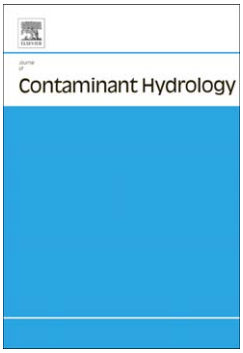

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The attenuation of microorganisms in on-site wastewater effluent discharged into highly permeable subsoils

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ABSTRACT

An extensive field study on percolation areas receiving both septic tank and secondary treated on-site effluents from single houses in Ireland was carried out to investigate the attenuation capacity of highly permeable subsoils with respect to *E.coli* bacteria and spiked bacteriophages (MS2, ΦX174 and PR772). The development of biomats across the percolation areas receiving the secondary effluent was restricted compared to the percolation area receiving septic tank effluent, promoting a much higher areal hydraulic loading which created significant differences in the potential microbiological loading to groundwater. Greatest *E.coli* removal in the subsoil occurred within the first 0.35 m of unsaturated subsoil for all effluent types. Analysis showed, however, that more evidence of faecal contamination occurred at depth in the subsoils receiving secondary treated effluents than that receiving

septic tank effluent, despite the lower bacterial influent load. All three bacteriophages were reduced to their minimum detection limit (< 10 PFU/mL) at a depth of 0.95 m below the percolation trenches receiving septic tank effluent, although isolated incidences of ΦX174 and PR772 were measured below one trench. However again, slightly higher breakthroughs of MS2 and PR772 contamination were detected at the same depth under the trenches receiving secondary treated effluent.

Keywords: on-site wastewater treatment and disposal, septic tank effluent, microbial contamination, *E.coli*, bacteriophage, groundwater, biomat.

1. Introduction

Groundwater is a natural resource facing an ever-increasing risk of pollution from a number of anthropogenic activities, including the diffuse threat posed by inadequately designed or dysfunctioning on-site wastewater treatment systems. The infiltration and percolation of such effluent through sufficiently permeable subsoil in the vadose zone is critical for the effective treatment of on-site wastewater prior to groundwater discharge (Siegrist and Boyle, 1987; Beal et al., 2005). Bacteria and viruses in the effluent pose a serious contaminant threat to groundwater, as they can migrate significant distances in the subsurface posing significant health risks and potential outbreaks of waterborne disease (Macler and Merkle, 2000). The effective attenuation of microbial pathogens is mainly carried out in the vadose zone due to contact between the pathogens, soil particles and associated biofilms, allowing an adequate period for treatment processes to occur (Van Cuyk et al., 2001). Conditions that contribute to unsaturated flow and the maximisation of effluent residence time include uniform effluent distribution, well drained soils, moisture deficits, and the development of a biomat layer (Cave and Kolsky, 1999). The importance of the biomat layer in particular cannot be

overstated, especially in a highly permeable medium such as sand or gravel, where it acts as the first line of defence against the migration of pathogens in the subsoil.

Field and laboratory studies have shown that pathogens can be dramatically reduced through 0.6 to 0.9 m of unsaturated subsoil (Kristiansen, 1981; Van Cuyk et al., 2004) yielding near complete removal of faecal coliform bacteria and greater than 4 log reduction in viruses (Gerba et al., 1981; Stevik et al., 1999; Van Cuyk et al., 2001). Studies in laboratory columns have also shown bacterial attenuation is greatest in soil with high percentages of fine particles, i.e. clay and silt (Tare and Bokil, 1982). Most of the biological activity has been shown to occur within the initial zone of percolation. For example, previous field research in Ireland into the attenuation of on-site effluent at four sites showed a 3 to 4 log reduction in enteric coliform concentrations over a distance of less than the first 0.3 m depth of unsaturated subsoil (Gill et al., 2007). Virus transport and fate in soils is predominantly a function of advection, inactivation, sorption and desorption (Schijven and Hassanizadeh, 2000). Column experiments on different porous media in the laboratory are commonly used to characterise virus transport (e.g. Sobsey et al., 1995; Jin et al., 1997; Guan et al., 2003) and many field studies have been carried out into ascertaining the extent of virus transport and removal in both unsaturated and saturated soils and groundwater (Bales et al., 1991; Woessner et al., 2001; Van Cuyk and Siegrist, 2007).

Most the aforementioned on-site research, however has been carried out in continental climates on sandy subsoils or under controlled laboratory conditions, with little field research previously performed in a temperate maritime climate or on the heterogeneous subsoils of north-western Europe, which are largely as a result of recent glaciation. Documented results are also difficult to extrapolate to an Irish context due to indeterminate percolation rates.

Microbial contamination of groundwater has been heavily documented in Ireland in recent times with the Irish EPA finding in its most recent national survey of water quality that, of 2718 samples collected at groundwater monitoring points between 2007 and 2009, positive faecal coliform counts were detected in 945 samples (34.8%) (EPA, 2010). No national studies on subsurface viral transport have been carried out to date. However, given that 26% of all water supplies in the country are provided by groundwater (EPA, 2009a), the protection of groundwater resources from microbial contamination by domestic wastewater effluent is imperative. The Irish and US EPA's current design guidelines for single-house wastewater treatment systems (EPA, 2009b; US EPA, 1980) specify a minimum 1.2 m depth of unsaturated subsoil to be present below the base of the percolation trench when receiving effluent directly from a septic tank. Alternatively, the depth of subsoil required for disposal of effluent which has passed through a secondary treatment process (e.g. package plant, sand filter etc.) has recently been increased from 0.6 m to 0.9 m in Ireland (EPA, 2009b), as discussed later. Due to the concern that inadequate treatment of contaminants was being achieved in highly permeable sandy or gravely subsoils, research was carried out to examine and compare the attenuation capacity of such subsoils in Ireland with respect to contaminant removal in treating septic tank effluent, secondary treated effluent from a horizontal flow constructed wetland, and secondary treated effluent provided by a packaged treatment system. The sites were chosen with subsoil classified at the upper limit of the allowed permeability range with respect to the Irish EPA's Code of Practice (EPA, 2009b).

2. Methods

2.1 Site Selection, design and construction

Two sites, A and B, were selected on the basis of having high permeability subsoil as well as a minimum 1.2 m of unsaturated subsoil below the proposed invert of the percolation trenches. In Ireland, an on-site standardized falling head percolation test, the so-called T-test, is used to determine subsoil permeability which is based on the average time for a 25 mm water level drop (Mulqueen and Rodgers, 2001). The sites were chosen with subsoil in the high permeability range of T-values between 1 and 5 which corresponds to field saturated hydraulic conductivities $4.2 \leq k_{fs} \leq 0.84$ m/d (Elrick and Reynolds, 1986). The subsoil on Site A (Kilrainy in County Kildare) was till derived from limestone and glacio-fluvial sands and gravels of depth between 5 to 10 m sitting on Dinantian pure unbedded limestone bedrock. XRD analysis showed the subsoil contained quartz, feldspars, chlorite, calcite and manganoan calcite. The subsoil on Site B (Redcross in County Wicklow) was till derived from Lower Palaeozoic rock greater than 3 m depth sitting on Ordovician metasediments. XRD analysis showed the subsoil contained quartz, feldspars, chlorite, ferroan and mica. The porosity of field samples of the subsoil on both sites was measured using a gravimetric method.

Three new on-site treatment systems (*I*, *II* and *III*) were constructed on the two sites by the research team at the beginning of the study in order to assess the contaminant attenuation in highly permeable subsoil to loading from different strength effluents (Table 1). On Site A, wastewater from the household was treated in a two-chambered septic tank of capacity 3785 L before the septic tank effluent (STE) was evenly split two-ways via a distribution device: half to a set of three parallel percolation trenches (each 20 m long), the other half to a constructed horizontal sub-surface flow (SSF) reed bed with dimensions 5.8 m x 2.6 m x 0.6 m depth. The treated effluent from the reed bed (RBE) was thereafter discharged to a set

of three percolation trenches (each 16 m long) to provide further polishing treatment of the percolate. Hence, the site was analysed as two separate treatment systems as shown in Fig. 1.

System I - a conventional septic tank system discharging STE to the percolation area

System II - a septic tank and reed bed system discharging RBE to the percolation area

On Site B, a rotating biological contactor, RBC (Biodisc®, Klargester) was installed to treat the household's raw wastewater and provide secondary-treated effluent (SE) for subsequent polishing. Effluent from the RBC was split via a distribution device, with of the flow directed to two parallel 16 m long percolation trenches (*System III*). The other half the flow was directed to a reed bed that was not part of this study.

The percolation trenches receiving effluent from all three processes were constructed at 2.45 m centres built to EPA specifications (EPA, 2009b) consisting, in each case, of 110 mm diameter perforated PVC pipe sitting on a bed of washed gravel (20–30 mm diameter) of 250 mm thickness in a 450 mm wide trench at a slope of 1:200. The achievement of an equal loading rate on each trench was achieved by a series of gravity-fed distribution devices that were being tested in parallel as a sub-element of this project (see Patel et al., 2008). In order to monitor the hydraulic efficiency and flow profile of these devices in distributing the effluent, tipping bucket instrumentation with datalogger (Model 6506H-Unidata, Australia) were installed underneath each of the four device outlets at each site.

