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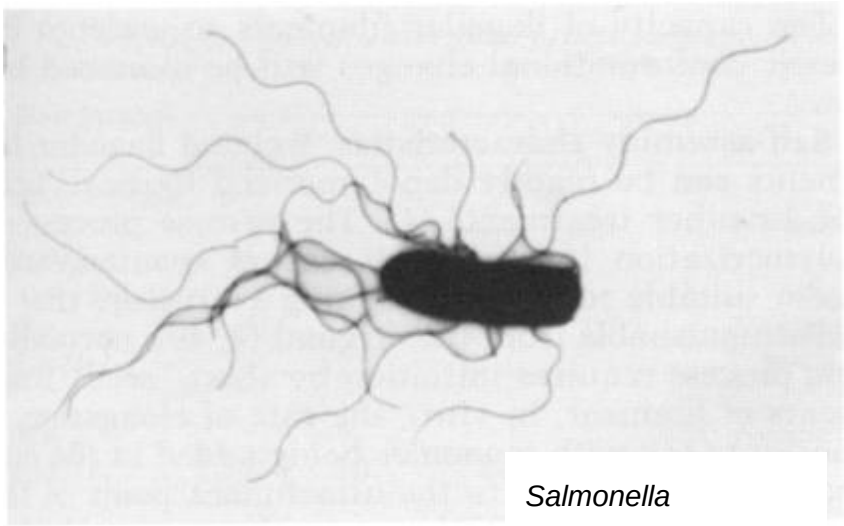

Salmonella

Organic Materials Guidelines - Pathogens Review

Jacqui Horswell and Joanne Hewitt

Peer reviewed by Wendy Williamson and Jennifer Prosser

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Organic Materials Guidelines - Pathogens Review

Jacqui Horswell and Joanne Hewitt

Centre for Integrated Biowaste Research (CIBR)

c/- ESR

P.O. Box 50-348

Porirua 5240

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Centre for Integrated Biowaste Research

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EXECUTIVE SUMMARY

Unlike many other waste streams, there are good prospects for alternative, beneficial end-use options for organic wastes because they can be carbon-rich and contain high concentrations of valuable nutrients. However, because organic wastes can contain potentially pathogenic microorganisms their beneficial reuse requires management and regulation to ensure minimal environmental/public health risks and maximum value.

This report provides a review of current research findings and management experiences with respect to the occurrence and fate of potentially pathogenic organisms in organic wastes. It reviews the protection of public and environmental health provided by the *Ministry for the Environment, New Zealand Water and Wastewater Association (2003) Guidelines for the Safe Application of Biosolids to Land in New Zealand* and regulations/guidelines on animal wastes, and gives recommendations for improvements where appropriate.

The key findings of the review are listed below:

- The reliability of waste treatment processes in reducing pathogens is essential for public health protection. This is especially true for products that will be sold and/or handled by the general public. Pathogen reduction requirements for such products (e.g. Grade A products) should be performance based as opposed to process based and be required to prove pathogen destruction performance. Flexibility to allow alternative treatment processes to be used should be retained provided that it can be demonstrated through process verification and routine monitoring that any proposed treatment method meets an equivalent pathogen standard. Performance testing should also involve measurement of the microbial indicator in the waste before treatment, and in the final product, to determine the capacity of the treatment to reduce pathogens.

- There is no justification to reduce the number of microbial indicators currently required for verification testing to produce wastes that will be directly sold and/or handled by the public (e.g. Grade A products), except for helminths. Limits should reflect most up to date analytical detection limits. Research findings from the Sydney Water QMRA study indicate that the test methods for *Cryptosporidium* are still not sufficiently reliable to determine infectivity, thus it is recommended that, at this stage, *Cryptosporidium* is not included in any new guideline. For helminths, Sydney Water do not view these pathogens as a significant risk – it should be noted however that Australian risk assessments are based on human health in most cases and it is recommended that in New Zealand the Steering Group members should take direction from MPI on inclusion/exclusion of the helminth in any new guideline. It would also be useful to undertake a survey to determine how prevalent helminth ova are in New Zealand biosolids and other organic wastes. The key contact at MPI will be Emil Murphy in the Animal products team.

- It is recognised that substantial regrowth of pathogenic microorganisms can occur in treated organic wastes (especially biosolids), pathogen regrowth testing should

be conducted annually, using three samples for all products that may be directly sold and/or handled by the public.

- There is some justification to reduce the number of samples required for verification sampling for products that may be directly sold and/or handled by the public (i.e. Grade A products), from 15 to 7 grab samples as per the *Western Australian guidelines for biosolids management* (2012).

- Due to their prevalence and concentration in sewage and sewage sludge, potential viral indicator candidates are human enteroviruses and human adenoviruses. The suitability of F-RNA and/or somatic bacteriophages (coliphages) as indicators in this context is still largely unknown. As human pathogens, the use of human enteroviruses or human adenoviruses is more informative than the use of bacteriophages. It is recommended that human adenovirus is measured as the virus indicator and that the methodology in the current *Guidelines for the Safe Application of Biosolids to Land in New Zealand* (2003) be modified. Although molecular analyses do not report on viability, qPCR could be useful for end product verification of Grade A composted biosolids, where the objective is to verify that viruses are removed. The Sydney Water QMRA supports the selection of human adenoviruses as the viral indicator in organic materials and also the integrated culture-PCR (C-PCR) methodology. The preliminary data from the QMRA indicate that human adenoviruses carry the greatest risk to human health, thus inclusion of human adenovirus in any new guideline is fully justified.

- There is no justification for increasing product monitoring requirements for organic wastes that are not directly sold and/or handled by the public (e.g. Grade B products). Management controls in the current *Guidelines for the Safe Application of Biosolids to Land in New Zealand (2003)* provide sufficient public and environmental health protection. However, before lifting site restrictions soil testing should be carried out to ensure that there has been no cumulative increase in microorganisms due to waste application. *Escherichia coli* is recommended for soil monitoring as it is neither arduous nor expensive, and may provide useful information on pathogen die-off in receiving soils.

- Agricultural wastes such as animal manures can potentially contain pathogens, the types of which are similar to those found in human sludge. There appear to be limited safeguards to protect public and animal health from potential pathogens in land applied animal wastes, risks are reduced by “good husbandry and management practices”. It is recommended that as for biosolids, these wastes undergo some form of process to reduce pathogens so that they do not pose a threat to public health and the environment.

INTRODUCTION AND CONTEXT

Purpose

The purpose of this report is to:

1. Summarise existing knowledge on potentially pathogenic organisms in organic wastes relative to the current *Guidelines for the Safe Application of Biosolids to Land in New Zealand (2003)* (Table 4.2).
2. In consultation with the Ministry of Health, Ministry for Primary Industries and Massey University, review the justification for the inclusion of the following pathogens: *E. coli, Campylobacter; Salmonella*; enteric viruses; helminth ova, which are in the current Biosolids Guidelines and to:
    a. Provide recommendations about which pathogens should and should not be in a new guideline, including supporting logic.
    b. Determine if other organic wastes contain additional pathogens of concern that should be included in a new guideline.
3. Review the recommended detection methods for pathogens in biosolids (Appendices 1 and 2 of the *Guidelines for the Safe Application of Biosolids to Land in New Zealand* (2003)); and provide recommendations for new methods for organic wastes with supporting logic.

Background

Organic wastes comprise more than 50% of the total wastes going to landfill in New Zealand. These wastes produce high greenhouse gas emissions and leach contaminants, including high levels of nitrogen. Unlike many other waste streams, there are good prospects for alternative, beneficial end-use options for organic wastes because they can be carbon-rich and contain high concentrations of valuable nutrients that can be used to bolster soil nutrient reserves, thereby reducing dependence on artificial fertilisers.

However, organic wastes can contain potentially pathogenic microorganisms that originate from tissues of diseased animals and people, and from healthy carriers who excrete infectious pathogens in faeces and urine. Many of these pathogens are zoonotic, i.e. they may cause infections in both animals and people and readily transfer between animals and human hosts. The biosecurity risk associated with the land application of organic wastes as fertilisers is hard to assess, and management requires technical guidance and regulation to ensure minimal environmental/public health risk and maximum re-use value.

The range of pathogenic microorganisms that may be found in organic waste streams derived from faecal material has been widely discussed in the literature; and in various reviews carried out in New Zealand and Australia, (e.g. ‘Evaluation of the Contemporary Guidelines and Practices of Pathogen Identification, Screening and Treatment in Sewage Sludge to obtain Biosolids Products which are safe for Land Application in Western Australia’ UWA, 2012; ‘Pathogen Monitoring in Land Treatment Systems’ Horswell, J. and Aislabie, J. 2006; ANZBP ‘Pathogen Presence & Pathways Report’ Ang, R. 2013).

Pathogens include species of bacteria, viruses, parasites, protozoa, helminths and fungi. The most important pathogens in terms of human health risk assessments are those spread by the faecal-oral route and include organisms such as *Campylobacter* spp., *Salmonella* spp., *Cryptosporidium*, *Giardia*, and enteric viruses. Pathogens of concern will also vary depending on geographical location, for example New Zealand has the highest rate of campylobacteriosis in the developed world, and thus *Campylobacter* spp. are a priority pathogen in New Zealand.

Over the last decade, at least one new pathogen per year that can be transmitted through the environment has been recognized as a public health threat (WHO, 2003). In addition, there are emerging, re-emerging or newly discovered pathogenic microorganisms for which there are typically few data available about their transmission routes, virulence, minimum infective dose, survival outside of host, or disinfectant susceptibility. Thus, there is a constant need to review current literature and update information for waste managers and regulators on risks and hazards from microbial contaminants that could potentially be present in organic wastes. This type of information will allow rapid response to any potential problem soon after it develops, when it is most easily dealt with.

Land application of most organic wastes requires a resource consent under the Resource Management Act (1991) in order to *"avoid, remedy or mitigate any adverse effects of activities on the environment".*

The most comprehensive body of research has been undertaken on human wastes (biosolids). Through this research, strict guidelines and regulations are in place in many countries including Australia (e.g. NSW EPA, 1998), the United States (US EPA Part 503 Rule, 1993) and New Zealand (NZWWA, 2003). These guidelines recommend appropriate treatment processes to reduce pathogen levels as well as practices for the safe handling, storage and application of biosolids. Thus, for the purposes of this report we mainly focus on biosolids guidelines, which serve as a model for management of organic material for beneficial reuse.

