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Improving the Food Safety of Louisiana Strawberry Industry

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IMPROVING THE FOOD SAFETY OF LOUISIANA STRAWBERRY INDUSTRY

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I dedicate my dissertation to my devoted parents Juan Brandao and Alicia Delgado who supported me every time without losing their belief in me.

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ABSTRACT

Escherichia coli O157:H7, *Salmonella sp.*, and *Listeria monocytogenes* have been linked to foodborne outbreaks in produce. The most recent outbreaks in produce have been associated with irrigation water due to infiltration of well water or water run-off from contaminated sources. The Food Safety Modernization Act (FSMA) requires all irrigation water to be safe for use on produce, as a strategy to reduce foodborne illnesses. A surfactant modified zeolite (SMZ) filtration system could provide produce farmers with a sustainable low-cost system for high-quality and safe irrigation water. The objective of this study was to evaluate the effectiveness of hexadecyltrimethylammonium bromide (HDTMABr) to develop a SMZ filtration system capable of removing *Escherichia coli* O157:H7, *Salmonella* serotypes, and *Listeria monocytogenes* from irrigation water. A liter of inoculated water with *Escherichia coli O157:H7*, *Salmonella* typhimurium, or *Listeria monocytogenes* at counts of 6 log CFU/ml was filtrated through a 20g column of SMZ. The SMZ at concentration higher than 20% w/w of HDTMABr removed > 6 log CFU/ml of *Escherichia coli* O157:H7 and *Listeria monocytogenes*, and > 2 log *Salmonella sp.* The SMZ was tested in a strawberry field using a filtration system with regular sand and with the SMZ operating at 25 GPM. Two controls were used to prove the effectivity of the SMZ—a positive control of the pond water and a filter system with only regular sand. An *Escherichia coli* non-pathogenic surrogate was used to spike pond water, which naturally contains fecal material, to concentrations higher than 6 log CFU/ml. The SMZ filtration system had a removal capacity of more than 99.99% of the *Escherichia coli* in comparison to the two controls that did not remove these bacteria from the system. SMZ modified with HDTMABr could be a viable solution for farmers to comply with new FSMA regulations and provide a way to reduce foodborne outbreaks.

CHAPTER 1. INTRODUCTION

As the produce industry expands to meet growing market demands for fresh locally grown produce so does the demand for high quality water. Produce production is water intensive and production requirements are met by drawing water from ground and surface sources. The quality of surface water is variable, and runoff has put the quality of surface water into question (1). In addition, the microbial quality of water used to irrigate produce crops has increasingly come under scrutiny due to recent foodborne illness. Outbreaks associated with fresh fruit and vegetable consumption had been reported. A variety of fecal contaminants have been isolated from irrigation water, associated sediments, and linked to outbreaks especially of *E. coli* O157:H7 in produce (1, 2).

The United States Center for Diseases Control and Prevention (CDC) estimates that: "each year roughly 1 in 6 Americans (48 million people) get sick, 128,000 are hospitalized and 3,000 dies of foodborne diseases." (3) In order to reduce the risk of foodborne outbreaks, the Food Safety Modernization Act (FSMA) was enacted by the United States government and new food safety policies were created. The FSMA regulation includes a provision for water, the standards in this regulation requires farmers to ensure that agricultural water is "safe" and "of adequate sanitary quality for its intended use."

The rule divides the use of water for produce into two standards based on intended use: a) water for food contact situations, have a limit of no detectable generic (non-pathogenic) *Escherichia coli*, b) water used for irrigation in which set a numerical criteria: a geometric mean (GM) <126 CFU/100ml generic *Escherichia coli* with a statistical threshold value (STV) of less than 410 CFU/100ml of generic *Escherichia coli* (4). This rule is addressing water as a possible risk and based on the source of water imposing stricter regulation to the producer that will have to

been able to prove that their water will not compromise the safety of their product. However, this standard raised concerns about the ability of farmers to comply. The principal concern is the limited ability of small farmers to quickly adapt and understand the new water rule standards, particularly in situations where the use of surface water is the only irrigation alternative.

Zeolites are known as molecular sieves and are widely used in water treatment industry for removal of metals from water. Zeolites are characterized for their low cost, strong ion-exchange property and large adsorption capacity (5). The use of a surfactant modified zeolite (SMZ) filtration system could provide produce farmers with a sustainable low-cost system for high quality and safe irrigation water. The overall goal of the project will be the reduction of foodborne pathogens in irrigation water used in strawberries by the development of a filtration system using SMZ. Zeolite filtration system is used in several industries. This will become a solution for famers that use water with high loads of bacteria without having to use chemical treatments or incur on expensive filtration systems.

The use of a Surfactant modified zeolite (SMZ) filtration system could provide produce farmers with a sustainable low-cost system for high quality and safe irrigation water. The modified zeolite filtration system is a safe alternative to produce water free of potential foodborne pathogens.

CHAPTER 2. LITERATURE REVIEW

2.1 Agricultural Water Circumstances

The United States Department of Agriculture (USDA), reported that irrigation water accounted for roughly 40% ($118.5 billion) of the value of the United States agricultural production. From that 40%, 52% of irrigation water originates from surface water sources and 48% is pumped from wells drawn from local and regional aquifers. However, dryland states have three times the average use of irrigation water than states with mostly wetlands. The production of fruit and vegetables is water intensive and requirements are met by drawing water from the ground and surface sources. Furthermore, out of the farms which use irrigation water, only 10% have advanced technology associated to their water irrigation system. The USDA also refers to the water used in the farm having an impact in the quality of the basin-level watershed. (6)

The quality of surface water can be contaminated by different sources of fecal bacteria and viruses, including soil runoff, flooding, wildlife and rain. In a study of a small urban sub-watershed in California, researchers were able to track fecal sources of bacteria from: a) wildlife (birds, rabbits, domestic dogs, cats, and unidentified wild animals) b) soil and c) sewage; to the sub-watershed finding wildlife as the principal source of water contamination (2). In addition, the microbial quality of water used to irrigate produce crops has increasingly come under scrutiny owing to recent foodborne outbreaks associated with fresh fruit and vegetables consumption. In Ontario, Canada, a study followed the water quality of 27 irrigation water sources on 17 farms, finding that 98.2% were acceptable for irrigation. However, the non-acceptable samples were extremely high in fecal indicators. The study found a direct correlation between the recent rain and a higher level of fecal indicators in the irrigation water sources(1).

Three recent outbreaks of *Escherichia coli* O157:H7 and Salmonella Saintpaul in fresh spinach (7), lettuce (8), and jalapeño peppers (9) were correlated to irrigation water as the possible source of the contamination. The outbreak in 2006 associated with fresh spinach, *Escherichia coli* O157:H7 was found in infected patients and the farm irrigation water. This foodborne outbreak occurred in 26 states, with over 200 cases reported and 3 deaths (7). During the outbreak investigation, several state and governmental agencies undertook an environmental study to determine how the spinach became contaminated. They found that the hydrogeological conditions at the fresh spinach farm contributed to the contamination of *Escherichia coli* O157:H7 from cattle and wild pigs into surface water of the San Juanito River to the farm well used for irrigation water. This investigation highlighted the importance of keeping rigorous safety controls of surrounding water sources and served as one of the principal sources for discussion in the FSMA water rule decisions(10). The lettuce outbreak that occurred in 2008 in Sweden was associated with *Escherichia coli* O157 contamination. The farm that produced the lettuce used irrigation water from a stream that tested positive for the same verotoxin-producing *Escherichia coli* strain (8). In the 2008 outbreak, *Salmonella* Saintpaul was associated with jalapeño peppers and other produce that caused 1,442 illnesses and 2 deaths; in 43 states, the District of Columbia and Canada. The FDA and other agencies from Mexico traced the Salmonella Saintpaul back to a farm in Tamaulipas, Mexico. A sample of the water from a holding pond used for irrigation was positive for the same *Salmonella sp*. serotype (11). These outbreaks clearly indicate the need for development of methods to control pathogens in the irrigation water supplied by ground and surface water.

2.2 Pathogenic Foodborne Bacteria in Irrigation Water

Naturally, there are diverse microorganisms in water bodies. Some of these microorganisms include bacteria like *Escherichia coli, Listeria monocytogenes* and *Salmonella sp.;* all of which are considered common pathogenic microorganisms associated with foodborne outbreaks. A 2017 study, using pyrosequencing in 17 water bodies used for irrigation found evidence that based on the kind of production system. Commercial, small scale or home garden had a pathogenic *Escherichia coli* at 16.3%, 1.3% and 1.9% in irrigation water, respectively and in all three *Salmonella sp.* was present in less than1%. This is an expected result because *Escherichia coli* is the most commonly associated bacteria in irrigation water in produce that causes outbreaks (12) , even though *Listeria monocytogenes* was not found in the study.

2.2.1 *Escherichia coli*

Escherichia coli usually presents itself as a non-pathogenic strain, gram-negative, facultative anaerobic, rod-shaped; this bacterium has a symbiotic relationship with the host. However, an estimated of 10% of the strains are pathogenic bacteria capable of causing foodborne outbreaks. These pathogenic strains of *Escherichia coli* have different pathways to cause illnesses. Based on these factors they are divided in five pathotypes: enteropathogenic *Escherichia coli* (EPEC), Shiga toxin-producing *Escherichia coli/* enterohemorrhagic *Escherichia coli* (STEC/EHEC), Shigella enteroinvasive *Escherichia coli* (EIEC), enteroaggregative *Escherichia coli* (EAEC), and enterotoxigenic *Escherichia coli* (ETEC) (13). According to the CDC 387, outbreaks were caused by *Escherichia coli* in food and water between 1998–2016, with 9,716 illnesses reported, 1,421 hospitalizations and 29 confirmed deaths (Figure 1) (3), Furthermore, *Escherichia coli* STEC caused 90% of the foodborne outbreaks and 95% of the deaths compared to the other pathogenic *Escherichia coli* (14).

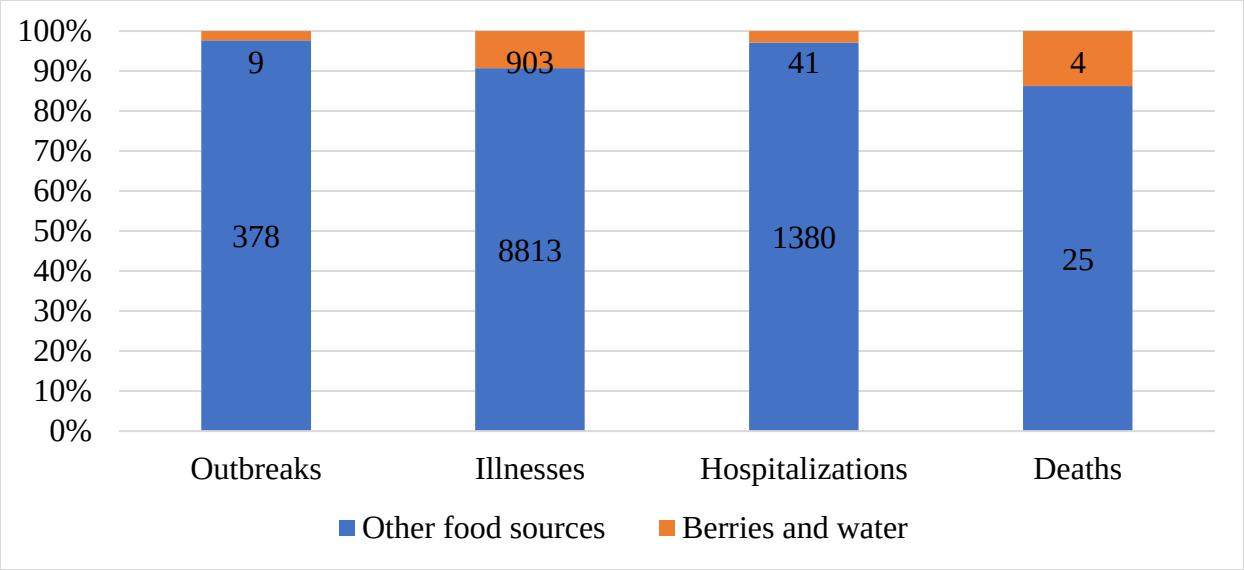


Figure 1. Estimated outbreaks data for *Escherichia coli*.
Source: Centers for Disease Control and Prevention, (CDC). National Outbreak Reporting System (NORS)

2.2.2 *Salmonella sp.*

Salmonella sp. is another pathogen that has caused foodborne outbreaks in fresh produce. An outbreak associated with tomatoes was related to Salmonella Newport in irrigation water (15). Based on these outbreaks, the CDC conducted a multistate effort to identify the pathways that this pathogen contaminates the produce. In relation to irrigation water, a study about tomatoes suggested that there are low chances of getting a *Salmonella sp*. infection from irrigation water. However, *Salmonella sp*. has been found to grow and survive on the surface of the produce (16). The CDC, estimates that between 1998–2016, there were about 1,695 reported outbreaks related to *Salmonella* sp. resulting in: 43,711 illnesses, 5,210 hospitalizations, and 70 deaths (3)(Figure 2). Most of these outbreaks are related to animal sources. However, outbreaks related to other sources such as produce is increasing. In 2018, data indicates that 26% of all major foodborne outbreaks occurring in the United States are related to the produce (17).

