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Article

Tracer Dyes and Microbial Indicators Suggest Mobilization of Fecal Waste Flows from Latrines Following Rainfall in Kibera, Kenya

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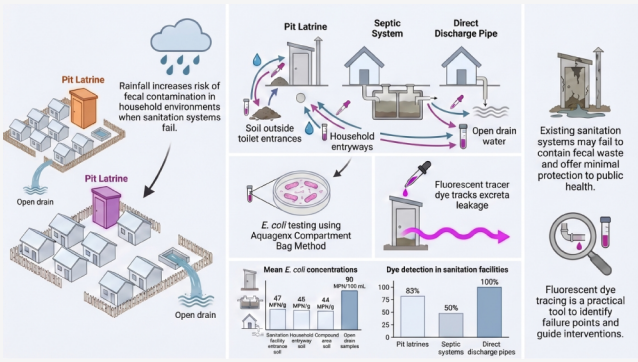
ABSTRACT: Malfunctioning sanitation systems can increase fecal contamination and exposure risks during rainfall, yet rapid methods for assessing system performance remain limited. This study evaluated sanitation system performance in low-income urban areas of Nairobi, Kenya, using *Escherichia coli* as a fecal indicator and fluorescent tracer dye to assess excreta flow. We collected 144 soil and 48 open drain water samples before and after rainfall and tested them for *E. coli* A fluorescent dye was introduced into sanitation systems to track leakage into surrounding environments. *E. coli* was detected in 75% of samples, with mean concentrations of 47 Most Probable Number per gram (MPN/g) in facility entrance soil, 45 MPN/g at household entryways, 44 MPN/g in compound soil, and 90 MPN/100 mL in open drains. Concentrations were lower immediately after rainfall, although the difference was not statistically significant (p = 0.097). Dye was detected outside sanitation systems in 56% of cases, rising to 78% following rainfall. Detection varied by system type: 83% of pit latrines, 50% of septic systems, and all direct discharge systems showed evidence of leakage. These findings reveal frequent containment failures and highlight the value of tracer dyes for identifying contamination pathways and informing targeted public health interventions.



KEYWORDS: *Dye tracing, Escherichia coli, Rainfall, Sanitation failure*

INTRODUCTION

Sanitation systems are intended to contain human waste and protect public and environmental health;[1] however, their performance in dense urban informal settlements is shaped by multiple interacting factors, including infrastructure design, maintenance, and aging. In these settings, systems often operate under constrained conditions, where limited space, high user loads, and inadequate maintenance can compromise functionality. Environmental stressors such as rainfall further influence system performance, particularly when infrastructure operates near or beyond its effective capacity. While drainage and sanitation systems are typically designed to accommodate rainfall events up to a specified return period, in informal settlements, these systems may operate differently from formal design assumptions due to blockages, informal connections, or limited maintenance, which can affect effective system capacity.[2,3]

Rainfall is commonly associated with an increased risk of sanitation system disruption through mechanisms such as overflow, leakage, and the mobilization of fecal contamination.[4] Damage to sanitation infrastructure due to rainfall is well documented, particularly in low- and middle-income countries (LMICs), where systems are often poorly designed, aging, or inadequately maintained.[5] Heavy or prolonged rainfall increases the risk of structural failures, including latrine collapse, damaged pipes, overloaded containments, and submerged or inaccessible facilities.[6−8] In sewered systems, overflow events and pump failures caused by power outages are common during heavy rainfall, highlighting how sanitation systems respond under real-world conditions, including rainfall events that may exceed design thresholds. In informal settlements, drainage systems may operate differently from formal design assumptions due to blockages, informal connections, or limited maintenance, which can affect effective system capacity.[2] Waterlogging and flooding can also disrupt critical support services such as road networks and electricity, hindering access to resources needed for system operation, repair, and maintenance.[9] These disruptions often drive users to adopt lower-service options, such as open defecation or sharing facilities,[7] representing a regression along the sanitation service ladder. Such setbacks suggest that

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rainfall-related impacts can reverse hard-won gains in sanitation access.

Beyond system disruption, rainfall plays a critical role in shaping fecal contamination patterns in the household environment. Failing sanitation systems are widely recognized as major contributors to environmental fecal contamination.[10] Studies have explored the link between rainfall-induced fecal contamination and health outcomes, with diarrheal diseases peaking seasonally, particularly during rainy seasons in tropical and subtropical climates.[11,12] In Ecuador, researchers found that using unimproved sanitation offered limited protection during rainfall events and was associated with a higher risk of diarrheal infection (OR = 2.9, 95% CI: 1.3−6.6), suggesting that heavy rainfall may amplify exposure to fecal contamination where sanitation is inadequate.[13] A systematic review and meta-analysis of 50 water, sanitation, and hygiene (WASH) trials found that sanitation systems can be overwhelmed by heavy rainfall, reducing their effectiveness in blocking enteric pathogen transmission. The meta-analysis showed that WASH interventions reduced the risk of diarrhea by 33% (RR = 0.67; 95% CI: 0.59−0.76), but the effect dropped to 18% during rainy seasons (RR = 0.82; 95% CI: 0.71−0.95).[14] Stormwater runoff has been shown to transport contaminants from latrines, drains, and open defecation sites into residential areas.[15]

However, the effects of rainfall on fecal contamination are not unidirectional. While rainfall can mobilize contaminants and expose system vulnerabilities, it can also dilute contaminant concentrations at specific locations while simultaneously redistributing contamination across the surrounding environment. In household and urban sanitation systems, dilution often occurs when rainfall infiltrates latrine pits, septic tanks, and wastewater networks, temporarily lowering microbial concentrations in these confined environments. Observational work in informal settlements has shown that short, intense rainfall events can reduce *E. coli* concentrations in heavily contaminated household soils, such as at toilet entrances, by roughly 40−50% within an hour, consistent with a dilution and wash-off effect at those microsites.[16] At the same time, reductions at the source location are offset by redistribution of pathogens to adjacent compound and household entrance areas, where post-rainfall *E. coli* levels often remain unchanged or show less pronounced declines.[17] These patterns are consistent with the "concentration−dilution" hypothesis from diarrhea epidemiology, in which rainfall following already wet conditions can dilute pathogen concentrations at specific exposure points, at times effectively spreading exposure risks rather than eliminating them.[18] As a result, observed contamination patterns across domestic environments reflect a combination of mobilization, dilution, and interaction with existing infrastructure conditions, including maintenance and aging. Understanding these interacting processes is critical for interpreting observed contamination patterns and for identifying pathways of exposure under real-world conditions.

Despite growing evidence linking rainfall to fecal contamination in household environments, studies have failed to demonstrate unambiguously that specific failing sanitation systems are the primary contributors. Traditional microbial methods, like fecal indicator bacteria, provide valuable information on the presence and magnitude of contamination but offer limited insight into the pathways through which contamination spreads.[19] In complex, high-density environments, contamination may originate from multiple sources and follow heterogeneous transport pathways, making it difficult to distinguish between local contamination and contamination transported through the environment.[20,21] While source-specific microbial tracking methods can address this limitation, they are often resource-intensive and require specialized expertise, limiting their applicability in low-income settings.[22]

Tracer-based approaches offer practical and complementary methods for addressing these gaps. Fluorescent dye tracing has been widely used in hydrological studies to visualize flow pathways, estimate transport distances, and identify system connectivity.[23,24] In sanitation monitoring, dye tracing can provide direct evidence of how fecal waste moves through containment systems and into surrounding environments, particularly under dynamic conditions such as rainfall. Despite its potential, dye tracing remains underutilized in informal settlements, where an understanding of transport pathways is critical for identifying system failures and targeting interventions. Combining microbial indicators with tracer-based methods provides a more comprehensive understanding of contamination dynamics. While *E. coli* measurements quantify the presence and extent of fecal contamination, dye tracing enables visualization of transport pathways and system connectivity. Together, these methods allow for a more complete characterization of contamination processes, helping to distinguish between contamination generated locally and that transported through environmental pathways and to identify system vulnerabilities that may not be detectable through microbial sampling alone.

