MICROBIOLOGICAL FOOD SAFETY RISKS ASSOCIATED WITH FLOOD-AFFECTED SOILS

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Authors Nicola King and Peter Cressey
Institute of Environmental Science and Research (ESR), Ilam, Christchurch

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Reviewed by: Graham Fletcher (Plant & Food Research), Rob Lake (ESR) and Phil Bremer (NZFSSRC)

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New Zealand Food Safety Science & Research Centre
Hopkirk Institute, Massey University, Tennant Drive, Palmerston North 4442
Phone: +64 (0) 6 356 9099

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ABBREVIATIONS

CFU	colony forming units
CI	Confidence interval
DNA	Deoxyribose nucleic acid (genetic material)
DW	Dry weight
ESR	Institute of Environmental Science and Research
FIB	Faecal indicator bacteria
FPSC	Fresh Produce Safety Centre (Australia & New Zealand)
FSANZ	Food Standards Australia New Zealand
IFPA	International Fresh Produce Association
LOD	Limit of detection
MPN	Most probable number
MST	Microbial source tracking
NZFS	New Zealand Food Safety
NZFSSRC	New Zealand Food Safety Science and Research Centre
PCR	Polymerase chain reaction
PFR	Plant & Food Research
qPCR	Quantitative Polymerase Chain Reaction
SD	Standard deviation
STEC	Shiga toxin-producing *Escherichia coli*
USFDA	United States Food & Drug Administration

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SUMMARY

Cyclone Gabrielle (13-14 February 2023) caused extensive damage to the east coast of New Zealand's North Island, including key horticultural production areas in Hawkes Bay, Gisborne/Tairāwhiti and Northland. This incident highlighted the limited information available to growers to assist their decision-making as to when flood-affected soils could be replanted with food crops without compromising food safety. The horticulture industry has an immediate need for guidance, to ensure both food security and safety.

To support future guidance, this project focused on:

- Compiling information that shows how flooding affects faecal indicators and pathogenic microorganisms in horticulture soils;
- Identifying sampling and testing methods that can be used for assessing the microbial safety of horticulture soils and flood silt; and
- Finding out if certain faecal indicators and genetic markers in soil samples are useful indicators for safe planting.

To understand if a post-flood soil presents a risk for food safety it is necessary to know what a normal soil profile looks like. *Escherichia coli* is often used as a marker for faecal contamination but these bacteria can be present in soils located away from agricultural and other human activities. Reported concentrations in such areas, when *E. coli* was detected, can be as high as 1000 CFU or MPN per gram of soil. Very limited data from crop growing soils suggest *E. coli* numbers are similar. *Listeria monocytogenes* is also a widespread environmental bacteria and can be found in horticulture soils, irrespective of flooding events, although the prevalence is likely to be 10% or less (i.e. 1 in 10 samples positive). Irrigation and rainfall tend to increase the likelihood of *L. monocytogenes* being detected in soils. *Salmonella* spp., Shiga toxin-producing *E. coli* (STEC), *Yersinia* spp. and *Campylobacter* spp. would not normally be expected to be present in soil without faecal contamination but can be introduced with windborne dust and water. *Salmonella* spp. and STEC have been isolated from soils in horticulture areas although many of these isolations are from soils that have received animal manures as fertiliser.

Studies of pathogenic microorganisms and faecal indicator bacteria (FIB) such as *E. coli* in post-flood soils are scarce. Available data suggest that initially, the concentration of *E. coli* and pathogens can be elevated after flooding but their numbers decrease during subsequent days or weeks. Higher soil moisture support their persistence. Pathogenic microorganisms are more likely to be present in soil if they are also present in nearby surface waters.

Field studies of the persistence of FIB and pathogenic microorganisms in horticulture soils provide some useful information on what might occur in soils if flooding leaves behind faecal-contaminated soils. However, most data come from studies where animal manure or slurries have been added to soils. A common observation under field conditions is an initial rapid decrease in bacteria numbers after contamination, followed by a longer period of slower reduction, stabilisation or fluctuation (tail-end survivors). Pathogenic bacteria can survive for a month or more under field conditions, but numbers will decrease over time. Temperature is a major driver for bacterial die-off, with higher temperatures increasing the rate of reduction. Higher soil moisture will prolong survival. Survival is generally poorer in sandy soils. For *L. monocytogenes*, which can be well adapted to living in soil, soil chemistry is important for survival.

Soil remediation activities can affect FIB and pathogenic microorganisms in horticulture soils. Tillage breaks up the flood-compacted soils, incorporating flood-deposited microorganisms into the

soil layer and increasing soil aeration, both of which improve microbial survival/growth. On outdoor experimental plots, covering the soil with polyethylene was more effective for killing *Salmonella* spp. in the soil that leaving the soils undisturbed or growing cover crops. The plastic cover generated high temperatures in the soil.

If growers decided they wanted to test their post-flood soils before replanting, it would be useful to have guidance on how to sample and what to test for. A range of soil sampling methods have been used. Most approaches involved core or grab samples of soils taken along a transect or pre-determined sampling pattern. Others involved walking around a site dragging a swab or wearing disposable boot covers. These latter methods were useful for assessing a wider area but were less sensitive compared to soil sampling (probably due to them only testing the soil surface). Overall, there are no standard or accepted methods so the approach selected should be guided by the purpose of the work.

To help determine whether testing soils for *E. coli*, genetic markers of faecal contamination (as used for Microbial Source Tracking, MST) or *Listeria* spp. was useful for assessing risk, 20 soil/silt samples taken by Landwise from horticultural areas soon after the 2023 floods were analysed. The results showed that:

- An *E. coli* concentration of >1000 MPN/g signals there might be faecal material present, but this was not consistent. However, this small survey suggests that *E. coli* concentration alone is not reliable as a sole indicator for the safety of soils for replanting edible crops.
- MST markers can indicate the presence of faecal material in soil, and the source of this material. They do not provide evidence that pathogenic microorganisms are present in soil, nor indicate which of these microorganisms might be present. However, in combination with *E. coli* results and wider knowledge from a landowner about their environment and the impact of the flood event (e.g. identifying any upstream or nearby sources of contamination), they provide useful additional information to support decision-making.
- *L. monocytogenes* might be more common in flood-affected soils than unaffected soils, although there is no evidence that the presence of these bacteria in soils is related to faecal contamination. Detection of *L. monocytogenes* in horticultural soils may indicate the need for greater vigilance in the processing environment.

On the basis of the available literature and the small field study conducted, it was concluded that:

- *E. coli* is an appropriate indicator of faecal contamination of soils but is not sufficient, on its own, for food safety decision making. MST or equivalent technology provides information on the presence and source of faecal material, including identifying if human faeces is present (which represents the highest food safety risk).
- A 60-day period between floodwater receding and replanting horticultural land is consistent with available evidence on pathogen survival and recommendations by overseas agencies.
- Reduction of the 60-day replanting period may be possible if appropriate testing is conducted.
- Detection of *L. monocytogenes* in horticultural soils should be viewed as a prompt for increased vigilance in the post-harvest environment but the absence of this organism is not an appropriate criterion for shortening replanting periods. It is not recommended to use *Listeria* spp. in soils as an indicator for the presence of *L. monocytogenes*.
- While some soil remediation activities might increase the rate of pathogen inactivation, remediation efforts are probably best focussed on soil health.

1 INTRODUCTION

Cyclone Gabrielle (13-14 February 2023) adversely impacted horticultural land in important growing areas of Hawkes Bay, Gisborne/Tairāwhiti and Northland. These highly productive areas were impacted by inundation with floodwater and deposition of flood-carried sediment. Both phenomena potentially introduced food safety hazards into horticultural areas.

During flooding events, pathogenic microorganisms can be transferred to horticultural land by flood waters carrying:

- Overflow from human sewerage holding and treatment facilities (septic tanks, wastewater treatment plants) and/or damaged sewerage infrastructure (pipes, pumps, etc.),
- Animal faecal material from pastoral lands or areas where animal faeces can become concentrated (e.g. feedlots, dairy sheds, effluent ponds, barns, abattoirs), and
- Already contaminated sediments re-suspended from upstream areas.

Unless there is gross contamination or an obvious, nearby contamination source, it is not easy to determine if pathogenic organisms could be present in flooded areas after the waters have receded. Other sources of microbial (microbiological) contamination might also be introduced such as dead animals and refuse but, unlike the three listed above, these tend to be more visually obvious, and their removal is a clear mitigation step.

There is also potential for microbial contaminants to be removed from horticultural land by flowing floodwaters. The level of contamination of horticultural land post-flood is therefore determined by the volume and flows of the floodwaters themselves and the land use characteristics of surrounding and upstream areas.

Following flooding, environmental factors such as the texture and mineralogy of the sediment, soil composition (availability of nutrients), soil pH and solar irradiation will have an impact on the persistence of pathogenic microorganisms. Their survival and potential for multiplication (growth) also depend on their biological characteristics and their ability to survive in the presence of the wider soil microbiota. Human interventions, such as sediment removal or incorporation of sediment deposits into soil, will also impact the survival of remaining pathogenic microorganisms.

In the months following the 2023 cyclones, growers of rotational food crops were not only concerned about returning their soil to productive levels ready for replanting, but also concerned that there could be residual microbial contaminants in these soils that might become a food safety issue in harvested produce. Assessing flood-associated changes in soils is generally hampered by the lack of suitable baseline (pre-flooding) information on soil microbial status. What does the microbial profile of a ‘risky’ soil look like compared to normal?

1.1 PROJECT RATIONALE AND OBJECTIVES

Investigations since the February 2023 floods have failed to locate readily available data that tells us what a ‘normal’ soil profile looks like from a microbial food safety perspective, and thus what a ‘risky’ soil profile looks like. This prevents anyone from being able to offer good advice in response to the question “is the soil safe for planting?”

There is a need for guidance for the reuse of horticultural land following flooding events, from a microbiological food safety perspective. Ideally, this would provide guidance on approaches for assessing the microbial safety of horticultural soils, and decision trees to support grower decision-making. An evidence base is needed to draft such guidance, which is not readily available.

There are four project objectives:

1. To compile available evidence on how flooding affects faecal indicators and pathogenic microorganisms in horticulture soils, to inform recovery from future flood events (Section 4).
2. To identify sampling and testing methods that can be used for assessing the microbial safety of horticulture soils and flood silt (Section 5).
3. To find out if certain faecal indicators and genetic markers in soil samples are useful indicators for safe planting (Section 5).
4. To communicate the findings to industry through a report that would support draft guidelines and a short industry-focused document (this document).

1.2 RESEARCH FOCUS

When considering post-flood risks, there are two main types of flooding, these being pooled rainwater (low risk if there are no sources of contamination within the flooded area) and flood water flowing into/across properties from other locations. This research focusses on the latter but is relevant to situations of where floods caused by pooled rainwater may have introduced contamination (e.g. through field toilets or broken wastewater infrastructure).

This work has focused on the risk from faecal contamination of horticultural soil since this presents the greatest microbial food safety risk because of the pathogenic bacteria, viruses and parasites that can be found in faeces. Testing soils (or any substrate) for pathogenic microorganisms is expensive and complex, so faecal indicator bacteria (FIB, such as *E. coli*, enterococci or faecal coliforms; see Section 3) are often used to indicate whether faecal material is present, and thus whether faecal-associated pathogens might also be present. However, *E. coli* are also naturally found in soils, so what concentration of *E. coli* in a soil clearly signals faecal contamination has occurred? "*For soil to be considered "safe" to grow vegetables, it is therefore uncertain whether it needs to contain no detectable E. coli or 1 CFU/g, 10 CFU/g or even 100 CFU/g soil*." (Ekman *et al.*, 2021). Other techniques could be applied to silt/sediment samples to provide context to FIB results. Microbial source tracking (MST) techniques can be used to provide information on the presence and source of faecal contamination, while metagenomic methods can provide an overview of the microbial populations present.

One bacterial genus, *Listeria*, contains environmental microorganisms that are associated with decaying plant matter, with some species able to cause disease in humans and animals. *L. monocytogenes* is a pathogenic species within the *Listeria sensu stricto* group of *Listeria* species (*Listeria* spp.). Fresh produce suppliers usually need to manage *Listeria* to mitigate risk from *L. monocytogenes*. Because of these horticulture-specific concerns, this project has also focused on *L. monocytogenes*.

To ensure that the work remained focused on gathering the information needed to support grower guidelines, a template for a grower decision tree was drafted. This is reproduced in Figure 1, with the red shading indicating the areas this current work addresses. Mapped to this are six high priority questions that were used to direct this research.

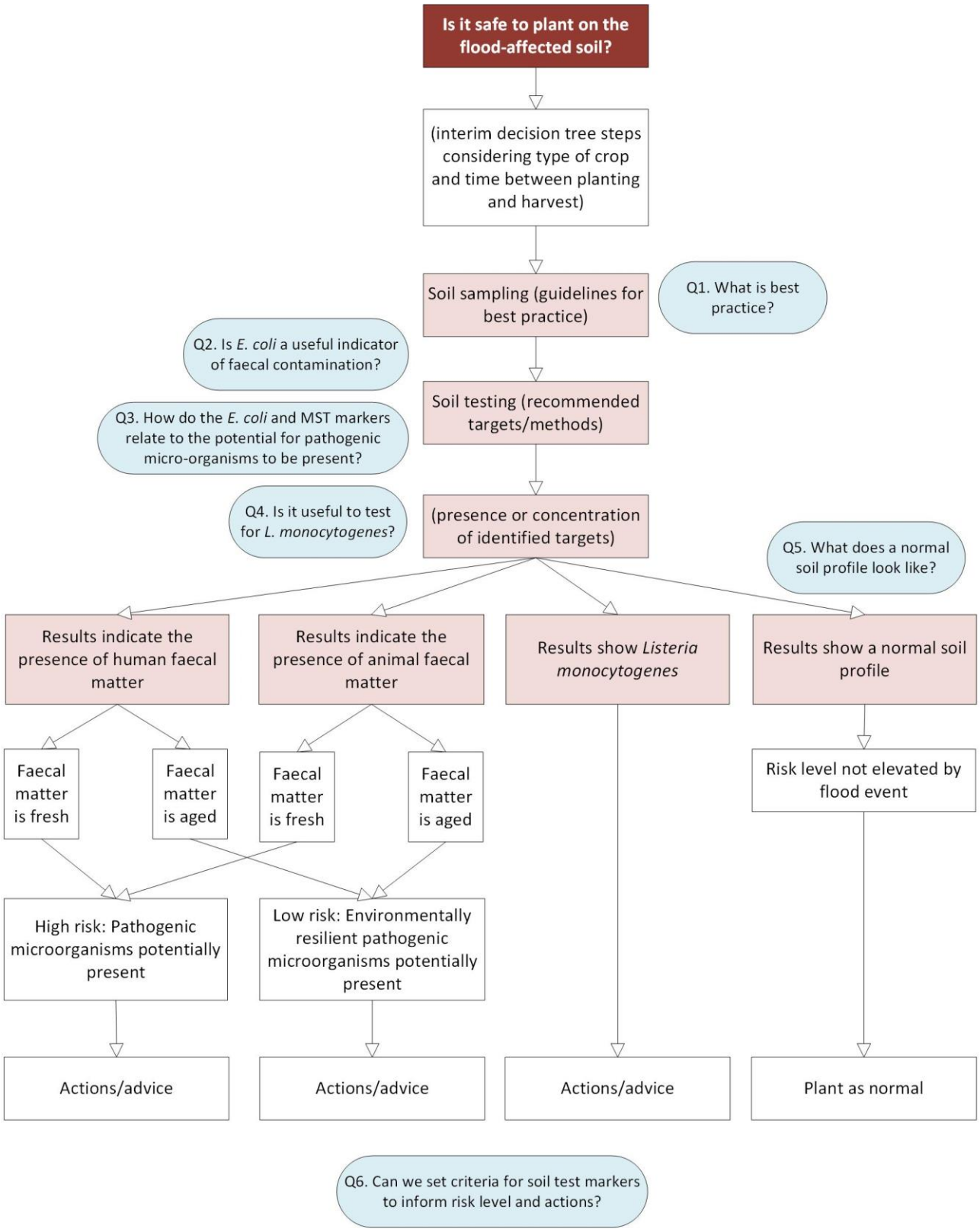

Figure 1. Grower decision-tree template used to guide this research

Red boxes indicate the focus areas for this research.
Blue boxes show the key research questions.

2 METHODS

This work was completed through literature review and laboratory analyses of sediment samples taken from horticulture land affected by the 2023 floods. Appendix A provides detail on the methods.

2.1 LITERATURE REVIEW

Relevant information was compiled from the scientific and grey literature. A range of material was considered to answer the six questions shown in Figure 1. The general topic areas were:

- The microbiology of horticulture soil, considering faecal indicators and pathogenic microorganisms.
- The microbiology of flood-affected soil, considering faecal indicators and pathogenic microorganisms.
- The persistence of faecal indicators and pathogenic microorganisms in field soils.

2.2 SOIL SAMPLE ANALYSES

Landwise, a charitable organisation supporting sustainable fruit and vegetable production, rapidly launched a horticultural silt and soil testing programme soon after the February 2023 floods, in the Northland, Hawke's Bay and Gisborne/Tairāwhiti areas.[1] Their work focused on soil productivity indicators (nutrients, texture, ecology, organic matter). They tested some samples for the faecal indicator bacteria *E. coli* (a range of concentrations was detected) and some chemicals (metals and pesticides; no concerning contamination was detected). Their work complements this current research and they kindly offered support through providing silt/soil samples for further analytical testing plus connections for further sampling, if required.

Twenty samples were selected from those taken by Landwise after the 2023 flood waters receded. These included the 14 samples from sites where additional samples had been tested for *E. coli* (all from the Hawke's Bay) and a further six samples from the Hawke's Bay, Wairoa and Gisborne. All 20 samples were analysed as shown in Figure 2. Full details of the analytical methods used are included in Appendix A.2. All were analysed for *L. monocytogenes* and faecal markers (MST method). Further analyses for four important pathogenic bacteria (*Salmonella* spp., *Campylobacter* spp., *Yersinia* spp. and Shiga toxin-producing *E. coli* [STEC]) were carried out depending on the MST results.[2]

Planning for this current research included resampling of a small number of 'risky' sites, as indicated by the presence of markers for human faecal material, the DNA from *Salmonella*, *Campylobacter*, STEC and/or *Yersinia*, and/or the presence of *L. monocytogenes* (live cells).

