CPS 2022 RFP – FINAL PROJECT REPORT

Project Title:
Microbiological risk assessment using QMRA in preharvest agriculture water treatment systems for leafy greens

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Objectives:

1. Determine the die-off or log-reduction of Shiga-toxigenic *E. coli* (STEC) and generic *E. coli* surrogates pre-established on leaf surfaces and in soil following agricultural water treatment with commonly used water treatment sanitizers (PAA and calcium hypochlorite).
2. Conduct in-field evaluations of water treatment variability or "breakthrough" using traditional grab sampling techniques for microbiological indicators (generic *E. coli* and total coliform bacteria) coupled with real-time in-line monitoring for various physical and chemical parameters.
3. Use real-world collected data from research Objectives 1 and 2 to conduct a QMRA for STEC in leafy greens (romaine and spinach).

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

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Summary of Findings and Recommendations

Findings:

- **Agricultural Water Treatment Effectiveness:** Treatments using peracetic acid (PAA) and calcium hypochlorite reduced microbial contamination in all evaluated scenarios, demonstrating a beneficial impact on bacteria present in water, soil, and plant surfaces.
- **Animal Intrusion Poses the Highest Risk:** The fecal slurry (FC) scenario had the highest risk and the least reduction in contamination due to the presence of organic matter and elevated bacterial concentrations, making it the most challenging to mitigate.
- **Calcium Hypochlorite Demonstrated Greater Efficacy:** In field trials, calcium hypochlorite resulted in higher microbial risk reductions compared to PAA, particularly in controlling *E. coli* on plant surfaces and in soil.
- **Variability in Treatment Success:** Risk reduction varied across contamination types, emphasizing the need for targeted treatment strategies to address different contamination scenarios effectively.
- **QMRA Findings:** The Quantitative Microbial Risk Assessment (QMRA) model demonstrated that *E. coli* concentration was the most critical factor influencing microbial risk. Sensitivity analysis indicated that microbial load in irrigation water must be minimized to effectively reduce contamination risks.
- **Persistence and Regrowth Observations:** Some bacteria exhibited regrowth post treatment, with *E. coli* surviving in soil for up to 72 hours post exposure to treated water. Further study is needed to understand persistence in different environmental conditions.
- **Partial Reduction on Romaine Lettuce:** While treated irrigation water significantly reduced *E. coli* levels on romaine lettuce, neither PAA nor chlorine treatments completely eliminated the bacteria. The level of reduction varied depending on sanitizer type and concentration, with PAA typically having a greater impact on immediate reductions but chlorine showing more sustained effectiveness.
- **Risk Per Exposure Analysis:** The highest individual exposure risk was observed in the animal intrusion scenario (2.28×10^{-8}), equating to a 1 in 43.9 million chance of infection per exposure. However, risk compounds over repeated exposures, emphasizing the need for ongoing mitigation.

Recommendations:

- **Enhance Water Treatment Strategies:** Optimize treatment approaches based on contamination sources, ensuring appropriate sanitizer residuals to maximize microbial reduction and limit treatment variability.
- **Focus on High-Risk Contamination Scenarios:** Develop enhanced mitigation strategies specifically targeting animal intrusion scenarios, where bacterial loads are highest and most persistent.
- **Implement Real-Time Monitoring & Response Systems:** Continuous monitoring of water quality and sanitizer effectiveness is needed to promptly identify and address treatment inconsistencies and breakthrough events.
- **Improve Understanding of Bacterial Persistence:** Further research is required on bacterial regrowth in soil and plant surfaces post treatment to enhance QMRA accuracy and optimize treatment application strategies.

- **Evaluate Long-Term Treatment Efficacy:** Investigate the prolonged impact of sanitizer application on microbial loads in soil and plant surfaces beyond the initial 72-hour period.
- **Enhance Grower Adoption of Best Practices:** Provide industry stakeholders with data-driven guidance on effective water treatment protocols to improve compliance with Leafy Greens Marketing Agreement (LGMA) metrics and reduce contamination risks.

Abstract

Agricultural water treatment research has largely focused on the efficacy of various chemistries and devices in reducing microbiological indicators, such as generic *Escherichia coli* and Total Coliform bacteria, in water. While significant progress has been made in understanding pathogen reduction within water, a critical knowledge gap remains regarding the impact of treated water on pathogens already present on plant surfaces or in soil. This study addressed these gaps through laboratory and field evaluations combined with Quantitative Microbial Risk Assessment (QMRA). Pre- and post-treatment sampling of soil and plant tissue demonstrated that agricultural water treatments using peracetic acid (PAA) and calcium hypochlorite (chlorine) effectively reduced contamination risks. One day post treatment (dpt), *E. coli* detection on inoculated lettuce heads varied by contamination scenario: 0% (0/90) for Atmospheric Deposition, 40% (36/90) for Animal Intrusion, and 4% (4/90) for Failed Treatment with PAA. In contrast, chlorine treatment resulted in 26% (24/90), 46% (42/90), and 0% (0/90) positive detections for the same scenarios. No bacteria were detected in composite or whole lettuce samples 7 days post treatment for either chemical, highlighting the sustained impact of water treatment on bacteria present on leaf surfaces and in soil. Although overall risk reduction was observed, contamination from animal intrusion posed the highest risk and showed the least reduction post treatment. This is likely due to increased organic matter and elevated bacterial concentrations in feces, warranting further attention. Additionally, variability was observed when comparing sanitizer impact on the die-off of various historical *E. coli* O157:H7 outbreak strains, with a range in die-off from 0.25 to 1.5 log reduction within 72 hours post treatment (hpt) on inoculated romaine leaves. These findings provide growers and regulators with valuable insights into the role of agricultural water treatment in mitigating microbial risks, ultimately enhancing consumer safety.

Background

In response to recent outbreaks linking *E. coli* O157:H7, in which agricultural water was suspected as the source, the California and Arizona Leafy Greens Marketing Agreement(s) adopted revised metrics in an attempt to address the possible role of irrigation water on crop contamination. These revised metrics now require growers to treat their irrigation water when using surface water for overhead irrigation within 21 days of harvest. In order to support industry, the EPA and FDA worked together to draft an Agricultural

Water Treatment protocol (https://www.fda.gov/media/140640/download) which dictates a 3-log reduction requirement for any sanitizer used in an agricultural setting. While much research has been done on efficacy of commonly used sanitizers including peracetic acid (PAA), calcium/sodium hypochlorite, and chlorine dioxide to reduce pathogens/indicators in water, little research has focused on if there is an added benefit of these sanitizers on established pathogens on crop surface or in soil. Until recently, the variability of successful treatment had not been properly characterized in an agricultural setting. Industry guidance is needed to better understand the cumulative impact of antimicrobial treatments commonly used by industry on pathogens/indicators in water, on plants, and in soil as well as the resulting range in reduction in human health risk to consumers.

Recent research (CPS Ag Water Treatment – Southwest Region), focused on the variability of agricultural water treatment at the field scale, has led to realization that water treatment, while successful at reducing risks of microbiological hazards, is somewhat "imperfect" and highly dynamic. Scientific data generated by our team, identified that this variability is due to physical and practical implementation barriers to agricultural water treatment at the field level. This phenomenon, or "breakthrough", was first documented by our research team as a potential reduction in grower confidence in meeting agricultural water treatment log reduction goals. This was demonstrated by periods of agricultural water treatment during an irrigation event resulting in greater than 3-log reduction of bacterial pathogens/indictors, and other periods of time resulting in nearly zero reduction during the same irrigation event. This was identified in multiple field trials, regardless of chemistry, flow rate, or ranch size. Additionally, as noted above, agricultural water treatment strategies have largely focused on treatment of pathogens/indicators in water with less focus on understanding the cumulative beneficial impact(s) of agricultural water treatment on established pathogen persistence, decay, and die-off on plant tissue and in soil over the course of the growing season. This project specifically addresses CPS (2022 RFP) research priority 4. Agriculture, b. Impact of preharvest water treatment on established foodborne pathogen persistence.

Research Methods and Results

Impacts of treatment on contamination events in the field. In the field experiments of growing and harvesting lettuce, three inoculum scenarios were explored for potential contamination and the subsequent impacts of chemical treatment within the irrigation system. These scenarios are defined in **Table 1** (see Appendices). Phase one of the study focused on evaluating the impact of two agricultural water treatment chemistries on the reduction of inoculated surrogates/pathogens on leaf surfaces of

romaine lettuce, and in soil. Various "contamination" events were modeled in consultation with leafy greens growers to represent the establishment of pathogens due to 1) aerial/atmospheric deposition (AD), 2) animal intrusion fecal slurry (FC), and 3) non-treated (prior to 21 days to harvest) agricultural water irrigation event (NT). All experiments were conducted in triplicate and occurred at the Maricopa and/or Yuma Agricultural Centers for field studies using the surrogate index organism (*E. coli* TVS353). For the field evaluations, following contamination events, agricultural water treatment(s) were applied according to industry standards and timelines within 21 days to harvest and included both positive and negative controls. For this study, the research and extension team evaluated two agricultural water treatment chemistries – peracetic acid (PAA) and calcium hypochlorite (chlorine) – aiming at a residual of 7 and 10 ppm for PAA and 2 and 4 ppm for free chlorine. Plant tissue and soil samples were taken for the background concentration of *E. coli* after inoculation within 12 hours, and prior to the addition of chemical treatment and irrigation of the field. The percent positives of these "before" and "after" samplings are illustrated for the implementation of PAA and for calcium hypochlorite (n=18 or n=90 depending on the sample type) (**Figure 1**). The research team then used cultural techniques to track microbial contaminant presence, persistence, and die-off over time on plant tissue and in soil and assigned a range of log-reduction potentials based on each agricultural water treatment, sample type, and surrogate/pathogen combination. In all scenarios evaluated, the highest detections were for the FC (animal intrusion fecal slurry) scenario, with over 40% positive samples detected after agricultural water treatment was applied for both sanitizers evaluated, followed by atmospheric deposition (AD) post calcium hypochlorite treatment, and non-treated (NT) irrigation water post PAA treatment (**Table 2**). While there was variability seen in the data for percent positive, post treatment for both sanitizers evaluated, it was clearly evident that residual bacterial contamination from animal intrusion/fecal contamination severely limited the success of agricultural water treatment sanitizers' effectiveness. To determine this variability in bacterial degradation and die-off, recorded data were then fed into a QMRA analysis.

Quantitative Microbial Risk Assessment (QMRA). QMRA is a process that allows an assessment in a quantitative fashion for estimation of the risk of infection and illness from a pathogenic microorganism. It has seen widespread application in water and food (Rock et al., 2019; Sunger et al., 2019; Stine et al., 2005; Jaykus, 1996; Haas et al., 1995; Regli et al., 1991). QMRA involves four basic steps: (1) hazard identification (the pathogen in question), (2) dose-response (the probability of infection from ingestion of a given number of organisms), (3) exposure assessment (the number of organisms ingested), and (4) risk characterization (estimation of the probability on infection or illness). Our team has previously used QMRA to estimate the probability of infection from hepatitis A virus and *Salmonella* in irrigation waters

used to irrigate cantaloupe and iceberg lettuce by various irrigation methods (Stine et al., 2005) as well as *E. coli* in irrigation waters on leafy greens (Rock et al., 2019).

Of particular interest is the hypothesis that historical agricultural water treatment failure/breakthrough events have resulted in outbreaks. We aimed to assess the plausibility of this hypothesis by including the treatment breakthrough probability and timing variables in the Monte Carlo analysis and evaluating to what extent these variables drive risk via a sensitivity analysis. Using in-field experimental data generated on treatment performance over time, we used maximum likelihood estimation to fit a distribution to for use in the Monte Carlo model.

Due to the nature of the data, two risk assessments were performed in parallel to illustrate the impacts of disinfection on potential *E. coli* contamination or presence in irrigation water. First, a point-estimate QMRA approach was used to show the relative risk and risk reduction of different treatments for the three contamination scenarios defined above. Then, based on absolute counts of generic *E. coli* present in the irrigation water before and after disinfection, a probabilistic risk assessment was performed to determine the impacts of treatment on potential bacteria contamination in the water system and subsequent lettuce ingestion.

Point estimate risk assessment: Impacts of chemical treatment on contamination scenarios using field data. To better understand and quantify the impacts on utilizing PAA or calcium hypochlorite, the results of the *E. coli* prevalence data were applied to a risk assessment framework. First, the most probable number (MPN) of *E. coli* on the lettuce head was estimated based on the percent positives according to Equation 1. This resulted in one single concentration estimate for each scenario. Due to the small number of calculation variables and only one variable which could be defined as a distribution in this scenario, a point estimate calculation was performed.

$$MPN = -\frac{1}{V}\ln\left(\frac{n-P}{n}\right) \qquad \textbf{Equation 1.}$$

Where *V* is the sampled volume or mass (25 g lettuce sample), *n* is the total number of samples, and *P* is the number of positive samples. This equation was applied to all scenarios to estimate the MPN per contamination event before and after treatment for both PAA and calcium hypochlorite.

