Science of the Total Environment 562 (2016) 344–352

Contents lists available at ScienceDirect

Science of the Total Environment

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

Quantification and risks associated with bacterial aerosols near domestic greywater-treatment systems

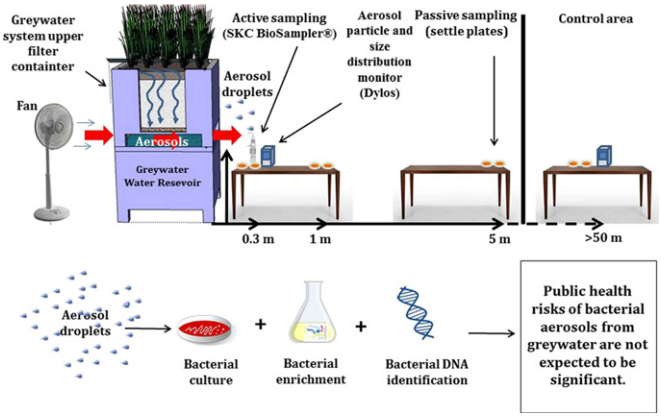
Maya Benami, Allison Busgang, Osnat Gillor *, Amit Gross *

Zuckerberg Institute for Water Research, Albert Katz International School for Desert Studies, The Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Sede Boqer Campus, Midreshet Ben Gurion 84990, Israel

HIGHLIGHTS

- Greywater aerosols had higher bacterial counts compared to background amounts.
- Low pathogen counts were detected on settle-plates from greywater aerosols.
- Before enrichment no bacteria were found in greywater aerosols, using a BioSampler®.
- After enrichment some pathogens were occasionally found in the greywater aerosols.
- QMRA results show that greywater aerosols were below safety limits for *S. aureus*.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 14 February 2016
Received in revised form 28 March 2016
Accepted 28 March 2016
Available online 19 April 2016

Editor: D. Barcelo

Keywords:
Enrichment
MPN-qPCR
Aerosols
Pathogens
Greywater
QMRA

ABSTRACT

Greywater (GW) reuse can alleviate water stress by lowering freshwater consumption. However, GW contains pathogens that may compromise public health. During the GW-treatment process, bioaerosols can be produced and may be hazardous to human health if inhaled, ingested, or come in contact with skin. Using air-particle monitoring, BioSampler®, and settle plates we sampled bioaerosols emitted from recirculating vertical flow constructed wetlands (RVFCW) – a domestic GW-treatment system. An array of pathogens and indicators were monitored using settle plates and by culturing the BioSampler® liquid. Further enumeration of viable pathogens in the BioSampler® liquid utilized a newer method combining the benefits of enrichment with molecular detection (MPN-qPCR). Additionally, quantitative microbial risk assessment (QMRA) was applied to assess risks of infection from a representative skin pathogen, *Staphylococcus aureus*.

According to the settle-plate technique, low amounts (0–9.7 × 10^4 CFU m^{-2} h^{-1}) of heterotrophic bacteria, *Staphylococcus* spp., *Pseudomonas* spp., *Klebsiella pneumoniae*, *Enterococcus* spp., and *Escherichia coli* were found to aerosolize up to 1 m away from the GW systems. At the 5 m distance amounts of these bacteria were not statistically different ($p > 0.05$) from background concentrations tested over 50 m away from the systems. Using the BioSampler®, no bacteria were detected before enrichment of the GW-aerosols. However, after enrichment, using an MPN-qPCR technique, viable indicators and pathogens were occasionally detected. Consequently, the QMRA results were below the critical disability-adjusted life year (DALY) safety limits, a measure of overall disease burden, for *S. aureus* under the tested exposure scenarios. Our study suggests that health risks from

* Corresponding authors.
E-mail address: amgross@exchange.bgu.ac.il (A. Gross).

http://dx.doi.org/10.1016/j.scitotenv.2016.03.200

aerosolizing pathogens near RVFCW GW-treatment systems are likely low. This study also emphasizes the growing need for standardization of bioaerosol-evaluation techniques to provide more accurate quantification of small amounts of viable, aerosolized bacterial pathogens.

1. Introduction

Onsite treatment and reuse of greywater (GW), domestically generated effluents excluding toilet and occasionally kitchen wastewater (WW), can increase water savings and alleviate water scarcity (Gross et al., 2008; Oron et al., 2014). Millions of onsite GW-treatment systems are in operation worldwide and are directly accessible to household inhabitants (Oron et al., 2014). Many GW-treatment systems, such as recirculating vertical flow constructed wetlands (RVFCW) (Gross et al., 2007, 2015), create bioaerosols which might compromise human health if critical amounts are inhaled, ingested, or come into contact with human skin.

1.1. Airborne pathogens and aerosol sampling techniques

Particles ranging from 0.01–50 μm in diameter that are suspended in air are categorized as aerosols (Gehr and Heyder, 2000). Between 80 and 90% of aerosols are <10 μm in diameter and of these, ~70% are of respirable size and can contain cultivable bacteria (Li et al., 2012). A significant fraction of particles from 0.5 μm up to 10 μm can enter into lung or gastrointestinal tissue (Thomas et al., 2008) and have thus been categorized by the US Environmental Protection Agency (US EPA, 2014) as particles that may not be filtered by the lungs but rather deposited there or ingested (Brunekreef and Holgate, 2002; Olin, 1999). Infectious pathogens, such as viruses and bacteria (SI: Table S1), are within the size range that can be carried in these aerosols (Bowers et al., 2011). Via exposure to skin, inhalation, or ingestion, they can have a potentially negative impact on human and animal health (Jeppesen, 1996; Li, 2013; Stellacci et al., 2010). The possibility of respirable air particles contaminated with fungi, bacteria, viruses, and other harmful organisms has been widely researched and reviewed (Baron and Willeke, 1986; Gralton et al., 2011; Lacey and Dutkiewicz, 1994).

Various environmental and physical factors affect airborne pathogen transport and their ability to remain infective over small or large distances (Dueker et al., 2012). In general, airborne bacteria and viruses can remain viable and travel further with increased wind velocity, increased relative humidity, lower temperature, or lower solar radiation. Other important factors include the sources and initial concentrations of pathogens in WW, duration of aerosolization, and droplet sizes (Asano, 1998; Li, 2013; Marthi et al., 1990; Teltsch and Katzenelson, 1978). Previous studies on WW systems have indicated that under ideal conditions, high concentrations of coliform bacteria are carried over distances of 90–130 m with a wind velocity of 1.5 m s^{-1} (Jeppesen, 1996), and that ideal conditions for bacterial survival are relative humidity levels of 70–80% and low temperatures (<12 °C) (Marthi et al., 1990). For most infectious agents, the aerosol research community has only rudimentary knowledge of the process of airborne disease transmission from WW sources to recipients due to the technical difficulties involved in obtaining quantitative estimates of excretion, distribution, stability, and probability of infection by exposure dose (Hermann et al., 2006). Part of these technical difficulties may stem from the type of biological aerosol sampling techniques employed.

Biological aerosol sampling is often performed using the settle-plate technique, which could simulate skin contact, and/or by utilizing a vacuum to draw air into a liquid impinger or impaction onto agar plates, which could simulate the action of inhalation/ingestion (Pasquarella et al., 2000). All air-collection methods have their limitations but also advantages, as described in depth by Napoli et al., 2012. It is known that in passive sampling using the settle-plate technique, microbial quantification is weakly (if at all) correlated with counts by other quantitative methods. This is because gravity, motion of the surrounding atmosphere, and other depositional dynamics due to particle size and shape affect what falls onto the agar plates. The volume of collected aerosols is unknown due to these factors, and larger particles may be inherently selected as they are more likely to settle (Napoli et al., 2012).

