Sediment re-suspension as a potential mechanism for viral and bacterial contaminants

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Abstract

Pathogenic enteric viruses and bacteria tend to occur in higher concentrations and survive longer in aquatic sediments than suspended in the water column. Re-suspension of these organisms can result in a significant degradation of overlying water quality. Additionally, the re-suspension of microbial pathogens in artificial irrigation canals could endanger the consumption of fresh and ready-to-eat produce. Irrigation water has been implicated in numerous fresh produce outbreaks over the last 30 years. This study aimed to quantify the proportions of bacterial and viral re-suspension from sediment in a recirculating flume with varying velocities. MS2 coliphage and *Escherichia coli* were found to re-suspend at rates that were not significantly different, despite organism size differences. However, *E. coli* re-suspension rates from sand and clay were significantly different. This suggests that likely sediment-associated particles were recovered with the organisms attached. Similar re-suspension rates are hypothesized to be due to the dynamics of sediment transport, rather than that of the organisms themselves. This study also indicated that the re-suspension of sediment at very low velocities (e.g. less than 10 cm/s), could impact the microbiological quality of the overlaying water. Results from this study conclude that sediment could be a viable mechanism for irrigation water contamination.

Keywords: Irrigation water quality; health-related water microbiology; food safety; fate and transport; sediment re-suspension

Introduction

Between the years of 1998 and 2013, 972 outbreaks were associated with consumption of raw produce in the United States. During this same time period, the percentage of outbreaks attributed to fresh produce rose consistently from 8% (1998-2001) to 16% (2010-2013).[1] The three most common pathogens associate with these outbreaks were *Escherichia coli, Salmonella enterica* and norovirus.[1] Between 2003 and 2012, *E. coli* O157, specifically, was linked to 255 foodborne outbreaks in the US, with at least 28 of those attributed to a fresh produce source.[2] Over a fourteen-year period (1998-2012) norovirus was implicated in over 400 fresh produce outbreaks.[3]

Fresh produce are especially vulnerable to contamination during the farm-to-fork chain, due to their minimal processing. Contamination can occur on-farm from a wide range of sources, including irrigation water.[4–7] Irrigation water has been cited as the source or potential source for a long list of fresh produce outbreaks throughout the world.[5,6,16–18,8–15] Open irrigation sources and canals are at risk for microbiological contamination from a wide range of natural and anthropogenic sources. Contamination can stem from point- and non- point sources such as run-off, animal contact or septic seepage.[5,7,19] Depending on the source and type of contamination event, high concentrations of fecal indicator bacteria (FIB) and potential pathogens can be introduced.[7] As irrigation water is used for a range of crops, including fresh and ready to eat produce, characterizing the microbiological quality is especially important. It is difficult to pinpoint sources of contamination in irrigation water, and often regulatory sampling does not come up over the maximum limit (varies by country).[6]

Historically, microbiological water quality monitoring for public health has focused on measuring and culturing FIB suspended in the water column. However, multiple studies have

found significantly higher concentrations of fecal bacteria in bottom sediments, as compared to the overlying water column. When sediments in a Northern Arizona watershed were sampled, membrane filtration showed fecal coliforms were on average >3-logs greater than the overlying water.[20] Similar results have been found in other watersheds and bodies of water. In an irrigation setting, sediment contained 10-1000 times more *E. coli* than the overlying water. [21] Byappanahalli et al.[22] found greater levels of *E. coli* in sediments of artesian springs. In this study, sediments from streams, banks and springs had consistently higher *E. coli* levels than their corresponding water sources by between 1-3 $\log_{10}$. Another study conducted in an estuary reported exponentially higher concentrations of FIB in sediments as compared to waters.[23] Other studies have also shown the same phenomena in various environmental and irrigation water sources, showing that this is a common occurrence throughout the world.[21,24–28]

Sediments in rivers, lakes and marine bodies have been sampled for a wide range of microorganisms, with high variability regularly reported for the same sites or water sources.[29] Presence of FIB and pathogens in sediment could pose a threat to human health if sediments are being re-suspended or direct contact is made to the sediments themselves. *Vibrio vulnificus*, *Salmonella* spp., fecal coliforms, shiga toxin-producing *E. coli* (STEC) and non-STEC *E. coli* have been found in fresh and marine water sand and sediments used for irrigation, estuaries, recreation and other agricultural purposes.[21,22,30–35] Potentially pathogenic human viruses have also been identified through molecular methods in sediments from similar sources.[36–39] While free-floating pathogens are exposed to stressed conditions and unlikely to persist in water, sedimentation and attachment to biofilms extend survival in stressful aquatic environments. In a 1987 study, the survival times for bacteria in sediment were found to correlate with particle size and increased from multiple days to multiple weeks, noting that *Escherichia coli* and *Salmonella*

Newport survival was greatest in sediments with at least 25% clay.[40] A range of survival times in sediments have been observed and vary with sediment composition and other environmental factors. Van Donsel and Geldreich[28] saw complete inactivation of *Salmonella* and fecal coliforms after 7 days, while another study found that over 80 days were required for inactivation of fecal coliforms in sediments.[41] Enteric viruses also experience longer survival times when adsorbed to sediment particles. [42] Survival time for poliovirus and echovirus increased from ~1 day to 6 days when the virus was attached to sediment. The same study observed poliovirus survival in polluted waters to be 1 hour, while in the same source sediment it was increased to over 4 days.[36] Significantly extended survival for echovirus, poliovirus 1, and Coxsackieviruses B9 and A9 has also been observed in sediment of seawater.[43]

With the established knowledge that sediment-borne pathogens and FIB are ubiquitous, the risk for their re-suspension and subsequent human exposure are inherent. In order to accurately evaluate these risks, factors which control re-suspension of these organisms needs to be investigated. This study aimed to do this by evaluating the influence of steady velocity changes on the re-suspension of sediment-associated *Escherichia coli* and MS2 coliphage. Shear stress and system equilibrium were previously evaluated,[44] however the focus of this study is on the effect of velocity alone. An understanding of organism re-suspension behavior with respect to water velocity can help inform predictive risk models using parameters more easily measured in the field. The objectives of this study were to quantify the proportion of organism re-suspension in a recirculating flume, under varying velocities, and to determine the potential impact on public health by generating re-suspension data applicable to quantitative microbial risk assessments (QMRA) for food- and waterborne human pathogens.

Materials and Methods

Field sample measurements and analyses

In addition to modeling an irrigation canal system in a flume, field samples (sediment; water and suspended sediment) and hydraulic and physicochemical measurements were taken from five canals in the Yuma Valley (Figure 1). Field samples were collected under normal conditions for the region (arid to semi-arid; 30.5ºC) with no rainfall in the previous 72 hrs. The purpose of these samples and measurements was to determine flume settings, and subsequent applicability to field conditions. They included water depth, flow rate, velocity, and sediment characteristics. The water velocity was measured by acoustic Doppler velocimetry (ADV) at the middle of each cross section in the canal. This was used as the mean velocity to calculate the flow rate of the canal by multiplying the area of cross section.

Irrigation canal bed sediment samples were dried at 110ºC for 24 hours and sieved to determine size. A set of sieves with openings of 25.4 mm, 12.7 mm, 8 mm, 4.75 mm, 2 mm, 1mm, 0.85 mm, 0.425 mm, 0.18 mm, and 0.075 mm were shaken for 15 min on a mechanical shaker to separate by size. Re-suspended sediment concentration from water samples was determined by comparing weight of samples before and after filtering. Due to the relatively small concentration of sediment in each sample, the pre-filtered samples were assumed to have the same density as water. After filtration (8 µm pore), filter papers were dried at 70ºC for 24 hours. The mass of the filtered sediments was measured and the suspended sediment concentration was calculated by dividing the sediment mass by the original sample volume.