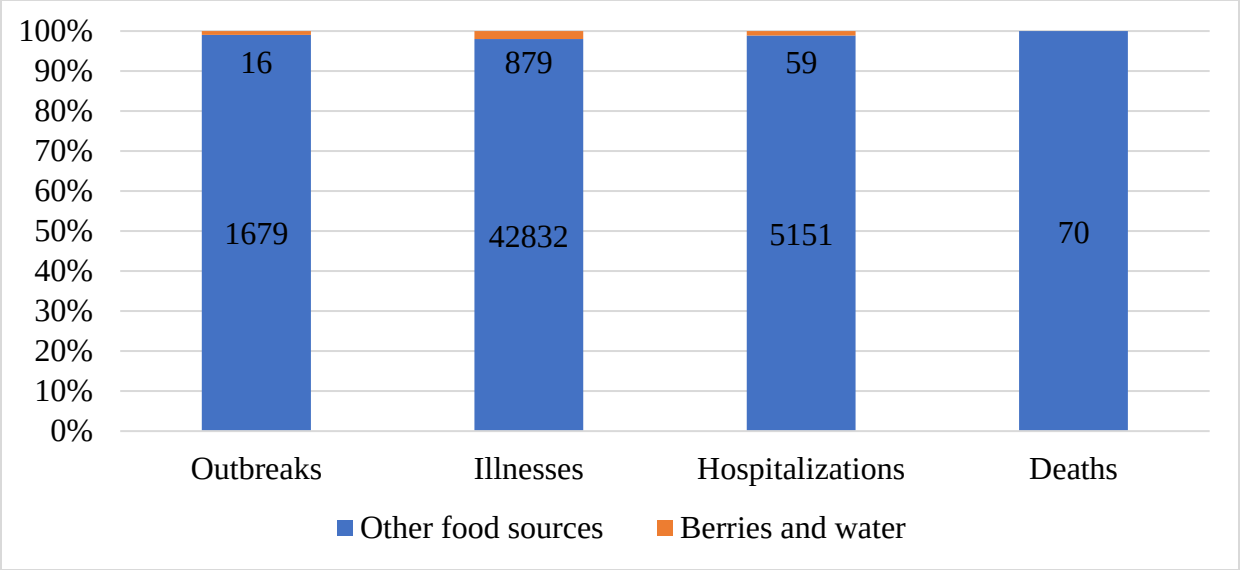


Figure 2. Estimated outbreaks data for *Salmonella sp.*
Source: Centers for Disease Control and Prevention, (CDC). National Outbreak Reporting System (NORS)

2.2.3 *Listeria monocytogenes*

Listeria monocytogenes is also a pathogen closely related to fresh produce outbreaks since it is naturally found in soil, water and manure (18). It is a gram-positive bacterium that can grow at refrigeration temperatures. *Listeria monocytogenes* causes listeriosis (19), a life threatening infection usually presented with headaches, stiff neck, confusion, loss of balance, and convulsions, in addition to fever and muscle aches. Pregnant woman could experience miscarriages, stillbirth, premature delivery, or life-threatening infections of the newborn (20). In the last 10 years, *Listeria monocytogenes* has caused 39 foodborne outbreaks resulting in: 554 illnesses, 448 hospitalizations, and 324 deaths (Figure 3) (21). In the last few years, several of these outbreaks were related to produce. Research has found that the principal source for the contamination of produce was from soil or water. The prevalence of *Listeria monocytogenes* was 4% and *Salmonella sp*. was 9% in treated water bodies and municipal water used for irrigation. In open water bodies such as lakes or trenches not used for irrigation, the prevalence was as high as 59% for *Listeria monocytogenes* and 39% for *Salmonella sp.* (22).

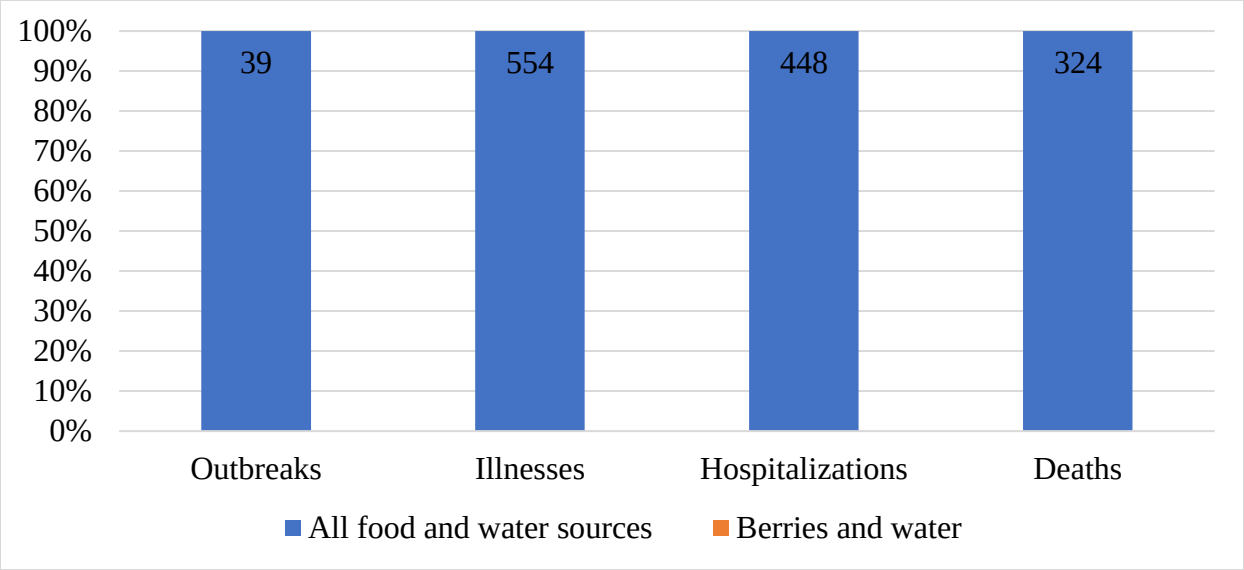


Figure 3. Estimated outbreaks data for *Listeria monocytogenes*.
Source: Centers for Disease Control and Prevention, (CDC). National Outbreak Reporting System (NORS)

2.3 Viruses with Implication to Waterborne Diseases

Illnesses caused by viruses amount to 49% of the foodborne outbreaks. Enteric viruses such as Hepatitis A (HAV) and Noroviruses (NoV) cause an estimated 19-21 million illnesses and contributes to 56,000–71,000 hospitalizations and 570–800 deaths. (3) Diseases caused by enteric viruses do not discriminate between race, gender, occupation or age. In the past, strawberries have been linked to several enteric virus outbreaks. Human enteric viruses pose a greater health risk than enteric bacteria due to their higher contingency, and lower infectious dose which can be as little as ten virions (23).

Hepatitis A virus (HAV) cause an inflammation of the liver and lead to its malfunction. HAV causes around 2,700 (1,650- 4,370) new infections each year and approximately 100 deaths per year (3). The main cause of infections with hepatitis A are related to fecal contamination if those who handle food do not wash their hands properly before preparing, and consuming food or after using the washroom, and through the consumption of raw or undercooked shellfish that came

from waters polluted by sewage. Most of the hepatitis outbreaks could be prevented with correct good manufacture practices and the provision of sick leaves to workers (20).

In recent years, HAV infections have decreased due to vaccinations that provide lifelong immunity. However, vulnerable populations are still present in the country and limited research is available on how HAV contamination is transmitted in the environment. In 48% of the HAV cases reported, the mode of transmission was unknown, principally because people are unable to recall what food they had consumed weeks prior to the onset of symptoms, making it hard for investigators to link the symptoms to a source. Therefore, HAV infections are more likely to be controlled through vaccinations and prevention (23).

2.4 Treatments for Control of Pathogens in Irrigation Water

With the enactment of the Food Safety Modernization Act (FSMA), remedial solutions for water that do not comply with the regulation has become a necessity. One of the options to comply with FSMA is the treatment of surface water, to attain high quality water for the irrigation of high-risk vegetables. Furthermore, the water used for pre-harvest practices of high-risk vegetables should meet the requirements of the 2012 Environmental Protection Agency (EPA) Recreational Water Quality Criteria (24). Several on-farm water treatment practices, including chlorine dioxide and ultraviolet light, have been investigated to reduce the risk of pre-harvest contamination of fresh produce (25, 26). These treatments, like chlorine dioxide, show promise in reducing the food safety risks associated with the surface waters used for irrigation. However, these same on-farm studies have found smaller lettuce heads during irrigation with chlorine dioxide treated water, as the long-term effects of chlorine compounds on soil health have not been adequately assessed (27).

There is a high interest in the industry to have the option of non-chemical treatments for irrigation water. Traditionally sand and carbon are widely used for the filtration of irrigation water,

especially when the removal of organic matter is beneficial. However, the use of chemicals like chlorine can create toxicity in the soil (28). Methods like activated carbon are commonly used in water reclamation for the removal of organic matter removal, and its large surface area grants enough absorption. However, its flow rates make it not as effective in irrigation water (29-32). Recently, there has been an increasing interest in using Zeolite to improve the efficiency of filtration-based water-waste treatment.

2.5 Zeolite Properties and Functionalities

The zeolites are a unique group of micro-porous rocks rich in minerals, which occur naturally in abundance and can also be synthetically made. They are usually composed of complex frameworks, one of which is made of aluminosilicates, based on the amount of aluminosilicates on which the zeolite functionalities are impacted (33). Since silica is uncharged, the aluminum creates a negative net charge. Therefore from these the ion-exchange capacity of the zeolite mineral which can be modified with surfactants for its ability to attract and form a cationic exchange system (34). Zeolites work as a sieve and are used in water treatment for the removal of metals due to low cost, strong ion-exchange property and their huge absorption capacity (35-37).

Zeolite has proven itself to be able to absorb cationic surfactants into their negatively charge structure. The use of cationic bonding surfactant in high concentration creates a hydrophobic bonding, which allows the Zeolite to form a bilayer (Figure 4). One of the proposed materials that Zeolite can be modified is hexadecyl (trimethyl) ammonium bromide (HDTMABr). HDTMABr changes the polarity from negative to positive, making zeolite more suitable to attach pathogenic bacteria onto the surface (38).

Surfactants like HDTMABr, have a long quaternary ammonia chain that have cations which are too large to enter channels with zeolite-like structure or internal exchange position,

making all the sorption of the cationic surfactant to occur on the external uniform tetrahedral surface of the zeolite (39). The amount of cationic surfactants that can be absorbed by the zeolite is determined by the amount of aluminosilicates or clinoptilolite present in the zeolite (40).

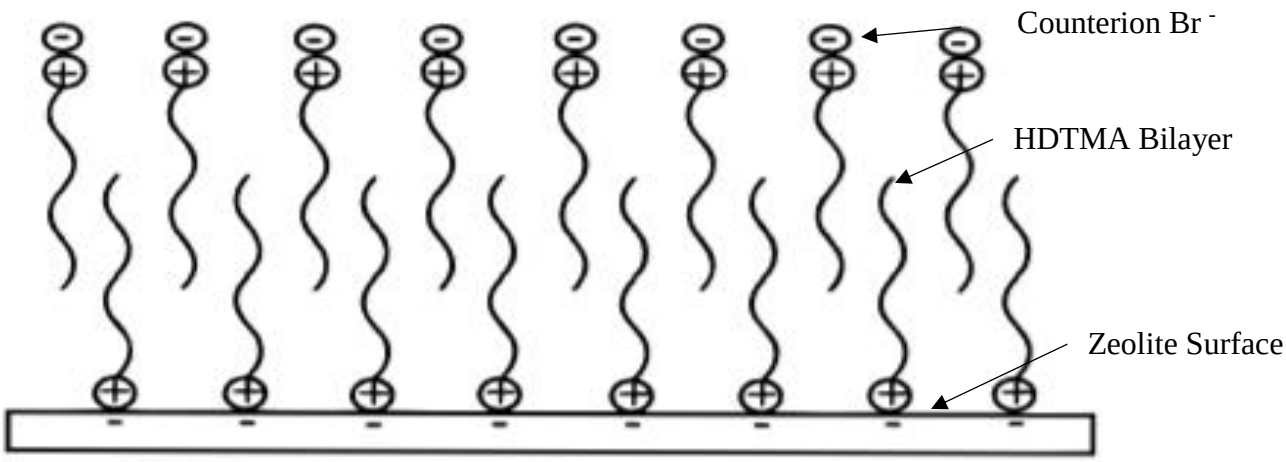


Figure 4. Bilayer of $HDTMA^{+}Br^{-}$ molecules in the surface of Zeolite

A field study was conducted using a modified zeolite filter pack to remove indicator bacteria and viruses from ground water. Results of the experiment showed a 99% removal of indicator viruses and 100% removal of indicator *Escherichia coli* from ground drinking water with the zeolite filter pack. After 5 months, the zeolite filter pack was still removing 100% of the indicator *Escherichia coli* from the ground water. All work conducted using zeolite was with the removal of the indicator generic *Escherichia coli* (Gram negative) and indicator viruses from drinking water (38).