Active sampling techniques, using a vacuum to draw air into an impinger or onto an agar plate, have a variety of designs and require calibration for each microorganism and each nutrient medium used. Thus, results obtained by these devices are variable and can often be difficult to interpret (Napoli et al., 2012). A significant limitation to active sampling methods includes the loss of sampling liquid through evaporation and re-aerosolization of bacterial droplets. This often reduces collection efficiency of liquid impingers along with the force of impact of the bacteria onto the liquid or agar medium surface which reduces bacterial viability (Lin et al., 2000).

1.2. Estimating health risks of aerosolized bacteria from GW systems

Quantitative microbial risk assessment (QMRA) is a promising modeling tool for predicting health risks associated with specific pathogens in water sources (Ashbolt et al., 2010; Till et al., 2008). In recent years, a few studies have applied or promoted the use of QMRA to estimate the health risks of GW use (Busgang et al., 2015; Maimon et al., 2010; O'Toole et al., 2014; Ottoson and Stenström, 2003). QMRA is based on a paradigm of four discrete steps: (1) hazard identification to describe the effects of the pathogens of concern on human health; (2) exposure assessment to determine the size and nature of the population which may be exposed via route, amount, and duration of exposure; (3) dose-response modeling to characterize the relationship between the exposure to specific doses of a pathogen and the probability of a negative outcome; and (4) risk characterization to determine the annual probability of illness and the maximum acceptable risk via the integration of information from the previous three steps (Haas et al., 1999). No study thus far has applied QMRA to evaluate the risks of bacterial pathogens that may aerosolize from GW systems. This may be due to the difficulties in obtaining accurate pathogen data for risks of ingestion and inhalation infectivity along with the limitations of various aerosol collection techniques (Marthi et al., 1990; Napoli et al., 2012; Oliver, 2010; O'Toole et al., 2014; Schmidt and Emelko, 2011).

Reuse of GW is widely practiced, yet the potential risks associated with the transport of pathogens found in GW via aerosols have not been thoroughly investigated. It has been shown that there are increased concentrations of pathogenic bacteria near contaminated water sources such as WW-treatment systems (Dutkiewicz et al., 2003; Haas et al., 2010), but little is known about the amounts or types of aerosolized bacteria in residential areas (Bowers et al., 2011).

Microbial characteristics of raw and treated GW from RVFCW treatment systems have been studied and pathogenic microorganisms have often been found (Benami et al., 2013, 2015; Gross et al., 2006) (SI: Table S1, Table S2). The transfer of the microorganisms from water to air occurs mainly during the aeration stage of treatment (Bauer et al., 2002). Thus, the source of pathogenic bioaerosols from these systems could originate from the recirculation and aeration of raw GW and possibly from the contribution of detached biofilm microorganisms (Sklarz et al., 2009; Gross et al., 2006, 2007, 2008).

To the best of our knowledge, there has been no investigation of aerosolized pathogens where onsite domestic WW treatment is practiced. Therefore, we had two aims: 1) to quantify bacterial pathogens

commonly found in GW from aerosols generated from model onsite domestic GW-treatment systems, and 2) to evaluate the risks associated with aerosolized pathogens by comparison to current published infective dose standards and by applying QMRA.

2. Materials and methods

2.1. Experimental setup

The experiments were designed to test the spread of pathogenic bacteria via aerosols originating from GW-treatment systems. Aerosols were sampled from three RVFCWs ($N = 3$) and were each tested three times ($n = 3$) over the duration of nine months (June–February 2014–2015). In an alternating testing scheme, a replicate (n) from each system was tested once a month (one sampling per month in total). Sampling was conducted early in the morning right before sunrise when temperature and solar radiation were minimal and relative humidity was high, maximizing pathogen survival (Marthi et al., 1990).

All of the GW systems treated washroom (sink and shower) and laundry effluents. All systems were located in the yards of domestic residences in the arid Negev desert region of Israel (34°46′58.548″E; 30°51′6.588″N) and all systems had been operating for over four years (Gross et al., 2008; Maimon et al., 2014). Briefly, an RVFCW is composed of two 500-L plastic containers (1.0 m × 1.0 m × 0.5 m) placed one on top of the other. The top container acts as a vertical flow wetland holding a planted three-layer bed and is perforated at the bottom. The bed is composed of a 5 cm top layer of woodchips, followed by a 35 cm middle layer of tuff gravel and a 10 cm lower layer of limestone pebbles. The lower container functions as a reservoir. Raw GW is applied to the top of the bed and trickles through the layers into the lower container. The water is then pumped continuously from the reservoir back to the top at a rate of ~300 L h^{-1}.

A schematic representation of the RVFCW is depicted in Fig. 1. Microorganisms constitute a central component of the RVFCW's capacity for treating GW. In the RVFCW treatment layers, biofilms are formed. The kinetic interactions for mineralization of organic matter of GW biofilms have been previously characterized (Baban et al., 2010).

The treatment efficiencies of the RVFCW have been studied previously (Benami et al., 2015; Gross et al., 2007; Sklarz et al., 2009). For a wide range of highly contaminated raw GW qualities, the RVFCW has been found to effectively remove on average 10^2–10^3 CFU 100 mL^{-1} of *E. coli* and lower TSS and BOD levels to <10 mg L^{-1} (Benami et al., 2015; Zapater et al., 2011) (SI: Table S2).

To mimic a worst-case scenario, aerosols were sampled shortly after a batch of raw GW was introduced into the RVFCW. A fan was set up behind each RVFCW system to direct the air flow in one direction and create a maximum air flow of approximately 1 m s^{-1} air flow at 5 m away, as detected by an anemometer (Kimo AMI 300 Multifunction Data Logger, North Yorkshire, England) (Fig. 1). Further details of the experimental set-up are listed in SI: Experimental information.

2.2. Weather data

Solar radiation (kWh m^{-2} h^{-1}), wind speed (m s^{-1}), temperature (°C), and relative humidity (%) were recorded using a portable iMETOS® ET weather station (Pessl Instruments GmbH, Weiz, Austria) located onsite during each sampling. The weather parameters in Table 1 were monitored during the samplings and averaged 0.28 ± 0.26 kWh m^{-2} h^{-1} for solar radiation, 0.1 ± 0.2 m s^{-1} for wind speed, 20.0 ± 7.0 °C for temperature, and 71.3 ± 20.0% for relative humidity.

2.3. Aerosol sampling methods

Due to the fact that there is no standard method yet established for aerosol samplings (Napoli et al., 2012) and that limitations exist in sampling aerosols with only one technique (Section 1.2), several aerosol collection methods were employed. Using several methods allowed a more complete understanding of the presence, size, and viability of the aerosolized bacteria. Bioaerosols emitted from the RVFCW were monitored by an air-particle monitoring device (Dylos air particle monitor) which was utilized to measure the aerosol fraction contributed by the GW treatment systems. Bioaerosols were collected by an active collection method, the BioSampler® (SKC Inc., 225-9595, Eighty Four, PA), and via a passive collection method using settle plates. All GW and aerosol collection (BioSampler® and settle plates) and monitoring (Dylos air particle monitor) efforts were performed simultaneously for one hour, for each independent sampling event, for a total of nine samplings ($N = 3, n = 3$).

2.3.1. Aerosol particle count

Particles of two size distributions (0.5–2.5 μm and 2.5–10 μm) were monitored by Dylos DC1700 air-particle monitors (Dylos Corporation, Riverside, CA). During sampling events, monitors were located 0.3 m and >50 m (control) away from the systems (Jones et al., 2015; Northcross et al., 2013; Semple et al., 2015; Steinle et al., 2015). In total, 465 and 459 measurements were taken for the 0.5–2.5 μm and 2.5–10 μm size ranges, respectively. The difference between background measurements from 50 m away from the RVFCW treatment system and the measurements closer (0.3 m) to the system was considered to be the aerosol fraction contributed by the system.

