



**CPS-WCFS 2013 RFP
FINAL PROJECT REPORT**

Project Title

Use of zero valent iron (ZVI) in irrigation of tomatoes with manure-contaminated water at varying *E. coli* levels

Project Period

July 1, 2013 – June 30, 2014

Principal Investigator

Kali Kniel
Department of Animal and Food Sciences
University of Delaware
302-831-6513, kniel@udel.edu

Co-Principal Investigator

Dallas Hoover
Department of Animal and Food Sciences
University of Delaware
302-831-8772, dgh@udel.edu

Manan Sharma
USDA, ARS, BARC, EMFSL
301-504-9198, manan.sharma@ars.usda.gov

Objectives

Assess and optimize ZVI in the presence of organic material (manure) for its effectiveness in the removal of coliforms, fecal coliforms, and E. coli from water used to irrigate tomatoes. At the same time this study will assess the action of ZVI to alter chemical properties of surface water.

Funding for this project provided by the Center for Produce Safety through:
The Western Center for Food Safety

FINAL REPORT

Abstract

Zero-valent iron (ZVI) is used for groundwater remediation and for wastewater treatment. In this study we build upon the data to support the use of ZVI for removal and inactivation of microbial pathogens from agricultural water. ZVI is a reactive non-toxic metal, relatively abundant, inexpensive and easy to produce. Reduction of ZVI requires little maintenance; however, little is known about the efficiency of microbial removal and organic load. Field scale water filtration units were packed with 1:1 ZVI and sand combinations and used to remove generic *E. coli* from a manure slurry with a high organic load. *E. coli* was undetectable from filtrate from ZVI columns compared to sand columns treated in an identical manner. When this filtrate was used to irrigate tomato plants, *E. coli* levels remained at zero or low when plants became contaminated from environmental sources. The same ZVI field columns were able to remove > 10,000 *Salmonella* bacteria from pond water in one trial and then >100,000 *Salmonella* from pond water in a second trial. Efficacy of ZVI using laboratory-scale filtration units was assessed on two important water pathogens, norovirus and *Cryptosporidium*. ZVI filtration resulted in >4 log reduction of norovirus surrogates and >5 log reduction of *Cryptosporidium* oocysts. Filtration units containing ZVI can be made in various sizes and may be a useful tool to reduce contaminated irrigation water.

Background

Contaminated irrigation water can be a vehicle to disseminate bacterial foodborne pathogens to produce crops and cause human illness. In 2006, 71 infections of *Escherichia coli* O157:H7 were associated with the consumption of contaminated, shredded iceberg lettuce. The most likely source of *E. coli* O157:H7 was irrigation water that had been contaminated with dairy manure effluent containing the pathogen (CFERT, 2008). In 2005, lettuce contaminated with *E. coli* O157:H7 through irrigation water sickened 135 individuals in Sweden. Lettuce was irrigated with river water containing cattle feces (Soderstrom *et al.* 2008). Produce commodities have been responsible for high profile outbreaks involving several commodities, including leafy greens, peppers and sprouts over the past 5 years (CDC, 2008; CFERT, 2008; EFSA, 2011). The overall annual economic cost of foodborne illness in the United States is estimated to be \$154 billion, of which \$38 billion is attributed to contaminated produce (Scharff 2010). Infections with *E. coli* O157:H7 and *Salmonella* are estimated to cost \$3.1 billion (in 2010 dollars) per year in the United States as well (USDA-ERS 2011).

Irrigation water that does not contain pathogens or transmit them to growing produce crops is of utmost importance to produce growers and consumers. Irrigation water standards in the FDA Proposed Produce Safety Rule and in the California Leafy Green Marketing Agreement (LGMA) were developed in response to produce outbreaks, and these standards state that for foliar application water samples must meet specific *E. coli* standards; for example, no single sample may contain greater than 235 MPN *E. coli* per 100 ml (LGMA 2011). At times water sources may not meet these or other similar standards due to chemical or biological hazards that may occur seasonally or after environmental events. Upon these occasions water mitigation strategies are alternatives and should be applied to water sources in question as a pre-application treatment of surface water (Harris *et al.*, 2012).