Resources used

The most comprehensive regulation on biosolids is US EPA Part 503 Rule (1993). Development of the Australia/New Zealand guidelines has been strongly based on this US EPA guideline. For the purposes of this review we have primarily used the following resources:

- US EPA; US Environmental Protection Agency (1993) Part 503-Standards for the Use or Disposal of Sewage Sludge. Federal Register 58, 9387-9404.
- Australian and New Zealand Biosolids Partnership (ANZBP): Review of Biosolids Guidelines (2009). Paul Darvodelsky, Dominic Flanagan, Jim Bradley
- Western Australian guidelines for biosolids management (2012) Department of Environment and Conservation
- EU policy on sewage sludge utilization and perspectives on new approaches of sludge management. (2014). G. Mininni & A. R. Blanch & F. Lucena & S. Berselli, Environmental Science and Pollution Research. Online.

- A Quantitative microbial risk assessment (QMRA) study undertaken by Sydney Water.

Rationale for developing waste management guidelines

One of the key drivers for the development of guidelines for waste management is to protect public health.

Most guidelines around the world have the same basic structure and are made up of four parts. Together they combine to give the desired level of protection for the community and environment.

1. Contaminant controls
2. Pathogen and vector attraction reduction
3. Management controls
4. Sampling and monitoring

BASIC STRUCTURE OF INTERNATIONAL GUIDELINES

1. Contaminant controls

Directly refers to inorganic and organic contaminants and is dealt with by another Working Group report.

2. Pathogen and vector attraction reduction

In most guidelines regulation of pathogens is carried out in two ways. The first is on the basis of the performance of known processes; and the second is to reduce pathogens to the numerical standards set out in the guidelines.

Rationale for Processes Control

The reliability of sludge treatment processes in reducing pathogens is essential for public health protection.

Most guidelines (including those in Australia, New Zealand and the United States) list approved or specified treatment technologies that are known to reliably and consistently reduce pathogens (e.g. lime treatment, aerobic thermophilic digestion, anaerobic digestion, composting). Most guidelines also provide some degree of flexibility and allow alternative biosolids treatment processes to be used provided that it can be demonstrated through process verification and routine monitoring that proposed treatment method meets an equivalent pathogen standard.

Rationale for requirement for both process control and pathogen monitoring

A key finding of the Australian and New Zealand Biosolids Partnership: Review of Biosolids Guidelines (2009) was that:

"*Pathogen reduction requirements should be performance based as opposed to process based……..*", and that "*The requirement to prove pathogen destruction performance of unknown processes should be retained*".

This approach is important to ensure that the microbiological reductions expected as a result of the treatment process (e.g. time, temperature) have actually been attained. The performance testing generally uses final product evidence for quality control: low level *E. coli* and non-detect *salmonella*, helminth and enteric viruses as indicators of destruction of pathogenic organisms.

The importance of 'performance testing' is further explained in an email from the author of the ANZBP: Review of Biosolids Guidelines (2009), Paul Darvodelsky, on the 21st July 2014.

"*We also believe that there should be the facility to demonstrate a new process can meet a certain level of pathogen kill and hence be acceptable. I would expect in a guideline that it would have the following form:*

1. Proven processes which are accepted as reaching a certain standard. Regular testing required to demonstrate that the process is operating properly;

2. New processes which can be proven to reach a certain standard which would then require regular testing to demonstrate the process is operating properly. I would expect such a process would also have to demonstrate a mechanism by which it achieved pathogen kill, i.e. no magic processes; and

3. Processes which don't fall into either category (e.g. vermiculture) which would require testing for every batch to prove the product meets the standard claimed. This testing would be the same as process verification and regular monitoring analyses, as sensible, to ensure product quality."

In the current *Guidelines for the Safe Application of Biosolids to Land in New Zealand* (2003) the performance of a process is determined via verification sampling:

"*Verification sampling is required to demonstrate that a treatment process is producing a final product of consistent quality. This phase of monitoring is typified by a high-frequency sampling regime. Verification monitoring should occur not only when a new process is commissioned but also when changes are made to an existing process, and also if any of the routine samples exceed the limits set for pathogens or chemical contaminants; in other words, whenever there could be a possible change to the quality of the final product.*"

Below is Table 6.2: *Pathogen standards* which outlines the criteria for verification sampling and routine sampling for biosolids to meet *Grade A* from the *Guidelines for the Safe Application of Biosolids to Land in New Zealand* (2003).

Table 6.2: Pathogen standards[1]

Pathogen	Verification sampling	Routine sampling
E. coli	< 100 MPN[2]/g	< 100 MPN/g
Campylobacter	< 1/25 g	N/A[4]
Salmonella	< 1/25 g	N/A
Enteric viruses	< 1 PFU[3]/4g	N/A
Helminth ova	< 1/4g	N/A

(Source: *Guidelines for the Safe Application of Biosolids to Land in New Zealand* (2003))

Discussions with the NZ Ministry of Health, Paul Prendergast and John Harding on the 10th of June 2014, indicated a preference for more of a focus on 'process' rather than sampling and monitoring (i.e. performance testing or the verification testing that is currently in the guidelines).

A key difference between the *Guidelines for the Safe Application of Biosolids to Land in New Zealand* (2003) and the US EPA Part 503 rule (1993) is the US EPA rule requirement to measure the microbial indicators both in the sludge during the treatment train, and in the final biosolids, to determine the capacity of the treatment to reduce pathogens. The US EPA Part 503 (1993) provides justification for this:

"Testing for enteric virus and viable helminth ova can be complicated by the fact that they are sometimes not present in the untreated sludge. In this case an absence of the organisms in the treated sludge does not demonstrate that the process can reduce them to below detectable limits. Monitoring should be continued until enteric viruses and/or viable helminth ova are detected in the fed sewage sludge. The treated sewage can then be analysed to determine if these organisms survived treatment. Thus it is essential to validate the treatment process until adequate specified pathogens reduction has been successfully demonstrated"

The Western Australian guidelines for biosolids management (2012) also state that:

"*Treatment performance involves measurement of the microbial indicator in the sludge during the treatment train, and in the final biosolids, to determine the capacity of the treatment to reduce pathogens. The calculation of pathogen log removals, following treatment with a quality assurance/quality control program, is considered a better approach for microbial risk management rather than only end-point quality monitoring for microbial indicators.*"

However the Western Australian guideline also recognises that:

"*At present, only end product quality is included …..because there are insufficient data to determine the log removals that can be achieved for each one of the approved treatment methods. However, projects are required to monitor for microbial treatment performance.*"

In the *Guidelines for the Safe Application of Biosolids to Land in New Zealand (2003)* there is currently no requirement to demonstrate removal of microbial indicators by measuring them before and after treatment.

Recommendation

It is recommended that microbial indicators in the organic waste are measured during the treatment train, and that verification monitoring must be carried out until all microbial indicators are detected in the fed waste to prove the process can effectively remove them.

Most international guidelines, including the *Guidelines for the Safe Application of Biosolids to Land in New Zealand (2003)* allow for 'flexibility' of treatment process to allow for new technologies. If biosolids are produced using different methods than those listed in the guideline, it must be demonstrated through process verification and routine monitoring that any proposed treatment method meets an equivalent pathogen standard. In the

Guidelines for the Safe Application of Biosolids to Land in New Zealand (2003), Table 6.1: Stabilisation requirements it states that “other processes” must show:

“*Demonstration by agreed comprehensive process and product monitoring that the Grade A pathogen levels can be consistently met.*”

The US EPA Part 503 rule (1993) also allows such flexibility as show in Table 5-1 below.

TABLE 5-1
Summary of the Six Alternatives for Meeting Class A Pathogen Requirements

In addition to meeting the requirements in one of the six alternatives listed below, the requirements in Table 5-2 must be met for all six Class A alternatives.

Alternative 1: Thermally Treated Biosolids

Biosolids must be subjected to one of four time-temperature regimes.

Alternative 2: Biosolids Treated in a High pH-High Temperature Process

Biosolids must meet specific pH, temperature, and air-drying requirements.

Alternative 3: Biosolids Treated in Other Processes

Demonstrate that the process can reduce enteric viruses and viable helminth ova. Maintain operating conditions used in the demonstration after pathogen reduction demonstration is completed.

Alternative 4: Biosolids Treated in Unknown Processes

Biosolids must be tested for pathogens—*Salmonella* sp. or fecal coliform bacteria, enteric viruses, and viable helminth ova—at the time the biosolids are used or disposed, or, in certain situations, prepared for use or disposal.

Alternative 5: Biosolids Treated in a PFRP

Biosolids must be treated in one of the Processes to Further Reduce Pathogens (PFRP) (see Table 5-4).

Alternative 6: Biosolids Treated in a Process Equivalent to a PFRP

Biosolids must be treated in a process equivalent to one of the PFRPs, as determined by the permitting authority.

(Source: US EPA; US Environmental Protection Agency (1993) Part 503-Standards for the Use or Disposal of Sewage Sludge. Federal Register 58, 9387-9404.)

As noted above by Paul Darvodelsky in the email communication on 21st of July 2014, for some processes, verification or performance testing alone may not be adequate to ensure human health protection.

“*Processes which don’t fall into either category (e.g. vermiculture) which would require testing for every batch to prove the product meets the standard claimed. This testing*

would be the same as process verification and regular monitoring analyses, as sensible, to ensure product quality."

Recommendation

It is recommended that for processes that could potentially produce an inconsistent product, due to difficulties in controlling the process, every batch should be tested for the full range of microbial indicator organisms. Such processes would include vermicomposting where it is difficult to control the biological process to the same extent as traditional composting (where time and temperature can be more easily controlled.

Rationale for testing for pathogen re-growth potential

It is recognised that substantial regrowth of pathogenic bacteria in treated biosolids (even Class A) can occur (US EPA, 1993, Western Australian guidelines for biosolids management, 2012). However a key difference between the *Guidelines for the Safe and Application of Biosolids to Land in New Zealand* (2003), and the US EPA Part 503 rule (1993) and the *Western Australian guidelines for biosolids management* (2012) is their approach to testing of biosolids for re-growth potential, with the *Western Australian guidelines for biosolids management* (2012) taking a more cautious view.

