CPS 2015 RFP
FINAL PROJECT REPORT

Project Title
Microbial food safety risks of reusing tail water for leafy green production

Project Period
January 1, 2016 – December 31, 2017 (extended to January 31, 2018)

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Objectives
1. *Characterize chemical and physical and microbiological composition of tail water from coastal vegetable fields.*
2. *Evaluate risks of using tail water in lettuce and leafy green production by quantifying microbial survival during simulated application to soils used to grow leafy greens.*

[*3. Evaluate methods to pre-treat tail water that improve the efficacy of antimicrobial treatments aimed at minimizing microbial food safety risks for a range of reuse applications (e.g., pre-irrigation and dust control, basic irrigation).*]

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FINAL REPORT

Abstract

Ground water is the main source of irrigation water in the Salinas Valley of California. For conservation reasons and to minimize off-site water quality impairments, there is interest to reuse irrigation run-off or 'tail water' for applications such as pre-irrigation and germination. Many Central Coast ranches have infrastructure for using tail water for irrigating crops, including sediment basins, reservoirs, and pumping systems. Currently, growers are reluctant to irrigate crops with tail water due to a lack of information on microbial food safety risks. The overall goal of this project was to evaluate the food safety risks of using tail water for irrigating leafy green crops using conventional irrigation and production practices. Chemical, physical, and microbiological characteristics of tail water were evaluated at five sites in the Salinas Valley during a 1-year period. Chemical and physical characteristics of tail water in retention basins at each site were measured in situ monthly. Samples of tail water and well water were also collected from these sites and analyzed for nutrients, pH, salinity constituents, and dissolved organic carbon. Water samples were evaluated for generic *Escherichia coli*, and coliform bacteria. Moore swabs were collected after 3 days and were analyzed for pathogenic *E. coli* and *Salmonella*. Incubation studies were conducted to evaluate the effect of nutrients in tail water on the survival of generic and pathogenic *E. coli*. A growth chamber study investigated whether tail water influenced the survival of pathogenic *E. coli* and *Salmonella* on lettuce and in soil. Results showed that the pH of tail water was often greater than 8.0. Salinity of the tail water was higher at southern sites where the salt content of the well water tended to be high, and where evaporation was highest. Generic *E. coli* exceeded 126 CFU/100 ml in approximately 20% of tail-water reservoir samples and in two of 26 irrigation water samples. Differences were found in the concentration of generic *E. coli* among sites, but the differences could not be correlated to nutrient concentration of the water. The incubation studies demonstrated that elevated concentrations of ortho-P and nitrate did not increase the survival of generic and pathogenic *E. coli* in tail water. Generic *E. coli* survived longer in well water than in tail water. The growth chamber study showed that the concentration of pathogenic *E. coli* and *Salmonella* decreased rapidly when applied to lettuce leaves. Both pathogens survived longer in soil than on lettuce leaves. Survival of pathogenic *E. coli* and *Salmonella* in soil and on lettuce leaves was similar for tail water and well water.

Background

The Central Coast of California faces severe restrictions on water supplies caused by periodic droughts such as the most recent 4-year drought from 2013–2016. Growers in this region rely almost exclusively on ground water for irrigating crops. As ground water levels continue to decline near the coast, seawater intrusion into coastal aquifers has accelerated.

The irrigation of vegetable crops with sprinkler and furrow systems on the Central Coast often results in significant agricultural drainage, also known as tail water. Tail water transports nutrients, sediments, pesticides, and bacteria that degrade the quality of downstream creeks, rivers and coastal estuaries. Reusing tail water for irrigation of crops could help conserve ground water, thereby protecting underground aquifers from seawater contamination, and minimize offsite impacts of irrigation run-off.

Although many farms on the Central Coast have infrastructure, such as retention basins, reservoirs, and pumping plants, to capture and reuse tail water, most growers are reluctant to use tail water for irrigation of food crops due to concerns of food safety risks from microbial human pathogens such as Shiga toxin–producing *E. coli* and *Salmonella*.

Salinity and nutrients have been monitored in agricultural drainage water, but few studies have focused on the microbial ecology of this water. Most microbial studies have focused on sewage water that is recycled and has a disinfection step before the water is applied to food crops. Tail water from surface run-off typically is high in nutrients, such as nitrogen and phosphorus, has high turbidity and suspended solids, and can have significant concentrations of pesticides and microorganisms. Generic *E. coli* can be present in irrigation run-off but is infrequently correlated with the presence of foodborne pathogens.

The objectives of this project were to: 1) monitor, characterize, and quantify microbial populations in run-off water from Central Coast vegetable fields; and 2) evaluate the risk of using this water source for the production of lettuce and other leafy green crops by quantifying survival of microorganisms during simulated reuse applications.

Note: The approved project initially had three objectives but was amended to focus on objectives 1 and 2 due to a reduced level of matching funds available.

Research Methods and Results

Methods:

1. Characterize chemical and physical and microbiological composition of tail water from coastal vegetable fields

Sample collection

Five farms located in the Salinas Valley that represented the Central Coast vegetable production were selected for sampling tail and irrigation water. Six tail-water reservoirs were sampled monthly from March 2016 to 2017. Tail water was collected in sterile Nalgene 1-L bottles attached to a telescoping pole, as described in EPA method 1603. For each reservoir, two 1-L tail water bottles were combined for further analysis (total of 82 samples). Three Moore swabs were placed per reservoir and left for 3 days before being placed in 55-oz Whirl-Pak filter bags (total of 229 samples). During irrigation season, an automated peristaltic pump was used to sample water from the sprinkler pipe into a 1-L bottle and a bottle containing a Moore swab (total of 26 water and 26 Moore swabs). All samples were stored immediately after collection at 4°C until processing. Analyses were conducted less than 8 h after collection for *E. coli* and coliform enumeration and after 24 h for aerobic plate counts and pathogen detection.

Quantification of generic *E. coli* and coliforms

Samples were homogenized by vigorous shaking, as described in EPA method 1603. Water (100 ml) was filtered onto 0.45-µm disposable analytical filters (Nalgene, Rochester, NY). The membrane was removed and placed onto CHROMagar ECC plates (CHROMagar, Paris, France). In addition, 250 µl of water was plated in duplicate with an Autoplate 4000 (Advanced Instruments, Norwood, MA) on CHROMagar ECC. Plates were incubated at 37°C for 24 h. Colonies were counted using a ProtoCOL 2 automated plate counter running ProtoCOL 3 v.1.0.22.0 software (Synbiosis, Frederick, MD).

Total aerobic plate count

Fifty microliters of water were plated in duplicate on plate count agar (PCA) (BD Diagnostics, Franklin Lakes, NJ) using an Autoplate 4000. The plates were incubated for 24 h at 37°C, and colonies were counted using the ProtoCOL 2 automated plate counter.