Biosand filter zero-valent iron incorporated (ZVI) treatment has been used in permeable reactive barriers to remove a broad range of chemical contaminants in groundwater (Meggyes and Simon 2000). ZVI oxidizes continuously in water through reactions with dissolved oxygen and protons to form amorphous iron hydroxides that are subsequently converted into more stable oxides and oxyhydroxides, such as magnetite, goethite and lepidocrocite (Odziemkowski *et al.* 1998; Phillips *et al.* 2000). Iron hydroxides, oxides and oxyhydroxides have a relatively high pH_{pzc} (point of zero charge) and can strongly adsorb viruses and other negatively charged micro-organisms via electrostatic interactions. ZVI does not generate potentially harmful by-products like other chemical treatments. ZVI-based technology has achieved greater than 5-log removal of two model viruses (bacteriophages MS2 and ØX174) (You *et al.* 2005) and of the surrogate bacterial pathogen *E. coli* O157:H12 (Ingram *et al.*, 2012) in ground water. As water becomes a more critical resource in agriculture, ZVI treatment could provide an agriculturally sustainable approach to providing irrigation water of sufficient quality to produce growers and potentially allow more sources of water to become eligible to irrigate produce. This proposal seeks to address important questions that remain concerning the use of ZVI, including potential for use to reduce coliform levels in the presence of organic material like manure and reduction of water nitrate concentrations.

Research Methods and Results

ZVI-Treated Irrigation Water for Use on Tomatoes

Plant preparation

Tomato (BHN-602) seeds purchased from (Johnny Selected Seeds, Winslow, ME) were started in 2401 seed trays (Dillen Products, Middlefield, OH) containing ProMix (Premier Horticultural Inc. Quakertown, PA) They were placed in the University of Delaware greenhouse misting room (22-24°C, 85% humidity) until germination. Upon germination, plants were moved out to the main greenhouse (28-30°C, 60% humidity). Tomatoes were transplanted into 4" square traditional thin wall pots (Dillen Products, Middlefield, OH) at 3 weeks. Plants were fertilized using Peter's Fertilizer (21-5-20) at a concentration of 200ppm of Nitrogen. A Dosatron (Dosatron International, Clearwater, FL) set at a rate of 1:128 was used to apply fertilizer once per week while in green house. At 6 weeks, seedlings were moved outside where they were hardened for 1 week. Following this, they were transplanted into plastic-culture (Rain-Flo, East Earl, PA). Irrigation was performed using Drip Tape (Rain-Flo, East Earl, PA) until full maturity. Tomatoes were grown in single rows with stakes (Honduran Pine Stakes (Rain-Flo, East Earl, PA) every 4' and plants evenly spaced at 18" apart.

Preparation of manure slurry inoculum

A 222.22 g composite sample of Dairy Manure (UD Dairy Farm, Newark, DE) was placed into a Carboy (Nalgene, Rochester, NY) containing 22L of Deionized water The sample was agitated for 2 min, allowed to sit for approximately 10 min, agitated again and serially diluted. *E. coli* was enumerated on TBX agar (Oxoid, UK) incubated at 37°C for 24 hrs.

Filter preparation

Commercial HydrAid Biosand filters (Cascade Engineering, Grand Rapids, MI, USA) were built as described previously by Ingram *et al.*, (2012), each containing gravel, filtration gravel and 45.4 kg of filtration sand into a 20-L water column (Figure 1). Filters (referred to as columns) are 0.77 m high with a diameter of 0.14 m. Column casing weight is ~3.6 kg empty and 63.5 kg filled with sand and gravel. Columns were modified in the filtration layer to contain either a filtration sand layer only or a combination of ZVI and filtration sand (SI) at a 1:1 ratio by weight. ZVI (Peerless Metal Powder and Abrasives, Detroit, MI, USA) was incorporated into columns

without modification. We used particle sizes of sand and ZVI as defined previously by Ingram et al., (2012). Before use in this study, water was applied to the filters (~11-22 L) each day for a minimum of 14 days, as suggested by manufacturer's guidelines.

Filtration of manure slurry

Manure slurry (11L) was added to each filter followed by 11L samples of clean water. Organic load and *E. coli* enumeration was assessed in pre- and post-filter water samples using a portable environmental monitoring system (Sper Scientific, Scottsdale, AZ).

Plant inoculation

Hybrid BHN-602 tomatoes were used for the duration of the project. Plants (180 total, 12 groups of 15 with 4 inoculum levels in triplicate) were inoculated with manure water at 10^4 , 10^3 , 10^2 *E. coli* CFU/ml. Control plants were treated identically with uncontaminated deionized water. Plants were inoculated with 250 ml inoculum/plant, saturating top leaves and fruits of tomatoes and whole plants for leafy greens. Each plant was inoculated individually using a sterilized graduated cylinder.

Sampling and *E. coli* enumeration

Samples were collected on day 0 pre- and post-watering, and on days 1, 3, 5, 7, and 10. Samples are composites of four tomatoes taken from four random plants within each plot at each level of inoculation. Plants were numbered and numbers chosen at random in advance of sample collection. Fruit samples (4 tomatoes of 500-600g total from 4 plants per plot) and leaf samples (2 leaves from 4 plants per plot) were weighed upon collection and placed into 1-gallon Ziploc bags (SE Johnson, Chicago, IL). Each sample was chosen at random using a random number selection system for each plot. After 1 minute of shaking by hand, samples were serially diluted in 15-ml Falcon tubes containing 9ml of phosphate buffer saline (Sigma Aldrich, St. Louis, MO) and plated on TBX agar (Oxoid, UK) in duplicate and incubated at 37°C and 42.5°C for 24hrs. Samples were also assessed using a Colilert Quantitray (IDEXX, Westbrook, ME) then incubated at 37°C.

