# Effect of heating and aging of poultry litter on the persistence of enteric bacteria

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**ABSTRACT** Food-borne illnesses have rarely been associated with the reuse of poultry litter as an organic fertilizer and soil amendment in agriculture. Yet farming practices in many countries have come under increased scrutiny because of heightened consumer awareness of food safety and environmental issues. This study was conducted to determine whether simple on-farm management practices could improve the microbiological safety of poultry litter. First, the effects of heat and moisture on the survival of *Escherichia coli* and *Salmonella enterica* serovar Typhimurium in poultry litter were investigated under laboratory conditions. Second, the persistence and regrowth of enteric bacteria were examined in poultry litter that had been aged for up to 12 wk in either a turned or static (unturned) windrow. *Escherichia coli* and *Salmonella* counts in poultry litter were reduced by >99% in 1 h at 55 or 65°C under laboratory conditions. At 35°C, both persisted longer under moist (65% wt/wt, wet basis) than dry (30% wt/wt) conditions. Poultry litter aged for 3 wk in a turned windrow, and up to 6 wk in a static windrow, supported increased *E. coli* densities when incubated in the laboratory at 37°C for 21 d. Peak temperatures >65°C were observed in both windrows within the first 3 wk of aging; after this point, the turned windrow was more consistently exposed to temperatures >45°C than the static windrow. By 12 wk, however, *E. coli* counts were very similar (3 to 3.6 $\log_{10}$) in the outside edge of both windrows. This study highlights the need for a better understanding of the interrelationship between spontaneous heating in organic waste streams, organic matter stabilization, and pathogen reduction.

**Key words:** poultry litter, *Escherichia coli*, *Salmonella*, composting, pathogen reduction

2011 Poultry Science 90:10–18
doi:10.3382/ps.2010-01023

## INTRODUCTION

Poultry litter or broiler litter is commonly recovered from many poultry operations and recycled as an organic fertilizer or as a feed supplement for ruminants (Capucille et al., 2002; Rankins et al., 2002; Harapas et al., 2003). Common bedding materials are wood shavings, sawdust, rice and peanut hulls, straw, or other dry, absorbent, low-cost organic materials. In Australia, spent poultry litter is widely used for growing organic and conventional vegetable crops. Poultry litter may or may not have been aged or composted by the time it arrives on the vegetable farm. It could be used immediately or stored for some months, sometimes in close proximity to maturing vegetable crops. Practices differ widely among farms and between crops on an individual farm. It is commonly rotary-hoed into the soil before planting or side-dressed during crop growth, sometimes within 3 wk of harvest (Harapas et al., 2003).

Poultry litter is known to contain bacteria that have the potential to cause human illness, such as *Salmonella* and *Staphylococcus* (Martin et al., 1998; Terzich et al., 2000), and contamination of fresh produce with manure has been implicated as the cause of numerous bacteriological food poisoning outbreaks (Brackett, 1999; Doyle and Erickson, 2008). Although the use of poultry litter in commercial vegetable farming has rarely been associated with food-borne illnesses, heightened consumer awareness of food safety issues has increased the scrutiny of on-farm management practices in many countries, including Australia.

High temperature is the most frequently studied mechanism involved in the inactivation of human pathogens during organic waste treatment processes such as composting and deep stacking. However, other mechanisms are known to be important, including microbial antagonism (including antibiotic production and direct parasitism), production of organic acids, pH changes,


Received July 21, 2010.
Accepted October 3, 2010.
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desiccation, exposure to ammonia, and competition for nutrients (Epstein, 1997; Chaudhry et al., 1998).

Poultry litter is a relatively hostile environment for the persistence of pathogens because it is typically dry, heats up readily, and generates ammonia gas. Deep stacking or ensiling poultry litter is commonly recommended as a pretreatment to improve its safety and palatability as a feed for ruminants (Capucille et al., 2004; Bush et al., 2007). Bush et al. (2007) showed that despite the wide variation of temperature within the stacks, *Salmonella* was eliminated in 98.7% of all inoculated sites. Furthermore, *Salmonella* organisms were reduced by at least 5 log in the remaining sites where it was still viable. Ensiling animal waste is also effective in controlling pathogens. Although lactic acid production is thought to be the most important factor, it is not the only mechanism involved (Chaudhry et al., 1998).

Composting is often the method of choice for making organic wastes safe before application to land. One objective of composting is to manage temperatures actively so that all parts of the pile are sanitized by exposure to high temperatures (Epstein, 1997). On-farm composting has not been widely adopted because it is perceived to involve a significant investment in infrastructure, machinery, and labor. The use of complex, highly managed systems is usually driven by a desire for minimum processing time and maximum throughput in municipal- or industrial-scale waste treatment processes, where waste loading rates are high and time and land area are limiting factors. These constraints are usually of less concern in on-farm waste treatment processes. Less intensively managed waste treatment systems may perform satisfactorily given a better understanding of how to manage the microbiological risks associated with them.

This study was conducted to determine whether simple on-farm management practices could improve the microbiological safety of poultry litter. Two experiments were conducted to investigate 1) the effects of heat and moisture on the survival of *Escherichia coli* and *Salmonella enterica* serovar Typhimurium in poultry litter under laboratory conditions and b) the effects of aging in windrows, in the field, on the persistence and regrowth of enteric bacteria in poultry litter.

## MATERIALS AND METHODS

### *Experiment 1: Effect of Temperature and Moisture on the Survival of E. coli and Salmonella Typhimurium in Poultry Litter Under Laboratory Conditions*

Approximately 15 kg of fresh poultry litter was sourced from the poultry industry southeast of Melbourne. The litter consisted of manure and rice hulls collected from broiler production sheds. The litter was stored for 1 d before collection. Enough poultry litter was collected for the entire experiment and was stored in a sealed container at 4°C.

