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Comparison of methods to determine the microbial quality of alternative irrigation waters

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ABSTRACT

The availability of water for crop irrigation is decreasing due to droughts, population growth, and pollution. Implementation of the Food Safety and Modernization Act (FSMA) for irrigation water standards will discourage growers to use poor microbial quality water for produce crop irrigation. We evaluated the applicability of a novel concentrator method for assessment of microbiological quality of alternative waters including secondary-treated wastewater (STWW), roof-harvest rainwater (RHW), and creek water (CW) in comparison to the standard membrane filtration method. Water samples of 100 ml were filtered through a 0.45 µm membrane filter using a vacuum manifold or concentrated to ~250 µl using the innovative concentrator. Then they were directly enumerated on specific agars, or enriched to monitor the populations of fecal bacterial indicators (*Escherichia coli*, enterococci, total and fecal coliforms) and bacterial pathogens (*Salmonella*, *Listeria monocytogenes*, and *E. coli* O157:H7). Presumptive pathogens were confirmed by real-time quantitative PCR. In total, 25 samples of alternative water were analyzed including 7 STWW, 9 RHW, and 9 CW. No significant differences between both detection methods were observed when enumerating indicator bacterial populations and detecting the presence of pathogens in RHW and CW samples. Recovery of fecal coliforms in STWW samples by concentrator analysis was significantly lower than the membrane filtration technique. Results suggest that performance of the concentrator method is equivalent to membrane filtration method in determining the microbiological quality of CW and RHW waters; the type of the water sources may influence the accuracy and sensitivity of the concentrator analysis.

1. Introduction

Water scarcity has become a major worldwide problem because of climate change and increased urbanization. Climate change altered the weather patterns and resulted in a higher frequency and intensity of droughts in the world (Meehl et al., 2007). Moreover, the world population has increased from 2.5 billion in 1950 to 6.5 billion in 2009 with doubled irrigated area and tripled water withdrawals (Pardey et al., 2014; Schierhorn et al., 2014) which could result in water scarcity in future. Therefore, to meet the growing demand for irrigation water, alternative water sources are imperative (Teklehaimanot et al., 2015).

The use of alternative water sources such as wastewater and roof-harvested rainwater in agriculture has gained more attention lately as a way to overcome water scarcity. Wastewater is commonly used in water scarce regions, especially in Asia and Africa countries. In China, 7% of the nation's farmland (4.1 million ha) are irrigated with polluted water (Xie, 2009); In Pakistan, 26% of the vegetables are dependent on irrigation with wastewater (Pedrero et al., 2010). In Europe, countries such as Spain, France, Italy, and Greece have allowed the use of treated wastewater for agriculture irrigation if it would not lead to the pollution of surface water by chemical and biological contaminants from the wastewater (EU, 2007; EC, 2016).

The approval of the Clean Water Act in 1972 made the secondary treatment a requirement for all wastewater treatment plants in the United States. The United States Environmental Protection Agency reported that 70% of the 16,000 facilities did not provide tertiary treatment of wastewater (USEPA, 2004), which was later recommended as an advance treatment (USEPA, 2012b). Thus, secondary-treated wastewater (STWW) was evaluated for its potential as irrigation water in the current study. Wastewater treatment plants process 130 gigalitre/day of wastewater in the United Sates (Seiple et al., 2017). The reuse of wastewater minimizes the discharge of treated effluent directly into freshwater (Scott et al., 2004). Moreover, wastewater contains nitrogen and phosphorus that are natural fertilizers for crops, which reduce the need for supplemental mineral fertilizers (Jimenez et al., 2010; Mojid

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et al., 2010).

Roof-harvested rainwater (RHW) and creek water (CW) have also been considered as potential water sources for irrigation purposes (Ahmed et al., 2011; Chidamba and Korsten, 2015). Roof-harvested rainwater has been used as a potable- and nonpotable-water source in many countries such as Australia (Uba and Aghogho, 2000; Evans et al., 2006; Despins et al., 2009). Currently, applications of RHW for irrigation have not been well recognized and only 17 states have established guidelines to regulate the usage of RHW for agricultural irrigation in the United States (NCSL, 2017).

The consumption of fresh produce increased by 25% per capita during 1990s compared to 1970s due to changes in dietary trends and globalization (Pollack, 2001; Brandl, 2006). The proportion of all foodborne outbreak illnesses associated with raw fresh produce has also increased from < 1% to 12% during this period (Harris et al., 2003; Sivapalasingam et al., 2004). Approximately 48 million foodborne illnesses occur each year in the United States and ~46% of the illnesses are linked to fresh produce contamination (Painter et al., 2013). Studies have suggested that water is a significant source of contamination to fresh produce such as spinach, lettuce, and cabbage (Uyttendaele et al., 2015). The recently proposed Food Safety and Modernization Act (FSMA) for irrigation water standards discourage growers to use poor microbial quality water for produce crop irrigation (USFDA, 2013).

The microbiological quality of water is generally assessed by monitoring the fecal indicator bacteria, which are commonly found in the guts of the warm-blooded animals (Pinfold et al., 1993; Uba and Aghogho, 2000; Sazakli et al., 2007). The alternative water resources herein studied may contain high populations of coliforms, *Escherichia coli* or pathogenic bacteria including *Salmonella* spp., *Listeria monocytogenes*, and *E. coli* O157:H7 (Déportes et al., 1995; Steele and Odumeru, 2004; USEPA, 2012a). For instance, although microbial populations decrease during the wastewater reclamation process (Van der Steen et al., 2000), the secondary treated effluents may contain *Salmonella* (Maynard et al., 1999; Armon et al., 2002). Moreover, RHW might be contaminated by bird droppings on the roof (Ahmed et al., 2011). The creek water is also susceptible to contamination with pathogenic microorganisms by storm water runoff, animal fecal materials, and sewage discharges (Bagdasaryan, 1964; Alderisio and DeLuca, 1999; Steele and Odumeru, 2004).

