THESIS

EFFICACY AND IMPACT OF SANITIZERS IN CONTROLLING PATHOGENIC BACTERIA ON IRRIGATION WATER

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ABSTRACT

EFFICACY AND IMPACT OF SANITIZERS IN CONTROLLING PATHOGENIC BACTERIA ON IRRIGATION WATER

Irrigation water quality is crucial for fresh produce. However, the absence of practical microbial water standards and EPA-registered sanitizers poses challenges for growers, impacting food security and increasing the risk of foodborne outbreaks. This study compared the effectiveness of commercial sanitizers (sodium hypochlorite - Chlo, peroxyacetic acid - PAA) and novel chemistries (sodium acid sulfate - SAS, potassium acid sulfate - KAS) at different concentrations for achieving a 3-log reduction of *E. coli* O157:H7 (EHEC), *Salmonella* (SM), or *Listeria monocytogenes* (LM). Additionally, the study examined their corrosive effects on aluminum and stainless steel, as well as their impact on plant and soil chemical and physical properties. Sanitizer evaluations were conducted under varying turbidities (0, 50, 100, 150 NTU), contact times (CT: 3 or 5 minutes), and bacterial concentrations of EHEC, SM, or LM at 6 and 3 log CFU/mL per EPA 2020 guidelines. Treatments that achieved a 3-log reduction of the selected organisms within a 5-minute contact time, with no viable cells after enrichment, were considered the most effective and exceeded EPA requirements. These treatments were then evaluated for their corrosion capacity on stainless steel (SS304, SS316) and aluminum (AL3004) coupons, as well as their effects on soil and plant health (romaine lettuce) under greenhouse conditions. Chlo at 60 ppm, SAS at 0.60%, and PAA at 30 ppm achieved a 3-log reduction, with no viable cells after enrichment, for all strains after a 5-minute contact time ($P<0.05$). The corrosion capacity for the most effective treatments was tested at 14-day intervals at 23°C and 73°C for stainless steel (SS304, SS316) and aluminum (AL3004) coupons submerged in each

sanitizer solution. There was no significant difference in corrosion between SS304 and SS316 ($P<0.05$). AL3004 showed greater corrosion than SS, with the highest rate observed at 0.6% SAS (4.52E-02 cm/year). Greenhouse-grown romaine lettuce was spray-inoculated with a bacterial cocktail of LM/EHEC at 5 log CFU/mL. Bacterial recovery was performed at 0-, 4-, 8-, and 12-days post-inoculation using selective-differential media. Soil samples were collected from treated and non-treated samples to assess soil chemical and physical properties. Bacterial inactivation had a negative correlation with water turbidity and a positive correlation with contact time and sanitizer concentration ($P<0.05$). In the greenhouse trial PAA, Chlorine and SAS exhibited a higher bacterial reduction compared to a water-control, with marginal differences in reduction between sanitizers ($P<0.05$). The highest reduction was achieved by PAA (0.98 log CFU/gram-lettuce) reduction compared to the water control. SAS exhibited phytotoxicity in the lettuce leaves in the second greenhouse trial. All sanitizers caused marginal changes in the overall properties of soil physical and chemical properties, with sodium and electrical conductivity being the main factors affected by the constant application of each sanitizer.

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CHAPTER 1 - LITERATURE REVIEW

Population growth, food demand, and food security are reforming the agriculture system. Ensuring food security is crucial, and this requires having sufficient food available for the growing population (Miladinov 2023). By 2050, it is anticipated that agricultural production needs to increase by an estimated 60% to meet global food requirements (Miladinov 2023). Agricultural productivity is limited by the availability of resources including water, energy, and farmland. In the last two decades, there has been a significant rise in cases of foodborne illnesses, coinciding with increasing demand for food (Godfray et al. 2010) and the expansion of growing areas to locations in which the risk of contamination may increase due to previous land use history and adjacent land use. Produce fields near confined animal feed lot operations (CAFOs), animal production zones, or wildlife corridors increase the risk of produce contamination(Strawn et al. 2013). This highlights the need for immediate actions and changes in the agricultural system to guarantee the safe production of produce which may result in a reduction in the number of outbreaks associated with raw agricultural commodities (Hussain and Gooneratne 2017).

Multiple fresh produce commodities including leafy greens, carrots, cantaloupe, onion, mangos, and papayas have been implicated on several food borne outbreaks (J. L. Banach and Van Der Fels-Klerx 2020a) . Many of these outbreaks trace back to specific practices within the supply chain, often linked to contamination from worker health, animal intrusion, equipment, and irrigation water. Notably, there are no kill steps in growing, harvesting, and packing of fresh produce to prevent contamination. Hence, effective risk assessment, and management must prioritize prevention of contamination and the use of treatments that significantly reduce the risk of human pathogen contamination (EFSA 2013).

Throughout the cropping cycle of high-risk crops (including romaine lettuce, cantaloupe, spinach, and apples per FDA assessments (Macieira, Barbosa, and Teixeira 2021; Herman, Hall, and Gould 2015),

potential vectors and mechanisms of microbiological contamination may arise from contaminated water sources used for irrigation, as well as during harvest or packing activities. The critical concern lies in the ability of pathogenic bacteria to adhere, survive, grow, and potentially become internalized within plant tissues, exacerbated by inadequate sanitation practices (Gurtler and Gibson 2022a). Water sources contaminated with pathogenic bacteria such as *Salmonella, Escherichia coli* O157:H7, Shiga toxin-producing *E. coli (*STEC), and *Listeria monocytogenes* have been identified as a major contributor to the contamination of fresh produce (Liu, Whitehouse, and Li 2018; Truchado et al. 2018; Rakhmanin et al. 2019; Krishnan et al. 2021; Rodrigues, da Silva, and Dunn 2019) .

Water, with its inherent capacity to spread these organisms across various operations, poses a considerable risk for fruit and vegetable cross contamination along the cropping cycle and during postharvest activities (Esmael et al. 2023). Although epidemiologists have identified some primary risk factors during crop production, numerous outbreaks have been directly or indirectly linked to contaminated irrigation water (Uyttendaele et al. 2015b). Ensuring the safety of water sources in early production stages has gained considerable attention due to the ease of introducing pathogenic bacteria into the supply chain. This literature review provides an overview of concepts related to fresh produce contamination with associated with irrigation water, regulatory requirements, microbiological contaminants, and water treatments, related to irrigation water and how these variables impact pathogen survival and persistence and soil and plant health parameters within romaine lettuce production systems.

1. Irrigation Water

Irrigation involves the regulated utilization of multiple water sources at specifics times to enhance or maintain crop yield. The irrigation system involves all the growing season and extends to include water used in activities such as field preparation, pre-irrigation, weed control, harvesting, and the leaching of salts from the root zone(Dieter 2018). Worldwide, the most significant utilization of freshwater is attributed to irrigation of agricultural commodities (Sauvé and Desrosiers 2014), where water plays a crucial role in facilitating the delivery of pesticides, fertilizers, adjuvants, and supporting postharvest cooling and washing

operations. Irrigation water could be a potential point of produce contamination with human pathogens if the quality of water is not known and if contaminated water is not treated before it is used.

The United States utilizes approximately 118 million gallons of water per day for agricultural purposes. More than 50% of this irrigation water is sourced from surface water, including streams, rivers, lakes, ponds, ground water and wastewater (mainly on non-edible crops) (Hrozencik 2023). The availability of surface water is closely tied to the annual rainfall rate, leading to more frequent use of substandard sources of water such as runoff, irrigation ditches and other effluents that compromise the water quality used in irrigation systems including treated wastewater(Dieter 2018). Low-quality water has the potential to be an indirect or direct source of contamination of food crops and could potentially increase soil contamination (Singh et al. 2018). Access to high-quality water is a fundamental prerequisite for ensuring the safety of fresh produce production. However, the increased usage of irrigation water has impacted water quality, leading to the emergence of contaminants in irrigation water (Sagasta, Marjani Zadeh, and Turral 2017).

Ensuring the safety of food and protecting human health requires effective management of water quality parameters used in primary production. Evaluating the impact of these contaminants in water sources is essential to mitigate the associated risks. Malakar, Snow, and Ray detailed the main sources of irrigation water and the most common contaminants present in those sources impacting food, soil, and water quality (Figure 1).

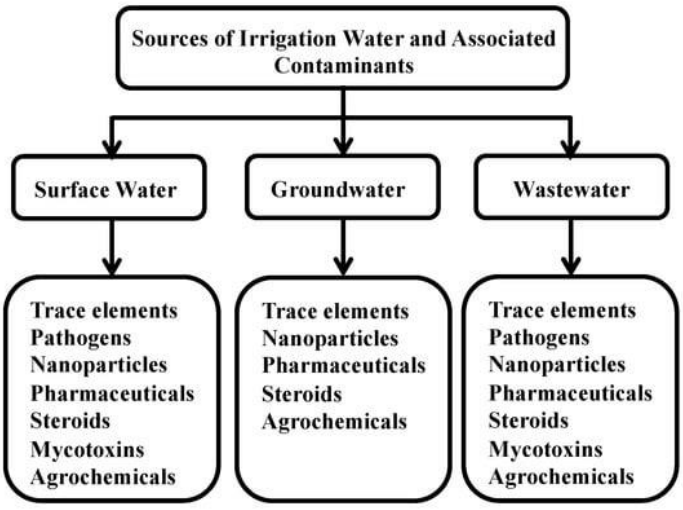

Figure 1. Main sources of irrigation water and the associated contaminants are present in each water source. Graphic obtained from Malakar, Snow, and Ray (2019).

The contaminants shown in Figure 1 are widespread in water sources, impacting irrigation systems, soil, and plant health. However, environmental factors, water sources, runoff, animal intrusion, and farm waste can modify and increase these contaminants further compromising water quality which will significantly impact crop safety. Water use in agricultural activities could be a carrier of physical, chemical, or biological hazards into production fields. These issues will compromise food quality and safety, highlighting the importance of knowing, protecting, and generating risk reduction practices that will reduce these contaminants from different agricultural production systems (National Research Council et al. 2015).

1.1 Microbiological Contaminants

Microbial contamination in water used for agriculture is a significant contributor to contamination in fresh produce. Irrigation water is a known vector for foodborne pathogen contaminations and its quality serves as an indicator of food safety. Bacterial pathogens followed by viral and parasitic pathogens represent the most common agents associated with contamination of fresh produce when contaminated water is used in farming activities in the United States and European Union (Callejón et al. 2015). However, there are pathogenic bacteria that are considered a high-priority food safety concern due to their physiological characteristics and natural presence in the environment, including *Listeria monocytogenes,*

Salmonella, E. coli O157:H7 and other STEC. These bacterial pathogens have been associated with 83 out of 85 multistate fresh produce outbreaks between 2010 and 2017 in the United States (Carstens, Salazar, and Darkoh 2019).

The risk of using contaminated water varies based on several factors including the geographical location of the irrigation source, weather conditions, land use, and surrounding activities near the water source. These factors are also linked to the prevalence of pathogenic bacteria and the incidence of foodborne diseases (Rodrigues, da Silva, and Dunn 2019). Additionally, the method of irrigation employed in agricultural production can either increase or decrease the risk of microbial contamination. For example, furrow irrigation presents a higher risk of contamination compared to the surface drip method for crops like cantaloupes, iceberg lettuce, and bell peppers (Stine et al. 2005). The principal sources of irrigation water include surface water—such as lakes, rivers, ponds, springs, and creeks—groundwater (typically from deep wells), municipal water, and, to a lesser extent, treated wastewater (Gil et al. 2015; DeSimone, McMahon, and Rosed 2015; Mullen, Yu, and Hoogenboom 2009). Surface water, which is commonly used for irrigation, poses a primary concern regarding water source management (Ewing 2011)due to its exposure to multiple contamination sources, thereby increasing the risk of contamination with human pathogens (Rodrigues, da Silva, and Dunn 2019).For instance, *Salmonella* has been detected in surface waters such as rivers, lakes, and ponds, and this pathogen can survive in aquatic environments through various mechanisms including entering a viable but nonculturable state or residing within free-living protozoa (Liu, Whitehouse, and Li 2018). On the other hand, *L. monocytogenes* has been found in water, soil, vegetation, and farm animal feces, with its frequency depending on several factors such as the season, surrounding land use, presence of animals, and physicochemical water parameters (Gartley et al. 2022a).

The prevalence and persistence of pathogenic bacteria are influenced by weather conditions, with climate change impacting bacterial survival in water sources. Microbial pathogens can survive for extended periods in water, with warmer temperatures facilitating the adaptation of aquatic populations. Increased rainfall can lead to more runoff of pathogens from adjacent lands (Bell et al. 2021). Several studies have

shown that microbial pathogens like *Salmonella, E. coli* O157:H7, and Enteroviruses are stable in groundwater (Schijven and Hassanizadeh 2000; Pang et al. 2004; John and Rose 2005; Nevecherya et al. 2005; Grisey et al. 2010; Bitton et al. 1983). Pathogen contamination in groundwater can result from agricultural practices, seepage, percolation, manure application, and the presence of straw and coarse organic matter (Y. Wang, Bradford, and Šimůnek 2014; Y. A. Pachepsky and Shelton 2011; Bradford et al. 2013).

1.1.1 Listeria monocytogenes

Listeria monocytogenes is a Gram-positive bacterium, categorized as a facultative anaerobic bacillus, and it does not produce spores or capsules. Growing conditions at temperatures of -2 to 45℃, with an optimum temperature between 30 and 37℃ facilitate growth. Capable of attaching to surfaces through biofilm formation and attachment genes, it can cause invasive infections(Cossart 2003). This pathogenic bacterium has been linked to numerous foodborne disease outbreaks over the past few decades, leading to listeriosis among human beings. The spectrum of symptoms caused by *L. monocytogenes* ranges from mild flu-like symptoms to more severe conditions such as meningitis, meningoencephalitis, septicemia, conjunctivitis, and pneumonia. In the United States, the incidence of disease caused by this organism was determined as 0.41 cases per 100,000 individuals. Notably, listeriosis carries a case mortality rate of 24%, and it poses a particular risk to pregnant women and immunocompromised individuals(FDA 2022).

Listeria monocytogenes can be found in moist and dry environments, soil, water, decaying vegetation, and animals (Osek, Lachtara, and Wieczorek 2022). It has been isolated from various sources such as water systems, vegetation, soil, farms, food contact surfaces, and the feces of animals (Vivant et al. 2015). This bacterium exhibits remarkable survival capabilities, even thriving under refrigeration conditions and tolerating broad pH ranges, making it difficult to control due to its resilience to harsh environments. The ability to survive varies among the 13 different serotypes and their respective environmental stressors; with serotypes 1/2a, 1/2b and 4b mostly associated with human disease (Doumith

et al. 2004). Previous research has indicated that *L. monocytogenes* shows increased survival in acidic bile under anaerobic conditions compared to aerobic environments(White et al. 2015).

Listeria monocytogenes can survive in water sources for extended periods. Botzler, Cowan, and Wetzler (1974) noted that this pathogen could persist for over eight weeks in pond water without showing signs of multiplication. Surface water, particularly in temperate climates, hosts a diverse population of *Listeria* and is considered a potential reservoir for *L. monocytogenes*, especially in rural agricultural watersheds (Stea et al. 2015). However, other studies indicate that levels of *L. monocytogenes* are higher in river waters at sites with lower water temperatures, and these levels decrease in winter and spring compared to the summer season (Sharma et al. 2020). Various studies have suggested that *L. monocytogenes* adopts multiple survival strategies in water environments, such as increasing heat resistance, adapting to acidic conditions, and coping with nutrient limitations. These adaptations also include altering gene expression to utilize available nutrients and withstand environmental stresses (Cacace et al. 2010; Tang et al. 2015; Gahan and Hill 2014; Bremer et al. 1998). The survival traits of *L. monocytogenes* pose a significant food safety concern, highlighting the necessity for proactive and preventive measures during the growing, harvesting, and processing of fresh produce to mitigate its presence and reduce the risk of contamination.

1.1.2 Salmonella

Salmonella, as a group, consists of facultatively anaerobic, non-spore-forming, Gram-negative bacilli, belonging to the family *Enterobacteriaceae* (Percival et al. 2014). *Salmonella* is found in the environment and is a well-known pathogen associated with poultry and meat products. They are known for their survival and persistence outside of a host animal, infecting a wide range of hosts, including domestic and wild animals, poultry, swine, cattle, birds, rodents, tortoises, turtles, cats, and even humans, serving as reservoirs (Liu, Whitehouse, and Li 2018; Lopez-Velasco et al. 2012). However, the occurrence of chronic carriers is rare in humans. A human can get infected by *Salmonella* through ingesting contaminated food, with the primary mode of transmission being the consumption of food contaminated with fecal matter

(Gianella 1996). This pathogenic bacterium can cause three clinical forms of infection in humans: gastroenteritis, enteric fever, and septicemia.

Salmonella has been found to survive for extended periods in various aquatic environments, including rivers, streams, and wells (Wright 1989). It is frequently detected in different water sources, with prevalence rates ranging from 2.5% to 79.2%, often influenced by seasonal variations and specific environmental conditions (Haley, Cole, and Lipp 2009). Research indicates that *Salmonella* utilizes diverse survival mechanisms in water, such as physiological and genetic responses that support energy production, cell structure biogenesis, ion transport, stress resistance, motility, and chemotaxis. Additional adaptations include filament formation, changes in gene expression related to virulence and stress response, and the ability to maintain a viable but non-culturable state (Spector and Kenyon 2012; Hills et al. 1997; Kingsley et al. 2018; Mandal and Kwon 2017). The contamination of irrigation water with *Salmonella* represents a significant public health concern. This is due to its prevalence, antibiotic resistance, and capacity to persist on crops and in the environment. Factors such as temperature, rainfall, and proximity to animal production facilities can influence contamination levels, underscoring the need for vigilant monitoring and control measures in agricultural practices (Y. Pachepsky et al. 2011; Malakar, Snow, and Ray 2019; Gurtler and Gibson 2022a; Alegbeleye and Sant'Ana 2021).

1.1.3 E. coli O157:H7

E. coli belongs to the family *Enterobacteriaceae*. It is a non-spore forming gram-negative bacterium. This bacterium can grow under aerobic and anaerobic conditions and some of its members do not produce enterotoxins (Jnani and Ray 2024). This bacterium is commonly present in the gastrointestinal tract of humans and animals. This bacterium can proliferate in a range of temperatures as low as 7 ℃ and as high as 46℃, with an optimum growing temperature of 37 ℃. *E. coli* can be transmitted via the oral-fecal route and there are seven principal pathotypes associated with this bacterium namely, Enterohaemorrhagic *E. coli* (EHEC), Shiga Toxin- producing *E. coli (STEC),* Enterotoxigenic *E. coli* (ETEC), Enteropathogenic *E. coli* (EPEC), Entero-invasive *E. coli* (EIEC), Enteroaggregative *E. coli*

(EAEC) and Diffusely Adherent *E. coli* (DAEC) (Osman et al. 2013). The severity of the symptoms varies based on the host and bacterial strain. For example, ETEC strains are host specific, while EPEC is commonly found in children in developing countries producing diarrhea, fever, and gastrointestinal problems (Kolenda, Burdukiewicz, and Schierack 2015). Of significant concern to human health are diseases caused by STEC and EHEC organisms, which produce toxins and possess virulent factors. These can lead to conditions ranging from diarrhea to life-threatening illnesses such as hemorrhagic colitis and hemolytic uremic syndrome (Gehua Wang, Clark, and Rodgers 2002). The persistence of *E. coli* O157:H7 in surface waters increases the risk of its spread, potentially fueling cycles of livestock re-infection and posing a threat to human health.

While previous research has explored its survival in various surface water environments, there remains a lack of data on how physico-chemical factors influence its longevity (Avery et al. 2008). *E. coli* O157:H7 employs a variety of survival strategies in water, including entering a viable but non-culturable state, attaching to container walls, optimizing membrane structure, growing at low temperatures, and resisting environmental stresses such as aeration, sterilization, and certain antimicrobial agents (Warburton et al. 1998; Y. Liu et al. 2009; Gehua Wang, Clark, and Rodgers 2002; Avery et al. 2008).

Possible routes of contamination include pre- and post-harvest activities, encompassing environmental factors (like septic tank leakage, runoff, and irrigation water), agricultural practices (such as cultivation, harvest methods, and crop management), and handling processes (including transportation and storage)(Bolten et al. 2023). Cross-contamination is connected to poor agricultural practices and lack of monitoring tools during field production (Carstens, Salazar, and Darkoh 2019). Sources of contamination include manure from livestock and wildlife contaminating water sources or soil when raw or poorly treated manure and compost is used to grow fresh fruits and vegetables. One of the principal routes of contamination identified during preharvest activities is irrigation water (Benjamin et al. 2013a).

1.2 Outbreaks Linked to Fresh Produce

In 2006, *E. coli* O157:H7, was responsible for a multi-state outbreak associated with bagged baby spinach grown in San Benito County, California - a region neighboring the Salinas Valley - identified through various samples taken on the property. These samples included those from cattle, feral swine, surface water, sediment, and soil (Grant et al. 2008). Suggesting that surrounding activities from the water source represent a high risk of contamination due to the possible runoff of pathogens. High precipitation levels may contribute to the spread of pathogens from livestock to the nearby water source, creating a cross-contamination process. From 2018 to 2019, Shiga toxin-producing *E. coli* was responsible for two multistate lettuce outbreaks in the Salinas Valley, California, both linked to a water source used for irrigation (Waltenburg et al. 2022).

Water contaminated with pathogenic bacteria like *E. coli* O157:H7 can lead to serious food safety issues when used for crop irrigation, as it is reported in the previous outbreaks mentioned. These bacteria can adhere to plant roots when introduced into the soil through contaminated irrigation water (Avery et al. 2008). Once present in the soil, the bacteria can persist and potentially contaminate subsequent crops grown in the same soil, even if the irrigation water source is cleaned or changed. The pathogenic bacteria can then transfer from the contaminated soil to the edible portions of crops, either through direct contact or the plant's vascular system (Macieira, Barbosa, and Teixeira 2021). To mitigate foodborne illness outbreaks, it is crucial to have a better understanding of the interactions between water contaminants, soil, and crop plants. This can inform strategies for monitoring and treating irrigation water, implementing soil management practices, developing crop varieties minimizing bacterial uptake, and enhancing food safety protocols for detecting and eliminating contaminated products before consumption (Iwu and Okoh 2019).

