CENTER for PRODUCE SAFETY

CPS 2023 Rapid Response
FINAL PROJECT REPORT

Project Title
CPS: Flood Rapid Response

Project Period
February 1, 2023 – April 30, 2023

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Objective
1. *To work alongside industry partners to conduct a longitudinal study on the impact of flood water(s) on the presence and persistence of microbiological indicators and pathogens on lands used to grow fresh produce.*

Funding for this project was provided through the CPS Campaign for Research.

FINAL REPORT

Abstract

Flooding poses significant challenges for crop production due to the introduction of microbiological, chemical, and physical hazards onto croplands. However, there is a lack of data on the presence and persistence of contamination post-flooding. This study aimed to address this knowledge gap by quantifying select microbiological indicators and pathogens in flooded fields over time.

The University of Arizona conducted a three-month (90 days) longitudinal study to evaluate the presence and persistence of microbiological indicators in soil samples from four different ranches. These ranches represented flood impacts from the Salinas River (Ranch S, T) or from creeks/tributaries (Ranch F, H). The study focused on three microbiological indicators: Total Coliform bacteria, Fecal Coliform bacteria, and generic *Escherichia coli*. Additionally, soil and water samples were analyzed for the presence of two pathogens: Shiga toxin–producing *E. coli* (STEC) and *Salmonella*.

Variable levels of Fecal Coliform bacteria were detected in soil samples across space and time, ranging from <1 to 1986.3 MPN/gram. The study observed log reductions ranging from -0.28 to 0.34 for Total Coliform bacteria, log reductions ranging from 0.04 to 0.80 for Fecal Coliform bacteria, and log reductions ranging from 0.00 to 0.95 for *E. coli* bacteria across all fields assessed over the 13-week study.

Comparing the presence of pathogens to indicator organisms, approximately 8% of soil samples were presumptive positive for STEC linked targets (stx + eae) using droplet digital PCR. For *Salmonella*, roughly 5% of soil samples were positive after the first flood, with 3% positive after the second flood, suggesting minimal impact of flood waters on the presence of *Salmonella* in soils. In contrast, the study recorded very few positive samples for STEC after the first flood. However, after the second flood, up to 47% of samples from all ranches combined showed presumptive-positive results for STEC in soil samples, as determined by culture.

These findings raise important questions about the prolonged flushing effect of flood waters, the force of flood waters on bacterial adhesion to soil particles, and the impact of nutrient loading on the survival of pathogens over time. Understanding these factors is crucial for developing effective strategies to mitigate microbiological contamination in flooded fields as well as appropriate metrics to ensure the safety of agricultural products.

Background

Although it is known that flooding can introduce microbiological, chemical, and physical hazards onto croplands, little data is available on the presence and persistence of contamination post-flooding over time. This rapid response project aimed to quantify select microbiological indicators and pathogens in flooded fields over time. While pathogens in the soil will usually die off rapidly over time due to drying conditions or fluctuations in temperature, floodwaters have the potential to contain large amounts of human sewage and runoff from animal production areas that could greatly impact die-off over time. Currently, LGMA recommends a waiting period of 60 days before replanting, to minimize the risk of pathogens persisting in the soil into the growing season. This waiting period can be shortened to 30 days with the inclusion of soil testing. In general, comprehensive testing for pathogens is not recommended for all flooding situations, but if there is a reason to believe that the soil is heavily contaminated with human pathogens, food crop producers may want to consider microbial testing following a flood. Depending on the flooding circumstances, pathogens of interest may include the following:

- Bacterial pathogens, such as *Salmonella*, *E. coli O157:H7*, other Shiga toxin-producing *E. coli*, and *Clostridium perfringens*
- Viral pathogens, such as hepatitis
- Parasites, such as *Cryptosporidium* and *Giardia*

Research Methods and Results

Over the course of the 90-day study, the University of Arizona worked alongside industry partners to conduct a longitudinal study on the impact of flood water(s) on the presence and persistence of microbiological indicators and pathogens on lands used to grow fresh produce.

Sample Types: The following sample types were collected in a manner to identify “hot spot” areas within production lands, including composite soil, discrete soil samples, flood/surface water grabs, and large volume ultra-filters for flood/surface waters adjacent to field(s) of concern.