Flume preparation

A closed-system flume (Figure 2) (Hydraulic Design and Product Co., Wayzata, MN, USA) which measured approximately 1.6 m in length and 0.15 m in width was used for this study. The entire flume and holding tank were disinfected by circulating a 10% sodium

hypochlorite solution, followed by a 10% sodium thiosulfate solution, to disinfect and subsequently neutralize any residual disinfectant. A base layer of 5-8 cm sediment (~20.5 kg) was then packed into the flume. An additional ~4.5 kg of sediment was dried at 121ºC for 15 min. After drying, the sediment was inoculated with one liter of sterile deionized water containing an average of 9.10 ± 0.42 log10 colony forming units (CFU) *Escherichia coli* (ATCC 25922) or 9.55 ± 0.52 log10 plaque forming unit (PFU) MS2 (ATCC 15597-B1) in a sterile stomacher bag (Seward Laboratory Systems Inc., Davie, FL, USA) and hand massaged to promote uniform and complete saturation. The sediment was dried at room temperature (27ºC) for 1~2 hours and a control sample was taken to ensure sediment was inoculated and determine starting concentration. The seeded sediment was then loosely packed on top of the base layer (1-2 cm) in the flume. The sediments used were classified as clayey sand (D50=0.05mm) and medium sand (D50=0.4 mm). For the purpose of this study, sediments are referred to as 'clay' and 'sand'. MS2 re-suspension was only tested in clay, whereas *E. coli* re-suspension was tested in both clay and sand as separate trials.

Following sediment preparation, the flume holding tank was filled with 247 L of tap water. The free chlorine residual was measured using a Pocket Chlorimeter II (Hach, Loveland, CO, USA) and neutralized with 10% sodium thiosulfate (Sigma-Aldrich, St. Louis, MO, USA). The pH (7.9) and temperature (25ºC) were measured using a pHTestr30 waterproof probe (Eutech Instruments, Vernon Hills, IL, USA). Experiments were varied by flow rate and velocities. A single flow rate (Q) was examined per experiment, and three trials corresponding to different velocities were examined within a single experiment (v1-v3)(Table 1). The flume tailgate was adjusted to control flow depth, and thus the cross-sectional area, which was used to vary the velocities while maintaining flow rate. Each trial began with circulation of holding tank

water through the flume. A control valve was adjusted to determine and set the flow rate. This was done at the start of each trial using a sharp-crested weir. Specific details regarding flume operation are described in Zhao et al. [44] Each velocity within a trial was run until equilibrium was reached, which was determined to be a period of 0.5 h. At the end of each trial, water samples were collected from the flume holding tank and the flume stream flow. Only water samples were analyzed for re-suspended organisms. Controls of sediment and water samples were taken before each trial to ensure sediment was inoculated, and there was no presence of target organisms in the water, respectively.

Microbial assays

Water samples from each trial were either analyzed for *E. coli* or MS2, depending on the target organism for the trial. *E. coli* was assayed by the spread plate method on MacConkey agar (Difco, Becton, Dickinson and Company, Franklin Lakes, NJ, USA).When necessary, serial 10-fold dilutions were made using sterile 0.05 M phosphate buffered saline (PBS) (pH 7.4) (Fisher Scientific, Waltham, MA, USA). Dilutions were plated in volumes of 100 µL in duplicates. The plates were then incubated at 37ºC for 24 h and *E. coli* colonies (pink) were enumerated. MS2 was assayed using the double agar overlay method. Briefly, 5 mL of sterile top agar was melted and held at 50ºC. A host of *Escherichia coli* ATCC 15597 was propagated in 125 mL of sterile tryptic soy broth (TSB) (Difco, Becton, Dickinson and Company, Franklin Lakes, NJ, USA) for approximately 4 hours at 37ºC with agitation. After exponential phase was achieved, 0.5 mL of host organism was combined with 1 mL of sample water in the sterile top agar. Serial 10-fold dilutions were also made for MS2 experiments using sterile PBS (pH 7.4); for dilutions, a volume of 0.1 mL was used. The tube was gently swirled and then poured onto a sterile tryptic soy agar plate (TSA) (Difco, Becton, Dickinson and Company, Franklin Lakes, NJ, USA). The

plates were solidified, then inverted and incubated for 24 h at 37ºC and plaques counted after 24 hours.[45]

Statistical analysis

All statistical analyses were performed in R.[46] Prior to statistical analysis, the total number of re-suspended organisms, determined from flume and holding tank water assays and the total volume of the system, were divided by the total initial sediment inoculum to determine the proportion re-suspended organisms. Beta regression, a method used for analyzing data restricted to a unit interval such as proportions, was used to test whether there were significant differences in mean re-suspended *E. coli* and MS2 inoculum proportion under the different treatments (velocities (cm/s), flow rates (L/s), sediment types) using the BetaReg package in R.[47] Significance of treatments and interactions between treatments was tested using a type II F-test in the car package.[48] A Tukey's HSD post-hoc test was used to determine significant differences between group means within treatments using the emmeans package.[49] Where relevant, estimated marginal (EM) means are plotted. EM is the mean response for each treatment level (group) adjusted for by other variables in the model. All plotting was done using the ggplot2 package.[50]

Results and Discussion

Statistical analyses comparing the proportions of re-suspended *E. coli* showed that the estimated mean proportion is significantly greater in clay than in sand sediment (χ^2= 18.2547, p= 1.932 x 10-5) (Figure 3). As the velocity increased, the mean proportion of re-suspended *E. coli* also increased (χ^2 = 32.5537, p = 1.474 x 10-6) (Figure 4). Once the velocity was greater

than 20 cm/s, the proportion of re-suspended *E. coli* was significantly greater than the 5 and 10 cm/s trials. At 20 cm/s, there was a threshold where the re-suspension increased but was not significantly different than the lower and higher velocity trials (Figure 4). The results were similar for both sediment types, and there was no significant interaction between sediment type and re-suspension ($p>0.05$) for *E. coli*, hence, re-suspension increased in both sediment types with increasing velocity but at lower proportions in sand. This is also evident in the reported re-suspended proportions (Tables 3-4).

There appears to be a point between 10 and 30 cm/s where re-suspension changes from a low range (<0.025 or 2.5%) to high range (>2.5%). This is confirmed by Zhou et al.[44] who show that a power function best described the relationship between *E. coli* fraction in water and bed shear stress. A similar trend was observed when MS2 re-suspension was evaluated (χ^2 = 35.591, p = 3.513 x 10-7) (Table 2). The lower velocities, 5 and 10 cm/s, were significantly different than 30 and 40 cm/s. However, in the MS2 trials, 20 cm/s was significantly different than 5 and 10 cm/s, but not 30 and 40 cm/s (Figure 5). This suggests that there is likely also threshold effect for MS2 but at a lower velocity not tested in this study (between 10 and 20 cm/s). While a power function describes the relationship between re-suspended *E. coli* and bed shear stress, a key advantage of the present study is that it allows for direct inference of the measured observations (proportion of re-suspended organisms) as opposed to fitting a deterministic model to colony forming units and shear stress. The analysis of proportions also provides terms more easily integrable into human health risk and exposure models.

When the overall estimated mean re-suspended proportions for both organisms were analyzed together in clay, the proportions were not as different as hypothesized (Figure 6). However, the interaction between organism and velocity shows that *E. coli* begin to re-suspend

in significantly higher proportions at 30 and 40 cm/s, when compared to 5 and 10 cm/s. The re-suspension observed for MS2 is not significantly different than *E. coli* at comparable flow velocities.

