Annotated Bibliography for Pathogen Survival in Soil Amended with Raw Manure

Supplemental material for the document:

Prepared by Marilyn Erickson, University of Georgia

Review. Based on various studies, migration of microorganisms occurs preferentially through macropores, worm holes, cracks, and fractures. Mathematical modeling of this phenomenon is limited because of the simplifying assumptions used in its development.

A significant increase in the number of E. coli cells passed through a soil column when macropores were present and the soil was wet. There was no passage of E. coli cells through a dry soil with macropores. No E. coli were eluted from columns without macropores even when the soils were wet. Simulated rainfall applied on the top of the soil columns caused bacteria to travel deeper into the soil.

Buffer width treatments were implemented by placing cattle fecal material containing known loads of C. parvum 0.1, 1.1 or 2.1 m up-slope of the runoff collector. Buffers with an increased percent land slope exhibited reduced retention efficiencies. The majority of C. parvum oocysts (2 to 5 log) were retained in the fecal matrix for the duration of the storm season, irrespective of the presence of a vegetated buffer. These results supported the assertion that grassland buffers are an effective method for reducing animal agricultural inputs of waterborne C. parvum.)

E. coli levels in freshly deposited faeces from all three species were similar (around 7 log CFU/g). Random 5 cm deep soil cores were taken. E. coli originating from cattle, sheep, and pigs had average decimal reduction times of 38, 36, and 26 days, respectively. The faster rate of E. coli decline when originating from pig feces may have been due to physiological differences in the E. coli strains and/or the chemistry of the feces.
After 14 days, the condition of the soil was different for each livestock species. The ground on which the pigs had been penned was substantially trampled, with no remaining intact grass, and very few individual fecal droppings visible, as they had mostly been incorporated into the soil. Although the grass in the cattle pen was also largely depleted, some intact fecal deposits were visible on the surface of this pen. The ground in the sheep pen still contained visible grass, with a root system and topsoil, and the sheep feces, appeared mostly intact.

Significantly higher numbers of S. Weltebreeden inoculated into manure and applied to soil before planting spinach were found in soil than in pot culture, where the pathogen had been inoculated directly into soil 14 days postplanting. May be attributed to a better developed spinach root system in the latter case, and leading to more pronounced effects of the rhizosphere on S. Weltebreeden stimulation. Moreover, the pathogen appeared to be mobilized from manure to spinach roots, as the number of contaminated pot cultures steadily increased throughout the evaluation period. When pathogen cells were added to the soil in a manure mixture, no Salmonella cells were recovered above the threshold level (4 log cells/g) on leaves when added to the soil in a manure mixture (used a molecular method for detection). When bacteria were added in saline solution and added

Prepared by Marilyn Erickson, University of Georgia
(https://extension.uga.edu/about/staff/index.cfm?pk_id=5686) 2-2-12
directly to soil 14 days after sowing and fertilization, cells were detected in all replicate pots on days 0 and 8 postinoculation. Moreover, *Salmonella* persisted on leaves in some of the replicates up to 21 days postinoculation.


Long-term persistence of the *Salmonella enterica* serovar Typhimurium DT12 clone occurred in the herd environment. Furthermore, when *Salmonella*-contaminated slurry was disposed of on the agricultural soil (a common waste disposal practice), the pathogen was isolated up to 14 days after the spread.


Manure spiked with *Salmonella enterica* serovar Senftenberg was applied to either the soil surface or injected 0.08 m into the soil to compare leaching over 36 d. Columns averaged 0.24 m height. The total recovery of leached *S. enterica* in drainage samples ranged from 0.08% to 13.8%. There was no statistically significant difference in the leaching concentration of *S. enterica* at each sampling time during the study period. In addition, comparison of enumerations by selective plating and real-time polymerase chain reaction yielded similar concentrations of *S. enterica*, indicating that mainly viable and culturable cells were leached from the columns. The profiles showed that the area covered by active pores ranged from 0.1% to 3.6%.


Most cells leached were viable. The loamy monoliths had significantly higher concentrations of *Salmonella* serovar Typhimurium cells in drainage water than the sandy monoliths. Pathogen remained culturable for at least a month under moist and cold conditions. Between 1.5% and 3.8% of the applied test strain (10 log cells applied to column) was still viable in the top 0.2 m after 28 days. The loamy soil is a well-structured soil with a clay content of 21 to 39%, whereas the sandy soil is less structured and contains 5% clay.


Soil isolates, as a group, were not genetically distinct from fecal isolates, with only 0.8% of genetic variation and no fixed mutations attributed to the isolate source. Controlling for the spatial pattern made it possible to detect environmental gradients of pH, moisture, and organic matter corresponding to the genetic structure of *E. coli* in soil. Therefore, while fecal deposition is the major predictor of *E. coli* distributions on the field scale, selection imposed by the soil environment has a significant impact on *E. coli* population structure and potentially amplifies the occasional introduction of stress-tolerant strains. Results suggested that *E. coli* genotypes displayed variation in persistence at different soil pH values.


An alternative management strategy than traditional pond storage and land application for disposal of feedlot includes vegetative treatment systems. A typical vegetative treatment system consists of a settling basin for solids collection and a vegetative treatment area for water and nutrient utilization. In this study, the liquid was discharged onto a 4.5-ha area of bromegrass which was harvested as hay. Populations of generic *E. coli* declined over time, as did the isolation frequencies of *E. coli* O157:H7 and *Campylobacter* spp. *E. coli* O157:H7 was isolated from only one of a total of 60 freshly-cut hay samples harvested from the vegetative area that had received basin runoff, before baling. Neither pathogen was recovered from hay following baling and storage.
Isolation of Cryptosporidium oocysts and Giardia cysts from vegetative samples was infrequent, indicating differences in sedimentation and/or transport in comparison to bacteria.


Review that addresses the pathogenic microorganisms in manure, storage and land application of manure, movement of microorganisms in soil and groundwater, impact on surface water, and management and treatment options.


Slurry was deposited by four different methods: (1) hose applicator on black soil followed by ploughing and harrowing; (2) hose applicator on black soil followed only by harrowing; (3) hose applicator on a field with winter-wheat seedlings without further soil treatment; (4) slurry injector on a field with winter-wheat seedlings without further soil treatment. *E. coli* and *Salmonella* could not be detected at all in soil following treatment (1). Following the other treatments, *E. coli* was not detected in soil samples after day 21 and *Salmonella* was no longer detected after day 7. Simulation results showed that clinical (4 log CFU/g) and sub-clinical *Salmonella* levels (2500 CFU/g) would fall below the detection limit within 10 or 5 days, respectively. Results show that ploughing and harrowing of soil amended with contaminated pig slurry was an effective means to reduce environmental exposure to *E. coli* and *Salmonella* on this clay-soil farm.


The purpose of this study was to determine the relative concentration and infectivity of *C. parvum* oocysts released from manure and leached through columns of undisturbed, macroporous karst soil. Manure (200 g containing 10^7 oocysts) was applied to the surface of each soil core. One liter of rain was applied from a distance of 2 cm to each column twice a week at a rate of 2.8 cm/h per simulation (approximates the 2-year, 1-h storm for the area). Two-thirds of the total oocysts were released in the first 7 days in all experiments. The short fall distance might have been a major factor accounting for the low oocyst release rates as the drops did not reach terminal velocities as natural raindrops would. At the beginning of leaching experiments, about 85% of oocysts were infective. After 12 weeks, oocysts leaching from soil columns (50 cm long) that had been maintained at 10°C were 20% infective. Substantially more oocysts leached from undisturbed soil columns than disturbed soil columns and the number of oocysts recovered in the leachate of the 10°C soil columns was about 1.5 times the number recovered in the leachate of the 25°C soil columns. Cool temperatures therefore appear to increase rates of release of oocysts from manure and leaching through soil.


Bovine feces were inoculated with *E. coli* O157:H7 (8–9 log CFU/g). A 4 to 5 log decrease in *E. coli* O157:H7 within 50 days was recorded in inoculated cow pats placed on grassland. After 50-57 days, the deposited material became integrated into the soil and was no longer detectable. The organism was still detectable in the surrounding soil for up to 99 d.


Observed that release of Cryptosporidium and Giardia from manure increased as water conductivity increased. Experimental observations indicate that only the surface layer of manure was depleted of finer manure materials and (oo)cysts and that the manure would act as a long-term source of contamination.

This report examined the distribution and survival of bacterial indicator organisms (fecal coliforms and fecal streptococci) on land used for disposal of piggery effluent. Persistence of fecal coliforms was greater in topsoil than pasture or subsoil. Times required for 90% reduction in topsoil ranged from 7 to 20 days. Autumn and winter conditions were conducive to the persistence of a survivor tail of these bacteria and 1 to 3 log cells/g topsoil. Fecal streptococci survived similarly on soil and pasture and were considered more suitable for survival in the environment than fecal coliforms. Variability in the number of indicator organisms in pig effluent had a bigger effect on the number of organisms in soil than did the range of application rates normally used on farms.


The first of four patients with *E. coli* O157:H7 infection had a diet almost exclusively of vegetables taken from a garden to which cow and calf manure had been applied repeatedly. No pathogen was detected in fecal specimens from these animals; however, they both had antibodies to *E. coli* O157 lipopolysaccharide. An isolate of *E. coli* O157:H7 was isolated from the garden soil and this isolate had a PFGE pattern identical to the pattern obtained from an isolate collected from the stool of the fourth patient.


The estimated average time required to reach undetectable concentrations of *E. coli* in sandy loam varied from 56 to 70 days, whereas the absence of *E. coli* was estimated at 77 days in loamy sand. The maximal *Salmonella* persistence in soil was 54 days. Soil samples were taken at a depth of 20 cm every 2 weeks after June application of organic and inorganic fertilizers.


