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Quantitative Microbial Risk Analysis for Various Bacterial Exposure Scenarios Involving Greywater Reuse for Irrigation

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Abstract: Greywater reuse can significantly reduce domestic water consumption. While the benefits are promising, risks are still under debate. Using a quantitative microbial risk-assessment model, we assessed the health risks associated with greywater reuse. The pathogens *Salmonella enterica*, *Shigella* spp., and *Staphylococcus aureus* were evaluated due to their possible prevalence in greywater and limited information regarding their potential risk with relation to greywater reuse for irrigation. Various exposure scenarios were investigated. Monte Carlo simulation was used and results were compared to the maximum "acceptable" limit of 10^{-6} disability-adjusted life years (DALY) set by the World Health Organization. Safe reuse was met for all worst-case exposure scenarios for *Staphylococcus aureus, Salmonella enterica and Shigella* spp. If their concentrations were kept below 10,000, 50 and 5 cfu/100 mL, respectively. For the best-practice (more realistic) scenarios, safe reuse was met for *Staphylococcus aureus* if its concentration was kept below 10^6 cfu/100 mL. *Salmonella enterica* met the safe reuse requirements if a maximum concentration of 500 cfu/100 mL was maintained and *Shigella* spp. if a maximum concentration was lower than 5 cfu/100 mL. Based on reported concentrations of these bacteria in greywater, proper treatment and disinfection are recommended.

Keywords: greywater; QMRA; DALY; risk assessment; pathogen

1. Introduction

Greywater reuse has been suggested as an alternative to freshwater for some uses, particularly in water-scarce regions [1–4]. Greywater includes all domestic effluent, excluding the wastewater stream generated by toilets (blackwater). It is typically used on a single-household level to irrigate gardens, and can save up to 50% of individual household freshwater demand [1]. It should be noted that other usages of greywater have been suggested but were not discussed in the current study. However, along with its potential benefits, greywater must be handled responsibly to eliminate potential environmental and health risks. Raw greywater quality is highly variable, and it often contains pathogens (Table 1). Typically, a reduction in microbial counts is recorded after biological treatment of greywater, and a further reduction is observed after disinfection (Table 1).

Table 1. Ranges of concentrations (cfu/100 mL) of selected pathogens in raw, biologically treated, and disinfected greywater. Values in parentheses represents mean order of magnitude.

Bacteria	Infection	Raw Greywater	Biologically Treated Greywater	Disinfected Greywater
Staphylococcus aureus [a]	Skin infections	10^4–10^6 (10^4)	n.d.–10^3 (10)	n.d.–10^3 (<10)
Shigella spp.	Some species cause diarrhea, inflammatory bacillary dysentery, or shigellosis	n.d. [b]	n.d.–10^4 (n.d.) [c]	n.d.
Salmonella [d] *enterica*	Some species cause salmonellosis, bacteremia, gastroenteritis, enteric fever	n.d. [e]–10^4	n.d.–10^3 (n.d.) [c]	n.d.

[a] [5–8]; [b] [9]; no mention of methodology or units; [c] [10]; [d] [11]; [e] [12]; n.d.—non-detectable.

Several studies focusing on *Rotavirus*, *Norovirus*, *Campylobacter*, and *Cryptosporidium* have defined the acceptable maximum levels of these pathogens and the pathogen-reduction requirements in wastewater and greywater [13,14]. These pathogens are all related to gastrointestinal diseases and are often used as model pathogens when investigating health risks in treated water, wastewater, and greywater. Other bacteria known to cause gastrointestinal diseases, such as *Salmonella enterica*, *Shigella* spp., and *Staphylococcus aureus* (the latter might also causes dermal infection), have also been found in greywater [5,6,10,15]. However, there are only a few limited quantitative studies in the literature on the risk of exposure to these pathogens in greywater [16–18].

Health risks associated with greywater reuse are largely unknown due to limited quantitative risk research [14,19–22]. Moreover, there are no reports connecting greywater reuse with actual public health issues such as disease outbreaks. These are likely to be the reasons for the wide discrepancy between countries' regulations for greywater reuse.

The main health concern associated with greywater reuse is mild to severe gastrointestinal diseases brought on by the possible ingestion of minute to significant amounts of greywater via various exposure pathways [14,21,23]. Quantitative microbial risk assessment (QMRA) is frequently used to define the human health risks of various practices involving wastewater reuse. It is comprised of four distinct steps: (i) hazard identification and enumeration of pathogens of concern; (ii) exposure assessment, which evaluates how and to what extent individuals might come into contact with the pathogens; (iii) dose-response modeling of the probability of illness based on the exposure to different dosages of pathogens; and (iv) risk characterization, to determine the annual probability of illness and, as a consequence, the maximum acceptable risk [24]. Overall, QMRA is used to determine the risks associated with specific exposure scenarios and to establish the required pathogen reduction in order to comply with (or be below) the maximum acceptable risk for a particular use. It is often used to create best-practice recommendations, guide law-makers, and inform policy [24].

