



The effect of a first flush rainwater harvesting and subsurface irrigation system on *E. coli* and pathogen concentrations in irrigation water, soil, and produce

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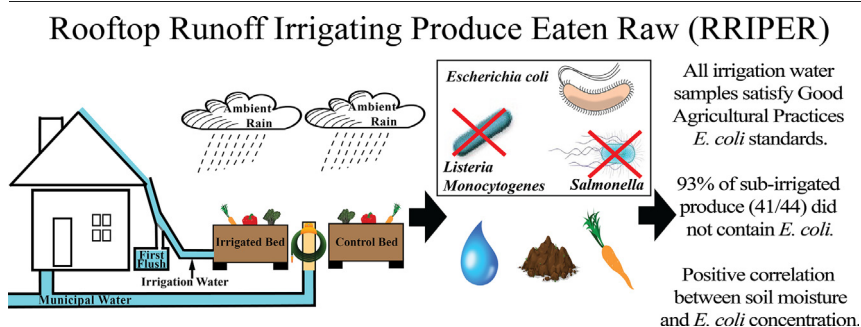
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HIGHLIGHTS

- Climate change stresses agriculture water sources, safe alternatives are needed.
- Harvested rainwater was delivered to produce through subsurface irrigation.
- No *Salmonella* or *Listeria* were detected in water samples.
- Only 7 % of produce from beds irrigated with harvested rainwater were *E. coli* positive.
- Rainwater collected with first flush system met current food safety standards.

GRAPHICAL ABSTRACT



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ABSTRACT

Climate change is stressing irrigation water sources, necessitating the evaluation of alternative waters such as harvested rainwater to determine if they meet water quality and food safety standards. We collected water, soil, and produce samples between June and August 2019 from two vegetable rain garden (VRG) sites in Frederick, Maryland that harvest rainwater using a first flush system, and deliver this water to produce through subsurface irrigation. The raised VRG beds include layers of gravel, sand, and soil that act as filters. We recorded the average surface soil moisture in each bed as well as antecedent precipitation. All water ($n = 29$), soil ($n = 55$), and produce ($n = 57$) samples were tested for generic *E. coli* using standard membrane filtration, and water samples were also tested for *Salmonella* spp. and *Listeria monocytogenes* by selective enrichment. No *Salmonella* spp. or *L. monocytogenes* isolates were detected in any water samples throughout the study period. Average *E. coli* levels from all harvested rainwater samples at both sites (1.2 and 24.4 CFU/100 mL) were well below the Good Agricultural Practices food safety guidelines. Only 7 % (3/44) of produce samples from beds irrigated with harvested rainwater were positive for *E. coli*. *E. coli* levels in soil samples were positively associated with average surface soil moisture ($r^2 = 0.13$, $p = 0.007$). Additionally, *E. coli* presence in water samples was marginally associated with a shorter length of antecedent dry period (fewer days since

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preceding rainfall) ($p = 0.058$). Our results suggest that harvested rainwater collected through a first flush system and applied to produce through subsurface irrigation meets current food safety standards. Soil moisture monitoring could further reduce *E. coli* contamination risks from harvested rainwater-irrigated produce. First flush and subsurface irrigation systems could help mitigate climate change-related water challenges while protecting food safety and security.

1. Introduction

As global temperatures continue to rise and populations grow exponentially, traditional irrigation water sources are becoming increasingly stressed. In 2016, 52 % of the world's population lived in areas of water scarcity, and this percentage is projected to increase to almost 57 % by 2050 (Boretti and Rosa, 2019). Climate change will reduce the quantity and quality of freshwater available for agriculture through increasing droughts, yet the growing population will increase demands for safe and reliable irrigation water (Dawadi and Ahmad, 2019; Hatfield et al., 2014; Malcolm et al., 2012). Climate change will also cause more variable precipitation. For example, seasonal drought and heavy precipitation are both projected to increase in frequency in the Northeast region of the United States (Melillo et al., 2014). Heavy precipitation events, which lead to reduced infiltration and cause runoff, increased by over 70 % in recent decades in this region (Melillo et al., 2014). These climate change impacts will affect plant growth, soil health, and food security and safety (Fahad et al., 2019, 2021a, 2021b; Sönmez et al., 2021). To address agricultural sustainability, it is becoming increasingly necessary to explore new sources of water for irrigation including harvested rainwater.

In addition to capturing water from extreme precipitation events, harvesting rainwater also conserves freshwater and reduces stormwater runoff (Steffen et al., 2013). Stormwater runoff is generated from rain and snowmelt that flows over impervious surfaces picking up harmful pollutants along the way, such as sediment, trash, and chemicals, which are then carried to local waterways (Li et al., 2009). Our study focused on rainwater harvested from roof surfaces at two urban garden sites. An Australian study showed that rainwater harvested from a roof surface could reduce the volume of stormwater runoff by 90 % (Tom et al., 2013). Harvesting rainwater may also replace city water connections and reduce water costs, especially for farms that do not have easily accessible water nearby or farms in urban areas. A Roanoke, Virginia case study found that nearly 442,934 m³/year of rainwater could be harvested from rooftops, providing all of the water needed for existing local agriculture (Parece et al., 2017).

Assessing the food safety risk of using harvested rainwater for produce irrigation is necessary because contaminated irrigation water has been identified as the cause of previous foodborne outbreaks. Multiple studies have found evidence of foodborne pathogens in freshwater sources intended for crop irrigation, although they did not investigate whether these pathogens were transmitted to the crops (Bell et al., 2015; Benjamin et al., 2013; Li et al., 2014; McEgan et al., 2014). Other studies have found evidence of transmission of foodborne pathogens from irrigation water to field crops, specifically pathogenic *Escherichia coli* and *Salmonella* spp. (Islam et al., 2004a, 2004b). Islam et al. (2004a) found that *Salmonella enterica* (2004b) and *E. coli* O157:H7 can survive for extended periods of time in soil and on the surface of produce, raising concerns about the safety of using contaminated irrigation water on edible produce. In contrast, a 2019 study found no conclusive evidence that irrigation water contaminated with *Salmonella enterica*, and *Listeria monocytogenes* transferred those pathogens to field soil and crops when drip irrigation was used (Allard et al., 2019). Further, subsurface irrigation—using perforated tubes or pipes under the ground surface to apply water to a crop's root zone—reduces microbial contamination of produce (Song et al., 2006).

