Science of the Total Environment 538 (2015) 230–239

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Epidemiological study for the assessment of health risks associated with graywater reuse for irrigation in arid regions

Allison Busgang [a], Eran Friedler [b], Ofer Ovadia [c], Amit Gross [a,*]

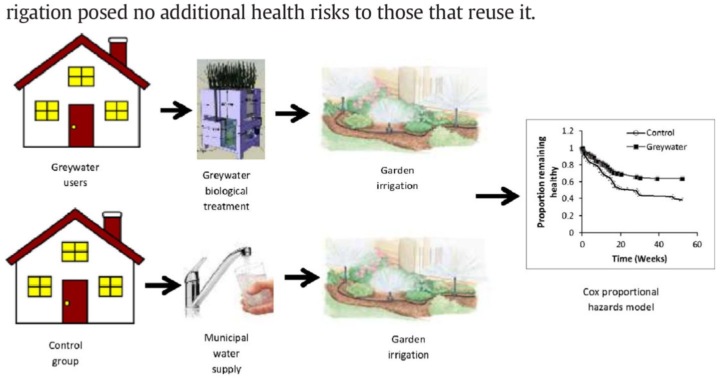
[a] Zuckerberg Institute for Water Research, Blaustein Institutes for Desert Research, Ben Gurion University of the Negev, Sede Boqer Campus, Midreshet Ben Gurion 84990, Israel
[b] Faculty of Civil & Environmental Engineering, Technion – Israel Institute of Technology, Haifa 3200003, Israel
[c] Department of Life Sciences, Ben Gurion University of the Negev, Beer Sheva 8410501, Israel

HIGHLIGHTS

- No additional burden of disease was found among graywater users in this study.
- Rate of illnesses found was less than the Israeli national rate for gastroenteritis.
- No specific exposures could be distinguished as a cause of illness.

GRAPHICAL ABSTRACT

Schematic representation of experimental design and main results suggesting that greywater use for garden irrigation posed no additional health risks to those that reuse it.



ARTICLE INFO

Article history:
Received 7 July 2015
Received in revised form 3 August 2015
Accepted 3 August 2015
Available online xxxx

Editor: D. Barcelo

Keywords:
Epidemiology
Graywater
Gastroenteritis
Risk analysis

ABSTRACT

Graywater reuse is rapidly gaining popularity as a viable source of reclaimed water, mainly for garden irrigation and toilet flushing. The purpose of this study was to determine, by epidemiological survey, the risk for gastroenteritis symptoms associated with graywater reuse. The study comprised a weekly health questionnaire answered by both graywater users and non-graywater users (control group) regarding their health status over a period of 1 year, and periodic sampling for graywater quality. Participants were also asked to respond to a one-time lifestyle questionnaire to assess their level of exposure to graywater or potable water used in garden irrigation. Graywater quality was typical and comparable to previous studies, with average fecal coliform concentration of 10^3 CFU 100 ml^{-1}. A Cox Proportional Hazards model indicated a somewhat higher health risk for the control group ($P < 0.05$), suggesting that there was practically no difference in the prevalence of water-related diseases between users of graywater and potable water. Since the concentration of pathogens in the current study was higher than that suggested by quantitative microbial risk assessment (QMRA), yet there was no difference in the prevalence of water-related diseases between control and graywater users, it was postulated that QMRA is conservative and can safely be used toward the establishment of regulations governing graywater reuse.

* Corresponding author.
E-mail addresses: abusgang@gmail.com (A. Busgang), eranf@tx.technion.ac.il (E. Friedler), oferovad@bgu.ac.il (O. Ovadia), amgross@bgu.ac.il (A. Gross).

http://dx.doi.org/10.1016/j.scitotenv.2015.08.009

1. Introduction

Recently, there has been growing interest in the separation and reuse of graywater as a water-saving strategy, particularly in water-scarce regions. Approximately 60% of domestic effluent is graywater, including effluents from washing, bathing and laundry, but excluding toilet water (Gilboa and Friedler, 2008). Graywater is typically used to irrigate gardens and flush toilets and can save up to 50% of household freshwater demand (Maimon et al., 2010; Ottoson and Stenström, 2003). Furthermore, graywater reuse can be economically feasible on both a national and household scale (Gross et al., 2015).

Alongside its potential water-saving benefits, graywater must be handled responsibly to eliminate potential environmental and health risks (Gross et al., 2015). Graywater is often found to be contaminated with various pathogens associated with fecal contamination, as well as opportunistic pathogens (Table 1).

Fecal contamination of graywater is usually the result of activities such as washing fecally contaminated laundry (e.g. diapers and underwear) and showering and washing hands after contact with potentially contaminated surfaces and objects (Ottoson and Stenström, 2003). Fecal contamination, which is commonly estimated by fecal coliform concentration, exhibits high variability, ranging from non-detectable to as many as 10^6–10^7 CFU 100 ml^{-1}. The skin and mucus pathogens *Pseudomonas aeruginosa* and *Staphylococcus aureus* have been found in graywater at concentrations of 10^2 and 10^5 CFU 100 ml^{-1}, respectively (Gilboa and Friedler, 2008). Occasionally gastrointestinal bacteria, such as *Salmonella enterica* and *Campylobacter*, can be introduced by food-handling in the kitchen (Ottoson and Stenström, 2003; Gilboa and Friedler, 2008). On-site treatment systems and the subsequent localized discharge of treated effluent can lead to public health and environmental concerns through direct contact with the effluent and contamination of groundwater resources (Levett et al., 2010). Given the prevalence of fecal related bacteria found in graywater, the health concerns often associated with graywater reuse are mild to moderate gastrointestinal diseases brought on by the possible ingestion of minute to significant amounts of graywater via various exposure pathways (O'Toole et al., 2012; Maimon et al., 2010).