Moore swab enrichment
(a) Primary enrichment:
Moore swabs were incubated in 250 ml of tryptic soy broth (TSB; EMD Millipore, Billerica, MA) for 2 h at 20°C with 200 rpm shaking, followed by incubation at 42°C for 8 h and 4°C for 12 h. Five 1-ml aliquots and one 50-ml aliquot of the enrichment broth were frozen in 15% glycerol at −80°C for 6 months before being used in the following processes.
(b) Secondary enrichment for detection of STEC or *Salmonella*:
100 µl of the primary enrichment was inoculated in 10 ml of Modified Buffered Peptone Water with pyruvate (mBPWp) (Neogen, Lansing, MI) supplemented with acriflavin (10 mg/L), cefsulodin (10 mg/L) and vancomycin (8 mg/L) for detection of STEC, or in 10 ml of Rappaport-Vassiliadis R10 broth (RV) (BD Diagnostics), and incubated at 42°C for 24 h before being analyzed by real-time PCR.

Detection of *Salmonella* and Shiga toxin–producing *E. coli* by real-time PCR
Aliquots (1 ml) of primary enrichment broth or secondary enrichment broth were centrifuged at 20,800 ×*g* for 2 min to pellet the cells. After discarding the supernatant, the cell pellet was suspended in 1 mL of 0.1% peptone water and centrifuged at 20,800 ×*g* to remove the enrichment medium. The cell pellet was finally suspended in 1 ml of MilliQ water and boiled for 10 min to lyse the cells. The lysate was centrifuged at 20,800 ×*g* for 2 min, and 100 µl of the supernatant was saved for use in real-time PCR.

Salmonella and Shiga toxin–producing *E. coli* were detected with a TaqMan assay designed to amplify *invA* for *Salmonella* and Shiga toxin *stx* genes for *E. coli*. Each TaqMan reaction (20 µl) contained 1 µl of DNA lysate, 0.9 µM of reverse and forward primers, 0.25 µM of probe, and 10 µl Premix Ex Taq Master Mix (TaKaRa Bio USA, Mountain View, CA). *invA* gene was amplified with SalINV forward and reverse primers and probe (**Table 1** – see Appendices), while *stx* genes were amplified with a multiplex reaction that included all four sets of *stx* primers and probes (Table 1). Amplification was conducted in a 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA) with one denaturation step at 95°C for 30 s followed by 40 cycles of 95°C for 3 s and annealing at 60°C for 30 s. Enrichment samples were identified as positive for the gene of interest when the cycle threshold (Ct) was lower than 38.

Isolation and culture of STEC and *Salmonella*
Aliquots of *stx* or *invA* positive water samples were thawed from storage at −80°C to retrieve potential STEC and *Salmonella* cells using 20 µl of magnetic beads conjugated with anti-O157 or *Salmonella* antibodies (Invitrogen/Dynal, Carlsbad, CA). The manufacturer-developed protocol was followed, and a Dynal BeadRetriever (Invitrogen/Dynal) was used to automate the IMS. The Dynabeads were suspended before spreading 25 µl onto CHROMagar O157 or CHROMagar STEC (CHROMagar, Paris, France) and Rainbow Agar O157 (Biolog, Hayward, CA) supplemented with novobiocin (Sigma-Aldrich, St. Louis, MO) (20 µg/ml) and tellurite (Sigma-Aldrich) (0.8 µg/ml) (NT-RA) to retrieve STEC or CHROMagar Salmonella plus (CHROMagar) to retrieve *Salmonella*. The agar plates with IMS beads were incubated for 24 h at 37°C. Colonies that produced a pure culture of colored colonies on one or both agars were tested for the presence of *stx* or *invA* genes by using the previously described TaqMan assay.

Salmonella and *E. coli* serogrouping
Salmonella serotyping was done by the California Animal Health & Food Safety Laboratory (http://www.cahfs.ucdavis.edu/). *E. coli* O serogrouping was done by PCR with primer sets that identified O26, O45, O103, O111, O121, O145 and O157. PCR reaction (25 µl) contained 2.5 µl 10X KAPA Taq Buffer A, 0.3 mM dNTP mix, 0.5 µM forward and reverse primers (**Table 2**), 0.8 U KAPA Taq DNA polymerase (Kapa Biosystems, MA) and 10 ng of purified genomic DNA. The thermocycling conditions were as follows: an initial denaturation at 95°C for 3 min, 25 cycles of

94°C for 30s, 58°C for 30 s, and 72°C for 1 min, followed by a final extension step at 72°C for 1 min. PCR products were analyzed in 1.5% agarose gels and 0.5 X Tris-borate-EDTA. DNA from known serogroups of O26, O45, O103, O111, O121, O145 and O157 were included as a positive control.

Data analyses

Statistical analyses were conducted with JMP Pro 12 on log-transformed data for *E. coli*, total coliforms and heterotrophic plate counts. Site and time effects on the *E. coli*, coliforms and heterotroph concentrations were assessed by Wilcoxon signed rank test and by analysis of variance (ANOVA) followed by a multiple comparison with a Tukey test for normally distributed data. Pearson's correlation coefficients were calculated to evaluate the correlations between chemical factors and *E. coli* and coliform concentrations and aerobic plate counts. Results with *P* values <0.05 were considered significant.

2. Evaluate risks of using tail water in lettuce and leafy green production by quantifying microbial survival during simulated application to soils used and lettuce

Inoculum preparation

Five *Salmonella* strains, five *E. coli* O157:H7 and four surrogate generic *E. coli* strains were mixed in a cocktail to inoculate the water (**Table 3**). For each strain, 20 µl of an overnight bacterial liquid culture was spread plated with an automated spiral plater (Autoplate 4000, Spiral Biotech Inc., Boston, MA) onto tryptic soy agar supplemented with rifampicin 50 µg/ml (TSAR) and incubated at 37°C for 12 h to produce a bacterial lawn. Cells were dislodged with 5 ml of 0.1% peptone, and strains were combined in equal volume to create the inoculum cocktail. The cocktails were diluted in tail water, irrigation water and MilliQ water to the desired concentration.

Tail water inoculation and sampling

Tail water was collected from all reservoirs on 10/3/2016 and 11/7/2016, stored on ice, and shipped for processing within 24–48 h. A 1-L tail water bottle was inoculated with *E. coli* O157:H7 or *Salmonella* cocktail to an approximate concentration of 10^5 CFU/100 ml and held at 19°C for 7 days. After shaking the sample vigorously in a 90° arc for at least 25 times, 50-µl or 250-µl samples were plated in duplicate onto TSAR plates with an automated spiral plater. When the pathogen level reached the limit of detection by plating, 100 ml of water was filtered onto disposable 0.45-µM analytical filters and the membranes were placed onto plates of CHROMagar O157 supplemented with rifampicin 50 mg/L to enumerate *E. coli* O157:H7 or on plates of Salmonella Chromogenic agar base (Oxoid) supplemented with rifampicin 50 mg/L to enumerate *Salmonella.* All plates were incubated at 37°C for 24 h.