In this context, this study applies a combined microbial and dye-tracing approach to investigate sanitation system performance in Kibera, Nairobi, a large informal settlement characterized by high population density and exposure to climate-related risks. Specifically, we assess contamination patterns before and after rainfall events to better understand how rainfall influences both the presence and movement of fecal contamination. The objectives of this study were to (1) assess fecal contamination levels in household environments before and after rainfall using microbial indicators; (2) use fluorescent dye tracers to identify potential transport pathways and compromised sanitation systems; (3) examine how contamination patterns vary with sanitation design features and operational conditions, including maintenance; and (4) evaluate the combined utility of microbial testing and dye tracing for detecting and interpreting sanitation system failures in complex urban environments. This study highlights the potential of tracer dyes to complement microbial indicators in improving the detection and interpretation of fecal contamination in complex environments.

METHODS

Study Setting and Design

The study was a cross-sectional assessment of fecal contamination from sanitation infrastructure in Kibera, Nairobi's largest informal settlement in Nairobi, Kenya. This study was conducted following a period of above-average rainfall (March−May 2024, with some areas receiving 111−200% of the long-term mean), which resulted in flooding that affected 147,061 people and displaced 20,968 households.[25] The study area was selected due to its diverse sanitation systems, recurring flood history, and documented impact of rainfall on fecal contamination.[26] Approximately 77.4% of Kibera's population lacks access to improved sanitation, with traditional pit latrines predominant.[27] Poorly designed facilities, inadequate maintenance, and limited fecal sludge management result in manual emptying or abandonment of full systems. This contributes to fecal pollution and recurring outbreaks of excreta-related diseases like cholera and dysentery.

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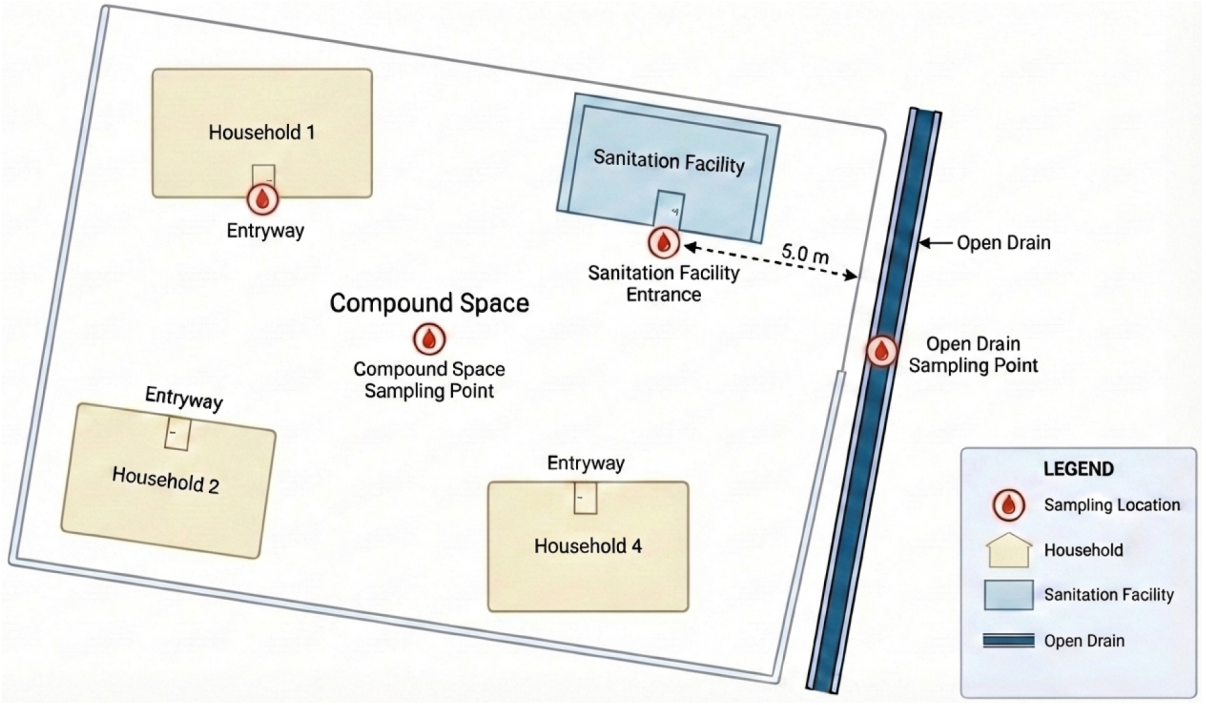

Figure 1. Schematic of targeted sampling locations within a household environment.

Sanitation Systems and Sampling Locations

The study evaluated active on-site sanitation systems that were shared among multiple households, including lined pit latrines, septic systems, urine-diverting dry toilets, and toilets with direct discharge pipes ("straight pipes"). Facilities were chosen to reflect variations in construction quality and flooding exposure, excluding abandoned, collapsed, or inaccessible systems. A cluster sampling approach was used to identify sanitation systems and environmental sampling locations. The study area was first divided into geographically defined subunits (clusters), corresponding to 14 villages. A random subset of seven villages was selected for inclusion in the study. Within each selected village, sanitation facilities were identified through systematic transect walks conducted along predefined routes. Transect walks were guided by local leaders to ensure full coverage and to identify sanitation systems in the study area. Because no complete inventory of sanitation facilities existed, all eligible systems encountered along the transects were screened for inclusion based on predefined criteria. Facilities were considered eligible if they were functional, in regular use at the time of the study, and accessible for environmental sampling. Sewer-connected systems were rare in the study area and were excluded as the study methods were not designed to capture their exposure pathways. Environmental samples were then collected at standardized locations around each eligible sanitation system, including the facility entrance, household entryway, shared compound area (targeting key areas where children play), and nearby open drains (the closest point within a 5-m radius of a sanitation facility). The sampling locations were evenly distributed along shared compounds that ranged in radius from 5 to 10 m on average (Figure 1).

A minimum sampling framework, guided by prior work in similar settings, was applied to ensure adequate representation across sanitation system types.[28] The sample size was estimated based on a stratified design using four sanitation technologies: pit latrines, septic systems, urine-diverting dry toilets, and direct discharge pipes. Six systems were sampled for each technology, resulting in a total of 24 systems. At each sanitation facility, samples were taken from four environmental locations: facility entrance, household entryway, compound area, and open drain, resulting in 96 samples. To account for temporal variability, sampling was done before and after rainfall, increasing the number of environmental samples to 192. Field controls were collected to check for contamination, including four pre-rainfall and four post-rainfall samples corresponding with each sanitation system type, adding eight samples. This brought the total to 200 samples (Table 1).

Table 1. Sampling Locations and Sample Types Collected during the Study

	Row total across sanitation systems	Sanitation systems			
		Pit latrines	Septic systems	Urine-diverting dry toilets	Black discharge pipes
Samples collected during prerainfall conditions					
Facility entrance	24	6	6	6	6
Household entryway	24	6	6	6	6
Shared compound area	24	6	6	6	6
Open drain	24	6	6	6	6
Control[a]	4	1	1	1	1
Total samples collected during prerainfall conditions	100	25	25	25	25
Samples collected during post-rainfall conditions					
Facility entrance	24	6	6	6	6
Household entryway	24	6	6	6	6
Shared compound area	24	6	6	6	6
Open drain	24	6	6	6	6
Control	4	1	1	1	1
Total samples collected during post-rainfall conditions	100	25	25	25	25

[a]A total of eight control samples were collected pre-rainfall (n = 4) and post-rainfall (n = 4) to represent household conditions surrounding each sanitation technology.

