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The Distribution of Fecal Contamination in an Urbanized Tropical Lake and Incidence of Acute Diarrheal Disease

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Cite This: https://doi.org/10.1021/acsestwater.2c00255 Read Online

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ABSTRACT: Aquatic ecosystems of tropical countries are vulnerable to fecal contamination that could cause spikes in the incidences of acute diarrheal disease (ADD) and challenge public health management systems. Vembanad lake, situated along the southwest coast of India, was monitored for one year (2018–2019). *Escherichia coli*, an indicator of fecal contamination, was prevalent in the lake throughout the year. Multiple antibiotic resistance among more than 50% of the *E. coli* isolates adds urgency to the need to control this contamination. The high abundance of *E. coli* and incidence of ADD were recorded during the early phase of the southwest monsoon (June–July), prior to the once-in-a-century floods that affected the region in the later phase (August). The extent of inundation in the low-lying areas peaked in August, but *E. coli* in the water peaked in July, suggesting that contamination occurred even prior to extreme flooding. During the COVID-19-related lockdown in March–May 2021, fecal contamination in the lake and incidence of ADD reached minimum values. These results indicate the need for improving sewage treatment facilities and city planning in flood-prone areas to avoid the mixing of septic sewage with natural waters during extreme climate events or even during the normal monsoon.

KEYWORDS: *Fecal contamination, Escherichia coli, COVID-19, remote sensing, flood, antimicrobial resistance*

1. INTRODUCTION

Aquatic ecosystems contaminated with septic sewage may serve as reservoirs of water-associated human pathogens, causing sporadic disease outbreaks.[1−3] Current public health management systems advocate washing hands and assuring clean drinking water and sanitation facilities to prevent the transmission of water-associated contagious diseases. The lives of approximately 0.3 million children (under the age of 5 years) were lost globally in just one year (2015) due to water-associated diseases triggered by the lack of access to clean drinking water and sanitation facilities.[4] High numbers of cases of enteric and respiratory infections are also linked to exposure to microbially polluted coastal or inland waters and to consumption of contaminated seafood.[5,6] Recent advances in our understanding of water-associated diseases have brought to the fore the need to consider environmental reservoirs of human pathogens in public health management plans to control transmission of water-associated diseases.[1−3] Such a multidisciplinary approach would include *in situ* monitoring of microbial pollution and water quality in natural water bodies and engagement of the general public through citizen science and other mechanisms, combined with satellite-based observations and modeling to identify potentially contaminated

Special Issue: Water Challenges and Solution Opportunities in South Asia, a Rapidly Developing Region of the World

Received: June 3, 2022
Revised: January 11, 2023
Accepted: January 11, 2023

Figure 1. Images captured from the study area, which shows interactions of people with Vembanad lake: waste disposal (a), washing and bathing (b), transportation of drinking water (c), transportation of people (d), different types of fishing (e, f), and tourism (g).

areas and to issue alerts to manage human interactions with such systems.[2,3,7,8] Even when the drinking water supply is safe, people exposed to contaminated water through various routes such as washing clothes, bathing, fishing, transport, and tourism are prone to infections.[5,6,9]

Extreme weather conditions associated with natural variability or climate change could broaden the geographic distribution of microbial pathogens and facilitate their proliferation in coastal and inland waters, enhancing the incidence of water-associated diseases.[10−16] A growing number of water-associated diseases are linked to extreme weather incidents,[17] which may be heavy rains, floods, and cyclones. Such events could promote the introduction of pathogens into natural bodies of water, as well as influence the survival, reproduction, and transmission of pathogens and vectors. Extreme weather incidents elevate the impacts of nonpoint sources of fecal contamination in aquatic ecosystems through disruption of sanitation infrastructure, overflowing of contaminated water from sewage treatment plants, and increased stormwater flow.[18] Many of the pathogens reaching aquatic ecosystems from leaking sewage treatment systems have multiple antibiotic resistance, which aggravate the intensity of the problem.[19] This is especially important in coastal areas where nearly 50% of the world's population lives.[20]

The floods that occurred in Kerala state, along the southwest coast of India, in 2018 is an example of an extreme event, which caused severe damage to the basic sanitation systems.[21] The floods, caused by extreme rainfall (>60% more precipitation than normal in the catchment area) and subsequent opening of sluice gates of dams across major rivers to avoid their collapse, caused severe damage to lives and assets (death toll of 440 and one million people shifted to shelters).[22] Vembanad lake (09°00′−10°40′ N latitude and 76°00′−77°30′ E longitude), which straddles Alappuzha, Ernakulam, and Kottayam districts of Kerala, was severely affected. Fecal pollutants enter Vembanad lake through nonpoint sources, such as drains of commercial and residential buildings opening into the lake, unlawful dumping of toilet waste and solid waste, and excessive number of tourist boats.[23−25] Many of these sources of pollution were blocked when a national lockdown was declared in India from March 24, 2020, to curtail the spread of COVID-19. Apart from controlling the spread of the pandemic, the lockdown had a significant role in reducing water and air pollution globally and in Vembanad lake.[26−33]

It is important to study the environmental reservoirs of pathogenic bacteria in Vembanad lake and the transmission of water-associated diseases through these reservoirs for several reasons: (1) This is a low-lying area, vulnerable to high impact from sea-level rise associated with climate change. (2) Nearly 1.6 million people interact with Vembanad lake and use its water for dumping waste, washing, fishing, recreation, and transportation (Figure 1). (3) Vembanad lake receives fresh water discharge from 10 major rivers covering ∼40% of the drainage area of the state. (4) The lake also receives ∼235 million liters of sewage from nonpoint sources of pollution.[34] (5) Fecal contamination of groundwater and surface water and associated disease burden are high in Kerala.[35,36] (6) Finally, the literacy rate, public awareness, human development index, and medical facilities in the area are high,[37] which could facilitate translation of scientific findings to policy action.

