ASSESSING LINKAGES BETWEEN *E. COLI* LEVELS IN STREAMBED SEDIMENT AND OVERLYING WATER IN AN AGRICULTURAL WATERSHED IN IOWA DURING THE FIRST HEAVY RAIN EVENT OF THE SEASON

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ABSTRACT. *This study involved field observations in Squaw Creek watershed, located in central Iowa, to investigate the impact of a heavy rain event (rainfall of 71 mm in 24 h) on* E. coli *levels in the streambed sediment and overlying water. We assessed relationships between streamflow and* E. coli *and nutrient levels in the water column and streambed sediment. The results showed that during a heavy rain event,* E. coli *levels in the water column varied considerably, ranging from 360 to 37,553 CFU per 100 mL with a mean of 7,598 CFU per 100 mL. Elevated streamflow resulted in greater levels of* E. coli *in the water column. Streambed sediment* E. coli *levels ranged from 896 to 6,577 CFU per 100 g with a mean of 3,355 CFU per 100 g. Regression analysis found exponential relationships between streamflow and* E. coli *levels in the water column (R^2 = 0.56) and between streamflow and* E. coli *levels in the streambed sediment (R^2 = 0.45). R^2 values of the exponential relationship between streamflow and water column* E. coli *levels increased considerably when regressions for the rising and falling limbs of the hydrograph were performed separately (R^2 = 0.64 and 0.94, respectively). The exponential relationship between total suspended solids (TSS) and water column* E. coli *levels yielded an R^2 of 0.38, while TSS and streamflow yielded an exponential relationship with an R^2 of 0.64. The results presented here provide information on in-stream bacteria dynamics of an agricultural watershed during the first heavy rain of the season. We anticipate that the results will improve the understanding of in-stream* E. coli *transport during rain events and provide insight for policy makers to allocate* E. coli *loads in impaired water bodies.*

Keywords. E. coli*, Streambed sediment, Suspended sediment, Water quality.*

According to the U.S. Environmental Protection Agency (USEPA), elevated levels of pathogens or fecal indicator bacteria (FIB) in streams are a major cause of water quality impairments in the U.S. (USEPA, 2012a). More than 480,000 km of streams and 2 million ha of lakes are currently listed as impaired due to elevated pathogen levels. Implementation of plans to remediate impaired waters, called total maximum daily loads (TMDL), are estimated to cost more than $4 billion annually (USEPA, 2011). The USEPA has developed recreational water quality criteria (RWQC) in order to protect the public from exposure to harmful levels of pathogens in surface waters. In the 2012 RWQC (based on the 1986 RWQC), the USEPA included two sets of recommended criteria for waters designated for primary contact recreation: geometric mean (GM) coliform density and a statistical threshold value (STV) of indicator organisms. The recommended GM and STV values for *E. coli* in fresh waters are 126 CFU per 100 mL and 410 CFU per 100 mL, respectively (USEPA, 2012b).

During rain events, *E. coli* levels in streams are influenced by fresh inputs from subsurface flow (including tile drainage) and overland flow, as well as the resuspension of legacy *E. coli* present in streambed sediments (Cho et al., 2010; Droppo et al., 2009; Jamieson et al., 2005; Kiefer et al., 2012; Nagels et al., 2002; Pandey et al., 2012a; Pandey and Soupir, 2013; Wu et al., 2009). Many studies have demonstrated that streambed sediments can harbor considerable levels of FIB (Bai and Lung, 2005; Goyal et al., 1977; Muirhead et al., 2004; Pandey et al., 2012a; Smith et al., 2008). During high flows, sediment-associated bacteria are released from the streambed to the water column, elevating FIB levels in the water column (Pandey and Soupir, 2013).

Understanding the impacts of flow on in-stream *E. coli* levels is important. A number of studies (Bai and Lung, 2005; McDonald et al., 1982; Muirhead et al., 2004; Nagels et al., 2002; Parajuli et al., 2009; Sherer et al., 1988) have used flood events to study the impacts of flow on resuspension of *E. coli*. Nagels et al. (2002) reported bacterial dynamics during flood events in a stream in Australia and found that *E. coli* concentrations in the water column rose by more than two orders of magnitude during the rising

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limb of a flood event. Similarly, Muirhead et al. (2004) studied fecal contamination in sediment during artificial floods in dry weather and found that, during high flows, *E. coli* concentrations increased approximately two orders of magnitude from a background concentration of 100 CFU per 100 mL. Jamieson et al. (2005) also reported increased *E. coli* levels in the water column with increased flows. In addition to *E. coli* levels, streamflow and precipitation events have been found to increase nitrate-nitrogen concentrations in stream water (Feng et al., 2012; Hartz et al., 2008; Schilling et al., 2009). Therefore, improved understanding is needed of how the combined effects of elevated nutrients and flow are related to in-stream *E. coli* levels.

In this study, we evaluated the variations in *E. coli* levels in streambed sediment and the water column during a heavy rain event. At elevated streamflow, the relationships among total suspended solids (TSS), nutrient concentrations, and water and sediment column *E. coli* levels were evaluated. The study was performed during nighttime hours, when temperature variations were minimal, in order to limit the impact of solar radiation and temperature changes on *E. coli* growth and decay.

Study Area

The watershed data used in this study included land cover, soil types, locations of confined animal feeding operations (CAFOs), and the stream network (fig. 1). The Natural Resources Geographic Information System (NRGIS) library was used to obtain the watershed data. The majority of the land in the watershed, 74% of the total watershed area, is under row-crop production, dominated by corn and soybean. The percentages of deciduous forest, ungrazed grass, grazed grass, Conservation Reserve Program (CRP) grassland, and alfalfa in the watershed are 2.7%, 10.9%, 2.52%, 1.7%, and 1.8%, respectively. Common/industrial, residential, and barren land areas are 1.6%, 1.2%, and 0.06% of the total watershed area, respectively. The soils in

