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Next generation toolbox for greener pharmaceuticals design and manufacturing towards reduced environmental impact

D7.1-Report on the outcomes of the ENVIROMED monitoring campaigns

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Executive Summary

Deliverable D7.1, *"Report on the outcomes of the ENVIROMED monitoring campaigns"*, provides an integrated overview of the preparation, execution, and preliminary results of the environmental monitoring activities conducted within Work Package 7 (WP7). The principal objectives of WP7 were to demonstrate and validate the performance of the ENVIROMED Wastewater Spectroscopic Analyser (WSA) under real operational conditions and to assess the occurrence, persistence, and behaviour of pharmaceutical micropollutants across different environmental compartments.

The work presented in this report includes Tasks T7.1 to T7.4, encompassing:

- The preparation and methodological definition of monitoring campaign activities and protocols.
- The implementation of the first environmental monitoring campaign in a major clinical facility, focusing on hospital effluents.
- The second campaign assessing pharmaceutical contaminants in the inlet and effluent of the Psyttalia wastewater treatment plant (WWTP).
- The third campaign investigating the marine environment surrounding the Athens WWTP outlet, focusing on water and biota samples.

Across these campaigns, the ENVIROMED consortium successfully demonstrated the operational reliability and analytical capability of the WSA at TRL 6–7, achieving 100% field operational success (7/7 cycles) and >90% quantitative agreement with confirmatory LC/GC-HRMS/MS analyses for selected pharmaceuticals. The WSA consistently detected key compounds such as diclofenac (DCF) and carbamazepine (CBZ) in both hospital and municipal effluents, with SPE enrichment factors exceeding 10,000x in some matrices. This validated its potential as a field-deployable, cost-efficient alternative to laboratory-based methods for near-real-time pharmaceutical monitoring.

Monitoring data revealed pronounced pharmaceutical hotspots in hospital discharges (up to >100,000 ng/L), limited removal efficiencies in conventional WWTP processes, and the persistence of target compounds in treated effluents reaching the marine environment. Preliminary ecotoxicological assessments performed independently by CNR confirmed cellular stress responses in marine organisms even at ng/L exposure levels, highlighting the environmental relevance of the detected concentrations.

The WSA's performance under field conditions confirmed its robustness and ability to operate autonomously within complex wastewater and environmental matrices. However, observed sensitivity drifts linked to temperature fluctuations, matrix interference, and microfluidic cell stability indicate areas for further optimization (e.g. enhanced optical cell configuration and adaptive signal correction).

Overall, the results presented in this deliverable demonstrate the feasibility of continuous, in-situ environmental monitoring using spectroscopic sensing technology and provide critical quantitative evidence supporting the main objectives of ENVIROMED: to advance the detection and understanding of pharmaceutical pollution across the urban water cycle, to validate cost-effective sensing solutions for regulatory monitoring, and to contribute to European policy implementation under the UWWTD (2024/3019) and related environmental directives.

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List of Acronyms

Table 1: Acronyms and abbreviations

Term	Definition
WWTP	WasteWater Treatment Plant
WSA	Wastewater Spectroscopic Analyser
API	Active Pharmaceutical Ingredient
ATR	Attenuated Total Reflectance
BC	Binding Capacity
CBZ	Carbamazepine
DL	Detection limit
DMF	Dimethylformamide
DMSO	Dimethyl Sulfoxide
EF	Enrichment Factor
FT	Fourier Transform
FTIR	Fourier Transform Infrared Spectroscopy
GA	Grant Agreement
GUI	Graphical User Interface
IR	Infrared
LOD	Limit Of Detection
LOQ	Limit of Quantification
BQL	Below the Quantification Limit
MBE	Molecular Beam Epitaxy
DCF	Diclofenac
PI	Phagocytic Index
PR	Phagocytic Rate
MIP	Molecularly Imprinted Polymer
QCL	Quantum Cascade Laser
TRL	Technology Readiness Level
MSF	Multi-Stage Filtration
SPE	Solid-Phase Extraction
EF	Enrichment Factor
KPI	Key Performance Indicators
UWWTD	Urban WasteWater Treatment Directive

Term	Definition
SDL	Screening Detection Limit
LMS	Lysosome Membrane Stability

1 Introduction

1.1 Purpose and scope of the deliverable

Deliverable D7.1 aims to document and critically evaluate the design, execution, and preliminary outcomes of the environmental monitoring campaigns conducted under ENVIROMED WP7. It synthesizes results from Tasks T7.1 to T7.4 and provides the technical and operational basis for the final assessment and policy feedback activities in Task T7.5.

The deliverable specifically reports on the configuration and deployment of the ENVIROMED Wastewater Spectroscopic Analyser (WSA), the development and harmonization of monitoring protocols, and the generation of validated field data across three pilot sites: a hospital wastewater stream, a municipal wastewater treatment plant, and a marine coastal area. These complementary environments represent successive stages of the urban water cycle, enabling direct observation of emission sources, treatment efficiencies, and environmental sinks. Beyond validating instrument performance, these campaigns generated a coordinated dataset that links technological performance with environmental observations providing empirical evidence of pharmaceutical behaviour and persistence in real wastewater and marine systems.

1.2 Relation to WP7 objectives and tasks

WP7 focuses on validating the ENVIROMED sensing platform through real-world environmental monitoring actions and on providing robust data to assess pharmaceutical pollution. The deliverable D7.1 captures the outcomes of:

- T7.1: Preparation and training activities, protocol development, and KPI definition.
- T7.2: Monitoring of hospital effluent at the MITERA clinical site.
- T7.3: Monitoring of WWTP influent and effluent at Psyttalia WWTP.
- T7.4: Monitoring of the marine environment near the WWTP discharge point.

Together, these four tasks establish the framework for demonstrating the functionality, accuracy, and resilience of the ENVIROMED system across diversified environments, paving the way for environmental data integration and regulatory communication under Task 7.5.

1.3 Structure of the document

This report is organized as follows:

- Chapter 2 introduces the environmental monitoring framework, including rationale, targeted micropollutants, and the general monitoring protocol.
- Chapter 3 presents the demonstration and validation of the WSA across all monitoring sites.
- Chapters 4 to 6 summarize the results and key findings from the three environmental campaigns.

- Chapter 7 provides an integrated analysis of cross-campaign data, identifying pollution trends and hotspots.
- Chapter 8 presents a decision support framework for pharmaceutical monitoring campaigns in wastewater systems.
- Chapter 9 concludes with main outcomes and next steps for WP7 and regulatory alignment activities.

2 Environmental Monitoring Framework

2.1 Rationale and scope of monitoring activities

Pharmaceutical micropollutants have emerged as a growing environmental concern due to their persistence, bioaccumulation potential, and diverse biological impacts. Conventional analytical approaches, though accurate, are expensive, time-consuming, and provide limited temporal resolution. The ENVIROMED project addresses this gap through the deployment of a novel Wastewater Spectroscopic Analyser capable of real-time, in situ measurement of targeted contaminants.

The WP7 monitoring activities are designed to validate the analyser's field performance, support calibration through laboratory cross-checks, and generate large-scale data on pharmaceutical contamination across distinct sectors of the urban water cycle. The selected pilot sites from hospital wastewater at origin to marine environmental sinks represent a continuum of pollution pathways and serve as real-world testbeds for the ENVIROMED technology.

2.2 Overview of monitoring sites

Three representative sites were selected for the ENVIROMED campaigns:

- Clinical wastewater (MITERA Hospital, Greece): A large healthcare facility serving as a model of hospital effluent monitoring.
- WWTP inlet and effluent (Psyttalia WWTP, Athens): One of the largest wastewater treatment plants in Europe, representing urban-scale wastewater purification.
- Marine environment (Saronic Gulf, Greece): The receiving environment of WWTP discharge, used to examine pollutant dispersion and bioaccumulation.

Each site poses unique challenges in hydrodynamics, pollutant load variability, and logistical implementation, offering an extensive validation landscape for the ENVIROMED technologies.

2.3 Targeted pharmaceutical micropollutants

The ENVIROMED environmental monitoring activities were designed to focus on a selected set of active pharmaceutical ingredients (APIs) representative of different therapeutic classes and environmental behaviours. Four compounds were originally targeted in the project's monitoring protocol: diclofenac (DCF), carbamazepine (CBZ), metoprolol (MTP), and hydrochlorothiazide (HCT). These substances were selected based on their frequent occurrence in wastewater systems, persistence in aquatic environments, and relevance as indicators of pharmaceutical pollution.

In addition, compound 1H-Benzotriazole (1H-BTR), a corrosion inhibitor commonly found in municipal wastewater, was included in the monitoring plan due to its environmental relevance and persistent behavior.

Table 2 presents the pharmaceutical compounds and the industrial additive, together with their respective abbreviations and their classification according to therapeutic/industrial use. The selection aligns with the overall objectives of the ENVIROMED project and ensures regulatory relevance, as several of these compounds are included in the updated Urban Wastewater Treatment Directive (UWWTD; 2024/3019) watch list.

Table 2: Targeted pharmaceutical compounds in ENVIROMED project and their classification regarding their use

Targeted compounds	Class/Use
Diclofenac – DCF	Nonsteroidal anti-inflammatory drug (NSAID) (used to treat pain and inflammatory diseases)
Carbamazepine - CBZ	Antiepileptic (used in the treatment of epilepsy, neuropathic pain, schizophrenia, and bipolar disorder)
Hydroxychlorothiazide - HCT	Diuretic (used in hypertension and oedema)
Metoprolol – MTP	Beta blocker (used to treat high blood pressure and abnormally fast heart rate)
1H-Benzotriazole - 1H-BTR	Corrosion inhibitor/industrial chemical

During field implementation of the monitoring campaigns, quantitative detection and reliable concentration estimates were consistently achieved for DCF and CBZ across all investigated sites, as these compounds were present at levels within the WSA's calibrated detection range. These two compounds therefore served as the primary performance tracers for validating the ENVIROMED WSA, enabling direct quantitative comparison with LC/GC-HRMS/MS reference measurements (91-92% agreement) under real-world environmental conditions.

MTP and HCT were included in the full analytical workflow (MSF→SPE→spectroscopy) but could not be quantitatively validated due to project timeline constraints limiting extended calibration efforts and the current prototype's instrumental sensitivity limitations for these specific analytes. Despite successful MIP-SPE enrichment (confirmed by laboratory analysis of B-fractions), their post-enrichment concentrations remained below the WSA's effective LOD, preventing statistically robust recovery assessment.

This focused validation strategy on DCF/CBZ provided a robust foundation for assessing the WSA's analytical capability while the comprehensive laboratory characterisation of all five target compounds (190 samples) through accredited methods ensured complete seasonal monitoring coverage per WP7 objectives. The complementary approach WSA for real-time validation, laboratory analysis for full compound profiling maximised scientific output within the available project timeframe.

2.4 Monitoring Protocol and Sampling Methodology

A harmonized monitoring protocol was developed during T7.1 to ensure comparability across sites and to comply with data quality requirements. The protocol covered:

- **Sampling frequency**: Periodic and event-based sampling, complemented by continuous WSA measurements.
- **Sample handling and storage**: Procedures ensuring stability and representativeness of samples.

- **Calibration and validation**: Internal calibration and validation were performed using conventional laboratory analyses to cross-verify WSA results. No third-party validation was conducted.
- **Data collection and management**: Centralized database ensuring traceability, quality assurance, and interoperability with ENVIROMED analytical tools.

This framework ensures that the monitoring campaigns produce comparable, high-quality datasets suitable for statistical analysis, cross-validation, and regulatory use.

3 Demonstration and Validation of the Wastewater Spectroscopic Analyser (WSA)

3.1 Description of the WSA Technology

The WSA is the integrated analytical platform developed within ENVIROMED to enable automated detection of pharmaceutical micropollutants in complex wastewater matrices. It combines three coordinated subsystems: (i) Multi-Stage Filtration (MSF), (ii) Solid-Phase Extraction (SPE), and (iii) mid-infrared spectroscopic analysis.

The full technical design, development rationale, and subsystem construction are documented in Deliverable D4.1 under Task 4.2 and are not repeated here. This section focuses on the demonstration and validation activities performed within WP7, including operator training, site deployment, real-environment demonstrations, and initial operational validation of the analyser.

3.2 Training material and operator preparation

Prior to deployment at the pilot sites, a structured training process was carried out to ensure safe and effective operation of the WSA by non-technical personnel. Training materials included:

- **The official WSA User Manual**, covering subsystem descriptions, safety instructions, setup procedures, maintenance steps (filter replacement, MIP replacement), and WebUI operation.
- **Hands-on demonstrations conducted at the Pilot sites,** providing operators with practical instruction on start-up procedures, sequence execution, system shutdown, and routine inspections.
- **Safety training**, particularly regarding handling of methanol, laser operation, and mechanical hazards.
- **Web-based User Interface training**, including interpretation of sequence status, absorption measurements, and automated logs.
- **Troubleshooting guidance** for common operational scenarios such as pressure anomalies, filter depletion and MIP cartridge replacement.
- **A demonstrative video of the WSA**, produced in the laboratory, showcasing the operation and interaction of the three subsystems, which served as an additional visual training and reference tool

Operators at both pilot sites reported that the training and documentation were clear, practical, and sufficient for independent operation of the WSA.

3.3 Laboratory demonstrations: spectroscopic and sample preparation sub-system performance

Laboratory demonstrations were completed for the WSA in two-parts: the initial demonstration exhibited the optical spectroscopic sub-system, and the final demonstration exhibited the multi-stage filtration sub-system and the SPE sub-system. The former had HORIBA present to

observe the operation of the system and obtain LOD values from spectroscopic measurements performance. The latter had Fraunhofer present to observe the fluidic operation of both SPE & multi-stage filtration sub-system in support of previously analysed results. Both demonstrations were detailed in Deliverable 4.1 and as such will only be summarised in this section.

The spectroscopic sub-system utilised a commercial (MIR-CAT) QCL as the frequency-tuned emission source in the mid-infrared with a VIGO detector used to monitor the transmitted power. A custom-built flow-cell was employed for interaction of the mid-infrared emission with the substance to be analysed. This consisted of an optical fibre running alongside a fluid through-pipe in a self-contained package. Monitoring the VIGO detector output as a function of the calibrated QCL frequency as it was tuned provided a spectroscopic reading on the fluid. Note that it was intended that the ALPES laser replace the commercial QCL for validation & Pilot activities.

The sub-system was demonstrated to observers from HORIBA to both provide training on operation and to provide comparative performance measurements alongside alternative techniques. Operation was demonstrated by performing a full spectral sweep and background subtraction of pure methanol samples. It should be noted that this technique was not that applied latterly after integration that relied on a single spectral point analysis. The exhibited performance was comparable to synchronous measurements applied to the same samples made by a commercial UV/Vis spectrometer. Further details on the performance are available in Deliverable 4.1.

The MSF and SPE sub-systems prepare the sample for spectral analysis by removing particulate contaminants and concentrating specific marker APIs by MIP technology. The MSF system allows for controlled filtering of an inlet sample to remove particulates on the scale of µm to prevent interference in the downstream sub-systems. The SPE sub-system is based upon controlled application of water dissolved APIs to a MIP which acts as a selective sieve, and then a solvent (methanol) is applied to extract the APIs in a smaller volume of fluid for a higher concentration of solution. There is also interlinking pumping stages to link to the spectroscopic sub-system and overall GUI control of the system.

Both sub-systems were demonstrated to an observer from Fraunhofer to provide training on operation and show the performance in principle. No verification of performance was provided on the date due to no spectroscopic sub-system. The system was observed to operate in the stages expected with a sample produced for spectral analysis subsequently to operation. Verification of previously produced samples was provided on the date; micro-plastic particles were shown to be removed using microscopy techniques prior to and after the multi-stage filtration process, and successful pre-concentration of APIs was shown by spectral analysis of SPE prepared samples.

Third-party validation was planned late in the project to provide comprehensive performance evaluation and operational approval. Given the late-stage integration of the ALPES QCL and the priority to demonstrate the complete WSA under real operational conditions at the MITERA and EYDAP pilot sites, it was not feasible to complete this additional validation within the project timeframe. This strategic decision does not compromise the objectives or validity of the present deliverable, as WP7 already provides field-based validation evidence through direct comparison of WSA measurements with accredited external laboratory analyses under authentic wastewater conditions. Instead, it reflects a deliberate focus on early operational deployment, which is particularly valuable at the current TRL stage. Beyond the

project, a dedicated full-system validation campaign, including independent third-party assessment, is recommended to further consolidate performance metrics and support future upscaling and certification activities.

3.4 Configuration and deployment at the pilot site

The WSA was deployed at two representative operational environments: a healthcare facility (MITERA Hospital) and a municipal wastewater treatment installation (EYDAP/Psytallia). At both locations, the complete analyzer comprising the MSF module, the SPE subsystem, and the mid-infrared spectroscopic unit was installed in situ, configured for on-site operation. The final deployed configurations at the two pilot sites are shown in Figure 1 (MITERA hospital) and Figure 2 (EYDAP/ Psytallia).

Figure 1: Installation of WSA at MITERA hospital

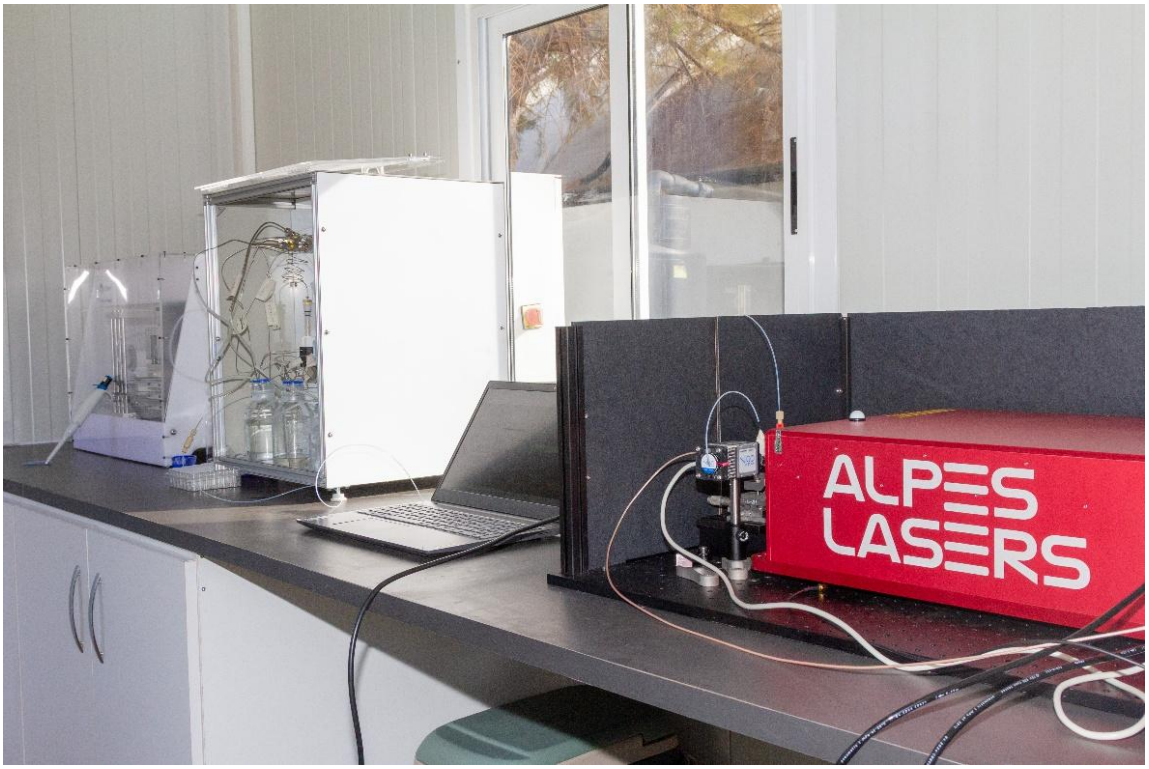

Figure 2: Installation of WSA at EYDAP PSYTALLIA ISLAND.

