# Water and Sediment Microbial Quality of Mountain and Agricultural Streams

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#### Abstract

Increased public health risk caused by pathogen contamination in streams is a serious issue, and mitigating the risk requires improvement in existing microbial monitoring of streams. To improve understanding of microbial contamination in streams, we monitored Escherichia coli in stream water columns and streambed sediment. Two distinct streams and their subwatersheds were studied: (i) a mountain stream (Merced River, California), which represents pristine and wild conditions, and (ii) an agricultural stream (Squaw Creek, Iowa), which represents an agricultural setting (i.e., crop, manure application, cattle access). Stream water column and sediment samples were collected in multiple locations in the Merced River and Squaw Creek watersheds. Compared with the mountain stream, water column E. coli concentrations in the agricultural stream were considerably higher. In both mountain and agricultural streams, E. coli concentrations in bed sediment were higher than the water column, and principal component analysis indicates that land use affected water column E. coli levels significantly (p < 0.05). The cluster analysis showed grouping of subwatersheds for each basin, indicating unique land use features of each watershed. In general, water column E. coli levels in the mountain stream were lower than the USEPA's existing water quality criteria for bacteria. However, the E. coli levels in the agricultural stream exceeded the USEPA's microbial water quality criteria by several fold, which substantiated that increased agricultural activities, use of animal waste as fertilizers, and combined effect of rainfall and temperature may act as potential determining factors behind the elevated E. coli levels in agriculture streams.

#### **Core Ideas**

• Watershed land use influences in-stream pathogen contamination and hence public health risk.

- The agricultural stream showed higher *E. coli* concentration than the mountain stream.
- Increased forest and grassland use showed lower *E. coli* concentrations in stream water.
- Increased agricultural land use showed higher *E. coli* concentrations in stream water.
- The agricultural stream water column *E. coli* concentrations far exceeded USEPA recommended values.

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J. Environ. Qual. doi:10.2134/jeq2017.12.0483 This is an open access article distributed under the terms of the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/) Received 15 Dec. 2017. Accepted 25 Apr. 2018. \*Corresponding author (pkpandey@ucdavis.edu). ATHOGENIC bacteria in ambient water such as rivers, lakes, reservoirs, coastal, and noncoastal waters present a major water quality concern. Contaminated streams pose risks to public and animal health, and mitigating the risks requires improved understanding of bacterial contamination in stream water columns and streambed sediment at the watershed scale (Allende et al., 2017; Cho et al., 2016; Jamieson et al., 2004; Pachepsky et al., 2016; Pandey et al., 2014). One of the major issues in evaluating microbial water quality of streams is limited microbial data. The lack of observed data for evaluating surface water microbial quality often diminishes the chances of implementing water quality plans successfully.

Nevertheless, increased public concerns and the demand for improved water quality for both drinking and recreational purposes call for improvements in monitoring stream water quality. Currently, more than 480,000 km// of rivers and shorelines and 2 million ha of lakes in the United States are impaired by elevated bacteria and pathogen indicators (USEPA, 2012). As not all ambient water bodies are monitored for microbial quality, the actual number of impairments is likely much higher than reported. Approaches such as total maximum daily loads (TMDLs) are being developed for indicator bacteria causing impairment of ambient waters in the United States, and these TMDLs require accounting of both point sources and nonpoint sources (e.g., from agricultural activities) for applicable water quality standards (USEPA, 2012). However, plans like TMDLs have yet to be implemented in many countries (Pandey et al., 2014).

A major challenge in developing TMDLs for bacteriaimpaired streams is the limited availability of microbial data. Further, these TMDLs are based solely on the water column water quality, and contamination in streambed sediment is often overlooked. Improved surveillance and reconnaissance of bacterial contamination in streams as well as in streambed sediment can potentially help improve surface water quality. The focus of this study was to monitor microbial quality of two streams in distinct regions (mountainous and agricultural) to understand the potential effects of land use on microbial quality of streams possessing distinct watershed characteristics.

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Abbreviations: CFU, colony-forming unit; GM, geometric mean; STV, statistical threshold value; TMDL, total maximum daily load.

Evaluating microbial quality of a river and understanding the potential sources of contamination can be challenging because of the complexities involved in assessing the impacts of streambed sediment on the water column (Cho et al., 2016; Jamieson et al., 2005; Kim et al., 2017; Pandey et al., 2016). Further, the microbial quality of streambed sediment is rarely monitored. The difficulties involved in sediment sample collection and analysis of sediment attached bacteria can be attributed as main reasons for the nonavailability of these data.

Although the existing methods of quantification of bacteria resuspension and understanding of the precise impacts of sediment on the water column need to be improved, the adverse effects of sediment contamination on the water column are well established (Bai and Lung, 2005; Bragina et al., 2017; Jamieson et al., 2005; Pandey et al., 2016; Park et al., 2017; Piorkowski et al., 2014). The negative effects of sediment may depend on the flow, stream characteristics, weather, and watershed land use characteristics (Pandey and Soupir, 2013; Pandey et al., 2012a).

