USING MANURES TO GROW VEGETABLES – A GUIDE TO REDUCING RISK

Manure – friend or foe?

Manures have been used to improve agricultural soil fertility for over 7,000 years. Manures add nutrients and organic matter, increase soil bulk density, enhance structure and water holding capacity and increase biodiversity. It is no accident that humans started keeping livestock at the same time as they started growing crops.

Unfortunately, manures can contain pathogenic bacteria such as *Escherichia coli* (*E. coli*), *Salmonella* spp., *Listeria monocytogenes*, *Campylobacter* spp., *Yersinia enterocolitica* and others. Even a small dose of some of these human pathogens – particularly some species of *Salmonella* spp. and types of *E. coli* – can cause severe illness and even death.

BACTERIA	DESCRIPTION	TEST
Escherichia coli (*E. coli*)	*E. coli* is relatively common in the environment. Although often used as an indicator that produce is contaminated, most *E. coli* strains are harmless; there are only a very few types (known as Shiga-toxin *E. coli*) that can make people sick. Vegetables with up to 100 CFU* of *E. coli* per gram are still considered acceptable for human consumption (FSANZ).	*E. coli* can be isolated from samples directly using selective media. Results are reported as CFU/g of product. The minimum detectable population is often 10 CFU/g.
Salmonella spp.	A number of species of *Salmonella* spp. can cause severe human illness. Symptoms include vomiting, diarrhoea and fever. As few as 100 bacteria can make people sick, so there is zero tolerance for *Salmonella* spp. on fresh vegetables. Any detection in a 25g sample will trigger product rejection.	The test usually used for *Salmonella* spp. relies on enrichment to stimulate growth. The bacteria can then be detected using bacteriophages. This method is able to detect a single bacterium in a sample. Results are presence/absence per 25g product.
Listeria monocytogenes	Unlike *E. coli*, *Salmonella* spp. and other gut bacteria, *Listeria* spp. can survive for long periods in the soil. Various species of *Listeria* are commonly found in a wide range of environments. However, only *Listeria monocytogenes* can cause listeriosis. Infection can cause miscarriages, and severe illness and death in the elderly or immunocompromised. Fatality rates may be up to 30% for this group.	Tests for *Listeria* use enrichment, allowing the bacteria to multiply. Results are presence/absence per 25g product.
Thermotolerant coliforms	Thermotolerant coliforms are relatively common in the environment. While they may be used to evaluate faecal contamination, the specific bacteria *E. coli* is a better indicator of risk.	Coliforms are isolated from samples directly using selective media.

Table 1. Bacteria that may be part of a testing program

** CFU – Colony forming unit, that is, a single bacterium.*

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Untreated manures used to grow vegetables can contaminate food with these bacteria. Contamination may occur directly through contact between vegetables and manure-amended soil, or indirectly if manures contaminate water used for irrigation or crop sprays.

However, most human pathogenic bacteria are naturally adapted to living inside the guts of warm-blooded mammals. The soil and plant surfaces are not their natural habitat. Populations of bacteria in these environments therefore tend to decline and eventually die out. Understanding how quickly this occurs is critical to ensure vegetables are safe to eat.

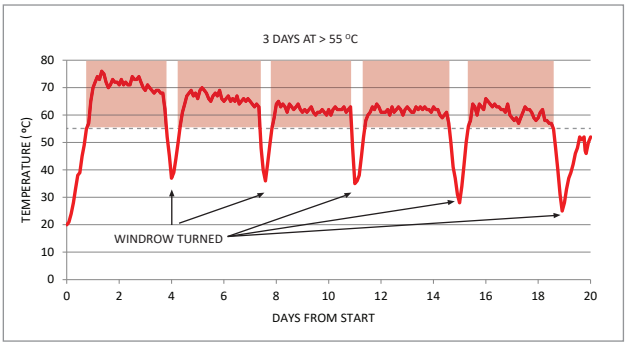

Figure 1. Temperature profile within a windrow

Reducing risk – composting

The best option to reduce risk is to use only manures that have been thoroughly composted. Heat generated during composting kills human pathogens present in the manure, along with weed seeds and plant diseases.