On the other hand, *L. monocytogenes* is commonly found in ready-to-eat (RTE) foods, such as salad mix, dairy and delicatessen meats (Zhu, Gooneratne, and Hussain 2017). Making this pathogen responsible for 19% of the total deaths due to the consumption of contaminated food in the United States (Scallan et al. 2011). Multiple outbreaks caused by *L. monocytogenes have* been linked to fresh vegetables. For example,

in Texas in 2010, there was an outbreak associated with chopped celery, causing 10 deaths from listeriosis(Gaul et al. 2013). In 2011 the Jensen Farms outbreak in Colorado, resulted in thirty people sick by consuming contaminated cantaloupe with *Listeria monocytogenes* (McCollum et al. 2013). In the same year, pre-packaged caramel apples caused a multistate listeriosis outbreak, resulting in thirty-five cases reported with 34 hospitalization and seven deaths(ANGELO et al. 2017). In 2021, Dole and Fresh Express brand packaged salads were investigated for two separate *L. monocytogenes* outbreaks. The Dole outbreak was linked back to contamination of harvesting equipment used for iceberg lettuce and other produce. However, it has been speculated that it could be multiple sources of contamination including produce from the field, not properly treated wash water, movement of workers, or pests (Beuchat 1997; Gartley et al. 2022)

Salmonella contamination in fresh produce is often linked to environmental factors prevalent in agricultural settings, such as tainted water sources and the presence of both domestic and wild animals (Hanning, Nutt, and Ricke 2009). Over the past decades, there have been numerous outbreaks associated with *Salmonella*, with a significant portion traced back to contaminated vegetables (Kowalska 2023). Notably, in 2020, *Salmonella* Newport triggered one of the largest multistate outbreaks in the United States in recent history, primarily originating from dry bulb onions. Field investigations highlighted various factors contributing to contamination, including polluted irrigation water, animal intrusion, and runoff from nearby livestock operations with inadequate sanitation practices at packinghouses (McCormic et al. 2022). *Salmonella* has been detected in diverse water sources, including surface and groundwater, consistently emerging as a leading cause of contamination events (Spector and Kenyon 2012; Hills et al. 1997; Liu, Whitehouse, and Li 2018). Multiple outbreaks linked to fresh produce have been traced back to irrigation water, as well as outbreaks involving romaine lettuce and cantaloupes in recent years, including a December 2020 incident that resulted in six fatalities and 407 illnesses (Saw et al. 2020). Numerous scientific articles point out the issues associated with irrigation water, treatments for inactivation of pathogens in irrigation water, and the lack of viable and reliable microbial water standards that align with food safety needs for

fresh produce (Gurtler and Gibson 2022b). Irrigation water is a common and essential requirement for crop production; however, its inherent risks will vary based on water source, method, and timing of irrigation, crop type and pathogen load (Benjamin et al. 2013b). However, this pathogen has not been only associated with fresh produce, has been responsible of an outbreak of gastroenteritis in 2014, causing a contamination of a town with 50,000 inhabitants in Croatia where the source of contamination was groundwater contaminated with *Salmonella* (Kovačić, Huljev, and Sušić 2017),

1.3 Risk Associated with the Irrigation System

Sources of irrigation water can be ranked by decreasing risk of microbiological hazard; these are raw or inadequately treated wastewater, surface water, groundwater from shallow wells, groundwater from deep wells, rain, and potable water (Y. Pachepsky et al. 2011). Surface water is the main source for agricultural irrigation systems. In the United States, the use of surface water by farmers has increased by 6.3% while the use of groundwater has decreased by 9.2% (NASS 2018). Surface water can be contaminated by environmental factors (wild animals, run off, poor agricultural practices, and livestock) representing a risk for food production. The water source used in crop production has great potential to compromise the microbial integrity of the irrigation system. Table 1 lists the different irrigation methods used in agriculture and compares them based on the risk associated with the water source (Uyttendaele et al. 2015a)

Table 1. Level of risk associated with different source water, irrigation methods and crop types (Uyttendaele et al. 2015a)

Level of risk	Source water	Irrigation Method	Crop type
Low	• Municipal potable water • Groundwater collected from deep wells/bores. • Rainwater (collected in closed systems) • Groundwater from shallow wells/bores	• Subsurface • Drip • Furrow	• Root crops • Low foliar • Off ground

High	• Adequately treated wastewater-rainwater (collected in closed systems) • Surface waters in proximity to animals/human habitation • Raw/poorly treated wastewater	• Spray • Surface irrigation with watering cans	• Fruit trees • Low foliar

Note: The table above is a modified version obtained from Uyttendaele et al. 2015. This resource explained the risk level of contamination based on the water source, irrigation method and crop type.

The irrigation system is responsible for the transportation of water from the source to the field. During transport, the irrigation system creates an ecological environment where different sources and routes of contaminations will impact the overall quality of the water (Jjemba et al. 2010). Among these sources, pipe corrosion, leaks, backflow, and the introduction of chemicals including fertilizers, pesticides and adjuvants bring the pathogen to the irrigation system. Water is susceptible to microbiological depreciation, depending on the transportation mode. Irrigation water transport via irrigation ditches or canals provides inherit microbial risks associated with bottom sediments, bank soils and algae, while water transport using pipes interact with biofilms generated by bacteria, leaks or poor design that prevents adequate flow, or physicochemical contaminants generated corrosion in the pipeline (Jjemba et al. 2010).

The storage of water for irrigation purposes can also influence pathogen contamination and transmission depending on water source and management of the reservoirs. Water storage in ponds can be rapidly contaminated by avian species or other wildlife (Higgins et al. 2009; McLain and Williams 2008; Field and Samadpour 2007). Although irrigation systems like check dams, impoundments, inter-basin transfer schemes, abstractions schemes and reservoirs serve as place for survival and proliferation of pathogenic bacteria(Abbasi 2001); the type of application used in the irrigation system has a significant impact on the risk of contamination. For instance, sprinkler irrigation poses a higher risk of contamination compared to furrow and subsurface drip irrigation systems. Depending on the crop being grown, furrow and drip irrigation systems have minimal contact with the edible part of the crop, reducing the risk of contamination (Jung, Jang, and Matthews 2014).

Sprinkler irrigation systems facilitate water contact with the surface area of the crop, creating a potential risk of microbial attachment. The application method influences the spread, distribution, and potential for internalization of some pathogens in fresh produce (Fonseca et al. 2011). Pathogen internalization although rarely observed on field grown fruits and vegetables, could increase when the organism is introduced by a sprinkler system as opposed to when the water is directly applied to the soil, such as with furrow or subsurface drip irrigation. Pathogen internalization has been documented under a few experimental conditions with limited observations related to commercial growing practices. It is influenced by environmental and external factors such as geographical location, rainfall events, irrigation regime (considering the time of day), season, and harvest time (Erickson 2012). Seasonality and rainfall events appear to have an impact on the transfer and pathogen concentration in water sources. Overall, irrigation water coming from surface water sources is likely to contain pathogenic bacteria at any time during the growing season (Alegbeleye and Sant'Ana 2021). This imminent and potential risk of contamination highlights the importance of implementing standards, treatments, and guidelines to reduce microbiological hazards impacting quality of irrigation water.

The presence of foodborne pathogens in irrigation water is a potential risk if the irrigated crop is consumed by humans. However, the true risk of disease caused by pathogens in water will depend on several variables such as pathogen load, persistence in the environment, ability to multiply outside of the host, infection dose and host response (Feachem David J Bradley Hemda Garelick and Duncan Mara 1983). The viability of pathogens in water is impacted by time within the body of water (Dechesne et al. 2006) and its survival varies depending on the type of system (water, sewage, and soil) and temperatures ranging from 20 to 30°C (Feachem David J Bradley Hemda Garelick and Duncan Mara 1983). Results from multiple investigations indicate that bacteria such as fecal coliforms, *Salmonella* spp., and *Shigella* spp. typically survive for fewer than 30 days in water and sewage, while *Entomoeba histolytica* cysts and enteroviruses survive for fewer than 15 to 50 days, respectively. In contrast, *Ascaris lumbricoides* eggs can survive for several months.

Moreover, bacteria and viruses are likely to persist longer in groundwater compared to surface water due to cooler temperatures, protection from sunlight and UV light, and lower microbiological and biological activity. In soil, bacteria like fecal coliforms, *Salmonella* spp., and *Shigella* spp. usually survive for fewer than 20 days, while *Entomoeba histolytica* cysts and enteroviruses survive for fewer than 10 and 20 days, respectively. However, *Ascaris lumbricoides* eggs can survive for many months. Factors affecting the persistence of pathogenic microorganisms in water, sewage, and soil include temperature, pH, moisture levels, antagonism from soil microflora, and exposure to sunlight (Feachem David J Bradley Hemda Garelick and Duncan Mara 1983).

The shorter survival times of pathogenic microorganisms on crops compared to water and soil are attributed to increased exposure to sunlight, desiccation on crop surfaces, lack of nutrient sources and competition with microorganisms colonizing the phylosphere (Mogren et al. 2018). Several studies have demonstrated a significant reduction in the levels of pathogenic microorganisms over time on vegetables surfaces irrigated with poor-quality water despite significant changes in surface desiccation. (Bastos and Mara 1995; Ayres et al. 1992; Armon et al. 1994). Contrary to these evaluations, (Bastos and Mara 1995) determined that rainfall was associated with an increase in pathogen survival and persistence, suggesting that microorganisms remained viable on surfaces and the addition of moisture, cooler temperatures and lower UV index would provide the necessary conditions for pathogen growth and further contamination of vegetables. For instance, *E. coli* O157:H7 survived on the surface of harvested lettuce for up to 15 days when stored at 4°C (Badawy, Gerba, and Kelley 1985). Several strategies can be implemented to reduce the risk associated with pathogenic bacteria in irrigation water, such as improving the microbial quality of water, restricting the use of poor water quality in fresh produce, following guidelines or recommendations to assess and control the microbial profile of the water, and being aware of external contaminants close to the water source, such as domestic or wildlife animals, and runoff.

1.4 Regulations for Microbial Quality of Irrigation Water

Regulations associated with the microbial quality of irrigation water are imperative to control and reduce microbiological hazards that will contaminate raw agricultural commodities. Establishing and enforcing regulatory standards for the microbiological quality of water before harvest can significantly help in controlling microbiological risks and ensuring food safety (Rock et al. 2019). The assessment of the microbiological quality of irrigation water should be conducted within the specific context of each farm. Some regions around the world have proposed standards and guidelines that require farmers to monitor their agricultural water systems, considering associated agroecosystem features and conditions that may introduce microbiological hazards to fresh produce through water. Guideline implementation to control and improve water quality is practical and may be beneficial for famers, to improve food safety due the imminent concern related to the presence of pathogens in irrigation water and the potential hazard for humans to ingest these pathogens via contaminated fresh produce, leading to illness.

1.5 Standards for Microbial Quality of Irrigation Water

Microbiological water quality standards (MWQS) are based on indicator organisms, used to potentially predict the presence of pathogenic bacteria. An indicator organism can be defined as a group or single organism that demonstrate the efficacy of a process, in this context the efficacy of actions taken to meet the microbial criteria to ensure food safety (Fewtrell and Bartram 2001). A diversity of microorganisms has been proposed as indicators; however, only a small number of them have been used for standards related to microbial quality. One main carrier of pathogens in water is fecal contamination; for this reason, microorganisms found in feces were selected as indicators for pathogen contamination (Goh et al. 2019).

Different standards have been implemented based on thermotolerant fecal coliforms (FC) and total coliforms (TC), which can grow and ferment lactose and produce acid and gas at 44.5°C. *E. coli* has been adopted as an indicator in the same manner as nematodes or helminth egg counts (Y. A. Pachepsky et al. 2014). Indicators can be tolerated in a high concentration in surface water when the irrigation water does

not meet the edible portion of the crop. There is not a clear correlation between the presence of indicator organisms and that of pathogens because it does not reflect the ecology of the microorganisms. For this reason, it has been suggested the use of multiple indicators for a better understanding of the types, occurrence, and concentration of pathogens might provide greater safety around the farm; however identifying these indicator organisms based on the presence of a specific pathogen have been extremely difficult to find, suggesting that an index approach in which a series of organisms are used to predict the presence of a human pathogen could represent a safer option for consumers. Significant research is lagging in this regard and current efforts focus on valid and reproducible water treatments that will limit negative environmental and plant impacts (Griffith et al. 2016; Motlagh and Yang 2019; Edberg et al. 2000).

MWQS should vary based on the irrigation water source, method of irrigation, type of crop, and land use. Especially for wastewater, which imposes restrictions on irrigation; for example, it must not be used on crops likely to be eaten raw. Water quality standards can vary significantly based on the country. In the United States, MWQS vary by state; some state standards specify maximum microbiological concentrations, while others do not (Blumenthal and Peasey 2002). The risk varies based on the water source and irrigation method. However, an imminent risk is observed when irrigation water has a concentration higher than 100,000 Total Coliform TC/ml, an amount exceeding permitted levels.

There is no available information on how microbiological water quality affects pathogen concentration in produce, and for this reason, federal water quality standards have been based on microbiological standards for recreational and drinking water (Scholz 2006). Recreational water standards, developed on the assumption of human health risks associated with full-body contact during swimming, do not account for the rapid die-off of pathogens during post-irrigation intervals or environmental stresses caused by crop production (Barker-Reid et al. 2009). These standards, along with those for drinking water, are based on epidemiological studies (Fujioka et al. 2015; Pruss 1998). However, the microbiological quality of irrigation water should be assessed within a farm context, focusing on the agricultural water system and the agro-ecosystem features that might introduce pathogenic bacteria through water (Rock et

al. 2019). There are no epidemiological studies conducted specifically for irrigation water due to obvious reasons, leading to the adoption of recreational water standards as the most acceptable option, despite significant scrutiny and criticism of its use (Uyttendaele et al. 2015a).

The FDA in the Produce Safety Rule (PSR), establish a Microbial Water Quality Profile (MWQP) for each source of untreated agricultural surface or groundwater in the initial round of rule publications. Until April 2024, they looked to define specific criteria to ensure water quality, stating that the microbial quality of the water must be assessed by either quantitative methods or by testing for the presence or absence of contaminants, ensuring under postharvest conditions that no detectable generic *E. coli* is present in 100 ml (0 CFU/100 ml) of agricultural water (Assar and Ravaliya 2015). However, for production water, multiple reiterations of the law, looked to find consensus among the industry, academia, and FDA as to the microbiological criterion used to evaluate its safety and the risk evaluation and mitigation strategies needed to keep this water safe.

To ensure an accurate assessment of the microbiological status of a water source or irrigation system, it is imperative to combine biological analyses of water samples with risk assessments that include physicochemical quality parameters, land use history, adjacent land conditions, and agronomic practices (Malakar, Snow, and Ray 2019; Osburn, Aylward, and Barrett 2021; Krishnan et al. 2021). This comprehensive approach generates valuable data that can help identify sources of pathogens. By examining parameters such as chemical oxygen demand (COD), biochemical oxygen demand (BOD), turbidity, water temperature, conductivity, total dissolved solids, and pH, insights can be gained into how both microbiological and physicochemical factors impact water quality.

1.5.1 Clean Water Act (F. D. and A. FDA 2018)

The Clean Water Act (CWA) establishes regulations for discharging pollutants into the waters of the United States and regulates quality standards for surface waters. Surface water needs to meet water

quality criteria, that is expressed as concentrations of pollutants, temperature, pH, turbidity units, and toxic units (Copeland 2016)

Table 2. Parameters for surface water based on the Clean Water Act.

Parameter	Description	Standard/ Regulation
Dissolved Oxygen	The amount of oxygen dissolved in water, essential for aquatic life.	Minimum levels vary based on the designated use of the water body.
pH	A measure of the acidity or basicity of water.	Typically, between 6.5 and 9.0 for the protection of aquatic life.
Turbidity	A measure of water clarity, affected by suspended particles.	Maximum levels vary based on the designated use of the water body.
Temperature	Affects the chemical and biological processes in water	Temperature limits are set to protect aquatic life.
Bacteria	Presence of harmful bacteria like *E. coli* or fecal coliforms.	Limits are set based on the designated use, such as recreation or drinking water.
Nutrients	Excessive levels of nutrients like nitrogen and phosphorus can lead to eutrophication.	Limits are set to control nutrient pollution and prevent algal blooms.
Toxic Substances	Harmful chemicals and pollutants like pesticides, heavy metals, and organic compounds	Limits are set to protect aquatic life and human health.
Biological Criteria	Assessment of the health and diversity of aquatic communities.	Used to evaluate the overall ecological condition of a water body.

Note: Parameters described for surface water based on the Clean Water Act.

The Clean Water Act (CWA) serves as a foundational regulatory framework in the United States. While it does not offer explicit directives for the quality of water used for irrigation purposes in fresh produce, its provisions indirectly influence irrigation water quality by overseeing the standards of surface water sources that could potentially be utilized for growing fruits and vegetables. The CWA delineates several key regulations pertaining to surface water quality, which inevitably have ramifications for farming. These include (Copeland 2016):

- **National Pollutant Discharge Elimination System (NPDES):** This program regulates the discharge of pollutants into navigable waters, ensuring that water bodies maintain a certain level of

cleanliness. While directly focused on curbing pollution, its effects extend to safeguarding the quality of water resources available for irrigation.

- **Water Quality Standards (WQS):** Establishing criteria for water purity. WQS ensures that water bodies meet specific quality benchmarks. Adherence to these standards indirectly contributes to maintaining the suitability of surface water for irrigation, as it guarantees a baseline level of cleanliness and safety.
- **Total Maximum Daily Load (TMDL):** TMDL programs set limits on the number of pollutants that water bodies can withstand while still meeting water quality standards. By controlling pollutant levels, TMDL initiatives help preserve the integrity of surface water sources, thereby indirectly impacting the suitability of such water for irrigation purposes.
- **Nonpoint Source Pollution Control:** This aspect of the CWA focuses on mitigating pollution originating from diffuse sources, such as agricultural runoff. By addressing the broader issue of nonpoint source pollution, this regulation indirectly contributes to enhancing the overall quality of surface water, which in turn affects its suitability for irrigation.

In essence, while the CWA does not provide explicit guidelines for irrigation water quality, its overarching regulations concerning surface water management play a crucial role in indirectly influencing and safeguarding the quality of water resources that may be utilized for irrigation purposes.

MWQS are regulatory requirements established by federal, state, local, or territory governments to protect and maintain the quality of surface water. These standards set specific criteria and thresholds for various parameters to ensure that water bodies meet designated uses, such as drinking water supply, recreation, aquatic habitat, and agriculture. To accomplish a water quality criterion, specific numerical values are defined in parameters such as dissolved oxygen, pH, temperature, nutrients (e.g., nitrogen and phosphorus), bacteria (e.g., *E. coli*), toxic substances (e.g., heavy metals, pesticides), and sedimentation. Furthermore, an antidegradation policy regulation aims to maintain and protect existing water quality in

high-quality waters and establish criteria and procedures for permitting activities that may degrade water quality.

1.5.2 World Health Organization (WHO) and Food and Agriculture Organization (FAO)

The quality of water used for drinking, irrigation or recreational use has a significant impact on human health. Recognizing this, the World Health Organization (WHO) has developed guidelines to control the health hazards present in water. The guidelines focus on the microbiological quality of treated wastewater that is reused for agriculture. The microbiological guidelines for wastewater have three main objectives: the absence of fecal indicator bacteria in the wastewater, the absence of excess cases of enteric disease in the exposed population, and a model-generated estimated risk below a defined acceptable level. Total and fecal coliform organisms are used as indicators to monitor treated water. In the United States, the guideline for wastewater specifies zero fecal coliform bacteria per 100 mL for water used to irrigate crops that are eaten raw. However, the 1989 WHO guideline for wastewater used in unrestricted irrigation, meaning crops that are likely to be eaten uncooked, allows for less stringent limits of <1000 fecal coliform bacteria/mL. For irrigation of commercially processed and fodder crops, the guideline limit is <200 fecal coliform bacteria / 100 mL (Blumenthal et al. 2000).

On the other hand, FAO guidelines were developed based on the risk of contact to the edible part of the crop and considering epidemiologic studies. FAO defined three categories for water quality (a) irrigation of crops likely to be eaten uncooked, sport fields, and public parks; (b) irrigation of cereal crops, industrial crops, fodder crops, pasture, and trees; and (c) localized irrigation. The indicator used for microbial quality is <1000 fecal coliforms/100 mL and physicochemical parameters are not included in these guidelines. Agronomic parameters are included such as, total dissolved solids over 2000 (TDS, mg/L), or electrical conductivity higher than 3 dS/m (ECw). FAO guidelines are mainly recommendations, and their application must be adjusted to local conditions(FAO and Food and Agriculture Organization 2023). The guidelines provided by FAO serve as an instrument to monitor and manage the water quality used in food production. Water quality, such as microbial and quality parameters serve as a reference to know the state of the water

source. Fecal coliforms, serve as a reference to know if the water source is contaminated with various foodborne pathogens (Field and Samadpour 2007) . . In fresh produce, maintaining a good quality level is essential because during harvest and post harvesting handling and packing, there are no kill (i.e., inactivation) steps to reduce microbial contamination. If contamination occurs at this stage, cleaning and washing steps will not be adequate to eliminate this risk.

1.1.1 Environmental Protection Agency (EPA) and Produce Safety Rule (PSR) (Stoeckel et al. 2019)

In the United States the Food Safety Modernization Act (FSMA), identifies water as one possible source of contamination of fresh produce, which has multiple applications including irrigation, pesticide application, and cooling. The FSMA includes the need to evaluate water through the Produce Safety Rule (PSR) agricultural water standards (Boys, Ollinger, and Geyer 2015). Agricultural water means the water used in covered activities on covered produce, or food contact surfaces, including water used in growing activities. Agricultural water regulations often focus on detecting fecal contamination, which can carry pathogenic bacteria. All agricultural water must be safe and of adequate sanitary quality for its intended use. These regulations typically revolve around monitoring the levels of *E. coli* in water resources used for irrigating fresh produce and that will be in direct contact with the harvestable portion of the crop (Gerdes et al. 2022). As of December 2022, farmers that are covered by the PSR must create a Microbial Water Quality Profile (MWQP) based on generic *E. coli*, with a geometric mean of 126 or less colony forming units (CFU) of generic E. coli per 100 ml of water and a statistical threshold value of 410 or less CFU of generic *E. coli* per 100 ml of water for untreated surface water or well water. Based on each source, the number of samples to be collected varies with source, with the requirement of these samples being representative of use and collected within a specific timeframe based on water source. Since January 2023, this MWQP, as well as other aspects within section E of the produce safety rule have been under revision and it is expected that multiple requirements stipulated in the rule will significantly change. At this moment, few statements can be provided regarding metrics and most requirements, however, there will be at least 6 mandates that will remain in place and those include, training needs, requirements when treating irrigation

water, inspection of the irrigation system, the generation of corrective actions that arise when the source of water is believed to be contaminated, the use of 19 different microbiological testing methods to determine the presence of generic *E. coli* in agricultural water and the evaluation of the risks of contamination of water sources and those practices that can reduce those risks.

In harvest and postharvest activities, water should not contain detectable levels of generic *E. coli*, in a water sample of 100 ml, and untreated surface waters cannot be used for any harvest and postharvest activities. If generic *E. coli* is found, the use of such water should be stopped immediately, and corrective measures must be implemented before reuse (F. D. and A. FDA 2022). The frequency of test agricultural water varies based on the water source used, however update of the microbial water quality profile annually, and the samples of agricultural water must be representative of use and must be collected as close in time as practicable to, but, prior to, harvest (21 CFR 112.45 2019).