Re-suspension of bottom sediments is a major contributor to overall water quality degradation. Previous studies have established that these sediments also harbor FIB and pathogens. [22,30–35,51]Sediment-associated FIB and pathogens can have serious downstream implications in an irrigation setting if released back into the water during irrigation, pumping or dredging. While literature exists on the re-suspension in natural waterways, little information is apparent for re-suspension in irrigation canals.[52] The introduction of the Agricultural Water Rule as part of the Food Safety Modernization Act (FSMA) focuses on *E. coli* suggested limits modeled after recreation water, however, the dynamics of irrigation waters and canal systems are much different than that of natural or recreational waterways.[53,54] This study simulated steady flow and discrete changes in re-suspension from sediments into the overlying water to model irrigation canals in a laboratory setting.

Results showed that sediment-associated organism re-suspension occurs at low velocities (5 and 10 cm/s) and significantly increases as velocity increases. At 5 and 10 cm/s, variable re-suspension was observed for *E. coli* and MS2. Field studies have also observed re-suspension of sediment-borne bacteria at low velocities. In one of these studies, re-suspension velocities ranged from ~2 x 10-4 to 1 x 10-3 cm/s for seeded, traceable *E. coli* from bed sediments measured after rainfall-induced stream flow increases.[55] Pandey et al.[56] observed a similar range of re-suspension velocities for *E. coli* (4 x 10-5 cm/s to 1 x 10-4 cm/s) in field-based measurements. While this study's lowest measured velocity was 5 cm/s, based on the in-field measured flow velocities from this study (Table 5) and from literature [52,55,56], it can be concluded that there is

likely constant particle exchange between sediment beds and water columns in streams with even negligible flow.

A threshold of re-suspension was observed in this study between low (5 and 10 cm/s) and high (30 and 40 cm/s) test velocities. *E. coli* appeared to reach a threshold of re-suspension at 20 cm/s, while MS2 experienced this at a lower velocity between 10 and 20 cm/s (untested in this study). Re-suspension thresholds occur naturally in streams and are influenced by multiple factors such as sediment cohesion, aggregate formation or other particle interactions.[57] The threshold observed with microorganisms is likely due, in part, to the sediment re-suspension threshold. Importantly, future studies should determine whether there is a significant change in contamination risk at velocities below the threshold compared to velocities above the thresholds. Results from this study also showed that MS2 and *E. coli* were re-suspending at rates that were not significantly different (Figure 6), despite clear organism size differences. This suggests that likely sediment-associated organisms were recovered. Similar re-suspension rates are hypothesized to be due to the dynamics of sediment transport, rather than that of the organisms themselves. While human enteric viruses have been isolated in sediment, and observed to have prolonged survival in sediment, there appears to be a lack of literature evaluating viral re-suspension from sediment. This research suggests that if these viruses are bound to sediment, a significant proportion can be re-suspended into the overlying water, and in turn, potentially pose a risk to public health.

Conclusions

This study concludes that re-suspension of potential pathogens in sediments can occur at low flow rates and not just after sediment disturbance events. The re-suspension could potentially be significant enough to be considered hazardous to microbiological quality of

irrigation water, based off measurements from this study. If sediments are harboring pathogenic microorganisms this could impact the microbiological quality and safety of the overlying water. This finding shows that sampling of sediment in irrigation canals is crucial to protecting public health from potential foodborne illness in fresh produce, and properly evaluating water quality used for irrigation. Results from this work can be used for targeted human health risk assessments associated with sediment-borne re-suspension in irrigation water. The results can also be used for recreational exposures from sediment-borne organisms. Future research should address how mixed sediments and mixed organism populations, and organic load influence organism re-suspension.

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References

[1] Bennett, S.D.; Sodha, S. V; Ayers, T.L.; Lynch, M.F.; Gould, L.H.; Tauxe, R. V. Produce-associated foodborne disease outbreaks , USA , 1998 – 2013. Epidemiol. Infect. **2018**, *146*, 1397–1406.

[2] Heiman, K.E.; Mody, R.K.; Johnson, S.D.; Griffin, P.M.; Gould, L.H. Escherichia coli O157 Outbreaks in the United States , 2003 – 2012. Emerg Infect Dis **2015**, *21*(8), 1293–1301.

[3] Wadamori, Y.; Gooneratne, R.; Hussain, M.A. Outbreaks and factors influencing microbiological contamination of fresh produce. J. Sci. Food Agric. **2017**, *97*(5), 1396–1403.

[4] LYNCH, M.F.; TAUXE, R. V.; HEDBERG, C.W. The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities. Epidemiol. Infect. **2009**, *137*(03), 307.

[5] Suslow, T.V.; Oria, M.P.; Beuchat, L.R.; Garrett, E.H.; Parish, M.E.; Harris, L.J.; Farber, J.N.; Busta, F.F. Production Practices as Risk Factors in Microbial Food Safety of Fresh and Fresh-Cut Produce. Compr. Rev. Food Sci. Food Saf. **2003**, *2*(s1), 38–77.

[6] Uyttendaele, M.; Jaykus, L.A.; Amoah, P.; Chiodini, A.; Cunliffe, D.; Jacxsens, L.; Holvoet, K.; Korsten, L.; Lau, M.; McClure, P.; Medema, G.; Sampers, I.; Rao Jasti, P. Microbial Hazards in Irrigation Water: Standards, Norms, and Testing to Manage Use of Water in Fresh Produce Primary Production. Compr. Rev. Food Sci. Food Saf. **2015**, *14*(4), 336–356.

[7] Gelting, R.J.; Baloch, M.A.; Zarate-Bermudez, M.; Hajmeer, M.N.; Yee, J.C.; Brown, T.; Yee, B.J. A systems analysis of irrigation water quality in an environmental assessment of an E. coli O157: H7 outbreak in the United States linked to iceberg lettuce. Agric. Water Manag. **2015**, *150*, 111–118.

[8] Edelstein, M.; Sundborger, C.; Ivarsson, S.; Insulander, M.; Jernberg, C. Barriers to trace-

back in a salad-associated EHEC outbreak, Sweden, June 2013 | barfblog. PLoS Curr. Outbreaks **2015**, (June 2013), 1–19.

[9] Hoang, L.M.N.; Fyfe, M.; Ong, C.; Harb, J.; Champagne, S.; Dixon, B.; Isaac-Renton, J. Outbreak of cyclosporiasis in British Columbia associated with imported Thai basil. Epidemiol. Infect. **2005**, *133*(1), 23–27.

[10] Behravesh, C.B.; Mody, R.K.; Jungk, J.; Gaul, L.; Redd, J.T.; Chen, S.; Cosgrove, S.; Hedican, E.; Sweat, D.; Chávez-Hauser, L.; Snow, S.L.; Hanson, H.; Nguyen, T.A.; Sodha, S. V.; Boore, A.L.; Russo, E.; Mikoleit, M.; Theobald, L.; Gerner-Smidt, P.; Hoekstra, R.M.; Angulo, F.J.; Swerdlow, D.L.; Tauxe, R. V.; Griffin, P.M.; Williams, I.T. 2008 Outbreak of Salmonella Saintpaul infections associated with raw produce. N. Engl. J. Med. **2011**, *364*(10), 918–927.

[11] Greene, S.K.; Daly, E.R.; Talbot, E.A.; Demma, L.J.; Holzbauer, S.; Patel, N.J.; Hill, T.A.; Walderhaug, M.O.; Hoekstra, R.M.; Lynch, M.F.; Painter, J.A. Recurrent multistate outbreak of salmonella newport associated with tomatoes from contaminated fields, 2005. Epidemiol. Infect. **2008**, *136*(2), 157–165.

[12] Nygård, K.; Lassen, J.; Vold, L.; Andersson, Y.; Fisher, I.; Löfdahl, S.; Threlfall, J.; Luzzi, I.; Peters, T.; Hampton, M.; Torpdahl, M.; Kapperud, G.; Aavitsland, P. Outbreak of Salmonella Thompson infections linked to imported rucola lettuce. Foodborne Pathog. Dis. **2008**, *5*(2), 165–173.

