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Fecal microbial pollution in river and coastal environments: Influence of weather conditions

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ABSTRACT

Coastal systems are under increasing threat from human activity and climate-driven disturbances, yet the specific impact of different weather conditions on microbial contamination remains insufficiently resolved. It was hypothesized that precipitation regimes, intensified by climate change, act as primary drivers of fecal microbial pollution and nutrient mobilization in riverine and coastal systems. To test this hypothesis, the Arzilla river (located in the western Adriatic Sea, Italy) and its adjacent coastal waters were investigated using an event-based spatial sampling design. Nutrients and total suspended matter were analysed, and the occurrence of *Escherichia coli* and intestinal enterococci were examined under contrasting weather conditions (ranging from drought to heavy rainfall). Despite its small size, the Arzilla watershed represents a model of a Mediterranean system where geomorphological, hydrological, and anthropogenic factors converge, making it representative for ecological and management considerations at a larger scale. Rainfall intensity has been demonstrated to exert a significant influence on the expression of microbial responses. An extreme precipitation event with levels in excess of 100 mm, resulted in elevated microbial loads exceeding regulatory thresholds at both river and marine stations. The range of microbial loads at the river stations was from 20 to >30,000 CFU/100 mL, while at the marine stations it was from <10 to >140,000 CFU/100 mL. These findings underscore, the vulnerability of coastal waters to climate change-related disturbances. An increase of nutrients and TSM concentrations during periods of wet conditions was observed, indicating an enhancement in runoff-driven transport. Enterococci exhibited greater persistence and delayed peaks compared to *E. coli*. The findings of this study provide mechanistic insight into the short-term dynamics linking precipitation regimes, microbial pollution, and nutrient mobilization at the land–sea interface. The findings emphasize the vulnerability of small Mediterranean catchments to climate-driven disturbances and underscore the need to integrate adaptive, event-based monitoring methodologies into European management frameworks.

1. Introduction

Coastal environments are increasingly subject to pressures that threaten their health and ecological balance (Cabral et al., 2019). These pressures arise as a consequence of both direct and indirect human activities, exerting significant impacts on marine ecosystems and water resources (Ansari and Matondkar, 2014). The main contributors to this phenomenon are urban discharges, overflow events that release untreated wastewater (Al Aukidy and Verlicchi, 2017), and diffuse inputs from industrial activities and landfills. These sources interact with hydrological processes influencing contaminant transport.

Urban runoff carries oils, metals, and pesticides, while agricultural activities introduce fertilizers and agrochemicals that degrade water quality and promote eutrophication (Eregno et al., 2016; Xu et al., 2019). Physical alterations to watercourses and the presence of illegal landfills have been demonstrated to further reduce ecosystem resilience

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(Sabater and Tockner, 2010).

These cumulative pressures are increasingly affecting water quality, including microbial contamination dynamics, thus highlighting the need for adaptive and targeted management policies (Bedri et al., 2016).

Microbial communities are sensitive indicators of environmental stress, pollution, and ecosystem dynamics (Ma et al., 2022). Their rapid responses to environmental change and sensitivity to contaminants make them ideal bioindicators (Nogales et al., 2011).

However, when assessing water quality for regulatory and public health purposes, fecal indicator bacteria (FIB) such as *Escherichia coli* and intestinal enterococci remain essential, as they provide direct evidence of fecal contamination and associated risks.

Rivers represent major pathways for the transport of contaminants from various sources, including agricultural, industry and urban areas, to coastal environments. Enteric pathogens originating from human and animal waste persist in these aquatic systems, with hydrological conditions influencing their movement downstream into coastal waters (Luna et al., 2019; Manini et al., 2022). Understanding interactions between pollution contamination sources and hydrological pathways is therefore crucial (Alegbeleye and Sant'Ana, 2020).

European regulatory frameworks including the MSFD, WFD and the Nitrates Directive provide robust guidelines for environmental protection. However, these models not fully account for the growing influence of climate change and extreme meteorological events (Lehmann et al., 2015; Rummukainen, 2012). The exacerbating frequency of extreme events, such as torrential rainfall, coastal storms, and flooding, has been well documented. The events are a direct consequence of climate change, and have been shown to exacerbate existing pollution issues (He et al., 2023).

Extreme rainfall events, in particular, have a significant impact on microbial water quality. High-intensity precipitation has been demonstrated to increase surface runoff, mobilise fecal material from both agricultural and urban areas, resuspend contaminated sediments, and rapidly transport pathogens into rivers and coastal zones (Valle-Levinson et al., 2020; Cho et al., 2022). Such events have been shown to overwhelm combined sewer systems and trigger CSO discharges (Brown and Peake, 2006). These mechanisms generate sharp, short-term peaks in FIB concentrations, often exceeding regulatory thresholds within hours, thereby challenging monitoring frameworks that are designed around routine rather than event-driven conditions.

Small Mediterranean basins are especially vulnerable to the consequences of flash-flooding and intense seasonal rainfall patterns. Recent studies have highlighted the vulnerability of these systems to rapid microbial spikes following storm events (Pérez-Gutiérrez et al., 2020; Campisano et al., 2021). However empirical event-based assessments remain limited, leaving key mechanisms insufficiently understood.

In this study, an event-focused approach is adopted to assess how rainfall and drought influence microbial contamination and nutrient dynamics in the Arzilla stream and adjacent coastal waters. Despite its small size, the Arzilla catchment represents a model Mediterranean system where geomorphological, hydrological, and anthropogenic factors converge, making it highly representative for ecological and management considerations at a broader scale. The study aims to quantify short-term environmental responses to contrasting meteorological conditions, providing evidence to address current scientific and regulatory gaps. Notably, this study does not assess health outcomes such as diarrhoeal or skin infections; instead, it focuses on the environmental dynamics and potential release of enteric pathogens under varying weather conditions.

2. Material and methods

2.1. Study area

The study area is located along the Arzilla stream and in the bathing waters off the coast of Fano, a densely urbanized town (north-western Adriatic Sea, Italy; Fig. 1). The Arzilla stream has a torrential flow regime and has been identified as a Site of Community Importance (SCI) with the code IT5310008. Its watershed covers approximately 110 km^2. Its longitudinal profile exhibits various irregularities due to the influence of the geological substrate on its hydrography. The flooding that affects the areas near the mouth of the Arzilla stream and some upstream zones is attributed to the reduced capacity of the channel to manage the water flow during intense weather events (Ferrarin et al., 2021). Although past interventions, such as channel widening and the construction of artificial levees designed to manage a flow rate of 400 m^3/s, were carried out to improve flood protection, these measures are now insufficient to ensure the safety of surrounding areas due to sediment accumulation and the relative lowering of the banks.

The mouth of the Arzilla is located near a very popular beach, where bathing is prohibited during significant discharge events due to the risk of bacterial contamination from sewage (Penna et al., 2021). Coastal water circulation in this area is mainly influenced by tidal forces, with a tidal range of about 40 cm, along with winds and southward longshore transport (Marini et al., 2015; Orlic et al., 1992).

2.2. Sampling strategy

The sampling design adopted in this study was developed on the basis of previous sampling activities carried out in the Arzilla River and its coastal zone (WATERCARE project 2019–2021; funded by the European Union under the Interreg Italy–Croatia CBC Programme). The related datasets, published in Manini et al. (2022) and Penna et al. (2023), provided a solid understanding of fecal contamination processes in the area. Building on this consolidated knowledge, the 2024 targeted sampling campaign was structured as an event-based spatial survey, focusing on different rainfall intensities in order to refine the understanding of short-term fecal contamination dynamics under representative meteorological conditions. An event-based spatial approach was chosen because dilution effects and post-event decay had already been investigated in previous high-frequency studies, which included 6-h interval sampling campaigns in the Arzilla watershed.

