



On-site treated wastewater disposal systems – The role of stratified filter medias for reducing the risk of pollution

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ABSTRACT

The transmission of pathogens from partially or fully treated wastewater to different water sources are a pervasive risk to public health. To reduce the risk, the integration of source separation, on-site greywater treatment system, and an efficient disposal scheme are the most critical approaches. This study intended to evaluate the removal of nutrient and microbial suspension in the filtration systems used for effluent disposal. The effluent from an on-site greywater treatment plant was loaded into the columns, and the effluent from the columns was monitored for nutrients, total coliform bacteria, *Escherichia coli*, and *Salmonella typhimurium* phage 28B (St28B) for one year. Thus, from the range of infiltration systems tested, column-B (15 cm layer of each, Filtralite, fine sand, and till soil) showed the highest removal of total coliforms and *E. coli*, 3–4 log₁₀ reduction, while the lowest removal observed in column-C (a layer of 25 cm crushed stone and 50 cm till soil), 2–3 log₁₀ reduction. The virus removal efficiency of the columns reduced from 19% to 70% during the simulation of a rainfall event. Moreover, the rise of St28B concentration after rainfall experiment may probably be the sign of detachment enhanced by low ionic strength rainwater.

1. Introduction

Centralized wastewater treatment systems for sparsely populated rural communities and recreational cabins are not economically and technically feasible. As an alternative, decentralised on-site wastewater treatment systems are commonly utilised with the intention to treat relatively small volumes of wastewater originated from individual dwellings, a cluster of homes or businesses, and institutional facilities, and dispose of the effluents in the vicinity close to its sources of generation (Wood et al., 2016). There are different types of on-site wastewater treatment systems designed to treat wastewater to various levels before it is disposed into the soil infiltration system (Ho and Anda, 2006; Meinzinger, 2010; Wood et al., 2015). Frequently, the effluent from on-site treatment system is infiltrated through unsaturated soil media and further treated by adsorption, chemical reaction and biodegradation before recharging to groundwater (Gill et al., 2009; Van Cuyk et al., 2001). On-site treated wastewater disposal systems are usually constructed as a soil infiltration trench with a variety of configurations, receive effluent through the perforated piping system. The type of configurations depends on the level of treatment, the sensitivity of the recipient, the regulation of the country, availability of materials, and hydrogeological setting of the area (Kaseva, 2004; Stevik et al.,

2004; Stevik et al., 1999; Van Cuyk and Siegrist, 2007).

Filtration of treated wastewater through a natural soil profile or constructed filter media is frequently considered as a post-treatment system and usually constructed from different materials such as crushed stones, different soil types, and marine sediments (Levine et al., 2008). When treated wastewater filtered through the filter media, the effluent receives further treatment attributed to the interaction of different processes including infiltration and percolation processes coupled with physical, chemical, and biological processes and the removal of microbial pathogens through the mechanisms of straining, adsorption, and inactivation (McCray et al., 2005; Stevik et al., 2004). Various factors determine the retention and transport of microorganisms in porous media. These factors include physicochemical property of filter media, clogging, biofilm, microbial cell size and shape, hydraulic loading, moisture content, organic matter content, temperature, pH, ionic strength and species, electrostatic charge on the cell surface, hydrophobicity, chemotaxis, concentration of microbial cell, microbial species and the presence of other microorganisms (Bradford et al., 2013; Stevik et al., 2004). However, there is a substantial variation in pollutant removal efficiency of filter materials depending on its origin, mineralogy, chemical composition, and physical properties (Chen, 2012; Cucarella and Renman, 2009; Lamy et al., 2008; Seelsaen et al., 2006).

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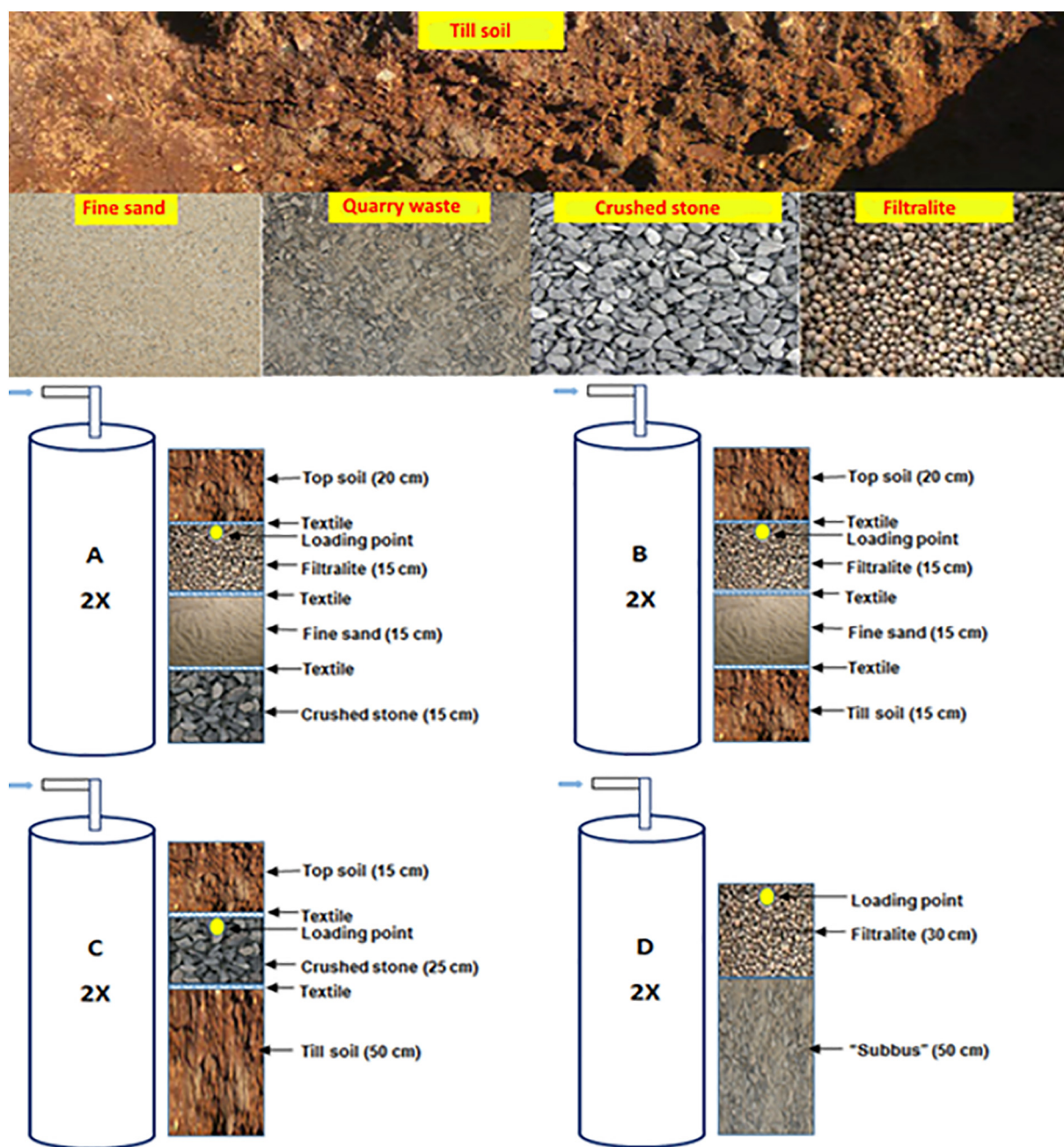