2.3.2. Active aerosol sampling

Air-sampling was done by a popular active sampling device called the BioSampler® (SKC Inc.) which was created to reduce evaporation, *re*-aerosolization, and impact of bacteria into the liquid medium—all factors that can reduce bacterial collection or viability (Lin et al., 2000). During each sampling event, aerosols were collected for 1 h by air suction at a rate of 12.5 L min^{-1} into the BioSampler® liquid impinger as suggested by the manufacturer. The sampler was located 0.3 m downwind of the RVFCW GW-treatment system (Gross et al.,

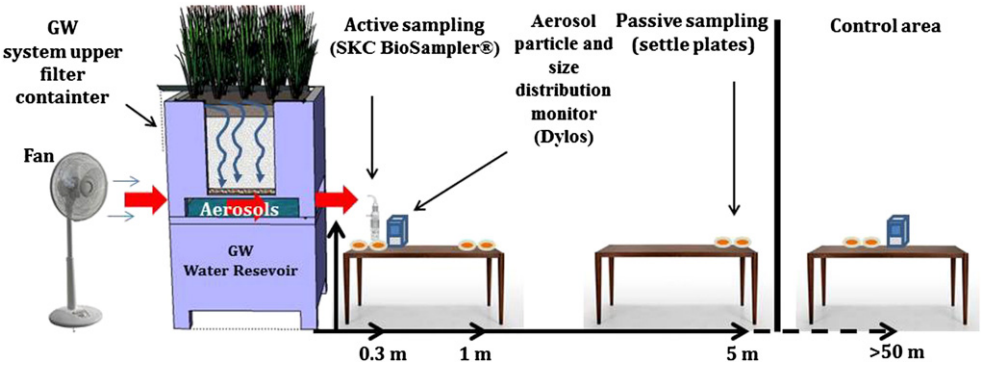


Fig. 1. Field setup for bioaerosol pathogen monitoring.

Table 1
Weather data collected during the samplings.

Sampling	Solar radiation ($kWh\ m^{-2}\ h^{-1}$)	Wind speed ($m\ s^{-1}$)	Temperature (°C)	Relative humidity (%)
1	0.79	0.1	26.0	48.8
2	0.58	0.0	26.9	61.1
3	0.04	0.0	21.1	48.8
4	0.28	0.1	26.7	55.7
5	0.21	0.5	21.9	84.6
6	0.34	0.2	22.9	74.7
7	0.08	0.0	14.7	100.0
8	0.13	0.1	13.6	68.3
9	0.18	0.0	6.5	100.0
Average	0.28 ± 0.26	0.1 ± 0.2	20.0 ± 7.0	71.3 ± 20.0

2008), reflecting the closest distance at which a person can comfortably sit or stand near a GW system.

2.3.3. Passive aerosol sampling

Settle plates with selective agars were placed at 0.3, 1, 5, and >50 m away from the systems for 1 h to evaluate total heterotrophic bacteria, indicators and pathogens as described in Section 2.4 and in SI: Table S3.

2.3.4. GW samples

During each sampling event GW was collected from the source of the aerosols, the reservoir of the RVFCW. Collection of the GW was performed immediately after the raw GW went through the treatment cycle one time, henceforth representing the "worst-case" or "most contaminated" form of treated GW. The treated GW was analyzed for microbial pathogens and indicators as described in Section 2.4.

At the end of each sampling, liquid and plate samples were immediately brought to the laboratory in a chilled cooler. The plates were incubated at the temperatures dictated by the microbial medium manufacturers' instructions (SI: Table S3). Liquid samples from the BioSampler® impinger were kept at 4 °C and processed within 24 h of collection. Bacteria were enumerated as described in Section 2.4. A more detailed description of the sampling methods and field setup is provided in the SI sections: Sampling information, Field setup, and Aerosol and bacterial sampling (Fig. 1).

2.4. Microbial quantification in aerosols and GW

An array of pathogens and indicators were tested using the settle plates and by culturing the BioSampler® liquid on selective agar plates. These two quantification methods were only able to detect culturable bacteria which grew from the aerosol samples on selective growth media. Additionally, a newer method combining the benefits of culture-dependent enrichment with molecular detection (MPN-qPCR) (Orlofsky et al., 2015) was employed to enumerate bacteria by distinguishing specific (detecting DNA) culturable (identified via enrichment) bacteria from the BioSampler® liquid impinger.

2.4.1. Culture-dependent methods

Colony-forming units (CFU) were enumerated using standard methods (APHA, 2012) after appropriate incubation times as described by manufacturer instructions for total heterotrophic bacteria, *Escherichia coli*, *Enterococcus* spp., *Klebsiella pneumoniae*, *Pseudomonas* spp., and *Staphylococcus* spp. (SI: Table S3). Settle-plate agars were supplemented with cycloheximide (Hi-Media Laboratories, Mumbai, India) at 100 $\mu g\ mL^{-1}$ to suppress fungal growth. Similarly, 1 mL samples were taken from the BioSampler® liquid impinger and the collected bacteria were enumerated for the same bacteria using the same selective agar plates (SI: Table S3).

2.4.2. Most probable number (MPN)-based culture-dependent method

GW samples from the RVFCW were analyzed by MPN-based enzymatic-based microbial kits (IDEXX, Westbrook, ME) for the detection of *Pseudomonas aeruginosa*, fecal coliforms, *E. coli*, and Enterococci (SI: Table S3).

2.4.3. Enrichment and molecular detection

Enrichment was applied to 5 mL aliquots taken from the BioSampler® liquid impinger to address (1) the reduction in bacterial viability and the release of free DNA (Zhen et al., 2013) that the BioSampler® active sampling might induce; and (2) the assumption that the GW systems may produce low amounts of aerosolizing pathogens. *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *S. aureus* were targeted using an MPN–qPCR enrichment and molecular detection method of pathogens in aerosols as described previously (Orlofsky et al., 2015). For a more robust DNA extraction to detect the targeted pathogens, the GeneAll® Exgene™ Genomic DNA kit (Biofrontier Technology, Seoul, S. Korea) was used according to the manufacturer's instructions in place of the technique cited in Orlofsky et al., 2015. Molecular (qPCR) detection of *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *S. aureus* followed protocols from Benami et al., 2013. Primers and probes used for molecular detection of the bacterial species in the MPN-qPCR method are listed in SI: Table S4.

2.5. Recovery of pathogens in aerosols

Calibration and recovery tests to assess the impact due to time, bacteria, collection and enrichment media using the BioSampler® were performed and are described in SI: Calibration tests, Table S5, and Figure S1.

2.6. QMRA

QMRA was used to estimate potential risks from possible exposure to GW aerosols and compare it to the tolerable risk as postulated in the World Health Organization (WHO) guidelines for GW reuse (Haas et al., 1999; WHO, 2006). *S. aureus* was tested as a representative skin pathogen (Nishijima et al., 1995) because this bacteria is commonly found and is postulated to be a risk in GW sources (SI: Table S1).

An exposure scenario was chosen to simulate average and worst-case scenarios for skin-infection risks from *S. aureus*. The settle-plate data was used to estimate the number of bacteria that might come in contact with the skin assuming, as a worst-case scenario, lounging 0.3 m away from the GW-treatment systems daily for 1 h. More details can be found in SI: QMRA Scenarios, Tables S6 and S7.

2.6.1. Dose-response modeling

The exponential dose-response model used for *S. aureus* scenario was acquired from Rose and Haas, 1999:

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

where d is the dose of microorganisms that a person may be exposed to (days × number [CFU] of pathogens per m^2), and $k = 1.31 \times 10^7$ is a shape factor that is characteristic of the process. To account for multiple exposures per year, the individual probabilities were summed as:

$$P = 1 - (1 - P_{inf})^n \quad (2)$$

where P is the probability of infection from n exposure events per year. All parameters related to these models can be found in SI: Tables S6 and S7. To estimate exposures as probability distributions, the model was run as Monte Carlo simulations in Matlab (Armstrong and Haas, 2007; Haas et al., 1999).