Following deployment, the WSA was operated under real wastewater conditions to verify core system performance, assess behavior in non-laboratory environments, and familiarize pilot-site personnel with the analyzer's workflow. During these sessions, the instrument executed fully automated sequences, including sample intake, MSF filtration, solid-phase extraction, washing and elution steps, and subsequent mid-infrared spectroscopic measurements. Real wastewater samples with varying suspended solids, turbidity, and chemical composition were processed, allowing direct observation of the stability and responsiveness of the filtration and extraction stages under authentic operational conditions.

Operators supervised the sequences via the Web-based User Interface, which provided real-time status information, sub-system activity indicators, and automated alerts where relevant. The system successfully recorded absorption signals during spiked demonstration runs, including sample–reference cycles and baseline measurements generated by the spectroscopic sub-system. These runs also enabled verification of sub-system synchronisation, in particular the coordination between MSF flow, SPE cartridge operation, and the spectroscopic acquisition window.

Overall, these pilot-site demonstrations confirmed that the WSA is capable of autonomous, end-to-end analytical operation and of completing full measurement cycles in the presence of complex wastewater matrices, providing a solid basis for the subsequent quantitative validation presented in Section 3.6.

3.5 Results from the pilots

3.5.1 Sampling strategy and sample characterization

Pilot validation of the WSA was conducted using seven wastewater samples collected from hospital (MITERA) and municipal (EYDAP/Psyttalia) (see Table 3). To assess both the effect of the MIP-SPE enrichment step and the agreement with external laboratory data, a systematic A/B sampling strategy was applied to each wastewater matrix:

- **Sample A**: Aliquot of the untreated wastewater matrix, analysed by the external laboratory to determine native pharmaceutical concentrations (ng/mL).
- **Sample B**: Aliquot of the same wastewater matrix, processed through complete WSA workflow (MSF → SPE using MIPs → mid-IR spectroscopic analysis), generating an enriched eluate in methanol for WSA measurement and external laboratory cross-check.

Table 3: Wastewater samples analysed with the WSA sensor during the monitoring campaign

Sample	Sampling date	Site	Origin	Analytical focus
1A/1B	19/11/2025	MITERA	Hospital	DCF (non-spiked sample)
2A/2B	19/11/2025	MITERA	Hospital	DCF (spiked with 35 ng/mL)
3A/3B	11/08/2025	MITERA	Hospital	DCF (non-spiked sample)
4A/4B	13/10/2025	MITERA	Hospital	CBZ (non-spiked sample)
5A/5B	06/10/2025	MITERA	Hospital	CBZ (non-spiked sample)
6A/6B	06/10/2025	MITERA	Hospital	CBZ (spiked with 50 ng/mL)
7A/7B	30/10/2025	EYDAP	Municipal	DCF (non-spiked sample)

In this context, "**enriched**" refers to the B-fraction eluates obtained after MIP-SPE pre-concentration, where APIs present in the A-fraction are selectively retained on the MIP and subsequently eluted in a smaller solvent volume, leading to significantly higher analyte concentrations than in the original wastewater. Approximately 1 mL of methanolic eluate was collected after SPE for mid-infrared evaluation and quantification of these enriched analytes.

The pilot campaign included both non-spiked and spiked matrices to evaluate WSA performance under authentic and controlled concentration conditions:

- ✓ **Samples 1 and 2** (MITERA, DCF): Originated from the same hospital wastewater matrix.
  - 1A/1B: Non-spiked hospital wastewater (A: native concentration; B: enriched eluate), used as non-spiked reference for DCF.
  - 2A/2B: Aliquots of the same matrix spiked with DCF (35 ng/mL) prior to SPE (A: spiked wastewater; B: enriched spiked eluate), enabling direct evaluation of enrichment and detection performance.

- ✓ **Samples 3 - 6** (MITERA, DCF and CBZ): Additional hospital wastewater matrices collected at different dates.
  - 3A/3B: Hospital wastewater focusing on DCF.
  - 4A/4B: Hospital wastewater focusing on CBZ (native levels).
  - 5A/5B: Non-spiked hospital wastewater focusing on CBZ.
  - 6A/6B: Same matrix as 5, spiked with CBZ (50 ng/mL) prior to SPE to assess enrichment and detection at higher CBZ levels.

- ✓ **Sample 7** (EYDAP/Psyttalia, DCF): Municipal wastewater matrix (7A/7B) used to evaluate WSA performance under mixed urban conditions.

This design allowed within-matrix comparisons (non-spiked vs spiked, A vs B fractions) to disentangle enrichment performance from instrumental sensitivity and to quantify WSA-laboratory agreement.

Selected A/B samples were also analysed by external laboratories using liquid and gas chromatography coupled to high-resolution mass spectrometry (LC/GC-HRMS/MS) to provide reference measurements for both native (A) and enriched (B) fractions. Table 4 summarizes the screening of wastewater samples and the concentrations detected by the external laboratory for the main target compounds, while Table 5 extends this to 12 pharmaceutical analytes, enabling multi-analyte assessment of the MIP-SPE enrichment performance.

Table 4: Wastewater sample screening - Concentrations detected by external laboratory (ng/mL)

Sample	CBZ (ng/mL)	MTP (ng/mL)	DCF (ng/mL)	HCT (ng/mL)	1-BTR[1] (ng/mL)	5-Me-BTR[2] (ng/mL)
1A	<LOD	0.0726	0.242	<LOD	88.3	0.19
2A	0.0690	<LOQ	24.5	<LOD	72.4	0.19
3A	14.9	1.07	2.27	0.539	0.34	6.52
4A	<LOD	0.07	3.20	<LOD	1.15	1.81
5A	<LOD	0.07	3.28	<LOD	0.268	15.3
6A	222	0.10	3.94	<LOD	0.332	15.9
7A	5.88	0.86	1.66	2.02	8.39	0.85

[1]1-BTR: 1H-Benzotriazole, [2]5-Me-BTR: 5-(or 6)-methyl-Benzotriazole.

Table 5: Comprehensive Analyte Screening (External Lab)

Concentration (ng/mL)	CBZ	MTP	DCF	HCT	CLR	AMS	VLF	CDS	IRB	CTL	1-BTR	5-Me-BTR
1A	<LOD	0.0726	0.242	<LOD	<LOQ	0.0388	0.0330	0.129	0.0511	0.06	88.3	0.19
2A	0.0690	<LOQ	24.5	<LOD	<LOQ	0.0210	<LOQ	<LOQ	0.0377	0.03	72.4	0.19
3A	14.9	1.07	2.27	0.539	<LOQ	0.0157	0.217	<LOQ	0.762	0.40	0.34	6.52
4A	<LOD	0.07	3.20	<LOD	<LOQ	0.0153	0.0502	0.193	0.0369	2.87	1.15	1.81
5A	<LOD	0.07	3.28	<LOD	<LOQ	0.0128	0.0675	<LOD	0.00342	0.837	0.268	15.3
6A	222	0.10	3.94	<LOD	<LOQ	0.0126	0.121	<LOQ	0.0294	0.806	0.332	15.9
7A	5.88	0.86	1.66	2.02	0.41	1.39	0.801	0.425	2.60	0.668	8.39	0.85
1B	360	92.6	3009	24.2	<LOQ	0.00401	<LOQ	<LOQ	0.0177	0.0210	42.9	<LOQ
2B	236	57.9	2386	13.9	<LOQ	0.00372	<LOQ	<LOQ	0.0226	0.0176	55.0	0.123
3B	307	48.1	2274	16.5	<LOQ	0.0141	0.141	0.214	8.61	8.92	10.9	18.1
4B	171	15.2	1484	5.28	<LOQ	0.0235	0.307	0.563	0.332	15.0	2.83	8.16
5B	318	49.0	5134	22.1	<LOQ	0.0978	0.814	<LOQ	0.118	10.1	1.50	50.2
6B	692	7.41	778	1.79	<LOQ	0.0441	0.441	3.00	0.0468	5.74	2.02	13.0
7B	53.1	10.4	846	31.4	9.33	11.0	3.45	0.201	40.9	11.4	24.5	69.1
Instrumental LOD	0.016	0.011	0.0708	0.0800	0.010	0.0020	0.0058	0.042	0.0032	0.0062	0.062	0.028

Carbamazepine (CBZ), Metoprolol (MTP), Diclofenac (DCF), Hydrochlorthiazide (HCT), Clarithromycin (CLR), Amisulpride (AMS), Venlafaxine (VLF), Candesartan (CDS), Irbesartan (IRB), Citalopram (CTL), 1H-Benzotriazole (1-BTR), 5-(or 6)-methyl-Benzotriazole, 5-Me-BTR

3.5.2 Comparative performance analysis: WSA vs External Laboratory

- **Quantification Results - DCF**

Table 6 presents the quantification of DCF obtained using the WSA sensor and the corresponding reference values measured by the external laboratory.

Very good agreement for samples 2B and 3B:

- **Sample 2B:** WSA = 2174.65 ng/mL vs. Lab = 2386 ng/mL (≈91% agreement).
- **Sample 3B:** WSA = 2616.61 ng/mL vs. Lab = 2274 ng/mL (≈92% agreement).

These results demonstrate a strong quantitative correlation between WSA measurements and external laboratory data for enriched eluates.

Samples 1B - same order of magnitude**:**

WSA = 1000 ng/mL vs. Lab = 3009 ng/mL. While the relative deviation is higher, both methods indicate the same concentration range after enrichment, confirming the ability of

the WSA to correctly capture strongly elevated DCF levels in non-spiked hospital wastewater.

Sample 7B - Discrepancy due to non-detection:

For Sample 7B, the external laboratory reported a concentration of 846 ng/mL, while the WSA did not detect DCF (n.d.).

This discrepancy is attributed to instrumental sensitivity limitations of the current WSA configuration for this specific matrix and concentration range. It indicates that, under certain conditions, the effective WSA LOD for DCF in enriched municipal eluates remains above ~800 ng/mL, whereas hospital matrices with higher levels were successfully quantified.

Table 6: DCF quantification: WSA sensor vs. external laboratory analysis

Sample	WSA MC[1] (ng/mL)	WSA Detection	External Lab MC (ng/mL)	Agreement	Notes
1A (non-spiked)	-	Not analyzed	0.242	-	Background level
1B (enriched)	1000	✓ Detected	3009	High enrichment	EF = 12433
2A (spiked +35 ng/mL)	-	Not analyzed	24.5	-	Background level
2B (enriched)	2174.65	✓ Detected	2386	Excellent	EF = 97.39
3A (hospital)	-	Not analyzed	2.27	-	Low native concentration
3B (enriched)	2616.61	✓ Detected	2274	Very Good	EF = 1004.76
7A (municipal)	-	Not analyzed	1.16	-	Low concentration
7B (enriched)	n.d.	Not detected	846	No detection	WSA limitation

[1]MC: Measured concentrations, EF: Enrichment Factor, [3]n.d.: not detected., [4]LOD: Limit of detection

Enrichment performance:

Figure 3 shows DCF concentrations before (A, blue bars) and after enrichment (B, orange bars) for four selected wastewater samples (1, 2, 3, 7). In all cases where B-fractions were analyzed, the enriched concentrations are substantially higher than the corresponding A-fraction levels measured by the external laboratory, confirming that the MIP-SPE extraction effectively concentrates DCF prior to WSA measurement.

The right panel of Figure 3 reports the calculated enrichment factor for each sample. Most samples exhibit high EF values (>700–10,000), indicating strong and reproducible pre-concentration performance of the MIP-SPE method. Sample 2 exhibits a notably lower EF (~97) compared to the other samples, which can be explained by its initially higher DCF concentration, likely due to matrix effects. When the starting concentration is already elevated, the relative increase achieved through the enrichment procedure appears smaller, resulting in a lower EF, even though the absolute DCF concentration after enrichment remains high.

The combined evaluation of pre- and post-enrichment concentrations and EF values demonstrates the high enrichment capability of the MIP-SPE process. At the same time, the relatively wide EF range (700–10,000) highlights some inter-sample variability, suggesting that future work should focus on refining calibration procedures and stabilizing enrichment efficiency to further strengthen quantitative consistency across diverse wastewater matrices.

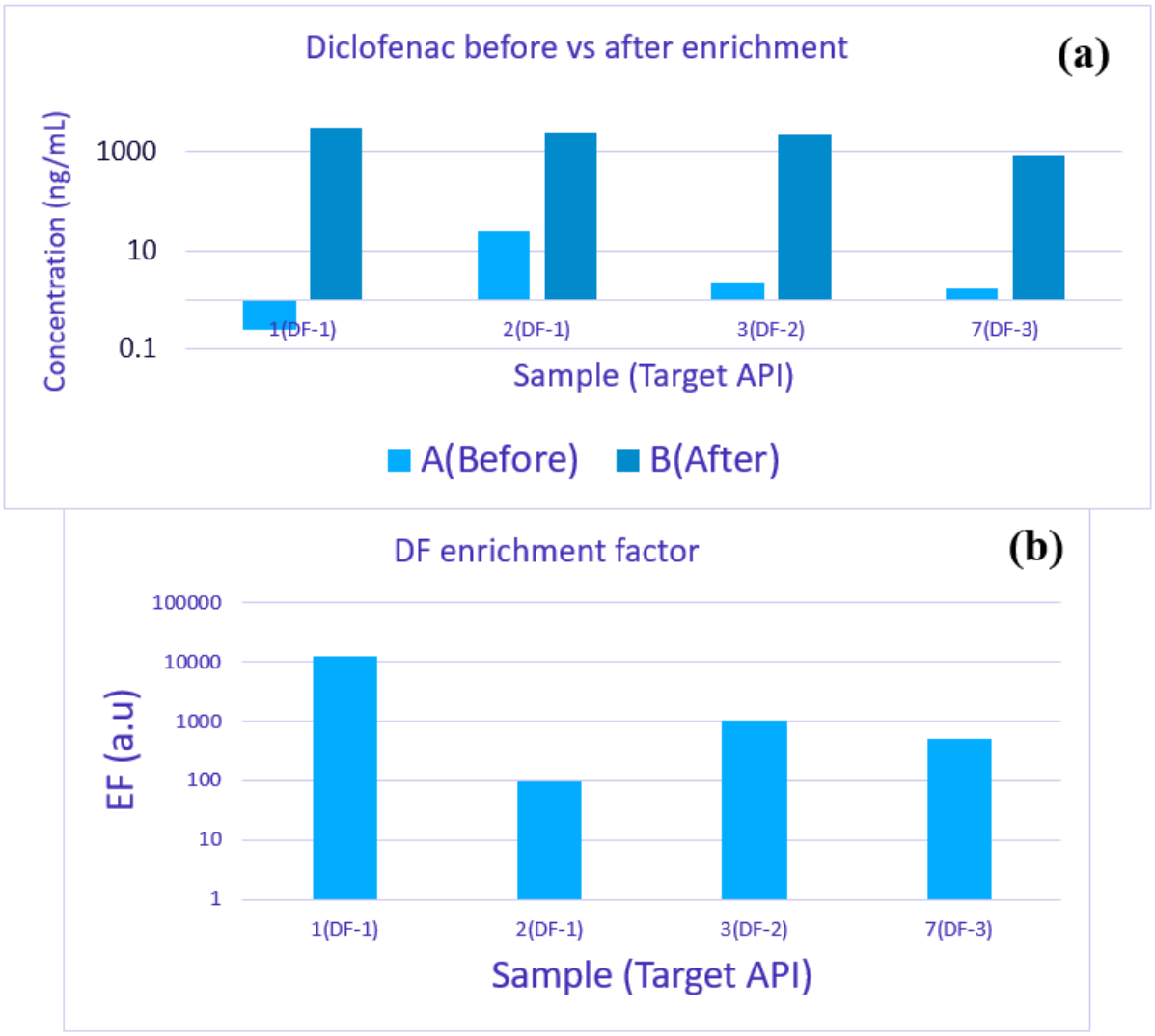

Figure 3: DCF pre- and post-enrichment concentrations (a) and enrichment factors across samples (b).

- **Quantification Results - CBZ**

Table 7 presents the quantification results for CBZ obtained using the WSA sensor, alongside the reference concentrations measured by the external laboratory.

Consistent non-detection by WSA:

Across all enriched fractions (B samples), the WSA sensor consistently reported *n.d.* for CBZ, whereas the external laboratory detected concentrations in the range of **171-692 ng/mL**. This pattern indicates a systematic detection limitation on the WSA side.

Instrumental limit of detection (LOD) constraints:

- **WSA instrumental LOD for CBZ:** 950 ng/mL (based on May 2025 laboratory demonstrations)
- **External laboratory LOD for CBZ:** 0.016 ng/mL
- **LOD discrepancy:** The WSA detection limit is approximately **59,000× higher** than that of the external laboratory. This substantial difference explains the inability of the WSA sensor to detect CBZ at the concentration levels present in the enriched samples.

The non-detection of CBZ by the WSA sensor is therefore attributed to its high instrumental LOD rather than to poor performance of the extraction/enrichment process. The enrichment factors obtained (up to **EF = 19875 times**) demonstrate that the MIP-SPE pre-concentration pathway performs effectively for CBZ.