Fig. 1. Filter media and the cross-sectional view of the columns.

These properties include particle size and shape, the porosity of the grains or aggregates define their specific surface area, and surface property (charge). The smaller the particle size, the larger the surface area to undergo high sorption of nutrient and viruses (Reddy et al., 1999). Also, the chemical property of filter media with the pH of infiltrated water determines the affinity or reactivity and the strength of the interaction (Khadhraoui et al., 2002).

When untreated, treated or partially treated wastewater is discharged into the environment, it joins the surface water systems or infiltrate into subsurface media and joins the groundwater zone. These discharges are the primary source of microbial and nutrient contaminants entering into different water bodies that are used as a source for drinking water, irrigation, and fishing (Abdel-Raouf et al., 2012; Geary and Whitehead, 2001; Naidoo and Olaniran, 2013; Tanner et al., 2012). Information about the treatment level of on-site treatment and disposal system, and the removal efficiency of subsurface media helps

us to establish safe setback distances between on-site disposal fields and to drinking water supply sources (e.g., wells, springs, reservoirs) (Blaschke et al., 2016; Charles et al., 2004; Yates and Yates, 1989). However, the current knowledge gap limits the performance of the established setback distance (Blaschke et al., 2016). Therefore, detailed studies are required to address these problems to set the safest setback distance and minimise the risk of contamination.

Microbes and nutrient removal efficiency of the infiltration media have studied at laboratory bench scale, and such experiments allow detailed investigation since it is based on well-controlled boundary conditions. However, it is difficult to extrapolate the significance of results from small laboratory scale to field scale studies (Oxarango et al., 2011). On the other hand, field-scale studies are rare (Ausland et al., 2002; Martin et al., 1996), and the experiments are challenged by the interference of unknown factors and diffused boundary conditions. Because of the complexity and high degree of spatial variations as one

moves from laboratory scale to field scale experiment, the evaluation of infiltration media efficiency could potentially be affected by different processes (Fellner et al., 2009; Oxarango et al., 2011). Considering the challenges of the two experimental scales (laboratory and field), this study conducted on “pilot-scale experiment”, which is expected to generate comparable results to that of a controlled experimental setup, even though it is costly, and space and time-consuming.

The objectives of this study were to identify an efficient treated greywater disposal system in removing nutrients, virus and bacteria under different environmental conditions as a post-treatment step. The results of this study will provide insights about the removal efficiency of different infiltration systems that help to improve treated greywater disposal system design and lead to a better assessment of microbial pollution risk of source water from treated greywater discharge.

2. Material and methods

2.1. Experimental set up and filter medias

The source separation wastewater treatment system at Kaya student dormitory in Norwegian University of Life Sciences (NMBU), Ås, collected greywater and blackwater separately and pumped into the Faculty of Science and Technology laboratory for different experiment and the detail of system is described in (Todt et al., 2015). In this experiment, source separated greywater was treated with on-site greywater treatment plant (biofilter system), which consists of a sequence of a primary settler, an unsaturated fixed-film biofilter and a secondary clarifier and then the effluent pumped into unsaturated infiltration column representing infiltration trench in the actual treated greywater disposal system. With the intention to investigate the treatment efficiency of soil infiltration systems, which is used as a post-treatment step for the on-site greywater treatment system, four different stratified infiltration columns (Column-A, B, C, and D) with two replicate were constructed. Each column represents a single hole in the actual perforated disposal pipe that placed on the top of the infiltration trench. A cylindrical polypropylene opaque pipe (length: 100 cm; internal diameter: 60 cm) was used for the construction of infiltration column, and the bottom was sealed with the same material. The filter media used for the construction of columns in this experiment include till soil (glacial till deposit), crushed granite stone, fine sand, lightweight clay aggregates (filtralite), and quarry waste “subbus”. The crushed stone (11–22 mm) and the quarry waste “Subbus” (0.2–16 mm) were originated from gneiss and amphibolites, obtained from Vinterbro quarry, Norway. The till soil was excavated from Nordby area, Norway and contained Iron oxide (9.2 g/kg) and Aluminium oxide (2.4 g/kg). Fine sand (0.2–1.0 mm) dominated by silicon dioxide in the form of quartz, and the lightweight aggregate LWA (2–4 mm) (Filtralite, Saint-Gobain Byggevarer AS, Alnabru, Norway) were utilised to form stratification in the infiltration columns (Fig. 1). Nonwoven geotextile fabrics were used to separate the layer. The columns were packed up to different height depending upon the specification of each column and care was taken to ensure uniform packing, and at the same time, preferential flow paths were avoided by pouring the media in small quantities during packing.

The packing was carried out by compacting with a 3.5 kg flat wooden pole that dropped from 60 cm height. Each column has a different stratification of infiltration media. Column-A comprises three layers, 15 cm of crushed stone at the bottom, an intermediate layer of 15 cm fine sand, and the upper 15 cm of filtralite used to distribute the hydraulic load uniformly and geotextile separated each layer. Column-B contains the same layer as column-A except for the bottom layer, which encompasses till soil, instead of crushed stone. Column-C represents the national standard for the construction of treated wastewater disposal system that consists of 50 cm till soil at the bottom and 25 cm of crushed stone at the top. Column-D is the new trial column constructed by 50 cm bottom layer with quarry waste “subbus” and the top 30 cm pack with Filtralite.