2.6.2. Risk characterization

The annual probability of infection was determined for the *S. aureus* exposure scenario and the risk was characterized as above or below the maximum tolerable risk suggested according to Disability Adjusted Life Years (DALY) (WHO, 2006) and from Havelaar et al., 2012 in combination with data from Adak et al., 2005. Further description of the DALY used in this research along with model assumptions are listed in SI: DALY, and Model assumptions.

2.7. Statistical analyses

For statistical analyses, microbial counts were log-transformed. Culture-dependent, MPN-qPCR techniques, and aerosol counts as a function of distance from aerosol source were compared by Student's *t*-test or one-way analysis of variance (ANOVA) with a significance level of $p < 0.05$, followed by Tukey's multiple comparison test when needed. If the data set did not pass the normality test, Mann-Whitney or one-way ANOVA on ranks (Kruskal-Wallis) test was used. Statistical analyses were conducted by SigmaPlot® 13.0 software.

To determine the reliability of the QMRA model predictions, the uncertainty and sensitivity in the model inputs were determined following a previously reported method (Xue et al., 2006). More information on the application of sensitivity and uncertainty analyses in relation to the *S. aureus* scenario is provided in SI: Uncertainty and sensitivity analyses and Tables S8 and S9. Spearman rank correlation was used for the uncertainty analyses.

3. Results and discussion

3.1. Particle enumeration by the Dylos air particle monitor

Dylos air-particle monitors were used to assess potential aerosols generated by the GW-treatment systems. Fig. 2 shows the background concentrations (>50 m away from GW system) and GW contributions (0.3 m away from the GW system) of particles that were in the 0.5–2.5 μm and 2.5–10 μm diameter ranges.

In the 0.5–2.5 μm diameter range, background particle results ranged from 1.7×10^1 to 1.7×10^2 with an average of $8.4 \times 10^1 \pm 5.5 \times 10^1$ particles m^{-3}. Closer to the GW systems (0.3 m distance) particles ranged from 4.0×10^1 to 2.1×10^3 particles m^{-3} with an average of $1.3 \times 10^2 \pm 2.3 \times 10^2$. Results were similar for the 2.5–10 μm diameter range, as background particle results ranged from 2.6×10^1 to 1.6×10^2 with an average of $6.9 \times 10^1 \pm 3.1 \times 10^1$ particles m^{-3}. Closer to the system (0.3 m distance), the particle amounts were usually higher, ranging 3.4×10^1 to 6.9×10^2 particles m^{-3} with an average of $1.4 \times 10^2 \pm 1.0 \times 10^2$.

On average, aerosolized particle counts at 0.3 m away were one to two orders of magnitude higher than the background counts (>50 m away) for both of the tested particle size ranges (Fig. 2). These results from the Dylos air particle monitor suggest that aerosols large enough to carry pathogenic bacteria are produced by GW-treatment systems. This coincides with studies that found increased particle counts within these size ranges next to WW-treatment systems (Li et al., 2011, 2012).

3.2. Bacteria in GW

In this study, pathogenic bacteria were found in all biologically treated GW samples. *P. aeruginosa* was, on average, the most frequently detected bacterium, ranging from 9.4×10^1 to 3.1×10^4 MPN 100 mL^{-1} and *Staphylococcus* spp., *E. coli*, and *K. pneumoniae* ranged from 0 to 4.1×10^3 MPN 100 mL^{-1} (Table 2). Fecal coliforms and *Enterococcus* spp. ranged from 0 to 2.4×10^4 MPN 100 mL^{-1} in the biologically treated GW samples (data not shown).

The presence of pathogenic bacteria in GW has been acknowledged (Benami et al., 2013, 2015; Friedler et al., 2006) and consequently, many GW regulations have addressed this issue by various means (Dixon et al., 1999; Maimon et al., 2010; WHO, 2006). Moreover, some reports have discussed risks associated with GW (Busgang et al., 2015; Maimon et al., 2010; O'Toole et al., 2012). Yet, little is known about the possible risks associated with GW aerosols and only a few reports were found to speculate on the risks (Blanky et al., 2015; Jeppesen, 1996) or to attempt pathogen quantification (Orlofsky et al., 2015) from GW aerosols.

3.3. Aerosolized bacterial enumeration by the BioSampler®

The BioSampler® was used as a direct active enumeration method for bacteria. No bacterial colonies grew following inoculation of the liquid impinger field samples (after 1 h operation) on selective agar plates (Table 2). However, after enrichment was applied to the BioSampler® impinger liquid viable indicators and pathogens were occasionally detected, ranging from 0 to 1.1×10^4 CFU m^{-3} h^{-1} (Orlofsky et al., 2015). On a presence/absence basis, after enrichment, *K. pneumoniae* was detected in 67% of the collected samples, *E. coli* in 44%, *P. aeruginosa* in 33% and *S. aureus* in 11% (Table 2).

As humans breathe in an estimated 0.6 to 1.5 m^3 of air per hour (US EPA, 1997), we estimated whether the tested bacteria fell within published infective dosage levels for ingestion, inhalation, or skin contact (Table 2) assuming that one would stand 0.3 m away from the GW systems for one hour. We considered a worst-case scenario that 100% of the selected bacteria had the ability to infect the digestive tract, lungs, or skin. Since no bacteria were detected before enrichment, in this scenario we assessed the infective risk using median values from the BioSampler® impinger liquid after enrichment. *K. pneumoniae* fell within published median infective dosage levels for ingestion. Using maximal bacterial values, *P. aeruginosa* also fell within the lower limit of the published median infective dosage levels for ingestion (Table 2). These bacteria are opportunistic pathogens and are generally known to cause infections on the skin, and inside the nose, ear, lungs, or in

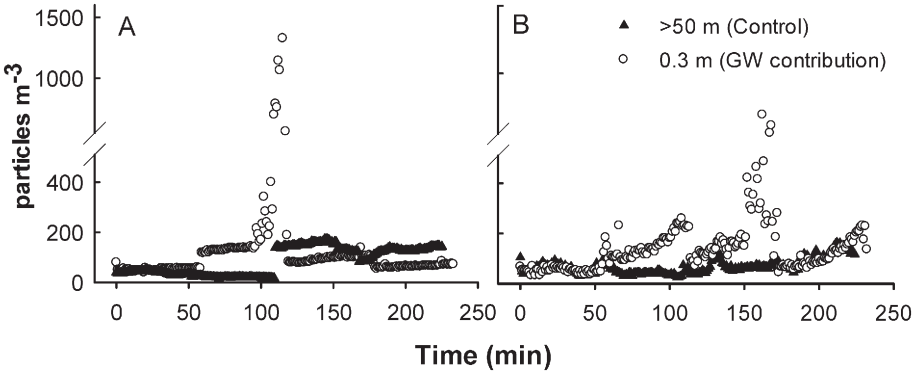


Fig. 2. Average number of particles per m^3 ranging in diameter from A) 0.5 to 2.5 μm; and B) 2.5 to 10 μm, measured 0.3 and 50 m away from the greywater treatment systems over time, as measured by a Dylos air-particle monitor ($N = 4$).

Table 2
Median infective doses and counts of bacterial concentrations in GW and GW-generated aerosols[a].