To date, the FDA has proposed criteria for recreational water standards based on EPA requirements, but these have limited relevance to agricultural environments. Good Agricultural Practices (GAPs) programs offer guidelines that extend beyond microbial water quality to include defining water sources, their use, application methods, and irrigation frequency concerning harvest activities(United Stated Department of Agriculture USDA 2022). While the FDA 2011 produce safety rule adopts these metrics, it lacks clear standards for water microbial quality, leaving growers to establish their metrics, resulting in the widespread use of chemical treatments to ensure water safety.

To meet this requirement, growers apply different treatment methods. The EPA mentions that the treatment method used must be effective to make the water safe and of adequate sanitary quality and meet the microbial quality criteria. However, there are no recommendations for water treatment, and there are no specific criteria to follow. Certainly, this has created significant challenges for farmers. Currently, three main challenges hinder fresh produce growers' access to safe water sources: (1) weather and climate change, which affect snow mass and the accumulation of groundwater; (2) the lack of a viable and reliable microbial

water standards that align with the food safety requirements in fresh produce; and (3) the need for effective chemical, physical, or mechanical treatments to render irrigation water safe for fresh produce.

In April 2020, the EPA released an addendum to pesticide quality standards, outlining the requirements for sanitizers to receive an EPA label for controlling human pathogens in irrigation water. Notably, there are currently no sanitizers with an EPA registration number specifically designated for the control of human pathogens in irrigation water. These acknowledges two challenges faced by growers: a lack of sanitizers with a label that will allow them to meet rule requirements and the difficulty of implementing treatments without clear guidance on working and operational limits (Environmental Protection Agency 2023). Chemical treatments, meanwhile, are effective only through a very narrow and intricate approach. Sanitizer use in irrigation water is influenced by factors such as concentration, organism, contact time, turbidity, organic matter, water pH, water hardness, and chemical properties.

FDA published the last update in May 2024, revised Subpart E of the FDA Food Safety Modernization Act (FSMA) Produce Safety Rule. This final rule presents new standards for assessing the microbial quality of water used before harvesting. The new rule proposes a systems-based approach for pre-harvest agricultural water assessments to identify hazards and guide risk mitigation strategies. Farms covered by the Produce Safety Rule, which use pre-harvest agricultural water, need to conduct evaluation on an annual basis. It is described that additional assessments must be performed whenever significant changes occur that could increase the likelihood of introducing known or foreseeable hazards onto produce or food contact surfaces.

The factors evaluated as part of the pre-harvest agricultural water assessment include:

- Agricultural water system.
- Agricultural water practices.
- Crop characteristics.
- Environmental conditions.

- Other relevant factors (FDA 2024).

Following their assessments, farms must determine whether corrective or mitigation measures are necessary to eliminate the risk of contamination associated with pre-harvest agricultural water. This includes implementing expedited measures to address hazards such as animal activity, biological soil amendments of animal origin (BSAAOs), or untreated or inadequately treated human waste on adjacent and nearby land. The FDA provides a table that outlines the required actions and corrective measures based on the assessment's findings (FDA 2024).

The new rule specifies that agricultural water and irrigation systems must be inspected at the beginning of the growing season and at least once annually to identify conditions likely to introduce hazards. According to Title 21, Subpart E §112.43, any treatment method must ensure the water is safe and meets relevant microbial quality standards. If the water does not meet these requirements, its use must be discontinued immediately. Before the water source can be reused, the entire affected agricultural water system must be re-inspected and treated in accordance with §112.43 (FDA 2024). This final rule provides enhanced guidance for managing pre-harvest agricultural water and reflects recent scientific findings from investigations of several produce-related outbreaks.

1.5.3 California Leafy Green Marketing Agreement (LGMA) (California LGMA 2023)

The California LGMA is a buyer driven food safety program established by California farmers who grow leafy greens such as lettuce, spinach, and kale. The LGMA was created due to food safety concerns and outbreaks related to leafy greens. It is a program that offers specific guidelines for growing, harvesting, and processing leafy greens. These guidelines include areas such as water quality, soil amendments, worker hygiene, equipment sanitation, and traceability. The LGMA food safety standards are updated periodically and are science-based. Regular audits of all growers enrolled in this program are conducted to ensure compliance with these standards.

The LGMA recognizes the risk associated with agricultural water, and the metrics established intended to mitigate the risk by providing practices to identify and correct nonconformities on time. The best practices for general agricultural management are:

- Evaluate and control the agricultural system (source, storage, and conveyance)
- Identify, and evaluate the hazards proximate to your agricultural water systems that may not be under your control.
- Perform an agricultural water assessment; water systems that convey untreated human or animal waste are never suitable for use in leafy greens, and review water records periodically.

The assessment of the agricultural water system involves evaluating the hazards associated with agricultural waters and considering the overall quality of the system. This assessment includes examining the source, storage, and conveyance methods of the water system, analyzing how the water is used, and the application methods employed, considering the timing of water usage, and considering environmental factors that could potentially lead to contamination.

LGMA categorizes water used in agricultural practices into two types, water type A and B.

- **Water type A:** Agricultural water that rarely contains indicators of fecal contamination due to environmental factors such as hydrogeologic filtration and state-regulated treatments set forth by the USEPA and state authorities. Examples of agricultural water systems may come from public and private providers and stored/conveyed in closed delivery systems. A baseline microbial assessment is not necessary for type A system using source water from a public/private provider. The initial microbial water quality assessment test water delivery systems collect three 100 ml samples during one irrigation event with at least one sample taken at the end of the delivery system and analyzed for generic *E. coli* using FDA allowed method. The acceptance criteria state that there should be no detectable generic *E. coli* in at least 2 of 3 samples and less than 10 MPN in the remaining sample. When five samples are collected, the rule states that it should be no detectable generic *E. coli* in at least 4 out 5 samples with

less than 10 MPN in the remaining samples. Corrective actions must be taken if generic *E. coli* is detected in more than 2 samples or if *E. coli* levels exceed 10 MPN /100 ml in a single sample. If the agricultural system does not meet these criteria, it is disqualified from being used as Type A water. This is particularly important because the water directly contacts edible leafy greens during irrigation.

- **Water type B:** This category includes carious types of agricultural water systems:

    1. Agricultural water from a third-party provider who certifies that the water meets the adequate microbial quality standards.
    2. Agricultural water that naturally achieves appropriate microbial quality by filtering through soil.
    3. Water sources that are vulnerable to contamination have not been treated to reduce microbes adequately. Such water must be used in ways that minimize the risk of crop contamination.

The examples for this agricultural water systems are ground chemigation, drip irrigation, furrow irrigation, and dust abatement. Regarding microbial analysis, the acceptance criteria is <126 MPN/100 ml geometric mean of 5 samples and <576 MPN/100 ml all single samples. The action level is required when >126 MPN/100 ml or >576 MPN/100 ml (any single sample). Remedial actions need to be taken when water does not meet microbial quality. Water does not directly contact edible leafy greens during irrigation.

For microbial quality assessment, the indicator of choice is generic *E. coli*, with an acceptance criterion of <126 MPN/100ml (geometric mean of 5 samples) and <235 MPN/100 ml (all single samples). Additionally, for overhead application, if the application of irrigation water is twenty-one or less days before harvest, indicators such as generic *E. coli* (<126 CFU/100 ml) and total coliforms are used (<1000 CFU/100 ml) (California LGMA 2023). Simultaneously, the testing of agricultural water systems is defined to protect leafy greens from contamination, and specific treatment requirements are mandated that reflect microbial conditions after treatment instead of using specific treatments. For example, treatments such as UV, ozonation, chlorine, or filtration are listed as appropriate methods. In

the case of chlorine treatments, the acceptance criterion includes maintaining a concentration of more than 1 ppm after application and ensuring the pH remains between 5.5 and 7.5. Additionally, other treatments approved by EPA for reducing human pathogens in water must also adhere to product-specific guidelines (California LGMA 2023).

1.5.5 Arizona Leafy Green Marketing Agreement (ALGMA) (Arizona LGMA 2023)

This document aims to provide specific guidance for lettuce and leafy green farmers, offering practices to address areas such as water, soil amendments, crop inputs, environmental factors, work practices, and field sanitation. ALGMA addresses the safety of leafy greens, understanding that they are mostly consumed raw, and emphasizes that the way they are grown, harvested, packed, held, processed, and distributed is crucial to minimizing the risk of human pathogen contamination. The metrics offered are intended to prioritize risk by classifying agricultural water systems for specific uses within leafy green operations.

ALGMA define the best practices for water management, defining that agricultural water systems encompass source, storage, and conveyance components, each requiring evaluation to ensure the quality of water used in leafy green operations meets prescribed standards. It is advised to assess and address food safety hazards near agricultural water systems not under control, with water from uncharacterized sources should never be used. An Agricultural Water Assessment, as outlined in Appendix A in the ALGMA document, should precede water usage, documenting system description and components. All controllable elements, including water source and distribution systems, must be managed to minimize human pathogen contamination. Testing water quality at the point of use is crucial. Surface water use after storms requires caution due to increased bacterial loads. Untreated human or animal wastewater conveyance systems are unsuitable for leafy green operations and must be separate. Water records must be regularly reviewed and authenticated by a supervisor or responsible party within a week of recording.

The ALGMA categorizes agricultural water into two types: Type A and B. Type A refers to water that is improbable to contain fecal contamination indicators, either due to natural conditions or because it is managed by a USEPA and state-regulated treatment regime. This is determined through an agricultural water system assessment detailed in Appendix A, microbial testing, and, if applicable, treatment verification. Type B includes all other agricultural water systems. It is important to know if the water source meets the microbial quality due to natural physical, chemical, and biological processes that filter water as it passes through the soil. It is important to mention that microbial water quality depends on the properties of the agricultural water system's components and how they are maintained.

ALGMA define steps to elaborate a hazard analysis for agricultural water, including:

(1) Assessment of agricultural water systems

(2) How is your agricultural water system being used?

(3) When is your agricultural water system being used?

These steps aim to evaluate and monitor the risks associated with irrigation water. For instance, in bullet point (3), the timing of water use plays a critical role as the risk of contamination of leafy greens is closely linked to when agricultural water is applied in the production environment. For this reason, the requirements for agricultural water that is aerially applied to leafy green crops vary depending on the timing of its application relative to the harvest date:

- Within 21 days of the harvest dates.
- More than 21 days before the scheduled harvest date

Additionally, ALGMA in Table 2A offers guidelines for irrigation water sourced from Type B agricultural water systems, including ground chemigation, drip irrigation, furrow irrigation, and dust abatement. Water designated for Type B purposes must adhere to microbial standards, with accepted values including a rolling geometric mean of 126 MPN/100 ml for generic *E. coli.* Acceptance criteria fluctuate based on the number of samples collected. Sampling procedures require collecting a 100 ml sample as close

as practical to the point of use, with frequencies specified for agricultural water sources. The table further details acceptance criteria, ranging from a single sample value of 126 MPN/100 ml to a rolling geometric mean of ,126 MPN/100 ml and 576 MPN/100 ml for any single sample. Investigation and remedial actions are mandated if test results surpass these levels. Additionally, records of water samples, analyses, and remedial actions must be meticulously documented and retained for verification purposes. The importance of conducting agricultural water assessments to pinpoint and mitigate contamination sources is underscored. Subsequent testing for pathogens such as STEC and Salmonella is imperative if follow-up testing indicates crop contamination before harvest. If agricultural water does not meet the requirements, remedial actions are required.

Irrigation water from Type A agricultural water systems, sourced from public/private providers, requires a microbial water quality assessment. This entails collecting three samples of 100 mL during one irrigation event at the end of the delivery system and analyzing them for generic *E. coli* using an FDA-allowed method. The acceptance criteria mandate no detectable generic *E. coli* in at least two of the three samples and <10 MPN in the remaining sample. The action level is defined by generic *E. coli* detected in more than two samples or levels exceeding >10 MPN/100 mL in a single sample. Follow-up testing involves conducting a root cause analysis and an agricultural water system assessment, as described in Appendix A, before the next irrigation event to identify and rectify any failures. After performing the analysis and assessment, retesting the water in five 100 mL samples collected during the next irrigation event is necessary (sampling locations can be at the end of any segment or node/branch within the irrigation system of concern). An agricultural water system is disqualified for Type A usage when the acceptance criteria are not met; however, the water can still be utilized as a Type B agricultural water system.

In the table below describes the similarities and slight difference in the definitions of type A and B water based on the CLGMA and ALGMA.

Table 3. Similarities and differences between California and Arizona Leafy Green Marketing Agreement.

		California Leafy Green Marketing Agreement	Arizona Leafy Green Marketing Agreement
Type A water	**Definition**	Agricultural water that is unlikely to contain indicators of fecal contamination either due to natural hydrogeologic filtration or through controlled USEPA and state regulated treatment regime as demonstrated by an agricultural water system assessment.	
	Application and usages	There are no differences between California and Arizona Leafy green Marketing Agreement for applications and usages, both agreements defined the followed application and usages: - Overhead irrigation and chemical application prior to (>) 21 days before scheduled harvest date - Germination - Ground chemigation. - Drip irrigation. - Furrow irrigation. - Dust abatement. - Non-food-contact farm equipment cleaning - Overhead applications (including irrigation pesticide spray, aerial chemigation) applied (<) 21 days of scheduled harveste date.	
	Remedial actions for type A and B -> A agricultural water systems. (Total coliform monitoring level failure)	5/5 samples with maximum level of 99 MPN in 100 ml in all water samples.	Not described in the Arizona Leafy Green Marketing Agreement.
Type B water	**Definition**	All other agricultural water systems.	
	Applications and usages	- Overhead irrigation and chemical application prior to (>21) days before schedule harvest date - Germination - Ground chemigation. - Drip irrigation. - Furrow irrigation. - Dust abatement. - Non-food-contact farm equipment cleaning	
	Metric of acceptance criteria	≤126 MPN /100 ml (rolling criteria mean n=5 and ≤ 576 MPN/100 ml for any single sample.	
	Irrigation water from treated Type B to A agricultural water systems	1. Routine verification of microbial water quality 2. Routine water treatment monitoring	1. Routine verification of microbial water quality 2. Routine water treatment monitoring 3. When adding crop nutrients and/or crop protection materials

		within 21 days to schedule harvest. Sampling need do not be conducted during irrigation events when crop nutrition/protection chemicals are being applied.

Note: LGMA and ALGMA share the same concept in defining the application, usage, and metrics for criteria referring to agricultural water types A and B. However, the difference between LGMA and ALGMA lies in their approach to type A agricultural water systems. For type A, it involves remedial actions in cases of total coliform monitoring level failure, while for type B, it relates to the transfer of irrigation water from treated type B to type A agricultural water systems.

1.6 Water Treatments

Multiples strategies have been proposed to control water quality during pre-harvest operations. Reducing the risk of produce contamination during the application of irrigation water has become a primary challenge for growers. Water treatment strategies should focus primarily on preventing cross-contamination from the irrigation system into the crop. To achieve this, the FDA and EPA have established standards and guidelines to mitigate the risk associated with the use of irrigation water in agricultural production such as the PSR (Produce Safety Rule), microbial quality standards for processed agricultural products, recreational water quality criteria, microbial risk assessment guidelines and water reuse guidelines. Many different treatments have been proposed to treat irrigation water including the use of sanitizers, ozone, ultraviolet (UV) light and filtration systems. However, many of them are not adapted to meet irrigation water requirements under commercial farming practices and many of them require elaborate procedures and costly investments that may not result in adequate control of human pathogens under the use of large volumes of water and that may also result in soil and plant contamination with hazardous byproducts (J. L. Banach and Van Der Fels-Klerx 2020b; J. Banach et al. 2015; J. L. Banach et al. 2020; Villanueva et al. 2015; Monarca et al. 2002).

Filters are used to remove clogging agents and inorganic materials like sando or clay in the water. The use of filters avoids clogging problems in the irrigations system and improves the water quality. There are diverse types of filters available for irrigation systems. However, the choice of filter depends on diverse factors such as water source, composition, desired level of filtration, and most importantly the volume of water needed for an irrigation event. Filters are composed of varying materials such as sand, gravel, a

combination of both or fabric, and be use alone or in combination with other water treatment methods (Milstein and Feldlite 2015). Sand filters are not effective at removing pesticides, there is a potential for partial microbial removal from contaminated water depending on the size of the particles being removed (Hedegaard and Albrechtsen 2014). Filters are effective in removing sediment, suspended solids, and larger microbial aggregates from water, thereby improving water quality, and reducing the risk of clogging irrigation equipment including drip tape. Regular maintenance and cleaning of filters are also critical to ensure proper operation and prevent clogging or impairment of the irrigation system. However, filters may not be effective in removing dissolved contaminants or microorganisms that are smaller than the filter pore size. The common filter size used for irrigation water is 178 and 214 μm, due to irrigation water volume requirements, which is not capable of removing bacteria (size range from 1 to 10 μm, making this treatment option unsuitable for reducing large particles and sediments (Zong et al. 2019) before the application of other treatments that can inactivate bacteria moving in the water column.

On the other hand, chemical treatments are effective against biological contaminants, highlighting the use of sanitizer as a suitable and effective option for water treatment. Chemical sanitation involves the use of chemical sanitizers at a specific concentration and contact time. There are many alternatives for treatment of irrigation water such as chlorine (Cl) based, peracetic acid (PAA), acidified water, copper, ozonation, ultraviolet (UV), membrane filtration, constructed wetlands, solar disinfection, and others. Highlighting action mode, challenges related to the sanitizers, and the bioproducts generated. The effectiveness of a sanitizer is drastically altered by impurities in water. Water hardness is the most important chemical property that has a direct effect on sanitizing efficacy. Water with 0–60 ppm (0–3.5 gpg) hardness is classified as soft water, while 60–120 ppm (3.5–7 gpg) is categorized as moderately hard. Hard water falls within the range of 120–180 ppm (7–10.5 gpg), whereas water with a hardness exceeding 180 ppm (>10.5 gpg) is considered very hard (Diggs and Parker 2009). Consequently, there is a significant need for the development of new water treatments that do not depend on these water chemistry parameters. Two of these new novel treatments are sodium acid sulfate (SAS) and potassium acid sulfate (KAS). These novels

sanitizers work by combining three modes of action based on changes in osmotic potential, protein/membrane oxidation, and pH alteration, offering a competitive advantage against traditional sanitizers, by avoiding the generation of carcinogens and other by-products that pollute the environmental.

1.6.1 Chlorine Based Sanitizers

The use of chlorine to treat water is one of the most widely used methods for microbial control. Chlorine has significant oxidizing capacity, making it a powerful antimicrobial agent. Chlorine based sanitizers are the primary treatment used to treat water, and its mode of action is based on oxidizing membranes which lead to destroying biological microorganisms (ARNOLD and COLFORD 2007). When added to irrigation water, this process is known as chlorination. The EPA has established that sanitation involves using chemicals to kill bacteria on surfaces, but it is not designed to eliminate viruses. In contrast, disinfection is defined as the use of chemicals to kill both viruses and bacteria on surfaces. However, products used for both sanitation and disinfection must be registered with the EPA (EPA 2023). Additionally, chlorine is permissible in organic crop production, organic food processing, and organic livestock management, provided the residual chlorine levels for wash water in direct contact with the crop or food contact surfaces, in flume water systems or for cleaning irrigation systems applied to crops or fields, do not exceed the maximum residual disinfectant limit set under the Safe Drinking Water Act of 4 mg/L expressed as Cl_2 (USDA 2024).

As a disinfectant, chlorine is available in three forms: chlorine gas ($Cl_{2(g)}$), hypochlorite (sodium or calcium), and chlorine dioxide ($ClO_{2)}$. Chlorine exists in water as either hypochlorous acid (HOCl) or hypochlorite (OCl^-), both referred to as free available chlorine. Hypochlorous acid is a faster and stronger oxidizer than hypochlorite. The amount of HOCl or OCl- present in the water is related to the pH of the solution. To maintain free chlorine at levels close to 95% or higher, the pH of the solution must be between 5.5 and 6.5 (Waters and Hung 2014). Careful attention must be placed to avoid high acidic conditions (< pH=4.0) and elevated temperatures (>40 C) that can result in gassing of chlorine. Chlorine demonstrates greater effectiveness at lower pH levels. At these lower pH levels, more hypochlorous ions are generated,

enhancing antimicrobial activity. Conversely, when the pH of water is at or above a pH of 7, the effectiveness of chlorination diminishes significantly by at least 50%, with significantly lower effectiveness at pH values higher than 8. If the water being used is very hard, it is essential to appropriately treat it before use (Centers for Disease Control and Prevention 2022). When chlorine is introduced into water either in its molecular forms or as hypochlorite, it undergoes hydrolysis to produce aqueous molecular chlorine, hypochlorous acid, and hypochlorite ion. Together, these are collectively known as free chlorine (FC). Both hypochlorous acid and hypochlorite ion act as antimicrobial agents, but hypochlorous acid (HOCl) is notably more effective than hypochlorite ion (OCl^-)(Tvrdá and Benko 2020).

Chlorine is a potent oxidizer that alters the chemical structure of bacteria by breaking the chemical bonds in their molecules, essentially 'burning' the membranes of unwanted microorganisms in water. Chlorine is used in high concentrations to obtain a rapid kill rate. Chlorine is a nonselective oxidant which reacts avidly with a variety of cellular components and affects metabolic processes (Shang and Blatchley 1999). The cytoplasmic membrane may be a key in bacterial inactivation by chlorine due to alterations in its permeability after chlorination. Membrane permeabilization has been observed when bacteria are exposed to chlorine concentrations as high as 50 ppm(L.-W. Luo et al. 2021). Chlorine induced a leakage of macromolecules from the bacteria cells indicating the permeability changes of the membrane (Figure 2) (Venkobachar, Iyengar, and Prabhakara Rao 1977). However, the reported mechanism of action is not consistent in the literature, specifically for DNA suggesting that chlorine can also cause direct damage to DNA. For this reason, other mode of actions has been suggested such as protein denaturation by oxidation of tryptophan and tyrosine(Ogata 2007)

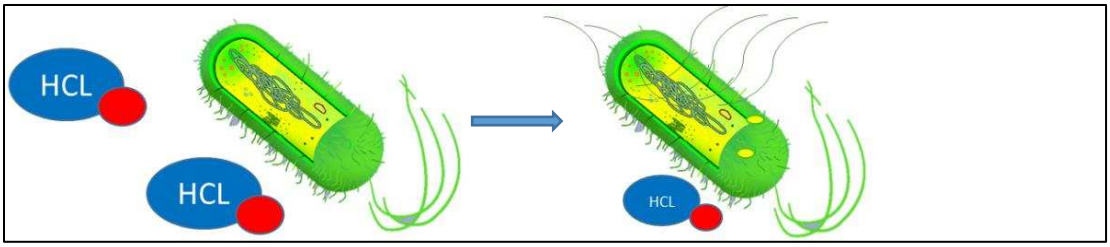

Figure 2. Action method of chlorine against pathogenic bacteria. Chlorine compounds penetrate the bacterial cell wall, disrupting the cell membrane and causing leakage of cellular components, while also oxidizing essential cellular components, leading to oxidative damage, and interfering with vital metabolic processes in the microorganism.