In this study, we monitored the distribution of *E. coli* at multiple locations in the Vembanad lake for one year at ∼20-day interval and investigated the antibacterial resistance of

https://doi.org/10.1021/acsestwater.2c00255
ACS EST Water XXXX, XXX, XXX−XXX

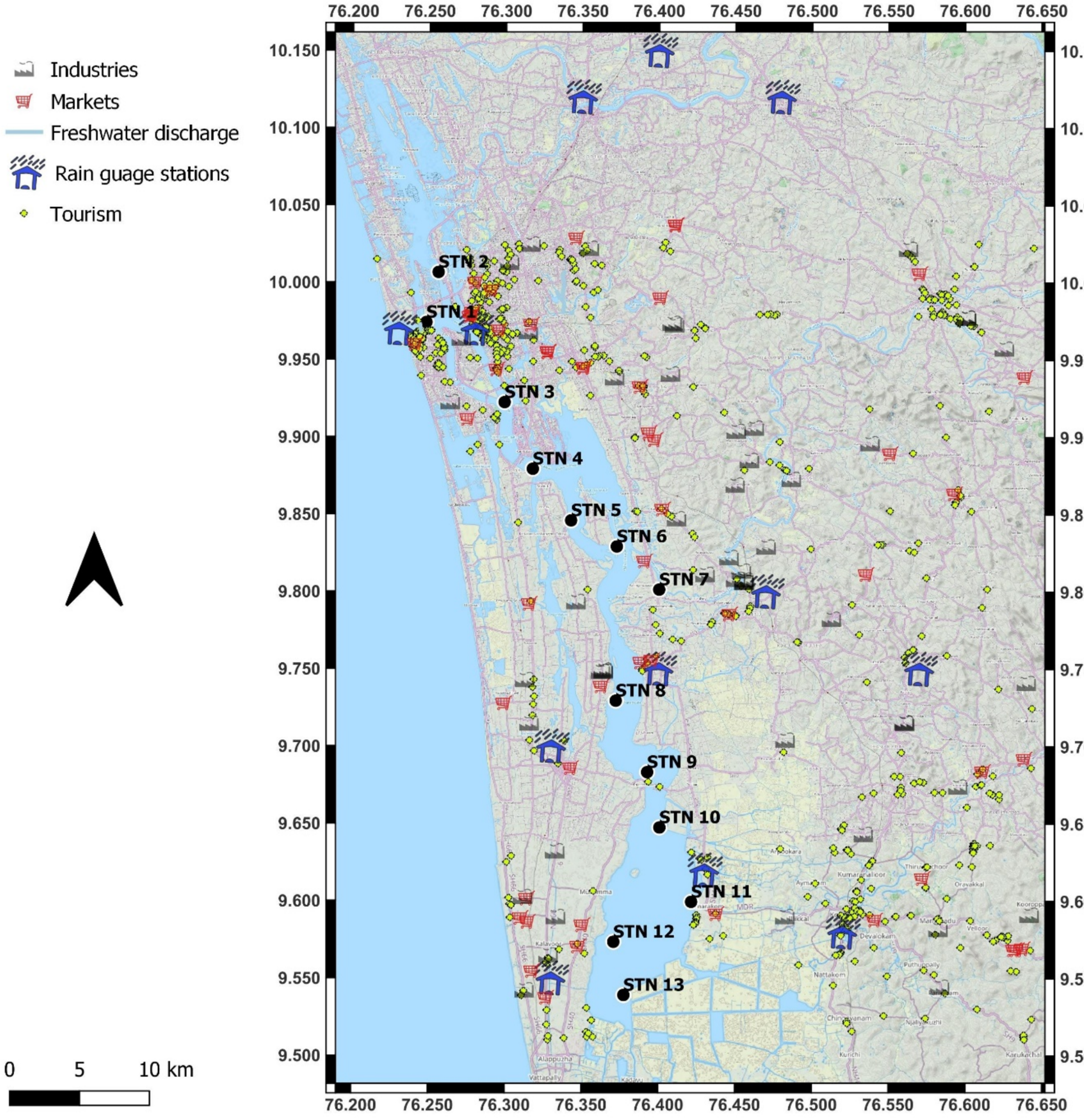

Figure 2. Map of study area showing sampling locations, rain gauge stations. and major sources of pollution. Stations 1−6 are treated as brackish water stations and 7−13, as fresh water stations.

E. coli isolates. Since the Vembanad lake had been monitored for a year just prior to the lockdown and immediately after lockdown, it became possible to examine the impacts of the once-in-a-century floods in 2018 and the pandemic lockdown in 2020 on the distribution of fecal contaminants in the lake. *In situ* and remote-sensing data on environmental variables and data on the incidence of acute diarrheal disease (ADD) were used to analyze the influence of rainfall, flooding, and lockdown on fecal contamination in the lake and on the prevalence of ADD.

2. METHODS

2.1. Study Area and Sample Collection. Water samples were collected from 0.5 m below the water surface at 13 locations in the Vembanad lake at an interval of ∼20 days from April 2018 to May 2019 (Figure 2). Of these, stations 1−6 are brackish water dominated during the nonmonsoon seasons but become fresh water dominated during the southwest monsoon season, whereas stations 7−13 are fresh water dominated most of the year. Water samples were also collected on 12th May 2020, 48 days after implementation of the national lockdown in India on 24th March 2020. A total of 234 water samples were collected during the study period using a 5 L capacity Niskin bottle. Salinity and temperature of the water column were measured using sensors attached to a Conductivity, Temperature, and Depth (CTD) rosette. Subsamples for microbiological analysis (250 mL) were transferred aseptically to a sterile polypropylene bottle and transported to the laboratory in an ice-chest for further analysis.

2.2. Enumeration of *Escherichia coli*. The *E. coli* were enumerated using the quantitative real-time polymerase chain reaction (qPCR) technique. The water sample (1 L) was filtered through a polycarbonate membrane filter with a pore size of 0.2 μm, and DNA was extracted following the method in ref 38 with small modifications as described in our previous publication.[39] Absolute qPCR was carried out in a Roche Lightcycler 96-well Real-time PCR system (Roche, USA) to enumerate the number of *E. coli* present in the water column. The recombinant plasmid (pJET; 2974bp) containing a portion of β-D-glucuronidase gene (187 bp) was linearized using restriction enzyme NcoI and used as the standard. The standard curve was prepared from different dilutions of linearized plasmid ranging from 10^8 to 10^1 copies of the β-D-glucuronidase gene. Copy number calculation of the standard DNA template was done using the online tool of the URI Genomics and Sequencing Centre (https://cels.uri.edu/gsc/cndna.html). The qPCR amplification was performed in a total volume of 10 μL, containing 2× SYBR Premix Ex *Taq*II (Tli RNase H Plus, Takara, USA), 10 ng of template DNA, and 10 pmμL^{-1} of each primer (UALF-ATGGAATTTCGCCG-ATTTTGC and UALR-ATTGTTTGCCTCCCTGCTGC). The qPCR program was initial denaturation at 95 °C for 10 min followed by 40 cycles of denaturation (95 °C for 10 s), annealing (60 °C for 10 s), and extension (72 °C for 10 s) and extension completion with a dissociation (melt curve) analysis of amplified products performed at the end of each cycle to confirm the amplification and detection of confirmed PCR product. The absolute quantification data obtained was analyzed with Roche Light cycler 96 software (Roche, USA).