Bacterial strains used for testing included a nonpathogenic strain of *E. coli* (NCTC 10418) and *Salmonella* Typhimurium. The latter bacterium was selected because this serovar had been isolated from poultry litter samples in previous studies undertaken at the Department of Primary Industries, Knoxfield, Australia. *Salmonella* Typhimurium was supplied by The Microbiological Diagnostic Unit, Department of Microbiology and Immunology, The University of Melbourne, Australia.

A laboratory-scale apparatus was used with different temperature conditions in 6 discrete chambers. Each chamber contained a water bath into which a 100-mm-diameter plastic container, constructed to hold four 50-mL plastic tubes, was immersed. The temperature of each water bath was regulated by a dial set above the chamber, with a possible range of 30 to 75°C. Temperature could be controlled independently for each discrete chamber. T-type thermocouples were inserted into each of the chambers to log temperature on an hourly basis. Sterile 50-mL plastic tubes containing 10 g of poultry litter inoculated with *E. coli* and *Salmonella* Typhimurium were used. The plastic tubes were placed in the chambers once the water bath had reached the desired temperature.

Twelve test runs were performed in the apparatus and were considered as 1 experiment for analysis. Bacterial survival was assessed for all combinations of 4 temperatures (35, 45, 55, and 65°C) and 3 moisture content levels (30, 50, and 65% wt/wt, wet basis). At 35 and 45°C, samples were assessed at 0, 2, 5, 8, and 24 h. Because the rate of thermal inactivation of the bacteria was expected to be faster at higher temperatures, the sampling protocol differed slightly at 55 and 65°C; these samples were assessed after 0, 1, 3, 5, and 24 h. Each combination of temperature and moisture content was replicated 6 times during the series of 12 runs. The order of the 12 runs was randomized, with the restriction that each group of 3 tests (1 to 3, etc.) had 1 of each moisture content.

The randomization of temperatures to chambers had the following features: 1) for any run, 2 of the 4 temperatures were represented once, and the other 2 were represented twice; 2) for any of the 3 moisture contents, the combination of temperatures was different for each run; 3) every combination of temperature and moisture content occurred exactly once in each of the 6 chambers; and 4) the first 6 runs and the second 6 runs each had 3 replicates of every combination of temperature and moisture content.

The poultry litter and bacterial inoculum were prepared the day before each run. For each run, a quantity of poultry litter was sieved through a 4.75-mm-aperture sieve to remove large lumps of manure and feathers to produce a 500-g sample. The moisture content of the

litter was determined by oven-drying at 105°C for 24 h. Moisture content was adjusted to the required level by spreading the litter across the base of a large plastic tray and wetting with deionized water. Ten grams of the wetted poultry litter was then placed into each of 35 sterile 50-mL plastic tubes with screw-top lids. The tubes were then stored at 4°C until inoculation with bacteria the following day.

*Escherichia coli* and *Salmonella* Typhimurium were cultivated at 37°C and at 120 rpm in an orbital incubator for 18 h. Cultures for each bacterium were inoculated from freshly grown nutrient agar plates into 2 separate 50-mL flasks containing 15 mL of sterile nutrient broth. After incubation and shaking for 18 h, contents of the 2 flasks were pooled and mixed. The approximate inoculum density for each organism was $10^9$ cfu/mL. A 0.1-mL aliquot of the combined inoculum was added to each tube containing the poultry litter. For each chamber of the bench-scale apparatus, there were 5 sample tubes, 4 of which were immersed into the water bath and 1 of which was set aside to determine initial *E. coli* and *Salmonella* Typhimurium levels at the beginning of the experimental run.

The 10-g poultry litter sample from each tube was emptied into 50 mL of sterile 0.1% peptone water in a jar. The jar was placed on an orbital shaker for 30 min set at 120 rpm and allowed to settle before 10-fold serial dilutions were prepared into sterile 0.1% peptone water and plated onto selective media.

Direct counts of *E. coli* were determined on *E. coli*-coliform agar plates (Petrifilm, 3M, St. Paul, MN) incubated at 37°C for 48 h. Final results were calculated as colony-forming units per gram of dry weight (cfu/g). For *Salmonella* Typhimurium, 0.1 mL of the serial dilutions was inoculated onto xylose lysine deoxycholate agar plates (Difco, Detroit, MI) incubated at 37°C for 24 h. Blue colonies with gas were considered *E. coli* on the Petrifilm, and pink or red colonies with black centers were considered *Salmonella* on the xylose lysine deoxycholate plates.

All results were transformed to base 10 logarithms and analyzed by residual maximum likelihood using GenStat for Windows, 6th edition (Lawes Agricultural Trust, VSN International Ltd., Oxford, UK). Residual maximum likelihood handles unbalanced data arising from unequal replication of treatments, missing values, or unbalanced experimental designs.

## Experiment 2: The Effects of Aging in Windrows

Two windrows were constructed from fresh poultry litter, collected from the same broiler production shed, at a location 40 km southeast of Melbourne. Each windrow had the following approximate dimensions: 3.3 m (width) × 1.5 m (height) × 20 m (length). One windrow was completely turned with a front-end loader every 7 to 10 d for the first 8 wk, and the other windrow was not turned.

Mean daily temperatures were measured in each windrow using T-type thermocouples linked to a data logger (DT500, Datataker Pty Ltd., Scoresby, Australia) recording every 2 h. Thirty-five thermocouples were spaced in a 300 × 300 mm grid pattern from near the base to near the top of each windrow to monitor temperature in the cross-section (Figure 1). One cross-section was monitored for each windrow at any one time, but the thermocouples were moved to a different part of the windrow every 7 to 10 d during the first 8 wk (corresponding to turning events in the turned windrow). Any exposed thermocouples on the surface of the windrows were re-covered at this time. Cross-sectional isotherms were mapped using Delta Graph 5 for Windows (Red Rock Software, Salt Lake City, UT.). The surface area of a cross-section falling into the following temperature categories, <35°C, 35 to 44°C, 45 to 54°C, 55 to 64°C, and >65°C, was calculated using image analysis software (SigmaScan Pro 3, Jandel Scientific, San Rafael, CA).

