ABSTRACT

Title of Document:	ASSESSMENT OF ZERO-VALENT IRON CAPABILITIES TO REDUCE FOOD-BORNE PATHOGENS VIA FILTRATION AND RESIDUAL ACTIVITIES IN IRRIGATION WATER
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Inadequate disinfection of contaminated freshwater that is used to irrigate food crops that are eaten raw can result in foodborne illnesses. Therefore, in this study we assessed the efficacy of a low-cost, water treatment technology, zero-valent iron (ZVI), in reducing microbiological contamination of synthetic irrigation water. Specifically, we compared the capabilities of a ZVI-sand filter versus a sand filter in reducing levels of *Salmonella* Newport MDD314 and *E. coli* TVS 353 through filtration or residual disinfection. Our data showed that ZVI-sand filtration was more effective than sand filtration alone in reducing levels of both of these microorganisms. Our results also showed that, after filtration, there seemed to be no

residual disinfection capabilities associated with either the ZVI-sand system or the sand system alone. Our findings suggest that ZVI-sand filtration can effectively reduce microbial contaminants in irrigation water; however, there seem to be no residual disinfection capabilities after filtration.

ASSESSMENT OF ZERO-VALENT IRON CAPABILITIES TO REDUCE FOOD-BORNE PATHOGENS VIA FILTRATION AND RESIDUAL ACTIVITIES IN IRRIGATION WATER

By

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Dedication

Dedicated to first God for guidance through this program, my family, and many friends including the USDA 201 crew, Shoshana Pemberton and Denesha Simmons for countless encouragement.

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First, I would like to give a special thanks to Jesus for helping me through life, in general. I'm appreciative of His mercy, grace and love He has shown every single day faithfully. Second, I would like to thank my family who constantly remind me that I can accomplish anything in life. I am truly blessed to be under their love and care.

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Chapter 1: Introduction

Freshwater Availability

Diminishing freshwater resources is a growing concern globally due to increased demand for freshwater and climate variability (Elliott et al., 2014). Freshwater makes up about 3% of the Earth's water, and of that, only 0.5% is usable for potable, agricultural and industrial purposes (UN Water, 2006). In the United States, approximately 355,000 million gallons of water is used per day, of which 85% is freshwater sequestration (Donnelly & Cooley, 2015). Furthermore, agriculture accounts for 70% of freshwater withdrawals in the United States (Maupin et al., 2014) (United States Geological Survey, 2016). As a result, many freshwater resources are under significant stress from overuse (Donnelly & Cooley, 2015). For example, irrigation heavily depends on groundwater, so much so that 60% of irrigation water used in the U.S. originates from groundwater aquifers (Scanlon et al., 2012). Moreover, California alone accounts for 10 percent of total freshwater withdrawals nationwide (Maupin et al., 2014). The aquifers that support irrigation in the California Central Valley and the High Plains were identified as hotspots for groundwater depletion (Scanlon et al., 2012). This contributes to current scenarios where the rate of natural groundwater recharge in some aquifers is much slower than the rate of withdrawal, so much so that in the next 30 years it is expected that 35% of the southern High Plains will be unable to support irrigation (Scanlon et al., 2012) (Pimentel et al., 2004). Because irrigation is a solution for spatial and temporal issues relating to water demand and supply, crops are being produced in semi-deserts, which may be subject to climate change (Scanlon et al., 2012).

In addition to increased demand for freshwater, global climate change has significantly exacerbated the growing issue of freshwater availability and quality (OA US EPA, 2016b). Increased temperatures resulting from climate change have influenced drought patterns throughout the United States. In 2015, up to 70% of the continental U.S. experienced abnormally dry conditions at some point during the year, and 2012 was deemed the driest year on record (OA US EPA, 2016a). This phenomenon has had significant negative influences in both Western and Southwestern states (OA US EPA, 2016a), especially with regard to food production (Steven Wallander & Mark Jekanowski, 2016).

Furthermore, human activity and climate variability have caused an increase in nonpoint source pollution that affects the quality of available freshwater (OA US EPA, 2016b) (OW US EPA, n.d.). This is a result of contaminated groundwater recharge associated with precipitation events, where higher risks of impairments often occur in shallow aquifers (Pandey, Kass, Soupir, Biswas, & Singh, 2014). Furthermore, these phenomena have led to widespread contamination of surface water resources, such that at least 40 % of U.S. surface waters have elevated levels of contamination that could potentially lead to adverse public health impacts if these water sources are used to produce food crops (Centers for Disease Control and Prevention, 2016b) (Pimentel et al., 2004). Pathogenic microorganisms are considered the leading cause of freshwater contamination compared to other forms of impairments (Pandey et al., 2014). For example, in a 2-year study conducted in Virginia, researchers discovered that *Salmonella* spp. was present in 25% of the 91 streams, ponds and sediment tested (Markland, Ingram, Kniel, & Sharma, 2017) (Bell

et al., 2015). The most prevalent isolates identified were *S.* Javiana and Newport, where *S.* Newport isolates were closely associated with isolates recovered from contaminated tomatoes (Bell et al., 2015). Due to groundwater and surface water interactions, climate variability increases the likelihood of further pathogenic contamination of freshwater sources (Pandey et al., 2014).

Contaminated Irrigation Water and Foodborne Illness

Inadequate disinfection of contaminated freshwater sources that are used to irrigate food crops has the potential to result in foodborne illness. It is estimated that there are about 48 million cases of foodborne illnesses annually, which resulted in 127,839 hospitalizations and 3,037 deaths (Scallan et al., 2011). Additionally, foodborne illnesses may cost the nation around $146 billion annually (Scharff, 2012.) More specifically, costs related to *Salmonella* spp. and *Listeria monocytogenes* infections (leading causes of foodborne illness) are estimated to be $11.4 billion and $2.04 billion, respectively (Robert Roos, 2012). Similarly, outbreaks associated with *Escherichia coli* (*E. coli*) O157:H7, alone, have been calculated by the USDA to cost $478.4 million each year (Robert Roos, 2012).

In 2006, for example, the CDC investigated an outbreak of 80 *E. coli* O157:H7 infections associated with the consumption of contaminated lettuce at Taco John's restaurants in Minnesota and Iowa. (The California Department of Public Health, 2008). Efforts to isolate the source of the organisms, led investigators to the Central Valley and Central Coast in California. In conjunction with the FDA, the California Department of Public Health collected 251 samples of water, soil

sediments, swabs, fecal matter, and product specimens from two locations: a farm in Buttonwillow and growers in Santa Maria (The California Department of Public Health, 2008). Special concern was given to the farm in Buttonwillow, due to the existence of neighboring dairies and an interconnected irrigation and dairy effluent conveyance system (The California Department of Public Health, 2008). There were 32 positive results, all of which originated from Buttonwillow, and 10 samples (31%) genetically matched the *E. coli* isolated from the Taco John's outbreak strain. Moreover, out of the 10 samples that were identical matches, 60% of the samples (two swabs, four water, three water and sediment, and one soil) were collected within close proximity to lettuce growing fields. The remaining 40% came from neighboring dairies facilities (The California Department of Public Health, 2008). The lack of a backflow mechanism caused pressure variations within the interconnected system, thus allowing *E. coli* O157:H7, shed from the dairy manure, to enter and mix into the irrigation water (Markland et al., 2017)

Zero Valent Iron Biosand Filtration

To combat this growing issue of poor irrigation water quality, as described previously and in Chapter 2, that could potentially result in increases in foodborne illness, researchers have been exploring zero valent iron (ZVI) biosand filtration methods to reduce contaminants in irrigation water sources that would otherwise be pathogenic to humans (Noubactep et al., 2009). The proposed research will assess the capability of ZVI biosand filtration to reduce bacterial contamination in freshwater sources, and shed light on how long the ZVI can remain efficient in this process.

