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# Survival of *Salmonella enterica* and *Listeria monocytogenes* in manure-based compost mixtures at sublethal temperatures

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## ABSTRACT

Aerobic composting of animal manures has been advocated as an effective management tool to inactivate resident zoonotic pathogens where the time at lethal temperatures is used to determine the effectiveness of the treatment. In the absence of meeting these process conditions, the relative contributions of other physical factors on growth and persistence of zoonotic pathogens is vague and therefore the required storage time necessary for elimination of pathogens cannot be adequately estimated. This study explored the influence of sublethal temperatures, moisture levels, and light exposure on the survival of *Salmonella* and *Listeria monocytogenes* in compost mixtures that were prepared with three different sources of manure (dairy cow, swine, and chicken). As ambient temperatures increased from 20°C to 40°C, persistence of both pathogens decreased, which was likely due to the increased competitive activity of the more dominant indigenous microflora. During storage at 30°C, evaporation of water from compost mixtures occurred rapidly. Under those conditions, populations of *L. monocytogenes* declined in cow compost mixtures throughout a 4-week storage period, whereas *Salmonella* populations increased. In chicken compost mixtures at 30°C, populations of both pathogens decreased only during the first week of storage, which was likely due to the antimicrobial properties of ammonia initially present in chicken manure. When stored at 20°C, *L. monocytogenes* populations decreased more rapidly when compost mixtures were exposed to more intense light conditions whereas no discernible differences in *Salmonella* populations occurred in swine or cow compost mixtures under the different light conditions. These results indicate that developing safety guidelines for times to hold compost mixtures at sublethal temperatures, prior to land application, will be challenging.

Keywords: *Salmonella*, *Listeria monocytogenes*, compost, dairy, swine, chicken, temperature, moisture, light, manure

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## INTRODUCTION

Livestock and poultry production are major enterprises worldwide that in addition to the production of food, waste by-products that include solid manure and manure slurries are also produced in large quantities. For example, the Environmental Protection Agency (EPA) estimated that 1.1 billion tons of manure was produced annually within the U.S. (US EPA, 2013), with cattle contributing the greatest proportion (83%), followed by swine (10%), and poultry (7%). Land application of these wastes has been one of the most cost effective approaches to dispose of such large quantities of manure, with 5% of all cropland (15.8 million acres) in 2006 reported as having been fertilized with livestock manure (MacDonald *et al.*, 2009). Zoonotic pathogens are sporadically resident within animal manure (Le Bouquin *et al.*, 2010; LeJeune *et al.*, 2006; Lomonaco *et al.*, 2009). Hence if manure is applied to land, these pathogens can contaminate the soil, crops grown in those fields, and waterways that collect runoff from the fields.

Aerobic composting of animal wastes can inactivate zoonotic bacterial pathogens while creating a stable amendment that improves soil quality and fertility (Berry *et al.*, 2013; Raviv, 2005). Heat generated from the metabolic activity of thermophilic microorganisms in manure piles that are self-insulating is the primary mechanism for inactivating zoonotic pathogens (Pell, 1997; Wichuk and McCartney, 2007). Hence, process conditions for composting manures in the U.S. are based on EPA's regulations for composting biosolids that includes either a minimum temperature of 55°C for 3 days in aerated static piles or in-vessel systems, or 55°C for 15 days in windrow systems. Moreover, in the windrow systems, the material must be turned a minimum of 5 times to ensure that all material is subjected to the necessary thermal conditions (US EPA, 1999a). Composting at 40°C for 120 h or more, during which time the temperature exceeds 55°C for 4 h, has also been designated by EPA in Appendix B of the 503 Regulations as a process to significantly reduce pathogens (US EPA, 1999b). Unfortunately, when these EPA criteria are not met (Wichuk and McCartney, 2007), as could occur during winter composting or if piles are not turned to expose the surface material to sufficient internal heat, the holding time of compost materials to ensure pathogen inactivation is uncertain.

Compared to lethal heat exposure, the contribution of other physical factors (e.g., non-lethal temperatures, light, and desiccation) to inactivation of zoonotic pathogens in manure-based compost mixtures has not been elucidated because biological (i.e., competition for nutrients) and chemical (e.g., ammonia, volatile acids or other antimicrobials) factors that affect pathogen inactivation are also likely affected by the physical parameter. Such is the case with soil systems in which increased temperatures, despite being near the organism's optimal growth temperature, led to greater inactivation of *Escherichia coli* O157:H7 as a result of an accompanying increase in competition by the dominant native microbial community (Semenov *et al.*, 2007). Similarly, the effect of moisture levels on the fate of pathogens (*Salmonella* and *E. coli* O157:H7) or their surrogates in soil systems has been dependent on the pathogen population levels relative to the levels of the indigenous microbial community (Lang *et al.*, 2007; Ongeng *et al.*, 2011) and likely play a similar role in compost mixtures. Ammonia that is generated during the composting process (Beck-Friis *et al.*, 2003) and has been shown to be an antimicrobial agent toward *Salmonella* and *Listeria monocytogenes* in chicken and cattle manure (Himathongkham and Riemann, 1999; Park and Diez-Gonzalez, 2003) is also affected by moisture levels, with drying of manure accelerating the volatilization of ammonia (Gotaas, 1956) and inhibiting the conversion of nitrogenous compounds to aqueous ammonia (Hutchison *et al.*, 2000). Considering the complex interactions that moisture and temperature exert on the activity of indigenous microbial communities, it is of interest to investigate the role of moisture levels on inactivation of pathogens in compost mixtures that would likely be populated with different indigenous microflora from the different nitrogen feedstocks.