Bacteria	Range and [median] of culturable bacteria in GW (MPN/CFU 100 mL^{-1})	Detected aerosolized culturable bacteria from BioSampler® before enrichment $n = 9$	Detected aerosolized culturable bacteria from BioSampler® after enrichment and MPN-qPCR $n = 9$	Range and [median] of aerosolized culturable bacteria detected (CFU m^{-3} h^{-1}) from BioSampler® after enrichment and MPN-qPCR $n = 9$	Published median infective dose in CFU (reference)
E. coli	6.3×10^0 4.1×10^3 [8.0×10^1]	0/9 (0%)	4/9 (44%)	0 9.5×10^3 [1.3×10^3]	Indicator bacteria
K. pneumoniae	0 4.1×10^2 [2.7×10^1]	0/9 (0%)	6/9 (67%)	0 2.4×10^5 [5.3×10^2]	10^2 to 10^8 (Malina et al., 1991)
P. aeruginosa	9.4×10^1 3.1×10^4 [8.5×10^2]	0/9 (0%)	3/9 (33%)	0 2.6×10^4 [0]	10^4 to 10^8 (Roser et al., 2014; Rusin et al., 1997)
Staphylococcus spp.[a]	1.2×10^2 4.1×10^3 [4.7×10^2]	0/9 (0%)	1/9 (11%)	0 2.4×10^2 [0]	10^3 to 10^6 (Leggett et al., 2012; Schmid-Hempel and Frank, 2007)

[a] Only the specific *S. aureus* species and not *Staphylococcus* spp. genera were checked for in the BioSampler® samples after enrichment and DNA detection (MPN-qPCR).

the blood (Atanassova et al., 2001; Malina et al., 1991; Roser et al., 2014). However, no published study to date has found critical thresholds for the risk of human infection via inhalation or skin infection of *K. pneumoniae* or *P. aeruginosa* and thus a similar estimation could not be applied to these pathogens.

Due to the fact that we were unable to detect culturable pathogens before enrichment, the MPN-qPCR enrichment technique was used to improve detection sensitivity (Table 2). We used MPN-qPCR as a way to increase detection of aerosolized bacteria on a presence/absence basis. It enhanced our ability to revive previously non-culturable bacteria, thus reducing the possibility of false negative results from the BioSampler® collected samples.

However, until more optimization is performed, results from this report do not support the use of the MPN-qPCR enrichment technique (Orlofsky et al., 2015) as a reliable method for quantitation of actively collected bacterial aerosols. This is because laboratory aerosolization recovery experiments showed an over or under-estimation of aerosolized bacterial quantities between 10^1–10^2 CFU along with a detection limit of $>10^3$ CFU mL^{-1} when aerosols were collected by the BioSampler® (SI: Detection limit and recovery of bacteria in aerosols, Figure S1). These detection limit and quantification range issues made us suspicious of the environmental aerosol results calculated by MPN-qPCR when collected via the BioSampler®, as the collected environmental bacterial aerosol quantities ranged between 0 to 10^5 CFU mL^{-1} (Table 2). More research needs to be done to appropriately quantify a variety of viable bacteria in actively collected aerosols using this technique and others: a notion also upheld by Orlofsky et al. (2015).

3.4. Aerosolized bacterial enumeration by passive sampling (settle plates)

Heterotrophic bacteria, *Pseudomonas* spp., *Staphylococcus* spp., *E. coli*, *K. pneumoniae*, and *Enterococcus* spp. were collected on settle plates at 0.3 m away from the GW systems. They collected at an average rate of 3.5×10^1–9.7×10^4 CFU m^{-2} h^{-1} (Fig. 3). At 1 m away these bacteria were detected at an average rate of 0–2.4×10^3 CFU m^{-2} h^{-1}. In comparison to the 0.3 m distance, there was a significant decrease ($p < 0.05$) in bacterial quantities at the 1 m distance (excluding *E. coli* that was rarely found at both distances) (Fig. 3).

A Mann-Whitney statistical test indicated that average aerosolized heterotrophic bacteria ($7.0 \times 10^3 \pm 8.6 \times 10^3$ CFU m^{-2} h^{-1}) and *Staphylococcus* spp. ($2.1 \times 10^3 \pm 2.8 \times 10^3$ CFU m^{-2} h^{-1}) found 5 m away from the GW systems were not significantly different ($p > 0.05$) from average airborne bacteria in the control (>50 m away) samples ($3.3 \times 10^3 \pm 2.2 \times 10^3$ and $2.0 \times 10^3 \pm 2.1 \times 10^3$ CFU m^{-2} h^{-1} for the heterotrophs and *Staphylococcus* spp., respectively; data not shown). *Pseudomonas* spp., *Enterococcus* spp., *K. pneumoniae* and *E. coli* were not detected on the settle plates at 5 m and >50 m away from the GW systems (data not shown). Hence, only the measured amounts of heterotrophs and *Staphylococcus* spp. at 0.3 and 1 m away from the systems were adjusted by reducing each sampling amount by 3.3×10^3 and 2.0×10^3 CFU m^{-2} h^{-1}, respectively, to reflect the actual amounts minus the background concentrations.

To explain the paucity of *E. coli* on the settle plates, we assumed that *E. coli* might not be easily cultured on settle plates after aerosolization, but may be successfully revived after enrichment (Table 2). In the field, the same trend was observed with the other fecal coliform bacterium *K. pneumoniae*—which was also non-culturable on settle plates but revived after enrichment (Table 2). These results support the notion that without enrichment, culture-dependent detection techniques may not provide an adequate evaluation of environmentally sourced pathogen burdens (Orlofsky et al., 2015). In addition, due to the fact that they might not survive aerosolization, enteric coliform bacteria such as *E. coli* and *K. pneumoniae* may not be good candidates as

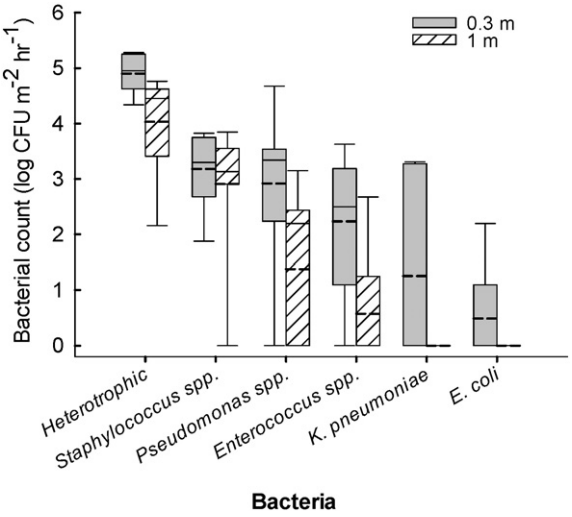


Fig. 3. Bacterial aerosols contributed from greywater treatment systems, collected at 0.3 m and 1 m away via the settle-plate method. Each plot represents three sampling campaigns from three similar GW treatment systems ($n = 9$). Whiskers refer to the 5th and 95th percentiles of the tested data, margins of the box to 25th and 75th percentiles, dashed black line in the box indicates mean and solid line indicates median.

universal indicators of aerosol contamination generated from GW. This observation is in contrast to previous work done on aerosol contamination from WW-treatment plants (Adams and Spendlove, 1970; Goff et al., 1973), but coincides with other GW studies which also proposed that enteric coliforms are not good indicators of GW contamination (Benami et al., 2015; Ottoson and Stenström, 2003; Ridderstolpe, 2004).

Bacteria from the genera *Pseudomonas*, *Staphylococcus* and *Enterococcus* may be either more prevalent in the outdoor air, as they are found in natural environments, or better adapted to the environmental stresses imposed by aerosolization compared to enteric bacteria (Alonso et al., 1999; Rathnayake et al., 2012). These results fall in line with other environmental air-quality and viability studies performed on these organisms (Byrd et al., 1991; Górny et al., 1999; Górny and Dutkiewicz, 2002; Heidelberg et al., 1997).

Although a significant difference was found between the aerosolized bacteria (excluding *E. coli* and *Staphylococcus* spp.) at 0.3 m vs. 1 m away from the GW systems, at both distances, the maximum number of bacteria that settled on the plates over the course of an hour did not reach the published median infective dosage levels for ingestion or skin infection for the pathogenic species within each genus (Table 2, Fig. 3) (Dixon et al., 1999; Malina et al., 1991; Rose and Haas, 1999; Roser et al., 2014). Therefore, for the average healthy individual, we assume little to no risk of infection from these pathogens aerosolizing from GW systems when evaluated by the settle-plate method. Furthermore, a QMRA was conducted using these data to further evaluate the health risks associated with skin infection from repeated exposures to *S. aureus*.

