Effects of Land Uses on Fecal Indicator Bacteria in the Water and Soil of a Tropical Watershed

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Effects of different land uses on densities of *Escherichia coli*, enterococci, and *Clostridium perfringens* in the water and soil of a tropical watershed were investigated. Densities of fecal indicator bacteria (FIBs) in the watershed exhibited a clear land-use dependency in the stream water. Significantly higher concentrations were detected in the urban portion of the stream (417, 420, and 44 CFU mL−1 for *E. coli*, enterococci, and *C. perfringens*, respectively) than in the forest portion (54, 32, and 5 CFU mL−1 for *E. coli*, enterococci, and *C. perfringens*, respectively). High concentrations of FIBs were also detected in the soil of the watershed with concentration ranges of 603–1,820,000, 69–17,000, and 0–525 CFU 100 g soil−1 for *E. coli*, enterococci, and *C. perfringens*, respectively, which however were not affected by the different land uses. Prior cumulative rainfall significantly correlated with concentrations of *E. coli* and enterococci in the urban stream water (r=0.73–0.87, P<0.05), but not with the alternative FIB *C. perfringens*. Poor correlations were observed in the forest reach of the stream for all FIBs. Furthermore, the concentration of *C. perfringens* only correlated strongly and significantly with *E. coli* and enterococci in stream water (r=0.70–0.82, P<0.05), but not in tropical soil, indicating different survival and transport behaviors.

**Key words:** Land use, watershed, water, soil, fecal indicator bacteria

The bacteriological quality of ambient waters in the United States is currently regulated based on the concentrations of common fecal indicator bacteria (FIBs), such as *Escherichia coli* and enterococci. Epidemiological studies conducted in the 1970s at multiple marine and freshwater bathing beaches in temperate regions revealed good correlations between swimming-associated gastroenteritis and water concentrations of *E. coli* and enterococci (32). Based on acceptable illness rates of 8 per 1,000 swimmers at fresh water beaches and 19 per 1,000 swimmers at marine beaches, water quality standards for the fecal indicators were determined to be 126 CFU 100 mL−1 for *E. coli* and 33 CFU 100 mL−1 for enterococci (32). In addition to *E. coli* and enterococci, state regulatory agencies have also adopted other indicator organisms; for example, the state of Hawaii uses *Clostridium perfringens* as an alternative indicator (15).

The use of fecal indicator bacteria for water quality monitoring comes with the fundamental assumptions that ideal indictor organisms do not persist outside of animal hosts for extended periods and do not grow in natural environments; however, recent research findings have challenged these assumptions. *E. coli* and enterococci were found to persist in various environmental matrices, including soil (5, 17), sediment (2, 6), beach sand (36, 37), and aquatic vegetation (4, 38). A number of studies have also reported the growth of FIBs under environmentally relevant conditions (7, 21, 40). Environmental reservoirs, where FIBs can persist and even grow, therefore need to be fully understood because of their potential to act as sources of FIBs to waterbodies. Since FIBs from environmental reservoirs may not closely associate with human pathogens, the assumed correspondence between illness rates and FIB concentrations in water may be significantly altered, which could undermine the reliability of current indicator-based water quality criteria.

Environmental reservoirs of FIBs in tropical regions may be more prominent than in temperate regions. Studies examining temporal fluctuations of FIBs in environmental reservoirs in temperate regions have shown that warmer temperatures usually corresponded to higher numbers. In a northern Minnesota watershed, Ishii et al. (2006) detected significantly higher concentrations of soil *E. coli*, up to 3,000 CFU g soil−1, during summer months than during winter months (17). Similar seasonal fluctuation patterns were also observed for *E. coli* in epilithic periphyton communities in Lake Superior (21) and in riverine sediments (26). In tropical environments where temperature is steadily maintained at high levels, Roll and Fujioka (1997) reported that soil from an urban watershed in Hawaii on average contained 460,000 CFU g−1 *E. coli* and 390,000 CFU g−1 enterococci (27).

