Literature review:

Legionella in recycled wastewater and greywater

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ABBREVIATIONS

BOD_5	5-Day biochemical oxygen demand
CFU	Colony-forming units
DALY	Disability-adjusted life years
DOC	Dissolved oxygen concentration
EMA	Ethidium monoazide
ESR	Institute of Environmental Science and Research
FAC	Free available chlorine
GC or GU	Genome copies or genome units
mgd	Million gallons per day
MPN	Most probable number
OTU	Operational taxonomic unit
QMRA	Quantitative microbial risk assessment
qPCR	Quantitative polymerase chain reaction
RO	Reverse osmosis
SDG	Sustainable Development Goal
spp.	species (plural)
TSS	Total suspended solids
US EPA	United States Environmental Protection Agency
UV	Ultraviolet
WHO	World Health Organization
WQMP	Water quality management plan
WWTP	Wastewater treatment plant

EXECUTIVE SUMMARY

Wastewater recycling, especially the reuse of greywater, is increasingly being seen as one approach to addressing the challenges posed by population growth, urbanisation, and climate change for accessing or conserving drinking water. However, there is a risk that the quest to find more sustainable solutions for water management could have unintended consequences if factors such as adequate wastewater treatment and storage are not addressed, or the water is utilised in a manner that increases the exposure risk to pathogens present in the water. There is empirical evidence that *Legionella* bacteria can survive and multiply in wastewater and greywater, and legionellosis cases have been associated with wastewater and wastewater treatment plants. However, there is significantly less evidence when only recycled greywater is considered, and there have been no published cases of legionellosis from these systems.

In New Zealand, *Legionella* bacteria have been isolated from wastewater treatment systems, including both sewage treatment plants and those used to process biological waste from industrial processes. *Legionella* bacteria have been found in both the treated effluent water and the sludge from waste treatment plants. However, there has only been anecdotal evidence of an association between legionellosis and wastewater (including greywater) and wastewater treatment systems. The lack of a conclusive link may be due in part to very limited sampling, as well as the intrinsic difficulty in recovering viable *Legionella* bacteria from wastewater samples.

Legionella bacteria are considered some of the most significant opportunistic waterborne pathogens and have been shown to persist and proliferate in both wastewater and recycled greywater systems. Recycled wastewater and greywater, along with its associated infrastructure, provides physicochemical and biological conditions that are favourable for the growth and persistence of *Legionella* bacteria. These include high levels of assimilable organic carbon, warm temperatures, difficulty in maintaining an effective biocide residual in treated effluent waters and associated plumbing infrastructure, and the frequent presence of amoeba hosts. Therefore, further studies using a quantitative microbial risk assessment approach are required to determine the true risk *Legionella* bacteria pose in recycled water systems.

There are currently no national standards or guidelines for wastewater and greywater reuse in New Zealand, with only disparate sources of information available. However, Australia has developed guidelines that have formed the basis of international guidelines in high-income countries, and the potential health risk from *Legionella* is specifically recognised in the 2020 guidelines from Alberta, Canada. These guidelines cover a wide range of uses and require the development of water quality management plans, which must include managing the growth of opportunistic pathogens such as *Legionella.*

Current regulations and standards frequently use faecal indicator bacteria to ensure water quality and safety, when taking a risk-based approach. However, this does not ensure the elimination of pathogenic viruses, protozoa, and non-enteric bacteria such as *Legionella*, which can proliferate in wastewater and greywater systems in the absence of adequate disinfection processes. There is also a need to address any concomitant risk associated with the use of recycled water, as some methods of discharge pose a greater risk of exposing the user to opportunistic pathogens than others. This risk can be managed by setting stricter treatment and monitoring criteria for recycled water where the infection risk is higher.

1. INTRODUCTION

1.1 DEFINITION OF GREYWATER AND WASTEWATER

Wastewater is the collective term for any waterborne waste originating from human activity and is generally categorised into stormwater, greywater and blackwater. Stormwater is runoff from hard surfaces (roads, paved areas, and roofs) following precipitation events. Greywater is defined as all household wastewater excluding toilet waste (faeces and urine). The term ‘light greywater’ is often used when referring to wastewater from bathroom showers and basins only, while ‘dark greywater’ refers to grey wastewater that contains more contaminated waste from laundry facilities, dishwashers, and kitchen sinks. However, the distinction between ‘light’ and ‘dark’ greywater based on organic load should not imply any significant difference in faecal indicator bacteria present (Leonard *et al.*, 2016). Toilet wastewater is frequently referred to as ‘blackwater’.

Since greywater does not include toilet waste, it is generally subjected to less microbial contamination and contains lower concentrations of organics and nutrients than mixed wastewater. However, the contaminating constituents of greywater vary depending on the source and influence both the makeup of the microbial population and the abundance of each species present. Some jurisdictions also exclude wastewater from food preparation areas and dishwashers from greywater to reduce the nutrient load and decrease the likelihood of bacteria such as *Salmonella* and *Campylobacter* being present in the waste stream (Busgang *et al.*, 2018; Ottoson and Stenström, 2003). This waste stream will contain oils and fats that can cause offensive odours and block pipework in untreated or simple treatment systems. Wastewater from dishwashers and laundries usually has a very high pH, which will influence the makeup of the microbiome (Bakare *et al.*, 2017).

The main differences between greywater and mixed wastewater are as follows:

- Greywater contains only about one-tenth of the total nitrogen compared with blackwater (Gray and Becker, 2000).
- Since mixed wastewater (containing faecal material) is excluded from greywater, there is a significantly lower organic matter content and pathogen load in greywater (Booker, 2001).
- The organic content of greywater decomposes more rapidly than that of blackwater (Oron *et al.*, 2014).
- Greywater can be applied directly to the root zone of plants, leading to the assimilation and further breakdown of organic matter (Andrews *et al.*, 2004; Prasad *et al.*, 2021).

1.2 GLOBAL AND DOMESTIC SETTINGS FOR WASTEWATER REUSE

Urbanisation, climate change, and natural disasters exacerbate both urban and rural water security concerns and cause municipalities and utilities to seek alternative drinking water sources and ways to reduce or reuse wastewater (Bint *et al.*,2019; Gholami-Shabani and Nematpour, 2024; Lee and Jepson, 2020). Climate change modelling for New Zealand predicts more frequent droughts, especially in eastern and northern regions, coupled with more frequent extreme rain events in western New Zealand and in the south of the South Island (EHINZ, 2024). In terms of natural disasters, the 2011 earthquake in Christchurch, New Zealand, caused considerable disruption to the reticulated drinking, waste and stormwater systems for many years, resulting in the city installing a greywater recycling and rainwater harvesting system for commercial buildings to provide water during and after a natural disaster (Bint *et al.*, 2019). Increasing urbanisation, coupled with limited access to reliable water sources, has seen water reuse being encouraged in New Zealand to reduce the demand on drinking water supplies, with many of the councils that promote wastewater or greywater reuse frequently experiencing seasonal potable water demand that outstrips availability, especially in the summer months, leading to water rationing.

In 2015, the United Nations set up 17 Sustainable Development Goals (SDGs) as part of their agenda for people, the planet, and prosperity through to 2030. The SDGs aim to stimulate action in areas of critical importance to humanity and the environment. Within the 17 SDGs, Goal 6 is titled 'Ensure access to water and sanitation for all'. This Goal has a number of key targets, including target 6.3: "By 2030, improve water quality by reducing pollution, eliminating dumping and minimizing release of hazardous chemicals and materials, halving the proportion of untreated wastewater and substantially increasing recycling and safe reuse globally"[1]. The SDGs bring together the water issues faced by the international community and demonstrate that even in a 'water-rich' country like New Zealand, there is a need to adjust current practices to meet increasing demand for access to safe drinking water.

The amount of domestic greywater produced in countries with developed economies ranges from around 35 to 200 litres/person/day compared to 20 to 30 litres/person/day in low and middle-income countries (Gross *et al*., 2015; Oteng-Peprah *et al.* 2018). The difference is most likely due to households having easier access to centralised water distribution systems in developed economies. Since greywater accounts for 40–70% of all water discharged to waste, developing and implementing systems for greywater reuse either as a single household, a neighbourhood or a community has significant potential as a water resource (Oron *et al.*, 2014).

In New Zealand, water usage ranges from 200 to 300 L/person/day, about 20–24% of which is used for toilet flushing (Whittaker *et al.*, 2022). This gives an average daily household production of 543 L of wastewater, approximately 70% by volume of which is greywater, demonstrating the scale of a resource that could potentially be recycled to reduce demand for treated drinking water. The amount of wastewater produced from the average New Zealand household is summarised in Table 1.