The bacterial attachment to a specific surface is related to the cell surface charge, hydrophobicity and external structures like flagella or extracellular polysaccharides. Most bacteria have a negative cell charge. However, this charge can vary significantly even between strains of the same species (41). As cationic surfactants can be used to modify materials like Zeolite, making them able to attach bacteria and a large Zeolite surface area, a modified material could increase the flow rate during filtration.

There is another explanation that a Surfactant Modified Zeolite (SMZ) filtration device would be effective in the gradual desorption of the surfactant (5). In the case of HDTMA, this could provide a constant release of the antimicrobial material into the filtrated water. The zeolite structure, owing to their negative charge can also entrap silver cations that have an antimicrobial effect. Then, the silver leech from the zeolite can kill bacteria over time. (42).

CHAPTER 3. REMOVAL OF FOODBORNE PATHOGENS USING SURFACTANT MODIFIED ZEOLITE (SMZ)

3.1 Introduction

The implementation of the Food Safety Modernization Act Water Rule comes with many challenges like the confusion about the indicator which must be used to predict better fecal pathogen contamination in water. However, the water rule settled that *Escherichia coli* was the best, based on the FDA literature review and studies. The other challenges are the type of water use: ground, surface or municipal water and the infiltration that can take place between surface and ground water. If the crop irrigation is directly applied onto the edible, part of a plant, such as spinach, or does not come into contact with the edible part of the plant. Strawberries are covered by plastic, and drip irrigation system is used during its cultivation, which prevents contact with the edible portion. Finally, the rules state that the water must comply with water test, with less than a geometric mean of 126 colony forming unit (CFU) of generic *Escherichia coli* and a statistical threshold of less than 410 CFU generic *Escherichia coli* (4).

If the water does not comply with the rule, the FDA has a few alternatives for the farmers: using others source of water, use a microbial die-off rate of 0.5 log per day until the standards are met or the treatment is supported for the research to be able to control the microbial rate and prove it by sampling. There are several treatments that can be used to control microbial rates. The most common is chemical treatment is adding chlorine to the water; however, non-chemical treatments, such as like UV lights, ozonation, reverse osmosis and solar radiation are also available. Some of these treatments are expensive and complicated to use. Physical removal is also an alternative to reach the microbial criteria. In this study, we focus in making a filtration material that can be used in commercially available filtration devices, costs less and is easy to use (43). To help produce farms comply with the new regulations, a novel filtration material based on Surfactant Modified

Zeolite (SMZ) was developed. The attachment and detachment of bacteria in a filtration system is the key part to its efficacy. However, when a filtration system is used, it only attaches the bacteria, making it possible for the bacteria to grow inside the filtration system. This problem could be solved by using a surfactant that has antimicrobial properties against the attached bacteria. Moreover, the Zeolite is especially of great interest in this regard due to the fact that it is an ion-exchange mineral for its ability to attract and bond the bacteria and viruses (36, 37). There are different kinds of forces involved in the attachment of bacteria. However, when the Zeolite is modified, scientific studies have found that surfactant modified zeolite enhances the hydrophobicity and ionic surface charge of zeolite, which in turn enhances the attachment of microorganisms to the surface of zeolite (38, 44-46).

3.2 Materials and Methods

3.2.1 Surfactant Modified Zeolite (SMZ)

A natural zeolite (Zeobrite Xtreme®) provided by Zeobrite® Corporation with a composition of more than 71% clinoptilolite was modified using hexadecyl(trimethyl)ammonium-bromide (HDTMABr). This is quaternary ammonia that works as a catatonic surfactant capable of modifying the zeolite surface polarity and increasing its efficiency in water treatment (47). The zeolite was modified by mixing it with solutions of HDTMABr and distilled water. The solutions were made at a concentration of HDTMABr 0, 5, 10, 15, 20, 25 and 30% (w/w) and mixed at a ratio of 0.6 ml/g of zeolite. The aqueous solution of zeolite with HDTMABr was agitated at 50 rpm for 24 hours, following which it was dried in a conventional oven at 150ºC for 30 minutes. The resulting Surfactant Modified Zeolite with hexadecyl (trimethyl) ammonium-bromide (SMZ-HDTMABr) was washed with tap water until all the frothing was removed, dried at 25°C

temperature in a desiccator chamber with t.h.e.® desiccant by Millipore Sigma and sieved to a particle size between 355 microns and 710 microns with ASTM-graded sieves (Figure 5).

Figure 5. Zeolite particle size classification. Left to right: unclassified or higher than 710 microns, below 355 microns, optimal size between 355-710 microns.

3.2.2 Microbial Culture Preparation

Three different pathogens were used to analyze the capabilities of the SMZ-HDTMABr to remove the bacteria, *Escherichia coli* O157:H7 *(*ATCC 43895*), Listeria* monocytogenes ½ a (Lm F4263, CDC, Atlanta) and a cocktail of 9 serotypes of *Salmonella sp*. The serotypes tested through the study were: Salmonella Albert (AR Bank #0401), Salmonella Cubana (AR Bank #0402), Salmonella Stanley (AR Bank #0403), Salmonella Heidelberg (AR Bank #0404), Salmonella Senftenberg (AR Bank #0405), Salmonella Corvallis (AR Bank #0406), Salmonella Concord (AR Bank #0407), Salmonella Typhimurium (AR Bank #0409) and Salmonella Infantis (AR Bank #0410). The bacteria cultures were reactivated from -80°C by growing them in successive transfers in a brain-heart infusion broth (BHI) for 24 hours at 37°C to achieve a concentration of 5–6 log CFU/ml. To assure the purity of the culture, the following selective media was used: *Escherichia*

coli O157:H7 was plated on Sorbitol MacConkey Agar supplemented with Cefixime-Tellurite (CT-SMAC), *Salmonella sp.* was plated on Xylose Lysine Deoxycholate Agar (XLD) and *Listeria monocytogenes* was plated on Modified Oxford Agar (MOX). All the plates were incubated at 37ºC for 48 hours, and a loop was used to inoculate the amount of BHI required for each experiment and incubated at 37ºC for 48 hours.

3.2.3 SMZ Bacterial Removal Testing

Laboratory scale filtration tests were conducted to determine the most effective concentration of HDTMABr to modify zeolite for the removal of *Escherichia coli* O157:H7, *Listeria monocytogenes* ½ a and *the Salmonella sp.* serotypes from Phosphate Buffer Saline (PBS). The filtration columns were prepared using a büchner funnel of 60 ml with 100g of the SMZ-HDTMABr modified with 0, 5, 10, 15, 20, 25 or 30% HDTMABr. The columns were compacted by filtering 500 ml of sterile distilled water and washed again before each filtration. Each filtration with the same material was set apart 48 hours. The material was stored in an aluminum foil at 25°C. The cultures were prepared by growing each bacteria in 50 ml of BHI for 24 hours at 37°C, followed by centrifuging it for 10 minutes at 4500RPM and the pellet was re-suspended in 1L of PBS buffer. The concentration of the inoculated buffer was plated and used as a positive control of the bacterial concentration. The inoculated PBS solution was filtered through the columns of each concentration of SMZ-HDTMABr and a sample of the effluent was collected to calculate the bacterial counts. All samples collected were serially diluted in PBS from 10^0 to 10^{-6} and 1ml was plated on selective media CT-SMAC, XLD and MOX, depending on the bacteria that was being tested. The spread plate technique was used, and all the bacteria were incubated at 37ºC for 48 hours. The experiment was repeated in duplicates for each bacteria.

3.2.4 Flow Cytometric Measurements

Flow cytometry analysis was performed using a BD FACScan flow cytometer with a light source of 15mW 488nm air cooled argon-ion laser. Two dyes were used in the experiment SYTO 9 and propidium iodide stains, bacteria with intact cell membranes stained fluorescent green, whereas bacteria with damaged membranes stained fluorescent red respectively. The excitation/emission maxima for these dyes are about 480/500nm for SYTO 9 stains, and 490/635 nm for propidium iodide. The bacterial stains were prepared as recommended by the manufacturer. All the treatments from SMZ-HDTMABr 0% to SMZ-HDTMABr 30% were analyzed, and a control of *Escherichia coli* and each dye were used to remove the autofluorescence. The data was analyzed using the software Flow Jo®. The bacteria were divided in four quadrants based on the fluorescent emitted: dead bacteria, altered dead bacteria, viable bacteria, altered viable bacteria. All the experiments were performed by duplicate.

3.2.5 Scanning Electronic Microscopy (SEM)

A scanning electronic microscopy where use the Quanta™ 3D DualBeam™ FEG FIB-SEM, which combines a Focused Ion Beam (FIB) with a high-resolution Field Emission Gun Scanning Electron Microscope (FEG-SEM). The pictures using the equipment were of treatments SMZ-HDTMABr 0%, SMZ-HDTMABr 10%, SMZ-HDTMABr 20% and SMZ-HDTMABr 30%.

3.2.6 Field Testing of SMZ Filtration

The SMZ-HDTMABr was tested in a field setting to account for other factors such as minerals, organic matter, natural bacterial population and the debris found naturally in pond water which can affect the filtration of the pathogen bacteria. The field was located at the LSU AgCenter Botanic Gardens in Baton Rouge, LA (Lat. 30° 24’ 32.1012’’N Long. 91° 6’ 21.0132”W). The crop selected was strawberries, an economic, cultural and susceptible crop in Louisiana that is

harvested directly from the field to the final container(48). The strawberries were grown and maintained through the growing practices recommended by the LSU AgCenter (49). The strawberry variety used was Strawberry Festival. Bareroots plats were transplanted in double drill rows with 0.4 m of spacing during the early fall, before the first frost in mid-October. The strawberry field was fertilized with 13-13-13 (10 lb/100 ft row), two weeks before transplanting, and fertilized weekly after the first frost through the drip irrigation system with 12-9-6 (16oz per 400 sq ft). The irrigation was supplemented at a rate of 25.4 mm per week if no rain was recorded. Frost protection was provided to the berries using floating row covers made by AgFabric® (Vista, California). Row covers were used intermittently during the winter due to the low temperature (<-5ºC) recorded during the season. Strawberries disease management was handled in a case by case basis, except for two applications of Captan® (2qrt per acre) used to control fungal infestations. The field dimension (Figure 6, a) was 30 x 22m further divided into nine plots having individual measure of 1.5 x 3m (Figure 6, a). Six plots were randomly assigned for each treatment in a split plot design. Each plot was separated by 3m to avoid cross contamination between the treatments.

3.2.7 Filtration System

The filtration system was developed using pool filters SandPro™ Model 50D purchased in Leslies pool supplies in Baton Rouge, LA. The treatments were as follows: a) control, which was pond water with no filtration system; b) sand treatment, composed of a filter filled with 22.68kg of 20 grain silica sand with a particle size of 0.45- 0.55 mm; a SMZ-HDTMABr composed of two consecutives filters: the first one filled with 22.68kg of silica sand as described above and a secondary filter filled with 11.34kg of SMZ-HDTMABr. The modification chosen for the field test was a medium concentration treatment at 20% (w/w) of HDTMABr.