It is commonly observed that various land uses affect water quality differently, as waterbodies near urban centers (22, 25, 31) and agricultural operations (1, 12, 23) have been found to often contain higher concentrations of *E. coli* and enterococci than other land uses. Studies on urban-affected watersheds have found those watersheds to contain one to two magnitude higher concentrations of FIBs (30) and that greater urbanization increased the probability of EPA water quality criteria being exceeded (25). Urbanization of watersheds may also negatively affect water quality in downstream areas, such as lagoons (9) and estuaries (19). Many previous land use studies, however, were prompted by pre-existing contamination issues and were often in areas affected by urban activities, such as wastewater effluent (13, 18) and storm runoff (28, 39), or by agricultural operations, such as pasture land (12, 23) and manure application (1, 25).
The aim of this study was to investigate the effects of different land uses (i.e., forest and urban) on densities of FIBs in the water and soil of the Manoa watershed on the island of Oahu, Hawaii. The urban and forest portions of the watershed were sampled over a ten-month period. Sampling events were conducted under relatively dry-weather conditions to minimize run-off derived complexities, and the data were used to estimate baseline concentrations of FIBs in water according to different land uses. Three FIBs, including the commonly used *E. coli* and enterococci as well as the secondary indicator *C. perfringens* used in Hawaii, were monitored and compared.

**Materials and Methods**

**Study sites**

Manoa watershed is a typical stream on the island of Oahu, Hawaii with a tropical climate (Fig. 1). The stream starts in the forest region of Manoa Falls, runs through the largely residential community of Manoa, and discharges into the Ala Wai Canal, a little over five kilometers from its origin. Annual flow rates of Manoa Stream average around 0.306 m/s and the stream rarely exceeds 3 meters wide and 1 meter deep from its origin to end. The watershed contains upstream forest regions and downstream urban areas, with a total drainage area slightly less than 16.2 km². Much of the rainfall in the Manoa Valley occurs at the stream's origin at the base of the Koolau Mountain Range in the forest part of the watershed. The watershed does not contain agricultural or livestock activities. Wild animals, such as feral pigs, inhabit the watershed, small birds populate the watershed in both urban and forest areas, and dogs and cats are present in the urban part of the watershed. The urban portion of the Manoa watershed is fully served by municipal sewer systems, and no on-site wastewater disposal systems are present in the forested region. Six sampling sites were selected along Manoa Stream, with three located in the forested reach and three in the urbanized reach (Table 1). Data for watershed geography and major land uses were obtained from Hawaii statewide geographic information system (http://hawaii.gov/dbedt/gis/download.htm) and processed using ArcGIS 9.3 software (ESRI; Redlands, CA). ArcGIS was used to draw vector model polygon drainage areas based on land topography from a raster data model of the site, and the ArcGIS polygon area calculator was subsequently used to determine total drainage areas and total areas of each land usage type.

**Sample collection**

Eight sampling events were conducted from May 2009 to February 2010 at six sampling sites under relatively dry-weather conditions. All samples were obtained early in the morning to avoid potential sun light inactivation of FIBs. Rainfall was determined from the previous 72 hours of cumulative rainfall data collected at the Manoa Lyon Arboretum gage by the National Oceanic and Atmospheric Administration and stream flow data collected at USGS gage number 16242500. Since the rainforest region receives frequent and sporadic precipitation, dry weather is defined in this study as the average daily hour rainfall of three days prior to each sampling being less than the average daily rainfall for the total 10-month sampling period. Water samples (one liter) were collected while standing on the stream bank with minimal disturbance to the water and underlying sediment. Approximately 200 g surface soil samples (0–4 cm from surface) at each site were collected using a sterile shovel from each of four locations that were approximately 0, 1, 3, and 5 m distance from the stream bank. Water and soil samples were kept in the dark and at 4°C during transport and processing. For bacterial enumeration, water samples were processed immediately upon returning to the laboratory and soil samples were processed within 24 hours of sampling.

**Bacterial enumeration**

Fecal indicator bacteria (*i.e.* *E. coli*, enterococci, and *C. perfringens*) in water and soil samples were enumerated using standard membrane filtration methods. Water samples (100 mL) were directly enumerated in duplicate using standard U.S. EPA membrane filtration methods (*i.e.* modified mTEC agar method for *E. coli* (35), mEI agar method for enterococci (34), and mCP agar method for *C. perfringens* (33)). For soil samples, bacterial cells were first extracted using ammonium phosphate buffer (APB) based on a previously described technique for tropical soils (20). Aliquots or dilutions of the soil extractants were then filtered and enumerated using the respective membrane filtration methods for *E. coli* (35), enterococci (34), and *C. perfringens* (33).

