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The question of pathogen quantification in disinfected graywater

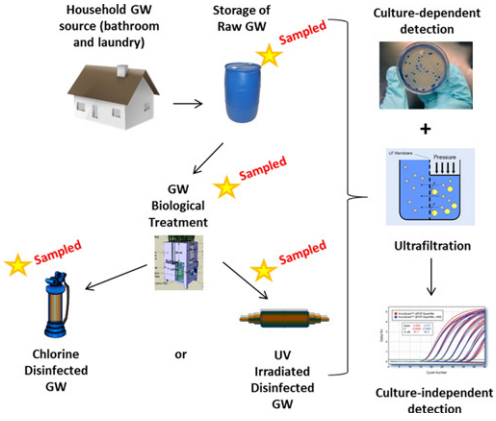
Maya Benami, Osnat Gillor *, Amit Gross *

Zuckerberg Institute for Water Research, Israel
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

- Biologically-treated graywater contains some indicators and pathogens.
- DNA-based qPCR detected no changes in graywater microbial counts after disinfection.
- On-site low pressure UV disinfection was less effective than chlorine.

GRAPHICAL ABSTRACT



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ABSTRACT

Graywater (GW) reuse for irrigation is recognized as a sustainable solution for water conservation. One of the major impediments to GW reuse is the presence of pathogenic microorganisms. This study monitored three similar on-site GW treatment systems bi-monthly over the course of a year to compare the presence of pathogens and indicators in raw, biologically treated, and biologically treated and disinfected [by chlorine and ultraviolet light (UV)] GW. The systems were designed to allow the testing of the same batch (collection) of water as it passed through the treatment chain. The samples were analyzed using standard culture-dependent methods and the data were compared to culture-independent DNA-based methods. Results suggested that the presence and abundance of fecal coliforms, *Escherichia coli*, *Salmonella enterica*, *Enterococcus* spp., *Staphylococcus aureus* and *Pseudomonas aeruginosa* differ among the various GW streams (e.g. raw, biologically treated, and disinfected). The culture-dependent analyses suggested that both chlorine and UV inactivate most of the bacteria tested in the biologically treated GW, albeit at different efficiencies. Conversely, the DNA-based analyses indicated no significant differences in pathogenic bacterial abundance between the biologically treated GW with or without disinfection. To better understand the discrepancies between the results, we repeated the analysis in the laboratory under controlled conditions using *Enterococcus faecalis* as a model bacterium and obtained similar results. We suggest that disinfection of biologically treated GW with chlorine or UV is effective for treating pathogens, but that the inactivation efficiency cannot be estimated by DNA-based qPCR.

* Corresponding authors at: Ben-Gurion University of the Negev, Sede Boqer Campus, Midreshet Ben Gurion 84990, Israel.
E-mail addresses: gilloro@bgu.ac.il (O. Gillor), amgross@bgu.ac.il (A. Gross).

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1. Introduction

The demand for water by a growing world population has prompted the search for new water sources. One such source is domestic water reclamation and reuse. Graywater (GW) is the non-toilet portion of the domestic wastewater stream that comes from bathing, laundry and in some cases, kitchen use (Friedler et al., 2005). GW composition and volume are highly variable, as they depend on sanitary standards, and on the lifestyle within a household such as resident age, family size, eating habits, and detergents used (WHO, 2006).

GW is often considered a rather harmless wastewater resource but world practice in its application varies. Raw GW is allowed for limited local reuse as irrigation water in several parts of Australia and USA while other countries only allow the use of GW following certain treatment requirements. A few countries ban GW use completely, primarily due to the unknown risks it may pose to public health (Finley et al., 2009; Maimon et al., 2010). Although world-wide practices may differ, as addressed by Maimon et al., 2010, academic consensus regards raw GW as a stream in need of treatment and disinfection to decrease possible health risks.

A main impediment to widespread GW use is the possible presence of pathogens (Benami et al., 2013), which might be directly or indirectly transferred to humans (Ottoson and Strenström, 2003; Toze, 2006). If pathogen concentrations exceed the stated health standards for treated wastewater reuse, disinfection is required to reduce possible infection (Gross et al., 2008). There is growing evidence that the elimination of pathogenic bacteria is essential to domestic GW reuse (Maimon et al., 2010). To that end, various disinfection methods have been suggested; among the most popular are chlorine and more recently, UV radiation (Gilboa and Friedler, 2008; Tchobanoglous et al., 2003). If it is to be used in small on-site systems, the disinfecting agent must be safe, simple, low-cost and reliable.

The ideal techniques for inactivating microorganisms depend upon the quality of the GW sources to be reused as well as their intended purpose after treatment (Beck et al., 2013). There are biological treatment systems, such as the recirculating vertical flow constructed wetlands (RVFCW) used in this study that can reliably provide high quality effluent with 5 days biochemical oxygen demand (BOD_5) and total suspended solids (TSS) concentrations of less than 10 mg L^{-1} (e.g. Alfiya et al., 2013; Sklarz et al., 2009). Such TGW effluent would require a markedly lower initial chlorine concentration to maintain a residual disinfectant concentration (Winward et al., 2008a).

