CENTER for PRODUCE SAFETY

CPS 2022 RFP
FINAL PROJECT REPORT

Project Title
Microbial risks during indoor leafy green production: current knowledge and future research needs

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Objectives

1. *Identify peer-reviewed literature, technical abstracts, and grey literature relevant to foodborne pathogen prevention and control in controlled environment agriculture (CEA).*
2. *Conduct semi-structured interviews with CEA leafy greens–producing operations to identify common themes related to microbial risks.*
3. *Collaborate with academic researchers and regulatory experts in food safety to further rank the most critical CEA research needs.*
4. *Synthesize the available knowledge to provide key takeaways, evidence-based practices, and knowledge gaps on microbial risks during indoor leafy green production.*

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FINAL REPORT

Abstract

Food crop production in controlled environments is an increasingly important sector of U.S. and global agriculture. Although controlled environment agriculture (CEA) helps exclude pests and diseases from produce, pathogen issues can still occur in these operations. This study aimed to assess microbial risks within indoor, soilless leafy green production to identify evidence-based best practices as well as future research needs to enhance CEA-grown produce safety. First, a scoping review of the peer-reviewed literature was conducted to determine knowledge gaps regarding the microbial risks of indoor leafy green production. Here, leafy greens are defined as any plant leaves that are commonly eaten as a vegetable, including the immature shoots of these leaf vegetables. Twenty-two and 36 studies addressing the food safety of microgreens and mature leafy greens, respectively, were identified. The review emphasized that the risk of microbial contamination will vary during CEA production depending on leafy green crop, growth media, and system selection. Overall, more data are needed regarding transfer, colonization, and survival of *L. monocytogenes* within indoor, soilless leafy green production systems. Limited knowledge and data are available about pathogen survival, spread, transfer, and elimination in/from materials in “ponic” systems, specifically within systems typical of a commercial-size operation. Preventive measures, mitigation strategies, and corrective actions are not defined specifically for CEA systems, resulting in the limited availability of outreach and education materials. Meanwhile, semi-structured interviews (n = 25) were conducted with producers of indoor, soilless leafy greens across the U.S. to gather information about current practices and potential food safety gaps. During the interviews, a trained interviewer introduced the study and asked 11 pre-determined questions, along with clarifying questions when necessary. The interviews revealed three major themes: i) contextual, ii) barriers to risk management and regulatory compliance, and iii) research needs. Thirteen subthemes were identified, and an example of a subtheme within each major theme, respectively, includes: worker hygiene and training; regulatory and certification environment; and risk assessments of individual issues. Overall, the growers expressed a strong desire for science-based risk assessments of individual issues within the industry, as opposed to receiving generalized advice. They felt that having access to data would enable them to make informed decisions about the risks they face and how to manage them effectively. Next, research and extension colleagues working in the field of CEA food safety were engaged. Specifically, during the USDA-funded “Strategizing to Advance Future Extension and Research in Controlled Environmental Agriculture” [S.A.F.E.R. CEA] Conference held April 13-14, 2023, several small group and breakout sessions were facilitated to enable identification of *what we already know* and *what we need to know more about* regarding CEA food safety. Attendees (n = 47) at the S.A.F.E.R. CEA Conference included 28 research and extension colleagues, 15 CEA industry members (operators and allied industries), and 4 regulatory officials (one in-person, three via Zoom). The following items were commonly identified as research needs: i) degree of pathogen survival, spread, transfer, and elimination in/from materials used in ponic systems; ii) validated practices related to the management of irrigation water and nutrient solution; iii) recommendations for effective sanitizers and validation of application within ponic systems; iv) preventive measures, mitigation strategies, and corrective actions specific for CEA systems; and v) specific guidance on best practices for CEA-grown produce. Finally, a list of short-, medium-, and long-term actionable items was developed. An example of each category, respectively, includes: standardization of definitions and terms for better communication between academia, regulatory agencies, and producers; defined food safety requirements for input materials including seeds and substrates along with certified suppliers; and pathogen control via manipulation of physicochemical and microbial community characteristics of nutrient solutions. To increase the food safety of CEA-grown leafy greens, action items should be approached using systems thinking across stakeholder groups.

Background

Food crop production in controlled environments is an increasingly important sector of the U.S. and global agriculture. According to the 2019 Census of Horticultural Specialties, sales from "food crops grown under protection" were roughly $700 million in the U.S. These crops include primarily tomatoes, lettuce, cucumbers, peppers, berries, and herbs, and account for 54% of the total production (cwt) in the U.S. (USDA-NASS, 2019). Controlled environment agriculture (CEA) takes advantage of technologies and automation to modify production climates, shield crops from biotic and abiotic stresses, and optimize environmental factors that maximize plant yield and quality. Greenhouses and indoor warehouses or shipping containers are common CEA structures, and hydroponics, soilless substrate culture, and vertical farming systems are common CEA growing systems. While CEA offers many advantages over traditional farming, such as increased yields, year-round production regardless of external weather conditions, reduced water use, and protection from pests, it also presents unique challenges related to food safety. Foodborne pathogens can enter and spread through CEA similar to field-grown crops via: (i) contaminated water or nutrient solution, (ii) unsanitary equipment, (iii) contaminated incoming materials such as seeds or plant materials, (iv) employees and staff, and (v) insects and animals. Microbial contamination can occur at various stages and from materials used during production including from water, growth substrate, air, and workers. Additional areas of concern include implementation of validated cleaning and sanitizing procedures, storage of soilless growth media, and documentation and record keeping of post-harvest practices.

Indoor leafy green production, especially in hydroponic systems, is widely preferred where environmental conditions are not favorable to conventional, field-based production (Bledsoe, 2020). Leafy greens are susceptible to contamination with pathogens such as *Escherichia coli*, *Salmonella*, and *Listeria monocytogenes* (Painter et al., 2013). In a study from 2012, hydroponically grown lettuce had lower numbers of thermotolerant coliforms, mesophilic aerobic bacteria, *Salmonella* serovars, and intestinal parasites compared to lettuce grown with traditional farming practices (Gomes Neto et al., 2012). Moreover, Arrais et al. (2020) reported that conventionally grown produce was 2.4 times more likely to be contaminated with *E. coli* than those grown hydroponically. Regardless, there is concern about the potential for the enclosed environment of CEA to create conditions, such as high humidity and temperature, that are favorable to the growth of pathogens (Ilic et al., 2017). The 2021 outbreak of *Salmonella* Typhimurium due to consumption of hydroponically grown lettuce and lettuce mixes provides evidence of the food safety risks in CEA, especially when preventive measures are not properly implemented or, more likely, not designed for CEA operations (US FDA, 2021). More recently, various packaged leafy green products from a U.S.-based farm using hydroponic production methods were recalled in April 2023 due to potential contamination with *L. monocytogenes* (US FDA, 2023). **Table 1** provides a list of the recalls and outbreak that have been reported due to potential contamination of leafy greens produced in indoor, soilless environments.

No standards or guidance have been established specifically for the leafy green CEA industry. As the volume of information on the microbial risks within indoor produce production grows, there is a need to understand the state of the science, industry practices, and current research efforts for the identification of evidence-based best practices as well as future research needs to enhance the safety of CEA-grown produce. This project extracted data and information from three sources: 1) peer-reviewed literature relevant to foodborne pathogen prevention and control in CEA were identified to evaluate knowledge gaps related to the microbial risks of indoor leafy green production; 2) semi-structured interviews with growers of indoor, soilless leafy greens were conducted to identify the specific needs and challenges of the industry; and 3) research and extension personnel from across the U.S. were brought together to discuss the needs of the CEA industry and to identify where research, outreach, and education could be developed to address these needs.

Research Methods and Results

Objective 1 Methods

Literature Search. Peer-reviewed journal articles and grey-literature (reports, dissertations, theses, and conference abstract databases) were assessed by extracting files from CAB Abstracts (Ebsco), Food Science and Technology Abstracts (FSTA), ScienceDirect, AGRICOLA, Web of Science, and other relevant databases. Google Scholar was utilized to obtain additional grey literature not extracted from databases (Haddaway et al., 2015), and backward citation searching of extracted reference lists was conducted. Search keywords were designed for inclusion of relevant CEA areas such as foodborne pathogens, food safety, pathogen internalization, pathogen persistence, endophytic, hydroponic, soilless, soil-free horticulture, greenhouse, indoor farm, growth chamber, deep water culture (DWC), nutrient film technique (NFT), leafy greens, lettuce, leafy vegetables, microgreens, and herbs. The extracted studies were reviewed and catalogued under pre-harvest and post-harvest areas of research to visually display metrics on past research focus areas in CEA-grown leafy greens such as pathogens of concern, production system/growing techniques, scale of operation (i.e., bench-top, pilot, commercial), produce type and cultivars/varieties evaluated, growth media type, contamination routes evaluated, environmental monitoring, hygienic design, among others.

Objective 1 Results

Literature Search Results. A scoping review was conducted to evaluate knowledge gaps related to the microbial risks of indoor leafy green production. Here, we defined leafy greens as any plant leaves that are commonly eaten as a vegetable, including the immature shoots of these leaf vegetables. Twenty-two and 36 studies addressing the food safety of microgreens and more mature leafy greens, respectively, were identified for this scoping review and included in our summary analysis. The data extracted from the studies relevant to the food safety aspects of microgreens and leafy greens are presented in **Table 2** and **Table 3**, respectively.

Summary of the Scoping Review. Produce grown under CEA is often assumed to have a reduced risk of pathogen contamination due to the low chance of exposure to outdoor contaminant factors. However, the recent outbreak and recalls (**Table 1**) show the possibility of pathogen introduction to produce during indoor production when there is a failure in the implementation of food safety management systems. Indoor production of commercial leafy greens including lettuce and microgreens is performed across a range of protective structures from primitive household setups to advanced and partially automatized growing systems. Indoor production systems include hydroponic, aquaponic, and aeroponic configurations. Hydroponic systems such as DWC and NFT with various engineering designs represent the main techniques used by growers. Depending on type of crop, growth substrates, and system selection, the risk of microbial contamination will vary during indoor production.

Leafy greens (i.e., baby, mature) can be contaminated during pre-harvest activities; however, the potential for contamination is relatively lower compared to microgreens due to several factors. These factors include the potential for direct contact with growth media, time in production, use of sophisticated technologies (e.g., automation), and ability to implement good agricultural practices. More specifically, more mature leafy greens from post-germination (i.e., placement in system) to harvest are less likely than microgreens to have direct contact with the growth media due to system setup and design. Regarding production time, microgreens can be harvested as early as 10 days so mitigation of pathogen risks can be limited compared to their mature leafy green counterparts.