3.5. QMRA

The presence of *S. aureus* in GW (Gilboa and Friedler, 2008; Zimmerman et al., 2014) is of potential concern due to its ability to infect oral, dermal, or respiratory tracts of individuals with weakened immune systems, children or the elderly, and quickly become resistant to antibiotics, particularly methicillin (Atanassova et al., 2001; Nishijima et al., 1995). The median infective dose of *S. aureus* has been recorded to be within the range of 10^3–10^6 CFU (Leggett et al., 2012; Schmid-Hempel and Frank, 2007). At 0.3 m away from the GW treatment systems, *Staphylococcus* spp. colonies were collected on settle plates ranging from 0 to 6.2×10^3 CFU m^{-2} h^{-1} (Fig. 3). Using this data for our QMRA scenarios (SI: Table S6 and Table S7), the model outputs resulted in the following information.

With an average annual probability of infection at 2.7×10^{-7} (5th–95th percentiles from 7.6×10^{-8} to 8.7×10^{-7}), *S. aureus* concentrations did not breach the DALY's limit of 3.9×10^{-4} (3.9 infections per 10,000 people) with respect to acceptable risks of infection from skin contact. In the worst-case scenario, $>3.2 \times 10^6$ CFU m^{-2} h^{-1} would need to be deposited to initiate dermal infection, which is 514 times more than the maximum number of *Staphylococcus* spp. colonies recorded in this study. The QMRA results confirmed that the critical DALY limit was not met for all exposure scenarios of *S. aureus* and for all concentration ranges in the GW aerosols as assessed by the settle plates. These results coincide with a recent epidemiological survey indicating that GW reuse is an insignificant human health risk (Busgang et al., 2015).

3.6. Understanding the risks

Overall risks were found to be negligible for the average healthy person at the maximum concentrations for the settle plate and QMRA exposure scenarios explored in this study. However, the time (duration and frequency) one stays near the GW systems were shown to be the most uncertain and sensitive parameters in the QMRA scenario (SI: Uncertainty and sensitivity analyses). Thus, in accordance with the results (Table S8 and Table S9), we recommend that immunocompromised individuals would distance themselves and decrease average contact frequency with the GW systems to lower the possible risk of skin infections from *S. aureus* and other aerosolized pathogens.

It should be noted that the bacterial counts detected in this study were expected to be the highest that can be collected (using the collection methods described above) from GW systems as samplings were performed immediately after the introduction of raw GW into the treatment systems. Over time, these systems remove bacterial indicators and pathogens. Thus, bacterial counts are expected to be lower (Sklarz et al., 2009) the longer the raw GW recirculates and, consequently, this reduces the concentration of aerosolizing bacteria. Additionally, a natural spread of the aerosols (dilution) into the air would play a role in reducing airborne microorganism concentrations as a function of distance from the GW treatment systems.

As discussed in Section 1.1, meteorological factors such as temperature, solar radiation, and humidity may also affect the viability of microorganisms during their dispersion (Dungan, 2014). While there is very little pathogen-specific information, previous laboratory and field studies on indicator organisms have shown that downwind concentrations of viable airborne microorganisms generally decrease with increased distance from the bacterial source, elevated temperatures and solar radiation or reduced relative humidity (Goff et al., 1973; Karra and Katsivela, 2007). In this study all samplings were conducted early in the morning right before sunrise when temperature and solar radiation were minimal and relative humidity was highest, enhancing pathogen survival. The weather data collected during the sampling period (Section 2.2; Table 1) were found to be beneficial for bacterial survival in accordance with previous studies (Asano, 1998; Li, 2013; Marthi et al., 1990; Teltsch and Katzenelson, 1978) and within the tested distances away from the GW treatment systems (0.3, 1, 5, >50 m).

This is the first study to assess specific and viable pathogen concentrations in aerosols originating from the treatment of raw GW. Although due diligence was done to standardize all parameters in this study, fluctuations in GW pathogen loads may affect pathogen aerosol concentrations and more research may be needed to investigate this possibility. On-site GW quality can vary significantly based on a large number of factors. However, from the numerous studies which tested raw GW, treated GW, and RVFCW removal efficiencies (Benami et al., 2013, 2015; Gross et al., 2007, 2008; Sklarz et al., 2009), the pathogen and indicator ranges mentioned in Section 3.2 fell within average recorded ranges (Table S2). Additionally, we consider the GW indicator and pathogen counts found in this study to be representative of typical GW quality as nine samplings from different private residences during different seasons were taken.

This research suggests that if risk-based analyses will be done on collected aerosol data, methodology choice (e.g. settle-plates for skin/surface; active-sampling impinger methods for ingestion/inhalation) should be taken into account and optimization on the chosen method should be performed to obtain more accurate results which may affect public health. Future risk analyses such as QMRA would be better served upon the standardization of bioaerosol collection and detection techniques. Additionally, more research is needed on the infectivity mechanisms and thresholds of various opportunistic pathogens common to GW such as *P. aeruginosa*, *S. aureus*, and *K. pneumoniae*, which cause infection upon ingestion, inhalation, and skin contact and were found to aerosolize from the GW systems (Table 2).

4. Conclusions

Aerosols near GW treatment systems (like the RVFCW tested here) contain elevated levels of pathogens which were similar to pathogens found in the GW. Using two common passive and active sampling techniques for aerosol collection (settle plates and BioSampler®, respectively), followed by bacterial enumeration on plates, results demonstrated that pathogens in aerosols from GW did not surpass QMRA DALY risk limits for *S. aureus* or median ingestion and skin infection thresholds for *P. aeruginosa*, *S. aureus*, or *K. pneumoniae*.

These findings suggest that the risks from bacterial aerosols to public health are expected to be negligible. However, it was also demonstrated that after application of enrichment using MPN-qPCR, bacterial counts could be underestimated suggesting a miscalculation of the potential risk. Using MPN-qPCR, *K. pneumoniae* and *P. aeruginosa* fell within published median infective dosage levels for ingestion (Table 2). Therefore, this research highlights the need for improvement and standardization of bioaerosol sampling and sensor technologies to better quantitatively evaluate the viable pathogen concentrations under ambient environmental conditions. Further investigation is recommended for the extended monitoring of many different types of bacteria, viruses, and endotoxins in domestic wastewater treatment systems which have aerosolization potential.

Acknowledgments

The authors acknowledge and thank Madeleine Tierney for her substantial help in the laboratory; Yaniv Kriger for his great patience and support during early morning field samplings; Boris Pritsker for his extensive advice, model parameter explanations, and general experimental planning; Ahuva Vonshak for her advice and lab assistance; and the families who contributed GW to this research. This study was supported by the Rosenzweig-Coopersmith Foundation Grant number 711560, Zuck Maccabi Fund, and the Israeli Water Authority Grant number 874130.

Appendix A. Supplementary data

Descriptions of testing set-ups, QMRA scenarios, and QMRA parameters are listed. Results from the uncertainty and sensitivity analyses related to the QMRA models are also reported. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.scitotenv.2016.03.200.