Sampling was carried out during the bathing season, from May to September 2024. The eight sampling events were not concentrated within a single month but were distributed across the entire period, each corresponding to a distinct meteorological condition recorded in real time at the two rainfall monitoring stations (St. SMA and St. PT1). This ensured that each event captured a unique hydrological scenario rather than repeated measurements under similar conditions.

Weather conditions were classified using rainfall ranges defined for the purposes of this study, in order to better characterize hydrological and microbial responses to precipitation events. These included drought (0 mm), light rain (0.1–5 mm), moderate rain (5–10 mm), heavy rain (10–5 mm) and extreme rainfall (>50 mm within 24 h).

To assess the water quality of the Arzilla River, its coastal receiving waters, samples were collected at five stations along the river and at three marine stations located along an offshore transect (50, 100 and 150 m from the shoreline; Fig. 1). The river stations (St. PT3, St. SMA; St. PT2; St. MT; St. PT1) were selected to capture the influence of different pollution sources and land-use conditions along the catchment (Fig. 2).

The PT3 station (43° 49′ 41.84″ N, 12° 50′ 32.10″ E) is located in the upper course of the river, in an area characterised by several small industrial activities (e.g., an oil mill, a furniture company, a frozen-food distributor), which may contribute to localized runoff and waste inputs. The SMA station (43° 50′ 23.35″ N, 12°53′40.34″ E) is located upstream of the wastewater treatment plant, about 20 km from the source and downstream of the wastewater treatment plant, in a narrow and dynamic floodplain with a gravel and silt substrate. The PT2 station (43° 49′ 9.16″ N, 12° 53′ 18.96″ E) receives water from a secondary stream influenced by an agro-livestock facility that operates a biogas treatment system. The MT station (43° 51′ 8.28″ N, 13° 0′ 6.98″ E) is located upstream of the spillway, enabling of water quality before overflow events

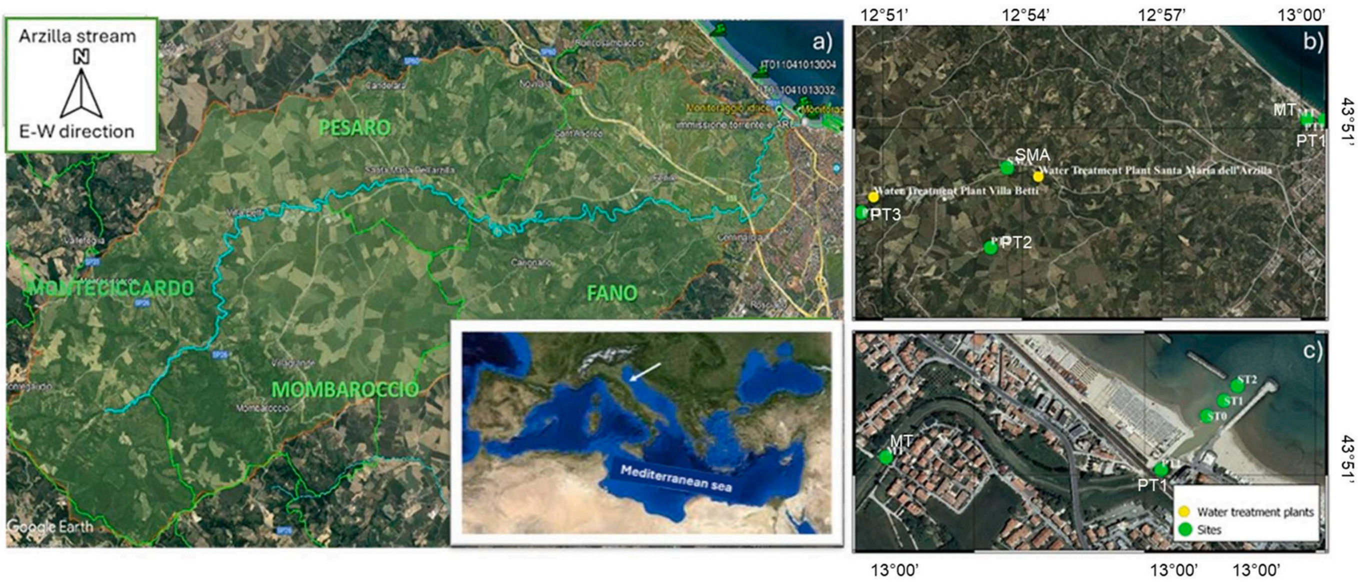

Fig. 1. Study area with an image of the watershed (a) and the sampling strategy, which includes five sampling stations positioned along the river: St. PT3, St. SMA; St. PT2; St. MT; St. PT1 (b) and three stations in the sea in front of the river mouth, (green dots) distributed along a transect at increasing distances from the shore (c). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

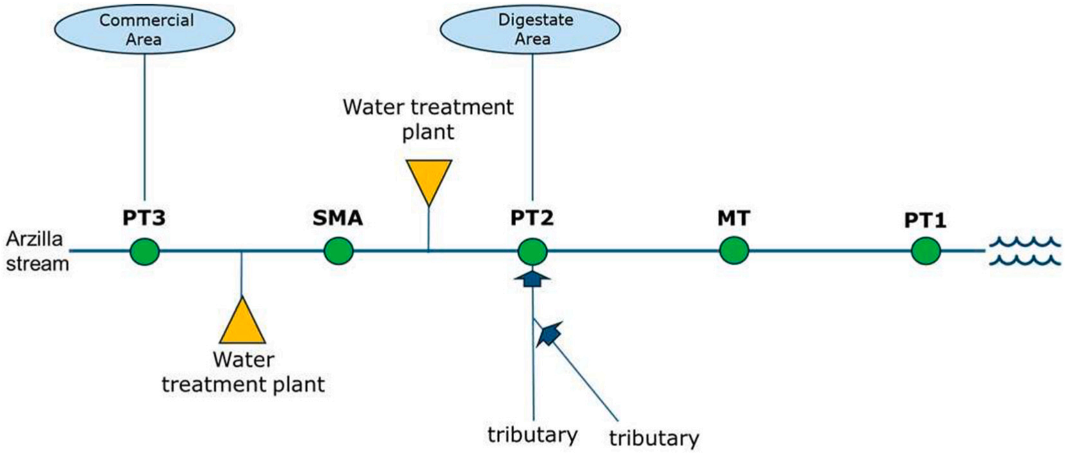

Fig. 2. Conceptual diagram of the Arzilla stream. The figure shows the anthropogenic inputs that influence its characteristics and environmental impacts along the five sampling stations.

occurring at St. PT1. The PT1 station (43° 51′ 7.52″ N, 13° 0′ 30.20″ E) is positioned at the river mouth to characterize the final freshwater outflow entering the coastal area.

Marine sampling was conducted at three stations: St.0 (43° 51′ 10.78″ N, 13° 00′ 34.07″ E; 70 cm depth), St.1 (43° 51′ 11.71″ N, 13° 00′ 35.49″ E; 120 cm depth), and St.2 (43° 51′ 12.61″ N, 13° 00′ 36.63″ E; 200 cm depth). These stations form a transect perpendicular to the shoreline and were selected to capture the dilution gradient and dispersion of river borne contaminants in the coastal bathing area.

2.3. Rainfall measurements and physico-chemical parameters

Rainfall data are collected by two automatic weather stations: one located at St. SMA, where the Civil Protection has installed a rain gauge at Santa Maria dell' Arzilla to monitor precipitation in the area, and one at St. PT1, equipped with a Campbell Scientific CLIMA VUE50 rain gauge.

This data (in mm) is collected in real-time and transmitted to a central system for analysis. Rainfall measurements are essential for assessing local weather conditions and their impact on river levels and water quality. An ultrasonic river-level sensor (Siemens SITRANS LU240) was installed at the river mouth and is used to calculate the flow rate of the river, measured in cubic meters per second (m^3/s).