Another physical factor that has received little attention for its involvement in inactivating pathogens in manure-based compost mixtures is sunlight. Due

to its inability to penetrate compost mixtures, sunlight would be lethal to pathogens primarily at the surface of compost mixtures as has occurred at the surfaces of natural waterways and lagoons (Davies and Evison, 1991; Maynard *et al.*, 1999). Hutchison *et al.* (2005) postulated that lack of surface contamination by *Salmonella*, *Listeria*, *Campylobacter*, or *E. coli* O157:H7 in composted static pile wastes after eight days was due to exposure of their surfaces to sunlight; however, the experiment lacked a control sample not exposed to sunlight. In contrast, Erickson *et al*. (2010) were able to detect both *Salmonella* and *Listeria* on the surface of static piles comprised of chicken litter and peanut hulls after composting for 14 and 56 days in the summer and winter, respectively. To gauge the potential impact of sunlight on pathogens in compost more specifically, results of a study on the survival of pathogens in beef cattle fecal pats is presented here for comparison (Meays *et al.*, 2005). In that experiment, *E. coli* survival under 4 different levels of solar exposure (controlled by using a shade cloth) was determined. After 45 days, fecal pats under the 0% shade cloth had the least surviving *E. coli*, followed by the 40%, 80%, and 100% treatments. A similar response in non-turned composting systems could result in longer recommended holding times for regions with a large number of overcast days compared to regions that are dominated by sunny days.

The purpose of this study was to determine the influence of several physical factors (i.e., temperature, level of light exposure, and moisture levels) on the inactivation of *Salmonella* and *L. monocytogenes* in compost mixtures that were stored in environmental chambers at temperatures ranging from 20°C to 40°C in amounts that would not be self-insulating. To account for the potential confounding influence of indigenous microflora on pathogen inactivation, this variable was addressed indirectly by utilizing manure in compost mixture formulations from different sources (dairy, chicken, and swine) that should have different microbial compositions.

## MATERIALS AND METHODS

### Pathogen Strains and Preparation

Three strains of green-fluorescent protein (GFP)-labeled *Salmonella enterica* serovar Enteritidis (ME-18, H4639, and H3353) and one strain of GFP-labeled *S.enterica* serovar Newport containing an ampicillin-resistant marker were selected from the culture collection at the University of Georgia, Center for Food Safety (Griffin, GA). Five strains of GFP-labeled *L. monocytogenes* containing an erythromycin-resistant marker (12443, H7550, G3982, 101M, and F6845) were also selected from the culture collection. Details on the construction of these GFP strains has been described by Ma *et al.* (2011) and they also reported that the loss of the GFP-plasmid after 20 generations, indicative of its stability, has ranged from 15 to 77% and 8 to 52% for the *Salmonella* and *L. monocytogenes* strains, respectively.

Frozen stock cultures of each GFP-labeled *Salmonella* strain and GFP-labeled *L. monocytogenes* strain were thawed and streaked onto tryptic soy agar (Difco, Becton Dickinson, Sparks, MD) containing 100 µg/ml ampicillin (TSA-A) and brain heart infusion agar (Becton Dickinson) containing 8 µg/ml erythromycin (BHIA-E), respectively. Following incubation at 37°C for 20 to 24 h, individual colonies from each plate were subsequently streaked onto a second plate that was incubated for another 20 to 24 h at 37°C. Individual *Salmonella* and *L. monocytogenes* colonies from these plates were then inoculated into 100 ml of tryptic soy broth (Becton Dickinson) containing 100 µg/ml of ampicillin (TSB-A) and 100 ml brain heart infusion broth (Becton Dickinson) containing 8 µg/ml erythromycin (BHIB-E), respectively. Broths were incubated at 37°C for 20 to 24 h with agitation (150 rpm) and bacteria were subsequently harvested by centrifugation (4,050 x g, 15 min, 4°C) with cell pellets being washed three times in 0.1% peptone water (Difco, Becton Dickinson). Reconstitution of the individual strains in 0.1% peptone water to an optical density of 0.5 (approximately $10^9$ CFU/ml) was made prior to combining equal volumes of each strain to comprise one four-strain mixture of *Salmonella* and one five-

strain mixture of *L. monocytogenes*. *Salmonella* and *L. monocytogenes* populations were determined by plating on TSA-A and modified Oxford agar (Acumedia Manufacturers, Lansing, MI) containing 8 µg/ml erythromycin (MOX-E), respectively. *Salmonella* transformed colonies emitted bright green fluorescence when viewed at 365 nm under a handheld UV light (Fotodyne Inc., Hartland, WI); however, visualization of fluorescent *L. monocytogenes* transformed colonies required use of a Leica MZ16 FA stereo fluorescence microscope (Bannockburn, IL).

## Compost Feedstocks and Chemical Analysis

Three sources of manure including dairy cow manure, swine manure, and broiler chicken litter were used as the primary nitrogen source for compost mixtures. These materials were collected from farms located near Griffin, GA, and upon arrival at the laboratory were frozen for at least 24 h to kill the majority of insect eggs (Sherman *et al.*, 2006). Carbon amendments in compost formulations (i.e., wheat straw and cottonseed meal) were purchased from a local feed supply store. To improve the homogeneity of compost formulations, wheat straw was shredded using a Flowtron Leaf Eater (Malden, MA) for lengths of 1 to 5 cm. Carbon, nitrogen, and moisture content analyses were conducted on all raw ingredients used in the compost mixtures (Erickson *et al.*, 2010) to assist in determining recipes for formulation of compost mixtures.