The physicochemical properties of representative samples of poultry litter were determined at wk 0, 3, 6, and 12. For each sampling event, 20 to 30 lots were taken with a spade from around the pile to a depth of up to 300 mm, mixed together, and quartered until a quantity of approximately 6 L was obtained. Samples were analyzed according to the methods described in the Australian Standard for Composts, Soil Conditioners and Mulches (AS4454, Standards Australia, 1999). Briefly, moisture content was determined by oven-drying at 105°C. The pH, nitrate-N, and ammonium-N were determined in 1:1.5 (poultry litter:deionized water) extracts. Bioassays were performed by incubating 10 radish (*Raphanus sativus* L. 'Long Scarlet') seeds at 25°C on the poultry litter sample and a reference sample known to be nontoxic. Germination and length of roots after 3 to 5 d incubation were determined as a percentage of the reference sample.

Sampling for enteric bacteria occurred near the outside edge of windrows, where temperatures were cooler

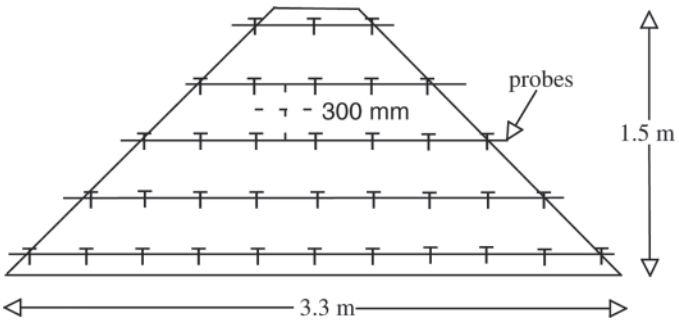


**Figure 1.** Schematic representation of the location of temperature probes in windrows with thermocouples spaced equidistantly in a 300 × 300 mm grid pattern.

and higher rates of survival could be expected. Sixteen samples of poultry litter were collected at 0- to 100-mm depths along the length of each windrow on wk 0, 3, 6, and 12. A portion of each sample was analyzed directly for enteric bacteria. *Escherichia coli* levels were determined using the same procedure described for experiment 1. For *Salmonella* spp., *Campylobacter* spp., and *Listeria* spp., a 25-g subsample was tested for their presence or absence in poultry litter, using the methods described in standards AS 1766.2.5 (Standards Australia, 1991b), AS 1766.2.13 (Standards Australia, 1991a), and AS/NZS 1766.2.16.1 (Standards Australia, 1998), respectively.

The remaining portion of each sample was divided into two 500-g subsamples and subjected to 1 of 2 pretreatments: 1) no change to moisture content or 2) moisture content adjustment to 50% (wt/wt, wet basis). Each treatment set was further divided into twenty-four 10-g samples, placed in 50-mL plastic tubes with screw-top lids, and incubated at 37°C for up to 21 d. For each treatment at each sampling time (0, 7, 14, and 21 d), 6 replicate tubes were analyzed for the presence of *E. coli* as described above in experiment 1. Bacterial count results were transformed to base 10 logarithms and analyzed by ANOVA using GenStat for Windows, 6th edition (Lawes Agricultural Trust).

Forty millimeters of rainfall was recorded during wk 10 of the trial, which resulted in pools of water lying at the base of the turned windrow pile. A 2-L sample of leachate was collected from there and analyzed for *Salmonella* and *Listeria*. Leachate was not available for collection from the static windrow.

## RESULTS AND DISCUSSION

### Experiment 1: Effect of Temperature and Moisture on the Survival of E. coli and Salmonella Typhimurium in Poultry Litter Under Laboratory Conditions

All treatment combinations resulted in a greater than 99% reduction in *E. coli* counts after 8 h (Figure 2), but at 35°C and 65% moisture content, more than 134,000 (5.13 $\log_{10}$) *E. coli* per gram were still present in the litter after 24 h of incubation (Figure 2a). The most rapid reduction in *E. coli* and *Salmonella* Typhymurium counts occurred at high temperatures, but 65°C was not more effective than 55°C. *Salmonella* Typhymurium was completely eliminated and *E. coli* counts were reduced by more than 99% after 1 h of exposure to these temperatures. These findings are in general agreement with other published accounts of the thermal death points of enteric bacteria (e.g., Golueke, 1991; Farrell, 1993; Epstein, 1997).

Incubation at 55°C and 50% moisture content for 5 h resulted in significantly lower *E. coli* counts compared with the same temperature conditions and 30% moisture content (Figure 2c). A similar effect was observed at 65°C, at which bacterial counts in samples incubated at 65% moisture content were significantly lower than at 30% (Figure 2d). After 24 h of incubation, no significant difference was found in *E. coli* counts between the different moisture contents at either 55 or 65°C.

The thermal sensitivity of organisms increases with increasing moisture content (de Bertoldi et al., 1991; Farrell, 1993), but at low moisture contents, desiccation may play a greater role in pathogen inactivation. Heat-drying at 80°C to 10% moisture content or lower is a recognized process for further reducing pathogens in the US Environmental Protection Agency's Part 503 regulations (US EPA, 2003). At lower temperatures (35 or 45°C), more effective reductions in *E. coli* and *Salmonella* Typhimurium counts were observed at 30% than at 65% moisture content. For *E. coli*, this effect was observed up until 5 h of exposure to 45°C (Figure 2b), but at 35°C, this was still the case after 24 h (Figure 2a).

