D.3.2.1. Generation of Sampling Data set

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INTRODUCTION

One suitable data set of microbial and environmental parameters measured in the pilot and target coastal areas has been generated and used in the WQIS for the alert system of bathing prohibition and safe recreational bathing.
In the pilot area of Fano and other target sites, at each emission points, the microbial and environmental sampling have been done along five transects at several points: at the emission of the discharge, then at 50, 100, 150, 200 and 300m from the coast within the recreational waters. The sampling has been done in 2019, 2020 and 2021 during the overflow period (based on the interval of 1-6 hours). The microbial sampling has then been used to analyse the distribution of faecal discharge.
The results of microbiological analyses are useful to assess the quality of recreational waters and to provide information concerning the health status of urban wastewater and coastal system through an innovative WQIS to forecast or provide an alert system for the safe recreational bathing along the coasts at the target sites of this project.

1. Microbial contamination indicators: Sampling Strategy

The analyzed microbial and environmental data were used to implement the Integrated Water Quality System (WQIS) proposed in the WATERCARE project. The aim of this section is to associate significant environmental variables indicating fecal load and contamination in water to microbial contamination (fecal contamination). The following guidelines on sampling strategy and analyses on various microbial and environmental variables will be useful for various stakeholders to implement new reliable sampling strategies and mitigation measures to avoid the closure of bathing areas for unnecessary periods of time such as now it occurs.

In this chapter, the following points have been focused: i) evaluation of the presence and quantification of fecal pollution and its space-time variability along a coastal area affected by river discharges; ii) analysis of the potential relationships of the abundance and distribution of fecal bacterial indicators with the main environmental variables; iii) identification of the origin and time of decay / persistence of microbial contamination during extreme rain events.

2. Pilot site Fano Arzilla River

2.1 Characterization of Fano pilot site

The study area of the pilot site is the Arzilla River and the bathing waters in front of the city of Fano (Marche Region, northern western Adriatic Sea). This area was selected as a pilot site since this site shows the typical characteristics of a medium urbanization city on the Adriatic Sea with a touristic and commercial harbor located near the mouth of the Arzilla River and on the coast which is characterized by numerous artificial barriers against the coastal erosion. The Arzilla stream collects sewage from the hinterland and CSO of Fano which is discharged into the sea, near beaches highly attended during the summer season. Intense rain events often cause the overflow of the local sewerage system that collects the microbial load from the municipality of Fano. Whenever there is a sewer run-off in the summer period, the bathing activity is closed based on the potential risk of fecal microbial contamination (Penna et al., 2021).

2.2 Sampling frequency of Fano pilot site

Two bathing seasons were considered from May to September 2019 and 2020, during which river and seawater samples were collected. Freshwater samples from the Arzilla River were collected in a 30 min time interval during rain events and every 6, 12 or 24 hours after rain events using the autosampler system located at the Arzilla estuary and in an upstream station before the CSO of the first rain collection tank (Fig. 6.3 -1). Samples were collected for determination of fecal bacteria, dissolved inorganic nutrients (ammonia-NH_4, nitrite-NO_2, nitrate-NO_3, total nitrogen, total phosphorus), total suspended solid (TSS), particulate organic matter (POM) and chlorophyll-a content characterizing the freshwater body that flowed into the sea (Table 1). At the end of each rainy event, surface seawater samples were manually collected with sterilized bottles in front of the Arzilla mouth, within 250 m from the coastline, for chemical and microbiological analyses. The sampling spatial scale followed a grid composed of three transects (Transects 1, 2 and 3) along with a point in the Arzilla mouth (Fig. 6.3 -2). Each transect includes points at 50, 100, 150, 200 and 250 m from coastline; the Transect 2 includes only three sampling points (i.e. 50, 100 and 150 m). This small spatial scale sampling strategy was adopted following the coastal morphology, bathymetry of the area, water currents and the presence of artificial barriers that influence the microbial dispersion. Further, at each site, temperature (°C), salinity, pressure, density (σ_T), oxygen concentration and saturation, pH, redox and Chl a were measured using a CTD multiparametric probe (Idronaut model Ocean Seven 316 Plus).

Figure 1. Study site and sampling strategy; a) two sampling stations are positioned along the Arzilla River, Arzilla outfall (where is located the automatic water sampling for microbiological and chemical analyses) and at Arzilla upstream; b) discharge point or CSO (combined sewer outflow) and automatic sampling station.

Figure 2. Map of seawater sampling in front of the Arzilla mouth. Sampling stations are distributed along three transects (transect 1 in red, transect 2 in blue, transect 3 in yellow) at increasing distance from the shore with different sampling points: 50m, 100m, 150m, 200m, 250m from the mouth.

2.3. Microbiological analysis

Field of river and seawater samples for microbiological analyses of fecal contamination (Escherichia coli and intestinal enterococci) were immediately carried out to the laboratory at in situ temperature in the dark and processed within a few hours after collection. E. coli and enterococci bacteria were analysed using culture-based methods. *E. coli* abundance was determined by membrane filtration. An appropriate volume of water (from 1 to 100 ml) was vacuum-filtered (pore size 0.22 μm, diameter 47 mm; Millipore) in triplicate and the filters were placed on m-FC agar plates and were incubated at 44.5 °C for 24 h. Only blue colonies were considered. The abundance was reported as CFU (Colony-Forming Units) 100 ml^{-1} of water. Enterococci abundance was assessed by membrane filtration and an appropriate volume (from 1 to 100 ml) were filtered in triplicate as described above and filters were placed on Slanetz Bartley agar plates. Plates were incubated at 37.5 °C for 48 h. Only red or reddish-brown colonies were considered as presumptive enterococci. The abundance was reported as CFU 100^{-1} ml of water filtered.