The experimental design was a 2 x 3 factorial. Poultry manure was surface applied at approximately 36.5 and 164 metric tons/ha to two types of soil (clay loam and sandy loam) contained in boxes (surface area of 0.0929 m² and depth of 40.6 cm) that were held in a controlled environmental chamber at 24.5°C. Soil samples were analyzed at intervals during a 30-day period. A soil sample consisted of the composite of two soil cores taken to a depth of 5 cm on a randomized grid from a specific treatment. An inactivation rate constant of 0.29/day was observed for fecal coliforms during the first seven days whereas an inactivation rate constant of 0.093/day was observed for fecal streptococci during the first 3 days. With both groups of organisms, the initial die-off was followed by a period of regrowth. Neither soil type nor manure application rate seemed to influence the decline in organism populations and was attributed to the fact that the majority of organisms were retained at the surface in a crust layer and did not interact directly with the soil.


This article addressed the effects of physical and chemical characteristics of the environment, mathematical modeling approaches of bacterial die-off, and a summary of past investigations of bacterial die-off in storage systems, soil and fresh/sea water environments.

Conducted experiments to measure bacterial and phage transport in runoff from fields amended with manure from animals fed either corn or 40\% wet distillers' grain rations under both till and no-till cropping conditions (manure applied to meet corn phosphorus requirements). Manure from animals fed distillers' grains has different physical, nutrient, and microbial characteristics from manure from animals fed traditional corn finishing diets including higher dissolved and total phosphorus levels and greater concentrations of some pathogenic bacteria. Rainfall was applied to soil field plots (0.75 x 1.5 m) for 30 min at an intensity of 70 mm/h. Afterwards, the entire volume of runoff was collected and subsamples taken for analysis. Based on the counts of bacteria in the source manure at the time of application, there was a relatively low percentage of bacteria transported in the runoff. No significant differences in transport of fecal indicator bacteria occurred in response to the different manure types; however, there was higher phage recovery in runoff from plots amended with manure from distillers' grain-fed animals.


Ellis, J.R., and T.M. McCalla. 1978. Fate of pathogens in soils receiving animal wastes – A review. ASAE Paper No. 76-2560. St. Joseph, MI: ASAE. This review discusses soil as a biological filter, survival after immobilization, and fate of specific microorganisms. It makes a statement in the abstract that with proper application of animal waste to land, there are rarely health problems for animals or humans and as such, it is a safe method of waste decontamination. Included in the review is a table summarizing reported pathogen survival in animal wastes and a table on reported pathogen survival in soil. It acknowledges that organic matter is one of several factors affecting removal and survival of bacteria, viruses, and helminthes in soil but does not address specifically the application of manure to soils.

Entry, J.A., R.K. Hubbard, J.E. Theis, and J.J Fuhrmann. 2000. The influence of vegetation in riparian filterstrips on coliform bacteria: II. Survival in soils. J. Environ. Qual. 29:1215-1224. Total and fecal coliform numbers were evaluated at 3 depths (0 to 5, 5 to 15, and 15 to 30 cm depths) after application of swine wastewater and were found to decline approximately 10-fold every 7 to 14 days in all seasons of the year. Total and fecal coliforms at the 3 soil locations correlated with temperature and moisture in a curvilinear relationship. Vegetation type did not affect survival of total and fecal coliform bacteria.

Erickson, M.C., C.C. Webb, J.C. Diaz-Perez, S.C. Phatak, J.J. Silvoy, L. Davey, A.S. Payton, J. Liao, L. Ma, and M.P. Doyle. 2010. Infrequent internalization of Escherichia coli O157:H7 into field-grown leafy greens. J. Food Prot. 73:500-506. E. coli O157:H7-contaminated compost was added to field plots to give a low level of contamination (1.2-2.2 log CFU/g) and a high level of contamination (3.7-4.0 log CFU/g). Smooth and savoy spinach, leafy green lettuce and parsley were subsequently transplanted into these field plots. Seven weeks later, only 1 of 40 soil samples from field plots contaminated at the lower level was found to be E. coli O157:H7-positive by enrichment, whereas 12 of 40 soil samples were positive by enrichment for this pathogen in plots contaminated at the higher level.

Fenlon, D.R., I.D. Ogden, A. Vinten, and I. Svoboda. 2000. The fate of Escherichia coli O157 in cattle slurry after application to land. J. Appl. Microbiol. 88S:149S-156S. Initially, almost all Escherichia coli were retained in the upper layers of the soil. Numbers steadily declined to less than 1\% of those applied by day 29, and E. coli O157 was only detected in the soil and on the grass for the
first week after application. Some bacteria were transported to deeper layers of the soil, but this was approximately 2% of the total; transport to drains over the same period was mainly associated with rainfall events and amounted to approximately 7% of applied \( E. coli \). The sandy soil was least conducive to survival. In loam and clay soils, survival times were considerably longer.


Simulated rainfall events of 55 mm/h for 30 min were applied to the bovine fecal pats. Transportation efficiency increased with decreasing size of the microorganism studied; \( Cryptosporidium \) oocysts were the least mobile followed by \( E. coli \) and then PRD1 phage. Rainfall events mobilized 0.5 to 0.9% of the \( Cryptosporidium \) oocysts, 1.3-1.4% of \( E. coli \) bacteria, and 0.03-0.6% of PRD1 and transported them a distance of 10 m. Subsequent rainfall events applied to aged fecal pats only mobilized 0.01-0.06% of the original \( Cryptosporidium \) oocyst load, between 0.04-15% of the \( E. coli \) load and 0.0006-0.06% of PRD1 bacteriophages, respectively.


Rotary tilled in farm yard manure and manual hoeing (Mech I), ploughed in farm yard manure (Mech II) and manual hoeing, rotary tilled in composted farm yard manure (Mech III) and manual hoeing. Used flame weeding (gas torch), plastic mulch, and straw mulch to control weeds. Soil samples were collected at two depths: 0–10 and 0–30 cm. \( E. coli \), \( Enterococcus \), and \( Salmonella \) were not detected in any soil sample prior to manure application. Enterobacteraceae counts ranged between 3.6 and 4.1 log CFU/g. \( E. coli \) not detected initially after application while detected approx. 8 weeks later. Supports the view that manure promotes microbial abundance including pathogenic groups. In order to clarify whether there is evidence for an accumulation of enteric pathogens in soils repeatedly amended with manure, soil samples from different agricultural fields (i.e. manure or mineral fertilizer application) were collected and analyzed. In none of the samples were \( Salmonella \) or \( E. coli \) detected while \( Enterococcus \) was randomly determined in low amounts in several soil samples.


Vegetative filter strips (VFS) composed of ryegrass used in conjunction with low, medium and high infiltration flow rates. Two experimental conditions explored: 1) diluted liquid swine manure runon, and 2) clean water runon 48 h afterward. The \( E. coli \) mass reductions ranged from 22 to 71% and were strongly correlated to infiltration or runoff reduction, which was dependent on the degree of flow concentration. Little to no effect of sedimentation on \( E. coli \) transport was observed. Hypothesized latter results were due to minimum \( E. coli \) attachment to sediment particles because the bacteria originated from manure sources.


The fate of \( E. coli \) O157:H7 in manure-amended soil was modeled per unit manure since it was most likely that pathogen cells introduced via manure would stay attached to manure particles and redistribute throughout the manure-amended soil mass. The density of \( E. coli \) O157:H7 numbers in manure-amended soil at time of planting lettuce seedlings was calculated with the Weibull decline model. A minimum storage time of 30 days and a minimum fertilization-to-planting interval of 60 days were most successful in reducing the risk. Sensitivity analysis revealed that the likelihood of contamination was most sensitive to the prevalence of contaminated manure, the manure storage time and the initial density of \( E. coli \) O157:H7 in naturally contaminated manure. Increasing the manure storage time (to a minimum of 30 days) and incorporating a fertilization-to-planting interval of at least 60 days were most successful in reducing the number of contaminated lettuce heads. Some specific organic farming practices involving manure and soil management were found to be risk reducing. The model estimated an average of 0.34 contaminated heads per hectare.

Review article that reported survival times of *E. coli* O157:H7 in manure amended soils range between several weeks and more than 6 months. Generally, survival has been reported to be prolonged in finer textured soils. However, most of these studies only included a limited number of different soils, which does not fully justify generalized conclusions on the effect of soil type. Few attempts have been made to link survival of *E. coli* O157:H7 with soil physico-chemical and biological variables. With respect to manure-amended soil, the survival time of *E. coli* O157:H7 in organic soils was best explained by the level of dissolved organic nitrogen (positive relation) and the Eubacterial species richness (negative relation). It has been hypothesized within macro-ecology that species richness and/or diversity will increase with increased resource heterogeneity and that a community becomes more susceptible to invasion whenever there is an increase in the amount of unused (available) resources because the invader will encounter less intense competition from resident species.


Fifty g inoculated portions of manure were added to 450 g of soil to give 7 log CFU/g and placed in 1 L pots and held in darkness at 16°C. The survival curves generally showed a concave curvature, indicating changes in biological stress over time but the calculated time to reach the detection limit (ttd) ranged from 54 to 105 days. Although the initial decline was faster in sandy soils, no significant differences were observed in ttd between both sandy and loamy soils; however, demonstrated that the survival of the copiotrophic *E. coli* O157:H7 in manure-amended soil was longer in more copiotrophic manure-amended soil systems. Concluded that *E. coli* O157:H7 populations declined faster under more oligotrophic soil conditions, which can be achieved by the use of organic fertilizer with a relatively high C/N ratio and consequently a relatively low rate of nutrient release. In addition, increased levels of easily available energy sources in DOC may (temporarily) decrease the competitive pressure between organisms and thus possibly allow increased persistence.


