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Strategies for the safe use of poultry litter in food crop production

Dr Kevin Wilkinson
VIC Department of Primary Industries

Project Number: VG01049

VG01049

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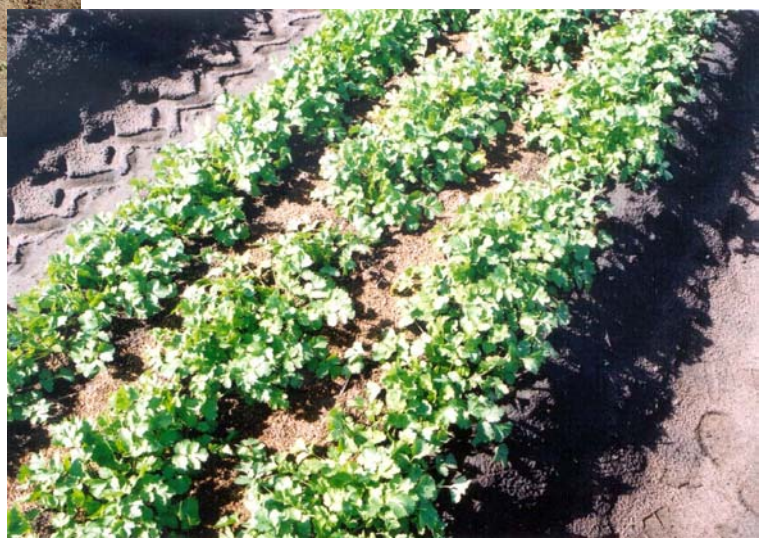

Strategies for the safe use of poultry litter in food crop production

Dr Kevin Wilkinson et al

Final report for project VG01049
Horticulture Australia Pty Ltd

September 2003

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Report purpose

This final report documents the research activities and outcomes achieved for project VG01049. It details studies that were conducted to evaluate the food safety risks associated with the current use of poultry litter in vegetable production and simple techniques for managing poultry litter for its safe use. These studies provided the basis for recommendations that will allow growers to continue to safely use poultry litter in food crop production.

Acknowledgments

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Contents

1. Media Summary

The use of poultry litter to grow vegetables is not known to have resulted in food poisoning outbreaks amongst consumers in Australia. To ensure that growers can continue to use it safely, studies were conducted to develop simple strategies to improve the microbiological safety of poultry litter.

Seven farms in the Melbourne area were surveyed over a 2-year period. No human pathogenic (disease causing) bacteria were found on any harvested crops to which poultry litter had been applied. On-farm poultry litter deliveries were variable in bacterial counts. A non pathogenic *Salmonella* sp. was found in some of the poultry litter deliveries and in a crop at harvest. Bacterial counts were shown to reduce in soil with time.

Injury to the leaves of Cos lettuce in the field was found to promote the persistence of *E. coli*, an indicator of the presence of pathogenic bacteria. Though no pathogenic bacteria were found on crops, it was shown that this could occur if fresh litter is side-dressed close to harvest.

Aging for 12 weeks and turning a heap only marginally improved the microbiological safety of poultry litter. Aged litter, therefore, is not equivalent to quality assured composted litter. Whilst controlled composting will undoubtedly produce the safest product from poultry litter, the results from this project suggest that it is not necessary to recommend it for all applications.

The following findings were made as a result of this research:

- Only use composted poultry litter for side-dressing,
- Store litter as far away as possible from maturing crops,
- Aged litter is not equivalent to fully composted litter,
- Where composted poultry litter is to be used, it must meet established quality assurance guidelines (e.g. for food safety and the Australian Standard™) as well as state environment protection guidelines. Suppliers of composted products must demonstrate that this is the case, and
- Further research is needed to develop processing guidelines for poultry litter that meet these guidelines without causing detriment to its value as a fertiliser.

2. Technical Summary

Studies were conducted to evaluate the food safety risks associated with the current use of poultry litter in vegetable production. Seven farms in the Melbourne area were surveyed. Low *Escherichia coli* counts and no *Campylobacter* or pathogenic *Salmonella* were detected on any crops at harvest. *E. coli* counts and *Salmonella* detections were variable in litter deliveries. No *Campylobacter* or *Listeria* were found in litter deliveries. A non-pathogenic salmonella, *S. enterica* serovar Sofia was detected on a coriander crop that could be traced back to fresh litter that had been side-dressed 18 days prior to harvest. Other growers surveyed didn't side dress so close to harvest time, used composted litter for side dressing or didn't side dress at all. An alternative source of the contamination may have been from the nearby litter stockpile which was only 50 m away from the crop. Whilst tested fields of the other growers were no closer than 130 m and most well over 300 m, all growers stored litter in close proximity to some crops.

Field and glasshouse trials showed that *E. coli* could persist longer on damaged Cos lettuce leaves than undamaged leaves. On the uninjured plants >99% reduction in *E. coli* counts occurred within the first 3 days, followed by a more gradual reduction thereafter. In contrast, the rate of reduction of bacteria on the injured leaves was relatively constant over time; after 3 days the *E. coli* count was reduced by only 37%, and a 99% reduction did not occur until day 23. The timing of injury in relation to contamination with enteric bacteria was found to be important for persistence to occur. When Cos lettuce shoots were injured 3 or more days prior to contamination, the persistence of *E. coli* was reduced to a similar level to the uninjured control. A similar response was found when shoots were injured 1 or more days after contamination with *E. coli.* An explanation for this effect is that *E. coli* is protected from desiccation and UV light in damaged tissue prior to the wound sealing over.

Small changes in the management of poultry litter, such as turning a heap and aging the litter resulted in only marginal improvements in pathogen control despite the sustained high temperatures achieved. *E. coli* counts did not reduce over time in the outer layers of either the turned windrow or static windrow. Periodic rainfall and temperatures in the 35-45 ºC range in the outer layers of the windrows could have provided ideal conditions for pathogens to multiply or persist. Laboratory trials showed that *E. coli* and *Salmonella* counts were reduced by over 99% within the first hour at 55-65 ºC. At 35 ºC both organisms persisted longer under moist (65% w/w) than dry (30% w/w) conditions. There was a marginal improvement in the suppression of pathogen re-growth with aging and turning. When 3-week-old litter from the turned windrow was incubated at 37 ºC in the laboratory, increased densities of *E. coli* were observed after 21 days, whereas counts in 6-week-old litter remained unchanged. In contrast, 3 or 6-week-old litter from the static pile supported increased *E. coli* densities following incubation. By 12 weeks, *E. coli* counts in the outside edge of both windrows were still as high as 31,600 cfu/g (4.5 log10), but upon incubation in the laboratory, remained unchanged for 7 days before reducing to zero after 14 or 21 days.

Whilst controlled composting will produce the safest product from poultry litter, the results from this project suggest that it is not necessary to recommend it for all applications. The following findings were made as a result of this research:

- Only use composted poultry litter for side-dressing,
- Store litter as far away as possible from maturing crops,
- Aged litter is not equivalent to fully composted litter with respect to microbial quality,
- Where composted poultry litter is to be used, it must meet established quality assurance guidelines (e.g. for food safety and the Australian Standard™) as well as state environment protection guidelines. Suppliers of composted products must demonstrate that this is the case, and
- Further research is needed to develop processing guidelines for poultry litter that meet these environment protection guidelines without causing detriment to its value as a fertiliser.

3. Introduction

3.1. Background to the project

For generations, vegetable growers have enjoyed the many advantages that poultry litter has to offer including its soil-conditioning ability, slow and fast rate of nutrient release, trace elements, low cost and ease of application. However, at the time this project began in July 2001, the use of poultry litter in vegetable production was a contentious issue due to concerns about food safety and the possibility of litter contaminating crops.

It was known that litter contains bacteria that have the potential to cause human illness, such as *Salmonella* (Riemann *et al.* 1998) and contamination of lettuce with manure had been implicated as the cause of bacteriological food poisoning outbreaks (Brackett 1999). Furthermore, glasshouse trials at the Department of Primary Industries, Knoxfield (formerly known as the Institute for Horticultural Development) had shown that slight damage to vegetable plants could allow *Escherichia coli* to persist for at least three weeks. It was not known at the time whether these bacteria could also survive on field grown crops.

While many growers were being discouraged from using poultry litter, the risk to human health was largely unknown. The future of this practice was thought to be uncertain unless the risks to the community were known and strategies for reducing the risk were developed.

3.2. Literature review

There have been several large, well-publicised outbreaks of food-borne illness associated with the cross-contamination of fresh produce with faecal matter from both domestic and wild animals (Brackett 1999). It should also be noted that other contributing factors may also be involved in such cases, including the use of contaminated water, unsanitised transportation vehicles and contamination by handlers on the farm and at retail outlets (Little *et al.* 1997; Thomas and Beirne 1994).

Nevertheless, the potential impact of contamination of produce or ground water from pathogens contained in manures could be catastrophic. A recent example was an incident in Walkerton, Canada in which 7 people died and 2300 people became ill (O'Connor 2002). The town water supply had been contaminated with the bacteria, *Escherichia coli* O157:H7. The source of the contamination was cow manure that had been spread on a farm adjacent to a well used as a collection point for the town's water.

Very little data is available on the incidence of pathogens in poultry litter in Australia. Work that has been done has shown that bacterial counts varied with the source of the litter and that the detection of *Salmonella*, in particular, was sporadic (Jaeger and Premier 2001). Two studies in the USA, however, showed that high levels of faecal coliforms (up to 3.8×10^6/ml) could be detected in run-off water from fields that had been amended with poultry manure (Giddens and Barnett 1980; McMurry *et al.* 1998).

The potential for pathogens from organic amendments to survive outside their host animal and to contaminate water supplies or crops depends upon a number of factors, including (Moss *et al.* 2002):

- Presence, quantity and viability of the organism;
- Method of application (e.g. surface application or incorporation);
- Solids content of the amendment (i.e. whether applied as a solid or liquid);
- Soil conditions such as permeability, infiltration, soil moisture, texture and pH;

- Climatic exposure, especially temperature and sunlight; and
- Soil biological conditions (i.e. competitive organisms).

The risk of pathogens surviving on produce is greatest when it is eaten raw or following limited heat treatment. Several studies have shown that pathogens can survive and grow on fresh produce (Piagentini *et al.* 1997; De Roever 1998; Berrang *et al.* 1989). Pathogens can contaminate crops directly when raw manures are side-dressed close to harvest, or contamination can occur indirectly via soil or soil water. Several researchers have studied the survival of pathogens in soil. Golueke (1983) and Olson *et al.* (1999) for example, reported on the survival of a number of parasites and pathogens in soil, in which the duration of survival ranged from 8 to about 180 days.

Composting is an effective mechanism for controlling pathogens in manures. Composting provides an opportunity to produce 'quality assured' organic amendments that comply with the Australian Standard for Composts, Soil Conditioners and Mulches (AS4454 1999). The principal mode of pathogen reduction during composting is the effect of sustained high temperatures. Pathogens such as *Salmonella* and *E. coli* are killed more quickly as temperature increases especially above 55 ºC (Farrell 1993).

Pereira-Neto *et al.* (1986) evaluated pathogen reduction using the aerated static pile method. Salmonellae were destroyed in 7-15 days; *E. coli* were reduced in number from 10 million to less than 100 colony forming units in 15 days; and fecal streptococci decreased from 10 million to less than 100 colony forming units in 30 days. During composting, temperatures frequently exceed 65 ºC for extended periods (weeks or months). For the production of quality assured compost, this temperature development is monitored and managed to provide a high degree of confidence that pathogens are eliminated.

The most common types of composting systems include the windrow method, aerated static pile and the in-vessel method. The choice of composting method depends on the waste being composted, the proposed site and environmental regulations (EPA Victoria 1996). Some composting systems are more effective at pathogen reduction within specific time-frames than others. Pereira-Neto *et al.* (1986) found that *Salmonellae* and *E. coli* were eliminated in an aerated static pile after 16 days, but were still detected in windrows after 60 days. For effective pathogen control, the entire mass of organic materials must be exposed to temperatures exceeding 55 ºC. In windrow systems this is usually achieved through frequent turning.