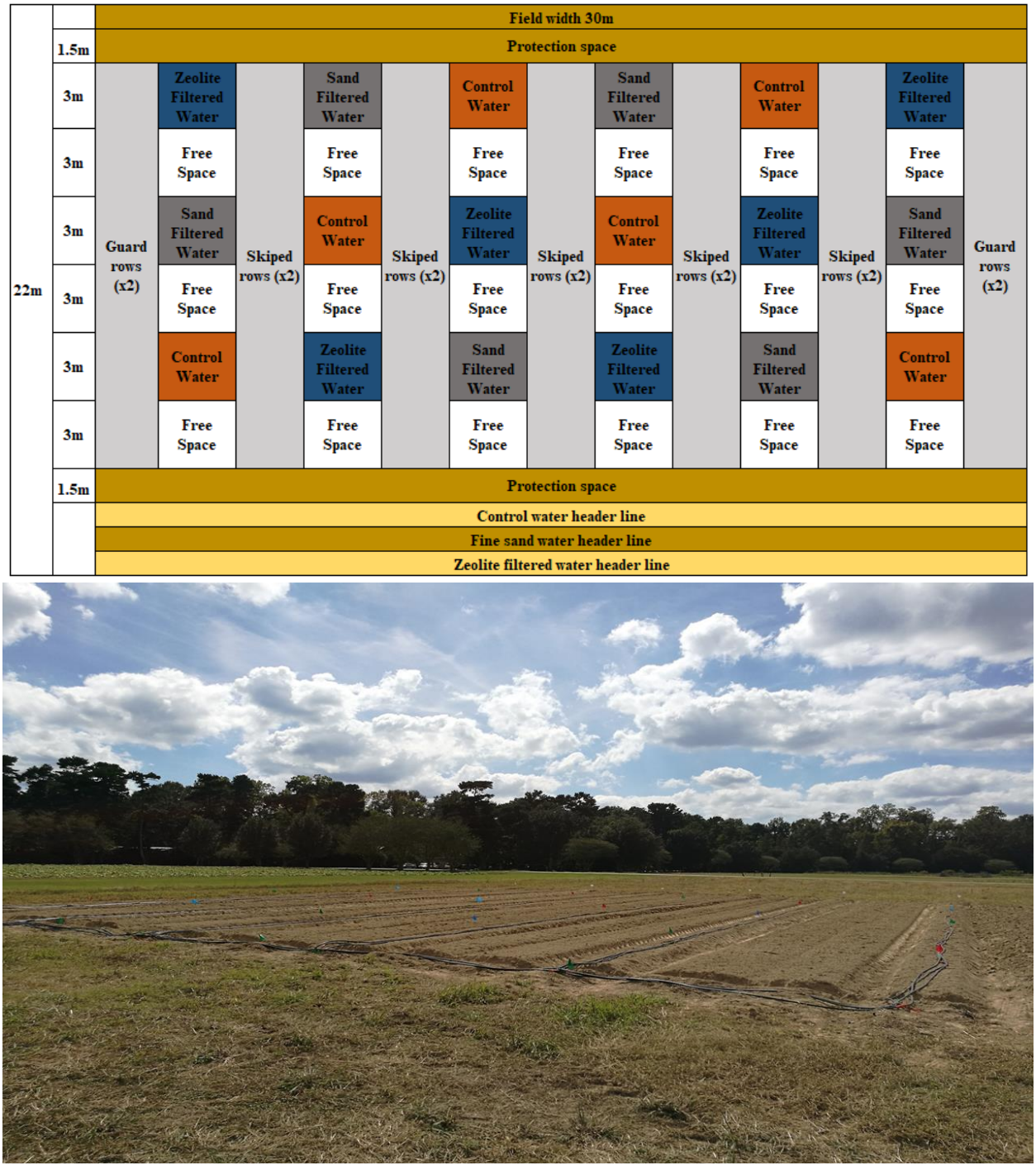


Figure 6. (a) Top, layout of the strawberry field. (b) Bottom, view of the strawberry field in October 2017.

The irrigation system had individual header lines for each treatment to avoid cross-contamination and to be able increase randomization in the field (Figure 6, b). The filtration system was used to irrigate the crops using pond water which was naturally contaminated with goose

feces. The pond water was additionally spiked with *Escherichia coli* ATCC 25922, a non-pathogenic strain, before sampling. One L of an overnight generic *Escherichia coli* culture, grown in BHI broth at 37ºC for 48 hours, was centrifuged, and the pellet was re-suspended into a 50 ml PBS buffer and added to a tank with 950L of pond water to achieve a final concentration of approximated 5-6 log CFU/ml of generic *Escherichia coli* (Figure 7).

Figure 7. Zeolite filtration arrangement. Tank with inoculated water, pool filtration system with sand zeolite.

3.2.8 Microbial Sampling

Each plot of strawberries was harvested monthly. Samples were removed immediately after irrigation with the three treatments. The water samples were taken from each header line into sampling cups of 100ml and refrigerated immediately at 4°C. The mature strawberry sample from each sub-plot was harvested individually and refrigerated immediately at 4°C. In the laboratory, each strawberry sample was pooled into a composite sample and two sub-samples of 25g were homogenized in buffered peptone water to control the effect of the acidity in the bacteria. The

strawberry and water samples were analyzed for Coliforms and *Escherichia coli* using 3M *E. coli*/coliform petrifilm™. The petrifilm analysis were perform using the AOAC™ Official Method 991.14(50).

3.2.9 Water Chemical Quality

Water testing was conducted at the LSU Soil Testing and Plant Analysis Laboratory to evaluate the changes by the zeolite filtration including alkalinity, calcium, chloride, conductivity, hardness (Ca, Mg) iron, magnesium, manganese, nitrate, pH, potassium, salts, sodium adsorption ratio (SAR), sulfur and bromide. The methods used at the farm were as follows: The alkalinity was tested by direct reading in the pH meter. The alkalinity was calculated after the sample was titrated to a pH of 4.5, using 0.02 N HCI. The conductivity was a direct reading in the conductivity meter, sodium, calcium, magnesium, potassium, iron, manganese, sulfur, chloride which was obtained by a direct reading on ICP, For nitrate, it was obtained by a direct reading on Hach DR900 Colorimeter and bromide, a direct reading in the Hanna Instruments HI96716 Photometer (conducted in LSU Food Microbiology laboratory).

3.2.10 Statistical Analysis

All the bacteriological methods were analyzed by the analysis of variances (ANOVA) and the difference between treatments were determined by pairwise comparison using tukey in the statistical package SAS® version 9.4. The statistical significance was used at $P<0.05$. All experiments were repeated 2 times with 2 replications per experiment.

3.3 Results and Discussion

This study evaluated the effectiveness of using a SMZ- HDTMABr in a filtration system to remove foodborne pathogens from irrigation water. An in-vitro study was conducted to determine the best concentration of SMZ- HDTMABr to use for removing *E. coli* 157:H7, *L.*

monocytogenes and *Salmonella* from sterile deionized water. In addition, the ability of the SMZ-HDTMABr to remove the foodborne bacterial from water after continuous filtration was investigated at 0, 48, and 72 hours.

SMZ- HDTMABr at concentrations from 15% to 30% removed more than 4.0 log CFU/ml of *E. coli* 0157:H7 from sterile deionized water (Table 1). This is consistent with the results from similar modifications of Zeolite with other surfactants that removed >1.5 log CFU/ml of *E. coli* using a Cu activated Zeolite biofilter (51). Another study using a zero-valent iron bios and filter with zeolite was able to remove > 6 log of *E. coli O157* in irrigation water (52). After reusing the SMZ-HDTMABr for three filtrations (72 hours), the *E. coli* counts began to increase in the sterile deionized water. The 100g of SMZ- HDTMABr might have started to reach the maximum filtration capacity after filtering 3L of 4.60 to 4.91 Log CFU/ml of *E. coli*. However, the SMZ-HDTMABr was still able to remove more than 4.0 log of *E. coli* O157:H7 from the sterile deionized water.

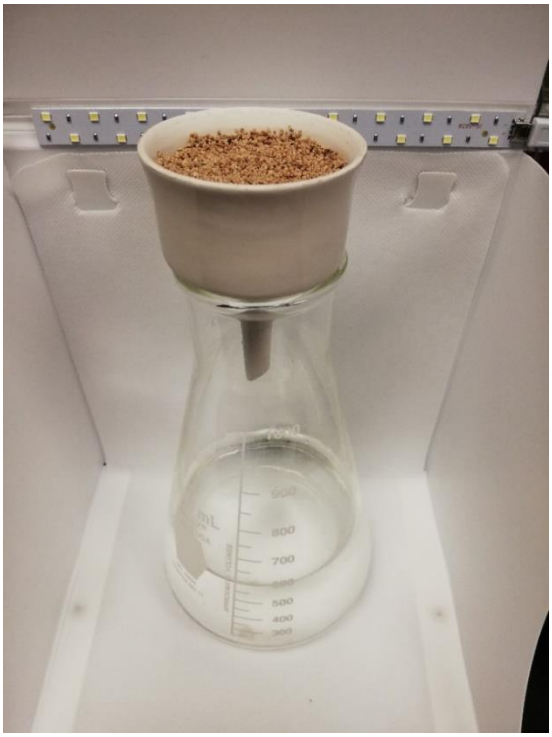


Figure 8. Filtration unit mounted with büchner funnel as support for the SMZ after filtration.

The same trend was observed at the 48hours filtration; however, the 96hous filtration presented a significant increase in the counts, suggesting that our filtration mount was reaching its filtration capacity for the heavy load of bacteria used in the study (Figure 8). Other study using a zero-valent iron biosand, which also have the ability to absorb bacteria due to its charged achieved a complete removal of *Escherichia coli* O157:H7; however, the filtration mechanism was deep bed and a lower concentration of bacteria 8log/100ml was used (52). Another study using a similar gravity bed filter modified zeolite with cooper, achieving consistency for 5 months after the removal of *Escherichia coli* from storm water (51). In our study, we were able to use the SMZ-HDTMABr for three filtrations (0, 48 and 72 hours) of *Escherichia coli* counts started to increase in the PBS. However, the SMZ-HDTMABr at concentrations higher than 20% was still able to remove more than 4.0 log CFU/ml of *Escherichia coli* O157:H7 from the sterile deionized water.

Table 1. *Escherichia coli O157:H7* counts[a] in sterile water after filtration through SMZ-HDTMABr.

Treatment	Filtration[b]		
% HDTMABr-SMZ	0hr	48hr	96hr
Control	4.91 ± 0.36^{Aa}	4.60 ± 0.52^{Ab}	4.63 ± 0.39^{Ab}
0	4.80 ± 0.34^{ABa}	4.79 ± 0.60^{ABa}	4.79 ± 0.45^{ABa}
5	4.69 ± 0.25^{Ba}	4.68 ± 0.67^{Ba}	4.55 ± 0.26^{Ba}
10	4.07 ± 0.99^{Cb}	4.02 ± 0.33^{Cb}	4.17 ± 0.02^{Ca}
15	1.62 ± 1.14^{Db}	2.71 ± 0.60^{Da}	2.63 ± 0.93^{Da}
20	1.34 ± 0.95^{Ea}	0.29 ± 0.42^{Eb}	1.22 ± 0.58^{Ea}
25	ND ^{Fb}	ND ^{Fb}	0.90 ± 0.73^{Fa}
30	ND ^{Fb}	ND ^{Fb}	0.29 ± 0.35^{Ga}

[a]Mean Log (CFU/ml) ± Standard Derivation. Population means within each column with different capital letters are significantly different ($P<0.05$). Population means within each row with different lowercase letters are significantly different ($P<0.05$).
[b]To test the filtration capacity of the SMZ *Escherichia coli O157:H7* inoculated water was filtered through the same material at different percentages of SMZ-HDTMABr at times 0, 48, 72 hr.

A study was conducted to determine how effective 30% HDTMABr-SMZ filtration was in removing different *Salmonella* serotypes from sterile deionized water (Table 2). No significant difference was found between the different serotypes of *Salmonella sp.* filtered through the 30%

HDTMABr-SMZ. Regardless of the *Salmonella sp.* Serotype, the bacterial count was reduced to non-detectable levels in filtered water at the higher concentration, 30% HDTMABr-SMZ. However, differences did occur at lower concentration with the *Salmonella* sp. serotypes.

The *Salmonella enterica* serotypes had no significant difference in removal rate from water using 30% HDTMABR-SMZ (Table 2). *Salmonella sp.* is a bacterium that has different attachment to surfaces as compared to other bacteria and moderate range of susceptibility against surfactants like HDTMABr. A study in poultry tissue found that different quaternary ammonia compounds were capable of inhibiting *Salmonella* Typhimurium even when used at low concentrations. However, significant difference was found between the quaternary ammonias, but the different strains were not tested (53).

Table 2. Salmonella serotypes counts[a] in sterile deionized water before and after filtration with SMZ-HDTMABr.

Salmonella Serotype	Treatment HDTMABr-SMZ %	Filtration 1	Filtration 2
S. Albert	0	5.84 ± 0.09^{A}	5.85 ± 0.01^{A}
	30	ND[b]^{B}	ND^{B}
S. Stanley	0	5.64 ± 0.07^{A}	5.85 ± 0.01^{A}
	30	ND^{B}	ND^{B}
S. Senftenberg	0	5.72 ± 0.03^{A}	5.93 ± 0.34^{A}
	30	ND^{B}	ND^{B}
S. Corvallis	0	5.88 ± 0.03^{A}	5.76 ± 0.15^{A}
	30	ND^{B}	ND^{B}
S. Cubana	0	5.97 ± 0.03^{A}	6.31 ± 0.07^{A}
	30	ND^{B}	ND^{B}
S. Concord	0	5.97 ± 0.14^{A}	5.60 ± 0.05^{A}
	30	ND^{B}	ND^{B}
S. Infantis	0	6.03 ± 0.02^{A}	6.25 ± 0.05^{A}
	30	ND^{B}	ND^{B}
S. Heidelberg	0	5.90 ± 0.08^{A}	6.13 ± 0.01^{A}
	30	ND^{B}	ND^{B}
S. Typhimurium	0	5.78 ± 0.08^{A}	5.93 ± 0.03^{A}
	30	ND^{B}	ND^{B}

[a]Mean log (CFU/ml) ± Standard Derivation. [b]ND= Non-detectable.
Each filtration is composed of 1l of inoculated water
Population means within each column with different capital letters are significantly different ($P<0.05$). No significance difference found between rows ($P<0.05$).