*E. coli* O157:H7 or *Salmonella* serovar Typhimurium were added to a final density of 7 log CFU/g dry weight manure then 60 g of manure mixed with 540 g soil (1:9). Pots incubated at 15°C in darkness. The pathogens' fate in manure-amended soil were monitored after they declined to relatively low and more realistic levels in manure (approximately 2 log CFU/gdw for *E. coli* O157:H7 and 4 log CFU/gdw for *Salmonella* serovar Typhimurium.) Survival of *E. coli* O157:H7 in the soils amended with both manures varied between 2 and 56 days. *E. coli* O157:H7 declined significantly faster in all organically managed soils than in the conventionally managed neighboring soils. *E. coli* O157:H7 disappeared exceptionally rapidly in the organic sandy soil. *Salmonella* serovar Typhimurium was in most cases still detected at 56 days after application of the manure to the soils. The two *Salmonella* serovar Typhimurium phenotypes showed quite different patterns of decline rate over the treatments. No consistent differences were found between organic and conventional soils.


The field survival of STEC was monitored in cowpats and underlying soils. A MPN-PCR stx assay was used to enumerate STEC populations. STEC levels ranged between 3.9 and 5.4 log CFU/g in fresh cowpats. In total, 8 PFGE-patterns were identified, corresponding to 6 distinct serotypes (i.e. O116:NM, O174:H21, O91:H10, O113:NM, O2:H27 and O175:H16. Serotypes O91:H10 and O113:NM were able to persist for at least 2 months in cowpats. The low concentrations of STEC found in soil implied that a small proportion of STEC populations
carried by faecal matter were likely to enter the soil and/or that the major part of STEC cells introduced into the soil were rapidly killed and/or leached into deeper soil layers. PFGE data analysis showed the 3 STEC strains isolated from water differed from those recovered from the cowpat and soil samples, indicating the existence of an alternate source of STEC contamination (wildlife) in the watershed and/or the presence of other circulating STEC clones in the dairy herd.


STEC 026 was initially present in manure-amended soil (two loam soils and one clay loam soil; 4 kg of soil to 200 g manure) at 6 log CFU/g and was stored at 4 or 20°C. STEC O26 was able to persist during extended periods in soil even in the presence of low moisture levels, i.e. less than 0.08 g water/g soil. Observed kinetics of STEC counts were correctly fitted by the log-linear model with tailing. In the one loam soil, STEC survived for 288 and 196 days at 4 and 20°C. In the other loam soil and in the clay loam soil, STEC survived for at least 365 days at both temperatures. The ambient temperature was significantly associated with the highest STEC count decline in all soils tested.


In this study, assessed the ability of potential pathogens (E. coli O157:H7 and total coliforms) to travel through soils with different textures. We also assessed whether manure trapped pathogens at the soil surface or was a source of nutrients that enhanced pathogen survival. Steady rainfall consisting of 16.5 mm/h was applied to 100 mm disturbed soil cores that were treated with manure and inoculated with Escherichia coli O157:H7 strain B6914. The level of B6914 in leachate was near the inoculum level each hour for 8 h, as was the level of B6914 at several soil depths after 24 h, indicating that there was a high rate of growth. Total B6914 levels exceeded the inoculum levels for all treatments except intact clay loam cores. The presence of manure often increased total B6914 leachate and soil levels in intact cores but had the opposite effect on disturbed soil cores. Soluble nitrogen may enhance transport.


In fallow soils, E. coli O157:H7 persisted for 25-41 days, on rye roots for 47-96 days and on alfalfa roots, in a silt loam soil, for 92 days, whereas on other legumes persistence ranged from 25-40 days, similar to fallow soil. Low persistence of E. coli O157:H7 on alfalfa and perhaps on other legume roots may be due to competition with a higher indigenous coliform population on these roots. Clay increased persistence and activity of E. coli O157:H7 and other coliforms. Manure did not enhance E. coli O157:H7 populations in fallow soils, potentially indicating that these soils contained nutrients similar to or resulting from previous applications of manure. Coliforms persisted longer in uninoculated treatments compared to similar treatments where E. coli O157:H7 was inoculated and indicated the pathogen's probable competition with indigenous flora for resources such as water, nutrients and niches.


Developed risk assessment models for seven pathogens, salmonellas, Listeria monocytogenes, campylobacters, Escherichia coli O157, Cryptosporidium parvum, Giardia, and enteroviruses. The models demonstrated the large potential impact of decay in the soil for predicting infections that could result from consumption of root crops on agricultural land to which treated sewage sludge had been applied in accordance with the Safe Sludge Matrix (12- or 30-month harvest intervals). Due to lack of sufficient data for decay of pathogens over the time intervals in the Matrix, linear reduction in the log-transformed counts over time was assumed. Using this assumption, it was stated that a 12-month harvest interval eliminated the risks from all seven pathogens and the highest risk was associated with C. parvum (one infection in the UK every 45 years).

Soil and manure-amended soil was inoculated with tetracycline-resistant *Salmonella* serovar Typhimurium at 1.5 x 10^8 cells/g soil. Over a period of 42 days at 5, 15, and 25°C, population densities were monitored by both plate counting and by molecular methods (DNA and *invA* mRNA). A detection limit of 10^6 *Salmonella* occurred with the *invA* mRNA analysis method. After 4 days, *invA* mRNA could no longer be detected in any of the soil scenarios. Higher values were found using DNA-based techniques than CFU levels. Based on plate count data, survival of *Salmonella* serovar Typhimurium was reduced by the addition of manure at 5 and 15°C while no significant differences were observed at 25°C. A negative correlation was found between *Salmonella* serovar Typhimurium CFU levels and protozoan most probably numbers. Soil samples were shaken with a phosphate buffer extractant to dislodge the pathogen from soil particles, hence, *Salmonella* within intracellular protozoan vesicles may have occurred and led to underestimation of the actual population of the pathogen.


Review article.


Swine manure was inoculated with *Salmonella anatum* (2.3 x 10^5 CFU/ml) and was applied to land at four rates: none, 0.5 x the agronomic rate of 37,000 L/ha, 1 x the agronomic rate, and 2x the agronomic rate. Immediately after application, the manure was incorporated to 10 cm by disking. Soil samples (2 cm deep) were collected over 144 days; however, freezing of the soil between days 39 and 130 occurred. Pathogen numbers were higher in soil as a result of manure application compared to the control treatment with no manure applied. *Salmonella*, fecal coliforms, and male-specific coliphages declined quickly in the runoff mixing zone and survival time did not relate to manure application rate. Somatic coliphages were more persistent with survival up to 143 days and survival positively correlated to the manure application rate.


The survival and transfer of *Listeria innocua* and *Clostridium sporogenes*, used as surrogates of the foodborne pathogens *Listeria monocytogenes* and *Clostridium botulinum*, were quantitatively assessed under field conditions. In the soil, spores of *C. sporogenes* declined by less than 0.7 log cycles within 16 months and were detected on parsley leaves throughout the experiment. In contrast, *L. innocua* in the soil declined by 7 log cycles in 90 days and was detected on leaves in low numbers (>0.04 MPN g(-1)) during the first 30 days. Rates of decline in soil were similar in the laboratory at 20 degrees C for two strains of *L. innocua* and *L. monocytogenes*; and in the field for *L. innocua* over two different years. *L. innocua* survived better in winter, indicating an important influence of temperature. The major cause of transfer of *L. innocua* from soil to parsley leaves was splashing due to rain and irrigation. As few as 1 CFU/g *Listeria* in soil led to contamination of parsley leaves. Internalisation of *Listeria* through parsley roots was not observed. Under the conditions of soil and climate studied, a delay of 90 days between application of potentially contaminated fertilizer and harvest should be sufficient to eliminate *L. monocytogenes*. 

Prepared by Marilyn Erickson, University of Georgia

(http://extension.uga.edu/about/staff/index.cfm?pk_id=5686) 2-2-12

Simulated at a microcosm level, a single application event of either cattle manure or 60-d anaerobically digested manure into both $\gamma$-irradiated and non-sterilised soils (80 kg N/ha), incubated these systems at 20°C for up to 3 months, and then analysed the survival of *E. coli*, *Salmonella*, and *Listeria*. Digested manure did not contain *E. coli* or *Salmonella* but did contain *Listeria* at $\sim$4 log CFU/g. *Listeria* was significantly more abundant in sterilized than in non-irradiated treatments indicating suppression by indigenous soil microbiota. *Listeria* populations decreased to control levels (3 log CFU/g) within 3 months.


Included in this review is a table itemizing outbreaks where manure has been implicated as the source of pathogens, a section discussing the effect of increased hog production, and a section reviewing the environmental survival of the major zoonotic pathogens from swine and cattle with subsections addressing survival in water, soil, and manure.


Bovine manure slurry was applied at a rate of 11.7 liters/m$^2$ to both bare and vegetated (blue clover and white clover mixture) plots (0.5 x 0.3 m) containing either sandy loam or clay loam soils and having a 20% slope. During 1-h rainfall simulations, where variations in rainfall intensity was approximated by varying the distance from the nozzle to the plot, concentrations of chloride ion, fecal coliforms, organic carbon, and phosphorus were measured in runoff collected from troughs at the edges of the plots at 5-min intervals. Kinetics of the fecal coliform release from manure was similar to the release kinetics of phosphorus and organic carbon.


Fecal coliforms were released from manure slurry applied on the top of the plots of bare sandy loam and clay loam plots and those that contained a 6-m vegetated filter strip (VFS). The VFS efficiency was found to be <95% in 25%, < 75% in 23%, and <25% in 20% of cases. Relatively long high-intensity rainfalls, low hydraulic conductivities, low net capillary drives of soil, and high soil moisture contents before rainfalls caused the partial failure of VFS to retain coliforms from the infiltration excess runoff.


Dairy manure was placed on turfgrass soil sod. Significant differences in release kinetics of *E. coli* and enterococci were found but not in the subsequent transport and this may affect the efficiency of using these organisms as indicators. A change from first-order release kinetics to zero-order kinetics after 1 h of rainfall simulation was observed.