Table 7: CBZ quantification: WSA sensor vs. external laboratory analysis

Sample	WSA MC[1] (ng/mL)	WSA Detection	External Lab MC (ng/mL)	Agreement	Notes
4A	-	Not analyzed	<LOD	-	-
4B (enriched)	n.d.[2]	Not detected	171	No detection	EF = 10687.5
5A	-	Not analyzed	<LOD	-	-
5B (enriched)	n.d.	Not detected	318	No detection	EF = 19875
6A (spiked +50 ng/mL)	-	Not analyzed	222	-	-
6B (enriched)	n.d.	Not detected	692	No detection	EF = 3.12

[1]MC: Measured concentrations, [2]n.d.: not detected. [4]LOD: Limit of detection (for CBZ from Laboratory demonstrations of WSA done in May 2025 see Table A2 (appendix)).

Recommendation for improved performance: To enable CBZ detection (LOD 950 ng/mL → target <200 ng/mL), mid-IR spectroscopic optimization is essential, specifically addressing flow-cell robustness issues observed during field deployment, biofouling accumulation, optical window degradation, and limited pathlength (5-10 cm), alongside lock-in amplification implementation and QCL stabilization for >10x SNR improvement. Enhanced flow-cell design with anti-fouling coatings, self-cleaning mechanisms, and extended path length (15-20 cm) will ensure stable long-term operation while boosting sensitivity for CBZ detection in enriched eluates.

Enrichment performance: Figure 4a compares CBZ concentrations before (A, blue) and after (B, orange) WSA processing (MSF filtration + MIP-SPE enrichment), showing effective analyte preconcentration from ng/L to ng/mL levels despite variable background concentrations. Figure 4b presents enrichment factors achieving 3.12 to 19,875 across samples,

with consistently high performance (>10,000 for samples 4B-5B) demonstrating robust MIP selectivity even in complex hospital wastewater.

The notably lower EF in sample 6B (spiked matrix) reflects saturation at high initial concentrations, confirming the finite selective binding capacity of the MIP. Overall, the EF trends indicate that while the MIP-SPE process is highly effective and reproducible at low-to-moderate analyte levels, enrichment efficiency decreases at elevated concentrations highlighting an area for ongoing optimization to further improve consistency and quantification reliability prior to IR spectroscopic measurement.

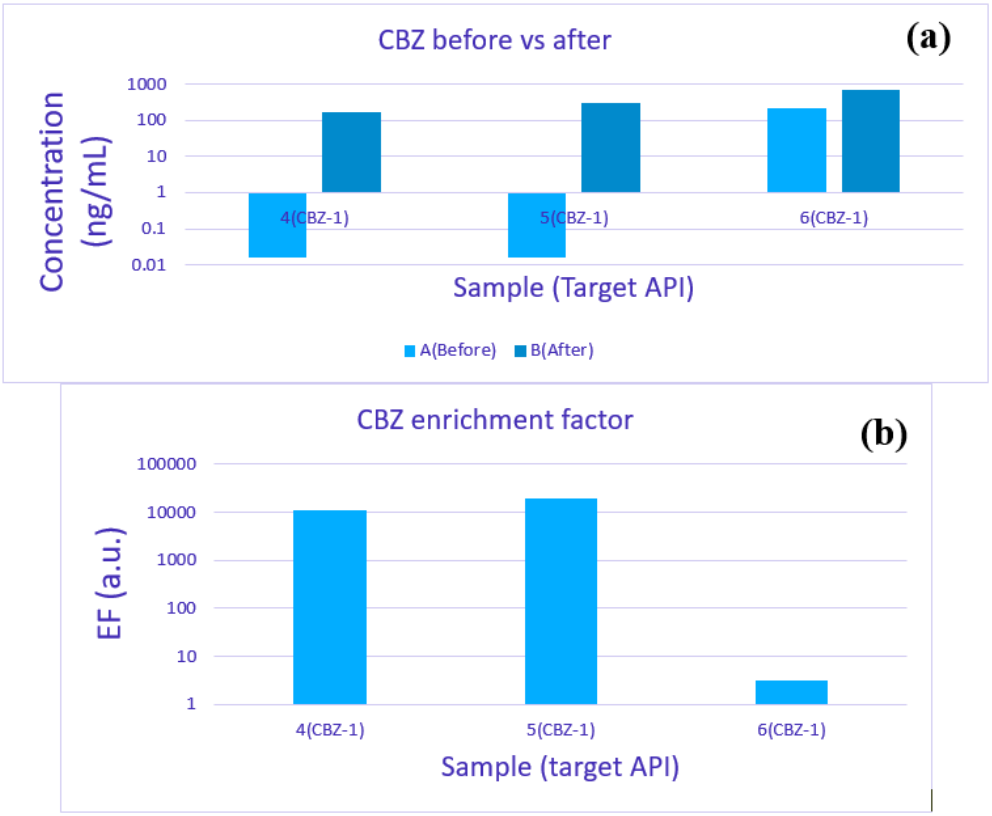

Figure 4: CBZ pre- and post-enrichment concentrations (a) and enrichment factors across samples (b). Concentrations reported as <LOD assumed to be equal to the LOD value.

Based on data from Table 5, Figure 5 shows the enrichment factors achieved for four target APIs (DCF, CBZ, MTP, and HCT) across different SPE samples. It demonstrates that the SPE device exhibits high selectivity and efficiency for DCF and CBZ, with enrichment factors often exceeding 10,000 in several samples, even in the presence of competing compounds MTP and HCT. In contrast, MTP and HCT show lower EF, indicating weaker interaction with the MIP-SPE phase or competitive effects.

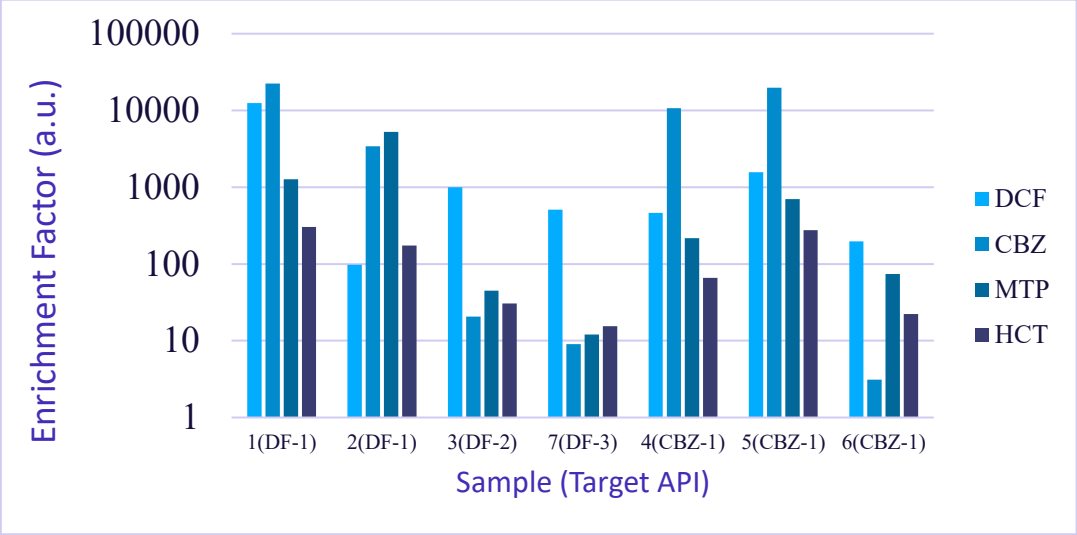

Figure 5: Multi-analyte enrichment factors achieved by WSA SPE process

Overall, the results highlight that the SPE system is particularly effective for the selective extraction and concentration of DCF and CBZ from complex mixtures containing multiple APIs.

3.6 Analytical Performance Indicators (WP7 KPIs)

The results obtained during the pilot demonstrations were evaluated against the key performance indicators (KPIs) defined within WP7, focusing on operational robustness, analytical functionality, and readiness for real-world deployment.

System robustness and operational stability: Across all seven pilot samples, the WSA successfully completed full automated analytical cycles without operator intervention. The system demonstrated stable performance under varying wastewater characteristics, including differences in turbidity, solids content, and chemical composition. No critical failures were observed during filtration, extraction, or spectroscopic acquisition, confirming the robustness of the integrated MSF–SPE–MIR workflow.

Analyte enrichment and matrix reduction: Comparison of raw samples (A fractions) with processed samples (B fractions) demonstrated effective concentration of the target APIs, as reflected by the EF obtained for DCF and CBZ. For DCF, EF values ranged from 97.39 (Sample 2B) to 12,433 (Sample 1B), with Samples 3B and 7B showing 1004.76 and 729.31, respectively, yielding a mean EF of approximately 3,566 and indicating excellent concentration performance. For CBZ, EF values were 10687.5 (Sample 4B), 19875 (Sample 5B), and 3.12 (Sample 6B), with a mean EF of ~11522, demonstrating exceptional concentration enrichment. Although CBZ was not detected by MIR spectroscopy due to instrumental limit of detection constraints, the high enrichment factors confirm that the SPE-MIP extraction pathway operated optimally, indicating that the observed analytical limitation is attributable to the IR detection sensitivity rather than the sample preparation process.

Selectivity and targeted detection: The use of dedicated MIP cartridges allowed selective extraction of specific APIs (DCF or CBZ) from complex wastewater matrices. Targeted analysis of DCF in Samples 2, 3, and 7, and CBZ in Samples 4–6, demonstrated the suitability of the WSA for compound-specific monitoring in mixed pharmaceutical environments.

Reproducibility and repeatability:
The system exhibited strong reproducibility in DCF extraction and detection. Samples 2B and 3B showed excellent inter-sample reproducibility, and A/B paired measurements supported internal verification of each analytical cycle. No significant instrumental drift or operator-related variability was observed. However, variations in enrichment performance were noted between samples, likely linked to differences in initial concentration and matrix composition. These concentration-dependent effects may influence the calibration curve and should be examined further to establish more robust quantitative calibration models.

Data traceability and validation:
For DCF (Samples 1B, 2B, 3B), excellent agreement was observed between WSA and external laboratory results with around 90% correlation for Samples 2B and 3B confirming traceability to LC/GC-HRMS/MS reference methods.

For CBZ (Samples 4B–6B), the WSA did not detect the analyte due to instrumental detection limits, yet the enrichment factors verified the integrity of the extraction step. Future improvements, particularly an enhancement of MIR sensitivity and calibration refinement considering enrichment variability, are recommended to strengthen quantitative reliability and extend compound coverage.

3.7 Validation under Real-World Conditions and TRL Advancement

The successful operation of the WSA in both hospital and municipal wastewater environments demonstrates its readiness for real-world deployment beyond laboratory conditions. The analyser operated autonomously, was handled by non-technical personnel following training, and processed authentic wastewater samples with minimal operational constraints.

These pilot results confirm that the WSA has reached a higher level of technological readiness, moving from laboratory validation to system demonstration in relevant operational environments. The outcomes of WP7 provide a solid foundation for further optimisation, extended validation campaigns, and potential future deployment for continuous monitoring of pharmaceutical micropollutants in wastewater.

3.8 Feedback from End-User

Operators at the pilot sites provided structured feedback based on their experience:

- The **WebUI was intuitive**, with clear step progress indicators and simple controls.
- Maintenance tasks such as **filter and MIP replacement** were manageable following the training.
- Users appreciated the **transparent view of subsystem activity**, including valves, pump states, and laser readiness.

This feedback directly informs refinements planned for future iterations of the analyzer.

3.9 Recommendations and Next Steps

To advance WSA capabilities, short-term priorities include further mid-infrared spectroscopic optimization specifically refining flow-cell design (increasing optical path length), implementing lock-in amplification to achieve over a tenfold improvement in signal-to-noise ratio, and improving QCL tuning precision to reduce the CBZ limit of detection below 200 ng/mL.

In parallel, a dedicated short-term study on EFs is recommended to better understand their variability and role in calibrating the technology for inlet water concentrations, thereby ensuring more accurate quantification across different matrices. Additional short-term actions involve developing multi-analyte SPE automation with rapid cartridge switching to enhance analytical throughput.

Medium-term developments should focus on integrating advanced data analytics such as machine learning for improved interpretation of complex wastewater spectra, implementing real-time alert mechanisms for threshold exceedance events, and engaging with regulatory bodies to contribute to the standardisation of field-based pharmaceutical monitoring.

In the long term, the deployment of continuous monitoring networks across wastewater treatment facilities, the creation of a centralised data repository for pharmaceutical occurrence trends, and the provision of evidence-based input to support environmental policy will enable large-scale adoption and regulatory impact of the WSA technology.

4 Environmental Monitoring Campaign 1 – Clinical Facilities Wastewater

4.1 Site Description and Operational Context

The first environmental monitoring campaign was conducted at MITERA Hospital, one of the largest hospitals in Greece (Figure 6). The hospital comprises 11 floors and covers a total area of approximately 30,000 m², housing three main clinics: General, Gynaecological, and Paediatric. Owing to its size and operational profile, MITERA serves as a representative case study of a large hospital operating in a European urban context. The hospital is located near the centre of Athens and is connected to the city's municipal sewage network.

Figure 6: MITERA Hospital Premises

4.2 Sampling Activities – Sample Collection and Preparation

To measure and assess the presence of pharmaceutical micropollutants in the wastewater stream of clinical facilities and to correlate concentration peaks and trends with seasonal variability (Task 7.2), wastewater samples were collected from the sewage system of MITERA Hospital. The results of the assessment provide insight into pharmaceutical levels in the hospital's wastewater and improve understanding of how effluents from large healthcare facilities contribute to pharmaceutical-related pollution. In addition, the findings support the validation of the novel WSA developed within the ENVIROMED project.

To achieve the objectives of Task 7.2, a total of fifty-six wastewater samples were collected between December 2024 and October 2025, following the sampling plan presented in Table 8. The sampling period was extended beyond M39 (August 2025), which was the initially anticipated end of Task 7.2. This extension aimed to generate a more comprehensive monitoring dataset of pharmaceutical compounds and to obtain more recent samples for

analysis with the WSA during the validation phase. The sampling frequency was intentionally set at a high level to enable the identification of potential seasonal variations throughout the monitoring period, spanning from winter 2024 to autumn 2025.

Table 8: Sampling plan of MITERA Hospital

Season	Dates	Season	Dates
Winter	02/12/2024	Summer	2/6/2025
	04/12/2024		2/6/2025
	06/12/2024		9/6/2025
	09/12/2024		16/6/2025
	23/12/2024		23/6/2025
	06/01/2025		30/6/2025
	20/01/2025		7/7/2025
	06/02/2025		7/7/2025
	6/2/2025		14/7/2025
	10/2/2025		16/7/2025
	17/2/2025		18/7/2025
	24/2/2025		21/7/2025
	26/2/2025		28/7/2025
	28/2/2025		4/8/2025
Spring	2/3/2025		11/8/2025
	3/3/2025		18/8/2025
	10/3/2025		25/8/2025
	17/3/2025	Autumn	1/9/2025
	24/3/2025		8/9/2025
	31/3/2025		15/9/2025
	7/4/2025		22/9/2025
	14/4/2025		29/9/2025
	21/4/2025		6/10/2025
	28/4/2025		13/10/2025
	5/5/2025		20/10/2025
	12/5/2025	Total Samples	56
	19/5/2025		
	21/5/2025		
	23/5/2025		
	25/5/2025		
	26/5/2025		

As mentioned already, monitoring of the pharmaceutical load focused on four target compounds: DCF, CBZ, HCT, and MTP. In addition, compound 1H-Benzotriazole (1H-BTR), a corrosion inhibitor commonly found in municipal wastewater, was included in the monitoring plan due to its environmental relevance and persistent behavior.

Following EYDAP's market research, the Laboratory of Analytical Chemistry of the Department of Chemistry at the National and Kapodistrian University of Athens (NKUA/TraMS) was selected to carry out the analysis of the selected samples.

According to what was agreed at Task 7.1, all wastewater samples were collected in high-density polyethylene bottles and stored in accordance with the established protocol for internal analysis using the WSA at the pilot site, as well as for parallel testing at the external Analytical Chemistry Laboratory of the National and Kapodistrian University of Athens (NKUA/TraMS).

4.3 Results and Discussion - External Laboratory (NKUA/TraMS) Results

A total of fifty-six samples (Table 9) were collected over the annual monitoring campaign carried out between December 2024 and October 2025.

Table 9: Detection Frequency of the targeted compounds in MITERA's wastewater during the overall sampling period

Compound	Detects	Total	Detect rate (%)	Non-detects
CBZ	41	56	73.2%	15
MTP	44	56	78.6%	12
DCF	55	56	98.2%	1
HCT	55	56	98.2%	1
1H-BTR	33	56	58.9%	23

Median concentrations of the five target compounds, calculated for each season as well as for the entire monitoring period, in MITERA's wastewater are presented in, while the complete dataset is provided in Table 10. The temporal trends of the target compounds are illustrated in Table 11. Results reported as below the LOD or below the LOQ were considered non-quantified. For time-series and seasonal trend analyses, these values were treated as non-detects (i.e. excluded from numerical summaries), to avoid introducing artificial bias from imputed low values.