<table>
<thead>
<tr>
<th>Site</th>
<th>Site description</th>
<th>Cumulative drainage area (ha)</th>
<th>% of total area</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Forest: steep (~60°) slope, densely vegetated; shaded</td>
<td>62.6</td>
<td>Forest 100 0 0</td>
</tr>
<tr>
<td>B</td>
<td>Forest: flat plateau; densely vegetated; shaded</td>
<td>128.2</td>
<td>Urban 100 0 0</td>
</tr>
<tr>
<td>C</td>
<td>Forest: flat plateau; densely vegetated; shaded</td>
<td>189.4</td>
<td>Commercial 100 0 0</td>
</tr>
<tr>
<td>D</td>
<td>Urban: adjacent to public park; tall grass</td>
<td>961.5</td>
<td>Forest 88 11 1</td>
</tr>
<tr>
<td>E</td>
<td>Urban: across street from shopping center; light shrubs; overhanging trees; shaded</td>
<td>1079.1</td>
<td>Urban 81 18 1</td>
</tr>
<tr>
<td>F</td>
<td>Urban: adjacent to apartments and public park; dry soil; overhanging trees</td>
<td>1227.5</td>
<td>Commercial 71 21 8</td>
</tr>
</tbody>
</table>

**Table 1.** Sampling sites, drainage areas, and land uses
Data analysis

Average concentrations of FIBs in the different land use regions of Manoa watershed were determined by calculating geometric means of concentrations from sampling sites within either the urban or forest parts of the watershed. Baseline concentrations of FIBs were determined by calculating their geometric means over the whole study period. The abundance ratio of FIBs between soil and water was calculated using the formula: \( R_{\text{soil/water}} = C_{\text{soil}}/C_{\text{water}} \), where \( C_{\text{soil}} \) is the indicator concentration in soil (CFU 100 g dry weight soil\(^{-1}\)) and \( C_{\text{water}} \) is the indicator concentration in water (CFU 100 mL\(^{-1}\)). Student’s \( t \) tests were performed in SigmaPlot (Systat Software, Chicago, IL, USA), with the default significance level at \( P \leq 0.05 \), unless stated otherwise. Linear regressions and the correlation coefficients were determined using Microsoft Excel with a statistical add-in (Statistixl, Australia).

Results

FIBs in Manoa Stream

The concentrations of \( E. coli \), enterococci, and \( C. perfringens \) in water exhibited a clear land-use dependency (Fig. 2); water samples from the same land-use type (i.e. forest or urban) contained similar levels of FIBs, and significantly higher concentrations were detected in water from the urban area compared to the forest area (\( P < 0.05 \)).

Over the ten-month sampling period, the abundance of \( E. coli \) at the urban sites was 2.5–143.3 times higher than at the forest sites, the abundance of enterococci at the urban sites was 3.2–59.8 times higher than at the forest sites, and the abundance of \( C. perfringens \) at the urban sites was 2.4–35.0 times higher than at the forest sites.

The range of FIB concentrations and their mean values over the ten-month study period are summarized in Table 2. Among the three FIBs, \( E. coli \) was always the most abundant in stream water with a mean of 54 CFU 100 mL\(^{-1}\) in the forest region, which meets the EPA recommended 126 CFU 100 mL\(^{-1}\) for freshwater, and 417 CFU 100 mL\(^{-1}\) in the urban region, which exceeded the standards. The geometric mean value of enterococci in the forest reach of the stream was 32 CFU 100 mL\(^{-1}\), lower than the EPA recommended standard of 33 CFU 100 mL\(^{-1}\), while enterococci in the urban reach of the stream was 420. \( C. perfringens \) was always the least abundant FIB with a mean of 5 CFU 100 mL\(^{-1}\) in the forest region and 44 CFU 100 mL\(^{-1}\) in the urban region.

Since sampling events were conducted during relatively dry-weather periods, the geometric means of FIB concentrations may represent baseline concentrations for each land use region. In the forest region of Manoa Stream, baseline concentrations were all well below currently adopted water quality standards, while concentrations in the urban region greatly exceeded current standards.

