

Improving produce safety by reducing pathogen load in soil using cover crops and developing
food safety educational resources for produce growers

by

Yeqi Zhao

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Department of Horticulture and Natural Resource
College of Agriculture

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Approved by:

Co-Major Professor
Dr. Eleni D. Pliakoni

Approved by:

Co-Major Professor
Dr. Manreet S. Bhullar

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Abstract

Fresh produce (commonly consumed raw) has been continually associated with multiple foodborne disease outbreaks, which cause both health concerns and economic losses.

Agricultural water and contaminated soil are suspected as common causative agents for recent produce-related foodborne illness outbreaks, which brought fresh produce safety to the forefront of public attention. New federal regulations by the U.S. Food and Drug Administration (FDA) as part of the FDA Food Safety Modernization Act (FSMA) established the Produce Safety Rule (PSR), which defined scientific criteria for growing, harvesting, storing, and packaging fresh produce. The PSR provides requirements on treating soil amendments and criteria for safer use of agricultural water.

There are several soil and water treatment methods and practices to reduce human pathogens in fresh produce. Specifically, for contaminated soils, cover crops have been recently explored for antimicrobial application in produce production, in addition to their soil health benefits. Cover crops are plants seeded before or after the growing season to improve soil health, increase water availability, reduce weed pressure, and prevent erosion. Cover crops also produce various antimicrobial secondary metabolites (i.e., glucosinolates, quercetin), yet the role of cover crops in moderating the population of human pathogens in the soil has rarely been investigated. However, with regards to the microbial quality of agricultural water, Kansas State University and University of Missouri Extension educators have been providing free water testing, training and information on agricultural water microbial quality to help produce growers reduce microbial risks from ag water sources.

The goal of this thesis is to study cover crops as potential antimicrobial interventions and understand grower needs to improve food safety measures and practices on a fresh produce farm.

To achieve this goal, there are two specific objectives: (1) soil treatment: determine the antimicrobial potential of three cover crop species to reduce the population of pathogenic soil microbiota which can minimize the possibility of cross-contamination of produce from agricultural soil; (2) agricultural water: determine knowledge gaps among Kansas and Missouri produce growers related to agricultural water quality and best practices and develop agricultural water safety handouts to improve growers' knowledge.

To complete the first part of this thesis, whole-plant samples of 4-week-old mustard greens (*Brassica juncea*, 'Kodiak'), sun hemp (*Crotalaria juncea*), or buckwheat (*Fagopyrum esculentum*) were chopped and mixed into autoclaved farm soil and inoculated with 7-logs of rifampicin-resistant generic *Escherichia coli* (*E. coli*). The surviving microbial population on days 0, 4, 10, 15, 20, 30, and 40 was enumerated using an agar-based methodology. All three cover crops significantly reduced the population of generic *E. coli* compared to the control ($p < 0.0001$); buckwheat resulted in the highest reduction (3.92 log CFU/g). An inhibitory effect on microbial growth was also observed in soils containing mustard greens ($p < 0.0001$) and sunn hemp ($p < 0.0001$). This study provides evidence for the bactericidal and bacteriostatic effects of cover crops. More information regarding the crops' secondary metabolite profile and potential as a soil bio-mitigation strategy to improve on-farm produce safety is warranted.

For objective 2, a survey was developed and administered to determine future extension outputs and activities to encourage growers to improve their practices related to water quality. The survey was distributed to Kansas and Missouri produce growers attending in-person or online produce safety-related events in late 2020 and early 2021. Also, it was distributed through email lists of produce growers from both states. Survey results ($n = 101$) indicate that 13.9% of the respondents tested their water for generic *Escherichia coli* (*E. coli*) more than once a year,

while 38.6% of the participants have never tested their water. Approximately half (59.3%) of respondents indicated they use municipal water for postharvest uses, while 6.7% indicated the use of untreated surface water for postharvest activities. Due to the concern of potential water contamination risks, researchers suggest that further training and educational resources would help growers improve practices related to water quality and produce safety.

Three handouts were developed to help growers better understand the current FSMA PSR water quality requirements (under consideration for further modifications) and best practices. These handouts include the following: (1) “New modified FSMA PSR agricultural water requirement proposal” handout summarizes the major modifications of the latest water proposal published in 2021; (2) “How to interpret water results” handout provides information to identify agricultural water uses and microbial safety criteria and best practices; and (3) “Back flush water fact sheet” discusses the hazards from back flush water and potential solutions.

In conclusion, this thesis focuses on improving fresh produce safety by minimizing the cross-contamination happening in agricultural soil and water. The reduction of microbial populations in the first part of the thesis demonstrated the potential of cover crops to be used as a sustainable antimicrobial treatment method for contaminated soil. Meanwhile, the survey results show produce growers have a basic awareness of agricultural water practices, but the safety of those practices can still be strengthened. The publication of relevant handouts can improve growers' knowledge and help them better comply with the latest regulations.

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Chapter 1 - Literature Review

Introduction

Significance of fresh produce in the human diet

Since fresh fruits and vegetables contain vitamin A, C, and E, carotenoids, folic acid, dietary fiber, and other minerals, they are one of the great resources to interrupt oxidative stress in the human body to minimize the risk of chronic disease, as well as reduce the concentration of harmful compounds and acid in feces (21). Researchers pointed out that higher fresh produce consumption levels will possibly reduce the risk of cancer (22, 123, 124). Moreover, research showed that consuming produce on a regular basis can reduce blood cholesterol levels (92), the risk of heart disease (66), and maintain blood pressure (118). The concept behind the connection between consuming produce and the risk of chronic disease is the balance of free radicals and phytochemical antioxidants (77) in the body. As the nutrients from produce are hard to replace with other foods, a balanced diet including fruits and vegetables is an important step in maintaining a healthy lifestyle. The USDA's MyPlate recommends eating approximately 2 cups of fruit and 3 cups of vegetables per person per day to maintain a healthy life (the specific recommendation also depends on gender and age) (80).

Besides the increase in health awareness, urban farming has become more popular in the last ten years in the U.S. with the aims of improving local food security, maximizing the use of vacant spaces, and improving agricultural sustainability. Although, due to the environmental impact, currently limited crops can grow on an urban farm, such as leaf greens (72), the model of

potential productivity of urban agriculture shows that small-scale systems are more productive than commercial farms (126). From 1990 to 2018, the value of fruit and vegetable production in the United States has increased each year (130). According to a report from Grand View Research, Inc., the U.S. fruit and vegetable market will reach approximately \$1.12 trillion, and the demand will be more than 243.2 million tons by 2025 (52). As a result, the rise of urban agriculture accelerated the produce market expansion but also added uncertainty to the produce safety, as many produce growers from urban areas own limited farmland and do not need to comply with the Produce Safety Rule (PSR) (annual monetary sales of a farm below \$25,000). So there is still a need for extension professionals to suggest them follow the Good Agricultural Practices (GAP) or PSR to ensure the produce safety during production.

Foodborne outbreaks related with produce

As the demand for fruits and vegetables increasing, more cases of foodborne disease outbreaks have been linked to produce in the past decades. These have highlighted produce safety concerns to the public. In recent years, food safety challenges have continued in an endless series, and there have even been about 3,000 deaths from foodborne causes annually in the US (131). Produce is one of the key vehicles for the transmission of foodborne pathogens in most parts of the world (78). From 2010 to 2017, a total of 85 multistate outbreaks associated with fresh produce with confirmed etiologies occurred in the United States, resulting in a known combined total of 4,658 known illnesses, 1,187 hospitalizations, and 55 deaths (29). *Escherichia coli* (*E. coli*), *Salmonella enterica* (*S. enterica*), and *Listeria monocytogenes* (*L. monocytogenes*)

are the three bacterial pathogens that caused the most multistate fresh produce outbreaks from 2010 to 2017 (29).

Economic impact of foodborne outbreaks

Foodborne outbreaks not only destroy human health but also lead to a huge economic burden. According to the USDA estimation, each year foodborne illnesses cost more than \$17.6 billion of loss (133). Of note, fresh produce is one of the major causes that is believed to account for 26% of the total economic loss (85). One foodborne disease outbreak can lead to \$6,330 to \$2.1 million in economic loss for a fast-casual restaurant (13). According to FDA rankings, the top most common pathogens that cause foodborne disease are *E. coli*, norovirus, *Salmonella spp.*, *Campylobacter*, Hepatitis A, and *Listeria* (138). Among these pathogens, outbreaks associated with *S. enterica*, *L. monocytogenes*, and *Campylobacter spp.* caused the highest total economic losses (113). In addition, the severity of the illness and the type of bacterial infection affect the amount of economic loss, and the cost also depends on the geographical region of the patient. California, Florida, and Texas were among the states with the highest economic losses per foodborne illness patient recorded in 2013 (113, 114).

At the same time, foodborne diseases will bring high economic losses to consumers and will also lead to economic and reputational losses to produce growers or processors. When certain pathogenic bacteria are detected in food products, all products sold are recalled as required by the U.S. Food & Drug Administration (FDA) (30). In this process, the producer has to pay all the costs associated with the recall, including the extra labor, wasted food, and recall costs. At the same

time, when a portion of food is recalled, the reputation of the food manufacturer will normally be damaged, and consumers often will discriminate against the brand. According to the Grocery Manufacturers Association (GMA), the average food recall costs the company about \$10 million per recall (129). For example, a large quantity of food (including safe produce) was dumped due to public health considerations during the 2018 large produce outbreak caused by contaminated romaine lettuce (32).

Thus, reducing the chances of food being infected by microorganisms and ensuring food safety is what every producer and consumer needs to understand and strive to do.

Contamination Factors on Produce Farms

Pathogen contamination of fresh produce can occur at any time during the production chain, although the likelihood of contamination is elevated during field operations such as species and harvest (48, 78). The introduction of human pathogens can occur via diverse routes, including agricultural water (14, 55) and biological soil amendments of animal origin (BSAAOs) (157). Once pathogens are introduced into the farm environment, they can survive and persist for long periods within the soil or water (26, 45, 88, 90, 122), which can cause subsequent spread to other areas of the fruit and vegetable production environment or even result in product contamination.

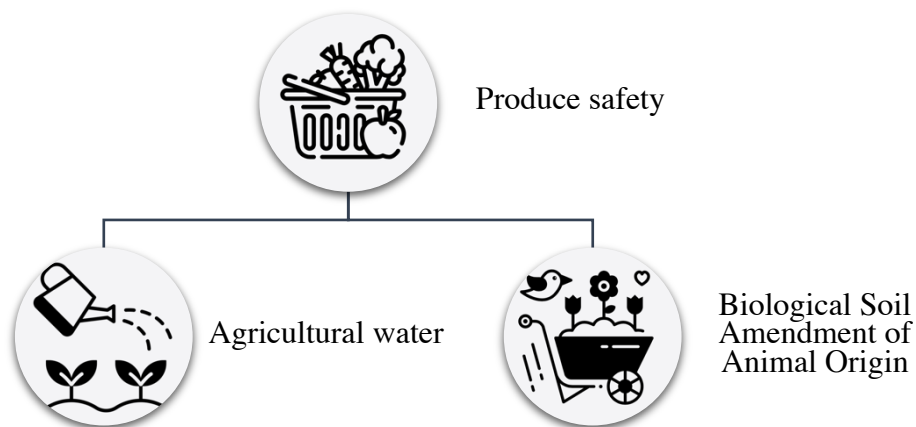


Figure 1.1 The two main contamination routes on a farm that cause produce safety issues.

Agricultural Water

Agricultural water is widely distributed and used in a produce farm which increases the opportunity for human pathogens to attach to the produce. The proximity of produce to contaminated water and the concentration of pathogens within the water are two risk factors contributing to the probability of produce contamination during field production (6). The contamination route always starts with fecal matter carrying human pathogens that drop into the water source (7). Then the transmission of human pathogens can occur at any time during field production (i.e., irrigation, flooding, agricultural runoff) and postharvest activities (washing, rinsing, cooling, etc.).

Biological Soil Amendments of Animal Origin (BSAAOs)

Agricultural soil is another reservoir that can transmit human pathogens to fresh produce. One of the major sources of these human pathogens that survive in the soil is from BSAAOs.

Without proper treatment (i.e., composting), BSAAOs can carry various human pathogens. Once these are applied to soil as fertilizer, they will contaminate the soil soon after. From soil, there are multiple opportunities for human pathogens to attach to the produce. For example, the transmission of human pathogens can occur during the germination of seeds or after the transplantation of seedlings in contaminated soil (28, 71). Therefore, in agricultural applications, it is necessary for growers to detect whether any unexpected animals or improper practices exist before, during, or after field production.

Farm Safety Modernization Act Produce Safety Rule

Overview of the regulation

The US implemented the FDA Food Safety Modernization Act (FSMA) in 2011, which was developed to shift the focus of the nation's food safety system from responding to foodborne illness to minimizing the number of these cases. It was designed to provide clear, specific actions that must be taken throughout the produce handling process to prevent contamination. Nine rules are included in FSMA, which include 1) Third-Party Certification Accreditation; 2) Current Good Manufacturing Practices and Hazard Analysis and Risk-Based Preventive Controls for Human Food and Animal Foods; 3) Foreign Supplier Verification Programs (FSVP); 4) Laboratory Accreditation; 5) Food Traceability; 6) Mitigation Strategies to Protect Food Against Intentional Adulteration; 7) Sanitary Transportation of Human and Animal Food; 8) Voluntary Qualified Importer Program (VQIP); and 9) Standards for the Growing, Harvesting, Packing and Holding of Produce for Human Consumption (10).

This last rule is also known as the Produce Safety Rule (PSR) and the final version was published on November 27th, 2015. It establishes science-based minimum standards for the safe growing, harvesting, packing, and holding of fruits and vegetables grown for human consumption. The compliance dates of PSR criteria are determined by the size of the farm in terms of annual sales and the risk of the commodities becoming a foodborne outbreak when they get contaminated. These include if the farm sells at least \$25,000 of produce annually (adjusted for inflation) and if the produce is likely to be eaten raw (e.g., leafy greens, cucumber, tomato, and most fruits) (137).

Table 1.1 The compliance dates for each rule are based on farm size (very small, small, and other businesses).

Note: ¹ The farm sizes are determined by the average annual monetary value of produce the farm sold during the previous 3-year period. The other businesses (not small) include those selling more than \$500,000 of produce; small size farms include annual monetary sales between \$250,000 to \$500,000; very small includes farms with annual monetary sales between \$25,000 to \$250,000; the annual monetary sales of a farm below \$25,000 are exempted from the PSR. ² The new proposed water requirements are modified based on the final rule which was published December 2nd, 2021 and caused the extended compliance dates. More information on these topics will be mentioned later in this review.

Farm Size¹			
/Compliance Date	Sprouts	Other Produce	Original Water Requirements
<i>(134)</i>			
Other Businesses (Not Small)	1/26/17	1/26/18	1/27/20
Small	1/26/18	1/28/19	1/26/21
Very Small	1/28/19	1/27/20	1/26/22

Under the PSR, there are six key requirements summarized by FDA as subparts, which include: agricultural water, biological soil amendments, sprouts, domesticated and wild animals, worker training and health and hygiene, and equipment, tools, and buildings (and post-harvest handling?) (135). Of note, maintaining the microbial quality of the water sources used on the farm is of primary importance in produce safety. As water is used in almost every aspect of production, once the water source is contaminated, there will be a higher risk of produce contamination.

Worker Training and Health and Hygiene

Workers are a part of the food safety concerns since they can carry multiple pathogens and spread them unintentionally via multiple routes (58, 65). FDA requires all farm workers who handle covered produce and/or food-contact surfaces and all supervisors to receive food safety training, including the principles of good hygiene and food safety; recognizing symptoms of foodborne illness and the importance of personal hygiene for all personnel and visitors; and other training relevant to the worker's job (137). Of note, water is a key component of these requirements in order to fulfill health and hygiene requirements, such as washing hands and mixing with sanitizer (35). Thus, the microbial quality of the water source which is used for hygiene contributes to the risk of cross-contamination concern on the farm.

Equipment, Tools, and Buildings

The equipment and tools that are used on farms can always directly contact produce. This part of the rule focuses on the standards of sanitation to prevent cross-contamination of produce.

It covers the building structure (i.e., greenhouses, germination chambers, toilets, and hand-washing facilities), the tools, and equipment that may touch or contact the produce. As in the health and hygiene section, water is also required in this part of the rule, especially during cleaning and sanitizing processes. Although the literature shows that the survival time of pathogenic bacteria partially depends on the surrounding environments (i.e., temperature, moisture, the attached surface material) (24, 46, 104), once pathogens are introduced to any equipment, tools, or building floor, the possibility of cross-contamination begins to increase.

Biological Soil Amendments

According to federal regulation § 112.3, biological soil amendments of animal origin (BSAAOs) represent a biological soil amendment that consists, in whole or in part, of materials of animal origin (137). BSAAOs are widely used to improve soil fertility and quality on organic farms and make a primary contribution to agriculture nutrient management (108, 116). However, if the BSAAOs are used improperly, they can lead to very high produce and water contamination risk since they are applied directly next to the produce. Further, pathogens (e.g., *E. coli*, *S. enterica*) have an extended survival length in a warmer and moist environment (91, 97). A scientifically valid controlled process is required from the PSR for treating BSAAOs to minimize the risk of contamination (137). Of note, the PSR and extension fact sheets highlight storage of the biological soil amendments away from water sources since that can cause potential contamination (19, 137).

Sprouts

Agricultural water quality is a key food safety concern in growing sprouts as water is the primary input in sprout production. In past decades, several foodborne outbreaks have been traced back to sprouts (15, 31, 49, 125), which have been contaminated by pathogens carried from sprout seeds and run-off water. As sprouts are grown in warm, moist, and nutrient-rich conditions, this is also the perfect environment for pathogens to survive. Unlike in field production systems, once a single pathogen is introduced into the sprouts system, it can easily spread to all the growing sprouts. Moreover, literature shows that because of the favorable growing conditions, sanitizer treatments (i.e., ozone and chlorine dioxide) cannot inactivate pathogen growth (2). Of note, water is required in most sprout growth steps. Therefore, cross-contamination can either happen during irrigation of sprout seeds in the field or during irrigating the sprouts during production (154). Based on the special growth environment, the PSR has established a separate section for sprout growers, which includes testing any microbes on or in the seeds, sprout irrigation water testing, and further corrective treatments.

Domesticated and Wild Animals

The fecal matter left behind on the field from grazing, working, and wild animals have a potential risk of containing human pathogens. The pathogens from their feces can directly contaminate the surrounding soil and produce (84). The PSR addresses this concern and requires taking all measures reasonably necessary to identify and not harvest produce that is likely to be contaminated. At a minimum, all covered farms are required to visually examine the growing

area and all the covered produce to be harvested (137). Furthermore, once any feces are dropped or flushed (i.e., rain and flood) into nearby water, it will contaminate the entire water source with pathogens from the fecal matter (18). This results in the water becoming a primary reservoir for human pathogens at the farm level.

Agricultural water

Water is an important part of agricultural production as well as a primary reservoir for many human pathogens on the farm. Accordingly, agricultural water microbial quality requirements have been written into the PSR. In current microbial water quality testing guidelines, fecal contaminations are determined by the number of generic *E. coli* in 100 mL of water. The presence of any strain of *E. coli* is a strong indication of fecal contamination, but it cannot be used as direct evidence of the presence of a human pathogen (127). In the PSR, generic *E. coli* is only used to measure microbial water quality and give growers information for future water management.



Figure 1.2 Schematic illustration of a worse condition fresh produce farm.

Originally Proposed Microbial Quality Criteria

Of note, during the thesis year (2020-2022), FDA proposed the new modified production water regulation and currently looking for feedback. Thus, this thesis includes both the current written agricultural water regulation and the new proposed production water regulation. To avoid any misleading, please check the current PSR for referencing.

In 2016, the PSR documented its minimum safety standards for agricultural water at that time. According to the rules clearly specified in PSR released in 2016, for surface water, twenty or more samples must be tested over a period of 2 to 4 years, with five or more samples rolled into the profile every year after the initial survey. The 2016 PSR requirements quantify the safety standards for agricultural water used directly on crops (other than sprouts) by using the

geometric mean (GM) and statistical threshold value (STV) of the water test results. The GM is the average of the water testing results and shows the central tendency of the quality of the water (117). The STV reflects the variability in the quality of the water samples over time.

Table 1.2 The standard for water microbial quality (GM and STV) for pre-harvest water included in the 2016 PSR.

	Geometric Mean	Statistical Threshold Value
Number of Generic <i>E. coli</i> (CFU/100mL)	≤ 126	≤ 410

The corrective actions for the water are required no longer than a year if the water testing results exceed the criteria number. During this period, growers can still use time water in some situations to keep flexibility for them (9, 23).

Microbial Water Testing Methods

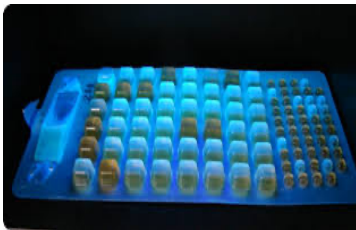
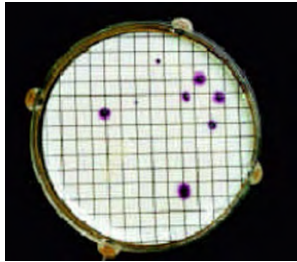
Produce growers need to collect water samples in bottles provided by the testing lab, with a sample size of no less than 100 milliliters. If a groundwater sample is collected, growers should let the water run for five minutes before the sample is collected. If the sample is surface water, keep the sampling bottle close to the center of the water body and try to avoid collecting impurities. After a successful collection, samples must be kept at a low temperature of 4 to 10 °C and sent to a nearby laboratory for analysis within 24 hours (20).

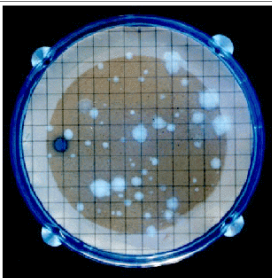
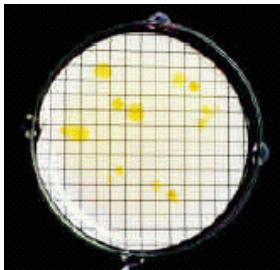
There are two sets of criteria for microbial water quality in the 2016 PSR water requirements that are based on the presence of generic *E. coli* in agriculture water. The microbial water quality for water that is used for post-harvest purposes (including any water that can directly

contact produce surfaces after harvest) must be 0 CFU/100mL, which means no detectable generic *E. coli* are allowed for post-harvest water. The other numerical critical that was proposed in the 2016 PSR water requirements are for water that is applied directly to growing produce (except sprouts). The microbial water quality value for production water in the 2016 PSR water requirements has to be below 126 CFU/100mL GM and 410 STV(132).

Currently, FDA states that there are eight methods considered scientifically valid and at least equivalent in accuracy, precision, and sensitivity in quantifying generic *E. coli* in agriculture water compared to the “EPA? Method 1603: *Escherichia coli* (*E. coli*) in Water by Membrane Filtration Using Modified membrane-Thermotolerant *Escherichia coli* Agar (Modified mTEC) (139)”. Based on the different types of methods, there are two units used in water test results: Colony Forming Unit (CFU) per 100 mL water sample and Most Probable Number (MPN) per 100 mL water sample.

Table 1.3 The eight methods are listed by name, description, result image, and reference.

Name of Method	Method Feature	Example	Reference
IDEXX Colilert/Colilert- 18 Test Kit (unit with MPN)	The Colilert method is used for 4-methylumbelliferyl-β-D-glucuronide (MUG) positive reaction, and the chemical has to be dissolved in the sample water to be tested so that the <i>E. coli</i> in the water can show the fluorescence effect under the ultraviolet light. After adding one packet of Colilert reagent into 100mL of sample water, it is sealed into a quantifying tray. Then it is incubated for 24 hours at 35°C; yellow wells may form to represent if the coliform is present. By checking the amount of blue fluorescent wells in the quanti-tray 2000 under a 6W, 365 nm UV light, the Most Probably Number (MPN) result can be calculated according to the table provided with the Quanti-Tray.		N/A
Method 1603 validated by EPA (unit with CFU)	This is a filtration method that follows the “U.S. EPA Method 1603 Protocol” by growing the <i>E. coli</i> that is left on the membrane in an m-TEC agar incubated for 2 hours at 35 °C and placed in a water bath for 22 hours at 44.5 °C. Counting the pink or purple color colonies formed in the agar provides the final result. This is a test that strictly adheres to FDA regulations; the results are precise and clear but include many disadvantages. The m-TEC agar used in this method is expensive, and it needs to be prepared before starting a water test. The filtration process is required for detection, and the turbidity level of the sample water directly determines the length of time of this process.		Method 1603: <i>Escherichia coli</i> (<i>E. coli</i>) in Water by Membrane Filtration Using Modified membrane-Thermotolerant <i>Escherichia coli</i> Agar (Modified mTEC) (September 2014)

Method 1604 validated by EPA (unit with CFU)	Filter 100mL water sample through a membrane filter and place it on MI agar on an absorbent pad saturated with 2-3 mL of MI broth and incubate at 35 °C for 24 hours. The <i>E. coli</i> will present with a blue color from the breakdown of IBDG by the <i>E. coli</i> enzyme β-glucuronidase; total coliform will fluoresce under longwave UV light from the breakdown of MUGal by the TC enzyme β-galactosidase.		Method 1604: Total Coliforms and <i>E. coli</i> in Water by Membrane Filtration Using a Simultaneous Detection Technique (MI Medium) (September 2002)
Method 1103.1 validated by EPA (unit with CFU)	Filter the water sample through the membrane and place it on the selective and differential medium, mTEC. Incubate at 35°C $\pm$ 0.5°C for 2 $\pm$ 0.5 hours and incubate at 44.5°C $\pm$ 0.2°C for 22 $\pm$ 2 hours. After incubation, transfer the membrane from mTEC agar to the absorbent pad saturated with urea substrate medium for 15 minutes is required for the final step. The color of the <i>E. coli</i> colonies in the final plate should be yellow or yellow-green under a fluorescent lamp.		Method 1103.1: <i>E. coli</i> in Water by Membrane Filtration Using membrane-Thermotolerant Escherichia coli Agar (mTEC) (March 2010)
9213 D validated by SM (unit with CFU)	Filter water sample through a membrane filter and place on mTEC agar. Incubate at 35°C for two h to resuscitate stressed bacteria, then incubate at 44.5°C for 22 h. Then transfer the membrane to a filter pad saturated with urea substrate and containing phenol red for 15 minutes. The result positive for <i>E. coli</i> should be fluorescence under the longwave UV lamp with yellow color.	No image available	Natural Bathing Beaches (2007). In: Standard Methods for the Examination of Water and Wastewater, 22nd Edition (Rice E.W., et al., Ed.), 9-46 – 9-48. Washington, DC: American Public Health Association. (2012).
9222 B validated by SM (unit with CFU)	In this method, the membrane will be placed on les-ENDO or m-ENDO agar which contains basic Fuchsin as the indicator. Filter water sample through a membrane filter and placed on the agar. After incubating the agar for 24 hours at 35 °C, the coliform will show up in red color. Transfer the membrane filter to a nutrient agar that contains MUG and incubate for 4 hours. The individual <i>E. coli</i> colonies will fluorescence under a longwave length UV lamp.	No image available	Standard Total Coliform Membrane Filter Procedure (1997), followed by 9222 G – MF Partition Procedures (1997) using NA-MUG media. In: Standard Methods for the Examination of Water and Wastewater, 21st Edition (Eaton A.D., et al., Ed.), 9-60 – 9-65, and 9-70 – 9-71, respectively. Washington,

**D 5392-93
validated by
ASTM (unit with
CFU)**

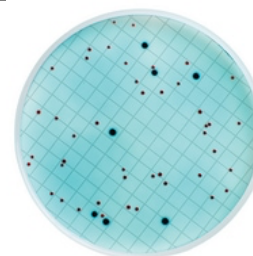
This is a two-step water testing procedure that provides a direct count of the colonies' numbers. Filter sample water through a membrane filter and place it on the selective medium, mTEC. The membrane on the medium is first incubated at 35°C for two h, and then the medium is incubated at 44.5°C for 22 h. Following incubation, the filter is transferred to a filter pad saturated with urea substrate. The yellow or yellow-brown colonies will be present after 15 minutes if the water sample is positive for *E. coli*. Colonies are counted with the aid of a 10 to 15 X magnifier and a fluorescent lamp.

No image available

Standard Test Method for Isolation and Enumeration of *E. coli* in Water by the Two- Step Membrane Filter Procedure. In: Annual Book of ASTM Standards, Volume 11.02. ASTM International. (1996, 1999, 2000).

**Hach Method
10029 for
Coliforms (unit
with CFU)**

This method was published by Hach Company. 100mL of the water sample is filtered through a 0.45-µm membrane filter and placed on the plate, which already added 2mL of the reagent onto a sterile pad on the plate. After incubating for 24 hours at 35 °C, the presence of red colonies indicates the coliforms by reducing the TTC. By hydrolyzing the enzyme-substrate BCIG, it shows blue color for *E. coli* colonies.



Total and *E. coli*, using m-ColiBlue24 Broth PourRite Ampules

Modified Agricultural Water Proposed Rule (proposed in December 2021)

After complying with the water microbial quality criteria for some time, some businesses reported that regulations are difficult to enforce because they are both expansive and complex (144). Thus, in December 2021, FDA revised and published proposed agricultural water requirements (136). The modified rule only impacts the production water regulation but does not change the originally published post-harvest water requirements. Instead of complying with microbial criteria and testing requirements for pre-harvest water (mentioned above), it emphasizes the use of an agricultural water assessment.

An agricultural water assessment is an evaluation that is required to be conducted by covered farms. The factors include agricultural water system(s), agricultural water practices, crop characteristics, environmental conditions, and other relevant factors. Based on the assessment, growers need to determine their water quality and take any actions (i.e., corrective measures, mitigation measures, inspect water system(s), and test water microbial quality) (155). This modification is intended to make it easier for more growers to comply. Large, covered farms were initially required to comply with the water requirements on January 26th, 2022, but FDA is exercising enforcement discretion for the pre-harvest water provisions for all farms while the water requirements are being finalized (as of July 2022).

Agricultural Water Treatment Methods

When the number of generic *E. coli* in the water source has been proved to exceed safe levels or the agricultural water assessment determines the water is not safe for use, corrective

measures are required before resuming the use of the water. Multiple methods can be used to improve the microbial water quality, including filtration, chlorine dioxide, ultraviolet (UV) radiation, and chemical oxidation (i.e., ozone, hydrogen peroxide, and peracetic acid), as shown in Table 1.4. Of note, another agricultural microbial water quality test is required after applying the treatments to confirm that the agricultural water can be used under PSR.

Table 1.4 Log count reduction of pathogens survival in water by different intervention technologies. Note: ZVI means zero-valent iron.

Process	Treatment	Target pathogen	Log reduction	Reference
Filtration	50% ZVI	<i>E. coli</i>	1.7	(67)
	50% sand			
	35% ZVI	<i>E. coli</i>	2.45	(81)
	65% sand			
	35% ZVI	<i>Listeria monocytogenes</i>	2.51	(81)
	65% sand			
	50% ZVI	<i>E. coli</i>	3.54	(8)
	50% sand			
Chlorine dioxide	10 mg/L	<i>E. coli</i>	7	(140)
	3 mg/L	<i>E. coli</i>	5	(57)
	5 ppm	<i>Salmonella</i>	7	(83)
	5 mg/L	<i>Staphylococcus aureus</i>	5	(95)
	1.2 mg/L	<i>Enterococcus faecalis</i>	6.3	(42)
UV	267 nm	<i>E. coli</i>	4	(94)
	275 nm	<i>E. coli</i>	3.6	(94)
	310 nm	<i>E. coli</i>	0.5	(94)

	265 nm	<i>E. coli</i>	4.3	(93)
	275 nm	<i>E. coli</i>	4	(93)
	289 nm	<i>E. coli</i>	2.42	(53)
Ozone	35 mg/L	<i>E. coli</i> O157:H7	3.98	(36)
	45 mg/L	<i>E. coli</i> O157:H7	3.9	(36)
	1 mg/L	<i>E. coli</i>	5	(50)
	1 mg/L	<i>Enterococcus faecalis</i>	6.4	(50)
Hydrogen peroxide	3%	<i>E. coli</i> O157:H7	0.77	(54)
	100 mg/L	<i>E. coli</i>	3.13	(47)
	100 mg/L	<i>Enterococcus faecalis</i>	2.45	(47)
Peracetic acid	8 mg/L	<i>E. coli</i>	3.52	(47)
	8 mg/L	<i>Enterococcus faecalis</i>	4.5	(47)
	3 mg/L	<i>E. coli</i>	3.57	(16)

Filtration

Filtration is a physical process that separates solid particles and liquid from water and reduces the turbidity of the water. The turbidity of water is caused by suspended particles and floating debris, which can provide a protective barrier for microorganisms' survival (147). Thus, reducing the level of turbidity of water before use is important. Filtration systems are widely applied and effective in removing particulate matter, algae, and most microorganisms. Different filtration strategies are used based on the different sizes of target microorganisms (Table 1.5).

Table 1.5 The application for different sizes of filtration strategy.

Filtration methods	Microfiltration Membrane	Ultrafiltration Membrane	Nanofiltration Membrane	Reverse Osmosis
Target Size	approximately 0.1 μm	approximately 0.01 μm	approximately 0.001 μm	<0.0005 μm
Applied for	Sediment and bacterial	Viruses	Microorganisms	Microorganisms

One of the commonly known filtrations is the slow sand filtration (SSF), which lets the water goes through various biological, physical, and chemical processes that finally removes the pathogenic organisms and turbidity effectively. Recently, a great deal of literature highlighted the efficiency and economics of the zero-valent iron (ZVI) bio-sand filtration system for reducing pathogen load in contaminated water (8, 67, 70, 81). Anderson-Coughlin et al. showed that zero-valent iron filtration could significantly reduce the *E. coli* population after going through the filtration system(from 13.13 MPN/ml to 9.59 MPN/ml) as well as shortening the die-off interval of the rest of the bacteria (from -0.48 MPN/day to -1.50 MPN/day) (8).

However, according to the FSMA PSR (21 CFR 112.43) (137), currently, sand-filtration is not considered a validated water treatment method that can be used for removing physical particles and requires a further treatment process to complete the corrective measures. Moreover, the backflush water from the filter can also be problematic as it contains most of the particles from the untreated water. Thus, how to handle the backflush water properly needs to be considered.

Chlorine Dioxide

Chlorine dioxide (ClO_2) is a chemical compound that forms as a gas when the temperature is above 11°C and forms as a liquid when temperatures are between 11°C and -59°C . Due to its chemical properties, it is primarily used for disinfecting drinking water (3). Chlorine dioxide can kill pathogens by breaking the chemical bonds in their molecules and results in pathogen inactivation. Combined chlorine can be found in most sanitizers, such as bleach, and can be used for produce cleaning purposes (59, 75). As shock chlorination is the most cost-effective method of disinfecting water which has been adopted by commercial growers, multiple organizations published shock chlorination guidelines to help growers use it properly (17, 43, 86).

In a lab trial, studies showed chlorine dioxide acted as an effective reagent that can kill more than 99% of plant pathogens in a short time (112). Also, Bel et al. found that 10 mg/L of chlorine can remove 7 logs of *E. coli* rapidly (140). Other research reported a 5 log reduction of *E. coli* populations with 3 mg/L of chlorine dioxide (57).

However, limitations of chlorine dioxide application are also present. It can only work for a short time period within the existence of chlorine dioxide in the water, which cannot be applied as a long-term solution.

Chemical Oxidation

Chemical oxidation is a chemical treatment used to eliminate organic materials in water and wastewater using oxidation, which is both efficient and environmentally friendly. This method has been widely used in the process of purifying and treating wastewater to increase the

use of water (47, 68, 79, 158) as reclaimed wastewater. The reaction of the process is based on a powerful oxidizing agent (e.g., ozone, hydrogen peroxide (H_2O_2), and peracetic acid (PAA)) and obtained at a sufficient concentration.

Ozone disinfection is known as a more effective disinfectant than chlorine against protozoan parasites in water systems (41). Since ozone is highly reactive and unstable, the cost of operation and maintenance are relatively higher than other similar water treatment methods. Hydrogen peroxide is another water treatment method that is environmentally friendly. Several studies show that hydrogen peroxide itself is a slow-acting water disinfectant (requires high dosages and long contact times), but its performance can be enhanced by combining with Cu^{2+} , Ag^+ , and Zn^{2+} (37, 106, 107, 141). Peracetic acid (PAA) is another common and strong oxidant that is applied to disinfect wastewater. It is a man-made chemical that can act against a wide spectrum of pathogens at a low concentration rapidly (12). Kitis et al. ranked the disinfection efficiency of PAA towards microorganisms as bacteria > viruses > bacterial spores > protozoan cysts (68). However, literature also shows the remaining decomposed PAA increases the organic content in the water, which potentially enhances the pathogen regrowth (68, 73, 76). Of note, all the chemical oxidation treatments can be combined with UV and applied in the water treatment process, which results are more efficient than when applied alone (16).

Ultraviolet (UV) Light Disinfection

UV light is a type of electromagnetic (EM) radiation, which has frequencies of about 8×10^{14} to 3×10^{16} hertz (Hz), and wavelengths of about 200 - 400 nanometers (nm). Based on the

wavelength, the UV light can be divided into three sub-bands: UV-A (320-400 nm), UV-B (280-320 nm), and UV-C (200-280 nm). The commercial UV lights for disinfection use UV-C wavelength (typically 254 nm), which is effective against most bacteria, protozoa, and viruses, by acting directly on the DNA of microorganisms and resulting in a germicidal effect (120).

As clearer water has a higher transmission rate, UV light is more effective in treating low turbidity water (more clear, higher transmission) than high turbidity water (UV light gets blocked) (142). Thus, filtration is always recommended, especially for surface water, before running through the UV system. Also, the effectiveness of a UV system depends on the sensitiveness of microorganisms to UV light. Research shows a 280 nm wavelength UV reactor results in a 4 log reduction in *E. coli* (96), while applying the same wavelength to mesophilic bacteria led to a 1.4 log reduction (34). On the other hand, literature shows the pathogen log inactivation does not follow the increase of the UV wavelength. Nyangaresi et al. tested multiple wavelengths of UV-LED on the inactivation of *E. coli* in water, and the 267 nm UV-LED resulted in a 4 log inactivation, whereas the 310 nm wavelength inactivated 0.5 logs of the *E. coli* population (94).

Other in-lab water treatments

There are some water treatments that have not been applied to agricultural water that are more in the experimental stage of research and development and have not been extensively applied commercially on fresh produce farms. (1) Ultrasound technology: The ultrasound system affects objects in a liquid medium by leading to cavitation, and the forces created by it influence the biological system. Thus, ultrasound technology kills microorganisms by enhancing mass

transfer through the biological membranes. Its application range is based on the type of cavitation, which can be broadly divided into transient and stable types. Research shows that it takes five days for ultrasound treatment to reach to 85% reduction (82). Thus, it needs to be applied combined with other biocides, such as chlorine, UV, and electrolysis. According to research, ultrasound treatment improves the effect of chlorination on reducing the bacterial population in raw stream water (102), reaching about a 4 log reduction of fecal coliforms when applied with UV (156).

Electrolysis also can work as a water pretreatment method to reduce microbial contamination. Electrolyzed water (EW) is created by passing an electric current through a dilute saltwater solution in an electrolysis chamber. The process uses a membrane cell to separate an anode and a cathode and produce acidic EW, which contains free chlorine. Thus, EW can be used to reduce the survival of *E. coli*, *Listeria*, and *Salmonella* (51, 64).

Agricultural Soil Treatment Methods

The transmission of human pathogens can occur during the germination of seeds or after the transplantation of seedlings in contaminated soil (28, 71). Also, the internalization of human pathogens, specifically enteric pathogens, through root uptake has been a point of debate due to the inconsistencies among numerous scientific studies (27, 40). Reducing pathogen load in potentially contaminated soil is important, especially in organic farms, which commonly use biological soil amendments of animal origin (BSAAOs) to improve soil fertility and quality (116).

Solarization

Soil solarization is a method of sterilizing soil using solar heating by covering the pre-irrigated soil with transparent plastic sheets, which has the advantages of simple manipulation and low cost. This method has been proven as one of the efficient ways of controlling plant pathogens in soil (121). Recently, using solarization to inactivate human pathogens (i.e., *E. coli* and *Salmonella*) has been confirmed effective both in the field (152) and in a greenhouse (151).

Cover Crops Application

The establishment of cover crops on organic farms is becoming a standard practice to improve soil health and nutrient management. Cover cropping's potential benefits include reduced soil erosion, weed suppression, soilborne and foliar plant disease suppression, reduction of plant-parasitic nematodes, soil improvement, and nutrient management through nitrogen fixation and carbon accumulation (60, 99, 115). Recently, cover crops have gained attention for their ability to suppress soilborne and foliar plant diseases (61, 74, 145); this antimicrobial activity has been attributed to the production of secondary metabolites which protect the plant from microbial invasion (amongst other roles). While research on the antimicrobial activity of cover crops to suppress plant pathogens has been well developed in past decades, studies investigating their antimicrobial activity towards human pathogens in the soil is still limited (109).

Secondary metabolites in cover crops and their antimicrobial activity

The secondary metabolites are the organic molecules that are neither involved with growth nor development but act as a defense mechanism in the plant to respond to the biotic and abiotic stressors (98). In previous literature, secondary metabolites have been widely studied against plant insects (33, 128), plant viruses (153), and plant fungus (63, 148). Until recent years, the antibacterial effect of secondary metabolites in cover crops has been noticed and proved. For example, many species in the *Brassicaceae* family produce glucosinolates which are water-soluble compounds resistant to degradation until they contact the enzyme myrosinase. Then it hydrolyzes them into isothiocyanates (I.C.T.s), nitriles, and other products (56), which inactivate pathogenic bacteria like *Staphylococcus aureus*, *Enterococcus faecalis*, *Listeria monocytogenes*, *E. coli*, and *Salmonella spp.* (4, 110, 111, 143). Flavonoids (i.e., rutin, quercetin, isoorientin) are another large group of secondary metabolites found in species such as buckwheat which inhibit both gram-negative and gram-positive bacterial survival (1, 11, 146). This research brought up the potential of using cover crops as an amendment to inactivate pathogenic bacteria in produce farms.

Extension Education

The USDA National Institute of Food and Agriculture (NIFA) defines agriculture extension as “Extension that provides non-formal education and learning activities to people throughout the country — to farmers and other residents of rural communities as well as to people living in urban areas. It emphasizes taking knowledge gained through research and education and bringing it directly to the people to create positive changes” (89). Agricultural

extension services can originally be traced back to World War I. It served to support war costs by helping farmers increase crop yields by relaying agricultural knowledge. A set of survey data collected in 1980 showed that farmers and all household members had a positive and significant effect after extension education (39). This study emphasized the necessity and significance of agricultural extension education. Nowadays, educators are bridging the gap between new knowledge and regulations from scientific research to growers and aim to solve farms' difficulties and increase the production, food security, and standard of living of farm families. Universities and colleges are also engaging in extension and outreach teams to help local agricultural producers.

In recent years, because PSR criteria require growers to receive training, “PSR training” has become a hot topic in the extension field. In 2010, Cornell University, FDA, and USDA created the Produce Safety Alliance (PSA), which developed a standardized curriculum and nation- wide education to help fresh produce growers to comply with the requirements outlined in the FSMA PSR. According to data from 2013, FDA estimated that 35,029 total produce farms in the U.S. are required to comply with the PSR, which are qualified as very small farms and above (150). In order to fulfill the national training need, in 2016, PSA launched a train-the-trainer program to provide the opportunity for more people to be qualified to deliver the PSA grower training course and to respond to growers' questions during the training sessions. By the end of March 2022, PSA has held 2,332 grower training courses with 46,361 participants and 2,325 people qualified as a trainer in the US (105).

Extension Education Theoretical Framework

In addition to PSA grower courses, extension agents also provide independent sessions on good agricultural practices and general science-based knowledge to both PSR- covered and exempt growers. Inspired by adult and youth education program planning frameworks, the extension education theoretical framework aims to help extension professionals plan effective training. Within the expectation of positive change in growers' behavior after the training, the social marketing theory and the theory of planned behavior are used to develop the extension training sessions. The outcomes will increase the likelihood of growers having behavior changes (25).

Social Marketing Theory

The social marketing theory is adopted from commercial marketing theory and includes the 4P's of marketing, which are the product, price, place, and promotion (149). Instead of the profit motive, like in commercial marketing, the primary goal of social marketing is to achieve a positive behavior change in a population. Kotler and Zaltman et al. defined social marketing as “a social influence technology involving the design, implementation, and control of programs aimed at increasing the acceptability of social ideas or practice in one or more groups of target adopters.” (69). There are two fundamental philosophies in social marketing Theory (62) :

- An organization's objective should be to meet the needs and demands of the customers.
- In order for an exchange to take place, the organization and the customer must engage as nearly equals, with each having something to contribute to the other and each being able

to gain from the transaction.

Since understanding the barriers to behavior change adoption among the target population is the keystone of social marketing theory (87), audience analysis is one of the most important elements of the whole process. The same technique is also applied in the agricultural extension area, known as research-based needs assessment activities. Based on the results from each activity, professionals can implement changes to help farmers overcome the barriers.

Theory of Planned Behavior

The Theory of Planned Behavior (TPB) (5) is an extension of the Theory of Reasoned Action (TRA) (44). It started in 1980 to predict an individual's intention to engage in a behavior at a specific time and place. The principle was designed to understand all activities for which people would exercise self-control. Behavioral intent is a critical component of this model; behavioral intentions are influenced by one's attitude about the probability that the behavior will produce the desired result and one's subjective assessment of the costs and benefits of that outcome (119).

According to the TPB, the behavioral achievement is dependent on both motivation (intention) and skill (behavioral control). It divides attitudes into three categories: behavioral, normative, and control. The TPB is made up of six constructs (attitudes, behavioral intention, subjective norms, social norms, perceived power, and perceived behavioral control) that together reflect a person's actual behavioral control (5).

TPB is considered in extension education to help planners understand the attitudes that growers hold about agriculture practices, the factors that influence their thoughts, and the necessity of measured behavioral intent before and after the training.

Survey Research

Surveys are one of the common measuring instruments to collect feedback and opinions from growers. It enables researchers to be more efficient by employing a sample of respondents to generalize to the entire community (38). The data results can help extension professionals to obtain information to learn the current growers' practices and knowledge. For example, surveys have previously been conducted to assess growers' produce safety knowledge (100, 103) and needs (101) to help them to comply with produce safety best practices.

Typical survey methods include telephone, paper, in-person, and web-based surveys (38). Among these methods, electronic surveys are more adopted in the recent era with the advantage of low cost and fast transmission (38). Of note, due to the COVID situation, public activities were largely canceled due to the concern for public health, so the surveys were largely web-based. Thus, the survey (chapter 3) in the thesis is mainly web-based.

Conclusion and Future Outlook

Soil contamination and improper BSAAOs practice always raise the possibility of cross-contamination, especially on organic farms which commonly use BSAAOs to treat the soil. Although using cover crops to reduce the pathogen load in soil has not been widely studied, the hands-on results demonstrated the potential of cover crops to be used as a sustainable and cost-

effective soil treatment. Further research is needed to compare the efficiency of pathogen reduction among different species of cover crops.

Meanwhile, water will always remain a key component of the food production system. Agricultural water as the primary reservoir of human pathogens is continuously suspected as a transmission route for produce-related foodborne illness outbreaks. One of the current challenges focuses on inappropriate agricultural water practices, including using surface water for postharvest use without validated treatment and misunderstanding some terminologies (i.e., water used for handwashing is considered as postharvest water). These practices might not directly lead to an outbreak but will raise the possibility of cross-contamination. Considering some high-tech agricultural water treatment methods (i.e., UV and chemical oxidation), some of these show promise of pathogen reduction but are not commonly used on farms. Further research and extension education is required to deliver the latest technology to growers.

Another challenge is the latest proposed modifications to the agricultural water rule. Although the new proposed rule is focused on simplifying the production water criteria, growers and extension professionals need more time to adopt the new modified rules after years of education on the current agricultural water regulation. During this transition period between the current version of the rule and the finalized new modified rule, growers may be confused with the requirements and the potential impact of on-farm water practices (i.e., water microbial quality test, agricultural water assessments, and water system inspection). Moreover, the new proposed rule requires growers to determine the water quality by themselves through different

factors. However, this requires growers to have more knowledge of potential agricultural water hazards and good practices. Although the FDA provides an online agricultural water assessment tool to help growers determine the safety of water sources, it still is a barrier for growers located in rural areas that do not have internet.

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Chapter 2 - Evaluating the Efficacy of Cover Crops to Reduce Bacterial Pathogen Load in Contaminated Soil

Abstract

Cover crops are plants seeded before or after cash crops to improve soil health, reduce weed pressure, and prevent erosion. Cover crops also produce various antimicrobial secondary metabolites (i.e., glucosinolates, quercetin), yet the role of cover crops in moderating the population of human pathogens in the soil has rarely been investigated. This study aims to determine the antimicrobial capacity of three cover crop species to reduce the population of pathogenic soil microbiota. Four-week-old mustard greens (*Brassica juncea*, ‘Kodiak’), sunn hemp (*Crotalaria juncea*), and buckwheat (*Fagopyrum esculentum*) were mixed into autoclaved soil and inoculated with 7 log rifampicin-resistant generic *Escherichia coli* (*E. coli*). The surviving microbial populations on days 0, 4, 10, 15, 20, 30, 40 were enumerated. All three cover crops significantly reduced the population of generic *E. coli* compared to control ($p < 0.0001$); buckwheat resulted in the highest reduction (3.92 log CFU/g). An inhibitory effect ($p < 0.0001$) on microbial growth was also observed in soils containing mustard greens and sunn hemp. This study provides evidence for the bactericidal and bacteriostatic effects of particular cover crops. More research regarding the secondary metabolites produced by certain cover crops and their potential as a bio-mitigation strategy to improve on-farm produce safety is warranted.

Introduction

Agricultural soils contain a dynamic microbiome that supports plant health and are home to organisms that produce secondary metabolites (a group of chemical compounds that defend the organisms from biotic and abiotic stress) (35). However, agricultural soils are also a potential route for the transmission of human pathogens to fresh fruits and vegetables. Pathogens like *E. coli* and *Salmonella* are shed in the fecal matter of animals, where they can be spread throughout the field soil by contaminated irrigation water (4, 18) or through weather-related events such as flooding (9, 36). Recent investigations indicate that many of these human pathogens can persist in agricultural soils for up to 100 days (25, 26, 31, 38), increasing the potential for spreading to other produce and to the growing environment. Commodities like watermelons and cantaloupes, in particular, are considered at high risk for contamination from agricultural soils as they are grown on the ground and often make direct contact with soil (8). For this reason, improving agricultural soil management practices is a topic emphasized by federal regulation (e.g., the Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR)) and guidelines for certifications (e.g., Good Agricultural Practices (GAPS) certification).

While such federal regulations and programs offer some preventative measures, there is little information available for growers on strategies to actively reduce the human pathogenic microbial risk of their soils. For example, one of the most pressing concerns in agricultural soil management is the application of biological soil amendments of animal origin (BSAOs), which can carry a high microbial risk since animals serve as the primary reservoir for many human

pathogens (23, 49). The FSMA PSR §112.56 states, “untreated BSAAOs must not be applied in a manner that directly contacts the harvestable portion of the crop and must be applied in a manner that minimizes the potential for contact with covered produce after application” (42). Otherwise, the soil management recommendations currently largely defer to the National Organic Program, which requires growers to apply a die-off interval for raw animal manure application of 120/90 days for edible produce directly/not directly in contact with the soil before harvest (7 CFR 205.203) (41). Of note, soil contamination from BSAAOs is of particular concern for organic produce growing operations, which largely rely on non-chemical (BSAAO-based) methods like manure-based composts to enrich the soil (28). Clearly, there is a need to expand the repertoire of pre-harvest strategies which can reduce the transmission of pathogens from agricultural soils to fresh produce.

Cover crops are typically utilized by growers to improve overall soil agronomic properties but show promise to also reduce the populations of plant and human pathogens in agricultural soils. Cover crops are cultivated between growing seasons to improve soil health and nutrient composition (e.g., carbon, nitrogen) (16, 34), reduce soil erosion (48), and conserve water (43). Plants used as cover crops include grasses (e.g., *Panicum maximum*), legumes (e.g., *Trifolium pretense*), and non-legume broadleaves (e.g., *Fagopyrum esculentum*), which are fast-growing, improve soil physical properties, and may or may not fix nitrogen (7, 12, 14). Recently, cover crops have gained attention for their ability to suppress soilborne and foliar plant diseases (19, 20, 46); this antimicrobial activity has been attributed to the production of secondary

metabolites, which are organic molecules that are not involved with growth or development, but aid in plant response to biotic and abiotic stressors (27). Amongst other roles, secondary metabolites can protect the plant from microbial invasion. For example, many species in the *Brassicaceae* family produce glucosinolates which are hydrolyzed to form cytotoxic compounds that inactivate pathogenic bacteria like *Staphylococcus aureus*, *Enterococcus faecalis*, *Listeria monocytogenes*, *E. coli*, and *Salmonella spp.* (2, 32, 33, 44). Flavonoids (i.e., rutin, quercetin, isoorientin) are another large group of secondary metabolites found in species such as buckwheat which inhibit both gram-negative and gram-positive bacterial survival (1, 3, 47). While research on the antimicrobial activity of cover crops to suppress plant pathogens has been well developed in past decades, studies investigating their antimicrobial activity towards human pathogens in the soil is limited.

A recent study by Reed-Jones et al. focused on the effects of cover crops (cereal rye, hairy vetch, crimson clover, and hairy vetch-rye) on long-term population variation of *E. coli* and *L. innocua* in the soil (29). The study found a suppressing effect of cover crops on *E. coli* and *L. innocua* based on the microorganisms' innate characteristics and soil type (i.e., loamy, clay). The authors also highlighted how soil temperature, tillage practices, and the weather could affect the bacterial population. While the authors observed potential microbial growth inhibition in fields containing cover crops, they raised concerns that the root exudates of living cover crops into the rhizosphere could also promote the growth of human pathogenic bacteria in the soil.

The objective of this study was to determine if there were antimicrobial capabilities of three common cover crops – mustard greens, buckwheat, and sunn hemp – in field soil inoculated with generic *E. coli*. This study will not only provide information on the feasibility of the three cover crops as a soil bio-mitigation method but also the effect of these crops on microbial survival and inform laboratory-based methodologies for such studies.

Material and Methods

Growing Conditions and Plant Biomass Harvesting

The experimental mustard greens (*Brassica juncea*, ‘Kodiak’) (True Leaf Market, Salt Lake City, UT, USA), sunn hemp (*Crotalaria juncea*) (Sow True Seed, Asheville, NC, USA), and buckwheat (*Fagopyrum esculentum*) (Albert Lea Seed Company, Albert Lea, MN) were grown in a greenhouse at the Olathe Horticulture Research and Extension Centre (OHREC) in Olathe, Kansas during the fall of 2021. Mustard greens and buckwheat were sown in large containers (229 cm × 73 cm × 10 cm). Sunn hemp was grown in 9.5-L nursery containers to account for their more expansive root system and larger overall biomass. The containers were filled with organic Plant Pro™ potting soil (Ohio Earth Food, Hartville, OH, USA) before seeding. The greenhouse was climate-controlled, with temperatures ranging from 12.2 °C during the night to 28.9 °C during the day. Overhead irrigation was applied to the crops every other day in order to provide ample soil moisture

Whole crops (including the underground biomass) were harvested at the flowering stage before producing seeds (27 days after propagation). The crops were gently removed from the

containers to avoid excess root loss, and the soil was removed. The plant material was then collected and immediately transported to the lab for further processing, which was performed on the same day.



Figure 2.1 The growing environment for buckwheat and mustard greens.

Soil preparation

The soil used for *E. coli* inoculation in this study was a chase silt loam (40) collected from a field at the OHREC, which had been in perennial grass for the past three years. The soil was collected from the field and transported to the lab. Prior to sterilization, the collected soil was separated into autoclave bags (Fisher Scientific, Waltham, MA, USA); large clumps of sediment were manually pulverized to facilitate handling after sterilization. The soil was then autoclaved at 121.0 °C and 15 psi for one hour; sterility was confirmed using a bioindicator (Fisher

Scientific, Waltham, MA, USA) placed in the center of the bagged soil. Flame-sterilized tongs were used to extract the bioindicator, and the soil was set aside to cool to room temperature before use in the study.

Bacterial strain

Plant roots and shoots are naturally colonized by a dynamic population of microbes that interact amongst themselves and the plant tissues (30). To avoid background interactions from non-target organisms in the phytobiomass, the soil used in the study was autoclaved first, and rifampicin-resistant generic *E. coli* (ATCC 25922) was acquired from the Food Safety Lab at Kansas State Olathe was used as the study microorganism. The strain was previously stored with 50% glycerol (Fisher Scientific, Waltham, MA, USA) in 1.5mL microcentrifugation tubes (Eppendorf North America, Enfield, CT, USA) at -80.0 °C. To initially propagate the bacteria, a loopful of frozen culture was transferred to a culture tube containing 10 mL tryptic soy broth (TSB; Remel Inc., San Diego, CA, USA) and incubated for 24 h at 35.0 °C.

The methodology of Bhullar et al. (6) was followed to induce rifampicin resistance. Briefly, 0.1 mL of the forementioned microbial culture was spread to a plate of tryptic soy agar (TSA; Remel Inc., San Diego, CA, USA) containing 20 µg/mL rifampicin (RIF; Fisher Scientific, Waltham, MA, USA); the TSA-RIF plate was then incubated for 24 h at 35.0 °C. The procedure was repeated using TSA-RIF plates with increasing concentrations of rifampicin (20, 40, 60 µg/mL) until a strain resistant to 80 µg/mL concentrations of rifampicin was generated on TSA-RIF plates.

To culture, the organism for experimentation, one colony of rifampicin-resistant *E. coli* was transferred to 10 mL of TSB containing 80 μ g/mL RIF (henceforth noted as TSB-RIF). The tube was incubated for 24 h at 35 °C and sub-cultured again in 30 mL of TSB-RIF. To prepare the cells for experimentation, the cells were washed twice with phosphate buffer saline (PBS; Fisher Scientific, Waltham, MA, USA) with centrifugation (Allegra X-30, Beckman-Coulter, Brea, CA, USA; 10,000 rpm, 10 min, 20.0 °C) occurring prior to and in between each wash. The final volume was adjusted to a minimum concentration of 7 log CFU/mL.

Sample Preparation and Inoculation

This study utilized a randomized complete block design with repeated measures. Three independent replications of each treatment (cover crop species) were performed. To mitigate the impact of the ‘rhizosphere effect’ and address food safety concerns raised by previous literature (29), we used chopped whole-plant biomass for this study. The whole-plant material (containing roots, shoots, and leaves) was chopped into 3-cm long pieces to imitate mowing and tilling and was manually homogenized in three large containers (one container per cover crop species). To simulate the soil-cover crop mixture, 200 g of the chopped plant tissue sample, 200 g of the sterile soil sample, and 75 mL of inoculum culture were combined in a 24 oz. Whirl-Pak bag (Whirl-Pak, Madison, WI, USA). The 1:1 ratio of cover crops and soil is based on the assumption that cover crops will only be incorporated into the upper 10 cm of soil. For the non-treated control, 400 g of the sterile soil sample and 75 mL of inoculum culture were combined. The ratio of the solid matter and inoculum was based on preliminary trials to ensure all solid

matter would be moistened by the inoculum. Each sample bag was shaken and massaged 50 times to homogenize the mixture before being transferred into a quart-sized container (1.4 L) with a plastic plant saucer (Mudeela, Seattle, WA, USA) placed underneath. The samples were stored at room temperature (average 20.6 ± 0.02 °C) and with an average relative humidity of $32.9 \pm 0.4\%$ for 40 days. An average of 30 mL of sterile DI (deionized) water was added to each container each week to prevent the soil from becoming too dry to facilitate bacterial survival.

Sample Collection and Microbial Enumeration

Before collecting the sample on each sampling day, each container was mixed using a sterile plastic spoon; the spoons were sterilized by soaking overnight in 10% bleach, followed by rinsing with distilled lab water. A 10-gram sample was collected from each container and transferred to a Whirl-Pak bag containing 90 mL of 0.1% buffered peptone water (BPW; Remel Inc., San Diego, CA, USA). The sample bag was manually massaged to homogenize the sample. Serial dilutions were prepared in 0.1% BPW, and 100 μ L of the sample solution was then spread-plated onto TSA-RIF before incubating for 24 h at 35 °C. Plates yielding 25-250 colonies were recorded for analysis. Samples were collected 0, 4, 10, 15, 20, 30, and 40 days after treatment.

Statistical analysis

The statistical treatment structure utilized was a one-way ANOVA. The whole study was evaluated using three blocks of four pots each on days 0, 10, 20, 30, and 40. The surviving bacterial population at each time point was subjected to log transformation in order to achieve the variance homogeneity assumption.

The bacteriostatic (phase I) rate was calculated based on the difference of log-transformed surviving bacterial populations between day 0 and day 10. The bactericidal (phase II) rate was calculated based on the difference in log-transformed surviving bacterial populations between day 10 and day 40. The bacteriostatic (phase I) and bactericidal (phase II) rates were analyzed separately using the linear mixed model. Fixed effects of the model included all treatments. Random effects were the block and the error term. The main effect per day was assessed via least squares means (LSMs) and their standard errors (SEMs). Pairwise comparisons were carried out using the two-sided test. The Holm-Tukey method was used to adjust for multiplicity. All tests were conducted at the 0.05 significance level. Statistical analysis was executed via Statistical Analysis Software (SAS version 9.4; Cary, NC) PROC MIXED with option DDFM=BETWITHIN in the MODEL statement.

Results and Discussion

Overall trends in cover crops microbial reduction

Overall, the data showed that certain cover crops could reduce the microbial risk of soils containing generic *E. coli* within 30 to 40 days following a simulated cover crop incorporation event (*Figure 2*). This microbial reduction was cover crop- ($p < 0.0001$) and time-dependent ($p < 0.0001$). By day 30, soils amended with buckwheat or mustard greens had achieved a maximum *E. coli* reduction of 3.92 ± 0.16 log CFU/g and 3.77 ± 0.16 log CFU/g, respectively. For soils containing macerated sunn hemp, *E. coli* was still detectable on day 30, only reaching a maximum microbial reduction of 3.70 ± 0.10 log CFU/g 40 days after treatment.

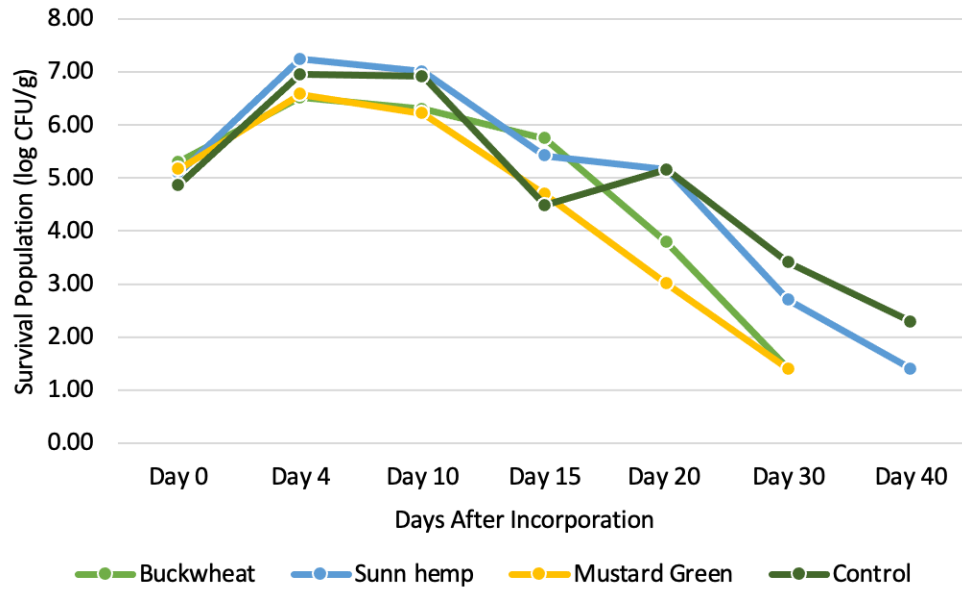


Figure 2.2 The surviving population of rifampicin-resistant *E. coli* in agricultural soil amended with macerated control, buckwheat, sunn hemp, or mustard greens

These overall findings provide potential insight into how these cover crops may be utilized to reduce human microbial risk and thus be used as a soil bio-mitigation strategy. For example, the persistence of *E. coli* in soils amended with sunn hemp could indicate that the secondary metabolites of buckwheat and mustard green tissues are more rapid in inducing microbial cytotoxicity compared to those in sunn hemp tissues. This observation is consistent with previous literature, which reports the cytotoxic activity of sunn hemp is predominantly in seeds rather than in root/shoot tissues (11, 24). In general, there were also no differences in *E. coli* survival between soils containing mustard greens or buckwheat throughout this study, despite their previously- documented different secondary metabolite composition and volume (5, 21).

A biphasic microbial variation curve (see Figure 2) was observed in the experiment, wherein statistically significant interaction effects ($p < 0.0001$) indicate unequal effects of the treatments

over time. The first phase (days 0 to 10) is characterized by a significant increase in the *E. coli* populations amongst all the treatments prior to their decline. From day 10 on, the microbial populations experienced a sharp decline until reaching levels below the detection limit. Topp et al. (39) reported observing a similar microbial growth curve and attributed the variation to the soil type and *E. coli* strains, amongst other factors. Of note, this previous study did not explore microbial survival in a chase silt loam as was utilized in this study, which is characterized by more than 50% silt and 12-27% clay (13), and is a soil composition (theoretically) more likely to have negligible influences on *E. coli* growth due to the relatively aerobic environment of low clay-content soils (37).

Modeling the surviving microbial population to study the biphasic survival curve

To further investigate the differences in microbial growth, the surviving microbial population was statistically modeled by fitting its survival to a nonlinear curve (*Figure 3; Equation 1*). The values used in the equation specific to each cover crop and the control are presented in *Table 1*. Overall, the models were able to explain a high degree (91% to 98%) of the observed variability in the microbial survival of each treatment.

$$y = \frac{x \cdot a + b}{1 + c \cdot x^d} \quad (1)$$

Equation 1, Where x is the days after initial inoculation

y is the total surviving microbial population

a is the rate of increase to the maximum peak

b is the multiplier that increases the height of the peak

c is the rate of decrease with respect to days after initial inoculation

d is the peak shape parameter

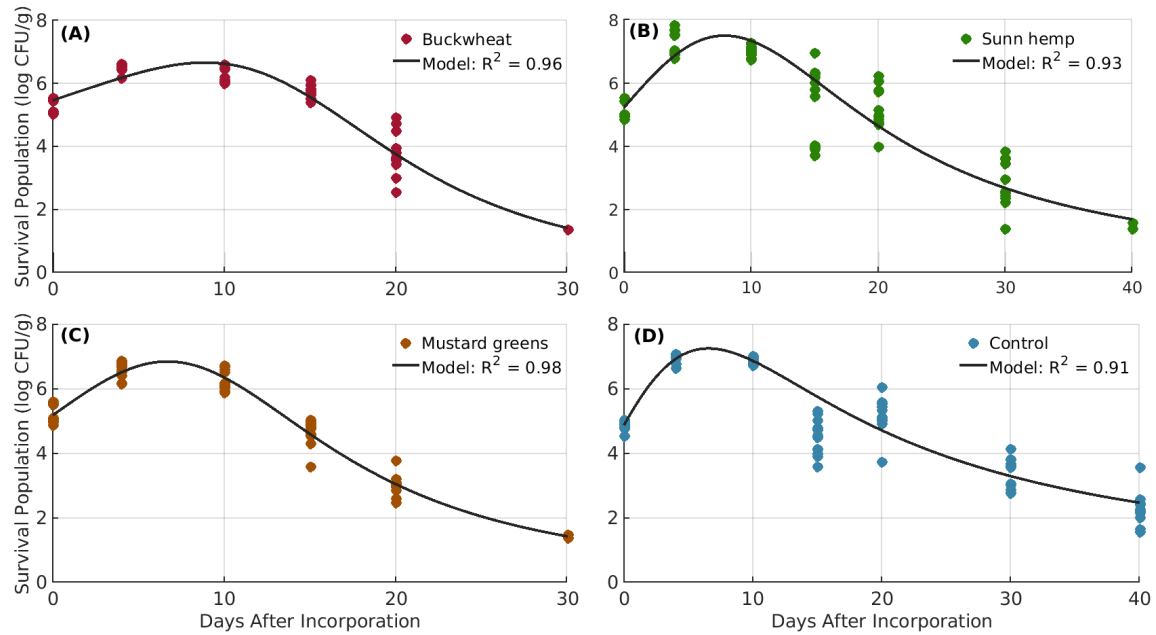


Figure 2.3 The fitted model estimating the surviving population of rifampicin-resistant *E. coli* in field soil supplemented with macerated control, buckwheat, sunn hemp, and mustard greens.

Table 2.1 Variable values by cover crops and control models.

	a	b	c	d	% Variance (R^2)
Control	0.7149	4.875	0.006.989	2.031	91
Buckwheat	0.1827	5.473	0.00001.497	3.827	96
Mustard greens	0.3761	5.184	0.0004.511	2.957	98
Sunn hemp	0.4654	5.227	0.0008.272	2.621	93

Bacteriostatic effects of cover crops during the exponential growth of *E. coli*

From days 0 to 4, there was a significant increase ($p < 0.05$) in the *E. coli* population in all treatments compared to their initial concentration. As evident by the value of a variable (Table 1), the control soils experienced a more rapid rate of increase to the maximum surviving population than the soils containing macerated cover crop tissue. Between the cover crops, sunn hemp showed a more rapid microbial population growth rate than buckwheat ($p < 0.0001$) or mustard greens ($p < 0.0001$). There were no significant differences between the initial growth rate (a) of the buckwheat and mustard greens ($p = 0.6178$) or the sunn hemp and control ($p = 0.2278$). After day 4, the soils containing macerated buckwheat ($p = 0.0021$), mustard greens ($p < 0.0001$) or sunn hemp ($p = 0.0010$) began experiencing a minor decrease in generic *E. coli* populations. In contrast, the microbial population of the control sample attenuated from days 4 to 10 as there were no statistically significant differences ($p = 0.6927$) detected during this time period.

These findings indicate that the exudated phytochemicals of all three macerated cover crops can impair the growth of *E. coli* during this initial growth phase, with buckwheat and mustard greens being the most effective. It is also likely that these results are reflective of an initial exudation of phytochemicals from the macerated cover crops stimulated by the artificial tillage. Divekar et al. note that many secondary metabolic production pathways are rapidly induced by physical stimuli (e.g., cutting of the plants) (15). Accordingly, using a rotary tiller or similar tool

to physically cut the cover crops (instead of only rotating them into the soil) may be critical to initiating their antimicrobial effect.

Bactericidal effects of cover crops during declining *E. coli* population

During the second phase (days 10 to 40), there was a more rapid, significant decrease ($p < 0.0001$) in the *E. coli* population in all treatments compared to their concentration on day 10. In this phase, the *E. coli* could be experiencing die-off due to limited nutrients available in the environment which is accelerated by the presence of cover crop tissues. As evidenced by the value of the c variable (Table 1), the control soil and soils containing macerated sunn hemp showed a slower microbial reduction rate than buckwheat or mustard greens ($p < 0.0001$). Buckwheat experienced the most rapid decline, but there were no significant differences detected between the microbial population of mustard greens and buckwheat ($p = 0.5626$).

The most rapid microbial decline for soil containing macerated sunn hemp occurred between days 20 to 30 which led to a 2.46 ± 0.15 log reduction of generic *E. coli*. Buckwheat also experienced the most rapid population decline (2.40 ± 0.15 log CFU/g) between days 20 and 30. Conversely, soils containing macerated mustard greens exhibited a microbial decline of 1.68 ± 0.26 log CFU/g between day 10 and day 20. There were no significant differences in the surviving population of *E. coli* between mustard greens and buckwheat except on day 20.

Interestingly, the results of this bactericidal phase do not necessarily align with the expectations from the literature. During this phase, it is conceivable that the more rapid decline is due to the cover crops beginning to degrade in the soils, further releasing exudates containing

anti-microbial secondary metabolites (10). Based on the degradation rate, sunn hemp should have the most rapid rate of degradation as the tissues decompose within two weeks post tillage (45). According to Wang et al., the phenolic compounds in buckwheat tissues typically degrade after 37 days, indicating a lower rate of metabolite exudation (47). As this study reports trends in antimicrobial activity which are opposite to what is expected (sunn hemp has less effective microbial reduction rate than buckwheat throughout the study), more investigation regarding the exudation and degradation of the principal secondary metabolites within the tissues of each cover crop is warranted.

Study Limitations

Though the data from this study fit the conventional assumptions for ANOVA, the statistical methodology needed to account for differences in the initial *E. coli* concentration between the control and cover crop-containing soils on day 0 (*Figure 1*). Accordingly, only inferences between the rate(s) of population growth/decline in each cover crop could be made. We believe this significant difference in *E. coli* populations on day 0 can be ascribed to the different compositions of the control samples. As no plant tissues were added to the control containers, 400g of farm soil was used as the control and was inoculated with the same volume of inoculum as other treatments. According to Fick's diffusion law, the diffusive flux goes from areas of high osmolyte concentration to areas of low osmolyte concentration (17). The lower moisture content of the control soils relative to the higher moisture content of the soils containing fresh cover crop tissues likely resulted in a more even distribution of the inoculum in the control soils. Thus,

when collecting the same weight of sample from each treatment, the control group resulted in a diluted generic *E. coli* population result.

The sole use of a gram-negative experimental pathogen presents another limitation of this study. This study evaluated the survival of non-pathogenic *E. coli* as a surrogate for pathogenic *E. coli* or other gram-negative species that may behave in a similar manner (i.e., *Salmonella* spp.), which are commonly found in the gut of wild and domestic animals. However, Locatelli et al. (22) reported higher stability and persistence of gram-positive bacteria (i.e., *Listeria monocytogenes*) in agricultural soils. When Neiunna et al. (29) inoculated cover crop-containing soils with *Listeria innocua*, the authors observed a lower antimicrobial efficacy against the pathogen compared to the gram-negative organisms. This reduced efficacy towards gram-positive organisms may not impact the application of cover crops in states dominated by cattle or poultry production, which have a higher risk of detecting gram-negative *E. coli* and *Salmonella* spp. in the soil. However, this could limit the applicability of the tested cover crops as a microbial risk mediation strategy in operations that struggle with high-risk sources of gram-positive organisms.

Conclusions

All three cover crop species resulted in a microbial reduction to non-detectable levels, while buckwheat and mustard greens also displayed a bacteriostatic effect on the growth of *E. coli* during the exponential microbial growth phase (days 0-10). Future studies should account for extrinsic and intrinsic environmental factors to provide more suggestions for the on-farm

applicability of cover crops as a bio-mitigation strategy. Factors like sunlight, temperature variation, and native soil microbiome interactions were eliminated in this experiment in order to determine if there was an effect of the cover crops on microbial populations. Moreover, the differential efficacy of the cover crops demonstrates the need for characterizing the mechanisms that drive the effects we observed in each of the different cover crop species, particularly including the secondary metabolite content and concentration within the plant tissues. A better understanding is needed of their corresponding environmental stability and exudation patterns as the crop decomposes into the soil. Overall, this is the first lab-trial study for human pathogen reduction on agricultural soil within cover crops amendments. The results demonstrated these cover crops as a promising, environmentally sustainable bio-mitigation strategy for human pathogens in soil that warrant more investigation to promote their use in produce growing operations.

