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Discharge of indicator bacteria from on-site wastewater treatment systems

Inga Herrmann*, Brenda Vidal, Annelie Hedström

Department of Civil, Environmental and Natural Resources Engineering, Luleå University of Technology, Laboratorievägen 14, 97 187 Luleå, Sweden, Tel. +46920492528; Fax: +46920491399; emails: Inga.Herrmann@ltu.se (I. Herrmann), Brenda.Vidal@ltu.se (B. Vidal), Annelie.Hedstrom@ltu.se (A. Hedström)

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ABSTRACT

Small-scale on-site wastewater treatment facilities present the risk of microbial pollution of groundwater used for drinking water and surface water used for recreational purposes. This study assessed the discharge of indicator bacteria, total coliform, *Escherichia coli*, intestinal enterococci and *Clostridium perfringens*, by flow-proportional sampling from 12 full-scale on-site treatment systems featuring biological treatment units (mainly sand filters) and alkaline filter beds for phosphorus treatment (P-filters). Correlations of effluent bacterial concentrations with pH, total and dissolved organic carbon, filter age and hydraulic load were evaluated. The bacterial concentrations in the effluents of the sand filters were considerable. The concentrations for excellent bathing water quality set in the EU bathing water directive, 200 and 500 colony forming units (cfu)/100 mL for intestinal enterococci and *E. coli*, respectively, were exceeded in three (intestinal enterococci) and one (*E. coli*) of the eight investigated sand filters. In one of the sand filters, effluent *E. coli* concentrations were high although no obvious malfunction of the filter was observed. In the effluent from the other investigated biological treatment units (a trickling fibre filter, two units with attached growth treatment and one aerated activated sludge technique), bacterial concentrations were very high (75,000 cfu/100 mL of *Clostridium perfringens* and 85,000 cfu per 100 mL of total coliform), possibly because of a shorter retention time of the wastewater in these facilities, missing aeration and little time between start-up and measurements. Three and four of the nine investigated P-filters exceeded excellent bathing water quality in coastal waters as stipulated by the EU bathing water directive in respect of *E. coli* and intestinal enterococci, respectively.

Keywords: Sand filter; Alkaline filter; Polonite; Total organic carbon; pH

1. Introduction

In Sweden, there are large areas of land that are sparsely populated, where about 11% of households are not connected to municipal sewerage [1]. On-site wastewater treatment is, therefore, widely used, potentially causing hygiene problems. Previous studies in different countries have shown that microbiological contaminants from on-site wastewater treatment impact the groundwater [2,3] and are a possible reason for elevated bacterial levels in streams [4–7]. Therefore, the discharge of pathogens from Swedish on-site wastewater treatment facilities is potentially problematic, because of the risk of contaminating the groundwater used for drinking water in rural areas and the surface water used for recreational purposes, especially in areas with summer houses.

On-site wastewater treatment facilities in Sweden typically consist of a primary treatment in a septic tank as well as a secondary treatment, achieved with soil-based systems such as sand filters or in mini-package treatment plants. To prevent eutrophication of the receiving water, the reduction requirements for phosphorus (P) are between 70% and 90%,

* Corresponding author.

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depending on the protection status of the area in question [8]. To comply with these regulations, many rural on-site facilities have recently been upgraded with a tertiary treatment unit, in the form of a filter bed that traps P (P-filter). The effluent from these facilities is either discharged to soil (infiltration) or, in many cases, directly discharged to receiving water bodies such as ditches, lakes, the sea or groundwater.

In previous pilot studies, the removal of bacteria from wastewater using sand filters was shown to be high, for example, in gravel-filled unplanted constructed wetlands [9] and sand filters treating primary effluent [10]. However, the treatment efficiency has been shown to depend on the filters' age [11] and effluent characteristics [12]. Bacterial discharge from full-scale sand filters has not yet been investigated. However, many on-site wastewater treatment systems, such as drain fields, distribute the wastewater to the ground for secondary treatment (instead of using constructed sand filters) and a number of studies have, therefore, dealt with bacterial reduction in soil [13–15]. Bacterial reduction in mini-package plants has rarely been studied. Results from grab sampling presented in a Swedish study indicated differing bacterial reduction in three mini-package plants (not including the ones investigated in this study) [16].