Clusters of three suction lysimeters (Soilmoisture Equipment Corporation Model 1 900) were installed at 5 metre intervals along the length of the trenches at nominal depths of 0.35, 0.65 and 0.95 m below the infiltrative surface as shown in Figs. 1 and 2. An additional set of lysimeters was installed at the 2.5 m distance point along the trenches of *System II* to provide

a more refined analysis of the biomat spread. Nine standard tensiometers (Soil Measurement Systems) were also installed along one trench on each system at the same three sampling depths as the lysimeters. This allowed the soil moisture tension below the percolation area to be monitored at each site visit. Automatic water samplers (Bühler Montec) were located downstream of the septic tank, RBC and reed bed to obtain a profile of the effluent entering the percolation trenches. Meteorological variables (rainfall, temperature, wind speed, relative humidity, solar radiation and sunshine hours) were recorded on site by a weather station (Campbell Scientific) and a rain gauge (Casella) in order to carry out appropriate mass balance analysis on the reed bed and examine the effect of dilution from recharge on the attenuation of the percolate in the subsoil.

2.2 Sampling methodology

On the morning preceding all sampling events, the lysimeters were put under a suction of 50 cbar using a vacuum-pressure hand pump. This value is well below the air-entry value of the lysimeter (2 bars) which helps to ensure the soil moisture solution is pulled horizontally from the vadose zone and the local soil does not dry out causing discontinuity in the sample. Once the vacuum was created by the application of the requisite pressure through the lysimeter's neoprene tubing, the latter was clamped with a plastic ring to maintain suction. The following day after 24 hours under suction, the lysimeter clamps were released and the samples were ready for extraction using a vacuum-pressure hand pump and a 1000 ml conical flask and rubber bung with an extraction tube attached. The total volume of sample collected was recorded before the samples were transferred to 70 ml sterilised plastic sample bottles (Sarstedt Ltd.). The samples were then stored in a cool box and taken directly to the laboratory for analysis within 4 hours.

2.3 Analysis of chemical and microbiological quality

Monitoring of the chemical and bacteriological constituents in the effluent took place on a bi-weekly basis over a 26 month period for both sites following an initial 6 month start-up period. All septic tank, RBC, reed bed and soil moisture samples were analysed for chemical oxygen demand (COD), ammonium (NH_4-N), nitrite (NO_2-N), nitrate (NO_3-N), orthophosphate (PO_4-P) and chloride (Cl) using a Merck Spectroquant Nova 60® spectrophotometer and associated US EPA approved reagent kits. Samples were also tested for total nitrogen (TN) using a Hach Lange LT200 thermostat and spectrophotometer DR2800 to ascertain the fraction of organic and inorganic-N present. The presence of *E.coli* in samples was detected using the Idexx Colilert®-18 test (IDEXX Laboratories Inc. Westbrook, Maine), as described in Standard Methods (American Public Health Association 2005). Enumeration was carried out using Idexx Quanti-Tray®/2000, a semi-automated quantification method based on the Standard Methods Most Probable Number (MPN) model. Given that the greatest volume of sample (100 mL) was required for zero-dilution bacteriological analysis, soil moisture samples were firstly transferred directly on site from a 1 L conical flask to disposable Colilert®-18 120 mL sterile vessels. If less than 100 mL volume was extracted from the lysimeters, appropriate serial dilutions were prepared in the laboratory.

The three bacteriophages (MS2, ΦX174 and PR772) used in the phage tracer studies (see Section 2.3) were obtained from the American Type Culture Collection (Manassas, VA, USA) and grown on their host *E. coli* lawns by the agar-overlay method while enumeration of the phages was performed by the plaque forming unit (PFU) method, described by Adams (1959). Assaying of the phage samples involved the following techniques. Agar plates were prepared and allowed to dry for 2 - 3 days, with the bottom agar for the phage assays

consisting of Trypticase soy broth (TSB) containing 15 g of agar per litre. The host bacteria grown in TSB in a conical flask and placed in a 37°C orbital shaker until mid-log phase (OD_{600nm} = 0.5) was reached. Top agar consisting of Trypticase soy broth containing (per litre) 7 g of agar and 1 mL of 1 M $CaCl_2$ was melted in a steamer after which 3.5 mL were aliquotted into a series of sterile culture tubes (1 for every dilution plus one blank). The sterile tubes were then placed in a water bath and maintained at a temperature of 45 °C. Serial dilutions of the phage sample were then prepared in TSB. The surface of the pre-warmed agar plates were overlaid with the 45 °C melted top agar from each of the sterile tubes, in which 100 µL of mid-log phase *Escherichia coli* host had been added. The plates were then incubated at room temperature for 1 - 5 minutes to allow the top agar to solidify. Phage enumeration was carried out using the Miles and Misera technique (Miles et al., 1938) with each plate split into four quarters. One drop of each phage dilution (50 µL) was spotted on the surface of each quarter of the prepared plates and allowed to cool for approximately 5 minutes. The plates were then inverted and incubated at 37 °C for 24 hours to allow bacterial growth and semiconfluent lysis of the bacterial lawn by the phage. Duplicates were carried out for each dilution and the average count reported. Only the dilutions that resulted in 10 - 300 plaques per quarter were counted. The minimum detection limit for all three phages was < 10 PFU/mL. Enumerations of the stock solutions of each phage were carried out in parallel in triplicate in order to quantify the standard error associated with the phage enumeration method, as reported later.

Parallel analysis of Br was carried out using a Dionex ICS-1500 Ion Chromatography System with an AS40 Automated Sampler. The column used was an Ion Pac AS14A 4 x 250 mm analytical column electrolytic suppression using an ASRS UltraII 4 mm suppressor. All reagents were AnalaR grade and the deionised water was 18 MOhm-cm, Type 1 water. The

eluant was 8.0 mM Sodium Carbonate, 1.0 mM sodium bicarbonate used at a flow rate of 1 mL/min. Standards were made from certified stocks and an independent QC was run alongside samples (every 10).

2.4 Phage tracer trials

To investigate the potential fate and transport of enteric viral pathogens in the subsurface environment of the percolation area, a multi-phage injection experiment was conducted using a selection of bacteriophages (MS2, ΦX174 and PR772) on each of the percolation trenches of *System I* (receiving STE) and the trenches of *System III* (receiving SE). The trials were carried out after 25 months of routine sampling analysis; the lysimeters results had shown that the biomat / effluent plume had reached an equilibrium length after 6 months. The rationale for using more than one bacteriophage was to investigate the effect of variations in size, hydrophobicity and isoelectric point among phages that might affect transport through soils. MS2 is a F-RNA coliphage while ΦX174 and PR772 are DNA somatic coliphages. A profile of each of the three phages is presented in Table 2.

At the same time as the phages, bromide (Br) was also spiked into the injection solution to act as a conservative tracer. This provided validation of the ongoing long-term Cl analysis (see previous section) as well as indicating potential breakthrough time of the phages. For each trial the lysimeters known through Cl analysis to be receiving effluent were primed in readiness for daily soil moisture collection. 2 L concentrated stocks of MS2, ΦX174, PR772 were brought to each site and added with the potassium bromide (KBr) to 10 L of distilled water (pH 7.08 for the *System I* trials and pH 7.23 for the *System III* trials). The diluted solution was then intermittently fed over an eight hour period to each percolation trench via the open vent pipe. The active effluent flows from the septic tank (*System I*) and *RBC* (*System*

III) to the trenches were simultaneously recorded, via tipping bucket measurement, to account for additional dilution of the injected solution. Soil moisture samples were collected once per day for four consecutive days during the trial for immediate phage and bromide analyses. Control experiments were run in the laboratory to determine that phage adsorption to lysimeter surfaces was negligible. In addition, control samples of both influent stocks were kept at each site from the time of injection (maintained at ambient weather conditions) and sampled once per day during the trial to assess the background rate of inactivation of each phage. Equally, samples of the unspiked effluent from the septic tank on *System, I* and RBC on *System III* were tested to see if they contained any background level of phages MS2, ΦX174 or PR772.

3. Results

3.1 Analysis of wetted percolation area and rainfall recharge

Chloride was employed as a crude tracer in the study to determine the distribution of the STE, RBE and SE within the percolation areas of *Systems I*, *II* and *III*, respectively and observe this dispersion of effluent along each instrumented trench with time to establish the extent of biomat development. From this analysis it could be decided (i) which lysimeters were sampling the percolate and (ii) the temporal quantification of dilution on the effluent due to the effects of rainfall recharge through the subsoil. A conceptual model (assuming homogeneous and isotropic subsoil properties and only taking account of matrix flow) based on the Cl data was subsequently derived for the analysis of the attenuation of the percolate. The concentrations of each constituent were then averaged accordingly across all trenches and all sampling positions where the percolate was known to have reached the 0.35, 0.65 and 0.95 m depth planes.

Results over the total duration of the monitoring showed significant differences between effluent dispersion on the basis of each effluent type corresponding to the differences in organic loads associated with each and the respective biomat development. A time-series plot of the mean Cl concentrations on *System I* measured at all five sample positions for the three depth planes (Fig. 3) showed that the highly concentrated STE had spread past the 0, 5 and 10 m sampling positions but not as far as the 15 or 20 m positions. Similar analysis on the trenches of *System II* showed the RBE to disperse to the 5 m sample position only. In contrast, low-organic SE from the RBC (see COD results in Tables 3 and 4) affected all three depth planes at only the initial (0 m) sampling position in the System III trenches; the effluent never reached the 5 m position (Fig. 4). Whilst there were geological differences between the subsoils on the two sites which may have contributed to the difference in effluent infiltration dynamics (the subsoils beneath *Systems I* and *II* contained significantly more fines compared to the subsoil beneath *System III* for example, as shown in Table 1), the falling head percolation tests carried out at the infiltrating layer of subsoil on both sites both retuned similar field saturated hydraulic conductivities.