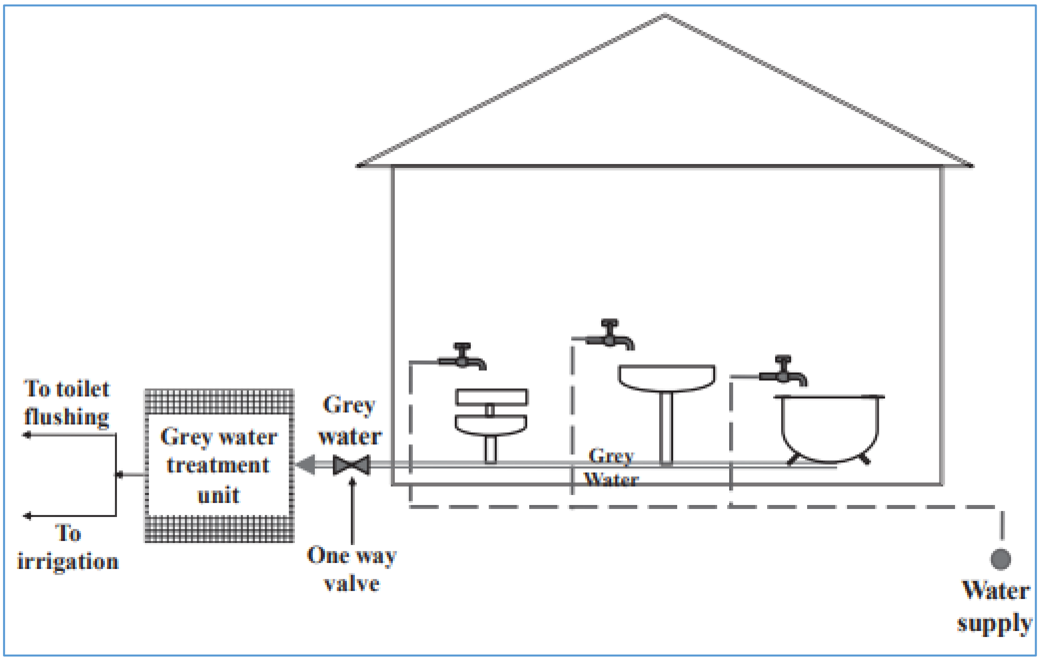
Table 1. Average daily wastewater production for a household in New Zealand*

Wastewater grade	%	Volume (L)	Source
Light greywater	31	168	Shower
	19	103	Tap
	7	38	Outdoor
Heavy greywater	13	71	Washing machine
	3	16	Dishwasher
Blackwater	24	130	Toilet
Other (including leaks)	3	16	Undefined
Total	**100**	**542**	
* Calculated from data supplied in Whittaker *et al.* [2022]			

[1] United Nations Sustainable Development Goals. https://www.un.org/sustainabledevelopment/water-and-sanitation/

Figure 1 shows a schematic of a typical single household greywater collection system where the treated greywater can be reused for toilet flushing or in areas close to the house for lawn or garden irrigation.

FIGURE 1: Schematic of Greywater collection system.



From Oron *et al.*, 2014. (Reproduce under Licence # 5803440619137)

1.3 WASTEWATER AND GREYWATER REUSE IN NEW ZEALAND

New Zealand has a high level of rainfall and a small population relative to other countries, which has meant that historically the reuse of wastewater has not been seriously considered. Greywater is generally perceived via psychosocial factors as being safer for reuse than sewage due to the exclusion of human faeces (Mohamed *et al*., 2019). Greywater is reused directly without further treatment in many low-income and water-deficient rural areas (Oh *et al*., 2018). In some parts of New Zealand, it is common practice to allow untreated greywater from laundries and bathrooms to be irrigated onto lawns or non-food producing vegetation via surface or subsurface driplines. However, the diversion of greywater for disposal purposes is often unregulated and compliance with regulatory requirements is lacking (Siggins *et al.*, 2016).

Greywater reuse is already a ‘permitted activity’ with some councils (e.g. Northland, Bay of Plenty, Hawke’s Bay, Tasman, Canterbury and Otago), but requirements vary greatly between regions and even between different councils within the same region (Garnet, 2013). Access to an alternative non-drinkable water supply is mandatory for new residential builds in the Kāpiti Coast District and is a permitted activity. Where untreated greywater reuse is permitted, it can only be collected from laundries and bathrooms and discharged via subsurface irrigation (KCDC, 2017). Additionally, Kāpiti District Council’s Code of Practice Guidelines state that before the greywater can be used for toilet flushing or surface irrigation, it must be treated, although no information is provided on what treatment methods are appropriate or how to measure compliance (KCDC, 2017).

The community-wide adoption of water recycling systems is seen as one way for Watercare to meet their 30-year goal of reducing drinking water consumption per capita. As part of this strategy, in April 2023, Auckland Watercare endorsed the use of the German Hydraloop® in-home water recycling system, which purportedly can reduce household potable water use by up to 45%. Greywater recycling requires the installation of a small storage tank (up to 160 L/person for a single dwelling) for 'short time' storage, along with chlorine or ultraviolet (UV) disinfection treatment and building consent from the Auckland City Council.

The environmental and economic benefits of reusing wastewater at both local and regional levels are expected to increase due to increasing population urbanisation, increasing freshwater scarcity and global weather change. These benefits are likely to drive the increased adoption of wastewater reuse practices. Many households in rural communities are already disposing of their wastewater on site using septic tank systems, and a few may use composting/waterless toilets where no centralised wastewater treatment system is available. Some of these households use greywater and blackwater separation, with untreated greywater being disposed of via subsurface irrigation, and since there is no aerosolisation of this water, the risk of infection via inhalation due to aerosolisation is considered to be negligible. Some of the drivers for greywater reuse globally are summarised in the box below.

Drivers for greywater reuse (global perspective)

- Address local water accessibility issues, including:
  - water scarcity
  - limited access to a reliable drinking water source
  - low resilience in dry periods
  - high infrastructure costs.
- Reduce wastewater burden by putting less water into:
  - municipal wastewater treatment systems
  - on-site treatment systems such as septic tanks.
- Address issues related to on-site limitation for sewage disposal, including:
  - poor site drainage
  - small discharge area available.

2. MICROORGANISMS IN REUSED GREYWATER OR WASTEWATER

2.1 PATHOGENS IN GREYWATER AND WASTEWATER

The health risks posed by greywater and wastewater reuse are usually evaluated using the faecal coliform *Escherichia coli* as an indicator of faecal contamination (Gassie and Englehardt, 2017; O'Toole *et al*., 2012). This is primarily because the tests for faecal coliforms, including *E. coli*, have been historically used as indicators of human faecal contamination of water and can be carried out relatively cheaply and quickly compared with measurements of other potential pathogens. However, faecal coliforms are a poor and inappropriate indicator of the levels of protozoa, viruses and opportunistic pathogens, including *Legionella*, in greywater and wastewater.

Since the microbial composition of domestic greywater is dependent on household factors, such as the age and number of household members, lifestyle and sanitation standards, the frequency of detergent and disinfectant use, and the volume of water usage, it can be extremely variable. Almost every study that has investigated the microbial risks in wastewater has focused primarily on faecal contaminants (Friedler *et al*., 2011; Ottoson and Stenström, 2003), with few studies having investigated the presence of skin and mucous tissue pathogens like *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Casanova *et al.*, 2001; Gilboa and Friedler, 2008; Winward *et al.*, 2008), and even fewer having considered opportunistic pathogens such as *Legionella* (Blanky *et al*., 2015; Jjemba *et al.*, 2010).

Because greywater includes water from bathing and clothes washing, it contains bacteria that are commensal to the skin and mucous membranes, such as *Staphylococcus aureus*, and when water from food preparation is included, *Salmonella, Campylobacter* and *Shigella* bacteria can also be introduced (Cogan *et al*., 1999). Pathogenic *E. coli* O157:H7 and enteric viruses (enteroviruses, noroviruses, rotaviruses) have also been found in greywater, primarily as a result of faecal contamination (O'Toole *et al*., 2012). However, while these pathogens can survive for extended periods in greywater, there is no evidence that they are able to replicate (Rose *et al*., 1991).

Even though greywater excludes wastewater from toilets, there is still a low risk of faecal-derived protozoa and helminths being present, as water is collected from baths and showers. Mature nematode eggs and the cysts of protozoan parasites can remain viable for 54 months in water, 18 months in soil and around 12 months in sand. Survival is dependent on several factors, such as climatic conditions, seasonal air temperatures, humidity or desiccation of the soil, and exposure to sunlight (Omarova *et al*., 2018; Storey and Phillips, 1985). Therefore, the subsequent disposal of egg- or cyst-contaminated water via surface or subsurface irrigation may cause those coming into contact with the water or spray either directly or indirectly via the soil, to become infected.

Any recycled water has the potential of harbouring environmental opportunistic human pathogens, including *Legionella*, *Mycobacterium*, *Acinetobacter* and *P. aeruginosa* – all of which can be introduced via contaminated feed water. Unlike many of the bacterial pathogens of human origin that do not replicate in the environment, these opportunistic pathogens are able to survive and replicate. However, very little attention has been given to these microorganisms in systems using recycled water (Blanky *et al*., 2015; Jjemba *et al.*, 2010).

Nagarkar *et al*. (2021) investigated how new molecular methods such as metagenomics can be used to describe the complex bacterial communities present in greywater and its associated infrastructure. In a preliminary case study using metagenomics to characterise the bacterial communities in potable water and greywater collected from a university college dormitory, the authors showed that different genera dominated at the different sample points based on DNA sequence reads. *Acidipropionibacterium acidipropionici* was the most abundant bacterium in the potable water but was present at a much lower relative abundance in the transient storage tank prior to treatment, whereas *Enterobacter cloacae* and *Pseudomonas* sp. HLS-5 were the most abundant bacteria (30–40% of all DNA reads) in the transient storage tank, but at very low abundance in the potable water and direct greywater samples. This perhaps indicates more suitable growth conditions in the storage tank environment for these bacteria. The authors also reported the detection of both enteric and respiratory pathogens in all greywater samples, although many were at very low abundances. These included *E. coli, P. aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae, Salmonella enterica, Shigella* spp., *Clostridium perfringens, Vibrio* spp., *Mycobacterium* spp., *Campylobacter jejuni* and *Legionella* spp. With the levels varying widely depending on the source inputs. The authors recognise the physicochemical characteristics of greywater will influence the survival, persistence, and proliferation of different bacterial taxa, and that the complex chemical and biological makeup of greywater varies greatly depending on the source inputs. They also argued that the use of metagenomics may improve understanding of the entire bacterial community, which in turn may allow the identification of surrogates for assessing the removal or neutralisation of metabolic and chemical pollutants, as well as for measuring the reduction in infection risk following treatment.

Table 2 summarises the risks posed by opportunistic pathogenic bacteria that have been isolated in greywater and wastewater treatment systems. *Legionella* is among those bacteria that can exist and replicate in wastewater, so recycled wastewater systems need to manage the risk potential created by the presence of this opportunistic pathogen.

Table 2. Opportunistic pathogenic bacteria found in greywater and wastewater treatment systems.