## Compost Mixture Formulation

Each type of manure was individually sprayed in a 28-L sanitized bowl with either GFP-labeled *Salmonella* or both GFP-labeled-*Salmonella* and *L. monocytogenes* to give initial cell populations of 3.3 to 7.5 log CFU/g. Carbon amendments and sterile deionized water were then added to the inoculated manure to comprise formulations having initial levels of 30% or 60% moisture and initial carbon:nitrogen (C:N) ratios of 20:1 to 40:1. Compost amendments and inoculated manure were mixed thoroughly for ca. 5 min in a Hobart mixer (model D320; ¾ h.p.) prior to distributing the mixtures into containers for experimental studies.

## Experimental Design

Four studies were conducted that varied in their experimental design. In the first experimental study investigating the role of temperature on survival of *Salmonella* in manure, no carbon amendment was added to the manure source (dairy cow manure and chicken litter) that were each obtained at two separate times. Dairy cow manure (2 kg) or chicken litter (1 kg) was sprayed with the *Salmonella* inoculum mixture (20 ml or 10 ml of 7 log CFU/ml, respectively) to obtain ca. 5 to 6 log CFU/g. Inoculated material (100 g) was placed into multiple square (12.7 $cm^2$) Ziplock plastic containers (S.C. Johnson & Sons, Racine, WI). With the first batch of dairy cow manure, three containers were held at 25°C and another three were held at 40°C. With the second batch of dairy cow manure, three containers were held at 35°C and another three were held at 40°C. For inoculated chicken litter, the first batch was stored in three separate containers only at 25°C whereas the second batch was filled into three containers that were stored at 40°C only. Samples were removed from each container initially and after 3 days of storage for analysis of *Salmonella* and mesophilic and thermophilic bacteria. Only one replicate trial of this experimental design was conducted.

The second experimental study investigated the role of temperature on survival of enteric pathogens in manure-based compost mixtures. Chicken litter was collected at six separate times, with each collection being used as an independent replicate trial. Each batch of chicken litter was sprayed with both an inoculum of *Salmonella* and an inoculum of *L. monocytogenes* before blending with wheat straw, cottonseed meal and sterile deionized water to obtain mixtures having an initial carbon:nitrogen (C:N) ratio of 40:1 and 60% moisture content Initial pathogen pop-

ulations in three of the batches was targeted at a low level (ca. 3.5 log CFU/g) while another three batches had a target at a higher level (ca. 6.7 log CFU/g). Duplicate samples from each inoculated mixture were obtained for pathogen and moisture content analysis prior to distributing compost mixtures into two uncovered translucent plastic cups (8.5 cm diameter x 5 cm height, ca. 45 g/cup). Cups containing the low level inoculum were placed in an environmental chamber at 30°C with a 12-h light and 12-h dark cycle whereas cups containing the high level inoculum were placed in a dark environmental chamber set to 20°C. In the lighted chamber, light was supplied by ten 400W Metal Halide MVR400/U bulbs (General Electric, Cleveland, OH) and ten 400W high pressure sodium lamps LU400/H/ECO, LUCALOS bulbs (General Electric). High pressure sodium lamps emit no ultraviolet (UV) light and while the metal halide bulbs emit a small band of long band UV light (ca. 375 nm), compost mixtures were not exposed as this UV light was filtered out by diffusive panels separating the lamps and chamber. The compost mixtures of all batches were held for two weeks at the specified temperature after which time the cups were removed and mixtures assayed for surviving pathogens.

For the third experimental study, both dairy cow manure and chicken litter were used as nitrogen sources to determine the role of moisture content on the survival of *Salmonella* and *L. monocytogenes*. Manure or litter was initially sprayed with an inoculum of *Salmonella* and an inoculum of *L. monocytogenes* and, then mixed with wheat straw, cottonseed meal, and sterile deionized water to obtain cow and chicken compost mixtures having an initial C:N ratio of 20:1, initial moisture contents of either 30% or 60%, and initial pathogen populations of ca. 3 to 4 log CFU/g. The cow and chicken compost mixtures were then distributed into small translucent cups (ca. 45 g/cup) used in study 2. The cups were stored uncovered in an environmental chamber at 30°C with a 12-h light (602 μmol/m$^2$/sec) and 12-h dark cycle for up to 4 weeks. Two cups from each treatment were removed initially and at weekly intervals and analyzed for pathogen populations, moisture content, and pH. In addition, half of the remaining cups were adjusted back to their initial weights and original moisture contents by spraying the sample with a light mist of sterile deionized water. Two cups whose moisture contents had been adjusted were also removed from each of the four treatments (manure source x target moisture content) at 2, 3, and 4 weeks for analysis of pathogen populations, moisture content, and pH. Three independent replicate trials in which samples were not adjusted for moisture content were conducted whereas two independent replicate trials were conducted for samples adjusted for moisture content. For each independent trial, the dairy cow manure and chicken litter were collected at separate times and were inoculated with a different batch of pathogen inocula.