To quantify the risk of infection for each scenario, the following exposure model (**Equation 2**) and dose-response model (**Equation 3**) were used:

$$d = CM \qquad \textbf{Equation 2.}$$

$$P(d) = 1 - \left(1 + \frac{d}{\beta}\right)^{-\alpha} \qquad \textbf{Equation 3.}$$

Where *C* is the concentration of *E. coli* on the lettuce, *M* is the amount of lettuce consumed per day, based on national averages, *d* is the total dose of *E. coli* consumed per day, and α and β are best-fit parameters for the chosen dose-response model to estimate the risk of infection. The values and sources for these parameters are listed in **Table 3**.

The infection risk before and after treatment (for the controlled inoculum concentrations in **Table 1** and not for indigenous contamination) for all scenarios is illustrated in **Figure 2**. While the risk was low ($<10^{-7}$) the differences before and after treatment of PAA for the fecal slurry (FC) scenario were less than for the other treatments. The fecal slurry also had the highest risk for the calcium hypochlorite addition (**Figure 2**). The higher risk (and prevalence of bacteria) for the FC scenario is likely due to disinfectant demand created by organic materials in the fecal slurry.

To better illustrate these treatment impacts and extrapolate beyond the specific laboratory inoculum conditions, the change in risk with treatment was estimated as a percent change (**Equation 4**) and plotted.

$$\%\ change = \left(\frac{P(d)_{\text{after}} - P(d)_{\text{before}}}{P(d)_{\text{before}}}\right) \times 100 \qquad \textbf{Equation 4.}$$

In all cases, there were fewer positive detections after treatment, resulting in lower risk (a negative percent change) (**Figure 3**). The PAA treatment had the best results for the atmospheric deposition (AD) scenario, resulting in a 100% reduction in risk (no positive detection after treatment). It is noted that there is no such thing as "zero risk", and therefore this primarily represents a reduction to below detection levels at the end of the field experiment. Calcium hypochlorite had the greatest reduction for the treatment failure (NT) scenario, also resulting in 100% reduction in risk. Fecal slurry (FC) had the highest detections in both treatment cases, with the lowest change in risk, although all scenarios did see a reduction in risk (percent change was negative for all).

Table 4 shows the overall risk values for an individual exposure for all scenarios evaluated. While the animal intrusion scenario represents the greatest risk of those evaluated (2.28×10^{-8}), overall these values presented here represent extremely low risk per single exposure event. It should be noted, however, that risk accumulates over multiple exposures and can therefore compound over days, weeks, years depending on average consumption of leafy greens in a given time period. The infection risk per exposure of 0.0000000228 (or 2.28×10^{-8}) means that for each individual exposure event, the probability of infection is 1 in ~43.9 million (1 / 0.0000000228 ≈ 43,859,649).

Probabilistic risk assessment: Impacts of treatment on irrigation water for field application. Additional water quality measurements were taken directly from the irrigation system (absolute counts of indigenous bacteria). *E. coli* was enumerated using IDEXX Colilert QuantiTray within 6 hours of sample collection. The "background" concentrations were sampled from the irrigation ditch prior to entering the distribution system. "First" refers to samples collected from the first irrigation point in the sequence, and "Last" the final irrigation riser. For each of the field experiments, a 2-acre plot was assessed. IDEXX *E. coli* (total *E. coli* in MPN/100mL) are illustrated in **Figure 4** for both chemical treatments. Post-treatment samples in both cases were primarily below the detection limit of 1 MPN/100mL, indicating the efficacy of adding both PAA and calcium hypochlorite to the water. While these counts do not indicate whether pathogenic *E. coli* strains are present, they demonstrate the impact of including disinfectants in the irrigation water to inactivate bacteria that may already be present prior to other contamination scenarios.

Risk assessment using irrigation water samples. A risk assessment was conducted using similar methodology to that described above, however using the empirical *E. coli* IDEXX absolute concentrations as a potential hazard. In this case, we assumed that the irrigation water contained the bacteria, and estimated an amount of water that would end up on the lettuce head. Variables used in the Monte Carlo simulation are described in **Table 3** (some variables are same as those used in lab data risk calculation).

Lognormal distributions were fitted to the groups of bacteria measurements (e.g., PAA applied, first riser) to capture the range and uncertainty of the concentrations. **Equation 5** was then used to estimate the exposure dose, followed by the same dose-response and risk characterization equations as the previous method (**Equations 1-4**).

$$d_{\mathrm{IDEXX}} = CWM \qquad \textbf{Equation 5}.$$

The results of the probability of infection for background and post-treatment irrigators are shown in **Figure 5.** Including chemical disinfectant reduced *E. coli* concentrations and thereby the microbial risk resulting from both disinfectants, as the risk between background and first riser location was reduced by over three orders of magnitude. Calcium hypochlorite had a higher reduction in risk, over 4.5 orders of magnitude difference between the background and last riser, for example.

A sensitivity analysis was conducted for this approach to assess the impact of each of the three parameters in **Equation 5** on the final risk estimate. The Spearman Rank correlation coefficient is plotted in **Figure 6** based on analysis of the infection risk using the parameter distributions in **Table 5** for the concentrations and risk results of the PAA background assessment. This risk model is most sensitive to the concentration of *E. coli* measured, with a ρ value of 0.94.

Analysis of laboratory-scale persistence trials. Laboratory trials measured concentrations of several *E. coli* strains in lettuce and on soil after mimicking an agricultural water treatment. The different conditions and treatments for these trials are listed in **Table 6**.

Escherichia coli survival in soils post exposure to treated irrigation water. The goal of these experiments was to understand the impact treated irrigation water had on eliminating established *E. coli* O157:H7 or generic *E. coli* populations in agricultural soils. Soils were inoculated with either high (10^4 CFU/g) or low (10^2 CFU/g) inoculums of either *E. coli* O157:H7 str. TW14359 (2006 spinach outbreak strain), *E. coli* O157:H7 str. REPEXH01 (2018 romaine lettuce outbreak strain), generic *E. coli* K12 str. MG1655, or generic *E. coli* str. TSV353 prior to any exposure to irrigation water. Next, inoculated soil microcosms were exposed to irrigation water treated with either 2 ppm chlorine, 4 ppm chlorine, 7 ppm PAA, 10 ppm PAA, or no treatment by mimicking overhead irrigation. Microcosms were then kept under either Yuma or Maricopa, AZ, conditions, which included the 5-year average amount of sunlight, temperature, and humidity for the month of January for each location. Samples were collected, serially diluted, and direct plated on selective media to quantify the amount of each *E. coli* strain at 0, 12, 24, 48, 72, 168, 336, and 504 hrs post-irrigation water exposure. All experiments were conducted in triplicate. The following results were found for the two different locations:

Yuma conditions. Neither *E. coli* O157:H7 strains were completed eliminated/killed immediately in soil by exposure to the treated irrigation water regardless of the type of sanitizer, sanitizer concentration, or inoculum dose of the strain. However, both high and low inoculums for both *E. coli* O157:H7 strains only

survived for 72 hrs in soils when exposed to treated irrigation water, but *E. coli* O157:H7 str. TW14359 also died at this time point when exposed to untreated irrigation water (Supplemental **Figure 1**). Whereas *E. coli* O157:H7 str. REPEXH01 survived until 168 hrs in soils when exposed to untreated irrigation water (Supplemental **Figure 2**). Overall, it suggests that treated irrigation water has very little impact on *E. coli* O157:H7 survival in soils regardless of the type or concentration of sanitizer used to treat the irrigation water. Interestingly, generic *E. coli* K12 str. MG1655 was unimpacted by exposure to irrigation water and survived for 504 hrs irrespective of the treatment conditions of the irrigation water (Supplemental **Figure 3**). On the other hand, generic *E. coli* str. TSV353 died immediately in the soil under Yuma conditions when exposed to irrigation water including the untreated irrigation water (Supplemental **Figure 4**).

Maricopa conditions. Similar to soil samples under Yuma conditions, *E. coli* O157:H7 str. TW14359 at low inoculums were not immediately eliminated by treated irrigation water, but also did not survive beyond 72 hrs post exposure including with untreated irrigation water. However, the high inoculum for the TW14359 survived for 504 hrs in the soil post irrigation water exposure under Maricopa conditions regardless of the type or concentration of the sanitizer (Supplemental **Figure 5**). The low inoculum *E. coli* O157:H7 str. REPEXH01 also survived for 72 hrs in soils after exposure to irrigation water including the untreated group, but both PAA concentrations (7 ppm and 10 ppm) actually survived until 168 hrs. The high inoculum REPEXH01 soil microcosms showed that both PAA concentrations and chlorine at 4 ppm eliminated REPEXH01 from the soil by 72 hrs compared to the untreated group that survived the entire 504 hrs (Supplemental **Figure 6**). Identical to the Yuma, AZ, conditions results, generic *E. coli* K12 str. MG1655 in soil were unimpacted by exposure to irrigation water and survived for 504 hrs irrespective of the treatment conditions of the irrigation water or size of the inoculum (Supplemental **Figure 7**). The generic *E. coli* str. TVS353 low inoculum group showed low levels of survival in all the treatment groups for 48 hrs, but 10 ppm PAA eliminated the strain at that timepoint, 7 ppm PAA eliminated it at 72 hrs, and both chlorine concentrations killed the strain by 168 hrs compared to the untreated irrigation water group that survived until 336 hrs post exposure (Supplemental **Figure 8**). Thus, the results for Maricopa, AZ, conditions were very similar to the results from Yuma, AZ, conditions.

Overall, treated irrigation water regardless of the sanitizer used or concentration fails to immediately eliminate/kill either *E. coli* O157:H7 or generic *E. coli* in soil, but the *E. coli* will typically kill it off by 72 hrs post exposure particularly at lower concentrations of the bacteria.

Escherichia coli survival on romaine lettuce post exposure to treated irrigation water. The goal of these experiments was to understand the impact treated irrigation water had on eliminating established *E. coli* O157:H7 or generic *E. coli* populations on romaine lettuce. Lettuce leaves were inoculated with either high (10^4 CFU/g) or low (10^2 CFU/g) inoculums of either *E. coli* O157:H7 str. TW14359 (2006 spinach outbreak strain), *E. coli* O157:H7 str. REPEXH01 (2018 romaine lettuce outbreak strain), generic *E. coli* K12 str. MG1655, or generic *E. coli* str. TSV353 prior to any exposure to irrigation water. Next, inoculated romaine lettuce were exposed to irrigation water treated with either 2 ppm chlorine, 4 ppm chlorine, 7 ppm PAA, 10 ppm PAA, or no treatment by mimicking overhead irrigation. Romaine lettuce was only kept under Yuma, AZ, conditions, which included the 5-year average for January for sunlight, temperature, and humidity for that location. For both inoculums, samples were collected, enriched following the FDA BAM protocol, and plated on selective media to assess the viable *E. coli* for all four strains at 0, 12, 24, 48, 72, and 168 hrs post-irrigation water exposure. Additionally, the high inoculum romaine lettuce was also serially diluted and direct plated to quantify the amount of each *E. coli* strain present on the romaine lettuce at the different timepoints. All experiments were conducted in triplicate. The following results were found for romaine lettuce under Yuma conditions:

High inoculum quantification. E. coli O157:H7 str. TW14359 on romaine lettuce leaves were reduced by 0.5 to 1.0 logs when exposed to treated irrigation water compared to untreated irrigation water with PAA at 7 ppm having the largest impact, but it did not eliminate/kill the pathogen off completely even after 168 hrs post exposure. Levels of the strain remained lower on romaine lettuce that was exposed to treated irrigation water compared to untreated irrigation water for the entire 168 hrs irrespective of the sanitizer type or concentration. However, there were differences in the amount of pathogen reduction between the different types of sanitizers and concentrations (Supplemental **Figure 9**). *E. coli* O157:H7 str. REPEXH01 had nearly identical results to TW14359 on romaine lettuce leaves, as there was an immediate 0.5–1.5 log reduction with PAA at 10 ppm have the biggest reduction compared to the untreated irrigation water. Additionally, the REPEXH01 levels remained reduced compared to the untreated irrigation water exposed group, but was never completely eliminated/killed by the treatment regardless of type or concentration of sanitizer (Supplemental **Figure 10**). The generic *E. coli* strains were impacted nearly identical to the *E. coli* O157:H7 strains, with an initial reduction that reduced the population but did not eliminate it for the duration of the experiment. Generic *E. coli* K12 str. MG1655 was reduced by 0.5 to 1.0 logs by exposure to treated irrigation water, with chlorine treated irrigation water having the strongest impact to the population. Again, these populations were reduced but not eliminated compared to the untreated irrigation water groups regardless of the type or concentration of the sanitizer (Supplemental

Figure 11). Generic *E. coli* str. TSV353 was impacted nearly identical to the two O157:H7 strains and K12, as there was the initial 0.25 to 1.0 log reduction compared to the untreated irrigation water with the strongest reduction due to 4 ppm chlorine or 10 ppm PAA treated irrigation water. While the population of TSV353 was reduced it was never eliminated across 168 hrs post exposure just like the other strains (Supplemental **Figure 12**).