P-filter media and P-filters have been extensively studied in laboratory-scale tests and pilot tests [17,18], but there have been only a few studies dealing with the reduction of microbiological contaminants. Many filter materials used in P-filters are alkaline, thus increasing the wastewater's pH so may therefore also help to decrease the bacterial content of the effluent. A pilot experiment by Nilsson et al. [19] showed the pH in the filter media Polonite to range from 9 to 12.3 and indicated a reduction of enterococci ranging from 52% to 91%. In a study with a pilot-scale P-filter using the filter media Filtra P, high log removal rates of *Escherichia coli*, enterococci and clostridia were measured [10]. Grab samples from full-scale P-filters using the filter media Filtralite P® indicated very low effluent bacterial concentrations [20]. Another Filtralite P® system completely removed bacteria during the first 3 years of operation, but removal efficiency decreased after the system's design capacity was exceeded [21].

In summary, the discharge of pathogens from on-site wastewater treatment facilities has not been comprehensively studied. In particular, facilities that discharge directly into receiving water bodies (instead of infiltration into soil), such as sand filters and mini-package plants, have been overlooked. Microbiological contaminant removal in sand filters has only been investigated at pilot-scale using grab sampling. The full-scale studies available focus on bacterial removal in soils. Bacterial removal by the P-filter media Polonite, which is widely used in Sweden, has not been investigated at full-scale. Therefore, the aim of this study was to investigate the discharge of indicator bacteria from full-scale on-site biological treatment units (mainly sand filter beds), and alkaline P-filters (mainly Polonite filters) using flow-proportional sampling. Knowledge about the discharge of indicator bacteria might help assessment of the potential risk of microbial pollution of natural water bodies from on-site wastewater treatment.

2. Materials and methods

2.1. Identification of on-site treatment facilities

In co-operation with seven Swedish municipalities, municipal databases were searched for on-site wastewater treatment facilities that used sand filters and/or P-filters. The operators of these facilities were contacted by telephone to obtain their approval. Thirty-four facilities were inspected for their suitability to the sampling intended to be carried out in this study. During inspection, the sand filter outlets were checked to ensure they were designed in a way that would enable flow-proportional sampling, that is, manual flow measurements during sampling. For P-filters, inlets and outlets were both checked for their accessibility for sampling and whether at least one of them was suitable for measuring the flow.

2.2. Investigated on-site treatment facilities

A description of the on-site wastewater treatment facilities investigated is given in Table 1. Eight sand filters, four other biological treatment units, namely a trickling filter with biological fibre material in a tank, two biofilm units without aeration and one aerated activated sludge unit, and nine P-filters were studied.

The sand filter beds that were constructed in accordance with the Swedish standard design (sand filters A and E) had an 80 cm thick layer of filter media (sand/gravel with particle sizes from 0 to 8 mm). A design load of 30–60 L m^{-2} d^{-1} is common in Sweden [22] and a sand filter for one household usually has a surface area of 25 m^2. The wastewater was spread over the filter through slotted pipes and collected in drainage pipes at the bottom of the filter. Both distribution and drainage pipes were embedded in a 30 cm thick gravel layer. Sand filters F–H deviated from the standard by having a layer of drainage baskets (biomodules) with triangular cross sections (width ca. 0.5 m) on the top (housing the distribution pipe); the sand layer was 60 cm and the top gravel layer 10 cm. Sand filters B and C were smaller and had a thinner sand layer but were improved with plastic crates (biomodules) increasing the hydraulic capacity, allowing the filter bed to be smaller compared with a bed built in accordance with the standard. All sand filters were covered with a 30 cm thick layer of soil.

In facilities I–L, mini-package plants were used instead of sand filters, followed by a P-filter. In facility I, the wastewater was biologically treated in a trickling filter made of fibre material (4evergreen by Biorock®). In facilities J–K, a Biop® unit with attached growth treatment was installed, however, there was no aeration. In facility L, an aerated activated sludge technique was used.

Among the nine investigated P-filters (Table 1), eight were bags filled with the filter material Polonite (supplier: Ecofiltration AB, Sweden) which were placed in a pit and operated in down-flow mode (D) or up-flow mode (F – L). The filter material Polonite provided by the company Ecofiltration Nordic AB is frequently used in P-filters installed in Swedish on-site wastewater treatment facilities. The material has grain sizes between 0.5 and 8 mm [23] and is produced by heating opoka rock [24]. In a pilot-scale test, pHs up to 12.3 have been measured [19]. P-filter E was a tank with two chambers, filled with the filter material Filtra P (supplier: Wavin-Labko Ltd., Tampere, Finland), where the water percolated downwards through the filter media in the