High concentrations of bacteria in streambed sediment have a potential to increase bacteria concentrations in the water column. Streambed sediment acts as a reservoir for bacteria, and resuspension of sediment particles can release particle-attached bacteria from the streambed to the water column (Cho et al., 2016; Droppo et al., 2009; Fries et al., 2008; Jamieson et al., 2005; Pandey and Soupir, 2013; Pandey et al., 2012b). Models that predict the effects of sediment water column interactions on water column bacteria concentrations have shown that the resuspension process plays an important role in increasing microbial contamination in the water column (Cho et al., 2016; Droppo et al., 2009; Jamieson et al., 2009; Jamieson et al., 2005; Pandey et al., 2012a; Rehmann and Soupir, 2009).

The lack of extensive observed data of bacteria in streambed sediment is a major hurdle when evaluating the microbial quality of a stream and developing a model useful for predicting bacteria concentrations of a stream under various flow and climate conditions. In many streams in the United States, limited bacteria data for evaluating the microbial quality of the water column do exist; however, for the streambed sediment, bacteria data are sparse. The goal of this study was to monitor water column and streambed sediment bacteria concentrations simultaneously to understand the microbial quality of agricultural and mountain streams.

This study builds on a previous study, which monitored bacteria concentrations in the water column and streambed sediment in a stream (Squaw Creek, Iowa) (Pandey et al., 2016). We extended the previous research by measuring Escherichia coli concentrations in streambed sediment and the water column of the Merced River, California. These two streams (Merced River and Squaw Creek) are distinct in nature. The Merced River passes through mountains and forested area, whereas Squaw Creek is an agricultural stream, mostly surrounded by corn (Zea mays L.) and soybean [Glycine max (L.) Merr.] crop fields. The majority of the watershed of the Merced River (particularly the upper watershed, near Yosemite National Park) is occupied by nonagricultural activities and mountains. The objectives of the study were (i) to evaluate the streambed sediment and water column E. coli concentrations in agricultural and mountain streams, (ii) understand the effects of an agricultural setting on stream bacteria concentrations, and (iii) perform a comparative analysis in terms of water quality for agricultural and mountain streams corresponding to the existing USEPA recreational microbial water quality criteria (USEPA, 2012). Having the data of these two distinct streams provided an opportunity to compare microbial health of agricultural and mountain streams. We anticipate that the observations provided here will provide additional insight with regards to assessing microbial quality of a stream.

# **Material and Methods**

# **Study Area**

The monitoring of a pathogen indicator (E. coli) in a streambed and water column was conducted in two watersheds: the Merced River Watershed, California, and Squaw Creek Watershed, Iowa (Fig. 1). In the Merced River, eight sampling locations were used to collect water and sediment samples for E. coli analysis. In Squaw Creek, water and sediment samples were collected in 16 locations for quantifying E. coli in the water column and sediment. Three sampling events were executed in each watershed. The sampling in Merced River was conducted between 2014 and 2017, whereas the sampling in Squaw Creek watershed was conducted between 2009 and 2011. The Merced River watershed area is approximately 2685 km<sup>2</sup>. The watershed is located in the central Sierra Nevada. The upper reaches of the Merced River are in Yosemite National Park. Flow in these reaches is unregulated, and many people visit the watershed when visiting the park (Hydra, 2017). One major hydroelectric project at New Exchequer Dam impounds 1.23 billion m<sup>3</sup> (1 million acre-foot) Lake McClure. The National Wild and Scenic Rivers System classifies total Merced River length of 197.14 km. River lengths classified as wild, scenic, and for recreational purposes are 114.26, 25.74, and 57.13 km, respectively (NWSRS, 2017). The Merced River flows from a glacially carved canyon (within Yosemite National Park) to Lake McClure Reservoir in the upper region (NPS, 2017). The first 127.13 km are protected with a wild and scenic river designation. In terms of vegetation, the riparian and meadow ecosystem within the river corridor includes alpine and subalpine meadows. About 647 ha (1600 acre) is designated as the Limestone Salamander Area of Critical Environmental Concern (NWSRS, 2017). From McClure Lake, the Merced River flows to the San Joaquin Valley. The lower Merced River drains into the San Joaquin River. The lower Merced River watershed represents an agricultural setting (crops, manure application, chemical use) (USGS, 2017).

The Squaw Creek watershed has a total drainage area of 592.39 km<sup>2</sup>. The main channel length is 60.46 km, and the total stream length within the watershed is 346.72 km. There are 75 first-order streams in the watershed. Watershed land use indicates 0.09, 0.17, and 0.05% of the watershed land area is water, wetland, and wetland forest, respectively. The watershed contains 20 confined animal feeding operation units. Deciduous forest, ungrazed grass, grazed grass, Conservation Reserve Program (CRP) grassland, and alfalfa make up 2.71, 10.87, 2.52, 1.70, and 1.84% of the watershed, respectively. Areas under corn, soybean, and other row crops are 41, 33, and 0.43% of the watershed, respectively. Common/industrial, residential, and barren land are 1.67, 1.27, and 0.06%, respectively.