The effluent from on-site greywater treatment plant (biofilter system) was feed into the infiltration columns using peristaltic pumps, which was synchronised with the biofilter system-dosing pump that was controlled by the level switch in the primary settling tank and the timer gives the plus interval. The actual flow rate of the peristaltic pumps were 2.51h^{-1} with the daily load variation 37.51d^{-1} to 44.81d^{-1} depending on the resting time of the treatment plant, which was fluctuating from 6 to 9 h a day, and results in 132 to $158\text{l m}^{-2}\text{d}^{-1}$ surface loading rate. The columns were loaded using a plastic tube with inner diameter 6 mm and placed at the centre of the column 7.5 cm deep from the top layer of either Filtralite in the case of column A, B, and D or crushed stone in the case of column C. The topsoil cover (15 cm to 20 cm) was for insulation as it is practised in the field.

The columns were placed in the laboratory room and exposed to a room temperature of 16 to 22 °C during summer and 10 to 15 °C during winter with minimum variation during day and night time. The raw greywater temperature has only minor variations between 18 and 22 °C due to a heating cable installed with the pipe to avoid freezing during winter. However, the water temperature reduced to 13–15 °C when it passed through the columns with minimum variation (± 1 °C) during winter and summer. The particle size distribution of the filter media on a weighted base was analysed in triplicate by standard operating procedure, LS 13320 Laser Diffraction Particle Size Analyser (Fraunhofer.rf780d optical model, Beckman Coulter, Inc. USA).

2.2. Tracer test using NaCl

A tracer test is an indirect method for estimating flow and characterizing filter media properties. NaCl is one of the tracers that permits measuring the breakthrough time of the aqueous solution and relates it to the (retarded) transport of either the bacterial or the viral tracers in the filter media. The tracer test in the unsaturated infiltration system studied by spiking the influent with sodium chloride (NaCl), which was added only on the initial pulse in the influent (electric conductivity (EC) of $950\ \mu\text{S}/\text{cm}$) and the EC was monitored from the column effluent every 20 min using EC meter in order to develop breakthrough curve.

2.3. Experimental setup for rainfall impact assessment

The rainfall simulation experiment was performed by collecting rainwater in an open plastic tank, transfer into the closed plastic container, and conductivity and pH were measured. In the sampled rains pH range of 6.6–7.2, with a mean value of pH = 6.8 and the conductivity ranged from 87 to $115\ \mu\text{S cm}^{-1}$. The experiment was carried out by mixing the rainwater with biofilter system effluent (1:4 rainwater to biofilter system effluent) and then pumped into the infiltration columns. The flow rate of the pump was increased from 2.51h^{-1} to 3.12h^{-1} to compensate for the additional water that comes from rainfall, considering the same will happen in the actual fields during rainfall. The objective of the rainfall simulation experiment in unsaturated columns was to test two conditions 1) to examine the detachment of virus particles from the infiltration media when virus shedding followed by rainfall after three days. 2) The virus removal efficiency of the infiltration systems when both virus shedding and rainfall happened simultaneously at the same time (rainfall simulation run for 17 h).

2.4. Microbial water quality analysis

The columns run for three weeks before each experiment conducted. Water samples from the inlet and outlets of the columns collected at once in a day (8:00 to 9:00 am) in 1-litre sterile bottles and three replicates for each parameter analysed within 1 h. The *Salmonella typhimurium* phage 28B (St28B) (Lilleengen, 1948) was propagated using a host culture of *S. typhimurium* type 5 in nutrient broth and analysed according to (Allestam and Carlander, 2000) and described in (Höglund

et al., 2002). The growth medium was prepared with distilled water, nutrient broth (0.8%), and yeast extract (0.05%). Chloroform (10 ml/l) was added to kill and lyse the host cells after incubation, and the suspension was then centrifuged for 10 min at 3000 rpm and filtered through a 0.45 µm filter. The final concentration of the propagation was 9.8×10^9 PFU/ml, and this stock kept at 4 °C until the time of usage. The St28b enumeration was carried out using a double-layer agar plaque assay. First, Petri dishes with 20 ml solid bottom-agar (growth medium with 1.5% w/v agar) were prepared. Then, 0.5 ml sample (after serial dilution in 0.9% NaCl, if needed), 0.5 ml exponential growth-phase host culture, and 4 ml molten top-agar (growth medium with 0.65% w/v agar) were mixed and poured over the solid agar in the Petri dishes. Finally, samples were incubated at 37 °C for 18 h and plaques were counted.

Enumeration of total coliforms (TC) and *Escherichia coli* (*E. coli*) were performed using Colilert-18 with Quanti-Tray/2000 (IDEXX Laboratories, USA) using the most probable number method (MPN) according to ISO 9308-2:2012.

2.5. Physicochemical water quality analysis

The samples were analysed for physicochemical parameters using standard methods: pH potentiometric measurement using probe according to NS-EN ISO 10523:2012; Conductivity: electrometric measurement using platina probe according to NS-ISO 7888:1993. For COD, total phosphorus (P), total nitrogen (N) spectrophotometric test kits (Hach-Lange, Berlin, Germany). Total suspended solids (TSS) were determined with 1.2 µm glass fibre filters (Whatman GF-C, GE Healthcare, and Little Chal-font, UK) and turbidity was measured with light scattering measurement at 860 nm.

2.6. Statistical analysis

The \log_{10} reduction of TC, *E. coli*, and St28B in the infiltration system, between the concentration of column influent and effluent, was calculated as $\text{Log}_{10} \text{ reduction} = -\text{Log}_{10} (C / C_0)$, where *C* is column effluent concentration, *C*₀ is influent column concentration, and the negative sign is to make the reduction positive. A one-way analysis of variance (ANOVA) with Tukey's post hoc test was used to examine whether the \log_{10} decrease of TC, *E. coli*, and St28B in the effluent of representative columns was a function of the infiltration system. The independent variable represented the four different types of infiltration system, columns- A, B, C, and D. The dependent variable was the \log_{10} reduction of TC, *E. coli*, and St28B. An alpha level of 0.05 was used to determine statistical significance for all analyses. All statistical analyses were performed using Minitab 17 statistical software (State College, PA: Minitab, Inc.).