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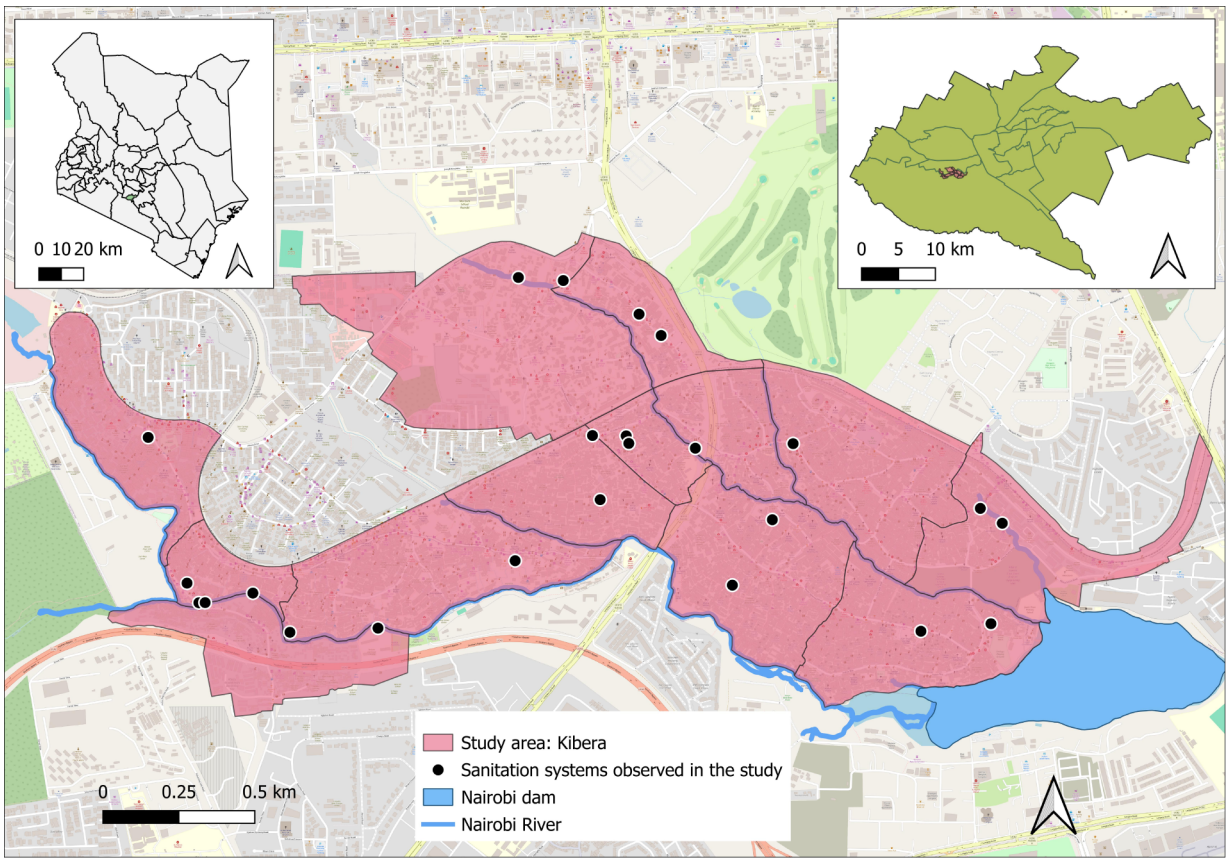

Figure 2. Map of the study area showing the location of the surveyed sanitation systems and sampling points. Basemap: OpenStreetMap contributors.

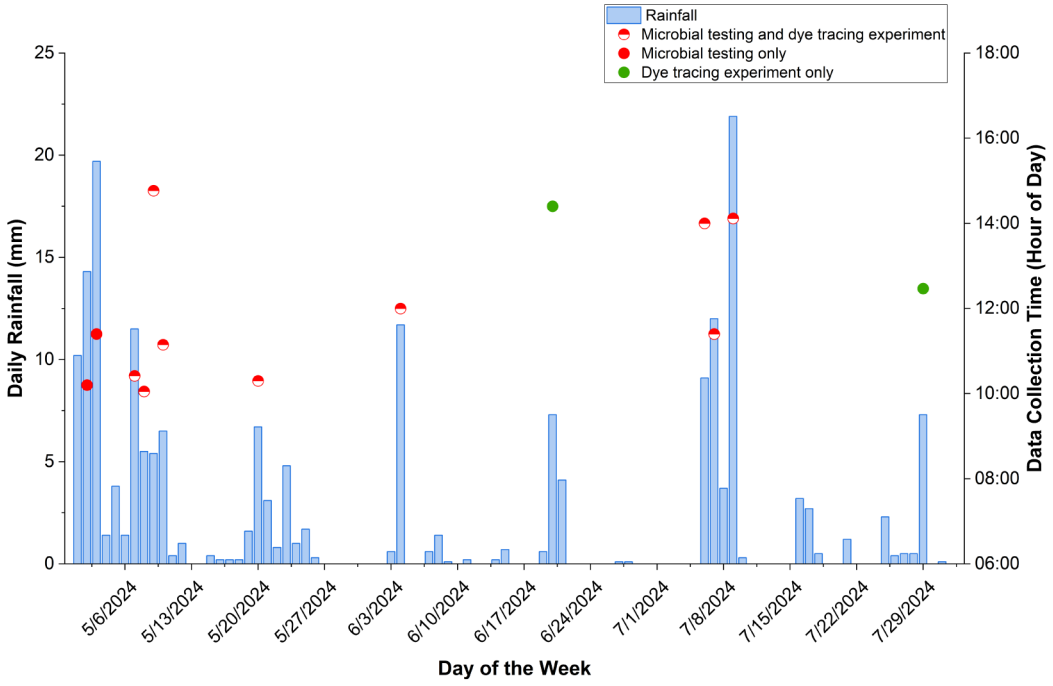

Figure 3. Schedule of sample collection versus daily rainfall for the period between May 1 and July 31, 2024.

Ethics

The Institutional Review Board of the University of North Carolina at Chapel Hill reviewed and approved the study protocols (24-1333). The Kenya National Commission for Science, Technology, and Innovation granted in-country approvals. Users, owners, or operators of the sanitation facilities granted verbal permission at least 24 h prior to the technical observations and environmental sampling for the detailed study. We selected a replacement sanitation facility using the determined sampling interval in cases of nonresponse or unwillingness to participate. The area chief and the Nairobi subcounty public health office provided oversight for the field implementation of the dye tracing

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experiment. We conducted a dye injection demonstration together with the oversight team and jointly reviewed the laboratory safety sheets for the product before the experiment. Additionally, the study team displayed a detailed flyer in Kiswahili at each sampling site to alleviate any community concerns about the dye's safety in the experiment.

Physical Assessment and Technical Evaluation of Sanitation Technologies

Visual inspections of sanitation systems and their immediate catchment areas were conducted, and measurements were taken as appropriate. Data were collected from 24 surveyed facilities (Figure 2), including design features, siting characteristics, nearby open defecation sites, child feces disposal practices, and household animal ownership. The physical dimensions of containment systems were measured using a retractable tape (25 ft; 7.62 m) and tank depth using a locally fabricated metallic dipping stick (12 mm in diameter). Facility emptying history was obtained from the owner, an adult user who had resided at the location for at least five years, or a designated toilet operator. Information about user numbers and shared facility use was also collected. All assessments were completed at the study onset, within 1 week prior to sample collection.

Sample Collection and Processing

At each sampling location, environmental samples were collected during two field conditions: prerainfall (cumulative rainfall of <2 mm over 24 h) and postrainfall (cumulative rainfall of >5 mm over 24 h). These thresholds were used operationally to distinguish dry and wet conditions and to better capture conditions relevant to runoff and contaminant transport. Rainfall occurrence and timing were monitored by using a combination of field-based rain gauge observations and local meteorological data. Four self-emptying rain gauges (Geevon Digital Rain Gauge, model 8703, Dusseldorf, Germany) were installed across the study area to capture spatial variation in rainfall. These gauges recorded rainfall continuously at a resolution of 0.1 mm and transmitted data to a real-time logging system. Sampling followed a reactive threshold-based approach. When cumulative rainfall exceeded 5 mm within a 24-h period, an automated alert was triggered, prompting field teams to initiate post-rainfall sampling as soon as feasible, typically within a few hours of the rainfall event. Due to logistical constraints, sampling was not simultaneous across all facilities, and both rainfall intensity and the exact timing of post-rainfall sampling varied between sites. As a result, observations reflect contamination dynamics under variable real-world rainfall conditions rather than controlled experimental rainfall scenarios. Sampling was conducted between May and July 2024 (Figure 3).

Soil and open drain samples were tested *in situ* using Aquagenx (CBT EC + TC MPN) test assays to quantify *E. coli* estimates in 100 mL samples via the Most Probable Number (MPN) method.[29−31] A growth medium dissolved in a 100 mL water sample was added to each of the five compartments of the Compartment Bags. After 48 h of incubation at ambient temperature, color changes signaled the presence of *E. coli*, and MPN concentrations were determined by comparing the color sequence to a reference table. The bags also quantified Total Coliforms (TC) through color changes under fluorescent light. This test is suitable for low-resource settings, requiring no extensive equipment, cold chain, or specialized expertise. Previous studies validated the *E. coli* MPN method against others like membrane filtration[32] and IDEXX Colilert,[33] showing consistent results within a 95% confidence interval.[30] Aquagenx Compartment Bags have been used in prior research to detect *E. coli* in drinking water, wastewater,[34] and floodwater,[35] but this study marks the first application to analyze soil.