References

Adak, G.K., Meakins, S.M., Yip, H., Lopman, B.A., O'Brian, S.J., 2005. Disease risks from foods, England and Wales, 1996–2000. Emerg. Infect. Dis. 11, 365–372.
Adams, A.P., Spendlove, J.C., 1970. Coliform aerosols emitted by sewage treatment plants. Science 169, 1218–1220.
Alonso, A., Rojo, F., Martinez, J.L., 1999. Environmental and clinical isolates of *Pseudomonas aeruginosa* show pathogenic and biodegradative properties irrespective of their origin. Environ. Microbiol. 1, 421–430.
APHA, 2012. Standard Methods for the Examination of Water and Wastewater. American Public Health Association, Washington, D.C.
Armstrong, T.W., Haas, C.N., 2007. A quantitative microbial risk assessment model for Legionnaires' disease: animal model selection and dose-response modeling. Risk Anal. 27, 1581–1596.
Asano, T., 1998. Wastewater Reclamation and Reuse: Water Quality Management Library. CRC Press, Washington, D.C.
Ashbolt, N.J., Schoen, M.E., Soller, J.A., Roser, D.J., 2010. Predicting pathogen risks to aid beach management: the real value of quantitative microbial risk assessment (QMRA). Water Res. 44, 4692–4703.
Atanassova, V., Meindl, A., Ring, C., 2001. Prevalence of *Staphylococcus aureus* and staphylococcal enterotoxins in raw pork and uncooked smoked ham—a comparison of classical culturing detection and RFLP-PCR. Int. J. Food Microbiol. 68, 105–113.
Baban, A., Hocaoglu, S.M., Atasoy, E.A., Gunes, K., Ayaz, S., Regelsberger, M., 2010. Grey water treatment and reuse by using RBC: A kinetic approach. Desalin. Water Treat. 23, 89–94.
Baron, P.A., Willeke, K., 1986. Respirable droplets from whirlpools: measurements of size distribution and estimation of disease potential. Environ. Res. 39, 8–18.
Bauer, H., Fuerhacker, M., Zibuschka, F., Schmid, H., Puxbaum, H., 2002. Bacteria and fungi in aerosols generated by two different types of wastewater treatment plants. Water Res. 36, 3965–3970.
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. 458–60, 298–302.
Benami, M., Gillor, O., Gross, A., 2015. The question of pathogen quantification in disinfected graywater. Sci. Total Environ. 506–7, 496–504.
Blanky, M., Rodríguez-Martínez, S., Halpern, M., Friedler, E., 2015. *Legionella pneumophila*: from potable water to treated greywater; quantification and removal during treatment. Sci. Total Environ. 533, 557–565.
Bowers, R.M., Sullivan, A.P., Costello, E.K., Collett, J.L., Knight, R., Fierer, N., 2011. Sources of bacteria in outdoor air across cities in the midwestern United States. Appl. Environ. Microbiol. 77, 6350–6356.
Brunekreef, B., Holgate, S.T., 2002. Air pollution and health. Lancet 360, 1233–1242.
Busgang, A., Friedler, E., Ovadia, O., Gross, A., 2015. Epidemiological study for the assessment of health risks associated with graywater reuse for irrigation in arid regions. Sci. Total Environ. 538, 230–239.
Byrd, J.J., Xu, H.S., Colwell, R.R., 1991. Viable but nonculturable bacteria in drinking water. Appl. Environ. Microbiol. 57, 875–878.
Dixon, A.M., Butler, D., Fewkes, A., 1999. Guidelines for greywater re-use: health issues. Water Environ. J. 13, 322–326.
Dueker, M.E., GD, O'Mullan, Juhl, A.R., Weathers, K.C., Uriarte, M., 2012. Local environmental pollution strongly influences culturable bacterial aerosols at an urban aquatic superfund site. Environ. Sci. Technol. 46, 10926–10933.
Dungan, R.S., 2014. Estimation of infectious risks in residential populations exposed to airborne pathogens during center pivot irrigation of dairy wastewaters. Environ. Sci. Technol. 48 (9), 5033–5042.
Dutkiewicz, J., Prazmo, Z., Krysinska-Traczyk, E., Skorska, C., Sitkowska, J., Cholewa, G., 2003. Exposure to bioaerosols in municipal sewage treatment plant. Ann. Agric. Environ. Med. 10, 241–248.
Friedler, E., Kovalio, R., Ben-Zvi, A., 2006. Comparative study of the microbial quality of greywater treated by three on-site treatment systems. Environ. Technol. 27, 653–663.
Gehr, P., Heyder, J., 2000. Particle-lung Interactions. Marcel Dekker, New York, Basel, pp. 473–489.
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.
Goff, G.D., Splendlove, J.C., Adams, A., Nicholes, P.S., 1973. Emission of microbial aerosols from sewage treatment plants that use trickling filters. Health Serv. Rep. 88, 640.
Górny, R.L., Dutkiewicz, J., 2002. Bacterial and fungal aerosols in indoor environment in Central and Eastern European countries. Ann. Agric. Environ. Med. 9, 17–23.
Górny, R.L., Dutkiewicz, J., Krysińska-Traczyk, E., 1999. Size distribution of bacterial and fungal bioaerosols in indoor air. Ann. Agric. Environ. Med. 6, 105–113.
Gralton, J., Tovey, E., McLaws, M.L., Rawlinson, W.D., 2011. The role of particle size in aerosolised pathogen transmission: a review. J. Infect. 62, 1–13.
Gross, A., Kaplan, D., Baker, K., 2006. Removal of microorganisms from domestic greywater using a recycling vertical flow constructed wetland (RVFCW). In: CN, Haas, JB, Rose, CP, Gerba (Eds.), Proceedings of the Water Environment FederationQuantitative Microbial Risk Assessment 6. John Wiley & Sons, New York, pp. 6133–6141 1999.
Gross, A., Shmueli, O., Ronen, Z., Raveh, E., 2007. Recycled vertical flow constructed wetland (RVFCW)—a novel method of recycling greywater for irrigation in small communities and households. Chemosphere 66, 916–923.
Gross, A., Sklarz, M.Y., Yakirevich, A., Soares, M.I.M., 2008. Small scale recirculating vertical flow constructed wetland (RVFCW) for the treatment and reuse of wastewater. Water Sci. Technol. 58 (2), 487.
Gross, A., Maimon, A., Alfiya, Y., Friedler, E., 2015. Greywater reuse. CRC Press, Boca Raton, Washington, D.C.
Haas, C.N., Rose, J.B., Gerba, C.P., 1999. Quantitative microbial risk assessment. John Wiley & Sons, New York.
Haas, D., Unteregger, M., Habib, J., Galler, H., Marth, E., Reinthaler, F.F., 2010. Exposure to bioaerosol from sewage systems. Water Air Soil Pollut. 207, 49–56.
Havelaar, A.H., Haagsma, J.A., Mangen, M.J.J., Kemmeren, J.M., Verhoef, L.P.B., Vijgen, S.M.C., Wilson, M., Friesema, I.H.M., Kortbeek, L.M., van Duynhoven, Y.T.H.P., van Pelt, W., 2012. Disease burden of foodborne pathogens in the Netherlands, 2009. Int. J. Food Microbiol. 156, 231–238.
Heidelberg, J.F., Shahamat, M., Levin, M., Rahman, I., Stelma, G., Grim, C., Colwell, R.R., 1997. Effect of aerosolization on culturability and viability of gram-negative bacteria. Appl. Environ. Microbiol. 63, 3585–3588.
Hermann, J.R., Hoff, S.J., Yoon, K.J., Burkhardt, A.C., Evans, R.B., Zimmerman, J.J., 2006. Optimization of a sampling system for recovery and detection of airborne porcine reproductive and respiratory syndrome virus and swine influenza virus. Appl. Environ. Microbiol. 72, 4811–4818.
Jeppesen, B., 1996. Domestic greywater re-use: Australia's challenge for the future. Desalination 106, 311–315.
Jones, M., Semple, S., Schofield, S., Bahaduri, S., Feary, J., Cullinan, P., 2015. The potential of a low-cost particle counter to quantify airborne particulate matter in a laboratory animal facility. J. Allergy Clin. Immunol. 135, AB76.
Karra, S., Katsivela, E., 2007. Microorganisms in bioaerosol emissions from wastewater treatment plants during summer at a Mediterranean site. Water Res. 41 (6), 1355–1365.
Lacey, J., Dutkiewicz, J., 1994. Bioaerosols and occupational lung disease. J. Aerosol Sci. 25, 1371–1404.
Leggett, H.C., Cornwallis, C.L., West, S.A., 2012. Mechanisms of pathogenesis, infective dose and virulence in human parasites. PLoS Pathog. 8 (2), e1002512.
Li, Y., 2013. Dispersion and risk assessment of bacterial aerosols emitted from rotating-brush aerator during summer in a wastewater treatment plant of Xi'an, China. Aerosol Air Qual. Res. 13 (6), 1807–1814.
Li, L., Gao, M., Liu, J., 2011. Distribution characterization of microbial aerosols emitted from a wastewater treatment plant using the orbal oxidation ditch process. Process Biochem. 46, 910–915.
Li, Y., Qui, X., Li, M., Ma, Z., Niu, T., Feng, Y., 2012. Concentration and size distribution of airborne actinomycetes in a municipal wastewater treatment plant. Pol. J. Environ. Stud. 21, 1305–1311.
Lin, X., Reponen, T., Willeke, K., Wang, Z., Grinshpun, S.A., Trunov, M., 2000. Survival of airborne microorganisms during swirling aerosol collection. Aerosol Sci. Technol. 32, 184–196.
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.