# *Escherichia coli* Observations, Stream Flow, and Temperature Data

Concentrations of *E. coli* in the streambeds and water columns were observed using culture-based methods described elsewhere

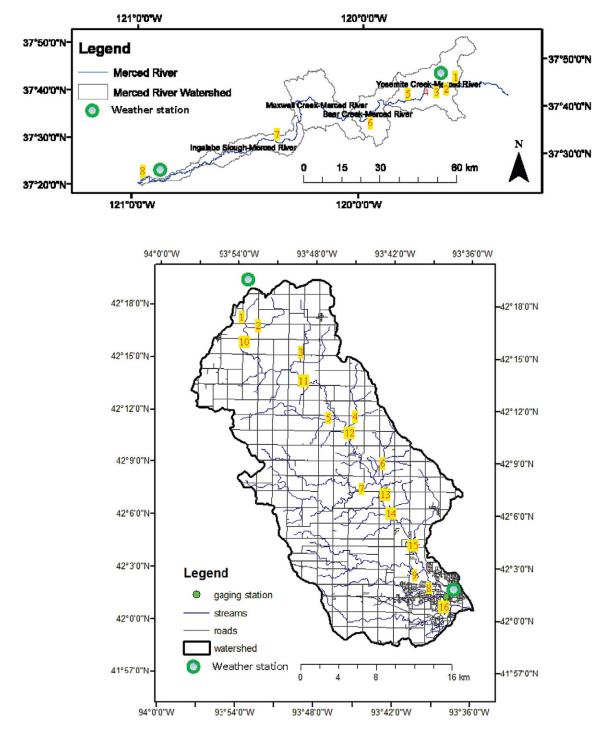


Fig. 1. Watershed maps and sampling locations: Merced River watershed, California (top) and Squaw Creek watershed, Iowa (bottom).

(Pandey and Soupir, 2013; Pandey et al., 2016). Both water and sediment samples were collected at the same locations. To collect water samples, we used a horizontal polycarbonate water bottle sampler (2.2 L, Forestry Suppliers, Inc.). Sediment samples were collected using a shallow water bottom dredge sampler (15- by 15-cm opening, Forestry Suppliers, Inc.). When the river reaches were easily accessible, samples were collected manually using sterile bottles (for water) and a spatula (for sediment). Water and sediment samples were stored at 4°C immediately after collection and analyzed within 24 h when possible (or at the earliest possible time) using membrane filtration techniques. To monitor *E. coli* in water samples, 100- to 500-mL samples were filtered

using 0.45- $\mu$ m filters. Sediment-attached *E. coli* concentrations were determined by stirring the sediment and deionized water mixture (1:1) for 15 min at 150 to 200 rpm. Subsequently, supernatant (0.5–2 mL) was filtered through 0.45- $\mu$ m filters. The filters were placed on membrane thermotolerant *E. coli* (mTEC) agar plates. Filters placed in agar were incubated at 44.5  $\pm$  0.2°C for 22 to 24 h, and red or magenta colonies appearing on the plates were counted as *E. coli* colonies. Each sample was analyzed in triplicate. This method conforms to the USEPA approved method 1603 (APHA, 1999).The *E. coli* levels in water were estimated in colony-forming units (CFU) per 100 milliliters. Sediment *E. coli* levels were estimated in CFU per 100 grams. To compare sediment and water *E. coli* concentrations, *E. coli* data were converted into CFU per cubic meter. Sediment bulk density of 1.26 g cm<sup>-3</sup> was used to determine *E. coli* levels in CFU per cubic meter (Pandey et al., 2012b).

River flow data were obtained from USGS gauging stations. For the Merced River (upper watershed), we used a gauging station (USGS 11264500) located at Happy Isles Bridge near Yosemite, CA. Water flow data for Squaw Creek watershed were obtained from a USGS gauging station (USGS 05470500) located at Ames, IA. Weather (temperature and rainfall) data for Ames were retrieved from Iowa Environment Mesonet (Iowa State University, 2018). Average daily weather data of Squaw Creek watershed were estimated using the daily data of Webster City (IA8806) and Ames (IA0200). Weather data for Merced River watershed were obtained from University of California Agriculture and Natural Resources, Integrated Pest Management Program (UC IPM, 2018) for two locations: the south entrance to Yosemite National Park (NCDC #8380) and Merced (CIMIS #148). The daily data of these two locations were used to estimate average daily data of precipitation and temperature for the Merced River watershed.

To understand the impacts of land use on *E. coli* levels in streams, the Merced River watershed was divided into four subwatersheds depending on streams and tributaries: (i) Beer Creek, ii) Maxwell Creek, (iii) Igalsbe Creek, and (iv) Yosemite

Creek. Similarly, the Squaw Creek watershed was divided into seven subwatersheds based on streams and tributaries: (i) Crooked Creek 1, (ii) Drainage Ditch 192, (iii) Crooked Creek 2, (iv) Lundys Creek, (v) Montgomery Creek, (vi) Onion Creek, and (vii) Worrel Creek.

A series of statistical tests was conducted to understand the relationships between land use, rainfall, temperature, and in-stream E. coli levels. The Pearson correlation matrix was estimated using land use of each subwatershed's and each watershed's average monthly temperature and cumulative monthly precipitation for the sampling month. Data were normalized to understand the variance. The multivariate analysis (principal component analysis) was conducted using land use, climate (rainfall and temperature), and E. coli observations of water and sediment. Using Past (3.0) software, we determined eigenvalues, loading factors, and clustering.