Whilst the *Guidelines for the Safe Application of Biosolids to Land in New Zealand* (2003), recognises that:

"*Ideally, monitoring the quality of biosolids should occur just prior to their use. This practice is in accordance with the USEPA, European Union, NSW EPA and the NRMMC guidelines. When determining the stabilisation grade, pathogen reduction monitoring should only be undertaken on the final product (just before sale), because pathogenic organisms may regrow after treatment has taken place*".

The US EPA Rule 503 (1993) considers that biosolids should be tested at:

"…*the last practical monitoring point before the biosolids is applied to the land or placed on a surface disposal site. Biosolids that are sold or given away cannot be monitored just prior to actual use or disposal; instead monitoring is required as it is prepared for sale or given away.*"

The Western Australian guidelines for biosolids management (2012) takes this one step further by requiring annual testing of a product for re-growth:

"*For biosolids or products containing biosolids of P1 and P2 grade, regrowth testing for E. coli and coliphages is required in order to demonstrate that the treatment process is working effectively and that, in combination with normal end-use management controls, re-growth has not occurred. Re-growth testing should be conducted annually, using three samples.*"

In New Zealand anecdotal evidence suggests that there may be potential for significant pathogen re-growth in biosolids products that may be stockpiled before release. An

example of this is composted biosolids that may be stockpiled uncovered and therefore be able to be re-wetted by rainfall or reinoculated with pathogens by birds or vermin

Recommendation

It is recommended that pathogen re-growth testing be conducted annually, using three samples in all Grade A products.

Rationale for numerical standards (Pathogen Grading)

Most biosolids guidelines contain pathogen grading that is dependent upon meeting:

- a treatment process known to reliably reduce pathogen levels;
- microbiological limits that demonstrate the effectiveness of the treatment process; and
- a vector attraction reduction control.

Ideally, to determine the microbiological quality of biosolids, testing should be undertaken for all pathogens, but this is not currently practicable. Consequently, microbial indicators are still used for routine evaluation of treatment performance and final biosolids quality. Indicator organisms are organisms believed to indicate the presence of a larger set of pathogens. An indicator organism should ideally have the following characteristics:

- consistently present in relatively high numbers in waste streams;
- standard methods exist to easily isolate and identify; and
- behave in a similar way to pathogenic organisms (because their life processes and ecological niches are similar), in both sewage treatment process and after land application.

The ANZBP: Review of Biosolids Guidelines (2009) undertook a comparison of the current pathogen requirements for Australia, New Zealand, the UK, EU and USA; *"Table 5.8 Stabilisation grade comparison"*.

Table 5.8 – Stabilisation grade comparison

	NSW, Qld, ACT	National	SA	Tas	Vic	WA	NZ	UK Safe Sludge Matrix	EU	USA
E. coli (MPN/g)	<100	<100	<100	<100	<100	<100	<100	<1000 + 6 log reduction	-	no criteria
Faecal coliforms (MPN/g)	<1,000	<100	-	<100	-	-	N/A	no criteria	-	<1000
salmonella sp. (/50g)	nd	<1	<1	<1/100 g	-	<1	<1/25g	absent from 2 g DS	-	or <3 per 4g DS
Enteric viruses (PFU/4g ds)	-	-	<1/50 g ds	-	-	-			-	
Helminth ova (PFU/4g ds)	-	-	<1/50 g ds	-	-	1			-	
Campylobacter							<1/25g		-	

(Source: Darvodelsky, P., Flanagan, D. and Bradley, J. (2009). Summary of Australian and New Zealand Biosolids Partnership: Review of Biosolids Guidelines)

Summary of Pathogen Grading in Western Australian guidelines for biosolids management (2012) Department of Environment and Conservation

Four pathogen grades are used: P1, P2, P3 and P4 and the standards are shown in *"Table 2: Approved treatment methods"* of the Western Australian guidelines.

Escherichia coli (E. coli) and coliphages are considered suitable microbial indicators of faecally derived bacteria and viruses respectively.

The highest grade of biosolids treatment (P1, equivalent to Grade A in the *Guidelines for the Safe and Application of Biosolids to Land in New Zealand*, 2003), must meet coliphages <10 PFU/10 g of dry final biosolids AND *E. coli* less than 100 counts per g of dry final biosolids. Strongyloides and Hookworm (viable ova) <1 per 50 g of dry final biosolids (only required north of the 26th parallel).

E. coli

E. coli is a bacterium from the thermo-tolerant coliforms group. *E. coli* monitoring can be used alone for pathogen grade P2 and P3 applications (that is, biosolids applications with low likelihood of human contact) and are equivalent to Grade B in the *Guidelines for the Safe Application of Biosolids to Land in New Zealand* (2003).

Coliphages

Coliphages are viruses that infect coliform bacteria (including *E. coli*) and their inclusion is a new parameter for routine evaluation of treatment performance and final P1 biosolids quality. The guidelines note that:

"*laboratories in WA are yet to validate analytical techniques and obtain NATA accreditation for this testing*".

There are many types of coliphages, usually one or both of two groups, somatic bacteriophages and F-RNA bacteriophages (e.g. MS2), are monitored. The somatic bacteriophages are usually more numerous in sludge and are more resistant to thermal inactivation than F-RNA bacteriophages. F-RNA bacteriophages are less prevalent in human faeces than somatic bacteriophages but they can be cultivated and enumerated to perform challenge tests to demonstrate the log removals a treatment can achieve.

Helminths

Helminths monitoring is only required for biosolids applications in endemic areas of WA (e.g. the Kimberley region). Specifically the guideline requires monitoring for Strongyloides and Hookworm with a viable ova limit of <1 per 50 grams of dry final biosolids.

The EU

Recycling sludge is a highly regulated activity in the EU. Current controls are based on the 1986 EU Sludge Directive (86/278/EEC). The sludge directive has no specific regulation and monitoring of microbial parameters to provide barriers on disease transmission. However, the transposition of the Directive in different member state legislation has led to some countries adopting additional health regulatory requirements on sludge treatment process and currently the international limit values on pathogens are placed in a rather wide range. This is the inevitable consequence of different attitudes towards sludge management practices in the member states.

The table below shows the standards for maximum concentrations of pathogens in sewage sludge in some EU countries (Mininni et al., 2014)

Table 1. Standards for maximum concentrations of pathogens in sewage sludge

	Salmonella	**Other pathogens**
Denmark (advanced treatment only)	No occurrence	Faecal streptococci, <100/g
France	8 MPN/10 g DM	Enterovirus, 3 MPCN/10 g DM Helminths eggs, 3/10 g DM
Finland	Not detected in 25 g	*Escherichia coli* <1,000 cfu
Italy	1,000 MPN/g DM	
Luxembourg		Enterobacteria, 100/g no eggs of worm likely to be contagious
Poland	Sludge cannot be used in agriculture	

	if it contains salmonella	

(Source: EU policy on sewage sludge utilization and perspectives on new approaches of sludge management. (2014). G. Mininni & A. R. Blanch & F. Lucena & S. Berselli, Environmental Science and Pollution Research. Online.)

In a recent review undertaken by Mininni et al. (2014) the authors reference a sludge directive revision working document draft from European Commission "Working Document on Sludge, 3rd Draft, Brussels, 27 April 2000, ENV.E.3/LM". This working document proposes that:

"…sludge to be used in agriculture without restrictions, should comply with the following requirement: being treated by advanced process, that fulfils the limits of E. coli <500 CFU/g and Salmonella <1/50 g and achieves 6 log reduction of a test organism such as Salmonella senftenberg".

However it is also noted that the feasibility and reliability of spiking *Salmonella senftenberg* is still amply debated.

The European Commission "Working Document on Sludge, 3rd Draft, Brussels, 27 April 2000, ENV.E.3/LM" goes on to suggest conventional indicators (e.g. *Escherichia coli*, faecal coliform bacteria, clostridia, somatic coliphages, etc.) and/or index pathogen (*Salmonella*) are used as surrogates of pathogen presence for routine evaluation of treatment plant performance and sludge microbial quality. Bacterial indicators present limitations for their role as surrogates of parasites and viruses. In order to overcome such limitations, spores of *Clostridium perfringens* have been proposed as alternative indicators of protozoan cysts in water treatments while bacteriophages of enteric bacteria had been advocated as surrogates of waterborne viruses in water quality control process.

However, an EU funded project "The ROUTES project (www.eu-routes.org), "Novel processing routes for effective sewage sludge management" has found that *C. perfringens* should be abandoned as a microbial indicator as they are not good indicators of the performance of anaerobic processes.

Finally, the Mininni et al (2014) review notes that:

"It does not seem that a new sludge directive is pending. Works in progress have not evidenced a health and environment impact due to sludge agricultural use although some attention is already paid by many member states on organic pollutants and pathogens."

UK

The EU Sludge Directive (86/278/EEC) was implemented in the UK in 1989 through *The Sludge (Use in Agriculture) Regulations*. The regulations are supported by *The Code of Practice for the Agricultural Use of Sewage Sludge* (1996), which details all aspects of sludge recycling to land; setting application rates, information requirements and guidelines for best practice.

It is anticipated revisions to the regulations and the accompanying Code of Practice will be introduced by the Department for Environment, Food and Rural Affairs (DEFRA) and will include statutory enhancement of *The Safe Sludge Matrix*. *The Safe Sludge Matrix* is currently a voluntary agreement between food retailers (British Retail Consortium) and UK water authorities and was developed to ensure the highest possible standards of food

safety and to provide a framework that gives all food industry stakeholders confidence that biosolids recycling to agricultural land is safe.

As a requirement of *The Safe Sludge Matrix*, sewage sludge is treated by processes to generate either conventional or enhanced biosolids products, which are suitable for recycling to agricultural land. Conventionally treated sludge has been subject to a defined treatment process and standards that ensure at least 99% of pathogens have been destroyed. Enhanced treated sludge will be free from *Salmonella* and will have been treated so as to ensure that 99.9999% pathogens have been destroyed (a 6 log reduction).

Summary of Pathogen Grading in US EPA Part 503 Rule (1993)

The 40 CFR Part 257 – *Criteria for classification of solid waste disposal facilities and practices* requires the use of specifically listed or approved treatment technologies to treat biosolids. Part 503 provides flexibility in how pathogen reduction and vector attraction reduction is met by giving 6 alternatives (*"Table 5-1 Summary of six alternatives for meeting Class A pathogen requirements") (page 9),* and requires microbial indicator measurements to be undertaken as shown below in "*Table 5-2 Pathogen requirements for all Class A Alternatives".*

TABLE 5-2
Pathogen Requirements for All Class A Alternatives

The following requirements must be met for ***all*** six Class A pathogen alternatives.