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Chapter 3 - Knowledge and Current Practices Related to Agricultural Water Microbial Quality among Kansas and Missouri Produce Growers

Abstract

Kansas State University and University of Missouri Extension educators have been providing training and information on agricultural water microbial quality to help produce growers reduce risk. However, we recognized the need to determine knowledge gaps among Kansas and Missouri growers related to agricultural water quality and best practices. A survey was developed to determine future extension outputs and activities to encourage growers to improve their practices related to water quality. The survey was distributed to Kansas and Missouri produce growers attending in-person or online produce safety-related events in late 2020 and early 2021 and was also distributed through email lists of produce growers from both states. Survey results ($n = 101$) indicate that 13.9% of the respondents tested their water for generic *Escherichia coli* (*E. coli*) more than once a year, while 38.6% of the participants have never tested their water. Approximately half (59.3%) of respondents indicated they use municipal water for postharvest uses, while 6.7% indicated the use of untreated surface water for postharvest activities. Due to the concern of potential water contamination risks, researchers suggest that further training and educational resources would help growers improve practices related to water quality and produce safety.

Introduction

In recent years, foodborne disease outbreaks resulting from fresh produce have continued to occur (4, 12). Because these commodities are often consumed raw (26, 32), it is imperative that

fresh produce growers use the safest practices possible. From 2010 to 2017, a total of 85 multistate outbreaks associated with fresh produce with confirmed etiologies occurred in the United States, resulting in a known combined total of 4,658 known illnesses, 1,187 hospitalizations, and 55 deaths (4). Further, before 2015, numerous fresh produce outbreaks have been attributed to contaminated agricultural water in commodities such as leafy greens (15, 22), tomatoes (2), and melons (33). Thus, water quality management practices became a major and critical aspect of the Food and Drug Administration's (FDA) Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR) (31).

The PSR, which was finalized in 2015 to take its place as one of the seven foundational rules under FSMA, is the first U.S. Federal regulation to have established science-based minimum standards for the safe growing, harvesting, packing, and holding of fruits and vegetables grown for human consumption. In December 2021, the FDA proposed a revision to Subpart E of the PSR that would change the preharvest agricultural water requirements for covered produce, other than sprouts (30). The FSMA PSR and the proposed revision state the considerations and requirements related to agricultural water management to improve the microbial safety of agricultural water and therefore of produce. In December 2021, FDA announced their intent to exercise enforcement discretion and also act to extend the compliance date for the agricultural water (31).

As per the FSMA PSR, "agricultural water means water used in covered activities on covered produce where water is intended to, or is likely to, contact covered produce or food contact surfaces." Agricultural water is classified into two categories based on the different usage periods: production water and postharvest water. Production water refers to water used during growing activities whereas postharvest water includes all uses of water during or after harvest (7,

31). Since the use of postharvest water is so close to consumption, microbial water quality is especially important (6). Thus, produce growers must meet the requirements of §112.44(a), i.e., no detectable generic *E. coli*/100mL water sample for water used postharvest. The proposed revisions to Subpart E focus on production (i.e., preharvest) uses of agricultural water. These revisions emphasize conducting agricultural water assessments for production water sources and uses, eliminating the federal requirement of an ongoing microbial water quality profile with periodic updates through water quality monitoring.

Each year, Kansas and Missouri growers produce approximately \$26 million (13) and \$81 million (7), respectively, of fruits and vegetables. Kansas State University and University of Missouri Extension educators have been providing training and information on agricultural water microbial quality, based on current and newly proposed FSMA PSR agricultural water requirements, to help produce growers from Kansas and Missouri reduce risk to produce from agricultural water. Although research shows that knowledge gain alone leads to limited behavior change, it can provide people with more informed choices that may or may not lead to behavior change (16). Pratt and Bowman (21) suggest that individually-relevant, problem-focused, and hands-on activities can help extension educators prompt personal behavior change. To help prompt this positive behavior change, educators need a better understanding of the gap between water quality best practices and produce growers' knowledge and actions related to agricultural microbial water quality.

Survey research (20) is one method that allows extension educators to easily understand a large number of growers' current water practices and knowledge gaps in a shorter time. Other surveys have previously been conducted to assess growers' general produce safety knowledge (17, 19) and needs (18) to help them to comply with produce safety best practices. Perry et al.

(17) found that North Central US produce growers have a knowledge gap in understanding agricultural water handling practices compared with other topics of the FSMA PSR, which highlights the needs for food safety extension educators to focus on delivering information on agricultural water best practices to produce growers.

This survey aims to determine gaps between safe water management practices (as outlined in the FSMA PSR requirements/ proposed requirements and other produce safety best practices) and (i) the current knowledge and (ii) the current practices of produce growers from Kansas and Missouri related to agricultural water quality. This will enable extension educators to develop additional training and resources on agricultural microbial water quality accordingly to better meet the needs of growers.

Materials and Methods

The project team developed a 14-question survey instrument based on similar surveys (18). Before implementing the survey, 4 extension experts reviewed the survey questions for face and content validity. The Kansas State University Produce Safety Extension Associate also performed cognitive interviews (14) with growers from the target population to further validate the survey. The Kansas State University Human Research Institutional Review Board (IRB) approved the use of the survey. The survey (*Appendix 1*) included questions on the growers' knowledge and practices related to microbial water quality, which was administered from October 2020 to August 2021. Due to in-person meeting restrictions related to the COVID-19 pandemic, produce growers mainly completed the survey online in Qualtrics ($n = 76$) during online produce safety-related trainings or by distribution of the online survey link to email listservs of produce growers in Kansas and Missouri. Other respondents ($n = 30$) completed paper copies of the survey during an in-person horticulture farm field day or in-person produce

safety training sessions. Two drawings for gift cards were utilized to incentivize participation in the survey both on-line and in-person. To optimize data collection, potential survey respondents were asked to only complete the survey if they were able to answer questions related to their operations' water management practices. Because most produce farms in Kansas and Missouri have only a few people working on them (28, 29), we are confident that most people filling out the survey are actively involved in the water management practices on their farm, and thus qualified to complete the survey.

The 14-question survey instrument included multiple choice questions with some open text response options designed to understand growers' knowledge and practices related to agricultural water use in both production and postharvest stages, as well as their understanding of the microbial quality of agricultural water. Questions included grower age, farm location, water testing frequency, and water usage for both production and postharvest activities.

The data were analyzed in Qualtrics using the cross-tabulation (crosstab) analysis method (10, 11) (also known as contingency table analysis) to identify the growers' knowledge of microbial risks related to different water sources and how they handle those risks. Cross-tabulation analysis compares the results of different questions from the same respondent to gain more insight into grower water quality management behaviors based on the risk of their water source. For example, growers who indicated that they did not treat their water source but indicated that they used a municipal water source (which is treated by a third-party company) were considered as having a safe water management practice. The responses from questions related to production or postharvest water uses, treatment application status, and microbial water quality test frequency were classified by use of different water sources (surface, ground, and municipal water). The data from any questions that allowed respondents to provide an open-

ended response were added to existing responses or a new category of responses was added to the question, based on the keywords in those responses. For example, a separate category was created for growers who wrote they used water from a rain barrel when selecting ‘other’ water for their water source.

Growers were asked if they were GAP (Good Agricultural Practices) certified (rather than if they were covered by the FSMA PSR) because the project team felt that growers would be more comfortable responding to the question of GAP certification, rather than FSMA PSR coverage. Accordingly, responses to the questions about water testing and water treatment were stratified based on their GAP certification status. Although GAP certification is generally a produce buyer requirement and is not a regulatory requirement, many growers view certification as an opportunity to improve their likelihood of compliance with the PSR. Thus, many growers covered by the PSR also obtain GAP certification.

Because some respondents did not answer all of the survey questions, the total number of responses to each question related to water sources in the cross-tab analysis may be different. Additionally, the total number of responses for questions regarding agricultural water source use could differ as growers may have reported using more than one water sources for one or more pre- or postharvest activities. To reduce item non-response bias, surveys having less than two-thirds of the questions completed were excluded from the final data analysis.

Results and Discussion

Results

A total of 106 survey responses were received. Of the 103 responses included in the final data analysis, 51 (49.5%) were from Kansas, 50 (48.5%) were from Missouri, one (1.0%) was marked as unknown, and one (1.0%) did not complete the question. The age range of growers

completing the survey were as follows: 18-29 ($n = 18$, 17.5%), 30-39 ($n = 15$, 14.6%), 40-49 ($n = 26$, 25.2%), 50-59 ($n = 19$, 18.5%), and above 60 ($n = 25$, 24.3%) years old. Most respondents ($n = 90$) were currently selling or planning to sell their produce to others. Most respondents ($n = 87$) were currently not GAP certified, as shown in Table 3.1.

Table 3.1 Reported microbial water quality testing ($n = 100$) status and GAP certification status ($n = 101$) by state.

	Kansas	Missouri	Total
Have tested water	16 (31.4%)	27 (55.1%)	43
Never tested water	25 (49.0%)	13 (26.5%)	38
Use third party test results	10 (19.6%)	9 (18.4%)	19
Total	51	49	100
GAP certified	2 (3.9%)	12 (24.0%)	14
Working towards being GAP certified in the future	7 (13.7%)	8 (16.0%)	15
Not GAP certified	42 (82.4%)	30 (60.0%)	72
Total	51	50	101

Of the participants which indicated they are currently growing fresh produce ($n = 101$), participants indicated their frequency of generic *E. coli* testing as more than once a year ($n = 14$, 13.9%), once a year ($n = 11$, 10.9%), 2-3 times in the last ten years ($n = 11$, 10.9%), once in last ten years ($n = 7$, 6.9%), never tested ($n = 39$, 38.6%), or that a third-party water source tests their water ($n = 19$, 18.8%). Between the two states (Table 3.1), 31.4% ($n = 16$) of Kansas growers said that they have tested their water in the past ten years compared to 55.1% ($n = 27$) of Missouri growers. Of the growers indicating they are GAP certified ($n = 14$), 10 (71.4%)

reported testing their water more than once a year and six (46.2%) reported that they treat their postharvest water sources. Of the non-GAP certified growers ($n = 87$), four (4.6%) growers test their water more than once a year and 15 (17.4%) growers treat their postharvest water sources (Table 3.2, Table 3.3). Because the questions inquiring about the type of water source used for production and postharvest applications allowed for more than one response, the total number of responses varied by question, as shown in *Figure 1*.

Table 3.2 The frequency of microbial water quality testing by GAP certification status (n = 101).

	More than once a year	Once a year	2–3 times in last 10 years	Once in last 10 years	Never test	Third party water test	Total
GAP certified	10 (71.4%)	0 (0.0%)	1 (7.1%)	1 (7.1%)	0 (0.0%)	2 (14.3%)	14
Working towards being							
GAP certified in the	1 (6.7%)	3 (20.0%)	1 (6.7%)	0 (0.0%)	8 (53.3%)	2 (13.3%)	15
future							
Not GAP certified	3 (4.2%)	8 (11.1%)	9 (12.5%)	6 (8.3%)	31 (43.1%)	15 (20.8%)	72

Table 3.3 Treatment application status for postharvest water based on GAP certification status (n = 99)

	Apply treatment	No treatment applied	Total
GAP certified	6 (46.2%)	7 (53.8%)	13
Working towards being			
GAP certified in the future	3 (21.4%)	11 (78.6%)	14
Not GAP certified	12 (16.7%)	60 (83.3%)	72

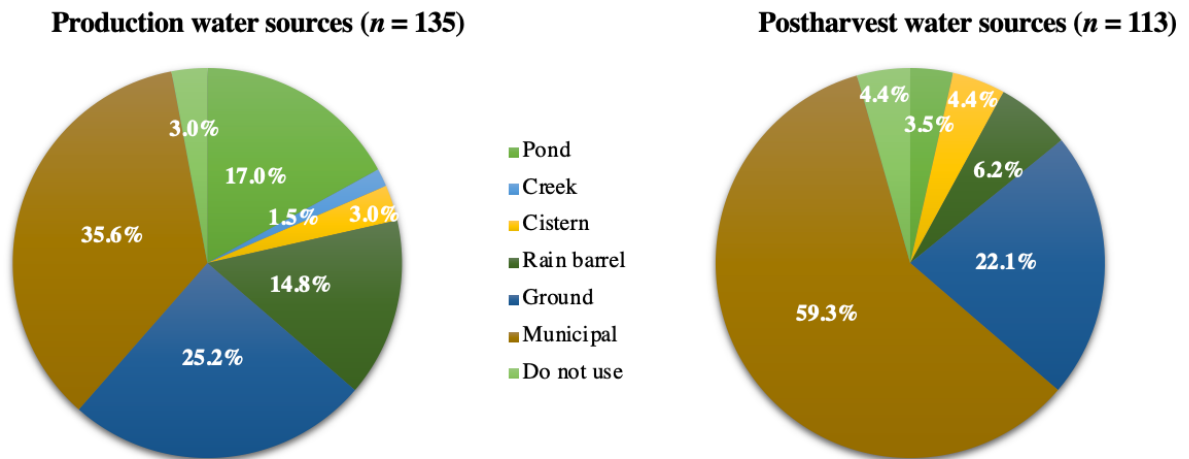


Figure 3.1 The percentages of different water sources that growers use for production ($n = 135$) and postharvest ($n = 113$) activities. (Note: among the 101 responses, several used more than one water type)

Municipal ($n = 48$, 35.6%), well ($n = 34$, 25.2%), pond ($n = 23$, 17.0%), and rain barrel ($n = 20$, 14.8%) water sources were the most commonly reported sources of production water (Figure 1). Municipal ($n = 67$, 59.8%) and well water ($n = 25$, 22.1%) were the most commonly reported water sources for postharvest water. Based on the number of responses for each water source, respondents appear to commonly utilize more sources of water for production applications ($n = 135$) than postharvest ($n = 113$) applications.

Participants also reported their applications of production and postharvest water (Figure 3.2). Of the survey respondents ($n = 101$), 90 (89.1%) indicated that they used production water in their operation. The three main reported uses of production water included irrigation ($n = 90$, 100%), mixing with crop sprays ($n = 38$, 42.2%), and mixing with fertilizers ($n = 38$, 42.2%). Of those growers that report using water for irrigation, 70 (77.8%) reported using drip irrigation, 37 (41.1%) reported using overhead irrigation, and 20 (22.2%) reported using drip irrigation under plastic. Furthermore, 90 (89.1%) indicated that they used postharvest water in their operation.

These growers reported their main uses of postharvest water to be for handwashing ($n = 77$, 85.6%), rinsing/washing produce ($n = 73$, 81.1%), and cleaning and sanitizing of food contact surfaces ($n = 68$, 75.6%). Other uses of postharvest water reported included ice making ($n = 7$, 7.8%), dunk tank cooling ($n = 6$, 6.7%), single pass/spray cooling ($n = 4$, 4.4%), and postharvest fungicide or wax applications ($n = 3$, 3.3%).

Of survey respondents who indicated that they used production water in their operation ($n = 90$), 75 (83.3%) reported that they do not treat their production water. Of the respondents who reported treating their production water, four (4.4%) reported using chemical sanitizers whereas 11 (11.1%) respondents indicated using physical methods such as sand filtration. Of survey respondents who indicated that they used postharvest water in their operation ($n = 90$), 68 (75.6%) reported that they do not treat their postharvest water. Fifteen respondents (16.7%) reported treating postharvest water with a chemical sanitizer and four (4.4%) reported using other treatments such as sand filtration or ultraviolet light. When comparing water treatment status and the water source used (shown in *Figure 3.3*), six (6.7%) respondents reported using surface water (ponds, cisterns, or rain barrels) for postharvest use without applying any treatment.

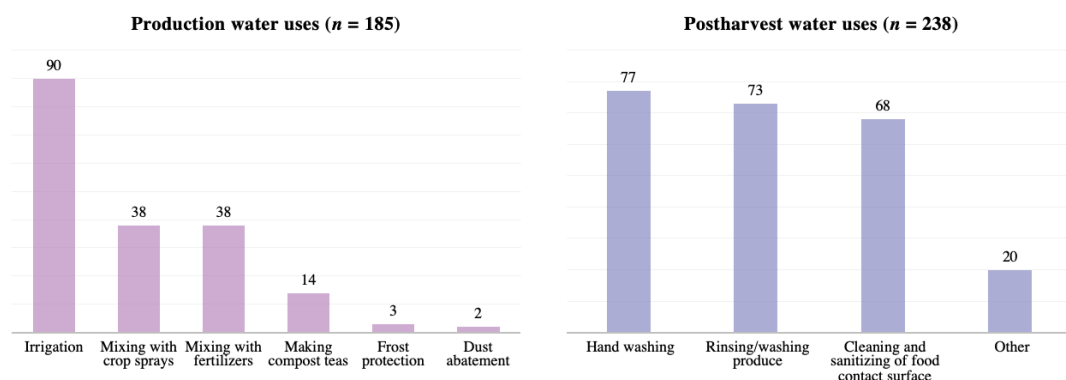


Figure 3.2 The different uses of production water ($n = 185$) and postharvest water ($n = 238$).

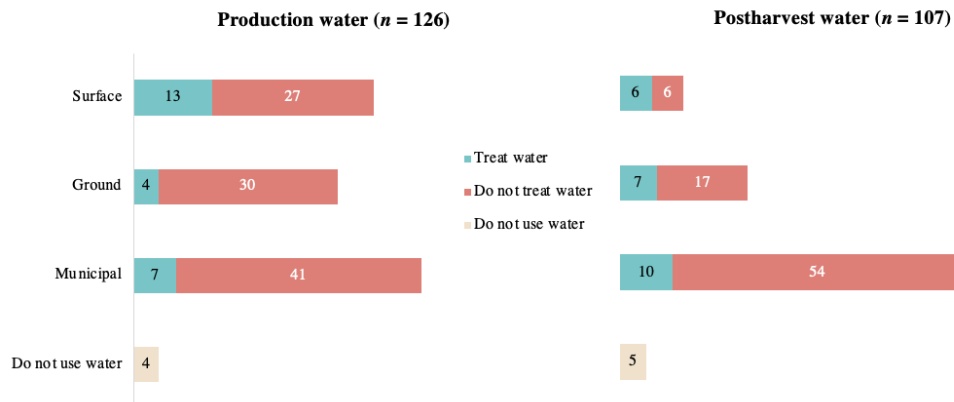


Figure 3.3 Treatment status for each water source that growers use for production (n = 126) and postharvest (n = 107) activities.

When asked what other practices (besides treatment) growers use to maintain/improve production water quality, most ($n = 70$, 77.8%) respondents using production water in their operation indicated that they did not use any other practices. Nineteen (21.1%) of the participants indicated that they conducted an annual evaluation of their water distribution system. When managing the quality of their postharvest water, 61 (67.8%) respondents who indicated that they used postharvest water in their operation ($n = 90$) reported that they do not use any additional practices, 17 (18.9%) reported routine cleaning and sanitation of tanks, 15 (16.7%) reported monitoring for buildup of organic material, 14 (15.6%) respondents reported conducting an annual evaluation, 10 (11.1%) reported monitoring water temperature during use, and nine (10.0%) reported using a routine water change schedule.

Discussion

Agricultural water quality remains a large concern for the fresh produce industry across the United States, particularly in the Midwest where previous studies indicate that agricultural water quality management is not well understood (17).

Most of the respondents demonstrated a preference for water sources with a lower microbial risk profile (such as municipal water) for postharvest activities, as compared to using surface water postharvest, which is higher risk. Specifically, the survey data indicated that – unlike production water, where municipal (35.6%) and surface water use (36.3%) were nearly equally reported – there is a higher use of municipal water (59.3%) and lower use of surface water (14.1%) for postharvest applications (*Figure 1*). As previous research has shown, microbial populations in surface water increase after events such as agricultural runoff (rain, melted snow, etc.) (3, 24, 27). Surface water is also more exposed to potential contamination from the surrounding open environment, and thus is considered to have a higher possibility of contamination (1, 8, 23).

According to the data collected from the survey, a minority of growers use specific postharvest water management practices that many would consider higher risk. Using surface water without any treatment for postharvest applications poses a higher risk of foodborne disease outbreaks, thus the FSMA PSR agricultural water criteria, GAP certification requirements and general produce safety best practices do not allow use of untreated surface water for postharvest activities (31). Interestingly, six survey respondents indicated that they were using untreated surface water for postharvest uses, and eight respondents indicated they treated their agricultural water using methods such as (bio)sand-filtration. Of note, sand-filtration is not a validated agricultural water treatment method according to the FSMA PSR (21 CFR 112.43) (31). However, growers do currently utilize sand filtration in irrigation systems to remove larger, physical particles, sometimes with the added misconception that it also can remove pathogens. This indicates that further education and training is needed to emphasize the significance of

microbial risk in postharvest water, as well as how to select an appropriate (validated) treatment method.

Conducting regular inspection and maintenance of the water source and distribution system is currently a requirement of the FSMA PSR (21 CFR 112.42) (31) and is a produce safety best practice to limit the risk of microbial contamination. By conducting regular inspections, growers can evaluate the risks to their water sources. The inspection includes identifying any broken part of the system structure, any wildlife evidence around open water sources, and other risks. Most of our survey respondents are not currently conducting an annual agricultural water system inspection. Thus, extension educators should provide practical information to growers on how to inspect their water sources regularly and encourage them to conduct a water system inspection at least annually, which is a requirement for growers covered by the FSMA PSR (21 CFR 112.42) (31). Of note, the proposed §112.43 is intended to supplement the requirements of the proposed §112.42 which will require a covered farm to regularly inspect and routinely maintain the components of its agricultural water system. While proposed §112.42 is focused on agricultural water system components, the proposed §112.43 would require covered farms to conduct a more comprehensive assessment of possible sources and routes by which known or reasonably foreseeable hazards are reasonably likely to be introduced into its preharvest agricultural water (30).

The FSMA PSR also highlights irrigation method as an important risk factor for contamination risk based on if the water contacts the harvestable portion of the produce. In most situations, overhead irrigation results in direct contact with the harvestable portion of the produce, which can result in a higher contamination risk than other irrigation methods. Conversely, drip irrigation (particularly drip irrigation under plastic) is considered to have a

relatively lower risk of contamination for crops grown above the ground. Of note, this characterization does not apply to root crops such as carrots, beets, and radishes (25). Most of the survey respondents indicated that their main use of production water was irrigation, namely drip irrigation (69.1%) or overhead irrigation (31.3%). However, the findings of the study call attention to particular agricultural water practices including the use of untreated surface water for direct water application during postharvest, and seldom testing the sanitary quality of agricultural water sources. Although not specifically measured by this study, further investigation and potentially extension efforts may need to occur to clarify how growers can reduce microbial risk by selecting irrigation methods that reduce the probability of water contact with the harvestable portion of the produce.

In the survey, the question about the frequency of conducting microbial water testing did not specify testing frequency of “production water” and “postharvest water” separately. Accordingly, crosstab analysis for the frequency of water testing for different sources of water used for different purposes (preharvest or postharvest) could not be conducted. Moreover, it was not possible to discern between treated and untreated sources when more than one source was indicated by the grower in this survey. We also did not include questions related to the location of their water source in relation to animals or other potential sources of contamination. These points could have been clarified by increasing the length of the survey. However, the expert panel reviewing the survey was concerned about the adverse effect of increasing the survey length on the response rate as some participants required more than 10 minutes to complete the survey. In a study of the effects of length on web-based survey completion, Galesic and Bosnjak (9) found that participants anticipating a longer survey completion time were less likely to finish the survey.

Conclusion

This survey was intended to provide information on the differences between best practices and the actual practices of Kansas and Missouri produce growers focusing on the use of agricultural water. Produce growers need to continue to improve their practices related to water quality to ensure that they are selling the safest produce possible. The survey responses will provide insight into the development of additional agricultural water safety educational materials for produce growers. The results indicated that efforts are needed to continue to encourage growers to test their water quality, to conduct at least an annual inspection of water sources and distribution system, and to use a validated surface water treatment method before using it for postharvest applications. The authors suggest that future extension trainings should emphasize the importance of microbial quality of agricultural water sources, and how risk to produce safety can be affected by selection of water source, decision to treat, and (in the case of production water) application method.

Acknowledge

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Chapter 4 - Agricultural Water Related Extension Education

Handouts

Based on the anecdotal evidence and survey responses from this thesis (Ch. 2), we identified the critical need to address a few important agricultural water safety-related topics that include the following:

New Modified FSMA PSR Agricultural Water Requirement Proposal

FSMA PSR published the agricultural water regulation in 2016, including using the different microbial quality criteria to value both production and postharvest water quality. However, after the implementation, the feedback from different farms focused on "the rules are too complicated and cost too much." Therefore, the FDA revised and published a proposed agricultural water requirement in December 2021 focused on production water. This handout summarized the major modification of the latest water requirement and aimed to help growers understand and comply with the proposed rule.

QUICK REFERENCE GUIDE

New Modified FSMA PSR Agricultural Water Requirement Proposal



In December 2021, the United States Food and Drug Administration (FDA) proposed a revision to Subpart E of the Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR) that would change the *pre-harvest agricultural water requirements* for covered produce, other than sprouts.

What is the proposed change?

The proposed revision to Subpart E modifies the previous requirement for produce growers to *replace the microbial criteria and testing requirements* for pre-harvest agricultural water with new provisions for *conducting pre-harvest agricultural water assessments*, providing more flexibility to produce growers in evaluating risks.

These aim for hazard identification (including consideration of agricultural water sources, distribution systems, and practices, as well as adjacent and nearby land uses, and other relevant factors), and using the results of the assessment to determine whether *corrective or mitigation measures* are needed to reduce the potential for contamination of produce or food contact surfaces with hazards associated with pre-harvest agricultural water.

What is an “agricultural water assessment” ?

It refers to an *evaluation*, conducted by a covered farm, of factors in its agricultural water system used during growing activities for non-sprout covered produce, its agricultural water practices for such pre-harvest water, crop characteristics, environmental conditions, and other relevant factors (including test results, where appropriate).

Factors	Description
Agricultural water system(s)	• Location and nature of the water source (including whether it is ground or surface water) • Water distribution system type (such as whether it is open or closed to the environment) • Degree to which the system is protected from possible sources of contamination
Agricultural water practices	• Water application method (overhead sprinkler, spray, drip, furrow, flood, seepage, etc.) • Time interval between the last direct water application and harvest of the covered produce
Crop characteristics	• Susceptibility of the covered produce to surface adhesion or internalization of hazards
Environmental conditions	• Frequency of heavy rain or extreme weather events may impact the agricultural water system or damage produce that can increase the susceptibility of produce to contamination. • Air temperatures • Sun (UV) exposure
Other relevant factors	• Including, if applicable, results of water testing that could inform the assessment



Questions, comments or concerns?

Contact your K-State Extension specialist **Londa Nwadike** (lnwadike@ksu.edu or nwadike@Missouri.edu) or **Cal Jamerson** (agri@ksu.edu) today!

New Modified FSMA PSR Agricultural Water Requirement Proposal

Corrective and mitigation measures

The proposed rule does **NOT** change the requirements for harvest and post-harvest water, but only impacts pre-harvest water. Under this proposed rule, a covered farm would *make a determination under the requirements, based on the outcome of its agricultural water assessment*, as to whether mitigation measures would be reasonably necessary to reduce the potential for contamination of covered produce (other than sprouts) or food contact surfaces with known or reasonably foreseeable hazards associated with its pre-harvest agricultural water.

Based on the assessment, if you determine...	Then you must...
that your agricultural water is not safe or is not of adequate sanitary quality for intended use(s).	- Immediately discontinue use(s) AND - Take corrective measures before resuming use of the water for pre-harvest activities
there is one or more known or reasonably foreseeable hazards related to animal activity, BSAAOs, or untreated or improperly treated human waste for which mitigation is reasonably necessary.	Implement mitigation measures promptly, and no later than the same growing season
there is one or more known or reasonably foreseeable hazards not related to animal activity, BSAAOs, or untreated or improperly treated human waste, for which mitigation is reasonably necessary.	- Implement mitigation measures as soon as practicable and no later than the following year Or - Test water as part of the assessment and implement measures, as needed, based on the outcome of the assessment
that there are no known or reasonably foreseeable hazards for which mitigation is reasonably necessary.	Inspect and adequately maintain the water system(s) regularly, and at least once each year

Exemptions:

A covered farm would be exempt from the requirement to conduct an assessment for pre-harvest agricultural water if the farm can demonstrate that the agricultural water meets these requirements, including:

- It meets certain requirements that apply for *harvest and post-harvest agricultural water* (such as the microbial quality criterion and testing requirements for untreated ground water);
- It is received from a *public water system or supply* that meets requirements established in the rule (provided that the farm has public water system results or certificates of compliance demonstrating that the water meets relevant requirements); or
- It is treated in accordance with the standards outlined in the Produce Safety Rule.

Prepared by: Yeqi Zhao Reviewed by: Manreet Bhullar, Londa Nwadike



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How to Interpret Water Results

Since 2016 FSMA PSR published agricultural water regulation, agricultural water has been separated into production water and postharvest water and from three main water sources (i.e., surface, ground, and municipal). Due to the different cross-contamination risks, the water criteria varied by different water activities. This handout aims to help Kansas and Missouri growers better understand the water regulation they need to comply with and interpret their water results within simple wording, images, and charts.

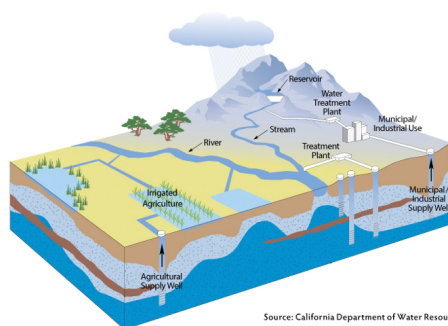
QUICK REFERENCE GUIDE

FSMA Produce Safety Rule Requirements for Agricultural Water

“ The **Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR)** states that all agricultural water **must** be of safe and adequate sanitary quality for its intended use (§112.41) ”

What is Agricultural Water?

Agricultural water is water that is intended or likely to contact the edible portions of produce or food contact surfaces during growing (production) or during/after harvest (post-harvest). The PSR outlines three common sources of agricultural water: **ground water**, **surface water**, and **municipal water**.



Public Water is water that is treated and monitored by a local water utility

Ground Water is water that is found beneath Earth's surface (ex. wells, aquifers)

Surface Water is water that is found or stored on the Earth's surface (ex. rivers, creeks, cistern, ponds supply tanks)

Risk of Contamination



What's the difference between Production and Postharvest Water?

Production Water is any water that is applied directly to produce during growing

- Irrigation
- Crop sprays
- Fertigation
- Frost protection
- Cooling



VS

Postharvest Water is any water that is used during and after field harvest of the produce

- Field Packing
- Washing
- Hydro-cooling
- Ice making
- Hand Washing



Note: water used for hand washing also counts as postharvest water since it can potentially contact the produce surface.

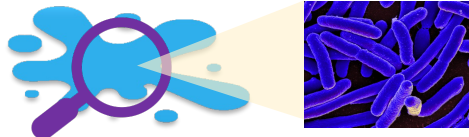


How do we determine water quality?

Agricultural Water Assessment The new modified agricultural water proposed rule uses an evaluation system to evaluate pre-harvest water quality. There are four main factors include agricultural water system(s), agricultural water practices, crop characteristics, and environmental conditions. Based on the assessment, you can determine whether your water is saved to use or not.

Tips: Agricultural Water Assessment Builder can help you evaluate your water quickly and easily!

Access from [link](#) or **QR-code**



Water Microbial Quality The Produce Safety Rule uses a bacteria called generic *E. coli* as an indicator of water quality. *E. coli* is a type of bacteria that is commonly found in animal waste (feces). Based on the number of generic *E. coli* in 100 ml, you can know whether your water is microbially safe to use or not.



Questions, comments or concerns?

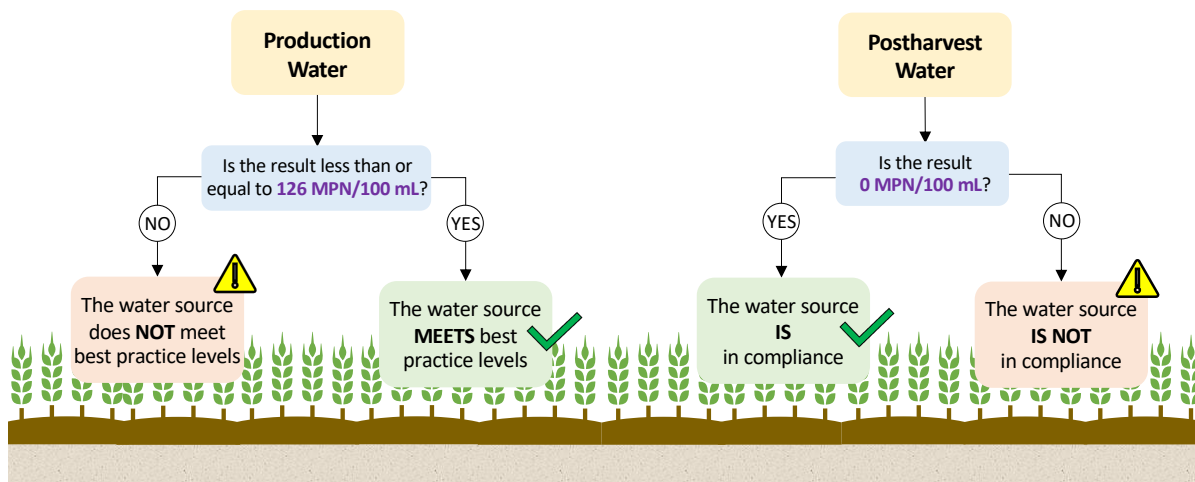
Contact your K-State Extension specialist **Londa Nwadike** (lnwadike@ksu.edu or nwadike1@Missouri.edu) or **Cal Jamerson** (agri@ksu.edu) today!

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How to Interpret Your Water Quality Test Results

Step 1: Determine if the water source meets best practice/ regulation levels

Intended Use:



Step 2: Determine the next steps if the water source **IS NOT best practices/ FSMA PSR compliant**

If the postharvest water is not compliant:

DISCONTINUE use as soon as practical (switch to municipal water or other reliable source) unless corrective measures are applied.

A few things you can do:

- Contact your local extension personnel for solution.
- Apply a **time interval** between the last irrigation and harvest.
- **Re-inspect** the entire affected agricultural water system for:
 - The extent it is under your control
 - Any conditions that are likely to contaminate produce surfaces
- Apply **validated treatments** to the water source.
 - The treatment must be effective to make the water safe and of adequate sanitary quality for its intended use (i.e., UV, chlorine).



Funding for this project is made possible in part by grant 1U18FD005895-02 (KS5895) from the FDA to KDA, as well as grant 1U18FD006145-01 from the FDA to MDA and USDA NIFA Grant 2019-70020-30358. The information and viewpoints do not necessarily reflect the viewpoints and policies of the supporting organizations, cooperating organizations, FDA or USDA.

Prepared by: Yeqi Zhao, Olivia Haley Reviewed by: Manreet Bhullar, Londa Nwadike

Backflush Water Fact Sheet

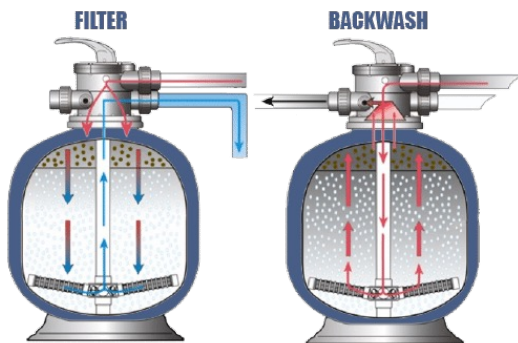
According to the survey results, the filtration system is the primary water treatment reported by the grower. The backflush water is the water used to clean the filter that contains various microorganisms and potentially includes human pathogens. Despite the use of filtration alone is not a validated agricultural water treatment, the handout relates to the filtration method, and backflush water is limited in the extension field. This handout aims to popularize the biohazard of backflush water and possible solutions.

QUICK REFERENCE GUIDE

Backflush Water Fact Sheet

Due to the produce safety concern, filtration systems are widely used in farms to catch small solid particles from original water sources (surface or ground water), and provide clearer water for agriculture purpose (irrigation, fertigation, cooling, etc.).

However, do you know the filtration system can also provide the “dirty” water called **backflush water**?

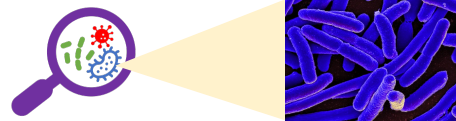


What is Backflush Water?

Backflush, also known as **backwash**, it is a mechanism always comes with a sand and Diatomaceous Earth (DE) filtration system acts as a **self-cleaning system**. It is a water flow that moves reversely through the filter tank to remove the accumulated particles from the filter.

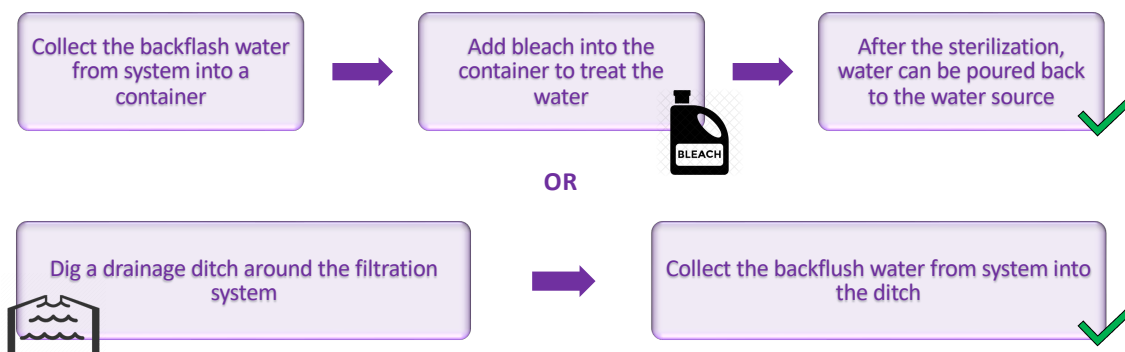
The Concerns That Backflush Water Brings Up:

As backflush water brings all the potential contaminated particles from the filter surface, the backflush water itself is considered as a **highly contaminated** water. If the backflush is not handled and/or stored properly, it can re-add the contamination to the original source or to other clean water sources on the farm.



How to Manage the Backflush Water in My Farm?

Following are several methods to manage your backflush water:



Purdue University Logo



Questions, comments or concerns?

Contact your K-State Extension specialist **Londa Nwadike** (phone number; email) or **Cal Jamerson** (phone number; email) today!

Chapter 5 - Conclusion

The thesis focused on improving the food safety practices reducing the risk of fresh produce outbreaks in the US, specifically expected outcomes on minimizing the cross-contamination chance in the fresh produce farms. The primary two reservoirs for transmitting human pathogens are the farm soil and agricultural water. Thus, to complete the goal, the thesis work included three objectives: (1) reducing the pathogen load in potentially contaminated agricultural soils using cover crops; (2a) accessing current practices and knowledge of Kansas and Missouri growers by using survey; (2b) developing extension material focused on managing agricultural water quality based on the survey results.

Soil Treatment

Several soil treatment methods reduce human pathogens in fresh produce fields. Specifically, cover crops have been recently explored for antimicrobial application in produce production, besides their soil health benefits for contaminated soils. Chapter 2 evaluates the efficacy of reducing pathogen load in contaminated soil with three different cover crop species (i.e., buckwheat, mustard greens, and sunn hemp). The results showed all three cover crops could reduce the bacteria population in contaminated soils after 30 to 40 days following artificial tillage within buckwheat did the best. However, the sole use of a gram-negative pathogen (generic *E. coli*), soil type, and selected three cover crops would be the primary limitations in this study. Considering this is the preliminary study, future research could study the antimicrobial efficacy on soil with different pathogens/soil types/cover crops to better understand the implications of these results. Nevertheless, these results demonstrated the potential of applying cover crops as an antimicrobial treatment in contaminated produce fields. The

outcomes will benefit the organic fresh produce growers as they use biological soil amendments to enrich the soil as a common practice.

Agricultural Water Safety

Unlike soil amendment contamination, agricultural water safety has always been a concern in all types of produce farms, as highlighted in multiple produce outbreaks. Within continuously providing training and information on agricultural water microbial quality to help produce growers reduce risk, the survey developed (chapter 3) is necessary to determine the current knowledge and practice gaps between produce growers and agricultural water management best practices. The surveys were sent out between late 2020 and early 2021 during the COVID-19 pandemic, in which most of the growers' in-person activities had been canceled due to public health concerns. Thus, most surveys were distributed through Qualtrics (an online survey instrument), and the total responses were lower than the expectation (106/200). Despite the dilemma, the survey results indicate the growers from Kansas and Missouri have a basic awareness of agricultural water practices. Still, more demanding practices must be strengthened (e.g., using validated water treatment methods).

Based on the survey responses, three handouts were developed (chapter 4), including: (1) "New modified FSMA PSR agricultural water requirement proposal," which summarized the significant modification of the latest water requirement published in 2021; (2) "How to interpret water results" provides information to identify the agricultural water sources and microbial safety criteria; (3) "Backflush water fact sheet" discussed the hazard from back flush water and solutions. These handouts aim to fulfill the produce growers' current needs and help them to mitigate risk from agricultural water cross-contamination.

In conclusion, the survey provided information on the gaps between best practices and the actual practices of Kansas and Missouri produce growers on agricultural water management; these gave extension professionals the directions for developing further outputs. In addition, in December 2021, the FDA published a new modified agricultural water requirement proposal. The new proposed rule will replace the produce water criteria once it is finalized. Thus, further study can focus on assessing the produce growers' knowledge of the new proposed rule and develop handouts based on the results.

Appendix A - Survey Questions

1. What is your age?

☐ 18-29

☐ 40-49

☐ 60+ years

☐ 30-39

☐ 50-59

2. Are you currently Good Agriculture Practices (GAP) certified?

☐ Yes

☐ No

☐ No, but working
towards being GAP
certified in the future

3. Are you currently raising produce (fruits, vegetables, herbs, mushrooms, nuts) for sale?

☐ Yes

☐ No

☐ No, but plan to in the future

4. In which state do you grow produce? (If more than one, mark the primary state)

☐ Kansas

☐ Missouri

☐ Other _____

5. Do you currently use water PRE-harvest in your produce operation? (check all that apply)

☐ For irrigation

☐ For dust abatement

☐ For mixing with crop sprays

☐ Frost protection

☐ For mixing with fertilizers

☐ Other _____

☐ For making compost teas

☐ No

6. If you use water for irrigation, how do you irrigate? (check all that apply)

☐ Drip irrigation

☐ Furrow irrigation

☐ Drip irrigation under plastic

☐ Other _____

☐ Overhead irrigation

☐ I do not irrigate

7. What sources of PRE-harvest water do you use? (check all that apply)

☐ Pond

☐ Cistern

☐ Creek

☐ Other (such as rain barrel, spring, or
others. Please list) _____

☐ Well

☐ Do not use water pre-harvest

☐ Municipal water

8. Do you currently use water POST-harvest in your operation? (check all that apply)

☐ Rinsing/ washing produce

☐ Single-pass/spray cooling

☐ Commodity movement (i.e. dump
tanks/ flumes)

☐ Dunk tank cooling

☐ Ice making

- ☐ Post-harvest fungicide or wax
- ☐ Handwashing
- ☐ Cleaning and sanitizing of food contact surfaces
- ☐ Other (please list) _____

9. What sources of POST- harvest water do you use? (check all that apply)

- ☐ Municipal water
- ☐ Well
- ☐ Cistern
- ☐ Pond
- ☐ Creek
- ☐ Other (such as rain harvest barrel or others. Please list) _____
- ☐ Do not use water post-harvest

10. Do you test your water used in your produce operation for generic *E. coli*?

- ☐ Yes, more than once a year
- ☐ Yes, once a year
- ☐ Yes, we have tested our water 2-3 times in the last 10 years or so
- ☐ Yes, we have tested our water once in the last 10 years or so
- ☐ No, we have never tested our water
- ☐ A third party (such as the municipal supplier)

11. Do you treat your water used PRE-harvest (such as in irrigation) to improve its microbial quality?

- ☐ Yes, we treat it with a sanitizer (list sanitizer used) _____
- ☐ Yes, we use a different treatment (list treatment- for example, sand filter, other) _____
- ☐ No

12. Do you treat your water used POST-harvest (such as in washing) to improve its microbial quality?

- ☐ Yes, we treat it with a sanitizer (list sanitizer used) _____
- ☐ Yes, we use a different treatment (list treatment- for example, sand filter, ozone, UV, reverse osmosis, other) _____
- ☐ No

13. What other practices (besides treatment) do you use to maintain/improve the quality of the water used PRE-harvest in your produce operation?

- ☐ We conduct an annual evaluation of our water distribution system
- ☐ Other (please list) _____
- ☐ None

14. What other practices (besides treatment) do you use to maintain/improve the quality of the water used POST-harvest in your produce operation?

- ☐ We conduct an annual evaluation of our water distribution system
- ☐ Routine water change schedule
- ☐ Routine cleaning and sanitation of tanks
- ☐ Monitoring for buildup of organic material
- ☐ Monitoring water temperature during post-harvest use
- ☐ Other (please list) _____
- ☐ None

Improving produce safety by reducing pathogen load in soil using cover crops and developing
food safety educational resources for produce growers

by

Yeqi Zhao

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College of Agriculture

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Approved by:

Co-Major Professor
Dr. Eleni D. Pliakoni

Approved by:

Co-Major Professor
Dr. Manreet S. Bhullar

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Abstract

Fresh produce (commonly consumed raw) has been continually associated with multiple foodborne disease outbreaks, which cause both health concerns and economic losses.

Agricultural water and contaminated soil are suspected as common causative agents for recent produce-related foodborne illness outbreaks, which brought fresh produce safety to the forefront of public attention. New federal regulations by the U.S. Food and Drug Administration (FDA) as part of the FDA Food Safety Modernization Act (FSMA) established the Produce Safety Rule (PSR), which defined scientific criteria for growing, harvesting, storing, and packaging fresh produce. The PSR provides requirements on treating soil amendments and criteria for safer use of agricultural water.

There are several soil and water treatment methods and practices to reduce human pathogens in fresh produce. Specifically, for contaminated soils, cover crops have been recently explored for antimicrobial application in produce production, in addition to their soil health benefits. Cover crops are plants seeded before or after the growing season to improve soil health, increase water availability, reduce weed pressure, and prevent erosion. Cover crops also produce various antimicrobial secondary metabolites (i.e., glucosinolates, quercetin), yet the role of cover crops in moderating the population of human pathogens in the soil has rarely been investigated. However, with regards to the microbial quality of agricultural water, Kansas State University and University of Missouri Extension educators have been providing free water testing, training and information on agricultural water microbial quality to help produce growers reduce microbial risks from ag water sources.

The goal of this thesis is to study cover crops as potential antimicrobial interventions and understand grower needs to improve food safety measures and practices on a fresh produce farm.

To achieve this goal, there are two specific objectives: (1) soil treatment: determine the antimicrobial potential of three cover crop species to reduce the population of pathogenic soil microbiota which can minimize the possibility of cross-contamination of produce from agricultural soil; (2) agricultural water: determine knowledge gaps among Kansas and Missouri produce growers related to agricultural water quality and best practices and develop agricultural water safety handouts to improve growers' knowledge.

To complete the first part of this thesis, whole-plant samples of 4-week-old mustard greens (*Brassica juncea*, 'Kodiak'), sun hemp (*Crotalaria juncea*), or buckwheat (*Fagopyrum esculentum*) were chopped and mixed into autoclaved farm soil and inoculated with 7-logs of rifampicin-resistant generic *Escherichia coli* (*E. coli*). The surviving microbial population on days 0, 4, 10, 15, 20, 30, and 40 was enumerated using an agar-based methodology. All three cover crops significantly reduced the population of generic *E. coli* compared to the control ($p < 0.0001$); buckwheat resulted in the highest reduction (3.92 log CFU/g). An inhibitory effect on microbial growth was also observed in soils containing mustard greens ($p < 0.0001$) and sunn hemp ($p < 0.0001$). This study provides evidence for the bactericidal and bacteriostatic effects of cover crops. More information regarding the crops' secondary metabolite profile and potential as a soil bio-mitigation strategy to improve on-farm produce safety is warranted.

For objective 2, a survey was developed and administered to determine future extension outputs and activities to encourage growers to improve their practices related to water quality. The survey was distributed to Kansas and Missouri produce growers attending in-person or online produce safety-related events in late 2020 and early 2021. Also, it was distributed through email lists of produce growers from both states. Survey results ($n = 101$) indicate that 13.9% of the respondents tested their water for generic *Escherichia coli* (*E. coli*) more than once a year,

while 38.6% of the participants have never tested their water. Approximately half (59.3%) of respondents indicated they use municipal water for postharvest uses, while 6.7% indicated the use of untreated surface water for postharvest activities. Due to the concern of potential water contamination risks, researchers suggest that further training and educational resources would help growers improve practices related to water quality and produce safety.

Three handouts were developed to help growers better understand the current FSMA PSR water quality requirements (under consideration for further modifications) and best practices. These handouts include the following: (1) “New modified FSMA PSR agricultural water requirement proposal” handout summarizes the major modifications of the latest water proposal published in 2021; (2) “How to interpret water results” handout provides information to identify agricultural water uses and microbial safety criteria and best practices; and (3) “Back flush water fact sheet” discusses the hazards from back flush water and potential solutions.

In conclusion, this thesis focuses on improving fresh produce safety by minimizing the cross-contamination happening in agricultural soil and water. The reduction of microbial populations in the first part of the thesis demonstrated the potential of cover crops to be used as a sustainable antimicrobial treatment method for contaminated soil. Meanwhile, the survey results show produce growers have a basic awareness of agricultural water practices, but the safety of those practices can still be strengthened. The publication of relevant handouts can improve growers' knowledge and help them better comply with the latest regulations.

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Chapter 1 - Literature Review

Introduction

Significance of fresh produce in the human diet

Since fresh fruits and vegetables contain vitamin A, C, and E, carotenoids, folic acid, dietary fiber, and other minerals, they are one of the great resources to interrupt oxidative stress in the human body to minimize the risk of chronic disease, as well as reduce the concentration of harmful compounds and acid in feces (21). Researchers pointed out that higher fresh produce consumption levels will possibly reduce the risk of cancer (22, 123, 124). Moreover, research showed that consuming produce on a regular basis can reduce blood cholesterol levels (92), the risk of heart disease (66), and maintain blood pressure (118). The concept behind the connection between consuming produce and the risk of chronic disease is the balance of free radicals and phytochemical antioxidants (77) in the body. As the nutrients from produce are hard to replace with other foods, a balanced diet including fruits and vegetables is an important step in maintaining a healthy lifestyle. The USDA's MyPlate recommends eating approximately 2 cups of fruit and 3 cups of vegetables per person per day to maintain a healthy life (the specific recommendation also depends on gender and age) (80).

Besides the increase in health awareness, urban farming has become more popular in the last ten years in the U.S. with the aims of improving local food security, maximizing the use of vacant spaces, and improving agricultural sustainability. Although, due to the environmental impact, currently limited crops can grow on an urban farm, such as leaf greens (72), the model of

potential productivity of urban agriculture shows that small-scale systems are more productive than commercial farms (126). From 1990 to 2018, the value of fruit and vegetable production in the United States has increased each year (130). According to a report from Grand View Research, Inc., the U.S. fruit and vegetable market will reach approximately \$1.12 trillion, and the demand will be more than 243.2 million tons by 2025 (52). As a result, the rise of urban agriculture accelerated the produce market expansion but also added uncertainty to the produce safety, as many produce growers from urban areas own limited farmland and do not need to comply with the Produce Safety Rule (PSR) (annual monetary sales of a farm below \$25,000). So there is still a need for extension professionals to suggest them follow the Good Agricultural Practices (GAP) or PSR to ensure the produce safety during production.

Foodborne outbreaks related with produce

As the demand for fruits and vegetables increasing, more cases of foodborne disease outbreaks have been linked to produce in the past decades. These have highlighted produce safety concerns to the public. In recent years, food safety challenges have continued in an endless series, and there have even been about 3,000 deaths from foodborne causes annually in the US (131). Produce is one of the key vehicles for the transmission of foodborne pathogens in most parts of the world (78). From 2010 to 2017, a total of 85 multistate outbreaks associated with fresh produce with confirmed etiologies occurred in the United States, resulting in a known combined total of 4,658 known illnesses, 1,187 hospitalizations, and 55 deaths (29). *Escherichia coli* (*E. coli*), *Salmonella enterica* (*S. enterica*), and *Listeria monocytogenes* (*L. monocytogenes*)

are the three bacterial pathogens that caused the most multistate fresh produce outbreaks from 2010 to 2017 (29).

Economic impact of foodborne outbreaks

Foodborne outbreaks not only destroy human health but also lead to a huge economic burden. According to the USDA estimation, each year foodborne illnesses cost more than \$17.6 billion of loss (133). Of note, fresh produce is one of the major causes that is believed to account for 26% of the total economic loss (85). One foodborne disease outbreak can lead to \$6,330 to \$2.1 million in economic loss for a fast-casual restaurant (13). According to FDA rankings, the top most common pathogens that cause foodborne disease are *E. coli*, norovirus, *Salmonella spp.*, *Campylobacter*, Hepatitis A, and *Listeria* (138). Among these pathogens, outbreaks associated with *S. enterica*, *L. monocytogenes*, and *Campylobacter spp.* caused the highest total economic losses (113). In addition, the severity of the illness and the type of bacterial infection affect the amount of economic loss, and the cost also depends on the geographical region of the patient. California, Florida, and Texas were among the states with the highest economic losses per foodborne illness patient recorded in 2013 (113, 114).

At the same time, foodborne diseases will bring high economic losses to consumers and will also lead to economic and reputational losses to produce growers or processors. When certain pathogenic bacteria are detected in food products, all products sold are recalled as required by the U.S. Food & Drug Administration (FDA) (30). In this process, the producer has to pay all the costs associated with the recall, including the extra labor, wasted food, and recall costs. At the same

time, when a portion of food is recalled, the reputation of the food manufacturer will normally be damaged, and consumers often will discriminate against the brand. According to the Grocery Manufacturers Association (GMA), the average food recall costs the company about \$10 million per recall (129). For example, a large quantity of food (including safe produce) was dumped due to public health considerations during the 2018 large produce outbreak caused by contaminated romaine lettuce (32).

Thus, reducing the chances of food being infected by microorganisms and ensuring food safety is what every producer and consumer needs to understand and strive to do.

Contamination Factors on Produce Farms

Pathogen contamination of fresh produce can occur at any time during the production chain, although the likelihood of contamination is elevated during field operations such as species and harvest (48, 78). The introduction of human pathogens can occur via diverse routes, including agricultural water (14, 55) and biological soil amendments of animal origin (BSAAOs) (157). Once pathogens are introduced into the farm environment, they can survive and persist for long periods within the soil or water (26, 45, 88, 90, 122), which can cause subsequent spread to other areas of the fruit and vegetable production environment or even result in product contamination.

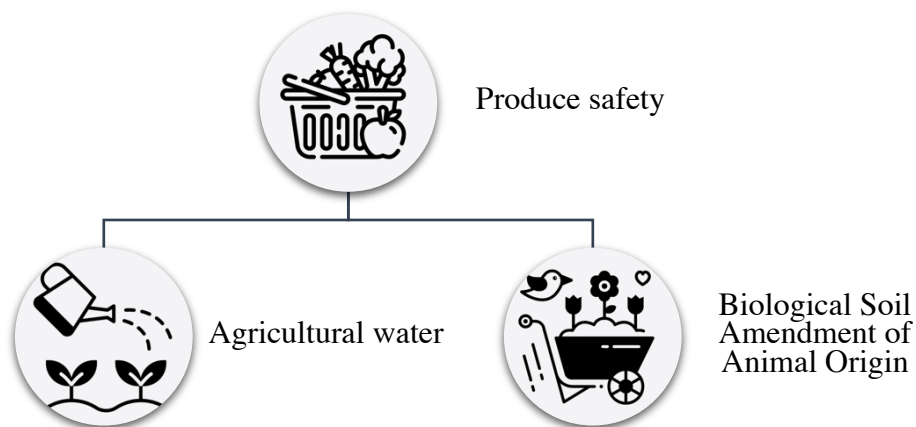


Figure 1.1 The two main contamination routes on a farm that cause produce safety issues.

Agricultural Water

Agricultural water is widely distributed and used in a produce farm which increases the opportunity for human pathogens to attach to the produce. The proximity of produce to contaminated water and the concentration of pathogens within the water are two risk factors contributing to the probability of produce contamination during field production (6). The contamination route always starts with fecal matter carrying human pathogens that drop into the water source (7). Then the transmission of human pathogens can occur at any time during field production (i.e., irrigation, flooding, agricultural runoff) and postharvest activities (washing, rinsing, cooling, etc.).

Biological Soil Amendments of Animal Origin (BSAAOs)

Agricultural soil is another reservoir that can transmit human pathogens to fresh produce. One of the major sources of these human pathogens that survive in the soil is from BSAAOs.

Without proper treatment (i.e., composting), BSAAOs can carry various human pathogens. Once these are applied to soil as fertilizer, they will contaminate the soil soon after. From soil, there are multiple opportunities for human pathogens to attach to the produce. For example, the transmission of human pathogens can occur during the germination of seeds or after the transplantation of seedlings in contaminated soil (28, 71). Therefore, in agricultural applications, it is necessary for growers to detect whether any unexpected animals or improper practices exist before, during, or after field production.

Farm Safety Modernization Act Produce Safety Rule

Overview of the regulation

The US implemented the FDA Food Safety Modernization Act (FSMA) in 2011, which was developed to shift the focus of the nation's food safety system from responding to foodborne illness to minimizing the number of these cases. It was designed to provide clear, specific actions that must be taken throughout the produce handling process to prevent contamination. Nine rules are included in FSMA, which include 1) Third-Party Certification Accreditation; 2) Current Good Manufacturing Practices and Hazard Analysis and Risk-Based Preventive Controls for Human Food and Animal Foods; 3) Foreign Supplier Verification Programs (FSVP); 4) Laboratory Accreditation; 5) Food Traceability; 6) Mitigation Strategies to Protect Food Against Intentional Adulteration; 7) Sanitary Transportation of Human and Animal Food; 8) Voluntary Qualified Importer Program (VQIP); and 9) Standards for the Growing, Harvesting, Packing and Holding of Produce for Human Consumption (10).

This last rule is also known as the Produce Safety Rule (PSR) and the final version was published on November 27th, 2015. It establishes science-based minimum standards for the safe growing, harvesting, packing, and holding of fruits and vegetables grown for human consumption. The compliance dates of PSR criteria are determined by the size of the farm in terms of annual sales and the risk of the commodities becoming a foodborne outbreak when they get contaminated. These include if the farm sells at least \$25,000 of produce annually (adjusted for inflation) and if the produce is likely to be eaten raw (e.g., leafy greens, cucumber, tomato, and most fruits) (137).

Table 1.1 The compliance dates for each rule are based on farm size (very small, small, and other businesses).

Note: ¹ The farm sizes are determined by the average annual monetary value of produce the farm sold during the previous 3-year period. The other businesses (not small) include those selling more than \$500,000 of produce; small size farms include annual monetary sales between \$250,000 to \$500,000; very small includes farms with annual monetary sales between \$25,000 to \$250,000; the annual monetary sales of a farm below \$25,000 are exempted from the PSR. ² The new proposed water requirements are modified based on the final rule which was published December 2nd, 2021 and caused the extended compliance dates. More information on these topics will be mentioned later in this review.

Farm Size¹			
/Compliance Date <i>(134)</i>	Sprouts	Other Produce	Original Water Requirements
Other Businesses (Not Small)	1/26/17	1/26/18	1/27/20
Small	1/26/18	1/28/19	1/26/21
Very Small	1/28/19	1/27/20	1/26/22

Under the PSR, there are six key requirements summarized by FDA as subparts, which include: agricultural water, biological soil amendments, sprouts, domesticated and wild animals, worker training and health and hygiene, and equipment, tools, and buildings (and post-harvest handling?) (135). Of note, maintaining the microbial quality of the water sources used on the farm is of primary importance in produce safety. As water is used in almost every aspect of production, once the water source is contaminated, there will be a higher risk of produce contamination.

Worker Training and Health and Hygiene

Workers are a part of the food safety concerns since they can carry multiple pathogens and spread them unintentionally via multiple routes (58, 65). FDA requires all farm workers who handle covered produce and/or food-contact surfaces and all supervisors to receive food safety training, including the principles of good hygiene and food safety; recognizing symptoms of foodborne illness and the importance of personal hygiene for all personnel and visitors; and other training relevant to the worker's job (137). Of note, water is a key component of these requirements in order to fulfill health and hygiene requirements, such as washing hands and mixing with sanitizer (35). Thus, the microbial quality of the water source which is used for hygiene contributes to the risk of cross-contamination concern on the farm.

Equipment, Tools, and Buildings

The equipment and tools that are used on farms can always directly contact produce. This part of the rule focuses on the standards of sanitation to prevent cross-contamination of produce.

It covers the building structure (i.e., greenhouses, germination chambers, toilets, and hand-washing facilities), the tools, and equipment that may touch or contact the produce. As in the health and hygiene section, water is also required in this part of the rule, especially during cleaning and sanitizing processes. Although the literature shows that the survival time of pathogenic bacteria partially depends on the surrounding environments (i.e., temperature, moisture, the attached surface material) (24, 46, 104), once pathogens are introduced to any equipment, tools, or building floor, the possibility of cross-contamination begins to increase.

Biological Soil Amendments

According to federal regulation § 112.3, biological soil amendments of animal origin (BSAAOs) represent a biological soil amendment that consists, in whole or in part, of materials of animal origin (137). BSAAOs are widely used to improve soil fertility and quality on organic farms and make a primary contribution to agriculture nutrient management (108, 116). However, if the BSAAOs are used improperly, they can lead to very high produce and water contamination risk since they are applied directly next to the produce. Further, pathogens (e.g., *E. coli*, *S. enterica*) have an extended survival length in a warmer and moist environment (91, 97). A scientifically valid controlled process is required from the PSR for treating BSAAOs to minimize the risk of contamination (137). Of note, the PSR and extension fact sheets highlight storage of the biological soil amendments away from water sources since that can cause potential contamination (19, 137).

Sprouts

Agricultural water quality is a key food safety concern in growing sprouts as water is the primary input in sprout production. In past decades, several foodborne outbreaks have been traced back to sprouts (*15, 31, 49, 125*), which have been contaminated by pathogens carried from sprout seeds and run-off water. As sprouts are grown in warm, moist, and nutrient-rich conditions, this is also the perfect environment for pathogens to survive. Unlike in field production systems, once a single pathogen is introduced into the sprouts system, it can easily spread to all the growing sprouts. Moreover, literature shows that because of the favorable growing conditions, sanitizer treatments (i.e., ozone and chlorine dioxide) cannot inactivate pathogen growth (*2*). Of note, water is required in most sprout growth steps. Therefore, cross-contamination can either happen during irrigation of sprout seeds in the field or during irrigating the sprouts during production (*154*). Based on the special growth environment, the PSR has established a separate section for sprout growers, which includes testing any microbes on or in the seeds, sprout irrigation water testing, and further corrective treatments.

Domesticated and Wild Animals

The fecal matter left behind on the field from grazing, working, and wild animals have a potential risk of containing human pathogens. The pathogens from their feces can directly contaminate the surrounding soil and produce (*84*). The PSR addresses this concern and requires taking all measures reasonably necessary to identify and not harvest produce that is likely to be contaminated. At a minimum, all covered farms are required to visually examine the growing

area and all the covered produce to be harvested (137). Furthermore, once any feces are dropped or flushed (i.e., rain and flood) into nearby water, it will contaminate the entire water source with pathogens from the fecal matter (18). This results in the water becoming a primary reservoir for human pathogens at the farm level.

Agricultural water

Water is an important part of agricultural production as well as a primary reservoir for many human pathogens on the farm. Accordingly, agricultural water microbial quality requirements have been written into the PSR. In current microbial water quality testing guidelines, fecal contaminations are determined by the number of generic *E. coli* in 100 mL of water. The presence of any strain of *E. coli* is a strong indication of fecal contamination, but it cannot be used as direct evidence of the presence of a human pathogen (127). In the PSR, generic *E. coli* is only used to measure microbial water quality and give growers information for future water management.



Figure 1.2 Schematic illustration of a worse condition fresh produce farm.

Originally Proposed Microbial Quality Criteria

Of note, during the thesis year (2020-2022), FDA proposed the new modified production water regulation and currently looking for feedback. Thus, this thesis includes both the current written agricultural water regulation and the new proposed production water regulation. To avoid any misleading, please check the current PSR for referencing.

In 2016, the PSR documented its minimum safety standards for agricultural water at that time. According to the rules clearly specified in PSR released in 2016, for surface water, twenty or more samples must be tested over a period of 2 to 4 years, with five or more samples rolled into the profile every year after the initial survey. The 2016 PSR requirements quantify the safety standards for agricultural water used directly on crops (other than sprouts) by using the

geometric mean (GM) and statistical threshold value (STV) of the water test results. The GM is the average of the water testing results and shows the central tendency of the quality of the water (117). The STV reflects the variability in the quality of the water samples over time.

Table 1.2 The standard for water microbial quality (GM and STV) for pre-harvest water included in the 2016 PSR.

	Geometric Mean	Statistical Threshold Value
Number of Generic <i>E. coli</i> (CFU/100mL)	≤ 126	≤ 410

The corrective actions for the water are required no longer than a year if the water testing results exceed the criteria number. During this period, growers can still use time water in some situations to keep flexibility for them (9, 23).

Microbial Water Testing Methods

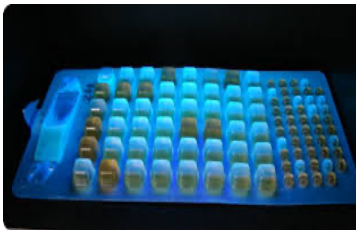
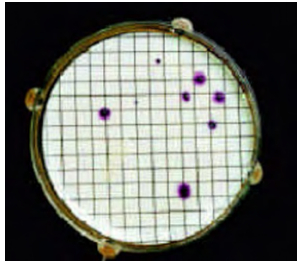
Produce growers need to collect water samples in bottles provided by the testing lab, with a sample size of no less than 100 milliliters. If a groundwater sample is collected, growers should let the water run for five minutes before the sample is collected. If the sample is surface water, keep the sampling bottle close to the center of the water body and try to avoid collecting impurities. After a successful collection, samples must be kept at a low temperature of 4 to 10 °C and sent to a nearby laboratory for analysis within 24 hours (20).

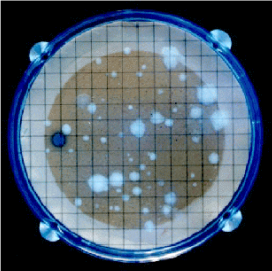
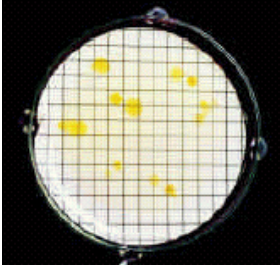
There are two sets of criteria for microbial water quality in the 2016 PSR water requirements that are based on the presence of generic *E. coli* in agriculture water. The microbial water quality for water that is used for post-harvest purposes (including any water that can directly

contact produce surfaces after harvest) must be 0 CFU/100mL, which means no detectable generic *E. coli* are allowed for post-harvest water. The other numerical critical that was proposed in the 2016 PSR water requirements are for water that is applied directly to growing produce (except sprouts). The microbial water quality value for production water in the 2016 PSR water requirements has to be below 126 CFU/100mL GM and 410 STV(132).

Currently, FDA states that there are eight methods considered scientifically valid and at least equivalent in accuracy, precision, and sensitivity in quantifying generic *E. coli* in agriculture water compared to the “EPA? Method 1603: *Escherichia coli* (*E. coli*) in Water by Membrane Filtration Using Modified membrane-Thermotolerant *Escherichia coli* Agar (Modified mTEC) (139)”. Based on the different types of methods, there are two units used in water test results: Colony Forming Unit (CFU) per 100 mL water sample and Most Probable Number (MPN) per 100 mL water sample.

Table 1.3 The eight methods are listed by name, description, result image, and reference.

Name of Method	Method Feature	Example	Reference
IDEXX Colilert/Colilert- 18 Test Kit (unit with MPN)	The Colilert method is used for 4-methylumbelliferyl-β-D-glucuronide (MUG) positive reaction, and the chemical has to be dissolved in the sample water to be tested so that the <i>E. coli</i> in the water can show the fluorescence effect under the ultraviolet light. After adding one packet of Colilert reagent into 100mL of sample water, it is sealed into a quantifying tray. Then it is incubated for 24 hours at 35°C; yellow wells may form to represent if the coliform is present. By checking the amount of blue fluorescent wells in the quanti-tray 2000 under a 6W, 365 nm UV light, the Most Probably Number (MPN) result can be calculated according to the table provided with the Quanti-Tray.		N/A
Method 1603 validated by EPA (unit with CFU)	This is a filtration method that follows the “U.S. EPA Method 1603 Protocol” by growing the <i>E. coli</i> that is left on the membrane in an m-TEC agar incubated for 2 hours at 35 °C and placed in a water bath for 22 hours at 44.5 °C. Counting the pink or purple color colonies formed in the agar provides the final result. This is a test that strictly adheres to FDA regulations; the results are precise and clear but include many disadvantages. The m-TEC agar used in this method is expensive, and it needs to be prepared before starting a water test. The filtration process is required for detection, and the turbidity level of the sample water directly determines the length of time of this process.		Method 1603: <i>Escherichia coli</i> (<i>E. coli</i>) in Water by Membrane Filtration Using Modified membrane-Thermotolerant <i>Escherichia coli</i> Agar (Modified mTEC) (September 2014)

Method 1604 validated by EPA (unit with CFU)	Filter 100mL water sample through a membrane filter and place it on MI agar on an absorbent pad saturated with 2-3 mL of MI broth and incubate at 35 °C for 24 hours. The <i>E. coli</i> will present with a blue color from the breakdown of IBDG by the <i>E. coli</i> enzyme β-glucuronidase; total coliform will fluoresce under longwave UV light from the breakdown of MUGal by the TC enzyme β-galactosidase.		Method 1604: Total Coliforms and <i>E. coli</i> in Water by Membrane Filtration Using a Simultaneous Detection Technique (MI Medium) (September 2002)
Method 1103.1 validated by EPA (unit with CFU)	Filter the water sample through the membrane and place it on the selective and differential medium, mTEC. Incubate at 35°C $\pm$ 0.5°C for 2 $\pm$ 0.5 hours and incubate at 44.5°C $\pm$ 0.2°C for 22 $\pm$ 2 hours. After incubation, transfer the membrane from mTEC agar to the absorbent pad saturated with urea substrate medium for 15 minutes is required for the final step. The color of the <i>E. coli</i> colonies in the final plate should be yellow or yellow-green under a fluorescent lamp.		Method 1103.1: <i>E. coli</i> in Water by Membrane Filtration Using membrane-Thermotolerant Escherichia coli Agar (mTEC) (March 2010)
9213 D validated by SM (unit with CFU)	Filter water sample through a membrane filter and place on mTEC agar. Incubate at 35°C for two h to resuscitate stressed bacteria, then incubate at 44.5°C for 22 h. Then transfer the membrane to a filter pad saturated with urea substrate and containing phenol red for 15 minutes. The result positive for <i>E. coli</i> should be fluorescence under the longwave UV lamp with yellow color.	No image available	Natural Bathing Beaches (2007). In: Standard Methods for the Examination of Water and Wastewater, 22nd Edition (Rice E.W., et al., Ed.), 9-46 – 9-48. Washington, DC: American Public Health Association. (2012).
9222 B validated by SM (unit with CFU)	In this method, the membrane will be placed on les-ENDO or m-ENDO agar which contains basic Fuchsin as the indicator. Filter water sample through a membrane filter and placed on the agar. After incubating the agar for 24 hours at 35 °C, the coliform will show up in red color. Transfer the membrane filter to a nutrient agar that contains MUG and incubate for 4 hours. The individual <i>E. coli</i> colonies will fluorescence under a longwave length UV lamp.	No image available	Standard Total Coliform Membrane Filter Procedure (1997), followed by 9222 G – MF Partition Procedures (1997) using NA-MUG media. In: Standard Methods for the Examination of Water and Wastewater, 21st Edition (Eaton A.D., et al., Ed.), 9-60 – 9-65, and 9-70 – 9-71, respectively. Washington,

**D 5392-93
validated by
ASTM (unit with
CFU)**

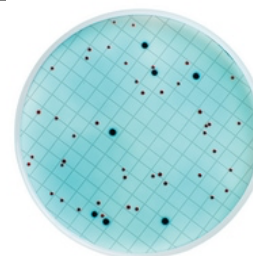
This is a two-step water testing procedure that provides a direct count of the colonies' numbers. Filter sample water through a membrane filter and place it on the selective medium, mTEC. The membrane on the medium is first incubated at 35°C for two h, and then the medium is incubated at 44.5°C for 22 h. Following incubation, the filter is transferred to a filter pad saturated with urea substrate. The yellow or yellow-brown colonies will be present after 15 minutes if the water sample is positive for *E. coli*. Colonies are counted with the aid of a 10 to 15 X magnifier and a fluorescent lamp.

No image available

Standard Test Method for Isolation and Enumeration of *E. coli* in Water by the Two- Step Membrane Filter Procedure. In: Annual Book of ASTM Standards, Volume 11.02. ASTM International. (1996, 1999, 2000).

**Hach Method
10029 for
Coliforms (unit
with CFU)**

This method was published by Hach Company. 100mL of the water sample is filtered through a 0.45-µm membrane filter and placed on the plate, which already added 2mL of the reagent onto a sterile pad on the plate. After incubating for 24 hours at 35 °C, the presence of red colonies indicates the coliforms by reducing the TTC. By hydrolyzing the enzyme-substrate BCIG, it shows blue color for *E. coli* colonies.



Total and *E. coli*, using m-ColiBlue24 Broth PourRite Ampules

Modified Agricultural Water Proposed Rule (proposed in December 2021)

After complying with the water microbial quality criteria for some time, some businesses reported that regulations are difficult to enforce because they are both expansive and complex (144). Thus, in December 2021, FDA revised and published proposed agricultural water requirements (136). The modified rule only impacts the production water regulation but does not change the originally published post-harvest water requirements. Instead of complying with microbial criteria and testing requirements for pre-harvest water (mentioned above), it emphasizes the use of an agricultural water assessment.

An agricultural water assessment is an evaluation that is required to be conducted by covered farms. The factors include agricultural water system(s), agricultural water practices, crop characteristics, environmental conditions, and other relevant factors. Based on the assessment, growers need to determine their water quality and take any actions (i.e., corrective measures, mitigation measures, inspect water system(s), and test water microbial quality) (155). This modification is intended to make it easier for more growers to comply. Large, covered farms were initially required to comply with the water requirements on January 26th, 2022, but FDA is exercising enforcement discretion for the pre-harvest water provisions for all farms while the water requirements are being finalized (as of July 2022).