A microscopic study revealed the presence of a coating on the surface of the Tyler sand, while this coating was absent from the BARC sand fractions. In the absence of manure colloids, bacterial attachment to soil, silt, and clay particles was much higher than the attachment to sand particles having no organic coating. The attachment to the coated sand particles was similar to the attachment to silt and clay. In the presence of manure colloids, a pronounced decrease occurred in the numbers of fecal coliforms attached in clay and silt fractions while a minor
decrease was noted in all sand fractions. The low attachment of bacteria to silt and clay particles in the presence of manure colloids may cause predominantly free-cell transport of manure-borne FC in runoff.


Suspensions of fecal coliforms (10³, 10⁴, or 10⁵ CFU/ml) in water or water-manure were prepared. To these suspensions, air-dried and water-saturated soil aggregates were submerged. The maximum association of fecal coliforms occurred with air-dried soil aggregates in the water-fecal coliform suspensions which were 300 times greater than the number of fecal coliforms associated with aggregates in the water-manure-fecal coliform suspensions and 2.5 times greater than fecal coliforms associated with water-saturated aggregates. The antecedent aggregate water content therefore was a significant factor affecting fecal coliform association with soil aggregates. It was conjectured that mechanisms of bacterial transport into aggregates were different with water-saturated and air-dried aggregates, Brownian diffusion and chemotaxis being the primary mechanism for the former case, and a capillary pressure gradient serving as the mechanism in the latter case. Clogging of intraaggregate fine pores by manure colloids and/or competition with other manure-borne organisms for attachment sites was offered as explanations for the substantially diminished fecal coliform interaction with air-dried aggregates in the presence of manure.


Spatial variability of bacterial concentrations in applied manure introduces high uncertainty in the predictive model predictions that estimate concentrations and total numbers of pathogen and indicator organisms leaving manure-fertilized fields. Faecal coliform (FC) concentrations in manure measured in 2004, 2005, 2007, and 2009 varied by 4 orders of magnitude each year. The smallest median FC concentration of 1.8 x 10⁸ CFU/g observed in 2005 was about 6 times less than the largest median concentration of 1.1 x 10⁵ CFU/g observed in 2007. The soil in this study was a coarse loamy sand in the top soil. The study area had been used for field corn production. Forty-six 20-g manure samples were taken randomly from the applied area and immediately after manure application to assess spatial variability of FC content in the applied manure. Runoff constituted a very small part of total precipitation in the observed events which was expected given the sandy texture of the studied soil. Higher rainfall intensity caused larger numbers of bacteria cells released from the manure and faster overland transport toward the flume. The droplet impact on soil surface increases with the rainfall rate and is likely to result in seal formation and macropore sealing that reduces hydraulic conductivity and becomes the infiltration-limiting factor. Monte Carlo simulations showed that using average FC concentrations led to substantial overestimation of the FC fraction reaching the edge of the field. Inaccurate representation of bacteria concentrations in manure with a small number of samples substantially distorted estimates of bacteria loss from the field in runoff. It was found that at least 15-20 samples were needed to correctly represent the uncertainty related to the uncertainty in applications.


Macropores can allow bacteria and pathogens to bypass the soil’s natural filter capacity and increase the risk of surface water and groundwater contamination. Micropores, mesopores, and macropores are defined as pore spaces with equivalent diameters of 5 to 30 µm, 30 to 75 µm, and larger than 75 µm, respectively. With macropores, wetting fronts propagate to significant depths by bypassing matrix pore space. Natural fractures originate from soil expansion and contraction or from geological processes. Biopores, on the other hand, are created by tunneling insects, small animals, nematodes, and decaying roots.

Significant differences in release kinetics of *Escherichia coli* and enterococci from each of the fecal matrices were determined. The order of release of *E. coli* from the fecal matrices (greatest to least, expressed as a percentage of the total present) was dairy cattle slurry > beef cattle FYM > beef-cattle feces > sheep feces. The relatively high percentage of *E. coli* released from all four fecal materials at the first analysis (day 1 or day 3) suggest they are probably associated with the more liquid fraction of animal manures. Generally, the sheep-fecal material remained relatively intact during the release phase whereas the beef-cattle feces tended to disaggregate more readily during the release phase.


Loamy sand and clay soils. Six-strain cocktail of *Salmonella* serovars (Agona, Hadar, Heidelberg, Montevideo, Oranienburg, and Typhimurium) were added to yield 5 log CFU/g directly to soil or to manure added to soil. Manure was mixed either throughout the soil or with the top kilogram of soil and the entire soil volumes adjusted to 60% or 80% of field capacity. Soil treatments were stored 180 d at temperature sequences representing winter to summer, spring to summer, or summer to winter. *Salmonella* numbers decreased during application to soil and the largest decreases occurred within the first week. Higher soil moisture, manure addition, and storage in the clay soil increased *Salmonella* survival. *Salmonella* survived longest in both soils during summer-winter exposure but was not isolated after 160 d from loamy sand soil exposed to other seasonal treatments. Soil samples were collected to 10 cm.


Wastes contaminated with *L. monocytogenes*, *Salmonella*, *Campylobacter*, *E. coli* O157, and *Cryptosporidium parvum* (approx. 6 log CFU/g) for bacterial pathogens and 8 log oocysts/plot were spread onto a grass pasture. There were no significant differences among the decimal reduction times for the bacterial pathogens as well as no significant differences between the rates of pathogen decline in liquid (slurry) and solid (farmyard manure) wastes. The mean bacterial reduction time was 1.94 days. The levels of most of the zoonotic agents had declined to below detectable levels by 64 days. However, for some waste types, 128 days was required for the complete decline of *L. monocytogenes* levels. The recovery of oocysts from plots was poor, and, typically, less than 0.005% of the oocysts inoculated were actually recovered over the course of the experiments.


For the batches of waste used in the present study, no zoonotic bacteria survived longer than 120 days (initial concentration of 6 log CFU/g). Bacterial decline was monitored over time and found to be significantly more rapid for all waste types when they were left on the soil surface. There were no significant differences in initial bacterial decline rates when wastes were spread in summer or winter. Leaving waste on the soil surface increases the likelihood of insect infestation and the spread of zoonotic agents to the wider environment. Furthermore, leaving wastes on the surface increases the possibility that rainfall heavy enough to cause surface runoff could wash pathogens and manures directly into watercourses where they are likely to last longer than those in terrestrial environments. Results indicated that not incorporating contaminated livestock wastes into soil was a potential intervention measure that may help to limit the spread of zoonotic agents further up the food chain.


Soils (collected from river beds) were fumigated with methyl bromide and methyl iodide. Both fumigants were effective in reducing *E. coli* O157:H7 concentrations in soil, and when fumigated soils were compared with nonfumigated soils, pathogen concentrations were significantly higher in the nonfumigated soils throughout the study. This resulted in a longer survival of the pathogen on the leaf surface especially in sandy soil than
observed in fumigated soils. Therefore, application of fumigant may play some roles in reducing the transfer of \( E. coli \) O157:H7 from soil to leaf.


Noncomposted bovine manure was applied to loamy sand, silt loam and silty clay loam prior to spring and summer planting of carrots, radishes, and lettuce. Soil and washed (30 s under running water) vegetables were analyzed for indigenous \( E. coli \). Within 90 days, the level of \( E. coli \) in manure-fertilized soil generally decreased by about 3 log CFU/g from initial levels of 4.2 to 4.4 log CFU/g. Low levels of \( E. coli \) generally persisted in manure-fertilized soil for more than 100 days and were detected in enriched soil from all three sites 132 to 168 days after manure application. For carrots and lettuce, at least one enrichment-negative sample was obtained ≤ 100 days after manure application for 63 and 88% of the treatments, respectively. The current ≥ 120-day limit provided an even greater likelihood of not detecting \( E. coli \) on carrots. The absolute absence of \( E. coli \) from vegetables harvested from manure-fertilized Wisconsin soils may not be ensured solely by adherence to the NOP ≥ 120-day limit.


Contaminated compost was applied to soil the day before lettuce and parsley seedlings were transplanted in late October 2002. \( E. coli \) O157:H7 persisted for 154 to 217 days in soils amended with contaminated composts and was detected on lettuce and parsley for up to 77 and 177 days, respectively, after seedlings were planted. Very little difference was observed in \( E. coli \) O157:H7 persistence based on compost type alone.


Compost inoculated to 7 \( \log \)/g and mixed with soil at a 1:5 ratio. Seedlings transplanted into pots. O157 decreased within 64 days by 3 \( \log \) CFU/g in soil and soil beneath the roots of green onions and by more than 2 \( \log \) CFU/g on onions. O157 survived better during the production of carrots with a 2.3 \( \log \) CFU/g reduction in soil and a 1.7 \( \log \) CFU/g reduction on carrots within 84 days. In general, the moisture content of soil surrounding green onions, as well as soil immediately beneath onion roots, was lower than that of soil for growing carrots. Onions reportedly contain more total phenolics than carrots.

Contaminated compost was applied to soil on day before lettuce and parsley transplanted. *Salmonella* was detected for up to 63 days and 231 days on lettuce and parsley, respectively. *Salmonella* persisted for 161 and up to 231 days in soils amended with contaminated composts. At 63 days when still detected on lettuce, soil had *Salmonella* populations of 2–4 log CFU/g.


O157 survived in soil samples for 154-196 days, and was detected for 74 and 168 days on onions and carrots, respectively. O157 survival was greatest in soil amended with poultry compost and least in soil containing alkaline-stabilized dairy manure compost. Survival profiles of *E.coli* O157:H7 on vegetables and soil samples, contaminated either by application of contaminated compost or irrigation water, were similar. Composts were applied to soil as a strip at a rate of 4.5 metric tons/ha before carrots and onions were sown.


Low amounts of bacterial indicators (1.9–4.7%) are released in runoff water from swine-slurry-amended soils, whereas greater amounts (1.1–28.3%) of these indicators are released in runoff water from cattle-manure-amended soils.