[13] Gibbs, R.; Pingault, N.; Mazzucchelli, T.; O'reilly, L.; Mackenzie, B.; Green, J.; Mogyorosy, R.; Stafford, R.; Bell, R.; Hiley, L.; Fullerton, K.; Buynder, P. Van. An outbreak of Salmonella enterica serotype Litchfield infection in Australia linked to consumption of contaminated papaya. J. Food Prot. **2009**, *72*(5), 1094–1098.

[14] Soderstrom, A.; Osterberg, P.; Lindqvist, A.; Johsson, B.; Lindberg, A.; Ulander, S.B.; Welinder-Olsson, C.; Lofdahl, S.; Kaijser, B.; Jong, B. De; Kuhlmann-Berenzon, S.; Boqvist, S.; Eriksson, E.; Szanto, E.; Andersson, S.; Allestam, G.; Henderstrom, I.; Ledet Muller, L.; Andersson, Y. A Large Escherichia coli O157 Outbreak in Sweden Associated with Locally Produced Lettuce. Foodborne Pathog. Dis. **2008**, *5*(3), 339–349.

[15] Ethelberg, S.; Lisby, M.; Böttiger, B.; Schultz, A.C.; Villif, A.; Jensen, T.; Olsen, K.E.; Scheutz, F.; Kjelsø, C.; Müller, L. Outbreaks of gastroenteritis linked to lettuce, Denmark, January 2010. Eurosurveillance **2010**, *15*(6), 1.

[16] SIVAPALASINGAM, S.; FRIEDMAN, C.R.; COHEN, L.; TAUXE, R. V. Fresh Produce: A Growing Cause of Outbreaks of Foodborne Illness in the United States, 1973 through 1997. J. Food Prot. **2004**, *67*(10), 2342–2353.

[17] California Food Emergency Response Team. Investigation of an Escherichia coli O157:H7 Outbreak Associated with Dole Pre-Packaged Spinach. Centers Dis. Control Prev. Natl. Cent. Environ. Heal. Heal. Serv. Branch **2007**, *95899*(997435), 1–51.

[18] Gelting, R.J.; Baloch, M.A.; Zarate-Bermudez, M.A.; Selman, C. Irrigation water issues potentially related to the 2006 multistate E. coli O157:H7 outbreak associated with spinach. Agric. Water Manag. **2011**, *98*(9), 1395–1402.

[19] Hilborn, E.D.; Mermin, J.H.; Mshar, P.A.; Hadler, J.L.; Voetsch, A.; Wojtkunski, C.; Swartz, M.; Mshar, R.; Lambert-Fair, M.-A.; Farrar, J.A.; Glynn, M.K.; Slutsker, L. A Multistate Outbreak of Escherichia coli O157:H7 Infections Associated with Consumption of Mesclun Lettuce. Arch. Intern. Med. **1999**, *159*, 1758–1764.

[20] Crabill, C.; Donald, R.; Snelling, J.; Foust, R.; Southam, G. The impact of sediment fecal coliform reservoirs on seasonal waterquality in Oak Creek, Arizona. Water Res. **1999**, *33*(9),

2163–2171.

[21] Benjamin, L.; Atwill, E.R.; Jay-Russell, M.; Cooley, M.; Carychao, D.; Gorski, L.; Mandrell, R.E. Occurrence of generic Escherichia coli, E. coli O157 and Salmonella spp. in water and sediment from leafy green produce farms and streams on the Central California coast. Int. J. Food Microbiol. **2013**, *165*(1), 65–76.

[22] Byappanahalli, M.; Fowler, M.; Shively, D.; Whitman, R. Ubiquity and Persistence of Escherichia coli in a Midwestern Coastal Stream †. Appl. Environ. Microbiol. **2003**, *69*(8), 4549–4555.

[23] Erkenbrecher, C.W. Sediment Bacterial Indicators in an Urban Shellfishing Subestuary of the Lower Chesapeake Bay. Appl. Environ. Microbiol. **1981**, *42*(3), 484–492.

[24] Goyal, S.M.; Gerba, C.P.; Melnick, J.L. Occurrence and Distribution of Bacterial Indicators and Pathogens in Canal Communities Along the Texas Coast. Appl. Environ. Microbiol. **1977**, *34*(2), 139–149.

[25] Lu, L.; Hume, M.E.; Sternes, K.L.; Pillai, S.D. Genetic diversity of Escherichia coli isolates in irrigation water and associated sediments: Implications for source tracking. Water Res. **2004**, *38*(18), 3899–3908.

[26] Obiri-Danso, K.; Jones, K. Distribution and seasonality of microbial indicators and thermophilic campylobacters in two freshwater bathing sites on the River Lune in northwest England. J. Appl. Microbiol. **1999**, *87*(6), 822–832.

[27] Roe, M.T.; Vega, E.; Pillai, S.D. Antimicrobial Resistance Markers of Class 1 and Class 2 Integron-bearing Escherichia coli from Irrigation Waters and Sediments. Emerg. Infect. Dis. **2003**, *9*(7), 5–9.

[28] Donsel, D.J. Van; Geldreich, E.E. RELATIONSHIPS OF SALMONELLAE TO FECAL

COLIFORMS IN BOTTOM SEDIMENTS. Water Res. **1971**, *5*, 1079–1087.

[29] Pachepsky, Y.A.; Shelton, D.R. Escherichia coli and fecal coliforms in freshwater and estuarine sediments. Crit. Rev. Environ. Sci. Technol. **2011**, *41*(12), 1067–1110.

[30] Alm, E.W.; Burke, J.; Spain, A. Fecal indicator bacteria are abundant in wet sand at freshwater beaches. Water Res. **2003**, *37*(16), 3978–3982.

[31] Chase, E.; Young, S.; Harwood, V.J. Sediment and Vegetation as Reservoirs of Vibrio vulnificus in the Tampa Bay Estuary and Gulf of Mexico. Appl. Environ. Microbiol. **2015**, *81*(7), 2489–2494.

[32] Fajri, J.A.; Yamada, T.; Setiyawan, A.S.; Li, F. Evaluation of Water and Sediment Quality in Open Channels that Receive Effluent from Johkasou Facilities. J. Water Environ. Technol. **2015**, *13*(3), 207–219.

[33] Jay, M.T.; Cooley, M.; Carychao, D.; Wiscomb, G.W.; Sweitzer, R. a.; Crawford-Miksza, L.; Farrar, J. a.; Lau, D.K.; O'Connell, J.; Millington, A.; Asmundson, R. V.; Atwill, E.R.; Mandrell, R.E. Escherichia coli O157:H7 in feral swine near spinach fields and cattle, central California coast. Emerg. Infect. Dis. **2007**, *13*(12), 1908–1911.

[34] Praveena, S.M.; Shamira, S.S.; Ismail, S.N.S.; Aris, A.Z. Fecal indicator bacteria in tropical beach sand: Baseline findings from Port Dickson coastline, Strait of Malacca (Malaysia). Mar. Pollut. Bull. **2016**, *110*(1), 609–612.

[35] Stephenson, A.G.R.; Rychert, R.C. Society for Range Management Bottom Sediment : A Reservoir of Escherichia coli in Rangeland Streams Bottom Sediment : a Reservoir of Escherichia coli in Rangeland Streams. J. Range Manag. **1982**, *35*(1), 119–123.

[36] Labelle, R.L.; Gerba, C.P. Influence of Estuarine Sediment on Virus Survival Under Field Conditions. **1980**, *39*(4), 749–755.

[37] Guyader, F. Le; Dubois, E.; Menard, D.; Pommepuy, M. Detection of hepatitis A virus, rotavirus, and enterovirus in naturally contaminated shellfish and sediment by reverse transcription-seminested PCR. Appl. Environ. Microbiol. **1994**, *60*(10), 3665–3671.