Table 10: Median concentrations (ng/L) of the targeted compounds in MITERA's wastewater during the overall sampling period and the discrete seasons

Sampling period	ng/L				
	CBZ	MTP	DCF	HCT	1H-BTR
Winter	100	2259	1107	2027	11705
Spring	97	421	481	2303	1181
Summer	33	5765	2936	1518	19297
Autumn	6497	1543	1857	1483	<LOD, <LOQ
Overall	97	2648	1669	1903	11705

Table 11: Concentrations (ng/L) of the targeted compounds in MITERA's wastewater during the overall sampling period

Sampling Date	ng/L				
	CBZ	MTP	DCF	HCT	1H-BTR
2/12/2024	<LOD	<LOD	<LOD	571	105924
4/12/2024	<LOD	<LOD	352	526	149041
6/12/2024	69.5	3030	1868	11234	11215
9/12/2024	<LOQ	<LOQ	1905	864	91090
23/12/2024	87.4	4373	1601	6199	25325
6/1/2025	<LOQ	631	577	1365	74033
20/1/2025	78.2	4296	3134	1660	4155
6/2/2025	68.4	3480	1107	11760	4079
6/2/2025	107	800	563	21738	596
10/2/2025	92	490	628	2033	LOD
17/2/2025	129	1488	303	2020	LOQ
24/2/2025	260	5151	8308	13444	LOD
26/2/2025	67	LOD	294	401	11705
28/2/2025	89	1159	1397	4448	747
2/3/2025	97	1728	805	2303	LOQ
3/3/2025	170	8481	1958	4394	1174
10/3/2025	92	2686	1118	5967	LOQ
17/3/2025	98	234	463	1903	LOQ
24/3/2025	91	7298	1132	3820	LOD
31/3/2025	96	754	1689	1052	23146
7/4/2025	98	190	481	4133	LOD
14/4/2025	93	183	650	5407	632
21/4/2025	14659	2730	21084	2377	LOD
28/4/2025	141	214	1165	6582	LOQ
5/5/2025	85	LOD	381	3036	LOQ
12/5/2025	LOD	181	236	168	1413
19/5/2025	LOD	187	234	105	LOQ
21/5/2025	LOD	LOD	298	161	1154
23/5/2025	LOD	421	304	154	1188

Sampling Date	ng/L				
	CBZ	MTP	DCF	HCT	1H-BTR
25/5/2025	LOD	LOD	172	154	1231
26/5/2025	LOQ	LOQ	186	1456	936
2/6/2025	LOQ	LOQ	153	2274	1258
2/6/2025	LOD	LOD	201	149	843
9/6/2025	LOQ	LOQ	238	97	882
16/6/2025	34	5765	107293	2544	832
23/6/2025	23	4384	2948	891	19337
30/6/2025	18	2799	1882	706	12347
7/7/2025	32	35773	17353	27860	104059
7/7/2025	21	4366	2936	800	19257
14/7/2025	35	21077	14174	2242	92970
16/7/2025	18	5984	4024	852	26396
18/7/2025	26	14517	6652	33916	41782
21/7/2025	36	39142	18988	1971	113861
28/7/2025	LOD	3637	2446	700	16045
4/8/2025	33	134483	63528	2404	421949
11/8/2025	<LOD	<LOQ	2814	ND	<LOD
18/8/2025	5919	1015	2281	3299	<LOD
25/8/2025	5766	1675	1719	1064	<LOD
1/9/2025	6239	1990	1511	1160	<LOQ
8/9/2025	9445	3003	1669	1495	<LOD
15/9/2025	4733	1075	1859	1816	<LOD
22/9/2025	3803	1095	1854	1102	<LOD
29/9/2025	6755	977	2285	7200	<LOD
6/10/2025	5379	965	2708	5287	<LOD
13/10/2025	10414	3356	1812	1471	<LOD
20/10/2025	8401	2610	2357	1255	<LOD
LOD	15	170	65	40	580

The relatively high proportion of non-detects for 1H-BTR and, to a lesser extent, CBZ and MTP indicate that seasonal comparisons must be interpreted cautiously.

Given the highly skewed distributions and the presence of episodic peaks and non-detects, median concentrations were used to characterize typical conditions.

Temporal behavior of individual compounds

The temporal evolution of selected pharmaceuticals during the monitoring period is summarized in Figure 7, which shows concentration trends and seasonal variations for key analytes.

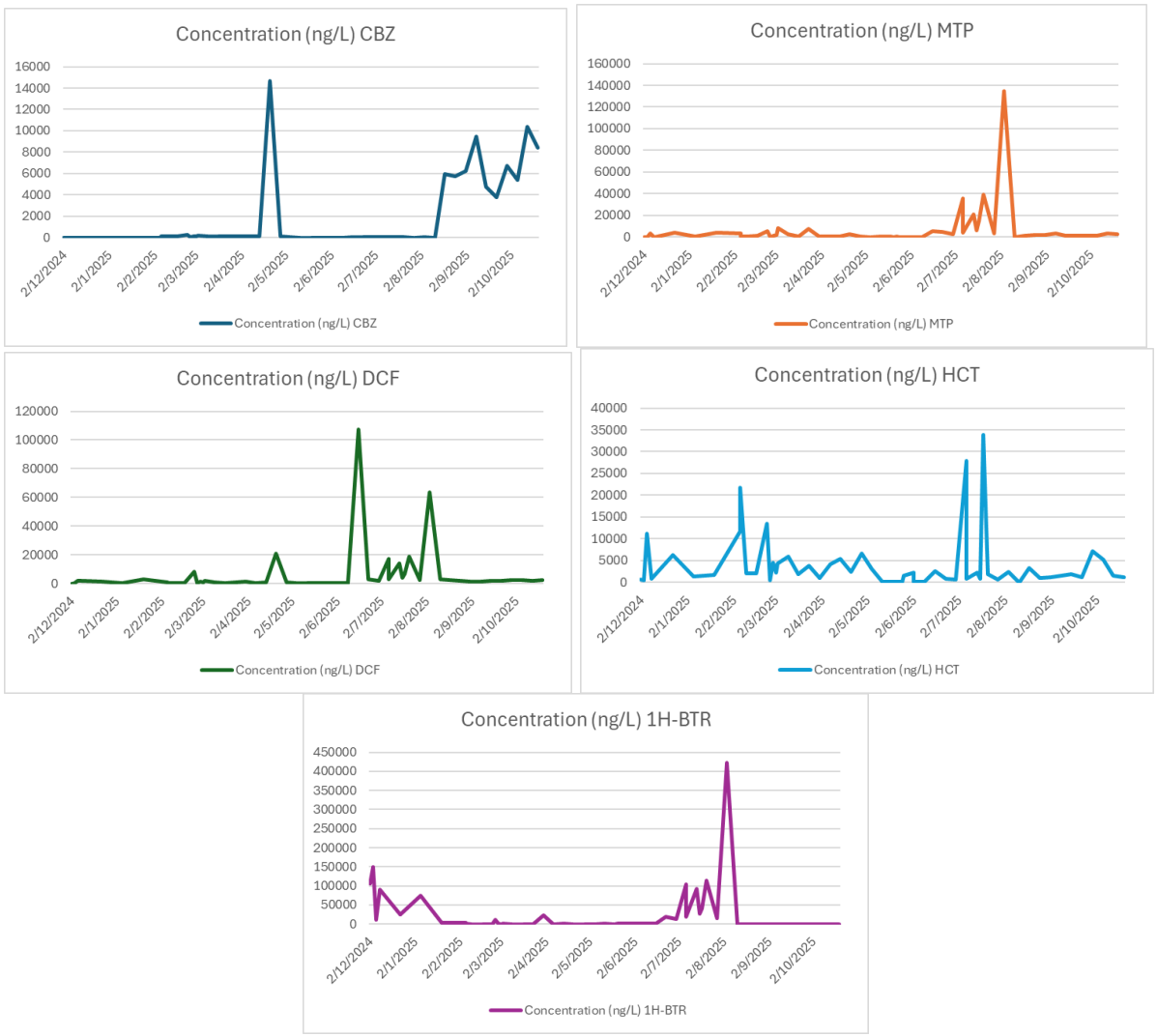

Figure 7: Concentrations (ng/L) of the targeted compounds in MITERA's wastewater during the overall sampling period.

Carbamazepine: CBZ ranged from 18 ng/L to 14659 ng/L. CBZ showed low and stable concentrations from winter to early summer, followed by a strong increase from August to October 2025, resembling a step change rather than a gradual seasonal trend. Monthly medians were ~78–120 ng/L from December to April, decreased to ~23–29 ng/L in June–July, and then increased sharply to ~5766–8401 ng/L in August–October. Several extreme events (>9000 ng/L) were observed.

This pattern indicates a change in source strength or episodic discharge toward the end of the monitoring period.

Metoprolol: The concentrations of MTP ranged between 181 ng/L and 134483 ng/L with the highest concentration value was reported in early August 2025. MTP exhibited very high sample-to-sample variability and strong summer dominance, with multiple extreme spikes in July–August (up to 134483 ng/L). No smooth monotonic trend was observed.

This behavior suggests episodic, high-strength inputs, possibly related to hospital activity or wastewater system dynamics, rather than uniform seasonal variation.

Diclofenac: DCF varied from 153 ng/L to 107293 ng/L with the highest value recorded in June 2025. DCF showed a clear summer signal, with monthly medians rising sharply in July and remaining elevated through August–October. Several large outliers were detected, including one extreme peak (>100000 ng/L).

This pattern is consistent with seasonal NSAID consumption combined with event-driven discharges or dilution effects.

Hydrochlorothiazide: The range of concentrations for HCT was between 97 ng/L and 33916 ng/L. HCT exhibited a bimodal seasonal pattern, with elevated concentrations in late winter–early spring and again in mid-summer, separated by a low-concentration period in late spring. Several large peaks (>20000 ng/L) were observed.

Compared with MTP and DCF, HCT showed a more stable baseline, indicating a combination of continuous medical use and episodic enrichment.

1H-Benzotriazole: Regarding 1H-BTR, concentrations ranged between 596 ng/L and 421949 ng/L. 1H-BTR displayed the highest proportion of non-detects and very strong seasonality. In this dataset, autumn samples were entirely below LOD/LOQ, whereas winter and summer showed very large episodic peaks (up to 421949 ng/L).

This pattern is typical of intermittent point-source discharges rather than population-level pharmaceutical use.

In addition, high-frequency weekly sampling campaigns were carried out during the monitoring period to investigate short-term concentration fluctuations of the target compounds and to gain preliminary insights into their consumption patterns. These campaigns took place in December 2024 and in February, May, and July 2025, thereby also supporting the assessment of seasonal variability. However, no consistent temporal trends were identified when comparing concentrations across the weekly campaigns, likely reflecting the widespread and continuous use of these substances.

During the weekly monitoring in December 2024, concentrations of CBZ, MTP, and DCF were generally below the LOD and LOQ, with the exception of 6 December, when elevated values were observed. In February, only one sampling day (Monday, 24 February) exhibited concentrations above the weekly average for all compounds except BTR. The May monitoring period was characterized by overall low concentrations, with several measurements below the LOD. In July, no clear concentration patterns were observed, although elevated levels of MTP, CT, and DCF were recorded on 18 July compared to the other days of the same week.

4.4 Preliminary Interpretation

Across all compounds, the dominant feature of the time series is high temporal variability driven by sporadic peaks. While seasonality is evident (especially for MTP, DCF and 1H-BTR), event-driven episodes largely determine overall concentration statistics. CBZ shows a unique late summer-autumn 2025 increase, which may indicate changes in hospital use, disposal practices, or upstream inputs. HCT shows dual seasonality, consistent with chronic medication use combined with hospital treatment cycles.

The magnitude of several peaks especially for MTP, DCF and 1H-BTR exceeds many reported values for municipal and hospital wastewater in the literature, suggesting that hospital sewer catchments can act as high-intensity point sources, even when total volumetric contribution is small.

5 Environmental Monitoring Campaign 2 – WWTP Inlet & Effluents

5.1 Site Description and Operational Context

The second ENVIROMED environmental monitoring campaign was implemented at the Psyttalia WWTP (EYDAP), the central treatment facility for the Athens Metropolitan area (Figure 8). The Psyttalia WWTP receives and treats approximately 730,000 m³/day of municipal wastewater under stable operational conditions throughout the year. The Psyttalia WWTP capacity is 5,600,000 p.e., being one of the biggest WWTPs in Europe and worldwide.

The WWTP has been in operation since 1994 and includes pre-treatment, primary and secondary treatment with advanced biological nitrogen removal, sludge treatment, and cogeneration of electrical and thermal energy. In detail, the Psyttalia WWTP facilities include wastewater pretreatment at Akrokeramos (on the Attica mainland) comprising debris removal, screening, grit removal, and odour control units. Pretreated wastewater is piped to Psyttalia Island, by means of submerged pipes, so as to undergo primary and advanced secondary treatment, achieving suspended solids and organic load reduction by about 93% and total nitrogen reduction by about 80% in comparison with influent loads. The Psyttalia WWTP final effluent is being received by the Saronic Gulf through gradual deep disposal by means of a system of submerged outfall pipes.

Figure 8: Aerial view of the Psyttalia WWTP

5.2 Sampling Activities – Sample Collection and Preparation

In order to implement the monitoring of the WWTP inlet and outlet quality (Task 7.3) in terms of pharmaceutical compounds, wastewater samples were collected from the inlet and the outlet of the Psyttalia WWTP. The results of the assessment program give insight to the levels of the pharmaceuticals in the inlet and outlet of the WWTP as well as to the removal efficiency of the plant while giving support to the validation of the WSA. To achieve the goal of Task 7.3, twenty four-hour composite samples of sewage influents (raw wastewater prior to treatment) and effluents (treated wastewater prior to marine discharge) have been collected for one year (from October 2024-M30 until October 2025-M41) according to the plan shown in Table 12.

Therefore, more than 90 samples have been collected during the sampling period. The sampling of the inlet and outlet of the WWTP was decided to be extended beyond M39 (August 2025) which was the expected end of Task 7.3. The aim of this extension of the sampling plan was to obtain annual and complete monitoring dataset of pharmaceuticals and to collect fresher samples in order to be analyzed with the WSA at the validation stage.

The sampling frequency was deliberately set at high level to enable the identification of potential seasonal variations throughout the monitoring period (Autumn 2024 - Summer 2025). In preparation for the commencement of the sampling activities, regular monthly meetings were held in the preceding months, during which the participating partners CYRIC, MITERA, EYDAP, and RISA discussed in detail and finalized the sampling plan and overall monitoring strategy.

Table 12: Sampling plan of the inlet and the outlet in the Psyttalia WWTP

Season	Sampling Point: Inlet	Sampling Point: Outlet
	Dates	
Autumn	20/10/2024	21/10/2024
	22/11/2024	22/11/2024
	25/11/2024	25/11/2024
	27/11/2024	27/11/2024
	29/11/2024	29/10/2024
Winter	9/12/2024	9/12/2024
	20/1/2025	20/1/2025
	3/2/2025	3/2/2025
	17/2/2025	17/2/2025
Spring	4/3/2025	4/3/2025
	5/3/2025	5/3/2025
	7/3/2025	7/3/2025
	7/3/2025	7/3/2025
	10/3/2025	10/3/2025
	17/3/2025	17/3/2025
	31/3/2025	31/3/2025
	14/4/2025	15/4/2025
	28/4/2025	29/4/2025
	12/5/2025	13/5/2025
	14/5/2025	15/5/2025
	16/5/2025	17/5/2025
	18/5/2025	19/5/2025
	26/5/2025	27/5/2025
Summer	9/6/2025	10/6/2025
	23/6/2025	24/6/2025
	7/7/2025	8/7/2025
	21/7/2025	22/7/2025
	23/7/2025	24/7/2025
	25/7/2025	26/7/2025
	27/7/2025	28/7/2025

Season	Sampling Point: Inlet	Sampling Point: Outlet
	Dates	
	4/8/2025	5/8/2025
	18/8/2025	19/8/2025
Autumn	2/9/2025	3/9/2025
	9/9/2025	10/9/2025
	15/9/2025	16/9/2025
	22/9/2025	23/9/2025
	24/9/2025	25/9/2025
	26/9/2025	27/9/2025
	1/10/2025	2/10/2025
	6/10/2025	7/10/2025
	12/10/2025	13/10/2025
	16/10/2025	17/10/2025
	20/10/2025	21/10/2025
	30/10/2025	31/10/2025
	8/11/2025	9/11/2025
	17/11/2025	18/11/2025
	24/11/2025	25/11/2025
Total Samples/ Analysis	**94**	

To support the objective of the project, EYDAP conducted market research to identify a qualified external laboratory for the aforementioned analyses of the samples collected during the campaign and until the validation phase of the WSA. Following this, the Laboratory of Analytical Chemistry, Department of Chemistry at the National and Kapodistrian University of Athens (NKUA/TraMS) was selected.

All wastewater samples were collected in high-density polyethylene bottles and stored according to the protocol for the internal analysis with WSA in the pilot and the parallel testing at the external laboratory of Analytical Chemistry at National and Kapodistrian University of Athens (NKUA/TraMS). Due to the delay in finalization of the WSA and its operation and validation at the pilots (Psyttalia WWTP and MITERA hospital) analyses have been performed only at the external laboratory until October 2025.

5.3 Measurement Results

5.3.1 Methodology-External Laboratory

5.3.1.1 Sample preparation method for the determination of contaminants in wastewater by LC-MS/MS

All samples were filtered through Glass Microfiber Filters Diameter 47 mm (Whatman), and the filtrate was acidified to pH 6.5 ± 0.2 with 0.1 N HCl solution. A 10 μL mixture of internal standards at a concentration of 1 mg/L (diclofenac-d6 and hydrochlorthiazide-d2) was added to 990 μL of each sample (Calibration, Unknown). Samples were analyzed with direct injection to LC-MS/MS system.

For the quantification, a 10-point spiked calibration plot was prepared in a wastewater matrix with concentration range 0.1- 250 ng/L (concentration in vial).

The identification criteria for the determination of compounds were the retention time (± 0.2 min) and the ion ratio of each analyte compared to the standard solution (±30%). Quantification was based on the ratio of the peak area of each analyte to the peak area of the corresponding deuterated internal standard.

5.3.1.2 Quality Assurance & Quality Control (QA/QC)

A thorough quality assurance and quality control (QA/QC) was applied during the sample preparation and instrumental analysis. A mix of internal standards was added into each sample prior to extraction to assure satisfactory recovery of the target compounds and samples spiked with a mix of known CECs were also analyzed in each batch of samples. Moreover, procedural blank (reagent blank) was prepared to assess any external contamination which might have been brought in during the sample preparation of the extracts and analysis. Also, a field blank was analyzed to trace any contamination from the sampling to instrumental analysis. A mix of known analytes (RTI calibrant substances) was used to assess the stability of retention time during instrumental analysis. A QC sample was running every 10 injections to ensure the good operation and high sensitivity of the instrument.

5.3.2 Results and Discussion - External Laboratory (NKUA/TraMS) Results

A total of forty-seven influent and forty-seven effluent wastewater samples were collected over the annual monitoring campaign. The mean concentrations of the five targeted compounds of each discrete season, as well as for the entire monitoring period, at both the inlet and the outlet of the Psyttalia WWTP are presented in Table 13 and Table 14.

Table 13 Mean concentrations (ng/L) of the targeted compounds in the inlet of the Psyttalia WWTP during the overall sampling period and the discrete seasons

Sampling period	ng/L				
	Carbamazepine CBZ	Metoprolol MTP	Diclofenac DCF	Hydrochlorothiazide HCT	1H-Benzotriazole 1H-BTR
Autumn	418	1156	1564	2737	8548
Winter	296	645	1019	3136	3862
Spring	268	1305	962	5619	3493
Summer	387	1256	869	3459	3647
Autumn 2025	634	802	1913	2251	5264
Overall	**426**	**1063**	**1317**	**3613**	**4605**

Table 14: Mean concentrations (ng/L) of the targeted compounds in the outlet of the Psyttalia WWTP during the overall sampling period and the discrete seasons

Sampling period	ng/L				
	Carbamazepine CBZ	Metoprolol MTP	Diclofenac DCF	Hydrochlorothiazide HCT	1H-Benzotriazole 1H-BTR
Autumn	475	711	944	2047	4622
Winter	319	539	968	2002	1662
Spring	389	1278	1366	2604	5352
Summer	293	735	987	3465	2531
Autumn 2025	271	826	673	1538	6323
Overall	**336**	**898**	**994**	**2318**	**4994**

Regarding the inlet samples (Table 14), all compounds were quantitatively identified in the analyzed wastewater samples, indicating their continuous presence in raw wastewater. The highest mean concentrations were observed for HCT and 1H-BTR, with comparable values of 3613 and 4605 ng/L, respectively. For DCF and MTP, the mean concentrations were approximately four-fold lower than those of the two most abundant compounds (1317 and 1063 ng/L, respectively), while the lowest mean concentration was recorded for Carbamazepine (426 ng/L).