ZVI's capability to form iron oxides and other reactive species may prove to be essential with regard to the further inactivation of bacterial pathogens after filtration. This process is brought about when ZVI reacts in the presence of oxygen (O_2), water (H_2O), organic materials and minerals (Shi et al., 2015). Studies using nanoscale ZVI (nZVI) suggest that the formation of both reactive oxygen species (ROS) and Fe^{2+} contributes significantly to cell toxicity (Lefevre, Bossa, Wiesner, & Gunsch, 2016). Precipitation of both nZVI and iron sub-species around and inside the bacterial cell have been suggested to cause denatured macromolecules and damage intercellular structures, thus inducing cell death (Lefevre et al., 2016). The formation of these free radicals and ROS may be present in the ZVI filtrate, and could prove useful in producing residual disinfection of contaminated irrigation water. This research will assess the residual bactericidal activity of ZVI in water that has been previously filtered. These findings would also provide a more comprehensive assessment of the microbial quality of ZVI-filtered water.

FDA Standard for Irrigation

The Food Safety Modernization Act (FSMA) Produce Safety Rule, enacted through the FDA, established the basis for agricultural practices, and will be used as a standard for this analysis. The first criterion under this law states that there should be no detectable generic *E. coli* within water used for hand washing throughout harvesting, and that water that comes in contact with produce, and water used for sprouts….something is missing here? (FDA Center for Food Safety and Applied Nutrition, 2017). The second set of criteria is based on two statistical values, which

are geometric mean (GM) and statistical threshold value (STV) (FDA Center for Food Safety and Applied Nutrition, 2017). These criteria state that the GM is an average of samples, which represents the central tendency of water quality and should not exceed 126 CFU/100mL of generic *E. coli.* The STV, used in adverse conditions such as extreme flooding, is 410 CFU/100 mL, where 90% of samples tested should not exceed that value. (FDA Center for Food Safety and Applied Nutrition, 2017). Evaluating the efficacy of ZVI to be a disinfectant even after filtration may be useful in reducing bacterial populations even during storage and distribution of irrigation water. Hence the objective and hypothesis of this research is as follows:

Objective: Assess the capability of ZVI to reduce foodborne pathogens via filtration and residual activities, and reevaluate the design of a ZVI system for small-scale applications.

Hypothesis: Residual properties of ZVI can improve the quality of water by further reducing *Escherichia coli* (*E. coli*) TVS 353 and *Salmonella Newport* MDD314 levels in ZVI filtered water.

Gaps in Knowledge

We are aware that reduction of pathogens occurs during ZVI Biosand filtration, but there is little knowledge on residual iron species activity in the filtrate. This was briefly addressed in the work of Banerjee et al., (2011), where populations of *E. coli* were reduced more when the pathogen was inoculated into ZVI-filtered compared to sand-filtered water (post-filtration). Disinfecting irrigation water during

storage, between storage and distribution are strategies to eliminate bacterial pathogens (Selma, Suslow, Uyttendaele, & Allende, 2015,).

Significance and Rational

Farmers use retention ponds to store freshwater before irrigating food crops. By simulating this process in a laboratory setting, after applying ZVI filtration, we will be able to assess whether residual iron species from ZVI filtration have the ability to further reduce pathogen levels even after filtration. This would prove useful in reducing the potential transfer of microbial pathogens to crops that could otherwise cause foodborne illness in the general population.

Chapter 2: Background

Introduction

Existing Knowledge

Properties of Zero-Valent Iron Filtration

In order to remediate contaminants from freshwater resources, researchers have explored the use of zero valent iron (ZVI). It is non-toxic, abundant, relatively cheap and easy to produce (Fu, Dionysiou, & Liu, 2014). ZVI has been used as a permeable reactive barrier to remediate trichloroethylene (TCE) and pentachloroethylene (PCE) from groundwater at a rate of 95 and 91 percent, respectively (Guan et al., 2015). ZVI filtration has also been shown to remediate contaminants such as nitro-aromatics, dyes, phenolic compounds, heavy metals, oxyanions, arsenite, nitrates, bromate, selenite and uranyl from freshwater sources (Fu et al., 2014). ZVI, being a reductant, is able to readily transfer electrons to toxic material and thus create a non-toxic stable product (Fu et al., 2014). Moreover, small-scale field experiments that inoculated water with 8.5 log CFU/mL *E. coli* O157 have illustrated that ZVI filters were able to reduce the pathogen levels to about 2.1 log CFU/mL in water used for irrigation (Ingram et al., 2011). This is the very reason why understanding the capabilities of ZVI technology is crucial in terms of the treatment of toxic contaminants and bacterial pathogens, such as *Salmonella* spp. and *E. coli*, in freshwater sources used for irrigation.

Salmonella spp.

Genus Description

Salmonella spp. are generally 2-5 microns long by 0.5-1.5 microns wide, gram-negative, rod-shaped bacteria with peritrichous flagella for mobility (Andino & Hanning, 2015) (WHO, 2016). They are part of the *Enterobacteriaceae* family and include two species, *S. enterica* and *S. bongori*, which are broken further down into 6 subspecies and over 2,579 serovars (Andino & Hanning, 2015). Moreover, *Salmonella* spp. have the capability to survive in a wide range of environmental conditions, including pH ranges from 3.8-9.5(Keerthirathne, Ross, Fallowfield, & Whiley, 2016).

Ecological Habitat and Distribution

Salmonella spp. are mainly found in the gastrointestinal (GI) tract of livestock, such as cattle and poultry, wild birds, reptiles, and some rare occasions in insects (Andino & Hanning, 2015). Having a wide array of hosts, *Salmonella* can pollute and persist in numerous environmental matrices including water, which create public health and food safety concerns (Andino & Hanning, 2015). Shedding of these bacteria occurs by defecation, and their presence in water or food denotes fecal contamination (Andino & Hanning, 2015).

