

Characterisation of Indicator Organisms and Pathogens in Domestic Greywater for Recycling

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Abstract Greywater from baths, showers and wash-basins was collected separately from all other domestic wastewater at a university block of flats with a dual reticulation system and analysed for a range of contaminants including indicator organisms and pathogens. Greywater flow and temperature were also monitored and a diurnal variation was observed. Physical and chemical water quality parameters were similar to previously published data, although measured COD and BOD levels appeared to be lower, possibly due to settlement or biodegradation in the storage tanks. Plate counts and indicator organism concentrations were consistently high suggesting a high level of human bacterial contamination necessitating biological treatment and disinfection if the water is to be used for recycling. However, these high levels of indicator organisms did not correlate to pathogen presence and should not be used as pathogen indicators in greywater. One positive count of *Salmonella veltereden* was observed as well as low levels of *Giardia*, *Cryptosporidium*, *Escherichia coli* O157:H7, enteroviruses and *Legionella* were not identified in any of the samples. The research also

highlighted a number of problems with the complexity of this type of sampling programme, such as identifying the most likely time to isolate pathogens and analysing an ‘unusual’ water source.

Keywords Domestic · Greywater · Microbiology · Pathogens · Reuse

1 Introduction

Of several alternative wastewater sources that can be reused, greywater is generally perceived to be more ‘acceptable’ than other domestic wastewater, such as blackwater, due to a common misconception that it has lower levels of contamination. However, greywater contains significant microbiological contamination (Birks et al. 2004; Lazarova et al. 2002; Rose et al. 1990; Surendran and Wheatley 1998). By characterising the level of contamination, assessment can be made of the potential health risks and implications of its use for recycling, the type of treatment that is necessary and the associated economic viability. Greywater can broadly be defined as all wastewater generated in the household, excluding toilet waste. Greywater can include wastewater from bathroom sinks, baths and showers (‘light grey’) and may also include more contaminated waste from laundry facilities, dishwashers and, in some instances, kitchen sinks (‘dark grey’). Table 1 highlights the variation in raw ‘light’ greywater quality that has been found in previous studies.

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Table 1 Characteristics of 'light' greywater from various studies

Parameter	Laine 2001	Birks et al. 2004; Smith et al. 2001	Surendran and Wheatley 1998	Rose et al. 1990	Holden and Ward 1998	Christova-Boal et al. 1996
Greywater type	B, S, W	W	B, S, W	B, S, W, L	B, S, W	S, B
BOD (mg/l)	129–155	5–142	216–252	^a	33	76–200
COD (mg/l)	367–587	21–355	424–433	^a	40	^a
SS (mg/l)	58–153	7–102	40–76	^a	^a	48–120
Turbidity (NTU)	60–164	^a	57	20–140	20	113
pH	7.3–7.5	^a	7.7	5–7	^a	6.4–8.1
Ammonia, as N (mg/l)	^a	^a	0.5–1.6	0.15–3.2	1.1	<0.1–15
TKN, as N (mg/l)	^a	0–23.3	^a	0.6–5.2	^a	4.6–20
Total phosphorous (mg/l)	0.3–0.4	^a	1.6–45.5	4–35	^a	0.11–1.8
Total coliforms (cfu/100 ml)	6.4×10^3 – 9.4×10^3	$>2.4 \times 10^3$ – 10^6	5×10^4 – 6×10^6	6.1×10^6	^a	500 – 2.4×10^7
Faecal coliforms (cfu/100 ml)	^a	^a	32–600	1.8×10^4 – 7.9×10^6	^a	170 – 3.3×10^3
<i>Escherichia coli</i> (cfu/100 ml)	10 – 1.5×10^3	0 – $>2.4 \times 10^6$	^a	^a	^a	^a
Faecal enterococci (cfu/ml)	$40 \times 2.1 \times 10^3$	0 – 2×10^4	^a	^a	^a	2.4×10^3
22 °C plate counts/ml	^a	0 – $>3.0 \times 10^5$	^a	^a	^a	^a
37 °C plate counts/ml	^a	0 – $>3.0 \times 10^5$	^a	^a	^a	^a

^aNot stated in the study.

B Bath, S shower, W washbasin, L laundry.