Organism	Origin	Mode of transmission	Survival in water	Growth in greywater & wastewater	Disease	Reference
***Acinetobacter* spp.**	Water or soil	Aerosol inhalation; contact with contaminated surfaces; person-to-person (respiratory aerosol)	Part of the natural aquatic and soil microflora; able to survive in extreme environments	Yes	Pneumonia; bacteraemia; endocarditis	Kisková *et al*., 2023
***Aeromonas* spp.**	Water and food	Contact with contaminated water; ingestion	Part of the natural aquatic microflora; known to survive and proliferate in low-nutrient waters	Yes	Gastroenteritis; wound infections; bacteraemia/ septicaemia	Fernández-Bravo *et al*., 2020
Campylobacter jejuni* and *Campylobacter coli	Zoonotic (cattle, chickens, birds, flies)	Ingestion of contaminated food or water; faecal–oral route	Can remain viable for >120 days at 4°C in stream water; rapid die-off (9-log decrease) in 3–12 days at 4°C in stream water	Not proven	Gastroenteritis	Strakova *et al*., 2022
Cyanobacteria	Water	Ingestion or inhalation	Part of the natural aquatic microflora; known to survive and proliferate in low-nutrient waters	Yes	Hepato-, derma- and neurotoxicity	Romanis *et al*., 2020
***Escherichia coli* and *Shigella* spp.**	Faecal material	Ingestion of contaminated food or water; faecal–oral route	Comparatively rapid die-off compared with other pathogens commonly demonstrated	Although environmentally adapted *E. coli* strains have been shown to replicate in the environment, commensal *E. coli* strains have not been shown to survive and persist in the environment	Gastroenteritis	Jang *et al*., 2017
***Legionella* spp.**	Water or soil	Aerosol inhalation (common) or ingestion (rare)	Part of the natural aquatic and soil microflora; known to survive and proliferate in low-nutrient waters	Yes	Legionellosis	Hamilton *et al*., 2018
Nontuberculous mycobacteria (NTM)	Water or soil	Aerosol inhalation (common) or ingestion (rare)	Part of the natural aquatic and soil microflora; known to survive and proliferate in low-nutrient waters	Unknown	Pulmonary infection	Guo *et al*., 2019
Pseudomonas aeruginosa	Water or soil	Contact with contaminated water; ingestion	Part of the natural aquatic and soil microflora; known to survive and proliferate in low-nutrient waters	Yes	Wound infections; bacteraemia/ septicaemia	Khalaphallah and Andres, 2012

***Salmonella enterica* serovar Typhi**	Faecal material	Ingestion of contaminated food or water; faecal–oral route	May persist in wastewater and soil due to an association with amoebae present in the environment	May persist in wastewater and soil due to an association with amoebae present in the environment	Typhoid fever	Douesnard-Malo and Daigle, 2011
Stenotrophomonas maltophilia	Water or soil	Aerosol inhalation; person-to-person (respiratory aerosol)	Part of the natural aquatic and soil microflora; known to survive and proliferate in low-nutrient waters	Yes	Respiratory and urinary tract infections; bacteraemia	Brooke, 2012

2.2 *LEGIONELLA* IN RECYCLED GREYWATER AND WASTEWATER

There is very little peer-reviewed literature available on the microbial health risks posed by *Legionella* in reused greywater or wastewater. However, there has been a recent review of the presence and risks of *Legionella* in wastewater treatment plants (WWTPs), which included a list of 25 peer-reviewed studies published since 1993 where *Legionella* bacteria were detected by either culture or molecular tests in either municipal or industrial WWTPs (Caicedo *et al.*, 2019). Where the *Legionella* bacteria were quantified, the concentrations ranged from 1.7 x 10^3 cells/L to 10^{10} cells/L within WWTPs. *Legionella* bacteria have been isolated from all parts of the waste treatment process and have been reported to proliferate in the waste systems that include aerobic biological treatment, such as activated sludge tanks, where the temperature and dissolved oxygen concentration (DOC) are in the optimal range for *Legionella* growth (temperature: 25–35°C; DOC: 0.5–2.0 mg/L).

A review of the potential microbial hazards from greywater reuse by Benami *et al*. (2016) gave an extensive list of bacterial, viral and protozoal pathogens of both human and system origin that were found in both the greywater and the receiving environment and cited two studies where *Legionella* bacteria have been identified (Birks *et al*., 2004; Blanky *et al*., 2015; see section 4.4).

In a study on the microbial quality of both the source waters and treated water in an on-site recycling water treatment plant, three separate reclaimed water sources were tested: roof-collected rainwater, greywater from handwash basins and groundwater from an on-site bore (Birks *et al.*, 2004). The recycled water passed though ultrafiltration and reverse osmosis (RO) membranes before chlorination and was used for toilet and urinal flushing. The pathogen makeup observed in the three water sources reflected the expected contamination incidents, with the rainwater containing *Giardia* and faecal coliforms, which were likely to be of avian origin, and the greywater contained faecal coliforms at a much higher concentration than in the other sources (absent from the groundwater samples) and also including *Legionella*, *Cryptosporidium* and *Giardia*. *Legionella* bacteria were only isolated from the greywater samples, but their origin could not be explained as the greywater was collected from bathroom handwash basins – although it can be assumed that they likely became resident in the surface biofilm within the pipework due to no active biocide control occurring until after the RO treatment.

In a recent study carried out in the USA where six separate water recycling plants were selected for the monitoring of *Legionella* bacteria, 50% of 115 samples tested positive for these bacteria by culture, while 80% tested positive by molecular testing (Johnson *et al*., 2018). The samples were collected from the effluent water immediately after treatment, from the storage facilities and from three points within the distribution system. A total of 12 different *Legionella* species were identified with 54% being *L. pneumophila*. Culture-positive effluent samples contained around five times less *Legionella* than those collected from the distribution system, indicating proliferation of *Legionella* when a sufficiently high free available chlorine (FAC) level was not maintained. The samples were collected seasonally, and both the presence and concentration of *Legionella* increased from their lowest levels in spring (March) to their highest levels in early autumn (September) before decreasing again in late autumn (November). The study found that culturable *Legionella* generally increased with increasing water resident time and decreasing levels of FAC residuals. With quantitative polymerase chain reaction (qPCR) testing, in samples where the FAC residual was >0.2 mg/L, the *Legionella* levels averaged 25 genome units (GU)/mL, whereas where the FAC residual concentration was <0.2 mg/L, the *Legionella* levels averaged 150 GU/mL. The study also used ethidium monoazide (EMA) treatment coupled with qPCR testing to determine the level of viable versus non-viable *Legionella* in each sample. This showed that between 50% and 91% of the DNA detected by qPCR originated from non-viable bacteria, indicating that relying on qPCR testing alone to detect *Legionella* may overestimate the exposure risk. This study also looked for the presence of thermophilic and mesophilic amoebae in the recycled water since these are recognised *Legionella* hosts. All samples from the six plants monitored were found to contain amoebae, with concentrations between 0.2 and 9.2 most probable number (MPN)/100 mL of mesophilic trophozoites and between 0.4 and 4.3 MPN/100 mL of thermophilic trophozoites. The lowest trophozoite concentration was observed in the plant that maintained a constant chloramine residual. However, this plant still had concentrations of *Legionella* at approximately 10^1 colony forming units (CFU)/mL, which was similar to levels in the two other plants where the FAC level was three times lower. This indicates that other factors play a role in controlling *Legionella* growth in water systems.

3. EPIDEMIOLOGY OF *LEGIONELLA* BACTERIA IN RECYCLED WATER

3.1 THE NATURAL HABITAT OF *LEGIONELLA* BACTERIA

Legionella bacteria are part of the natural microflora of waters and rich organic soils and as such are widely distributed in the environment (WHO, 2007). They have been isolated from ground water and terrestrial waters (lakes, rivers, drains, and other bodies of water), as well as estuarine and marine waters and wastewaters. *Legionella* bacteria are also abundant in organically rich matrices such as compost, garden soil, sewage sludge, and mulch derived from tree bark and other vegetative material. They have a complex life cycle and rarely exist as 'free-living' bacteria, usually being associated with other microorganisms in mixed population biofilms, or as intracellular parasites of freshwater and soil protozoa (Boamah *et al*., 2017; Declerck, 2010; Rowbotham, 1980). Free-living protozoa serve as hosts for legionellae – the bacterium can invade vegetative protozoa (trophozoites) and replicate in vacuoles within the trophozoite cytoplasm.

When trophozoites are subjected to environmental stress due to unfavourable conditions such as extreme temperatures, nutrient depletion, or the presence of disinfectants, encystment is triggered. This is a protozoan survival strategy, and the *Legionella* bacteria are either expelled in vesicles just before encystment or remain dormant inside the protozoan cysts (Richards *et al*., 2013). Both the vesicles and cysts provide a protective environment for the relatively fragile *Legionella* – vesicles are resistant to freezing, sonication and disinfection, while cysts are highly resistant to desiccation, disinfection, and temperature extremes. Thus, the *Legionella*–amoeba interaction aids the persistence and proliferation of *Legionella* in both soil and water ecosystems.

Because *Legionella* spp. grow over a wide temperature range between 20 and 46°C (Cervero-Aragó *et al*., 2019), warm water systems are thought to serve as a common source for *Legionella* growth, especially where there are inadequate control measures to mitigate their proliferation.

3.2 LEGIONELLOSIS: MODE OF TRANSMISSION

Legionellosis is the disease that occurs following infection with *Legionella* bacteria. The recognised route of human infection from *Legionella* is via the inhalation of dust or water aerosols containing the bacteria. Water aerosols that are ≤5 µm in diameter are able to reach the alveoli of the lower respiratory tract, and on transmission, the bacteria invade and replicate mainly within alveolar macrophages via the same mechanism that is used to invade and colonise their natural protozoan hosts (Winn and Myerowitz, 1981; Abu Kwaik *et al*., 1998).