Since no difference were found with the different *Salmonella sp.* serotypes filtered through the HDTMABR-SMZ, we used a cocktail of nine strains to do the *in-vitro* study. In the filtration of the cocktail through the SMZ-HDTMABr (Table 3), a reduction of 1 log CFU/ml at 0 hours occurred at 15 and 20% SMZ-HDTMABr, 2 log CFU/ml at 25% SMZ-HDTMABr and >4 log CFU/ml at 30% SMZ-HDTMABr. The same trend was not observed at 48 hours, where the SMZ-HDTMABr were only able to reduce 1 log CFU/ml at 20% SMZ-HDTMABr and approximately 2 log CFU/ml at 25 and 30%. At 72 hours, the only clear trend was 1 log CFU/ml at 30% SMZ-HDTMABr.

When the surfactant attaches to all the anions in the zeolite, a higher cationic charge will result. However, further increase in the concentration of the surfactant can create a by-layer of HDTMABr bonded by hydrophobic interaction, causing the surfactant to desorb from the surface of the SMZ and a subsequent reduction of the attachment of bacteria to the zeolite. Due to the antimicrobial actions of the surfactant, the total bacterial removal will not necessarily be adverse (33). *Salmonella sp.*, have a hydrophobicity of 0.42 and a negative surface charge(54), these characteristics make *Salmonella sp*. easier to be removed at higher concentrations, not only a higher net charge is achieved but also more hydrophobicity, due to the increase of the HDTMABr by-layer. Additionally we do not expect the HDTMABr desorption to be the only cause in the reduced removal of *Salmonella sp.* from the water; resistance to quaternary ammonia like HDTMABr can also be another factor (55). A study found that fractal silver nanoparticles supported on zeolites was able to completely remove *Escherichia coli* and greatly reduce the number of *Salmonella sp.* from sterile water. Their results were similar to the SMZ modified with HDTMABr used in this research, that supports the net charges and hydrophobicity as the principal sources for the bacterial removal (56).

Table 3. *Salmonella enterica* serotypes cocktail counts[a] in sterile water after filtration through SMZ-HDTMABr.

Treatment % HDTMABr-SMZ	Filtration[b] 0hr	48hr	96hr
Control	4.79 ± 0.18^{Aa}	4.76 ± 0.18^{Aa}	4.28 ± 0.07^{Ab}
0	4.63 ± 0.05^{ABb}	4.93 ± 0.03^{Aa}	4.22 ± 0.14^{Ac}
5	4.64 ± 0.05^{ABb}	4.91 ± 0.09^{Aa}	4.33 ± 0.26^{ABc}
10	4.44 ± 0.39^{Bb}	4.83 ± 0.03^{Aa}	4.15 ± 0.17^{ABc}
15	3.34 ± 1.44^{Cc}	4.74 ± 0.01^{Aa}	4.01 ± 0.04^{ABb}
20	3.34 ± 0.71^{Cb}	3.90 ± 0.12^{Ba}	3.93 ± 0.02^{ABa}
25	1.34 ± 1.34^{Dc}	2.99 ± 1.00^{Cb}	4.15 ± 0.03^{Ba}
30	0.33 ± 0.33^{Ec}	2.58 ± 0.48^{Db}	3.02 ± 1.08^{Ca}

[a]Mean Log (CFU/ml) ± Standard Derivation. Population means within each column with different capital letters are significantly different ($P<0.05$). Population means within each row with different lowercase letters are significantly different ($P<0.05$).
[b]To test the filtration capacity of the SMZ *Salmonella enterica* serotype cocktail inoculated water was filtered through the same material at different percentages of SMZ-HDTMABr at times 0, 48, 72 hours.

Listeria monocytogenes was reduced to non-detectable levels in sterile water when filtered through HDTMABr-SMZ at concentrations as low as 10% HDTMABr (Table 4). *Listeria monocytogenes* is sensitivity to the antimicrobial activity of quaternary ammonia compounds similar to HDTMABr (57). There is evidence that *Listeria monocytogenes* do not contain the resistance genes to protect them against surfactants like HDTMABr (58). In addition, we can see a trend over the decrease in effectiveness after subsequent filtrations in the reduction *Listeria monocytogenes* like what was found with *Salmonella sp.* and *Escherichia coli* O157:H7 using HDTMABr-SMZ. However, at the last filtration the removal of *Listeria monocytogenes* is far greater than the other bacteria. The HDTMABr at concentrations higher than 10% and 15% removed a 4.0 log of *Listeria monocytogenes* and *Escherichia coli* O157:H7 from the water.

Table 4. *Listeria monocytogenes* counts[a] in sterile water after filtration through SMZ-HDTMABr.

Treatment	Filtration[b]								
% HDTMABr-SMZ	0hr			48hr			96hr		
Control	5.78	±	0.14^{Aa}	5.84	±	0.09^{Ba}	5.61	±	0.13^{Ab}
0	5.71	±	0.25^{Ab}	6.02	±	0.03^{Aa}	5.67	±	0.19^{Ab}
5	2.59	±	1.50^{Ba}	1.92	±	1.61^{Cb}	0.44	±	0.44^{Bc}
10		ND	^{Cb}	0.03	±	0.03^{Db}	0.23	±	0.23^{Ca}
15		ND	^{Ca}		ND	^{Da}		ND	^{Da}
20		ND	^{Ca}		ND	^{Da}		ND	^{Da}
25		ND	^{Ca}		ND	^{Da}		ND	^{Da}
30		ND	^{Ca}		ND	^{Da}		ND	^{Da}

[a]Mean Log (CFU/ml) ± Standard Derivation. Population means within each column with different capital letters are significantly different (P<0.05). Population means within each row with different lowercase letters are significantly different (P<0.05).
[b]To test the filtration capacity of the SMZ *Listeria monocytogenes* inoculated water was filtered through the same material at different percentages of SMZ-HDTMABr at times 0, 48, 72 h.

Table 5. Kirby-bauer disk diffusion susceptibility test with HDTMA-Br.

	% (v/v) HDTMA-Br									
Bacteria	1	2	3	4	5	10	15	20	Gm[a]	Va[b]
S. Albert	6.90	7.03	7.59	7.88	8.34	9.55	9.26	10.68	<6	25.16
S. Stanley	6.71	7.05	7.49	7.78	8.18	9.18	9.56	10.22	<6	<6
S. Senftenberg	6.73	6.93	7.13	7.64	8.11	8.65	9.34	10.61	9.96	11.98
S. Corvallis	6.75	6.96	7.47	7.86	8.16	8.92	9.65	10.30	24.35	23.05
S. Cubana	6.85	7.13	7.52	7.74	8.14	8.89	10.43	10.82	26.33	22.11
S. Concord	6.64	6.87	7.41	7.94	8.12	9.03	9.55	10.45	6.60	6.53
S. Infantis	6.82	6.90	7.23	7.92	7.96	9.11	9.91	10.58	13.76	12.39
S. Heidelberg	6.74	7.16	7.38	7.64	7.41	8.81	9.72	10.10	24.61	27.45
S. Typhimurium	6.83	7.03	7.45	7.73	8.24	9.03	9.66	10.53	17.90	25.17
Escherichia coli O157:H7	7.31	7.56	8.82	8.99	9.22	9.82	10.23	10.60	<6	27.05
Listeria monocytogenes	6.62	7.00	7.32	7.68	7.90	9.25	10.35	10.86	<6	13.17

[a] Gentamicin. [b] Vancomycin.

Based on the results of the laboratory scale experiments, 20% HDTMABR was the more effective treatment and was selected to be used in the filtration system for the field trials. The results found that the HDTMABR-SMZ filtration device was able to remove more than 4.0 log CFU/ml of coliform and *Escherichia coli* (Table 6). over two production cycles without the

reduction of the overall effectivity and the use of sand was not able to remove bacteria. Only the use of the Zeolite filtration device was able to assure that the pond water achieved a level optimal for irrigation water after the remedial practice.

Table 6. Coliform and *Escherichia coli* Petrifilm counts[a] of inoculated pond water after filtered through sand or 20% HDTMABr-SMZ during field-testing on strawberry plants using Petrifilm.

	Control			Sand			Zeolite		
Coliforms									
February, 2017	4.95	±	0.02^{aAB}	3.39	±	0.04^{bC}	ND		^{cC}
March, 2017	5.41	±	0.52^{aA}	5.26	±	0.60^{aA}	0.86	±	0.67^{bB}
April, 2017	5.66	±	0.39^{aA}	5.36	±	0.45^{aA}	ND		^{bC}
February, 2018	4.31	±	0.07^{aB}	4.10	±	0.07^{aB}	1.97	±	0.10^{bA}
March, 2018	4.02	±	0.52^{aC}	3.86	±	0.60^{aB}	ND		^{bC}
April, 2018	4.87	±	0.39^{aB}	4.51	±	0.45^{aA}	1.15	±	0.26^{bB}
Escherichia coli									
February, 2017	4.62	±	0.12^{aB}	3.35	±	0.03^{bC}	ND		^{cC}
March, 2017	5.41	±	0.01^{aA}	5.26	±	0.11^{aA}	0.86	±	0.49^{bB}
April, 2017	5.66	±	0.12^{aA}	5.36	±	0.03^{aA}	ND		^{bC}
February, 2018	4.31	±	0.04^{aC}	4.10	±	0.22^{aB}	1.31	±	0.02^{bA}
March, 2018	4.02	±	0.03^{aC}	3.86	±	0.10^{aC}	ND		^{bC}
April, 2018	4.80	±	0.04^{aB}	4.51	±	0.14^{aB}	1.15	±	0.00^{bA}

[a]Mean Log (CFU/ml) ± Standard Derivation. ND= non detectable. Population means within each column with different capital letters are significantly different ($P<0.05$). Population means within each row with different lowercase letters are significantly different ($P<0.05$).

Two methods for detecting *Escherichia coli* in the filtered irrigation water were used during the field experiments. The two methods were the *Escherichia coli*/coliform Petrifilm method (Table 6) and EPA 1603 water filtration method (Table 7). During the two harvesting years, similar trends were found with both Petrifilm and EPA 1603 (Table 6 & 7). However, due to the high concentration of *Escherichia coli* in the control and sand, all the results were over the detection limit of the EPA 1603 method. However, in places where no detectable counts were obtained with the Petrifilm method, we were able to obtain the CFU of *Escherichia coli* with the EPA method. Moreover, the results obtained through the EPA 1603 method confirmed that using the HDTMABr-SMZ could reduce the levels of *Escherichia coli* under the regulation limit of <126

CFU/100ml of *Escherichia* coli (59). Similar to our results, a study done in the midwestern of USA found that when the EPA 1603 method is used as a standard, an accuracy of 82.07% is achieved (60).

Table 7. *Escherichia coli* levels inoculated into pond water after filtered through sand or 20% HDTMABr-SMZ during field-testing using the EPA 1603 method.

	Control	Sand	Zeolite
Escherichia coli			
February, 2017	>300^{Aa}	>300 ^{Aa}	10 ± 0.00^{Eb}
March, 2017	>300 ^{Aa}	>300 ^{Aa}	>300 ^{Aa}
April, 2017	>300 ^{Aa}	>300 ^{Aa}	3.88 ± 1.00^{Cb}
February, 2018	>300 ^{Aa}	>300 ^{Aa}	3.10 ± 1.08^{Cb}
March, 2018	>300 ^{Aa}	>300 ^{Aa}	4.00 ± 2.17^{CDb}
April, 2018	>300 ^{Aa}	>300 ^{Aa}	5.51 ± 1.08^{Db}

Geometric mean log (CFU/100 ml) ± Statistical threshold value.
[a]Mean Log (CFU/ml) ± Standard Derivation. Population means within each column with different capital letters are significantly different (P<0.05). Population means within each row with different lowercase letters are significantly different (P<0.05).

Strawberry samples were harvested during the first production cycle (2017) and analyzed for *Escherichia coli* and Coliform counts (Table 8). No significance difference were found between treatments. Strawberries in Louisiana are cultivated using a plastic cover that separates the water from fruit, resulting in no direct contact from the irrigation water to the fruit. However, Louisiana is a state prone to flooding, creating a high-risk situation if contaminated water is used to irrigate a field.

Table 8. Coliform and *Escherichia coli* levels of strawberry samples during the harvesting seasons.