At St. PT1 and at the three marine stations, temperature, salinity, dissolved oxygen, turbidity, pH, and redox were measured using a CTD probe as described by Penna et al. (2021) to evaluate fresh-seawater interaction. The data related to these physico-chemical parameters are reported in the Supplementary Materials (*see Table S1 in the Supplementary Material*).

2.4. Microbiological analysis

Water samples collected for fecal indicator bacteria analysis were placed in sterile bottles and transported to the laboratory as quickly as possible using portable refrigerators. Fecal Indicator Bacteria (FIB) are used to assess fecal contamination in water and estimate the risk of the presence of fecal-origin pathogens. The most important FIB are *Escherichia coli* (*E. coli*) and intestinal enterococci. These indicators are used in international regulations (e.g., the European Directive 2006/7/EC for bathing waters and EPA guidelines in the USA) to monitor water quality and protect public health. The abundance of *Escherichia coli* was determined using the membrane filtration method, adapted from Luna et al. (2019). Briefly, water samples from each station were serially diluted (1, 10, and 100 mL) and vacuum-filtered through 0.22 μm pore size, 47 mm diameter membrane filters (Millipore). The filters were then aseptically transferred onto Chromogenic Coliform Agar (CCA) plates and incubated at 36 ± 2C for 18–24 h. Only blue colonies were counted, and the results were expressed as colony forming units (CFU) per 100 mL of water (CFU 100 mL^{-1}). *E. coli* colonies were subjected to a purification and isolation process. Individual colonies from the CCA plates were streaked onto selective enrichment broth (Tryptic Soy Broth, Liofilchem Inc.) and subsequently streaked onto new fresh CCA agar plate through successive rounds of subculturing until a pure, isolated colony was obtained. The identity of *Escherichia coli* isolates was confirmed by Polymerase Chain Reaction (PCR) targeting the specific 623 bp fragment of the *uidA* gene (modified from McDaniels et al., 1996) followed by agarose gel electrophoresis to visualize amplification products. Similarly, enterococci abundance was determined by the membrane filtration method. Water samples were filtered in triplicate, using sample volumes ranging from 1 to 100 mL, following the same procedure as described for *Escherichia coli*. The filters were then aseptically transferred onto Slanetz-Bartley agar plates and incubated at 36 ± 2 °C for 48 ± 4 h. Colonies exhibiting a red or reddish-brown colour were considered presumptive enterococci and enumerated. Results were expressed as colony forming units (CFU) per 100 mL of water.

2.5. Nutrient and total suspended material analysis

Total suspended matter (TSM), calculated as the sum of particulate organic matter (POM) and particulate inorganic matter (PIM), and dissolved inorganic nutrient analysis (nitrite, nitrate, ammonia and inorganic phosphate) were measured. Water samples for the determination of dissolved inorganic nutrients were collected in dark bottles, immediately transferred to the laboratory, filtered through a 0.45 μm nitrocellulose membrane (Millipore 45 mm diameter), and stored at −20 °C in polyethylene bottles until analysis. Water samples for TSM (total suspended matter) were passed through pre-combusted and preweighed GF/F Whatman (0.7 μm) and Millipore nitrocellulose (0.45 μm) filters, respectively, and analysed immediately.

Nutrient concentrations were quantified using a UV-1700 Shimadzu model as described in (Strickland and Parsons, 1972). Total nitrogen (TN) and total phosphorus (TP) were measured in unfiltered water samples following the methodology of Valderrama (1981). Measurement accuracy was ±0.02 μmol L^{-1} for $N\text{-}NH_3$, $N\text{-}NO_2$, $N\text{-}NO_3$, TN, and TP. A five-point calibration curve was established using Merck certified reference standards. Method accuracy was subsequently verified by analyzing the standard. Accuracy was assessed on ten standard replicates, yielding: ± 0.001 μmol L^{-1} ($N\text{-}NH_3$), ± 0.006 μmol L^{-1} ($N\text{-}NO_2$), ± 0.005 μmol L^{-1} ($N\text{-}NO_3$). TSM concentrations were determined gravimetrically by filtering a known water volume through 0.7 μm GF/F membrane filters (Millipore, Bedford, MA, USA) according to Alpha Analytical Quality. Total suspended solids were dried at 103–105 °C and total volatile suspended solids were dried at 500 °C (APHA-AWWA-WPCF, 24th edition). The accuracy, evaluated on five replicates, had a coefficient of variation (CV) below 5 %. For PIM (particulate inorganic matter) and POM (particulate organic matter) analysis, the GF/F filters were subsequently combusted at 500 °C for 1 h following the APHA APHA-AWWA-WPCF (24th edition). POM values were obtained by subtracting PIM from TSM.

2.6. Statistical analysis

Statistical analyses were performed using R software (version 4.5.0 for Windows; R Core Team, 2015) [https://www.R-project.org/]. Relationships between numerical variables were assessed using Kendall's tau correlation coefficient (Hollander et al., 2014), a nonparametric method well-suited for small sample sizes and non-normal data distributions (Uyanto, 2022). To evaluate the importance of predictor variables influencing fecal indicator bacteria counts, we employed a Random Forest (RF) model (Breiman, 2001). RF was chosen for its robustness and flexibility in handling complex interactions and nonlinearities (Tyralis et al., 2019). The model was primarily used for inference, focusing on variable importance ranking and partial dependence analyses. Hyperparameters ntree and mtry were optimized using the tuneRF function from the randomForest package prior to model fitting. Differences in fecal indicator bacteria levels between river and marine stations were tested using the Wilcoxon rank-sum test (*see Table S3 in the Supplementary Material*), a nonparametric alternative to the *t*-test suitable for non-normal data (Hollander et al., 2014). Statistical significance was set at $p < 0.05$ for all tests.

3. Results

3.1. Fecal indicator bacteria

Microbial responses were analysed for each event and at each sampling station (*see Table S2 in the Supplementary Material*). River stations (PT3, SMA, PT2, MT and PT1) demonstrated higher sensitivity to precipitation, showing significant increases even after moderate rainfall. By contrast, marine stations (St.0, St.1 and St.2) exhibited a delayed and generally weaker response (Fig. 3). Under dry conditions or light rainfall (up to 5 mm), the average *Escherichia coli* concentration at river stations was generally below 300 CFU/100 mL. However, during Event 1, despite the absence of rainfall, the concentrations at stations PT3 and MT were above 600 CFU/100 mL. Furthermore, station MT exhibited values approaching 800 CFU/100 mL during Event 6, despite the continued absence of rainfall. During moderate rainfall events (between 5 mm and 10 mm), *E. coli* concentrations exceeded 1000 CFU/100 mL at all stations, except for Event 2. Apart from station SMA, which recorded high values of 9700 CFU/100 mL, the other stations showed concentrations below 600 CFU/100 mL. Event 8 was an extreme case, characterised by flooding and prolonged, widespread rainfall (198 mm) across the watershed. The microbiological impact was immediate and severe: all riverine stations exceeded 5000 CFU/100 mL of *E. coli,* with some measurements overcoming the upper detection limit. In general, *E. coli* values remained below 200 CFU/100 mL at marine stations during all Events (except for Event 8), both in the absence of rainfall and during periods of heavy precipitation (up to 54 mm). Only a significant increase was observed during Event 8, with average concentrations of around 120,600 CFU/100 mL. However, no significant differences were observed along the coast-to-offshore transect (from station 0 to station 2) during any of the sampling periods (Events 3–8). The results for intestinal enterococci did not follow the same trends as those observed for *E. coli*. The bacterial response appeared to be more delayed, providing a snapshot of the situation after the event (Manini et al., 2022).