Soil. Approximately 500g soil samples were collected at a depth of up to 6 inches using disposable sterile scoops, and deposited in individual sterile 24-oz Whirl-Pak bags. Soil samples were stored on ice at 4°C for up to 36 hours until transport to the laboratory. Sample locations were flagged, labeled, and GPS marked accordingly to track positive/negative result locations. Soil samples represented both discrete sample points collected in a grid-like pattern over the area of interest as well as composited samples collected from up to five locational transects representing 10 sub-samples each across identified field(s). Each field assayed was subdivided into five cross-sectional transects at the following distances from where flood waters entered or exited the field, 100 ft, 200 ft, 400 ft, 800 ft, and 1600 ft (**Figure 1**). Soil samples were shipped on ice to the University of Arizona Maricopa Agricultural Center where they were weighed and divided prior to

enrichment for select pathogens and indicators. In addition to soil microbiology, soil moisture was recorded for every sample collected. A subset of soil samples representative of each field of interest were also evaluated for heavy metals (As, Pb, Cd, Hg), soil salinity (water-soluble Ca, Mg, K and Na; SAR [sodium adsorption ratio]; ESP [exchangeable Na percentage]), and a Complete Soil Test (macronutrients including soil pH, N/P/K, micronutrients, and trace minerals including sulfur, manganese and magnesium).

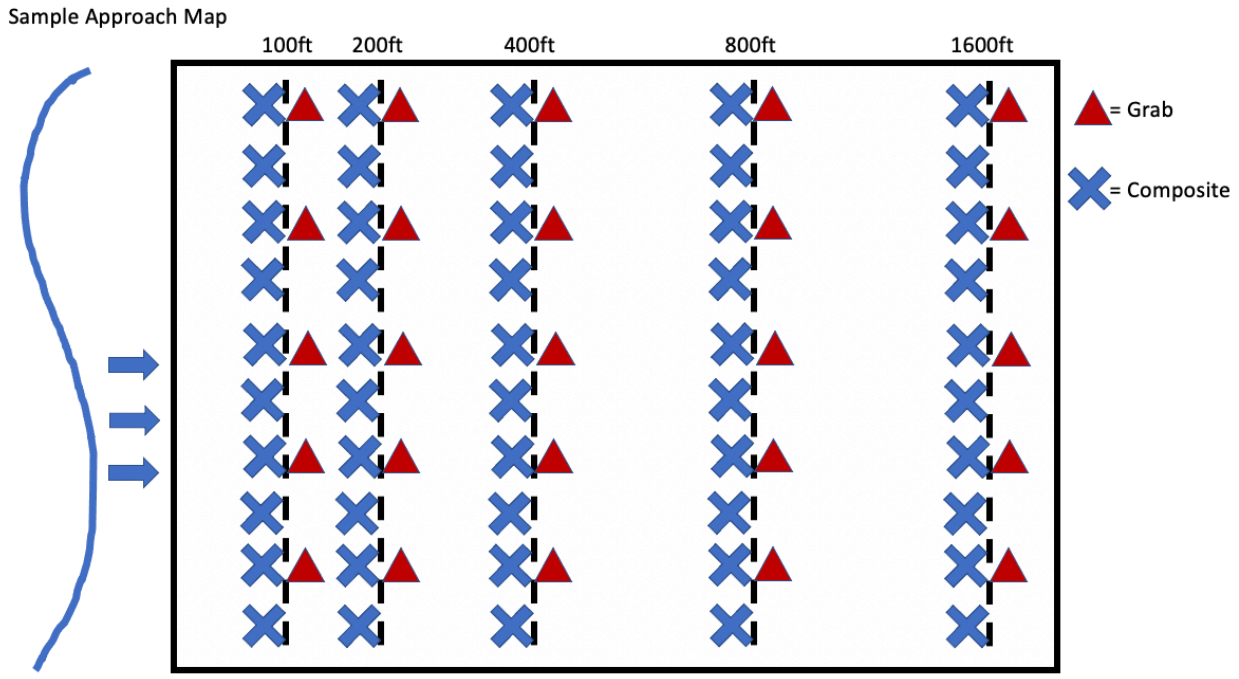

Figure 1. Sample approach map

Water. Grab water samples were collected in 1L sterile polypropylene containers and stored on ice and transported back to the lab for further analysis. Samples were analyzed for both generic *E. coli* as well as Enterococci, Total Coliform bacteria by IDEXX QuantiTray within 6 hours of collection. Appropriate dilutions were performed to achieve a countable value in MPN/100mL to avoid Too Numerous to Count (TNTC). Additionally, Fecal Coliform bacteria were quantified following Standard Methods for the Examination of Water and Wastewater (4). Grab water samples were analyzed for the following: pH, temperature, turbidity (NTU), dissolved oxygen (mg/L), and electrical conductivity (uS/cm).

Large volume hollow-fiber ultrafiltration (UF) was conducted on site in the field using the Rexeed-25S (Asahi Kasei Medical Co.). Samplers aimed to filter up to 100L, but due to increased turbidity and particulate matter, reduced sample volumes were collected. Filters were shipped to the University of Arizona Maricopa Agricultural Center within 24hrs on ice and were backflushed with a 500 mL solution of 0.5% Tween 80, 0.01% sodium polyphosphate, and 0.001% Antifoam Y-30

Emulsion. Following backflushing, filter backflush was partitioned for microbe-specific detection method or desired secondary concentration method.