The temporal trend of the target compounds in the influent samples is illustrated in Figure 9 and Table 15.

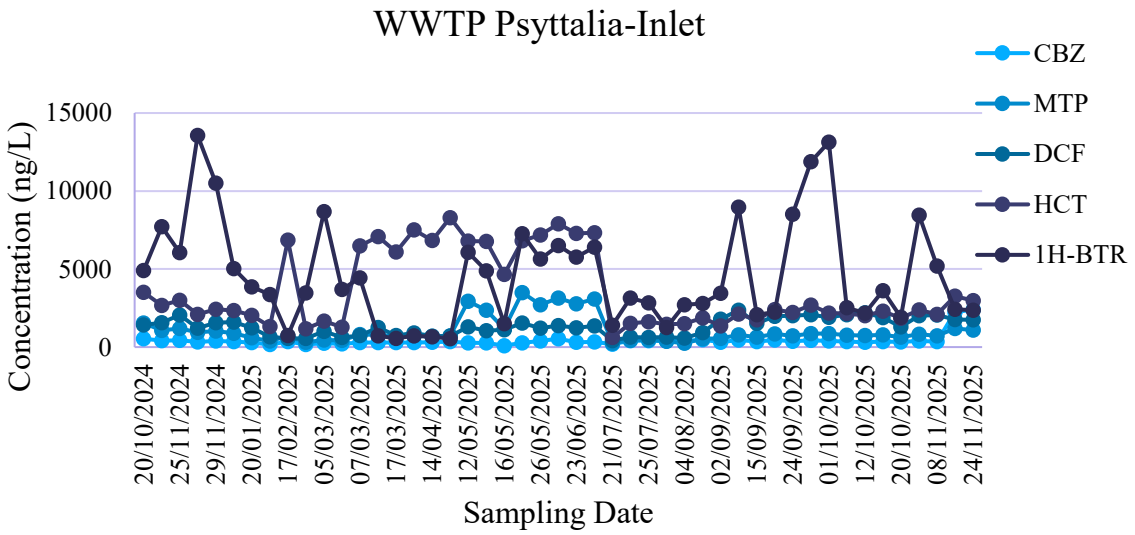

Figure 9: Concentrations (ng/L) of the targeted compounds in the inlet of the Psyttalia WWTP during the overall sampling period.

Table 15: Concentrations (ng/L) of the targeted compounds in the inlet of the Psyttalia WWTP during the overall sampling period.

Sampling Date	ng/L				
	Carbamazepine CBZ	Metoprolol MTP	Diclofenac DCF	Hydrochlorothiazide HCT	1H-Benzotriazole 1H-BTR
20/10/2024	534	1539	1414	3502	4899
22/11/2024	415	1063	1558	2669	7713
25/11/2024	428	1196	2065	2993	6063
27/11/2024	329	958	1217	2102	13563
29/11/2024	384	1024	1564	2417	10500
9/12/2024	350	890	1609	2350	5040
20/1/2025	298	596	1236	2027	3862
3/2/2025	176	387	668	1310	3361
17/2/2025	358	705	562	6858	733
4/3/2025	173	303	563	1175	3481
5/3/2025	251	464	1014	1672	8692
7/3/2025	216	426	625	1281	3695
7/3/2025	293	813	744	6487	4427
10/3/2025	284	775	1261	7084	719
17/3/2025	286	675	740	6093	565
31/3/2025	284	715	912	7516	714
14/4/2025	314	691	694	6828	665
28/4/2025	347	721	707	8297	546
12/5/2025	263	2930	1297	6799	6082
14/5/2025	275	2357	1044	6769	4893
16/5/2025	96	1171	1112	4657	1508
18/5/2025	278	3500	1550	6814	7266
26/5/2025	388	2722	1205	7189	5650
9/6/2025	540	3135	1388	7904	6509
23/6/2025	315	2779	1230	7289	5768
7/7/2025	331	3084	1365	7334	6402
21/7/2025	199	260	422	615	1400
23/7/2025	424	435	648	1519	3152

Sampling Date	ng/L				
	Carbamazepine CBZ	Metoprolol MTP	Diclofenac DCF	Hydrochlorothiazide HCT	1H-Benzotriazole 1H-BTR
25/7/2025	420	459	626	1619	2825
27/7/2025	388	377	644	1461	1265
4/8/2025	391	241	570	1512	2716
18/8/2025	474	531	928	1878	2787
2/9/2025	312	560	1797	1353	3455
9/9/2025	448	779	2369	2113	8970
15/9/2025	346	639	1527	1752	2072
22/9/2025	460	853	1969	2401	2249
24/9/2025	372	730	2015	2211	8525
26/9/2025	433	859	2073	2691	11883
1/10/2025	386	864	1907	2172	13136
6/10/2025	358	772	2068	2168	2522
12/10/2025	322	743	2201	2019	2187
16/12/2025	358	778	1888	2292	3613
20/10/2025	332	631	1300	1855	1908
30/10/2025	384	824	2018	2394	8451
8/11/2025	354	719	2054	2099	5188
17/11/2025	2237	1186	1772	3266	2431
24/11/2025	2403	1091	1740	2982	2363
LOD	16	100	50	50	410

LOD: Limit of Detection, LOQ: Limit of Quantification

The concentrations of MTP ranged between 241 ng/L and 3500 ng/L, and the highest concentration value was reported in May 2024. The concentrations observed in the present study exceed those reported by Cappelli et al. (2022), whose mean value approximated the lower end of the concentration range measured herein. Such discrepancies may reflect differences in local consumption patterns since all WWTPs examined employ similar conventional treatment processes. CBZ concentrations ranged from 96 ng/L to 2403 ng/L. The highest concentrations of CBZ (>2000 ng/L) were recorded in the final two samples collected in October 2025 and were approximately six-fold higher than the levels observed during the remaining annual monitoring campaign. The concentrations observed in this study were higher than those reported by Petromelidou et al. (2024). Slightly lower were documented the concentrations of the inlet in WWTPs examined by Kosma et al. (2014) and Fontanals et al. (2023).

Diclofenac varied from 422 ng/L to 2369 ng/L with the highest value recorded in September 2025. Comparable influent concentrations of DCF have been reported by Fontanals et al. (2023). The range of concentrations for HCT was between 615 ng/L and 8297 ng/L. In contrast, Morosini et al. (2017) reported significant lower concentrations of Hydrochlorothiazide, highlighting potential differences in consumption patterns of the population.

Regarding 1H-BTR, concentrations ranged between 546 ng/L and 13563 ng/L. Compared with the values reported by Cappelli et al. (2022), the levels detected in the present study were notably higher. That discrepancy could be attributed to the fact that Cappelli's study (2022) was conducted during the COVID-19 pandemic, a period with reduced industrial activities. In contrast to previous studies conducted by Stasinakis et al. (2013), our findings indicate that higher concentrations were reported during this monitoring campaign.

Concentrations of most target compounds remained comparable across the four sampling seasons in the inlet samples. Nevertheless, significantly elevated concentrations were observed for 1H-BTR during autumn 2024 (mean concentration: 8548 ng/L), for HCT during the spring period (mean concentration: 5619 ng/L), and for CBZ during the last sampling period, in October 2025 (mean concentration: 634 ng/L). In contrast, for MTP, a significant reduction in the mean inlet concentration was observed during winter period (645 ng/L).

In addition, high-frequency weekly sampling campaigns were conducted during the monitoring period in order to investigate potential concentration fluctuations of the target compounds and to derive preliminary insights into consumption patterns among the inhabitants of the Attica region. These campaigns were implemented in October 2024, as well as in March, July, and August 2025, thereby also supporting the assessment of seasonal variability. The temporal trend of the target compounds in the effluent samples is presented in Figure 10 and Table 16.

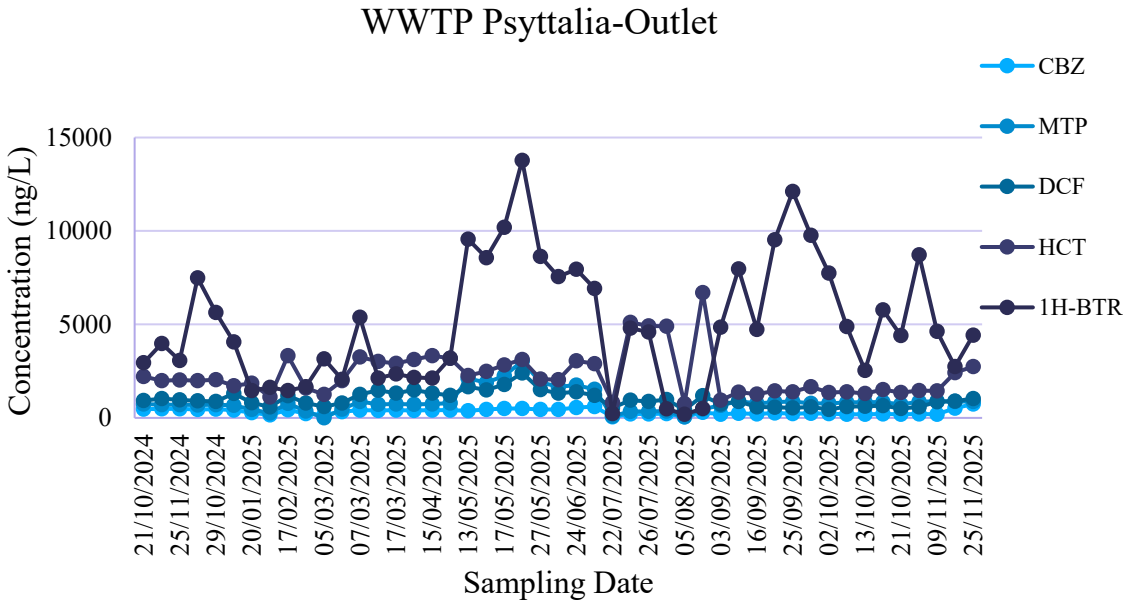

Figure 10: Concentrations (ng/L) of the targeted compounds in the outlet of the Psyttalia WWTP during the overall sampling period.

However, no consistent trend was identified based on the comparison of the respective concentrations. The concentrations of the target compounds exhibit no significant variations during the weeks examined, possibly due to their widespread and continuous usage. Only on May 16th did all compounds, with the exception of DCF, show notably lower concentration compared to the remaining days of the same week.

Table 16: Concentrations (ng/L) of the targeted compounds in the outlet of the Psyttalia WWTP during the overall sampling period.

Sampling Date	ng/L				
	Carbamazepine CBZ	Metoprolol MTP	Diclofenac DCF	Hydrochlorothiazide HCT	1H-Benzotriazole 1H-BTR
21/10/2024	473	741	932	2209	2957
22/11/2024	490	701	1027	1984	3974
25/11/2024	491	711	971	2023	3066
27/11/2024	453	688	911	1983	7480
29/10/2024	469	714	877	2035	5631
9/12/2024	411	664	1304	1718	4068
20/1/2025	278	450	802	1848	1463
3/2/2025	162	265	586	1121	1628
17/2/2025	425	775	1181	3321	1456
4/3/2025	223	370	798	1592	1662
5/3/2025	151	<LOQ	598	1250	3157
7/3/2025	324	464	797	1998	2061
7/3/2025	414	875	1254	3266	5374
10/3/2025	398	727	1481	3013	2123
17/3/2025	420	743	1320	2921	2346
31/3/2025	402	732	1493	3113	2169
15/4/2025	408	735	1323	3322	2122
29/4/2025	414	794	1210	3195	3194
13/5/2025	375	2104	1667	2262	9552
15/5/2025	444	1887	1495	2495	8566
17/5/2025	504	2244	1778	2837	10188
19/5/2025	506	3035	2405	3118	13780
27/5/2025	456	1901	1506	2080	8629

Sampling Date	ng/L				
	Carbamazepine CBZ	Metoprolol MTP	Diclofenac DCF	Hydrochlorothiazide HCT	1H-Benzotriazole 1H-BTR
10/6/2025	445	1665	1319	2045	7560
24/6/2025	544	1751	1388	3045	7951
8/7/2025	601	1525	1208	2891	6924
22/7/2025	58.4	87.4	543	821	224
24/7/2025	214	351	947	5109	4804
26/7/2025	203	340	880	4916	4594
28/7/2025	232	361	981	4899	484
5/8/2025	47	63.1	430	752	188
19/8/2025	294	473	1186	6707	497
3/9/2025	185	674	748	947	4857
10/9/2025	238	897	886	1375	7963
16/9/2025	213	780	585	1264	4738
23/9/2025	259	842	573	1447	9533
25/9/2025	229	871	528	1390	12112
27/9/2025	251	781	588	1657	9772
2/10/2025	222	782	454	1358	7752
7/10/2025	199	829	598	1393	4878
13/10/2025	195	842	624	1309	2545
17/10/2025	211	848	652	1507	5779
21/10/2025	186	728	495	1351	4404
31/10/2025	217	858	588	1455	8713
9/11/2025	201	897	864	1451	4636
18/11/2025	521	879	889	2423	2744
25/11/2025	741	884	1026	2739	4425
LOD	12	100	50	25	170

LOD: Limit of Detection, LOQ: Limit of Quantification

Concerning the treated wastewater (effluent), the targeted compounds were omnipresent in all samples throughout the monitoring period, with the exception of one sample in which MTP was measured below the LOQ. The highest mean concentration in the effluent samples of the Psyttalia WWTP was observed for 1H-BTR, whereas the lower was recorded for CBZ.

Specifically, the mean concentrations were 4994 ng/L for 1H-BTR, 2318 ng/L for HCT, 994 ng/L for DCF, 898 ng/L for MTP, and 336 ng/L for CBZ.

In effluent samples, CBZ ranged between 47 ng/L and 741 ng/L with the lowest value been recorded in August 2025. Comparable concentration ranges have been reported by Petromelidou et al. (2024), however Krakkó, et al. (2019) observed notably higher values. The mean concentration of CBZ measured in the present study was higher than those documented by Fontanals et al. (2023) and Petromelidou et al. (2024). MTP ranged from below the LOQ up to 3035 ng/L. Lower effluent concentrations were reported by Krakkó, et al. (2019) and Choi et al. (2022). The minimum concentration of DCF was 430 ng/L while the maximum concentration was 2405 ng/L, and it was recorded during May 2025. In contrast, Krakkó, et al. (2019), reported higher DCF concentrations compared with the results of the present study.

For the diuretic HCT, effluent concentrations ranged between 752 ng/L and 6707 ng/L. Morosini et al. (2017) reported higher effluent concentrations of HCT compared to the influent, a behavior that was not observed herein. The concentration range for 1H-BTR was 188 ng/L and 13780 ng/L. Significantly lower were the levels of 1H-BTR observed by Stasinakis et al. (2013). Furthermore, results from a study encompassing 90 European WWTPs indicated that the average concentrations of 1H-BTR were comparable to the mean concentration observed in the present monitoring study (Loos et al., 2013).

A comparison of the results obtained from the seasonal monitoring campaigns revealed deviations in mean concentrations of several compounds in the effluent, despite the fact that the procedures and the stages of the WWTP remained unvarying throughout the year. Indeed, MTP and DCF exhibited increased mean concentrations during the spring period (1278 ng/L and 1366 ng/L, respectively). On contrary, the mean concentration of 1H-BTR showed a decrease during winter and summer (1662 ng/L and 2531 ng/L, respectively) compared to the values recorded in the other seasons. Levels of Carbamazepine and Hydrochlorothiazide remained relative constant over the annual monitoring period.

The weekly sampling campaigns highlighted the overall stability of these compounds, reflecting the consistent performance and the efficacy of the treatment plant. Only on August 22nd were the values of these compounds notably lower compared to those recorded during the remaining weekly sampling.

5.3.3 Removal of target compounds during wastewater treatment

For the compounds that were detected both in influent and effluent wastewater samples, their removal efficiency during wastewater treatment and their fate in the Psyttalia WWTP were investigated.

According to the results, the five compounds presented inadequate removal during the treatment in the Psyttalia WWTP. Indeed, CBZ, DCF, MTP, and 1H-BTR were removed slightly or even increased after wastewater treatment with mean removals of -3%, 8%, 9% and -55%, respectively. In contrast, Hydrochlorothiazide presented partially removal (mean removal: 16%). It should be noted that in cases where apparent increases in effluent concentrations were observed, this may be attributed not to real formation or release phenomena, but to temporal mismatches between influent and effluent sampling relative to the hydraulic retention time of the plant. Short-term concentration fluctuations in the influent, combined with the time lag before corresponding effluent samples are collected, can lead to seemingly negative removal values. Such artefacts highlight the importance of synchronized or flow-proportional sampling strategies for more accurate removal assessment.

Figure 11 to Figure 15 illustrate the trend and the removal of the target compound over the monitoring period in the Psyttalia WWTP.

According to the literature, the removal of pharmaceutical compounds from wastewater occurs through a combination of concurrent mechanisms, including direct adsorption and/or absorption by activated sludge microorganisms, chemical transformation processes, and biodegradation (Mantovani et al., 2024). Furthermore, the persistence observed for several pharmaceutical compounds has been attributed to the formation of metabolites during wastewater treatment, which may subsequently be transformed back into their parent compounds, as well as to the potential desorption of compounds previously associated with the sludge phase (Skalska-Tuomi et al., 2025).

Removal efficiency is strongly influenced by the design characteristics and operational conditions of the treatment facility, in addition to the physicochemical properties of the wastewater matrix. Conventional wastewater treatment plants employing activated sludge or biofilm-based processes generally exhibit limited effectiveness in the removal of pharmaceutical compounds (Mailler et al., 2016, Park et al., 2020).

In Skalska-Tuomi's (2025) research, the concentration of diclofenac, carbamazepine, metoprolol in treated wastewater was at the same level in treated wastewater as in raw wastewater. According to Vieno & Sillanpää (2014), DCF is poorly biodegradable and only a small part is sorbed on activated sludge particles resulting in low removal yields over the conventional wastewater treatment processes. Similarly, Carbamazepine is almost completely persistent during conventional activated sludge treatment (Loos et al., 2013) and MTP presents low removal rates (between 0% and 36%) (Rubirola et al., 2014, Ja´en-Gil et al., 2019).