Section 5.3 provides details of the final analyses conducted and the results.

It is acknowledged that, due to the purpose of the sampling, the samples were not taken using strict aseptic techniques. Although care was taken, the samples may have been contaminated through collection and subsequent handling. However, this risk was outweighed by the opportunity to use these samples to generate useful data to inform future efforts.

[1] https://www.landwise.org.nz/2024/03/27/soil-recovery-after-cyclone-gabrielle-building-back-better/
[2] 'spp.' encompasses all species of a genera. For ease of reading, only the genera names will be used subsequently in this report unless 'spp.' is important to include to prevent uncertainty (e.g. *Listeria* spp. vs. *L. monocytogenes*).

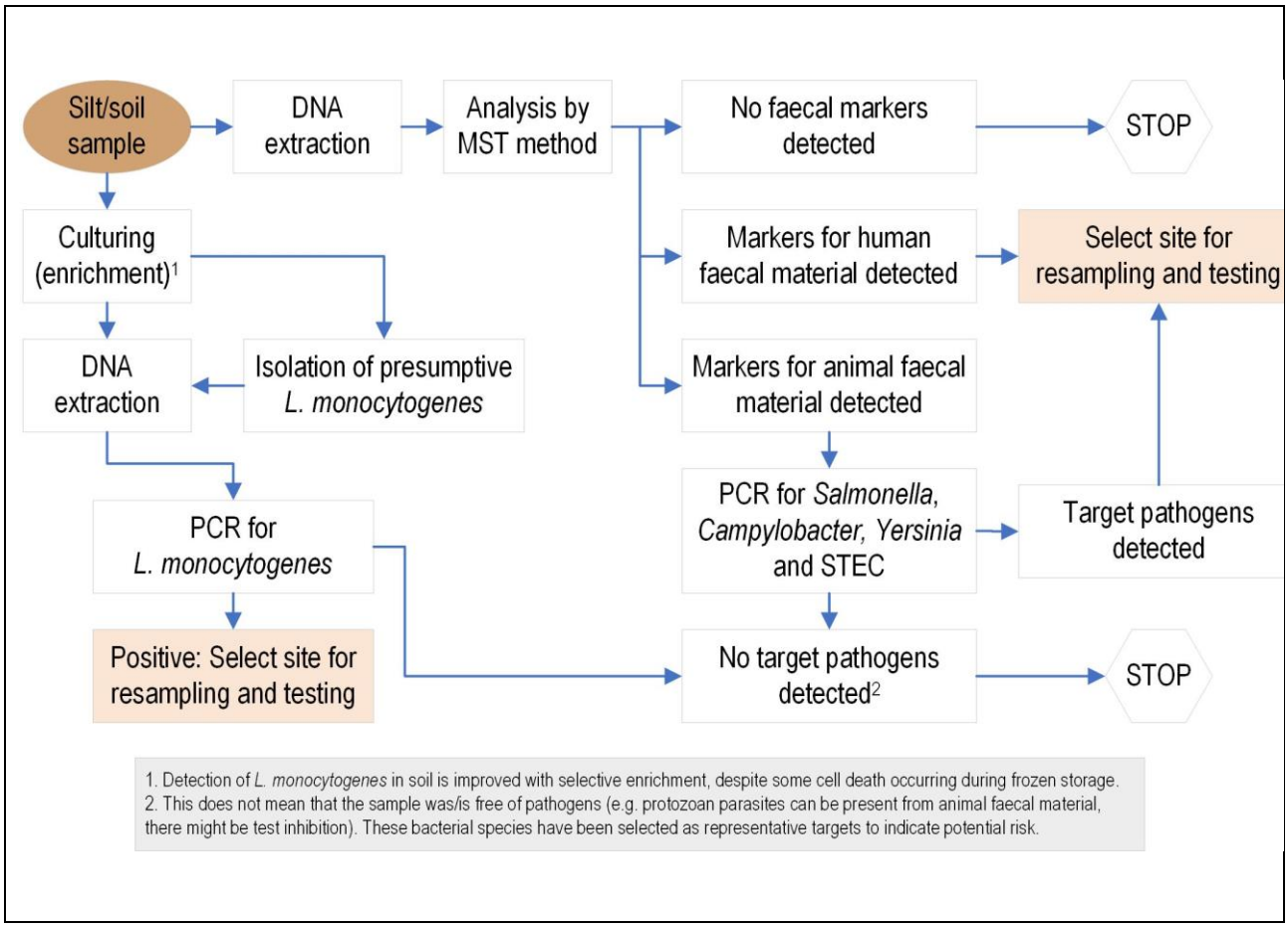

Figure 2. Summary of laboratory tests of soil/sediment samples

3 FAECAL INDICATORS AND PATHOGENIC MICROORGANISMS

This section provides an overview of faecal indicators and the pathogenic microorganisms of relevance to this current work.

3.1 FAECAL INDICATOR BACTERIA (FIB)

FIB are used to indicate a risk to human health from faecal contamination since routine monitoring for the presence of pathogenic microorganisms is impractical because a wide range of pathogen species could be present. Compared to FIB test methods, tests for pathogens can be complex, have low recovery rates and are expensive. *E. coli* and enterococci are commonly used FIB because they are consistently present in high concentrations in the faeces of warm-blooded animals and there are standard methods available for testing different types of environmental samples. Other FIBs include total coliforms (TC) and faecal coliforms (FC). Coliform is a collective term used for a large group of gram-negative, non-sporeforming, rod-shaped bacteria that all belong to the Enterobacteriaceae family. While the coliform group includes normal intestinal bacteria, it also includes environmental bacteria (Paruch and Mæhlum, 2012). FCs are a thermotolerant sub-group of TCs. In New Zealand, FIB testing is used routinely to monitor the safety of drinking and recreational waters.[3]

When using FIB as indicators of faecal contamination, there are some important considerations:

1. The presence of FIB does not confirm the presence of enteric (faecal) pathogens, only that these might be present. FIB are common inhabitants of the gastrointestinal tracts of mammals and birds, but their detection does not indicate the source of faecal matter nor confirms that pathogenic microorganisms are present (Korajkic *et al.*, 2018). Pathogenic microorganisms might only be present intermittently in faeces from individuals or groups of warm-blooded animals. Human enteric pathogens will be present in sewage if illness is circulating in the community.
2. FIB are indicators of faecal contamination but not indicators for opportunistic pathogenic microorganisms that are natural environmental inhabitants. Examples of opportunistic pathogens relevant to foodborne infection are *L. monocytogenes* and *Bacillus cereus*. Spore-forming clostridia, which can also cause foodborne illness, are naturally found both in faeces and the environment (Palmer *et al.*, 2019).
3. FIB survival in environmental samples can be different to pathogenic microorganisms. For this reason, FIB may be poor indicators for pathogenic microorganisms that survive longer in environmental samples. For example, parasites that are excreted with faeces but have environmentally resistant life stages, such as *Toxoplasma gondii, Giardia* spp. and *Cryptosporidium* spp., have longer survival times than FIB. This means that low concentrations of FIB do not necessarily imply a low risk to human health.
4. In some conditions, FIB can persist and replicate in the environment. This can trigger ‘false positive’ results.

Regarding point 4, the presence of naturalised (persistent) FIB populations in the environment has recently been reviewed from the perspective of recreational water quality monitoring (Devane *et al.*, 2020). The authors established that naturalised *Escherichia* and *Enterococcus* species have

[3] For further information on faecal indicators and water quality monitoring, see this LAWA factsheet https://www.lawa.org.nz/learn/factsheets/faecal-indicators/

been identified in environmental matrices including soil, and standard tests are unlikely to differentiate these 'non-enteric' strains from enteric (faecal) FIB. The naturalised strains may be considered as two groups: Those that are defecated into the environment and are able to persist under favourable conditions by adapting to a non-host lifestyle (i.e. indicators of past faecal contamination), and those that are truly environmental (separate lineages that lack the genes important for survival in the gut of animals). Laboratory methods are needed to distinguish between FIB from fresh faeces, aged faeces (i.e. faecal contamination in the past) and naturalised populations. Devane *et al.* (2020) propose that additional methods are needed to determine whether there is a health risk from environmental samples with elevated numbers of *E. coli* but no obvious source of faecal contamination.

3.2 GENETIC INDICATORS FOR FAECAL CONTAMINATION

New analytical techniques have been developed by molecular biologists that target genetic material from microorganisms. When these methods are designed to analyse all the genetic material in a sample, to determine which organisms are present, this is called metagenomics. Metagenomic methods address some of the FIB weaknesses noted in Section 3.1, since the methods can detect multiple microorganisms in one test and indicate the likely sources of contamination. Metagenomic Next Generation Sequencing (NGS) is an example of a high-throughput method that amplifies and sequences short pieces of extracted DNA or RNA in a sample. The sequences are compared with a library of genetic data to identify the microorganisms present and the relative abundance of different microorganisms can be calculated. Some weaknesses are:

- These molecular techniques only detect gene fragments and so do not indicate whether this genetic material came from viable microorganisms.
- It is difficult to quantify the different microorganisms in a sample (calculating relative abundance is achievable).
- The results are often not detailed enough to know whether the microorganisms present in a sample constitute a human health risk (e.g., analysis might reveal the presence of *Listeria* spp. but would usually not identify the pathogenic species such as *L. monocytogenes*).

MST uses qPCR (quantitative Polymerase Chain Reaction) to target specific host-associated genetic material from the gut of animals and humans. The concentration of these markers can be calculated. MST is used to identify the sources of faecal contamination by targeting and quantifying genetic markers of species-specific microorganisms or host-bacteria interactions. Some markers can additionally indicate aged faecal contamination. For example, the human marker crAssphage has a slower decay rate than both FIB and bacterial MST markers, and its continued presence when there are very low concentrations of other human MST markers is considered indicative of aged or treated sewage (Boehm *et al.*, 2018; Leonard *et al.*, 2021).

3.3 PATHOGENIC MICROORGANISMS

A range of pathogenic microorganisms can cause foodborne disease.[4] Their characteristics, and their relative importance in the context of fresh produce food safety, have recently been reviewed (King and Cressey, 2021). Four faecal-associated bacteria were selected as analytical targets for this current research:

[4] Pathogen data sheets are available from https://www.mpi.govt.nz/science/food-safety-and-suitability-research/food-risk-assessment/foodborne-hazard-data-sheets/

- *Campylobacter* spp., the most common bacterial cause of notified gastroenteritis in New Zealand.[5] The two species important for human illness (campylobacteriosis) are *Campylobacter jejuni* and *Campylobacter coli*. *Campylobacter* are commonly found in animal faeces, particularly that of ruminants and poultry, and are only found in soil contaminated with faecal material (directly or indirectly, e.g. with faecal-contaminated water).
- *Yersinia* spp., the second most common bacterial cause of notified gastroenteritis in New Zealand. The two species important for human illness (yersiniosis) are *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*. *Yersinia* can be found in faeces from a range of both well and sick animals. They are introduced to soil through faecal contamination.
- *Salmonella* spp., an important cause of foodborne disease (salmonellosis) and a cause of illness in animals. *Salmonella* are shed with faeces and will only be present in soil contaminated with faecal material. The group *Salmonella enterica* subsp. *enterica* contains multiple serotypes, all potential human pathogens, although some are more commonly isolated from human cases (e.g. serotypes Typhimurium and Enteritidis). Humans are the only hosts for serotypes Typhi and Paratyphi, which cause enteric fever (typhoid or paratyphoid fever). *S.* Typhi and *S.* Paratyphi are not endemic in New Zealand but have caused infections in people returning from countries where the disease is endemic, meaning there is a low risk of these being introduced into the New Zealand environment with human faeces. The non-typhoidal *Salmonella* serotypes are widespread in New Zealand and can be present in the faeces of a range of warm- and cold-blooded animals, and humans.
- Shiga toxin-producing *E. coli* (STEC), a subgroup of *E. coli* that can cause very serious illness, particularly in children. As introduced in Section 3.1, *E. coli* are natural inhabitants of the gut of humans and other warm-blooded animals (particularly ruminants), so are excreted with faeces. However, some strains are known to be opportunistic human pathogens, causing enteric or extraintestinal disease. Pathogenic *E. coli* can be grouped into pathotypes or described using serotypes or virulence genes (Pakbin *et al.*, 2021). STEC are strains of *E. coli* carrying one or more *stx* genes, which enable Shiga toxin production. The *E. coli* serotype O157:H7 is an example of an STEC since this serotype usually carries one or more *stx* genes. STEC will only be present in soil contaminated with faeces.

As introduced in Section 1.2, *L. monocytogenes* was also selected as an analytical target for this current research. This species of *Listeria* can be naturally present in the soil environment and associated vegetation. *L. monocytogenes* can cause serious foodborne illness, which can manifest as invasive listeriosis ("listeriosis") or non-invasive listeriosis ("febrile listeriosis" or "listerial gastroenteritis"). Older adults are particularly vulnerable to listeriosis and the invasive form can cause miscarriage in pregnant women (Horn *et al.*, 2023). *L. monocytogenes* can also be introduced into the environment with the faeces from animals, and with abortion or other bodily fluids from infected animals (listeriosis can cause abortions in pregnant livestock). Fresh produce growers need to manage *L. monocytogenes* if required by the food safety regulations that apply to their business and/or by the markets they are trading into.[6]

It should be noted that many other human pathogenic microorganisms may be present in human and animal faeces. While some of these are zoonotic – able to be passed between animals and humans, some are highly species specific. In particular, enteric viruses that affect humans, such as

[5] See New Zealand notifiable disease surveillance data provided by ESR, funded by the Ministry of Health https://www.esr.cri.nz/our-research/nga-kete/infectious-disease-intelligence/notifiable-diseases/

[6] Producers of horticultural food and horticultural packing operations (packhouses) are subject to National Programme Level 1, which requires operators to manage food safety hazards (https://www.mpi.govt.nz/food-business/running-a-food-business/national-programmes/). There are microbiological limits for *L. monocytogenes* in ready-to-eat foods set out in Standard 1.6.1 and Schedule 27 of the Australia New Zealand Food Standards Code (https://www.foodstandards.gov.au/food-standards-code/legislation).

rotavirus, norovirus and adenovirus, are human-specific and contamination of soils and silt with these pathogens will only occur as the result of human sewage entering the environment. Enteric viruses have been reported to survive for longer in soils than common bacterial pathogens (WHO, 2006). The presence of enteric viruses and other human-adapted pathogens is one reason that untreated human waste is not considered to be an appropriate manure for addition to soils.

4 HOW FLOODS AFFECT MICROBES

KEY FINDINGS

What does a normal soil profile look like?

- *E. coli* is not always detected but can be present in soils located away from agricultural and other human activities. Reported concentrations in such areas, where *E. coli* was able to be detected, were usually <100 CFU or MPN per gram of soil but sometimes exceeded 1000 CFU or MPN per gram of soil. Very limited data from crop growing soils suggest *E. coli* numbers have a similar range.
- *L. monocytogenes* can be found in horticulture soils although the prevalence is likely to be 10% or less (i.e. 1 in 10 samples positive). Irrigation and rainfall tend to increase the likelihood of these bacteria being detected in soils.
- *Salmonella*, STEC, *Yersinia* and *Campylobacter* would not normally be expected to be present in soil without faecal contamination but can be introduced with wind (contaminated dust) and contaminated water. *Salmonella* and STEC have been isolated from soils in horticulture areas although many results are from soils that have received animal manures as fertiliser.

How do floods affect faecal indicators and pathogenic microorganisms in horticulture soils?

- Studies of FIB and pathogenic microorganisms in post-flood soils are scarce. Available data suggest that initially, the concentration of *E. coli* and pathogens can be elevated after flooding but their numbers decrease during subsequent days or weeks. Higher soil moisture supports their persistence. Pathogenic microorganisms are more likely to be present in soil if they are also present in nearby surface waters.
- In areas affected by Cyclone Gabrielle, elevated *E. coli* concentrations were measured in flood-deposited silt near a wastewater treatment plant and two sites located elsewhere, described as being agricultural. Samples from all three sites contained *E. coli* at <1000 CFU/g.

What more can we learn from field studies of the persistence of faecal indicators and pathogenic microorganisms in horticulture soils?

- Most studies of the soil persistence of FIB and pathogenic microorganisms relate to the presence of animal manure or slurries. A common observation under field conditions is a rapid decrease in bacteria numbers followed by a longer period of slower reduction, stabilisation or fluctuation (tail-end survivors). Pathogenic bacteria can survive for a month or more under field conditions but numbers will decrease over time.
- Temperature is a major driver for bacterial die-off, with the rate of reduction increasing with increasing temperature. One study predicted that, under medium temperature field conditions (10-20°C), the *E. coli* or *Salmonella* concentration would decrease by 1-log after 13 and 16 days, respectively, but this might take as long as 75 days (Tran *et al.*, 2020). *E. coli* survived better if the soil was contaminated during dry conditions (possibly due to a desiccation stress response) but subsequent higher soil moisture usually prolonged survival and possibly permitted growth. Survival is generally poorer in coarser sandy soils.

- For *L. monocytogenes*, which can be well adapted to living in soil, soil chemistry is important for survival.

What do we know about the effect of soil remediation on faecal indicators and pathogenic microorganisms in horticulture soils that have been flooded?

- Only one study was located that explored this scenario, and only total coliforms and aerobic bacteria were measured. Post-flood, the concentrations of these indicator microorganisms were higher in plots that were tilled, planted with cover crops or covered with plastic mulch compared to undisturbed (control) plots. Tillage breaks up the flood-compacted soils, incorporating flood-deposited microorganisms into the soil layer and increasing soil aeration, both of which improve microbial survival/growth.
- On outdoor experimental plots, covering the soil with polyethylene was more effective for killing *Salmonella* in the soil than leaving the soils undisturbed or growing cover crops. The plastic cover generated high temperatures in the soil.