Covered in this review are a detailed summary of factors affecting survival of pathogens and indicator organisms in soil systems including moisture, soil type, temperature and pH, manure application rate and characteristics, nutrient availability, and biological competition. The review also covers transport of pathogens and indicator organisms in soils receiving manure and separates the studies addressing this issue into field studies and column studies. Manure management strategies to minimize bacterial leaching have been subdivided into those addressing manure storage and pretreatment, timing and rate of application, and manure application method.


The soil water potentials investigated did not affect oocyst inactivation at any temperature or with any of the 3 soil types (silty clay loam, silt loam, and loamy sand). Oocyst survival appeared to be significantly greater in the silt loam than in the two other soil types when incubated at 20°C. At 30°C, oocyst survival was significantly less in the silt clay loam than in the other two soil types. Thus, oocyst survival was affected by soil texture but temperature appeared to be the factor most affecting oocyst survival.


O157 cells survived for up to 77, > 226, and 231 days in manure-amended autoclaved soil held at 5, 15, and 21°C, respectively. Pathogen populations declined more rapidly in manure-amended unautoclaved soil under the same conditions, likely due to antagonistic interactions with indigenous soil microorganisms. O157 cells were inactivated more rapidly in both autoclaved and unautoclaved soils amended with manure at a ratio of 1 part manure to 10 parts soil than in soil samples containing dilute amounts of manure.

Initial *L. monocytogenes* cell numbers of 5 to 6 log CFU/g survived for up to 43, 43, and 14 days in manure-amended autoclaved soil at 5, 15, and 21°C, respectively. In manure-amended unautoclaved soil, the pathogen was detectable for up to 43, 21, and 21 days at 5, 15, and 21°C, respectively. *L. monocytogenes* was inactivated more rapidly in autoclaved soil amended with manure at a manure/soil ratio of 1:10 than in the more dilute (1:100) manure in soil samples at both 15 and 21°C. However, in manure-amended unautoclaved soil, *L. monocytogenes* survived longer in samples with ratios of 1:10 than in the more dilute (1:100) manure-amended soil.


Seedlings were transplanted into the beds after 3 weeks and kept at 15°C during light period of 20 h (150 to 250 µmol/m2/s) and 12°C for 4-h dark period. Watered with sprinkler but care was taken to avoid splash of soil onto the plants. Soil was fertilized with bovine manure that had been inoculated with 10⁴ CFU/g *E. coli* O157:H7. Samples of lettuce taken 2 and 7 weeks after transplanting (stomached cut pieces). After 1 week of application of manure, the *E. coli* O157:H7 numbers in the soil were below the detection limit of the enumeration method (<100 CFU/g) but detected through enumeration in 10 of 10, 3 of 10, and 2 of 10 samples of soil at 1, 4, and 8 weeks, respectively. *E. coli* O157:H7 was not isolated from any of the lettuce, outer leaves, or root samples analyzed.


The results show that significant amounts of bacteria can reach surface water by infiltrating through the soil and travelling through sub-surface tile drains to the receiving water. Rain shortly after manure application is suggested to be the most important indicator of bacterial contamination rather than spreading rate (volume applied per unit area) or condition of the field prior to spreading.


Bovine manure and bovine manure compost inoculated with *Salmonella* Newport (8.6 log CFU/g) was placed in a glass jar and covered with soil containing 50 nematodes (*Caenorhabditis elegans*)/g. Lettuce, strawberry, and carrots placed on top of the soil contained the bacterial pathogen on their surfaces 1 – 3 days later in the manure systems whereas it was found on the surfaces of the produce items, 1 – 7 days later in the manure compost systems. This study demonstrated that *C. elegans* had the potential for transporting *S. Newport* in soil to the surface of preharvest fruits and vegetables.


It was reported that manure affects oocyst attachment to soil in a complex manner. Initially, dilute manure (0.1%) enhanced oocyst attachment to soil particles but increased manure content (1%) led to less attachment. It was conjectured that some facilitating component in the manure was offset by higher biomass concentrations in the 1.0% manure suspension, which competed with oocysts for soil attachment sites. Resuspension of soil-manure-oocyst pellets in freshwater led to detachment of oocysts from soil particles and the magnitude of detachment was again the greatest with the 0.1% manure suspension compared to the 1.0% manure suspension. The extent of oocyst attachment to soil particles and the tendency to remain attached therefore appeared to be correlated with manure dilution. Consequently, rates of manure dissolution control not only oocyst "release" but also transient attachment to soil particles.

Population dynamics of *Salmonella enterica* var. Typhimurium, *E. coli* O157:H7, *Pseudomonas fluorescens* were investigated in their introduction to cattle excrements and subsequent to entering the soil, plants of cress, and migration through the GI tract of French snails. Started with an initial concentration of 10 log CFU/gdw. The survival of these bacteria in the excrements and soil was investigated at cyclically changing (day-night, 25-15°C) and constant (18°C) temperatures. The cyclically changing temperature adversely affected the survival of *E. coli* O157:H7 and *P. fluorescens*, but did not influence *S. enterica* var. Typhimurium. On the cress plants grown in a mixture of cattle excrements and soil, an increase in the number of the introduced bacteria was observed.


Silty clay loam (SCL) and loamy sand (LS) were mixed with fresh bovine manure, exposed daily to 10 h at 22°C/14 h at 9°C, and watered weekly for 12 weeks. Initial numbers of *E. coli* were ~ 5 log CFU/g. While *E. coli* numbers increased initially 1-2 log, then decreased <1 and 2 log CFU/g in SCL and LS, respectively. The two soil types differ markedly in water-holding capacity, with 100 g oven-dried LS and SCL absorbing an average of 35 and 64 ml water, respectively. Enterococci numbers rose less and then declined faster than those of *E. coli*. *E. coli* and enterococci may survive at least 19 weeks at 9-21°C in bovine manure/soil with *E. coli* surviving better.


Application of manure to pastures more than 2 wk in advance of storm-associated runoff was related to a >80% reduction of fecal coliform bacteria concentration and load compared to applications within 2 wk before a runoff event. For every 10 m of buffer length, a 24% reduction in fecal coliform bacteria concentration was documented. A 0.5 (75%), one (90%), and two (99%) log reduction in manure fecal coliform bacteria concentration was observed for manure holding times in manure management systems of approx. 20, 66, and 133 d, respectively.


Isogenic deletion mutants that were missing one of four virulence factors, *stx1, stx2, stxl–2*, and *eae* in *E. coli* O157:H7 EDL933 were constructed, and their growth in rich media and survival in soils with distinct texture and chemistry were characterized. The survival data were successfully analyzed using Double Weibull model, and the modeling parameters of the mutant strains were not significantly different from those of the wild type. The calculated *T d* (time needed to reach the detection limit, 100 CFU/g soil) for loamy sand, sandy loam, and silty clay was 32, 80, and 110 days, respectively. It was also found that *T d* was positively correlated with soil structure (e.g. clay content), and soil chemistry (e.g. total nitrogen, total carbon, and water extractable organic carbon). Finer textured soils (clayey) compared to coarser textured soils (sandy) may provide protective pore spaces to improve the survival of soil bacteria.


In this review article, model systems were used to determine the persistence of the organism in river water, feces, soil cores, and on stainless steel work surfaces. Survival of the organism was found to be greatest in soil cores containing rooted grass. In cattle feces, it remained detectable at high levels for more than 50 days. In contrast, the organism survived much less readily in cattle slurry and river water where it fell in numbers from more than 10^6/ml to undetectable levels in 10 and 27 days, respectively.

Following application of livestock waste to land, transfer of *Cryptosporidium parvum* through soil was monitored in the laboratory using simulated rainfall and intact soil cores. From an initial concentration of 8 log oocysts/core, oocysts were detected in low numbers in the leachates from clay loam and silty loam soils but not in that from a loamy sand soil. Analysis of soil cores revealed that the majority (73%) of oocysts were found in the top 2 cm of soil. Numbers decreased to 13%, 8% and 5% at depths of 10, 20, and 30 cm, respectively.


Cultural presence/absence tests for three pathogens (*Listeria* spp., *Campylobacter* spp., and *Salmonella* spp.) detected only *Listeria* spp., which did not differ inside (23% positive samples) and outside (28% positive). Molecular tests detected all three pathogens at low levels that were not different inside and outside. We found no evidence of cumulative buildup of *Campylobacter* spp., *Listeria* spp., or *Salmonella* spp. in spray field soils.


Poultry manure was uniformly distributed on top of sod-covered or tilled (upper 12.5 cm) soil blocks and the blocks were irrigated. The spatial distribution of drainage and fecal coliforms through the soil blocks was not uniform. Fecal coliforms appeared where most drainage flowed. Breakthrough of fecal coliforms through tilled blocks was delayed with respect to the breakthrough of fecal coliforms through sod-covered blocks. Rainfall on a well-structured soil will cause the preferential movement of fecal bacteria, even with unsaturated flow conditions, and could contribute to fecal coliform concentrations in shallow groundwater.


Lettuce plants, young (12 days old) or mature (30 days old) were grown in soil, manure-amended soil, or irrigated with water containing 1, 2, 3, or 4 log CFU/g or /ml of *E. coli* O157:H7. Composite soil samples (10 g) as well as lettuce plants (cut from the root systems 1 cm above the soil surface) were collected on each sample day for all treatments. The level of contamination of lettuce tissue by *E. coli* O157:H7 was extremely low in this study even though the pathogen persisted in the soil during the growth period. Following *E. coli* O157:H7-contamination of 12-day-old plants at levels of 2-4 log CFU/g, 0 of 18 lettuce samples and 2 of 18 lettuce samples were found positive for the pathogen at the end of the cultivation periods (30 days postexposure) in plants exposed in soil or manure-amended soil, respectively. 3 of 12 and 8 of 12 samples were found positive for the pathogen 15 days after exposure of mature plants to *E. coli* O157:H7 (3-4 log CFU/g) in soil or manure-amended soil, respectively.