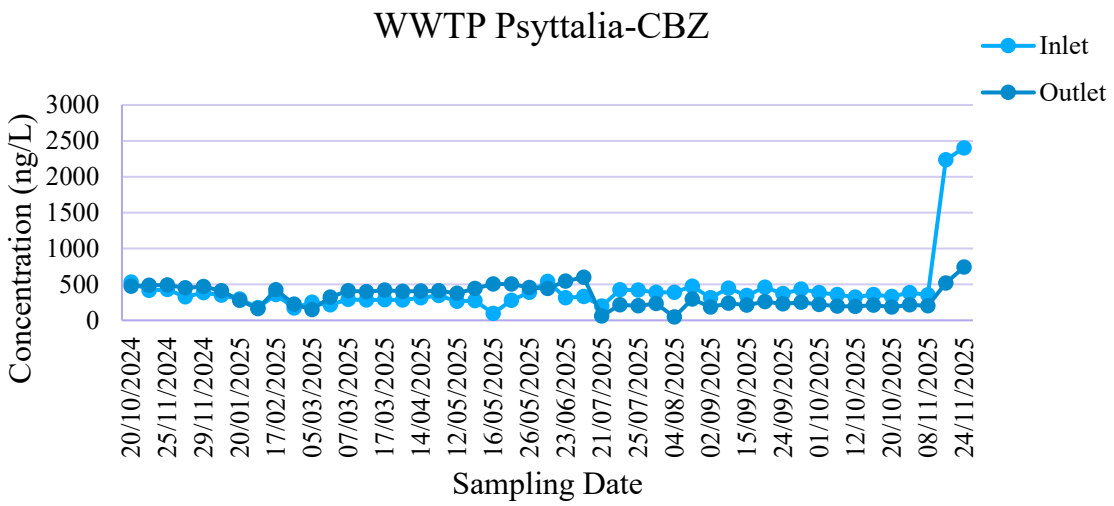

Figure 11: Trend of the Carbamazepine in influent and effluent of the Psyttalia WWTP over the monitoring period.

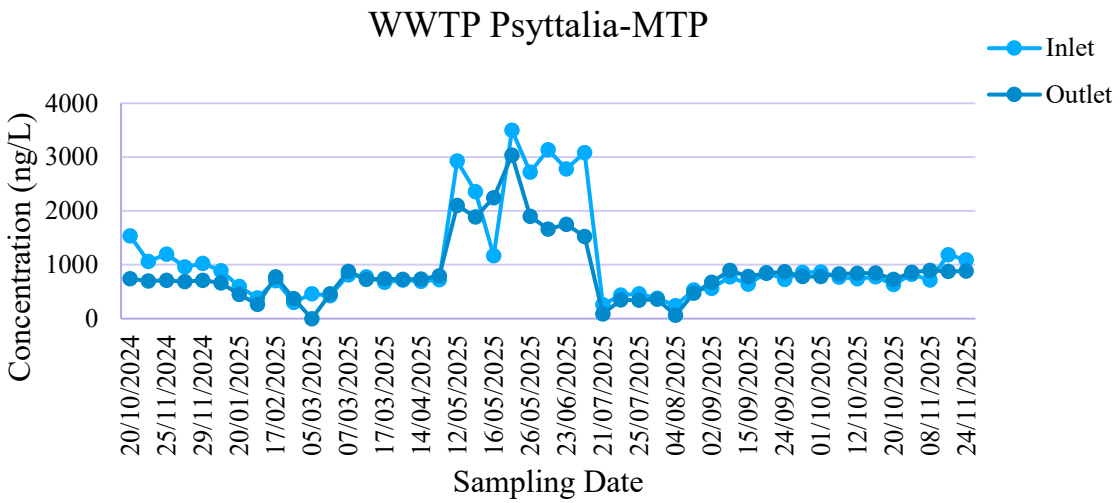

Figure 12: Trend of the Metoprolol in influent and effluent of the Psyttalia WWTP over the monitoring period.

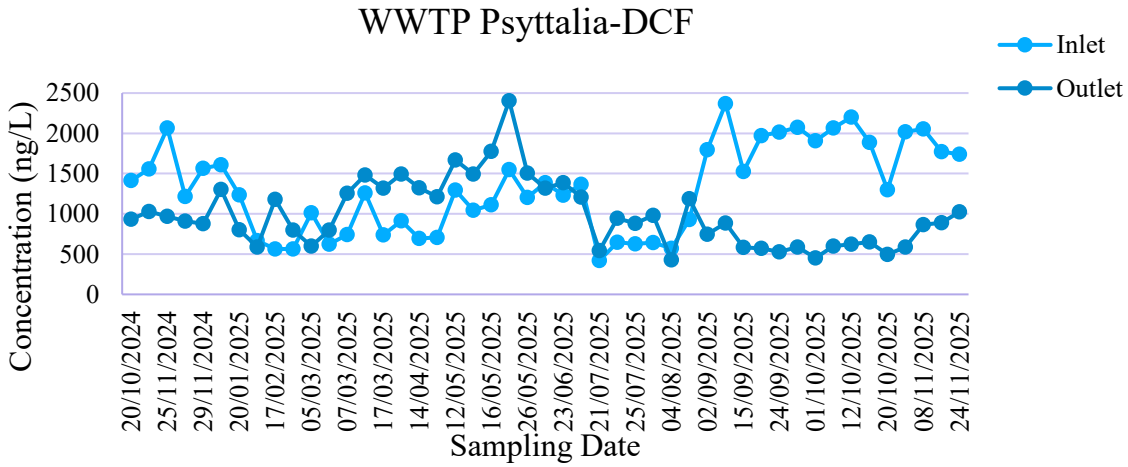

Figure 13: Trend of the Diclofenac (DCF) in influent and effluent of the Psyttalia WWTP over the monitoring period.

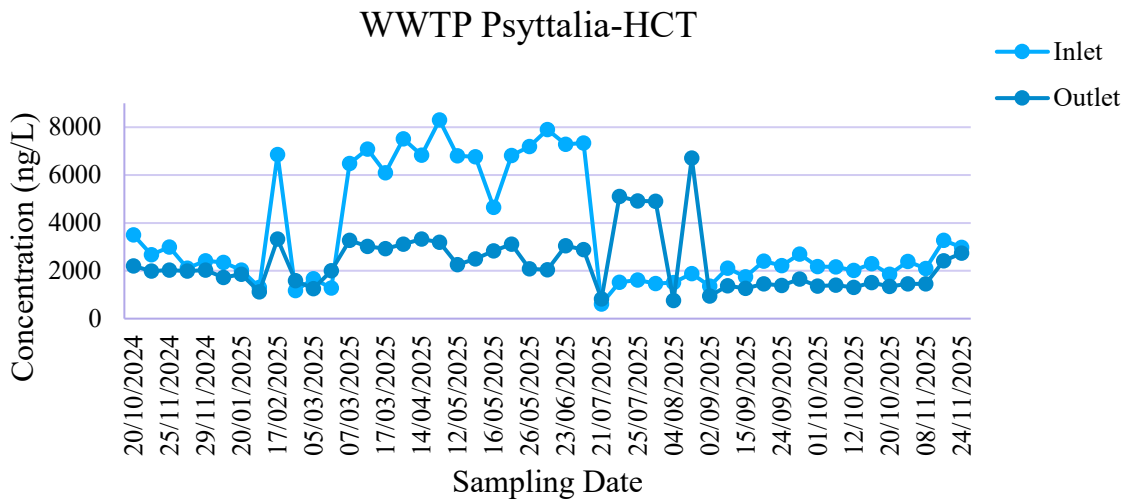

Figure 14: Trend of the Hydrochlorothiazide (HCT) in influent and effluent of the Psyttalia WWTP over the monitoring period.

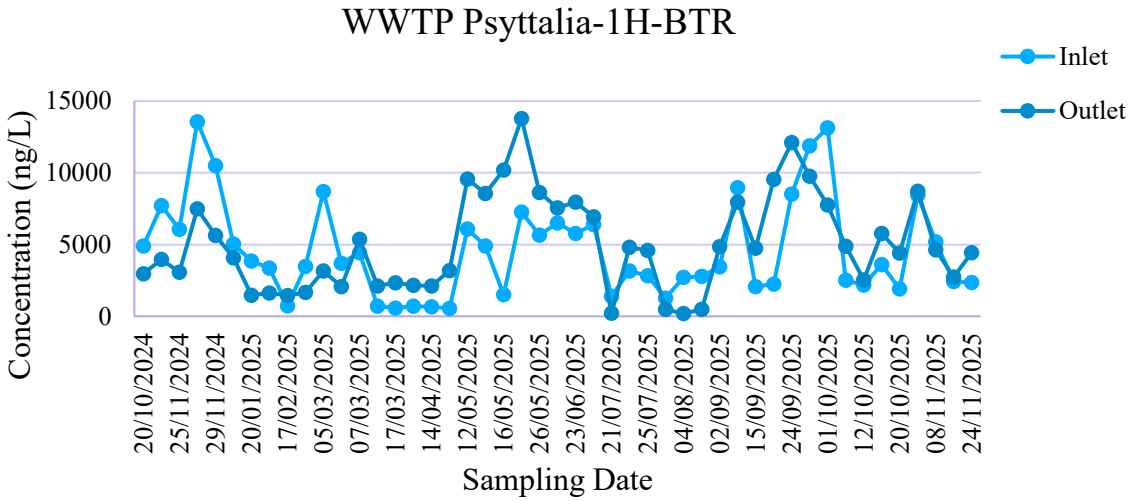

Figure 15: Trend of the 1H-Benzotriazole (1H-BTR) in influent and effluent of the Psyttalia WWTP over the monitoring period.

5.4 Preliminary Interpretation

The annual monitoring campaign implemented under Task 7.3 demonstrated the continuous presence of all targeted pharmaceutical compounds and the industrial additive in both influent and effluent wastewater samples. While seasonal variations were observed for selected compounds, overall concentrations remained relatively stable throughout the year, as confirmed by high-frequency sampling campaigns. The concentration levels observed were largely consistent with values reported for comparable European wastewater treatment plants. This finding highlights that conventional wastewater treatment processes were shown to provide limited removal of several compounds, which are included in the UWWTD watch list, resulting in their persistent detection in treated effluents.

In addition, these results underline the critical need for a systematic, long-term monitoring infrastructure an installed WSA capable of detecting pollutant fluctuations in near real time and providing actionable data for plant operators and regulators. Such an integrated WSA would not only enable early identification of treatment inefficiencies and emerging contaminants but also support data-driven optimization of advanced removal technologies and inform evidence-based regulatory frameworks.

6 Environmental Monitoring Campaign 3 - Marine environment

6.1 Sampling Activities - Seawater Sample Collection

In order to implement monitoring of the marine environment under Task 7.4, with a focus on pharmaceutical compounds, seawater samples were collected from the marine area surrounding the Psyttalia WWTP (EYDAP). The outcomes of the monitoring program provide an insight into the concentration levels and seasonal variability of pharmaceutical compounds in the marine environment. Furthermore, the collected data allows a preliminary assessment of the potential influence of the WWTP on the adjacent offshore marine ecosystem.

To achieve the objectives of Task 7.4, a total of forty seawater samples were collected during the annual monitoring campaign from seven sampling stations located in the vicinity of Psyttalia Island, as shown in Figure 16. Stations A1 and B1 are situated in closer proximity to Psyttalia Island, while stations A3 and A4 are positioned at the end point of the WWTP discharge pipelines. Station A2 is located at the midpoint of the primary effluent pipeline, whereas stations B2 and B3 are located near Atalanti Island, an island off the western coast of Attica region, between Salamis Island and the port of Athens, Piraeus. Additional information including the precise geographical coordinates of the sampling stations, is provided in Table 17.

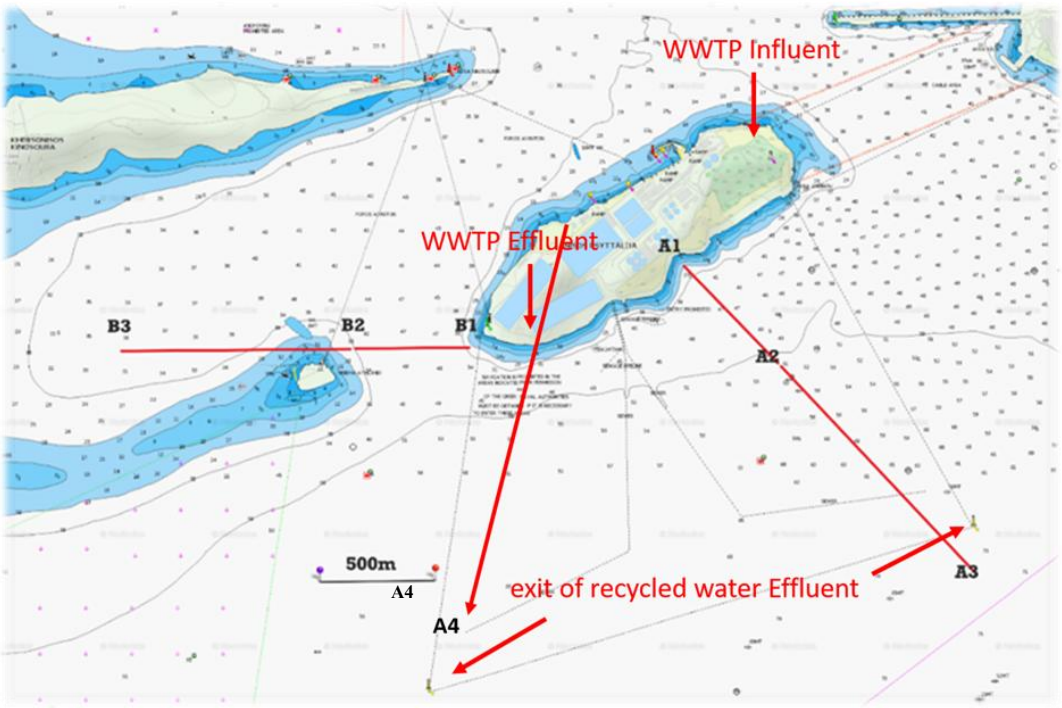

Figure 16: Sampling stations in marine environment adjacent to Psyttalia WWTP (EYDAP)

Table 17: Sampling stations, geographical coordinates, and station descriptions

Station	Latitude (N)	Longitude (E)	Description
A1	37°55.836′	23°35.340′	Eastern side of Psyttalia Island, near WWTP facilities
A2	37°55.734′	23°35.568′	Southeast of Psyttalia Island, coastal mixing zone
A3	37°55.542′	23°36.150′	Offshore eastern station, downstream of treated effluent dispersion 1
A4	37°55.458′	23°35.208′	Offshore Southern station, downstream of treated effluent dispersion 2
B1	37°55.746′	23°35.004′	Western side of Psyttalia Island
B2	37°55.758′	23°34.716′	Western offshore station close to Atalanti Island
B3	37°55.776′	23°34.368′	Furthest western offshore station, reference marine conditions

Dedicated coordination meetings were held between the partners involved (CNR, EYDAP, RISA, and IRES) in order to prepare the sampling plan and define all technical details of the sampling activities. The sampling cruises were carried out using an inflatable speedboat. Surface water samples were collected at all stations (hereafter denoted as station name followed by "S", e.g., A1S) while additional near the bottom-samples were collected from stations A3 and A4 during the four seasonal campaigns and from station B3 during the summer campaign (hereafter denoted as station name followed by "B", e.g., A3B) using an individual Niskin bottle in order to evaluate the distribution of the compounds in terms of depth.

This first sampling campaign was conducted in June 2024 and was considered as test campaign, intended to evaluate the sampling and shipment protocols and to allow for the implementation of any necessary adjustments. The second campaign took place in October 2024 (M29) and coincided with the first scheduled seasonal monitoring campaign (autumn period, as defined under Task 2.3). The third campaign was conducted in March 2025 (M34) and was carried out in alignment with the second seasonal campaign (winter period). The fourth environmental monitoring campaign was carried out in May 2025 (M36), which accounts for the third seasonal campaign (spring period). The following sampling campaign was conducted in July 2025 (M39) and that was the fourth seasonal campaign (summer period). An additional sampling campaign took place in October 2025, however samples were collected from only four stations (A1, A3, B1, B2). This supplementary sampling campaign provides an opportunity for comparative analysis and for the assessment of organic micropollutant concentration levels between October 2024 and October 2025 sampling periods.

To support the objective of Task 7.4 and the project overall, EYDAP conducted market research to identify a qualified external laboratory for the aforementioned analyses of the seawater samples collected during the annual monitoring campaign. Following this, the Laboratory of Analytical Chemistry, Department of Chemistry at the National and Kapodistrian University of Athens (NKUA/TraMS) was selected. All wastewater samples were collected in high-density polyethylene bottles and stored according to the protocol for the analyses at the external laboratory of Analytical Chemistry at National and Kapodistrian University of Athens (NKUA/TraMS).

6.2 Measurement Results

6.2.1 Sample preparation method for the determination of contaminants in marine samples by LC-HRMS/MS

All samples were filtered through Glass Microfiber Filters Diameter 47 mm (Whatman) and the filtrate was acidified to pH 6.5 ± 0.2 with 0.1 N HCl solution and 800 mL of each sample was spiked with mix of internal standards. Preparation step was achieved with SPE using Oasis HLB cartridges (Waters). The cartridges were conditioned with 3 mL MeOH and 3 mL H_2O then the sample was loaded and vacuum drying for 1 h. The elution step was followed by adding with 2x3 mL methanol. The extracts were evaporated to dryness with a gentle stream of N_2(g) at 40ºC and the residue was reconstituted in 200 µL ACN:H_2O 1:1, 0.1% formic acid, filtrated through 0.45 µm RC filters, and was ready for LC-HRMS/MS analysis.

For the quantification, a 4-point spiked calibration plot was prepared in wastewater matrix with concentration range 0.2-10 ng/L (concentration in sample). The identification criteria for the determination of compounds were the retention time (±0.2 min) and the ion ratio of each analyte compared to the standard solution (±30%). Quantification was based on the ratio of the peak area of each analyte to the peak area of the corresponding deuterated internal standard.

6.2.2 Results and Discussion - External Laboratory (NKUA/TraMS) Results

Overall, thirty-seven organic micropollutants from different chemical classes were detected in the twenty-two samples collected during the autumn (October 2024), winter, and October 2025 sampling periods. Based on the main use, class or application of the detected compounds, a classification is indicated, although some compounds may have multiple uses. Therefore, out of the thirty-six compounds, the thirty-one were classified due to their use as pharmaceuticals and the remaining five were coffee and tobacco related contaminants. Among the most abundant class, five compounds were categorized as antidepressants and antipsychotic drugs, four were antibiotics, and one was anti-hypertensive. In the eighteen samples collected in the spring and summer sampling period, thirty-six organic micropollutants were identified. In contrast to the other sampling periods, one pharmaceutical compound was not detected. The detected organic micropollutants and their classification according to their primary use are presented in Table 18.