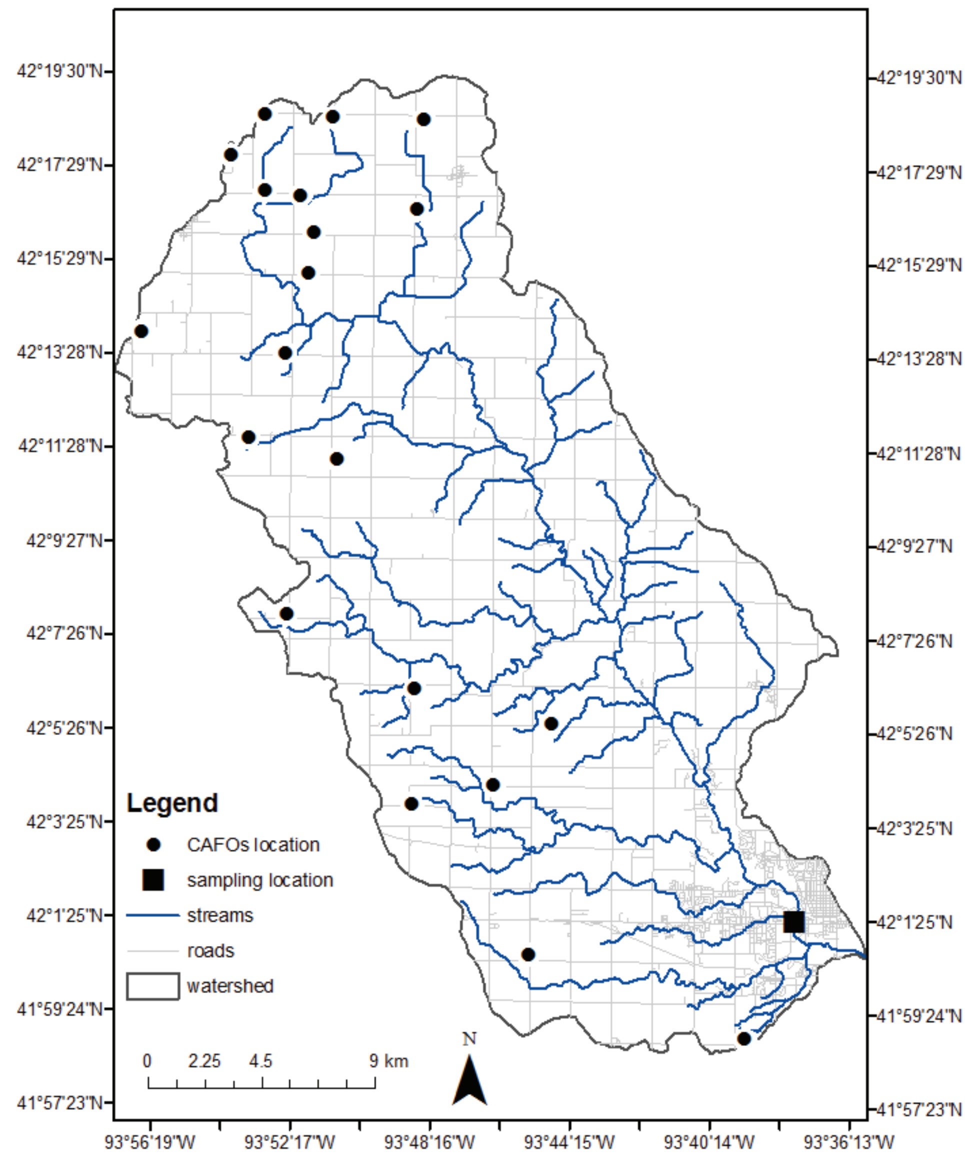

Figure 1. Squaw Creek watershed in central Iowa showing watershed boundary, CAFOs, sampling location, streams, and roads.

the Squaw Creek watershed consist of loamy Wisconsin glacial till and clayey lacustrine deposits, including loam, silty clay, clay loam, and silty clay loam. The total number of animals in all CAFOs (20 total) was 57,127. Hogs are the major livestock in the watershed, accounting for 80% of the total animals, followed by pigs (18%) and sows (2%). The Squaw Creek watershed, HUC 10 (0708010503) (fig. 1), has a total drainage area of 593 km^2. The basin length and perimeter of the watershed are 43.5 and 134.0 km, respectively, with an average land slope of 2.0%. The watershed contains 75 first-order streams. The main channel length is about 60.5 km. The total length of tributaries and streams within the watershed is approximately 346.7 km. The stream data were obtained from the NRGIS through the Iowa Department of Natural Resources. The main stream, Squaw Creek, passes through four counties (Story, Webster, Hamilton, and Boone) in central Iowa. Samples were collected at USGS gauging station 05470500 (42° 1′ 23″ N, 93° 37′ 49″ W) located in the city of Ames. Climate data (i.e., air temperature and precipitation) for Ames were obtained from the Iowa Environmental Mesonet (IEM).

Measurement and Analysis

Samples from the streambed sediment and the overlying water column were collected each hour during the heavy rain. The event (April 14-15, 2012) is listed in the storm event database of the National Oceanic and Atmospheric Administration (NOAA). The National Climatic Data Center reported this event as the first significant severe weather outbreak of the season (NOAA, 2014) in the study area. The cumulative rainfall of 71 mm occurred in 24 h. The amount of rainfall in that short period of time was a 100-year event (NOAA, 2014). Precipitation and streamflow are shown in figure 2a.