Table 1
Properties of the investigated on-site wastewater treatment facilities

Treatment facility	Biological treatment (time of start-up)	P-filter (time of start-up)	No. of users	Frequency of use
A	Sand filter, Swedish standard design (2009)	–	3	Year-round
B	Sand filter with biomodules (2013)	–	2	Year-round
C	Sand filter with biomodules (2015)	–	5	Year-round
D	Sand filter, Swedish standard design (2014)	Bag with Polonite, down-flow (2014)	2	Year-round
E	Swedish standard design (2009)	Wavin-Labko tank with Filtra P (2009)	5	Year-round
F	Swedish standard design, modified (Oct 2010)	Bag with Polonite, up-flow (May 2015)	2	Summer only
G	Swedish standard design, modified (2012)	Bag with Polonite, up-flow (2015)	2	Summer only
H	Swedish standard design, modified (Nov 2015)	Bag with Polonite, up-flow (spring 2016)	2	Year-round
I	Biological fibre material in a tank (2012)	Bag with Polonite, up-flow (2014)	2	Ca. 6 months/year
J	Biop®, biofilm treatment without aeration (2008)	Bag with Polonite, up-flow (June 2016)	14	Year-round
K	Biop®, biofilm treatment without aeration (2008)	Bag with Polonite, up-flow (June 2016)	14	Year-round
L	Activated sludge with aeration (June 2016)	Bag with Polonite, up-flow (June 2016)	2	Year-round

first chamber and upwards in the second chamber. In Filtra P, pH values up to 12.7 have been measured [25].

2.3. Indicator bacteria

Pathogenic organisms were not expected to be present in the investigated on-site wastewater treatment facilities due to the small number of users (often only two, Table 1). Therefore, indicator organisms were used as a surrogate. Four groups of indicator bacteria were investigated in this study: total coliforms, *E. coli*, intestinal enterococci and *Clostridium perfringens*. Each of these indicator organisms indicates faecal contamination and is used in establishing performance criteria for drinking water, freshwater and saltwater recreation [26].

2.4. Sampling and analyses

Twelve on-site wastewater treatment facilities (A–L, Table 1) were sampled, each on at least three occasions during ca. 3–4 h at different times of the day. The number of sampling events and the times when samples were taken differed between the investigated facilities (Table 2), because after sampling at the first sites chosen had started, the inspection of new facilities continued. At each sampling event, two samples were taken from the third chamber of the septic tank or from the distribution/pumping pit from where the wastewater was transferred to the biological treatment, from the outlet of the biological treatment unit and from the outlet of the P-filter. During sampling, the flow was measured manually either at the outlet of the sand filters, or the outlet or inlet of the P-filters by capturing the influent/effluent in a measuring container and recording the time taken to fill it. Samples from the outlet of the biological treatment units and P-filters were taken, proportional to the measured flow, generating two composite samples at each sampling event. Septic tank samples were taken, using grab samples, at the beginning and end of each sampling event.

Total suspended solids (TSS, not measured at all events), temperature and pH were measured in the samples in situ. TSS was determined using the European standard EN 872:2005 [27]. The pH was measured using a WTW pH330 pH meter with a WTW SenTix41 pH electrode.

Bacterial samples were stored in cooling bags and transferred to the laboratory for analysis directly after the sampling was completed. In some cases, when samples were taken in the late evening, they were transferred to the laboratory the following day. Bacterial analyses were carried out at two laboratories using the Swedish standard methods SS 028167-2 (modified) for *E. coli* and total coliforms, SS-EN ISO 7899-2 for intestinal enterococci and ISO/CD 14189/6461-2 for *C. perfringens*.

Samples for analysis of total and dissolved organic carbon (TOC and DOC) were frozen and stored for later analysis. Samples for analysis of DOC were filtered through 0.45 μm filters in situ (before freezing). TOC and DOC were analysed using IR detection (based on CSN EN 1484, CSN EN 16192, SM 5310).

2.5. Statistical analyses

The measured bacterial concentrations were weighted for the flow, that is, flow-weighted geometric means $\bar{x}^*$ were calculated:

$$\bar{x}_g^* = \left(\prod_{i=1}^{n} x_i^{w_i}\right)^{\frac{1}{\sum_{i=1}^{n} w_i}} \quad (1)$$

Table 2
Number of sampling events, bacterial samples taken from the biological treatment units and P-filters and the time when sampling was carried out

Facility	No. of sampling events	Time of sampling	Total duration of sampling (h)	Mean flow (L h^{-1})
A	7	Sept 2015–June 2016	25	47.8
B	3	May–June 2016	13	3.2
C	3	May–June 2016	9	10.5
D	6	Sept 2015–May 2016	17	9.5
E	6	Sept 2015–May 2016	17	15.7
F	3	Aug 2016	7	6.6
G	3	Aug 2016	8	17
H	4	Aug 2016	9	5.3
I	3	Aug 2016	8	68.1
J	3	Aug–Sept 2016	10	39.9
K	3	Aug–Sept 2016	11	29.4
L	3	Aug–Sept 2016	8	47.6

where $\bar{x}_g^*$ = flow-weighted geometric mean; n = number of observations; w_i = volume of wastewater making up the composite sample.