Epidemiology is an essential part of risk assessment. However, it may be limited by the sensitivity of the study, and seeking to associate very small risks to the background can prove to be challenging (O'Toole et al., 2012). Nevertheless, despite their limitations and difficulties, epidemiological studies can provide valuable information on the possible scale of water-related hazards, which complement the predictions obtained from quantitative microbial risk assessments (QMRAs). In addition, epidemiological studies are generally more readily understood and accepted by the public than theoretical models designed to predict disease risks (Sinclair et al., 2010). Interestingly, despite the increasing use of graywater worldwide, only a handful of studies have attempted to evaluate the health risks associated with graywater reuse by epidemiological methods (O'Toole et al., 2012; Sinclair et al., 2010). These studies were all performed in temperate regions where exposures are expected to differ from those in arid regions. Moreover, they were short-term, retrospective studies which were unable to account for seasonality and long-term effects.

In the study by O'Toole et al. (2012), members of an exposed (graywater-using) population in Australia were asked to respond to a questionnaire corresponding to the 2 weeks following graywater sampling in order to assess any link between the pathogens found in the graywater and cases of gastrointestinal illness within the household. Sinclair et al. (2010) performed an epidemiological study on a neighborhood in Australia with a dual reticulation system, where highly treated recycled wastewater was used for toilet flushing and other non-potable uses. The study compared the health status of residents from the dual reticulation area with that of residents of a nearby neighborhood with conventional water supply. Health-status determination was based on the reasons why the residents consulted local general physicians. There were no differences between the exposed group and the control group in either study.

The objectives of this study were, first, to determine whether long-term reuse of graywater for garden irrigation in an arid region leads to a higher incidence of gastrointestinal illnesses compared to a control group, and second, to compare the results of the epidemiological study to published results of health risks determined by QMRA, a common tool used by policy-makers to define risks.

Table 1
Concentrations of pathogens and indicator bacteria found in raw, biologically treated and disinfected graywater by different studies and various enumeration techniques (Numbers in brackets are typical mean orders of magnitude).

Bacteria	Units	Raw graywater	Biologically treated graywater	Disinfected graywater
Total coliforms[a]	Log CFU 100 ml^{-1}	5–7 (5)	2–7 (<2)	n.d.–2 (<1)
Fecal coliforms[a]	Log CFU 100 ml^{-1}	1–7 (4)	1–5 (2)	0–5 (<0)
Pseudomonas aerigunosas[a]	Log CFU 100 ml^{-1}	3–5 (3)	n.d.–4 (2)	n.d.–4 (<1)
Staphylococcus aureus[a]	Log CFU 100 ml^{-1}	4–6 (4)	n.d.–3 (1)	n.d.–3 (<1)
Shigella spp.[b,e]	Log gene copies 100 ml^{-1}	n.d.[b,c]	n.d.–4 (n.d.)[e]	n.d.
Salmonella enterica[d,e]	Log gene copies 100 ml^{-1}	n.d.[d]	n.d.–3 (n.d.)[e]	n.d.

n.d. — non-detectable.
[a] Gilboa and Friedler, 2008; Winward et al., 2008; Boyjoo et al., 2013.
[b] Jefferson et al., 2004.
[c] No mention of methodology or units.
[d] Birks and Hills, 2007.
[e] Benami et al., 2013.

2. Materials and methods

2.1. Study population and climate

Exposed (graywater-using) and control (non-graywater-using) populations from the Ramat Negev regional council, Central Negev, Israel were asked about their health status on a weekly basis for a period of 1 year from December 2013 until December 2014. In Israel, graywater reuse is not prevalent due to legal issues. No official registry of graywater users exists, limiting the sample size in this study. The exposed group consisted of all known families recycling graywater in the region, i.e., 20 families totaling 75 individuals, and a matched control group consisting of 17 families with a total of 73 individuals. The control group was matched based on region and age. The ages of the study participants ranged from several months to a maximum 62 years at the start of the study (Fig. C.1 in Appendix C). The age distribution was similar for both groups ($\chi^2 = 7.525$, DF = 5, $P = 0.184$).

The climate in the Central Negev, Israel is arid desert with an average yearly precipitation of <100 mm over a period of 41 days per year, mostly during the winter months (December to March) with only few rain events in fall and spring and no rainfall at all during the summer months. During the hot summer months (May through September), the average temperature is 32 °C and during the colder winter months, the average temperature is 18 °C (data represent trends from 1970–2000) (Israel Meteorological Service, 2015).

2.2. Graywater quality

Graywater samples from participating houses were sampled at least five times during the study and analyzed for fecal coliforms, total suspended solids (TSS) and biological oxygen demand (BOD) to identify the quality spectrum of the graywater used. Samples were taken back to the laboratory and analyzed for fecal coliforms by membrane-filtration technique using TBX selective agar (Merck, 2000), TSS by gravimetric method and BOD by standard method within less than 24 h. All analyses followed standard procedures (APHA, 2005).

The quality of the graywater being reused by participants in this study was determined in order to understand the potential risks involved and to insure that graywater systems were functioning properly and should not impact the study in unexpected ways.

The quality varied according to the different treatment systems used by the landlords. Generally, four types of graywater systems were used: a) direct bucket irrigation with raw graywater from the kitchen sink and shower, b) recirculating vertical flow constructed wetlands (RVFCW) following the design of Sklarz et al. (2009) either being maintained professionally or c) by the homeowner, and d) a custom-made closed slow sand filter and settling tank.

The water being used to irrigate the control gardens was standard municipal tap water which is frequently sampled by municipal authorities and subject to strict Israeli drinking water regulations. The microbiological standards require that there be <1 CFU 100 mL^{-1} and <1 NTU turbidity. Sampling is carried out once every month by the Ministry of Health (Israel Ministry of Health, 2013).