Properly composted litter has several major advantages: it has a lower risk of pathogens being present, it is less odorous and composting transforms the organic matter into humus which is beneficial for soil conditioning purposes (Stratton and Rechcigl 1998). One major drawback to composting is the high cost associated with the set-up of a facility that meets environmental protection regulations and the higher costs of processing. Since composting is not seen as a viable option at present, studies are needed to determine whether small changes in the management of poultry litter, such as turning a heap and aging the litter could effectively control enteric bacteria.

3.3. Aims of the project

- To evaluate the food-safety risks associated with the current use of poultry litter on vegetable farms,
- To investigate the persistence of enteric bacteria on leafy vegetables in the glasshouse and field, and
- To investigate the persistence of enteric bacteria in poultry litter during aging and heat treatment.

4. Materials and Methods

4.1. Risk associated with current use of poultry litter on vegetable farms

4.1.1. Current use of poultry litter

Six properties on the Mornington Peninsula about 60 km South East of Melbourne (Farms A – E and Farm G) and one property from Bacchus Marsh approximately 50 km North West of Melbourne (Farm F) were selected for the study. Vegetable growers were interviewed to establish how they used poultry litter and to identify whether particular practices could be linked to bacterial contamination of crops at harvest.

In face to face interviews, growers were asked questions to identify:

- Where they got their litter,
- Whether it was fresh, stockpiled or composted,
- How long it was kept before application,
- Where it was stored. A map was provided or drawn noting especially the proximity of litter to crops,
- When and how it was applied,
- Whether they side-dressed, and
- What was their source of irrigation water.

4.1.2. Incidence of enteric bacteria in litter, soils and crops

The same 7 properties were used to track the incidence of bacteria from litter deliveries through to crops at harvest. At each farm, sequential samples were taken of:

- A poultry litter delivery (one just arrived),
- Soil prior to treatment with the litter delivery,
- Soil after treatment with the litter delivery, and
- Crops at harvest growing in the litter treated soils.

These samples were tested for *Escherichia coli*, as an indicator of enteric bacterial pathogens and for human pathogenic bacteria known to be associated with poultry, *Salmonella* and *Campylobacter* spp. Crops that are usually eaten raw were chosen for this study because the risk of food-borne illness occurring is likely to be highest in the absence of cooking.

Sampling method - poultry litter pile

The pile was sampled within two days of delivery. Samples were taken from four sectors or quarters of the pile. Two sub-samples per sector were taken from each side about a quarter and half way up the pile. Then depending on the size of the pile, up to 3 equidistant samples was taken also from the top of the pile. A long handle narrow trench shovel was used to take sub-samples. This was thoroughly washed and disinfected with 70% ethanol between piles and "rinsed" between quarters by deeply inserting it several times into each quarter away from the actual sampling sites. Fresh, clean, disposable gloves were worn per quarter. Chicken litter from 50 to 100 cm deep was dug out from each sampling site, pooled and thoroughly mixed with all other sub-samples of the quarter. Approximately two thirds of a clean 27 by 40 cm doubled polyethylene bag (one placed in another) was aseptically filled with each quarter's sample. These press sealable bags were then placed in an esky with frozen Willow ® Ice Bricks™ covering its bottom and transported to a 4°C cool room within 3 hours.

Sampling method - soil and soil + litter

The area of soil that was sampled ranged between approximately 1300 to 15,500 m^2 depending on the area of the farms that were to be treated with a specific litter delivery. This area was sampled from four longitudinal quarters, parallel to the planting rows or where the rows were to be formed. From each quarter, ten to twenty equidistance sub-samples were aseptically scooped with a sterile 500 ml polyethylene wide jar, to a depth of about 10 cm. The jar was replaced for each quarter and the pooled sub-samples were bagged and transported as above.

Sampling method - plant material

Sampling was done on mature crops up to a week before harvest and in the case of Grower D on the remnant material three days after the harvest. It was in the same quarters and similar positions as for soil. A single plant (Iceberg lettuce or celery) was aseptically cut close to the ground at each sub-sample site. The knife was wiped thoroughly with 70% ethanol at each quarter. The sampled material was sealed in large clean autoclave bags with no mixing of plants from different quarters. These bags were transported in an air-conditioned car to a 4°C cool room within 3 hours. For crops, which were small enough to be broken off by hand near the soil, three hand-fulls were removed at each sub-sample site. Fresh, clean disposable gloves were used for each quarter. These sub-samples were bagged and transported in the same fashion as the litter.

Bacteriological testing

Plant samples were aseptically chopped into pieces no larger than 2cm and then re-bagged. Prior to testing all samples were thoroughly mixed within their bags.

Samples from each quarter were tested with the exception of pathogen testing for Growers A, C, and non-harvest samples for Growers B and D. In these cases, pathogen testing was done on a composite sample of all quarters.

Testing or submission of samples to the private microbiology laboratory was usually completed within six hours. Samples were kept at 4°C until tested.

Testing for *Salmonella*, *Campylobacter* and *Listeria* was performed by Silliker Microtech Pty. Ltd. (NATA accredited) using the Australian Standard™ Methods (Standards Australia Committee on Food Microbiology, 1998). Direct counts of presumptive *E. coli* were determined with 3M Petrifilm™ *E.coli* / Coliform count plates incubated at 44°C in an air incubator for 48 hours. Blue colonies producing gas were deemed to be presumptive *E. coli*. Twenty-five grams of plant material per sample was stomached for 2 minutes in 250 ml of 0.1% peptone or 10 g of soil or litter were shaken in a 500ml jar with 100ml of the 0.1% peptone for 10 minutes at 150 rpm.

Enrichment was used to increase the sensitivity of presumptive *E. coli* detection. Samples were agitated as described above in Tryptone Soya Broth (Oxoid Ltd., UK) instead of 0.1% peptone and incubated at 37°C for 18 to 24 hours prior to plating out as described for direct counts.

Counts were expressed per gram of the dry weight for soil and litter samples (dried at 60°C until constant weight) and fresh weight for plant samples.

Data analysis

Analysis of variance was performed using GenStat for Windows, Release 4.2, Fifth edition (Lawes Agricultural Trust, VSN International Ltd, Oxford, U.K.).

4.2. Persistence of enteric bacteria on leafy vegetables – effect of leaf injury under field conditions

4.2.1. Site description and field trial set-up

The trial was located at the Department of Primary Industries, Knoxfield (formerly the Institute for Horticultural Development) on a gently sloping site of approximately 880 m^2. Soil type was an acidic (pH in water of 5.25), yellowish, greyish-brown clay loam. Irrigation was by overhead sprinklers with water supplied from a nearby dam. Soil samples were taken and analysed by the State Chemistry Laboratory (Department of Primary Industries), Werribee to determine rates of fertiliser application. Based on these results, lime was added at the rate of 10 t/ha on 13 September 2001 and incorporated by rotary hoe to raise the pH above 6.0. NPK fertiliser ('Apex' formulation, Incitec Ltd.) at rates of 2.55 kg/ha N, 2.85 kg/ha P and 3.0 kg/ha K was spread by hand on 12th November 2001 and rotary hoed in 2 days later.

Cos lettuce seedlings ('Junior' variety) were transplanted on 19th and 20th November 2001 in two rows spaced 15 cm apart and 10 cm between plants within the same row.

Trial design

Plants were subjected to the following four treatments:

- Dosed and injured,
- Dosed and uninjured,
- Undosed and injured, and
- Undosed and uninjured

Each treatment was replicated four times. Each of these replicates (16) were subjected to individual plots in a 4 by 4 latin square design (randomised, blocked and latinised).

Each plot consisted of 3 rows of 30 plants that were separated by about 15 cm. Plots within the same row were separated by 2.8 m and the rows were separated by 2.7 m.

Within each plot, were subplots which were randomised for removal for testing at 1, 3, 7,16, 23 and 37 days post *E. coli* dosing. These subplots consisted of 3 rows of 2 plants (6). These subplots were separated by a buffer subplot of plants that were not removed in order to reduce any "edge" effect.

Site maintenance

After transplanting, "Defender" ™ Snail and Slug Pellets were applied. The herbicide "Kerb" (Rohm and Haas Australia Pty. Ltd.) was applied on 21st November 2001. In late November the following chemicals were applied at the recommended rates, the fungicide ManKocide® DF (Griffin Corporation Australia Pty. Ltd.) and the insecticide Malathion (Arthur Yates and Co. Limited).

4.2.2. *E. coli* dose preparation

Escherichia coli^{Nal} culture number NCTC 9001 (ACM 1803) was provided by Dr Patricia Desmarchelier and Lesley Duffy of Food Science Australia. This nalidixic acid resistant mutant was prepared by repeated subculturing on media containing increasing amounts of antibiotic until it grew well on medium containing 20 µg/ml of nalidixic acid.

Escherichia coli culture number NCTC 9001 was used as the non-nalidixic acid resistant strain. *Enterobacter aerogenes*, culture number ACM 4982, was used as a negative control culture for the Tryptone Bile X-Glucuronide (TBX) (Oxoid Ltd., UK) agar plates and 3M

Petrifilm™ *E.coli* / Coliform count plates. These last 2 cultures were purchased from the Centre of Bacterial Diversity and Identification, Department of Microbiology, University of Queensland, Australia.

The day before the suspensions were needed, Tryptone Soya Agar (TSA) plates were streaked with *E. coli*^{Nal} and incubated overnight at 37°C. After incubation, colonies from 2 plates were emulsified using a surface sterilised glass rod and 6ml 0.1% peptone each. The resultant peptone/bacterial suspension was then dispersed into 3.2 litres of deionised water and dispensed into a sprayer bottle. The sprayer bottle was calibrated to deliver 5ml per 6 sprays. The 'blank' suspension was prepared in the same manner from non-streaked plates. After transferral to the sprayers, viable counts of the suspension and blank were performed using Tryptone Bile X-glucuronide with added nalidixic acid (TBXN) plates and 3M Petrifilm™ *E.coli* / Coliform count plates.

4.2.3. Infliction of injury to plants and application of *E. coli* suspensions

Plants were injured and sprayed using the appropriate *E. coli* suspension or deionised water blank on the 10th December 2001. Plants were injured by pressing the 3 central leaves together between the thumb and forefinger using disposable gloves. Within a few minutes of injury, the appropriate spray was applied to plants from calibrated sprayers. This was done by spraying twice about 10 cm directly above the centre of each plant.

4.2.4. Testing lettuce plants for persistence of *E. coli* and total aerobic bacterial counts (TAC)

The experimental unit at each sampling time was six plants harvested at soil level. Care was taken to prevent contamination of the shoots by soil. The knife was sprayed with ethanol and wiped with fresh paper towelling between each experimental unit. The very outer leaves of harvested plants were removed and the remainder placed in large, sealable plastic bags, then weighed and stored at 4ºC until testing.

During testing, the plant material from each experimental unit was aseptically chopped and mixed. Twenty-five grams of this was aseptically weighed out to which 225 ml of 0.1% peptone was added followed by stomaching at high speed for 2 minutes. Serial dilutions to 10^4 were performed and the suspensions were inoculated onto 3M Petrifilm™ *E.coli* / Coliform count plates, 3M Petrifilm™ Total Aerobic Count (TAC) plates and TBXN agar plates. The 3M Petrifilm™ *E.coli*/Coliform and TAC plates were inoculated with 1 ml of the suspension and 0.1 ml was spread onto the TBXN agar plates. All were incubated at 37^{o}C for 48 hours, except for the TAC plates, which were incubated for 24 hours

4.2.5. Data analysis

Data were analysed by ANOVA using GenStat for Windows, Release 4.2, Fifth edition (Lawes Agricultural Trust, VSN International Ltd, Oxford, U.K.).

4.3. Persistence of enteric bacteria on leafy vegetables – the effect of timing of leaf injury in relation to bacterial contamination

4.3.1. Trial design

Two separate trials were conducted to test:

- The effect of injury prior to bacterial contamination (Trial 1), and
- The effect of injury after bacterial contamination (Trial 2).

Both trials were conducted in a glasshouse with Cos lettuce. For Trial 1, pots were arranged in a randomised completed block design with four replicates per treatment. The following treatments were imposed:

- Injury at 14, 7, 3, 1 and 0 days prior to dosing with *E.coli* suspension.
- Two negative controls – uninjured and dosed; uninjured and undosed.

For Trial 2, the design is described in the table below and 5 replicates were used. The *CycDesign* Program[1] was used to create a resolvable, latinised by column design using two replicate groups since the five replicates were split over two glasshouse benches.