Numerous studies have analyzed harvested rainwater for the presence of fecal indicator bacteria, such as *E. coli* (Abbasi and Abbasi, 2011; Amin et al., 2013; Bae et al., 2019; Clark et al., 2019; Gikas and Tsihrintzis, 2017; Gikas and Tsihrintzis, 2012; Hamilton et al., 2018; Hamilton et al., 2019; Kim et al., 2016). As outlined in a review by Hamilton et al., out of 19 studies analyzing *E. coli* in rainwater collection tanks, at least one tank

in all 19 studies exceeded World Health Organization drinking water guidelines of no *E. coli* per 100 mL of water (2019). In addition, several researchers have investigated the occurrence and concentration of microbial pathogens in harvested rainwater identifying *Campylobacter*, *Salmonella*, *Shigella*, *Vibrio*, pathogenic *E. coli*, *Acinetobacter*, *Aeromonas*, *Citrobacter*, *Klebsiella*, *Legionella*, *Mycobacterium*, *Pseudomonas*, *Staphylococcus*, *Yersinia*, as well as several pathogenic protozoa and viruses (Hamilton et al., 2019). To address the noted microbial risks from harvested rainwater, a few studies have evaluated the effectiveness of a range of treatment systems, including first flush systems—a system that collects the first gallons of runoff water at the beginning of a rainfall event—on the microbial quality of harvested rainwater (Gikas and Tsihrintzis, 2012; Mendez et al., 2011; Lee et al., 2012). First flush systems have been found to reduce the concentration of total coliforms and turbidity in harvested rainwater (Mendez et al., 2011; Lee et al., 2012). Despite the breadth of studies evaluating the presence of microorganisms in harvested rainwater, there is a lack of research on the impact of first flush systems and subsurface irrigation on pathogens in harvested rainwater intended for agricultural irrigation. In addition, to our knowledge, no previous studies have coupled an evaluation of the quality of harvested rainwater collected through a first flush system with an analysis of the produce subsurface-irrigated with this water. One study estimated the risk of foodborne illness from consuming lettuce irrigated with harvested rainwater using a risk assessment model but did not collect actual produce samples. The addition of the produce analysis in the present study provides important information for human health risks from the use of harvested rainwater for produce irrigation. Yin et al. conducted a controlled field experiment where harvested rainwater was used to spray irrigate spinach, but the harvested rainwater did not undergo any additional treatments before use (Yin et al., 2019). Because food safety guidelines and regulations, such as the U.S. Food and Drug Administration (FDA) Food Safety Modernization Act (FSMA), encourage the use of irrigation techniques that reduce contact between irrigation water and produce, it is increasingly important to understand the risk for transfer of bacteria between harvested rainwater supplied by drip or subsurface irrigation to crops.

The purpose of this study was to assess the presence and concentration of *E. coli*, *Salmonella* spp., and *L. monocytogenes* in rainwater harvested from a rooftop surface using a first flush system, as well as soil and produce samples irrigated with this water through a subsurface system. In addition, we analyzed the correlation between the presence and concentration of *E. coli* with soil moisture levels and antecedent dry periods. This study serves as a first step in evaluating the feasibility and microbial safety of using a first flush system combined with subsurface delivery to collect harvested rainwater for produce irrigation.

2. Material and methods

2.1. Site description and field design

Water, soil, and produce samples were collected between June and August 2019 at two sites in Frederick, Maryland, USA (Fig. 1): the Religious Coalition for Emergency Human Needs (RC) (Fig. 1A) and Hood College (HC) (Fig. 1B). Frederick is located approximately 32 km northwest of Washington, D.C., and receives an average of 91 cm of precipitation each year.

The roof surface area available for rainwater capture was approximately 190 and 70 m² at the RC and HC locations, respectively. The roofs at both locations consisted of asphalt shingles. The RC building used vinyl gutters and aluminum downspouts, while the HC location was equipped with

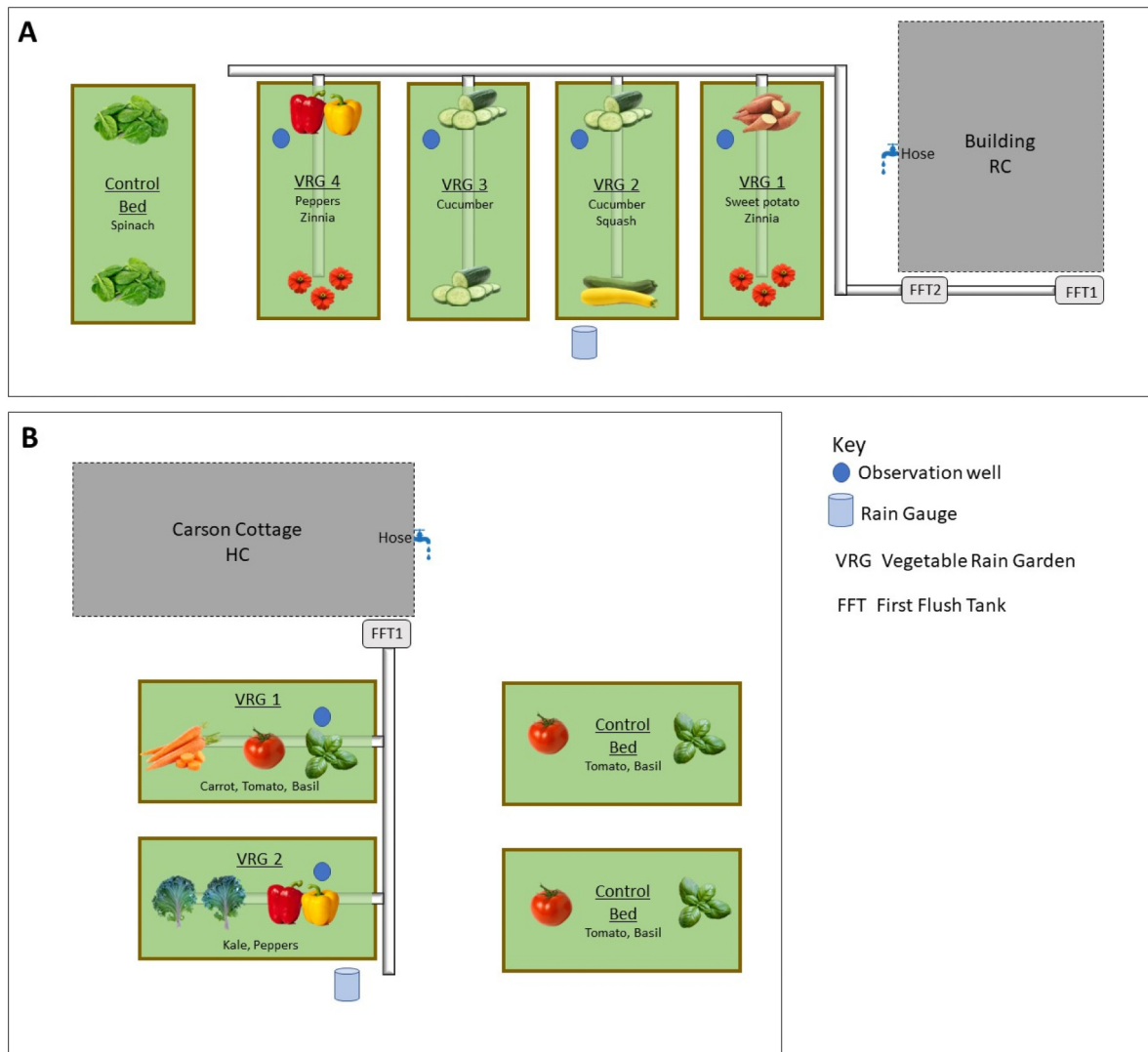


Fig. 1. Study area location and sampling sites in Frederick, MD. A) Overhead schematic of layout at Religious Coalition for Emergency Human Needs (RC) site. B) Overhead schematic of layout at Hood College (HC) site. Vegetable Rain Garden (VRG) beds were subsurface irrigated with harvested rainwater. Beds and buildings are not drawn to scale.

galvanized steel gutters and downspouts. Six experimental vegetable rain garden (VRG) beds were sampled in the study, four at the RC and two at the HC location. The VRG beds were constructed based on a design adapted from Melbourne Water (Fig. 2) (Richards et al., 2015). VRG beds were sub-irrigated by harvested rainwater. Before entering VRG beds, harvested rainwater was directed into a 159-liter (35-gal) first flush tank by gutters and downspouts. This corresponds to the first 0.06 and 0.07 in. during a 1-in. rain event being diverted at RC and HC respectively. Once the first flush tank was filled, a ball valve stopped collection in the first flush tank and redirected additional harvested rainwater into the VRG bed reservoirs via a perforated pipe 3 f. below the soil surface. At the end of each rain event the first flush tank was drained.