Enrichment assays. In order to assess if the different *E. coli* strains were actually completely eliminated or killed off by the different treated irrigation water, inoculated romaine lettuce leaves were enriched to evaluate if the strains were present even in extremely low concentrations. Enrichment assays of *E. coli* O157:H7 strain TW14359 found that at high inoculums there was no difference in the presence of the pathogen on the romaine lettuce leaves after exposure to different treated irrigation water compared to untreated. However, at the low inoculum levels both concentrations of PAA decreased but did not completely eliminate the number of positives samples by 48 hrs post exposure compared to untreated irrigation water (Supplemental **Table 1**). Similarly, *E. coli* O157:H7 strain REPEXH01 saw no difference at the higher inoculum but also had a reduced number of positive samples when exposed to PAA treated irrigation water at 48 hrs post exposure compared to untreated irrigation water (Supplemental **Table 2**). Generic *E. coli* K12 str. MG1655 was also unimpacted by the treated irrigation water versus untreated irrigation water at the higher inoculum. However, PAA-treated irrigation water did reduce the number of positive samples compared to untreated irrigation water, but not until 168 hrs post exposure compared to 48 hrs for the two O157:H7 strains (Supplemental **Table 3**). Whereas the generic *E. coli* str. TSV353 had a reduced number of positive samples at 24 hrs post exposure when exposed to PAA-treated irrigation water compared to the untreated irrigation water. Although TSV353 also did not have a difference regardless of treatment or concentration in the number of positive samples for the romaine lettuce that was exposed to the higher inoculum (Supplemental **Table 4**). Chlorine-treated irrigation water did not impact the number of positive *E. coli* samples after exposure for any of the four strains tested compared to the untreated irrigation water groups.

Overall, these results demonstrate that exposure to treated irrigation water will reduce the population of *E. coli* O157:H7 or generic *E. coli* established on romaine lettuce leaves, but it will not eliminate it completely regardless of the type of sanitizer used, concentration of the sanitizer, or amount of *E. coli* present on the plant. However, the amount of reduction will be different depending on the sanitizer and/or concentration with PAA treated irrigation water typically having more of an impact than chlorine treated irrigation water.

The results of all trials are plotted in the supplemental figures and illustrate the different strains over time. **Figure 7** provides an example of the survival rates of *E. coli* O157:H7 strain REPEXH01 (2018 romaine lettuce outbreak) in soils kept under Maricopa, AZ, conditions (light, temperature, and humidity) after exposure to different types and concentrations of sanitizers in irrigation water with high (10^4 CFU/g) or low (10^2 CFU/g) inoculums.

Outcomes and Accomplishments

Over the course of this project, the research and extension team used both field and laboratory data modeled after real-world industry scenarios to develop QMRA outcomes on the impact of sanitizer use on risk. The results, presented above, have been (and will be) shared during the annual CPS Research Symposium in 2023, 2024, and 2025 (*June*). The project team will use the collected data to inform over 200 industry participants of the most effective strategies towards agricultural water treatment performance to meet LGMA metrics requirements as well as to minimize the risk of produce contamination by foodborne pathogens, which could be present in irrigation waters, on plant surfaces, or in soil. It is anticipated that 3 to 6 months after the 2025 CPS Research Symposium, the research team will survey stakeholder participants to assess change in knowledge based on project information and findings presented. Also, the team will aim to conduct industry interviews to assess adoption of technologies and techniques evaluated in the project.

To date, the research and extension team have been actively sharing research findings to local grower stakeholder participants with the goal of 200 professionals reached. The experimental data collected and the resulting QMRA will be used to monitor the project's success in increasing food safety knowledge through the number of stakeholders that gained knowledge about control and/or intervention food safety practices.

Summary of Findings

- Agricultural water treatment using PAA and/or calcium hypochlorite reduced risk for all contamination scenarios evaluated indicating the added beneficial impact of water treatment on bacteria that may be present on leaf surface or in soil.

- The animal intrusion/fecal slurry (FC) scenario demonstrated the highest risk and the least reduction with the addition of agricultural water treatment likely due to the presence of additional organic matter as well as elevated concentrations of bacteria typically found in feces.
- Calcium hypochlorite was more successful as a disinfectant, with higher reductions in risk than PAA for field trials.
- The QMRA point-estimate approach demonstrates the relative risk reduction of applying disinfectants to the different contamination scenarios, highlighting the most effective applications to reduce positive detections in each case
- The probabilistic QMRA method evaluated used a Monte Carlo simulation to capture variability in bacteria concentrations and exposure, specifically for *E. coli* that were measured in the irrigation system.
- The risk assessment demonstrated that *E. coli* concentration was the most sensitive parameter, highlighting the importance of reducing bacterial load in irrigation water for irrigation of produce, as well as the importance of treating the water for bacterial loads which may already be present regardless of contamination events.
- Persistence trials demonstrated regrowth in many cases and non-linear or first order reduction, and should be further examined prior to inclusion in QMRA risk estimates incorporating field persistence factors.
- Overall, treated irrigation water regardless of the sanitizer used or residual concentration (ppm) will typically kill off *E. coli* O157:H7 and/or generic *E. coli* in soil within 72 hrs post exposure particularly at lower concentrations of the bacteria.
- Exposure to treated irrigation water will significantly reduce the population of *E. coli* O157:H7 and/or generic *E. coli* established on romaine lettuce leaves, but it will not eliminate it completely regardless of the type of sanitizer used, concentration of the sanitizer, or amount of *E. coli* present on the plant.
- The amount of reduction may be variable depending on the sanitizer type and/or concentration residual (ppm).

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APPENDICES

Publications and Presentations

Presentations:

1. *Rock, C., Hamilton, K., Cooper, K. Quon, H., Obergh, V., Bowen, D., Gerba, C., and Bright, K.* **Microbiological risk assessment using QMRA in preharvest agriculture water treatment systems for leafy greens.** Center for Produce Safety Research Symposium 2023 (poster)

2. *Rock, C., Hamilton, K., Cooper, K. Quon, H., Obergh, V., Bowen, D., Gerba, C., and Bright, K.* **Microbiological risk assessment using QMRA in preharvest agriculture water treatment systems for leafy greens.** Center for Produce Safety Research Symposium 2024 (poster & oral presentation)

3. **Rock, C., Hamilton, K., Cooper, K. Quon, H., Obergh, V., Bowen, D., Gerba, C., and Bright, K.* **Microbiological risk assessment using QMRA in preharvest agriculture water treatment systems for leafy greens.** Center for Produce Safety Research Symposium 2025 (oral presentation) **future presentation*

Budget Summary

This project was awarded $407,697 in research funds. Over the course of the study, the research and extension team utilized funds to support students and research staff salaries at both the University of Arizona and Arizona State University. Additionally, funds were used to conduct both field and laboratory evaluation of agricultural water treatment impact on bacterial die-off over time, with most of the funds used towards cultural and molecular media and reagents to determine bacterial concentrations both before and after treatment on lettuce, soil, and in agricultural water. Travel funds were used to support the project PI and Co-PIs, students, and staff to conduct field experiments and/or attend the annual Center for Produce Safety Research Symposium in 2023, 2024 and 2025.

Tables and Figures & Supplemental Figures and Tables (see below)

Table 1. Field Contamination Scenarios

Scenario label	**Definition and description**	**Inoculum concentration (CFU/mL)**
AD	*Atmospheric deposition*. TVS353 was added to lettuce through direct application though calibrated back-pack sprayer with known volume per time.	64
FC	*Fecal slurry (animal intrusion).* TVS353 was added, mimicking animal intrusion (combining known volumes of animal fecal matter with a buffer and the inoculum at a certain level, similar to cow or chicken manure).	68,000
NT	*Treatment failure.* TVS353 was added, to mimic a non-successful water treatment episode, assuming *E. coli* is already present in the water supply.	60.5

Table 2. Percent positive lettuce and soil samples at 1 and 7 days post treatment (dpt)

CONTAMINATION TYPE	DAYS POST PAA TREATMENT (DPT)					
	1 day	7 days	1 day	7 days	1 day	7 days
	Lettuce Composite		Lettuce Head		Soil Composites	
Atmospheric Deposition (AD)	0% (0/18)	0% (0/18)	0% (0/90)	- -	11% (2/18)	0% (0/18)
Animal Intrusion (FC)	100% (18/18)	0% (0/18)	40% (36/90)	- -	22% (4/18)	33% (6/18)
Failed Treatment (NT)	22% (4/18)	0% (0/18)	4% (4/90)	- -	0% (0/18)	11% (2/18)

CONTAMINATION TYPE	DAYS POST CALCIUM HYPOCHLORITE TREATMENT (DPT)					
	1 day	7 days	1 day	7 days	1 day	7 days
	Lettuce Composite		Lettuce Head		Soil Composites	
Atmospheric Deposition (AD)	0% (0/18)	0% (0/18)	26% (24/90)	- -	0% (0/18)	0% (0/18)
Animal Intrusion (FC)	100% (18/18)	0% (0/18)	46% (42/90)	- -	78% (14/18)	67% (12/18)
Failed Treatment (NT)	0% (0/18)	0% (0/18)	0% (0/90)	- -	0% (0/18)	0% (0/18)

Table 3. Model Parameters and Definitions

Definition	Model parameter	Value or distribution	Units	Source
Concentration of *E. coli* on lettuce or in irrigation water	C	Empirical data (point estimate)	MPN/g	This study
Mass lettuce consumed per day	*M*	8.5 (point estimate)	g/d	(USDA, 2024)
Dose response (pooled *E. coli* model, infection endpoint)	Beta-Poisson model, α, β	α = 0.1778 β = 1.7796×10^6 (point estimate)	Unitless	(Haas et al., 1999)

Table 4. Total infection risk per exposure and percent change in risk for each scenario before and after treatment at the given inoculum levels (**Table 1**).

	Peracetic acid			Calcium hypochlorite		
	Before Treatment	After Treatment	Percent change	Before Treatment	After Treatment	Percent change
Atmospheric Deposition (AD)	7.58E-09	0	-100%	1.74E-08	4.86E-09	-71.99%
Animal Intrusion (FC)	1.83E-08	1.74E-08	-5.23%	3.73E-08	2.28E-08	-38.91%
Treatment failure (NT)	1.74E-08	1.54E-09	-91.10%	7.58E-09	0	-100%

Table 5. Model parameters for risk assessment model of IDEXX *E. coli* water concentrations.

Definition	Model parameter	Value or distribution		Units	Source
Concentration of *E. coli* on lettuce or in irrigation water	*C*	Lognormal distributions fit to empirical data:		MPN/100mL	This study
		PAA BG	μ = 2.034, σ = 0.617		
		PAA First	μ = -5.95, σ = 6.018		
		PAA Last	μ = -4.53, σ = 6.094		
		Calcium hypochlorite BG	μ = 3.772, σ = 0.644		
		Calcium hypochlorite First	μ = -14.7, σ = 11.669		
		Calcium hypochlorite Last	μ = -6.743, σ = 8.418		
Mass lettuce consumed per day	*M*	Uniform (7,10)		g/d	(USDA, 2024)
Water holding on lettuce	*W*	Normal (μ = 0.108, σ = 0.019)		mL/g	(Hamilton et al., 2006)
Dose response (pooled *E. coli* model, infection endpoint)	Beta-Poisson model, α, β	α = 0.1778 $\beta = 1.7796 \times 10^6$		Unitless	(Haas et al., 1999)

Table 6. Data types and measurements for persistence trials.