Further confirmation of the biomat development was carried out via a CCTV study whereby a special camera was sent down each percolation pipe. The resulting images clearly showed how far the effluent had travelled down the pipe before percolating into the gravel below. The lengths measured with the camera closely matched the lysimeter results which are reported as "active" lengths in Table 3. The extent of percolation area deemed active in all three systems outlined (i.e. active length /constructed length) was 50% - *System I*, 41% - *System II*, 3% - *System III* which is compared with respect to organic loads in the effluent in Table 3 (and see Table 4 for effluent organic quality). The biomat zone forms at the soil-gravel interface along the base and wetted sides of the percolation trenches. Reduced

percolation rates through the biomat due to clogging as a result of anaerobic activity can cause the effluent to pond above the biomat but leaves unsaturated conditions below, for aerobic degradation processes to operate on percolating effluent; (Siegrist and Boyle, 1987; Beal et al., 2008). Variations in influent flow to the percolation pipes at different times of day will mean that during periods of high flow the effluent is more likely to make it farther down the length of the percolation pipe. However, the saturated conditions above the biomat will also promote mixing as effluent flows longitudinally along the percolation trench acting to mute plug flow movement of any contaminant peaks down through the subsoil. It should be noted that it was not possible to discern any significant consistent differences in hydraulic loading along the length of the trenches where the effluent plume was known to be percolating from either the tensiometers results or soil moisture analyses.

Quantification of the effect of dilution on effluent attenuation was calculated by determining the zone of contribution around each trench from recorded rainfall and evapotranspiration data on each site. A more detailed description of this procedure is provided in Gill et al. (2009). After dilution factors were calculated at each depth plane a simple mass balance approach was adopted to estimate the zone of contribution of effective rainfall at each depth plane, the dimensions of which are shown on Table 5. As seen, the horizontal spreading of the plume with depth was expected as it highlights the dispersion of the effluent plume below each percolation trench. It should also be noted that the biomats at the base of each of the trenches had spread to the lengths recorded in Table 5 by the time monitoring of the trenches had begun (approximately 6 months following site start-up) and remained relatively constant throughout the duration of the project i.e. the effluent was not seen to have dispersed further along the trench over time.

In order to corroborate the (i) findings on dilution effects and (ii) dispersion of the effluent, the response of the tensiometers against effective rainfall at three strategic sample positions (front, middle and back) longitudinally along the trenches was examined. As demonstrated for *System I* in Figure 5, the tensiometers at the three depth planes at the 17.5 m sample position, where no effluent was recorded, reacted significantly to the variation in effective rainfall over the total sampling period. During periods of zero effective rainfall (e.g. September 2007), as the soil dries, water became less available and the water potential became more negative. In contrast, when the soil became more saturated during periods of effective rainfall (e.g, January 2007) the soil moisture tension was seen to increase. In comparison, while the tensiometers at the 2.5 m sample position (Figure 6) showed some response during high rainfall events, the readings were seen to be much less changeable across the year due to the more consistent influence of the percolating effluent rather than the contribution of recharge. Hence, any reductions in contaminant concentrations under the percolation trenches as a result of the effect of pure dilution of the effluent from effective rainfall are likely to be very small.

3.2 Pollutant loading on percolation area

The mean on-site wastewater effluent quality recorded across the duration of the trials entering the three different systems' percolation trenches is shown on Table 4. This along with Table 3 shows the higher organic loading in the STE compared to either of the secondary treated effluents in *Systems II* and *III*, as well as the influent indicator bacteria concentrations in each of the influents.

3.3 Bacteriological analysis

Table 6 presents the results of sample analysis for *E. coli* which show that, allowing for the

factor of safety introduced due to any sample dilutions, almost complete removal of enteric bacteria is achieved within *System I* by the 0.95 m plane. However, isolated incidences of faecal contamination were encountered with depth. The elevated *E. coli* concentrations at a number of sample positions on two separate dates (in May and December 2007) coincided with high levels of rainfall which could have promoted temporarily saturated conditions thereby facilitating microbial transport and breakthrough at depth. However, there was no evidence of any denitrification on these dates which would have been indicative of saturated conditions in the subsoil. This is corroborated by the response of the tensiometers on these dates (see Fig. 6) which did not indicate the prevalence of saturated conditions in the subsoil.

Analysis of the *E. coli* data measured at the three depth planes of *System II* receiving effluent from the reed bed shows (Table 7) of the 164 samples soil moisture samples collected, 18 were found to contain significant concentrations of enteric bacteria (>10 MPN/100 mL). While this may suggest the occurrence of only isolated incidences of bacterial migration with depth, there were two occasions on the 0.95 m depth plane when concentrations detected were greater than 100 MPN/100 mL and moreover a further two samples where recovery was greater than 1 000 MPN/100 mL. Such incidences again appear to coincide with periods of increased hydraulic load to the percolation area owing to significant effective rainfall events. Nevertheless, these events highlight the fact that despite the reduced bacterial count in the influent to these trenches, owing to treatment in the horizontal flow reed bed, episodic faecal contamination at the 0.95 m plane has been shown to be more frequent than that measured at the corresponding depth in the same subsoil treating STE. Results from the lysimeters located outside the effluent treatment zone showed zero *E. coli* concentrations and the following average Total coliforms concentrations reflecting the native coliform bacteria in the soil: 3.1 x 10^3 (± 2.1 x 10^3) MPN/100 mL, 1.7 x 10^3 (± 1.2 x 10^3) MPN/100 mL and 1.3 x 10^3 (± 6.1 x 10^2) MPN/100 mL at the 0.35, 0.65 and 0.95 m depth planes respectively.

The installation of the RBC packaged treatment system (*System III*) greatly reduced bacterial loading on the percolation area (see Table 4) compared to *System I* with concentrations of *E. coli* in the SE of the same order of magnitude to that contained in the wetland effluent on *System II*. In allowing for the factor of safety introduced due to the sample dilutions, it is clear from Table 8 that almost complete enteric bacterial removal was achieved within the system. Moreover, the first 0.35 m of subsoil appeared to be responsible for the majority of bacterial attenuation over the sampling period. Nevertheless, there was some evidence of faecal contamination with depth as recorded on a number of sampling events.

An elevated *E. coli* contamination (1 624 MPN/100 mL) recorded at the front of one of the two trenches (trench 1) in August 2007 was an isolated incident. The split in the effluent from the distribution device was particularly poor on this date with approximately twice the mean flow recorded on this trench over the duration of the project and over three times the flow received on trench 2 on this date. However, with nitrification clearly occurring at depth on this date along with the contribution of effective rainfall being zero around this period, it would suggest unsaturated conditions were dominant in the subsoil. Nevertheless, the absence of an established biomat coupled with such high infiltration of effluent over a short length of the trench would have had the effect of reducing effluent residence time in the vadose zone and thus increasing the possibility of bacterial migration episodically. It was noted that at the corresponding lysimeter on the second trench, which was receiving much smaller flow, no faecal coliform contamination was recorded at any of the three depth planes. Again, results from the lysimeters located outside the effluent treatment zone showed zero *E. coli* concentrations and the following average native Total coliforms concentrations in the soil: 1.3×10^3 ($\pm$ 1.7×10^2) MPN/100 mL, 1.8×10^3 ($\pm$ 9.7×10^2) MPN/100 mL and 1.6×10^3 ($\pm$ 5.8×10^2) MPN/100 mL at the 0.35, 0.65 and 0.95 m depth planes respectively.

3.4 Results of phage spiking trials

The multi-bacteriophage spiking (and parallel bromide injection) trials were carried out 25 months after both sites became operational in order to investigate phage transport and attenuation in the highly permeable subsoils when receiving septic tank and secondary treated on-site effluents. It was recognised that given the permeability of the subsoils on both sites, any initial breakthrough was likely to be swift. As such the trials were restricted to the estimated time of effluent travel to the greatest sample depth plane below the infiltrative surface.

In order to ascertain the approximate travel time for the effluent to infiltrate and percolate to each of the three target depth planes the following relationship was used:

$$T_{dp} = \frac{A_s d\, \eta}{Q} \qquad (1)$$

where, T_{dp} = effluent travel time to reach the target depth plane (days), d = depth to plane of interest, A_s = infiltrative surface area (m^2), η = effective porosity (v/v), Q = mean daily flow (m^3/d).

This relationship assumes uniform application and infiltration by plug flow under unsaturated conditions with no hydrodynamic dispersion or heterogeneous preferential flow and thus gave a first approximation of travel times. For *System I,* A_{is} was determined by the product of the estimated biomat length, taken as the mean across trenches 2 and 3 (9.5 m), and the percolation trench width (0.45 m). The mean daily flow rate to the trenches was measured as 0.397 m^3/d while η was estimated to be 0.20 v/v. For these conditions the estimated time required for the STE to percolate to between the 0.35 and 0.95 m depth plane was between 2 and 4 days. The infiltrative surface on *System III,* at 0.16 m^2, was found to be significantly

less than that of *System I* owing to the reduced biomat length. The effective porosity was again estimated to be 0.20 v/v while the mean daily flow rate up to the date of the trial was 0.131 m^3/d. From Eqn. 1 this yielded approximate travel times to both the 0.35 and 0.95 m planes of less than 1 day. The lysimeters known to be within the zone of effluent contribution from the Cl analysis, could thus be primed in readiness for daily soil moisture collection having regard to the expected effluent travel times.