For the fourth experimental study, three different sources of manure (dairy cow manure, chicken litter, and swine manure) were inoculated and incorporated into compost mixtures to examine the effect of light on survival of *Salmonella* and *L. monocytogenes*. Wheat straw and cottonseed meal served as the carbon amendments and were mixed with the *Salmonella*- and *L. monocytogenes*-inoculated manure sources and sterile deionized water to obtain cow-, chicken-, and swine-compost mixtures having an initial C:N ratio of 30:1, an initial moisture content of 60%, and pathogen populations of ca. 6.7 to 7.5 log CFU/g. Duplicate samples from each of the three contaminated compost mixtures were analyzed for initial pathogen populations, moisture content, and pH. The remainder of each compost mixture was then distributed into the small translucent cups (ca. 45 g/cup) used in studies 2 and 3 described above. The cups for each treatment group were then divided into three groups. One group was placed in a dark environmental chamber, the second group was placed in an environmental chamber where all bulbs were turned on to simulate daily "bright" sunny conditions (12 h at 524-573 μmol/m$^2$/sec and 12 h in the dark), and the third group was placed in an environmental chamber where only half the bulbs were turned on to simulate daily "cloudy" conditions (12 h at 289-359 μmol/m$^2$/sec and 12 h in the dark). All chambers were at 20°C. Duplicate samples from each treatment at five sample times over the course of 4 weeks,

12 weeks, and 18 weeks for swine, chicken, and cow compost mixtures, respectively, were enumerated for pathogens and assayed for moisture content and pH. Using the above experimental design, three independent replicate trials were conducted with each trial using manure collected at separate times and a different batch of pathogen inoculum cultured for inoculation of the manure.

### Microbiological and pH Analyses

Both direct plating and selective enrichment culture was used for detection of GFP-labeled *Salmonella* and *L. monocytogenes*. In direct plating assays, compost sample (5 g) was added to 45 ml of 0.1% peptone water in a sterile Whirl-Pak bag and mixed in a stomacher. Ten-fold serial dilutions of this homogenate were made prior to spreading on TSA-A or MOX-E plates for enumeration of GFP-labeled *Salmonella* or *L. monocytogenes*, respectively. Selective enrichment culture for *Salmonella* and *L. monocytogenes* consisted of adding compost sample (5 g) directly to 45 ml of TSB-A or BHIB-E, respectively, and incubating at 37°C for 24 h. These enriched samples were then streaked on TSA-A or MOX-E plates to determine the presence or absence of *Salmonella* or *L. monocytogenes*, respectively, at a detection limit of 20 cells/100 g.

To determine initial levels of mesophilic and thermophilic microbial populations in chicken and cow manure, ten-fold serial dilutions of the stomached homogenates were spread onto Difco plate count agar (Becton Dickinson, Sparks, MD). Colonies of mesophilic and thermophilic bacteria were counted after overnight incubation at 30°C and 55°C, respectively.

Measurement of pH was determined with an Acumet Basic pH meter (Fisher Scientific, Pittsburgh, PA) on 5-g compost samples dispersed in 250 ml of deionized water. Compost mixtures were analyzed for moisture using the same procedure described for moisture analysis of feedstuffs.

### Statistical Analyses

*Salmonella* and *L. monocytogenes* populations in samples for each independent trial were converted to logarithmic values prior to determining differences from initial population levels. Logarithmic pathogen decreases, % moisture content, and pH values were subjected to the general linear- and one-way analysis of variance (ANOVA) test using the StatGraphics Centurion XV software package (StatPoint, Inc., Herndon, VA). When statistical differences were observed ($P < 0.05$) with the ANOVA test, sample means were differentiated with the least significant difference test ($P = 0.05$).

## RESULTS AND DISCUSSION

Several studies have previously addressed the survival of zoonotic pathogens in manure at ambient temperatures (Himathongkham *et al*., 1999a, b; 2000; Sinton *et al*., 2007); however, this type of study was repeated in our preliminary study (first experimental study) with locally-obtained manure to give some baseline information on the fate of *Salmonella* and other indigenous microflora in the absence of a carbon amendment. Different responses were observed for *Salmonella* and the indigenous microflora depending on the manure source and storage temperatures. Following a 3-day storage period, no changes in populations of the indigenous microflora (mesophilic and thermophilic bacteria) occurred when present in cow manure and stored at 25°C (Table 1). In contrast, the populations of both mesophilic and thermophilic bacteria increased in cow manure stored at 35°C or 40°C, but decreased in chicken litter stored at 40°C for a similar time period. *Salmonella* decreases occurred in both manure sources when held at 40°C, but reductions were substantially greater in chicken litter than in cow manure. *Salmonella* populations also decreased in chicken litter held at 25°C, but increased in cow manure held at 25°C or 35°C. Transient increases in *Salmonella* population have been observed previously in cow

Table 1. Indigenous bacterial populations in chicken and cow manure and fate of *Salmonella* when stored for 3 days at temperatures between 25°C and 40°C[1].