The VRG beds consisted of three layers: gravel (46 cm), sand (10 cm), and topsoil (36 cm) (Fig. 2). Water moved vertically from the gravel to the sand and soil layer via sand wicks. The soil layer in each VRG bed at the RC was filled with topsoil provided by Bussard Brothers (Monrovia, Maryland) and amended with compost in the spring.

There were slight differences in the VRG bed design at each location. The sides of the VRG beds at the RC were lined with pond liner, with the bottom of the bed left unlined while the HC VRG beds were fully lined. In the fully lined HC VRG beds, the continued flow of harvested rainwater

allowed the gravel layer to become saturated with water and act as a reservoir. In the unlined RC beds, harvested rainwater infiltrated back into the ground. In addition, the HC VRG beds were built with a reservoir overflow positioned at the top of the gravel layer of each bed, while the RC VRG beds were built with an overflow pipe at the terminal VRG bed. A device was installed at the RC site to capture post-flush water at each of the first flush tanks to allow for easy access for sampling of irrigation water (Fig. 2). Each location also contained control beds that were watered with municipal water supplied via a hose; one control bed at RC and two at HC. Control beds differed slightly at each location due to the differences in garden designs used at each site. Control beds at the HC location consisted of two in-ground beds amended with compost. The RC control bed was a traditional raised bed built from the same lumber as the VRG beds and filled with topsoil provided by Bussard Brothers (Monrovia, Maryland).

Seeds of multiple crops (cucumber, basil, carrot, kale, pepper, and cherry tomatoes) were chosen by the site garden managers and planted in the VRG and control beds in the spring of 2019 (Fig. 1). Crops grown in the VRG, and control beds were grown using organic practices. Seedlings were maintained following USDA Good Agricultural Practices (GAPs) at both locations and watered by hose during dry periods to ensure their survival.

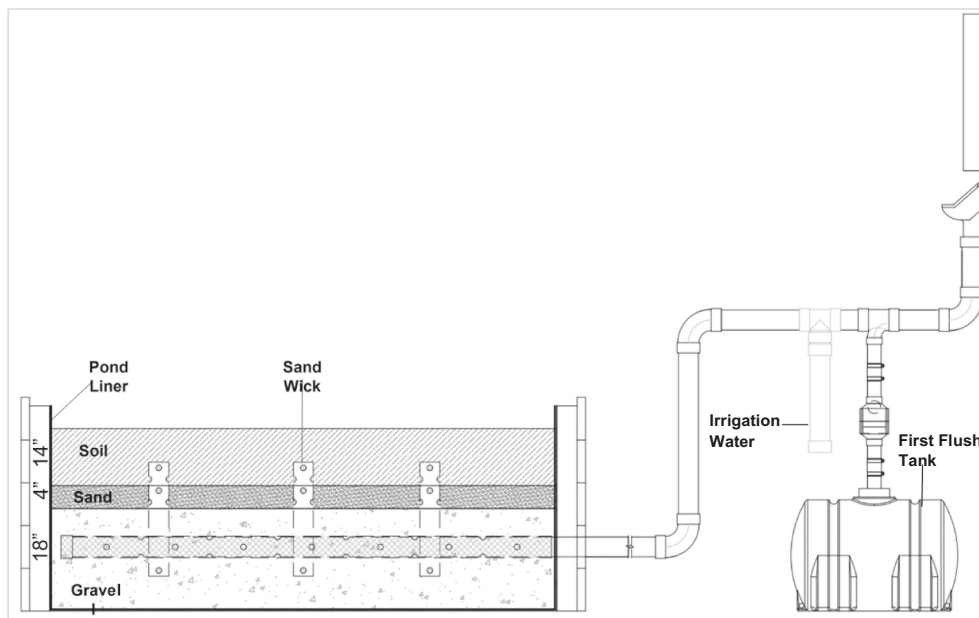


Fig. 2. Diagram of the Vegetable Rain Garden (VRG) bed design (adapted from Richards et al., 2015). Rainwater filled a 159-liter (35-gallon) “first-flush” tank before diverting water into the perforated pipe embedded in the gravel layer. In fully lined VRG beds, the gravel layer becomes saturated and acts as a reservoir (HC). Infiltration of harvested rainwater back into the ground occurred in beds where the bottom was unlined (RC). Post-rainfall, water moved vertically from the gravel reservoir to the sand and soil layer via sand wicks.

2.2. Sample collection

2.2.1. Water samples

Samples were collected from the first flush tank and water entering the VRGs (“Irrigation Water”) from both study sites. Irrigation water at RC was collected from a post-first flush collection device (Fig. 2), while at HC, water was retrieved aseptically using sterile tubing and a hand pump from an observation well. Municipal water was also collected from a hose at the RC site which was used to water the control bed. A total of 29 grab samples were collected between June 2019 and August 2019: 21 samples from RC; 8 samples from HC (Table 1). Samples were collected in sterile 1 L Nalgene® polypropylene bottles (Thermo Fisher Scientific, Waltham, MA) for generic *E. coli* and pathogen analysis and transported in coolers containing ice packs at 4 °C to the laboratory for processing within 24 h.

2.2.2. Soil samples

Two soil samples were collected from each VRG and control bed on all sampling dates ($n = 55$); 40 samples from RC and 15 from HC (Table 1). Two equidistant sampling zones were selected within each VRG and control

bed and marked with flags for future sampling dates. Soil from the top 4 cm layer of each bed was collected using sterile scoops, placed into sterile Whirl-Pak® bags, and transported in coolers containing ice packs at 4 °C to the laboratory for processing within 24 h.

2.2.3. Soil moisture

Soil moisture was measured using a Delta T Devices Dynamax probe (SM150T) equipped with a handheld portable meter (HH150). Soil moisture was measured in VRG and control beds on eight sampling dates between June and August 2019. Surface readings were obtained to calculate average soil moisture for each bed.

2.2.4. Produce samples

Two produce samples ($n = 57$; 40 from RC, 17 from HC), consisting of either two leaves or two small fruits/vegetables (approximately 20 g), were collected from each VRG or control bed using sterile sampling scissors and placed into sterile Whirl-Pak® bags. Produce samples were collected as close to the soil sampling zones as possible. Produce samples were transported in coolers containing ice packs at 4 °C to the laboratory for processing within 24 h.

Table 1

Average concentration and distribution of *E. coli*-positive water, soil, and produce samples by location, water or bed type, and sampling month.