Results

Manure slurry (11 L) was filtered through ZVI and sand columns during two separate trials and tomato plants were irrigated with these water samples (Figure 1-2) in August 2013 and August 2014. Levels of *E. coli* were analyzed on tomato fruits and leaf tissue over 5 or 10 days. The trial in 2013 ended at day 5 post-inoculation due to heavy flooding and deterioration of the plants. The data collected is shown in Tables 1-3. The fields used were also being used for a larger study where *E. coli* was introduced into the field; therefore, while no *E. coli* was detected in ZVI-treated water, field samples tested positive for *E. coli*. There was a great amount of rain during trial one, while there was none during trial two. This likely influenced the large number of samples where *E. coli* was noticeably absent.

Fourteen days after completion of the first trial, a second addition of manure slurry (11 L) was filtered through the ZVI and sand filters. The removal efficiency of *E. coli* by ZVI was impacted by the presence of residual manure in the filter or by fouling to the filter (data not shown), with removal of *E. coli* reduced to ~2 logs compared to the >4 log removal in the primary studies.

Use of ZVI to reduce *Salmonella* from Surface Water

Filtration and use of Pond Water

Surface water samples (125L) were collected from Papen Farms (Dover, DE) ponds within

areas of active irrigation during the early spring of 2014. Samples were held at 4°C prior to use. Samples were monitored for dissolved solids, *E. coli*, total coliforms, *Salmonella*, and nitrate levels. Water dissolved solids and nitrate levels were determined using a portable environmental monitoring system following EPA guidelines (Sper Scientific, Scottsdale, AZ). Water samples were inoculated with varying levels of *Salmonella* Newport (environmental isolates from VA Eastern shore, collection of the University of Delaware Department of Animal and Food Sciences) prior to filtration. Filtrate was used to water 3-4 week old tomato plants under greenhouse conditions, where fruit and leaf samples were collected. *Salmonella* was enumerated on XLT-4 media and enriched as necessary following procedures established by the FDA BAM using RV broth. Water samples were gravity-fed over ZVI and sand filtration units, as described above.

Results

In two separate trials surface water (11 L) was inoculated with one of two different isolates of *Salmonella* Newport with 10^4 - 10^5 cells/ml of surface water and applied to columns containing ZVI or sand. Prior to inoculation, surface water samples contained 260 ppm total dissolved solids (TDS) with 95.8 *E. coli* MPN/100 ml. ZVI filtration successfully removed 4-5 logs of *Salmonella* from the surface water during the two trials (Figure 3). Filtrate water from sand columns was diluted to <1 log cfu/ml *S. Newport* in order to compare the behavior of similar concentrations of bacteria in irrigation water on tomato plants and fruit. There was no significant difference on plants or on fruits following detection of *Salmonella*.

Inactivation of Bacteria by ZVI

Results of column deconstruction

Sand and ZVI columns that were used for field irrigation studies were taken apart and the amount of *E. coli* enumerated. Each column was separated into four equal sections and 25 g samples were enumerated in triplicate from each section. Viable *E. coli* were detected in each layer of the sand filter. Approximately the same number of bacteria were detected in each of the four sections of the column from the surface to the bottom. The sections of the sand column contained *E. coli* averages of 4.9, 5.6, 1.8, and 4.3 log cfu/g from the surface to the bottom of the sand region of the column. In contrast 9.7 log cfu/g *E. coli* was detected at the surface of the ZVI column. The middle and bottom layers of the ZVI column contained 2.2, 1, and <1 log cfu/g *E. coli*. Several samples from the lowest layer of the ZVI column did not have detectable *E. coli* present. This supports previous hypothesis that ZVI can inactivate bacteria.

Effectiveness of ZVI on Norovirus and *Cryptosporidium parvum*

Norovirus remains the leading cause of acute gastroenteritis. Two laboratory surrogates are used to understand the inactivation and persistence of human norovirus since human norovirus cannot be cultivated under laboratory conditions. By comparing the two most viable norovirus surrogates, murine norovirus (MNV) and Tulane virus (TV), we can appreciate how human norovirus may respond to similar situations (Kniel, 2014). MNV is perhaps more genetically similar to human norovirus, and TV shares great similarity in terms of capsid structure. *Cryptosporidium parvum* is a protozoan parasite that causes a potent gastroenteritis that may be self-limiting after several days or may lead to chronic illness in immunocompromised individuals. The presence of *C. parvum* oocysts have increased from 2006 to 2008 in municipal water supplies and outbreaks of cryptosporidiosis associated with recreational water have also increased in the past several years (Yoder et al., 2010).