Chlorination is usually an efficient and reliable disinfectant for most pathogens but being a strong oxidizer, it must be handled with care. The amount of free available chlorine in a sample is positively correlated with its inactivation, or lethality effects on microorganisms, but overdosing might result in the formation of toxic byproducts such as halomethanes (Edberg et al., 2000). Being an unstable molecule that breaks down when exposed to air, light or metals, its impact diminishes over time (Abadias et al., 2011; Edberg et al., 2000). UV light (UV–C: 200–280 nm) inactivates microorganisms via the formation of lacerations in the DNA that interfere with its ability to proliferate. On-site disinfection with low-pressure UV is safe to handle, does not leave residual disinfection byproducts, and is usually reliable, and it has therefore been proposed as an alternative to chlorine for disinfection (Chang et al., 1985; Muraca et al., 1987). Both UV and chlorination, however, require maintenance and are negatively impacted by the presence of organic matter, pH levels, and suspended solids (Qualls et al., 1983, 1985; Supplementary information: GW Quality and Disinfection).

Separate studies have tested the disinfection efficiency of chlorine or UV to reduce selected pathogen viability in GW systems. In general results of these studies vary based on GW quality, disinfection dosages, and bacteria targeted. However, when considering fecal coliforms as the sole indicator of disinfection efficiency (as virtually practiced by all standards for irrigation) it has been demonstrated that both chlorine and UV disinfection can reduce average fecal coliform concentrations to below 10 CFU 100 mL^{-1} in biologically treated domestic effluents (Friedler et al., 2005; Sklarz et al., 2009).

The required level of disinfection of pathogens relate to their infective dosages (Supplementary information: Infective dose). This is a complex issue as infective dose varies between people, type of contact (e.g. aerosols vs. ingestion), and specific pathogen properties (Leggett et al., 2012; Roser et al., 2014; Rusin et al., 1997; Schmid-Hempel and Frank, 2007). A complete inactivation of pathogens would be ideal but impractical. Therefore, the concept of acceptable risk was developed in which it was determined that a certain infection in a population is reasonable. Recent GW standards base their allowed indicator concentration for a certain use (e.g. irrigation) after quantitatively analyzing the potential risk for infection using infective dose standards (Maimon et al., 2010; Ottoson and Strenström, 2003).

Pathogens and/or indicators are generally characterized by culture-dependent methods that are considered the "gold standard" for pathogen quantification (Friedler and Gilboa, 2010; Gilboa and Friedler, 2008; Winward et al., 2008a,b). Culture-independent technologies, primarily real-time quantitative PCR (qPCR), have been successfully applied for the detection of pathogens in various environmental waters (Francy et al., 2009; Girones et al., 2010; Shannon et al., 2007). Correlations between culture-dependent and -independent methods are still under debate, however, with some researchers confirming the agreement between methods (Brinkman et al., 2003; Castillo et al., 2006; Whitman et al., 2010), while others challenge that agreement (Huggett et al., 2005; Noble et al., 2010; Pietarinen et al., 2008). The sensitivity and specificity of these methods primarily depend on the background microflora, sample matrix, presence of non-culturable cells, and inhibitory substances (e.g. fats, proteins, polysaccharides, heavy metals, antibiotics, and organic compounds) and with this the agreement between both detection methods may fluctuate (Girones et al., 2010; Maurer, 2011). This heightens the need for more investigation of the effects the matrix might have on the recovery of microorganisms, viability of the targeted microorganisms, and on qPCR reaction conditions. To our knowledge no study has compared the accuracy or agreement of qPCR and culture-based detection on a variety of pathogens and indicators in differing types of environmental waters.

The aims of the current study are to establish disinfection efficiency of GW by using multiple tests on samples of: (1) on-site raw GW, (2) biologically treated GW (TGW), and (3) TGW disinfected by either chlorine or UV. We hypothesized that both disinfection methods would consistently lower the culturable microorganism concentrations, on average, to below published infective dosage levels (Beck et al., 2013; Gilboa and Friedler, 2008). Moreover, we expected high inactivation efficiency due to the high quality effluent after the biological treatment that by itself inactivates significant amounts of microorganisms (Gross et al., 2008). We also hypothesized that culture-dependent and -independent methods would report similar pathogen concentrations in all types of GW, excluding disinfected TGW. Potentially higher detection sensitivity would exist for culture-independent, molecular-based technologies because of their ability to quantify already inactivated as well as viable but non-culturable pathogens (Keer and Birch, 2003; Oliver, 2010; Sen et al., 2011).

2. Material and methods

2.1. Field setup, sample collection and processing

Raw, biologically treated, and disinfected (UV or chlorine) GW effluent (excluding kitchen effluents) from three recirculating vertical flow constructed wetland (RVFCW) sites (Alfiya et al., 2013) were collected bi-monthly over the course of 1 year (Fig. 1). Briefly, an RVFCW is composed of two 500 L plastic containers (1.0 m × 1.0 m × 0.5 m) placed atop each other. The top container acts as a vertical flow wetland holding a planted three-layer bed and was perforated at the bottom. The bed is composed of 5 cm top layer of woodchips, followed by a 35 cm middle