3. Result and discussion

3.1. Filter media

The grain-size distribution plots were used to estimate *D*₁₀ and *D*₆₀, which is used to calculate the uniformity of the particle size distribution (uniformity coefficient, *C*_u), as the ratio of *D*₆₀ to *D*₁₀, and the effective grain size of filter media, which is *D*₁₀ strongly correlated with permeability (Fig. 2). *D*₁₀ is termed as the effective particle size it means that 10% of the particles are finer and 90% of the particles are coarser than that particular particle size *D*₁₀. Similarly, *D*₆₀ means the diameter of the filter media for which 60% of the particles are finer, and 40% of the particles are coarser than *D*₆₀. The higher the uniformity coefficient, the broader range of particle size in the filter media (Alyamani and Şen, 1993). Also, to secure an adequate hydraulic conductivity and to minimise the risk of clogging, the effective grain size *D*₁₀ should be in the range of 0.3 to 2.0 mm, *D*₆₀ in the range of 0.5 to 8.0 mm, and the uniformity coefficient should be < 4 (Brix et al., 2001) (Table 1). This

recommendation is intended to avoid clogging at higher loading rates and in this study, the indexes are out of the range except in the case of Filtralite, and clogging observed in column-A after eight months.

As we can see from Fig. 2, steep curves, in the case of Filtralite indicate the filter material with a narrow range of particle sizes, poorly graded filter. On the other hand, gentle slope curves, such as till soil, contain a wide range of particle sizes, well-graded filter. The treatment efficiency of filter media increases with decreasing the particle size of the filter media, which indicates the importance of small interstices between particles and larger surface area that allows more adsorption to take place (Jenkins et al., 2011). However, having a filter media with too fine grain size will lead to rapid clogging (Nam et al., 2000). Therefore, proper filter size selection is crucial.

3.2. Tracer experiments

Tracer tests were conducted to determine the flow behaviour and residence time distribution in each of the columns that permits measuring the breakthrough time of the aqueous solution and relates it to the (retarded) transport of either the bacterial or the viral tracers in the filter media. The high hydraulic retention time improves removal performance of the filter media by exposing the microbes for different removal processes. The tracer transport was characterised by their breakthrough curve, plotting the NaCl concentration at the outlet (µS/cm) against the time taken to travel (Fig. 3). Consistent with the difference in the grain size distribution of infiltrations system and travelling distance (depth of columns), column-C had longer time (360 min) to the peak tracer appearance and a lower peak concentration as compared with the other columns. Column-A exhibited a shorter time (60 min) to peak as compared with the other columns, but the peak concentration was almost the same as column-B and column-D. The average residence time for the influent in the case of column-D had 160 min, relatively lower as compared with the other columns travelling distance.

3.3. Physico-chemical parameters

Water quality assessment (raw greywater, biofilter system effluent, and columns effluent) of the system from 22 observations for each parameter presented in Table 2. The average total phosphorus (P) in the untreated greywater was 0.95 ± 0.25 mg/l, and the concentration in the effluent of the biofilter system effluent was 34.7% lower than the influent concentration (raw grey water). Furthermore, P in the effluent of the columns were 73.7% to 82.1% lower than the untreated greywater concentration. The average level of total nitrogen (N) at the columns effluent was 53.0% to 61.3% lower than the concentration in the raw greywater. On the other hand, the concentration of nitrate in the untreated greywater was very low 0.33 mg/l and it increases to 3.08 mg/l in the biofilter system effluent and further increases from 4.68 to 6.20 mg/l in the columns effluent. The increasing trend of nitrate in the columns effluent is an indication of nitrification, which is the process of converting ammonia into nitrate in the presence of oxygen and nitrifying bacteria under unsaturated flow condition. The concentration of BOD in the raw greywater was 137 ± 38 mg O₂/L, and 80% removal was observed in the biofilter system effluent. Whereas, BOD was below the detection limit in all columns effluent. The concentration of COD in the raw greywater was 274 ± 87 mg O₂/L and 90.9% to 96.1% was reduced in the column effluent. Suspended solids and turbidity in the untreated greywater were highly varied with time, and the average suspended solids and turbidity were 32.47 ± 29 mg/l and 40.33 ± 39 FNU respectively. The infiltration column was effective in reducing both suspended solids and turbidity, and the reduction reached up to 90.9% in suspended solids and 87.9% in turbidity. The nutrients removal efficiency of on-site greywater treatment plant coupled with unsaturated infiltration systems were significant, and it should be noticed that the variation depended on the

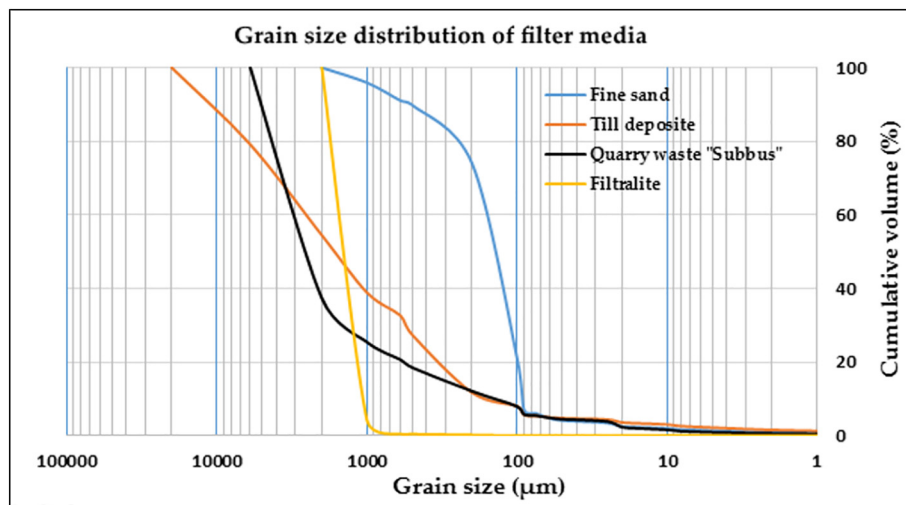


Fig. 2. Particle size distribution plot for different filter media.

Table 1
Particle size distribution of filter materials.

Particle size distribution	Particle size (mm)			
	Filtralite	Quarry waste "subbus"	Till deposit	Fine sand
D ₉₀	1.90	5.42	13.30	0.50
D ₆₀	1.61	3.47	2.90	0.17
D ₅₀	1.48	2.82	1.75	0.15
D ₁₀	1.06	0.15	0.15	0.01
Coefficient of uniformity (Cu)	1.5	23.1	19.3	17.0

difference in filter media; however, there was no single infiltration system universally efficient to remove all pollutants.

