



Faecal contamination of greywater and associated microbial risks

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Abstract

The faecal contamination of greywater in a local treatment system at Vibyåsen, north of Stockholm, Sweden was quantified using faecal indicator bacteria and chemical biomarkers. Bacterial indicator densities overestimated the faecal load by 100–1000-fold when compared to chemical biomarkers. Based on measured levels of coprostanol, the faecal load was estimated to be $0.04 \text{ g person}^{-1} \text{ day}^{-1}$. Prevalence of pathogens in the population and the faecal load were used to form the basis of a screening-level quantitative microbial risk assessment (QMRA) that was undertaken for rotavirus, *Salmonella typhimurium*, *Campylobacter jejuni*, *Giardia lamblia* and *Cryptosporidium parvum*. The different exposure scenarios simulated—direct contact, irrigation of sport fields and groundwater recharge—gave unacceptably high rotavirus risks ($0.04 < P_{\text{inf}} < 0.60$) despite a low faecal load. The poor reduction of somatic coliphages, which were used as a virus model, in the treatment was one main reason and additional treatment of the greywater is suggested. Somatic coliphages can under extreme circumstances replicate in the wastewater treatment system and thereby underestimate the virus reduction. An alternative QMRA method based on faecal enterococci densities estimated similar risks as for rotavirus. Growth conditions for *Salmonella* in greywater sediments were also investigated and risk modelling based on replication in the system increased the probability of infection from *Salmonella* 1000-fold, but it was still lower than the risk of a rotavirus infection.

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1. Introduction

The interest in the separation and reuse of different wastewater fractions (i.e. storm-, grey- and blackwater) has increased in recent years, largely due to economical, structural and ecological considerations [1,2]. Greywater, here defined as wastewater without input from toilets (i.e. wastewater from laundries, showers, bathtubs, hand basins and kitchen sinks) is often extensively

treated in the combined systems or separately in spread settlings. The later treatment often consists of a settling tank followed by a soil infiltration system, a sandfilter trench or a subsurface flow wetland providing a 0.7–3 log reduction of thermotolerant coliforms [3]. The high-grade treatment of greywater has been questioned since it constitutes a large fraction of the actual wastewater flow, but has a low degree of faecal contamination [4] and local systems are often ill adapted for reuse. Attention has been paid to the possibility of reusing greywater, especially in arid areas [5–7]; however, the potential risk with such reuse need to be systematically addressed. Greywater contains many chemicals used in households of which some may lead to soil degradation. Christova-Boal et al. [8] have

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addressed the risks of a pH-rise and zinc accumulation in the soil as well as excessive phosphorus leakage to groundwater in sandy soils. Furthermore, a risk of increased transmission of hazardous microorganisms may also occur depending on the faecal content and contamination of the greywater.

Since toilet waste is not included in greywater, faecal contamination should be minimal. Some activities, such as washing faecally contaminated laundry (i.e. diapers), childcare and showering may add minor amounts. Occasionally gastro-intestinal bacteria, such as *Salmonella* and *Campylobacter*, can be introduced by food-handling in the kitchen [9]. Greywater may have an elevated load of easily degraded organic material, which may favour growth of enteric bacteria such as faecal indicators and such growth in wastewater systems has been reported [10]. Hence, focus on bacterial indicator numbers will lead to an over-estimation of faecal loads and thus risk. Several studies have reported high numbers of traditional faecal indicators in greywater, that from “a regulatory point-of-view” would indicate substantial faecal contamination (Table 1).

If greywater should be reused, there is a need to differentiate between the actual faecal loads and potential regrowth of indicators if actual risks should be assessed.

Potential areas of greywater reuse are irrigation of golf courses and parks as well as fertilisation of crops [17], toilet flushing [18] and groundwater recharge [1]. The guidelines based on thermotolerant coliform-counts range from <1 to 10,000 colony forming units (c.f.u.) 100 mL⁻¹ depending on its designated use [19–21]. These guidelines result in a substantial treatment requirement of the greywater, which does not necessarily reflect the potential risks of faecal contamination.

A risk estimate approach has been discussed for the evaluation of reuse alternatives of wastewater with quantitative microbial risk assessment (QMRA) [22–24], where it is easier to measure or calculate the potential pathogen load than in greywater. Since indicator bacteria may overestimate the faecal load and thereby the risks of enteric pathogens, chemical biomarkers, such as coprostanol, may be used as an alternative faecal indicator also applicable to other systems [25]. Coprostanol is formed by the intestinal microflora from the cholesterol. Both substances are excreted with the faeces [26]. The risk models however need to take into account the potential regrowth of some pathogenic bacteria such as *Salmonella* and *Campylobacter*.