For *Salmonella* Typhimurium, all moisture levels at 35°C resulted in a reduction of more than 99% in counts within 24 h; at 45°C, the same level of control was achieved after 8 h (Figure 3). However, after 24 h of exposure to both temperatures, *Salmonella* Typhimurium counts were significantly higher at 65% moisture content compared with 30% moisture content. At 35°C, about 40 times more *Salmonella* Typhimurium were enumerated at 65% moisture content than at 30% ($P < 0.05$). At 45°C, 50 times more were enumerated at 65% moisture content compared with 30%, although, in general, counts at this temperature were significantly lower than those at 35°C ($P < 0.05$).

These findings suggest that moisture content may have little direct effect on pathogen inactivation in the center of piles or windrows provided that high temperatures can be sustained for long periods. But moisture content has a critical impact on microbiological activity in organic materials and therefore temperature development (Epstein, 1997), which in turn determines the efficacy of pathogen inactivation. Where temperatures are lower on the outside of piles, the persistence of enteric bacteria may actually be facilitated by moist conditions.

### Experiment 2: Effects of Aging in Windrows

*Listeria* and *Campylobacter* were not detected in any poultry litter sample collected from either windrow. However, *Listeria monocytogenes*, *Listeria innocua*, and *Salmonella enterica* serovar Agona were detected in the leachate sample collected at the base of the turned windrow on wk 10. In addition, no effective change was found in *E. coli* counts in the outer layers of either windrow over the course of the trial. Counts ranged between about 3 to 3.6 $\log_{10}$ for both windrows over the 12-wk period of monitoring (data not shown). *Salmo-*

*nella* species were detected more frequently in the static windrow. In the turned windrow, *Salmonella* was no longer detected after 6 wk, but was detected in 2 out of 16 samples in the static windrow (data not shown). *Salmonella enterica* serovar Sofia was the most frequently detected serovar in both windrows. Two pathogenic serovars were detected in the turned window (*Salmonella enterica* serovar Hvittingfoss and *Salmonella enterica* serovar Mbandaka) after 3 wk.

The persistence of *E. coli* in the outer layers of both windrows is consistent with the findings from experiment 1. Approximately 180 mm of rainfall was recorded during the 12-wk monitoring period, and the edges of both windrows periodically became wet. In addition, temperatures above 45°C were seldom recorded in this zone. The outer edges of the windrows may constitute a reservoir of bacteria that could potentially multiply and recontaminate other portions of the poultry litter. Regrowth of enteric bacteria has been previously associated with rainfall events in soil amended with biosolids (Pepper et al., 1993) and in piles of biosolids stored outdoors (Gibbs et al., 1997). The occurrence of *Listeria* and *Salmonella* in the leachate from the turned windrow at wk 10 also suggests that, in this case, periodic turning of poultry litter did not greatly improve its microbiological safety.

Turning incorporates the cooler outer layers of piles into the center, where they are then exposed to thermophilic conditions. It also fluffs up and aerates the material, resulting in higher temperatures sustained for longer periods (Epstein, 1997). These principles were clearly demonstrated in this experiment, with poultry