At each sampling point, the researchers used a sterilized 20 × 20 cm stencil to guide the excavation of soil to a depth of 2 cm using a sterilized metal scoopula. The soil was homogenized by mixing it for 30 s with the scoopula before a sterile plastic spoon was used to transfer 1 g into a 100 mL Whirl-Pack bag filled with water up to the 100 mL mark. The mixture was dissolved in the reagent medium and poured into a compartment bag. Each bag was sealed with a two-piece plastic clip to isolate the compartments and incubated at ambient temperature for 24−48 h before recording the MPN readings. The same procedure was used to process 100 mL of drain water in Whirl-Pack bags. Eight controls were collected by rinsing a sterilized scoopula at the start of sample collection, collecting the rinse water in a 100 mL Whirl-Pack bag, and using the same testing procedure. In addition to soil and open drain samples, measurements were taken of soil pH, moisture, and light at each sampling location using a Sonkir MS02 Soil pH Tester. In total, 200 samples from soil (n = 144), open drains (n = 48), and controls (n = 8) were collected and processed.

Dye Tracing Experiment

The dye experiment was conducted at the same sanitation facilities and locations used for *E. coli* sampling to ensure comparability. A fluorescein dye solution was prepared by diluting 50 mL of stock dye with water to a final volume of 1 L (5% v/v). Injection volumes (100−300 mL per facility) were determined based on estimated containment volume, reported use, and desludging history, following guidance from Field.[36] The dye was injected into the sanitation system through drop holes or flush holes, depending on facility design. Facilities received an initial dose during prerainfall conditions to establish background fluorescence and identify any compounds fluorescing at similar wavelengths. At least 2 weeks later, facilities received a second dose at the onset of a forecasted rainfall event to assess dye mobilization under postrain conditions.

Observations commenced at the time of dye injection and continued for up to 1 h postinjection. Observation points were positioned 0.2 m apart, equidistant from each other, downstream of the facility under observation in the direction of dye plume dispersion, extending to the point where the plume dissipated. At each observation point, 1 g of soil was collected. The soil samples were placed in a beaker and eluted with five parts of distilled water according to the method described in Mull et al.[37] Tracer signals were identified through visual inspection of the eluate for the characteristic tracer color, and dye concentration in the preprepared sample was quantified using a portable spectrophotometer (721, LDC Digital Lab visible spectrophotometer). Detailed procedures for the dye experiment are provided in the Supporting Information.

Statistical Analyses

Statistical analyses were conducted in Stata/SE 13.1 (StataCorp). Summary statistics were used to describe the distribution of *E. coli* concentrations (MPN/100 mL). Pearson's chi-square tests assessed associations between the presence or absence of *E. coli* (binary outcome) and sanitation technology and sample type.

For concentration-based analyses, *E. coli* values were $\log_{10}$-transformed for analysis and visualization; however, results are presented in original units (CFU) in the text to aid interpretation. Nondetects in undiluted 100 mL surface water samples were assigned a value of 0.5 MPN/100 mL, corresponding to the lower detection limit, to allow log transformation. Differences in log-transformed *E. coli* concentrations across sanitation technologies, sample types, and other covariates were evaluated using the nonparametric Kruskal−Wallis test.

Dye injection volumes were adjusted in the field to account for variations in containment size and expected flow conditions across sanitation systems. To enable comparability across sites, measured dye concentrations were normalized by the injected volume prior to analysis. Dye tracer results were summarized descriptively as presence or absence. Measured absorbance values were log-transformed and compared across groups using the Kruskal−Wallis test, with concentrations estimated from standard calibration curves (Supporting Information). Because both *E. coli* and dye data were non-normally distributed, their association was assessed using the Wilcoxon signed-rank test. Pearson's correlation coefficient (r) was calculated to describe the direction and strength of the relationship. Statistical significance was defined as $p < 0.05$.

Because of the field-based nature of the study, rainfall events could not be standardized across sites. To account for this variability, rainfall depth was included as a covariate in regression analyses. The timing of dye injection and postrainfall sampling relative to rainfall onset also varied across sites and was accounted for in the analysis.

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Table 2. Variation in *E. coli* Detection and Concentrations across Sample Types, Sanitation Technologies, and Rainfall Conditions[a,b]

	Pre-rainfall conditions				Post-rainfall conditions				Total samples				
	Positivity		MPN[a] Count		Positivity		MPN Count		Positivity		MPN Count		
	n	%	Mean	SD	n	%	Mean	SD	n	%	Mean	SD	*p*
Sanitation technology													
Pit latrines	23	92	78	41	16	64	46	47	39	78	62	47	.008
Septic systems	20	80	44	43	19	76	59	49	39	78	51	46	.408
UDDTs[c]	19	76	54	49	16	64	40	47	35	70	47	48	.298
Discharge pipes	19	76	58	47	17	68	55	47	36	72	56	46	.697
Sample type													
Household entryway soil	18	72	48	46	16	67	60	46	34	69	45	46	.738
Sanitation facility entryway soil	20	87	62	46	14	58	44	47	34	72	47	46	.020
Compound area soil	19	79	46	46	15	63	52	47	34	71	44	46	.498
Open drain water	23	96	90	29	22	92	63	45	45	94	90	29	.971

[a]MPN refers to the Most Probable Number of *E. coli* colony-forming units per sample. [b]*p*-value calculated from the Kruskal–Wallis test to determine differences across rainfall conditions. Statistical significance is $p > 0.05$. [c]UDDTs refer to urine-diverting dry toilets, a type of water-less sanitation system.

RESULTS

Sample Characteristics

A total of 200 environmental samples were collected for *E. coli* analysis and 144 samples for dye tracing across dry and wet weather conditions, sourced from 24 on-site sanitation systems representing the range of technologies in the study area, including pit latrines ($n = 6$), septic systems ($n = 6$), urine-diverting dry toilets ($n = 6$), and direct discharge ("black water") pipes ($n = 6$). Sample types collected were soil from the sanitation facility entrance ($n = 48$), soil from the household entryway ($n = 48$), soil from a central compound area ($n = 48$), and water from open drains ($n = 48$). Of the surveyed open drains, 19% were lined with cement, 24% were visibly clogged, and most contained trash or sediment, with an average width of approximately 30 cm. Although no visible human excreta were observed in drains, used diapers were frequently present. Other soil parameters (pH, light, and moisture levels) were also documented for each sampling location. On average, light intensity at sampling locations rose from 900 lx during prerain conditions to 1,500 lx during postrain conditions. Soil and open drain pH values ranged from 5 to 8. Under prerain conditions, mean soil moisture was 4% water fraction by volume (wfv), increasing to 8–10% wfv following rainfall.

Surveyed Sanitation Systems

The surveyed sanitation systems represent the diversity of technologies and management conditions in the study area. Most facilities (88%) were shared, serving an average of eight households per unit. Twenty-eight percent were located within shared residential compounds, while 72% were situated outside residential plots, with users reporting an average walking time of 6 min to access a facility. The construction quality varied widely. Thirty-six percent of facilities were elevated above ground level, typically to reduce flood exposure. Structural deficiencies were common: 48% exhibited visible cracks or signs of partial collapse, 9% had damaged pipework, and 17% discharged to effluent receivers (e.g., soak pits or open drains) with overflow or leakage. Additionally, 16% of facilities appeared to be full and in need of emptying. Facilities averaged 45 months of age. Users reported emptying containment systems approximately four times over five years, although professional emptying services were rarely utilized (6%). Common coping strategies included abandoning full systems, constructing new pits or tanks, sharing neighboring facilities, or using public toilets for a fee. Open defecation sites and domestic animals were observed near the sampling locations.

Fecal Contamination in Household Environments

Across all 200 samples, *E. coli* was detected in 75% of observations (Table 2). Detection rates were highest in open drain water (94%) and similar across soil locations, including household entryways (69%), sanitation facility entryways (72%), and compound soils (71%). Detection frequencies varied by rainfall conditions, with higher positivity observed during prerain conditions (81%) compared to postrainfall conditions (68%). Presence–absence analyses indicated significant variation in *E. coli* detection by sample type and rainfall condition ($p < 0.001$), but not by sanitation technology ($p = 0.359$). Overall, 26% of samples fell below the lower detection limit, while 49% exceeded the upper detectable limit. The mean (SD) for *E. coli* concentration across all samples was 56[46] MPN/100 mL. Mean *E. coli* concentrations differed by sanitation technology and sample type. Across all conditions, samples associated with pit latrines and direct discharge pipes exhibited the highest mean concentrations (62 and 56 MPN/100 mL, respectively), followed by septic systems (51 MPN/100 mL) and urine-diverting dry toilets (47 MPN/100 mL). Open drain water consistently showed the highest concentrations across sampling periods (mean: 90 MPN/100 mL).