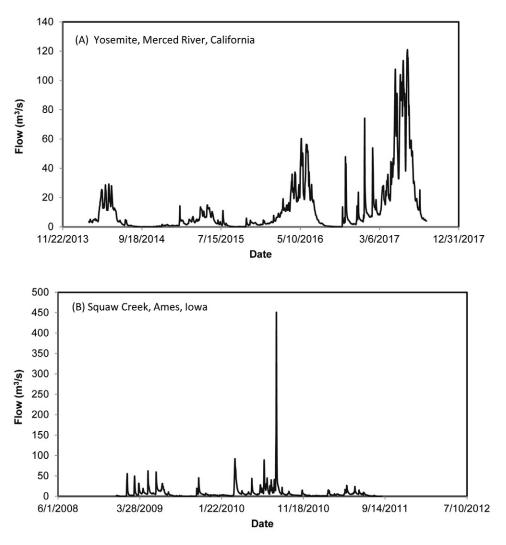
# **Results and Discussion** Stream Flow and Temperature

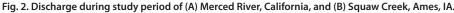
Stream flow in the Merced River watershed varied from 0.06 to

120.91 m<sup>3</sup> s<sup>-1</sup>, whereas the flow at Squaw Creek watershed varied from 0.02 to 450.23 m<sup>3</sup> s<sup>-1</sup> (Fig. 2; Table 1). The minimum flow for both rivers was comparable (0.02–0.06 m<sup>3</sup> s<sup>-1</sup>); however, the peak flow of the Squaw Creek watershed was 3.7 times higher than that of the Merced River. The peak flow in Squaw Creek occurred between August and September 2010. The peak flow conditions in the Merced River occurred between May and July 2017. Daily temperature of the Merced River watershed varied from –7.0 to 24.30°C, and daily rainfall varied from 0.0 to 22.34 mm (Fig. 3). For the Squaw Creek watershed, daily temperature was between –25.30 and 30.03°C. Daily precipitation varied from 0.0 to 79.75 mm. Average daily temperatures for the Squaw Creek watershed and the Merced River watershed were 9.21 and 12.77°C, respectively (Fig. 3).

# Water Column Escherichia coli Concentrations

Longitudinal changes in water column *E. coli* in the Merced River are shown in Fig. 4. Locations 7 and 8 in the Merced River represent conditions downstream, where agriculture predominates. In contrast, locations 1 to 6 are in the upper region of the watershed, where the flows are unregulated and the landscape is forested mountains including Yosemite National Park. Overall, this is a more pristine environment, although still tread by many people. The *E. coli* levels in the water column were relatively low





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Table 1. Descriptive	statistics of temperature	e, precipitation, flo	w, and E. coli.

Parameters	Minimum	Maximum	Average	Standard deviation				
Yosemite, Merced River, California								
Temperature (°C)	-0.7	24.30	12.77	6.3				
Precipitation (mm d <sup>-1</sup> )	0.0	22.3	1.8	2.4				
Flow (m <sup>3</sup> s <sup>-1</sup> )	0.06	120.91	11.96	19.78				
Water <i>E. coli</i> (CFU m⁻³)†	$1.2 \times 10^{4}$	7.5 × 10⁵	$1.4 \times 10^{5}$	2.6 × 10⁵				
Sediment <i>E. coli</i> (CFU m <sup>-3</sup> )	5.7 × 10⁵	$2.9 \times 10^{8}$	$6.2 \times 10^{7}$	$1.1 \times 10^{8}$				
Squaw Creek, Iowa								
Temperature (°C)	-25.30	30.03	9.21	11.97				
Precipitation (mm $d^{-1}$ )	0.0	79.75	2.4	6.74				
Flow (m <sup>3</sup> s <sup>-1</sup> )	0.02	450.23	8.21	20.14				
Water <i>E. coli</i> (CFU m⁻³)	2.2 × 10 <sup>6</sup>	$2.2 \times 10^{7}$	$5.4 \times 10^{6}$	$4.9 \times 10^{6}$				
Sediment <i>E. coli</i> (CFU m <sup>-3</sup> )	$7.8 \times 10^{6}$	$1.0 \times 10^{8}$	$4.0 \times 10^{7}$	$2.8 \times 10^{7}$				

+ CFU, colony-forming unit.

(<80 CFU 100 mL<sup>-1</sup>). Average *E. coli* levels in the water column were 14 CFU 100 mL<sup>-1</sup>. The samples with higher concentrations of *E. coli* (74 and 25 CFU 100 mL<sup>-1</sup>) were obtained from the lower regions of watershed, mainly located in the San Joaquin Valley.

To compare the bacterial quality of the Merced River with the USEPA's recreational water quality criteria (USEPA, 2012), recreational water quality criteria are plotted with the observations. For example, the USEPA recommends two criteria for *E. coli* in fresh water for meeting recreational water quality standards. The first recommendation suggests that the *E. coli* geometric mean (GM) concentration should be  $\leq 126$  CFU 100 mL<sup>-1</sup> and the recommended statistical threshold value (STV) should be 410 CFU 100 mL<sup>-1</sup>. The second recommendation suggests *E. coli* GM and STV values of 100 and 320 CFU 100 mL<sup>-1</sup>, respectively. The *E. coli* concentrations in the Merced River were well below the USEPA's recreational water quality criteria.

