# Virus transport from on-site wastewater treatment systems

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# Abstract

Viral pathogens present in wastewater, discharged to land from domestic wastewater systems, can contaminate groundwater and drinking-water supply wells. By understanding how viral pathogens move through soils, we can optimise assessment of the risk of groundwater contamination. This is particularly pertinent in areas where drinking-water supply wells are situated near wastewater discharge. Free draining soils such as those in much of Canterbury, New Zealand, may increase the risk of groundwater contamination. Current bacterial indicators do not provide a good indication of viral contamination. This research investigates the transport of pathogenic viruses in free draining Canterbury soils. Intact soil cores of silty and sandy loam overlying sandy gravels were used to carry out saturated virus transport experiments dosed with on-site wastewater.

Analysis of intact soil cores revealed heterogeneous soil structure and macropore characteristics. Hydraulic loading of secondary treated wastewater resulted in successive clogging of intact soil cores under saturated conditions. Hydraulic conductivity of both intact soil cores decreased dramatically over a period of 56 days with wastewater conditioning. Saturated virus transport experiments conducted every two weeks in intact soil cores showed that male-specific-2 coliphage (MS2 phage) was a more conservative indicator of virus transport and removal than rotavirus and rotavirus surrogate from on-site wastewater treatment systems (OWTS). Mass recovery of MS2 phage was substantially higher than that of rotavirus and rotavirus surrogate. Consequently, log reduction values (LRVs) for MS2 phage through intact soil cores were also lower than rotavirus and rotavirus surrogate. Rotavirus surrogate better represented rotavirus transport and removal than MS2 phage, however, MS2 phage still provides a conservative indication of viral contamination from OWTS. The similarity of rotavirus to rotavirus surrogate during the intact core experiments showed that rotavirus surrogate is a useful tool for predicting virus transport and removal in wastewater experiments. However, caution must be exercised when using rotavirus surrogate for separation distances from drinking water supply wells as it does not provide a conservative indication of viral contamination as MS2 phage does. The Guidelines for separation distances from OWTS to drinking water supply wells used in New Zealand appear to be too conservative having been modelled using data based on conservative indicators. Determining appropriate separation distances from pathogenic virus, virus surrogate, and indicator virus data would allow the modelling of a more realistic scenario. However, further investigation into the transport and removal of viruses from OWTS is first required to provide data in different soil types and under various conditions to enable the improvement of current separation distance guidelines.

# Acknowledgements

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To Richard Lamb and Hazel Pearson for generating my interest in wastewater of all things! I respect your tenacity to answer the scientific questions in your work in on-site wastewater. This gave me opportunities to learn lots, problem solve, and gave me a foot up when I came back to study. Andrew Dakers, you keep myself and much of the on-site wastewater industry here in New Zealand aware of the risks of viral contamination from on-site wastewater and pressed for research in this area. Liping Pang, you went out of your way to help me with my analysis and modelling. I am so grateful to you for that. To Judith, Susan, Phil, Kurt, Bron, Erin and Margaret at ESR, cheers for all your help, I certainly couldn't have done it without you. Particularly thank you, Judith Webber, for teaching me micro methods and having my kit in your lab. Annabelle, you've been awesome. Thanks for teaching me lots at ESR and for being a great friend here. Thanks so much Risi, Mum, Dad, and my friends and flatmates for being wonderful. You're all heaps of fun, we have lots of fun, and that kept me happy and healthy this year.

# Glossary of terms

| | |
|---|---|
| **Adsorption** | The physical or chemical attachment of substances to the surface of particles |
| **Biofilm** | The layer of biological growth and inorganic constituents that develops at the on the infiltrative surface of the soil |
| **Biological oxygen demand (BOD)** | A gross measurement of the concentration of biodegradable organic impurities in wastewater expressed in milligrams per litre |
| **Design loading rate** | The loading rate that applies to the distribution of wastewater to the disposal field area, expressed as $L/m^2/day$. The DLR is derived from AS/NZS 1547:2012, based on the long-term acceptance rate of the soil, and reduced by a safety factor |
| **Disposal field** | The area of land and distribution system employed to discharge primary of secondary treated on-site wastewater to the soil |
| **Domestic wastewater** | Wastewater derived from a domestic households or from staff and residents facilities, but excluding commercial and industrial wastewater and stormwater flows |
| **Effluent** | Sewage, water, or other liquid derived from waste, treated or partially treated |
| **Groundwater** | A body of water in the soil below the soil surface in which all pores are saturated. |
| **Hydraulic conductivity** | The ability for a soil or other medium to transmit water in liquid form through pores |
| **Male-specific-2 coliphage (MS2 phage)** | A virus which infects host bacteria *Escherichia coli* and *Salmonella typhimurium* commonly used as a tracer for human enteric viruses |
| **Macropore** | Pores or cavities in the soil matrix larger than larger than 75 µm |
| **On-site wastewater treatment system (OWTS)** | On-site wastewater treatment system unit which treats wastewater to a primary or secondary level on site where the source is not connected to municipal sewerage infrastructure |
| **Pathogenic** | Disease-causing, generally applied to microorganisms that cause disease. |
| **Secondary treatment** | The aerobic biological wastewater treatment process following primary treatment to a treatment level of ≤20 mg/L $_cBOD_5$ and ≤20 mg/L TSS |
| **Septic tank** | A buried, watertight tank designed to receive treat wastewater to a primary level. Settleable solids are retained in the bottom of the tank while floating materials form a scum layer at the top, creating an anaerobic environment. |
| **Separation distance** | The distance that wastewater discharge must be situated from any boundary, water body, or other limiting factor |
| **Straining** | Straining is the retention of a particle or microorganism when its diameter is too large to filter through a pore |

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|---|---|
| **Rotavirus** | A double-stranded virus in the Reoviridae family that causes gastroenteritis. Rotavirus was selected for this study as the conservative pathogenic for virus transport |
| **Rotavirus surrogate** | DNA labelled silica nanoparticles designed to mimic rotavirus transport |
| **Wastewater** | The contaminated water produced from domestic dwelling activities, institutions, commercial or public facilities |
| **Water table** | The upper surface of groundwater at which the hydraulic pressure is zero and below which the soil is permanently saturated |

# Abbreviations and units

| | | | |
|---|---|---|---|
| **$_cBOD_5$** | five day carbonaceous biological oxygen demand | **mg** | milligram |
| **°C** | Degrees Celsius | **MR** | mass recovery relative to potassium bromide |
| **cm** | centimetre | **mV** | millivolt |
| **d** | day | **pfu** | plaque forming units |
| **DO** | dissolved oxygen | **ppm** | parts per million |
| **DOC** | Dissolved organic carbon | **qPCR** | quantitative polymerase chain reaction |
| ***E. coli*** | *Escherichia coli* | **SD** | Standard deviation |
| **ESR** | The Institute of Environmental Science and Research, New Zealand | **TSS** | total suspended solids |
| **g** | gram | **µg** | microgram |
| **LRV** | Log reduction value | **µL** | microlitre |
| **m** | meter | | |
| **mm** | millimetre | | |
| **MS2 phage** | male-specific-2 coliphage | | |
| **OWTS** | on-site wastewater treatment system | | |
| **PVC** | polyvinyl chloride | | |
| ***K*** | hydraulic conductivity | | |
| ***$K_{sat}$*** | saturated hydraulic conductivity | | |
| **KBr** | potassium bromide | | |
| **L** | litre | | |

# 1 Introduction

## 1.1 Contamination of drinking water from on-site wastewater discharge

### *1.1.1 Waterborne disease*

Waterborne disease is caused by the consumption of microbial pathogens in contaminated drinking water (Borchardt et al., 2012; Benedict, 2017). Microbial pathogens are microscopic organisms such as bacteria, viruses, and protozoa, that are disease-causing. Waterborne disease is estimated to cause 2.2 million deaths each year with 1.8 billion people worldwide drinking water from water supplies that suffer from faecal contamination (Bain et al., 2014; Ramírez-Castillo et al., 2015).

Viruses are infectious agents that infect living cells of humans or other organisms and they are the smallest of the microbial pathogens. They are responsible for disease such as influenza, gastroenteritis, and immunodeficiency disorders. Viral pathogens from contaminated drinking water can cause diarrhoea, gastrointestinal disease and systematic illnesses and are the leading cause of childhood gastroenteritis worldwide (Ramírez-Castillo et al., 2015). New Zealand has some of the highest reported notifiable disease rates in the Organisation for Economic Co-operation and Development (OECD) countries. Over the past year, there have been 440 notifiable cases of gastroenteritis in New Zealand (Institute of Environmental Science and Research [ESR], 2017).

Wastewater which contains viral pathogens can be discharged to land and is a potential source of drinking water supply contamination. For instance, deep water supply wells from 220-300m were found to be contaminated by viral pathogens sourced from wastewater seepage (Bradbury et al., 2013). Waterborne disease outbreak studies show that viral pathogens are widespread in groundwater systems (Abbaszadegan et al., 2003; Borchardt et al. 2003, 2007, 2012; Bradbury et al., 2013). Bradbury et al.'s (2013) survey of drinking water wells around Wisconsin, USA, revealed that 46% of groundwater wells tested positive for viruses and many wells were positive for virus infectivity. Detected viruses in Bradbury et al.'s (2013) study included adenovirus, enterovirus, rotavirus and norovirus. Virus presence is variable through time and season, and Bradbury et al. (2013) found there was significant correlation between outbreaks of specific virus serotypes in municipal sewerage and subsequent well contamination. Sampling from other sites in the United States revealed widespread viral contamination of groundwater also (Abbaszadegan et al., 2003; Fout et al., 2003). In all of these studies, wastewater was suggested as a potential source of contamination.

### *1.1.2 Contamination from on-site wastewater treatment systems*

On-site wastewater treatment systems (OWTS) are small discharge domestic wastewater treatment systems that serve a single dwelling, which is not serviced by a municipal sewerage system. OWTS generally consist of an enclosed treatment process followed by a disposal system that discharges the treated wastewater to land. OWTS in New Zealand most commonly consist of either a primary (septic), or a primary and secondary (aerobic) treatment unit. From this unit, treated wastewater is discharged to the disposal field either by gravity or dose-loading to soil in the subsurface (Auckland Regional Council [ARC], 2004).

OWTS that discharge wastewater to land can contaminate nearby drinking water supply wells. Discharge of domestic wastewater from OWTS releases pathogens including viruses into soil via disposal fields and land application systems (Moore. et al., 1981; Pang et al., 2006; Morrissey et al., 2015). Viral pathogens entering the soil can be transported through the soil profile into groundwater which may be consumed as drinking water, posing a significant risk to public health. This is particularly concerning in areas where wastewater is discharged in close proximity to drinking water supply wells. There are well over 270,000 OWTS in New Zealand, mostly in rural areas, serving 20% of the population in some regions (Ministry for the Environment [MfE], 2008). Many households that have an OWTS also have a drinking water supply well. Figure 1 illustrates this scenario whereby wastewater containing pathogens is applied to soil on a rural property. The household's drinking water supply well is in close proximity, and down-gradient of the disposal field.

Most of the 50,000 drinking water supply wells registered in New Zealand Regional Council databases have no form of microbial treatment i.e. filtration or disinfection to eliminate microorganisms (MfE, 2008). In addition to private wells, approximately two-thirds of small community drinking water supplies (serving less than 500 people) do not have microbial treatment, nor are they required to under New Zealand legislation at the present time.

The risk of contamination posed by the lack of drinking water treatment is exacerbated by the current state of OWTS operation and management. An alarming proportion of OWTS do not comply with regulatory guidelines in areas of maintenance and management, and many do not provide sufficient treatment (Levett et al., 2010; Prince, 2017). The poor performance of OWTS is widely due to inadequate installation and maintenance, poor public awareness, deficient resources from regulatory authorities, as well as the inadequate adoption of standards, procedures, and guidelines

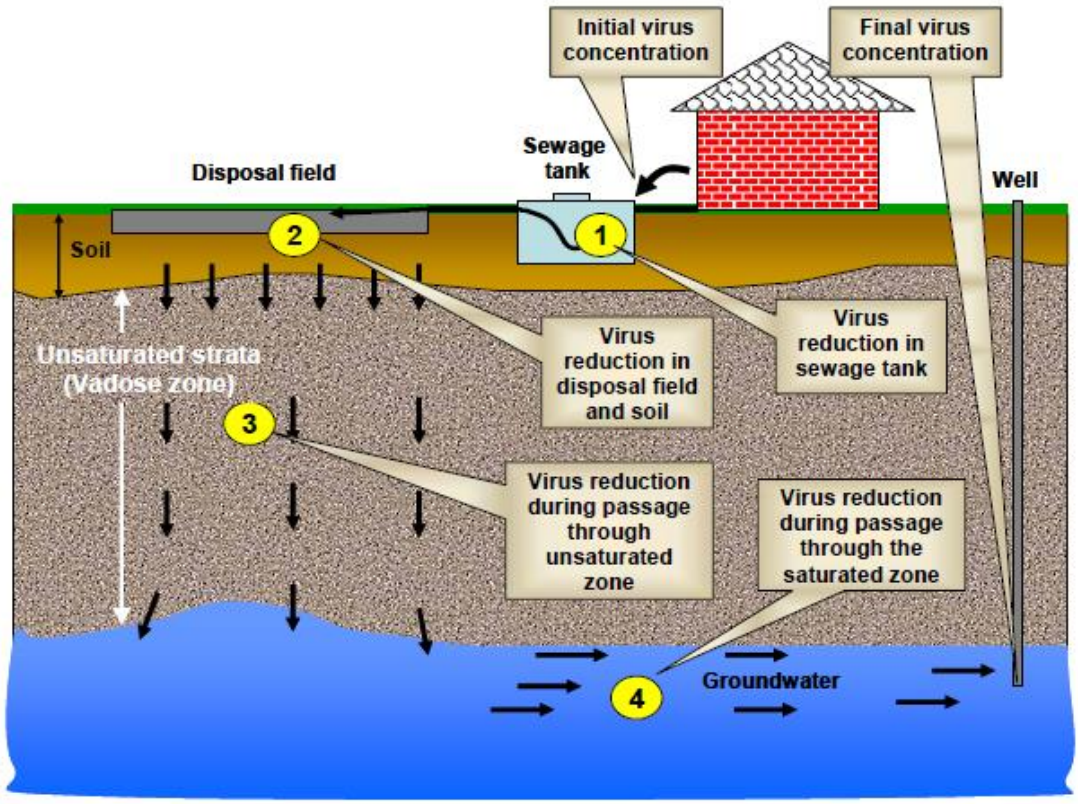


**Figure 1. Components of virus removal between the OWTS and groundwater well, from Moore et al., 2010.**

(Gunady et al., 2015). In an assessment of 31 aerated OWTS in South Australia, 97% of aerobic OWTS did not comply with guidelines for wastewater parameters which included faecal indicator bacteria, *Escherichia coli* (*E. coli*) (Levett et al., 2010). These issues with OWTS result in an increased risk of drinking water contamination. Prince et al.

Multiple instances of contaminated drinking water have been attributed to domestic wastewater discharge (Lipp et al., 2001; Carroll et al., 2005; Li et al., 2011). OWTS have been identified as the probable source of drinking water well contamination as far as 35m to 100m down-gradient of the discharge (Carroll et al., 2005; Hanson et al., 2006). Increased densities of OWTS can increase the risk of contamination of water sources (Carroll et al., 2005). While land disposal can provide some additional removal of viruses, better understanding of virus removal through the soil profile is imperative to mitigate drinking water contamination.

### *1.1.3 Separation distances to drinking water wells*

Separating OWTS discharge from groundwater decreases the risk of drinking water contamination. When wastewater is discharged to land, the underlying soil provides additional treatment and removal of pathogens as the wastewater percolates toward underlying groundwater (figure 1). Appropriate separation distances can, therefore, help mitigate the risk of contamination of drinking water wells

(Moore et al., 2010; Blaschke et al., 2016; Schijven et al., 2017). Separation distances can be both vertical or horizontal setbacks between the bottom of the disposal field and the seasonal high groundwater table or drinking water supply well. Horizontal separation distances from OWTS have been recommended as great as 100 meters from down-gradient drinking water wells to mitigate faecal contamination. The New Zealand Standard 1547 (2012) for on-site domestic wastewater management, sets out design requirements for OWTS including separation distances and treatment requirements (Standards New Zealand, 2012). More recently, guidelines for separation distance based on virus transport between OWTS and wells have become available to designers of wastewater systems and regulatory authorities by the Institute of Environmental Science and Research (ESR) (Moore et al., 2010).