Wherever air and water mix there is a potential for water aerosols to be generated. Agitation of either the air or water at an air–water interface will create turbulence, causing aerosols to be generated. Thus, water flowing from a pipework outlet into air where there is a pressure differential will generate aerosols, as will the impaction of flowing water onto a hard surface (Mbaye *et al*., 2023). The creation of aerosols requires an energy transfer to overcome surface tension and fluid viscosity to break water up into smaller droplets, and the amount of aerosols generated will be influenced by the size of the opening, the water flow rate, the size of any pressure drop, the water temperature and the air humidity.

Any aerosol-generating device will transmit *Legionella* bacteria if present in the feed water. Cooling towers are perhaps the most widely known source of legionellosis among such devices, with the contaminated aerosols having been shown to spread more than 10 km under favourable humidity and airflow conditions (Nygard *et al*., 2008). Another important source of bioaerosols is WWTPs, as mechanical agitation of the wastewater generates a large amount of bioaerosols (Sánchez-Monedero *et al*., 2008) that potentially contain *Legionella* bacteria.

In any domestic setting, there is a range of common activities involving moving water (e.g. garden watering, toilet flushing, turning on a tap or showering, vehicle washing, water blasting) that will generate aerosols – and when the source water contains *Legionella*, there is a potential of aerosol spread with an increased risk of legionellosis. The aerosols from a *Legionella*-contaminated water supply will contain *Legionella*, and the inadvertent inhalation of the contaminated aerosolised water will potentially result in legionellosis for any susceptible person exposed to that aerosol.

3.3 *LEGIONELLA* IN WASTEWATER AND GREYWATER

No extensive studies have been undertaken to determine the presence and level of *Legionella* in wastewaters in New Zealand. However, there have been incidental findings during source tracing investigations for *Legionella*. Source tracing and risk assessment activities have isolated *Legionella* bacteria from dairy factory wastewater treatment systems, pulp mill wastewater treatment systems, waste stabilisation ponds and dewatered sewage sludge, as well as aeration pond water prior to spray irrigation onto pasture (Graham *et al*., 2023; see section 4.1). There have also been anecdotal reports of legionellosis resulting from exposure to spray drift from these wastewater sources, although no definite link has been established in any case.

Culture analysis of wastewater to identify the presence of pathogenic bacteria can be difficult when the bacterium of interest is present in relatively low numbers or has fastidious growth requirements. This often means that either enrichment or exclusion steps, or a combination of these, need to be included to improve the recovery efficacy for the pathogen of interest when processing samples prior to culture. However, this can result in a loss of recovery of the target organism, leading to an underestimation of the true number present. Even when methods are robust, the inability to process large sample volumes restricts any useful lower level of detection limit. The slower growing *Legionella* bacteria are quickly overgrown by the more abundant heterotrophic bacteria in environmental water samples and by faecal bacteria in sewage system wastewater samples. By contrast, while molecular methods are frequently not constrained by high levels of background microflora, they cannot distinguish between live and dead bacteria and so could potentially overestimate the levels of viable bacteria present in a sample.

4. OCCURRENCE AND DISPERSION OF *LEGIONELLA* FROM WASTEWATER AND GREYWATER

4.1 WASTEWATER TREATMENT PLANTS

Both centralised and industrial WWTPs are primarily designed to reduce the organic and nutrient load in the effluent feed using a combination of chemical, physical and biological processes before discharge into the environment. Depending on the sensitivity of the receiving environment, the wastewater treatment system may require pathogen removal prior to discharge, although this is not currently a common goal for WWTPs in New Zealand. Urban WWTPs contain a vast and diverse list of bacteria, some of which are human pathogens derived from the waste stream (bacteria shed in faeces) or are complicitly intrinsic to the environmental waters. The major human bacterial pathogens that are found in urban wastewater include *Salmonella* spp., *Escherichia* spp., *Shigella* spp., *Yersinia* spp., *Klebsiella* spp., *Leptospira* spp., *Vibrio cholerae*, *Aeromonas hydrophila*, *Legionella* spp., *Mycobacterium* spp. and *Pseudomonas* spp. (Amha *et al*., 2017; Cai and Zhang, 2013; Douesnard-Malo and Daigle, 2011; Maynard *et al*., 2005; Stevik *et al*., 2004). Because standard bacterial culture methods have limitations for successfully isolating *Legionella* and some other pathogenic microorganisms from wastewater and greywater samples, primarily due to the extremely high numbers of heterotrophic bacteria present (often >10^8 CFU/mL) (Casanova *et al.*, 2001), other detection strategies that can more readily detect *Legionella* are now used, such as metagenomics (Cai and Zhang, 2013; Numberger *et al*., 2019).

4.1.1 Using metagenomics to follow pathogens of interest in WWTPs

Numberger *et al.* (2019) used full-length 16S rRNA gene sequence reads to compare the taxonomic makeup of the bacterial communities in influent and effluent samples of a WWTP in Berlin, Germany. Untreated raw influent and treated effluent samples were collected every 2–3 months between February and October, and next generation sequence analysis of the PCR products was carried out using the PacBio Platform (Pacific Biosciences, USA). This provided full length 16S rRNA gene sequence reads, expressed as operational taxonomic units (OTUs), and allowed for taxonomic identification to the genus and species level. The authors found that the dominant OTUs varied both seasonally and spatially, with the WWTP treatment process efficiently reducing the relative abundance of all disease-associated bacterial groups in the effluent except for *Legionella* spp. and *Leptospira* spp., the relative proportion of which increased from the inflow to the effluent. This indicates that while WWTPs can effectively reduce the number of enteric bacteria, they may inadvertently increase the number of potentially pathogenic bacteria, which will be subsequently released into the environment.

In a separate study, Cai and Zhang (2013) assessed the potential environmental risks from human pathogens in two WWTPs in Hong Kong using a metagenomics approach to avoid the necessity of unreliable culture methods. The authors used 24 metagenomic DNA datasets derived from a high-throughput shotgun sequencing technique to analyse samples collected from each plant's influent, activated sludge and effluent and found that *Legionella pneumophila* was present in all parts of the waste treatment systems. However, for both WWTPs, the *Legionella* levels were significantly higher in the effluent stream than in the activated sludge and were relatively negligible in the influent stream. By contrast, the

Escherichia coli levels showed the opposite trend, with significantly higher levels in the influent stream and negligible levels in the effluent stream. This implies that *Legionella* can replicate within these systems and may pose a risk to workers exposed to aerosols from the activated sludge process.

4.1.2 Case control study of *Legionella* dispersion and transmission from WWTPs

In a Dutch case control study undertaken over a 6-year period following two legionellosis outbreaks that had been traced back to WWTPs, Vermeulen *et al.* (2021) found that there was a statistically significant association between legionellosis cases and the calculated annual average aerosol concentrations originating from WWTPs (odds-ratio: 1.32 [1.06–1.63]). The atmospheric dispersion model they used to calculate annual average aerosol concentrations originating from 776 WWTPs (449 industrial plants and 327 sewage treatment plants) was based on the Gaussian plume model, and the robustness of their model was tested using case data from the WWTP-related outbreaks. There was a close similarity between the distance of transmission from the outbreak site and the calculated dispersion map from that location. However, the authors noted that better data were needed to reduce the assumptions in their model, which included that:

- *Legionella*-containing aerosols from WWTPs behave like primary particulate matter up to 10 µm
- all WWTPs emit equal amounts of aerosols
- the average annual aerosol concentration at the residential address of each case or control is related to the exposure level
- WWTPs are continuous sources of *Legionella*-containing aerosols
- there is no reduction in the concentration of *Legionella* in air.

In a follow-up study by the same group (van den Berg *et al*., 2023), a risk matrix was developed for industrial WWTPs based on factors including the:

- source of the waste influent (organically rich wastewaters from industrial sources such as food and wood processing, petrochemical, and animal rendering)
- biological treatment
- temperature of the wastewater through the treatment process
- use of aeration basins or tanks.

The risk matrix was used to categorise the WWTPs as either high, moderate, low or very low risk for *Legionella* growth and subsequent emission to air or discharge to surface waters. In a follow-up exercise, *Legionella* bacteria were cultured from 13 (18.3%) of 71 industrial WWTPs, and it was found that rates of culture positivity were lower in systems where the water temperature was between 25 and 29°C compared with those between 30 and 38°C. The *Legionella* concentrations ranged from 1 x 10^5 to 3 x 10^8 CFU/L, which are consistent with the ranges found in similar studies (Blatny *et al*., 2011). Mitigation steps suggested by the authors to reduce the level of *Legionella* in the effluent included using a prolonged anaerobic treatment and disinfecting the effluent using UV radiation or chemical biocides. The authors also mentioned further steps to reduce aerosol emissions, including covering aeration ponds and tanks or using pure oxygen or fine bubble aeration – fine bubble aeration has been shown to produce significantly lower levels of aerosol emissions than horizontal rotor aeration or mechanical agitation (Brandi *et al*., 2000; Han *et al*., 2020).

4.1.3 Pulp mill WWTPs

A series of samples were repeatedly collected from set sampling points in the effluent stream of a pulp mill waste treatment system in New Zealand over a 6-year period, and *Legionella* bacteria were frequently isolated from the sampling sites within the secondary waste treatment steps (primary and secondary activated sludge ponds and the final clarifier) but not the primary steps (roughing tower, dissolved air flotation tank and pre-stage treatment) (Institute of Environmental Science and Research [ESR], unpublished data, 2016–2022). This suggests that conditions suitable for the proliferation of *Legionella* bacteria exist later within the treatment process. Due to the very high levels of heterotrophic bacteria present in the wastewater samples interfering with the isolation of *Legionella*, the lower limit of detection for *Legionella* isolation was high (2,500 CFU/L). This meant when *Legionella* was detected it was at extremely high levels and may pose a risk to workers exposed to aerosols from the activated sludge process. It was also noted that the secondary activated sludge pond was located adjacent to a major highway (<100 m), although it was screened by trees, which may mitigate some exposure risk. The treated effluent was discharged to a river under the company's resource consent conditions.