3.4. Bacteria removal efficiency

In this study, the concentration of indicator bacteria in the raw greywater was exhibited high variability, and the average concentration of total coliforms and *E. coli* in the untreated greywater were $5.4 \times 10^6 (\pm 2.8 \times 10^6)$ and $1.0 \times 10^6 (\pm 9.2 \times 10^6)$ MPN/100 ml respectively. The on-site greywater treatment plant (biofilter system) reduced total coliforms and *E. coli* to $3.8 \times 10^5 (\pm 5.8 \times 10^5)$ and

$9.2 \times 10^4 (\pm 1.4 \times 10^5)$ MPN/100 ml respectively. The effluent from on-site greywater treatment system was pumped into columns, and the effluent from each column was collected and analysed on a daily basis for four to five days during the sampling periods, T1 (March 2016), T2 (April 2016), T3 (June 2016), T4 (August 2016), and T5 (February 2017). The concentration of total coliforms and *E. coli* in the effluent were monitored and compared with the concentration in the influent to determine the removal efficiency of the columns (Fig. 4).

It is difficult to differentiate the mechanisms responsible for the microbial reduction in this setting, but it could be the combination of die-off straining and sorption. Also, the process of bacterivory (consumption of bacteria by bacterivores) would be another possible mechanism for bacterial removal. Regardless of the mechanisms, the average log₁₀ reduction of *E. coli* ranges from 2.6 to 3.0 in period 1 (T1) and *E. coli* removal efficiency of all columns improved through time and reached from 2.9 to 3.4 log₁₀ reduction in the fourth period (T4), however, the reduction decline to 0.4 to 3.0 at period 5 (T5). Considerable improvement in total coliform and *E. coli* removal efficiency of columns were noted until the fourth period, but after one year, the removal efficiency decline, specifically significant removal efficiency decline was observed in column-A and column-C. It could be explained by biofilm dispersion, which can happen due to stress, conditions like alteration in nutrient availability, oxygen fluctuations, an increase in toxic products (Kostakioti et al., 2013; Rowe et al., 2010;

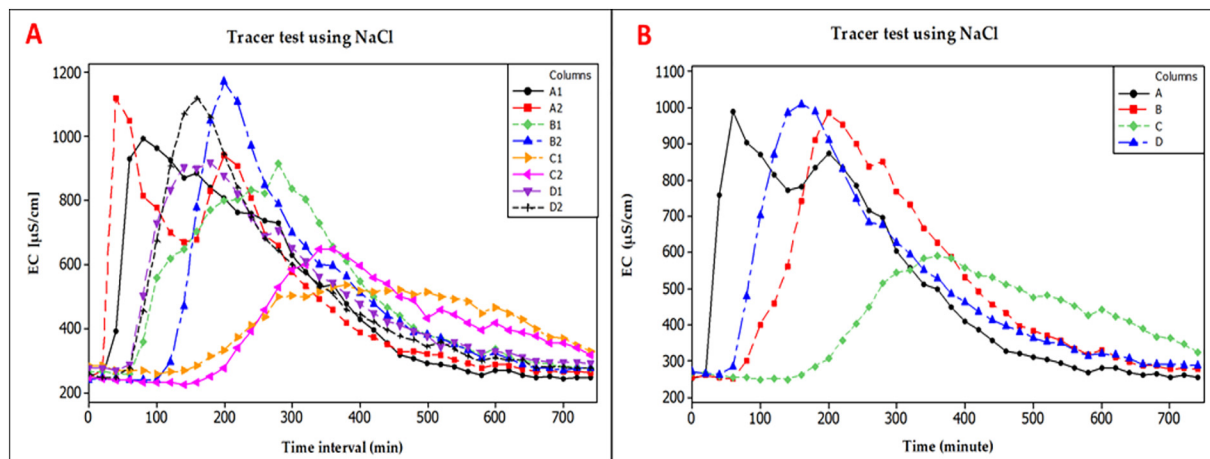


Fig. 3. Breakthrough curve for NaCl tracer in columns with different stratified filter media. A) For each replicate column. B) The average value for the representative columns.

Table 2
Quantified water quality parameters in raw greywater, biofilter system effluent, and columns effluent ($n = 22$).

Water quality parameters	Unit	Statistical value	Raw greywater, biofilter system effluent, and columns effluent									
			Raw greywater	Biofilter system effluent	A1	A2	B1	B2	C1	C2	D1	D2
P_{total}	mg/l	Mean	0.95	0.62	0.25	0.23	0.13	0.12	0.17	0.17	0.21	0.20
		StDev	0.25	0.23	0.20	0.16	0.06	0.05	0.04	0.09	0.16	0.15
N_{total}	mg/l	Mean	10.4	6.22	4.14	4.06	4.81	4.89	4.06	4.04	4.18	4.02
		StDev	4.38	2.81	2.25	2.34	3.40	3.54	2.37	2.23	2.18	2.21
NO ₃ -N (nitrate)	mg/l	Mean	0.33	3.08	4.68	5.08	5.61	6.20	5.61	5.58	5.29	5.26
		StDev	0.03	0.55	0.61	0.63	0.38	0.46	0.27	0.09	0.37	0.30
COD	mg/l O ₂	Mean	274	77.8	25.0	19.3	10.7	11.3	23.5	20.2	11.5	12.0
		StDev	87.5	24.4	15.7	7.75	0.59	1.37	11.0	4.30	4.34	3.90
Suspended solid	mg/l	Mean	32.47	14.53	6.63	5.58	3.51	2.94	5.01	5.44	6.78	5.98
		StDev	29.55	9.62	14.03	9.45	4.74	2.86	2.91	6.63	14.19	12.14
Turbidity	FNU	Mean	40.3	20.7	5.26	4.86	5.20	6.60	15.4	14.2	5.69	4.21
		StDev	39.0	20.5	3.73	3.33	2.58	2.76	10.8	7.08	8.60	5.26
EC	µS/cm	Mean	305	319	343	339	324	317	291	287	361	361
		StDev	76.1	90.1	101	88.6	81.1	79.2	83.6	80.0	110	110
pH		Mean	7.04	7.15	7.50	7.55	7.39	7.36	6.95	7.05	7.78	7.83
		StDev	0.20	0.16	0.18	0.21	0.20	0.22	0.25	0.33	0.22	0.16

Sauer et al., 2004), however, to confirm the real causes, further in-depth study is required. Regarding the overall microbial removal efficiency of the columns, column-B (15 cm layer of each, Filtralite, fine sand, and till soil) showed the highest removal of total coliforms and *E. coli* [3–4 log reduction (99.9–99.99%)], whereas, the poorest removal observed in column-C (a layer of 25 cm crushed stone and 50 cm till soil) [2–3 log reduction (99–99.9%)]. Thus, the stratified layer of filtralite, fine sand, and till soil in the case of column-B that could favor the formation of biofilm and the layer could resist biofilm stress conditions that favour dispersion.