2.3. Study questionnaire

At the start of the study period, an exposure-assessment questionnaire was sent out to the participants to estimate probable relevant exposure pathways (Appendix A). Questions were related to activities in the family garden, including amount of time spent in the garden and the type and timing of irrigation employed. Other questions, such as the presence of pets in the household and the end use of the graywater, were also asked.

To get reliable data on sickness, a questionnaire (Appendix B) was sent out via weekly email for a period of 52 weeks to one representative family member who was instructed to respond if someone in the family had been sick with symptoms of gastroenteritis, including upset stomach, diarrhea, nausea, vomiting or fever, during the previous week. If an individual was sick, they were then instructed to fill out the remainder of the questionnaire regarding the activities of the individual who was sick during the week preceding the illness to ascertain his/her exposures. If individuals failed to respond to the weekly email, a phone call was made to the specific household and the questionnaire was filled out over the phone.

2.4. Data and statistical analysis

To test for the effect of graywater use on rate of illness, a Cox Proportional Hazards model (Cox, 1972) was used, with graywater exposure (control, i.e., non-graywater-using = 0, and exposed, i.e., graywater-using = 1), sex (female = 0, and male = 1), age (in years), and the respective interaction terms as covariates. Time-to-event data are commonly analyzed using this method, which enables the evaluation of effects of different covariates on the rate at which the event in focus (e.g., illness) occurs independent of the time-varying background hazard rate (e.g., illness rate) (Kalbfleisch and Prentice, 2002). Using the Cox Proportional Hazards model allows for the estimation of a coefficient (β) for each one of the covariates and tests for its significance. The exponent of the coefficient (i.e., hazard ratio), $\exp(\beta)$, estimates the expected change in the event occurrence rate per one unit change in the covariate, when the other covariates are held constant (Kalbfleisch and Prentice, 2002). Hence, a negative coefficient ($\beta < 0$ or $\exp(\beta) < 1$) corresponds to the reduction in hazard for a one-unit increase in the covariate, and, conversely, a positive coefficient ($\beta > 0$ or $\exp(\beta) > 1$) corresponds to the increase in hazard for a one-unit increase in the covariate. To control for repeated measurements on the same subject, a robust jackknife variance estimator grouped by observations per participant was used (Lin and Wei, 1989). This statistical analysis was performed using S-PLUS 2000 (MathSoft, Inc., Cambridge, MA, USA).

To analyze the potential impacts of exposure factors (i.e. irrigation time, irrigation method, time spent in the garden, etc.) on the

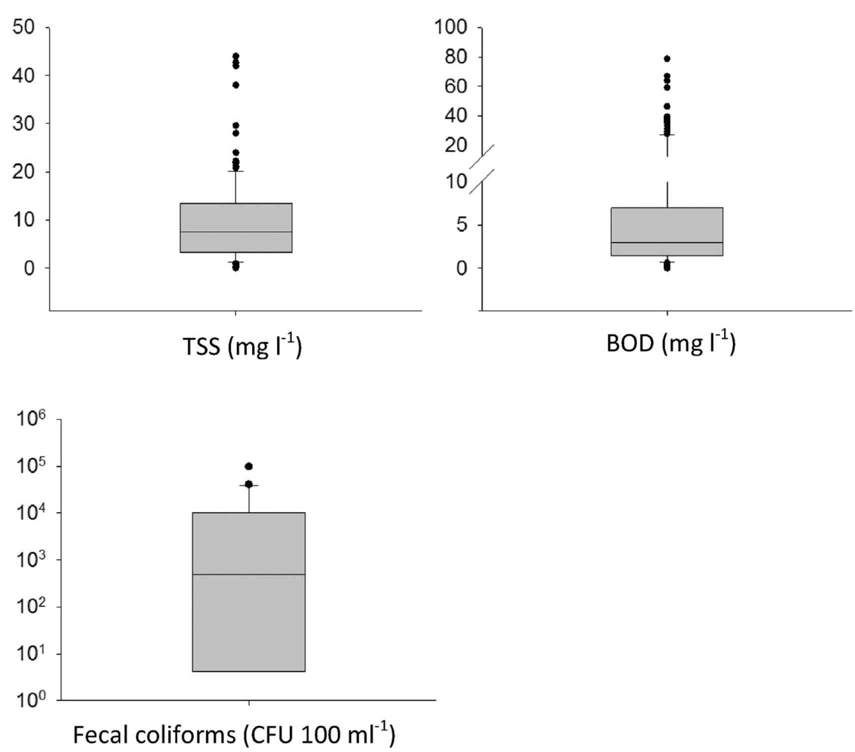


Fig. 1. Graywater quality parameters – TSS, BOD and fecal coliform concentration – in graywater of varying quality ($n_{samples} = 180$).