The maximum acceptable risk is based on the World Health Organization's [25] standard measure of disability-adjusted life years (DALYs). The DALY is a measure of global disease burden expressed as the number of years lost due to illness, disability, or early death. The basic principle of this measure is to weigh each health outcome caused by a specific agent for its severity (between 0 and 1), with death being the most severe outcome (i.e., a value of 1). This weight is then multiplied by the duration of the health effect, and by the number of people in a population affected by the particular outcome. Summarizing all health outcomes caused by one agent will result in an estimate of the burden of disease attributable to this agent [26]. The WHO has set a maximum acceptable level of risk at 10^{-6} DALYs per person per year (PPPY) for all water-related illnesses [25]. Hence, [13] estimated that for a community, a maximum of 0.1% of the population may become ill each year.

The increasing practice of greywater reuse worldwide in recent years and the potential health risks involved, combined with a lack of significant risk evaluation, suggest the need for quantitative tools to evaluate health risks associated with greywater reuse. The focus of this research is the evaluation of quantitative microbial risk associated with the best-practice and worst-case scenarios

of on-site greywater reuse for garden irrigation. Greywater due to its origin may contain skin- and mucous-tissue pathogens, such as *Staphylococcus aureus* [5]. Greywater originating from the kitchen sink and dishwasher may contain pathogens introduced by food handling such as *Salmonella sp*. and *Shigella* spp. Therefore, *Salmonella enterica*, *Shigella* spp., and *Staphyloccocus aureus* were identified as pathogens of concern for which risk assessment is of interest with respect to greywater reuse. Each bacterium was investigated individually for the maximum acceptable pathogen concentration that yields a risk below DALY limit for the examined scenarios.

2. Materials and Methods

2.1. Development of the QMRA Model

2.1.1. Hazard Identification

Salmonella enterica, *Shigella* spp., and *Staphylococcus aureus* were chosen as representative of the expected risks from greywater for this study. *Salmonella enterica* is a common pathogen associated with food handling in kitchen wastewater that might, on rare occasion, be included in greywater-reuse schemes. *Shigella* is an opportunistic pathogen that has been found in previous studies in treated greywater [9,10,27]. *Salmonella enterica* and *Shigella* spp. represent the oral route of ingestion and possible infection, which might result in symptoms of mild to severe gastroenteritis. *Staphylococcus aureus* is another common opportunistic pathogen found in greywater [5,6,10] that can cause skin infections from dermal contact. It is shed during bathing activities and may enter the greywater stream through the bath and shower effluent [6,10].

2.1.2. Exposure Assessment

The major exposure routes associated with greywater reuse for garden irrigation were identified as follows. The scenarios associated with the worst-case exposures were: (i) accidental drinking; (ii) garden irrigation that generate aerosols (e.g., sprinkler irrigation); (iii) garden work and lounging that might lead to hand-to-mouth contact with the greywater, such as after gardening, playing, or lounging in the irrigated area; (iv) food crop consumption of crops irrigated with greywater; and (v) accidental handwashing using the greywater. The best-practice exposure scenarios that one might be exposed to are modifications of the worst-case exposures to represent more realistic scenarios that consider practice guidelines, such as washing hands after being in the garden and avoiding the irrigation of vegetable gardens. Since in the best-practice exposure scenarios the residents are considered aware of the potential risks and professionals are the ones to maintain the greywater systems, scenarios such as of accidental drinking and garden irrigation were not analyzed. The scenarios associated with the best-practice exposures were (vi) system maintenance might lead to aerosols ingestion by the system technician; (vii) garden work and lounging might lead to hand-to-mouth contact when this scenario considers the reduction from handwashing; (viii) herb crop consumption considering reduction of microorganisms from washing herbs; and (ix) dermal contact during greywater system maintenance.

All parameters and detailed descriptions regarding the worst-case and best-practice exposure scenarios can be found in Table 2, with additional parameters found in Appendix A (Table A1). The approach taken was identification of exposure scenarios that are relevant for on-site greywater reuse (based on literature and the research team's long-term experience in a close monitoring of 20 greywater systems for over 8 years in 4 climatic zones in Israel [28]). The chosen volumes and frequencies are based on literature that dealt with such estimations (Table 2). From this literature, the ranges were stated, and a representative middle value was used. It should be noted that all values are estimations and not based on direct observation from on-site greywater systems. All of the parameters were assumed to follow a triangular distribution [29]. Exposure scenarios (i) through (iv); and (vi) through (viii) (Table 2), were investigated with respect to *Salmonella enterica* and *Shigella* spp.

The scenarios investigated for *Staphylococcus aureus* were (v) and (ix), which are related to dermal contact with the greywater. Therefore, exposure scenarios associated with *Staphylococcus aureus* were not based on volume of water ingested but instead on the amount of time spent with one's hands submerged and the film thickness of the water remaining on the hands after washing while air drying [30]. All exposure scenarios were also associated with an estimated frequency of occurrence per year, because most of these activities can occur multiple times per year. Some of the scenarios' frequencies were taken from previous studies and others were collected in an epidemiological study that we performed, in which dozens of people were asked questions regarding their habits and possible contact with greywater on a weekly basis for a year, as detailed in [31]. Scenario (vii) (garden work and lounging) differs from scenario (iii) in that it includes the transfer efficiencies of microorganisms to various surfaces and hands. Scenarios (vii) and (ix) (dermal contact) additionally consider pathogen reduction from handwashing with soap after the exposure activity is completed (Table A1).