The COD and BOD of greywater can be relatively high, even in 'light' greywater (see Table 1), but is much increased if 'dark' greywater (e.g., from kitchen sinks, washing machines) is included (Surendran and Wheatley 1998). Greywater generally contains less suspended solids and nitrogen than typical domestic sewage, as toilet water is excluded. Generally, ammonia and total Kjeldahl nitrogen concentrations in greywater are about 10 times lower than in domestic wastewater. However, greywater can contain more phosphorus, although this primarily originates from detergents in washing powder typically found in 'dark' greywater.

Greywater can contain high levels of bacteria (see Table 1). The presence of faecal coliforms, *Escherichia coli* and faecal enterococci may indicate a pathogenic risk in potable water and these organisms have previously been used to assess the safety of greywater recycling (Rose et al. 1990). However their

absence does not necessarily signify that water is pathogen free. This depends both on the type of treatment that has been used and also the reliability of the indicator organisms at predicting the presence of pathogens, which has previously been questioned (Gerba and Rose 2003).

It is relatively rare to have access to a large 'real' source of greywater for detailed analysis. A source was available for this study, with sampling carried out at a block of flats with a dual reticulation system at Cranfield University in the south of England. The university is a postgraduate only university and the flats house primarily married couples, therefore it was assumed that the greywater would be relatively typical of a standard block of flats. The greywater collected from the flats is not currently used for recycling but has been used for a number of pilot trials at the university. The primary objective of the

sampling programme was to characterise ‘real’ raw greywater with specific reference to microbiology. This was achieved by analysing for basic physical and chemical parameters, microbiological indicator organisms and a range of waterborne pathogens at various times both during the day and, also, the year.

2 Materials and Methods

2.1 Greywater collection

At Fedden flats hall of residence, ‘light’ greywater is collected via dedicated pipework from the baths, showers and handbasins of 18 of the 72 flats and directed to a subsurface collection sump (Tank A) in the grounds of the halls of residence (see Fig. 1). This is then pumped to a collection tank (Tank B) in an adjacent portacabin where samples can be taken. When the greywater volume in Tank B drops below a certain level, a low level float switch in the tank activates the sump pump in Tank A to pump more greywater into Tank B. The greywater in Tank B flows to drain via an outlet pipe near the bottom of the tank when the tank is full. There is also an

overflow at the top of the Tank B in the case of very high greywater flows.

2.2 Greywater flow and temperature monitoring

The greywater flow from the flats was measured using a flowmeter (‘M’ in Fig. 1) on the outlet pipe near the bottom of Tank B. The pipework after the flowmeter was the height of the top of the tank (see Fig. 1) so that greywater would only flow through the flowmeter and out of the tank when the tank was full. The greywater temperature in both Tanks A and B was measured using two floating data loggers, which logged the temperature every 5 min. Both the temperature and flow data were used to predict when the greywater was likely to be ‘fresh’ and when ‘stagnant’ so the two extremes could be sampled each day during the sampling programme.

2.3 Greywater sampling

Greywater samples were taken from Tank B (see Fig. 2). The sampling rationale was for it to be carried out in three phases with a review of the results after each phase. This is summarized in Table 2.

