Resuspension of *Escherichia coli* and MS2 Bacteriophage from Bed Sediment in Irrigation Canals

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Abstract: Sediments are known to be potential reservoirs of pathogenic microorganisms that can influence the microbial quality of the overlaying water. A set of laboratory experiments was conducted to investigate the resuspension of *Escherichia coli* and the MS2 virus from bed sediment to the overlaying water in irrigation canals. Consequently, their concentration in moving water is dependent on flow properties (e.g., velocity, shear stress) and the size of bed sediment. When bed material is sandy loam, their quantity in water increases with the shear stress on bed surface. However, for a sandy bed, their presence in water has no apparent correlation with flow properties. The amount of MS2 virus in water was greater at low flow velocity and shear stress than *Escherichia coli* because the size of the MS2 virus is much smaller. Finally, an empirical relation was formulated for calculating the maximum allowable *Escherichia coli* concentration in sandy loamy bed sediment. **DOI: 10.1061/(ASCE)IR.1943-4774.0001169.**

Author keywords: Sediments; *Escherichia coli*; Virus; Coliphage; MS2; Irrigation water; Produce; Resuspension.

Introduction

The microbial quality of irrigation water is critical for the safety of fresh produce. Studies have shown that irrigation water is a source of microbiological contamination in fresh produce (Pachepsky et al. 2011; Olaimat and Holley 2012; Gil et al. 2015). Irrigation water carrying pathogenic microorganisms significantly increases the risk of produce contamination (Stine et al. 2005). From 1998 to 2008, a total of 26,735 illnesses was reported from 684 food-borne disease outbreaks involved with the consumption of contaminated produce in the United States (Uyttendaele et al. 2015).

Even when potable municipal water is used, these organisms can enter into an irrigation system from storm water or animals. Studies have found that particle surfaces serve to support attached microorganisms in freshwater, which allows organisms to settle, stay, and then resuspend into the water column (Bitton and Marshall 1980). Although water in an irrigation canal may be free of pathogenic microorganisms or may satisfy the *Escherichia coli* (*E. coli*) standard (USEPA 2012), when water starts to flow for irrigation, the organisms in the sediment can be resuspended into the water column, resulting in degradation of microbial water quality (Pachepsky and Shelton 2011).

Escherichia coli is a member of the fecal coliform group and is found as a commensal organism in the intestine of warm-blooded animals; however, some strains are pathogenic and cause gastrointestinal disease (diarrhea, vomiting, nausea, fever, and so on). Other pathogenic strains have the ability to cause urinary tract infections, meningitis, bacteremia, and hemolytic uremic syndrome (Murray et al. 1999). Pathogenic *Escherichia coli* has been associated with many foodborne outbreaks. From 1982 to 2002, over 400 cases of foodborne outbreaks in the United States were caused by one strain of *Escherichia coli*, O157:H7 (Rangel et al. 2005; Heiman et al. 2015).

Numerous studies have focused on *Escherichia coli* as an indicator for enteric pathogens (de Beauwere et al. 2014). Water temperature, turbidity, and flow rate have been found to influence the resuspension of *Escherichia coli* from sediments (Christeensen 2002; Skraber et al. 2002; Parkhurst et al. 2005; He and He 2008; Ge and Frick 2009; David and Haggard 2011; Francy et al. 2013). Temperature and natural competition are the major influences on the survival of *Escherichia coli* in water, which may survive for 5 to 7 days or longer (Flint et al. 1987). Many studies have assessed its decay rate in water and sediment (Vinten et al. 2004; Grant et al. 2005; Stretch and Mardon 2005; Hellweger 2007; Riou et al. 2007; Baffaut and Sadeghi 2010). However, only a few studies have examined the effect of flow and sediment properties on its resuspension. Collins and Rutherford (2004) correlated the resuspension rate with the average daily flow discharge when modeling the fate of *Escherichia coli* throughout a watershed in New Zealand. Bai and Lung (2005) calculated the resuspension flux using a reference erosion rate and the bed shear stress in the modified environment fluid dynamics code (EFDC) model (Hamrick 1992) for simulating the transport of *Escherichia coli*. Sanders et al. (2005) developed a resuspension model using the dimensional analysis, assuming the resuspension rate of *Escherichia coli* is proportional to the shearing rate. Cho et al. (2010) computed the resuspension flux by multiplying the *Escherichia coli* concentration in the sediment and the resuspension rate. Kim et al. (2010) calculated the resuspension rate using the simplified Bagnold's stream power equation. Pandey et al. (2012) correlated the resuspension rate to bed shear stress and the critical stress for both noncohesive and cohesive sediment. Regardless of the methods adopted in these studies, a threshold value, or the critical shear stress, was used to determine when bed sediment starts to be entrained, which is a key parameter

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for assessing *Escherichia coli* concentration in water. However, there is no consensus on the critical shear stress for sediment entrainment, especially for sandy loam–sized bed sediment (Steets and Holden 2003; Bai and Lung 2005; Jamieson et al. 2005). The uncertainty of critical shear stress is one of the challenges in determining *Escherichia coli* concentration in water (Pachepsky and Shelton 2011). The amount of microorganisms adsorbed to sediment surfaces directly affects the cohesion between sediment particles. Also, the critical shear stress varies with this cohesive force. Therefore, the amount being entrained into the water by sediment resuspension is dependent on bed shear stress. This study examined the critical shear stress for *Escherichia coli* entrainment, and the influence of sediment size on the concentration of *Escherichia coli* in water.