Human health risks are dependent on both the source of the pathogens, the treatment applied and the exposure routes. In the current study, a pond system is included as post treatment where direct human exposure to the water is possible. Since the water may be recirculated for irrigation purposes or used for groundwater recharge, these scenarios have also been considered in an evaluation of the microbial risks. The coliform indicator bacteria probably overestimate the risks due to their potential regrowth within the system. We have therefore chosen to make a comparative validation of the greywater system based on three different approaches: (1) evaluation of faecal load based on coprostanol concentrations and using epidemiological data to estimate the pathogen load, (2) based on faecal enterococci numbers and a dose–response model derived from bathing water exposure [27] and (3) based on faecal enterococci as an index organism for *Salmonella*. The overall aim of this study is to provide a broad view on microbial risks associated with reuse of source-separated greywater based on a screening level QMRA approach.

Table 1
Reported numbers of indicator bacteria in grey wastewater (log₁₀/100 mL)

Wastewater origin	Total coliforms	Thermotolerant coliforms	<i>E. coli</i>	Faecal enterococci	Reference
Bath, hand basin			4.4	1.0–5.4	[11]
Laundry	3.4–5.5	2.0–3.0		1.4–3.4	[8]
Shower, hand basin	2.7–7.4	2.2–3.5		1.9–3.4	[8]
Greywater	7.9	5.8		2.4	[5]
Shower, bath	1.8–3.9	0–3.7		0–4.8	[12]
Laundry, wash	1.9–5.9	1.0–4.2		1.5–3.9	[12]
Laundry, rinse	2.3–5.2	0–5.4		0–6.1	[12]
Greywater	7.2–8.8				[13]
Hand basin, kitchen sink		5.0		4.6	[2]
Greywater		5.2–7.0	3.2–5.1		[14]
Greywater, 79% shower	7.4	4.3–6.9			[15]
Kitchen sink		7.6	7.4	7.7	[16]
Greywater		5.8	5.4	4.6	[16]

2. Materials and methods

2.1. The investigated system

Södra Vibyåsen in Sollentuna, Stockholm, is a housing area consisting of 85 terraces (row houses), with 212 inhabitants of which 17 (8%) are children under the age of three. Household wastewater is separated into blackwater and greywater. Blackwater is treated, stored and used for agricultural purposes on a nearby farm, whereas the greywater is treated locally in a pond system as part of an aesthetic landscaping approach. The average greywater flow is 13,800 L day⁻¹ or 65 L person⁻¹ day⁻¹. The water is treated in settling tanks, by activated sludge and then passed through a biofilter before it is released into a pond where exposure to humans may occur (Fig. 1).

The reported reduction of thermotolerant coliforms in such treatment are: settling tanks 0–30%, activated sludge treatment 85–97% [28], biofilters 96–98.5% [3] and ponds 95–99% [29] with the latter depending on system design, retention time and dilution [29,30]. The treatment performance on other parameters at Vibyåsen has shown to be: Tot-N 75%, Tot-P 98%, COD 82% and BOD 95%.

2.2. Sampling

Water samples (500 mL) were taken monthly over a year for bacterial indicators and coliphages. From sampling point CW, time proportional samples (30 mL, 30 times h⁻¹) were taken with a continuous sampler whereas grab samples were taken from sampling points P_{in} and P_{out} (Fig. 1). For the coprostanol and cholesterol analyses, 5 L of water were taken with the continuous sampler daily between 2001.10.09 and 2001.10.18. From these daily samples, faecal enterococci densities were also

determined for correlation purposes. To obtain particulate matter, samples for the sterol analyses, the water was filtered through glass fibre filters (\varnothing 15 cm, Schleicher and Schuell No. 8, nominal pore size 0.5 μ m) [31].

2.3. Analyses

Bacterial numbers were estimated using spread plate techniques and Swedish standard methods; total coliforms (SS 02816:2, mEndoagar-LES, 44 h, 35°C), presumptive *Escherichia coli* (SS 02816:2, mfc agar, 24 h, 44°C with further confirmation in LTL5B 44°C) and faecal enterococci (SS 028179:1, mEnterococcus agar, 44 h, 35°C with further confirmation on eskulina-gar 44°C). For sulphite-reducing anaerobes (SS-EN 26 461:2) the samples were heated (75°C, 15 min) and thereafter analysed (Perfringens agar base, 44 h, 37°C) with the pour plate method. Somatic coliphages were analysed by plaque assay according to ISO 10750-2 using *E. coli* CN, ATCC 13706, as the host strain.