Flow-weighted arithmetic means $\bar{x}^*$ were calculated for TOC, DOC and TSS as well as the H^+ ion activity (pH):

$$\bar{x}^* = \frac{\sum_{i=1}^{n} x_i w_i}{\sum_{i=1}^{n} w_i} \quad (2)$$

where $\bar{x}^*$ = flow-weighted arithmetic mean; n = number of observations; w_i = volume of wastewater making up the composite sample.

To determine whether there was a difference between influent and effluent bacterial concentration, a t-test was carried out. Pearson correlations of the measured parameters were calculated using the statistical software Minitab [28].

3. Results and discussions

3.1. Identification of on-site wastewater treatment facilities

The Swedish Agency for Marine and Water Management recently suggested the initiation of regular checks (including sampling) of the function and regulatory compliance of on-site wastewater treatment facilities by competent authorities [29]. In this study, however, only 12 of 34 inspected facilities could be sampled, thus meaning about 65% of facilities were not suitable for sampling. The main reasons were that there was no flow in the outlet pipe or the outlet pipe was not accessible. Furthermore, contacting the operators (private property owners) was time consuming and the long distances between the facilities made visiting impractical. These experiences show that monitoring of installed on-site treatment systems would be challenging.

3.2. Discharge of bacteria from sand filters

The bacterial concentrations in the effluents of the sand filters (facilities A–H) were, in some cases, considerable (Fig. 1). For example, in the effluent of sand filter B, concentrations of intestinal enterococci were as high as >100,000 cfu/100 mL and in the effluent of sand filter H, average *E. coli* concentrations were 1,192 cfu/100 mL (Fig. 1). These concentrations were many times higher than those in a study by Kauppinen et al. [10] who investigated a pilot-scale sand filter similar to the Swedish standard design at temperatures between 0°C and 15°C, and found effluent concentrations of *E. coli* and intestinal enterococci to be 180 and 4 cfu/100 mL, respectively. The concentrations set for excellent bathing water quality are 200 and 100 cfu/100 mL for intestinal enterococci and 500 and 250 cfu/100 mL for *E. coli* in inland and coastal waters, respectively [30]. The geometric mean concentration of intestinal enterococci of three of the investigated sand filters exceeded the concentration set for inland waters (Fig. 1). For *E. coli*, this was the case in one sand filter (H). For irrigation of food crops, coliform indicators should be below 10^3 cfu/100 mL [31]. Two of the studied sand filters (B and H) exceeded this value in respect of total coliform and one (H) in respect of *E. coli*.

The mean bacterial concentrations in the outlets of the sand filters differed considerably between facilities (Fig. 1). There are several possible reasons for this. Rolland et al. [32] found that the level of compaction of the sand affected the treatment efficiency of sand filters. The particle size distribution of the coarse sand that the authors investigated was in the same range as recommended for sand filters in Swedish guidelines. It is possible that the sand filters with elevated bacterial outlet concentrations were not well compacted during construction. As bacteria adsorption is an important removal mechanism in sand filters which depends on the adsorption capacity of the sand [33], the observed differences in removal efficiency could also be due to different properties of the sand used in the investigated filters. Filter age has been shown to affect the number of bacteria present in the filter [34] and can, therefore, potentially affect pathogen treatment efficiency. Seeger et al. [11] observed more efficient bacterial reduction in a sand filter after 1.5 years, probably due to the development of a microbial community in the top layer of the filter (schmutzdecke) that needs time to develop. In this study, however, no correlation between filter age and removal of indicator bacteria was found, possibly because