Regional regulatory authorities also provide additional rules for OWTS in some regions of New Zealand. The New Zealand Standard 1547 (2012) requires a minimum vertical separation distance of 0.6 from the bottom of the wastewater disposal bed to the seasonal high groundwater table, and a minimum horizontal separation distance of 20m to the nearest drinking water supply well (Standards New Zealand, 2012). This standard does not consider virus contamination in groundwater but is based upon the faecal bacterial indicator *E. coli*. *E. coli* does not provide a good indicator of virus presence or concentration in environmental waters (see section 1.4). Regulatory authorities are increasingly becoming better informed of the risks of viral contamination and this has resulted in increasing uptake of the Guidelines as a tool to mitigate viral contamination. However, this recent uptake of these guidelines by designers and regulatory authorities have highlighted issues with the implementation of the recommended separation distances.

### *1.1.4 The Guidelines*

To mitigate the risk of viral contamination from OWTS, ESR established the '*Guidelines for separation distances based on virus transport between on-site domestic wastewater systems and wells'* referred to hereafter as "the Guidelines" (Moore et al., 2010). The Guidelines enable the user to calculate separation distances for OWTS based on virus transport and removal processes through the treatment and discharge of wastewater to land. The Guidelines were developed using New Zealand and international data available for virus concentrations in septic tank effluent and groundwater, as well as virus transport data in soil and vadose zone materials (Moore et al., 2010). Monte Carlo modelling was used to calculate predicted virus reductions in a range of soil, vadose, and groundwater conditions. The authors recognise the limitations and the paucity of the data used for these calculations in the Guidelines. They highlight the conservative nature of the separation distances, particularly in some hydrogeological settings where the separation distances become impractical due

to the distance required. These limitations have been confirmed by users of the Guidelines, especially by regional councils, district councils, and engineers who design OWTS. Difficulty applying the Guidelines limits their usefulness to users and subsequently their potential to the mitigate risks posed to human health by viral contamination.

The Guidelines follow a four-step approach to determine the reduction in virus concentrations as follows:

1) Virus removal in the OWTS
2) Virus removal in the disposal field and underlying soil
3) Virus removal in the unsaturated (vadose) zone above the water table, and
4) Virus removal in the groundwater aquifer

The overall reduction in the necessary virus concentration is determined by the input concentration into the OWTS from the household, the output from the OWTS, and then subsequent removal in the disposal field and soil. The removal of viruses in New Zealand soils in the Guidelines is based on two studies of *Salmonella* bacteriophage (McLeod et al., 2008; Pang et al., 2008). These studies reported spatial removal rates of bacteriophage in specific New Zealand soil types. These data were then used to estimate virus removal in generic New Zealand soil classifications. Leaching experiments in four soil types were undertaken by McLeod et al. (2001) which used either secondary treated municipal waste or dairy shed effluent. While this data provides insights into leaching of dairy shed effluent, the conditions do not reflect OWTS discharge to land. To properly understand the specific virus transport capacities from OWTS wastewater we must consider the conditions to which wastewater is discharged from these units. These conditions include virus input concentrations, design loading rates, distribution system type, and the receiving soil's capacity to transport and remove viruses (Van Cuyk et al., 2007; Morales et al., 2014).

## 1.2 Virus transport from OWTS

### *1.2.1 Pathogenic viruses in OWTS*

Virus concentrations in human wastewaters are highly variable. Domestic on-site wastewater exhibits even greater variability than municipal wastewater because virus concentrations are not homogenised by population size (Hewitt et al., 2011). Drinking water supply wells are more likely to be contaminated with viruses than bacteria. This is because: a) viruses can have higher persistence than bacteria in wastewater and groundwater, b) fewer virus particles are required to infect an individual compared to some bacterial pathogens, and c) viruses are an order of magnitude smaller

than bacteria (Gerba et al., 1978; Rose et al., 1991). This high infectivity of viruses allows them to cause disease in smaller numbers than bacteria (Moore et al., 2010). In New Zealand, there are five notifiable waterborne and potentially waterborne disease-causing viruses: adenoviruses, enteroviruses, noroviruses, rotaviruses, all of which cause gastroenteritis, as well as hepatitis A virus, which causes liver disease.

### *1.2.2 Virus concentration in OWTS*

Virus concentrations in OWTS discharge are determined by the virus input concentration in the raw household wastewater and subsequent reduction through the treatment process (figure 2). While virus occurrence is less frequent in OWTS than in municipal wastewater, when outbreaks do occur concentrations can be much higher due to less dilution (as described above). Moore et al. (2010) developed an algorithm in the Guidelines for determining possible virus concentrations in domestic wastewater from a single dwelling based on virus concentrations in human faeces.

Of the five waterborne and potentially waterborne viruses identified by Moore et al. (2010), rotavirus is shed at the highest concentrations. Concentrations of rotavirus can be shed at $10^7$ to $10^{12}$ per gram of faeces for up to 39 days during infection. The Guidelines use shedding concentration, dwelling occupancy, stool mass, and water usage to calculate virus concentration in the OWTS to the 95% percentile for any given viral outbreak. i.e., 1 in 20 outbreaks is expected to exceed this concentration. Note that there is likely to be none of these waterborne pathogenic viruses present if there is no viral outbreak in the dwelling.

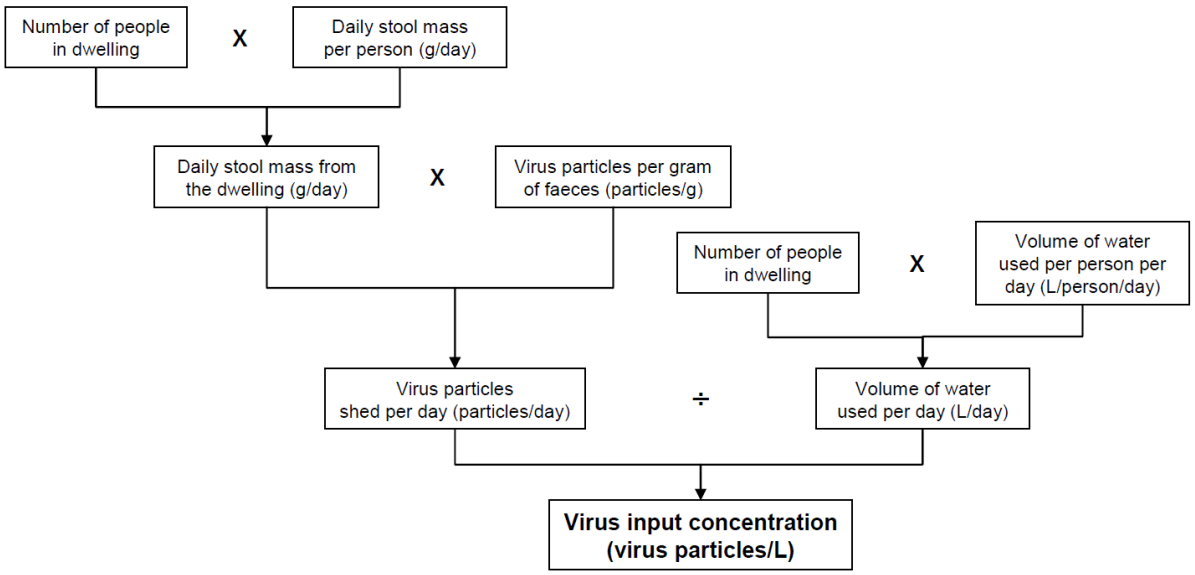


**Figure 2. Data inputs and their relationship in the calculation of virus concentration entering an OWTS from an infected household, from Moore et al., 2010.**

### *1.2.3 Virus removal rates in OWTS*

There is limited reporting of virus concentrations in OWTS wastewater discharge. Removal of viruses in municipal wastewater has been reported in the literature but the data is highly variable. For example, Payment et al. (1986) observed an average virus reduction of 75% by primary treatment. Rao et al. (1984) determined virus removal in primary treatment to be seasonal, dropping from 63% average removal to 29% in the monsoon months. Conversely, Morris (1984) found that primary treatment had no significant effect on virus removal. In a study of municipal wastewater processes in the Netherlands, viruses were reduced significantly all except for rotaviruses following treatment (Lodder, 2005). Observed log reductions in of virus in Lodder et al.'s (2005) study were: F-specific phage (1.6), somatic phage (1.1), enterovirus (1.4), reovirus (1.3), norovirus (1.8). Rotavirus only showed a 0.2 log reduction. Treatment processes in their study consisted of primary settlement, biological activated sludge treatment, and phosphorus removal.

The Guidelines present values for virus removal in OWTS depends on the level of treatment obtained prior to discharge to land. Primary treatment of wastewater within the septic tank is estimated to achieve a log reduction of 0.6 in virus concentration. Secondary treatment of wastewater by an aerobic treatment unit is estimated to achieve a log reduction of 1.0 in virus concentration. If tertiary treatment is present (i.e. UV irradiation or chlorination) then a further 1.0 log reduction can be achieved. Moore et al. (2010) used reported reductions in virus concentrations from the literature to estimate a representative log reduction by primary treatment of 0.6 log.

### *1.2.4 Virus removal in disposal fields*

After primary or secondary treatment in the septic tank or aerobic treatment unit, wastewater is commonly discharged to land via a disposal field or drip irrigation field (figure 3). This may be dose-loaded or gravity fed. Disposal fields are primarily designed to discharge treated domestic wastewater to land in a way which enables long-term soakage into underlying soils. This process, often gravity or dose-loading to a sand bed or conventional trench provides some additional treatment as the wastewater seeps into the underlying soil (Barnett et al., 2009; Standards New Zealand, 2012). The effectiveness of this stage of disposal and treatment may depend on the upkeep and function of the OWTS and disposal field. Research shows that some additional treatment is provided by disposal field media before wastewater enters the receiving soil (Axler, 2005). Virus removal may be achieved in disposal fields in the order of 0.36 – 0.49 log for pea gravel and sand respectively (Moore et al., 2010). Discharge of wastewater from OWTS either via disposal field or directly into the soil via subsurface drip irrigation is governed by interactions with receiving soil.

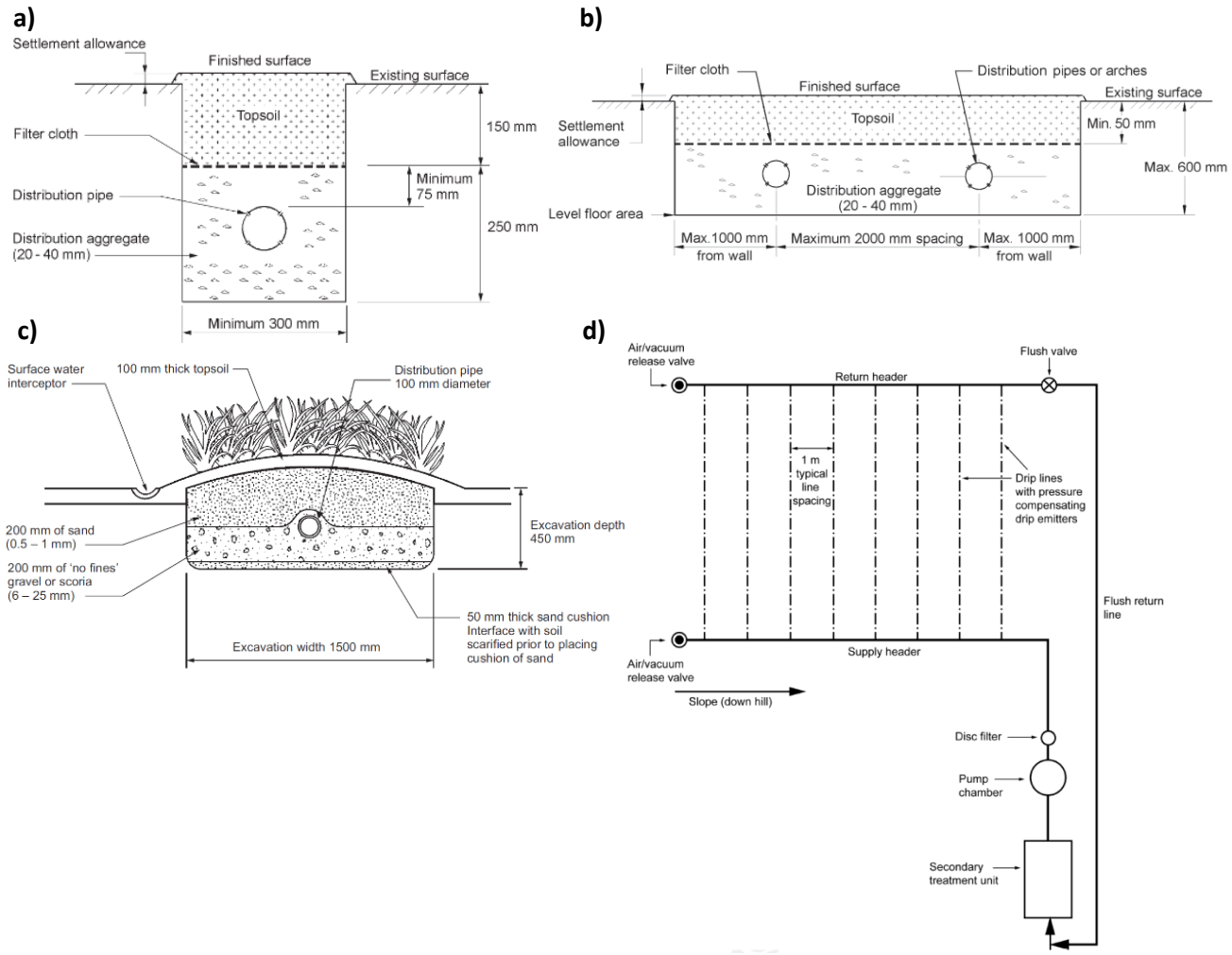


**Figure 3. Examples of wastewater disposal systems commonly used in New Zealand following primary or secondary OWTS. a) conventional piped trench, b) conventional bed, c) evapotranspiration system, d) drip irrigation system showing secondary OWTS, from AS/NZS 1547: 2012.**

## 1.3 Virus removal in the subsurface

Virus removal in the subsurface is governed by complex attachment/detachment, straining and inactivation processes (Schijven et al., 2017; Bradford et al., 2015; Hijnen et al., 2005). These processes are determined by an array of physical, chemical and biological properties of the soil, virus properties and wastewater composition. Soil properties that influence virus removal include soil structure, surface chemistry, hydraulic conductivity, organic content, and macropore characteristics (Vaughn et al., 1981; Gerba et al., 1991; Powelson et al., 1993; McLeod et al., 2004; Hijnen et al., 2005; Sinton et al., 2005; Pang et al., 2008; Yusong et al., 2013; Morales et al., 2014). This complex array of variables in the subsurface are broken down in this section to explain how each can affect virus removal.

### *1.3.1 Virus properties*

Virus properties determine how specific viruses react with the subsurface environment. These properties include virus type, surface properties, size and isoelectric point (Jansons et al., 1989; Powelson et al., 1994; Dowd et al., 1998; Hijnen et al., 2005). Table 1 shows the difference in virus size and isoelectric point that affect rates of transport and removal in the subsurface of waterborne and potentially waterborne viruses in New Zealand. These are compared with commonly used indicator, male-sepcific-2 bacteriophage (MS2 phage), and a DNA-labelled surrogate available to mimic rotavirus in transport experiments.

**Table 1. Properties of the 5 notifiable waterborne and potentially waterborne viruses in New Zealand, MS2 phage and rotavirus surrogate (Pang et al., 2014; Michen et al., 2010)**

| | | Size (nm) | Isoelectric point (pI) |
|---|---|---|---|
| Notifiable waterborne or potentially waterborne virus | Adenovirus | 80 - 90 | 3.5 – 4.0 |
| | Enterovirus | 20 - 30 | 4.0 – 6.4 |
| | Hepatitis A virus | 20 - 30 | 2.8 |
| | Norovirus | 25 - 40 | 5.5 – 6.9 |
| | Rotavirus | 60 - 80 | 4.5 |
| Bacteriophage | MS2 phage | 20 - 30 | 3.9 |
| DNA-labelled surrogate | Rotavirus surrogate | 70 | 2.5 |

### *1.3.2 Attachment, straining & die-off*

Virus attachment in soil depends on the virus surface charge, the charge of the receiving soils, and of the chemistry of the water (Bradford et al., 2004; Harvey et al., 2004; Torkzaban et al., 2006a). Studies in colloid transport theory have reported attachment as the dominant process for the removal of colloids including viruses, with straining also playing an important role (Attinti et al., 2010; Bradford et al., 2003; Stevik et al., 1999). Attachment theory predicts an exponential decrease in concentration of particles such as viruses over distance in porous media determined by first order kinetics. While this theory is applicable for clean homogeneous porous media transporting inert colloids, attachment of microorganisms adds complexity which disobeys this first-order attachment (Bradford et al., 2003). Attachment rates have been shown to be higher in sandy loam than in clayey soils, and this is attributed to the presence of preferential flow paths in clayey soils (Sinton et al., 2005). The detachment of virus particles is less important than attachment and has been considered a largely

irreversible process, however, this too is not well understood in heterogenous environments (Pieper et al., 1997).