Investigations on the food safety risks in soilless production systems have typically focused on the potential for pathogens to internalize within edible portion of the leafy greens. Based on the literature review, ***there is no clear answer regarding the risk of internalization***. Briefly, pathogen internalization through roots to the edible part of leafy greens can depend on the starting population of the pathogen, root damage, and pathogen type. For example, Wang et al. (2020) reported the presence of Shiga toxin-producing *E. coli* (STEC) in the water of both hydroponic and aquaponic systems growing lettuce and basil. Subsequent analysis of the root surfaces confirmed the presence of STEC, but no internalization into the edible portion of the plant was detected. Conversely, root damage of leafy greens such as basil, cilantro, kale, and lettuce grown in aquaponics resulted in the internalization of *E. coli* O157:H7 through roots and subsequent spread to the leaves (Wang et al., 2021). Similarly, internalization of *E. coli* O157:H7 from plant growth nutrient solution was also observed in two spinach cultivars when there was root damage present (Macarisin et al., 2014). Despite no definitive conclusion on the risk factors for pathogen internalization in leafy green, the ***variety of crop, technology, system type, and growth media used in CEA leafy green production as well as general horticultural management practices impact pathogen uptake***.

The efficacy of pre- and post-harvest treatment of microgreens and leafy greens to reduce and/or eliminate pathogens is demonstrated to some extent in the extracted studies shown in **Table 2** and **Table 3**. However, the fragile nature of these crops and the possible undesirable sensory changes reduce the likelihood that growers would adopt currently available treatment technologies or that consumers would accept a product of diminished quality.

During the previous decade, the number of peer-reviewed research articles related to the food safety of CEA-grown crops has steadily increased. However, more research and knowledge are needed to 1) better characterize best practices for risk mitigation and 2) allow for risk assessments to be developed, especially in hydroponic systems. ***More data are sought regarding transfer, colonization, and survival of L. monocytogenes*** as one of the primary causes of current recalls related to leafy greens. Specifically, most peer-reviewed journal articles have focused on *Salmonella* serovars (n = 22) and STEC (n = 23) compared to *Listeria* spp./*L. monocytogenes* (n = 12). Limited knowledge and data are available about pathogen survival, spread, transfer, and elimination in/from materials in “ponic” systems, specifically within systems representative of a commercial-size operation. Currently, no specific food safety standards and guidelines are proposed for produce grown in CEA, which is likely due to both a lack of data and the lower production quantities using indoor soilless methods compared to open field agriculture. Similarly, preventive measures, mitigation strategies, and corrective actions are not defined specifically for CEA systems, resulting in the limited availability of outreach and education materials.

Objective 2 Methods

Survey and semi-structured interviews with CEA leafy greens–producing operations

Ethics statement and recruitment. The University of Arkansas Institutional Review Board (Protocol No: 2302455435) reviewed the study and granted an exemption. Prior to conducting semi-structured interviews, participants were emailed a consent letter and asked if they had reviewed the consent letter and then a verbal “yes” was given in order to proceed with the interview. Convenience sampling was used to recruit participants from a comprehensive list of CEA growers. The final list included 210 CEA operations across 47 states and the District of Columbia. Growers were contacted via email, company website contact forms, or direct message on Instagram (Meta Platforms, Menlo Park, CA) or LinkedIn (LinkedIn Corporation, Sunnyvale, CA) to gauge their interest in participating in the study. To qualify for the study,

growers had to have an active indoor, soilless leafy green growing operation and sell their product to customers (i.e., not growing solely for personal use).

Survey structure. The Qualtrics platform (Qualtrics, Provo, Utah) was utilized to conduct a survey with 18 items (**Table 4**). The survey was designed to include both open-ended and closed-ended questions, with the option to enter responses under "other." It is worth noting that the survey was developed using items from a survey validated previously by Misra and Gibson (2021) and utilized by Hamilton and colleagues (2023).

Semi-structured interviews. All interviews were conducted by a Ph.D.-trained interviewer via Zoom (Zoom Video Telecommunications, San Jose, CA), Microsoft Teams (Microsoft Corporation, Redmond, WA), or telephone, depending on the grower's preference and internet availability. The interviewer provided an introduction to the study and asked the 11 questions listed in **Table 5**, along with additional clarifying questions when necessary. Zoom and Microsoft Teams recordings were auto-transcribed using audio transcription services available on each software platform. Telephone interviews were recorded using Rev Call Recorder for iOS version 2.6 (Rev, Austin, TX), and audio files were then sent to Scribie (Scribie, San Francisco, CA) for automated verbatim transcription. The audio and video recordings were deleted after the transcription was completed.

Data analysis and interpretation. The analysis of the survey data was conducted using R Studio (R Studio, 2020; R Core Team, 2022). The transcripts from the semi-structured interviews were analyzed using the emergent thematic approach to identify key themes. Two independent researchers coded the transcripts and identified themes into non-mutually exclusive categories (Lune and Berg, 2017). The researchers then met to discuss and merge the identified themes. A constant comparison approach was used to identify broad themes across all interviews. The themes were divided into three categories: *contextual* (physical and operational attributes that could impact implementation of practices), *barriers to risk management and regulatory compliance* (physical or education barriers to safely growing produce or complying with regulations), and *research needs* (information desired by the growers). The survey and the semi-structured interviews were completed by twelve participants, while twenty-five participants completed only the semi-structured interviews. The study included CEA produce growers from across the continental United States (**Figure 1**).

Objective 2 Results

Survey. Results of the survey instrument are listed in **Table 4**. Notably, only 12 of 25 growers completed the survey, so a good portion of data is missing. For example, from the interviews, it is known that at least four growers had aquaponic systems, yet none completed the survey. The most commonly grown leafy green was “lettuce” (n=5; 27.8%) (there were many varieties), followed by herbs (such as basil) (n=3; 16.7%) and arugula (n=3; 16.7%), and then microgreens (n=2; 11.1%), and kale (n=2; 11.1%). Leafy greens were not the only agricultural product grown by the majority of respondents (n=7; 58.3%); all respondents who completed the follow-up question grew crops in addition to leafy greens (n=6; 100%).

Leafy greens growers were somewhat uncertain if their produce was subject to the PSR: “I don’t know” (n=5; 45.5%). Most growers used either vertical farming techniques (n=5; 45.5%) or some variety of greenhouse (n=4; 36.4%). The most frequently held certification was the “Good Agricultural Practices (GAP) Audit” (n=5; 29.4%) followed by “None of these” (n=4; 23.5%). Leafy greens were sold through a variety of avenues, with most growers selling via more than one type of venue (n=7; 63.63%). The most common venues were “Commercial Restaurants” (n=7; 20.0%), “Grocery Stores” (n=7; 20.0%), “Institutional Foodservice

Establishments (hospitals, schools, childcare, long-term care)" (n=6; 17.1%), and "Wholesaler/Distributers" (n=6; 17.1%).

Respondents were most frequently the owner of the operation (n=4; 33.3%), and gross revenue was evenly distributed between "Less than $25,000" to "Greater than $500,000." Most leafy green production areas were built for indoor farming (n=7; 63.6%), and the median number of employees working in the production area was 8 persons (min = 2 persons; max = 220 persons). The median production area was 3000 sq ft (min = 360 sq ft; max = 124000 sq ft), and the median harvesting frequency was 3 days per week (min = 1 day/week; max = 7 days/week).

Semi-structured Interviews. The average interview lasted approximately 45 minutes (range: 23.5-63.3 min; mean: 43.9±12.4 min; median: 45.1 min), and three major themes were isolated from the 11 interview questions: *contextual* (worker hygiene and training; agricultural water; growth substrates and nutrients; pests and biocontrol; harvesting, storage, and transportation; and sanitizer selection and use), *barriers to risk management and regulatory compliance* (business upgrades; regulatory and certification environment; traceability), and *research needs* (algae control; post-harvest storage, treatment, and washing; risk assessments of individual issues; and training program development).

Contextual Themes. A major area of concern for many growers was related to labor factors, particularly ***worker training and compliance, worker retention, and developing a culture of food safety***. A majority of growers faced challenges with handwashing and gloving compliance, which was a widely required practice at the operation level. Notably, a related issue that was frequently reported was finding the time to properly train new employees and struggling with employee accountability. The employment of disabled individuals was reported to cause hurdles with personal protective equipment (PPE) compliance, overall training, and consistently performing food safety related tasks. Many growers expressed concern about the difficulty of implementing food safety culture and the need for additional resources to support these efforts. Additionally, some growers reported difficulty retaining employees due to the high turnover rate in the agriculture industry, which further compounded their labor-related challenges.

Most grower participants obtained their agricultural water from wells or municipal sources. However, when it comes to water testing, many growers had a negative perception due to three primary reasons: ***(1) they didn't understand the reason behind the testing, (2) the cost of testing was deemed too high, and (3) they feared false positives leading to a recall that could potentially ruin their reputation.*** Despite this, many produce growers still utilized some form of water filter, such as sediment, activated carbon, UV, and/or reverse osmosis, to mitigate waterborne contaminants. Some growers added hydrogen peroxide to their water supply for sanitization or pest control purposes. Additionally, many farms incorporated ZeroTol® into their practices to control plant pathogens, which was frequently added to their water supply.

Mold was identified as the primary issue with growth substrates, as it could compromise product quality. However, most growers were unsure if moldy substrate posed a food safety risk to consumers. Growers reported several issues with pests, specifically aphids (family: *Aphididae*) being a common problem. Growers were concerned about the damage that aphids can cause to their plants, and many reported releasing adult ladybugs (family: *Coccinellidae*) as a common solution. Mice and other rodents were also reported as problematic by select growers, and a majority of farms reported using commercial extermination services to address this issue. While a few growers reported pests such as deer and raccoons at outdoor waste piles, they clarified that these waste piles were far from the indoor growing facility and posed no threat to their crops.

Controlling climate factors such as temperature and humidity was identified as a significant challenge in the industry. Temperature was also identified as a critical factor for

maintaining the cold chain during the storage and transportation of produce. One grower specifically mentioned that produce farmers have a more challenging time maintaining the cold chain than other industries such as meat or dairy, due to the lack of thermal mass in produce. This meant that ***any temperature fluctuations during storage or transportation could have a significant impact on the quality and shelf-life of the produce***, which could cause their entire shipment to be rejected by retailers. To address these challenges, growers discussed implementing advanced monitoring systems to keep track of environmental conditions. Overall, growers were ***keen to find innovative solutions to these challenges to ensure the long-term sustainability and profitability of their operations and relatively less concerned about food safety***.

Sanitizer selection was a major concern for many growers due to the varied options available in the market. While some growers were comfortable using bleach, many were hesitant to use it because of the potential harmful effects on the environment and worker safety. As a result, they turned to alternative sanitizers such as ZeroTol®, soap, boiling water, peroxide, various acids, and alcohol wipes. However, there was no consensus on which sanitizer was the best for a particular operation, and some growers indicated not cleaning or sanitizing certain equipment such as water recirculating pumps or hydroponic tanks. The confusion around sanitizer selection was compounded by the fact that different sanitizers had different directions for use, and growers were not always clear on how to properly use them. Many growers were concerned about using "harsh" chemicals that might harm their workers or the environment, so they were hesitant to try new sanitizers. This was particularly true for organic operations who wanted to avoid synthetic chemicals altogether. ***Some growers were open to trying new sanitizers, but they were unsure of how to evaluate them and what factors to consider when selecting one.***

Barriers to Risk Management and Regulatory Compliance. In terms of business upgrades, growers sought a variety of improvements to enhance their operations. One of the most common upgrades was to acquire equipment to improve cold chain compliance, such as refrigerated vehicles or additional refrigeration space. Many growers also expressed interest in automation to help monitor climate factors, with some seeking upgrades for every step of the growing, harvesting, and packaging process. Aspects of automation that growers were interested in varied greatly, but many expressed the desire for software or technology to help with tasks such as tracking inventory, managing crops, and monitoring employees. Another common request was for someone to manage the various administrative tasks and food safety monitoring required of their operations. Many growers were overwhelmed by the amount of paperwork and bureaucracy involved in running a successful produce business and felt that having a dedicated person or team to handle these tasks would be a valuable asset. In addition to upgrades to equipment and administrative tasks, some growers ***expressed interest in expanding their growing space or providing additional training resources for their employees***. With a growing demand for locally sourced produce, many growers saw potential for expanding their operations and increasing their yield but felt that they needed more space or training to do so effectively.