	Control	Sand	Zeolite
Coliforms			
February, 2017	1.73 ± 0.49	1.66 ± 0.58	2.09 ± 0.69
March, 2017	2.28 ± 0.61	2.04 ± 0.70	2.60 ± 0.39
April, 2017	1.90 ± 0.59	1.59 ± 0.57	1.63 ± 0.49
Escherichia coli			
February 2017	1.66 ± 0.28	1.30 ± 0.00	ND
March 2017	1.45 ± 0.49	1.48 ± 0.50	2.02 ± 0.67
April 2017	ND	ND	ND

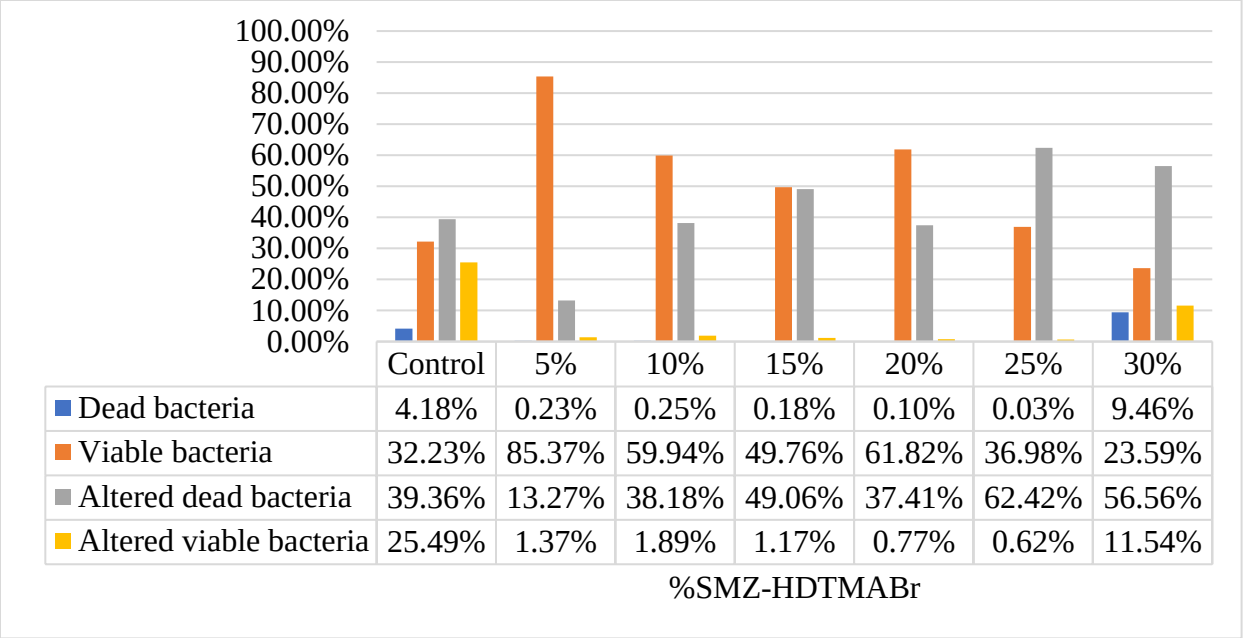
Mean log (CFU/g) ± Standard Derivation. ND = No significance differences

Table 9. Overall chemical results of irrigation water after filtration through different filtration treatments.

Parameter, unit	Control				Sand				Zeolite			
	2017		2018		2017		2018		2017		2018	
Alkalinity	30.91 ± 2.82		31.72 ± 3.45		60.19 ± 26.88		41.48 ± 10.35		78.08 ± 17.08		41.48 ± 3.45	
Calcium	9.56 ± 0.36		7.28 ± 0.33		16.08 ± 2.01		6.98 ± 0.14		9.67 ± 3.46		7.02 ± 0.23	
Chloride, ppm	9.97 ± 0.85		14.06 ± 0.86		11.35 ± 1.18		14.25 ± 0.08		19.13 ± 0.23		13.78 ± 4.18	
Conductivity, µmho	200.97 ± 21.81		130.65 ± 2.12		206.03 ± 31.19		129.65 ± 0.49		263.20 ± 44.80		130.5 ± 0.07	
Hardness (Ca, Mg)	29.10 ± 1.04		24.13 ± 1.26		45.49 ± 5.45		23.47 ± 0.29		28.67 ± 10.55		23.91 ± 1.03	
Iron, ppm	0.07 ± 0.03		0.46 ± 0.02		0.07 ± 0.05		0.31 ± 0.09		0.20 ± 0.16		0.20 ± 0.01	
Magnesium, ppm	1.27 ± 0.04		1.44 ± 0.10		1.30 ± 0.17		1.47 ± 0.01		1.10 ± 0.47		1.55 ± 0.11	
Manganese, ppm	0.00 ± 0.00		0.02 ± 0.00		0.00 ± 0.00		0.01 ± 0.00		0.01 ± 0.01		0.01 ± 0.00	
Nitrate, ppm	1.33 ± 0.00		1.77 ± 0.63		2.07 ± 1.35		1.33 ± 0.00		1.99 ± 0.22		1.33 ± 0.00	
pH	8.23 ± 0.98		7.67 ± 0.23		9.31 ± 0.24		7.60 ± 0.07		8.13 ± 0.15		7.64 ± 0.13	
Potassium, ppm	4.10 ± 1.96		3.99 ± 1.50		3.41 ± 0.88		5.40 ± 0.35		4.34 ± 0.10		7.62 ± 2.41	
Salts, ppm	128.62 ± 13.96		83.62 ± 1.36		131.86 ± 19.96		82.98 ± 0.32		168.45 ± 28.67		83.52 ± 0.05	
SAR	0.77 ± 0.26		0.65 ± 0.00		0.80 ± 0.28		0.66 ± 0.03		4.39 ± 2.51		0.66 ± 0.00	
Sodium, ppm	9.57 ± 3.14		7.35 ± 0.20		12.50 ± 4.94		7.37 ± 0.24		47.31 ± 20.20		7.37 ± 0.14	
Sulfur, ppm	0.64 ± 0.26		0.57 ± 0.02		0.76 ± 0.13		0.51 ± 0.02		1.92 ± 0.60		0.48 ± 0.07	
Bromide	0.15 ± 0.13		0.16 ± 0.14		0.24 ± 0.17		0.20 ± 0.16		0.05 ± 0.05		0.07 ± 0.05	

Mean ± Standard Derivation.

In the chemical profile of the field study all the parameters where fit to plant strawberries the chemical results do not suggest any chemical leak or problem for using the filtration device, especially the levels of bromine remained lower in the filter results, this is excepted due to bromide being the counter ion of the SMZ-HDTMABr keeping it as a stable molecule with ion exchange.



	Control	5%	10%	15%	20%	25%	30%
Dead bacteria	4.18%	0.23%	0.25%	0.18%	0.10%	0.03%	9.46%
Viable bacteria	32.23%	85.37%	59.94%	49.76%	61.82%	36.98%	23.59%
Altered dead bacteria	39.36%	13.27%	38.18%	49.06%	37.41%	62.42%	56.56%
Altered viable bacteria	25.49%	1.37%	1.89%	1.17%	0.77%	0.62%	11.54%

%SMZ-HDTMABr

Figure 9. Viable non culturable bacteria analysis through flowcytometry.

Viable but not culturable are a known problem in food safety bacteria in the figure 9 we observed a flow cytometry analysis which is not a precise technique yet in live/death bacteria, however provide us with a estimated of how many viable but not culturable bacteria are being produced at each level of the HDTMABr in this case of *Escherichia coli O157:H7*. The results tend to decrease the viable bacteria and increased the amount of affected membranes the more concentration of HDTMABr we have, however due to the live stain (green) being in both live and death bacteria this is just an approximated.

The SEM FIB pictures demonstrate the means of action of the SMZ-HDTMABr at lower concentrations figure 10 a and b, we can appreciate a great number of bacteria with intact surfaces with attachment. Though at 20% of SMZ-HDTMABr the bacteria have greatly affected membrane due to the bactericide action of the quaternary ammonia and no bacteria were visible at the higher concentration of 30%.

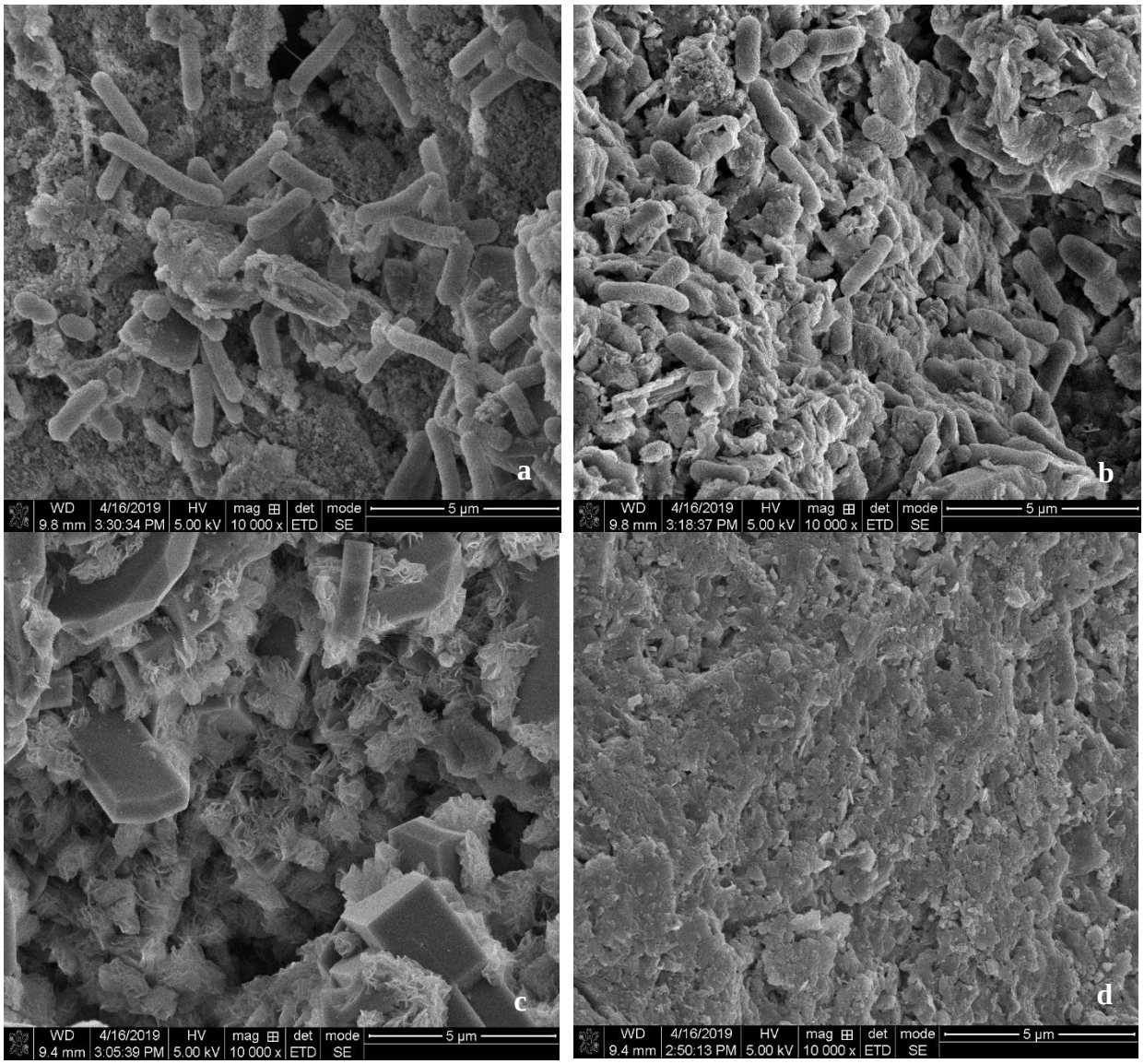


Figure 10. SEM-FIB pictures of a) SMZ-HDTMA 0%, b) SMZ-HDTMA 10%, c) SMZ-HDTMA 20%, d) SMZ-HDTMA 30%.

3.4 Conclusion

The *in-vitro* HDTMABr-SMZ filtration reduced *Listeria monocytogenes* to non-detectable levels with 10% HDTMABr, *Escherichia coli* O157:H7 to non-detectable levels with 25% HDTMABr, and *Salmonella sp*. to < 1 log CFU/ml with 30% HDTMABr in sterilized water. Even though *Salmonella sp.* had, a lower removal rate from water using the *in-vitro* SMZ-HDTMABr filtration compared to *E, coli* O157:H7 and *Listeria monocytogenes,* it was significantly better than

conventional agricultural sand. The use of SMZ-HDTMABr in the field during two harvesting seasons using a pre-filtration system of sand to remove the organic matter and specific zeolite size that was modified with HDTMABr could remove foodborne bacteria from irrigation water with enough efficacy to comply with the current water rule regulations. Farmers can easily adapt the filtration system if they use drip irrigation.

CHAPTER 4. ASSESSMENT OF LOUISIANA STRAWBERRY'S FOOD SAFETY: MICROBIAL COUNTS OF LOUISIANA STRAWBERRY RETAIL AND FOOD SAFETY GAPS OF PRODUCE HANDLERS IN LOUISIANA FARMERS.