Rainfall-related differences in *E. coli* concentrations were observed for some but not all sanitation systems and sample types. Concentrations associated with pit latrines were significantly higher during prerainfall conditions compared with postrainfall conditions ($p = 0.008$). Similarly, sanitation facility entryway soils showed a significant decrease in concentrations following rainfall ($p = 0.020$). In contrast, concentrations in household entryway soils, compound soils, septic systems, urine-diverting dry toilets, and open drains did not differ significantly between dry and wet conditions. Moreover, total coliforms were detected in 86% of samples collected during prerainfall conditions and 82% of samples collected post-rainfall; detailed results are provided in the Supporting Information.

Nonparametric analyses were used to compare $\log_{10}$-transformed *E. coli* concentrations before and after rainfall across

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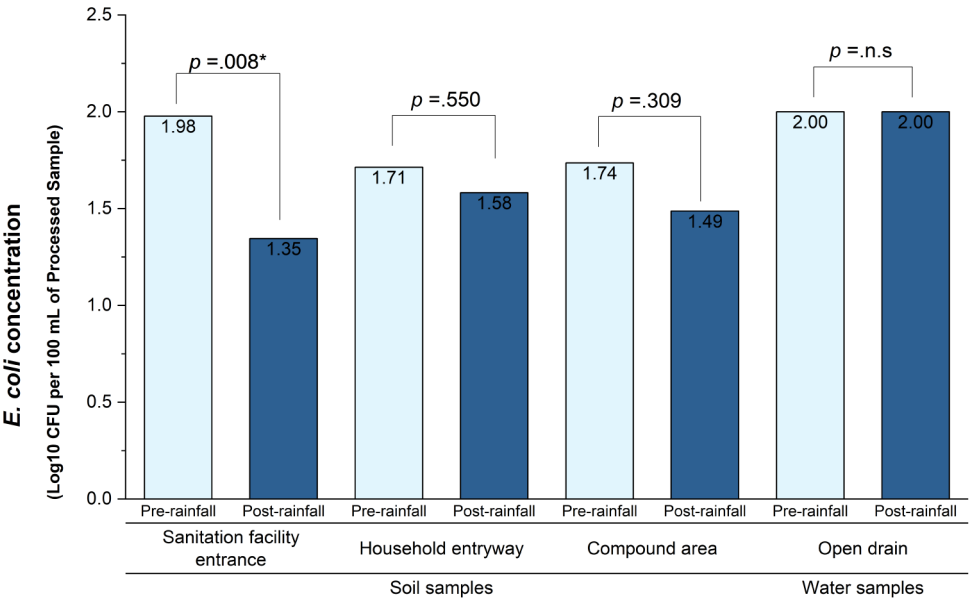

Figure 4. Mean $\log_{10}$-transformed *E. coli* concentrations by sample type and rainfall conditions. Error bars represent standard deviation. MPN = most probable number.

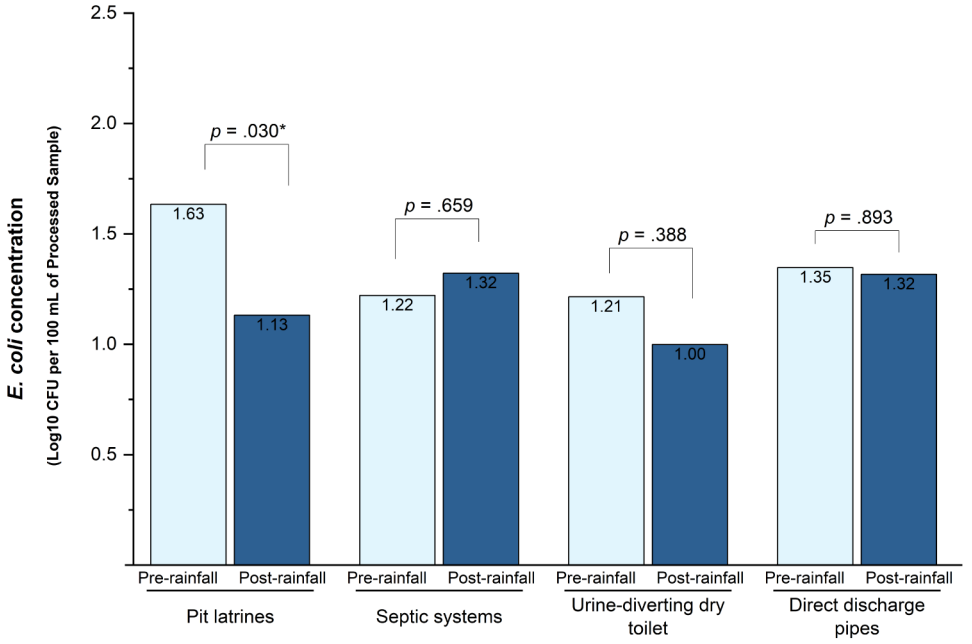

Figure 5. Mean $\log_{10}$ *E. coli* concentrations by sanitation system and rainfall conditions. Error bars represent standard deviation. MPN = most probable number.

sample types and sanitation technologies. On average, fecal contamination levels were slightly lower following rainfall, with mean concentrations of 32 MPN/100 mL (1.5 $\log_{10}$ MPN/100 mL) during prerainfall conditions compared to 25 MPN/100 mL (1.4 $\log_{10}$ MPN/100 mL) post-rainfall; however, this overall difference was not statistically significant ($p = 0.097$).

The findings indicate that *E. coli* concentrations varied by sample type and were influenced by rainfall conditions (Figure 4). Soil samples collected at sanitation facility entrances showed the largest decrease in mean *E. coli* concentrations following rainfall, with reductions of approximately 0.4 $\log_{10}$ MPN/100 mL (~2.5-fold decrease), consistent with dilution or surface washout effects. Smaller decreases were observed in household entrance soil and compound soil (approximately 0.1 $\log_{10}$ MPN/100 mL, 1.25-fold decrease). In contrast, *E. coli* concentrations in open drain water showed little change between pre- and post-rainfall conditions, indicating relatively stable contamination levels regardless of rainfall. Wilcoxon signed-rank tests indicated statistically significant differences between pre- and post-rainfall concentrations for sanitation facility entrance soils ($p = 0.020$) and samples associated with pit latrines ($p = 0.008$). No significant rainfall-related differences were detected for household entrance soils, compound soils, or open drain samples, suggesting that rainfall influences fecal contamination unevenly across environmental compartments,

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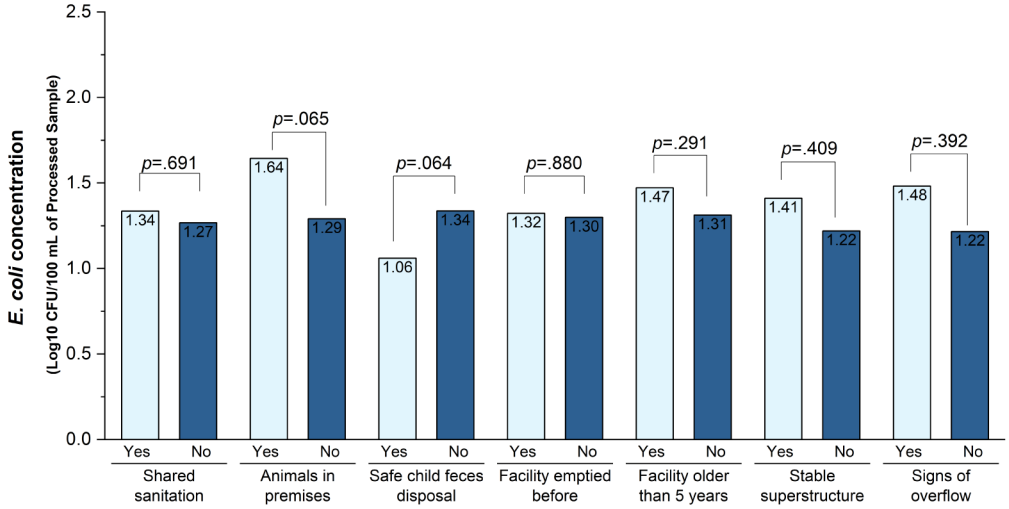

Figure 6. *E. coli*concentrations influenced by sanitation practices, infrastructure conditions, and household environmental factors.