Straining is the retention of a particle or microorganism when its diameter is too large to filter through a pore, and is therefore inversely proportional to soil particle size (Torkzaban et al., 2008). In OWTS disposal fields, factors such as biofilm formation, wastewater chemistry, hydraulic loading rate and dosing regimen all influence attachment of viruses (Van Cuyk et al., 2004, 2007).

The inactivation of viruses may also contribute to their reduction through the soil, however, pathogenic viruses have been shown to survive for months in groundwater, so this mechanism is not likely to significantly reduce viral contamination. Verma (2008) studied the survival of indicator microorganisms *E. coli* and Enterococci in soil and found that temperature significantly affected inactivation rates. This has implications for OWTS with large annual temperature differentials. The presence of predator organisms, as well as available nutrients, affected inactivation rates also.

### *1.3.3 Water chemistry*

Chemistry of the wastewater or receiving water affects virus removal too. Domestic wastewater contains organic matter and nutrients and generally exhibits a higher pH than drinking water (8.5 to 9). An investigation into how solution chemistry affects the transport of viruses was conducted by Torkzaban et al. (2006b). Using virus tracers MS2 phage and fX174 phage, experiments showed greater retention of the viruses at a lower pH and higher ionic strength. This was attributed to greater electrostatic interactions at the soil-water interface. Yusong et al. (2013) support these findings also, where in their study microorganisms including X174 showed increased retention as ionic strength increased. Schulze-Makuch et al. (2003) observed faster breakthrough of MS2 phage at a higher pH and delayed breakthrough at a reduced pH in saturated aquifer laboratory experiments. Increasing the level of organic contaminants has shown a lower removal rate of microorganisms including MS2 phage in experiments conducted by Weaver et al. (2013). This was attributed to competition for sites on the media and the authors suggest that prolonged contamination with organic matter, such as that from wastewater, may cause faster and longer transport of pathogens.

The constituents present in wastewater, including nutrients and microorganisms, change the physical characteristics of the disposal field media and receiving soils by eventually forming a clogging biofilm layer at the receiving surface. Aimin et al. (2011) found that the retention of microorganisms increased with the addition of nutrients to stimulate biofilm growth in laboratory sand aquifer experiments. The retention of microorganisms was mostly at the surface and within the biofilm layer. This was supported by a field analysis of OWTS disposal fields by Gill et al. (2009), where reduced biofilm

development occurred with the application of secondary treated effluent with lower nutrient loadings, than with primary effluent loading. Percolation through the less developed biofilm in systems receiving secondary effluent resulted in a greater transport of nutrients to underlying groundwater.

### *1.3.4 Hydraulic properties*

The biofilm layer affects virus removal mechanisms and changes soils hydraulic conductivity (Beal et al., 2008; Jiang et al., 2010). The hydraulic conductivity of soil is the measure of how much water can travel through the pore space of soil or porous media in either saturated or unsaturated conditions. Radcliffe et al. (2009) demonstrated using modelling, that biofilm restricts OWTS disposal field bottom flux, in the range of 1 to 57% of the soil's initial hydraulic conductivity. In 12 different soil types, disposal field bottom flux ranged from 2.92 to 10.43 cm/d despite initial soil hydraulic conductivity values ranging 8.18 – 642.98 cm/d. Severe clogging of soils and biofilm growth resulting in continuous ponding, when loaded with septic tank effluent, has been reported by Siegrist (1987), whereas clogging by tap-water treatment was negligible in the same study. Saturated hydraulic conductivity values averaging 241 cm/d were observed, reducing to less than 1% after loading with septic tank effluent at rates of 1.3 – 5.2 cm/d (Siegrist, 1987).

Hydraulic loading rates of wastewater from OWTS vary from 0.2 cm/d to 5.0 cm/d depending on the receiving soil type, system design, treatment level, and other site constraints that determine the design loading rate. Nicosia et al. (2001) found log removals of PRD1 phage in OWTS disposal fields were 1.43-1.91 for high load cells (6.3cm/d) and 2.21 for lower hydraulic loading (3.2cm/d). With 125 mm of rainfall, a 1.2 log increase in PRD1 phage was detected at a 0.6 m depth below the disposal field area. Clogging rates are attributed to the mass loading of biological oxygen demand and total suspended solids (Siegrist, 1987). A layer of organic and inorganic material forms above the infiltrative surface within the disposal field as well as clogging of pores resulting in reduced pore size. Clogged soil surfaces had significantly high organic carbon and nitrogen concentrations compared to unclogged surfaces (Siegrist, 1987). Higher water contents have been observed in the clogged soils also. Therefore, by lowering the hydraulic loading rate of the disposal field, soil clogging can be slowed. Van Cuyk et al. (2007) observed higher removal of MS2 phage & PRD1 phage at higher loading rates of 250mm/d compare to loading at 50mm/d. Also, a more frequent dosing regimen spread out the hydraulic loading rate of effluent and resulted in better virus removal. McCray's (2009) review of soil treatment from OWTS recommended that lowering hydraulic loading rates for the purpose of reducing water content could improve virus removal.

### *1.3.5 Porosity & macropore flow*

Porosity and macropore flow both determine hydraulic conductivity in heterogeneous soil and consequently influence virus removal processes. Porosity is the volume of the space between soil particles, which determines the amount of water the soil can hold when saturated. Porosity in soil science is generally expressed as a value between 0 and 1, where 0 is no pore space and 1 is 100% pore space. Typical porosities of soils range from 0.20 for gravels to 0.60 for fine silty clays or organic clays. Morales et al. (2014) found that soil porosity and grain size significantly affected virus attachment rates.

Macropores are large pores that have been formed by cracking, or by burrowing animals such as worms. Water flow in pores of all sizes is determined by gravity, capillary pressure, and friction from the solid surfaces and water. However, the larger the macropores, the greater gravity dominates flow, thereby resulting in preferential flow paths (Šimůnek et al., 2003; Jarvis, 2007). Natusch et al. (1996) demonstrated that macropores formed by worms and roots can rapidly transmit microbes. In soils considered poorly drained, McLeod et al. (2001) observed rapid transmission of percolating water through large soil cracks. McLeod et al.'s (2001) study also shows the contrast of inter-pedal flow in poorly drained soils compared to flow through wet sand in sandy soils. Both mechanisms resulted in high rates of *Salmonella* phage transport.

Techniques to assess the effect of macropore flow have included transport experiments and modelling. The use of x-ray computed tomography has proven a useful tool for the analysis of soil structure and soil hydro-physical properties in recent years (Taina et al., 2008). First used in soil science by Petrovic et al. (1982) for bulk density analysis, image analysis by X-ray computed tomography (X-ray CT) scanning has developed to enable pore network extract from soil cores (Luo et al., 2010; Lamandé et al., 2013). Solute transport can be predicted by using image analysis to quantify pore size distribution and connectivity (Köhne et al., 2011). X-ray CT imaging has also been used to show how macroporosity can vary significantly, even is soils with spatially similar structure (Naveed et al., 2015). Naveed et al.'s (2015) study found that macropore network characteristics correlated strongly with saturated hydraulic conductivity. Image reconstruction of intact soil cores has also been used to extract macropore network density, surface area and length (Luo et al., 2010). Luo et al.'s (2010) research shows that soil type and land use significantly impact macropore characteristics. To understanding virus removal in specific soil types, it is therefore essential to gain an understanding of soil structure, and where possible, porosity and macropore flow characteristics.

### *1.3.6 Soil type & structure*

Hydraulic conductivity, soil structure and texture largely define the soil type. Soil structure and texture are determined by particle size, bulk density, porosity, organic carbon content and chemical constituents and biological interactions (Gupta et al., 1979). Hence, soil type has widely been reported as the governing mechanism of virus removal (Van Cuyk et al., 2007; McLeod et al., 2008; Pang, 2009). Soils can be vastly heterogeneous spatially (Schijven et al., 2000), thus, determining the soil type for a disposal field area is critical to assessing how viruses can be removed. Pang et al. (2008) modelled virus breakthrough in various New Zealand soil types using data from lysimeter experiments. Clayey soils and soils with cracks transported the most viruses and bacteria, followed by silt loam over gravel, silt loam, sandy soil, dune sand soil, pumice soil and allophanic soil. The low transport rate of allophanic soil has been attributed to its high organic matter content, and this theory is consistent with Schijven et al. (2000, 2002).

McLeod et al. (2008) used data from virus transport experiments in New Zealand soils to predict the risk of virus transport by macropore flow. Soils with the highest virus transport were those with larger soil peds. They suggest that an effective rapid method for identifying the soil's ability to transport viruses via macropore flow could be achieved by sieving the soil for ped-size analysis. Their analysis of low, medium and high transport of microorganisms via macropore flow was then applied to generic New Zealand soils from the New Zealand Land Resource Inventory (figure 4) (McLeod et al., 2008).

Pang et al. (2009) developed a database using available peer reviewed research data for microbial transport in the subsurface. Their database summarises microbial removal rates per meter for various soil types. Most of the research used to compile this table used bacterial indicators. Some virus data, mostly for *Salmonella* phage, is also presented and summarised by Schijven et al. (2017) and shows the efficiencies of virus removal in different soil types (table 2). As discussed, previous studies of virus transport in New Zealand are in the context of dairy farm run-off and irrigation. There is limited understanding of virus transport and removal in the context of domestic on-site wastewater, or risks that viruses pose to contamination of groundwater (Gerba et al., 1991; Powelson et al., 1994; Van Cuyk et al., 2007; Morales et al., 2014;). Moreover, to our knowledge, no investigation of virus transport or removal in New Zealand soils from on-site wastewater systems has been undertaken. Virus transport studies in New Zealand soils have primarily been in volcanic derived North Island soils (McLeod et al., 2001, 2003, 2004; Pang et al., 2008). Pang et al. (2008) did study virus transport in two Canterbury soils (i.e. Lismore shallow silt loam over gravels and Templeton silt loam), but again this was in the context of dairy shed effluent. Their results found virus attachment and removal to be attributed to

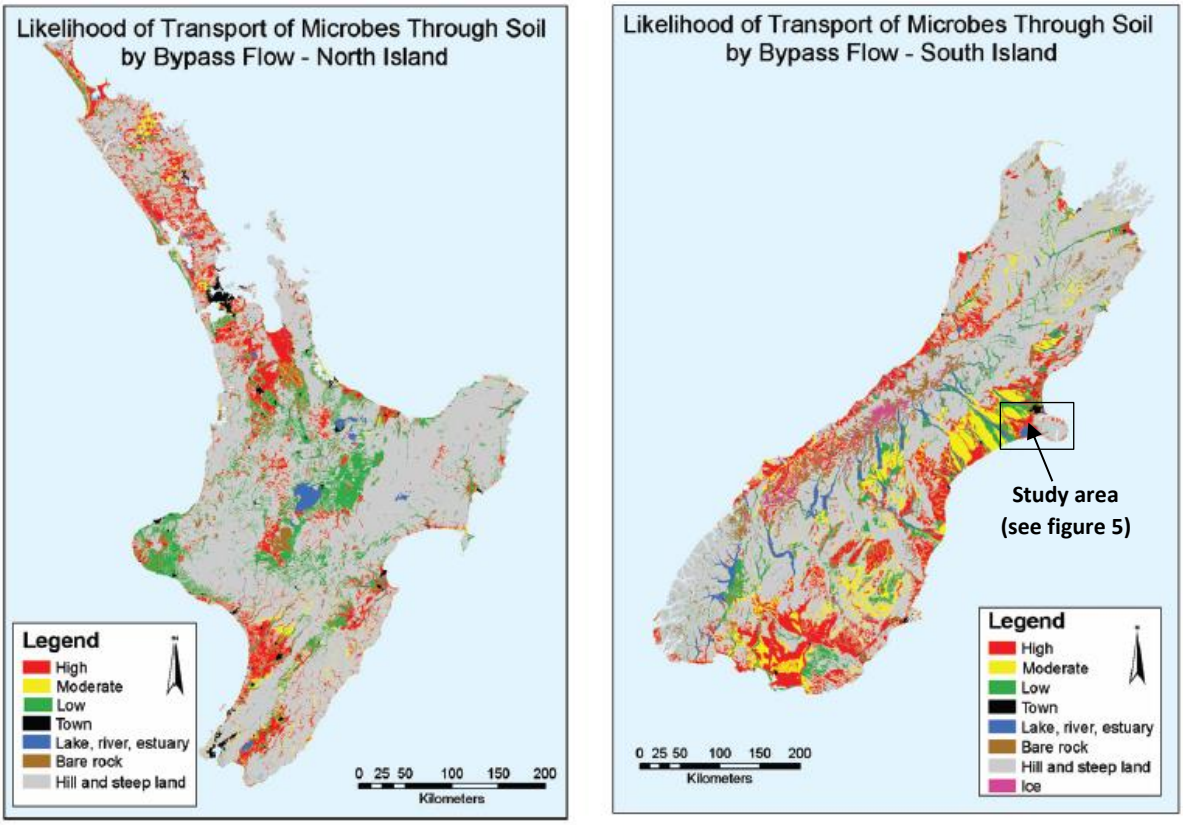


**Figure 4. Likelihood of transport of microbes through soil by preferential flow (by-pass flow) on flat to rolling land in New Zealand, from McLeod et al., 2008.**

soil chemistry and lithology. This finding, in conjunction with international literature, highlights the need to investigate the virus transport and removal capacity of specific soil types to better evaluate the risk of contamination of viruses in underlying groundwater. Lack of virus removal studies may be due to their pathogenicity restricting their use in field and laboratory experiments. The database of microbial removal adapted from Pang et al. (2009) by Schijven et al. (2017) indicates the wide range of removal capacities both between soil types and between different indicator microorganisms (table 2).

**Table 2. Efficiencies of virus removal in different soil types from Schijven et al., 2017.**

| Soil type | Contamination source | Microorganism | Removal Rate ($Log_{10}$/m) | | |
|---|---|---|---|---|---|
| | | | mean | min | max |
| Allophanic soil | Dairy shed effluent | Salmonella phage | Complete removal | | |
| Clay loam | Dairy shed effluent | Salmonella phage | 1.8 | 1.59 | 2.15 |
| Clayey soil | Dairy shed effluent | Salmonella phage | 0.97 | 0.12 | 2.08 |
| Deep silt | Dairy shed effluent | Salmonella phage | 1.99 | 1.56 | 2.56 |
| Fine – very fine sand | Sewage | PRD1 phage | 9.19 | 5.02 | 13.68 |
| Fine sandy loam | Sewage sludge | Poliovirus | 5.26 | 4.97 | 5.54 |
| Fine sandy loam | Dairy shed effluent | Salmonella phage | 2.98 | 2.4 | 3.28 |
| Loamy sand | Microbial tracer | Salmonella phage | 3.76 | 2.74 | 4.87 |
| Pumice soil | Dairy shed effluent | Salmonella phage | 16.61 | 15.75 | 17.46 |
| Recent sandy soil | Dairy shed effluent | Salmonella phage | 2.46 | 2.08 | 2.89 |
| Shallow silt loam over gravel | Dairy shed effluent | Salmonella phage | 1.98 | 0.99 | 2.53 |
| Silt loam | Dairy shed effluent | Salmonella phage | 2.3 | 2.07 | 2.69 |
| Silty clay loam | Dairy shed effluent | Salmonella phage | 2.8 | 1.87 | 4.18 |
| Silty sands and gravel | Sewage | f2 bacteriophage | 2.19 | 1.31 | 2.86 |

## 1.4 Viral indicators and tracers

Virus removal is determined by various physical and chemical characteristics of both the organisms and the receiving environment. Therefore, the different properties of viruses compared to indicator bacteria cause them to exhibit different transport and removal mechanisms. Viruses have been reported to have longer survival times in water (Skraber et al., 2004; Ramírez-Castillo et al., 2015). Enteric viruses have been detected in waters that met criteria for faecal indicator bacteria, reported by Gerba et al. (1996) and Pusch et al. (2005).