4.1 THE MICROBIOLOGY OF HORTICULTURE SOILS

From a microbiological perspective, soils are complex in terms of their physical and chemical properties, and are inhabited by a diverse community of microbes that interact with each other, their environment and the wider flora and fauna existing there (Luan *et al.*, 2023).

This section considers information that indicates what concentrations of FIB or pathogenic microorganisms might be considered 'normal' in horticulture soils. It should be noted that, while the summarised studies report mean concentrations of microbial species or type within an environment, concentrations and microbial community structures are likely to be highly variable across a site and across seasons and other events that impact on environmental conditions.

4.1.1 Faecal coliforms

Faecal coliform concentrations were determined in soils from three vegetable farms in southeast Spain (Selma et al., 2007). Samples were taken from three farms employing either: traditional crop rotation with moderate fertiliser inputs, intensive with inputs from uncomposted chicken manure or intensive with inputs from composted olive waste. The mean faecal coliform concentrations were similar in soil from all three farms (approximately 1.3-1.5 $\log_{10}$ CFU/g).

Faecal coliforms were determined in soil from three areas in the Rhode River catchment (Maryland, USA) (Faust, 1982). Soils were from pasture, cornfield or forest sites. Samples were also taken at different depths. The mean faecal coliform concentrations were highest in soil from pasture (3-396 MPN/g or 0.5-2.6 $\log_{10}$ MPN/g) compared to cornfield (4-26 MPN/g or 0.6-1.4 $\log_{10}$ MPN/g) or forest (6-27 MPN/g or 0.8-1.4 $\log_{10}$ MPN/g). In soil under pasture, faecal coliform concentrations were highest in surface soil (0-1 cm), with concentrations decreasing with depth down to 25 cm. Faecal coliform concentrations in soil under pasture were highest in Autumn, which the author of the study ascribed to cattle being grazed in the pasture in that season.

4.1.2 *E. coli*

E. coli is considered to be a marker of faecal contamination in soils. However, during faecal-oral transmission the bacteria will often need to survive for a period of time in habitats outside a host (Dusek *et al.*, 2018). As discussed in Section 3.1, *E. coli* can become adapted for growth outside a warm-blooded host. Isolates of *E. coli* from various "extra-host" habitats have been shown to exhibit phenotypes and population structures that differ from faecally- or clinically-derived strains.

Some relevant studies were located that measured *E. coli* in soils from horticulture areas:

- In a study carried out across cropping farms in Virginia, USA, soils were aseptically sampled on four occasions to a depth of approximately 15 cm (Cook *et al.*, 2023). Generic *E. coli* was not detected (limit of detection (LOD) 1 $\log_{10}$ CFU/g) in 55% of samples. Across three farms, *E. coli* was detected in 67.5, 43.8 and 22.5% of samples. In samples where *E. coli* was detected, the mean concentration was 1.53 ± 0.77 $\log_{10}$ CFU/g (mean ≈34 CFU/g), with minor differences between farms (1.43-1.61 $\log_{10}$ CFU/g). No association was found between *E. coli* concentrations and presence of pathogens (*L. monocytogenes* and *Salmonella*). The mean *E. coli* concentration in *Salmonella*-negative soils (1.54 $\log_{10}$ CFU/g) was similar to that in *Salmonella*-positive soils (1.35 $\log_{10}$ CFU/g).
- Soil samples were aseptically collected from three horticultural farms in Florida, USA (Diekman *et al.*, 2024). Samples were taken from the top 15 cm of soil, with 20 random samples collected per farm on each of four occasions from October 2021 to April 2022. *E. coli* was detected in 27 of 239 samples (11.3%), with a mean concentration in positive samples of 1.56 $\log_{10}$ CFU/g. The prevalence of positive samples ranged from 1.3% to 22.8% across the three farms.
- In an intensive survey of two lettuce fields on one USA farm, *E. coli* was not detected in any soil samples (<0.5 CFU/g) (Wu *et al.*, 2023). Soils were sampled by three methods: soil adhering to boot covers after walking through sections of the fields (*n* = 28), composite grab samples (*n* = 28) and single grab samples (*n* = 72).
- A study in Vietnam focused on horticulture soils receiving irrigation water from contaminated sources (Prayoga *et al.*, 2021). Partially treated municipal wastewater was discharged to a river that was directly connected to irrigation channels. The concentration of *E. coli* in the soil samples was highly variable but most values were $<10^3$ CFU/g (3 $\log_{10}$ CFU/g) soil, even during the wet season. While the wet season increased contamination from municipal wastewater, this was countered by the dilution effect due increased water volume in the irrigation channels, from run-off of rainfall. As a result, the concentration of *E. coli* in the soil samples was not significantly different between the wet and dry seasons.
- Two studies of kiwifruit orchards in Shaanxi Province, China, found *E. coli* in 72/104 (69%) and 28/55 (51%) of soil samples (Feng *et al.*, 2014; Feng *et al.*, 2015).
- *E. coli* was detected in 37% of soil samples (*n* = 276) collected from lettuce farms in the West Flanders region of Belgium, with a median concentration of 1.2 $\log_{10}$ CFU/g and a maximum of 3.2 $\log_{10}$ CFU/g (Holvoet *et al.*, 2014). Samples with quantifiable concentrations of *E. coli* were associated with higher ambient temperatures.

Other studies provide further information on the prevalence and concentration of *E. coli* that might be expected in horticulture soils.

Soil samples (*n* = 1430) were collected around the Buffalo River in Minnesota, USA and tested for *E. coli* (Dusek *et al.*, 2018). *E. coli* isolates were obtained from 581 soils (41%). There was a greater prevalence of *E. coli* in soils from deciduous forest, pasture and wooded wetlands compared to croplands, grasslands and scrublands. The distinction made between pasture and grasslands in this study is unclear, with the latter perhaps being non-cultivated, natural grasslands. Concentrations of *E. coli* in soils were only reported for forest and pasture samples and were in the range 1-3 $\log_{10}$ CFU/g.

To determine the relative contributions of pasture and cattle to the soil *E. coli* population, cattle were removed from a grazing pasture approximately one year before initiation of a study (NandaKafle *et al.*, 2017). Soil cores (4 cm) were taken before reintroduction of cattle, with *E. coli* concentrations found in the range 0 to 25 CFU/g. After grazing, concentrations up to 100 CFU/g (2 $\log_{10}$ CFU/g) were detected.

Although not farm soils, information on patterns of *E. coli* in soils was provided by a study of three sites adjacent to Lake Superior in the USA (Ishii *et al.*, 2006). Sampling sites ranged from locations that were frequently inundated to sites that remained predominantly dry. *E. coli* concentrations were highest in summer and autumn and lower during winter and spring. The maximum concentration of *E. coli* detected was 3.48 $\log_{10}$ CFU/g. However, the two sites at which this concentration was detected were quite different in character; one was on a hillside, which was rarely saturated and the other was at the waterline. *E. coli* was most frequently detected at a site that was saturated almost year round, with concentrations in the range 5 to 1150 CFU/g (0.7-3.06 $\log_{10}$ CFU/g).

Soil surveys in riparian areas located in tropical, subtropical and temperate regions showed that *E. coli* is common in these soils, even in areas relatively undisturbed by humans (Byappanahalli *et al.*, 2012). The maximum concentrations reported were in the order of 3 $\log_{10}$ MPN or CFU per gram of soil. Genotyping showed wide diversity among *E. coli* strains within and between locations.

4.1.3 *Listeria* spp. and *L. monocytogenes*

As introduced in Section 3.3, *L. monocytogenes* can be found in soils and in faeces from animals and humans. Several relevant studies were located to indicate the *L. monocytogenes* prevalence and concentration values that might be expected in horticulture soils. However, *L. monocytogenes* is nearly always found at low numbers in soils and detection methods often need to include selective enrichment (Smith *et al.*, 2018).

- In New York State (USA), soil samples were collected from five fresh produce farms over two years, and tested for *L. monocytogenes* (Strawn *et al.*, 2013a). The samples were preferentially taken from fields used for growing commodities intended for raw consumption. Faecal deposits (e.g. from wildlife) were not observed in the majority of these fields on each sampling occasion, although manure slurries were applied at three farms between cropping cycles. The prevalence of *L. monocytogenes* was 16/178 (9%), with positive samples being more often collected during winter months. The likelihood of detecting *L. monocytogenes* was influenced by the soil available water storage (AWS – a measure of the quantity of water that the soil is able to store for use by plants), temperature and proximity to pasture and surface water. Cooler, moist soils located closer to pasture or further from surface waters were more likely to be positive.
- In a follow-up study of 263 fields on 21 fresh produce farms in New York State, *L. monocytogenes* was detected in 30/263 (11%) soil samples and 21/263 (8%) drag swabs, giving an overall prevalence of *L. monocytogenes*-positive fields of 46/263 (17.5%) (Strawn *et al.*, 2013b). Based on the combined results of soil and drag swab testing, a multivariate model indicated that *L. monocytogenes* was more likely to be present if the field had been irrigated or wildlife had been observed in the field within 3 days of sample collection, or if soils had been cultivated within 7 days of sample collection.
- After completion of a longitudinal study of spinach fields, the overall prevalence of *Listeria* spp. and *L. monocytogenes* in soil samples (n = 1092) was 12% and 8%, respectively (Weller *et al.*, 2015). *L. monocytogenes* was more likely to be detected 24 hours after irrigation or rainfall events.
- A longitudinal study of 19 organic farms in the USA, where untreated animal manure was used for fertilisation, detected *L. monocytogenes* in 101/2150 (5%) of soil samples (Pires *et al.*, 2023).
- A study conducted in Poland detected *L. monocytogenes* in 9/36 soil samples taken 150 m from “areas near food processing” (with all positive samples being near the entrance to a

meat factory), but did not detect *L. monocytogenes* in 18 soil samples from orchards (Szymczak *et al.*, 2014).

- *Listeria monocytogenes* was detected at a concentration of 4 MPN/g in a composite sample from one of 13 carrot fields in Nova Scotia, Canada (Dowe *et al.*, 1997). *Listeria* spp. were detected in six of 13 surrounding uncultivated meadows, with four isolates identified as *L. monocytogenes* and two as *L. ivanovii/seeligeri*. Concentrations in the uncultivated meadows were in the range 4-90 MPN/g.
- In a study of three different fresh produce farms in Canterbury, New Zealand, two soil samples were collected on two visits to each farm, and each sample tested for *Listeria* spp. (Zhu, 2015). All 12 soil samples were positive, with the average counts of *Listeria* spp. for each farm being 4.67, 4.30 and 3.89 $\log_{10}$ CFU/g. *L. monocytogenes* was not detected.

Additional soil studies only tested for *Listeria* spp., which are easier to detect compared to *L. monocytogenes*. However, most members of this genus are non-pathogenic. The presence of *Listeria* spp. indicates the potential for *L. monocytogenes* to be present but does not in itself suggest pathogenic *Listeria* are present in the soil. These studies suggest *Listeria* spp. are commonly found in soils at concentrations of 1,000 CFU or MPN per gram of soil.

- *Listeria* spp. concentrations were determined in soils from vegetable farms in southeast Spain (Selma *et al.*, 2007). Samples were taken from three farms under different management practices: traditional crop rotation with moderate fertiliser inputs (TR), intensive with inputs from uncomposted chicken manure (INT-A), and intensive with inputs from composted olive waste (INT-B). Testing of the soil amendments (chicken manure and olive waste) showed these contained mean concentrations of *Listeria* spp. of approximately 4 $\log_{10}$ CFU/g. However, the highest mean soil concentration of *Listeria* spp. was on TR (approximately 5.5 $\log_{10}$ CFU/g), followed by INT-A (approximately 5.0 $\log_{10}$ CFU/g) and INT-B (approximately 3.5 $\log_{10}$ CFU/g).

4.1.4 *Salmonella*, STEC, *Yersinia* and *Campylobacter*

Salmonella, STEC, *Yersinia* and *Campylobacter* would not normally be expected to be present in soil without faecal contamination but windblown or waterborne deposition can occur. Evidence from a five-year survey of an almond orchard suggested that some *Salmonella* strains can persist in the environment, since in this study, the same *Salmonella* Enteritidis phage type was detected in samples from the orchard across the whole 5-year period (Uesugi *et al.*, 2007).

Some relevant studies of horticulture soils are available.

- A multi-country study of leafy green and strawberry growing areas in Belgium, Brazil, Egypt, Norway and Spain included 1037 soil samples (Ceuppens *et al.*, 2015). Of these, 11/599 (2%) were positive for *Salmonella* and 5/587 (1%) were confirmed as containing viable STEC (6% were PCR positive for STEC). *Campylobacter* was not detected. Concentrations of generic *E. coli* were also measured but there was no significant correlation between these values and the presence of STEC in soil. However, there was a correlation between the concentration of generic *E. coli* and the presence of *Salmonella* in soil, such that soil samples containing ≥100 CFU/g (≥2 $\log_{10}$ CFU/g) *E. coli* were more likely to contain *Salmonella* (among the *Salmonella* positive soils, the mean *E. coli* concentration was approximately 1000 CFU/g or 3 $\log_{10}$ CFU/g). Some *Salmonella*-negative soil samples contained *E. coli* at concentrations exceeding 1000 CFU/g. This is not unexpected since not all faecal material contains *Salmonella*. Considering soil samples with <100 CFU/g *E. coli*, there was only one outlier that also contained *Salmonella*.

- A survey in the US tested 120 soil samples from eight produce farms in Southern Georgia, and only detected *Salmonella* DNA in one sample (no *Salmonella* isolates were obtained) (Lee *et al.*, 2019).
- Soil samples were aseptically collected from three horticultural farms in Florida, USA (Diekman *et al.*, 2024). Samples were taken from the top 15 cm of soil, with 20 random samples collected per farm on four occasions from October 2021 to April 2022. *Salmonella* was detected in 1 of 239 samples (0.4%), with presumptive *Salmonella* colonies confirmed by PCR.
- In the study of soils from five fresh produce farms in New York State (USA) described in Section 4.1.3, the soil samples were also tested for *Salmonella* and STEC (Strawn *et al.*, 2013a). The prevalence of both was low: 4/178 (2%) *Salmonella* positive and 3/178 (2%) STEC positive. In a follow up study of 21 fresh produce farms in New York State, also described previously, *Salmonella* was detected in 13/263 (5%) of soil samples, or 16/263 (6%) fields sampled (Strawn *et al.*, 2013b). *Salmonella* was more likely to be present if manure had been applied within 365 days of sampling, although this finding was derived from the combined results of both drag swab and soil testing of fields.
- A longitudinal study of 19 organic farms in the USA recovered non-O157 STEC from 174/2150 (8%) soil samples, *Salmonella* from 15/2150 (0.7%) soil samples and *E. coli* O157 from one sample (Pires *et al.*, 2023). A companion paper described findings from a larger set of 2461 soil samples, although the prevalence values were similar (Ramos *et al.*, 2021). Untreated animal manure was used for fertilising these sites and the presence of non-O157 STEC in cattle manure increased the odds of the soil being positive after manure application. Soil moisture was also positively associated with the presence of non-O157 STEC.

Few data were located to indicate the expected prevalence of *Yersinia* in horticulture soils. A small study of salad vegetable growing farms in Finland isolated *Yersinia pseudotuberculosis* from 1/51 samples (Niskanen *et al.*, 2003). *Yersinia* was not isolated from 14 samples of soil from seven dairy farms in Australia (McAuley *et al.*, 2014).

4.2 THE IMPACT OF FLOODING ON THE MICROBIOLOGY OF HORTICULTURE SOILS

Floodwater models can be developed to predict flood events. At a basic level, these use rainfall-runoff models that focus on the effect of rainfall on the capacity of existing water storage areas (lakes, rivers, streams, subsurface storage), and combine these with hydrodynamic models that simulate how water moves across the land (Collender *et al.*, 2016). Extra components need to be added to such models to predict the mobilisation of microbiological hazards from their existing source, their transport with runoff or floodwater flows, and where they might settle. Methods are available to do this, although applications have usually focussed on FIB (Collender *et al.*, 2016). In New Zealand, these methods have been used to predict in-stream microbial mobilisation during high flow events (e.g. Drummond *et al.* (2022) and Hong *et al.* (2018)). Regional models could show areas of higher risk for microbial contamination during flood events, but developing and validating such models takes a lot of time and money. Even if such models were available, there is still the need for growers to understand their local post-flood soil conditions.

Studies of post-flood soils tend to focus on total biological activity and markers of soil productivity rather than evaluating the persistence of microorganisms of relevance to food safety. Laboratory, greenhouse and controlled field studies show that, in general, the microbiological profile of soils is affected by floods, although the microbial populations can be resilient and return to pre-flood levels within several weeks if the flood events are not too severe (duration of flooding < 5 weeks) (Shah *et al.*, 2021; Unger *et al.*, 2009).

The overall changes in the microbiological community of soils can be observed through measuring the metabolic utilisation of different carbon sources. Using this approach, studies in microcosms and under natural conditions found that water stagnation (i.e. slow drainage after flooding) was detrimental to the microbiological community, as indicated by a decrease in overall microbiological metabolic activity (Furtak *et al.*, 2022). The authors suggested that this was due to decreased oxygen diffusion into the soils and the subsequent death of obligatory aerobic microorganisms, however flooding causes other chemical changes in the soil that can also alter bacterial metabolism (Furtak and Wolińska, 2023). The results of Furtak *et al.* (2022) also suggested the emergence of anaerobic microorganisms. In these experiments, the soil pH also significantly decreased after flooding, with monitoring up to 56 days after the flooding event, although the authors noted that this was not consistently observed in other studies and is influenced by soil type.