2.4. Environmental parameters analyses

River and seawater samples for nutrient analyses were filtered (nitrocellulose Millipore, 0.45 μm) and stored at −20 °C in polyethylene bottles until analysis, whereas water samples for TSS (total suspended solid), POM (particulate organic matter) and Chl a were filtered on GF/F Whatman, 0.7 μm and on nitrocellulose Millipore, 0.45 μm filters, respectively, and immediately processed. Nutrient concentrations were measured using a UV-1700, Shimadzu model following Strickland and Parsons (1972). N-TOT and P-TOT were determined on unfiltered water samples according to the method of Valderrama (1981). Accuracy was ± 0.02 μmol l^{-1} for $N\text{-}NH_4$, $N\text{-}NO_2$, $N\text{-}NO_3$, N-TOT, P-TOT. A calibration curve was made with 5 levels of Merck standards and the accuracy was tested using a standard as sample. The precision was tested on 10 replicates of the standard and were: ±0.001 μmol l^{-1} ($N\text{-}NH_4$), ±0.006 μmol l^{-1} ($N\text{-}NO_2$), ±0.005 μmol l^{-1} ($N\text{-}NO_3$). TSM concentrations were determined gravimetrically by filtration of a known volume of water sample through 0.7 μm pre-combusted and pre-weighed GF/F membrane filters (Millipore, Bedford, MA, USA) following APHA (2017). For PIM and POM determination the filters were then ashed at 500°C for 1 h following the APHA Loss On Ignition (LOI) method. POM concentrations were calculated by difference between TPM (total particulate matter) and PIM (particulate inorganic matter). The precision estimated on 5 replicates by coefficient of variation was < 10%. Chl a concentration was determined in 90% acetone homogenates of particulate matter collected on filters as described above. The Chl a was analysed spectrophotometrically.

PARAMETERS	Method (Manual, Automatic, Lab)	
	Riverine	Seawater
Meteorological data		
Rainfall (mm/m^2 and length of time)	√	
Wind (speed and direction)	√	
Solar Radiation (%)	√	
Sea water current	N--NE--E--SE--S--SW--W--NW	
Sea state (waves)	√	
Chemical/physical data		
Salinity	√	√
Temperature (°C)	√	√
Redox (mV)	√	√
pH	√	√
Conductibility (mS/cm)	√	√
BOD_5 (mg/L)	√	
COD (mg/L)	√	
Turbidity (NTU)	√	√
Dissolved O_2 (% sat)	√	√
Dissolved O_2 (mg/L)	√	√
Chlorophyll *a* (µg/L)	√	√
TSS (mg/L)	√	√
POM (mg/L)	√	√
Ammonium $N\text{-}NH_4$ (µM)	√	√
Nitrates $N\text{-}NO_3$ (µM)	√	√
Nitrites $N\text{-}NO_2$ (µM)	√	√
TN (µM)	√	√
TP (µM)	√	√
Ortho-phosphate $P\text{-}PO_4$ (µM)	√	√
Microbiological data		
Faecal Indicator Bacteria (*Escherichia coli* and *Enterococcus*)	√	√

Table 1. List of meteorological, physical, chemical, and microbial parameters analysed in river and seawater in Italian target sites.

2.5 Conclusion

The faecal microbial contamination was related to the rainy events with high flow of wastewater, with recovery times for the microbiological indicators varying between 24 and 72 hours and related to dynamic dispersion. The positive correlation between ammonium and faecal bacteria at the Arzilla River and the consequences in seawater can provide a theoretical basis for controlling the ammonium in rivers and monitoring the potential risk of the bathing waters pathogen pollution. The hypothesis that individual characteristics of beaches may influence pathogen concentrations and manifest serious health risks is supported by obtained data. It is therefore highly suggested the use of specific data for a location for management decisions and our results provide the basis for such planning.

3. Pescara River

3.1. Characterization of Pescara site

The area under study is rather limited, it affects the Abruzzo coast close to the Pescara port area, the terminal stretches and the mouth of the Pescara River, the area surrounding the breakwater built to protect access to the port.

Figure 3. Location of Pescara site

The sampling plan took into account the data relating to currents and the geomorphology and bathymetry of the seabed, previously analyzed by the client. (Table 2) from which we try to verify and create models of the distribution of water following rainy events.
1) The inclination of 25 ° NNW with respect to the geographic north of the coast line of the submarine pipeline, due to the presence of a coastal oil terminal.
2) The characterization of the sediment accumulation areas, near the breakwater with an orientation almost EAST – WEST.
3) The formation of currents which interacting with these structures have modified the dilution modalities or the increases in concentration of the chemical and organic substances under study.

Sampling stations	Latitude	Longitude
PPS 1	42.470902	14.227755
PPS 2	42.470172	14.224811
PPS 3	42.472041	14.225916
PPS 4	42.473306	14.229538
PPS 5	42.471478	14.233852
PPS 6	42.470416	14.222372
PPS 7	42.470322	14.236976
PPS 8	42.472295	14.217908
PPS 9	42.463456	14.238191
PPR 1	42.445957	14.186422
PPR 2	42.461686	14.210923
PPR 3	42.465148	14.221217
PPR 4	42.469744	14.229630
AS Automatic sampling	42.466327	14.224545
IT013068028012	42,470249	14,220312

Table 2. Sampling stations with indication of GPS coordinates

In relation to the foregoing, and to understand the area of influence of the waters leaving the port, in the context of a preliminary survey of the area concerned, nine sampling stations at sea, 4 sampling stations on the river were selected. The installation of an automatic sampler always on the river and a station near the shoreline. The stations are located at progressive distances from each other to uniformly cover the area for the creation of the distribution model.