2.3. Isolation of *E. coli* and Antibiotic Resistance Profile. Water samples (10 mL) filtered through a glass fiber filter with a 3 μm pore size were added to a sterile tube containing alkaline peptone water (APW; 90 mL) and incubated overnight at 28 ± 2 °C. The cell lysate was prepared from the enrichment broth by boiling,[40] which was then directly used as the DNA template for screening for the *E. coli* specific β-D-glucuronidase gene using a primer set (UAL). The PCR reaction mixture consisted of Emerald Amp GT PCR master mix (2×) (Takara), forward and reverse primers (10 pmol μL^{-1} each), and 1 μL of the crude DNA template. The reaction was performed in a Takara thermal cycler with the following temperature and cycling conditions: initial denaturation (95 °C for 2 min), followed by cycle denaturation (at 94 °C for 30 s), annealing (for 30 s at 60 °C), and extension (at 72 °C for 60 s) for a total of 25 cycles and a final extension for 7 min at 72 °C. The PCR products were loaded onto agarose gel (1%) impregnated with ethidium bromide after which electrophoresis was carried out with 1× TAE buffer, and the result was visualized using a gel documentation system (BioRAD, USA).

Bacteria showing positive reactions to the UAL were isolated from the APW to sterile plates containing Chromcult coliform agar (Merck) by a spread plate technique. The plates were incubated at 37 ± 2 °C overnight, and colonies with dark blue to violet color were isolated and preserved. Sensitivity of these bacteria to 12 antibiotics was tested following a standard disc diffusion technique for 200 randomly selected isolates. Muller Hinton (MH) agar plates were swabbed with overnight grown bacterial suspension cultures, and commercially available antibiotic discs (HiMedia, India) were placed on the agar and incubated at 28 ± 2 °C for 24 h. The antibiotics tested were Amoxycylin (30 μg), Meropenem (10 μg), Ampicillin (25 μg), Imipenem (10 μg), Nalidyxic acid (30 μg), Norfloxacin (10 μg), Moxifloxacin (5 μg), Gatifloxacin (5 μg), Trimethoprim (10 μg), Gentamicin (10 μg), Tetracycline (30 μg), and Erythromycin (15 μg). The zone of inhibition was recorded using an antibiotic susceptibility scale (Himedia, India), and antibiotic resistance profiles of each isolate were calculated following the standard antimicrobial zone size interpretation chart.[41] The multiple antibiotic resistance (MAR) index was calculated as the ratio of the number of antibiotics to which an organism is resistant to the total number of antibiotics tested.[42] Bacteria were classified into low (<0.25), medium (0.25−0.5), high (0.5−0.75), and extreme (>0.75) antibiotic resistant groups based on their MAR index.

2.4. *In Situ* and Satellite Remote-Sensing Data on Precipitation and Flooding. Daily precipitation data from 19 rain gauges placed around the Vembanad lake (Ernakulam, Alapuzha, and Kottayam districts of Kerala) were retrieved from the Indian Meteorology Department (IMD) for the period of April 2018 to March 2019 and May 2020. Average rainfall in each month in the region was calculated. The surface images of Vembanad lake before and after the incidence of floods were retrieved from Sentinel-1A and -1B synthetic-aperture radar (SAR) images available from the Copernicus Open Access Hub (https://scihub.copernicus.eu; 2018−2020 data accessed on 22 April 2021). Level-1 Ground Range Detected (GRD) SAR products were processed at 10 m spatial resolution using the Sentinel Application Platform (SNAP) from the European Space Agency (ESA)[43] as recommended by the United Nations Space-based Information for Disaster Management and Emergency (SPIDER) portal (https://www.un-spider.org/advisory-support/recommended-practices/recommended-practice-radar-based-flood-mapping).

The SAR images obtained were calibrated through radiometric correction to obtain the radar backscatter coefficients, after which images were filtered to remove noise using a speckle filter (Lee, 7 × 7) in SNAP. Processed images were reprojected (WGS84) using a range-Doppler terrain correction algorithm, and multiple tiles covering Vembanad lake were combined and cropped using the mosaicking tool in SNAP. To separate water from nonwater pixels, a threshold of 0.04 was selected for the Vembanad lake region based on analyses of the histograms of the radar backscatter coefficients. Binary maps of water (backscatter coefficient <0.04) and nonwater pixels (backscatter coefficient >0.04) were created using the band maths tool in SNAP. For flood mapping, a base map of Vembanad lake was developed from five S1 images of May 2018, as well as of December to March 2019 (06−05−2018, 07−12−2018, 31−12−2018, 19−03−2019, 31−03−2019), all during the dry season when we may expect a minimum extent of Vembanad lake with limited influence from rainfall and/or agricultural activities (i.e., intentional flooding of rice cultivation fields). The base map of Vembanad lake was compared with the binary water/nonwater S1 images to create flood maps, marking pixels that fall out of the minimum extent of Vembanad lake as flooded. For each image, the area (number of pixels) marked as flooded was also calculated.

2.5. Incidence of Acute Diarrheal Diseases. Primary data on the incidence of acute diarrheal disease were collected from the Directorate of Health, Government of Kerala, India, by submitting a request under the Right to Information Act of the Government of India.[44] Monthly incidence of acute diarrheal disease reported from the three districts that border the Vembanad lake, Ernakulam, Kottayam, and Alappuzha districts, were retrieved for the period of May 2018 to March 2019 and May 2020.

2.6. Data Analysis. All data on salinity, temperature, and abundance of *E. coli* were subjected to Shapiro−Wilk and Anderson−Darling tests for normality of sampled population. Pearson and Spearman rank correlations were chosen for normally and non-normally distributed variables, respectively. Pairwise correlations of *E. coli* with salinity and temperature and monthly averages of rainfall and incidence of ADD were calculated by Spearman or Pearson rank at a significance level of $p < 0.05$. All statistical analyses were done using RStudio platform (version 1.4.1717).[45]