The scenarios of food and herb crop consumption (iv and viii) were represented by Equation (1), adapted from [14]:

$$\mathrm{d} = \mathrm{IVc}10^{-\mathrm{w}}\mathrm{e}^{-\mathrm{kt}} \quad (1)$$

in which d is the daily dose of bacteria that a person could be exposed to (cfu/day), I is the average amount of produce consumed by the Israeli public per person per day (g/day), V is the volume of water which might cling to the plant surface (mL/g), c is the concentration of bacteria in the greywater (cfu/mL), w is the $\log_{10}$ reduction in bacterial concentration from washing the produce, k is the kinetic decay constant (per day), and t is the withholding period (days). For the worst-case scenarios, it is assumed that no protective measures are taken with regard to irrigation practices. Thus, w and t in Equation (1) are considered to be zero. For the best-practice scenarios, it is assumed that the produce is washed and a withholding period is applied (Table A1). It should be noted that in this case, the food and herb crop consumption were calculated according to the Israeli data; however, it can easily be changed for each region around the word.

Table 2. Potential worst-case and best-practice scenarios of exposure to greywater under investigation in this study. Additional relevant parameters associated with the best-practice exposure scenarios are found in Appendix A, Table A1.

Exposure Scenario	Activity	Route of Exposure	Volume (mL) [a]	Frequency (PPPY) [a,b]	Comment	References
i	Accidental drinking	Accidental consumption of greywater	100 (50–200)	1 (0.5–2)	Child/third party unknowingly drinking from the garden hose or system if exposed.	[20,32]
ii	Garden irrigation	Aerosols from irrigation	0.1 (0.05–0.2)	180 (52–365)	This scenario assumes that the residents are always present in the garden during irrigation events and that aerosols may be ingested during this time.	[20,31,32]
iii	Garden work and lounging	Ingestion due to contact with plants, soil, pipes	1 (0.5–2)	20 (4–35)	Occurs by hand-to-mouth transfer of microorganisms.	[20,31,32]
iv	Food crop consumption	Ingestion of crops irrigated with greywater	425 (300–550) g 0.1 (0.05–0.2) mL	7 (0–14)	This scenario examines the possibility of food crops becoming contaminated with pathogens from direct irrigation with greywater.	[17,20,31–33]
v	Hand-washing	Washing hands with greywater	20 (10–30) µm 40 (20–60) s	1 (0.5–2)	Scenario associated with accidental dermal contact through handwashing. Instead of volume, these values are film thickness in µm of the water remaining after handwashing (s represents washing time in seconds).	[34,35]
vi	System maintenance	Aerosols during system maintenance	0.1 (0.05–0.2)	4 (3–6)	This scenario assumes that the greywater system technician is performing maintenance every 2–4 months.	[36]
vii	Garden work and lounging	Indirect ingestion (contact with plants or soil)	0.1 (0.05–0.2)	16 (8–28)	Occurs by hand-to-mouth transfer of microorganisms. The transfer efficiencies of microorganisms to surfaces and hands, and the reduction from handwashing is considered in this scenario.	[20,37,38]
viii	Herb crop consumption	Ingestion of greywater-irrigated herbs	5 (0–20) g 0.1 (0.05–0.2) mL	7 (0–14)	This scenario examines the possibility of herbs irrigated by drip irrigation becoming contaminated with pathogens from irrigation with greywater; environmental decay, reduction from washing herbs, and a withholding period are considered.	[14,17,20,39]
ix	Dermal contact	Hands contacting greywater	20 (10–30) µm 40 (20–60) s	4 (3–6)	Accidental dermal contact during greywater system maintenance. Instead of volume, these values are film thickness in µm of the water remaining after handwashing (s represents washing time in seconds). This scenario also considers reduction from handwashing.	[34–37]

[a] The values of volume and frequency presented in the table are averages, and in parentheses are the minimum and maximum expected exposures that are ±50% of the published average value; [b] frequency is defined as the number of possible occurrences PPPY. Most exposure scenarios are likely to occur more than once a year.

2.1.3. Dose-Response Modeling

The dose-response model is used to determine the risk of infection at various doses of exposure to the microorganism under investigation as described by [24]. Previous studies used the beta-Poisson dose-response model for *Salmonella enterica* [17,18,40,41] and *Shigella* spp. [24,42]:

$$P_{inf} = 1 - (1 + \frac{d}{N_{50}}\left(2^{\frac{1}{\alpha}} - 1\right))^{-\alpha} \quad (2)$$

in which P_{inf} is the probability of infection from a single exposure (based on d, the dose of microorganisms consumed (number of pathogens)), N_{50} is the median infective dosage (number of pathogens), and α is a shape factor. The beta-Poisson dose-response model is widely used in QMRA studies, including risk assessment of greywater [17,18,40,41].