Experiment	Inoculum	Treatment	Time (hours)	Bacteria Strain	Location
Lettuce head	Low (10^2 CFU), High (10^4 CFU)	Irrigation control, Calcium hypochlorite 2 ppm, Calcium hypochlorite 4 ppm, PAA 7 ppm, PAA 10 ppm	0, 12, 24, 48, 72, 168	TW14359[a], REPEXH01[b], K-12 sub-strain MG1655[c], TVS353[d]	Yuma, AZ
Soil	Low (10^2 CFU), High (10^4 CFU)	Irrigation control, Calcium hypochlorite 2 ppm, Calcium hypochlorite 4 ppm, PAA 7 ppm, PAA 10 ppm	0, 12, 24, 48, 72, 168, 336, 504	TW14359, REPEXH01, K-12 sub-strain MG1655, TVS353, K-12[e]	Yuma, AZ, Maricopa, AZ

[a]pathogenic *E. coli* strain from spinach; [b]pathogenic *E. coli* strain from romaine lettuce; [c]generic *E. coli* strain; [d]generic *E. coli*, rifampicin resistant; [e]generic *E. coli* strain, isolated from stool sample.

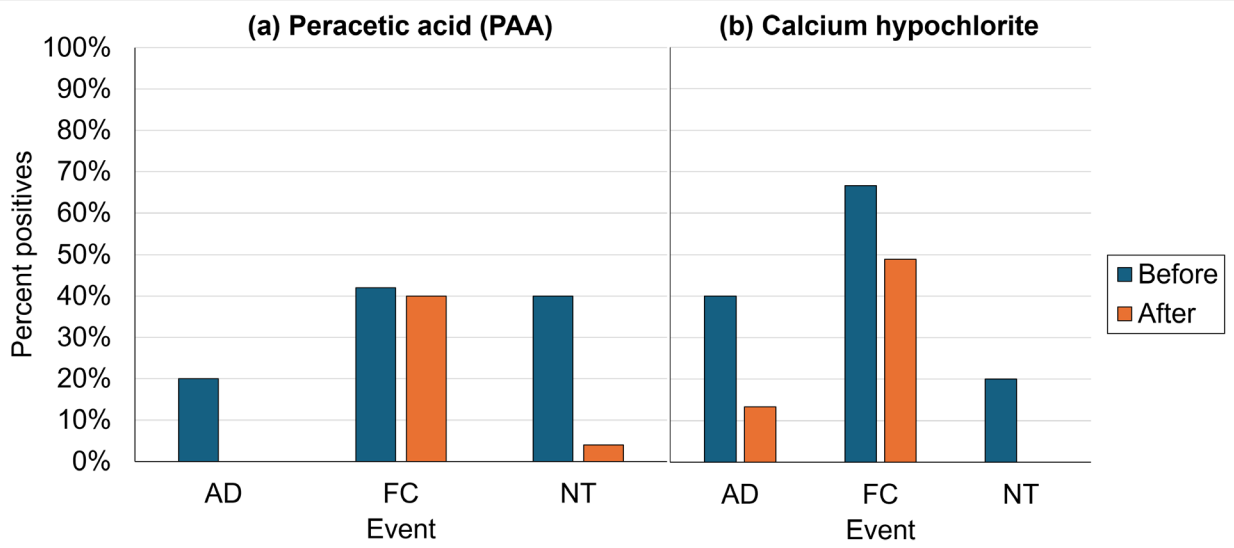


Figure 1. Percent positive detections for *E. coli* on lettuce head field samples based on background (before) and post treatment of the indicated chemical application (after).

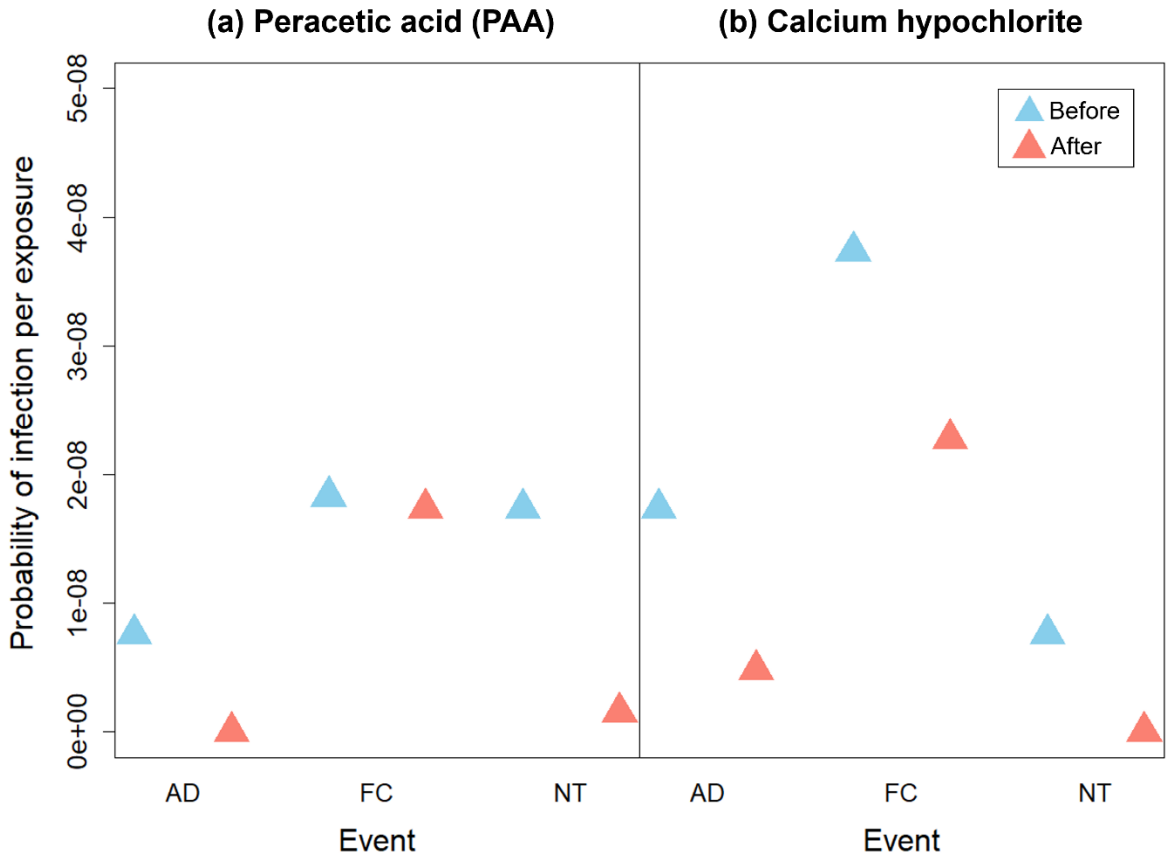


Figure 2. Total infection risk per exposure for each scenario before and after treatment at the given inoculum levels (**Table 1**).

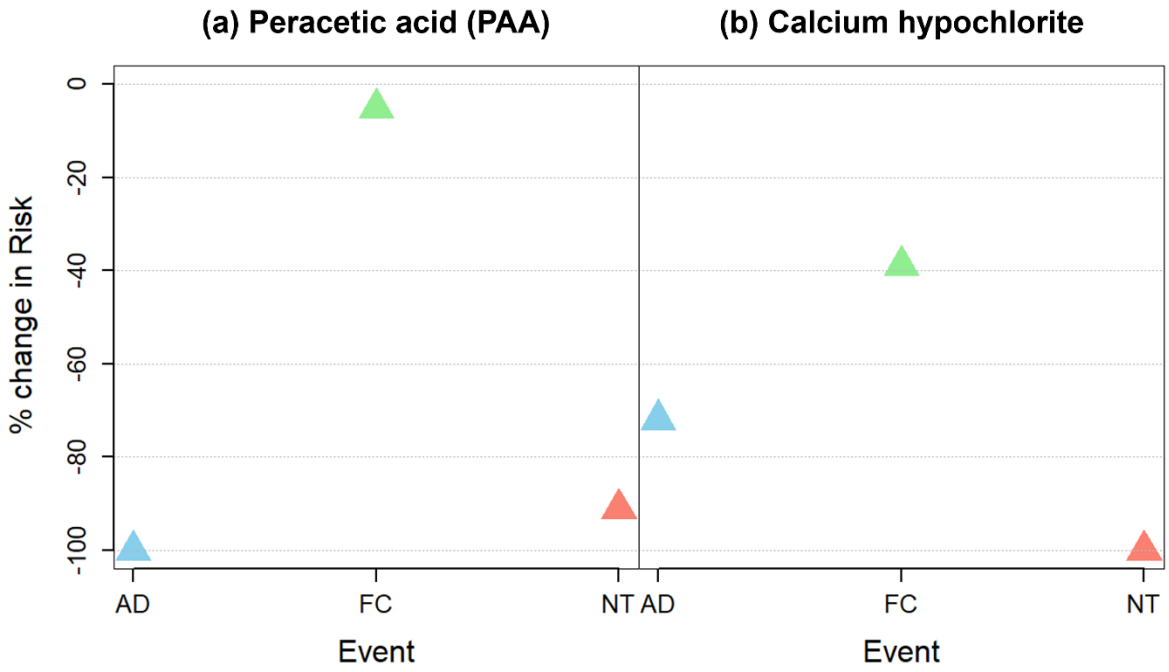


Figure 3. Percent change in risk from treatment after inoculation events. Note that "100%" refers to non-detect observations only present for that scenario after the disinfectant was applied (MPN formula does not address "non-detect" limits of detection; this is addressed for continuous data in the scenario that follows.

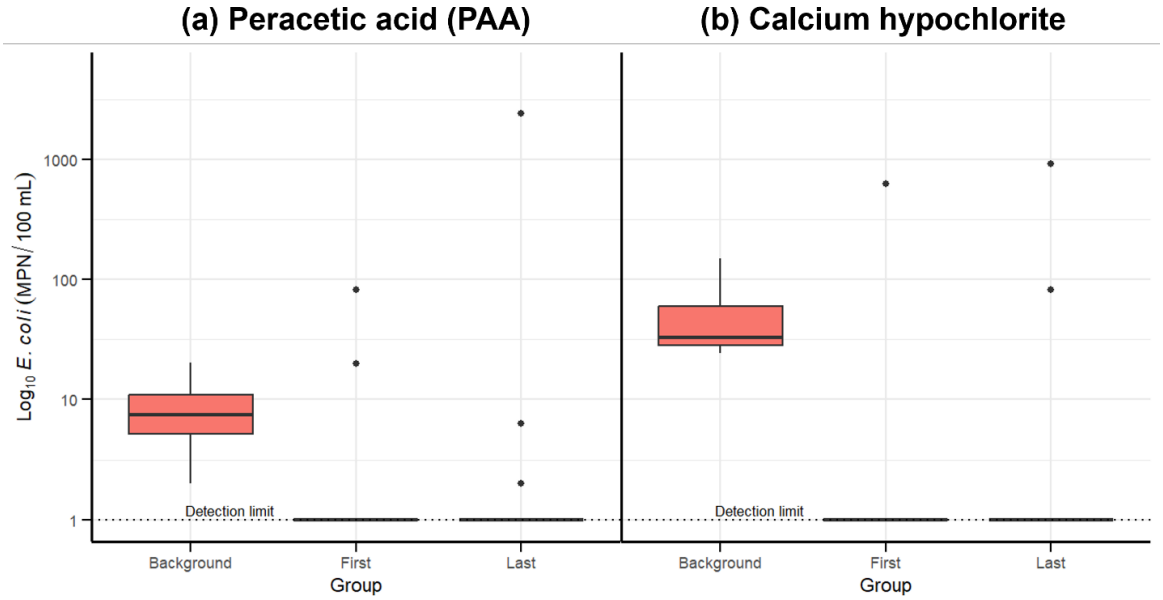


Figure 4. IDEXX *E. coli* results for irrigation water samples. Samples below the detection limit of 1 MPN/100mL were plotted at this limit (n=11/13 first, 10/13 last for PAA, 9/10 first, 8/10 last for calcium hypochlorite).

