method simulated the possibility that the pathogen survived composting process Approach #2)

Season	MC (%)	Time 0		48h		96 h	
		Trt	Control	Trt	Control	Trt	Control
Summer	40	5.88 ± 0.14 (Aa*)	6.02 ± 0.08 (Aa*)	ND	ND	5.08 ± 0.30 (Aa*)	5.95± 0.08 (Aa*)
	30	6.04 ± 0.03 (Aa*)	6.02 ± 0.07 (Aa δ)	ND	ND	4.66 ± 0.08 (Aa*)	5.23 ± 0.9(Aa*)
	20	5.76 ± 0.29 (Aa*)	6.21± 0.11 (Aa*)	ND	ND	4.31 ± 0.21 (Aa*)	5.1 ± 0.03 (Aa δ)
Fall	40	5.46 ± 0.13 (Aa*)	5.49 ± 0.14(Aa*)	4.40 ± 0.02(Aa*)	4.49± 0.08(Aa*)	4.09± 0.23 (Ab*)	4.34 ± 0.09(Ab*)
	30	5.50 ± 0.09 (Aa*)	5.39 ± 0.17(Aa*)	4.39 ± 0.06 (Aa*)	4.42 ± 0.07(Aa*)	3.68 ± 0.35 (Aa*)	4.16 ± 0.04(Ab*)
	20	5.58 ± 0.14 (Aa*)	5.43± 0.12 (Aa*)	4.27± 0.05 (Aa*)	4.36 ± 0.04 (Aa*)	3.63 ± 0.10 (Aa*)	3.74 ± 0.14 (Ab*)
Winter	40	5.58± 0.24 (Aa*)	5.79± 0.55 (Aa*)	4.60± 0.41(Aa*)	5.09 ± 0.29 (Aa*)	4.24 ± 0.17 (Aa*)	4.92± 0.04(Ab*)
	30	5.50± 0.12 (Aa*)	5.59 ± 0.40 (Aa*)	4.90± 0.15 (Aa*)	5.02 ± 0.17(Aa*)	4.35± 0.35 (Aa*)	4.86 ± 0.14 (Aa*)
	20	5.58±0.13(Aa*)	5.52± 0.43 (Aa*)	4.62± 0.33 (Aa*)	4.99 ± 0.12 (Aa*)	4.13 ± 0.23 (Aa*)	4.79 ± 0.13 (Aa*)

Samples consisted of composts with established moisture content (MC) levels. Means of treatment and controls are compared, within each combination numbers followed by different capital letters are significantly different ($P < 0.05$), different small letters indicate significant differences between seasons for the same conditions (treatment, MC) and star symbol indicate comparison between moisture levels within the same season and treatment. For the data points at 48h factors season and moisture levels included for analysis only Fall and Winter trials. Means of treatment and controls are compared, within each treatment numbers followed by different letters are significantly different ($P < 0.05$).