Wat. Res. Vol. 25, No. 1, pp. 37–42, 1991
Printed in Great Britain.

0043-1354/91 $3.00 + 0.00

MICROBIAL QUALITY AND PERSISTENCE OF ENTERIC PATHOGENS IN GRAYWATER FROM VARIOUS HOUSEHOLD SOURCES

JOAN B. ROSE[1]Ⓜ, GWO-SHING SUN[2], CHARLES P. GERBA[2,*]Ⓜ and NORVAL A. SINCLAIR[2]

[1]Department of Environmental and Occupational Health, College of Public Health, University of South Forida, Tampa, FL 33612 and [2]Departments of Microbiology and Immunology, and Nutrition and Food Science, University of Arizona, Tucson, AZ 85721, U.S.A.

(First received December 1989; accepted in revised form June 1990)

Abstract—This study was designed to evaluate the microbial quality and safety of graywater for reuse purposes. The microbial and chemical composition of graywater from shower/bath, wash cycle and rinse cycle of a clothes washing machine was determined. Graywater composed from all sources within a house was also monitored each week over a 2–3-month time period. Samples were taken from a diverse group of families with children (18 months–9 years of age) and without children. Standard plate count bacteria (SPC) ranged from 10^5 to 10^{10} colony forming units (cfu) per 100 ml for shower and bath water, and an average of 10^4 to 10^6 cfu per 100 ml for total coliforms. Families with small children produced wash cycle graywater containing 10^6 cfu per 100 ml of fecal coliforms. During investigations on storage of graywater, it was found that total bacterial SPC and coliform baceria increased one order of magnitude. *Salmonella atyphimurium* and *Shigella dysenteriae* seeded into graywater were found to persist for at least several days. Poliovirus type 1 added to graywater decreased 99 and 90% at 25 and 17°C, respectively, after 6 d of storage in graywater. These data imply that there may be some risk associated with reuse of graywater when these pathogenic bacteria or viruses are being excreted by an individual producing the graywater.

Key words—graywater, reuse, indicator bacteria, coliform, survival, *Salmonella*, *Shigella*, poliovirus, viruses, water quality

INTRODUCTION

Water is a limited and valuable resource, particularly in arid environments such as found in the Southwestern United States. Increases in the population within the last decade have placed a major burden on the current water supplies. Conservation methods have been implemented in many states. Treated domestic sewage is currently being reused for large scale irrigation as one means of conserving the better quality water for other purposes. However, for the individual homeowner or apartment complex, very few options are available which can aid in water conservation. Graywater reuse may be a viable option for these cases. However, the quality of graywater must first be assessed to determine any potential health risk associated with reuse.

Graywater is defined as all wastewaters generated in the household, excluding toilet wastes (Ingham, 1980), and includes wastewater from bathroom sinks, baths, showers, laundry facilities, dishwashers and, in some instances, kitchen sinks, Siegrist (1976) estimated that 65% of all wastewater generated in a household is graywater. Although approx. 29.4 gallons per capital per day are generated, volumes of graywater produced vary from area to area. For example, Foster and DeCook (1986) reported that about 31 gallons of graywater were produced by one Tucson resident per day; Ingham (1980) estimated that up to 59 gallons of graywater were produced by one California resident per day; and for a family of four, 1300 gallons of graywater may be produced in 1 week.

It is obvious that large volumes of graywater may be available for reuse including lawn, landscape and garden irrigation, or even toilet bowl flushing. In arid regions, all of the household landscape irrigation needs can be met with graywater generated within the household (Rose *et al.*, 1988). However, there are problems encountered with this type of reuse. One of the concerns is microbial content. The presence of *Eschericia coli* and other enteric organisms in water indicates fecal contamination and the possible presence of intestinal pathogens such as *Salmonella* or enteric viruses. Fecal coliforms are a pollution indicator and may be used to assess the relative safety of graywater. Generally, a high fecal coliform count is undesirable and implies a greater chance for human illness to develop as a result of contact during graywater reuse.