One-way analysis of variance for log reduction of total coliforms and *E. coli* revealed that at least one column was significantly different from the others ($p > 0.05$) and column-C1 is the most different one. Post hoc comparison using Tukey test indicates that total coliform and *E. coli* removal efficiency of column B and column C were significantly different.

3.5. Virus removal efficiency

The virus removal efficiency of columns was investigated under three conditions. 1) the removal efficiency of columns under normal circumstance, 2) the removal efficiency of columns when the virus is shedding simultaneously with rainfall, and 3) virus detachment from saturated filter media during the recession (declining trend) due to the effect of rainfall. To estimate the removal of virus in the infiltration system, St28B, were added into the column influent and the concentration in the effluent was analysed.

3.5.1. Virus removal efficiency under normal condition (without rainfall)

St28B was mixed with treated greywater and continuously fed into the columns for 15 h, and three samples with one-hour interval were taken from the columns effluent after 15 h to calculate the removal efficiency of virus from each column. These experiments were repeated two times, T1-April 2016, and T2-February 2017, with an average inlet concentration of 1.0×10^5 and 4.5×10^5 PFU/ml respectively. The average log₁₀ reduction of St28B during the two periods were 1.8, 1.4, 1.1, and 1.9 in the representative column-A, B, C, and D respectively (Fig. 4E and F). The initial (T1) virus removal efficiency of columns has been relatively higher with the variation from 1.4 to 2.4 log₁₀ reduction in the case of column-C2 and column-A2 respectively. While during the second period (T2), the removal efficiency of columns ranged from 0.56 to 1.94 log₁₀ reduction in the case of column-C2 and column-D1 respectively. The virus removal efficiency of all columns declined during the second period (T2), and the reduction of removal efficiency could be explained by the saturation of adsorption surface of filter media, due

to biofilm dispersion, or a combination of different factors. The infiltration system using quarry west “subbus” (column D), which was dominantly characterised by granite and amphibolite, have been relatively efficient in removing the virus. However, statistical analysis result showed that there was not a significant difference ($p < 0.05$) in the removal of a virus in the columns.

3.5.2. Attachment and detachment of St28B during and after rainfall

This experiment was conducted by mixing rainwater, and treated greywater with a 1:4 ratio, and added St28B into the mix. At the same time, the pumping rate was increased by 25% to compensate for the additional rainwater that can infiltrate in the column during the rainfall event. During this experiment, the EC and the pH measurement of the influent were 235 µS/cm and 7.02 respectively. Besides, the temperature in the effluent ranges from 14.4 to 14.8 °C in both cases of with and without rainfall experiment. Fig. 6 illustrated the St28B removal efficiencies of columns with and without rainfall condition. The change in the influent water due to the addition of rainwater and the higher loading rate resulted in lowering the St28B removal efficiency of the columns. The highest removal efficiency was 1.02 log₁₀ reduction in column-A, and the lowest reduction was 0.19 log₁₀ reduction in column-C. The overall St28B removal efficiency of column A, B, C and D reduced by 19.3%, 57.7%, 70.8%, and 40.4% respectively as compared with the removal efficiency without rainfall (Fig. 5).

Rainfall can adversely affect the performance of the treated greywater disposal system by placing an additional hydraulic load on the infiltration scheme, changing active-solid water interface and changing the water chemistry. The effect of rainfall on the detachment of St28B from the infiltration columns was an extension of the virus removal experiment. With the intention to investigate the tendency of virus saturated infiltration system whether retaining or detach virus particle during the rainfall event, virus spiked greywater feed into the column during virus removal experiment, then only grey water without virus was used to feed the columns after that rainwater (EC 23 µS/cm and pH 6.94) was introduced to the columns for 1 h to simulate the rainfall event. The concentration of virus particle in the effluent was monitored throughout the experiment to evaluate concentration change, and plotted with the time frame of the experiment on the same graph (Fig. 6).

The downturn curve in the case of column A1 has gentle slope due to clogging that resulted in delayed infiltration, whereas the recession curve of the other columns has a similar pattern. The concentration of St28B after the application of rainfall increased and it could be the sign of detachment enhanced during the application of low ionic strength rainwater. As the EC of the influent water reduced, the surface potential