4.1 Introduction

The produce can become contaminated with foodborne pathogens at any step along the food production chain. The FDA with the new FSMA regulation has sought to identify the key points of contamination. Trying to address these points in a new set of regulations: the produce safety rule (PSR) which focuses on six topics: a) agricultural water; b) biological soil amendments; c) worker training, health, and hygiene; d) domesticated and wild animals; e) sprouts; and f) equipment, tools, and buildings (61). Before the FSMA regulation, produce food handlers had some notions of food safety due to the use of Good Agricultural Practices (GAP). These voluntary regulations managed by the U.S. Department of Agriculture (USDA) covers topics to avoid problems such as cross-contamination, temperature abuse, animal manure and irrigation water that have caused some of the foodborne outbreaks associated with produce (62). Good Agricultural Practices (GAP) for minimal processed produce were the primary regulations that farmers use to train and operate under the food safety regulations (63).

The state of Louisiana has several farm practices which increase the risk of the contamination of strawberries with enteric viruses and foodborne bacteria such as consumer handpicking from the field. This practice is the found in 10% of strawberry market in Louisiana (48). To prevent foodborne outbreaks, strawberry growers and harvesters in Louisiana need to be educated in food safety practices. A study that evaluated the perception of farmers related to food regulations. They found that the key conclusions to address these problems were: a) the farmers need training related to their operational size, b) FSMA and GAP can be confusing and redundant, c) help to support farmers with new regulation with time and money (64), providing them with the

resources to comply more readily with the new regulations. Additionally, there is proof that before these new regulations, consumers and farmers have been seeking to increase their food safety using third party certification (65).

To determine the situation of Louisiana strawberry food safety industry, two studies were conducted: a) Study of the microbiological level of indicator bacteria and the prevalence of foodborne pathogens in strawberries purchased from retailers located in Baton Rouge, LA; and b) a cross-sectional study of the knowledge of produce growers with a survey created using a needs assessment model (66).

4.2 Materials and Methods

4.2.1 Level of Indicator Bacteria and Pathogens in Louisiana Retailer Strawberry

Prevalence of indicator bacteria and foodborne pathogens in strawberries purchased from retailers were analyzed during the months of January to May 2018. The strawberries (n=100) were collected from retailers in Baton Rouge and the surrounding cities of Louisiana. Strawberries were purchased from the following retailers: Walmart, Alberston, Winn Dixie, Trader Joe's, Southside Food Market and the 47th Annual Ponchatoula Strawberry Festival between April 13th–15th, 2018. From each sample, descriptive information, such as the date, brand or farm (Louisiana farms are codified to protect their identities), retailer, kind of production, organic or conventional, and place of origin, were collected.

The strawberries were purchased, placed into ice-chests and transported to the LSU AgCenter Food Microbiology and Safety Laboratory. All samples were analyzed the same day. A sample of 25g was chosen randomly from each container and homogenized in Buffered Peptone Water (BPW) to control the natural strawberry acidity. The microbiological indicators analyzed were the aerobic plate count (APC) using 3M Petrifilm™ Aerobic Count Plate and 3M Petrifilm™

E. coli/Coliform Count Plate. *Listeria sp.* was evaluated by the enrichment of the sample for 24 hours at 30ºC in Buffered *Listeria* Enrichment Broth (BLEB). Afterwards, it was plated onto Modified Oxford Agar (MOX) for 24 h incubation at 35°C. Positive *Listeria sp.* like colonies were isolated in Blood Agar Plates (BAP) at 25ºC for 24 hours and further tested with Microgen® *Listeria* latex test was performed. *Salmonella sp*. was analyzed by enriching the sample in Tetratione Broth Hajna for 24 hours at 35ºC and plated in XLD 48 hours at 35ºC. Black colonies with expected morphology were further tested with Microgen® *Salmonella* latex test. The results were reported as colony forming units per gram (CFU/g) and the pathogen results as prevalence.

$$\text{Prevalence} = \frac{\text{\# of positive samples}}{\text{Total number of samples}} \times 100$$

4.2.2 Food Handlers Survey

A survey with 33 questions was developed containing 13 demography questions and 20 questions, following a needs assessment model. The questionnaire was given to produce farmers to determine their knowledge gap in the proper handling of the produce to prevent foodborne pathogens contamination. A needs assessment is a systematic set of procedures that determine needs, examine their nature and causes, and set priorities for future action. (67)

The principal target of the survey were farmers with an emphasis in strawberry production practices. The actual population of strawberry farmers in Louisiana is estimated to be 100 farmers and the crop has an estimated of 300 cultivated acres in the state. The minimum amount of positive responses is n=10 based on the population of strawberry farmers. However, the survey was open to any produce farmer and specific questions were allocated only for strawberry farmers. The survey was analyzed using descriptive statistics. The results of the analysis and the needs assessment model provided information about the principal weakness in safety training in the produce industry in the state of Louisiana.

4.3 Results and Discussion

4.3.1 Bacterial Indicators

An important factor to Louisiana consumers is to purchase locally grown strawberries. The supply of strawberries in the state is constant during the year, and it even increases during Louisiana strawberry season. However, most of the suppliers of strawberries in the country come from California and Florida (68). This study aims to develop a framework of the food microbiological state of the retailers' strawberry as a measure of food safety.

The study was conducted from January to May 2018. During this time, each sample had its date of purchase recorded, and the dates were clustered into months for a more concise analysis. Table 10 shows a tendency of increase in the bacterial counts during the months of March and April (APC 5.54-5.13 log CFU/ml and Coliforms 1.87-0.61 log CFU/ml), dates of the Louisiana strawberry harvest. However, coliform bacteria, a closer indicator of pathogens presence, were higher in the months of February and March (1.05 and 1.87 log CFU/ml), when strawberry from other states are still a primary source. On the other hand, the counts of coliform the principal indicator for produce were lower, at around 0.61 log CFU/ml in the months, where a higher prevalence of pathogens was found in the strawberries.

Table 10. Bacterial indicator counts and prevalence levels in strawberry by month.

Month	n	APC	Coliform	% Prevalence		
				Escherichia coli	*Salmonella sp.*	*Listeria sp.*
January	16	4.46 ± 1.10^{bc}	0.88 ± 0.86^{b}	nd	nd	2
February	15	3.53 ± 1.36^{d}	1.05 ± 1.09^{ab}	nd	nd	nd
March	19	5.54 ± 0.74^{a}	1.87 ± 0.88^{a}	2	nd	nd
April	26	5.13 ± 0.47^{ab}	0.61 ± 0.78^{b}	nd	1	2
May	24	4.31 ± 0.69^{cd}	0.65 ± 0.87^{b}	nd	nd	nd

Mean log (CFU/g) ± Standard Derivation. nd = non detectable

The strawberries' place of origin (Table 11) was recorded to determine if this influenced the pattern of the food safety. During the sampling, 44% of strawberries were from Louisiana

farms. These strawberries had the higher microbial counts for APC. In addition, a higher prevalence for *Escherichia coli* and *Salmonella sp.* was found. However, no statistical differences were found in the origin sources for coliforms, while in APC, Louisiana had high counts at 5.29 log CFU/ml and prevalence of *Salmonella sp.* and *Listeria sp.*, considering that the strawberries had no post-harvest treatment. This demonstrates a problem with Louisiana Strawberries. Furthermore, the foreign strawberries had one of the lowest means in APC 4.32 log CFU/ml and coliform 0.70 log CFU/ml. However, *Listeria sp.* was found in the foreign sample, the pathogen with the highest mortality rate in the immunocompromised people. These results indicated that it is necessary to enforce tighter regulations of verification programs from foreign suppliers contained in the FSMA regulation.

Table 11. Bacterial indicator counts and prevalence levels in strawberry by place of origin.

Place of origin		n	APC	Coliform	% Prevalence		
					Escherichia coli	*Salmonella sp.*	*Listeria sp.*
Foreign	Mexico	18	4.32 ± 1.17^{b}	0.70 ± 0.88^{a}	nd	nd	2
Domestic	USA*	7	4.68 ± 0.90^{ab}	1.32 ± 1.25^{a}	nd	1	nd
	LA	44	5.29 ± 0.63^{a}	1.23 ± 0.99^{a}	2	nd	2
	AR	11	3.58 ± 1.52^{b}	0.72 ± 0.97^{a}	nd	nd	nd
	CA	20	4.17 ± 0.64^{b}	0.63 ± 0.84^{a}	nd	nd	nd

Mean log (CFU/g) ± Standard Derivation. nd = non detectable. *Unknown state

The study focused on retailers, (Table 12), as retailers like Retailer 5 and Alberston are expected to follow GAP with GFSI programs in place, at their supplier farms. Other suppliers from smaller retailers are only required to have basic food safety programs such as GAP-based or family practices. This will be the scenario for the Louisiana farmers. As explained before, this expected trend was observed in this study. Retailers with stricter food safety programs like Retailer 5 had the lowest of APC and Coliforms counts with 4.10 and 0.74 log CFU/ml, respectively. The strawberry festival and the famers market had the highest counts at 5.54 to5.11 log CFU/ml APC

and 1.84 to 0.69 log CFU/ml coliforms, which can be directly correlated to fewer food safety requirements to sell their product.

Table 12. Bacterial indicator counts and prevalence levels in strawberry by retailer.

Retailer	n	APC	Coliform	% Prevalence		
				Escherichia coli	*Salmonella sp.*	*Listeria sp.*
Retailer 1	2	4.59 ± 1.25^{abc}	0.69 ± 0.98^{ab}	nd	nd	1
Retailer 2	23	5.11 ± 0.48^{ab}	0.69 ± 0.79^{b}	nd	2	nd
Retailer 3 (Farmers market)	19	5.54 ± 0.74^{a}	1.87 ± 0.88^{a}	2	nd	nd
Retailer 4	14	4.23 ± 0.85^{bc}	0.80 ± 0.96^{b}	nd	nd	nd
Retailer 5	35	4.10 ± 1.22^{c}	0.74 ± 0.95^{b}	nd	1	1
Retailer 6	7	4.48 ± 0.78^{abc}	0.95 ± 0.90^{ab}	nd	nd	nd

Mean log (CFU/g) ± Standard Derivation. nd = non detectable

The individual farm and brand information was recorded for the strawberries; each farm has their own food safety practices in place, as even with standard regulations, they are responsible for developing a specific food safety plan for their own operation. Our study found that most of the farms in Louisiana require more food safety training, support and a review of their food safety practices to increase microbial quality in strawberries as they had higher APC counts on the strawberries compared to out of state farms (Table 13). There was a variability in the sample size between each farm as we were unable to sample constantly from every farm due to their specific dates of harvest. A longitudinal annual study could help to understand how the microbial counts of strawberries fluctuates between farms located in Louisiana and other brands sold in the state to consumers.

Table 13. Bacterial indicator counts and prevalence levels in strawberry by brand or farm.

Brand	n	APC	Coliform	% Prevalence		
				Escherichia coli	*Salmonella sp.*	*Listeria sp.*
Brand 1	9	4.35 ± 0.64^{abc}	0.59 ± 0.89^{ab}	nd	nd	nd
Brand 2	2	3.14 ± 0.56^{abc}	0.44 ± 0.63^{ab}	nd	nd	nd
Louisiana Farm A	6	5.26 ± 0.52^{ab}	1.07 ± 0.96^{ab}	nd	nd	nd
Louisiana Farm B	19	5.54 ± 0.74^{a}	1.87 ± 0.88^{a}	2	nd	nd
Louisiana Farm C	5	4.95 ± 0.39^{abc}	0.69 ± 0.53^{ab}	nd	nd	nd
Brand 3	2	5.86 ± 0.00^{abc}	1.02 ± 1.44^{ab}	nd	nd	nd
Brand 4	2	4.57 ± 1.17^{abc}	0.58 ± 0.81^{ab}	nd	nd	nd
Brand 5	2	4.97 ± 0.02^{abc}	nd^{b}	nd	nd	nd
Brand 6	11	3.58 ± 1.52^{c}	0.72 ± 0.97^{ab}	nd	nd	nd
Louisiana Farm D	6	4.93 ± 0.35^{abc}	0.77 ± 0.94^{ab}	nd	1	nd
Louisiana Farm E	2	4.96 ± 0.21^{abc}	1.50 ± 0.28^{ab}	nd	nd	nd
Mainland Farms	2	3.83 ± 0.77^{abc}	0.98 ± 1.38^{ab}	nd	nd	nd
Louisiana Farm F	6	5.28 ± 0.62^{ab}	0.21 ± 0.51^{b}	nd	1	nd
Brand 7	2	4.36 ± 0.36^{abc}	2.31 ± 0.44^{ab}	nd	nd	nd
Brand 8	3	3.82 ± 0.82^{abc}	1.21 ± 1.05^{ab}	nd	nd	nd
Brand 9	1	5.70 ± 0.00^{abc}	0.00 ± 0.00^{b}	nd	nd	1
Brand 10	10	4.18 ± 1.34^{bc}	1.11 ± 1.06^{ab}	nd	2	nd
Brand 11	8	4.06 ± 0.36^{bc}	0.46 ± 0.71^{b}	nd	nd	nd
Brand 12	2	4.98 ± 0.00^{abc}	nd^{b}	nd	nd	nd

Mean log (CFU/g) ± Standard Derivation.
nd = non detectable

Organically grown strawberries (Table 14) did not have any impact in the bacteriological loads and no significance was found compared to regularly grown strawberries, even though there was a higher expectation in the count of strawberries from organic farms due to the use of practices like manure fertilization. However, the results show that either kind of production can produce similar food safety, and this is not a concerning factor.