| | | Chicken manure[2] | | Cow manure[3] | | |
|---|---|---|---|---|---|---|
| | Day | 25°C | 40°C | 25°C | 35°C | 40°C |
| Mesophilic bacteria populations (log CFU/g)[5] | 0 | ND[4] | 9.38 ± 0.13 a | 9.22 ± 0.10 a | 6.88 ± 0.41 b | 6.88 ± 0.06 b |
| | 3 | 7.65 ± 0.14 | 5.86 ± 0.33 b | 9.51 ± 0.16 a | 8.88 ± 0.41 a | 8.44 ± 0.64 a |
| Thermophilic bacteria populations (log CFU/g)[5] | 0 | ND | 7.08 ± 0.02 a | 8.36 ± 0.08 a | 6.35 ± 0.01 b | 6.35 ± 0.01 b |
| | 3 | 8.32 ± 0.25 | 5.86 ± 0.06 b | 8.54 ± 0.17 a | 8.46 ± 0.38 a | 8.19 ± 0.83 a |
| *Salmonella*'s fate after 3 days of storage (Δ log CFU/g)[6] | | ↓[7] 1.21 | ↓ 5.56 | ↑[8] 1.88 | ↑ 1.11 | ↓ 0.89 |

[1] Data collected from first experimental study.

[2] Mesophilic and thermophilic bacteria population levels are mean ± S.D., n = 3.

[3] Mesophilic and thermophilic bacteria population levels are mean ± S.D., n = 3 for 25°C and 35°C samples, n = 6 for 40°C samples.

[4] Not determined.

[5] Values for this parameter within each column followed by a different letter are significantly different ($P < 0.05$).

[6] *Salmonella* initial populations in manure samples ranged from 5 to 6 log CFU/g.

[7] Decrease in population.

[8] Increase in population.

manure held at ambient temperatures (Himathongkham *et al.*, 1999a; Sinton *et al.*, 2007) and a minimal water content of 80% was a prerequisite (Sinton *et al.*, 2007). *Salmonella* die-off in chicken manure has also been documented and has been ascribed to the generation of ammonia (Himathongkham *et al.*, 1999b; 2000) which is an antimicrobial (Himathongkham and Riemann, 1999). For the current study, it is plausible that the production of ammonia by the indigenous microflora could be stimulated by the presence of bedding material that was included during collection of the chicken manure.

For the second experimental set of studies, temperature was the main variable of interest, but chicken litter was mixed with the carbon amendments, wheat straw and cottonseed meal, to create compost mixtures prior to their storage. When compost mixtures were formulated to an initial moisture content of 60% and an initial C:N ratio of 40:1, storage for 2 weeks at 20°C in the dark led to *L. monocytogenes* reductions of 0.97 ± 0.66 log CFU/g. In contrast, *Salmonella* populations remained relatively constant (increase of 0.16 ± 0.56 log CFU/g) over the same time period. Storage of chicken compost mixtures at 30°C for the same time interval but under lighted conditions, however, resulted in population decreases for both *L. monocytogenes* (1.87 ± 1.22 log CFU/g loss) and *Salmonella* (0.89 ± 1.54 log CFU/g loss). This trend of increased pathogen inactivation with increasing ambient temperatures

Table 2. Comparison of *Salmonella* and *L. monocytogenes* losses in chicken and cow manure-based compost mixtures[1] stored for up to 4 weeks at 30°C[2].

| | Cumulative pathogen reduction (log CFU/g)[3, 4] | | | |
|---|---|---|---|---|
| | *L. monocytogenes*[5] | | *Salmonella*[6] | |
| Week | Chicken manure compost mixture | Cow manure compost mixture | Chicken manure compost mixture | Cow manure compost mixture |
| 1 | 2.09 ± 0.67 a | 0.59 ± 1.27 b | 1.36 ± 1.62 a | -0.35 ± 1.73 a-c |
| 2 | 1.87 ± 1.22 a | 0.60 ± 1.25 b | 0.89 ± 1.54 ab | -0.82 ± 1.44 bc |
| 3 | 2.11 ± 0.93 a | 1.15 ± 1.22 ab | 1.11 ± 1.50 ab | -1.31 ± 2.10 c |
| 4 | 2.11 ± 0.93 a | 2.21 ± 0.82 a | 0.90 ± 1.64 ab | -0.58 ± 1.56 bc |

[1] Compost mixtures were formulated with carbon amendments to give an initial carbon:nitrogen ratio of 20:1 and pathogen populations of ca. 3.5 log CFU/g.

[2] Data collected from third experimental study.

[3] Pathogen data collected from treatments evaluating compost mixtures formulated to either an initial moisture content of 30% or 60% and then either readjusted to the original moisture content on a weekly basis or left undisturbed were not significantly different. The data were therefore pooled prior to statistical analysis and displaying the data in this table by the manure source used in the compost mixture.

[4] Pathogen reductions were calculated by subtracting the population level at each time period from the initial population level.

[5] Values for this pathogen (mean ± S.D.), across both rows and columns, followed by a different letter are significantly different ($P < 0.05$).

[6] Values for this pathogen (mean ± S.D.), across both rows and columns, followed by a different letter are significantly different ($P < 0.05$).

is similar to the patterns in soil of pathogen survival previously documented (Lang *et al.*, 2007; Semenov *et al.*, 2007). Based on those studies, the investigators suggested that increases in temperature, despite being close to the pathogen's optimal growth temperature, increased the competitive activity of the more dominant indigenous microflora which adversely affected the pathogen's survival (Lang *et al.*, 2007). This explanation may be the basis for the decreased pathogen persistence we observed in chicken compost mixtures as exposure temperatures increased.