Days after dosing	Injury	Dosing with *E. coli*
14	Yes	Yes
14	No	Yes
14	Yes	No
7	Yes	Yes
7	No	Yes
7	Yes	No
3	Yes	Yes
3	No	Yes
3	Yes	No
1	Yes	Yes
1	No	Yes
1	Yes	No
0	Yes	Yes
0	Yes	No
0	No	Yes

4.3.2. Trial set-up and maintenance

Six week old Cos lettuce seedlings, cv. Junior, were transplanted into pasteurised potting mix on 11th June 2002 and 22nd October 2002 for Trial 1 and 2 respectively, and fertilised with Osmocote® Controlled Release Plant Food Plus[2] at the recommended rate. Irrigation was initially by way of sprayers timed to operate for between 1 and 2 minutes once or twice per day, depending on requirements. After dosing, the sprayers were replaced with drip irrigators and these were timed to operate twice per day for 3 minutes. A blue tarpaulin was installed under the pots to collect run off for disinfection after the trial. Plants were kept under a tent

[1] A package for the computer generation of experimental designs, Version 2 Feb – 2002, © D. Whitaker, E. R. Williams* and K. A. John, Department of Statistics, University of Waikato, Hamilton, New Zealand, and *CSIRO Forestry and forest Products, Canberra, Australia.

[2] 15% Nitrogen (N) - 7.9% nitrate nitrogen, 7.1% ammonical nitrogen, 4.4% Phosphorous (P)- 3.3% P water soluble, 10.0% Potassium (K) - water soluble (chlorine free), 2.5% Sulphur (S), 1.2% Magnesium (Mg), 0.02% Boron (B), 0.05% Copper (Cu), 0.4% iron (Fe) – 0.20% present as iron sulphate, 0.02% present as iron EDTA, 0.06% Manganese (Mn), 0.02% Molybdenum (Mo), 0.015% Zinc (Zn), 6.0%

frame covered with clear polyethylene, to prevent interference from leaks and adjacent irrigation systems.

4.3.3. Infliction of injury to plants prior / after dosing with bacterial suspension

For Trial 1, plants were first injured 14 days prior to dosing (27th July 2002). On this occasion, 3 large inner leaves from all plants in the experiment were selected for injury by marking them with a pen. On each occasion when plants were to be injured, clean disposable gloves were worn and the marked leaves were folded in half longitudinally and horizontally and pressed together between the thumb and forefinger.

For Trial 2, plants were injured in a similar manner to Trial 1 but injury was imposed after dosing with the bacterial suspension.

4.3.4. Preparation of inoculum for dosing

Escherichia coli culture number NCTC 10418 was obtained from Eleonora Puglia of the School of Microbiology, The University of Melbourne. This is non-pathogenic and non-engineered with wild type characteristics. *Enterobacter aerogenes*, culture number ACM 4982, was used as a negative control culture for the 3M Petrifilm™ *E.coli* / Coliform count plates. It was purchased from the Centre of Bacterial Diversity and Identification, Department of Microbiology, University of Queensland.

The inocula were prepared from 1 day-old colonies growing on TSA plates, as follows. The colonies from one plate were emulsified with 6 ml of 0.1% peptone using a surface sterilised bent glass rod. This suspension was poured into 35 ml centrifuge tubes and topped up with 0.1% peptone. Controls were prepared in the same manner using blank (uninoculated) plates. The tubes were centrifuged at 3000 rpm for 10 minutes after which the supernatants were decanted and the pellets were re-suspended in fresh 0.1% peptone. The tubes were centrifuged again and the pellets were re-suspended in deionised water. The volume of each suspension was made up to 1.2 litres and mixed well. Live suspensions were transferred to calibrated sprayers delivering 5.8 ml per 6 sprays while the controls ('blanks') were transferred to calibrated sprayers delivering 5.6 ml per 10 sprays. After transferring to the sprayers, viable counts of each suspension were performed using serial dilutions on 3M Petrifilm™ *E.coli* / Coliform count plates.

4.3.5. Dosing with bacterial suspensions

Suspensions were delivered by holding the sprayer 10-15 cm from each side of the marked leaf at a 45 degree angle and spraying twice. Dosing occurred for each treatment block over a 1-2 hour period in succession. Dosing was done per block for statistical reasons.

For Trial 2, dosing with bacterial suspension occurred on the 29th of November 2002.

4.3.6. Testing for persistence of *E. coli*

Marked leaves were processed 3 days and 5 days after dosing for Trial 1 and 3 days after injury for Trial 2. Marked leaves were removed with sterilised scissors, cut up and weighed. To each plant sample was added 250 ml 0.1% peptone followed by stomaching at high speed for 2 minutes. Serial dilutions were prepared and suspensions were applied to 3M Petrifilm™ *E.coli* / Coliform count plates and incubated at 37 ºC. Plates were assessed at 24 and 48 hrs. For total aerobic bacterial counts, stomached samples were plated out as above onto 3M Petrifilm™ TAC plates and incubated at 37°C for 24 hours.

4.3.7. Data analysis

Data were analysed by ANOVA using the statistical package GenStat for Windows, Release 4.2, Fifth edition (Lawes Agricultural Trust, VSN International Ltd, Oxford, U.K.).

4.4. Persistence of enteric bacteria in poultry litter – the effect of temperature and moisture under laboratory conditions

4.4.1. Poultry litter

Approximately 15 kg of fresh poultry litter was sourced from the storage depot of a poultry litter contractor, South East of Melbourne. The litter consisted of manure and rice hulls collected from the cleaning of broiler (poultry meat birds) production sheds. The litter was stockpiled at the depot for a day prior to collection. This one batch of poultry litter was used for all experimental runs conducted in this trial.

Analysis of poultry litter

Moisture content of the poultry litter was determined prior to the commencement of the experiment. Four 150 g sub samples were sieved with a 4.75 mm aperture sieve to remove large lumps of manure and feathers. The samples were then oven dried at 105^{o}C for 24 hours.

A sample of the poultry litter was sent to the State Chemistry Laboratory (Department of Primary Industries) for analysis. Tests performed were according to the Australian Standard™ for composts, soil conditioners and mulches (AS4454-1999).

Storage of poultry litter

The poultry litter was stored in a 25 litre domestic garbage bin, placed in a 4^{o}C cool room, for the entire trial period.

4.4.2. Bacterial strains

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

4.4.3. Description of bench scale apparatus

A laboratory scale composting apparatus was employed with different temperature conditions in six discrete chambers (Appendix, Plate 1). Each chamber contained a water bath into which a 10 cm diameter plastic container, constructed to hold four 50 ml plastic tubes, was immersed (Appendix, Plate 2). The temperature of the water bath was regulated by a dial set above each chamber, with a possible range of 30 – 75^{o}C. Temperature could be controlled independently for each discrete chamber. Probe-type thermometers were inserted into each of the chambers to log temperature.

4.4.4. Trial design

Sterile 50 ml plastic tubes containing 10 g of poultry litter inoculated with approximately 10^{9} cfu/ml of *E. coli* and *S.* Typhimurium were used. The plastic tubes were placed in the chambers, once the water bath had reached the desired temperature.

A plan of the chamber arrangement is shown in Figure 1 (Appendix) where the chambers are numbered from 1 to 6. The trial design protocol shown in Table 1 illustrates this arrangement

(Appendix). The 12 test runs were considered as one experiment for analysis. Pathogen survival was assessed for all combinations of four temperatures (35, 45, 55 and 65^{o}C) and three moisture content levels (30, 50 and 65% w/w). The sampling protocol is outlined in Table 2 (Appendix).

Each combination of temperature x moisture content was replicated six times during the series of 12 test runs. The order of the 12 tests was randomised, with the restriction that each group of three tests (1-3 etc) had one of each moisture content.

The randomisation of temperatures to chambers had the following features:

- For any test, two of the four temperatures were represented once, and the other two, twice;
- For any of the three moisture contents, the combination of temperatures was different for each test;
- Every combination of temperature and moisture content occurred exactly once in each of the six chambers; and
- The first six tests and the second six tests each had three replicates of every combination of temperature and moisture content.

4.4.5. Preparation and inoculation of poultry litter samples

The poultry litter and bacterial inoculum were prepared the day before each test run.

Preparation of poultry litter

For each test run, a quantity of poultry litter was sieved with a 4.75 mm aperture sieve to remove large lumps of manure and feathers to produce a 500 g sample. The moisture content of the litter was determined and then adjusted to the required level by spreading the litter across the base of a large plastic tray and wetting with de-ionised water.

Ten grams of the wetted poultry litter was then placed into each of 35 sterile 50 ml plastic tubes with screw-top lids. The tubes were then stored in a 4^{o}C cool room until inoculation with the bacteria strains the following day.

Preparation of bacterial inoculum

E. coli and *S.* Typhimurium were cultivated at 37^{o}C and at 120 rpm in an orbital incubator for 18 hours. Cultures for each bacterium were inoculated from freshly grown nutrient agar plates into two separate 50 ml flasks containing 15 ml of sterile nutrient broth. After incubation and shaking for 18 hours, contents of the two flasks were pooled and mixed.

Inoculation of poultry litter samples

To each of the 35 sample tubes containing the poultry litter, 0.1 ml of the combined *E. coli* and *S.* Typhimurium inoculum was added.

For each chamber of the bench scale apparatus, there were 5 sample tubes; 4 of which were immersed into the water bath and one set aside to determine initial *E. coli* and *S.* Typhimurium levels at the start of the test run.

4.5.6. Sampling after incubation and bacteriological testing

One sample tube was removed from the relevant chamber, according to the sampling protocol listed in Table 2 (Appendix).

After retrieval, the 10 g poultry litter sample was emptied from the tube into 50 ml of sterile 0.1% peptone water in a jar. The jar was placed on an orbital shaker for 30 minutes set at 120 rpm and allowed to settle before ten-fold serial dilutions were prepared into sterile 0.1% peptone water and plated onto 3M Petrifilm™ *E. coli* / Coliform count plates and XLD agar plates.

Tests for E. coli

Direct counts of *E. coli* were determined by 3M Petrifilm™ *E. coli* / Coliform count plates incubated at 37°C for 48 hours. Blue colonies producing gas were deemed to be *E. coli*. Final results were calculated as colony forming units per gram of fresh weight (CFU/g).

Tests for Salmonella enterica serovar Typhimurium

The Microbiological Diagnostic Unit (Department of Microbiology and Immunology, The University of Melbourne) provided XLD agar plates for the determination of *S.* Typhimurium. 0.1 ml of the serial dilutions were inoculated onto the plates, which were then incubated at 37°C for 24 hours and sent to The Microbiological Diagnostic Unit for identification and determining the number of colony forming units.

4.5.7. Data analysis

All results were transformed to base 10 logarithms and analysed by residual maximum likelihood (REML) using GenStat for Windows, 6th Edition (Lawes Agricultural Trust, VSN International Ltd, Oxford, U.K.).

4.6. Persistence of enteric bacteria in poultry litter – the effects of aging in windrows

4.6.1. Windrow management

Two windrows were constructed from fresh poultry litter, collected from the same broiler production shed, at a location 40 km South East of Melbourne. Each windrow had the following approximate dimensions: 3 m (width) x 1.6 m (height) x 20 m (length).

Turned windrow

This windrow was completely turned with a front-end loader every 7-10 days for 8 weeks. In the final four weeks no turning occurred.

Static windrow

The second windrow remained undisturbed for the same 12-week trial period, apart from sampling activities.

4.6.2. Temperature monitoring

Mean daily temperatures were measured in each windrow using t-type thermocouples taped to dowelling poles and linked to a datalogger recording every 2 hours. Thermocouples were spaced in a 30 cm by 30 cm grid pattern from the base to the top of each windrow, so that a cross-sectional profile of internal temperatures could be generated using Delta Graph 5 for Windows (Red Rock Software and SPSS Inc.). The surface area of a cross-section falling into the following temperature categories, <35 ºC, 35-44 ºC, 45-54 ºC, 55-64 ºC and >65 ºC was calculated using image analysis software (SigmaScan Pro 3, Jandel Scientific).