Food safety programs such as Freshcare, SQF and HARPS do not restrict use of manures if they are certified as having been treated. This means they must be composted in accordance with Australian Standard 4454 "Composts, soil conditioners and mulches". Two methods can be used to ensure food safety:

WINDROW COMPOSTING

- Raw materials formed into windrows or piles
- Composted for at least 15 days with five turnings
- The centre of each windrow (or pile) must heat to over 55°C for at least three days between turnings.

IN-VESSEL COMPOSTING

- Raw materials placed inside a vessel or contained space
- Aeration provided by injection or turning
- All materials must heat to over 55°C for at least three days.

Unless manures are certified as being treated in accordance with AS4454, they are considered the same as untreated manure. This includes manures that have been partially composted or aged, or supplied by a non-certified compost producer.

Figure 2. Windrows must be turned to ensure all of the material is thoroughly treated.

Reducing risk – storing safely

If products containing fresh, semi-composted or aged manures are used, it is essential they are stored so as to minimise the risk of contaminating crops or water sources. Contamination can potentially occur through direct application to the crop, wind drift or water.

Runoff from stored or recently applied manures can readily contaminate neighbouring sites and water sources. Storage sites need to be located, constructed and maintained so as to prevent this from occurring. Manures should not be added to soil if there is a chance rain or irrigation will wash through the area onto existing vegetable crops. Incorporating manures, rather than simply spreading them on top of soil, is another way to reduce the risk of this occurring.

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Figure 3. Storing manure uphill from farm dams allows runoff to contaminate irrigation water (left) while storing piles next to cropping areas potentially allows dust to contaminate harvested product. (right) Photo: R. Bennett

Reducing risk – limiting persistence of human pathogens

The key to reducing risk from manures is reducing survival of any human pathogenic bacteria they contain. Unfortunately, persistence is difficult to predict, as it is influenced by many factors in the soil and environment. The two most important factors reducing survival are low soil moisture (either continuous or intermittent) and high temperatures. For example, populations of both *E. coli* and *Salmonella* spp. decline 1.4 times faster at temperatures over 20°C compared to temperatures below 10°C. A range of other factors are also associated with reduced persistence. Some of these are listed in Table 2.

FACTOR ASSOCIATED WITH REDUCED SURVIVAL OF *E. COLI*	
Soil properties	Low soil moisture generally, or intermittent dry spells
	Sandy soil with low clay content
	High population and diversity of soil microbes, particularly protozoans and fungi
	Aerobic conditions in soil/good soil structure
	Saline conditions (high EC values)
	High pH (alkaline, lime added)
Manure properties	Manure previously aerated by turning
	Animals fed on a low fibre diet or one high in tannins
	High ammonia (nitrogen) content in poultry litter
Application method	Solid waste applied rather than slurry
	Manure left on surface rather than incorporated into soil
	Growing site fallow at the time of application
	Applied to an open field, not underneath protected cropping
Climate	High and/or fluctuating temperatures
	Strong ultraviolet light
	Low rainfall

Table 2. Factors that can reduce the survival of human pathogenic bacteria in manure amended soil

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Reducing risk – withholding periods

Apart from the factors listed in Table 2, the key element reducing risk is **time**. Many food safety standards mandate withholding periods between application of untreated (or semi-treated) manures and harvest. These time intervals are intended to allow human pathogens in soil or on plant surfaces to return to normal environmental levels.

The Guidelines for Fresh Produce Food Safety recommend:

- **90 days** between addition of manure to soil and harvest for high risk products such as lettuce and other salad greens (grown in or close to the soil AND may be eaten uncooked)
- **45 days** between addition of manure to soil and harvest for lower risk products (NO contact with soil OR always eaten cooked OR with inedible shell).