Although the membrane plays a significant role in the inactivation of bacteria by chlorine there are other factors, such as uncoupling of the electron chain or enzyme inactivation either in the membrane or in the cell interior. A recent study highlights the different reactions between Gram-negative and Gram-positive bacteria treated with chlorine. The findings demonstrate that membrane structures play an important role in bacterial resistance to chlorine in the presence of organic matter. This resistance is related to the accessibility of chlorine to targets within the cell (Virto et al. 2005). Organic matter is a problematic factor when chlorine is used as a disinfectant, due to the production of by-products that can cause harmful health effects.

When chlorine reacts with organic substances it leads to the creation of harmful and mutagenic halogenated by-products (chlorinated and/or brominated). If the water treated with chlorine has organic matter, a complex mixture of chlorine byproducts is formed. Additionally, only approximately half of the total organic halides produced can be detected and measured (Villanueva et al. 2015). The two main classes of byproducts found in water treated with chlorine or chlorination are halogenated trihalomethanes (TH) and haloeacetic acids (HA). The formation and concentration of TH and HA in chlorinated water depends on the composition of the water, and on the occurrence of residual chlorine in the distribution system. Other bioproducts of chlorination are trihalomethanes viz. chloroform, dichlorobromomethane, dibromochloromethane and bromoforms (Rodriguez, Sérodes, and Levallois 2004). The formation of these compounds depends on factors such as temperature, pH, sanitizer concentration, contact time, inorganic

compounds, and natural organic matter in the water source. Highlighting the importance of the physicochemical quality of the water when chlorine treatments are implemented.

In the United States, the most common chlorine products are aqueous solutions containing 5.25 to 12.5% sodium hypochlorite. In vitro suspension tests have shown that solutions with approximately 140 ppm of chlorine dioxide can achieve a 6-log reduction in *Staphylococcus aureus* within one minute and in *Bacillus atrophaeus* spores within two and a half minutes in the presence of 3 g/L bovine albumin. Low concentrations of free available chlorine have biocidal effects on mycoplasma (25 ppm) and vegetative bacteria (<5 ppm) within seconds, even without organic matter (D. H. Lee, Miles, and Perry 1985; Dychdala 2001). Research by Hulkower et al. (2011) demonstrated that 25 different viruses could be inactivated within 10 minutes using 200 ppm of available chlorine. Additionally, according to the AOAC Use-Dilution Method, 100 ppm of free chlorine was found to kill between 10^6 and 10^7 *Staphylococcus aureus*, *Salmonella choleraesuis*, and *Pseudomonas aeruginosa* in less than 10 minutes (Rutala and Weber 1997).

Other study shows that in fresh produce chlorine concentrations between 3 and 100 ppm are effective in reducing microbial populations without significantly altering the produce (Praeger, Herppich, and Hassenberg 2018). Fu et al. (2018) assessed the effectiveness of chlorine in post-harvest washing of cut lettuce, finding that factors such as temperature and organic matter significantly influence performance. A chlorine concentration of 10 or 20 ppm was effective in preventing *E. coli* O157:H7 cross-contamination when washing lettuce in tap water, while 30 ppm was necessary to prevent cross-contamination during industrial-scale washing.

Table 4. Chlorine concentration used for sanitation processes.

Chlorine concentration	Usage	Reference
50 ppm	Treat drip irrigation systems	Storlie (1997)
<200 ppm available chlorine	Sanitizer in food processing operations	Mcglynn (1997)
Residual chlorine concentration 10, 30, 60 and 90 mg/L	Lettuce and strawberry produce washing process	Chen and Hung (2018)

0.5 ppm free chlorine	Inactivate *E. coli* O157:H7 in suspension below the detection level.	Y. Luo et al. (2011)
75 to 150 ppm	Squash (75-100 ppm), spinach and lettuce (75-150 ppm), cucumber, broccoli, melons sweet potatoes (100-150ppm)	Gutierrez-Rodriguez (2014)
25 ppm free chlorine	Inactivation of *Listeria monocytogenes* in simulated apple dump tank water	Wang et al. (2023)
50 to 150 mg/L	Inactivation of *Salmonella* Thyphimurium in fresh-cut lettuce during chlorine washing	(Possas et al. 2023)

Note: Chlorine concentrations recommended based on scientific investigations.

The primary advantage of this method is that chlorine is relatively inexpensive, and automated systems and generators may not necessitate a significant capital investment. This is mainly applicable to the hypochlorite versions of the sanitizers. For generation of chlorine gas or chlorine dioxide significantly higher investments are needed with an added risk of explosion if not managed accordingly. To achieve effective disinfection, it is important to maintain a residual concentration of at least 0.5 mg/L of free chlorine for a minimum of 30 minutes (pH<7). When chlorine is used in lower concentrations, microorganisms that survive the treatment may remain injured in a state known as viable but not culturable rather than inactivated (LeChevallier, Evans, and Seidler 1981) which allows the organisms to remain viable for prolonged periods of times and in the long run create resistance to such treatments (Adefisoye and Olaniran 2022). Although chlorine disinfection is an effective method to treat contaminated water, it faces various challenges, particularly in farm applications. When chlorine is applied to water, it reacts with organic matter, metals, and other elements affecting the disinfection capacity of chlorine (Gopal et al. 2007). Other factors that influence the difference between free residual chlorine and the chlorine added in the system are temperature, pH, turbidity, and the chemical composition of the water. (Nielsen et al. 2022). High pH or water hardness decrease the bactericidal activity of chlorine against microorganisms such as *L. monocytogenes* and *E. coli* O157:H7 (Pangloli and Hung 2013) and currently there are a limited number of systems that can adjust water pH and hardness to treat large volumes of water before being treated with chlorine-based sanitizers at the necessary volumes of water needed for irrigation. Thus, some growers have

focus on the use of chlorine dioxide or the generation of chlorinated gas to treat water which cost and operation wise, represent a significant challenge for consistent use on different farms or locations within the farm (Zheng, Cayanan, and Dixon 2008). The efficacy of chlorine can be affected by multiple environmental factors. Table 3 describes the impact of these factors on chlorine efficacy.

Table 5. Effect of environmental factors on the efficacy of chlorine.

Environmental Factor	Effect
Temperature	A temperature range between 15 and 25 ℃, exhibit optimal results for chlorination treatments (Park and Kang 2018).
pH	Chlorine is more effective as a sanitizer in a pH range between 6.5 and 7.5. Higher pH levels can lead to a decrease in the concentration of free chlorine available for disinfection and when the pH is it the optimum range the level of free chlorine increases enhancing disinfection efficiency (Marín et al. 2020).
Suspended solids, turbidity	There is an interrelationship between elevated turbidities and the efficiency of chlorine, levels below 5 NTU are recommended (LeChevallier, Evans, and Seidler 1981).
Organic matter	Higher levels of organic matter results in higher demands of chlorine, due the chlorine inactivation caused by organic matter (Chen and Hung 2017).

Note: NTU (Nephelometric turbidity unit). Environmental factors like temperature, pH, suspended solids, turbidity, and organic matter may reduce the effectiveness of chlorine concentrations.

Further, there have been documented explosion incidents in CA linked to the use of these systems which highlight the complexity of use and the need of significant training of personnel to use them as intended by the manufacturer. Several incidents involving chlorine gas leaks or releases from storage tanks and chlorination systems have been reported. For instance, in August 2003, a chlorine gas leak at a water treatment plant in Alameda County prompted nearby residents to shelter in place (SFGate 2003). In August 2015, an accidental release of chlorine gas from a cylinder at Pacific Steel and Recycling in East Spokane sickened workers and affected more than a dozen people at a nearby city building (Alexander 2015). Most recently, in December 2023, a chlorine leak in a San Diego pool resulted in the hospitalization of 13 individuals (Domenick Candelieri 2023). These incidents typically stem from accidental releases or leaks involving chlorine storage tanks, tanker trucks, or chlorination systems in water treatment facilities, industrial plants, or other applications. Causes often include equipment failures, human error, or accidents during the transportation or handling of chlorine. It is crucial to acknowledge the hazards associated with

chlorine gas and understand that implementing proper safety measures, thorough training, and a robust emergency response plan can significantly mitigate the risks of handling chlorine gas.

1.6.2 Peracetic Acid (PAA)

Peracetic acid is an oxidizing disinfectant agent used for the inactivation of various microorganisms. The molecule is simply acetic acid with extra oxygen. PAA, is soluble in water, colorless and has a pungent acetic acid odor (Moondra et al. 2018). PAA can be used in conventional or organic productions; PAA is approved as a synthetic substance that is allowed in certified organic production (7 CFR Sec. 205.601).

The mode of action of this disinfectant is based on release of active oxygen species and the formation of free hydroxy radicals (OH), which oxidize or disrupt thiol groups in enzymes and increase cell wall permeability by disrupting sulfhydryl (-SH) and sulfur (S-S) bonds (McDonnell 2007). PAA disrupts the chemiosmotic function of the lipoprotein cytoplasmic membrane through dislocation or rupture of cell walls, oxidize essential enzymes, may inactivate catalase, and acts on the bases of the DNA. PAA has the advantage of being free from decomposition by peroxidases and remains relatively active in the presence of organic loads up to a certain level typically below 150 NTUs (Maunuksela 2016; Nerandzic et al. 2016). PAA is commercially available in the form of a quaternary equilibrium mixture containing acetic acid, hydrogen peroxide (HP), PAA and water. A general recommendation to use PAA solutions is immediately use after preparation, because it loses half percent of its strength after 24 hours (Schoeb and Eaton 2017).

PAA is capable of inactivating gram-positive and gram-negative bacteria, fungi, and yeasts in less than 5 minutes at concentrations lower than 100 ppm. However, in the presence of organic matter higher concentrations of 200 to 500 ppm are needed (Centers for disease Control and Prevention 2008). In the case of spores, PAA at 1% can provide a 5-log reduction after 1 minute (Malyshev et al. 2021). PAA disinfection capacity is more dependent on dosage that the contact time, and it seems to exhibit a higher inactivation for *E. coli* than total coliform bacteria (Mezzanotte et al. 2007). Despite both groups being part of the same *Enterobacteriaceae* family, which further highlights the complexity of using some of these organisms as

indicators of the presence of a human pathogen, when within the same family simple divergences between groups can create a significant response to a treatment. PAA exhibits potent oxidizing and bactericidal properties while generating less byproducts that can cause environmental pollution when compared to chlorinated solutions and the lower range of concentrations effective at controlling human pathogens in water. PAA has been shown to be less impacted by suspended solids or organic materials up to a certain range, showing significant bacterial inactivation when water has been previously filtered (De Velásquez et al. 2008).

However, its susceptibility to decomposition necessitates careful storage in cool, dark places to prevent mass loss. PAA effectively inactivate waterborne microorganisms, although higher doses may be required for resilient strains (Zhang et al. 2022). As with all other sanitizers, the concentration of PAA during water treatments must balance the needs for safety, water quality, and economic factors. The water matrix influences the oxidation capacity of PAA, requiring control over the concentrations during its usage. PAA is degraded by environmental factors such as temperature, turbidity, and organic matter or impurities in the water, and is less impacted by fluctuating water pH up to a pH range of about 7.5 to 8 (Luukkonen et al. 2014) (Luukkonen et al. 2014). Table 2 describes the effect on environmental factors in the efficiency of peracetic acid (Luukkonen et al. 2014).

Table 6. Effect of environmental factors on the efficacy of PAA.

Environmental Factor	Effect
Temperature	Disinfection efficiency increases with temperature. Active between 0 and 100°C (Stampi, De Luca, and Zanetti 2001; Baldry et al. 1995).
pH	More effective in acidic conditions. Above pH 9 the efficiency starts to decrease. The non-dissociated form (CH_3COOOH) is thought to be a more active disinfectant than the dissociated form (CH_3COOO-)
Suspended solids, turbidity	Slight decrease in the disinfection efficiency as suspended solids or turbidity increases. This can be seen in the required dose for different effluents: primary > secondary > tertiary
Organic matter	High organic load may increase the required dose, however it is less affected than other chemical disinfection methods. No significant formation of harmful byproducts in reactions with organic matter

Note: Table obtained and modified from Luukkonen et al. (2014).

By-products generated by the decomposition of the disinfectant in water, in the case of PAA are acetic acid, hydrogen peroxide, oxygen and water, but it also generates halogenated trihalomethanes (TH) and haloeacetic acids (HA) as described by Terao (2014). When PAA is consumed in an aqueous solution three reaction take place: spontaneous decomposition, hydrolysis, and transition-metal-catalyzed decomposition. Spontaneous decomposition of the acetic acid and oxygen takes place when the solution reaches a pH range of 5.5 to 8.2 (Gehr and Cochrane 2002). The by-products isolated from water sources treated with PAA are primarily carboxylic acids, which are not recognized as mutagenic (Monarca et al. 2000, 2003). Monarca et al. (2002) noted that a significant advantage of PAA is the absence of halogen-containing by-products in treated water. However, this finding contradicts other studies; for example, W.-N. Lee and Huang (2019) evaluated the formation of disinfection by-products (DBPs) in a lettuce washing system using sodium hypochlorite and PAA. They found that PAA generated fewer trihalomethanes than sodium hypochlorite. Although PAA has a lower tendency to form chlorinated DBPs compared to chlorine, the potential for DBP formation still exists with PAA. Additionally, an earlier study by Crathorne et al. (1991) investigated the formation of aldehydes when PAA was used to treat wastewater. Generally, while PAA does produce by-products when used as a sanitizer in water treatment, this risk must be considered when choosing this sanitizer. Moreover, the use of this disinfectant incurs high costs, attributed to its limited production capacity globally (Kitis 2004).

1.6.3 Sodium Acid Sulfate (SAS) and Potassium Acid Sulfate (KAS)

SAS and KAS are novel antimicrobial agents that have been used in the food industry as ingredients in sauces, sport drinks, chocolate, and other products in part due to their ability to reduce the need for adding salts. Currently SAS is recognized as GRAS and listed under the “safer choice antimicrobial” for its safety regarding human health and the environment by EPA (Environmental Protection Agency 2024). They have been used as a processing aid, acidulant and anti-browning agent (Fan et al. 2009). There is no information regarding the formation of by-products due to the use of these antimicrobial agents as sanitizers treatment

in water. SAS has been tested as an antimicrobial agent in food (chicken drumsticks, carcasses, and apples); however, there is no literature on the use of KAS as an antimicrobial agent (Sheng et al. 2020; Dittoe et al. 2019). SAS has been shown to be an effective sanitizer even in the presence of high organic matter, making it an excellent choice for washing systems with high organic matter (Sheng et al. 2020). SAS and KAS exhibit three modes of action identified for the inactivation of both gram-negative and gram-positive bacteria. This multifaceted mode of action is based on changes in osmotic potential, protein/membrane oxidation, and pH alteration, making them competitive antimicrobials against traditional sanitizers such as chlorine or PAA (McDaniel et al. 2021). It is imperative to know that the mechanism of inactivation varies based on the characteristics of the target microorganism.

When SAS and KAS are dissolved in water, they dissociate into potassium or sodium hydrogen sulfate, and bisulfate ions (Na^+, or K^+, H^+, and HSO_4^-) without reacting with organic matter and production of halogenated trihalomethanes (TH), haloeacetic acids (HA), trihalomethanes viz. chloroform, dichlorobromomethane, dibromochloromethane and bromoforms. The initial chemical change in water treated with SAS or KAS is a decrease in pH, followed by alterations in osmotic potential between the solution and the bacterial cell cytoplasm(McDaniel et al. 2021). KAS and SAS create an osmotic potential that induces hypoosmotic shocks in the bacteria, followed by consistent exposure to hyperosmolarity preventing the formation of viable but not culturable cells. These changes lead to the inactivation or leakage of proteins and macromolecules, resulting in a loss of nutrient uptake, inhibition of DNA replication, and increased ATP accumulation due to the inhibition of macromolecular biosynthesis. The combined effects of acidity, osmolarity, and oxidation-reduction potential contribute to the inactivation of pathogenic bacteria (McDaniel et al. 2021).

The mode of action of the sanitizer has a significant impact in its ability to effectively reduce microbials contaminants. Water quality plays an important role in performing an adequate disinfection(Bhagwat 2019). The ease of use coupled with costs associated with capital investments, personnel training and environmental impact, influences sanitizer use by farmers. The lack of clear

guidelines developed by federal and state agencies to support farmers to choose an effective sanitizer to treat water, has become an issue for food safety. These sanitizers guidelines or standards should vary based on the crop, target pathogen, and water quality, considering the side effects of a sanitizer treatment such as corrosive effects and formation of carcinogenic compounds that impact soil and crop health, and the long-term sustainability of farming fresh produce.

1.7 Lettuce (Lactuca sativa)

Leafy greens, such as romaine lettuce, spinach, iceberg lettuce, kale, and spring mix have been associated with multiple human pathogens responsible for multiple food borne outbreaks in the United States and Canada (Marshall et al. 2020). The main producers of leafy greens in the United States are California and Arizona(United States Department of Agriculture 2023). Leafy greens are mainly consumed raw and are considered an important source of lutein, being part of the dietary guidelines for Americans (Li et al. 2021). However, besides their health benefits leafy green have been often implicated in foodborne diseases, becoming the most common cause of foodborne illness in the United States (Painter et al. 2013)(Wendel et al. 2009) (Herman, Hall, and Gould 2015). A food borne outbreak can be defined as an event in which two or more people become ill due to the ingestion of a contaminated food item (Herman, Hall, and Gould 2015). Between 2014 to 2021, a total of 78 foodborne disease outbreaks linked to leafy greens, mainly lettuce and spinach, were reported to the Centers for Disease Control and Prevention (CDC) (Center for Disease Control and Prevention 2023). Lettuce is primarily used in salads, consumed raw, and its contamination has been attributed to different environmental factors, agricultural practices, and potentially to crop characteristics (Center for Disease Control and Prevention 2023)

Lettuce (*Lactuca sativa*) is an annual or biennial leaf vegetable, from the family Asteraceae. Lettuce exhibits widespread adaptability to diverse climate conditions, making them a highly sought-after crop with significant demand. Their ability to be rotated within the same year further contributes to their popularity (Ferreira et al. 2009). Lettuce is adapted to cool growing conditions with an optimum temperature range of 60 to 65 °F. At 70 to 80 °F, the plants flower and produce seed. Lettuce is low in fat and contains essential

polyunsaturated fatty acids for health such as omega-6, linoleic acid, omega-3, and α-linolenic acid (Kaur, Chugh, and Gupta 2014).This vegetable has a higher water requirement, the moisture content of the soil has a great impact on head quality. For this reason, irrigation practices are fundamental to keep cultivar quality(Sanders 2019).

Lettuce is divided into two categories: head (iceberg) and leaf (romaine, butterhead, baby and other leaf types), and there are the most valuable United States grown vegetables (Agricultural Marketing Resource Center (AgMRC) 2021). Lettuce production varies based on state, and it is measured as lettuce head productions (cwt), California 33.17 M, and Arizona 10.3 M (WPR 2024). In Florida it is estimated that 1000 acres is dedicated to lettuce production (National IPM Data Base 2004). At the same time, new practices for food production have emerged in the last few years. For example, Texas has one of the largest lettuces greenhouses, with an area of 20 acres and providing a water saving compared to conventional agriculture of 380 million gallons annually (Revol Greens 2024). For example, California leads lettuce production in the summer, fall, and spring, while in the winter, the main production takes place in Arizona (Davis and Lucier 2021). The acreage used for lettuce production surpasses 375,000 acres, making this leafy green one of the most important and consumed vegetables in the United States.

Lettuce is a main ingredient for salads or sandwiches, creating an important economic income for farmers. In 2022, lettuce comprised almost 20% of the $21.8 billion in cash receipts received by United States growers from the sale of vegetables and melons. Sales of romaine lettuce reached $1.54 billion, iceberg lettuce sales amounted to $1.33 billion, and leaf lettuce sales trailed at $1.25 billion. Highlighting, the importance of romaine lettuce for daily consumption and economic impact (U.S. Department of Agriculture USDA and Weber 2023). However, despite its high consumption rate, romaine lettuce also poses a risk as it can serve as a potential vehicle for transmitting diseases. From 2009 to 2021, the CDC reported that romaine lettuce was responsible for 39 foodborne outbreaks, resulting in 5 deaths (Centers for Disease Control and Prevention 2023). This highlights the importance of understanding the agricultural

practices employed in growing this vegetable and identifying the environmental factors and sources introducing bacterial contaminants during preharvest and postharvest activities.

1.7.1 Agricultural Practices for Lettuce

Lettuce demonstrates versatility in their cultivation methods, which encompass various horticulture practices. These methods include conventional, organic, open, or sheltered field, and hydroponic (sheltered cultivation) systems, each exhibiting distinct characteristics concerning management, yield, and vegetable quality (Barbosa et al. 2015). In the case of romaine lettuce, its growth may be influenced by season (sensitive to heat and cold).

Lettuces undergoes six distinct developmental stages: seed, cotyledon, seedling, rosette, cupping, and heading periods (David Kerns et al. 2020). The seed stage encompasses the period from pre-planting to emergence. For successful germination, seeds require adequate exposure to water and appropriate temperatures. The germination stage can vary, lasting from 7 hours to 12 days, depending on the lettuce variety. Following germination, the cotyledon stage commences, during which the roots establish and grow a few inches. The seedling stage starts with the appearance of the first true leaf and continues until the plant forms a distinct circular cluster of leaves known as a rosette. The rosette stage lasts approximately 25 to 50 days, depending on the season. At this stage is in which most lettuce cultivars are transplanted to the field and the stage in which significant susceptibility to desiccation can result in the loss of the entire crop. At this stage sprinkler irrigation is needed to keep moisture levels high to avoid losing the crop. Such practice could pose a significant risk of contamination if water quality is not controlled. Subsequently, the cupping stage begins, and head formation usually takes 7 days for fall-planted lettuce and 14 days for winter-planted varieties. At this stage furrow and drip irrigation approaches are the typical industry practices targeting reduce water consumption and pest incidence. Finally, in the heading stage, the plant continues to grow until it reaches maturity for harvest (D l Kerns, Palumbo, and Sanches 1999). The time it takes to reach harvest maturity depends on the cultivation method used, with hydroponic cultivation taking six to eight weeks,

greenhouse cultivation taking five to seven weeks, and open field cultivation taking seven to ten weeks (Islam et al. 2021)

Furthermore, activities occurring during each season, such as the movement of domestic and wild animals, can affect the level of bacterial contamination. For instance, field contamination with *E. coli* O157:H7, a pathogenic bacterium found in manure, is one of the many *EHEC* or *STEC* serogroups. Domesticated livestock like cows and wild animals such as deer have the potential to contaminate nearby produce fields or water sources (Astill et al. 2020). Irrigation water has been identified in multiple outbreak investigations as the route of contamination, carrying bacterial pathogens like *Salmonella, L. monocytogenes, and EHEC or STEC organisms*. This results in cross-contamination through the irrigation system spreading contaminants on the external surface of the product during the growing season (Percival et al. 2014; Gartley et al. 2022; Y. Pachepsky et al. 2011). The water source plays an important role in identifying potential risks because different contaminants may be introduced from environmental sources such as agricultural runoff.