A Norwegian group studied the dispersion of *Legionella*-containing aerosols from the surface of aeration ponds at a pulp mill following three legionellosis outbreaks that were linked to a wet scrubber at the mill (Blatny *et al*., 2011). The waste treatment process consisted of an anaerobic reactor followed by two aeration ponds operated at 36–38°C. The wet scrubber was located within 200 m of the ponds, and these ponds were considered to be the source of the *Legionella* contaminating the scrubber. The study looked at the aerosol dispersion patterns from the aerated ponds to 500 m downwind of them. Air was pumped into each aerated pond at a rate of 3.0×10^4 m^3/hour, resulting in large amounts of aerosols being produced. The *Legionella* concentration had been measured at up to 10^{10} CFU/L in the pond water and at 43 CFU/m^3 directly above the aeration ponds. Aerosol dispersion modelling showed that the aerosol plumes generated from the aerated ponds only increased slowly in width from 150 to 180 m at distances of 150 and 350 m, respectively, downwind from the ponds. The modelling also showed that the aerosol plume remained relatively close to the ground, with maximum aerosol concentrations occurring at 5–10 m above ground level. The dilution effect of the *Legionella*-containing particles was estimated to be 1/50 of the concentration above the ponds at 500 m downwind, but contaminated aerosols could easily reach distances of up to 200 m downwind from the aerated ponds. The authors also projected that the estimated aerosol concentration was 1/1500 of the initial level at 10 km downwind from the ponds.

4.1.4 Dairy factory WWTPs used for spray irrigation onto pasture

Field testing was undertaken by ESR to ascertain the risk caused by the presence of *Legionella* bacteria in the wastewater effluent from a dairy factory following its primary and secondary treatment. *Legionella feeleii* was isolated from several sites within a wastewater reticulation system that was being used for irrigating pasture. Culture-positive water samples were collected from the final oxidation pond, the storage pond and the pipework being used for spray-irrigating the wastewater. Although the application was being undertaken in a rural area, sprayed aerosols can travel a considerable distance and often farm workers are moving the irrigators while in operation. There has also been a report of cattle being infected with *Legionella* – although this is thought to have originated from contaminated water being used to prepare calf feed (Fabbi *et al*., 1998) – which suggests that livestock may also be infected with *Legionella* in the same way as humans.

4.1.5 Spray irrigation of treated wastewater

Point-of-entry and point-of-use water samples were collected from three non-potable recycled wastewater distribution systems in the western USA that were served by four WWTPs (Fahrenfeld *et al*., 2013). Although the focus of the study was on reclaimed wastewater as a reservoir of antibiotic resistance genes, the authors also used *Escherichia coli*, *Legionella pneumophila* and *Pseudomonas aeruginosa* as waterborne and faecal indicators. Quantitative PCR screening for *E. coli* and *L. pneumophila* using the *gad*AB and *mip* targets, respectively, resulted in positive detections for 48% of the samples: 35% for *gad*AB and 17% for *mip*. By contrast, *P. aeruginosa* screening using the *ecrf*X/*gyr*B target was below the detection limit in all samples. The authors also noted that recreational fields are commonly spray-irrigated with treated wastewater, so human contact with the aerosols and soil is likely to occur. The level of the *Legionella mip* gene detected in the wastewater effluent was comparable to that observed by the same authors in an earlier study on opportunistic pathogens found in chlorinated drinking water distribution systems (Wang *et al.*, 2012).

4.1.6 Municipal water reclamation plants

Ajibode *et al.* (2013) monitored the water in two separate wastewater reclamation facilities in the arid state of Arizona, USA, for both chemical and biological markers over a 15-month period. The two facilities used different methods of treatment and disinfection and produced either Class A+ or Class A recycled water, where Class A+ water contains <10 mg/L of total nitrogen and Class A water has no nitrogen restriction.

Facility A was a tertiary filtration plant that produced 10 million gallons per day (mgd) (37,850 m^3/day) of Class A recycled water using chlorine disinfection. The plant consisted of an effluent booster station, a chlorine contact chamber and a chlorine booster station some 18 km downstream, with 40 km of pipeline. The average chlorine concentration was 3.9 mg/L at the point where the water left the facility but rapidly dissipated with increasing distance to a level of <0.1 mg/L at the most distal point, despite the chlorine booster station. There was a strong inverse correlation between the chlorine level and both the heterotrophic and *Legionella* bacteria concentrations at each sampling point, and the injection of more chlorine at the 18 km station led to an approximately 2-log reduction in the heterotrophic plate count and a 1.3-log reduction in the *Legionella* count, although both rebounded to pre-chlorination levels within another 5 km.

Facility B produced 4 mgd of Class A+ recycled water using UV light as the primary means of disinfection with a contact time of 1.2 min using low-pressure lamps (wavelength 254 nm) at a dosage rate of >0.1 J/cm^2. This facility could treat up to 10.6 m^3/min and up to 4 mgd (15,140 m^3/day) and had 13 km of associated pipeline.

The reclaimed water from both facilities was used to irrigate grassed areas, including parks, golf courses and schools. Water samples were collected at monthly intervals at discrete locations along the distribution system. Heterotrophic bacteria and *Aeromonas*, were found to be present at high frequencies (>80% of samples) in both facilities. Coliforms were detected in 60% of samples from Facility A but in more than 90% of samples from Facility B. This is likely to be due to the different treatment methods.

Legionella bacteria were detected in approximately 40% of all samples from both facilities, while *Mycobacterium* bacteria were found in 40% of samples from facility A and 56% of samples from facility B. Although amoebic activity was observed in about a third of samples, it did not correlate with the presence of any of the indicators or with the presence of *Legionella*, *Mycobacterium* or *Aeromonas*, suggesting that amoeba growth may require different water conditions from opportunistic pathogen and indicator regrowth. Furthermore, *Legionella*, *Mycobacterium* and *Aeromonas* were frequently detected in both systems, with no statistically significant difference between seasons at either facility, with *Legionella* concentrations varying from approximately $10^{1.7}$/100 mL (spring) to $10^{3.6}$/100 mL (summer) at Facility A and approximately $10^{2.5}$/100 mL (spring and autumn) to 10^3/100mL (winter and summer) at Facility B. By contrast, waterborne indicators such as *Escherichia coli* and *Enterococcus* were rarely detected, and where they were detected, were only present at low concentrations (<10^1/100 mL). Therefore, the authors concluded that new indicators of water-based pathogens need to be developed for recycled wastewater systems.

4.2 DECENTRALISED (DOMESTIC) GREYWATER RECYCLING SYSTEMS

Treated domestic greywater is primarily used for garden irrigation and toilet flushing (Gross *et al*., 2007; March *et al*., 2004). No reports were identified in the scientific literature indicating that a *Legionella* infection had occurred as a result of exposure to treated domestic greywater. However, studies have shown that *Legionella* bacteria can persist in these water systems, indicating that they potentially pose an infection risk.

In 2015, Blanky *et al.* (2015) investigated *Legionella* levels in four different potable and greywater systems in single and multiunit dwellings in Israel. They found that untreated greywater had a *Legionella* concentration of 1.2 x 10^5 CFU/L, while treated and chlorinated greywater had concentrations of 2.5 x 10^4 CFU/L and 6.3 x 10^3 CFU/L, respectively. While this equates to a 95% reduction in viable *Legionella* across the entire treatment process, this is significantly lower than the reduction seen for faecal coliforms (99.7% reduction). The untreated greywater contained concentrations of *Legionella* that were 2-3 logs higher than in the potable source water, indicating that *Legionella* bacteria were able to survive and replicate in the greywater prior to treatment. However, it was concluded that greywater did not pose a higher risk than the potable source water in these systems when the greywater was treated and chlorinated. It should be noted though that the concentration of *Legionella* in greywater will have been underestimated due to the introduction of a pre-filtration step to remove coarse matter, which will also have excluded any *Legionella* adsorbed onto the solids from the analysis. The authors also observed a seasonal change in the concentrations of *Legionella* in both the potable water and greywater, with an almost inverse relationship between the two: for the potable water, *Legionella* concentrations were highest in summer (approximately 10^2 CFU/L) and lowest in winter (approximately 10^{-1} CFU/L), while for the untreated greywater, concentrations were highest in autumn (approximately 10^5 CFU/L) and lowest in summer (approximately 10^3 CFU/L). However, no explanation for this was given.

In a follow-up study by the same group (Blanky *et al*., 2017), a four-step quantitative microbial risk assessment (QMRA) was carried out for *Legionella* exposure from the use of recycled greywater for garden irrigation and toilet flushing. The four steps included hazard identification (using *Legionella* culture data from their 2015 study), exposure assessment (using parameters such as exposure duration, inhalation rates and aerosol generation), dose response modelling and risk characterisation for each scenario (sprinkler irrigation or toilet flushing). The authors estimated that using either untreated or treated but not chlorinated greywater for irrigation would present an exposure risk to *Legionella* that was 1–2 logs higher than using potable water. By contrast, when greywater was treated and chlorinated for sprinkler irrigation, the average annual infection risk (8.5 x 10^{-7} to 1.3 x 10^{-8}) was approximately 2–4 logs lower than the tolerable risk of infection (10^{-4} cases per person per year), indicating that greywater is safe to use for garden irrigation when properly treated and disinfected. The cumulative annual risk of disease from *Legionella* when using untreated greywater for toilet flushing was estimated to be 2–3 logs higher than using potable water. Similarly, when reusing treated and chlorinated greywater for toilet flushing the annual infection risk was estimated to be 1.3 orders of magnitude higher than using potable water. Therefore, the study concluded that sprinkler irrigation and toilet flushing should be avoided if the recycled greywater is not treated and chlorinated.