However, guidelines on withholding periods vary between standards. For example:

- **Freshcare** – 90 days for products that are grown in or close to the soil and may be eaten uncooked; 45 days for other products
- **GLOBALG.A.P.** – 60-day withholding period for tree crops, but 60 days before planting for vegetable crops
- **USDA** – 120 days / 90 days for high and low risk products respectively
- **HARPS** – 365 days if the harvestable part of the crop is grown in or within 1m of the ground, or is harvested from the ground, and may be eaten uncooked
- The **Fresh Salad Producers group** – 365-day withholding period
- The **California Leafy Greens Marketing Agreement** prohibits all use of manures.

Why 90/45 days?

These exclusion periods are based on an overview of die-off rates published in the scientific literature. However, results vary widely between environments and with different agricultural practices. Researchers have reported die-off times ranging from a few days to many months. Most research has been conducted in the USA or Europe where temperatures, UV radiation and soil types are very different from Australian conditions.

The project "Pathogen persistence from paddock to plate" (VG16042) has examined how quickly populations of human pathogens (*E. coli*, *Salmonella* spp. and *Listeria* spp.) return to environmental levels when added to sandy or clay loam soil. Cattle manure and fresh poultry litter were incorporated into the soil at a high rate (20t/ha) at the start of spring (trial A), summer (trial B) and autumn (trial C) crops of cos lettuce.

As the manures did not contain detectable human pathogens, *E. coli* was added (inoculated) so as to achieve approximately 100,000 bacteria/g manure. *Salmonella* spp. and *Listeria innocua* were also added to poultry litter and cattle manure respectively, achieving approximately 2,500 bacteria/g manure.

Additional plots were amended with manure alone (no inoculated pathogens) or left as untreated controls (no manure). Soil samples were taken for testing at intervals during crop production, and mature lettuce were tested from the summer and autumn crops.

Figure 4. The project trial site in Cobbitty, NSW. Raised boxes were filled with clay or sandy soil. Poultry litter or cattle manure inoculated with high populations of human pathogens was incorporated at a rate of 20t/ha before planting the boxes with cos lettuce.

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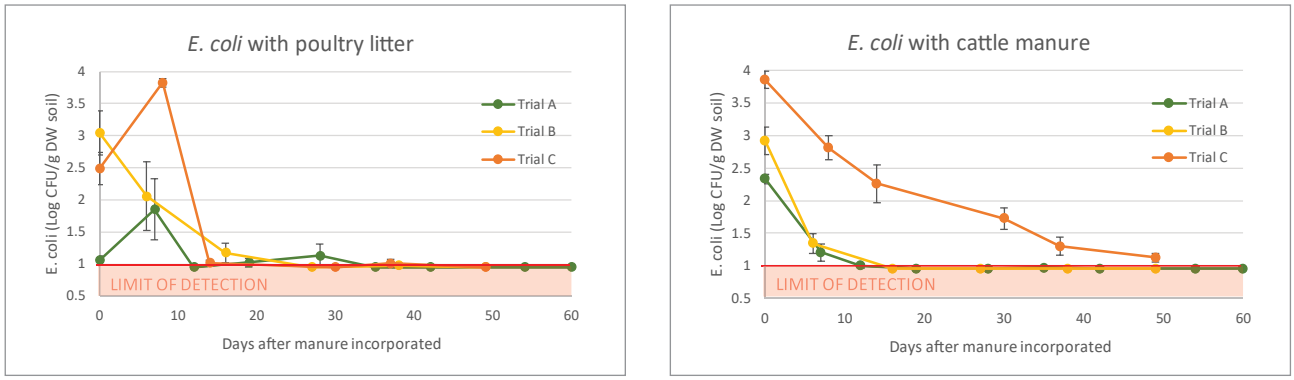

Figure 5. Average populations of *E. coli* in soil amended with 20t/ha of poultry litter or cattle manure during growth of three lettuce crops in Cobbitty, NSW. Values are combined from two soil types (sandy and clay loam). Bars indicate standard error of each value.

Persistence of bacteria in the soil

The highest populations of *E. coli* were recorded within two weeks after inoculated manure was added to soil. Occasional high detections (>100 CFU *E. coli*/g soil) continued for up to 30 days in soil containing inoculated cattle manure or poultry litter. Although it had been expected that the bacteria would survive longer in clay compared to sandy soil, this did not occur in these trials.