The lettuce industry has adopted Good Agricultural Practices (GAPs) as part of its normal production operations. These GAPs provide general food safety guidance on critical production steps during the growing, harvesting, transportation, cooling, packing, and storage of fresh produce (Tarlengco 2024). GAP guidance includes evaluating potential microbiological hazards associated with land history, adjacent land use, water quality, worker hygiene, pesticides and fertilizer use, equipment sanitation, harvesting and handling produce, traceability and recall, training, product transportation, documents, and records (FAO 2016). GAP practices highly recommended are for incremental reduction of microbial contaminants include the following: (Sinkel and Daniel 2015):

- Develop and enact protocols to prevent pest infestation in irrigation pipes and drip tapes.
- Verify that all water utilized in pre- and post-harvest activities adheres to microbial water standards. This encompasses water utilized for hydrovac cooling.
- Ensure regular cleaning and sanitation of any cooling equipment employed for lettuce.

- Avoid the application of raw animal manure on or near lettuce crops.
- Note that chlorine alone is inadequate for eliminating pathogens in lettuce latex post-cutting and coring; it is advisable to use a more potent FDA-approved sanitizing agent.

However, it has been recognized that an important risk of contamination in these steps can also compromise the entire food supply chain. For this reason, the FSMA produce safety rule and third-party certification GAP practices and buyer driven requirements focused on reducing risks at the farm, following a combination of principles including Hazard Analysis of Critical Control Point (HACCP), GAPs, Sanitation Practices and risk assessment programs (FMI 2022).

During lettuce production on farms, there are specific agricultural practices that can be a potential source of contamination as the use of raw manure for fertilization or irrigation practices using water sources with an unknown microbial quality (Khanal et al. 2024; Ramos et al. 2021). A 2017 study evaluated the efficacy of implementing food safety and hygienic practices among lettuce farmers. The results revealed that 53% of lettuce farmers used raw poultry droppings, and 70% applied raw cow dung, both of which pose a microbial risk to human health. The study also showed that most lettuce farmers operated under high microbial risk conditions (Oyinlola et al. 2017).

In 2019, the FDA conducted an analysis of factors that might have contributed to the contamination of romaine lettuce implicated in the fall 2018 multi-state outbreak of *E. coli* O157:H7. This outbreak declared over in January 9, 2019, affected 16 states, resulting in 62 reported illnesses, 25 hospitalizations, and two cases of hemolytic uremic syndrome (HUS). Investigative teams focused on several areas, including agricultural water, equipment, tools, sanitation, soil amendments, growing and harvesting practices, animal intrusion, adjacent land use, and employee health and hygiene practices. An *E. coli* O157:H7, sharing the same genetic fingerprint as the outbreak strain, was recovered from the sediment of an on-farm water reservoir in Santa Maria, which supplied water for the romaine lettuce consumed by the ill individuals. This finding indicated that the water reservoir contained *E. coli* O157:H7, although this pathogen was previously undetected. It is known that this strain can survive in water for extended periods (Guodong Wang and Doyle

1998). However, the investigative team could not determine how the reservoir became contaminated with the outbreak strain. The FDA suggests that the most likely route of contamination was using water from this reservoir.

The investigation revealed that the farm had procedures to collect and test reservoir water for generic *E. coli* and to treat the water with sanitizer before use. However, records did not indicate that sanitizer levels were maintained during irrigation, post-harvest handling, or equipment washing/rinsing. Traceback investigations further revealed that additional ranches under the same ownership, as well as other farms, might have distributed contaminated romaine lettuce or other produce. These farms did not use water from the reservoir where the *E. coli* O157:H7 strain was detected, and the FDA could not identify a potential source of contamination.

This outbreak underscores the necessity of adhering to Good Agricultural Practices (GAP), such as verifying that all water used in pre- and post-harvest activities meets microbial water standards, ensuring water quality, and consistently applying sanitizer treatments throughout the growing process. Monitoring activities surrounding the water source is crucial to determine if an open water source is vulnerable to contamination due to environmental factors. Maintaining records of actions taken to mitigate risks is essential. Despite food safety concerns associated with outbreaks, adherence to GAP and ongoing monitoring of water quality, soil health, and other risk factors can help prevent such incidents.

CHAPTER 2 – EFFICACY AND IMPACT OF SANITIZERS IN CONTROLLING PATHOGENIC BACTERIA ON IRRIGATION WATER

1. Introduction

Over the past three decades, foodborne illnesses associated with human pathogens in fresh produce have emerged as a significant concern for public health (Hussain & Gooneratne, 2017; Kowalska, 2023a). Outbreaks commonly involve leafy greens, root vegetables, and vine-grown fruits. Many of these incidents can be traced back to certain practices within the supply chain, often related to contamination from worker health issues, animal intrusion, poor equipment sanitation, and irrigation water (Bintsis, 2018; Iwu & Okoh, 2019; Rao & Ravishankar, 2019). Notably, the processes of growing, harvesting, and packing fresh produce lack steps to fully eliminate pathogens, making prevention of contamination critical (Kowalska, 2023b).

During the cropping cycle potential vectors and mechanisms for microbiological contamination can arise from the use of contaminated water sources for irrigation, as well as during harvest and packing activities (Ling et al., 2014; Rakhmanin et al., 2019; Rodrigues et al., 2019). A major concern is the ability of pathogenic bacteria to adhere to and become internalized within plant tissues, a situation worsened by inadequate sanitation practices (Erickson, 2012). Water, crucial for spreading these organisms across various operations, is recognized as a significant risk by both the FDA and consumers of raw fruits and vegetables (FDA, 2024; Hopper & Rogers, 2023; Truitt et al., 2018). Although the exact sources of crop contamination are often not identified, numerous outbreaks have been directly or indirectly linked to contaminated irrigation water (FDA, 2024) highlighting the importance of ensuring water safety at early production stages to avoid introducing human pathogens into the supply chain.

Water not only plays a pivotal role in the delivery of pesticides, fertilizers, and adjuvants but also supports critical postharvest operations such as cooling and washing. Access to high-quality water is

essential for ensuring crop safety throughout the cropping cycle. Nevertheless, the quality of water can be compromised by environmental factors, animal intrusion, and farm waste, significantly affecting multiple crop rotations (Giri and Qiu 2016). Preharvest agricultural water is recognized as a route of contamination for foodborne pathogens during the production of fruits and vegetables (Jay et al. 2007; R. Gelting et al. 2005; R. J. Gelting et al. 2011; Bos, Carr, and Keraita, n.d.; F. D. and A. FDA 2024; Truchado et al. 2018). Although various strategies, including preharvest water treatments, have been proposed to mitigate this risk (Gurtler & Gibson, 2022b; Krishnan et al., 2021; Matthews, 2023), there remains a gap in the literature concerning the specific sanitizer concentrations, contact times, and water chemistry parameters, to effectively inactivate *Salmonella (*SM*)*, *E. coli* O157:H7(EHEC)*,* and *Listeria monocytogenes* (LM*)* in irrigation water.

Currently, fresh produce growers face three main challenges in accessing safe water sources: 1) weather and climate change impacts on snow mass and groundwater accumulation; 2) the absence of a reliable microbial water standard that meets food safety requirements for fresh produce; and 3) the need for effective chemical, physical, or mechanical treatments to ensure irrigation water safety. Managing water quality during pre-harvest operations has become a primary challenge, particularly when physical and mechanical treatments fail to provide the necessary volumes of safe irrigation water needed to maintain yields and plant quality (Beuchat et al., 2001a; Chen et al., 2021; De Luca et al., 2008; López-Gálvez et al., 2017; Van Boxstael et al., 2013). While several chemical treatments have been considered effective, they require a precise and complex approach for handling, using, and monitoring their effectiveness. Further, their suggested effectiveness lacks standardization and benchmark parameters that could be used to assess effectiveness across an array of water chemistries, making the selection of one of these sanitizers a significant gamble. Furthermore, under the Produce Safety Rule (PSR), the use of an EPA labeled sanitizer for treatment of irrigation water is mandatory (PSA 2024), however, currently, there are no sanitizers with such a label.

To date, the FDA has considered adopting microbial recreational water standards developed by the EPA for use in irrigation water. However, these standards have limited applicability in agricultural settings. Good Agricultural Practices (GAPs) provide guidelines that cover more than microbial water quality, including the definition of water sources, usage, application methods, and irrigation frequency relative to harvest activities (Hopper & Rogers, 2023; Van Boxstael et al., 2013). Although the FDA's 2011 PSR adopts these metrics, it lacks clear standards for microbial water quality, leaving growers to establish their own metrics and potential mitigation treatments. This led to the widespread use of unregistered chemical treatments for water safety. In April 2020, the EPA updated pesticide quality standards, setting requirements for sanitizers to control pathogens in irrigation water. Two challenges were highlighted: the lack of effective sanitizers and the difficulty of implementing treatments without clear guidelines and benchmarks. Factors influencing sanitizer use in irrigation water include concentration, type of organism, contact time, turbidity, organic matter, pH, hardness, and chemical properties (Lechevallier et al., 1981; Murphy et al., 2023; Pangloli & Hung, 2013; Preston et al., 2010; Waters & Hung, 2014). This information, crucial for global growers, will become increasingly important once the FDA implements the final mandates of section E of the PSR focused on preharvest agricultural water.

Before the FDA's 2011 PSR, farmers applied sanitizers to control the microbial quality of irrigation water using chemistries that may not have reflected the accurate effectiveness against human pathogens in these systems. Guidelines, standards, and health assessments address this need by providing scientifically based information to meet food safety requirements. However, further research is needed to fully understand the best approach to ensure water quality throughout the entire cropping cycle (LaBorde 2018; Younes et al. 2022, Fewtrell and Bartram 2001). Understanding the impact of water chemistry on pipe corrosion, soil and crop health, and the formation of carcinogenic compounds by sanitizers is essential for supporting agriculture (Monarca et al., 2002; Villanueva et al., 2015). However, the overall effectiveness of these treatments remains uncertain, and critical aspects are often not thoroughly described in the literature, making it difficult to establish clear benchmarks. The effects of water sanitation treatments on

plant health, soil microbiome, pH, electrical conductivity, and pipeline corrosion are not well-explored. Much of this scientific information is proprietary, limiting access for fresh produce growers. These challenges represent a global issue for the fresh produce industry (Van Boxstael et al., 2013), with potential significant economic and health consequences if not addressed before new regulations take effect.

The absence of effective microbial standards for irrigation water represents a significant obstacle in ensuring its quality and safety for use on fruits and vegetables (Mathews, 2023). Moreover, the effectiveness of current sanitizer solutions in controlling human pathogens in irrigation water remains questionable, which is critical for minimizing fresh produce contamination. The interaction of water chemical treatments, such as peracetic acid (PAA) and chlorine-based sanitizers, with organic matter in irrigation water can lead to the formation of carcinogenic by-products (Lee & Huang, 2019) and the formation of viable but not culturable (VBNC) cell lines (Pazos-Rojas et al. 2023). This not only exacerbates contamination issues but also poses risks for outbreaks and farmland integrity. The lack of EPA-registered sanitizers, employing these or newer chemistries effectively to eliminate human pathogens from irrigation water, represents a further challenge. This project aims to achieve two main objectives: (1) generate information to support the use of current and novel water chemistry treatments for the elimination of SM, EHEC and LM in accordance with 2020 EPA requirements and (2) provide information regarding the impact of sanitizers that meet EPA requirements on pipe corrosion, plant health, and soil chemical and physical properties in pre-harvest environments.

2. Material and Methods

2.1 Strain Collection

Strains associated with fresh produce outbreaks were selected, including *Listeria monocytogenes* from a cantaloupe outbreak, *Salmonella* Newport from a tomato outbreak, *E. coli* O157:H7 from a lettuce outbreak, and environmental generic *E. coli* W778 (ECW77) isolated from a water source in Salinas Valley, California, along with surrogate strains. These strains, resistant to rifampicin at 50 µg/mL (Rif50), were acquired from Dr. Trevor Suslow at the Department of Plant Sciences, University of California Davis. The

collection included *L. monocytogenes* strain 390-6 (LM390), *L. innocua* ATCC 33090 LOT: 57765700 (LI330), three *Salmonella* serovars; S. Typhimurium SPN452 (ST452), S. Montevideo PTVS 285 (SM285), and S. Newport PTVS067 (SN067), and four *E. coli* strains; *E. coli* O157:H7 PTVS016L (EHEC016; this strain is resistant to 50 µg/mL kanamycin [KAM]), generic *E. coli* W778 (GECW77), and two avirulent *E. coli* O157:H7 strains; ATCC 4388 (AEHEC438) and ATCC 700728 (AEHEC700). For the sanitizer evaluations, separate inoculation events used cocktails of two *Listeria* (LM390 and LI330)-LMIC, three *Salmonella* (ST452, SM285 and SN067)-SMC, and four *E. coli* strains (EHEC016, AEHEC438, AEHEC700 and GECW77)-EHEC4, while a combined cocktail of two *Listeria* (LMIC) and three *E. coli* (AEHEC438, AEC700 and GECW77)-EHEC3 strains was employed for all greenhouse evaluations.

2.2 Cocktail Preparation for Sanitizer Evaluation and Greenhouse Sanitizer Comparison

Cocktails were prepared from cultures grown on trypticase soy agar (TSA) plates with Rif50, following a modified version of the protocol by Gutiérrez-Rodríguez et al. (2012). Using an L-shaped spreader, cells were scraped from the agar and suspended in 1 mL of 0.01 M K2PO4 buffer at pH 7. This cell suspension was then transferred into 40 mL of the same buffer. Three plates, yielding a total of 3 mL of cell suspension, were combined with the 40 mL buffer, and the suspension was adjusted to an OD600 of 0.75, indicating a theoretical bacterial concentration of 9.0 log CFU/ml. Once the target OD was obtained, the specific bacteria strains were combined and diluted in K2PO4 to create two distinct cocktails. Cocktail concentrations were confirmed using differential/selective media based on the microorganisms. For water sanitizer evaluations, two bacterial concentrations were used; one at 6 log CFU/ml and another at 3 log CFU/ml, while for greenhouse evaluations, a final cocktail of EHEC3 and LMIC at 5 log CFU/ml was utilized.

2.3 Sanitizer Evaluation

The experimental design involved a comprehensive evaluation of four commercial sanitizers: sodium hypochlorite (Lab Alley; Lot. No. 21L17651F1/Solution3-EPA Reg. No.70925-1) commonly

known as chlorine, peracetic acid (PAA) (SaniDate 5.0; EPA Reg. No.70299-19), potassium acid sulfate (KAS) (Jones Hamilton; Lot. No.WF10602), and sodium acid sulfate (SAS) (pHase; Lot. No. WG221X2). Each sanitizer was tested at three concentrations across four water turbidity levels and two contact times. The study aimed to assess their efficacy in reducing bacterial populations of the *E. coli* cocktail-EHEC4, *Salmonella* cocktail-SMC, and *Listeria* cocktail-LMIC, at concentrations of 3 and 6 log CFU/ml. All sanitizer concentrations were chosen based on industry practices.

Additional experimental treatment details are provided in Table 7. The top three treatment combinations capable of providing a 3-log reduction of the selected organisms at a contact time of 5 min with no viable cells after enrichment were considered the most effective and were used to study the corrosive impact of sanitizers on pipelines, their impact on plant and soil health, and their potential to reduce human pathogens in romaine lettuce.

Table 7. Experimental approach to test sanitizer efficacy of Chlorine, PAA, SAS and KAS.

	Concentration	**Turbidity (NTU)**	**Contact time (minutes)**	**Pathogenic Bacteria**	**Bacteria concentration**
Sodium Hypochlorite	15 ppm (Low) 30 ppm (Medium) 60 ppm (High)	0 50 100 150	3 5	LMIC SMC EHEC4	3 log CFU/ml 6 log CFU/ml
Peracetic Acid (PAA)	10 ppm (Low) 20 ppm (Medium) 30 ppm (High)				
Potassium Acid Sulfate (KAS)	0.40% (Low) 0.50% (Medium) 0.60% (High)				
Sodium Hydrogen Sulfate (SAS)	0.40% (Low) 0.50% (Medium) 0.60% (High)				

Note: This experiment has a factorial design. Each treatment was evaluated in triplicates. The combination evaluated were defined by Sanitizer (1) x Sanitizer Concentration (3) x Turbidity Level (4) x Contact time (2) x Bacteria (3) x Bacteria Concentration (2), possible treatments evaluated per sanitizer 144, each treatment was evaluated in triplicates. Total of samples evaluated per sanitizer 432 samples. NTU (Nephelometric Turbidity Unit). LMIC (*L. monocytogenes* cocktail), SMC (*Salmonella* cocktail), EHEC4 (*E. coli* cocktail).

2.3.1 Preparation of Water Turbidity

To simulate common irrigation water turbidity, soil was collected from the Agriculture Research, Development, and Education Center South (ARDEC), located north of Fort Collins, CO, USA. This soil was used to prepare water samples with turbidities of 0, 50, 100, and 150 NTU in 5-liter flasks of deionized water (DW). Autoclaved soil was added incrementally to achieve these turbidity levels. Water turbidity was measured using a calibrated portable turbidimeter (HACH 2100Q01 2100Q). The water was then transferred into 100 ml sterile specimen cups (Fisher-Cat.No.14375152) to initiate further testing and sample preparation.

2.3.2 Sanitizer Preparation

Sanitizer concentrations were prepared in 1 L flasks of DW. Chlorine solutions were adjusted to a pH of 5.5 and 6 using 15% citric acid (Oakwood Chemical, Cat.102738). The pH adjustments for the other sanitizers were not necessary (Beuchat et al., 2001b; López-Gálvez et al., 2017; Zanetti et al., 2007). Sanitizer concentrations were monitored using test strips for chlorine (NOBLE Chemical Inc, Lot.224422) and PAA (Bartovation, PPA03V100), and through acid-sulfate titration for KAS and SAS. All sanitizers were prepared and used on the same day.

2.3.3 Preparation and Treatment of Contaminated Water

To simulate contaminated irrigation water, a 99 ml specimen cup containing water was inoculated with 1 ml of a cocktail of each selected bacterial pathogen to achieve final concentrations of 6 and 3 log CFU/ml. A 1 ml aliquot of the respective sanitizer was then added and mixed using a sterile wooden stick. Contact times of 3 and 5 minutes were maintained and controlled using a timer. Each treatment was evaluated in triplicates, obtaining a total of 432 samples per sanitizer (chlorine, PAA, SAS and KAS).

After treatment, each sanitizer solution was neutralized to stop further pathogen inactivation. For chlorine, 7 mg of sodium thiosulfate (HACH, Cat.101649) was used per ppm to inactivate the solution. PAA was neutralized using a neutralizing buffer (HiMedia, Cat. M1334A), while SAS and KAS were

inactivated with sodium hydroxide (VWR, Cat.MK7708-10). The effectiveness of inactivation was verified using chlorine and PAA strips; pH strips confirmed the inactivation for SAS and KAS to a final pH of 5.0.

Following neutralization, 100 µL of each sample was plated in duplicate on selective and differential media to determine viable pathogen populations: LMIC populations on Listeria CHROMAgar™/Rif50, SMC on XLT4-Agar/Rif50/Penta Chloronitrobenzene (PCNB), and EHEC4 on CHROMAgar™ O157/Rif50/PCNB and CHROMAgar™ O157/KAM/PCNB (counts from both media were combined and reported as EHEC4). To assess the extent of bacterial damage caused by the sanitizer, an enrichment process was carried out to determine whether it achieved complete inactivation of bacteria at concentrations of 3 or 6 logs.

The enrichment process was conducted for both low (15 ppm Chlorine, 10 ppm PAA, KAS and 0.4% SAS) and high sanitizer concentrations (60 ppm Chlorine, 30 ppm PAA, KAS and 0.6% SAS). After the samples were plated, 50 ml of each sample was mixed with 50 ml of 2X Universal Pre-enrichment Broth (UPB) (VWR, Tryptone to 2X YT Broth, Life Technologies Cat. 90001-755) in a 1:1 ratio, and then incubated overnight at 35°C. For LMIC enrichment, 10 ml of the 2X UPB solution was transferred to 90 ml of Buffered Listeria Enrichment (BLE) (HiMedia, SKU GM1578) and incubated for 48 hours at 30°C. The mixture was then streaked on Listeria CHROMAgar™/Rif50. For SMC enrichment, 10 ml of the 2X UPB was added to 90 ml of BD Difco™ dehydrated culture media: fluid Tetrathionate (TBB) (Fisher Scientific, Cat. DF0104176) and incubated for 6 hours at 42°C. Subsequently, 20 ml of the TBB solution was transferred to 180 ml of mBroth and incubated overnight at 37°C. The mixture was then streaked on XLT4-Agar/Rif50/PCNB. For EHEC4 enrichment, 10 ml of the 2X UPB was mixed with 90 ml of mEHEC and incubated for 18 hours at 37°C. The resulting mixture was streaked on CHROMAgar™ O157/Rif50/PCNB. The results of the enrichment are reported based on the number of positives found out of the total replicates.

Finally, the effect of each neutralizer on the potential inactivation of the inoculated cocktails was also assessed in two separate experimental trials. Under those conditions, specimen cups were inoculated

with the bacterial cocktails, followed by the addition of sodium thiosulfate (0.105-, 0.210-, and 0.420g) for chlorine concentrations of 10, 30 and 60 ppm respectively, neutralizing buffer (1-, 2-, and 2 ml) for PAA concentrations of 10, 20, and 30 ppm, and sodium hydroxide for SAS (3.730, 4.630 and 5.130 ml) and KAS (3.040, 3.630 and 4.355 ml) to inactivate concentrations of 0.4, 0.5, and 0.6%. Bacterial survival was tested by plating each strain on selective and differential media to determine the impact of the neutralizer on bacterial survival. The addition of neutralizer agents did not affect bacterial survival (data not shown).

2.3.4 Corrosive Impact of Sanitizers on Pipelines

The top three sanitizer treatments, which included 60 ppm Chlorine, 30 ppm PAA and 0.6% SAS, were selected to assess their corrosive effects on stainless steel 304, stainless steel 316, and aluminum alloy 3004/3003, commonly used in irrigation lines. The assessment was conducted in triplicate samples using metal coupons (Pacific Sensor) to estimate corrosion rates in cm per year, employing a method developed by Dunn. The experiment was divided into two phases, each lasting 14 days, at temperatures of 21°C and 73°C, totaling 30 days. Sanitizer treatments were prepared, and 100 ml of each was poured into a glass container with a lid. Metal coupons were weighed using an analytical balance (OHAUS, Explorer-EX 124), submerged in the sanitizer solutions, and the containers were partially closed and placed in a dark area at room temperature. After 14 days, the solutions were discarded, and the coupons were dried and weighed. New sanitizer solutions were prepared, and the same coupons were submerged for an additional 14 days at 72°C. After this period, the coupons were dried and weighed again to estimate their corrosion capacity using Dunn's established procedure.