4.6.3. Poultry litter analysis

Each windrow was sampled at weeks 0, 3, 6 and 12 for chemical analysis by the State Chemistry Laboratory (Department of Primary Industries). Tests performed were according to the Australian Standard™ for composts, soil conditioners and mulches (AS4454-1999). For each sampling event, 20 to 30 spade fulls were taken from around the pile to a depth of 5 – 30 cm, mixed together, and quartered until a quantity of approximately 6 litres was obtained.

4.6.4. Bacterial monitoring

Samples of poultry litter were collected from each pile for analysis of enteric pathogenic bacteria at weeks 0, 3, 6 and 12. Four samples were collected from four locations along the length of the composting pile, totalling 16 samples for each pile at each sampling event. Each sample consisted of 6 trowel-fulls (approximately 1.5 kg) of poultry litter taken from a depth of 5 – 10cm. Samples were analysed for *E. coli*, *Salmonella* spp., *Campylobacter* spp. and *Listeria* spp.

During week 10 of the trial, a 2 litre sample of leachate was collected from the base of the turned windrow pile, following heavy rainfall. The sample was sent to The Microbiological Diagnostic Unit (Department of Microbiology and Immunology, The University of Melbourne) for analysis of *Salmonella* spp., *Campylobacter* spp and *Listeria* spp.

Bacteriological testing

Approximately 0.5 kg of poultry litter from each sample was set aside for *E. coli* testing and the remaining sample was sent to The Microbiological Diagnostic Unit (Department of Microbiology and Immunology, The University of Melbourne) for testing of the other bacteria.

Tests for E. coli

For each sample, 10 g of poultry litter was added to 50 ml of sterile 0.1% peptone water in a jar. The jar was placed on an orbital shaker for 30 minutes set at 120 rpm and allowed to settle before ten-fold serial dilutions were prepared into sterile 0.1% peptone water The same procedures were followed as for *E. coli* testing in the bench scale trial.

Tests for Salmonella spp., Campylobacter spp. and Listeria spp.

For *Salmonella* spp., *Campylobacter* spp. and *Listeria* spp., a 25 g sub-sample was tested and quoted as presence or absence in 25 g of poultry litter. The methods used to test for each bacterium were, respectively, Australian Standard™ AS 1766.2.5 – 1991, AS 1766.2.13 – 1991 and AS / NZS 1766.2.16.1 – 1998.

4.6.5. Re-growth and persistence of enteric bacteria in aged litter

A quantity of poultry litter was collected from each of the windrows at weeks 0, 3, 6 and 12 to determine the potential of pathogen re-growth or persistence in the aged poultry litter. Material collected was either left untreated or modified prior to incubating at 37ºC for up to 21 days. *E. coli* was selected as the indicator organism for the purposes of the trial.

Sample preparations

At each sampling event, 12 grab samples of poultry litter were taken from around each windrow at a depth of 5 – 10 cm, with a total of approximately 6 kg collected.

Each sample was then divided into three 500 g sub-samples and were treated as follows:

- Untreated,
- Deionised water added to adjust moisture to 50% (w/w) or
- Deionised water added to adjust moisture to 50% (w/w) and inoculated with *E. coli*.

Procedures for adjusting the moisture content to 50% and inoculating with *E. coli* were followed according to those used in the bench scale trial.

Each treatment set was further divided into twenty-four 10 g samples placed in 50 ml plastic tubes with screw top lids. Eighteen of the 10 g samples were then incubated at 37ºC for up to 21 days (Appendix, Plate 4). The remaining six 10 g samples were tested for *E. coli*, which provided baseline levels (ie at 0 day).

Trial design

A summary of the parameters tested for these trials are listed in Table 3 (Appendix).

Sampling and tests for E. coli

Six 10 g samples were removed from the incubator at 7, 14 and 21 days. Each sample, was added to 50 ml of sterile 0.1% peptone water in a jar. The same procedures were followed as for *E.coli* testing in the bench scale composting trials. Final results were calculated as colony forming units per gram of dry weight (CFU/g).

4.6.6. Data analysis

All bacterial count results were transformed to base 10 logarithms. Tables of means were constructed for the bacteria (*E. coli* and *Salmonella* spp.) detected in the windrows. An analysis of variance (ANOVA) was used to analyse the data from the pathogen persistence and re-growth trial. GenStat for Windows, 6th Edition (Lawes Agricultural Trust, VSN International Ltd, Oxford, U.K.) was used for all statistical analysis.

5. Results

5.1. Risk associated with current use of poultry litter on farms

5.1.1. Current use of poultry litter

An overview of the practices used is shown in Figure 1. Litter arrives on the farm either fresh, aged or 'composted[3]'. It is then stored for some months before use or it is used immediately. Litter was stored on all farms within 50 m of the nearest crop. Poultry litter can be used before planting or as mulch just after planting. Used before planting, the litter is usually spread on the surface of the soil and then rotary hoed in. This is often done within days but may be done much later. Litter may also be applied at a late stage of crop growth as a side-dressing on the surface of the soil.

5.1.2. Incidence of enteric bacteria in litter, soils and crops

Although presumptive *E. coli* was present on crops at harvest the counts were all below 1 log(cfu/g+1) or <10 *E. coli* per gram (Table 1). *E. coli* counts of litter and litter treated soil were as high as 3.56 log(cfu/g+1) or 3630 *E. coli* per gram on Farm A early in the crop growing period but then dropped to low counts by harvest time. The highest count in the soil at harvest was only 1.81 log(cfu/g+1) on Farm E. Farm F had no direct counts from any samples, and soil and litter samples for Farms B, D and G either had no direct counts or were not statistically different from their crop's counts (Table 1). *Salmonella enterica* serovar Typhimurium was detected in the litter on Farm A, though it was not detected at all after that (Table 1).

S. enterica serovar Sofia was detected twice in litter deliveries (Farms E & G), but only once on a crop at harvest (Farm E) (Table 1). In the case of Farm E, the *Salmonella* that was detected on coriander almost certainly came from the litter, as it was traced back right through to the litter delivery (Table 1). The coriander on Farm E was side-dressed with litter 18 days prior to harvest. Other growers surveyed did not side-dress that close to harvest time, used 'composted' litter for side-dressing, or didn't side dress at all (Table 1). The stockpile that was sampled on Farm E was within 50 m of the coriander field from which *S. enterica* serovar Sofia was detected.

Litter deliveries on Farms D to G were tested for *Listeria* and none was detected.

[3] Though some growers reported that they were using composted litter, it is not clear whether it was actually composted (as per established best practice guidelines such as AS4454 1999; EPA Victoria 1996; Wilkinson *et al.* 2001) or aged for a given length of time.

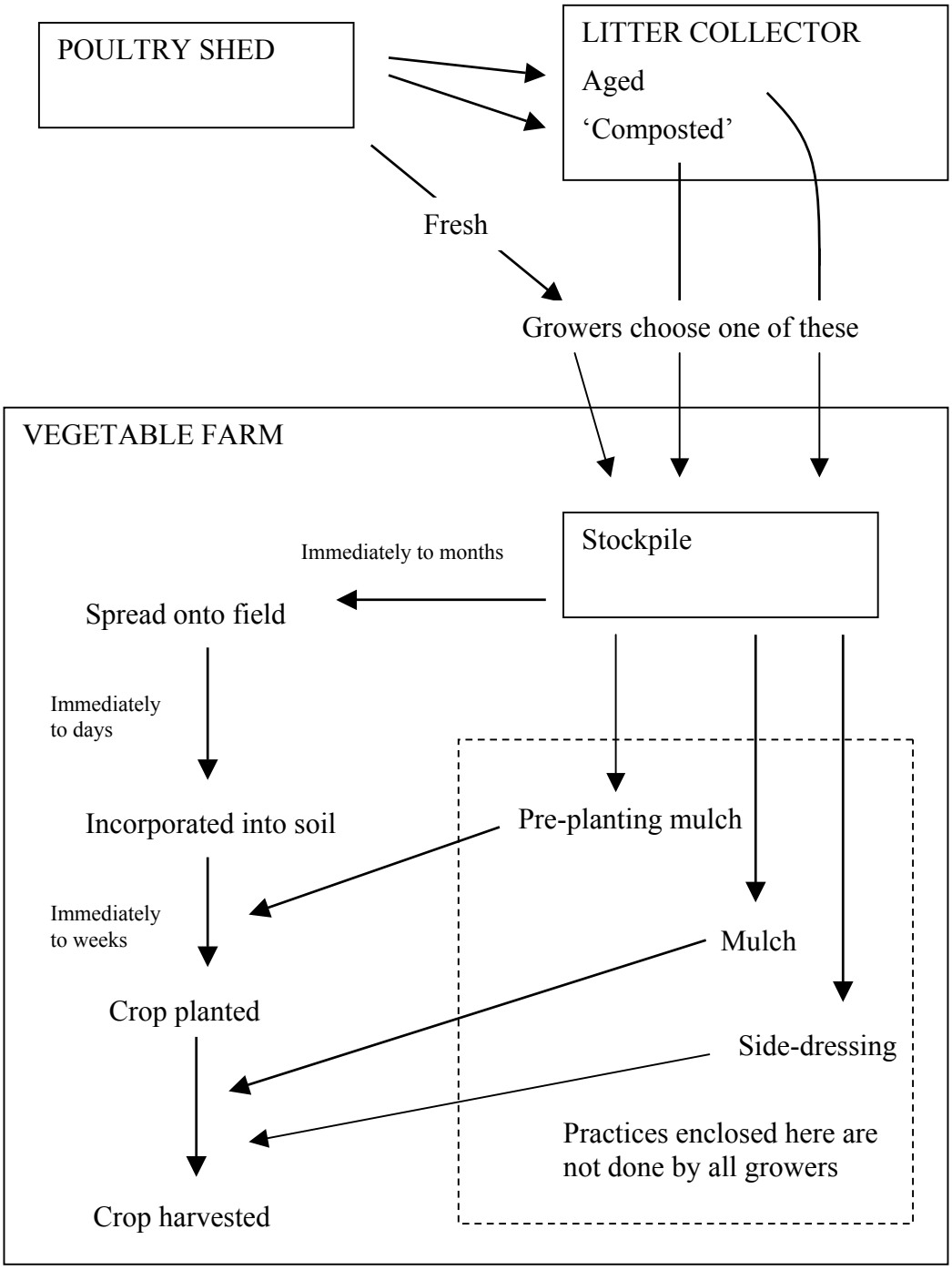


Figure 1: **Current use of poultry litter in vegetable production. 'Composted' litter may or may not be fully composted (see footnote on previous page).**

Table 1: **Incidence of enteric bacteria[1] in litter soils and crops from seven farms surveyed.**

Event		Farm						
		A	**B**	**C**	**D**	**E**	**F**	**G**
Litter delivered	Date	28.8.2001	19.9.2001	19.9.2001	26.10.2001	4.9.2002	~20.10.2002	17.9.2002
Litter type		Supposably aged	Aged	Fresh	Fresh	Obviously fresh	Obviously aged	Probably fresh
Litter delivery sampled	Date	28.8.2001	20.9.2001	21.9.2001	26.10.2001	4.9.2002	21.10.2002	19.9.2002
	E. coli	3.56	0.41	+	1.23	1.91	ND	0.49
	Pathogen	*S.* Typhimurium	ND	ND	ND	*S.* Sofia subsp. II	ND	*S.* Sofia subsp. II
Pre-litter soil sampled	Date	28.8.2001	17.9.2001	21.9.2001	26.10.2001	4.9.2002	21.10.2	Not tested
	E. coli	+	+	0.54	0.41	+	+	
	Pathogen	*Campylobacter*[2]	ND	ND	ND	ND	ND	
Litter dug into soil	Date	29.8.2001	26.9.2001	22.9.2001	26.10.2001		~29.10.2002	7.10.2002
Soil+litter sampled	Date	19.9.2001	26.9.2001	27.9.2001	1.11.1		12.12.2	8.10.2
	E. coli	0.34	1.49	2.02	1.81		+	0.53
	Pathogen	ND	ND	ND	ND		ND	ND
Crop planted	Date	30.8.2001	27.10.2001	26.9.2001	30.11.2001	3.9.2002	~14.11.2002	~18.11.2002
Litter[3] applied as mulch	Date				30.11.2001	5.9.2002		
Litter[3] side dressing	Date		4.11.2001[4]					
Soil + litter sampled	Date		8.11.2001		4.12.2001	10.9.2002		18.12.2002
	E. coli		0.73		1.00	+		+
	Pathogen		ND		ND	*S.* Sofia subsp. II		ND
Litter[3] side dressing	Date					11.10.2002		
Soil+ litter sampled	Date					14.10.2002		
	E. coli					3.88		
	Pathogen					*S.* Sofia subsp. II		
Soil+litter sampled at harvest	Date	8.10.2001	6.2.2002	19.12.2001	21.1.2002	28.10.2002	7.1.2003	24.2.2003
	E. coli	+	0.28	1.18	+	1.81	+	+
	Pathogen	*S.* Sofia subsp. II	ND	ND	ND	ND	ND	ND
Crop sampled at harvest	Date	8.10.2001	6.2.2002	19.12.2001	21.1.2002	29.10.2002	7.1.2003	24.2.2003
	E. coli	+	0.26	+	0.59	+	+	+
	Pathogen	ND	ND	ND	ND	*S.* Sofia subsp. II	ND	ND
	Crop	Salad brassicas	Celery	Celery	Iceberg lettuce	Coriander	Mixed lettuce	Celery
L.S.D. (*P* = 0.05) for *E. coli*		1.07	1.62	1.07	1.49	0.59		1.77

[1]*E. coli* counts were means of four quarters expressed as log(cfu/g + 1); + *E. coli* detected after enrichment from at least one quarter; ND not detected.
[2]Species not identified.
[3]Litter different to original delivery - it was not tested and unless specified was probably fresh.
[4]Litter different to original delivery – it was 'composted' according to the grower.