Location	Sample type (n)	Water or bed type (n)	Average <i>E. coli</i> (CFU/100 mL) (CFU/g) ^a	No. Positive Samples (%)			Total positive samples (%)
				June 2019	July 2019	August 2019	
Religious coalition (RC)	Water (21)	First Flush (8)	1.8	0/2 (0)	4/4 (100)	2/2 (100)	6/8 (75)
		Irrigation Water (6)	0.5	0/2 (0)	3/4 (75)	–	3/6 (50)
		Municipal (7)	0.0	0/2 (0)	0/3 (0)	0/2 (0)	0/7 (0)
	Soil (40)	Control (8)	0.0	0/2 (0)	0/4 (0)	0/2 (0)	0/8 (0)
		Irrigated (32)	177.6	1/8 (12)	3/16 (19)	0/8 (0)	4/32 (12)
	Produce (40)	Control (8)	0.0	0/2 (0)	0/4 (0)	0/2 (0)	0/8 (0)
		Irrigated (32)	53.1	0/8 (0)	1/16 (6)	0/8 (0)	1/32 (3)
Hood College (HC)	Water (8)	First Flush (2)	0.5	–	1/2 (50)	–	1/2 (50)
		Irrigation Water (6)	32.3	–	4/4 (100)	1/2 (50)	5/6 (83)
		Control (5)	147.5	–	2/3 (67)	0/2 (0)	2/5 (40)
	Soil (15)	Irrigated (10)	837.5	–	5/6 (83)	1/4 (25)	6/10 (60)
		Control (5)	0.0	–	0/3 (0)	0/2 (0)	0/5 (0)
	Produce (17)	Irrigated (12)	0.2	–	2/8 (25)	0/4 (0)	2/12 (17)
		Control (5)	0.0	–	0/3 (0)	0/2 (0)	0/5 (0)

^a *E. coli* were measured in CFU/100 mL for water samples, and in CFU/g for both soil and produce samples.

2.2.5. Rainfall data

During each sampling event the local weather conditions were recorded, including the ambient temperature (°F) and the total rainfall (in) in the past 24 h as measured by the onsite rain gauges. In addition, historic daily levels of precipitation (in) between June 1–August 30, 2019, were retrieved from the closest weather station to both study sites (HC and RC) in Frederick, MD from the local weather forecast service (wunderground.com). These data were used to compute the length of the antecedent dry period for each sampling date, i.e., the number of days without rainfall.

2.3. Microbiological analysis

2.3.1. *E. coli* isolation

Water samples were processed for *E. coli* using U.S. EPA Standard Method 1604 (US EPA, 2002). Briefly, 10, 100, and 500 mL of each sample were vacuum filtered through 0.45 µm, 47 mm mixed cellulose filters (Millipore, Billerica, MA). Filters were plated on MI Agar to isolate *E. coli* (BD, Franklin Lakes, NJ). Plates were incubated at 37 °C for 24 h, then all blue colonies under ambient light were counted as presumptive *E. coli*. Presumptive colonies were purified on MacConkey agar and archived in Brucella broth (Becton, Dickinson, and Company) with 15 % glycerol at –80 °C. *E. coli* ATCC 8739 was used as a positive control and phosphate buffered saline was used as a negative control throughout the isolation process.

2.3.2. *Salmonella* and *Listeria* isolation

Analyses for both pathogens were based on methods used in Sharma et al. (2020). Each of the 1 L water samples were gravity-filtered through a modified Moore swab (MMS) composed of grade #90 cheesecloth (Lions Services, Inc., USA). Prior to sampling, the MMS was autoclaved and aseptically inserted into a polyvinyl chloride (PVC) cartridge (16 cm × 4 cm) that was disinfected by soaking in 10 % commercial hypochlorite (bleach) solution overnight (modified from Sbodio et al. (2013)). After gravity-filtration, the MMS was then placed in a sterile Whirl-Pak bag and 100 mL of Universal Pre-enrichment Broth (UPB) (Accumedia, Lansing, MI, USA) were added and massaged for 1 min. The MMS in UPB was then incubated at 37 °C overnight (18–24 h). After incubation, the sample bags were massaged for 1 min, and 40 mL of UPB enrichment were aseptically transferred into a sterile 50 mL conical tube. To determine the presence of *Salmonella*, enrichment liquid was vortexed, and 1 mL and 0.1 mL were transferred into tubes containing 9 mL of tetrathionate (TT) broth (Accumedia) and 10 mL of Rappaport-Vassiliadis (RV) broth (Accumedia), respectively, which were then incubated overnight at 42 °C. For *L. monocytogenes* detection, 1 mL of enriched UPB was transferred to a tube containing 10 mL Buffered Listeria Enrichment Broth (BLEB; Accumedia) supplemented with 0.1 % sodium pyruvate (Sigma-Aldrich, St. Louis, MO, USA) and incubated at 37 °C for 24 h. Selective enrichment broths (1 µL) for *Salmonella* and *L. monocytogenes* were plated on XLT4 (Accumedia) and RAPID'L mono (BioRad, Hercules, CA, USA) agar, respectively, and incubated at 37 °C for up to 48 h. Previous work by Sharma et al. showed that this method has a detection limit of 1 MPN/L (Sharma et al., 2020).

2.4. Confirmation of *E. coli* isolates

A quick lysis method was used to extract DNA from one colony of each isolate as previously described (Micallef et al., 2016). For confirmation of *E. coli*, the DNA was tested by PCR using primers that amplify the β-glucuronidase gene (*uidA*) as previously described by Yoshitomi et al. (2003). Each 25 µL reaction consisted of 2.5 µL of 10× standard Taq reaction buffer (New England Biolabs, Ipswich, MA), 0.2 mM of each dNTP (Amresco, Solon, OH), 2 mM MgCl₂, 0.6 mM of each primer (Integrated DNA Technologies, Coralville, IA), and 2 µL of template DNA. PCR amplification consisted of an initial denaturing step of 95 °C for 3 min, 35 amplification cycles of 95 °C for 30 s, 51 °C for 30 s, and 72 °C for 1 min, and a final extension at 72 °C for 5 min. We separated amplification products by electrophoresis on 2 % agarose gels with EzVision One Loading Dye (Amresco)

and 1× Tris-Borate-EDTA (Alpha Aesar, Ward Hill, MA) at 150 V. A Gel Doc XR+ (BioRad) was used to visualize gels.

2.5. Statistical analysis

The log₁₀ (concentration CFU + 1) transformation of *E. coli* was used to normalize the data before statistical analysis. Geometric means were calculated using the average of the log₁₀-transformed data, which were then back-transformed and approximated to the closest integer. Geometric means were compared to the GAPs recommendation allowing a maximum of one water sample to have ≤235 CFU/100 mL generic *E. coli* and all water samples to have a rolling geometric mean of <126 CFU/100 mL of generic *E. coli* (Cornell, 2022). A two-sample *t*-test was used to evaluate differences between sample types and location. For data that were not approximately normal, or for which the sample size was <20, the Mann-Whitney test was used to evaluate the differences between water or bed types, and each location separately. Moreover, a non-parametric one-way analysis of variance (Kruskal-Wallis test), and the pairwise two-way multiple comparison analysis were used to determine significant differences between *E. coli* concentrations among water types at the RC site.

Correlation and regression analyses were applied to evaluate the association between the *E. coli* levels in soil samples and the average surface soil moisture content (%), as well as the *E. coli* levels in water samples and the length of the antecedent dry period (the number of preceding days without rainfall). The Mann-Whitney Test was also used to evaluate the differences between the detection of *E. coli* in soil or water samples for both locations, and the average surface soil moisture content as well as the length of the antecedent dry period (the number of days without rainfall). Given the relative proximity of the sampling locations, water samples from HC and RC were combined for correlation analyses between *E. coli* and precipitation. All statistical analyses were performed using SAS 9.4 (Cary, NC USA).