For each point the analyzes are aimed at evaluating the horizontal variation of the concentrations of both the chemical and organic characteristics (Fig. 6.3-4).

Figure 4. Location of sampling points

3.2. Sampling frequency

The sampling schedule to be carried out considered the samplings already foreseen for the ordinary monitoring activities carried out during the bathing season by ARTA Abruzzo.

They were carried out over a period in stable conditions and in adverse weather conditions.

The following table shows the dates and number of samplings performed.

SAMPLING PLAN				
WEATHER CONDITION	CALENDAR	REPETITIONS	AUTOMATIC SAMPLING	SAMPLES OF THE SEAWATER AND RIVERWATER
NORMAL WEATHER	12/07/2021 26/07/2021 09/08/2021 06/09/2021	4x during the bathing season	1 sample	1 sample for each points: 4 river points 9 sea points
HEAVY RA	20/07/2021		14 samples	

	26/08/2021	6x during the bathing season		1 sample for each points: 4 river points 9 sea points
	31/08/2021			
	29/09/2021			

Table 3: Dates and Numbers of samplings performed

3.3 Data base

The analytical method for enterococci [CFU/100ml] was the IRSA-CNR n.7040 - C - membrane filtration method (MF). This method allows to count the number of colonies grown on a membrane placed on agar medium.
The determination of the number of colonies of Escherichia coli [CFU/100ml] was done by using the ISRA-CNR method n.7030/F.
The total nitrogen was determined by the UNI 11658 method. Total Phosphorus was determined using the Macherey-Nagel method. Ammonia nitrogen was measured with the Hanna HI3826 method.

3.4. Conclusion

The monitoring actions carried out with the Watercare project on the Port site on the Pescara River confirmed and highlighted the determinants of the quality of the marine bathing waters of the port side areas.
In particular, the modelling described the propagation to the north of river waters during the most important meteorological events with the related repercussions and exceedances of the values that have determined the decay of the state of the coastal water body over the years.
The port mouth system is also undergoing further modification both from a structural point of view (completion of the opening of the Breakwater and extension of the port arms) and functional (construction of the first rain basin) and therefore the monitoring carried out and the sampling will be used as a baseline scenario for subsequent evaluation of future settings.
The maintenance and implementation of alert mechanisms of the monitoring system created by Watercare in Pescara will ensure the issuance of more effective protocols for the management of bathing with targeted and specific bans on the stretches of the Pescara coast. The system implemented in the reading parameters and with the alert application will also be able to provide useful support in the emergency management of accidental and occasional events involving the spread of pollutants.
At the end of the project and the sampling activities, it was also highlighted the opportunity of the replicability of the system on other river systems which, due to different characteristics, require

specific monitoring connected to bathing water both during intense meteorological phenomena and in ordinary outflows.

The most important cases are:

- The mouth of the Feltrino river, in the province of Chieti, which implies to a lesser extent the criticalities highlighted in Pescara (armed mouth and coastal defence works that result in a difficult change of water)
- The mouth of the rivers Alento (CH) and Saline (PE), which are sites of regional interest (they are subjected to upstream depollution actions). The Watercare system allows the continuous reading of the quality of the water in the mouth during the upstream removal / safety measures of the elements of danger (landfills, contaminated sites, etc.)
- The mouth of the Tordino (TE) River and the Vibrata (TE) River where the quality of the water must be monitored in conjunction with the activities of the construction of systems for the collection and treatment of first rainwater and of the flooded flows together with a progressive rationalization and control of discharges along the riverbed.

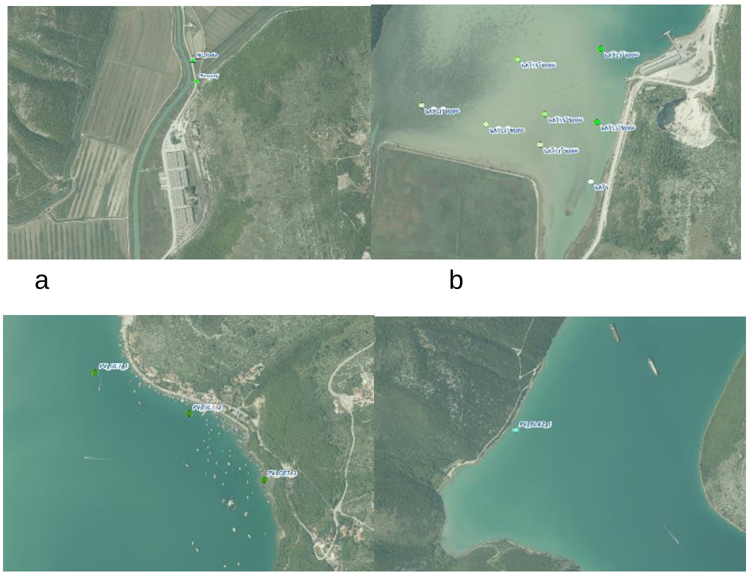
4. Raša River

4.1. Description of pilot site Raša

Site location is Crpna stanica Štalije upstream from mouth of river Raša. It is located on river Raša in Raša Bay. The river Raša is 23 km long, the river basin covers 279 km². It flows through the Raška Valley (12 km long, up to 1 km wide) and flows into the sea at Rasa Bay. From the overflow of the Rakonek spring to the mouth it is under the influence of the sea and shows salinity-dependent salinization. In rainy periods, the flow increases significantly, because in the middle part of the stream receives water from several abundant permanent springs (Bolobani, Sveti Anton, Šumber, Grdak, Rakonek, Mutvica, Kokoti and Fonte Gaja), occasional larger springs (Sušnica, Sušak) and several small unnamed occasional sources.