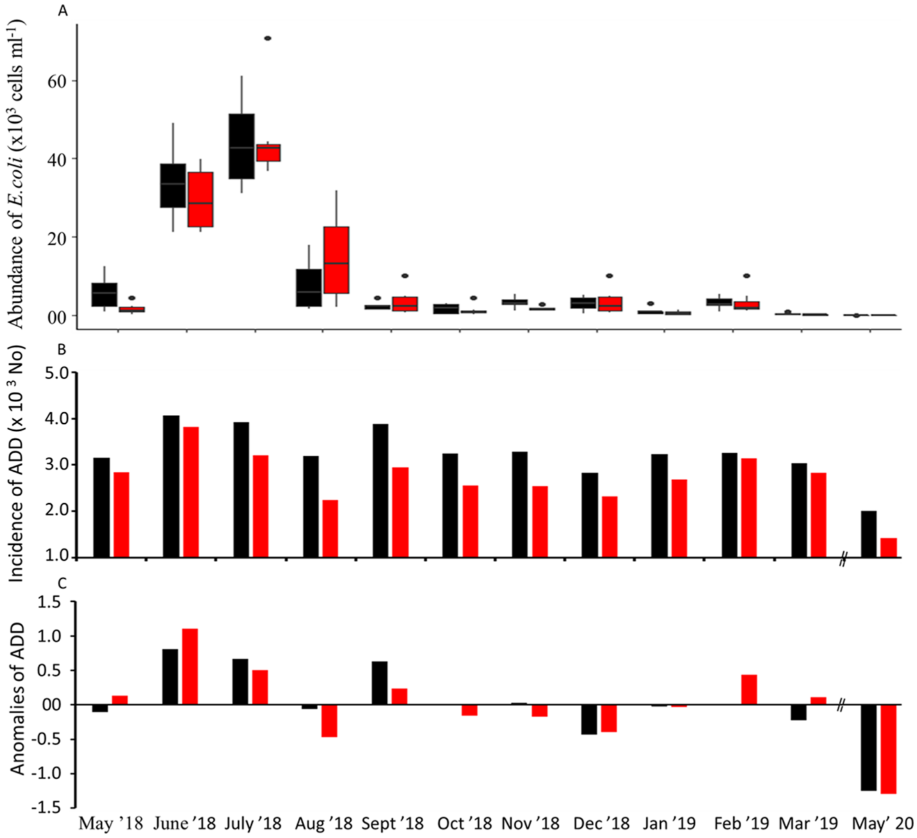

Figure 3. Temporal variation in the abundance of *E. coli* in the water column of Vembanad lake (A); incidence of acute diarrheal disease (ADD) (B); the anomalies in the incidence of ADD, computed as the difference from the mean value for all available observations (C) in the brackish water (black) and fresh water (red) regions of Vembanad lake. Diseases data for Ernakulam district are assigned to the brackish water side of the lake, and the sum of data from Alappuzha and Kottayam districts are assigned to the southern, fresh water side of the lake.

3. RESULTS AND DISCUSSION

Avoiding interactions of people with contaminated water bodies along with access to clean water and safe sanitation measures are essential to achieve the global target of preventing transmission of water-associated diseases by 2030.[3,46] Oral−fecal transmission of water-associated diseases is well understood and can be prevented to a large extent by practicing water, sanitation, and hygiene (WaSH) protocols recommended by WHO.[47] At the same time, it is important to understand factors that determine environmental reservoirs of human pathogens under climate change and associated extreme weather incidents, as it can have long-term implications on public health.

3.1. Concentration of *E. coli* in the Water Column and Reports of Acute Diarrheal Disease.

Analyses of water samples collected from 13 locations in the Vembanad lake at 20-day intervals confirmed that fecal contamination is prevalent in the lake throughout the year with an increase in abundance during June and July 2018, associated with the southwest monsoon rain (Figure 3A). The highest abundance of *E. coli* was found in the water sample collected in July 2018 from station 4 (61×10^3 cells mL^{-1}) in the brackish water regions of the lake, and the next highest value was found in the same month at station 11 (71×10^3 cells mL^{-1}) on the fresh water side (Suppl. Figure 1). Additional surges in *E. coli* counts were found in Station 11 in August and November 2018. Minor spikes in the abundance were also observed in station 13 at multiple times during the study period (Suppl. Figure 1). The abundance of *E. coli* was less than 200 cells mL^{-1} in all stations in March 2019 and May 2020.

There was a significant Spearman correlation between the abundance of *E. coli* in Vembanad lake and anomalies in the number of cases of ADD in Ernakulam, Alappuzha, and Kottayam districts combined ($r = 0.58$, $p < 0.05$) (Suppl. Figure 2). The incidence of ADD was high during the first half of the southwest monsoon season with ∼4000 cases being reported from both brackish and fresh water areas of the lake in June 2018. The numbers then reduced gradually to a minimum of ∼2800 and 2330 cases in brackish water and fresh water regions, respectively, in December 2018 (Figure 3B). Interestingly, the number of reported cases of ADD after two months of lockdown in May 2020 was ∼2000 and ∼1400 in brackish water and fresh water areas, respectively, which was approximately 40% and 50% less than the number of incidences reported from the same region in May 2018. There was not even a single instance during the study period when the abundance of *E. coli* in the water column of Vembanad lake dropped below detectable limits or when there were no reports of ADD, but the anomalies in the incidence of ADD (around average values of 3262 for brackish and 2712 for fresh water regions) were high and positive during the initial phase of the southwest monsoon in June and July (Figure 3C).

Given the high number density of *E. coli* cells and their broad prevalence, we also investigated their antibacterial resistance properties.

3.2. Antibiotic Resistance Profile of *E. coli*.

The *E. coli* cells grown as dark blue to violet-colored colonies in the

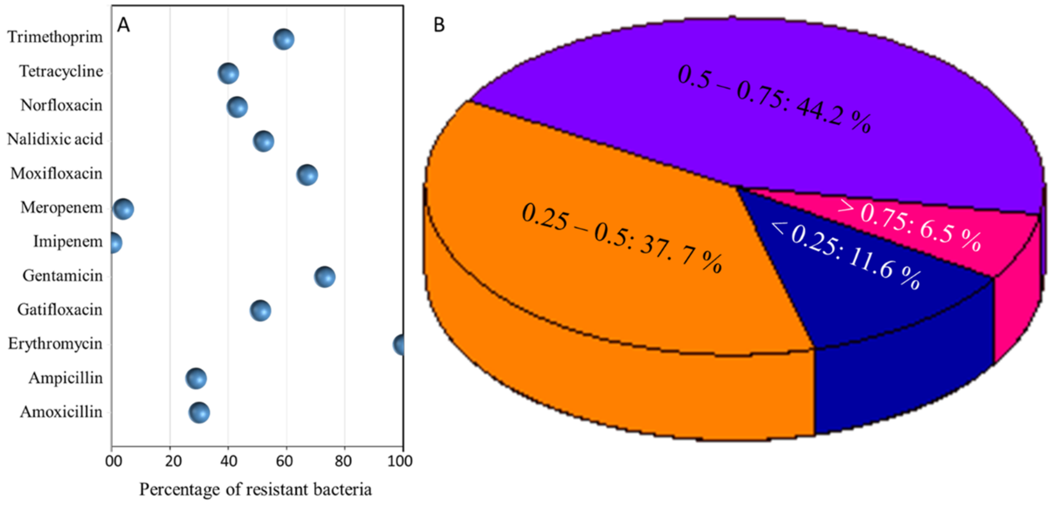

Figure 4. Antibiotic resistance profile of *E. coli* (A) and their multiple antibiotic resistance (MAR) index (B). MAR index groups are indicated as low (<0.25), medium (0.25–0.5), high (0.5–0.75), and extreme (>0.75) antibiotic resistant.

Chromcult coliform agar (HiMedia, India) and isolated (200 samples) showed a pattern of varying resistance against 12 antibiotics. All the isolates showed resistance to Erythromycin, and none was resistant to the new generation antibiotic Imipenem (Figure 4). More than 50% of the isolates showed resistance to Nalidixic acid (52%), Moxifloxacin (67%), Gatifloxacin (51%), Trimethoprim (59%), and Gentamycin (73%). The multiple antibiotic resistance index also varied between samples of *E. coli*, with 50% of the isolates showing an index of more than 0.5.