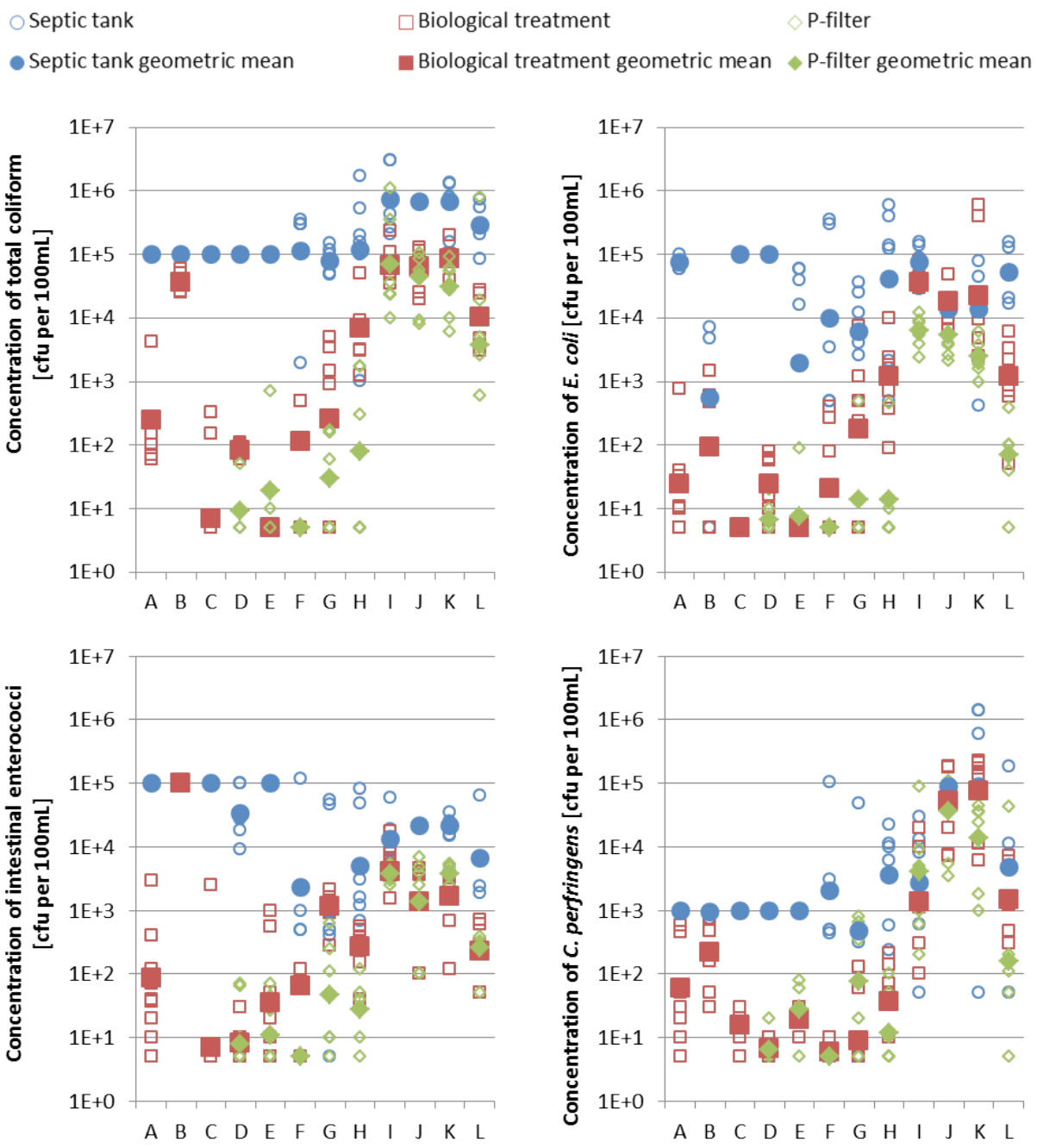

Fig. 1. Measured concentrations (hollow markers) and flow-weighted geometric mean concentrations (filled markers) of total coliform (upper left), *E. coli* (upper right), intestinal enterococci (lower left) and *C. perfringens* (lower right) in the outlets of the septic tanks, biological treatment units and P-filters of the investigated facilities.

most investigated sand filters were at an age (Table 1) where the schmutzdecke was already fully developed and other factors were predominant. For example, sand filter B was only 3 years old but performed poorly both in respect of bacteria (Fig. 1) and biological parameters (Table 3), probably due to clogging as the effluent was dark in colour with a strong smell and also because the outflow from this filter was very small (Table 2). Clogging can be caused by, among other factors, filamentous particles originating from toilet paper [35] and quickly increases when ponding of water does not disappear between feed batches [36].