3.4.1 System I - percolation trenches receiving STE

Individual stock solutions of phages MS2, ΦX174 and PR772 at concentrations of 7.8×10^4 ($\pm$ 9.1×10^3) PFU/mL, 9.2×10^4 ($\pm$ 7.4×10^3) PFU/mL and 9.9×10^6 ($\pm$ 1.2×10^6) PFU/mL, respectively were added to 10 L of distilled water. The diluted solution was then spiked into two percolation pipes over an eight hour period, during which time a total of 12.8 L of STE flow was discharged to the two trenches being used in the experiment. Accounting for the dilutions, the reduced mean input phage concentrations over the 8 hour period for both trenches to was 5.6×10^3 PFU/mL (MS2), 6.6×10^3 PFU/mL (ΦX174) and 7.1×10^5 PFU/mL (PR772). The daily adjusted flows to the trenches recorded over the four day trial were 174.7 L/d, 162.1 L/d, 204.2 L/d and 131.7 L/d. This equated to a mean daily flow over the trial of 168.2 L/d or an equivalent hydraulic loading rate (HLR) over the wetted infiltrative surface area of 19.7 mm/d. No rainfall was recorded on the site over the duration of the trial.

Inspection of the bromide results from the injection trial shows the effluent to have percolated to the 0.65 m depth plane along the first 5 m of trench 2 within 2 days. The heterogeneity of the natural treatment system of the subsoil with 3-dimensional variations in the consistency, moisture content, packing of the natural porous medium, as well as the heterogeneous

biologically active (biomat) zone will have influenced variations in the distribution of flow paths and travel times of the bromide tracer from the point of application to the individual sampling points on the same depth plane. Percolate retention times in the soil beneath the percolation trenches under examination were also constantly varying owing to differing hydraulic loading rates.

Breakthrough of bromide to the 0.95 m depth plane was detected at trench 2 on day 3 at the 0 m and 5 m sample positions. No bromide was detected at the 10 m sample position over the duration of the trial. On trench 1, the effluent had percolated to the 0.65 m plane by day 3 but further travel time to the 0.65 m plane was left inconclusive due to the failure in drawing any sample from this sampler during the trial.

Fig. 7 illustrates the minimum planar log removal of each bacteriophage detected at all sample positions over the duration of the trial. The results are further summarised in terms of phage concentrations in Table 9. Phage values considered were only those seen to coincide with incidences of bromide evidence at that point. It is important to note that the input concentration of PR772 was two orders of magnitude greater than that of MS2 and ΦX174 (see Table 9). The background rates of inactivation of the phages as quantified from the control samples was not considered to be significant when compared to the overall removal rate and so wasn't factored into the removal results. The background rates of inactivation were similar between the three phages and also similar to the values reported for MS2 and ΦX174 by Van der Wielen et al. (2008), i.e. ~ 0.1 log over the 4 days of sampling. Greatest removal in the initial 0.35 m below the infiltrative surface was achieved by phage MS2 (1.1 log-unit), followed by PR772 (0.6 log-unit ~ 78%) and ΦX174 (0.6 log-unit ~ 75%). With the majority of the MS2 now retained by the 0.3 m depth, the most significant removal of ΦX174

and PR772 phages occurred between the 0.3 and 0.65 m depth planes with a further 1.6 and 2.9 log units attenuated, respectively. By the time the percolate had reached the 0.95 m depth plane on day 3 (as evidenced by bromide detection) MS2 had been reduced to their minimum detection limit of < 10 PFU/mL, whilst ΦX174 and PR772 recorded 20 PFU/mL on one trench and < 10 PFU/mL at the expected peak time of arrival. This equates to an overall percentage removal of > 99.82% (MS2), > 99.77% (ΦX174) and > 99.99% (PR772) between the infiltrative surface and 0.95m depth plane. It should be noted that the 8 to 12% uncertainty in observed phage concentrations spiked into the trenches would have minimal effect on the interpretation of the relative log removal results reported. Hence, in summary, almost complete removal of the three phages was achieved over an unsaturated subsoil depth of 0.95 m on *System I*.

3.4.2 System III - percolation trenches receiving SE

The phage tracer experiment carried out on the percolation trenches receiving SE from the RBC packaged plant was identical to that on *System I*. Although Cl analysis suggested the effluent to have only been present at the very front of the trenches (within 1 m distance), samples from 5 m sampling position were also collected and analysed as a precautionary measure. Concentrated stock solutions of MS2**,** ΦX174 and PR772 at concentrations of 1.4 x 10^5 (± 1.1 x 10^4) PFU/mL, 5.1 x 10^5 (± 6.3 x 10^4) PFU/mL and 2.5 x 10^7 (± 1.1 x 10^6) PFU/mL, respectively were added to 10 L of distilled water on site. The mixed solution was then spiked into each percolation pipe over an eight hour period, during which time a total of 12.95 L of SE flow was fed to both percolation trenches. Accounting for the dilutions, the reduced mean input phage concentrations for both trenches was 9.7 x 10^3 PFU/mL (MS2), 3.5 x 10^4 PFU/mL (ΦX174) and 1.7 x 10^6 PFU/mL (PR772). The daily flows to both trenches over the three day trial were measured at 130.7 L/d, 72.7 L/d and 150.3 L/d, respectively. This equates to a mean daily flow over the trial of 117.9 L/d or an equivalent HLR over the

infiltrative surface area of 749 mm/d. The calculations take into account the extra dilution resulting from 0.4 mm of rainfall recorded on day 3 of the trial.

Examining the bromide from the soil moisture samples at the front of the trenches (0 m sampling position) highlights the rapid travel time of the percolating effluent: all three depth planes were seen to be receiving effluent after 1 day of the trial, due to the poor biomat spread and resulting high HLR (almost 20 times that of Site A) and the highly permeable sandy GRAVEL. Bromide continued to be detected at all three depths at the front of the trenches over the 3 day trial.

Minimum removal rates of the three phages across the three depth planes over the duration of the trial are plotted in Figure 8 while Table 10 expresses the results in terms of phage concentrations. Again, phage values were considered for those sampling positions that coincided with evidence of bromide tracer. PR772 was injected into the trenches two orders of magnitude greater than ΦX174 and three orders of magnitude greater than MS2. Initial removal of all three phages over the first 0.35 m were found to be similar at rates of 1.3 log-unit, 1.6 log-unit and 0.9 log-unit for MS2, ΦX174 and PR772, respectively. Subsequent attenuation of the phages over the remaining 0.6 m resulted in all three achieving a further 1.2, 1.9 ad 3.9 log-unit removal for MS2, ΦX174 and PR772, respectively. Averaging the results from the three phages produces a mean phage removal of at least 3.6 log-units between the infiltrative surface and the 0.95 m plane. Again, the 4 to 12% uncertainty in observed phage concentrations spiked into the trenches would have minimal effect on the interpretation of the relative log removals calculated. While the majority of phage concentrations recorded at the critical 0.95 m depth plane were below the limit of detection (< 10 PFU/mL), MS2 was detected at a concentration of 50 PFU/mL on day 1 while PR772,

at 20 and 30 PFU/mL, was picked up at two samples point a day later. With analysis of chemical determinants appearing to rule out any preferential flow at this sampling position, phage detection at this depth over such a short-term trial highlights a very real risk of viral contamination in the underlying aquifer.

4. Discussion of microorganism attenuation

4.1 Bacteria attenuation

The results from the *E. coli* analysis at the three different depth planes for all three systems have shown that the majority of attenuation of enteric bacteria was achieved within the first 0.35 m depth plane, indicating the significant effect of the clogged interstitial pore spaces of the biomat that has prevented significant transport of bacteria. As discussed earlier, the reductions in contaminant concentrations under the percolation trenches as a result of pure dilution of the effluent from effective rainfall would be very small and therefore the prominent attenuation mechanisms operating in the vadose zone must be physical, chemical and biological processes.

The comparison between the *E. coli* data measured at the three depth planes of *Systems I and II* in the same subsoil but receiving different quality effluent (from the septic tank and reed bed respectively) has shown that despite lower concentrations of bacteria entering the percolation trenches downstream of the reed bed, episodic faecal contamination at the 0.95 m plane was more frequent than that measured at the corresponding depth in the same subsoil treating STE. Hence, it would appear that it was the biological processes in the biomat, and not the influent strength, that played the dominant role in coliform retention. Equally, the RBC packaged treatment system on *System III* greatly reduced bacterial loading on the

percolation area compared to the STE on *System I* but again evidence of faecal contamination with depth was recorded on a number of sampling events, which would appear to be due the poor biomat development, which would also have the effect of increasing the infiltration rate or hydraulic load per unit area, thus compromising treatment capacity. Also, the high sand content of the coarse textured subsoil in the vadose zone would have played an instrumental role in facilitating the movement of bacteria through the subsoil.