Few studies have addressed the microbial content of graywater. In one such study an average of 215 total coliforms 100 per ml and 107 fecal coliforms per 100 ml were found in laundry wash water, while bathing water contained 1810 total coliforms per 100 ml and 1210 fecal coliforms per 100 ml (EPA,

*Author to whom all correspondence should be addressed.

1984). Hypes (1974) found that graywater containing household garbage had 1.27×10^8 and 1.88×10^7 cfu per 100 ml of standard plate count and total coliform bacteria, respectively. The heterogeneity in graywater composition based on the limited information available makes it difficult for public health officials to assess the risks associated with graywater reuse.

Since 1980, Arizona has implemented new regulations and guidelines protecting not only the quality but also the quantity of limited ground and surface water sources. These regulations have encouraged wastewater reuse and led to the development of new water quality standards (Arizona, 1984). Graywater reuse is also governed by these standards, which include a geometric mean of less than 25 fecal coliforms per 100 ml and 5 NTU of turbidity. Information on the microbial quanity of graywater will further aid in establishing guidelines for the implementation of graywater reuse and in assessing treatment requirements prior to reuse.

During this study, the microbial quality of graywater was evaluated. Indicator microorganisms and total bacterial populations were used as an index of contamination. Survival of *Salmonella*, *Shigella* and poliovirus in graywater was also examined.

MATERIALS AND METHODS

Sample collection

Graywater was collected from various locations using sterile, wide-mouthed plastic bottles. For showers and baths, drains were stoppered and 500 ml of water was taken from the colletion basin. Samples from washing machines were obtained from the wash as well as the rinse cycles. Nine to 10 samples were collected from each of the six families participating in the study. Sets included two young couples (20–30 years with no children), three families with children ranging in age from 18 months to 9 years of age and a family with young (17–20 years) adults. Combined graywater for one family (with a child 18 months of age) was obtained from a storage sump which collected waste water from all sources within the house, excluding the toilet and the kitchen sink. Graywater was held in the storage sump before being pumped to a treatment system. Residence time in the sump varies, depending on production of garywater in the household, but was usually less than 24 h. Samples were collected directly from the top of the storage sump once a week for $2\frac{1}{2}$ months. The graywater was transported on ice (4°C) and processed within 4 h. Tapwater was collected from outside taps. Tapwater was not chlorinated, since it was obtained from deep wells.

Bacterial enumeration

Standard plate count (SPC) bacteria were enumerated using plate count agar (Difco, Detroit, Mich.) according to *Standard Methods* (APHA *et al.*, 1975). Total and fecal coliforms were numerated by membrane filtration (0.45 μm pore size, Gelman, Gelman Sciences Inc., Ann Arbor, Mich.) as previously described (APHA *et al.*, 1985). M-Endo agar LES (Difco, Detroit, Mich.) was used for the former and M-Fc agar (Difco, Detroit, Mich.) was used for the latter.

Bacterial growth and survival

Bath water samples were incubated in sterilized polypropylene bottles at 25°C. Plate count bacteria and coliforms were enumerated as described, immediately after collection and thereafter every other day for 12 days.

Survival of Salmonella and Shigella in graywater

Salmonella typhimurium and *Shigella dysenteriae* (obtained from the culture collection of the Department of Microbiology and Immunology, University of Arizona, Tucson, Ariz.) were cultured in nutrient broth (Difco, Detroit, Mich.) at 35°C for 24 h. Cultures were centrifuged (1600 *g* for 10 min), washed 2 × sterile saline (0.85%) and finally suspended in a volume of 20 ml with sterile saline. Ten ml each of *Salmonella* and *Shigella* suspension were seeded into 40 ml of combined graywater from the sump tank and held at 25°C for 12 days. In addition, a 50 ml volume of non-seeded graywater was held under identical conditions.

Hektone enteric agar (BBL, Cockeysville, Md) was used to enumerate *Salmonella* and *Shigella* by the spread plate technique. Control plates were prepared with non-seeded graywater samples and positive control plates were also prepared by streaking the agar with the *Salmonella* and *Shigella* strains used in the test. All plates were incubated at 37°C for 24 h and typical colonies were counted with a Quebec colony counter (New Brunswick Scientific Co., New Brunswick, N.J.).