Use of Laboratory-Scale Columns for analysis of Virus and Protozoa

Using laboratory-scale filters that have been used previously (Shi et al., 2012), relatively small volumes of sterile water were inoculated with known concentrations of three different organisms. Each organism was tested independently of the others. Columns were flushed with bleach prior to use and between trials and rinsed thoroughly. Column materials are identical to those described previously used in the large filtration systems. Sand and ZVI columns were packed with 1 cm of sand on the bottom and top of each column, with the middle composed of 1:1 mix of ZVI:sand based on volume. The sand at the top and bottom acts to retain the ZVI:sand mix. In each test one ZVI column and one sand column were tested in duplicate runs. Input suspensions per column included 100 ml of inoculum, followed by a 300 ml flush. Liquids were delivered at one ml per min. During this time, 80 fractions of 5 ml each were collected. MNV, TV, and *C. parvum* were evaluated by RT-qPCR for 18 of the 80 fractions. Break-through curves were generated using every fifth fraction collected during the input and flush.

Virus recovery using quantitative RT-PCR

Virus preparation

MNV was propagated in confluent 24-h RAW 264.7 cells incubated at 37°C and 5% CO₂ for approximately 48 h in Dulbecco's Modification of Eagle's Medium (DMEM, Mediatech, Inc., Manassas, VA) containing 4.5 g l⁻¹ of glucose, L-glutamine and sodium pyruvate amended with fetal bovine serum (FBS, HyClone, Logan, UT) at 10% v/v, 4 mM glutamax (Invitrogen), sodium bicarbonate (Mediatech), and antibiotic/antimycotic (100 U penicillin, 100 µg ml⁻¹ streptomycin, 0.25 µg ml⁻¹ amphotericin B, HyClone). MNV was recovered from cells by three freeze/thaw cycles (-80°C/25 to 37°C) and centrifugation (233 x g). The virus-containing supernatant was aliquoted and stored at -80°C until further use. Tulane virus (TV) was propagated in 24-h confluent LLCMK2 cells, incubated for approximately 55 h at 37°C with 5% CO₂ in Medium 199/EBSS (M199 with Earles Balanced Salts and L-glutamine, HyClone) amended with 10% FBS and antibiotic/antimycotic mix as previously described. TV was collected from LLCMK2 cells as described for MNV and stored at -80°C until use.

Virus enumeration

Viral RNA was extracted from column fractions according to manufacturer instructions for the QIAamp® Viral RNA Kit (Qiagen, Hilden, Germany). Genomic RNA was quantified by reverse transcription (Sensiscript® RT Kit, Qiagen), real-time polymerase chain reaction (qPCR) (QuantiTect® SYBR® Green PCR Kit, Qiagen) on a Rotor-Gene Q thermocycler and detection system (Qiagen). MNV-1 primers (MNV-F, 5'-CTTCGCAAGACACGCCAATTTTCAG and MNV-R, 5'-GCATCACAATGTCAGGGTCAACTC, Sigma-Aldrich® Corp., St. Louis, MO, USA) targeted the polymerase gene (Hsu *et al.*, 2005). TV primers (TV-F, 5'-TCGCGCAGCGCACTTA and TV-R, 5'-CAAGAATCCAGAACAACCAATATCA) were specific for nonstructural polyprotein region. Reverse transcription conditions were 37°C for 60 min. Amplification conditions included a hot start of 95°C for 15 min, 40 cycles of denaturation at 94°C for 15 sec, annealing at 59°C for 30 sec, and extension at 72°C for 30 sec, and confirmatory melt. Genomic copies in samples were determined by comparison to viral standard curves with the lowest level of detection designated as one RT-qPCR unit.

Virus recovery

Both trials of MNV were nearly identical, with <10 genomic copies detected in less than half of the samples. No virus was detected in the other fractions. The break-through curve is depicted in Figure 5 where ≥1,000 virus particles were removed. The two trials for TV showed conflicting

data, with removal of >1,000 virus particles in one trial and <100 virus particles in the second trial (data not shown).