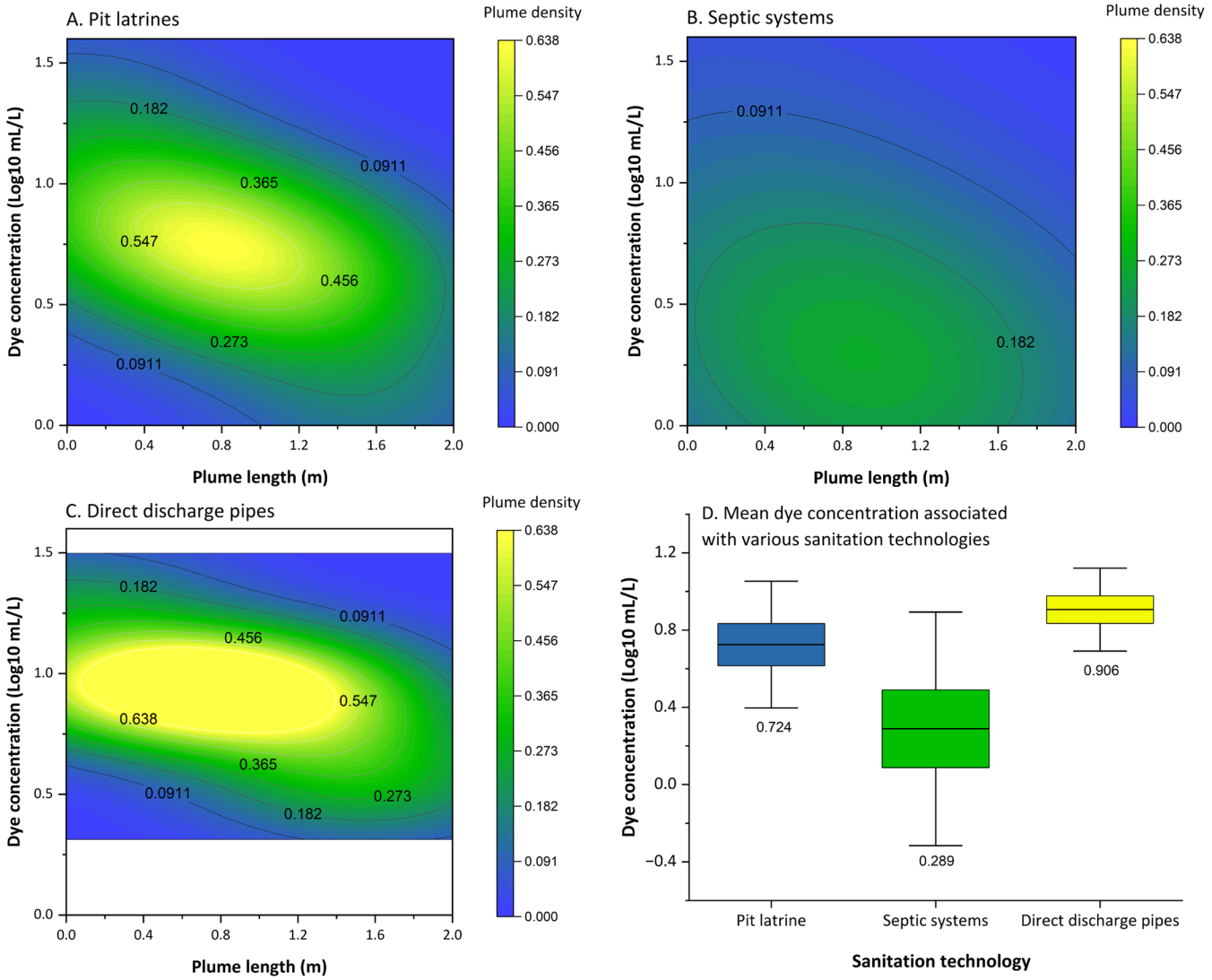

Figure 7. Cross-sectional profiles of dye plume for (A) pit latrines, (B) septic systems, and (C) direct discharge pipes. The *x*-axis represents plume length in meters, while the *y*-axis shows log-transformed dye concentrations. Color gradients represent relative dye signal intensity, with higher values near the injection point and decreasing intensity with distance, consistent with dilution and dispersion processes. Subfigure (D) displays the mean log-transformed dye concentrations across the various sanitation technologies.

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ACS EST Water XXXX, XXX, XXX−XXX

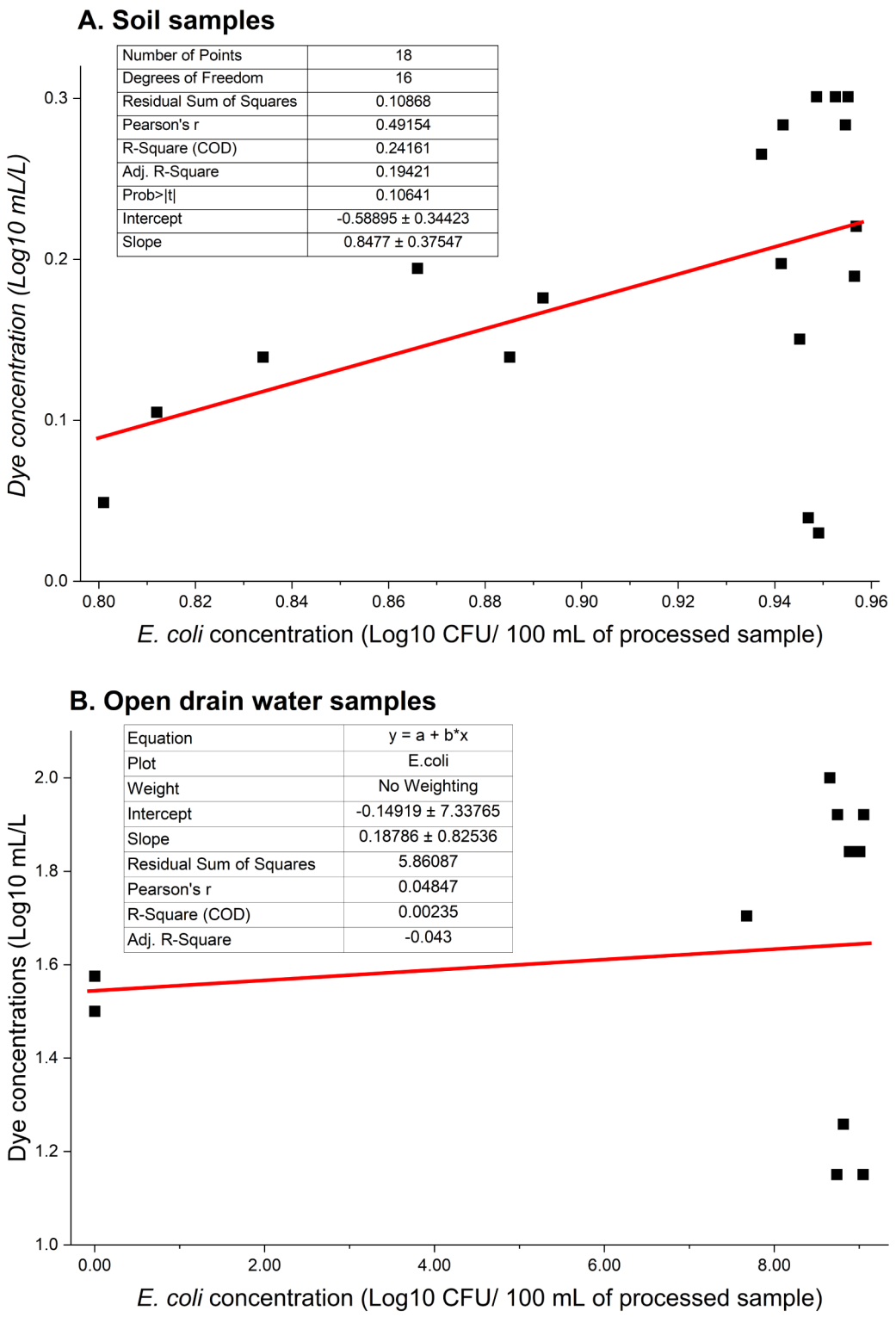

Figure 8. Correlation between *E. coli* and dye concentrations for (A) soil and (B) open drain water samples.

with surface soils near sanitation facilities showing the highest sensitivity to rainfall.