Water column *E. coli* concentrations in Squaw Creek Watershed (Fig. 5) showed *E. coli* levels between 222 and 3084 CFU 100 mL<sup>-1</sup>. The average *E. coli* concentration of 16 samples was 607 ( $\pm$  702) CFU 100 mL<sup>-1</sup>. The *E. coli* levels in Squaw Creek exceeded the USEPA's limit of bacterial concentration (for enterococci and *E. coli* shown Table 2) in recreational water by 2- to 18-fold. The average *E. coli* concentration was 382% higher than the USEPA's Recommendation 1 (GM  $\leq$  126 CFU 100 mL<sup>-1</sup>) and 507% higher than Recommendation 2 (GM  $\leq$ 100 CFU 100 mL<sup>-1</sup>) (Table 2).

## Sediment Escherichia coli Concentrations

Sediment *E. coli* concentrations were higher when compared to water column *E. coli* concentrations in both watersheds (Merced River and Squaw Creek) (Fig. 6). In the Merced River, the average *E. coli* concentration in sediment was 3330 ( $\pm$  5702) CFU 100 g<sup>-1</sup> ( $\approx$  6.2 × 10<sup>7</sup> ± 1.1 × 10<sup>8</sup> CFU m<sup>-3</sup>), with variations from 30 to 15,533 CFU 100 g<sup>-1</sup> ( $\approx$  5.7 × 10<sup>5</sup> ± 2.9 × 10<sup>8</sup> CFU m<sup>-3</sup>). In the Squaw Creek watershed, the average *E. coli* concentration in sediment was 4040 ( $\pm$  2985) CFU 100 g<sup>-1</sup> ( $\approx$  3.96 × 10<sup>7</sup> ± 2.8 × 10<sup>8</sup> CFU m<sup>-3</sup>), with variations from 784 to 10,289 CFU 100 g<sup>-1</sup> ( $\approx$  7.8 × 10<sup>6</sup> ± 1.0 × 10<sup>8</sup> CFU m<sup>-3</sup>). Comparison between water column *E. coli* and sediment *E. coli* levels is shown in Fig. 6. In the Merced River watershed, the average water *E. coli* level was 1.4 × 10<sup>5</sup> ± 2.6 × 10<sup>5</sup> CFU m<sup>-3</sup>, and in the Squaw Creek watershed, the average water *E. coli* level was 5.4 × 10<sup>6</sup>

 $(\pm 4.9 \times 10^6)$  CFU m<sup>-3</sup>. Comparing water and sediment *E. coli* concentrations, the average sediment *E. coli* concentration in the Merced River watershed was 433 times higher than water column *E. coli* concentrations. In the Squaw Creek watershed,

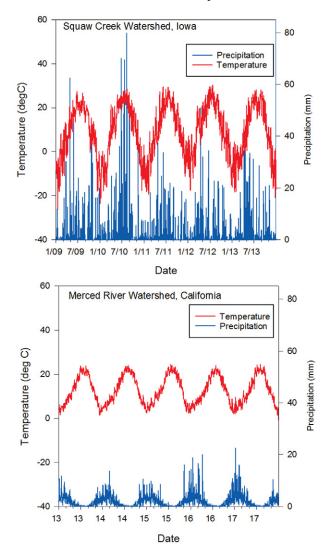


Fig. 3. Air temperature and precipitation data: Merced River, California (top) and Squaw Creek, Ames, IA (bottom). Temperature and precipitation shown are an average of two weather stations (Merced City and Yosemite National Park) in the Merced River watershed and an average of two locations (Webster City and Ames City) in the Squaw Creek watershed.

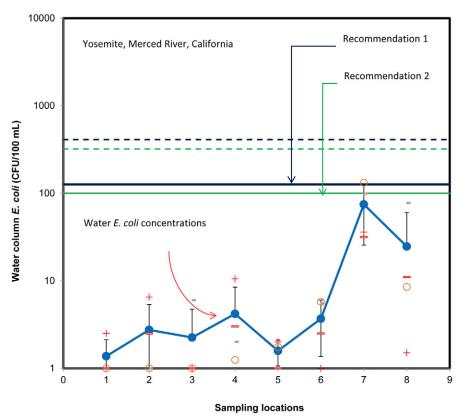
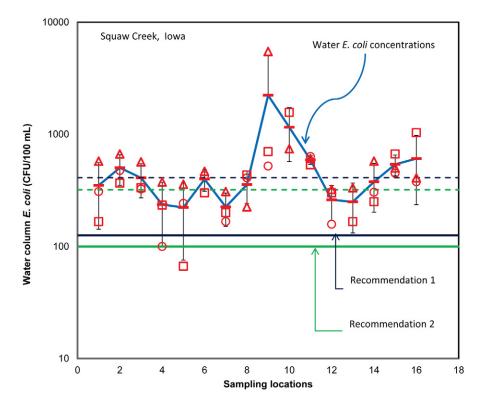
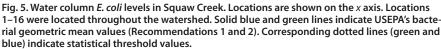


Fig. 4. Water column *E. coli* levels in the Merced River. Locations are shown on the *x* axis. Locations 1–6 indicate the upper Merced River. Locations 7 and 8 indicate the lower Merced River. Green solid line indicates USEPA's water quality bacterial geometric mean values (Recommendation 2); solid blue line indicates USEPA's bacterial geometric mean values (Recommendation 1). Corresponding dotted lines (green and blue) indicate statistical threshold values (STV).





sediment *E. coli* levels were seven times higher than water column *E. coli* levels.