Table 14. Bacterial indicator counts and prevalence levels in strawberry by brand or farm.

Brand	n	APC	Coliform	% Prevalence		
				Escherichia coli	*Salmonella sp.*	*Listeria sp.*
Regular	85	4.75 ± 1.10	1.01 ± 1.00	2	nd	2
Organic	15	4.16 ± 0.74	0.69 ± 0.89	nd	2	nd

Mean log (CFU/g) ± Standard Derivation. nd = non detectable

4.3.2 Food Handlers Knowledge Survey

The survey was sent to 500 producers using post mail, but we did not receive a response from any of the farmers. A person-to-person survey was conducted, in which 33 surveys were collected. The survey was given at the Pontachoula strawberry festival and food safety GAP training workshops. The population surveyed were composed principally by male Caucasian producers (Table 14). The survey was divided in three parts: a) Demographic and food safety record information like outbreaks in their farms, b) food safety knowledge questions to determine their reediness in produce safety based on GAP and c) questions related to the filtration device developed in the previous study.

The farmers had an overall knowledge score of 76.15%. The principal gaps of the farmer's knowledge were the inability to recognize the symptoms related to foodborne illnesses, except for vomiting and diarrhea, correct procedures to avoid cross-contamination after handwashing and understanding that foodborne diseases can spread even after the person has recovered. In addition, the strawberry handlers scored high in activities intrinsic to the strawberry industry. In this industry, strawberries are packaged while they are still in the field. Thus, the importance of protecting the packaging from foreign material like soil or vegetative parts from surroundings was explained.

In other questions related to temperature and storage of the strawberries, the food handlers had average scores of 32.35%, the lowest score obtained in the survey, which highlights a knowledge gap in safe post-harvest practices. However, the food handlers scored high in questions related to basic hand washing and sanitization, fresh markets, festivals and street vendors.

Table 15. Food Handler Survey.

1. What is your age:	Answer	n
a. 18-24 years old	5.88%	2
b. 25-34 years old	14.71%	5
c. 35-44 years old	14.71%	5
d. 45-54 years old	29.41%	10
e. 55-65 years old	35.29%	12
2. With what gender do you identify yourself:		
a. Male	73.53%	25
b. Female	26.47%	9
3. Ethnicity origin (or Race):		
a. Caucasian/White	70.59%	24
b. Hispanic or Latino	14.71%	5
c. African American or Black	0.00%	0
d. Asian or Pacific Islander	2.94%	1
e. Native American or American Indian	2.94%	1
f. Other	8.82%	3
4. What is the highest degree or level of school you have completed? If currently enrolled, highest degree received.		
a. No school attended	0.00%	0
b. Some school, no diploma	5.88%	2
c. High school graduate	41.18%	14
d. Associate degree	11.76%	4
e. Bachelor's degree or more	41.18%	14
5. What languages are you proficient to understand signs and directions:		
a. English	85.29%	29
b. Spanish	0.00%	0
c. Spanish and English	14.71%	5
d. Other	0.00%	0
6.Have you received food safety training?		
a. Yes	88.24%	30
b. No	11.76%	4
7. Do you or your workers go to work with a sore throat or cough?		
a. Yes	44.12%	15
b. No	55.88%	19
8. Have you or your workers ever worked with diarrhea?		
a. Yes	20.59%	7
b. No	79.41%	27

(Table continued)

9. Have you or your workers ever worked with an infected wound?		
a. Yes	26.47%	9
b. No	73.53%	25
10. Have you ever been employed at a farm during a foodborne outbreak?		
a. Yes	23.53%	8
b. No	76.47%	26
11. Is it better to wash your hands with?		
a. Warm/hot water	76.47%	26
b. Cold water	14.71%	5
c. I don't know	8.82%	3
12. Do you need to wash your hands if you are going to wear gloves during food handling situations?		
a. Yes	85.29%	29
b. No	14.71%	5
13. How much time is needed to properly wash your hands?		
a. 20 seconds	61.76%	21
b. 15 seconds	11.76%	4
c. 30 seconds	26.47%	9
14. At least when strawberries should be washed?		
a. After harvesting	8.82%	3
b. Before processing	8.82%	3
c. Before eating	82.35%	28
15. If you only urinate you don't need to wash your hands with soap		
a. Yes	11.76%	4
b. No	88.24%	30
16. What are corrects procedures to exit a bathroom?		
a. Open the door only after finish cleaning and drying complete your hands	29.41%	10
b. Using a paper towel to open and close the door	70.59%	24
c. Leave the door open	0.00%	0
17. It is true that if strawberries are already in the primary containers (e.g. clamshells, pint baskets, etc.) it doesn't matter if they are in contact with the soil		
a. True	17.65%	6
b. False	82.35%	28
18. Can you take harvesting tools (glove) into the bathroom if you clean and sanitized them toughly before using again?		
a. True	17.65%	6
b. False	82.35%	28

(Table continued)

19. You must wash your hands after?		
a. Using the bathroom:	100.00%	30
b. Eating or drinking:	86.67%	26
c. Smoking:	86.67%	26
d. Having contact with soil:	86.67%	26
20. Below what temperature at least must be stored perishable fruit?		
a. 40°F	32.35%	11
b. 45°F	8.82%	3
c. 50°F	32.35%	11
d. 35°F	20.59%	7
e. I don't know	5.88%	2
21. It is a real concern that employees can spread food related illness several days after the recover?		
a. True	58.82%	20
b. False	41.18%	14
22. All employees including those that are not related to food handling need to have knowledge of base sanitation and hygiene principles?		
a. True	100.00%	34
b. False	0.00%	0
23. If a lesion is fully covered a worker can be allowed to continue harvesting?		
a. True	73.53%	25
b. False	20.59%	7
N/A	5.88%	2
24. Visitor and customer that comply with hand washing don't have to comply with all the lesser established hygienic practices?		
a. True	14.71%	5
b. False	85.29%	29
25. What kind of water source do you use?		
a. Municipal water	70.59%	24
b. Well water	26.47%	9
c. Surface water	2.94%	1
26. Will you be willing to use a new filtration system capable to remove bacteria?		
a. Yes	70.59%	24
b. No	29.41%	10
27. What will be the principal reason that will impede you from implement a new filtration system to reduce bacteria from irrigation water?		
a. Price	50.00%	17
b. Do not need it	32.35%	11
c. Power Draw	14.71%	5

4.4 Conclusion

The results of this study show that farmers had an overall score in the survey of 76.15% in food safety knowledge and lacked specific safety information in the proper handling of the strawberries during the harvest and processing to prevent cross-contamination of strawberries. Every question about the handling of strawberries received scores around an average of 80%. Based on these results, training workshops should address handling situations during harvesting and processing to prevent the cross-contamination of strawberries.

CHAPTER 5. CONCLUSION

The SMZ was able to reduce the concentration of *Escherichia coli, Listeria monocytogenes* and *Salmonella sp.* from water because of the HDTMA-Br modification. The modification created a bipolar layer on the surface of the Zeolite giving properties to catch bacteria and because of its bactericide effect. Neither the control nor the regular sand filtration system showed a reduction in the concentration of bacteria during the different harvest. The strawberries showed no significant difference using any of the filtration systems, this was because the plastic used in the strawberry field avoided the direct contact between the fruit and the water source. However, in the second harvest there was an increase in the concentration of bacteria in all the treatments because of a flooding in the field, this made possible the direct contact of bacteria with the strawberries. The strawberry industry in Louisiana presented similar trends in bacterial counts that the rest of the country, however the results suggest that training and more studies could be done to enhance the score of the food safety industry.

APPENDIX. SURVEY OF FOOD SAFETY KNOWLEDGE OF LOUISIANA FOOD HANDLERS

Dear Fruit and Vegetable Producers,

We are mailing this survey to determine fruit and vegetable handlers understanding of Good Agricultural Practices (GAP). Your participation is voluntary. This survey is targeting any farm workers between the ages of 18 and 65 years old. We hope to gain information about food safety gaps that will allow the LSU AgCenter to develop educational materials. Survey responses are anonymous, and every effort will be made to maintain the confidentiality of workers responses. All questionnaires will be kept in a sealed box until the survey process is over. Results of the study may be published; however, no information that can identify statistically or specific individuals or farms will be used.

Investigators: The following investigators are available for questions about this study,
M-F, 8:00 a.m. - 2:30 p.m.
Dr. Marlene Janes, Professor, 225-342-5812, mjanes@agenter.lsu.edu
Jose Brandao, Research Assistant, 225-276-7945, jbran19@lsu.edu

Please mail your response back to Jose Brandao at **287 Knapp Hall Baton Rouge LA 70803**
Or scan and email your survey to Jose Brandao at jbran19@lsu.edu

Thank you for your time and participation. Please mail responses back by July 15, 2018.

1. What is your age:
    a. 18-24 years old
    b. 25-34 years old
    c. 35-44 years old
    d. 45-54 years old
    e. 55-65 years old

2. With what gender do you identify yourself:
    a. Male
    b. Female

3. Ethnicity origin (or Race):
    a. Caucasian/White
    b. Hispanic or Latino
    c. African American or Black
    d. Asian or Pacific Islander
    e. Native American or American Indian
    f. Other
4. What is the highest degree or level of school you have completed? If currently enrolled, highest degree received.
    a. No school attended
    b. Some school, no diploma

c. High school graduate
d. Associate degree
e. Bachelor's degree or more

5. What languages are you proficient to understand signs and directions?
    a. English
    b. Spanish
    c. Spanish and English
    d. Other_____

6. Have you received food safety training?
    a. Yes
    b. No

7. Do you or your workers go to work with a sore throat or cough?
    a. Yes
    b. No

8. Have you or your workers ever worked with diarrhea?
    a. Yes
    b. No

9. Have you or your workers ever worked with an infected wound?
    a. Yes
    b. No

10. Have you ever been employed at a farm during a foodborne outbreak?
    a. Yes
    b. No

11. Is it better to wash your hands with?
    a. Warm/hot water
    b. Cold water
    c. I don't know

12. What are acceptable ways to dry your hands after washing them? (Check all that apply: _x_)
    a. Forced air: _____
    b. Paper towel: _____
    c. Reusable cloth towel: _____

13. Do you need to wash your hands if you are going to wear gloves during food handling situations?
    a. Yes
    b. No

14. How much time is needed to properly wash your hands?
    a. At least wash hands with water and soap for 20 seconds
    b. At least wash hands with water and soap for 15 seconds
    c. At least wash hands with water and soap for 30 seconds

15. If you only urinate then you do not need to wash your hands with soap.
    a. True
    b. False

16. What are corrects procedures to exit a bathroom? (Check all that apply: _x_)
    a. Open the door only after you finish cleaning and drying completely your hands: ____
    b. Using a paper towel to open and close the door: _____
    c. Leave the door open: _____

17. You can take harvest tools (gloves) into the bathroom if you clean and sanitized them thoroughly before using them again?
    a. True
    b. False

18. You must wash your hands after? (Check all that apply: _x_)
    a. Using the bathroom: _____
    b. Eating or drinking: _____
    c. Smoking: _____
    d. Having contacted the soil: ____

19. Employees are able to spread food related illness several days after they recovery?
    a. True
    b. False

20. All employees including those that are not related to food handling need to have knowledge of basic sanitation and hygiene principles?
    a. True
    b. False

21. If a lesion is fully covered, a worker can be allowed to continue harvesting?
    a. True
    b. False

22. Visitors and customers that comply with hand washing don't have comply with all the lesser established hygienic practices?
    a. True
    b. False

23. What kind of irrigation source do you use?
    a. Municipal water
    b. Well water
    c. Surface water

24. Would you be willing to use a new filtration system capable to removing bacteria from irrigation water, to ensure safety of your produce?
    a. Yes
    b. No

25. What reason would impede you from using new technology (irrigation filtration systems) to ensure safety of your produce? (Choose any answers that apply)
    a. Price (average costs ranges between $500-$5,000 depending on number of acres irrigated)
    b. I do not need it because I irrigate with municipal water
    c. Other _____

THANK YOU FOR ANSWERING THE QUESTIONS. PLEASE CONTINUE IF YOU PRODUCE <u>STRAWBERRIES</u> ON YOUR FARM.

26. When should strawberries be washed?
    a. After harvesting
    b. Before processing
    c. Before eating

27. It is true that if strawberries are contained in the primary containers (e.g. clamshells, pint baskets, etc.) the containers can sit directly on soil.
    a. True
    b. False
28. What is the ideal storage temperature for strawberries?
    a. 40°F
    b. 45°F
    c. 50°F
    d. 35°F
    e. I don't know

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