As evidenced by the above study, oxygen (and nitrogen) diffusion into saturated or silt-covered soils can be limited and this creates an environment unsuitable for strictly aerobic microorganisms (Furtak and Wolińska, 2023). However, *E. coli* and the pathogenic bacteria considered in this review are facultative anaerobes, meaning they can survive in low oxygen or anaerobic environments (they are adapted to live in the intestines of humans and animals). Notwithstanding this ability, it has been reported that the biomass of gram-negative bacteria, which includes *E. coli*, *Salmonella*, *Yersinia* and *Campylobacter*, tends to reduce in soils in response to flooding (Wagner *et al.*, 2015). Gram-positive bacteria tend to be less affected (Furtak and Wolińska, 2023; Wagner *et al.*, 2015). Microbial biomass of soils has been observed to return to pre-flood levels within weeks of a flood (González Macé *et al.*, 2016).

Some studies were located in which the effect of flooding on pathogenic microorganisms and FIB in horticulture soils was measured. Below, we focus on longitudinal studies under field conditions. Most only considered post-flood samples so there is no ability to compare with conditions prior to flooding. Despite the possibility of using predictive models, a flooding event is difficult to predict and it is usually not possible for researchers to plan when and where pre-flood samples should be taken (Furtak *et al.*, 2022). If a fresh produce growing area is not flooded by waters crossing major sources of microbiological contamination, pathogenic bacteria can be difficult to detect after the flood waters subside. For example, after flooding in Victoria, Australia, researchers collected 15 soil drag samples from production fields but only detected STEC in one of these (Singh, 2023). However, they also found STEC on a produce sample and in harvest bins and drains in the processing facility. This highlights that, while soils might have low numbers of pathogens, these can be transferred by equipment, vehicles and workers into post-harvest processing areas. *Salmonella* and *L. monocytogenes* were also detected on non-contact surfaces in the processing facility receiving harvested produce from these fields, noting that these pathogens had not been detected through environmental sampling of these facilities during the years prior to the flood event. The source of the *Salmonella* and *L. monocytogenes* contamination was not identified.

Relevant studies on the impact of flooding on soil microbiology are summarised below.

Post-flood changes to faecal indicators and pathogens in lettuce fields

Following flooding in the south-east of Spain during 2012, four fields of iceberg lettuce (*Latuca sativa*) were sampled 1, 2, 3, 5 and 7 weeks after flooding. Surface soil (0-5 cm depth), lettuces and irrigation water were sampled on each occasion. Total coliforms and *E. coli* were enumerated in all samples, while *Enterococcus* was additionally enumerated in water samples. All samples were analysed for *Salmonella*, pathogenic *E. coli*

Castro-Ibáñez *et al.* (2015)

- *E. coli* concentrations were elevated in soils one week after flooding but were <2 $\log_{10}$

(O157:H7, O26, O103, O111, O145) and *L. monocytogenes* using enrichment and PCR detection, followed by culture confirmation.

Indicator organisms

Irrigation water

No details of the source of the irrigation water was given. An earlier study by the same group in the same region specified creek water as the source of irrigation water (Selma *et al.*, 2007). At week 1 post-flooding coliform and *E. coli* concentrations in irrigation water were elevated, at approximately 5 and 4 $\log_{10}$ CFU/100 mL, respectively. Three weeks after flooding coliform concentrations had decreased to 1-2 $\log_{10}$ CFU/g, while *E. coli* was no longer detectable.

Soil

Total coliform concentrations were approximately 5 $\log_{10}$ CFU/g at week 1. From week 2 onwards, concentrations were in the range 3-4 $\log_{10}$ CFU/g. These levels were described as normal for the area. The concentrations of *E. coli* at week 1 were 3-4 $\log_{10}$ CFU/g. *E. coli* was not detected in soils at weeks 3 and 5 (<2 $\log_{10}$ CFU/g). *E. coli* was detected in some soil samples at week 7, with the highest concentration being approximately 2.8 $\log_{10}$ CFU/g. The authors of the study concluded that this was unrelated to the flooding event and was possibly due to sporadic faecal contamination from wildlife.

Lettuce

Coliform and *E. coli* concentrations on lettuce samples followed a similar pattern to that observed in irrigation water and soil. At week 1 the coliform concentrations were approximately 6 $\log_{10}$ CFU/g, and the mean *E. coli* concentration was approximately 3.4 $\log_{10}$ CFU/g. *E. coli* was not detected at any later time points.

Pathogens

One week after the flood most samples of irrigation water, soil and lettuce were positive for *Salmonella* by PCR. However, only 2/4 soil samples and 1/4 irrigation water samples were culture positive for *Salmonella.* After 3 weeks, 4 of 18 samples (22%) were positive for *Salmonella* by PCR only, while at 5 and 7 weeks all samples were negative. The only positive soil samples were at week 1.

Pathogenic *E. coli* (STEC O145, O111, O103 and O126) was detected by PCR in soil samples taken at week 1, 3 and 7 but viable bacteria were not recovered from any of these samples. No irrigation water samples tested positive and of the lettuce samples, only those tested from week 1 were PCR-positive.

L. monocytogenes was only detected in two lettuce samples taken 3 weeks after the flooding and the authors concluded that flood water was unlikely to have been a source of *L. monocytogenes* contamination. Soils were not tested for *L. monocytogenes*.

CFU/g at three weeks.

- Viable *Salmonella* was detected in soils one week after flooding but all soils were negative thereafter.
- Pathogenic *E. coli* was detected in soils by PCR at 1, 5 and 7 weeks post-flood but viable microorganisms could not be recovered.

Post-flood changes to *E. coli* and *Salmonella* in produce field soils

Bergholz *et al.* (2016)

– *E. coli* and *Salmonella* are more likely to be present in moist soils.

Samples (water, surface soil and drag swabs) were taken on two fresh produce farms in New York State affected by flooding as a result of Hurricane Irene in August 2011. Qualitative testing (presence/absence) was carried out for *E. coli* and *Salmonella* by enrichment cultures, followed by PCR confirmation of presumptive colonies. Fields were sampled at 21, 44 and 238 days post-flood. *E. coli* prevalence in all sample types decreased between 21 and 44 days but only the prevalence in drag swabs decreased appreciably between 44 and 238 days. While *E. coli* was not enumerated, the genetic diversity among isolates at each time point reduced, suggesting die-off of less resilient strains. Sites closest to nearby surface water (canal, river, stream) were more likely to yield *E. coli*. *Salmonella* was detected in 4/209 drag swab samples and 2/90 soil samples, with all positive samples being from visibly moist soils. From combined soil and water sampling, it was observed that the proportion of samples positive for *Salmonella* decreased from day 21 (4.7%) to day 44 (2.5%) but increased again by day 238 (4.6%) after a period of rainfall. The lack of quantitative data on microbial concentrations and the lack of pre-flood information makes the information from this study of limited use.

Post-flood changes to faecal indicators and pathogens in soils

Casteel et al. (2006)

– *E. coli* concentrations were not elevated in post-flood soils (concentrations in the range 3-4 $\log_{10}$ MPN/100 g dry soil)

– *C. perfringens* spore concentrations were elevated in post-flood soils

The Center for Environmental Farming Systems (CEFS), North Carolina, USA was affected by flooding during Hurricanes Dennis, Floyd and Irene in September-October 1999. Soil samples (<15 cm depth, n = 29) had previously been taken from CEFS in March 1999. A further 28 samples were taken in October 1999. Table 1 summarises the results of microbiological analyses of the two sets of soil samples. Coliphage are used as indicators for human enteric viruses.

Table 1. Comparison of soil microbiology at Center for Environmental Farming Systems, North Carolina before and after a series of flooding events

Microbiological parameter	Frequency of detection, positive/total (percent)		Mean $\log_{10}$ MPN/100 g dry soil (95% confidence interval	
	March 1999	October 1999	March 1999	October 1999
Total coliforms	27/29 (93)	19/28 (68)	4.0 (3.7-4.1)	4.1 (3.7-4.3)
Faecal coliforms	2/29 (7)	1/28 (4)	3.5 (2.6-3.7)	4.0 (3.2-4.6)
E. coli	2/29 (7)	1/28 (4)	3.5 (2.6-3.7)	4.0 (3.2-4.6)
Somatic coliphage	0/29 (0)	3/28 (11)	-	1.1 (<0.47-1.7)
Male-specific coliphage	4/29 (14)	0/28 (0)	0.9 (<0.47-1.1)	-
Clostridium perfringens spore	29/29 (100)	24/28 (86)	4.7 (4.5-4.8)	6.6 (6.1-6.8)

Of the various indicators of potential faecal (human or animal) contamination, most prevalence and concentration values were lower after

flooding. Somatic coliphage were detected in more post-flood samples compared to pre-flood samples, and the *C. perfringens* spore count was significantly higher in post-flood samples. It is of interest to note that the pre- and post-flood values for *E. coli*, a standard indicator for faecal contamination, were similar between pre- and post-flood soil samples.

Post-flood changes to faecal indicators and pathogens in soils

Rock *et al.* (2023)

Over a 90-day period following flooding in the Salinas growing area of California, four ranches were visited six times and soil samples obtained from these sites were tested for total coliforms, faecal coliforms, *E. coli*, STEC and *Salmonella*. During this time, a second flood occurred. The full results of this study were not available but the available data showed that the concentrations of all three FIB generally decreased over time, although the actual reduction in numbers was not large (<1 log) nor always significant. From the examples shown it could be seen that the concentration of faecal coliforms in some samples was >100 MPN/g soil and *E. coli* concentrations were in the range 1-100 MPN/g soil. The concentration of *E. coli* was found to be positively correlated with soil moisture.

- Higher moisture soils had higher *E. coli* concentrations.
- STEC was found in soils flooded by tributaries that were also STEC-positive.
- Faecal coliform bacteria are not good indicators of food safety risks.

Salmonella was detected in approximately 5% of soil samples collected after the first flood, and 3% after the second flood. The prevalence of STEC was higher among samples collected after the second flood compared to those collected after the first flood. Soils flooded by tributaries or creeks through overland flow were more likely to be STEC-positive compared to sites adjacent to the Salinas River. Water samples confirmed that STEC was more likely to be present in these tributaries. The researchers concluded that faecal coliform bacteria were not suitable for indicating pathogen risk because their concentrations were variable across space and time and not correlated to the presence of STEC or *Salmonella*.

Strawberry mesocosm study: *E. coli* changes with simulated flooding

Shiraz *et al.* (2020)

In an outdoor mesocosm[7] study using raised beds containing strawberry plants, five treatments were applied:

- Control. Exposure to 890 L of floodwater (strawberries fully submerged) will no *E. coli* inoculum added.
- High flood high contamination (HFHC). Exposure to 890 L of floodwater (strawberries fully submerged) containing 6 $\log_{10}$ CFU/L of a mixture of three strains of generic *E. coli*.
- High flood low contamination (HFLC). As for HFHC, except the *E. coli* concentration was 2 $\log_{10}$ CFU/L.
- Low flood high contamination (LFHC). Exposure to 600 L of floodwater (strawberries did not come into direct contact with floodwater) containing 6 $\log_{10}$ CFU/L of a mixture of three strains of generic *E. coli*.

- *E. coli* was detected in soil samples two days after flooding but not at four days.

[7] A mesocosm is a trial plot formed under field conditions where normal conditions are allowed to exert an effect over the experiment. A microcosm is an enclosed, controlled system that might be completely isolated from any external influence, or only open to selected external influences (e.g. natural light or temperature). A useful image is provided by Furtak *et al.* (2022).

- Low flood low contamination (LFLC). As for LFHC, except the *E. coli* concentration was 2 $\log_{10}$ CFU/L.

The floodwater was municipal water with the added *E. coli* plus fresh cow manure mixed in at a concentration of approximately 0.4 g/L. Each flooding event lasted for 4 hours. Strawberries were harvested at maturity and stored at 4°C and tested for *E. coli* at 0, 48, 96 and 144 hours. Soil samples were tested every 48 hours for one week.

Generic *E. coli* was not detected on strawberries at any time point from any of the mesocosms. Coliforms were detected on mature strawberries from HFHC (1.0 $\log_{10}$ CFU/g), HFLC (1.1 $\log_{10}$ CFU/g) and LFHC (1.5 $\log_{10}$ CFU/g) at 0 hours, but not at later storage times.

E. coli was detected in soil from all mesocosms at 0 hours (1.1-1.6 $\log_{10}$ CFU/g), except for the control, and was still detectable in the HC, but not the LC mesocosms after 48 hours (1.5 $\log_{10}$ CFU/g). *E. coli* was not detectable in any soil samples at 96 hours. Coliforms were detected in all soil samples, including control, at all time points and the presence of these microorganisms appeared to be unrelated to the experimental treatments.

Further to the above, a multi-country study evaluating the impact of agronomy and environmental factors on pathogen presence in soil, water and fresh produce found that *Salmonella* was 10.9 times more likely to be present in samples taken within one week of a flooding event, and STEC was 7.7 times more likely (Ceuppens *et al.*, 2015). Note that this analysis considered all samples together.

4.2.1 Information from the aftermath of Cyclone Gabrielle

Approximately 280 silt samples were collected, mainly by staff from the Napier City Council, analysed for faecal coliforms and *E. coli* in the aftermath of Cyclone Gabrielle (Dr Emily Frost, Napier City Council, personal communication). These were collected from residential and industrial areas. None of the microbial analyses were on samples from horticultural sites.

Concentrations ranged from not detected to >16,000 MPN/g. Repeated analyses were only available for 23 sites, which were sampled and tested for FIB on 9 March 2023 and again on 16 March 2023.[8] Table 2 gives the *E. coli* results for these 23 sites. It should be noted that in most cases the faecal coliform and *E. coli* results were identical.

Table 2. *Escherichia coli* concentrations in flood silt samples collected on 9 and 16 March 2023

Site code	Site type	Location	*E. coli* concentration (MPN/g)	
			9 March 2023	16 March 2023
A2-1	Residential	Awatoto	9200	1600
A3-1	Residential	Awatoto	9200	920
A6-1	Residential	Awatoto	5400	1600
U2-1	Residential (rural)	Awatoto	5400	2400
U5-1	Residential (rural)	Awatoto	>16,000	5400

[8] Cyclone Gabrielle struck on 13-14 February 2024, so the first sampling was approximately 3 weeks after the cyclone. However, it is unknown at what date the flood waters had fully receded from these sites.

U9-1	Residential (rural)	Awatoto	9200	2400
U13-1	Residential (rural)	Awatoto	16,000	3500
B1-1	Industrial	Awatoto	5400	2400
D1-1	Industrial	Awatoto	5400	3500
D1-2	Industrial	Awatoto	5400	2400
F1-1	Industrial	Awatoto	3500	3500
G1-1	Industrial	Awatoto	16,000	2400
G1-2	Industrial	Awatoto	16,000	2400
G1-3	Industrial	Awatoto	16,000	1600
I1-1	Industrial	Awatoto	5400	2400
J1-1	Industrial	Awatoto	5400	5400
J1-2	Industrial	Awatoto	3500	350
L1-1	Industrial	Awatoto	9200	2400
L1-2	Industrial	Awatoto	16,000	5400
N1-1	Industrial	Awatoto	5400	2400
P1-1	Industrial	Awatoto	1100	3500
P1-2	Industrial	Awatoto	540	2400
Q1-1	Industrial	Awatoto	5400	2400

Except for site P1, the *E. coli* concentration remained unchanged or decreased in the week between sampling events. Decreases in bacterial concentrations ranged between 35 and 90%.

While no analyses for specific pathogens or faecal source markers were carried out on these samples, the proximity of the sampling locations to the Napier wastewater treatment plant (WWTP) suggests that the WWTP may have contributed to the elevated *E. coli* concentrations. It should be noted that industrial sites G1 and L1 were two of the closest sites to the WWTP and had the highest initial *E. coli* concentrations of the industrial sites.

In the complete set of microbiological results, almost all *E. coli* results exceeding 5000 MPN/g (3.7 $\log_{10}$ MPN/g) were from sites within approximately 500 m of the WWTP. However, it should be noted that this was a zone that was intensively sampled in the immediate aftermath of the cyclone. Silt samples with *E. coli* concentrations <100 MPN/g (<2 $\log_{10}$ MPN/g) were mostly from silt repositories that were established during the recovery phase after the cyclone. Sampling at these sites did not begin until early April 2023.

Assessment of silt samples from the Tairāwhiti region taken during 1-18 March 2023 was carried out (Haira *et al.*, 2023). Two of the sites, in Uawa, were described as ‘agricultural’ and mean *E. coli* concentrations of 980 and 640 MPN/g (2.99 and 2.81 log10 MPN/g) were measured. Samples were also analysed for faecal source tracking markers. No human faecal markers were detected and only very low concentrations of ruminant faecal markers. The report noted that, although the *E. coli* results could be indicative of potential faecal contamination, any contamination from human or ruminant sources was not recent, as indicated by the absence or very low numbers of human and ruminant faecal markers.

4.3 PERSISTENCE OF PATHOGENIC MICROORGANISMS AND FAECAL MARKERS IN SOIL

4.3.1 Studies of pathogenic microorganisms in soils

Once faecal-associated pathogenic microorganisms are outside the animal host and into the soil, the conditions are generally unfavourable for their survival. Their survival time depends on many variables. Aside from the characteristics of the microorganisms themselves, and strain variations, survival is affected by factors that help survival (e.g. adequate nutrients and water) and those that are bactericidal (e.g. UV exposure, predation by protozoa). Because enteric pathogens can tolerate acidic conditions, survival is better in low pH soils (Hoagland *et al.*, 2018). Survival is also better in soils with low microbiological diversity but worse in soils with a higher sand content. Because of all these factors, and more (e.g. temperature, humidity, soil management, plant coverage, rhizosphere community) it is difficult to make general rules about how soil properties affect the survival of enteric pathogens (Hoagland *et al.*, 2018).

Studies of the survival of enteric pathogens in soils tend to involve the intentional application of faeces/manure, to inform farm management practices, and there are reviews of these data (Alegbeleye *et al.*, 2018; Ongeng *et al.*, 2015). These are less relevant to considering flood affected soils but provide some general information for worse-case scenarios (e.g. flood sediment containing a high concentration of faecal material, or pooled floodwaters heavily contaminated with slurries or sewage). Some examples of findings from longitudinal field studies are provided below.

