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Persistence of *Salmonella* during Dairy Manure Compost at Different Sampling Events and Thermal Conditions

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Abstract. *Application of animal manure to agricultural land is considered as a viable option in exchange of commercial fertilizer application considering the environmental impacts. However, animal manure can be a source of several pathogenic bacteria such as Salmonella which can survive in manure or manure-amended soil for extended period of time. Considering the economic and environmental significance associated with animal manure application into cropland, a laboratory scale bench top study was conducted to determine the inactivation trend in dairy manure compost. Composted solid samples were collected at various sampling frequencies and different thermal conditions for two weeks to get a holistic understanding about the inactivation of Salmonella in initial phase of composting process. Collected samples were analyzed according the Bacteriological Analytical Manual (BAM) suggested by USFDA. Results from the analysis found almost 5 orders of magnitude reductions from initial Salmonella concentration of 1.6 x 10^9 cfu/ gm in compost at 37°C over the period of the experiment. While evaluating the effects of sampling time (between morning and evening) during the first three days of experiment, there was significant interaction between day and time of sampling in the compost samples. In addition to 37°C composting study, an intensive heat stress study was carried out at 48 and 58°C by collecting samples at 30 mins interval for 2 hrs (0, 30, 60, 90, and 120 mins) to observe the effect of elevated temperatures on inactivation of Salmonella during the 10^{th} day of the experiment and the results showed that the change in Salmonella levels was inconsistent in composted material. We anticipate that the results will help in improving dairy manure management, and also provide scientific insight to the policy makers and farm owners, while using composting as a method of animal manure treatment.*

Keywords. *Compost, Dairy, Manure, Salmonella, Temperature.*

Introduction

Approximate 0.9 billion tons of cattle manure is generated in the United States (US) annually, which can potentially fertilize around 5% of total USA croplands (USDA, 2009). Applications of animal manure to crop land are seen as an alternative to chemical fertilizers for replenishing soil nutrients. A disadvantage of applying animal manure into cropland can contribute to pathogenic pollution in the environment. The presence of pathogens such as *E.coli* O157:H7, *Salmonella spp.*, *Campylobacter*, *Clostridium perfringens*, *Listeria monocytogens* etc in manure can potentially contaminate food and water (Zhao et al. 1995, Pell 1997). Several studies found pathogen contamination in vegetables grown in the cropland receiving manure (Natvig et al. 2002, Islam et al. 2004 a,b).

Stored manure, feces, lagoon water, and bedding are considered as the major reservoir of pathogens in dairy farms (Toth et al. 2013, Murinda et al. 2004). Previous research reported that these pathogens can survive in solid manure or slurry for extended period of time (Heinonen-Tanski et al. 1998, Himathongkham and Riemann 1999, Himathongkham et al. 1999, Jiang et al. 2002, You et al. 2006, Shepherd et al. 2009, Erickson et al. 2009, Erickson et al. 2014). Treating animal manure prior to land application can potentially help in controlling pathogen influx into cropland.

Among the pathogens, *Salmonella* is the second most confirmed single-etiology outbreaks and illnesses in the U.S. according to CDC (2014). *Salmonella* alone causes more than1.2 million infections each year that accounts for $365 million in direct medical cost (CDC, 2014). It is not only a human health hazard but also responsible for the clinical disease in cattle like fever and diarrhea (Smith 2002, Toth et al. 2011).

Thus, it is import to understand the inactivation mechanisms of *Salmonella* in manure piles before applying it to the field. In order to identify the survival characteristics of *Samonella enterica* in dairy farm environment, Toth et al. (2011) found that pathogen can survive >137 days. Baloda et al. (2001) showed that *Salmonella* could survive up to 299 days in a terrestrial microcosm study.