Agricultural Water Treatment Methods

When the number of generic *E. coli* in the water source has been proved to exceed safe levels or the agricultural water assessment determines the water is not safe for use, corrective

measures are required before resuming the use of the water. Multiple methods can be used to improve the microbial water quality, including filtration, chlorine dioxide, ultraviolet (UV) radiation, and chemical oxidation (i.e., ozone, hydrogen peroxide, and peracetic acid), as shown in Table 1.4. Of note, another agricultural microbial water quality test is required after applying the treatments to confirm that the agricultural water can be used under PSR.

Table 1.4 Log count reduction of pathogens survival in water by different intervention technologies. Note: ZVI means zero-valent iron.

Process	Treatment	Target pathogen	Log reduction	Reference
Filtration	50% ZVI	<i>E. coli</i>	1.7	(67)
	50% sand			
	35% ZVI	<i>E. coli</i>	2.45	(81)
	65% sand			
	35% ZVI	<i>Listeria monocytogenes</i>	2.51	(81)
	65% sand			
	50% ZVI	<i>E. coli</i>	3.54	(8)
	50% sand			
Chlorine dioxide	10 mg/L	<i>E. coli</i>	7	(140)
	3 mg/L	<i>E. coli</i>	5	(57)
	5 ppm	<i>Salmonella</i>	7	(83)
	5 mg/L	<i>Staphylococcus aureus</i>	5	(95)
	1.2 mg/L	<i>Enterococcus faecalis</i>	6.3	(42)
UV	267 nm	<i>E. coli</i>	4	(94)
	275 nm	<i>E. coli</i>	3.6	(94)
	310 nm	<i>E. coli</i>	0.5	(94)

	265 nm	<i>E. coli</i>	4.3	(93)
	275 nm	<i>E. coli</i>	4	(93)
	289 nm	<i>E. coli</i>	2.42	(53)
Ozone	35 mg/L	<i>E. coli</i> O157:H7	3.98	(36)
	45 mg/L	<i>E. coli</i> O157:H7	3.9	(36)
	1 mg/L	<i>E. coli</i>	5	(50)
	1 mg/L	<i>Enterococcus faecalis</i>	6.4	(50)
Hydrogen peroxide	3%	<i>E. coli</i> O157:H7	0.77	(54)
	100 mg/L	<i>E. coli</i>	3.13	(47)
	100 mg/L	<i>Enterococcus faecalis</i>	2.45	(47)
Peracetic acid	8 mg/L	<i>E. coli</i>	3.52	(47)
	8 mg/L	<i>Enterococcus faecalis</i>	4.5	(47)
	3 mg/L	<i>E. coli</i>	3.57	(16)

Filtration

Filtration is a physical process that separates solid particles and liquid from water and reduces the turbidity of the water. The turbidity of water is caused by suspended particles and floating debris, which can provide a protective barrier for microorganisms' survival (147). Thus, reducing the level of turbidity of water before use is important. Filtration systems are widely applied and effective in removing particulate matter, algae, and most microorganisms. Different filtration strategies are used based on the different sizes of target microorganisms (Table 1.5).

Table 1.5 The application for different sizes of filtration strategy.

Filtration methods	Microfiltration Membrane	Ultrafiltration Membrane	Nanofiltration Membrane	Reverse Osmosis
Target Size	approximately 0.1 μm	approximately 0.01 μm	approximately 0.001 μm	<0.0005 μm
Applied for	Sediment and bacterial	Viruses	Microorganisms	Microorganisms

One of the commonly known filtrations is the slow sand filtration (SSF), which lets the water goes through various biological, physical, and chemical processes that finally removes the pathogenic organisms and turbidity effectively. Recently, a great deal of literature highlighted the efficiency and economics of the zero-valent iron (ZVI) bio-sand filtration system for reducing pathogen load in contaminated water (8, 67, 70, 81). Anderson-Coughlin et al. showed that zero-valent iron filtration could significantly reduce the *E. coli* population after going through the filtration system(from 13.13 MPN/ml to 9.59 MPN/ml) as well as shortening the die-off interval of the rest of the bacteria (from -0.48 MPN/day to -1.50 MPN/day) (8).

However, according to the FSMA PSR (21 CFR 112.43) (137), currently, sand-filtration is not considered a validated water treatment method that can be used for removing physical particles and requires a further treatment process to complete the corrective measures. Moreover, the backflush water from the filter can also be problematic as it contains most of the particles from the untreated water. Thus, how to handle the backflush water properly needs to be considered.

Chlorine Dioxide

Chlorine dioxide (ClO_2) is a chemical compound that forms as a gas when the temperature is above 11°C and forms as a liquid when temperatures are between 11°C and -59°C . Due to its chemical properties, it is primarily used for disinfecting drinking water (3). Chlorine dioxide can kill pathogens by breaking the chemical bonds in their molecules and results in pathogen inactivation. Combined chlorine can be found in most sanitizers, such as bleach, and can be used for produce cleaning purposes (59, 75). As shock chlorination is the most cost-effective method of disinfecting water which has been adopted by commercial growers, multiple organizations published shock chlorination guidelines to help growers use it properly (17, 43, 86).

In a lab trial, studies showed chlorine dioxide acted as an effective reagent that can kill more than 99% of plant pathogens in a short time (112). Also, Bel et al. found that 10 mg/L of chlorine can remove 7 logs of *E. coli* rapidly (140). Other research reported a 5 log reduction of *E. coli* populations with 3 mg/L of chlorine dioxide (57).

However, limitations of chlorine dioxide application are also present. It can only work for a short time period within the existence of chlorine dioxide in the water, which cannot be applied as a long-term solution.

Chemical Oxidation

Chemical oxidation is a chemical treatment used to eliminate organic materials in water and wastewater using oxidation, which is both efficient and environmentally friendly. This method has been widely used in the process of purifying and treating wastewater to increase the

use of water (47, 68, 79, 158) as reclaimed wastewater. The reaction of the process is based on a powerful oxidizing agent (e.g., ozone, hydrogen peroxide (H_2O_2), and peracetic acid (PAA)) and obtained at a sufficient concentration.

Ozone disinfection is known as a more effective disinfectant than chlorine against protozoan parasites in water systems (41). Since ozone is highly reactive and unstable, the cost of operation and maintenance are relatively higher than other similar water treatment methods. Hydrogen peroxide is another water treatment method that is environmentally friendly. Several studies show that hydrogen peroxide itself is a slow-acting water disinfectant (requires high dosages and long contact times), but its performance can be enhanced by combining with Cu^{2+} , Ag^+ , and Zn^{2+} (37, 106, 107, 141). Peracetic acid (PAA) is another common and strong oxidant that is applied to disinfect wastewater. It is a man-made chemical that can act against a wide spectrum of pathogens at a low concentration rapidly (12). Kitis et al. ranked the disinfection efficiency of PAA towards microorganisms as bacteria > viruses > bacterial spores > protozoan cysts (68). However, literature also shows the remaining decomposed PAA increases the organic content in the water, which potentially enhances the pathogen regrowth (68, 73, 76). Of note, all the chemical oxidation treatments can be combined with UV and applied in the water treatment process, which results are more efficient than when applied alone (16).

Ultraviolet (UV) Light Disinfection

UV light is a type of electromagnetic (EM) radiation, which has frequencies of about 8×10^{14} to 3×10^{16} hertz (Hz), and wavelengths of about 200 - 400 nanometers (nm). Based on the

wavelength, the UV light can be divided into three sub-bands: UV-A (320-400 nm), UV-B (280-320 nm), and UV-C (200-280 nm). The commercial UV lights for disinfection use UV-C wavelength (typically 254 nm), which is effective against most bacteria, protozoa, and viruses, by acting directly on the DNA of microorganisms and resulting in a germicidal effect (120).

As clearer water has a higher transmission rate, UV light is more effective in treating low turbidity water (more clear, higher transmission) than high turbidity water (UV light gets blocked) (142). Thus, filtration is always recommended, especially for surface water, before running through the UV system. Also, the effectiveness of a UV system depends on the sensitiveness of microorganisms to UV light. Research shows a 280 nm wavelength UV reactor results in a 4 log reduction in *E. coli* (96), while applying the same wavelength to mesophilic bacteria led to a 1.4 log reduction (34). On the other hand, literature shows the pathogen log inactivation does not follow the increase of the UV wavelength. Nyangaresi et al. tested multiple wavelengths of UV-LED on the inactivation of *E. coli* in water, and the 267 nm UV-LED resulted in a 4 log inactivation, whereas the 310 nm wavelength inactivated 0.5 logs of the *E. coli* population (94).

Other in-lab water treatments

There are some water treatments that have not been applied to agricultural water that are more in the experimental stage of research and development and have not been extensively applied commercially on fresh produce farms. (1) Ultrasound technology: The ultrasound system affects objects in a liquid medium by leading to cavitation, and the forces created by it influence the biological system. Thus, ultrasound technology kills microorganisms by enhancing mass

transfer through the biological membranes. Its application range is based on the type of cavitation, which can be broadly divided into transient and stable types. Research shows that it takes five days for ultrasound treatment to reach to 85% reduction (82). Thus, it needs to be applied combined with other biocides, such as chlorine, UV, and electrolysis. According to research, ultrasound treatment improves the effect of chlorination on reducing the bacterial population in raw stream water (102), reaching about a 4 log reduction of fecal coliforms when applied with UV (156).

Electrolysis also can work as a water pretreatment method to reduce microbial contamination. Electrolyzed water (EW) is created by passing an electric current through a dilute saltwater solution in an electrolysis chamber. The process uses a membrane cell to separate an anode and a cathode and produce acidic EW, which contains free chlorine. Thus, EW can be used to reduce the survival of *E. coli*, *Listeria*, and *Salmonella* (51, 64).

Agricultural Soil Treatment Methods

The transmission of human pathogens can occur during the germination of seeds or after the transplantation of seedlings in contaminated soil (28, 71). Also, the internalization of human pathogens, specifically enteric pathogens, through root uptake has been a point of debate due to the inconsistencies among numerous scientific studies (27, 40). Reducing pathogen load in potentially contaminated soil is important, especially in organic farms, which commonly use biological soil amendments of animal origin (BSAAOs) to improve soil fertility and quality (116).

Solarization

Soil solarization is a method of sterilizing soil using solar heating by covering the pre-irrigated soil with transparent plastic sheets, which has the advantages of simple manipulation and low cost. This method has been proven as one of the efficient ways of controlling plant pathogens in soil (121). Recently, using solarization to inactivate human pathogens (i.e., *E. coli* and *Salmonella*) has been confirmed effective both in the field (152) and in a greenhouse (151).

Cover Crops Application

The establishment of cover crops on organic farms is becoming a standard practice to improve soil health and nutrient management. Cover cropping's potential benefits include reduced soil erosion, weed suppression, soilborne and foliar plant disease suppression, reduction of plant-parasitic nematodes, soil improvement, and nutrient management through nitrogen fixation and carbon accumulation (60, 99, 115). Recently, cover crops have gained attention for their ability to suppress soilborne and foliar plant diseases (61, 74, 145); this antimicrobial activity has been attributed to the production of secondary metabolites which protect the plant from microbial invasion (amongst other roles). While research on the antimicrobial activity of cover crops to suppress plant pathogens has been well developed in past decades, studies investigating their antimicrobial activity towards human pathogens in the soil is still limited (109).

Secondary metabolites in cover crops and their antimicrobial activity

The secondary metabolites are the organic molecules that are neither involved with growth nor development but act as a defense mechanism in the plant to respond to the biotic and abiotic stressors (98). In previous literature, secondary metabolites have been widely studied against plant insects (33, 128), plant viruses (153), and plant fungus (63, 148). Until recent years, the antibacterial effect of secondary metabolites in cover crops has been noticed and proved. For example, many species in the *Brassicaceae* family produce glucosinolates which are water-soluble compounds resistant to degradation until they contact the enzyme myrosinase. Then it hydrolyzes them into isothiocyanates (I.C.T.s), nitriles, and other products (56), which inactivate pathogenic bacteria like *Staphylococcus aureus*, *Enterococcus faecalis*, *Listeria monocytogenes*, *E. coli*, and *Salmonella spp.* (4, 110, 111, 143). Flavonoids (i.e., rutin, quercetin, isoorientin) are another large group of secondary metabolites found in species such as buckwheat which inhibit both gram-negative and gram-positive bacterial survival (1, 11, 146). This research brought up the potential of using cover crops as an amendment to inactivate pathogenic bacteria in produce farms.

Extension Education

The USDA National Institute of Food and Agriculture (NIFA) defines agriculture extension as “Extension that provides non-formal education and learning activities to people throughout the country — to farmers and other residents of rural communities as well as to people living in urban areas. It emphasizes taking knowledge gained through research and education and bringing it directly to the people to create positive changes” (89). Agricultural

extension services can originally be traced back to World War I. It served to support war costs by helping farmers increase crop yields by relaying agricultural knowledge. A set of survey data collected in 1980 showed that farmers and all household members had a positive and significant effect after extension education (39). This study emphasized the necessity and significance of agricultural extension education. Nowadays, educators are bridging the gap between new knowledge and regulations from scientific research to growers and aim to solve farms' difficulties and increase the production, food security, and standard of living of farm families. Universities and colleges are also engaging in extension and outreach teams to help local agricultural producers.

In recent years, because PSR criteria require growers to receive training, "PSR training" has become a hot topic in the extension field. In 2010, Cornell University, FDA, and USDA created the Produce Safety Alliance (PSA), which developed a standardized curriculum and nation- wide education to help fresh produce growers to comply with the requirements outlined in the FSMA PSR. According to data from 2013, FDA estimated that 35,029 total produce farms in the U.S. are required to comply with the PSR, which are qualified as very small farms and above (150). In order to fulfill the national training need, in 2016, PSA launched a train-the-trainer program to provide the opportunity for more people to be qualified to deliver the PSA grower training course and to respond to growers' questions during the training sessions. By the end of March 2022, PSA has held 2,332 grower training courses with 46,361 participants and 2,325 people qualified as a trainer in the US (105).

Extension Education Theoretical Framework

In addition to PSA grower courses, extension agents also provide independent sessions on good agricultural practices and general science-based knowledge to both PSR- covered and exempt growers. Inspired by adult and youth education program planning frameworks, the extension education theoretical framework aims to help extension professionals plan effective training. Within the expectation of positive change in growers' behavior after the training, the social marketing theory and the theory of planned behavior are used to develop the extension training sessions. The outcomes will increase the likelihood of growers having behavior changes (25).

Social Marketing Theory

The social marketing theory is adopted from commercial marketing theory and includes the 4P's of marketing, which are the product, price, place, and promotion (149). Instead of the profit motive, like in commercial marketing, the primary goal of social marketing is to achieve a positive behavior change in a population. Kotler and Zaltman et al. defined social marketing as “a social influence technology involving the design, implementation, and control of programs aimed at increasing the acceptability of social ideas or practice in one or more groups of target adopters.” (69). There are two fundamental philosophies in social marketing Theory (62) :

- An organization's objective should be to meet the needs and demands of the customers.
- In order for an exchange to take place, the organization and the customer must engage as nearly equals, with each having something to contribute to the other and each being able

to gain from the transaction.

Since understanding the barriers to behavior change adoption among the target population is the keystone of social marketing theory (87), audience analysis is one of the most important elements of the whole process. The same technique is also applied in the agricultural extension area, known as research-based needs assessment activities. Based on the results from each activity, professionals can implement changes to help farmers overcome the barriers.

Theory of Planned Behavior

The Theory of Planned Behavior (TPB) (5) is an extension of the Theory of Reasoned Action (TRA) (44). It started in 1980 to predict an individual's intention to engage in a behavior at a specific time and place. The principle was designed to understand all activities for which people would exercise self-control. Behavioral intent is a critical component of this model; behavioral intentions are influenced by one's attitude about the probability that the behavior will produce the desired result and one's subjective assessment of the costs and benefits of that outcome (119).

According to the TPB, the behavioral achievement is dependent on both motivation (intention) and skill (behavioral control). It divides attitudes into three categories: behavioral, normative, and control. The TPB is made up of six constructs (attitudes, behavioral intention, subjective norms, social norms, perceived power, and perceived behavioral control) that together reflect a person's actual behavioral control (5).

TPB is considered in extension education to help planners understand the attitudes that growers hold about agriculture practices, the factors that influence their thoughts, and the necessity of measured behavioral intent before and after the training.

Survey Research

Surveys are one of the common measuring instruments to collect feedback and opinions from growers. It enables researchers to be more efficient by employing a sample of respondents to generalize to the entire community (38). The data results can help extension professionals to obtain information to learn the current growers' practices and knowledge. For example, surveys have previously been conducted to assess growers' produce safety knowledge (100, 103) and needs (101) to help them to comply with produce safety best practices.

Typical survey methods include telephone, paper, in-person, and web-based surveys (38). Among these methods, electronic surveys are more adopted in the recent era with the advantage of low cost and fast transmission (38). Of note, due to the COVID situation, public activities were largely canceled due to the concern for public health, so the surveys were largely web-based. Thus, the survey (chapter 3) in the thesis is mainly web-based.

Conclusion and Future Outlook

Soil contamination and improper BSAAOs practice always raise the possibility of cross-contamination, especially on organic farms which commonly use BSAAOs to treat the soil. Although using cover crops to reduce the pathogen load in soil has not been widely studied, the hands-on results demonstrated the potential of cover crops to be used as a sustainable and cost-

effective soil treatment. Further research is needed to compare the efficiency of pathogen reduction among different species of cover crops.

Meanwhile, water will always remain a key component of the food production system. Agricultural water as the primary reservoir of human pathogens is continuously suspected as a transmission route for produce-related foodborne illness outbreaks. One of the current challenges focuses on inappropriate agricultural water practices, including using surface water for postharvest use without validated treatment and misunderstanding some terminologies (i.e., water used for handwashing is considered as postharvest water). These practices might not directly lead to an outbreak but will raise the possibility of cross-contamination. Considering some high-tech agricultural water treatment methods (i.e., UV and chemical oxidation), some of these show promise of pathogen reduction but are not commonly used on farms. Further research and extension education is required to deliver the latest technology to growers.

Another challenge is the latest proposed modifications to the agricultural water rule. Although the new proposed rule is focused on simplifying the production water criteria, growers and extension professionals need more time to adopt the new modified rules after years of education on the current agricultural water regulation. During this transition period between the current version of the rule and the finalized new modified rule, growers may be confused with the requirements and the potential impact of on-farm water practices (i.e., water microbial quality test, agricultural water assessments, and water system inspection). Moreover, the new proposed rule requires growers to determine the water quality by themselves through different

factors. However, this requires growers to have more knowledge of potential agricultural water hazards and good practices. Although the FDA provides an online agricultural water assessment tool to help growers determine the safety of water sources, it still is a barrier for growers located in rural areas that do not have internet.

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Chapter 2 - Evaluating the Efficacy of Cover Crops to Reduce Bacterial Pathogen Load in Contaminated Soil

Abstract

Cover crops are plants seeded before or after cash crops to improve soil health, reduce weed pressure, and prevent erosion. Cover crops also produce various antimicrobial secondary metabolites (i.e., glucosinolates, quercetin), yet the role of cover crops in moderating the population of human pathogens in the soil has rarely been investigated. This study aims to determine the antimicrobial capacity of three cover crop species to reduce the population of pathogenic soil microbiota. Four-week-old mustard greens (*Brassica juncea*, ‘Kodiak’), sunn hemp (*Crotalaria juncea*), and buckwheat (*Fagopyrum esculentum*) were mixed into autoclaved soil and inoculated with 7 log rifampicin-resistant generic *Escherichia coli* (*E. coli*). The surviving microbial populations on days 0, 4, 10, 15, 20, 30, 40 were enumerated. All three cover crops significantly reduced the population of generic *E. coli* compared to control ($p < 0.0001$); buckwheat resulted in the highest reduction (3.92 log CFU/g). An inhibitory effect ($p < 0.0001$) on microbial growth was also observed in soils containing mustard greens and sunn hemp. This study provides evidence for the bactericidal and bacteriostatic effects of particular cover crops. More research regarding the secondary metabolites produced by certain cover crops and their potential as a bio-mitigation strategy to improve on-farm produce safety is warranted.

Introduction

Agricultural soils contain a dynamic microbiome that supports plant health and are home to organisms that produce secondary metabolites (a group of chemical compounds that defend the organisms from biotic and abiotic stress) (35). However, agricultural soils are also a potential route for the transmission of human pathogens to fresh fruits and vegetables. Pathogens like *E. coli* and *Salmonella* are shed in the fecal matter of animals, where they can be spread throughout the field soil by contaminated irrigation water (4, 18) or through weather-related events such as flooding (9, 36). Recent investigations indicate that many of these human pathogens can persist in agricultural soils for up to 100 days (25, 26, 31, 38), increasing the potential for spreading to other produce and to the growing environment. Commodities like watermelons and cantaloupes, in particular, are considered at high risk for contamination from agricultural soils as they are grown on the ground and often make direct contact with soil (8). For this reason, improving agricultural soil management practices is a topic emphasized by federal regulation (e.g., the Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR)) and guidelines for certifications (e.g., Good Agricultural Practices (GAPS) certification).

While such federal regulations and programs offer some preventative measures, there is little information available for growers on strategies to actively reduce the human pathogenic microbial risk of their soils. For example, one of the most pressing concerns in agricultural soil management is the application of biological soil amendments of animal origin (BSAOs), which can carry a high microbial risk since animals serve as the primary reservoir for many human

pathogens (23, 49). The FSMA PSR §112.56 states, “untreated BSAAOs must not be applied in a manner that directly contacts the harvestable portion of the crop and must be applied in a manner that minimizes the potential for contact with covered produce after application” (42). Otherwise, the soil management recommendations currently largely defer to the National Organic Program, which requires growers to apply a die-off interval for raw animal manure application of 120/90 days for edible produce directly/not directly in contact with the soil before harvest (7 CFR 205.203) (41). Of note, soil contamination from BSAAOs is of particular concern for organic produce growing operations, which largely rely on non-chemical (BSAAO-based) methods like manure-based composts to enrich the soil (28). Clearly, there is a need to expand the repertoire of pre-harvest strategies which can reduce the transmission of pathogens from agricultural soils to fresh produce.

Cover crops are typically utilized by growers to improve overall soil agronomic properties but show promise to also reduce the populations of plant and human pathogens in agricultural soils. Cover crops are cultivated between growing seasons to improve soil health and nutrient composition (e.g., carbon, nitrogen) (16, 34), reduce soil erosion (48), and conserve water (43). Plants used as cover crops include grasses (e.g., *Panicum maximum*), legumes (e.g., *Trifolium pretense*), and non-legume broadleaves (e.g., *Fagopyrum esculentum*), which are fast-growing, improve soil physical properties, and may or may not fix nitrogen (7, 12, 14). Recently, cover crops have gained attention for their ability to suppress soilborne and foliar plant diseases (19, 20, 46); this antimicrobial activity has been attributed to the production of secondary

metabolites, which are organic molecules that are not involved with growth or development, but aid in plant response to biotic and abiotic stressors (27). Amongst other roles, secondary metabolites can protect the plant from microbial invasion. For example, many species in the *Brassicaceae* family produce glucosinolates which are hydrolyzed to form cytotoxic compounds that inactivate pathogenic bacteria like *Staphylococcus aureus*, *Enterococcus faecalis*, *Listeria monocytogenes*, *E. coli*, and *Salmonella spp.* (2, 32, 33, 44). Flavonoids (i.e., rutin, quercetin, isoorientin) are another large group of secondary metabolites found in species such as buckwheat which inhibit both gram-negative and gram-positive bacterial survival (1, 3, 47). While research on the antimicrobial activity of cover crops to suppress plant pathogens has been well developed in past decades, studies investigating their antimicrobial activity towards human pathogens in the soil is limited.

A recent study by Reed-Jones et al. focused on the effects of cover crops (cereal rye, hairy vetch, crimson clover, and hairy vetch-rye) on long-term population variation of *E. coli* and *L. innocua* in the soil (29). The study found a suppressing effect of cover crops on *E. coli* and *L. innocua* based on the microorganisms' innate characteristics and soil type (i.e., loamy, clay). The authors also highlighted how soil temperature, tillage practices, and the weather could affect the bacterial population. While the authors observed potential microbial growth inhibition in fields containing cover crops, they raised concerns that the root exudates of living cover crops into the rhizosphere could also promote the growth of human pathogenic bacteria in the soil.

The objective of this study was to determine if there were antimicrobial capabilities of three common cover crops – mustard greens, buckwheat, and sunn hemp – in field soil inoculated with generic *E. coli*. This study will not only provide information on the feasibility of the three cover crops as a soil bio-mitigation method but also the effect of these crops on microbial survival and inform laboratory-based methodologies for such studies.

Material and Methods

Growing Conditions and Plant Biomass Harvesting

The experimental mustard greens (*Brassica juncea*, ‘Kodiak’) (True Leaf Market, Salt Lake City, UT, USA), sunn hemp (*Crotalaria juncea*) (Sow True Seed, Asheville, NC, USA), and buckwheat (*Fagopyrum esculentum*) (Albert Lea Seed Company, Albert Lea, MN) were grown in a greenhouse at the Olathe Horticulture Research and Extension Centre (OHREC) in Olathe, Kansas during the fall of 2021. Mustard greens and buckwheat were sown in large containers (229 cm × 73 cm × 10 cm). Sunn hemp was grown in 9.5-L nursery containers to account for their more expansive root system and larger overall biomass. The containers were filled with organic Plant Pro™ potting soil (Ohio Earth Food, Hartville, OH, USA) before seeding. The greenhouse was climate-controlled, with temperatures ranging from 12.2 °C during the night to 28.9 °C during the day. Overhead irrigation was applied to the crops every other day in order to provide ample soil moisture

Whole crops (including the underground biomass) were harvested at the flowering stage before producing seeds (27 days after propagation). The crops were gently removed from the

containers to avoid excess root loss, and the soil was removed. The plant material was then collected and immediately transported to the lab for further processing, which was performed on the same day.



Figure 2.1 The growing environment for buckwheat and mustard greens.

Soil preparation

The soil used for *E. coli* inoculation in this study was a chase silt loam (40) collected from a field at the OHREC, which had been in perennial grass for the past three years. The soil was collected from the field and transported to the lab. Prior to sterilization, the collected soil was separated into autoclave bags (Fisher Scientific, Waltham, MA, USA); large clumps of sediment were manually pulverized to facilitate handling after sterilization. The soil was then autoclaved at 121.0 °C and 15 psi for one hour; sterility was confirmed using a bioindicator (Fisher

Scientific, Waltham, MA, USA) placed in the center of the bagged soil. Flame-sterilized tongs were used to extract the bioindicator, and the soil was set aside to cool to room temperature before use in the study.

Bacterial strain

Plant roots and shoots are naturally colonized by a dynamic population of microbes that interact amongst themselves and the plant tissues (30). To avoid background interactions from non-target organisms in the phytobiomass, the soil used in the study was autoclaved first, and rifampicin-resistant generic *E. coli* (ATCC 25922) was acquired from the Food Safety Lab at Kansas State Olathe was used as the study microorganism. The strain was previously stored with 50% glycerol (Fisher Scientific, Waltham, MA, USA) in 1.5mL microcentrifugation tubes (Eppendorf North America, Enfield, CT, USA) at -80.0 °C. To initially propagate the bacteria, a loopful of frozen culture was transferred to a culture tube containing 10 mL tryptic soy broth (TSB; Remel Inc., San Diego, CA, USA) and incubated for 24 h at 35.0 °C.

The methodology of Bhullar et al. (6) was followed to induce rifampicin resistance. Briefly, 0.1 mL of the forementioned microbial culture was spread to a plate of tryptic soy agar (TSA; Remel Inc., San Diego, CA, USA) containing 20 µg/mL rifampicin (RIF; Fisher Scientific, Waltham, MA, USA); the TSA-RIF plate was then incubated for 24 h at 35.0 °C. The procedure was repeated using TSA-RIF plates with increasing concentrations of rifampicin (20, 40, 60 µg/mL) until a strain resistant to 80 µg/mL concentrations of rifampicin was generated on TSA-RIF plates.

To culture, the organism for experimentation, one colony of rifampicin-resistant *E. coli* was transferred to 10 mL of TSB containing 80 $\mu\text{g/mL}$ RIF (henceforth noted as TSB-RIF). The tube was incubated for 24 h at 35 °C and sub-cultured again in 30 mL of TSB-RIF. To prepare the cells for experimentation, the cells were washed twice with phosphate buffer saline (PBS; Fisher Scientific, Waltham, MA, USA) with centrifugation (Allegra X-30, Beckman-Coulter, Brea, CA, USA; 10,000 rpm, 10 min, 20.0 °C) occurring prior to and in between each wash. The final volume was adjusted to a minimum concentration of 7 log CFU/mL.

Sample Preparation and Inoculation

This study utilized a randomized complete block design with repeated measures. Three independent replications of each treatment (cover crop species) were performed. To mitigate the impact of the ‘rhizosphere effect’ and address food safety concerns raised by previous literature (29), we used chopped whole-plant biomass for this study. The whole-plant material (containing roots, shoots, and leaves) was chopped into 3-cm long pieces to imitate mowing and tilling and was manually homogenized in three large containers (one container per cover crop species). To simulate the soil-cover crop mixture, 200 g of the chopped plant tissue sample, 200 g of the sterile soil sample, and 75 mL of inoculum culture were combined in a 24 oz. Whirl-Pak bag (Whirl-Pak, Madison, WI, USA). The 1:1 ratio of cover crops and soil is based on the assumption that cover crops will only be incorporated into the upper 10 cm of soil. For the non-treated control, 400 g of the sterile soil sample and 75 mL of inoculum culture were combined. The ratio of the solid matter and inoculum was based on preliminary trials to ensure all solid

matter would be moistened by the inoculum. Each sample bag was shaken and massaged 50 times to homogenize the mixture before being transferred into a quart-sized container (1.4 L) with a plastic plant saucer (Mudeela, Seattle, WA, USA) placed underneath. The samples were stored at room temperature (average 20.6 ± 0.02 °C) and with an average relative humidity of $32.9 \pm 0.4\%$ for 40 days. An average of 30 mL of sterile DI (deionized) water was added to each container each week to prevent the soil from becoming too dry to facilitate bacterial survival.

Sample Collection and Microbial Enumeration

Before collecting the sample on each sampling day, each container was mixed using a sterile plastic spoon; the spoons were sterilized by soaking overnight in 10% bleach, followed by rinsing with distilled lab water. A 10-gram sample was collected from each container and transferred to a Whirl-Pak bag containing 90 mL of 0.1% buffered peptone water (BPW; Remel Inc., San Diego, CA, USA). The sample bag was manually massaged to homogenize the sample. Serial dilutions were prepared in 0.1% BPW, and 100 μ L of the sample solution was then spread-plated onto TSA-RIF before incubating for 24 h at 35 °C. Plates yielding 25-250 colonies were recorded for analysis. Samples were collected 0, 4, 10, 15, 20, 30, and 40 days after treatment.

Statistical analysis

The statistical treatment structure utilized was a one-way ANOVA. The whole study was evaluated using three blocks of four pots each on days 0, 10, 20, 30, and 40. The surviving bacterial population at each time point was subjected to log transformation in order to achieve the variance homogeneity assumption.

The bacteriostatic (phase I) rate was calculated based on the difference of log-transformed surviving bacterial populations between day 0 and day 10. The bactericidal (phase II) rate was calculated based on the difference in log-transformed surviving bacterial populations between day 10 and day 40. The bacteriostatic (phase I) and bactericidal (phase II) rates were analyzed separately using the linear mixed model. Fixed effects of the model included all treatments. Random effects were the block and the error term. The main effect per day was assessed via least squares means (LSMs) and their standard errors (SEMs). Pairwise comparisons were carried out using the two-sided test. The Holm-Tukey method was used to adjust for multiplicity. All tests were conducted at the 0.05 significance level. Statistical analysis was executed via Statistical Analysis Software (SAS version 9.4; Cary, NC) PROC MIXED with option DDFM=BETWITHIN in the MODEL statement.

Results and Discussion

Overall trends in cover crops microbial reduction

Overall, the data showed that certain cover crops could reduce the microbial risk of soils containing generic *E. coli* within 30 to 40 days following a simulated cover crop incorporation event (*Figure 2*). This microbial reduction was cover crop- ($p < 0.0001$) and time-dependent ($p < 0.0001$). By day 30, soils amended with buckwheat or mustard greens had achieved a maximum *E. coli* reduction of 3.92 ± 0.16 log CFU/g and 3.77 ± 0.16 log CFU/g, respectively. For soils containing macerated sunn hemp, *E. coli* was still detectable on day 30, only reaching a maximum microbial reduction of 3.70 ± 0.10 log CFU/g 40 days after treatment.

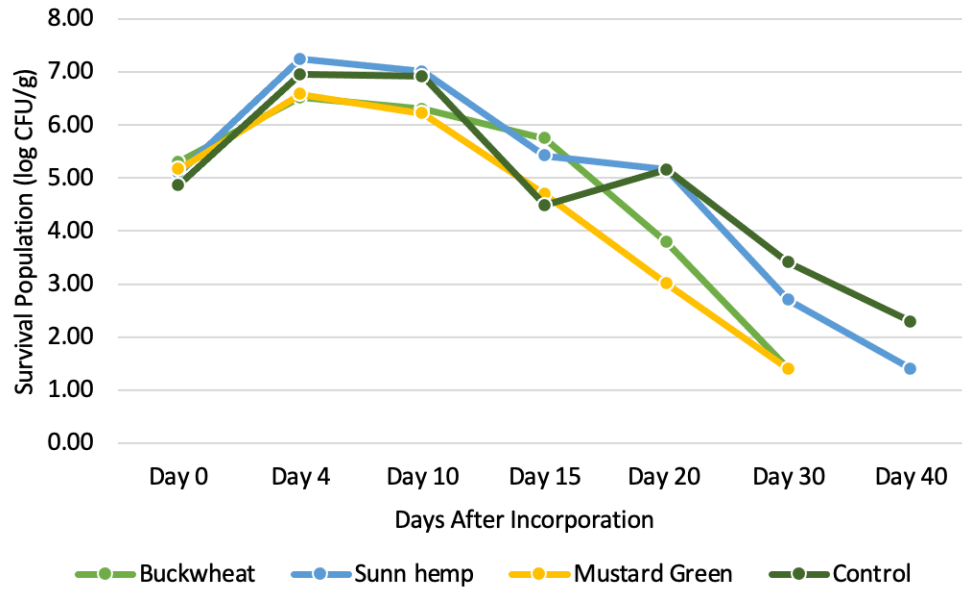


Figure 2.2 The surviving population of rifampicin-resistant *E. coli* in agricultural soil amended with macerated control, buckwheat, sunn hemp, or mustard greens

These overall findings provide potential insight into how these cover crops may be utilized to reduce human microbial risk and thus be used as a soil bio-mitigation strategy. For example, the persistence of *E. coli* in soils amended with sunn hemp could indicate that the secondary metabolites of buckwheat and mustard green tissues are more rapid in inducing microbial cytotoxicity compared to those in sunn hemp tissues. This observation is consistent with previous literature, which reports the cytotoxic activity of sunn hemp is predominantly in seeds rather than in root/shoot tissues (11, 24). In general, there were also no differences in *E. coli* survival between soils containing mustard greens or buckwheat throughout this study, despite their previously- documented different secondary metabolite composition and volume (5, 21).

A biphasic microbial variation curve (see Figure 2) was observed in the experiment, wherein statistically significant interaction effects ($p < 0.0001$) indicate unequal effects of the treatments

over time. The first phase (days 0 to 10) is characterized by a significant increase in the *E. coli* populations amongst all the treatments prior to their decline. From day 10 on, the microbial populations experienced a sharp decline until reaching levels below the detection limit. Topp et al. (39) reported observing a similar microbial growth curve and attributed the variation to the soil type and *E. coli* strains, amongst other factors. Of note, this previous study did not explore microbial survival in a chase silt loam as was utilized in this study, which is characterized by more than 50% silt and 12-27% clay (13), and is a soil composition (theoretically) more likely to have negligible influences on *E. coli* growth due to the relatively aerobic environment of low clay-content soils (37).

Modeling the surviving microbial population to study the biphasic survival curve

To further investigate the differences in microbial growth, the surviving microbial population was statistically modeled by fitting its survival to a nonlinear curve (*Figure 3; Equation 1*). The values used in the equation specific to each cover crop and the control are presented in *Table 1*. Overall, the models were able to explain a high degree (91% to 98%) of the observed variability in the microbial survival of each treatment.

$$y = \frac{x \cdot a + b}{1 + c \cdot x^d} \quad (1)$$

Equation 1, Where *x* is the days after initial inoculation

y is the total surviving microbial population

a is the rate of increase to the maximum peak

b is the multiplier that increases the height of the peak

c is the rate of decrease with respect to days after initial inoculation

d is the peak shape parameter

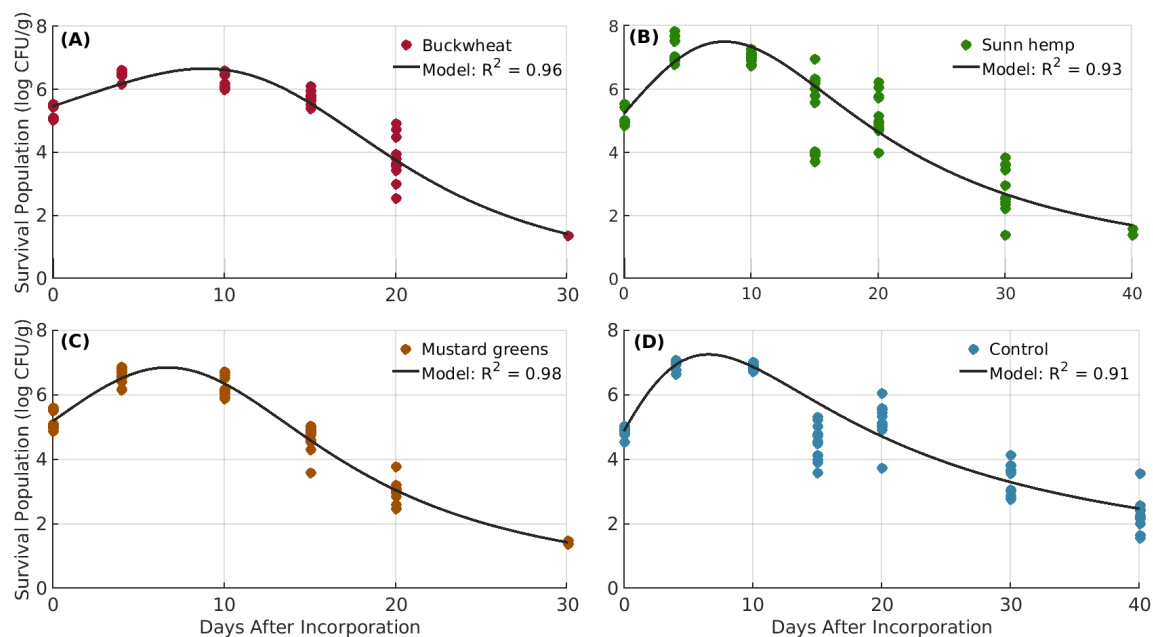


Figure 2.3 The fitted model estimating the surviving population of rifampicin-resistant *E. coli* in field soil supplemented with macerated control, buckwheat, sunn hemp, and mustard greens.

Table 2.1 Variable values by cover crops and control models.

	a	b	c	d	% Variance (R^2)
Control	0.7149	4.875	0.006.989	2.031	91
Buckwheat	0.1827	5.473	0.00001.497	3.827	96
Mustard greens	0.3761	5.184	0.0004.511	2.957	98
Sunn hemp	0.4654	5.227	0.0008.272	2.621	93

Bacteriostatic effects of cover crops during the exponential growth of *E. coli*

From days 0 to 4, there was a significant increase ($p < 0.05$) in the *E. coli* population in all treatments compared to their initial concentration. As evident by the value of a variable (Table 1), the control soils experienced a more rapid rate of increase to the maximum surviving population than the soils containing macerated cover crop tissue. Between the cover crops, sunn hemp showed a more rapid microbial population growth rate than buckwheat ($p < 0.0001$) or mustard greens ($p < 0.0001$). There were no significant differences between the initial growth rate (a) of the buckwheat and mustard greens ($p = 0.6178$) or the sunn hemp and control ($p = 0.2278$). After day 4, the soils containing macerated buckwheat ($p = 0.0021$), mustard greens ($p < 0.0001$) or sunn hemp ($p = 0.0010$) began experiencing a minor decrease in generic *E. coli* populations. In contrast, the microbial population of the control sample attenuated from days 4 to 10 as there were no statistically significant differences ($p = 0.6927$) detected during this time period.

These findings indicate that the exudated phytochemicals of all three macerated cover crops can impair the growth of *E. coli* during this initial growth phase, with buckwheat and mustard greens being the most effective. It is also likely that these results are reflective of an initial exudation of phytochemicals from the macerated cover crops stimulated by the artificial tillage. Divekar et al. note that many secondary metabolic production pathways are rapidly induced by physical stimuli (e.g., cutting of the plants) (15). Accordingly, using a rotary tiller or similar tool

to physically cut the cover crops (instead of only rotating them into the soil) may be critical to initiating their antimicrobial effect.

Bactericidal effects of cover crops during declining *E. coli* population

During the second phase (days 10 to 40), there was a more rapid, significant decrease ($p < 0.0001$) in the *E. coli* population in all treatments compared to their concentration on day 10. In this phase, the *E. coli* could be experiencing die-off due to limited nutrients available in the environment which is accelerated by the presence of cover crop tissues. As evidenced by the value of the c variable (Table 1), the control soil and soils containing macerated sunn hemp showed a slower microbial reduction rate than buckwheat or mustard greens ($p < 0.0001$). Buckwheat experienced the most rapid decline, but there were no significant differences detected between the microbial population of mustard greens and buckwheat ($p = 0.5626$).

The most rapid microbial decline for soil containing macerated sunn hemp occurred between days 20 to 30 which led to a 2.46 ± 0.15 log reduction of generic *E. coli*. Buckwheat also experienced the most rapid population decline (2.40 ± 0.15 log CFU/g) between days 20 and 30. Conversely, soils containing macerated mustard greens exhibited a microbial decline of 1.68 ± 0.26 log CFU/g between day 10 and day 20. There were no significant differences in the surviving population of *E. coli* between mustard greens and buckwheat except on day 20.

Interestingly, the results of this bactericidal phase do not necessarily align with the expectations from the literature. During this phase, it is conceivable that the more rapid decline is due to the cover crops beginning to degrade in the soils, further releasing exudates containing

anti-microbial secondary metabolites (10). Based on the degradation rate, sunn hemp should have the most rapid rate of degradation as the tissues decompose within two weeks post tillage (45). According to Wang et al., the phenolic compounds in buckwheat tissues typically degrade after 37 days, indicating a lower rate of metabolite exudation (47). As this study reports trends in antimicrobial activity which are opposite to what is expected (sunn hemp has less effective microbial reduction rate than buckwheat throughout the study), more investigation regarding the exudation and degradation of the principal secondary metabolites within the tissues of each cover crop is warranted.

Study Limitations

Though the data from this study fit the conventional assumptions for ANOVA, the statistical methodology needed to account for differences in the initial *E. coli* concentration between the control and cover crop-containing soils on day 0 (*Figure 1*). Accordingly, only inferences between the rate(s) of population growth/decline in each cover crop could be made. We believe this significant difference in *E. coli* populations on day 0 can be ascribed to the different compositions of the control samples. As no plant tissues were added to the control containers, 400g of farm soil was used as the control and was inoculated with the same volume of inoculum as other treatments. According to Fick's diffusion law, the diffusive flux goes from areas of high osmolyte concentration to areas of low osmolyte concentration (17). The lower moisture content of the control soils relative to the higher moisture content of the soils containing fresh cover crop tissues likely resulted in a more even distribution of the inoculum in the control soils. Thus,

when collecting the same weight of sample from each treatment, the control group resulted in a diluted generic *E. coli* population result.

The sole use of a gram-negative experimental pathogen presents another limitation of this study. This study evaluated the survival of non-pathogenic *E. coli* as a surrogate for pathogenic *E. coli* or other gram-negative species that may behave in a similar manner (i.e., *Salmonella* spp.), which are commonly found in the gut of wild and domestic animals. However, Locatelli et al. (22) reported higher stability and persistence of gram-positive bacteria (i.e., *Listeria monocytogenes*) in agricultural soils. When Neiunna et al. (29) inoculated cover crop-containing soils with *Listeria innocua*, the authors observed a lower antimicrobial efficacy against the pathogen compared to the gram-negative organisms. This reduced efficacy towards gram-positive organisms may not impact the application of cover crops in states dominated by cattle or poultry production, which have a higher risk of detecting gram-negative *E. coli* and *Salmonella* spp. in the soil. However, this could limit the applicability of the tested cover crops as a microbial risk mediation strategy in operations that struggle with high-risk sources of gram-positive organisms.

Conclusions

All three cover crop species resulted in a microbial reduction to non-detectable levels, while buckwheat and mustard greens also displayed a bacteriostatic effect on the growth of *E. coli* during the exponential microbial growth phase (days 0-10). Future studies should account for extrinsic and intrinsic environmental factors to provide more suggestions for the on-farm

applicability of cover crops as a bio-mitigation strategy. Factors like sunlight, temperature variation, and native soil microbiome interactions were eliminated in this experiment in order to determine if there was an effect of the cover crops on microbial populations. Moreover, the differential efficacy of the cover crops demonstrates the need for characterizing the mechanisms that drive the effects we observed in each of the different cover crop species, particularly including the secondary metabolite content and concentration within the plant tissues. A better understanding is needed of their corresponding environmental stability and exudation patterns as the crop decomposes into the soil. Overall, this is the first lab-trial study for human pathogen reduction on agricultural soil within cover crops amendments. The results demonstrated these cover crops as a promising, environmentally sustainable bio-mitigation strategy for human pathogens in soil that warrant more investigation to promote their use in produce growing operations.

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Chapter 3 - Knowledge and Current Practices Related to Agricultural Water Microbial Quality among Kansas and Missouri Produce Growers

Abstract

Kansas State University and University of Missouri Extension educators have been providing training and information on agricultural water microbial quality to help produce growers reduce risk. However, we recognized the need to determine knowledge gaps among Kansas and Missouri growers related to agricultural water quality and best practices. A survey was developed to determine future extension outputs and activities to encourage growers to improve their practices related to water quality. The survey was distributed to Kansas and Missouri produce growers attending in-person or online produce safety-related events in late 2020 and early 2021 and was also distributed through email lists of produce growers from both states. Survey results ($n = 101$) indicate that 13.9% of the respondents tested their water for generic *Escherichia coli* (*E. coli*) more than once a year, while 38.6% of the participants have never tested their water. Approximately half (59.3%) of respondents indicated they use municipal water for postharvest uses, while 6.7% indicated the use of untreated surface water for postharvest activities. Due to the concern of potential water contamination risks, researchers suggest that further training and educational resources would help growers improve practices related to water quality and produce safety.

Introduction

In recent years, foodborne disease outbreaks resulting from fresh produce have continued to occur (4, 12). Because these commodities are often consumed raw (26, 32), it is imperative that

fresh produce growers use the safest practices possible. From 2010 to 2017, a total of 85 multistate outbreaks associated with fresh produce with confirmed etiologies occurred in the United States, resulting in a known combined total of 4,658 known illnesses, 1,187 hospitalizations, and 55 deaths (4). Further, before 2015, numerous fresh produce outbreaks have been attributed to contaminated agricultural water in commodities such as leafy greens (15, 22), tomatoes (2), and melons (33). Thus, water quality management practices became a major and critical aspect of the Food and Drug Administration's (FDA) Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR) (31).

The PSR, which was finalized in 2015 to take its place as one of the seven foundational rules under FSMA, is the first U.S. Federal regulation to have established science-based minimum standards for the safe growing, harvesting, packing, and holding of fruits and vegetables grown for human consumption. In December 2021, the FDA proposed a revision to Subpart E of the PSR that would change the preharvest agricultural water requirements for covered produce, other than sprouts (30). The FSMA PSR and the proposed revision state the considerations and requirements related to agricultural water management to improve the microbial safety of agricultural water and therefore of produce. In December 2021, FDA announced their intent to exercise enforcement discretion and also act to extend the compliance date for the agricultural water (31).

As per the FSMA PSR, "agricultural water means water used in covered activities on covered produce where water is intended to, or is likely to, contact covered produce or food contact surfaces." Agricultural water is classified into two categories based on the different usage periods: production water and postharvest water. Production water refers to water used during growing activities whereas postharvest water includes all uses of water during or after harvest (7,

31). Since the use of postharvest water is so close to consumption, microbial water quality is especially important (6). Thus, produce growers must meet the requirements of §112.44(a), i.e., no detectable generic *E. coli*/100mL water sample for water used postharvest. The proposed revisions to Subpart E focus on production (i.e., preharvest) uses of agricultural water. These revisions emphasize conducting agricultural water assessments for production water sources and uses, eliminating the federal requirement of an ongoing microbial water quality profile with periodic updates through water quality monitoring.

Each year, Kansas and Missouri growers produce approximately \$26 million (13) and \$81 million (7), respectively, of fruits and vegetables. Kansas State University and University of Missouri Extension educators have been providing training and information on agricultural water microbial quality, based on current and newly proposed FSMA PSR agricultural water requirements, to help produce growers from Kansas and Missouri reduce risk to produce from agricultural water. Although research shows that knowledge gain alone leads to limited behavior change, it can provide people with more informed choices that may or may not lead to behavior change (16). Pratt and Bowman (21) suggest that individually-relevant, problem-focused, and hands-on activities can help extension educators prompt personal behavior change. To help prompt this positive behavior change, educators need a better understanding of the gap between water quality best practices and produce growers' knowledge and actions related to agricultural microbial water quality.

Survey research (20) is one method that allows extension educators to easily understand a large number of growers' current water practices and knowledge gaps in a shorter time. Other surveys have previously been conducted to assess growers' general produce safety knowledge (17, 19) and needs (18) to help them to comply with produce safety best practices. Perry et al.

(17) found that North Central US produce growers have a knowledge gap in understanding agricultural water handling practices compared with other topics of the FSMA PSR, which highlights the needs for food safety extension educators to focus on delivering information on agricultural water best practices to produce growers.

This survey aims to determine gaps between safe water management practices (as outlined in the FSMA PSR requirements/ proposed requirements and other produce safety best practices) and (i) the current knowledge and (ii) the current practices of produce growers from Kansas and Missouri related to agricultural water quality. This will enable extension educators to develop additional training and resources on agricultural microbial water quality accordingly to better meet the needs of growers.

Materials and Methods

The project team developed a 14-question survey instrument based on similar surveys (18). Before implementing the survey, 4 extension experts reviewed the survey questions for face and content validity. The Kansas State University Produce Safety Extension Associate also performed cognitive interviews (14) with growers from the target population to further validate the survey. The Kansas State University Human Research Institutional Review Board (IRB) approved the use of the survey. The survey (*Appendix 1*) included questions on the growers' knowledge and practices related to microbial water quality, which was administered from October 2020 to August 2021. Due to in-person meeting restrictions related to the COVID-19 pandemic, produce growers mainly completed the survey online in Qualtrics ($n = 76$) during online produce safety-related trainings or by distribution of the online survey link to email listservs of produce growers in Kansas and Missouri. Other respondents ($n = 30$) completed paper copies of the survey during an in-person horticulture farm field day or in-person produce

safety training sessions. Two drawings for gift cards were utilized to incentivize participation in the survey both on-line and in-person. To optimize data collection, potential survey respondents were asked to only complete the survey if they were able to answer questions related to their operations' water management practices. Because most produce farms in Kansas and Missouri have only a few people working on them (28, 29), we are confident that most people filling out the survey are actively involved in the water management practices on their farm, and thus qualified to complete the survey.

The 14-question survey instrument included multiple choice questions with some open text response options designed to understand growers' knowledge and practices related to agricultural water use in both production and postharvest stages, as well as their understanding of the microbial quality of agricultural water. Questions included grower age, farm location, water testing frequency, and water usage for both production and postharvest activities.

The data were analyzed in Qualtrics using the cross-tabulation (crosstab) analysis method (10, 11) (also known as contingency table analysis) to identify the growers' knowledge of microbial risks related to different water sources and how they handle those risks. Cross-tabulation analysis compares the results of different questions from the same respondent to gain more insight into grower water quality management behaviors based on the risk of their water source. For example, growers who indicated that they did not treat their water source but indicated that they used a municipal water source (which is treated by a third-party company) were considered as having a safe water management practice. The responses from questions related to production or postharvest water uses, treatment application status, and microbial water quality test frequency were classified by use of different water sources (surface, ground, and municipal water). The data from any questions that allowed respondents to provide an open-

ended response were added to existing responses or a new category of responses was added to the question, based on the keywords in those responses. For example, a separate category was created for growers who wrote they used water from a rain barrel when selecting ‘other’ water for their water source.

Growers were asked if they were GAP (Good Agricultural Practices) certified (rather than if they were covered by the FSMA PSR) because the project team felt that growers would be more comfortable responding to the question of GAP certification, rather than FSMA PSR coverage. Accordingly, responses to the questions about water testing and water treatment were stratified based on their GAP certification status. Although GAP certification is generally a produce buyer requirement and is not a regulatory requirement, many growers view certification as an opportunity to improve their likelihood of compliance with the PSR. Thus, many growers covered by the PSR also obtain GAP certification.

Because some respondents did not answer all of the survey questions, the total number of responses to each question related to water sources in the cross-tab analysis may be different. Additionally, the total number of responses for questions regarding agricultural water source use could differ as growers may have reported using more than one water sources for one or more pre- or postharvest activities. To reduce item non-response bias, surveys having less than two-thirds of the questions completed were excluded from the final data analysis.

Results and Discussion

Results

A total of 106 survey responses were received. Of the 103 responses included in the final data analysis, 51 (49.5%) were from Kansas, 50 (48.5%) were from Missouri, one (1.0%) was marked as unknown, and one (1.0%) did not complete the question. The age range of growers

completing the survey were as follows: 18-29 ($n = 18$, 17.5%), 30-39 ($n = 15$, 14.6%), 40-49 ($n = 26$, 25.2%), 50-59 ($n = 19$, 18.5%), and above 60 ($n = 25$, 24.3%) years old. Most respondents ($n = 90$) were currently selling or planning to sell their produce to others. Most respondents ($n = 87$) were currently not GAP certified, as shown in Table 3.1.

Table 3.1 Reported microbial water quality testing ($n = 100$) status and GAP certification status ($n = 101$) by state.

	Kansas	Missouri	Total
Have tested water	16 (31.4%)	27 (55.1%)	43
Never tested water	25 (49.0%)	13 (26.5%)	38
Use third party test results	10 (19.6%)	9 (18.4%)	19
Total	51	49	100
GAP certified	2 (3.9%)	12 (24.0%)	14
Working towards being GAP certified in the future	7 (13.7%)	8 (16.0%)	15
Not GAP certified	42 (82.4%)	30 (60.0%)	72
Total	51	50	101

Of the participants which indicated they are currently growing fresh produce ($n = 101$), participants indicated their frequency of generic *E. coli* testing as more than once a year ($n = 14$, 13.9%), once a year ($n = 11$, 10.9%), 2-3 times in the last ten years ($n = 11$, 10.9%), once in last ten years ($n = 7$, 6.9%), never tested ($n = 39$, 38.6%), or that a third-party water source tests their water ($n = 19$, 18.8%). Between the two states (Table 3.1), 31.4% ($n = 16$) of Kansas growers said that they have tested their water in the past ten years compared to 55.1% ($n = 27$) of Missouri growers. Of the growers indicating they are GAP certified ($n = 14$), 10 (71.4%)

reported testing their water more than once a year and six (46.2%) reported that they treat their postharvest water sources. Of the non-GAP certified growers ($n = 87$), four (4.6%) growers test their water more than once a year and 15 (17.4%) growers treat their postharvest water sources (Table 3.2, Table 3.3). Because the questions inquiring about the type of water source used for production and postharvest applications allowed for more than one response, the total number of responses varied by question, as shown in *Figure 1*.

Table 3.2 The frequency of microbial water quality testing by GAP certification status (n = 101).

	More than once a year	Once a year	2–3 times in last 10 years	Once in last 10 years	Never test	Third party water test	Total
GAP certified	10 (71.4%)	0 (0.0%)	1 (7.1%)	1 (7.1%)	0 (0.0%)	2 (14.3%)	14
Working towards being							
GAP certified in the	1 (6.7%)	3 (20.0%)	1 (6.7%)	0 (0.0%)	8 (53.3%)	2 (13.3%)	15
future							
Not GAP certified	3 (4.2%)	8 (11.1%)	9 (12.5%)	6 (8.3%)	31 (43.1%)	15 (20.8%)	72

Table 3.3 Treatment application status for postharvest water based on GAP certification status (n = 99)

	Apply treatment	No treatment applied	Total
GAP certified	6 (46.2%)	7 (53.8%)	13
Working towards being			
GAP certified in the future	3 (21.4%)	11 (78.6%)	14
Not GAP certified	12 (16.7%)	60 (83.3%)	72

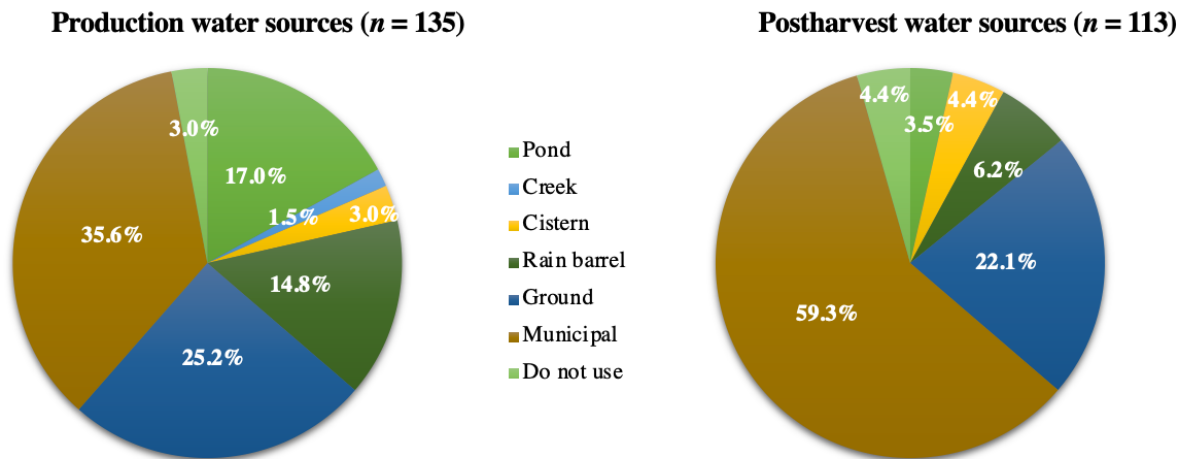


Figure 3.1 The percentages of different water sources that growers use for production ($n = 135$) and postharvest ($n = 113$) activities. (Note: among the 101 responses, several used more than one water type)

Municipal ($n = 48$, 35.6%), well ($n = 34$, 25.2%), pond ($n = 23$, 17.0%), and rain barrel ($n = 20$, 14.8%) water sources were the most commonly reported sources of production water (Figure 1). Municipal ($n = 67$, 59.8%) and well water ($n = 25$, 22.1%) were the most commonly reported water sources for postharvest water. Based on the number of responses for each water source, respondents appear to commonly utilize more sources of water for production applications ($n = 135$) than postharvest ($n = 113$) applications.

Participants also reported their applications of production and postharvest water (Figure 3.2). Of the survey respondents ($n = 101$), 90 (89.1%) indicated that they used production water in their operation. The three main reported uses of production water included irrigation ($n = 90$, 100%), mixing with crop sprays ($n = 38$, 42.2%), and mixing with fertilizers ($n = 38$, 42.2%). Of those growers that report using water for irrigation, 70 (77.8%) reported using drip irrigation, 37 (41.1%) reported using overhead irrigation, and 20 (22.2%) reported using drip irrigation under plastic. Furthermore, 90 (89.1%) indicated that they used postharvest water in their operation.

These growers reported their main uses of postharvest water to be for handwashing ($n = 77$, 85.6%), rinsing/washing produce ($n = 73$, 81.1%), and cleaning and sanitizing of food contact surfaces ($n = 68$, 75.6%). Other uses of postharvest water reported included ice making ($n = 7$, 7.8%), dunk tank cooling ($n = 6$, 6.7%), single pass/spray cooling ($n = 4$, 4.4%), and postharvest fungicide or wax applications ($n = 3$, 3.3%).

Of survey respondents who indicated that they used production water in their operation ($n = 90$), 75 (83.3%) reported that they do not treat their production water. Of the respondents who reported treating their production water, four (4.4%) reported using chemical sanitizers whereas 11 (11.1%) respondents indicated using physical methods such as sand filtration. Of survey respondents who indicated that they used postharvest water in their operation ($n = 90$), 68 (75.6%) reported that they do not treat their postharvest water. Fifteen respondents (16.7%) reported treating postharvest water with a chemical sanitizer and four (4.4%) reported using other treatments such as sand filtration or ultraviolet light. When comparing water treatment status and the water source used (shown in *Figure 3.3*), six (6.7%) respondents reported using surface water (ponds, cisterns, or rain barrels) for postharvest use without applying any treatment.

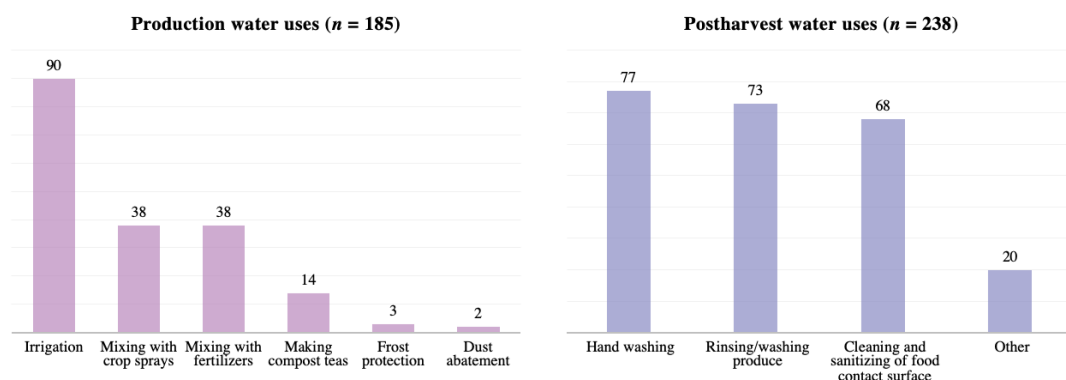


Figure 3.2 The different uses of production water ($n = 185$) and postharvest water ($n = 238$).

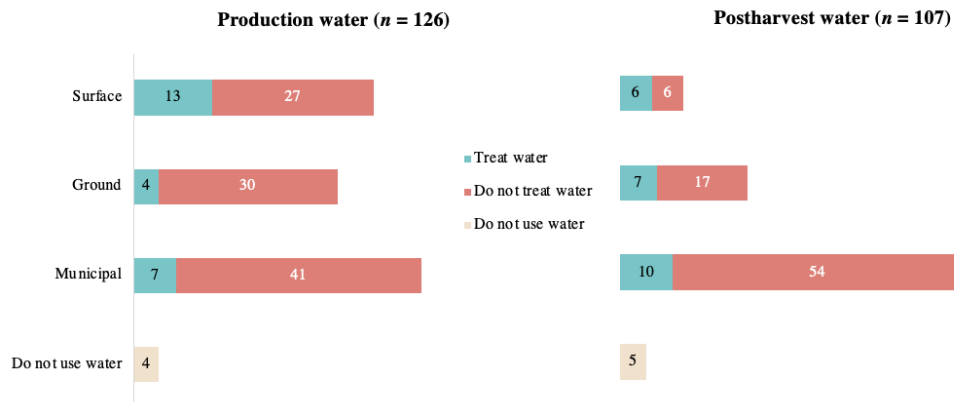


Figure 3.3 Treatment status for each water source that growers use for production (n = 126) and postharvest (n = 107) activities.

When asked what other practices (besides treatment) growers use to maintain/improve production water quality, most ($n = 70$, 77.8%) respondents using production water in their operation indicated that they did not use any other practices. Nineteen (21.1%) of the participants indicated that they conducted an annual evaluation of their water distribution system. When managing the quality of their postharvest water, 61 (67.8%) respondents who indicated that they used postharvest water in their operation ($n = 90$) reported that they do not use any additional practices, 17 (18.9%) reported routine cleaning and sanitation of tanks, 15 (16.7%) reported monitoring for buildup of organic material, 14 (15.6%) respondents reported conducting an annual evaluation, 10 (11.1%) reported monitoring water temperature during use, and nine (10.0%) reported using a routine water change schedule.

Discussion

Agricultural water quality remains a large concern for the fresh produce industry across the United States, particularly in the Midwest where previous studies indicate that agricultural water quality management is not well understood (17).

Most of the respondents demonstrated a preference for water sources with a lower microbial risk profile (such as municipal water) for postharvest activities, as compared to using surface water postharvest, which is higher risk. Specifically, the survey data indicated that – unlike production water, where municipal (35.6%) and surface water use (36.3%) were nearly equally reported – there is a higher use of municipal water (59.3%) and lower use of surface water (14.1%) for postharvest applications (*Figure 1*). As previous research has shown, microbial populations in surface water increase after events such as agricultural runoff (rain, melted snow, etc.) (3, 24, 27). Surface water is also more exposed to potential contamination from the surrounding open environment, and thus is considered to have a higher possibility of contamination (1, 8, 23).

According to the data collected from the survey, a minority of growers use specific postharvest water management practices that many would consider higher risk. Using surface water without any treatment for postharvest applications poses a higher risk of foodborne disease outbreaks, thus the FSMA PSR agricultural water criteria, GAP certification requirements and general produce safety best practices do not allow use of untreated surface water for postharvest activities (31). Interestingly, six survey respondents indicated that they were using untreated surface water for postharvest uses, and eight respondents indicated they treated their agricultural water using methods such as (bio)sand-filtration. Of note, sand-filtration is not a validated agricultural water treatment method according to the FSMA PSR (21 CFR 112.43) (31). However, growers do currently utilize sand filtration in irrigation systems to remove larger, physical particles, sometimes with the added misconception that it also can remove pathogens. This indicates that further education and training is needed to emphasize the significance of

microbial risk in postharvest water, as well as how to select an appropriate (validated) treatment method.

Conducting regular inspection and maintenance of the water source and distribution system is currently a requirement of the FSMA PSR (21 CFR 112.42) (31) and is a produce safety best practice to limit the risk of microbial contamination. By conducting regular inspections, growers can evaluate the risks to their water sources. The inspection includes identifying any broken part of the system structure, any wildlife evidence around open water sources, and other risks. Most of our survey respondents are not currently conducting an annual agricultural water system inspection. Thus, extension educators should provide practical information to growers on how to inspect their water sources regularly and encourage them to conduct a water system inspection at least annually, which is a requirement for growers covered by the FSMA PSR (21 CFR 112.42) (31). Of note, the proposed §112.43 is intended to supplement the requirements of the proposed §112.42 which will require a covered farm to regularly inspect and routinely maintain the components of its agricultural water system. While proposed §112.42 is focused on agricultural water system components, the proposed §112.43 would require covered farms to conduct a more comprehensive assessment of possible sources and routes by which known or reasonably foreseeable hazards are reasonably likely to be introduced into its preharvest agricultural water (30).

The FSMA PSR also highlights irrigation method as an important risk factor for contamination risk based on if the water contacts the harvestable portion of the produce. In most situations, overhead irrigation results in direct contact with the harvestable portion of the produce, which can result in a higher contamination risk than other irrigation methods. Conversely, drip irrigation (particularly drip irrigation under plastic) is considered to have a

relatively lower risk of contamination for crops grown above the ground. Of note, this characterization does not apply to root crops such as carrots, beets, and radishes (25). Most of the survey respondents indicated that their main use of production water was irrigation, namely drip irrigation (69.1%) or overhead irrigation (31.3%). However, the findings of the study call attention to particular agricultural water practices including the use of untreated surface water for direct water application during postharvest, and seldom testing the sanitary quality of agricultural water sources. Although not specifically measured by this study, further investigation and potentially extension efforts may need to occur to clarify how growers can reduce microbial risk by selecting irrigation methods that reduce the probability of water contact with the harvestable portion of the produce.

In the survey, the question about the frequency of conducting microbial water testing did not specify testing frequency of “production water” and “postharvest water” separately. Accordingly, crosstab analysis for the frequency of water testing for different sources of water used for different purposes (preharvest or postharvest) could not be conducted. Moreover, it was not possible to discern between treated and untreated sources when more than one source was indicated by the grower in this survey. We also did not include questions related to the location of their water source in relation to animals or other potential sources of contamination. These points could have been clarified by increasing the length of the survey. However, the expert panel reviewing the survey was concerned about the adverse effect of increasing the survey length on the response rate as some participants required more than 10 minutes to complete the survey. In a study of the effects of length on web-based survey completion, Galesic and Bosnjak (9) found that participants anticipating a longer survey completion time were less likely to finish the survey.

Conclusion

This survey was intended to provide information on the differences between best practices and the actual practices of Kansas and Missouri produce growers focusing on the use of agricultural water. Produce growers need to continue to improve their practices related to water quality to ensure that they are selling the safest produce possible. The survey responses will provide insight into the development of additional agricultural water safety educational materials for produce growers. The results indicated that efforts are needed to continue to encourage growers to test their water quality, to conduct at least an annual inspection of water sources and distribution system, and to use a validated surface water treatment method before using it for postharvest applications. The authors suggest that future extension trainings should emphasize the importance of microbial quality of agricultural water sources, and how risk to produce safety can be affected by selection of water source, decision to treat, and (in the case of production water) application method.

Acknowledge

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Chapter 4 - Agricultural Water Related Extension Education

Handouts

Based on the anecdotal evidence and survey responses from this thesis (Ch. 2), we identified the critical need to address a few important agricultural water safety-related topics that include the following:

New Modified FSMA PSR Agricultural Water Requirement Proposal

FSMA PSR published the agricultural water regulation in 2016, including using the different microbial quality criteria to value both production and postharvest water quality. However, after the implementation, the feedback from different farms focused on "the rules are too complicated and cost too much." Therefore, the FDA revised and published a proposed agricultural water requirement in December 2021 focused on production water. This handout summarized the major modification of the latest water requirement and aimed to help growers understand and comply with the proposed rule.

QUICK REFERENCE GUIDE

New Modified FSMA PSR Agricultural Water Requirement Proposal



In December 2021, the United States Food and Drug Administration (FDA) proposed a revision to Subpart E of the Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR) that would change the *pre-harvest agricultural water requirements* for covered produce, other than sprouts.

What is the proposed change?

The proposed revision to Subpart E modifies the previous requirement for produce growers to *replace the microbial criteria and testing requirements* for pre-harvest agricultural water with new provisions for *conducting pre-harvest agricultural water assessments*, providing more flexibility to produce growers in evaluating risks.

These aim for hazard identification (including consideration of agricultural water sources, distribution systems, and practices, as well as adjacent and nearby land uses, and other relevant factors), and using the results of the assessment to determine whether *corrective or mitigation measures* are needed to reduce the potential for contamination of produce or food contact surfaces with hazards associated with pre-harvest agricultural water.

What is an “agricultural water assessment” ?

It refers to an *evaluation*, conducted by a covered farm, of factors in its agricultural water system used during growing activities for non-sprout covered produce, its agricultural water practices for such pre-harvest water, crop characteristics, environmental conditions, and other relevant factors (including test results, where appropriate).

Factors	Description
Agricultural water system(s)	• Location and nature of the water source (including whether it is ground or surface water) • Water distribution system type (such as whether it is open or closed to the environment) • Degree to which the system is protected from possible sources of contamination
Agricultural water practices	• Water application method (overhead sprinkler, spray, drip, furrow, flood, seepage, etc.) • Time interval between the last direct water application and harvest of the covered produce
Crop characteristics	• Susceptibility of the covered produce to surface adhesion or internalization of hazards
Environmental conditions	• Frequency of heavy rain or extreme weather events may impact the agricultural water system or damage produce that can increase the susceptibility of produce to contamination. • Air temperatures • Sun (UV) exposure
Other relevant factors	• Including, if applicable, results of water testing that could inform the assessment



Questions, comments or concerns?

Contact your K-State Extension specialist **Londa Nwadike** (lnwadike@ksu.edu or nwadike@Missouri.edu) or **Cal Jamerson** (agri@ksu.edu) today!

New Modified FSMA PSR Agricultural Water Requirement Proposal

Corrective and mitigation measures

The proposed rule does **NOT** change the requirements for harvest and post-harvest water, but only impacts pre-harvest water. Under this proposed rule, a covered farm would *make a determination under the requirements, based on the outcome of its agricultural water assessment*, as to whether mitigation measures would be reasonably necessary to reduce the potential for contamination of covered produce (other than sprouts) or food contact surfaces with known or reasonably foreseeable hazards associated with its pre-harvest agricultural water.

Based on the assessment, if you determine...	Then you must...
that your agricultural water is not safe or is not of adequate sanitary quality for intended use(s).	- Immediately discontinue use(s) AND - Take corrective measures before resuming use of the water for pre-harvest activities
there is one or more known or reasonably foreseeable hazards related to animal activity, BSAAOs, or untreated or improperly treated human waste for which mitigation is reasonably necessary.	Implement mitigation measures promptly, and no later than the same growing season
there is one or more known or reasonably foreseeable hazards not related to animal activity, BSAAOs, or untreated or improperly treated human waste, for which mitigation is reasonably necessary.	- Implement mitigation measures as soon as practicable and no later than the following year Or - Test water as part of the assessment and implement measures, as needed, based on the outcome of the assessment
that there are no known or reasonably foreseeable hazards for which mitigation is reasonably necessary.	Inspect and adequately maintain the water system(s) regularly, and at least once each year

Exemptions:

A covered farm would be exempt from the requirement to conduct an assessment for pre-harvest agricultural water if the farm can demonstrate that the agricultural water meets these requirements, including:

- It meets certain requirements that apply for *harvest and post-harvest agricultural water* (such as the microbial quality criterion and testing requirements for untreated ground water);
- It is received from a *public water system or supply* that meets requirements established in the rule (provided that the farm has public water system results or certificates of compliance demonstrating that the water meets relevant requirements); or
- It is treated in accordance with the standards outlined in the Produce Safety Rule.

Prepared by: Yeqi Zhao Reviewed by: Manreet Bhullar, Londa Nwadike



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How to Interpret Water Results

Since 2016 FSMA PSR published agricultural water regulation, agricultural water has been separated into production water and postharvest water and from three main water sources (i.e., surface, ground, and municipal). Due to the different cross-contamination risks, the water criteria varied by different water activities. This handout aims to help Kansas and Missouri growers better understand the water regulation they need to comply with and interpret their water results within simple wording, images, and charts.

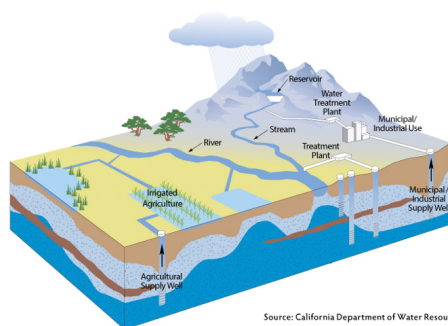
QUICK REFERENCE GUIDE

FSMA Produce Safety Rule Requirements for Agricultural Water

“ The **Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR)** states that all agricultural water **must** be of safe and adequate sanitary quality for its intended use (§112.41) ”

What is Agricultural Water?