Norovirus is a human enteric virus that causes approximately 67% of foodborne illnesses in the United States (Gerba and Kayed 2003). Human norovirus has not been successfully cultured in laboratories for application to environmental studies, making it difficult to study in the environment. Studying norovirus characteristics in the environment is typically done using a surrogate (MS2, murine norovirus, or feline calicivirus) (Gerba and Kayed 2003; Richards 2012). Norovirus is a nonenveloped, icosahedral virus, which is approximately 30 nm in diameter (Gerba and Kayed 2003). Norovirus can spread through the fecal-oral transmission route, and it has been reported to remain viable outside of a host for over 2 months in water (Lopman et al. 2012).

The recent EPA regulation in their 2012 Recreational Water Quality Criteria only recommended *Escherichia coli* and enterococci for evaluating the microbiological contamination in water (USEPA 2012). However, Jurzik et al (2010) pointed out that *Escherichia coli* might not be a good indicator for viruses. Since bacteria and viruses may act differently in water and sediment, EPA is now considering adding coliphages as possible indicators (USEPA 2015). Therefore, the resuspension of both bacteria and viruses were studied.

Adsorption (or desorption) is the fundamental mechanism by which *Escherichia coli* attaches to (or detaches from) sediment, but it is difficult to quantify the adsorption/desorption because of the complexity of the transformation process (Gao et al. 2011). A partition constant was often used to represent the relationship between the adsorbed and the free *Escherichia coli* in the water (Bai and Lung 2005; Kim et al. 2010; Gao et al. 2011). With this approach, the total *Escherichia coli* in water is a function of the absorbed *Escherichia coli*, which is dependent on flow properties. Therefore, the objectives of this study were (1) to derive a relationship between the total amount of *Escherichia coli* and MS2 virus in water and flow properties (e.g., bed shear stress) and (2) to quantify the critical shear stress for the sediments adsorbed with *Escherichia coli* or MS2.

Experimental Setup

Experiments were conducted in a 15.2-cm-wide, 160.7-cm-long flume to mimic an irrigation canal, as shown in Fig. 1. The flume is rectangular and has a bottom slope of 0.001. A tank provides water to the flume, and also receives water from the end of the flume, resulting in a self-recirculating system. At the beginning of each experimental run, 246 L of water was added to the tank. Water temperature was measured after the tank was filled up, ranging from 23 to 27°C. A valve was used to control flow rate, and a tailgate at the end of the flume was placed to control water depth so that different velocities could be reached at a certain discharge. The flow rate was dependent on the valve opening,

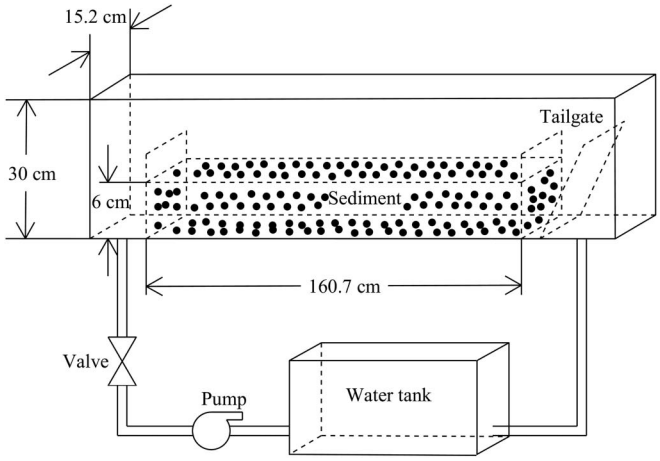

Fig. 1. Sketch for the flume

which was predetermined by using a sharp-crested weir installed at the flume end. Although the flume was short, a steady quasiuniform flow reach was achieved during each experimental run by regulating the tailgate at the flume end. The criterion for steady quasiuniform flow is to make sure the flow depths at the front, middle, and end of the reach are nearly the same (e.g., within a 1-mm difference). Two types of sediment were used. One had a mean size, $D_{50} = 0.4$ mm, and uniformity coefficient $C_u = 2.25$, and the other, $D_{50} = 0.05$ mm, $C_u = 5.35$. The gradations of both sediments were shown in Fig. 2. The sediments are classified as medium sand and sandy loam, respectively, based on the USDA textural soil classification system (USDA 1987). The sandy loam consisted of 53.3% sand and 46.7% silt.

The *Escherichia coli* and MS2 coliphage were obtained from the American Type Culture Collection (ATCC) (Manassas, Virginia). The strains of *Escherichia coli* and MS2 used in the experiments were ATCC 25922 and ATCC 15597-B1, respectively. Before each experiment, *Escherichia coli* was grown up in sterile tryptic soy broth (TSB) (BD Biosciences, Franklin Lakes, New Jersey) at 37°C and agitated overnight (18–22 h). MS2 was propagated every 2 weeks, using the method described by Sassi et al. (2015), and stored at 4°C. The organisms were inoculated into sterile deionized water, and the water was then added to the sediment until saturated.