Controlling animal waste pathogens is essential due to the health risk associated with food borne pathogens. Considering the growing number of *Salmonella* related foodborne outbreaks, and previously proven linkages between animal manure borne pathogens and public health risk, we designed a laboratory scale bench top study to identify the optimum thermal conditions required for inactivating *Salmonella* in dairy manure piles. The specific objectives of the study were to: i)determine the decay pattern of *Salmonella* levels at 37°C, ii) evaluate the impact of sampling event (morning vs evening) on *Salmonella* count, iii) understand critical die-off time of *Salmonella* during heat stress , and iv) compare the efficiency of the temperatures to control the *Salmonella* levels in animal manure.

Material and Methods

The fresh dairy manure sample was collected from the University of California, Davis dairy facility located on the campus. After collection, manure samples were stored at -20°C before starting the experiment. Prior to start the experiment, feedstock was prepared by thawing the manure sample. About 4.27 kg of dairy manure was thawed and mixed with 4.5L of deionized water where the final pH of the mixture was 7.27. It was then sieved through a 850 µm (ASTM #20) mesh to separate the residue from the liquid slurry (pH=7.7 at 37 °C). The sieved residue (solid manure) was then used for the compost study.

Pure strain of *Salmonella typhimurium LT2* (ATCC # 700720) was grown overnight in Luria-Bertani (LB) broth (Difco LB Broth Miller; Becton, Dickinson and Company, Sparks, MD, USA) on a bench top incubator shaker (MaxQ 4000, Thermo Scientific, Ohio, USA) at 100 rpm and 37 ^{0}C for 24 hrs. To ensure the quality control, a negative control of the respective growth media was used. The initial concentration of *Salmonella* was enumerated from the inoculum. Afterwards, 30 ml pure culture of *Salmonella* was centrifuged at 10,000 rpm for 15 min to form the pellet. After draining the supernatant, the pellet was dissolved in 5 ml phosphate-buffered saline (PBS) before adding with the dairy solid manure. Initial samples were collected to enumerate the initial concentrations of pathogens in solid manure. The manure was then placed inside an open heating chamber to ensure the constant heating at 37°C. Daily mixing was provided to the manure pile manually. The experiment

was conducted for two weeks. While samples were collected twice (morning and evening) during the first three days, subsequently dairy sampling was performed for the next eleven days of the incubation.

Salmonella enumeration from the compost was conducted using the Bacteriological Analytical Manual (BAM) (FDA, 2014). Xylose Lysine Desoxycholate (XLD) agar (Difco, Becton, Dickinson and Company, Sparks, MD, USA) was used for the isolation and differentiation of *Salmonella*. It appears as red-yellow with black centers in the agar plates. Slurry samples were serially diluted in PBS, platted, and incubated for 24 hrs at 35°C to ensure the growth before enumeration.

Compost samples were plated in duplicate and enumerated to determine the change in *Salmonella* concentration during the experiment. Statistical analysis was conducted to evaluate the significant change in *Salmonella* concentrations in compost over the time of experiment. Additionally, effects of sampling time was evaluated between morning and evening at 37°C for the first three days of experiment. Subsequently, an intensive heat stress study was carried out at elevated temperatures and samples were collected at 30 mins interval for 2 hrs (0, 30, 60, 90, and 120 mins) to observe the thermal (increased temperature) effect on inactivation of *Salmonella* during the day 10 of experiment. Finally, a comparative study was carried out among the three (37, 48, and 58°C) temperatures to evaluate the inactivation during the last 4 days of experiment. All the statistical analysis was done using PROC GLIMMIX in SAS (Little et al, 1996). An alpha level of $\alpha = 0.05$ was used to identify significant differences among treatments by "least significant difference" methods.