2.4 Greenhouse Sanitizer Comparison

2.4.1 Romaine Lettuce Growth Conditions

Two cultivars of romaine lettuce, red cv. Ezbruke and green cv. Coastal Star, were grown by the commercial nursery Dursants until they reached the 3 to 4 leaf stage. The lettuces were then transplanted

into smart pods (3-gallon capacity) filled with ARDEC soil and placed in a biosafety level 2 greenhouse. Conditions in the greenhouse were maintained at an average temperature of 20 to 22℃ during the day and 18 to 21 ℃ at night, with a 17:7-hour light-dark cycle. The duration of the experiment was 10 weeks until the lettuce reached the mature stage. Lettuce plants were watered daily to maintain consistent irrigation; the amount of water was determined based on field capacity to ensure homogeneous growth. The experiment was conducted from May to June and repeated from September to October 2024.

2.4.2 Greenhouse Inoculation and Sanitizer Application

Once the romaine lettuce reached the mature stage, each plant was spray-inoculated with a Log 5 combined cocktail of LMIC and EHEC3. A total of 180 plants were inoculated in each experiment trial The spray inoculation was carried out in the greenhouse using a 1-gallon automatic sprayer (RYOBI One+ 18V) with the nozzle adjusted to deliver larger droplet sizes and a constant pressure of 320 PSI. Each pot with a single romaine lettuce received a 50 ml aliquot of the pathogenic cocktail. Twenty-four hours post inoculation, the top three sanitizers were prepared (60 ppm chlorine, 30 ppm PAA and 0.6% SAS) and applied to each plant using the same automatic sprayer, delivering a 50 ml dose per plant. All lettuce plants inside the greenhouse were divided into four groups, each with a total of 48 plants inside plastic trays (130x255cm), with an equal mix of red and green cultivars per tray to ensure homogeneous sampling. Each tray was sprayed with a specific sanitizer, and one tray served as a control and was sprayed with DI water. The entire lettuce plant (excluding the roots) was evaluated at day 1 (n= 30), 24 hours after the inoculum application, same day the sanitizer concentration was applied on the plant and the next sampling days were evaluated; 4 (n=48), 8 (n=48), and 12 (n=52) days after sanitizer application. Before harvest, plant health was assessed visually to identify any changes in the leaves, such as color, injuries, discoloration, or death.

After the first experiment ended, plants roots were removed from each pod. For 7 weeks each pod was watered with 800 ml of the specific sanitizer concentration every other day. Each pod receives the same sanitizer during the whole experiment. The constant sanitizer application took place before the start of the next growing period from September to October. During this interval between experiments, each pot

received a total of 5.6 L of sanitizer solution at the end of the 7 weeks interval during experiments. The constant sanitizer application was conducted to evaluate the impact of sanitizer concentrations in soil physiological and nutritional content.

2.4.3 Bacterial Recovery and Enumeration

For bacterial recovery, lettuce plants excluding roots were harvested at four different times. At each sampling, the same number of plants from each cultivar was harvested. The samples collected were as follows: 32 samples on day 0, 48 samples on day 4 - 8, and 52 samples on day 12. Each romaine lettuce was placed in a sterile filtered bag (Whirl-Pak 25.4 x 38.1 cm), weighed on a scale (OHAUS Compass™ CX) and transported to the produce safety laboratory located at the Nutrien building. Each bag was filled with 75 ml of 0.01 M K2PO4 buffer containing tween20 and homogenized using a stomacher for 30 seconds. The supernatant was then transferred to a 50 ml sterile centrifuge tube. Each tube was vortexed for 5 seconds to further homogenize the samples, which were then plated on selective and differential media for bacterial enumeration LMIC populations were plated on Listeria-CHROMAgar™/Rif50 and EHEC3 populations on CHROMAgar™ O157/Rif50/PCNB, the results of EHEC3 were divided into generic *E. coli* (GEC) and avirulent *E. coli* O157:H7 (AEHEC), this bacterial differentiation was made based on morphological differences on the bacterial growth on the agar such as colony color (Gutiérrez-Rodríguez et al. 2019; Lopez-Velasco et al. 2012).

2.4.4 Soil Chemical and Physical Properties

After both trials in the greenhouse experiment, soil samples were collected from each pod according to the soil sampling instructions provided by the Soil, Water, and Plant Testing Laboratory at CSU Spur – Department of Soil and Crop Sciences. The test performed was "Soil: S1 Complete Package 2023S3658-3693." A total of 36 samples were evaluated, with 6 samples evaluated per treatment: 60 ppm chlorine, 30 ppm PAA, 0.6% SAS, DI water, and control samples from pods that did not receive any treatment or irrigation application during the whole experiment. The test evaluated parameters such as pH, soluble salts (EC), organic matter (OM), lime, cation exchange capacity (CEC), base saturation percentage, nitrate-N

(NO3), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), sulfate-sulfur (SO4-S), zinc (Zn), iron (Fe), manganese (Mn), copper (Cu), and boron (B). The soil parameters measured included pH, electrical conductivity (EC) at a 1:1 ratio, phosphorus (P) concentration measured via the Olsen Bicarbonate method, sulfur (S) concentration measured by the M-3 method, sodium (Na) concentration measured using ammonium acetate, sodium percentage, calcium percentage, and copper (Cu) concentration measured using the Hot Water-DTPA method The laboratory results were evaluated, and an ANOVA test was conducted to identify changes in the parameters between treatments.

2.5 Statistical Analysis

2.5.1 Water Quality and Sanitation Effect on Human Pathogens

A factorial design was employed for this experiment. Evaluating a total of 144 treatments per sanitizer. The treatments were calculated based on the number of possible combinations of the variables evaluated. Each treatment was evaluated in triplicates.

Statistical analyses were performed in RStudio (2023.06.1). A no-parametric test “Shapiro test” was employed to identify statistical differences between the sanitizers, and Kruskal Wall test was performed to identify the treatments that achieved the highest bacteria reduction. Spearman correlation was performed to identify the variables related to the bacterial reduction. Bacterial reduction is defined as the log CFU/ml reduction for all microorganisms evaluated.

2.5.2 Corrosion Effects in Stainless Steel and Aluminum Coupons

Statistical analyses were performed in RStudio (2023.06.1). A no-parametric test “Shapiro test” was employed to identify statistical differences between the sanitizers, and Kruskal Wall test was used to evaluate the corrosive impact per sanitizer on the coupons.

2.5.3 Impact of Sanitizers Solution on Plant, Soil Chemical – Physical Properties and Inactivation of Pathogenic Bacteria from Romaine Lettuce Leaf.

A slip-plot design with repeated measures over time was used for the greenhouse experiment. R Studio was employed to conduct the Shapiro test and Kruskal-Wall analysis to evaluate the bacterial reduction achieved by the sanitizer. The impact of the sanitizers on soil chemical and physical properties was evaluated in R Studio using ANOVA and Tukey tests to identify the differences between the variables

3. Results and Discussion

3.1 Water Quality and Sanitation Effect on Human Pathogens

This study found a positive correlation ($p<0.05$) between bacterial reduction and several variables: contact time (0.12), sanitizer concentration (0.25), and inoculum dose (0.77). Conversely, there was a negative correlation with turbidity (-0.08), with all correlations exhibiting significant differences per statistical method (Table 8). Bacterial reduction was defined by the combined reduction obtained from the three microorganisms evaluated. Reductions were combined to evaluate the overall impact of sanitizer treatments in gram positive and negative bacteria. The strongest correlation was determined between bacterial reduction and inoculum dose, the bacterial reduction is positively correlated with the initial inoculum level present in the sample. Contact time and sanitizer concentration, exhibit a positive correlation with sanitizer concentration suggesting that longer exposure times and higher sanitizer concentrations could increase the sanitizer effectiveness increasing the capacity of bacterial reduction. On the other hand, the negative correlation found with the turbidity level suggests that increasing turbidity levels in the sample negatively affects the sanitizer's effectiveness, resulting in lower bacterial reductions.

Table 8. Correlation matrix between bacterial reduction and multiple variables.

	Bacterial Reduction	**Turbidity**	**Contact Time**	**Sanitizer Concentration**	**Inoculum Dose**
Bacterial Reduction	1.0000	-0.086**	0.1286**	0.2540*	0.7760**
Turbidity	-0.086**	1.0000	0.0000	0.0000	0.0000
Contact Time	0.1286**	0.0000	1.0000	0.0000	0.0000

Sanitizer Concentration	0.2540*	0.0000	0.0000	1.0000	0.0000
Inoculum Dose	0.7760**	0.0000	0.0000	0.0000	1.0000

Note: The bacterial reduction is defined by the reduction of SMC, LMIC and EHEC4 results combined. This table reports the correlations between the regression variables. Variables are defined as bacterial reduction, turbidity, contact time, and sanitizer concentration ∗∗, and ∗ indicate significance at 0.01, and 0.05 levels, respectively.

The most critical factor in achieving higher bacterial reduction was the initial bacterial load in the sample, followed by the sanitizer concentration and contact time. The inoculum dose 6 log CFU/ml presents higher bacterial reductions, which is explained by the number of bacterial cells present in the sample compared to 3 log CFU/ml, higher inoculum concentration led to higher bacterial reductions. Interestingly, the presence of higher bacterial loads did not affect the effectiveness of sanitizers, although the literature on the impact of bacterial load is limited (Al Ali et al., 2020; Vandegrift et al., 2017). Previous studies have mainly focused on the effects of sanitizer type, contact time, and organic matter for the optimal timing for sanitizer use to reduce bacterial loads in specific sources. For example, Stefanello et al. (2021) noted that factors such as sanitizer type, contact time, sanitizer concentration, and organic load influence the antimicrobial action of sanitizers, aligning with the findings of this study. According to Scheusner et al. (1971), a higher concentration of sanitizer results in a greater proportion of injured or dead bacteria, reducing the likelihood of bacterial recovery and proliferation, and enhancing the effectiveness of the treatment. However, because these other studies had no predetermine benchmarks for water chemistries and bacterial inactivation it is difficult to assess the overall similarity between studies and their outcomes.

For example, Neo et al. (2013) evaluated the efficacy of chlorine and PAA at different concentrations for reducing natural microflora, EHEC, SM, and LM on mung bean sprouts. They found that higher sanitizer concentrations provided greater bacterial reduction, with the most effective results achieved using PAA (Neo et al., 2013). This suggests that PAA may be an effective alternative to chlorine as a sanitizer treatment. The Neo et al. (2013) study primarily focused on bacterial reduction without evaluating the impact of water chemistry on sanitizer effectiveness. While PAA has become a favorable option for sanitation, chlorine remains widely used for disinfection, particularly in water treatment,

although it can be inactivated by high levels of organic matter as with PAA (How et al., 2017; Teng et al., 2018).

Turbidity plays a crucial role in irrigation water, as it can increase with the distance traveled through agricultural pipelines (McCoy & Olson, 1986). A study by Lechevallier et al. (1981) observed a negative correlation between turbidity and bacterial reduction resulting from sanitizer treatments, suggesting that maintaining turbidity levels between 1 and 5 NTU could ensure adequate water quality; however, under commercial fresh produce practices with varying water sources, this level of turbidity might not be achieved with current sand filter technologies and when using surface water resources. Turbidity, often used to measure water cloudiness, has been associated with microbiological contamination. Higher turbidity in water treated with sanitizers reduces their effectiveness ((McCoy and Olson 1986). Figure 3 illustrates how sanitizer effectiveness decreases at higher turbidity levels. In this study, turbidity was found to have a negative impact (-0.086) on bacterial reduction, indicating that elevated turbidity levels reduce sanitizer efficacy, regardless of the sanitizer used. Further studies corroborate the impact of different turbidity levels on the efficacy of sanitizer treatments (Mohamed et al., 2015; Nielsen et al., 2022; Preston et al., 2010).However, the findings in this study, that higher turbidity levels reduce sanitizer efficacy, contradict several studies that reported that turbidity levels or organic matter do not affect the efficacy of sanitizer treatments (J. Banach et al., 2015; Gehr et al., 2003; Irakoze et al., 2022). This discrepancy could be attributed to the lack of standards and benchmarks previously available to assess sanitizer efficacy when treating irrigation water.

Under consistent benchmarks as those provided by the 2020 EPA standards, our findings suggest that it is necessary to select an effective sanitizer treatment that remains effective even at higher turbidity levels to prevent bacterial contamination in crops (Figure 3). Previous research has shown that high turbidity levels are directly correlated with higher coliform content in water, indicating that water with a 5 NTU turbidity will have more than one coliform per 100 ml, reducing the efficacy of sanitizers (Lechevallier et al., 1981). As shown in Table 8, higher bacterial loads in the water negatively affect the

effectiveness of sanitizers, aligning with the findings of Lechevallier et al. (1981). However, higher bacterial load or turbidity level will depend on the water source (Brainwood et al., 2004). Water quality can change throughout the seasons or be influenced by activities conducted around water sources, such as agriculture or livestock practices. High turbidity has been related to acute gastrointestinal illness, which highlights the relationship between turbidity levels and microbiological contamination in natural water sources (De Roos et al., 2017). However, our study does not find a correlation between bacteria concentration and turbidity levels. This can be explained by the fact that the inoculation was performed in a laboratory, and DI water was used to conduct the experiment, eliminating the environmental impact and interactions between water sources, environmental factors, and bacteria present. Nevertheless, our findings indicate that turbidity will significantly contribute to bacterial survival due to its impact on reducing sanitizer effectiveness.

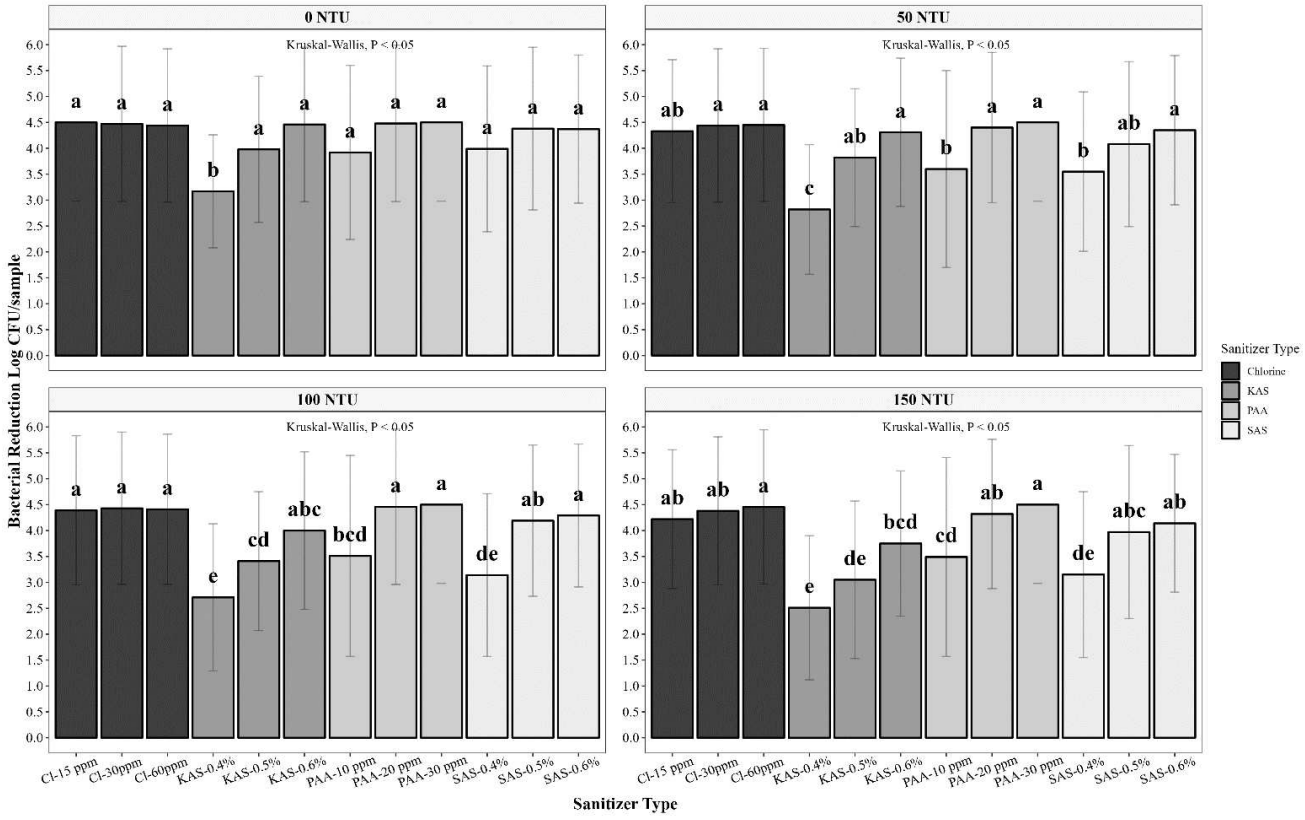

Figure 3. Overall effectiveness of sanitizer concentrations against four turbidity levels.

Note: The bacterial reduction is defined by the reduction of SMC, LMIC and EHEC4 results combined. NTU is defined as 'Nephelometric Turbidity Units'. Results are exhibited as log CFU/sample, where sample means 100 ml. The statistical analysis was conducted to define the differences between sanitizers within each turbidity level: 0, 50, 100, and 150 NTU. Treatments with different letters indicate statistical differences between treatments. Error bars represent standard deviation.

While farmers concentrate on controlling water quality within their irrigation systems, having complete control over external factors affecting water sources to promptly address bacterial contamination is often impractical. This study reiterates the importance of understanding bacteria concentration and turbidity levels in water sources to assist farmers in effectively treating their irrigation water, optimizing sanitizer effectiveness, and minimizing the potential risks associated with using contaminated agricultural water on fresh produce.

The correlation matrix also identified contact time as the third key factor affecting bacterial reduction, following sanitizer concentration. Numerous studies have shown that increasing contact time

enhances bacterial reduction when using chlorine and PAA as sanitizers (Ghostlaw et al., 2020; Hassenberg et al., 2017; Rossi et al., 2007). Longer contact times increase the likelihood that sanitizers will damage bacteria in water samples, highlighting the rationale behind the 5-minute contact time requirement established by the EPA method published in 2020, (EPA, 2020). However, this benchmark, may not be reflective of most contact times achieved within different commercial irrigation systems, in which adjacent fields to irrigation systems would receive and use treated or untreated water with less than 5-minute contact time. Such condition highlights a potential risk of contamination in those fields if the necessary contact time to inactivate SMC, LMC, and EHEC is not achieved as described under these experimental conditions.

Previous research by Ghostlaw et al. (2020) highlighted that the effectiveness of PAA, and chlorine is significantly related to contact times and critical in driving treatment effectiveness for greater *Salmonella* reduction in pre-harvest agricultural water. In agricultural settings, contact time is influenced by various factors, including the flow rate of the irrigation system, the size of the farm, the diameter of the pipelines used, and the time elapsed between the application of the sanitizer and its arrival at the irrigation sprinklers. Under our experimental conditions, the most effective sanitizer concentrations per sanitizer used on corrosion and soil and plant health studies and data analysis focused on the sanitizer's effectiveness at a turbidity level of 150 NTU, an inoculum dose of 6 log CFU/ml, and comparing contact times of 3 and 5 minutes (Figure 4). Notable differences were observed across different sanitizer concentrations: low (15 ppm chlorine, 10 ppm PAA, 0.4% SAS, and KAS), medium (30 ppm chlorine, 20 ppm PAA, 0.5% SAS, and KAS), and high (60 ppm chlorine, 30 ppm PAA, 0.6% SAS, and KAS) concentrations at those specific contact times. At medium and high concentrations, all sanitizers achieved a 3-log reduction in both 3 and 5 minutes.

At low concentrations, only KAS failed to achieve the 3-log reduction standard set by the EPA's 2020 addendum within 3 minutes, suggesting that this sanitizer concentration should be avoided. Based on EPA requirements we could infer that all medium and high concentrations for Chlorine, PAA, KAS, and SAS were able to achieve a 3-log reduction within 3 to 5 minutes of contact time, however, when the

viability of cells was assessed after enrichment multiple samples tested positive after enrichment for medium concentrations of Chlorine, KAS and PAA (Table 9). For this reason, we decided to consider only the high concentrations of Chlorine, PAA, KAS and SAS as the most effective treatments (60 ppm chlorine, 30 ppm PAA, 0.6% SAS, and KAS).

Within irrigation systems of many leafy greens including romaine lettuce, both plastic and aluminum pipelines remained exposed to the environment allowing temperatures to rise to 60-70℃. At these temperatures Chlorine and PAA will become inactive, and no residual effect will remain inside the pipelines. This inactivation has not been associated with SAS and KAS sanitizers, potentially highlighting an advantage over these other chemistries. Although a 3-log reduction is a significant approach to reducing the risk of crop contamination, having viable cells in water could be a significant risk even at those elevated temperatures. It is essential to use effective sanitizer concentrations and adequate contact times to ensure appropriate treatments while also considering the potential soil and plant health effects when using continuous applications of sanitizers for the long-term sustainability of farming communities.