Agricultural water is water that is intended or likely to contact the edible portions of produce or food contact surfaces during growing (production) or during/after harvest (post-harvest). The PSR outlines three common sources of agricultural water: **ground water**, **surface water**, and **municipal water**.



Public Water is water that is treated and monitored by a local water utility

Ground Water is water that is found beneath Earth's surface (ex. wells, aquifers)

Surface Water is water that is found or stored on the Earth's surface (ex. rivers, creeks, cistern, ponds supply tanks)

Risk of Contamination



What's the difference between Production and Postharvest Water?

Production Water is any water that is applied directly to produce during growing

- Irrigation
- Crop sprays
- Fertigation
- Frost protection
- Cooling



VS

Postharvest Water is any water that is used during and after field harvest of the produce

- Field Packing
- Washing
- Hydro-cooling
- Ice making
- Hand Washing



Note: water used for hand washing also counts as postharvest water since it can potentially contact the produce surface.

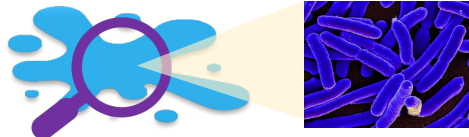


How do we determine water quality?

Agricultural Water Assessment The new modified agricultural water proposed rule uses an evaluation system to evaluate pre-harvest water quality. There are four main factors include agricultural water system(s), agricultural water practices, crop characteristics, and environmental conditions. Based on the assessment, you can determine whether your water is saved to use or not.

Tips: Agricultural Water Assessment Builder can help you evaluate your water quickly and easily!

Access from [link](#) or **QR-code**



Water Microbial Quality The Produce Safety Rule uses a bacteria called generic *E. coli* as an indicator of water quality. *E. coli* is a type of bacteria that is commonly found in animal waste (feces). Based on the number of generic *E. coli* in 100 ml, you can know whether your water is microbially safe to use or not.



Questions, comments or concerns?

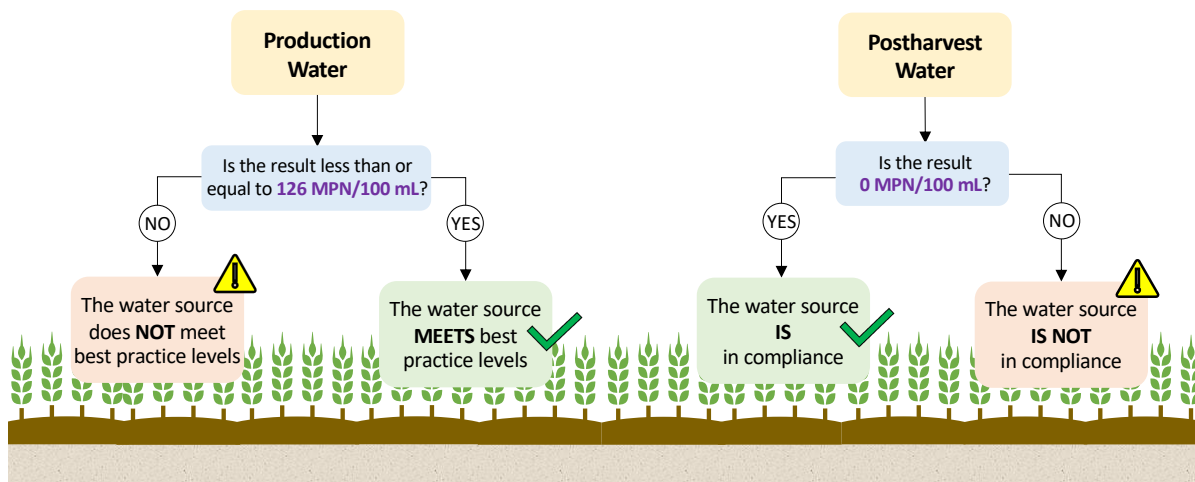
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How to Interpret Your Water Quality Test Results

Step 1: Determine if the water source meets best practice/ regulation levels

Intended Use:



Step 2: Determine the next steps if the water source IS NOT best practices/ FSMA PSR compliant

If the postharvest water is not compliant:

DISCONTINUE use as soon as practical (switch to municipal water or other reliable source) unless corrective measures are applied.

A few things you can do:

- Contact your local extension personnel for solution.
- Apply a **time interval** between the last irrigation and harvest.
- **Re-inspect** the entire affected agricultural water system for:
 - The extent it is under your control
 - Any conditions that are likely to contaminate produce surfaces
- Apply **validated treatments** to the water source.
 - The treatment must be effective to make the water safe and of adequate sanitary quality for its intended use (i.e., UV, chlorine).

[Kansas State University](https://www.k-state.edu/)

Name: Katelynn Stull

E-mail: kjstull@ksu.edu

Cell-phone: 913-307-7394

[University of Missouri](https://www.missouri.edu/)

Name: Dr. Londa Nwadike

E-mail: nwadikel@Missouri.edu

Cell-phone: 913-307-7391

Prepared by: Yeqi Zhao, Olivia Haley Reviewed by: Manreet Bhullar, Londa Nwadike



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Backflush Water Fact Sheet

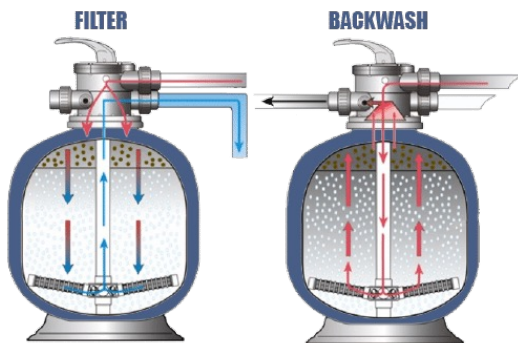
According to the survey results, the filtration system is the primary water treatment reported by the grower. The backflush water is the water used to clean the filter that contains various microorganisms and potentially includes human pathogens. Despite the use of filtration alone is not a validated agricultural water treatment, the handout relates to the filtration method, and backflush water is limited in the extension field. This handout aims to popularize the biohazard of backflush water and possible solutions.

QUICK REFERENCE GUIDE

Backflush Water Fact Sheet

Due to the produce safety concern, filtration systems are widely used in farms to catch small solid particles from original water sources (surface or ground water), and provide clearer water for agriculture purpose (irrigation, fertigation, cooling, etc.).

However, do you know the filtration system can also provide the “dirty” water called **backflush water**?

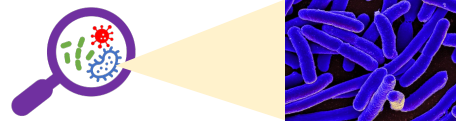


What is Backflush Water?

Backflush, also known as **backwash**, it is a mechanism always comes with a sand and Diatomaceous Earth (DE) filtration system acts as a **self-cleaning system**. It is a water flow that moves reversely through the filter tank to remove the accumulated particles from the filter.

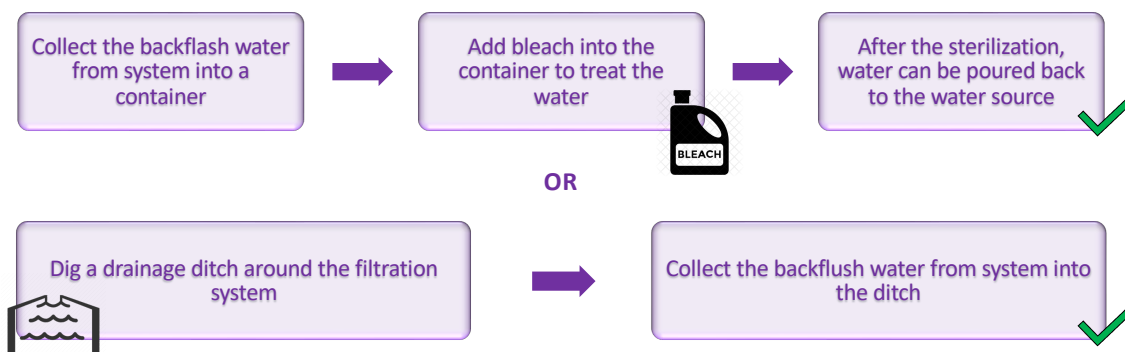
The Concerns That Backflush Water Brings Up:

As backflush water brings all the potential contaminated particles from the filter surface, the backflush water itself is considered as a **highly contaminated** water. If the backflush is not handled and/or stored properly, it can re-add the contamination to the original source or to other clean water sources on the farm.



How to Manage the Backflush Water in My Farm?

Following are several methods to manage your backflush water:



Purdue University Logo



Questions, comments or concerns?

Contact your K-State Extension specialist **Londa Nwadike** (phone number; email) or **Cal Jamerson** (phone number; email) today!

Chapter 5 - Conclusion

The thesis focused on improving the food safety practices reducing the risk of fresh produce outbreaks in the US, specifically expected outcomes on minimizing the cross-contamination chance in the fresh produce farms. The primary two reservoirs for transmitting human pathogens are the farm soil and agricultural water. Thus, to complete the goal, the thesis work included three objectives: (1) reducing the pathogen load in potentially contaminated agricultural soils using cover crops; (2a) accessing current practices and knowledge of Kansas and Missouri growers by using survey; (2b) developing extension material focused on managing agricultural water quality based on the survey results.

Soil Treatment

Several soil treatment methods reduce human pathogens in fresh produce fields. Specifically, cover crops have been recently explored for antimicrobial application in produce production, besides their soil health benefits for contaminated soils. Chapter 2 evaluates the efficacy of reducing pathogen load in contaminated soil with three different cover crop species (i.e., buckwheat, mustard greens, and sunn hemp). The results showed all three cover crops could reduce the bacteria population in contaminated soils after 30 to 40 days following artificial tillage within buckwheat did the best. However, the sole use of a gram-negative pathogen (generic *E. coli*), soil type, and selected three cover crops would be the primary limitations in this study. Considering this is the preliminary study, future research could study the antimicrobial efficacy on soil with different pathogens/soil types/cover crops to better understand the implications of these results. Nevertheless, these results demonstrated the potential of applying cover crops as an antimicrobial treatment in contaminated produce fields. The

outcomes will benefit the organic fresh produce growers as they use biological soil amendments to enrich the soil as a common practice.

Agricultural Water Safety

Unlike soil amendment contamination, agricultural water safety has always been a concern in all types of produce farms, as highlighted in multiple produce outbreaks. Within continuously providing training and information on agricultural water microbial quality to help produce growers reduce risk, the survey developed (chapter 3) is necessary to determine the current knowledge and practice gaps between produce growers and agricultural water management best practices. The surveys were sent out between late 2020 and early 2021 during the COVID-19 pandemic, in which most of the growers' in-person activities had been canceled due to public health concerns. Thus, most surveys were distributed through Qualtrics (an online survey instrument), and the total responses were lower than the expectation (106/200). Despite the dilemma, the survey results indicate the growers from Kansas and Missouri have a basic awareness of agricultural water practices. Still, more demanding practices must be strengthened (e.g., using validated water treatment methods).

Based on the survey responses, three handouts were developed (chapter 4), including: (1) "New modified FSMA PSR agricultural water requirement proposal," which summarized the significant modification of the latest water requirement published in 2021; (2) "How to interpret water results" provides information to identify the agricultural water sources and microbial safety criteria; (3) "Backflush water fact sheet" discussed the hazard from back flush water and solutions. These handouts aim to fulfill the produce growers' current needs and help them to mitigate risk from agricultural water cross-contamination.

In conclusion, the survey provided information on the gaps between best practices and the actual practices of Kansas and Missouri produce growers on agricultural water management; these gave extension professionals the directions for developing further outputs. In addition, in December 2021, the FDA published a new modified agricultural water requirement proposal. The new proposed rule will replace the produce water criteria once it is finalized. Thus, further study can focus on assessing the produce growers' knowledge of the new proposed rule and develop handouts based on the results.

Appendix A - Survey Questions

1. What is your age?

☐ 18-29

☐ 40-49

☐ 60+ years

☐ 30-39

☐ 50-59

2. Are you currently Good Agriculture Practices (GAP) certified?

☐ Yes

☐ No

☐ No, but working
towards being GAP
certified in the future

3. Are you currently raising produce (fruits, vegetables, herbs, mushrooms, nuts) for sale?

☐ Yes

☐ No

☐ No, but plan to in the future

4. In which state do you grow produce? (If more than one, mark the primary state)

☐ Kansas

☐ Missouri

☐ Other _____

5. Do you currently use water PRE-harvest in your produce operation? (check all that apply)

☐ For irrigation

☐ For dust abatement

☐ For mixing with crop sprays

☐ Frost protection

☐ For mixing with fertilizers

☐ Other _____

☐ For making compost teas

☐ No

6. If you use water for irrigation, how do you irrigate? (check all that apply)

☐ Drip irrigation

☐ Furrow irrigation

☐ Drip irrigation under plastic

☐ Other _____

☐ Overhead irrigation

☐ I do not irrigate

7. What sources of PRE-harvest water do you use? (check all that apply)

☐ Pond

☐ Cistern

☐ Creek

☐ Other (such as rain barrel, spring, or
others. Please list) _____

☐ Well

☐ Do not use water pre-harvest

☐ Municipal water

8. Do you currently use water POST-harvest in your operation? (check all that apply)

☐ Rinsing/ washing produce

☐ Single-pass/spray cooling

☐ Commodity movement (i.e. dump
tanks/ flumes)

☐ Dunk tank cooling

☐ Ice making

- ☐ Post-harvest fungicide or wax
- ☐ Handwashing
- ☐ Cleaning and sanitizing of food contact surfaces
- ☐ Other (please list) _____

9. What sources of POST- harvest water do you use? (check all that apply)

- ☐ Municipal water
- ☐ Well
- ☐ Cistern
- ☐ Pond
- ☐ Creek
- ☐ Other (such as rain harvest barrel or others. Please list) _____
- ☐ Do not use water post-harvest

10. Do you test your water used in your produce operation for generic *E. coli*?

- ☐ Yes, more than once a year
- ☐ Yes, once a year
- ☐ Yes, we have tested our water 2-3 times in the last 10 years or so
- ☐ Yes, we have tested our water once in the last 10 years or so
- ☐ No, we have never tested our water
- ☐ A third party (such as the municipal supplier)

11. Do you treat your water used PRE-harvest (such as in irrigation) to improve its microbial quality?

- ☐ Yes, we treat it with a sanitizer (list sanitizer used) _____
- ☐ Yes, we use a different treatment (list treatment- for example, sand filter, other) _____
- ☐ No

12. Do you treat your water used POST-harvest (such as in washing) to improve its microbial quality?

- ☐ Yes, we treat it with a sanitizer (list sanitizer used) _____
- ☐ Yes, we use a different treatment (list treatment- for example, sand filter, ozone, UV, reverse osmosis, other) _____
- ☐ No

13. What other practices (besides treatment) do you use to maintain/improve the quality of the water used PRE-harvest in your produce operation?

- ☐ We conduct an annual evaluation of our water distribution system
- ☐ Other (please list) _____
- ☐ None

14. What other practices (besides treatment) do you use to maintain/improve the quality of the water used POST-harvest in your produce operation?

- ☐ We conduct an annual evaluation of our water distribution system
- ☐ Routine water change schedule
- ☐ Routine cleaning and sanitation of tanks
- ☐ Monitoring for buildup of organic material
- ☐ Monitoring water temperature during post-harvest use
- ☐ Other (please list) _____
- ☐ None

Improving produce safety by reducing pathogen load in soil using cover crops and developing
food safety educational resources for produce growers

by

Yeqi Zhao

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College of Agriculture

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Approved by:

Co-Major Professor
Dr. Eleni D. Pliakoni

Approved by:

Co-Major Professor
Dr. Manreet S. Bhullar

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Abstract

Fresh produce (commonly consumed raw) has been continually associated with multiple foodborne disease outbreaks, which cause both health concerns and economic losses.

Agricultural water and contaminated soil are suspected as common causative agents for recent produce-related foodborne illness outbreaks, which brought fresh produce safety to the forefront of public attention. New federal regulations by the U.S. Food and Drug Administration (FDA) as part of the FDA Food Safety Modernization Act (FSMA) established the Produce Safety Rule (PSR), which defined scientific criteria for growing, harvesting, storing, and packaging fresh produce. The PSR provides requirements on treating soil amendments and criteria for safer use of agricultural water.

There are several soil and water treatment methods and practices to reduce human pathogens in fresh produce. Specifically, for contaminated soils, cover crops have been recently explored for antimicrobial application in produce production, in addition to their soil health benefits. Cover crops are plants seeded before or after the growing season to improve soil health, increase water availability, reduce weed pressure, and prevent erosion. Cover crops also produce various antimicrobial secondary metabolites (i.e., glucosinolates, quercetin), yet the role of cover crops in moderating the population of human pathogens in the soil has rarely been investigated. However, with regards to the microbial quality of agricultural water, Kansas State University and University of Missouri Extension educators have been providing free water testing, training and information on agricultural water microbial quality to help produce growers reduce microbial risks from ag water sources.

The goal of this thesis is to study cover crops as potential antimicrobial interventions and understand grower needs to improve food safety measures and practices on a fresh produce farm.

To achieve this goal, there are two specific objectives: (1) soil treatment: determine the antimicrobial potential of three cover crop species to reduce the population of pathogenic soil microbiota which can minimize the possibility of cross-contamination of produce from agricultural soil; (2) agricultural water: determine knowledge gaps among Kansas and Missouri produce growers related to agricultural water quality and best practices and develop agricultural water safety handouts to improve growers' knowledge.

To complete the first part of this thesis, whole-plant samples of 4-week-old mustard greens (*Brassica juncea*, 'Kodiak'), sun hemp (*Crotalaria juncea*), or buckwheat (*Fagopyrum esculentum*) were chopped and mixed into autoclaved farm soil and inoculated with 7-logs of rifampicin-resistant generic *Escherichia coli* (*E. coli*). The surviving microbial population on days 0, 4, 10, 15, 20, 30, and 40 was enumerated using an agar-based methodology. All three cover crops significantly reduced the population of generic *E. coli* compared to the control ($p < 0.0001$); buckwheat resulted in the highest reduction (3.92 log CFU/g). An inhibitory effect on microbial growth was also observed in soils containing mustard greens ($p < 0.0001$) and sunn hemp ($p < 0.0001$). This study provides evidence for the bactericidal and bacteriostatic effects of cover crops. More information regarding the crops' secondary metabolite profile and potential as a soil bio-mitigation strategy to improve on-farm produce safety is warranted.

For objective 2, a survey was developed and administered to determine future extension outputs and activities to encourage growers to improve their practices related to water quality. The survey was distributed to Kansas and Missouri produce growers attending in-person or online produce safety-related events in late 2020 and early 2021. Also, it was distributed through email lists of produce growers from both states. Survey results ($n = 101$) indicate that 13.9% of the respondents tested their water for generic *Escherichia coli* (*E. coli*) more than once a year,

while 38.6% of the participants have never tested their water. Approximately half (59.3%) of respondents indicated they use municipal water for postharvest uses, while 6.7% indicated the use of untreated surface water for postharvest activities. Due to the concern of potential water contamination risks, researchers suggest that further training and educational resources would help growers improve practices related to water quality and produce safety.

Three handouts were developed to help growers better understand the current FSMA PSR water quality requirements (under consideration for further modifications) and best practices. These handouts include the following: (1) “New modified FSMA PSR agricultural water requirement proposal” handout summarizes the major modifications of the latest water proposal published in 2021; (2) “How to interpret water results” handout provides information to identify agricultural water uses and microbial safety criteria and best practices; and (3) “Back flush water fact sheet” discusses the hazards from back flush water and potential solutions.

In conclusion, this thesis focuses on improving fresh produce safety by minimizing the cross-contamination happening in agricultural soil and water. The reduction of microbial populations in the first part of the thesis demonstrated the potential of cover crops to be used as a sustainable antimicrobial treatment method for contaminated soil. Meanwhile, the survey results show produce growers have a basic awareness of agricultural water practices, but the safety of those practices can still be strengthened. The publication of relevant handouts can improve growers' knowledge and help them better comply with the latest regulations.

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Chapter 1 - Literature Review

Introduction

Significance of fresh produce in the human diet

Since fresh fruits and vegetables contain vitamin A, C, and E, carotenoids, folic acid, dietary fiber, and other minerals, they are one of the great resources to interrupt oxidative stress in the human body to minimize the risk of chronic disease, as well as reduce the concentration of harmful compounds and acid in feces (21). Researchers pointed out that higher fresh produce consumption levels will possibly reduce the risk of cancer (22, 123, 124). Moreover, research showed that consuming produce on a regular basis can reduce blood cholesterol levels (92), the risk of heart disease (66), and maintain blood pressure (118). The concept behind the connection between consuming produce and the risk of chronic disease is the balance of free radicals and phytochemical antioxidants (77) in the body. As the nutrients from produce are hard to replace with other foods, a balanced diet including fruits and vegetables is an important step in maintaining a healthy lifestyle. The USDA's MyPlate recommends eating approximately 2 cups of fruit and 3 cups of vegetables per person per day to maintain a healthy life (the specific recommendation also depends on gender and age) (80).

Besides the increase in health awareness, urban farming has become more popular in the last ten years in the U.S. with the aims of improving local food security, maximizing the use of vacant spaces, and improving agricultural sustainability. Although, due to the environmental impact, currently limited crops can grow on an urban farm, such as leaf greens (72), the model of

potential productivity of urban agriculture shows that small-scale systems are more productive than commercial farms (126). From 1990 to 2018, the value of fruit and vegetable production in the United States has increased each year (130). According to a report from Grand View Research, Inc., the U.S. fruit and vegetable market will reach approximately \$1.12 trillion, and the demand will be more than 243.2 million tons by 2025 (52). As a result, the rise of urban agriculture accelerated the produce market expansion but also added uncertainty to the produce safety, as many produce growers from urban areas own limited farmland and do not need to comply with the Produce Safety Rule (PSR) (annual monetary sales of a farm below \$25,000). So there is still a need for extension professionals to suggest them follow the Good Agricultural Practices (GAP) or PSR to ensure the produce safety during production.

Foodborne outbreaks related with produce

As the demand for fruits and vegetables increasing, more cases of foodborne disease outbreaks have been linked to produce in the past decades. These have highlighted produce safety concerns to the public. In recent years, food safety challenges have continued in an endless series, and there have even been about 3,000 deaths from foodborne causes annually in the US (131). Produce is one of the key vehicles for the transmission of foodborne pathogens in most parts of the world (78). From 2010 to 2017, a total of 85 multistate outbreaks associated with fresh produce with confirmed etiologies occurred in the United States, resulting in a known combined total of 4,658 known illnesses, 1,187 hospitalizations, and 55 deaths (29). *Escherichia coli* (*E. coli*), *Salmonella enterica* (*S. enterica*), and *Listeria monocytogenes* (*L. monocytogenes*)

are the three bacterial pathogens that caused the most multistate fresh produce outbreaks from 2010 to 2017 (29).

Economic impact of foodborne outbreaks

Foodborne outbreaks not only destroy human health but also lead to a huge economic burden. According to the USDA estimation, each year foodborne illnesses cost more than \$17.6 billion of loss (133). Of note, fresh produce is one of the major causes that is believed to account for 26% of the total economic loss (85). One foodborne disease outbreak can lead to \$6,330 to \$2.1 million in economic loss for a fast-casual restaurant (13). According to FDA rankings, the top most common pathogens that cause foodborne disease are *E. coli*, norovirus, *Salmonella spp.*, *Campylobacter*, Hepatitis A, and *Listeria* (138). Among these pathogens, outbreaks associated with *S. enterica*, *L. monocytogenes*, and *Campylobacter spp.* caused the highest total economic losses (113). In addition, the severity of the illness and the type of bacterial infection affect the amount of economic loss, and the cost also depends on the geographical region of the patient. California, Florida, and Texas were among the states with the highest economic losses per foodborne illness patient recorded in 2013 (113, 114).

At the same time, foodborne diseases will bring high economic losses to consumers and will also lead to economic and reputational losses to produce growers or processors. When certain pathogenic bacteria are detected in food products, all products sold are recalled as required by the U.S. Food & Drug Administration (FDA) (30). In this process, the producer has to pay all the costs associated with the recall, including the extra labor, wasted food, and recall costs. At the same

time, when a portion of food is recalled, the reputation of the food manufacturer will normally be damaged, and consumers often will discriminate against the brand. According to the Grocery Manufacturers Association (GMA), the average food recall costs the company about \$10 million per recall (129). For example, a large quantity of food (including safe produce) was dumped due to public health considerations during the 2018 large produce outbreak caused by contaminated romaine lettuce (32).

Thus, reducing the chances of food being infected by microorganisms and ensuring food safety is what every producer and consumer needs to understand and strive to do.

Contamination Factors on Produce Farms

Pathogen contamination of fresh produce can occur at any time during the production chain, although the likelihood of contamination is elevated during field operations such as species and harvest (48, 78). The introduction of human pathogens can occur via diverse routes, including agricultural water (14, 55) and biological soil amendments of animal origin (BSAAOs) (157). Once pathogens are introduced into the farm environment, they can survive and persist for long periods within the soil or water (26, 45, 88, 90, 122), which can cause subsequent spread to other areas of the fruit and vegetable production environment or even result in product contamination.

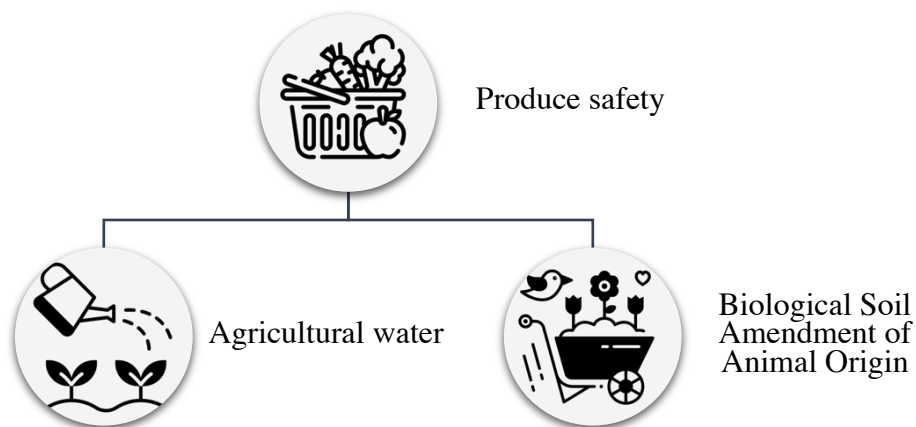


Figure 1.1 The two main contamination routes on a farm that cause produce safety issues.

Agricultural Water

Agricultural water is widely distributed and used in a produce farm which increases the opportunity for human pathogens to attach to the produce. The proximity of produce to contaminated water and the concentration of pathogens within the water are two risk factors contributing to the probability of produce contamination during field production (6). The contamination route always starts with fecal matter carrying human pathogens that drop into the water source (7). Then the transmission of human pathogens can occur at any time during field production (i.e., irrigation, flooding, agricultural runoff) and postharvest activities (washing, rinsing, cooling, etc.).

Biological Soil Amendments of Animal Origin (BSAAOs)

Agricultural soil is another reservoir that can transmit human pathogens to fresh produce. One of the major sources of these human pathogens that survive in the soil is from BSAAOs.

Without proper treatment (i.e., composting), BSAAOs can carry various human pathogens. Once these are applied to soil as fertilizer, they will contaminate the soil soon after. From soil, there are multiple opportunities for human pathogens to attach to the produce. For example, the transmission of human pathogens can occur during the germination of seeds or after the transplantation of seedlings in contaminated soil (28, 71). Therefore, in agricultural applications, it is necessary for growers to detect whether any unexpected animals or improper practices exist before, during, or after field production.

Farm Safety Modernization Act Produce Safety Rule

Overview of the regulation

The US implemented the FDA Food Safety Modernization Act (FSMA) in 2011, which was developed to shift the focus of the nation's food safety system from responding to foodborne illness to minimizing the number of these cases. It was designed to provide clear, specific actions that must be taken throughout the produce handling process to prevent contamination. Nine rules are included in FSMA, which include 1) Third-Party Certification Accreditation; 2) Current Good Manufacturing Practices and Hazard Analysis and Risk-Based Preventive Controls for Human Food and Animal Foods; 3) Foreign Supplier Verification Programs (FSVP); 4) Laboratory Accreditation; 5) Food Traceability; 6) Mitigation Strategies to Protect Food Against Intentional Adulteration; 7) Sanitary Transportation of Human and Animal Food; 8) Voluntary Qualified Importer Program (VQIP); and 9) Standards for the Growing, Harvesting, Packing and Holding of Produce for Human Consumption (10).

This last rule is also known as the Produce Safety Rule (PSR) and the final version was published on November 27th, 2015. It establishes science-based minimum standards for the safe growing, harvesting, packing, and holding of fruits and vegetables grown for human consumption. The compliance dates of PSR criteria are determined by the size of the farm in terms of annual sales and the risk of the commodities becoming a foodborne outbreak when they get contaminated. These include if the farm sells at least \$25,000 of produce annually (adjusted for inflation) and if the produce is likely to be eaten raw (e.g., leafy greens, cucumber, tomato, and most fruits) (137).

Table 1.1 The compliance dates for each rule are based on farm size (very small, small, and other businesses).

Note: ¹ The farm sizes are determined by the average annual monetary value of produce the farm sold during the previous 3-year period. The other businesses (not small) include those selling more than \$500,000 of produce; small size farms include annual monetary sales between \$250,000 to \$500,000; very small includes farms with annual monetary sales between \$25,000 to \$250,000; the annual monetary sales of a farm below \$25,000 are exempted from the PSR. ² The new proposed water requirements are modified based on the final rule which was published December 2nd, 2021 and caused the extended compliance dates. More information on these topics will be mentioned later in this review.

Farm Size¹			
/Compliance Date <i>(134)</i>	Sprouts	Other Produce	Original Water Requirements
Other Businesses (Not Small)	1/26/17	1/26/18	1/27/20
Small	1/26/18	1/28/19	1/26/21
Very Small	1/28/19	1/27/20	1/26/22

Under the PSR, there are six key requirements summarized by FDA as subparts, which include: agricultural water, biological soil amendments, sprouts, domesticated and wild animals, worker training and health and hygiene, and equipment, tools, and buildings (and post-harvest handling?) (135). Of note, maintaining the microbial quality of the water sources used on the farm is of primary importance in produce safety. As water is used in almost every aspect of production, once the water source is contaminated, there will be a higher risk of produce contamination.

Worker Training and Health and Hygiene

Workers are a part of the food safety concerns since they can carry multiple pathogens and spread them unintentionally via multiple routes (58, 65). FDA requires all farm workers who handle covered produce and/or food-contact surfaces and all supervisors to receive food safety training, including the principles of good hygiene and food safety; recognizing symptoms of foodborne illness and the importance of personal hygiene for all personnel and visitors; and other training relevant to the worker's job (137). Of note, water is a key component of these requirements in order to fulfill health and hygiene requirements, such as washing hands and mixing with sanitizer (35). Thus, the microbial quality of the water source which is used for hygiene contributes to the risk of cross-contamination concern on the farm.

Equipment, Tools, and Buildings

The equipment and tools that are used on farms can always directly contact produce. This part of the rule focuses on the standards of sanitation to prevent cross-contamination of produce.

It covers the building structure (i.e., greenhouses, germination chambers, toilets, and hand-washing facilities), the tools, and equipment that may touch or contact the produce. As in the health and hygiene section, water is also required in this part of the rule, especially during cleaning and sanitizing processes. Although the literature shows that the survival time of pathogenic bacteria partially depends on the surrounding environments (i.e., temperature, moisture, the attached surface material) (24, 46, 104), once pathogens are introduced to any equipment, tools, or building floor, the possibility of cross-contamination begins to increase.

Biological Soil Amendments

According to federal regulation § 112.3, biological soil amendments of animal origin (BSAAOs) represent a biological soil amendment that consists, in whole or in part, of materials of animal origin (137). BSAAOs are widely used to improve soil fertility and quality on organic farms and make a primary contribution to agriculture nutrient management (108, 116). However, if the BSAAOs are used improperly, they can lead to very high produce and water contamination risk since they are applied directly next to the produce. Further, pathogens (e.g., *E. coli*, *S. enterica*) have an extended survival length in a warmer and moist environment (91, 97). A scientifically valid controlled process is required from the PSR for treating BSAAOs to minimize the risk of contamination (137). Of note, the PSR and extension fact sheets highlight storage of the biological soil amendments away from water sources since that can cause potential contamination (19, 137).

Sprouts

Agricultural water quality is a key food safety concern in growing sprouts as water is the primary input in sprout production. In past decades, several foodborne outbreaks have been traced back to sprouts (*15, 31, 49, 125*), which have been contaminated by pathogens carried from sprout seeds and run-off water. As sprouts are grown in warm, moist, and nutrient-rich conditions, this is also the perfect environment for pathogens to survive. Unlike in field production systems, once a single pathogen is introduced into the sprouts system, it can easily spread to all the growing sprouts. Moreover, literature shows that because of the favorable growing conditions, sanitizer treatments (i.e., ozone and chlorine dioxide) cannot inactivate pathogen growth (*2*). Of note, water is required in most sprout growth steps. Therefore, cross-contamination can either happen during irrigation of sprout seeds in the field or during irrigating the sprouts during production (*154*). Based on the special growth environment, the PSR has established a separate section for sprout growers, which includes testing any microbes on or in the seeds, sprout irrigation water testing, and further corrective treatments.

Domesticated and Wild Animals

The fecal matter left behind on the field from grazing, working, and wild animals have a potential risk of containing human pathogens. The pathogens from their feces can directly contaminate the surrounding soil and produce (*84*). The PSR addresses this concern and requires taking all measures reasonably necessary to identify and not harvest produce that is likely to be contaminated. At a minimum, all covered farms are required to visually examine the growing

area and all the covered produce to be harvested (137). Furthermore, once any feces are dropped or flushed (i.e., rain and flood) into nearby water, it will contaminate the entire water source with pathogens from the fecal matter (18). This results in the water becoming a primary reservoir for human pathogens at the farm level.

Agricultural water

Water is an important part of agricultural production as well as a primary reservoir for many human pathogens on the farm. Accordingly, agricultural water microbial quality requirements have been written into the PSR. In current microbial water quality testing guidelines, fecal contaminations are determined by the number of generic *E. coli* in 100 mL of water. The presence of any strain of *E. coli* is a strong indication of fecal contamination, but it cannot be used as direct evidence of the presence of a human pathogen (127). In the PSR, generic *E. coli* is only used to measure microbial water quality and give growers information for future water management.



Figure 1.2 Schematic illustration of a worse condition fresh produce farm.

Originally Proposed Microbial Quality Criteria

Of note, during the thesis year (2020-2022), FDA proposed the new modified production water regulation and currently looking for feedback. Thus, this thesis includes both the current written agricultural water regulation and the new proposed production water regulation. To avoid any misleading, please check the current PSR for referencing.

In 2016, the PSR documented its minimum safety standards for agricultural water at that time. According to the rules clearly specified in PSR released in 2016, for surface water, twenty or more samples must be tested over a period of 2 to 4 years, with five or more samples rolled into the profile every year after the initial survey. The 2016 PSR requirements quantify the safety standards for agricultural water used directly on crops (other than sprouts) by using the

geometric mean (GM) and statistical threshold value (STV) of the water test results. The GM is the average of the water testing results and shows the central tendency of the quality of the water (117). The STV reflects the variability in the quality of the water samples over time.

Table 1.2 The standard for water microbial quality (GM and STV) for pre-harvest water included in the 2016 PSR.

	Geometric Mean	Statistical Threshold Value
Number of Generic <i>E. coli</i> (CFU/100mL)	≤ 126	≤ 410

The corrective actions for the water are required no longer than a year if the water testing results exceed the criteria number. During this period, growers can still use time water in some situations to keep flexibility for them (9, 23).

Microbial Water Testing Methods

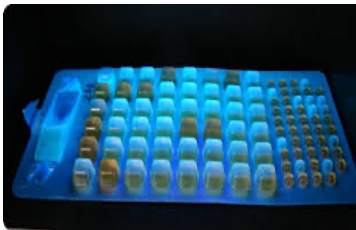
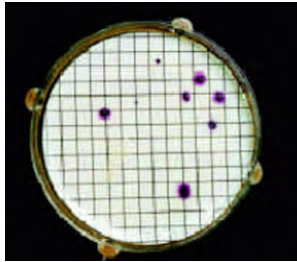
Produce growers need to collect water samples in bottles provided by the testing lab, with a sample size of no less than 100 milliliters. If a groundwater sample is collected, growers should let the water run for five minutes before the sample is collected. If the sample is surface water, keep the sampling bottle close to the center of the water body and try to avoid collecting impurities. After a successful collection, samples must be kept at a low temperature of 4 to 10 °C and sent to a nearby laboratory for analysis within 24 hours (20).

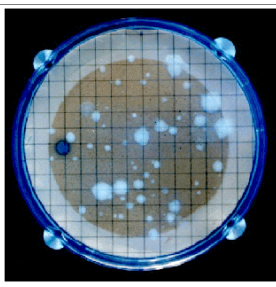
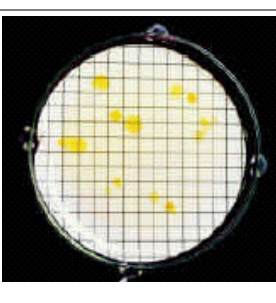
There are two sets of criteria for microbial water quality in the 2016 PSR water requirements that are based on the presence of generic *E. coli* in agriculture water. The microbial water quality for water that is used for post-harvest purposes (including any water that can directly

contact produce surfaces after harvest) must be 0 CFU/100mL, which means no detectable generic *E. coli* are allowed for post-harvest water. The other numerical critical that was proposed in the 2016 PSR water requirements are for water that is applied directly to growing produce (except sprouts). The microbial water quality value for production water in the 2016 PSR water requirements has to be below 126 CFU/100mL GM and 410 STV(132).

Currently, FDA states that there are eight methods considered scientifically valid and at least equivalent in accuracy, precision, and sensitivity in quantifying generic *E. coli* in agriculture water compared to the “EPA? Method 1603: *Escherichia coli* (*E. coli*) in Water by Membrane Filtration Using Modified membrane-Thermotolerant *Escherichia coli* Agar (Modified mTEC) (139)”. Based on the different types of methods, there are two units used in water test results: Colony Forming Unit (CFU) per 100 mL water sample and Most Probable Number (MPN) per 100 mL water sample.

Table 1.3 The eight methods are listed by name, description, result image, and reference.

Name of Method	Method Feature	Example	Reference
IDEXX Colilert/Colilert- 18 Test Kit (unit with MPN)	The Colilert method is used for 4-methylumbelliferyl-β-D-glucuronide (MUG) positive reaction, and the chemical has to be dissolved in the sample water to be tested so that the <i>E. coli</i> in the water can show the fluorescence effect under the ultraviolet light. After adding one packet of Colilert reagent into 100mL of sample water, it is sealed into a quantifying tray. Then it is incubated for 24 hours at 35°C; yellow wells may form to represent if the coliform is present. By checking the amount of blue fluorescent wells in the quanti-tray 2000 under a 6W, 365 nm UV light, the Most Probably Number (MPN) result can be calculated according to the table provided with the Quanti-Tray.		N/A
Method 1603 validated by EPA (unit with CFU)	This is a filtration method that follows the “U.S. EPA Method 1603 Protocol” by growing the <i>E. coli</i> that is left on the membrane in an m-TEC agar incubated for 2 hours at 35 °C and placed in a water bath for 22 hours at 44.5 °C. Counting the pink or purple color colonies formed in the agar provides the final result. This is a test that strictly adheres to FDA regulations; the results are precise and clear but include many disadvantages. The m-TEC agar used in this method is expensive, and it needs to be prepared before starting a water test. The filtration process is required for detection, and the turbidity level of the sample water directly determines the length of time of this process.		Method 1603: <i>Escherichia coli</i> (<i>E. coli</i>) in Water by Membrane Filtration Using Modified membrane-Thermotolerant <i>Escherichia coli</i> Agar (Modified mTEC) (September 2014)

Method 1604 validated by EPA (unit with CFU)	Filter 100mL water sample through a membrane filter and place it on MI agar on an absorbent pad saturated with 2-3 mL of MI broth and incubate at 35 °C for 24 hours. The <i>E. coli</i> will present with a blue color from the breakdown of IBDG by the <i>E. coli</i> enzyme β-glucuronidase; total coliform will fluoresce under longwave UV light from the breakdown of MUGal by the TC enzyme β-galactosidase.		Method 1604: Total Coliforms and <i>E. coli</i> in Water by Membrane Filtration Using a Simultaneous Detection Technique (MI Medium) (September 2002)
Method 1103.1 validated by EPA (unit with CFU)	Filter the water sample through the membrane and place it on the selective and differential medium, mTEC. Incubate at 35°C $\pm$ 0.5°C for 2 $\pm$ 0.5 hours and incubate at 44.5°C $\pm$ 0.2°C for 22 $\pm$ 2 hours. After incubation, transfer the membrane from mTEC agar to the absorbent pad saturated with urea substrate medium for 15 minutes is required for the final step. The color of the <i>E. coli</i> colonies in the final plate should be yellow or yellow-green under a fluorescent lamp.		Method 1103.1: <i>E. coli</i> in Water by Membrane Filtration Using membrane-Thermotolerant Escherichia coli Agar (mTEC) (March 2010)
9213 D validated by SM (unit with CFU)	Filter water sample through a membrane filter and place on mTEC agar. Incubate at 35°C for two h to resuscitate stressed bacteria, then incubate at 44.5°C for 22 h. Then transfer the membrane to a filter pad saturated with urea substrate and containing phenol red for 15 minutes. The result positive for <i>E. coli</i> should be fluorescence under the longwave UV lamp with yellow color.	No image available	Natural Bathing Beaches (2007). In: Standard Methods for the Examination of Water and Wastewater, 22nd Edition (Rice E.W., et al., Ed.), 9-46 – 9-48. Washington, DC: American Public Health Association. (2012).
9222 B validated by SM (unit with CFU)	In this method, the membrane will be placed on les-ENDO or m-ENDO agar which contains basic Fuchsin as the indicator. Filter water sample through a membrane filter and placed on the agar. After incubating the agar for 24 hours at 35 °C, the coliform will show up in red color. Transfer the membrane filter to a nutrient agar that contains MUG and incubate for 4 hours. The individual <i>E. coli</i> colonies will fluorescence under a longwave length UV lamp.	No image available	Standard Total Coliform Membrane Filter Procedure (1997), followed by 9222 G – MF Partition Procedures (1997) using NA-MUG media. In: Standard Methods for the Examination of Water and Wastewater, 21st Edition (Eaton A.D., et al., Ed.), 9-60 – 9-65, and 9-70 – 9-71, respectively. Washington,

**D 5392-93
validated by
ASTM (unit with
CFU)**

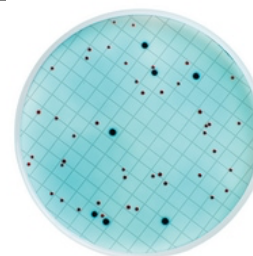
This is a two-step water testing procedure that provides a direct count of the colonies' numbers. Filter sample water through a membrane filter and place it on the selective medium, mTEC. The membrane on the medium is first incubated at 35°C for two h, and then the medium is incubated at 44.5°C for 22 h. Following incubation, the filter is transferred to a filter pad saturated with urea substrate. The yellow or yellow-brown colonies will be present after 15 minutes if the water sample is positive for *E. coli*. Colonies are counted with the aid of a 10 to 15 X magnifier and a fluorescent lamp.

No image available

Standard Test Method for Isolation and Enumeration of *E. coli* in Water by the Two- Step Membrane Filter Procedure. In: Annual Book of ASTM Standards, Volume 11.02. ASTM International. (1996, 1999, 2000).

**Hach Method
10029 for
Coliforms (unit
with CFU)**

This method was published by Hach Company. 100mL of the water sample is filtered through a 0.45-µm membrane filter and placed on the plate, which already added 2mL of the reagent onto a sterile pad on the plate. After incubating for 24 hours at 35 °C, the presence of red colonies indicates the coliforms by reducing the TTC. By hydrolyzing the enzyme-substrate BCIG, it shows blue color for *E. coli* colonies.



Total and *E. coli*, using m-ColiBlue24 Broth PourRite Ampules

Modified Agricultural Water Proposed Rule (proposed in December 2021)

After complying with the water microbial quality criteria for some time, some businesses reported that regulations are difficult to enforce because they are both expansive and complex (144). Thus, in December 2021, FDA revised and published proposed agricultural water requirements (136). The modified rule only impacts the production water regulation but does not change the originally published post-harvest water requirements. Instead of complying with microbial criteria and testing requirements for pre-harvest water (mentioned above), it emphasizes the use of an agricultural water assessment.

An agricultural water assessment is an evaluation that is required to be conducted by covered farms. The factors include agricultural water system(s), agricultural water practices, crop characteristics, environmental conditions, and other relevant factors. Based on the assessment, growers need to determine their water quality and take any actions (i.e., corrective measures, mitigation measures, inspect water system(s), and test water microbial quality) (155). This modification is intended to make it easier for more growers to comply. Large, covered farms were initially required to comply with the water requirements on January 26th, 2022, but FDA is exercising enforcement discretion for the pre-harvest water provisions for all farms while the water requirements are being finalized (as of July 2022).

Agricultural Water Treatment Methods

When the number of generic *E. coli* in the water source has been proved to exceed safe levels or the agricultural water assessment determines the water is not safe for use, corrective

measures are required before resuming the use of the water. Multiple methods can be used to improve the microbial water quality, including filtration, chlorine dioxide, ultraviolet (UV) radiation, and chemical oxidation (i.e., ozone, hydrogen peroxide, and peracetic acid), as shown in Table 1.4. Of note, another agricultural microbial water quality test is required after applying the treatments to confirm that the agricultural water can be used under PSR.

Table 1.4 Log count reduction of pathogens survival in water by different intervention technologies. Note: ZVI means zero-valent iron.

Process	Treatment	Target pathogen	Log reduction	Reference
Filtration	50% ZVI	<i>E. coli</i>	1.7	(67)
	50% sand			
	35% ZVI	<i>E. coli</i>	2.45	(81)
	65% sand			
	35% ZVI	<i>Listeria monocytogenes</i>	2.51	(81)
	65% sand			
	50% ZVI	<i>E. coli</i>	3.54	(8)
	50% sand			
Chlorine dioxide	10 mg/L	<i>E. coli</i>	7	(140)
	3 mg/L	<i>E. coli</i>	5	(57)
	5 ppm	<i>Salmonella</i>	7	(83)
	5 mg/L	<i>Staphylococcus aureus</i>	5	(95)
	1.2 mg/L	<i>Enterococcus faecalis</i>	6.3	(42)
UV	267 nm	<i>E. coli</i>	4	(94)
	275 nm	<i>E. coli</i>	3.6	(94)
	310 nm	<i>E. coli</i>	0.5	(94)

	265 nm	<i>E. coli</i>	4.3	(93)
	275 nm	<i>E. coli</i>	4	(93)
	289 nm	<i>E. coli</i>	2.42	(53)
Ozone	35 mg/L	<i>E. coli</i> O157:H7	3.98	(36)
	45 mg/L	<i>E. coli</i> O157:H7	3.9	(36)
	1 mg/L	<i>E. coli</i>	5	(50)
	1 mg/L	<i>Enterococcus faecalis</i>	6.4	(50)
Hydrogen peroxide	3%	<i>E. coli</i> O157:H7	0.77	(54)
	100 mg/L	<i>E. coli</i>	3.13	(47)
	100 mg/L	<i>Enterococcus faecalis</i>	2.45	(47)
Peracetic acid	8 mg/L	<i>E. coli</i>	3.52	(47)
	8 mg/L	<i>Enterococcus faecalis</i>	4.5	(47)
	3 mg/L	<i>E. coli</i>	3.57	(16)

Filtration

Filtration is a physical process that separates solid particles and liquid from water and reduces the turbidity of the water. The turbidity of water is caused by suspended particles and floating debris, which can provide a protective barrier for microorganisms' survival (147). Thus, reducing the level of turbidity of water before use is important. Filtration systems are widely applied and effective in removing particulate matter, algae, and most microorganisms. Different filtration strategies are used based on the different sizes of target microorganisms (Table 1.5).

Table 1.5 The application for different sizes of filtration strategy.

Filtration methods	Microfiltration Membrane	Ultrafiltration Membrane	Nanofiltration Membrane	Reverse Osmosis
Target Size	approximately 0.1 μm	approximately 0.01 μm	approximately 0.001 μm	<0.0005 μm
Applied for	Sediment and bacterial	Viruses	Microorganisms	Microorganisms

One of the commonly known filtrations is the slow sand filtration (SSF), which lets the water goes through various biological, physical, and chemical processes that finally removes the pathogenic organisms and turbidity effectively. Recently, a great deal of literature highlighted the efficiency and economics of the zero-valent iron (ZVI) bio-sand filtration system for reducing pathogen load in contaminated water (8, 67, 70, 81). Anderson-Coughlin et al. showed that zero-valent iron filtration could significantly reduce the *E. coli* population after going through the filtration system (from 13.13 MPN/ml to 9.59 MPN/ml) as well as shortening the die-off interval of the rest of the bacteria (from -0.48 MPN/day to -1.50 MPN/day) (8).

However, according to the FSMA PSR (21 CFR 112.43) (137), currently, sand-filtration is not considered a validated water treatment method that can be used for removing physical particles and requires a further treatment process to complete the corrective measures. Moreover, the backflush water from the filter can also be problematic as it contains most of the particles from the untreated water. Thus, how to handle the backflush water properly needs to be considered.

Chlorine Dioxide

Chlorine dioxide (ClO_2) is a chemical compound that forms as a gas when the temperature is above 11°C and forms as a liquid when temperatures are between 11°C and -59°C . Due to its chemical properties, it is primarily used for disinfecting drinking water (3). Chlorine dioxide can kill pathogens by breaking the chemical bonds in their molecules and results in pathogen inactivation. Combined chlorine can be found in most sanitizers, such as bleach, and can be used for produce cleaning purposes (59, 75). As shock chlorination is the most cost-effective method of disinfecting water which has been adopted by commercial growers, multiple organizations published shock chlorination guidelines to help growers use it properly (17, 43, 86).

In a lab trial, studies showed chlorine dioxide acted as an effective reagent that can kill more than 99% of plant pathogens in a short time (112). Also, Bel et al. found that 10 mg/L of chlorine can remove 7 logs of *E. coli* rapidly (140). Other research reported a 5 log reduction of *E. coli* populations with 3 mg/L of chlorine dioxide (57).

However, limitations of chlorine dioxide application are also present. It can only work for a short time period within the existence of chlorine dioxide in the water, which cannot be applied as a long-term solution.

Chemical Oxidation

Chemical oxidation is a chemical treatment used to eliminate organic materials in water and wastewater using oxidation, which is both efficient and environmentally friendly. This method has been widely used in the process of purifying and treating wastewater to increase the

use of water (47, 68, 79, 158) as reclaimed wastewater. The reaction of the process is based on a powerful oxidizing agent (e.g., ozone, hydrogen peroxide (H_2O_2), and peracetic acid (PAA)) and obtained at a sufficient concentration.

Ozone disinfection is known as a more effective disinfectant than chlorine against protozoan parasites in water systems (41). Since ozone is highly reactive and unstable, the cost of operation and maintenance are relatively higher than other similar water treatment methods. Hydrogen peroxide is another water treatment method that is environmentally friendly. Several studies show that hydrogen peroxide itself is a slow-acting water disinfectant (requires high dosages and long contact times), but its performance can be enhanced by combining with Cu^{2+} , Ag^+ , and Zn^{2+} (37, 106, 107, 141). Peracetic acid (PAA) is another common and strong oxidant that is applied to disinfect wastewater. It is a man-made chemical that can act against a wide spectrum of pathogens at a low concentration rapidly (12). Kitis et al. ranked the disinfection efficiency of PAA towards microorganisms as bacteria > viruses > bacterial spores > protozoan cysts (68). However, literature also shows the remaining decomposed PAA increases the organic content in the water, which potentially enhances the pathogen regrowth (68, 73, 76). Of note, all the chemical oxidation treatments can be combined with UV and applied in the water treatment process, which results are more efficient than when applied alone (16).

Ultraviolet (UV) Light Disinfection

UV light is a type of electromagnetic (EM) radiation, which has frequencies of about 8×10^{14} to 3×10^{16} hertz (Hz), and wavelengths of about 200 - 400 nanometers (nm). Based on the

wavelength, the UV light can be divided into three sub-bands: UV-A (320-400 nm), UV-B (280-320 nm), and UV-C (200-280 nm). The commercial UV lights for disinfection use UV-C wavelength (typically 254 nm), which is effective against most bacteria, protozoa, and viruses, by acting directly on the DNA of microorganisms and resulting in a germicidal effect (120).

As clearer water has a higher transmission rate, UV light is more effective in treating low turbidity water (more clear, higher transmission) than high turbidity water (UV light gets blocked) (142). Thus, filtration is always recommended, especially for surface water, before running through the UV system. Also, the effectiveness of a UV system depends on the sensitiveness of microorganisms to UV light. Research shows a 280 nm wavelength UV reactor results in a 4 log reduction in *E. coli* (96), while applying the same wavelength to mesophilic bacteria led to a 1.4 log reduction (34). On the other hand, literature shows the pathogen log inactivation does not follow the increase of the UV wavelength. Nyangaresi et al. tested multiple wavelengths of UV-LED on the inactivation of *E. coli* in water, and the 267 nm UV-LED resulted in a 4 log inactivation, whereas the 310 nm wavelength inactivated 0.5 logs of the *E. coli* population (94).

Other in-lab water treatments

There are some water treatments that have not been applied to agricultural water that are more in the experimental stage of research and development and have not been extensively applied commercially on fresh produce farms. (1) Ultrasound technology: The ultrasound system affects objects in a liquid medium by leading to cavitation, and the forces created by it influence the biological system. Thus, ultrasound technology kills microorganisms by enhancing mass

transfer through the biological membranes. Its application range is based on the type of cavitation, which can be broadly divided into transient and stable types. Research shows that it takes five days for ultrasound treatment to reach to 85% reduction (82). Thus, it needs to be applied combined with other biocides, such as chlorine, UV, and electrolysis. According to research, ultrasound treatment improves the effect of chlorination on reducing the bacterial population in raw stream water (102), reaching about a 4 log reduction of fecal coliforms when applied with UV (156).

Electrolysis also can work as a water pretreatment method to reduce microbial contamination. Electrolyzed water (EW) is created by passing an electric current through a dilute saltwater solution in an electrolysis chamber. The process uses a membrane cell to separate an anode and a cathode and produce acidic EW, which contains free chlorine. Thus, EW can be used to reduce the survival of *E. coli*, *Listeria*, and *Salmonella* (51, 64).

Agricultural Soil Treatment Methods

The transmission of human pathogens can occur during the germination of seeds or after the transplantation of seedlings in contaminated soil (28, 71). Also, the internalization of human pathogens, specifically enteric pathogens, through root uptake has been a point of debate due to the inconsistencies among numerous scientific studies (27, 40). Reducing pathogen load in potentially contaminated soil is important, especially in organic farms, which commonly use biological soil amendments of animal origin (BSAAOs) to improve soil fertility and quality (116).

Solarization

Soil solarization is a method of sterilizing soil using solar heating by covering the pre-irrigated soil with transparent plastic sheets, which has the advantages of simple manipulation and low cost. This method has been proven as one of the efficient ways of controlling plant pathogens in soil (121). Recently, using solarization to inactivate human pathogens (i.e., *E. coli* and *Salmonella*) has been confirmed effective both in the field (152) and in a greenhouse (151).

Cover Crops Application

The establishment of cover crops on organic farms is becoming a standard practice to improve soil health and nutrient management. Cover cropping's potential benefits include reduced soil erosion, weed suppression, soilborne and foliar plant disease suppression, reduction of plant-parasitic nematodes, soil improvement, and nutrient management through nitrogen fixation and carbon accumulation (60, 99, 115). Recently, cover crops have gained attention for their ability to suppress soilborne and foliar plant diseases (61, 74, 145); this antimicrobial activity has been attributed to the production of secondary metabolites which protect the plant from microbial invasion (amongst other roles). While research on the antimicrobial activity of cover crops to suppress plant pathogens has been well developed in past decades, studies investigating their antimicrobial activity towards human pathogens in the soil is still limited (109).

Secondary metabolites in cover crops and their antimicrobial activity

The secondary metabolites are the organic molecules that are neither involved with growth nor development but act as a defense mechanism in the plant to respond to the biotic and abiotic stressors (98). In previous literature, secondary metabolites have been widely studied against plant insects (33, 128), plant viruses (153), and plant fungus (63, 148). Until recent years, the antibacterial effect of secondary metabolites in cover crops has been noticed and proved. For example, many species in the *Brassicaceae* family produce glucosinolates which are water-soluble compounds resistant to degradation until they contact the enzyme myrosinase. Then it hydrolyzes them into isothiocyanates (I.C.T.s), nitriles, and other products (56), which inactivate pathogenic bacteria like *Staphylococcus aureus*, *Enterococcus faecalis*, *Listeria monocytogenes*, *E. coli*, and *Salmonella spp.* (4, 110, 111, 143). Flavonoids (i.e., rutin, quercetin, isoorientin) are another large group of secondary metabolites found in species such as buckwheat which inhibit both gram-negative and gram-positive bacterial survival (1, 11, 146). This research brought up the potential of using cover crops as an amendment to inactivate pathogenic bacteria in produce farms.

Extension Education

The USDA National Institute of Food and Agriculture (NIFA) defines agriculture extension as “Extension that provides non-formal education and learning activities to people throughout the country — to farmers and other residents of rural communities as well as to people living in urban areas. It emphasizes taking knowledge gained through research and education and bringing it directly to the people to create positive changes” (89). Agricultural

extension services can originally be traced back to World War I. It served to support war costs by helping farmers increase crop yields by relaying agricultural knowledge. A set of survey data collected in 1980 showed that farmers and all household members had a positive and significant effect after extension education (39). This study emphasized the necessity and significance of agricultural extension education. Nowadays, educators are bridging the gap between new knowledge and regulations from scientific research to growers and aim to solve farms' difficulties and increase the production, food security, and standard of living of farm families. Universities and colleges are also engaging in extension and outreach teams to help local agricultural producers.

In recent years, because PSR criteria require growers to receive training, "PSR training" has become a hot topic in the extension field. In 2010, Cornell University, FDA, and USDA created the Produce Safety Alliance (PSA), which developed a standardized curriculum and nation- wide education to help fresh produce growers to comply with the requirements outlined in the FSMA PSR. According to data from 2013, FDA estimated that 35,029 total produce farms in the U.S. are required to comply with the PSR, which are qualified as very small farms and above (150). In order to fulfill the national training need, in 2016, PSA launched a train-the-trainer program to provide the opportunity for more people to be qualified to deliver the PSA grower training course and to respond to growers' questions during the training sessions. By the end of March 2022, PSA has held 2,332 grower training courses with 46,361 participants and 2,325 people qualified as a trainer in the US (105).

Extension Education Theoretical Framework

In addition to PSA grower courses, extension agents also provide independent sessions on good agricultural practices and general science-based knowledge to both PSR- covered and exempt growers. Inspired by adult and youth education program planning frameworks, the extension education theoretical framework aims to help extension professionals plan effective training. Within the expectation of positive change in growers' behavior after the training, the social marketing theory and the theory of planned behavior are used to develop the extension training sessions. The outcomes will increase the likelihood of growers having behavior changes (25).

Social Marketing Theory

The social marketing theory is adopted from commercial marketing theory and includes the 4P's of marketing, which are the product, price, place, and promotion (149). Instead of the profit motive, like in commercial marketing, the primary goal of social marketing is to achieve a positive behavior change in a population. Kotler and Zaltman et al. defined social marketing as “a social influence technology involving the design, implementation, and control of programs aimed at increasing the acceptability of social ideas or practice in one or more groups of target adopters.” (69). There are two fundamental philosophies in social marketing Theory (62) :

- An organization's objective should be to meet the needs and demands of the customers.
- In order for an exchange to take place, the organization and the customer must engage as nearly equals, with each having something to contribute to the other and each being able

to gain from the transaction.

Since understanding the barriers to behavior change adoption among the target population is the keystone of social marketing theory (87), audience analysis is one of the most important elements of the whole process. The same technique is also applied in the agricultural extension area, known as research-based needs assessment activities. Based on the results from each activity, professionals can implement changes to help farmers overcome the barriers.

Theory of Planned Behavior

The Theory of Planned Behavior (TPB) (5) is an extension of the Theory of Reasoned Action (TRA) (44). It started in 1980 to predict an individual's intention to engage in a behavior at a specific time and place. The principle was designed to understand all activities for which people would exercise self-control. Behavioral intent is a critical component of this model; behavioral intentions are influenced by one's attitude about the probability that the behavior will produce the desired result and one's subjective assessment of the costs and benefits of that outcome (119).

According to the TPB, the behavioral achievement is dependent on both motivation (intention) and skill (behavioral control). It divides attitudes into three categories: behavioral, normative, and control. The TPB is made up of six constructs (attitudes, behavioral intention, subjective norms, social norms, perceived power, and perceived behavioral control) that together reflect a person's actual behavioral control (5).

TPB is considered in extension education to help planners understand the attitudes that growers hold about agriculture practices, the factors that influence their thoughts, and the necessity of measured behavioral intent before and after the training.

Survey Research

Surveys are one of the common measuring instruments to collect feedback and opinions from growers. It enables researchers to be more efficient by employing a sample of respondents to generalize to the entire community (38). The data results can help extension professionals to obtain information to learn the current growers' practices and knowledge. For example, surveys have previously been conducted to assess growers' produce safety knowledge (100, 103) and needs (101) to help them to comply with produce safety best practices.

Typical survey methods include telephone, paper, in-person, and web-based surveys (38). Among these methods, electronic surveys are more adopted in the recent era with the advantage of low cost and fast transmission (38). Of note, due to the COVID situation, public activities were largely canceled due to the concern for public health, so the surveys were largely web-based. Thus, the survey (chapter 3) in the thesis is mainly web-based.

Conclusion and Future Outlook

Soil contamination and improper BSAAOs practice always raise the possibility of cross-contamination, especially on organic farms which commonly use BSAAOs to treat the soil. Although using cover crops to reduce the pathogen load in soil has not been widely studied, the hands-on results demonstrated the potential of cover crops to be used as a sustainable and cost-

effective soil treatment. Further research is needed to compare the efficiency of pathogen reduction among different species of cover crops.

Meanwhile, water will always remain a key component of the food production system. Agricultural water as the primary reservoir of human pathogens is continuously suspected as a transmission route for produce-related foodborne illness outbreaks. One of the current challenges focuses on inappropriate agricultural water practices, including using surface water for postharvest use without validated treatment and misunderstanding some terminologies (i.e., water used for handwashing is considered as postharvest water). These practices might not directly lead to an outbreak but will raise the possibility of cross-contamination. Considering some high-tech agricultural water treatment methods (i.e., UV and chemical oxidation), some of these show promise of pathogen reduction but are not commonly used on farms. Further research and extension education is required to deliver the latest technology to growers.

Another challenge is the latest proposed modifications to the agricultural water rule. Although the new proposed rule is focused on simplifying the production water criteria, growers and extension professionals need more time to adopt the new modified rules after years of education on the current agricultural water regulation. During this transition period between the current version of the rule and the finalized new modified rule, growers may be confused with the requirements and the potential impact of on-farm water practices (i.e., water microbial quality test, agricultural water assessments, and water system inspection). Moreover, the new proposed rule requires growers to determine the water quality by themselves through different

factors. However, this requires growers to have more knowledge of potential agricultural water hazards and good practices. Although the FDA provides an online agricultural water assessment tool to help growers determine the safety of water sources, it still is a barrier for growers located in rural areas that do not have internet.

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Chapter 2 - Evaluating the Efficacy of Cover Crops to Reduce Bacterial Pathogen Load in Contaminated Soil

Abstract

Cover crops are plants seeded before or after cash crops to improve soil health, reduce weed pressure, and prevent erosion. Cover crops also produce various antimicrobial secondary metabolites (i.e., glucosinolates, quercetin), yet the role of cover crops in moderating the population of human pathogens in the soil has rarely been investigated. This study aims to determine the antimicrobial capacity of three cover crop species to reduce the population of pathogenic soil microbiota. Four-week-old mustard greens (*Brassica juncea*, ‘Kodiak’), sunn hemp (*Crotalaria juncea*), and buckwheat (*Fagopyrum esculentum*) were mixed into autoclaved soil and inoculated with 7 log rifampicin-resistant generic *Escherichia coli* (*E. coli*). The surviving microbial populations on days 0, 4, 10, 15, 20, 30, 40 were enumerated. All three cover crops significantly reduced the population of generic *E. coli* compared to control ($p < 0.0001$); buckwheat resulted in the highest reduction (3.92 log CFU/g). An inhibitory effect ($p < 0.0001$) on microbial growth was also observed in soils containing mustard greens and sunn hemp. This study provides evidence for the bactericidal and bacteriostatic effects of particular cover crops. More research regarding the secondary metabolites produced by certain cover crops and their potential as a bio-mitigation strategy to improve on-farm produce safety is warranted.

Introduction

Agricultural soils contain a dynamic microbiome that supports plant health and are home to organisms that produce secondary metabolites (a group of chemical compounds that defend the organisms from biotic and abiotic stress) (35). However, agricultural soils are also a potential route for the transmission of human pathogens to fresh fruits and vegetables. Pathogens like *E. coli* and *Salmonella* are shed in the fecal matter of animals, where they can be spread throughout the field soil by contaminated irrigation water (4, 18) or through weather-related events such as flooding (9, 36). Recent investigations indicate that many of these human pathogens can persist in agricultural soils for up to 100 days (25, 26, 31, 38), increasing the potential for spreading to other produce and to the growing environment. Commodities like watermelons and cantaloupes, in particular, are considered at high risk for contamination from agricultural soils as they are grown on the ground and often make direct contact with soil (8). For this reason, improving agricultural soil management practices is a topic emphasized by federal regulation (e.g., the Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR)) and guidelines for certifications (e.g., Good Agricultural Practices (GAPS) certification).

While such federal regulations and programs offer some preventative measures, there is little information available for growers on strategies to actively reduce the human pathogenic microbial risk of their soils. For example, one of the most pressing concerns in agricultural soil management is the application of biological soil amendments of animal origin (BSAOs), which can carry a high microbial risk since animals serve as the primary reservoir for many human

pathogens (23, 49). The FSMA PSR §112.56 states, “untreated BSAAOs must not be applied in a manner that directly contacts the harvestable portion of the crop and must be applied in a manner that minimizes the potential for contact with covered produce after application” (42). Otherwise, the soil management recommendations currently largely defer to the National Organic Program, which requires growers to apply a die-off interval for raw animal manure application of 120/90 days for edible produce directly/not directly in contact with the soil before harvest (7 CFR 205.203) (41). Of note, soil contamination from BSAAOs is of particular concern for organic produce growing operations, which largely rely on non-chemical (BSAAO-based) methods like manure-based composts to enrich the soil (28). Clearly, there is a need to expand the repertoire of pre-harvest strategies which can reduce the transmission of pathogens from agricultural soils to fresh produce.

Cover crops are typically utilized by growers to improve overall soil agronomic properties but show promise to also reduce the populations of plant and human pathogens in agricultural soils. Cover crops are cultivated between growing seasons to improve soil health and nutrient composition (e.g., carbon, nitrogen) (16, 34), reduce soil erosion (48), and conserve water (43). Plants used as cover crops include grasses (e.g., *Panicum maximum*), legumes (e.g., *Trifolium pretense*), and non-legume broadleaves (e.g., *Fagopyrum esculentum*), which are fast-growing, improve soil physical properties, and may or may not fix nitrogen (7, 12, 14). Recently, cover crops have gained attention for their ability to suppress soilborne and foliar plant diseases (19, 20, 46); this antimicrobial activity has been attributed to the production of secondary

metabolites, which are organic molecules that are not involved with growth or development, but aid in plant response to biotic and abiotic stressors (27). Amongst other roles, secondary metabolites can protect the plant from microbial invasion. For example, many species in the *Brassicaceae* family produce glucosinolates which are hydrolyzed to form cytotoxic compounds that inactivate pathogenic bacteria like *Staphylococcus aureus*, *Enterococcus faecalis*, *Listeria monocytogenes*, *E. coli*, and *Salmonella spp.* (2, 32, 33, 44). Flavonoids (i.e., rutin, quercetin, isoorientin) are another large group of secondary metabolites found in species such as buckwheat which inhibit both gram-negative and gram-positive bacterial survival (1, 3, 47). While research on the antimicrobial activity of cover crops to suppress plant pathogens has been well developed in past decades, studies investigating their antimicrobial activity towards human pathogens in the soil is limited.

A recent study by Reed-Jones et al. focused on the effects of cover crops (cereal rye, hairy vetch, crimson clover, and hairy vetch-rye) on long-term population variation of *E. coli* and *L. innocua* in the soil (29). The study found a suppressing effect of cover crops on *E. coli* and *L. innocua* based on the microorganisms' innate characteristics and soil type (i.e., loamy, clay). The authors also highlighted how soil temperature, tillage practices, and the weather could affect the bacterial population. While the authors observed potential microbial growth inhibition in fields containing cover crops, they raised concerns that the root exudates of living cover crops into the rhizosphere could also promote the growth of human pathogenic bacteria in the soil.

The objective of this study was to determine if there were antimicrobial capabilities of three common cover crops – mustard greens, buckwheat, and sunn hemp – in field soil inoculated with generic *E. coli*. This study will not only provide information on the feasibility of the three cover crops as a soil bio-mitigation method but also the effect of these crops on microbial survival and inform laboratory-based methodologies for such studies.

Material and Methods

Growing Conditions and Plant Biomass Harvesting

The experimental mustard greens (*Brassica juncea*, ‘Kodiak’) (True Leaf Market, Salt Lake City, UT, USA), sunn hemp (*Crotalaria juncea*) (Sow True Seed, Asheville, NC, USA), and buckwheat (*Fagopyrum esculentum*) (Albert Lea Seed Company, Albert Lea, MN) were grown in a greenhouse at the Olathe Horticulture Research and Extension Centre (OHREC) in Olathe, Kansas during the fall of 2021. Mustard greens and buckwheat were sown in large containers (229 cm × 73 cm × 10 cm). Sunn hemp was grown in 9.5-L nursery containers to account for their more expansive root system and larger overall biomass. The containers were filled with organic Plant Pro™ potting soil (Ohio Earth Food, Hartville, OH, USA) before seeding. The greenhouse was climate-controlled, with temperatures ranging from 12.2 °C during the night to 28.9 °C during the day. Overhead irrigation was applied to the crops every other day in order to provide ample soil moisture

Whole crops (including the underground biomass) were harvested at the flowering stage before producing seeds (27 days after propagation). The crops were gently removed from the

containers to avoid excess root loss, and the soil was removed. The plant material was then collected and immediately transported to the lab for further processing, which was performed on the same day.



Figure 2.1 The growing environment for buckwheat and mustard greens.

Soil preparation

The soil used for *E. coli* inoculation in this study was a chase silt loam (40) collected from a field at the OHREC, which had been in perennial grass for the past three years. The soil was collected from the field and transported to the lab. Prior to sterilization, the collected soil was separated into autoclave bags (Fisher Scientific, Waltham, MA, USA); large clumps of sediment were manually pulverized to facilitate handling after sterilization. The soil was then autoclaved at 121.0 °C and 15 psi for one hour; sterility was confirmed using a bioindicator (Fisher

Scientific, Waltham, MA, USA) placed in the center of the bagged soil. Flame-sterilized tongs were used to extract the bioindicator, and the soil was set aside to cool to room temperature before use in the study.

Bacterial strain

Plant roots and shoots are naturally colonized by a dynamic population of microbes that interact amongst themselves and the plant tissues (30). To avoid background interactions from non-target organisms in the phytobiomass, the soil used in the study was autoclaved first, and rifampicin-resistant generic *E. coli* (ATCC 25922) was acquired from the Food Safety Lab at Kansas State Olathe was used as the study microorganism. The strain was previously stored with 50% glycerol (Fisher Scientific, Waltham, MA, USA) in 1.5mL microcentrifugation tubes (Eppendorf North America, Enfield, CT, USA) at -80.0 °C. To initially propagate the bacteria, a loopful of frozen culture was transferred to a culture tube containing 10 mL tryptic soy broth (TSB; Remel Inc., San Diego, CA, USA) and incubated for 24 h at 35.0 °C.

The methodology of Bhullar et al. (6) was followed to induce rifampicin resistance. Briefly, 0.1 mL of the forementioned microbial culture was spread to a plate of tryptic soy agar (TSA; Remel Inc., San Diego, CA, USA) containing 20 µg/mL rifampicin (RIF; Fisher Scientific, Waltham, MA, USA); the TSA-RIF plate was then incubated for 24 h at 35.0 °C. The procedure was repeated using TSA-RIF plates with increasing concentrations of rifampicin (20, 40, 60 µg/mL) until a strain resistant to 80 µg/mL concentrations of rifampicin was generated on TSA-RIF plates.

To culture, the organism for experimentation, one colony of rifampicin-resistant *E. coli* was transferred to 10 mL of TSB containing 80 μ g/mL RIF (henceforth noted as TSB-RIF). The tube was incubated for 24 h at 35 °C and sub-cultured again in 30 mL of TSB-RIF. To prepare the cells for experimentation, the cells were washed twice with phosphate buffer saline (PBS; Fisher Scientific, Waltham, MA, USA) with centrifugation (Allegra X-30, Beckman-Coulter, Brea, CA, USA; 10,000 rpm, 10 min, 20.0 °C) occurring prior to and in between each wash. The final volume was adjusted to a minimum concentration of 7 log CFU/mL.

Sample Preparation and Inoculation

This study utilized a randomized complete block design with repeated measures. Three independent replications of each treatment (cover crop species) were performed. To mitigate the impact of the ‘rhizosphere effect’ and address food safety concerns raised by previous literature (29), we used chopped whole-plant biomass for this study. The whole-plant material (containing roots, shoots, and leaves) was chopped into 3-cm long pieces to imitate mowing and tilling and was manually homogenized in three large containers (one container per cover crop species). To simulate the soil-cover crop mixture, 200 g of the chopped plant tissue sample, 200 g of the sterile soil sample, and 75 mL of inoculum culture were combined in a 24 oz. Whirl-Pak bag (Whirl-Pak, Madison, WI, USA). The 1:1 ratio of cover crops and soil is based on the assumption that cover crops will only be incorporated into the upper 10 cm of soil. For the non-treated control, 400 g of the sterile soil sample and 75 mL of inoculum culture were combined. The ratio of the solid matter and inoculum was based on preliminary trials to ensure all solid

matter would be moistened by the inoculum. Each sample bag was shaken and massaged 50 times to homogenize the mixture before being transferred into a quart-sized container (1.4 L) with a plastic plant saucer (Mudeela, Seattle, WA, USA) placed underneath. The samples were stored at room temperature (average 20.6 ± 0.02 °C) and with an average relative humidity of $32.9 \pm 0.4\%$ for 40 days. An average of 30 mL of sterile DI (deionized) water was added to each container each week to prevent the soil from becoming too dry to facilitate bacterial survival.