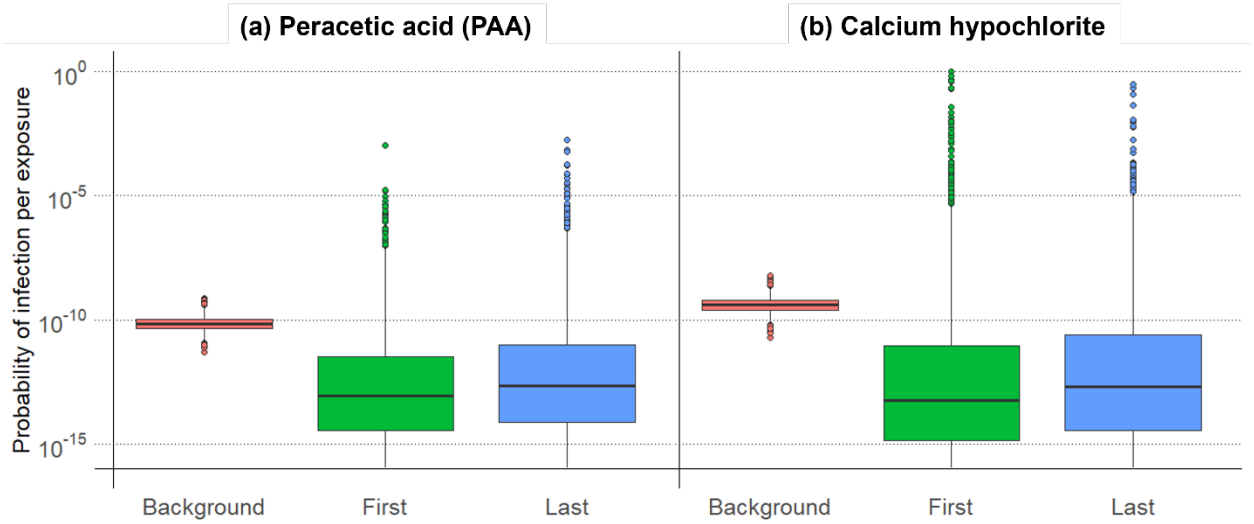


Figure 5. Infection risk per exposure based on measured concentrations of generic *E. coli* from the water used for irrigation.

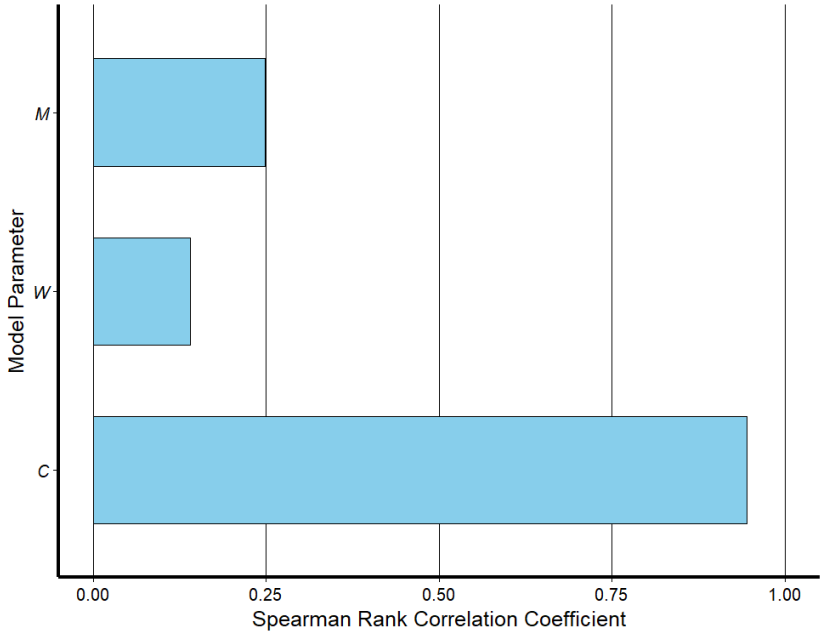


Figure 6. Spearman Rank correlation coefficient (ρ) for the Monte Carlo simulated values for the IDEXX risk assessment (**Table 3, Equation 5**). The concentration distribution is shown for the PAA background condition. The trend matches the other conditions evaluated as part of this study.

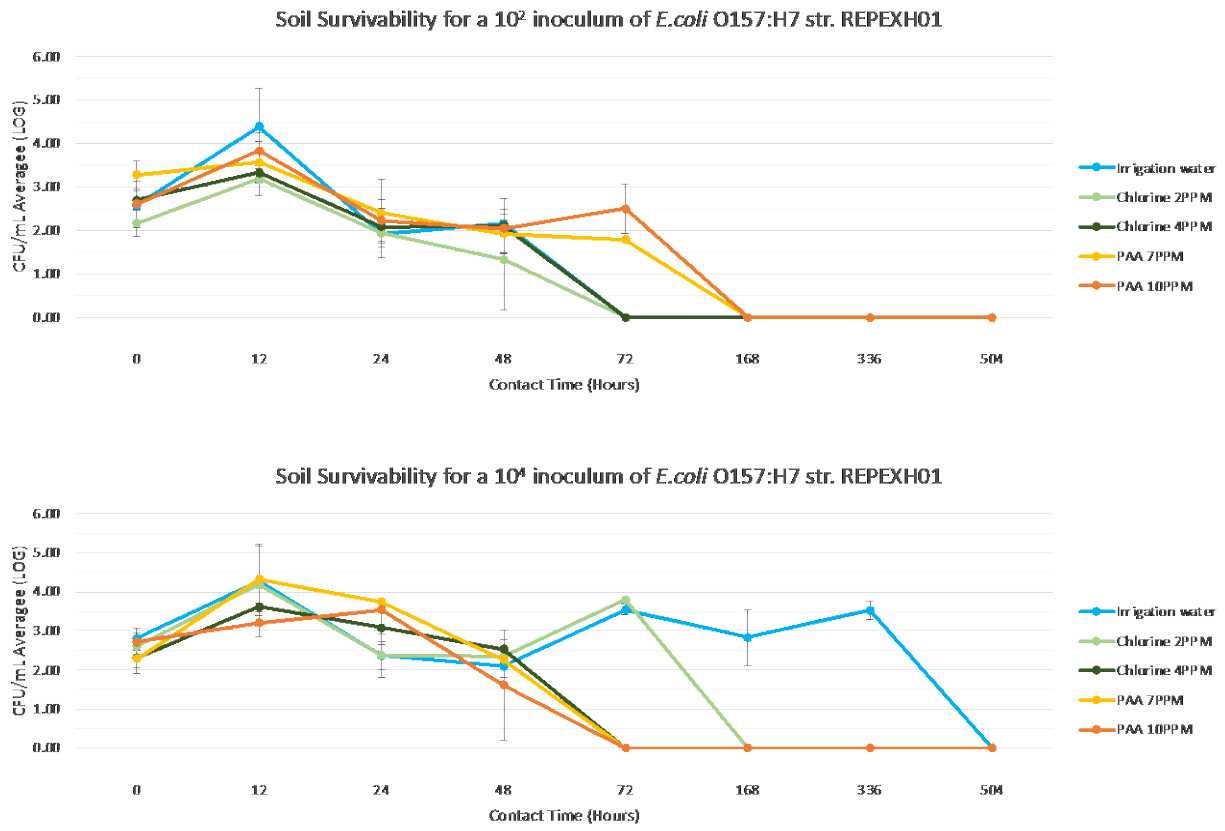
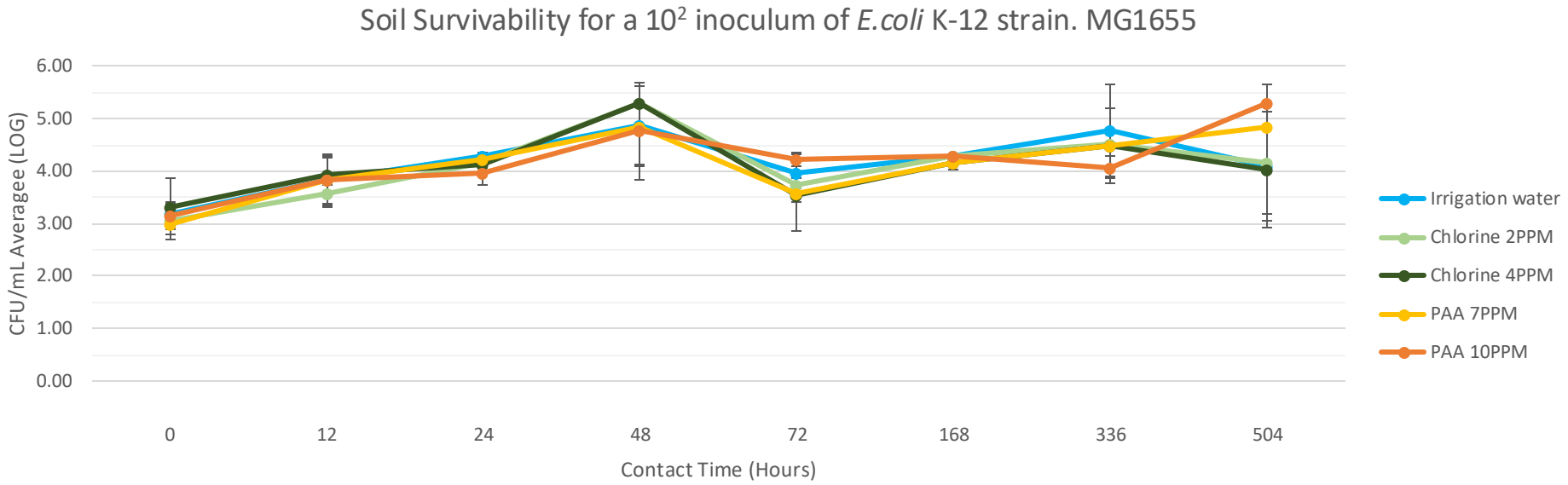
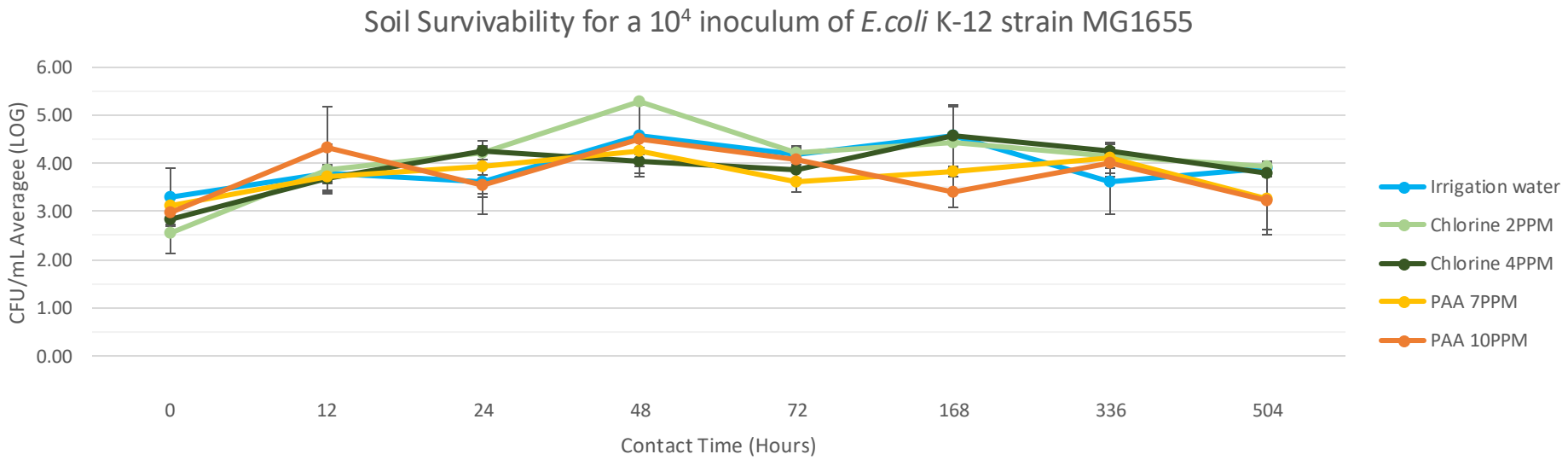


Figure 7. Survival rates of *E. coli* O157:H7 strain REPEXH01 (2018 romaine lettuce outbreak) in soils kept under Maricopa, AZ, conditions (light, temperature, and humidity) after exposure to different types and concentrations of sanitizers in irrigation water with high (10^4 CFU/g) -*bottom figure*- or low (10^2 CFU/g) –*top figure*- inoculums.