Either:

the density of fecal coliform in the biosolids must be less than 1,000 most probable numbers (MPN) per gram total solids (dry-weight basis),

or

the density of *Salmonella* sp. bacteria in the biosolids must be less than 3 MPN per 4 grams of total solids (dry-weight basis).

Either of these requirements must be met at one of the following times:

- when the biosolids are used or disposed;
- when the biosolids are prepared for sale or give-away in a bag or other container for land application; or
- when the biosolids or derived materials are prepared to meet the requirements for EQ biosolids (see Chapter 2).

Pathogen reduction must take place before or at the same time as vector attraction reduction, except when the pH adjustment, percent solids vector attraction, injection, or incorporation options are met.

(Source: US EPA; US Environmental Protection Agency (1993) Part 503-Standards for the Use or Disposal of Sewage Sludge. Federal Register 58, 9387-9404.)

If thermal treatment or high pH/temperature are used (alternatives 1 or 2, Table 5-1, page 9) then Part 503 requires monitoring for faecal coliforms and a density of less than 1,000 MPN faecal coliform per gram of total solid sewage sludge (dry weight basis) to be Class A. Part 503 also allows *Salmonella spp*. to be monitored instead of faecal coliforms. The density of the *Salmonella* must be below detection limits of 3 MPN/4 g of total sewage sludge solids (dry weight). The limit of 1,000 MPN faecal coliform is based on experimental evidence carried out by Yanko (1987) which demonstrated that this level of faecal coliform correlated with a very low level of *salmonellae* detection in composted sludge.

If alternatives 3 – 6 (in Table 5-1) (page 9) are used (other or unknown processes), then according to Table 5-2 the biosolids must also be monitored for enteric virus with a limit of less than the detection limits of 1 CFU/ 4 g; and 1 viable helminth ova /4g total solids.

Sydney Water Quantitative microbial risk assessment (QMRA)

Sydney Water is currently undertaking a Quantitative Microbial Risk Assessment (QMRA). A QMRA is a framework and approach that brings information and data together with mathematical models to address the spread of microbial agents through environmental exposures and to characterize the nature of the adverse outcomes. The Sydney Water QMRA has been monitoring pathogen reduction in four of their waste water treatment plants. The treatments include:

- Primary anaerobic digestion
- WAS anaerobic digestion
- WAS aerobic digestion
- Liquid sludge (lagoon for 6 months)

Twelve months (monthly interval) of pathogen data in both raw wastewater (influent) and biosolids have been measured in the most comprehensive risk assessment undertaken to date. The pathogens enumerated were: adenovirus (CC-PCR), Cryptosporidium (IFA/DAPI), Giardia (IFA/DAPI), Salmonella (MPN) and indicators, E. coli, faecal coliforms, and enterococci. Significant resources have been dedicated to development/improvement of pathogen detection methods including recovery data for the organisms in the complex media that is biosolids. At the time of writing this review, the raw data was being processed in the QMRA model. On the 21st of November, 2014, Jacqui Horswell met with representatives from Sydney Water to discuss the QMRA study and preliminary research findings.

Below is a brief summary of results to date:

- The QMRA model ranks exposure in the following order: treatment plant workers > farmers > consumers.
- The Technical Specialists at Sydney Water have selected human adenovirus as the most appropriate indicator of viruses in biosolids and use the CC-PCR method for detection. The preliminary data from the QMRA indicate that human adenoviruses carry the greatest risk to human health.
- The main driver for inclusion of Cryptosporidium in the QMRA was the detection in the Greater Metropolitan Sydney drinking water of Cryptosporidium in 1998.
- Sydney Water has developed an in-house method for enumeration of viable Cryptosporidium based on the infection of cells in tissue culture. This method is similar to other methods used internationally for this purpose. The standard DAPI staining indicates that the organism's nucleus is intact but this does not necessarily mean that the organism is infective. Sydney Water use the additional infectivity test with HCT8 cells and have determined that oocysts from natural surface waters generally have low infectivity (1 to 10%), unless recently contaminated with infected faecal material such as sewage effluent or farm runoff, and the upper limit seems to be ~35% in freshly shed oocysts. Discussion with Sydney Water Technical Specialists suggests that although the methodology has been significantly improved, there are still difficulties recovering organisms from complex media such as biosolids and viability testing is not straightforward. While it is expensive in itself to do the cell culture viability test, it is comparable in cost to the standard Cryptosporidium and Giardia

enumeration and may add considerable value where a risk assessment needs to clarify the true health risk. At present it is primarily a tool for research and major projects where it can add value. Sydney Water has on-going discussions with laboratories using similar methods in research to ensure clients and the industry in general get the best value from using the test and its results appropriately.

- Potentially infective Cryptosporidium has been detected in biosolids at low levels in the Sydney Water study. It should be noted that there was limited scope for testing infectivity by the cell culture infectivity test in the course of the study. While it is possible to detect a single infectious oocyst by this method, it was felt that results would be equivocal if less than 10 oocysts total could be inoculated into per test, both to allow quantitation of the inoculum and because in general only a minor fraction of oocysts are found to be infectious (see above). Fewer than hoped of the samples reached this criterion: only three samples were tested, and no infectious oocysts were detected in biosolids. In the opinion of the Sydney Water Technical Specialist only one treatment works (Picton aged sludge) was well enough examined to place an upper bound on infectivity (< 1.5% approx.).

- Monitoring of helminths is not seen as a priority by Sydney Water due to the low prevalence of helminths in the catchment area and also the end-use of the biosolids which is predominantly the agricultural sector. Sydney Water are currently developing an in-house detection method for helminths which may improve reliability of the data.

- Levels of Salmonella detected in the biosolids were low and not identified as a risk in the preliminary QMRA.

Recommendation

The drivers for organisms selected in the Sydney Water QMRA study may be different to New Zealand, this is likely the case for *Cryptosporidium* and helminths. It is recommended that monitoring of *Cryptosporidium* in Grade A biosolids is still not required due to continued difficulties with measurements for viability/infectivity. The preliminary QMRA results have provided justification for the continued inclusion of viral indicators in NZ guidelines.

Summary of New Zealand Guidelines

Two pathogen standards are used, A and B. Standards have been set for Grade 'A' for faecal coliforms, *salmonella spp., campylobacter spp.*, enteric viruses and helminths and limits are shown in *Table 6.2: Pathogen standards* above (page 7).

Grade A biosolids are effectively "pathogen free' and microbiological criteria specified in various guidelines are based on detection limits for a particular pathogen, however there is some disagreement between guidelines on detection limits. Grade 'A' biosolids are considered to be of significantly high quality that they can be safely handled by the public and applied to land without significant risk of adverse effects.

Faecal indicators and pathogens in biosolids

E. coli

Many countries, including the USA, still use faecal coliforms in their regulations and guidelines. This is because they have large historical data sets based on faecal coliform levels in biosolids. The US is currently building up *E. coli* data bases and may switch to measuring *E. coli* in the future (pers com. J. E. Smith, US EPA). Current limits in the *Guidelines for the Safe Application of Biosolids to Land in New Zealand (2003)*, are based on US EPA Part 503 (1993) limit of < 1,000 MPN/g for faecal coliforms, the justification for this limit is based on work undertaken by Yanko (1987) who demonstrated that this level of coliforms correlated with low numbers of *salmonella*. In general *E. coli* levels are ten-fold lower than faecal coliforms, hence the current *E. coli* limit of < 100 MPN/g is set in the *Guidelines for the Safe Application of Biosolids to Land in New Zealand (2003)*.

It has been widely demonstrated that coliform bacteria do not adequately reflect the occurrence and survival of pathogens in treated sewage and wastewater (Harwood et al. 2005; Moce-Llivina et al. 2003, Sidu and Toze, 2009), and it is thus important to monitor a suite of organisms including a sub-set of pathogens, this is the case for most international guidelines including US EPA Part 503 and the Australian guidelines as shown in *"Table 5.8 Stabilisation grade comparison"* (above, page 12) taken from the ANZBP: *Review of Biosolids Guidelines* (2009).

Campylobacter

The Guidelines for the *Safe Application of Biosolids to Land in New Zealand* (2003), are unique in requiring monitoring for *Camplylobacter*. They state that:

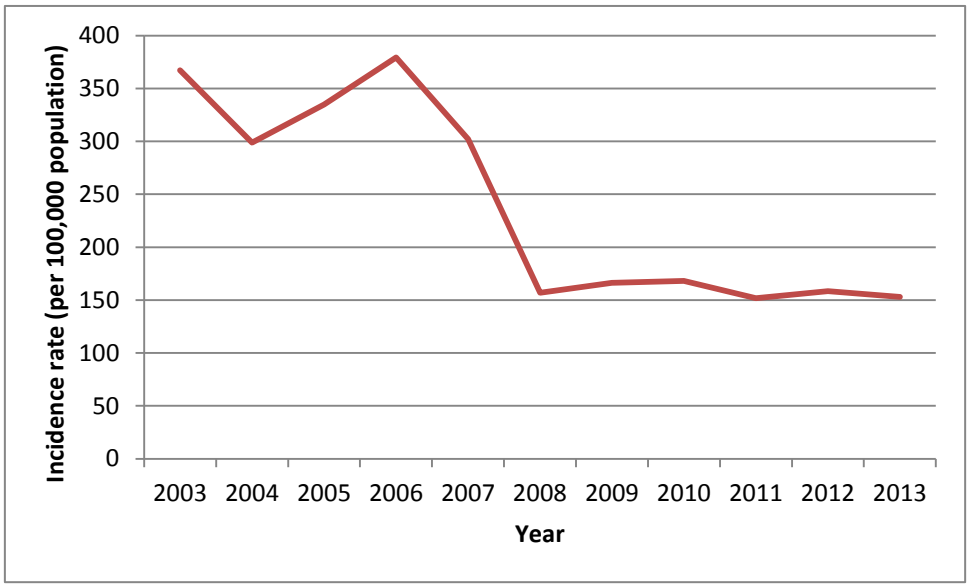
"*Whereas Salmonella spp. are traditionally used as an indicator for pathogen removal, the high incidence of campylobacter infection in the New Zealand community makes it a greater risk. For this reason campylobacter is required for verification sampling.*"

The incident rate of campylobacteriois has declined since 2003. Figure 1 below shows the incidence of campylobacteriois from 2003 – 2013 (New Zealand Public Health Surveillance Reports (NZPHS)). However, this rate is still significantly higher than the rest of the developed world (e.g. in Australia the rate is only 78 per 100,000 population (Notifiable Infectious Disease Reports, WA Department of Health, 16 June 2014)). Therefore, there is no justification for removing *Camplylobacter* as a microbial indicator in a New Zealand guideline.