Samples were processed for the enrichment of STEC or *Salmonella* as described below using a modified version of the protocol in the Bacteriological Analytical Manual (1, 2). Sample enrichment ratios for STEC are shown in **Table 1**. For *Salmonella*, samples were weighed and mixed with 2x Universal Pre-enrichment Broth. After incubation at 35°C for 18 hrs, sample aliquots were transferred for enrichment in Tetrathionate broth and incubated at 42°C for 6 hrs. Samples were then transfered to M Broth (incubated at 37°C for 18hrs) and plated on XLT4 agar (incubated at 37°C for up to 48 hrs) for colony identification selection. Recovery efficiency evaluation was conducted for each sample type/location to confirm recovery.

Table 1. Enrichment broth ratios for STEC

Ultrafilter or other liquid samples	Sediment or other solid samples
225 mL sample plus 225 mL 2x mBPWp	25 g sample plus 225 mL 1x mBPWp

While the research team focused on both indicator organisms and pathogens, we aimed to build datasets that support clarity of the current LGMA guidance of soil test results: Fecal coliforms <100 MPN/gram of total solids, negative for *Salmonella*, and negative for STEC. In addition to presence/absence data for the above-mentioned organisms, our team divided sample extracts/backflush to be analyzed by droplet digital PCR (ddPCR) to produce a quantitative value for both STEC and various SerO groups using the Bio-Rad dd-Check STEC kit. The dd-Check STEC kit uses ddPCR technology to detect double-positive linked virulence genes (*stx* and *eae*) in a single bacterium from samples containing bacteria with single-positive or unlinked virulence genes, reducing the number of false-positive STEC results. This detection and linkage verification of targets in a single bacterium enhances the accuracy of pathogenic *E. coli* testing. Pathogenic STEC is defined as a single *E. coli* bacterium carrying both the *stx* genes and an intimin-coding gene such as *eae*. One of the challenges with STEC screening using traditional PCR or ELISA-based methods is the inability to distinguish the presence of virulence factors coming from a single *E. coli* bacterium (true positive) or from multiple *E. coli* bacteria (false positive). This can lead to a high rate of presumptive positives that cannot be culturally confirmed. The sample extracts are available for future metagenomic analysis by the project team.

Sampling frequency continued every 5 to 7 days for the first 30 days and then every 2 weeks up to 90 days or until microbiological values tailed off and dropped to below the detection limit for the method. It should be noted that the second flood event shifted the sampling timeline from what

was originally proposed. Sampling was initiated the week of February 6th, and concluded the week of April 24th, 2023.

Outcomes and Accomplishments

Over the course of the 90-day study the research team conducted a total of 6 sampling campaigns to the Salinas growing region. During each sampling campaign the research team was able to assess four separate ranches that were selected for the study based on their geographic features and relation to flood water type. The research team categorized each of these ranches as follows: (1) flooding from adjacent creek, grazing operations adjacent land, (2) flooding from adjacent creek, tributary grazing, (3) flooding from Salinas River, adjacent neighbor ranch, and (4) flooding from Salinas River, grazing operations adjacent land. Ranches were located across the growing region and included the following areas: Gilroy/Holloway, Salinas, Spence, and King City. By evaluating four different ranch/flood types this allowed the research team to better understand the variability in flood source waters and if differences were identified in resulting soil pathogen and indicator data.

In total, the research team collected 440 soil samples that represent over 2,300 sample analyses for pathogens, indicators, and physical chemical parameters. Also, 22 water samples were collected to add to our understanding flood water contribution to soil quality and potential human health risks to fresh produce.

Summary of Findings and Recommendations

Over the course of the study, our team was able to calculate log reductions of Total Coliform bacteria, Fecal Coliform bacteria, and generic *E. coli* in soil samples collected in each of the four ranches evaluated as part of the longitudinal study. Log reductions across all fields assayed over the 13-week study ranged from -0.28 to 0.34 for Total Coliform bacteria, 0.04 to 0.80 for Fecal Coliform bacteria, and 0.00 to 0.95 for *E. coli* bacteria. While these reductions may seem minimal, it is important to recognize that initial bacterial concentrations were not orders of magnitude above those anticipated, thus indicating that at the start of the study concentrations had already declined to relatively “low” values, or that that the impact of flooding on indicators was not as detrimental as originally thought.

The following figures (**Figures 2–5**) show the average concentrations of each of the three indicator organisms evaluated on each sampling date across the four fields sampled. Fields “T” and “S” are indicative of Salinas River flooding while fields “F” and “H” are representative of tributary/creek flooding from adjacent lands. The date of January 15th, 2023 on each of the graphs below identifies the date of initial flood water receeding from each ranch evaluated.