While the presence of enteric bacteria at the 0.35m depth plane would not pose an immediate threat to groundwater contamination and its associated health implications, the sporadic migration of the pathogen to the 0.95 m depth plane on all three systems is a concern and emphasises the negative influence of highly permeable subsoil in facilitating pathogenic transport to groundwater. Such isolated incidences of faecal contamination with depth were generally encountered during periods of high effective rainfall which would have facilitated such breakthrough, conditions which may have also promoted reversible adsorption (desorption) of the enteric bacteria from the thin layer of clay particles at shallow depths.

4.2 Phage attenuation

MS2 and ΦX174 have commonly been used in many environmental studies under both saturated and unsaturated subsoil conditions (Jin et al., 1997; Woessner et al., 2001; Guan et al., 2003; Zhuang and Jin, 2003). MS2 has been used as a surrogate for coxackievirus and norovirus, given its similarities in structure and IEP whilst ΦX174 has been suggested (Jin et al., 2007) as a good model for poliovirus due to its same IEP and attachment behaviour. Less is known about the response of PR772 in such studies, other than that it is very closely related (97%) to PRD-1 at the genome level (Lute et al., 2004), the latter of which is a commonly-used surrogate for subsurface viral fate and transport studies because of its stability in

aqueous and geologic media (Yahya et al., 1993). For example, recent studies by Charles et al. (2008) on mound systems showed higher removals of MS2 compared to PRD-1 when dosed onto an on-site mound system. Members of the *Tectiviridae* group are also thought to be good indicators of the larger enteric viral pathogens such as rotavirus and adenovirus (the two most commonly diagnosed waterborne enteric viruses) as they are similar in terms of genome replication, capsid architecture and vertex organisation (Lute et al., 2004).

The controlling factors and mechanisms surrounding the mobility of viral pathogens through different soils and aquifer materials have been shown to be highly variable, depending on their properties (clay fraction, metal oxides, organic matter content, temperature, pH) and the degree of water saturation (saturated or unsaturated). With unsaturated conditions predominating in the test sites, it would have caused the phages to move through the soil in thin films of water and be drawn nearer to the soil particles, increasing their potential for adsorption.

The biomat plays a very important role in maintaining unsaturated conditions by controlling the infiltration rate, as well as performing a viral attenuation process in its own right. The biomat contains a complex mix of biofilm and biological material which offer a huge number and diversity of potential sorption sites. In addition, the biomat acts to significantly decrease flow rates and increase retention times, thus increasing opportunities for attachment of viral-sized particles. Interestingly, the function of the biomat has often been overlooked and not incorporated in the many transport models (Corapcioglu and Haridas, 1984; Sim and Chrysikopoulos, 1999; Anders and Chrysikopoulos, 2005) for viral pathogens in the subsurface environment. Hence, a sizeable fraction of the removal and retardation of the phages observed above the 0.35 m depth plane on *System I* may be attributed to the active

biomat. The development of the biomat is closely related to the organic matter content in the effluent. Schijven and Hassanizadeh (2000) have pointed out that the enhancing and attenuating effects of organic matter (both in dissolved form in the effluent and bonded form on the media) are likely to produce significant variations with respect to virus removal and transport in porous media. The presence of organic matter has been shown to reduce virus attachment and thus facilitate virus transport by providing additional negative charges, covering positively charged sites, or competing with viruses for attachment sites (Zhuang and Jin, 2003). This hydrophilic blocking of virus sorption sites, as well as an increase of virus-medium electrostatic repulsion arising from modification of the soil and virus surface by organic matter, may have been responsible for the facilitated transport. For example, Van Cuyk et al. (2004) found increased virus concentration directly below the infiltrative surface at field test sites, suggesting a more biogeochemically active zone for virus survival whilst Sobsey et al. (1980) and Moore et al. (1981) found that the complex organics found in natural soil materials were comparatively poor sorbents for poliovirus. The organic matter content of the natural soil matrix for *System I* was determined at the three depth planes (before any effluent infiltration) by the loss on ignition method (BS 1377: Part 3) and showed 3.7% to be present at 0.35 m below the infiltrative surface. ΦX174 showed the least attenuation through the first 0.35 m depth of subsoil which perhaps would not have been expected given that it has an IEP of 6.6 and therefore should have minimal negative charge at circumneutral pH. Hence, its attachment behavior should be less adversely affected by the blocking of positive sorption sites by negatively charged organics compared to either MS2 or PR772 which have similar IEPs, at or below 4. It might also have been expected that MS2 and PR772 would show similar enhanced transport through a zone where negatively charged organics block good sorption sites, which was not the case; MS2 showed much higher removal through the first 0.35 m (1.1 log-unit) compared to PR772 (0.6 log-unit). Hence, it is unclear as to why

there was such significant differences in removal between the three phages at the 0.35m plane in *System I,* indicative of the complicated transport mechanisms in this zone. However, what can be noted is that the reduction in organics with depth appears to have been favorable for phage removal.

The greater retention of phage MS2 above the 0.35 m depth plane with respect to the other phages tested may be due to a number of factors. This phage's low IEP value of 3.5 – 3.9 compared to the soil pH might have indicated a reduced likelihood of sorption to soils or other surfaces, although the relevance of such a parameter determined in controlled ionic conditions to the conditions in an effluent adsorption field is questionable. The presence of the AWI (air-water interface) under unsaturated conditions has been suggested by several researchers to be the dominant removal mechanism responsible for the increased removal of colloidal particles, including viruses, in unsaturated systems (Powelson and Mills, 1996; Jin et al., 2000). Powelson et al. (1990) have shown MS2, in particular, to be strongly influenced by this and ultimately inactivated at the AWI. Hence, this mechanism may have played a significant early role in the MS2 inactivation between the infiltrative surface and the 0.35 m depth plane, particularly given the disparity in recorded concentrations between MS2 and the other two phages in this trial. ΦX174, unlike MS2, has been shown not to undergo any inactivation at the AWI as it has been found to be extremely hydrophilic and is thus resistant to forces at the hydrophobic AWI (Thompson et al., 1998). The multi-log removal of all three phages between the 0.35 and 0.95 m planes is most likely due to adsorption to the soil matrix which, although dominated by sand and gravel, contains 15 – 30% clay fraction. The reduction in organic matter content with depth would have also facilitated a greater number of sorption sites for phage adhesion. X-ray diffraction (XRD) analysis of the subsoils at the different depth bands was also carried out to determine their mineral content, as detailed in

Section 2.1. This found no evidence of any significant Al, Fe and Mn oxides on either site that may have promoted microbial attachment to the subsoil media. It should also be noted that due to the relatively low flows from the on-site systems during the 8 hour periods of phage spiking, the average ionic strength in the diluted effluent passing to trenches would have been reduced which could have facilitated phage transport.

The removal efficiencies of MS2, ΦX174 and PR772 were more similar in the subsoil environment of *System III* than in that of *System I*. In addition, all three phages exhibited greater removal at the 0.35 m depth plane over the trial than the corresponding plane on *System I*. Distinct differences existed, however, between the hydraulic and organic loading conditions on both sites which could explain the differences in phage transport. Firstly, the STE on *System I* typically contained an organic (COD) load nine times that of the SE discharged into the trenches of *System III*. The greatest phage removal detected at the 0.35 m depth plane was that of the hydrophylic ΦX174 (1.6 log removal) which has the largest IEP of the three phages which is just under the pH of the percolating medium. In contrast to the organic matter on *System I* facilitating increased vertical transport of the phages, the low organic matter content at the 0.35 m depth plane (measured at 0.9%) on *System III* meant that this interaction would have been minimal. Removal of all three phages appeared fairly uniform with depth, possibly indicating consistent adsorption to the soil particles under unsaturated conditions. Finally, the muted development of the biomat for *System III* promoted a flow per unit surface area that was ~ 37 times greater than that of *System I*. This difference in hydraulic loading may have contributed to the differences in phage transport. For example, Van Cuyk *et al*. (2007) demonstrated somewhat counter-intuitively that a higher HLR (250 mm/d as opposed to 50 mm/d) promoted greater removal of MS2 and PRD-1 at the infiltrative surface.

4.3 Implications to on-site regulation

The results from the continual monitoring of enteric bacteria across the percolation areas over 2 year periods as well as the results from the phage tracer studies have shown that 0.9 m of unsaturated subsoil may not be fully effective in removing bacteria and viruses where the subsoil is highly permeable. This was important from an Irish regulatory context as previous regulation had stated that the minimum unsaturated depth of subsoil required for secondary treated effluent was 0.6 m. This research contributed to the decision to increase the minimum unsaturated subsoil depth from 0.6 to 0.9 m under percolation areas receiving secondary effluent in the new regulatory Code of Practice document for Ireland (EPA, 2009b); the required depth of unsaturated subsoil for septic tank (primary) effluent was maintained at 1.2 m. In addition, the research also led to the accepted limit of field saturated hydraulic conductivities (k_{fs}) for subsoils receiving either septic tank or secondary treated effluent to be reduced from 4.2 to 1.4 m/d (T-value 1 to 3), as sites with more permeable subsoil than the sites studied in this research would clearly present higher risks of microbial breakthrough at depth.

5. Conclusions

The percolation trenches treating septic tank effluent showed that 50% (or 30 m) of percolation trench had been utilised after 32 months of system operation. This was in contrast to the significantly muted biomat development, approximately 1 m in length only, on the percolation trenches receiving secondary effluent over the same period of time.