Analysis of a garden soil was undertaken when a child became ill following playing in the plots one day after raw manure had been applied to the plots and radishes planted. Isolates obtained from the patient and the garden plots had indistinguishable PFGE patterns. The qualitative method used for detection of *E. coli* O157:H7 involved blending 25 g of soil in 225 ml tryptic soy broth. Following a 6-h enrichment and immunomagnetic concentration, as few as 10 cells per 25 g of sample could be detected. Nineteen days after the soil was amended with manure, approx. 3.4 log CFU/g of *E. coli* O157:H7 was present in the plots. The pathogen was present in two of the four garden plots on day 42, one of the plots on day 69 and none of the plots on day 92. When radishes were ripe (day 69), none of the four composite samples of radishes tested positive for *E. coli* O157:H7.

*Salmonella* (3 strains) inoculated into manure-fertilized soil (silty clay loam and loamy sand) to give 4 to 5 log CFU/g. (Contaminated manure was approx. 7 log CFU/g). Simulated environmental conditions for Wisconsin growing season of March to August and June to September. Vegetables were radishes, arugula, and carrots. If contaminated manure was applied in March, vegetables harvested in August were not contaminated, however, the pathogen could still be detected in the soils. *Salmonella* declined in both soils from initial levels of ca. 4.8 to 1.7 log CFU/g between March application and planting of vegetables 9 weeks later. It was detected in both soils by enrichment when vegetables were harvested 21 weeks later. Repeated freeze-thaw cycles were detrimental to the survival of *Salmonella* and *E. coli* in manure-fertilized soil. If contaminated manure was applied in June, radishes and arugulo were contaminated at harvest and were more likely to be contaminated when harvested from silty clay loam soils than from loamy sand soils. Vegetables had been planted approximately 9 and 11 weeks after manure applications in March and June, respectively.


A 1-log reduction in the oocysts infectivity in saturated soil was observed at 30°C. Incubation for 10 days in dry loamy soil at 32°C resulted in a 3-log reduction in their infectivity while no change of oocysts viability was recorded. Previous die-off studies of *C. parvum* used viability tests that do not necessarily reflect the oocyst infectivity. Desiccation and high temperatures enhance the loss of infectivity of *C. parvum*. Incubation of *C. parvum* oocysts with an extracellular *Pseudomonas alcaligenes* extract resulted in 100% loss of infectivity. Incubation of *C. parvum* with an extracellular *B. subtilis* extract resulted in partial inactivation of the oocysts. Outer two layers of the cell wall are digested (cleaving antigenic components necessary for infection), however, the inner wall protects the DNA. Positive results obtained by PCR may indicate that *C. parvum* oocysts are present in the environmental samples. However, these results cannot be used for assessing the infectivity state of the oocysts.


Initial inoculum levels: 2.7-5.2 log CFU/ml or g for *E. coli* O157:H7; 3.2-4.5 log CFU/ml or g for *Salmonella*; 2.2-4.9 log CFU/ml or g for *Listeria*; 2.1-4.2 log CFU/ml or g for *Campylobacter*. *E. coli* O157, *Salmonella* and *Campylobacter* survived (June-December) in stored slurries and dirty water for up to 3 months with *Listeria* surviving for up to 6 months. In contrast, all these pathogens survived for less than one month in solid manure heaps where temperatures greater than 55°C were obtained. Following manure spreading to land, *E. coli* O157, *Salmonella* and *Campylobacter* generally survived in the soil for up to one month after application to both the sandy arable and clay loam grassland soils, whereas *Listeria* commonly survived for more than one month.


Two agricultural soils (sandy loam and silty clay) were amended with poultry manure, cattle manure slurry or human urine in a two year study. Because of differences in nitrogen content, the actual amounts applied per lysimeter were 40 g poultry manure, 50 g urine, or 150 g cattle slurry. Manure mixed with the top 5 cm of soil in lysimeter. Soil plugs of 5 cm depth analyzed. For soil A (sandy loam), the average initial plate count for *E. coli* O157:H7 was 6.0 and 6.3 in CFU/gdw in year 1 and 2, respectively. For soil B (silty clay), the average initial plate count as 5.7 and 6.2 CFU/gdw in year 1 and 2. *E. coli* O157:H7 was detected for up to 90 days by the spread plate technique (detection limit 2 log CFU/gdw) and the longest inactivation times were seen in soil amended with poultry manure. The average initial plate count of *Salmonella* in soil A was 6.5 and 6.6 log CFU/gdw for year 1 and 2, respectively. For soil B, the average plate count was 6.3 and 6.6 log CFU/gdw for...
Salmonella was detected up to 60 or 90 days in both soils during year 1 and 2, respectively, and the longest inactivation times were also seen in soils amended with poultry manure.


To achieve a sufficient reduction in Salmonella, the calcium hydroxide had to be applied at a sufficient rate, and the amount required varied because of manure:soil ratio and incubation temperature. The results showed that a pH above 11 was needed and that a high pH had to be maintained for up to 7 days. In addition, a high manure:soil ratio in combination with a higher incubation temperature was found to rapidly neutralize the pH and to increase the risk of Salmonella regrowth.


Examined both clay loam and sandy loam soils. Soil cores stored at either 5°C or 15°C. Measured over a 4-week period after cattle slurry application (natural contamination). Leaching losses were between 0.2% and 10% of total E. coli and were dependent on rainfall. Recovery of E. coli in grass and soil declined with approximately first order kinetics. The die off of the susceptible pool was linear with a half-life of 3-4 days, and was faster at the higher temperature and lowest moisture content. The resistant pool was not strongly affected by temperature or moisture and had a half-life for die off between 18 and 24 days. The die off rate of E. coli O157 was the same or slightly faster than that of the commensal E. coli population, indicating that the field behavior of E. coli O157 can be studied by monitoring the total population of E. coli applied with slurry. If weather conditions are dry after the application on well-drained sandy soils, it is unlikely that any significant losses of bacteria to drains will occur. Under these conditions, microorganisms may remain on the soil/grass where their numbers will decline due to desiccation and exposure to natural UV radiation. E. coli O157 numbers decline at similar or slightly faster rates than the general E. coli population.


The objective of this study was to determine the survival of E. coli O157:H7 and S. Typhimurium in bovine manure and in manure-amended bulk soil (1:9) as affected by inoculation density (4 and 7 log CFU/g) and moisture (one set left undisturbed while other set watered to maintain $\geq$ 80% RH) under tropical field and screen house conditions. Soil was dispensed into 2 L pots and was subjected to fluctuating conditions of temperature between a minimum of 16 and a maximum of 42°C. Maintaining the matrices at high moisture level promoted the persistence of high-density inocula and enhanced the decline of low-density inocula in the screen house, but moisture condition did not affect survival in the field. The two enteric bacteria survived longer in manure-amended soil than in manure. The 7 log CFU/g E. coli O157:H7 and S. Typhimurium survived for 49-84 days and 63-98 days, while at 4 log CFU/g, persistence was 21-28 and 35-42 days, respectively.


Three-week old cabbage seedlings were transplanted and cultivated for 120 days on manure-amended soil inoculated with 4 or 7 log CFU/g non-virulent E. coli O157:H7 and S. Typhimurium. Cabbage rhizosphere did not affect survival of either E. coli O157:H7 or S. Typhimurium when manure-amended soil was inoculated at 4 log CFU/g. Across all sampling points, CFU number in the rhizosphere of S. Typhimurium was also not significantly different from that observed in bulk soil when initially inoculated at a level of 7 log CFU/g. In contrast, CFU counts of E. coli O157:H7 were significantly higher in the rhizosphere than in bulk soil of treatments initially inoculated at a level of 7 log CFU/g.

A model to predict the survival of *Salmonella enterica* serovar Typhimurium under dynamic temperature conditions in soil in the field was developed. The working hypothesis was that the inactivation phenomena associated with the survival kinetics of an organism in an agricultural matrix under dynamic temperature conditions is for a large part due to the cumulative effect of inactivation at various temperatures within the continuum registered in the matrix in the field. The modelling approach followed included (i) the recording of the temperature profile that the organism experiences in the field matrix, (ii) modelling the survival kinetics under isothermal conditions at a range of temperatures that were registered in the matrix in the field; and (iii) using the isothermal-based kinetic models to develop models for predicting survival under dynamic conditions. Manure was inoculated first to give 8 log CFU/g then mixed in a 1:9 ratio with soil to give 7 log CFU/g. Five g of the inoculated matrices placed in 10 ml bottle which was submerged in glass jars containing a small quantity of water at designated temperature of 16, 25, 37 or 42°C. The time needed for 7 log CFU/g *S. Typhimurium* in manure and manure-amended soil to reach the detection limit of the enumeration method (2 log CFU/g) under tropical conditions was predicted to be 61–68 days and corresponded with observed CFU of about 2.2–3.0 log CFU/g, respectively.


*E. coli* O157:H7- and *Salmonella* Typhimurium was inoculated into fresh bovine manure and the manure was then incorporated into the soil to give 4 and 7 log CFU/g either on the day of transplantation or 56, or 105 days post-transplantation of cabbage seedlings in 6 L plastic pots. The pots were set randomly in an open field until 120 days post-transplantation when cabbage plants were sampled. Soil samples in the vicinity of the roots were taken throughout cultivation. Persistence of 4 log CFU/g *E. coli* O157:H7 and *S. Typhimurium* in the soil was limited with these pathogens being present in the soil and on the surface of plants at harvest only when the contaminated manure was applied 105 days post-transplantation. In contrast, *E. coli* O157:H7 and *S. Typhimurium* survived in the soil throughout the cultivation period when inoculated at 7 log CFU/g. All plants examined for leaf contamination were positive for *E. coli* O157:H7 and *S. Typhimurium* at harvest irrespective of the time of manure application. Internalized pathogens were present in surface-sterilized cabbage leaves at harvest only when the plants had been exposed on the day of transplantation with 7 log CFU/g.