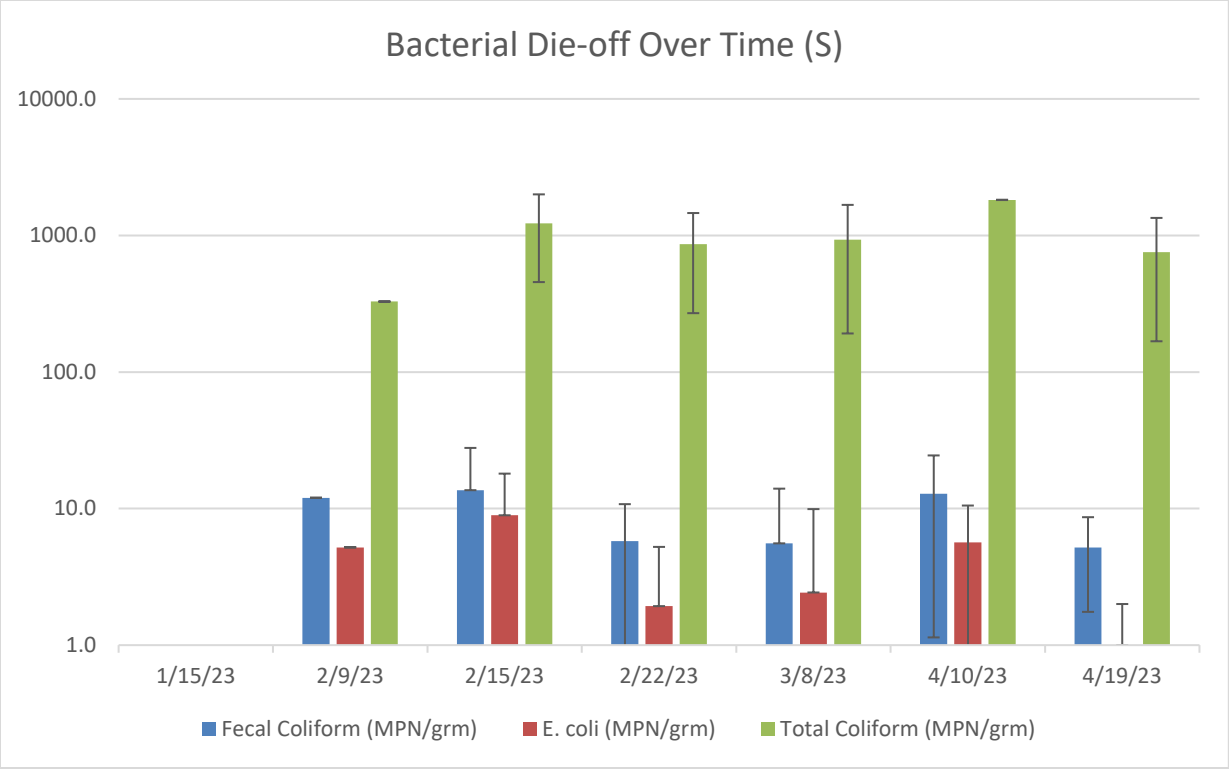

Figure 2. Bacterial die-off over time at Ranch S impacted by the Salinas River.