Staphylococcus aureus follows an exponential dose-response model [30]:

$$P_{inf} = 1 - \ exp^{(-d/k)} \quad (3)$$

in which d is the dose of microorganisms that a person may be exposed to, similar to the beta-Poisson model, with the units of days × number of pathogens per cm^2, and k is a characteristic of the process.

To account for multiple exposures per year, the individual probabilities are summed over a specified period of the year as follows:

$$P = 1 - \left(1 \ - P_{inf}\right)^{n} \quad (4)$$

in which P is the probability of infection from n exposure events per year.

All parameters related to the model can be found in Table 3.

Table 3. Dose-response parameters for all bacteria under investigation.

Parameter	Distribution or Point Estimates, Mean	References
Disease burden (B)	DALYs per case of illness	
Salmonella spp.	49×10^{-3}	[43]
* *Staphylococcus aureus*	2.6×10^{-3}	[43]
Shigella spp.	26×10^{-3}	[44]
Dose-response models		
Salmonella enterica	Beta-Poisson: $\alpha_{se} = 0.3136$, $N_{50se} = 2.4 \times 10^4$	[17,18,40,41,45,46]
Staphylococcus aureus	Exponential: $k_{sa} = 1.31 \times 10^7$	[30]
Shigella spp.	Beta-Poisson: $\alpha_s = 0.162$, $N_{50s} = 1.127 \times 10^3$	[24,42]

* The value of disease burden for *Staphylococcus aureus* is a "composite" value which is based on major infection pathways (and not distinctively for skin contact).

All exposure scenarios were simulated using Monte Carlo simulation. Each scenario ran 500,000 times, in which the frequency of volume and events were randomly changed according to the corresponding distribution, for each run. The pathogen concentrations were also modified between the limits appear in Table 1. The output of the model generated 500,000 pathogen concentrations compatible with annual probabilities of infection, for each scenario. The output data was statistically analyzed. The model was developed using Matlab, 2013.

2.1.4. Risk Characterization

The annual probability of infection was determined for each exposure scenario, and the risk was characterized as above or below the maximum tolerable risk suggested according to the DALY [25]. The DALY can be converted to a probability of illness or infection by the following equation:

$$\text{DALY} = P_{ill} \times B \times S_f \quad (5)$$

in which P_{ill} is the probability of symptomatic illness occurring, B is the pathogen-specific burden of disease, and S_f is the susceptible fraction of the population. To keep the analysis more conservative, it was assumed that the probability of infection is equal to the probability of illness ($P_{inf} = P_{ill}$). The entire population was also assumed to be susceptible to infection by all pathogens ($S_f = 1$); no immunity development was considered, and there was no reduction (die off) or increase (regrowth) in the concentration of pathogens present in the greywater between production (or treatment) and human contact. Using this equation, we calculated for each bacterium the maximum allowable probability of infection per person per year that was used as the maximum limit at which the risk is unacceptable.

2.1.5. Uncertainty and Sensitivity Analyses

To determine the reliability of the QMRA model predictions, uncertainty and sensitivity in the model input were determined following the method of [47]. In principle, the more uncertain a variable is, the higher the associated error may be, and the less sensitive the variable, the more stable it is to changes in the model.

The sensitivity analysis was performed for the examined pathogens and is demonstrated for the worst-case scenarios of *Shigella* spp. (Table A2). The analysis considers the ratios of the output results when the model was run using the 25th, 50th, and 75th percentiles of each input variable, respectively, while holding the remaining input variables at the 50th percentile.

A second sensitivity of the DALY was evaluated by running the model with changed burdens of disease within +10% of the values in the literature.

3. Results and Discussion

3.1. Risk Assessment

The maximum acceptable probability of infection based on the limit of 10^{-6} DALYs PPPY of each bacterium under investigation was calculated according to Equation (5) and was in the range of 10^{-4} to 10^{-5}. Each bacterium was investigated individually for the maximum acceptable pathogen concentration that yields a risk below this DALY limit for both the worst-case and best-practice scenarios described in Table 2.

The risk of infection from a specific pathogen is related to the actual dose received by a person and not to its concentration in the greywater (Equations (2) and (3)). It is therefore clear that different exposure scenarios will result in the reception of different quantities of bacteria; hence, the probability of infection will vary among scenarios. The results from exposure to *Salmonella enterica* in greywater showed that under all worst-case exposure scenarios, the risk was much higher than the DALY limit (2.04×10^{-5} PPPY for *Salmonella*, Figure 1). Exposure scenarios of accidental drinking and garden work had the highest and lowest risk for *Salmonella* infection, respectively. *Salmonella* concentration of 50 cfu/100 mL resulted in crossing the threshold of the *Salmonella* DALY limit for the accidental drinking scenario, whereas for the garden work and lounging scenarios (iii and vii), the DALY limit was surpassed when *Salmonella* concentration was 500 cfu/100 mL.

