Pathogen Occurrence in Treated Domestic Wastewater intended for Irrigation

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The goal of this project was to apply the latest methods for the detection of microbial pathogens in water to assess the performance of these facilities and the suitability of the treated wastewater for crop irrigation. Samples of treated wastewater were collected multiple times from six different wastewater treatment facilities in Arizona. Treatment at four of the plants involved advanced Bardenpho processes followed by chlorination and de-chlorination. Two of the other facilities used ultraviolet light as the primary disinfection process. The plants using UV light and one of the plants using chlorination filtered the treated wastewater. Samples were tested for human enteric viruses, viral indicators, *Cryptosporidium*, *Giardia*, *Cyclospora*, *Salmonella enterica* and *Listeria monocytogenes*. Enteric viruses were assessed by an integrated cell culture real time polymerase chain reaction (ICC-qPCR) assay and protozoan parasites by USEPA Method 1623 with some modifications for the analysis of *Cyclospora*. S. *enterica* and *L. monocytogenes* were recovered by filtration through Moore Swab followed by non-selective/selective enrichments and plating on selective media. Presumptive positives were confirmed using PCR/qPCR. None of the samples tested were positive for *S. enterica* or *L. monocytogenes. A*denovirus, enterovirus, and reovirus were detected in a facility after chlorination (sample size 40 to 100 liters) but not in the effluent while viral indicators were detected by qPCR in most facilities. No protozoa were detected in 20 – 60 liters. The results of this study indicate that in most cases pathogen levels were below detection meeting current standards in Arizona for irrigation of food crops.

Green Sanitizers: Improved Safety and Shelf-life of Iceberg Lettuce Washed with Plant-based Antimicrobial Microemulsions

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The produce industry currently uses chlorine to wash vegetables post-harvest. However, chlorine is disadvantageous because it is not sustainable to the environment, not user friendly, corrosive to equipment, and loses efficacy with organic matter. Consumers prefer natural over chemical sanitizers. Alternatives for wash sanitizers must be employed to better suit the needs of consumers and for improving food safety. Plant-based antimicrobials in the wash water were evaluated for their efficiency against a foodborne pathogen *Salmonella enterica* and a spoilage organism *Lactobacillus casei* on Iceberg lettuce. Wash microemulsions that were assessed included oregano oil, lemongrass oil, and cinnamon oil along with a plant emulsifier for improved solubility of the oil in water. Iceberg lettuce (10 g) was washed thoroughly and inoculated with either *L. casei* (6.0 log CFU/g) or *S. enterica* serotype Newport (6.0 log CFU/g). The leaves were treated with 0.1%, 0.3%, or 0.5% of one of the treatment solutions, stored at 4^{o}C, observed, and analyzed for surviving populations of *L. casei* and *S.* Newport on days 0, 3, 7, 10, 14, 21, and 28. The efficacies of the antimicrobials were concentration- and storage-time dependent. The essential oil microemulsions exhibited a 0.5-5 log CFU/g reduction in *S.* Newport population throughout the various time points during days 0-28. The essential oil microemulsions were also effective against *L. casei* resulting in 1-3 log CFU/g reduction during storage at days 0-28. The essential oil microemulsions provide natural, eco-friendly, and effective alternatives to chemicals for produce decontamination, along with lasting residual activity and recyclability.

Cyclospora: Potential Reservoirs and Occurrence in Irrigation Waters

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Cyclospora cayetanensis has been associated with cyclosporiasis outbreaks from produce imported from Mexico since the mid '90s and more recently in the US. This study examined the occurrence of *C. cayetanensis* in irrigation waters, raw sewage, and treated wastewater from produce growing regions of Yuma, Arizona, the Upper Rio Grande Valley of Texas/New Mexico, and El Paso, Texas. *C. cayetanensis* was concentrated using Gelman Envirochek high-volume (HV) filters, by filtering 100-liter samples of irrigation water, treated wastewater and collecting 1 Liter of raw sewage samples. A TaqMan assay probe targeting the internal transcriber 2 gene (ITS2) was developed and tested for detection of *C. cayetanensis* in environmental water samples using quantitative PCR. A total of 181 irrigation water samples were collected over a 2-year period from both regions and 243 wastewater treatment plant (WWTP) samples were collected from six WWTPs (three each in AZ and TX). Samples tested presumptively positive for *C. cayetanensis* in irrigation waters. Results were 15/119 (~13%), 3/62 (~5%) in AZ and TX, respectively. In WWTP samples, results yielded 76/165 (~46%), and 28/78 (~36%) in AZ and TX, respectively. This study showed that *C. cayetanensis* is presumptively present in irrigation waters in Arizona and Texas and a complete a risk assessment study should be performed to determine the risk of produce being contaminated. The WWTP findings indicate that there is a presumptive incidence of *C. cayetanensis* infection among both the Arizona and Texas districts. Further studies are needed to confirm presumptive positive samples by sequencing or alternative.