FIBs in soil of Manoa watershed

Stream bank soil in the Manoa watershed contained high concentrations of FIBs at all sampling sites throughout the study period (Fig. 3). The concentration ranges detected were 603–1,820,000 CFU 100 g soil\(^{-1}\) for \( E. coli \), 69–17,800 CFU 100 g soil\(^{-1}\) for enterococci, and 0–525 CFU 100 g soil\(^{-1}\) for \( C. perfringens \). Although the abundance rank among the three FIBs in soil was the same as in stream water (i.e. \( E. coli > \) enterococci > \( C. perfringens \)), the concentration differences observed between the urban and forest reaches of the

stream water did not exist in soil. In fact, soil in the urban region appeared to contain slightly lower concentrations of FIBs than soil from the forest region of the watershed.

![Fig. 2. Water concentrations of \( E. coli \) (a), enterococci (b), and \( C. perfringens \) (c) at the six sampling sites in Manoa Stream over the 8-month experimental period. Closed symbols are for Urban sites: A (▲), B (●), and C (■); while open symbols are for Forest sites: D (△), E (○), F (□). Upper and lower dashed lines represent the geometric means of fecal indicator concentrations in the urban and forest regions, respectively.](image)

![Table 2. Range of fecal indicator concentrations in the forest and urban reaches of Manoa Stream](image)

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Forest region (CFU 100 mL(^{-1}))</th>
<th>Urban region (CFU 100 mL(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>( E. coli )</td>
<td>6–166</td>
<td>409–1326</td>
</tr>
<tr>
<td>Enterococci</td>
<td>8–237</td>
<td>80–1210</td>
</tr>
<tr>
<td>( C. perfringens )</td>
<td>0–19</td>
<td>20–100</td>
</tr>
</tbody>
</table>

\( ^{a} \) Current U.S EPA recommended standards for freshwater are 126 CFU 100 mL\(^{-1}\) for \( E. coli \) and 33 CFU 100 mL\(^{-1}\) for enterococci; the Hawaii water quality standard for \( C. perfringens \) is 50 CFU 100 mL\(^{-1}\).

\( ^{b} \) Geometric means of the concentration in all sampling sites within the region.

\( ^{c} \) Mean concentrations were calculated as geometric means over the 8-month sampling period.
In order to compare the concentrations of FIBs in soil (CFU 100 g soil\(^{-1}\)) with those in stream water (CFU 100 mL\(^{-1}\)), soil-water fecal indicator ratios \(R_{\text{soil/water}}\) at the sampling sites were calculated (Fig. 4). Comparatively, soil in the Manoa watershed contained much higher levels of fecal indicators than water; the highest \(R_{\text{soil/water}}\) for \(E.\ coli\), enterococci, and \(C.\ perfringens\) were \(10^{3.91}\), \(10^{3.93}\), and \(10^{3.95}\). The \(R_{\text{soil/water}}\) values for the forest sites were significantly higher than those at the urban sites \((P<0.05)\), which indicates that either the exchange of FIBs between the forest soil and water was severely hampered kinetically or that the forest soil retained higher numbers of fecal indicators at equilibrium.

Effects of precipitation and stream flow

Since a potential transport route of FIB from soil to water is by the infiltration of rain water through soil, the time-series concentration data of FIBs in the stream water was correlated with three-day cumulative rainfall and stream flow. Interestingly, the relationship also exhibited a clear land-use dependency, which was more apparent for \(E.\ coli\) and enterococci than for \(C.\ perfringens\), as indicated by Pearson’s product moment correlation coefficients (Table 3). The only statistically significant correlations \((P<0.05)\) were observed between the concentrations of \(E.\ coli\) and enterococci and prior cumulative rainfall in the urban reach of the stream \((r=0.73–0.87)\); however, the same correlations were not statistically significant in the forest reach of the stream \((P=0.69)\). The FIB concentrations did not exhibit a statistically significant correlation with stream flow in either the forest or urban reach of the stream. The alternative FIB \(C.\ perfringens\) exhibited no correlations with either stream flow or prior cumulative rainfall (Table 3).