A few studies have reported the risks of *Salmonella enterica* in greywater [19,48]; however, the presence of this pathogen in greywater is questionable, because a thorough confirmation procedure is needed. Moreover, the major source of *Salmonella enterica* in households is food handling in the kitchen [49]; thus, it is thought to enter the greywater via the kitchen effluent [9]. The relatively low volume associated with kitchen effluent in comparison to other greywater sources, its high contamination with organic matter, and the potential presence of pathogens such as *Salmonella enterica* have led to recommendations by various researchers to exclude kitchen water from greywater-reuse schemes [1,8,50,51].

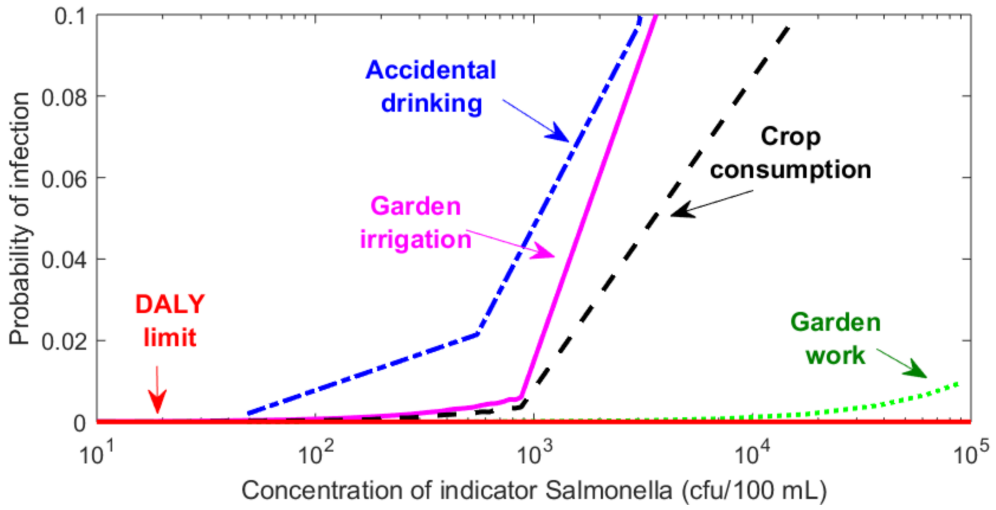

Figure 1. Probability of infection from *Salmonella enterica* in greywater under four worst-case exposure scenarios. The red solid horizontal line represents the DALY limit of acceptable risk as suggested by the WHO [25].

The potential exposure from hand-to-mouth contact during garden work and lounging demonstrates an acceptable risk up to a concentration of 500 cfu/100 mL (Table 4), whereas maximum acceptable risk associated with herb consumption and with the potential infrequent exposure to aerosols during system maintenance was estimated as 2×10^4 cfu/100 mL.

Table 4. Best-practice scenarios for maximum tolerable concentration of the tested pathogens above which the DALY limits are compromised.

Bacteria	Maximum Tolerable Concentration in the Best-Practice Scenarios			
	Ingestion Due to System Maintenance	Ingestion Due to Garden Work and Lounging	Ingestion Due to Herb Crop Consumption	Dermal Contact
Salmonella enterica (cfu/100 mL)	2×10^4	5×10^2	2×10^4	N.R.
Shigella spp. (cfu/100 mL)	<5	<5	<5	N.R.
Staphylococcus aureus (cfu/100 mL)	N.R.	N.R.	N.R.	5×10^6

N.R.—not relevant, as *S. aureus* can be harmful via dermal contact whereas the others cannot (and *S. aureus* is not harmful in other scenarios).

Given the low quantities of *Salmonella enterica* typically found in biologically treated greywater, it does not represent a significant gastrointestinal risk (Table 1). Regardless, the maximum concentration of *Salmonella enterica* in greywater should not exceed 50 cfu/100 mL. This can be achieved through biological treatment, preferably followed by disinfection.

A concentration of 5 cfu/100 mL was enough to cross the maximum acceptable probability of infection of *Shigella* spp. for the garden work scenario, while for the other scenarios the acceptable threshold was crossed at concentration below 1 cfu/100 mL (Figure 2). These results suggest that *Shigella* inactivation is required, and exposure to greywater should be minimized to prevent contact and ingestion of this microorganism. Yet, it is important to note that *Shigella* spp. has only been found in a few greywater samples [9].