Growers frequently ***expressed uncertainty about the regulatory requirements for their operations***. They indicated that it would be ideal to have a clear understanding of the recommended best practices, standards, and legal requirements. They often felt overwhelmed by the number of possible certifications available from various agencies and organizations. Differences between USDA (i.e., USDA GAP audit) and FDA (i.e., Produce Safety Rule [PSR]) requirements and conflicts with local state agencies and inspectors added to their confusion. Many growers also ***expressed frustration with the lack of consistency in regulatory requirements and inspections, leading to confusion and uncertainty***. Despite these challenges, all growers shared a desire for compliance, indicating their willingness to adhere to

regulatory standards. However, the lack of clear and concise information was a common obstacle in achieving this goal. Some growers also mentioned the need for a streamlined certification process that is both affordable and applicable to their specific operations. They suggested that regulatory agencies work more closely with growers to understand their unique needs and provide guidance on achieving compliance. Growers emphasized that regulatory compliance is not only important for consumer safety but also for the success and reputation of their businesses.

In the area of traceability, many growers expressed a desire to improve their systems. Some growers mentioned that they had a learning curve when it came to traceability, but that it became easier with time. Most growers had some sort of system in place, such as digital barcoding or spreadsheets, to track specific produce lots. However, there was some variation in the level of sophistication of the traceability systems between different growers. Some growers were not convinced of the value of mock recalls, which are required by certain certifications. They felt that a real recall would be significantly different and were unsure of how to prepare for it. The biggest challenge for many growers was determining how granular their traceability systems needed to be, and what was *required* versus what was *recommended*.

Research Needs. The issue of managing algae growth was a common theme among the growers. They expressed a ***strong need for implementing standardized methods to control algae growth***, which was found to be a significant challenge for many of them. Some growers expressed interest in exploring alternative methods, such as the use of beneficial microbes or the implementation of UV light treatment systems. It was clear from their responses that they were mainly interested in these practices for their potential impact on profitability, rather than food safety concerns. The growers understood that by increasing the shelf life of their products, they would be able to reduce waste and potentially increase their profits. Despite the fact that food safety was not the primary motivation behind these post-harvest treatments, it is worth noting that they could still have a positive impact on reducing the risk of microbial contamination and improving the overall quality of the produce.

The growers in the study expressed a ***strong desire for science-based risk assessments of individual issues within the industry, as opposed to receiving generalized advice***. They felt that having access to data would enable them to make informed decisions about the risks they face and how to manage them effectively. However, many growers were ***concerned that the industry was moving too quickly without proper consideration of the potential risks involved***. As a result, they wanted to understand which factors were important for food safety in order to avoid making mistakes that could lead to pathogen outbreaks or recalls. The growers suggested that there needs to be a concerted effort to gather and disseminate this information to prevent future problems. Overall, the growers emphasized the importance of proactive measures to address food safety issues and a desire to learn from past mistakes in order to avoid future ones. Specifically, many growers expressed a ***desire to learn from other growers' mistakes***. They suggested that information about the causes of past recalls and pathogen outbreaks should be publicly available and easily accessible. By understanding what factors contributed to these incidents, growers could take steps to avoid similar situations and improve their own food safety practices. However, at present, there appears to be a lack of transparency and communication about the causes of food safety incidents in the industry. Growers expressed frustration that they did not have access to this information, and that it was not always clear what steps were being taken to prevent similar incidents from occurring in the future. By making information about past incidents more readily available, the industry could facilitate a more collaborative and proactive approach to food safety. Growers could learn from each other's experiences, and the industry as a whole could work to identify and address common risk factors. This could help to prevent future incidents and ensure the safety of the food supply for consumers.

Growers unanimously expressed a strong need for concise, standardized training programs to ensure that their staff understands and follows best practices for food safety. The growers acknowledged that ***food safety practices cannot be applied in a "one size fits all" approach, but they emphasized that the lack of a standardized program can be challenging***. The growers want a turnkey, concise gold standard that can be easily implemented for staff with varying backgrounds. They also highlighted that free training programs would be beneficial in ensuring that all growers, including smaller ones, have access to the information they need to maintain food safety standards. Many of the growers have noted that the existing training seminars come at a high cost, which can be prohibitive for them and their employees.

Objective 3 Methods

Discussions with Academic and Regulatory Personnel. To further understand ongoing research efforts within food safety and CEA production systems, we reached out to academic colleagues (research and extension) and regulatory experts working in the field of CEA food safety. These discussions were more informal than the semi-structured interviews described in Objective 2. Specifically, during the USDA-funded "Strategizing to Advance Future Extension and Research in Controlled Environmental Agriculture" [S.A.F.E.R. CEA] conference held at the Ohio Controlled Environment Agriculture Research Center (https://foodsafety.uada.edu/2023-safer/), several small group and breakout sessions were facilitated to enable identification of ***what we already know*** and ***what we need to know more about*** regarding food safety in CEA. These groups also included a relatively smaller number of industry and allied industry members. Opinions and thoughts of academics and regulatory experts were noted to further rank the most critical CEA research needs.

Objective 3 Results

There were approximately 47 attendees at the S.A.F.E.R. CEA Conference, including 28 research and extension colleagues, 15 CEA industry members (operators and allied industries), and 4 regulatory affiliates (one in person, three via Zoom). It is important to note that regulatory members were actively recruited to attend, but due to government travel restrictions and the truncated timeline between conference announcement and the meeting dates, their attendance was minimal.

Several issues and needs were mentioned by research and extension colleagues. During discussions and presentations, all agreed about the following statements:

(1) Pathogens can enter CEA systems via agricultural water, contaminated nutrient solutions, contaminated seeds, air, growth media/substrates, and other supplies.
(2) Pathogens can survive long enough and spread through CEA systems to cause foodborne disease outbreaks.
(3) Peer-reviewed publications can be a resource to understand potential food safety risks; however, specific studies are needed to target CEA industry concerns.
(4) Produce Safety Rule standards designed for open field production do not apply for all contamination risks and may be insufficient for CEA systems due to the high risk of pathogen spread once introduced into the operation.

Unknowns and research needs were also highlighted by researchers and regulatory experts. In summary, the following items were commonly identified as CEA industry needs:

(1) Degree of pathogen survival, spread, transfer, and elimination in/from materials used in ponic systems.

(2) Validated practices related to the management of irrigation water and nutrient solution including identification of effective water treatment techniques for recirculating systems.
(3) Recommendations for effective sanitizers and validation of application within ponic systems.
(4) Preventive measures, mitigation strategies, and corrective actions specific for CEA systems.
(5) Specific guidelines for produce grown in CEA (e.g., some of the general PSR requirements may apply to CEA, but risk factors can differ from field conditions).

Objective 4 Methods

The findings extracted in Objectives 1, 2, and 3 were further analyzed to identify major factors driving food safety risk management in the indoor, soilless leafy green production industry. Furthermore, we addressed the identified needs by categorizing actionable items into three groups: short-term, medium-term, and long-term.

Objective 4 Results

Indoor, soilless produce production is a new research area being introduced to the CPS research portfolio. We believe that the mixed methods approach resulting in a scoping review, identification of research priorities for the CEA industry, and qualitative assessment of microbial risks can serve as a tool for the CPS Technical Committee to identify future research priorities and to inform stakeholders of actionable items. Overall, we have surmised that implementation of food safety risk management practices in CEA leafy green growing operations is driven by key factors, including:

(1) type of crops and required production materials;
(2) diversity among production systems and technologies;
(3) food safety knowledge and awareness;
(4) available infrastructure of small producers and family-owned farms; and
(5) willingness to invest more in safety by small versus large companies.

As the culminating objective of this project, the actionable items have been categorized under three titles based on a broad timeframe. Short-term actionable items include development and adoption of currently available risk assessment tools, standard operating procedures (SOPs), safe handling practices, and currently applicable training materials. The application of short-term items can be easily developed in collaboration with CEA industry, research and extension specialist, and regulatory officials based on experience. Adaptation of these items and implementation of food safety risk management practices can be achieved within a reasonable time with modifications depending on the unique needs of small and large producers.

Short-term Actionable Items

- Development/adoption of current risk assessment tools.
- Development/adoption of current cleaning/training SOPs.
- Development/adoption of best practices for nutrient solution preparation and handling.
- Development/adoption of accessible food safety training materials, fact sheets, and GAPs relevant to CEA.
- Creation/adoption of food safety culture around CEA production systems.
- Development/adoption of effective communication with CEA growers for food safety.
- Standardization of definitions and terms for better communication between research, extension, government/regulatory agencies, and producers.

Considerations regarding product flow (e.g., building design for separation of production, harvest, and packing) and handling with food safety in mind as well as developed of new, validated training materials to improve foods safety management systems in CEA are categorized as medium-term actionable items. Also, microbial safety standards for input materials and water sources can be proposed until validated science-based limits and standards are determined for indoor, soilless production systems.

Medium-term Actionable Items

- Development/adoption of appropriate harvesting practices depending on type of product and production technology/systems.
- Development/adoption of accessible food safety training materials, fact sheets, and GAPs specific to ponic production technology/systems.
- Development/adoption of accessible preventive measures, mitigation strategies, and corrective actions specific for type of product and production technology/systems.
- Define food safety requirements for input materials including seeds and substrates and certified suppliers.
- Adaptation of PSR agricultural water testing standards and measurable indicators and limits associated with type of source to the CEA industry.

Science-based actions requiring data curation will take time to plan, for the research to be conducted, and for recommendations to be generated. The behavior or response of pathogens can be evaluated based on CEA production technology and system type. Also, specific data can be generated to propose risk-based standards for microbiological water quality and input materials (i.e., seeds, growth media).

Long-term Actionable Items

- Characterize pathogen survival, spread, transfer, and elimination risks within water, nutrient solutions, growth media substrates, and production materials (e.g., porous and non-porous surfaces).
- Determine the effect and possible use of non-chemical treatment techniques that balance system and plant health.
- Investigate pathogen control via manipulation of physicochemical and microbiome characteristics of nutrient solutions.
- Develop algae control strategies and tolerable growth limits while maintaining food safety.
- Develop/optimize water testing standards, measurable indicators, and limits associated with source type.
- Develop/optimize material input safety standards for supplier certification.

