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

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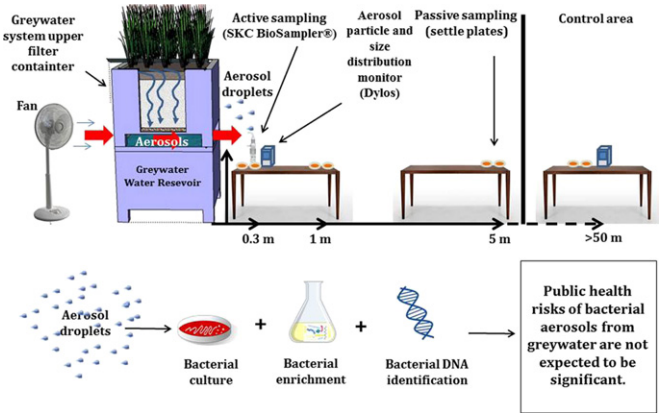
HIGHLIGHTS

- 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



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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

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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