5.2. Persistence of bacteria on leafy vegetables – the effect of leaf injury under field conditions

E. coli counts on uninjured plants were reduced by more than 99% within the first 3 days (Fig. 2). In contrast, the rate of reduction of bacteria on the injured leaves was relatively constant over time; after 3 days the *E. coli* count was reduced by 37%, and a 99% reduction did not occur until day 23 (Fig. 2). Leaf injury had no affect on total aerobic counts (data not presented).

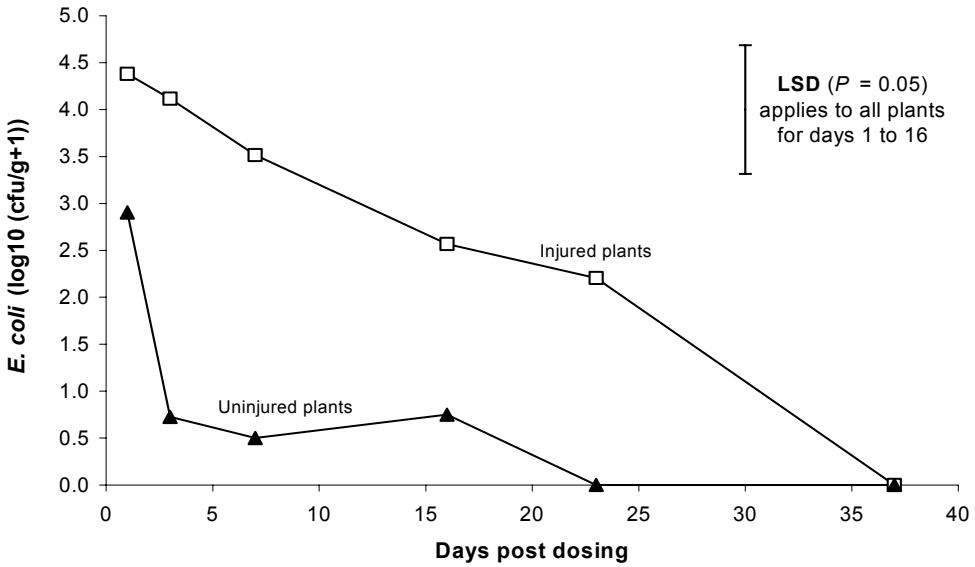


Figure 2: The effect of leaf injury on the persistence of *E. coli*^{Nal} on field Cos lettuce

5.3. Persistence of bacteria on leafy vegetables – the effect of timing of leaf injury in relation to bacterial contamination

E. coli counts reduced rapidly with increasing time between bacterial contamination and leaf injury (Fig. 3 & 4). *E. coli* counts were highest when leaf injury occurred less than 1 day prior to, or less than 3 days after, bacterial contamination (Fig. 3 & 4). When the duration between bacterial contamination and leaf injury exceeded these times, *E. coli* counts were not significantly more to those found in uninjured plants.

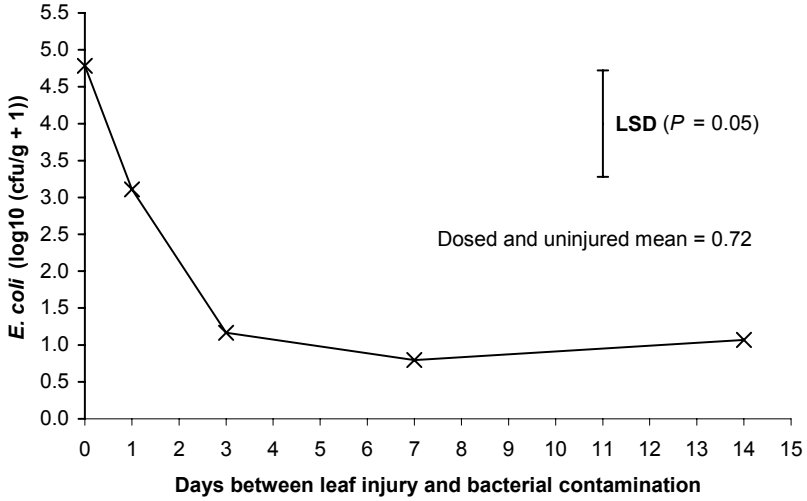


Figure 3: ***E. coli*** **counts from Cos lettuce with increasing time between leaf injury and bacterial contamination. Injury was applied at the indicated times prior to inoculation with *E. coli*. Counts were determined 3 days after inoculation.**

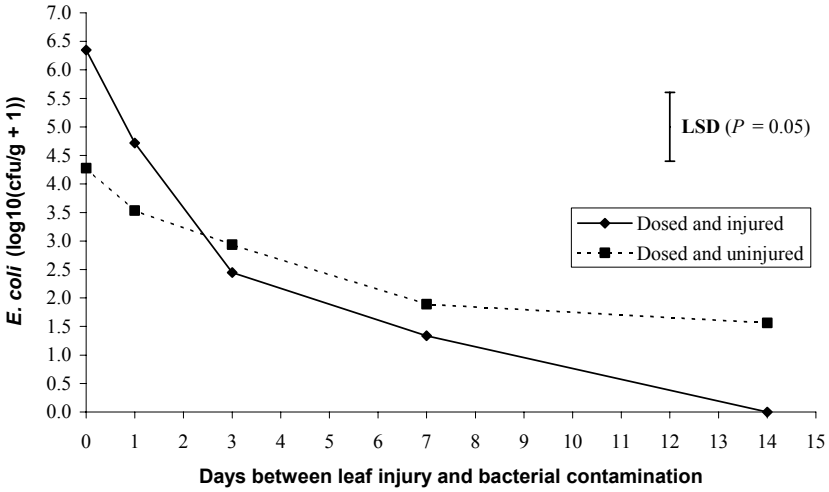
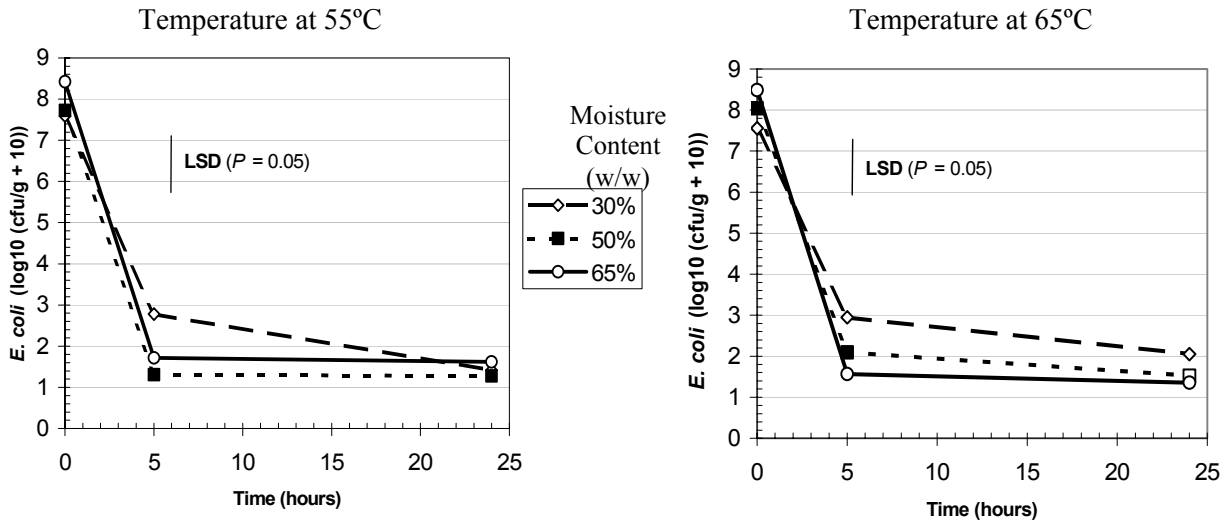


Figure 4: *E. coli* counts from Cos lettuce with increasing time between leaf injury and bacterial contamination. Inoculation with *E. coli* occurred at the indicated times after leaf injury. Counts were determined 3 days after inoculation.

5.4. Persistence of enteric bacteria in poultry litter – effect of temperature and moisture under laboratory conditions

The most rapid reduction in *E. coli* and *S.* Typhymurium counts occurred at high temperatures, but 65ºC was not more effective than 55ºC. *S.* Typhymurium was completely eliminated and *E. coli* counts were reduced by more than 99% after 1 hr exposure to these temperatures.

Incubation at 65ºC and 65% moisture content for 5 hrs resulted in significantly lower *E. coli* counts compared to the same temperature conditions and 30% moisture content (Fig. 5 & 6). Similar effects were observed at 55ºC between 65% and 50% moisture content. At 24 hrs incubation, there was no significant difference in *E. coli* counts between the different moisture contents.



Figures 5 & 6: Effect of high temperature (55-65ºC) and moisture content (30-65% w/w) on persistence of *E. coli* in poultry litter.

All treatment combinations resulted in greater than a 99% reduction in *E. coli* counts after 8 hrs, but at 35ºC/65% more than 134,000 (5.13 log10) *E coli* were still present in the litter after 24 hrs incubation (Fig. 7). At lower temperatures (35-45ºC), more effective reduction in *E. coli* and *S.* Typhimurium counts was observed at 30% moisture content than at 65% (Figs. 7-9). For *E. coli*, this effect was observed up until 5 hrs exposure to 45ºC, but at 35ºC this was still the case after 24 hrs (Fig. 7).

All moisture levels at 35ºC resulted in more than a 99% reduction in *S.* Typhimurium counts within 24 hrs; at 45ºC the same level of control was achieved after 8 hrs (Figs. 8 & 9). However, after 24 hrs exposure to both temperatures, *S.* Typhimurium counts were significantly higher at 65% moisture content compared to 30% (Figs. 8 & 9). At 35ºC, there were about 40 times more *S.* Typhimurium enumerated at 65% moisture content than at 30% (p=0.05). At 45ºC there were 50 times more at 65% moisture content compared to 30%, though in general, counts at this temperature were significantly lower than at 35ºC (p=0.05).