Field studies of *Salmonella* survival in soils with growing crops

Outdoor plots containing mature cabbages were spray-inoculated with a solution of *Salmonella* Typhimurium and samples collected for eight days, with irrigation occurring every second day. *Salmonella* was not detected in soil samples taken at day 8. In a second trial, *Salmonella* was applied to the soil between mature kale and collard greens (avoiding the plants) with subsequent irrigation only coming from natural rainfall events. The concentration in soils decreased approximately 4 $\log_{10}$ CFU/g soil over 10 days.

Lee *et al.* (2019)

- *Salmonella* rapidly died during a 10-day period in fresh produce growing soils

Field studies of pathogen survival in horticulture soils with added manures

Salmonella Sofia (and *E. coli*) were inoculated into non-composted poultry litter and this mix incorporated into the topsoil in outdoor raised gardens containing soils typically used for vegetable growing in New South Wales, Australia. In parallel, another plot of soil was treated with non-composted dairy cow manure containing an inoculum of *Listeria innocua* (and *E. coli*). Lettuces were planted one week after soil preparation and testing of soils and lettuces continued for 50 days, under warm conditions (maximum temperatures in the range 20-40°C). Soil testing results showed that the concentration of *Salmonella* decreased over time (average 4.4 days for 1 $\log_{10}$ reduction) but these bacteria were still detectable at 50 days. Similar observations were reported for *Listeria*. *Salmonella* was not detected on any lettuces, and *Listeria* spp. was detected on two immature lettuces from the plots with cow manure but none of the mature lettuces were positive.

Ekman *et al.* (2021)

- *Salmonella* and *Listeria* can persist in soils with faecal matter but do not necessarily contaminate mature crops
- Faster die-off at higher temperatures

Field studies of pathogen survival with added manures

Nicholson *et al.* (2005)

Using arable soil in the UK, the survival of *E. coli* O157, *L. monocytogenes*, *C. jejuni* and *Salmonella* (Enteritidis or Typhimurium) was measured over time when these pathogens were inoculated into animal faeces and the mixes were incorporated into two soils (sandy and clay loam). Different faecal materials were applied in separate plots: manure slurries from beef cattle, dairy cattle and pigs, and solid manures from these animals plus sheep and broiler litter. In a separate set of experiments, the same pathogen/manure mixes were spread on the surface of grassland soils (not incorporated). The starting concentrations of the pathogens were in the range of 2-4 $\log_{10}$ CFU/g soil. The presence of the pathogens in the soils was monitored up to 32 days, with pathogen/soil combinations still positive at 32 days assessed again after 9 months. No pathogens were detected in the 9 month samples. As would be expected the survival times differed between pathogens and treatments. In general, *Campylobacter* survived for shorter periods compared to the other pathogens but were still detectable after one month in some treatments. *E. coli* O157 and *Salmonella* were more likely than *Campylobacter* to still be detectable at one month after treatment, with *Listeria* surviving longer. Survival of all pathogens was greater in the clay loam soil.

- Pathogenic bacteria can survive for a month or more under field conditions.
- Sandy soils are less supportive of pathogen survival compared to clay loam soils.

Factors affecting pathogen survival in soils with manure (field studies)

Hort Innovation (2021)

Outdoor mesocosms were prepared with either a sandy or a clay loam soil, with poultry or cattle manure added, with poultry manure containing 5 $\log_{10}$ CFU/g *E. coli* and 3.4 $\log_{10}$ CFU/g *Salmonella* Sofia, or cattle manure containing 5 $\log_{10}$ CFU/g *E. coli* and 3.4 $\log_{10}$ CFU/g *L. innocua.* Trials were conducted in spring, summer and autumn. Maximum daily temperatures during the first three weeks of each trial were 20-25, 30-40 and 25-35°C, respectively, with stronger solar radiation during the spring and summer trials. *L. innocua* concentrations were not quantified and results were expressed as the proportion of mesocosms positive at each time point. *L. innocua* was not detected in any mesocosm after 6 days under summer conditions but was still detected in a proportion of mesocosms after 50-60 days under spring and autumn conditions. *Salmonella* Sofia was more persistent in the spring trial, with low concentrations detected up to 60 days. In the other two trials, *S.* Sofia was not detected after 15 days, with attenuation most rapid under summer conditions.

- *Listeria* and *Salmonella* survival is shorter under summer conditions compared to spring and autumn.

Factors affecting *Salmonella enterica* survival in soils with manure (laboratory and field studies)

Hort Innovation (2018)

Under controlled conditions, a cocktail of *Salmonella enterica* serovars was inoculated into soils (clay loam or sandy) with or without added chicken manure. The initial soil concentration of *S. enterica* was approximately 6 $\log_{10}$ CFU/g. In sandy soil, the decline in *S. enterica* concentrations appeared to be monophasic, with the organism no longer detectable by 28

- *Salmonella* survival is better in clay loam soils compared to sandy soils.

days. In clay loam soil, the decline was biphasic, with an initial 3 log_{10} CFU/g decrease in the first 28 days followed by an approximately 1 log_{10} CFU/g increase, with *S. enterica* concentrations at approximately 4 log_{10} CFU/g at 50 days. The presence of 2% w/w chicken manure in clay loam soil increased the survival time of *S. enterica*. The rate of decline in *S. enterica* concentrations in clay loam soil was faster with increasing temperature. Decline was monophasic at 37°C, with *S. enterica* undetectable by about 30 days. At 5 or 21°C, the decline was biphasic, with little further decrease in concentrations after 28 days. The addition of 2% chicken manure improved the survival of *S. enterica* at all temperatures. Fluctuating soil moisture reduced the survival of *S. enterica* compared to constant soil moisture.

Similar results were observed in a field study. In sandy soils, *S. enterica* concentrations decreased to low or undetectable levels within a few days and remained at those levels to the end of the trial (98 days). In clay loam soil, *S. enterica* concentrations declined more slowly, with concentrations near the LOD, but still able to be measured at 98 days. The use of cover crops was trialled, on the basis that they would increase microbial diversity and decrease *S. enterica* concentrations. However, none of the cover crops was effective in this respect.

- *Salmonella* survival is better in the presence of faecal matter, cooler temperatures and/or constant soil moisture.
- *Salmonella* can survive in soil for several months.

Persistence of *Salmonella* in horticulture soils in the presence of poultry litter (field studies)

Suslow *et al.* (2013)

Chicken manure pellets and untreated chicken litter were inoculated with *Salmonella* and this mixture incorporated into field plots. The concentration of *Salmonella* in the soil decreased over time, with most of the die-off occurring during the first month. In some trials, these bacteria were still detectable 73 days after soil inoculation. This trend was consistent for control plots and for plots where cover crops were planted and later incorporated into the soil.

- In the presence of poultry litter, *Salmonella* can survive for longer than one month.

Some studies were located that inform on factors that affect *L. monocytogenes* survival.

Persistence of *L. monocytogenes* in different soils (laboratory study)

Locatelli *et al.* (2013)

Using laboratory microcosms, 100 soil samples representing different soils found across France were each adjusted to 80% of the water field capacity and inoculated with *L. monocytogenes* before storage at 20°C. *L. monocytogenes* survived for approximately three months in most (71%) of the soils tested, but relatively shorter periods (up to two weeks) in the other soils. Soil chemistry strongly affected survival in the short term (and was the most important factor overall), with Basic Cation Saturation Ratio (an indicator of the availability of cations such as calcium) explaining most of the variance in survival at days 7 and 14. Soil texture was important for driving longer term survival, with greater survival in fine-grained soils, those with a high proportion of clay. Climate and land use were not found to be statistically significant.

- *L. monocytogenes* can survive for months.
- Soil chemistry affects survival.

Persistence of different *L. monocytogenes* strains in soil (laboratory study)

Sevellec *et al.* (2022)

The survival of 216 *L. monocytogenes* strains, collected over a period of 30 years from animals, foods and environments, was compared in microcosms prepared from soil from one location. Survival was measured over three days at 25°C and, at the end of this period, the population of the majority of strains had reduced by 95%. This signals that some strains of *L. monocytogenes* are well adapted to soil survival, while many are not (noting that these experiments were conducted under controlled conditions). There was no correlation between the origin of the strain and its ability to survive. The best surviving strain was isolated from an animal. Further analyses showed that soil fitness was improved through many small, cumulative genomic variations.

- Not all *L. monocytogenes* strains survive well in soil

4.3.2 Studies of faecal markers in soils

The survival of FIB in soils is generally prolonged under cooler, stable temperatures with low sunlight, higher moisture and good nutrient availability. However, soils and soil communities are complex, and survival (or growth) is affected by the total environment including other factors such as soil type and characteristics (like pH, Luan *et al.* (2023)) and the presence of competing microorganisms or macroflora that feed on microorganisms (Ishii *et al.*, 2010).

Similarly to pathogenic microorganisms, studies to inform survival of FIB under field conditions tend to focus on manure-amended soils. One study was located that measured the survival of one strain of *E. coli* in horticulture soils in the absence of manure (Islam *et al,* 2004; see below). Other examples of longitudinal field studies are included below.

Models to predict the survival of *E. coli* (and other bacteria) in manure-amended soils have been published, but these do not account for dynamic environmental conditions nor changing bacterial concentrations (Pang *et al.*, 2020). Using data generated by Sharma *et al.* (2019; see below), a new model was developed to predict viable *E. coli* concentrations over time in manure-amended soils under different agricultural and environmental conditions (Pang *et al.*, 2020). The model did not predict any statistically significant difference between survival of generic *E. coli* and the pathogenic serotype *E. coli* O157:H7. Sensitivity testing showed that, of the environmental variables, *E. coli* survival was most influenced by the number of days of rain since the previous sampling day and the soil moisture content, with these being positively correlated.

Persistence of pathogenic *E. coli* in fresh produce field soils

Islam *et al.* (2004)

As part of a larger study, outdoor plots growing parsley or lettuces were sprayed with water containing a non-toxigenic strain of *E. coli* O157:H7. From a starting concentration of approximately 3.5 $\log_{10}$ CFU/g, the numbers of *E. coli* O157:H7 in soil samples in both plots initially decreased during the first month, stabilised for 2-3 months, then decreased further during the following 1-2 months to reach concentrations not detected by enrichment.

- *E. coli* slowly die off in horticulture soils without added faecal material but can survive for 5 months

Factors affecting *E. coli* survival in soils with manure (field studies)

Sharma *et al.* (2019)

This publication summarised 324 survival profiles for non-pathogenic *E. coli* (gEc) and attenuated *E. coli* O157 (attO157) in soils that were either natural or amended with poultry, horse or dairy manure. Manures were

- Survival time depends on

applied to the surface of fields and then sprayed with an inoculum of gEc or attO157. Spatiotemporal factors (site, year and season) affected microbial survival to a greater extent than weather variables (temperature, rainfall). Microbial survival time (dpi100mort)[9] was strongly affected by the year of the trial, with survival times in the 2013 year consistently greater than in 2011, 2012 or 2014. Other than in 2013, dpi100mort values were mostly less than 90 days, however, in 2013 dpi100mort values for both test organisms reached 210 days. Initial soil moisture content was the individual factor that accounted for the greatest percentage of variability in survival duration, with lower initial soil moisture content (11.2-12.1%) supporting longer survival times. The authors of this study noted that this finding was consistent with other studies and suggested that the phenomenon may be due to specific desiccation stress responses in the *E. coli* strains.

combinations of factors.

– Soils that were dryer when *E. coli* was inoculated supported longer survival times.

Field studies of pathogen survival in horticulture soils with added manures

Ekman *et al.* (2021)

E. coli was inoculated into either non-composted poultry litter or non-composted dairy cow manure, and each mix incorporated into the topsoil of separate outdoor raised gardens containing soil types typically used for vegetable growing in NSW, Australia. Lettuces were planted one week after soil preparation and testing of soils and lettuces continued for 50 days, under warm conditions (maximum temperatures in the range 20-40°C). The concentration of *E. coli* in cow manure-amended soils decreased over time, initially taking 5-10 days to reduce by 1 $\log_{10}$ CFU/g DW (dry weight) soil. Survival was better during trials when soil moisture was more consistent, and daily temperatures and solar radiation levels were lower. *E. coli* did not persist well in poultry litter-amended soils and these bacteria were intermittently detected in both treated and control soils. When data from all trials were combined, it was predicted that the concentration of *E. coli* would decrease at least 3 log over 50 days. Separately, the time taken for a 1 log reduction ranged from 2.5 to 8.8 days, averaging 5.7 days across three trials and two soil types. *E. coli* was detected on three lettuces, with the highest concentration being a mature lettuce harvested from a control plot (4.6 $\log_{10}$ CFU/g). The authors offered no explanation for this somewhat surprising result.

– *E. coli* can persist in soils not containing added faecal matter but are likely to be present at higher concentrations in soils with faecal matter incorporated.

– Low soil moisture and high air temperatures increase die-off rate.

Survival of *E. coli* in manure-amended soils

Hodgson *et al.* (2016)

E. coli survival in randomised plots was examined after application of dairy slurry by either surface broadcast (BC) or shallow injection (SI) at a test site in Devon, UK. Experiments were repeated in each of three seasonal periods (May, July, October). In control plots (no slurry), *E. coli* concentrations were generally below the LOD (1 $\log_{10}$ CFU/g DW). In all three trials, *E. coli* concentrations declined more quickly in BC plots, with half-lives (the time for the concentration to decrease by 50%) of 6-24 days

– Higher temperatures and UV levels increased the die-off rate.

[9] Dpi100mort was defined as the number of days post-inoculation (dpi) in which the predicted $\log_{10}$ CFU (MPN)/gram dry weight (gdw) value reached 0.11 $\log_{10}$ MPN/gdw, as a threshold of viability.

for BC and 10-34 days for SI. Decreases, by either application method, were most rapid in May (Spring). Mean temperatures (15.1°C, compared to 14.9°C in July and 10.7°C in October) and UV levels (236 wm^2, compared to 130 wm^2 in July and 61 wm^2 in October) were higher during this period. For the SI application, *E. coli* was detected up to 100 days in the July and October trials but was not detected beyond 50 days in the May trial. For the BC application, *E. coli* was not detected after 10 days in the May and July trials and approximately 50 days in the October trial.

– Bacteria survive better in the soil than on the surface.

Survival of *E. coli* and faecal coliforms in manure-amended soils

Park *et al.* (2016)

Literature data were used to develop kinetic models for the thermal inactivation of indicator organisms in manure-amended soils. Survival was considered to be biphasic, with an initial rapid decrease in bacterial concentrations, followed by a markedly slower decrease, although under some conditions only a single phase was apparent. Higher (>15°C) or lower (<8°C) temperatures favoured biphasic kinetics, while intermediate temperatures favoured a single phase. Soil texture (coarse, loam, fine) and soil moisture (wet, intermediate, dry), study type (laboratory, field), manure type (bovine, swine) and manure application state (slurry, solid, liquid) also affected survival kinetics, although patterns were complex. Higher inactivation rates were favoured by coarser soil texture, higher soil moisture, liquid application of manure, surface application of manure (rather than mixing in) and in field studies compared to laboratory studies.

– Soil temperature affected survival.
– There is often an initial rapid decrease in soil concentrations followed by a longer phase of slower die-off.

Factors affecting *E. coli* survival in soils (meta-analysis)

Tran *et al.* (2020)

In a meta-analysis of inactivation curves for *E. coli* and *Salmonella*[10] in manure amended soils from 42 published laboratory or field studies, temperature (air and soil combined) was identified as the main factor influencing survival of both organisms under laboratory conditions, but only *E. coli* under field conditions. The time required for a log reduction of *E. coli* under field conditions was significantly longer at low (<10°C) compared with high (>20°C) temperatures. Survival was also longer when manure was incorporated into soil compared to when manure was surface applied, presumably due to the antibacterial effect of solar irradiation. Both microorganisms survived longer under laboratory conditions compared to field conditions.

– Lower temperatures prolonged *E. coli* survival in soils.
– *E. coli* survival was not significantly affected by manure or soil type, and moisture.

Based on 62 field studies, the estimated mean time for *E. coli* to reduce by 1 log_{10} under medium temperature field conditions (10-20°C) was 13 days (standard deviation (SD) 11 days, maximum 38 days). For *Salmonella*, under the same conditions and based on 23 field studies, the estimated mean period for a 1-log reduction was 16 days (SD 20 days, maximum 75 days). Median and 95% confidence interval (CI) values were also estimated, and the study authors used these to assess *E. coli* and *Salmonella* reductions during exclusion periods of 45, 60, 90, 120 and 265

– On average, *E. coli* populations reduce by 1 log over two weeks but the time for a 1 log reduction can be several months.

[10] The information on *Salmonella* is not presented in the previous section because the authors did not derive strong findings relevant to field conditions. However, it is useful to include the information here for comparison to the findings for *E. coli*.

days. While these estimates were based on a lot of assumptions, including that the decline rates were linear, the results indicated that a 45-day exclusion period was sufficient to reduce environmentally relevant concentrations to negligible levels. At 45 days, *E. coli* was predicted to have decreased by 6 $\log_{10}$ CFU/g soil and *Salmonella* by 8 $\log_{10}$ CFU/g soil.

Factors affecting *E. coli* survival in soils with manure (field studies)

Hort Innovation (2021)

Mesocosms were prepared with either a sandy or a clay loam soil and added poultry or cattle manure seeded with *E. coli*, *Salmonella* and/or *L. innocua*. The poultry manure contained 5 $\log_{10}$ CFU/g *E. coli* and 3.4 $\log_{10}$ CFU/g *Salmonella* Sofia. The cattle manure contained 5 $\log_{10}$ CFU/g *E. coli* and 3.4 $\log_{10}$ CFU/g *L. innocua.* Trials were conducted in spring, summer and autumn. Maximum daily temperatures during the first three weeks of each trial were 20-25, 30-40 and 25-35°C, respectively, with stronger solar radiation during spring and summer. In soils amended with cattle manure, *E. coli* concentrations decreased to close to the LOD (1 $\log_{10}$ CFU/g) within 10 days in the spring and summer trials, but persisted to about 50 days in the autumn trial. In soil amended with poultry litter, *E. coli* concentrations decreased to the LOD within 3 weeks in all three trials, although occasional high numbers were observed at later time points.