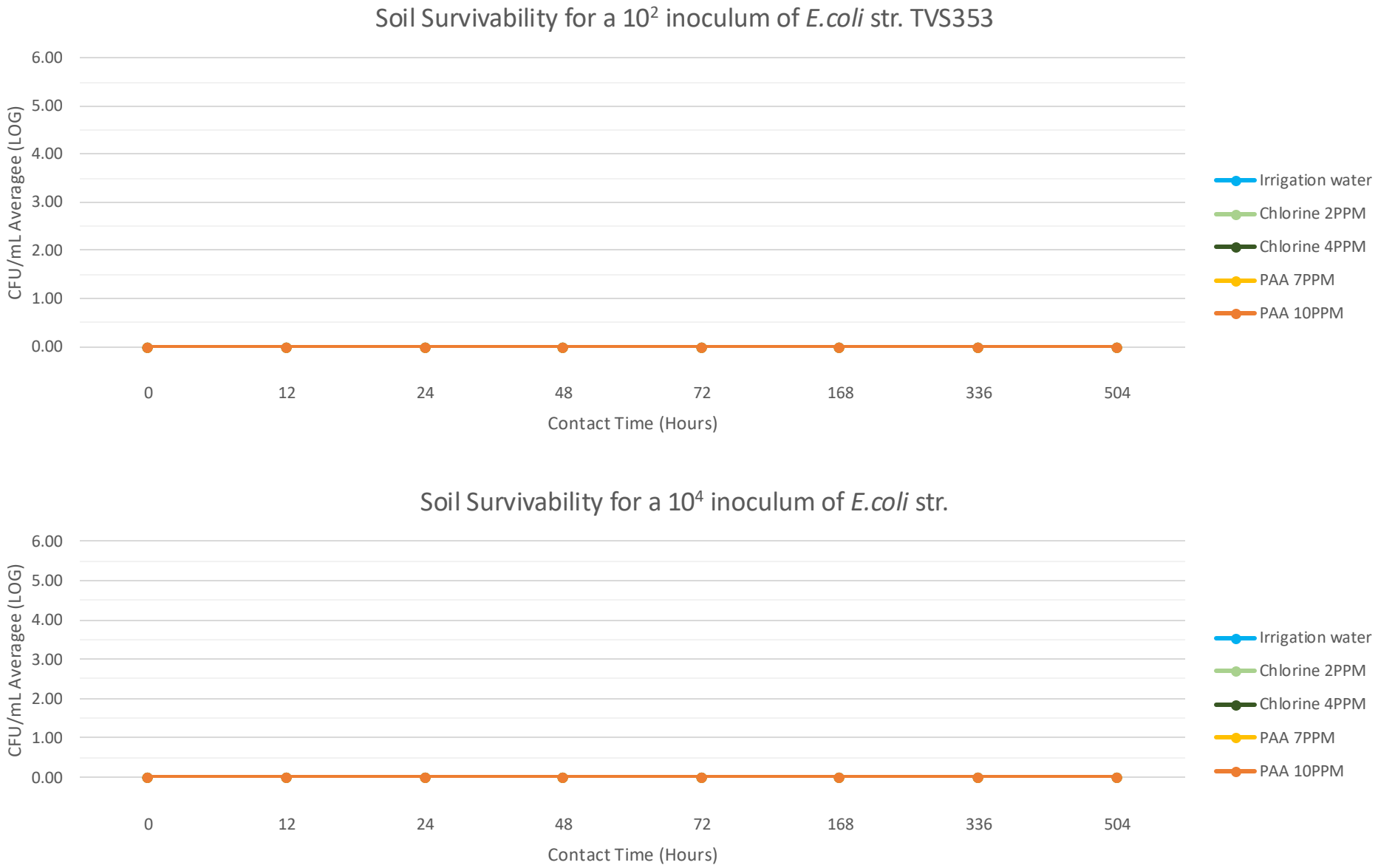
Supplemental Figure 1. Survival rates of *E. coli* O157:H7 strain TW14359 (2006 spinach outbreak) in soils under Yuma, AZ conditions (light, temperature, and humidity) after exposure to different types and concentrations of sanitizers in irrigation water with high (10^4 CFU/g) or low (10^2 CFU/g) inoculums.

Supplemental Figure 2. Survival rates of *E. coli* O157:H7 strain REPEXH01 (2018 romaine lettuce outbreak) in soils kept under Yuma, AZ conditions (light, temperature, and humidity) after exposure to different types and concentrations of sanitizers in irrigation water with high (10^4 CFU/g) or low (10^2 CFU/g) inoculums.

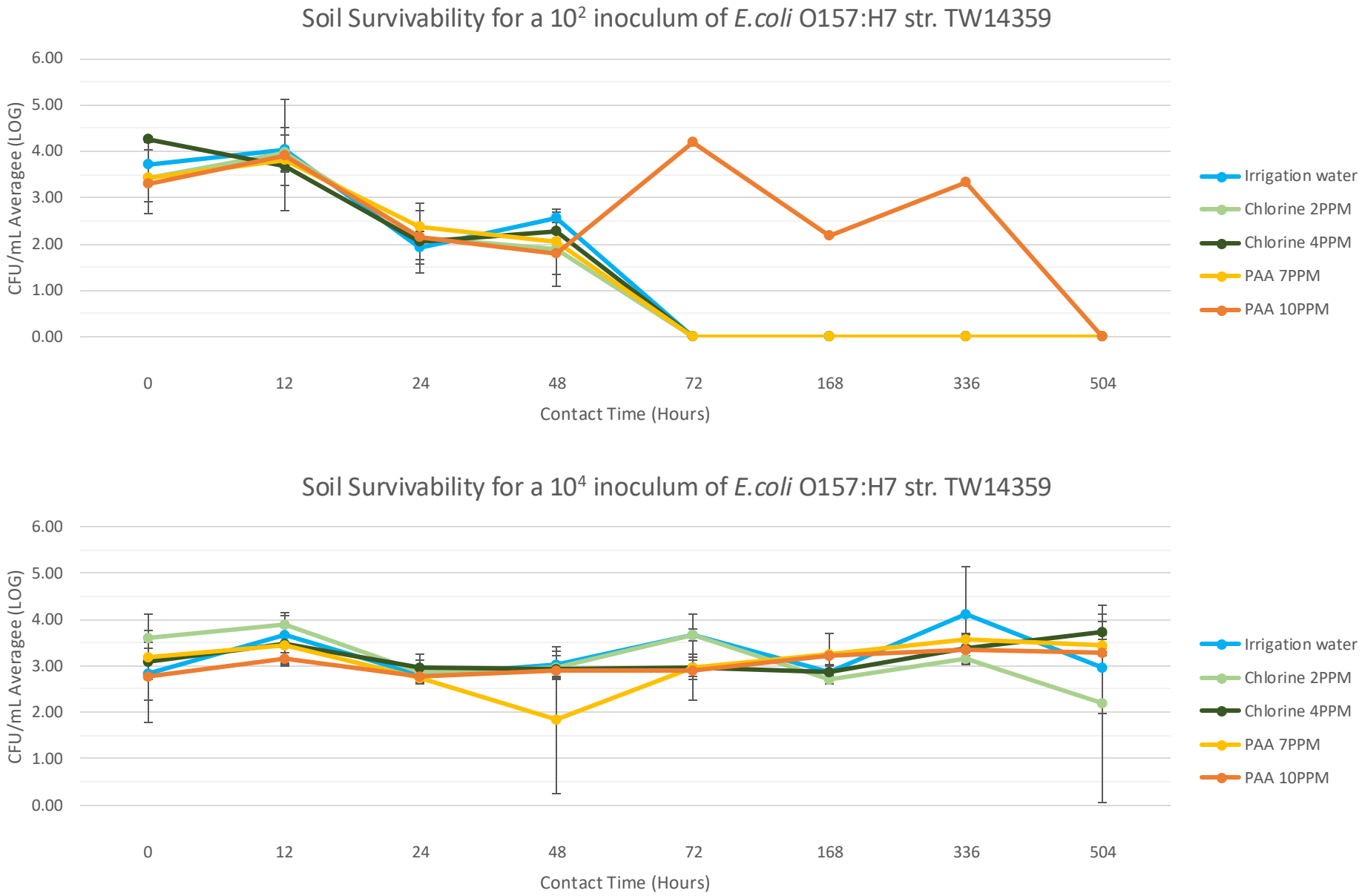




Supplemental Figure 3. Survival rates of generic *E. coli* K12 strain MG1655 in soils kept under Yuma, AZ conditions (light, temperature, and humidity) after exposure to different types and concentrations of sanitizers in irrigation water with high (10^4 CFU/g) or low (10^2 CFU/g) inoculums.

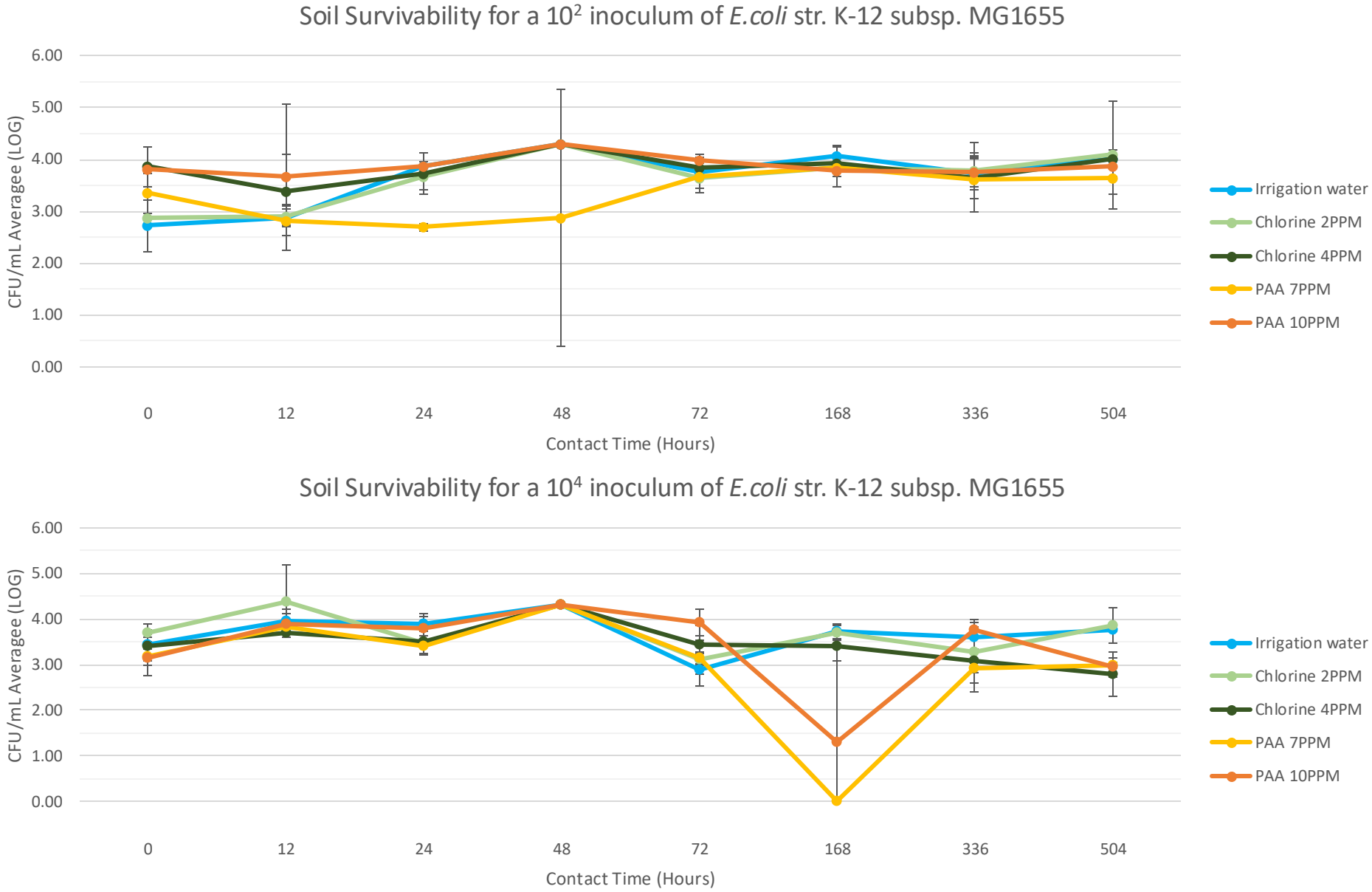


Supplemental Figure 4. Survival rates of generic *E. coli* strain TVS353 in soils kept under Yuma, AZ conditions (light, temperature, and humidity) after exposure to different types and concentrations of sanitizers in irrigation water with high (10^4 CFU/g) or low (10^2 CFU/g) inoculums.