Microbial indicators including faecal coliforms, Enterococci*, Clostridium perfringens*, and F-specific bacteriophage were proven ineffective as indicators of presence and inactivation of human enteric viruses through municipal wastewater treatment processes in New Zealand (Simpson et al., 2003). The high infectivity of viruses allows them to cause disease in smaller numbers than bacteria (Moore et al., 2010). New Zealand's drinking water standards require less than one *E. coli* per 100mL but do not specify a virus limit (Ministry of Health [MOH], 2008).

Various virus strains are present in domestic wastewater so appropriate viral indicators must be selected to effectively evaluate viral pathogenic risks in water and wastewater samples (Lin et al., 2013). Though some research suggests a good correlation between faecal bacteria and viruses, others contradict these findings. Recent research stresses the need to use viruses themselves as indicators of pathogenic viruses rather than bacteria (Azadpour-Keeley et al., 2003; Simpson et al., 2003; Skraber et al., 2004; McCray, 2009; Lin et al., 2013). A widespread survey of wells by Abbaszadegan et al. (2003) found that of 400 samples, 4.8% had infective pathogenic human viruses. No direct correlations between viruses and microbial indicators were found. Additionally, Borchardt et al. (2003) report positive virus samples in the absence of bacterial indicators. The World Health Organisation [WHO] (2016) also recognise that the fate and transport of viruses are very different to faecal indicator bacteria and that outbreaks of waterborne disease have occurred in absence of faecal indicator bacteria.

The Guidelines use rotavirus as the representative virus of choice to calculate separation distances (Moore et al., 2010). Due to the high concentrations shed in faeces, separation distance to satisfactorily remove rotavirus is greater than for the other waterborne viruses. Rotavirus can also persist in groundwater, remaining infective for up to 7 months (Carter, 2005; Espinosa et al., 2008). The Guidelines recognise that using rotavirus as an indicator virus is conservative and using it may result in impractical separation distances. Therefore, hepatitis A virus is also used as an indicator virus in the Guidelines, because it causes more severe illness than rotavirus. Rotavirus is recommended as the default virus of choice when implementing the guidelines to provide a high level of protection. Hepatitis A virus could be alternatively used should the user believe the separation distance to be “excessive” or “impracticable”.

By understanding how viral pathogens move through soil, we can optimise assessment of the risk of groundwater contamination. This is particularly pertinent in areas where drinking water supply wells are situated near wastewater discharge. Free draining soils overlaying groundwater such as those in much of Canterbury, New Zealand, may increase the risk of groundwater contamination. Current bacterial indicators do not provide a good indication of viral contamination.

## 1.5 Objectives

This research aims to investigate the transport of pathogenic viruses in free draining Canterbury soils using intact soil cores overlaying sandy gravels. As rotavirus is the relevant pathogenic virus used in the Guidelines, experiments will be conducted using rotavirus along with a relevant indicator virus and virus surrogate in OWTS virus transport experiments.

The research objectives of this study are:

a) Review separation distance guidelines for OWTS and methods for determining these guidelines.

b) Determine relevant hydraulic properties and macropore characteristics of free draining Canterbury soils.

c) Investigate virus transport through intact soil cores under a worst-case saturated OWTS scenario to;
    a) Understand transport behaviour of pathogenic virus, indicator virus, and virus surrogate; and
    b) Determine pathogenic virus, indicator virus, and virus surrogate removal capacity of intact soil cores.

d) Compare pathogenic virus transport and removal capacity in intact soil cores with indicator virus and virus surrogate.

# 2 Methodology

## 2.1 Canterbury soil

Canterbury has widespread free draining soils over-laying extensive groundwater aquifers (figure 5). This geology makes the region especially susceptible to groundwater contamination from OWTS. Canterbury free draining silty and sandy gravels and sand have been selected in this study to represent the most at-risk soil types, in terms of contaminant transport, that overlie Canterbury's vast groundwater system. Previous studies of viral contaminant transport through soils in New Zealand have been reported for a variety of soil types in the North Island and a select few in the Southland region of the South Island (McLeod et al., 2001, 2003, 2004). These soils, however, are markedly different in structure and composition to Canterbury soils. The alluvial derived soils in the study area are highly heterogeneous and consist of three major textural types; various silty & sandy gravel mixtures (~90%), sand lenses (~5%) and open framework gravels, often with associated secondary fine silts on the upper and lower edges (~5%).

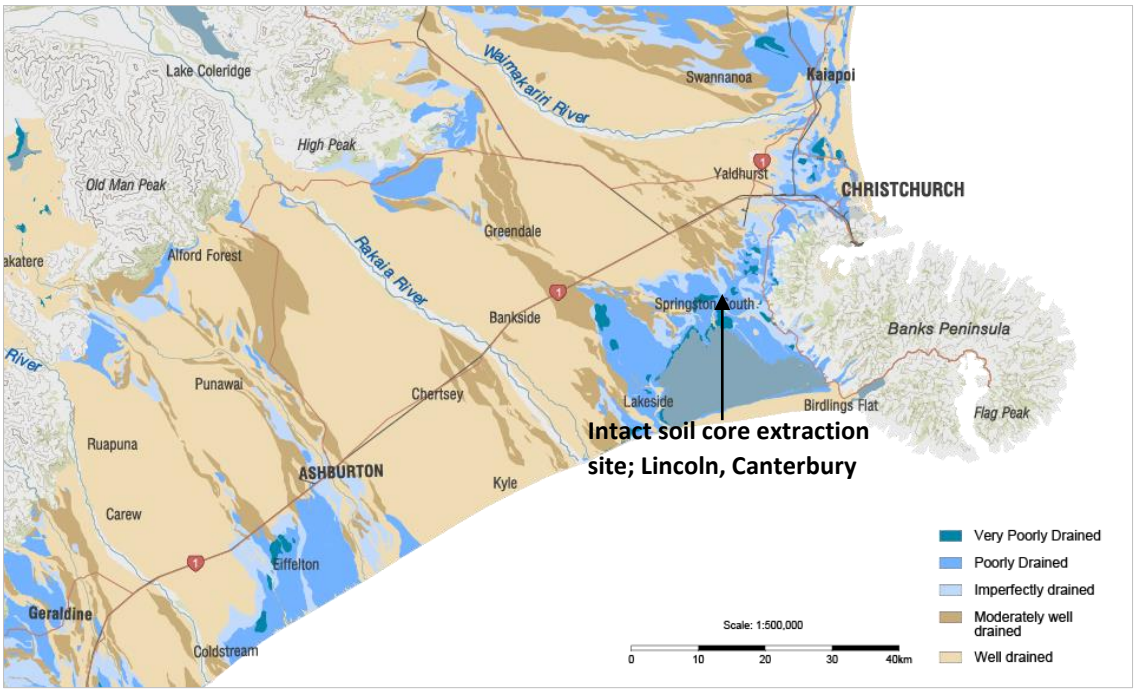


**Figure 5. Soil drainage in Canterbury, New Zealand. Map sourced from Landcare Research 2016**

## 2.2 Intact soil cores

Intact cores of Lismore soils were collected by ESR from the study area in order to carry out controlled laboratory experiments (figure 6). By collecting the soil in its intact form, soil structure, macropores and heterogeneity were maintained. Two cores were excavated from a site near Lincoln on the Canterbury Plains (figure 6, a & b). Prior to excavation, grass and topsoil was removed then cores were excavated intact from a depth of 100-500mm. The cores were cylindrical, 400mm in height and 300mm in diameter. They were contained in a PVC outer shell (figure 6. c). The space between the soil core and PVC shell was filled with petroleum jelly to prevent preferential flow down the perimeter of the cores. The cores in an unsaturated, dry condition when they were collected. Core 1 consisted of sandy-silty loam 0-110mm, sandy loam 110-190mm, and sandy gravel 190-400mm. Core 2 consisted of sandy-silty loam

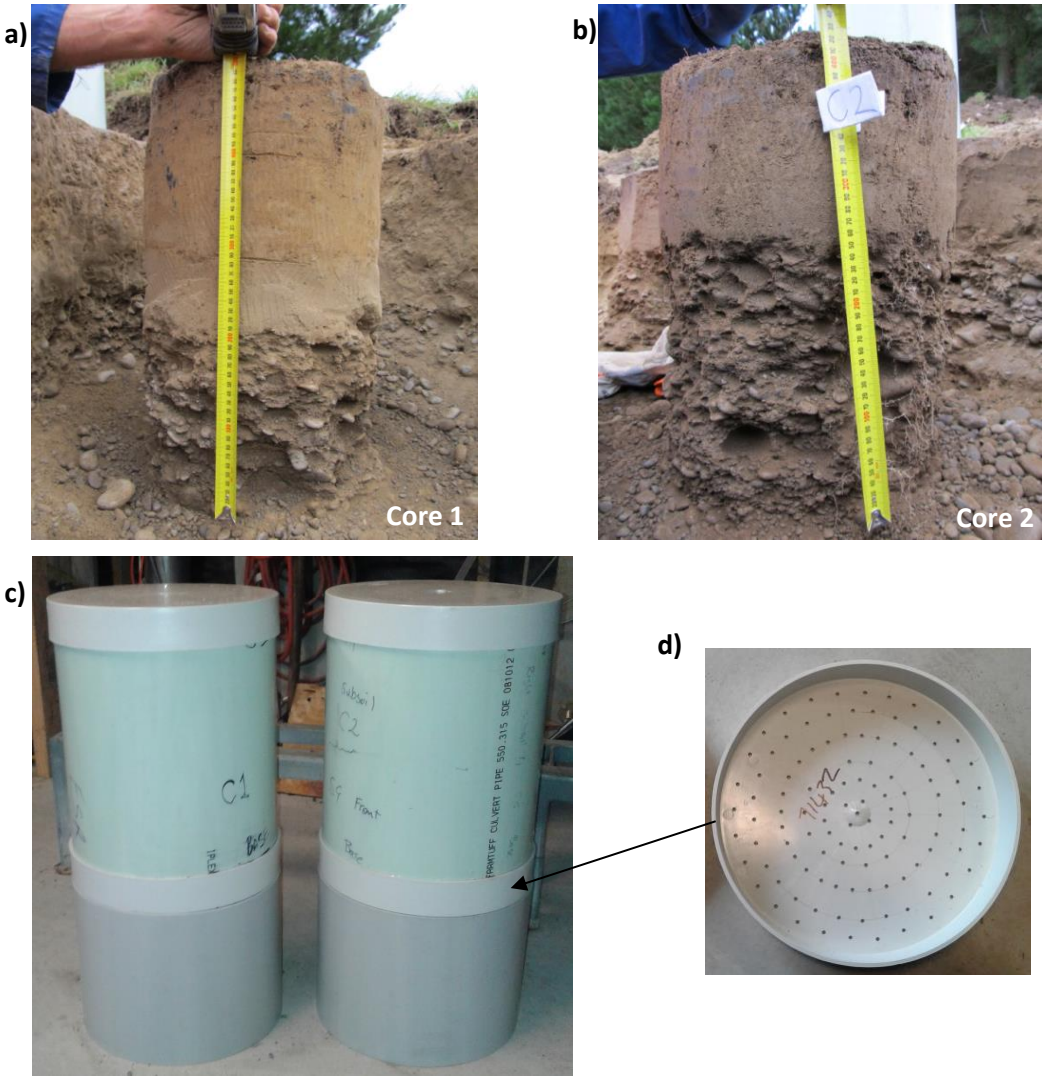


**Figure 6. a & b) intact soil cores during excavation consisting of two soil horizons of silty sandy loam and sand gravels. c) cores 1 & 2 encased in PVC. d) perforated base plate at bottom of cores for drainage through dome (dome not visible in picture).**

0-200mm, and sandy gravel 200-400mm. Despite being excavated from the same site, the soil was visually heterogeneous. Colour, macropore size, density, and soil horizon depth differed across the site also.

After cleaning of excess petroleum jelly, a 10mm layer of fine coarse sand was laid onto the bottom of each core. This enabled contact with the base plates and prevented loss of material from the bottom of the cores. The sand used was first washed with a chlorine-based detergent for sterilisation and then rinsed. Perforated PVC base-plates and PVC domes were fixed to the base of each core (figure 6. d).

### *2.2.1 X-ray computed tomography*

The soil cores were scanned using X-ray CT (figure 7). This process involved scanning each core to produce a series of spinal (vertical) and coronal (horizontal) images, producing one image at every 0.6mm. Image data was then used to analyse the soil structure, heterogeneity, and macroporosity of each core.

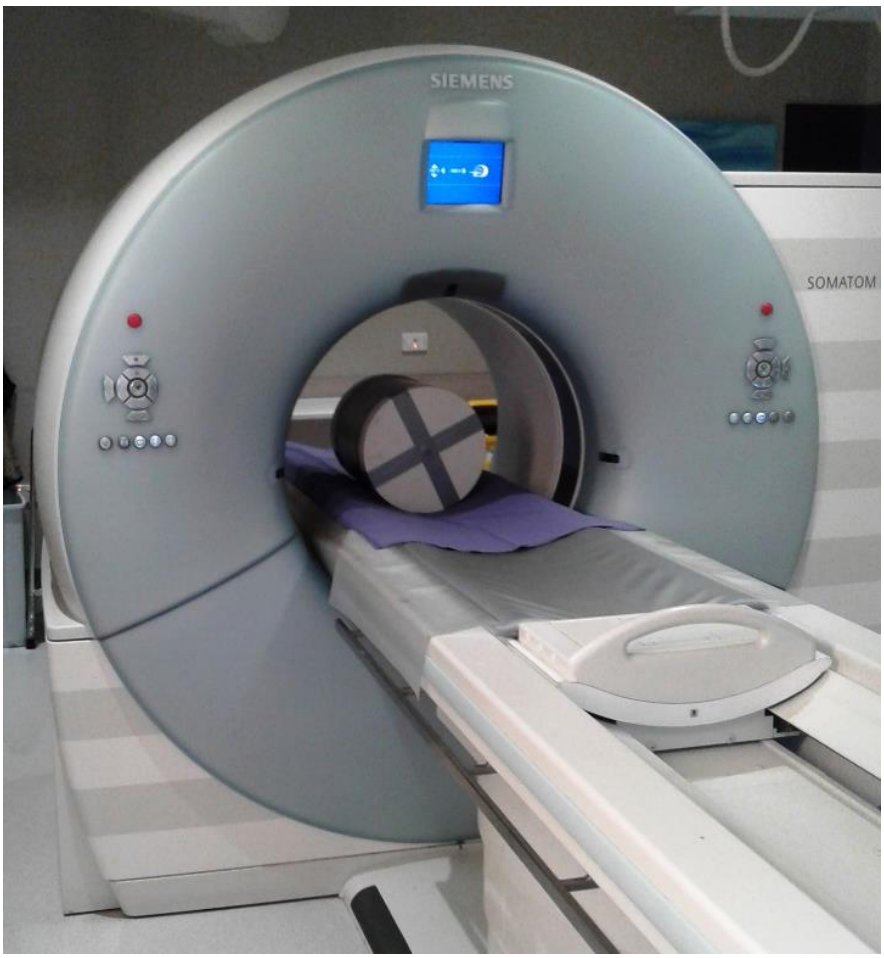


**Figure 7. X-ray computed tomography scanning intact soil core 1 in PVC casing.**

### *2.2.2 Image analysis*

Images were analysed using ImageJ® (Rasband) to determine macroporosity & average macropore size. The images were converted to 8-bit greyscale, before being cropped to exclude varied edges and the petroleum jelly casing. A median filter with a radius of 3 pixels was applied to the images, and the contrast curves stretched (normalization) to 50 minimum 204 maximum pixel values to better enable segmentation. The images were segmented using a method adapted from Kulkarni et al. (2012) and could then be analysed to obtain macroporosity data. Segmentation was performed by using a subsample to determine a threshold where air space and solid material divide. This threshold was set at an pixel intensity value of 100 and applied to the spinal image sequence for each core (figure 8). Segmented images were then analysed using the ImageJ plugin *analyse particles* to determine macropore characteristics.