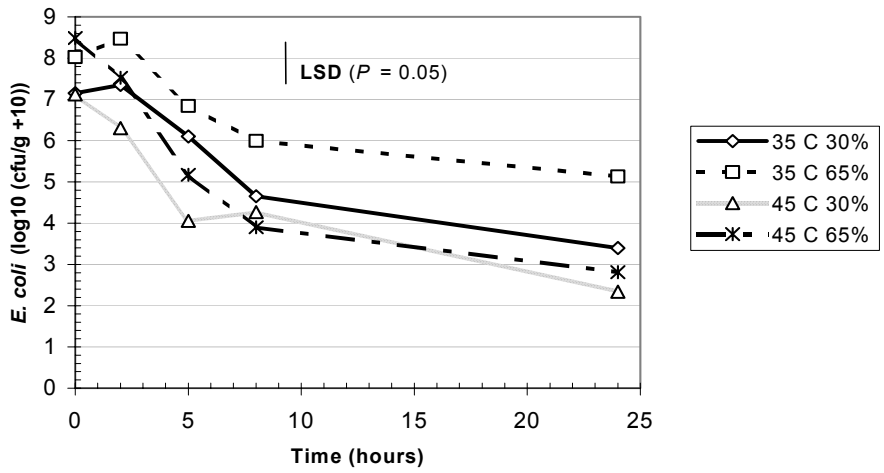
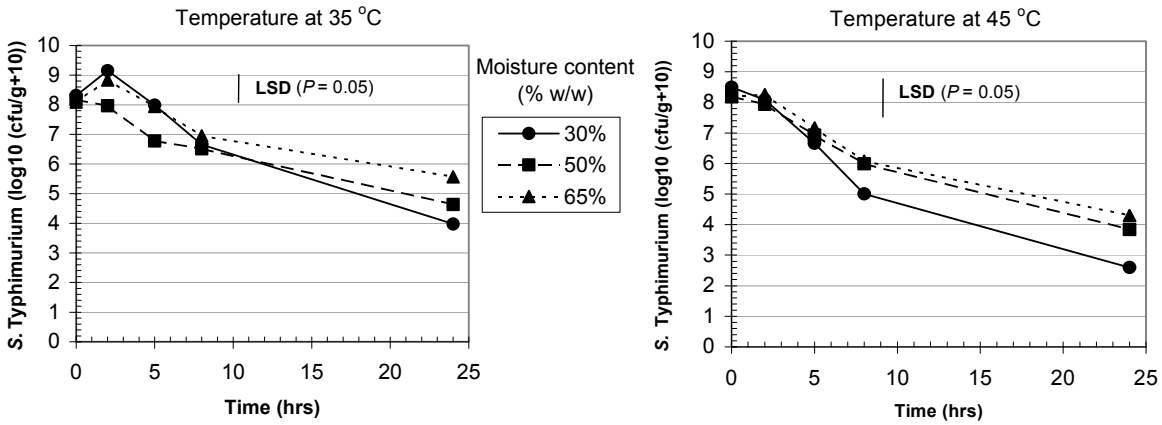


Figure 7: Effect of low temperature (35-45ºC) and moisture content (30 & 65% w/w) on persistence of *E. coli* in poultry litter. Data for 50% moisture content are not shown as these were not significantly different to the 30% treatment combinations (p>0.05).



Figures 8 & 9: Effect of temperature (35 & 45ºC) and moisture content (30, 50 & 65% w/w) on persistence of *Salmonella enterica* serovar Typhimurium in poultry litter. Data for treatment combinations at 55 and 65ºC are not presented as *S.* Typhimurium was eliminated within 1 hr (first sampling) at these temperatures.

5.5. Persistence of enteric bacteria in poultry litter – effects of aging in windrows

5.5.1. Temperature and poultry litter analysis

More of the poultry litter in the turned windrow was consistently exposed to higher temperatures over the course of the trial than in the static windrow (Figs. 10 & 11). In the first 3 weeks of the trial, the two windrows followed a similar pattern of temperature distribution; temperatures peaked above 65ºC during this time (Figs. 10 & 11). Temperatures also fell away gradually in both windrows after about 9 weeks.

Temperatures in the 35-44ºC range predominated in the static windrow during the middle period (3-9 weeks) of the trial (Fig. 10). During this period, the average portion (% surface area) of litter exposed to the 35-44ºC range was approximately 60%. Only 7% of the litter was exposed to temperatures of 45ºC and above during this period. In contrast, about 54% of litter was exposed to temperatures of 45ºC and above in the turned windrow during the same period. From 3-9 weeks, about equal portions of litter in the turned windrow was exposed to the 35-44ºC and 45-54ºC temperature ranges (Fig. 11). Temperatures above 55ºC were also more common in the turned windrow.

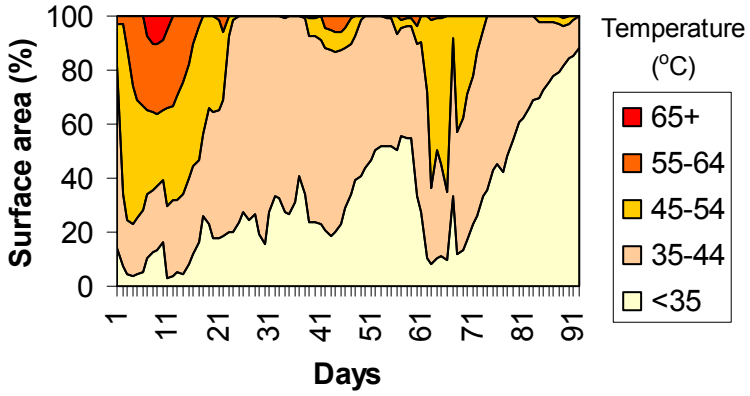


Figure 10: Percent surface area of litter exposed to a given temperature range over a 12 week aging process in a static windrow. Temperature ranges are average daily temperatures taken in a 30 cm by 30 cm grid through one cross-section of the windrow.

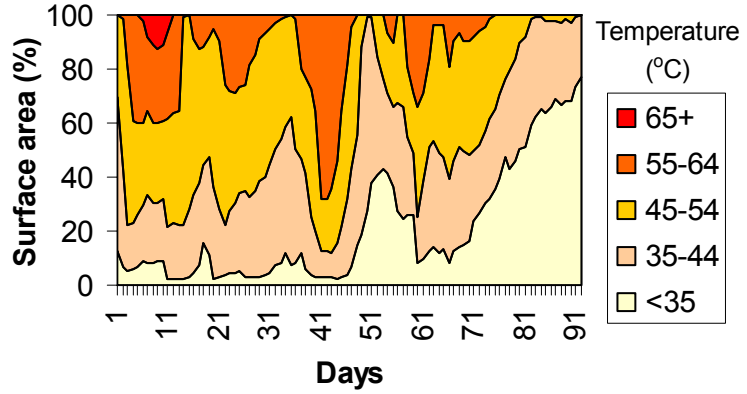


Figure 11: Percent surface area of litter exposed to a given temperature range over a 12 week aging process in a turned windrow. Temperature ranges are average daily temperatures taken in a 30 cm by 30 cm grid through one cross-section of the windrow.

High temperatures were achieved in both windrows despite the poultry litter being very dry. Moisture content of the litter ranged from a low of 16 to a maximum of 24% (w/w) by week 6 (Table 2). The poor germination and toxicity test results are probably due to the high levels of ammonium that was present in both windrows at each sampling occasion (Table 2). Both windrows emitted a distinct odour of ammonia gas during the course of the trial.

The ammonium/nitrate ratio peaked at 54.6 after 6 weeks for the turned and 38.2 for the static windrow (Table 2). After 12 weeks, the ratio for either windrow was still not below 10, an indication that the poultry litter was still not stable or mature.

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

Attribute[1]		Week 0		Week 3		Week 6		Week 12	
		Static	Turned	Static	Turned	Static	Turned	Static	Turned
Moisture	% (w/w)	16	18	22	22	22	24	21	23
pH		8.2	8.3	7.6	8.1	8.4	8.3	8.4	8.6
C:N		10	9.4	9.2	9.1	10	9.4	8.5	8.3
Ammonium-N	mg/L	390	380	450	370	420	530	580	600
Nitrate-N	mg/L	69	46	44	26	11	9.7	58	37
Ammonium/Nitrate ratio[2]		5.6	8.2	10.2	14.2	38.2	54.6	10	16.2
Germination test	%	ND	ND	0	0	0	0	10	0
Toxicity index[2]		ND	ND	0	0	0	0	7	0

[1]tested according to AS4454 (1999); Full analysis available in Appendix; ND - test not done.
[2]critical values for a mature product are <1 for ammonium/nitrate ratio (Bernal *et al.* 1998; Pare *et al.*1998) and >60 for toxicity index (AS4454 1999)

5.5.2. Detection of enteric bacteria in windrows

Listeria and *Campylobacter* were not detected in any sample collected from either windrow. However, *L. monocytogenes*, *L. innocua* and *Salmonella enterica* serovar Agona were detected in leachate from a puddle of water lying at the base of the turned windrow following heavy rainfall.

There was no effective change in *E. coli* counts in the outer layers of either windrow over the course of the trial (Fig. 12).

Salmonella species were detected more frequently in the static windrow. In the turned windrow, *Salmonella* was no longer detected after 6 weeks, but was detected in 2 out of 16 samples in the static windrow (Table 3). *S.* Sofia was the most frequently detected serovar in both windrows. Two pathogenic serovars were detected in the turned window (*S*. Hvittingfoss, *S*. Mbandaka).

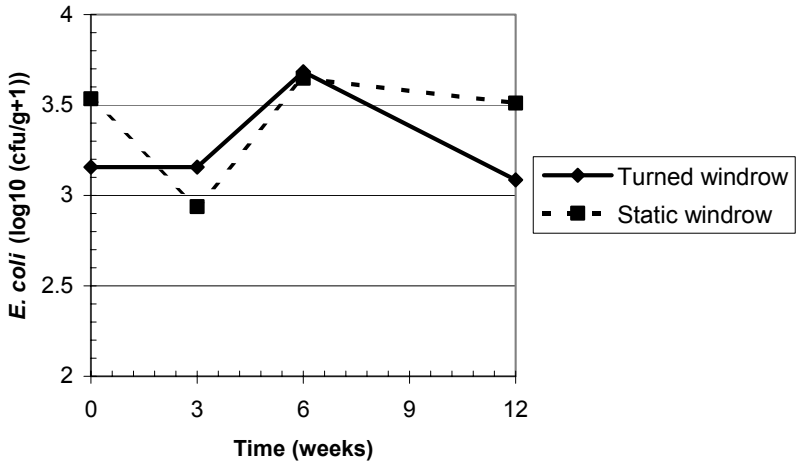


Figure 12: *E. coli* counts in poultry litter from two windrows aging with time. Data are means of 16 samples taken 5-10 cm below the surface of the windrows on each sampling occasion.

Table 3: Type and frequency of *Salmonella* detected in poultry litter windrows

	Turned windrow		Static windrow	
Sampling (wk)	**Frequency[1] (*n*/16)**	***Salmonella* serovar detected**	**Frequency[1] (*n*/16)**	***Salmonella* serovar detected**
0	7	all S.Sofia	11	all S.Sofia
3	2	S.Hvittingfoss S.Mbandaka	7	all S.Sofia
6	0	-	2	all S.Sofia
12	0	-	0	-

[1] Frequency of detection in 16 samples collected at each sampling time from each windrow

5.5.3. Re-growth and persistence of enteric bacteria in aged litter

Re-growth of *E. coli* occurred in poultry litter aged up to 3 weeks in the turned windrow and up to 6 weeks in the static windrow (Tables 4 & 5). Spiking the litter with additional *E. coli* increased initial counts but did not increase the incidence of re-growth in either windrow; in these treatments all counts were reduced after 21 days of incubation.

In the turned windrow, *E. coli* counts increased more than 1900 fold in unamended fresh litter (0 weeks old) after 21 days incubation (Table 4). An eleven hundred-fold increase was also observed in 3 week old litter that had been adjusted to 50% moisture content prior to incubation for 21 days (Table 4).

Counts in 6 week old litter from the turned windrow were not significantly lower after 21 days incubation in the unamended treatment and after 14 days in the litter adjusted to 50% moisture content (Table 4). In 12 week old unamended litter, counts were not reduced significantly until 14 days of incubation. Though counts in the 50% moisture content treatment of 12 week old litter were not significantly different with incubation time, *E. coli* could not be detected by day 14 (Table 4).

Table 4: Re-growth and persistence of *E. coli* in poultry litter of different ages from turned windrow. Data are mean counts of *E. coli* (log_{10} (cfu/g + 10)).