Recommendation

It is recommended that the Steering Group members take direction from the Ministry of Health on inclusion/exclusion of *Campylobacter* in any new guideline.

Figure 1. Incidence of campylobacteriosis in New Zealand



(Source: New Zealand Public Health Surveillance Reports (NZPHS))

Salmonella

Salmonella sp. are monitored as indicators of the impact of treatment processes on bacterial pathogens. They are an important human pathogen, with salmonellosis ranking 5th in a ranking of New Zealand notifiable diseases in 2013 (summarised from appendices to the 2013 ESR Public Health Surveillance report: https://surv.esr.cri.nz/PDF_surveillance/AnnualRpt/AnnualSurv/2013/2013AnnualSurvTables.pdf)

The limits given in US EPA Part 503 (1993) rule for *Salmonella* spp. are 3 MPN/4 g which according to the guidelines, are the detection limit for *Salmonella spp*. The current limit in the *Guidelines for the Safe Application of Biosolids to Land in New Zealand (2003)* is based on the requirement of the Living Earth Joint Venture (LEJV) consent figure of 1/25g. At the time, this resource consent, to produce composted biosolids from Wellington sewage sludge, contained the most comprehensive microbial risk assessment information.

Discussions with Sunita Raju, Team leader for microbiology at Eurofins in Wellington, indicated that their detection limit for *Salmonella* spp. is <2 MPN /g (pers comm 21July 2014).

Recommendation

It is recommended that the limit for *Salmonella* be reduced to the analytical detection limit for the MPN method for *Salmonella*; <2 MPN/g.

Enteric viruses

Human enteric viruses include many virus types/ groups including enteroviruses, adenoviruses, noroviruses (described in the current NZ guidelines and/ or by given in US EPA Part 503 (1993) rule, as small round structured viruses and/or Norwalk virus), astroviruses, sapoviruses and hepatitis A and E virus. Therefore a virus indicator or representative is required as it is not possible to detect all virus types using any one assay. When information on viability is required, there are only a few enteric virus candidates, due to difficulties with the culture of many of the enteric virus groups.

The current *Guidelines for the Safe Application of Biosolids to Land in New Zealand* (2003), refer to the quantification of enteric viruses with limits derived from the US EPA Part 503 (1993) rule of < 1 plaque forming unit (PFU) enteric viruses/4 g (dry solids). The methodology given in the current *Guidelines for the Safe Application of Biosolids to Land in New Zealand* (2003), is for human adenoviruses, the rationale being that human adenoviruses are generally more resistant to physical and chemical agents than other enteric viruses. For example, human adenoviruses have been shown to be more resistant to UV inactivation than enteroviruses (reviewed by Sidhu and Toze, 2009).

The US EPA specify the detection of enteroviruses as representative of enteric viruses, whilst the *Western Australian Guidelines for Biosolids Management* (2010) and the directive revision working document draft from European Commission (2000), specify the measurement of bacteriophages (coliphages) as possible indicators of enteric viruses due to their prevalence in human sewage and relative ease of culture (Pillai et al., 2011). A class I pathogen product must contain < 10 PFU/g (dry weight) of F-RNA or somatic bacteriophages in the WA guidelines.

What is the best viral indicator?

Due to their prevalence and concentration in human sewage, potential viral candidates are human enteroviruses and human adenoviruses. These viruses both contain types that are culturable, and hence their viability can be readily determined. Human enteroviruses and human adenoviruses are almost always present in untreated biosolids generated from municipal wastewater, and at concentrations between 10^1 and 10^4 per gram (Sidhu and Toze, 2009; J Hewitt, PhD thesis submitted 2014). If not present, at low concentrations in the untreated product, or at levels below the limit of detection this may be problematic if process efficiency requires evaluating as it may not be possible to determine removal efficiencies.

Methodology

Detection by culture

For culture, a number of different approaches may be used – based on culture, integrated culture and PCR and PCR alone:

- Culturable human enterovirus types readily show cytopathic effect (CPE) and can be quantified using a monolayer or agar cell suspension plaque assay using BGM cells or similar.

- Culturable human adenoviruses, particularly types 40 and 41 that are the most common human adenovirus types in wastewater, do not always readily demonstrate CPE and so plaque assays are not necessarily suitable. Methods such as integrated culture-PCR (C-PCR) that do not rely on the appearance of CPE to detect viral replication can be used to detect viable viruses. This approach has been successful in detecting human adenoviruses that are otherwise difficult to culture. This method is generally a presence/absence assay but could be easily adapted to a quantitative assay by using multiple assays/dilutions and applying a MPN approach to estimate virus concentrations. However, it is recognised that this is more costly and requires further validation before being used routinely.

The current *Guidelines for the Safe Application of Biosolids to Land in New Zealand* (2003), specify that C-PCR is used to enumerate human adenoviruses - this is the same approach as used in a current QMRA project by Sydney Water to monitor pathogen reduction in waste water treatment plants. In that project, human adenoviruses and not human enteroviruses are to be monitored.

The appendix of the current *Guidelines for the Safe Application of Biosolids to Land in New Zealand* (2003) describes methods to recover and to detect human adenoviruses in biosolids and is split into two parts. The first part describes the processing of biosolids to recover enteric viruses. The second part describes the detection of adenoviruses using C-PCR. This method has been extensively evaluated as part of a recently submitted PhD thesis (J Hewitt, PhD thesis submitted) and a number of issues were identified:

1. In the first part, an important concentration step has been (probably inadvertently) omitted. In the second part, the use of BGM cells is recommended for the C-PCR assay. While BGM cells are frequently used for the detection of human enteroviruses, they are not optimal for the detection of human adenoviruses. Instead, HEK-293 cells are better suited for the detection of human adenoviruses, particularly for types 40 and 41. These types are the most prevalent human adenoviruses in sewage and sewage sludge (J Hewitt, PhD thesis submitted; Hewitt et al., 2011). The primers recommended for the detection of adenoviruses detect all adenovirus types, and not just adenovirus types 40 and 41 as indicated in the *Guidelines for the Safe Application of Biosolids to Land in New Zealand* (2003). Work carried out by ESR showed that the integration of real-time quantitative PCR (qPCR) (instead of PCR) in the C-PCR assay was quick, sensitive and integrated well with the workflow.

2. The *Guidelines for the Safe Application of Biosolids to Land in New Zealand* (2003) limit of < 1 PFU enteric viruses /4 g originates from the US EPA methods (US EPA Appendix H: Method for the Recovery and Assay of Enteroviruses from Sewage Sludge). For this to be achieved, at least 4 g of dry weight solids needs to be tested. For the detection of human enteroviruses and adenoviruses, multiple tests are hence required. For an enterovirus plaque assay, this can be readily easily achieved, albeit multiple plates and dilutions are required. For adenoviruses, only 0.25 g biosolids can be tested per assay (depending on the concentration factor), and so as for enteroviruses, multiple replicates are needed so that a total of 1 g can be assayed. This would result in a limit of quantitation of < 1 infectious unit /g. More sample can be tested but this significantly increases the amount of work required and hence cost. The unit ‘infectious unit’ is more appropriate than PFU – a term solely used for viruses that form ‘plaque forming units‘- and is more suited to enteroviruses or bacteriophages.

Detection by PCR

Human enteroviruses and human adenoviruses can both also be readily detected using PCR and/or qPCR methods but this does not give any information on viability. Concentrations of viruses by qPCR are usually 2-4 $\log_{10}$ higher than those determined by culture.

Human adenoviruses have been proposed as molecular indices for environmental samples impacted by human wastewater due to their high concentrations, for example in receiving waters (Hewitt et al., 2013). In a New Zealand study, qPCR titres in dewatered anaerobic sludges (n=9) ranged between 10^5-10^6 genome copies/g for human adenoviruses and between 10^3-10^5 genome copies/g for human enteroviruses (Hewitt et al., unpublished). A small study on virus presence in New Zealand composted biosolids samples (n=3) showed that final human adenoviruses and enteroviruses were not detected by qPCR and so this approach may be useful for end product verification. This could be a preferred methodology over enteric virus cell culture assays for verification of Grade A composted biosolids, where the objective is to verify that viruses are removed. However, more data on viral composition of composted samples would be required to confirm that such an approach is valid.

Detection of coliphages

Viable bacteriophages (coliphages) can be quantified using a standardised (and US EPA approved) plaque assay (e.g. The APHA 2005 9224 describes 'Methods using coliphages to monitor the microbial quality of water and wastewaters). Methods include the somatic coliphages assay (9224B), male-specific coliphage assay using E. coli Famp (9224C), Salmonella typhimurium WG49 (D), single-agar-layer method (9224E), and membrane filter method (9224F).

Coliphages such as male specific F+RNA bacteriophages are present in concentrations between 10^2 and10^4/g untreated biosolids. F-RNA bacteriophages are generally present in lower concentrations in the environment than somatic coliphages. Although coliphages are better indicators of human viruses than faecal bacterial indicators, particularly in relation to the evaluation of process effectiveness, there are uncertainties around their comparative susceptibilities to inactivation processes and in relation to human enteric viruses. The *Western Australian guidelines* (2012) for biosolids management suggest that coliphages are used as enteric virus indicators. However, the suitability of F-RNA and/or somatic bacteriophage as enteric virus indicators is still largely unknown and their suitability as reliable viral indicators is still to be fully assessed. This is supported by the recent review "A review of coliphages as indicators of enteric virus risk in sewage sludges and biosolids" by Robert Humphries and Benjamin Currell (Water Corporation, WA, Australia). The authors sought expert opinion on the rationale for the inclusion of coliphages in the *Western Australian Guidelines for Biosolids Management* (2012). The authors concluded in a summary email to interested parties that

"*while coliphages are a useful indicator of enteric virus risk, the Western Australian Biosolids Guideline value is too conservative, and is not scientifically based. To our knowledge no other public health or environmental jurisdiction has adopted coliphages, and there seems to be no evidence that the conventional suite of bacterial indicators has failed. Adoption of excessively conservative indicator values threatens the reuse and recycling of biosolids and other organic wastes that may contain human pathogens*."