Raša Bay is situated on the eastern coast of Croatian Istria peninsula, southwest of the City of Labin. It is the lower part of the former valley of the river Raša, which is submerged by the young postglacial sea level rise. It is about 12 km long, with an average width of about 1 km. The depth of the bay varies from 44 m at the entrance to the bay to 10 m near the port of Bršica; further towards the mouth, shoals with depths of less than 3 m continue.

With its deposits, Raša gradually fills the bay, which is especially noticeable along the west coast. The sides of the Raša Bay are steep and inaccessible, built mostly of limestone, and overgrown with sparse Mediterranean vegetation.



a b

c d

Figure 5. *Location of the automatic sampling station (AP) at river Raša and channel Krapanj (a). Locations of measuring stations at Raša river mouth – transects (b). Locations of measuring stations at Raša bathing sea waters – GET 1, 2, 3 (c). Locations of measuring stations at Raša bathing sea waters – BLAZ 1 (d).*

4.2. Frequency of sampling

SUNNY PERIOD	HEAVY RAIN PERIOD	
AS Raša – 1 sample Krapanj channel – 1 sample Bathing sea water-12 samples	*AS Raša – 14 samples*	*Bathing sea water-12 samples*
18.09.2020	22.9.-1.10.2020	
	22.-27.09.2020	29.09.2020 30.09.2020 01.10.2020
04.05.2021	13-22.5.2021	
	13-20.5.2021	20.05.2021 21.05.2021 22.05.2021
29.06.2021	4-5.7.2021	
	04.07.2021	05.07.2021
	13-16.7.2021	
	13.- 14.07.2021	15.07.2021 16.07.2021
14.09.2021	16.-21.09.2021	
	16.-20.09.2021	20.09.2021 21.09.2021
	26-28.9.2021	
	26-28.9.2021	28.9.2021

Table 4. Performed sampling plan for Raša pilot site

4.3. Data base creation

Upon the completion of each sampling all analysed data was entered int the database. Data was checked before making the final report.

4.4. Analysed data

List in table below shows parameters analysed.

PARAMETERS	River	Seawater
Physical-chemical		
Air temp (°C)	AS probe	Probe
Water temp (°C)	AS probe	Probe
pH	AS probe	Probe
Redox (mV)	AS probe	Probe
Conductivity (mS/cm)	AS probe	Probe
Mutnoća (NTU)	AS probe	Probe
Salinity (PSU)	AS probe	Probe
Oxygen saturation (%O_2)	AS probe	Probe
Dissolved O_2 (mg/L)	AS probe	Probe
BOD_5 (mg/L)	LAB	NO
COD_{Mn} (mg/L)	LAB	NO
Ammonia (mgN/L)	LAB	NO
TN (mgN/L)	LAB	NO
TP (mgP/L)	LAB	NO
Mikrobiological		
Escherichia coli (CFU/100 ml)	LAB	LAB
Fecal enterokok (CFU/100 ml)	LAB	LAB

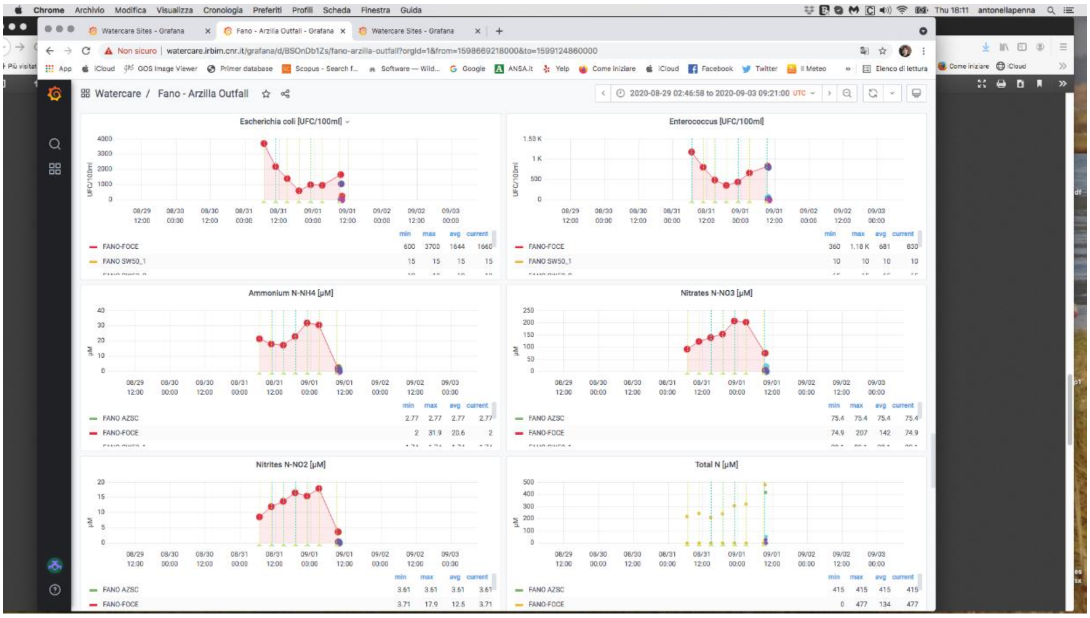
Table 5. List of monitored parameters at all Croatian target sites