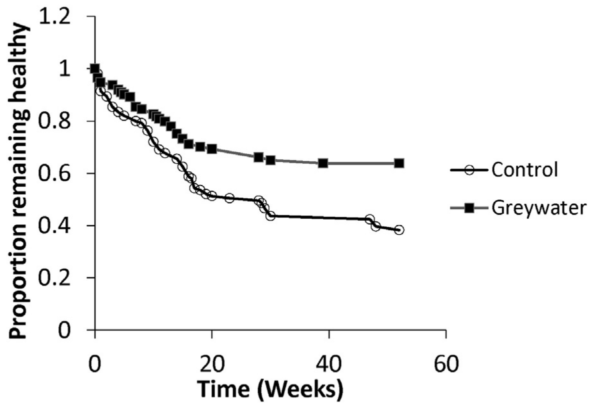


Fig. 2. Kaplan–Meier (Kalbfleisch and Prentice, 2002) illness rate curves for the control (non-graywater-using) and exposed (graywater-using) groups.

occurrence of illnesses, Chi square tests of independence were performed. The severities of the reported illnesses were ranked as follows: 1 – illnesses lasting up to 3 days and not requiring any time off from regular activities; 2 – illnesses lasting up to 3 days and requiring time off from regular activities; 3 – illnesses lasting more than 4 days and requiring time off from regular activities. A Chi square test of independence was performed to test for differences in the severity of reported illnesses between the control group and the exposed group. These analyses were conducted in STATISTICA, v12.0 (StatSoft, Ltd., Tulsa, OK, USA). A power analysis was also conducted to ensure a sufficient number of participants and to determine the number of subjects that would need to be sick at least once throughout the study period to provide sufficient power (results in Appendix D).

3. Results and discussion

3.1. Graywater quality

In this study, the quality of the graywater subjected to various treatments was typical, with TSS ranging from below the detection limit to 50 mg l^{-1}, BOD from below the detection limit to 80 mg l^{-1} and fecal coliforms from non-detectable to 10^5 CFU 100 ml^{-1}; all results were highly representative of graywater quality (Fig. 1). As expected, the range of results for all measured parameters was quite large as graywater quality changes between and within sources over time (Boyjoo et al., 2013; Gross et al., 2015). The results also showed that unless regulations are imposed and enforced, potential pollution from graywater, including fecal contamination, can be expected to vary considerably and often exceed graywater standards (e.g. British (for spray application) and Canadian standards).

3.2. Epidemiological study results

The rate of illness for the graywater and control treatments was compared by the Cox Proportional Hazards model. Results revealed a somewhat higher risk for the control group ($Z = -2.31$, $P = 0.021$) (Fig. 2), suggesting that the main exposure to gastrointestinal disease-causing bacteria is likely not from exposure to pathogens in graywater, but rather from the many other potential sources, both inside and outside of the household. This result coincides with those found in previous studies by O'Toole et al. (2012) and Sinclair et al. (2010), who undertook epidemiological studies and found no connection between illness and graywater or wastewater reuse. In addition, the rate of illness decreased significantly with age ($Z = -2.12$, $P = 0.034$), and tended to be higher in males than in females ($Z = 1.93$, $P = 0.053$), however, none of the interaction terms were significant.

As described in the methods, the severities of the reported illnesses were ranked on a scale of 1 to 3, ranging from mild with no disruption of daily activities, to severe with an over 4-day disruption of daily activities. The severities of the reported illnesses were of interest for comparison against the national average. It was found that 0.05% of the total surveyed population reported being severely ill one or more times during the study period while the remainder of the population was mildly or moderately sick throughout the study period (Fig. 3A). The Israeli national reported incidence rate of severe gastrointestinal disease was 0.138% in 2012 (Israel Ministry of Health, 2014). The rate found in this study was lower, demonstrating the adequacy of the results, despite the small sample size. Israeli national rates for mild and moderate illness events are not recorded anywhere and as expected, in this study, accounted for most of the illnesses reported. No statistically significant differences were found between the responses (mild, moderate, severe) of the graywater users and the control group ($\chi^2 = 0.362$, DF $= 2$, $P = 0.83$).

Although, it was not the focus of the study, it was noted that most of the recorded illnesses, in general, occurred during the colder months of the year, i.e., fall, winter and spring, and the fewest illnesses occurred during the summer with no statistical difference between the treatment groups ($\chi^2 = 3.25$, DF $= 3$, $P = 0.35$), as depicted in Fig. 3B. Summertime is the period when graywater reuse is expected to be highest due to increased irrigation needs, yet the fewest illnesses (in both groups) occurred during this period. This could be due to climatic reasons, as the study took place in an arid region with high solar radiation that can lead to higher rates of pathogen inactivation and faster drying of irrigated surfaces. Due to the high temperatures, it is also possible that most people avoid spending too much time outdoors, thereby reducing

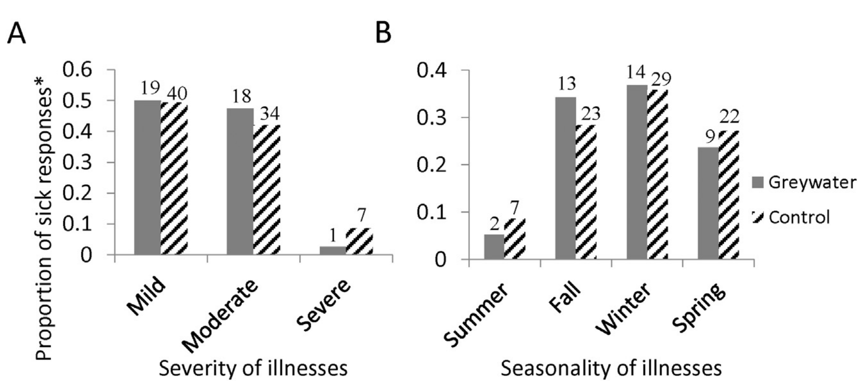


Fig. 3. A) Severity of illnesses reported and B) seasonality of illnesses reported. *Proportion of the population that reported being ill at least once. Numbers above bars represent the number of responses per group.