Survival of poliovirus in graywater

Samples of graywater from the sump were collected and held in the dark at 17 and 25°C. The graywater was seeded with approx. 10^6 plaque forming unit (pfu) ml^{-1} of poliovirus type 1 (LSc). Subsamples were taken daily for 8 days. The virus was enumerated on BGM cell monolayers using an agar overlay procedure (Melnick *et al.*, 1979). After 48–72 h incubation at 37°C in 5% CO_2 the agar was removed. Plaques were visualized using a 0.5% crystal violet stain in 20% ethanol.

Physical and chemical water quality tests

Several physical and chemical determinants of graywater were measured including: pH (Model PHI meter 70, Beckman Co., Irvine, Calif.); turbidity (Model 2100 A turbidimeter, Hach Co., Loveland, Colo.); phosphate, sulfates, nitrate-nitrogen and ammonia-nitrogen (Model DR/IA colorimeter, Hach Co., Loveland, Colo.); chlorides, total hardness and alkalinity (digital titrator Model CD-DT Hach Co., Loveland, Colo.).

RESULTS

Comparison of the microbial quality of shower water, bath water, wash and rinse cycle of laundry water were made for all households (Fig. 1). Total bacterial populations (SPC) were not significantly different from one source to the other and averaged between 10^7–10^8 colony forming units (cfu) per

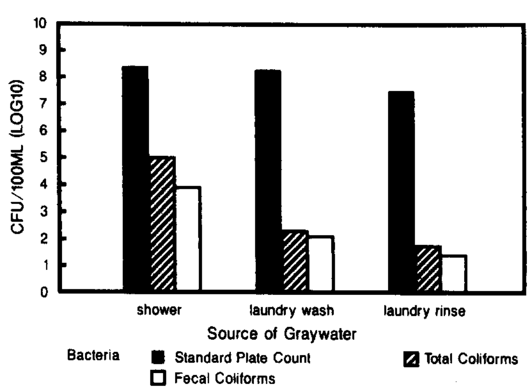

Fig. 1. Bacterial concentrations in shower and laundry graywater.

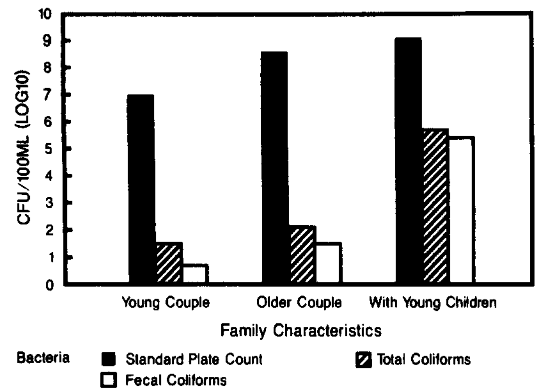

Fig. 2. Bacterial concentrations in graywater generated by a variety of families.

100 ml. Total coliforms and fecal coliforms were higher in the shower water (10^5 and 6×10^3 cfu per 100 ml, respectively) than in laundry wash and rinse water. In the laundry and rinse water, 199 and 56 cfu per 100 ml total coliforms were enumerated and 126 and 25 cfu per 100 ml fecal coliforms, respectively.

Data for microbial quality from all graywater samples for individual family sets are shown in Fig. 2. Total SPC bacterial numbers were high; i.e. 10^7–3×10^8 cfu per 100 ml, and differences beween graywater samples for young couples, older couples and couples with young children were not significant. Total coliforms and fecal coliforms were low in the graywater from families without childlren and averaged between 6 and 80 cfu per 100 ml. In contrast, however, fecal coliform and total coliform counts were significantly higher in graywater from families with young children and averaged 1.5×10^3 and 3.2×10^5 cfu per 100 ml, respectively.