The filters with the captured sterols were treated by direct saponification in 95% ethanol and 10 M NaOH (2:1) and extracted twice in hexane as described in Midtvedt et al. [32]. The blown down material was silylated with bis(trimethylsilyl)trifluoroacetamide and analysed as in Leeming et al. [33] with 5 α -cholestane as internal standard. The GC-MS (Hewlett Packard 5890 and 5970 mass-selective detector fitted with a direct capillary inlet, split/splitless injector and a 50 m fused-silica capillary column) was operated in scan acquisition mode described in Nichols et al. [34].

2.4. Sediment experiments

Enterococcus faecalis (ATCC 29212) and *Salmonella typhimurium* (ATCC 14028) were added to original and sterilised (121°C, 15 min) sediment collected from the

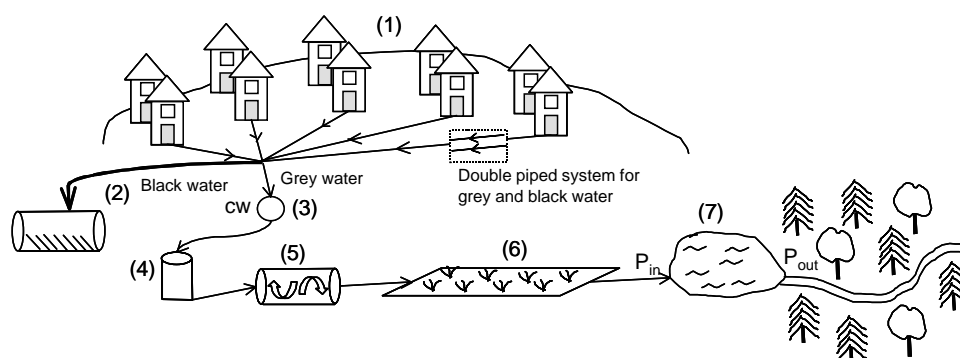


Fig. 1. Schematic picture of the greywater treatment of Vibyåsen. In the houses there are separated pipes for grey and blackwater (1). The blackwater is collected in a tank (2). The individual greywater lines are connected in a well (3) whereafter particulate material is reduced in settling tanks (4) treated by activated sludge (5) and in filter beds (6) before released into a pond system (7). Samples have been taken from the connection well, CW, before, P_{in} , and after, P_{out} , the pond. Modified from Palmqvist [62].

settling tank of the greywater treatment system (Fig. 1), and their persistence followed during a 50-day period in room temperature. Particle associated organisms were extracted as in Långmark et al. [35] and analysed by the spread plate method on mEnterococcus agar (Difco) and Brilliant green agar (Oxoid).

2.5. Risk assessment

2.5.1. Exposure scenarios

Four different exposure scenarios were validated for the three applied risk estimate approaches in the QMRA.

1. Accidental ingestion of 1 mL treated greywater, P_{out} .
2. Accidental ingestion of 1 mL treated greywater, P_{in} .
3. Yearly risk from direct exposure after irrigation with greywater, assuming 1 mL intake day⁻¹, 26 days year⁻¹.
4. Yearly risk from drinking groundwater recharged from the pond as described in Asano et al. [22] with modifications on the environmental die-off data and the water intake [36].

The three different approaches used were:

1. Measuring faecal contamination in greywater with coprostanol concentrations and using epidemiological data to assess risks as in Höglund et al. [37].

2. Using a dose–response model derived from occurrence of faecal enterococci in marine waters [27] assuming an exponential probability of infection.
3. Using faecal enterococci as an index organism for the *Salmonella* occurrence in greywater based on sediment experiments.

2.5.2. Densities of pathogens in greywater

Faecal contamination of greywater was calculated based on coprostanol values and assumed to be lognormally distributed. Epidemiological data were then used to estimate the likely prevalence of the respective pathogen in the faeces taking into account under-reporting (Table 2). The estimated pathogen density (mL⁻¹) is thus: faecal load (g person⁻¹ day⁻¹) × yearly incidence × excretion time (days) × pathogen density in faeces (g⁻¹)/365 (days) × flow (mL person⁻¹ day⁻¹).

Since pathogenic bacterial growth is possible, an exposure scenario using faecal enterococci as an index organism for *Salmonella* occurrence in greywater was simulated based on growth experiments.