Figure 5 presents the mean $\log_{10}$ *E. coli* concentrations under pre- and post-rainfall conditions across sanitation technologies. A significant reduction in concentrations following rainfall was observed for pit latrines (p = 0.030), indicating a measurable rainfall effect for this system type. In contrast, no statistically significant differences between dry and wet conditions were detected for septic systems, UDDTs, or direct discharge pipes, suggesting that contamination associated with septic systems, dry toilets, and direct discharge remains relatively stable across the rainfall conditions.

Samples associated with shared sanitation facilities, structurally compromised sanitation systems, household environments with domestic animals, and households where discarded diapers were observed had higher arithmetic mean *E. coli* concentrations

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ACS EST Water XXXX, XXX, XXX−XXX

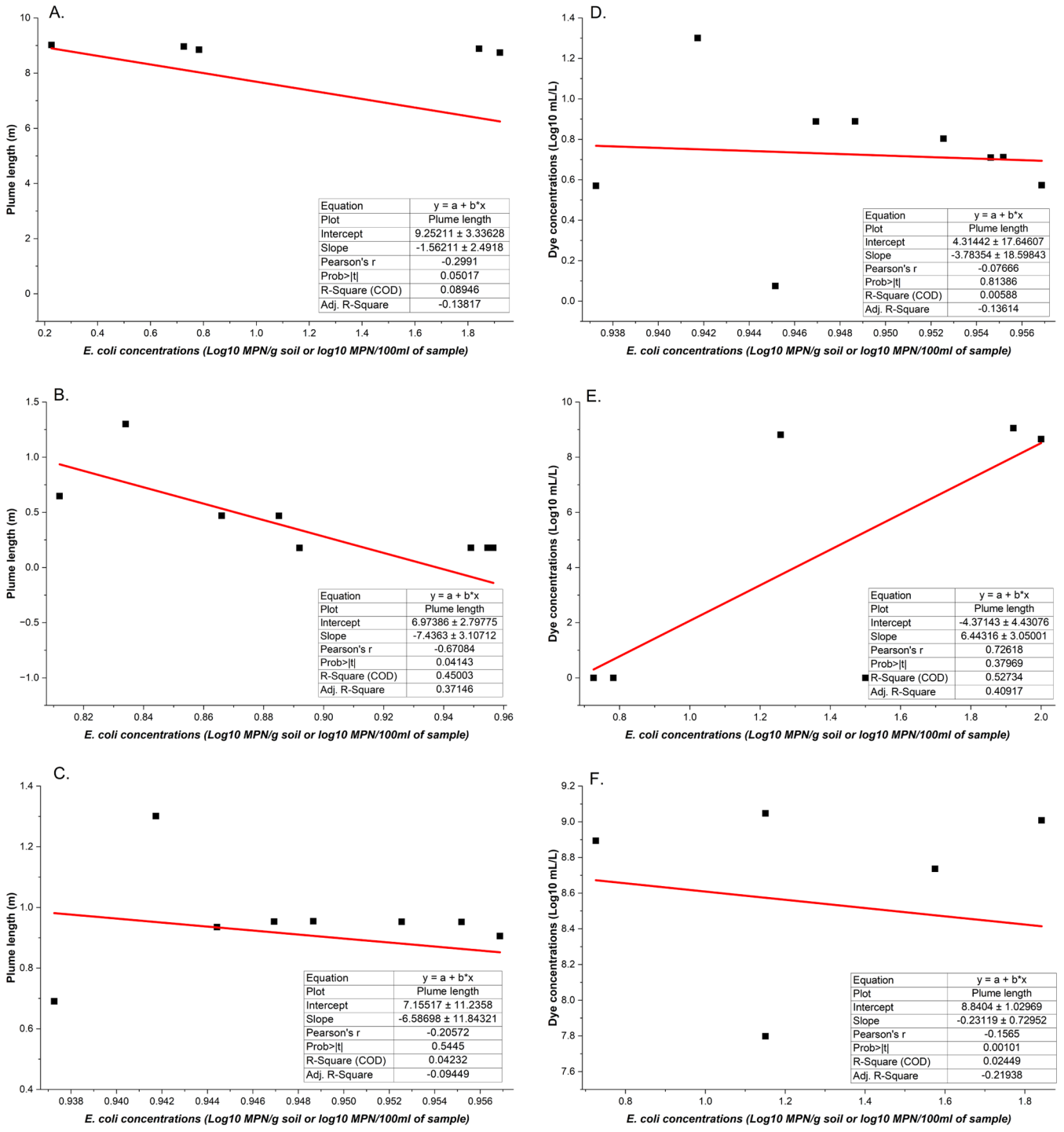

Figure 9. Sanitation system-specific correlation between fecal indicator concentrations, dye concentrations, and plume length. A. Pit latrines: correlation between *E. coli* concentrations and dye plume length. B. Septic systems: correlation between *E. coli* concentrations and dye plume length. C. Direct discharge pipes: correlation between *E. coli* concentrations and dye plume length. D. Pit latrines: correlation between *E. coli* concentrations and dye concentrations. E. Septic systems: correlation between *E. coli*concentrations and dye concentrations. F. Direct discharge pipes: correlation between *E. coli* concentrations and dye concentrations.

(Figure 6). Additionally, higher *E. coli* concentrations were found in samples linked to sanitation technologies that were more than five years old, showed signs of overflowing effluent receivers, or had not been emptied in the last five years prior to the study.

Tracer Presence and Concentrations

Tracer presence was observed in the facility's surrounding environment within 1 h of introduction across all sampled sanitation technology types. Dye was detected in the surrounding soils of 83% of pit latrines, 50% of septic systems, and all direct discharge pipes. Direct discharge pipes exhibited the highest tracer concentrations, with a mean of 7.6 mL/L and a

https://doi.org/10.1021/acsestwater.6c00135
ACS EST Water XXXX, XXX, XXX−XXX

peak of 9.0 mL/L, resulting in a plume averaging 1.8 m in length. Pit latrines showed a mean tracer concentration of 5.1 mL/L and a peak of 7.8 mL/L, producing a relatively shorter plume. Septic systems had the lowest tracer concentrations, with a mean of 2.1 mL/L and a peak of 2.9 mL/L, generating the smallest plume. Distance zero marked the point of tracer introduction via flushing from the residential toilet. Plume length corresponded to the final visible observation of dye in the soil up to 1 h after injection. Cross-sectional profiles of dye plumes showed that dye signal intensity decreased with increasing distance from the injection point, consistent with dilution and dispersion processes (Figure 7).

Association between Dye Tracer and Fecal Microbial Contamination

Associations between *E. coli* and dye concentrations were assessed through correlation analyses. Results indicated a positive association between dye concentrations and *E. coli* concentrations overall ($r = 0.194$, $p = 0.106$), although this relationship was not statistically significant, suggesting that higher tracer intensity may correspond to elevated fecal contamination (Figure 8). However, when disaggregated by sample type, distinct patterns emerged. In open drain water samples, the positive relationship was more apparent, indicating that the dye concentration more closely reflects direct transport and accumulation of fecal contamination in surface pathways. In contrast, soil samples showed a weaker association, likely due to underlying transport processes, such as filtration and adsorption, that reduce the strength of the relationship between tracer and microbial concentrations.

However, when the correlation between dye and *E. coli* concentrations was stratified by sanitation system type, distinct patterns emerged. For pit latrines, *E. coli* concentrations were negatively correlated with plume length ($r = -0.30$, $p = 0.050$), indicating that longer tracer migration distances do not correspond to increased microbial concentrations near the source (Figure 9). Septic systems showed a stronger negative association ($r = -0.67$, $p = 0.041$), suggesting that greater plume dispersion may coincide with dilution of fecal contamination. No significant association was observed for direct discharge pipes ($r = -0.21$, $p = 0.545$). Overall, findings from correlation analyses suggest that dye tracers capture some, but not all, pathways of fecal contamination in these environments, but the relationship varied by sanitation system and sample type, suggesting system-specific differences in hydraulic connectivity and subsurface transport processes rather than a uniform contamination signal.