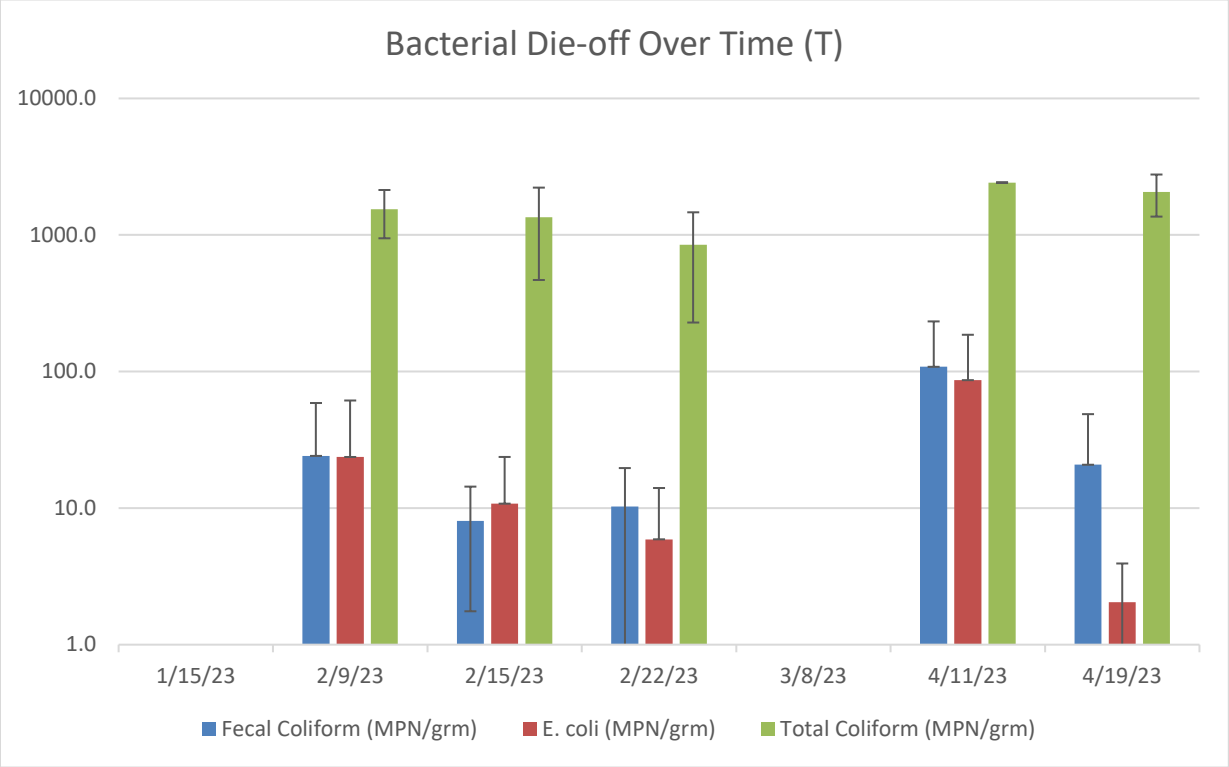

Figure 3. Bacterial die-off over time at Ranch T impacted by the Salinas River.* (**Samples collected on 03/08/23 were lost in the mail upon shipping from California to Arizona.*)

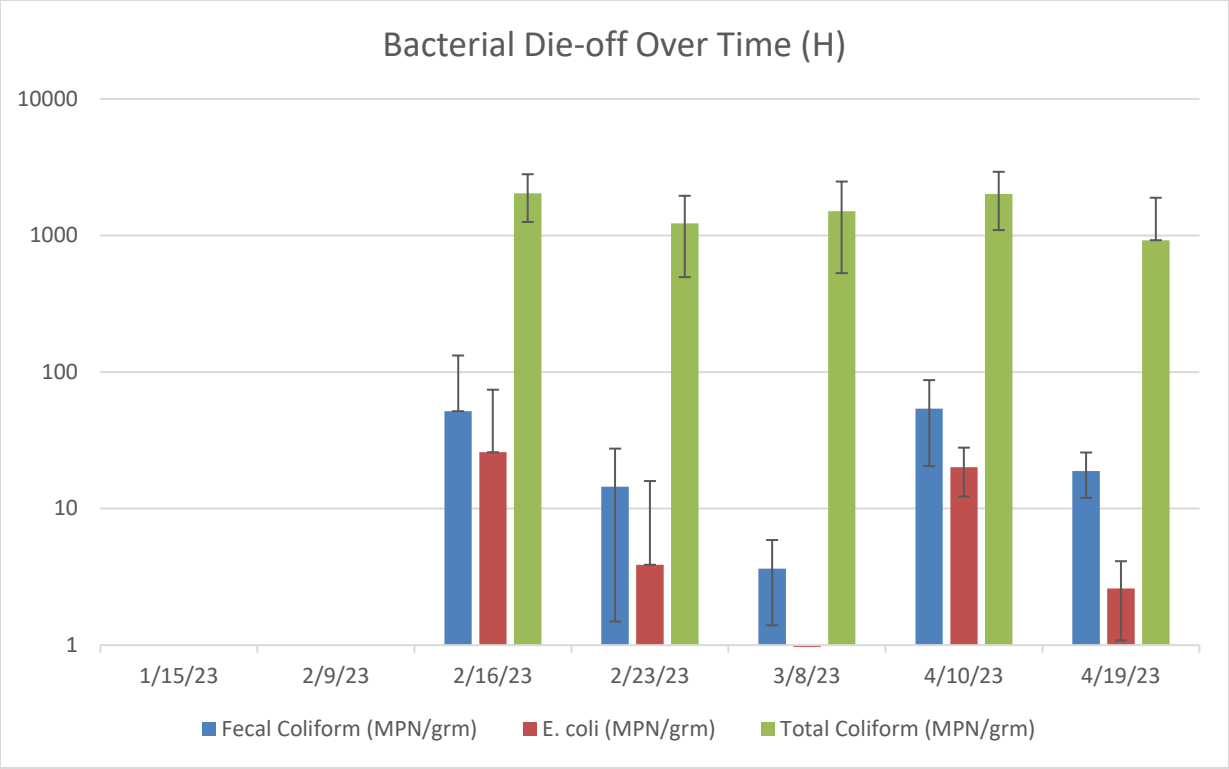

Figure 4. Bacterial die-off over time at Ranch T impacted by a tributary or creek.