Epidemiology and Pathogenicity

In the United States, *Salmonella* spp. is one of the leading causes of foodborne illnesses, which costs an estimated $3.7 billion, 19,336 hospitalizations, 379 deaths

and over a million total cases annually (Andino & Hanning, 2015) (CDC, 2017). Although outbreaks of *Salmonella* spp. are widely associated with consumption of contaminated poultry, egg or meat products, in recent years there has been isolation of the organism from melons, sprouts, tomatoes, peppers, mangoes and leafy greens (Bell et al., 2015) (Andino & Hanning, 2015).

The pathogenicity of *Salmonella* spp. depends on and is determined by a number of factors including the host immunity status and the specific isolate (van Asten & van Dijk, 2005). Severity of diseases that are caused by the genus is relative to the serovar, which is broken into two groups: those that cause enteric fever and non-typhoid *Salmonellae* (NTS), in which both groups produce endotoxins and exotoxins that have the ability to affect mammalian cells (Andino & Hanning, 2015) (van Asten & van Dijk, 2005). The virulence factors of *Salmonella* spp. are located in gene clusters known as *Salmonella* pathogenicity islands (SPI), and differences in these regions cause varying disease severities (van Asten & van Dijk, 2005) (Andino & Hanning, 2015). Salmonellosis induced by *S. enterica* serovars *Typhi* and *Paratyphi* cause gastroenteritis, septicemia and or enteric fevers (Andino & Hanning, 2015). NTS differ from typhoid salmonellae in that they cause gastroenteritis, nausea, vomiting and diarrhea (Andino & Hanning, 2015). However, NTS are classified as the leading cause of hospitalization and deaths, and are not restricted to mammalian organisms (Andino & Hanning, 2015). The global burden of typhoid-related *Salmonella* is commonly observed in developing countries, whereas NTS occurs worldwide (Gal-Mor, Boyle, & Grassl, 2014).

In regards to irrigation water, 26 cases of *Salmonella* infections were associated with a multistate outbreak of *Salmonella* Muechen or Kentucky [Add Ref]. During the outbreak event, the FDA and Kansas Department of Agriculture conducted inspections at the Sweetwater farms and obtained both sprout samples and water used for irrigation (Centers for Disease Control and Prevention, 2016a). Isolates of *S.* Kentucky and *S.* Cubana were found in the water samples, and the source of the *S.* Muenchen was traced back to sprout seed lots. In the seed lots, investigators sampled seedlings, and found the indicated strains of *Salmonella* with similar typing to water samples (Centers for Disease Control and Prevention, 2016a).

E. coli

Genus Description

E. coli are a large group of bacteria that are rod-shaped, gram-negative organisms, which are 2 microns in length and 0.25–1.0 microns in diameter (Centers for Disease Control and Prevention, 2015) (Huang, Mukhopadhyay, Wen, Gitai, & Wingreen, 2008) (Gu et al., 2016). As described by the CDC, most *E. coli* are harmless and in some cases important for healthy intestinal processes. However, other strains are of public health concern and can be pathogenic (Centers for Disease Control and Prevention, 2015). Pathogenic *E. coli* are grouped and categorized into 6 intraintestinal pathotypes and 1 extraintestinal pathotype: 1) Shiga toxin-producing *E. coli* (STEC) or Enterohemmorrhagic *E.coli* (EHEC); 2) Enterotoxigenic *E.coli* (ETEC); 3) Enteropathogenic *E. coli* (EPEC); 4) Enteroaggregative *E. coli* (EAEC); 5) Enteroinvasive *E. coli* (EIEC); 6) Diffusely adherent *E. coli* (DAEC); and 7)

Extraintestinal Pathogenic *E. coli* (ExPEC) (Centers for Disease Control and Prevention, 2015) (Köhler & Dobrindt, 2011) .

Ecological Habitat and Distribution

Similar to *Salmonella* spp., *E. coli* can be found in the gastrointestinal (GI) tract of warm-blooded animals, mainly livestock. Their presence is often used as an indicator of fecal contamination, and their persistence in secondary environmental habitats, such as water sources, depends heavily on temperature and nutrient availability. Though being commensal or mutualistic in most cases, certain *E. coli* do exhibit pathogenic traits and are of concern to public health (Tenaillon, Skurnik, Picard, & Denamur, 2010).

Epidemiology and Pathogenicity

Of the major *E. coli* pathotypes, STEC or EHEC are the most commonly associated with foodborne outbreaks, and of major public health concern (Centers for Disease Control and Prevention, 2015). EHECs differ from STEC because they have the *eae* gene that codes for intimin, which allows the bacterium to attach to the host intestines (Loukiadis, Kérourédan, Beutin, Oswald, & Brugère, 2006). STEC have the ability to cause hemolytic uremic syndrome (HUS), via the production of Shiga toxin. These toxins halt protein synthesis in the host intestinal epithelial cells, vascular and renal cells, which results in the initiation of apoptotic cell death. Although non-O157 strains of EHECs are able to produce Shiga toxin, *E. coli* O157:H7 are the largest

contributors to STEC-related foodborne outbreaks (Melton-Celsa, Mohawk, Teel, & O'Brien, 2012).

Objectives of This Thesis Project

In this study, we evaluated the efficacy of ZVI to reduce *Salmonella enterica* serovar *Newport* MDD314 and *E. coli* TVS 353 populations via filtration and residual disinfection activities within the filtrate by performing pre- and post-inoculation of filtered water over a course of 7 days at room temperature.

Chapter 3: Efficacy of ZVI in Reducing Foodborne Bacterial Pathogens in Irrigation Water via Filtration and Residual Activities

Abstract

Due to climate change and human activity, many freshwater resources are under stress and the quality of these sources is becoming a concern for public health. For instance, increased precipitation events often lead to non-point source pollution and can cause contaminated groundwater recharge. Inadequate disinfection of contaminated water sources that are used to irrigate food crops has the potential to result in foodborne illnesses and outbreaks. The purpose of this study was to assess whether zero-valent iron (ZVI) filtration can be used as a treatment for irrigation water, and to evaluate residual disinfecting properties in the filtrate. A one-pass ZVI filter was assembled containing 25%/75% ZVI/sand mixture (v/v), which was evaluated against a 100% sand filter and a synthetic water control. Two treatment methods were performed on both the ZVI-sand mixture and sand-only apparatuses. Synthetic water was ultimately inoculated either pre- or post-filtration to achieve 5 log CFU/mL each of *Escherichia coli* TVS 353 and *Salmonella enterica* subspecies *enterica* serovar Newport MDD314. In the first treatment, the synthetic water was pumped through each filter column, collected, and inoculated. These samples were stored at 25°C and analyzed on days 0, 1, 2, 4, and 7 post-inoculation. In the second treatment, the synthetic water was inoculated **before** filtration and analyzed before and after filtration, which included similar storage and sampling conditions as treatment 1. In the first treatment, we observed that the ZVI reduced the *E. coli* and *S.* Newport populations by 0.34 and 1.29 log CFU/mL respectively, compared to sand

that only reduced by 0.65 and 1.25 log CFU/mL(p-values= 0.80 and 0.56) over the sample period. In treatment 2, ZVI reduced *E. coli* and *S.* Newport populations by 0.10 and 0.19 log CFU/mL, compared to sand reductions which were 0.26 and 0.63 (p-values=0.147 and 0.96865). In regards to filtration efficacy, ZVI significantly reduced *E. coli* and *S.* Newport populations from initial inoculation levels by 1.75 and 1.89 log CFU/mL (p-value=0.04953). Whereas sand only reduced 0.83 and 1.48 log CFU/mL (p-value=0.2752). ZVI's main mode of reduction seems to be via filtration and not residual reactive species within the filtrate. Findings support that ZVI is a useful tool to remediate pathogens, and may be more potent depending on the organism.