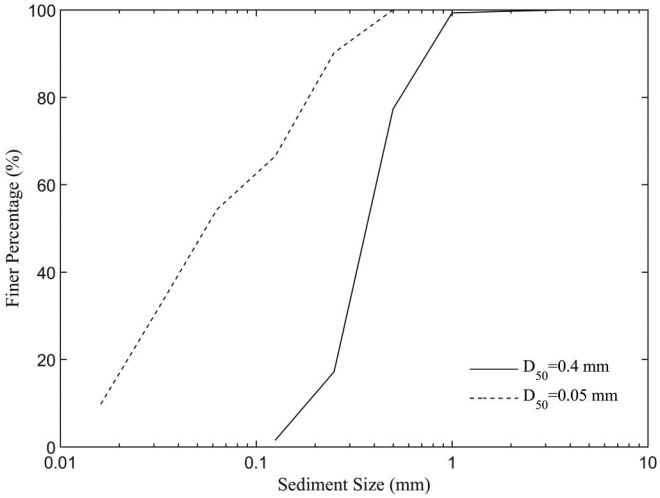

Fig. 2. Sediment cumulative distribution curve

Samples were collected using sterile 50-mL polypropylene conical tubes (BD Biosciences). To determine the concentration of the organisms in sediment samples, 5 mL of sterile phosphate buffer saline (PBS) (0.05 M, pH 7.4) was added to each sediment sample. The samples were agitated for 30 min to elute the organism.

Experimental Runs

Prior to each experimental run, 5 cm of clean sediment was evenly placed on the bottom of the flume, weighing approximately 20.43 kg (45 lb). Then, 4.086 kg (9 lb) of inoculated sediment of the same size was placed evenly on the top of the clean sediment. Next, the pump was started, and the valve was gradually opened to a desired location to reach a given discharge. The first series of experiments was to determine how long it took the *Escherichia coli* or MS2 to reach the equilibrium concentration in the water column. A flow rate of 0.73 L/s and velocity of 20 cm/s was selected for *Escherichia coli*. The sediment size was $D_{50} = 0.05$ mm. This experiment was duplicated to verify the initial results. During each experiment, water samples were collected from the flume and the tank after 30 min, 1 h, 2 h, 4 h, and 6 h. Fig. 3 shows the results from the first series of experiments, which shows that the amount of *Escherichia coli* in water remained approximately constant after 0.5 h. The mean *Escherichia coli* counts after 0.5 h are shown, respectively, as a solid and a dashed line for Trial 1 and Trial 2 data. Even though the amount of *Escherichia coli* increased in the water column slightly after 0.5 h, the differences from the mean value were in the range of one order of magnitude. For bacterial assays, one order difference in bacterial numbers is within the assay error range, so it was considered to have reached the equilibrium condition. Therefore, it is assumed that the entrainment of *Escherichia coli* into water reaches the equilibrium condition in approximately 0.5 h.

Because MS2 virus is smaller than *Escherichia coli*, it is easier to be entrained than *Escherichia coli*. Therefore, MS2 is assumed to reach the maximum concentration within 0.5 h. An experimental run was conducted to verify this assumption, and the total experiment duration was shortened to 4 h. As shown in Fig. 4, the amount of MS2 remained nearly constant after 0.5 h as well. Similarly, the mean value after 0.5 h was plotted. It is apparent that the deviations from the mean were within one order of magnitude. Thus, a sampling period of 0.5 h was also used for MS2.

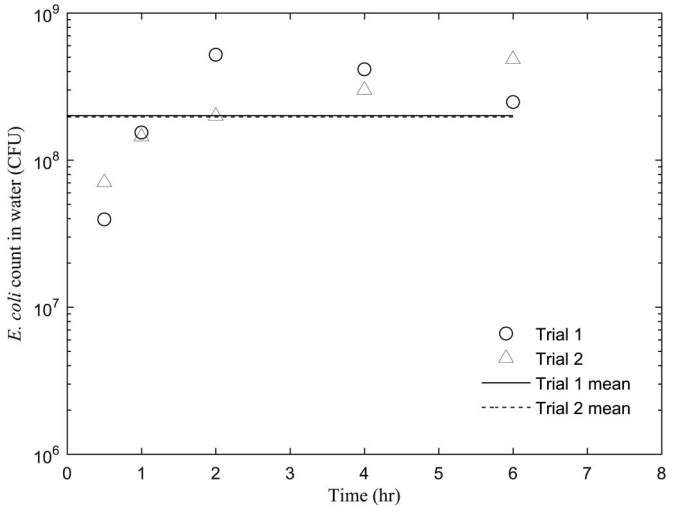

Fig. 3. Changes of *Escherichia coli* counts in water with time

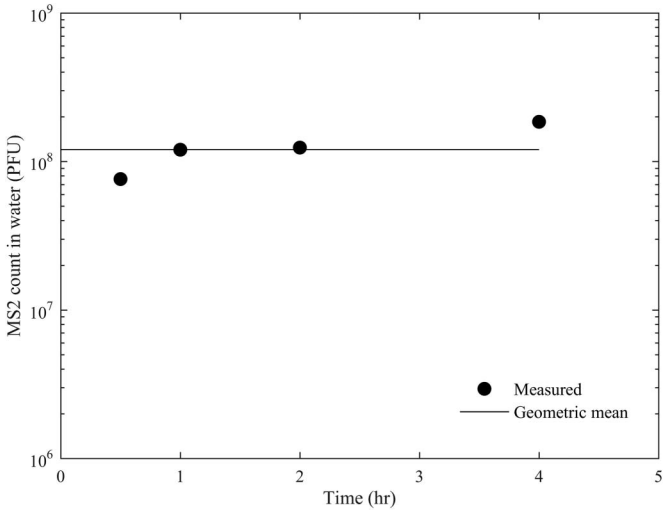

Fig. 4. Changes of MS2 counts in water with time

A second series of experiments was conducted to determine the correlation between the equilibrium concentration of *Escherichia coli* in water and flow properties (e.g., velocity, shear stress). At first, sediment inoculated with *Escherichia coli* was placed on the top of the clean sediment in the flume. Prior to each experimental run, sediment samples were collected at the front, middle, and end of the flume. During each run, flow discharge was fixed, and the tailgate was adjusted to obtain a desired flow depth in the flume. Flow depth was measured using a fine-scaled ruler glued to the side of the flume, while flow discharge was determined based on the opening of the valve. Flow velocity was calculated using flow discharge and flow depth.