(Pers comm Dr Robert Humphries Email 29th July 2014).

One rationale for the selection of coliphages over human enteric virus detection by culture methods is that setting up and maintaining a laboratory that has the capability to perform coliphage (F-RNA and/or somatic bacteriophages) assays is simpler than to establish the capability for enteric virus culture. In addition, once established and in routine use, assays for bacteriophages are cheaper than for enteric viruses.

Summary of information

Overall, information on enteric virus infectivity of biosolids is limited with most available biosolids infectivity data relating to enteroviruses, reflecting the established US EPA guidelines. There are few reports on the viability of human adenoviruses, and F-RNA or somatic bacteriophages in biosolids. Furthermore, there is a lack of available data on comparative survival properties through product treatment processes of human enteroviruses, human adenoviruses and coliphages/ bacteriophages. For these reasons, the choice of the indicator is difficult, even without considering the comparative ease of setting up the laboratory and costs involved in testing. As human pathogens, the use of human enteroviruses or human adenoviruses is more informative than the use of bacteriophages. However, the detection of enteric viruses by culture is comparatively expensive as the methods are time consuming and require specialised skills, reagents and equipment, and as such may not be suitable for routine or extensive monitoring.

Recommendation

It is recommended that adenovirus is measured as the virus indicator and that the methodology in the current *Guidelines for the Safe Application of Biosolids to Land in New Zealand* (2003) is modified to reflect the correct adenovirus methodology. It is also recommended that there be an option to analyse samples for human enterovirus as an alternative to human adenovirus. Further investigation of the suitability of using qPCR for end product verification is recommended.

Cryptosporidium/Giardia

In the current *Guidelines for the Safe Application of Biosolids to Land in New Zealand* (2003) it was identified that *Cryptosporidium*/*Giardia* are *"a known problem in New Zealand"*. Indeed Cryptosporidiosis and Giardiasis rank 4th and 3rd respectively in a ranking of New Zealand notifiable diseases in 2013 (summarised from appendices to the 2013 ESR Public Health Surveillance report:

https://surv.esr.cri.nz/PDF_surveillance/AnnualRpt/AnnualSurv/2013/2013AnnualSurvTables.pdf)

However, at the time of writing of the *Guidelines for the Safe Application of Biosolids to Land in New Zealand* (2003) it was decided that:

"*Current test methods are not yet sufficiently reliable to warrant setting standards for biosolids*".

However, discussions with MPI personnel Gillian Anderson and Andrew Pearson on 2nd of July 2014, raised concerns about *Cryptosporidium* and the prevalence of this organism in cows.

Recommendation

Current test methods for *Cryptosporidium* are still not sufficiently reliable to warrant setting standards for biosolids or other organic wastes.

Helminth ova

The limit of < 1/4g helminth ova in the current *Guidelines for the Safe Application of Biosolids to Land in New Zealand (2003)* was derived from the US EPA Part 503 rule (1993) and was determined to be the detection limit at the time (i.e. 1993). The US EPA Part 503 Rule also requires a viability test. The most up to date biosolids guidelines are those from Western Australian and they require that Strongyloides and Hookworm are monitored with a viable ova limit of <1 per 50 grams of dry final biosolids, but only in certain regions of Western Australia.

Discussions with Sunita Raju, Team leader for microbiology at Eurofins in Wellington, have confirmed the detection limit of < 1/4g for the helminth test (pers comm 21st July 2014). Discussions and email correspondence with Marina Fisher, Senior Laboratory Analyst Microbiology at Watercare on the 22 July 2014 also confirmed the detection limit, and further that they do not undertake the viability testing unless specifically asked as it takes up to 4 weeks. Additionally, the methodology used does not generally identify species of helminth, just 'ova'. Marina also commented that "*we enumerate nematodes and cestodes. We don't see a lot in biosolids samples*". The potential low incidence of helminths in organic wastes may be problematic if verification monitoring has to be continued until they are detected to prove the treatment process can effectively remove them – under these circumstances an option may be to add helminth to pre-treated waste.

In the ANZBP:Review of Biosolids Guidelines (2009)" it was suggested that:

"…*the helminth criteria be removed*".

Justification for the removal of helminth criteria (email from Paul Darvodelsky), 21 July 2014 was:

"*The recommendation to remove helminth criteria is two-fold. Firstly the risk for most communities is very low because helminths are rarely present. This is not the case however for many indigenous communities in northern Australia. Secondly there are no recognised testing protocols for helminths and testing that has been done to date by seeding sludge with helminths and testing kill rates has been successful, but not what you would call very scientific. Another factor that I would see is that life cycle of helminths is probably pretty well known and if you have a process which meets certain time-temperature criteria then I would expect you could reasonably predict helminth inactivation – is that so?*"

Although there may well be justification to remove helminths as microbial indicators from biosolids monitoring, this might not be the case for other organic wastes that could be included in a new *Organic Wastes Guideline*. Meat works waste and animal manures for example, may well contain higher levels of helminths which could be a potential risk to livestock.

Discussions with the NZ Ministry of Health, Paul Prendergast and John Harding on the 10 June 2014, indicated the inclusion of monitoring for helminth ova removal was originally put forward by MPI (or MAF as they were at the time). Discussion with MPI personnel Emil Murphy once again indicated support for inclusion of helminths in any guideline.

Recommendation

It is recommended that the Steering Group members take direction from MPI on inclusion/exclusion of the helminth in any new guideline. It is also suggested that a survey is undertaken to determine how prevalent helminth ova are in biosolids in New Zealand.

3. Management Controls

Grade B

Grade B biosolids products have no set recommended maximum pathogen levels in the *Guidelines for the Safe Application of Biosolids to Land in New Zealand (2003)*. However, they must undergo processing such as anaerobic digestion to reduce vector attraction and reduce pathogens to levels that they do not pose a threat to public health and the environment as long as actions are taken to prevent exposure to the biosolids after their use or disposal.

Due to the higher levels of pathogens potentially present in a Grade B biosolids, adequate time must be allowed for the biosolids to remain in, or on, the land for natural attenuation to further reduce the pathogens before use of the land for cropping or public access. BUT natural attenuation relies on environmental factors such as temperature, UV, and indigenous microbial competition; these will vary from site-to-site and can't be controlled or predicted. This means that there must be management practices in place that, where possible, provide the "best" abiotic conditions for natural die-off.

Over the last 5 years several New Zealand specific studies have investigated microbial fate and survival in land applied biosolids. Below is a summary of key findings and recommendations.

A study investigating the survival of *E. coli* and *Salmonella* spp. in biosolids applied to a *Pinus radiata* forest (Horswell et al., 2007) found longer survival times of pathogens in cool wet conditions. Thus, they concluded that biosolids' microbes do not like warm dry conditions; apply biosolids when it is warm and dry NOT when it is wet and cold.

The above study also found that withholding periods of greater than 6 months are sufficient to reduce microbial contaminants to background levels. However, a PhD study undertaken by Jason Levitan, (2010) "Die-off of pathogens and assessment of risks following biosolids application in pine plantations" (Murdoch University) found that pathogen re-growth can occur if the conditions are right up to 1.5 years after biosolids were applied to forestry.

A study investigating the mobility and survival of *Salmonella Typhimurium* and human adenovirus from spiked sewage sludge applied to soil columns (Horswell et al., 2010) found that if transported below the top layers of the soil, pathogens can survive for extended periods of time; and that biosolids may enhance mobility and survival, possibly due to enhanced microsite habitat and the addition of nitrogen, thus groundwater contamination from vertical movement of pathogens is a potential risk.

The ANZBP recently produced an update of new literature assessing the risks/hazards and pathogens of primary concern for both Australia and New Zealand (ANZBP 'Pathogen Presence & Pathways Report' Ang, R. 2013), including information on survival and fate of pathogens in soil and vegetation.

"*Table 2: Survival and fate of pathogens from wastewater/biosolids in soil and vegetation"* below, illustrates that pathogens can survive for extended periods of time in soils.

Table 2: Survival and fate of pathogens from wastewater/biosolids in soil and vegetation.

Organism	**Survival in soil**	**Survival in vegetation**	**Notes**
Bacteria	Days - months. Can survive as long as a year in soil.	Days - months. Can survive as long as 6 months on plants.	Depends on temperature and moisture. Can multiply under appropriate conditions in biosolids, soil, or on vegetation. Much variation in survival time between species. *E. coli* or thermotolerant coliforms have similar survival dynamics as *Salmonella*, *Campylobacter*, and *Shigella* spp., and can therefore be used as reliable indicator organisms for these pathogens. However, *Legionella* spp., *Leptospira* spp., *P. aeruginosa*, *V. cholera*, and *Yersinia* spp. can persist far longer than *E. coli*.
Helminths	Weeks - years. Can survive as long as 7 years in soil.	Days - months. Can survive as long as 5 months on plants.	Obligatory parasites, therefore unable to multiply in biosolids, soil, or on vegetation.
Viruses	Days – months.Can survive as long as 6 months in soil.	Days - months; generally for shorter periods than in soil, and depends on precipitation, type of vegetation etc. For example, smooth-surfaced tomatoes provided a more favourable environment than on cabbages.	Obligatory parasites, therefore unable to multiply in biosolids, soil, or on vegetation. Temperature is the main controlling factor, with colder conditions favouring survival.
Protozoa	Up to 10 weeks in normal soil; >12 weeks in sterile, autoclaved soil.	Up to 5 days.	Obligatory parasites, therefore unable to multiply in biosolids, soil, or on vegetation. Depends on temperature and desiccation, and variations exist between species.
Fungi and yeasts	At least a year.	Little information.	Affected by pH; they grow better in slightly acid conditions ~pH 5-7.

(Source: ANZBP 'Pathogen Presence & Pathways Report' Ang, R. 2013).