Introduction

Agriculture is one of the largest users of freshwater and accounts for roughly 330 million acres of land in the United States (Markland et al., 2017) (Maupin et al., 2014) (United States Geological Survey, 2016). Furthermore, in 2010, over 126 billion gallons of freshwater was used for agricultural practices including irrigation (Markland et al., 2017). As a result, many freshwater sources are under stress, and the demand for more water is expected to increase by 2050 (Donnelly & Cooley, 2015) (Scanlon et al., 2012) (Pimentel et al., 2004).

The main supplier of water for irrigation is groundwater, which supplies up to 60% of the water needed for agricultural processes in the United States (Scanlon et al., 2012). Other sources, as described by Markland et al., include surface water sources such as ponds reservoirs and lakes. Because the supply of water comes from environmental sources, irrigation water is prone to becoming polluted with various contaminants (Markland et al., 2017). The quality of water used for irrigation is further threatened by human activity and climate variability (OA US EPA, 2016b) (OW US EPA, n.d.). Non-point source pollution stemming from farms, industrial sites, feedlots, and barnyards all have the potential to impair sources used for irrigation in the U.S, and is of public health concern (Centers for Disease Control and Prevention, 2016b) (Pimentel et al., 2004) (Markland et al., 2017). Additionally, due to surface water and groundwater interactions, increased precipitation events can cause contaminated recharge of groundwater sources, where greater risk is associated with shallow aquifers (Pandey et al., 2014).

For instance, *Salmonella* Newport (*S.* Newport) has been associated with outbreaks, since 2002, in regards to tomatoes grown in the Virginian Eastern Shore, one of the largest producers of fresh produce located in the Delmarva Peninsula (Markland et al., 2017). Environmental assessments in this region have found that *S.* Newport isolates from irrigation pond water had the same PFGE pattern as isolates associated with outbreaks that occurred in 2002, 2005, 2006, and 2010. Although the source of contamination is uncertain, the likely contamination of tomatoes occurred during pre-harvest using polluted irrigation water (Markland et al., 2017).

Under the Food Safety Modernization Act (FSMA), mandated by the FDA, the standards for proper protocol for growing, harvesting, packing and holding are described in the Produce Safety Rule (PSR) (Markland et al., 2017). Within the PSR, all water sources obtained from a nonpublic source must be tested and evaluated (FDA Center for Food Safety and Applied Nutrition, 2017) (Markland et al., 2017). The testing requirements extend to water used for harvesting or water that comes in contact (direct and indirect) with produce (including ice) (FDA Center for Food Safety and Applied Nutrition, 2017). Furthermore, the PSR mandates that the concentrations of generic *E. coli* should not exceed 126 CFU/mL in the geometric mean (average) of samples, and the standard threshold (adverse events) value should not surpass 410 CFU/mL in 100mL. If water does not meet this standard, the producer must allow sufficient time between irrigation and harvest for the appropriate die-off to occur, or treat the water that would allow for 0.5 log/day of bacteria prior harvest (Markland et al., 2017) (FDA Center for Food Safety and Applied Nutrition, 2017).

Zero-Valent Iron may prove to be useful in reducing bacterial populations to the FSMA standards. The proposed research will assess the capability of ZVI Biosand filtration to reduce bacterial contamination in freshwater sources, and shed light on how long the ZVI can remain effective in its bactericidal activity. ZVI's capability to form iron oxides and other species may prove to be essential with regard to the further inactivation of bacterial pathogens after filtration. This process is brought about when ZVI reacts in the presence of oxygen (O_2), water (H_2O), organic materials and minerals (Shi et al., 2015). Studies using nanoscale ZVI (nZVI) suggest that the formation of both reactive oxygen species (ROS) and Fe^{2+} contributes significantly to cell toxicity (Lefevre et al., 2016). Precipitation of both nZVI and iron sub-species around and inside the bacterial cell have been suggested to cause denatured macromolecules and damage intercellular structures, thus inducing cell death (Lefevre et al., 2016). This research will assess the effectiveness of residual ZVI concentrations at a granular level in reducing bacterial populations, providing additional information as to whether or not ZVI Biosand filtration systems could be an effective irrigation water treatment system.

Materials and Methods

ZVI Apparatus Framework

For this experiment, three filtration treatments were evaluated: 1) ZVI 2) sand and 3) a synthetic water control, which was not treated by filtration. Each filter apparatus was assembled using a 2 inches (diameter) by 2 feet (length) Charlotte PVC Sch. 40 plain-end pipe, which is equivalent to an interior volume of about 1.245 L. A Charlotte 2 inch male adapter, stuffed with an O-ring, a porous plastic hard-backing

made from Commercial HydrAid Biosand filters (Cascade Engineering, Grand Rapids, MI, USA), and sun shade screen mesh, was glued to the Charlotte 2 inch by 2 feet PVC pipe using Oatey PVC purple primer and clear PVC cement. A 2 in. x ¾ in. PVC Sch. 40 Reducer bushing was primed and glued to a 2 in PVC Hub x FIPT Female adapter, which was used to screw onto the male ends of the filter column. The glue was allowed to set for 24 hours, before continuing with the assembly. Each filter module was filled to the total volume of each column using a 25%/75% ZVI/sand mixture (v/v) or 100% sand. After filling each column either with ZVI/sand mixture or sand alone, another Charlotte 2 inch male adapter, was attached to the opposite side of the filter. The size of ZVI and sand particles ranged from was 425-600μm. The ZVI and sand were ordered from Peerless Metals Powders & Abrasive (Detroit, MI, USA) and Filtersil Filtration Sands and Gravel (Ottawa, MN), respectively. The male threads of the filter columns were primed with Real-Tuff liquid plumbers tape to create a liquid-proof system, and then the female reducer was attached to the columns. Following this process, a ¾ inch Hex brass nipple adapter was treated with the Real-Tuff liquid plumbers tape, and threaded onto the reducer brushing on both ends of the columns. Similarly, a Watts ¾ inch Brass FIP x FIP Full Port Threaded Ball Valve was threaded onto the open ends of the columns. This valve allowed us to control the flow of water, and kept the filter systems hydrated. A ¾ inch SharkBite Pex pipe was fitted with either a SharkBite 1/2 inch x 3/4 inch Brass Push-to-Connect x Female Pipe thread adapter or a SharkBite 1/2 inch x 3/4 inch Brass Push-to-Connect x Male Pipe thread adapter, which was used to attach the columns to the Shurflo pump model 4008-101-A65. The pump was positioned at the end the filter in

order to create a pressurized system, and draw water as a pull system. An illustration of the filter system assembly is shown in **Figure 1.**