Sample Collection and Microbial Enumeration

Before collecting the sample on each sampling day, each container was mixed using a sterile plastic spoon; the spoons were sterilized by soaking overnight in 10% bleach, followed by rinsing with distilled lab water. A 10-gram sample was collected from each container and transferred to a Whirl-Pak bag containing 90 mL of 0.1% buffered peptone water (BPW; Remel Inc., San Diego, CA, USA). The sample bag was manually massaged to homogenize the sample. Serial dilutions were prepared in 0.1% BPW, and 100 μ L of the sample solution was then spread-plated onto TSA-RIF before incubating for 24 h at 35 °C. Plates yielding 25-250 colonies were recorded for analysis. Samples were collected 0, 4, 10, 15, 20, 30, and 40 days after treatment.

Statistical analysis

The statistical treatment structure utilized was a one-way ANOVA. The whole study was evaluated using three blocks of four pots each on days 0, 10, 20, 30, and 40. The surviving bacterial population at each time point was subjected to log transformation in order to achieve the variance homogeneity assumption.

The bacteriostatic (phase I) rate was calculated based on the difference of log-transformed surviving bacterial populations between day 0 and day 10. The bactericidal (phase II) rate was calculated based on the difference in log-transformed surviving bacterial populations between day 10 and day 40. The bacteriostatic (phase I) and bactericidal (phase II) rates were analyzed separately using the linear mixed model. Fixed effects of the model included all treatments. Random effects were the block and the error term. The main effect per day was assessed via least squares means (LSMs) and their standard errors (SEMs). Pairwise comparisons were carried out using the two-sided test. The Holm-Tukey method was used to adjust for multiplicity. All tests were conducted at the 0.05 significance level. Statistical analysis was executed via Statistical Analysis Software (SAS version 9.4; Cary, NC) PROC MIXED with option DDFM=BETWITHIN in the MODEL statement.

Results and Discussion

Overall trends in cover crops microbial reduction

Overall, the data showed that certain cover crops could reduce the microbial risk of soils containing generic *E. coli* within 30 to 40 days following a simulated cover crop incorporation event (*Figure 2*). This microbial reduction was cover crop- ($p < 0.0001$) and time-dependent ($p < 0.0001$). By day 30, soils amended with buckwheat or mustard greens had achieved a maximum *E. coli* reduction of 3.92 ± 0.16 log CFU/g and 3.77 ± 0.16 log CFU/g, respectively. For soils containing macerated sunn hemp, *E. coli* was still detectable on day 30, only reaching a maximum microbial reduction of 3.70 ± 0.10 log CFU/g 40 days after treatment.

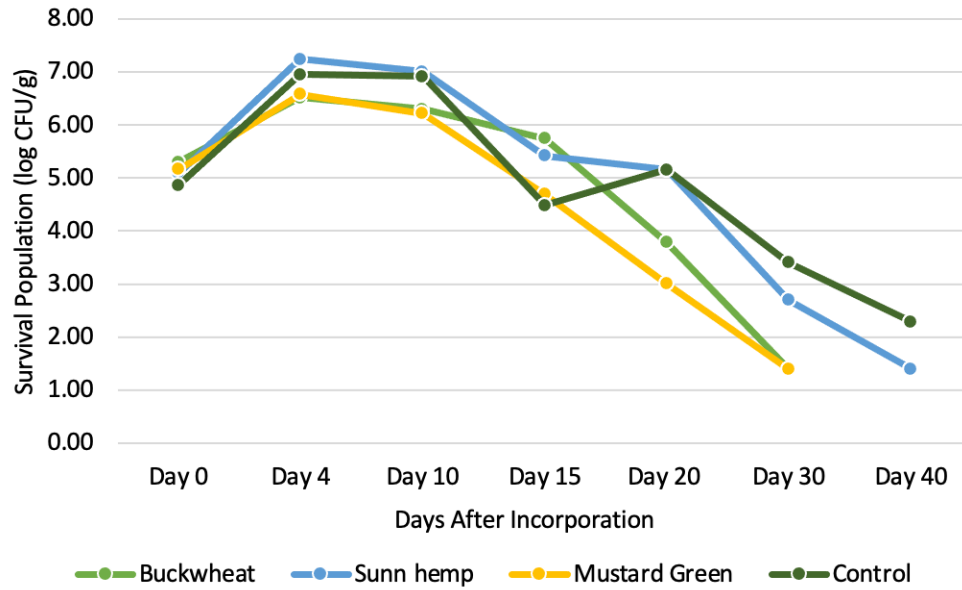


Figure 2.2 The surviving population of rifampicin-resistant *E. coli* in agricultural soil amended with macerated control, buckwheat, sunn hemp, or mustard greens

These overall findings provide potential insight into how these cover crops may be utilized to reduce human microbial risk and thus be used as a soil bio-mitigation strategy. For example, the persistence of *E. coli* in soils amended with sunn hemp could indicate that the secondary metabolites of buckwheat and mustard green tissues are more rapid in inducing microbial cytotoxicity compared to those in sunn hemp tissues. This observation is consistent with previous literature, which reports the cytotoxic activity of sunn hemp is predominantly in seeds rather than in root/shoot tissues (11, 24). In general, there were also no differences in *E. coli* survival between soils containing mustard greens or buckwheat throughout this study, despite their previously- documented different secondary metabolite composition and volume (5, 21).

A biphasic microbial variation curve (see Figure 2) was observed in the experiment, wherein statistically significant interaction effects ($p < 0.0001$) indicate unequal effects of the treatments

over time. The first phase (days 0 to 10) is characterized by a significant increase in the *E. coli* populations amongst all the treatments prior to their decline. From day 10 on, the microbial populations experienced a sharp decline until reaching levels below the detection limit. Topp et al. (39) reported observing a similar microbial growth curve and attributed the variation to the soil type and *E. coli* strains, amongst other factors. Of note, this previous study did not explore microbial survival in a chase silt loam as was utilized in this study, which is characterized by more than 50% silt and 12-27% clay (13), and is a soil composition (theoretically) more likely to have negligible influences on *E. coli* growth due to the relatively aerobic environment of low clay-content soils (37).

Modeling the surviving microbial population to study the biphasic survival curve

To further investigate the differences in microbial growth, the surviving microbial population was statistically modeled by fitting its survival to a nonlinear curve (*Figure 3; Equation 1*). The values used in the equation specific to each cover crop and the control are presented in *Table 1*. Overall, the models were able to explain a high degree (91% to 98%) of the observed variability in the microbial survival of each treatment.

$$y = \frac{x \cdot a + b}{1 + c \cdot x^d} \quad (1)$$

Equation 1, Where x is the days after initial inoculation

y is the total surviving microbial population

a is the rate of increase to the maximum peak

b is the multiplier that increases the height of the peak

c is the rate of decrease with respect to days after initial inoculation

d is the peak shape parameter

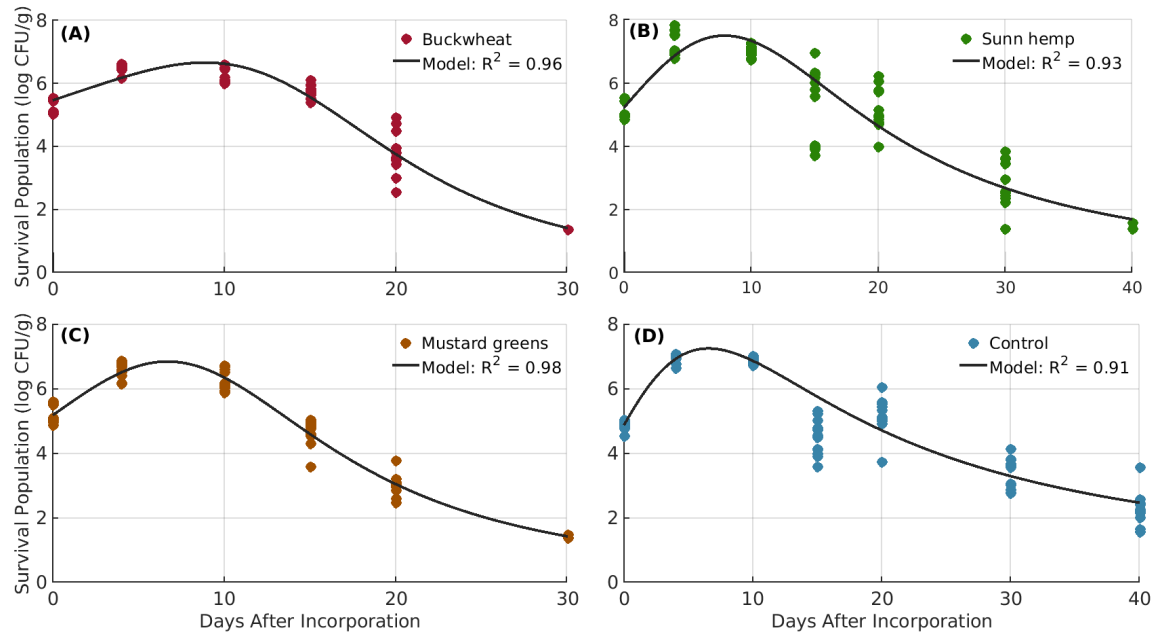


Figure 2.3 The fitted model estimating the surviving population of rifampicin-resistant *E. coli* in field soil supplemented with macerated control, buckwheat, sunn hemp, and mustard greens.

Table 2.1 Variable values by cover crops and control models.

	a	b	c	d	% Variance (R^2)
Control	0.7149	4.875	0.006.989	2.031	91
Buckwheat	0.1827	5.473	0.00001.497	3.827	96
Mustard greens	0.3761	5.184	0.0004.511	2.957	98
Sunn hemp	0.4654	5.227	0.0008.272	2.621	93

Bacteriostatic effects of cover crops during the exponential growth of *E. coli*

From days 0 to 4, there was a significant increase ($p < 0.05$) in the *E. coli* population in all treatments compared to their initial concentration. As evident by the value of a variable (Table 1), the control soils experienced a more rapid rate of increase to the maximum surviving population than the soils containing macerated cover crop tissue. Between the cover crops, sunn hemp showed a more rapid microbial population growth rate than buckwheat ($p < 0.0001$) or mustard greens ($p < 0.0001$). There were no significant differences between the initial growth rate (a) of the buckwheat and mustard greens ($p = 0.6178$) or the sunn hemp and control ($p = 0.2278$). After day 4, the soils containing macerated buckwheat ($p = 0.0021$), mustard greens ($p < 0.0001$) or sunn hemp ($p = 0.0010$) began experiencing a minor decrease in generic *E. coli* populations. In contrast, the microbial population of the control sample attenuated from days 4 to 10 as there were no statistically significant differences ($p = 0.6927$) detected during this time period.

These findings indicate that the exudated phytochemicals of all three macerated cover crops can impair the growth of *E. coli* during this initial growth phase, with buckwheat and mustard greens being the most effective. It is also likely that these results are reflective of an initial exudation of phytochemicals from the macerated cover crops stimulated by the artificial tillage. Divekar et al. note that many secondary metabolic production pathways are rapidly induced by physical stimuli (e.g., cutting of the plants) (15). Accordingly, using a rotary tiller or similar tool

to physically cut the cover crops (instead of only rotating them into the soil) may be critical to initiating their antimicrobial effect.

Bactericidal effects of cover crops during declining *E. coli* population

During the second phase (days 10 to 40), there was a more rapid, significant decrease ($p < 0.0001$) in the *E. coli* population in all treatments compared to their concentration on day 10. In this phase, the *E. coli* could be experiencing die-off due to limited nutrients available in the environment which is accelerated by the presence of cover crop tissues. As evidenced by the value of the c variable (Table 1), the control soil and soils containing macerated sunn hemp showed a slower microbial reduction rate than buckwheat or mustard greens ($p < 0.0001$). Buckwheat experienced the most rapid decline, but there were no significant differences detected between the microbial population of mustard greens and buckwheat ($p = 0.5626$).

The most rapid microbial decline for soil containing macerated sunn hemp occurred between days 20 to 30 which led to a 2.46 ± 0.15 log reduction of generic *E. coli*. Buckwheat also experienced the most rapid population decline (2.40 ± 0.15 log CFU/g) between days 20 and 30. Conversely, soils containing macerated mustard greens exhibited a microbial decline of 1.68 ± 0.26 log CFU/g between day 10 and day 20. There were no significant differences in the surviving population of *E. coli* between mustard greens and buckwheat except on day 20.

Interestingly, the results of this bactericidal phase do not necessarily align with the expectations from the literature. During this phase, it is conceivable that the more rapid decline is due to the cover crops beginning to degrade in the soils, further releasing exudates containing

anti-microbial secondary metabolites (10). Based on the degradation rate, sunn hemp should have the most rapid rate of degradation as the tissues decompose within two weeks post tillage (45). According to Wang et al., the phenolic compounds in buckwheat tissues typically degrade after 37 days, indicating a lower rate of metabolite exudation (47). As this study reports trends in antimicrobial activity which are opposite to what is expected (sunn hemp has less effective microbial reduction rate than buckwheat throughout the study), more investigation regarding the exudation and degradation of the principal secondary metabolites within the tissues of each cover crop is warranted.

Study Limitations

Though the data from this study fit the conventional assumptions for ANOVA, the statistical methodology needed to account for differences in the initial *E. coli* concentration between the control and cover crop-containing soils on day 0 (*Figure 1*). Accordingly, only inferences between the rate(s) of population growth/decline in each cover crop could be made. We believe this significant difference in *E. coli* populations on day 0 can be ascribed to the different compositions of the control samples. As no plant tissues were added to the control containers, 400g of farm soil was used as the control and was inoculated with the same volume of inoculum as other treatments. According to Fick's diffusion law, the diffusive flux goes from areas of high osmolyte concentration to areas of low osmolyte concentration (17). The lower moisture content of the control soils relative to the higher moisture content of the soils containing fresh cover crop tissues likely resulted in a more even distribution of the inoculum in the control soils. Thus,

when collecting the same weight of sample from each treatment, the control group resulted in a diluted generic *E. coli* population result.

The sole use of a gram-negative experimental pathogen presents another limitation of this study. This study evaluated the survival of non-pathogenic *E. coli* as a surrogate for pathogenic *E. coli* or other gram-negative species that may behave in a similar manner (i.e., *Salmonella* spp.), which are commonly found in the gut of wild and domestic animals. However, Locatelli et al. (22) reported higher stability and persistence of gram-positive bacteria (i.e., *Listeria monocytogenes*) in agricultural soils. When Neiunna et al. (29) inoculated cover crop-containing soils with *Listeria innocua*, the authors observed a lower antimicrobial efficacy against the pathogen compared to the gram-negative organisms. This reduced efficacy towards gram-positive organisms may not impact the application of cover crops in states dominated by cattle or poultry production, which have a higher risk of detecting gram-negative *E. coli* and *Salmonella* spp. in the soil. However, this could limit the applicability of the tested cover crops as a microbial risk mediation strategy in operations that struggle with high-risk sources of gram-positive organisms.

Conclusions

All three cover crop species resulted in a microbial reduction to non-detectable levels, while buckwheat and mustard greens also displayed a bacteriostatic effect on the growth of *E. coli* during the exponential microbial growth phase (days 0-10). Future studies should account for extrinsic and intrinsic environmental factors to provide more suggestions for the on-farm

applicability of cover crops as a bio-mitigation strategy. Factors like sunlight, temperature variation, and native soil microbiome interactions were eliminated in this experiment in order to determine if there was an effect of the cover crops on microbial populations. Moreover, the differential efficacy of the cover crops demonstrates the need for characterizing the mechanisms that drive the effects we observed in each of the different cover crop species, particularly including the secondary metabolite content and concentration within the plant tissues. A better understanding is needed of their corresponding environmental stability and exudation patterns as the crop decomposes into the soil. Overall, this is the first lab-trial study for human pathogen reduction on agricultural soil within cover crops amendments. The results demonstrated these cover crops as a promising, environmentally sustainable bio-mitigation strategy for human pathogens in soil that warrant more investigation to promote their use in produce growing operations.

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Chapter 3 - Knowledge and Current Practices Related to Agricultural Water Microbial Quality among Kansas and Missouri Produce Growers

Abstract

Kansas State University and University of Missouri Extension educators have been providing training and information on agricultural water microbial quality to help produce growers reduce risk. However, we recognized the need to determine knowledge gaps among Kansas and Missouri growers related to agricultural water quality and best practices. A survey was developed to determine future extension outputs and activities to encourage growers to improve their practices related to water quality. The survey was distributed to Kansas and Missouri produce growers attending in-person or online produce safety-related events in late 2020 and early 2021 and was also distributed through email lists of produce growers from both states. Survey results ($n = 101$) indicate that 13.9% of the respondents tested their water for generic *Escherichia coli* (*E. coli*) more than once a year, while 38.6% of the participants have never tested their water. Approximately half (59.3%) of respondents indicated they use municipal water for postharvest uses, while 6.7% indicated the use of untreated surface water for postharvest activities. Due to the concern of potential water contamination risks, researchers suggest that further training and educational resources would help growers improve practices related to water quality and produce safety.

Introduction

In recent years, foodborne disease outbreaks resulting from fresh produce have continued to occur (4, 12). Because these commodities are often consumed raw (26, 32), it is imperative that

fresh produce growers use the safest practices possible. From 2010 to 2017, a total of 85 multistate outbreaks associated with fresh produce with confirmed etiologies occurred in the United States, resulting in a known combined total of 4,658 known illnesses, 1,187 hospitalizations, and 55 deaths (4). Further, before 2015, numerous fresh produce outbreaks have been attributed to contaminated agricultural water in commodities such as leafy greens (15, 22), tomatoes (2), and melons (33). Thus, water quality management practices became a major and critical aspect of the Food and Drug Administration's (FDA) Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR) (31).

The PSR, which was finalized in 2015 to take its place as one of the seven foundational rules under FSMA, is the first U.S. Federal regulation to have established science-based minimum standards for the safe growing, harvesting, packing, and holding of fruits and vegetables grown for human consumption. In December 2021, the FDA proposed a revision to Subpart E of the PSR that would change the preharvest agricultural water requirements for covered produce, other than sprouts (30). The FSMA PSR and the proposed revision state the considerations and requirements related to agricultural water management to improve the microbial safety of agricultural water and therefore of produce. In December 2021, FDA announced their intent to exercise enforcement discretion and also act to extend the compliance date for the agricultural water (31).

As per the FSMA PSR, "agricultural water means water used in covered activities on covered produce where water is intended to, or is likely to, contact covered produce or food contact surfaces." Agricultural water is classified into two categories based on the different usage periods: production water and postharvest water. Production water refers to water used during growing activities whereas postharvest water includes all uses of water during or after harvest (7,

31). Since the use of postharvest water is so close to consumption, microbial water quality is especially important (6). Thus, produce growers must meet the requirements of §112.44(a), i.e., no detectable generic *E. coli*/100mL water sample for water used postharvest. The proposed revisions to Subpart E focus on production (i.e., preharvest) uses of agricultural water. These revisions emphasize conducting agricultural water assessments for production water sources and uses, eliminating the federal requirement of an ongoing microbial water quality profile with periodic updates through water quality monitoring.

Each year, Kansas and Missouri growers produce approximately \$26 million (13) and \$81 million (7), respectively, of fruits and vegetables. Kansas State University and University of Missouri Extension educators have been providing training and information on agricultural water microbial quality, based on current and newly proposed FSMA PSR agricultural water requirements, to help produce growers from Kansas and Missouri reduce risk to produce from agricultural water. Although research shows that knowledge gain alone leads to limited behavior change, it can provide people with more informed choices that may or may not lead to behavior change (16). Pratt and Bowman (21) suggest that individually-relevant, problem-focused, and hands-on activities can help extension educators prompt personal behavior change. To help prompt this positive behavior change, educators need a better understanding of the gap between water quality best practices and produce growers' knowledge and actions related to agricultural microbial water quality.

Survey research (20) is one method that allows extension educators to easily understand a large number of growers' current water practices and knowledge gaps in a shorter time. Other surveys have previously been conducted to assess growers' general produce safety knowledge (17, 19) and needs (18) to help them to comply with produce safety best practices. Perry et al.

(17) found that North Central US produce growers have a knowledge gap in understanding agricultural water handling practices compared with other topics of the FSMA PSR, which highlights the needs for food safety extension educators to focus on delivering information on agricultural water best practices to produce growers.

This survey aims to determine gaps between safe water management practices (as outlined in the FSMA PSR requirements/ proposed requirements and other produce safety best practices) and (i) the current knowledge and (ii) the current practices of produce growers from Kansas and Missouri related to agricultural water quality. This will enable extension educators to develop additional training and resources on agricultural microbial water quality accordingly to better meet the needs of growers.

Materials and Methods

The project team developed a 14-question survey instrument based on similar surveys (18). Before implementing the survey, 4 extension experts reviewed the survey questions for face and content validity. The Kansas State University Produce Safety Extension Associate also performed cognitive interviews (14) with growers from the target population to further validate the survey. The Kansas State University Human Research Institutional Review Board (IRB) approved the use of the survey. The survey (*Appendix 1*) included questions on the growers' knowledge and practices related to microbial water quality, which was administered from October 2020 to August 2021. Due to in-person meeting restrictions related to the COVID-19 pandemic, produce growers mainly completed the survey online in Qualtrics ($n = 76$) during online produce safety-related trainings or by distribution of the online survey link to email listservs of produce growers in Kansas and Missouri. Other respondents ($n = 30$) completed paper copies of the survey during an in-person horticulture farm field day or in-person produce

safety training sessions. Two drawings for gift cards were utilized to incentivize participation in the survey both on-line and in-person. To optimize data collection, potential survey respondents were asked to only complete the survey if they were able to answer questions related to their operations' water management practices. Because most produce farms in Kansas and Missouri have only a few people working on them (28, 29), we are confident that most people filling out the survey are actively involved in the water management practices on their farm, and thus qualified to complete the survey.

The 14-question survey instrument included multiple choice questions with some open text response options designed to understand growers' knowledge and practices related to agricultural water use in both production and postharvest stages, as well as their understanding of the microbial quality of agricultural water. Questions included grower age, farm location, water testing frequency, and water usage for both production and postharvest activities.

The data were analyzed in Qualtrics using the cross-tabulation (crosstab) analysis method (10, 11) (also known as contingency table analysis) to identify the growers' knowledge of microbial risks related to different water sources and how they handle those risks. Cross-tabulation analysis compares the results of different questions from the same respondent to gain more insight into grower water quality management behaviors based on the risk of their water source. For example, growers who indicated that they did not treat their water source but indicated that they used a municipal water source (which is treated by a third-party company) were considered as having a safe water management practice. The responses from questions related to production or postharvest water uses, treatment application status, and microbial water quality test frequency were classified by use of different water sources (surface, ground, and municipal water). The data from any questions that allowed respondents to provide an open-

ended response were added to existing responses or a new category of responses was added to the question, based on the keywords in those responses. For example, a separate category was created for growers who wrote they used water from a rain barrel when selecting ‘other’ water for their water source.

Growers were asked if they were GAP (Good Agricultural Practices) certified (rather than if they were covered by the FSMA PSR) because the project team felt that growers would be more comfortable responding to the question of GAP certification, rather than FSMA PSR coverage. Accordingly, responses to the questions about water testing and water treatment were stratified based on their GAP certification status. Although GAP certification is generally a produce buyer requirement and is not a regulatory requirement, many growers view certification as an opportunity to improve their likelihood of compliance with the PSR. Thus, many growers covered by the PSR also obtain GAP certification.

Because some respondents did not answer all of the survey questions, the total number of responses to each question related to water sources in the cross-tab analysis may be different. Additionally, the total number of responses for questions regarding agricultural water source use could differ as growers may have reported using more than one water sources for one or more pre- or postharvest activities. To reduce item non-response bias, surveys having less than two-thirds of the questions completed were excluded from the final data analysis.

Results and Discussion

Results

A total of 106 survey responses were received. Of the 103 responses included in the final data analysis, 51 (49.5%) were from Kansas, 50 (48.5%) were from Missouri, one (1.0%) was marked as unknown, and one (1.0%) did not complete the question. The age range of growers

completing the survey were as follows: 18-29 ($n = 18$, 17.5%), 30-39 ($n = 15$, 14.6%), 40-49 ($n = 26$, 25.2%), 50-59 ($n = 19$, 18.5%), and above 60 ($n = 25$, 24.3%) years old. Most respondents ($n = 90$) were currently selling or planning to sell their produce to others. Most respondents ($n = 87$) were currently not GAP certified, as shown in Table 3.1.

Table 3.1 Reported microbial water quality testing ($n = 100$) status and GAP certification status ($n = 101$) by state.

	Kansas	Missouri	Total
Have tested water	16 (31.4%)	27 (55.1%)	43
Never tested water	25 (49.0%)	13 (26.5%)	38
Use third party test results	10 (19.6%)	9 (18.4%)	19
Total	51	49	100
GAP certified	2 (3.9%)	12 (24.0%)	14
Working towards being GAP certified in the future	7 (13.7%)	8 (16.0%)	15
Not GAP certified	42 (82.4%)	30 (60.0%)	72
Total	51	50	101

Of the participants which indicated they are currently growing fresh produce ($n = 101$), participants indicated their frequency of generic *E. coli* testing as more than once a year ($n = 14$, 13.9%), once a year ($n = 11$, 10.9%), 2-3 times in the last ten years ($n = 11$, 10.9%), once in last ten years ($n = 7$, 6.9%), never tested ($n = 39$, 38.6%), or that a third-party water source tests their water ($n = 19$, 18.8%). Between the two states (Table 3.1), 31.4% ($n = 16$) of Kansas growers said that they have tested their water in the past ten years compared to 55.1% ($n = 27$) of Missouri growers. Of the growers indicating they are GAP certified ($n = 14$), 10 (71.4%)

reported testing their water more than once a year and six (46.2%) reported that they treat their postharvest water sources. Of the non-GAP certified growers ($n = 87$), four (4.6%) growers test their water more than once a year and 15 (17.4%) growers treat their postharvest water sources (Table 3.2, Table 3.3). Because the questions inquiring about the type of water source used for production and postharvest applications allowed for more than one response, the total number of responses varied by question, as shown in *Figure 1*.

Table 3.2 The frequency of microbial water quality testing by GAP certification status (n = 101).

	More than once a year	Once a year	2–3 times in last 10 years	Once in last 10 years	Never test	Third party water test	Total
GAP certified	10 (71.4%)	0 (0.0%)	1 (7.1%)	1 (7.1%)	0 (0.0%)	2 (14.3%)	14
Working towards being							
GAP certified in the	1 (6.7%)	3 (20.0%)	1 (6.7%)	0 (0.0%)	8 (53.3%)	2 (13.3%)	15
future							
Not GAP certified	3 (4.2%)	8 (11.1%)	9 (12.5%)	6 (8.3%)	31 (43.1%)	15 (20.8%)	72

Table 3.3 Treatment application status for postharvest water based on GAP certification status (n = 99)

	Apply treatment	No treatment applied	Total
GAP certified	6 (46.2%)	7 (53.8%)	13
Working towards being			
GAP certified in the future	3 (21.4%)	11 (78.6%)	14
Not GAP certified	12 (16.7%)	60 (83.3%)	72

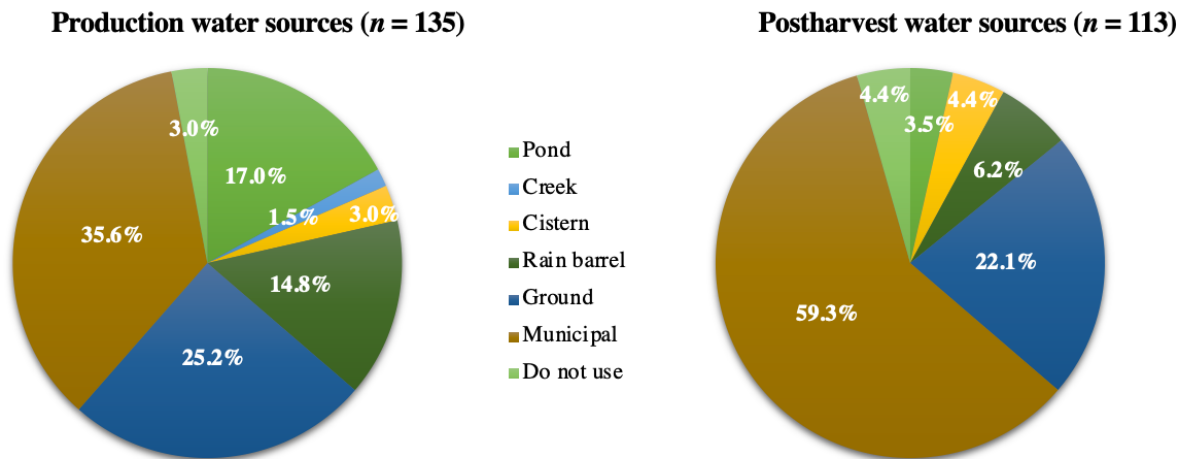


Figure 3.1 The percentages of different water sources that growers use for production ($n = 135$) and postharvest ($n = 113$) activities. (Note: among the 101 responses, several used more than one water type)

Municipal ($n = 48$, 35.6%), well ($n = 34$, 25.2%), pond ($n = 23$, 17.0%), and rain barrel ($n = 20$, 14.8%) water sources were the most commonly reported sources of production water (Figure 1). Municipal ($n = 67$, 59.8%) and well water ($n = 25$, 22.1%) were the most commonly reported water sources for postharvest water. Based on the number of responses for each water source, respondents appear to commonly utilize more sources of water for production applications ($n = 135$) than postharvest ($n = 113$) applications.

Participants also reported their applications of production and postharvest water (Figure 3.2). Of the survey respondents ($n = 101$), 90 (89.1%) indicated that they used production water in their operation. The three main reported uses of production water included irrigation ($n = 90$, 100%), mixing with crop sprays ($n = 38$, 42.2%), and mixing with fertilizers ($n = 38$, 42.2%). Of those growers that report using water for irrigation, 70 (77.8%) reported using drip irrigation, 37 (41.1%) reported using overhead irrigation, and 20 (22.2%) reported using drip irrigation under plastic. Furthermore, 90 (89.1%) indicated that they used postharvest water in their operation.

These growers reported their main uses of postharvest water to be for handwashing ($n = 77$, 85.6%), rinsing/washing produce ($n = 73$, 81.1%), and cleaning and sanitizing of food contact surfaces ($n = 68$, 75.6%). Other uses of postharvest water reported included ice making ($n = 7$, 7.8%), dunk tank cooling ($n = 6$, 6.7%), single pass/spray cooling ($n = 4$, 4.4%), and postharvest fungicide or wax applications ($n = 3$, 3.3%).

Of survey respondents who indicated that they used production water in their operation ($n = 90$), 75 (83.3%) reported that they do not treat their production water. Of the respondents who reported treating their production water, four (4.4%) reported using chemical sanitizers whereas 11 (11.1%) respondents indicated using physical methods such as sand filtration. Of survey respondents who indicated that they used postharvest water in their operation ($n = 90$), 68 (75.6%) reported that they do not treat their postharvest water. Fifteen respondents (16.7%) reported treating postharvest water with a chemical sanitizer and four (4.4%) reported using other treatments such as sand filtration or ultraviolet light. When comparing water treatment status and the water source used (shown in *Figure 3.3*), six (6.7%) respondents reported using surface water (ponds, cisterns, or rain barrels) for postharvest use without applying any treatment.

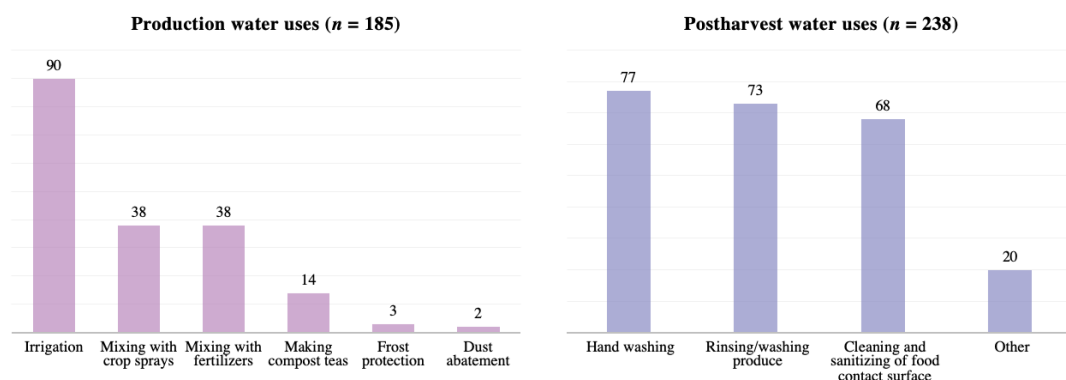


Figure 3.2 The different uses of production water ($n = 185$) and postharvest water ($n = 238$).

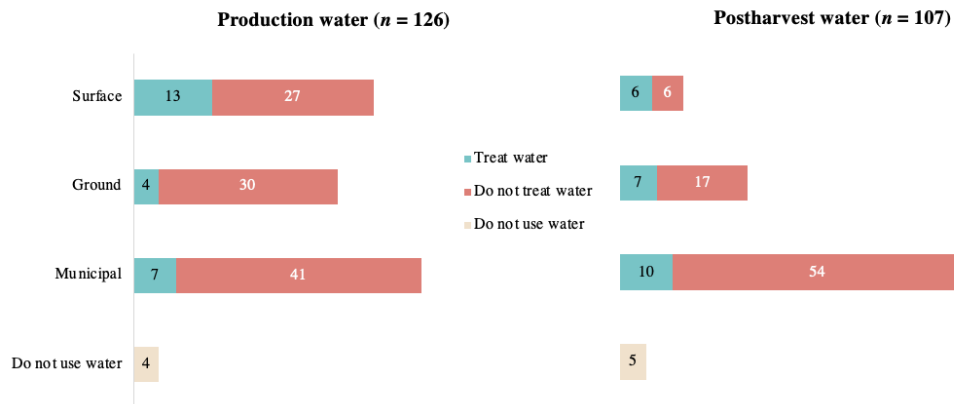


Figure 3.3 Treatment status for each water source that growers use for production (n = 126) and postharvest (n = 107) activities.

When asked what other practices (besides treatment) growers use to maintain/improve production water quality, most ($n = 70$, 77.8%) respondents using production water in their operation indicated that they did not use any other practices. Nineteen (21.1%) of the participants indicated that they conducted an annual evaluation of their water distribution system. When managing the quality of their postharvest water, 61 (67.8%) respondents who indicated that they used postharvest water in their operation ($n = 90$) reported that they do not use any additional practices, 17 (18.9%) reported routine cleaning and sanitation of tanks, 15 (16.7%) reported monitoring for buildup of organic material, 14 (15.6%) respondents reported conducting an annual evaluation, 10 (11.1%) reported monitoring water temperature during use, and nine (10.0%) reported using a routine water change schedule.

Discussion

Agricultural water quality remains a large concern for the fresh produce industry across the United States, particularly in the Midwest where previous studies indicate that agricultural water quality management is not well understood (17).

Most of the respondents demonstrated a preference for water sources with a lower microbial risk profile (such as municipal water) for postharvest activities, as compared to using surface water postharvest, which is higher risk. Specifically, the survey data indicated that – unlike production water, where municipal (35.6%) and surface water use (36.3%) were nearly equally reported – there is a higher use of municipal water (59.3%) and lower use of surface water (14.1%) for postharvest applications (*Figure 1*). As previous research has shown, microbial populations in surface water increase after events such as agricultural runoff (rain, melted snow, etc.) (3, 24, 27). Surface water is also more exposed to potential contamination from the surrounding open environment, and thus is considered to have a higher possibility of contamination (1, 8, 23).

According to the data collected from the survey, a minority of growers use specific postharvest water management practices that many would consider higher risk. Using surface water without any treatment for postharvest applications poses a higher risk of foodborne disease outbreaks, thus the FSMA PSR agricultural water criteria, GAP certification requirements and general produce safety best practices do not allow use of untreated surface water for postharvest activities (31). Interestingly, six survey respondents indicated that they were using untreated surface water for postharvest uses, and eight respondents indicated they treated their agricultural water using methods such as (bio)sand-filtration. Of note, sand-filtration is not a validated agricultural water treatment method according to the FSMA PSR (21 CFR 112.43) (31). However, growers do currently utilize sand filtration in irrigation systems to remove larger, physical particles, sometimes with the added misconception that it also can remove pathogens. This indicates that further education and training is needed to emphasize the significance of

microbial risk in postharvest water, as well as how to select an appropriate (validated) treatment method.

Conducting regular inspection and maintenance of the water source and distribution system is currently a requirement of the FSMA PSR (21 CFR 112.42) (31) and is a produce safety best practice to limit the risk of microbial contamination. By conducting regular inspections, growers can evaluate the risks to their water sources. The inspection includes identifying any broken part of the system structure, any wildlife evidence around open water sources, and other risks. Most of our survey respondents are not currently conducting an annual agricultural water system inspection. Thus, extension educators should provide practical information to growers on how to inspect their water sources regularly and encourage them to conduct a water system inspection at least annually, which is a requirement for growers covered by the FSMA PSR (21 CFR 112.42) (31). Of note, the proposed §112.43 is intended to supplement the requirements of the proposed §112.42 which will require a covered farm to regularly inspect and routinely maintain the components of its agricultural water system. While proposed §112.42 is focused on agricultural water system components, the proposed §112.43 would require covered farms to conduct a more comprehensive assessment of possible sources and routes by which known or reasonably foreseeable hazards are reasonably likely to be introduced into its preharvest agricultural water (30).

The FSMA PSR also highlights irrigation method as an important risk factor for contamination risk based on if the water contacts the harvestable portion of the produce. In most situations, overhead irrigation results in direct contact with the harvestable portion of the produce, which can result in a higher contamination risk than other irrigation methods. Conversely, drip irrigation (particularly drip irrigation under plastic) is considered to have a

relatively lower risk of contamination for crops grown above the ground. Of note, this characterization does not apply to root crops such as carrots, beets, and radishes (25). Most of the survey respondents indicated that their main use of production water was irrigation, namely drip irrigation (69.1%) or overhead irrigation (31.3%). However, the findings of the study call attention to particular agricultural water practices including the use of untreated surface water for direct water application during postharvest, and seldom testing the sanitary quality of agricultural water sources. Although not specifically measured by this study, further investigation and potentially extension efforts may need to occur to clarify how growers can reduce microbial risk by selecting irrigation methods that reduce the probability of water contact with the harvestable portion of the produce.

In the survey, the question about the frequency of conducting microbial water testing did not specify testing frequency of “production water” and “postharvest water” separately. Accordingly, crosstab analysis for the frequency of water testing for different sources of water used for different purposes (preharvest or postharvest) could not be conducted. Moreover, it was not possible to discern between treated and untreated sources when more than one source was indicated by the grower in this survey. We also did not include questions related to the location of their water source in relation to animals or other potential sources of contamination. These points could have been clarified by increasing the length of the survey. However, the expert panel reviewing the survey was concerned about the adverse effect of increasing the survey length on the response rate as some participants required more than 10 minutes to complete the survey. In a study of the effects of length on web-based survey completion, Galesic and Bosnjak (9) found that participants anticipating a longer survey completion time were less likely to finish the survey.

Conclusion

This survey was intended to provide information on the differences between best practices and the actual practices of Kansas and Missouri produce growers focusing on the use of agricultural water. Produce growers need to continue to improve their practices related to water quality to ensure that they are selling the safest produce possible. The survey responses will provide insight into the development of additional agricultural water safety educational materials for produce growers. The results indicated that efforts are needed to continue to encourage growers to test their water quality, to conduct at least an annual inspection of water sources and distribution system, and to use a validated surface water treatment method before using it for postharvest applications. The authors suggest that future extension trainings should emphasize the importance of microbial quality of agricultural water sources, and how risk to produce safety can be affected by selection of water source, decision to treat, and (in the case of production water) application method.

Acknowledge

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Chapter 4 - Agricultural Water Related Extension Education

Handouts

Based on the anecdotal evidence and survey responses from this thesis (Ch. 2), we identified the critical need to address a few important agricultural water safety-related topics that include the following:

New Modified FSMA PSR Agricultural Water Requirement Proposal

FSMA PSR published the agricultural water regulation in 2016, including using the different microbial quality criteria to value both production and postharvest water quality. However, after the implementation, the feedback from different farms focused on "the rules are too complicated and cost too much." Therefore, the FDA revised and published a proposed agricultural water requirement in December 2021 focused on production water. This handout summarized the major modification of the latest water requirement and aimed to help growers understand and comply with the proposed rule.

QUICK REFERENCE GUIDE

New Modified FSMA PSR Agricultural Water Requirement Proposal



In December 2021, the United States Food and Drug Administration (FDA) proposed a revision to Subpart E of the Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR) that would change the *pre-harvest agricultural water requirements* for covered produce, other than sprouts.

What is the proposed change?

The proposed revision to Subpart E modifies the previous requirement for produce growers to *replace the microbial criteria and testing requirements* for pre-harvest agricultural water with new provisions for *conducting pre-harvest agricultural water assessments*, providing more flexibility to produce growers in evaluating risks.

These aim for hazard identification (including consideration of agricultural water sources, distribution systems, and practices, as well as adjacent and nearby land uses, and other relevant factors), and using the results of the assessment to determine whether *corrective or mitigation measures* are needed to reduce the potential for contamination of produce or food contact surfaces with hazards associated with pre-harvest agricultural water.

What is an “agricultural water assessment” ?

It refers to an *evaluation*, conducted by a covered farm, of factors in its agricultural water system used during growing activities for non-sprout covered produce, its agricultural water practices for such pre-harvest water, crop characteristics, environmental conditions, and other relevant factors (including test results, where appropriate).

Factors	Description
Agricultural water system(s)	• Location and nature of the water source (including whether it is ground or surface water) • Water distribution system type (such as whether it is open or closed to the environment) • Degree to which the system is protected from possible sources of contamination
Agricultural water practices	• Water application method (overhead sprinkler, spray, drip, furrow, flood, seepage, etc.) • Time interval between the last direct water application and harvest of the covered produce
Crop characteristics	• Susceptibility of the covered produce to surface adhesion or internalization of hazards
Environmental conditions	• Frequency of heavy rain or extreme weather events may impact the agricultural water system or damage produce that can increase the susceptibility of produce to contamination. • Air temperatures • Sun (UV) exposure
Other relevant factors	• Including, if applicable, results of water testing that could inform the assessment



Questions, comments or concerns?

Contact your K-State Extension specialist **Londa Nwadike** (lnwadike@ksu.edu or nwadike@Missouri.edu) or **Cal Jamerson** (agri@ksu.edu) today!

New Modified FSMA PSR Agricultural Water Requirement Proposal

Corrective and mitigation measures

The proposed rule does **NOT** change the requirements for harvest and post-harvest water, but only impacts pre-harvest water. Under this proposed rule, a covered farm would *make a determination under the requirements, based on the outcome of its agricultural water assessment*, as to whether mitigation measures would be reasonably necessary to reduce the potential for contamination of covered produce (other than sprouts) or food contact surfaces with known or reasonably foreseeable hazards associated with its pre-harvest agricultural water.

Based on the assessment, if you determine...	Then you must...
that your agricultural water is not safe or is not of adequate sanitary quality for intended use(s).	- Immediately discontinue use(s) AND - Take corrective measures before resuming use of the water for pre-harvest activities
there is one or more known or reasonably foreseeable hazards related to animal activity, BSAAOs, or untreated or improperly treated human waste for which mitigation is reasonably necessary.	Implement mitigation measures promptly, and no later than the same growing season
there is one or more known or reasonably foreseeable hazards not related to animal activity, BSAAOs, or untreated or improperly treated human waste, for which mitigation is reasonably necessary.	- Implement mitigation measures as soon as practicable and no later than the following year Or - Test water as part of the assessment and implement measures, as needed, based on the outcome of the assessment
that there are no known or reasonably foreseeable hazards for which mitigation is reasonably necessary.	Inspect and adequately maintain the water system(s) regularly, and at least once each year

Exemptions:

A covered farm would be exempt from the requirement to conduct an assessment for pre-harvest agricultural water if the farm can demonstrate that the agricultural water meets these requirements, including:

- It meets certain requirements that apply for *harvest and post-harvest agricultural water* (such as the microbial quality criterion and testing requirements for untreated ground water);
- It is received from a *public water system or supply* that meets requirements established in the rule (provided that the farm has public water system results or certificates of compliance demonstrating that the water meets relevant requirements); or
- It is treated in accordance with the standards outlined in the Produce Safety Rule.

Prepared by: Yeqi Zhao Reviewed by: Manreet Bhullar, Londa Nwadike



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How to Interpret Water Results

Since 2016 FSMA PSR published agricultural water regulation, agricultural water has been separated into production water and postharvest water and from three main water sources (i.e., surface, ground, and municipal). Due to the different cross-contamination risks, the water criteria varied by different water activities. This handout aims to help Kansas and Missouri growers better understand the water regulation they need to comply with and interpret their water results within simple wording, images, and charts.

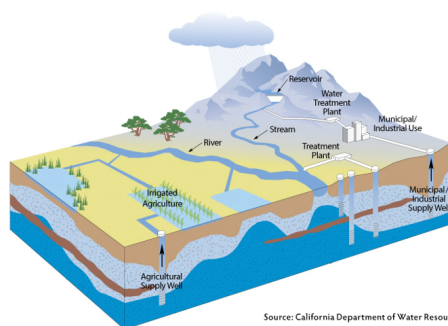
QUICK REFERENCE GUIDE

FSMA Produce Safety Rule Requirements for Agricultural Water

“ The **Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR)** states that all agricultural water **must** be of safe and adequate sanitary quality for its intended use (§112.41) ”

What is Agricultural Water?

Agricultural water is water that is intended or likely to contact the edible portions of produce or food contact surfaces during growing (production) or during/after harvest (post-harvest). The PSR outlines three common sources of agricultural water: **ground water**, **surface water**, and **municipal water**.



Public Water is water that is treated and monitored by a local water utility

Ground Water is water that is found beneath Earth's surface (ex. wells, aquifers)

Surface Water is water that is found or stored on the Earth's surface (ex. rivers, creeks, cistern, ponds supply tanks)

Risk of Contamination



What's the difference between Production and Postharvest Water?

Production Water is any water that is applied directly to produce during growing

- Irrigation
- Crop sprays
- Fertigation
- Frost protection
- Cooling



VS

Postharvest Water is any water that is used during and after field harvest of the produce

- Field Packing
- Washing
- Hydro-cooling
- Ice making
- Hand Washing



Note: water used for hand washing also counts as postharvest water since it can potentially contact the produce surface.

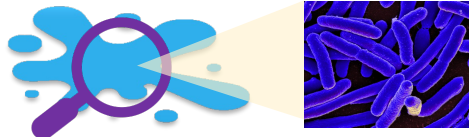


How do we determine water quality?

Agricultural Water Assessment The new modified agricultural water proposed rule uses an evaluation system to evaluate pre-harvest water quality. There are four main factors include agricultural water system(s), agricultural water practices, crop characteristics, and environmental conditions. Based on the assessment, you can determine whether your water is saved to use or not.

Tips: Agricultural Water Assessment Builder can help you evaluate your water quickly and easily!

Access from [link](#) or **QR-code**



Water Microbial Quality The Produce Safety Rule uses a bacteria called generic *E. coli* as an indicator of water quality. *E. coli* is a type of bacteria that is commonly found in animal waste (feces). Based on the number of generic *E. coli* in 100 ml, you can know whether your water is microbially safe to use or not.



Questions, comments or concerns?

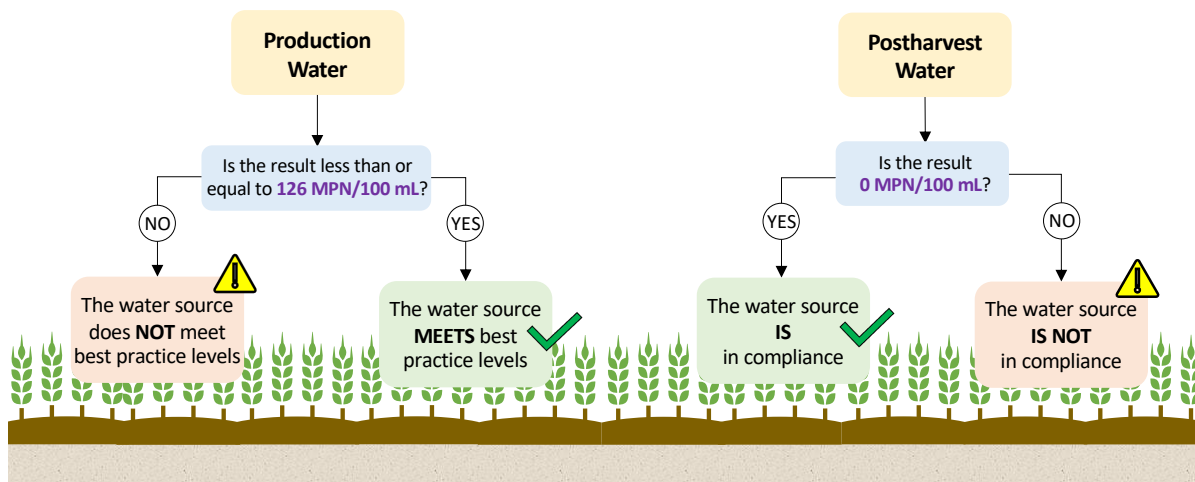
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How to Interpret Your Water Quality Test Results

Step 1: Determine if the water source meets best practice/ regulation levels

Intended Use:



Step 2: Determine the next steps if the water source IS NOT best practices/ FSMA PSR compliant

If the postharvest water is not compliant:

DISCONTINUE use as soon as practical (switch to municipal water or other reliable source) unless corrective measures are applied.

A few things you can do:

- Contact your local extension personnel for solution.
- Apply a **time interval** between the last irrigation and harvest.
- **Re-inspect** the entire affected agricultural water system for:
 - The extent it is under your control
 - Any conditions that are likely to contaminate produce surfaces
- Apply **validated treatments** to the water source.
 - The treatment must be effective to make the water safe and of adequate sanitary quality for its intended use (i.e., UV, chlorine).

[Kansas State University](https://www.k-state.edu)

Name: Katelynn Stull

E-mail: kjstull@ksu.edu

Cell-phone: 913-307-7394

[University of Missouri](https://www.missouri.edu)

Name: Dr. Londa Nwadike

E-mail: nwadikel@Missouri.edu

Cell-phone: 913-307-7391

Prepared by: Yeqi Zhao, Olivia Haley Reviewed by: Manreet Bhullar, Londa Nwadike



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Backflush Water Fact Sheet

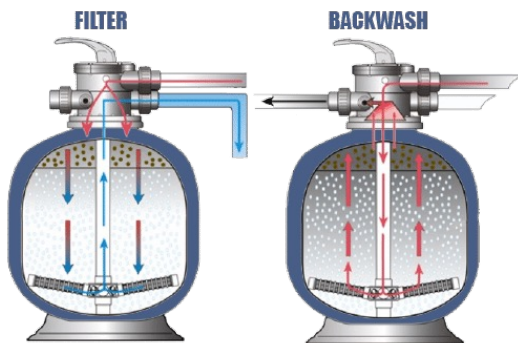
According to the survey results, the filtration system is the primary water treatment reported by the grower. The backflush water is the water used to clean the filter that contains various microorganisms and potentially includes human pathogens. Despite the use of filtration alone is not a validated agricultural water treatment, the handout relates to the filtration method, and backflush water is limited in the extension field. This handout aims to popularize the biohazard of backflush water and possible solutions.

QUICK REFERENCE GUIDE

Backflush Water Fact Sheet

Due to the produce safety concern, filtration systems are widely used in farms to catch small solid particles from original water sources (surface or ground water), and provide clearer water for agriculture purpose (irrigation, fertigation, cooling, etc.).

However, do you know the filtration system can also provide the “dirty” water called **backflush water**?

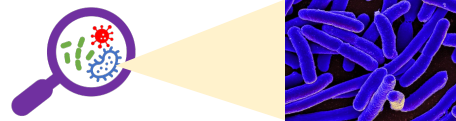


What is Backflush Water?

Backflush, also known as **backwash**, it is a mechanism always comes with a sand and Diatomaceous Earth (DE) filtration system acts as a **self-cleaning system**. It is a water flow that moves reversely through the filter tank to remove the accumulated particles from the filter.

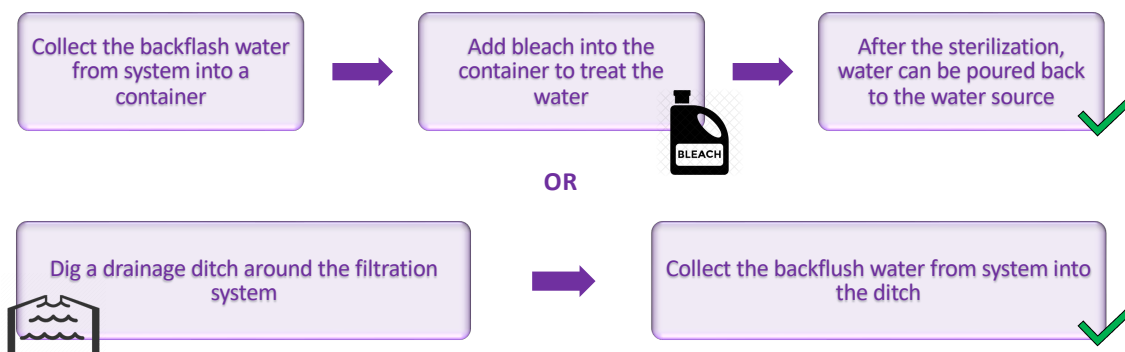
The Concerns That Backflush Water Brings Up:

As backflush water brings all the potential contaminated particles from the filter surface, the backflush water itself is considered as a **highly contaminated** water. If the backflush is not handled and/or stored properly, it can re-add the contamination to the original source or to other clean water sources on the farm.



How to Manage the Backflush Water in My Farm?

Following are several methods to manage your backflush water:



Purdue University Logo



Questions, comments or concerns?

Contact your K-State Extension specialist **Londa Nwadike** (phone number; email) or **Cal Jamerson** (phone number; email) today!

Chapter 5 - Conclusion

The thesis focused on improving the food safety practices reducing the risk of fresh produce outbreaks in the US, specifically expected outcomes on minimizing the cross-contamination chance in the fresh produce farms. The primary two reservoirs for transmitting human pathogens are the farm soil and agricultural water. Thus, to complete the goal, the thesis work included three objectives: (1) reducing the pathogen load in potentially contaminated agricultural soils using cover crops; (2a) accessing current practices and knowledge of Kansas and Missouri growers by using survey; (2b) developing extension material focused on managing agricultural water quality based on the survey results.

Soil Treatment

Several soil treatment methods reduce human pathogens in fresh produce fields. Specifically, cover crops have been recently explored for antimicrobial application in produce production, besides their soil health benefits for contaminated soils. Chapter 2 evaluates the efficacy of reducing pathogen load in contaminated soil with three different cover crop species (i.e., buckwheat, mustard greens, and sunn hemp). The results showed all three cover crops could reduce the bacteria population in contaminated soils after 30 to 40 days following artificial tillage within buckwheat did the best. However, the sole use of a gram-negative pathogen (generic *E. coli*), soil type, and selected three cover crops would be the primary limitations in this study. Considering this is the preliminary study, future research could study the antimicrobial efficacy on soil with different pathogens/soil types/cover crops to better understand the implications of these results. Nevertheless, these results demonstrated the potential of applying cover crops as an antimicrobial treatment in contaminated produce fields. The

outcomes will benefit the organic fresh produce growers as they use biological soil amendments to enrich the soil as a common practice.

Agricultural Water Safety

Unlike soil amendment contamination, agricultural water safety has always been a concern in all types of produce farms, as highlighted in multiple produce outbreaks. Within continuously providing training and information on agricultural water microbial quality to help produce growers reduce risk, the survey developed (chapter 3) is necessary to determine the current knowledge and practice gaps between produce growers and agricultural water management best practices. The surveys were sent out between late 2020 and early 2021 during the COVID-19 pandemic, in which most of the growers' in-person activities had been canceled due to public health concerns. Thus, most surveys were distributed through Qualtrics (an online survey instrument), and the total responses were lower than the expectation (106/200). Despite the dilemma, the survey results indicate the growers from Kansas and Missouri have a basic awareness of agricultural water practices. Still, more demanding practices must be strengthened (e.g., using validated water treatment methods).

Based on the survey responses, three handouts were developed (chapter 4), including: (1) "New modified FSMA PSR agricultural water requirement proposal," which summarized the significant modification of the latest water requirement published in 2021; (2) "How to interpret water results" provides information to identify the agricultural water sources and microbial safety criteria; (3) "Backflush water fact sheet" discussed the hazard from back flush water and solutions. These handouts aim to fulfill the produce growers' current needs and help them to mitigate risk from agricultural water cross-contamination.

In conclusion, the survey provided information on the gaps between best practices and the actual practices of Kansas and Missouri produce growers on agricultural water management; these gave extension professionals the directions for developing further outputs. In addition, in December 2021, the FDA published a new modified agricultural water requirement proposal. The new proposed rule will replace the produce water criteria once it is finalized. Thus, further study can focus on assessing the produce growers' knowledge of the new proposed rule and develop handouts based on the results.

Appendix A - Survey Questions

1. What is your age?

☐ 18-29

☐ 40-49

☐ 60+ years

☐ 30-39

☐ 50-59

2. Are you currently Good Agriculture Practices (GAP) certified?

☐ Yes

☐ No

☐ No, but working
towards being GAP
certified in the future

3. Are you currently raising produce (fruits, vegetables, herbs, mushrooms, nuts) for sale?

☐ Yes

☐ No

☐ No, but plan to in the future

4. In which state do you grow produce? (If more than one, mark the primary state)

☐ Kansas

☐ Missouri

☐ Other _____

5. Do you currently use water PRE-harvest in your produce operation? (check all that apply)

☐ For irrigation

☐ For dust abatement

☐ For mixing with crop sprays

☐ Frost protection

☐ For mixing with fertilizers

☐ Other _____

☐ For making compost teas

☐ No

6. If you use water for irrigation, how do you irrigate? (check all that apply)

☐ Drip irrigation

☐ Furrow irrigation

☐ Drip irrigation under plastic

☐ Other _____

☐ Overhead irrigation

☐ I do not irrigate

7. What sources of PRE-harvest water do you use? (check all that apply)

☐ Pond

☐ Cistern

☐ Creek

☐ Other (such as rain barrel, spring, or
others. Please list) _____

☐ Well

☐ Do not use water pre-harvest

☐ Municipal water

8. Do you currently use water POST-harvest in your operation? (check all that apply)

☐ Rinsing/ washing produce

☐ Single-pass/spray cooling

☐ Commodity movement (i.e. dump
tanks/ flumes)

☐ Dunk tank cooling

☐ Ice making

- ☐ Post-harvest fungicide or wax
- ☐ Handwashing
- ☐ Cleaning and sanitizing of food contact surfaces
- ☐ Other (please list) _____

9. What sources of POST- harvest water do you use? (check all that apply)

- ☐ Municipal water
- ☐ Well
- ☐ Cistern
- ☐ Pond
- ☐ Creek
- ☐ Other (such as rain harvest barrel or others. Please list) _____
- ☐ Do not use water post-harvest

10. Do you test your water used in your produce operation for generic *E. coli*?

- ☐ Yes, more than once a year
- ☐ Yes, once a year
- ☐ Yes, we have tested our water 2-3 times in the last 10 years or so
- ☐ Yes, we have tested our water once in the last 10 years or so
- ☐ No, we have never tested our water
- ☐ A third party (such as the municipal supplier)

11. Do you treat your water used PRE-harvest (such as in irrigation) to improve its microbial quality?

- ☐ Yes, we treat it with a sanitizer (list sanitizer used) _____
- ☐ Yes, we use a different treatment (list treatment- for example, sand filter, other) _____
- ☐ No

12. Do you treat your water used POST-harvest (such as in washing) to improve its microbial quality?

- ☐ Yes, we treat it with a sanitizer (list sanitizer used) _____
- ☐ Yes, we use a different treatment (list treatment- for example, sand filter, ozone, UV, reverse osmosis, other) _____
- ☐ No

13. What other practices (besides treatment) do you use to maintain/improve the quality of the water used PRE-harvest in your produce operation?

- ☐ We conduct an annual evaluation of our water distribution system
- ☐ Other (please list) _____
- ☐ None

14. What other practices (besides treatment) do you use to maintain/improve the quality of the water used POST-harvest in your produce operation?

- ☐ We conduct an annual evaluation of our water distribution system
- ☐ Routine water change schedule
- ☐ Routine cleaning and sanitation of tanks
- ☐ Monitoring for buildup of organic material
- ☐ Monitoring water temperature during post-harvest use
- ☐ Other (please list) _____
- ☐ None

Improving produce safety by reducing pathogen load in soil using cover crops and developing
food safety educational resources for produce growers

by

Yeqi Zhao

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College of Agriculture

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Approved by:

Co-Major Professor
Dr. Eleni D. Pliakoni

Approved by:

Co-Major Professor
Dr. Manreet S. Bhullar

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Abstract

Fresh produce (commonly consumed raw) has been continually associated with multiple foodborne disease outbreaks, which cause both health concerns and economic losses.

Agricultural water and contaminated soil are suspected as common causative agents for recent produce-related foodborne illness outbreaks, which brought fresh produce safety to the forefront of public attention. New federal regulations by the U.S. Food and Drug Administration (FDA) as part of the FDA Food Safety Modernization Act (FSMA) established the Produce Safety Rule (PSR), which defined scientific criteria for growing, harvesting, storing, and packaging fresh produce. The PSR provides requirements on treating soil amendments and criteria for safer use of agricultural water.

There are several soil and water treatment methods and practices to reduce human pathogens in fresh produce. Specifically, for contaminated soils, cover crops have been recently explored for antimicrobial application in produce production, in addition to their soil health benefits. Cover crops are plants seeded before or after the growing season to improve soil health, increase water availability, reduce weed pressure, and prevent erosion. Cover crops also produce various antimicrobial secondary metabolites (i.e., glucosinolates, quercetin), yet the role of cover crops in moderating the population of human pathogens in the soil has rarely been investigated. However, with regards to the microbial quality of agricultural water, Kansas State University and University of Missouri Extension educators have been providing free water testing, training and information on agricultural water microbial quality to help produce growers reduce microbial risks from ag water sources.

The goal of this thesis is to study cover crops as potential antimicrobial interventions and understand grower needs to improve food safety measures and practices on a fresh produce farm.

To achieve this goal, there are two specific objectives: (1) soil treatment: determine the antimicrobial potential of three cover crop species to reduce the population of pathogenic soil microbiota which can minimize the possibility of cross-contamination of produce from agricultural soil; (2) agricultural water: determine knowledge gaps among Kansas and Missouri produce growers related to agricultural water quality and best practices and develop agricultural water safety handouts to improve growers' knowledge.

To complete the first part of this thesis, whole-plant samples of 4-week-old mustard greens (*Brassica juncea*, 'Kodiak'), sun hemp (*Crotalaria juncea*), or buckwheat (*Fagopyrum esculentum*) were chopped and mixed into autoclaved farm soil and inoculated with 7-logs of rifampicin-resistant generic *Escherichia coli* (*E. coli*). The surviving microbial population on days 0, 4, 10, 15, 20, 30, and 40 was enumerated using an agar-based methodology. All three cover crops significantly reduced the population of generic *E. coli* compared to the control ($p < 0.0001$); buckwheat resulted in the highest reduction (3.92 log CFU/g). An inhibitory effect on microbial growth was also observed in soils containing mustard greens ($p < 0.0001$) and sunn hemp ($p < 0.0001$). This study provides evidence for the bactericidal and bacteriostatic effects of cover crops. More information regarding the crops' secondary metabolite profile and potential as a soil bio-mitigation strategy to improve on-farm produce safety is warranted.

For objective 2, a survey was developed and administered to determine future extension outputs and activities to encourage growers to improve their practices related to water quality. The survey was distributed to Kansas and Missouri produce growers attending in-person or online produce safety-related events in late 2020 and early 2021. Also, it was distributed through email lists of produce growers from both states. Survey results ($n = 101$) indicate that 13.9% of the respondents tested their water for generic *Escherichia coli* (*E. coli*) more than once a year,

while 38.6% of the participants have never tested their water. Approximately half (59.3%) of respondents indicated they use municipal water for postharvest uses, while 6.7% indicated the use of untreated surface water for postharvest activities. Due to the concern of potential water contamination risks, researchers suggest that further training and educational resources would help growers improve practices related to water quality and produce safety.

Three handouts were developed to help growers better understand the current FSMA PSR water quality requirements (under consideration for further modifications) and best practices. These handouts include the following: (1) “New modified FSMA PSR agricultural water requirement proposal” handout summarizes the major modifications of the latest water proposal published in 2021; (2) “How to interpret water results” handout provides information to identify agricultural water uses and microbial safety criteria and best practices; and (3) “Back flush water fact sheet” discusses the hazards from back flush water and potential solutions.

In conclusion, this thesis focuses on improving fresh produce safety by minimizing the cross-contamination happening in agricultural soil and water. The reduction of microbial populations in the first part of the thesis demonstrated the potential of cover crops to be used as a sustainable antimicrobial treatment method for contaminated soil. Meanwhile, the survey results show produce growers have a basic awareness of agricultural water practices, but the safety of those practices can still be strengthened. The publication of relevant handouts can improve growers' knowledge and help them better comply with the latest regulations.

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Chapter 1 - Literature Review

Introduction

Significance of fresh produce in the human diet

Since fresh fruits and vegetables contain vitamin A, C, and E, carotenoids, folic acid, dietary fiber, and other minerals, they are one of the great resources to interrupt oxidative stress in the human body to minimize the risk of chronic disease, as well as reduce the concentration of harmful compounds and acid in feces (21). Researchers pointed out that higher fresh produce consumption levels will possibly reduce the risk of cancer (22, 123, 124). Moreover, research showed that consuming produce on a regular basis can reduce blood cholesterol levels (92), the risk of heart disease (66), and maintain blood pressure (118). The concept behind the connection between consuming produce and the risk of chronic disease is the balance of free radicals and phytochemical antioxidants (77) in the body. As the nutrients from produce are hard to replace with other foods, a balanced diet including fruits and vegetables is an important step in maintaining a healthy lifestyle. The USDA's MyPlate recommends eating approximately 2 cups of fruit and 3 cups of vegetables per person per day to maintain a healthy life (the specific recommendation also depends on gender and age) (80).

Besides the increase in health awareness, urban farming has become more popular in the last ten years in the U.S. with the aims of improving local food security, maximizing the use of vacant spaces, and improving agricultural sustainability. Although, due to the environmental impact, currently limited crops can grow on an urban farm, such as leaf greens (72), the model of

potential productivity of urban agriculture shows that small-scale systems are more productive than commercial farms (126). From 1990 to 2018, the value of fruit and vegetable production in the United States has increased each year (130). According to a report from Grand View Research, Inc., the U.S. fruit and vegetable market will reach approximately \$1.12 trillion, and the demand will be more than 243.2 million tons by 2025 (52). As a result, the rise of urban agriculture accelerated the produce market expansion but also added uncertainty to the produce safety, as many produce growers from urban areas own limited farmland and do not need to comply with the Produce Safety Rule (PSR) (annual monetary sales of a farm below \$25,000). So there is still a need for extension professionals to suggest them follow the Good Agricultural Practices (GAP) or PSR to ensure the produce safety during production.

Foodborne outbreaks related with produce

As the demand for fruits and vegetables increasing, more cases of foodborne disease outbreaks have been linked to produce in the past decades. These have highlighted produce safety concerns to the public. In recent years, food safety challenges have continued in an endless series, and there have even been about 3,000 deaths from foodborne causes annually in the US (131). Produce is one of the key vehicles for the transmission of foodborne pathogens in most parts of the world (78). From 2010 to 2017, a total of 85 multistate outbreaks associated with fresh produce with confirmed etiologies occurred in the United States, resulting in a known combined total of 4,658 known illnesses, 1,187 hospitalizations, and 55 deaths (29). *Escherichia coli* (*E. coli*), *Salmonella enterica* (*S. enterica*), and *Listeria monocytogenes* (*L. monocytogenes*)

are the three bacterial pathogens that caused the most multistate fresh produce outbreaks from 2010 to 2017 (29).

Economic impact of foodborne outbreaks

Foodborne outbreaks not only destroy human health but also lead to a huge economic burden. According to the USDA estimation, each year foodborne illnesses cost more than \$17.6 billion of loss (133). Of note, fresh produce is one of the major causes that is believed to account for 26% of the total economic loss (85). One foodborne disease outbreak can lead to \$6,330 to \$2.1 million in economic loss for a fast-casual restaurant (13). According to FDA rankings, the top most common pathogens that cause foodborne disease are *E. coli*, norovirus, *Salmonella spp.*, *Campylobacter*, Hepatitis A, and *Listeria* (138). Among these pathogens, outbreaks associated with *S. enterica*, *L. monocytogenes*, and *Campylobacter spp.* caused the highest total economic losses (113). In addition, the severity of the illness and the type of bacterial infection affect the amount of economic loss, and the cost also depends on the geographical region of the patient. California, Florida, and Texas were among the states with the highest economic losses per foodborne illness patient recorded in 2013 (113, 114).

At the same time, foodborne diseases will bring high economic losses to consumers and will also lead to economic and reputational losses to produce growers or processors. When certain pathogenic bacteria are detected in food products, all products sold are recalled as required by the U.S. Food & Drug Administration (FDA) (30). In this process, the producer has to pay all the costs associated with the recall, including the extra labor, wasted food, and recall costs. At the same

time, when a portion of food is recalled, the reputation of the food manufacturer will normally be damaged, and consumers often will discriminate against the brand. According to the Grocery Manufacturers Association (GMA), the average food recall costs the company about \$10 million per recall (129). For example, a large quantity of food (including safe produce) was dumped due to public health considerations during the 2018 large produce outbreak caused by contaminated romaine lettuce (32).

Thus, reducing the chances of food being infected by microorganisms and ensuring food safety is what every producer and consumer needs to understand and strive to do.

Contamination Factors on Produce Farms

Pathogen contamination of fresh produce can occur at any time during the production chain, although the likelihood of contamination is elevated during field operations such as species and harvest (48, 78). The introduction of human pathogens can occur via diverse routes, including agricultural water (14, 55) and biological soil amendments of animal origin (BSAAOs) (157). Once pathogens are introduced into the farm environment, they can survive and persist for long periods within the soil or water (26, 45, 88, 90, 122), which can cause subsequent spread to other areas of the fruit and vegetable production environment or even result in product contamination.

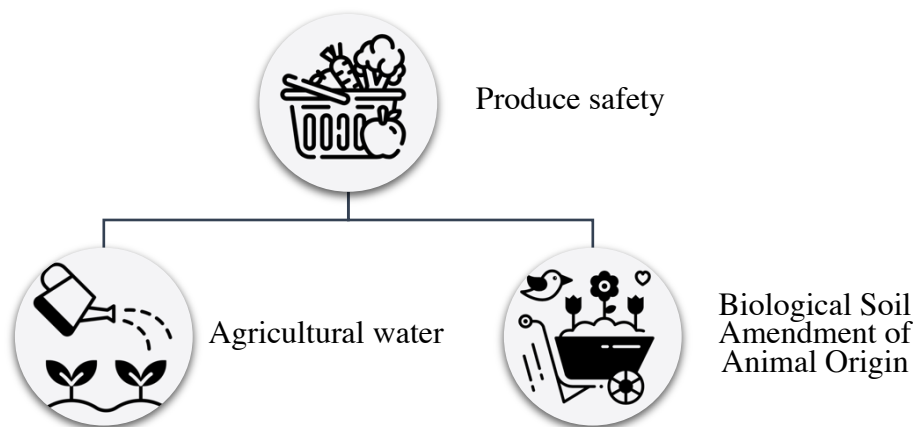


Figure 1.1 The two main contamination routes on a farm that cause produce safety issues.

Agricultural Water

Agricultural water is widely distributed and used in a produce farm which increases the opportunity for human pathogens to attach to the produce. The proximity of produce to contaminated water and the concentration of pathogens within the water are two risk factors contributing to the probability of produce contamination during field production (6). The contamination route always starts with fecal matter carrying human pathogens that drop into the water source (7). Then the transmission of human pathogens can occur at any time during field production (i.e., irrigation, flooding, agricultural runoff) and postharvest activities (washing, rinsing, cooling, etc.).

Biological Soil Amendments of Animal Origin (BSAAOs)

Agricultural soil is another reservoir that can transmit human pathogens to fresh produce. One of the major sources of these human pathogens that survive in the soil is from BSAAOs.

Without proper treatment (i.e., composting), BSAAOs can carry various human pathogens. Once these are applied to soil as fertilizer, they will contaminate the soil soon after. From soil, there are multiple opportunities for human pathogens to attach to the produce. For example, the transmission of human pathogens can occur during the germination of seeds or after the transplantation of seedlings in contaminated soil (28, 71). Therefore, in agricultural applications, it is necessary for growers to detect whether any unexpected animals or improper practices exist before, during, or after field production.

Farm Safety Modernization Act Produce Safety Rule

Overview of the regulation

The US implemented the FDA Food Safety Modernization Act (FSMA) in 2011, which was developed to shift the focus of the nation's food safety system from responding to foodborne illness to minimizing the number of these cases. It was designed to provide clear, specific actions that must be taken throughout the produce handling process to prevent contamination. Nine rules are included in FSMA, which include 1) Third-Party Certification Accreditation; 2) Current Good Manufacturing Practices and Hazard Analysis and Risk-Based Preventive Controls for Human Food and Animal Foods; 3) Foreign Supplier Verification Programs (FSVP); 4) Laboratory Accreditation; 5) Food Traceability; 6) Mitigation Strategies to Protect Food Against Intentional Adulteration; 7) Sanitary Transportation of Human and Animal Food; 8) Voluntary Qualified Importer Program (VQIP); and 9) Standards for the Growing, Harvesting, Packing and Holding of Produce for Human Consumption (10).

This last rule is also known as the Produce Safety Rule (PSR) and the final version was published on November 27th, 2015. It establishes science-based minimum standards for the safe growing, harvesting, packing, and holding of fruits and vegetables grown for human consumption. The compliance dates of PSR criteria are determined by the size of the farm in terms of annual sales and the risk of the commodities becoming a foodborne outbreak when they get contaminated. These include if the farm sells at least \$25,000 of produce annually (adjusted for inflation) and if the produce is likely to be eaten raw (e.g., leafy greens, cucumber, tomato, and most fruits) (137).

Table 1.1 The compliance dates for each rule are based on farm size (very small, small, and other businesses).

Note: ¹ The farm sizes are determined by the average annual monetary value of produce the farm sold during the previous 3-year period. The other businesses (not small) include those selling more than \$500,000 of produce; small size farms include annual monetary sales between \$250,000 to \$500,000; very small includes farms with annual monetary sales between \$25,000 to \$250,000; the annual monetary sales of a farm below \$25,000 are exempted from the PSR. ² The new proposed water requirements are modified based on the final rule which was published December 2nd, 2021 and caused the extended compliance dates. More information on these topics will be mentioned later in this review.

Farm Size¹			
/Compliance Date <i>(134)</i>	Sprouts	Other Produce	Original Water Requirements
Other Businesses (Not Small)	1/26/17	1/26/18	1/27/20
Small	1/26/18	1/28/19	1/26/21
Very Small	1/28/19	1/27/20	1/26/22

Under the PSR, there are six key requirements summarized by FDA as subparts, which include: agricultural water, biological soil amendments, sprouts, domesticated and wild animals, worker training and health and hygiene, and equipment, tools, and buildings (and post-harvest handling?) (135). Of note, maintaining the microbial quality of the water sources used on the farm is of primary importance in produce safety. As water is used in almost every aspect of production, once the water source is contaminated, there will be a higher risk of produce contamination.