Soil inoculation and sampling

Tail and irrigation water samples were collected from farm C, and stored on ice for 6 h before being aliquoted into 1-L bottles and frozen at −20°C. When necessary, water was thawed for 24 h at room temperature prior to inoculation. One liter of tail or irrigation or MilliQ water was inoculated at a concentration of 10^6 CFU/100 ml (high). Soil collected from farm C was sieved through 0.17 mm mesh. Water (600 ml of tail, irrigation, or MilliQ) inoculated at 10^6 CFU/100 ml was added per 2 kg of soil in a 12 ×12 bag to reach 100% saturation or 30% water holding capacity (WHC). The inoculated soil was mixed thoroughly and distributed in four plastic containers (500 g/container). Twenty seeds of Sure Shot iceberg lettuce (Seminis) were planted in two out of four containers. Containers were placed in the growth chamber with humidity and temperature similar to those measured in spring in the Salinas Valley (**Figure 1**). Soil moisture level was monitored by measuring the decrease in container weight during the first days after inoculation and then with a moisture analyzer (HG63, Mettler Toledo). MilliQ water was added every 2 or 3 days to maintain an average soil water saturation of 62%. Soil (1.3 g or 1 g dry

weight) was sampled at day 0, 2, 7, 14, 21, and 28, and then added to 10 ml of Butterfield phosphate buffer (BPB). After vortexing for 1 min and resting for 30 min, 50-µl and 250-µl samples were plated on TSAR and incubated at 37°C overnight.

Plant inoculation and sampling
Two-month-old iceberg lettuce plants (Sure shot, Seminis) were transferred from the campus facility environmental chamber into the laboratory growth chamber with humidity and temperature similar to those measured in spring in the Salinas Valley (**Figure 1**). Individual leaves were inoculated with 10 × 2-µl drops of 6 log CFU/ml cocktail inoculum serial diluted in tail, irrigation, or MilliQ water, and then sampled at day 0, 1, 2, 3, and 7. Plants inoculated with the same type of organism and water were grouped in one container to avoid cross-contamination. For sampling, inoculated leaves were separated from the plants by hand and transferred into a 24-oz Whirl-Pak filter bag with 50 ml of 0.1 % peptone water per bag. Bacteria were recovered by pummeling the leaves in a Smasher for 1 min at fast speed. Aliquots of 250 µl were plated on TSAR for enumeration. The remaining samples were also filtered onto disposable 0.45-µM analytical filters, and the membranes were placed onto plates of CHROMagar O157 with rifampicin 50 mg/L to enumerate *E. coli* O157:H7, or plates of Salmonella Chromogenic agar base (Oxoid) with rifampicin 50 mg/L to enumerate *Salmonella*, or plates of CHROMagar ECC with rifampicin 50 mg/L to enumerate *E. coli*. If enumeration by filtering reached 0 CFU, leaves were enriched in 50 mL of TSBR for 24 h at 37°C. Enrichment broth was plated on the appropriate selective media to confirm the presence of *Salmonella*, *E. coli* or *E. coli* O157:H7.

Nutrients effects on survival of generic E. coli in tail and well water
An incubation study was conducted to evaluate if high nutrient levels in tail and well water influenced the survival of generic *E. coli*. Samples of tail and well water were collected from one of the Salinas Valley monitoring sites in October 2017. Either sodium nitrate (100 ppm NO_3-N) or sodium phosphate (30 ppm ortho-P), or a combination of both were added to the tail and well water samples. Water samples were inoculated with a native rifampicin-resistant strain of generic *E. coli* at a concentration of log 5.5 CFU/100 ml and incubated at 16°C for 6 weeks. Subsamples were evaluated for *E. coli* concentration by plating on selective media 0, 2, 7, 15, 21, and 42 days after inoculation.

Results:

1. *Characterize chemical and physical and microbiological composition of tail water from coastal vegetable fields*

Aerobic plate counts were not significantly different between tail and irrigation water (**Figure 2**). and varied between 2.43 to 5.74 with an average of 3.78 log CFU/ml in the tail water samples and between 1.00 to 5.98 with an average of 3.86 log CFU/ml in the irrigation water samples. Coliforms were detected at all time points in tail water collected from all the sites, with concentrations that varied between 0.78 to 5.30 and an average of 3.90 log CFU/100 ml. In irrigation water, coliform concentrations ranged from 0 to 4.94 log CFU/100 ml, with an average of 3.52 log CFU/100 ml. Coliform concentration in tail water was significantly lower at site A and higher at site D2 than at all the other sites (Figure 2).

E. coli concentration was significantly higher at site D2 than at the other sites (**Figure 3**), and all the samples tested positive. *E. coli* was not detected in 42%, 38%, 31%, 23%, and 8% of the samples collected at site E, A, C, D1, and B, respectively. The *E. coli* geometric mean remained below 126 CFU/100 ml for all sites except D2 (1,016 CFU/100 ml). Single samples collected at site B, C, and D1 were equal or below 235 CFU/100 ml and met the GPAS metric

for water applied to edible plants. Single samples collected at site A and E were below or equal to 576 CFU/100 ml.

Salmonella could not be recovered from primary enrichment, but four out of 229 Moore swab secondary enrichment samples tested positive by real-time PCR (**Table 4**). *Salmonella* was retrieved from samples collected in reservoir D2 in August, September and October 2016 and in reservoir B in October 2016. *E. coli* O45 was retrieved from one primary enrichment in reservoir B in June 2016. After a secondary enrichment of the 229 Moore swabs, three different STEC were retrieved: two O145 from reservoir B in June and July 2016 and one O157 from reservoir D2 in February 2017.

Seasonal variation had no influence on the microbiological composition of the tail water. The major factor that influenced *E. coli* concentration in tail water was the location of the reservoir. Physicochemical water characteristics were measured at each sampling time to evaluate a possible relationship with the microbial composition; data are shown in **Table 5**. For all reservoirs sampled, the tail water had a basic pH and low turbidity, with a range of 6 to 212 mg/L of suspended solids. Salinity varied according to the geographical location, with a higher electrical conductivity for the reservoirs located in the south of the Salinas Valley (D1, D2, E) than in the north (A, B, C). The concentration of soluble phosphate (PO_4^-) was correlated with the concentration of generic *E. coli* in tail-water reservoirs.

2. Evaluate risks of using tail water in lettuce and leafy green production by quantifying microbial survival during simulated application to soils used and lettuce.