Populations of *E. coli* in soil amended with inoculated poultry litter fell from up to 20,000 CFU/g soil to below the level of detection (10 CFU/g) within 50 days in all seasons. This indicates a mortality of over 99.9%. Even though no *E. coli* was detected in the poultry litter itself, there were occasional detections in the soil amended with poultry litter alone. These mainly occurred within the first two weeks after the manure was added. After 50 days, *E. coli* in these blocks was also below the level of detection.

Populations of *E. coli* in soil amended with inoculated cattle manure declined below the level of detection within three weeks in trials A and B. However, the bacteria survived longer when it was added to the soil in autumn (trial C). This was a period when weather was relatively mild and soil moisture stayed high. In trial C, the average population after 50 days was 13 CFU/g soil, suggesting at least 98% of *E. coli* died over this time period. There were no significant detections of *E. coli* in soil amended with cattle manure alone.

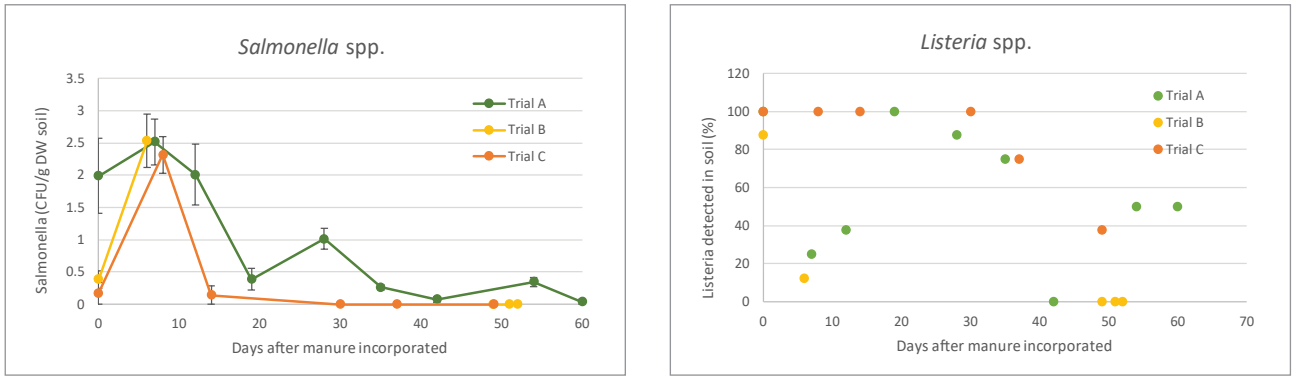

Figure 6. Average populations of *Salmonella* spp. in soil amended with 20t/ha poultry litter and percentage of soil samples positive for *Listeria* spp. during spring, summer and autumn lettuce crops in Cobbitty, NSW. Values are combined from two soil types (sandy and clay loam).

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Salmonella spp. populations also declined rapidly after addition to soil. In trials B and C, the bacteria could not be detected in samples taken two weeks or more after incorporation of poultry litter. However, the bacteria survived significantly longer during trial A. Even though the population was low (<3 bacteria/gram), there was still a 50:50 chance of detecting *Salmonella* spp. in soil after 50 days. Similar results have been reported in Victoria, with small populations of *Salmonella* spp. remaining detectable for up to 70 days after addition to soil.

In trials A and C half of all blocks were still positive for *Listeria* spp. 50 days after the manure was added. *Listeria* spp. only fell below detectable levels over the summer trial.

Species of *Listeria* spp. can persist in soil for extended periods, with many found there naturally. For example, one Victorian study found *Listeria* spp. in up to 60% of farm soils even without addition of manure. However, the risk from a positive detection of *Listeria* spp. in soil is unclear. It is very difficult to count the population, so there could be a single bacteria in a soil sample, or many thousands.