DISCUSSION

The findings of this study highlight the limitations of existing sanitation systems in low-income urban settings, where fecal contamination is widespread and containment performance varies significantly across technologies. While pit latrines and direct-discharge pipes showed the highest dye concentrations and *E. coli* levels in the environment, indicating greater leakage or exfiltration, septic systems and urine-diverting dry toilets exhibited lower localized *E. coli* levels, suggesting that containment effectiveness is highly technology- and context-dependent. This reinforces concerns about the ability of existing sanitation systems to isolate waste, particularly in high-density settings. Consistent with evidence from the literature,[4,5,38,39] results further implicate poor construction, aging infrastructure, or inadequate maintenance as factors in poorly performing systems, with structural issues, high user loads, unsafe diaper disposal, and leaking effluent discharges identified as key contributors to contamination. While the results do not imply that any single technology is inherently "safe," they do highlight that commonly used systems often fail to prevent fecal contamination of adjacent environments. This is particularly important in informal settlements where multiple contamination pathways coexist and where households are exposed to fecal pollution through soil, drains, and shared spaces. The implication is that in these contexts, sanitation systems frequently do not function as intended and therefore offer limited protection to residents. Further, the data challenges conventional sanitation hierarchies that assume uniform performance across technologies, supporting broader messaging from other researchers,[40] suggesting that such rankings may not hold universally, especially in settings where construction quality, maintenance, and environmental conditions vary widely.

Rainfall introduces multiple and sometimes opposing processes that shape fecal contamination dynamics. While it can mobilize contaminants and expose sanitation system vulnerabilities, it can also dilute contaminant concentrations at specific locations. In this study, lower *E. coli* concentrations were observed following rainfall, particularly in soils near latrines and household entryways, consistent with dilution and surface washout processes, sometimes described as a "flush effect" reported elsewhere.[41] These findings align with a broader but mixed body of evidence. Some studies report increased contamination following heavy rainfall due to mobilization and redistribution of fecal material,[42−45] while others observe lower concentrations attributable to dilution.[16,18] Together, these results highlight that rainfall does not uniformly increase or decrease contamination but instead reshapes contamination patterns through a combination of mobilization, dilution, and interaction with existing infrastructure conditions. This complexity underscores the limitations of relying solely on fecal indicator bacteria to infer sanitation system failure and resulting contamination.

The study provides insights into the role of dye tracers in understanding contamination pathways, particularly in settings where microbial indicators such as *E. coli* alone provide an incomplete picture. While dye tracing has been applied in limited studies to track wastewater contamination,[46−49] its use in characterizing household sanitation systems in high-burden environments remains underexplored. Overall, the relationship between dye concentration and *E. coli* levels was positive but not statistically significant, suggesting that dye intensity does not consistently predict microbial contamination across all contexts. However, patterns varied by the environmental compartment and sanitation system. In open drain water samples, a clearer positive association between dye and *E. coli* concentrations was observed, indicating that dye tracers may more effectively capture direct transport and accumulation of fecal contamination in surface pathways. In contrast, the weaker associations observed in soil samples likely reflect subsurface processes, such as filtration, adsorption, and retention, which decouple tracer movement from microbial persistence. Analysis by the sanitation system type further highlights this heterogeneity. Negative correlations between plume length and *E. coli* concentrations for pit latrines and septic systems suggest that greater tracer migration distances are associated with dilution and dispersion rather than increased microbial contamination. This indicates that the plume extent reflects the reach of transport pathways rather than the intensity of contamination at a given location. No

https://doi.org/10.1021/acsestwater.6c00135
ACS EST Water XXXX, XXX, XXX−XXX

consistent relationship was observed for direct discharge systems, underscoring the variability of transport dynamics across system types. These findings suggest that dye tracers capture aspects of fecal contamination pathways but do not provide a direct proxy for microbial concentration. As such, dye tracing is best used as a complementary tool alongside microbial testing, rather than as a substitute. Its value lies in identifying potential leakage points and transport pathways that may not be evident from the microbial indicators alone. In complex, high-density environments where multiple contamination pathways coexist, dye tracing can enhance monitoring efforts by identifying where contamination is likely to move, even when microbial concentrations alone do not clearly indicate system failure.

This study has several limitations that should be acknowledged. The sample size was insufficient to detect statistically significant differences in *E. coli* concentrations and dye signals across technologies or to explore subneighborhood and environmental factors in detail. Additionally, data collection occurred shortly after intense regional flooding, which may have influenced both microbial and dye signals despite accounting for antecedent conditions. Rainfall events were not standardized across sites, and variability in rainfall intensity and timing, as well as in the timing of dye injection and sampling, may have influenced observed contamination patterns. The use of *E. coli* as an indicator of fecal contamination presents further challenges, as it can persist in the environment for extended periods (up to 2−4 months in soils)[50] and includes nonpathogenic strains, which limits its direct relevance to immediate public health risks. Moreover, the CBT method used to quantify *E. coli* has an upper detection limit of 48.3 MPN/100 mL, potentially underestimating contamination levels in high-burden environments like informal settlements. To address this, we included additional indicators such as the binary presence/absence of*E. coli*. The dye tracing method also faced potential limitations, including interference from background signals such as soil moisture and fluorescing contaminants, as well as photochemical degradation. However, validation tests conducted by applying the dye under different laboratory simulations confirmed the method's effectiveness in this context.

Despite these limitations, the study provides novel evidence of fecal contamination flows across sanitation technologies in high-burden, low-income urban settings. The findings highlight the complex interplay among rainfall, sanitation system integrity, and fecal contamination, suggesting that current systems often fail to prevent the spread of fecal material into household environments and do not adequately protect public health. These insights underscore the need for improved sanitation infrastructure and monitoring approaches tailored to the challenges of dense, resource-constrained urban environments.

IMPLICATIONS

The potential of fluorescent dye tracing as a real-time, low-cost tool for identifying sanitation system failures suggests that it should be integrated into routine inspections alongside microbial testing. Establishing standard operating procedures for dye applications could strengthen regulatory oversight, enabling faster detection of leaks and illegal discharges, particularly in informal settlements where conventional microbial testing is not feasible. This method could also serve as an early warning system in high-contamination environments, improving response times and public health outcomes. These findings emphasize the need for evidence-based, targeted interventions to improve sanitation systems and protect public health in vulnerable urban areas.

A key finding from this study is that the sanitation technologies evaluated were not effective at containing human excreta or protecting public health. Policymakers should focus on investing in infrastructure that can withstand climatic challenges such as heavy rainfall to reduce fecal contamination. Factors such as unstable superstructures, domestic animals, unsafe diaper disposal, shared sanitation, aging latrines, and leaking effluent discharge must be addressed through regular maintenance, repairs, and better hygiene practices. There was little evidence that specific sanitation technologies were more resilient to the impacts of rain.

The study also highlights the need for stricter infrastructure standards to prevent wastewater−stormwater mixing, as the persistent presence of *E. coli* in open drains and soil underscores this issue. Promoting alternative sanitation technologies, such as urine-diverting dry toilets and septic systems, which showed lower fecal contamination under rainfall, could provide more sustainable options in similar contexts. Additionally, formalizing fecal sludge emptying services, setting licensing requirements for providers, and introducing financing mechanisms like subsidies or microlenders could improve access to safe sanitation and waste collection for low-income households.

CONCLUSION

This study contributes to growing evidence on how sanitation systems in low-income urban environments respond to rainfall and extreme weather events, with implications for fecal contamination and public health risks. In settings where climate change is expected to intensify rainfall, sanitation services that can effectively contain excreta under wet conditions are critical for reducing household and environmental exposure. Findings show that most sanitation systems failed to fully contain fecal waste, and failure was shaped by technology type, structural conditions, and maintenance practices.

While the tracer dye did not capture all fecal contamination dynamics, it proved useful for identifying sanitation system failures and likely routes of contamination spread, particularly following rainfall events. Together, these findings underscore the need for sanitation infrastructure, management, and monitoring approaches that are resilient to rainfall and flooding. Although this study focused on low-income urban areas of Nairobi, the insights are likely relevant to other rapidly urbanizing, flood-prone settings and can inform targeted climate-resilient sanitation interventions and public health responses.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsestwater.6c00135.

Additional experimental details and methods for the dye tracing experiment, including photographs of the experimental setup and results, as well as rainfall estimates during the study period (PDF)

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ACS EST Water XXXX, XXX, XXX−XXX

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Notes

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ACS EST Water XXXX, XXX, XXX–XXX