Combined graywater samples from a single family unit were tested for SPC, total and fecal coliforms. Ten weekly collections were made for 2.5 months. Results are shown in Fig. 3. Numbers of SPC fluctuated only slightly and averaged 6.1×10^8 cfu per 100 ml over the entire period. Results were similar for total coliforms which averaged 2.8×10^7 cfu per 100 ml. In contrast, numbers of fecal coliforms varied more than 2.5 logs ranging from 1.82×10^4 to 7.94×10^6 cfu per 100 ml.

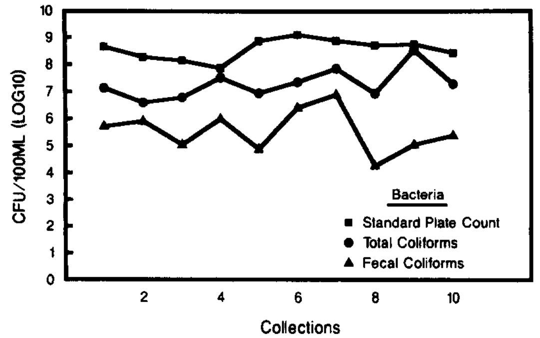

Fig. 3. Bacterial variations in combined graywater from a single family.

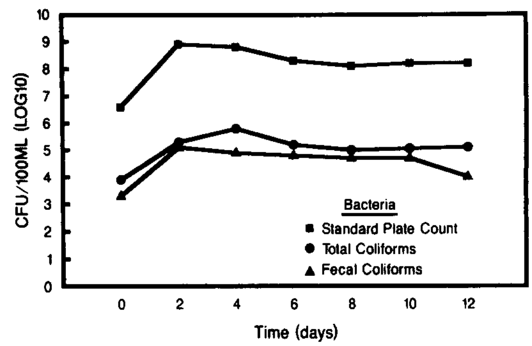

Fig. 4. Bacterial growth and survival in bath graywater.

Growth and survival of SPC, total and fecal coliforms were determined in bath water from families with young children. The results are shown in Fig. 4. The numbers of SPC, total coliform and fecal coliform bacteria increased one to two logs within the first 48 h and then declined slowly. Even after 12 d, however, numbers remained higher than those initially present.

To determine the fate of pathogens in graywater, two pathogenic enteric bacteria, *S. typhimurium* and *S. dysenteriae*, were seeded into combined graywater from the sump and held at 25°C. The results are shown in Fig. 5. In both cases numbers decreased, and after 4 d incubation *S. typhimurium* decreased 0.6 $\log_{10}$ and *S. dysenteriae* decreased 1.7 $\log_{10}$. After 8–10 d incubation, population size stabilized for both agents, with a 3.5 $\log_{10}$ reduction.

Poliovirus survival was also examined in combined graywater from the sump (Fig. 6). After 2 d storage, no change in virus numbers was observed in graywater stored at 17°C. A 1.0 $\log_{10}$ reduction was observed at a 25°C storage temperature. After 8 d incubation, poliovirus decreased 1.35 $\log_{10}$ and 1.7 $\log_{10}$ at temperatures of 17 and 25°C, respectively.

The physical and chemical characteristics of graywater samples from the time series study are shown in Table 1. For comparative purposes, tapwater was also analyzed. Degradation of tapwater quality after use in the home was evident by slight increases in ammonia and phosphate and an approximate 100-fold increase in turbidity.

The average values for ammonia-nitrogen, and turbidity of graywater from different families and

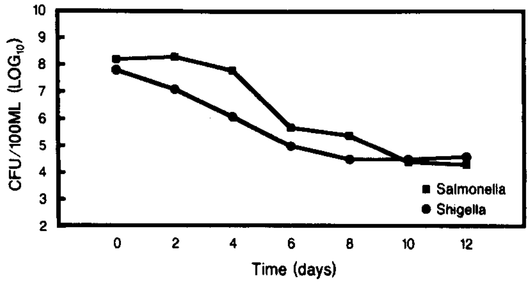

Fig. 5. Survival of *Salmonella typhimurium* and *Shigella dysenteriae* in graywater.