4.3 MIXED REUSE (IRRIGATION, TOILET FLUSHING, COOLING TOWER)

A year-long study on water quality after storage in four different geographically distant wastewater recycling plants in the USA (California, Florida, Massachusetts, and New York) showed that opportunistic water-borne pathogens (*Aeromonas, Legionella, Mycobacterium*, and *Pseudomonas*) occurred more frequently than the standard indicator bacteria (enterococci, coliforms, and *Escherichia coli*) (Jjemba *et al*., 2010). Samples were collected from the plant effluent, the reservoir, and three points in the distribution system over four consecutive days each season for a year. One system used UV treatment to disinfect the water, while three of the systems were chlorinated, although an active disinfection residual was not maintained throughout these systems and, in most cases, the water temperature was above 20°C. Both factors would contribute to the growth of *Legionella* and the other opportunistic pathogens. The end use of the recycled water was the irrigation of sports fields, toilet flushing and use in cooling towers, all of which are activities that produce aerosols that would increase the exposure risk to the pathogens present in the water.

The same research group undertook QMRA to evaluate the *Legionella* risks associated with using recycled water for toilet flushing, spray irrigation and cooling towers (Hamilton *et al*., 2018). Their QMRA approach used four parameters: hazard identification, exposure assessment, dose–response assessment and risk characterisation. Information on pathogen presence was obtained using culture-based methods, qPCR, and EMA-qPCR, from 19 reclaimed water utilities in the USA. This data was used to quantify *Legionella* in the source water and in turn had a major influence on the exposure parameters used to estimate annualised infection risk for each exposure scenario modelled. The modelling showed that the median annual infection risk and annual clinical severity infection risk for *Legionella* from toilet flushing exceeded the United States Environmental Protection Agency's (US EPA's) annual infection benchmark of 10^{-4} infection cases/person/year (US EPA, 2012) for some aerosol exposure estimation methods, while the 95th percentile risk exceeded the benchmark for all methods. Furthermore, using long range dispersion models *Legionella* exposure risks were potentially still significant at large distances from cooling towers while sprinklers operating under typical conditions would necessitate setback distances of greater than 75 m. The concentration of *Legionella* in the reclaimed water had the greatest effect on risk, highlighting the importance of controlling levels of these bacteria. However, several other factors were also important, including the type of population at risk (residential or occupational), the operating conditions (drift eliminator performance or stack height for cooling towers) and the meteorological conditions (for cooling towers and sprinklers), as well as the dose response model used (infection or clinical severity of infection) and the detection method used (culture-based, qPCR or EMA-qPCR). The authors concluded that risks to public health could be reduced by implementing management practices such as closing toilet lids, using more efficient drift eliminators for cooling towers, and using wind breaks for cooling towers and sprinklers.

4.4 AGRICULTURAL IRRIGATION

With the practice of reusing reclaimed wastewater for agricultural irrigation increasing globally, there is a need to ascertain the exposure risk to potential pathogens from the aerosols that are generated during sprinkler or spray application, especially when there is inadequate or no treatment of the water prior to use. Different modes of application of the water to vegetation will also present different risks – for example, spray irrigation is known to generate aerosols and so may represent a severe health risk to the nearby population if *Legionella* bacteria are present in the water.

Few studies have investigated the risks associated with exposure to *Legionella* bioaerosols originating from agricultural plots irrigated with wastewater, although there have been attempts to assess the risks for other enteric bacteria and viruses using a QMRA approach (Courault *et al.*, 2017). One of the most common methods for modelling irrigation is the Gaussian plume model (Dungan, 2014; Mori *et al.*, 2020), which models the concentrations of water in the air in a specified area from a source over a given period of time. Gaussian plume models have mainly been used to predict infection risks from enteric pathogens, but they can be used for any aerosolised pathogen (Dungan, 2014).

Massiot *et al.* (2023) monitored the levels of *Legionella* bacteria in two corn fields in the southwest of France that had been irrigated with wastewater treated in two different ways (ultra-filtration and UV radiation). They found that levels of *Legionella* in the water were high (up to 10^6 genome copies/L) even after standard wastewater treatment followed by the UV treatment. The authors then used the data to update a general Bayesian network model in a QMRA to monitor the risk of *Legionella* infection in the vicinity of the irrigated fields and used the model to simulate different exposure scenarios with respect to the length of exposure, distance from the emission source and climate. They found that the median annual risk of *Legionella* infection from the two corn fields did not exceed the US EPA's annual infection benchmark of 10^{-4} infection cases/person/year (US EPA, 2012) for any of the populations at risk (maximum estimated disability-adjusted life years [DALYs] = 10^{-5}). The authors cautioned, however, that it was still worth monitoring the risk.

In another study in the USA, Mori and Smith (2023) used a QMRA approach to look at the risk of legionellosis for residents exposed to wastewater being spray irrigated onto neighbouring farmland. Their risk modelling used several scenarios from both low-pressure and high-pressure irrigation systems and included four input parameters: hazard identification, exposure assessment, dose–response assessment and risk characterisation. They identified the aerosol concentration, inhalation rate, source concentration of bacteria and exposure duration as important determinants of risk based on their relatively high sensitivity scores using the Sobol sensitivity analysis. They also found that the mean risk of infection for a single exposure event exceeded the safety threshold of 10^{-6} infections/exposure up to 1 km from a low-pressure irrigator and up to 2 km from a high-pressure irrigator, although the median risk of infection did not exceed the threshold for any distance or irrigator pressure. The authors concluded that the health risk posed by *Legionella pneumophila* to individuals spending time outside downwind of an irrigator spraying diluted municipal wastewater was minimal, except in the unlikely scenario where the weather, exposure and irrigation conditions that support bacterial viability coincide.

4.5 SPRAY-IRRIGATION OF PARKLAND AND GOLF COURSES

In arid regions, reclaimed wastewater is commonly used for the irrigation of parks, golf courses, playgrounds, and home lawns. When spray or sprinkler irrigation is used as the method of application, it creates an opportunity for the aerosolisation of *Legionella* where present and subsequent exposure to these bacteria by inhalation.

Pepper and Gerba (2018) applied QMRA to estimate the risk of infection from *Legionella* associated with the spray irrigation of recycled water onto lawns or parkland. Their risk assessment used four basic steps: hazard identification, exposure assessment, dose–response assessment and risk characterisation. The exposure assessment was based on variables such as the concentration of *Legionella* in the reclaimed water, the size of the aerosols produced during irrigation (based on the spray application method), the distance of spray drift from the source, and the duration and frequency of exposure. The dose–response model used data from guinea pig studies to predict the effects on humans (Muller *et al*., 1983) and the risk of infection was based on data from a previous study by the group (Ajibode *et al*., 2013). Three different scenarios that typified park user exposure to *Legionella*-contaminated spray-irrigated water were used: a one-time event, once per week for 12 weeks (summer), and once per week for 1 year. The findings showed that there was a direct log-linear relationship between infection probability and the concentration of *L. pneumophila* in the recycled water. For example, the calculated risk of infection from one 10-minute exposure event per week for 1 year increased 10,000-fold from 1.4 x 10^{-7} to 1.4 x 10^{-3} when the *Legionella* concentration rose from 10 CFU/100 mL to 100,000 CFU/100 mL. Furthermore, when the *Legionella* concentration exceeded 1000 CFU/100 mL, the annual one-time exposure risk of infection exceeded the recommended US EPA guidelines for drinking water of 10^{-4} (US EPA, 2002). The authors concluded that routine monitoring of reclaimed water spray irrigation systems would be prudent and that remedial action should be taken when *Legionella* levels exceed 1000 CFU/mL.

4.6 VEHICLE WASH FACILITIES

Vehicle wash stations consist of three different setup types: fully automated, full service (where the vehicle is manually washed by attendants) and self-serve (where members of the public have access to the facility to manually wash their vehicle). The exposure risk potential to *Legionella* for users differs between the first type and the other two types, primarily due to differences in the contact with aerosols generated by the devices. Where the water is a single pass and drains to waste, the *Legionella* exposure risk is much lower than where the water is recycled, and where water is recirculated in an open system that allows for the accumulation of dirt and grime, bacterial contamination is inevitable. Conditions that encourage *Legionella* growth in these systems include water stagnation, the lack of an active biocide residual, and the accumulation of sediment and sludge.

In 2012, as part of source tracing for a community-acquired case of legionellosis, *Legionella jordanis* and *Legionella pneumophila* were isolated from the recycled water reservoir of a self-serve vehicle wash in Auckland (ESR, unpublished data). Similarly, in 2008, a legionellosis outbreak in Victoria, Australia, that involved seven cases was traced to a self-serve vehicle wash facility where the water was recycled without adequate disinfection (McGarry *et al*., 2008). The Victorian Department of Health has since published brief guidance for commercial car washes on managing the risks associated with *Legionella*, which includes the following standard risk-reduction steps for any water system:

- Do not store water at temperatures between 20°C and 60°C.
- Replace warm water storage with instantaneous units.
- Replace rubber hosing with poly tubing, metal tubing or copper tubing.
- Regularly disinfect the system with a chlorine-based disinfectant.

A recent study to evaluate the risk of legionellosis from car washes in Italy showed the presence of *Legionella* bacteria in both water and aerosol samples collected from the facilities (Laganà *et al*., 2023). A total of 120 samples were collected from 30 different car washes over a five-month period, with sampling taking place on a Monday prior to system startup after 36 hours of stagnation and again the following Thursday. None of the car washes sampled used water recycling, but 7 of the 60 water samples collected on a Monday were culture-positive for *Legionella* compared with only one from the Thursday sampling. The aerosol sampling showed a similar trend, with 3 of the 60 air samples collected on a Monday being culture-positive for *Legionella* compared with none from the Thursday sampling. These findings emphasise the risk posed by water stagnation that allows for the buildup of *Legionella* prior to discharge via an aerosol-generating device.