2.5.3. Reduction of pathogens

Faecal enterococci were used as an index organism for the bacterial pathogens reduction, *Clostridium perfringens* spores for the parasitic protozoa and coliphages for rotavirus. Reduction data from the greywater treatment

Table 2

Literature data used to calculate microbial health risks from various exposures to treated greywater. The log₁₀ normal distribution has been applied for the excretion time, excretion density and reduction in saturated aquifers, mean and standard deviation given

	Faecal enterococci	<i>Campylobacter jejuni</i>	<i>Salmonella</i>	<i>Cryptosporidium parvum</i>	<i>Giardia lamblia</i>	Rotavirus
Incidence of infection (%)		15.6 ^{a,b}	9.00 ^{a,b}	0.31 ^b	0.84 ^{a,b}	0.95 ^c
Excretion time (days)		(1.18, 0.325) ^d		(1.48, 0.173) ^e		(1.0, 0.30) ^{d,f}
Excretion density (no g ⁻¹ faeces)		(8, 1) ^d	(8, 1) ^d	(7,1) ^g	(7,1) ^h	(10,1) ^{f,i,j}
Dose–response model	Exponential ^k	Beta-Poisson ^l	Beta-Poisson ^m	Exponential ⁿ	Exponential ^o	Beta-Poisson ^p
	$k = 177$	$N_{50} = 896, \alpha = 0.145$	$N_{50} = 23,600, \alpha = 0.3126$	$k = 238.6$	$k = 50.23$	$N_{50} = 5.6, \alpha = 0.265$

^a SMI [38].

^b Mead et al. [39].

^c Wheeler et al. [40].

^d Faechem et al. [12].

^e Stehr-Green et al. [41].

^f Gerba et al. [23].

^g Girdwood and Smith [42].

^h Jakubowski et al. [43].

ⁱ Bishop [44].

^j Ward et al. [45].

^k Derived from Key et al. [27].

^l Medema et al. [46].

in Vibyåsen were used, sometimes supplemented with literature data (Table 2). Viral reduction data during groundwater recharge, in the environment and in the saturated zone, are based on Asano et al. [22] (0.7 m^{-1}), Badawy et al. [50] (0.07 h^{-1}) and Yates et al. [51] $\log_{10}\text{norm}$ (0.10; 0.05) day^{-1} respectively. These data have been applied for all organisms.

2.5.4. Dose–response

The Beta Poisson dose–response models were used for *Campylobacter jejuni* [46], *Salmonella* spp [47] and rotavirus [24]. Exponential models were used for the recreational water exposure (derived from [27]), *Giardia* [49] and *Cryptosporidium* [48], (Table 2).

2.6. Analytical tools

Statistical analyses and diagrams were made in Sigma plot 2000 (SPSS Inc., Chicago). The exposure models were developed in Excel 2000 (Microsoft Corporation, Redmond) spreadsheets and run using @Risk 3.5.2. (Palisade Corporation, Newfield), an add-on to Excel. The input distributions were sampled using Latin Hypercube simulations in @Risk with 10,000 iterations per simulation. Chemstation (Agilent Technologies, Palo Alto, CA) was used to operate the GC-MS, process and quantify chromatography data.

3. Results

3.1. Faecal contamination

High and variable numbers of the different groups of faecal indicator bacteria were found in the greywater (Table 3). The variability could not be explained by seasonality. The data fitted $\log_{10}\text{normal}$ distributions (except for total coliforms), which were applied in the risk analysis, rather than normal distributions. Somatic coliphages were present indicating either a direct faecal input or propagation in the system due to regrowth of *E. coli*.

Table 3
Faecal indicators at the collection point CW in Vibyåsen greywater system (\log_{10} 100 mL^{-1} or $\mu\text{g L}^{-1}$)

Organism/biomarker	Mean	Std	Min	Max
Coliform	8.1	0.78	5.5	8.7
<i>E. coli</i>	6.0	0.60	4.3	6.8
Faecal enterococci	4.4	0.48	3.0	5.1
<i>C. perfringens</i> spores	3.3	0.61	2.3	4.8
Somatic coliphages	3.3	0.63	1.4	4.0
Coprostanol ($\mu\text{g L}^{-1}$)	8.6	4.4	3.1	14.9
Cholesterol ($\mu\text{g L}^{-1}$)	17.3	8.4	7.4	31.6

Coprostanol was detected in all samples in amounts equivalent to a mean faecal load of $0.04\text{ g person}^{-1}\text{ day}^{-1}$. The mean concentration in the greywater was $8.6\mu\text{g L}^{-1}$, compared to an average of $10,000\mu\text{g L}^{-1}$ expected in wastewater. The mean values of selected faecal indicators have been used to estimate the total faecal load in the greywater, giving a faecal load of between 0.04 and $65\text{ g person}^{-1}\text{ day}^{-1}$ (Table 4). For the QMRA, the faecal load in greywater was based on coprostanol concentrations as a conservative biomarker, since coprostanol is not produced within the system.