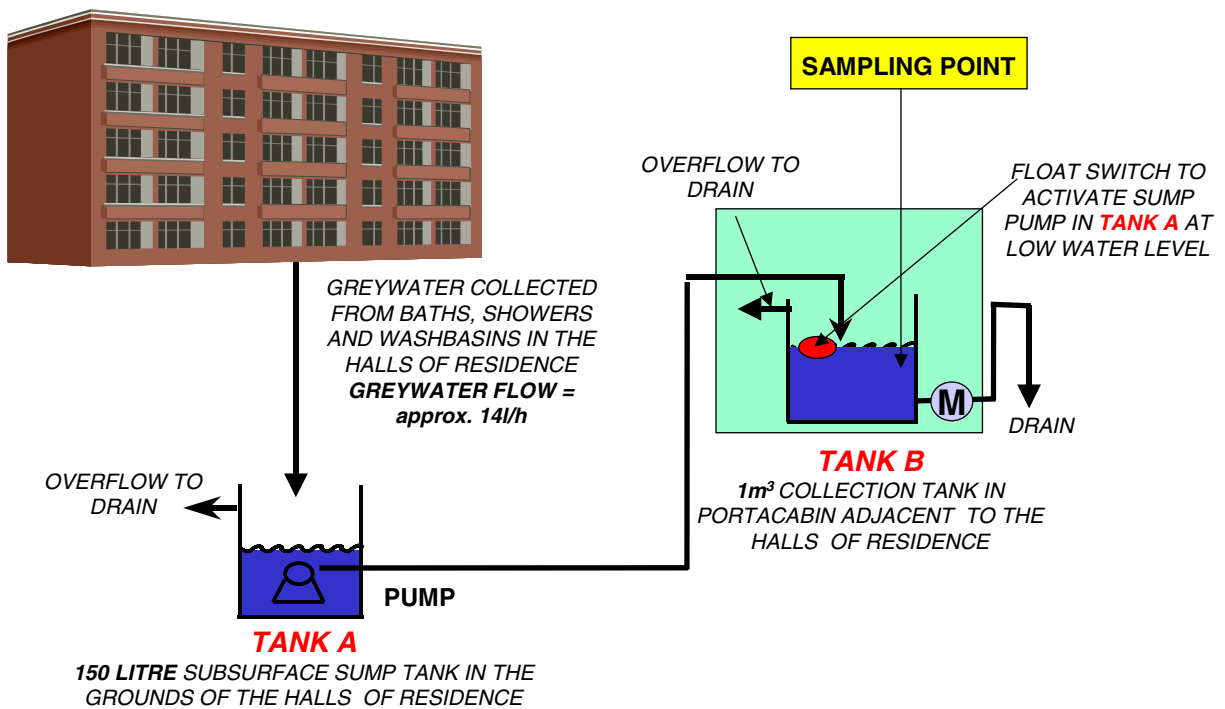


Fig. 1 Schematic of the greywater collection system at Fedden flats

Table 2 Summary of the sampling programme

Phase	Frequency	Duration	Determinands	No. of repeats/sample
1	Twice a day (8 A.M. and 3 P.M.) every day	2 weeks	Indicators (<i>E. coli</i> , total coliforms, faecal enterococci, 22 & 37 °C plate counts)	3
Review results				
2a	3 times a week (2 in week, 1 at weekend)	3 months	Indicators (as above); physical & chemical (BOD, soluble BOD, COD, SS, pH, turbidity, conductivity, TKN, P)	1
2b	3 times a week (2 in week, 1 at weekend)	3 months	Pathogens (<i>Salmonella</i> , <i>Giardia</i> , <i>Cryptosporidium</i> , <i>E. coli</i> O157:H7, enteroviruses and <i>Legionella</i>)	1
Review results				
3	3 times a week (2 in week, 1 at weekend)	1 week	Indicators; physical and chemical; pathogens (all as above)	1

Phase 1 was an intense 2 weeks of sampling during which samples were taken in triplicate daily (at 8 A.M. in the morning and 3 P.M. in the afternoon). From analysis of the temperature and flow data it was believed at 8 A.M. there would be ‘fresh’ greywater and at 3 P.M., more ‘stagnant’ greywater, so that the two extremes of water quality could be analysed for. During Phase 1, the greywater was monitored for indicator organisms and general physical and chemical parameters. The water quality results of Phase 1 of sampling were reviewed and used to design Phase 2 of the sampling programme.

Phase 2 sampling was carried out for 3 months, with less frequent samples taken than in Phase 1 and greywater analysed for indicator organisms, general physical and chemical parameters and some pathogens. Sampling times were based on the results of Phase 1.

In Phase 3, indicator organisms, general physical and chemical parameters and pathogens were analysed for one week, later in the year, as a replication of Phase 2. The complexity and high cost of the pathogen analysis meant that samples in Phase 2 and 3 were analysed for pathogens only when there was a