Acknowledgements:
- This research was funded by Center for Product Safety (CPS)
- Western Alliance to Expand Student Opportunities (WAESO)

Identification of sources of fecal contamination in irrigation waters for production of leafy green crops in Arizona

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Irrigation water has been implicated as a major source of pathogen contamination in leafy greens. This study investigates potential sources of contamination with foodbome pathogens in irrigation waters using genetic markers associated with fecal bacteria unique to cattle, swine, birds, and humans. The presence of Salmonella was also investigated as a major foodborne pathogen along with generic *E. coli*, the microbial standard established on the Food and Drug Administration (FDA) Food Safety Modernization Act (FSMA) for irrigation waters. Currently, one hundred twenty nine water samples for irrigation canals have been collected from two major water irrigation districts in Arizona. Membrane filtration is used for concentration of fecal bacteria and *Salmonella* while generic *E. coli* is evaluated with Colilert® defined substrate technology. Out the 129 samples collected, 10 of them (8%) has exceeded 126 colony forming units (CFU)/100 mL (FSMA microbial standard), with four confirmed *Salmonella* species by API strip and 16S rDNA sequencing. Quantitative real time polymerase chain reaction assays will be used for detection and quantification of host-specific bacterial genomes, which will help us to estimate levels and sources of fecal contamination. The assessment of fecal genetic markers will aid in the accurate identification of the type of host inputs (e.g., sewage, wildlife, agricultural) and consequently the reservoirs or sources of foodbome pathogens like Salmonella that will result in a more accurate assessment of the risks to human health.

Presentation Title: Efficacy of Peracetic Acid (PAA) on Agricultural Irrigation Water to Reduce Microbiological Pathogens and Indicators

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In response to recent *Escherichia coli* (*E. coli*) O157:H7 outbreak(s) involving romaine lettuce in which agricultural water was suspected as the source, new food safety metrics demand surface water used for overhead irrigation be treated to limit transfer to produce eaten fresh. These rapid changes have left the fresh produce industry with many unresolved questions as to how to meet new food safety standards to protect public health. It is now required that water used for overhead irrigation within 21 days of harvest must be treated to non-detectable levels of generic *E. coli* and $\leq$ 99 MPN/100 mL of Total Coliform Bacteria in two of three samples using an US EPA approved sanitizer following label specifications, guidelines for use, and consideration of environmental impacts; however, specific guidance on disinfection procedures and products that ensure appropriate microbial die-off is lacking.

Sanitizers using peracetic acid and hydrogen peroxide (PAA) could play an important role in disinfecting irrigation water to meet LGMA requirements. In order to address industry needs, our team is investigating the disinfection efficacy of five PAA-based sanitizers on irrigation water subject to a broad suite of microbial pathogens and indicators including Total Coliform bacteria, *E. coli* (TVS353), Salmonella, and *E. coli* O157:H7. To date, all sanitizers evaluated, ranging from 27 to 3 ppm of PAA, demonstrated between 2.17 and 3.91 total log reductions. The results of this study will provide industry with the guidance they need to meet LGMA requirements, potentially reducing contamination in fresh produce, and protect public health.

Evaluation of methods for recovery of hepatitis A virus from fruit purée

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Hepatitis A virus (HAV) has been implicated in many foodborne outbreaks and can pose a significant health risk to consumers. Extensive food handling during production, processing, or packaging can result in virus contamination. However, foods are seldom tested for viral contamination, oftentimes due to a lack of proficient methods. In this study, several methods for the recovery of spiked HAV at varying concentrations from fruit purée were evaluated. Methods ranged from precipitation using polyethylene glycol to elution with glycine buffer. Recovered viral RNA was extracted and reverse transcription quantitative polymerase chain reaction (RT-qPCR) was used for selective amplification and quantification of HAV genomes. HAV recovery efficiencies were calculated using the following equation: recovery efficiency = 100% x genome copies recovered from the purée/genome copies of virus spike. The efficiency of HAV recovery from seeded fruit purée was relatively low and highly variable. An optimized glycine buffer elution method resulted in the highest average recovery of 4.47%, accounting for qPCR inhibition, with a method reporting limit of 5.2×10^3 genome copies per 5 grams of sample. This study highlights the difficulties and limitations of virus monitoring in the oftentimes complex matrices of food products.