The concentration of the two chemical biomarkers correlated with each other ($r^2 = 0.81$) ($p = 0.0004$) while a significant correlation between faecal enterococci and coprostanol ($r^2 = 0.25$) ($p = 0.14$) and cholesterol ($r^2 = 0.23$) ($p = 0.16$) could not be confirmed (Fig. 2).

3.2. Reduction over treatment

Greywater treatment ($\text{CW}-P_{\text{in}}$) caused a 0.7–1 log reduction of bacteria and 0.2–0.3 log reduction of somatic coliphages and spores of sulphite reducing clostridia (Fig. 3). The bacteria were substantially reduced in the pond ($P_{\text{in}} - P_{\text{out}}$), from 2.3 logs for faecal enterococci to 3 logs for *E. coli*. The reduction was lower for the somatic coliphages and *Clostridia* spores, 1.2 and 1 log respectively (Fig. 3). Degradation of coprostanol and cholesterol were not measured in this study.

3.3. Sediment experiments

In sterilised sediment, both *Salmonella* and *Enterococci* could grow to a density of about $10^7\text{ c.f.u. g}^{-1}$ whereas they died-off in less than 50 days when incubated in the presence of indigenous microflora (Fig. 4).

Based on this experiment, the faecal enterococci: *Salmonella*-index is expected to be 1 in the risk assessment analyses, i.e. the densities of *Salmonella* are supposed to equal the density of faecal enterococci, due to their similar behaviour in the sediments.

3.4. Risk characterisation

The median risk of infection related to the exposure scenarios is summarised in Table 5. The highest risk using the faecal load and epidemiological data, method (1), always emanates from virus infections. *Campylobacter* poses the highest risk of the bacterial pathogens and *Giardia* of the parasitic, because of their higher prevalence and lower infectious doses. By using the faecal enterococci index for *Salmonella* occurrence, method (3), the risk of infection increased by a magnitude of 1000 for the bacterial pathogen. Still the risk was 100 times lower than estimated from rotavirus by method (1). Using faecal enterococci densities and

Table 4

Indicator occurrence, measured as excreted organisms person⁻¹ day⁻¹, and the corresponding faecal load (g person⁻¹ day⁻¹) in greywater (flow 64.9 L person⁻¹ day⁻¹)

Organism	Indicators in greywater (person ⁻¹ day ⁻¹)	Excretion rate (g ⁻¹ faeces)	Faecal load (g person ⁻¹ day ⁻¹)
<i>E. coli</i>	10E8.8 c.f.u.	10E7 ^a c.f.u.	65
Faecal enterococci	10E7.2 c.f.u.	10E6.5 ^a c.f.u.	5.4
Coprostanol	0.56 mg	12.74 ^b mg	0.04
Cholesterol	1.1 mg	5.08 ^b mg	0.22

^aGeldreich [52].

^bLeeming [26].

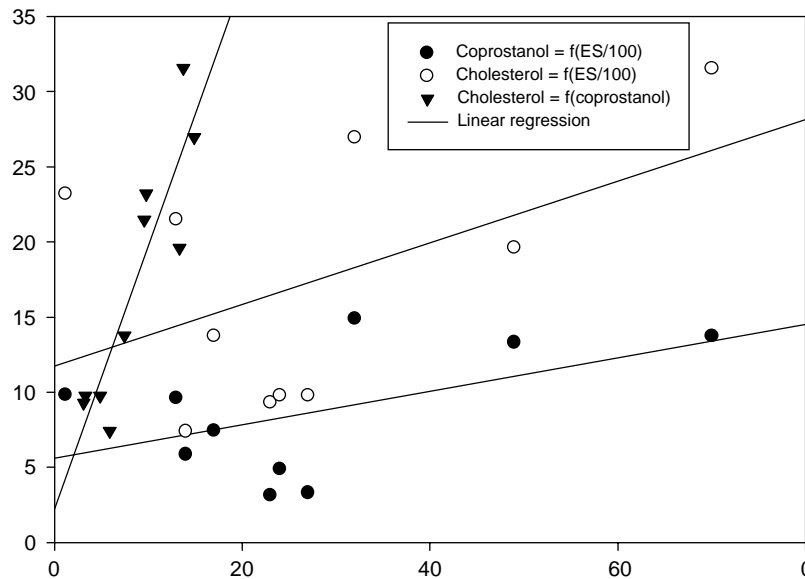


Fig. 2. Faecal enterococci densities [$\times 10^{-3}$ c.f.u. mL⁻¹] as functions of coprostanol and cholesterol concentrations [$\mu\text{g L}^{-1}$] and cholesterol concentration as a function of coprostanol.