Outcomes and Accomplishments

The outcomes of this research have been discussed in the methods and results sections in detail. All objectives have been completed as proposed, and results from Objectives 1 and 2 have been converted to manuscripts for publication. As a product of objective 1, a scoping review with comprehensive tables has been prepared and will be submitted to a respected journal in a short time. In this review, science-based pathogen contamination risks and mitigation strategies for indoor production of microgreens and leafy greens are discussed during both pre-harvest and post-harvest stages of production. The second objective has also been completed with survey and semi-structured interviews of producers of indoor, soilless leafy greens from 20 states throughout the U.S. The data revealed that producers are presented with

a variety of challenges, barriers, and information needs related to food safety in their operations depending on size and infrastructure. Regardless of the volume of production and type of production system, the common themes for safety challenges and barriers were similar. Common concerns included food safety training and awareness, creating a culture of food safety, lack of standards/guidelines, proper use of sanitizers and treatment techniques, and the need for certified supplies and sustainability. The third objective indicated that areas of limited science-based data relevant to food safety in CEA-grown leafy greens mostly aligned with producers' concerns. Finally, the fourth objective presented short-, medium-, and long-term actionable items to address microbial risks during indoor leafy green production based on the available knowledge, key takeaways, and current practices generating from Objectives 1-3.

Summary of Findings and Recommendations

Finding 1: Investigations on the food safety risks in soilless production systems have typically focused on the potential for pathogens to internalize within edible portion of the leafy greens. Based on the literature review, there is no clear answer regarding the risk of internalization.

Recommendation 1: We recommend a concerted research effort to tease apart the proposed risk factors for pathogen internalization in leafy greens, including cultivar/variety selection, pathogen type (e.g., selection of serotypes relevant to indoor, soilless production), growth media selection, root colonization, root zone health (i.e., protect from damage), and role of microbial community.

Finding 2: Published research has focused predominately on risks related to STEC and *Salmonella* serovars, with relatively fewer (50% less) peer-reviewed articles addressing the risks of *L. monocytogenes* within indoor, soilless leafy green production. Meanwhile, *L. monocytogenes* is the primary pathogen responsible for CEA-grown leafy green product recalls.

Recommendation 2: We recommend that additional research be conducted to characterize the transfer, colonization, and persistence of *L. monocytogenes* within indoor production systems, along with the critical routes responsible for the introduction of *L. monocytogenes* into these environments.

Finding 3: There is little information regarding microbial risks associated with material selection and reuse within indoor, soilless leafy green production. For instance, DWC systems often utilize Styrofoam rafts that are reused until deemed unsuitable via visual inspection versus NFT systems that utilize gutters composed of vinyl (food-grade PVC, but there are DIY options that suggest retro-fitting gutters from home improvement stores). However, there is no guidance on material selection and reuse.

Recommendation 3: We recommend a characterization of common surfaces within indoor, soilless production systems to evaluate cleanability, appropriate sanitizer chemistries, environmental monitoring strategies, and indicators/signs indicating a given material should be replaced.

Finding 4: The use of hydrogen peroxide is a common application for both sanitization and plant health by producers, with no standards along with limited support of scientific data.

Recommendation 4: Immediate studies should be conducted to assess the use of hydrogen peroxide within the CEA environment, with a focus on control of human pathogens. Limits and safe application procedures should be a top priority.

Finding 5: Risk of contamination is dependent on CEA system type (e.g., DWC, shallow water culture, NFT, vertical, etc.) in addition to management practices.

Recommendation 5: Risk-based approach to system selection and management should be the goal of the CEA industry.

Finding 6: The training and educational needs of the CEA industry are not currently being met. Although, recently, new training and outreach materials have become available, these materials are either not reaching the end users or are perceived as too generic or not applicable to the workforce.

Recommendation 6: More CEA-specific training and outreach materials should be developed with the help of academia, producers, and regulatory experts and shared as open access. In addition, materials should be optimized for the target workforce employed by CEA operations with socially driven missions (e.g., many employ disabled and neurodiverse persons).

Finding 7: CEA producers are not aware of the Cooperative Extension Service and the types of activities provided by universities to improve CEA food safety.

Recommendation 7: Extension and outreach programs of universities should be increased for CEA leafy green production, and producers should be contacted to inform about these programs.

APPENDICES

Publications

Allyson N. Hamilton, Zeynal Topalcengiz, Kristen E. Gibson (2023). Growing Safer Greens: Exploring Food Safety Practices and Challenges in Controlled Environment Agriculture Through Thematic Analysis of Grower Interviews. *(In preparation)*

Zeynal Topalcengiz, Sahaana Chandran, Kristen E. Gibson (2023). A Scoping Review of Microbial Risks During Indoor, Soilless Leafy Green Production. *(In preparation)*

Budget Summary

The following is a summary of the funds expended as of April 31, 2023:

Category	Budget	Funds Expended to Date
Salary and wages	$13,750.00	$13,750.02*
Fringe benefits	$3,873.00	$4,234.99*
Travel	$4,656.00	$1,296.97
Other direct costs	$4,050.00	$71.12
Indirect costs	$1,410.00	$1,410.00
Total	$27,739.00	$20,763.10

*Overspent categories are primarily due to change in fringe benefit rates and will be corrected at grant close out through rebudget of other direct costs.

Tables 1–5 and Figure 1 (see below)

Table 1. Outbreaks and recalls of foodborne disease associated with leafy greens and microgreens grown in CEA in North America.

Product	Outbreak/Recall	Pathogen	Location	Recall date	Reference
Arugula Microgreens, Broccoli Microgreens, Fresh Microgreen Mix, Sweet & Crunchy Microgreen Mix, Spicy Microgreen Mix, Pea Shoots Microgreens, Sunflower Microgreens, Wheatgrass, Spring Pea Microgreen Mix	Recall	*L. monocytogenes*	Alberta, British Columbia, Canada; WA, USA	4/24/18 and 4/30/18	CFIA, 2018a
Broccoli Microgreens, Spicy Microgreen Mix	Recall	*L. monocytogenes*	Ontario, Canada	08/25/18	CFIA, 2018b
Broccoli Microgreens, Radish microgreens, Spicy micro & lettuce mix	Recall	*L. monocytogenes*	Ontario, Canada	09/22/18	CFIA, 2018c
Sweet Pea Shoots, Pea Shoots	Recall	*L. monocytogenes*	Alberta, British Columbia, Canada	06/07/18	CFIA, 2018d
Daikon Radish (microgreens)	Recall	*L. monocytogenes*	New Brunswick, Nova Scotia, Prince Edward Island, Canada	06/28/18	CFIA, 2018e
Mix Spicy Microgreens	Recall	*L. monocytogenes*	Quebec, Canada	05/22/19	CFIA, 2019a
Sweet Pea Shoots, Pea Shoots	Recall	*L. monocytogenes*	Alberta, British Columbia, Saskatchewan, Canada	04/19/19	CFIA, 2019b
Arugula Microgreens	Notification	*Salmonella*	Quebec, Canada	06/29/18	CFIA, 2019c
Arugula Microgreens, Broccoli Microgreens, Coriander Microgreens	Recall	*Salmonella*	New Brunswick, Quebec, Canada	08/20/20	CFIA, 2020
Packaged leafy greens including romaine lettuce, spinach, and various mixes	Outbreak*	*Salmonella* (Typhimurium)	IL, MI, PA, WI, USA	05/10/21 and 08/18/21	USFDA, 2021
Micro Greens, Baby Kale & Baby Spinach with Sweet Pea Leaves, Cat Grass	Recall	*Salmonella*	NY, PA, MA, NJ, VA, MD, NC, USA	12/23/2022	USFDA, 2022a
Krunch, Butter and Romaine whole head variety lettuce	Recall	*Salmonella*	FL, USA	11/03/22	USFDA, 2022b
Packaged various products of romaine lettuce and mixes	Recall	*L. monocytogenes*	MI, OH, IN, IL, KY, WI, USA	04/07/2023	USFDA, 2023

* This outbreak caused 31 cases with 4 hospitalizations.

Table 2. Summary of published studies investigating partly or completely microbiological aspects of microgreens.

Microgreen	Trial/Treatment	Monitored/Inoculated Microorganisms	Treatment/Inoculation conditions	Storage/Growth conditions	Harvest/ Sampling time	Comments/Highlights	Reference
Common sunflower, Radish, Arugula, Common beet, Red cabbage, Brown mustard, Broccoli, Spinach, Cress	Market survey	Shiga toxin-producing *E. coli* (STEC), *Salmonella* spp., and *Listeria* spp.		The samples originated from 8 countries, 10 manufacturers, 6 local producers, and 5 retailers and comprised 19 crop species		No viable STEC; *Salmonella* and *L. monocytogenes* detected in microgreens or seeds used for microgreens	Bergšpica et al., 2020
'Tah Tasai' Chinese cabbage	Post-harvest wash followed by storage	Aerobic mesophilic bacteria count (AMBC) and Coliform	(1) Dipping: tap water, 100 ppm chlorinated water, or 0.25% (w/v) each of citric and ascorbic acid mixture for 2 min (2) Dipping + Spray: 0.5% (w/v) citric acid solution (2 min) followed by 50% (v/v) ethanol spray	Stored in 35 µm PE or PP bags in a dark room at 5°C.	Day 0, 3, 5, 7 and 9	Reduction in AMBC population during initial period of storage and higher reduction effect of chlorinated water compared to other treatments	Chandra et al., 2012
Sunflower and Pea Shoot	Pre-harvest contamination from growth media	*L. monocytogenes* (strain F2365) and *S. enterica* Javiana (ATCC BAA1593), AMBC	Growth media inoculation (10^5–10^7 CFU/g) followed by seeding on Day 3	Grown on no-drainage tray filled with 3.5 mm thick biopolymer natural fiber blend mat or a Canadian *sphagnum* peat and vermiculite mix	Day 10 for both microgreen and growth media sampling	Growth media and microgreen variety effected pathogen transfer to microgreens	Deng et al., 2021
Sunflower and Pea Shoot	Pre-harvest contamination of microgreen leaves	Tulane virus (TV) as Human norovirus (HuNoV) surrogate	Inoculation of adaxial side of leaf surfaces (10^4–10^5 PFU/g) on Day 7	Grown on tray filled with Canadian *sphagnum* peat and vermiculite mix	Day 7, 8, 9 and 10	Microgreen variety on leaf surface impacted persistence of a HuNoV surrogate	Deng and Gibson, 2022
Sunflower and Pea Shoot	Pre-harvest contamination from growth media	TV as HuNoV surrogate	1) Growth media inoculation (~10^7 PFU /g) followed by seeding 2) growth media inoculation on Day 7	Grown on tray filled with Canadian *sphagnum* peat and vermiculite mix	Day 10 for micro-greens and Day 0, 1, 3,5,10 for growth media sampling	TV persistence for 10 days with differences between microgreen variety but no internalization	Deng and Gibson, 2023