[38] Staggemeier, R.; Bortoluzzi, M.; Silva Heck, T.M. da; Luz, R.B. da; Fabres, R.B.; Soliman, M.C.; Rigotto, C.; Baldasso, N.A.; Spilki, F.R.; Matos Almeida, S.E. de. Animal and human enteric viruses in water and sediment samples from dairy farms. Agric. Water Manag. **2015**, *152*, 135–141.

[39] Staggemeier, R.; Bortoluzzi, M.; Silva Heck, T.M. da; Silva, T. da; Spilki, F.R.; Matos Almeida, S.E. de. Molecular detection of human adenovirus in sediment using a direct detection method compared to the classical polyethylene glycol precipitation. J. Virol. Methods **2015**, *213*, 65–67.

[40] Burton, G.A.; Gunnison, D.; Lanzal, G.U.Y.R. Survival of Pathogenic Bacteria in Various Freshwater Sediments. **1987**, *53*(4), 633–638.

[41] Davies, C.M.; Long, J.A.H.; Donald, M.; Ashbolt, N.J. Survival of Fecal Microorganisms in Marine and Freshwater Sediments. Appl. Environ. Microbiol. **1995**, *61*(5), 1888–1896.

[42] Gerba, C.P. Chapter 5 Virus Occurrence and Survival in the Environmental Waters. Perspect. Med. Virol. **2007**, *17*(07), 91–108.

[43] Smith, E.M.; Gerba, C.P.; Melnick, J.L. Role of Sediment in the Persistence of Enteroviruses in the Estuarine Environment. Appl. Environ. Microbiol. **1978**, *35*(4), 685–689.

[44] Zhou, K.; Sassi, H.P.; Morrison, C.M.; Duan, J.G.; Gerba, C.P. Resuspension of Escherichia coli and MS2 bacteriophage from bed sediment in irrigation canals. J. Irrig. Drain. Eng. **2017**, *143*(6).

[45] Kropinski, A.M.; Mazzocco, A.; Waddell, T.E.; Johnson, R.P. Bacteriophages. **2009**, *501*,

69–76.

[46] Ihaka, R.; Gentleman, R. R: A Language for Data Analysis and Graphics. J. Comput. Graph. Stat. **1996**, *5*(3), 299–314.

[47] Cribari-Neto, F.; Zeileis, A. BetaReg. J. Stat. Softw. **2010**, *34*(2), 1–24.

[48] Fox, J.; Weisberg, S. An {R} Companion to Applied Regression. 2011.

[49] Lenth, R. emmeans: Estimated Marginal Means, aka Least Square Means. 2018.

[50] Wickham, H. ggplot2: Elegant Graphics for Data Analysis. 2016.

[51] Benjamin, L.; Atwill, E.R.; Jay-Russell, M.; Cooley, M.; Carychao, D.; Gorski, L.; Mandrell, R.E. Occurrence of generic Escherichia coli, E. coli O157 and Salmonella spp. in water and sediment from leafy green produce farms and streams on the Central California coast. Int. J. Food Microbiol. **2013**, *165*(1), 65–76.

[52] Pandey, P.K.; Soupir, M.L. Assessing the Impacts of E. coli Laden Streambed Sediment on E. coli Loads over a Range of Flows and Sediment Characteristics. J. Am. Water Resour. Assoc. **2013**, *49*(6), 1261–1269.

[53] United States Department of Health and Human Services; United States Food and Drug Administration. FDA Food Safety Modernization Act. **2013**, 1–5.

[54] Lothrop, N.; Bright, K.R.; Sexton, J.; Pearce-Walker, J.; Reynolds, K.A.; Verhougstraete, M.P. Optimal strategies for monitoring irrigation water quality. Agric. Water Manag. **2018**, *199*, 86–92.

[55] Jamieson, R.C.; Joy, D.M.; Lee, H.; Kostaschuk, R.; Gordon, R.J. Resuspension of Sediment-Associated in a Natural Stream. J. Environ. Qual. **2005**, *34*(2), 581.

[56] Pandey, P.K.; Soupir, M.L.; Rehmann, C.R. A model for predicting resuspension of Escherichia coli from streambed sediments. Water Res. **2012**, *46*(1), 115–126.

[57] Droppo, I.G.; Ongley, E.D. Flocculation of Suspended Sediment in Rivers of Southeastern Canada. Water Res. **1994**, *28*(8), 1799–1809.

Table 1: Flow rates and correlating velocities. L/s: Liters per second; cm/s: Centimeters per second

Test Flow Rates and Velocities			
Q (L/s)	**v1 (cm/s)**	**v2 (cm/s)**	**v3 (cm/s)**
0.41	5	10	20
0.73	10	20	30
1.46	20	30	40

Q: flow rate; v: velocity

Table 2: Proportion of resuspension of MS2 from Clay (N=27)

MS2 from Clay				
v (cm/s)	**Mean**	**SD**	**Min**	**Max**
5	2.41×10^{-3}	1.38×10^{-3}	8.44×10^{-4}	3.48×10^{-3}
10	4.56×10^{-3}	2.85×10^{-3}	2.67×10^{-3}	1.02×10^{-2}
20	1.81×10^{-2}	1.29×10^{-2}	4.21×10^{-3}	4.71×10^{-2}
30	2.58×10^{-2}	7.73×10^{-3}	1.30×10^{-2}	3.65×10^{-2}
40	4.37×10^{-2}	3.60×10^{-2}	2.07×10^{-2}	8.52×10^{-2}

v: velocity; SD: standard deviation; Min: minimum; Max: maximum

Table 3: Proportion of resuspension of *E. coli* from Clay (N=27). ND: Non-detect

***E. coli* from Clay**				
v (cm/s)	**Mean**	**SD**	**Min**	**Max**
5	1.97×10^{-5}	3.41×10^{-5}	ND	5.90×10^{-5}
10	6.21×10^{-4}	1.19×10^{-3}	ND	3.02×10^{-3}
20	2.82×10^{-2}	2.74×10^{-2}	1.76×10^{-4}	7.10×10^{-2}
30	9.49×10^{-2}	9.65×10^{-2}	2.18×10^{-2}	2.77×10^{-1}
40	1.34×10^{-1}	9.75×10^{-2}	2.26×10^{-2}	2.05×10^{-1}

v: velocity; SD: standard deviation; Min: minimum; Max: maximum

Table 4: Proportion of resuspension of *E. coli* from Sand (N= 24). ND: Non-detect

***E. coli* from Sand**				
v (cm/s)	**Mean**	**SD**	**Min**	**Max**
5	3.88×10^{-4}	6.66×10^{-4}	ND	1.16×10^{-3}
10	2.19×10^{-3}	2.22×10^{-3}	ND	4.97×10^{-3}
20	1.28×10^{-2}	2.14×10^{-2}	ND	5.75×10^{-2}
30	1.09×10^{-2}	1.15×10^{-2}	ND	2.55×10^{-2}
40	3.85×10^{-2}	4.48×10^{-2}	4.26×10^{-3}	7.27×10^{-2}

v: velocity; SD: standard deviation; Min: minimum; Max: maximum

Table 5: Results from field analyses. NA: Not available.