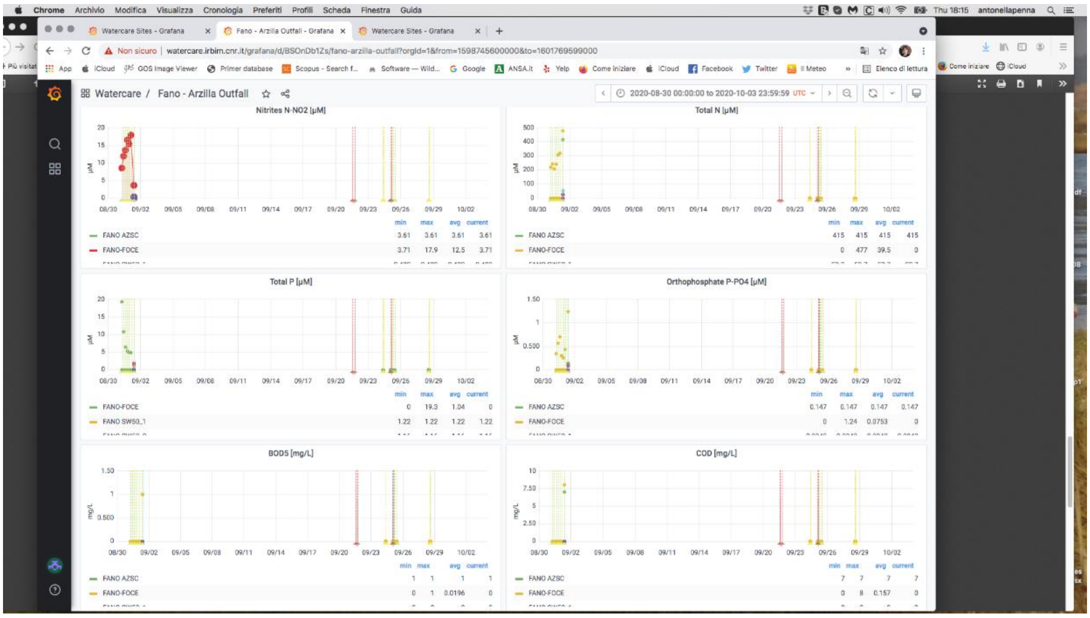
4.5. Microbial dispersion and nutrients in seawater

Microbial, nutrients, hydrological, BOD5 and COD data produced from 2019 were downloaded into grafana web database. Any meteorological event was registered in grafana web database as shown below.


Watercare / Pola - Rasa River
2020-09-07 04:56:48 to 2020-10-22 16:27:18 UTC
Water Temperature [°C]
RASA River - Exo3
RASA PV0
min max avg current
11.7 °C 25 °C 18.3 °C 14.0 °C
16.8 °C 22.3 °C 18.5 °C 16.8 °C
Water Salinity [ppt]
RASA River - Exo3
RASA PV0
min max avg current
0 ppt 28.7 ppt 10.6 ppt 1.52 ppt
11.8 ppt 37 ppt 20.3 ppt 11.8 ppt
Water Dissolved Oxygen [mg/L - %]


Watercare / Fano - Arzilla Outfall
2020-08-22 23:50:45 to 2020-09-10 20:32:17 UTC
Water Temperature [°C]
Water Temperature [°C] Fano Outfall
min max avg current
19.500 °C 27.640 °C 23.347 °C 20.680 °C
Water Salinity [ppt]
Foce Exo2 Salinity [ppt]
min max avg current
0.9200 22.8900 8.5956 1.0300
Water Dissolved Oxygen
ODO Sat Local [%] (right-y)
min max avg current
12.570 86.140 47.991 71.220





For the purpose of implementing project WP3 activities PP8 made a probe sampling. Sampling points were corrected on field and adjusted to real conditions and delivered to CW partner (field work). PP8 performed first normal weather and heavy rain weather sampling in bathing season 2020 for WP3 activities and gathered first laboratory data. Rain alarm works and also automatic sampling.

Three sampling tips were made within this period and following are their reports all given in the PP8 PR4 Report.

After all the analyses were done, every result was inserted in the agreed interface: https://watercare.irbim.cnr.it/wsa

All meteorological data from River Raša site can be found in real-time on Grafana portal.

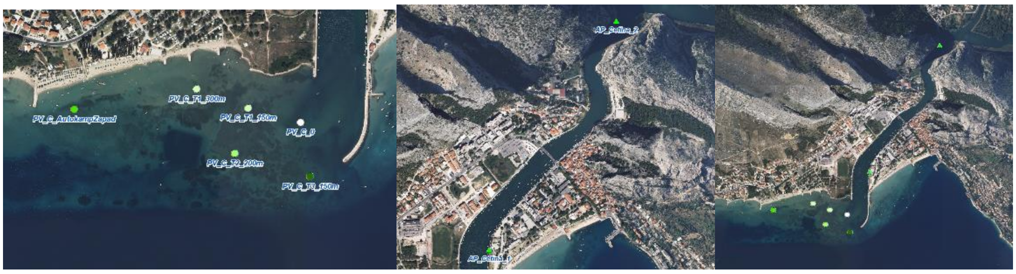
4.6. Conclusion

During stable weather conditions microbiological pollution on all stations in rivers, channel and bathing sea waters is low, but during rainy events microbial contamination is evident in all locations, depending on rain intensity and rain continuity. Largest microbial pollutions are evident at Krapanj channel, especially during summer periods and low flows when percentage of waste waters in channel is on the rise. Nutrients have an increase after rainy events at intervals of a few days after the onset of rainfall.