To evaluate the effect of different levels of nitrate and phosphate on the survival of *Salmonella* and *E. coli* O157:H7, tail water collected from all the reservoirs was inoculated with a cocktail of 5 log CFU/100 ml of *Salmonella* and *E. coli* O157:H7 and held for 7 days at 19°C or the average temperature recorded in the tail-water reservoirs between March and October 2016. Both *Salmonella* and *E. coli* O157:H7 populations declined in tail water over 7 days (**Figure 4**). The slowest rate of decline was recorded in tail water collected from reservoir E and the highest rate of decline was recorded in tail water collected from reservoir A (Figure 4). Concentrations of phosphate and nitrate were lowest in water collected from reservoir A and E. Although water from reservoir D2 had the highest level of phosphate and from reservoir D1 had the highest level of nitrate, *Salmonella* and *E. coli* O157 populations in D1 and D2 water were lower after 7 days than in E water.

The incubation experiment with tail and well water collected from site C also showed that the addition of nutrients (N and P) had no significant effect on the survival of generic *E. coli*. Generic *E. coli* populations declined rapidly in tail water during the first week after inoculation (**Figure 5**). In contrast, generic *E. coli* populations declined more slowly in inoculated well water, presumably due to less competition from other microbial species.

In soil, *Salmonella*, generic *E. coli* and *E. coli* O157:H7 had the same rate of decline regardless of the water carrier (MilliQ, irrigation or tail water) used for inoculation (**Figure 6**). Seeds, planted in the contaminated soil, germinated to reach cotyledon stage at day 28, with an extremely low rate of germination (0 to 1%). Cotyledons were collected and pooled from each pot separately without touching the soil. The inoculated pathogen could not be enumerated in any of the cotyledon samples and was rarely detected by enrichment.

E. coli, *E. coli* O157:H7 and *Salmonella* populations declined more rapidly on the plant surface than in soil, and with higher variability at each sampling time point (**Figure 7**). When the inoculated bacteria were suspended in MilliQ water, they survived slightly longer on the plant surface than those suspended in the other water sources, with an average decline of 2 log CFU/leaf after 7 days; survival for bacteria suspended in tail and irrigation water was identical, with an average decline of 3 log CFU/leaf per 7 days.

Outcomes and Accomplishments

- The physical, chemical, and microbial properties of tail water from five representative Salinas Valley farms were characterized during an entire year.
- Using Moore swabs the presence of pathogenic *E. coli* (STEC, O157:H7) and *Salmonella* was evaluated in 229 samples from tail-water reservoirs and 26 samples from operating sprinkler systems.
- Incubation studies were conducted to evaluate if tail water enhances the survival of *E. coli* and *Salmonella* compared with well water.
- Incubation studies were conducted to evaluate if elevated concentrations of phosphate and nitrate prolong the survival of *E. coli* in tail water.
- A growth chamber study was conducted to evaluate if tail water prolongs the survival of pathogenic *E. coli* and *Salmonella* in the soil and on lettuce leaves.

Summary of Findings and Recommendations

- *E. coli* concentration was low in five out of six tail-water reservoirs, with a geometric mean that remained below 126 CFU/100 ml.
- Prevalence of *Salmonella* (1.8%), STEC (1.8%) and *E. coli* O157 (0.4%) was lower than previously described for the Central Coast watershed (Cooley et al., 2014) or California reservoirs (Partyka et al., 2018).
- Elevated concentrations of phosphate and nitrate did not increase the survival of *Salmonella*, generic *E. coli* or *E. coli* O157:H7 in water.
- Tail water did not increase the survival of *Salmonella* or *E. coli* O157:H7 in soil or on growing lettuce plants compared with well water.
- Tail water that meets CLGMA metrics for application to edible crops could be used for pre-irrigation and germination of leafy green vegetables and would not be expected to increase food safety risks more than irrigating with well water.

References cited:

Cooley M., Quinones B., Oryang D., Mandrell R.E. and Gorski L. 2014. Prevalence of Shiga toxin producing *Escherichia coli*, *Salmonella enterica* and *Listeria monocytogenes* at public access watershed sites in a California Central Coast agricultural region. Front. Cell. Infect. Microbiol. 4:30. doi: 10.3389/fcimb.2014.00030

Partyka M., Bond R., Chase J. and Atwill R. 2018. Spatial and temporal variability of bacterial indicators and pathogens in six California reservoirs during extreme drought. Water Res. 129:436–446

APPENDICES

Publications and Presentations

Presentations:

Cahn M., Harris L., Moyne A., Koike S., Murphy L. (2016) Microbial food safety risks of reusing tail water for leafy green production. CPS Research Symposium, June 28-29, Seattle, WA.

Cahn M., Harris L., Moyne A., Koike S., Murphy L. (2017) Microbial food safety risks of reusing tail water for leafy green production. CPS Research Symposium, June 20-21, Denver, CO.

Moyne A.L., Murphy L.A., Cahn M.D., Koike S.T. and Harris L.J. (2017) Microbial quality of tail water in the California Central Coast Salinas Valley. Abstract P3-230. Annual International Association for Food Protection (IAFP) meeting, July 9-12, Tampa, FL.

Budget Summary

All funds allocated to this project will be utilized.

Tables and Figures

Table 1: Primers and probes used in real-time PCR to detect *Salmonella* or and Shiga toxin–producing *E. coli* (STEC).

Name	Sequence (5'-3')	Organism detected
SalINV Forward	AACGTGTTTCCGTGCGTAAT	*Salmonella*
SalINV Reverse	TCCATCAAATTAGCGGAGGC	*Salmonella*
SalINVA Probe	6-FAM/TGGAAGCGCTCGCATTGTGG/8-QSY7	*Salmonella*
Stx1Forward	CATCGCGAGTTGCCAGAAT	STEC
Stx1 Reverse	TCCCACGGACTCTTCCATCT	STEC
Stx1 Probe	56-JOEN/ATCTGATGA/ZAN/TTT/CCTTCTATGTGTCCG/3IBkFQ	STEC
Stx2abc Forward	GGACCACATCGGTGTCTGTTATT	STEC
Stx2abc Reverse	CCCTCGTATATCCACAGCAAAAT	STEC
Stx2abc Probe	56-FAM/CCACACCCC/ZEN/ACCGGCAGT/3IABkFQ	STEC
Stx2f Forward	CGCTGTCTGAGGCATCTC	STEC
Stx2f Reverse	TCCTCTGTACTCTGGAAGAACATTAC	STEC
Stx2f Probe	5TexRd-XN/TTATACAATGACGGCTCAGGATGTTGACCTTACC/3IAbRQSp	STEC
Stx2ex Forward	GAAACTGCTCCTGTTTATACGATGAC	STEC
Stx2ex Reverse	CCGGAAGcACATTGCTGAT	STEC
Stx2ex Probe	5Cy3/CCCCCAGTTCAGAGTGAGGTCCACG/3IAbRQSp	STEC

Table 2: Primers used for serogrouping.