At each flow depth, the experimental run lasted for at least 30 min before water samples were collected from the flume and the tank. Then, the velocity was increased by lowering the tailgate. After the flow became steady and quasiuniform, which typically took more than 30 min, water samples were collected again from the flume and the tank. This procedure continued until flow velocity reached about 40 cm/s. At each discharge, three different velocities were tested. After this series of experimental runs, flow was stopped, and water was drained out of the flume. Sediment was immediately resampled at the same locations as those before the run. All the samples were immediately placed in an iced cooler and sent to the microbiology lab to enumerate the *Escherichia coli* or MS2. Each experiment was triplicated to ensure the results were consistent. After collecting the samples, the flume was cleaned with liquid bleach to remove any remaining microorganisms in the system.

The third series of experiments was designed to determine the correlation between MS2 in water and flow properties. Sediment inoculated with MS2 instead of *Escherichia coli* was placed in the flume prior to each experiment. Then, flow was introduced to the flume, and the tailgate was adjusted to reach desired flow depths. Flow rate and depths were measured using the same method as in the second series. Sediment samples were collected before and after each experiment, and analyzed. Table 1 summarizes flow conditions for all the experimental runs. The duplicates of each experiment nearly had the same flow properties as the original. The same flow discharges, depths, and velocities were used in the experiments for *Escherichia coli* and MS2 in sandy loamy bed. For sandy bed sediment, only *Escherichia coli* was tested using the similar flow conditions as those for the sandy loamy bed.

Table 1. Experimental Flow Conditions

Series number	Organism	Sediment	Flow rate (L/s)	0–30 min		30–60 min		60–90 min	
				Depth (cm)	Velocity (cm/s)	Depth (cm)	Velocity (cm/s)	Depth (cm)	Velocity (cm/s)
Second	*Escherichia coli*	Sand	0.41	5.4	5	2.7	10	1.4	20
Second	*Escherichia coli*	Sand	0.41	5.4	5	2.7	10	1.4	20
Second	*Escherichia coli*	Sand	0.41	5.4	5	2.7	10	1.4	20
Second	*Escherichia coli*	Sand	0.73	4.8	10	2.4	20	1.6	30
Second	*Escherichia coli*	Sand	0.73	4.8	10	2.4	20	1.6	30
Second	*Escherichia coli*	Sand	0.73	3.2	15	1.9	25	1.6	30
Second	*Escherichia coli*	Sand	1.46	4.8	20	3.2	30	2.4	40
Second	*Escherichia coli*	Sand	1.46	4.8	20	3.2	30	2.4	40
Second	*Escherichia coli*	Sand	1.46	3.8	25	2.8	35	2.4	40
Second	*Escherichia coli*	Sandy loam	0.41	5.4	5	2.7	10	1.4	20
Second	*Escherichia coli*	Sandy loam	0.41	5.4	5	2.7	10	1.4	20
Second	*Escherichia coli*	Sandy loam	0.41	5.4	5	2.7	10	1.4	20
Second	*Escherichia coli*	Sandy loam	0.73	4.8	10	2.4	20	1.6	30
Second	*Escherichia coli*	Sandy loam	0.73	4.8	10	2.4	20	1.6	30
Second	*Escherichia coli*	Sandy loam	0.73	4.8	10	2.4	20	1.6	30
Second	*Escherichia coli*	Sandy loam	1.46	4.8	20	3.2	30	2.4	40
Second	*Escherichia coli*	Sandy loam	1.46	4.8	20	3.2	30	2.4	40
Second	*Escherichia coli*	Sandy loam	1.46	4.8	20	3.2	30	2.4	40
Third	MS2	Sandy loam	0.41	5.4	5	2.7	10	1.4	20
Third	MS2	Sandy loam	0.41	5.4	5	2.7	10	1.4	20
Third	MS2	Sandy loam	0.41	5.4	5	2.7	10	1.4	20
Third	MS2	Sandy loam	0.73	4.8	10	2.4	20	1.6	30
Third	MS2	Sandy loam	0.73	4.8	10	2.4	20	1.6	30
Third	MS2	Sandy loam	0.73	4.8	10	2.4	20	1.6	30
Third	MS2	Sandy loam	1.46	4.8	20	3.2	30	2.4	40
Third	MS2	Sandy loam	1.46	4.8	20	3.2	30	2.4	40
Third	MS2	Sandy loam	1.46	4.8	20	3.2	30	2.4	40

Data Processing

Water and sediment samples with *Escherichia coli* were spread plated on MacConkey Agar (BD Biosciences, Franklin Lakes, New Jersey) in the volumes of 100 μL and 1 mL. These samples were serially diluted (10-fold) using sterile phosphate buffered saline (PBS) (pH 7.4) and incubated at 37°C overnight. After incubation, pink colonies were enumerated. Water and sediment samples with MS2 were processed using a double-agar overlay method (Kropinski et al 2009). The host organism (*Escherichia coli* ATCC 15597) was propagated in sterile TSB (BD Biosciences) and allowed to reach an exponential growth phase in approximately 4 h with agitation at 37°C. Then, 0.5 mL of the host organism was combined with two volumes of sample, 1 mL or 100 μL, in 5 mL of sterile, melted top agar. The tube was gently swirled to mix and poured over sterile tryptic soy agar (BD Biosciences) and incubated overnight at 37°C. Plaques (clearings in the bacterial lawn) were enumerated the next day. The total number of *Escherichia coli* and MS2 in the water was calculated as

$$N_w = C_f V_f + C_t(247{,}000 - V_f) \quad (1)$$

where N_w = number of *Escherichia coli* or MS2 in the water with the units of colony-forming units (CFU) for *Escherichia coli* and plaque-forming units (PFU) for MS2, respectively; C_f = concentration of *Escherichia coli* or MS2 in the flume (CFU/mL or PFU/mL); V_f = volume of water in the flume (mL); and C_t = concentration of *Escherichia coli* or MS2 in the tank (CFU/mL or PFU/mL). The total water volume was 247,000 mL, which consists of 246,000 mL originally in the tank, and 1,000 mL added to the flume with the inoculated sediment.