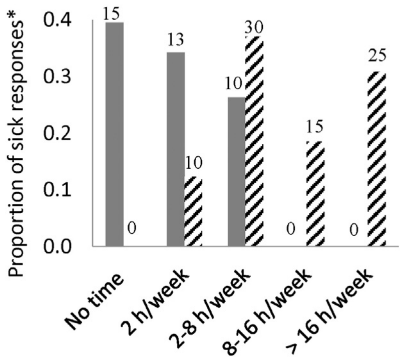


Fig. 4. The proportion of individuals who reported being ill at least once in the study classified by the amount of time spent in the garden per week, with 118 observations. *Proportion of the population that reported being ill at least once.

exposure to graywater during that period of the year. These results also fall in line with the survey of Sinclair et al. (2010), where it was demonstrated that fewer illnesses occur during the summer months despite the increased exposure to recycled wastewater, even when used (against practice recommendations) in swimming pools. This result further stresses the unlikely contribution of graywater reuse as a significant source of illness.

3.3. Relationship between potential exposure and risk

The relationship between various behavioral habits and their effects on exposure and subsequent disease risk was investigated. Participants were asked about exposures such as the end use of the graywater, the type and timing of irrigation, and the amount of time spent in the garden. Alongside these graywater-specific exposures, participants were asked about any unrelated exposures they may have experienced prior to the onset of their symptoms, such as swimming in a public pool or at the beach, and eating food from a restaurant (Fig. C.2, Appendix C).

In general, the three most common activities prior to illness onset were eating food from a restaurant (dine-in or take-away), swimming at the local public pool and being near sick people (e.g. in the household, at school, or work). For about 50% of the control group and 20% of the graywater users, none of the activities listed as potential exposures were relevant before the onset of illness (Fig. C.2, Appendix C). Since only the sick populations, and not the healthy individuals, were asked to respond regarding their exposures during the week prior to illness, links between the exposure activities and the onset of illnesses are uncertain and only considered as possible indications.

3.3.1. Exposure to graywater through time spent in the garden

All of the graywater users who were ill at least once responded that they spent between 0 and 8 h per week in their garden (this could

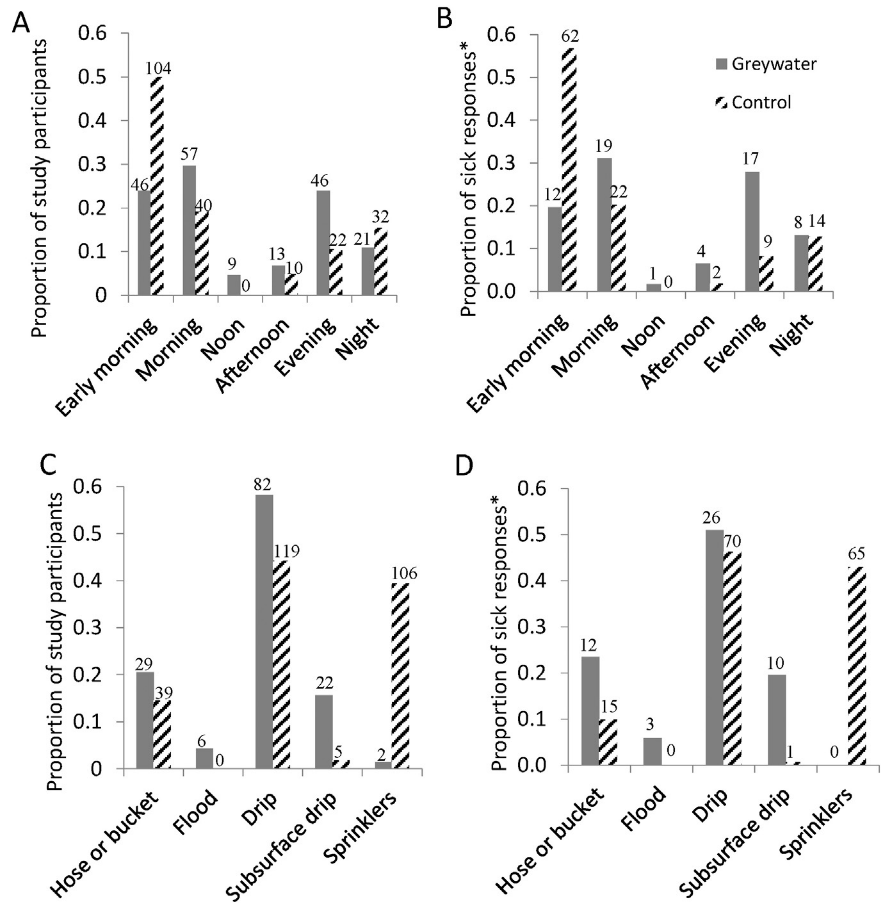


Fig. 5. The proportion of individuals in the study grouped according to time of irrigation, with 192 (graywater) & 208 (control) observations (A), and the proportion of sick individuals during the study period grouped according to irrigation time, with 61 (graywater) & 109 (control) observations (B). The proportion of individuals in the study grouped according to the various types of irrigation, with 141 (graywater) & 269 (control) observations (C), and the proportion of the sick individuals during the study period grouped according to irrigation type, with 51 (graywater) & 151 (control) observations (D). A portion of the respondents used multiple types of irrigation regimes and irrigated at various times of the day accounting for the differing amounts of observations for each figure. *Proportion of the population that reported being ill at least once.

involve sitting, playing, having a meal, etc.), while the respondents who were ill at least once from the control group responded that they had spent between 2 and 16 or more hours in their gardens per week, with zero responses of spending no time in the garden ($\chi^2 = 57.76$, DF = 4, $P < 0.001$) (Fig. 4). This significant difference of amount of time spent in the garden between the exposed and control groups suggests that there is perhaps some difference between these groups that is affecting their behavior not associated with graywater reuse. It is postulated that families with younger children may tend to spend more time in the garden than those with older children. Moreover, the number of young children (<13 years old) was slightly higher in the control group (Fig. C.1) possibly accounting for the population in the control group that reported spending more than 8 h per week in their garden. Whereas, there were fewer families with young children in the graywater group possibly accounting for the population that reported spending no time in their garden.