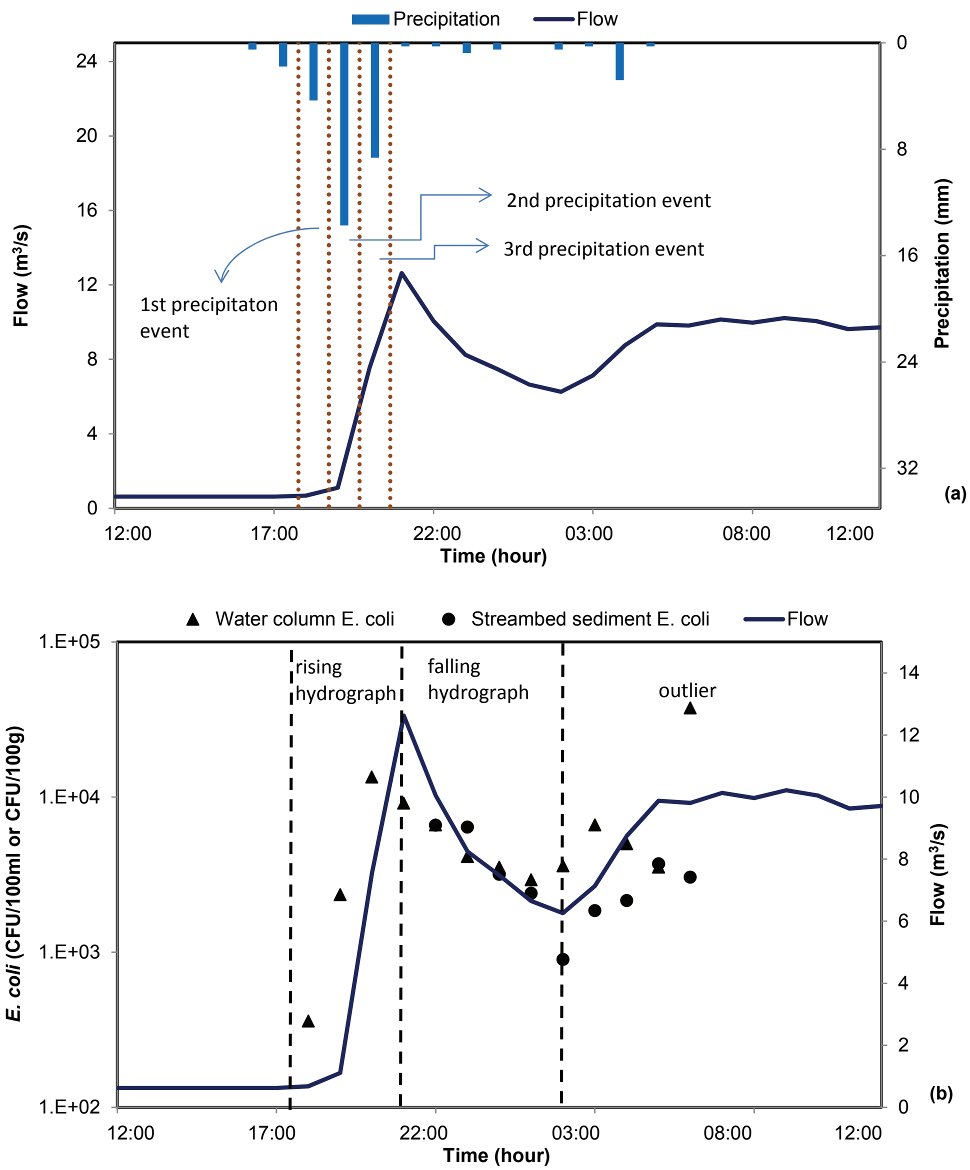

Figure 2. Streamflow, precipitation, and *E. coli* levels in the water column and streambed sediment: (a) precipitation and streamflow, and (b) streamflow and *E. coli* levels.

While water sampling began at 18:00 h on April 14 and ended at 06:00 h on April 15, sediment sample collection began 22:00 h on April 14 and ended at 06:00 h on April 15. The initial plan was to study the changes in *E. coli* levels during heavy rain in the stream water column only. However, as the event advanced, the initial sampling strategy was modified, and we began streambed sediment sampling because heavy rainfall can potentially impact *E. coli* levels in the streambed sediment. Water samples were collected from the center of the stream using a horizontal polycarbonate water bottle sampler (2.2 L, Forestry Suppliers, Inc., Jackson, Miss.) by lowering the instrument from a bridge into the center of the stream at the sampling location. Sediment samples were collected from the top 2 to 3 cm of the streambed using a shallow water bottom dredge sampler (15 cm × 15 cm opening, Forestry Suppliers, Inc., Jackson, Miss.) at the same location as water sample collection. The water samples were collected prior to sediment samples to avoid streambed sediment disturbance. Samples were stored at 4°C and analyzed within 24 h.

The *E. coli* attached to streambed sediment particles were detached by stirring 80 g of sediment diluted with 80 mL of purified water for 15 min at approximately 150 to 225 rpm using a magnetic stir bar, as described by Pandey et al. (2012a). The mixed solution was used to enumerate the *E. coli* concentration (CFU g^{-1}) in the sediment. The *E. coli* concentrations in the sediment and the water were determined by membrane filtration (EPA Method 1603) and enumerated on modified mTEC agar (Difco modified mTEC agar, Becton, Dickinson and Co., Sparks, Md.) (APHA, 1999). *E. coli* in the water and sediment samples were analyzed in triplicate. *E. coli* concentrations in the water column were measured over a 12 h period (18:00 h to 06:00 h), while *E. coli* concentrations in the sediment were measured over an 8 h period (22:00 h to 06:00 h) on April 14-15, 2012.

TSS concentrations were estimated gravimetrically using EPA Method 160.2. Polycarbonate filters (Material No. 1215637, GE Water & Process Technologies, Chicago, Ill.) were used to filter 200 mL water samples, and filters with separated solids were subsequently dried overnight at 103°C to 105°C. A filter pore size of 8 µm was selected to avoid clogging due to the elevated levels of fine particles in the stream water during the elevated flow conditions. The nitrate-nitrogen and orthophosphate concentrations of water column samples were analyzed using an AQ2 discrete auto analyzer (Seal Analytical, Mequon, Wisc.) following EPA Method 352.2. A spectrophotometer (DR 2800, Hach Co., Loveland, Colo.) was used to analyze water samples for total nitrogen and total phosphorus following Hach Methods 10071 and 8190, respectively. Total organic carbon (TOC) concentrations were estimated using the persulfate-ultraviolet oxidation method (Phoenix 8000 UV-persulfate TOC analyzer, Teledyne Dohrmann, Mason, Ohio).

The organic matter content of streambed sediment samples was determined using a loss-on-ignition (LOI) method (ASTM 2974-00D) (Sutherland, 1998). The total nitrogen and total carbon concentrations of the streambed sediment samples were determined using a C:N analyzer (Carter and Gregorich, 2007). The grain size curve was estimated using the AASTHO Soil Classification System (ASTM D422). Streamflow data were obtained from USGS gauging station 05470500 on Squaw Creek in Ames, Iowa, which is the same location where the samples were collected.