Results and Discussions

Results from the compost sample analysis found significant change in concentration over the period of 14 days experiment period when the initial concentration of *Salmonella* in compost manure went from 1.6×10^9 cfu/ gm to 3.2×10^5 cfu/ gm by the end of experiment (Figure 1). An overall decreasing trend was observed during the first 9 days of experiment when the concentration of *Salmonella* went to lowest (1.5×10^5 cfu/ gm) and then showed periodic rise and fall. The moisture content in the compost sample went from 78% to 16% during that time of experiment. When Toth et al. (2013) surveyed the 13 dairy operations in Pennsylvania, the authors found the presence of *Salmonella* in fresh fecal samples as well as in stored manure. In a previous study, Toth et al (2011) found that *Salmonella* Newport was eliminated in the static compost pile after 18 hrs of incubation at 64°C. Very few studies reported complete elimination of *Salmonella* in compost piles. When *Salmonella* was inoculated in dairy manure, Nicholson et al. (2005) observed that it survived for 4 days. Ahmed and Sorensen (1995) found a 2.5 days for log reduction time from biosolid compost at 38°C. While composting fecal matter with food waste, Vinneras (2007) reported a 2.4 log reduction over initial concentration after 5 days of composting inside a 90 L reactor. In addition to decay of *Salmonella* over the time during composting, the increase in *Salmonella* levels during the initial phase of composting has also been reported. As an example, Russ and Yanko (1981) observed an increase in pathogen concentration in the initial phase of digestion. The experiment was carried out at a relatively low temperature (28 and 36°C). After an initial increase, pathogen levels went below detection limit when the compost temp was reached to 44°C.

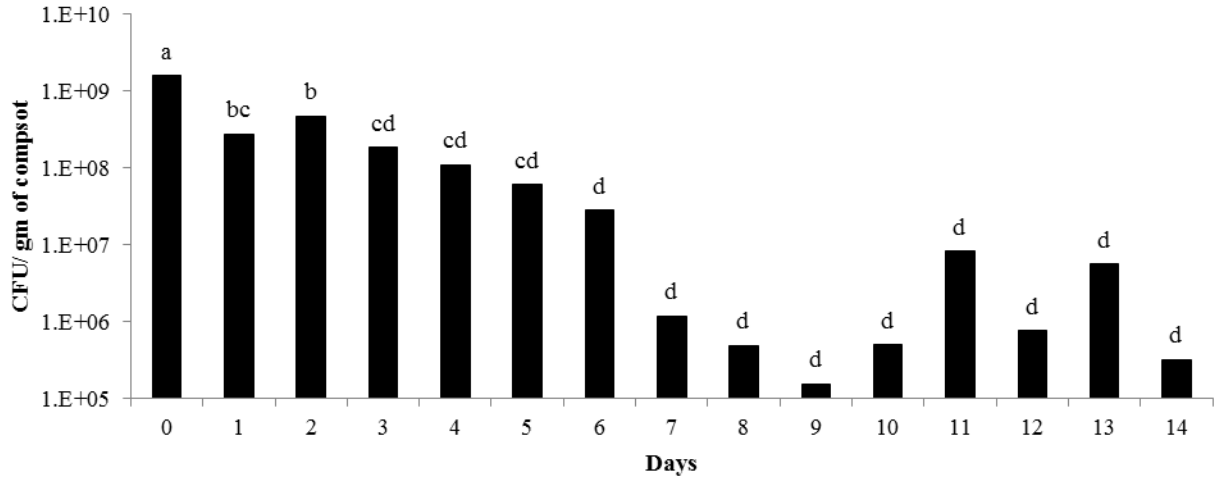

Figure 1. Change in concentration of *Salmonella* in compost at 37°C (similar letters on top of bars show no significant differences at α=0.05)

While analyzing the samples collected at morning and evening during the first three days of experiment, there was a significant day and time interaction effect (p=0.0029) on the concentration of *Salmonella* (Figure 2). The count of *Salmonella* was different between morning and evening sampling event during the first two days of experiment but not on day 3.