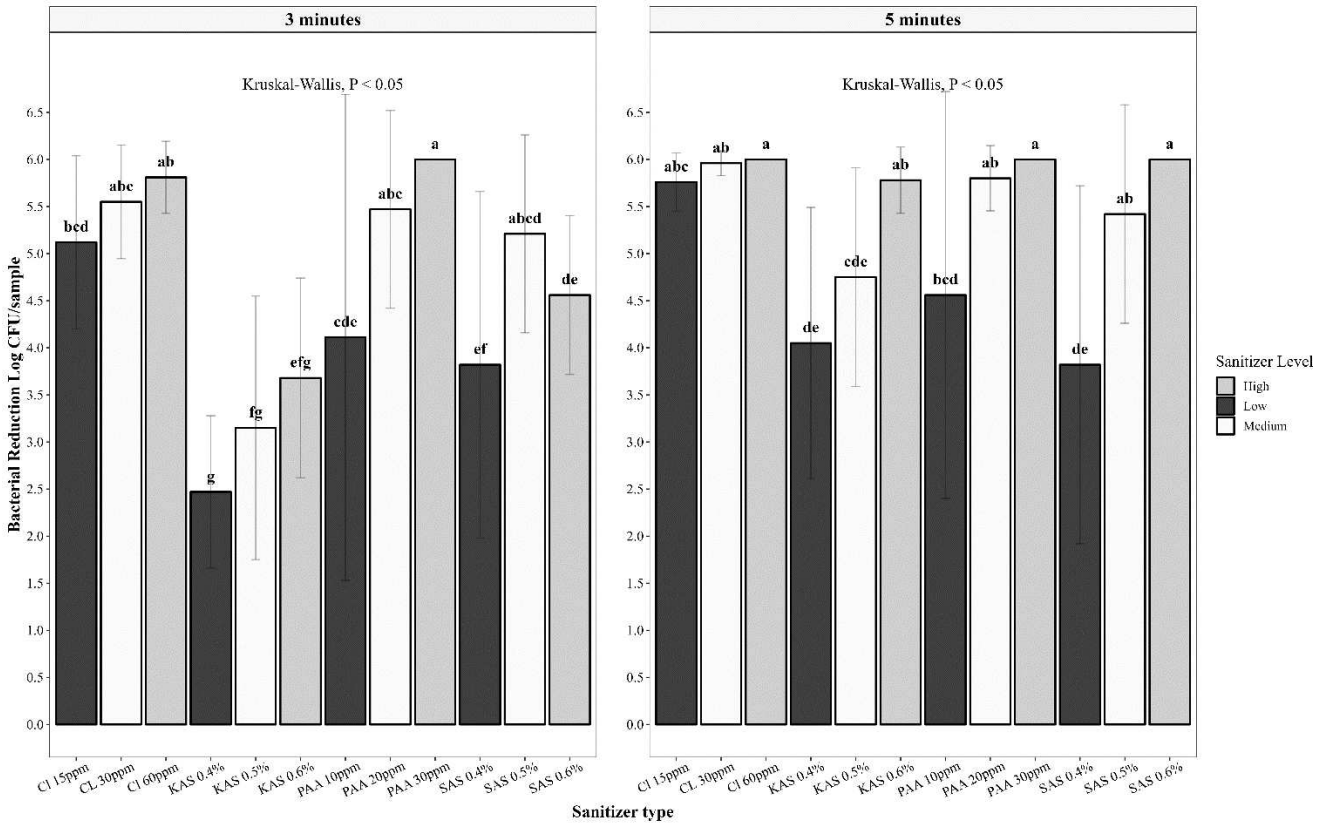

Figure 4. Sanitizer Efficacy: Reducing 6 logs- turbidity level of 150 NTU.

Note: Results are presented as log CFU/sample, where sample means 100 ml. The statistical analysis was conducted to define the differences between sanitizers within each contact time. Treatments with different letters indicate statistical differences between treatments ($p<0.05$). Error bars represent standard deviation. Sanitizer concentrations are divided into three categories: low (Chlorine 15 ppm, PAA 10 ppm, SAS, and KAS 0.40%), medium (Chlorine 30 ppm, PAA 20 ppm, SAS, and KAS 0.50%), and high (Chlorine 60 ppm, PAA 30 ppm, SAS, and KAS 0.60%).

Sanitizer effectiveness varies based on target microorganism to reduce. Figure 5 shows the bacterial reduction achieved by sanitizer concentration at 5 minutes, and a turbidity lever of 150 NTU per microorganism. LMIC (*Listeria* cocktail) exhibits the lowest bacterial reduction compared to the other bacteria, suggesting that this microorganism presents a higher survival rate compared to the other bacteria evaluated. This difference in survival may be attributed to the physiological and genetic differences between the microorganisms (Guerreiro, Arcari, and O'Byrne 2020; Tasara and Stephan 2007; Wiktorczyk-Kapischke et al. 2023; Feroz 2013). Understanding that the sanitizer efficacy may vary based on the target pathogen to reduce, is fundamental to improve disinfection and cleanness protocols based on the risk assessment analysis of the production facility.

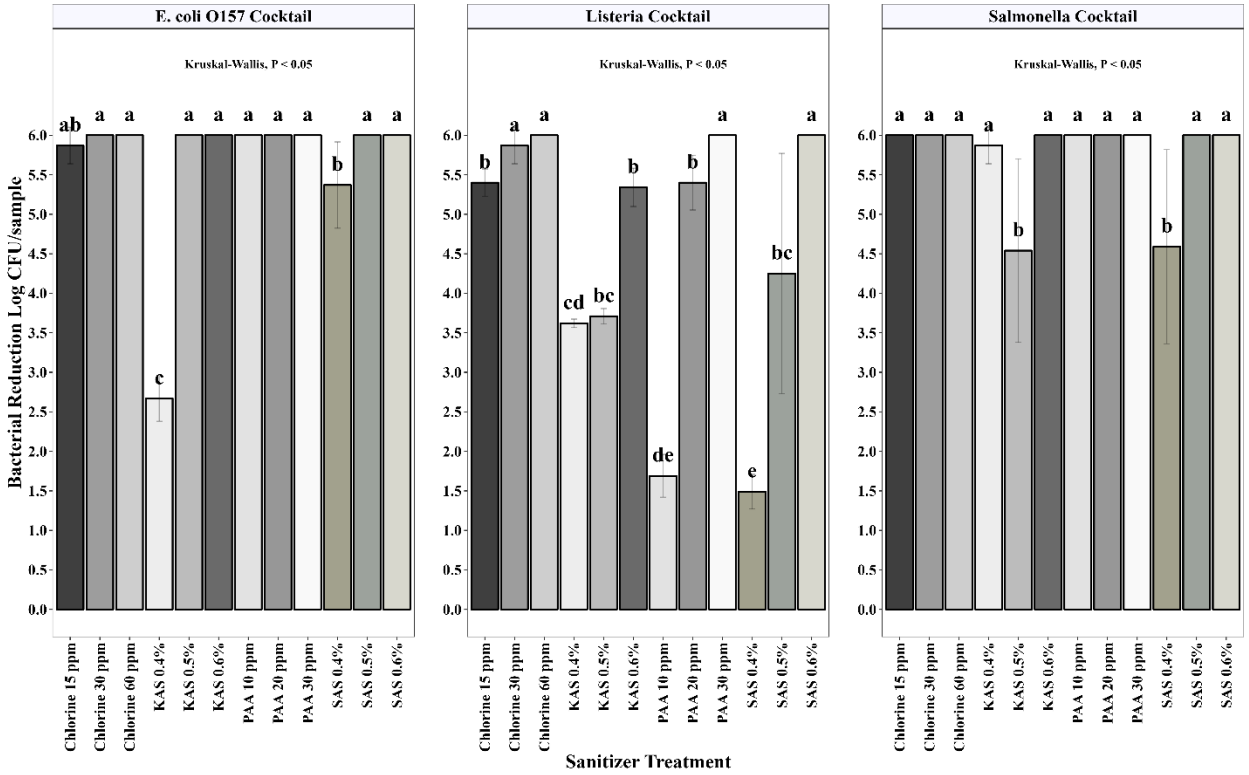

Figure 5. Bacterial reduction achieved sanitizer treatment divided based on the microorganism evaluated.

Note: Results are presented as log CFU/sample, where sample means 100 ml. The statistical analysis was conducted to define the differences between sanitizers within microorganisms evaluated. Treatments with different letters indicate statistical differences between treatments ($p<0.05$). Error bars represent standard deviation. Bacterial reduction achieved at contact time of 5 minutes and a turbidity level of 150 NTU.

Table 9 lists the number of positive sanitizer-treated water samples after the enrichment process. This information is critical to assess the true effectiveness of sanitizer treatments, as it describes which samples continue to have viable cells after the sanitizer treatment. The enrichment was performed for both high and low concentrations of the sanitizer treatments since both will indicate the overall spectrum of effectiveness of each treatment. For a 5-minute contact time SAS at 0.6%, chlorine at 60 pm and PAA at 30 ppm provided the 3-log reduction and no viable cells after enrichment (Table 9), indicating that these treatments produce irreversible injury to bacterial cells making them more effective and safer options for treating irrigation water. Injured cells are potentially as significant as healthy pathogen cells because they can recover and become functionally normal if they are in a favorable environment (BUSTA, 1976). Therefore, determining the presence of sub-lethally injured cells vs irreversibly injured cells is critical to the quality and safety of the selected treatments (Bozoglu et al., 2004; BUSTA, 1976; Wu et al., 2001, WU,

2008). Such results lack further understanding of the effects of these concentrations on metal corrosion, soil and plant health, and these other variables must be understood before selecting a specific treatment.

Table 9. Number of positive samples after each sanitation treatment.

Sanitizer Type and concentration	Turbidity (NTU)	Positive results after enrichment											
		SMC Log 6		SMC Log 3		LMIC Log 6		LMIC Log 3		EHEC4 Log 6		EHEC4 Log 3	
		3 minutes	5 minutes	3 minutes	5 minutes	3 minutes	5 minutes	3 minutes	5 minutes	3 minutes	5 minutes	3 minutes	5 minutes
KAS-0.4%	0	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	2 of 3
	50	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3
	100	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3
	150	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3
KAS-0.6%	0	3 of 3	0 of 3	0 of 3	0 of 3	3 of 3	0 of 3	0 of 3	0 of 3	3 of 3	3 of 3	0 of 3	2 of 3
	50	3 of 3	1 of 3	1 of 3	0 of 3	3 of 3	3 of 3	1 of 3	1 of 3	3 of 3	2 of 3	2 of 3	2 of 3
	100	3 of 3	3 of 3	3 of 3	1 of 3	3 of 3	2 of 3	3 of 3	2 of 3	3 of 3	3 of 3	3 of 3	3 of 3
	150	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3
SAS-0.4%	0	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	0 of 3	0 of 3	0 of 3
	50	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	1 of 3	3 of 3	1 of 3
	100	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	2 of 3
	150	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3
SAS-0.6%	0	3 of 3	3 of 3	0 of 3	0 of 3	3 of 3	3 of 3	0 of 3	0 of 3	3 of 3	0 of 3	0 of 3	0 of 3
	50	3 of 3	3 of 3	0 of 3	0 of 3	3 of 3	3 of 3	1 of 3	0 of 3	3 of 3	0 of 3	0 of 3	0 of 3
	100	3 of 3	3 of 3	3 of 3	0 of 3	3 of 3	3 of 3	1 of3	0 of 3	3 of 3	1 of 3	0 of 3	0 of 3
	150	3 of 3	3 of 3	3 of 3	0 of 3	3 of 3	3 of 3	0 of 3	0 of 3	3 of 3	1 of 3	0 of 3	0 of 3
PAA-10ppm	0	3 of 3	1 of 3	1 of 3	0 of 3	3 of 3	3 of 3	3 of 3	1 of 3	0 of 3	0 of 3	0 of 3	0 of 3
	50	3 of 3	2 of 3	1 of 3	1 of 3	3 of 3	3 of 3	3 of 3	0 of 3	0 of 3	0 of 3	0 of 3	0 of 3
	100	3 of 3	2 of 3	1 of 3	1 of 3	3 of 3	3 of 3	3 of 3	0 of 3	0 of 3	0 of 3	0 of 3	0 of 3
	150	3 of 3	3 of 3	2 of 3	1 of 3	3 of 3	3 of 3	3 of 3	0 of 3	3 of 3	3 of 3	0 of 3	0 of 3
PAA-30ppm	0	0 of 3	0 of 3	0 of 3	0 of 3	0 of 3	0 of 3	0 of 3	0 of 3	0 of 3	0 of 3	0 of 3	0 of 3
	50	0 of 3	0 of 3	0 of 3	0 of 3	0 of 3	0 of 3	0 of 3	0 of 3	0 of 3	0 of 3	0 of 3	0 of 3
	100	0 of 3	1 of 3	0 of 3	0 of 3	0 of 3	0 of 3	0 of 3	0 of 3	0 of 3	0 of 3	0 of 3	0 of 3
	150	0 of 3	1 of 3	0 of 3	0 of 3	0 of 3	0 of 3	0 of 3	0 of 3	0 of 3	0 of 3	0 of 3	0 of 3
Chlorine-15ppm	0	3 of 3	3 of 3	0 of 3	0 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	2 of 3	1 of 3	0 of 3
	50	3 of 3	3 of 3	2 of 3	0 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	1 of 3	0 of 3
	100	3 of 3	3 of 3	2 of 3	0 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	1 of 3	0 of 3
	150	3 of 3	3 of 3	3 of 3	0 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	3 of 3	1 of 3	0 of 3
Chlorine-60ppm	0	3 of 3	3 of 3	0 of 3	0 of 3	3 of 3	3 of 3	3 of 3	0 of 3	3 of 3	2 of 3	0 of 3	0 of 3
	50	3 of 3	3 of 3	0 of 3	0 of 3	3 of 3	3 of 3	3 of 3	0 of 3	3 of 3	3 of 3	0 of 3	0 of 3
	100	3 of 3	3 of 3	0 of 3	0 of 3	3 of 3	3 of 3	3 of 3	0 of 3	3 of 3	3 of 3	2 of 3	0 of 3
	150	3 of 3	3 of 3	0 of 3	0 of 3	3 of 3	3 of 3	3 of 3	0 of 3	3 of 3	3 of 3	2 of 3	0 of 3

Note: SMC (*Salmonella* Cocktail), LMIC (*Listeria* Cocktail), EHEC (*E. coli* cocktail). Number of positive samples out of three replicates per treatment evaluated. Chlorine at 60 ppm, most of samples were positive after enrichment for LMIC, exhibiting potential possibility of recover of injured bacteria. EHEC at 6 log, was not completely inactivated for chlorine solutions. On the other hand, PAA, chlorine and SAS did not exhibit positive samples after enrichment at higher concentrations.

3.2 Corrosion Effects in Stainless Steel and Aluminum Coupons

This study evaluated the corrosive effects of effective sanitizer treatments on pipelines and food contact surfaces made from common materials such as aluminum and stainless steel, respectively. No significant difference was observed between the corrosion rates of 304 and 316 stainless steel for all sanitizers ($p>0.05$). Previous studies focusing on the corrosion susceptibility of stainless-steel types 304 and 316 in various environments—including marine-urban settings, exposure to hypochlorite ions, oilfield biofilms, and sulfuric acid solutions—suggest that type 304 is more susceptible to corrosion (Martins et al., 2014; Nishimura, 1993; Wang et al., 2021). Contrary to these findings, our results show no significant difference in corrosion resistance between stainless steel types 304 and 316 when exposed to sanitizers such as chlorine, PAA, and SAS, despite earlier studies indicating that the nickel content in type 316 might confer increased corrosion resistance when compared to type 304. This could be attributed to the low concentrations used in these evaluations and the evaluation temperature of 23°C.

However, the corrosion rate was influenced by both the temperature and the type of sanitizer treatment. At the highest tested temperature of 73°C, chlorine and water treatments showed increased corrosion rates in stainless steel (Table 10). Robertson (1991) study on the corrosion of stainless steel at high temperatures in water revealed that in hot water devoid of oxygen, the corrosion rate is primarily driven by the movement of iron particles through small channels within the oxide layer that encases the steel. This layer forms in two stages: the inner layer thickens as water penetrates tiny openings in the oxide, while the outer layer expands as metal ions migrate through it. In our experiments, where the glass jar was sealed to prevent spills and limit oxygen exposure, this mechanism likely contributed to the observed corrosion at 73°C. Furthermore, chlorine showed a significantly higher corrosion rate at 73°C compared to 23°C and despite the known inactivation of Chlorine at temperatures above 50°C (Cervero-Aragó et al. 2015). Although no previous studies on the behavior of sodium chlorite at 73°C has been determined during the development of this project, McKinley (1964) research on the interaction between nickel and chlorine at extremely high temperatures (1000°C and 1600°C) found that chlorine reacts with nickel to form NiCl

and NiCl2, both of which then evaporate, consuming a significant portion of the chlorine present. The differences between the evaluated temperatures yield comparisons difficult to extrapolate, however, similar chemical process could potentially occur at lower temperatures, where the presence of chloride ions might accelerate corrosion through an anodic reaction (Johansson, 1990). This chemical process may explain the corrosion rate observed by chlorine at 60 ppm at 73 °C. The known propensity of chlorides to induce pitting corrosion in metals such as aluminum and stainless steel in atmospheric conditions supports this hypothesis (Wranglen, 1985), offering a plausible explanation for the increased corrosion observed when metal samples are exposed to chlorine.

Corrosion rates for stainless steel varied from 1.68E-04 to 8.15E-05 cm/year at 73C, the highest rates were recorded for untreated water and the lowest for SAS and PAA treatments (Table 10). In the food industry water lines tend to have a wall thickness that ranges from 0.127 to 0.635 cm. For this type of system, total corrosion durations for chlorine and water treatments ranged from 758 to 2998 years, while under PAA and SAS treatments, durations extended from 1582 to 7911 years and 1558 to 7791 years, respectively. The untreated water condition showed both the highest corrosion rate and shortest duration of 600 to 2998 years. These findings emphasize the effectiveness of PAA and SAS treatments in reducing corrosion rates and extending the life of stainless-steel pipelines, with thicker walls offering enhanced corrosion resistance. This corrosion effect could also be extrapolated to food contact surfaces in which stainless-steel is used with identical results. Stainless steel is highly resistant to corrosion, a trait attributed to its chemical composition, microstructure, and the presence of elements like chromium and molybdenum (Nascimento et al., 2019; Sjögren et al., 2011; Steel Construction Institute, 2002). This resistance aligns with the current study's findings that water treatments have a minimal corrosive effect and do not significantly reduce pipeline lifespan. While there are no studies specifically on agricultural corrosion in stainless steel caused by water treatments, stainless steel's longevity and resistance are well-recognized in the food industry (Rossi et al., 2024). Corrosion remains a critical concern for farmers and food processors, in equipment or food contact surfaces using other types of metals besides stainless steel as it can diminish

their lifespan and compromise processing systems. Therefore, employing water sanitizers with a minimal impact on pipelines, equipment or food contact surfaces is crucial to prevent the introduction of contaminants to the environment.

For aluminum evaluations, our findings indicate that corrosion rates for aluminum coupons were not significantly affected by variations in temperature (Figure 6). No statistical differences ($p>0.05$) were observed between different temperatures, however, each sanitizer treatment impacted corrosion in a different manner. The type of sanitizer treatment plays a crucial role in determining the corrosion rate on aluminum coupons. Specifically, SAS at 0.6% exhibited higher corrosion rates compared to all other treatments (Figure 6). Aluminum pipelines, commonly used for freshwater transportation are made from plastic or from aluminum alloy 3004. The latter are particularly susceptible to pitting corrosion, which can detrimentally affect their tensile properties, bursting strength, and hydraulic efficiency (Booth et al., 1965). The 0.6% SAS treatment could exacerbate pitting in aluminum, leading to increased corrosion irrespective of temperature changes. This same effect was not observed at 23 and 73 ℃ for all other sanitizers. At 73℃, both PAA and Chorine based sanitizers undergo a self-accelerating decomposition reaction and gassing off and this was also determined under our experimental conditions (data not shown, supplemental materials).

Table 10. Corrosion rate of stainless-steel coupons with respect to sanitizers and temperatures employed.

Treatment	Temperature	Corrosion cm/year	SD	P-Value
Chlorine 60 ppm	22.5 ℃	4.02E-05[b]	4.11-05	<0.05
PAA 30 ppm		1.11E-04[a]	1.09E-04	<0.05
SAS 0.6%		1.03E-05[a]	4.57E-05	<0.05
Water - Control		4.03E-05[b]	3.66E-05	<0.05
Chlorine 60 ppm	73 ℃	1.68E-04[a]	4.01E-05	<0.05
PAA 30 ppm		8.03E-05[b]	3.65E-05	<0.05
SAS 0.6%		8.15E-05[b]	5.33E-05	<0.05
Water - Control		2.12E-04[a]	1.26E-04	<0.05

Note: SD (Standard deviation). Letters (a, b, c) denote significant differences among treatments based on Kruskal-Wallis ($p<0.05$).

Water Treatment	Corrosion (cm/year)	Total pipeline corrosion (years)	
		Wall Thickness 0.12 cm	Wall Thickness 0.635 cm
SAS	4.52E-02[a]	3	14
PAA	3.96E-04[b]	321	1603
Chlorine	1.24E-04[b]	1010	5051
Water	- 5.64E-03[c]	-23	-113

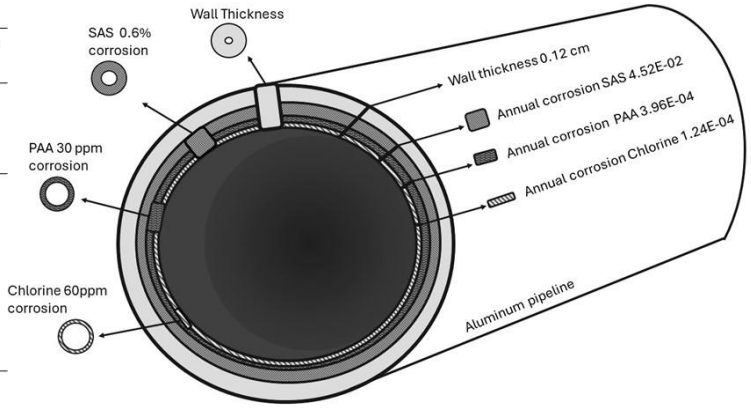

Figure 6. The corrosion rate of aluminum coupons with respect to the sanitizer employed, and corrosion levels based on the wall thickness of irrigation pipelines.

Note: Letters (a, b, c) denote significant differences among treatments based on Kruskal-Wallis ($p<0.05$). The graphic offers a visualization of corrosion caused by different sanitizer treatments in an irrigation aluminum pipeline with a wall thickness of 0.12 cm. Each color/pattern represents a sanitizer treatment.

Extensive research has identified hydrochloric acid (HCl) and sodium chloride (NaCl) as primary corrosive agents for aluminum, largely due to aluminum's vulnerability to acid solutions. The corrosion rate is influenced by the concentration of these chemicals and the presence of other specific ions in the solution (Chaoyang et al., 2019; Gusti Ayu Arwati, 2017; Kamarska, 2019; Zuchry et al., 2020) at 23 ℃. At this ambient temperature, all sanitizers present important corrosion effects on aluminum, however, SAS presented the highest corrosion rates due to the acidic environment created by this solution and its ability to remain active at this temperature when compared to all other treatments.

Figure 6 details the influence of sanitizer treatments on the lifespan of aluminum and stainless-steel pipelines, considering variations in wall thickness. For aluminum, environmental temperature does not significantly impact corrosion, underscoring the critical role of water treatment in determining pipeline lifespan. Typically, an aluminum pipeline can last on average 40 years, influenced by factors such as corrosion protection, operational conditions, maintenance, wall thickness, and regular inspections (Miscow et al., 2004; Potter, 1990). Among these, operational conditions and maintenance are key determinants of pipeline longevity with wall integrity impacting the overall longevity of the units. While wall thickness can range from 0.127 to 0.635 cm the SAS 0.6% water treatment has significant corrosion, potentially reducing

the lifespan of aluminum irrigation and potentially introducing metal residues into the water system. At 23℃ both Chlorine and PAA solutions also presented corrosion of aluminum coupons; however, their effect was as elevated as with the SAS 0.6% solution. Overall, all sanitizer treatments, meeting 2020 EPA requirements, will leach metal residues into the agricultural system.

3.3 Impact of Sanitizers Solution on Plant, Soil Chemical – Physical Properties and Inactivation of Pathogenic Bacteria from Romaine Lettuce Leaf.