Table 18: Detected compounds in marine environment during the sampling campaigns and their classification regarding their use.

ORGANIC MICROPOLLUTANTS	CLASS
ANABASINE	Tobacco related chemical
ANATABINE	Tobacco related chemical
AMISULPRIDE	Antidepressant & Antipsychotic Drugs
CAFFEINE	Stimulants
CLINDAMYCIN	Pharmaceuticals
CLOPIDOGREL	Pharmaceuticals
CLOPIDOGREL COOH	Pharmaceutical metabolite
IRBESARTAN	Pharmaceutical
LIDOCAINE	Pharmaceutical
MEMANTINE	Pharmaceutical

ORGANIC MICROPOLLUTANTS	CLASS
MEFENAMIC ACID	Pharmaceutical
MINOXIDIL	Pharmaceutical
NICOTINE	Tobacco related chemical
NORFLOXACIN	Antibiotic
TELMISARTAN	Anti-hypertensive
THEOPHYLLINE	Pharmaceutical
TRAMADOL	Pharmaceutical
TRIMETHOPRIM	Pharmaceutical
VENLAFAXINE	Antidepressant & Antipsychotic Drugs
VIGABATRIN	Pharmaceutical
AZITHROMYCIN	Pharmaceutical
CARBAMAZEPINE	Pharmaceutical
CETIRIZINE	Pharmaceutical
CIMETIDINE	Pharmaceutical
CITALOPRAM	Antidepressant & Antipsychotic Drugs
CLARITHROMYCIN	Antibiotic
LEVOFLOXACIN	Antibiotic
METFORMIN	Pharmaceutical
MIRTAZAPINE	Antidepressant & Antipsychotic Drugs
NIFLUMIC ACID	Pharmaceutical
O-DESMETHYL-TRAMADOL	Pharmaceuticals TPs
O-DESMETHYL-VENLAFAXINE	Pharmaceuticals TPs
OFLOXACIN	Antibiotic
COTININE	Tobacco related chemical
QUETIAPINE	Antipsychotic Drugs
TORSEMIDE	Pharmaceutical
BENZOYLECGONINE	Metabolite of cocaine

TP: Transformation Products

Concerning the five target pharmaceutical compounds monitored in influent and effluent samples from the Psyttalia WWTP under the scope of Task 7.3 (Diclofenac, Carbamazepine, Hydrochlorothiazide, Metoprolol, and 1H-Benzotriazole), only Carbamazepine was detected in seawater samples, probably due to the dilution effect.

Furthermore, six compounds (Amisulpride, Irbesartan, Venlafaxine, Carbamazepine, Citalopram, and Clarithromycin) out of the thirty-seven are included in the watch list of the updated Urban Wastewater Treatment Directive (UWWTD; 2024/3019). In addition, three pharmaceuticals (Azithromycin, Carbamazepine, and Clarithromycin) are included in the watch list of the Proposal for a Directive of the European Parliament and of the Council amending Directive 2000/60/EC establishing a framework for Community action in the field of water policy, Directive 2006/118/EC on the protection of groundwater against pollution and deterioration and Directive 2008/105/EC on environmental quality standards in the field of water policy - Analysis of the final compromise text with a view to agreement.

The concentrations of the detected compounds, expressed in ng/L, at the different sampling points during the annual monitoring campaign are presented in Table 19 to Table 23. For each detected compound, the corresponding LOD and LOQ are also reported in the aforementioned tables.

Table 19: Concentrations (ng/L) of the detected compounds at sampling stations in the marine area surrounding the Psyttalia WWTP during the campaign conducted on 21 October 2024.

21/10/2024											
ANALYTES (ng/L)	Stations									*LOQ*	*LOD*
	A1S	A2S	A3S	A3B	A4S	A4B	B1S	B2S	B3S		
ANABASINE	34	5.6	6.8	11	3.2	6.5	2.9	1.4	2	0.87	0.26
ANATABINE	23	13	1.2	22	9.4	13	5.9	17	10	0.86	0.26
AMISULPRIDE	1.1	2	0.8	0.8	0.9	1	1	1.1	0.64	0.1	0.032
CAFFEINE	44	30	28	46	16	27	17	12	16	1.8	0.53
CLINDAMYCIN	2.8	4.4	3.4	5.8	<LOQ	<LOQ	<LOQ	<LOQ	<LOD	0.74	0.25
CLOPIDOGREL	<LOQ	<LOQ	0.32	1.1	<LOD	<LOQ	<LOQ	<LOD	<LOQ	0.16	0.054
CLOPIDOGREL COOH	34	254	43	30	72	74	115	45	56	5	1.7
IRBESARTAN	0.79	1.4	1.1	0.62	0.69	0.84	2.6	0.87	0.44	0.1	0.033
LIDOCAINE	0.74	1.5	0.51	0.43	1.6	1.4	3.2	1.4	<LOQ	0.28	0.092
MEMANTINE	15	1.6	15	16	0.16	0.27	0.53	0.33	0.23	0.091	0.03
MEFENAMIC ACID	1.7	1.5	3.9	1.7	2	1.7	4.9	2.8	1.7	0.5	0.17
MINOXIDIL	4.6	5.8	4.3	1	2.2	15	6.2	2.8	16	0.18	0.062
NICOTINE	45	12	11	16	7.6	10	8	5.1	5.9	0.65	0.22
NORFLOXACIN	144	24	28	39	105	50	52	43	116	0.26	0.088
TELMISARTAN	4.2	2.1	2.7	2	9.4	10	4.6	5.3	6.7	0.2	0.067
THEOPHYLLINE	7.9	22	13	5.1	33	63	17	12	8.8	0.18	0.059
TRAMADOL	0.29	2	0.21	0.18	0.14	0.32	0.64	0.44	0.41	0.05	0.016
TRIMETHOPRIM	14	3.2	1.5	2.6	4.9	10	4.6	6	24	0.26	0.088
VENLAFAXINE	6.2	2.4	2.1	2.1	1.2	1.4	0.72	1	1.4	0.1	0.033
VIGABATRIN	80	85	67	87	76	83	82	80	84	0.73	0.24
AZITHROMYCIN	2.4	1.3	0.8	1.6	<LOQ	<LOQ	<LOQ	<LOQ	<LOD	0.71	0.24
CARBAMAZEPINE	1	2.3	6.8	1.5	1.3	1.4	2	1.3	1.1	0.0063	0.0021
CETIRIZINE	<LOD	<LOQ	<LOD	<LOQ	1.1	1.3	1.5	<LOQ	1	1	0.33
CIMETIDINE	5	<LOD	1.2	<LOQ	17	7.6	3.8	3.1	14	1.2	0.4
CITALOPRAM	1.9	1.9	2.9	2.7	3.2	3.9	2.9	4.1	4	0.17	0.055
CLARITHROMYCIN	0.9	0.9	0.7	0.5	1.8	0.7	2.8	0.7	1.1	0.1	0.035
LEVOFLOXACIN	2.1	2.1	1.6	1.8	5.4	3.3	4	4.2	3.4	0.04	0.013
METFORMIN	18	18	11	23	25	57	67	24	58	0.25	0.083
MIRTAZAPINE	<LOQ	<LOQ	<LOQ	0.35	<LOQ	0.7	0.2	<LOQ	<LOQ	0.2	0.066
NIFLUMIC ACID	1.2	1.2	1.5	1.4	1.4	1.2	1.5	1.3	1.4	0.21	0.071
O-DESMETHYL-TRAMADOL	0.15	0.15	0.08	<LOQ	0.136	0.17	0.34	0.48	0.49	0.077	0.026
O-DESMETHYL-VENLAFAXINE	0.63	0.63	0.22	0.38	0.21	1.8	1	0.9	2	0.056	0.019
OFLOXACIN	1.4	1.4	0.87	0.94	2.1	1.7	2.4	1.7	1.5	0.038	0.013
COTININE	53	53	28	61	43	51	37	36	41	0.41	0.14
QUETIAPINE	0.057	0.057	0.029	0.031	0.03	0.073	0.082	0.097	0.084	0.024	0.008
TORSEMIDE	1.3	1.3	1.7	1.8	0.17	<LOQ	0.38	<LOQ	<LOQ	0.059	0.02
BENZOYLECGONINE	0.13	0.13	0.11	0.17	0.12	0.28	0.18	0.34	0.35	0.054	0.018
CUMULATIVE CONCENTRATION LEVELS	**552.7**	**558.5**	**291.4**	**388.7**	**448.3**	**501.4**	**456.7**	**317.2**	**480.7**	-	-

LOQ: Limit of Quantification, LOD: Limit of Detection

Table 20: Concentrations (ng/L) of the detected compounds at sampling stations in the marine area surrounding the Psyttalia WWTP during the campaign conducted on 6 March 2025

6/3/2025											
ANALYTES (ng/L)	Stations									*LOQ*	*LOD*
	A1S	A2S	A3S	A3B	A4S	A4B	B1S	B2S	B3S		
ANABASINE	17	4.1	15	12	5.8	8.7	5	11	2.6	0.87	0.26
ANATABINE	50	4.2	7.7	11	8.2	20	6.3	17	11	0.86	0.26
AMISULPRIDE	18	2.3	2	1.6	4.7	2.4	1.3	1.9	0.87	0.1	0.032
CAFFEINE	184	20	25	19	32	25	36	56	28	1.8	0.53
CLINDAMYCIN	7.9	<LOQ	<LOQ	<LOQ	<LOQ	<LOD	<LOQ	<LOQ	0.79	0.74	0.25
CLOPIDOGREL	11	0.17	0.25	0.21	0.55	0.47	<LOQ	0.37	<LOQ	0.16	0.054
CLOPIDOGREL COOH	706	211	167	41	188	81	77	83	90	5	1.7
IRBESARTAN	120	46	33	16	124	83	3.5	54	4.8	0.1	0.033
LIDOCAINE	18	2.7	1.2	0.92	3	2.2	0.95	1.8	0.83	0.28	0.092
MEMANTINE	3.7	0.4	0.38	0.3	0.34	0.33	1.1	0.39	1.4	0.091	0.03
MEFENAMIC ACID	20	30	19	10	104	48	3.1	32	1.7	0.5	0.17
MINOXIDIL	18	1.9	1.5	2	3.8	2.8	1.5	1.8	1.1	0.18	0.062
NICOTINE	22	9.1	20	14	12	12	8.6	15	6.1	0.65	0.22
NORFLOXACIN	10	0.84	1.2	3.7	1.2	1.2	1.8	1.4	1.8	0.26	0.088
TELMISARTAN	39	22	14	10	27	27	2.4	18	3.8	0.2	0.067
THEOPHYLLINE	156	31	70	19	94	25	31	118	8	0.18	0.059
TRAMADOL	10	2.8	1.5	0.7	3.4	1.1	1	2.3	0.8	0.05	0.016
TRIMETHOPRIM	22	50	24	18	11	3.9	8	2.5	3.7	0.26	0.088
VENLAFAXINE	34	1.7	1.2	2	2.5	1.4	1.5	1.8	2.2	0.1	0.033
VIGABATRIN	84	82	78	86	86	91	84	88	89	0.73	0.24
AZITHROMYCIN	37	2.6	2.2	1.1	2.4	1.8	1.1	2.4	2.1	0.71	0.24
CARBAMAZEPINE	14	5	3.6	2.6	8.1	10	1.2	4.6	1.3	0.0063	0.0021
CETIRIZINE	15	1	1.1	1.1	1.9	1.1	<LOQ	1.2	<LOQ	1	0.33
CIMETIDINE	17	7.7	8.6	62	22	3.3	27	2.9	13	1.2	0.4
CITALOPRAM	11	4.7	2.9	4.4	8	11	1.3	5.1	1.4	0.17	0.055
CLARITHROMYCIN	30	18	11	10	26	5.5	1.2	8.4	2.6	0.1	0.035
LEVOFLOXACIN	4	4.8	3.7	3.1	6.2	3.9	2.2	3.3	2	0.04	0.013
METFORMIN	443	209	135	76	257	122	62	167	62	0.25	0.083
MIRTAZAPINE	1	<LOQ	<LOQ	0.65	0.28	1	0.23	<LOQ	<LOQ	0.2	0.066
NIFLUMIC ACID	2.6	1.4	1.5	1.6	1.6	1.6	1.5	1.5	1.5	0.21	0.071
O-DESMETHYL-TRAMADOL	6.2	0.74	0.51	0.41	1.5	0.4	0.59	0.64	0.33	0.077	0.026
O-DESMETHYL-VENLAFAXINE	18.8	6.2	2.4	1.7	4.9	1.5	1.5	3.3	1.2	0.056	0.019
OFLOXACIN	2.6	1.1	2	1.7	1.2	2.2	0.85	1.4	0.88	0.038	0.013
COTININE	54	32	32	42	35	44	40	45	37	0.41	0.14
QUETIAPINE	0.79	0.22	0.12	0.085	0.39	0.092	0.088	0.14	0.072	0.024	0.008
TORSEMIDE	1.2	<LOQ	0.51	<LOQ	0.92	0.63	0.34	0.6	0.24	0.059	0.02
BENZOYLECGONINE	8.9	0.65	0.64	0.39	1.5	0.51	0.31	0.79	0.32	0.054	0.018
CUMULATIVE CONCENTRATION LEVELS	**2217.7**	**817.8**	**690.2**	**476.7**	**1090.8**	**647.4**	**416.4**	**755.0**	**385.1**	-	-

LOQ: Limit of Quantification, LOD: Limit of Detection

Table 21: Concentrations (ng/L) of the detected compounds at sampling stations in the marine area surrounding the Psyttalia WWTP during the campaign conducted on 29 May 2025.

29/5/2025											
ANALYTES (ng/L)	Stations									*LOQ*	*LOD*
	A1S	A2S	A3S	A3B	A4S	A4B	B1S	B2S	B3S		
ANABASINE	21.5	28.7	14.6	9.49	18.3	15.7	20.6	10.5	11.6	0.87	0.26
ANATABINE	43.5	47.9	32	21.3	47.8	22.2	42.7	26.6	34.7	0.86	0.26
AMISULPRIDE	0.97	0.174	0.153	0.535	0.21	1.81	0.234	0.22	0.193	0.1	0.032
CAFFEINE	7.06	3.5	<LOQ	<LOQ	4.19	2.05	3.63	<LOQ	1.88	1.8	0.53
CLINDAMYCIN	1.26	<LOQ	<LOQ	<LOQ	<LOQ	1.65	0.842	<LOQ	0.905	0.74	0.25
CLOPIDOGREL	2.45	1.42	0.423	0.678	0.767	2.09	0.595	1.23	1.22	0.16	0.054
CLOPIDOGREL COOH	38.2	13.9	11.6	15.9	24.3	83.5	30.4	23.6	27.5	5	1.7
IRBESARTAN	12.1	1.55	1.7	1.66	2.16	3.35	2.53	1.54	1.14	0.1	0.033
LIDOCAINE	1.76	0.466	<LOQ	<LOQ	0.437	1.85	0.928	0.639	0.432	0.28	0.092
MEMANTINE	16.3	10.6	5.77	3.78	9.45	14.3	9.69	7.28	10.5	0.091	0.03
MEFENAMIC ACID	3.09	25.9	13.7	20	17.1	10.6	34.37	70.04	15.57	0.5	0.17
MINOXIDIL	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.18	0.062
NICOTINE	64.5	96	36.6	31.7	56.6	60	67.9	34.2	35.8	0.65	0.22
NORFLOXACIN	17.6	45.9	20.2	7.77	12.2	8.61	19.1	17.2	15.8	0.26	0.088
TELMISARTAN	3.03	0.727	1.35	1.84	1.59	1.17	0.82	1.11	0.835	0.2	0.067
THEOPHYLLINE	7.97	3.87	2.98	3.72	8.66	6.7	3.85	4.72	1.93	0.18	0.059
TRAMADOL	0.49	0.08	<LOQ	0.07	0.09	0.39	0.16	0.13	0.19	0.05	0.016
TRIMETHOPRIM	1.49	1.68	1.25	0.5	0.38	0.87	0.65	0.75	0.64	0.26	0.088
VENLAFAXINE	3.1	2.36	1	0.74	1.04	1.44	0.95	1.04	1.59	0.1	0.033
VIGABATRIN	30.2	27.8	23.8	37.8	24.3	27.3	34.1	27	26.4	0.73	0.24
AZITHROMYCIN	1.41	0.99	1.05	1	1.12	1.39	0.73	1.22	1.22	0.71	0.24
CARBAMAZEPINE	22.2	11.1	6.72	11.7	9.3	21.99	13.5	13.3	15.4	0.006	0.00
CETIRIZINE	10.8	3.96	3.83	2.29	4.02	6.42	7.45	8.87	7.86	1	0.33
CIMETIDINE	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1.2	0.4
CITALOPRAM	12.3	3.68	5.54	4.63	5	6.53	3.71	3.92	6.03	0.17	0.055
CLARITHROMYCIN	0.214	<LOQ	<LOQ	<LOD	<LOD	0.151	<LOD	0.044	<LOD	0.1	0.035
METFORMIN	8.4	1.34	1.38	1.19	1.36	1.43	2.38	2.28	2.08	0.25	0.083
MIRTAZAPINE	<LOD	<LOD	<LOD	0.221	<LOD	1.21	<LOD	<LOD	<LOD	0.2	0.066
NIFLUMIC ACID	0.802	0.253	0.285	<LOQ	0.224	1.1	0.463	0.409	0.426	0.21	0.071
O-DESMETHYL-TRAMADOL	0.158	0.091	<LOQ	0.142	<LOQ	0.149	0.081	0.081	0.517	0.077	0.026
O-DESMETHYL-VENLAFAXINE	1.54	0.203	0.0579	0.0666	0.0696	0.63	0.122	0.31	0.341	0.056	0.019
OFLOXACIN	0.86	0.266	0.27	1.14	0.1	0.673	0.901	0.201	0.195	0.038	0.013
COTININE	25	10	6.77	6.67	8.78	7.04	13.2	4.87	3.77	0.41	0.14
QUETIAPINE	0.100	0.027	0.006	0.033	0.023	<LOQ	0.024	<LOQ	<LOD	0.024	0.008
TORSEMIDE	<LOQ	<LOQ	0.631	0.387	<LOQ	<LOQ	0.303	<LOQ	0.607	0.059	0.02
BENZOYLECGONINE	0.287	0.078	0.068	<LOQ	0.122	0.045	0.091	0.096	0.0909	0.054	0.018
CUMULATIVE CONCENTRATION LEVELS	**360.7**	**345.0**	**195.3**	**188.5**	**260.1**	**314.4**	**317.0**	**264.7**	**227.4**	-	-

LOQ: Limit of Quantification, LOD: Limit of Detection

Table 22: Concentrations (ng/L) of the detected compounds at sampling stations in the marine area surrounding the Psyttalia WWTP during the campaign conducted on 24 July 2025.