	TRIAL B (SUMMER, 120 TESTS/TREATMENT)			TRIAL C (AUTUMN, 80 TESTS/TREATMENT)		
	E. coli	*Salmonella* spp.	*Listeria* spp.	*E. coli*	*Salmonella* spp.	*Listeria* spp.
Control (no manure)	**0.8%**			**1.2%**		
Poultry litter	**0.8%**			0%		
Poultry litter + inoculant	0%	0%		0%	0%	
Cattle manure	0%			0%		
Cattle manure + inoculant	**0.8%**	0%	0%	0%		**3.8%**

Table 3. Positive detections of *E. coli* and *Listeria* spp. on lettuce in summer and autumn trials

Contamination of lettuce

To simulate a "worst case" scenario, mature lettuce were tested with the dirty outer leaves still attached. One lettuce grown with poultry litter and another grown with inoculated cattle manure had unacceptable levels (>100CFU/g) of *E. coli* in trial B. However, by far the highest level of *E. coli* recorded, at 40,000 bacteria/g, was on a lettuce from an unamended plot.

There were no detections of *E. coli* during trial C, but there were three detections of *Listeria* spp. on lettuce grown with inoculated cattle manure, but there were three detections of *Listeria* spp. on lettuce grown with inoculated cattle manure.

There is usually zero tolerance for *L. monocytogenes* on foods. While *Listeria* spp. is unable to grow on dry surfaces, fresh cut products provide the nutrients bacteria need to multiply. Even if products are kept cool, populations can potentially increase to levels causing serious illness and death. Detections on these foods (e.g. fresh cut lettuce) are, therefore, extremely serious.

Listeria spp. is very rarely found in poultry litter but can be associated with cattle manure. Manure from intensive animal operations (e.g. feedlots, saleyards) can potentially contain very high populations of *L. monocytogenes*, especially if cattle are stressed.

Figure 7. To simulate a 'worst case' scenario, lettuces were harvested for testing with outer leaves and dirt still attached.

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Conclusions

Manures offer many benefits for soil health. They add nutrients and organic matter, building soil fertility, improving structure and stimulating microbial activity. However, they can also contain human pathogenic bacteria. If these contaminate the harvested product, consumers may become extremely sick and even die.

Most human pathogens do not survive for extended periods in soil. However, die-off rates are highly variable, being affected by temperature, soil type, soil moisture and many other environmental factors. For this reason, withholding periods between application of manure and harvest are conservative, assuming a 'worst case' scenario.

In these three trials, high initial populations of *E. coli* and *Salmonella* spp. in soil fell below or close to detectable levels within 50 days of application.

However, the results must be considered cautiously. These trials were conducted in a single location over a relatively short time period. Moreover, *L. innocua* persisted in the soil during both the autumn and spring cropping cycles. While this result does not necessarily mean that the human pathogen *L. monocytogenes* would also persist in soil under these conditions, it does demonstrate the variability between microbial populations.

While human pathogens in soil may be considered potential contaminants, it is their presence on the harvested vegetables that is most important. Even though lettuces were tested with soil attached, they were rarely positive for human pathogens.

However, detections of *Listeria* spp. on a number of lettuce does raise concerns. There is zero tolerance for *L. monocytogenes* on harvested produce, so any detection will trigger a product withdrawal. Moreover, processing then storing this lettuce could potentially allow small populations of bacteria to grow and multiply, creating a significant food safety risk.

Recommendations

- The best way to reduce risk from manures is to apply only products that have been treated (e.g. composted in accordance with AS4454) to kill any human pathogens present
- High temperatures, dry conditions and other environmental factors reduce survival of human pathogens in soil, but effects are variable
- Withholding periods between application of manure and harvest provide an alternative method to for reducing risk:
    - In these trials, populations of *E. coli* and *Salmonella* spp. consistently fell to barely detectable levels within 50 days after addition to soil
    - Populations of *Listeria* spp. can potentially survive longer than 50 days.
- The length of withholding periods used will therefore depend on:
    - Whether the product is grown in or close to the soil and may be eaten uncooked (i.e. high risk)
    - Whether a risk reduction step or other processes are applied after harvest
    - On-farm risk assessments and customer mandated requirements.