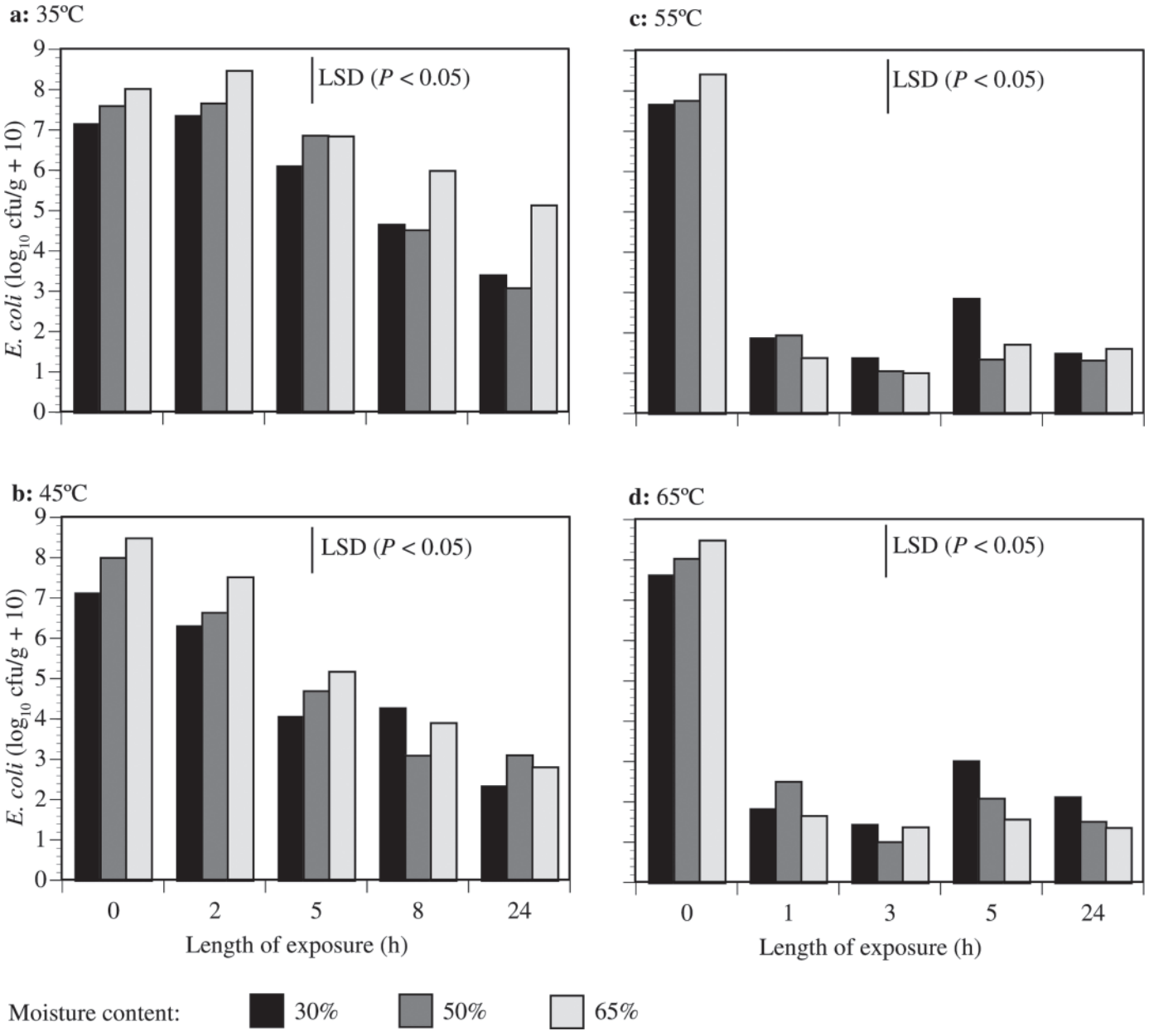


**Figure 2.** Effect of temperature (35, 45, 55, and 65°C) and moisture content (30, 50, and 65% wt/wt, wet basis) on persistence of *Escherichia coli* in poultry litter.

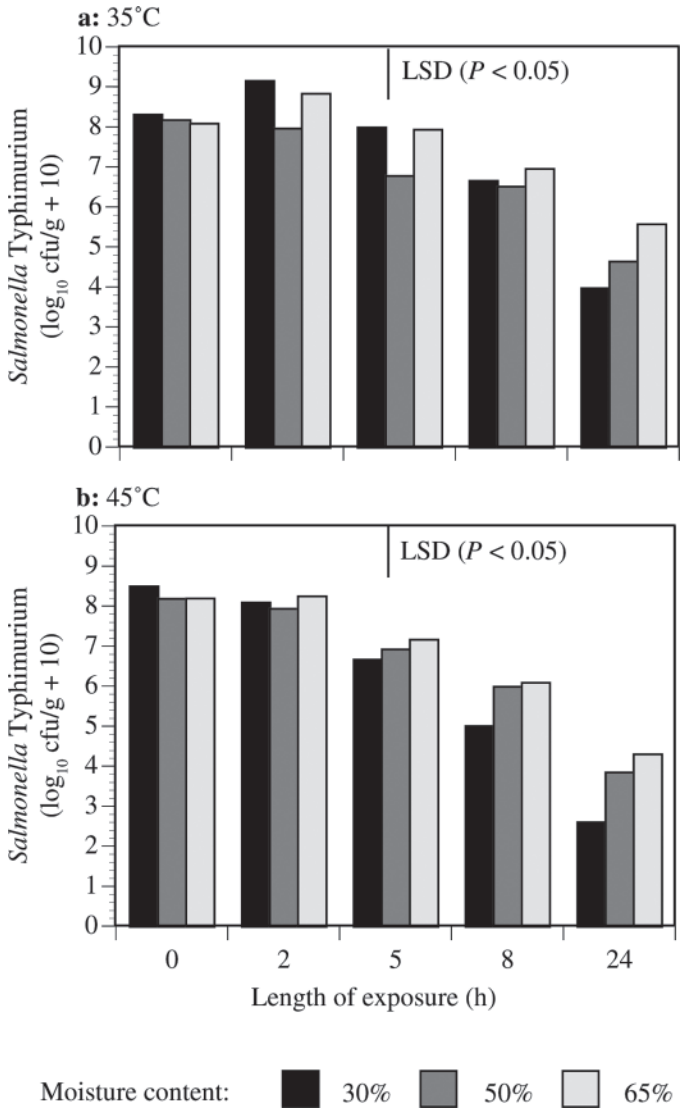


**Figure 3.** Effect of temperature (35 and 45°C) and moisture content (30, 50, and 65% wt/wt, wet basis) on persistence of *Salmonella enterica* serovar Typhimurium in poultry litter. Data for treatment combinations at 55 and 65°C are not presented because *Salmonella* Typhimurium was eliminated within 1 h (first sampling) at these temperatures.

litter in the static windrow consistently being lower in temperature (Figure 4a) than that in the turned windrow (Figure 4b), particularly after 21 d. During the first 21 d, the 2 windrows followed a similar pattern of temperature distribution, and both windrows peaked above 65°C. Temperatures in the 35 to 44°C range predominated in the static windrow during the middle period (21 to 63 d) of the trial (Figure 4a). In the static windrow, 7% of the litter (estimated from percentage of surface area in the cross-section) was exposed to temperatures of 45°C and above during this period, compared with about 54% in the turned windrow. From 21 to 63 d, about equal portions of litter in the turned windrow were exposed to the 35 to 44°C and 45 to 54°C temperature ranges (Figure 4b).

The fact that *E. coli* persisted under these conditions even in the turned windrow either suggests that the prescribed time-temperature requirements were inadequate or reflects the difficulty of ensuring that the time-temperature requirements were met by all compost particles (Gale, 2002; Wichuk and McCartney, 2007). Although turning apparently improved thermophilic conditions, it may not have been enough to prevent regrowth of bacteria, especially when significant portions of the litter could have bypassed high-temperature conditions.