O-genotype	Name	Sequence (5'-3')
O 26	Og26-F	GGGGGTGGGTACTATATTGG
	Og26-R	AGCGCCTATTTCAGCAAAGA
O 45	Og45-F	GTCCCCAGGGTTTGTGTATG
	Og45-R	AATAAGGGAGCCCGCGAT
O 103	Og103-F	TAAGTACGGGGGTGCTTTTT
	Og103-R	AAGCTCCCGAGCACGTATAA
O 111	Og111-F	CAAGAGTGCTCTGGGCTTCT
	Og111-R	AACGCAAGACAAGGCAAAAC
O 121	Og121-F	CAAATGGGCGTTAATACAGCC
	Og121-R	TTCCACCCATCCAACCTCTAA
O 145	Og145-F	TTCGCGCACAGCATGGTTAT
	Og145-R	TACAATGCACCGCAAACAGT
O 157	Og157-F	CAGGTGAAGGTGGAATGGTTGTC
	Og157-R	TTAGAATTGAGACCATCCAATAAG

Table 3: Generic *E. coli*, *E. coli* O157:H7 and *Salmonella* rifampicin-resistant strains used for inoculation.

LJH#	Strain designation	Parent	Origin
614	*Salmonella* Montevideo	519 or G4639	Patient from 1993 tomato outbreak
636	*Salmonella* PT30	608	Raw almonds
1219	*Salmonella* Agona	517	Alfalfa sprout
1260	*Salmonella* Newport	MDD114	Tomato outbreak 2008
1261	*Salmonella* Saintpaul	1210	Clinical, tomato jalapeno outbreak 2008
1153	*Escherichia coli* O157:H7	537 or H1730	Clinical, lettuce outbreak
1215	*Escherichia coli* O157:H7	F4546 or 538	Clinical, sprout outbreak
1256	*Escherichia coli* O157:H7	EC4045 or 1187	Spinach plant, spinach outbreak
1258	*Escherichia coli* O157:H7	EC4191 or 1188	Spinach plant, spinach outbreak
1382	*Escherichia coli* O157:H7	NML# 11-1865	Human, walnut-associated outbreak, Ontario
1247	*Escherichia coli*	TVS 354	Lettuce (Salinas Valley, CA)
1612	*Escherichia coli*	TVS 353	Irrigation water (Salinas Valley, CA)
1613	*Escherichia coli*	TVS 355	Sandy loam soil (Salinas Valley, CA)
1614	*Escherichia coli*		River in Hawaii

Table 4: Prevalence of *Salmonella* and STEC in tail and irrigation water.

Sampling source	Number of samples with recovered pathogen/ Total number of samples	
	Salmonella	STEC
Tail-water reservoir	4/229	4/229
Irrigation water	0/26	0/26

Table 5: Chemical characteristics of tail water collected in each reservoir at different sites.

	Site[a]					
	A	**B**	**C**	**D1**	**D2**	**E**
pH	8.89 ± 0.38^{A}	8.80 ± 0.33^{A,B}	8.56 ± 0.40^{A,B}	8.59 ± 0.31^{A,B}	8.37 ± 0.64^{A,B}	8.35 ± 0.27^{B}
EC µS cm^{-1}	0.71 ± 0.21^{B}	1.04 ± 0.33^{B}	0.70 ± 0.27^{B}	3.00 ± 1.07^{A}	2.92 ± 1.15^{A}	3.54 ± 0.52^{A}
SAR	2.69 ± 0.37^{C}	2.12 ± 0.29^{C}	0.89 ± 0.29^{D}	4.15 ± 1.01^{A,B}	4.96 ± 1.41^{A}	3.98 ± 0.43^{B}
Ca^{2+} meq/L	1.73 ± 0.75^{D}	3.50 ± 1.29^{D}	3.15 ± 1.38^{D}	11.05 ± 4.45^{B}	6.87 ± 2.68^{C}	14.01 ± 2.10^{A}
Mg^{2+} meq/L	1.39 ± 0.46^{C}	2.38 ± 0.85^{C}	2.04 ± 0.87^{C}	7.44 ± 3.03^{B}	8.68 ± 4.22^{A}	9.23 ± 1.17^{A,B}
Na^{+} meq/L	3.29 ± 0.90^{B}	3.61 ± 0.92^{B}	1.48 ± 0.68^{B}	12.90 ± 5.31^{A}	14.30 ± 6.67^{A}	13.58 ± 2.18^{A}
Cl^{-} meq/L	2.32 ± 0.60^{B}	2.94 ± 0.76^{B}	1.07 ± 0.53^{B}	12.82 ± 5.42^{A}	13.41 ± 6.08^{A}	16.34 ± 2.13^{A}
B^{3+} meq/L	0.11 ± 0.03^{B}	0.05 ± 0.01^{B}	0.04 ± 0.01^{B}	1.28 ± 0.52^{A}	1.10 ± 0.42^{A}	1.50 ± 0.18^{A}
HCO_3^{-} meq/L	1.69 ± 0.48^{A}	1.81 ± 0.55^{A}	1.82 ± 0.85^{A}	1.59 ± 0.66^{A}	1.84 ± 0.72^{A}	1.89 ± 0.82^{A}
CO_3^{2-} meq/L	0.35 ± 0.25^{A}	0.32 ± 0.16^{A}	0.17 ± 0.11^{A}	0.24 ± 0.15^{A}	0.34 ± 0.23^{A}	0.23 ± 0.11^{A}
TOC mg/L	15.44 ± 12.69^{B}	10.89 ± 5.24^{B}	11.49 ± 3.30^{B}	13.41 ± 7.17^{B}	29.19 ± 14.54^{A}	7.88 ± 3.19^{B}
Total N mg/L	20.02 ± 9.51^{C}	50.73 ± 26.65^{B}	18.36 ± 9.70^{C}	74.61 ± 31.30^{A}	14.71 ± 6.51^{C}	32.95 ± 20.03^{B}
NH_4^{+} mg/L	0.42 ± 0.58^{A}	1.19 ± 2.31^{A}	0.92 ± 1.03^{A}	0.96 ± 1.35^{A}	1.23 ± 1.98^{A}	0.96 ± 2.22^{A}
NO_3^{-} mg/L	15.52 ± 9.29^{C}	43.86 ± 24.01^{B}	14.18 ± 8.63^{C}	70.01 ± 33.66^{A}	9.21 ± 5.95^{C}	28.26 ± 16.51^{B}
PO_4^{3-} mg/L	0.35 ± 0.21^{B}	0.72 ± 0.50^{B}	0.25 ± 0.21^{B}	0.51 ± 0.43^{B}	4.69 ± 5.91^{A}	0.26 ± 0.15^{B}
Total P mg/L	0.68 ± 0.27^{B}	1.14 ± 0.38^{B}	0.57 ± 0.40^{B}	0.71 ± 0.41^{B}	7.18 ± 6.34^{A}	0.38 ± 0.14^{B}
SO_4^{-} mg/L	18.00 ± 6.17^{C}	21.85 ± 8.15^{C}	49.56 ± 22.85^{C}	181.2 ± 69.23^{B}	228.20 ± 94.42^{A,B}	257.00 ± 40.29^{A}
TSS mg/L	90.86 ± 41.74^{A}	70.21 ± 43.60^{A,B}	54.50 ± 50.20^{A,B}	39.36 ± 24.34^{B}	61.43 ± 37.14^{A,B}	35.92 ± 24.26^{B}
DOC mg/L	5.79 ± 2.56^{B}	5.26 ± 1.45^{B}	6.17 ± 2.17^{B}	7.52 ± 1.64^{B}	14.79 ± 7.06^{A}	4.90 ± 1.17^{B}

[a] Results represent the mean ± standard deviation (n=14). Different letters within the same row denote significant difference ($P < 0.05$) among the different sites.
DOC = dissolved organic carbon, EC = electrical conductivity, SAR = sodium absorption ratio,
TOC = total organic carbon, TSS = total suspended solids.