Overall, enterococci values were low during periods of minimal rainfall, not exceeding 300 colony-forming units (CFU/100 mL), with a few exceptions like those seen for *E. coli.* For example, during Event 1, stations PT3 and SMA recorded 1935 and 410 CFU/100 mL, respectively. In Event 6, Station MT recorded 612 CFU/100 mL. No consistent trend was observed along the upstream-to-mouth gradient during more intense rainfall events, with values ranging from 39 CFU/100 mL at station PT1 (river mouth) to 9400 CFU/100 mL at station PT3 (Event 6). Very high concentrations were recorded during heavy rainfall, with levels reaching 48,500 CFU/100 mL at the PT1 station. Along the coastal transect in front of the river mouth, enterococci levels were higher than *E. coli* levels during low-rainfall events. Enterococci values were approximately three times higher in Event 3, twice as high in Event 4 and around four times higher in Event 5 (with rainfall amounts of 3.5 mm, 0.9 mm and 1 mm respectively). During Event 6, when there was no

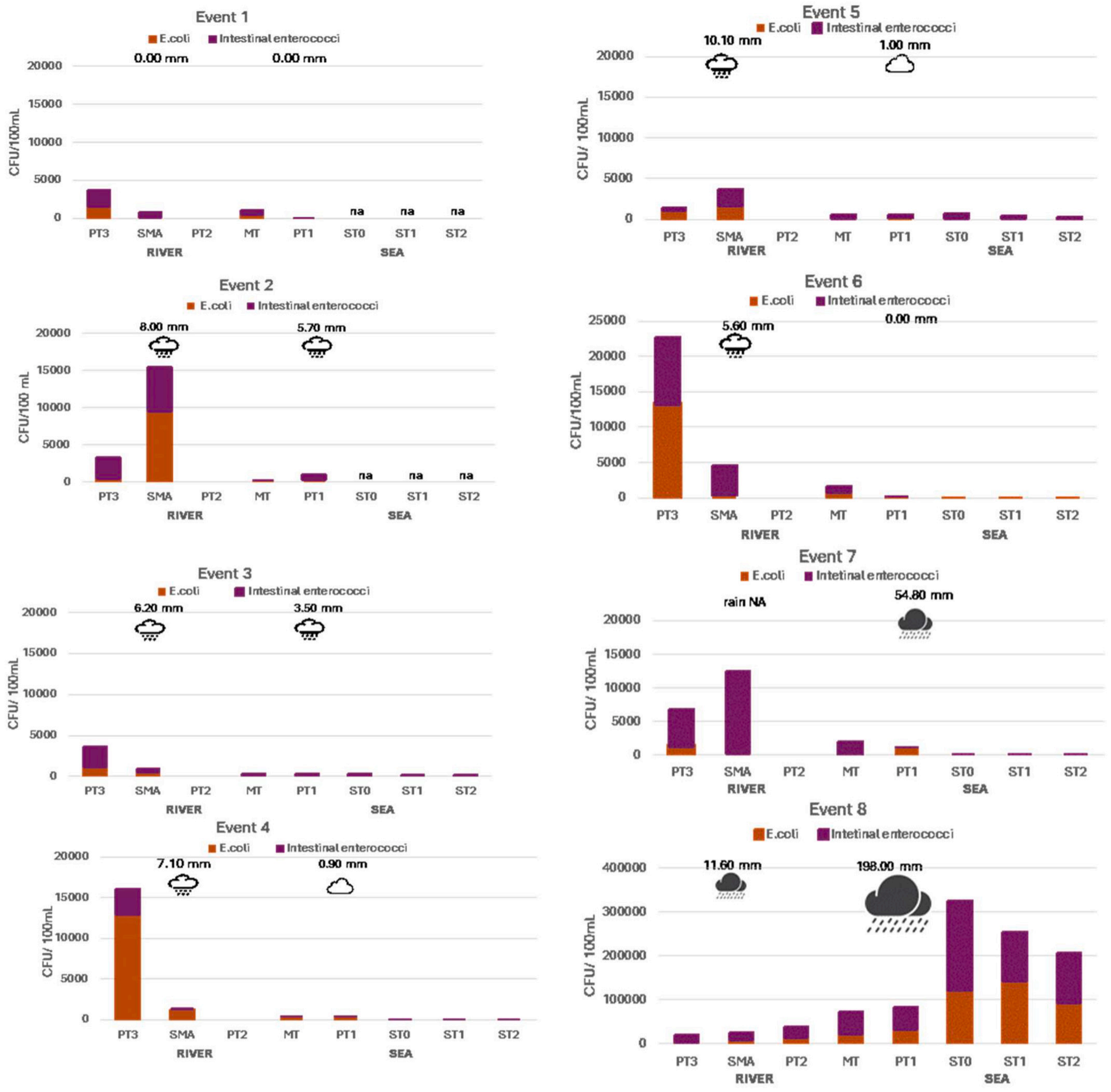

Fig. 3. Trend of fecal microbial contamination (*E. coli* and intestinal enterococci) along the river basin and the adjacent coastal area under different weather conditions. The icons on the graph indicate precipitation levels, classified for better understanding as follows: light rain (from 0.1 to 5 mm), moderate rain (from 5 mm to 10 mm), heavy rain (from 10 mm to 15 mm), extreme rainfall (>50 mm), and drought (0 mm), recorded at the two rain monitoring stations (St. SMA and St. PT1). The data are based on 8 sampling campaigns carried out from May to September 2024.

rainfall, no enterococci were detected at any of the sampled stations. During Event 7, despite intense rainfall (54 mm), enterococci concentrations were very low, averaging 7.3 CFU/100 mL. Event 8 was characterised by extreme rainfall and average enterococci concentrations exceeded 100,000 CFU/100 mL at all three coastal stations.

3.2. *Nutrients and suspended material*

The spatial and temporal variability of nitrogen (N-TOT, N–NO_3, N–NO_2, N–NH_3), phosphorus (P-TOT and P-PO_4), and total suspended matter (TSM, PIM and POM), was analysed at the Arzilla river sampling stations (PT3, SMA, PT2, MT, PT1) and the seawater stations (St.0, St.1, St.2) during dry conditions (Event 1) and under different rainfall intensities (Events 2–8) (Fig. 4; *see Tables S1 and S2 in the Supplementary Material*). N-TOT and N–NO_3 were the predominant nitrogen across all stations and Events. In river waters, N-TOT ranged from 55.09 to 2848.7 μM, whereas seawater concentrations ranged from 20.75 to 214.16 μM. Similarly, N–NO_3 concentrations ranged between 74.9 and 217.58 μM in the river and 1.23 and 70.07 μM in the seawater. Ammonia concentration ranged from 0.39 to 41.67 μM in the river and 0.24 to 14.41 μM in the seawater. River P-TOT ranged from 0.42 to 0.92 μM, while seawater values ranged between 11.07 and 23.9 μM. P-PO4 concentrations were

Fig. 4. Concentrations of dissolved inorganic nutrients: nitrite (NO_2^-), nitrate (NO_3^-), ammonia (NH_3), and inorganic phosphate (PO_4^{3-}), measured in the river basin and adjacent coastal area during eight sampling events conducted under different weather conditions.