Correlation between different FIB concentrations

Time-series concentrations in water were used to test the relationships between the traditional FIBs (\(E.\ coli\) and enterococci) and the alternative FIB \(C.\ perfringens\) (Table 4). The two traditional indicators, \(E.\ coli\) and enterococci, showed a strong positive correlation in their water concentrations \((r=0.82, P<0.05, n=48)\), and also correlated with \(C.\ perfringens\) with slightly smaller correlation coefficients \((r=0.70–0.75, P<0.05, n=48)\), indicating that \(C.\ perfringens\) is a compatible alternative indicator for the watershed. In
soil, significant correlations were observed between \( E. \ coli \) and enterococci and between \( E. \ coli \) and \( C. \ perfringens \). The correlation between enterococci and \( C. \ perfringens \) was not statistically significant (Table 4).

### Discussion

Although it is usually expected that stream water under an urban influence contains higher levels of FIBs than more pristine forest regions, only a few direct comparison studies have been conducted to date (25, 31). Previously, it was shown that \( E. \ coli \) concentrations were significantly higher in urban than forest portions of a temperate watershed (31), and the same was also observed for enterococci (26). The present study examined \( E. \ coli \), enterococci, and \( C. \ perfringens \) in the tropical climate of the Manoa watershed, and consistently detected higher concentrations in urban waters than forest waters over a 10-month sampling period.

The fecal indicator organisms \( E. \ coli \), enterococci, and \( C. \ perfringens \) were consistently detected at high abundance levels in the soil of the Manoa watershed regardless of land use, which indicates that soil is an important environmental reservoir of indicator organisms. Previous studies have shown that soils found in both temperate regions and tropical regions were capable of supporting the growth of \( E. \ coli \) (5, 8, 10). The presence of \( E. \ coli \) and enterococci in the soils of Hawai‘i’s tropical climate was previously reported in areas under direct urban influence, and the abundance levels were on average \( 4.6 \times 10^6 \) CFU 100 g\(^{-1} \) \( E. \ coli \) and \( 3.9 \times 10^5 \) CFU 100 g\(^{-1} \) enterococci (5, 27). The present study observed lower concentrations of FIBs in the soil of the Manoa Stream watershed. The difference may be attributable to watershed characteristics, such as soil nutrients and indigenous microbial communities (3, 24, 29). \( C. \ perfringens \) was also consistently detected in soil from the urban region of the Manoa watershed, an observation that has not been reported previously for tropical soils, but is not surprising because of the ability of \( C. \ perfringens \) to produce spores and survive under various environmental conditions.

The present study also showed that land uses did not significantly affect the abundance of FIBs in tropical soil. Throughout the study period, all three FIBs were consistently enumerated from not only urban soil, but also the presumably more pristine forest soil. The abundance levels of FIBs in forest soils were actually slightly higher than in urban soils, albeit not statistically significant according to the \( t \) test. Since fecal indicators were consistently detected at high abundance levels in all soil samples collected at different times and from different locations, this strongly indicates that FIBs are autochthonous members of tropical soil microbial communities rather than originating from exogenous animal fecal sources.

Given the high levels of FIBs in the soil of the Manoa watershed, it may be logical to conclude that soil is a potential source of FIBs for water (16), in addition to other potential fecal sources, such as broken sewer lines, domesticated animals, and wild birds. The exchange between soil and water is undoubtedly a major transport route to stream water during heavy precipitation and runoff events when soil particles are directly carried into the stream; however, the interaction between soil and water is less obvious under relatively dry weather conditions, such as in this study. The 72-hour cumulative rainfall and stream flow exhibited a good positive correlation with fecal indicator concentrations in the urban region, but not in the forest region of the watershed (Table 3). In addition, it was observed that high \( R_{\text{soil/water}} \) values were observed in the forest soil, while \( R_{\text{soil/water}} \) values in the urban soil were much smaller and approached 1. When considered together, it appears that soils in the forest and urban regions of the watershed may differ in terms of the exchange of fecal indicators between soil and water, implicating a land-use effect on the transport of FIBs from environmental reservoirs to waterbodies. Indeed, physicochemical analyses of soil samples indicated that forest soil contains significantly higher levels of moisture (47.0±7.2%) and organic matter (5.9±1.6%) than urban soil (46.8±7.3%, 3.4±1.3%, respectively. Further investigations are needed to fully understand the mechanisms of soil water interactions and to determine the contribution of soil-sourced FIBs for water in various land uses.