Compared with the Merced River, differences in sediment and water column E. coli concentrations were narrower in Squaw Creek. This could be attributed to the influx of suspended particles in the water column from agricultural land during runoff and storm events, as well as sediment characteristics including cohesive and organic matter in the streambed (Pandey and Soupir, 2013). Additional factors such as the difference between a temperate and semiarid climate regime that causes differences in flow variance and the difference in livestock populations have a potential to affect the bacterial population of these two streams. Squaw Creek passes through agricultural land, and substantial amounts of clay particles and particle-attached E. coli are transported from the crop land to streams during runoff and storm events (Pandey and Soupir, 2013; Pandey et al., 2016).

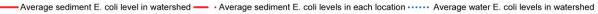
The effects of streambed sediment on increasing water column bacteria concentrations are often determined by the use of mathematical models. Higher concentrations of bacteria in streambed sediment of mountain streams is a concern (Allende et al., 2017; Hellberg and Chu, 2016; Hotaling et al., 2017; Kim et al., 2017; Pachepsky et al., 2016) because many of these streams are used for recreational purposes, and recreational activities enhance the likelihood of releasing bacteria from streambed sediment to the water column through resuspension. The increased concentration of bed sediment bacteria is reported to be linked with organic content and flow conditions (Jamieson et al., 2004; Pandey and Soupir, 2013). Survival of bacteria is increased when these bacteria are attached to soil and sediment particles (Cho et al., 2016). Further, the bacteria in sediments are stored for a long time compared with the water column (Bai and Lung, 2005; Jamieson et al., 2005). In many ambient waters, including estuaries, prolonged persistency of pathogens (e.g., E. coli, Enterococcus spp., fecal indicator bacteria) in sediment is observed (Fries et al., 2008).

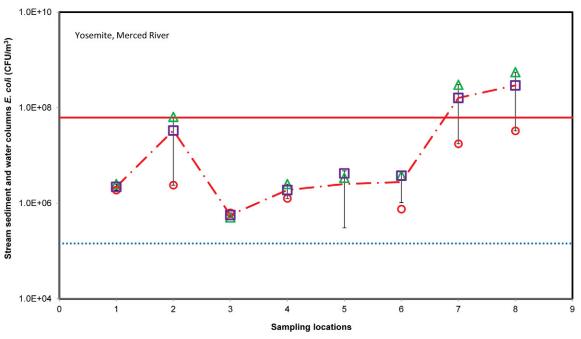
Land use of subwatersheds of the Merced River and Squaw Creek watersheds is shown in Fig. 7. In the Squaw Creek watershed, agriculture use varied from 54 to 87% among subwatersheds, while grassland use varied from 11 to 17%.

Table 2. USEPA microbial water quality criteria and recommendations.†

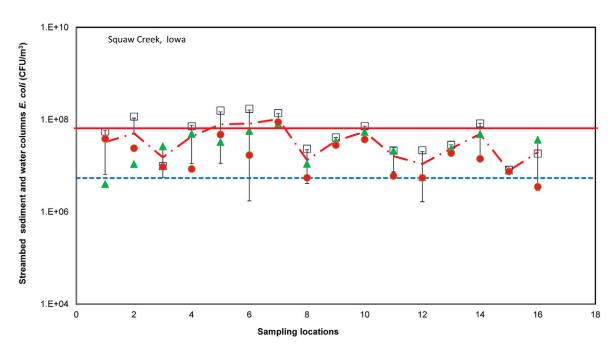
Recommendations	Indicator bacteria			
	Enterococci		E. coli	
	Geometric mean	Statistical threshold	Geometric mean	Statistical threshold
Recommendation 1	35	130	126	410
Recommendation 2	30	110	100	320

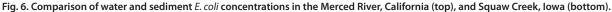
+ Recommendations and water quality criteria are based on USEPA's 2012 recreational water quality criteria for fresh water (USEPA, 2012). In the Merced River watershed, agriculture use varied from 0 to 37% among subwatersheds; grassland use varied from 27 to 76%. Relationships between watershed land uses, temperature, rainfall, and microbial water quality of streams were assessed using the correlation matrix shown in Fig. 8. The figure shows Pearson correlation coefficients among land use, climate, water *E. coli* levels, and sediment *E. coli* levels. An increase in forest and grassland reduced water *E. coli* levels, whereas increases in agriculture and temperature resulted in higher water *E. coli* levels in water. In terms of sediment *E. coli* levels, the linkages among *E. coli*,





- Average sediment E. coli at each location - Average sediment E. coli at watershed ----Average water E. coli at watershed





land use, and climate data were relatively poor (Fig. 8), which can be attributed to complex characteristics and nonhomogeneous sediment matrix.

Principal component analysis is shown in Fig. 9. The eigenvalues (explained variance) of each component is shown in scree plot (Fig. 10), indicating that >80% variance was explained by the first three components. Scatter plot among the two largest components resulted in a distinct feature of these two watersheds (Fig. 9). The subwatersheds of the Merced River were clustered together on the left side of the coordinate, whereas all subwatersheds of Squaw Creek were clustered in the right side of the coordinate, which indicates their unique characteristics. Subwatersheds that appear closer together on the scatter plot are more highly correlated.