recreational water exposure, method (2), underestimated the virus risk slightly. Regression analyses between the risks from the two methods gave the following relation, $f(x) = 0.0069 + 0.67x$ ($r^2 = 0.93$), ($p = 0.0075$).

4. Discussion

If the investigated greywater system is judged according to the traditional indicator bacteria analysis the conclusion would be that a substantial, although highly variable, faecal input had taken place, which is consistent with several other studies (Table 1). However, bacterial indicator growth, particularly of the coliform group, may occur in the system overestimating the faecal load of greywater substantially. Such a growth has been shown to occur in several similar systems based on the degradation of organic carbon [10]. Regrowth of the faecal coliforms and for some traditional bacterial

pathogens, like *Salmonella* readily occurs [53]. Growth of these and other bacterial pathogens, such as *E. coli* O157:H7, can even occur on improperly stored vegetables and may thus create a substantial input due to kitchen handling in greywater systems [54]. *Salmonella* and other enteric bacterial pathogens can also survive in traditional water closets, where they have been assumed to cause transmission [55]. It is similarly likely that they will survive and potentially grow in household piping installations.

Of the bacterial indicators, faecal enterococci seem to be the most appropriate to use since the overestimation of the faecal load is not as high as for the use of coliform bacteria, although they also seem to have the ability to regrow within a greywater system. Another way to measure the faecal contamination is by using chemical biomarkers. The measured coprostanol concentrations in this study gave a faecal load of 0.04 g faeces person⁻¹ day⁻¹ in greywater, compared with the 5.4

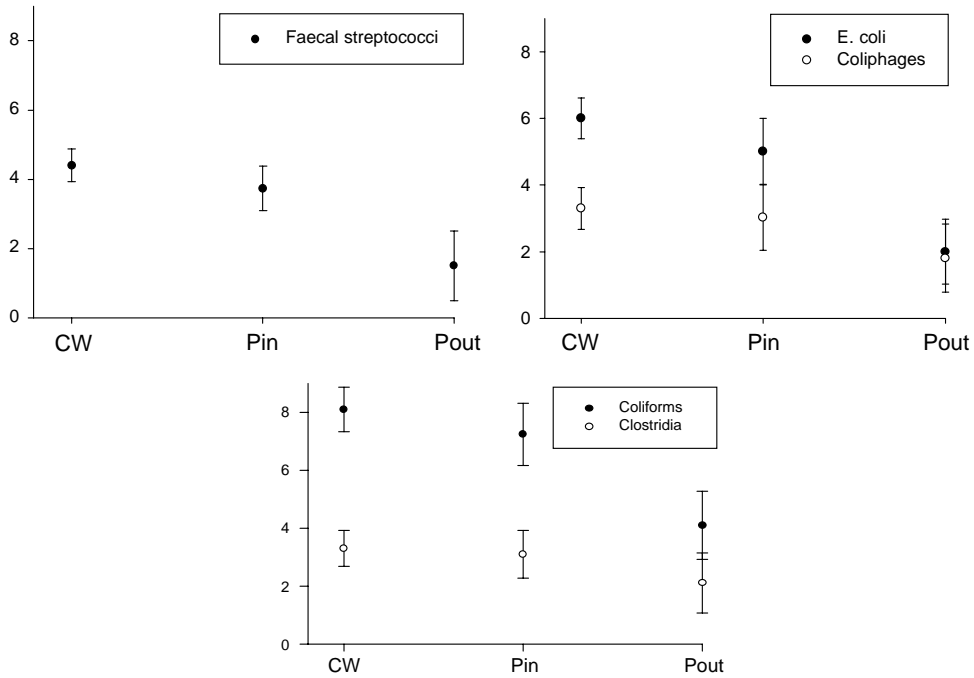


Fig. 3. Faecal indicators in Vibyåsen greywater treatment. The densities are measured as densities sampling point CW [\log_{10} norm 100 mL^{-1}], densities sampling point CW-log reduction ($CW-P_{in}$) and densities sampling point CW-log reduction ($CW-P_{out}$). The log reductions are measured from paired samples and normally distributed.