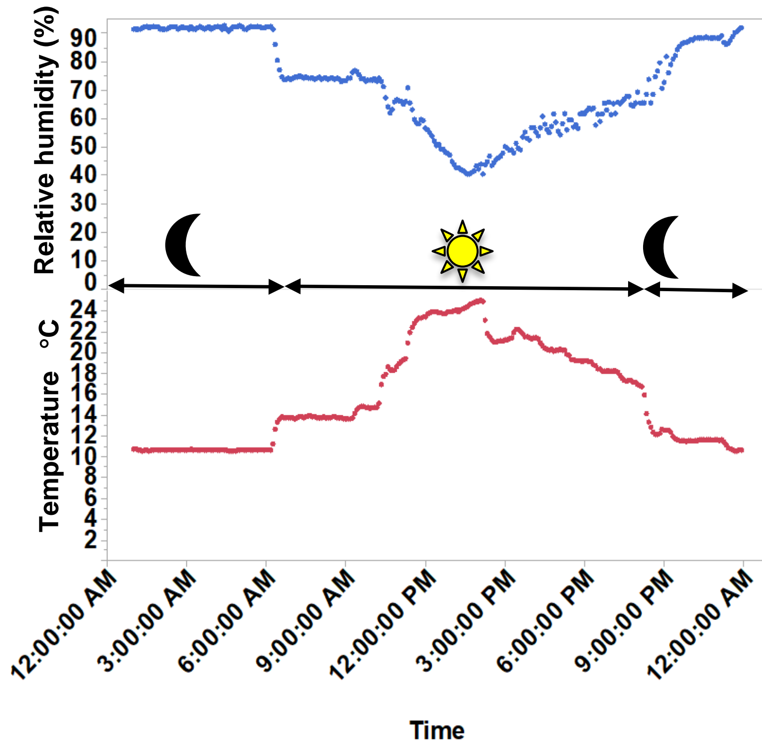

Figure 1: Daily recorded relative humidity and temperature in the growth chamber.

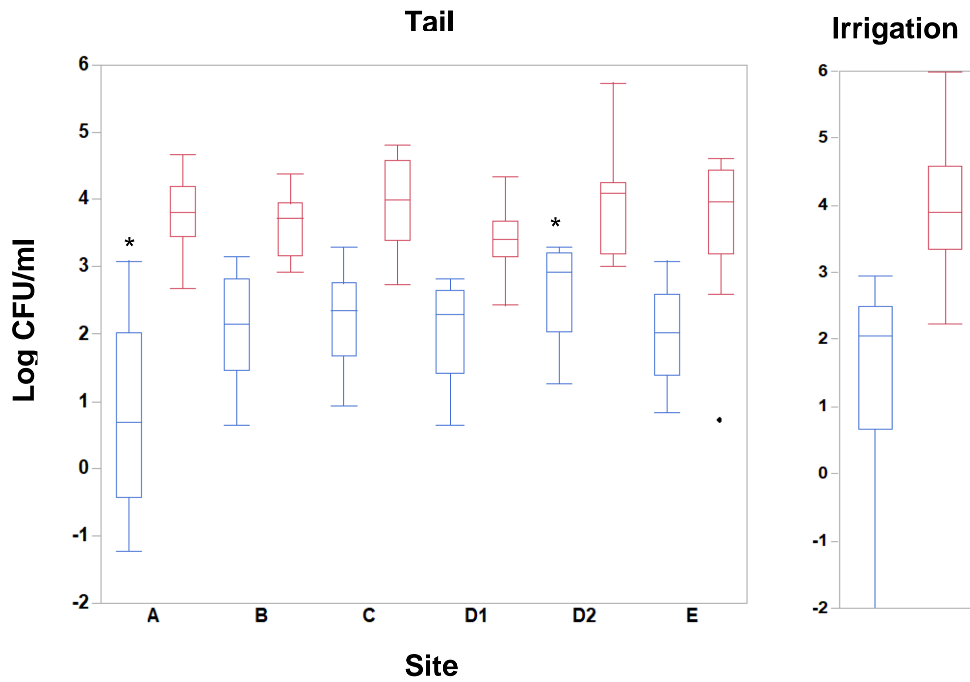

Figure 2: Concentration of coliforms (blue) and aerobic plate count (red) in tail water for each site and in irrigation water. Data are presented in a boxplot graph where the bottom and top of the box are the 25th and 75th percentile, respectively, and the end of the whiskers are the minimum and maximum of the data. A star indicates significant differences as determined with a Tukey's HSD test ($P < 0.05$).