3. Results

3.1. Presence and concentration of *E. coli*

E. coli were detected at both the Religious Coalition (RC) and Hood College (HC) locations in all sample types. Between both locations, *E. coli* was present in 52 % (15/29) of all water, 22 % of soil (12/55), and 5 % (3/57) of produce samples from all beds (irrigated and control). Looking at only harvested rainwater used for irrigation and soil and produce in beds irrigated with harvested rainwater, 67 % (8/12) of harvested rainwater, 24 % (10/42) of soil, and 7 % (3/44) of irrigated produce samples positive for *E. coli*. The distribution of *E. coli*-positive samples differed by location, water type, bed type, and month (Table 1). We observed the highest percentage of *E. coli*-positive harvested rainwater samples during the month of July, at both RC (88 %, 7/8) and HC (83 %, 5/6) (Table 1).

At the RC site, 64 % (9/14) of the harvested rainwater samples (first flush and irrigation water) were positive for *E. coli*, with an average concentration of 1.2 CFU/100 mL (Table 1). A greater percentage of first flush samples at the RC site were positive for *E. coli* and had higher concentrations of *E. coli* than that of irrigation water samples. No *E. coli* was detected in any municipal water samples.

At the HC site, 75 % (6/8) of harvested rainwater samples (first flush and irrigation water) were positive for *E. coli*, with an average concentration of 24.4 CFU/100 mL. Unlike at the RC site, first flush samples at HC had a lower percentage of *E. coli*-positive samples and a lower concentration of *E. coli*, compared to irrigation water samples, however these differences were not significant.

For soil samples, at the RC site, *E. coli* were detected in 12 % (4/32) of irrigated bed soil samples. No *E. coli* were detected in RC control bed soil samples. At HC, a greater percentage of soil samples from irrigated beds were positive for *E. coli* and concentrations were higher compared to control bed soil samples. For both sites, the highest percentage of *E. coli*-positive soil samples from irrigated beds were collected during July, with 19 % (3/16) at RC and 83 % (5/6) at HC (Table 1).

No *E. coli* were detected in produce samples collected from control beds at either site (Table 1). At RC, *E. coli* was only detected in one produce sample from all sampling dates from the irrigated beds. At HC, the mean *E. coli* concentration across all irrigated beds was 0.2 CFU/g, and 17 % (2/12) of irrigated beds were positive for *E. coli*. The number of produce samples which contained *E. coli* across both sites was low, and the only *E. coli*-positive samples were collected in July (Table 1).

Average *E. coli* concentrations from all water samples were below the GAPs guidelines (geometric mean < 126 CFU/100 mL; maximum one sample 235 CFU/100 mL) (Fig. 3a). The average *E. coli* concentration from water samples at the HC location was 3.09 CFU/100 mL greater than the levels observed at RC, but this difference was not statistically significant. At the RC location, mean *E. coli* concentrations were higher in first flush water samples compared to irrigation water samples, but this difference was not statistically significant. No *E. coli* were identified in municipal water samples at the RC location. At the HC location, mean *E. coli* concentrations in irrigation water samples were higher compared to first flush collections, but this difference was not significant (Fig. 3a).

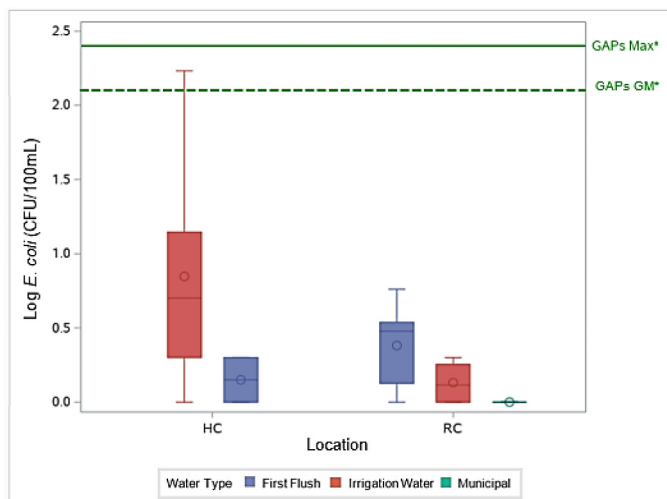
On average, *E. coli* concentrations from soil surface samples from control beds were lower than those collected from irrigated beds at both

sites, however, this difference was not statistically significant (Fig. 3b). At the RC site, no *E. coli* were detected in soil from control beds, and only two samples had *E. coli* concentrations above 5 CFU/g in irrigated beds. There were no statistically significant differences between *E. coli* levels in irrigated beds and control beds at HC (Fig. 3b).

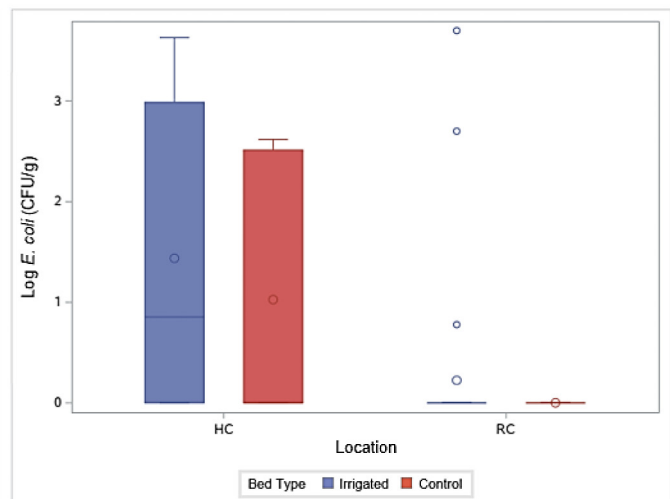
In produce samples, *E. coli* were not detected in control beds at either location and there were only three positive samples from irrigated beds, with an average concentration of 0.2 CFU/g at HC and 53.1 CFU/g at RC (Fig. 3c). In addition, there were no statistically significant differences between bed types or between sites. Of the three *E. coli* positive produce samples, two samples had concentrations at or below 1 CFU/g (carrot tops and bell pepper), and one sample collected from an irrigated bed at RC had a mean *E. coli* concentration of 1700 CFU/g (cucumber) (Fig. 3c).

As expected, *E. coli* concentrations were lower on produce commodities compared to soil and harvested water (Fig. 3d). The highest mean *E. coli* concentrations were detected in irrigated soil samples from HC (837.5 CFU/g). At this site, soil *E. coli* levels were not significantly different from produce or water samples. At the RC site, *E. coli* concentrations were relatively low across all sample types, but *E. coli* levels differed significantly

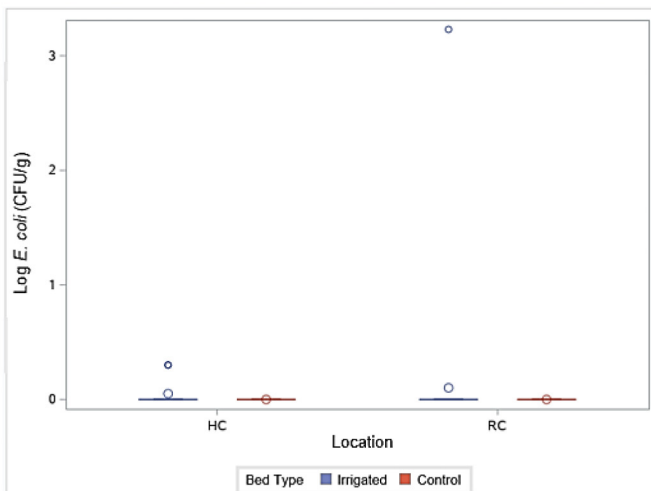
a) Water Samples



b) Soil Samples



c) Produce Samples



d) Water, Soil and Produce Samples (Irrigated Beds Only)