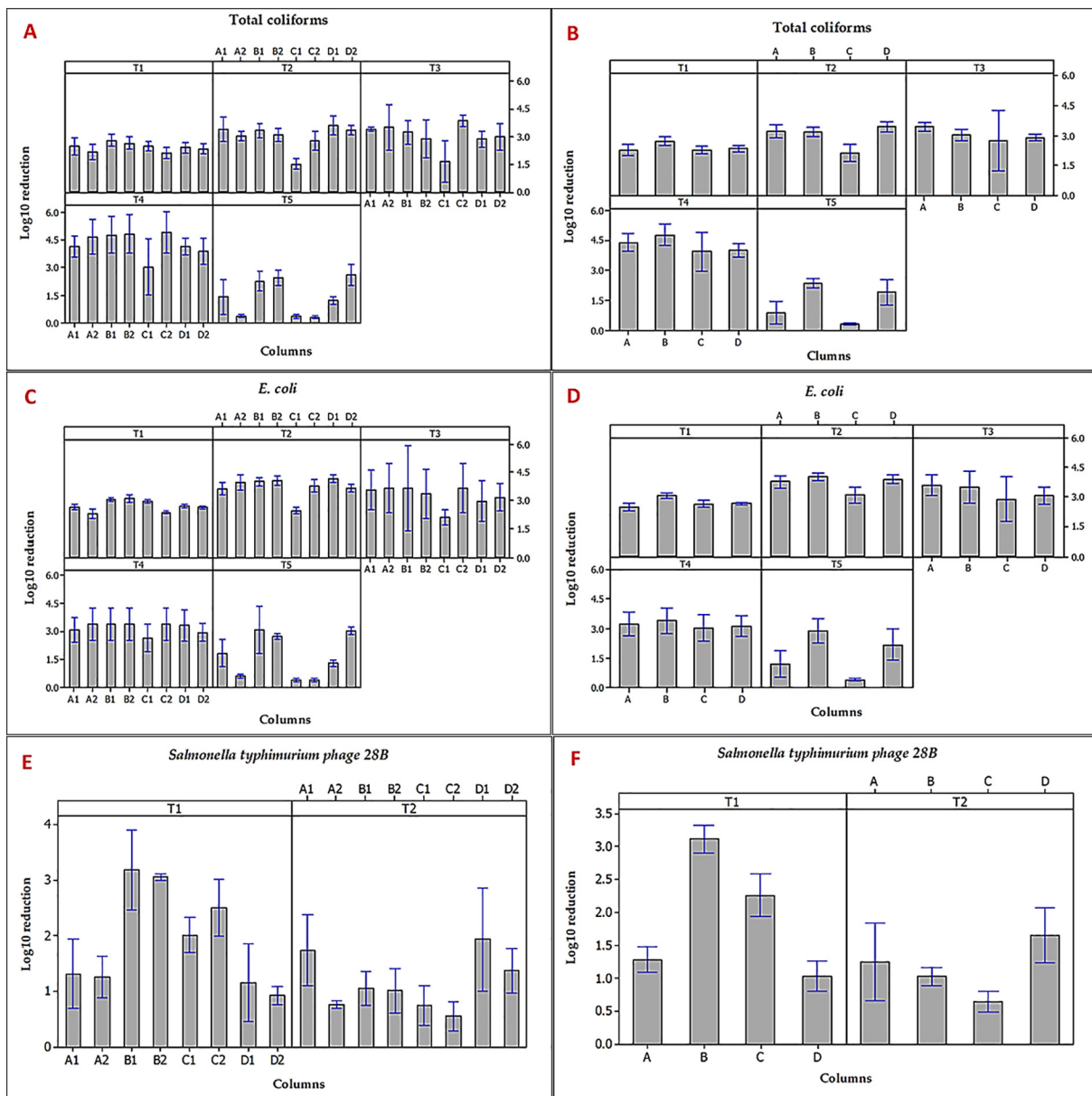


Fig. 4. Mean \log_{10} reduction A) total coliforms in replicate columns, B) total coliforms in representative columns, C) *E. coli* in replicate columns, D) *E. coli* in representative columns, during monitoring period T1, T2, T3, T4 and T5, and E) *Salmonella typhimurium* phage 28B in replicate columns, F) *Salmonella typhimurium* phage 28B in representative columns during monitoring period T1 and T2. (Error bar represent 95% confidence interval for the mean).

of the adsorbed St28B most likely increased due to the expansion of electrostatic double layer surrounding the virus particle and collector surface, leading to an increase in virus detachment and then the detached virus particles re-enter into the infiltrated water and leached out during rainfall event (Penrod et al., 1996; Quanrud et al., 2003).

4. Conclusion

The construction of an on-site treated greywater disposal system from selected filter media with different stratification operate as a post-treatment step and significantly reduced microbial pathogens, nutrients, organic load and also brought a degree of denitrification. To estimate the removal of microbial pathogens and nutrients in unsaturated and saturated flow conditions, necessary to conduct the experiment in the appropriate design, scale, and test the performance of different filter media with different stratifications. This study intends to address this issue by considering the Norwegian guideline for the

construction of treated wastewater disposal system. Hence, such quantitative information enabled us to identify more efficient treated greywater disposal system and contribute to the computation of the safest setback distance between greywater disposal system and drinking water sources.

The evidence from this study indicated that on-site treated greywater effluent received significant treatment after infiltrating through different unsaturated infiltration systems. Indicator bacteria removal efficiency of all infiltration systems improved through time; however, the efficiency suddenly reduced after one-year, and the causes for the poor performance of the infiltration systems will need more investigation in the future. Also, the removal of total coliforms and *E. coli* in all infiltration system was relatively higher as compared to St28B.

The rainfall experiment result demonstrated that St28B removal efficiency of the infiltration systems significantly reduced when virus shedding simultaneously with the rainfall event and the magnitude of efficiency reduction ranges from 19% to 70% depending on the