Worker Training and Health and Hygiene

Workers are a part of the food safety concerns since they can carry multiple pathogens and spread them unintentionally via multiple routes (58, 65). FDA requires all farm workers who handle covered produce and/or food-contact surfaces and all supervisors to receive food safety training, including the principles of good hygiene and food safety; recognizing symptoms of foodborne illness and the importance of personal hygiene for all personnel and visitors; and other training relevant to the worker's job (137). Of note, water is a key component of these requirements in order to fulfill health and hygiene requirements, such as washing hands and mixing with sanitizer (35). Thus, the microbial quality of the water source which is used for hygiene contributes to the risk of cross-contamination concern on the farm.

Equipment, Tools, and Buildings

The equipment and tools that are used on farms can always directly contact produce. This part of the rule focuses on the standards of sanitation to prevent cross-contamination of produce.

It covers the building structure (i.e., greenhouses, germination chambers, toilets, and hand-washing facilities), the tools, and equipment that may touch or contact the produce. As in the health and hygiene section, water is also required in this part of the rule, especially during cleaning and sanitizing processes. Although the literature shows that the survival time of pathogenic bacteria partially depends on the surrounding environments (i.e., temperature, moisture, the attached surface material) (24, 46, 104), once pathogens are introduced to any equipment, tools, or building floor, the possibility of cross-contamination begins to increase.

Biological Soil Amendments

According to federal regulation § 112.3, biological soil amendments of animal origin (BSAAOs) represent a biological soil amendment that consists, in whole or in part, of materials of animal origin (137). BSAAOs are widely used to improve soil fertility and quality on organic farms and make a primary contribution to agriculture nutrient management (108, 116). However, if the BSAAOs are used improperly, they can lead to very high produce and water contamination risk since they are applied directly next to the produce. Further, pathogens (e.g., *E. coli*, *S. enterica*) have an extended survival length in a warmer and moist environment (91, 97). A scientifically valid controlled process is required from the PSR for treating BSAAOs to minimize the risk of contamination (137). Of note, the PSR and extension fact sheets highlight storage of the biological soil amendments away from water sources since that can cause potential contamination (19, 137).

Sprouts

Agricultural water quality is a key food safety concern in growing sprouts as water is the primary input in sprout production. In past decades, several foodborne outbreaks have been traced back to sprouts (*15, 31, 49, 125*), which have been contaminated by pathogens carried from sprout seeds and run-off water. As sprouts are grown in warm, moist, and nutrient-rich conditions, this is also the perfect environment for pathogens to survive. Unlike in field production systems, once a single pathogen is introduced into the sprouts system, it can easily spread to all the growing sprouts. Moreover, literature shows that because of the favorable growing conditions, sanitizer treatments (i.e., ozone and chlorine dioxide) cannot inactivate pathogen growth (*2*). Of note, water is required in most sprout growth steps. Therefore, cross-contamination can either happen during irrigation of sprout seeds in the field or during irrigating the sprouts during production (*154*). Based on the special growth environment, the PSR has established a separate section for sprout growers, which includes testing any microbes on or in the seeds, sprout irrigation water testing, and further corrective treatments.

Domesticated and Wild Animals

The fecal matter left behind on the field from grazing, working, and wild animals have a potential risk of containing human pathogens. The pathogens from their feces can directly contaminate the surrounding soil and produce (*84*). The PSR addresses this concern and requires taking all measures reasonably necessary to identify and not harvest produce that is likely to be contaminated. At a minimum, all covered farms are required to visually examine the growing

area and all the covered produce to be harvested (137). Furthermore, once any feces are dropped or flushed (i.e., rain and flood) into nearby water, it will contaminate the entire water source with pathogens from the fecal matter (18). This results in the water becoming a primary reservoir for human pathogens at the farm level.

Agricultural water

Water is an important part of agricultural production as well as a primary reservoir for many human pathogens on the farm. Accordingly, agricultural water microbial quality requirements have been written into the PSR. In current microbial water quality testing guidelines, fecal contaminations are determined by the number of generic *E. coli* in 100 mL of water. The presence of any strain of *E. coli* is a strong indication of fecal contamination, but it cannot be used as direct evidence of the presence of a human pathogen (127). In the PSR, generic *E. coli* is only used to measure microbial water quality and give growers information for future water management.



Figure 1.2 Schematic illustration of a worse condition fresh produce farm.

Originally Proposed Microbial Quality Criteria

Of note, during the thesis year (2020-2022), FDA proposed the new modified production water regulation and currently looking for feedback. Thus, this thesis includes both the current written agricultural water regulation and the new proposed production water regulation. To avoid any misleading, please check the current PSR for referencing.

In 2016, the PSR documented its minimum safety standards for agricultural water at that time. According to the rules clearly specified in PSR released in 2016, for surface water, twenty or more samples must be tested over a period of 2 to 4 years, with five or more samples rolled into the profile every year after the initial survey. The 2016 PSR requirements quantify the safety standards for agricultural water used directly on crops (other than sprouts) by using the

geometric mean (GM) and statistical threshold value (STV) of the water test results. The GM is the average of the water testing results and shows the central tendency of the quality of the water (117). The STV reflects the variability in the quality of the water samples over time.

Table 1.2 The standard for water microbial quality (GM and STV) for pre-harvest water included in the 2016 PSR.

	Geometric Mean	Statistical Threshold Value
Number of Generic <i>E. coli</i> (CFU/100mL)	≤ 126	≤ 410

The corrective actions for the water are required no longer than a year if the water testing results exceed the criteria number. During this period, growers can still use time water in some situations to keep flexibility for them (9, 23).

Microbial Water Testing Methods

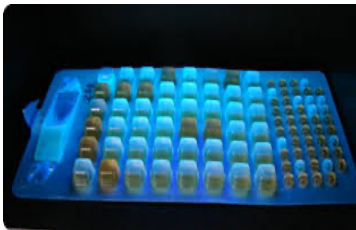
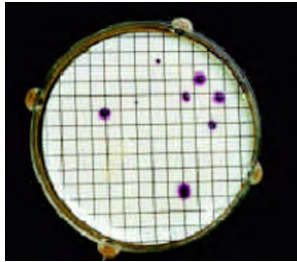
Produce growers need to collect water samples in bottles provided by the testing lab, with a sample size of no less than 100 milliliters. If a groundwater sample is collected, growers should let the water run for five minutes before the sample is collected. If the sample is surface water, keep the sampling bottle close to the center of the water body and try to avoid collecting impurities. After a successful collection, samples must be kept at a low temperature of 4 to 10 °C and sent to a nearby laboratory for analysis within 24 hours (20).

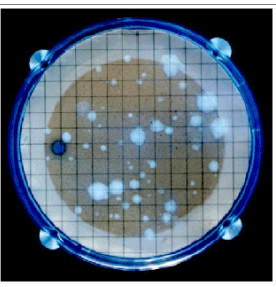
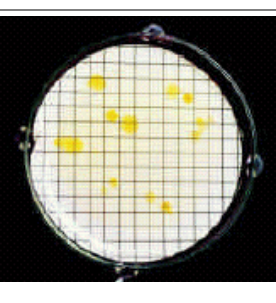
There are two sets of criteria for microbial water quality in the 2016 PSR water requirements that are based on the presence of generic *E. coli* in agriculture water. The microbial water quality for water that is used for post-harvest purposes (including any water that can directly

contact produce surfaces after harvest) must be 0 CFU/100mL, which means no detectable generic *E. coli* are allowed for post-harvest water. The other numerical critical that was proposed in the 2016 PSR water requirements are for water that is applied directly to growing produce (except sprouts). The microbial water quality value for production water in the 2016 PSR water requirements has to be below 126 CFU/100mL GM and 410 STV(132).

Currently, FDA states that there are eight methods considered scientifically valid and at least equivalent in accuracy, precision, and sensitivity in quantifying generic *E. coli* in agriculture water compared to the “EPA? Method 1603: *Escherichia coli* (*E. coli*) in Water by Membrane Filtration Using Modified membrane-Thermotolerant *Escherichia coli* Agar (Modified mTEC) (139)”. Based on the different types of methods, there are two units used in water test results: Colony Forming Unit (CFU) per 100 mL water sample and Most Probable Number (MPN) per 100 mL water sample.

Table 1.3 The eight methods are listed by name, description, result image, and reference.

Name of Method	Method Feature	Example	Reference
IDEXX Colilert/Colilert- 18 Test Kit (unit with MPN)	The Colilert method is used for 4-methylumbelliferyl-β-D-glucuronide (MUG) positive reaction, and the chemical has to be dissolved in the sample water to be tested so that the <i>E. coli</i> in the water can show the fluorescence effect under the ultraviolet light. After adding one packet of Colilert reagent into 100mL of sample water, it is sealed into a quantifying tray. Then it is incubated for 24 hours at 35°C; yellow wells may form to represent if the coliform is present. By checking the amount of blue fluorescent wells in the quanti-tray 2000 under a 6W, 365 nm UV light, the Most Probably Number (MPN) result can be calculated according to the table provided with the Quanti-Tray.		N/A
Method 1603 validated by EPA (unit with CFU)	This is a filtration method that follows the “U.S. EPA Method 1603 Protocol” by growing the <i>E. coli</i> that is left on the membrane in an m-TEC agar incubated for 2 hours at 35 °C and placed in a water bath for 22 hours at 44.5 °C. Counting the pink or purple color colonies formed in the agar provides the final result. This is a test that strictly adheres to FDA regulations; the results are precise and clear but include many disadvantages. The m-TEC agar used in this method is expensive, and it needs to be prepared before starting a water test. The filtration process is required for detection, and the turbidity level of the sample water directly determines the length of time of this process.		Method 1603: <i>Escherichia coli</i> (<i>E. coli</i>) in Water by Membrane Filtration Using Modified membrane-Thermotolerant <i>Escherichia coli</i> Agar (Modified mTEC) (September 2014)

Method 1604 validated by EPA (unit with CFU)	Filter 100mL water sample through a membrane filter and place it on MI agar on an absorbent pad saturated with 2-3 mL of MI broth and incubate at 35 °C for 24 hours. The <i>E. coli</i> will present with a blue color from the breakdown of IBDG by the <i>E. coli</i> enzyme β-glucuronidase; total coliform will fluoresce under longwave UV light from the breakdown of MUGal by the TC enzyme β-galactosidase.		Method 1604: Total Coliforms and <i>E. coli</i> in Water by Membrane Filtration Using a Simultaneous Detection Technique (MI Medium) (September 2002)
Method 1103.1 validated by EPA (unit with CFU)	Filter the water sample through the membrane and place it on the selective and differential medium, mTEC. Incubate at 35°C $\pm$ 0.5°C for 2 $\pm$ 0.5 hours and incubate at 44.5°C $\pm$ 0.2°C for 22 $\pm$ 2 hours. After incubation, transfer the membrane from mTEC agar to the absorbent pad saturated with urea substrate medium for 15 minutes is required for the final step. The color of the <i>E. coli</i> colonies in the final plate should be yellow or yellow-green under a fluorescent lamp.		Method 1103.1: <i>E. coli</i> in Water by Membrane Filtration Using membrane-Thermotolerant Escherichia coli Agar (mTEC) (March 2010)
9213 D validated by SM (unit with CFU)	Filter water sample through a membrane filter and place on mTEC agar. Incubate at 35°C for two h to resuscitate stressed bacteria, then incubate at 44.5°C for 22 h. Then transfer the membrane to a filter pad saturated with urea substrate and containing phenol red for 15 minutes. The result positive for <i>E. coli</i> should be fluorescence under the longwave UV lamp with yellow color.	No image available	Natural Bathing Beaches (2007). In: Standard Methods for the Examination of Water and Wastewater, 22nd Edition (Rice E.W., et al., Ed.), 9-46 – 9-48. Washington, DC: American Public Health Association. (2012).
9222 B validated by SM (unit with CFU)	In this method, the membrane will be placed on les-ENDO or m-ENDO agar which contains basic Fuchsin as the indicator. Filter water sample through a membrane filter and placed on the agar. After incubating the agar for 24 hours at 35 °C, the coliform will show up in red color. Transfer the membrane filter to a nutrient agar that contains MUG and incubate for 4 hours. The individual <i>E. coli</i> colonies will fluorescence under a longwave length UV lamp.	No image available	Standard Total Coliform Membrane Filter Procedure (1997), followed by 9222 G – MF Partition Procedures (1997) using NA-MUG media. In: Standard Methods for the Examination of Water and Wastewater, 21st Edition (Eaton A.D., et al., Ed.), 9-60 – 9-65, and 9-70 – 9-71, respectively. Washington,

**D 5392-93
validated by
ASTM (unit with
CFU)**

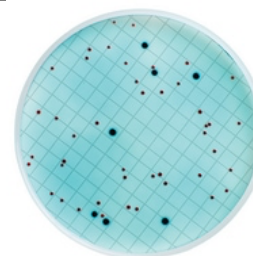
This is a two-step water testing procedure that provides a direct count of the colonies' numbers. Filter sample water through a membrane filter and place it on the selective medium, mTEC. The membrane on the medium is first incubated at 35°C for two h, and then the medium is incubated at 44.5°C for 22 h. Following incubation, the filter is transferred to a filter pad saturated with urea substrate. The yellow or yellow-brown colonies will be present after 15 minutes if the water sample is positive for *E. coli*. Colonies are counted with the aid of a 10 to 15 X magnifier and a fluorescent lamp.

No image available

Standard Test Method for Isolation and Enumeration of *E. coli* in Water by the Two- Step Membrane Filter Procedure. In: Annual Book of ASTM Standards, Volume 11.02. ASTM International. (1996, 1999, 2000).

**Hach Method
10029 for
Coliforms (unit
with CFU)**

This method was published by Hach Company. 100mL of the water sample is filtered through a 0.45-µm membrane filter and placed on the plate, which already added 2mL of the reagent onto a sterile pad on the plate. After incubating for 24 hours at 35 °C, the presence of red colonies indicates the coliforms by reducing the TTC. By hydrolyzing the enzyme-substrate BCIG, it shows blue color for *E. coli* colonies.



Total and *E. coli*, using m-ColiBlue24 Broth PourRite Ampules

Modified Agricultural Water Proposed Rule (proposed in December 2021)

After complying with the water microbial quality criteria for some time, some businesses reported that regulations are difficult to enforce because they are both expansive and complex (144). Thus, in December 2021, FDA revised and published proposed agricultural water requirements (136). The modified rule only impacts the production water regulation but does not change the originally published post-harvest water requirements. Instead of complying with microbial criteria and testing requirements for pre-harvest water (mentioned above), it emphasizes the use of an agricultural water assessment.

An agricultural water assessment is an evaluation that is required to be conducted by covered farms. The factors include agricultural water system(s), agricultural water practices, crop characteristics, environmental conditions, and other relevant factors. Based on the assessment, growers need to determine their water quality and take any actions (i.e., corrective measures, mitigation measures, inspect water system(s), and test water microbial quality) (155). This modification is intended to make it easier for more growers to comply. Large, covered farms were initially required to comply with the water requirements on January 26th, 2022, but FDA is exercising enforcement discretion for the pre-harvest water provisions for all farms while the water requirements are being finalized (as of July 2022).

Agricultural Water Treatment Methods

When the number of generic *E. coli* in the water source has been proved to exceed safe levels or the agricultural water assessment determines the water is not safe for use, corrective

measures are required before resuming the use of the water. Multiple methods can be used to improve the microbial water quality, including filtration, chlorine dioxide, ultraviolet (UV) radiation, and chemical oxidation (i.e., ozone, hydrogen peroxide, and peracetic acid), as shown in Table 1.4. Of note, another agricultural microbial water quality test is required after applying the treatments to confirm that the agricultural water can be used under PSR.

Table 1.4 Log count reduction of pathogens survival in water by different intervention technologies. Note: ZVI means zero-valent iron.

Process	Treatment	Target pathogen	Log reduction	Reference
Filtration	50% ZVI	<i>E. coli</i>	1.7	(67)
	50% sand			
	35% ZVI	<i>E. coli</i>	2.45	(81)
	65% sand			
	35% ZVI	<i>Listeria monocytogenes</i>	2.51	(81)
	65% sand			
	50% ZVI	<i>E. coli</i>	3.54	(8)
	50% sand			
Chlorine dioxide	10 mg/L	<i>E. coli</i>	7	(140)
	3 mg/L	<i>E. coli</i>	5	(57)
	5 ppm	<i>Salmonella</i>	7	(83)
	5 mg/L	<i>Staphylococcus aureus</i>	5	(95)
	1.2 mg/L	<i>Enterococcus faecalis</i>	6.3	(42)
UV	267 nm	<i>E. coli</i>	4	(94)
	275 nm	<i>E. coli</i>	3.6	(94)
	310 nm	<i>E. coli</i>	0.5	(94)

	265 nm	<i>E. coli</i>	4.3	(93)
	275 nm	<i>E. coli</i>	4	(93)
	289 nm	<i>E. coli</i>	2.42	(53)
Ozone	35 mg/L	<i>E. coli</i> O157:H7	3.98	(36)
	45 mg/L	<i>E. coli</i> O157:H7	3.9	(36)
	1 mg/L	<i>E. coli</i>	5	(50)
	1 mg/L	<i>Enterococcus faecalis</i>	6.4	(50)
Hydrogen peroxide	3%	<i>E. coli</i> O157:H7	0.77	(54)
	100 mg/L	<i>E. coli</i>	3.13	(47)
	100 mg/L	<i>Enterococcus faecalis</i>	2.45	(47)
Peracetic acid	8 mg/L	<i>E. coli</i>	3.52	(47)
	8 mg/L	<i>Enterococcus faecalis</i>	4.5	(47)
	3 mg/L	<i>E. coli</i>	3.57	(16)

Filtration

Filtration is a physical process that separates solid particles and liquid from water and reduces the turbidity of the water. The turbidity of water is caused by suspended particles and floating debris, which can provide a protective barrier for microorganisms' survival (147). Thus, reducing the level of turbidity of water before use is important. Filtration systems are widely applied and effective in removing particulate matter, algae, and most microorganisms. Different filtration strategies are used based on the different sizes of target microorganisms (Table 1.5).

Table 1.5 The application for different sizes of filtration strategy.

Filtration methods	Microfiltration Membrane	Ultrafiltration Membrane	Nanofiltration Membrane	Reverse Osmosis
Target Size	approximately 0.1 μm	approximately 0.01 μm	approximately 0.001 μm	<0.0005 μm
Applied for	Sediment and bacterial	Viruses	Microorganisms	Microorganisms

One of the commonly known filtrations is the slow sand filtration (SSF), which lets the water goes through various biological, physical, and chemical processes that finally removes the pathogenic organisms and turbidity effectively. Recently, a great deal of literature highlighted the efficiency and economics of the zero-valent iron (ZVI) bio-sand filtration system for reducing pathogen load in contaminated water (8, 67, 70, 81). Anderson-Coughlin et al. showed that zero-valent iron filtration could significantly reduce the *E. coli* population after going through the filtration system(from 13.13 MPN/ml to 9.59 MPN/ml) as well as shortening the die-off interval of the rest of the bacteria (from -0.48 MPN/day to -1.50 MPN/day) (8).

However, according to the FSMA PSR (21 CFR 112.43) (137), currently, sand-filtration is not considered a validated water treatment method that can be used for removing physical particles and requires a further treatment process to complete the corrective measures. Moreover, the backflush water from the filter can also be problematic as it contains most of the particles from the untreated water. Thus, how to handle the backflush water properly needs to be considered.

Chlorine Dioxide

Chlorine dioxide (ClO_2) is a chemical compound that forms as a gas when the temperature is above 11°C and forms as a liquid when temperatures are between 11°C and -59°C . Due to its chemical properties, it is primarily used for disinfecting drinking water (3). Chlorine dioxide can kill pathogens by breaking the chemical bonds in their molecules and results in pathogen inactivation. Combined chlorine can be found in most sanitizers, such as bleach, and can be used for produce cleaning purposes (59, 75). As shock chlorination is the most cost-effective method of disinfecting water which has been adopted by commercial growers, multiple organizations published shock chlorination guidelines to help growers use it properly (17, 43, 86).

In a lab trial, studies showed chlorine dioxide acted as an effective reagent that can kill more than 99% of plant pathogens in a short time (112). Also, Bel et al. found that 10 mg/L of chlorine can remove 7 logs of *E. coli* rapidly (140). Other research reported a 5 log reduction of *E. coli* populations with 3 mg/L of chlorine dioxide (57).

However, limitations of chlorine dioxide application are also present. It can only work for a short time period within the existence of chlorine dioxide in the water, which cannot be applied as a long-term solution.

Chemical Oxidation

Chemical oxidation is a chemical treatment used to eliminate organic materials in water and wastewater using oxidation, which is both efficient and environmentally friendly. This method has been widely used in the process of purifying and treating wastewater to increase the

use of water (47, 68, 79, 158) as reclaimed wastewater. The reaction of the process is based on a powerful oxidizing agent (e.g., ozone, hydrogen peroxide (H_2O_2), and peracetic acid (PAA)) and obtained at a sufficient concentration.

Ozone disinfection is known as a more effective disinfectant than chlorine against protozoan parasites in water systems (41). Since ozone is highly reactive and unstable, the cost of operation and maintenance are relatively higher than other similar water treatment methods. Hydrogen peroxide is another water treatment method that is environmentally friendly. Several studies show that hydrogen peroxide itself is a slow-acting water disinfectant (requires high dosages and long contact times), but its performance can be enhanced by combining with Cu^{2+} , Ag^+ , and Zn^{2+} (37, 106, 107, 141). Peracetic acid (PAA) is another common and strong oxidant that is applied to disinfect wastewater. It is a man-made chemical that can act against a wide spectrum of pathogens at a low concentration rapidly (12). Kitis et al. ranked the disinfection efficiency of PAA towards microorganisms as bacteria > viruses > bacterial spores > protozoan cysts (68). However, literature also shows the remaining decomposed PAA increases the organic content in the water, which potentially enhances the pathogen regrowth (68, 73, 76). Of note, all the chemical oxidation treatments can be combined with UV and applied in the water treatment process, which results are more efficient than when applied alone (16).

Ultraviolet (UV) Light Disinfection

UV light is a type of electromagnetic (EM) radiation, which has frequencies of about 8×10^{14} to 3×10^{16} hertz (Hz), and wavelengths of about 200 - 400 nanometers (nm). Based on the

wavelength, the UV light can be divided into three sub-bands: UV-A (320-400 nm), UV-B (280-320 nm), and UV-C (200-280 nm). The commercial UV lights for disinfection use UV-C wavelength (typically 254 nm), which is effective against most bacteria, protozoa, and viruses, by acting directly on the DNA of microorganisms and resulting in a germicidal effect (120).

As clearer water has a higher transmission rate, UV light is more effective in treating low turbidity water (more clear, higher transmission) than high turbidity water (UV light gets blocked) (142). Thus, filtration is always recommended, especially for surface water, before running through the UV system. Also, the effectiveness of a UV system depends on the sensitiveness of microorganisms to UV light. Research shows a 280 nm wavelength UV reactor results in a 4 log reduction in *E. coli* (96), while applying the same wavelength to mesophilic bacteria led to a 1.4 log reduction (34). On the other hand, literature shows the pathogen log inactivation does not follow the increase of the UV wavelength. Nyangaresi et al. tested multiple wavelengths of UV-LED on the inactivation of *E. coli* in water, and the 267 nm UV-LED resulted in a 4 log inactivation, whereas the 310 nm wavelength inactivated 0.5 logs of the *E. coli* population (94).

Other in-lab water treatments

There are some water treatments that have not been applied to agricultural water that are more in the experimental stage of research and development and have not been extensively applied commercially on fresh produce farms. (1) Ultrasound technology: The ultrasound system affects objects in a liquid medium by leading to cavitation, and the forces created by it influence the biological system. Thus, ultrasound technology kills microorganisms by enhancing mass

transfer through the biological membranes. Its application range is based on the type of cavitation, which can be broadly divided into transient and stable types. Research shows that it takes five days for ultrasound treatment to reach to 85% reduction (82). Thus, it needs to be applied combined with other biocides, such as chlorine, UV, and electrolysis. According to research, ultrasound treatment improves the effect of chlorination on reducing the bacterial population in raw stream water (102), reaching about a 4 log reduction of fecal coliforms when applied with UV (156).

Electrolysis also can work as a water pretreatment method to reduce microbial contamination. Electrolyzed water (EW) is created by passing an electric current through a dilute saltwater solution in an electrolysis chamber. The process uses a membrane cell to separate an anode and a cathode and produce acidic EW, which contains free chlorine. Thus, EW can be used to reduce the survival of *E. coli*, *Listeria*, and *Salmonella* (51, 64).

Agricultural Soil Treatment Methods

The transmission of human pathogens can occur during the germination of seeds or after the transplantation of seedlings in contaminated soil (28, 71). Also, the internalization of human pathogens, specifically enteric pathogens, through root uptake has been a point of debate due to the inconsistencies among numerous scientific studies (27, 40). Reducing pathogen load in potentially contaminated soil is important, especially in organic farms, which commonly use biological soil amendments of animal origin (BSAAOs) to improve soil fertility and quality (116).

Solarization

Soil solarization is a method of sterilizing soil using solar heating by covering the pre-irrigated soil with transparent plastic sheets, which has the advantages of simple manipulation and low cost. This method has been proven as one of the efficient ways of controlling plant pathogens in soil (121). Recently, using solarization to inactivate human pathogens (i.e., *E. coli* and *Salmonella*) has been confirmed effective both in the field (152) and in a greenhouse (151).

Cover Crops Application

The establishment of cover crops on organic farms is becoming a standard practice to improve soil health and nutrient management. Cover cropping's potential benefits include reduced soil erosion, weed suppression, soilborne and foliar plant disease suppression, reduction of plant-parasitic nematodes, soil improvement, and nutrient management through nitrogen fixation and carbon accumulation (60, 99, 115). Recently, cover crops have gained attention for their ability to suppress soilborne and foliar plant diseases (61, 74, 145); this antimicrobial activity has been attributed to the production of secondary metabolites which protect the plant from microbial invasion (amongst other roles). While research on the antimicrobial activity of cover crops to suppress plant pathogens has been well developed in past decades, studies investigating their antimicrobial activity towards human pathogens in the soil is still limited (109).

Secondary metabolites in cover crops and their antimicrobial activity

The secondary metabolites are the organic molecules that are neither involved with growth nor development but act as a defense mechanism in the plant to respond to the biotic and abiotic stressors (98). In previous literature, secondary metabolites have been widely studied against plant insects (33, 128), plant viruses (153), and plant fungus (63, 148). Until recent years, the antibacterial effect of secondary metabolites in cover crops has been noticed and proved. For example, many species in the *Brassicaceae* family produce glucosinolates which are water-soluble compounds resistant to degradation until they contact the enzyme myrosinase. Then it hydrolyzes them into isothiocyanates (I.C.T.s), nitriles, and other products (56), which inactivate pathogenic bacteria like *Staphylococcus aureus*, *Enterococcus faecalis*, *Listeria monocytogenes*, *E. coli*, and *Salmonella spp.* (4, 110, 111, 143). Flavonoids (i.e., rutin, quercetin, isoorientin) are another large group of secondary metabolites found in species such as buckwheat which inhibit both gram-negative and gram-positive bacterial survival (1, 11, 146). This research brought up the potential of using cover crops as an amendment to inactivate pathogenic bacteria in produce farms.

Extension Education

The USDA National Institute of Food and Agriculture (NIFA) defines agriculture extension as “Extension that provides non-formal education and learning activities to people throughout the country — to farmers and other residents of rural communities as well as to people living in urban areas. It emphasizes taking knowledge gained through research and education and bringing it directly to the people to create positive changes” (89). Agricultural

extension services can originally be traced back to World War I. It served to support war costs by helping farmers increase crop yields by relaying agricultural knowledge. A set of survey data collected in 1980 showed that farmers and all household members had a positive and significant effect after extension education (39). This study emphasized the necessity and significance of agricultural extension education. Nowadays, educators are bridging the gap between new knowledge and regulations from scientific research to growers and aim to solve farms' difficulties and increase the production, food security, and standard of living of farm families. Universities and colleges are also engaging in extension and outreach teams to help local agricultural producers.

In recent years, because PSR criteria require growers to receive training, “PSR training” has become a hot topic in the extension field. In 2010, Cornell University, FDA, and USDA created the Produce Safety Alliance (PSA), which developed a standardized curriculum and nation- wide education to help fresh produce growers to comply with the requirements outlined in the FSMA PSR. According to data from 2013, FDA estimated that 35,029 total produce farms in the U.S. are required to comply with the PSR, which are qualified as very small farms and above (150). In order to fulfill the national training need, in 2016, PSA launched a train-the-trainer program to provide the opportunity for more people to be qualified to deliver the PSA grower training course and to respond to growers' questions during the training sessions. By the end of March 2022, PSA has held 2,332 grower training courses with 46,361 participants and 2,325 people qualified as a trainer in the US (105).

Extension Education Theoretical Framework

In addition to PSA grower courses, extension agents also provide independent sessions on good agricultural practices and general science-based knowledge to both PSR- covered and exempt growers. Inspired by adult and youth education program planning frameworks, the extension education theoretical framework aims to help extension professionals plan effective training. Within the expectation of positive change in growers' behavior after the training, the social marketing theory and the theory of planned behavior are used to develop the extension training sessions. The outcomes will increase the likelihood of growers having behavior changes (25).

Social Marketing Theory

The social marketing theory is adopted from commercial marketing theory and includes the 4P's of marketing, which are the product, price, place, and promotion (149). Instead of the profit motive, like in commercial marketing, the primary goal of social marketing is to achieve a positive behavior change in a population. Kotler and Zaltman et al. defined social marketing as “a social influence technology involving the design, implementation, and control of programs aimed at increasing the acceptability of social ideas or practice in one or more groups of target adopters.” (69). There are two fundamental philosophies in social marketing Theory (62) :

- An organization's objective should be to meet the needs and demands of the customers.
- In order for an exchange to take place, the organization and the customer must engage as nearly equals, with each having something to contribute to the other and each being able

to gain from the transaction.

Since understanding the barriers to behavior change adoption among the target population is the keystone of social marketing theory (87), audience analysis is one of the most important elements of the whole process. The same technique is also applied in the agricultural extension area, known as research-based needs assessment activities. Based on the results from each activity, professionals can implement changes to help farmers overcome the barriers.

Theory of Planned Behavior

The Theory of Planned Behavior (TPB) (5) is an extension of the Theory of Reasoned Action (TRA) (44). It started in 1980 to predict an individual's intention to engage in a behavior at a specific time and place. The principle was designed to understand all activities for which people would exercise self-control. Behavioral intent is a critical component of this model; behavioral intentions are influenced by one's attitude about the probability that the behavior will produce the desired result and one's subjective assessment of the costs and benefits of that outcome (119).

According to the TPB, the behavioral achievement is dependent on both motivation (intention) and skill (behavioral control). It divides attitudes into three categories: behavioral, normative, and control. The TPB is made up of six constructs (attitudes, behavioral intention, subjective norms, social norms, perceived power, and perceived behavioral control) that together reflect a person's actual behavioral control (5).

TPB is considered in extension education to help planners understand the attitudes that growers hold about agriculture practices, the factors that influence their thoughts, and the necessity of measured behavioral intent before and after the training.

Survey Research

Surveys are one of the common measuring instruments to collect feedback and opinions from growers. It enables researchers to be more efficient by employing a sample of respondents to generalize to the entire community (38). The data results can help extension professionals to obtain information to learn the current growers' practices and knowledge. For example, surveys have previously been conducted to assess growers' produce safety knowledge (100, 103) and needs (101) to help them to comply with produce safety best practices.

Typical survey methods include telephone, paper, in-person, and web-based surveys (38). Among these methods, electronic surveys are more adopted in the recent era with the advantage of low cost and fast transmission (38). Of note, due to the COVID situation, public activities were largely canceled due to the concern for public health, so the surveys were largely web-based. Thus, the survey (chapter 3) in the thesis is mainly web-based.

Conclusion and Future Outlook

Soil contamination and improper BSAAOs practice always raise the possibility of cross-contamination, especially on organic farms which commonly use BSAAOs to treat the soil. Although using cover crops to reduce the pathogen load in soil has not been widely studied, the hands-on results demonstrated the potential of cover crops to be used as a sustainable and cost-

effective soil treatment. Further research is needed to compare the efficiency of pathogen reduction among different species of cover crops.

Meanwhile, water will always remain a key component of the food production system. Agricultural water as the primary reservoir of human pathogens is continuously suspected as a transmission route for produce-related foodborne illness outbreaks. One of the current challenges focuses on inappropriate agricultural water practices, including using surface water for postharvest use without validated treatment and misunderstanding some terminologies (i.e., water used for handwashing is considered as postharvest water). These practices might not directly lead to an outbreak but will raise the possibility of cross-contamination. Considering some high-tech agricultural water treatment methods (i.e., UV and chemical oxidation), some of these show promise of pathogen reduction but are not commonly used on farms. Further research and extension education is required to deliver the latest technology to growers.

Another challenge is the latest proposed modifications to the agricultural water rule. Although the new proposed rule is focused on simplifying the production water criteria, growers and extension professionals need more time to adopt the new modified rules after years of education on the current agricultural water regulation. During this transition period between the current version of the rule and the finalized new modified rule, growers may be confused with the requirements and the potential impact of on-farm water practices (i.e., water microbial quality test, agricultural water assessments, and water system inspection). Moreover, the new proposed rule requires growers to determine the water quality by themselves through different

factors. However, this requires growers to have more knowledge of potential agricultural water hazards and good practices. Although the FDA provides an online agricultural water assessment tool to help growers determine the safety of water sources, it still is a barrier for growers located in rural areas that do not have internet.

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Chapter 2 - Evaluating the Efficacy of Cover Crops to Reduce Bacterial Pathogen Load in Contaminated Soil

Abstract

Cover crops are plants seeded before or after cash crops to improve soil health, reduce weed pressure, and prevent erosion. Cover crops also produce various antimicrobial secondary metabolites (i.e., glucosinolates, quercetin), yet the role of cover crops in moderating the population of human pathogens in the soil has rarely been investigated. This study aims to determine the antimicrobial capacity of three cover crop species to reduce the population of pathogenic soil microbiota. Four-week-old mustard greens (*Brassica juncea*, ‘Kodiak’), sunn hemp (*Crotalaria juncea*), and buckwheat (*Fagopyrum esculentum*) were mixed into autoclaved soil and inoculated with 7 log rifampicin-resistant generic *Escherichia coli* (*E. coli*). The surviving microbial populations on days 0, 4, 10, 15, 20, 30, 40 were enumerated. All three cover crops significantly reduced the population of generic *E. coli* compared to control ($p < 0.0001$); buckwheat resulted in the highest reduction (3.92 log CFU/g). An inhibitory effect ($p < 0.0001$) on microbial growth was also observed in soils containing mustard greens and sunn hemp. This study provides evidence for the bactericidal and bacteriostatic effects of particular cover crops. More research regarding the secondary metabolites produced by certain cover crops and their potential as a bio-mitigation strategy to improve on-farm produce safety is warranted.

Introduction

Agricultural soils contain a dynamic microbiome that supports plant health and are home to organisms that produce secondary metabolites (a group of chemical compounds that defend the organisms from biotic and abiotic stress) (35). However, agricultural soils are also a potential route for the transmission of human pathogens to fresh fruits and vegetables. Pathogens like *E. coli* and *Salmonella* are shed in the fecal matter of animals, where they can be spread throughout the field soil by contaminated irrigation water (4, 18) or through weather-related events such as flooding (9, 36). Recent investigations indicate that many of these human pathogens can persist in agricultural soils for up to 100 days (25, 26, 31, 38), increasing the potential for spreading to other produce and to the growing environment. Commodities like watermelons and cantaloupes, in particular, are considered at high risk for contamination from agricultural soils as they are grown on the ground and often make direct contact with soil (8). For this reason, improving agricultural soil management practices is a topic emphasized by federal regulation (e.g., the Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR)) and guidelines for certifications (e.g., Good Agricultural Practices (GAPS) certification).

While such federal regulations and programs offer some preventative measures, there is little information available for growers on strategies to actively reduce the human pathogenic microbial risk of their soils. For example, one of the most pressing concerns in agricultural soil management is the application of biological soil amendments of animal origin (BSAOs), which can carry a high microbial risk since animals serve as the primary reservoir for many human

pathogens (23, 49). The FSMA PSR §112.56 states, “untreated BSAAOs must not be applied in a manner that directly contacts the harvestable portion of the crop and must be applied in a manner that minimizes the potential for contact with covered produce after application” (42). Otherwise, the soil management recommendations currently largely defer to the National Organic Program, which requires growers to apply a die-off interval for raw animal manure application of 120/90 days for edible produce directly/not directly in contact with the soil before harvest (7 CFR 205.203) (41). Of note, soil contamination from BSAAOs is of particular concern for organic produce growing operations, which largely rely on non-chemical (BSAAO-based) methods like manure-based composts to enrich the soil (28). Clearly, there is a need to expand the repertoire of pre-harvest strategies which can reduce the transmission of pathogens from agricultural soils to fresh produce.

Cover crops are typically utilized by growers to improve overall soil agronomic properties but show promise to also reduce the populations of plant and human pathogens in agricultural soils. Cover crops are cultivated between growing seasons to improve soil health and nutrient composition (e.g., carbon, nitrogen) (16, 34), reduce soil erosion (48), and conserve water (43). Plants used as cover crops include grasses (e.g., *Panicum maximum*), legumes (e.g., *Trifolium pretense*), and non-legume broadleaves (e.g., *Fagopyrum esculentum*), which are fast-growing, improve soil physical properties, and may or may not fix nitrogen (7, 12, 14). Recently, cover crops have gained attention for their ability to suppress soilborne and foliar plant diseases (19, 20, 46); this antimicrobial activity has been attributed to the production of secondary

metabolites, which are organic molecules that are not involved with growth or development, but aid in plant response to biotic and abiotic stressors (27). Amongst other roles, secondary metabolites can protect the plant from microbial invasion. For example, many species in the *Brassicaceae* family produce glucosinolates which are hydrolyzed to form cytotoxic compounds that inactivate pathogenic bacteria like *Staphylococcus aureus*, *Enterococcus faecalis*, *Listeria monocytogenes*, *E. coli*, and *Salmonella spp.* (2, 32, 33, 44). Flavonoids (i.e., rutin, quercetin, isoorientin) are another large group of secondary metabolites found in species such as buckwheat which inhibit both gram-negative and gram-positive bacterial survival (1, 3, 47). While research on the antimicrobial activity of cover crops to suppress plant pathogens has been well developed in past decades, studies investigating their antimicrobial activity towards human pathogens in the soil is limited.

A recent study by Reed-Jones et al. focused on the effects of cover crops (cereal rye, hairy vetch, crimson clover, and hairy vetch-rye) on long-term population variation of *E. coli* and *L. innocua* in the soil (29). The study found a suppressing effect of cover crops on *E. coli* and *L. innocua* based on the microorganisms' innate characteristics and soil type (i.e., loamy, clay). The authors also highlighted how soil temperature, tillage practices, and the weather could affect the bacterial population. While the authors observed potential microbial growth inhibition in fields containing cover crops, they raised concerns that the root exudates of living cover crops into the rhizosphere could also promote the growth of human pathogenic bacteria in the soil.

The objective of this study was to determine if there were antimicrobial capabilities of three common cover crops – mustard greens, buckwheat, and sunn hemp – in field soil inoculated with generic *E. coli*. This study will not only provide information on the feasibility of the three cover crops as a soil bio-mitigation method but also the effect of these crops on microbial survival and inform laboratory-based methodologies for such studies.

Material and Methods

Growing Conditions and Plant Biomass Harvesting

The experimental mustard greens (*Brassica juncea*, ‘Kodiak’) (True Leaf Market, Salt Lake City, UT, USA), sunn hemp (*Crotalaria juncea*) (Sow True Seed, Asheville, NC, USA), and buckwheat (*Fagopyrum esculentum*) (Albert Lea Seed Company, Albert Lea, MN) were grown in a greenhouse at the Olathe Horticulture Research and Extension Centre (OHREC) in Olathe, Kansas during the fall of 2021. Mustard greens and buckwheat were sown in large containers (229 cm × 73 cm × 10 cm). Sunn hemp was grown in 9.5-L nursery containers to account for their more expansive root system and larger overall biomass. The containers were filled with organic Plant Pro™ potting soil (Ohio Earth Food, Hartville, OH, USA) before seeding. The greenhouse was climate-controlled, with temperatures ranging from 12.2 °C during the night to 28.9 °C during the day. Overhead irrigation was applied to the crops every other day in order to provide ample soil moisture

Whole crops (including the underground biomass) were harvested at the flowering stage before producing seeds (27 days after propagation). The crops were gently removed from the

containers to avoid excess root loss, and the soil was removed. The plant material was then collected and immediately transported to the lab for further processing, which was performed on the same day.



Figure 2.1 The growing environment for buckwheat and mustard greens.

Soil preparation

The soil used for *E. coli* inoculation in this study was a chase silt loam (40) collected from a field at the OHREC, which had been in perennial grass for the past three years. The soil was collected from the field and transported to the lab. Prior to sterilization, the collected soil was separated into autoclave bags (Fisher Scientific, Waltham, MA, USA); large clumps of sediment were manually pulverized to facilitate handling after sterilization. The soil was then autoclaved at 121.0 °C and 15 psi for one hour; sterility was confirmed using a bioindicator (Fisher

Scientific, Waltham, MA, USA) placed in the center of the bagged soil. Flame-sterilized tongs were used to extract the bioindicator, and the soil was set aside to cool to room temperature before use in the study.

Bacterial strain

Plant roots and shoots are naturally colonized by a dynamic population of microbes that interact amongst themselves and the plant tissues (30). To avoid background interactions from non-target organisms in the phytobiomass, the soil used in the study was autoclaved first, and rifampicin-resistant generic *E. coli* (ATCC 25922) was acquired from the Food Safety Lab at Kansas State Olathe was used as the study microorganism. The strain was previously stored with 50% glycerol (Fisher Scientific, Waltham, MA, USA) in 1.5mL microcentrifugation tubes (Eppendorf North America, Enfield, CT, USA) at -80.0 °C. To initially propagate the bacteria, a loopful of frozen culture was transferred to a culture tube containing 10 mL tryptic soy broth (TSB; Remel Inc., San Diego, CA, USA) and incubated for 24 h at 35.0 °C.

The methodology of Bhullar et al. (6) was followed to induce rifampicin resistance. Briefly, 0.1 mL of the forementioned microbial culture was spread to a plate of tryptic soy agar (TSA; Remel Inc., San Diego, CA, USA) containing 20 µg/mL rifampicin (RIF; Fisher Scientific, Waltham, MA, USA); the TSA-RIF plate was then incubated for 24 h at 35.0 °C. The procedure was repeated using TSA-RIF plates with increasing concentrations of rifampicin (20, 40, 60 µg/mL) until a strain resistant to 80 µg/mL concentrations of rifampicin was generated on TSA-RIF plates.

To culture, the organism for experimentation, one colony of rifampicin-resistant *E. coli* was transferred to 10 mL of TSB containing 80 μ g/mL RIF (henceforth noted as TSB-RIF). The tube was incubated for 24 h at 35 °C and sub-cultured again in 30 mL of TSB-RIF. To prepare the cells for experimentation, the cells were washed twice with phosphate buffer saline (PBS; Fisher Scientific, Waltham, MA, USA) with centrifugation (Allegra X-30, Beckman-Coulter, Brea, CA, USA; 10,000 rpm, 10 min, 20.0 °C) occurring prior to and in between each wash. The final volume was adjusted to a minimum concentration of 7 log CFU/mL.

Sample Preparation and Inoculation

This study utilized a randomized complete block design with repeated measures. Three independent replications of each treatment (cover crop species) were performed. To mitigate the impact of the ‘rhizosphere effect’ and address food safety concerns raised by previous literature (29), we used chopped whole-plant biomass for this study. The whole-plant material (containing roots, shoots, and leaves) was chopped into 3-cm long pieces to imitate mowing and tilling and was manually homogenized in three large containers (one container per cover crop species). To simulate the soil-cover crop mixture, 200 g of the chopped plant tissue sample, 200 g of the sterile soil sample, and 75 mL of inoculum culture were combined in a 24 oz. Whirl-Pak bag (Whirl-Pak, Madison, WI, USA). The 1:1 ratio of cover crops and soil is based on the assumption that cover crops will only be incorporated into the upper 10 cm of soil. For the non-treated control, 400 g of the sterile soil sample and 75 mL of inoculum culture were combined. The ratio of the solid matter and inoculum was based on preliminary trials to ensure all solid

matter would be moistened by the inoculum. Each sample bag was shaken and massaged 50 times to homogenize the mixture before being transferred into a quart-sized container (1.4 L) with a plastic plant saucer (Mudeela, Seattle, WA, USA) placed underneath. The samples were stored at room temperature (average 20.6 ± 0.02 °C) and with an average relative humidity of $32.9 \pm 0.4\%$ for 40 days. An average of 30 mL of sterile DI (deionized) water was added to each container each week to prevent the soil from becoming too dry to facilitate bacterial survival.

Sample Collection and Microbial Enumeration

Before collecting the sample on each sampling day, each container was mixed using a sterile plastic spoon; the spoons were sterilized by soaking overnight in 10% bleach, followed by rinsing with distilled lab water. A 10-gram sample was collected from each container and transferred to a Whirl-Pak bag containing 90 mL of 0.1% buffered peptone water (BPW; Remel Inc., San Diego, CA, USA). The sample bag was manually massaged to homogenize the sample. Serial dilutions were prepared in 0.1% BPW, and 100 μ L of the sample solution was then spread-plated onto TSA-RIF before incubating for 24 h at 35 °C. Plates yielding 25-250 colonies were recorded for analysis. Samples were collected 0, 4, 10, 15, 20, 30, and 40 days after treatment.

Statistical analysis

The statistical treatment structure utilized was a one-way ANOVA. The whole study was evaluated using three blocks of four pots each on days 0, 10, 20, 30, and 40. The surviving bacterial population at each time point was subjected to log transformation in order to achieve the variance homogeneity assumption.

The bacteriostatic (phase I) rate was calculated based on the difference of log-transformed surviving bacterial populations between day 0 and day 10. The bactericidal (phase II) rate was calculated based on the difference in log-transformed surviving bacterial populations between day 10 and day 40. The bacteriostatic (phase I) and bactericidal (phase II) rates were analyzed separately using the linear mixed model. Fixed effects of the model included all treatments. Random effects were the block and the error term. The main effect per day was assessed via least squares means (LSMs) and their standard errors (SEMs). Pairwise comparisons were carried out using the two-sided test. The Holm-Tukey method was used to adjust for multiplicity. All tests were conducted at the 0.05 significance level. Statistical analysis was executed via Statistical Analysis Software (SAS version 9.4; Cary, NC) PROC MIXED with option DDFM=BETWITHIN in the MODEL statement.

Results and Discussion

Overall trends in cover crops microbial reduction

Overall, the data showed that certain cover crops could reduce the microbial risk of soils containing generic *E. coli* within 30 to 40 days following a simulated cover crop incorporation event (*Figure 2*). This microbial reduction was cover crop- ($p < 0.0001$) and time-dependent ($p < 0.0001$). By day 30, soils amended with buckwheat or mustard greens had achieved a maximum *E. coli* reduction of 3.92 ± 0.16 log CFU/g and 3.77 ± 0.16 log CFU/g, respectively. For soils containing macerated sunn hemp, *E. coli* was still detectable on day 30, only reaching a maximum microbial reduction of 3.70 ± 0.10 log CFU/g 40 days after treatment.

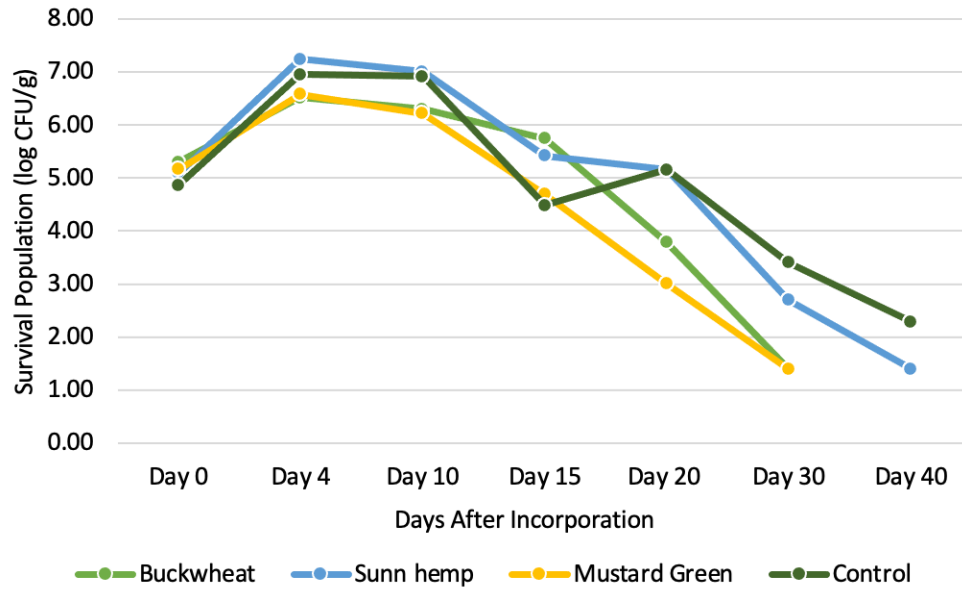


Figure 2.2 The surviving population of rifampicin-resistant *E. coli* in agricultural soil amended with macerated control, buckwheat, sunn hemp, or mustard greens

These overall findings provide potential insight into how these cover crops may be utilized to reduce human microbial risk and thus be used as a soil bio-mitigation strategy. For example, the persistence of *E. coli* in soils amended with sunn hemp could indicate that the secondary metabolites of buckwheat and mustard green tissues are more rapid in inducing microbial cytotoxicity compared to those in sunn hemp tissues. This observation is consistent with previous literature, which reports the cytotoxic activity of sunn hemp is predominantly in seeds rather than in root/shoot tissues (11, 24). In general, there were also no differences in *E. coli* survival between soils containing mustard greens or buckwheat throughout this study, despite their previously- documented different secondary metabolite composition and volume (5, 21).

A biphasic microbial variation curve (see Figure 2) was observed in the experiment, wherein statistically significant interaction effects ($p < 0.0001$) indicate unequal effects of the treatments

over time. The first phase (days 0 to 10) is characterized by a significant increase in the *E. coli* populations amongst all the treatments prior to their decline. From day 10 on, the microbial populations experienced a sharp decline until reaching levels below the detection limit. Topp et al. (39) reported observing a similar microbial growth curve and attributed the variation to the soil type and *E. coli* strains, amongst other factors. Of note, this previous study did not explore microbial survival in a chase silt loam as was utilized in this study, which is characterized by more than 50% silt and 12-27% clay (13), and is a soil composition (theoretically) more likely to have negligible influences on *E. coli* growth due to the relatively aerobic environment of low clay-content soils (37).

Modeling the surviving microbial population to study the biphasic survival curve

To further investigate the differences in microbial growth, the surviving microbial population was statistically modeled by fitting its survival to a nonlinear curve (*Figure 3; Equation 1*). The values used in the equation specific to each cover crop and the control are presented in *Table 1*. Overall, the models were able to explain a high degree (91% to 98%) of the observed variability in the microbial survival of each treatment.

$$y = \frac{x \cdot a + b}{1 + c \cdot x^d} \quad (1)$$

Equation 1, Where x is the days after initial inoculation

y is the total surviving microbial population

a is the rate of increase to the maximum peak

b is the multiplier that increases the height of the peak

c is the rate of decrease with respect to days after initial inoculation

d is the peak shape parameter

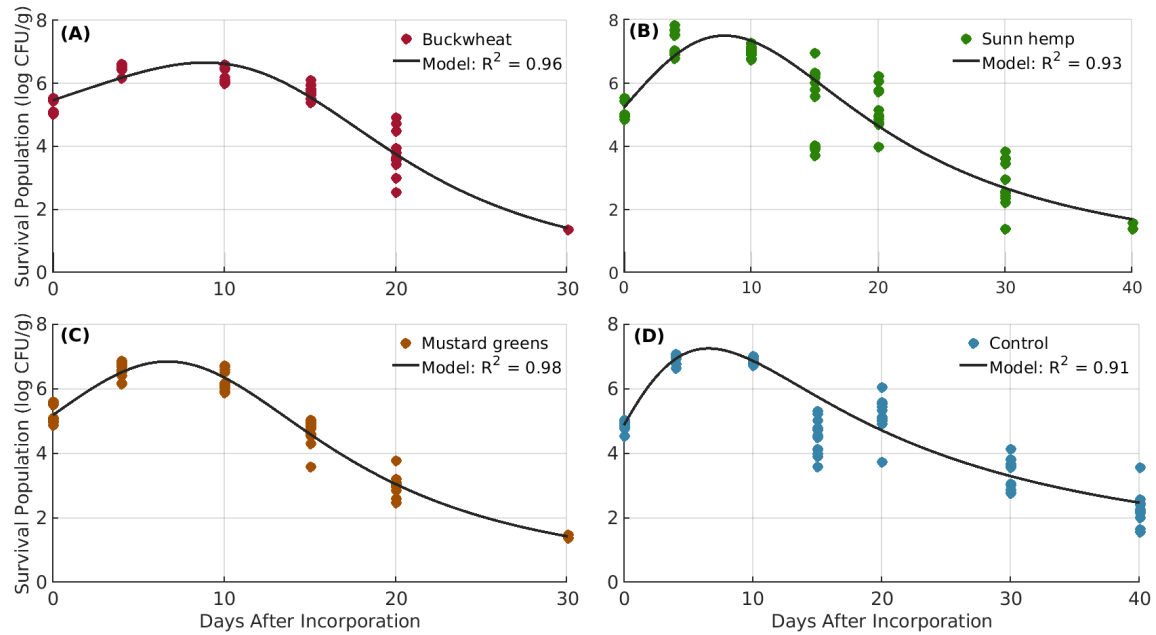


Figure 2.3 The fitted model estimating the surviving population of rifampicin-resistant *E. coli* in field soil supplemented with macerated control, buckwheat, sunn hemp, and mustard greens.

Table 2.1 Variable values by cover crops and control models.

	a	b	c	d	% Variance (R^2)
Control	0.7149	4.875	0.006.989	2.031	91
Buckwheat	0.1827	5.473	0.00001.497	3.827	96
Mustard greens	0.3761	5.184	0.0004.511	2.957	98
Sunn hemp	0.4654	5.227	0.0008.272	2.621	93

Bacteriostatic effects of cover crops during the exponential growth of *E. coli*

From days 0 to 4, there was a significant increase ($p < 0.05$) in the *E. coli* population in all treatments compared to their initial concentration. As evident by the value of a variable (Table 1), the control soils experienced a more rapid rate of increase to the maximum surviving population than the soils containing macerated cover crop tissue. Between the cover crops, sunn hemp showed a more rapid microbial population growth rate than buckwheat ($p < 0.0001$) or mustard greens ($p < 0.0001$). There were no significant differences between the initial growth rate (a) of the buckwheat and mustard greens ($p = 0.6178$) or the sunn hemp and control ($p = 0.2278$). After day 4, the soils containing macerated buckwheat ($p = 0.0021$), mustard greens ($p < 0.0001$) or sunn hemp ($p = 0.0010$) began experiencing a minor decrease in generic *E. coli* populations. In contrast, the microbial population of the control sample attenuated from days 4 to 10 as there were no statistically significant differences ($p = 0.6927$) detected during this time period.

These findings indicate that the exudated phytochemicals of all three macerated cover crops can impair the growth of *E. coli* during this initial growth phase, with buckwheat and mustard greens being the most effective. It is also likely that these results are reflective of an initial exudation of phytochemicals from the macerated cover crops stimulated by the artificial tillage. Divekar et al. note that many secondary metabolic production pathways are rapidly induced by physical stimuli (e.g., cutting of the plants) (15). Accordingly, using a rotary tiller or similar tool

to physically cut the cover crops (instead of only rotating them into the soil) may be critical to initiating their antimicrobial effect.

Bactericidal effects of cover crops during declining *E. coli* population

During the second phase (days 10 to 40), there was a more rapid, significant decrease ($p < 0.0001$) in the *E. coli* population in all treatments compared to their concentration on day 10. In this phase, the *E. coli* could be experiencing die-off due to limited nutrients available in the environment which is accelerated by the presence of cover crop tissues. As evidenced by the value of the c variable (Table 1), the control soil and soils containing macerated sunn hemp showed a slower microbial reduction rate than buckwheat or mustard greens ($p < 0.0001$). Buckwheat experienced the most rapid decline, but there were no significant differences detected between the microbial population of mustard greens and buckwheat ($p = 0.5626$).

The most rapid microbial decline for soil containing macerated sunn hemp occurred between days 20 to 30 which led to a 2.46 ± 0.15 log reduction of generic *E. coli*. Buckwheat also experienced the most rapid population decline (2.40 ± 0.15 log CFU/g) between days 20 and 30. Conversely, soils containing macerated mustard greens exhibited a microbial decline of 1.68 ± 0.26 log CFU/g between day 10 and day 20. There were no significant differences in the surviving population of *E. coli* between mustard greens and buckwheat except on day 20.

Interestingly, the results of this bactericidal phase do not necessarily align with the expectations from the literature. During this phase, it is conceivable that the more rapid decline is due to the cover crops beginning to degrade in the soils, further releasing exudates containing

anti-microbial secondary metabolites (10). Based on the degradation rate, sunn hemp should have the most rapid rate of degradation as the tissues decompose within two weeks post tillage (45). According to Wang et al., the phenolic compounds in buckwheat tissues typically degrade after 37 days, indicating a lower rate of metabolite exudation (47). As this study reports trends in antimicrobial activity which are opposite to what is expected (sunn hemp has less effective microbial reduction rate than buckwheat throughout the study), more investigation regarding the exudation and degradation of the principal secondary metabolites within the tissues of each cover crop is warranted.

Study Limitations

Though the data from this study fit the conventional assumptions for ANOVA, the statistical methodology needed to account for differences in the initial *E. coli* concentration between the control and cover crop-containing soils on day 0 (*Figure 1*). Accordingly, only inferences between the rate(s) of population growth/decline in each cover crop could be made. We believe this significant difference in *E. coli* populations on day 0 can be ascribed to the different compositions of the control samples. As no plant tissues were added to the control containers, 400g of farm soil was used as the control and was inoculated with the same volume of inoculum as other treatments. According to Fick's diffusion law, the diffusive flux goes from areas of high osmolyte concentration to areas of low osmolyte concentration (17). The lower moisture content of the control soils relative to the higher moisture content of the soils containing fresh cover crop tissues likely resulted in a more even distribution of the inoculum in the control soils. Thus,

when collecting the same weight of sample from each treatment, the control group resulted in a diluted generic *E. coli* population result.

The sole use of a gram-negative experimental pathogen presents another limitation of this study. This study evaluated the survival of non-pathogenic *E. coli* as a surrogate for pathogenic *E. coli* or other gram-negative species that may behave in a similar manner (i.e., *Salmonella* spp.), which are commonly found in the gut of wild and domestic animals. However, Locatelli et al. (22) reported higher stability and persistence of gram-positive bacteria (i.e., *Listeria monocytogenes*) in agricultural soils. When Neiunna et al. (29) inoculated cover crop-containing soils with *Listeria innocua*, the authors observed a lower antimicrobial efficacy against the pathogen compared to the gram-negative organisms. This reduced efficacy towards gram-positive organisms may not impact the application of cover crops in states dominated by cattle or poultry production, which have a higher risk of detecting gram-negative *E. coli* and *Salmonella* spp. in the soil. However, this could limit the applicability of the tested cover crops as a microbial risk mediation strategy in operations that struggle with high-risk sources of gram-positive organisms.

Conclusions

All three cover crop species resulted in a microbial reduction to non-detectable levels, while buckwheat and mustard greens also displayed a bacteriostatic effect on the growth of *E. coli* during the exponential microbial growth phase (days 0-10). Future studies should account for extrinsic and intrinsic environmental factors to provide more suggestions for the on-farm

applicability of cover crops as a bio-mitigation strategy. Factors like sunlight, temperature variation, and native soil microbiome interactions were eliminated in this experiment in order to determine if there was an effect of the cover crops on microbial populations. Moreover, the differential efficacy of the cover crops demonstrates the need for characterizing the mechanisms that drive the effects we observed in each of the different cover crop species, particularly including the secondary metabolite content and concentration within the plant tissues. A better understanding is needed of their corresponding environmental stability and exudation patterns as the crop decomposes into the soil. Overall, this is the first lab-trial study for human pathogen reduction on agricultural soil within cover crops amendments. The results demonstrated these cover crops as a promising, environmentally sustainable bio-mitigation strategy for human pathogens in soil that warrant more investigation to promote their use in produce growing operations.

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Chapter 3 - Knowledge and Current Practices Related to Agricultural Water Microbial Quality among Kansas and Missouri Produce Growers

Abstract

Kansas State University and University of Missouri Extension educators have been providing training and information on agricultural water microbial quality to help produce growers reduce risk. However, we recognized the need to determine knowledge gaps among Kansas and Missouri growers related to agricultural water quality and best practices. A survey was developed to determine future extension outputs and activities to encourage growers to improve their practices related to water quality. The survey was distributed to Kansas and Missouri produce growers attending in-person or online produce safety-related events in late 2020 and early 2021 and was also distributed through email lists of produce growers from both states. Survey results ($n = 101$) indicate that 13.9% of the respondents tested their water for generic *Escherichia coli* (*E. coli*) more than once a year, while 38.6% of the participants have never tested their water. Approximately half (59.3%) of respondents indicated they use municipal water for postharvest uses, while 6.7% indicated the use of untreated surface water for postharvest activities. Due to the concern of potential water contamination risks, researchers suggest that further training and educational resources would help growers improve practices related to water quality and produce safety.

Introduction

In recent years, foodborne disease outbreaks resulting from fresh produce have continued to occur (4, 12). Because these commodities are often consumed raw (26, 32), it is imperative that

fresh produce growers use the safest practices possible. From 2010 to 2017, a total of 85 multistate outbreaks associated with fresh produce with confirmed etiologies occurred in the United States, resulting in a known combined total of 4,658 known illnesses, 1,187 hospitalizations, and 55 deaths (4). Further, before 2015, numerous fresh produce outbreaks have been attributed to contaminated agricultural water in commodities such as leafy greens (15, 22), tomatoes (2), and melons (33). Thus, water quality management practices became a major and critical aspect of the Food and Drug Administration's (FDA) Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR) (31).

The PSR, which was finalized in 2015 to take its place as one of the seven foundational rules under FSMA, is the first U.S. Federal regulation to have established science-based minimum standards for the safe growing, harvesting, packing, and holding of fruits and vegetables grown for human consumption. In December 2021, the FDA proposed a revision to Subpart E of the PSR that would change the preharvest agricultural water requirements for covered produce, other than sprouts (30). The FSMA PSR and the proposed revision state the considerations and requirements related to agricultural water management to improve the microbial safety of agricultural water and therefore of produce. In December 2021, FDA announced their intent to exercise enforcement discretion and also act to extend the compliance date for the agricultural water (31).

As per the FSMA PSR, "agricultural water means water used in covered activities on covered produce where water is intended to, or is likely to, contact covered produce or food contact surfaces." Agricultural water is classified into two categories based on the different usage periods: production water and postharvest water. Production water refers to water used during growing activities whereas postharvest water includes all uses of water during or after harvest (7,

31). Since the use of postharvest water is so close to consumption, microbial water quality is especially important (6). Thus, produce growers must meet the requirements of §112.44(a), i.e., no detectable generic *E. coli*/100mL water sample for water used postharvest. The proposed revisions to Subpart E focus on production (i.e., preharvest) uses of agricultural water. These revisions emphasize conducting agricultural water assessments for production water sources and uses, eliminating the federal requirement of an ongoing microbial water quality profile with periodic updates through water quality monitoring.

Each year, Kansas and Missouri growers produce approximately \$26 million (13) and \$81 million (7), respectively, of fruits and vegetables. Kansas State University and University of Missouri Extension educators have been providing training and information on agricultural water microbial quality, based on current and newly proposed FSMA PSR agricultural water requirements, to help produce growers from Kansas and Missouri reduce risk to produce from agricultural water. Although research shows that knowledge gain alone leads to limited behavior change, it can provide people with more informed choices that may or may not lead to behavior change (16). Pratt and Bowman (21) suggest that individually-relevant, problem-focused, and hands-on activities can help extension educators prompt personal behavior change. To help prompt this positive behavior change, educators need a better understanding of the gap between water quality best practices and produce growers' knowledge and actions related to agricultural microbial water quality.

Survey research (20) is one method that allows extension educators to easily understand a large number of growers' current water practices and knowledge gaps in a shorter time. Other surveys have previously been conducted to assess growers' general produce safety knowledge (17, 19) and needs (18) to help them to comply with produce safety best practices. Perry et al.

(17) found that North Central US produce growers have a knowledge gap in understanding agricultural water handling practices compared with other topics of the FSMA PSR, which highlights the needs for food safety extension educators to focus on delivering information on agricultural water best practices to produce growers.

This survey aims to determine gaps between safe water management practices (as outlined in the FSMA PSR requirements/ proposed requirements and other produce safety best practices) and (i) the current knowledge and (ii) the current practices of produce growers from Kansas and Missouri related to agricultural water quality. This will enable extension educators to develop additional training and resources on agricultural microbial water quality accordingly to better meet the needs of growers.

Materials and Methods

The project team developed a 14-question survey instrument based on similar surveys (18). Before implementing the survey, 4 extension experts reviewed the survey questions for face and content validity. The Kansas State University Produce Safety Extension Associate also performed cognitive interviews (14) with growers from the target population to further validate the survey. The Kansas State University Human Research Institutional Review Board (IRB) approved the use of the survey. The survey (*Appendix 1*) included questions on the growers' knowledge and practices related to microbial water quality, which was administered from October 2020 to August 2021. Due to in-person meeting restrictions related to the COVID-19 pandemic, produce growers mainly completed the survey online in Qualtrics ($n = 76$) during online produce safety-related trainings or by distribution of the online survey link to email listservs of produce growers in Kansas and Missouri. Other respondents ($n = 30$) completed paper copies of the survey during an in-person horticulture farm field day or in-person produce

safety training sessions. Two drawings for gift cards were utilized to incentivize participation in the survey both on-line and in-person. To optimize data collection, potential survey respondents were asked to only complete the survey if they were able to answer questions related to their operations' water management practices. Because most produce farms in Kansas and Missouri have only a few people working on them (28, 29), we are confident that most people filling out the survey are actively involved in the water management practices on their farm, and thus qualified to complete the survey.

The 14-question survey instrument included multiple choice questions with some open text response options designed to understand growers' knowledge and practices related to agricultural water use in both production and postharvest stages, as well as their understanding of the microbial quality of agricultural water. Questions included grower age, farm location, water testing frequency, and water usage for both production and postharvest activities.

The data were analyzed in Qualtrics using the cross-tabulation (crosstab) analysis method (10, 11) (also known as contingency table analysis) to identify the growers' knowledge of microbial risks related to different water sources and how they handle those risks. Cross-tabulation analysis compares the results of different questions from the same respondent to gain more insight into grower water quality management behaviors based on the risk of their water source. For example, growers who indicated that they did not treat their water source but indicated that they used a municipal water source (which is treated by a third-party company) were considered as having a safe water management practice. The responses from questions related to production or postharvest water uses, treatment application status, and microbial water quality test frequency were classified by use of different water sources (surface, ground, and municipal water). The data from any questions that allowed respondents to provide an open-

ended response were added to existing responses or a new category of responses was added to the question, based on the keywords in those responses. For example, a separate category was created for growers who wrote they used water from a rain barrel when selecting ‘other’ water for their water source.

Growers were asked if they were GAP (Good Agricultural Practices) certified (rather than if they were covered by the FSMA PSR) because the project team felt that growers would be more comfortable responding to the question of GAP certification, rather than FSMA PSR coverage. Accordingly, responses to the questions about water testing and water treatment were stratified based on their GAP certification status. Although GAP certification is generally a produce buyer requirement and is not a regulatory requirement, many growers view certification as an opportunity to improve their likelihood of compliance with the PSR. Thus, many growers covered by the PSR also obtain GAP certification.

Because some respondents did not answer all of the survey questions, the total number of responses to each question related to water sources in the cross-tab analysis may be different. Additionally, the total number of responses for questions regarding agricultural water source use could differ as growers may have reported using more than one water sources for one or more pre- or postharvest activities. To reduce item non-response bias, surveys having less than two-thirds of the questions completed were excluded from the final data analysis.

Results and Discussion

Results

A total of 106 survey responses were received. Of the 103 responses included in the final data analysis, 51 (49.5%) were from Kansas, 50 (48.5%) were from Missouri, one (1.0%) was marked as unknown, and one (1.0%) did not complete the question. The age range of growers

completing the survey were as follows: 18-29 ($n = 18$, 17.5%), 30-39 ($n = 15$, 14.6%), 40-49 ($n = 26$, 25.2%), 50-59 ($n = 19$, 18.5%), and above 60 ($n = 25$, 24.3%) years old. Most respondents ($n = 90$) were currently selling or planning to sell their produce to others. Most respondents ($n = 87$) were currently not GAP certified, as shown in Table 3.1.

Table 3.1 Reported microbial water quality testing ($n = 100$) status and GAP certification status ($n = 101$) by state.

	Kansas	Missouri	Total
Have tested water	16 (31.4%)	27 (55.1%)	43
Never tested water	25 (49.0%)	13 (26.5%)	38
Use third party test results	10 (19.6%)	9 (18.4%)	19
Total	51	49	100
GAP certified	2 (3.9%)	12 (24.0%)	14
Working towards being GAP certified in the future	7 (13.7%)	8 (16.0%)	15
Not GAP certified	42 (82.4%)	30 (60.0%)	72
Total	51	50	101

Of the participants which indicated they are currently growing fresh produce ($n = 101$), participants indicated their frequency of generic *E. coli* testing as more than once a year ($n = 14$, 13.9%), once a year ($n = 11$, 10.9%), 2-3 times in the last ten years ($n = 11$, 10.9%), once in last ten years ($n = 7$, 6.9%), never tested ($n = 39$, 38.6%), or that a third-party water source tests their water ($n = 19$, 18.8%). Between the two states (Table 3.1), 31.4% ($n = 16$) of Kansas growers said that they have tested their water in the past ten years compared to 55.1% ($n = 27$) of Missouri growers. Of the growers indicating they are GAP certified ($n = 14$), 10 (71.4%)

reported testing their water more than once a year and six (46.2%) reported that they treat their postharvest water sources. Of the non-GAP certified growers ($n = 87$), four (4.6%) growers test their water more than once a year and 15 (17.4%) growers treat their postharvest water sources (Table 3.2, Table 3.3). Because the questions inquiring about the type of water source used for production and postharvest applications allowed for more than one response, the total number of responses varied by question, as shown in *Figure 1*.

Table 3.2 The frequency of microbial water quality testing by GAP certification status (n = 101).

	More than once a year	Once a year	2–3 times in last 10 years	Once in last 10 years	Never test	Third party water test	Total
GAP certified	10 (71.4%)	0 (0.0%)	1 (7.1%)	1 (7.1%)	0 (0.0%)	2 (14.3%)	14
Working towards being							
GAP certified in the	1 (6.7%)	3 (20.0%)	1 (6.7%)	0 (0.0%)	8 (53.3%)	2 (13.3%)	15
future							
Not GAP certified	3 (4.2%)	8 (11.1%)	9 (12.5%)	6 (8.3%)	31 (43.1%)	15 (20.8%)	72

Table 3.3 Treatment application status for postharvest water based on GAP certification status (n = 99)

	Apply treatment	No treatment applied	Total
GAP certified	6 (46.2%)	7 (53.8%)	13
Working towards being			
GAP certified in the future	3 (21.4%)	11 (78.6%)	14
Not GAP certified	12 (16.7%)	60 (83.3%)	72

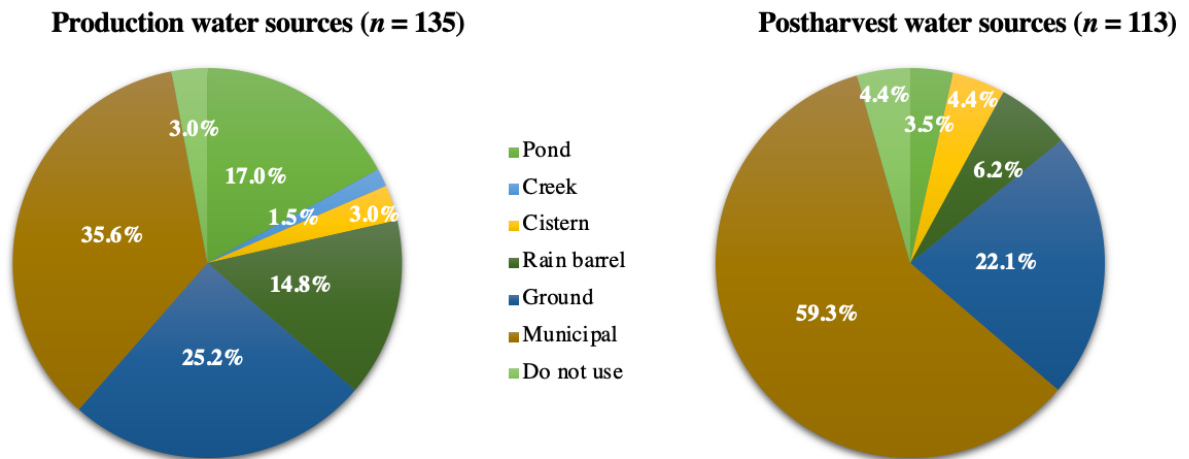


Figure 3.1 The percentages of different water sources that growers use for production ($n = 135$) and postharvest ($n = 113$) activities. (Note: among the 101 responses, several used more than one water type)

Municipal ($n = 48$, 35.6%), well ($n = 34$, 25.2%), pond ($n = 23$, 17.0%), and rain barrel ($n = 20$, 14.8%) water sources were the most commonly reported sources of production water (Figure 1). Municipal ($n = 67$, 59.8%) and well water ($n = 25$, 22.1%) were the most commonly reported water sources for postharvest water. Based on the number of responses for each water source, respondents appear to commonly utilize more sources of water for production applications ($n = 135$) than postharvest ($n = 113$) applications.

Participants also reported their applications of production and postharvest water (Figure 3.2). Of the survey respondents ($n = 101$), 90 (89.1%) indicated that they used production water in their operation. The three main reported uses of production water included irrigation ($n = 90$, 100%), mixing with crop sprays ($n = 38$, 42.2%), and mixing with fertilizers ($n = 38$, 42.2%). Of those growers that report using water for irrigation, 70 (77.8%) reported using drip irrigation, 37 (41.1%) reported using overhead irrigation, and 20 (22.2%) reported using drip irrigation under plastic. Furthermore, 90 (89.1%) indicated that they used postharvest water in their operation.

These growers reported their main uses of postharvest water to be for handwashing ($n = 77$, 85.6%), rinsing/washing produce ($n = 73$, 81.1%), and cleaning and sanitizing of food contact surfaces ($n = 68$, 75.6%). Other uses of postharvest water reported included ice making ($n = 7$, 7.8%), dunk tank cooling ($n = 6$, 6.7%), single pass/spray cooling ($n = 4$, 4.4%), and postharvest fungicide or wax applications ($n = 3$, 3.3%).