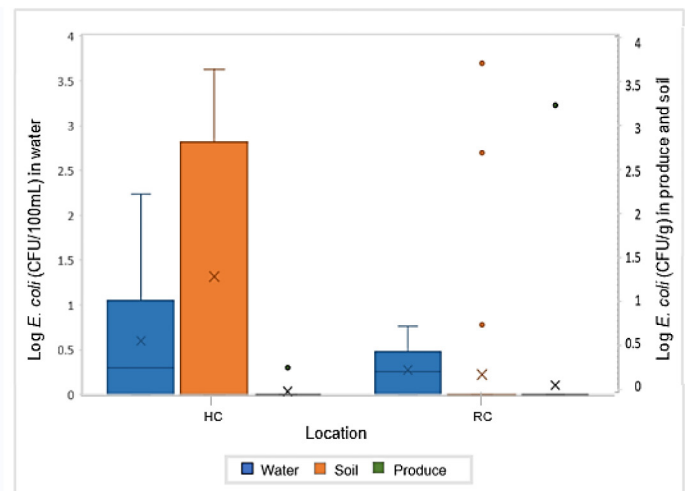


Fig. 3. *E. coli* concentrations by sample type, location, and water or bed type. Whiskers are drawn from the 75th percentile to the upper adjacent value and the 25th percentile to the lower adjacent value, the midline is the median (when absent the median = 0). The circles outside of the bars represent the outliers, while the circles or crosses within the bars represent the mean. *The marked GAPs guidelines correspond to the geometric mean (GM) guideline of ≤ 2.1 log CFU/100 mL (126 CFU/100 mL) and the maximum recommended *E. coli* value for 1 water sample (Max) guideline of ≤ 2.4 log CFU/100 mL (235 CFU/100 mL).

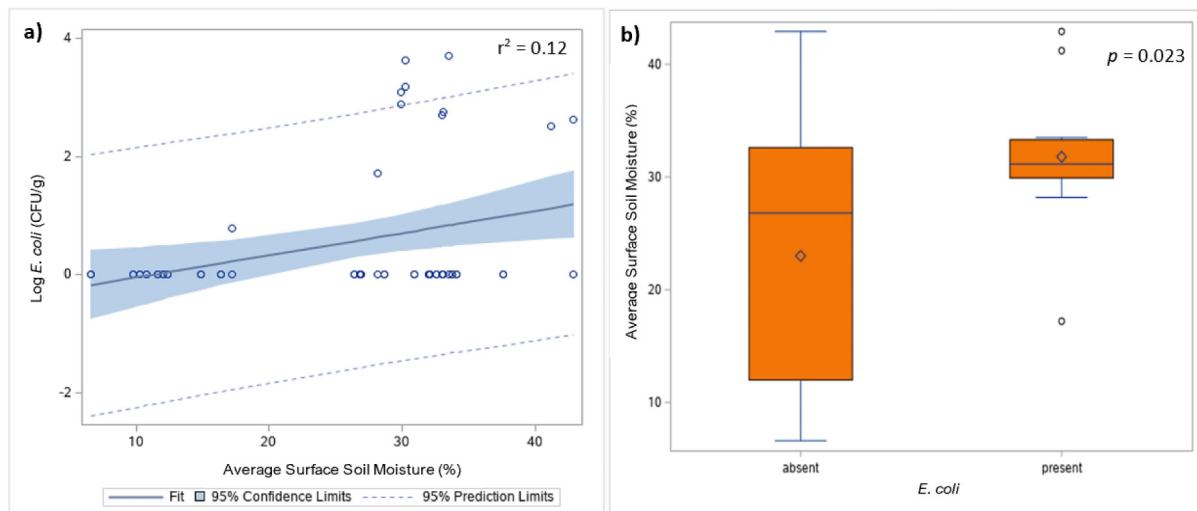


Fig. 4. Association between a) Average surface soil moisture (%) and *E. coli* concentration (log CFU/g) in soil samples from both locations and b) Average surface soil moisture percentage and presence/absence of *E. coli* in all soil samples. Statistical significance was determined by the Mann-Whitney test.

between produce and water samples ($p < 0.0001$), but not compared to soil samples (Fig. 3d).

3.1.1. Association between soil moisture and *E. coli* in soil

Average surface soil moisture did not differ significantly between locations. *E. coli* concentrations from soil samples pooled from both sites were positively correlated with average surface soil moisture content, although the relationship was weak ($r^2 = 0.12$, $p = 0.008$) (Fig. 4a). Nonetheless, for every 10 % increase in soil moisture, there was an average increase of *E. coli* by 2.4 CFU/g in the soil (Fig. 4a). Moreover, the presence of *E. coli* in soil samples was associated with higher average surface soil moisture (32 %) compared to samples with no *E. coli* (23 %; $p = 0.023$) (Fig. 4b).

3.1.2. Association between precipitation and *E. coli* in water

E. coli concentrations in water samples from both study sites were negatively associated with the length of the antecedent dry period (number of days without rainfall), but this relationship was not statistically significant (Fig. 5a). However, the presence of *E. coli* in water samples was marginally associated with the length of the antecedent dry period ($p = 0.058$) (Fig. 5b).

3.2. Pathogens

No *Salmonella* spp. or *L. monocytogenes* isolates were detected in water samples from either the HC or RC site, throughout the study period.

4. Discussion

Previous studies have recognized the environmental and economic benefits of harvesting rainwater, however, there is still a dearth of information concerning the safety of consuming raw produce grown with this type of water (Angrill et al., 2012; Campisano et al., 2017; Santo et al., 2021). In particular, the presence of microbiological contaminants from avian and animal fecal matter may require additional treatment to ensure that harvested rainwater meets food safety standards for irrigation (Abbasi and Abbasi, 2011; Amin et al., 2013; Bae et al., 2019; Clark et al., 2019; Gikas and Tsihrintzis, 2017; Gikas and Tsihrintzis, 2012; Kim et al., 2016). In the present study, we evaluated bacterial fecal indicator and pathogen concentrations in ambient rainwater and harvested rainwater collected through two first flush systems connected to VRG beds in Frederick,