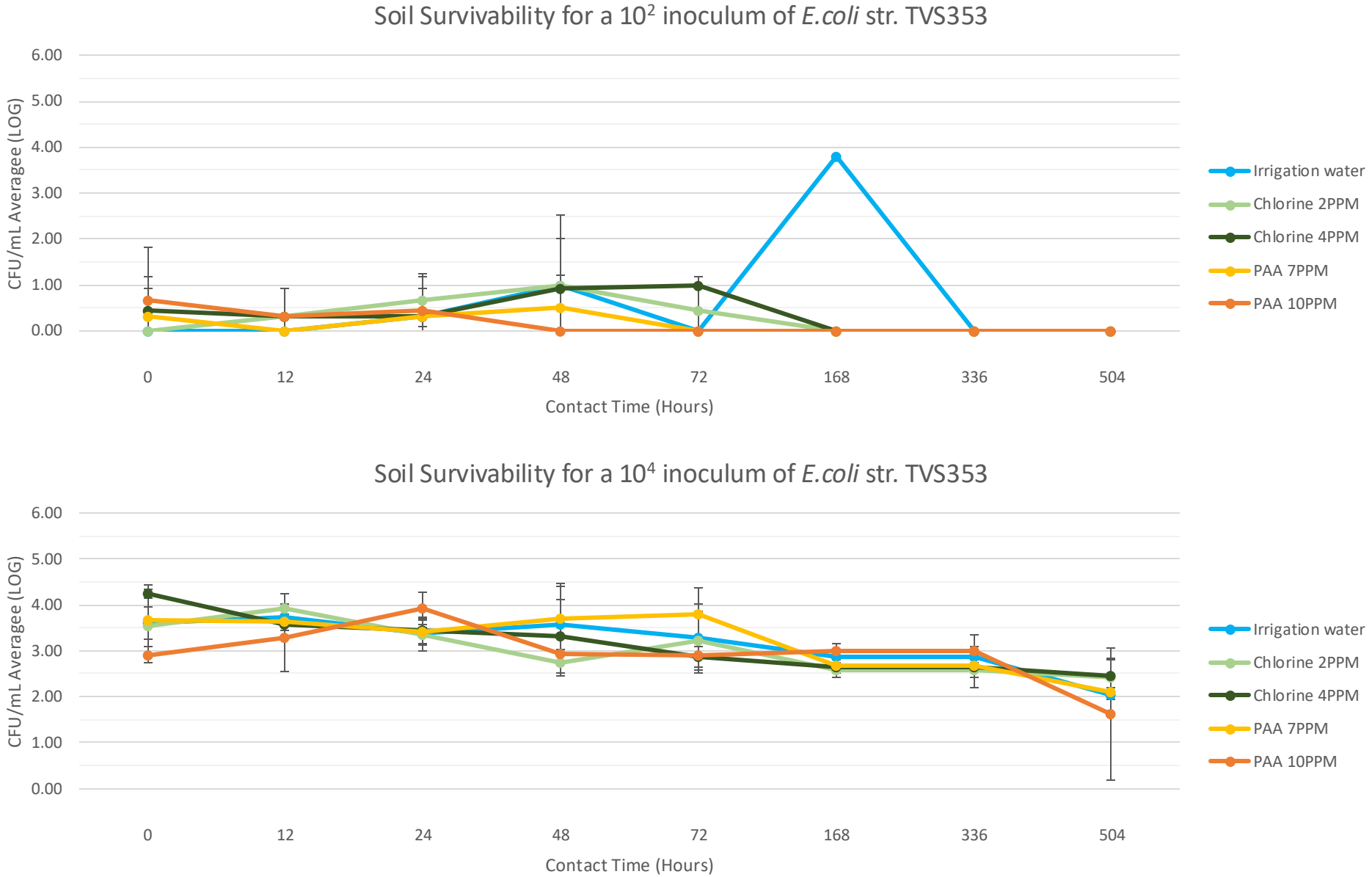


Supplemental Figure 5. Survival rates of *E. coli* O157:H7 strain TW14359 (2006 spinach outbreak) in soils under Maricopa, AZ conditions (light, temperature, and humidity) after exposure to different types and concentrations of sanitizers in irrigation water with high (10^4 CFU/g) or low (10^2 CFU/g) inoculums.

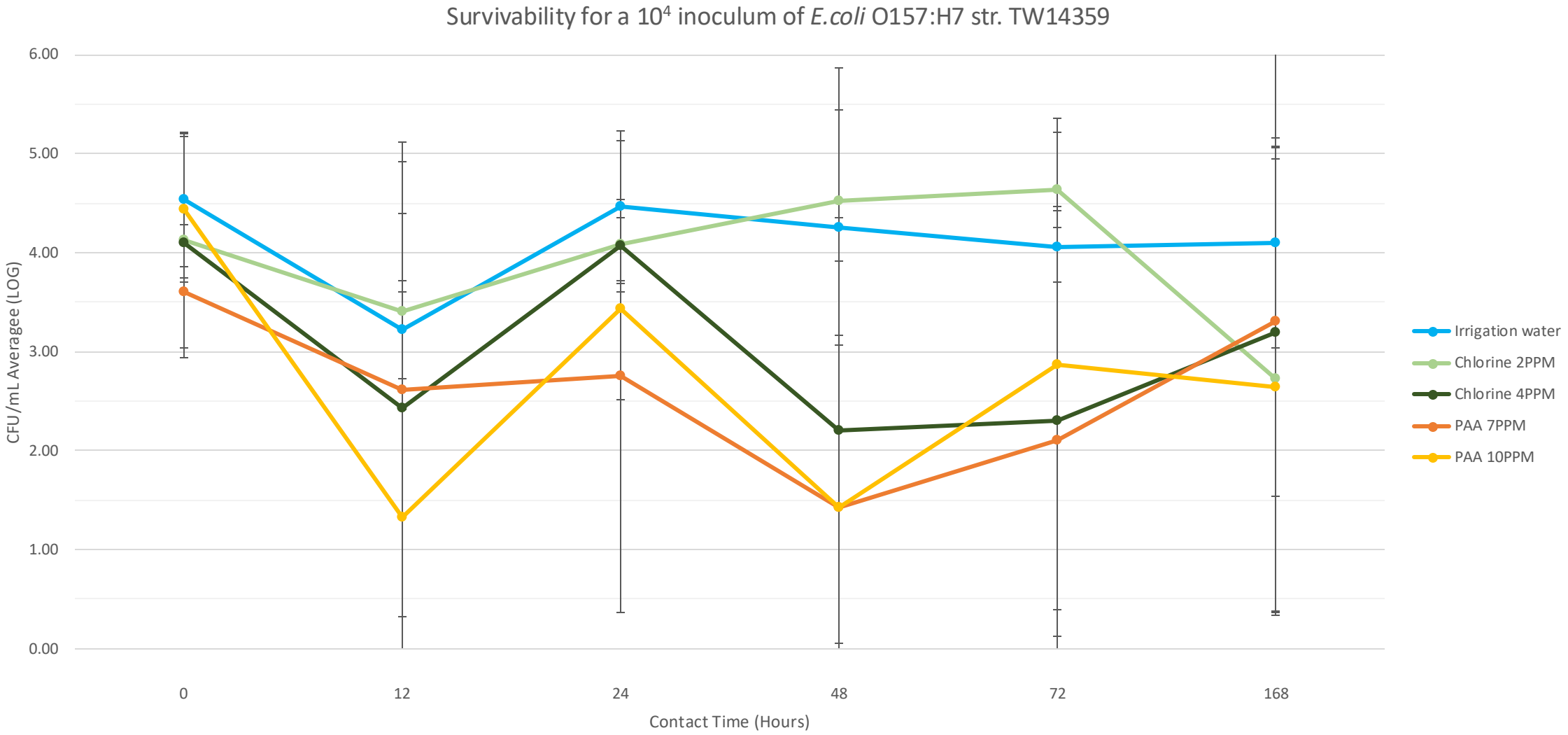
Supplemental Figure 6. Survival rates of *E. coli* O157:H7 strain REPEXH01 (2018 romaine lettuce outbreak) in soils kept under Maricopa, AZ conditions (light, temperature, and humidity) after exposure to different types and concentrations of sanitizers in irrigation water with high (10^4 CFU/g) or low (10^2 CFU/g) inoculums.



Supplemental Figure 7. Survival rates of generic *E. coli* K12 strain MG1655 in soils kept under Maricopa, AZ conditions (light, temperature, and humidity) after exposure to different types and concentrations of sanitizers in irrigation water with high (10^4 CFU/g) or low (10^2 CFU/g) inoculums.



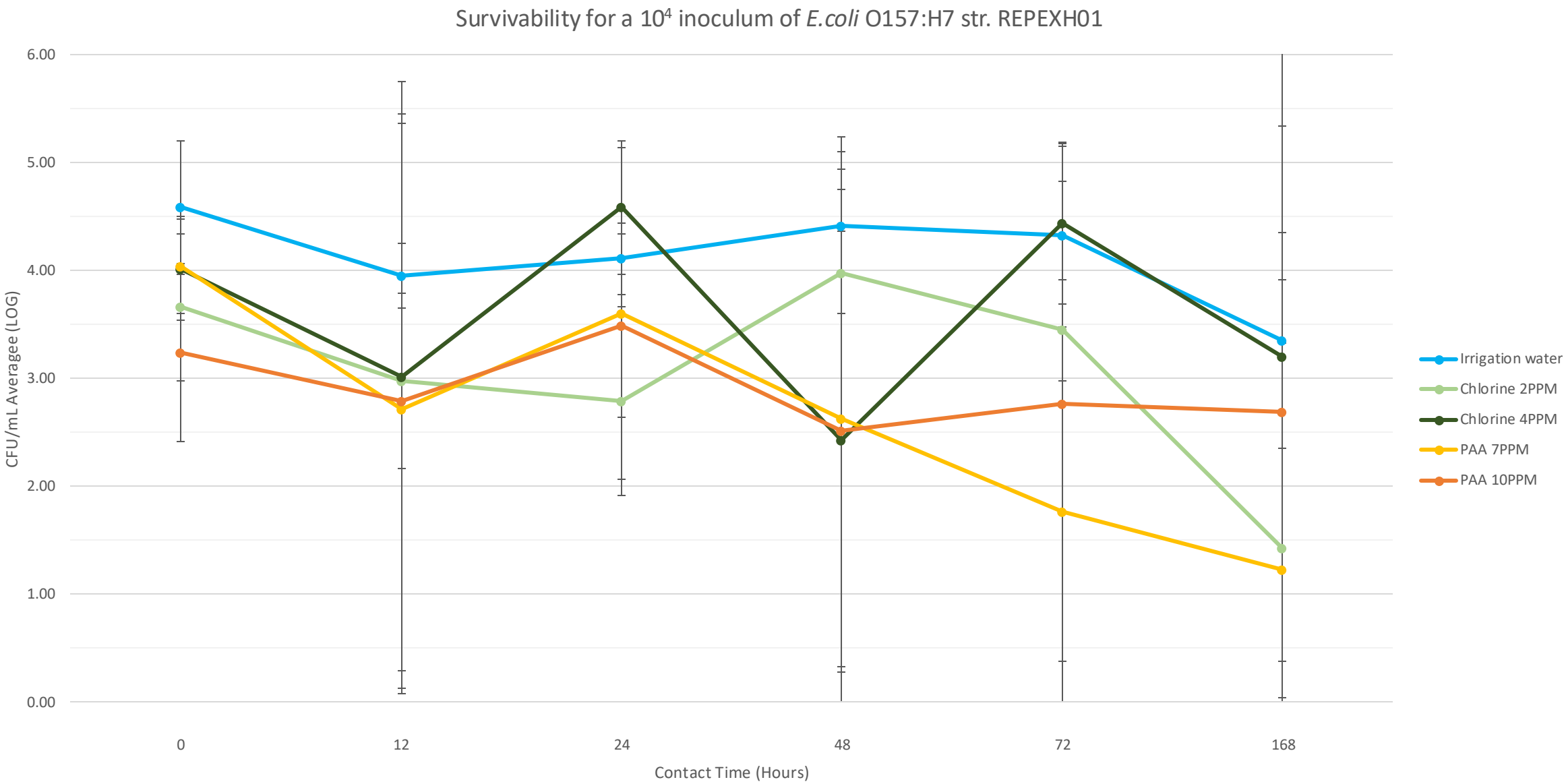
Supplemental Figure 8. Survival rates of generic *E. coli* strain TVS353 in soils kept under Maricopa, AZ conditions (light, temperature, and humidity) after exposure to different types and concentrations of sanitizers in irrigation water with high (10^4 CFU/g) or low (10^2 CFU/g) inoculums.



Supplemental Figure 9. Survival rates of *E. coli* O157:H7 strain TW14359 (2006 spinach outbreak) on romaine lettuce under Yuma, AZ conditions (light, temperature, and humidity) after exposure to different types and concentrations of sanitizers in irrigation water with high (10^4 CFU/g) inoculum.