Incubation (days)[2]	Treatment prior to incubation of litter[1] Unamended	50% moisture (w/w)	50% moisture + *E. coli*
	Poultry litter 0 weeks old		
0	1.46	3.68	6.83
7	3.44	1.58	6.60
14	2.16	2.85	4.88
21	4.56	3.22	3.97
	Poultry litter 3 weeks old		
0	3.47	1.51	8.43
7	4.79	3.50	7.11
14	2.16	2.13	10.02
21	3.85	4.50	5.38
	Poultry litter 6 weeks old		
0	3.35	4.55	8.48
7	5.25	6.86	7.71
14	1.66	2.85	4.82
21	1.78	1.00	3.46
	Poultry litter 12 weeks old		
0	4.50	3.88	8.72
7	3.67	3.34	6.45
14	1.00	1.00	1.00
21	1.00	1.00	1.00

LSD = 2.71 (P=0.05)

[1] Unamended – litter incubated 'as is'; 50% moisture – moisture content of litter amended to 50% (w/w); 50% moisture + E. coli - litter inoculated with *E. coli* and moisture content amended to 50% (w/w).

[2]Poultry litter incubated after treatment at 37ºC for up to 21 days before *E. coli* counts were assessed.

E. coli could not be detected in 12 week old litter from both windrows that had been incubated for 14 or 21 days (Table 4 & 5). In contrast, incubation for 7 days did not reduce *E. coli* counts for any treatment combination in either windrow. Overall, litter from the static

windrow reacted in a similar manner to that from the turned windrow. However, incubation of unamended 6 week old litter or litter adjusted to 50% moisture content resulted in a significant increase in *E. coli* counts (Table 5). In the litter amended to 50% moisture content, this increase amounted to more than a 6-fold increase in population after every 24 hrs of incubation.

Table 5: Re-growth and persistence of *E. coli* in poultry litter of different ages from static windrow. Data are mean counts of *E. coli* (log_{10} (cfu/g + 10)).

Incubation (days)[2]	Treatment prior to incubation of litter[1] Unamended	50% moisture (w/w)	50% moisture + *E. coli*
	Poultry litter 0 weeks old		
0	4.06	4.17	8.03
7	2.79	2.55	2.44
14	1.79	2.01	3.55
21	2.20	4.65	4.17
	Poultry litter 3 weeks old		
0	2.85	2.84	8.32
7	2.53	4.94	8.06
14	2.83	3.49	3.26
21	1.99	6.29	5.50
	Poultry litter 6 weeks old		
0	3.12	1.80	8.35
7	5.96	7.52	6.07
14	4.70	1.00	7.45
21	1.55	3.04	4.04
	Poultry litter 12 weeks old		
0	4.43	3.40	8.52
7	3.23	4.14	7.91
14	1.00	1.00	1.00
21	1.00	1.00	1.64

LSD = 2.58 (P=0.05)

[1] Unamended – litter incubated 'as is'; 50% moisture – moisture content of litter amended to 50% (w/w); 50% moisture + E. coli - litter inoculated with *E. coli* and moisture content amended to 50% (w/w)

[2]Poultry litter incubated after treatment at 37ºC for up to 21 days before *E. coli* counts were assessed.

6. Discussion

6.1. Risk associated with current use of poultry litter on vegetable farms

The clearest contrast in the way litter was used and the subsequent bacteriological results occurred between Growers E and F. Grower F used aged litter and didn't side dress and had favourable bacteriological results. In contrast, Grower E used obviously fresh litter, did side dress and had higher *E. coli* counts and *Salmonella* was detected.

E. coli was present on crops at harvest but the counts recorded were not particularly high for produce (Sagoo *et al.* 2001; IFST 1997). The levels of bacteria on the produce would be further reduced after washing or disinfection, as is the usual practice of growers before shipment. *E. coli* counts of litter and litter treated soil were high for some of the growers surveyed early in the crop growing period, but these dropped to low counts by harvest time.

It cannot be certain whether the presence of salmonellae and *E. coli* counts was due to litter or another source of contamination. Low levels of *E. coli* contamination is also likely to occur in open fields due to wild animals and the use of contaminated irrigation water. However, in the case of *S. enterica* serovar Sofia on the coriander for Grower E, the bacterium almost certainly arose from fresh litter used as aside dressing or from contamination from the stock pile. The detection of *S. enterica* serovar Sofia is cause for concern, even though this species hasn't been associated with human disease (Jay *et al.*1997). If poultry litter can contaminate a crop with *S. enterica* serovar Sofia, then it may also do the same with human pathogenic salmonellae associated with poultry. These include *S.* Typhimurium found in the litter delivery of one of the growers surveyed, *S.* Hvittingfoss and *S.* Mbandaka detected in the turned windrow, *S.* Virchow found in Victorian litter by Jaeger and Premier (2001), and *S.* Enteritidis associated with chickens overseas (Riemann *et al.*1998).

In the case of the contaminated coriander, it had been side-dressed with fresh litter 18 days prior to harvest. Other growers surveyed didn't side dress so close to harvest time, used composted litter for side dressing, or didn't side dress at all. An alternative source of the contamination, may have been from the nearby litter stockpile. The closest edge of the coriander field was only 50 m away from the litter stockpile, whilst tested fields of the other growers were no closer than 130 m, and most well over 300 m. Being so close to the coriander made cross contamination possible via rain, wind, turbulence from passing vehicles, and movement of litter for treatment of other fields.

Despite extensive sampling, the great bulk of each field was not tested. Thus, even if no pathogens or *E. coli* were detected, these organisms could still be present in isolated pockets in the field. Although *E. coli* levels are used as an indicator of faecal contamination, they aren't the ultimate guide because even when *E. coli* could only be detected by enrichment, *Salmonella* and *Campylobacter* were detected on one farm. Litter deliveries to four growers were tested for *Listeria* and none was detected. Further, no *Listeria* was found in eight poultry farm litter-collections studied by Jaeger and Premier (2001).

6.2. Persistence of enteric bacteria on leafy vegetables

Shoot injury caused the persistence of *E. coli* on plants in the field. *E. coli* counts from both injured and uninjured plants fell with time but the rate of reduction was faster for the uninjured plants.

On the uninjured plants the most rapid reduction in *E. coli* counts occurred within the first 3 days (>99% reduction), followed by a more gradual reduction thereafter. This pattern of mortality is typical of pathogen populations (Feachem *et al.* 1983a). In contrast, the rate of reduction of bacteria on the injured leaves was relatively constant over time. After 3 days the *E. coli* count was reduced by 37% and a 99% reduction did not occur until day 23.

Injured tissue may provide essential nutrients and/or protection that allow the *E. coli* to persist. Other studies have shown that damaged food crop tissue allows the multiplication of enteric bacteria. For example, an increase in *E. coli* 0157:H7 was observed on injured apples (Dingman 2000) and higher *Salmonella* counts were recorded from vegetables with soft rots compared to produce in good condition (Wells and Butterfield 1997). Brandl *et al.* (2002) investigated the localization of *Salmonella* serovar Thompson on coriander plants that developed lesions 9 days after inoculation due to infection by a component of the natural leaf microflora. *Salmonella* serovar Thompson was not detected in every lesion of a particular leaf but it was present at extremely high densities in some lesions. The bacterium was also observed on the cuticle of healthy tissue and found within damaged tissue, apparently gaining entry through the disrupted leaf cuticle.

In our study, the indigenous microflora (indicated by total aerobic counts) on Cos lettuce were unaffected by shoot injury. This suggests that the persistence of *E.coli* was not due to the supply of essential nutrients. Instead, shoot injury could have protected the *E. coli* from desiccation or from UV light. A wide range of bacteria can multiply on wet, uninjured plant parts. *E. coli*, *S. enterica* serovar Typhimurium and *Aeromonas hydrophilia* multiplied on corn and bean plants held under high relative humidity for 48 hours and then declined after another 72 hours under dry conditions (O'Brien *et al.* 1989). A clear correlation between high humidity and high CFU of *Salmonella enterica* serovar Thompson inoculated on to coriander shoots was shown by Brandl *et al.* (2002). The population of this bacterium did not increase on coriander plants (in a growth chamber) if the relative humidity was kept at 40 to 50% for 4 days but under wet conditions populations increased.

Our results have shown the importance of the timing of injury in relation to contamination with enteric bacteria. When Cos lettuce shoots were injured 3 or more days prior to contamination, the persistence of *E. coli* was reduced to a similar level to the uninjured control. A similar response was found when shoots were injured 1 or more days after contamination with *E. coli.* Perhaps *E. coli* is better able to shelter in damaged tissue prior to the wound sealing over when damage and contamination occur concurrently.

In contrast to conditions on plant surfaces, where faecal bacteria are susceptible to sunlight and desiccation, soil conditions may be relatively sheltered and supportive microhabitats where moisture, shade, and nutrient availability permit survival for many weeks (Feachem *et al.* 1983a). Bogosian *et al* (1996) found that *E. coli* plate counts did not exhibit any decline even after 100 days at 4, 20 or 37°C in autoclaved soil. In another study, *E. coli* 0157:H7 took over 220 days to drop from about 7.5 $\log_{10}$ CFU/g to about 4 $\log_{10}$ CFU/g at 15°C in manure amended autoclaved-soil (Jiang *et al.* 2002). Whilst persistence of enteric bacteria in soil was not the particular object of our study, only low levels of *E. coli* were detected in soil at harvest. In addition, only two sites were found with *Salmonella* (a non-pathogenic strain) at harvest. At one site the *Salmonella* was found in the soil while at the other site it was detected in the crop. It is not known how long enteric bacteria might survive under the soil conditions found in this study if they were introduced with poultry litter in high densities.

As only non-pathogenic *E. coli* was used, the degree of persistence of actual enteric bacterial pathogens might be different to that found in this study. The characteristics of different species and strains of enteric pathogenic bacteria are known to differ. For example, *E. coli* 0157:H7 strains were found to adhere less strongly to alfalfa sprouts than non-pathogenic *E. coli* strains (Barak *et al.* 2002).

6.3. Persistence of enteric bacteria in poultry litter during aging and heat treatment

The main objective of the windrow field trial was to determine whether small changes in the management of poultry litter, such as turning a heap and aging the litter would effectively control enteric bacteria. This approach was taken because controlled composting was not seen to be a viable option at present due to the higher set-up and processing costs that are involved at government-regulated composting facilities.

Effective pathogen control was not achieved in the space of 12 weeks despite the sustained high temperatures achieved in the two windrows tested. Temperatures in the turned windrow were sustained for longer and a greater portion of the litter was consistently exposed to higher temperatures compared to the static windrow. Turning oxygenates the litter and also ensures that the edge of a pile is mixed into the centre and subjected to the high temperatures required for pathogen reduction. Despite almost weekly turning for the first 8 weeks and the higher temperatures achieved *E. coli* counts did not reduce over time in the outer layers of the turned windrow. The outer edge of both windrows could have provided ideal conditions for pathogens to multiply or persist. The edge of the windrows were wet periodically from rainfall and temperatures above 45 ºC were seldom recorded in this zone.

The bench scale trials showed that both *E. coli* and *Salmonella* Typhimurium persisted longer under conditions of low temperature and high moisture. At 35 ºC, reductions in both organisms were faster under dry conditions (30% w/w moisture) than under moist conditions (65% w/w moisture). *E. coli* and *Salmonella* counts were reduced by over 99% within the first hour at 55-65 ºC, a result that confers generally with other studies (Farrell 1993). At 55-65 ºC the moisture content of litter was found to be less important than the time of exposure, but the data also showed that *E. coli* counts were lower after 5 hours exposure to moisture contents of 50 or 65% than at 35% (w/w). For *Salmonella* the effect of moisture content at high temperatures could not be determined because it was eliminated within the first hour at 55-65 ºC.

Turning reduced re-growth, but not the persistence of *E. coli* in incubated litter; increased densities of *E. coli* were observed in 3-week-old litter, whereas counts in 6-week-old litter remained unchanged. In contrast, 3 or 6-week-old litter from the static pile supported increased *E. coli* densities following incubation. By 12 weeks, *E. coli* counts in both windrows were unchanged after 7 days and not detected after 14 days incubation. The incubation conditions of the litter that were used (37ºC incubation in dry or moist conditions) were similar to the conditions that would have frequently occurred in the outer layers of windrows or in piles stored on farms.

Persistence and re-growth of salmonellae in composted sewage sludge was studied by Hussong *et al.* (1985). Growth and death rates were found to be dependent on moisture and the microflora of the compost. In irradiation-sterilised composts inoculated with salmonellae, the salmonellae grew at a rate of 0.65 doublings per hour for over 24 hours. They concluded that the active indigenous microflora of compost establishes a homeostatic barrier to colonisation by salmonellae, and in the absence of competing microflora, reinoculated salmonellae may grow to potentially hazardous levels. In our trials, increases in *E. coli* counts were observed in both dry (12-35% w/w) and moist (50% w/w) litter of different ages. These differences may reflect changes in the indigenous microflora.