Transport of manure-borne bacteria in soils was hypothesized to be similar to colloid-facilitated transport and was tested by collecting effluent from lysimeters filled with undisturbed stony soil and surface coated with bovine manure in a potassium bromide solution to which simulated rainfall (7.1 cm/h) was applied for 5 h. The average velocity of bacteria and manure colloids was about 7 times larger than the average pore water velocity. Average values for the release rate constants were not significantly different for fecal coliforms and manure colloids. A convective-dispersive equation that included adsorption/exclusion and first-order removal/re-growth terms could be used as a model for the fecal coliform transport in soil.

Clay loam soil was fractionated to obtain particles of coarse sand, medium sand, fine sand, silt, and clay sizes. These fractions were then exposed to a manure suspension containing 2-3 log CFU/ml of fecal coliforms. *E. coli* attached to the different fractions was then fingerprinted using rep PCR techniques. Preferential attachment of different strains to particles of different size classes was attributed to differences in both particle surface and bacteria surface properties. Concern was expressed that differences in attachment of pathogenic and non-pathogenic *E. coli* may exist and would subsequently affect overland transport of the two groups.


By analyzing published data from field experiments and large intact soil cores, an extensive database of microbial removal rates was established in this review article for a wide range subsurface media. High microbial removal rates were found in volcanic rocks, pumice, sand, fine sand, and highly weathered aquifer rocks. Low removal rates were found in structured clayey soils, stony soils, coarse gravel aquifers, fractured rocks, and karst limestones. In general, clay particles are every effective at filtering microbial contaminants under ideal matrix flow conditions, but clay soils under field conditions are susceptible to shrinking and cracking, often lowering removal rates in comparison with sandy soils. For the same media, the removal rates for viruses are in the same order of magnitude as they are for bacteria, and can be lower or higher (due to possible removal with associated large colloids). Removal rates were lower for enteroviruses than for other human viruses; for waste-associated microbes than for those cultivated in the laboratory. Unfavorable attachment conditions due to the presence of organic matter, heterogeneous attachment conditions (due to heterogeneity in the properties of microbial contaminants, change in solution chemistry, or detachment), and physical straining may have caused the discrepancies from the linear pattern predicted from traditional transport models and filtration theory.


In this review article that was based on the published data obtained for water, soil, and feces, *C. parvum* oocysts exhibited a first-order die-off. Temperature appeared to be the most lethal factor affecting oocysts in the environment. Unlike temperature, the other factors affecting oocyst survival in the environment have not been defined clearly and need further study.


No-till, an effective conservation practice, often results in soil having higher water infiltration and percolation rates than conventional tillage. Applied 1 L of liquid dairy manure containing 5 log *C. parvum* oocysts/ml to test the effect of tillage and rainfall on oocyst transport. The blocks were subjected to rainfall treatments consisting of 5 mm or 30 mm in 30 min. Even before any rain was applied, approx. 300 ml of water from the liquid manure (30% of that applied) was transported through the no-till soil, but none through the tilled blocks. After rain was applied, a greater number and percentage of first leachate samples from the no-till soil and blocks compared to the tilled blocks tested positive for *Cryptosporidium* oocysts. In contrast to leachate, greater numbers of oocysts were recovered from the tilled soil, itself, than from the no-till soil.


In this review article, major transport processes reviewed were leaching and surface runoff for land areas receiving animal wastes, and pastures and rangeland watersheds where animals distribute waste directly to the land. Also included in this article were calculated die-off rate constants for pathogens and indicator organisms that was based on literature data. Die-off rates ranged from 0.08-0.91/day for fecal coliforms, from 0.05-3.87/day for fecal streptococci, from 0.21-6.93/day for *Salmonella*, from 0.62-0.74/day for *Shigella* sp., and 0.04-
3.69/day for viruses. With a 10°C rise in temperature, die-off rates doubled while die-off rates also increased with a decrease in soil moisture and were minimal in a pH range of 6-7.


*E. coli* O157:H7 was recoverable from soils (25 g sample) for >120 days following challenge by all conditions except for swine manure-amended soils at 25°C and a moisture content of 80% field capacity (55 to 120 days). *S. enterica* serovar Typhimurium was cultivable from swine manure-amended soils for 55 to 120 days at 10°C and 25°C but only for 25 to 55 days from beef manure-amended soils at these temperatures. Linear regression analysis indicated that the relationships between paired measurements of viable *E. coli* O157:H7 versus *stx1* and *S. enterica* serovar Typhimurium versus *ttrRSBCA* were highly predictable. This suggests that these qPCR genetic markers may be reliable conservative surrogates for monitoring fecal pollution from manure-amended land. Used a biphasic decay model to describe data. In this study, k2 was not affected by temperature or moisture content but only by the matrix. This may be a clue to the mechanisms that lead to extended persistence.


This report summarized the literature regarding the pathways for the release of zoonotic agents and antimicrobial-resistant bacteria endemic in animals confined to concentrated animal feeding operations (CAFO) and the potential of those pathogens to persist in different environments, including soils. In addition to discussing the state of knowledge on these issues, the gaps in the research that need to be addressed were identified.


Farm dairy effluent inoculated at 5 log CFU/ml with *C. jejuni* was applied to intact soil cores at a rate of 2 L/m². Cores were incubated at 10°C for up to 32 days. *C. jejuni* had declined to the limit of detection (2/100 g) by 25 days in Hamilton (clay) and Taupo (pumice/sandy) soils and by 32 days in Waihou soil (allophanic). In contrast, in Horotiu (allophonic) soil, the decline was only 3 orders of magnitude after 32 days. Its survival could not be statistically linked to any of the soil properties measured (clay content, organic matter, CEC and nitrogen). Simulated heavy rainfall was applied 4 and 11 days after application and only about 1% of the applied *C. jejuni* were recovered in leachates. A higher percentage of *C. jejuni* (relative to that of the 3 other soils) was washed from Horotiu soil both 4 and 11 days after application. *E. coli* were only recovered only down to 5 cm but *C. jejuni* were recovered down to 15 cm. Speculate that *C. jejuni* actively moved through soil water films as these bacteria are very motile and able to move towards favorable environments.


Measured only total and fecal coliform bacteria. The pig slurry amendment induced the highest initial and also persistent presence of total and faecal coliform bacteria. The higher application rate (210 k N/ha) seemed to induce a long-lasting persistence of the total, but especially the faecal coliform, population in the amended soils compared to the lower rate (150 kg N/ha). In most cases, an increase in the total coliforms content was observed in the soils amended with mineral fertilizer compared to controlled soils.

In this review article the following conclusions were made: 1) soil moisture favors the survival of viruses and bacteria with reductions in bacterial and viral population densities being observed under dry soil conditions; 2) clays favor the adsorption of microorganisms to soil particles and this further reduces the die-off rates by creating a barrier against microbial predators and parasites. Thus, the rates of enteric pathogens survival are lower in sandy soils with a low water-holding capacity; 3) pH affects the adsorption characteristics of cells so inactivation rates in acidic soils are lower. Increases in cation concentrations also result in increased adsorption rates, consequently affecting microbial survival; 4) soil texture controls, in part, the movement of microorganisms, because fine-grained soils avoid movement while coarse-grained soils promote it.


Temperature did not affect the release of (oo)cysts from manure, however, the rates of release of (oo)cysts were faster from cow manure than from calf manure. Dripping water, as opposed to misting, resulted in a higher release rate of (oo)cysts from manure due to the increased mechanical forces associated with droplet impact.

Although this article only addressed manure, the results would be applicable to manure deposited on land and thus contamination of land.


Liquid swine manure, inoculated with E. coli at ca. 7 log CFU/ml, was applied to intact soil cores (20 x 30 cm) 4, 8, or 16 d before the first rainfall event (50.8 mm over a 4-h period) and each core received one to three rainfall events. There was no effect on leaching when different application methods (no-till surface-broadcast, broadcast and incorporated, and tilled before broadcast) were employed; however, there was greater survival in soils when the manure had been incorporated. The greatest degree of E. coli leached occurred following the first rainfall event but an increasing time between manure application and that first rainfall event decreased the amount leached. Subsequent rainfall events led to decreasing levels of E. coli in the leachate. Significant leaching and survival in soil was possible even if the first rain occurred 16 d after manure application.


All soil-manure mixtures inoculated with E. coli O157:H7 (7 log CFU/gdw) were incubated for 60 days at 16°C in plastic pots. On average, the decline of E. coli O157:H7 was more irregular in conventional and loamy soils than in organic and sandy soils. Multiple regression analysis of irregularity of E. coli O157:H7 survival on 13 soil characteristics showed a positive relation with the ratio of copiotrophic/oligotrophic bacteria (counted CFU on C-rich and C-poor media, respectively, suggesting greater instability at higher available substrate concentrations. Generally, organically managed soils show a higher diversity of bacteria and higher microbial biomass than conventionally managed soils. Such characteristics make them less susceptible to invasive pathogens and increase resilience and resistance to stress.


The initial calculated inoculum densities of E. coli O157:H7 and Salmonella serovar Typhimurium were 7.8 and 8.1 log CFU/gdw of soil (for upper 10 cm), respectively, both for manure and slurry treatments. More pathogen cells percolated to greater depths after slurry than after manure application. Survival of E. coli O157:H7 was significantly longer in soil with slurry than in that with manure, while survival of Salmonella serovar Typhimurium was equally high with manure and slurry. The densities of the pathogens were not different in the rhizosphere compared to the bulk soil with manure, while the densities were higher in the rhizosphere than in bulk soil after slurry application. Results suggest that surface application of manure may decrease the risk of
contamination of groundwater and lettuce roots compared to injection of slurry. Estimated survival time of *Salmonella* serovar Typhimurium was significantly higher than that of *E. coli* O157:H7 at all depths except at 40 cm.