The multiple antibiotic resistance seen among the *E. coli* isolates also underlines the anthropogenic role on the prevalence of fecal contaminants in the lake. Similar resistance patterns of bacteria have been reported from elsewhere, mostly from clinical isolates, veterinary sources, and sewage treatment systems.[48−51] The resistance among the majority of isolates (>60%) to Erythromycin, Gentamicin, and Moxifloxacin found in this study is not surprising as a similar resistance to these antibiotics has been reported earlier in *E. coli* isolated from both clinical and environmental sources from various locations and is attributed to the common use of these antibiotics.[52−54] However, resistance to the Carbapenem class of antibiotics (Meropenem) among a small number (<10%) of isolates is a matter of concern, as estuaries can function as a medium for gene exchange and increase the risk of spreading antibiotic-resistant bacteria in the environment and then back to humans.[55] These high levels of multiple antibiotic resistance, combined with a high prevalence of *E. coli* in the lake waters, points to the importance of containing this contamination. This requires an understanding of the factors responsible for the fecal contamination of the lake waters and for its seasonality in Vembanad lake. To this end, we investigated the relationship between *E. coli* in the water and the seasonal variability in hydrographical conditions.

3.3. Salinity, Temperature, Rainfall, and Flooding. The study area received heavy southwest monsoon rainfall in 2018 compared with 2019 with a cumulative rainfall of ∼60,000 and ∼50,000 mm from April to November in each year, respectively (Figure 5A). In 2018, the average precipitation in May (335 ± 138 mm), June (665 ± 228 mm), July (795 ± 302 mm), and August (596 ± 145 mm) was substantially higher than that in the corresponding months of 2019, which were 52 ± 27, 386 ± 97, 487 ± 95, and 781 ± 175 mm, respectively. The average rainfall for May in 2018 (335 ± 138 mm) and 2020 (332 ± 91.2) was comparable. The monthly average rainfall reached a maximum of 795 mm in July 2018, but the continued heavy rainfall, along with the opening of sluice gates of dams due to overload, caused flooding in August 2018.[56] More than 400 km^2 of land near the lake was affected by the flood (Figure 5D). In Kuttanad, the traditional rice farming region below sea level in the vicinity of the fresh water region of the lake, water levels rose to 2.4–4.6 m.[57]

Salinity and temperature were highly dynamic in the Vembanad lake during the study period (Figure 5B,C). The entire lake was dominated by fresh water during June to August 2018, and the salinity increased with the withdrawal of the southwest monsoon in the northern, brackish water regions of the lake. The saline water influx was more prominent up to station 6, with average salinity reaching up to 21.8 ± 6.9 psu in February 2019. The southern stations (7–13) remained fresh water dominated and recorded a maximum salinity of 11.1 ± 5.1 psu in May and below 5 psu for most of the year. Unlike salinity, seasonal variability in temperature was comparable in the two parts of the lake, throughout the year. The temperature minimum was observed in July 2018 (26.6 ± 1.1 °C in brackish water and 26.8 ± 0.8 °C in fresh water region) corresponding to the rainfall maximum, and the maximum was observed in April 2019 (32.3 ± 0.9 °C in brackish water and 32.9 ± 1.5 °C in fresh water region) at the peak of the Indian summer.

Salinity and temperature had a negative correlation with the abundance of *E. coli* in Vembanad lake (Figure 6A,B). Low salinity (<10 psu) with warm temperature (25–30 °C) was seen to be favorable to *E. coli* in most of the cases for Vembanad lake (Figure 6C). In fact, we see (Figure 6A,C) that *E. coli* cell abundance greater than 20 × 10^3 cells mL^{-1} is rare (only 3 instances) when salinity is greater than 10 psu, whereas this threshold is breached frequently, when salinity is close to zero. The *E. coli* counts plotted as a function of temperature (Figure 6B) also reveal high counts at low temperatures, which in turn corresponds to the southwest monsoon season (Figure 5A,C).