Greatest *E.coli* removal in the subsoil occurred above the 0.35 m depth plane on all three system types. Analysis showed, however, that more evidence of faecal contamination

occurred at depth in the subsoils receiving secondary treated effluents (13% and 15% of samples at a depth of 0.95 m under the percolation trenches on two sites detected the presence of *E.coli*) than that receiving septic tank effluent (7% of samples), despite the lower bacterial influent load. This was due to the higher areal hydraulic loading rate acting on the base of the trenches receiving secondary effluent owing to the absence of a biomat at its infiltrative surface, and the high sand content present, which facilitated free movement of the bacteria. This highlights the influence of flow conditions on bacterial transport and emphasises the risks posed by discharging secondary effluent by gravity flow into permeable sand/gravel subsoils.

All three bacteriophages were reduced to their minimum detection limit (< 10 PFU/mL) at a depth of 0.95 m below the percolation trenches receiving septic tank effluent, although isolated incidences of ΦX174 and PR772 were measured below one trench. Slightly higher breakthroughs of MS2 and PR772 contamination, however, were detected at the same depth under the trenches receiving secondary effluent. As the bacteriophage studies were single spiking trials, more comprehensive and long-term research is needed to investigate the exact mechanisms of attenuation and impact of other environmental factors such as rainfall on these processes, with all the inherent heterogeneities and associated uncertainties in such field studies. This will help to understand the hydrogeological formations affecting groundwater vulnerability to viral contamination and the physical and chemical properties governing viral fate and transport in the vadose zone.

This research contributed to the decision to increase the minimum unsaturated subsoil depth from 0.6 to 0.9 m under percolation areas receiving secondary effluent in the new regulatory Code of Practice document for Ireland (EPA, 2009b), as well as reducing the accepted limit

of subsoil hydraulic conductivity deemed suitable for discharge of on-site effluent. However, as shown by this research, 0.9 m of unsaturated subsoil may not be fully effective in removing bacteria and viruses where the subsoil is highly permeable. As such it is recommended that in subsoils with particularly high permeability, an unsaturated subsoil depth of 1.2 m be maintained to reduce any contamination risks.

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Fig. 1. Plan view and instrumentation layout of (a) *Systems I* and *II* on Site A and (b) *System III* on Site B (percolation trenches). The reed bed also visible at Site B was not part of this study.

Fig. 2. Cross section of percolation trench including suction lysimeter and tensiometer profile.

Fig. 3. Mean Cl concentrations measured for the three depth planes at the five sample positions on *System I* trenches.

Fig. 4. Mean Cl concentrations measured for the three depth planes at the four sample positions on *System III* trenches.

Fig. 5.
Soil moisture tension plotted against effective rainfall for the 17.5 m (back) sample position on *System I* percolation trenches.

Fig. 6.
Soil moisture tension plotted against effective rainfall for the 2.5 m (front) sample position on *System I* percolation trenches.

Fig. 7. Phage log-unit removal (*System I*) recorded at each depth plane (0.35 0.65, 0.95 m) at all sample positions over the duration of trial. Note, MS2 recorded all samples as less than the limit of detection at the 0.95 m plane, hence the error bar to show the uncertainty in removal.

Fig. 8. Phage log-unit removal (*System III*) recorded at each depth plane (0.35, 0.65, 0.95 m) at all sample positions over the duration of trial. Note, MS2 and ΦX174 recorded samples as

less than the limit of detection at the 0.95 m plane, hence the error bar to show the uncertainties in removals.

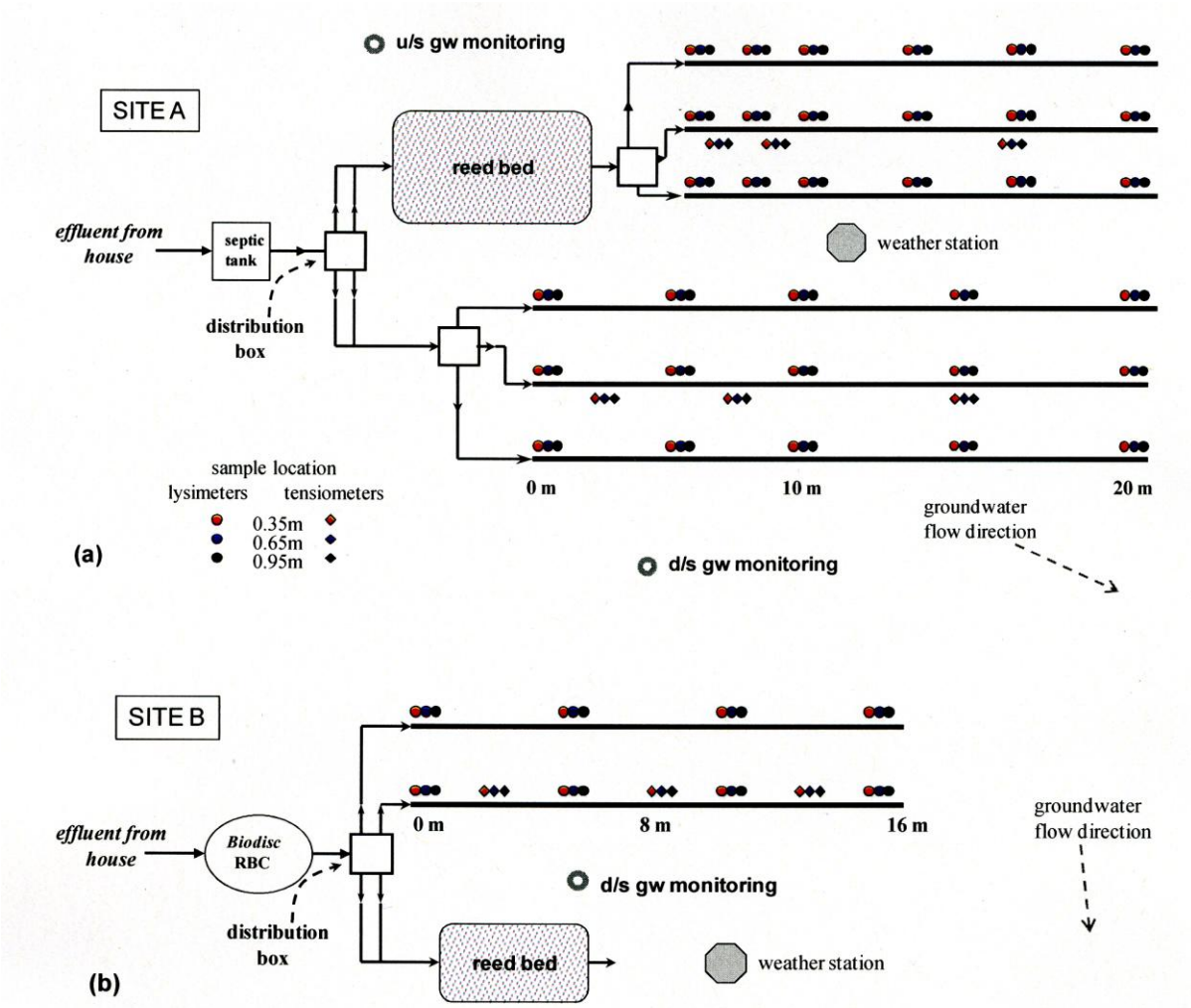

Fig. 1. Plan view and instrumentation layout of (a) *Systems I* and *II* on Site A and (b) *System III* on Site B (percolation trenches). The reed bed also visible at Site B was not part of this study.

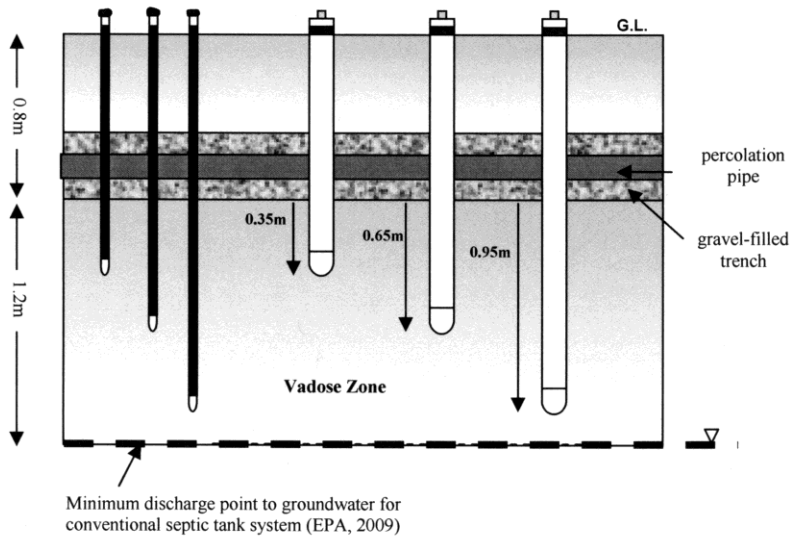

Fig. 2. Cross section of percolation trench including suction lysimeter and tensiometer profile.