While the effluent from sand filter B was dark in colour with a strong smell, no such observations were made at sand filter H where the effluent was clear and effluent TOC concentrations were at an acceptable level (Table 3), despite high effluent bacterial concentrations (Fig. 1). This shows that bacterial concentrations can be high even though there is no obvious malfunction of the sand filters observed. Recently

Table 3
Flow-weighted arithmetic means of pH, TOC and DOC measured in the effluents of the biological treatment units and P-filters

Facility	Weighted mean pH		Weighted mean TOC		Weighted mean DOC	
	Biological treatment units	P-filters	Biological treatment units	P-filters	Biological treatment units	P-filters
	[ ]	[ ]	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}
A	4.5		9.7		9.9	
B	6.9		160		131	
C	4.8		9.3		7.6	
D	6.2	9.4	8.2	8.6	8.9	8.8
E	4.2	6.0	13.2	11.9	12.4	12.2
F	7.4	12.1	19.8	12.7	22.8	11.2
G	7.2	10.5	12.0	11.0	13.3	10.2
H	6.8	9.9	20.1	14.6	18.5	13.9
I	7.2	9.6	39.8	31.3	29.9	25.4
J	7.3	8.6	25.3	22.4	17.6	17.4
K	7.5	8.8	22.0	19.0	29.3	17.1
L	8.0	9.7	29.3	26.6	25.6	22.9

proposed new legislation for on-site wastewater treatment suggests regular check-ups of on-site facilities [29], however, but does not give details on how these check-ups are to be carried out. The results of the current study suggest that visual inspection will not be sufficient to assess the function of a facility in terms of microbiological contaminant discharge.

In sand filters, bacteria are retained by adsorption, physical straining and biological action. Bacterial retention has been shown to depend on several factors [37], inter alia, the organic matter content, pH, temperature, concentration of bacteria and hydraulic loading. Average TOC, DOC and pH measured in the sand filter effluents are shown in Table 3. The concentrations of total coliform, *C. perfringens* and intestinal enterococci (but not *E. coli*) measured in the effluents of the sand filters were strongly positively correlated with the concentrations of TOC and DOC in the effluents, which was indicated by large Pearson correlation values of ≥0.96 ($p = 0.000$). With a high concentration of organic carbon, less bacteria were retained in the filters, possibly because of competition for adsorption sites as was suggested by Sélas et al. [12]. Furthermore, high effluent concentrations of TOC and DOC indicate low degradation of organic matter in the filter which may have resulted in low bacterial removal.

The pH, however, was not an important effluent parameter governing the bacterial concentration in the outlets of the sand filters, as these did not significantly correlate with the measured pH (Pearson correlations insignificant). Similarly, there were no significant correlations between bacterial concentrations and outlet TSS concentrations.

3.3. Discharge of bacteria from other biological treatment units

In the effluent of the biological treatment units other than sand filters (facilities I–L), bacterial concentrations were very high (Fig. 1). For example, at facility K, *C. perfringens* and total coliform concentrations of 75,000 and 85,000 cfu/100 mL, respectively, were observed. At facility I, the Biorock® fibre filter was much smaller (1.15 × 1.15 m) compared with the investigated sand filters that commonly had a surface area of 25 m^2, suggesting a considerably shorter retention time of the wastewater in the filter which could be the reason for the high bacterial concentrations in the effluent. The Biop® attached growth treatment used at facility J and K was not aerated as intended by the manufacturer. Therefore, the treatment was probably insufficient with regard to bacteria (Fig. 1). No malfunction of the activated sludge unit at facility L was visually observed, however, the facility was recently put into operation (around 2 months before sampling) and so it is possible that the activated sludge was not fully developed when the samples were taken.

The effluent concentrations of intestinal enterococci significantly positively correlated with the effluent concentrations of TSS (Pearson correlation value = 0.98, $p = 0.02$), indicating that these bacteria were attached to particles. Probably, the mini-package plants did not remove TSS as efficiently as the sand filters, thus discharging larger numbers of bacteria.

3.4. Discharge of bacteria from P-filters

The concentrations of indicator bacteria in the outlets of the P-filters (facilities D–L) differed considerably between the filters and were in some cases high, especially in the P-filters at facilities I–L (Fig. 1). Three and four of the nine investigated P-filters exceeded excellent bathing water quality in coastal waters as stipulated by the EU bathing water directive [30] with regard to *E. coli* and intestinal enterococci, respectively. P-filter effluent bacterial concentrations (not *C. perfringens*) were dependent on the flow observed through the filters (positive significant Pearson correlations of 0.82 [$p = 0.007$] for total coliform, 0.77 [$p = 0.02$] for *E. coli* and 0.68 [$p = 0.045$] for intestinal enterococci). For example, the flow through P-filters I–L was higher compared with most of the other P-filters (Table 2) and the effluent bacterial concentrations had the same trend (Fig. 1). This shows the importance of the flow conditions and residence time of the wastewater in the P-filters for bacterial reduction, as shown by Sélas et al. [12].