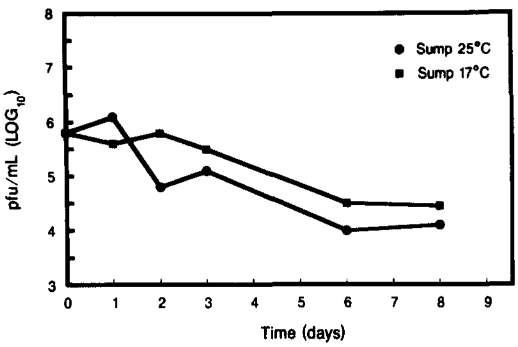

Fig. 6. Survival of poliovirus in graywater.

sources are shown in Table 2. Both ammonia-nitrogen which ranged in concentration from a low of 0.06 mg l^{-1} to a high of 3.47 mg l^{-1}, and turbidity ranged from a low of 14 NTU to a high of 297 NTU. In addition, both of these determinants were invariably higher in wash water than rinse water, as might be expected.

Table 2. Ammonia and turbidity values from a variety of graywater sources and families

Housing situation	Ammonia nitrogen (mg/l)	Turbidity (NTU)
Young couples		
Bath/shower	0.342	96
Laundry wash	0.1	296*
Laundry rinse	0.06	29
Families with young children		
Bath/shower	0.37	28
Laundry wash	3.47	54
Laundry rinse	0.08	18
Family with young adults		
Bath	0.11	43
Laundry wash	0.44	39
Laundry rinse	0.33	14

*These couples engaged in outdoor activities to a greater degree than the other families, which may explain the higher solids content of the laundry wash water.

DISCUSSION

The collection and use of graywater may result in a significant reduction of fresh water consumption for residential irrigation in arid regions (Foster and DeCook, 1986; Rose *et al.*, 1988). In Tucson, Arizona, it has been estimated that approx. 31 gallons of graywater can be collected per day per person, which amounts to 33,945 gallons of graywater per year for a family of 3. An effective graywater system in addition to other water-saving devices may not only conserve municipal water for home consumption but may also reduce the need for increased capital investment in municipal water distribution systems and energy costs.

The quality of graywater and its reuse application will define the appropriate guidelines to reduce health risks associated with potential exposure. When addressing graywater quality, the analyses performed in this study indicate that turbidity and microbial contamination will be of the most concern.

A wide variety of microbial profiles can be obtained from graywater. This may depend on family characteristics such as the number and ages of children, hygienic lifestyles and activities such as gardening or use of cloth diapers. The quality of graywater can be influenced by the source (i.e. bath vs laundry) of the water. The proportion of graywater from each fixture has been estimated at 5–6% for the bathroom sink, 42–79% for the bathtub/shower, 10–17% from kitchen sink or dishwasher and 5–23% from laundry facilities (Winneberger, 1976; Popkin, 1978).

The most significant amount of graywater is generated from showers or baths and may also contribute the greatest number of microorganisms. Both total and fecal coliform concentrations were greater in shower or bath water than in laundry waters for all families. The one exception was a collection from one laundry wash cycle containing a load of cloth diapers. Similar results have been previously reported (EPA, 1984). Total and fecal coliform numbers were approx. ten times greater in bathing water than in laundry water.

Levels of coliforms were also comparable between this study and a previous investigation (EPA, 1984) for laundry waters. In both or shower graywater, total coliforms were 100 times greater in this study while fecal coliform numbers were only five times greater. Surprisingly, microbial populations in the combined graywater collected from the sump were

Table 1. Ranges of values for the physical and chemical characteristics of graywater and tap water from a single family

Variable		Ranges in storage sump tank*	Averages in storage tank*	Averages in tap water†
pH		5–7	6.54	6.6
Turbidity	NTU	20–140	76.3	0.8
Phosphate	mg/l	4–35	9.3	3.1
Sulfate	mg/l	12–40	22.9	28.3
Ammonia nitrogen	mg/l	0.15–3.2	0.74	0
Nitrate	mg/l	0–4.9	0.98	1.0
Total nitrogen	mg/l	0.6–5.2	1.7	1.0
Chloride	mg/l	3.1–12	9.0	10
Hardness	mg/l	112–152	144	142
Alkalinity	mg/l	149–198	158	131

*Average of 10 samples.
†Average of 8 samples.