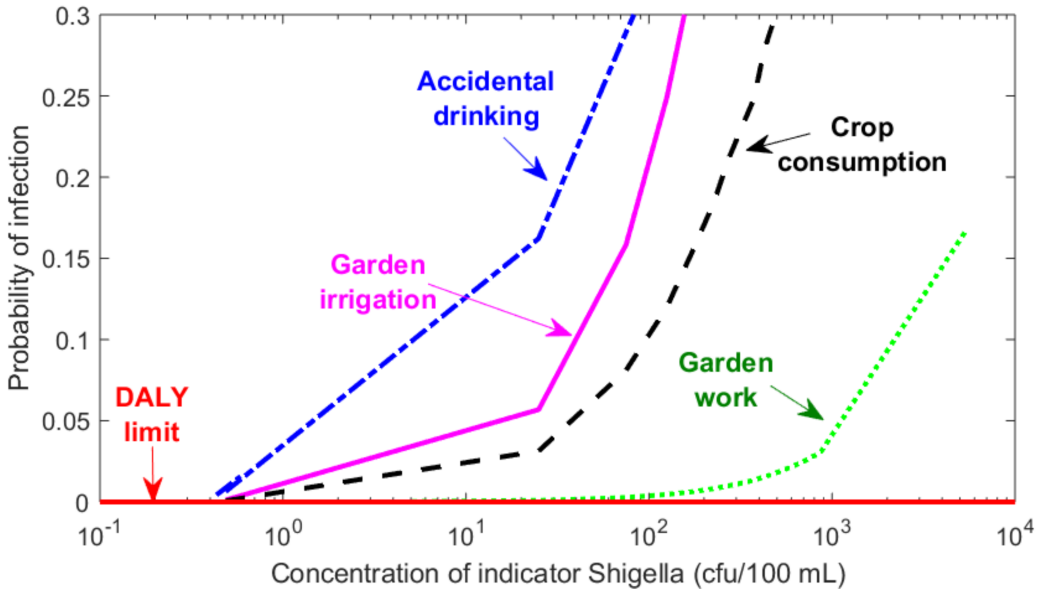

Figure 2. Probability of infection from *Shigella* spp. In greywater under four worst-case exposure scenarios (Table 2). The red solid horizontal line represents the DALY limit of acceptable risk as suggested by the WHO [25].

Similar to the results of the worst-case scenarios, concentration below 5 cfu/100 mL of *Shigella* spp. was found to produce acceptable risk in the best-practice scenarios (Table 4). The probabilities of infection were always above the DALY limit for all scenarios investigated, and the maximum acceptable concentration for all examined scenarios was 5 cfu/100 mL. This supports the worst-case scenario results, which suggest that disinfection to non-detectable concentrations is required for safe reuse of greywater containing *Shigella* spp.

Chlorine tablets and low-pressure UV irradiation have been shown to be good disinfectants against all of the examined pathogens, with up to 8 log reductions recorded [16,52–54]. Given that *Salmonella enterica* and *Shigella* spp. are found only rarely, at concentrations of up to 4 log gene copies/100 mL (Table 1 [1,17]), the use of chlorine could reduce these bacteria to non-detectable levels [52].

The results from exposure to *Staphylococcus aureus* during handwashing (worst-case scenario) were found to be acceptable up to a concentration of 10^6 cfu/100 mL. This concentration is greater than that usually found in greywater (Figure 3).

The result of the best-practice exposure scenario for dermal contact during maintenance of the greywater treatment system was similar to that obtained from the worst-case scenario with acceptable risk of up to 10^6 cfu/100 mL of *Staphylococcus aureus* (Table 4). The reason for the similar results between worst-case and best-practice scenarios stems from lower exposure frequency in the worst-case scenario than in the best-practice one. In other words, the worst-case scenario is considered a rare accident, whereas the best-practice exposure is expected to occur much more frequently.

In this study, it was determined that accidental drinking posed the highest risk to human health for all pathogens examined.

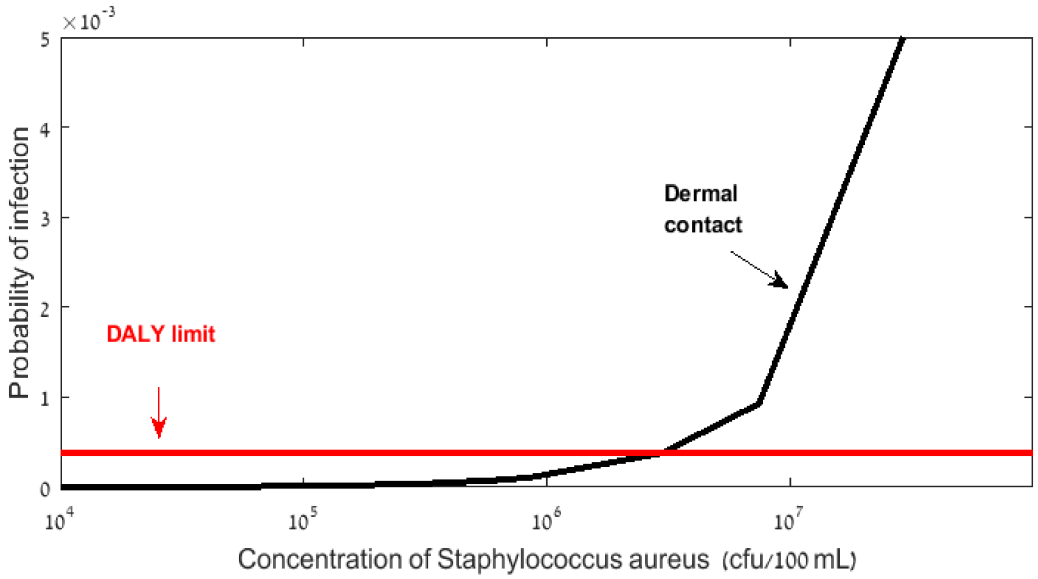

Figure 3. Probability of skin infection from worst-case exposure to *Staphylococcus aureus* due to accidentally washing hands in greywater. The red solid horizontal line represents the DALY limit of acceptable risk as suggested by the WHO [25].