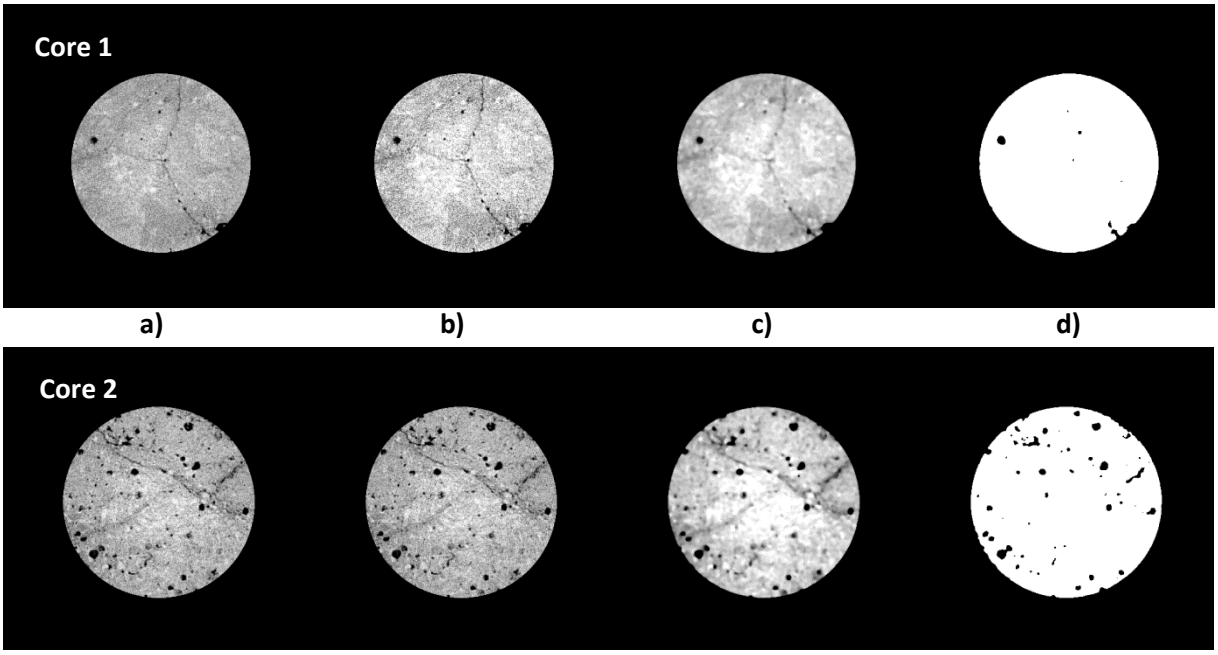


**Figure 8. Image segmentation process of X-ray CT spinal scans examples slices from cores 1 & 2. a) cropped image, b) normalization, c) median filter, d) thresholding.**

### *2.2.3 Hydraulic conductivity*

Hydraulic conductivity of the soil is the measure of the soil's ability to transmit water. Determining the hydraulic conductivity of the soil cores provides an indication of the amount of wastewater a disposal field can transmit (Barnett et al., 2009). The hydraulic conductivity ($K$) of the soil cores were measured under saturated conditions to determine the saturated hydraulic conductivity ($K_{sat}$). Saturated conditions were used to depict the worst-case scenario where the disposal field is saturated either by

rainfall, high groundwater, or a failing wastewater disposal system where the disposal field is in the saturated zone.

To enable saturation in the laboratory, Tygon® 17 tubing was fixed to the outlet at the base of the dome to allow for saturation and/or drainage (figure 9). The soil cores were slowly saturated from the bottom up with deaerated water to reduce air trapping. Deaerated water was constantly supplied over a period of 48 hours, with an overflow to reduce pressure. The deaerated water supply was then turned off and the cores were allowed to stand saturated for a further 48 hours to allow any remaining air bubbles within the cores to dissolve.

A dosing system consisting of a Masterflex® peristaltic pump and Tygon® tubing constantly supplied deaerated water to the top of the core. The water was distributed through 5 evenly spaced T-junctions on the surface of the gravel layer. The cores were kept saturated at a constant head of 5cm above the surface of the soil, maintained by an overflow at this level. The cores were allowed to drain from the outlet at the bottom through a weir system that prevented air locking. The weir system also allowed the head to be adjusted.

Saturated hydraulic conductivity experiments were conducted by way of a Darcy experiment, whereby the difference in head between inlet and outlet were measured. Head difference was 40cm and flow was calculated as per the following equation:

$$q = -K\frac{dh}{dl} \tag{1}$$

Where $q$ is the outflow from the column in terms of flux; this is calculated by the below equation, where $Q$ is the flow rate per unit time and $A$ is the cross-sectional area of the column.

$$q = \frac{Q}{A} \tag{2}$$

The length of the column, $dl$, remains constant and is equal to $l_1 - l_2$ where $l_1$ is the top of the column and $l_2$ is the bottom of the column. The head difference, $dh$, remains constant and is equal to $h_1 - h_2$ where $h_1$ is the water level in the column, and $h_2$ is the water level measured by the manometer at the bottom of the column.

## 2.3 Wastewater application

The core saturation apparatus was modified to mimic OWTS disposal field conditions in the laboratory (figure 9). Secondary treated wastewater was sourced from an aerated OWTS in Fernside, on the Canterbury Plains. The OWTS was an Oasis 2000 serving a family of four. The residence was constantly occupied over the collection regimen. Wastewater was collected from the pump chamber of the wastewater system using a peristaltic pump approximately every two weeks. Wastewater parameters were monitored for pH, carbonaceous biological oxygen demand ($_cBOD_5$), total suspended solids (TSS), and dissolved oxygen (DO), both at the time of collection, during use and storage.

The cores were conditioned by wastewater application for two weeks prior, and for the period during which virus transport experiments were conducted (42 days). The wastewater design loading rate was scaled down to account for the surface area of the cores. 3.53L were applied daily, equating to a loading rate of 50mm/$m^2$/d, based on maximum values of design criteria for on-site domestic wastewater management (Standards New Zealand, 2012). This reflects the maximum design loading rate for secondary treated effluent in free draining soils (Standards New Zealand, 2012). The design loading rate was delivered evenly over 3 doses during a 24 hour period to mimic an OWTS pump chamber dosing system. Wastewater was supplied to the reservoir supplying the cores and was constantly mixed using a magnetic stirrer to prevent it becoming anaerobic and keep the constituents evenly distributed. The wastewater was dosed to the top of the core from a distribution system buried within 20mm of sterilised fine gravel/coarse sand to reflect a primary disposal field. Wastewater was evenly distributed through a perforated tube at 6 evenly-spaced locations (insert picture). Deaerated groundwater was constantly supplied to the top of the core above the gravel maintain saturation by the apparatus described in the previous chapter.


**Figure 9. Virus transport experiment intact soil core apparatus**

## 2.4 Virus transport experiments

Virus transport experiments were carried out using the intact soil cores to determine the relative transport and removal of rotavirus, rotavirus surrogate, and MS2 phage. After being conditioned with wastewater for 14 days (described in section 2.3), three virus transport experiments were conducted on each core every 2 weeks over a period of 6 weeks. During each experiment, one regular wastewater dosing event 1.176L (one third of the design loading rate) was spiked with potassium bromide (KBr), rotavirus, rotavirus surrogate and MS2 phage, and applied as per the regular wastewater dosing regimen described above in section 2.3. The wastewater injection solution was spiked with 10ppm KBr, $10^5$ plaque forming units (pfu) per mL MS2 phage, $10^5$ copies per mL rotavirus, and $10^5$ particles per mL rotavirus surrogate. Injection solution was dosed at 300ml/minute to mimic dosing of OWTS disposal field.

Time series samples were taken for a period of 1 minute over the course of the experiments which lasted up to 8 hours. Aliquots (200ul) were sub-sampled for molecular analysis of rotavirus and rotavirus surrogates. Samples were stored in 100ml glass Schott bottles, refrigerated at 4°C for KBr and MS2 phage plaque analysis.

### *2.4.1 Conservative tracer analysis*

From the time series samples, 10ml subsamples were taken to analyse KBr concentration. Millivolt (mV) values were obtained using a bromide ion selective probe and converted to parts per million (ppm) using a calibration curve (Walshe et al., 2010; Weaver et al., 2013).

### *2.4.2 Rotavirus*

Rotavirus is a pathogenic virus which needs a host in order to replicate and survive and is capable of causing severe gastroenteritis. It is a spherical virus with a diameter 60-80nm with an isoelectric point of ~4.5 (Gutierrez et al., 2009). Rotavirus (ATCC® VR1516™) was sourced from the American Type Culture Collection. Rotavirus stock was prepared by growing in cell lines on a MA104 cell monolayer to reach confluent growth (adapted from Rutjes et al., 2009, and Li et al., 2010). Infected cell lines were washed and freeze-thaw cycled prior to being stored at -80°C. Before addition to the injection solution the rotavirus stock was thawed over ice at 4°C then sonicated in a waterbath for 2 minutes then vortexed to mix. 120µL of $10^9$ copies/mL rotavirus stock was pipetted into the wastewater volume of 1,200mL to achieve a concentration of $10^5$ copies/mL.

### *2.4.3 MS2 phage*

MS2 phage is commonly used as an indicator organism as it is non-pathogenic and present at consistent concentrations in faecal material (Gerba et al., 1982; Havelaar et al., 1993). MS2 phage is an icosahedral virus with a diameter of approximately 20-30nm (Havelaar et al., 1993). MS2 phage stock culture was obtained from the American Type Culture Collection (Product No. 15597-B1). MS2 phage infects coliform bacteria such as Salmonella and *E. coli*. MS2 phage was propagated by growing plaques to confluence on overlay Tyrptone yeast-extract glucose agar plates with host bacteria *Salmonella typhimurium* WG 49 (American Public Health Association [APHA], 2005). The MS2 phage was scraped from the overlay agar and was then separated via shaking and centrifugation, and filtration. The stock was stored at -20°C for use (Weaver et al., 2013). Before addition to the injection solution the MS2 phage stock was thawed over night at 4°C, sonicated in a waterbath for 2 minutes then vortexed to mix. 120µL of the $10^9$ pfu/mL MS2 phage stock was pipetted into 1,200mL of wastewater injection solution to achieve a concentration of $10^5$ pfu/mL.

### *2.4.4 Rotavirus surrogates & amplification inhibition experiments*

The rotavirus surrogates are 70nm silica nanoparticles developed to mimic rotavirus transport (figure 10), (Pang et al., 2014). Rotavirus surrogate was synthesized by covalently coating glycoprotein and a double-stranded 302 base-pair synthetic DNA onto 70 nm carboxylated silica beads (Pang et al., 2014). They are non-pathogenic so can be safely used as field tracers. The rotavirus surrogates have specific proteins and a DNA marker for sensitive detection. The surrogates remain stable in size, surface charge and can be easily detected. While preliminary experiments during their development suggested the particles are detectable in environmental waters and wastewaters, this is the first instance that the particles have been used directly in wastewater. Therefore, experiments to test for amplification inhibitors were also required.

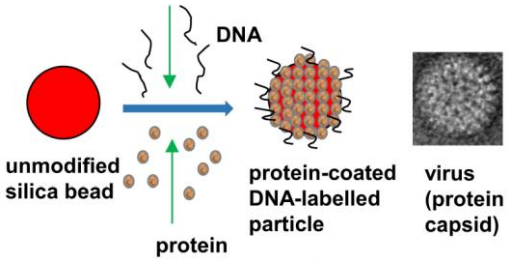


**Figure 50. Development of virus mimicking rotavirus labelled silica nanoparticles (rotavirus surrogate) from Pang et al 2012.**

Wastewater can contain contaminants which can inhibit DNA amplification during quantitative polymerase chain reaction (qPCR) (Schrader et al., 2012). These inhibitors include humic acids, fats,

proteins, polyphenols and heavy metals that can be found in wastewater samples (Shieh et al., 1995). Rotavirus surrogate samples were prepared both in, and in the absence of wastewater in 1mL aliquots at concentrations within the expected range of detection, $C_{max}$ ranging $10^4$ - $10^8$, and enumerated by qPCR. Bovine serum albumin (BSA) was added to the samples which provides some resistance to amplification inhibitors such as humic and fulvic acids (Comey et al., 1994). Samples were then analysed using real-time qPCR. The amplification of the rotavirus tag was performed using KAPA SYBR FAST Universal Kit and a LightCycler 480 Real-Time qPCR System. Positive and negative controls were included in every experiment. Each sample was analysed in duplicate.

## 2.5 Rotavirus and rotavirus surrogate analysis

### *2.5.1 Rotavirus analysis*

From the virus transport experiments, 200µL aliquots from the time series samples were taken and stored at -80°C for up to 1 week. Samples were defrosted and rotavirus RNA was extracted using a High Pure Viral Nucleic Acid Kit. Purified RNA was reverse-transcribed used the Invitrogen SuperScript VILO cDNA synthesis kit (Invitrogen) and then amplified using 2-step real-time PCR and Platinum Quantitative PCR SuperMix-UDG (adapted from Pang et al., 2004). Rotavirus counts were calculated as copies per mL.

### *2.5.2 Rotavirus surrogate analysis*

From the virus transport experiments, 200µL aliquots from the time series samples were taken and stored at -80°C for up to 1 week. Rotavirus surrogate samples were then defrosted for analysis. Bovine serum albumin (BSA) was added to the samples which provides some resistance to amplification inhibitors (Comey et al., 1994). Samples were analysed using qPCR as per the inhibition experiments described above in section 2.4.4. Rotavirus surrogate counts were calculated as copies per mL.

### *2.5.3 MS2 phage analysis*

Time series samples from the virus transport experiments were refrigerated in glass Schott bottles overnight. MS2 phage was analysed using the overlay pour plating method (APHA, 2005). 1mL subsamples and subsequent serial dilutions were plated to obtain the necessary dilution for enumeration. *E. coli* (F-amp) was the host strain used (American Type Culture Collection Product No. 13706). Plaques were enumerated by eye counts and expressed as pfu/per mL with a detection limit of 1 pfu/mL. MS2 phage stock of $10^9$ pfu/mL was used as the positive control. Deaerated groundwater was used as the negative control.

## 2.6 Data analysis

The fraction of mass recovery (MR) of KBr, MS2 phage, rotavirus and rotavirus surrogates from the virus transport experiments was calculated by integrating a breakthrough curve and normalising it to the total mass injected:

$$MR = \frac{\int_0^{\infty} Q(t)C(t)dt}{\int_0^{t_0} Q(t)C_0(t)dt} \quad (3)$$

where Q is the flow rate, $C_0$ and C is the concentration of the injection solution and outflow, respectively, t is the time, and $t_0$ is the duration of the pulse.

Log removal values (LRVs) were calculated by peak breakthrough concentration relative to injection concentration of MS2 phage, rotavirus and rotavirus surrogate:

$$-\log_{10}\left(\frac{C_{max}}{C_0}\right) \quad (4)$$

Where $C_{max}$ is the peak concentration of MS2 phage, rotavirus or rotavirus surrogate, and $C_0$ is the input concentration of MS2 phage, rotavirus or rotavirus surrogate in the injection solution.

## 2.7 Virus batch test experiments

Batch test experiments were conducted simultaneously with the transport experiments to assess degradation of rotavirus and rotavirus surrogates for the duration of each core experiment (Weaver et al., 2013). A sub-sample of injection solution was stored in the dark at the same temperature as the core. Aliquot samples were taken hourly and frozen at -80°C. Samples were later analysed for rotavirus and rotavirus surrogate as per above qPCR methods described above.

## 2.9 Modelling virus transport data

### *2.9.1 Advection-dispersion equilibrium model*

The transport of viruses and KBr through the saturated intact soil cores under steady flow was modelled using STANMOD computer programme using an advection–dispersion equilibrium model that incorporates a first-order removal term (Šimůnek et al., 1999):

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} - V\frac{\partial C}{\partial x} - \lambda C \qquad (5)$$

Where, *C* is the outflow concentration, *D* is the dispersion coefficient (cm $h_{-1}$), *V* is the average pore-water velocity (cm $h_{-1}$), x is the distance (cm), and λ is a first-order removal rate (h).

# 3 Results

## 3.1 Soil structure and macroporosity

Image analysis of the intact soil cores revealed heterogeneous structure and macropore characteristics. Figure 11 shows spinal and coronal slices of cores 1 and 2 through the middle of the cores. Notable cracks are visible through the silty-loam and become less visible in the sandy loam layer of core 1. Some large macropores are also visible in the silty-loam layer of core 1. Many large macropores are visible in the silty-sandy loam layer of core 2 with some cracks also visible. Figure 12 shows macroporosity values derived from image analysis. Core 2 had substantially more macropore area than core 1 in the top silty-sandy loam (table 3). Macroporosity is dramatically higher in the top 180 to 200mm of both cores. The sandy gravel layer in both cores had very few macropores, and hence very little macropore area. Average macropore size was greatest in the silty-sandy loam layer and smallest in the sandy gravel layers for both cores.