Average *E. coli* levels were estimated from triplicate samples of the water column and sediment. We used parametric methods for statistical analysis. The normality of the data (*E. coli* levels in water column and streambed sediment, and TSS concentrations) was checked using SAS JMP (JMP Pro 11, SAS Institute, Inc., Cary, N.C.) with the Shapiro-Wilk W test. The normal quantile plot, outlier box plot, and goodness-of-fit test were evaluated for normality analysis. Parametric methods, such as linear and exponential regression analyses, were performed using Microsoft Excel 2010 to relate *E. coli* concentrations with water quality parameters and streamflow. Coefficients of determination (R^2) were used as an indicator to evaluate the model fit.

RESULTS AND DISCUSSION

Figure 2 shows the precipitation (mm), cumulative precipitation (mm), and streamflow (m^3 s^{-1}) at the sampling location. The hourly changes in flow and precipitation for 24 h (from noon on April 14 to noon on April 15) are shown in figure 2a. During sampling, the streamflow varied from 0.7 m^3 s^{-1} (at 18:00 h) to 10.2 m^3 s^{-1} (at 06:00 h). At 18:00 h (during the first sample collection), streamflow was relatively low at 0.7 m^3 s^{-1}; however, flow increased 11-fold within a 2 h period. Cumulative precipitation was 6.6 mm at 18:00 h and 28.9 mm by 20:00 h. No considerable precipitation occurred after 22:00 h, and the cumulative precipitation at 06:00 h of the next day (April 15) remained at a total of 34.6 mm (fig. 2a). The impacts of precipitation and flow on the *E. coli* levels in the stream water and sediment are shown in figure 2b.

RELATIONSHIPS BETWEEN *E. COLI* CONCENTRATIONS AND STREAMFLOW

In general, *E. coli* levels in the water column and streambed sediment increased with the increase in streamflow (fig. 2b). For instance, the streamflow at 18:00 h was 0.7 m^3 s^{-1}, and it increased 18-fold at 21:00 h. The *E. coli* level in the water column was 360 CFU per 100 mL at 18:00 h, and it had increased 25-fold by 21:00 h. The flow receded slightly after 22:00 h, and it leveled off to 4.8 m^3 s^{-1} at 01:00 h. During this period, the water column *E. coli* decreased by 55%.

Although no additional considerable precipitation was observed at the gauging station (in Ames) after 22:00, streamflow ascended slightly between 02:00 h (4.8 m^3 s^{-1}) and 06:00 h (10.2 m^3 s^{-1}). This increase in flow resulted in a 10-fold increase in *E. coli* levels in the water column at 06:00 h when compared to the levels at 02:00 h. The concentration of *E. coli* in the first streambed sediment sample was 6,577 CFU per 100 g at 22:00 h. After 4 h (02:00 h on April 15), the concentration had decreased by 86%. During this time, the water column *E. coli* levels decreased by 45%.

Since the observations were performed overnight, the potential for *E. coli* growth and decay due to changes in temperature and solar radiation was likely negligible. The average air temperature during the observation period was 15.9°C ±0.6°C. The air temperature was 17.0°C at 18:00 h on April 14 and 15.9°C at 06:00 h on April 15. The variations in *E. coli* levels in the water column and streambed sediment were assumed to be driven primarily by the hydrologic factors streamflow and precipitation.

The normality tests of water column and streambed sediment *E. coli* levels and TSS concentrations resulted in p-values greater than 0.05. Therefore, the null hypothesis (i.e., the data are normally distributed) was not rejected. The p-value (Prob < W) by Shapiro-Wilk W test for water column *E. coli* levels was 0.13, while the p-value of streambed sediment *E. coli* levels was 0.17. The p-value of TSS concentrations was 0.17. These results indicated that the water column and streambed sediment *E. coli* levels and the TSS concentrations were normally distributed.

The relationship between streamflow and *E. coli* levels was expected, as previous studies have also shown correlations between water quality (i.e., *E. coli*, pH, sulfate, nutrients, metals) and watershed characteristics including rainfall (Krometis et al., 2007; Pandey et al., 2012b; Rothwell et al., 2010). Crowther et al. (2002), Kay et al. (2008), and Wilkes et al. (2011) reported that the levels of fecal indicators in stream waters increased considerably during rainfall events, potentially due to inputs from diffuse sources.

In addition to the *E. coli* influx from the watershed through runoff, another potential source of elevated *E. coli* levels in the water column could be the resuspension of particle-attached *E. coli* from the streambed sediment to the water column. Similar to this study, previous studies have observed high levels of indicator organisms in streambed sediments (Davies et al., 1995; Kim et al., 2010). These particle-attached microorganisms can be released from the streambed to the water column during high flow conditions. For example, Bai and Lung (2005) created a series of artificial high flow events in a stream by releasing reservoir water, which eliminated the possibility of bacteria contributions from overland flow (i.e., runoff from the watershed) to understand the *E. coli* resuspension process. They found that *E. coli* concentrations during peak flow (4.5 $m^3 s^{-1}$) were 14,000 to 16,000 times greater than during the base flow conditions.

Figure 3 compares the streambed sediment *E. coli* levels (CFU per 100 g) and water column *E. coli* levels (CFU per 100 mL). In approximately 55% of the collected samples, *E. coli* concentrations in the sediment exceeded the concentrations in the water column, while 44% of the samples had higher levels of *E. coli* in the water column. In 66% of the samples, the differences in *E. coli* levels between water and sediment were within a factor of 2, and in 33% of the samples the difference was greater than a factor of 2 (fig. 3). In one sample, which was collected at 06:00 h on April 15, the *E. coli* level in the water was one order of magnitude greater than that of the sediment. These results vary significantly from the multiple studies that have reported 10 to 10,000 times higher levels of FIB in streambed sediment samples when compared to the water column (Bai and Lung, 2005; Pandey et al., 2012a).

The changes in sediment and water column *E. coli* levels with flow signify that flow conditions have a considerable influence on *E. coli* levels. For example, on April 12, 2012 (at 12:00 h), when streamflow was 0.5 $m^3 s^{-1}$, the *E. coli* level in the water column was 27 (±12) CFU per 100 mL, and the *E. coli* level in the streambed sediment was 5,441(±585) CFU per 100 g. At the same sampling location on April 17, 2012, the streamflow increased 12.7-fold (at 12:00 h), and the *E. coli* level in the water column increased 88-fold. However, the *E. coli* level in the streambed sediment increased only 1.6-fold. A summary of the chemical properties of the stream water and sediment during the heavy rain event is shown in table 1.