Table 2. Results from the First Series of Experiments

Organism	Sediment	Sample time (h)	Flume (CFU/mL or PFU/mL)	Tank (CFU/mL or PFU/mL)	Starting inoculum (CFU or PFU)
Escherichia coli	Sandy loam	0.5	370	155	3.46×10^9
Escherichia coli	Sandy loam	1	425	630	3.46×10^9
Escherichia coli	Sandy loam	2	2,080	2,105	3.46×10^9
Escherichia coli	Sandy loam	4	1,675	1,675	3.46×10^9
Escherichia coli	Sandy loam	6	1,420	995	3.46×10^9
Escherichia coli	Sandy loam	0.5	261	286	3.66×10^9
Escherichia coli	Sandy loam	1	511	585	3.66×10^9
Escherichia coli	Sandy loam	2	790	806	3.66×10^9
Escherichia coli	Sandy loam	4	1,044	1,216	3.66×10^9
Escherichia coli	Sandy loam	6	1,664	1,964	3.66×10^9
MS2	Sandy loam	0.5	299.5	308.5	1.44×10^{11}
MS2	Sandy loam	1	452	486	1.44×10^{11}
MS2	Sandy loam	2	563	500	1.44×10^{11}
MS2	Sandy loam	4	781	748	1.44×10^{11}

The concentration of *Escherichia coli* in the water and sediment was calculated as

$$C_w = N_w/247{,}000 \quad (2)$$

where C_w = concentration of *Escherichia coli* or MS2 in the water (CFU/mL or PFU/mL). The numbers of *Escherichia coli*, N_w, changes with the concentration of suspended sediment; therefore the concentration of *Escherichia coli* in the water varies with flow velocity and depth. If N_{tot} represents the total number of inoculum in CFU or PFU (*Escherichia coli* or MS2), and the mass of the inoculated sediment for each experimental run remains the same, 4.086 kg, then the concentration of *Escherichia coli* or MS2 in the sediment at the beginning of each run is

$$C_s = N_{tot}/4.086 \quad (3)$$

where C_s = concentration of *Escherichia coli* or MS2 in the sediment (CFU/kg or PFU/kg). As flow depth and velocity increase, the fraction of *Escherichia coli* or MS2 in the water, calculated as

Table 3. Results from the Second Series of Experiments

Organism	Sediment	Flow rate (L/s)	Velocity (cm/s)	Flume (CFU/mL)	Tank (CFU/mL)	Starting inoculum (CFU)
Escherichia coli	Sand	0.41	5	0.3	0	6.00×10^8
Escherichia coli	Sand	0.41	10	2.6	1.7	6.00×10^8
Escherichia coli	Sand	0.41	20	82.4	72	6.00×10^8
Escherichia coli	Sand	0.41	5	44[a]	0	5.02×10^8
Escherichia coli	Sand	0.41	10	1	2	5.02×10^8
Escherichia coli	Sand	0.41	20	1	0	5.02×10^8
Escherichia coli	Sand	0.41	5	0	0	5.00×10^8
Escherichia coli	Sand	0.41	10	0	0	5.00×10^8
Escherichia coli	Sand	0.41	20	0	0	5.00×10^8
Escherichia coli	Sand	0.73	10	49	19.5	2.50×10^9
Escherichia coli	Sand	0.73	20	126.5	284.5	2.50×10^9
Escherichia coli	Sand	0.73	30	409.5	117	2.50×10^9
Escherichia coli	Sand	0.73	10	31	43	2.06×10^9
Escherichia coli	Sand	0.73	20	30	5.5	2.06×10^9
Escherichia coli	Sand	0.73	30	87.5	166	2.06×10^9
Escherichia coli	Sand	0.73	15	0	0	2.16×10^9
Escherichia coli	Sand	0.73	25	0	0	2.16×10^9
Escherichia coli	Sand	0.73	30	2.5	0	2.16×10^9
Escherichia coli	Sand	1.46	20	27	14.5	2.60×10^9
Escherichia coli	Sand	1.46	30	127	97	2.60×10^9
Escherichia coli	Sand	1.46	40	879	762.5	2.60×10^9
Escherichia coli	Sand	1.46	20	0	0	6.00×10^7
Escherichia coli	Sand	1.46	30	0	0	6.00×10^7
Escherichia coli	Sand	1.46	40	2.5	1	6.00×10^7
Escherichia coli	Sand	1.46	25	0	0.5	2.90×10^9
Escherichia coli	Sand	1.46	35	0	0.5	2.90×10^9
Escherichia coli	Sand	1.46	40	5	10	2.90×10^9
Escherichia coli	Sandy loam	0.41	5	0	0	1.06×10^9
Escherichia coli	Sandy loam	0.41	10	0	0	1.06×10^9
Escherichia coli	Sandy loam	0.41	20	96	224	1.06×10^9
Escherichia coli	Sandy loam	0.41	5	0	0	6.60×10^8
Escherichia coli	Sandy loam	0.41	10	0	0	6.60×10^8
Escherichia coli	Sandy loam	0.41	20	122	173.5	6.60×10^8
Escherichia coli	Sandy loam	0.41	5	0	0.5	1.98×10^9
Escherichia coli	Sandy loam	0.41	10	2	3.5	1.98×10^9
Escherichia coli	Sandy loam	0.41	20	147.5	158	1.98×10^9
Escherichia coli	Sandy loam	0.73	10	0	0	1.40×10^9
Escherichia coli	Sandy loam	0.73	20	6	13	1.40×10^9
Escherichia coli	Sandy loam	0.73	30	152.5	162.5	1.40×10^9
Escherichia coli	Sandy loam	0.73	10	43.5	41	3.36×10^9
Escherichia coli	Sandy loam	0.73	20	794	972	3.36×10^9
Escherichia coli	Sandy loam	0.73	30	1,030	1,726	3.36×10^9
Escherichia coli	Sandy loam	0.73	10	0.5	2	1.76×10^9
Escherichia coli	Sandy loam	0.73	20	61	135	1.76×10^9
Escherichia coli	Sandy loam	0.73	30	1,517	1,982.5	1.76×10^9
Escherichia coli	Sandy loam	1.46	20	0	1	1.34×10^9
Escherichia coli	Sandy loam	1.46	30	107	118.5	1.34×10^9
Escherichia coli	Sandy loam	1.46	40	290	118.5	1.34×10^9
Escherichia coli	Sandy loam	1.46	20	420	430	4.72×10^9
Escherichia coli	Sandy loam	1.46	30	1,425	945	4.72×10^9
Escherichia coli	Sandy loam	1.46	40	4,120	3,915	4.72×10^9
Escherichia coli	Sandy loam	1.46	20	46	35	2.58×10^9
Escherichia coli	Sandy loam	1.46	30	686.5	696.5	2.58×10^9
Escherichia coli	Sandy loam	1.46	40	1,746	1,812	2.58×10^9

