Technical Note Agreements # 65-9104-0-816 (817)

Evaluation of Microbial Communities in Sediment Basins and Algal Mats and their Impact on Food Safety

Introduction

Sediment basins (Figure 1) on irrigated agricultural sites are an important Conservation Practice (CP) meant to capture and detain sediment laden runoff for a sufficient length of time for sediment settling prior to discharge. Accumulated sediment may be periodically removed and redistributed to adjacent fields, the regularity of which is determined by the basin's sediment holding capacity. It is well established that sediments associated with standing water are microbiologically rich, capable of harboring bacteria in far excess of the overlying water. The purpose of this study was to examine the potential microbiological impact a properly installed sediment basin may have on nearby produce fields. Additionally we examined whether algal mats formed along the edges of sediment basins and/or drainage ditches were additional reservoirs of pathogenic bacteria. Over the course of one year we sampled sediment basins and tail-water recovery systems, both NRCS and non-NRCS engineered, to determine which factors may contribute to the occurrence and persistence of bacterial pathogens in sediment and algal mats.

Figure 1. Example of a California sediment basin used during this study.

Project Field Methods

The Western Institute for Food Safety and Security research team (WIFSS) enlisted branch offices of the NRCS and other stakeholders in three states (California, Mississippi and Florida) to secure access to farms with constructed sediment basins and tail-water recovery systems. To preserve grower cooperation and confidentiality, no record of farm ownership or specific location was included in the dataset. Outside California, all of the enrolled farms used tail-water recovery systems. We adapted a high-volume filtration method (Polaczyk et al., 2008) to detect the presence of pathogens (*E. coli* O157:H7, *Salmonella*) enabling us to analyze a greater volume of water (20 L) than is done under normal sampling regimes (100 mL) (Figure 2). A

Figure 2. Field Sampling w/ water collection pump in foreground and dredge sampler in background

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secondary water sample was taken (1000 mL) to enumerate indicator *E. coli*. Sediment was collected using a modified dredge sampler and analyzed for the presence of pathogens (*E. coli* O157:H7, *Salmonella*) using a customized laboratory method (Weber and Legge, 2010). Samples were cooled (4°C) and shipped to WIFSS and analyzed within 24 hours of collection. A blank (potable water in a sterile container) was used as a negative control to assure quality control throughout sampling. Algal mats were sampled at a sub-set of California basin sights.

Project Laboratory Methods

Water/Sediment

Water samples were concentrated from 20L to 300-1000 mL retentate samples using high-volume ultrafiltration. Retentates were processed *for E. coli* O157:H7 and *Salmonella* using a protocol described by Cooley et al. (2007) with the following modifications: 30 g/L of tryptic soy broth (TSB) powder was added directly into the Nalgene bottle containing the retentate and enriched for 2 hours at 25°C and 100 rpm, 8 hours at 42°C and 100 rpm and held at 4°C until processed with immunomagnetic separation the presence/absence of *E. coli* O157:H7 and *Salmonella* (Figure 3). All presumptive positive samples were biochemically confirmed.

Figure 3. UV image of agar plates confirming the presence of pathogenic bacteria in a sample.

Indicator *E. coli* was enumerated by membrane filtration using 47mm, 0.45mm pore sterile filter, incubated on Chromagar EC agar for 24 to 48 hours. Two colonies were selected for biochemical confirmation.

Algae

Algae was processed for *E. coli* O157:H7 and *Salmonella* using a protocol as described by Cooley et al. (2007), with the following modifications: 50 g of algae was placed into a whirl-pack bag containing 450 mL of TSB supplemented with cis-2-decenoic acid to a final concentration of 110 nM and enriched for 2 hours at 25°C and 100rpm, 8 hours at 42°C and 100 rpm and held at 4°C until processed with immunomagnetic separation for the presence/absence of *E. coli* O157:H7 and *Salmonella*. All presumptive positive samples were biochemically confirmed

Indicator *E. coli* was enumerated by membrane filtration using 47mm, 0.45mm pore sterile filter, incubated on Chromagar EC agar for 24 to 48 hours. Two colonies were selected for biochemical confirmation.