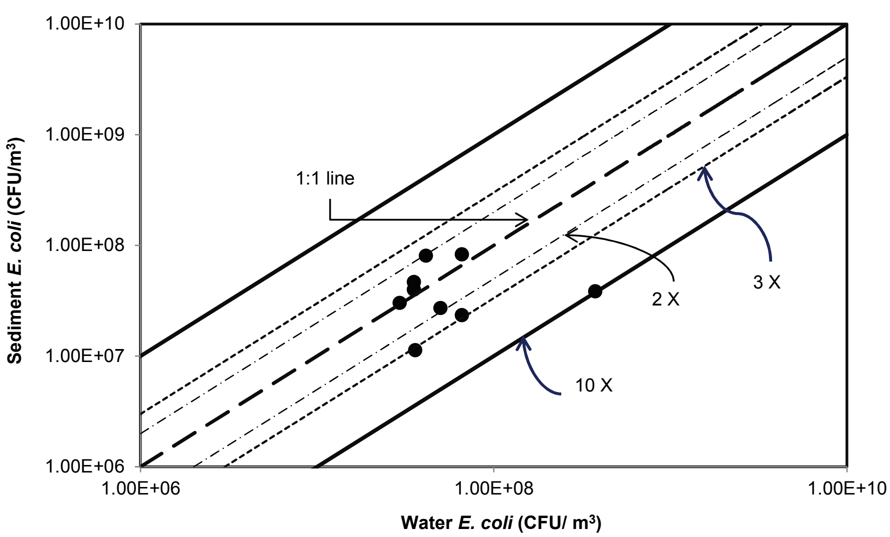

Figure 3. Comparison between streambed sediment *E. coli* and water column *E. coli* levels. The dashed line indicates the 1:1 line, the thick lines indicate a relationship within a factor of 2, the dotted lines indicate a relationship within a factor of 3, and the thin solid lines indicate one order of magnitude. *E. coli* levels in the water column and sediment were converted to CFU per 100 m^3. Sediment *E. coli* levels were estimated by multiplying the *E. coli* in the sediment (CFU g^{-1}) by the bulk density of the sediment (1.26×10^6 g m^{-3}; Pandey et al., 2012a).

Table 1. Statistics of *E. coli* levels and nutrient concentrations in water and sediment samples.

Parameter[a]	Mean	Median	SD	Min.	Max.
Stream water					
E. coli (CFU per 100 mL)	7,598	4,133	9,593	360	37,553
Streamflow (m^3 s^{-1})	7.4	7.5	3.4	0.7	12.6
TSS (ppm)	418	390	257	70	770
pH	7.8	7.7	0.1	7.7	7.9
Total nitrogen (ppm)	7.1	5.8	3.9	4.0	16.8
ORP (mV)	228	226	5.6	222	236
Nitrate-nitrogen (ppm)	3.6	2.4	3.2	1.4	11.8
Total phosphorous (ppm)	2.2	2.2	1.2	0.5	4.4
Orthophosphate (ppm)	0.1	0.1	0.1	0.1	0.2
TOC (ppm)	2.4	2.5	0.3	2	2.8
Streambed sediment					
E. coli (CFU per 100 g)	3,354	3,049	1,954	897	6,577
Total nitrogen (ppm)	550	535	298	260	856
Total carbon (%)	2.1	2.2	0.6	1.4	2.6
Organic matter (%)	0.4	0.4	0.2	0.3	0.6

[a] TSS = total suspended solids, ORP = oxygen reduction potential, and TOC = total organic carbon.

IMPACTS OF STREAMBED SEDIMENT PROPERTIES ON *E. COLI* LEVELS

Grain size analyses of the sediment samples are shown in figure 4a. The proportions of fine particles in sample 1 were greater than in samples 2 and 3. The D_{10} at 03:00 h increased 12-fold compared to the D_{10} at 22:00 h. In sample 1, approximately 25% of the particles were less than 0.6 mm in diameter. In sample 3 (collected 5 h after sample 1), only 0.2% of the particles were smaller than 0.6 mm. Figure 4c shows the proportions of sand, silt, and clay materials. In sample 1, the percentages of fine silt and fine clay were 1.3% and 1.5%, respectively, and the percentage of fine sand was 14.3%. In sample 2, the fine sand percentage decreased to 1.8%, while the fine silt and fine clay percentages decreased to 0% and 0.7%, respectively. In sample 3, most of the particles (>70%) were fine gravel, the fine clay percentage was 0.5%, and fine sand and fine silt were undetectable. The concentration of *E. coli* in the sediment decreased with decreasing percentages of smaller particles (i.e., silt and clay). For example, in sample 2, the *E. coli* concentration was 48% of the concentration in sample 1, while in sample 3, the *E. coli* concentration was 28% of the concentration in sample 1.

Comparison of the *E. coli* levels and sediment characteristics of the three samples taken at 22:00 h., 00:00 h, and 03:00 h indicated that *E. coli* levels in the streambed sediment and the proportions of fine particles in the sediment decreased over time. A potential reason for the decreased *E. coli* levels in the streambed sediment could be in-stream resuspension (Bai and Lung, 2005). Streambed sediment characteristics play an important role in regulating resuspension (Pandey et al., 2012a), which can be influenced by many factors, such as particle size, mineralogical composition, organic matter, temperature, pH, ionic strength, and streamflow (Droppo and Ongley, 1994; Mehta et al., 1989). Researchers such as Black et al. (2002) and Muirhead et al. (2004) have reported that most bacteria attach to cohesive particles and fine sediments, which appears evident in our results as well. Particles smaller than 8 μm (clay and very fine silt) showed stronger cohesion than larger silt particles (8 to 62 μm) (van Rijn, 2007).