Supplemental Table 1. Number of romaine lettuce samples kept under Yuma, AZ conditions that were positive for *E. coli* O157:H7 strain TW14359 following enrichment post exposure to sanitizer treated irrigation water

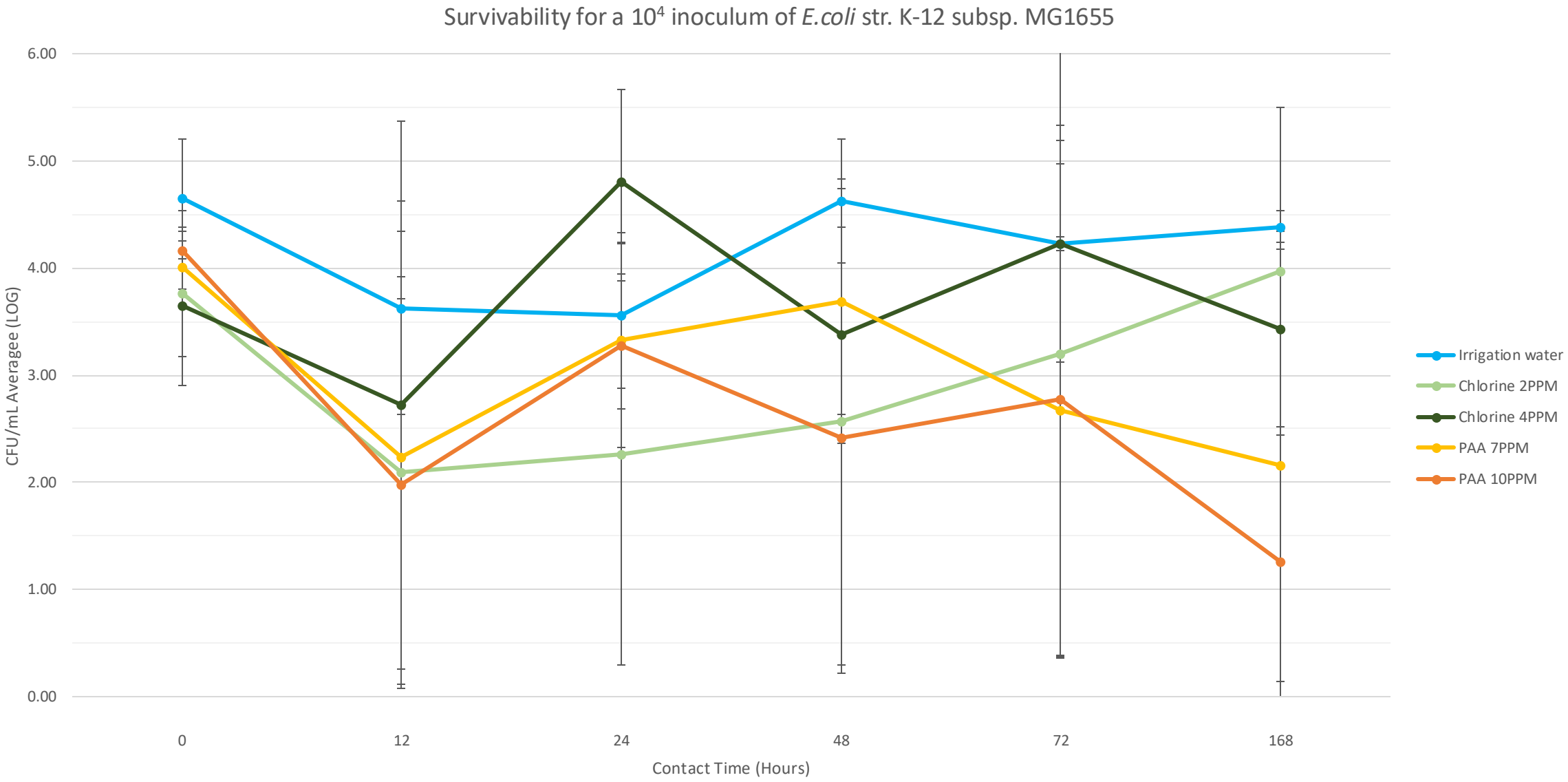
Inoculum	Sanitizer	0 hours	12 hours	24 hours	48 hours	72 hours	168 hours
10^2	Irrigation water	3/3	3/3	3/3	2/3	2/3	2/3
	Chlorine 2PPM	3/3	3/3	3/3	3/3	3/3	1/3
	Chlorine 4 PPM	2/3	3/3	3/3	2/3	2/3	2/3
	PAA 7 PPM	3/3	2/3	2/3	1/3	1/3	1/3
	PAA 10PPM	2/3	3/3	2/3	1/3	1/3	1/3
10^4	Irrigation water	3/3	3/3	3/3	3/3	3/3	3/3
	Chlorine 2PPM	3/3	3/3	3/3	2/3	2/3	3/3
	Chlorine 4 PPM	3/3	3/3	3/3	3/3	3/3	3/3
	PAA 7 PPM	3/3	3/3	3/3	2/3	2/3	2/3
	PAA 10PPM	3/3	3/3	3/3	3/3	3/3	3/3



Supplemental Figure 10. Survival rates of *E. coli* O157:H7 strain REPEXH01 (2018 romaine lettuce outbreak) on romaine lettuce under Yuma, AZ conditions (light, temperature, and humidity) after exposure to different types and concentrations of sanitizers in irrigation water with high (10^4 CFU/g) inoculum.

Supplemental Table 2. Number of romaine lettuce samples kept under Yuma, AZ conditions that were positive for *E. coli* O157:H7 strain REPEXH01 following enrichment post exposure to sanitizer treated irrigation water

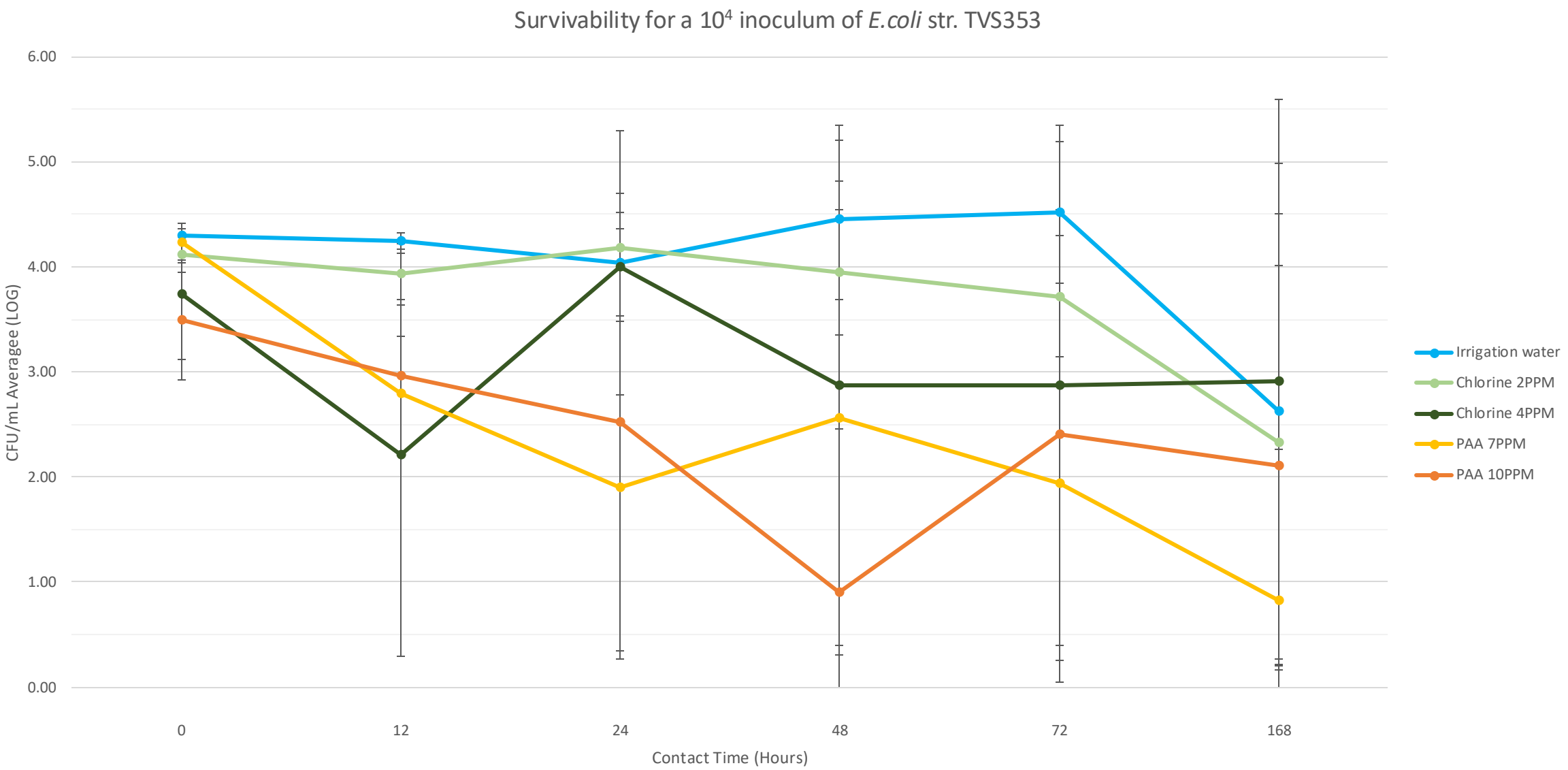
Inoculum	Sanitizer	0 hours	12 hours	24 hours	48 hours	72 hours	168 hours
10^2	Irrigation water	3/3	3/3	3/3	3/3	3/3	3/3
	Chlorine 2PPM	3/3	3/3	3/3	3/3	3/3	3/3
	Chlorine 4 PPM	3/3	3/3	3/3	2/3	2/3	2/3
	PAA 7 PPM	3/3	3/3	2/3	1/3	1/3	1/3
	PAA 10PPM	3/3	3/3	1/3	0/3	1/3	1/3
10^4	Irrigation water	3/3	3/3	3/3	3/3	3/3	3/3
	Chlorine 2PPM	3/3	3/3	3/3	3/3	3/3	3/3
	Chlorine 4 PPM	3/3	3/3	3/3	3/3	2/3	2/3
	PAA 7 PPM	3/3	3/3	3/3	2/3	3/3	2/3
	PAA 10PPM	3/3	3/3	3/3	2/3	3/3	3/3



Supplemental Figure 11. Survival rates of generic *E. coli* K12 strain MG1655 on romaine lettuce under Yuma, AZ conditions (light, temperature, and humidity) after exposure to different types and concentrations of sanitizers in irrigation water with high (10^4 CFU/g) inoculum.

Supplemental Table 3. Number of romaine lettuce samples kept under Yuma, AZ conditions that were positive for generic *E. coli* K12 strain MG1655 following enrichment post exposure to sanitizer treated irrigation water

Inoculum	Sanitizer	0 hours	12 hours	24 hours	48 hours	72 hours	168 hours
10^2	Irrigation water	3/3	2/3	3/3	3/3	2/3	3/3
	Chlorine 2PPM	3/3	1/3	3/3	3/3	2/3	3/3
	Chlorine 4 PPM	3/3	2/3	2/3	2/3	0/3	2/3
	PAA 7 PPM	3/3	3/3	2/3	2/3	2/3	1/3
	PAA 10PPM	3/3	3/3	3/3	3/3	3/3	1/3
10^4	Irrigation water	3/3	3/3	3/3	3/3	3/3	3/3
	Chlorine 2PPM	3/3	3/3	3/3	3/3	3/3	3/3
	Chlorine 4 PPM	3/3	3/3	3/3	3/3	3/3	3/3
	PAA 7 PPM	3/3	3/3	3/3	3/3	3/3	3/3
	PAA 10PPM	3/3	3/3	3/3	1/3	1/3	2/3



Supplemental Figure 11. Survival rates of generic *E. coli* K12 TVS353 on romaine lettuce under Yuma, AZ conditions (light, temperature, and humidity) after exposure to different types and concentrations of sanitizers in irrigation water with high (10^4 CFU/g) inoculum.

Supplemental Table 4. Number of romaine lettuce samples kept under Yuma, AZ conditions that were positive for generic *E. coli* strain TVS353 following enrichment post exposure to sanitizer treated irrigation water

Inoculum	Sanitizer	0 hours	12 hours	24 hours	48 hours	72 hours	168 hours
10^2	Irrigation water	3/3	3/3	2/3	3/3	3/3	2/3
	Chlorine 2PPM	3/3	3/3	2/3	3/3	2/3	2/3
	Chlorine 4 PPM	3/3	2/3	3/3	3/3	3/3	2/3
	PAA 7 PPM	3/3	2/3	1/3	1/3	3/3	0/3
	PAA 10PPM	3/3	3/3	0/3	1/3	2/3	0/3
10^4	Irrigation water	3/3	3/3	3/3	3/3	3/3	2/3
	Chlorine 2PPM	3/3	3/3	3/3	3/3	3/3	2/3
	Chlorine 4 PPM	3/3	3/3	3/3	3/3	3/3	2/3
	PAA 7 PPM	3/3	3/3	3/3	3/3	3/3	2/3
	PAA 10PPM	3/3	3/3	3/3	3/3	2/3	2/3