3.3.2. Exposure to graywater related to irrigation

The effects of irrigation type and timing on possible exposure and the proportion of the population that reported being ill at least once are summarized in Fig. 5. In general, most of the study participants tended to irrigate their gardens in the early morning, morning, evening, and night (Fig. 5A). This makes sense as the study took place in the desert, where evaporation is extremely high at noon, making irrigation very inefficient during the noon and afternoon hours. The time of irrigation was also important for exposure in that people are generally not going to be in their gardens in the early morning and night hours. Moreover, it was observed that most participants used drip irrigation; however, there was also a large proportion of the control group that used sprinklers as well (Fig. 5C). In terms of exposure, households that use flood, drip, and subsurface drip irrigation will not be exposed to any risk from aerosols as these methods do not produce any. Results showed no pattern between timing and means of irrigation and the occurrence of sickness in each group ($\chi^2_{\text{graywater, type of irrigation}} = 1.79$, DF = 4, $P = 0.77$, $\chi^2_{\text{control, type of irrigation}} = 2.95$, DF = 3, $P = 0.40$, $\chi^2_{\text{graywater, time of irrigation}} = 1.97$, DF = 5, $P = 0.85$, $\chi^2_{\text{control, time of irrigation}} = 3.06$, DF = 4, $P = 0.55$) (Fig. 5B & D).

3.3.3. Exposure to graywater via irrigated plants

Almost 60% of the participants used graywater to irrigate an ornamental garden, including flowers, trees and shrubs that have no fruit or herbal uses; 40% of graywater users irrigated fruit trees and only 4% irrigated herbs that were used in teas (not raw as in salads) (Fig. 6).

The irrigation of fruit trees has been shown to pose virtually no risk (Finley et al., 2009). Those authors found that when using drip irrigation, any fecal contamination originating in the irrigation water or soil remains close to the soil surface and does not travel up the plants to the fruits themselves. This suggests that the irrigation of fruit trees should not pose any risk to the individuals eating the fruit if drip irrigation is used. The only way the fruit might become contaminated is by use of sprinkler irrigation, where the irrigation water could come into direct contact with the edible fruit. In this study, only 0.02% of graywater users used sprinkler irrigation, and none of them became sick during the study period (Fig. 5C & D).

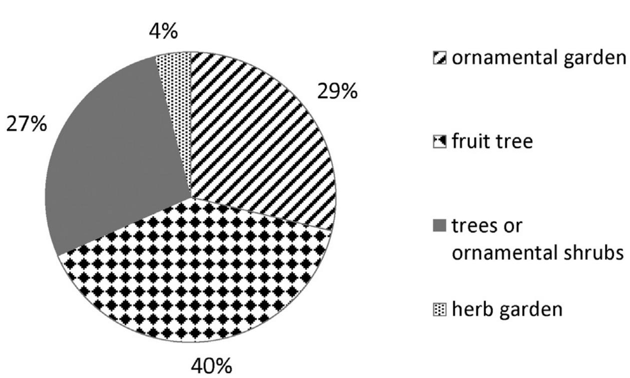


Fig. 6. Graywater uses as reported by participants, based on 174 observations.

3.4. Comparison to QMRA results

QMRA models are typically used to empirically define risks to human health from the use of particular water sources. They are used by policy-makers to develop standard practice guidelines and regulations (Maimon et al., 2010). This model is often considered to be highly conservative: the worst-case risks are usually presented due to inherently high variability and uncertainty within the model (Haas et al., 1999).

Mara et al. (2007) considered the risks of *Rotavirus* infection from wastewater reuse for restricted agriculture (due to accidental ingestion of soil while working or playing in the wastewater-irrigated field) under labor-intensive and highly mechanized conditions. Safe use was met up to a concentration of 10^5 CFU *Escherichia coli* 100 mL^{-1} for labor-intensive agriculture, whereas for highly mechanized agriculture, this limit could be relaxed to 10^6 CFU *E. coli* 100 mL^{-1}. However, if children (under 15 years of age) were present on the farm, the maximum exposure was set at 10^4 CFU *E. coli* 100 mL^{-1}. In addition, good agreement was found between the QMRA results and a small epidemiological survey of the farmers.

Maimon et al. (2010) used *E. coli* as a surrogate for *Rotavirus* and considered the risks of *Rotavirus* infection from graywater reuse for garden irrigation. The authors reported a maximum tolerable concentration of *E. coli* ranging between 10^2 and 10^4 CFU 100 mL^{-1}.

In the current study, graywater contained on average 10^3 CFU 100 mL^{-1} with an upper limit of 10^5 CFU 100 mL^{-1}, and yet no excess illnesses were reported among the graywater users. This supports the notion that QMRA is sometimes on the “conservative side” and therefore can be used as a safe and reliable tool for modeling risks.

4. Conclusions

No additional burden of disease was found among graywater users in this study, suggesting that graywater is not a major source of gastrointestinal disease. Moreover, based on the survey, the majority of the suspected exposures which occurred prior to the onset of illness included activities that were not related to graywater reuse. Previously reported QMRA results were more conservative than the findings from the current epidemiological survey. This implies that QMRA is a conservative tool and can be used reliably for modeling risks associated with graywater reuse. Further research involving a larger study population is recommended as it would enhance the statistical power, and enable elaboration on the possible connections between gastrointestinal illnesses and other potential illnesses and exposures associated with graywater.

Acknowledgments

This research was funded by the Rosenzweig–Coopersmith Foundation (RCF) and Zuck Macabi Foundation. The authors would like to thank the study participants, Ms. Rina Miaskovski for the laboratory assistance and Dr. Ruthi Berger for her assistance with the epidemiological questionnaire.