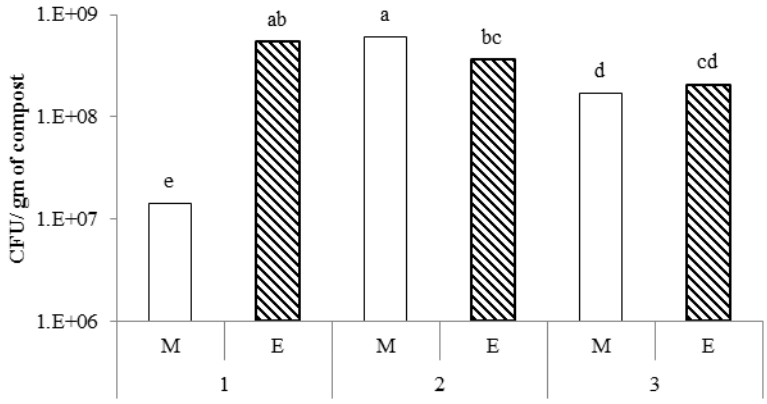

Figure 2. Change in *Salmonella* concentration in morning and evening sampling events during the first three days of experiment at 37°C (similar letters on top of bars show no significant differences at α=0.05)

The results from the heat stress study during the 10th day of experiment are shown in Figure 3. During the heat stress study, when the samples were collected at 30 mins interval for 2 hrs at 48 and 58 °C, statistical analysis showed a significant interaction between time and temperature (p<0.0001). There was almost an order of magnitude increase in concentration within 30 minutes over the initial concentration after the start of study at 48°C and almost two orders of magnitudes increase in concentration at 58°C (within 30 minutes). Although the concentration went down to two order or magnitude at 58°C during the next two sampling events, pathogen levels kept increasing at 48°C at 60 min sampling event and then went down at 90 min sampling event. Again the concentration went up to 1.97×10^7 cfu/ gm during the last sampling event at 48°C. At 58°C, the concentration increased to 1.34×10^7 cfu/ gm during the final sampling event. A quick fluctuation of *Salmonella* concentration in compost was noticed (Fig. 3).

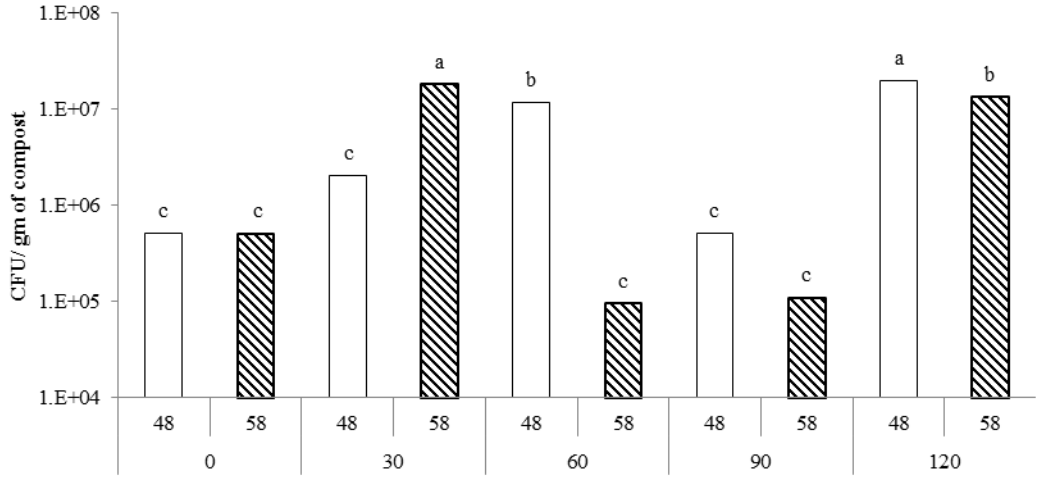

Figure 3. Heat stress study at 48°C and 58°C during the Day 10 of experiment (similar letters on top of bars show no significant differences at α=0.05)

While comparing the three temperatures (37, 48, and 58 °C) during the last four days (11, 12, 13, and 14) of experiment, there was a significant difference in *Salmonella* concentration in compost samples of 37°C compared to 48 and 58°C at day 11 and day 13 (Figure 4). However, there was no significant difference in concentrations among the samples incubated at three temperatures at day 12 and day 14 of experiment. It can be summarized from the heat stress and comparative thermal study that higher temperature alone may not be the confirming indicator parameter to assess the inactivation of *Salmonella* in composted dairy manure.