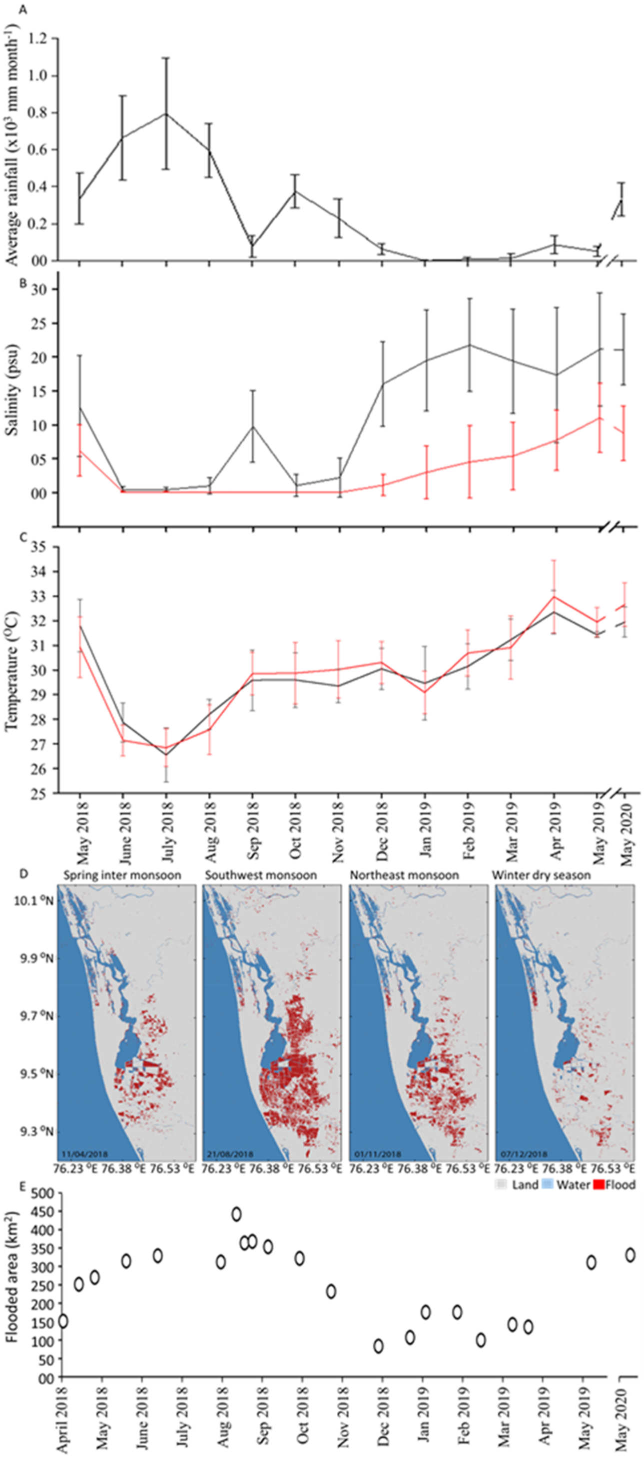

Figure 5. *In situ* observations of hydrographic variables averaged over the brackish water (black) and fresh water (red) stations of Vembanad lake (left panels) and the extent of water-covered areas in and around Vembanad lake from Sentinel-1 remote-sensing data during various months of the study. (A) Average rainfall (× 10^3 mm per month) from a total of 12 rain gauges near Vembanad lake, for both fresh water and brackish water regions combined; (B) average salinity (psu); (C) average temperature (°C). (D) Water-covered areas, with red pixels indicating water pixels beyond the regular extent of Vembanad lake, which is shown in blue. Four maps are provided for 2018 with an example day given for each season, from left to right: April 2018 representing the spring inter monsoon season (March–May); August 2018 representing the southwest monsoon (June–September); November 2018 representing the northeast monsoon

Figure 5. continued

(October–November); December 2018 representing the winter dry season (December–February). (E) The flooded area (total area of red pixels in km^2) throughout the study period. Note that Vembanad lake covers over 287 km^2 according to the Sentinel-1 base map and that paddy cultivation fields typically fall outside the normal water extent of the lake and thus might appear as "flooded" on the maps when waterlogged.

All these results point to heavy rains as a prominent factor that influences the abundance of *E. coli* in the waters of Vembanad lake. In fact, the average monthly *E. coli* counts in Vembanad Lake remain below 10×10^3 cells mL^{-1} while monthly average rainfall stays below a threshold of 400 mm and increases rapidly for higher monthly rainfall (Figure 7A). An exponential function ($y = 635 \times \exp(0.0054)$) fits the observations well with $r^2 = 0.93$. When the *E. coli* counts are plotted as a function of cumulative rainfall, starting from May 2018 (Figure 7B), we see that the steepest increase in cell counts is from May to June, with the counts peaking in July 2018, prior to the floods in August and September 2018. This is contrary to any expectation that the contamination levels might have been highest during the floods. A possible explanation for this observation is that fecal contamination in the soil from surrounding areas gets washed down into the lake either through direct land drainage or through river transport in the initial days of the heavy monsoon rain. This could be potentially augmented by contamination of groundwater from septic tanks, when the water table rises in the region, during the rains, bringing groundwater up to, or above, the level of the septic tanks (Figure 8). In the later stages of the monsoon, which includes the period of floods, it is possible that the *E. coli* load from nonpoint sources has already been washed out by the earlier rains, which when combined with an increase in current and reduced residence time of the waters in the lake leads to a rapid drop in *E. coli* counts, as seen in Figure 7B.

Rain and associated floods have been shown to contribute directly to elevated levels of fecal contaminants in coastal waters elsewhere.[14] However, in the system studied here, with sustained rain for up to four months, the increase in the *E. coli* load is associated with only the first two months of southwest monsoons, with the concentrations falling off during the subsequent two months of rain (Figure 7b). A plausible inference from the evidence presented here is that enhanced fecal contamination does not emerge with floods: the problem is present well before the floods and is probably associated with land drainage and a rise in groundwater levels and consequent contamination of rivers and groundwater with fecal material. In Vembanad lake, heavy rain could have caused mixing of septic tanks and sewage treatment systems with terrestrial runoff, which in turn could have led to the observed increase in the abundance of *E. coli* in the water in the first phase of the southwest monsoon.

The sewage treatment plan of Kerala is decentralized, wherein every house, residential complex, and commercial building has individual septic tanks.[58] In other words, leaking sewage treatment systems may function as multiple sources of fecal pollution and cause severe problems during heavy rainfall and associated elevation of the water table, especially during southwest monsoon, even in the absence of floods. It has been reported that fecal contamination was also present in nearly 65% of samples collected from rivers and wells after the floods

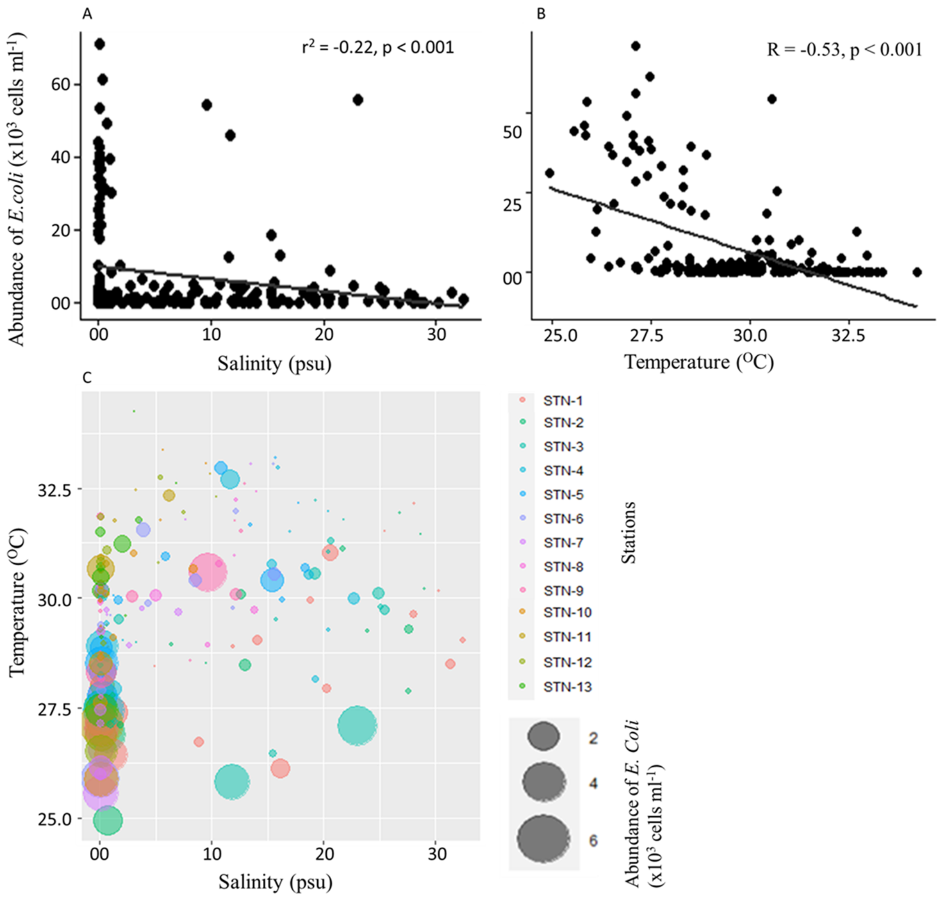

Figure 6. Pairwise correlations of *E. coli* cell abundance with (A) salinity (Spearman correlation, *n* = 205), (B) temperature (Pearson correlation, *n* = 205), and (C) distribution of *E. coli* with respect to variation in salinity and temperature in Vembanad lake (*n* = 205).