	Canal Characteristics				**Sediment Characteristics**	
Canal	Water Depth (m)	Canal Width (m)	Velocity (cm/s)	Flow Rate (L/s)	D50 (mm)	D84 (mm)
1	0.76	9.33	0.61	39.79	0.36	1.87
2	0.09	3.35	NA	NA	15.9	NA
3	0.88	9.33	60.7	4500	11.2	24.5
4	0.91	2.74	21	349.9	0.17	1.44
5	0.41	NA	0.02	NA	1.45	15.9

D50: average particle size; D84: 84th percentile of size

Figure Captions:

Figure 1. Map of field sampling sites

Figure 2. Flume water cycling with sample locations denoted by diamonds

Figure 3. Comparison of mean proportion of re-suspended *E. coli* from sand sediment (n=27) and clay sediment (n=24). Different letters show significant differences between means based on Tukey's HSD test ($\alpha = 0.05$). 95% confidence levels with Sidak correction are also shown. EM: estimated marginal

Figure 4. Comparison of mean proportion of re-suspended *E. coli* between different velocities (n=51). Different letters show significant differences between means based on Tukey's HSD test ($\alpha = 0.05$). Where letters are the same there is no significant difference. 95% confidence levels with Sidak correction are also shown. EM: estimated marginal

Figure 5. Comparison of mean proportion of re-suspended MS2 bacteriophage between different velocities (n=27) in clay. Different letters show significant differences between means based on Tukey's HSD test ($\alpha = 0.05$). Where letters are the same there is no significant difference. 95% confidence levels with Sidak correction are also shown. EM: estimated marginal

Figure 6. Comparison of mean proportion of re-suspended *E. coli* and MS2 between different velocities in the clay treatment (n=78). Different letters show significant differences between means based on Tukey's HSD test ($\alpha = 0.05$). Where letters are the same there is no significant difference. 95% confidence levels with Sidak correction are also shown. EM: estimated marginal

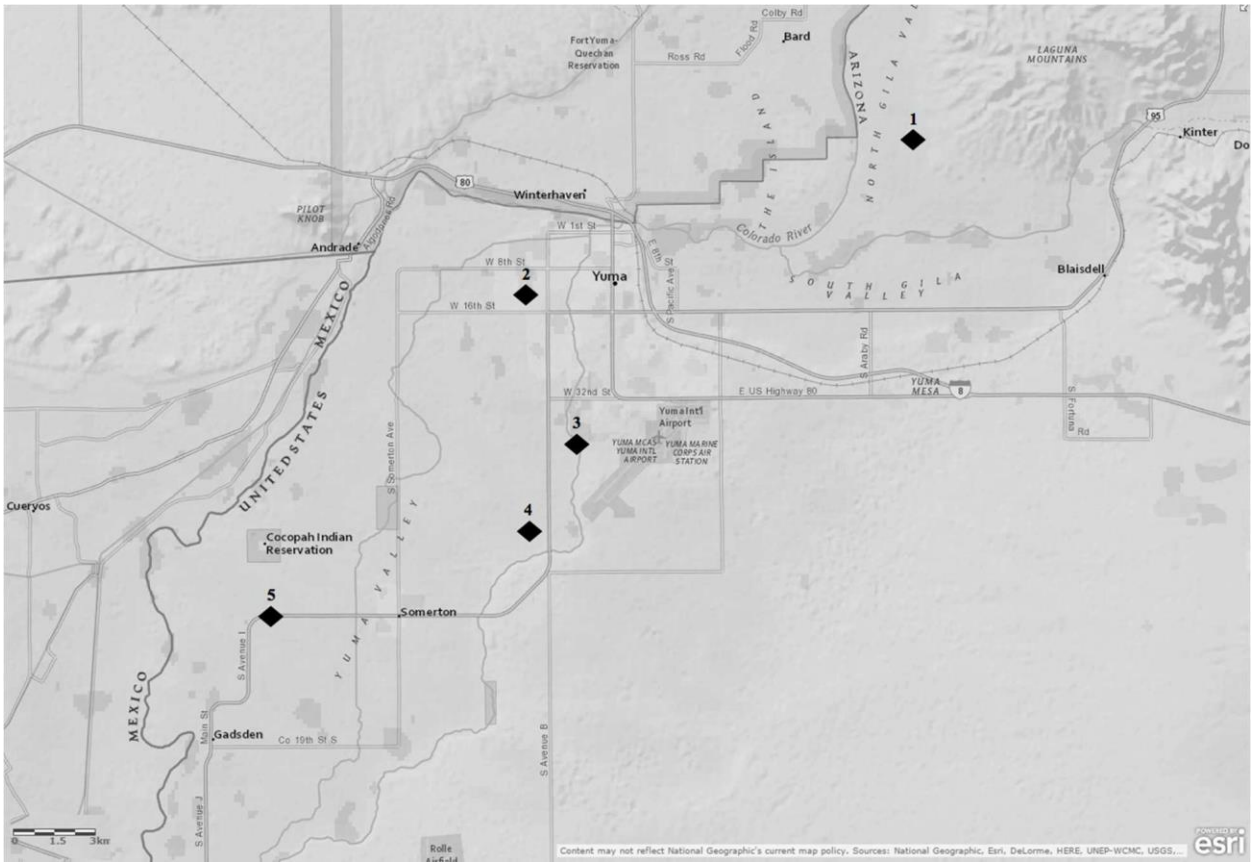
Fort Yuma-Quechan Reservation
Colby Rd
Ross Rd
Flood Rd
Bard
ARIZONA
NORTH GILA VALLEY
LAGUNA MOUNTAINS
1
95
Kinter
THE ISLAND
Winterhaven
80
PILOT KNOB
Andrade
Laguna Rd
W 1st St
Colorado River
W 8th St
2
Yuma
E 8th St
S Pacific Ave
SOUTH GILA VALLEY
Blaisdell
W 16th St
MEXICO
UNITED STATES
S Araby Rd
W 32nd St
E US Highway 80
YUMA MESA
8
S Fortuna Rd
Yuma Intl Airport
3
YUMA MCAS-YUMA INTL AIRPORT
YUMA MARINE CORPS AIR STATION
S Somerton Ave
Cuervos
4
Cocopah Indian Reservation
YUMA VALLEY
5
Somerton
S Avenue I
MEXICO
Main St
Gadsden
Co 10th St S
S Avenue B
S Avenue J
0 1.5 3km
Rolle Air Field
Content may not reflect National Geographic's current map policy. Sources: National Geographic, Esri, DeLorme, HERE, UNEP-WCMC, USGS,...
esri

Figure 1

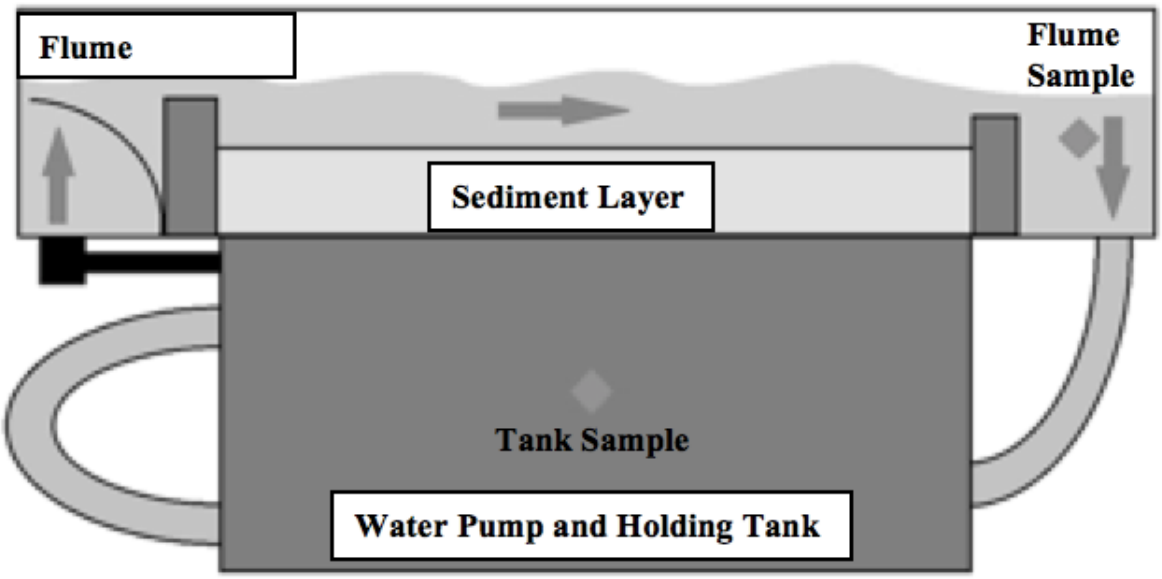
Flume
Flume Sample
Sediment Layer
Tank Sample
Water Pump and Holding Tank