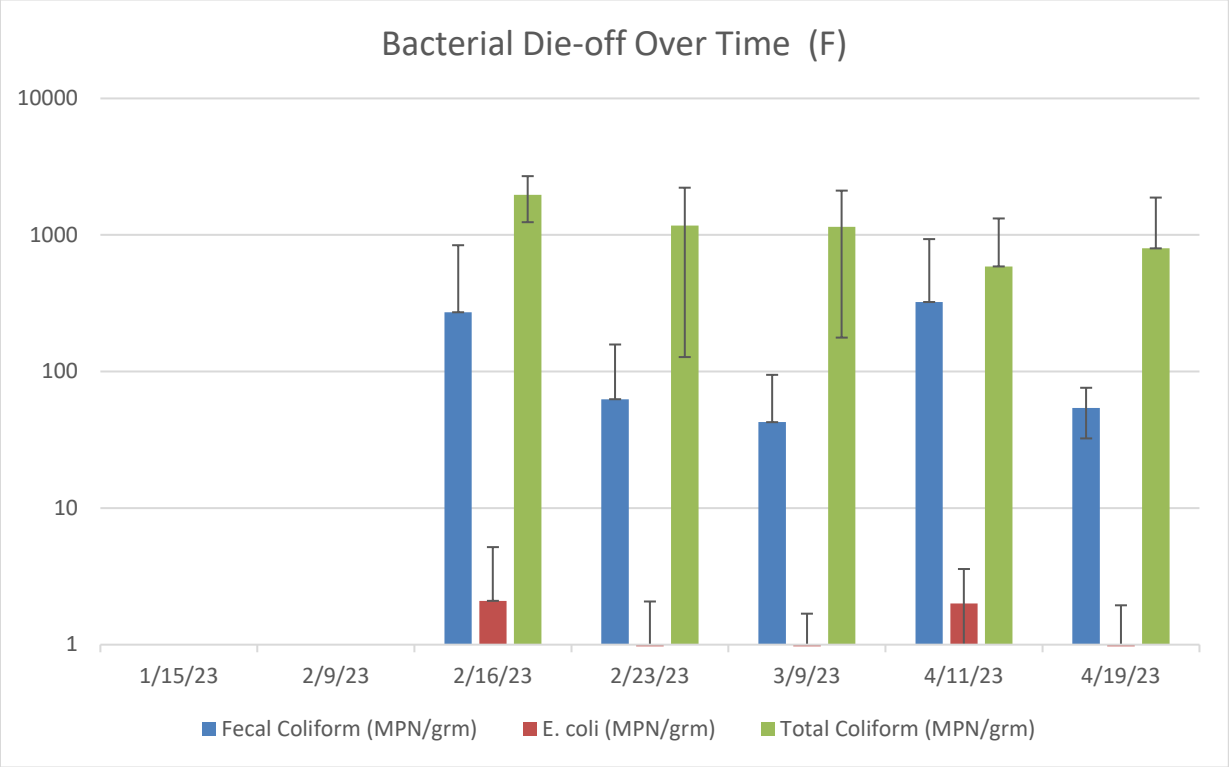

Figure 5. Bacterial die-off over time at Ranch F impacted by a tributary or creek*. (**Samples were not collected on 02/09/23 due to the ranch still being saturated.*)

In each of the four figures above it can be easily seen that as time passes, with each week of sampling all bacterial indicator organisms decline. With the additional flooding event that occurred after the week of 03/08/23, one can visually note the increase in all parameters measured, followed by a period of decreasing concentration. One important observation is that the variability in the Fecal Coliform bacteria across any individual field is quite high. This was observed across all four ranches evaluated.

Figure 6 below represents a heat-map of Fecal Coliform bacteria measured in individual or composite soil samples collected at field transects of 100, 200, 400, 800, or 1600 feet on each individual sampling date. Blocks indicated as red signifiy higher concentrations of Fecal Coliform bacteria, while blocks highlighted in green indicate a lower concentration of Fecal Coliform bacteria. As a note to the reader, the current LGMA guideline for acceptable criateria to re-plant a previously flooded field is <100 MPN fecal coliform bacteria per gram of soil. Variable levels of Fecal Coliform bacteria were detected in soil samples across space and time, ranging from <1 to 1986.3 MPN/gram. Alternatively, looking at the distribution of generic *E. coli* from the same ranch (F) over the same sampling period (**Figure 7**), it is quickly seen that MPN values are much more consistant across space and time. When evaluating generic *E. coli*, the threshold value of 10 MPN/gram of soil has been suggested as an alternative criteria for acceptance to re-plant previously flooded fields. We do not see significant differences in grab sample versus composite sample strategies.