- *E. coli* survival is shorter under spring and summer conditions compared to autumn.
- *E. coli* does not survive as well in the presence of poultry litter compared to cattle manure.

Survival of manure-deposited *E. coli* on grassland (field study)

Avery *et al.* (2004)

After groups of healthy, adult cattle, pigs or sheep were penned on grassland for two weeks, *E. coli* from the resulting faecal-contaminated soils was enumerated for 218 days. The starting concentration of *E. coli* was (on average) 5.3, 4.3 and 5.0 $\log_{10}$ CFU/g in samples from the cattle, sheep and pig pens, respectively. The concentrations decreased over time, reaching 0.5 $\log_{10}$ CFU/g or less during a period of approximately 3.5 months. The control soil samples were reported to contain *E. coli* at approximately 0.5 $\log_{10}$ CFU/g. It took approximately one month for the concentration to reduce by 1 log. The soil temperature at 8 cm depth during the study averaged 6.5°C (range 0.4-15.6°C).

- *E. coli* decrease by approx. 1 log per month in faecal-contaminated soil.
- Without faeces, *E. coli* can be present at ~0.5 $\log_{10}$ CFU/g soil.

Survival of *E. coli* in soils treated with urban wastewater sludge

Estrada *et al.* (2004)

The survival of *E. coli* was monitored after treated sludge was incorporated into 300 kg of soil and the containers carrying each mixture were stored outside for 80 days. The sludge had been treated by anaerobic digestion and mechanical hydration. Additional samples had also received a final heat-drying step. Faecal coliforms and *E. coli* were not detected in soils without added sludge, at any point during the trial, and their concentrations remained low in soils treated with the heat-dried sludge. In soils treated with the non-heat-dried sludge, the concentrations of both indicators increased during the first 10 days but had returned to low or undetectable levels by 80 days. Moisture levels were also highest during days 5-10 and

- *E. coli* and faecal coliforms can grow in soils containing sewage sludge
- Growth is encouraged by moisture

it was concluded that moisture and nutrient availability were most important for supporting microbial growth.

Persistence of *E. coli* in agricultural soils treated with sewage sludge

Miguel *et al.* (2020)

Aerobically digested sewage sludge from an urban wastewater treatment plant was applied to two 4 ha areas with different soil types (one clayey, one sandy). After sludge application, the soil was tilled and two days later, corn and sunflower crops were planted. Before sludge application, *E. coli* was detected in the soils at mean values of 3.3 $\log_{10}$ CFU/g DW for the clay soil and 4.7 $\log_{10}$ CFU/g DW for the sandy soil. During the first five weeks after sludge application, the concentration of *E. coli* in the clay soil increased from 3 to 5 $\log_{10}$ CFU/g DW but an increase was not observed in the sandy soil. By 42 weeks (after crop harvest) the *E. coli* concentrations were lower than that measured in the soils prior to sludge application. It should be noted that the water used for irrigating the crops also contributed *E. coli* to the soils.

- Treated sewage sludge contributes *E. coli* to soils.
- *E. coli* persists longer in clayey soils compared with sandy soils.

4.3.3 Studies of the effects of soil remediation activities

Water-saturated soils become oxygen-depleted and are likely to have lost nitrogen and other elements essential for growing healthy crops. Growers must decide if, when and how they will begin the process of returning post-flood agricultural soils to a healthy state. This might involve silt removal or incorporation of the silt into the soil through soil tillage, sowing cover crops (which might then be mulched into the soil) and/or adding soil improvers (fertilisers, composts, probiotic mixes, etc.). The flooded land might be left for some time before any activities are undertaken, particularly if the soil remains saturated.

Only one study was located that evaluated the effect of post-flood soil remediation activities on the presence and concentration of indicator microorganisms, although FIB and pathogenic bacteria were not measured in this study.

Researchers in the US studied the effects of five soil remediation treatments on the concentrations of coliforms and aerobic bacteria in soils after part of a research farm was inundated by a flooded river for 40 days (Monroe *et al.*, 2015). The control plots were left undisturbed, other than when soil samples were collected, but all treatment plots were tilled 15 days after flood waters receded, then again two days later (17 days after flooding). The five treatments were:

1. Cover crop: Oats sown after tilling.
2. Incorporation of collards: Collards sown after tilling, then grown collards incorporated into soil 68 days after sowing.
3. Black plastic mulch laid over soil.
4. Clear plastic mulch laid over soil.
5. Tillage only: Plots were tilled a third time, 58 days after flood waters receded.

One herbicide treatment was applied to all plots 22 days after the flood water receded, to control weeds. Soil samples were taken at 3, 14, 24, 36, 52, 67, 78, and 107 days after the flood waters receded.

At day 3, the concentration of total coliforms was in the range 2.5-5.3 $\log_{10}$ CFU/g soil, and the concentration of total aerobes was in the range 6.6-7.0 $\log_{10}$ CFU/g soil. Over time, the

concentration of total coliforms decreased and the concentration of total aerobic bacteria increased in all plots (including the control plots). Based on total coliforms, there was no significant difference between the five treatments. However, the concentration of total coliforms decreased faster in the control plots compared to the treatment plots. The total aerobic plate count results showed that aerobic bacteria concentrations were higher in treatment plots compared to control plots. Some samples were also tested for yeasts and moulds, which were present at higher concentrations in soils from treatment plots compared to control plots. Overall, the researchers found that the concentrations of these indicator microorganisms were lowest in undisturbed (control) plots. They proposed that waterborne microorganisms are deposited on the soil surface as the flood waters recede, and subsequent dehydration and sunlight causes microbial cell death. Tillage breaks up the flood-compacted soils, incorporating flood-deposited microorganisms into the soil layer and increasing soil aeration, both of which improve microbial survival/growth. They recommended soils be left undisturbed and bare (with the use of herbicides).

While not deployed on previously flooded land, another study investigated soil treatments and produced relevant findings (Suslow *et al.*, 2013). After a mixture of poultry manure, poultry litter and *Salmonella* was incorporated into soils, different plots were either left fallow (controls), seeded with a cover crop that was later tilled into the soil, or covered with clear polyethylene (to initiate solarisation, i.e. a greenhouse effect). In these field trials, the use of cover crops did not significantly affect *Salmonella* survival compared to the control. However, solarisation was significantly more effective at killing *Salmonella* compared to the control. Temperatures at 6 cm under the polyethylene covers exceeded 40°C while the non-covered soils only reached a maximum of 36°C.

5 SOIL SAMPLING AND TESTING

KEY FINDINGS

Are there suitable sampling and testing methods that can be used for assessing the microbial safety of horticulture soils and flood silt?

- No standard or accepted methods were identified for sampling horticultural soils and flood silt, although testing soil/silt rather than using swabs or boot covers appears to be more sensitive.
- The approach should be guided by the purpose of the work.

Are faecal indicators and genetic markers in soil samples useful indicators for safe planting?

The small analytical study of post-cyclone soil samples indicates that:

- An *E. coli* concentration of >1000 MNP/g signals there might be faecal material present, but this was not consistent. Data from WWTP contaminated silt (Section 4.2.1) support that *E. coli* concentrations measuring in the thousands per gram are likely to be contaminated with faecal matter. However, this small survey suggests that *E. coli* concentration alone is not reliable as a sole indicator for the safety of soils for replanting edible crops.
- MST markers can indicate the presence of faecal material in soil, and the source of this material. They do not provide evidence that pathogenic microorganisms are present in soil, nor indicate which of these microorganisms might be present. However, in combination with *E. coli* results and wider knowledge from a landowner about their environment and the impact of the flood event (e.g. identifying any upstream or nearby sources of contamination), they provide useful additional information to support decision-making.
- *L. monocytogenes* might be more common in flood-affected soils although there is no evidence that the presence of these bacteria in soils is related to faecal contamination. Detection of *L. monocytogenes* in horticultural soils may indicate the need for greater vigilance in the processing environment.

5.1 SUITABLE SOIL/SEDIMENT SAMPLING METHODS

If samples of soil/sediment from previously flooded land are to be tested for FIB or other measures to indicate food safety risks then it is important to consider how those samples should be selected. The results from the samples will be used for decision-making so the samples selected need to either be representative of the area of interest or show where contamination might be at its worst. There is also a need to minimise the number of samples required (to minimise cost) while still taking sufficient samples to answer the questions that prompted testing.

5.1.1 Sampling patterns

In New Zealand, soil testing is widely used to manage soil nutrients and sampling along transects is a standard approach (Knowles and Dawson, 2018). A transect might run across the whole area of interest, or if the site is non-homogenous, several transects might be set up within the area of interest to capture this variability. Grid sampling might be used where in-depth knowledge of a particular area is required. This involves dividing an area up into equal sized cells and either

preparing a composite of subsamples from within each cell or collecting a sample at equidistant points based on the grid pattern.

5.1.2 Selection of sampling sites

The selection of sample locations can be based on judgement or probability (Pennock *et al.*, 2006). Judgement sampling relies on someone having good knowledge of the sample region and selecting sites that suit a particular purpose. Judgement sampling would be useful if the intent is to sample the worst flood affected areas, or locations nearest possible contamination sources (e.g. closest to animal rearing operations or stream/river banks). Statistics such as mean values can be calculated from the test results generated through judgement sampling but the samples are not representative of the whole sample region.

Probability sampling relies on methods that maximise randomness. Random sampling is rarely practical for field studies so transects or grids are usually used (as introduced above), and zig-zag lines might also be set up (Pennock *et al.*, 2006). The location of the transect should ideally be random but usually transects or grids are set up in a way that most effectively answers a research question.

5.1.3 Sample collection

The flooding of livestock facilities, sewage treatment plants, septic tanks, as well as overland discharges from sewers, can be a significant source of pathogenic microbes. Concerns over pathogens decrease upon drying of the soil/sediment deposits, due to the impact of desiccation and solar irradiation on pathogen survival (Presley *et al.*, 2006; Provin *et al.*, 2008). The microbial biomass of a normal soil is highest in the upper 0-10 cm layer and around the roots of plants (Unger *et al.*, 2009). The following paragraphs highlight the diversity of sampling methods used or, in some cases, the lack of information provided on how sampling was carried out, in studies of the microbiology of flood debris/silt.

In a study in the USA, researchers investigated correlations between the type of land cover and environmental measures such as air temperature and soil moisture (Dusek *et al.*, 2018). Based on their findings, these researchers recommended collecting 10–20 soil samples from croplands to maximise the chance of detecting *E. coli* in a single sample. They also recommended collecting these samples from sites where the soil surface was moist or saturated with water. To generate these findings their soil sampling methods at each site involved collecting 10 surface (0–4 inches in depth) samples, each of approximately 300 g. Each sample was collected using a sterile, disposable plastic scoop and stored in a sterile bag. The locations of individual soil samples were pseudo-randomly selected at the site (i.e. a transect was not used).

In a longitudinal study of pathogens in fresh produce growing soils in New York State, each field was sampled through preparing a composite soil sample consisting of five subsamples of topsoil from five locations in each field, and a drag swab sample (Strawn *et al.*, 2013a). For the latter, a premoistened drag swab was dragged around the field perimeter and diagonally back and forth for ≥10 min, covering an average field area of 0.75 ha. The prevalence values for *L. monocytogenes*, *Salmonella* and STEC were similar between the drag swabs and composite soil samples. However, in a follow-up study the soil samples yielded higher microbiological prevalence values compared to the drag swabs, e.g. *Salmonella* prevalence of 5% vs. 2% for soil and swab samples, respectively (Strawn *et al.*, 2013b).

To monitor the spatial and temporal differences in *E. coli* and *Salmonella* in produce fields after flooding, one group of researchers deployed both drag swabs and core samples at each sample site (Bergholz *et al.*, 2016). A sample site was a 30 m by 30 m square (“quadrangle”). These squares were positioned at 76 m intervals along multiple transects. A drag swab sample was

collected by taking a sterile swab pre-moistened with laboratory media, attaching a sterile string, dragging the swab for five passes through the square and then around the perimeter of the square, then replacing the swab back into its bag. Core samples were collected to 4 cm deep, from one point in the square. The corer was not sanitised between use, instead “To minimize cross-contamination, the soil probe was conditioned to each quadrangle by first extruding and discarding five soil cores prior to collection of soil for analysis.” The *E. coli* prevalence values from the core samples were higher than those from the drag swabs. Thus, although the drag swabs covered a wider area at each site, the core samples were more sensitive.

In another post-flood study of crop growing areas, researchers selected sampling sites in each field by following a zig-zag pattern starting from one of the field’s sides (Castro-Ibáñez *et al.*, 2015). At each sampling site, the top 5 cm of soil was collected from an area of 20 cm diameter, using a spade.

In another post-flood study, the researchers collected both composite soil samples, collected from up to five locational transects representing 10 subsamples each across identified field(s), and single grab samples, collected in a grid-like pattern at pre-set locations across each study area (Rock *et al.*, 2023). Approximately 500 g of soil samples were collected at a depth of up to 15 cm using disposable sterile scoops and deposited in individual sterile Whirl-Pak bags. Soil samples were stored on ice at 4°C for up to 36 hours until transport to the laboratory. No differences between the two methods were observed upon testing for microbiological measures.

A study of soil sampling methods in non-flooded lettuce growing fields compared results from soil grab samples with those from soiled boot covers (Wu *et al.*, 2023). The boot covers were worn on the feet of a person who walked through the area of interest on a pre-set path. Boot covers have been used for environmental sampling of animal rearing operations. The results showed that soil samples collected on the boot covers were comparable to grab samples in terms of enumerated indicators (aerobic plate count, total coliforms) and the phyla and genera present in the samples.

In a New Zealand study on flooded-affected horticultural land (Graham Fletcher, Plant and Food Research, personal communication) composite samples were created by collecting five small samples from a 1 m x 1 m plot. A diagonal line was taken across the longest axis of the field from one corner to another (adapting to the field boundaries as needed). Samples were taken at the start and end of this line, then at three points of equal distance along the transect. All samples were collected using sterile equipment. The samples were packed with ice packs and sent to the laboratory within 24 hours of collection.

5.1.4 Summary

It is clear from the previous sections that there is no ‘standard’ approach to the sampling of flood deposited silt and sediment. However, the literature highlights certain issues that should be considered:

Selection of sampling sites. Available information on potential sources of contamination within the affected zone should be reviewed. The selection of sampling sites should also consider the direction of floodwater flow from potential sources of contamination to potential locations of deposition. Unless existing information is available on baseline or background levels of contamination, if possible control sites should also be identified that have not been affected by flooding and silt deposition.

Sampling patterns. There does not appear to be any preference in previous studies for particular sampling patterns. However, those responsible for the design of a sampling plan should be aware of the various options (grid, transect, zigzag, etc.) and consider which is most appropriate.

Sample type. Samples may be single soil/silt samples or composites of sub-samples from a particular location. While composite samples are a good means of ensuring that the sample taken is representative of a particular location, the turbulence of flood water and the substantial mixing that will occur suggest that the composition of the samples should be reasonably uniform at a particular location, depending on the depth of sample taken. Swabs and boot covers are possible alternatives to taking soil samples, but these only test the silt/soil surface and the studies above show that they are not as sensitive as soil samples.

Sampling technique. The site might contain distinct layers of silt and soil, or not. If the purpose of the sampling is to determine whether the silt is contaminated, then the sampling technique applied should be such that it can discriminate between the deposited silt and the underlying soil. Sampling equipment should either be sterile (possibly disposable), with one set used at each distinct site, or consideration should be given to sanitising or conditioning of sampling equipment between sampling locations, to ensure that there is no cross-contamination between samples from different sites.

Sample containers should be appropriate for the intended analytes and discussion with the intended analytical laboratory should be undertaken prior to sampling. The time between sampling and delivery of samples to the analytical laboratory should be as short as practically possible. Chiller packs should be used to prevent microbiological growth and to slow microbiological metabolism.

5.2 RESULTS FROM SOIL SAMPLING ANALYSES

Table 3 summarises the results for 20 soil samples taken from previously flooded sites after the flood waters had subsided. Of the 20 samples selected for analysis, 14 had previously been tested for *E. coli* concentrations.

5.2.1 Microbial Source Tracking (MST)

Based on the generic faecal marker GenBac3, 14/20 (70%) of the samples contained some faecal material. GenBac3 is an indicator used to screen samples for faecal contamination (fresh and aged), although GenBac3 can occasionally be present from non-faecal sources. Of the 14 GenBac3-positive samples, six contained markers of ruminant faecal contamination but none contained markers of human faecal contamination.

For the remaining eight GenBac3-positive samples, either the concentration of ruminant or human faecal markers were below the detection limit for the assays, or the faecal material was from another source (e.g. other non-ruminant species such as wild birds, poultry, pigs and carnivorous pets). These eight samples were subsequently screened for avian faecal markers but were negative.

5.2.2 Pathogen-specific PCR

Six samples that were positive for the ruminant faecal markers were further tested for *Salmonella* spp., *Campylobacter* spp., *Yersinia* spp. and STEC. These bacterial pathogens were not detected.

5.2.3 *Listeria* spp. and *L. monocytogenes*

Listeria spp. was detected in 7/20 soil samples and *L. monocytogenes* was detected in 4/20 (20%) soil samples. Full details of the methods used are included in Appendix A.2. This is higher than generally observed from studies of ‘normal’ horticulture soils (10% or less). All of the positive samples in this current survey were identified through PCR analysis of soil enrichments. There were 11 presumptive *L. monocytogenes* isolates identified through post-enrichment isolation steps.

The confirmation test showed that two of these isolates were *Listeria* spp. but none were *L. monocytogenes*.

Two of the four sites which were PCR-positive for *L. monocytogenes* were resampled approximately one year after the cyclone. These were sites from which samples 14 and 16 were taken. *Listeria* spp. was not detected in these soil samples.