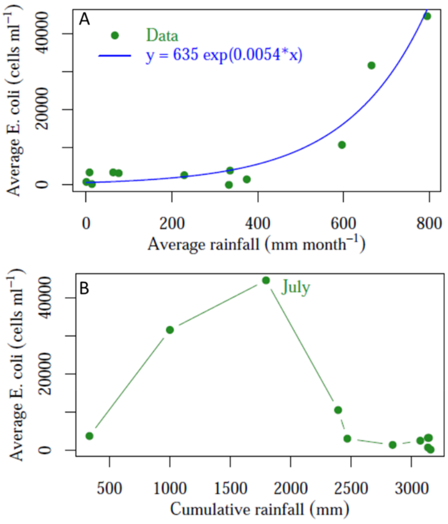

Figure 7. Relationship of *E. coli* with monthly average rainfall (A) and cumulative rainfall (B). Green dots and lines are observations; the blue line in the top panel is the exponential function fitted to the data. The cumulative rainfall data are only plotted for the 11 sequential months (datum for May 2020 not included).

in 2018,[59] but the reduction in the abundance of *E. coli* in August is unexpected. A possible reason could be the increased flushing rate of the lake waters during the southwest monsoon,[60] which could in turn have washed some of the pollutants out into the Arabian Sea by August, especially during the floods, when the inflow of river waters was further increased subsequent to the opening of the sluice gates of multiple dams.

It is possible that the low salinity and low temperature that prevailed in the lake during the southwest monsoon also favored the survival of *E. coli*. It has been reported that high-saline water in estuarine and coastal regions may diminish the growth of *E. coli* through membrane depolarization and altering of the expression of nearly 300 genes, thereby affecting many cellular processes.[61−63] Salinity-induced reductions in *E. coli* were reported in rivers and adjoining coastal waters of Costa Rica, where more than 85% of the samples collected from rivers exceeded the desirable level of fecal contamination (5 colonies mL^{-1}) in recreational waters,[64] while it reduced to less than 50% in samples collected from coastal waters.[9] These arguments run counter to the high abundance of *E. coli* found in Vembanad lake even at high salinity conditions, with the fecal contamination never dropping below detectable limit or even below the desirable level for recreational waters. This could be attributed to a continuous supply of new fecal contaminants through nonpoint sources, such as leaking septic sewage treatment systems.[23,25] Septic tank leakage appears to be a probable source of contamination since Kerala state has been declared to be free of open defecation. A high abundance of fecal contaminants in coastal waters of tropical countries in general, which receive runoff from streams and storm drains, has been reported earlier,[65] indicating the widespread nature of the problem.

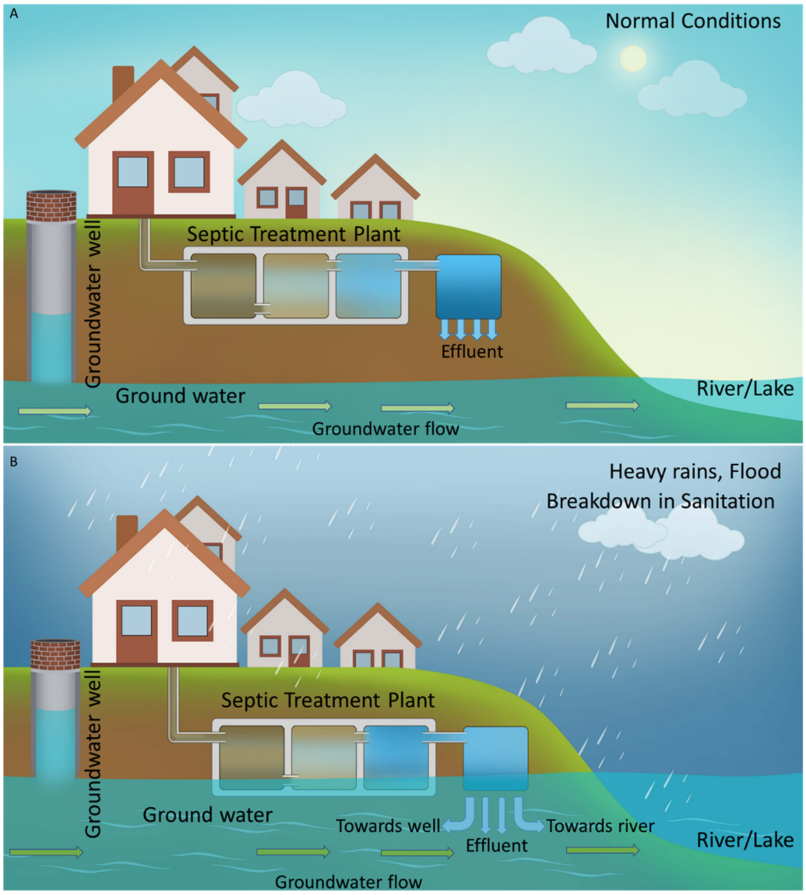

Figure 8. Schematic diagram showing how changes in groundwater and open water levels following heavy rains can lead to contamination of lakes, rivers, and wells. Note that contamination can occur even before there are floods.

3.4. The *E. coli* Pollution Budget of Vembanad Lake.

According to the guidelines of WHO for coastal and fresh water,[66] there is a 10% chance of gastrointestinal illness from exposure to water exceeding *E. coli* abundance of 5 colonies mL^{-1}. *E. coli* counts in Vembanad lake are consistently higher than this threshold, irrespective of season. If we take an average abundance of *E. coli* of all the months of 2018, except for June−September that were affected by the southwest monsoon rains, we get a figure of 3,142 cells mL^{-1}. This is the minimum value that might be expected in the lake, on average. Even this number is much higher than the threshold of 5 colonies of *E. coli* (mL^{-1}) used by WHO to assess risks, by a huge factor of 628. The implication is that the pollution level in Vembanad lake must be reduced by this factor, to bring disease risk to less than 10%.

The first step in achieving this target is to understand the sources of pollution, with the object of eliminating those sources. The data collected in this study allow us to propose a preliminary budget of *E. coli* pollution in Vembanad lake, by partitioning the total pollution load into three components:

1. The basic component associated with the basic survival activities of the people who live on the shores of the lake.
2. The component added to the basic component from the economic activities over the lake, such as tourism and illegal dumping of sewage into the lake.
3. The monsoonal component, which accounts for an additional supply of *E. coli* through land drainage from surrounding areas, which includes leakage from septic tanks when the water table rises, as well as river influx, which drains from an extensive area surrounding the lake.