Turning did appear to have a marginal effect on regrowth of *E. coli* when poultry litter was sampled from the windrows and incubated in laboratory conditions. Overall, litter from the static windrow reacted in a manner similar to that from the turned windrow. However, incubation of unamended 6-wk-old static-windrow lit-

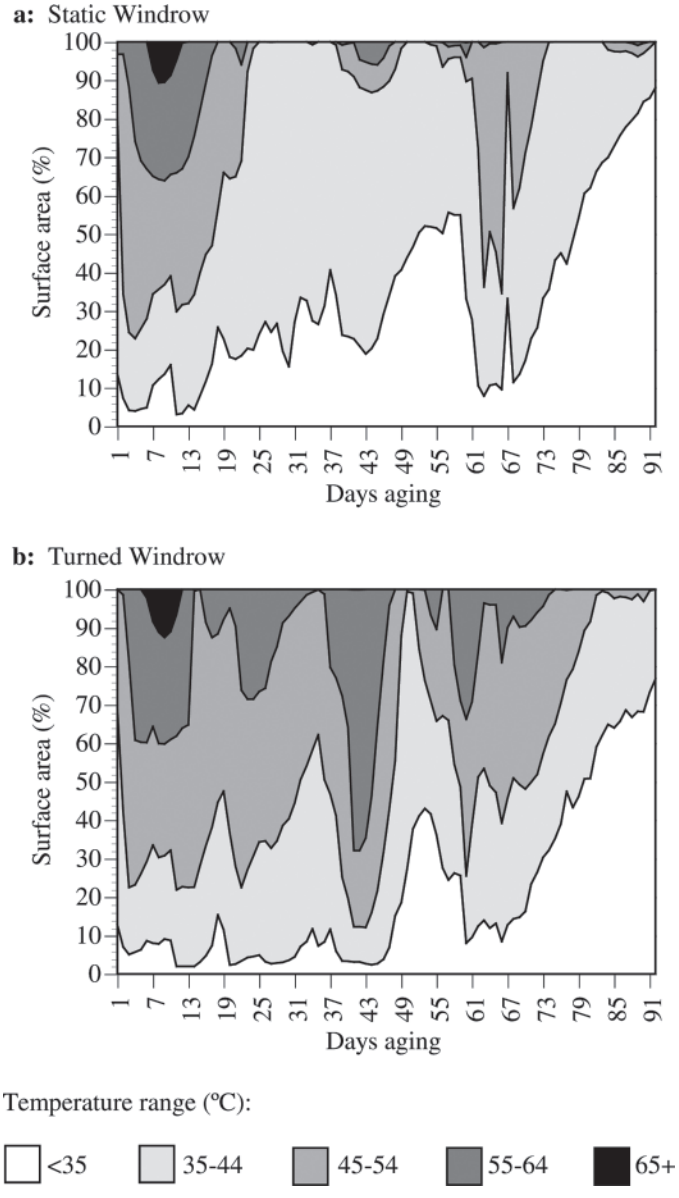


**Figure 4.** Percentage of surface area of litter exposed to a given temperature range over a 12-wk aging process in the static and turned windrows. Temperature ranges are average daily temperatures taken in the grid pattern, as shown in Figure 1.

**Table 1.** Regrowth and persistence of *Escherichia coli* in poultry litter of different ages from turned and static windrows

| Litter age (wk) | Incubation period[1] (d) | *E. coli* [$\log_{10}$ (cfu/g + 10)] | | | |
|---|---|---|---|---|---|
| | | Pretreatment of litter from turned windrow[2] | | Pretreatment of litter from static windrow[2] | |
| | | None | Amended | None | Amended |
| 0 | 0 | 1.46[b] | 3.68[a] | 4.06[a] | 4.17[ab] |
| | 7 | 3.44[ab] | 1.58[a] | 2.79[a] | 2.55[ab] |
| | 14 | 2.16[ab] | 2.85[a] | 1.79[a] | 2.01[b] |
| | 21 | 4.56[a] | 3.22[a] | 2.20[a] | 4.65[a] |
| 3 | 0 | 3.47[a] | 1.51[b] | 2.85[a] | 2.84[b] |
| | 7 | 4.79[a] | 3.50[ab] | 2.53[a] | 4.94[ab] |
| | 14 | 2.16[a] | 2.13[ab] | 2.83[a] | 3.49[b] |
| | 21 | 3.85[a] | 4.50[a] | 1.99[a] | 6.29[a] |
| 6 | 0 | 3.35[ab] | 4.55[ab] | 3.12[bc] | 1.80[b] |
| | 7 | 5.25[a] | 6.86[a] | 5.96[a] | 7.52[a] |
| | 14 | 1.66[b] | 2.85[bc] | 4.70[ab] | 1.00[b] |
| | 21 | 1.78[b] | 1.00[c] | 1.55[c] | 3.04[b] |
| 12 | 0 | 4.50[a] | 3.88[a] | 4.43[a] | 3.40[ab] |
| | 7 | 3.67[ab] | 3.34[ab] | 3.23[ab] | 4.14[a] |
| | 14 | 1.00[b] | 1.00[b] | 1.00[b] | 1.00[b] |
| | 21 | 1.00[b] | 1.00[b] | 1.00[b] | 1.00[b] |

[a–c]Means differ at $P < 0.05$ when common superscripts are lacking for each litter pre-treatment (column) and litter age (i.e., 0, 3, 6 or 12 wk) data set.

[1]Incubation at 37°C for up to 21 d before *E. coli* counts were assessed.

[2]Pretreatment of litter before incubation: none = litter incubated without moisture content adjustment; amended = moisture content of litter amended to 50% (wt/wt, wet basis).

ter or litter adjusted to 50% (wet basis) moisture content resulted in a significant increase in *E. coli* counts after 7 d (Table 1). A similar trend was observed in the turned windrow, but the results were not statistically significant. Increased *E. coli* counts were observed only after 21 d of incubation in litter aged up to 3 wk in the turned windrow ($P < 0.05$).

After 21 d of incubation, *E. coli* counts increased by more than 1,900-fold in unamended fresh litter (0 wk old) from the turned windrow (Table 1). Adjusting the moisture content to 50% increased *E. coli* counts in litter from both windrows after 21 d of incubation, but only in 3-wk-old litter. In 12-wk-old unamended litter from both windrows, counts were not reduced significantly until 14 d of incubation, when *E. coli* could no longer be detected (Table 1).