between 0.01 and 0.21 µM in the river and from 0.37 to 8.27 µM in the seawater. During the dry period (Event 1), the lowest of N-TOT and N-NO$_3$ values were found at station PT3 (89.8 and 74.9 µM, respectively), with relatively consistent higher values observed throughout the river (mean 251.3 ± 11.78 µM for N-TOT and 223.54 ± 7.1 µM forN-NO$_3$). Slightly lower values of N-TOT were observed at the MT station. During moderate rainfall events of 5 and 10 mm (Events 2–6), N-TOT and N-NO$_3$ values remained lowest atPT3 (mean 84.79 ± 18.2 µM and 33.82 ± 28.05 µM, respectively), highest at PT1 (mean 247.18 ± 62.22 µM and 169.78 ± 59.37 µM, respectively), and intermediate at SMA (mean 177.61 ± 28.05 µM and 122.79 ± 21.55 µM, respectively). No significative differences in N and P nutrients were found between dry and moderate rainfall conditions. Heavy rainfall Events (>10 mm; Events 7 and 8) showed a similar pattern with lowest values of N-TOT and N-NO$_3$ at PT3 (mean 223.94 ± 131.65 µM and 143.71 ± 142.34 µM, respectively). Higher concentrations were observed at PT1 during Event 7 (277.37 µM and 193.83 µM, respectively) and PT2 during Event 8 (2848.75 µM and 326.41 µM, respectively). SMA showed intermediate values for both Events (mean 648.54 ± 639 μM and 242.5 ± 147.54 μM, respectively). Statistical analysis indicated no significative nutrient differences between moderate and heavy rainfall events overall, except for N-TOT between Events 3 and 8 ($p < 0.05$) and $N\text{-}NH_3$ between Events 6 and 8 ($p < 0.01$). Similarly, no significative differences were observed between dry and heavy rainfall events for most nutrients. In seawater, the lowest values occurred during Event 7 (29.16 ± 10.92 μM), despite heavy rainfall during Event 7 (54.8 mm). Higher N-TOT values appeared during moderate and heavy rainfall Events 5 and 8, respectively (mean 169.04 ± 25.26 μM). Intermediate values were registered during moderate rainfall Events 3 and 4 (mean 45.87 ± 21.7 μM). $N\text{-}NO_3$ was lowest during Events 5–7 (mean 5.93 ± 3.25 μM) with the highest during Event 8 (69 ± 1.82 μM). Intermediate values were found during moderate rainfall Events 3 and 4 (mean 45.87 μM ± 21.7). Nutrient concentrations generally decreased along the coastal transect, except for Event 3. Dry conditions were found in seawater in Event 6. No significant

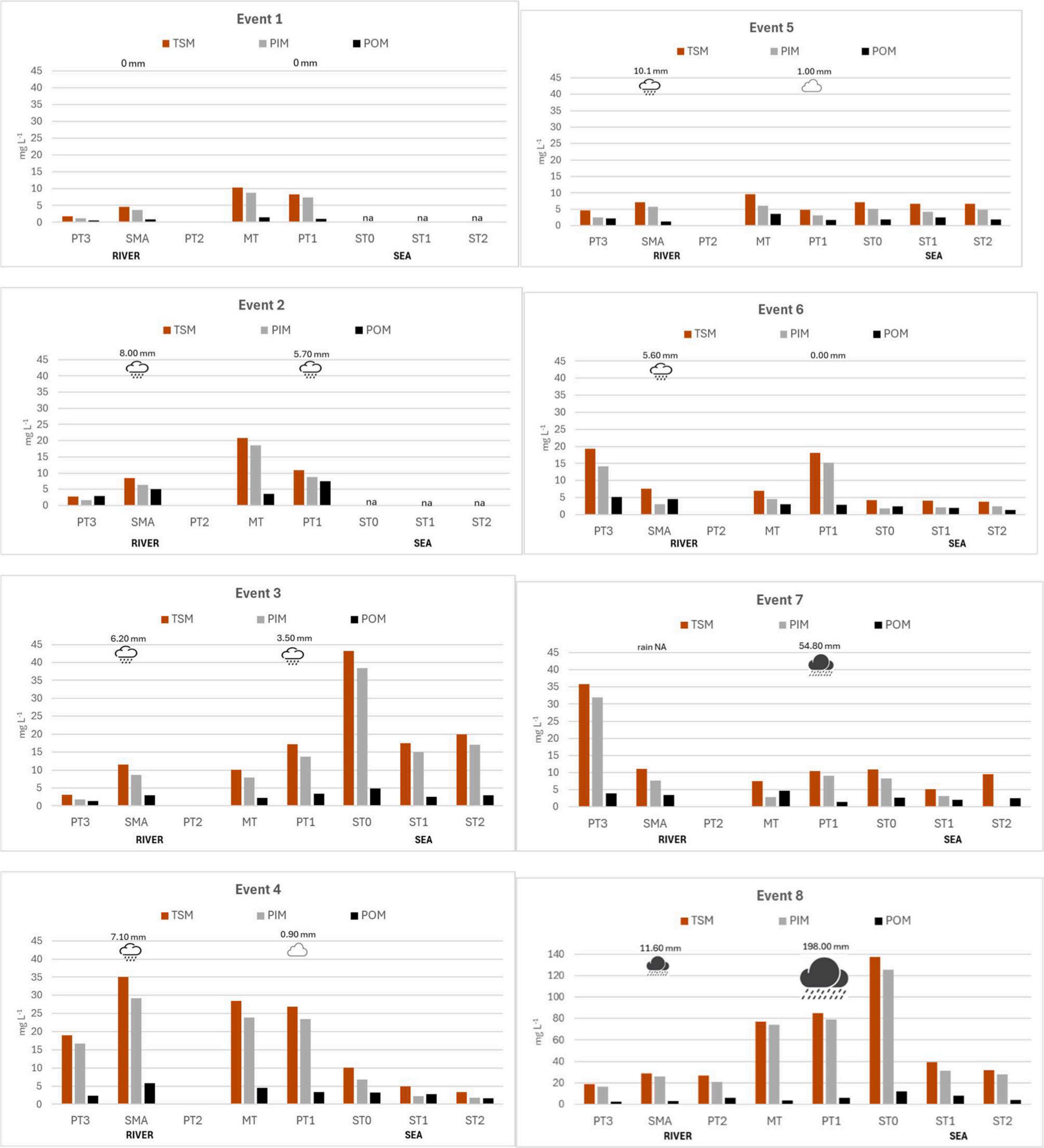

Fig. 5. Total suspended material (TSM), total particulate organic matter (POM) and particulate inorganic matter (PIM) in the river basin and in the adjacent coastal area under different weather conditions during the eight events.

nutrient differences were detected along the coastal transect between dry (Event 6) and moderate rainfall events (Events 3–5), except for N-TOT in Event 5 ($p < 0.01$)or t between moderate and heavy rainfall events, except for significative differences in N-TOT, N-NO$_3$ N-NH$_3$, P-PO$_4$ and P-TOT across some events ($p < 0.01$).