Broccoli raab	Pre-harvest contamination from growth media	AMBC and Yeast & Mold counts, *Enterobacteriaceae*, *E. coli*		Grown on trays filled with a textile-fiber mat, 100% biodegradable mat, a mixture (50:50 v/v) of fine black and white peat, and Sure to Grow mats at 16.9°C	Day 11	Significant effect of different growing media use on microbiological population on microgreens	Di Gioia et al., 2016
Arugula	Pre-harvest contamination from growth media	Rotavirus (RV) and TV	Inoculation of plant growth nutrient solution (feed water) for internalization (10^6–10^7 PFU/mL) and inoculation of leaf surfaces (10^6–10^7 PFU/g)	Grown on tray filled with water supplemented with plant growth nutrient solution under mesh screen	Day 1, 2, 3, 5, 6 and 7	Both the type and location of virus in arugula may impact virus inactivation during post-harvest treatment vegetables	Fuzawa et al., 2021
	Post-harvest treatment followed by storage		Exposure of virus internalized and virus-inoculated leaves to peroxyacetic acid at 30 ppm (pH 2.5) and 80 ppm (pH 2.8) for 30 s to 3 min			Reduction up to 1.5 interior and 5 log PFU/g on leaves	
Romaine lettuce and Cherry belle radishes	Pre-harvest contamination from growth media	Generic *E. coli* (ATCC 25922) and *E. coli* O157:H7 (ATCC 35150), AMBC and Yeast & Mold counts	Growth media inoculation (10^5–10^6 CFU/g) on seeding day followed by spray and bottom irrigation	Grown on no-drainage clamshell containers filled with peat moss alone and perlite supplemented with plant growth nutrient solution	Lettuce: Day 17 for Perlite, Day 21 for peat moss. Radish: Day 10	Higher pathogen transfer levels to edible part of radish microgreens than lettuce counterparts and no effect of irrigation type	Işık et al., 2020
Cherry belle radishes	Pre-harvest contamination from growth media	*S. enterica* Typhimurium (ATCC 14028), *E. coli*	Growth media inoculation (10^5–10^6 CFU/g) on seeding day	Grown on no-drainage clamshell containers filled with perlite supplemented with	Day 10	Pathogen transfer levels to edible part of radish microgreens	Işık et al., 2022

	Pre-harvest treatment	O157:H7 (ATCC 35150), and Generic *E. coli* (ATCC 25922), and AMBC and Yeast & Mold counts	Sprayed with chlorinated water at concentrations of 0.50, 1.00, and 2.00 ± 0.05 ppm free chlorine once (day 9), twice (day 8 and 9), three (day 7, 8, and 9), and four times (day 6, 7, 8, and 9)	plant growth nutrient solution		Limited reduction of pathogens on radish microgreens after spray application of chlorinated water during growth	
	Pre-harvest contamination from growth media	Abiotic surrogate (GloGerm)	Growth media and seed inoculation on seeding day	Grown on no-drainage clamshell containers filled with perlite and abiotic surrogate supplemented with plant growth nutrient solution		Abiotic surrogate spread on cotyledon and upper hypocotyl of radish microgreen plants regardless of seed or growth media inoculation	
Buckwheat	Storage Temperature	AMBC		Stored in PE bags prepared with 16.6 pmol/(m^2 s Pa) OTR film at 1, 5, 10, 15, or 20 °C	Days 0, 3, 6, 10 and 14	Reduction in AMBC population during initial period of storage and accelerated growth for the rest of storage	Kou et al., 2013
	Packaging film followed by storage			Stored in PE bags prepared with 8.0, 16.6, 21.4 and 29.5 pmol/(m^2 s Pa) OTR films at 5°C	Days 0, 4, 7, 14 and 21		
	Post-harvest wash followed by storage		Dipping: 100 ppm and 50 ppm chlorinated water with pH adjusted to 6.5 using citric acid for 30 s followed by 1 min rinse and drying	Stored in PE bags prepared with 16.6 pmol/(m^2 s Pa) OTR film at 5°C	Days 0, 4, 7, 14 and 21		

Broccoli	Pre-harvest treatment followed by storage	AMBC	Sprayed daily with H_2O (pH 5.6 acidified water) only, 1, 10, and 20 mM $CaCl_2$ or $MgCl_2$ with calcium chelator 5 mM EGTA for 10 days	Grown on tray filled with hydroponic growth pads and stored in PE bags prepared with 16.6 pmol/(m^2 s Pa) OTR film at 5°C	Days 0, 4, 7, 14 and 21	Reduction in AMBC population during initial period of storage and accelerated growth for the rest of storage	Kou et al., 2014
Broccoli cultivar Arcadia	Pre-harvest treatment followed by storage	AMBC	Sprayed daily with tap water (pH 5.5–6.0) only; 1, 10, or 20 mM/L Ca Amino Acid or Ca-lactate; or 10 mM/L $CaCl_2$ after sowing the seeds	Grown on tray filled with hydroponic growth pads and stored in sealed PE bags prepared with 16.6 pmol/(m^2 s Pa) OTR film at 5°C	Days 0, 4, 7, 11 and 14	Reduction in AMBC population during initial period of storage and accelerated growth for the rest of storage for all treatments except for pre-harvest $CaCl_2$ spray with no dipping	Kou et al., 2015
	Post-harvest treatment followed by storage		Sprayed daily with tap water during growth followed by dipping: 0, 25, 50, or 100 mM/L Ca-lactate + 100 ppm chlorinated water (pH 6.5) mixture for 30 s				
	Pre/Post-harvest treatment followed by storage		(1) Sprayed daily with tap water during growth followed by dipping in chlorinated water (100 ppm) or Ca- lactate (50 mM/L) solution for 30 s (2) Sprayed daily with $CaCl_2$ (10 mM/L) during growth followed by dipping in chlorinated water (100 ppm) for 30 s (3) Sprayed daily with tap water during growth followed by dipping in Ca-lactate (50 mM/L) with chlorinated water (100 ppm) for 30 s				

'Tah Tasai' Chinese cabbage	Post-harvest wash followed by storage	AMBC	Dipping: In cold (5°C) and warm (25°C) chlorinated water with 0, 50 or 100 ppm free chlorine for 90 s	Stored in PP bags in a dark room for at 15°C	Day 8	Reduction in AMBC population during initial period of storage	Lee et al., 2009
Garden cress	Pre-harvest contamination	*S. enterica* Newport (MET-S1-166), *E. coli* O157:H7 (MET-K1-30), *E. coli* O104:H4 *(*MET-A1-80), and *E. coli* O78:H2 (MET-A1-90)	Seed inoculation (10^6–10^8 MPN/g) by dipping and water inoculation (~10^8 MPN/mL) by spraying	Grown on tray filled with autoclaved peat	Day 30	Biofilm formation of tested pathogen and serotypes on cress leaves grown using both contaminated seeds and irrigation water	Namli et al., 2022
Roselle	Pre-harvest treatment of seeds	Generic *E. coli*, Total coliform, AMBC and Yeast & Mold counts	Seed treatment with 5% hydrogen peroxide (H_2O_2), UV-C (36 watts), advanced oxidation process (AOP; H_2O_2 + UV-C) and improved AOP by combination with microbubbles (MBs) (H_2O_2 + MBs and H_2O_2 + UV-C + MBs)	Grown in a wetted sponge	Day 7	Limited reduction success and questionable microbiological analysis on tested microorganisms	Phornvillay et al., 2022
Swiss chard	Pre-harvest contamination from seed and water	*S. enterica* Cubana strain CFSAN055271, *S*. Hartford strain NY20	Seed inoculation (~10^1 and ~10^2 CFU/g) and water inoculation (~0.02, ~0.2, ~2, ~20, and ~2,000 CFU/g)	Grown on tray filled with potting soil A or B or hydroponic growth pads	Day 14	Significant effect of different growth media used and inoculation level on pathogen survival and growth	Reed et al., 2018
Kale and Mustard	Pre-harvest contamination from water	HuNoV surrogate (murine norovirus [MNV]), Total coliforms and *E. coli*	Water inoculation on day 8 (~3.5 log PFU/mL) circulated in hydroponic system.	Grown on tray filled with hydroponic grow pads soaked in circulating water supplemented with plant nutrient solution	Hour 0, 2, 4, 8, and 12; Day 8, 9, 10, 11, and 12 for survival and internalization	Transfer of MNV to edible part of microgreens from root	Wang and Kniel, 2016

Amaranath var. Red army, Broccoli, Kale var. Red Russian, Mustard red frill, Coriander, Rocket var. Victoria, Basil var. Purple dark opal, Parsley var. Italian plain leaved, Radish var. Sangria	Pre-harvest contamination from growth media	*E. coli* O157:H7	Matting substrate (GrowFelt Purple, Reco or White) inoculation (~10^3 CFU/mL) or seed inoculation (~10^3, ~10^5, ~10^7 CFU/mL)	Grown on PE containers lined with dry matting pad (purple, white, reco) on perlite	Day 6: Kale, Rocket, Radish Day 9: Mustard Day 12: Amaranath, Broccoli, Coriander Day 19: Basil, Parsley	Various colonization level depending on plant tissue type, source of contamination (water>seed), inoculation level, and environmental factors	Wright and Holden, 2018
Alfalfa, Broccoli, Coriander, Lettuce (Curled and Oak leaf, 'Lollo-Rossa'), Parsley (Italian Plain Leaved) and Rocket (Victoria)	Pre-harvest contamination from growth media	*E. coli* O157:H7	The matting substrate (GrowFelt Purple) inoculation by called as 'watered' (~10^3 CFU/mL) or seed inoculation by dipping called as 'soaked' (~10^7 CFU/mL)	Grown on trays filled with commercial compost after germination and signs of cotyledon and root emergence (4–11 days)	N/A	Lower colonization on true leaves compared to cotyledon and species	Wright et al., 2022
Daikon radish	Storage Temperature	AMBC and Yeast & Mold counts		Stored in PE bags prepared with 16.6 pmol/(m^2 s Pa) OTR film at 1, 5, or 10°C in a dark room	Day 0, 3, 7, 10 and 14	Negative effect of temperature increase on storage and reduction in AMBC population during initial period of storage with accelerated growth for the rest of storage after chlorine treatment	Xiao et al., 2014a
	Packaging film followed by storage			Stored in PE bags prepared with 8.0, 11.6, 16.6, 21.4, or 29.5 pmol/(m^2 s Pa) OTR films at 1°C in a dark room	Day 0, 7, 14, 21 and 28		
	Post-harvest wash followed by storage		Dipping: 50, or 100 ppm free chlorinated water at pH 6.5 adjusted with citric acid solution for 1 min followed by 1 min rinse	Stored in polyethylene film bags prepared with 29.5 pmol/(m^2 s Pa) with OTR film at 1°C in a dark room	Day 0, 7, 14, 21 and 28		
Daikon radish	Pre-harvest contamination from seed	*E. coli* O157: H7 strains ATCC 43888, ATCC 43895, and EC415 (cocktail), *E. coli* O104:H4 strain TW16133 (individual)	Seed inoculation at low (~1 log) and high (~4 log) concentrations (CFU/g)	Grown on tray filled with commercial germination mix at 25°C/18°C (day/night) with daily irrigation with sterile distilled water	Day 7	Significant effect of inoculation level and proliferation on pathogen transfer	Xiao et al., 2014b

Daikon radish	Pre-harvest contamination from seed	*E. coli* O157:H7 strains ATCC 43888, ATCC 43895, and EC415 (cocktail)	Seed inoculation at low (3 to 4 log CFU/g) and high (5 to 6 log CFU/g) levels on seeds	Grown on tray filled with commercial germination mix or hydroponic growing pads at 25°C/18°C (day/night) with daily spray or bottom irrigation with sterile distilled water	Day 7	Significant effect of inoculation level on pathogen transfer to edible part microgreens and no effect of irrigation type	Xiao et al., 2015

Storage/Growth conditions: Polyethylene (PE); Polypropylene (PP); Oxygen Transmission Rate (OTR).