The total nitrogen, total carbon (TC), and organic matter (OM) in the streambed sediment are shown in figure 4b. The percentages of total nitrogen, total carbon, and organic matter were greater in sample 1 than in samples 2 and 3. The increased percentages were likely caused by the greater presence of finer material in sample 1. In sample 1, the percentages of total carbon and organic matter were 2.6% and 0.6%, respectively. In sample 3, total carbon and organic matter decreased to 82% and 50% of the values in sample 1. In sample 1, the total nitrogen level was 856 ppm, while in samples 2 and 3 total nitrogen was 62% and 30% of the concentration in sample 1, respectively. Pachepsky and Shelton (2011) reported positive relationships between sediment *E. coli* levels and organic matter during runoff events. Although our results suggest that the *E. coli* levels followed the same trends as the nutrient concentrations in the streambed sediment, we suggest further studies to understand the relationships between sediment nutrient concentrations and *E. coli* levels because the sediment nutrient data in this study are limited. Previous studies reported that sediment organic matter and nutrient concentrations provide suitable conditions for bacterial growth (Garzio-Hadzick et al., 2010; Jamieson et al., 2005). However, Banning et al. (2003) reported that an increase in sediment nutrients may have little effect on *E. coli* persistence because of increased competition for nutrients by other microflora.

INTERACTIONS OF STREAMBED AND WATER COLUMN

As the $D_{50,}$ and D_{10} of the streambed sediment increased during the heavy rain event, the TSS and *E. coli* levels in the water column also increased, likely the result of resuspension of fine particles and *E. coli* attached to these particles. Over time, the proportion of fine particles in the sediment decreased. Increased TSS and *E. coli* levels in the water column with increased flow were reported elsewhere (Cho et al., 2010; Hipsey et al., 2008; Jamieson et al., 2005). However, simultaneous monitoring of streambed sediment properties was unavailable in these studies. In addition to resuspension, another likely source of increased TSS and *E. coli* during high flows was the influx of sediment and particle-attached *E. coli* from the watershed via surface runoff (Dorner et al., 2006; Fries et al., 2006; Kronetis et al., 2007). However, Dorner et al. (2006) reported that resuspension of legacy *E. coli* might have a greater impact on water column *E. coli* levels than fresh *E. coli* influx (via runoff) from the watershed.

Figure 5a shows the levels of *E. coli* and TSS in the water column, and figure 5b shows the linear and exponential relationships between TSS and *E. coli*. In the first water sample (collected at 18:00 h on April 14), TSS was 100 mg L^{-1}; it peaked at 760 mg L^{-1} at 21:00 h during the peak flow of 12.6 m^3 s^{-1}. During this period, the *E. coli* levels increased 25-fold. The TSS level dropped to 120 mg L^{-1} at 01:00 h on April 15 (16% of the peak), and at this time *E. coli* concentrations in the water column decreased to 22% of the peak. At 06:00 h on April 15, an exceptionally high *E. coli* level (37,533 CFU per 100 mL) was observed in the water column (shown as an outlier in fig. 2b), which was excluded from the regression analysis. We were unable

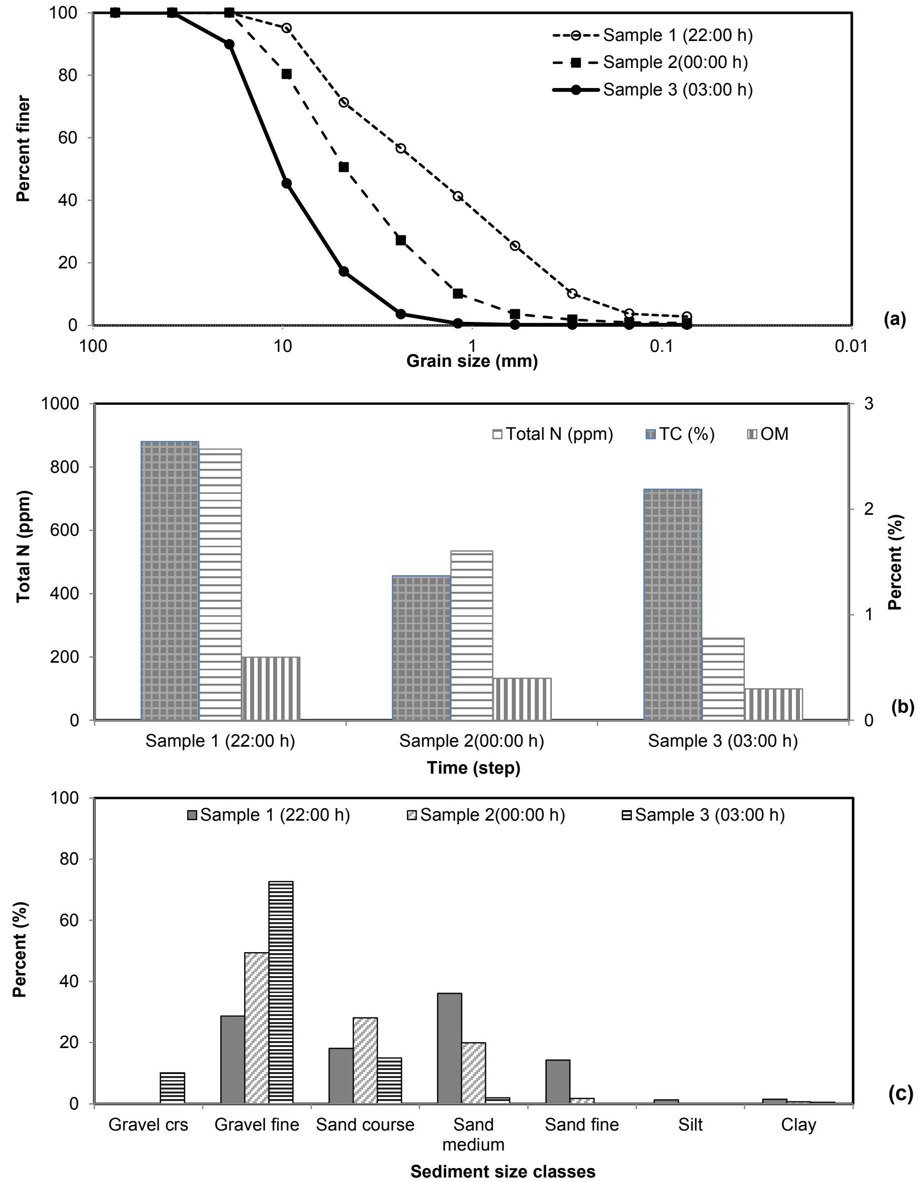

Figure 4. Streambed sediment size classes: (a) grain size of sediment samples; (b) total nitrogen, total carbon (TC), and organic matter (OM) content of samples 1, 2, and 3; and (c) percentage of gravel, sand, silt and clay of samples 1, 2, and 3.