Of survey respondents who indicated that they used production water in their operation ($n = 90$), 75 (83.3%) reported that they do not treat their production water. Of the respondents who reported treating their production water, four (4.4%) reported using chemical sanitizers whereas 11 (11.1%) respondents indicated using physical methods such as sand filtration. Of survey respondents who indicated that they used postharvest water in their operation ($n = 90$), 68 (75.6%) reported that they do not treat their postharvest water. Fifteen respondents (16.7%) reported treating postharvest water with a chemical sanitizer and four (4.4%) reported using other treatments such as sand filtration or ultraviolet light. When comparing water treatment status and the water source used (shown in *Figure 3.3*), six (6.7%) respondents reported using surface water (ponds, cisterns, or rain barrels) for postharvest use without applying any treatment.

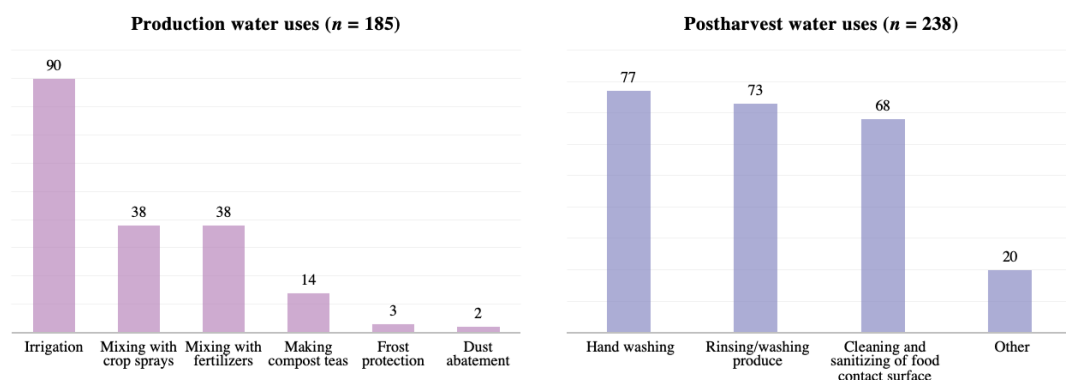


Figure 3.2 The different uses of production water ($n = 185$) and postharvest water ($n = 238$).

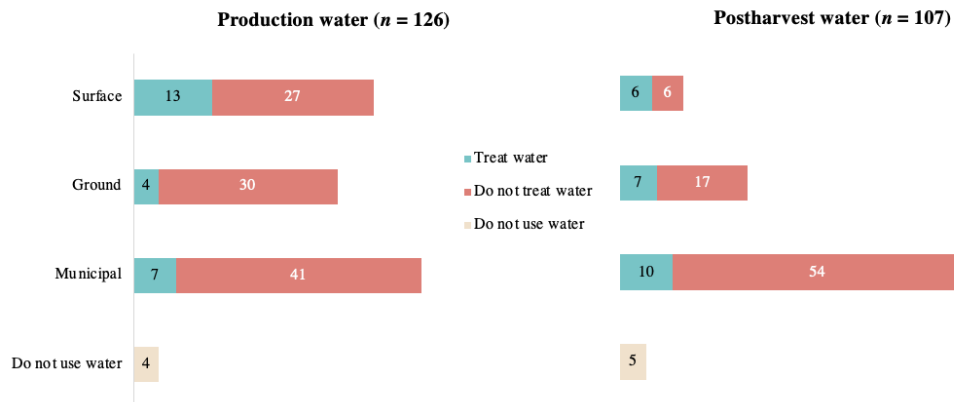


Figure 3.3 Treatment status for each water source that growers use for production (n = 126) and postharvest (n = 107) activities.

When asked what other practices (besides treatment) growers use to maintain/improve production water quality, most ($n = 70, 77.8\%$) respondents using production water in their operation indicated that they did not use any other practices. Nineteen (21.1%) of the participants indicated that they conducted an annual evaluation of their water distribution system. When managing the quality of their postharvest water, 61 (67.8%) respondents who indicated that they used postharvest water in their operation ($n = 90$) reported that they do not use any additional practices, 17 (18.9%) reported routine cleaning and sanitation of tanks, 15 (16.7%) reported monitoring for buildup of organic material, 14 (15.6%) respondents reported conducting an annual evaluation, 10 (11.1%) reported monitoring water temperature during use, and nine (10.0%) reported using a routine water change schedule.

Discussion

Agricultural water quality remains a large concern for the fresh produce industry across the United States, particularly in the Midwest where previous studies indicate that agricultural water quality management is not well understood (17).

Most of the respondents demonstrated a preference for water sources with a lower microbial risk profile (such as municipal water) for postharvest activities, as compared to using surface water postharvest, which is higher risk. Specifically, the survey data indicated that – unlike production water, where municipal (35.6%) and surface water use (36.3%) were nearly equally reported – there is a higher use of municipal water (59.3%) and lower use of surface water (14.1%) for postharvest applications (*Figure 1*). As previous research has shown, microbial populations in surface water increase after events such as agricultural runoff (rain, melted snow, etc.) (3, 24, 27). Surface water is also more exposed to potential contamination from the surrounding open environment, and thus is considered to have a higher possibility of contamination (1, 8, 23).

According to the data collected from the survey, a minority of growers use specific postharvest water management practices that many would consider higher risk. Using surface water without any treatment for postharvest applications poses a higher risk of foodborne disease outbreaks, thus the FSMA PSR agricultural water criteria, GAP certification requirements and general produce safety best practices do not allow use of untreated surface water for postharvest activities (31). Interestingly, six survey respondents indicated that they were using untreated surface water for postharvest uses, and eight respondents indicated they treated their agricultural water using methods such as (bio)sand-filtration. Of note, sand-filtration is not a validated agricultural water treatment method according to the FSMA PSR (21 CFR 112.43) (31). However, growers do currently utilize sand filtration in irrigation systems to remove larger, physical particles, sometimes with the added misconception that it also can remove pathogens. This indicates that further education and training is needed to emphasize the significance of

microbial risk in postharvest water, as well as how to select an appropriate (validated) treatment method.

Conducting regular inspection and maintenance of the water source and distribution system is currently a requirement of the FSMA PSR (21 CFR 112.42) (31) and is a produce safety best practice to limit the risk of microbial contamination. By conducting regular inspections, growers can evaluate the risks to their water sources. The inspection includes identifying any broken part of the system structure, any wildlife evidence around open water sources, and other risks. Most of our survey respondents are not currently conducting an annual agricultural water system inspection. Thus, extension educators should provide practical information to growers on how to inspect their water sources regularly and encourage them to conduct a water system inspection at least annually, which is a requirement for growers covered by the FSMA PSR (21 CFR 112.42) (31). Of note, the proposed §112.43 is intended to supplement the requirements of the proposed §112.42 which will require a covered farm to regularly inspect and routinely maintain the components of its agricultural water system. While proposed §112.42 is focused on agricultural water system components, the proposed §112.43 would require covered farms to conduct a more comprehensive assessment of possible sources and routes by which known or reasonably foreseeable hazards are reasonably likely to be introduced into its preharvest agricultural water (30).

The FSMA PSR also highlights irrigation method as an important risk factor for contamination risk based on if the water contacts the harvestable portion of the produce. In most situations, overhead irrigation results in direct contact with the harvestable portion of the produce, which can result in a higher contamination risk than other irrigation methods. Conversely, drip irrigation (particularly drip irrigation under plastic) is considered to have a

relatively lower risk of contamination for crops grown above the ground. Of note, this characterization does not apply to root crops such as carrots, beets, and radishes (25). Most of the survey respondents indicated that their main use of production water was irrigation, namely drip irrigation (69.1%) or overhead irrigation (31.3%). However, the findings of the study call attention to particular agricultural water practices including the use of untreated surface water for direct water application during postharvest, and seldom testing the sanitary quality of agricultural water sources. Although not specifically measured by this study, further investigation and potentially extension efforts may need to occur to clarify how growers can reduce microbial risk by selecting irrigation methods that reduce the probability of water contact with the harvestable portion of the produce.

In the survey, the question about the frequency of conducting microbial water testing did not specify testing frequency of “production water” and “postharvest water” separately. Accordingly, crosstab analysis for the frequency of water testing for different sources of water used for different purposes (preharvest or postharvest) could not be conducted. Moreover, it was not possible to discern between treated and untreated sources when more than one source was indicated by the grower in this survey. We also did not include questions related to the location of their water source in relation to animals or other potential sources of contamination. These points could have been clarified by increasing the length of the survey. However, the expert panel reviewing the survey was concerned about the adverse effect of increasing the survey length on the response rate as some participants required more than 10 minutes to complete the survey. In a study of the effects of length on web-based survey completion, Galesic and Bosnjak (9) found that participants anticipating a longer survey completion time were less likely to finish the survey.

Conclusion

This survey was intended to provide information on the differences between best practices and the actual practices of Kansas and Missouri produce growers focusing on the use of agricultural water. Produce growers need to continue to improve their practices related to water quality to ensure that they are selling the safest produce possible. The survey responses will provide insight into the development of additional agricultural water safety educational materials for produce growers. The results indicated that efforts are needed to continue to encourage growers to test their water quality, to conduct at least an annual inspection of water sources and distribution system, and to use a validated surface water treatment method before using it for postharvest applications. The authors suggest that future extension trainings should emphasize the importance of microbial quality of agricultural water sources, and how risk to produce safety can be affected by selection of water source, decision to treat, and (in the case of production water) application method.

Acknowledge

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Chapter 4 - Agricultural Water Related Extension Education

Handouts

Based on the anecdotal evidence and survey responses from this thesis (Ch. 2), we identified the critical need to address a few important agricultural water safety-related topics that include the following:

New Modified FSMA PSR Agricultural Water Requirement Proposal

FSMA PSR published the agricultural water regulation in 2016, including using the different microbial quality criteria to value both production and postharvest water quality. However, after the implementation, the feedback from different farms focused on "the rules are too complicated and cost too much." Therefore, the FDA revised and published a proposed agricultural water requirement in December 2021 focused on production water. This handout summarized the major modification of the latest water requirement and aimed to help growers understand and comply with the proposed rule.

QUICK REFERENCE GUIDE

New Modified FSMA PSR Agricultural Water Requirement Proposal



In December 2021, the United States Food and Drug Administration (FDA) proposed a revision to Subpart E of the Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR) that would change the *pre-harvest agricultural water requirements* for covered produce, other than sprouts.

What is the proposed change?

The proposed revision to Subpart E modifies the previous requirement for produce growers to *replace the microbial criteria and testing requirements* for pre-harvest agricultural water with new provisions for *conducting pre-harvest agricultural water assessments*, providing more flexibility to produce growers in evaluating risks.

These aim for hazard identification (including consideration of agricultural water sources, distribution systems, and practices, as well as adjacent and nearby land uses, and other relevant factors), and using the results of the assessment to determine whether *corrective or mitigation measures* are needed to reduce the potential for contamination of produce or food contact surfaces with hazards associated with pre-harvest agricultural water.

What is an “agricultural water assessment” ?

It refers to an *evaluation*, conducted by a covered farm, of factors in its agricultural water system used during growing activities for non-sprout covered produce, its agricultural water practices for such pre-harvest water, crop characteristics, environmental conditions, and other relevant factors (including test results, where appropriate).

Factors	Description
Agricultural water system(s)	• Location and nature of the water source (including whether it is ground or surface water) • Water distribution system type (such as whether it is open or closed to the environment) • Degree to which the system is protected from possible sources of contamination
Agricultural water practices	• Water application method (overhead sprinkler, spray, drip, furrow, flood, seepage, etc.) • Time interval between the last direct water application and harvest of the covered produce
Crop characteristics	• Susceptibility of the covered produce to surface adhesion or internalization of hazards
Environmental conditions	• Frequency of heavy rain or extreme weather events may impact the agricultural water system or damage produce that can increase the susceptibility of produce to contamination. • Air temperatures • Sun (UV) exposure
Other relevant factors	• Including, if applicable, results of water testing that could inform the assessment



Questions, comments or concerns?

Contact your K-State Extension specialist **Londa Nwadike** (lnwadike@ksu.edu or nwadike@Missouri.edu) or **Cal Jamerson** (agri@ksu.edu) today!

New Modified FSMA PSR Agricultural Water Requirement Proposal

Corrective and mitigation measures

The proposed rule does **NOT** change the requirements for harvest and post-harvest water, but only impacts pre-harvest water. Under this proposed rule, a covered farm would *make a determination under the requirements, based on the outcome of its agricultural water assessment*, as to whether mitigation measures would be reasonably necessary to reduce the potential for contamination of covered produce (other than sprouts) or food contact surfaces with known or reasonably foreseeable hazards associated with its pre-harvest agricultural water.

Based on the assessment, if you determine...	Then you must...
that your agricultural water is not safe or is not of adequate sanitary quality for intended use(s).	- Immediately discontinue use(s) AND - Take corrective measures before resuming use of the water for pre-harvest activities
there is one or more known or reasonably foreseeable hazards related to animal activity, BSAAOs, or untreated or improperly treated human waste for which mitigation is reasonably necessary.	Implement mitigation measures promptly, and no later than the same growing season
there is one or more known or reasonably foreseeable hazards not related to animal activity, BSAAOs, or untreated or improperly treated human waste, for which mitigation is reasonably necessary.	- Implement mitigation measures as soon as practicable and no later than the following year Or - Test water as part of the assessment and implement measures, as needed, based on the outcome of the assessment
that there are no known or reasonably foreseeable hazards for which mitigation is reasonably necessary.	Inspect and adequately maintain the water system(s) regularly, and at least once each year

Exemptions:

A covered farm would be exempt from the requirement to conduct an assessment for pre-harvest agricultural water if the farm can demonstrate that the agricultural water meets these requirements, including:

- It meets certain requirements that apply for *harvest and post-harvest agricultural water* (such as the microbial quality criterion and testing requirements for untreated ground water);
- It is received from a *public water system or supply* that meets requirements established in the rule (provided that the farm has public water system results or certificates of compliance demonstrating that the water meets relevant requirements); or
- It is treated in accordance with the standards outlined in the Produce Safety Rule.

Prepared by: Yeqi Zhao Reviewed by: Manreet Bhullar, Londa Nwadike



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How to Interpret Water Results

Since 2016 FSMA PSR published agricultural water regulation, agricultural water has been separated into production water and postharvest water and from three main water sources (i.e., surface, ground, and municipal). Due to the different cross-contamination risks, the water criteria varied by different water activities. This handout aims to help Kansas and Missouri growers better understand the water regulation they need to comply with and interpret their water results within simple wording, images, and charts.

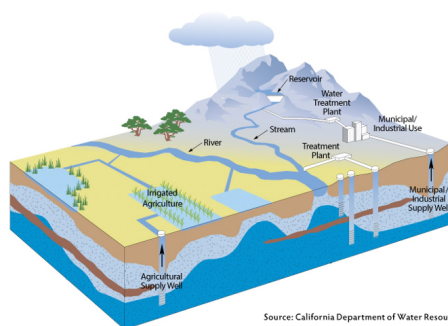
QUICK REFERENCE GUIDE

FSMA Produce Safety Rule Requirements for Agricultural Water

“ The **Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR)** states that all agricultural water **must** be of safe and adequate sanitary quality for its intended use (§112.41) ”

What is Agricultural Water?

Agricultural water is water that is intended or likely to contact the edible portions of produce or food contact surfaces during growing (production) or during/after harvest (post-harvest). The PSR outlines three common sources of agricultural water: **ground water**, **surface water**, and **municipal water**.



Public Water is water that is treated and monitored by a local water utility

Ground Water is water that is found beneath Earth's surface (ex. wells, aquifers)

Surface Water is water that is found or stored on the Earth's surface (ex. rivers, creeks, cistern, ponds supply tanks)

Risk of Contamination



What's the difference between Production and Postharvest Water?

Production Water is any water that is applied directly to produce during growing

- Irrigation
- Crop sprays
- Fertigation
- Frost protection
- Cooling



VS

Postharvest Water is any water that is used during and after field harvest of the produce

- Field Packing
- Washing
- Hydro-cooling
- Ice making
- Hand Washing



Note: water used for hand washing also counts as postharvest water since it can potentially contact the produce surface.

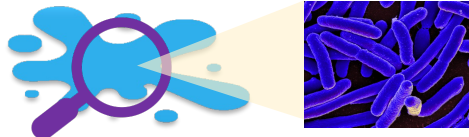


How do we determine water quality?

Agricultural Water Assessment The new modified agricultural water proposed rule uses an evaluation system to evaluate pre-harvest water quality. There are four main factors include agricultural water system(s), agricultural water practices, crop characteristics, and environmental conditions. Based on the assessment, you can determine whether your water is saved to use or not.

Tips: Agricultural Water Assessment Builder can help you evaluate your water quickly and easily!

Access from [link](#) or **QR-code**



Water Microbial Quality The Produce Safety Rule uses a bacteria called generic *E. coli* as an indicator of water quality. *E. coli* is a type of bacteria that is commonly found in animal waste (feces). Based on the number of generic *E. coli* in 100 ml, you can know whether your water is microbially safe to use or not.



Questions, comments or concerns?

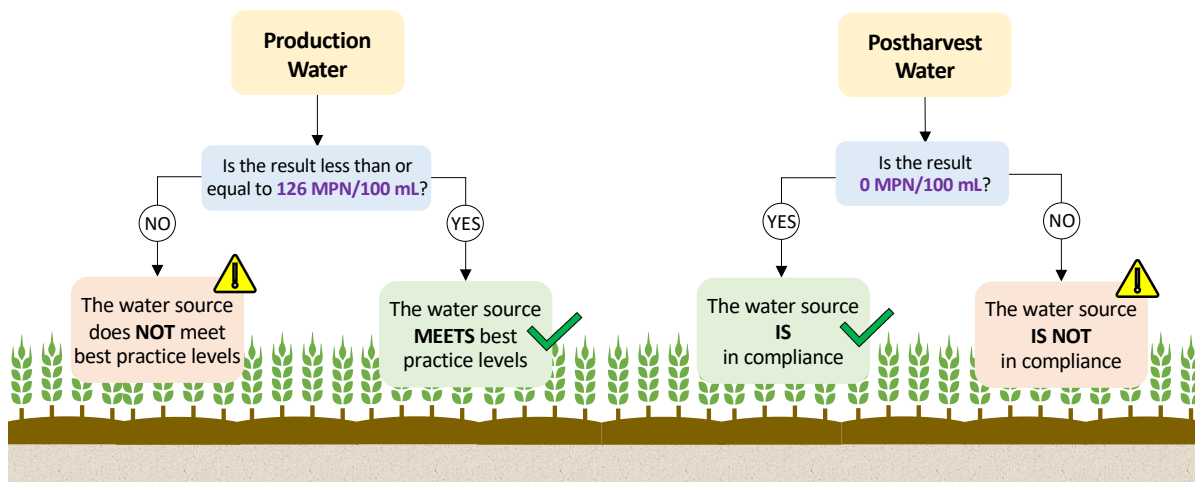
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How to Interpret Your Water Quality Test Results

Step 1: Determine if the water source meets best practice/ regulation levels

Intended Use:



Step 2: Determine the next steps if the water source IS NOT best practices/ FSMA PSR compliant

If the postharvest water is not compliant:

DISCONTINUE use as soon as practical (switch to municipal water or other reliable source) unless corrective measures are applied.

A few things you can do:

- Contact your local extension personnel for solution.
- Apply a **time interval** between the last irrigation and harvest.
- **Re-inspect** the entire affected agricultural water system for:
 - The extent it is under your control
 - Any conditions that are likely to contaminate produce surfaces
- Apply **validated treatments** to the water source.
 - The treatment must be effective to make the water safe and of adequate sanitary quality for its intended use (i.e., UV, chlorine).

[Kansas State University](https://www.k-state.edu/)

Name: Katelynn Stull

E-mail: kjstull@ksu.edu

Cell-phone: 913-307-7394

[University of Missouri](https://www.missouri.edu/)

Name: Dr. Londa Nwadike

E-mail: nwadikel@Missouri.edu

Cell-phone: 913-307-7391

Prepared by: Yeqi Zhao, Olivia Haley Reviewed by: Manreet Bhullar, Londa Nwadike



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Backflush Water Fact Sheet

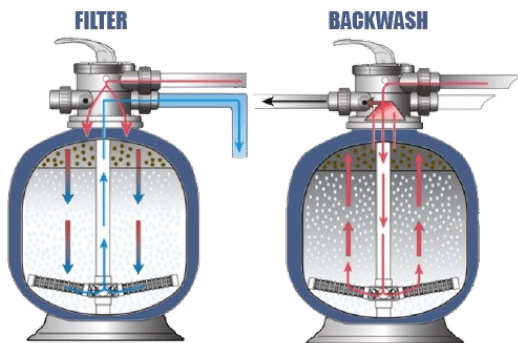
According to the survey results, the filtration system is the primary water treatment reported by the grower. The backflush water is the water used to clean the filter that contains various microorganisms and potentially includes human pathogens. Despite the use of filtration alone is not a validated agricultural water treatment, the handout relates to the filtration method, and backflush water is limited in the extension field. This handout aims to popularize the biohazard of backflush water and possible solutions.

QUICK REFERENCE GUIDE

Backflush Water Fact Sheet

Due to the produce safety concern, filtration systems are widely used in farms to catch small solid particles from original water sources (surface or ground water), and provide clearer water for agriculture purpose (irrigation, fertigation, cooling, etc.).

However, do you know the filtration system can also provide the “dirty” water called **backflush water**?

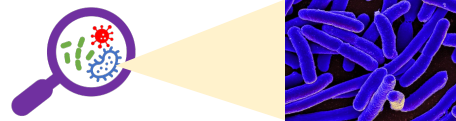


What is Backflush Water?

Backflush, also known as **backwash**, it is a mechanism always comes with a sand and Diatomaceous Earth (DE) filtration system acts as a **self-cleaning system**. It is a water flow that moves reversely through the filter tank to remove the accumulated particles from the filter.

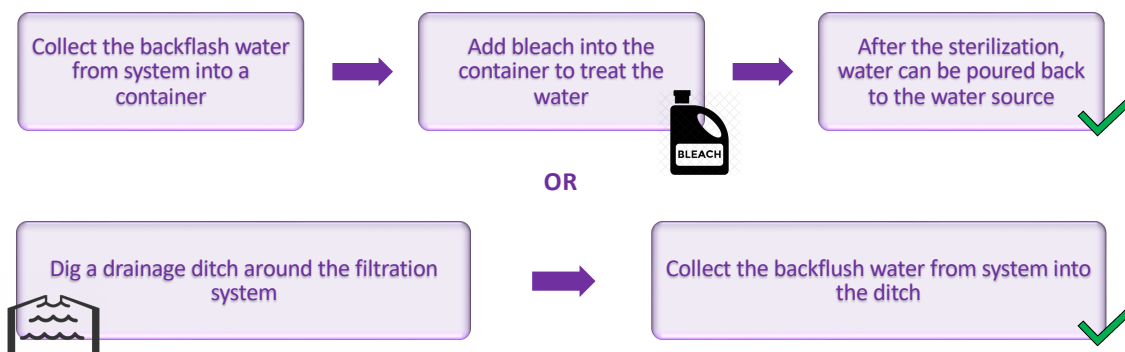
The Concerns That Backflush Water Brings Up:

As backflush water brings all the potential contaminated particles from the filter surface, the backflush water itself is considered as a **highly contaminated** water. If the backflush is not handled and/or stored properly, it can re-add the contamination to the original source or to other clean water sources on the farm.



How to Manage the Backflush Water in My Farm?

Following are several methods to manage your backflush water:



Purdue University Logo



Questions, comments or concerns?

Contact your K-State Extension specialist **Londa Nwadike** (phone number; email) or **Cal Jamerson** (phone number; email) today!

Chapter 5 - Conclusion

The thesis focused on improving the food safety practices reducing the risk of fresh produce outbreaks in the US, specifically expected outcomes on minimizing the cross-contamination chance in the fresh produce farms. The primary two reservoirs for transmitting human pathogens are the farm soil and agricultural water. Thus, to complete the goal, the thesis work included three objectives: (1) reducing the pathogen load in potentially contaminated agricultural soils using cover crops; (2a) accessing current practices and knowledge of Kansas and Missouri growers by using survey; (2b) developing extension material focused on managing agricultural water quality based on the survey results.

Soil Treatment

Several soil treatment methods reduce human pathogens in fresh produce fields. Specifically, cover crops have been recently explored for antimicrobial application in produce production, besides their soil health benefits for contaminated soils. Chapter 2 evaluates the efficacy of reducing pathogen load in contaminated soil with three different cover crop species (i.e., buckwheat, mustard greens, and sunn hemp). The results showed all three cover crops could reduce the bacteria population in contaminated soils after 30 to 40 days following artificial tillage within buckwheat did the best. However, the sole use of a gram-negative pathogen (generic *E. coli*), soil type, and selected three cover crops would be the primary limitations in this study. Considering this is the preliminary study, future research could study the antimicrobial efficacy on soil with different pathogens/soil types/cover crops to better understand the implications of these results. Nevertheless, these results demonstrated the potential of applying cover crops as an antimicrobial treatment in contaminated produce fields. The

outcomes will benefit the organic fresh produce growers as they use biological soil amendments to enrich the soil as a common practice.

Agricultural Water Safety

Unlike soil amendment contamination, agricultural water safety has always been a concern in all types of produce farms, as highlighted in multiple produce outbreaks. Within continuously providing training and information on agricultural water microbial quality to help produce growers reduce risk, the survey developed (chapter 3) is necessary to determine the current knowledge and practice gaps between produce growers and agricultural water management best practices. The surveys were sent out between late 2020 and early 2021 during the COVID-19 pandemic, in which most of the growers' in-person activities had been canceled due to public health concerns. Thus, most surveys were distributed through Qualtrics (an online survey instrument), and the total responses were lower than the expectation (106/200). Despite the dilemma, the survey results indicate the growers from Kansas and Missouri have a basic awareness of agricultural water practices. Still, more demanding practices must be strengthened (e.g., using validated water treatment methods).

Based on the survey responses, three handouts were developed (chapter 4), including: (1) "New modified FSMA PSR agricultural water requirement proposal," which summarized the significant modification of the latest water requirement published in 2021; (2) "How to interpret water results" provides information to identify the agricultural water sources and microbial safety criteria; (3) "Backflush water fact sheet" discussed the hazard from back flush water and solutions. These handouts aim to fulfill the produce growers' current needs and help them to mitigate risk from agricultural water cross-contamination.

In conclusion, the survey provided information on the gaps between best practices and the actual practices of Kansas and Missouri produce growers on agricultural water management; these gave extension professionals the directions for developing further outputs. In addition, in December 2021, the FDA published a new modified agricultural water requirement proposal. The new proposed rule will replace the produce water criteria once it is finalized. Thus, further study can focus on assessing the produce growers' knowledge of the new proposed rule and develop handouts based on the results.

Appendix A - Survey Questions

1. What is your age?

☐ 18-29

☐ 40-49

☐ 60+ years

☐ 30-39

☐ 50-59

2. Are you currently Good Agriculture Practices (GAP) certified?

☐ Yes

☐ No

☐ No, but working
towards being GAP
certified in the future

3. Are you currently raising produce (fruits, vegetables, herbs, mushrooms, nuts) for sale?

☐ Yes

☐ No

☐ No, but plan to in the future

4. In which state do you grow produce? (If more than one, mark the primary state)

☐ Kansas

☐ Missouri

☐ Other _____

5. Do you currently use water PRE-harvest in your produce operation? (check all that apply)

☐ For irrigation

☐ For dust abatement

☐ For mixing with crop sprays

☐ Frost protection

☐ For mixing with fertilizers

☐ Other _____

☐ For making compost teas

☐ No

6. If you use water for irrigation, how do you irrigate? (check all that apply)

☐ Drip irrigation

☐ Furrow irrigation

☐ Drip irrigation under plastic

☐ Other _____

☐ Overhead irrigation

☐ I do not irrigate

7. What sources of PRE-harvest water do you use? (check all that apply)

☐ Pond

☐ Cistern

☐ Creek

☐ Other (such as rain barrel, spring, or
others. Please list) _____

☐ Well

☐ Do not use water pre-harvest

☐ Municipal water

8. Do you currently use water POST-harvest in your operation? (check all that apply)

☐ Rinsing/ washing produce

☐ Single-pass/spray cooling

☐ Commodity movement (i.e. dump
tanks/ flumes)

☐ Dunk tank cooling

☐ Ice making

- ☐ Post-harvest fungicide or wax
- ☐ Handwashing
- ☐ Cleaning and sanitizing of food contact surfaces
- ☐ Other (please list) _____

9. What sources of POST- harvest water do you use? (check all that apply)

- ☐ Municipal water
- ☐ Well
- ☐ Cistern
- ☐ Pond
- ☐ Creek
- ☐ Other (such as rain harvest barrel or others. Please list) _____
- ☐ Do not use water post-harvest

10. Do you test your water used in your produce operation for generic *E. coli*?

- ☐ Yes, more than once a year
- ☐ Yes, once a year
- ☐ Yes, we have tested our water 2-3 times in the last 10 years or so
- ☐ Yes, we have tested our water once in the last 10 years or so
- ☐ No, we have never tested our water
- ☐ A third party (such as the municipal supplier)

11. Do you treat your water used PRE-harvest (such as in irrigation) to improve its microbial quality?

- ☐ Yes, we treat it with a sanitizer (list sanitizer used) _____
- ☐ Yes, we use a different treatment (list treatment- for example, sand filter, other) _____
- ☐ No

12. Do you treat your water used POST-harvest (such as in washing) to improve its microbial quality?

- ☐ Yes, we treat it with a sanitizer (list sanitizer used) _____
- ☐ Yes, we use a different treatment (list treatment- for example, sand filter, ozone, UV, reverse osmosis, other) _____
- ☐ No

13. What other practices (besides treatment) do you use to maintain/improve the quality of the water used PRE-harvest in your produce operation?

- ☐ We conduct an annual evaluation of our water distribution system
- ☐ Other (please list)_____
- ☐ None

14. What other practices (besides treatment) do you use to maintain/improve the quality of the water used POST-harvest in your produce operation?

- ☐ We conduct an annual evaluation of our water distribution system
- ☐ Routine water change schedule
- ☐ Routine cleaning and sanitation of tanks
- ☐ Monitoring for buildup of organic material
- ☐ Monitoring water temperature during post-harvest use
- ☐ Other (please list)_____
- ☐ None

Improving produce safety by reducing pathogen load in soil using cover crops and developing
food safety educational resources for produce growers

by

Yeqi Zhao

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College of Agriculture

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Approved by:

Co-Major Professor
Dr. Eleni D. Pliakoni

Approved by:

Co-Major Professor
Dr. Manreet S. Bhullar

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Abstract

Fresh produce (commonly consumed raw) has been continually associated with multiple foodborne disease outbreaks, which cause both health concerns and economic losses.

Agricultural water and contaminated soil are suspected as common causative agents for recent produce-related foodborne illness outbreaks, which brought fresh produce safety to the forefront of public attention. New federal regulations by the U.S. Food and Drug Administration (FDA) as part of the FDA Food Safety Modernization Act (FSMA) established the Produce Safety Rule (PSR), which defined scientific criteria for growing, harvesting, storing, and packaging fresh produce. The PSR provides requirements on treating soil amendments and criteria for safer use of agricultural water.

There are several soil and water treatment methods and practices to reduce human pathogens in fresh produce. Specifically, for contaminated soils, cover crops have been recently explored for antimicrobial application in produce production, in addition to their soil health benefits. Cover crops are plants seeded before or after the growing season to improve soil health, increase water availability, reduce weed pressure, and prevent erosion. Cover crops also produce various antimicrobial secondary metabolites (i.e., glucosinolates, quercetin), yet the role of cover crops in moderating the population of human pathogens in the soil has rarely been investigated. However, with regards to the microbial quality of agricultural water, Kansas State University and University of Missouri Extension educators have been providing free water testing, training and information on agricultural water microbial quality to help produce growers reduce microbial risks from ag water sources.

The goal of this thesis is to study cover crops as potential antimicrobial interventions and understand grower needs to improve food safety measures and practices on a fresh produce farm.

To achieve this goal, there are two specific objectives: (1) soil treatment: determine the antimicrobial potential of three cover crop species to reduce the population of pathogenic soil microbiota which can minimize the possibility of cross-contamination of produce from agricultural soil; (2) agricultural water: determine knowledge gaps among Kansas and Missouri produce growers related to agricultural water quality and best practices and develop agricultural water safety handouts to improve growers' knowledge.

To complete the first part of this thesis, whole-plant samples of 4-week-old mustard greens (*Brassica juncea*, 'Kodiak'), sun hemp (*Crotalaria juncea*), or buckwheat (*Fagopyrum esculentum*) were chopped and mixed into autoclaved farm soil and inoculated with 7-logs of rifampicin-resistant generic *Escherichia coli* (*E. coli*). The surviving microbial population on days 0, 4, 10, 15, 20, 30, and 40 was enumerated using an agar-based methodology. All three cover crops significantly reduced the population of generic *E. coli* compared to the control ($p < 0.0001$); buckwheat resulted in the highest reduction (3.92 log CFU/g). An inhibitory effect on microbial growth was also observed in soils containing mustard greens ($p < 0.0001$) and sunn hemp ($p < 0.0001$). This study provides evidence for the bactericidal and bacteriostatic effects of cover crops. More information regarding the crops' secondary metabolite profile and potential as a soil bio-mitigation strategy to improve on-farm produce safety is warranted.

For objective 2, a survey was developed and administered to determine future extension outputs and activities to encourage growers to improve their practices related to water quality. The survey was distributed to Kansas and Missouri produce growers attending in-person or online produce safety-related events in late 2020 and early 2021. Also, it was distributed through email lists of produce growers from both states. Survey results ($n = 101$) indicate that 13.9% of the respondents tested their water for generic *Escherichia coli* (*E. coli*) more than once a year,

while 38.6% of the participants have never tested their water. Approximately half (59.3%) of respondents indicated they use municipal water for postharvest uses, while 6.7% indicated the use of untreated surface water for postharvest activities. Due to the concern of potential water contamination risks, researchers suggest that further training and educational resources would help growers improve practices related to water quality and produce safety.

Three handouts were developed to help growers better understand the current FSMA PSR water quality requirements (under consideration for further modifications) and best practices. These handouts include the following: (1) “New modified FSMA PSR agricultural water requirement proposal” handout summarizes the major modifications of the latest water proposal published in 2021; (2) “How to interpret water results” handout provides information to identify agricultural water uses and microbial safety criteria and best practices; and (3) “Back flush water fact sheet” discusses the hazards from back flush water and potential solutions.

In conclusion, this thesis focuses on improving fresh produce safety by minimizing the cross-contamination happening in agricultural soil and water. The reduction of microbial populations in the first part of the thesis demonstrated the potential of cover crops to be used as a sustainable antimicrobial treatment method for contaminated soil. Meanwhile, the survey results show produce growers have a basic awareness of agricultural water practices, but the safety of those practices can still be strengthened. The publication of relevant handouts can improve growers' knowledge and help them better comply with the latest regulations.

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Chapter 1 - Literature Review

Introduction

Significance of fresh produce in the human diet

Since fresh fruits and vegetables contain vitamin A, C, and E, carotenoids, folic acid, dietary fiber, and other minerals, they are one of the great resources to interrupt oxidative stress in the human body to minimize the risk of chronic disease, as well as reduce the concentration of harmful compounds and acid in feces (21). Researchers pointed out that higher fresh produce consumption levels will possibly reduce the risk of cancer (22, 123, 124). Moreover, research showed that consuming produce on a regular basis can reduce blood cholesterol levels (92), the risk of heart disease (66), and maintain blood pressure (118). The concept behind the connection between consuming produce and the risk of chronic disease is the balance of free radicals and phytochemical antioxidants (77) in the body. As the nutrients from produce are hard to replace with other foods, a balanced diet including fruits and vegetables is an important step in maintaining a healthy lifestyle. The USDA's MyPlate recommends eating approximately 2 cups of fruit and 3 cups of vegetables per person per day to maintain a healthy life (the specific recommendation also depends on gender and age) (80).

Besides the increase in health awareness, urban farming has become more popular in the last ten years in the U.S. with the aims of improving local food security, maximizing the use of vacant spaces, and improving agricultural sustainability. Although, due to the environmental impact, currently limited crops can grow on an urban farm, such as leaf greens (72), the model of

potential productivity of urban agriculture shows that small-scale systems are more productive than commercial farms (126). From 1990 to 2018, the value of fruit and vegetable production in the United States has increased each year (130). According to a report from Grand View Research, Inc., the U.S. fruit and vegetable market will reach approximately \$1.12 trillion, and the demand will be more than 243.2 million tons by 2025 (52). As a result, the rise of urban agriculture accelerated the produce market expansion but also added uncertainty to the produce safety, as many produce growers from urban areas own limited farmland and do not need to comply with the Produce Safety Rule (PSR) (annual monetary sales of a farm below \$25,000). So there is still a need for extension professionals to suggest them follow the Good Agricultural Practices (GAP) or PSR to ensure the produce safety during production.

Foodborne outbreaks related with produce

As the demand for fruits and vegetables increasing, more cases of foodborne disease outbreaks have been linked to produce in the past decades. These have highlighted produce safety concerns to the public. In recent years, food safety challenges have continued in an endless series, and there have even been about 3,000 deaths from foodborne causes annually in the US (131). Produce is one of the key vehicles for the transmission of foodborne pathogens in most parts of the world (78). From 2010 to 2017, a total of 85 multistate outbreaks associated with fresh produce with confirmed etiologies occurred in the United States, resulting in a known combined total of 4,658 known illnesses, 1,187 hospitalizations, and 55 deaths (29). *Escherichia coli* (*E. coli*), *Salmonella enterica* (*S. enterica*), and *Listeria monocytogenes* (*L. monocytogenes*)

are the three bacterial pathogens that caused the most multistate fresh produce outbreaks from 2010 to 2017 (29).

Economic impact of foodborne outbreaks

Foodborne outbreaks not only destroy human health but also lead to a huge economic burden. According to the USDA estimation, each year foodborne illnesses cost more than \$17.6 billion of loss (133). Of note, fresh produce is one of the major causes that is believed to account for 26% of the total economic loss (85). One foodborne disease outbreak can lead to \$6,330 to \$2.1 million in economic loss for a fast-casual restaurant (13). According to FDA rankings, the top most common pathogens that cause foodborne disease are *E. coli*, norovirus, *Salmonella spp.*, *Campylobacter*, Hepatitis A, and *Listeria* (138). Among these pathogens, outbreaks associated with *S. enterica*, *L. monocytogenes*, and *Campylobacter spp.* caused the highest total economic losses (113). In addition, the severity of the illness and the type of bacterial infection affect the amount of economic loss, and the cost also depends on the geographical region of the patient. California, Florida, and Texas were among the states with the highest economic losses per foodborne illness patient recorded in 2013 (113, 114).

At the same time, foodborne diseases will bring high economic losses to consumers and will also lead to economic and reputational losses to produce growers or processors. When certain pathogenic bacteria are detected in food products, all products sold are recalled as required by the U.S. Food & Drug Administration (FDA) (30). In this process, the producer has to pay all the costs associated with the recall, including the extra labor, wasted food, and recall costs. At the same

time, when a portion of food is recalled, the reputation of the food manufacturer will normally be damaged, and consumers often will discriminate against the brand. According to the Grocery Manufacturers Association (GMA), the average food recall costs the company about \$10 million per recall (129). For example, a large quantity of food (including safe produce) was dumped due to public health considerations during the 2018 large produce outbreak caused by contaminated romaine lettuce (32).

Thus, reducing the chances of food being infected by microorganisms and ensuring food safety is what every producer and consumer needs to understand and strive to do.

Contamination Factors on Produce Farms

Pathogen contamination of fresh produce can occur at any time during the production chain, although the likelihood of contamination is elevated during field operations such as species and harvest (48, 78). The introduction of human pathogens can occur via diverse routes, including agricultural water (14, 55) and biological soil amendments of animal origin (BSAAOs) (157). Once pathogens are introduced into the farm environment, they can survive and persist for long periods within the soil or water (26, 45, 88, 90, 122), which can cause subsequent spread to other areas of the fruit and vegetable production environment or even result in product contamination.

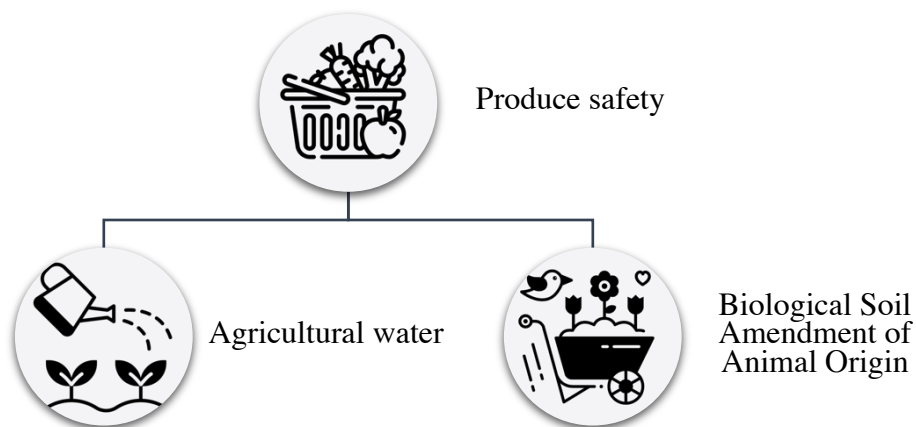


Figure 1.1 The two main contamination routes on a farm that cause produce safety issues.

Agricultural Water

Agricultural water is widely distributed and used in a produce farm which increases the opportunity for human pathogens to attach to the produce. The proximity of produce to contaminated water and the concentration of pathogens within the water are two risk factors contributing to the probability of produce contamination during field production (6). The contamination route always starts with fecal matter carrying human pathogens that drop into the water source (7). Then the transmission of human pathogens can occur at any time during field production (i.e., irrigation, flooding, agricultural runoff) and postharvest activities (washing, rinsing, cooling, etc.).

Biological Soil Amendments of Animal Origin (BSAAOs)

Agricultural soil is another reservoir that can transmit human pathogens to fresh produce. One of the major sources of these human pathogens that survive in the soil is from BSAAOs.

Without proper treatment (i.e., composting), BSAAOs can carry various human pathogens. Once these are applied to soil as fertilizer, they will contaminate the soil soon after. From soil, there are multiple opportunities for human pathogens to attach to the produce. For example, the transmission of human pathogens can occur during the germination of seeds or after the transplantation of seedlings in contaminated soil (28, 71). Therefore, in agricultural applications, it is necessary for growers to detect whether any unexpected animals or improper practices exist before, during, or after field production.

Farm Safety Modernization Act Produce Safety Rule

Overview of the regulation

The US implemented the FDA Food Safety Modernization Act (FSMA) in 2011, which was developed to shift the focus of the nation's food safety system from responding to foodborne illness to minimizing the number of these cases. It was designed to provide clear, specific actions that must be taken throughout the produce handling process to prevent contamination. Nine rules are included in FSMA, which include 1) Third-Party Certification Accreditation; 2) Current Good Manufacturing Practices and Hazard Analysis and Risk-Based Preventive Controls for Human Food and Animal Foods; 3) Foreign Supplier Verification Programs (FSVP); 4) Laboratory Accreditation; 5) Food Traceability; 6) Mitigation Strategies to Protect Food Against Intentional Adulteration; 7) Sanitary Transportation of Human and Animal Food; 8) Voluntary Qualified Importer Program (VQIP); and 9) Standards for the Growing, Harvesting, Packing and Holding of Produce for Human Consumption (10).

This last rule is also known as the Produce Safety Rule (PSR) and the final version was published on November 27th, 2015. It establishes science-based minimum standards for the safe growing, harvesting, packing, and holding of fruits and vegetables grown for human consumption. The compliance dates of PSR criteria are determined by the size of the farm in terms of annual sales and the risk of the commodities becoming a foodborne outbreak when they get contaminated. These include if the farm sells at least \$25,000 of produce annually (adjusted for inflation) and if the produce is likely to be eaten raw (e.g., leafy greens, cucumber, tomato, and most fruits) (137).

Table 1.1 The compliance dates for each rule are based on farm size (very small, small, and other businesses).

Note: ¹ The farm sizes are determined by the average annual monetary value of produce the farm sold during the previous 3-year period. The other businesses (not small) include those selling more than \$500,000 of produce; small size farms include annual monetary sales between \$250,000 to \$500,000; very small includes farms with annual monetary sales between \$25,000 to \$250,000; the annual monetary sales of a farm below \$25,000 are exempted from the PSR. ² The new proposed water requirements are modified based on the final rule which was published December 2nd, 2021 and caused the extended compliance dates. More information on these topics will be mentioned later in this review.

Farm Size¹			
/Compliance Date <i>(134)</i>	Sprouts	Other Produce	Original Water Requirements
Other Businesses (Not Small)	1/26/17	1/26/18	1/27/20
Small	1/26/18	1/28/19	1/26/21
Very Small	1/28/19	1/27/20	1/26/22

Under the PSR, there are six key requirements summarized by FDA as subparts, which include: agricultural water, biological soil amendments, sprouts, domesticated and wild animals, worker training and health and hygiene, and equipment, tools, and buildings (and post-harvest handling?) (135). Of note, maintaining the microbial quality of the water sources used on the farm is of primary importance in produce safety. As water is used in almost every aspect of production, once the water source is contaminated, there will be a higher risk of produce contamination.

Worker Training and Health and Hygiene

Workers are a part of the food safety concerns since they can carry multiple pathogens and spread them unintentionally via multiple routes (58, 65). FDA requires all farm workers who handle covered produce and/or food-contact surfaces and all supervisors to receive food safety training, including the principles of good hygiene and food safety; recognizing symptoms of foodborne illness and the importance of personal hygiene for all personnel and visitors; and other training relevant to the worker's job (137). Of note, water is a key component of these requirements in order to fulfill health and hygiene requirements, such as washing hands and mixing with sanitizer (35). Thus, the microbial quality of the water source which is used for hygiene contributes to the risk of cross-contamination concern on the farm.

Equipment, Tools, and Buildings

The equipment and tools that are used on farms can always directly contact produce. This part of the rule focuses on the standards of sanitation to prevent cross-contamination of produce.

It covers the building structure (i.e., greenhouses, germination chambers, toilets, and hand-washing facilities), the tools, and equipment that may touch or contact the produce. As in the health and hygiene section, water is also required in this part of the rule, especially during cleaning and sanitizing processes. Although the literature shows that the survival time of pathogenic bacteria partially depends on the surrounding environments (i.e., temperature, moisture, the attached surface material) (24, 46, 104), once pathogens are introduced to any equipment, tools, or building floor, the possibility of cross-contamination begins to increase.

Biological Soil Amendments

According to federal regulation § 112.3, biological soil amendments of animal origin (BSAAOs) represent a biological soil amendment that consists, in whole or in part, of materials of animal origin (137). BSAAOs are widely used to improve soil fertility and quality on organic farms and make a primary contribution to agriculture nutrient management (108, 116). However, if the BSAAOs are used improperly, they can lead to very high produce and water contamination risk since they are applied directly next to the produce. Further, pathogens (e.g., *E. coli*, *S. enterica*) have an extended survival length in a warmer and moist environment (91, 97). A scientifically valid controlled process is required from the PSR for treating BSAAOs to minimize the risk of contamination (137). Of note, the PSR and extension fact sheets highlight storage of the biological soil amendments away from water sources since that can cause potential contamination (19, 137).

Sprouts

Agricultural water quality is a key food safety concern in growing sprouts as water is the primary input in sprout production. In past decades, several foodborne outbreaks have been traced back to sprouts (15, 31, 49, 125), which have been contaminated by pathogens carried from sprout seeds and run-off water. As sprouts are grown in warm, moist, and nutrient-rich conditions, this is also the perfect environment for pathogens to survive. Unlike in field production systems, once a single pathogen is introduced into the sprouts system, it can easily spread to all the growing sprouts. Moreover, literature shows that because of the favorable growing conditions, sanitizer treatments (i.e., ozone and chlorine dioxide) cannot inactivate pathogen growth (2). Of note, water is required in most sprout growth steps. Therefore, cross-contamination can either happen during irrigation of sprout seeds in the field or during irrigating the sprouts during production (154). Based on the special growth environment, the PSR has established a separate section for sprout growers, which includes testing any microbes on or in the seeds, sprout irrigation water testing, and further corrective treatments.

Domesticated and Wild Animals

The fecal matter left behind on the field from grazing, working, and wild animals have a potential risk of containing human pathogens. The pathogens from their feces can directly contaminate the surrounding soil and produce (84). The PSR addresses this concern and requires taking all measures reasonably necessary to identify and not harvest produce that is likely to be contaminated. At a minimum, all covered farms are required to visually examine the growing

area and all the covered produce to be harvested (137). Furthermore, once any feces are dropped or flushed (i.e., rain and flood) into nearby water, it will contaminate the entire water source with pathogens from the fecal matter (18). This results in the water becoming a primary reservoir for human pathogens at the farm level.

Agricultural water

Water is an important part of agricultural production as well as a primary reservoir for many human pathogens on the farm. Accordingly, agricultural water microbial quality requirements have been written into the PSR. In current microbial water quality testing guidelines, fecal contaminations are determined by the number of generic *E. coli* in 100 mL of water. The presence of any strain of *E. coli* is a strong indication of fecal contamination, but it cannot be used as direct evidence of the presence of a human pathogen (127). In the PSR, generic *E. coli* is only used to measure microbial water quality and give growers information for future water management.



Figure 1.2 Schematic illustration of a worse condition fresh produce farm.

Originally Proposed Microbial Quality Criteria

Of note, during the thesis year (2020-2022), FDA proposed the new modified production water regulation and currently looking for feedback. Thus, this thesis includes both the current written agricultural water regulation and the new proposed production water regulation. To avoid any misleading, please check the current PSR for referencing.

In 2016, the PSR documented its minimum safety standards for agricultural water at that time. According to the rules clearly specified in PSR released in 2016, for surface water, twenty or more samples must be tested over a period of 2 to 4 years, with five or more samples rolled into the profile every year after the initial survey. The 2016 PSR requirements quantify the safety standards for agricultural water used directly on crops (other than sprouts) by using the

geometric mean (GM) and statistical threshold value (STV) of the water test results. The GM is the average of the water testing results and shows the central tendency of the quality of the water (117). The STV reflects the variability in the quality of the water samples over time.

Table 1.2 The standard for water microbial quality (GM and STV) for pre-harvest water included in the 2016 PSR.

	Geometric Mean	Statistical Threshold Value
Number of Generic <i>E. coli</i> (CFU/100mL)	≤ 126	≤ 410

The corrective actions for the water are required no longer than a year if the water testing results exceed the criteria number. During this period, growers can still use time water in some situations to keep flexibility for them (9, 23).

Microbial Water Testing Methods

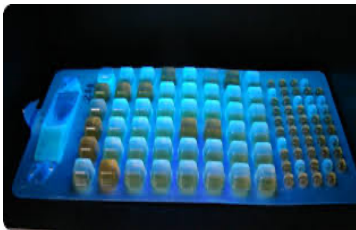
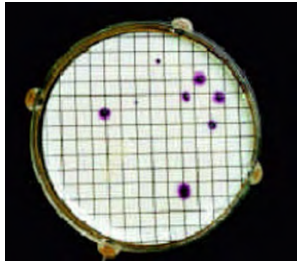
Produce growers need to collect water samples in bottles provided by the testing lab, with a sample size of no less than 100 milliliters. If a groundwater sample is collected, growers should let the water run for five minutes before the sample is collected. If the sample is surface water, keep the sampling bottle close to the center of the water body and try to avoid collecting impurities. After a successful collection, samples must be kept at a low temperature of 4 to 10 °C and sent to a nearby laboratory for analysis within 24 hours (20).

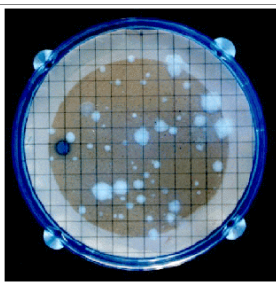
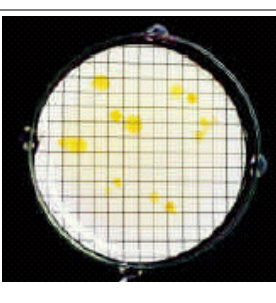
There are two sets of criteria for microbial water quality in the 2016 PSR water requirements that are based on the presence of generic *E. coli* in agriculture water. The microbial water quality for water that is used for post-harvest purposes (including any water that can directly

contact produce surfaces after harvest) must be 0 CFU/100mL, which means no detectable generic *E. coli* are allowed for post-harvest water. The other numerical critical that was proposed in the 2016 PSR water requirements are for water that is applied directly to growing produce (except sprouts). The microbial water quality value for production water in the 2016 PSR water requirements has to be below 126 CFU/100mL GM and 410 STV(132).

Currently, FDA states that there are eight methods considered scientifically valid and at least equivalent in accuracy, precision, and sensitivity in quantifying generic *E. coli* in agriculture water compared to the “EPA? Method 1603: *Escherichia coli* (*E. coli*) in Water by Membrane Filtration Using Modified membrane-Thermotolerant *Escherichia coli* Agar (Modified mTEC) (139)”. Based on the different types of methods, there are two units used in water test results: Colony Forming Unit (CFU) per 100 mL water sample and Most Probable Number (MPN) per 100 mL water sample.

Table 1.3 The eight methods are listed by name, description, result image, and reference.

Name of Method	Method Feature	Example	Reference
IDEXX Colilert/Colilert- 18 Test Kit (unit with MPN)	The Colilert method is used for 4-methylumbelliferyl-β-D-glucuronide (MUG) positive reaction, and the chemical has to be dissolved in the sample water to be tested so that the <i>E. coli</i> in the water can show the fluorescence effect under the ultraviolet light. After adding one packet of Colilert reagent into 100mL of sample water, it is sealed into a quantifying tray. Then it is incubated for 24 hours at 35°C; yellow wells may form to represent if the coliform is present. By checking the amount of blue fluorescent wells in the quanti-tray 2000 under a 6W, 365 nm UV light, the Most Probably Number (MPN) result can be calculated according to the table provided with the Quanti-Tray.		N/A
Method 1603 validated by EPA (unit with CFU)	This is a filtration method that follows the “U.S. EPA Method 1603 Protocol” by growing the <i>E. coli</i> that is left on the membrane in an m-TEC agar incubated for 2 hours at 35 °C and placed in a water bath for 22 hours at 44.5 °C. Counting the pink or purple color colonies formed in the agar provides the final result. This is a test that strictly adheres to FDA regulations; the results are precise and clear but include many disadvantages. The m-TEC agar used in this method is expensive, and it needs to be prepared before starting a water test. The filtration process is required for detection, and the turbidity level of the sample water directly determines the length of time of this process.		Method 1603: <i>Escherichia coli</i> (<i>E. coli</i>) in Water by Membrane Filtration Using Modified membrane-Thermotolerant <i>Escherichia coli</i> Agar (Modified mTEC) (September 2014)

Method 1604 validated by EPA (unit with CFU)	Filter 100mL water sample through a membrane filter and place it on MI agar on an absorbent pad saturated with 2-3 mL of MI broth and incubate at 35 °C for 24 hours. The <i>E. coli</i> will present with a blue color from the breakdown of IBDG by the <i>E. coli</i> enzyme β-glucuronidase; total coliform will fluoresce under longwave UV light from the breakdown of MUGal by the TC enzyme β-galactosidase.		Method 1604: Total Coliforms and <i>E. coli</i> in Water by Membrane Filtration Using a Simultaneous Detection Technique (MI Medium) (September 2002)
Method 1103.1 validated by EPA (unit with CFU)	Filter the water sample through the membrane and place it on the selective and differential medium, mTEC. Incubate at 35°C $\pm$ 0.5°C for 2 $\pm$ 0.5 hours and incubate at 44.5°C $\pm$ 0.2°C for 22 $\pm$ 2 hours. After incubation, transfer the membrane from mTEC agar to the absorbent pad saturated with urea substrate medium for 15 minutes is required for the final step. The color of the <i>E. coli</i> colonies in the final plate should be yellow or yellow-green under a fluorescent lamp.		Method 1103.1: <i>E. coli</i> in Water by Membrane Filtration Using membrane-Thermotolerant Escherichia coli Agar (mTEC) (March 2010)
9213 D validated by SM (unit with CFU)	Filter water sample through a membrane filter and place on mTEC agar. Incubate at 35°C for two h to resuscitate stressed bacteria, then incubate at 44.5°C for 22 h. Then transfer the membrane to a filter pad saturated with urea substrate and containing phenol red for 15 minutes. The result positive for <i>E. coli</i> should be fluorescence under the longwave UV lamp with yellow color.	No image available	Natural Bathing Beaches (2007). In: Standard Methods for the Examination of Water and Wastewater, 22nd Edition (Rice E.W., et al., Ed.), 9-46 – 9-48. Washington, DC: American Public Health Association. (2012).
9222 B validated by SM (unit with CFU)	In this method, the membrane will be placed on les-ENDO or m-ENDO agar which contains basic Fuchsin as the indicator. Filter water sample through a membrane filter and placed on the ager. After incubating the agar for 24 hours at 35 °C, the coliform will show up in red color. Transfer the membrane filter to a nutrient agar that contains MUG and incubate for 4 hours. The individual <i>E. coli</i> colonies will fluorescence under a longwave length UV lamp.	No image available	Standard Total Coliform Membrane Filter Procedure (1997), followed by 9222 G – MF Partition Procedures (1997) using NA-MUG media. In: Standard Methods for the Examination of Water and Wastewater, 21st Edition (Eaton A.D., et al., Ed.), 9-60 – 9-65, and 9-70 – 9-71, respectively. Washington,

**D 5392-93
validated by
ASTM (unit with
CFU)**

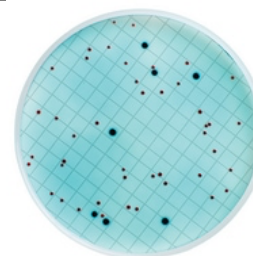
This is a two-step water testing procedure that provides a direct count of the colonies' numbers. Filter sample water through a membrane filter and place it on the selective medium, mTEC. The membrane on the medium is first incubated at 35°C for two h, and then the medium is incubated at 44.5°C for 22 h. Following incubation, the filter is transferred to a filter pad saturated with urea substrate. The yellow or yellow-brown colonies will be present after 15 minutes if the water sample is positive for *E. coli*. Colonies are counted with the aid of a 10 to 15 X magnifier and a fluorescent lamp.

No image available

Standard Test Method for Isolation and Enumeration of *E. coli* in Water by the Two- Step Membrane Filter Procedure. In: Annual Book of ASTM Standards, Volume 11.02. ASTM International. (1996, 1999, 2000).

**Hach Method
10029 for
Coliforms (unit
with CFU)**

This method was published by Hach Company. 100mL of the water sample is filtered through a 0.45-µm membrane filter and placed on the plate, which already added 2mL of the reagent onto a sterile pad on the plate. After incubating for 24 hours at 35 °C, the presence of red colonies indicates the coliforms by reducing the TTC. By hydrolyzing the enzyme-substrate BCIG, it shows blue color for *E. coli* colonies.



Total and *E. coli*, using m-ColiBlue24 Broth PourRite Ampules

Modified Agricultural Water Proposed Rule (proposed in December 2021)

After complying with the water microbial quality criteria for some time, some businesses reported that regulations are difficult to enforce because they are both expansive and complex (144). Thus, in December 2021, FDA revised and published proposed agricultural water requirements (136). The modified rule only impacts the production water regulation but does not change the originally published post-harvest water requirements. Instead of complying with microbial criteria and testing requirements for pre-harvest water (mentioned above), it emphasizes the use of an agricultural water assessment.

An agricultural water assessment is an evaluation that is required to be conducted by covered farms. The factors include agricultural water system(s), agricultural water practices, crop characteristics, environmental conditions, and other relevant factors. Based on the assessment, growers need to determine their water quality and take any actions (i.e., corrective measures, mitigation measures, inspect water system(s), and test water microbial quality) (155). This modification is intended to make it easier for more growers to comply. Large, covered farms were initially required to comply with the water requirements on January 26th, 2022, but FDA is exercising enforcement discretion for the pre-harvest water provisions for all farms while the water requirements are being finalized (as of July 2022).

Agricultural Water Treatment Methods

When the number of generic *E. coli* in the water source has been proved to exceed safe levels or the agricultural water assessment determines the water is not safe for use, corrective

measures are required before resuming the use of the water. Multiple methods can be used to improve the microbial water quality, including filtration, chlorine dioxide, ultraviolet (UV) radiation, and chemical oxidation (i.e., ozone, hydrogen peroxide, and peracetic acid), as shown in Table 1.4. Of note, another agricultural microbial water quality test is required after applying the treatments to confirm that the agricultural water can be used under PSR.

Table 1.4 Log count reduction of pathogens survival in water by different intervention technologies. Note: ZVI means zero-valent iron.

Process	Treatment	Target pathogen	Log reduction	Reference
Filtration	50% ZVI	<i>E. coli</i>	1.7	(67)
	50% sand			
	35% ZVI	<i>E. coli</i>	2.45	(81)
	65% sand			
	35% ZVI	<i>Listeria monocytogenes</i>	2.51	(81)
	65% sand			
	50% ZVI	<i>E. coli</i>	3.54	(8)
	50% sand			
Chlorine dioxide	10 mg/L	<i>E. coli</i>	7	(140)
	3 mg/L	<i>E. coli</i>	5	(57)
	5 ppm	<i>Salmonella</i>	7	(83)
	5 mg/L	<i>Staphylococcus aureus</i>	5	(95)
	1.2 mg/L	<i>Enterococcus faecalis</i>	6.3	(42)
UV	267 nm	<i>E. coli</i>	4	(94)
	275 nm	<i>E. coli</i>	3.6	(94)
	310 nm	<i>E. coli</i>	0.5	(94)

	265 nm	<i>E. coli</i>	4.3	(93)
	275 nm	<i>E. coli</i>	4	(93)
	289 nm	<i>E. coli</i>	2.42	(53)
Ozone	35 mg/L	<i>E. coli</i> O157:H7	3.98	(36)
	45 mg/L	<i>E. coli</i> O157:H7	3.9	(36)
	1 mg/L	<i>E. coli</i>	5	(50)
	1 mg/L	<i>Enterococcus faecalis</i>	6.4	(50)
Hydrogen peroxide	3%	<i>E. coli</i> O157:H7	0.77	(54)
	100 mg/L	<i>E. coli</i>	3.13	(47)
	100 mg/L	<i>Enterococcus faecalis</i>	2.45	(47)
Peracetic acid	8 mg/L	<i>E. coli</i>	3.52	(47)
	8 mg/L	<i>Enterococcus faecalis</i>	4.5	(47)
	3 mg/L	<i>E. coli</i>	3.57	(16)

Filtration

Filtration is a physical process that separates solid particles and liquid from water and reduces the turbidity of the water. The turbidity of water is caused by suspended particles and floating debris, which can provide a protective barrier for microorganisms' survival (147). Thus, reducing the level of turbidity of water before use is important. Filtration systems are widely applied and effective in removing particulate matter, algae, and most microorganisms. Different filtration strategies are used based on the different sizes of target microorganisms (Table 1.5).

Table 1.5 The application for different sizes of filtration strategy.

Filtration methods	Microfiltration Membrane	Ultrafiltration Membrane	Nanofiltration Membrane	Reverse Osmosis
Target Size	approximately 0.1 μm	approximately 0.01 μm	approximately 0.001 μm	<0.0005 μm
Applied for	Sediment and bacterial	Viruses	Microorganisms	Microorganisms

One of the commonly known filtrations is the slow sand filtration (SSF), which lets the water goes through various biological, physical, and chemical processes that finally removes the pathogenic organisms and turbidity effectively. Recently, a great deal of literature highlighted the efficiency and economics of the zero-valent iron (ZVI) bio-sand filtration system for reducing pathogen load in contaminated water (8, 67, 70, 81). Anderson-Coughlin et al. showed that zero-valent iron filtration could significantly reduce the *E. coli* population after going through the filtration system(from 13.13 MPN/ml to 9.59 MPN/ml) as well as shortening the die-off interval of the rest of the bacteria (from -0.48 MPN/day to -1.50 MPN/day) (8).

However, according to the FSMA PSR (21 CFR 112.43) (137), currently, sand-filtration is not considered a validated water treatment method that can be used for removing physical particles and requires a further treatment process to complete the corrective measures. Moreover, the backflush water from the filter can also be problematic as it contains most of the particles from the untreated water. Thus, how to handle the backflush water properly needs to be considered.

Chlorine Dioxide

Chlorine dioxide (ClO_2) is a chemical compound that forms as a gas when the temperature is above 11°C and forms as a liquid when temperatures are between 11°C and -59°C . Due to its chemical properties, it is primarily used for disinfecting drinking water (3). Chlorine dioxide can kill pathogens by breaking the chemical bonds in their molecules and results in pathogen inactivation. Combined chlorine can be found in most sanitizers, such as bleach, and can be used for produce cleaning purposes (59, 75). As shock chlorination is the most cost-effective method of disinfecting water which has been adopted by commercial growers, multiple organizations published shock chlorination guidelines to help growers use it properly (17, 43, 86).

In a lab trial, studies showed chlorine dioxide acted as an effective reagent that can kill more than 99% of plant pathogens in a short time (112). Also, Bel et al. found that 10 mg/L of chlorine can remove 7 logs of *E. coli* rapidly (140). Other research reported a 5 log reduction of *E. coli* populations with 3 mg/L of chlorine dioxide (57).

However, limitations of chlorine dioxide application are also present. It can only work for a short time period within the existence of chlorine dioxide in the water, which cannot be applied as a long-term solution.

Chemical Oxidation

Chemical oxidation is a chemical treatment used to eliminate organic materials in water and wastewater using oxidation, which is both efficient and environmentally friendly. This method has been widely used in the process of purifying and treating wastewater to increase the

use of water (47, 68, 79, 158) as reclaimed wastewater. The reaction of the process is based on a powerful oxidizing agent (e.g., ozone, hydrogen peroxide (H_2O_2), and peracetic acid (PAA)) and obtained at a sufficient concentration.

Ozone disinfection is known as a more effective disinfectant than chlorine against protozoan parasites in water systems (41). Since ozone is highly reactive and unstable, the cost of operation and maintenance are relatively higher than other similar water treatment methods. Hydrogen peroxide is another water treatment method that is environmentally friendly. Several studies show that hydrogen peroxide itself is a slow-acting water disinfectant (requires high dosages and long contact times), but its performance can be enhanced by combining with Cu^{2+} , Ag^+ , and Zn^{2+} (37, 106, 107, 141). Peracetic acid (PAA) is another common and strong oxidant that is applied to disinfect wastewater. It is a man-made chemical that can act against a wide spectrum of pathogens at a low concentration rapidly (12). Kitis et al. ranked the disinfection efficiency of PAA towards microorganisms as bacteria > viruses > bacterial spores > protozoan cysts (68). However, literature also shows the remaining decomposed PAA increases the organic content in the water, which potentially enhances the pathogen regrowth (68, 73, 76). Of note, all the chemical oxidation treatments can be combined with UV and applied in the water treatment process, which results are more efficient than when applied alone (16).

Ultraviolet (UV) Light Disinfection

UV light is a type of electromagnetic (EM) radiation, which has frequencies of about 8×10^{14} to 3×10^{16} hertz (Hz), and wavelengths of about 200 - 400 nanometers (nm). Based on the

wavelength, the UV light can be divided into three sub-bands: UV-A (320-400 nm), UV-B (280-320 nm), and UV-C (200-280 nm). The commercial UV lights for disinfection use UV-C wavelength (typically 254 nm), which is effective against most bacteria, protozoa, and viruses, by acting directly on the DNA of microorganisms and resulting in a germicidal effect (120).

As clearer water has a higher transmission rate, UV light is more effective in treating low turbidity water (more clear, higher transmission) than high turbidity water (UV light gets blocked) (142). Thus, filtration is always recommended, especially for surface water, before running through the UV system. Also, the effectiveness of a UV system depends on the sensitiveness of microorganisms to UV light. Research shows a 280 nm wavelength UV reactor results in a 4 log reduction in *E. coli* (96), while applying the same wavelength to mesophilic bacteria led to a 1.4 log reduction (34). On the other hand, literature shows the pathogen log inactivation does not follow the increase of the UV wavelength. Nyangaresi et al. tested multiple wavelengths of UV-LED on the inactivation of *E. coli* in water, and the 267 nm UV-LED resulted in a 4 log inactivation, whereas the 310 nm wavelength inactivated 0.5 logs of the *E. coli* population (94).

Other in-lab water treatments

There are some water treatments that have not been applied to agricultural water that are more in the experimental stage of research and development and have not been extensively applied commercially on fresh produce farms. (1) Ultrasound technology: The ultrasound system affects objects in a liquid medium by leading to cavitation, and the forces created by it influence the biological system. Thus, ultrasound technology kills microorganisms by enhancing mass

transfer through the biological membranes. Its application range is based on the type of cavitation, which can be broadly divided into transient and stable types. Research shows that it takes five days for ultrasound treatment to reach to 85% reduction (82). Thus, it needs to be applied combined with other biocides, such as chlorine, UV, and electrolysis. According to research, ultrasound treatment improves the effect of chlorination on reducing the bacterial population in raw stream water (102), reaching about a 4 log reduction of fecal coliforms when applied with UV (156).

Electrolysis also can work as a water pretreatment method to reduce microbial contamination. Electrolyzed water (EW) is created by passing an electric current through a dilute saltwater solution in an electrolysis chamber. The process uses a membrane cell to separate an anode and a cathode and produce acidic EW, which contains free chlorine. Thus, EW can be used to reduce the survival of *E. coli*, *Listeria*, and *Salmonella* (51, 64).

Agricultural Soil Treatment Methods

The transmission of human pathogens can occur during the germination of seeds or after the transplantation of seedlings in contaminated soil (28, 71). Also, the internalization of human pathogens, specifically enteric pathogens, through root uptake has been a point of debate due to the inconsistencies among numerous scientific studies (27, 40). Reducing pathogen load in potentially contaminated soil is important, especially in organic farms, which commonly use biological soil amendments of animal origin (BSAAOs) to improve soil fertility and quality (116).

Solarization

Soil solarization is a method of sterilizing soil using solar heating by covering the pre-irrigated soil with transparent plastic sheets, which has the advantages of simple manipulation and low cost. This method has been proven as one of the efficient ways of controlling plant pathogens in soil (121). Recently, using solarization to inactivate human pathogens (i.e., *E. coli* and *Salmonella*) has been confirmed effective both in the field (152) and in a greenhouse (151).

Cover Crops Application

The establishment of cover crops on organic farms is becoming a standard practice to improve soil health and nutrient management. Cover cropping's potential benefits include reduced soil erosion, weed suppression, soilborne and foliar plant disease suppression, reduction of plant-parasitic nematodes, soil improvement, and nutrient management through nitrogen fixation and carbon accumulation (60, 99, 115). Recently, cover crops have gained attention for their ability to suppress soilborne and foliar plant diseases (61, 74, 145); this antimicrobial activity has been attributed to the production of secondary metabolites which protect the plant from microbial invasion (amongst other roles). While research on the antimicrobial activity of cover crops to suppress plant pathogens has been well developed in past decades, studies investigating their antimicrobial activity towards human pathogens in the soil is still limited (109).

Secondary metabolites in cover crops and their antimicrobial activity

The secondary metabolites are the organic molecules that are neither involved with growth nor development but act as a defense mechanism in the plant to respond to the biotic and abiotic stressors (98). In previous literature, secondary metabolites have been widely studied against plant insects (33, 128), plant viruses (153), and plant fungus (63, 148). Until recent years, the antibacterial effect of secondary metabolites in cover crops has been noticed and proved. For example, many species in the *Brassicaceae* family produce glucosinolates which are water-soluble compounds resistant to degradation until they contact the enzyme myrosinase. Then it hydrolyzes them into isothiocyanates (I.C.T.s), nitriles, and other products (56), which inactivate pathogenic bacteria like *Staphylococcus aureus*, *Enterococcus faecalis*, *Listeria monocytogenes*, *E. coli*, and *Salmonella spp.* (4, 110, 111, 143). Flavonoids (i.e., rutin, quercetin, isoorientin) are another large group of secondary metabolites found in species such as buckwheat which inhibit both gram-negative and gram-positive bacterial survival (1, 11, 146). This research brought up the potential of using cover crops as an amendment to inactivate pathogenic bacteria in produce farms.

Extension Education

The USDA National Institute of Food and Agriculture (NIFA) defines agriculture extension as “Extension that provides non-formal education and learning activities to people throughout the country — to farmers and other residents of rural communities as well as to people living in urban areas. It emphasizes taking knowledge gained through research and education and bringing it directly to the people to create positive changes” (89). Agricultural

extension services can originally be traced back to World War I. It served to support war costs by helping farmers increase crop yields by relaying agricultural knowledge. A set of survey data collected in 1980 showed that farmers and all household members had a positive and significant effect after extension education (39). This study emphasized the necessity and significance of agricultural extension education. Nowadays, educators are bridging the gap between new knowledge and regulations from scientific research to growers and aim to solve farms' difficulties and increase the production, food security, and standard of living of farm families. Universities and colleges are also engaging in extension and outreach teams to help local agricultural producers.

In recent years, because PSR criteria require growers to receive training, “PSR training” has become a hot topic in the extension field. In 2010, Cornell University, FDA, and USDA created the Produce Safety Alliance (PSA), which developed a standardized curriculum and nation- wide education to help fresh produce growers to comply with the requirements outlined in the FSMA PSR. According to data from 2013, FDA estimated that 35,029 total produce farms in the U.S. are required to comply with the PSR, which are qualified as very small farms and above (150). In order to fulfill the national training need, in 2016, PSA launched a train-the-trainer program to provide the opportunity for more people to be qualified to deliver the PSA grower training course and to respond to growers' questions during the training sessions. By the end of March 2022, PSA has held 2,332 grower training courses with 46,361 participants and 2,325 people qualified as a trainer in the US (105).

Extension Education Theoretical Framework

In addition to PSA grower courses, extension agents also provide independent sessions on good agricultural practices and general science-based knowledge to both PSR- covered and exempt growers. Inspired by adult and youth education program planning frameworks, the extension education theoretical framework aims to help extension professionals plan effective training. Within the expectation of positive change in growers' behavior after the training, the social marketing theory and the theory of planned behavior are used to develop the extension training sessions. The outcomes will increase the likelihood of growers having behavior changes (25).

Social Marketing Theory

The social marketing theory is adopted from commercial marketing theory and includes the 4P's of marketing, which are the product, price, place, and promotion (149). Instead of the profit motive, like in commercial marketing, the primary goal of social marketing is to achieve a positive behavior change in a population. Kotler and Zaltman et al. defined social marketing as “a social influence technology involving the design, implementation, and control of programs aimed at increasing the acceptability of social ideas or practice in one or more groups of target adopters.” (69). There are two fundamental philosophies in social marketing Theory (62) :

- An organization's objective should be to meet the needs and demands of the customers.
- In order for an exchange to take place, the organization and the customer must engage as nearly equals, with each having something to contribute to the other and each being able

to gain from the transaction.

Since understanding the barriers to behavior change adoption among the target population is the keystone of social marketing theory (87), audience analysis is one of the most important elements of the whole process. The same technique is also applied in the agricultural extension area, known as research-based needs assessment activities. Based on the results from each activity, professionals can implement changes to help farmers overcome the barriers.