Initial moisture contents (30% or 60%) and weekly adjustment of the moisture contents of chicken- and cow compost mixtures were the two variables of interest in our third experimental study in determining their influence on *L. monocytogenes* and *Salmonella* inactivation in compost mixtures stored for up to 4 weeks at 30°C. However, under these conditions, populations of either pathogen were not affected by the initial moisture contents nor did weekly additions of water to return the compost mixtures to their original moisture contents affect the reduction of pathogens ($P > 0.05$). Moisture analysis of the compost mixtures revealed that water was lost very quickly from the samples stored in uncovered containers and equilibrated to approximately the same percentage of moisture (9.7 ± 2.7%) regardless of the initial moisture content or when weekly additions of water were applied to the mixtures. These conditions were therefore likely responsible for the failure of moisture content to have an effect on patho-

Table 3. pH of chicken and cow manure-based compost mixtures[1] following storage at 30°C for 4 weeks when initial moisture contents were either 30% or 60%[2].

| | pH[3] | | | |
|---|---|---|---|---|
| | Chicken manure compost mixture | | Cow manure compost mixture | |
| Week | 30% moisture | 60% moisture | 30% moisture | 60% moisture |
| 0 | 7.25 ± 0.66 a-f | 7.58 ± 1.22 a-d | 6.84 ± 0.33 d-f | 6.64 ± 0.34 f |
| 1 | 7.06 ± 0.32 b-f | 7.85 ± 0.66 a | 6.80 ± 0.29 ef | 7.46 ± 0.21 a-e |
| 2 | 6.97 ± 0.34 c-f | 7.63 ± 0.59 a-c | 6.77 ± 0.22 ef | 7.09 ± 0.10 b-f |
| 3 | 6.99 ± 0.42 c-f | 7.75 ± 0.34 ab | 6.81 ± 0.16 ef | 7.32 ± 0.29 a-f |
| 4 | 6.87 ± 0.41 d-f | 7.74 ± 0.24 ab | 6.77 ± 0.08 ef | 7.45 ± 0.22 a-e |

[1] Compost mixtures were formulated with carbon amendments to give an initial carbon:nitrogen ratio of 20:1.

[2] Data collected from third experimental study.

[3] Values within the table (mean ± S.D.) followed by a different letter are significantly different ($P < 0.05$).

gen inactivation. Hence, the data were pooled to determine the changes that occurred in pathogen populations in the chicken and cow compost mixtures (Table 2). As with dairy manure in the absence of a carbon amendment and held at 25°C or 35°C (Table 1), the populations of *Salmonella* in the cow compost mixtures increased from initial populations after holding of the materials at 30°C and remained at these higher levels over the 4-week trial (Table 2). In contrast, *L. monocytogenes* populations declined in cow compost mixtures after only one week of storage at 30°C and additional significant decreases occurred up to 4 weeks of storage ($P < 0.05$). Over all time periods, there were significantly greater reductions in *Salmonella* and *L. monocytogenes* populations in the chicken compost mixtures compared to the cow compost mixtures ($P < 0.05$). Interestingly, the decreases in pathogen populations in the chicken compost mixtures occurred during the first week of storage but not after additional storage (Table 2).

Monitoring the pH of the chicken- and cow manure-based compost mixtures during storage at 30°C for 4 weeks (third experimental study) revealed that initially the chicken compost mixtures were approximately 0.5 pH units higher than the cow compost mixtures (Table 3). Although not determined in this study, the ammonia present that has previously been associated with higher pH values in chicken manure (Himathongkham *et al*., 1999b; 2000) could have been the principal factor responsible for the die-off of pathogens in the chicken compost mixtures. It appears, however, that pH alone may not be used as an indicator of a compost mixture's capacity to sustain viable pathogen populations. In the cow compost mixtures having an initial moisture content of 60%, the pH increased to values approximating those detected in the chicken compost mixtures (Table 3) yet *Salmonella* grew in those mixtures (Table 2). Based on these results, it is likely that *Salmonella* is susceptible to ammonia that is present in chicken

Table 4. *Salmonella* and *L. monocytogenes* reductions during storage at 20°C under different light conditions in compost mixtures prepared with different manure sources[1].