***Cryptosporidium* recovery using a cell culture infectivity assay coupled with quantitative RT-PCR**

In vitro cell culturing of *C. parvum* and oocyst treatment

Human ileocecal adenocarcinoma (HCT-8; ATCC CCL-244) was maintained in RPMI 1640 medium (Mediatech Cellgro, Herndon, VA) supplemented with L-glutamine (300 mg/L; Mediatech Cellgro), HEPES (25mM; Mediatech Cellgro), and fetal bovine serum (pH 7.2; Biofluids, Inc., Rockville, MD). For normal cell maintenance medium was supplemented with 10% fetal bovine serum and decreased to 2% fetal bovine serum for parasite infection. HCT-8 cells were cultivated in 6-well cell culture plates and incubated at 37°C in a 5% CO₂ humidified incubator to allow the development of 85 to 90 % confluence in medium for 48 hours. The cell monolayer in each well of the plate was considered a single replicate.

Inoculation of monolayers with oocysts

Prior to infection of oocysts on the cell monolayer, the cell growth media was removed and cells were washed with HBSS to remove any cellular debris. Fresh cell maintenance medium (2% FBS, 500 µl) was added in each well. Samples of control oocysts or treated oocysts (100 µl) were added to wells of culture plates in duplicate. The plates were placed in the incubator at 37°C for 1 hour to allow for infection of the cells. The inoculum was then removed, and 2 ml of fresh media was added in each well. The plates were placed in the incubator at 37°C with 5% CO₂ for 48 hours for replication. Media was removed, and each well was rinsed by 1 ml HBSS 3 times. 500 µl accutase (BD Biosciences, San Jose, CA) was added in each well and plates were incubated for 10 min at 37°C or until cell layer became detached as determined by microscopy. Media, HBSS, as well as detached cells were collected for each well for DNA extraction.

DNA extraction from oocysts and cell culture monolayers

DNA extraction was performed using QIAmp DNA Mini Kit (Qiagen, Hilden, Germany). In the last step the DNA were eluted from the column with 20 µl elution buffer and store at 4°C for a maximum of 1 week until PCR amplification.

Real-time PCR Quantification

Real-time PCR quantification was performed. PCR primers specific for the *C. parvum* heat shock protein gene (*hsp70*) were designed as follows: forward primer (5'-GTGTCTGCTGTTGATAAG -3') and reverse primer (5'-AGAGGTAGTTCTCCAAAG -3'). Each 20-µl reaction contains 10 µl of TaqMan Master Mix (2X), 1µl of TaqMan forward and reverse primers, 3 µl of sterile RNase DNase free water, and 6.0 µl of DNA template. Amplification conditions were as follows: UDG Incubation at 50°C for 2 min and Enzyme Activation at 95°C for 10 min, and 40 cycles of denaturation at 95°C for 15s, annealing at 60°C for 1min, followed by a dissociation step at 60 °C for 15 s and 90 °C for 15 s. Signal of green florescence was obtained during annealing step. Each sample was performed in duplicate. Purified DNA that was extracted from log 6 *C. parvum* oocysts down to log 1 oocyst/ml was used to create a standard curve. RNase DNase free water replacing template DNA served as a negative control. The *C. parvum* standard curve obtained was used to determine log number of oocysts of each sample on the basis of the C_T values.

***Cryptosporidium* oocyst recovery**

Twenty or sixty fractions were collected during the inoculum pulse and the flush, respectively. A fraction is collected automatically every 5 ml. Every fifth fraction was analyzed for the

presence of *C. parvum* oocysts, which were detected in the sand column flush samples beginning at fraction 5. Where 2.4 log oocysts were detected. The number of oocysts released from the sand column increased over time through the sixtieth fraction collected. No oocysts were detected in any of the fractions collected from the ZVI column, indicating complete removal of > 6 logs of *C. parvum* oocysts (Figure 6). It is likely that removal of oocysts is a combination of physical and chemical factors. Oocysts are 4-5µm in size and could be retained in the column.

Outcomes and Accomplishments

ZVI has great potential for use in agriculture to modify existing irrigation water. While we had previously shown that ZVI has the ability to remove bacterial pathogens from water, we had not yet examined water with an organic load. Here we describe the positive outcome in reduction of generic *E. coli* along with physical changes in water clarity. This study assessed a worst case scenario for irrigation water, likely with a higher organic load than what would be encountered in surface water on a general basis, but could be similar to random instances. We also showed that ZVI is effective on removal and inactivation of *Salmonella* using pond water that has more of the typical amount of dissolved solids that might be present in irrigation water.

Summary of Findings and Recommendations

There is a growing body of literature on the use of ZVI for wastewater treatment (Fu et al., 2014). This knowledge can be used to enhance the development of ZVI for irrigation water. The flow rates used to generate the data in this report were 800 ml/min in gravity fed columns. This is greater flow than used previously, and suggests that retention time not as great of an issue as previously thought. In this study, high levels of organics were able to foul filter material and reduce the efficacy of ZVI removal of bacteria. We have also shown that ZVI is useful on multiple types of microorganisms. One of the greatest threats to water safety is the protozoan parasite *Cryptosporidium parvum* which is highly resistant to normal chlorine levels. ZVI here was also effective to some degree in removal and inactivation of norovirus surrogates. Certainly ZVI is not right for every situation, but it is a useful tool to reduce contaminated irrigation water and to create usable irrigation water to meet specific safety standards.