Appendix A. Initial lifestyle questionnaire

Questionnaire on health and lifestyle habits

Name _______________ Email __________ Date ______________

1. Does your home have a greywater system: Yes / No (if no, skip ahead to question 15)

2. What kind of system: ________

3. When was the system installed? (approximate date): ___________

4. Which of the following water sources are connected to the system (you can mark more than one answer):

Bath / shower / bathroom sink / washing machine / kitchen sink / dishwasher / other: ________

5. What is the graywater used for (you can mark more than one answer):

Flushing toilet / ornamental garden irrigation / vegetable garden irrigation / fruit trees or bushes irrigation / trees or ornamental shrubs irrigation / ornamental ponds / other: ______________

6. What is, in your estimation, the amount of water passing through the system: ______

7. Have you noticed a change in plants or soil since the use of the system: Yes / No.

Please elaborate: ____

8. Does water accumulate on the ground after irrigation: Yes / No.

9. Have you noticed any unpleasant odors from the system or irrigation line: Yes / No

10. Have any insects been found in the system water:

Mosquitoes / mosquito larvae / cockroaches / mites / other: _______

11. What is the frequency of maintenance on the system, on average:

Once a week / every other week / once a month / every two months / 2-5 times a year / once a year or less / other: ___________

12. How long does maintenance of the system take and what kind of maintenance is required

____

13. Does someone from the family maintain the greywater system: Yes / No, or is there a professional who maintains the system: Yes / No.

14. Are any of the following measures used during maintenance of the system (mark an X where appropriate):

	Almost always/ Always	Often	Rarely	Never
Gloves				
Hand washing				

15. What form of irrigation is used in the garden (you can mark more than one answer):

Hose or bucket / flood / covered conduits / drip / subsurface drip / sprinklers / other: ______

16. What is, to the best of your knowledge, the area of irrigated land: __________

17. When is irrigation usually carried out:

Early morning / morning / noon / afternoon / evening / night

18. How often is the garden irrigated:

Every day / every other day / every week / every other week / once a month / less than once a month / other: ______________________

19. Does someone from the family maintain the garden: Yes / No, or is there a gardener: Yes / no.

20. Is the gardening done with the use of any of the following (mark an X where appropriate):

	Almost always/ Always	Often	Rarely	Never
Gloves				
Hand washing				

21. How much time, on average, do the members of the household spend in the irrigated area?

Two hours per week / 2-8 hours per week / 8-16 hours per week / over 16 hours a week

22. Age and gender of individuals living in the household (including year of service and soldiers):

1. M / F____ 2. M / F____ 3. M / F____ 4. M / F____ 5. M / F______ 6. M / F _____ 7. M / F _____ 8. M / F______ 9. M / F_____

23. Do you have pets in the house: dog / cat / rodent / bird / reptile / other ___________

24. Do family members regularly visit (at least once a week) petting zoos, farms or stables? Yes / no

Appendix B. Weekly health questionnaire

Weekly health questionnaire

Please indicate if anyone in the family has been sick with the following symptoms in the last week. If no one in the family was sick then there is no need to answer any of the questions below, simply reply to the email by saying "no one was sick".

Please fill in **for each family member** if they suffered from any of the following symptoms:

	Diarrhea	Vomiting	Nausea	Stomach pain	Fever
Gender and age					
Duration (days)					
Gender and age					
Duration (days)					
Gender and age					
Duration (days)					
Gender and age					
Duration (days)					

If you answered yes to any of the symptoms listed above then **please answer for each sick family member the following questions**:

1. Did the sick family member visit a doctor as a result of his symptoms? Yes / no / I don't know
2. Did the symptoms cause the sick family member to miss school / work / neither? (please indicate which)
3. Did the symptoms cause anyone else in the family to miss school / work / neither? (please indicate which)
4. Did the sick family member eat food during the week before his symptoms started from a restaurant / take away / neither?
5. Did the sick family member eat raw fruits, vegetables, herbs from your garden during the week before the symptoms started? Yes / no / I don't know
6. Did the sick family member go swimming during the week before his symptoms started? Yes / no / I don't know. Please indicate if it was a public pool / private pool / beach
7. Was the sick family member doing any gardening during the week before his symptoms started? Yes / no / I don't know
8. Was the sick family member taking antibiotics during the week before his symptoms started? Yes / no / I don't know
9. Did the sick family member drink (accidentally or purposely) from the water used to irrigate the garden during the week before his symptoms started? Yes / no / I don't know
10. Was the sick family member exposed to any sick people during the week before his symptoms started at school / work / neither / I don't know?

Appendix C. Additional relevant figures

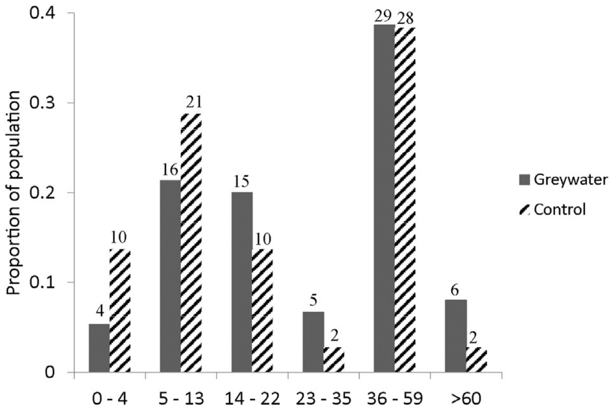


Fig. C.1. Participant age distribution was consistent between the graywater and control group ($\chi^2 = 7.525$, DF = 5, $P = 0.184$). The number of participants (n) in each age group is shown above each column, $N_{total} = 148$.