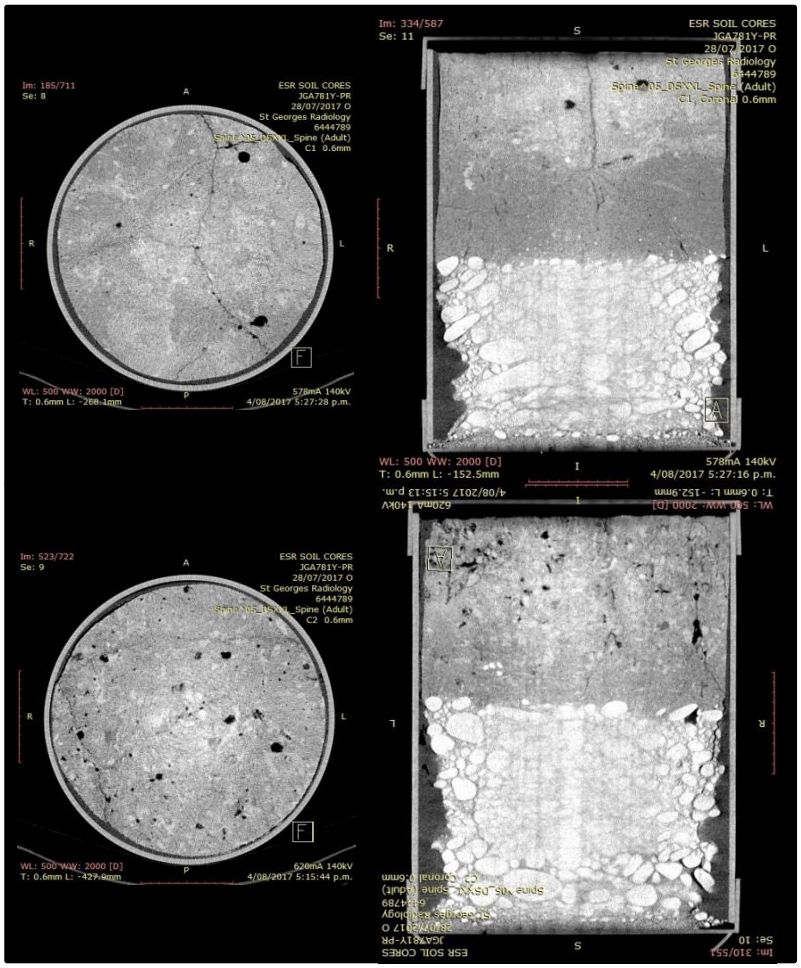


**Figure 11. X-ray CT image spinal and coronal images of intact soil cores.**

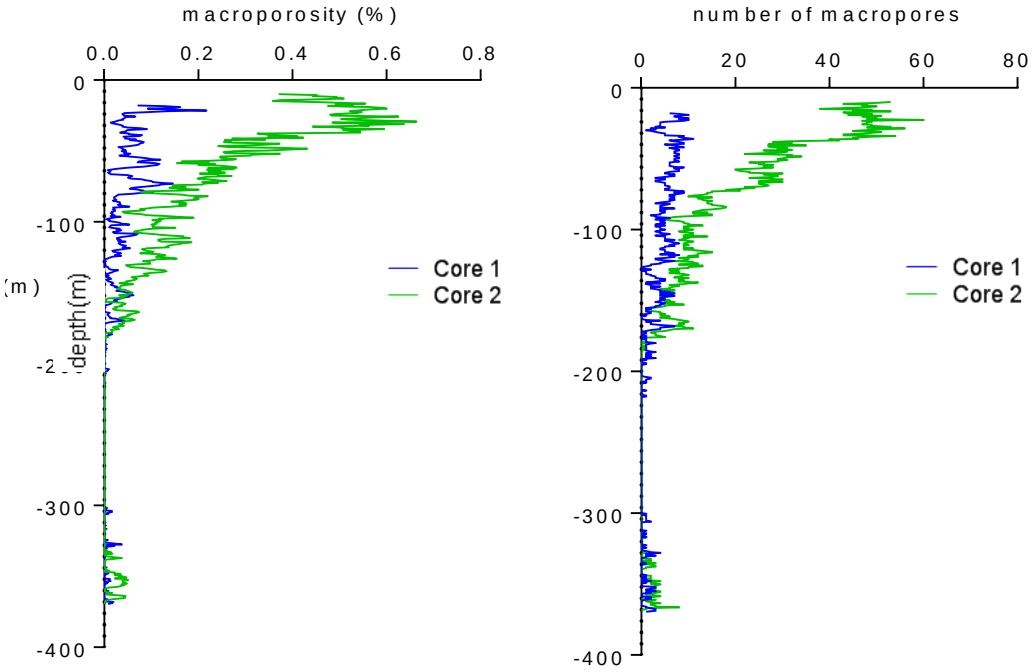


**Figure 12. Macropore analysis of X-ray CT imagery. a) average macropore area (%) relative to area of 0.6mm slice. b) number of macropores per core 0.6mm slice.**

**Table 3. Average macropore area, number and size for intact soils cores 1 and 2.**

| | Layer | Average macropore area per slice (%) | Average number of macropores per slice | Average macropore size per slice ($cm^2$) |
|---|---|---|---|---|
| **Core 1** | Silty-sandy loam | 0.068 | 8.6 | 1.25 |
| | Sandy loam | 0.012 | 2.1 | 0.75 |
| | Sand gravel | 0.008 | 1.6 | 0.66 |
| **Core 2** | Silty-sandy loam | 0.168 | 16.0 | 1.49 |
| | Sandy gravel | 0.004 | 0.50 | 1.26 |

## 3.2 Hydraulic conductivity

The initial saturated hydraulic conductivity test was repeated three times for each intact soil core. The average saturated hydraulic conductivity for cores 1 and 2 was 53 cm $d^{-1}$ and 55 cm $d^{-1}$ respectively (table 4). Saturated hydraulic conductivity reduced dramatically over the course of the experiments to 16 cm $d^{-1}$ and 23 cm $d^{-1}$ for cores 1 and 2 respectively.

**Table 4. Saturated hydraulic conductivity of intact soil cores**

| | Core 1 (cm $d^{-1}$) | Core 2 (cm $d^{-1}$) |
|---|---|---|
| **Initial ($K_{sat}$)** | 53 | 55 |
| **Final ($K_{sat}$)** | 16 | 23 |

## 3.3 Wastewater parameters

The wastewater used throughout the course of conditioning and running experiments consistently met secondary wastewater parameters of ≤20 mg $L^{-1}$ $_cBOD_5$ and ≤20 mg/L TSS. The average $_cBOD_5$ was 8.5mg $L^{-1}$ (SD = 3.5). The average TSS was 8.3mg $L^{-1}$ (SD = 3.8) (table 5). DO and pH remained consistent with average values of 6.0 mg/L and 8.4mg/L respectively (table 5).

**Table 5. Concentration of wastewater during conditioning and virus transport experiments.**

| | Average | Standard deviation | NZ standard for secondary wastewater* |
|---|---|---|---|
| **$_cBOD_5$ (mg/L)** | 8.5 | 3.5 | ≤20 |
| **TSS (mg/L)** | 8.3 | 3.8 | ≤20 |
| **DO (mg/L)** | 6.0 | 1.1 | - |
| **pH** | 8.4 | 0.8 | - |

*from AS/NZS:1547, 2012.

## 3.4 Injection solution parameters

Table 6 shows the injection concentrations of KBr, MS2 phage, rotavirus and rotavirus surrogate for the virus transport experiments. KBr concentrations ranged from 10.3 to 17.2 mg/L. MS2 phage concentrations ranged from 6.2 x $10^4$ to 2.5 x $10^5$ PFU/mL. Rotavirus concentrations ranged from 1.4 x $10^7$ to 2.3 x $10^8$ copies/mL. Rotavirus surrogate ranged from 5.8 x $10^8$ to 2.0 x $10^{10}$ copies/mL.

**Table 6. Injection solution concentrations of KBr, MS2 phage, rotavirus, and rotavirus surrogate for virus transport experiments.**

| | Day | KBr (mg/L) | MS2 phage (pfu/mL) | Rotavirus (copies/mL) | Rotavirus surrogate (copies/mL) |
|---|---|---|---|---|---|
| **Core 1** | 14 | 14.3 | $1.2 \times 10^5$ | $1.4 \times 10^7$ | - |
| | 28 | 10.3 | $2.0 \times 10^5$ | $2.3 \times 10^8$ | - |
| | 42 | 11.7 | $2.5 \times 10^5$ | $2.8 \times 10^7$ | $5.0 \times 10^8$ |
| **Core 2** | 14 | 12.5 | $1.8 \times 10^5$ | $2.6 \times 10^7$ | $1.3 \times 10^{10}$ |
| | 28 | 17.2 | $8.0 \times 10^4$ | $3.4 \times 10^7$ | $2.0 \times 10^{10}$ |
| | 28 | 11.8 | $6.2 \times 10^4$ | $2.6 \times 10^7$ | $5.0 \times 10^9$ |

## 3.5 Virus transport experiments

Figure 13 shows the breakthrough curves for KBr, MS2 phage, rotavirus and rotavirus surrogates during the experiments for cores 1 and 2 carried out every two weeks during wastewater conditioning. The breakthrough curves for all inputs clearly show a decrease in the transport of KBr, virus and rotavirus surrogate through the intact soil cores as wastewater conditioning progresses. Rotavirus and rotavirus surrogate had a greater velocity than MS2 phage and KBr, generally arriving earlier through both cores. MS2 phage showed greater velocity through the cores than KBr, arriving earlier in all experiments except day 42 for core 2.

Table 7 shows the breakthrough peak time of KBr, virus and rotavirus surrogate. Core 2 consistently showed earlier breakthrough of KBr, MS2 phage and rotavirus compared with core 1. KBr breakthrough peaks increased from 200 to 480 minutes during core 1 experiments, and from 80 to 480 minutes during core 2 experiments. MS2 phage breakthrough peaks increased from 150 to 420 minutes during core 1 experiment and increased from 60 to 480 minutes during core 2 experiments. Rotavirus breakthrough peaks were 180, 300, and 180 minutes for day 14, 28 and 42 experiments respectively for core 1 and increased from 50 to 300 minutes for core 2 experiments. The rotavirus surrogate breakthrough peak for core 1 during the day 42 experiment occurred at 195 minutes and increased from 40 to 300 minutes during core 2 experiments.

Table 7 shows mass recovery of MS2 phage, rotavirus and rotavirus surrogate relative to KBr during all virus transport experiments. Mass recovery of KBr decreased dramatically from 104.6% to 4.0% for

core 1 and 49.6% to 12.5 % for core 2 during the conditioning period. KBr mass recovery on day 28 of conditioning of core 2 was higher than day 14 (54.0%). Mass recovery of MS2 phage relative to KBr decreased from 0.485 to 0.289 for core 1 experiments but increased from 0.312 to 1.143 for core 2 during the conditioning period. Rotavirus & rotavirus surrogate mass recovery was variable during all experiment for cores 1 and 2 but remained substantially lower than mass recovery of MS2 phage.

Table 7 shows the log removal values (LRVs) of MS2 phage, rotavirus and rotavirus surrogate for the virus transport experiments. MS2 phage LRVs ranged from 0.74 – 1.99 during core 1 experiments and 0.93 – 1.47 during core 2 experiments. Rotavirus LRVs ranged from 2.24 – 3.69 during core 1 experiments and 1.50 – 3.36 during core 2 experiments. Rotavirus surrogate LRV was 3.34 during the day 42 experiment for core 1 and ranged from 1.34 to 3.41 during core 2 experiments. Figure 14 shows the log reduction of MS2 phage, rotavirus and rotavirus surrogate during all experiments relative to injection concentrations. Log reduction of MS2 phage is generally less than rotavirus and rotavirus surrogate throughout the duration of the experiments. Rotavirus surrogate log reduction trends are similar to rotavirus, with the exception of the day 28 experiments for core 2.

Figure 15 shows flow rates and pH during the virus transport experiments. Flow rates during each virus transport experiment were stable for each virus transport experiment, apart from a notable decrease in flow during the core 1, day 28 experiment. Flow rates dramatically decreased over the 42 days of wastewater conditioning. Increase in pH was observed following injection of wastewater injection solution. Recovery of pH is seen in clearly in day 14 experiments when flow rates are substantially higher.

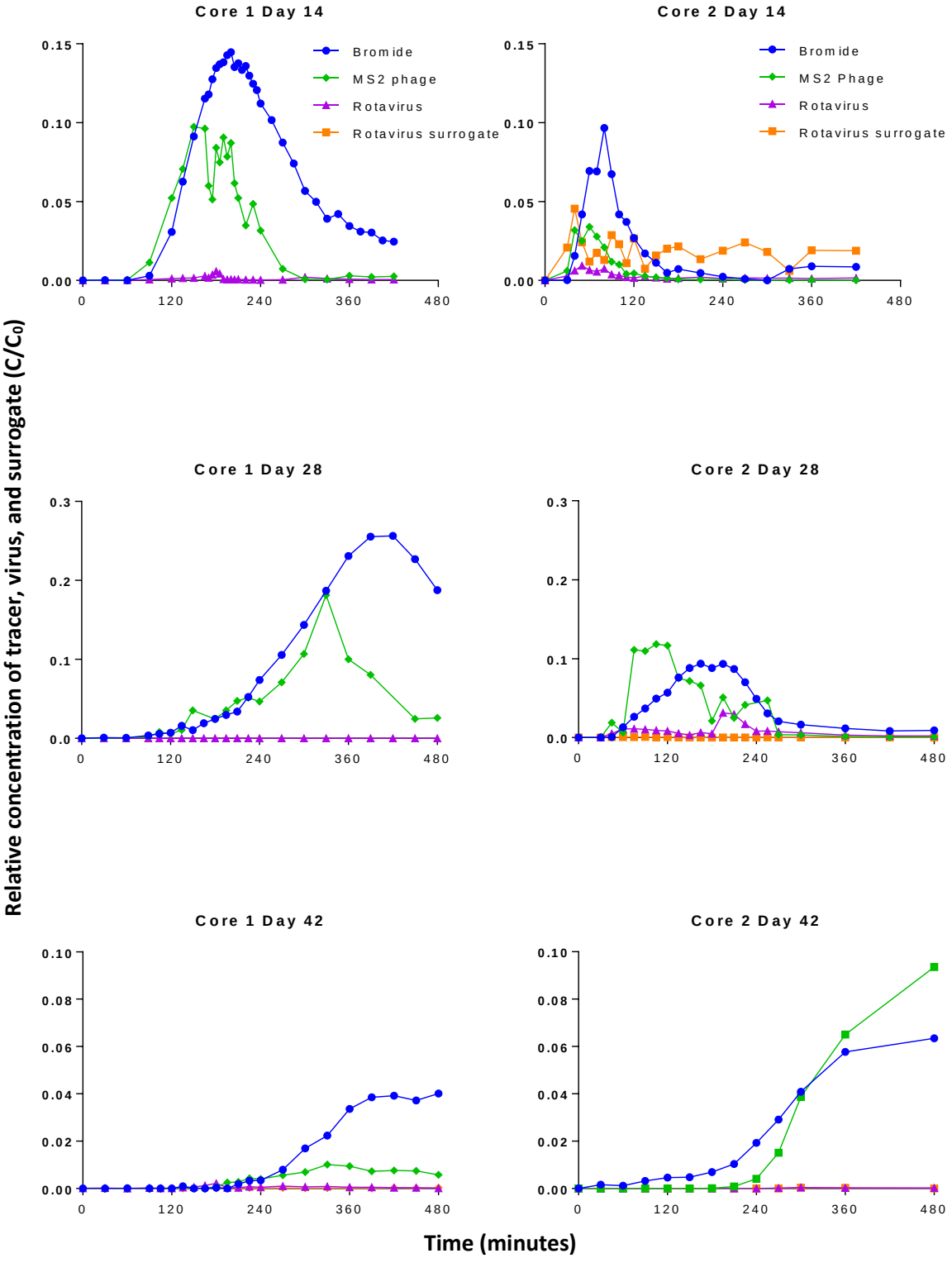


**Figure 13. Concentration of time series samples from virus transport experiments after 14, 28 and 42 days of conditioning with secondary treated wastewater for KBr, MS2 phage, rotavirus and rotavirus surrogate. No data for rotavirus surrogate for core 1, day 14 and 28 experiments.**