Table 3. Summary of published studies investigating partly or completely microbiological aspects of leafy greens.

Leafy green	Trial/Treatment	Monitored/Inoculated Microorganisms	Treatment/Inoculation conditions	Storage/Growth* conditions	Harvest/ Sampling time	Comments/Highlights	Reference
Lettuce	Post-harvest analysis - Assessment of microbiological profile of harvested plants	Shiga toxin-producing *E. coli* (STEC), *E. coli,* and *Salmonella* spp.	Hydroponically and conventionally grown lettuce were bought from supermarket	N/A	N/A	Conventionally grown plants were 2.4 times more likely to be contaminated with *E. coli*; *Salmonella* was present in 16.67% of the samples studied.	Arraris et al., 2020
Lettuce	Pre-harvest contamination	Coxsackievirus B2	Growth media inoculation at 9.62 log GC/L (9.30 log MPN/L) and 7.62 log GC/L (7.30 log MPN/L)	Grown in 2-in. plug trays in greenhouse for 20 days after germination and moved to a hydroponic system with plant growth nutrient solution	Day 1, 2, 3, and 4	Absorption of enterovirus occurs through the roots in hydroponically grown plants.	Carducci et al., 2015
Lettuce	Pre-harvest contamination	Fecal coliform bacteria, yeast, and mold	Water, peat moss plugs (substrate), and lettuce were collected to evaluate microbiological populations	Grown in peat moss substrate in NFT systems.	N/A	Substrates are a potential source of contamination and can transfer microbes to harvested leaves.	Dankwa et al., 2020
Romaine lettuce	Pre-harvest contamination	Human norovirus (HuNoV) (GII.4), Tulane virus (TV) and Murine norovirus (MNV)	Growth media inoculation: 1 x 10^6 RNA copies/mL of HuNoV and 1 x 10^6 to 2 x 10^6 PFU/mL of TV and MNV	Grown in 2-in. plug trays followed by insertion in hydroponic growth system twenty days after germination.	Day 0, 1, 2, 3, 7, and 14.	HuNoV and its surrogates internalized via roots and spread to the shoots and leaves.	DiCaprio et al., 2012
Leafy greens (spinach, leafy lettuce and parsley)	Pre-harvest contamination	*E. coli* O157:H7 (K3995, F4546 and K4492) and Shiga toxin-negative *E. coli* strains (CV267, 6980-2 and 6982-2)	Growth media inoculation: 25 ml of inoculum having either 6.5, 7.5, or 8.5 log CFU/mL	Grown in Tifton Sandy Loam soil.	Day 0, 1, 2, and 3	Internalization increased in water-saturated soil.	Erickson et al., 2013
			Growth media inoculation: 25 ml of inoculum having 6 log CFU/mL + water saturated soil		Day 0, 2, 4 and 7		
			Growth media inoculation: 25 ml of inoculum having 8 log CFU/mL + either moistened or water saturated soil		Day 0, 3, and 6		
Curly lettuce	Pre-harvest analysis of nutrient media	*E. coli, Salmonella* spp., total coliforms, thermotolerant fecal coliforms and endoparasites	Eight different nutrient solutions – four mineral and four organomineral solutions were used for cultivation	Grown in hydroponic systems with NFT in greenhouse.	After 24 days	(1) No *E. coli* and *Salmonella* detected in any plants.	Filho et al., 2017

						(2) Low levels of parasitological contamination observed.	
Lettuce seedlings	Pre-harvest contamination from growth media	*E. coli* O157:H7 (B6-914) and *S. enterica* Typhimurium (strains MAE 110 and 119)	Growth media inoculation: 3.39 x 10^7 CFU/ml in nutrient solution and 3 ml of 1 x 10^9 CFU/ml in soil	Grown in plant growth nutrient solution and potting soil.	Day 18 for plants grown in nutrient solution and day 35 for plants grown in soil	Internalization of *S.* Typhimurium MAE 119 occurred at high densities in the nutrient solution. In soil system, the presence of *E. coli* was significantly higher than *Salmonella*.	Franz et al., 2006
	Post-harvest treatment		Surface sterilization of shoot and root by dipping in 1) 1% silver nitrate solution for 10 s and two washes in demineralized water for 10 s or 2) 1% sodium hypochlorite for 5 s and 5 s in 70% ethanol followed by two washes in demineralized water for 10 s			Silver nitrate treatment was significantly better compared to sodium hypochlorite treatment.	
Iceberg lettuce	Post-harvest analysis and post-harvest	Thermotolerant fecal coliforms, mesophilic aerobic bacteria, *Salmonella* sp. and intestinal parasites	Treatment of 25g of lettuce with 100 ppm of sodium hypochlorite and 1% acetic acid	Lettuce that was grown traditionally, organically and in hydroponic systems were purchased from supermarkets.	N/A	(1) High numbers of coliforms, mesophilic bacteria and parasites found in lettuce grown organically and in soil. (2) Both sodium chlorite and acetic acid treatment were effective in reducing bacterial counts.	Gomes Neto et al., 2012
Lettuce, Baby spinach, Red lettuce and Ricola	Contamination during harvest	*E. coli* (ATCC 35218)	Petiole of the plants were cut using: (1) Metal scalpel at 25°C and 200°C (2) Metal scissor at 25°C and 200°C After cutting, the petiole was placed in bacterial inoculum (10^6 CFU/mL).	Plants purchased from grocery store and were also grown on plant growth nutrient solution and soil.	1.5 hours	Cutting leaves using scissors at 200°C significantly reduces bacterial uptake.	Guerra et al., 2022

Spinach var. Barbados and Avenger	Pre-harvest contamination	Generic *E. coli* (TVS 353, 354, and 355) and *E. coli* O157:H7 (ATCC 700728 and ATCC 43888)	(1) Hydroponic condition – Spray inoculation of leaves (log 4 CFU/mL) (2) Field condition – Spray inoculation of leaves (log 1.45 and log 3.4 CFU/m^2)	(1) Grown in plant growth nutrient solution and coir-vermiculite horticultural mix in greenhouse. (2) Grown in fields in 152 cm wide raised seedbeds (commercial practice method).	(1) Day 4, 7, 9, 14 and 21 for plants grown in nutrient solution (2) Day 1, 7, 14 and 21 for plants grown in soil.	Strain source, water availability and localization within plant have significant impact on persistence of generic *E. coli* and *E. coli* O157:H7.	Gutiérrez-Rodríguez et al., 2012
Lettuce	Pre-harvest contamination and attachment	*L. monocytogenes* (ATCC 19111, strain Pirie) and *S. enterica* Typhimurium LT2 (strain JSG626)	Growth media inoculation: sporadic (~10^4 CFU/mL) and extreme (~10^4 CFU/mL)	Grown in plant growth nutrient solution in NFT Systems	Day 0.5, 1, 7, 14, 21 and 28	Pathogen survival in commercial NFT systems	Ilic et al., 2022
Lettuce and Spinach	Pre-harvest contamination	*E. coli* O157:H7 (C9490), *S. enterica* Typhimurium (SA941256), *L. monocytogenes* (CRIFS23074)	Seed inoculation at 10^2 CFU/mL for 20 min	(1) Sterile filter paper discs in Petri dishes for ≤ 10 days study (2) Solidified hydroponic nutrient solution for > 10 days study	(1) Germinating seeds for ≤ 10 days study (2) Day 9 and 49	*E. coli* O157:H7 and *L. monocytogenes* present in high levels on seedlings	Jablasone et al., 2005
	Co-inoculation study with representative endogenous bacteria	*Enterobacter cloacae* and *Chryseomonas luteola*				*E. cloacae* reduce *E. coli* O157:H7 and *L. monocytogenes* levels by 1 log CFU/g on lettuce.	
Spinach	Pre-harvest contamination	*E. coli* O157:H7 (ATCC 35150, ATCC 43889, ATCC 43895, ATCC 51657, ATCC 700378, ATCC BAA-460), *S. enterica* Enteritidis (ATCC BAA-708, ATCC 4931), *S. enterica* Typhimurium (ATCC 29057, ATCC 29629, ATCC 29630), and *L. monocytogenes* (ATCC 19111, ATCC 19117, ATCC 19118, ATCC 13932, ATCC 15313, ATCC 35152).	(1) Inoculation of growth media (800 mL) in plastic containers having 10 weeks old plants with either 10^6 or 10^3 CFU/ml. (2) Leaf surface inoculation with 100 μl (10-15 spots) of inoculum at levels of 10^6 and 10^3 CFU/leaf	Grown in hydroponic system with NFT in a greenhouse maintained at 20°C. After inoculation, plants/leaves were held at 23°C and 50% relative humidity.	After 48 h for plants grown in nutrient solution and after 24 h for leaf inoculation	The probability of contamination is promoted through root and high inoculum levels.	Koseki t al., 2011

Lettuce var. Tamburo, Nelly and Cancan	Pre-harvest association	*S. enterica* serotypes Dublin, Typhimurium, Enteritidis, Newport, and Montevideo	Soil inoculation at 10^7 CFU/g.	Grown in manure-amended soil	After 6 weeks	Endophytic colonization of *S.* Dublin in lettuce Tamburo	Klerks et al., 2007
	Pre-harvest colonization		Lettuce seedling root inoculation (10^7 CFU/mL of each *S. enterica* serovar)	Grown in plant growth nutrient solution	After 7 days	Differential interaction between *S. enterica* serovars and lettuce cultivars.	
Lettuce, Spinach and Celery	Pre-harvest contamination	*S. enterica* Typhimurium (LT2, S1 and ATCC 14028)	Inoculation of irrigation water - 1 ml of 10^7-10^8 CFU/mL	Grown in soil	After 21 days	All *Salmonella* strains were good endophytic colonizers of the plant roots.	Kljujev et al., 2018
Lettuce	Pre-harvest contamination and attachment	*L. monocytogenes* (strains O8A06, O8A07 and O8A08)	Lettuce leaf inoculation (~10^5 CFU/mL) followed by 1 s, 10 s, 30 s, 60 s, 2 min and 5 min exposure for attachment before rinsing	Grown in soil and plant growth nutrient solution	N/A	Rapid attachment of L. to lettuce leaves regardless of soil or hydroponic grown lettuce extract	Kyere et al., 2019
Lettuce	Pre-harvest contamination and biofilm formation	*L. monocytogenes* (strains O8A06, O8A07 and O8A08)	Lettuce extract inoculation (~10^5 CFU/mL)	Grown in soil and plant growth nutrient solution	Day 2, 4, 6, 8 and 10	Improved survival and biofilm formation due to lettuce extract regardless of soil or hydroponic grown lettuce extract	Kyere et al., 2020
Lettuce	Seed contamination	*S. enterica* Typhimurium (ATCC 14028)	Seed inoculation – 50 seeds mixed in 1 mL of bacterial suspension (6 log CFU/mL) and dried for 30 min.	Grown in plant growth nutrient solution in hydroponic systems.	Week 0, 1, 2, 3, 4, 5, 6, and 7	*Salmonella* was present in lettuce plants and hydroponic systems throughout the growth period.	Li et al., 2021
	Seed and seedlings treatment		(1) Inoculated seeds mixed with 100 μmol/L rose Bengal solution (25 seeds in 2 mL) for 30 mins at 25°C followed by 30 mins illumination with fiber illuminator at 80% its maximum intensity. (2) Seedlings from inoculated seeds sprayed with 10 and 100 μmol/L rose Bengal solution (4 mL for 50 seedlings) followed by 30 mins illumination with fiber illuminator at 80% its maximum intensity.			Rose-bengal mediated photo dynamic inactivation resulted in a reduction of ~1 log CFU/plant.	