5.2.4 Combined results

The laboratory phase of this current work was not designed to provide statistically robust evidence for relationships between *E. coli* soil concentrations and the presence of faeces or pathogenic bacteria. However, it was expected that the results would indicate possible relationships or trends and inform on suitable test methods. Figure 3 depicts the results from all analyses. The most notable observation from this figure is the lack of strong trends or relationships, but there are some useful observations:

- The generic faecal marker (GenBac3) was detected in all but six of the samples. Where known, the concentrations of *E. coli* in the GenBac3-negative samples were all <400 MPN/g (<2.6 $\log_{10}$ MPN/g) but there were also GenBac3-positive samples with similar *E. coli* concentrations. However, soil with *E. coli* concentrations greater than 500 MPN/g (2.7 $\log_{10}$ MPN/g) were all GenBac3-positive. Data from WWTP contaminated silt (Section 4.2.1) support that *E. coli* concentrations measuring in the thousands are likely to be contaminated with faecal matter. Three of the four samples with *E. coli* concentrations >1000 MPN/g (3 $\log_{10}$ MPN/g) contained ruminant faecal markers. The cause of the higher *E. coli* concentration and presence of GenBac3 in sample 2 was not identified.
- The ruminant faecal marker was detected in samples with higher *E. coli* concentrations (>500 MPN/g) but the number of positive samples were few and the relationship might not be consistent. Sample 18 was not tested for *E. coli*.
- Despite the higher concentrations of *E. coli* and the presence of ruminant faecal markers in samples 4, 9 and 12, faecal-associated pathogenic bacteria were not detected. Other microbial pathogens (including viruses and parasites) could have been present.
- Landwise reported the presence of birds on flooded lands. While their faeces can be a source of post-flood contamination, no avian faecal markers were detected in samples that were positive for the generic faecal marker, but negative for the ruminant faecal marker.
- There does not appear to be a relationship between the presence of *Listeria* spp. or *L. monocytogenes* with the other markers. However, this was not unexpected since *Listeria* spp. are natural environmental inhabitants. The prevalence of *L. monocytogenes* (20%) is higher than reported in studies of 'normal' horticulture soils (10% or less, Section 4.1.3). This suggests that *L. monocytogenes* might be more common in flood-affected soils, perhaps due to decomposing vegetable matter. Two repeat samples from sites positive for *L. monocytogenes* (samples 14 and 16) were tested for *Listeria* spp. one year after the first samples were taken; *Listeria* spp. were not detected.

Noting the limitations of this work, the results suggests that *E. coli* concentration can be a reasonable indicator for the presence of faecal contamination. However, it is difficult to define a microbial limit on which to base this conclusion. A hypothetical limit derived from this work, but requiring further studies to confirm, is: Soils with *E. coli* at a concentration of >2.7 $\log_{10}$ MPN/g might contain faecal matter, but soils containing *E. coli* at >3 $\log_{10}$ MPN/g are likely to contain faecal material. However, d(Cook *et al.*, 2023, Section 4.2.3) detected *Salmonella* in soils with lower *E. coli* concentrations (mean of approximately 34 CFU/g or 1.5 $\log_{10}$ CFU/g). Thus *E. coli* alone is not reliable as a sole indicator for the safety of soils for replanting edible crops.

MST markers can indicate the presence of faecal material in soil, and the source of this material. They do not provide evidence that pathogenic microorganisms are present in soil, nor indicate which of these microorganisms might be present. However, in combination with *E. coli* results and wider knowledge from a landowner about their environment and the impact of the flood event (e.g. identifying any upstream or nearby sources of contamination), they provide useful additional information to support decision-making.

Based on PCR testing, *L. monocytogenes* can be detected in horticultural soils. The absence of *Listeria* spp. at two retested sites suggests the situation may be dynamic, with *L. monocytogenes* establishing temporary, rather than permanent populations. *L. monocytogenes* was not cultured from any of the seven samples that were PCR-positive. Although obtaining isolates can be difficult, this level of failure is unusual (Graham Fletcher, personal communication) and may indicate that the detected DNA was from dead or viable but non-culturable (VBNC) cells. Otherwise it may just be that other microflora outcompeted *Listeria* cells in the culture method, preventing the latter from being able to be isolated.

As a final note, with the above methods being dependant on PCR-based approaches, potential exists for false negatives due to PCR inhibition. Clays and humic substances in soils and sediments are known to interfere with PCR reactions. To reduce this effect, samples can be diluted and/or an internal recovery standard can be added (to measure inhibition) (Bott *et al.*, 2023).

Table 3. Summary of soil sample test results

Sample number[1]	Region	Sediment depth[2] (cm)	*E. coli* (MPN/g)[3]	Presence/absence of faecal markers[4]				Presence/ absence of pathogens[5]	*Listeria* presence/absence[4]	
				Generic	Human	Ruminant	Avian		*Listeria* spp.	*L. monocytogenes*
1	Hawke's Bay	<5	350	Not detected	Not tested	Not tested	Not tested	Not tested	Not detected	Not detected
2	Hawke's Bay	5-20	1600	Detected	Not detected	Not detected	Not detected	Not tested	Not detected	Not detected
3	Hawke's Bay	>20	350	Not detected	Not tested	Not tested	Not tested	Not tested	Not detected	Not detected
4	Hawke's Bay	20	>1600	Detected	Not detected	Detected	Not tested	Not detected	Not detected	Not detected
5	Hawke's Bay	>20	920	Detected	Not detected	Detected	Not tested	Not detected	Detected	Not detected
6	Hawke's Bay	5-20	240	Detected	Not detected	Not detected	Not detected	Not tested	Not detected	Not detected
7	Hawke's Bay	5-20	540	Detected	Not detected	Detected	Not tested	Not detected	Detected	Not detected
8	Hawke's Bay	5-20	70	Not detected	Not tested	Not tested	Not tested	Not tested	Not detected	Not detected
9	Hawke's Bay	>20	1600	Detected	Not detected	Detected	Not tested	Not detected	Detected	Not detected
10	Hawke's Bay	5-20	350	Detected	Not detected	Not detected	Not detected	Not tested	Not detected	Not detected
11	Hawke's Bay	<5	<180	Not detected	Not tested	Not tested	Not tested	Not tested	Detected	Detected
12	Hawke's Bay	>20	1700	Detected	Not detected	Detected	Not tested	Not detected	Not detected	Not detected
13	Hawke's Bay	>20	22	Not detected	Not tested	Not tested	Not tested	Not tested	Detected	Detected
14[(6)]	Hawke's Bay	>20	110	Detected	Not detected	Not detected	Not detected	Not tested	Detected	Detected
15	Hawke's Bay	>20	Not tested	Detected	Not detected	Not detected	Not detected	Not tested	Not detected	Not detected
16[(6)]	Hawke's Bay	<5	Not tested	Detected	Not detected	Not detected	Not detected	Not tested	Detected	Detected
17	Wairoa	>20	Not tested	Detected	Not detected	Not detected	Not detected	Not tested	Not detected	Not detected

Sample number[1]	Region	Sediment depth[2] (cm)	*E. coli* (MPN/g)[3]	Presence/absence of faecal markers[4]				Presence/ absence of pathogens[5]	*Listeria* presence/absence[4]	
				Generic	Human	Ruminant	Avian		*Listeria* spp.	*L. monocytogenes*
18	Gisborne/ Tairāwhiti	5-20	Not tested	Detected	Not detected	Detected	Not tested	Not detected	Not detected	Not detected
19	Gisborne/ Tairāwhiti	5-20	Not tested	Not detected	Not tested	Not tested	Not tested	Not tested	Not detected	Not detected
20	Gisborne/ Tairāwhiti	>20	Not tested	Detected	Not detected	Not detected	Not detected	Not tested	Not detected	Not detected
Number of positive samples/number tested:				**14/20**	**0/14**	**6/14**	**0/8**	**0/6**	**7/20**	**4/20**

Notes:

[1] All samples collected March–May 2023.

[2] The depth of the sediment along the transect. The samples were collected using a core sampler or grab samples where the sediment was too wet.

[3] Analysis conducted on freshly collected composite samples.

[4] Analysis conducted on frozen samples from single point along the site transect. The same sample was tested for faecal markers and *Listeria* spp.

[5] Sample tested by PCR for *Salmonella* spp., *Campylobacter* spp., *Yersinia* spp. and STEC. Only samples positive for the ruminant faecal marker were tested for these bacteria.

[6] Approximately one year after these samples were taken, new samples were collected from the same site and tested for *Listeria* spp., which was not detected.

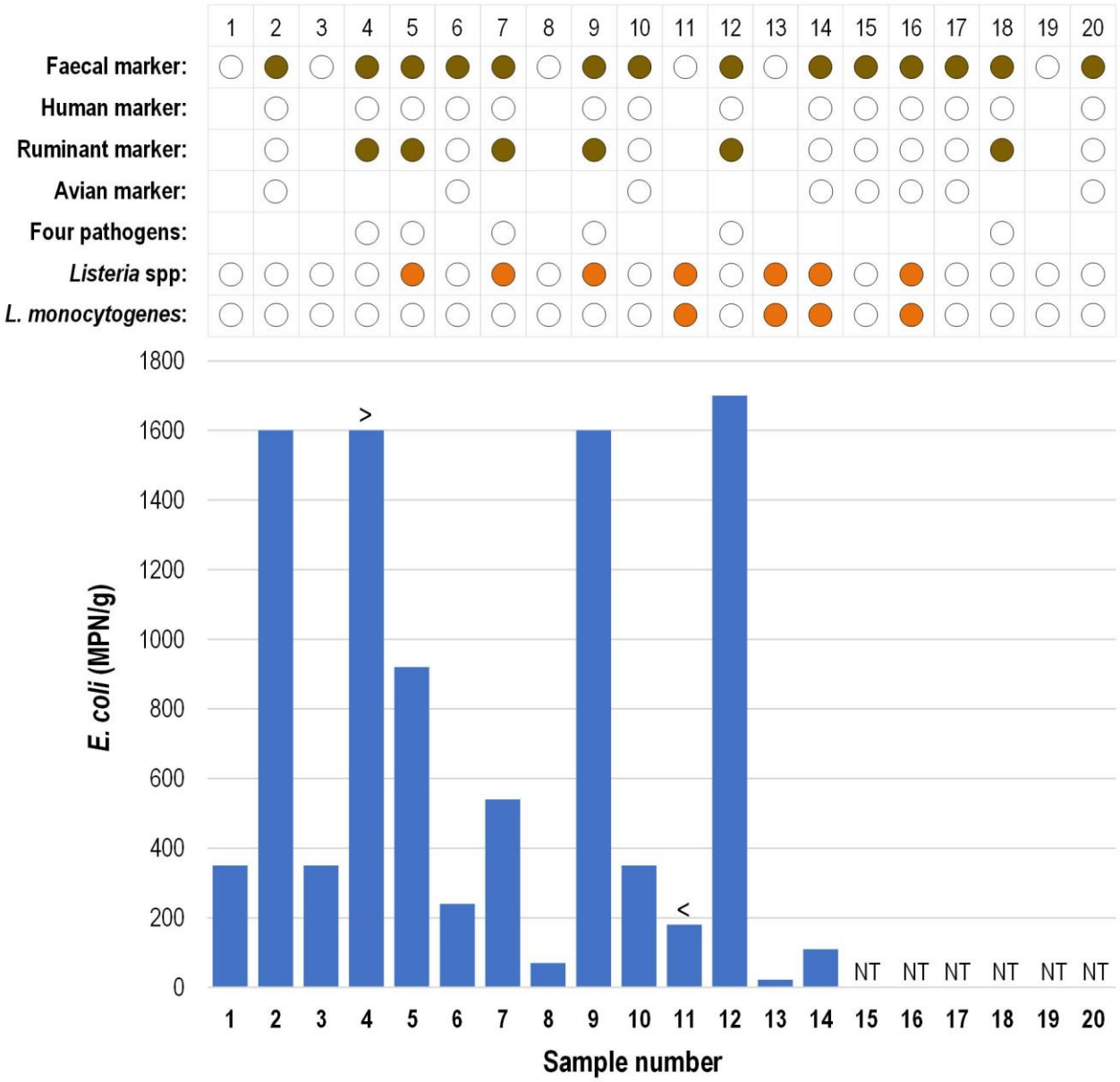

Figure 3. Summary of soil sample test results

Notes: Circles indicate which samples were tested for the faecal markers, pathogens (*Salmonella* spp., *Campylobacter* spp., *Yersinia* spp. and STEC), *Listeria* spp. or *L. monocytogenes*. Open circles show samples where these targets were not detected, brown and orange dots indicate that the target was detected (see Table 3). Some samples were not tested (NT) for *E. coli*, and some *E. coli* results were reported as being greater than (>) or less than (<) the value shown.

6 CONCLUSIONS AND DATA GAPS

KEY FINDINGS

- *E. coli* is an appropriate indicator of faecal contamination of soils but is not sufficient, on its own, for food safety decision making. MST or equivalent technology provides information on the presence and source of faecal material, including identifying if human faeces are present (which may represent a higher food safety risk due to the presence of species-specific enteric viruses and parasites).
- A 60-day period between floodwater receding and replanting horticultural land is consistent with available evidence on pathogen survival, and overseas recommendations for horticulture.
- Reduction of the 60-day replanting period may be possible if appropriate testing is conducted.
- Detection of *L. monocytogenes* in horticultural soils should be viewed as a prompt for increased vigilance in the post-harvest environment but the absence of this organism is not an appropriate criterion for shortening replanting periods. It is not recommended to use *Listeria* spp. in soils as an indicator for the presence of *L. monocytogenes*.
- While some soil remediation activities might increase the rate of pathogen inactivation, remediation efforts are probably best focussed on soil health.

Flood events, such as those associated with Cyclone Gabrielle, have the potential to cause relocation of microbial pathogens. Food safety concerns arise when microbes, particularly those of human or ruminant faecal origin, are relocated to horticultural land. The saturation of soils may also provide conditions suitable for growth of aerobic or anaerobic microbes already present in soil. However, it should be noted that the huge volume of water passing across land also has the potential to remove microbes. For example, peak flow estimates from sites across the Hawke's Bay show high volumes of flowing flood water. One of the highest peak flows measured, by the Ngaruroro River gauge at Fernhill, was 5,398 m^3/s (Lane, 2024).

6.1 EVIDENCE-BACKED ADVICE

In the aftermath of Cyclone Gabrielle the primary measure taken to ascertain the microbiological status of silts and soil was the concentration of generic *E. coli*. These organisms have been shown to be a natural inhabitant of some soils, with concentrations usually in the range 0-100 CFU/g and occasionally more than 1000 CFU/g. Field studies conducted as part of the current study indicated that soil *E. coli* concentrations greater than 500 CFU/g were nearly always associated with a positive result for the general marker of faecal contamination and often associated with the marker of ruminant faecal contamination. However, the reverse was not always observed since faecal markers were not always associated with elevated *E. coli* concentrations. It seems reasonable to view a soil *E. coli* concentration of greater than 500 CFU/g as indicative of faecal contamination, with concentrations of 1000 CFU/g or higher as being a stronger indicator. However, concentrations below 500 CFU/g cannot be taken as definitive evidence of the absence of any microbial hazards. For example, the work of Ceuppens *et al.* (2015) showed that *Salmonella* was present in horticulture soils containing 100 CFU/g *E. coli*.

Longer time, higher temperature, low water activity and solar irradiation have all been shown to contribute to the decline of pathogen and FIB concentrations in soils. The degree of soil saturation and the associated oxygen status of the soil will also have an impact on the microbial composition of the soil. In this context, the 60-day period between floodwater receding and replanting proposing by the California Leafy Green Products Handler Marketing Agreement (California LGMA (2021), see Appendix B) appears to be consistent with the available evidence, noting the proviso that the land must have dried out during that period. The Fresh Produce Safety Centre Australia & New Zealand reiterates the 60 day standdown period (FPSC (2023b), see also Appendix B).

STEC and *Salmonella* are the pathogens that have occasionally been shown to be present following flooding events. However, this knowledge is driven by methodologies designed to test for these microorganisms. Other zoonotic pathogens, such as *Campylobacter* and *Yersinia*, have not been reported although these are not usually targeted in test methods. The California LGMA guidance allows for a shortening of the post-flood replant period from 60 to 30 days if testing demonstrates the absence of STEC and *Salmonella* and a faecal coliform concentration of less than 100 CFU/g (Appendix B.6). While we consider that *E. coli* would be a more appropriate FIB than faecal coliforms, and that a higher microbiological limit might be adopted, these criteria are otherwise consistent with the available evidence. The Fresh Produce Safety Centre Australia & New Zealand recommends various exclusion periods before harvesting after soils have been subjected to untreated animal manures, with the minimum being 45 days under certain conditions, including persistently warm daily temperatures (Appendix B.3). This advice becomes more relevant if post-flood soils are grossly contaminated with animal faecal material, e.g. overflows from dairy effluent ponds.

The available literature does not provide any information on viral contamination of soils following flooding. Given the high degree of species specificity of pathogenic viruses, the absence of human faecal material in flood debris would indicate a low level of viral risk. On this basis, we consider that a further criterion for a reduction in the replanting period should be demonstration of the absence of human faecal material by MST or equivalent technology.

The available evidence does not suggest that the presence of *L. monocytogenes* in horticultural soils will necessarily be related to flooding events, although flooding may assist in creating suitable ecological niches for this organism, such as, through the deposition of organic material. Detection of *L. monocytogenes* in horticultural soils should be viewed as a prompt for increased vigilance in the post-harvest environment but the absence of this organism is not an appropriate criterion for shortening replanting periods. The data collated in Section 4.1.3 show that *Listeria* spp. are commonly detected in soils at concentrations of 1,000 CFU or MPN per gram of soil. In comparison, *L. monocytogenes* is difficult to find in soil samples due comparatively lower prevalence values (~10%) and presumably lower concentrations (as indicated by the need to use enrichment methods to detect this species). Based on these data, it is not recommended to use *Listeria* spp. in soils as an indicator for the presence of *L. monocytogenes*.