The presence of various environmental reservoirs of FIBs means that there are potentially certain numbers (i.e. baseline concentrations) of FIBs in waterbodies even in the absence of actual fecal contamination. When such baseline concentrations are relatively low, current indicator-based water quality standards may still be valid for detecting actual fecal contaminations and associated health risks; however, when baseline concentrations are very high, as in the Manoa Stream, it is possible that contributions from environmental reservoirs alone could cause contaminations in stream water to reach the set thresholds and trigger false positive contamination alarms. Therefore, it may be necessary to establish baseline concentrations of FIBs and incorporate them into the development of water quality criteria. The present study monitored FIBs in stream water under relatively dry-weather conditions over a 10-month period and determined the average concentrations of FIBs in different land-use regions (Table 2). Assuming the absence of actual fecal contamination propagated through rainfall-derived runoff, these concentration levels could be used to establish watershed-specific water quality standards.

\( E. \ coli \) and enterococci are recommended by the U.S. EPA as common FIBs for freshwater based on the correlation between illness rates and indicator concentrations in water (32). Since linear regression was used to determine threshold concentrations for water quality criteria (32), a positive correlation between the concentrations of \( E. \ coli \) and enterococci in water should therefore exist. Such a correlation

### Table 4. Correlation among the concentrations of \( E. \ coli \), enterococci, and \( C. \ perfringens \)

<table>
<thead>
<tr>
<th></th>
<th>Water (n=48)</th>
<th>Soil (n=144)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( r )</td>
<td>( P )</td>
</tr>
<tr>
<td>( E. \ coli ) vs. enterococci</td>
<td>0.82</td>
<td>6.7×10(^{-12} )</td>
</tr>
<tr>
<td>( E. \ coli ) vs. ( C. \ perfringens )</td>
<td>0.75</td>
<td>8.1×10(^{-10} )</td>
</tr>
<tr>
<td>Enterococci vs. ( C. \ perfringens )</td>
<td>0.70</td>
<td>4.3×10(^{-8} )</td>
</tr>
</tbody>
</table>

\( ^* \) Statistically significant correlations (\( P<0.05 \)) were underlined. \( ^{**} \) Samples with fecal indicators below detection limits were given a value of 1 during correlation analysis.
has been detected between \textit{E. coli} and enterococci in a freshwater lake ($r=0.51$) (11) and in urban storm water runoff ($r=0.42$) (28). The present study showed a very strong correlation between \textit{E. coli} and enterococci in stream water ($r=0.82$, Table 4). Although positive correlations between environmental concentrations of \textit{E. coli} and enterococci in water were found in this and other studies (11, 28), the trend should be considered cautiously when applied to other watersheds with different land use patterns. For example, Fisher and Dillard (2003) found no correlations between \textit{E. coli} and enterococci in a watershed with multiple land uses (14).

Since \textit{C. perfringens} has been previously suggested as a more accurate indicator of fecal contamination in Hawaii (15), Manoa Stream water and soil FIB data were also used to examine the relationships among the relative abundance of \textit{C. perfringens}, \textit{E. coli}, and enterococci. Positive correlations in concentration were observed between \textit{C. perfringens} and the other two traditional FIBs in water samples; however, poor correlations among \textit{C. perfringens}, \textit{E. coli}, and enterococci were observed in soil samples. The observed contrast may be associated with the different survival and/or growth behaviors of the different indicators, which requires further investigation.

In conclusion, our results indicate that FIB concentrations in Manoa stream water exhibited a clear land-use dependency between the forest and urban reaches. Significantly higher concentrations of all three FIBs, including \textit{E. coli}, enterococci, and \textit{C. perfringens}, were detected in the urban reach of Manoa Stream than in the forest reach. High concentrations of FIBs were constantly detected in the soil throughout the watershed, and the forest and urban soil of the watershed contained comparable levels of FIBs. Statistically significant correlations were observed between cumulative rainfall in the watershed and concentrations of \textit{E. coli} and enterococci in stream water at the urban reach, indirectly supporting the notion that tropical soil is a potential source of common FIBs for stream water. In contrast, the alternative FIB \textit{C. perfringens} in the urban stream water did not correlate significantly with cumulative rainfall. Although the concentration of \textit{C. perfringens} exhibited significant correlations with concentrations of the two common FIBs in stream water, correlations in soil were weak, suggesting different survival and transport behaviors of \textit{C. perfringens}.

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