5. Cetina pilot site

5.1. Description of Cetina pilot site

Site location is mouth of river Cetina, downstream part of the riverbed where two automatic sampling stations are positioned. The Cetina River is a typical karst watercourse in the deep and well-developed Dinaric karst. Cetina has a length of about 105 km, and it is the longest and most water-rich river in Dalmatia. Its basin covers an area of 1,463 km2. From its source in Dinara Mountain, at the height of 385 meters above sea level, Cetina flows into the Adriatic Sea in the town of Omis.



a b c

Figure 6. Locations of measuring stations at Cetina River mouth and bathing sea waters – transects (a). Locations of the automatic sampling stations (AP) at Cetina River (b). Locations of all measuring stations at Cetina pilot site (c).

5.2. Frequency of sampling

<table>
<tr><th>SUNNY PERIOD</th><th colspan="2">HEAVY RAIN PERIOD</th></tr>
<tr><td>AS Cetina 1 – 1 sample
AS Cetina 2 – 1 sample
Bathing sea water-6 samples</td><td>AS Cetina 1 – 14 samples
AS Cetina 2 – 14 samples</td><td>Bathing sea water-6 samples</td></tr>
<tr><td>26.05.2021</td><td colspan="2">08.06.2021</td></tr>
<tr><td>28.06.2021</td><td colspan="2">06.-07.07.2021</td></tr>
<tr><td></td><td>06.07.2021</td><td>06.07.2021
07.07.2021</td></tr>
<tr><td>07.09.2021</td><td colspan="2">26.-27.08.2021</td></tr>
</table>

	26.08.2021	26.08.2021 27.08.2021
	20.-22.09.2021	
	20.09.2021	21.09.2021 22.09.2021
	29.09.-01.10.2021	
19.10.2021	29.09.2021	30.09.2021 01.10.2021

Table 6. Performed sampling plan for Cetina pilot site

5.3. Data base creation

Upon the completion of each sampling all analyzed data was entered int the database. Data was checked before making the final report.

5.4. Analysed data

List in table 5 shows parameters analysed.

5.5. Conclusion

During stable weather conditions microbiological pollution on majority of stations in the river and bathing sea waters is low. During rainy events there was evidence of increasing microbial concentrations, but majority of samples were in good status, only 2 samples of the bathing sea water closest to the river mouth were assessed as insufficient after one rain event in July.

6. Neretva River pilot site

6.1. Description of pilot site Neretva

Site location is mouth of river Neretva, downstream part of the riverbed (near the city of Ploče). The Neretva River basin is shared by Bosnia and Herzegovina (about 10,100 km2) and Croatia (about 280 km2). It is about 220 kilometers long, and only the final 20 kilometers are in Croatia forming an extensive delta with large reedbeds, lakes, wet meadows, lagoons, sandbanks, sandflats and saltmarshes. Neretva Delta is surrounded with karst hills rich with underground water that supplies numerous springs, streams, and lakes. The river mouth area is characterized with many drainage channels, and it represents ecologically unique area. In the Neretva Delta, at least 313 bird species have been registered. Neretva and its tributaries are also exceptionally rich in fish species. The Delta is the most fertile area of the middle Dalmatia oriented on commercial agricultural production (mostly tangerine plantations and vegetable greenhouses).

a b c

Figure 7. Locations of measuring stations at Neretva River mouth – transects (a). Locations of the automatic sampling stations (AP) at river Neretva and bathing sea waters (b). Locations of all measuring stations at Neretva pilot site (c).

6.2. Frequency of sampling

SUNNY PERIOD	**HEAVY RAIN PERIOD**	
AS Neretva – 1 sample Bathing sea water-11 samples	*AS Neretva – 14 samples*	*Bathing sea water-11 samples*
27.05.2021	07.-08.06.2021	
	07.-08.06.2021	08.06.2021
19.07.2021	05.-06.07.2021	
	05.-06.07.2021	06.07.2021
08.09.2021	24.-26.08.2021	
	24.-26.08.2021	26.08.2021
28.09.2021	18.-20.09.2021	
	18.-20.09.2021	20.09.2021
	01.-03.11.2021	
	01.-03.11.2021	03.11.2021
	03.-05.11.2021	

	03.-05.11.2021	05.11.2021

Table 7. Performed sampling plan for Neretva pilot site

6.3. Data base creation

Upon the completion of each sampling all analysed data was entered int the database. Data was checked before making the final report.

6.4. Analysed data

List in table 5 shows parameters analysed.

6.5. Conclusion

During stable weather conditions elevated concentrations of E. coli were registered in multiple stations in the sea along with river channel in September, during summer periods and low flows. During rainy events microbial contamination with E coli is evident in river water samples at the end of the 24-hr sampling period, locations of bathing sea water and most evident in November probably due to intensive rains at the end of October. With every increase of microbial parameters evident is an increase in ammonia concentrations indicating anthropological pollution.