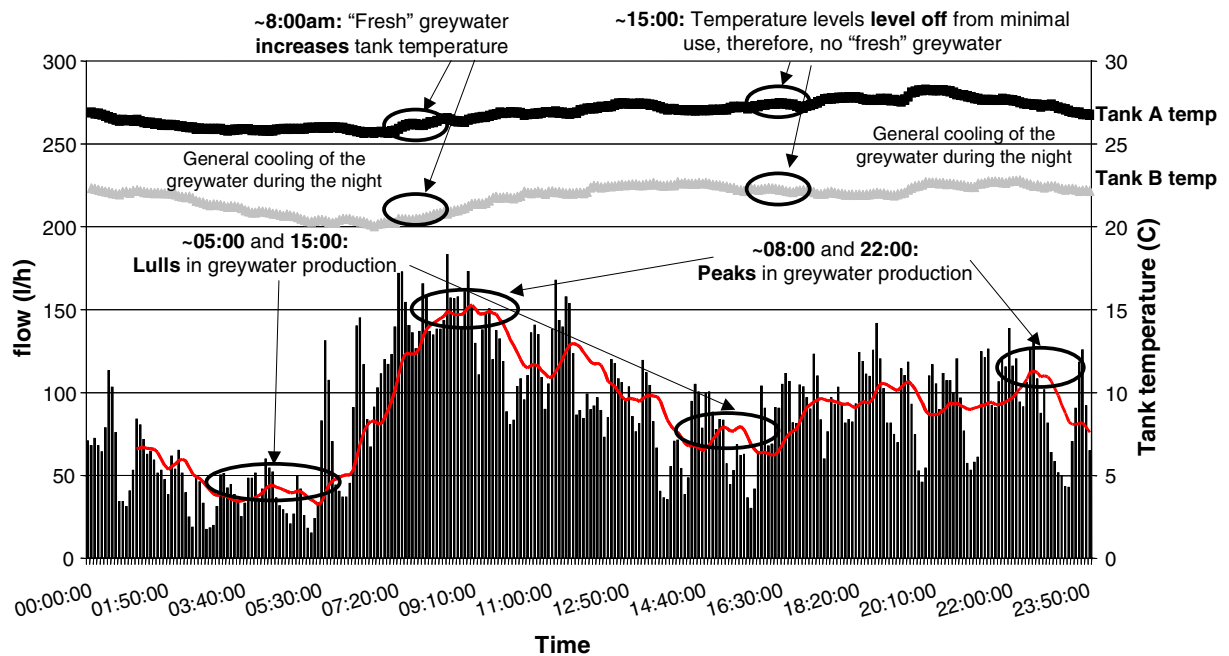


Fig. 2 Diurnal variation in greywater flow and temperature

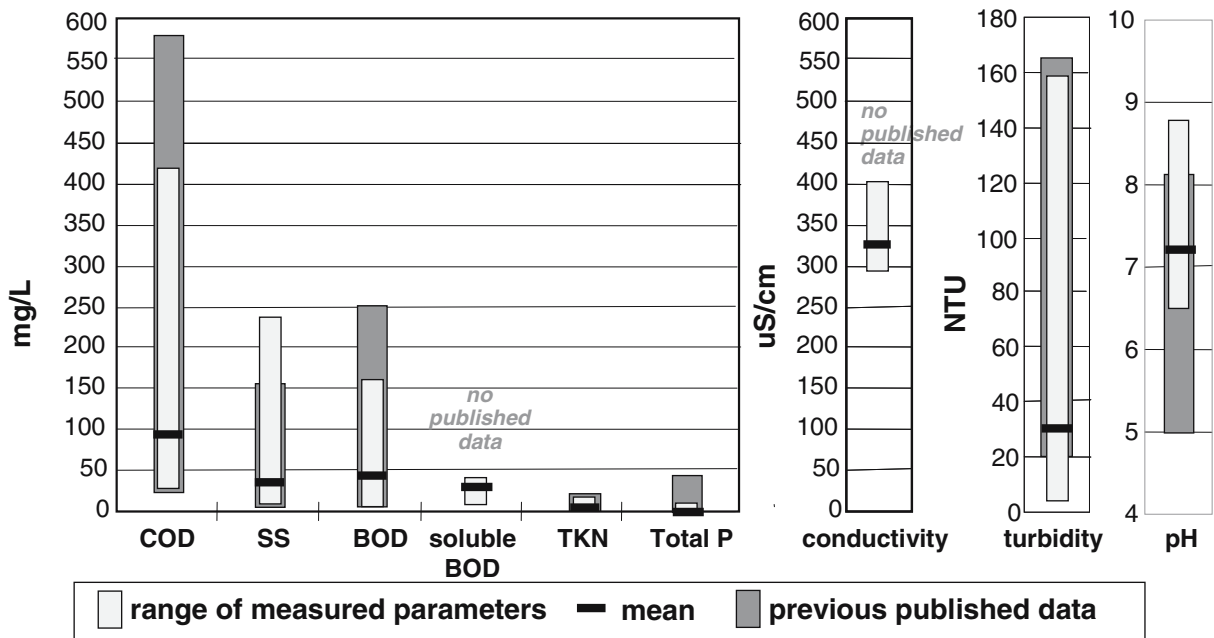


Fig. 3 Greywater quality (from Phase 1, 2 and 3)—physical and chemical properties

very high probability that there would be a positive count (i.e., when it was predicted that there would be high levels of indicator organisms in the greywater).

3 Results and Discussion

3.1 Greywater flow and temperature

Diurnal variation in greywater production was observed with peak flows at 8 A.M. and 10 P.M. and lulls at 5 A.M. and 3 P.M. (see Fig. 2). Temperature data followed a similar pattern to flow, with lower temperatures observed during times of low greywater flow and high temperatures when high volumes of ‘fresh’ greywater were being produced (see Fig. 2). The occupants were primarily married couples and the average per capita consumption (pcc) was comparable to Thames Water data for two-person households at 185 l/p/d (Thames Water internal data from 2003 for two-person households gives an average pcc of 187 l/p/d).

3.2 Greywater quality—physical and chemical properties

Physical and chemical water quality parameters were similar to previously published data for ‘light’ grey-

water, although measured COD and BOD levels appeared to be lower (see Fig. 3), possibly due to settlement or biodegradation in the storage tanks. Concentrations of turbidity and suspended solids increased during the sampling period (from 25 to 30 NTU and 30 to 52 mg/l respectively), possibly from deterioration in the cleanliness of the storage tanks (see Fig. 4). Levels of phosphorous were consistently low (<4 mg/l) as this ‘light’ greywater did not contain any detergents from washing machine powders. Table 3 summarises the results of the physical and chemical parameter analysis.

3.3 Greywater quality—microbiological properties

The plate counts and indicator organism concentrations were high (see Fig. 5) probably due to biodegradation in the tanks and multiplication in a nutrient rich environment (mean total coliforms at 2.2×10^7 cfu/100 ml, *E. coli* at 3.9×10^5 cfu/100 ml and faecal enterococci at 2.5×10^3 cfu/100 ml, see Table 3). The 37 °C plate counts were generally as high as the 22 °C plate counts (mean of 1.3×10^7 cfu/ml and 1.5×10^7 cfu/ml respectively, see Table 3) suggesting a high level of human bacterial contamination (from bacteria on the skin, faecal contamination etc.). For both plate counts and indicator organisms, the