Spinach var. Waitiki and Space	Pre-harvest contamination	*E. coli* O157:H7 (86-24, curli-deficient 86-24Δ*csgA* mutant and curli-overexpressing 86-24*csgD*^{c} mutant)	(1) Nutrient solution inoculation at 7 log CFU/mL and 5 log CFU/mL (2) Soil inoculation at 5 log CFU/mL	Grown in organic garden soil in 4-inch pots and hydroponic trays filled with nutrient solution kept in controlled environmental chamber at 22°C and 70-72% humidity.	Day 0, 7, 14, 21 and 35	Internalization of *E. coli* O157:H7 is dependent on root damage.	Macarisin et al., 2013
Romaine lettuce	Post-harvest analysis- Assessment of microbiological profile of harvested plants	Aerobic plate count (APC), fecal coliforms, yeasts, molds, *Salmonella, E. coli* O157:H7, *L. monocytogenes,* and *S. aureus*	Hydroponically grown, organically grown and conventionally grown lettuce were bought from supermarket	NA	N/A	No significant difference in microbiological profile between lettuce grown by different farming practices.	Mohammad et al., 2022
Lettuce cultivar Red Sails	Pre-harvest analysis of nutrient media	*E. coli* and total coliforms	NA	Grown in aquaponics	Week 0, 2, 4 and 6	(1) No internalization of coliforms or *E. coli* was found. (2) Coliform bacteria detected reflected their normal presence in the environment.	Moriarty et al., 2018
	Pre-harvest treatment		UV treatment of water between 180 mJ/cm^2 at 26 L/min and 30 mJ/cm^2 at 170 L/min.			UV treatment reduced bacterial counts by 1.5 log in water and 3 log on lettuce samples.	
Lettuce Red sails	Pre-harvest contamination	*E. coli* O157:H7 (35150)	Inoculation of growth media at 5 log CFU/mL followed by root damage of plants by cutting either two or three times	Grown in custom built hydroponic system consisting of Pentair polystyrene transplant tray filled with vermiculite.	Day 73	*E. coli* internalization observed in all plant and root injury did not significantly affect bacterial concentration.	Moriarty et al., 2019
Lettuce	Pre-harvest contamination	*E. coli* O157:H7 (ATCC 43888), *L. monocytogenes* (403T12B) and *S. enterica* Senftenberg	Inoculation of growth media (180 g) at 5 log CFU/g (compost or anaerobic liquid)	Grown in peat amended with compost and anaerobic digestion liquid	Day 1, 7, 14, 21, 28, 35, 42 and 50	Internalization of *S.* Senftenberg and *E. coli* observed in lettuce plants but not *L. monocytogenes.*	Murphy et al., 2016
Lettuce	Assessment of microbiological pathogens present in water and leaf samples	*Bacillus, Mycobacterium, Aeromonas, Pseudomonas* and *Enterobacter.*	Lettuce leaves and irrigation water from two different hydroponic facilities were tested.	Grown in plant growth nutrient solution in hydroponic system.	N/A	Presence of various bacterial genera was confirmed, and water samples had higher level of bacterial contamination than leaves.	Rivera et al., 2015

Butter lettuce, Romaine lettuce, Basil, Cilantro and Spinach	Stomatal response to post-harvest environmental conditions	*E. coli* O157:H7 (86-24) and *S. enterica* Typhimurium (SL1344)	(1) For stomatal bioassay, leaves were floated in bacterial inoculum (10^8 CFU/mL) and kept at a relative humidity of either 65% or 95% in dark at 4°C or at 25°C with 12 hours of light. (2) For bacterial inoculations, plants were dipped in bacterial inoculum (10^8 CFU/mL) and incubated at either 65% or 95% relative humidity in dark at 4°C at 25°C with 12 hours of light.	Hydroponically grown produce was purchased from local store.	(1) For stomatal bioassay, 2 and 4 h. (2) For bacterial inoculations, day 1, 3 and 7	(1) *E. coli* induced strong stomatal immunity in all plants regardless of relative humidity. (2) Neither of the two bacteria multiplied inside the plants.	Roy and Melotto, 2019
Lettuce var. Green Star and Salad Bowl	Pre-harvest contamination with antimicrobials and bacteria	*S. enterica* Infantis	Inoculation of irrigation water (wastewater) with bacteria (5 or 7 log CFU/mL) and antimicrobials (oxytetracycline, sulfamethoxazole and lincomycin – 1ppm)	Grown in soil sub-irrigated with wastewater.	Day 24, 35 and 46	(1) Internalization of *Salmonella* was low. (2) Antimicrobial accumulation was dependent on lettuce cultivar.	Sallach et al., 2015
Radish	Pre-harvest contamination	*Citrobacter freundii* (PSS60), *Enterobacter* spp. (PSS11), *E. coli* (PSS2), *Klebsiella oxytoca* (PSS82), *Serratia grimesii* (PSS72), *Pseudomonas putida* (PSS21) *Stenotrophomonas maltophilia* PSS52 and *L. monocytogenes* (ATCC 19114)	Growth media inoculation (~10^6 CFU/mL)	Grown in mineral nutrient solution filled hydroponic systems placed in green house.	Sampled every seven days during entire spring season	(1) All bacteria survived in the nutrient solution throughout the crop cycle of radish. (2) *C. freundii*, *Enterobacter* spp., and *K. oxytoca* internalized into radish plants.	Settani et al., 2012
Butterhead lettuce	Pre-harvest contamination	*E. coli* O157:H7 (ATCC 35150), *L. monocytogenes* (ATCC 19115), *S. enterica* Typhimurium	Inoculation of nutrient solution used for watering seeding trays filled with soilless substrate (10^5 CFU/mL)	Grown in vermiculite and plant growth nutrient solution.	Day 3, 5, 7, 14, 21 and 28	Internalization of all four bacteria was observed in both lettuce seedling and pants grown hydroponically.	Standing et al., 2013

		(ATCC 14028), *S. aureus* (ATCC 12600)	Inoculation of nutrient solution used for watering plants placed in hydroponic system (10^5 CFU/mL)		Before inoculation and day 7, 14, 21 and 28 days		
Lettuce	Antibiotic resistance after internalization	*S. enterica* Infantis	Inoculation of nutrient solution with bacteria (10^{10} CFU/mL) and antibiotic (Oxytetracycline - 0.1g/10 mL)	Grown in 200 mL glass jars filled with 50 mL of plant growth nutrient solution	Day 21, 35, and 48	No difference in antibiotic resistance levels observed between plants grown in media with the antibiotic and without the antibiotic.	Thomas, 2014
Chicory varieties, Lettuce varieties, Dandelion, Rocket, varieties, Pak choi, Mizuna, Mustard, Swiss card, Red card, Spinach, Sorrel (30 baby leaves)	Pre-harvest contamination and attachment	Generic *E. coli* (ATCC 35218)	Inoculation of adaxial side of leaves (~10^7 CFU/mL) followed by incubation for 1.5 h at 25°C before rinsing	Grown in plant growth nutrient solution	N/A	Positive correlation of attachment with roughness and water content	Truschi et al., 2023
	Post-harvest treatment		UV Treatment for 5 min with a distance of 10 cm (UVC ≥ 90% with 108.4 µW/cm2 from 0.5 m)			Negative correlation between UV reduction and roughness	
Butterhead lettuce cultivar *Buttercrunch*	Post-harvest transfer	*S. enterica* Enteritidis strain ptvs177	Inoculation of roots by immersing in 1 mL of inoculum (6.5 log CFU/mL) in 400 mL of BPW for 10 mins and stored at 4 or 12°C for 18 days.	Grown in continuous flow NFT system and harvested after 46-56 days for experiment. After inoculation of roots, excess water was removed by squeezing and roots were wrapped forming a knot and transferred to container.	Day 0, 1, 2, 3, 6, 9, 12, 15 and 18.	High levels of transfer rate were observed from contaminated gloves and roots to leaves.	Waitt et al., 2013
			Inoculation of gloves with 1 mL of inoculum (6.6 log CFU/mL)	Living lettuce purchased from local supermarket. Inoculated gloves were used to transfer three lettuce heads consecutively to containers.	Immediately to lettuce heads after inoculation		

Lettuce and Basil	Pre-harvest contamination	Shiga toxin-producing *E. coli* (STEC), *L. monocytogenes* and *Salmonella* spp.	Recirculating water, roots and edible portions of lettuce and basil were harvested for microbiological analyses.	Grown in greenhouse-based aquaponic and hydroponic systems	(1) Lettuce - Day 30 after transplanting (2) Basil – Day 60 after transplanting	(1) STEC was present in the water of both the systems and on the surface of the roots of all plants. (2) No internalization was observed in any plants.	Wang et al., 2020
Basil cultivar Genovese, Cilantro, Lettuce var. Cherokee and Kale	Pre-harvest contamination	Shiga toxin-producing *E. coli* (STEC)	(1) Root damage treatment on seedlings before transplanting (2) Root damage treatment of mature plants at three weeks after transplanting.	Grown in aquaponic and hydroponic systems	(1) Hydroponic systems – no STEC found. (2) Aquaponic systems – STEC internalized in the roots of all plants. STEC present in leaves of plants that received root damage during seedling stage.	Seedling root damage causes internalization of STEC to the edible parts of the plant	Wang et al., 2021
Spinach	Pre-harvest contamination	Bioluminescent *E. coli* P36	(1) Seed (20g) inoculation (10^7 CFU/mL) for 20 min followed by drying at room temperature for 8 hours. (2) Nutrient solution inoculation ($10^3 – 10^2$ CFU/mL) (3) Soil inoculation (10^2 CFU/g)	Grown in soil microcosms (compost) placed on tray with irrigation water and maintained in greenhouse at 20-26°C and NFT hydroponic system in a greenhouse.	(1) Day 12, 14, 16, 20, 23, 25, 32 and 35 for plants grown in soil (2) Day 16 for plants grown in nutrient solution	Internalization of *E. coli* in plants grown in hydroponic solution but not in plants grown in soil.	Warriner et al., 2003
Lettuce	Pre-harvest contamination from growth media	*S. enterica* Enteritidis (NCTC 5188)	Growth media inoculation in low (~10^3 CFU/mL) and high (~10^6 CFU/mL) level	Grown in plant growth nutrient solution at pH levels of 5, 6, 7 and 8 in Deep Flow Technique (DFT) system	Day 14 and 21 for small-medium plants and Day 28 and 29 for old plants	No presence of *Salmonella* on leaves and possible controlling the survival and growth of *S.* Enteritidis by pH with effect of plant growth	Xylia et al., 2022
Radish, and Romaine lettuce	Pre-harvest contamination	Tulane virus (TuV)	Nutrient solution inoculation of radish with TuV at 10^6 PFU/mL Soil inoculation of 20 mL of 10^6 PFU/mL to the root zone of radish and Romaine lettuce	Grown in soil and plant growth nutrient solution	Day 0, 1, 3, 7 and 14	Plant type, growth matrix and inoculum level influence internalization and dissemination.	Yang et al, 2017

NFT – Nutrient Film Technique

Table 4. Qualtrics survey questions with response summary.