Synthetic Water Framework

Modified synthetic stream water was prepared as described by Shelton et al., (2014) with modifications. The following nutrients were dissolved in Elga water to the create a stock solution with concentrations: 1.708 g/L Humic acid (Alfa Aesar, Ward Hill, MA, USA), 6.3 g/L $(NH_4)_2SO_4$ (Thermo Fisher Scientific, Fair Lawn, NJ, USA), and 0.878 g/L KH_2PO_4 (Sigma Aldrich, St. Louis, MO, USA), which was added to approximately 19 L of sterile deionized water in a sterile 20 L carboy with a Carbon to Nitrogen to Phosphorus ratio of 5:1:0.01, respectively (Shelton et al., 2014). The final chemical concentrations in the 20 liter carboy were 0.2563 grams/ liters humic acid, 0.0944g/ liters ammonium sulfate, and 0.000878g/ liters potassium phosphate monobasic. After allowing the mixture to aerate over a 24 hour period, 1.20 g of $CaSO_4 \cdot 2H_2O$ (Acros Organics, NJ, USA) was added to 1 L of deionized water and stirred until calcium sulfate was stirred until fully suspended (US EPA, 2002). The 1 L mixture was then added to the 19 L mixture indicated previously, and the pH was adjusted to 6.4-7.5 using 6M NaOH as needed (Shelton et al., 2014). This synthetic water was used in the filters that are described above for the analysis of ZVI residual potential.

Treatments and Inoculation Preparation

Two treatment methods were performed on both the ZVI-sand and sand-filters, and are further illustrated in **Table 1**. Synthetic water was inoculated either pre- or post-filtration with 5 logs CFU/mL of *E. coli* TVS 353 and *Salmonella* Newport MDD314. To begin, strains were struck from frozen stocks onto MacConkey agar

(Neogen Accumedia, Lansing, MI, USA) supplemented with 80 μμg/mL of rifampicin (Sigma, USA) (MAC-R) or Xylose Lysine Deoxycholate agar (Neogen Accumedia, Lansing, MI, USA) supplemented with 80 μμg/mL of rifampicin (Sigma-Aldrich, St. Louis, MO, USA) (XLD-R), respectively. Two sterile round-bottom centrifuge tubes were filled with 30mL of Tryptic Soy Broth (Neogen Accumedia, Lansing, MI, USA) containing 80mg/mL rifampicin (TSBR). Using biomass from plates grown up previously, one centrifuge tube was inoculated with *E. coli* TVS 353 and the other with *S.* Newport MDD314, and allowed to incubate for 24 h at 37°C with shaking at 125 RPM. After incubating cultures for 24 h, the tubes were vortexed vigorously, and then centrifuged at 5,000 x g for 10 minutes using the Allegra 25R centrifuge (Beckman Coulter, Fullerton, CA, USA). The supernatant was removed from each tube and the cell pellet was washed in 30 mL of Phosphate-Buffered-Saline (PBS) (Sigma-Aldrich, St. Louis, MO, USA) twice, and the cell pellet was re-suspended in 30 mL of synthetic water. This suspension was then used for serial dilution and subsequent inoculation of synthetic water, as described in the next sections. In each of the following treatments, filtrates (treatment 1), or synthetic water (treatment 2 and synthetic water control) were inoculated to target 5 log CFU/mL for each strain.

Treatment 1: Post-Filtration

In the first treatment, the synthetic water was pumped through either the ZVI or the sand filter columns, collected, and inoculated with the inoculum mentioned above. These samples were stored at 25°C and analyzed on days 0, 1, 2, 4, and 7 post-inoculation. To begin, filters were first flushed using 5 L of sterile water followed by 5 L of uninoculated synthetic water. Flushed synthetic water was collected and stored to be analyzed for iron species and other metals. Following hydration and flushing of

the filter columns, an additional 10 L of uninoculated synthetic water was pumped through each filter column and collected in separate 10 L carboys. The average flow rate for synthetic water through ZVI filters was 1.25 L /min, and for synthetic water through the sand-only filter was 1.13 liters/minute, respectively. A series of tenfold dilutions was performed from the prepared washed cells, as mentioned previously, to achieve target concentration of 7 log CFU/mL for each strain. Ten mL of *E. coli* TVS 353 and 10mL of *S.* Newport MDD314 were added to sterile 2 L bottles containing 980 mL of the ZVI filtrate, sand filtrate, and the synthetic water control. Immediately after, the inoculated filtered water was shaken vigorously, 13 mL were removed for Day 0 measurements, and inoculated filtered water were stored at 25°C for up to 7 days. Serial dilutions of each filtered water sample were made in PBS, and 100 µL were spiral plated on MAC-R and XLD-R, in duplicate, for each medium). Inoculated MAC-R and XLD-R were incubated for 18-24 hours at 42°C and 37°C, respectively. Samples were analyzed on d, following a similar fashion.

Treatment 2: Post-Filtration

In the second treatment, the synthetic water was inoculated in the same manner above, but before filtration, and bacterial populations were recovered before and immediately after filtration. Filtered waters were also stored for up to 7 days at 25^{o}C and enumerated on days 0, 1, 2, 4, and 7). Treatment 2 used the same filters above following the collection of the filtrates in Treatment 1. Fifteen liters of inoculated synthetic water were filtered through each filter type, and the filtrate was collected in 1 L fractions. Fractions 1, 3, 5, 7, 9, 11, 13, and 15 L were analyzed separately, and fractions 2, 4, 6, 8, 10, 12, and 14 L were combined and analyzed as a

composite sample. This was done for the first replicate experiment to assist in assay optimization. For the second and third replicates, the odd fractions were combined to form one composite, and this was analyzed in addition to the even composite.

Statistical Analysis

Statistical analysis used for this specific study was a repeated measures ANOVA (lme package) using R version 3.4.0. After performing the repeated measures ANOVA, the Kruskal Wallis test was used to determine differences in *E. coli* and *Salmonella* populations based on filtration type and within filter type, length of storage, e [something missing here]. In all cases, p-values ≤ 0.05 were defined as statistically significant.

Results

Treatment 1: Post Inoculation of E. coli TVS 353 and S. Newport

The population of both *E. coli* TVS 353 and *S. Newport* were analyzed independent of each other in regards to the three filter conditions (ZVI, sand, or not filtered in treatment 1). In regards to both bacterial populations, including the data for 3 replicates, we collected 45 data points from days 0-2, 4, and 7.