As with the sand filters, P-filter effluent concentrations of total coliform and *E. coli* correlated positively with effluent TOC concentrations, which were higher in the effluents of P-filters I and L than in the other P-filter effluents (Pearson correlations of 0.77 (p = 0.01) for total coliform and 0.73 (p = 0.03) for *E. coli*). Furthermore, with increasing influent bacterial concentrations, effluent bacterial concentrations increased (Pearson correlations between inlet and outlet concentrations were 0.78 (p = 0.014) for *C. perfringens*, 0.87 (p = 0.002) for total coliform, 0.93 (p = 0.000) for *E. coli* and 0.85 (p = 0.004) for intestinal enterococci) while log removal rates (Fig. 2) were not affected.

Log removal of bacteria varied between bacteria type and P-filters, with negative removal observed in several cases (Fig. 2). Due to the high data variability, paired t-tests showed no significant (α = 0.05) difference between the average influent and effluent concentrations of either bacteria type, measured at facilities D–L. This means that the collected data did not generally confirm a further reduction of the bacterial content of the wastewater in the P-filters, despite their high pH (Table 3) and despite a strong correlation between the log removal of total coliform and pH (Pearson correlation were 0.75, p = 0.02). Possibly, the pH in many of the P-filters was not high enough to support removal of bacteria other than coliforms. The overall ineffective reduction of bacteria in the P-filters could be due to the fact that the P-filters were flow-saturated, thus decreasing the attachment of bacteria to the filter particles, as suggested by Cooper et al. [38], who observed that increased moisture content likely reduced bacterial attachment to soil. However, P-filters D, F, H and J removed all four bacteria types (positive log removal, Fig. 2) indicating a potential of the P-filters to serve as a cleaning step not only for P but also for bacteria. Possibly, the number of P-filters investigated in this study was not large enough to prove their efficiency.

P-filter media need to be changed after a certain time in use because they become saturated with P. Although the necessary change intervals are uncertain, it is usually recommended to change the material after 2 years of use. In terms of bacterial reduction, the age of the filter was not observed to be a decisive factor as the outlet bacterial concentrations did not correlate with filter age, and only the log removal rates of *E. coli* correlated with filter age (Pearson correlation of –0.73, p = 0.03).

3.5. Seasonal differences in bacterial reduction

Facilities A, D and E were sampled during the cold season (autumn 2015) as well as in warmer weather (spring to autumn 2016). Below-zero air temperature during sampling was measured at facilities A and D. At facility A, the temperature was –1°C when sampling on 15th October 2015. At facility D, the temperature was –0.6°C during the evening sampling event on 13th October 2015. In the sand filter effluents of facilities A and D, the geometric mean concentration of *E. coli* was lower during the cold sampling events (9 and 8 cfu/100 mL at A and D, respectively) compared with the other sampling events (30 and 37 cfu/100 mL at A and D, respectively). This contradicts findings of previous studies where the adsorption of bacteria had been reported as reducing with decreasing temperature while the survival of *E. coli* increases down to 5°C [37]. However, as air temperatures during sampling were low, the temperatures of the water when samples were taken were, on average, 4.8°C and 4.9°C at sand filters A and D, respectively. Further cooling during outside storage in the cooling bag is possible. Thus, possibly, *E. coli* did not survive the handling of the samples indicating that this indicator bacteria type is unsuitable for assessing on-site wastewater systems at cold temperatures.

Differences in concentration of intestinal enterococci were not as distinct. The effluent concentration of intestinal enterococci of sand filter A was much lower at cold (<10 cfu/100 mL) compared with warmer temperatures (140 cfu/100 mL). For sand filter D, however, no such pronounced difference was observed (10 cfu/100 mL at the cold temperature sampling event compared with 8 cfu/100 mL during all other events).