The results of this study provide evidence indicating that increased grassland and forest uses results in lower in-stream *E. coli* levels, whereas increased agricultural, animal–agriculture, and manure uses have the potential to increase microbial contamination in streams. The relationships between land use and water *E. coli* concentrations were significant, whereas relationships between sediment *E. coli* and land use were not significant, indicating that additional studies are needed to understand how land use potentially affects sediment *E. coli* levels. Previous studies

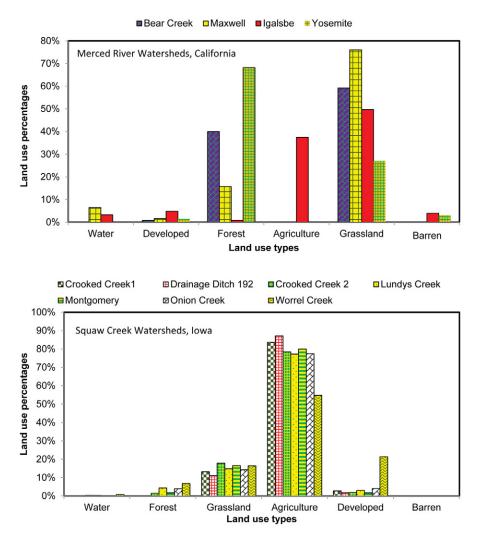


Fig. 7. Land use of watersheds: Merced River watershed, California (top), and Squaw Creek watershed, Iowa (bottom).

reported that the streambed sediment E. coli concentrations in a stream could be several times (100-10,000) higher than that of water column concentrations (Bai and Lung, 2005; Bragina et al., 2017; Pandey et al., 2012a), depending on stream and watershed characteristics. In agricultural streams, comparison between sediment and water column E. coli concentrations showed that both sediment and water column E. coli can be influenced by land use and increased animal-agriculture system (Bai and Lung, 2005; Jamieson et al., 2005; Pandey et al., 2012b). In general, livestock and manure application as a fertilizer are reported to be main factors influencing bacterial contamination in agricultural streams. For example, the most contaminated bed sediment was observed in stream with unrestricted cattle access (Bragina et al., 2017). Even in streams that are restricted for cattle, sediment E. coli concentrations were higher than water column E. coli (Bragina et al., 2017). These previous observations align with the results of this study. In general, cattle and other livestock are excluded from the Merced River; nevertheless, the sediment samples of the Merced River showed considerably higher concentrations of E. coli compared with the water column, which could be as an effect of runoff and in-stream transport processes. Additional studies are needed to improve understanding of major sources of E. coli in Merced River.

Restricting livestock manure entering into streams is considered to be effective in controlling bacteria loads to streams (Gotkowska-Płachta et al., 2016; Myers et al., 2017; Pachepsky et al., 2016; Soupir et al., 2006). However, additional measures may be needed for reducing the levels of sediment-attached bacteria in a stream. Various pathogenic bacteria are attached to particles, and the influx of manure and particle-attached bacteria can increase the bacteria levels in stream bed sediment for prolonged periods of time (Pachepsky et al., 2006; Pandey et al., 2014). These particle-attached bed sediment pathogens can be resuspended into the water column in the event of high flow and rainfall (Pandey et al., 2014, 2016). Under adverse conditions, these disease-causing pathogens in water can cause serious illness and death in humans (Pachepsky et al., 2006; Pandey et al., 2014). Studies have shown that in many areas of southeast Asia, changes in land use have resulted in higher runoff, soil erosion, and suspended sediment in streams. Increased turbidity and suspended sediment have been found to be related to higher fecal coliform counts in surface water and the spread and outbreaks of diseases (Wilson and Everard, 2017). In countries where surface water is a major source of water for drinking by humans and animals and for irrigation, the prevalence of pathogens in surface water increases risks not only to public health but also to animal health (Stea et

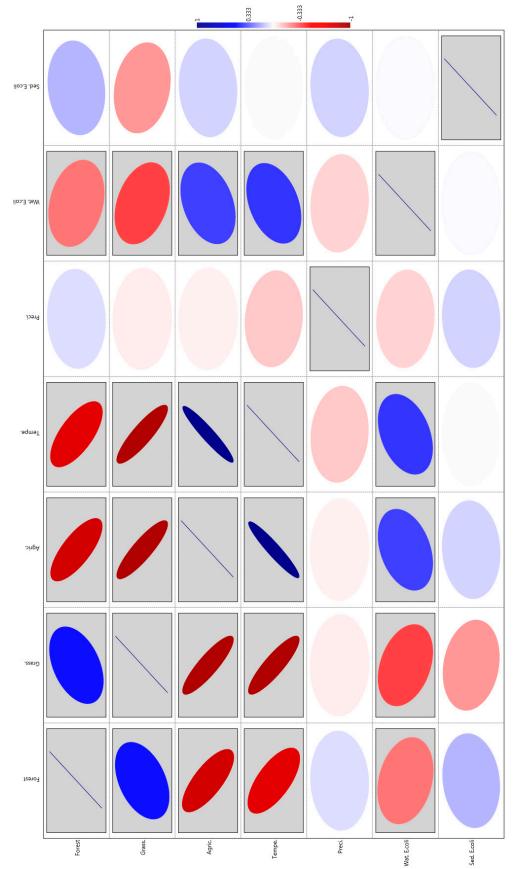


Fig. 8. Pearson correlation coefficient matrix relating land use with stream water and sediment *E. coli* levels; ellipses with gray box showed significant correlation (p < 0.05).

al., 2015a, 2015b). Previous studies showed that the increased pathogen levels in surface water and its subsequent use by the public in drought conditions was linked with diarrhea outbreaks

among the public (Boithias et al., 2016; Pandey et al., 2014; Rochelle-Newall et al., 2015).