3.2. Uncertainty and Sensitivity Analyses

Using Spearman's rank correlation, pathogen concentration was found to be the most uncertain variable (Figure 4). Spearman's rank correlation is used to determine the dependence of a result on a variable. A higher correlation suggests a higher dependence, and therefore importance in the uncertainty. This result suggests that pathogen concentration has the widest range in the model input data, and that more information on this variable is required.

Pathogen concentration was found to be the most important parameter in the sensitivity analysis (Table A2). This suggests that small changes in pathogen concentration will result in large changes in the output of the model, or in other words, in the probability of infection. Reducing the pathogen concentration is, consequently, the most effective way of reducing the risk of infection from greywater. Simple procedures, such as use of disinfectants (e.g., chlorine, UV light, etc.), drip and subsurface irrigation, and other physical barriers that prevent the spread of pathogens can be put in place to reduce potential risks associated with greywater reuse.

Few studies have considered the sensitivity of the DALY [55,56]. Nevertheless, the evidence suggests that it is a robust measure overall [56]. Variation in the disability weights for diseases that are both mild and frequent might lead to considerable effects on the calculated burden of disease, because the relative impact of a small difference (e.g., 0.1) is much larger at the mild end of the severity scale [55]. DALYs for mild and frequent diseases should be given special attention, and the uncertainty should be evaluated on a case-by-case basis.

The second sensitivity of the DALY indicated that the final result—the point at which the microbial concentration was deemed acceptable—was recorded, and the percent differences between each resulting concentration were determined. The differences in the end results were shown to vary between 9 and 12% (data not shown). This is not a significant variation, and it was therefore postulated that the DALYs used in this model are robust.

It is also important to recall that the model is highly conservative because of the assumptions that were made regarding the input variables, i.e., that the entire population is susceptible to infection by these pathogens and that every infection results in illness. We therefore postulate that QMRA can be used safely, despite the uncertainty in the DALYs.