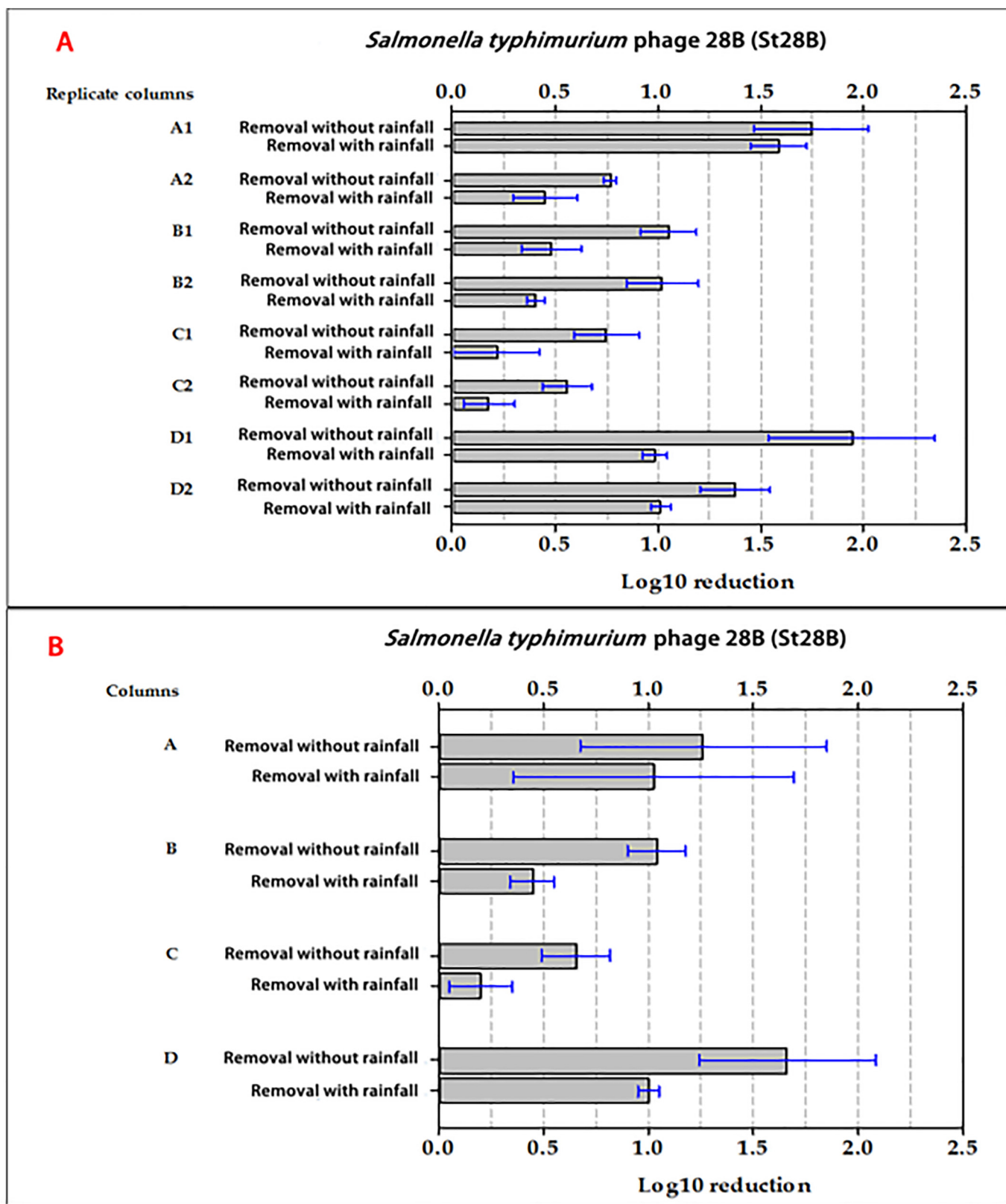


Fig. 5. Variations of St28B removal efficiency when shedding simultaneously with rainfall and without rainfall in A) replicate columns, and B) representative columns.

infiltration system. For the range of infiltration system tested, column with 30 cm filtralite at the top and 50 cm quarry waste “subbus” at the bottom (Column-D), and filtralite - fine sand - till soil stratified filtration system (Column-B) provided comparably better treatment

performance with respect to total coliforms, *E. coli*, St28B, nutrients and organic load removal efficiency without clogging problem within the experimental period.

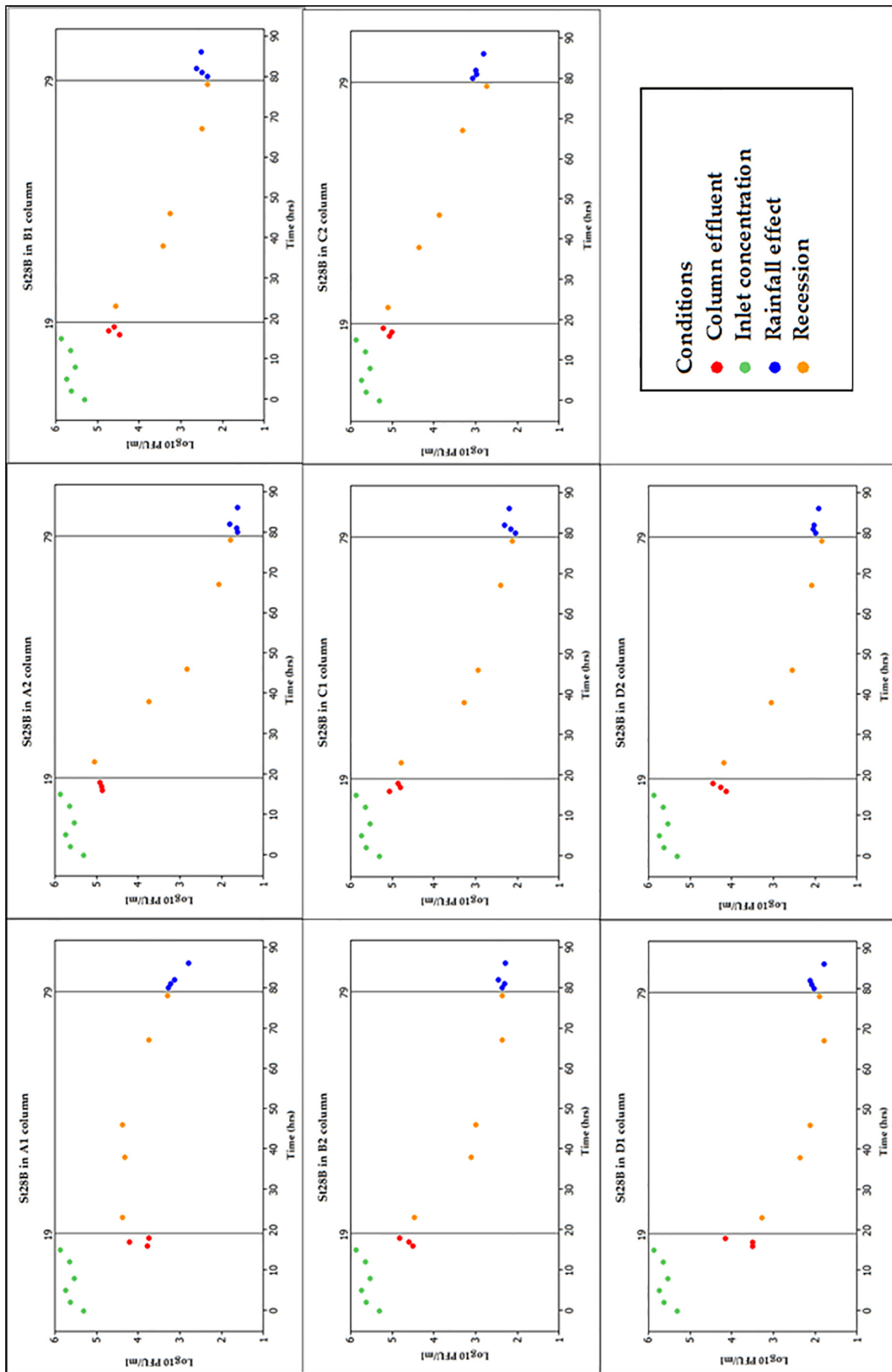


Fig. 6. St28B concentration at the inlet of the columns for 15 h (green dots), St28B concentration in the columns effluent after saturation (red dots), St28B concentration in the columns effluent at 79th hour (blue dots). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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