The linear mixed model used to analyze the results of E. coli survival and S. Newport survival in filtered water were significant (p=0.0077 and 0.0349, respectively).

With regards to Treatment 1, there were no reductions in both *E. coli* TVS 353 and *S.* Newport population due to the residual filtration process. In sand filtration, *E. coli* and *S.* Newport populations were reduced by 0.65 and 1.25, respectively, while

ZVI filtration reduced populations by 0.34and 1.29 log CFU/mL, respectively. Moreover, when comparing 1. Sand to synthetic water, 2. ZVI to sand, and 3. ZVI to the synthetic water, we observed there were no significant differences for both *E. coli* TVS 353 (1. p=0.995, 2. p=0.797, and 3. p=0.742) and *S.* Newport population decline (1.p=0.758, 2. p=0.560, and 3. p=0.192).

Moreover, using the Kruskal-Wallis test, we observed that there were no statistically significant difference for the *E. coli* and *S.* Newport populations within ZVI filtrate based on the log CFU/mL values over the sampling period(p=0.6929 and p=0.09701). Additionally, in the sand filtrate, we observed significant difference only for the *S.* Newport populations (p=0.03932) and for the *E. coli* populations there were no significant difference (p=0.05402) based on the log CFU/mL values over the sampling period.

Treatment 2: Pre- Inoculation of E. coli TVS 353 and Salmonella Newport

Likewise, population of *E. coli* TVS 353 and *S. Newport* were analyzed independent of each other in regards to the three filter conditions (ZVI, sand, or not filtered in treatment 2). We collected 45 data points from days 0-2, 4, and 7, similar to that of treatment 1.

The linear mixed model used to analyze the results of E. coli survival were significant (p>0.0000), but not for S. Newport survival (p=0.7451) in filtered water. In treatment 2, there were no differences in both *E. coli* TVS 353 and *S.* Newport population between the ZVI and sand filtrate residual condition. In sand filtration, *E. coli* and *S.* Newport populations were reduced by 0.26 and 0.63 log CFU/mL, respectively, while ZVI filtration reduced populations of *E. coli* and *S.* Newport by

0.10 and 0.19, respectively. Comparing the following: 1. Sand to synthetic water, 2. ZVI to sand, and 3. ZVI to the synthetic water, we observed there were no significant differences for *E. coli* TVS 353 (1. p=0.883, 2. p=0.147, and 3. p=0.343), but significance in *S.* Newport population decline (1.p=0.00200, 2. p=0.96865, and 3. p=0.00479).

Furthermore, using the Kruskal-Wallis test, we observed that there were no statistically significant difference for the *E. coli* and *S.* Newport populations within ZVI filtrate based on the log CFU/ml values over the sampling period(p=0.9047 and p=0.7135). We also noticed that there were no significant reduction for the *E. coli* and *S.* Newport populations within sand filtrate based on the log CFU/ml values over the sampling period (p=0.6929 and p=0.7095).

With Treatment 2, there were reductions in both *E. coli* TVS 353 and *S.* Newport population due to the actual filtration process. In sand filtration, *E. coli* and *S.* Newport populations were reduced by 0.83 and 1.48 log CFU/mL, respectively, while ZVI filtration reduced populations of *E. coli* and *S.* Newport by 1.75 and 1.86 log CFU/mL, respectively. The Kruskal-Wallis revealed that there were significant reductions from the initial inoculation levels for ZVI (p=0.04953) on day 0, but not for the sand filtration (p=0.2752).

Discussion

As described previously, we sought to evaluate the efficacy of ZVI in disinfecting microbial contamination of synthetic irrigation water through filtration and residual activities. As shown in the treatment 1, there was no residual effect on bacterial population concentrations in the ZVI filtrate. Moreover, for treatment 2

there was no observed residual effect as well. Although we observed significant differences between *Salmonella* populations in the ZVI filtrate and the synthetic water control, there was no statistically significant difference between the populations of *Salmonella* on each sampling day for the ZVI filter condition.

However, there was an initial reduction in bacterial populations from filtering through the ZVI-sand filter compared to the sand filter alone. This further supports ZVI as a possible tool to remove harmful pathogens from irrigation water. Moreover, based on the results observed, the effects of ZVI may vary amongst different organisms, and biological differences, such as cell surface charge, may cause a greater reduction rate associated with this water treatment approach.

4.1 Limitations

There were certain limitations that may have contributed to the results we observed. For instance, the potential for contact time to ZVI treatment was reduced due to design complications. As described in the methods, only one column of a filter apparatus was used in this study. Shown in previous studies, contact with ZVI was a crucial component that is needed to reduce bacterial contaminants from irrigation water.

Moreover, we are uncertain as to whether there were viable-but-non-culturable *E.coli* or *Salmonella* cells remaining within the filter column. Additionally, the functionality of the organisms was not evaluated. We are not sure if the cells recovered are able to still persist on leafy greens and other produce, in addition to causing infectious responses in hosts. Although, this was beyond the scope of this

study, evaluating the viability, functionality and infectivity of bacterial pathogens post ZVI treatment is important for both agriculture and public health.

Also, we did not have any crop or plant models in this particular study. We are uncertain whether there are reactive oxygen species and other by-products in the filtrate that potentially influence plant development, which includes growth, functionality, and yield.

4.2 Future Assessments

As stated previously, there may not have been sufficient enough contact time with the ZVI particles within the filter set up to have created reactive oxygen species and other essential by-products to reduce pathogenic bacteria. To further this research, studies should increase the number of ZVI columns to each filter. Additionally, increasing the percentage of ZVI in each column may prove useful in creating a sufficient residual reactive species to cause disinfection. The filters should be built in parallel with connectors. To assess viability, propidium monoazide (PMA) treatment and real-time PCR should be used as described in Truchado, Gil, Kostic, & Allende, 2016. Additionally, plant models should be use to track the effect of using ZVI filtered water on development. Little is known about how ZVI would affect plants and the symbiotic microbial relationship that they have with organisms in the environment.

4.3 Public Health Significance

As described previously, ZVI may prove to be essential in reducing pathogens and contamination from irrigation water. Although there was no residual activity in this specific analysis, we are aware that reduction of pathogens occurs during ZVI Biosand filtration. Moreover, our findings are in agreement with previous studies that show that water should be filtered before use (Carey et al., 2016) (Jjemba, Weinrich, Cheng, Giraldo, & LeChevallier, 2010). Adequately treating irrigation water during storage, between storage and during distribution are still strategies to eliminate bacterial pathogens (Selma et al., 2015, p.).

Conclusions

Our study represents one step further with regard to developing potential, cost-effective, feasible treatments for irrigation water. This study confirmed that ZVI filtration is capable of removing bacterial contamination, such as *Salmonella Newport*, but showed that the residual disinfecting capabilities of this technology may be limited. More studies are needed to assess the various capabilities of this technology.

Table 1. Illustration of treatment 1 and 2 that was used to assess ZVI residual capabilities.