	100 ft	200 ft	400 ft	800 ft	1600 ft
2/16/23	43.3	91.4	37.2	64.0	277.8
	39.8	108.1	53.5	9.6	39.8
	1986.3	387.7	25.4	396.8	107.7
	20.3	13.8	866.4	18.1	14.7
	11.3	16.7	26.0	791.5	27.8
2/23/23	416.0	88.8	52.0	30.5	21.1
	231.0	35.9	55.1	7.1	4.1
	89.1	111.8	22.8	7.5	6.1
	49.0	13.5	304.4	3.0	2.0
	15.6	14.5	44.5	4.0	3.0
3/9/23	40.8	32.5	37.6	21.8	13.8
	16.1	26.0	43.0	16.9	19.6
	9.3	22.9	144.5	10.1	0.0
	8.2	104.4	51.2	10.3	14.5
	248.9	36.8	108.6	46.3	4.1
4/11/23	1413.6	73.3	49.1	64.0	14.5
4/19/23	60.8	73.3	70.8	46.0	20.2

Figure 6. Fecal Coliform heat-map for Ranch F

	100 ft	200 ft	400 ft	800 ft	1600 ft
2/16/23	2.0	3.0	1.0	0.0	2.0
	1.0	5.2	3.1	3.0	0.0
	1.0	1.0	9.7	1.0	0.0
	2.0	1.0	2.0	0.0	0.0
	2.0	2.0	2.0	1.0	1.0
2/23/23	0.0	0.0	2.0	0.0	0.0
	0.0	3.0	0.0	1.0	0.0
	1.0	2.0	0.0	0.0	0.0
	0.0	0.0	0.0	1.0	0.0
	0.0	1.0	0.0	1.0	0.0
3/9/23	0.0	0.0	0.0	0.0	0.0
	0.0	0.0	0.0	6.3	1.0
	0.0	0.0	2.0	0.0	0.0
	0.0	0.0	0.0	1.0	0.0
	0.0	0.0	1.0	0.0	0.0
4/11/23	2.0	1.0	4.0	3.0	0.0
4/19/23	0.0	3.0	0.0	0.0	0.0

Figure 7. Generic *E. coli* heat-map for Ranch F

When evaluating the presence of pathogens in soil samples when compared to indicator organisms, 8% of soil samples in total were considered presumptive positive for STEC linked targets by ddPCR. Of the total samples collected, roughly 5% of soil samples were positive for *Salmonella* after the first flood, with 3% positive after the second flood, indicatiing little impact of flood waters on the presence of *Salmonella* in soils. Alternatively, after the first flood the research team recorded very few STEC positive samples (**Table 2**). However, after the second flood, up to 47% of samples from collective ranches had soil samples presumptive positive for STEC by culture. This raises questions regarding the impact of sustained flushing over an extended period of time, the potential force of flood waters on bacterial adhesion to soil particles, and the impact of nutrient loading of pathogen survival over time.

Table 2. Prevalence of STEC and genetic markers in soil samples over time post flood

Days postflood 1	Days postflood 2	% prevalence (no. of positive samples/total samples)			
		stx	eae	STEC linkage	STEC culture
35	-	48 (15/31)	55 (17/31)	3 (1/31)	19 (6/31)
42	-	16 (14/90)	31 (28/90)	3 (3/90)	8 (7/90)
49	-	29 (31/108)	34 (37/108)	10 (11/108)	11 (12/108)
63	-	26 (19/72)	33 (24/72)	6 (4/72)	11 (8/72)
Days postflood 1	Days postflood 2				
98	28	53 (10/19)	79 (15/19)	21 (4/19)	47 (9/19)
105	35	42 (8/19)	37 (7/19)	16 (3/19)	42 (8/19)

Results indicate that fields adjacent to creeks/tributaries with overland flow had increased likelihood of detecting pathogens than those adjacent to the Salinas River. In total, the research team evaluated five different water sources adjacent to flooded fields, including the Salinas River, Pajaro River, Miller Creek, Alisal Creek, and an unnamed drain. In the evaluation of water sources, *Salmonella* was found in all sources, whereas STEC was detected exclusively in the creeks/tributaries and rivers. The prevalence of pathogens was significantly higher in the two creeks/tributaries, with positive samples comprising 80% of the total. In contrast, the two rivers showed a lower incidence, accounting for only 20% of positive samples. Notably, no STEC was detected in the collected drain samples, offering further insights into the pathogen distribution across the different water sources.