to verify the potential reasons for this elevated *E. coli* level, as it was the last sample of the precipitation event. Further water sampling (i.e., after 06:00 h) was needed to confirm if this trend continued or if it was truly an outlier. An exponential relationship between TSS and *E. coli* levels was estimated (fig. 5b) by fitting an exponential trend line between TSS (*x*-axis) and *E. coli* level (*y*-axis in log scale). The R^2 of the exponential function was 0.38. Previous studies, such as Fries et al. (2006) and Krometis et al. (2007), also reported an increase in *E. coli* levels in the water column with increased TSS. Krometis et al. (2007) suggested that fecal coliform, enterococci, and TSS are greatest in the period soon after rain begins. The first flush event is the leading cause of influx of bacteria and particles from the watershed to the streams.

Regression analyses among streamflow, *E. coli* concentrations in the water column and streambed sediment, and TSS are shown in figure 6. The exponential regression between streamflow and *E. coli* levels was a better fit than the linear regression. For example, the R^2 values between the water column *E. coli* and streamflow in exponential and linear regressions were 0.56 and 0.31, respectively. The R^2 values for exponential and linear regressions between streambed sediment *E. coli* and streamflow were 0.52 and 0.45, respectively. When linear regressions were performed separately for the rising limb (flow from 0.7 to 12.6 $m^3 s^{-1}$) and falling limb (flow from 12.6 to 6.3 $m^3 s^{-1}$) of the hydrograph (fig. 2b), the R^2 value increased considerably. The R^2 was 0.64 for the rising limb and 0.94 for the falling limb. One reason for the increased R^2 values for the separate rising and falling limbs could be the identical patterns of flow and *E. coli* levels in both limbs (fig. 2b). Another possibility is the reduced number of samples (n = 4 for rising limb; n = 6 for falling limb) when analyzing the hydro-

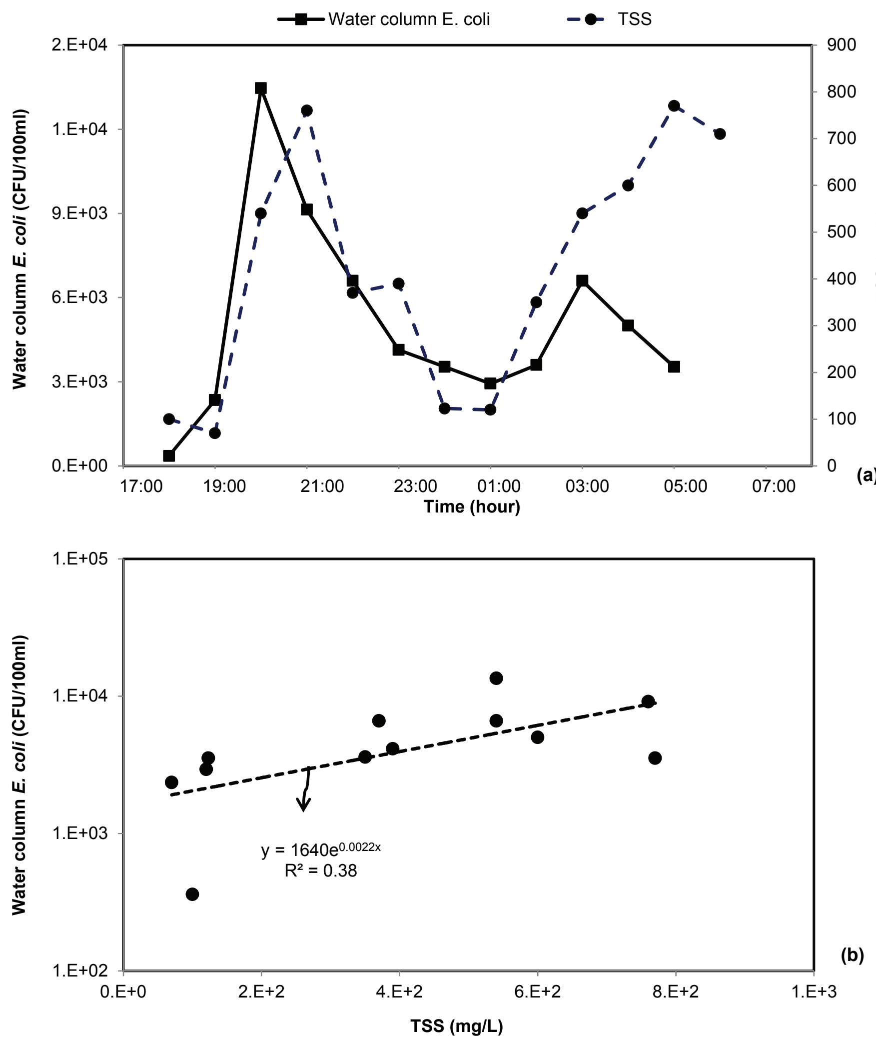

Figure 5. Linkages between total suspended solids (TSS) and *E. coli* levels: (a) *E. coli* levels and TSS concentrations in the water column, and (b) exponential relationship between TSS and *E. coli* levels. The total number of observations was 12.

graph limbs separately. The regressions between streamflow and TSS yielded greater R^2 values. In the exponential regression, the R^2 value between TSS and streamflow was 0.64.