The current study revealed that all tested water treatments exhibited phytotoxic effects in the second greenhouse trial, with sodium acid sulfate (SAS) at 0.6% showing higher phytotoxicity compared to chlorine and peroxyacetic acid (PAA) (Figure 7). Phototoxicity symptoms were not observed during the first trial, and this can be explained by the changes in soil parameters caused in the soil after the sanitizer application during phase 1. Phytotoxicity refers to the inhibition or negative changes in plants caused by chemical compounds, insects, oxidative stress, or external factors in the growth environment (Hyder et al., 2023; Purcell, 2009). In our observations white indents in tissue were observed for all treatments with 0.6% SAS presenting larger leaf depressions. This observation was not consistent across all treated plants (Figure 7)

These observations align with previous research from Marschner (1986) which suggested that sodium sulfate (Na2SO4) could induce leaf injuries due to the accumulation of sodium ions. Bie et al. (2004) examined the effects of sodium sulfate and sodium bicarbonate on lettuce, finding that sodium sulfate decreased water uptake, leading to osmotic stress, potential leaf necrosis, and injuries, though these symptoms varied depending on the lettuce cultivar. However, in this study similar yield was observed across all the sanitizers evaluated, independent of the phytotoxicity signals observed in some treatments. This can be explained due to all plants having received and the same content of water during the irrigation process, leading to homogenous evapotranspiration and the marginal effects determined for all soil chemical and physical properties evaluated in this study (Table 11). Cahn et al. (2022), mentioned that when the crops are exposed to the same irrigation amount meeting water requirements, yields will be uniform. At the same

time, when the evapotranspiration is near 100% it can reduce environmental impacts associated with nitrate leaching and surface runoff.

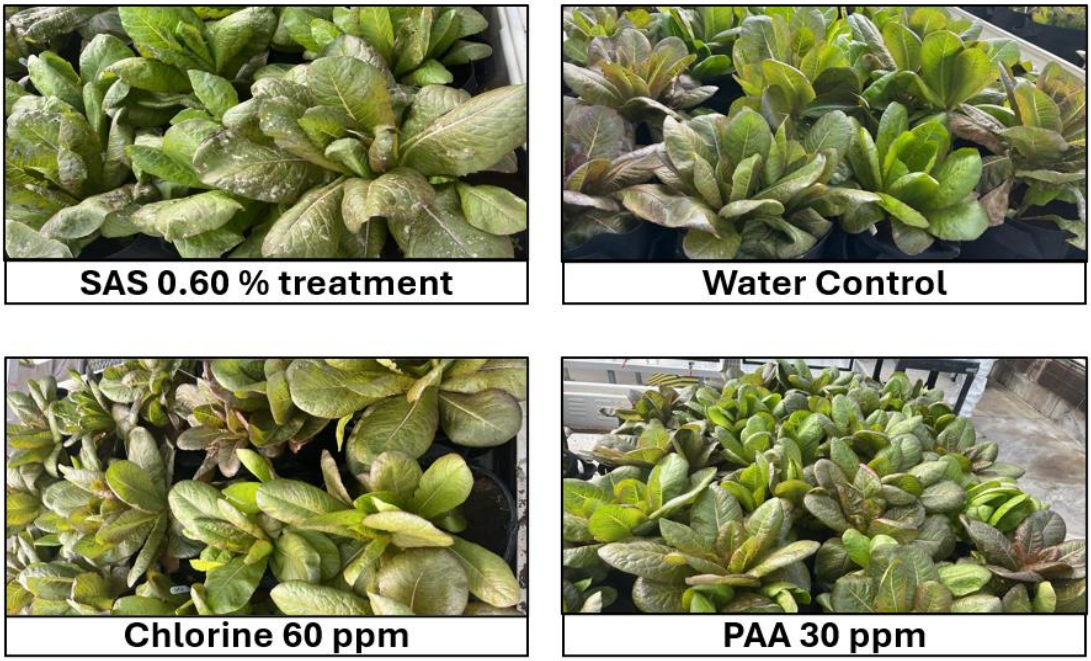

Figure 7. Phytotoxicity signals caused by irrigation water treatments.
Note: Phytotoxicity symptoms were defined by leaf damage or coloration changes in romaine lettuce after sanitizer application on day 12, second greenhouse trial

Further, Okubo & Utsunomiya (1996) indicate that leaf injuries in plants, such as romaine lettuce, can occur due to the accumulation of sodium ions from SAS water treatments. Additional studies, such as those by Abd El-Samad & Shaddad (1996), evaluated the effects of sodium carbonate, sodium sulfate, and sodium chloride on pea plant growth, concluding that a plant's salt tolerance significantly influences its reaction to these compounds. This is due to variations in osmotic pressure and the accumulation of sodiumharides, proteins, amino acids, and prolines, which differ based on the sodium sulfate or chloride levels. These reactions could be happening with SAS and Chlorine sanitizer applications, although the phytotoxic effect with chlorinated solutions is much lower due to significant differences in sodium ion concentrations between treatments. These findings are not in agreement with observations from those reported by Unruh et al., 2021; Maher et al 2020, Jacob Robert Jenott, Professor Sara Gragg, 2014; Maher, 2013 and Jenott et al 2017 in which all treated fresh cut romaine lettuce and spinach with similar

concentrations of SAS and found no phytotoxicity in any of these leafy greens. These differences could be associated with known differences in leaf morphological properties between greenhouse and field grown leafy greens; in which the latter has a robust cell wall and epidermis when compared to greenhouse grown plants. Similar observations in the difference of the tensile strength of leaves were reported by Gutierrez-Rodriguez et al, 2011 in which differences in leaf strength were associated with nitrogen inputs and growing practices for spinach leaves.

PAA has been widely used in the post-harvest stage to improve sanitizing methods, exhibiting effectiveness in microbial reduction, and causing less browning, chemical changes, or physical damage in leafy greens (J. L. Banach et al., 2020; de Moraes Motta Machado et al., 2022; Pahariya et al., 2022). On the other hand, PAA in the pre-harvest stage is used as an alternative to reduce foliar or mold diseases to improve crop quality and yield. Yang et al. (2022) evaluated the effect of PAA on strawberries, finding that this sanitizer reduced the microbial population and is a feasible alternative for maintaining the quality of fresh strawberries. PAA has been widely used as a disinfectant against fungal plant pathogens, being effective without causing changes in the plant or visible damage (Copes & Ojiambo, 2023). While PAA in the pre-harvest stage has not been primarily evaluated as an alternative to reduce human pathogen bacteria, this sanitizer has commonly been used to reduce fungal plant pathogens without causing damage or changes in vegetable or fruit crops. Under our experimental conditions, PAA treatment romaine plants showed no significant signs of phytotoxicity at the pre-harvest stage. McGehee & Raudales, 2023, explored the phytotoxic effects of chlorine on lettuce in hydroponic systems, revealing that chlorine-treated water, when used to prepare sanitizer solutions, causes phytotoxicity within two days and reduces plant biomass as chlorine concentration increases. These phytotoxic effects were specific to hydroponically grown bib lettuce systems, where chlorine interacted with nutritional solutions, unlike in this study, where chlorine was applied directly to the crop. This previous study suggests that when crops are fertilized and treated with chlorine, phytotoxic reactions can occur due to interactions with other chemicals.

3.4 Soil Chemical and Physical Properties

Table 11 presents the results of different sanitizer treatments on various soil chemical and physical parameters. The treatments included a control (soil not used for plant growth or irrigation applied during the whole experiment), tap water, chlorine at 60 ppm, PAA at 30 ppm, and SAS at 0.6%. Each sanitizer treatment exhibited distinct effects on these soil parameters in which EC, sodium and sulfur and copper concentrations were the main parameters impacted by sanitizer treatments. For both the control and tap water treatments, the soil chemical and physical properties remained identical except for the phosphorus and sulfur concentrations (Table 11).

The concentration of phosphorus increased for all tap water treated soils while the concentration of sulfur for this same treatment was reduced when compared to the control. When comparing the effects of continuous application of sanitizer water treatments for soil chemical and physical properties, the most significant differences were obtained for sulfur, EC, and sodium concentrations. The 0.6% SAS treatments presented the highest concentrations of these parameters when compared to Chlorine and PAA treatments. PAA treated soils presented the highest concentrations of copper when compared to all other treatments. All pipelines used under these experimental conditions were plastic and no corrosion was expected from any treatment. No known source of copper was determined for any of the treatments.

Table 11. Impact of sanitizers treatments on soil chemical and physical parameters

Sanitizer Treatment	Soil Parameter							
	pH	EC 1:1 mmho/cm	Olsen Bicarbonate	M- 3	Ammonium Acetate	Base Saturation		Hot Water - DTPA
			P (ppm)	S (ppm)	Na (ppm)	Na %	Ca%	Cu (ppm)
Control	7.82^{b}	2.42^{b}	14.91^{b}	93.16^{b}	106.50^{b}	1.7^{c}	71.08^{bc}	1.06^{b}
Water	7.88^{ab}	2.29^{b}	19.66^{a}	78.50^{b}	106.33^{b}	2.0^{bc}	70.83^{bc}	1.15^{b}
Chlorine	7.93^{ab}	2.11^{b}	12.16^{b}	75.50^{b}	117.83^{b}	1.6 ^{c}	72.0 ^{ab}	1.31^{ab}
PAA	7.95^{a}	2.01^{b}	13.16^{b}	89.6^{b}	120.66^{b}	3.0 ^{b}	72.83^{a}	1.53^{a}

SAS	7.83[ab]	3.87[a]	14.16[b]	171.3[a]	303.0[a]	4.6 [a]	70.83 [c]	1.13[b]

Note: Values presented are means of measured soil parameters under various treatments. pH values are reported to two decimal places, while other parameters are reported to two significant figures. Letters (a, b, c) denote significant differences among treatments based on Tukey-test ($p<0.05$).EC (Electrical Conductivity, unit: mmho/cm)

The increase in the sodium concentration in soil from the SAS 0.6% treatment could be potentially associated with some of the phytotoxicity determined in experiment 2 (Figure 7). Vicente-Sánchez et al. (2014) evaluate the impact of Na in iceberg lettuce leaves and reported that elevated levels of Na affect the leaf and may cause growth problem in the crop. The chlorine and PAA treatments resulted in an increase in the amount of Na in the soil however minimal phytotoxicity signals were observed in the romaine lettuce (Figure 7). The abundance of sodium within the soil can displace crucial nutrients like calcium, magnesium, and potassium, which are fundamental for plant growth. Consequently, this displacement may lead to nutrient inadequacies in plants, causing a decrease in crop yield (Alkharabsheh et al. 2021).Cahn and Ajwa (2004) mentioned that an EC superior of 1.4 dS/m in irrigation water and 2.1 dS/m in soil extract may cause a 10% yield loss in lettuce production. This was not observed under our experimental conditions. EC is an important factor in lettuce growth, and this parameter has been principally studied in hydroponics systems suggesting an EC of 1.8 for optimal dry weight, however in the range of 1.8 to 2.4 not yield differences were observed (Samarakoon et al., 2019).

Understanding the effect of the sanitizers treatments on soil and plant health besides corrosion rates and human pathogen inactivation, is imperative to maintain optimal conditions during the growing seasons. The main changes observed under these evaluations were associated with Na concentrations and EC after sanitizer application, however it is important to highlight that the control soil that was not used for plant growth exhibited different soil parameters compared to water, indicating that the presence of a vegetable crop can also be responsible for natural changes in soil parameters.

When evaluating the efficacy of the sanitizer treatments in reducing the population of AEHEC, LMIC, and GEC from romaine lettuce leaves it was evident that a significant reduction was observed when compared to the control (water) treatment (Figure 8). There was no statistic difference in the bacterial

survival between the two cultivars evaluated ($p>0.05$). After 12 days post inoculation and application of sanitizer treatments results from greenhouse trial 1 and 2 (Data combined between trial 1 and 2) show a statistical difference in the survival of AEHEC, LMIC, and GEC over time. The use of sanitizer treatment provides a higher bacterial reduction compared to the control treatment. PAA at 30 ppm showed an average 0.98 log CFU/g reduction when compared to the control treatment (water) for all pathogenic bacteria. Chlorine at 60 ppm and SAS at 0.60% exhibited marginal differences compared to PAA, with reductions of approximately 0.3 and 0.5 log CFU/g respectively (Figure 8). These findings suggest that the application of any irrigation water treatments will aid in pathogen reduction from the surface of romaine lettuce, with PAA treatments providing the highest Log reduction 12 days post-inoculation for AEHEC, LMIC, and GEC.

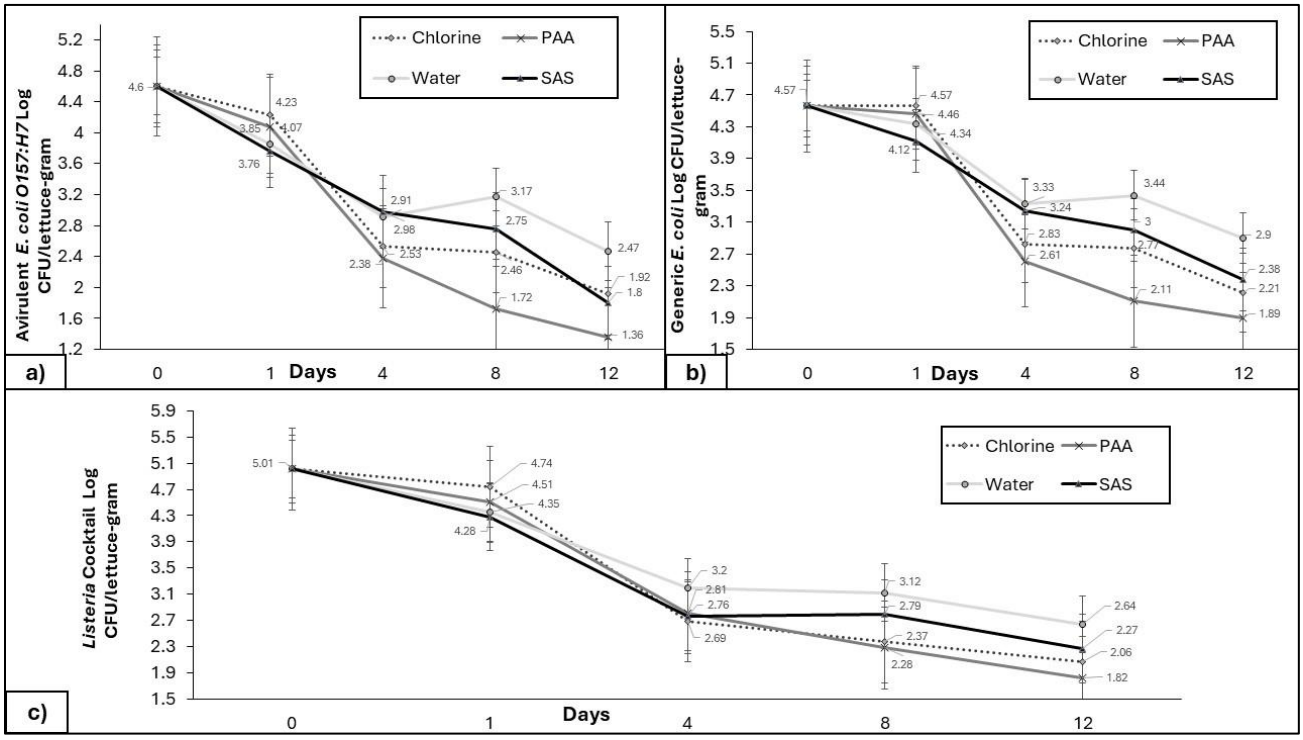

Figure 8. Bacterial survival on romaine lettuce plants over 12 days post inoculation based on irrigation water sanitizer treatments in greenhouse.

Note: a) Avirulent *E. coli* O157:H7 reduction over time based on sanitizer treatment ($p<0.05$). b) Generic *E. coli* reduction overtime based on sanitizer treatment ($p<0.05$. c) Listeria Cocktail reduction based on sanitizer treatment ($p<0.05$). Sanitizer Treatments Chlorine 60 ppm, PAA 30 ppm, SAS 0.6%, water – control treatment. Bar errors show standard deviation.

Previous research indicates that pathogenic bacteria die-off rates can vary significantly due to microbial gene responses to environmental stress (L. L. Dunn et al., 2020; Hu et al., 2022; Simpson Beauchamp et al., 2012). Environmental factors like weather, water quality, and resident aquatic microbiota are known to influence these rates, which often follow a biphasic pattern: comprised of an initial rapid decline followed by a prolonged tailing phase (Choi et al., 2011; Jeamsripong et al., 2019; Medina-Martínez et al., 2015; Weller et al., 2017).

Table 12 describes the linear die-off rates for the sanitizer treatments evaluated in this study. Among the treatments, PAA at 30 ppm consistently exhibited the highest efficacy across all three bacterial inoculate, with linear die-off rates ranging from 0.138 to 0.269 log CFU/day. Chlorine at 60 ppm also showed notable effectiveness, particularly against avirulent *E. coli* O157:H7, with die-off rates ranging from 0.222 to 0.246. SAS at 0.6% had similar die-off rates for the chlorine treatment with linear die-off rates ranging from 0.195 to 0.222 log CFU/day, while water, serving as the control, exhibited the lowest die-off rates ranging from 0.138 to 0.198 log CFU/day. Water has been identified as a significant route of contamination during pre-harvest activities. Studies have explored the survival, presence, and risk of pathogenic bacteria in water, informing guidelines like those from the produce safety rule, which suggest the use of a die-off rate of 0.5 logs per day (FAO & Food and Agriculture Organization, 2023; Gartley et al., 2022; Malakar et al., 2019; Y. Pachepsky et al., 2011; Y. A. Pachepsky et al., 2014). However, our findings suggest that this rate varies with the type of bacteria, indicating that this die-off rate cannot be universally applicable, especially for LMIC and AEHEC in greenhouse-produced vegetables.

Table 12. Die-off magnitude, pathogenic bacteria.

	Linear die-off rates (log CFU/day)		
Sanitizer Treatment	***Listeria* Cocktail**	**Generic *E. coli***	**Avirulent *E. coli* O157:H7**
Chlorine 60 ppm	0.246^{ab}	0.195^{abc}	0.222^{acb}
PAA 30 ppm	0.267^{a}	0.222^{acb}	0.269^{a}
SAS 0.60%	0.228^{ab}	0.181^{abc}	0.233^{ab}
Water - Control	0.198^{abc}	0.138^{c}	0.176^{bc}

Note: Data represent an average of 30 samples replicates per treatment, with comparison made by die-off rate of all pathogenic bacteria evaluated. Letters (a, b, c) denote significant differences among treatments based on Kruskal-Wallis ($p<0.05$).

The different magnitudes of die-off rates observed for each microorganism in this study underscore the complexity of pathogen persistence dynamics in greenhouse environments or field production, emphasizing the need for customized mitigation strategies to ensure food safety in lettuce production. Notably, the generic *E. coli* strain used in this study could serve as a surrogate in experimental setups for pathogenic *E. coli* strains due to its lower die-off rate, providing a conservative estimate for treatment effectiveness. Current, die-off rates proposed by the produce safety rule may not reflect die-off rates for all pathogenic bacteria, necessitating additional measures to mitigate the use of potentially contaminated water in the pre-harvest stage. Sanitizers in water treatments have proven effective, with PAA at 30 ppm achieving a 0.98 log reduction when compared to the control treatment (water) if pathogenic bacteria are present on crop surfaces (Figure 8). While both SAS at 0.6% and chlorine achieve a 0.3 and 0.5 log greater reduction than water, concerns about chlorine and PAA by-products, such as halogenated trihalomethanes and haloeacetic acids—which are potential carcinogens (Gopal et al., 2007)—highlight the need for safer alternatives.

Research indicates that PAA and chlorine-based sanitizers in postharvest food processing can control foodborne pathogens effectively (Simpson & Mitch, 2022). However, the presence of high organic matter can diminish the efficacy of both treatments (How et al., 2017; Teng et al., 2018). In contrast, SAS does not produce harmful by-products, making it a safer option for pre-harvest stages. The antimicrobial efficacy of SAS against foodborne pathogens such as *E. coli, Salmonella,* and *L. monocytogenes*—damaging cell components and increasing leakage of nucleic acids and proteins—supports its use as a water treatment (McDaniel et al., 2021). However, the potential for phytotoxic effects on lettuce surfaces warrants further investigation to assess environmental impacts under commercial practices instead of greenhouse conditions where it is known that leaf morphology changes drastically when compared to field grown romaine lettuce. Studies from Unruh et al., 2021; Maher et al 2020 and Jenott et al 2017 have not found any phytotoxicity for field grown romaine lettuce or spinach during washing and refrigerated storage.

Krishnan et al. (2023) report that PAA is more effective than sodium hypochlorite at low concentrations in reducing pathogens in both surface and groundwater. At higher concentrations, no statistical difference was observed between the sanitizers, which may be attributed to the absence of organic matter in the tested water. This aligns with other findings that show PAA as highly effective in preharvest applications for leafy greens, offering superior microbial reduction compared to conventional treatments (Krishnan et al., 2021; Mohammad et al., 2023; Pahariya et al., 2022). However, the formation of carcinogenic compounds in the presence of organic matter (Dubey et al. 2020) is of concern for the long-term sustainability of farm soil.

4. Conclusions

The findings of this study underscore the importance of sanitizer concentration, contact time, and water turbidity in water sanitation. The use of chlorine, PAA, SAS, and KAS at medium and high concentrations meets EPA standards for a minimum 3-log bacterial reduction. However, PAA at 30 ppm, chlorine at 60 ppm, and SAS at 0.6% exhibit better performance in higher turbidity levels and longer exposure times. These were the same treatments evaluated for their impact on corrosion and on soil and plant health.

Regarding corrosion rates, stainless steel 304 and 316 show no difference in corrosion rate between sanitizers, but corrosion is influenced by sanitizer treatment and temperature. Conversely, aluminum exhibited higher corrosion rates for all sanitizers, with SAS at 0.6% presenting the highest rates. Both PAA and chlorine sanitizers become inactivated at temperatures above 50°C. Under our experimental conditions for corrosion, elevated temperatures inside the pipelines will result in no residual effects for the inactivation of human pathogens for PAA and chlorine sanitizers when compared to SAS. Further studies are needed to define the overall impact of SAS at 0.6% on the longevity of aluminum pipeline corrosion.

In the greenhouse, PAA at 30 ppm, SAS at 0.6% and chlorine at 60 ppm were able to provide a significant log reduction in the populations of AEHEC, LMIC, and GEC over time from the surface of romaine lettuce plants. Linear die-off rates varied by sanitizer treatment and inoculated organism, with all

die-off rates not meeting current produce safety rule rates listed at 0.5 Log CFU per day in the field. All three inoculated organisms (AEHEC, LMIC, and GEC) had identical die-off slopes within the first 12 days post-inoculation, with differences only associated with the magnitude of the final die-off rate.

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