5. *LEGIONELLA* AND GREYWATER REUSE GUIDELINES

Several guidelines and standards have been developed by various national and international organisations to address the biological and chemical health risks associated with wastewater reuse. Wastewater treatment systems are primarily designed to remove or significantly reduce biological and chemical hazards in the waste stream prior to discharge into the receiving environment. The major health concern when considering recycled greywater or wastewater is primarily focused on exposure to inherent human-derived pathogens at any point from pre-treatment collection to post-treatment discharge. A recent review of these was prepared by ESR for Health New Zealand – Te Whatu Ora (Leonard *et al*., 2023). This section reviews some of the same guidelines with a focus on *Legionella* bacteria persistence, exposure risk and mitigation steps, as the survival and proliferation of opportunistic environmental bacteria like *Legionella* are frequently overlooked when developing health-based reduction targets.

5.1 NEW ZEALAND

There are currently no national greywater or wastewater reuse guidelines in New Zealand that outline the requirements for wastewater reuse as a discharge to land managed by the regional or territorial councils. Some information for subsurface irrigation of greywater may be obtained from the relevant sections of AS/NZS 1547:2012 *On-site domestic wastewater management*, although it is noted that greywater diversion devices and surface irrigation with greywater are excluded, as well as guidelines issued by councils for the on-site disposal of wastewater to land. Under the Building Regulations 1992, the installation of permanent wastewater reuse systems must comply with the Building Code. Greywater disposal to land may be a permitted activity, i.e. no resource consent is necessary, if it meets the requirements set out in Regional Plan Rules. Another source of guidance information is the Building Research Association of New Zealand (BRANZ), which has published several articles detailing on-site wastewater treatment and greywater recycling.[2]

5.1.1 AS/NZS 1547:2012 – On-site domestic wastewater management

The joint Australian–New Zealand standard AS/NZS 1547:2012 provides the requirements for on-site domestic wastewater treatment systems and application of the treated effluent to land. This Standard aims to protect public health and the environment using sustainable and effective systems and covers all-waste septic tank systems as well as greywater systems. It sets out the performance objectives and system design requirements, along with construction, installation, operation, and maintenance requirements. Most of the performance criteria appear to be contingent on the ‘site-and-soil evaluation’ and include a ‘risk management’ component. The Standard covers on-site wastewater systems, including the primary and secondary treatment and disinfection of wastewater originating from household activities. Waste discharge from toilets, urinals, kitchens, bathroom sanitary fittings (including showers, washbasins, baths, spa baths) and laundries is included. However, discharge from spa pools and hot tubs is not covered, despite these systems having been identified as the most common source of legionellosis outbreaks in New

[2] BRANZ: https://www.level.org.nz/water/wastewater/

Zealand (Graham *et al*., 2023). The Standard also excludes systems that are used to treat wastewater from commercial or industrial sources or stormwater.

The waste treatment systems covered in AS/NZS 1547:2012 are normally designed for domestic wastewater flows up to 14,000 L/week from a population equivalent of up to 10 persons. The Standard recommends that an all-waste septic tank has a minimum capacity of 3000 L to provide a minimum 24-hour settling volume based on 200 L/person/day, while the minimum capacity for a greywater septic tank is recommended to be 1800 L to allow for a minimum 24-hour settling volume based on 600 L/day flow for a 1–5 person household. Accumulated sludge is required to be removed at specific time intervals (every 3–5 years) to prevent a reduction in the 24-hour settling period.

The Standard defines greywater as 'the domestic wastes from a bath, shower, basin, laundry and kitchen, but excluding toilet and urinal wastes which are described as black water'. The application to land methods for greywater include disposal via shallow subsurface irrigation. The Standard covers surface drip and spray irrigation of disinfected secondary treated effluent, not greywater, over soil or non-food vegetated areas, but notes that small treatment systems can have inconsistent performances and that some regulatory authorities do not permit spray irrigation. Disinfection must be continuous and can be carried out using either UV radiation or chlorine. The Standard specifies a FAC residual level of 0.5 to 2.0 mg/L, while UV treatment should be delivered in the range of 250 to 270 nm. There is a brief mention of the environmental and health impacts to be aware of when using chlorine disinfection in wastewaters, including the production of disinfection byproducts, such as trihalomethanes and dioxins. It also mentions that UV treatment can be compromised by elevated levels of suspended solids, turbidity, a change in colour or poor maintenance of the UV light source. The Standard also provides guidance on how to incorporate appropriate setback distances based on factors including effluent quality, application method, soil geology and groundwater depth (refer to Appendix R of AS/NZS 1547:2012).

The Standard makes no explicit reference to the control of *Legionella* bacteria, but it is assumed that where all performance measures are met, including the appropriate treatment and disposal conditions, any potential risk of exposure to opportunistic pathogens such as *Legionella* should be minimised. Although the Standard lists the mitigation steps to limit aerosol production and dispersion to lessen the health risks from spray application, there is no microbiological assessment of the effluent other than that the average *Escherichia coli* levels should be no greater than 10 CFU/100 mL (refer to Appendix M of AS/NZS 1547:2012).

5.1.2 Auckland Council guideline document, GD2021/006

The Auckland Council guideline document, GD2021/006 *On-site Wastewater Management in the Auckland Region* (Chen and Silyn Roberts, 2021), which was published as a draft in 2021, provides technical guidance for the design, installation, and management of on-site wastewater systems in the Auckland region. This latest guideline updates and replaces the previous guidance provided in *On-site wastewater systems: Design and management manual* (Ormiston AW, Floyd RE. 2004.) and aims to safeguard against any public health risks and to minimise harmful environmental effects that may result from the failure of on-site wastewater treatment systems. The guideline references AS/NZS 1547:2012 for many of its recommendations and is intended for single-dwelling households as well as institutions (such as schools, commercial and public facilities) in the Auckland region, with the system capacity scaled based on a wastewater discharge allowance of up to 200 L/person/day for a population equivalent of 15 persons. This can be further divided into a blackwater flow of 25–

45 L/person/day and a greywater flow of 100–140 L/person/day, with the variance resulting from premise water-saving plumbing fixtures (refer to Section C of GD2021/006).

The guideline specifies monitoring parameters for assessing the performance of the wastewater treatment system and for verifying the effluent quality, with the testing frequency being dependent on the potential for adverse effects in the receiving environment. Both biological and chemical parameters are listed, including the organic load, measured as the 5-day biochemical oxygen demand (BOD_5), total suspended solids (TSS), faecal coliform or *E. coli* level, pH and nutrient level. Certification of performance is also required. The frequency for testing is not prescribed and is determined based on the complexity of the on-site wastewater systems and perceived risks of failure.

Section D1.8 of GD2021/006 states that only treated greywater originating from bathroom washbasins, showers, baths and laundries is considered acceptable for reuse. Reuse options are limited to flushing toilets (where the water must be disinfected), carrying out subsurface irrigation of gardens, including fruit trees and bushes but not root crops, and filling ornamental ponds where there is no direct human contact.

Greywater that is reused for toilet flushing must undergo advanced secondary treatment and disinfection as a minimum (section D1.8.3 of GD2021/006) and must maintain a FAC residual level of ≥ 0.5 g/m^3 (0.5–1.0 mg/L) at a pH between 6.5 and 8.5. When treated to this level, the greywater is also deemed suitable for other purposes with potential public health risks if there is continuous on-line chlorine monitoring, but how compliance is ensured is not indicated. *Escherichia coli* levels are used as the only microbial indicator of the effectiveness of the treatment system, with the median level set at ≤10 CFU/100 mL, and testing for turbidity is also required when UV treatment is used. The frequency of testing for *E. coli* is not clearly indicated, although there is mention that factors such as high turbidity (suspended solids) and elevated BOD_5 (organic matter) can compromise disinfection with both chlorine and UV radiation.

The guideline includes spray application as a 'non-conventional land application' method (section E1.1.4.2 of GD2021/006) for the discharge of advanced secondary treated and disinfected wastewater, which must maintain a FAC residual of 0.5–1.0 mg/L (section F3.4 of GD2021/006). The guideline recommends that spray irrigator heads are no higher than 500 mm above the ground surface and that the wetted surface area is restricted to a maximum of 2.0 m from each spray head. This is most likely designed to reduce aerosol drift, but spray irrigation systems are not recommended due to higher environmental and health risks.

The guideline makes no explicit reference to the control of *Legionella* bacteria, with the efficacy of any disinfection process measured using faecal coliforms or *E. coli* as indicators of microbial health risk. Both spray irrigation and toilet flushing can produce large amounts of aerosols, increasing the exposure risk to pathogens in the water. Factors such as retention time post disinfection, water stagnation, and biofilm development on tanks and pipework can influence the growth of opportunistic pathogens in the effluent waters.

5.2 AUSTRALIA

5.2.1 Australian National Guidelines for Water Recycling: Managing Health and Environmental Risks (Phase1)

The *Australian National Guidelines for Water Recycling* (NRMMC-EPHC, 2006) were published in 2006 and use a risk management framework that is designed to protect the health of both the public and the environment. Although each Australian state and territory has its own guidelines regarding greywater or wastewater reuse, national guidelines were adopted so there was a consistent approach when dealing with either centralised or decentralised recycled wastewater systems. The national guidelines focus on water recycled from treated sewage (both centralised sewage treatment plants and on-site systems) and greywater systems. Reuse purposes include garden watering, toilet flushing, and car and clothes washing in a domestic setting, as well as reuse in a wider setting, such as in cooling towers, for fire control or for agricultural and parkland irrigation.

In undertaking a risk management approach for water recycling, the guidelines use a stepwise approach to identify the hazards present, estimate the likelihood of an adverse event resulting from exposure to those hazards, assess any impact from that adverse event, and identify the preventative or harm reduction measures that can be implemented as part of controlling or managing risk.