**Table 7. Transport parameters for virus transport experiments.**

| | | Core1 | | | Core 2 | | |
|---|---|---|---|---|---|---|---|
| | | **Day 14** | **Day 28** | **Day 42** | **Day 14** | **Day 28** | **Day 42** |
| Q (mL/min) | | 55.95 | 13.24 | 6.85 | 90.08 | 40.79 | 9.81 |
| SD (mL/min) | | 2.57 | 5.07 | 0.67 | 1.90 | 2.90 | 0.45 |
| pH | | 7.29 | 7.07 | 6.71 | 6.84 | - | - |
| SD | | 0.31 | 0.42 | 0.21 | 0.30 | - | - |
| Tpeak (min) | KBr | 200 | 420 | 480 | 80 | 165 | 480 |
| Tpeak (min) | MS2 phage | 150 | 330 | 420 | 60 | 105 | 480 |
| Tpeak (min) | rotavirus | 180 | 300 | 180 | 50 | 195 | 300 |
| Tpeak (min) | rotavirus surrogate | NA | NA | 195 | 40 | 90 | 300 |
| LRV | KBr | 0.84 | 0.59 | 1.40 | 1.02 | 1.03 | 1.20 |
| | MS2 phage | 1.01 | 0.74 | 1.99 | 1.47 | 0.93 | 1.03 |
| | rotavirus | 2.24 | 3.69 | 2.66 | 2.03 | 1.50 | 3.36 |
| | rotavirus surrogate | | | 3.34 | 1.34 | 3.11 | 3.41 |
| M (%) | KBr | 104.6 | 61.5 | 4.0 | 49.6 | 54.0 | 12.5 |
| MR | MS2 phage | 0.485 | 0.390 | 0.289 | 0.312 | 0.837 | 1.143 |
| | rotavirus | 0.019 | 0.001 | 0.037 | 0.154 | 0.217 | 0.005 |
| | rotavirus surrogate | | | 0.010 | 0.002 | 0.006 | 0.003 |

**Where, *Q* denotes flow rate, SD standard deviation, $C_{max}$ maximum breakthrough concentration, $C_0$ injection concentration, M mass recovery, MR mass recovery relative KBr, and - no data.**

## 3.6 Modelling virus transport through intact soil cores

Figure 16 shows how breakthrough curves simulated with the one-dimensional equilibrium advection-dispersion equation using STADMOD compare against the observed KBr, MS2 phage, rotavirus and rotavirus surrogate data. The model appears to provide reliable simulation of KBr transport and MS2 phage but not of the measured rotavirus and rotavirus surrogate transport, which showed significant variability. The velocity of MS2 phage relative to KBr is consistent across core 1 experiments ranging from 0.84 to 0.89, with more variability for core two ranging from 0.65 to 1.06. The velocity of rotavirus relative to KBr is variable ranging from 0.56 to 1.54 across cores 1 and 2. The velocity of rotavirus surrogate relative to KBr is consistent during core 1 experiments ranging from 0.63 to 0.80, but more variable for core 2 experiments ranging from 0.10 to 1.51. The removal rates of MS2 phage are generally one order of magnitude lower than rotavirus for all experiments. The removal rates of rotavirus surrogate are more similar to the removal rates of rotavirus across the experiments for both cores.

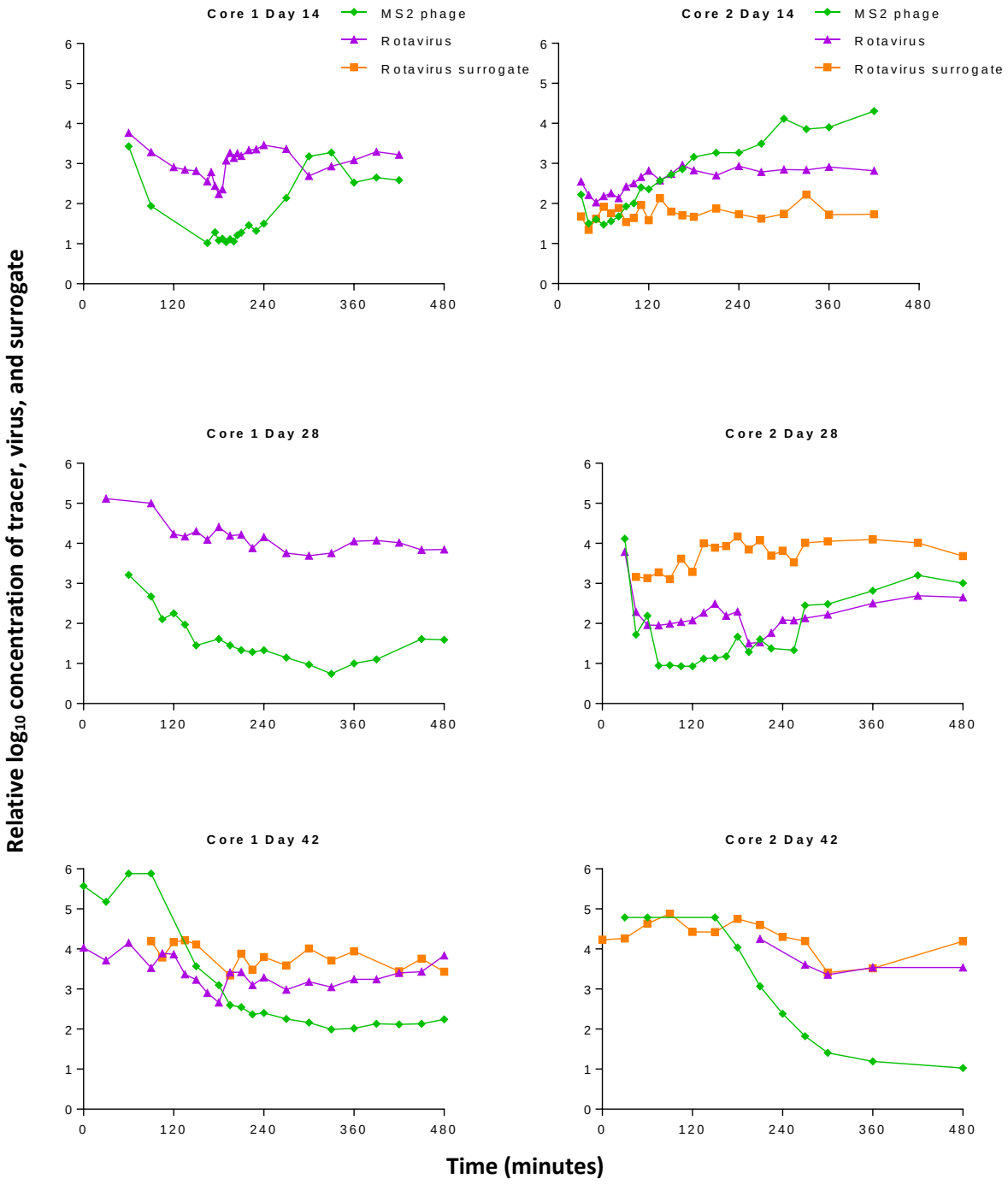


**Figure 64. $Log_{10}$ of MS2 phage, rotavirus & rotavirus surrogate relative to injection concentrations during virus transport experiments. No data for rotavirus surrogate for core 1, day 14 and 28 experiments.**

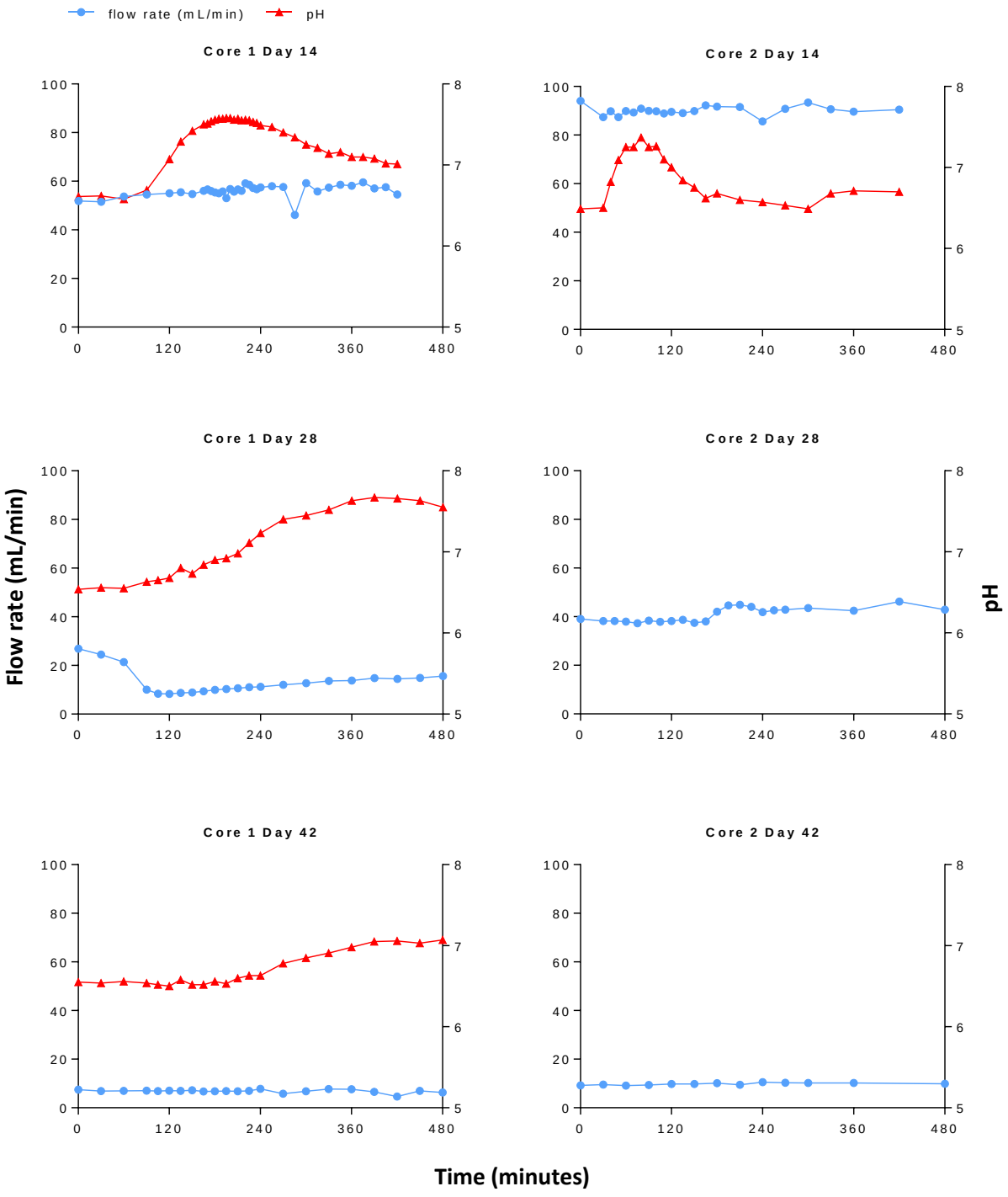


**Figure 15. pH and flow rate of outflow during virus transport experiments.**

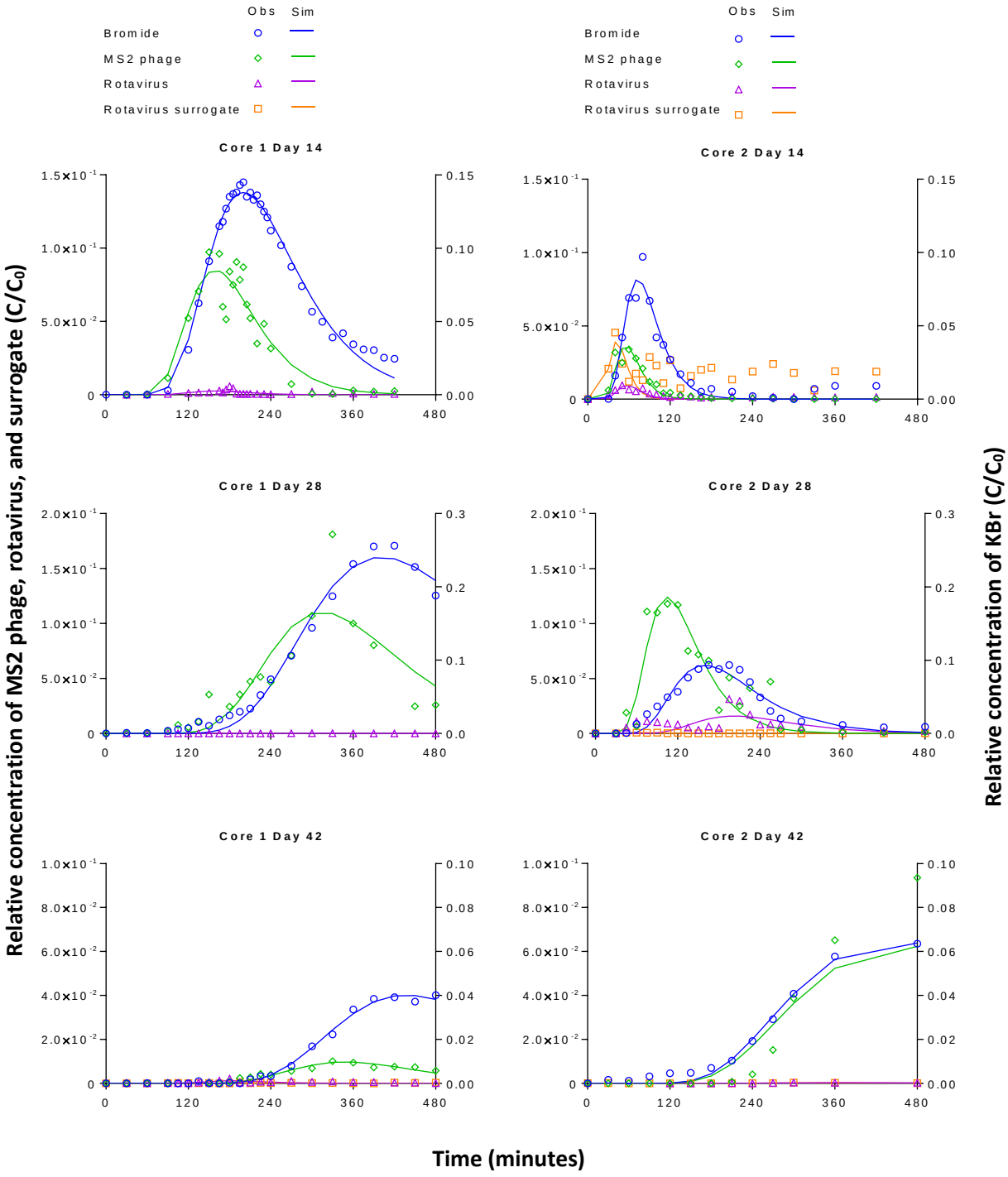


**Figure 16. Observed and model simulated particle concentrations from the virus transport experiments. Comparison of KBr, MS2 phage, rotavirus and rotavirus surrogate for each experiment. No data for rotavirus surrogate for core 1, day 14 and 28 experiments.**

| | Core 1 | | | | | | Core 2 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Day 14 | | Day 28 | | Day 42 | | Day 14 | | Day 28 | | Day 42 | |
| | Value | S.E Coeff. | Value | S.E Coeff. | Value | S.E Coeff. | Value | S.E Coeff. | Value | S.E Coeff. | Value | S.E Coeff. |
| **Velocity relative to KBr** | | | | | | | | | | | | |
| **MS2 phage** | 0.84 | 0.02 | 0.85 | 0.03 | 0.89 | 0.02 | 0.84 | 0.02 | 0.65 | 0.02 | 1.06 | 0.07 |
| **rotavirus** | 1.13 | 0.10 | 1.37 | 0.06 | 0.56 | 0.04 | 0.96 | 0.04 | 1.54 | 0.14 | 1.54 | 0.08 |
| **rotavirus surrogate** | - | - | - | - | 0.80 | 0.06 | 0.63 | 0.08 | 0.69 | 0.04 | 1.51 | 0.10 |
| **Removal rate ($min^{-1}$)** | | | | | | | | | | | | |
| **MS2 phage** | 0.004 | 0.00 | 0.003 | 0.00 | 0.004 | 0.00 | 0.015 | 0.00 | 0.001 | 0.00 | 0.000 | 0.00 |
| **rotavirus** | 0.025 | 0.00 | 0.027 | 0.00 | 0.011 | 0.00 | 0.035 | 0.00 | 0.010 | 0.00 | 0.016 | 0.00 |
| **rotavirus surrogate** | - | - | - | - | 0.015 | 0.00 | 0.018 | 0.00 | 0.045 | 0.00 | 0.018 | 0.00 |
| **R2 value** | | | | | | | | | | | | |
| **MS2 phage** | 0.89 | | 0.78 | | 0.93 | | 0.90 | | 0.84 | | 0.88 | |
| **rotavirus** | 0.29 | | 0.00 | | 0.05 | | 0.50 | | 0.17 | | 0.80 | |
| **rotavirus surrogate** | - | | - | | -0.16 | | - * | | 0.31 | | 0.64 | |

**Table 8.Simulated transport outputs estimated by STANMOD for KBr, MS2 phage, rotavirus and rotavirus surrogate.** * R2 value not generated**.**

# 4 Discussion

## 4.1 Macroporosity and hydraulic soil characteristics

### *4.1.1 Macropore characteristics*

Analysis of X-ray CT images revealed heterogeneous structure and macropore characteristics within and between intact soil cores. Heterogeneity was also observed by Luo et al. (2010) analysis of macropore characteristics within and between soil types by similar image analysis. Land-use has been shown to significantly affect macropore characteristics; including macropore surface area and macropore density in the Luo et al. (2010) study. Soil compaction can also decrease macroporosity (Kim et al., 2010). Kim et al. (2010) measured a 69% reduction in macroporosity in soils that underwent compaction. Studies have shown a strong correlation of macropore characteristics with macropore flow (Luo et al., 2010; Naveed et al., 2015). Naveed et al. (2015) showed that mean macropore diameter best predicted macropore flow, while other macropore network characteristics were also strongly correlated with hydraulic conductivity, air permeability, and gas diffusivity in the soil. Kim et al. (2010) also used X-ray CT image analysis to explain variation in hydraulic conductivity, with macroporosity being most strongly correlated with hydraulic conductivity. This is consistent with the higher hydraulic conductivity associated with the greater average macropore area observed in core 2. Despite the cores being extracted at the same site, this heterogeneity highlights the need for local site-specific soil analysis to determine hydraulic properties of *in situ* soil used for wastewater disposal.