Antibiotic Resistance and Susceptibility of Bacterial Isolates from Various Irrigation Water Sources

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Alternative water sources are being investigated for irrigation of food crops in areas where drought is prevalent. These alternative water sources could be contaminated with bacteria. Considering the rise of antibiotic-resistance among bacteria, it is important to understand their prevalence in alternative sources of irrigation water so their spread can be prevented. This will help in selecting the best alternative source with the least resistant isolates for irrigating food crops. We investigated the antibiotic resistance and susceptibility of various bacterial isolates (*Enterococcus faecalis*, *Escherichia coli*, and *Salmonella)* found in reclaimed and return flow water samples collected from various regions in Arizona. These bacterial isolates were screened against 13 antibiotics using a disk diffusion assay on Mullen Hinton agar (MHA). A lawn of each isolate was made on MHA and antibiotic disks were placed on the agar followed by incubation at 37^{o}C for 24 hours. Zones of inhibition were measured; resistance and susceptibility were determined based on the breakpoints given by the manufacturer. Results showed a varying degree of antibiotic resistance depending on the bacteria and water source. In both water sources among all bacterial isolates, the most resistance was found for bacitracin and penicillin and the least resistance was found for chloramphenicol and trimethoprim. *E. coli* was the most resistant among the three bacteria tested. However, among the two alternate water sources, reclaimed water was found to harbor less antibiotic resistant bacteria. Hence, reclaimed water may be the better option for alternative source of irrigation water for food crops.

Efficacy of Ozone Against *Salmonella* Newport in Recycled and Non-recycled Spinach Wash Water

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Recycling the water used in agricultural production may be essential in regions where drought conditions are likely to occur in the future. Appropriate treatment technologies are needed to decontaminate the water prior to recylcing. The objective was to investigate the efficacy of ozone treatment against *Salmonella* Newport in recycled and non-recycled spinach wash water. The non-recycled spinach wash water was prepared by adding spinach and tap water in a stomacher bag and shaking gently for 2 min and then removing the spinach. Recycled wash waters were prepared by washing either 2 or 3 batches of spinach in the same tap water in a stomacher bag. For 3 batches, 0.1 g soil was also added into the bag. Each wash water was inoculated with 100 microliters of *Salmonella* and mixed thoroughly. The different spinach wash waters were treated with ozone (5.5 mg/L) for 30 sec, 1 min, and/or 2 min. Untreated wash waters were also included as controls. Wash waters were diluted and plated on xylose lysine desoxycholate agar for enumeration of surviving *Salmonella*. In both non-recycled and recycled (two and three times) wash waters, 2 min, 1 min, and 30 sec ozone treatments were very effective in reducing *Salmonella* population to below the detection limit (1 log CFU/mL). Overall, there was a reduction in *Salmonella* population to below detection levels with different ozone treatment times both in recycled and non-recycled spinach wash waters. This indicates that ozone can potentially be used as a treatment for effectively decontaminating produce wash water.

Prevalence of *Cyclospora cayetanesis* in irrigation water in the Southern region of the US

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Cyclospora cayetanensis is a coccidian parasite which causes enteric illness in humans and is of major public health concern. *C. cayetanensis* has been implicated in outbreaks from produce imported from South America since the 1990s and more recently in produce grown in the US. Further studies should be performed in order to better understand the transmission of this pathogen into produce. On a previous study, irrigation canal water and influent and effluent wastewater treatment plant samples were collected from Arizona and Texas during a two-year period. *C. cayetanensis* presence was tested used a developed qPCR assay targeting the ITS-2 region. It was determined that *C. cayetanensis* was presumptively present in these areas, requiring further confirmation of samples with sequencing. The current study further asses the prevalence of this organism in Arizona by collecting 17 samples monthly for one year from different water districts in the region. Briefly, *C. cayetanensis* is concentrated using Gelman Envirochek high-volume (HV) filters, by filtering 100-liter samples of irrigation water. DNA extraction and qPCR assay are done as specified in BAM 19B (with modifications), an FDA approved qPCR protocol for detection of *C. cayetanensis* in produce published in 2018 (BAM 19B) targeting the 18S region. This study is currently being completed and will be finalized in January 2020. Results should help determine the prevalence of *C. cayetanensis* in this region and will aid in developing an accurate risk assessment to determine the risk of contamination of produce.