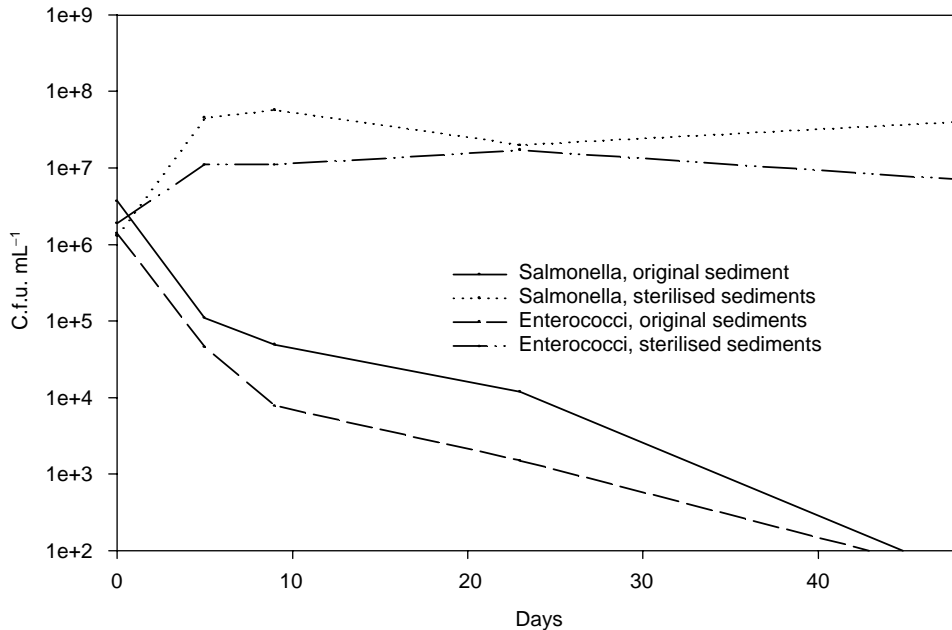


Fig. 4. Growth and reduction of *Salmonella* and *Enterococci* as a function of time in original and sterilised sediments from a settling tank in Vibyåsen.

and 65 g from the bacterial indicators. This measurement may also have some limitations, underestimating the risks in systems to which many infants are

connected. Not all young children excrete coprostanol, since they lack the intestinal bacterial flora needed to convert cholesterol to coprostanol [56]. One major input

Table 5
Median risk of infection based on 6 exposure scenarios and 3 methods

Method	Organism	Exposures and risks					
		Groundwater ^a			Direct contact		Irrigation ^a
		1 ^b	3 ^b	6 ^b	P_{in}	P_{out}	
(1) Faecal load	Rotavirus	10 ^{-0.2}	10 ^{-6.0}	n.r. ^c	10 ^{-0.6}	10 ^{-1.4}	10 ^{-0.2}
	<i>Salmonella</i>	10 ^{-5.0}	10 ⁻¹¹	n.r.	10 ^{-5.3}	10 ^{-7.9}	10 ^{-5.0}
	<i>Campylobacter</i>	10 ^{-2.5}	10 ^{-8.5}	n.r.	10 ^{-2.8}	10 ^{-5.4}	10 ^{-2.5}
	<i>Cryptosporidium</i>	10 ^{-7.1}	n.r.	n.r.	10 ^{-4.6}	10 ^{-5.8}	10 ^{-3.7}
	<i>Giardia</i>	10 ^{-7.9}	n.r.	n.r.	10 ^{-5.5}	10 ^{-6.6}	10 ^{-4.5}
(2) Faecal enterococci	Total gastroenteritis	10 ^{-0.4}	10 ^{-6.3}	n.r.	10 ^{-0.6}	10 ^{-2.7}	10 ^{-0.4}
(3) Bacterial growth	<i>Salmonella</i>	10 ^{-2.0}	10 ^{-8.0}	n.r.	10 ^{-2.2}	10 ^{-4.5}	10 ^{-2.0}

^aYearly risk.

^bRetention time in aquifer [months].

^cNegligible risk.

of faeces in the greywater may come from washing diapers and soiled infant clothes as well as the babies themselves and an underestimation of the faecal load is thus possible. Since 8% of the connected people are young children and possible low converters of cholesterol, this investigation also included the measurements of cholesterol for comparisons, giving a faecal load of 0.22 g person⁻¹ day⁻¹. This is an overestimation of the faecal load since the cholesterol also may emanate from the kitchen sink and other sources. The measurement of the two different sterols, however, gives a concentration span of potential faecal input that is more realistic than when taking into account the indicator bacteria. Other substances may also be used to detect anthropogenic influence from greywater systems in the environment, such as detergents [57] or bile- and fatty acids [58]. The possibility of quantifying the faecal load from analyses of these elements are limited though.