[a]This datum is likely to be contaminated by the sediment stirred up by the sampler.

N_{tot}/N_t, increases. To determine the correlation between the fraction of *Escherichia coli* or MS2 in water and bed shear stress, the nondimensional shear stress is used

$$\tau_* = \frac{\rho_w u_*^2}{(\rho_s - \rho_w) g D_{50}} \quad (4)$$

where τ_* = nondimensional shear stress; u_* = shear velocity, calculated from $(u/u_*) = (1/\kappa)\ln(z/k) + A_r$ (m/s); ρ_w = water density (kg/m^3); ρ_s = sediment density (kg/m^3); g = gravitational acceleration (m/s^2); and D_{50} = medium diameter of sediment (m).

Experimental Results

The results for the first, second, and third series of experiments are summarized in Tables 2–4, respectively. These tables show the experimental flow discharge and velocity as well as the concentration of microorganisms in water and sediment. Sand-sized sediment was only used in the second series of experiments for *Escherichia coli*. Empirical relations were derived based on these data.

Escherichia Coli versus Bed Shear Stress

Fig. 5 shows the relationship between the fraction of *Escherichia coli* in water and the bed shear stress for sediment with $D_{50} = 0.05$ mm. Since triplicate experiments were performed at each flow condition, there are two or three measured data at each shear stress (Fig. 5). This is attributed to the randomness and error of the measured *Escherichia coli* counts. In the first series of experiments, one order of magnitude was considered as the range of measurement error. Therefore, an error bar was added to the mean *Escherichia coli* fraction at each shear stress. The upper and lower limits of the error bar are the possible range of *Escherichia coli* in water at a given shear stress. Although the error range is large, it is undeniable that the fraction of *Escherichia coli* in water increases with shear stress. The best curve fitting between the fraction of *Escherichia coli* and bed shear stress is a power function. The correlation coefficient $R^2 = 0.682$, and the functional relation is

$$N_w/N_{tot} = 0.640\tau_*^{1.82} \quad (5)$$

Since the total volume of water, the total mass of bed sediment, and the inoculated sediment remained as constants throughout the experiments, the concentration of *Escherichia coli* in water (CFU/mL) and the concentration of *Escherichia coli* in sediment (CFU/kg) can be calculated by using Eqs. (2) and (3), respectively. The correlation among the ratio of *Escherichia coli* concentration in water, that in sediment, and the bed shear stress was formulated using curve fitting. The power relationship, Eq. (6), was found, similar to Eq. (5) except for the coefficient, but it is written in terms of *Escherichia coli* concentration in water and sediment as

$$C_w/C_s = 1.06 \times 10^{-5}\tau_*^{1.82} \quad (6)$$

When shear stress is less than 0.1, the percentage can be as low as 1%, which is negligible. Therefore, the critical shear stress for *Escherichia coli* entrainment was set as $\tau_* = 0.1$, which is greater than the constant value 0.047 for noncohesive sediment. This indicates that sediment with *Escherichia coli* is harder to be entrained than the clean sediment. This phenomenon is caused by the cohesion from the biofilms between particles created by *Escherichia coli*.