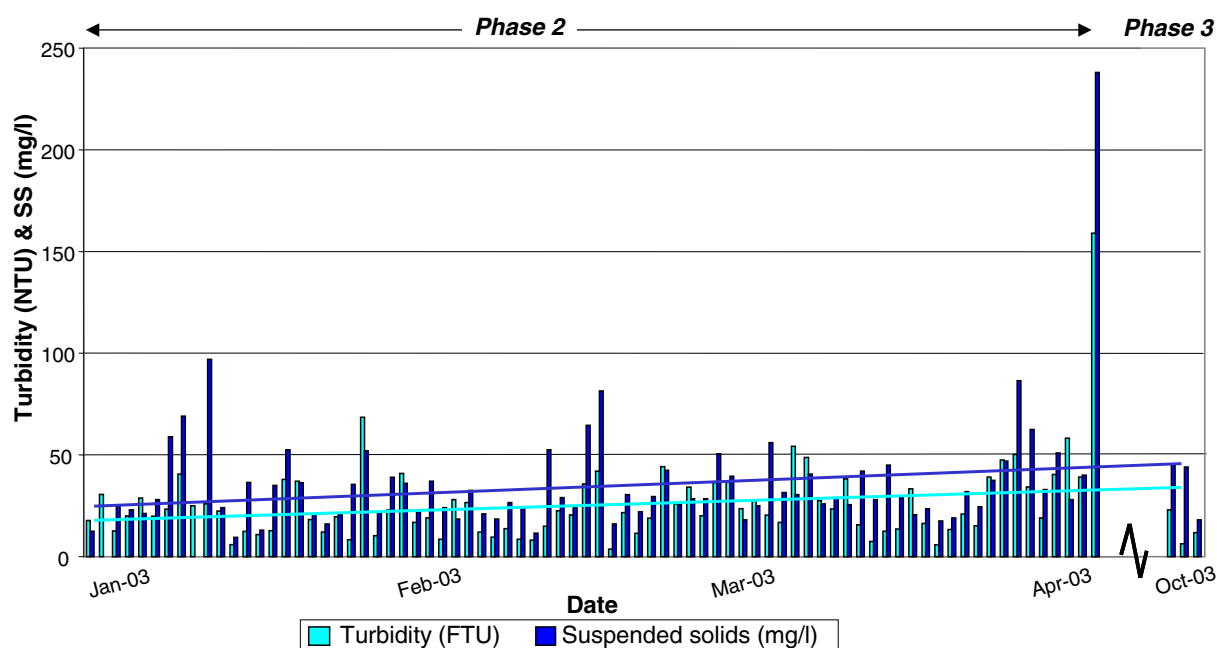


Fig. 4 Levels of turbidity and SS in the greywater (mg/l) (from Phase 2 and 3)

levels of contamination were higher than for previously published data (see Fig. 5). However, for plate counts, the previous published data available was from washbasins only (Birks et al. 2004, see Table 1) and not shower and bath water, as in this study, so this would be expected. The presence of total coliforms, *E. coli* and faecal enterococci shows faecal contamination, indicating the possible presence of pathogens.

3.4 Greywater quality—pathogens

The untreated greywater was also analysed for a range of pathogens and only two were identified. In all of the samples taken, one positive count of *Salmonella* (*Salmonella veltereden*) was found (see Table 4). This is a common species associated with food poisoning from partially cooked meat and shellfish and presumably passed into the greywater by an infected person

Table 3 Summary of mean and SD of the physical, chemical and microbiological parameters compared to the previously published data shown in Table 1

Physical and chemical parameters				Microbiological parameters			
Parameter	Mean	SD	Published data	Parameter	Mean	SD	Published data
pH	7.2	0.23	5–8.1	22 °C plate counts/ml	8.0×10^6	1.5×10^7	$0 \rightarrow 3.0 \times 10^5$
Turbidity, NTU	26.5	21	20–164	37 °C plate counts/ml	6.3×10^6	1.3×10^7	$0 \rightarrow 3.0 \times 10^5$
SS, mg/l	36.8	29.4	7–153	Total coliforms/100 ml	2.2×10^7	9.0×10^7	$>2.4 \times 10^3$ – 2.4×10^7
TKN, mg/l	4.6	2.8	0–20	<i>Escherichia coli</i> /100 ml	3.9×10^5	2.4×10^6	$0 \rightarrow 2.4 \times 10^6$
Total P, mg/l	0.86	0.82	0.11–45.5	Faecal enterococci/ 100 ml	2.5×10^3	4.8×10^3	$0 \rightarrow 2 \times 10^4$
BOD, mg/l	46.4	26.6	5–252				
Soluble BOD, mg/l	31.2		N/A				
COD, mg/l	96.3	52.6	21–587				
Conductivity, $\mu\text{S}/\text{cm}$	327	22.7	N/A				

N/A No data available.