The treatment efficiency of the investigated system was low, less than 1 log for bacteria and only 0.24 log for somatic coliphages, compared to approximately 2 log reduction expected based on results from other studies [28]. Phages and spores were not as effectively removed as were bacterial indicators in the pond (1.2 and 1 log, respectively). With regard to virus reduction and potential exposure this is not enough as seen in the risk estimations. The reduction of bacteria in the pond was 2.3–3 log, largely due to sedimentation. A similar situation of lower viral reduction has also been seen in surface-flow wetlands, where however, higher reduction efficiency occurs [59]. A system like this needs to be fenced and could not be seen just as a scenic input of landscaping if direct exposure may occur. After the pond treatment, the risk may still be unacceptably high for viruses (10^{-1.4}) and additional treatment is necessary due to its use or discharge. Suggestions for additional treatment are ozonation or chemical precipitation in the beginning of the system. The reuse for irrigation

purposes was in many cases above the 10⁻³ risk addressed by Haas [60], despite the low faecal input. Further treatment should be conducted before use, or the time between irrigation and public access to the field extended. In this exposure scenario the withholding time between irrigation and public access was 12 h nighttime and the risk based on 26 days of exposure. For a single event, the rotavirus risk was 10^{-1.5} (data not shown). Groundwater exposure assessments were also based on repeated events, giving unacceptably high risk of infections from drinking water with just 1-month retention time in the saturated zone. There are proposed guidelines for groundwater recharge in California [1] but otherwise the recommendation is that site-specific considerations should be taken due to different circumstances of the aquifer [20]. The risk calculations of this study indicated that 3 months would be enough, given the conditions for environmental die-off. In addition to the microbial contamination of soil and groundwater, the impact from household chemicals as well as phosphorus and nitrogen contamination must be considered, which might be a problem of larger magnitude [8].

The first QMRA method, using faecal load and epidemiological data is perhaps the most appropriate one. For a simpler screening-level QMRA, other methods could be useful. By method (2) (faecal enterococci and recreational water exposure) the risk of infection correlated fairly well to the rotavirus risk for all exposure scenarios, except for exposure at P_{out} . The larger difference at this exposure point could be explained by the significantly higher bacterial than viral reduction over the pond. Assuming *Salmonella* densities are equal to faecal enterococci (method 3) increased the risk of the bacterial pathogen a 1000 fold. Still *Salmonella* was not the main threat to human health because of its high infectious dose. If *Campylobacter* could grow to the same densities in this system, the risk

would be much higher ($0.006 < P_{\text{inf}} < 0.81$), but before making that assumption additional experiments have to be carried out. Another system approach to reduce risks from bacterial pathogens is to separate waste from the kitchen sink from the greywater, as proposed by Christova-Boal et al. [8], decreasing the growth factors in the system significantly [16].

This screening level QMRA has interpreted the risks in a conservative way. For example, the reduction in the saturated zone was measured at temperatures up to 12°, which probably is higher than in Sweden, leading to a higher die-off rate than in the actual situation. But as stated for groundwater recharge, site-specific considerations should be considered and pathogen reduction in soil and the aquifer is a key issue for safe water management. Further, the environmental die-off was in several situations assumed to be the same for bacterial and parasitic pathogens as for enteric viruses, which may not always be the case. The high risk of rotavirus infections is partly due to the low reduction of somatic coliphages, which were used as a model for virus reduction. Somatic coliphages can under extreme circumstances replicate in natural unpolluted waters [61], and may also replicate in the treatment system underestimating the virus reduction. Investigation with other virus models may give additional information.

In conclusion we suggest that guidelines for greywater recirculation and reuse should not be based on thermotolerant coliforms as a hygienic parameter, because of the large input of non-faecal coliforms and/or growth of coliforms. The overestimation of the faecal load, and thus risk, that the indicator bacteria give is however to some degree compensated for by the higher susceptibility to treatment and environmental die-off. The risk model based on faecal enterococci densities correlated well to the risk from viruses, which is supposed to be the most prominent in a system without disinfection due to their high excretion figures, environmental persistence and low infectious doses. If guidelines should be based on bacterial densities, faecal enterococci are preferred.

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