| | | Cumulative pathogen reductions (log CFU/g)[2] | | | | | |
|---|---|---|---|---|---|---|---|
| | | *L. monocytogenes*[3] | | | *Salmonella*[3] | | |
| Manure source | Week | Dark | Cloudy[4] | Sunny[5] | Dark | Cloudy | Sunny |
| Chicken | 2 | 0.86 ± 0.01 e | 1.37 ± 0.74 e | 1.37 ± 0.61 e | 0.32 ± 0.65 e | 0.48 ± 0.26 e | 1.01 ± 0.76 de |
| | 6 | 2.70 ± 0.27 d | 4.71 ± 1.01 c | 5.83 ± 0.30 ab | 1.10 ± 1.02 c-e | 1.39 ± 0.63 b-e | 3.07 ± 1.44 ab |
| | 8 | 4.38 ± 1.08 c | 5.83 ± 0.30 ab | 5.83 ± 0.30 ab | 1.07 ± 0.56 de | 2.09 ± 0.38 a-e | 3.41 ± 1.14 a |
| | 10 | 5.01 ± 1.12 bc | 5.83 ± 0.30 ab | 6.03 ± 0.13 a | 1.89 ± 0.81 a-e | 2.11 ± 0.79 a-e | 2.90 ± 1.91 a-c |
| | 12 | 5.93 ± 0.24 ab | 5.83 ± 0.30 ab | 5.93 ± 0.28 ab | 3.14 ± 1.92 ab | 2.45 ± 0.72 a-d | 2.99 ± 1.53 ab |
| Dairy cow | 2 | 0.36 ± 0.81 d | 1.07 ± 1.85 d | 2.03 ± 2.12 cd | -0.31 ± 1.08 a | 0.23 ± 2.04 a | 1.08 ± 3.43 a |
| | 6 | 1.04 ± 0.81 d | 2.39 ± 2.90 cd | 2.43 ± 1.61 cd | 0.44 ± 1.75 a | 0.58 ± 2.40 a | 1.01 ± 2.45 a |
| | 10 | 1.28 ± 0.26 d | 2.87 ± 2.50 b-d | 2.90 ± 2.73 b-d | 0.71 ± 0.89 a | 1.63 ± 2.94 a | 1.75 ± 2.84 a |
| | 14 | 2.25 ± 0.39 cd | 4.09 ± 1.46 a-c | 5.29 ± 0.70 ab | 0.93 ± 1.96 a | 1.78 ± 2.81 a | 1.67 ± 2.06 a |
| | 18 | 4.70 ± 1.71 a-c | 4.29 ± 1.27 a-c | 6.27 ± 1.02 a | 1.61 ± 2.96 a | 1.90 ± 2.72 a | 2.09 ± 2.56 a |
| Swine | 0.3 | -0.57 ± 0.45 de | -0.64 ± 0.56 e | -0.08 ± 0.62 c-e | -0.82 ± 0.37 f | -0.75 ± 0.52 ef | -0.25 ± 0.51 c-f |
| | 1 | -0.26 ± 0.48 c-e | -0.27 ± 0.55 c-e | -0.01 ± 0.46 c-e | -0.44 ± 0.53 d-f | -0.43 ± 1.02 d-f | -0.11 ± 0.75 b-f |
| | 2 | 0.07 ± 0.41 c-e | 0.14 ± 0.56 b-d | 0.44 ± 0.11 bc | -0.36 ± 0.69 c-f | 0.02 ± 0.53 a-f | 0.16 ± 0.36 a-e |
| | 3 | 0.10 ± 0.25 b-e | 0.22 ± 0.24 bc | 0.86 ± 0.45 ab | 0.13 ± 0.46 a-f | 0.36 ± 0.62 a-d | 0.74 ± 0.48 ab |
| | 4 | 0.39 ± 0.17 bc | 0.39 ± 0.36 bc | 1.38 ± 0.66 a | 0.42 ± 0.41 a-d | 0.56 ± 0.68 a-c | 0.87 ± 0.38 a |

[1] Data were collected from fourth experimental study.
[2] Reductions were determined relative to initial values in compost mixtures.
[3] Values (mean ± S.D.) within each manure source for this pathogen followed by a different letter are significantly different ($P < 0.05$).
[4] Compost mixtures were exposed daily to light conditions of 289 to 359 μmol/m$^2$/sec for 12 h and to dark conditions for 12 h.
[5] Compost mixtures were exposed daily to light conditions of 524 to 573 μmol/m$^2$/sec for 12 h and to dark conditions for 12 h.

compost mixtures initially but the low-moisture conditions present in these mixtures inhibit the indigenous microflora from generating additional ammonia. The low moisture conditions, however, do not directly contribute to inactivation of desiccation-resistant *Salmonella* (Pedersen *et al.*, 2008; Tamura *et al.*, 2009), whereas a substantial proportion of the *L. monocytogenes* population is susceptible to either ammonia or desiccation stress.

The last study addressed the influence of light in the visible and infrared spectrum on inactivation of pathogens in manure-based compost mixtures held at sublethal temperatures. Compost mixtures prepared with chicken litter, dairy cow manure or swine manure and held at 20°C were exposed to one of three lighting conditions simulating dark, sunny, or cloudy conditions. Preliminary studies were conducted with each of these compost mixtures to determine the approximate time interval over which samples should be taken to obtain measurable pathogen reductions and determine whether light conditions could significantly affect their inactivation. Unfortunately, the storage time intervals selected for swine compost mixture were underestimated and the greatest pathogen reduction was only slightly greater than 1 log CFU/g (Table 4). Despite this limitation, significant trends were determined for the swine compost mixture data. In particular, both pathogen populations increased during the first week of storage under all lighting conditions in swine compost mixtures. Following 2 weeks of storage, *Salmonella* remained at elevated populations for the swine compost mixtures that were held in the dark, whereas under cloudy or sunny conditions, *Salmonella* populations decreased slightly. Further reductions in *Salmonella* populations occurred during the next two weeks of storage, but there were no statistical differences in response to light exposure ($P > 0.05$). For *L. monocytogenes* in swine compost mixtures, only sunny conditions at week 4 had significantly greater reductions of this pathogen compared to mixtures held under cloudy or dark conditions ($P < 0.05$). The inability to detect significant differences in the reduction of either pathogen under dark and cloudy conditions is likely due to the relatively minimal reductions that occurred during the short time period that was examined for swine compost mixtures. In contrast, over all time periods, reductions in *L. monocytogenes* populations were statistically significant for both cloudy and sunny conditions compared to dark conditions for chicken compost mixtures stored for 6 weeks or in cow compost mixtures stored for 14 weeks (Table 4, $P < 0.05$).