Theory of Planned Behavior

The Theory of Planned Behavior (TPB) (5) is an extension of the Theory of Reasoned Action (TRA) (44). It started in 1980 to predict an individual's intention to engage in a behavior at a specific time and place. The principle was designed to understand all activities for which people would exercise self-control. Behavioral intent is a critical component of this model; behavioral intentions are influenced by one's attitude about the probability that the behavior will produce the desired result and one's subjective assessment of the costs and benefits of that outcome (119).

According to the TPB, the behavioral achievement is dependent on both motivation (intention) and skill (behavioral control). It divides attitudes into three categories: behavioral, normative, and control. The TPB is made up of six constructs (attitudes, behavioral intention, subjective norms, social norms, perceived power, and perceived behavioral control) that together reflect a person's actual behavioral control (5).

TPB is considered in extension education to help planners understand the attitudes that growers hold about agriculture practices, the factors that influence their thoughts, and the necessity of measured behavioral intent before and after the training.

Survey Research

Surveys are one of the common measuring instruments to collect feedback and opinions from growers. It enables researchers to be more efficient by employing a sample of respondents to generalize to the entire community (38). The data results can help extension professionals to obtain information to learn the current growers' practices and knowledge. For example, surveys have previously been conducted to assess growers' produce safety knowledge (100, 103) and needs (101) to help them to comply with produce safety best practices.

Typical survey methods include telephone, paper, in-person, and web-based surveys (38). Among these methods, electronic surveys are more adopted in the recent era with the advantage of low cost and fast transmission (38). Of note, due to the COVID situation, public activities were largely canceled due to the concern for public health, so the surveys were largely web-based. Thus, the survey (chapter 3) in the thesis is mainly web-based.

Conclusion and Future Outlook

Soil contamination and improper BSAAOs practice always raise the possibility of cross-contamination, especially on organic farms which commonly use BSAAOs to treat the soil. Although using cover crops to reduce the pathogen load in soil has not been widely studied, the hands-on results demonstrated the potential of cover crops to be used as a sustainable and cost-

effective soil treatment. Further research is needed to compare the efficiency of pathogen reduction among different species of cover crops.

Meanwhile, water will always remain a key component of the food production system. Agricultural water as the primary reservoir of human pathogens is continuously suspected as a transmission route for produce-related foodborne illness outbreaks. One of the current challenges focuses on inappropriate agricultural water practices, including using surface water for postharvest use without validated treatment and misunderstanding some terminologies (i.e., water used for handwashing is considered as postharvest water). These practices might not directly lead to an outbreak but will raise the possibility of cross-contamination. Considering some high-tech agricultural water treatment methods (i.e., UV and chemical oxidation), some of these show promise of pathogen reduction but are not commonly used on farms. Further research and extension education is required to deliver the latest technology to growers.

Another challenge is the latest proposed modifications to the agricultural water rule. Although the new proposed rule is focused on simplifying the production water criteria, growers and extension professionals need more time to adopt the new modified rules after years of education on the current agricultural water regulation. During this transition period between the current version of the rule and the finalized new modified rule, growers may be confused with the requirements and the potential impact of on-farm water practices (i.e., water microbial quality test, agricultural water assessments, and water system inspection). Moreover, the new proposed rule requires growers to determine the water quality by themselves through different

factors. However, this requires growers to have more knowledge of potential agricultural water hazards and good practices. Although the FDA provides an online agricultural water assessment tool to help growers determine the safety of water sources, it still is a barrier for growers located in rural areas that do not have internet.

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Chapter 2 - Evaluating the Efficacy of Cover Crops to Reduce Bacterial Pathogen Load in Contaminated Soil

Abstract

Cover crops are plants seeded before or after cash crops to improve soil health, reduce weed pressure, and prevent erosion. Cover crops also produce various antimicrobial secondary metabolites (i.e., glucosinolates, quercetin), yet the role of cover crops in moderating the population of human pathogens in the soil has rarely been investigated. This study aims to determine the antimicrobial capacity of three cover crop species to reduce the population of pathogenic soil microbiota. Four-week-old mustard greens (*Brassica juncea*, ‘Kodiak’), sunn hemp (*Crotalaria juncea*), and buckwheat (*Fagopyrum esculentum*) were mixed into autoclaved soil and inoculated with 7 log rifampicin-resistant generic *Escherichia coli* (*E. coli*). The surviving microbial populations on days 0, 4, 10, 15, 20, 30, 40 were enumerated. All three cover crops significantly reduced the population of generic *E. coli* compared to control ($p < 0.0001$); buckwheat resulted in the highest reduction (3.92 log CFU/g). An inhibitory effect ($p < 0.0001$) on microbial growth was also observed in soils containing mustard greens and sunn hemp. This study provides evidence for the bactericidal and bacteriostatic effects of particular cover crops. More research regarding the secondary metabolites produced by certain cover crops and their potential as a bio-mitigation strategy to improve on-farm produce safety is warranted.

Introduction

Agricultural soils contain a dynamic microbiome that supports plant health and are home to organisms that produce secondary metabolites (a group of chemical compounds that defend the organisms from biotic and abiotic stress) (35). However, agricultural soils are also a potential route for the transmission of human pathogens to fresh fruits and vegetables. Pathogens like *E. coli* and *Salmonella* are shed in the fecal matter of animals, where they can be spread throughout the field soil by contaminated irrigation water (4, 18) or through weather-related events such as flooding (9, 36). Recent investigations indicate that many of these human pathogens can persist in agricultural soils for up to 100 days (25, 26, 31, 38), increasing the potential for spreading to other produce and to the growing environment. Commodities like watermelons and cantaloupes, in particular, are considered at high risk for contamination from agricultural soils as they are grown on the ground and often make direct contact with soil (8). For this reason, improving agricultural soil management practices is a topic emphasized by federal regulation (e.g., the Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR)) and guidelines for certifications (e.g., Good Agricultural Practices (GAPS) certification).

While such federal regulations and programs offer some preventative measures, there is little information available for growers on strategies to actively reduce the human pathogenic microbial risk of their soils. For example, one of the most pressing concerns in agricultural soil management is the application of biological soil amendments of animal origin (BSAOs), which can carry a high microbial risk since animals serve as the primary reservoir for many human

pathogens (23, 49). The FSMA PSR §112.56 states, “untreated BSAAOs must not be applied in a manner that directly contacts the harvestable portion of the crop and must be applied in a manner that minimizes the potential for contact with covered produce after application” (42). Otherwise, the soil management recommendations currently largely defer to the National Organic Program, which requires growers to apply a die-off interval for raw animal manure application of 120/90 days for edible produce directly/not directly in contact with the soil before harvest (7 CFR 205.203) (41). Of note, soil contamination from BSAAOs is of particular concern for organic produce growing operations, which largely rely on non-chemical (BSAAO-based) methods like manure-based composts to enrich the soil (28). Clearly, there is a need to expand the repertoire of pre-harvest strategies which can reduce the transmission of pathogens from agricultural soils to fresh produce.

Cover crops are typically utilized by growers to improve overall soil agronomic properties but show promise to also reduce the populations of plant and human pathogens in agricultural soils. Cover crops are cultivated between growing seasons to improve soil health and nutrient composition (e.g., carbon, nitrogen) (16, 34), reduce soil erosion (48), and conserve water (43). Plants used as cover crops include grasses (e.g., *Panicum maximum*), legumes (e.g., *Trifolium pretense*), and non-legume broadleaves (e.g., *Fagopyrum esculentum*), which are fast-growing, improve soil physical properties, and may or may not fix nitrogen (7, 12, 14). Recently, cover crops have gained attention for their ability to suppress soilborne and foliar plant diseases (19, 20, 46); this antimicrobial activity has been attributed to the production of secondary

metabolites, which are organic molecules that are not involved with growth or development, but aid in plant response to biotic and abiotic stressors (27). Amongst other roles, secondary metabolites can protect the plant from microbial invasion. For example, many species in the *Brassicaceae* family produce glucosinolates which are hydrolyzed to form cytotoxic compounds that inactivate pathogenic bacteria like *Staphylococcus aureus*, *Enterococcus faecalis*, *Listeria monocytogenes*, *E. coli*, and *Salmonella spp.* (2, 32, 33, 44). Flavonoids (i.e., rutin, quercetin, isoorientin) are another large group of secondary metabolites found in species such as buckwheat which inhibit both gram-negative and gram-positive bacterial survival (1, 3, 47). While research on the antimicrobial activity of cover crops to suppress plant pathogens has been well developed in past decades, studies investigating their antimicrobial activity towards human pathogens in the soil is limited.

A recent study by Reed-Jones et al. focused on the effects of cover crops (cereal rye, hairy vetch, crimson clover, and hairy vetch-rye) on long-term population variation of *E. coli* and *L. innocua* in the soil (29). The study found a suppressing effect of cover crops on *E. coli* and *L. innocua* based on the microorganisms' innate characteristics and soil type (i.e., loamy, clay). The authors also highlighted how soil temperature, tillage practices, and the weather could affect the bacterial population. While the authors observed potential microbial growth inhibition in fields containing cover crops, they raised concerns that the root exudates of living cover crops into the rhizosphere could also promote the growth of human pathogenic bacteria in the soil.

The objective of this study was to determine if there were antimicrobial capabilities of three common cover crops – mustard greens, buckwheat, and sunn hemp – in field soil inoculated with generic *E. coli*. This study will not only provide information on the feasibility of the three cover crops as a soil bio-mitigation method but also the effect of these crops on microbial survival and inform laboratory-based methodologies for such studies.

Material and Methods

Growing Conditions and Plant Biomass Harvesting

The experimental mustard greens (*Brassica juncea*, ‘Kodiak’) (True Leaf Market, Salt Lake City, UT, USA), sunn hemp (*Crotalaria juncea*) (Sow True Seed, Asheville, NC, USA), and buckwheat (*Fagopyrum esculentum*) (Albert Lea Seed Company, Albert Lea, MN) were grown in a greenhouse at the Olathe Horticulture Research and Extension Centre (OHREC) in Olathe, Kansas during the fall of 2021. Mustard greens and buckwheat were sown in large containers (229 cm × 73 cm × 10 cm). Sunn hemp was grown in 9.5-L nursery containers to account for their more expansive root system and larger overall biomass. The containers were filled with organic Plant Pro™ potting soil (Ohio Earth Food, Hartville, OH, USA) before seeding. The greenhouse was climate-controlled, with temperatures ranging from 12.2 °C during the night to 28.9 °C during the day. Overhead irrigation was applied to the crops every other day in order to provide ample soil moisture

Whole crops (including the underground biomass) were harvested at the flowering stage before producing seeds (27 days after propagation). The crops were gently removed from the

containers to avoid excess root loss, and the soil was removed. The plant material was then collected and immediately transported to the lab for further processing, which was performed on the same day.



Figure 2.1 The growing environment for buckwheat and mustard greens.

Soil preparation

The soil used for *E. coli* inoculation in this study was a chase silt loam (40) collected from a field at the OHREC, which had been in perennial grass for the past three years. The soil was collected from the field and transported to the lab. Prior to sterilization, the collected soil was separated into autoclave bags (Fisher Scientific, Waltham, MA, USA); large clumps of sediment were manually pulverized to facilitate handling after sterilization. The soil was then autoclaved at 121.0 °C and 15 psi for one hour; sterility was confirmed using a bioindicator (Fisher

Scientific, Waltham, MA, USA) placed in the center of the bagged soil. Flame-sterilized tongs were used to extract the bioindicator, and the soil was set aside to cool to room temperature before use in the study.

Bacterial strain

Plant roots and shoots are naturally colonized by a dynamic population of microbes that interact amongst themselves and the plant tissues (30). To avoid background interactions from non-target organisms in the phytobiomass, the soil used in the study was autoclaved first, and rifampicin-resistant generic *E. coli* (ATCC 25922) was acquired from the Food Safety Lab at Kansas State Olathe was used as the study microorganism. The strain was previously stored with 50% glycerol (Fisher Scientific, Waltham, MA, USA) in 1.5mL microcentrifugation tubes (Eppendorf North America, Enfield, CT, USA) at -80.0 °C. To initially propagate the bacteria, a loopful of frozen culture was transferred to a culture tube containing 10 mL tryptic soy broth (TSB; Remel Inc., San Diego, CA, USA) and incubated for 24 h at 35.0 °C.

The methodology of Bhullar et al. (6) was followed to induce rifampicin resistance. Briefly, 0.1 mL of the forementioned microbial culture was spread to a plate of tryptic soy agar (TSA; Remel Inc., San Diego, CA, USA) containing 20 µg/mL rifampicin (RIF; Fisher Scientific, Waltham, MA, USA); the TSA-RIF plate was then incubated for 24 h at 35.0 °C. The procedure was repeated using TSA-RIF plates with increasing concentrations of rifampicin (20, 40, 60 µg/mL) until a strain resistant to 80 µg/mL concentrations of rifampicin was generated on TSA-RIF plates.

To culture, the organism for experimentation, one colony of rifampicin-resistant *E. coli* was transferred to 10 mL of TSB containing 80 μ g/mL RIF (henceforth noted as TSB-RIF). The tube was incubated for 24 h at 35 °C and sub-cultured again in 30 mL of TSB-RIF. To prepare the cells for experimentation, the cells were washed twice with phosphate buffer saline (PBS; Fisher Scientific, Waltham, MA, USA) with centrifugation (Allegra X-30, Beckman-Coulter, Brea, CA, USA; 10,000 rpm, 10 min, 20.0 °C) occurring prior to and in between each wash. The final volume was adjusted to a minimum concentration of 7 log CFU/mL.

Sample Preparation and Inoculation

This study utilized a randomized complete block design with repeated measures. Three independent replications of each treatment (cover crop species) were performed. To mitigate the impact of the ‘rhizosphere effect’ and address food safety concerns raised by previous literature (29), we used chopped whole-plant biomass for this study. The whole-plant material (containing roots, shoots, and leaves) was chopped into 3-cm long pieces to imitate mowing and tilling and was manually homogenized in three large containers (one container per cover crop species). To simulate the soil-cover crop mixture, 200 g of the chopped plant tissue sample, 200 g of the sterile soil sample, and 75 mL of inoculum culture were combined in a 24 oz. Whirl-Pak bag (Whirl-Pak, Madison, WI, USA). The 1:1 ratio of cover crops and soil is based on the assumption that cover crops will only be incorporated into the upper 10 cm of soil. For the non-treated control, 400 g of the sterile soil sample and 75 mL of inoculum culture were combined. The ratio of the solid matter and inoculum was based on preliminary trials to ensure all solid

matter would be moistened by the inoculum. Each sample bag was shaken and massaged 50 times to homogenize the mixture before being transferred into a quart-sized container (1.4 L) with a plastic plant saucer (Mudeela, Seattle, WA, USA) placed underneath. The samples were stored at room temperature (average 20.6 ± 0.02 °C) and with an average relative humidity of $32.9 \pm 0.4\%$ for 40 days. An average of 30 mL of sterile DI (deionized) water was added to each container each week to prevent the soil from becoming too dry to facilitate bacterial survival.

Sample Collection and Microbial Enumeration

Before collecting the sample on each sampling day, each container was mixed using a sterile plastic spoon; the spoons were sterilized by soaking overnight in 10% bleach, followed by rinsing with distilled lab water. A 10-gram sample was collected from each container and transferred to a Whirl-Pak bag containing 90 mL of 0.1% buffered peptone water (BPW; Remel Inc., San Diego, CA, USA). The sample bag was manually massaged to homogenize the sample. Serial dilutions were prepared in 0.1% BPW, and 100 μ L of the sample solution was then spread-plated onto TSA-RIF before incubating for 24 h at 35 °C. Plates yielding 25-250 colonies were recorded for analysis. Samples were collected 0, 4, 10, 15, 20, 30, and 40 days after treatment.

Statistical analysis

The statistical treatment structure utilized was a one-way ANOVA. The whole study was evaluated using three blocks of four pots each on days 0, 10, 20, 30, and 40. The surviving bacterial population at each time point was subjected to log transformation in order to achieve the variance homogeneity assumption.

The bacteriostatic (phase I) rate was calculated based on the difference of log-transformed surviving bacterial populations between day 0 and day 10. The bactericidal (phase II) rate was calculated based on the difference in log-transformed surviving bacterial populations between day 10 and day 40. The bacteriostatic (phase I) and bactericidal (phase II) rates were analyzed separately using the linear mixed model. Fixed effects of the model included all treatments. Random effects were the block and the error term. The main effect per day was assessed via least squares means (LSMs) and their standard errors (SEMs). Pairwise comparisons were carried out using the two-sided test. The Holm-Tukey method was used to adjust for multiplicity. All tests were conducted at the 0.05 significance level. Statistical analysis was executed via Statistical Analysis Software (SAS version 9.4; Cary, NC) PROC MIXED with option DDFM=BETWITHIN in the MODEL statement.

Results and Discussion

Overall trends in cover crops microbial reduction

Overall, the data showed that certain cover crops could reduce the microbial risk of soils containing generic *E. coli* within 30 to 40 days following a simulated cover crop incorporation event (*Figure 2*). This microbial reduction was cover crop- ($p < 0.0001$) and time-dependent ($p < 0.0001$). By day 30, soils amended with buckwheat or mustard greens had achieved a maximum *E. coli* reduction of 3.92 ± 0.16 log CFU/g and 3.77 ± 0.16 log CFU/g, respectively. For soils containing macerated sunn hemp, *E. coli* was still detectable on day 30, only reaching a maximum microbial reduction of 3.70 ± 0.10 log CFU/g 40 days after treatment.

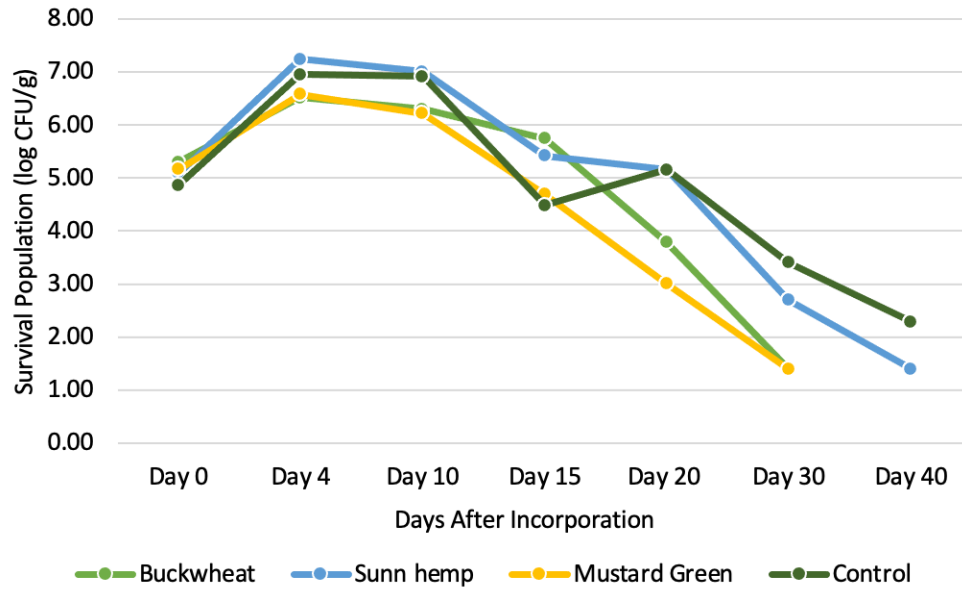


Figure 2.2 The surviving population of rifampicin-resistant *E. coli* in agricultural soil amended with macerated control, buckwheat, sunn hemp, or mustard greens

These overall findings provide potential insight into how these cover crops may be utilized to reduce human microbial risk and thus be used as a soil bio-mitigation strategy. For example, the persistence of *E. coli* in soils amended with sunn hemp could indicate that the secondary metabolites of buckwheat and mustard green tissues are more rapid in inducing microbial cytotoxicity compared to those in sunn hemp tissues. This observation is consistent with previous literature, which reports the cytotoxic activity of sunn hemp is predominantly in seeds rather than in root/shoot tissues (11, 24). In general, there were also no differences in *E. coli* survival between soils containing mustard greens or buckwheat throughout this study, despite their previously- documented different secondary metabolite composition and volume (5, 21).

A biphasic microbial variation curve (see Figure 2) was observed in the experiment, wherein statistically significant interaction effects ($p < 0.0001$) indicate unequal effects of the treatments

over time. The first phase (days 0 to 10) is characterized by a significant increase in the *E. coli* populations amongst all the treatments prior to their decline. From day 10 on, the microbial populations experienced a sharp decline until reaching levels below the detection limit. Topp et al. (39) reported observing a similar microbial growth curve and attributed the variation to the soil type and *E. coli* strains, amongst other factors. Of note, this previous study did not explore microbial survival in a chase silt loam as was utilized in this study, which is characterized by more than 50% silt and 12-27% clay (13), and is a soil composition (theoretically) more likely to have negligible influences on *E. coli* growth due to the relatively aerobic environment of low clay-content soils (37).

Modeling the surviving microbial population to study the biphasic survival curve

To further investigate the differences in microbial growth, the surviving microbial population was statistically modeled by fitting its survival to a nonlinear curve (*Figure 3; Equation 1*). The values used in the equation specific to each cover crop and the control are presented in *Table 1*. Overall, the models were able to explain a high degree (91% to 98%) of the observed variability in the microbial survival of each treatment.

$$y = \frac{x \cdot a + b}{1 + c \cdot x^d} \quad (1)$$

Equation 1, Where *x* is the days after initial inoculation

y is the total surviving microbial population

a is the rate of increase to the maximum peak

b is the multiplier that increases the height of the peak

c is the rate of decrease with respect to days after initial inoculation

d is the peak shape parameter

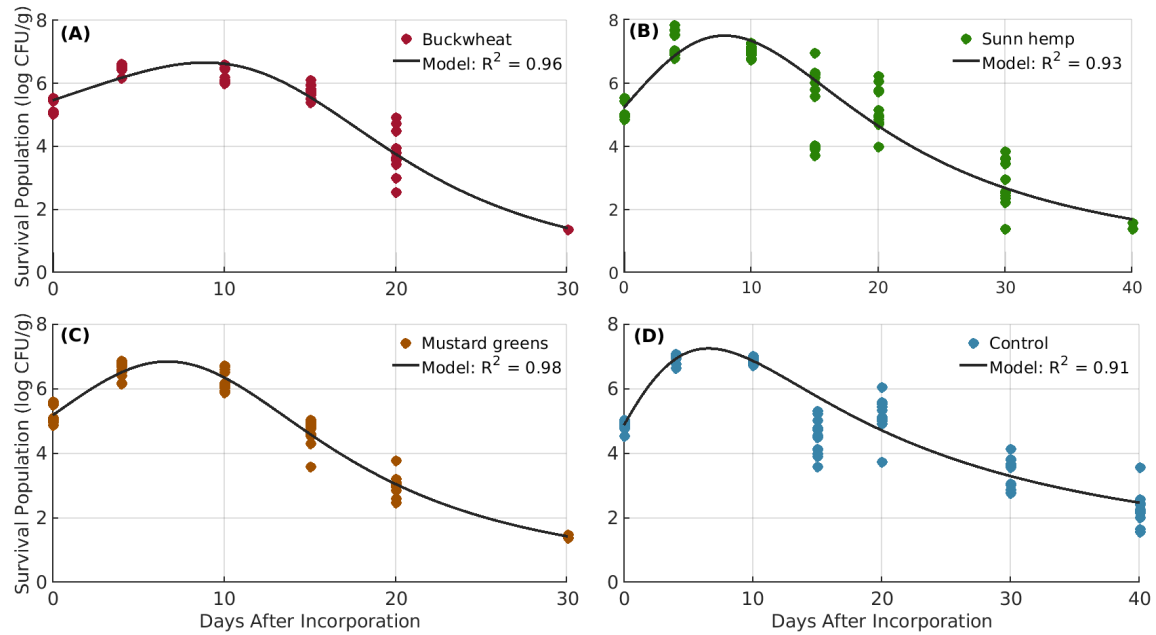


Figure 2.3 The fitted model estimating the surviving population of rifampicin-resistant *E. coli* in field soil supplemented with macerated control, buckwheat, sunn hemp, and mustard greens.

Table 2.1 Variable values by cover crops and control models.

	a	b	c	d	% Variance (R^2)
Control	0.7149	4.875	0.006.989	2.031	91
Buckwheat	0.1827	5.473	0.00001.497	3.827	96
Mustard greens	0.3761	5.184	0.0004.511	2.957	98
Sunn hemp	0.4654	5.227	0.0008.272	2.621	93

Bacteriostatic effects of cover crops during the exponential growth of *E. coli*

From days 0 to 4, there was a significant increase ($p < 0.05$) in the *E. coli* population in all treatments compared to their initial concentration. As evident by the value of a variable (Table 1), the control soils experienced a more rapid rate of increase to the maximum surviving population than the soils containing macerated cover crop tissue. Between the cover crops, sunn hemp showed a more rapid microbial population growth rate than buckwheat ($p < 0.0001$) or mustard greens ($p < 0.0001$). There were no significant differences between the initial growth rate (a) of the buckwheat and mustard greens ($p = 0.6178$) or the sunn hemp and control ($p = 0.2278$). After day 4, the soils containing macerated buckwheat ($p = 0.0021$), mustard greens ($p < 0.0001$) or sunn hemp ($p = 0.0010$) began experiencing a minor decrease in generic *E. coli* populations. In contrast, the microbial population of the control sample attenuated from days 4 to 10 as there were no statistically significant differences ($p = 0.6927$) detected during this time period.

These findings indicate that the exudated phytochemicals of all three macerated cover crops can impair the growth of *E. coli* during this initial growth phase, with buckwheat and mustard greens being the most effective. It is also likely that these results are reflective of an initial exudation of phytochemicals from the macerated cover crops stimulated by the artificial tillage. Divekar et al. note that many secondary metabolic production pathways are rapidly induced by physical stimuli (e.g., cutting of the plants) (15). Accordingly, using a rotary tiller or similar tool

to physically cut the cover crops (instead of only rotating them into the soil) may be critical to initiating their antimicrobial effect.

Bactericidal effects of cover crops during declining *E. coli* population

During the second phase (days 10 to 40), there was a more rapid, significant decrease ($p < 0.0001$) in the *E. coli* population in all treatments compared to their concentration on day 10. In this phase, the *E. coli* could be experiencing die-off due to limited nutrients available in the environment which is accelerated by the presence of cover crop tissues. As evidenced by the value of the c variable (Table 1), the control soil and soils containing macerated sunn hemp showed a slower microbial reduction rate than buckwheat or mustard greens ($p < 0.0001$). Buckwheat experienced the most rapid decline, but there were no significant differences detected between the microbial population of mustard greens and buckwheat ($p = 0.5626$).

The most rapid microbial decline for soil containing macerated sunn hemp occurred between days 20 to 30 which led to a 2.46 ± 0.15 log reduction of generic *E. coli*. Buckwheat also experienced the most rapid population decline (2.40 ± 0.15 log CFU/g) between days 20 and 30. Conversely, soils containing macerated mustard greens exhibited a microbial decline of 1.68 ± 0.26 log CFU/g between day 10 and day 20. There were no significant differences in the surviving population of *E. coli* between mustard greens and buckwheat except on day 20.

Interestingly, the results of this bactericidal phase do not necessarily align with the expectations from the literature. During this phase, it is conceivable that the more rapid decline is due to the cover crops beginning to degrade in the soils, further releasing exudates containing

anti-microbial secondary metabolites (10). Based on the degradation rate, sunn hemp should have the most rapid rate of degradation as the tissues decompose within two weeks post tillage (45). According to Wang et al., the phenolic compounds in buckwheat tissues typically degrade after 37 days, indicating a lower rate of metabolite exudation (47). As this study reports trends in antimicrobial activity which are opposite to what is expected (sunn hemp has less effective microbial reduction rate than buckwheat throughout the study), more investigation regarding the exudation and degradation of the principal secondary metabolites within the tissues of each cover crop is warranted.

Study Limitations

Though the data from this study fit the conventional assumptions for ANOVA, the statistical methodology needed to account for differences in the initial *E. coli* concentration between the control and cover crop-containing soils on day 0 (*Figure 1*). Accordingly, only inferences between the rate(s) of population growth/decline in each cover crop could be made. We believe this significant difference in *E. coli* populations on day 0 can be ascribed to the different compositions of the control samples. As no plant tissues were added to the control containers, 400g of farm soil was used as the control and was inoculated with the same volume of inoculum as other treatments. According to Fick's diffusion law, the diffusive flux goes from areas of high osmolyte concentration to areas of low osmolyte concentration (17). The lower moisture content of the control soils relative to the higher moisture content of the soils containing fresh cover crop tissues likely resulted in a more even distribution of the inoculum in the control soils. Thus,

when collecting the same weight of sample from each treatment, the control group resulted in a diluted generic *E. coli* population result.

The sole use of a gram-negative experimental pathogen presents another limitation of this study. This study evaluated the survival of non-pathogenic *E. coli* as a surrogate for pathogenic *E. coli* or other gram-negative species that may behave in a similar manner (i.e., *Salmonella* spp.), which are commonly found in the gut of wild and domestic animals. However, Locatelli et al. (22) reported higher stability and persistence of gram-positive bacteria (i.e., *Listeria monocytogenes*) in agricultural soils. When Neiunna et al. (29) inoculated cover crop-containing soils with *Listeria innocua*, the authors observed a lower antimicrobial efficacy against the pathogen compared to the gram-negative organisms. This reduced efficacy towards gram-positive organisms may not impact the application of cover crops in states dominated by cattle or poultry production, which have a higher risk of detecting gram-negative *E. coli* and *Salmonella* spp. in the soil. However, this could limit the applicability of the tested cover crops as a microbial risk mediation strategy in operations that struggle with high-risk sources of gram-positive organisms.

Conclusions

All three cover crop species resulted in a microbial reduction to non-detectable levels, while buckwheat and mustard greens also displayed a bacteriostatic effect on the growth of *E. coli* during the exponential microbial growth phase (days 0-10). Future studies should account for extrinsic and intrinsic environmental factors to provide more suggestions for the on-farm

applicability of cover crops as a bio-mitigation strategy. Factors like sunlight, temperature variation, and native soil microbiome interactions were eliminated in this experiment in order to determine if there was an effect of the cover crops on microbial populations. Moreover, the differential efficacy of the cover crops demonstrates the need for characterizing the mechanisms that drive the effects we observed in each of the different cover crop species, particularly including the secondary metabolite content and concentration within the plant tissues. A better understanding is needed of their corresponding environmental stability and exudation patterns as the crop decomposes into the soil. Overall, this is the first lab-trial study for human pathogen reduction on agricultural soil within cover crops amendments. The results demonstrated these cover crops as a promising, environmentally sustainable bio-mitigation strategy for human pathogens in soil that warrant more investigation to promote their use in produce growing operations.

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Chapter 3 - Knowledge and Current Practices Related to Agricultural Water Microbial Quality among Kansas and Missouri Produce Growers

Abstract

Kansas State University and University of Missouri Extension educators have been providing training and information on agricultural water microbial quality to help produce growers reduce risk. However, we recognized the need to determine knowledge gaps among Kansas and Missouri growers related to agricultural water quality and best practices. A survey was developed to determine future extension outputs and activities to encourage growers to improve their practices related to water quality. The survey was distributed to Kansas and Missouri produce growers attending in-person or online produce safety-related events in late 2020 and early 2021 and was also distributed through email lists of produce growers from both states. Survey results ($n = 101$) indicate that 13.9% of the respondents tested their water for generic *Escherichia coli* (*E. coli*) more than once a year, while 38.6% of the participants have never tested their water. Approximately half (59.3%) of respondents indicated they use municipal water for postharvest uses, while 6.7% indicated the use of untreated surface water for postharvest activities. Due to the concern of potential water contamination risks, researchers suggest that further training and educational resources would help growers improve practices related to water quality and produce safety.

Introduction

In recent years, foodborne disease outbreaks resulting from fresh produce have continued to occur (4, 12). Because these commodities are often consumed raw (26, 32), it is imperative that

fresh produce growers use the safest practices possible. From 2010 to 2017, a total of 85 multistate outbreaks associated with fresh produce with confirmed etiologies occurred in the United States, resulting in a known combined total of 4,658 known illnesses, 1,187 hospitalizations, and 55 deaths (4). Further, before 2015, numerous fresh produce outbreaks have been attributed to contaminated agricultural water in commodities such as leafy greens (15, 22), tomatoes (2), and melons (33). Thus, water quality management practices became a major and critical aspect of the Food and Drug Administration's (FDA) Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR) (31).

The PSR, which was finalized in 2015 to take its place as one of the seven foundational rules under FSMA, is the first U.S. Federal regulation to have established science-based minimum standards for the safe growing, harvesting, packing, and holding of fruits and vegetables grown for human consumption. In December 2021, the FDA proposed a revision to Subpart E of the PSR that would change the preharvest agricultural water requirements for covered produce, other than sprouts (30). The FSMA PSR and the proposed revision state the considerations and requirements related to agricultural water management to improve the microbial safety of agricultural water and therefore of produce. In December 2021, FDA announced their intent to exercise enforcement discretion and also act to extend the compliance date for the agricultural water (31).

As per the FSMA PSR, "agricultural water means water used in covered activities on covered produce where water is intended to, or is likely to, contact covered produce or food contact surfaces." Agricultural water is classified into two categories based on the different usage periods: production water and postharvest water. Production water refers to water used during growing activities whereas postharvest water includes all uses of water during or after harvest (7,

31). Since the use of postharvest water is so close to consumption, microbial water quality is especially important (6). Thus, produce growers must meet the requirements of §112.44(a), i.e., no detectable generic *E. coli*/100mL water sample for water used postharvest. The proposed revisions to Subpart E focus on production (i.e., preharvest) uses of agricultural water. These revisions emphasize conducting agricultural water assessments for production water sources and uses, eliminating the federal requirement of an ongoing microbial water quality profile with periodic updates through water quality monitoring.

Each year, Kansas and Missouri growers produce approximately \$26 million (13) and \$81 million (7), respectively, of fruits and vegetables. Kansas State University and University of Missouri Extension educators have been providing training and information on agricultural water microbial quality, based on current and newly proposed FSMA PSR agricultural water requirements, to help produce growers from Kansas and Missouri reduce risk to produce from agricultural water. Although research shows that knowledge gain alone leads to limited behavior change, it can provide people with more informed choices that may or may not lead to behavior change (16). Pratt and Bowman (21) suggest that individually-relevant, problem-focused, and hands-on activities can help extension educators prompt personal behavior change. To help prompt this positive behavior change, educators need a better understanding of the gap between water quality best practices and produce growers' knowledge and actions related to agricultural microbial water quality.

Survey research (20) is one method that allows extension educators to easily understand a large number of growers' current water practices and knowledge gaps in a shorter time. Other surveys have previously been conducted to assess growers' general produce safety knowledge (17, 19) and needs (18) to help them to comply with produce safety best practices. Perry et al.

(17) found that North Central US produce growers have a knowledge gap in understanding agricultural water handling practices compared with other topics of the FSMA PSR, which highlights the needs for food safety extension educators to focus on delivering information on agricultural water best practices to produce growers.

This survey aims to determine gaps between safe water management practices (as outlined in the FSMA PSR requirements/ proposed requirements and other produce safety best practices) and (i) the current knowledge and (ii) the current practices of produce growers from Kansas and Missouri related to agricultural water quality. This will enable extension educators to develop additional training and resources on agricultural microbial water quality accordingly to better meet the needs of growers.

Materials and Methods

The project team developed a 14-question survey instrument based on similar surveys (18). Before implementing the survey, 4 extension experts reviewed the survey questions for face and content validity. The Kansas State University Produce Safety Extension Associate also performed cognitive interviews (14) with growers from the target population to further validate the survey. The Kansas State University Human Research Institutional Review Board (IRB) approved the use of the survey. The survey (*Appendix 1*) included questions on the growers' knowledge and practices related to microbial water quality, which was administered from October 2020 to August 2021. Due to in-person meeting restrictions related to the COVID-19 pandemic, produce growers mainly completed the survey online in Qualtrics ($n = 76$) during online produce safety-related trainings or by distribution of the online survey link to email listservs of produce growers in Kansas and Missouri. Other respondents ($n = 30$) completed paper copies of the survey during an in-person horticulture farm field day or in-person produce

safety training sessions. Two drawings for gift cards were utilized to incentivize participation in the survey both on-line and in-person. To optimize data collection, potential survey respondents were asked to only complete the survey if they were able to answer questions related to their operations' water management practices. Because most produce farms in Kansas and Missouri have only a few people working on them (28, 29), we are confident that most people filling out the survey are actively involved in the water management practices on their farm, and thus qualified to complete the survey.

The 14-question survey instrument included multiple choice questions with some open text response options designed to understand growers' knowledge and practices related to agricultural water use in both production and postharvest stages, as well as their understanding of the microbial quality of agricultural water. Questions included grower age, farm location, water testing frequency, and water usage for both production and postharvest activities.

The data were analyzed in Qualtrics using the cross-tabulation (crosstab) analysis method (10, 11) (also known as contingency table analysis) to identify the growers' knowledge of microbial risks related to different water sources and how they handle those risks. Cross-tabulation analysis compares the results of different questions from the same respondent to gain more insight into grower water quality management behaviors based on the risk of their water source. For example, growers who indicated that they did not treat their water source but indicated that they used a municipal water source (which is treated by a third-party company) were considered as having a safe water management practice. The responses from questions related to production or postharvest water uses, treatment application status, and microbial water quality test frequency were classified by use of different water sources (surface, ground, and municipal water). The data from any questions that allowed respondents to provide an open-

ended response were added to existing responses or a new category of responses was added to the question, based on the keywords in those responses. For example, a separate category was created for growers who wrote they used water from a rain barrel when selecting ‘other’ water for their water source.

Growers were asked if they were GAP (Good Agricultural Practices) certified (rather than if they were covered by the FSMA PSR) because the project team felt that growers would be more comfortable responding to the question of GAP certification, rather than FSMA PSR coverage. Accordingly, responses to the questions about water testing and water treatment were stratified based on their GAP certification status. Although GAP certification is generally a produce buyer requirement and is not a regulatory requirement, many growers view certification as an opportunity to improve their likelihood of compliance with the PSR. Thus, many growers covered by the PSR also obtain GAP certification.

Because some respondents did not answer all of the survey questions, the total number of responses to each question related to water sources in the cross-tab analysis may be different. Additionally, the total number of responses for questions regarding agricultural water source use could differ as growers may have reported using more than one water sources for one or more pre- or postharvest activities. To reduce item non-response bias, surveys having less than two-thirds of the questions completed were excluded from the final data analysis.

Results and Discussion

Results

A total of 106 survey responses were received. Of the 103 responses included in the final data analysis, 51 (49.5%) were from Kansas, 50 (48.5%) were from Missouri, one (1.0%) was marked as unknown, and one (1.0%) did not complete the question. The age range of growers

completing the survey were as follows: 18-29 ($n = 18$, 17.5%), 30-39 ($n = 15$, 14.6%), 40-49 ($n = 26$, 25.2%), 50-59 ($n = 19$, 18.5%), and above 60 ($n = 25$, 24.3%) years old. Most respondents ($n = 90$) were currently selling or planning to sell their produce to others. Most respondents ($n = 87$) were currently not GAP certified, as shown in Table 3.1.

Table 3.1 Reported microbial water quality testing ($n = 100$) status and GAP certification status ($n = 101$) by state.

	Kansas	Missouri	Total
Have tested water	16 (31.4%)	27 (55.1%)	43
Never tested water	25 (49.0%)	13 (26.5%)	38
Use third party test results	10 (19.6%)	9 (18.4%)	19
Total	51	49	100
GAP certified	2 (3.9%)	12 (24.0%)	14
Working towards being GAP certified in the future	7 (13.7%)	8 (16.0%)	15
Not GAP certified	42 (82.4%)	30 (60.0%)	72
Total	51	50	101

Of the participants which indicated they are currently growing fresh produce ($n = 101$), participants indicated their frequency of generic *E. coli* testing as more than once a year ($n = 14$, 13.9%), once a year ($n = 11$, 10.9%), 2-3 times in the last ten years ($n = 11$, 10.9%), once in last ten years ($n = 7$, 6.9%), never tested ($n = 39$, 38.6%), or that a third-party water source tests their water ($n = 19$, 18.8%). Between the two states (Table 3.1), 31.4% ($n = 16$) of Kansas growers said that they have tested their water in the past ten years compared to 55.1% ($n = 27$) of Missouri growers. Of the growers indicating they are GAP certified ($n = 14$), 10 (71.4%)

reported testing their water more than once a year and six (46.2%) reported that they treat their postharvest water sources. Of the non-GAP certified growers ($n = 87$), four (4.6%) growers test their water more than once a year and 15 (17.4%) growers treat their postharvest water sources (Table 3.2, Table 3.3). Because the questions inquiring about the type of water source used for production and postharvest applications allowed for more than one response, the total number of responses varied by question, as shown in *Figure 1*.

Table 3.2 The frequency of microbial water quality testing by GAP certification status (n = 101).

	More than once a year	Once a year	2–3 times in last 10 years	Once in last 10 years	Never test	Third party water test	Total
GAP certified	10 (71.4%)	0 (0.0%)	1 (7.1%)	1 (7.1%)	0 (0.0%)	2 (14.3%)	14
Working towards being							
GAP certified in the	1 (6.7%)	3 (20.0%)	1 (6.7%)	0 (0.0%)	8 (53.3%)	2 (13.3%)	15
future							
Not GAP certified	3 (4.2%)	8 (11.1%)	9 (12.5%)	6 (8.3%)	31 (43.1%)	15 (20.8%)	72

Table 3.3 Treatment application status for postharvest water based on GAP certification status (n = 99)

	Apply treatment	No treatment applied	Total
GAP certified	6 (46.2%)	7 (53.8%)	13
Working towards being			
GAP certified in the future	3 (21.4%)	11 (78.6%)	14
Not GAP certified	12 (16.7%)	60 (83.3%)	72

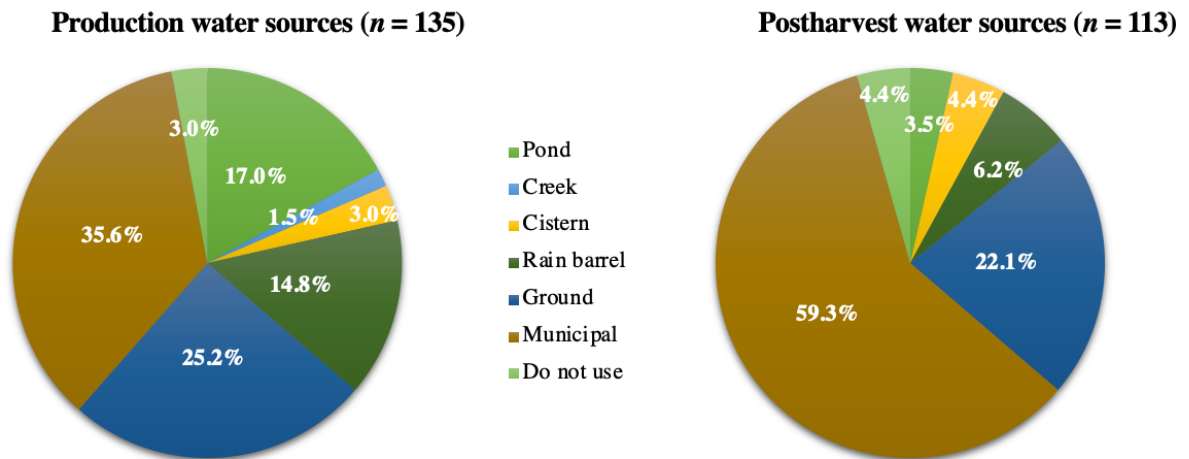


Figure 3.1 The percentages of different water sources that growers use for production ($n = 135$) and postharvest ($n = 113$) activities. (Note: among the 101 responses, several used more than one water type)

Municipal ($n = 48$, 35.6%), well ($n = 34$, 25.2%), pond ($n = 23$, 17.0%), and rain barrel ($n = 20$, 14.8%) water sources were the most commonly reported sources of production water (Figure 1). Municipal ($n = 67$, 59.8%) and well water ($n = 25$, 22.1%) were the most commonly reported water sources for postharvest water. Based on the number of responses for each water source, respondents appear to commonly utilize more sources of water for production applications ($n = 135$) than postharvest ($n = 113$) applications.

Participants also reported their applications of production and postharvest water (Figure 3.2). Of the survey respondents ($n = 101$), 90 (89.1%) indicated that they used production water in their operation. The three main reported uses of production water included irrigation ($n = 90$, 100%), mixing with crop sprays ($n = 38$, 42.2%), and mixing with fertilizers ($n = 38$, 42.2%). Of those growers that report using water for irrigation, 70 (77.8%) reported using drip irrigation, 37 (41.1%) reported using overhead irrigation, and 20 (22.2%) reported using drip irrigation under plastic. Furthermore, 90 (89.1%) indicated that they used postharvest water in their operation.

These growers reported their main uses of postharvest water to be for handwashing ($n = 77$, 85.6%), rinsing/washing produce ($n = 73$, 81.1%), and cleaning and sanitizing of food contact surfaces ($n = 68$, 75.6%). Other uses of postharvest water reported included ice making ($n = 7$, 7.8%), dunk tank cooling ($n = 6$, 6.7%), single pass/spray cooling ($n = 4$, 4.4%), and postharvest fungicide or wax applications ($n = 3$, 3.3%).

Of survey respondents who indicated that they used production water in their operation ($n = 90$), 75 (83.3%) reported that they do not treat their production water. Of the respondents who reported treating their production water, four (4.4%) reported using chemical sanitizers whereas 11 (11.1%) respondents indicated using physical methods such as sand filtration. Of survey respondents who indicated that they used postharvest water in their operation ($n = 90$), 68 (75.6%) reported that they do not treat their postharvest water. Fifteen respondents (16.7%) reported treating postharvest water with a chemical sanitizer and four (4.4%) reported using other treatments such as sand filtration or ultraviolet light. When comparing water treatment status and the water source used (shown in *Figure 3.3*), six (6.7%) respondents reported using surface water (ponds, cisterns, or rain barrels) for postharvest use without applying any treatment.

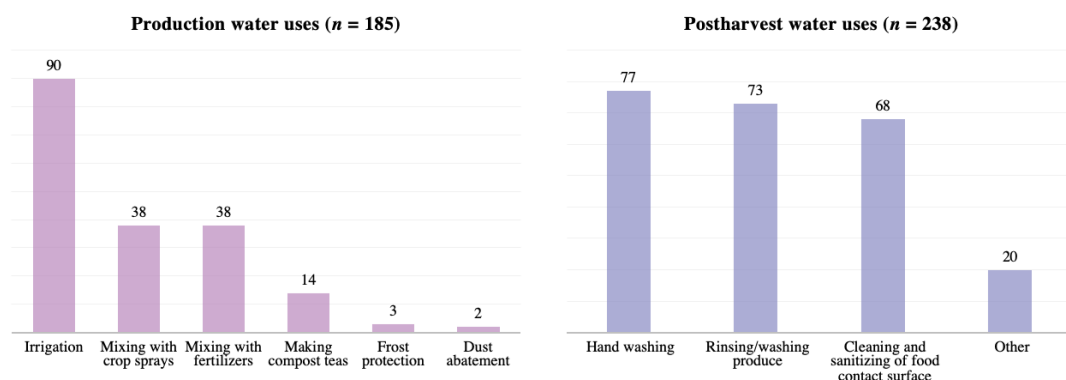


Figure 3.2 The different uses of production water ($n = 185$) and postharvest water ($n = 238$).

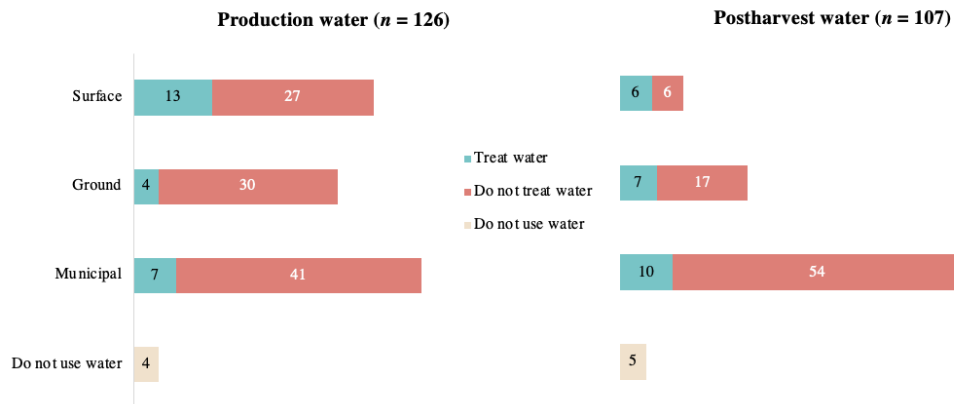


Figure 3.3 Treatment status for each water source that growers use for production (n = 126) and postharvest (n = 107) activities.

When asked what other practices (besides treatment) growers use to maintain/improve production water quality, most ($n = 70$, 77.8%) respondents using production water in their operation indicated that they did not use any other practices. Nineteen (21.1%) of the participants indicated that they conducted an annual evaluation of their water distribution system. When managing the quality of their postharvest water, 61 (67.8%) respondents who indicated that they used postharvest water in their operation ($n = 90$) reported that they do not use any additional practices, 17 (18.9%) reported routine cleaning and sanitation of tanks, 15 (16.7%) reported monitoring for buildup of organic material, 14 (15.6%) respondents reported conducting an annual evaluation, 10 (11.1%) reported monitoring water temperature during use, and nine (10.0%) reported using a routine water change schedule.

Discussion

Agricultural water quality remains a large concern for the fresh produce industry across the United States, particularly in the Midwest where previous studies indicate that agricultural water quality management is not well understood (17).

Most of the respondents demonstrated a preference for water sources with a lower microbial risk profile (such as municipal water) for postharvest activities, as compared to using surface water postharvest, which is higher risk. Specifically, the survey data indicated that – unlike production water, where municipal (35.6%) and surface water use (36.3%) were nearly equally reported – there is a higher use of municipal water (59.3%) and lower use of surface water (14.1%) for postharvest applications (*Figure 1*). As previous research has shown, microbial populations in surface water increase after events such as agricultural runoff (rain, melted snow, etc.) (3, 24, 27). Surface water is also more exposed to potential contamination from the surrounding open environment, and thus is considered to have a higher possibility of contamination (1, 8, 23).

According to the data collected from the survey, a minority of growers use specific postharvest water management practices that many would consider higher risk. Using surface water without any treatment for postharvest applications poses a higher risk of foodborne disease outbreaks, thus the FSMA PSR agricultural water criteria, GAP certification requirements and general produce safety best practices do not allow use of untreated surface water for postharvest activities (31). Interestingly, six survey respondents indicated that they were using untreated surface water for postharvest uses, and eight respondents indicated they treated their agricultural water using methods such as (bio)sand-filtration. Of note, sand-filtration is not a validated agricultural water treatment method according to the FSMA PSR (21 CFR 112.43) (31). However, growers do currently utilize sand filtration in irrigation systems to remove larger, physical particles, sometimes with the added misconception that it also can remove pathogens. This indicates that further education and training is needed to emphasize the significance of

microbial risk in postharvest water, as well as how to select an appropriate (validated) treatment method.

Conducting regular inspection and maintenance of the water source and distribution system is currently a requirement of the FSMA PSR (21 CFR 112.42) (31) and is a produce safety best practice to limit the risk of microbial contamination. By conducting regular inspections, growers can evaluate the risks to their water sources. The inspection includes identifying any broken part of the system structure, any wildlife evidence around open water sources, and other risks. Most of our survey respondents are not currently conducting an annual agricultural water system inspection. Thus, extension educators should provide practical information to growers on how to inspect their water sources regularly and encourage them to conduct a water system inspection at least annually, which is a requirement for growers covered by the FSMA PSR (21 CFR 112.42) (31). Of note, the proposed §112.43 is intended to supplement the requirements of the proposed §112.42 which will require a covered farm to regularly inspect and routinely maintain the components of its agricultural water system. While proposed §112.42 is focused on agricultural water system components, the proposed §112.43 would require covered farms to conduct a more comprehensive assessment of possible sources and routes by which known or reasonably foreseeable hazards are reasonably likely to be introduced into its preharvest agricultural water (30).

The FSMA PSR also highlights irrigation method as an important risk factor for contamination risk based on if the water contacts the harvestable portion of the produce. In most situations, overhead irrigation results in direct contact with the harvestable portion of the produce, which can result in a higher contamination risk than other irrigation methods. Conversely, drip irrigation (particularly drip irrigation under plastic) is considered to have a

relatively lower risk of contamination for crops grown above the ground. Of note, this characterization does not apply to root crops such as carrots, beets, and radishes (25). Most of the survey respondents indicated that their main use of production water was irrigation, namely drip irrigation (69.1%) or overhead irrigation (31.3%). However, the findings of the study call attention to particular agricultural water practices including the use of untreated surface water for direct water application during postharvest, and seldom testing the sanitary quality of agricultural water sources. Although not specifically measured by this study, further investigation and potentially extension efforts may need to occur to clarify how growers can reduce microbial risk by selecting irrigation methods that reduce the probability of water contact with the harvestable portion of the produce.

In the survey, the question about the frequency of conducting microbial water testing did not specify testing frequency of “production water” and “postharvest water” separately. Accordingly, crosstab analysis for the frequency of water testing for different sources of water used for different purposes (preharvest or postharvest) could not be conducted. Moreover, it was not possible to discern between treated and untreated sources when more than one source was indicated by the grower in this survey. We also did not include questions related to the location of their water source in relation to animals or other potential sources of contamination. These points could have been clarified by increasing the length of the survey. However, the expert panel reviewing the survey was concerned about the adverse effect of increasing the survey length on the response rate as some participants required more than 10 minutes to complete the survey. In a study of the effects of length on web-based survey completion, Galesic and Bosnjak (9) found that participants anticipating a longer survey completion time were less likely to finish the survey.

Conclusion

This survey was intended to provide information on the differences between best practices and the actual practices of Kansas and Missouri produce growers focusing on the use of agricultural water. Produce growers need to continue to improve their practices related to water quality to ensure that they are selling the safest produce possible. The survey responses will provide insight into the development of additional agricultural water safety educational materials for produce growers. The results indicated that efforts are needed to continue to encourage growers to test their water quality, to conduct at least an annual inspection of water sources and distribution system, and to use a validated surface water treatment method before using it for postharvest applications. The authors suggest that future extension trainings should emphasize the importance of microbial quality of agricultural water sources, and how risk to produce safety can be affected by selection of water source, decision to treat, and (in the case of production water) application method.

Acknowledge

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Chapter 4 - Agricultural Water Related Extension Education

Handouts

Based on the anecdotal evidence and survey responses from this thesis (Ch. 2), we identified the critical need to address a few important agricultural water safety-related topics that include the following:

New Modified FSMA PSR Agricultural Water Requirement Proposal

FSMA PSR published the agricultural water regulation in 2016, including using the different microbial quality criteria to value both production and postharvest water quality. However, after the implementation, the feedback from different farms focused on "the rules are too complicated and cost too much." Therefore, the FDA revised and published a proposed agricultural water requirement in December 2021 focused on production water. This handout summarized the major modification of the latest water requirement and aimed to help growers understand and comply with the proposed rule.

QUICK REFERENCE GUIDE

New Modified FSMA PSR Agricultural Water Requirement Proposal



In December 2021, the United States Food and Drug Administration (FDA) proposed a revision to Subpart E of the Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR) that would change the *pre-harvest agricultural water requirements* for covered produce, other than sprouts.

What is the proposed change?

The proposed revision to Subpart E modifies the previous requirement for produce growers to *replace the microbial criteria and testing requirements* for pre-harvest agricultural water with new provisions for *conducting pre-harvest agricultural water assessments*, providing more flexibility to produce growers in evaluating risks.

These aim for hazard identification (including consideration of agricultural water sources, distribution systems, and practices, as well as adjacent and nearby land uses, and other relevant factors), and using the results of the assessment to determine whether *corrective or mitigation measures* are needed to reduce the potential for contamination of produce or food contact surfaces with hazards associated with pre-harvest agricultural water.

What is an “agricultural water assessment” ?

It refers to an *evaluation*, conducted by a covered farm, of factors in its agricultural water system used during growing activities for non-sprout covered produce, its agricultural water practices for such pre-harvest water, crop characteristics, environmental conditions, and other relevant factors (including test results, where appropriate).

Factors	Description
Agricultural water system(s)	• Location and nature of the water source (including whether it is ground or surface water) • Water distribution system type (such as whether it is open or closed to the environment) • Degree to which the system is protected from possible sources of contamination
Agricultural water practices	• Water application method (overhead sprinkler, spray, drip, furrow, flood, seepage, etc.) • Time interval between the last direct water application and harvest of the covered produce
Crop characteristics	• Susceptibility of the covered produce to surface adhesion or internalization of hazards
Environmental conditions	• Frequency of heavy rain or extreme weather events may impact the agricultural water system or damage produce that can increase the susceptibility of produce to contamination. • Air temperatures • Sun (UV) exposure
Other relevant factors	• Including, if applicable, results of water testing that could inform the assessment



Questions, comments or concerns?

Contact your K-State Extension specialist **Londa Nwadike** (lnwadike@ksu.edu or nwadike@Missouri.edu) or **Cal Jamerson** (agri@ksu.edu) today!

New Modified FSMA PSR Agricultural Water Requirement Proposal

Corrective and mitigation measures

The proposed rule does **NOT** change the requirements for harvest and post-harvest water, but only impacts pre-harvest water. Under this proposed rule, a covered farm would *make a determination under the requirements, based on the outcome of its agricultural water assessment*, as to whether mitigation measures would be reasonably necessary to reduce the potential for contamination of covered produce (other than sprouts) or food contact surfaces with known or reasonably foreseeable hazards associated with its pre-harvest agricultural water.

Based on the assessment, if you determine...	Then you must...
that your agricultural water is not safe or is not of adequate sanitary quality for intended use(s).	- Immediately discontinue use(s) AND - Take corrective measures before resuming use of the water for pre-harvest activities
there is one or more known or reasonably foreseeable hazards related to animal activity, BSAAOs, or untreated or improperly treated human waste for which mitigation is reasonably necessary.	Implement mitigation measures promptly, and no later than the same growing season
there is one or more known or reasonably foreseeable hazards not related to animal activity, BSAAOs, or untreated or improperly treated human waste, for which mitigation is reasonably necessary.	- Implement mitigation measures as soon as practicable and no later than the following year Or - Test water as part of the assessment and implement measures, as needed, based on the outcome of the assessment
that there are no known or reasonably foreseeable hazards for which mitigation is reasonably necessary.	Inspect and adequately maintain the water system(s) regularly, and at least once each year

Exemptions:

A covered farm would be exempt from the requirement to conduct an assessment for pre-harvest agricultural water if the farm can demonstrate that the agricultural water meets these requirements, including:

- It meets certain requirements that apply for *harvest and post-harvest agricultural water* (such as the microbial quality criterion and testing requirements for untreated ground water);
- It is received from a *public water system or supply* that meets requirements established in the rule (provided that the farm has public water system results or certificates of compliance demonstrating that the water meets relevant requirements); or
- It is treated in accordance with the standards outlined in the Produce Safety Rule.

Prepared by: Yeqi Zhao Reviewed by: Manreet Bhullar, Londa Nwadike



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How to Interpret Water Results

Since 2016 FSMA PSR published agricultural water regulation, agricultural water has been separated into production water and postharvest water and from three main water sources (i.e., surface, ground, and municipal). Due to the different cross-contamination risks, the water criteria varied by different water activities. This handout aims to help Kansas and Missouri growers better understand the water regulation they need to comply with and interpret their water results within simple wording, images, and charts.

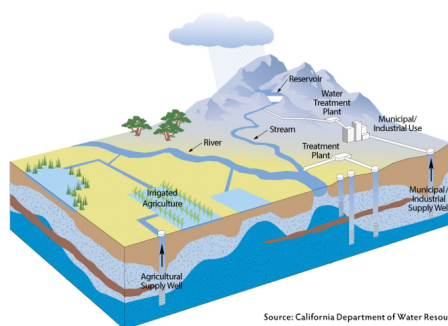
QUICK REFERENCE GUIDE

FSMA Produce Safety Rule Requirements for Agricultural Water

“ The **Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR)** states that all agricultural water **must** be of safe and adequate sanitary quality for its intended use (§112.41) ”

What is Agricultural Water?

Agricultural water is water that is intended or likely to contact the edible portions of produce or food contact surfaces during growing (production) or during/after harvest (post-harvest). The PSR outlines three common sources of agricultural water: **ground water**, **surface water**, and **municipal water**.



Public Water is water that is treated and monitored by a local water utility

Ground Water is water that is found beneath Earth's surface (ex. wells, aquifers)

Surface Water is water that is found or stored on the Earth's surface (ex. rivers, creeks, cistern, ponds supply tanks)

Risk of Contamination



What's the difference between Production and Postharvest Water?

Production Water is any water that is applied directly to produce during growing

- Irrigation
- Crop sprays
- Fertigation
- Frost protection
- Cooling



VS

Postharvest Water is any water that is used during and after field harvest of the produce

- Field Packing
- Washing
- Hydro-cooling
- Ice making
- Hand Washing



Note: water used for hand washing also counts as postharvest water since it can potentially contact the produce surface.

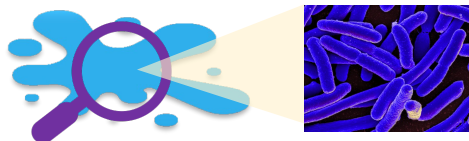


How do we determine water quality?

Agricultural Water Assessment The new modified agricultural water proposed rule uses an evaluation system to evaluate pre-harvest water quality. There are four main factors include agricultural water system(s), agricultural water practices, crop characteristics, and environmental conditions. Based on the assessment, you can determine whether your water is saved to use or not.

Tips: Agricultural Water Assessment Builder can help you evaluate your water quickly and easily!

Access from [link](#) or **QR-code**



Water Microbial Quality The Produce Safety Rule uses a bacteria called generic *E. coli* as an indicator of water quality. *E. coli* is a type of bacteria that is commonly found in animal waste (feces). Based on the number of generic *E. coli* in 100 ml, you can know whether your water is microbially safe to use or not.



Questions, comments or concerns?

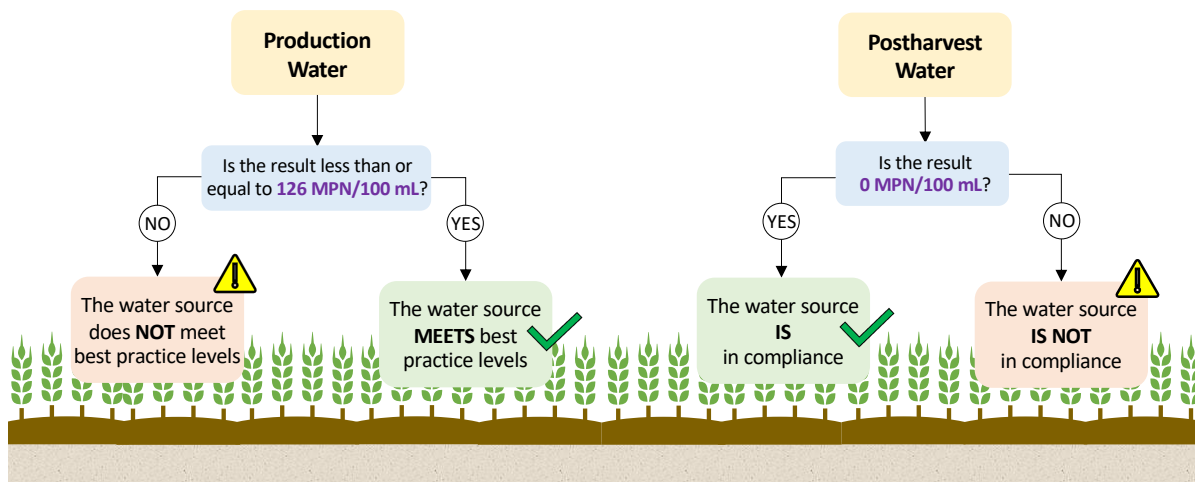
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How to Interpret Your Water Quality Test Results

Step 1: Determine if the water source meets best practice/ regulation levels

Intended Use:



Step 2: Determine the next steps if the water source IS NOT best practices/ FSMA PSR compliant

If the postharvest water is not compliant:

DISCONTINUE use as soon as practical (switch to municipal water or other reliable source) unless corrective measures are applied.

A few things you can do:

- Contact your local extension personnel for solution.
- Apply a **time interval** between the last irrigation and harvest.
- **Re-inspect** the entire affected agricultural water system for:
 - The extent it is under your control
 - Any conditions that are likely to contaminate produce surfaces
- Apply **validated treatments** to the water source.
 - The treatment must be effective to make the water safe and of adequate sanitary quality for its intended use (i.e., UV, chlorine).

[Kansas State University](https://www.k-state.edu/)

Name: Katelynn Stull

E-mail: kjstull@ksu.edu

Cell-phone: 913-307-7394

[University of Missouri](https://www.missouri.edu/)

Name: Dr. Londa Nwadike

E-mail: nwadikel@Missouri.edu

Cell-phone: 913-307-7391

Prepared by: Yeqi Zhao, Olivia Haley Reviewed by: Manreet Bhullar, Londa Nwadike



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Backflush Water Fact Sheet

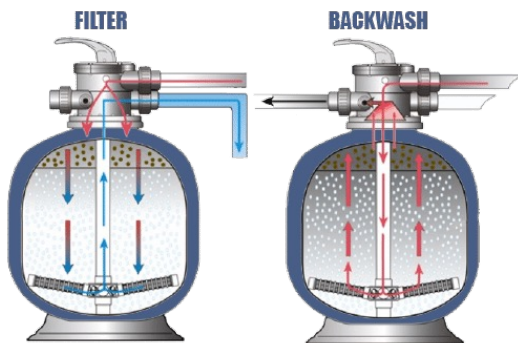
According to the survey results, the filtration system is the primary water treatment reported by the grower. The backflush water is the water used to clean the filter that contains various microorganisms and potentially includes human pathogens. Despite the use of filtration alone is not a validated agricultural water treatment, the handout relates to the filtration method, and backflush water is limited in the extension field. This handout aims to popularize the biohazard of backflush water and possible solutions.

QUICK REFERENCE GUIDE

Backflush Water Fact Sheet

Due to the produce safety concern, filtration systems are widely used in farms to catch small solid particles from original water sources (surface or ground water), and provide clearer water for agriculture purpose (irrigation, fertigation, cooling, etc.).

However, do you know the filtration system can also provide the “dirty” water called **backflush water**?

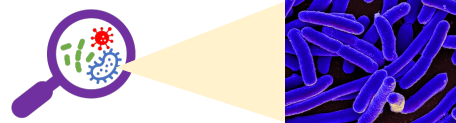


What is Backflush Water?

Backflush, also known as **backwash**, it is a mechanism always comes with a sand and Diatomaceous Earth (DE) filtration system acts as a **self-cleaning system**. It is a water flow that moves reversely through the filter tank to remove the accumulated particles from the filter.

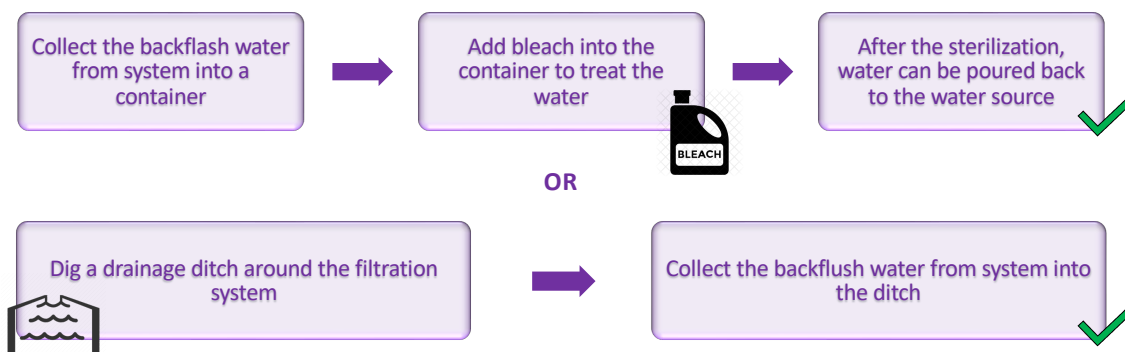
The Concerns That Backflush Water Brings Up:

As backflush water brings all the potential contaminated particles from the filter surface, the backflush water itself is considered as a **highly contaminated** water. If the backflush is not handled and/or stored properly, it can re-add the contamination to the original source or to other clean water sources on the farm.



How to Manage the Backflush Water in My Farm?

Following are several methods to manage your backflush water:



Purdue University Logo



Questions, comments or concerns?

Contact your K-State Extension specialist **Londa Nwadike** (phone number; email) or **Cal Jamerson** (phone number; email) today!

Chapter 5 - Conclusion

The thesis focused on improving the food safety practices reducing the risk of fresh produce outbreaks in the US, specifically expected outcomes on minimizing the cross-contamination chance in the fresh produce farms. The primary two reservoirs for transmitting human pathogens are the farm soil and agricultural water. Thus, to complete the goal, the thesis work included three objectives: (1) reducing the pathogen load in potentially contaminated agricultural soils using cover crops; (2a) accessing current practices and knowledge of Kansas and Missouri growers by using survey; (2b) developing extension material focused on managing agricultural water quality based on the survey results.

Soil Treatment

Several soil treatment methods reduce human pathogens in fresh produce fields. Specifically, cover crops have been recently explored for antimicrobial application in produce production, besides their soil health benefits for contaminated soils. Chapter 2 evaluates the efficacy of reducing pathogen load in contaminated soil with three different cover crop species (i.e., buckwheat, mustard greens, and sunn hemp). The results showed all three cover crops could reduce the bacteria population in contaminated soils after 30 to 40 days following artificial tillage within buckwheat did the best. However, the sole use of a gram-negative pathogen (generic *E. coli*), soil type, and selected three cover crops would be the primary limitations in this study. Considering this is the preliminary study, future research could study the antimicrobial efficacy on soil with different pathogens/soil types/cover crops to better understand the implications of these results. Nevertheless, these results demonstrated the potential of applying cover crops as an antimicrobial treatment in contaminated produce fields. The

outcomes will benefit the organic fresh produce growers as they use biological soil amendments to enrich the soil as a common practice.

Agricultural Water Safety

Unlike soil amendment contamination, agricultural water safety has always been a concern in all types of produce farms, as highlighted in multiple produce outbreaks. Within continuously providing training and information on agricultural water microbial quality to help produce growers reduce risk, the survey developed (chapter 3) is necessary to determine the current knowledge and practice gaps between produce growers and agricultural water management best practices. The surveys were sent out between late 2020 and early 2021 during the COVID-19 pandemic, in which most of the growers' in-person activities had been canceled due to public health concerns. Thus, most surveys were distributed through Qualtrics (an online survey instrument), and the total responses were lower than the expectation (106/200). Despite the dilemma, the survey results indicate the growers from Kansas and Missouri have a basic awareness of agricultural water practices. Still, more demanding practices must be strengthened (e.g., using validated water treatment methods).

Based on the survey responses, three handouts were developed (chapter 4), including: (1) "New modified FSMA PSR agricultural water requirement proposal," which summarized the significant modification of the latest water requirement published in 2021; (2) "How to interpret water results" provides information to identify the agricultural water sources and microbial safety criteria; (3) "Backflush water fact sheet" discussed the hazard from back flush water and solutions. These handouts aim to fulfill the produce growers' current needs and help them to mitigate risk from agricultural water cross-contamination.

In conclusion, the survey provided information on the gaps between best practices and the actual practices of Kansas and Missouri produce growers on agricultural water management; these gave extension professionals the directions for developing further outputs. In addition, in December 2021, the FDA published a new modified agricultural water requirement proposal. The new proposed rule will replace the produce water criteria once it is finalized. Thus, further study can focus on assessing the produce growers' knowledge of the new proposed rule and develop handouts based on the results.

Appendix A - Survey Questions

1. What is your age?

☐ 18-29

☐ 40-49

☐ 60+ years

☐ 30-39

☐ 50-59

2. Are you currently Good Agriculture Practices (GAP) certified?

☐ Yes

☐ No

☐ No, but working
towards being GAP
certified in the future

3. Are you currently raising produce (fruits, vegetables, herbs, mushrooms, nuts) for sale?

☐ Yes

☐ No

☐ No, but plan to in the future

4. In which state do you grow produce? (If more than one, mark the primary state)

☐ Kansas

☐ Missouri

☐ Other _____

5. Do you currently use water PRE-harvest in your produce operation? (check all that apply)

☐ For irrigation

☐ For dust abatement

☐ For mixing with crop sprays

☐ Frost protection

☐ For mixing with fertilizers

☐ Other _____

☐ For making compost teas

☐ No

6. If you use water for irrigation, how do you irrigate? (check all that apply)

☐ Drip irrigation

☐ Furrow irrigation

☐ Drip irrigation under plastic

☐ Other _____

☐ Overhead irrigation

☐ I do not irrigate

7. What sources of PRE-harvest water do you use? (check all that apply)

☐ Pond

☐ Cistern

☐ Creek

☐ Other (such as rain barrel, spring, or
others. Please list) _____

☐ Well

☐ Do not use water pre-harvest

☐ Municipal water

8. Do you currently use water POST-harvest in your operation? (check all that apply)

☐ Rinsing/ washing produce

☐ Single-pass/spray cooling

☐ Commodity movement (i.e. dump
tanks/ flumes)

☐ Dunk tank cooling

☐ Ice making

- ☐ Post-harvest fungicide or wax
- ☐ Handwashing
- ☐ Cleaning and sanitizing of food contact surfaces
- ☐ Other (please list) _____

9. What sources of POST- harvest water do you use? (check all that apply)

- ☐ Municipal water
- ☐ Well
- ☐ Cistern
- ☐ Pond
- ☐ Creek
- ☐ Other (such as rain harvest barrel or others. Please list) _____
- ☐ Do not use water post-harvest

10. Do you test your water used in your produce operation for generic *E. coli*?

- ☐ Yes, more than once a year
- ☐ Yes, once a year
- ☐ Yes, we have tested our water 2-3 times in the last 10 years or so
- ☐ Yes, we have tested our water once in the last 10 years or so
- ☐ No, we have never tested our water
- ☐ A third party (such as the municipal supplier)

11. Do you treat your water used PRE-harvest (such as in irrigation) to improve its microbial quality?

- ☐ Yes, we treat it with a sanitizer (list sanitizer used) _____
- ☐ Yes, we use a different treatment (list treatment- for example, sand filter, other) _____
- ☐ No

12. Do you treat your water used POST-harvest (such as in washing) to improve its microbial quality?

- ☐ Yes, we treat it with a sanitizer (list sanitizer used) _____
- ☐ Yes, we use a different treatment (list treatment- for example, sand filter, ozone, UV, reverse osmosis, other) _____
- ☐ No

13. What other practices (besides treatment) do you use to maintain/improve the quality of the water used PRE-harvest in your produce operation?

- ☐ We conduct an annual evaluation of our water distribution system
- ☐ Other (please list)_____
- ☐ None

14. What other practices (besides treatment) do you use to maintain/improve the quality of the water used POST-harvest in your produce operation?

- ☐ We conduct an annual evaluation of our water distribution system
- ☐ Routine water change schedule
- ☐ Routine cleaning and sanitation of tanks
- ☐ Monitoring for buildup of organic material
- ☐ Monitoring water temperature during post-harvest use
- ☐ Other (please list)_____
- ☐ None