While controlling the levels of bacteria in streams can be a long-term plan, requiring considerable resources, improved

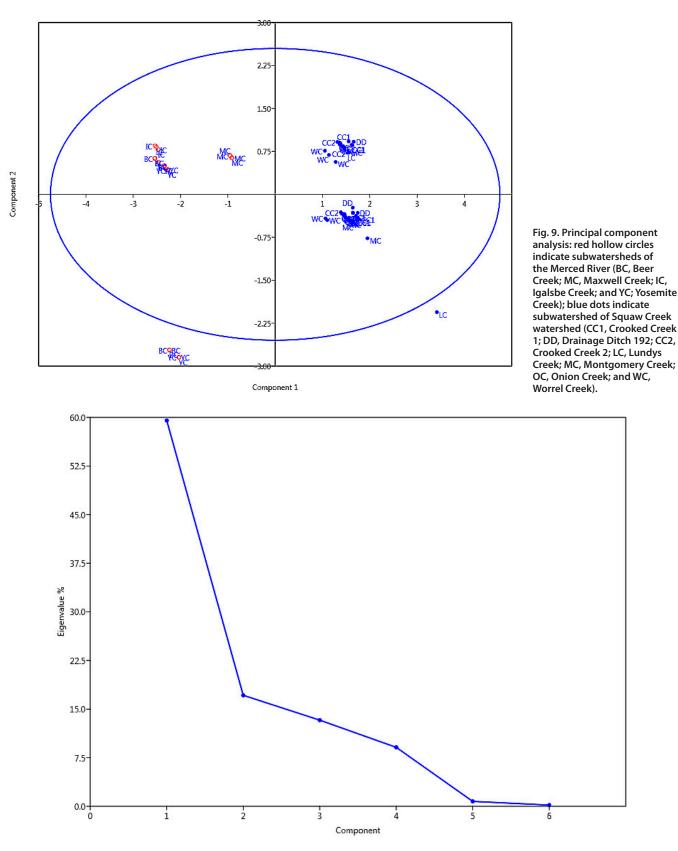


Fig. 10. Scree plot showing eigenvalues (variance explained) and principal component components (components include land use and weather). First three components (1–3) explained the variance more than 80% (i.e., eigenvalue > 80%).

monitoring of bacterial water quality has a potential to reduce the health risk to the public and animals through the avoidance of direct contact and ingesting contaminated water. Growing concern with regard to bacterial quality of water used for recreation and drinking by human and animal as well as for irrigation (Allende et al., 2017; Boithias et al., 2016; Boithias et al., 2014; Rochelle-Newall et al., 2015) will likely impel increased microbial surveillance of ambient water.

Improved monitoring of E. coli in water column and sediment has the potential to identify the sources of contamination and enhance measures capable of controlling bacterial loads in stream water and sediment. The limited data availability of sediment E. coli concentrations is one of the major hindrances to develop the relationships among sediment characteristics, sediment E. coli, and water column E. coli. The resuspension process, which causes the release of bacteria from streambed sediment to the water column, depends on sediment characteristics and initial E. coli loads of bed sediment (Cho et al., 2016; Droppo et al., 2009; Pandey et al., 2016; Rehmann and Soupir, 2009). Higher E. coli loads in bed sediment followed by resuspension process under increased flow conditions releases E. coli from streambed to the water column, which results in higher E. coli loads in streams (Jamieson et al., 2005). The effects of the resuspension process in releasing bacteria from streambed sediment to the water column varies to a large extent, particularly during the first storm event (Jamieson et al., 2005; Pandey and Soupir, 2013). Even during base flow conditions, water column E. coli levels are increased substantially by resuspension (Park et al., 2017). Sediment E. *coli* strains were reportedly present in all water samples in a study describing the significant role of streambed bacteria contamination on the water column of streams (Piorkowski et al., 2014). This infers that understanding of streambed sediment bacteria loads and relationships among land use, stream water column bacteria, and particle-attached bacteria is important for evaluating stream health and consequential public health risks.

# Conclusions

This study assessed the relationships among water column *E*. coli levels, streambed E. coli levels, land use, and climate of the watersheds. Two distinct watersheds-the Merced River watershed and the Squaw Creek watershed-featuring unique land use characteristics were used as study areas. The observations of water and sediment E. coli levels were made at multiple locations in the subwatersheds of each watershed. These E. coli observations were used to determine the impact of land use on in-stream E. coli concentrations. The observations of water column and streambed sediment E. coli of agricultural and mountain streams indicates higher concentrations of E. coli in sediments of both types of streams. However, E. coli concentrations in the water column were found to be considerably higher in the agricultural stream than mountain stream. In the mountain stream, water E. coli concentrations were lower than the recommended recreational water quality standards. In the agricultural stream, however, water column E. coli levels were considerably greater than the recommended recreational water quality standards. While land use was found to be related to water column E. coli, the relationship between land use and sediment E. coli concentrations was not significant. The insights gained here enhance the existing understanding of in-stream E. coli contamination and the impacts of land use on surface water E. coli levels.

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