Treatment	**Time of Inoculation**	**Sampling Time**
1	Post-Filter	Post-Filter (days = 0, 1, 2, 4, and 7)
2	Pre-Filter	Pre- and Post-Filter (days = 0,1, 2, 4, and 7)

Figure 1. Average *E. coli* TVS 353 Populations for Treatment 1: Post -Inoculation Treatment for ZVI, Synthetic Water, and Sand

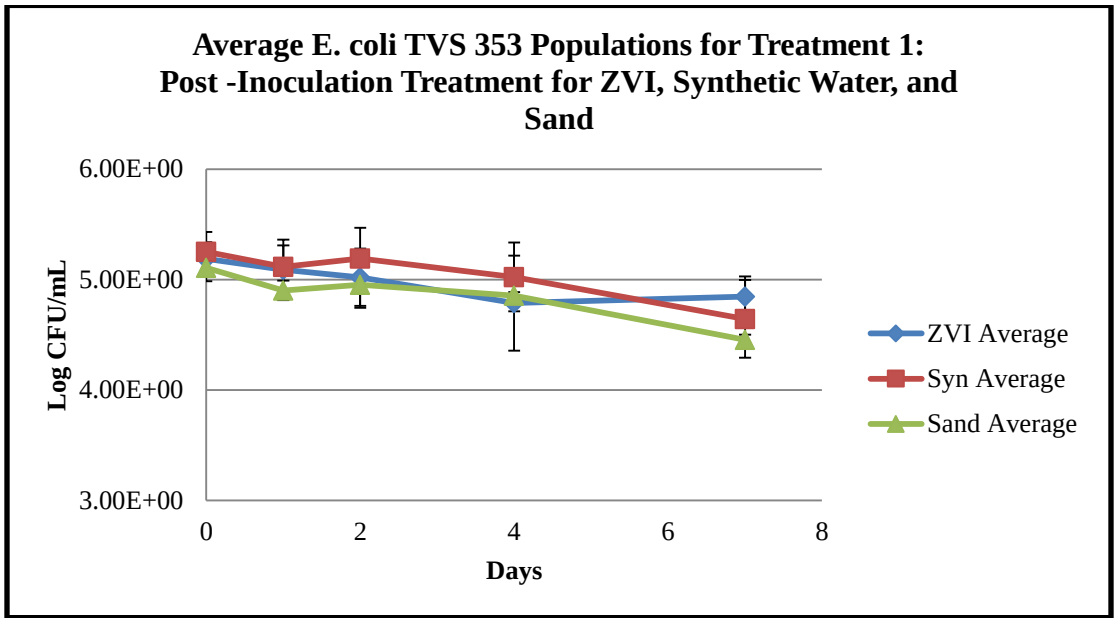


Figure 2. Average *Salmonella Newport* Populations for Treatment 1:Post-Inoculation Treatment for ZVI, Synthetic Water, and Sand.

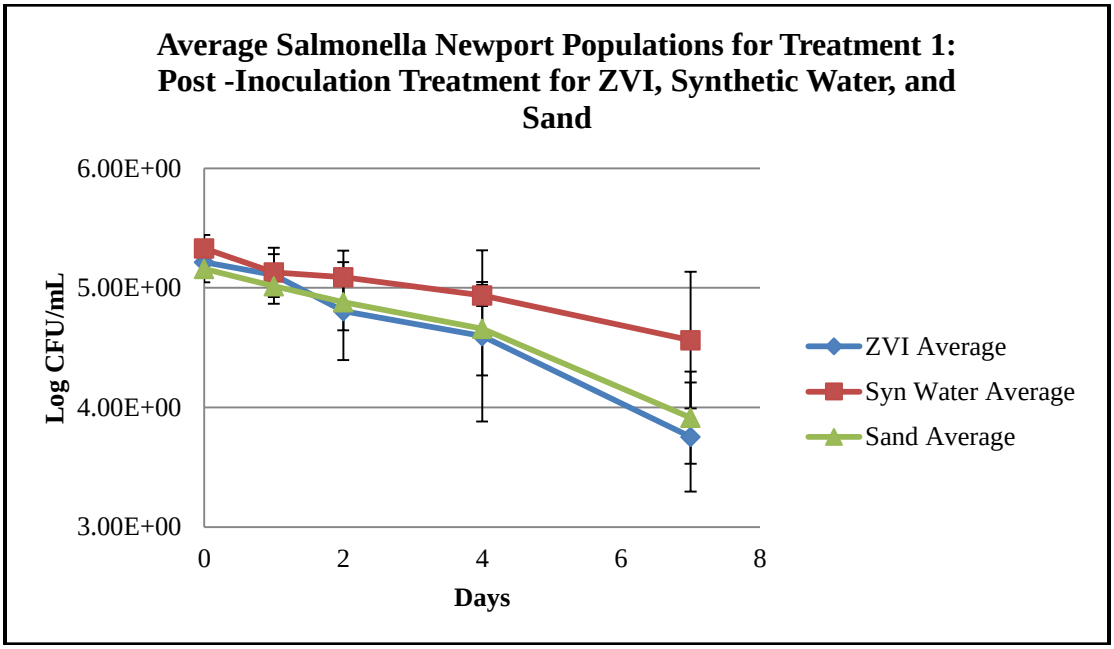


Figure 3. Average *E. coli* TVS 353 Populations for Treatment 2: Pre-Inoculation Treatment for ZVI, Synthetic Water, and Sand.