It is currently unclear whether horticultural practices can be used to increase the rate of microbial inactivation. Tillage breaks up the flood-compacted soils, incorporating flood-deposited microorganisms into the soil layer and increasing soil aeration, both of which improve microbial survival/growth (Monroe *et al.*, 2015). Monroe *et al.* (2015) recommended that soils be left undisturbed and bare (with the use of herbicides) following flooding events. However, soil remediation to a productive form will be a priority, including aeration of the soil to stimulate growth of microbes/fungi of importance to plant health, to prepare the soil adequately for supporting crops again. The evidence for sufficient benefits from soil remaining undisturbed is lacking and soil remediation should focus on returning the soil to a productive status.

6.2 DATA GAPS

With the exception of the North Carolinian study of Casteel *et al.* (2006), studies on the microbial status of flood-affected soils generally lack a baseline to compare analytical results against. The lack of knowledge of the 'normal' concentrations of FIBs and human pathogens of New Zealand horticultural soils is a major data gap.

Testing for pathogenic microorganisms is expensive and can be difficult for soils. Studies using alternative methods that measure microbial communities (e.g. metagenomics), where the focus is on identifying a suite of pathogens in soils, would yield useful information if conducted alongside tests for FIB.

As highlighted above, it is unknown whether measures to improve soil health following flooding events improves or exacerbates the situation with respect to microbial hazards.

6.3 POTENTIAL RESEARCH PROJECTS

A research project to provide information on microbial characteristics of New Zealand horticultural soils would address the most urgent data gap. From an analytical perspective, such a project should include at least FIBs (probably *E. coli*), faecal source markers and *Listeria monocytogenes.*

Additional data on post-flood soils under New Zealand conditions would provide robust evidence to support (or change) the conclusions above. A project that focuses on being prepared for rapid deployment after the next flood event would enable this to happen (e.g. ensuring preparedness of equipment, methodology and those who would be involved in field work and analyses).

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APPENDIX A: METHOD DETAILS

A.1 LITERATURE REVIEW

Relevant material was compiled from the scientific and grey literature, identified through structured Boolean searches in Web of Science, PubMed and the internet. This was a comprehensive review whereby a range of search terms were used on an as-needed basis and all publications were considered, rather than a systematic review imposing strict search strings and accept/reject criteria.

Information on the following topics were sought (references to Q1-Q6 show the relationship between this material and the six priority questions in Figure 1):

- Recommended soil sampling methods for pathogenic microorganisms to identify a suitable method to for use in future sampling events (sampling procedures, sample spacing, depth, etc.). **Q1**
- Sampling and testing of flood-affected soils for pathogenic microorganisms, FIB, MST markers. Preference was given to longitudinal studies of post-flood soils and flood-deposited sediments that measure microbiological survival over time. **Q1–Q4, Q6**
- Any available guidance documents, community studies (e.g. metagenomic profiles) or specific surveys (measuring FIB, MST markers or pathogenic microorganisms) that describe the microbiology of horticulture soils, particularly those that indicate what a ‘healthy’ or ‘normal’ soil profile should look like. **Q5**
- The impact of flooding events on the pathogen status of soils (before vs. after), particularly seeking information on pathogenic microorganisms that have been observed to increase in soils due to flooding events. **Q5**
- Examples of mitigation or remediation measures applied to flood-impacted land to decrease the contaminant content of soils. **Q6**
- The persistence of pathogenic microorganisms, FIB and MST markers in soils and the factors influencing their persistence/survival (noting that much of the available information is derived through the intentional application of biosolids (e.g. human biosolids, animal manure, compost) to land, i.e. worse-case scenarios). This part of the review was restricted to longitudinal field studies measuring both pathogenic microorganisms and FIB and/or MST markers (laboratory studies or studies using sterilised soils were excluded), plus field studies measuring *Listeria* in horticulture soils. **Q1–Q4, Q6**

All references were managed through the citation software, Endnote.

A.2 SOIL SAMPLE ANALYSIS

Sample selection: Post-flood

In the months following the 2023 floods, as flood waters receded and sites became accessible, Landwise collected 155 samples from 116 sites across Hawke’s Bay, Gisborne/Tairāwhiti and Northland. The majority of these samples were from horticulture areas including field cropping (fresh vegetables, process vegetables or arable cropping) and orchards. Sites were affected differently by flooding although they could be grouped into three main impact types: (i) soil eroded and stripped leaving subsoils exposed, (ii) soil impacted by sediment, and (iii) areas inundated with water for an extended period. Where possible, a 50 m transect was established across a site and three individual samples were taken along that transect (0, 25 and 50 m). The samples were taken

at different depths and captured both flood sediments (silt) and soil. All samples were stored in a frozen state.

For 14 Hawke's Bay sites, Landwise additionally prepared a composite sample from all transect points. These composite samples were subjected to a range of laboratory analyses that included *E. coli* concentrations, then were discarded. Due to the availability of *E. coli* results, these 14 sites were also selected for inclusion in this current research. Using their frozen sample stocks, during January 2024 Landwise selected one transect sample from each of the 14 sites.

Landwise selected an additional six samples based on the possibility of these being contaminated (e.g. the floodwater flowed across animal farming areas prior to reaching horticultural land), but also considering sites outside the Hawke's Bay and a history of good engagement with the landowners.

Sample selection: Re-sampling from higher risk sites

As shown in Figure 1, the presence of markers for human faecal material, the DNA from *Salmonella, Campylobacter*, STEC and/or *Yersinia*, and/or the presence of *L. monocytogenes* (live cells) were all considered as indicators of a 'risky' soil. It has been months since the original samples were taken and growers are likely to have modified the soil since, and possibly also grown crops. From the initial 20 sites, two 'risky' sites were resampled, with assistance from Landwise (Figure 1). The sites were two of the four that had tested positive for *L. monocytogenes* by PCR. Samples were collected approximately 12-14 months after Cyclone Gabrielle.

Sampling

The post-flood samples were taken preferentially using a core but in some cases a scoop was necessary where the substrate was still too wet. All samples were taken by samplers wearing gloves, and each transect sample was immediately placed in an individual plastic bag, then chilled until it could be placed into frozen storage.

Sample handling

Landwise (Hastings) sent all unopened, post-flood samples in a frozen state by overnight courier to PFR (Auckland), where they continued to be stored frozen (-18°C) until testing could commence. At PFR, each sample was defrosted and homogenised before a subsample was aseptically taken for *L. monocytogenes* testing. All defrosted samples were resealed, kept chilled and sent on the same day by overnight courier to ESR (Christchurch). Upon receipt at ESR, subsamples were aseptically taken for the MST and pathogen tests. Any remaining samples were resealed, refrozen and sent back to Landwise.

Testing for *L. monocytogenes*

A culture-dependent polymerase chain reaction (PCR) method was selected as preferable to direct PCR assays on soil extracts due to the risk of PCR inhibition from soil components.

Listeria enrichment: For each soil sample, 270 mL of buffered *Listeria* enrichment broth (BLEB) (Acumedia, Fort Richard, Auckland, New Zealand) was added to a 30 g portion of the soil in a sterile Ziploc bag. Soil samples were then mixed thoroughly by hand massaging and then incubated for 48 h at 30°C (first enrichment). At the end of this enrichment step, an aliquot of 1 ml from each sample was diluted 100 times by adding it to 99 mL of BLEB and further incubated for 48 h at 30°C (second enrichment).

Presumptive *L. monocytogenes* isolation: Upon completion of the first and second enrichments, samples were streaked onto CHROMagar *Listeria* (CHROMagar™, Paris 75006, France) and incubated at 37°C for 48 h. This agar shows presumptive *L. monocytogenes* colonies as blue-coloured colonies with a halo. Any presumptive *L. monocytogenes* colonies identified on the

CHROMagar plates were purified by re-streaking each onto individual CHROMagar *Listeria* plates. Each pure colony was then inoculated into tryptic soy broth with yeast (BD, Becton, Dickinson & Company, Sparks, MD 21152, USA) and incubated at 37°C for 48 h to obtain liquid cultures of pure, presumptive *L. monocytogenes* isolates.

PCR analysis: The first and second enrichments from all soil samples, plus a total of 11 presumptive *L. monocytogenes* isolates from eight soil samples, were taken forward for PCR analysis.

Bacterial genomic DNA extractions were obtained using the QIAamp DNA mini kit (Qiagen, Auckland, New Zealand). A real-time PCR method (USFDA, 2018) was used to identify *Listeria* spp. and *L. monocytogenes*. The PCR analysis was performed using a LightCycler 480 instrument (Roche Diagnostics, Mannheim, Germany) with a reaction volume of 10 µL, consisting of 5 µL of LightCycler 480 SYBR Green I Master mix, 1.5 µL of each primer (0.5 µM) and 2 µL of DNA template (sample or positive control) or water (negative control).

Testing for MST markers and microbial pathogen DNA

DNA was extracted from the silt samples using a method based on that reported by Gallard-Gongora *et al.* (2022) as follows. Slurries were created by adding 30 g of sand to 270 mL of phosphate-buffered solution, pH 7.0, in sterile bottles, shaking by hand for 3 min and allowing the sand to settle for 1 min (Anderson *et al.*, 2005). Slurry supernatant was filtered (25–100 mL) to refusal using mixed cellulose ester filter, 47 mm diameter 0.45 µm pore size (Millipore). Slurry filtrates were subjected to DNA extraction with a PowerWaterTM DNA extraction kit (Qiagen).

All 20 samples were tested for the faecal marker GenBac3 using a quantitative polymerase chain reaction (qPCR) assay based on Shanks *et al.* (2010). GenBac3 indicates the presence of bacteria in the Order *Bacteroidales*, which are abundant in the intestines of warm-blooded animals and humans and are not able to grow in the environment. Samples with a positive result for GenBac3 were tested using the human duplex qPCR based on Ahmed *et al.* (2019) and human *Bifidobacteria* qPCR based on Matsuki *et al.* (2004). GenBac3-positive samples were also tested using the ruminant BacR qPCR based on Reischer *et al.* (2006). Samples that tested positive for the ruminant qPCR were tested by qPCR assays targeting *Salmonella* (Hoorfar *et al*., 2000), *Campylobacter* (Best *et al.*, 2003), *Yersinia* (ISO18867)[11] or STEC (Derzelle *et al.*, 2011).

In a follow-up study, samples that were GenBac3-positive but BacR-negative were screened for the presence of avian faecal material by the method of Green *et al.* (2012). The method has been shown to detect faecal material from ducks, geese, gulls and chickens, but may be indicative of a wider range of avian types.

[11] International Organization for Standardization. Polymerase chain reaction (PCR) for the detection of food-borne pathogens: Detection of pathogenic *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*.

APPENDIX B: EXISTING GROWER GUIDANCE

Guidance for growers affected by flooding has been published. These resources focus on decision-making over harvesting flood-affected fresh produce, and few contain information to support growers to make decisions about replanting fields with new crops. Most guidance considers two main types of flooding, these being pooled rainwater (low risk if there are no sources of contamination within the flooded area) and flood water flowing into/across properties from other locations. Guidance related to soil contamination is usually based on studies of the survival of microbiological pathogens in raw manure applied to soils, so represents a worse-case event (gross faecal contamination). In general, an exclusion (stand-down) period of 1-2 months is recommended. Some guidance suggests undertaking soil testing but details on the test targets or how to interpret the results are not included.

B.1 THE MINISTRY FOR PRIMARY INDUSTRIES

Following the 2023 floods, MPI published guidance for harvesting flood-affected produce for human consumption (MPI, 2023). Growers were advised to destroy any mature crops that had been in contact with flood water. Any underground crops (e.g. carrots, potatoes) or alliums that were still in the early stages of growth with at least four weeks until maturity could be harvested when mature.

B.2 HORTICULTURE NEW ZEALAND

Horticulture New Zealand has published a decision tree to guide growers of flood-affected vegetable/ground crops, based on guidance published by MPI (HortNZ, 2023).

B.3 FRESH PRODUCE SAFETY CENTRE (AUSTRALIA & NEW ZEALAND)

The Fresh Produce Safety Centre (FPSC) Australia & New Zealand has published a grower guide relevant to flood-affected soils (FPSC, 2023b). This states: “Over time, contamination of the flooded site will naturally decrease. Ask your local agronomist to organise soil tests to check the level of contamination still present in the flooded fields before replanting. Wait at least 60 days before replanting. The soil must be sufficiently dried out.”

The FPSC has also published information that considers the survival of pathogenic microorganisms in soil after soil amendments and fertilisers are applied. In general, they advise that untreated (raw) animal manure is not used for growing short-term crops such as leafy vegetables and herbs (FPSC, 2023a). If untreated manure is incorporated into soil, advice on exclusion periods between manure application and harvest has been developed by the FPSC Technical Group (FPSC, 2022). The shortest exclusion period is 45 days, for fresh products grown in areas where the mean maximum temperature during that period is ≥20°C and where these products meet other requirements such as the harvested product being grown without soil contact, always being eaten cooked or being subjected to a validated pathogen reduction post-harvest process. The longest exclusion period is 180 days (6 months), assuming the mean maximum temperature during this period is <20°C and the harvested product is higher risk (e.g. is eaten raw and has an edible skin and/or is grown in contact with soil).

B.4 FOOD STANDARDS AUSTRALIA NEW ZEALAND (FSANZ)

Under the Australia New Zealand Food Standards Code, Standard 4.2.8–8 pertaining to leafy vegetable production considers weather events, stating that “A primary horticulture producer and a primary horticulture processor must take appropriate remedial action to ensure that leafy

vegetables adversely affected by weather conditions are not unacceptable."[12] This standard only applies in Australia, although New Zealand fresh produce growers are also required to ensure that the foods they produce are safe and suitable.[13] FSANZ provides some additional information on good practice, which focuses on ensuring harvested produce is not contaminated rather than assessing the safety of field soils prior to replanting.[14]

B.5 INTERNATIONAL FRESH PRODUCE ASSOCIATION

The International Fresh Produce Association (IFPA) has published advice relevant to soils flooded prior to the planting cycle (IFPA, 2023). They suggest a waiting period of 60 days before replanting but acknowledge this interval is affected by a range of factors including soil type, temperature and weather. They refer to guidance published by the US Food & Drug Administration (USFDA), who recommend waiting for the soil to dry and assessing risk factors such as the crop selection and the source of the flood waters (USFDA, 2011). The USFDA suggests testing the soil for "the presence of microorganisms of significant public health concern or appropriate indicator microorganisms" to generate information about relative risks but acknowledge that "sampling by itself does not guarantee that all raw agricultural commodities grown within the formerly flooded production area are free of the presence of human pathogens." They refer to waiting periods recommended by other experts before replanting (30-60 days). According to Kulhanek and Doohan (2015), one of the reference texts cited by the USFDA, these waiting periods are based on studies of raw manure being applied to soil. Tillage, or some form of soil reworking, is also recommended (Kulhanek and Doohan, 2015; USFDA, 2011).

B.6 CALIFORNIA LEAFY GREENS MARKETING AGREEMENT (LGMA)

The California Leafy Greens Marketing Agreement (LGMA) is a membership-based organisation of companies that ship and sell California-grown lettuce, spinach and other leafy greens products. At the heart of LGMA is a set of food safety practices that are implemented on leafy greens farms throughout the state. LGMA has developed guidelines for the safe production and harvest of lettuce and leafy greens, which includes specific guidance on the issue of flooding (California LGMA, 2021).

With respect to the time interval before planting can commence following floodwaters receding, the LGMA specifies:

- 60 days prior to planting provided that the soil has sufficient time to dry out.
- Appropriate soil testing can be used to shorten this period to 30 days prior to planting. This testing must be performed in a manner that accurately represents the production field and indicates soil levels of microorganisms lower than the recommended standards for processed compost. Suitable representative samples should be collected for the entire area suspected to have been exposed to flooding.

Appropriate mitigation and mitigation strategies recommended include:

- Prior to replanting or soil testing, the designated food safety professional for the grower shall perform a detailed food safety assessment of the production field. This designated professional will be responsible for assessing the relative merits of testing versus observing

[12] Australia New Zealand Food Standards Code – Standard 4.2.8 – Primary Production and Processing Standard for Leafy Vegetables. https://www.legislation.gov.au/F2022L01062/latest/text (accessed 21 May 2024).
[13] As required by the *Food Act* 2014 and associated regulations and notices.
[14] https://www.foodstandards.gov.au/business/food-safety-horticulture/weather-events (accessed 21 May 2024).

the appropriate time interval for planting, and also will coordinate any soil testing plan with appropriate third-party consultants and/or laboratories that have experience in this type of testing.

- Evaluate the source of flood waters (e.g., drainage canal, river, irrigation canal, etc.) for potential significant upstream contributors of human pathogens at levels that pose a significant threat to human health.
- Allow soils to dry sufficiently and be reworked prior to planting subsequent crops on formerly flooded production ground.
- Do not replant formerly flooded production ground for at least 60 days following the receding of floodwaters. This period or longer and active tillage of the soil provide additional protection against the survival of pathogenic organisms.
- If flooding has occurred in the past on the property, soil clearance testing may be conducted prior to planting leafy greens. Soil testing may be used to shorten the clearance period to 30 days. If performed, **testing must indicate soil levels of microorganisms lower than the standards for processed compost.** Suitable representative samples should be collected for the entire area suspected to have been exposed to flooding.
- Sample previously flooded soil for the presence of microorganisms of significant public health concern or appropriate indicator microorganisms. Microbial soil sampling can provide valuable information regarding relative risks; however, sampling by itself does not guarantee that crops grown within the formerly flooded production area will be free of the presence of human pathogens.
- Evaluate the field history and crop selection on formerly flooded production ground.
- Assess the time interval between the flooding event, crop planting, and crop harvest. Comparative soil samples may be utilized to assess relative risk if significant reductions in indicator microorganisms have occurred within this time interval.

The compost standards referred to are:

Target organism(s)	**Acceptance criteria**
Faecal coliforms	< 100 MPN/g of total solids (dry weight basis)
Salmonella spp.	Negative or <Detection limit (DL) (< 1 MPN / 30 grams)
STEC	Negative or < DL (per methodology used)

The criteria are reported to be based on a US Food and Drug Administration (USFDA) assessment of D- and Z-values for key pathogens of concern in foods.