Before lifting site restrictions it is sensible to carry out soil testing to ensure that there has been no cumulative increase in microorganisms due to biosolids application. *Escherichia*

coli is recommended for soil monitoring as it is neither arduous nor expensive, and may provide useful information on pathogen die-off in receiving soils. Control samples (i.e. from an adjacent site that has not had any biosolids applied to it) should be taken before application and at the end of the restraint period to determine 'background' *E. coli* numbers as these may fluctuate naturally (with season), high background levels could also indicate input from feral animals, or from birds. If numbers of *E. coli* are found to be 100 fold higher than background counts, decisions about further restricted access or land-use should be made on a case-by-case basis after consultation with the local Medical Officer of Health (Health Act, 1956). For example, if access is required to fell trees, forest workers could be exposed to elevated levels of pathogens in dust particles. The above approach is also supported in the Western Australian Guidelines (2012) in some circumstances:

"Depending upon the location of the application site, regulatory agencies may request monitoring at the site by the supplier for a specified period. Monitoring after application is to ensure that there are no adverse effects on public health or the local environment."

Most guidelines state that application of biosolids during heavy rainfall should be avoided.

The Western Australian guidelines for biosolids management (2012) state that:

"*The application of biosolids should not occur during rainfall events or when heavy rains are forecast*".

This is also recommended in the current *Guidelines for the Safe Application of Biosolids to Land in New Zealand (2003)*. This is primarily to avoid nutrient run-off but will also reduce the risks of pathogen run-off and the potential to contaminate surface and ground water. Heavy application rates of wastes to soil can increase soil saturation, which can also increase pathogen mobility and decrease moisture loss, and these can increase survival times.

Soil type is a critical factor in determining the potential for microbial leaching from biosolids applied to land – some soils are more high risk than others. This is acknowledged in the *Guidelines for the Safe Application of Biosolids to Land in New Zealand (2003)*, and in section "5.1.2 Soil type" of the Western Australian guidelines for biosolids management (2012). Landcare Research has produced a large body of research on the potential of bypass flow in New Zealand soils (McLeod et al., 2008).

Recommendations

Before lifting site restrictions, carry out soil testing to ensure that there has been no cumulative increase in microorganisms due to biosolids application.

Where possible organic wastes that may contain pathogens should be land applied when the weather is warm and dry.

As activity constraints and withholding periods do not protect against surface-runoff and leaching – it is essential to ensure adequate buffers are in place between the application site and receiving environments (e.g. surface water and groundwater) including food crops in adjoining fields.

Be precautionary when applying large amounts of biosolids to a site (e.g. forest sites where large amount can be applied bi-annually).

Avoid soils with high by-pass flow potential - leaching can be greater on these soils. Even in winter when soil cracks are closed the cracks provide preferential pathways for the movement of water and entrained microbes.

Soil incorporation

Both the *Guidelines for the Safe Application of Biosolids to Land in New Zealand* (2003), and the *Western Australian guidelines for biosolids management* (2012) recommend soil incorporation for biosolids. Soil incorporation is an important risk management strategy as it can:

- Reduce exposure to pathogens
- Remove vector access to pathogens
- Reduce risk of surface run-off
- Increase die-off
- Reduce chances of public contact

The *Western Australian guidelines for biosolids management* (2012) state that:

"…*biosolids applied to land in a rural setting should be spread evenly and then incorporated into the topsoil within 36 hours. Incorporation reduces odour problems, vector attraction, nitrogen loss through volatilisation and surface losses due to erosion, and improves the availability of phosphorus.*"

Recognising that under some circumstances:

"*Soil incorporation may not be compatible with all farming systems (for example, no till agriculture) and certain soil and weather conditions can cause significant environmental harm (for example, soil compaction, dust and erosion). Agricultural application of biosolids without the requirement for incorporation within 36 hours will only be considered for times of year where soil conditions are not suitable for incorporation at the time of conducting the biosolids application.*"

The review of the ANZBP: Review of Biosolids Guidelines (2009), determined that soil incorporation was flexible in some states. For example New South Wales, Queensland and Western Australia have a 36 hour incorporation rule; in Tasmania there should be incorporation where possible but not required in all circumstances.

The *Guidelines for the Safe Application of Biosolids to Land in New Zealand* (2003), clearly state that:

"*For pastoral land, soil incorporation is a vital risk management tool. As noted in sections 2.2.3 and 2.4, there is concern about the potential to contaminate meat or dairy produce via direct ingestion of pasture and/or surface soil. Consequently, consent conditions relating to discharges of biosolids to pastoral land should also include a requirement for soil incorporation (biosolids can be applied to pastoral land when pasture is resown).*"

The *Guidelines for the Safe Application of Biosolids to Land in New Zealand* (2003), go on to state that:

"…consent authorities should attach a condition requiring soil incorporation (pre-planting) on all consents relating to discharges of biosolids to horticultural or cropping land. This will negate the undesirable practice of applying biosolids to leaf, salad, or root crops where there is a risk of direct transfer to animals or humans."

A need for this requirement will depend on the vector attractant reduction criteria (for example in WA flies are particular vectors of concern), land use (e.g. horticultural land, stock grazing), climate (high rainfall areas will have increased risk of surface run-off if the waste is not incorporated), solids content of the waste (for some wastes a very low solids content may increase risks for leaching/by pass flow if ploughed in). In addition, the implications of this risk management control for the application of other organic wastes such as meat works sludge etc must be assessed as in general resource consents for this type of organic waste do not require soil incorporation.

4. Sampling and monitoring

Verification sampling

The minimum number of samples that should be taken in each monitoring phase and for each grade are detailed in "Table 8.1. Stabilisation grade sampling frequencies" of the *Guidelines for the Safe Application of Biosolids to Land in New Zealand* (2003),

Table 8.1: Stabilisation grade sampling frequencies

Grade	Monitoring type	Sampling regime	Parameters to be monitored
A	Product verification[1,2]	≥ 15 evenly dispersed grab samples per month for a 3-month period with ≤ 3 failures. If > 3 failures then the 15 following consecutive grab samples must comply.	■ *E. coli* ■ *Salmonella* ■ *Campylobacter* ■ enteric viruses ■ helminth ova ■ VAR
	Routine sampling	≥ 1 grab sample per week	■ *E. coli* ■ VAR
B	Product verification[2]	Not applicable for pathogen testing	■ VAR[3]
	Routine sampling	Not applicable for pathogen testing	■ VAR[3]

(Source: Guidelines for the Safe Application of Biosolids to Land in New Zealand (2003))

The 15 grab samples mentioned in the above table are not designed to result in statistically representative data.

The *Western Australian guidelines for biosolids management* (2012), specify collecting only seven samples for pathogen and volatile solids reduction monitoring, the default routine sampling regime is one sample per 300 dry tonnes. For pathogen re-growth, the default sampling regime is three samples tested annually.

In an email (30th July, 2014) offering more explanation, Nancy Penny, Biosolids and Sludge Management Section Leader at The Water Corporation in WA, stated that:

"*The WA biosolids guidelines don't actually have a number of samples to be taken as such for verification of a process. This is typically agreed with the DoH and is dependent on if the process is a new technology (for WA) or if we have existing parameters / concentration to work to. We do have a minimum number of samples to be taken once a process is established and for continuous processes this is based on 1 sample per 300 dry tonne which equates to 1 sample per week. This is also the case for pathogens (E.*

coli) which we report as a log reduction for processes that have digesters. To allow for variation we do a rolling geometric mean of 7 samples. Although we do weekly sampling we are only required to report exception through the year with all data reported end of year."

The US EPA Part 503 Rule (1993) does not specify a number of samples but states in "Table 6-1 Summary of Biosolids Sampling Considerations"

How Should Sampling Be Done and How Many Samples Should Be Taken?	Take either: Grab samples[b] (individual samples) for pathogens and percent volatile solids determinations, or Composite samples[b] (several grab samples combined) for metals. No fixed number of individual samples required (except for Class B pathogens, Alternative 1, take 7 samples). Enough material must be taken for the sample to be representative. Take a greater number of samples if there is a large amount of biosolids or if characteristics of biosolids vary a lot. See Table 6-4 for guidance (e.g., continuous, instantaneous, or monthly averages required).

(Source: US EPA; US Environmental Protection Agency (1993) Part 503-Standards for the Use or Disposal of Sewage Sludge. Federal Register 58, 9387-9404.)

Routine sampling

The current *Guidelines for the Safe Application of Biosolids to Land in New Zealand* (2003), require > 1 grab sample per week for *E. coli*.

In an email communication from Paul Darvodelsky on 25 July 2014, he related a conversation with Al Rubin from the USA:

"*In general they require a minimum of about 1 sample per year. The logic however was that there are three parts of the guidelines which protect human health and the environment. These are contaminant/pathogen levels, management practices and sampling and monitoring (knowing what's happening). The US EPA's view was that the levels set when combined with the management practices gave such a conservative approach that there was little need to also add the burden of a high level of sampling and monitoring. It was deemed unlikely that many utilities would not follow the guidelines and therefore the risk to community was therefore very low. Because of the cost of sampling and monitoring they relaxed this section of regulation.*"

However the requirement in the current *Guidelines for the Safe Application of Biosolids to Land in New Zealand (2003)* of > 1 grab sample per week for *E. coli* is not expensive or arduous bearing in mind that sampling for other chemical parameters is likely also being undertaken at the same time.

Recommendation

It is recommended that number of samples required for verification sampling be reduced from 15 to 7, in line with the Western Australian guidelines for biosolids management (2012).

PRIMARY SECTOR RELATED ORGANIC WASTE

Wastes included in this category are agricultural wastes such as meat works waste, manure (e.g. chicken, pig) and animal bedding (poultry industry wood shavings and bark).

Pathogens of primary concern in animal wastes

The major source of pathogens in animal manure that are of risk to humans are similar to those found in human sludge and are summarised in the table below.

Table 3. Select pathogens found in animal manure.