Fig. 6 shows the relationship between the fraction of *Escherichia coli* in water and the bed shear stress for sandy sediment with $D_{50} = 0.4$ mm. Similarly, an error bar was shown in Fig. 6 to illustrate the potential errors of measurements. Comparing with Fig. 5, the measured data are much more scattered than those in Fig. 5, and thus the correlation coefficient ($R^2 = 0.028$) is much smaller. Even in still water, the majority (>90%) of bacteria will exist as unattached in the sandy sediment, rather than attached to the sand through adsorption (Bitton and Marshall 1980). During the

Table 4. Results from the Third Series of Experiments

Organism	Sediment	Flow rate (L/s)	Velocity (cm/s)	Flume (PFU/mL)	Tank (PFU/mL)	Starting inoculum (PFU)
MS2	Sandy loam	0.41	5	6	4.5	1.34×10^9
MS2	Sandy loam	0.41	10	55	55.5	1.34×10^9
MS2	Sandy loam	0.41	20	219	256	1.34×10^9
MS2	Sandy loam	0.41	5	14.5	21.5	1.50×10^9
MS2	Sandy loam	0.41	10	24	27.5	1.50×10^9
MS2	Sandy loam	0.41	20	176	172.5	1.50×10^9
MS2	Sandy loam	0.41	5	94	131	1.10×10^{10}
MS2	Sandy loam	0.41	10	191.5	164	1.10×10^{10}
MS2	Sandy loam	0.41	20	515	560	1.10×10^{10}
MS2	Sandy loam	0.73	10	43	33	3.10×10^9
MS2	Sandy loam	0.73	20	220	269	3.10×10^9
MS2	Sandy loam	0.73	30	745	367.5	3.10×10^9
MS2	Sandy loam	0.73	10	306	285.5	2.22×10^{10}
MS2	Sandy loam	0.73	20	1,565	1,320	2.22×10^{10}
MS2	Sandy loam	0.73	30	3,410	2,170	2.22×10^{10}
MS2	Sandy loam	0.73	10	5.5	9	7.14×10^8
MS2	Sandy loam	0.73	20	29	31	7.14×10^8
MS2	Sandy loam	0.73	30	68	72.5	7.14×10^8
MS2	Sandy loam	1.46	20	42.5	49	1.30×10^9
MS2	Sandy loam	1.46	30	118.5	136.5	1.30×10^9
MS2	Sandy loam	1.46	40	148.5	132.5	1.30×10^9
MS2	Sandy loam	1.46	20	180.5	225	3.74×10^9
MS2	Sandy loam	1.46	30	752	546	3.74×10^9
MS2	Sandy loam	1.46	40	1,300	1,290	3.74×10^9
MS2	Sandy loam	1.46	20	346.5	272.5	1.62×10^{10}
MS2	Sandy loam	1.46	30	1,050	844	1.62×10^{10}
MS2	Sandy loam	1.46	40	1,965	1,340	1.62×10^{10}

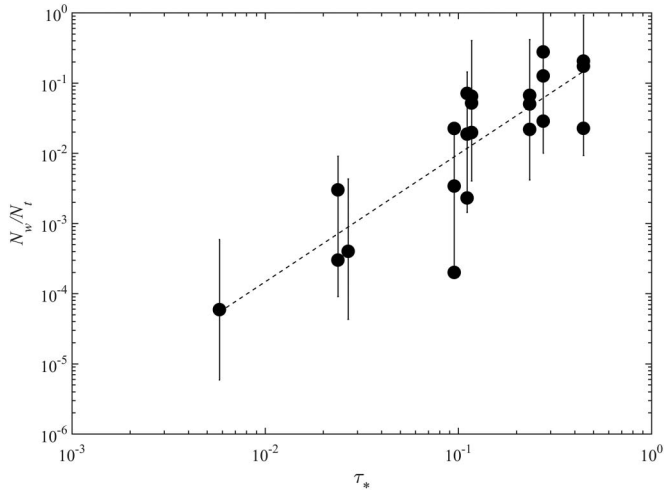

Fig. 5. Fraction of *Escherichia coli* in the water versus bed shear stress in sandy loamy bed

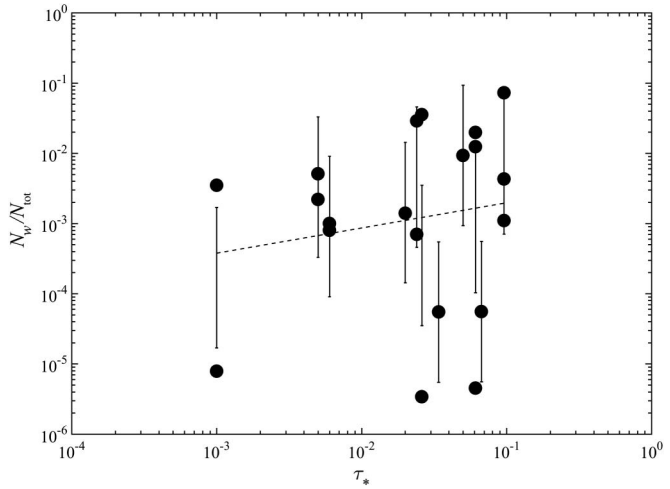

Fig. 6. Fraction of *Escherichia coli* in the water versus bed shear stress in sandy bed

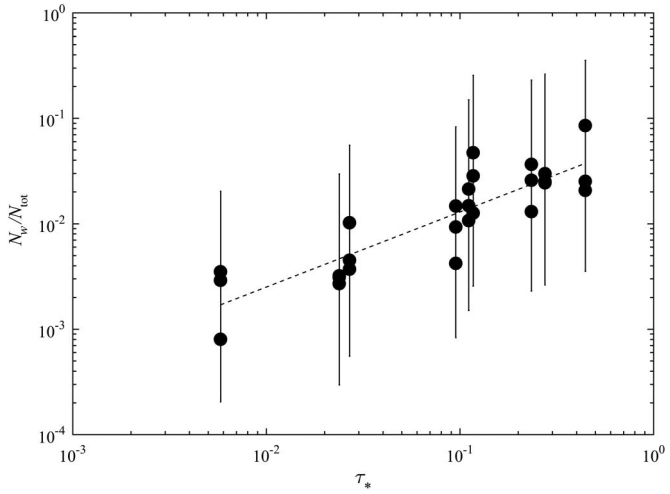

Fig. 7. Fraction of MS2 in the water versus bed shear stress in sandy loamy bed

experiment, *Escherichia coli* actually was present unattached to the sandy sediment. However, Bitton and Marshall (1980) found that more bacteria can be adsorbed to silt- and clay-sized sediment. Therefore, *Escherichia coli* concentration has less dependence on the resuspension of sediment from the sandy bed.