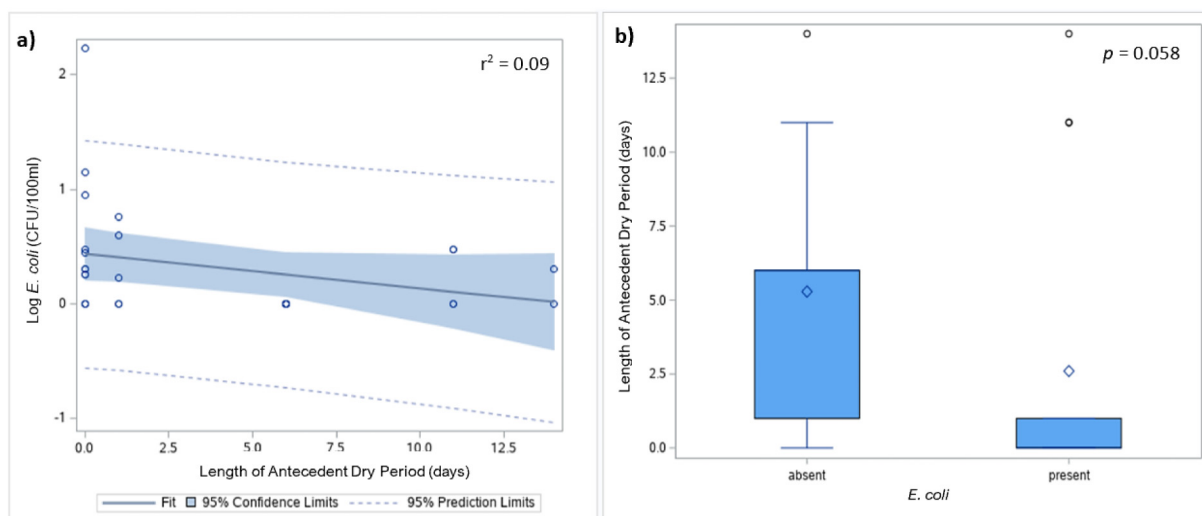


Fig. 5. Association between a) Length of antecedent dry period (number of days without rainfall) and *E. coli* concentration (log CFU/100 ml) in water samples and b) Length of the antecedent dry period and presence/absence of *E. coli* in water samples. Statistical significance was determined by the Mann-Whitney test.

Maryland, as well as in the soil and produce grown in these beds. We found that *E. coli* concentrations were lower on produce commodities compared to soil and harvested rainwater, and *E. coli* concentrations were generally low across all sample types. These results highlight the effectiveness of combining treatment technologies such as subsurface irrigation, biological filter layers, and a first flush device when irrigating fresh produce primarily eaten raw.

4.1. *E. coli* in harvested rainwater

Generic *E. coli* is used as an indicator organism in the GAPs guidelines to determine the risk of foodborne illness from agricultural water sources. We found that despite the differences in VRG bed designs used at each study location, the harvested rainwater and subsurface irrigation water sampled in this study met GAPs guidelines (GM < 126 CFU/100 mL; maximum one sample of 235 CFU/100 mL).

The low concentrations of *E. coli* in water samples from our study could be due to the multiple treatments used in the rainwater harvesting, garden bed, and irrigation system designs. The use of a first flush tank was an important feature of the RC and HC's rainwater harvesting systems. First flush diverters channel the initial volume water away from the rainwater harvesting storage system. The system used in this study utilized a ball valve to trap the initial 159 L (35 gal) in the first flush tank; any water collected after this volume was redirected into the VRG bed reservoirs. A 1-in. rainstorm equates to 0.623 gal/sq. ft. roof surface of harvested rainwater available. For the RC location with its 190 m² roof, a 1-in. rainstorm would produce approximately 1274 gal (4823 L) of harvested rainwater. According to Abbasi and Abbasi (2011), rooftop contaminants such as organic acids and toxic heavy metals are more concentrated in the first flush runoff, and findings indicate that subsequent runoff water is considerably safer. In addition, other works have demonstrated that first flush waters will often contain greater levels of *E. coli* and other bacterial pathogens (Amin et al., 2013; Gikas and Tsihrintzis, 2017; Gikas and Tsihrintzis, 2012). In this study, the average *E. coli* concentrations at the RC site were higher, although not significantly higher, in the first flush water samples compared to the irrigation water, as expected. However, the same pattern was not observed at the HC site, where the irrigation waters had higher *E. coli* concentrations (32.3 CFU/100 mL) than the first flush water (0.5 CFU/100 mL), although this difference was not significant. These differences between the study sites suggest that small modifications to the VRG bed design and study location have the potential to impact the bacterial levels found in harvested rainwater and warrant further analysis. Fully lined VRG beds at the HC site, which retained water in the beds as a type of reservoir, may have created a favorable environment for *E. coli* survival and periodic growth. Similarly, Campisano et al. (2017) reported that the quality of harvested rainwater may vary considerably between locations and can be affected by ambient weather conditions (e.g., local rainfall, wind, temperature, etc.), the surrounding wildlife, and small differences in garden bed design and rooftop material.

The VRG beds used at both the HC and RC sites were constructed with gravel, fine sand, and a layer of topsoil that acted as a biological filter. The application of similar treatments in irrigation systems (e.g., slow sand filters (SSFs) and zero-valent iron (ZVI)-sand filters) have shown varying degrees of success in the removal of *E. coli*. Among these studies, Ingram et al. (2011) and Kim et al. (2020) found that ZVI sand-filtered water contained significantly less *E. coli* and that ZVI filters were more effective at inactivating *E. coli* populations than SSFs. Anderson-Coughlin et al. (2021) also noted that *E. coli* levels were significantly reduced in ZVI-sand filtered irrigation water compared to unfiltered water; however, another study indicated that water quality parameters may affect *E. coli* reductions mitigated by ZVI-sand filtration (Kim et al., 2021). In contrast, a study by Marik et al. (2019) did not find a significant reduction of *E. coli* populations on lettuce leaves following irrigation with ZVI-sand filtered water. Ultimately, harvested rainwater treated with biological filters appears to be a cost-effective mitigation strategy that helps reduce *E. coli* abundance.

Nonetheless, the quality of the source water and the specific bed design may play an important role in its overall effectiveness.

4.2. Pathogens in harvested rainwater

Previous studies have identified pathogens including *Salmonella* spp., *Giardia lamblia*, *Legionella pneumophila*, and *Campylobacter jejuni* in roof-harvested rainwater samples, even when first flush systems were present (Ahmed et al., 2010, 2012). In our study, neither *Salmonella* spp. nor *L. monocytogenes* were detected in any water samples. Nevertheless, the unique VRG design and subsurface irrigation system might limit the generalizability of our findings for rainwater harvesting to this specific design. However, studies have shown that SSFs also significantly reduce human opportunistic pathogens such as *Sphingobacteriia*, *Flavobacteriia*, and *Clostridium perfringens* spores from harvested rainwater to levels safe for drinking water purposes (Seeger et al., 2016; Zhao et al., 2019). In addition, Marik et al. (2019) applied ZVI biosand filters to treat surface waters used for irrigation of produce crops; their results showed a significant reduction of *L. monocytogenes* populations on lettuce plants irrigated with ZVI filtered water.

4.3. *E. coli* in soil and produce

Although GAPs guidelines only address *E. coli* in agricultural water and not soil or produce, assessing the presence of *E. coli* in soil and produce provides a more holistic picture of possible human exposure pathways. Both the HC and RC sites used subsurface irrigation, in which the water enters the base of the garden bed and is taken up by the root zone through capillary action. This type of irrigation has been applied in other experimental studies (Richards et al., 2015; Tom et al., 2013), and has been shown to reduce evapotranspiration and plant stress, minimizing the need for additional irrigation. Subsurface irrigation may also prevent fresh produce crops from being splashed with soil bacteria during watering (Song et al., 2006; Stine et al., 2005). However, an important distinction between the VRG beds at each location is that the beds were fully lined at HC, while they were only partially lined at RC. Consequently, there were instances when the irrigation water accumulated and was stored in the gravel layer of the HC beds but there was no apparent holding time at the RC site. The storage of irrigation water prior to root uptake might have created favorable conditions for *E. coli* survival, as wetter environments may harbor more bacteria (Allard et al., 2019; Gikas and Tsihrintzis, 2017; Song et al., 2006; Tom et al., 2013). As a result, the difference in lining may have contributed to the greater prevalence of *E. coli* positive soil samples at the HC site compared to the RC, especially during the warmer month of July. Fully lined beds have also been shown to allow for pore spaces to remain filled with water which may lead to poor hydrologic performance for runoff management, as well as minimal benefits in terms of vegetable yield (Li et al., 2009; Tom et al., 2013).