TSM concentrations ranged from 6.23 to 47.32 mg/L (mean 16.87 ± 13.88 mg/L) in river and 4.08 to 69.44 mg/L (mean 20.33 ± 25.46 mg/L) in seawater (Fig. 5). POM values ranged between 4.36 and 43.29 mg/L (mean 14.41 ± 13.08 mg/L) in river and 2.17 to 61.42 mg/L (mean 16.92 ± 23.16 mg/L) in seawater. PIM values ranged from 0.97 to 4.02 mg/L (mean 2.84 ± 1.13 mg/L) in river and 1.92 to 8.02 mg/L (mean 3.41 ± 2.32 mg/L) in seawater. The percentage contribution of POM to TSM was high, ranging from 66.43 % to 91.49 % (mean 79.63 ± 8.13 %) in river and from 53.06 % to 88.45 % (mean 71.4 ± 14.46 %) in seawater. The contribution percentage of POM to TSM was found to range from 8.51 to 33.57 % (mean 20.37 ± 9.17 %) in river and from 11.55 to 46.93 % (mean 28.6 ± 14.46 %) in seawater. During dry conditions (Event 1), the lowest TSM PIM and POM values were recorded at PT3 (1.77, 1.23 and 1.54 mg/L, respectively), increasing toward MT station (1031, 8.85 and 1.46 mg/L, respectively), then slightly decreasing toward PT1. Moderate rainfall events (Events 2–6) showed similar spatial patterns with elevated values at MT at MT station (e.g., 2 20.78, 18.5 and 2.28 mg/L) and A at SMA station, during Event 3 and 4 (Event 4: 35.00, 29.14 and 5.86 mg/L). Event 6, saw higher values at PT3 and PT1 stations (mean: 18.77 ± 0.89, 14.71 ± 0.72 and 4.06 ± 1.61 mg/L. No significant differences in TSM, PIM and POM were detected between dry and moderate rainfall events, except for TSM between Events 1 and 4 ($p < 0.05$) and POM between Events 1 and 6 ($p < 0.01$). During heavy rainfall events (Events 7 and 8) spatial trends of TSM, PIM and POM varied. Event 7 had higher values at PT3 (35.85, 31.98 and 3.87 mg/L), whereas Event 8 had elevated levels at MT and PT1 (mean: 81.13 ± 5.48, 76.63 ± 3.71 and 4.50 ± 1.77 mg/L). Significant differences were observed between moderate and heavy rainfall for TSM and PIM (Events 5 and 8; $p < 0.01$). Differences were also detected between dry and heavy rainfall for TSM, PIM, and POM at certain stations ($p < 0.05$ to 0.01). In seawater, dry conditions were observed during Event 6, with TSM, PIM and POM concentrations exhibiting low values and a similar decreasing trend along the St.0, St.1 and St.2 coastal transect (mean 4.08 ± 0.25, 2.17 ± 0.34 and 1.92 ± 0.59 mg/L, respectively). Higher concentrations of TSM, PIM and POM were recorded during moderate rainfall events (Events 3–5), especially during Event 3, which showed maximum concentrations of 43.25, 38.38 and 4.88 mg/L respectively, in Station St.0. No significant differences in TSM, PIM and POM values were found at the river stations between dry (Event 6) and moderate (Events 3–5) rainfall events, except for PIM in Event 3 ($p < 0.01$) and POM in Event 1 ($p < 0.01$). During heavy rainfall Events, such as Events 7–8, TSM, PIM and POM showed higher values, especially in Event 8 at the St.0 (137.5, 125.38 and 12.13 mg/L, respectively). No significant differences in TSM, PIM and POM values were found at the river stations between moderate (Events 3–5) and heavy (Events 7–8) rainfall events, except for TSM and PIM in Events 4–5 ($p < 0.05$), and for POM in Event 5 ($p < 0.05$). Significant differences were found across river stations for TSM, PIM and POM values between dry (Event 6) and heavy rainfall events (Events 7–8), with $p < 0.01$ for TSM and PIM, and $p < 0.05$ for POM.

3.3. Correlation analysis between nutrient concentrations and fecal indicator bacteria

In the river (Fig. 6a; *see Tables S4 and S5 in the Supplementary Material*), a strong positive correlation was observed between *E. coli* and Enterococci ($\tau \approx 0.559$, $p < 0.001$), suggesting a common origin and similar environmental behaviour consistent with their role as indicators of fecal contamination. Both microbiological parameters also showed a strong positive correlation with rainfall, indicating that precipitation events were associated with increased concentrations of fecal indicators. This pattern likely reflected the influence of surface runoff, which mobilized and transported fecal contaminants from surrounding land areas into the river system.

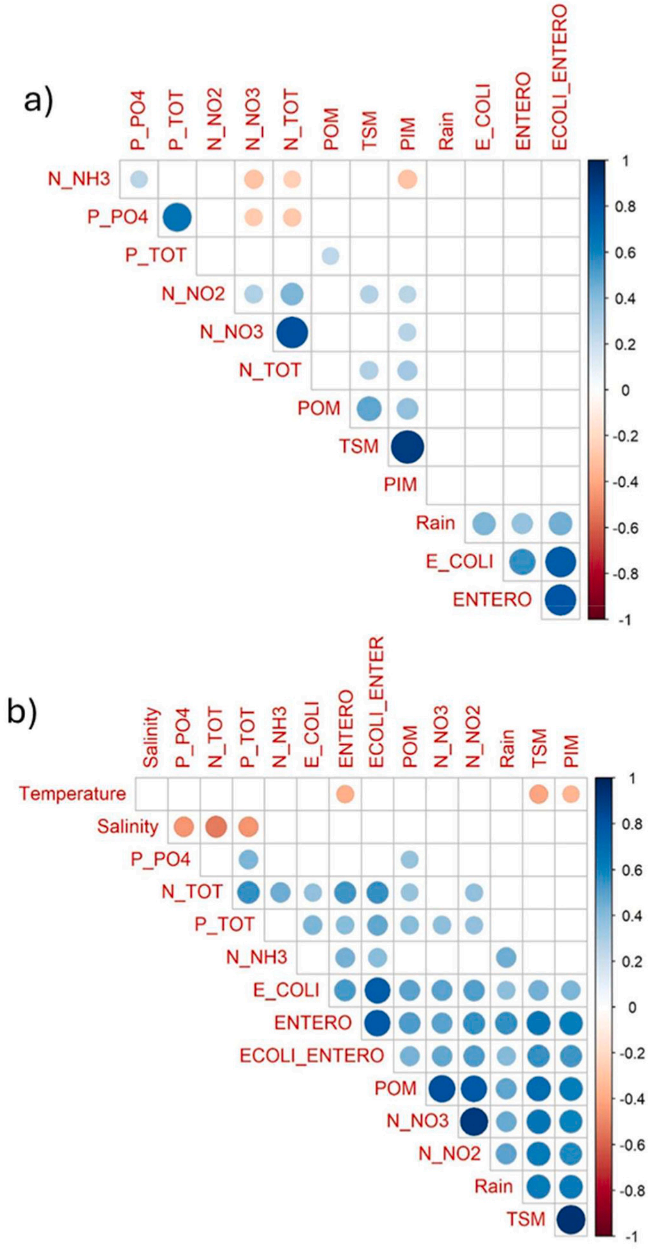

Fig. 6. Correlations between environmental, nutrient and microbiological parameters for two environments, calculated using the Kendall's tau (τ) coefficient: (a) river and (b) sea.

Among the physico-chemical parameters, a positive correlation was observed between total suspended matter (TSM) and particulate inorganic matter (PIM), highlighting the significant contribution of inorganic particles to overall turbidity. In addition, moderate positive correlations were found between nitrogen species (particularly N-NO$_2$ and N-NO$_3$), and between total phosphorus (PTOT) and phosphate (P-PO$_4$; $p < 0.001$), suggesting the co-occurrence of these nutrients potentially linked to common sources or shared biogeochemical processes.

In the sea (Fig. 6b; *Tables S4 and S5 in the Supplementary Material*), the strong positive correlation between *E. coli* and enterococci persists, similar to the pattern observed in the river, reinforcing the hypothesis of shared fecal contamination sources or mechanisms in both environments.

Stronger interrelationships among nitrogen species (N-NO_2, N-NO_3, and NTOT) were evident in the marine environment, suggesting a more interconnected nitrogen cycle in coastal waters. Furthermore, temperature showed a negative correlation with salinity and phosphate, possibly reflecting the effects of freshwater input, which typically lowered salinity and may influence nutrient concentrations. In contrast to the river, rainfall does not show a significant correlation with fecal indicator bacteria in the marine environment, although it correlated positively with some nutrient species, indicating potential land-derived nutrient enrichment following precipitation events.

The correlation results were also confirmed by the Random Forest model analysis (Fig. 7), where the relative importance of environmental and chemical variables in influencing fecal indicator bacteria concentrations was expressed as the percentage increase in Mean Squared Error (%IncMSE) when each variable was removed or added.

In the river system (Fig. 7a), *E. coli* concentrations were most strongly influenced by rainfall, PIM and TSM, followed by ammoniacal nitrogen (N-NH_3) and the occurrence of any event (e.g., storm or discharge). In this case, the percent of variance explained was 47.54.

In the sea (Fig. 7b), *E. coli* concentrations were most influenced by rainfall event and phosphorus-related variables (PTOT and P-PO_4). The influence of nitrogen species (N-NO_2 and N-NO_3^-) and salinity was also notable. In contrast, station location and temperature were among the least influential variables (percent of variance explained was 83.27).