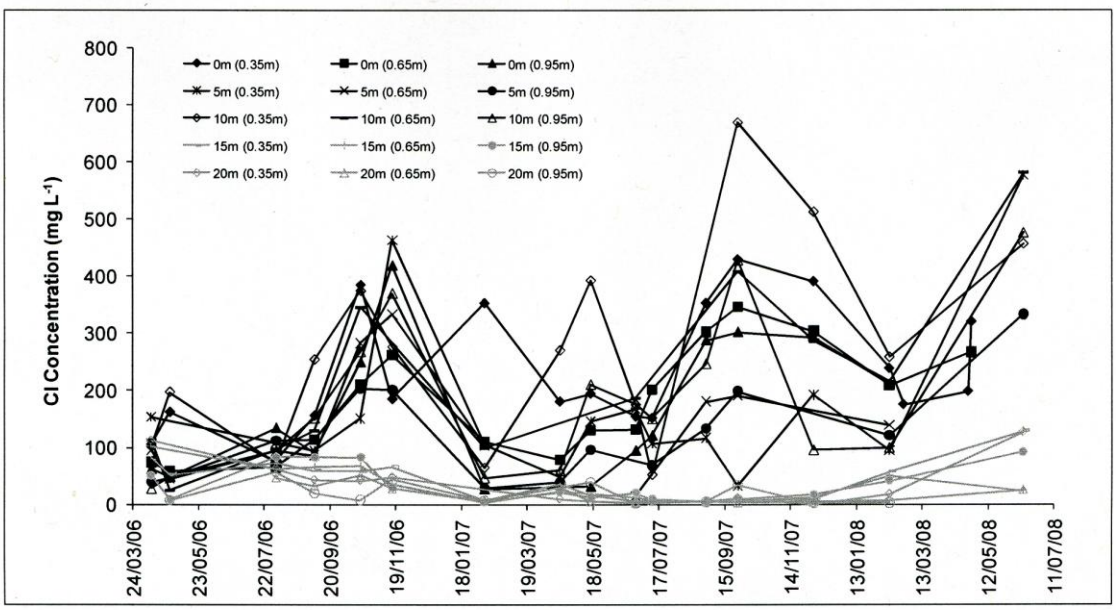

Fig. 3. Mean Cl concentrations measured for the three depth planes at the five sample positions on *System I* trenches.

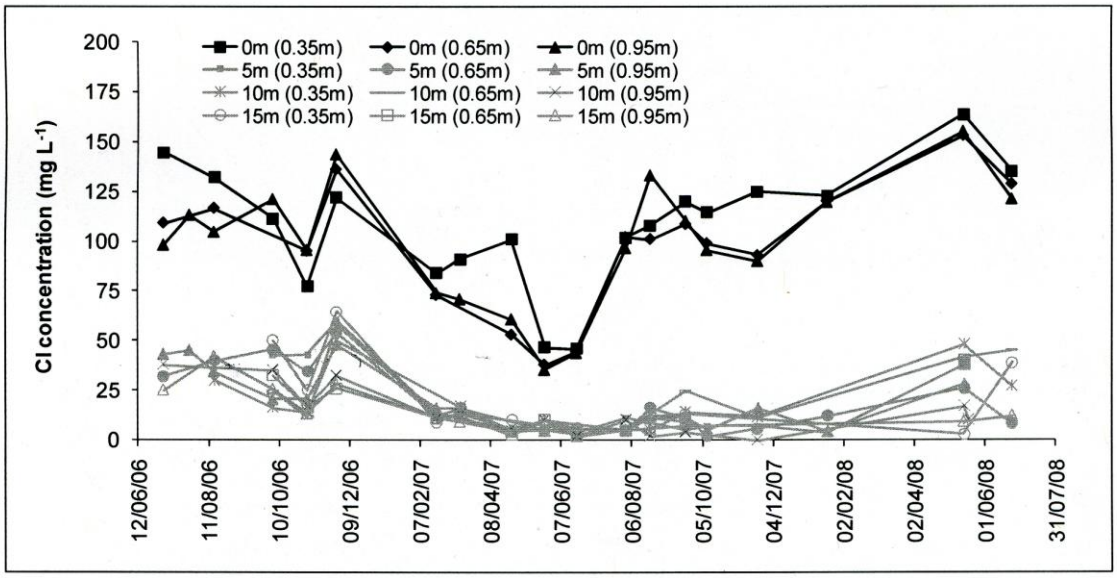

Fig. 4. Mean Cl concentrations measured for the three depth planes at the four sample positions on *System III* trenches.

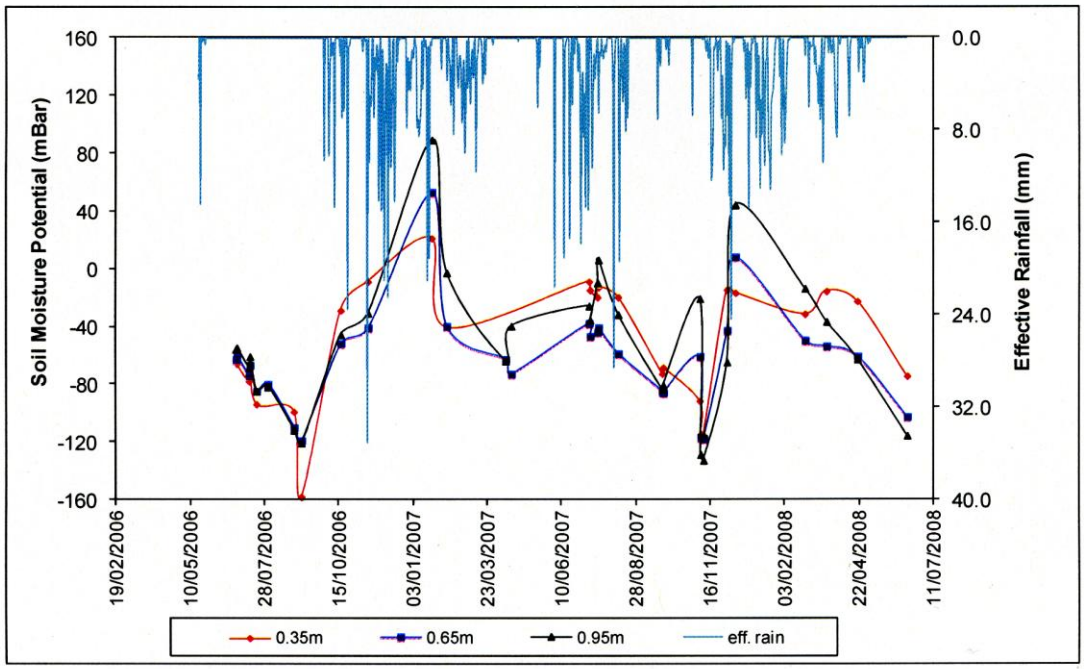

Fig. 5. Soil moisture tension plotted against effective rainfall for the 17.5 m (back) sample position on *System I* percolation trenches.

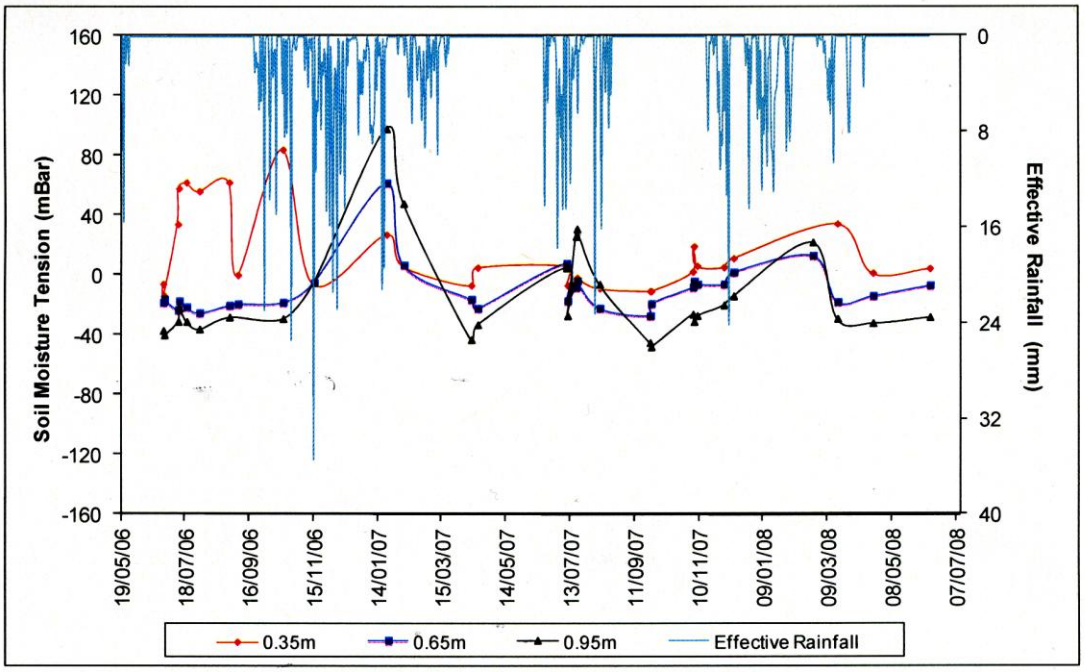

Fig. 6. Soil moisture tension plotted against effective rainfall for the 2.5 m (front) sample position on *System I* percolation trenches.