7. TROGIR, KAŠTELA AND SPLIT AREA

7.1. Characterization of sampling site

The study was conducted at 11 coastal sites in the coastal area of the central Adriatic Sea (Figure 1). Most of the sites are in the densely populated area of Kaštela Bay, which has a developed industrial infrastructure, marinas and popular beaches, as well as on the shores of Split, the second largest city in the Republic of Croatia and an important tourist centre.

Most of the coastline of the monitored area is karstified, which means that precipitation quickly disappears underground. Precipitation may not have enough time to adequately purify itself and may reach the surface through the abundant coastal and submerged freshwater sources. According to historical data, the average number of rainy days during the bathing season in study area is not high, but precipitation is often short-lived and heavy, which can result in short-term pollution of coastal recreational waters through leaching.

Study area is subject to different meteorological conditions on a small scale. This means that although bathing sites are not too far away, they can be subject to very different weather conditions at the same time, from local rain showers to wind events. The selected sampling stations cover many geographically diverse areas to better determine if precipitation levels can affect FIB concentrations in coastal waters. Depending on the meteorological conditions, the stations are grouped in two areas, Split and Kaštela.

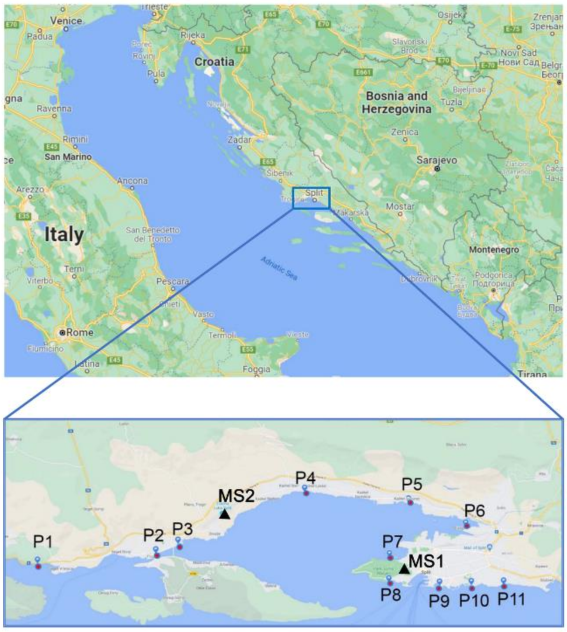


Figure 8. Study area with meteorological stations (MS1 and MS2) and sampling stations

Station P1 was selected as the reference station because it is far from settlements and faces the open sea. The station is rocky and difficult to access; therefore, the number of bathers is low during the bathing season. In addition, the area is heavily influenced by fresh water during the rainy season. The other stations are in urban areas and on popular beaches in these areas. Station P2 is located next to the parking lot in the city of Trogir and P7 is located near the large marina in the urban area of Split. Other stations are located on popular beaches where the number of bathers is high during the bathing season. Stations P4 and P5 are in the Bay of Kaštela on beaches under the influence of small creeks which can be a source of pollution, while stations in the urban area of Split are not directly affected by creeks and rivers, except for station P6, which is located near the mouth of the river Jadro. Stations P8-P11 are located next to the popular pebble beaches around Split coastline.

7.2. Frequency of sampling

Sampling was conducted in two bathing seasons, from June to October 2020 and from April to October 2021. The total of 51 sampling campaigns were conducted fortnightly during the bathing season, as required by the Croatian Regulation on the quality of bathing water [6]. Additional sampling campaigns were conducted after a rain event of more than 2 mm or precipitation. In the case of a rain event, sampling was carried out immediately the next morning, 24 and 72 hours after the first sampling to determine the possible influence of precipitation on the changes in the concentration of indicators of microbiological pollution.

Samples were collected at the sites, from 30 cm depth, using sterile 500-mL screw-cap bottles attached to a sampling rod. Samples were stored in a portable refrigerator to avoid exposure to sunlight and processed immediately upon arrival at the laboratory.

7.3. Data base creation

The data for the sampling campaigns organized in 2020 and 2021 bathing seasons were checked and uploaded to the Watercare cloud (WaterCare/WP3/Data/Split Area).

http://cloud.irbim.an.ismar.cnr.it/index.php/apps/files?dir=/WaterCare/WP3/Data/Split%20Area&fileid=339298

7.4. Analysed data

A total of 51 sampling campaigns were conducted fortnightly during the bathing season, as required by the Croatian Regulation on the quality of bathing water. In the case of a rain event higher than 2mm of precipitation, sampling was carried out immediately the next morning, 24 and 72 hours after the first sampling to determine the possible influence of precipitation on the changes in the concentration of indicators of microbiological pollution. The concentrations of *Escherichia coli* and intestinal enterococci (FIB) were determined at 605 samples in total. Abiotic parameters like temperature, salinity and pH value and meteorological conditions of the sampling sites were also monitored.

Both faecal indicator bacteria were determined using a membrane filtration method. For enumeration of E. coli, the modified method ISO 9308-1:2014 was used. Chromogenic Coliform Agar (CCA) was incubated for 4 hours at 36±2°C and then 20 hours at 44±0.5°C to increase selectivity without negative impact on recovery [13]. For enumeration of intestinal enterococci, the standard method ISO 7899-2:2000 was used [14]. The incubation on Slanetz & Bartley agar at

36°C ±2°C for 44±4 hours, was followed by additional incubation on prewarmed (44°C) Bile Aesculin Azide Agar and incubated at 44±0.5°C for 2 hours.