Figure 2

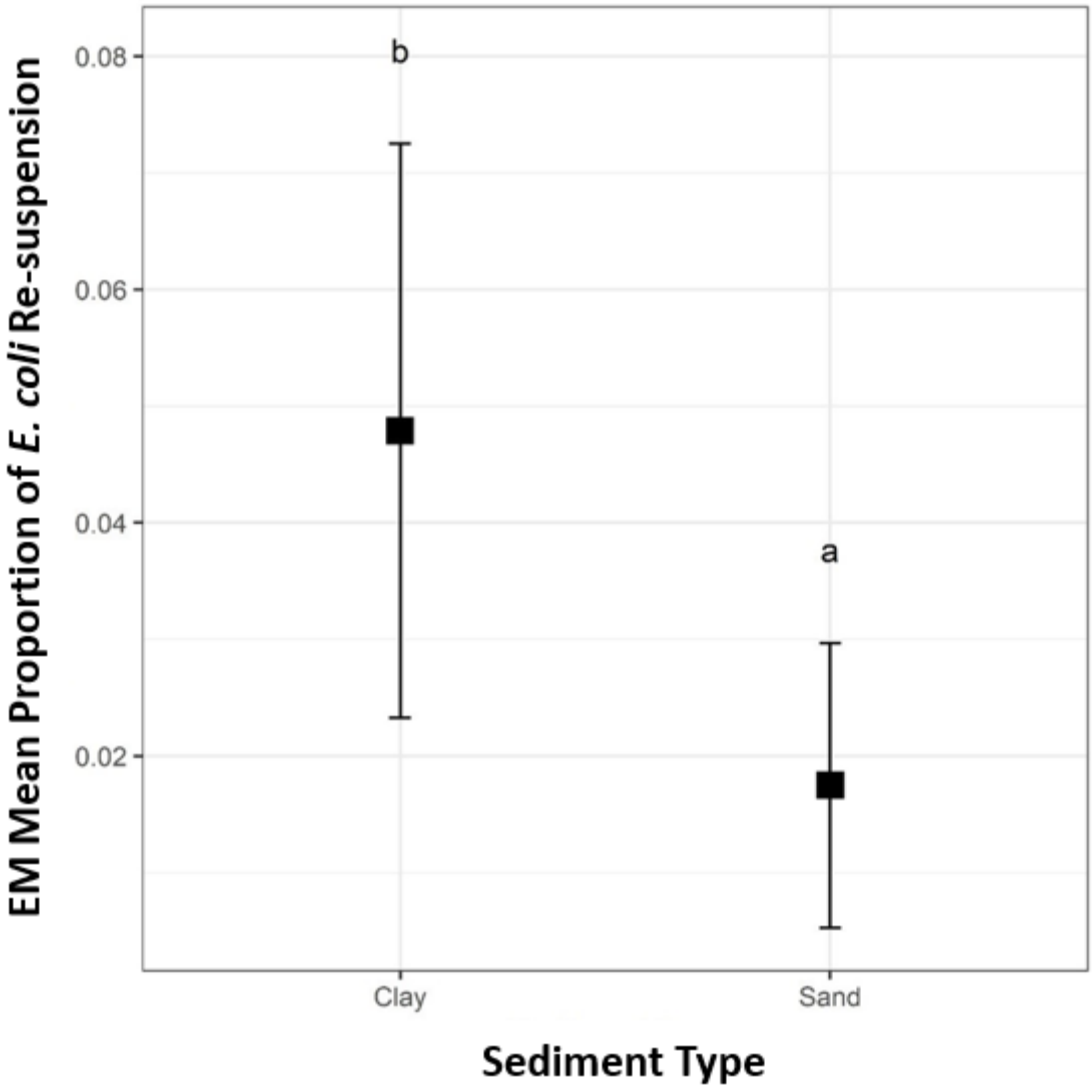
EM Mean Proportion of E. coli Re-suspension
0.08
0.06
0.04
0.02
b
a
Clay
Sand
Sediment Type

Figure 3

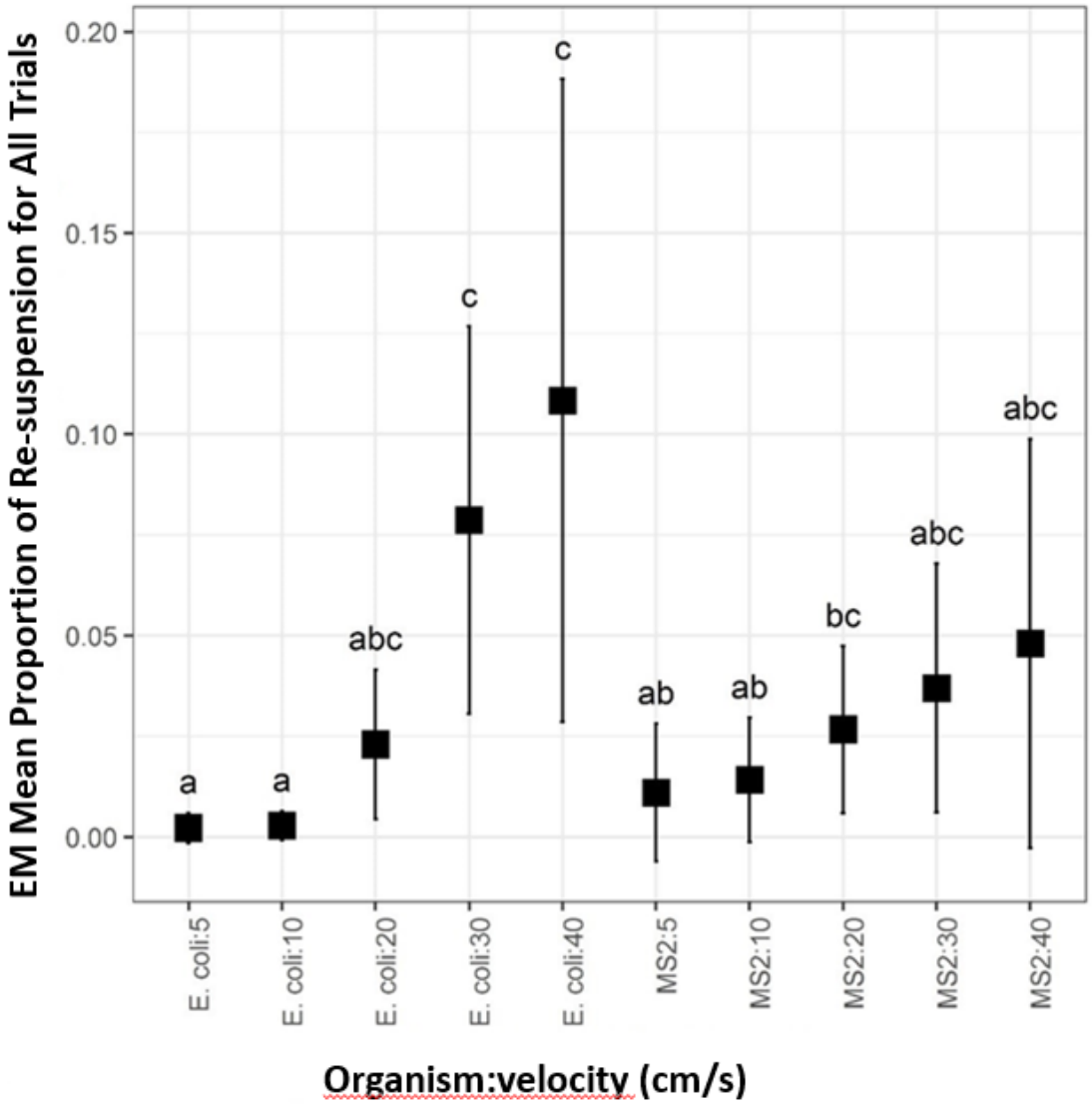
EM Mean Proportion of Re-suspension for All Trials
0.20
0.15
0.10
0.05
0.00
a
a
abc
c
c
ab
ab
bc
abc
abc
E. coli:5
E. coli:10
E. coli:20
E. coli:30
E. coli:40
MS2:5
MS2:10
MS2:20
MS2:30
MS2:40
Organism:velocity (cm/s)