100–1000 times greater than at any of the individual graywater collection sites.

It has been suggested that physical and chemical properties of graywater may contribute to the growth of microorganisms stored in graywater. The increases in phosphates, ammonia and turbidity in graywater indicate that nutrients may be available for microorganisms. Brandes (1976) surmised that higher numbers of coliforms in graywater, opposed to black water, may be due to the large amounts of material present which have undergone little biological degradation. Lower numbers of total coliforms in toilet water may directly relate to the amount of biologically unusable material present, as black water contains material that has undergone considerable microbial and enzymatic breakdown in the human digestive tract (McClelland, 1978).

The results presented in this study showed that the total aerobic count, coliform and fecal coliform bacteria stored in graywater increased during the first 48 h and then became fairly stable for the next 12 days. Similarly, Hypes (1974) found that the number of organisms in graywater containing household garbage was about $1.27 \times 10^8\ \mathrm{ml}^{-1}$ for total aerobic bacteria and $1.88 \times 10^7\ \mathrm{cfu\ ml}^{-1}$ for total coliform bacteria; but after 24 h of storage, the numbers increased to 2.17×10^9 cfu per 100 ml for total aerobic bacteria and $5.40 \times 10^8\ \mathrm{cf\ ml}^{-1}$ for total coliform bacteria. Laak (1974) also reported that, after a 24-h holding time, combined graywater contained 2.1×10^{10}/100 ml total aerobic bacteria and 5.4×10^6/100 ml total coliform bacteria. The concentrations of microorganisms from the combined graywater in this study are in agreement with those numbers reported by Hypes and Laak. The data indicate that the microorganisms are growing in the storage tank.

Graywater may contain microbial agents which present a public health hazard with reuse. The microbial profiles indicate that graywater can support a high concentration of aerobic heterotrophic mircoorganisms and total and fecal coliforms are readily detected in such waters. These last two types of microbes, particularly fecal coliforms, are indicators of fecal contamination. Although plant material, soil and food debris can contribute to the coliform population, concentrations reaching as high as 10^5 fecal coliforms indicate that enteric pathogens if being excreted by an individual in the household, would also be found in graywater.

Depending on the number of family members infected and the number of family units producing the graywater, a wide variety of enteric pathogens might be recovered from graywater. Regrowth in graywater of *Salmonella* or *Shigella* was not observed in the seeded survival studies as was seen for the coliform bacteria. However, *Salmonella* numbers remained stable for 2 d in graywater, while a more rapid decrease was observed for *Shigella*. Poliovirus, representing the enteric viruses, was found to have a similar survival rate in graywater, during the first 3–4 d, as *Salmonella*. After 8 d of incubation, concentrations had decreased only by $1.39\ \log_{10}$ while *Salmonella* numbers had decreased by $3.6\ \log_{10}$. Due to the low infectious dose of viruses, even low concentrations would be of concern.

Graywater reuse in Arizona currently falls under the regulations governing wastewater reuse. A level of 25 fecal coliform per 100 ml and 5 NTU of turbidity are two of the standards on record (Arizona, 1984). The levels of microorganisms and turbidity found in graywater are much greater than these standards. Although the possible health risks associated with exposure to graywater is undefined, it is prudent to take a cautious approach. In addition to the potential hazard, aesthetic and management concerns would mandate some level of graywater quality.

For the practical application of a graywater reuse system, treatment prior to reuse would be necessary. The major concerns appear to be turbidity, microbial concentration and the potential presence of pathogens. Possible treatments include storage, sedimentation, filtration, biological treatment and disinfection. Currently in Tucson, a water conservation and reuse demonstration house, "Casa del Agua," is under investigation (Rose *et al.*, 1988). The facility was built to evaluate the practicality and overall effects of implementing graywater and rain water harvesting sytems to supplement household needs. Various treatment systems for graywater are being evaluated to reduce microbial numbers before reuse.

Acknowledgements—We wish to thank B. C. Weimer and R. S. Silverman for their excellent technical assistance.

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