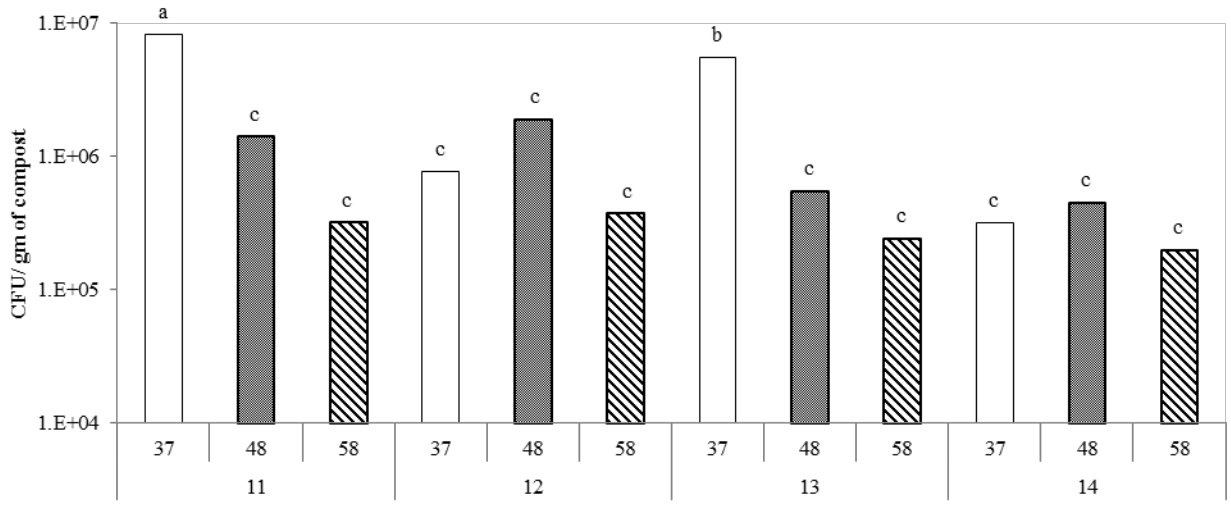

Figure 4. Comparative thermal study at 37, 48 and 58°C during the last three days of experiment (similar letters on top of bars show no significant differences at α=0.05)

Conclusion or Summary

Identifying the optimum time and temperature is required to inactivate pathogens in a compost pile to ensure food safety before the compost is being applied to the crop land. Several previous studies reported that a temperature of 55°C for 2-3 days will be sufficient to destroy the pathogens in biosolid compost (Burge et al. 1982, Zaleski et al. 2005). According to the regulation of USEPA for composting biosolids, a minimum temperature of 55°C is required for 3 days in aerated static piles or in-vessel systems (at 55°C), and 15 days in windrow systems. Thus the objective of the study was to assess the *Salmonella* inactivation at multiple temperatures. The results showed that the inactivation process of *Salmonella* varied according to time (morning vs evening), temperature (heat stress and comparative study), and duration of study in compost. During composting, both decay and growth was observed. Results indicate that the elevated temperature reduced the *Salmonella* levels considerably; however, the recurrence of *Salmonella* in solid manure can be a concern. Further studies are required to improve understanding of *Salmonella* inactivation dairy manure. On-farm studies focused on understanding the effects of changing temperature (i.e., seasonality) on *Salmonella* growth and decay can help in improving the animal waste management and thus minimizing the risk of pathogens to water and food.

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