Results

In all, 13 growers throughout California, Florida and Mississippi agreed to participate in this study, yielding 28 tail-water capture/sediment basin systems on 20 separate properties (Table 1). Sediment samples were only collected in California (Table 2). No samples, water, sediment or algae, tested positive for *E. coli* O157:H7. Nearly 45 % of water samples were positive for *Salmonella* (all states) while only 15 % sediment samples were positive for *Salmonella* (CA). *Salmonella* occurrence in Florida water samples was 79%, while all water samples from Mississippi were positive (Table 1). Only one algae sample (n=1 of 18) tested positive for *Salmonella*, precluding further analysis (Table 3).

In California, only 23.9% of water samples were positive for *Salmonella* (Table 1); however, nearly 1/3 of the enrolled basins (n=7 of 18) were regularly treated with copper sulfate (bluestone). While bluestone is typically used for algae inhibition, it is also an effective antimicrobial agent; the average indicator *E. coli* counts in water samples were nearly 20 times higher in non-bluestone basins. In California, 39% of sampled sediment basins were dried down during the sampling period (>3 months) and of those the occurrence of *Salmonella* in submerged sediments was 17% while only 1 out of 19 samples (5%) from dewatered basins were positive for *Salmonella* (Table 4). In Florida and Mississippi tail-water recovery systems were not allowed to dry down and were used primarily as reservoirs of recirculated irrigation water. Water from these basins was sent through a filtration system prior to field reapplication to reduce the risk of microbial contamination of crops. While 70% of samples that exceeded the industry standard of water quality (indicator *E.* coli > 235 CFU/100 mL) were positive for *Salmonella*, ~85% of *Salmonella* positives were not associated with an exceedance of the industry standard.

In water samples, the presence of overhanging trees; bank vegetation coverage >25%; and the presence of animal agricultural operations within 200 m all contributed to an increased likelihood of *Salmonella* occurrence. Additionally, both the use of surface water as an irrigation source (reservoir or canal) and a basin water level >50% full were significant predictors ($p<.05$) of *Salmonella* in water samples. Interestingly, the presence of mammals (wildlife) was negatively associated with *Salmonella* presence, meaning the basins where mammals were present were ~80% less likely to have *Salmonella* in water samples than basins without mammalian wildlife. Similarly to the water samples, sediment samples from basins where surface water was applied as irrigation were nearly 5 times more likely to have *Salmonella* than basins where ground water was applied. However, basin fill level (>50%) was negatively associated with *Salmonella* presence as was sediment exposure due to dewatering. In short, as basins were dewatered, sediment that remained submerged had a higher likelihood of containing *Salmonella* while sediments exposed following dewatering had a lower likelihood of containing *Salmonella* (Figure 4).

Impacts and Recommendations

The use of sediment basins for the recovery of irrigation water is effective in reducing sediment loading of waterways, however; sediment and water within the basins are microbiologically rich. If the water or undried sediment is reapplied to ready to eat produce fields it could pose a risk to human health. While the presence of wildlife habitat (overhanging trees, bank vegetation) increased the likelihood of *Salmonella* occurrence in water samples, these risk factors do not appear to contribute to *Salmonella* occurrence in sediment samples. Dewatering sediment basins or tail-water recovery systems and allowing sufficient time for sediment drying (weather and soil type dependent) decreases the likelihood of pathogen presence. Treatment of sediment basins with bluestone appears to be effective at decreasing the occurrence of algal mats and microbial contamination of both water and sediments (Nies, 1999). However; research has shown elevated copper levels can be physiologically stressful to aquatic animals (Garcia-Munoz et al., 2010) and vegetation (Yan et al., 2013).

Future Publication

Bond, R.F., M.L. Partyka, E.R. Atwill, J.A. Carabez, L.E. Kiger. (2013). Evaluation of microbial communities in agricultural sediment basins and their impact on food safety. Will be submitted to Agricultural Water Management late summer.

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Tables and Figures

Table 1. Summary of water sample collections and microbial water quality.

State	Sample #	Visits	Indicator *E. coli*/100mL[a]	*E. coli* O157:H7[b]	*Salmonella*[b]
California	71	5	341.5	0	17
Florida	24	4	68.1	0	19
Mississippi	12	3	62.8	0	12
Total	107	12	248.9	0	48

[a] average *E. coli* CFU
[b]number of positive samples

Table 2. Summary of sediment samples and microbial sediment quality.

CA County	Sample #	Indicator *E. coli* /1g[a]	*E. coli* O157:H7[b]	*Salmonella*[b]
Imperial	9	396.1	0	5
Monterey	42	160.0	0	1
Yolo	38	86.0	0	7
Total	89	152.3	0	13

[a] average *E. coli* CFU
[b]number of positive samples

Table 3. Summary of algal mat samples and microbial contamination.