A completely different set of responses to light was observed for *Salmonella* in chicken- or cow compost mixtures. In chicken compost mixtures, only sunny conditions led to statistically greater reductions in populations than dark conditions, and these occurred midway through the storage trial. In contrast but similar to the response in swine compost mixtures, light conditions did not affect the reductions in *Salmonella* populations in cow compost mixtures at any sampling time (Table 4).

A number of factors could contribute to light-mediated inactivation of *L. monocytogenes* in the compost mixtures. As a component of sunlight, both long wave (UVA, 315 to 400 nm) and medium wave (UVB, 280 to 315 nm) ultraviolet light has been shown to damage the genetic material of microorganisms (Davies and Evison, 1991; Jagger, 1985; Jiang *et al.*, 2009); however, in our environmental chambers, ultraviolet light was filtered out by the diffusive ceiling light panels and hence had no role. Alternatively, exogenous sensitizers in the compost materials such as humic substances (Chien *et al.*, 2007) may be activated by visible light energy. Such a mechanism has been demonstrated for inactivation of the Gram-positive, *Enterococcus faecalis* in waste stabilization pond water whereas the Gram-negative *E. coli* was inherently less susceptible to this pathway (Kadir and Nelson, 2014). Although *L. monocytogenes* has previously displayed some desiccation resistance, surviving for three months in a simulated food processing environment (Vogel *et al.*, 2010), it is not as resistant as *Salmonella* based on the lower recoveries of *L. monocytogenes* compared to *Salmonella* in aerosols of meat processing plants (Okraszewska-Lasica *et al.*, 2014). Hence, a third mechanism by which increased intensities of light may have led to increased inactivation of the *L.*

Table 5. % Moisture content and pH in compost mixtures held at 20°C and exposed to different light conditions across all five storage time periods examined with each type of manure[1, 2].

| | % Moisture[3] | | | pH[3] | | |
|---|---|---|---|---|---|---|
| Manure source | Dark | Cloudy[4] | Sunny[5] | Dark | Cloudy | Sunny |
| Chicken | 45.6 ± 24.4 a | 33.8 ± 17.1 b | 25.0 ± 16.8 b | 9.5 ± 0.3 a | 9.3 ± 0.3 ab | 9.2 ± 0.6 b |
| Dairy cow | 32.1 ± 19.9 a | 21.4 ± 13.4 b | 12.3 ± 5.7 c | 8.7 ± 0.6 a | 8.1 ± 0.8 b | 8.3 ± 0.5 b |
| Swine | 35.1 ± 17.2 a | 29.9 ± 12.5 a | 19.9 ± 13.5 b | 9.0 ± 0.5 a | 9.0 ± 0.5 a | 9.0 ± 0.4 a |

[1] Data were collected from fourth experimental study.

[2] Swine, chicken, and dairy cow compost mixtures were stored for 4, 12, and 18 weeks, respectively.

[3] Values in each row of an attribute followed by a different letter are significantly different ($P < 0.05$).

[4] Compost mixtures were exposed daily to light conditions of 289 to 359 µmol/m$^2$/sec for 12 h and to dark conditions for 12 h.

[5] Compost mixtures were exposed daily to light conditions of 524 to 573 µmol/m$^2$/sec for 12 h and to dark conditions for 12 h.

*monocytogenes* isolates used in this study may be through localized heating, additional dehydration at surface locations, and in turn increased desiccation stress. In support of this explanation, the moisture content in the compost mixtures decreased as the mixtures were exposed to higher levels of light and the highest levels of dehydration occurred in the dairy compost mixtures followed by the swine compost mixtures (Table 5). In addition to affecting the moisture content of the compost mixtures, light exposure led to decreased pH in the chicken and cow compost mixtures (Table 5). *L. monocytogenes* survival could have been improved under less alkaline conditions; however, that response was likely to be minimal in these compost mixtures due to the concurrent stress imposed by low moisture contents and light exposure. In the case of *Salmonella*, however, it is known that it is extremely resistant to desiccation (Pedersen *et al.*, 2008; Tamura *et al.*, 2009). Given that dehydration has induced cross-tolerance to a number of other stressors (Gruzdev *et al.*, 2011), such a state could also have been responsible for our inability to discern an effect of light on inactivation of *Salmonella* in the swine or cow compost mixtures.

In summary, both *Salmonella* and *L. monocytogenes* may survive in compost mixtures that are exposed to sublethal temperatures for extended periods of time. As ambient temperatures increased, the persistence of pathogens decreased which may be attributed to increased competitive activity by the more dominant indigenous microflora. Attempts to maintain the moisture content of compost mixtures on a weekly basis was challenging because rapid evaporation resulted in very dry mixtures in most cases. Under these conditions, *L. monocytogenes* appeared to be more susceptible to desiccation stress than *Salmonella* based on their relative reduction in populations in chicken and cow compost mixtures over time. *L. monocytogenes* populations also decreased more rapidly when compost mixtures were exposed to light conditions, described as sunny or cloudy, compared to dark conditions, but

this response did not occur with *Salmonella* in cow or swine compost mixtures. It is suggested that the drier conditions encountered in light-exposed cow and swine compost mixtures may have induced a cross-tolerance response by *Salmonella* to the light stress. If cross-tolerance responses by *Salmonella* are generated in low moisture compost mixtures held at sublethal temperatures, it will therefore be important to apply an intervention treatment to those compost mixtures prior to the activation of that response.

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