7.5. Conclusion

The impact of precipitation on the quality of coastal bathing waters was not found in the Split region or Kaštela, probably due to the low amount of precipitation. The quality of bathing waters in the Kaštela area was significantly worse than in the Split area, which is due to the condition of the sewage system in these areas and not to the precipitation effect. It was also found that bathing water quality depends on the timing of sampling and the indicator against which it is assessed. Escherichia coli proved to be a better indicator for early morning sampling, while intestinal enterococci were better for late morning sampling.
Further research should be carried out to develop appropriate and site-specific predictive models in areas where water quality exceedances were recorded. The spatio-temporal variations of FIB must be considered to make the model as reliable as possible. Once the models are developed and validated, they should be offered to the local authority, which can use them to provide early warnings to bathers of potential bathing water pollution.

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Annexes - Description of Croatian sites

Croatian sampling site description

Nr.	Pilot site	Stat_code	X_HTRS	Y_HTRS	LAT	LONG
1.	Cetina	AP_Cetina_1	515172	4811276	43,44124	43,44124
2.		AP_Cetina_2	515766	4812338	43,45079	43,45079
3.		PV_C_0 m	514985	4810921	43,43805	43,43805
4.		PV_C_T1_150m	514840	4810960	43,4384	43,4384
5.		PV_C_T1_300m	514699	4811011	43,43886	43,43886
6.		PV_C_T2_200m	514804	4810837	43,43729	43,43729
7.		PV_C_T3_150m	515011	4810773	43,43671	43,43671
8.		PV_C_Autokamp Zapad	514502	4810976	43,43855	43,43855
1.	Neretva	AP Neretva	577282,3	4764963	43,02055	17,44818
2.		PV_N_0m	576900	4764885	43,01989	17,44348
3.		PV_N_T1_200m	576856,1	4765022	43,02113	17,44296
4.		PV_N_T2_200m	576740,2	4764812	43,01925	17,44151
5.		PV_N_T2_400m	576560,6	4764690	43,01817	17,43929
6.		PV_N_T3_200m	576963,7	4764659	43,01785	17,44423
7.		PV_N_SPRUD 1	576709	4764396	43,01551	17,44107
8.		PV_N_SPRUD 2	576582,1	4764377	43,01535	17,43951
9.		PV_N_PLOČE UŠĆE 1	577778,5	4764907	43,02	17,45426
10.		PV_N_PLOČE UŠĆE 2	577137,7	4764694	43,01815	17,44637

11.		PV_N_PLOČE UŠĆE 3	577165,7	4764672	43,01795	17,44671
12.		PV_N_PLOČE UŠĆE 4	577277,3	4764685	43,01805	17,44808
1.	Raša	AP_Raša	307005,2	4992833	45,04919	14,04994
2.		Krapanj	307025,6	4992700	45,048	14,05025
3.		PV_0	306818,4	4990986	45,03253	14,04828
4.		PV_T1_200m	306654,7	4990867	45,03142	14,04625
5.		PV_T1_400m	306479,9	4990801	45,03078	14,04406
6.		PV_T1_600m	306271,4	4990740	45,03017	14,04144
7.		PV_T2_200m	306669	4990768	45,03053	14,04647
8.		PV_T2_400m	306582,4	4990591	45,02892	14,04544
9.		PV_T3_200m	306838,6	4990794	45,03081	14,04861
10.		PV_T3_400m	306851,1	4990558	45,02869	14,04886
11.		PV_GET_1	307076,2	4989732	45,02133	14,05203
12.		PV_GET_2	307372,1	4989603	45,02025	14,05583
13.		PV_GET_3	307609,5	4989395	45,01844	14,05892
14.		PV_BLAZ_1	306242,7	4988129	45,00669	14,04208

List of meteorological, physical, chemical, and microbial parameters analysed in river and seawater in Croatian target sites

	Method (Manual, Automatic, Lab)	
PARAMETERS	**River**	**Seawater**
Physical chemical		
Air temperature (°C)	AS probe	CTD
Water temperature (°C)	AS probe	CTD
pH	AS probe	CTD
Redox (mV)	AS probe	CTD
Conductivity (mS/cm)	AS probe	CTD
Turbidity (NTU)	AS probe	CTD
Salinity (PSU)	AS probe	CTD
Oxygen saturation (%O_2)	AS probe	CTD
Dissolved O_2 (mg/L)	AS probe	CTD
BOD_5 (mg/L)	LAB	NO
COD_{Mn} (mg/L)	LAB	NO
Ammonium N-NH_4 (µM)	LAB	NO
TN (µM)	LAB	NO
TP (µM)	LAB	NO
Microbiological		
Escherichia coli (CFU/100 ml)	LAB	LAB
Fecal enterococci (CFU/100 ml)	LAB	LAB

Sampling Strategy for Croatian sites

No rainy events	4 samplings	One riverine water sample from the automatic sampling station. RAŠA: 2 monitoring stations. CETINA: 2 monitoring stations. NERETVA: 1 monitoring station.	Seawater samples. RAŠA: 12 monitoring stations CETINA: 6 monitoring stations NERETVA: 11 monitoring stations
Heavy rain events	6 samplings	14 riverine water samples from the automatic sampling station. RAŠA: automatic monitoring station (14 samples), and channel Krapanj (1 sample). CETINA: 2 automatic monitoring stations. NERETVA: 1 automatic monitoring station.	One seawater sample per stations. RAŠA: 12 automatic monitoring stations. CETINA: 6 automatic monitoring stations. NERETVA: 11 automatic monitoring stations.

Raša river sampling was done in in bathing seasons 2020 and 2021.
Cetina and Neretva sites were sampled in bathing season 2021.