In this study, the presence of *E. coli* in 22 % of soil samples did not translate to a similar percentage of *E. coli* positive produce samples, in fact only 5 % of all produce samples (from both irrigated and control beds) had detectable *E. coli*. Further, only three out of 44 produce samples (7 %) from beds irrigated with harvested rainwater were positive for *E. coli* and these were all collected during the month of July. Recent studies have noted that *E. coli* levels in irrigation water tend to be higher when ambient temperatures are greater, which may also lead to an increased presence of *E. coli* in produce (Decol et al., 2017; Holvoet et al., 2014; Park et al., 2015). In addition to warmer temperatures, wildlife defecation in combination with rainfall the day of or before sample collection, may have led to contaminated produce. The VRG beds at both study sites were uncovered, which means animal and avian feces, as well as ambient rain, could have been introduced to the soil and produce resulting in the one produce (cucumber) sample with a high number of *E. coli*. Yin et al. similarly observed higher *E. coli* levels in harvested rainwater and irrigated spinach during the warmer summer months (2019). Micallef et al. (2012, 2013) also observed that for tomato plants, the distance from the ground was an important

factor in determining the presence of *Enterococcus* spp. and *Salmonella enterica*. As the sample with the highest *E. coli* concentration was a cucumber, it would have grown on the soil surface. In the Micallef et al. studies, contaminated soil and irrigation water in farms were found to be important factors in tomato plant contamination with enterococci (Micallef et al., 2012, 2013).

4.4. Impact of soil moisture and precipitation on *E. coli*

Another important aspect of bacterial contamination appears to be related to soil moisture and the length of time since the last rainfall period. In our study, we observed that garden beds with wetter soil were more likely to have *E. coli* present compared to drier soils, and this trend was similar with precipitation. The fewer days since the last rainfall event, the more likely that *E. coli* were detected in the soil. While this association was marginal, other studies have observed increased levels of generic and pathogenic *E. coli* strains in soil and produce samples following precipitation events, especially with heavy rainfall (Dobrowsky et al., 2014; Holvoet et al., 2014; Park et al., 2015). Other studies also have shown that the number of days of rainfall between sampling events, and soil moisture content of soils, are highly weighted predictors of *E. coli* survival durations in soils (Pang et al., 2020). In a study by Litt et al., more *E. coli* were transferred to cucumbers from soil in years with more rainfall during the growing season, with greater soil moisture levels in wetter seasons facilitating longer survival durations of *E. coli* in soils, and rainfall-induced splash events potentially creating more opportunities for soil-splash contamination of cucumbers (Litt et al., 2021). Increased levels of *E. coli* on produce and in soils may also be attributed to a greater dispersal of bacterial contaminants during rainfall events (Blaustein et al., 2016; Hellberg and Chu, 2016) and could have implications for the spread of enteric bacterial pathogens on produce as sudden rainfall events become more frequent and severe with climate change (IPCC, 2014; Melillo et al., 2014).

Our results appear to support the delay of produce collection for raised vegetable garden beds irrigated with harvested rainwater until the rain has stopped for several days and follows the current (as of February 2022) FDA FSMA microbial die-off suggestions (i.e., equivalent to a 2 log₁₀ die-off over 4 days) (US: US FDA, n.d.). For the cucumber sample with the high *E. coli* level from RC, this would result in 17 CFU/g after the assumed 2 log₁₀ die-off. However, as shown by Belias et al. (2020) there are other important factors that may determine microbial die-off on produce commodities that comply with the FSMA assumed die-off rates, such as relative humidity and the type of produce being harvested. Therefore, the timing of harvest may need to consider environmental conditions to further reduce the risk of foodborne illness.

4.5. Limitations

Overall, this study supports the use of harvested rainwater collected through a first flush system and applied to produce through a subsurface irrigation system. However, a larger number of samples over a longer period are needed to further ascertain the safety of the harvested water for use in produce eaten raw. As previous studies have pointed out, harvested rainwater quality might differ by geographic region, emphasizing the need for additional studies in multiple regions to determine the generalizability of our results. Although our study took place in a U.S. city, the results have broad implications for the global use of harvested rainwater to irrigate produce. Despite these limitations, this preliminary study helps address a considerable knowledge gap about the safety of harvested rainwater for produce irrigation.

4.6. Future research

To build upon the results of the study presented here, there are several areas of future research that could further confirm the safety and appropriateness of using harvested rainwater for produce irrigation. Our results

found that a first flush system combined with subsurface irrigation resulted in water that met food safety guidelines for *E. coli*, no detectable pathogens in water, and a low percentage of produce with any detectable *E. coli*. As our study is the first to evaluate food safety associated with harvested rainwater undergoing treatment before irrigation, a comparison between produce grown with harvested rainwater collected with and without multiple treatment technologies could provide valuable information to gardeners and farmers about the most effective strategies to improve harvested rainwater quality before irrigation. Future studies are also needed to investigate bacterial microbiomes of both harvested rainwater and produce grown with this water.

5. Conclusions

Limited information exists concerning the safety of using harvested rainwater to irrigate produce crops that are primarily eaten raw. In addition, no known studies have evaluated the use of harvested rainwater collected through a first flush system and applied through subsurface irrigation to produce. Our study provides preliminary data that can help improve the safety of harvested rainwater used to irrigate produce. Our results support the use of rooftop rainwater collected through a first flush system and supplied to produce via subsurface irrigation. This type of system could also be an important tool to mitigate water challenges stemming from climate change impacts. Although *E. coli* were detected in some harvested rainwater samples, the concentrations fell far below current irrigation water guidelines. Importantly, no *Salmonella* spp. or *L. monocytogenes* were detected in any water samples in this study. In addition, only 3 of 44 produce samples irrigated with harvested rainwater were positive for *E. coli*. Because we found that *E. coli* presence was associated with soil moisture and shorter antecedent dry period, monitoring soil moisture, and delaying produce harvest after rain events might further decrease the risk of *E. coli* contamination.

CRediT authorship contribution statement

Michele E. Morgado: Data curation, Formal analysis, Writing - Original Draft, Visualization, Writing - Review & Editing. **Claire L. Hudson:** Investigation, Resources, Writing - Original Draft, Writing - Review & Editing. **Suhana Chattopadhyaya:** Investigation, Writing - Review & Editing. **Kaitlin Ta:** Investigation, Writing - Review & Editing. **Cheryl East:** Investigation, Writing - Review & Editing. **Nathan Purser:** Visualization, Writing - Review & Editing. **Sarah Allard:** Methodology, Investigation, Writing - Review & Editing. **M. Drew Ferrier:** Writing - Review & Editing, Funding acquisition. **Amy R. Sapkota:** Resources, Writing - Review & Editing, Funding acquisition. **Manan Sharma:** Methodology, Investigation, Resources, Writing - Review & Editing. **Rachel Rosenberg Goldstein:** Conceptualization, Methodology, Investigation, Writing - Original Draft, Writing - Review & Editing, Supervision.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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