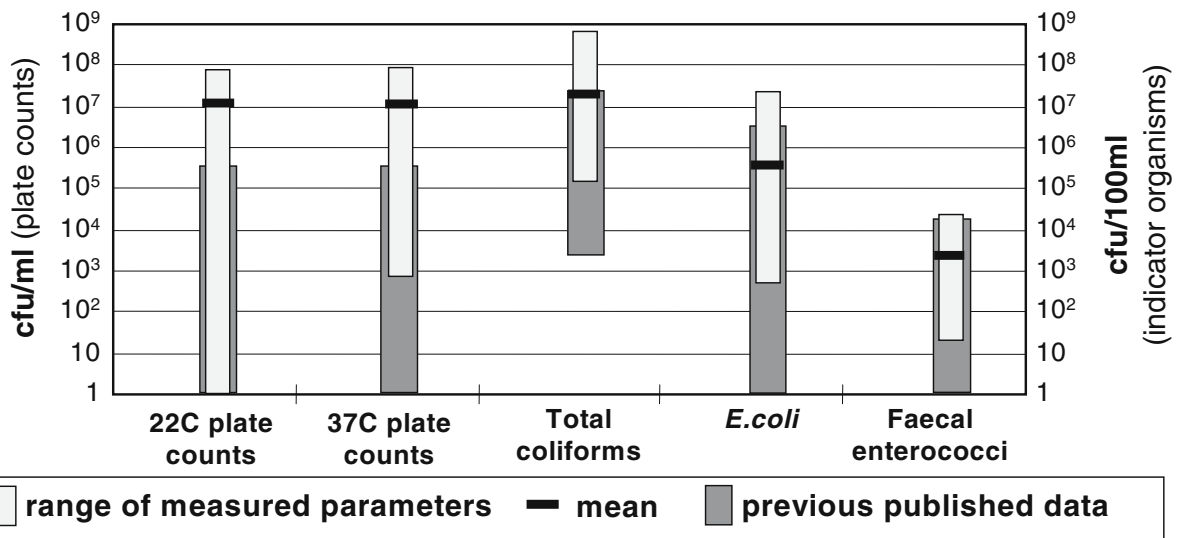


Fig. 5 Greywater quality (from Phase 1, 2 and 3)—microbiological properties

washing or possibly the washing of uncooked meat in a bathroom washbasin. Interestingly, this was at a time when the levels of indicator organisms were at the lower end of the range identified.

Giardia was also identified in 63% of the samples analysed (see Table 4), although these were not at levels to be of cause for concern as they were below the infective does of 10 to 25 cysts (positive *Giardia* samples were all <1.5 cysts/litre). *Cryptosporidium*, *E. coli* O157:H7 and *Legionella* were not identified in the samples; however, the likelihood of their presence is low as they are not common causes of illness (compared to pathogens such as *Salmonella*). Enteroviruses were not identified, despite being one of the more common groups of pathogens.

3.5 General trends in the greywater quality

Some trends in the greywater quality were observed during Phase 1, 2 and 3, such as the concentrations of turbidity and suspended solids increasing during the sampling period (see Fig. 4). However, no significant difference was observed between the week and the weekend samples for indicator organisms or 37 °C plate counts, although 22 °C plate counts were significantly higher in the week than at the weekend (*t* test at the 95% confidence level). It is unclear as to why the concentrations of environmental microorganisms would be higher in the week compared to the weekend and may simply be a data anomaly. There was also no significant difference in the

Table 4 Summary of results of the pathogen^a analysis

Pathogen	Positive samples (%)	Range identified	Pathogen	Positive samples (%)	Range identified
<i>Giardia</i> /l	63%	0.5–1.5	<i>Legionella pneumophila</i> , sg 1,2–14	0	0
<i>Salmonella</i> , MPN/l	13%	^b	<i>Legionella</i> sp. (non-pneumophila)	0	0
<i>Cryptosporidium</i> /l	0	0	Enterovirus enumeration/10 l	0	0
<i>Campylobacter</i> /l	0	0	<i>Escherichia coli</i> O157:H7/l	0	0

^a Each pathogen shown analysed eight times during the sampling programme.

^b Identified as *Salmonella weltevreden*.