For Enterococci in the river (Fig. 7c), the most important predictors were rainfall, TSM and PIM with N_NH_3 also contributing substantially (percent of variance explained was 80.40).

In the marine system (Fig. 7d), Enterococci concentrations were primarily influenced by phosphorus (PTOT and P-PO_4) and nitrogen species (N-NO_3 and N-NO_2). Events and rainfall were also important, though to a slightly lesser extent than in the river system (percent of variance explained was 74.36).

4. Discussion

The findings of this study demonstrated the key role of meteorological conditions, particularly intense rainfall, in driving fecal microbial contamination in riverine and coastal environments. These observations were consistent with those of Malham et al. (2014), who identified extreme weather events, especially intense rainfall, as major contributors to microbial pollution in aquatic systems. Climate change amplifies these pressures by increasing the frequency and severity of contamination episodes, thereby posing growing challenges to the resilience of freshwater and marine ecosystems.

The Arzilla river catchment, subject to various anthropogenic pressures including urban discharges, agricultural runoff and livestock effluents, exhibited strong responses in fecal indicator bacteria (FIB) concentrations during rainfall events, with the most critical conditions observed during the extreme rainfall event (Event 8). In parallel, Event 8 also recorded the highest concentrations of total nitrogen (N-TOT) and nitrate (N–NO_3) in the river, confirming a significant nutrient mobilization under intense precipitation (Brown and Peake, 2006; Eregno et al., 2016). In contrast, the coastal marine area displayed a more diluted and attenuated response, highlighting the differing dynamics of contaminant transport and dispersion between the two systems. Although nutrient levels In the sea were generally lower than in the river, Event 8 still showed the highest N–NO_3 concentration, indicating a downstream transfer of land-derived inputs under extreme conditions (Alegbeleye and Sant'Ana, 2020).

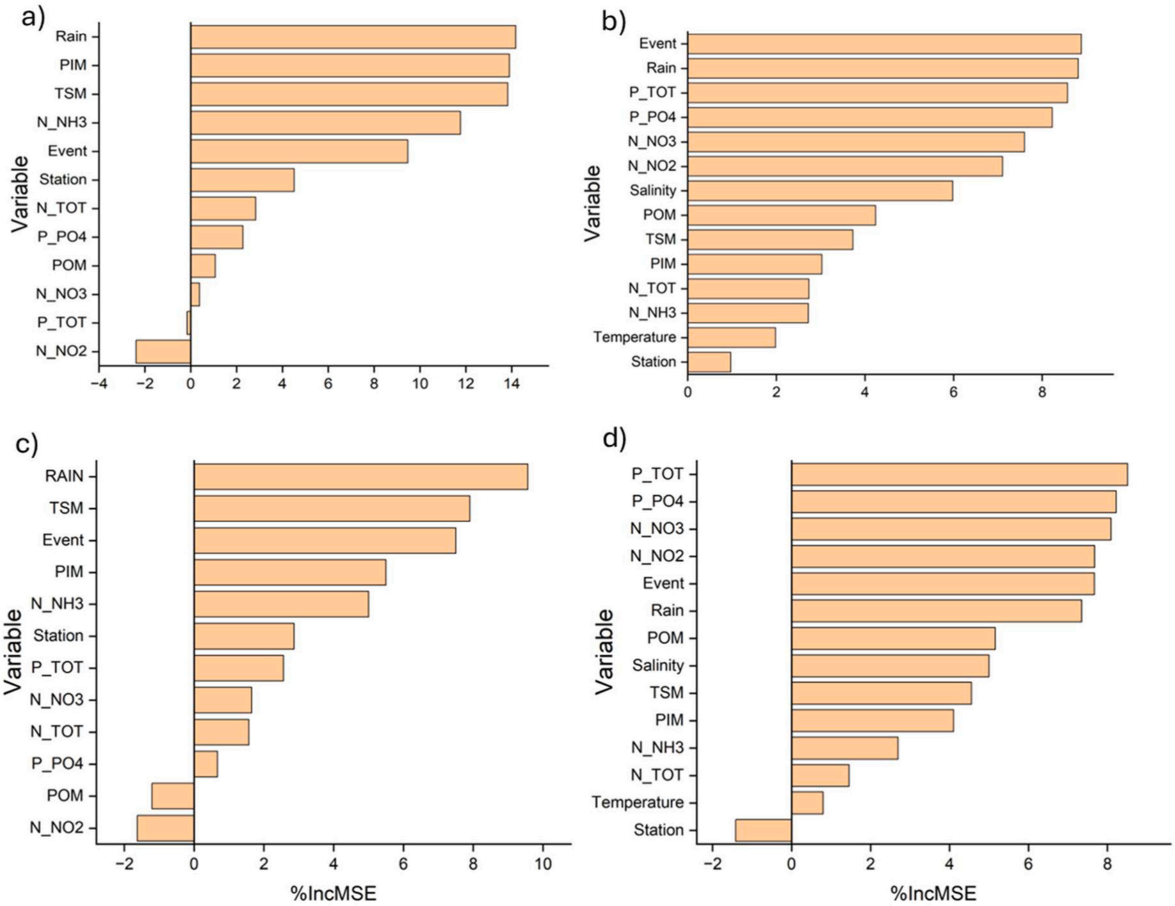

Fig. 7. Random forest model. Feature importance bar chart: a) *E. coli* in the river b) *E. coli* in the sea c) Enterococci in the river d) Enterococci in the sea.

These observational patterns were further corroborated by the Random Forest analysis, which provided robust support for the correlation–based findings and offered a more nuanced understanding of the relative importance of environmental and chemical drivers of fecal indicator bacteria. By isolating the key predictors through %IncMSE, the model strengthened confidence in the conclusions and highlighted the distinct dynamics between freshwater and marine systems.

In the river system, the dominance of rainfall, TSM, and PIM as predictors for both *E. coli* and Enterococci reinforced the hypothesis that precipitation-driven runoff and particulate transport are the primary mechanisms behind fecal contamination. Conversely, the negligible or even negative importance of nutrient species such as N-NO_2 and PTOT suggested that they may not be reliable indicators of microbial risk in rivers, at least not in the short term or at the resolution used in this study.

In the marine environment, however, a shift was observed: phosphorus-related variables (PTOT and P-PO_4) emerged as leading predictors, alongside rainfall and nitrogen species, indicating a more complex interaction between nutrient loading and microbial dynamics. The diminished role of station location and temperature implies spatial uniformity or a weaker influence of thermal gradients compared to land-derived inputs. Particularly interesting was the emerging importance of nutrient concentrations, especially phosphorus, in coastal waters, pointing to indirect processes such as nutrient-fuelled phytoplankton blooms that can alter bacterial survival or transport mechanisms. This finding suggests a potentially different contamination pathway in marine environments, where nutrient enrichment may influence bacterial persistence more than immediate runoff does.