### *4.1.2 Progressive clogging of intact soil cores*

The hydraulic loading of secondary treated wastewater resulted in progressive clogging of both intact soil cores over the course of the experiments. This clogging process is evident by the dramatic decrease in outflow from the cores observed over the 42 days of wastewater conditioning, during which time the virus transport experiments were conducted. Hydraulic loading rates in disposal fields are dependent on soil type and design parameters, and affect mass loading, clogging rate, and biofilm development in the soil. Higher hydraulic loading rates increase the mass loading and biofilm development (Beal et al., 2008). Loading with wastewater constituents and the resulting build-up of biofilm leads to the reduction of hydraulic conductivity in soils (Siegrist, 1987). Radcliffe et al. (2009) demonstrated that biofilm restricts OWTS disposal field bottom flux, in the range of 1 – 57% of the soil's hydraulic conductivity. In 12 different soil textural classes, trench bottom flux ranged from 2.92 to 10.43cm/d despite $K$ values ranging 0.081 – 6.43m/d. This is supported by the observations of

Siegrist (1987), where soil hydraulic conductivity values averaging 2.4m/d reduced to less than 1% after loading with septic tank effluent at rates of 1.3 – 5.2cm/d. Siegrist (1987) observed severe clogging of soils resulting in continuous ponding when loaded with septic tank effluent at 2.6 and 5.2cm/d, whereas clogging in tap-water treatments was negligible. Clogging rates were attributed to mass loading of biological oxygen demand and total suspended solids. A layer of organic and inorganic material was observed above the infiltrative surface, as well as clogging of pores resulting in reduced pore size. Higher water contents were also observed in the clogged soils. Clogged soil surfaces had significantly higher organic carbon and nitrogen concentrations too, which was attributed to blocking of soil pores with organic matter. Carbon and nitrogen concentrations below the clogged infiltrative surfaces were 1 log lower than the clogged area. Muhammad et al. (2003) used Total Kjeldahl Nitrogen (TKN) content in the biofilm layer of wastewater sand filtration units as a method to measure active biomass. A strong correlation between TKN and heterotrophic plate count was observed. Higher biomass was observed in the top 30mm of the sand filter, and steadily decreased down to a depth of 90mm below the infiltrative surface. Unstable flow rates were observed during transport experiments conducted on day 28 of conditioning for both the intact soil cores. Physically higher loading during wastewater dosing and the progressive formation of the clogging layer in the disposal field media may have led to a pressure change causing the observed variable bottom flux during the day 28 experiments of cores 1 and 2. Further analysis of the clogged surface including TKN, carbon content, and microbial analysis could enable better understanding of biofilm formation and the progressive clogging.

## 4.2 Virus transport through saturated intact soil cores

### *4.2.1 Conservative tracer (KBr)*

Substantial variability in transport patterns and LRVs were observed in the virus transport experiments through the intact soil cores over the wastewater conditioning period. The conservative tracer, KBr, showed a different breakthrough pattern for viruses and rotavirus surrogate. The variability within and between virus species is unsurprising and is consistent with the recent review of virus transport in the subsurface by Schijven et al. (2017). The difference in macroporosity and decrease in hydraulic conductivity, noted above, influence the transport and mass recovery of each species during the virus transport experiments. Previous research using dairy shed effluent in New Zealand shows a similar pattern of transport to that observed in these experiments (McLeod et al., 2003, 2004; Pang et al., 2008; Jiang et al., 2010). Pang et al. (2008) shows significant tailings in the KBr breakthrough curves in a variety of New Zealand soil types particularly in clayey and silty soils. Pang et al. (2008) suggest the long tailings are attributed to preferential flow regions or dual permeabilities. This along with the

higher macroporosity of the silty-sandy soil in the intact soil cores suggest preferential flow is occurring in this layer. The lesser macroporosity in the lower sandy-gravel suggests dual permeability between the upper and lower layers of the intact soil cores.

### *4.2.2 Virus and virus surrogate transport*

Earlier breakthrough of virus than the KBr was expected and is consistent with previous research (McLeod et al., 2003, 2004; Pang et al., 2008, 2014). The size and surface properties of MS2 phage, rotavirus and rotavirus surrogate differ to the KBr. The saturated virus transport experiments showed MS2 phage was a conservative indicator of pathogenic rotavirus, consistent with Pang et al. (2014). There is a notable difference in the transport behaviour of MS2 phage compare to rotavirus and rotavirus surrogate. The larger size of rotavirus and rotavirus surrogate constitutes faster transport through the intact soil cores as they only have access to larger pores compared with KBr. Preferential flow through macropores in the silty-sandy layer of the intact cores suggest velocity enhancement of viruses in the upper layers. Though MS2 phage breakthrough was earlier than KBr, it is slower than that observed in structured soils previously seen by Pang et al. (2008). Pang et al. (2008) showed earlier breakthrough of *Salmonella* bacteriophages in clayey soil and silt loam compared to sandy soils and gravels. The presence of a sandy-gravel layer in the intact soil cores with a lower macroporosity than the upper layers may have restricted velocity of viruses in the lower half of the intact soil cores, thereby delaying breakthrough. Also, the macropores, while enhancing preferential flow, may have become blocked over the duration of the experiments with wastewater application, as was observed by Natusch et al. (1996). Previous research shows that organic matter contamination influences virus transport by increasing velocity and competition for attachment sites (Wall et al., 2008, Weaver et al., 2013).

Organic loading can also influence transport of viruses (Weaver et al., 2013). Weaver et al. (2013) found increasing mass recoveries of MS2 phage in sand columns with higher dissolved organic matter conditioning. Weaver et al. (2013) also showed recovery of MS2 phage could exceed input concentrations following a conditioning event, suggesting the conditioning event caused detachment of MS2 phage from previous experiments. Conditioning with wastewater over the course of the experiments may have led to competition for attachment sites over time, influencing peak breakthrough time and mass recovery of viruses. However, the decreasing flow rate through the cores over time may have prevented any increase in virus transport velocity by this mechanism to be offset.

The low mass recovery of MS2 phage, rotavirus and rotavirus surrogate indicates a significant portion of viruses were retained in the intact cores. Rotavirus and rotavirus surrogate, however, was below the detection limit in the disposal field media following the virus transport experiments. Burbery et al.

(2015) attribute substantial attachment of MS2 phage under saturated conditions to surface-water interactions, particularly irreversible adsorption to plastic apparatus. During the intact core experiments, efforts were made to reduce the amount of virus attachment to experimental apparatus by avoiding plastic equipment and using glass fittings and Tygon® tubing to minimise attachment of particles. Therefore, this is an unlikely cause of virus attachment during these experiments.

The detachment of viruses may have also influenced mass recovery from the intact cores. Nutrient availability, hydrodynamic shear, and collision with suspended particles may all affect virus detachment processes (Tufenkji, 2007). The detachment of viruses can also be triggered when a change of pH occurs (Schijven et al., 1999; Hijnen et al., 2005; Walshe et al., 2010). Ionic strength and colloid concentration can also affect attachment of viruses (Walshe et al., 2010). Torkzaban et al. (2006) found that virus attachment increases as pH decreases in saturated sand column experiments. Walshe et al. (2010) showed that mineral colloids can assist in transporting viruses by increasing velocity and peak-concentration time, while also attaching to aquifer media and reducing mass recovery in aquifer media. The detachment of viruses in the intact cores may have occurred during the experiments due to disruption of attached viruses or further competition for binding sites, leading to the variability observed in the mass recovery of viruses.

### *4.2.3 Mass recovery of virus and virus surrogate*

Mass recovery of MS2 phage was substantially higher than that of rotavirus and rotavirus surrogate. This is consistent with Pang et al. (2014) who also showed greater recovery of MS2 phage in clean column experiments. Pang et al. (2014) found MS2 phage over predicted concentrations of rotavirus in clean saturated sand columns by 2 orders of magnitude. Farkas et al. (2015) investigated hydrophobicity of MS2 phage compared to rotavirus, finding that rotavirus surrogate generally better mimicked rotavirus adsorption to both unmodified and hydrophobic sand. Despite the greater hydrophobicity of MS2 phage, it had a significantly higher adsorption to the hydrophobic sand than both rotavirus and rotavirus surrogate. Determination of the mechanisms which attenuated viruses in the intact soil cores was beyond the scope of this study, however, the greater recovery of MS2 phage compared to rotavirus and rotavirus surrogate could be attributed to size difference and virus properties. The greater mass recovery of MS2 phage compared with rotavirus and rotavirus surrogate has also been attributed to MS2 phage having a lower isoelectric point by Pang et al. (2014). The greater recovery of MS2 phage compared to rotavirus and rotavirus surrogate may also indicate that straining of larger virus particles was occurring through the soil profile. This is comparable to Burbery et al. (2015) where in coral sand recovery of the larger organism *E. coli* was significantly less than recovery of MS2 phage during saturated column experiments. Tufenkji (2007) highlights difficulties in

obtaining estimates of straining mechanisms in the field. Intact soil cores too present a difficult medium to determine these straining and attachment processes due to their heterogeneous nature. While outside the scope of this research, imaging techniques or destruction of intact soil cores may provide insight into these processes. In addition to straining and attachment mechanisms, the review by Tufenkji (2007), demonstrates the complexities affecting virus inactivation in the subsurface. Research suggests that attachment of viruses to geological material could either protect viruses from inactivation, or accelerate inactivation. Contrasting evidence is also reported by Tufenkji (2007), suggesting that sterile conditions aid or reduce virus persistence in the subsurface. Understanding virus transport and removal processes should be considered contextually, and the complexities highlight the importance of *in situ* studies before assuming behaviour of viruses in the field.

### *4.2.4 Log reduction of virus and virus surrogate*

Virus removal rates in the subsurface have clear inverse relationships with virus transport velocity, hydraulic conductivity of soil and hydraulic loading (Schijven et al., 2017). LRVs for rotavirus and rotavirus surrogate through intact soil cores were higher than MS2 phage. Van Cuyk et al. (2007) found increased removal of both MS2 phage and PRD1 phage as wastewater conditioning continued over time. Initially, both viruses showed greater removal when dosed with septic tank effluent compared with artificial groundwater. As conditioning continued, this led to a greater removal in both treatments. Interestingly, Van Cuyk et al. (2007) also showed that a higher hydraulic loading rate of wastewater actually improved virus removal. Van Cuyk et al. (2007) conclude that after several weeks, virus removal is significantly higher than during the start-up period. However, the intact core experiments do not show any significant trend in virus removal as conditioning progresses. This may be attributed to the progressive reduction of available attachment sites over time, or by saturated conditions reducing attachment of viruses. Previous research has shown that overall virus reduction is generally higher in unsaturated soils compared with saturated soils (Powelson et al., 1994; Blanc et al., 1996). Powelson et al. (1994) showed that saturated flow transports viruses three times faster than unsaturated flow during wastewater application to soil.

## 4.3 Comparison of virus and virus surrogate

The LRVs, mass recoveries, and relative transport of MS2 phage compared to rotavirus in the intact soil cores during the experiments supports the use of MS2 phage as a conservative indicator organism of viral contamination. It can be expected that if MS2 phage are not recovered then rotavirus will not be present, and that a greater log reduction of rotavirus will be achieved compared with the log

reduction of MS2 phage. The rotavirus surrogate provides a closer representation to that of pathogenic rotavirus. The similarity between rotavirus surrogate and rotavirus is shown particularly in the low-flow experiments. Rotavirus surrogate is a useful tracer to use in OWTS laboratory and field studies to better understand the transport and removal of rotavirus without the need to use pathogenic virus. Additionally, the stability of rotavirus surrogate in secondary treated wastewater also demonstrates that it is a useful tool to evaluate rotavirus in other wastewater applications. However, it must be highlighted that rotavirus surrogate does not provide a conservative indication of viral contamination as MS2 phage does.

## 4.4 Implications for separation distances from OWTS

The results from the virus transport experiment in intact soil cores dosed with OWTS wastewater have important implications for current separation distance guidelines. Conservative transport of rotavirus compared with virus tracers used to determine separation distance guidelines can result in overly conservative calculations. Data used to establish the current guidelines for separation distances of OWTS to wells in New Zealand assumes reduction of viruses in soil based on previous research using conservative indicator viruses such as MS2 phage and *Salmonella* phage (Moore et al., 2010). The results from these intact soil core experiments confirms the conservative nature of MS2 phage and shows that rotavirus surrogate gives a more similar representation to rotavirus transport and removal from OWTS.

Further experimental research is needed to determine the removal rate of pathogenic viruses in soils from OWTS to enable better model simulation. Further data from intact soil core experiments using composite sampling over longer time periods would provide better data to model attachment-detachment of viruses in soil. Conducting unsaturated flow experiments, would also be required to better predict the removal of pathogenic viruses from OWTS. A broad range of soil types and environmental conditions are encountered in the field where OWTS disposal fields operate. Using various soil types, along with different loading rates, failure scenarios, and climate stresses would all better the understanding of virus transport from OWTS and enable more accurate estimation of appropriate separation distances from OWTS to drinking water supply wells.

# 5 Conclusions

Analysis of intact soil cores revealed heterogeneous soil structure and macropore characteristics. Hydraulic loading of secondary treated wastewater resulted in successive clogging of intact soil cores under saturated conditions. Hydraulic conductivity of both intact soil cores decreased dramatically over a period of 56 days with wastewater conditioning. Saturated virus transport experiments conducted every two weeks in intact soil cores showed that MS2 phage was a more conservative indicator of virus transport and removal than rotavirus and rotavirus surrogate from OWTS. Mass recovery of MS2 phage was substantially higher than that of rotavirus and rotavirus surrogate and consequently LRVs for MS2 phage through intact soil cores were also lower than rotavirus and rotavirus surrogate. Rotavirus surrogate better represented rotavirus transport and removal than MS2 phage, however, MS2 phage still provides a conservative indication of viral contamination from OWTS. The similarity of rotavirus to rotavirus surrogate during the intact core experiments shows that rotavirus surrogate is a useful tool for predicting virus transport and removal in wastewater experiments. However, caution must be exercised when using rotavirus surrogate for separation distances from drinking water supply wells as it does not provide a conservative indication of viral contamination as MS2 phage does. The Guidelines for separation distances from OWTS to drinking water supply wells used in New Zealand appear to be too conservative having been modelled using data based on conservative indicators. Determining appropriate separation distances from pathogenic virus, virus surrogate, and indicator virus data would allow the modelling of a more realistic scenario. However, further investigation into the transport and removal of viruses from OWTS is first required to provide data in different soil types and under various conditions to enable the improvement of current separation distance guidelines.

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