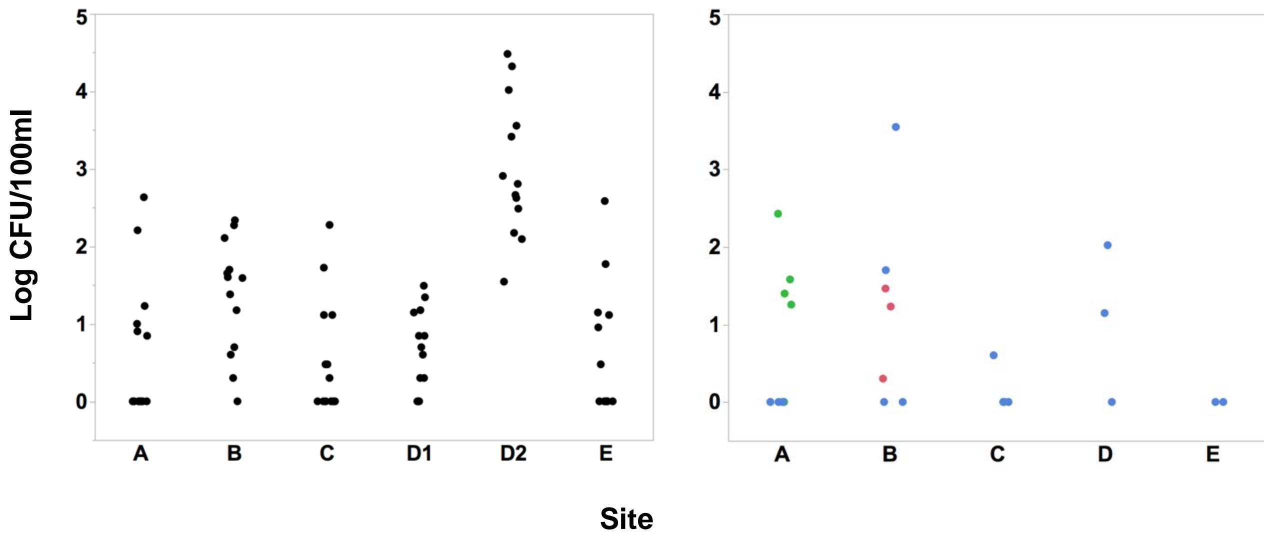

Figure 3: Generic *E. coli* concentration in water collected from reservoir or irrigation lines at each site. Water sampled from irrigation line was from well (blue dots) or tail water (green dots) or a combination of well and tail water (red dots). Samples were processed less than 8 h after collection; the limit of detection is 1 CFU/100 ml.

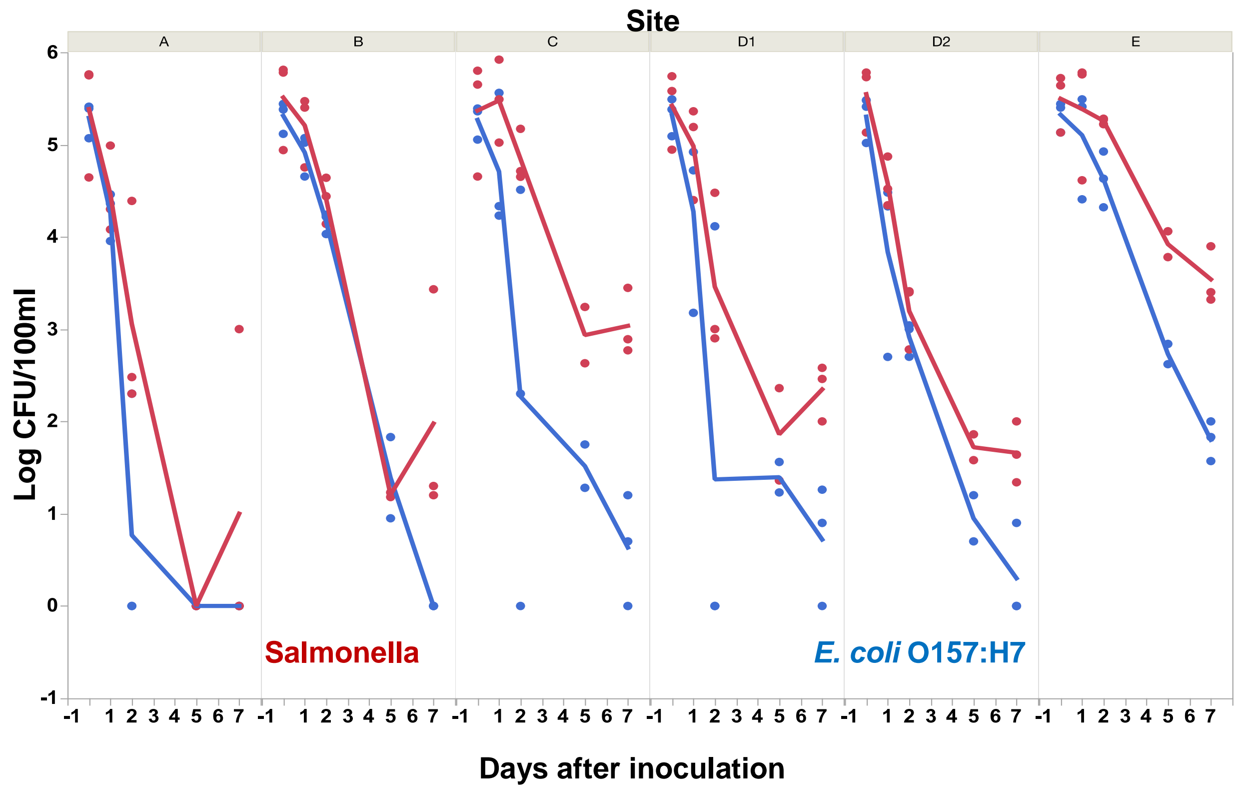

Figure 4: Survival of *Salmonella* and *E. coli* O157:H7 at 19°C for 7 days in tail water collected from reservoir A, B, C, D1, D2, and E. Each point represents one sample concentration. Means between two combined experiments (October and November, n=3) are connected by a line.

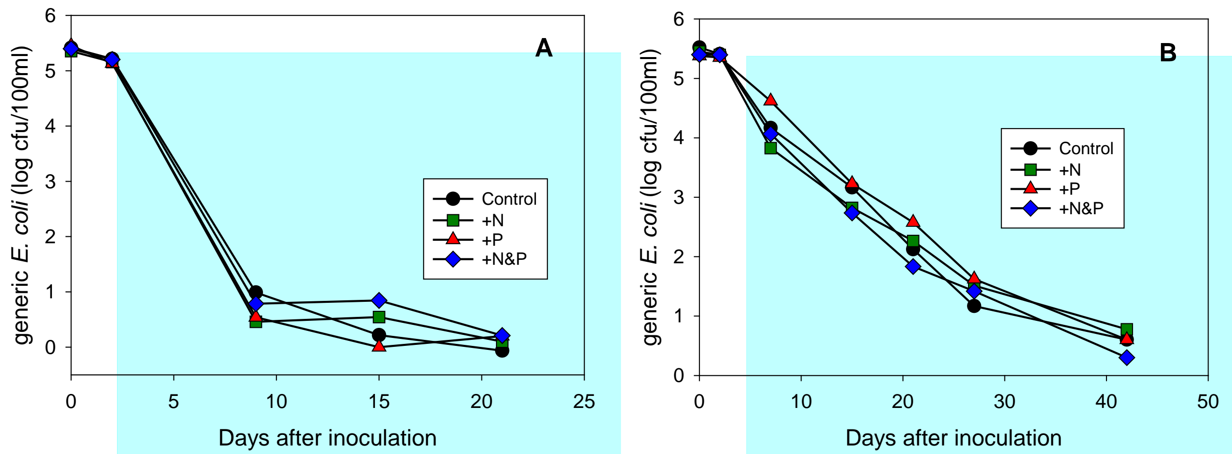

Figure 5: Effect of nutrients (N and P) on the survival of generic *E. coli* at 16°C in tail (A) and well water (B) collected from site C. Symbols represent the mean of 3 replications.