Precipitation acted as the primary driver of microbial pollution, with riverine stations showing marked increases in *E. coli* and enterococci concentrations in response to rainfall, especially when linked to point sources such as wastewater treatment plant overflows and agricultural effluent discharges (Al Aukidy and Verlicchi, 2017; Amorim et al., 2014). These findings were consistent with those of Baral et al. (2018), who observed that stormwater runoff was the dominant source of microbial contamination in an urban stream, with microbial loads increasing by up to 300 times during wet weather events compared to dry conditions. According to Baral et al. (2018), street runoff alone contributed over 70 % of the microbial load in storm drain outfalls, highlighting the central role of rainfall-driven wash-off from urban surfaces in fecal contamination. Similarly, Ahmed et al. (2019) reported significantly elevated FIB concentrations in urban stormwater during rainfall events, further confirming the central role of run-off from impervious surfaces in driving microbial pollution. The marine system, due to its higher hydrodynamic energy and dilution capacity, responded less dramatically to contamination events, except in extreme cases, when microbial loads exceeded regulatory thresholds. This highlighted the vulnerability of coastal waters to climate-driven perturbations (Rummukainen, 2012; Cabral et al., 2019). Significant differences were observed between the two fecal indicator bacteria (FIB). *E. coli* responded rapidly to rainfall, making it a reliable proxy for recent contamination, while enterococci displayed delayed and more persistent signals, even in the absence of recent precipitation. This pattern suggested differing environmental persistence and transport mechanisms, with enterococci likely being associated with sediments and particulate matter (Stocker et al., 2019). This association contributed to their seasonal persistence and delayed transport compared to *E. coli*, allowing prolonged mobilization along the river and into the sea (Luna et al., 2019; Manini et al., 2022). This was especially evident in the coastal transect, where *Enterococci* concentrations often exceeded those of *E. coli* during low-rainfall conditions, reinforcing the need to monitor both indicators for comprehensive risk assessment (Li et al., 2021; Quero et al., 2024).

This distinction is consistent with the findings of Baral et al. (2018), who reported that microbial taxa associated with Enterococcaceae and sediment bound bacteria had higher persistence in streambed sediment, whereas *E. coli* levels peaked rapidly after rainfall, acting as a tracer for recent contamination inputs. Ahmed et al. (2019) further support this observation, noting that enterococci tend to persist longer in the environment and are often particle-associated, allowing microbial signals to be sustained even during dry weather. These findings supported our interpretation of Enterococci as indicators of legacy contamination and delayed transport. Similarly, Malham et al. (2014) observed that enterococci and other FIB can persist in marine environments longer than previously assumed, especially under conditions of low UV radiation or reduced salinity following rainfall. Such conditions can enhance bacterial survival, reinforcing our interpretation of prolonged contamination signals along the coastal transect.

A key finding of this study was the identification of the river not only as a conduit but also as a temporary reservoir of microbial pollution. Even under dry conditions, elevated FIB values were observed at certain riverine stations, indicating that the Arzilla stream was capable of accumulating and gradually releasing microbial loads. This conservative behaviour was likely mediated by sediment resuspension, low flow rates, and the presence of diffuse sources along the watershed (Sabater and Tockner, 2010; Nogales et al., 2011). Similar behaviour was reported by Baral et al. (2018), who found that microbial communities in streambed sediments and embankment soils can act as reservoirs of fecal bacteria, particularly enterococci, contributing to delayed or persistent contamination during and after storm events. This interpretation was further supported by Malham et al. (2014), who emphasized the role of riverbed sediments and catchment soils as temporary reservoirs for fecal bacteria, leading to delayed peaks of contamination following initial events. Their study highlighted the importance of legacy pollution and resuspension processes in prolonging microbial presence, even during dry conditions.

The elevated levels of total suspended matter (TSM), particulate inorganic matter (PIM), and particulate organic matter (POM) recorded during heavy rainfall events, particularly at PT3 and MT in the river, and at St.0 in the sea during Event 8, further supported the role of surface runoff in transporting both microbial and chemical contaminants. In the river, POM accounted approximately 80 % of TSM on average, suggesting a relevant contribution of organic matter to turbidity and its potential role in microbial transport (Ma et al., 2022). In contrast, the marine system showed faster attenuation of microbial contamination, primarily due to wave action and coastal currents. However, during exceptional events, even this system became temporarily overwhelmed, as shown by the microbial peaks recorded at all marine stations during Event 8.

The ecological functioning of river systems is central for understanding contaminant dynamics at the land–sea interface. Rivers act not only as conduits for water but also as ecological filters, regulating nutrient and pathogen fluxes from upstream human activities to downstream coastal waters. This dual role becomes especially critical under extreme weather conditions, which are in frequency and intensity due to climate change (He et al., 2019; Bolan et al., 2024). The European Water Framework Directive (WFD, 2000/60/EC) is the main legislative framework promoting integrated water management at the river basin scale, aiming to achieve good ecological and chemical status for surface and groundwater. However, it does not explicitly address microbiological risks associated with diffuse pollution and climate-driven extreme events. Our results suggested the need to revise existing monitoring frameworks by incorporating microbial indicators as essential ecological metrics, particularly in urbanized and climate-sensitive basins (Caruso et al., 2016; Yuan et al., 2021).

In this context, regional governance tools offer valuable opportunities to strengthen participatory watershed management and incorporate microbial pollution control into catchment-scale planning. These frameworks can support the implementation of *Nature-Based Solutions* (NBS; Robotham, 2023), such as buffer strips alongside rivers (Zhang et al., 2010), artificial wetlands (Vymazal, 2011), and vegetated channels (Bedri et al., 2016).These solutions can prevent and break down microbial contaminants before they reach aquatic ecosystems. Additionally, constructing stormwater retention basins can mitigate the impact of intense rainfall by temporarily storing and gradually releasing contaminated water, thereby reducing microbial peaks and protecting the quality of water downstream.

From a management perspective, this study highlighted the importance of adopting integrated and adaptive strategies to mitigate fecal contamination. In this regard, Malham et al. (2014) advocate for a more holistic and proactive approach to management, combining real-time monitoring, predictive modelling, and *nature-based solutions* to anticipate microbial risks and protect public health. Specifically, we recommend the systematic integration of microbiological contamination and the use of molecular tools to improve source tracking and identify dominant pollution pathways. Adopting molecular techniques, such as 16S rRNA sequencing and Source Tracker analysis, as demonstrated by Baral et al. (2018), allows for the quantification of microbial source contributions. This provides essential insights into dominant contamination pathways and supports targeted management interventions. Similarly, Ahmed et al. (2019) emphasized the importance of combining FIB with Microbial Source Tracking markers to accurately differentiate between human and animal contamination sources in complex urban catchments. This combined approach provides essential insights into dominant contamination pathways and supports targeted management interventions. Moreover, adaptive monitoring in coastal areas is essential for capturing delayed or sustained microbial inputs, especially from persistent indicators such as enterococci. These strategies are particularly important in densely populated coastal catchments, where land and sea interactions are intensified (Ansari and Matondkar, 2014).

5. Conclusion

Our study shows that the microbial contamination in watersheds (such as the Arzilla river, our study site) highlight the complexity of land–sea interactions and underscore the importance of adaptive strategies to safeguard water quality. Future planning must recognise the dual ecological function of rivers, as both vectors and regulators of pollution, while also enhancing the resilience of coastal systems to climate variability through site-specific adaptation strategies and vulnerability assessments. Incorporating microbial study, molecular source tracking and predictive modelling into environmental policies will be crucial to safeguard ecosystem integrity and public health in a changing climate.

Finally, the implementation of integrated and adaptive strategies requires the active involvement of stakeholders, including local communities, municipal planners, and health and environmental authorities. By translating scientific evidence into participatory management practices, these actors can play a crucial role in reducing microbial risks, strengthening resilience to extreme weather events and informing policy debates on sustainable coastal management.

CRediT authorship contribution statement

Angela Freddi: Writing – original draft, Methodology, Formal analysis, Data curation. **Fabio Ricci:** Methodology, Formal analysis. **Manuela Coci:** Writing – original draft, Methodology, Formal analysis. **Stefano Guicciardi o Guizzardi:** Methodology, Formal analysis. **Silvia Casabianca:** Writing – original draft, Methodology. **Pierluigi Penna:** Methodology, Data curation. **Luigi Bolognini:** Conceptualization. **Mauro Marini:** Writing – original draft, Conceptualization. **Antonella Penna:** Writing – review & editing, Writing – original draft, Conceptualization. **Elena Manini:** Writing – review & editing, Writing – original draft, Validation, Methodology, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2025.119156.

Data availability

The data supporting the findings of this study are included in the published article and/or its supplementary materials

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