APPENDICES

References

CDC (Centers for Disease Control) (2008) Outbreak of Salmonella serotype saintpaul infections associated with multiple raw produce items – United States, 2008. *MMWR Morb Mortal Wkly Rep* 57, 929–934.

CFERT (California Food Emergency Response Team) (2008) Investigation of the Taco John's Escherichia coli O157:H7 outbreak associated with iceberg lettuce.

EFSA (European Food Safety Authority) (2011) Shiga toxin-producing E. coli (STEC) O104:H4 2011 outbreaks in Europe: taking stock. *EFSA J* 9, 2390–2412.

Fu, F., Dionysiou, D., Liuc, H., (2014) The use of zero-valent iron for groundwater remediation and wastewater treatment: A review. *Journal of Hazardous Materials* 267:194– 205.

Harris, L.J., Bender, J., Bihn, E.A., Blessington, T., Danyluk, M.D., Delaquis, P., Goodridge, L., Ibekwe, A.M., Ilic, S., Kniel, K., Lejeune, J.T., Schaffner, D.W., Stoeckel, D., Suslow, T.V. 2012. A Framework for Developing Research Protocols for Evaluation of Microbial Hazards and Controls during Production That Pertain to the Quality of Agricultural Water Contacting Fresh Produce That May Be Consumed Raw. *J. Food Protection*. 75:2251-2273.

Ingram, D.T., Callahan, M.T., Ferguson, S., Hoover, D.G., Chiu, P.C., Shelton, D.R., Millner, P.D., Camp, M.J., Patel, J.R., Kniel, K.E., Sharma, M. (2012) Use of zero-valent iron biosand filters to reduce Escherichia coli O157:H12 in irrigation water applied to spinach plants in a field setting. *J Appl Microbiol*. 2012 Mar;112(3):551-60. doi: 10.1111/j.1365-2672.2011.05217.x. Epub 2012 Jan 11.

Kniel, K.E. (2014) The makings of a good human norovirus surrogate. *Curr Opin Virol*. Feb;4:85-90. Epub 2014 Feb 1.

LGMA (Leafy Greens Marketing Agreement) (2011) Commodity specific food safety guidelines for the production and harvest of lettuce and leafy greens. July 22, 2011. Available at: <http://www.caleafygreens.ca.gov/sites/default/files/LGMAAcceptedFoodSafetyPractices207.22.11.pdf> (accessed 29 September 2011).

Meggyes, T. and Simon, G. (2000) Removal of organic and inorganic pollutants from groundwater using permeable reactive barriers Part2. Engineering of permeable reactive barriers. *Land Contam Reclam* 8, 175–187.

Odziemkowski, M.S., Schuhmacher, T.T., Gillham, R.W. and Reardonk, E.J. (1998) Mechanism of oxide film formation on iron in simulating groundwater solutions: Raman spectroscopic studies. *Corros Sci* 40, 371–389.

Phillips, D.H., Gu, B., Watson, D.B., Roh, Y., Liang, L. and Lee, S.Y. (2000) Performance evaluation of a zero-valent iron reactive barrier: mineralogical characteristics. *Environ Sci Technol* 34, 4169–4176.

Scharff, R.L. (2010) Health-related costs from foodborne illness in the United States. Available at: <http://www.producesafetyproject.org/admin/assets/files/Health-Related-Foodborne-Illness-Costs-Report.pdf-1.pdf> (accessed 29 September 2011).

Shi, C., Wei, J., Jin, Y., Kniel, K. E. and Chiu*, P. C. (2012) Removal of Viruses and Bacteriophages from Drinking Water Using Zero-Valent Iron. *Separation&Purification Technology*, 84, 72-78.

Soderstrom, A., Osterberg, P., Lindqvist, A., Jonsson, B., Lindberg, A., Blide Ulander, S., Welinder-Olsson, C., Lofdahl, S. et al. (2008) A large Escherichia coli O157:H7 outbreak in Sweden associated with locally produce lettuce. *Foodborne Pathog Dis* 5, 339–349

USDA-ERS (*United States Department of Agriculture, Economic Research Service*) (2011) Foodborne illness cost calculator. Available at: <http://www.ers.usda.gov/Data/Foodbornellness/> (accessed 29 September 2011).

Yoder, J., Harral, C., Beach, M. (2010) Cryptosporidiosis Surveillance --- United States, 2006—2008. 59(SS06);1-14 *MMWR*.