Maimon, A., Friedler, E., Gross, A., 2014. Parameters affecting greywater quality and its safety for reuse. Sci. Total Environ. 487, 20–25.
Malina, J., Hofmann, J., Franěk, J., 1991. Informative value of a mouse model of *Klebsiella pneumoniae* infection used as a host-resistance assay. Folia Microbiol. (Praha) 36, 183–191.
Marthi, B., Fieland, V.P., Walter, M., Seidler, R.J., 1990. Survival of bacteria during aerosolization. Appl. Environ. Microbiol. 56, 3463–3467.
Napoli, C., Marcotrigiano, V., Montagna, M., 2012. Air sampling procedures to evaluate microbial contamination: a comparison between active and passive methods in operating theatres. BMC Public Health 12, 594.
Nishijima, S., Namura, S., Kawai, S., Hosokawa, H., Asada, Y., 1995. *Staphylococcus aureus* on hand surface and nasal carriage in patients with atopic dermatitis. J. Am. Acad. Dermatol. 32, 677–679.
Northcross, A.L., Edwards, R.J., Johnson, M.A., Wang, Z.M., Zhu, K., Allen, T., Smith, K.R., 2013. A low-cost particle counter as a realtime fine-particle mass monitor. Environ. Sci.: Processes Impacts 15, 433–439.
Olin, S.S., 1999. Exposure to Contaminants in Drinking water: Estimating Uptake Through the Skin and by Inhalation. CRC Press, Boca Raton, Washington, D.C.
Oliver, J.D., 2010. Recent findings on the viable but nonculturable state in pathogenic bacteria. FEMS Microbiol. Rev. 34, 415–425.
Orlofsky, E., Benami, M., Gross, A., Dutt, M., Gillor, O., 2015. Rapid MPN-qPCR screening for pathogens in air, soil, water and agricultural produce. Water Air Soil Pollut. 226 (9), 1–10.
Oron, G., Adel, M., Agmon, V., Friedler, E., Halperin, R., Leshem, E., Weinberg, D., 2014. Greywater use in Israel and worldwide: standards and prospects. Water Res. 58, 92–101.
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.
O'Toole, J., Sinclair, M., Fiona Barker, S., K., Leder, 2014. Advice to risk assessors modeling viral health risk associated with household graywater: perspective. Risk Anal. 34, 797–802.
Ottoson, J., Stenström, T.A., 2003. Faecal contamination of greywater and associated microbial risks. Water Res. 37, 645–655.
Pasquarella, C., Pitzurra, O., Savino, A., 2000. The index of microbial air contamination. J. Hosp. Infect. 46, 241–256.
Rathnayake, I.U., Hargreaves, M., Huygens, F., 2012. Antibiotic resistance and virulence traits in clinical and environmental *Enterococcus faecalis* and *Enterococcus faecium* isolates. Syst. Appl. Microbiol. 35, 326–333.
Ridderstolpe, P., 2004. Indroduction to Greywater Management. Stockholm Environment Institute, Stockholm.
Rose, J.B., Haas, C.N., 1999. A risk assessment framework for the evaluation of skin infections and the potential impact of antibacterial soap washing. Am. J. Infect. Control 27, S26–S33.
Roser, D.J., Van Den Akker, B., Boase, S., Haas, C.N., Ashbolt, N.J., Rice, S.A., 2014. *Pseudomonas aeruginosa* dose response and bathing water infection. Epidemiol. Infect. 142, 449–462.
Rusin, P.A., Rose, J.B., Haas, C.N., Gerba, C.P., 1997. Risk assessment of opportunistic bacterial pathogens in drinking water. Rev. Environ. Contam. Toxicol. 152, 57–83.
Schmid-Hempel, P., Frank, S.A., 2007. Pathogenesis, virulence, and infective dose. PLoS Pathog. 3, e147.
Schmidt, P.J., Emelko, M.B., 2011. QMRA and decision-making: are we handling measurement errors associated with pathogen concentration data correctly? Water Res. 45, 427–438.
Semple, S., Ibrahim, A.E., Apsley, A., Steiner, M., Turner, S., 2015. Using a new, low-cost air quality sensor to quantify second-hand smoke (SHS) levels in homes. Tob. Control. 24, 153–158.
Sklarz, M.Y., Gross, A., Yakirevich, A., Soares, M.I.M., 2009. A recirculating vertical flow constructed wetland for the treatment of domestic wastewater. Desalination 246, 617–624.
Steinle, S., Reis, S., Sabel, C.E., Semple, S., Twigg, M.M., Braban, C.F., Leeson, S.R., Heal, M.R., Harrison, D., Lin, C., Wu, H., 2015. Personal exposure monitoring of PM2.5 in indoor and outdoor microenvironments. Sci. Total Environ. 508, 383–394.
Stellacci, P., Liberti, L., Notarnicola, M., Haas, C.N., 2010. Hygienic sustainability of site location of wastewater treatment plants. Desalination 253, 106–111.
Teltsch, B., Katzenelson, E., 1978. Airborne enteric bacteria and viruses from spray irrigation with wastewater. Appl. Environ. Microbiol. 35, 290–296.
Thomas, R.J., Webber, D., Sellors, W., Collinge, A., Frost, A., Stagg, A.J., Bailey, S.C., Jayasekera, P.N., Taylor, R.R., Eley, S., Titball, R.W., 2008. Characterization and deposition of respirable large- and small-particle bioaerosols. Appl. Environ. Microbiol. 74, 6437–6443.
Till, D., McBride, G., Ball, A., Taylor, K., Pyle, E., 2008. Large-scale freshwater microbiological study: rationale, results and risks. J. Water Health 6 (4), 443–460.
US EPA, 1997. Exposure factors handbook (1997 final report)—environmental assessment. United States Environmental Protection Agency.
US EPA, 2014. Particulate Matter (PM). United States Environmental Protection Agency.
WHO, 2006. Guidelines for the Safe Use of Wastewater, Excreta and Greywater: Policy and regulatory aspects. World Health Organization.
Xue, J., Zartarian, V.G., Ozkaynak, H., Dang, W., Glen, G., Smith, L., Stallings, C., 2006. A probabilistic arsenic exposure assessment for children who contact chromated copper arsenate (CCA)-treated playsets and decks, part 2: sensitivity and uncertainty analyses. Risk Anal. 26, 533–541.
Zapater, M., Gross, A., Soares, M.I.M., 2011. Capacity of an on-site recirculating vertical flow constructed wetland to withstand disturbances and highly variable influent quality. Ecol. Eng. 37, 1572–1577.
Zhen, H., Han, T., Fennell, D.E., Mainelis, G., 2013. Release of free DNA by membrane-impaired bacterial aerosols due to aerosolization and air sampling. Appl. Environ. Microbiol. 79, 7780–7789.
Zimmerman, B.D., Ashbolt, N.J., Garland, J.L., Keely, S., Wendell, D., 2014. Human mitochondrial DNA and endogenous bacterial surrogates for risk assessment of graywater reuse. Environ. Sci. Technol. 48, 7993–8002.