Additionally, the research team was able to confirm STEC SerO groups more often in samples collected from fields adjacent to flooded creeks/tributaries. While some enteric bacterial strains cause acute outbreaks linked to specific sources, other strains—referred to as reoccurring, emerging, or persisting (REP) strains—can reoccur and periodically cause acute outbreaks. They can also emerge and increase in frequency or persist and cause illnesses over periods of months or years, despite investigation and prevention efforts. It is important to note that O157:H7 was not confirmed from any sample collected in the study and that none of the previously reported REP strains, REPEXH01 and REPEXH02, were identified (https://www.cdc.gov/ncezid/dfwed/outbreak-response/rep-strains.html).

When confirming the presumptive pathogens, the following SerO groups were identified as predominant from soil samples (see **Table 3**).

Table 3. STEC SerO groups confirmed from soil samples

Flood Description	STEC SerO group
Adjacent Ranch/Salinas River	not detected
Adjacent Ranch/Salinas River	not detected
Adjacent Ranch/Salinas River	O26,O103
Salinas River	O26, O103, O45, O121
Salinas River	O111, O26, O103, O45, O121
Tributary	not detected
Tributary	O103, O45
Tributary	O103, O45
Tributary	O145, O103, O45, O121
Salinas River	O103, O45, O121
Salinas River	O45
Tributary	O145, O103, O45, O121
Tributary	O45
Tributary	O103, O45
Tributary	O103, O45, O121
Tributary	O45, O121

As mentioned above, physical and chemical parameters were also evaluated for soil samples collected in the study. One parameter that was identified as particularly useful for industry is the gravimetric water content (GWC). Gravimetric water content (%) is the mass of water per mass of dry soil. It is measured by weighing a soil sample, drying the sample to remove the water, then weighing the dried soil. When analyzing the data of GWC collected across all samples and comparing values with those of indicator organisms, the Pearson correlation coefficient (r) = 0.54 for *E.coli* and gravimetric water content % was calculated. This indicated that as soil moisture increased, *E. coli* MPN also increased (positive correlation). While notable for this study, this finding has been reported previously in the literature, as soil pH and moisture content are primary drivers of *E. coli* O157 survival (5). It is important to consider that gravimetric water content is an inexpensive and relatively straightforward parameter to monitor and could be a possible additional monitoring parameter used by LGMA and industry to inform field re-entry post flood.

Table 4 outlines ranges in various soil property measurements collected across all four unique ranches. Little to no sodicity-salinity problems were detected at the 0–6" profile. While high levels of sodium (Na) were detected, no 'sodicity' problem was detected; higher levels of Calcium and Magnesium possibly helped to 'neutralize' the sodium. It was identified that the pH at two ranches was very high, which can indicate that those ranches may be more prone to Na-problems in the future. However, it should be noted that flooding seemed to help with salinity, indicating that water pushed the salts (Na) down and away from the root zone.

Table 4. Soil physical and chemical analysis

Soil Properties	Ranch S	Ranch F	Ranch H	Ranch T
pH	7.93 – 8.38	7.47 – 7.95	7.44 – 8.19	7.79
Soluble salts (dS/m)	0.22 – 0.85	0.20 – 0.87	0.21 – 0.83	0.26
Sodium, Na (ppm)	91.8 – 111	266 – 274	119 – 165	183
Exchangeable Na percentage (ESP) %	1.44 – 3.76	1.7 – 2.86	1.4 – 2.6	2.55
Sodium adsorption ratio (SAR)	0.7 – 0.8	1.7 – 1.9	1.4	1.3

Grower Key Findings

- Fecal Coliform bacteria may not be the best indictor of pathogen risk.
  - Highly variable across space and time
  - Not correlated to STEC or *Salmonella*
- Generic *E.coli* are much more consistent across space and time.
- Not all flood waters are equal risk.
- Flood waters from adjacent creeks/tributaries indicated a greater likelihood of detecting pathogens (STEC) in soils.
- Bacterial indicator numbers declined or "recovered" before 30-day interval in all ranches.
- LGMA guidance on re-planting post flood at 60 days is conservative.

References cited

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APPENDICES

Publications and Presentations

1. CPS Rapid Response Industry Contributors Request for Participation, February 3rd, 2023
2. CPS Rapid Response Industry Contributors Research Update, March 23rd, 2023
3. Center for Produce Safety Advisory Board, April 27th, 2023
4. CPS Rapid Response Industry Contributors Final Research Update, May 26th 2023
5. CPS Rapid Response Industry Contributors Final Research Update, June 7th, 2023

Budget Summary

The project was awarded a total of $148,983; all funds were spent.

	Expenditures to date
Personnel Services	60,595.85
General Expenses	54,959.38
Travel	28,580.86
IDC	4,847.66
Total	***148,983.75***