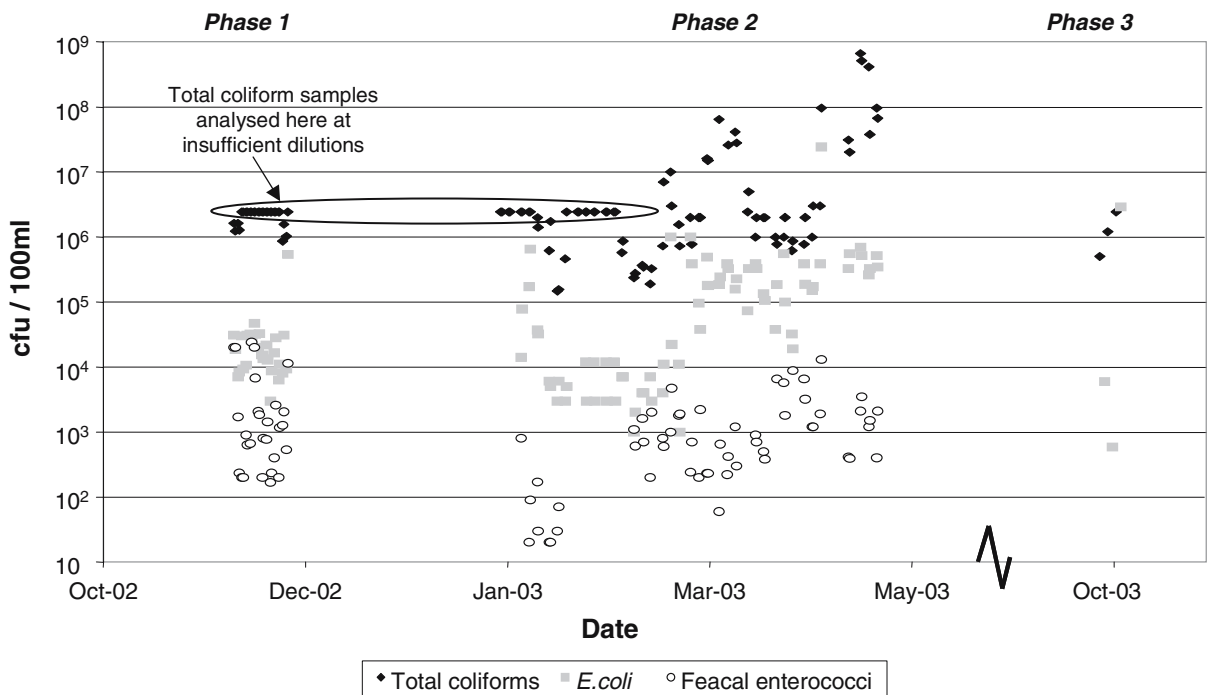


Fig. 6 Trends in indicator organism concentration over the sampling period

physical and chemical parameters analysed for in the week and weekend samples, with the exception of total phosphorous which was significantly higher in the samples taken during the week (at the 95% confidence level).

Figure 6 shows the concentrations of indicator organisms found throughout the sampling period. No significant difference was observed between the morning and afternoon samples for plate counts, total coliforms or any of the physical and chemical parameters analysed. However, *E. coli* and faecal enterococci levels were significantly higher in the afternoon samples, when it was thought that the greywater would have been stored for longer, compared to the morning samples predicted to contain fresher greywater (*t* test at the 95% confidence level). This indicates that more biodegradation had occurred in the greywater which had been left for a few hours. However, as this did not correlate with the BOD analysis (i.e., there was no significant difference in the BOD morning and afternoon samples), no firm conclusions can be drawn from this trend without further greywater quality analysis to confirm or refute the trend.

It should be noted that, although the best attempts were made to predict times during the day when the greywater was the most 'fresh' and most 'stagnant'

from the temperature and flow data this may have been inaccurately estimated. Therefore, variations in the greywater quality from being stored for different lengths of time may have been inadvertently missed.

4 Conclusions

The research showed that consistently high levels of potable water indicator organisms (total coliforms, *E. coli* and faecal enterococci) can be found in raw untreated greywater. However, these high levels of 'indicators' do not necessarily signify the presence of pathogens and, therefore, should not be used for pathogen indication in greywater. The potential for human pathogens to be present in detectable numbers in greywater was confirmed.

The high levels of microorganisms and BOD necessitate biological treatment to reduce contamination, if greywater is to be reused. Disinfection is also essential to eliminate pathogens and to provide a residual to minimise biofilm growth in the pipework.

This research has highlighted a number of difficulties and the complexity of the sampling programme, particularly, the expense of analysis for pathogens, identifying the most likely time to isolate pathogens,

analysing an ‘unusual’ water source and estimating when the greywater will be ‘fresh’ or ‘stagnant’. There was much variation in the results for each microbiological contaminant. However, the variation was much less during Phase 1 of the sampling when the analysis was carried out in triplicate, due to the increased accuracy of a larger sample. However, a larger sample obviously incurs an additional cost, which must be considered in any sampling programme.

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