Sites	Sample #	Indicator *E. coli* /100mL[a]	*E. coli* O157:H7[b]	*Salmonella*[b]
1	3	60.6	0	1
2	6	86.1	0	0
3	5	42.0	0	0
4	3	22.3	0	0
Total	17	57.4	0	1

[a] average *E. coli* CFU
[b]number of positive samples

Table 4. Microbial contamination, average indicator bacteria counts in 1g of sediment and *Salmonella* occurrence, in submerged sediments and sediments exposed to the air following basin dewatering.

Exposure	Indicator *E. coli*/g[a]	*Salmonella*[a]
Submerged	181.20	12.00
Exposed	45.74	1.00

[a] average *E. coli* CFU
[b]number of positive samples

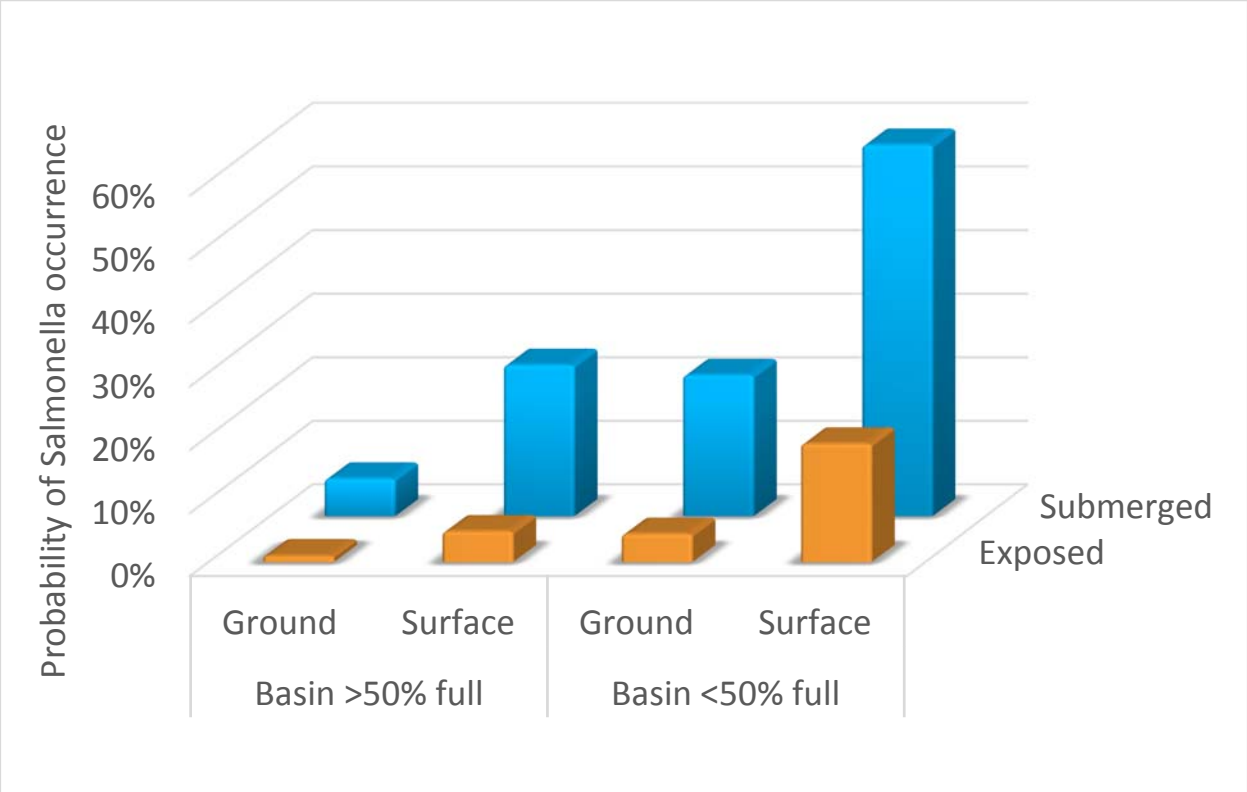

Figure 4. Chart depicting the changes in probability of *Salmonella* detection in sediment samples as a function of sediment exposure to air (submerged or exposed), basin fill level (more or less than half full), and irrigation water source (ground or surface water).