The litter that was collected for enteric bacteria testing was taken from the outside edge (5-10 cm deep) of the windrows. This zone is not representative of the whole pile and is the portion of litter where enteric bacteria are most likely to be present in highest numbers. The edge of a pile would normally be mixed with the internal contents of the pile as it is spread on the farm; thus the average density of enteric bacteria would be lowered. Assuming that the edge represents 5% of a pile, complete mixing of litter should result in an ‘average’ count that is up

to 20-times lower. Considering that the counts detected in the outside edge of a windrow at 12 weeks were as high as 31,600 cfu/g (4.5 log10; see Table 4), a 20-fold reduction in *E. coli* will result in an average count of 1,580 cfu/g. Composts that are used for side-dressing should contain less than 100 cfu *E. coli* /g (Behrsing and Premier 2002). In practice, mixing will not be conducted evenly, so that some portions of litter applied to soils and crops will contain higher bacterial loads than other portions from the same pile. This could explain the overall 'patchy' nature of bacterial counts in litter sampled in this study and others (e.g. Jaeger and Premier 2001).

High *E. coli* counts were still detected in 12 week-old litter despite the fact that the re-growth experiment showed that *E. coli* counts in litter of this age reduced to zero after incubation for a further 14 days or more. Counts were not reduced, though, within 7 days of incubation. Thus the management of either windrow was not effective at pathogen reduction because continual re-contamination and persistence of pathogens could occur on the outside edge of the pile.

Pathogen control is related to the rate of decomposition of organic materials (Stentiford 1993). Though high temperatures were achieved in the inner portions of each windrow, they were probably not the result of high rates of decomposition because the moisture content of the litter (16-24% w/w) was well below optimal levels (50-65% w/w) required for an active compost microflora (Epstein 1997). High temperatures can still be achieved when rates of decomposition are low because heat will build up as a result of the insulating properties of the litter. In addition to the heat generated by low levels of microbial activity, chemical oxidation can be the cause of rising temperatures in organic materials, which in some cases leads to spontaneous combustion in dry conditions (Buggeln and Rynk 2002). The low rate of decomposition effectively extends the time required to reach stabilisation and maturity and subsequently results in poor pathogen control. The ammonium to nitrate ratio of the litter in both windrows was 10 or more by week 12, higher than at the start of the trial. According to Bernal *et al.* (1998) and Paré *et al.* (1998), a stable and mature product is indicated by an ammonium to nitrate ratio of less than 1.

Manures that are stable and mature have several major advantages over fresh products. They are less likely to adversely affect plant growth (Avnimelech *et al.* 1993; Cooperband *et al.* 2003; Zucconi *et al.* 1981), be less odorous (Eggen and Veth 2001; Wiles *et al.* 2001), be properly sanitised (Tiquia *et al.* 2002) and more likely to prevent pathogen re-growth (Finstein *et al.* 1987; Hussong *et al.* 1985).

There are many recent examples of manure composting studies reported in the literature (e.g. Keener *at al.* 2001; Raviv *et al.* 1999; Robertson and Morgan 1995; Tiquia and Tam 2000, 2002; Vuorinen 1999), but these usually involve co-composting with other organic materials. Though the poultry litter used in our study contained rice hulls as the bedding material, the pungent ammonia odour it emitted indicated that it would need to be blended with additional bulking agent (e.g. green waste or sawdust) for it to be composted successfully. Elwell *et al.* (1998) characterised the composting process for unamended chicken layer manure and found that emissions of ammonia were much higher than is typically observed under normal composting conditions. Other studies have shown that losses of 38-50% of the initial nitrogen content of litter can occur during composting (Kirchmann and Witter 1989; Tiquia and Tam 2000, 2002). The loss of ammonia from litter is not only odorous but also results in loss of valuable nitrogen, thus reducing its value as a fertiliser. On the other hand, high levels of ammonia in manures can result in reductions in plant growth. Keener *et al.* (2001) found that symptoms of ammonium toxicity were observed in Deutzia plants when a container medium was amended with 8-16% composted pig manure. In contrast, plant growth was increased in the 4% mix compared to the control.

6.4. Conclusions

Bacteriological testing on all 7 farms showed low *E. coli* counts and no *Campylobacter* or pathogenic *Salmonella* on any crops at harvest. Litter deliveries were variable in *E. coli* counts and the presence of *Salmonella*. No *Campylobacter* or *Listeria* was found in litter deliveries either.

Non-pathogenic *Salmonella* was found on a coriander crop that could be traced back to fresh litter that had been side-dressed 18 days prior to harvest. The coriander field was only 50 metres away from the poultry litter stockpile. Contamination via rain, wind, turbulence from vehicles passing by and movement of litter for treatment of other fields may also have occurred.

Field and glasshouse trials showed that *E. coli* could persist longer on damaged Cos lettuce leaves than undamaged leaves when damage and contamination occurred close together. If contamination was delayed until 3 days after the damage occurred, the survival of *E.coli* (measured three days after application) was similar to that of undamaged plants. This result indicates that farm practices that may contaminate plants, such as side dressing with fresh litter, should not be done after practices or weather conditions that could cause minor injury to the plants.

Aging for 12 weeks and turning the heap was found to marginally improve the hygienic condition of poultry litter. The process of aging occurs under sub-optimal conditions resulting in low rates of decomposition. Turning the heap is ineffective under these conditions because the progress of stabilisation is too slow to prevent continual re-contamination and persistence of pathogens on the outside edge of the pile. An aging process is not equivalent to controlled composting which is characterised by rapid rates of decomposition achieved through stringent, documented process control.

Whilst controlled composting will undoubtedly produce the safest product from poultry litter, our results suggest that it is not necessary to recommend it for all applications. However, in the medium term, poultry litter recycling is likely to come under closer state environment protection regulation to bring it in line with other organic recycling activities (e.g. green waste composting sites). The likely result of this will be the establishment of controlled composting facilities for poultry litter. To ensure that poultry litter will be a viable option for vegetable growers in the medium term, further studies are needed to develop poultry litter processing guidelines that meet QA and environment protection guidelines without causing detriment to its value as a fertiliser. As composting will increase the cost of poultry litter, these studies are urgently needed to minimise the cost of processing and maximise the return for the grower.

7. Technical Transfer

An industry steering committee was formed at the commencement of the project with representatives including 7 vegetable growers from the South East of Melbourne and 3 poultry litter contractors. Several meetings were held with the steering committee, which provided opportunities to obtain feedback on the work program, discuss trial results and gain industry approval. On-going informal discussions regarding trial findings and the implications for the industry were also held with growers and poultry litter contractors assisting in the trial work.

The following communication activities were conducted over the duration of the project:

- Project results were presented at the "Fresh" Conference held during October 2001 in Melbourne.

- Data from trials investigating the effect of shoot injury on *E. coli* persistence was presented at the Costa's Postharvest Handling Course for quality control staff, held at the Victorian Department of Primary Industries, Knoxfield, August 2002.

- Article entitled "Strategies for the safe use of poultry litter in food crop production", in the Lettuce Leaflet newsletter, Issue No 7, September 2002. Victorian Department of Primary Industries.

- Poster entitled "Shoot injury to vegetable plants causes persistence of *Escherichia coli*" was presented at the Australian Society of Horticultural Science Conference, The University of Sydney, 29 September – 2 October 2002.

- Article entitled "The safe use of poultry litter in food crop production" in Shaping the Future, Access to Asian Vegetables newsletter, Issue 54, November 2002. Department of Natural Resources & Environment (now Victorian Department of Primary Industries) and Rural Industries Research & Development Corporation.

- Paper entitled "Strategies for the safe use of poultry litter in food crop production" was presented at the *Orbit* conference, Perth, 30 April - 2 May 2003.

- Project outcomes were presented to vegetable industry leaders, Department of Primary Industry researchers and HAL representatives at a vegetable research, development and extension forum held at the Department of Primary Industries, Knoxfield, 12th August 2003.

8. Recommendations

8.1. Recommendation 1

To avoid the risk of pathogens contaminating and persisting on crops, it is recommended that fresh litter should not be used as a side-dressing. Only fully composted poultry litter should be used for side-dressing.

8.2. Recommendation 2

Whilst it has not been possible to determine the minimum distance required to prevent cross contamination, storage of litter as far away as possible from mature crops is recommended.

8.3. Recommendation 3

Aged litter should not be regarded as equivalent to a fully composted product. Where fully composted litter is appropriate or desired, the supplier should demonstrate that it was produced under documented process control following established best practice and HACCP guidelines (e.g. AS 4454-1999; EPA Victoria 1996; Behrsing and Premier 2001; Wilkinson *et al.* 2001).

8.4. Recommendation 4

Develop processing guidelines for poultry litter that meet QA and environment protection guidelines without causing detriment to its value as a fertiliser.

9. Acknowledgments

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11. Appendix

(A) (B)

Plate 1: Bench scale composting apparatus shown with the lids of three chambers open (A) and close-up view of a chamber (B).

Plate 2: Inside chamber – plastic pot with sample tubes in water bath.

Plate 3: Inside incubator (37^{o}C)- samples for pathogen re-growth trial.

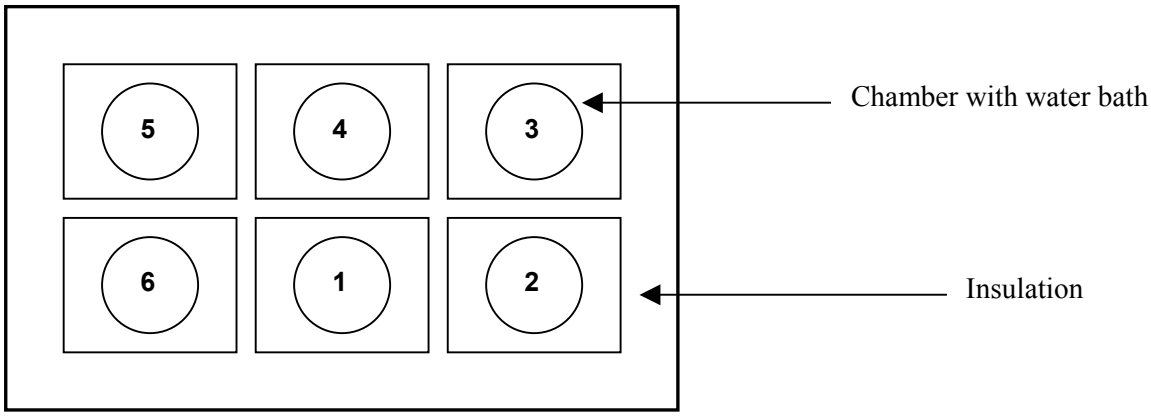

Figure 1: Plan of the arrangement of six compost chambers in the laboratory scale compost simulator.

Table 1: Trial design protocol for the assessment of pathogen survival at various temperatures and moisture content.

Test No.	Moisture content (%fw)	Randomised temperature settings for each test in 6 chambers
1	30	65 55 55
		35 45 35
2	65	65 35 55
		45 45 55
3	50	55 65 35
		65 55 45
4	30	55 35 45
		45 65 65
5	50	35 55 45
		45 65 35
6	65	35 55 45
		35 65 65
7	50	45 45 65
		55 35 65
8	65	45 65 35
		55 55 35
9	30	35 45 35
		65 55 45
10	65	55 45 65
		65 35 45
11	30	45 65 65
		55 35 55
12	50	65 35 55
		35 45 55

Table 2: Sampling protocol for assessment of pathogen survival at various temperatures

Temperature (oC)	**Sampling (hours from start)***				
35	0	2	5	8	24
45	0	2	5	8	24
55	0	1	3	5	24
65	0	1	3	5	24

(* Each chamber would contain four sample tubes at the start of the test run)

Table 3 : Parameters tested for the persistence and re-growth of pathogens in poultry litter collected from the turned and static windrows.

Litter age	**Treatment**	**Incubation time**	**No. of samples**
0	Untreated	0 day	6 x 10 g sample
3	50% moisture content	7 days	for each
6	50% moisture content & inoculated	14 days	treatment &
12	with E.coli	21 days	incubation time