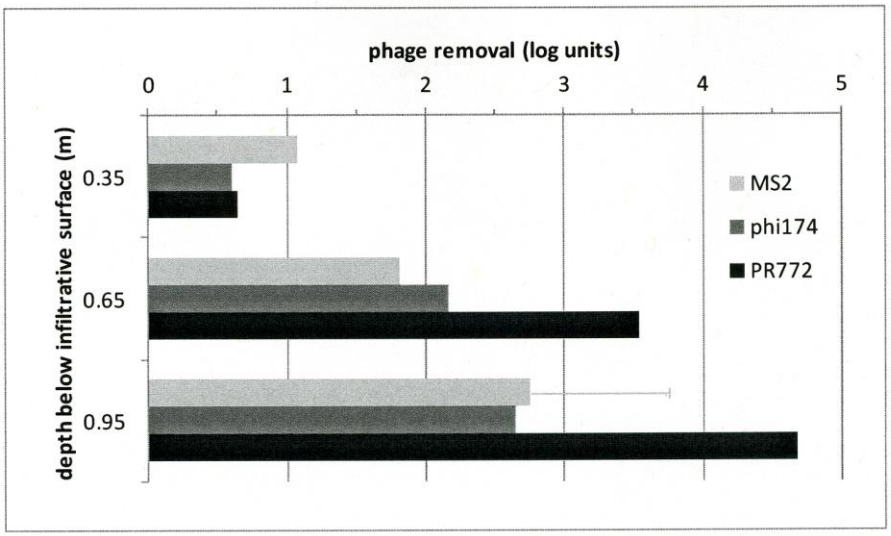

Fig. 7. Phage log-unit removal (*System I*) recorded at each depth plane (0.35 0.65, 0.95 m) at all sample positions over the duration of trial. Note, MS2 recorded all samples at less than the limit of detection at the 0.95 m plane, hence the error bar to show the uncertainty in removal.

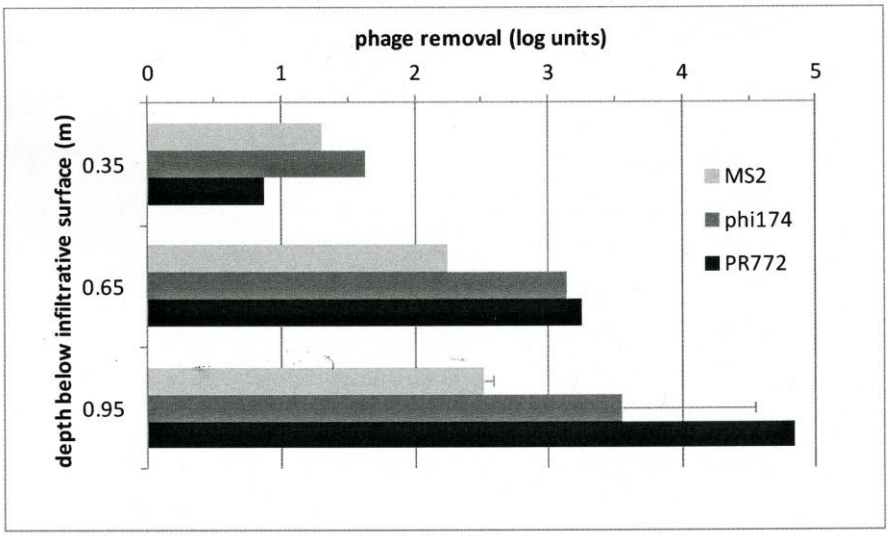

Fig. 8. Phage log-unit removal (*System III*) recorded at each depth plane (0.35, 0.65, 0.95 m) at all sample positions over the duration of trial. Note, MS2 and ΦX174 recorded samples at less than the limit of detection at the 0.95 m plane, hence the error bar to show the uncertainties in removals.

Table 1. Summary of site characteristics.

	Site A				Site B			
System	I		II		III			
Effluent	STE		RBE		SE			
No. residents	3		3		2			
Subsoil T-value	3.7				1.27			
Field sat. hyd. cond., k_{fs} (m/d)[b]	1.27				0.93			
Subsoil classification[a]	sandy, gravely SILT				sandy GRAVEL			
Particle size dist. (%) at depth	*gravel*	*sand*	*silt*	*clay*	*gravel*	*sand*	*silt*	*clay*
1.0 m	19	28	33	20	45	34	10	11
1.5 m	23	20	34	23	62	27	7	4
2.0 m	28	35	25	12	57	35	6	2

a Below proposed invert level of percolation trenches. See BS5930 (British Standards Institution, 1999) for method of classification

b Field saturated hydraulic conductivity – see Mulqueen and Rodgers (2001)

Table 2. Characteristics of selected phage surrogates (adapted from IAWPRC, 1991, Lute et al., 2004 and Collins et al., 2006).

Phage	Type	Family	Size (nm)	Symmetry	Isoelectric point (IEP)	Genetic material
MS2	Male specific F-RNA	Leviviridae	26	Icosahedral	3.5 - 3.9	Linear ss-RNA
ΦX174	Somatic	Microviridae	25-27	Icosahedral	6.6	Linear ss-DNA
PR772	Somatic	Tectiviridae	63	Icosahedral	3.8 - 4.2	Linear ds-DNA

Table 3. Comparison of total lengths of percolation trench in use after 32 months of system operation, based on design Long Term Acceptance Rates (LTARs) of 20 L/m^2.d for STE and 25 L/m^2.d for SE.

System	Influent strength	Mean COD load per trench (g/d)	Mean daily hydraulic load (L/d)	Total perc. trench length (m)	
				Constructed	Active length
System I	STE	216	395.4	60	30
System II	RBE	61	349.9	48	19.5
System III	SE	26	132.1	32	1

Table 4. Average effluent quality entering percolation trenches on *Systems I, II* and *III*.

Pollutant	*System I* STE mg/l (±sd)	*System II* RBE mg/l (±sd)	*System III* SE mg/l (±sd)
COD	547 (±186)	220 (±120)	199 (±85)
BOD_5	185 (±62)	48 (±37)	101 (±53)
NH_4-N	75.0 (±25)	60.2 (±24)	19.4 (±21)
NO_3-N	0.8 (±0.6)	4.3 (±2.0)	35.7 (±28.1)
TN	107 (±26)	78 (±23)	96 (±53)
PO_4-P	16.6 (±10.8)	8.8 (±6.0)	26.4 (±9.3)
pH	7.2 (±0.3)	7.4 (±0.2)	7.4 (±0.4)
		MPN/100ml	
Total Coliforms[a]	$1.28x10^7$	2.07×10^5	$2.88x10^5$
E. coli[a]	$7.44x10^5$	2.80×10^4	$1.33x10^4$

[a] average reported as geometric mean for coliforms

Table 5. Estimated biomat length and size and shape of the plume in 3 dimensions for percolation trenches of *Systems I*, *II* and *III*.

			Estimated width of plume (m) from each side of trench at depth plane		
System ID	Influent strength	Average length of biomat per trench (m)	0.35 m	0.65 m	0.95 m
System I	STE	10	0.1	0.17	0.3
System II	RBE	6.5	0.15	0.25	0.3
System III	SE	0.4	0.05	0.1	0.35

Table 6. Mean concentrations of *E. coli* across the three depth planes from the 0, 5 and 10 m sampling positions for *System I.*

Depth (m)	No. of samples	No. of samples with concentration (MPN/100mL)			
		< 10	10 – 100	101 – 1 000	> 1 000
0.35	59	53	4	2	0
0.65	46	39	4	3	0
0.95	54	50	4	0	0

Table 7. Mean concentrations of *E. coli* across the three depth planes from the 0 and 5 m sampling positions for *System II*.

Depth (m)	No. of samples	No. of samples with concentration (MPN/100 mL)			
		< 10	10 – 100	101 – 1 000	> 1 000
0.35	54	48	6	0	0
0.65	58	54	3	1	0
0.95	52	44	5	1	2

Table 8. Mean concentrations of *E. coli* across the three depth planes from the 0 m sampling positions for *System III*.

Depth (m)	No. of samples	No. of samples with concentration (MPN/100 mL)			
		< 10	10 – 100	101 – 1 000	> 1 000
0.35	33	28	4	0	1
0.65	29	25	4	0	0
0.95	31	27	3	1	0

Table 9. Maximum phage concentrations at each depth plane in the zone of effluent contribution over the 4-day trial on *System I.*

	Maximum phage conc. recorded across depth plane (PFU/mL)			
Depth below infiltrative surface (m)	MS2	ΦX174	PR772	Mean phage log-unit removal
0	5.6×10^3	6.6×10^3	7.1×10^5	-
0.35	4.7×10^2	1.7×10^3	1.6×10^5	0.77
0.65	87	45	2.1×10^2	2.50
0.95[a]	<10 & <10	< 10 & 20	< 10 & 20	>3.35

[a] results from both samples shown for 0.95 m depth plane

Table 10. Maximum phage concentrations at each depth plane in the zone of effluent contribution over the 4-day trial on *System* III.

	Maximum phage conc. recorded across depth plane (PFU/mL)			
Depth below infiltrative surface (m)	MS2	ΦX174	PR772	Mean phage log-unit removal
0	9.7×10^3	3.5×10^4	1.7×10^6	-
0.35	4.8×10^2	8.2×10^2	2.3×10^5	1.27
0.65	55	25	9.6×10^2	2.88
0.95[a]	<10 & 50	<10 & <10	20 & 30	>3.63

[a] results from both samples shown for 0.95 m depth plane

Highlights

- field study on percolation areas in permeable subsoils receiving on-site effluents
- focus on *E.coli* and spiked bacteriophages (MS2, ΦX174 and PR772)
- biomats receiving secondary effluent restricted compared to those receiving septic tank effluent
- more evidence of faecal contamination in subsoils receiving secondary treated effluents compared to septic tank effluent
- all three bacteriophages reduced to minimum detection limit at a depth of 0.95 m