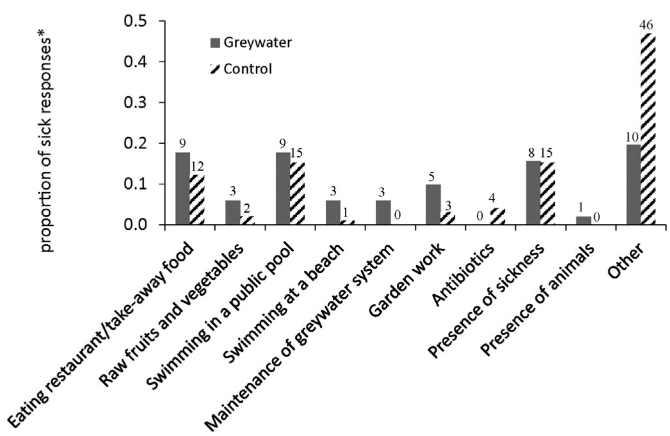


Fig. C.2. Proportion of the total sick population's reported activities the week prior to onset of illness, with 149 observations. 'Other' refers to instances in which none of the listed exposure activities were taken part in during the week prior to illness. *Proportion of the population that reported being ill at least once.

Appendix D. Power analysis

A one-sided power test estimating the required sample size for a Cox regression set at 80% power was performed. The result of this analysis determined that the required sample size is 128 participants, of which 52 should become ill at least once. In this study, the total number of participants was actually 148, of which 61 graywater using individuals were sick at least once during the study period. Indeed, the power calculated for the observed data in this study was 84%.

References

APHA, 2005. Standard Methods for the Examination of Water and Wastewater. 21st edition. American Public Health Association, Washington, D.C.

Benami, M., Gross, A., Herzberg, M., Orlofsky, E., Vonshak, A., Gillor, O., 2013. Assessment of pathogenic bacteria in treated graywater and irrigated soils. Sci. Total Environ. 458–460, 298–302.

Birks, R., Hills, S., 2007. Characterisation of indicator organisms and pathogens in domestic greywater for recycling. Environ. Monit. Assess. 129, 61–69.

Boyjoo, Y., Pareek, V., Ang, M., 2013. A review of greywater characteristics and treatment processes. Water Sci. Technol. 67, 1403–1424.

Cox, D.R., 1972. Regression models and life-tables (with discussion). J. R. Stat. Soc. Ser. B 34, 187–220.

Finley, S., Barrington, S., Lyew, D., 2009. Reuse of domestic greywater for the irrigation of food crops. Water Air Soil Pollut. 199, 235–245.

Gilboa, Y., Friedler, E., 2008. UV disinfection of RBC-treated light greywater effluent: kinetics, survival and regrowth of selected microorganisms. Water Res. 42, 1043–1050.

Gross, A., Maimon, A., Alfiya, Y., Friedler, E., 2015. Greywater Reuse. CRC Press, New York, pp. 1–258.

Haas, C.N., Rose, J.B., Gerba, C.P., Charles, P., 1999. Quantitative Microbial Risk Assessment. Wiley, New York.

Israel Meteorological Service, 2015. Retrieved June 21, 2015, from http://ims.gov.il/IMSENG/All_Tahazit/homepage.htm.

Israel Ministry of Health, 2013. Public health regulations — the sanitary quality of potable water and potable water facilities. State of Israel Official Gazette 7262pp. 1394–1424 (in Hebrew).

Israel Ministry of Health, 2014. Infectious Diseases. Health 2013. The Israel Center for Disease Control (Chapter 5).

Jefferson, B., Palmer, A., Jeffrey, P., Stuetz, R., Judd, S., 2004. Grey water characterisation and its impact on the selection and operation of technologies for urban reuse. Water Sci. Technol. 50, 157–164.

Kalbfleisch, J.D., Prentice, R.L., 2002. The Statistical Analysis of Failure Time Data. John Wiley & Sons, Hoboken, New York.

Levett, K., Vanderzalm, J., Page, D., Dillon, P., 2010. Factors affecting the performance and risks to human health of on-site wastewater treatment systems. Water Sci. Technol. 62, 1499–1509.

Lin, D.Y., Wei, L.J., 1989. The robust inference for the Cox proportional hazards model. J. Am. Stat. Assoc. 84, 1074–1079.

Maimon, A., Tal, A., Friedler, E., Gross, A., 2010. Safe on-site reuse of greywater for irrigation — a critical review of current guidelines. Environ. Sci. Technol. 44, 3213–3220.

Mara, D., Sleigh, P., Blumenthal, U., Carr, R., 2007. Health risks in wastewater irrigation: comparing estimates from quantitative microbial risk analyses and epidemiological studies. J. Water Health 5, 39–50.

Merck, 2000. Microbiology Manual. Laboratory Products Division, Merck, Darmstadt, Germany.

O'Toole, J., Sinclair, M., Malawaraarachchi, M., Hamilton, A., Barker, S.F., Leder, K., 2012. Microbial quality assessment of household greywater. Water Res. 46, 4301–4313.

Ottoson, J., Stenström, T., 2003. Faecal contamination of greywater and associated microbial risks. Water Res. 37, 645–655.

Sinclair, M., O'Toole, J., Forbes, A., Carr, D., Leder, K., 2010. Health status of residents of an urban dual reticulation system. Int. J. Epidemiol. 39, 1667–1675.

Sklarz, M., Gross, A., Yakirevich, A., Soares, M., 2009. A recirculating vertical flow constructed wetland for the treatment of domestic wastewater. Desalination 246, 617–624.

Winward, G.P., Avery, L.M., Frazer-Williams, R., Pidou, M., Jeffrey, P., Stephenson, T., Jefferson, B., 2008. A study of the microbial quality of grey water and an evaluation of treatment technologies for reuse. Ecol. Eng. 32, 187–197.