You, Y., Han, J., Chiu, P.C. and Jin, Y. (2005) Removal and inactivation of waterborne viruses using zerovalent iron. *Environ Sci Technol* 39, 9263–9269.

Publications and Presentations

A manuscript, based on this Final Report, is in development for submission to a peer reviewed journal. Abstracts will be submitted for poster presentations at the NoroCORE and IAFP meetings in 2015.

Budget Summary

Personnel (graduate and undergraduate students) and Fringe: \$31, 731
Supplies: \$11,639
Tuition fees: \$5,596
Indirect Costs: \$21,042

Tables and Figures

Table 1. Removal of *E.coli* from manure slurry by sand and ZVI filtration.

	Trial 1 Log cfu/ml <i>E. coli</i>	Trial 2 Log cfu/ml <i>E. coli</i>
Manure Slurry (not filtered)	4.1 ± 0.2	4.8 ± 1.1
Sand Filtered	3.4 ± 0.2	3.6 ± 0.07
ZVI Filtered	ND	ND*

ND: None detected

*No *E.coli* was detected in filtrate water through 6 days of flushing water through the unit

Table 2. Presence of *E. coli* on tomatoes following irrigation with filtered manure slurry (MS)

Irrigation Water Source	Log cfu/g <i>E. coli</i> ± std dev											
	Day 0+		Day 1		Day 3		Day 5		Day 7		Day 10	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Unfiltered MS	2.2 ± 1.1	2.2 ± 0.5	0.4 ± 0.6	1.1 ± 1.8	0.2 ± 0.17	ND	ND	0.8 ± 0.9	-	0.4 ± 0.7	-	0.3 ± 0.4
Sand filtered MS	2.3 ± 1.6	0.2 ± 0.2	0.08 ± 0.2	ND	0.58 ± 1.2	ND	ND	ND	-	ND	-	ND
ZVI- filtered MS	0.9 ± 1.7	ND	ND	0.38 ± 0.35	ND	ND	ND	ND	-	ND	-	ND

Table 3. Presence of *E. coli* on leaves of tomato plant following irrigation with filtered manure slurry (MS)

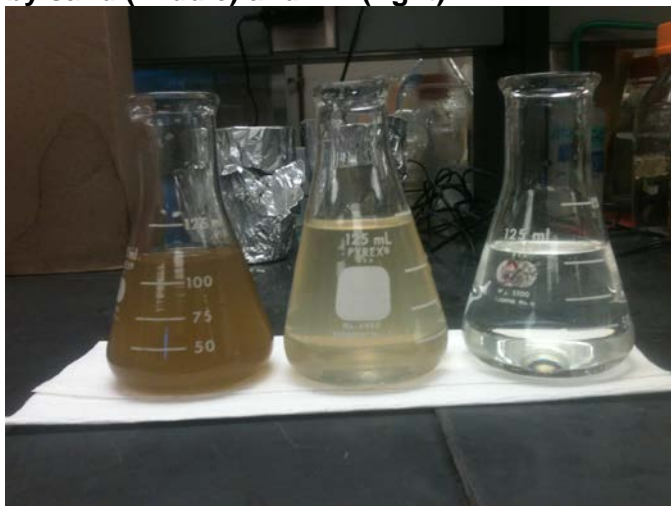
Irrigation Water Source	Log cfu/g <i>E. coli</i> ± std dev											
	Day 0+		Day 1		Day 3		Day 5		Day 7		Day 10	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Unfiltered MS	2.8 ± 1.5	5.1 ± 0.6	ND	ND	ND	ND	ND	ND	-	0.5 ± 0.9	-	0.1 ± 0.3
Sand filtered MS	3.1 ± 2.5	1.7 ± 0.3	0.1 ± 0.2	ND	ND	ND	ND	ND	-	0.1 ± 0.1	-	0.3 ± 0.8
ZVI-filtered MS	4.1* ± 2.1	ND	0.1 ± 0.2	ND	ND	ND	ND	ND	-	ND	-	0.1 ± 0.2

* Fields were being used for a larger study and *E. coli* not necessarily from irrigation water.

Figure 1. Hydraid Sand and ZVI filters (A) and the undergraduate and graduate student researchers who benefited from this funding and performed the research (B).



Figure 2. Observed changes in color and clarity of manure slurry (left) following filtration by sand (middle) and ZVI (right).



Kali Kniel, University of Delaware

Use of zero valent iron (ZVI) in irrigation of tomatoes with manure-contaminated water at varying *E. coli* levels

Figure 3. Filtration of pond water inoculated with *Salmonella* Newport (Trial 1 of 2 shown here).