Figure 6

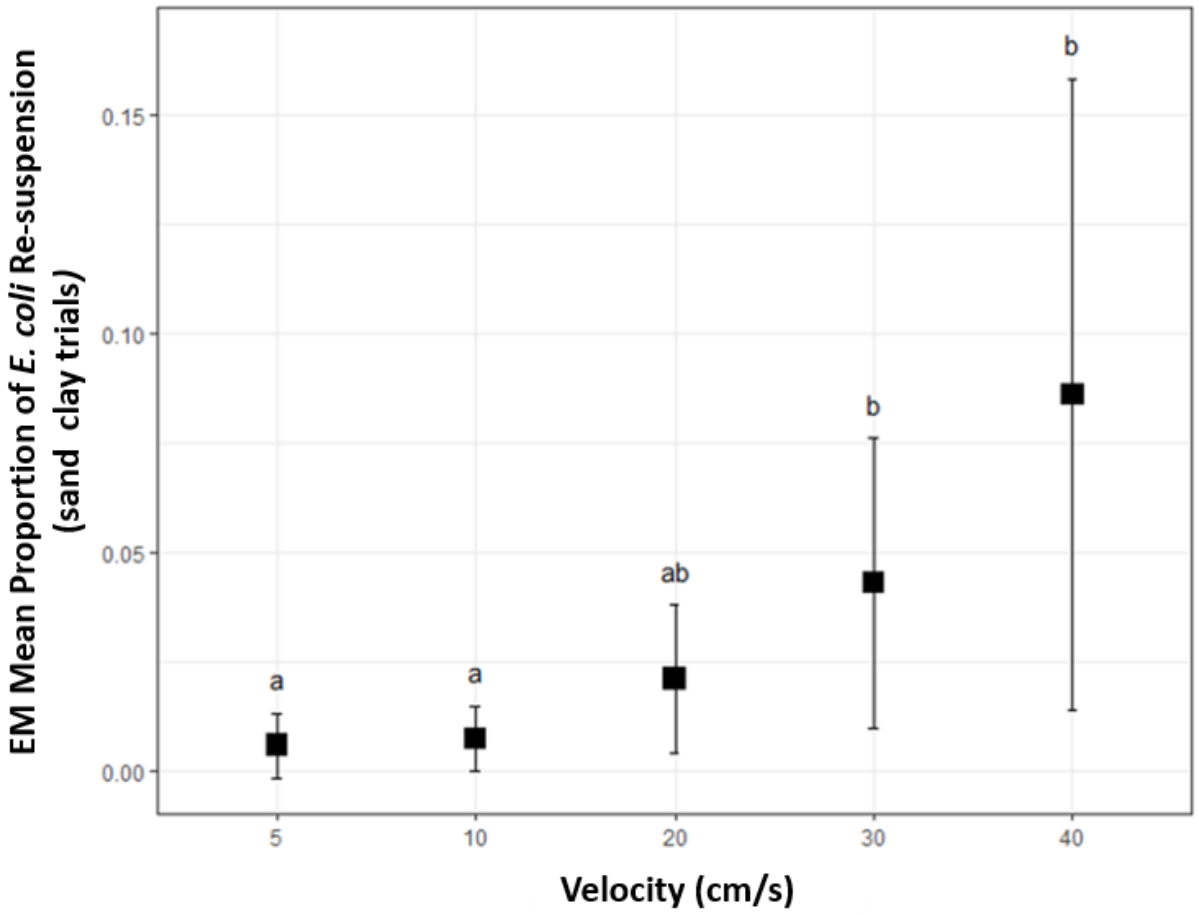
EM Mean Proportion of E. coli Re-suspension
(sand clay trials)
0.15
0.10
0.05
0.00
a
a
ab
b
b
5
10
20
30
40
Velocity (cm/s)

Figure 4

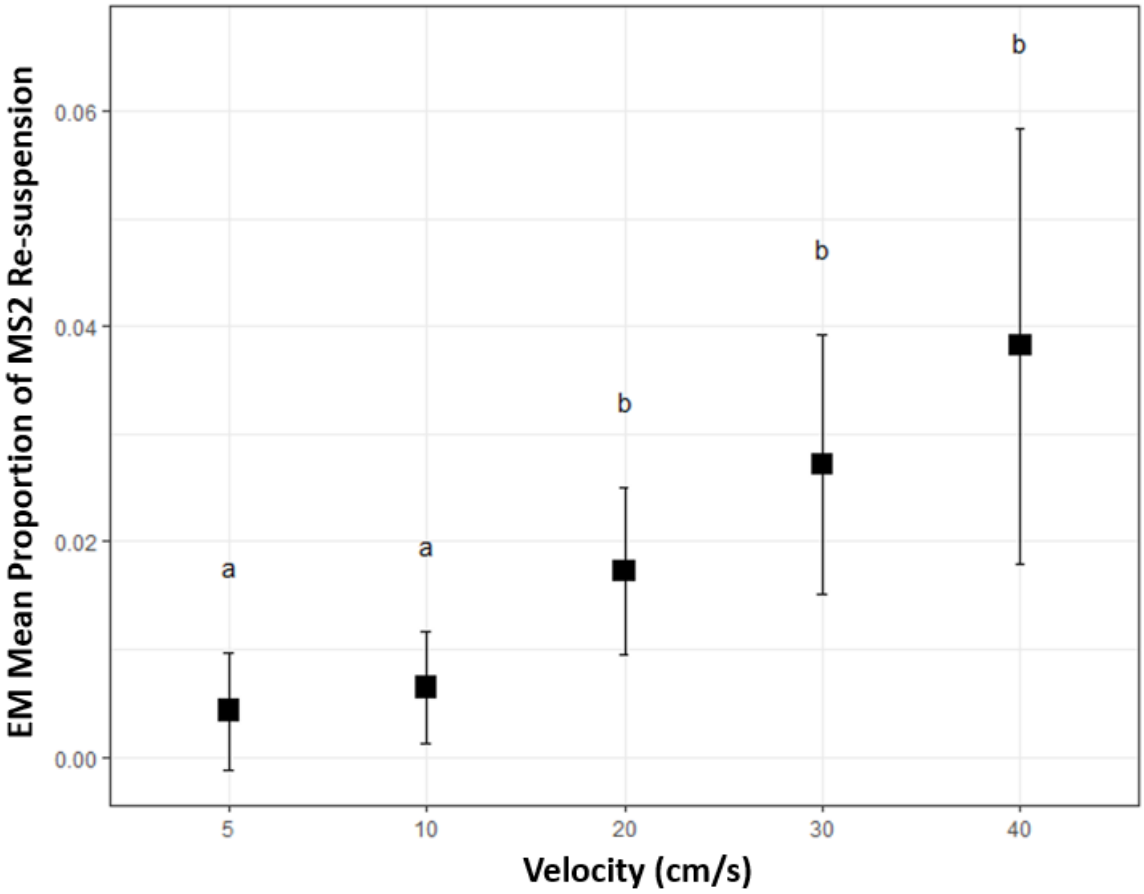
EM Mean Proportion of MS2 Re-suspension
0.06
0.04
0.02
0.00
a
a
b
b
b
5
10
20
30
40
Velocity (cm/s)

Figure 5