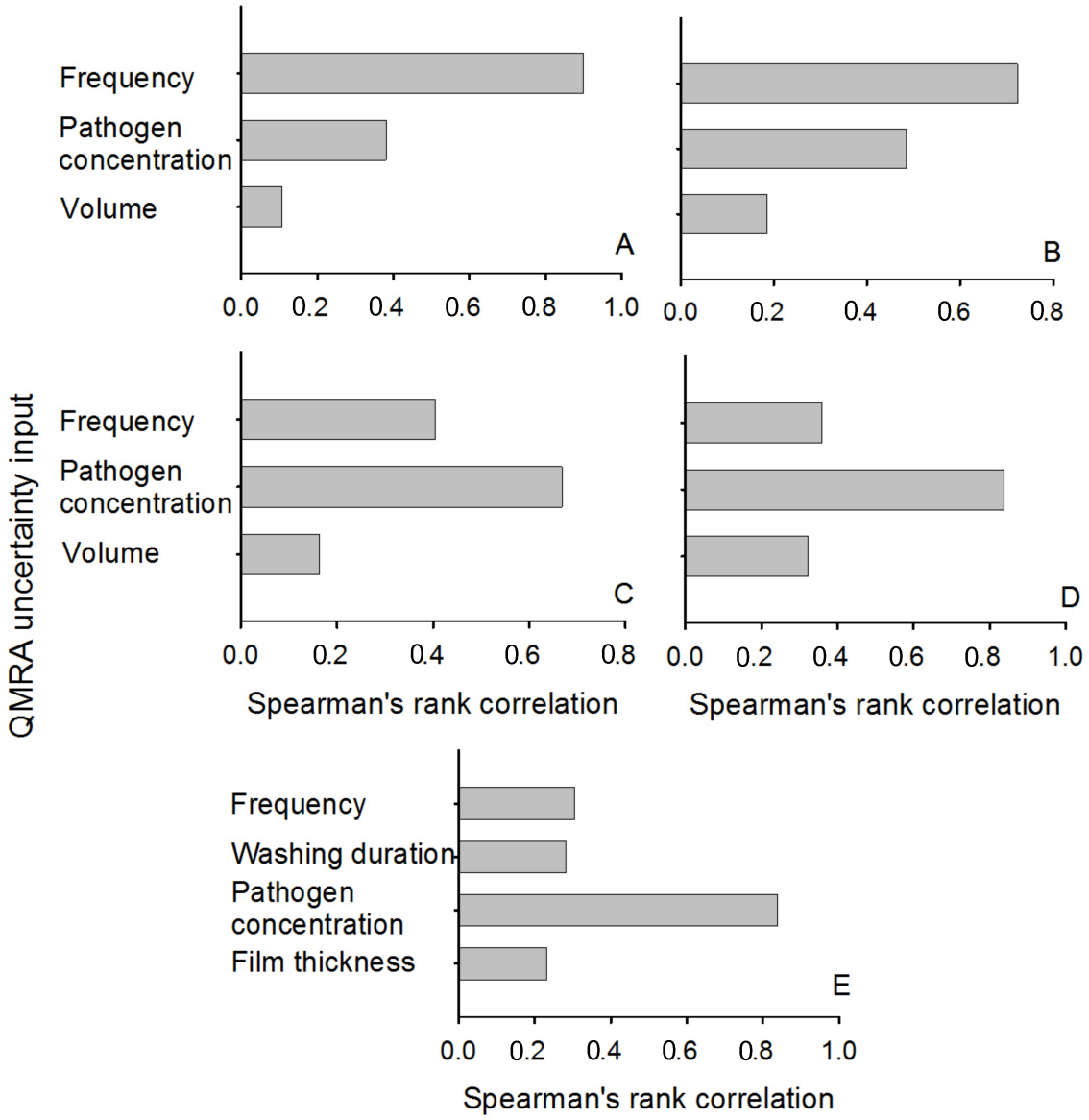

Figure 4. Uncertainty as determined by Spearman's rank correlation for each worst-case scenario using *Shigella* spp. as the representative bacteria. (**A**) Scenario (i)—accidental ingestion (drinking); (**B**) Scenario (ii)—aerosol exposure; (**C**) Scenario (iii)—garden work; (**D**) Scenario (iv)—crop consumption; and (**E**) Scenario (v)—handwashing.

4. Conclusions

Raw greywater, and often, biologically treated greywater, were shown to exceed the maximum level of acceptable risk at concentrations above 50 cfu/100 mL and 1 cfu/100 mL for *Salmonella enterica* and *Shigella* spp., respectively, under all worst-case exposure scenarios, excluding exposure to *Staphylococcus aureus* from handwashing (10^6 cfu/100 mL). Moreover, a good agreement between the results of the worst-case and best-practice exposure scenarios with respect to the maximum tolerable bacterial concentrations was found. It is therefore postulated that disinfection prior to reuse of greywater is recommended to meet the tolerable limit of risk.

Pathogen concentration was deemed the most important parameter in the model, suggesting that special effort should be made to minimize it in any reuse scheme.

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Author Contributions: A.B. has written the matlab code and conducted most of the research as part of her MSc studies. A.G. and E.F. initiated and directed the study and the approach taken as the academic supervisors of Allison. They also supplied much of the relevant data for the model. Y.G. upgraded the matlab code and edited the final drafts. All co-authors contributed to completing and improving the text of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix

Table A1. Additional relevant parameters associated with best-practice exposure scenarios.

Additional Parameters Used in Exposure Assessment	Avg (Min–Max)	Reference
Pathogen transfer efficiency to hands (%)	48 (46–50)	[37]
Pathogen transfer efficiency to surfaces (%)	55 (51–60)	[37]
Pathogen reduction from handwashing (%)	42 (31–51)	[36]
Shigella reduction from handwashing (%)	59 (38–73)	[36]
Pathogen reduction on produce from washing under a continuous stream (log reduction)	1.5 (0.3–2.2)	[38]
Withholding period (days)—uniform distribution	(0–2)	[14]
Decay rate (per day)—normal distribution	(0.8107, 0.3008)	[47]

Table A2. Sensitivity analysis inputs, outputs, and stepwise rank for each scenario.

Exposure Scenario	Parameter	Input Values			Ratios of Output Values			Stepwise Rank
		p25	p50	p75	p50:25	p75:50	p75:25	
i	Volume [a]	93.61	113.91	139.64	1.22	1.23	1.49	2
	Pathogen concentration [b]	2.50	5.00	7.56	2.00	1.51	3.02	1
	Frequency	0.93	1.13	1.39	1.21	1.22	1.48	3
ii	Volume [a]	0.93	1.13	1.39	1.22	1.23	1.49	3
	Pathogen concentration [b]	2.50	5.00	7.56	2.00	1.51	3.02	1
	Frequency	15.11	19.74	24.23	1.31	1.23	1.60	2
iii	Volume [a]	0.09	0.11	0.14	1.22	1.22	1.48	3
	Pathogen concentration [b]	2.50	5.00	7.56	2.00	1.51	3.02	1
	Frequency	152.96	196.03	245.29	1.28	1.25	1.60	2
iv	Volume [a]	9.39	11.36	13.85	1.21	1.22	1.48	3
	Pathogen concentration [b]	2.50	5.00	7.56	2.00	1.51	3.02	1
	Frequency	152.23	196.17	245.99	1.29	1.25	1.62	2
v	Film thickness [c]	0.0017	0.002	0.0023	0.63	1.17	0.74	2
	Pathogen concentration [b]	2.50×10^5	4.99×10^5	7.51×10^5	0.63	2.02	1.28	1
	Duration [d]	3.94×10^{-4}	4.60×10^{-4}	5.27×10^{-4}	0.87	1.54	1.34	3
	Frequency [a]	0.93	1.35	1.39	1.07	1.38	1.48	4

[a] Volume in mL; [b] concentration in cfu/100 mL; [c] film thickness in µm; [d] duration in seconds.

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