Traditionally, the membrane filtration method has been used for the examination of bacterial populations from environmental waters (USEPA, 2002a,b; USEPA, 2012b). However, this method may be inappropriate for water with high turbidity due to clogging of the membrane filter by particulate matter (SIS, 1996; Eckner, 1998; Köster et al., 2003). In this study, a rapid, innovative biological concentrator developed for general microbiology purpose was introduced for irrigation water analysis. This is the first report on the evaluation of an innovative biological concentrator to determine the microbial quality of alternative irrigation waters in comparison with the traditional membrane filtration method.

2. Materials and methods

2.1. Sampling locations and sample collection

Sampling locations included water collected from rain-barrels of local households, creeks (Little Paint Branch creek, Beltsville, MD; Little Cove creek, Chambersburg, PA), and a wastewater treatment plant (Arlington, VA). All water samples were collected from November 2016 to January 2017. Water samples (4 liters/sample) were collected in sterile, labelled plastic bottles (Fisher Scientific, Fair Lawn, NJ), stored at 4 °C, and analyzed within 24 h.

Secondary-treated wastewater (STWW) was collected at the Arlington Water Pollution Control Plant (AWPCP) that purifies ~115 million liters of wastewater each day from residences and businesses through 5 wastewater treatments and solids handling systems (Preliminary treatment, Primary treatment, Secondary treatment, Tertiary treatment/Chemical addition, and Treatment of solids). A total of 7 STWW samples were collected at the location where the waste water is passed through six-10 million liters, four-pass aeration tanks, configured for biological nutrient removal.

Seven creek water samples (CW) were collected from the Little Paint Branch creek (Beltsville, MD) and 2 CW samples were obtained from Little Cove Creek (Chambersburg, PA). In addition, 9 roof-harvest rainwater samples (RHW) were obtained from rain-barrels of local households located in the state of Maryland.

2.2. Indicator bacteria enumeration

Indicator bacteria including total coliforms, fecal coliforms, *E. coli*, and enterococci from each water sample were enumerated by the membrane filtration method and the concentrator method.

For the membrane filtration method, each water sample with appropriate dilution (total volume 100 ml) was filtered through a 0.45 µm (47 mm diameter) nitrocellulose membrane (Fisher Scientific) using vacuum manifold (Thermo Scientific, Waltham, MA). Immediately after filtration, membrane filters with trapped bacteria from the water samples were transferred onto specific agar plates and then transferred to incubator with appropriate incubation conditions. The specific agar media used were Violet Red Bile agar (VRB; Fisher Scientific), mFC agar (Fisher Scientific), mTEC agar (Neogen, Lansing, MI), and m-enterococcus agar (mE; Neogen) for the enumeration of total coliforms, fecal coliforms, *E. coli*, and enterococci, respectively. The VRB and mE agars were incubated at 35 °C for 24 h and 48 h, respectively, and mTEC and mFC agars were first incubated at 35 °C for 2 h, followed by at 44 °C for 24 h.

For the concentrator method, each water sample with appropriate dilution (total volume 100 ml) was concentrated using a bio-concentrator (InnovaPrep, Drexel, MO) and a 0.45 µm concentrating pipette (InnovaPrep) to a ~250 µl concentrate, and the entire ~250 µl concentrate was spread plated onto the aforementioned agars with the appropriate incubation conditions as previously prescribed.

For both methods, number of bacterial colonies between 20 and 60 per plate (USEPA, 2002a,b) from an original or diluted water sample was counted and expressed as log colony-forming units (CFU) per 100 ml. Plates with < 20 colonies were counted when counts were low in undiluted water sample. Detection thresholds of both detection methods for all water samples were 1 CFU/100 ml.

2.3. Anaerobic bacteria enumeration

Populations of anaerobic bacteria including *Clostridium perfringens* and *Bacteroides fragilis* from water samples were determined. Individual water samples (100 ml) were filtered or concentrated as previous described. Then the membrane and the ~250 µl concentrate were placed and spread plated onto Tryptose Sulfite Cycloserine agar (TSC; Sigma Aldrich) or Bacteroides Bile Esculin agar (BBE; Hardy Diagnostics, Santa Maria, CA) for the isolation of *C. perfringens* or *B. fragilis*, respectively. Agar plates were incubated anaerobically at 36 °C for 48 h (Bisson and Cabelli, 1979).

To detect low level contamination by these bacteria (< 1 CFU/100 ml), one additional membrane and a ~250 µl concentrate were separately transferred to a tube containing 10 ml of fluid thioglycollate broth (FTB, Fisher Scientific) and incubated anaerobically at 36 °C for 48 h for enrichment. After incubation, a loopful of FTB was streaked on TSC and BBE agars, and incubated at 36 °C for 24 h to detect these bacteria following enrichment.

2.4. Detection of pathogenic bacteria

Salmonella spp., *L. monocytogenes*, and *E. coli* O157:H7 were detected in water samples using primary enrichment in full-strength