RELATIONSHIPS BETWEEN *E. COLI* CONCENTRATIONS AND WATER QUALITY

The descriptive statistics of water quality parameters including TSS, nitrate-nitrogen, total nitrogen, total phosphorous, orthophosphate, and total organic carbon are shown in table 1. The nitrate-nitrogen and total phosphorus concentrations varied from 1.4 to 11.8 mg L^{-1} (with a mean of 3.6 ±3.2 mg L^{-1}) and from 0.5 to 4.4 mg L^{-1} (with a mean of 2.2 ±1.2 mg L^{-1}), respectively. The orthophosphate concentration range was 0.08 to 0.19 mg L^{-1} (with a mean of 0.14 ±0.04 mg L^{-1}). The variations in pH, ORP, and total organic carbon during the heavy rain event were relatively low (table 1). *E. coli* concentrations in the water column varied from 360 to 37,553 CFU per 100 mL with a mean of 7598 ±9593 CFU per 100 mL. A scatter plot and regression analysis were performed to obtain the best trend line fit. Performing linear, exponential, and logarithmic regression analyses, and excluding the outlier shown in figure 2b, resulted in slightly better R^2 values. The exponential relationship between orthophosphate and water *E. coli* levels yielded an R^2 of 0.45, while the exponential relationship between total phosphorus and water *E. coli* levels yielded an R^2 of 0.37. The logarithmic relationship between total organic carbon and water *E. coli* levels yielded an R^2 of 0.51, while the same relationship between nitrate-nitrogen and water *E. coli* levels yielded an R^2 of 0.24.

In-stream water quality (i.e., nutrients, *E. coli*, and TSS) in an agricultural watershed can be considerably impacted by runoff water influx. The influx of nutrients through surface runoff and drained water (via tile drains) can influence in-stream nutrient concentrations (Schilling, 2002; Schilling and Helmers, 2008; Schilling and Lutz, 2004). Although previous studies, such as Henis et al. (1989), reported that elevated nutrients in fresh waters increased *E. coli* levels, understanding the relationships between stream water nutrients and indicator organism levels can be challenging because microorganisms can proliferate even in environments with low nutrient levels (Egli, 2010; van Elsas et al., 2011; Vital et al., 2008). The results presented in our study improve understanding of the relationships between a heavy rain event and in-stream *E. coli* levels. Interactions

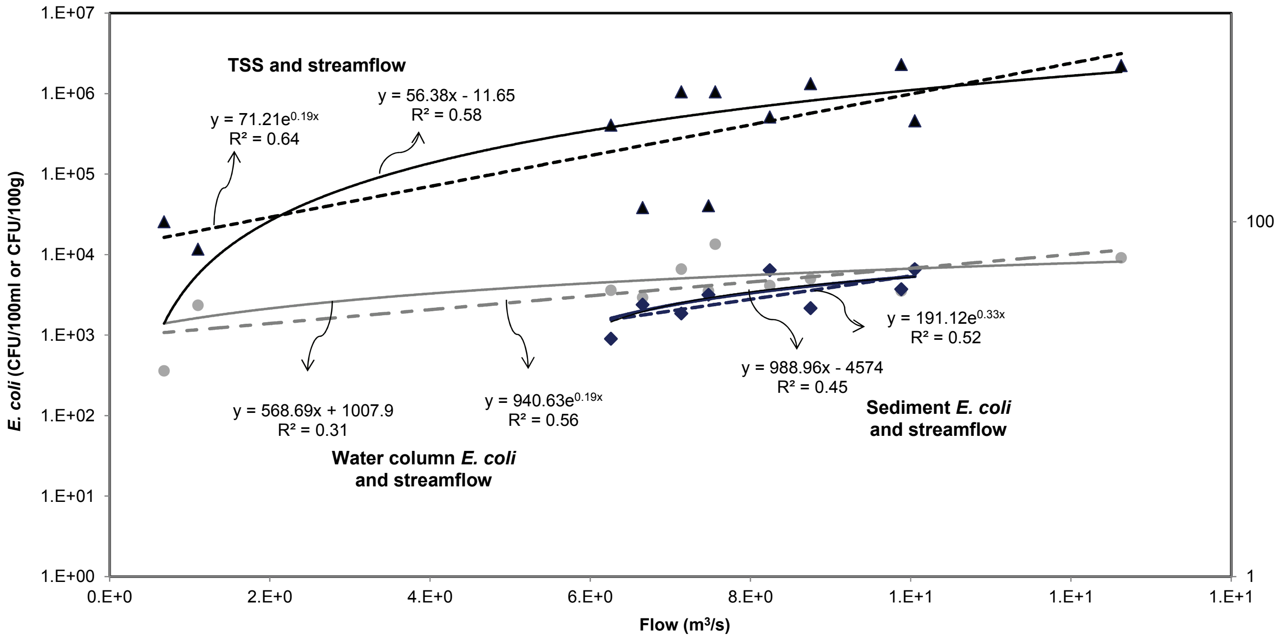

Figure 6. Relationships between streamflow, *E. coli* levels, and total suspended solids (TSS). Triangles indicate relationships between TSS and streamflow, circles indicate relationships between water column *E. coli* and streamflow, and diamonds indicate relationships between streambed sediment *E. coli* and streamflow. Solid lines indicate linear relationships, and dashed lines indicate exponential relationships. The total number of observations for water column *E. coli* levels and TSS was 12, and the total number of observations for sediment *E. coli* levels was 8.

between streamflow and *E. coli* levels in the water column and streambed sediment were analyzed. We suggest advancing this study by including additional experiments during multiple heavy rain events across a range of watershed characteristics to develop robust relationships between streamflow, in-stream nutrients, and *E. coli* levels during rain events. Additionally, extending observations to include the variations in *E. coli* levels from pre-rain to post-rain events, including base flow conditions, would further improve understanding of in-stream *E. coli* levels.

Conclusions

In this study, we attempted to improve understanding of how in-stream *E. coli* levels can vary during a heavy rain event in an agricultural watershed dominated by nonpoint sources of pollution. The focus was to understand the relationships between streamflow and *E. coli* levels in the water column as well as in the streambed sediment. Results showed that *E. coli* levels, suspended sediment, and nutrient concentrations varied considerably within a short period of time during the first heavy rain event of the season. The potential sources of *E. coli* and nutrients observed in the stream were likely a combination of runoff water from the agricultural watershed and resuspension from the streambed. Quantifying the contribution from the streambed through the resuspension process will require additional observations from multiple heavy rain events and from base flow conditions. We recommend that additional work include observations from multiple rain events and multiple watersheds with different landscape characteristics. In addition, combining laboratory-scale studies with field studies will help enhance our understanding of in-stream bacteria processes.

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