Pathogen	**Disease**
Bacillus anthracis	Anthrax
Vero cytotoxin-producing *Escherichia coli* O157 (VTEC O157)	VTEC O157 is found in the faeces of healthy livestock and is not necessarily associated with disease or production loss. The organism is excreted in both faeces and saliva and is a potential risk to people working in close contact with, or visiting, farm animals and their environment. Excretion is intermittent and variable and the organism can survive for long periods of time in the environment (several months), which is thought to be important in maintaining infection within the herd through re-infection.
Leptospira pomona	Leptospirosis
Listeria monocytogenes	Listerosis
Campylobacter species	*C. jejuni* and *C. coli* rarely, if at all, cause disease in animals under natural conditions. Nevertheless, surveys indicate that the intestinal carriage rate is high in healthy farm animals, poultry, pets, and wild birds and environmental contamination with *Campylobacter* species from faecal material is frequent.
Salmonella species	When livestock, particularly poultry and pigs, become infected with Salmonella, they frequently become carriers of the infection without showing any clinical signs of ill health. Infection, however, may occasionally result in disease such as enteritis, abortion, septicaemia, or death. Some serotypes or strains may cause particularly severe illness, especially in ruminants. Livestock are normally kept in groups, so one infected animal may pass the organisms to others within the group. In many cases, carriage of *Salmonella* in groups of farm animals resolves spontaneously but cycling of infection between different groups of animals may prolong the persistence of infection on some farms.
Clostirdum tetani	Tetanus

Histoplasma capsulatum	Histoplasmosis
Microsporum and Trichophyton	Ringworm
Giardia lamblia	Giardiasis
Cryptosporidium species	Infection may be found in clinically normal livestock. When disease occurs, it is most often seen in young animals, particularly calves, but also lambs and occasionally piglets. In lambs an infection with *Cryptosporidium* is often asymptomatic despite excreting in excess of 5 x 10^6 oocysts per gram of faeces. These non-clinical, highly infectious cases pose a risk to both the public, and the veterinary health of other livestock as there are no indications to isolate these animals from other livestock or human contact. Co-infection with other pathogens such as rotavirus and *E. coli* may also be present during clinical disease outbreaks. Clinical signs include diarrhoea, weight loss and anorexia.

(Source: WHO. 2012. Animal Waste, Water Quality and Human Health. A Dufour, J Bartram, R Bos, et al (eds). Published on behalf of the World Health Organization by IWA Publishing, UK. 489 pp. http://www.who.int/water_sanitation_health/publications/2012/animal_waste/en/; Ministry of Health. 2013. Guidelines for Drinking-water Quality Management for New Zealand 2013. Third edition. Wellington: Ministry of Health.)

Dairy Shed Effluent (DSE)

Discharge of DSE is a permitted activity and is well controlled by both Regional/District Councils and Dairy NZ. There are a number of good management practice guidelines available from the Dairy NZ website.

Recommendation

Dairy Shed Effluent should not be considered under this guideline.

Animal wastes that are treated to meet Grade A

The microbial indicators required for verification testing under the current *Guidelines for the Safe Application of Biosolids to Land in New Zealand (2003)* also represent the major group of risk organisms potentially found in animal manures and meat works wastes.

Recommendation

If a product is to be sold and/or handled by the public then it should effectively be pathogen free and be subject to the same treatment as a Grade A biosolids.

Animal wastes that are treated to meet Grade B

The land application of wastes such as piggery manure is a controlled activity under the Resource Management Act and requires resource consent.

In general there are currently no requirements to process agricultural wastes to reduce pathogens prior to land application. For these wastes the main risk mitigation is "good husbandry practices" and ensuring that wastes from pigs, poultry and dairy cows should be free of major diseases. Management controls such as:

- Proximity to groundwater: do not apply effluent to land within 50m of any wells or bore used for water supply purposes.
- Proximity to surface water: do not apply effluent to land within 25m of a surface water body.
- Suitable withholding periods prior to grazing (allowing maximum exposure to sunlight)
- Application well away from public places, cropping paddocks and horticultural blocks as disease causing micro-organisms may live in the effluent and can pose a risk to both animal and human health
- (Sources: Appendix VIIIC Taranaki Regional Freshwater Plan; Dairy NZ Effluent Resources, DNZ40-001.)

The Biosecurity (Ruminant Protein) Regulations (1999) place certain restrictions on the disposal of wastewater from the meat processor and rendering plants on land where ruminant animals may graze. The following advice is given for the surface application of materials to pasture and also applies to farmland generally:

- Paunch contents and manure may be applied to pasture.
- Ruminant animals may graze on land where such paunch contents and/or manure have been applied provided there are no visible signs of gut material/ ruminant protein. Ideally the paunch material will have been composted first.
- Before applying to pasture, slaughterhouse and rendering plant wastewater must be treated to remove float materials and sediments, and screened to the extent that it is suitable for spray irrigation. Ruminants may graze on pasture where wastewater has been applied provided the land and the vegetation are not visibly contaminated by the wastewater.
- Pasture may be harvested for feeding to ruminants provided the land and the vegetation are not visibly contaminated by the wastewater.
- Slaughterhouse and rendering wastewater treatment plant sludge may be applied to pasture provided the floating debris and settled solids in the wastewater were removed prior to treatment. Ruminant animals may graze on land where such sludge has been applied.

Poultry litter management

Bedding material for meat chickens, turkeys, ducks and layer hens consists of wood shavings and bark. The poultry industry generally removes litter from their operations regularly though-out the year and the used litter (thousands of tonnes) is generally spread on fields such as general land/farming applications, spread on dairy pasture and spread on mushroom/maize fields; a very small amount is composted.

(Source: Poultry management in New Zealand: production, manure management and emission estimations for the commercial chicken, turkey, duck and layer industries within New Zealand MAF Technical Paper No: 2012/15 Report prepared for Ministry of Agriculture and Forestry By Poultry Association of New Zealand and Egg Producers Federation of New Zealand April 2012).

Recommendation

There appear to be limited safeguards to protect public and animal health from potential pathogens in land applied animal wastes. It is recommended that as for biosolids these wastes must undergo some form of process to reduce pathogens so that they do not pose a threat to public health, animal health or the environment; if these process controls are combined with management practices (as set out under the *Guidelines for the safe application of Biosolids to land in New Zealand* (2003) with respect to buffer distances, with-holding periods etc) the risk of microbiological hazards impacting livestock or human health is low.

RECOMMENDATIONS AND CONCLUSIONS

Many organic wastes have commonality in terms of the pathogens that they contain and in eventual end-use (e.g. soil compost or conditioner). This review provides recommendations for microbial quality criteria for beneficial re-use of organic wastes based on protection of public health.

The *Guidelines for the Safe Application of Biosolids to Land in New Zealand* (2003) are the most comprehensive guideline with respect to microbial quality criteria for organic wastes. Strongly based on the US 40CFR503 rule (1993), they provide detailed descriptive guidance to potential biosolids users and describe "good practice". Since the development and release of the US regulations there has been a significant development of understanding of biosolids use, and the scientific understanding of the impacts and benefits of biosolids (and organic wastes as a whole) has progressed. In addition, there have also been a number of studies undertaken that can provide more information specific to New Zealand's soils, climate and production systems. This review aims to summarise recent advances in biosolids practice and research and provides recommendations for a new 'Organic Waste Materials Guideline'.

The opportunities for improving and rationalising organic waste regulation, based on an expansion of the *Guidelines for the Safe Application of Biosolids to Land in New Zealand* (2003) to integrate other wastes are discussed below in the form of a number of questions.

What is the justification for the inclusion of the following pathogens: *E. coli, Campylobacter; Salmonella;* enteric viruses; helminth ova, in the current *Guidelines for the Safe Application of Biosolids to Land in New Zealand* (2003)?

Any new guideline should retain the two pathogen grades A and B. The highest level of pathogen treatment should produce a product that is effectively 'pathogen free' and able to be directly handled by the public with minimal public health risks.

Pathogen reduction requirements for Grade A products should be performance based as opposed to process based. The microbial indicators of a range of pathogens are required to prove pathogen destruction; monitoring *E.* coli alone is not adequate. The microbial indicators in the current *Guidelines for the Safe Application of Biosolids to Land in New Zealand* (2003) are justified and should be retained with respect to: *E. coli, Salmonella spp.*, *Campylobacter spp.* and enteric virus. *E. coli* are used internationally as a biological indicator of faecal pollution; *Salmonella* spp. are traditionally used as an indicator for bacterial removal; *Campylobacter spp.* are required in New Zealand due to the high incidence of campylobacter infection. Human adenoviruses and enteroviruses are suitable indicators for removal of virus by treatment processes. Decisions on retention of helminth ova monitoring should made by The Ministry for Primary Industry based on risks to live stock.

Are there any other pathogens that should and should not be in a new guideline?

For *Cryptosporidium*, there is some justification for inclusion of this organism in microbial monitoring for Grade A products, due to prevalence and impacts on livestock, and high

disease rate in humans. Data from a comprehensive study undertaken by Sydney Water, monitoring the efficiency of sewage treatment processes on removal of a number of microbes, including *Cryptosporidium,* will aid decision making**.**

Do other organic wastes contain additional pathogens of concern that should be included in a new guideline?

The microbial indicators recommended for monitoring in organic wastes derived from human wastes (discussed above) represent the major group of risk organisms potentially found in animal manures and meat works wastes and will adequately assess any potential risks.

Are the detection methods/limits for pathogens in the current *Guidelines for the Safe Application of Biosolids to Land in New Zealand* (2003)) adequate?

Over the last ten years there has been significant method development in the area of environmental microbiology. For the microbial indicators that must be measured for verification monitoring to produce a product that is safe to be handled by the public (i.e. Grade A), limits should be based on methodological detection limits.

The current methods described for the recovery and detection of human adenoviruses in biosolids in the appendix of the *Guidelines for the Safe Application of Biosolids to Land in New Zealand* (2003) has been extensively evaluated as part of a recently submitted PhD thesis (J Hewitt, PhD thesis submitted). An important concentration step has been (probably inadvertently) omitted There are also modifications required in the detection step, including choice of cell line. Hence the methods need to be amended. An option to analyse samples for human enterovirus as an alternative to human adenovirus would also be useful. Molecular methods such as qPCR, although unable to report on viability, could be relevant for end product verification.

Methods sourced from the Sydney Water, once available, should be reviewed especially for *Cryptosporidium* and helminths.

What additional controls are important for land application of organic wastes that may still contain pathogens (e.g. Grade B)?

Management restrictions and guidance in *Guidelines for the Safe Application of Biosolids to Land in New Zealand* (2003) should be retained in a new 'Organic Waste Materials Guideline' with respect to managing public health risks from the land application of organic wastes that have lower levels of treatment (e.g. Grade B). Adequate time must be allowed for the product to remain in, or on, the land for natural attenuation to further reduce the pathogens before use of the land for cropping, stock grazing or public access. Restrictions such as buffer zones must be in place to prevent contamination of ground and surface waters.

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