	Question	Response Type	Levels
Geographical Location			
1	Please provide the 5-digit zip code where indoor leafy greens are produced.	Numerical	Numerical
Agricultural Practices, Other Agricultural Products, and the Produce Safety Rule			
2	Do you grow indoor leafy greens?	Binary	“Yes” (n=12; 100%) “No” (n=0; 0%)
3	Are indoor leafy greens the only agricultural product that you grow?	Binary	“Yes” (n=5; 41.7%) “No” (n=7; 58.3%)
4	Please indicate type(s) of indoor produced crop. (Name of crops)	Open response	"Lettuce” (n=5; 27.8%) “Herbs” (n=3; 16.7%) “Microgreens” (n=2; 11.1%) “Kale” (n=2; 11.1%) “Chard” (n=1; 5.6%) “Arugula” (n=3; 16.7%) “Mizuna Mix” (n=1; 5.6%) “Spinach” (n=1; 5.6%)
5	What are the other types of agricultural products on your farm?	3-Level Factor	“Crops” (n=6; 100%) “Livestock” (n=0; 0.0%) “Both” (n=0; 0.0%)
6	Do you grow any produce covered by the Produce Safety Rule?	3-Level Factor	“Yes” (n=5; 45.5%) “No” (n=1; 9.0%) “I don’t know” (n=5; 45.5%)
7	What type of livestock do you raise? Select all that apply.	5-Level Factor with open response	“Cattle” (n=0; 0%) “Swine” (n=0; 0.0%) “Small ruminants (sheep/goats)” (n=0; 0.0%) “Fish” (n=0; 0.0%) “Poultry” (n=0; 0.0%) “Other” (n=0; 0.0%)
8	In which type of system do you produce half or more of your indoor leafy greens?	6-Level Factor	“Hybrid facilities (Indoor growing operation without vertical growing systems. Mid-tech glass/poly greenhouse with vertical growing systems. Greenhouse with outdoor operations.)” (n=0; 0.0%) “Container farm (Self-contained growing units that use vertical farming and artificial lighting. In contrast to custom-designed warehouses, container farms strive for standardization.)” (n=1; 9.0%) “Indoor vertical farm (Any fully enclosed and opaque room with a vertical hydroponic,

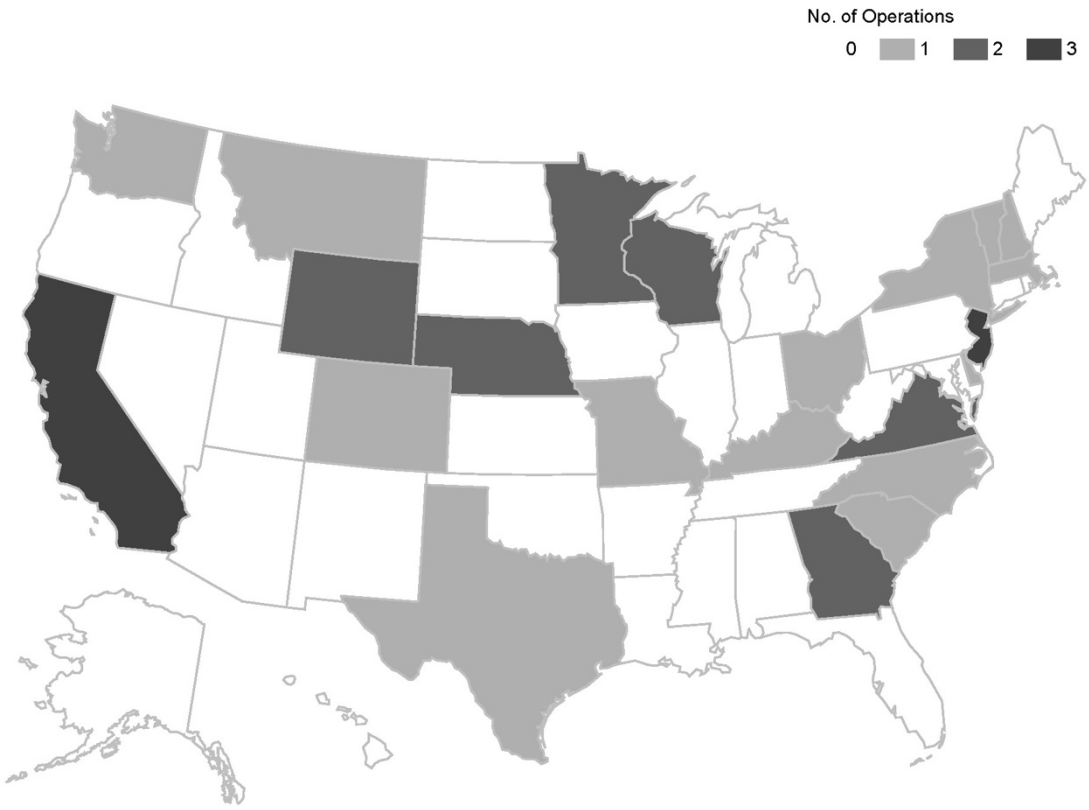
			aeroponic, and/or aquaponic system. Artificial lights are used.)" (n=5; 45.5%) "Low-tech high tunnel (Semi-circular, tunnel-shaped structure made of steel and polythene. Little to no automation.)" (n=1; 9.0%) "Mid-tech glass/ploy greenhouse (Transparent, enclosed structure made of glass or polycarbonate. Has more automation than high tunnel production but not to the full extent possible.)" (n=2; 18.2%) "High tech glass greenhouse (Transparent enclosed structure made of glass. Highly dependent on automation and technology systems.)" (n=2; 18.2%)
9	How often do you harvest leaf greens? (e.g., number of days per week)	Numerical	Mean (3.6±2.1 days/week) Median (3 days/week) Minimum (1 day/week) Maximum (7 days/week)
10	How would you classify your indoor farm production system?	5-Level Factor	"Use of organic practices (not certified)" (n=5; 45.5%) "Natural" (n=2; 18.2%) "I am not sure" (n=0, 0.0%) "Conventional" (n=3; 27.3%) "Certified Organic" (n=1; 9.0%)
Business Dynamics			
11	Please indicate your role in your company.	Open Response	"Sales and Service" (n=1; 8.3%) "Greenhouse Manager" (n=1; 8.3%) "Owner" (n=4; 33.3) "VP of Supply Chain" (n=1; 8.3%) "Co-Founder" (n=1; 8.3%) "Grower/Food Safety Officer (n=1; 8.3%) "R&D and Head Grower" (n=1; 8.3%) "Director of Food Safety and Quality Assurance" (n=1; 8.3%) "VP of Food Safety and Compliance" (n=1; 8.3%)
12	Each year, approximately how much (gross) revenue do you bring in from growing indoor leafy greens?	5-Level Factor	"Less than $25,000" (n=2; 18.2%) "$25,000 - $99,999" (n=3; 27.3%) "$100,000 - $249,999" (n=2; 18.2%) "$250,000 - $499,999" (n=1; 9.0%) "Greater than $500,000" (n=3; 27.3%)
13	Does your farm have any of the following certifications? (Check all that apply)	4-Level Factor with open response	"Certified Organic" (n=1; 5.9%) "Good Agricultural Practices (GAP) Audit" (n=5; 29.4%) "A third-party sustainability certification" (n=2; 11.8%) "None of these" (n=4; 23.5%)

			"Other (list) - 'GMP' (n=2; 11.8%), 'Produce Safety' (n=1; 5.9%), GFSI audit (PrimusGFS) for GAP' (n=1; 5.9%), 'Non-GMO Project Verification Certification' (n=1; 5.9%)"
14	How do you measure your farm's indoor leafy green production?	5-Level Factor with open response	"ounces" (n=0; 0%) "pounds" (n=4; 33.3%) "kilograms" (n=2; 16.7%) "heads" (n=5; 41.7%) "pallets" (n=1; 8.3%) "Other (list)" (n=0; 0%)
15	To whom do you sell your indoor leafy greens?	8-Level Factor with open response	"Farmer's Markets" (n=3; 8.6%) "U-Pick Sales" (n=0; 0.0%) "Food Cooperative (Co-op)" (n=3; 8.6%) "Community Supported Agriculture (CSA)" (n=2; 5.7%) "Institutional Foodservice Establishments (hospitals, schools, childcare, long-term care)" (n=6; 17.1%) "Commercial Restaurants" (n=7; 20.0%) "Grocery Stores" (n=7; 20.0%) "Wholesaler/Distributers" (n=6; 17.1%) "Other (list) - "'Food Bank' (n=1; 2.9%)"
16	What is the indoor leafy green production area built for?	2-Factor Response	"Built for indoor farming" (n=7; 63.6%) "Converted for indoor farming" (n=4; 36.4%)
17	How many personnel do you have working in the production area?	Numerical	Mean (38.5±66.8 persons) Median (8 persons) Minimum (2 persons) Maximum (220 persons)
18	What is the size of production area? (Acreage/Building/Space)	Open Response	Mean (22193±41241.1 sq ft) Median (3000 sq ft) Minimum (360 sq ft) Maximum (124000 sq ft)

Table 5. Semi-structured interview discussion guide questions.

Question No.	Question Content
1	Can you identify the top three biggest safety challenges during hydroponic of leafy greens?
2	Risk management practices for worker health and hygiene.
3	Risk management practices for agricultural water.
4	Risk management practices for soilless substrates.
5	Risk management practices for domesticated and wild animals.
6	Risk management practices for harvesting and packing activities.
7	Risk management practices for storage and transportation activities.
8	Risk management practices for equipment, tools, and building.
9	Risk management practices for traceability.
10	What would you do more to ensure the safety of crops in your production environment if you had unlimited resources?
11	Is there anything that you want to add?

Figure 1. Geographical locations of indoor, soilless leafy green operations (n = 25) who participated in semi-structured interviews. Two operations have locations in multiple states.



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