The mean *E. coli* load during the nonmonsoon months of 3,142 cells mL^{-1} represents the sum of the basic component (1) and the economic activities component (2). In the lockdown period, the *E. coli* abundance in May 2020 dropped to 33 ± 36 cells mL^{-1}, which represents a decrease of 99.8%, relative to the corresponding value in 2018, which was $39 \pm 107 \times 10^2$ cells mL^{-1}. We can accept 33 cells mL^{-1} to be our best estimate of component (1) and 3,109 (= 3,142−33) to be representative of component (2). Finally, component (3) can be estimated as the average of 19,773 cells mL^{-1} during the 4 monsoon months, from which the baseline of the sum of components (1) and (2) is subtracted, yielding 16,631 cells mL^{-1} on average during the monsoon months.

Such basic information on the *E. coli* budget of the lake can be used to outline measures for revival of Vembanad lake to its pristine condition. The evidence presented here points to the fact that even the torrential rains and resultant high flow of water through the lake and out into the Arabian Sea are only able to reduce the pollution load, not eliminate it fully. This implies that the pollution is accumulating faster than it can be flushed out of the system by the circulation pattern of the lake and that, in the absence of remedial measures, the pollution load is likely to accumulate over time. This could explain the high microbial pollution levels in the lake. However, shutting down all economic activities during the lockdown months, which occurred during the nonmonsoon months, did bring down the pollution levels considerably.

However, shutting down all economic activities cannot be the long-term solution to improving water quality. The evidence presented here points instead to the need to render septic tanks resilient to leaks, especially during heavy rains and at times of an increasing water table: the lake contamination peaks prior to the occurrence of any floods. These requirements are over and above that of maintaining the open defecation-free status of the entire drainage area of the lake. Furthermore, dumping of waste in the lake must be curtailed stringently, whether it be from houses or from industries.

3.5. Relationship between *E. coli* Abundance and ADD Cases. Though we were unable to establish significant relationships between the number of cases of ADD and hydrological conditions in the lake, a Spearman correlation analysis showed that the anomalies in the incidence of ADD have a significant correlation with *E. coli* abundance in the lake ($r = 0.58$, $p < 0.05$) (Suppl. Figure 2). An increase in the number of cases of ADD during the southwest monsoon and its reduction during lockdown (May 2020) indicate a potential connection between fecal contamination in the Vembanad Lake and the occurrences of ADD. Women and children interact frequently and closely with waterbodies for washing clothes and recreation, respectively. Hence, the chances of acquiring water-associated disease through contact with contaminated water are high among them. Upsurges in fecal contamination and subsequent incidence of water-associated diseases are reported from coastal regions across the globe because of natural disasters, such as storm surges and floods.[67−73] The abundance of *E. coli* in Vembanad lake decreased after August, though never below detection limits. There was a corresponding decrease in ADD cases. Continuous monitoring of water quality of inland and coastal waters using *in situ* measurements and remote sensing and preparation of risk maps indicating contaminated areas would motivate the public to practice environmental hygiene, and citizen science can be an effective mode of collecting data and also of societal engagement.[2,3,7] We also recognize the importance of combining observations with hydrological and hydrodynamic models that deal with transport mechanisms and flushing rates of the lake[60] to understand the movement of pathogenic bacteria in the water. Ideally, such models would account for the growth and decay dynamics of the different types of bacteria under the altered environmental conditions.

Our inference on the impact of nonpoint sources of fecal contamination on the water quality of the lake is supported by the much-reduced number of fecal contaminants and disease incidence during the COVID-19 pandemic, when the public was encouraged to follow the WaSH protocol. During lockdown, many activities over the lake, such as recreation and transportation, were stopped, and illegal dumping of sewage became difficult. Furthermore, many nonpoint sources of fecal contamination, such as markets, house boats, and public places, were nonfunctional during lock down, contributing to the reduced number of incidences of ADD in May 2020. Studies have also reported improvement in air and water quality after lockdown.[27,28,74] Our observations toward the end of the COVID-19 lockdown period provided evidence that, at least during the dry season and when the socioeconomic activities in and around the lake are suspended, there is a considerable decrease in the *E. coli* pollution levels in the lake. Our results point to the need for better sewage treatment systems for preventing the mixing of septic sewage with the aquatic ecosystem during storm surges and floods. This requirement is even more acute in low-lying tropical areas under threat of increasing sea level and more frequent and higher-intensity storms related to climate change.

4. CONCLUSION

We conducted a year-long study in a tropical lake that served to understand the influence of the southwest monsoon, the century's largest flood in 2018, and the COVID-19 related lockdown in 2020 on the distribution of *E. coli* in Vembanad lake as well as its relation to the incidence of acute diarrheal disease (ADD). We found that rainfall has a strong influence on the distribution of *E. coli* in Vembanad lake and that the presence of these fecal contaminants is associated with low salinity and low temperature. Multiple antibiotic resistance of the *E. coli* isolates also confirmed an anthropogenic influence on the fecal contamination of the lake and highlighted the importance of eliminating it. The anomalies in the incidence of ADD showed a strong correlation with the abundance of *E. coli* in Vembanad lake. Our observations have helped to put in place the first, broad-brush budget of the sources of *E. coli* pollution in the lake, but refining the budget and tracking changes call for continuous monitoring of the water bodies for a longer time period, using both *in situ* and remote sensing observations. This study, together with previous findings from this lake and from elsewhere, points to the need for revamping the sewage treatment engineering and city planning to avoid the mixing of septic sewage with natural waters during extreme climate events such as heavy rains and floods or even during the natural monsoon cycles of heavy rains. As our understanding of the ecosystem becomes better, we can improve the risk maps[2] that can be produced to alert the local population against the threats from waterborne diseases and to help plan remedial actions. As we work toward such a holistic solution, the importance of personal hygiene cannot be overstated, as an interim measure to minimize health risks from contaminated water. Our study has revealed a complex and integrated path that has to be followed to achieve the global target[75] of eliminating the transmission of water associated disease by 2030.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsestwater.2c00255.

> Supplementary Figure 1: Spatiotemporal variation in the distribution of *E. coli* in the water column of Vembanad lake; Supplementary Figure 2: Spearman's rank correlation showing the correlation of monthly average abundance of *E. coli* in the water column of Vembanad lake with anomalies in the incidence of ADD (PDF)

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Funding

This work was implemented with the financial support of the Department of Science and Technology, India (DST/TM/INDO-UK/2K17/64 (C)) and Natural Environmental Research Council (UK) under India-UK water quality research program for the project REVIVAL. Additional support from the National Centre for Earth Observation of the UK and European Space Agency (ESA) under the WIDGEON are gratefully acknowledged.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We acknowledge the Directors of CSIR-NIO, ICAR-CMFRI, NERCI, and PML and Scientist-in-Charge of CSIR-NIO RCK for their support and encouragement.

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