Other studies have shown that pathogens can regrow in fresh manure initially, but after storage for a few days, numbers decline rapidly. For example, Himathongkham and Riemann (1999) showed that *E. coli* O157:H7 and *L. monocytogenes* were able to multiply by as much as 100-fold for a period of 2 d in fresh chicken manure at 20°C while *Salmonella* Typhimurium densities remained stable. Prolonging the storage time to 6 d resulted in a 1- to 2-log decrease of *Salmonella* Typhimurium compared with the initial count and a 3- to 4-log decrease of *E. coli* O157:H7. At the same time, numbers of *L. monocytogenes* did not decrease below the initial count. Similarly, when fecal coliforms were added to 5 stacked poultry litter samples obtained in Georgia, numbers decreased from >7.00 $\log_{10}$ cfu/g of litter to below detectable levels within 8 d (Hartel et al., 2000). These samples had been incubated at 28°C. When the incubation temperature was lowered to 18°C, the fecal coliform count decreased more slowly to 2.87 $\log_{10}$ cfu/g after 16 d.

Moisture, organic carbon availability, and microbiological competition are the key factors that influence the regrowth of pathogens in organic materials (Russ and Yanko, 1981; Hussong et al., 1985; Millner et al., 1987; Soares et al., 1995). Thus, persistence or regrowth of bacteria, or both, occurred in our study, possibly as a result of the slow progress of stabilization accompanying the aging process. Organic carbon availability was not directly determined in our study, although there were indications that the process of stabilization was far from complete after 12 wk of aging. The ammonium-to-nitrate ratio of the litter in both windrows was 10 or more by wk 12, higher than at the beginning of the experiment (Table 2). According to Bernal et al. (1998) and Paré et al. (1998), a stable and mature product is indicated by an ammonium-to-nitrate ratio of <1. The moisture content of the litter (16 to 24%) was also well below the optimal levels (50 to 65%) required for rapid stabilization (Epstein, 1997). The germination and toxicity test results were also consistent with an immature compost-like product, probably as a result of the high levels of ammonium present (Table 2).

High temperatures could still be achieved in poultry litter despite low rates of microbial decomposition, probably because the litter had low thermal conductivity and low gas permeability. In addition to the heat

**Table 2.** Changes in the physicochemical properties of poultry litter during an aging process in a static and turned windrow

| | wk 0 | | wk 3 | | wk 6 | | wk 12 | |
|---|---|---|---|---|---|---|---|---|
| Property[1] | Static | Turned | Static | Turned | Static | Turned | Static | Turned |
| Moisture (%, wt/wt, wet basis) | 16 | 18 | 22 | 22 | 22 | 24 | 21 | 23 |
| pH | 8.2 | 8.3 | 7.6 | 8.1 | 8.4 | 8.3 | 8.4 | 8.6 |
| C:N | 10 | 9.4 | 9.2 | 9.1 | 10 | 9.4 | 8.5 | 8.3 |
| Ammonium-N (mg/L) | 390 | 380 | 450 | 370 | 420 | 530 | 580 | 600 |
| Nitrate-N (mg/L) | 69 | 46 | 44 | 26 | 11 | 10 | 58 | 37 |
| Ammonium:nitrate ratio | 5.6 | 8.2 | 10.2 | 14.2 | 38.2 | 53 | 10 | 16.2 |
| Germination test (%) | ND[2] | ND | 0 | 0 | 0 | 0 | 10 | 0 |
| Toxicity index[3] (%) | ND | ND | 0 | 0 | 0 | 0 | 7 | 0 |

[1]Tested according to standard AS 4454 (Standards Australia, 1999).

[2]ND = test not done.

[3]Ten radish seeds were germinated on the poultry litter sample and a reference sample that is known to be nontoxic. Data shown are the length of roots after 3 to 5 d of incubation as a percentage of the reference samples.

generated by microbial activity, chemical oxidation can be the cause of increasing temperatures in organic materials, which in some cases leads to spontaneous combustion in dry conditions (Buggeln and Rynk, 2002). Sometimes a compost-like product appears to be stable, but it is only so because it is too dry to support high rates of microbiological activity. On rewetting of these materials, an ideal environment can be provided for pathogens to repopulate (Soares et al., 1995).

## ACKNOWLEDGMENTS

This work was financially supported by Horticulture Australia Pty Ltd. (Sydney, Australia), VegFed (Wellington, New Zealand), T. D. & E. C. Ould Pty Ltd. (Cranbourne, Australia), Lightowler Fowl Manure Pty Ltd. (Somerville, Australia), and C. L. & A. K. Warlan Pty Ltd. (Winchelsea, Australia). The authors thank Tom Glanville (Iowa State University, Ames) for critically reviewing this paper.

## REFERENCES

Bernal, M. P., A. F. Navarro, M. A. Sanchez-Monedero, A. Roig, and J. Cegarra. 1998. Influence of sewage sludge compost stability and maturity on carbon and nitrogen mineralization in soil. Soil Biol. Biochem. 30:305–313.

Brackett, R. E. 1999. Incidence, contributing factors, and control of bacterial pathogens in produce. Postharvest Biol. Technol. 15:305–311.

Buggeln, R., and R. Rynk. 2002. Self-heating in yard trimmings: Conditions leading to spontaneous combustion. Compost Sci. Util. 10:162–182.

Bush, D. J., M. H. Poore, G. M. Rogers, and C. Altier. 2007. Effect of stacking method on *Salmonella* elimination from recycled poultry bedding. Bioresour. Technol. 98:571–578.

Capucille, D. J., M. H. Poore, C. Altier, and G. M. Rogers. 2002. Evaluation of *Salmonella* shedding in cattle fed recycled poultry bedding. Bovine Pract. 36:15–21.