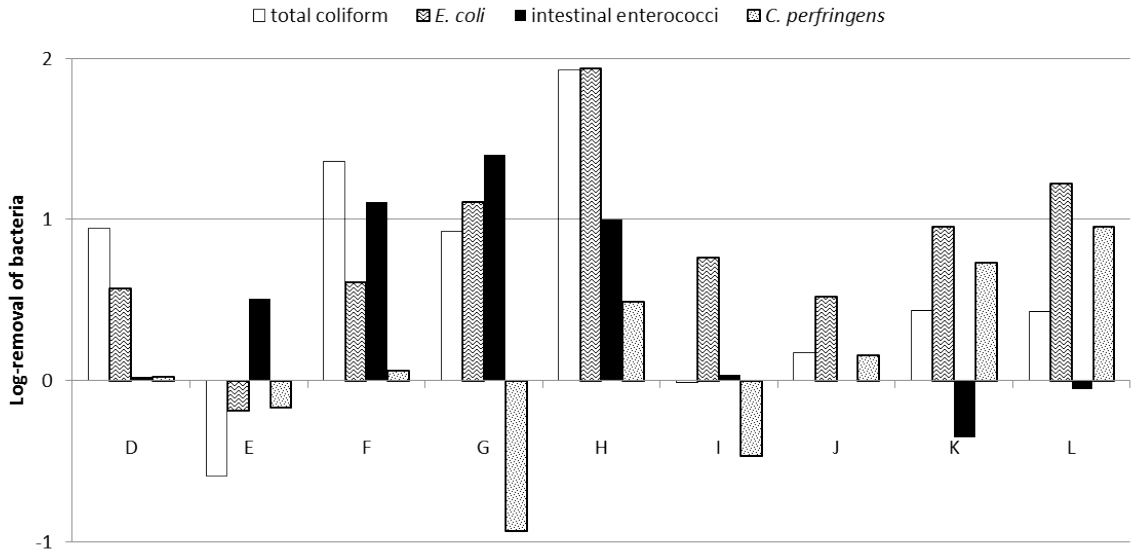

Fig. 2. Log removal of indicator bacteria in the investigated P-filters (D–L).

3.6. Risk of pathogen discharge from on-site wastewater systems

In this study, only facilities with an above-ground outlet were investigated. However, at many facilities, the effluent is infiltrated into the ground (below-ground outlet) with the potential risk of contaminating the groundwater. In Sweden, about 1,200,000 people use their own private wells for drinking water supply [22], underlining the importance of clean groundwater in areas with on-site wastewater treatment. Although soil has been found to reduce microbiological contaminants effectively [14,15], also in cold climates [13], contamination of drinking water wells [39] and the groundwater [2,40,41] has been reported as occurring. It has also been stressed that it is important that an effective reduction of bacteria is achieved in the unsaturated zone because, in saturated soil, bacteria are spread faster and over longer distances [42]. Therefore, the relatively high effluent concentrations of indicator bacteria observed in this study (Fig. 1) confirm the risk to the groundwater created by Swedish on-site wastewater treatment systems, especially if the vadose zone below the infiltration is not deep enough.

As many Swedish on-site facilities are located by lakes and watercourses used for recreational purposes, pathogen discharge (Fig. 1) is an important issue because it represents a risk to good bathing water quality as stipulated in the EU bathing water directive [30] or the US Recreational Water Quality Criteria, thus posing a risk to human health [43]. This problem has also been reported in other countries, such as Australia [44]. Health risks are especially great if the facilities directly discharge into small watercourses used for human activities where the effluent is hardly diluted and microbiological contamination can reach high levels. On the other hand, at times, cool temperatures in Sweden possibly contribute to a concentration decrease of certain bacteria after discharge.

4. Conclusions

The bacterial concentrations in the effluents of the sand filters were considerable. Three and one of the eight investigated sand filters exceeded the criteria set for excellent water quality by the EU bathing water directive with regard to intestinal enterococci and *E. coli*, respectively. In one sand filter, effluent *E. coli* concentrations were high although no obvious malfunction was observed. The effluent concentrations of total coliform, *C. perfringens* and intestinal enterococci (but not *E. coli*) measured in the effluents of the sand filters were strongly positively correlated with the effluent concentrations of TOC and DOC. However, effluent bacterial concentrations did not significantly correlate with pH and filter age. Unexpectedly, *E. coli* concentrations in the effluents of the sand filters decreased during a sampling event carried out at sub-zero temperatures, possibly due to sample handling. This indicates that *E. coli* is unsuitable for assessing on-site wastewater systems at cold temperatures.

In the effluents of the other investigated biological treatment units (a trickling fibre filter, two units with attached growth treatment and one aerated activated sludge technique), bacterial concentrations were very high, possibly due to reasons such as a shorter retention time of the wastewater in these facilities, missing aeration and little time between start-up and measurements.

The theory that P-filters used as a cleaning step after the biological treatment would further reduce the bacterial content of the treated water was not generally confirmed. Results from P-filters investigated in this study showed that, on average, the filters did not further reduce the bacterial content. However, data variability was high, making this result somewhat uncertain. Reduction in bacterial concentration was higher in P-filters with low hydraulic load as well as low effluent TOC.

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