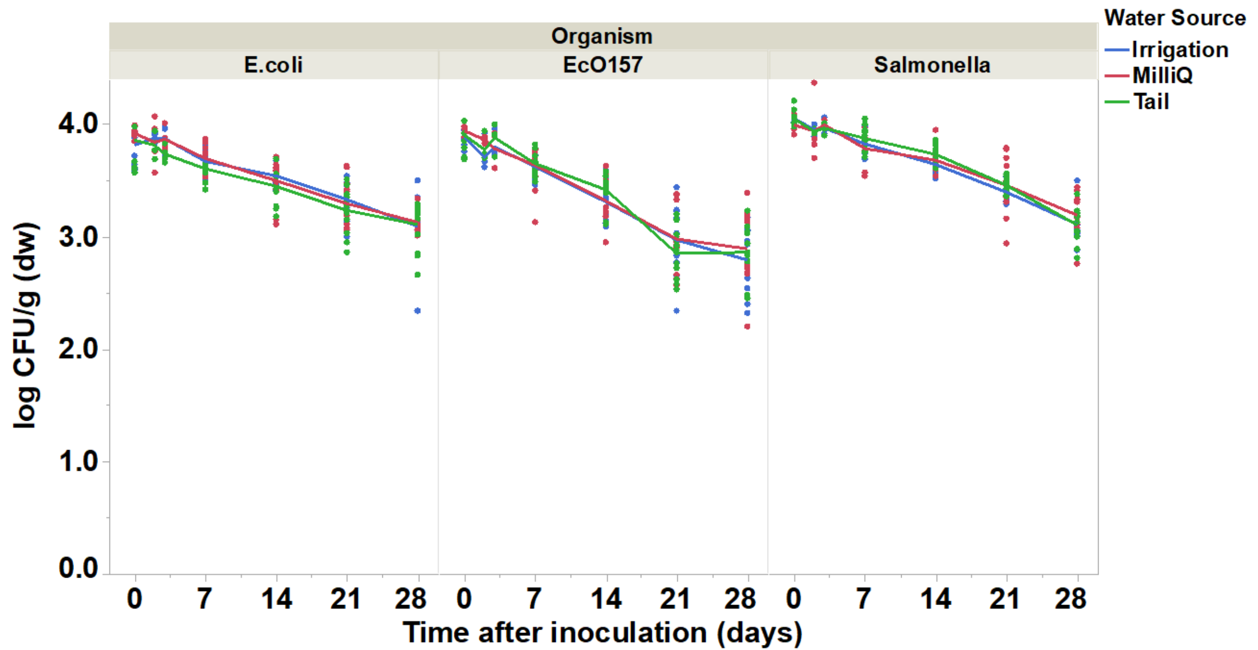

Figure 6: Survival of *Salmonella*, generic *E. coli* and *E. coli* O157:H7 in soil after inoculation with tail, irrigation or MilliQ water. Soil samples were incubated in the growth chamber with a temperature and humidity cycle similar to Salinas Valley spring weather conditions. Each point represents one sample concentration. Mean of two separate experiments (n=8) are connected by a line.

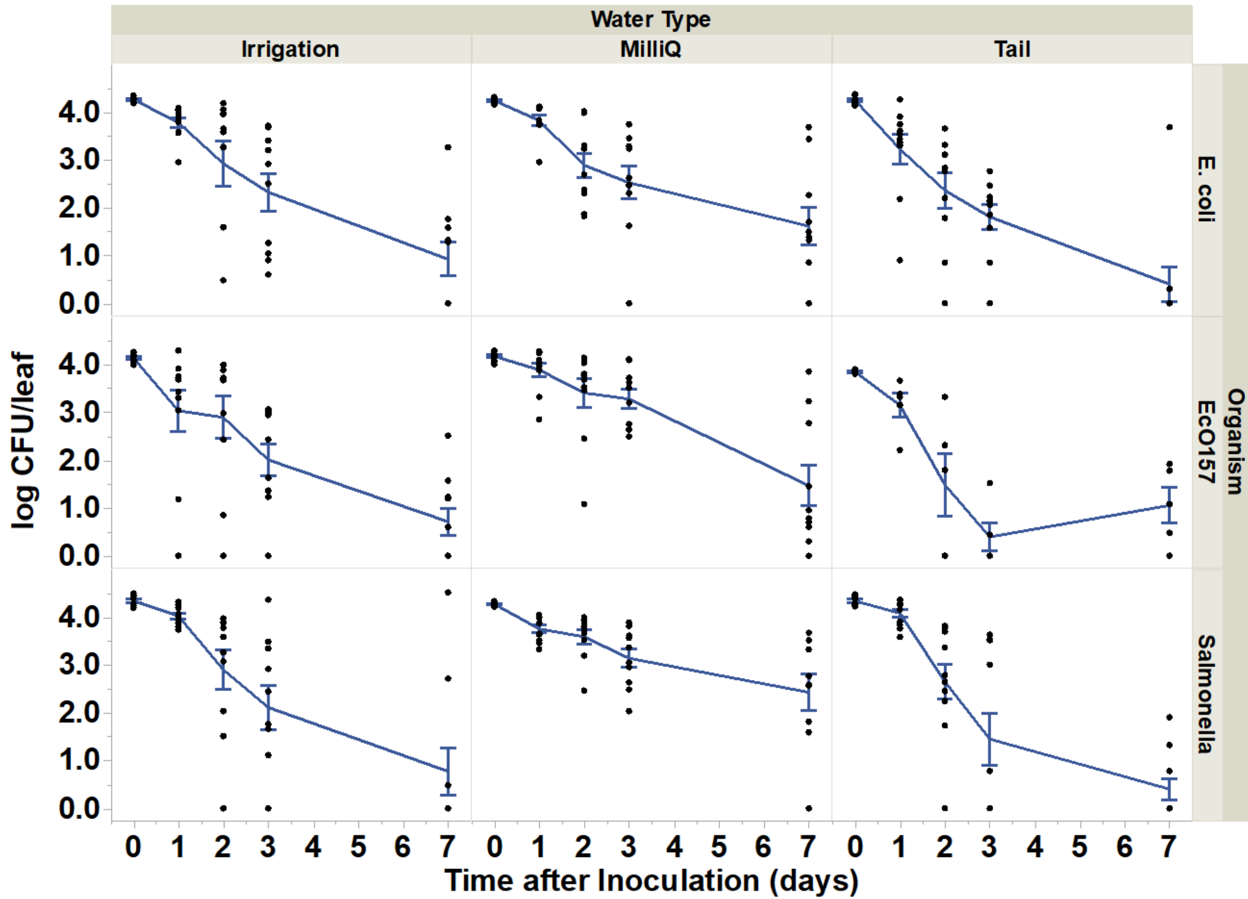

Figure 7: Survival of *Salmonella*, generic *E. coli* and *E. coli* O157:H7 on lettuce leaves after inoculation with tail, irrigation or MilliQ water. Plants were incubated in a growth chamber with a temperature and humidity cycle similar to Salinas Valley spring weather conditions. Each point represents one sample concentration. Mean of two separate experiments (n=10) are connected by a line.