Capucille, D. J., M. H. Poore, and G. M. Rogers. 2004. Growing and finishing performance of steers when fed recycled poultry bedding during the growing period. J. Anim. Sci. 82:3038–3048.

Chaudhry, S. M., J. P. Fontenot, and Z. Naseer. 1998. Effect of deep stacking and ensiling broiler litter on chemical composition and pathogenic organisms. Anim. Feed Sci. Technol. 74:155–167.

de Bertoldi, M., F. Zucconi, and M. Civilini. 1991. Temperature, pathogen control and product quality. Pages 195–199 in The BioCycle Guide to the Art and Science of Composting. The JG Press Inc., Emmaus, PA.

Doyle, M. P., and M. C. Erickson. 2008. Summer meeting 2007—The problems with fresh produce: An overview. J. Appl. Microbiol. 105:317–330.

Epstein, E. 1997. The Science of Composting. Technomic Publishing AG, Basel, Switzerland.

Farrell, J. B. 1993. Fecal pathogen control during composting. Pages 282–300 in Science and Engineering of Composting: Design, Environmental, Microbiological and Utilization Aspects. H. A. J. Hoitink and H. M. Keener, ed. Renaissance Publications, Worthington, OH.

Gale, P. 2002. Risk Assessment: Use of Composting and Biogas Treatment to Dispose of Catering Waste Containing Meat. Department for Environment, Food and Rural Affairs, London, UK.

Gibbs, R. A., C. J. Hu, G. E. Ho, and I. Unkovich. 1997. Regrowth of faecal coliforms and *Salmonellae* in stored biosolids and soil amended with biosolids. Water Sci. Technol. 35:269–275.

Golueke, C. G. 1991. When is compost safe? Pages 220–229 in The Biocycle Guide to the Art and Science of Composting. J. Goldstein, ed. The JG Press Inc., Emmaus, PA.

Harapas, D., R. Tomkins, K. Wilkinson, P. Franz, and R. Premier. 2003. Strategies for the safe use of poultry litter in food crop production. Proc. 4th Int. Conf. ORBIT Assoc., Perth, Western Australia, Australia. ORBIT e.V., Weimar, Germany.

Hartel, P. G., W. I. Segars, J. D. Summer, J. V. Collins, A. T. Phillips, and E. Whittle. 2000. Survival of fecal coliforms in fresh and stacked broiler litter. J. Appl. Poult. Res. 9:505–512.

Himathongkham, S., and H. Riemann. 1999. Destruction of *Salmonella typhimurium*, *Escherichia coli* O157:H7 and *Listeria monocytogenes* in chicken manure by drying and/or gassing with ammonia. FEMS Microbiol. Lett. 171:179–182.

Hussong, D., W. D. Burge, and N. K. Enkiri. 1985. Occurrence, growth, and suppression of *Salmonellae* in composted sewage sludge. Appl. Environ. Microbiol. 50:887–893.

Martin, S. A., M. A. McCann, and W. D. Waltman II.. 1998. Microbiological survey of Georgia poultry litter. J. Appl. Poult. Res. 7:90–98.

Millner, P. D., K. E. Powers, N. K. Enkiri, and W. D. Burge. 1987. Microbially mediated growth suppression and death of *Salmonella* in composted sewage sludge. Microb. Ecol. 14:255–265.

Paré, T. H., H. Dinel, M. Schnitzer, and S. Dumontet. 1998. Transformations of carbon and nitrogen during composting of animal manure and shredded paper. Biol. Fertil. Soils 26:173–178.

Pepper, I., K. Josephson, R. Bailey, M. Burr, and C. Gerba. 1993. Survival of indicator organisms in Sonoran Desert soil amended with sewage sludge. J. Environ. Sci. Health A Environ. Sci. Eng. Toxicol. 28:1287–1302.

Rankins, D. L., M. H. Poore, D. J. Capucille, and G. M. Rogers. 2002. Recycled poultry bedding as cattle feed. Vet. Clin. North Am. Food Anim. Pract. 18:253–266.

Russ, C. F., and W. A. Yanko. 1981. Factors affecting *Salmonellae* repopulation in composted sludges. Appl. Environ. Microbiol. 41:597–602.

Soares, H. M., B. Cárdenas, D. Weir, and M. S. Switzenbaum. 1995. Evaluating pathogen regrowth in biosolids compost. Biocycle 36:70–74.

Standards Australia. 1991a. AS 1766.2.13: Food Microbiology—Examination for Specific Organisms—*Campylobacter*. Standards Australia, Sydney, Australia.

Standards Australia. 1991b. AS 1766.2.5: Food Microbiology—Examination for Specific Organisms—*Salmonellae*. Standards Australia, Sydney, Australia.

Standards Australia. 1998. AS/NZS 1766.2.16.1: Food Microbiology—Examination for Specific Organisms—Food and Animal Feeding Stuffs—Horizontal Method for the Detection and Enumeration of *Listeria monocytogenes*—Detection Method. Standards Australia, Sydney, Australia.

Standards Australia. 1999. AS 4454: Composts, Soil Conditioners and Mulches. Standards Australia, Sydney, Australia.

Terzich, M., M. J. Pope, T. E. Cherry, and J. Hollinger. 2000. Survey of pathogens in poultry litter in the United States. J. Appl. Poult. Res. 9:287–291.

US EPA. 2003. Control of Pathogens and Vector Attraction in Sewage Sludge (Including Domestic Septage) Under 40 CFR Part 503. Environmental Regulations and Technology. US Environmental Protection Agency, Cincinnati, OH.

Wichuk, K. M., and D. McCartney. 2007. A review of the effectiveness of current time-temperature regulations on pathogen inactivation during composting. J. Environ. Eng. Sci. 6:573–586.