A simulation model was developed to investigate the relative effects of temperature, oxygen concentration, substrate content, and competition by autochthonous microbial community on the oscillatory behavior and survival of *E. coli* O157:H7 in manure and manure-amended soil. Oscillations of bacterial populations were attained by the relationships between relative growth and death rates with readily available substrate content. The relative effects of changes in temperature on simulated survival time of *E. coli* O157:H7 were more pronounced than changes in oxygen condition. At the surface of a heap with unturned manure, simulated survival time was the longest (2.4 times longer than inside the same heap).


Treatments included two methods of litter application (surface broadcast and subsurface banding), commercial fertilizer, and control. Simulated rainfall was applied to treatments. Increasing the time between litter application and the first runoff event helped decrease nutrient and *E. coli* losses from surface broadcast litter, but those losses generally remained significantly greater than controls and subsurface banded, regardless of runoff timing. This study shows that subsurface litter banding into perennial grassland can substantially reduce nutrient and pathogen losses in runoff compared to the traditional surface-broadcast practice.


Determined the reductions of hepatitis A, poliovirus 1, echovirus 1, and the indicator virus MS@ in 10-cm deep, miniature soil columns (coarse sandy, loamy sand, clay loam, and organic muck) dosed twice weekly with 2.5 cm of water or wastewater and incubated at 5 or 25°C for 16 weeks. Few or no viruses were detected in clay loam column effluents whereas with organic muck columns, virus reductions ranged from 30-98%. In the sandy soil columns, viruses were retained generally better in loamy sand than in coarse sand. Virus reductions were greater in sandy soil columns dosed with groundwater than with wastewater.


Types of pathogens potentially present in the manure of swine and other agricultural animals, the levels of these pathogens in animal wastes, the potential for release of pathogens to sites off-farm, and the ability of current or proposed management practices to reduce the levels in animal wastes is reviewed in this white paper.


Three soils with different textures (silty loam, silty clay loam, and loamy fine sand) were treated with standard cowpats. Then rainfall was applied to the plots until saturation-excess flow occurred for 30 min, and samples were collected 10, 20, and 30 min after initiation of the runoff event. Percentage of *E. coli* and enterococci attached to particulates in runoff ranged from 28 to 49% with few statistically significant differences in attachment among the 3 soils. At least 50% of all attached *E. coli* and enterococci were associated with particles in the 8- to 62-µm particle size category. The results indicate that the majority of fecal bacteria attach to and are transported with manure colloids in sediment-laden flow regardless of the soil texture.

Manure significantly increased fecal bacteria in leachate compared with unmanured treatments. Manure application to no-tillage soil in spring did not accelerate water contamination by fecal coliforms relative to fall manure applications. No-tillage did not accelerate water contamination by fecal coliforms relative to tilled soils.


Sentinel chambers (small cylinder capped with semi-permeable porous membranes) containing silt loam soil and dairy manure mixtures inoculated with *Salmonella* Newport (7 log CFU/g) were placed in 3 dairy environments: 1) static compost pile 30 cm from surface; 2) dairy lagoon immersed 30 cm beneath surface; and 3) grass border of an agricultural field 10 cm below surface. Survival of *S.* Newport in the static compost pile (64°C) was less than 18 h whereas in the dairy lagoon and in the field soil, the pathogen survived for > 137 d and 276 d, respectively. Survival patterns in the latter two systems followed an increase or plateau during the first couple of days, then a log-linear decline for 6 to 13 weeks, and finally a long tailing phase for 4 to 9 months.


The bacteria were fingerprinted by enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC-PCR). A representative isolate from one ERIC group which increased in abundance in soil (designated strain C279) and one which decreased (designated strain C278) were chosen for comparison. These strains persisted comparatively when inoculated into loam soil. However, when added into a loam soil or a sandy soil supplemented with 10% (v/v) swine manure slurry, strain C279 increased in abundance 10-fold, whereas strain C278 did not. Strains able to proliferate in manured soils can have a selective advantage. The swine manure slurry had a pH of 8.5 and a dry matter content of 1%.


Review article. Based on literature, it was concluded that the majority of pathogens in manures applied to land will decline below detectable limits after 3 months. It was suggested that the factors likely to affect the rate at which microorganisms are detached may include the number of microorganisms on the soil surface; whether or not they are protected by soil or biological material; how strongly they are adhered to particle surfaces and to each other; the kinetic energy of the rainfall; and the overland flow velocity and depth. Therefore more pathogens are likely to be transported to a watercourse if an overland flow event occurs soon after fecal waste is applied to land. The way that fecal wastes are applied to the land may also have a bearing on the survival and hence the chances of pathogen transport to watercourses. Small scale rainfall simulation experiments conducted by the authors however suggest that there is approximately a 10-fold increase in fecal coliform transport if waste is surface-applied rather than incorporated. Measures designed to trap microorganisms once entrained, such as vegetative filter strips, have been shown to be highly variable in their effectiveness. These figures compare with a much narrower range for sediment (95-99%). The lower bacterial trapping efficiencies of the vegetative filter strips support the argument that because of their lower density, deposition is less likely for free-floating microorganisms once entrained than it would be for a soil particle of similar size.

Review article. Microbial community compositions had been modified by fumigation at different intensities. A clear effect of fumigation depth on the survival of the invading strain T (a non-toxigenic \textit{E. coli} O157:H7 derivative) was noted, as a progressive increase of depth coincided with a progressively enhanced inoculant survival rate. This was consistent with the hypothesis that soil systems with reduced biological complexity offer enhanced opportunities for invading microbial species to establish and persist. A loamy sand soil was used.


Bacterial PCR-DGGE fingerprints were grouped into two clusters. The irregularity (defined as the intensity of irregular dynamic changes in a population over time) was higher for cluster 1, which consisted primarily of soils that had received liquid manure and artificial fertilizer. This cluster with the higher irregularity was characterized by higher bacterial diversity and evenness. No significant differences in the survival time of \textit{E. coli} O157:H7 was found. The consequence of a high temporal irregularity is a lower accuracy of predictions of population behavior, which results in higher levels of uncertainty associated with the estimates of model parameters when modeling the behavior of \textit{E. coli} O157:H7 in the framework of risk assessments.


Survival evaluated in 2 types of silty clay loam soil (high carbon and low carbon) placed in trays and exposed to field conditions. Initial plate counts of \textit{E. coli} O157:H7 for high-and low-carbon soils were 1.5 x 10^6 and 1.6 x 10^6 CFU/g, respectively. Differences existed in survival of \textit{E. coli} O157:H7 in low- and high-carbon soil at all temperatures, indicating an important role of soil composition on the survival of this pathogen. The highest death rate of \textit{E. coli} O157:H7 in sterile soil occurred in the low-carbon soil at 4°C whereas in nonsterile soil, the highest death rate was observed in the low-carbon soil at 22°C. In the nonsterile system lethality was owing to inhibition by indigenous soil microorganisms and starvation. High mortality rate periods could be correlated with soil drying suggesting that desiccation has a significant impact on \textit{E. coli} O157:H7 survival.


After 4 weekly irrigation events, fecal coliform, \textit{E. coli} and enterococci densities were analyzed in leachate from soil columns (20 x 30 cm) receiving simulated fall and spring manure applications at 168 kg N/ha and 336 kg N/ha. Fall soil columns were frozen for 7 weeks between manure application and irrigation. Less bacterial leaching was observed in fall manure-applied columns as compared to the spring manure-applied columns.


The pathogen survived in soil for over 5 weeks, although at significantly greater numbers in soil receiving stomach content waste in comparison to cattle slurry. Persistence of the pathogen in soil was unaffected by the presence of a rhizosphere. This study has shown that whilst survival of \textit{E. coli} O157:H7 in waste-amended soil is not significantly affected by the presence or absence of a maize rhizosphere, it may vary significantly with waste type.


Initial \textit{Salmonella} concentrations in inoculated dairy cow manure-soil mixtures averaged 6.86 log CFU/g. Samples were stored at 24°C and moisture losses were made up on a weekly basis. Initial population increases
occurred during the first day of storage, then decreased in all samples until day 107 in the manure-amended nonsterilized soil and day 158 in the manure-amended sterilized soil. MPN monitoring data indicated that the organisms persisted for 184, 332, and 405 days in manure, manure-amended nonsterilized soil, and manure-amended sterilized soil, respectively.


Manure loading rates equivalent to 37.5 and 75 Mg/ha were used. Manure loading rates had no effect on mortality rates. The rates for the first 2 weeks of incubation were significantly greater in subsoil than topsoil for total coliforms, fecal coliforms, and fecal streptococci. Bacterial cell numbers decreased to, or close to, detection levels (3 colony forming units/g soil) after 8 weeks of incubation. Mortality rates were adequately described by a two-stage exponential decay model. The rates for the first 2 weeks of incubation were significantly greater in subsoil than topsoil for total coliforms (0.31 log cells/day vs 0.20 log cells/day), fecal coliforms (0.33 log cells/day vs 0.22 log cells/day), and fecal streptococci (0.31 log cells/day vs 0.24 log cells/day).


Salmonella typhimurium was inoculated into a clay and a fine sandy loam using cattle manure slurry and saline as inoculum carriers. Soil samples were incubated at 3 temperatures (5, 22 and 39°C) and 3 moistures (0, 0.5 and >22 atm tension). Survival was monitored over 12 weeks. Flooded samples were prepared by adding sufficient distilled water to cover the soil with a 0.5-cm depth of water. The only set of conditions that consistently led to death of all the salmonellae within 1 week was the incubation temperature of 39°C and the dry soil condition. For other soil conditions, factors interacted to such an extent that it wasn't possible to predict the survival of the organism. Soil moisture and temperature interacted as did soil moisture and inoculation method. Survival at 5 and 22°C was comparable and usually longer than at 39°C. In flooded soil samples held at 22°C, growth occurred during the first 3 days but declined afterward.