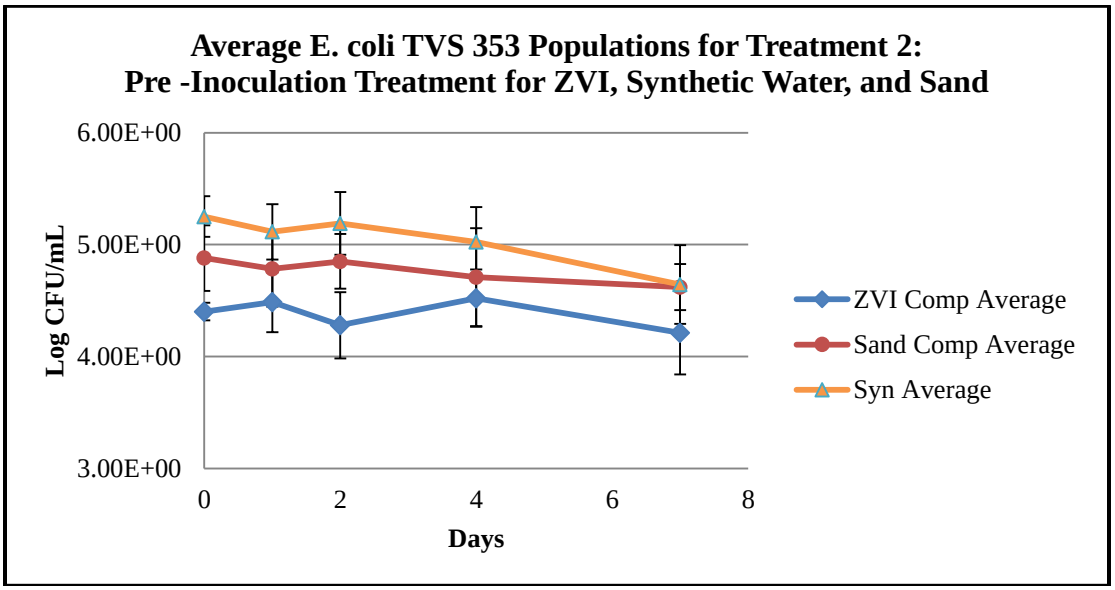
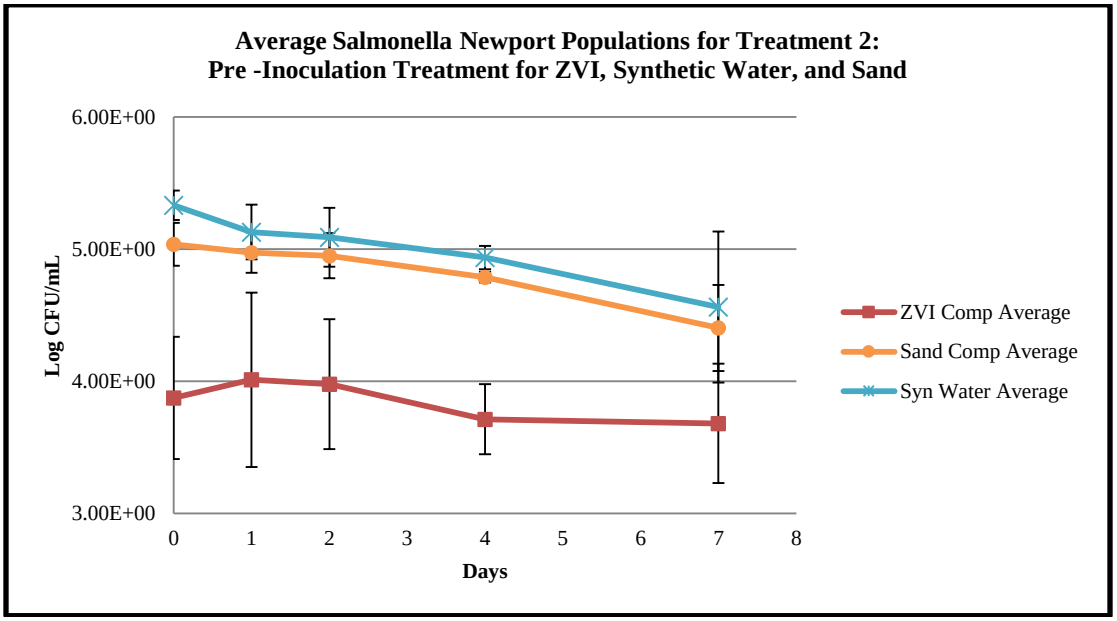


Figure 4. Average *Salmonella* Newport Populations for Treatment 2: Pre -Inoculation Treatment for ZVI, Synthetic Water, and Sand.



Chapter 4: Public Health Implications and Conclusions

As described previously, ZVI may prove to be essential in reducing pathogens and contamination from irrigation water. Although there was no residual activity detected in this specific analysis, we are aware that reduction of pathogens occurs during ZVI Biosand filtration. Moreover, our findings were in agreement with previous studies that demonstrated that water should be filtered before use (Carey et al., 2016) (Jjemba et al., 2010). Adequately treating irrigation water during storage, between storage and during distribution are still effective strategies to eliminate bacterial pathogens (Selma et al., 2015, p.).

Conclusions

Our study represents one step further with regard to the development of potential, cost-effective, feasible treatments for irrigation water. This study confirmed that ZVI filtration is capable of removing bacterial contamination, including *Salmonella Newport.* More studies are needed to assess the various capabilities of this technology. Furthermore there is a need to evaluate the effect of ZVI on plant development and the various symbiotic microbes that live alongside food crops that are essential for growth.

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WORK EXPERIENCE

UNITED STATES DEPARTMENT OF AGRICULTURE, Beltsville, MD
Graduate Research Intern, Jan 2016 - Present

- Recruited by the USDA for demonstrating hard work ethics and good integrity
- Researched and developed Zero-Valent Iron filtration method to assess for potential remediation of food-borne bacterial pathogens in irrigation water.
    - Additionally, performed bacterial recovery from sample water bodies and analyze them for generic *E. coli* and other bacterial pathogens.

UNIVERSITY OF MARYLAND, College Park, MD
Teacher's Assistant, Aug 2016 – Dec 2016

- Support in teaching AASP 189I HIV/AIDS in a Global Perspective course by overseeing online discussion sessions, grading course material, and occasionally proctoring lecture sessions.

UNIVERSITY OF MARYLAND, College Park, MD
Student Researcher, Jun 2014 – Jun 2015

- Assessed water quality of reclaimed water to investigate whether it was safe for groundwater recharge.
- Performed qualitative analysis of survey responses from a water quality of private well system and education study in Maryland.

UNIVERSITY OF THE VIRGIN ISLANDS, St. Thomas, USVI
Student Researcher, Jan 2014 – Aug 2015

- Evaluated pathogenicity of *E. coli* recovered from storm water runoff that form during high precipitation events, and its impact on Brewers Bay beach on St. Thomas, USVI near the University of the Virgin Islands. Used GIS to map the watershed on the western end of St. Thomas near the University.

EDUCATION

UNIVERSITY OF THE VIRGIN ISLANDS, St. Thomas, USVI
Biology, B.S., May 2015
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REPORTS

K. Bibb, **R. Bradshaw**, N. Boonchaisri, M. DeSantiago, L. Kavi, Y. Khan, Bernadette Kilcer, Winnie Mutunga, Suraj Panthi; **Flint, Michigan Water Crisis Risk Assessment Report;** *Dec* 2016; Developed as a course report in response to the Flint Water Crisis under Dr. Abdel-Razak M. Kadry

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K. Altalib, **R. Bradshaw**, J. Chopyk, H. Koka, M. Nnaji. **South Carolina Intermodal Health Impact Assessment.** *Dec 2015.*

AWARDS

2011 EDC Scholarship
2014 Certified Peer Educator for Substance Abuse and HIV Awareness
2014 Citi Certification for Human Subject Research
2014 Emerging Caribbean Scientist Program
2014 Assistive Employee Award

CONFERENCES

University of the Virgin Islands Fall Symposium *Oct 2014*
Annual Biomedical Research Conference for Minority Students *Nov 2014*
American Society for Microbiology *Jun 2015*
Association of Collegiate Schools of Architecture Fall Conference *Sep 2016*
The Washington DC Branch of the American Society for Microbiology and Capital Area Food Protection
Association Joint Fall Meeting *Dec 2016*
Society of Toxicology March *2017*