MS2 versus Bed Shear Stress

Only in the sandy loamy bed did the fraction of *Escherichia coli* in water show a significant correlation with bed shear stress. Gerba and Schaiberger (1975) found that viruses poorly adsorb to the sand. For example, when the sediment concentration in water was 10,000 mg/L, the virus adsorption rate was 9% measured; as the concentration was reduced to 1,000 mg/L, the adsorption amount was zero (Bitton and Marshall 1980). Therefore, only the resuspension of MS2 from the fine sediment bed, sandy loam was studied. Fig. 7 shows the relationship between the fraction of MS2 in water and the bed shear stress for $D_{50} = 0.05$ mm sandy loam. The error bar shows the range of error for the mean value at each shear stress. The relation between the fraction of MS2 in water and bed shear stress is also best fitted to a power function

$$N_w/N_{\text{tot}} = 0.0664\tau_*^{0.712} \tag{7}$$

Similarly, using the concentrations of MS2 in water and sediment calculated by Eqs. (2) and (3), the relation between the fraction of MS2 in water and the bed shear stress in terms of their concentrations is

$$C_w/C_s = 1.10 \times 10^{-6}\tau_*^{0.712} \tag{8}$$

The correlation coefficient for Eqs. (7) and (8) is $R^2 = 0.753$. Comparing Eqs. (5) and (7), when the shear stress is small, more MS2 can be entrained into water than *Escherichia coli*. The possible reason is that when the shear stress is small, the entrainment of *Escherichia coli* or MS2 into water is through a physical diffusion process. The size of *Escherichia coli* (1-μm scale) is much larger than MS2 (0.01-μm scale); therefore, it is more difficult for *Escherichia coli* to enter the water through the diffusion process.

Application to Irrigation Canals

Both Eqs. (6) and (8) are only applicable to sandy loamy bed sediment. For field applications, *Escherichia coli* in sediment ranged from 10^4 to 5.0×10^5 CFU/g (Kim et al. 2010, Pachepsky and Shelton 2011). In this study, the concentration of *Escherichia coli* in inoculated sediment varied from 1.5×10^4 to 1.2×10^5 CFU/g within the range of field observations. The concentration of *Escherichia coli* and MS2 in water in the sandy bed channel has no apparent relation with flow properties. Since the maximum shear stress on a canal bed surface is limited by its slope and size, the maximum allowable *Escherichia coli* concentration in sandy loamy bed sediment is limited by the maximum bed shear stress. Using CFU/mL as the unit for the concentration of *Escherichia coli* in sediment, Eq. (6) was revised for the maximum *Escherichia coli* concentration in sediment as

$$C_{s,E.coli}^{\max} = 9.45 \times 10^4 C_{c,E.coli}\tau_*^{-1.82} \tag{9}$$

where $C_{s,E.coli}^{\max}$ in CFU/mL = maximum allowable *Escherichia coli* concentration in sediment and $C_{c,E.coli}$ = regulated criteria of *Escherichia coli* in water (or maximum allowable *Escherichia coli* in water). The Food and Drug Administration (FDA) requires that water used for produce irrigation have less than a geometric average of 126 CFU *Escherichia coli* per 100 mL of water (USFDA 2015).

Then, the maximum allowable *Escherichia coli* concentration in bed sediment can be calculated by substituting the criteria into Eq. (9)

$$C_{s,E.coli}^{\max} = 1.19 \times 10^5 \tau_*^{-1.82} \quad (10)$$

To avoid irrigation water that exceeds the standard for *Escherichia coli*, the concentration of *Escherichia coli* in sandy loamy bed sediment must be measured periodically to ensure it is smaller than the maximum allowable value calculated by Eq. (10). Since Eqs. (9) and (10) were derived from Eq. (6), which was based on experiments of sandy loamy bed material, these two equations can only be applied to sandy loamy bed. For sandy bed, the majority of *Escherichia coli* and MS2 are not adsorbed to the sandy sediment.

Conclusions

Three series of laboratory experiments in a flume were conducted to study the entrainment of *Escherichia coli* and MS2 from bed sediment in irrigation canals. The first series found that *Escherichia coli* and MS2 in water reached the equilibrium concentration in about 30 min under a given flow condition. The second and third series of experiments showed that both *Escherichia coli* and MS2 had a significant correlation with bed shear stress in sandy loamy bed. The correlation between *Escherichia coli* and bed shear stress is weak in sandy bed sediment. The critical shear stress for *Escherichia coli* entrainment from sandy loamy bed sediment is 0.1, greater than that for clean sediment. The concentrations of *Escherichia coli* or MS2 in water are dependent on their concentrations in sandy loamy bed sediment and bed shear stress. Although this research is the first that studied the entrainment of *Escherichia coli* and MS2 in a hydraulic research facility, more experiments of nonuniform sediment at various flow conditions are needed to better quantify the correlations between *Escherichia coli* and MS2 concentrations and bed shear stress.

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