The guidelines recognise the impracticality of setting human health-based targets for all microorganisms that might be present in recycled water and suggest that a more pragmatic approach is to choose appropriate reference pathogens that represent a worst-case scenario, covering issues such as high occurrence, high concentration in the raw wastewater, high pathogenicity, low removal efficiency with treatment and long survival in the environment. An example given for viruses is a combination of rotaviruses and adenoviruses, using dose–response data for rotaviruses and occurrence data for adenoviruses. Candidates for bacterial references included *E. coli* O157:H7, *Campylobacter*, *Shigella* and *Salmonella*.

The guidelines use DALYs to quantify the burden of disease caused by microbiological, chemical and physical hazards. The tolerable risk adopted in the guidelines is 10^{-6} DALYs per person per year (equivalent to one illness per 1000 people per year) (section 3.1.1). This is considered a stringent value and was adopted from the World Health Organization (WHO) drinking water guidelines (WHO, 2008). Microbial health-based performance targets can also be used to identify the risk of infection or disease from the recycled water, but a lack of data for many pathogens limits the ability to set meaningful targets for these.

Hazard removal must be achieved by the effective treatment of wastewater before reuse, while exposure reduction is achieved by restricting access to land application areas and controlling the method of application. The guidelines show indicative log reductions that have been achieved for indicator microorganisms or pathogens using different treatment processes (e.g. primary or secondary treatment, membrane filtration, chlorination, UV treatment) that range from 0 to greater than 6 logs. Two of the examples provided are chlorination and membrane filtration, which can achieve a 6-log reduction. The guidelines state that ‘Conservative estimates suggest that drip irrigation will reduce exposure by at least 2 logs compared with spray irrigation, and that subsurface irrigation will provide a further 2-log reduction’ (see Controlling methods of application, p96.)

The guidelines identify that a major route of exposure to microbial hazards from recycled water is the ingestion of pathogens present in reused greywater. These can be aerosolised by either toilet flushing or spray irrigation, but the guidelines make no reference to mitigating the exposure risk via inhalation of aerosolised pathogens such as *Legionella*. The guidelines provide values for achievable log-reduction targets for enteric viruses, enteric protozoa and enteric bacteria for recycled water, but make no reference to other opportunistic pathogens that are potentially present in the recycled water.

Generally, on-site restrictions for domestic greywater from a single household can reduce risk where the level of greywater treatment is low – for example, by only allowing subsurface irrigation and not allowing irrigation of vegetables. For higher exposure risks (e.g. toilet flushing), more extensive (and expensive) treatment is required. As in New Zealand, some people reuse greywater after treatment while others reuse it without any treatment, such as bucketing bath water to the garden.

5.3 CANADA

5.3.1 Canadian Guidelines for Domestic Reclaimed Water for Use in Toilet and Urinal Flushing (January 2010)

These guidelines provide a management framework for the collection, treatment and reuse of greywater from single households and multi-unit residential or commercial buildings, and for community clustering. They state that 'the potential health risks associated with decentralized domestic reclaimed water treatment systems mean that there is a need for a high level of treatment reliability and oversight' (see Management framework, p27). The guidelines take a risk-based approach in setting water quality parameters including BOD_5, TSS, turbidity, *Escherichia coli*, thermotolerant coliforms and FAC residuals. All domestic recycled water that is to be used for toilet and urinal flushing requires disinfection by any chemical, physical or biological means that will result in the destruction, inactivation or removal of microorganisms.

Like the Australian National Guidelines, the Canadian guidelines use a risk-based assessment comprising four components: hazard identification, exposure assessment, hazard characterisation and risk characterisation – indeed, the Canadian guidelines are based on the Australian guidelines.

As part of the hazard identification, the guidelines mention that opportunistic pathogens including *Pseudomonas aeruginosa*, *Aeromonas* spp., *Legionella* spp. and *Mycobacterium avium* are potentially present in recycled water, along with human enteric pathogens. The guidelines also mention that due to the diversity of pathogens that are potentially present in wastewater and greywater, it is impractical to monitor for all of them. Therefore, like the Australian guidelines, the Canadian guidelines suggest using reference pathogens based on criteria such as high occurrence, high concentrations, high pathogenicity, low removal efficiency on treatment and long survival in the environment for evaluating either treatment success or exposure risk. Some candidate reference viral pathogens include noroviruses, enteroviruses and rotaviruses, as humans are the only natural hosts for these viruses and they do not replicate in wastewater systems. The enteric protozoa *Cryptosporidium parvum* and *Giardia lamblia* are most often associated with waterborne diseases, and both species only multiply in the gastrointestinal tract of their mammalian hosts. The guidelines state that *Cryptosporidium* spp. are less readily removed by water treatment processes than *G. lamblia* and are also less sensitive to most types of disinfection, making *C. parvum* a useful choice as the reference pathogen for protozoan hazards. The bacterial candidates listed are the usual enteric bacteria (*E. coli*, *C. jejuni*, *Shigella* spp. and *Salmonella* spp.), but the guidelines make no direct reference to the monitoring of opportunistic pathogens that may be present in the recycled water. Hazard identification should also include chemical hazards, such as the creation of disinfection byproducts because of chlorine disinfection.

The exposure assessment includes accidental ingestion of the recycled water due to issues such as cross-connection with the potable water supply, along with respiratory illness because of the aerosolisation of microorganisms that are present in the water.

5.3.2 Public Health Guidelines for Water Reuse and Stormwater Use, 2020 (Health Guidelines), Government of Alberta

These guidelines (Alberta Health, 2021), which were published in 2021 by the Alberta Provincial Government, are designed to provide a health risk-based assessment process and performance targets for the reuse of alternative water sources, i.e. those not supplied by fresh surface water, reservoirs or groundwater. The alternative sources include municipal wastewater, greywater, stormwater, roof-collected rainwater, and vehicle wash water. End uses for the recycled water include ornamental water features, the irrigation of food and non-food crops, vehicle washing, clothes washing, toilet and urinal flushing, and recreational water use (e.g. swimming, boating, canoeing or kayaking).

The guidelines broaden the scope of the 2010 Canadian guidelines for the reuse of water for toilet flushing and provide a process for developing a water quality management plan (WQMP) when using an alternative water source that is based on water safety plan principles. A WQMP is required for each water system using recycled or non-fresh water to consider source water attributes, the intended end use of the recycled water, identification of hazards to the health of the end user (either microbial or chemical) and the removal or reduction of these hazards to safe levels. The WQMP also requires the setting of pathogen log-reduction targets and treatment processes, as well as the required system monitoring to ensure targets are met.

Of note, the guidelines recommend that the WQMP should manage the growth of opportunistic pathogens including *Legionella pneumophila*, *Pseudomonas aeruginosa* and *Mycobacterium avium* in post-treatment water storage and distribution infrastructure.

5.4 USA

5.4.1 United States Environmental Protection Agency. 2012 Guidelines for Water Reuse

In the USA, water reuse regulations have been developed at the state level with no federal regulations. The United States *Guidelines for Water Reuse* were last updated in 2012 by the US EPA and provide information about different treatment technologies, recycled water quality, types of water reuse applications, and system design and management considerations to protect the health of users of the recycled water (US EPA, 2012). The guidelines state that as part of addressing public health considerations, the most significant goal is to mitigate microbial and chemical contaminants in the recycled water. This involves both proper treatment and appropriate end use of the recycled water. Basically, as there are increasing levels of human exposure, there must be a concomitant increase in the level of treatment and monitoring to assess treatment efficacy.

The guidelines list *Legionella* amongst the pathogenic bacteria that are potentially present in wastewater (table 6.2 of the US EPA 2012 guidelines). The guidelines also recognise that aerosolisation of inappropriately treated recycled water is of concern due to the potential inhalation of pathogenic bacteria and viruses. Aerosol inhalation may pose a direct exposure risk from sprinkler irrigation systems spraying recycled water, or an indirect risk where the recycled water is reused as make-up water in cooling towers that are not adequately controlled.

The guidelines also provide a case study of a risk assessment undertaken for *Legionella* spp. in reclaimed water at Tossa de Mar, Costa Brava, Spain. The recycled wastewater is supplied to water distributed to fire hydrants and is used for activities such as street cleaning and landscape irrigation. The parameters used to assess water quality include parasitic helminth eggs, *Escherichia coli*, suspended solids and turbidity, and *Legionella* is also purportedly measured where the end use of the recycled water warrants this, although specific details are not provided. The FAC residual level is constantly monitored to ensure it is maintained at an appropriate level throughout the system.

6. SUMMARY

Legionella bacteria are ubiquitous in water and soil ecosystems and are also regularly isolated from recycled wastewater systems when they are specifically tested for. The established primary mode of transmission for *Legionella* bacteria is via inhalation of aerosolised contaminated water or soil. Therefore, any process that involves water or dust containing *Legionella* bacteria poses a risk to a susceptible population (e.g. people who are immunocompromised, have chronic respiratory disease, have had recent surgery or are elderly). The *Legionella* risk associated with cooling towers, reticulated drinking water systems and recreational waters is widely recognised, and many jurisdictions have developed guidelines and regulations to manage or limit the exposure risk. However, recycled wastewater and greywater has been shown to contain *Legionella* bacteria and current wastewater treatment systems do not remove them from the effluent stream. In fact, studies have shown that *Legionella* is able to survive and replicate throughout the waste treatment process and within the infrastructure used for treated effluent disposal.

The reuse of recycled wastewater and greywater as an alternative water resource is potentially an emerging health issue as it becomes more widely adopted and will bring concomitant health risks that will require careful management to prevent illness in the exposed and susceptible population. Even in waste treatment systems with a high level of pathogen removal, *Legionella* can persist and the exposure risk to it can be increased with aerosolisation of the treated waste.

Current risk models are unable to accurately establish the intrinsic infection risk from the exposure to aerosolised recycled wastewater and greywater due to the many assumptions that need to be made with these models. Some limitations result from not knowing the infectious dose, its survival under different environment conditions, and the variability in recovery rates with different detection methods. There is yet no standardised QMRA methodology for *Legionella*, even though this approach is frequently used to establish criteria for exposure risk mitigation.

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