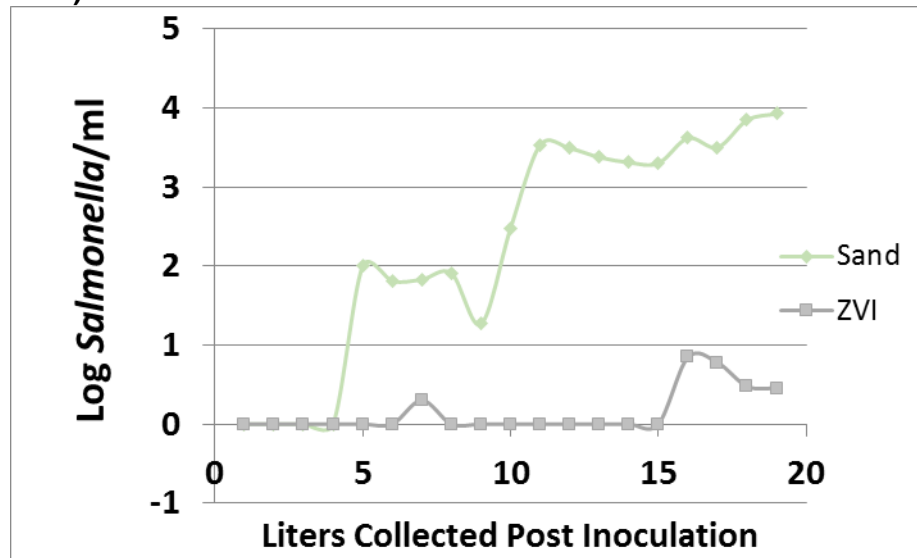


Figure 4. Laboratory-scale ZVI (A) and sand (B) filtration units attached to fraction collectors (C).

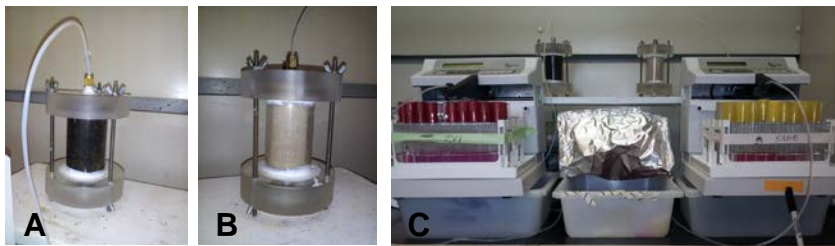


Figure 5. Removal of mouse norovirus (MNV) by filtration. Initial inoculum was 3873 RT-qPCR copies of MNV and 80 fractions were collected and every fifth was analyzed.

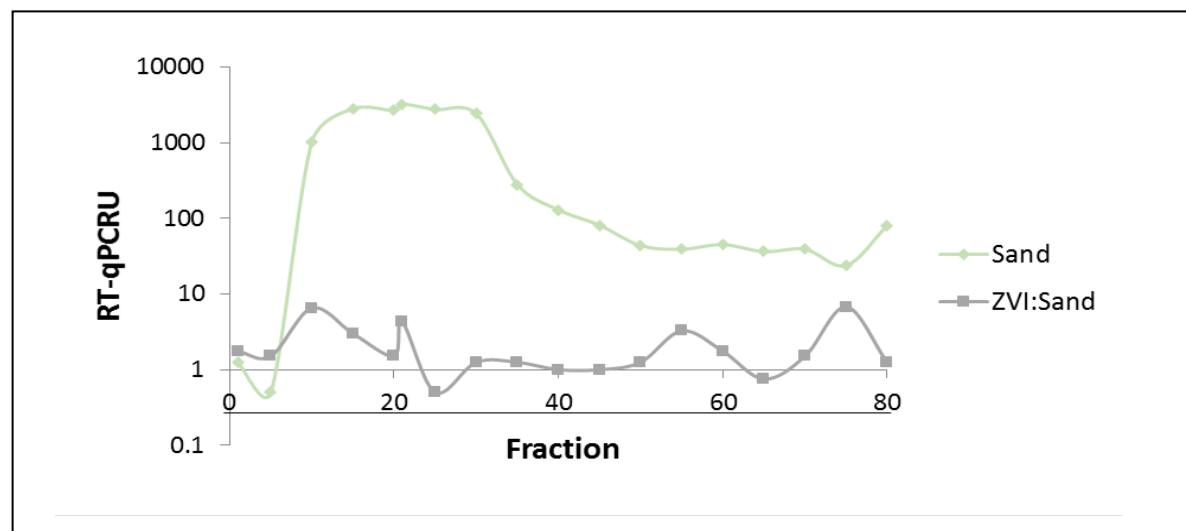


Figure 6. Removal of *Cryptosporidium parvum* oocysts by filtration. Every fifth fraction was analyzed from 60 fractions collected.