24/7/2025											
ANALYTES (ng/L)	Stations									*LOQ*	*LOD*
	A1S	A2S	A3S	A3B	A4B	B1S	B2S	B3S	B3B		
ANABASINE	3.01	4.15	1.82	2.66	2	7.95	2.84	6.08	3.57	0.87	0.26
ANATABINE	6.96	8.77	6.65	14.3	18	17.4	4.96	19	11.7	0.86	0.26
AMISULPRIDE	6.16	0.256	0.568	0.971	2.59	<LOD	<LOD	0.192	1.23	0.1	0.032
CAFFEINE	5.95	1.94	6.72	3.64	2.45	10.3	2.19	8.77	2.53	1.8	0.53
CLINDAMYCIN	<LOD	<LOD	<LOD	<LOQ	1.41	<LOQ	<LOD	<LOQ	<LOQ	0.74	0.25
CLOPIDOGREL	0.557	0.246	0.172	<LOQ	0.471	0.332	0.216	<LOQ	0.232	0.16	0.054
CLOPIDOGREL COOH	40.9	<LOQ	<LOD	26.4	56.1	10.5	<LOQ	<LOQ	22.9	5	1.7
IRBESARTAN	19.3	4.42	2.6	4.25	2.93	2.98	2.27	2.85	10.4	0.1	0.033
LIDOCAINE	<LOQ	<LOD	<LOD	0.371	0.979	0.565	<LOQ	0.704	0.338	0.28	0.092
MEMANTINE	2	2.03	1.89	3.08	8.67	2.41	1.69	3.77	4.47	0.091	0.03
MEFENAMIC ACID	17.9	18.8	21.9	17.7	28.1	28.6	20.8	32.1	26.2	0.5	0.17
MINOXIDIL	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.18	0.062
NICOTINE	8.45	11.7	4.47	6.69	5.2	22.6	7.22	18.2	8.11	0.65	0.22
NORFLOXACIN	89.1	198	19.1	65.45	71.3	166	36.7	280	82.3	0.26	0.088
TELMISARTAN	2.85	1.06	2.91	0.95	1.79	1.01	0.79	1.14	2.12	0.2	0.067
THEOPHYLLINE	6.77	2.95	2.45	2.85	3.29	13.2	3.75	19	2.01	0.18	0.059
TRAMADOL	3.55	0.07	<LOD	0.845	1.51	0.186	0.104	0.096	0.513	0.05	0.016
TRIMETHOPRIM	1.41	1.38	1.46	2.81	1.95	0.962	0.526	0.838	1.56	0.26	0.088
VENLAFAXINE	332.91	2.29	1.96	1.56	13.04	1.87	0.67	3.2	2.86	0.1	0.033
VIGABATRIN	188	146	185	200	202	92	135	125	142	0.73	0.24
AZITHROMYCIN	<LOQ	<LOQ	<LOQ	<LOQ	0.91	<LOQ	<LOQ	<LOQ	<LOQ	0.71	0.24
CARBAMAZEPINE	7.81	3.11	2.29	1.8	6.73	4.6	0.965	5.06	13.5	0.0063	0.0021
CETIRIZINE	3.83	15.84	13.75	24.11	6.3	15.6	0.874	5.04	4.09	1	0.33
CIMETIDINE	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1.2	0.4
CITALOPRAM	13.41	5.1	10.6	4.31	7.47	5.64	4.04	4.73	11	0.17	0.055
CLARITHROMYCIN	<LOD	0.324	0.279	1.48	2.02	0.31	<LOD	0.309	1.22	0.1	0.035
METFORMIN	2.7	1.8	2.22	1.25	1.52	1.21	2.18	1.37	1.44	0.25	0.083
MIRTAZAPINE	<LOD	<LOQ	<LOD	0.873	0.2	<LOD	<LOD	<LOD	<LOQ	0.2	0.066
NIFLUMIC ACID	0.501	<LOD	0.293	<LOD	0.46	<LOD	<LOQ	0.212	<LOQ	0.21	0.071
O-DESMETHYL-TRAMADOL	2	0.156	0.14	0.229	0.678	0.271	0.284	0.154	0.144	0.077	0.026
O-DESMETHYL-VENLAFAXINE	11.6	0.332	1.09	1.41	2.66	1.81	0.207	1.24	1.15	0.056	0.019
OFLOXACIN	0.703	0.307	0.415	0.922	0.716	0.333	0.205	0.271	1.09	0.038	0.013
COTININE	10.2	5.61	16.3	7.46	8.34	10.6	6.49	13.5	12.8	0.41	0.14
QUETIAPINE	0.125	0.0756	0.0259	0.0781	0.142	0.0515	<LOQ	0.0823	0.088	0.024	0.008
TORSEMIDE	11.7	6.15	0.597	1.06	2.38	12.6	8.84	19.4	10.1	0.059	0.02
BENZOYLECGONINE	0.0609	0.108	0.0777	<LOD	0.109	0.0854	<LOQ	0.13	0.0856	0.054	0.018
CUMULATIVE CONCENTRATION LEVELS	**800.9**	**445.9**	**308.1**	**400.3**	**464.4**	**432.7**	**247.0**	**575.7**	**382.7**	-	-

LOQ: Limit of Quantification, LOD: Limit of Detection

Table 23: Concentrations (ng/L) of the detected compounds at sampling stations in the marine area surrounding the Psyttalia WWTP during the campaign conducted on 2 October 2025.

2/10/2025						
ANALYTES	Stations				LOQ	LOD
	A1S	A3S	B1S	B2S		
ANABASINE	43.5	82.2	76.4	70.8	0.87	0.26
ANATABINE	31.8	57.4	55.3	47.3	0.86	0.26
AMISULPRIDE	0.625	3.71	0.461	0.679	0.1	0.032
CAFFEINE	14.9	22.0	5.00	7.07	1.8	0.53
CLINDAMYCIN	<LOD	<LOD	<LOD	<LOD	0.74	0.25
CLOPIDOGREL	0.179	0.388	0.308	0.256	0.16	0.054
CLOPIDOGREL COOH	8.93	37.8	13.5	12.0	5	1.7
IRBESARTAN	4.21	15.7	4.52	3.20	0.1	0.033
LIDOCAINE	0.528	1.98	0.448	0.434	0.28	0.092
MEMANTINE	0.202	0.736	0.296	0.134	0.091	0.03
MEFENAMIC ACID	37.5	6.91	16.7	8.62	0.5	0.17
MINOXIDIL	<LOD	<LOD	<LOD	<LOD	0.18	0.062
NICOTINE	4.70	7.68	6.71	6.96	0.65	0.22
NORFLOXACIN	7.58	10.6	13.6	29.9	0.26	0.088
TELMISARTAN	0.898	1.11	0.764	0.539	0.2	0.067
THEOPHYLLINE	22.1	29.7	6.24	11.7	0.18	0.059
TRAMADOL	0.556	1.52	0.478	0.440	0.05	0.016
TRIMETHOPRIM	1.41	1.86	1.52	1.73	0.26	0.088
VENLAFAXINE	1.08	2.17	1.68	1.80	0.1	0.033
VIGABATRIN	7.03	10.2	8.03	7.66	0.73	0.24
AZITHROMYCIN	<LOQ	1.64	<LOQ	<LOQ	0.71	0.24
CARBAMAZEPINE	1.04	1.15	0.993	0.718	0.006	0.002
CETIRIZINE	22.5	24.3	32.6	18.4	1	0.33
CIMETIDINE	<LOD	<LOD	<LOD	<LOD	1.2	0.4
CITALOPRAM	0.938	3.09	1.98	1.21	0.17	0.055
CLARITHROMYCIN	<LOD	<LOD	<LOD	<LOD	0.1	0.035
LEVOFLOXACIN	0.547	0.895	0.548	0.477	0.04	0.013
METFORMIN	43.3	90.2	88.7	84.6	0.25	0.083
MIRTAZAPINE	0.300	0.301	0.202	0.325	0.2	0.066
NIFLUMIC ACID	<LOQ	<LOQ	<LOQ	<LOQ	0.21	0.071
O-DESMETHYL-TRAMADOL	0.280	0.761	0.537	0.389	0.077	0.026
O-DESMETHYL-VENLAFAXINE	5.03	6.80	5.52	4.47	0.056	0.019
OFLOXACIN	1.57	2.33	1.81	1.86	0.038	0.013
COTININE	32.6	39.5	50.9	44.4	0.41	0.14
QUETIAPINE	0.142	0.625	0.206	0.151	0.024	0.008
TORSEMIDE	0.744	1.61	1.10	3.73	0.059	0.02
BENZOYLECGONINE	0.230	0.977	0.286	0.377	0.054	0.018
CUMULATIVE CONCENTRATION LEVELS	**297.9**	**468.1**	**398.3**	**373.2**	-	-

LOQ: Limit of Quantification, LOD: Limit of Detection

In addition, the cumulative concentration levels at the respective sampling points are provided. The stacked bar plots (Figure 17 to Figure 21) summarize the occurrence of organic micropollutants and their contribution to the cumulative concentration at the different sampling stations and associated depth.

6.2.2.1 Levels of organic micro-pollutants during autumn sampling campaign

Figure 17 illustrates the cumulative concentrations of the 37 organic compounds at each sampling station, for both surface and near-bottom seawater samples collected during the first seasonal sampling campaign conducted in October 2024. Each bar corresponds to a sampling point, while the coloured segments indicate the contribution of individual compounds to the total concentration.

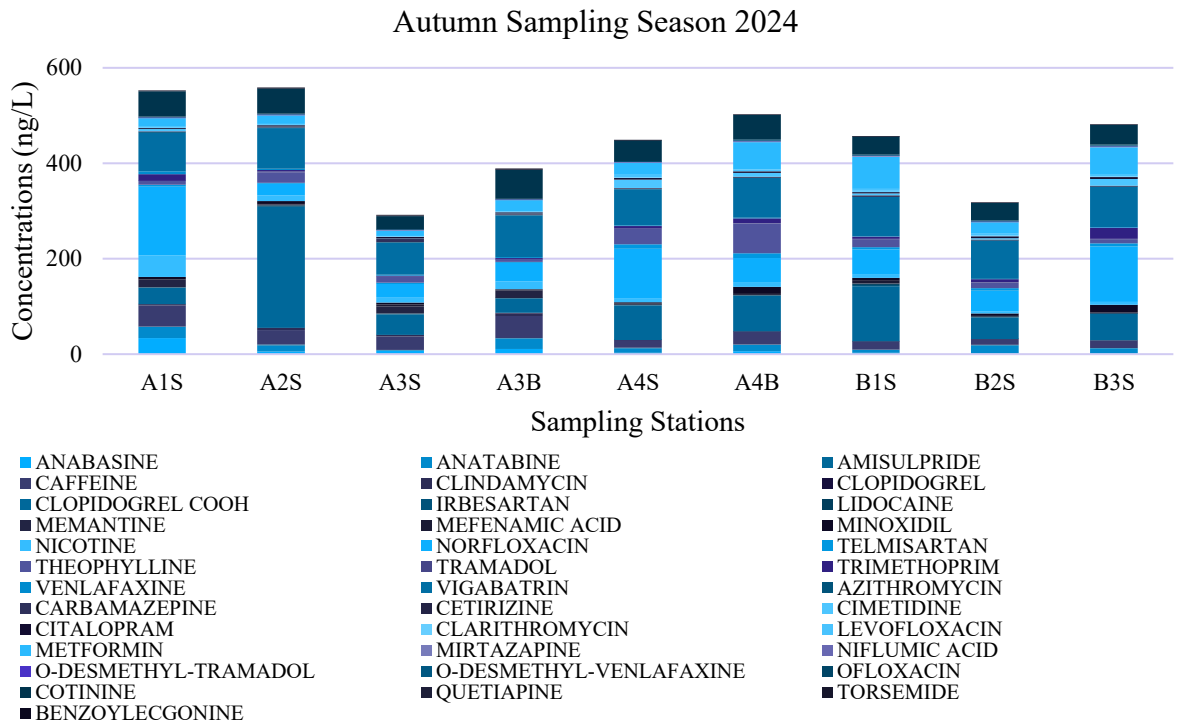

Figure 17: Cumulative levels of the target compounds displayed as a stacked bar plot at the different sampling stations during monitoring in autumn period

Surface samples collected from stations A1 and A2 exhibited the highest overall cumulative concentrations (552.7 ng/L and 558.5 ng/L, respectively) and various detected compounds reached their peak concentration levels in these sampling stations, suggesting a stronger influence from the water circulation currents as these stations are further from the discharging points. In contrast, stations A3 (surface sample) and B2 showed considerably lower concentration levels (291.4 ng/L and 317.2 ng/L, respectively). Cumulative concentrations in the sea surface samples of the stations A3 and A4 (448.3 ng/L) were lower than the ones in the near-bottom depth, possibly due to the proximity to the discharge pipeline (388.7 ng/L and 501.4 ng/L, respectively). Stations B1 and B3 exhibited comparable cumulative levels of organic micropollutants (455.9 ng/L and 479.6 ng/L, respectively) despite the fact that station B3 is located further of the WWTP and its discharge pipelines. This could be attributed to the seawater circulation.

Regarding compound distribution during the first seasonal sampling campaign, certain therapeutic categories, such as antibiotics, for epilepsy treatment, for reduction of heart disease and stroke risk, and coffee and tobacco related contaminants, contribute substantially across most stations. Indeed, Norfloxacin (antibiotic), Vigabatrin (pharmaceutical-used for epilepsy treatment), Cotinine, Nicotine, and Caffeine (coffee and tobacco related contaminants) were abundant at all sampling points with mean concentrations of 60.6 ng/L, 80.0 ng/L, 45.3 ng/L, 14.3 ng/L, and 27.5 ng/L, respectively (Table 24).

The metabolite of the antiplatelet drug Clopidogrel (Clopidogrel COOH) was contributing mainly to station A2 (254 ng/L) and the antidiabetic agent Metformin was in high levels at near-bottom of station A4 (57 ng/L) and at surface seawater of stations B1 and B3 (67 ng/L and 58 ng/L, respectively). A consistent trend was observed when examining the relationship between concentration levels of these compounds and seawater depth. Specifically, for all five compounds, with the exemption of Norfloxacin, an increase in concentration levels was observed from the near bottom to the surface.

6.2.2.2 Levels of organic micropollutants during winter sampling campaign

Figure 18 highlights the cumulative contribution of the detected compounds to total concentrations at the sampling stations and water depths during the second seasonal sampling campaign conducted in March 2025. The highest cumulative concentration levels occurred at the sampling station A1 (2217.7 ng/L), where most of the detected compounds such as the metabolite Clopidogrel COOH and Metformin showed the highest concentration compared to the remaining sampling points. At station A1, the cumulative concentration was twofold higher than the concentration recorded at station A4 (1090.8 ng/L), the second more polluted station regarding the micropollutant levels.

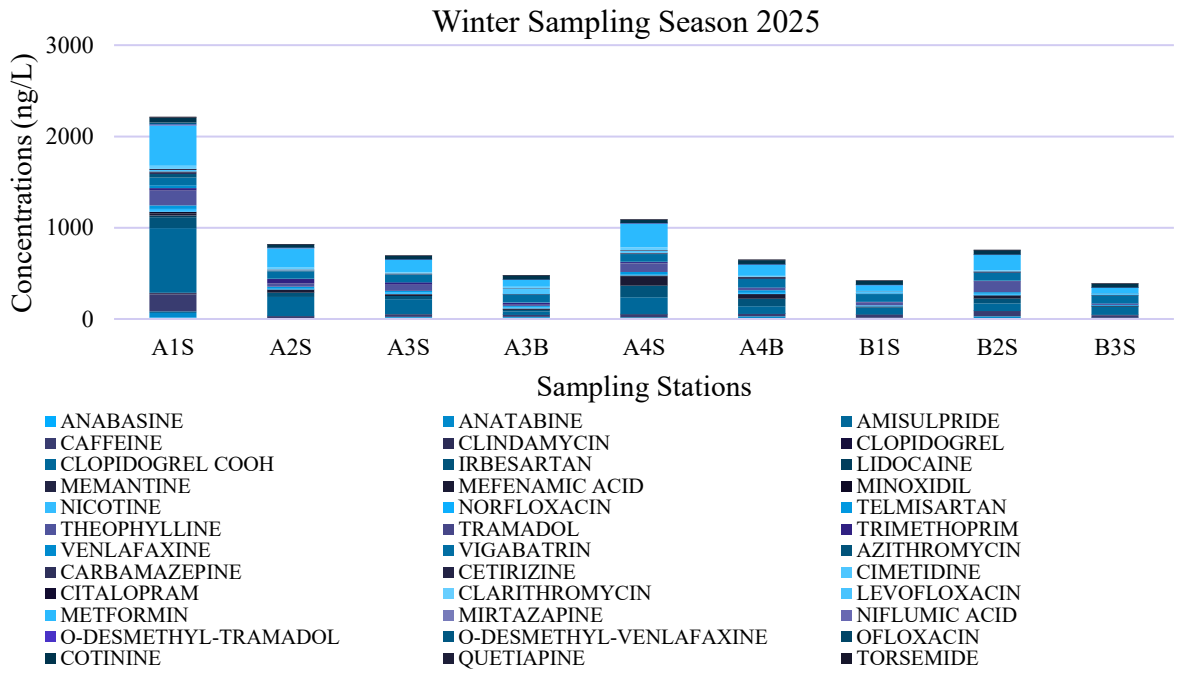

Figure 18: Cumulative levels of the target compounds displayed as a stacked bar plot at the different sampling stations during monitoring in winter period

Stations A1 and A2 showed again high cumulative concentrations mainly due to the prevailing water circulation. The third most polluted sampling station was A2 (817.8 ng/L).

In contrast to the previous sampling campaign, an opposite pattern was observed regarding the concentration levels of the detected compounds at stations A3 and A4. In fact, in spite of the proximity to the discharge pipeline, the near-bottom samples exhibited lower concentrations (476.7 ng/L and 647.4 ng/L, respectively) than the corresponding surface samples (690.2 ng/L and 1090.8 ng/L, respectively), probably due to the prevailing circulation conditions. Stations B1 and B3 exhibited again comparable cumulative levels of organic compounds (416.4 ng/L and 385.1 ng/L, respectively).

Concerning compound distribution during the second seasonal sampling campaign, antibiotics, antidiabetic agents, drugs for epilepsy treatment, for respiratory diseases, for reduction of heart disease and stroke risk, and coffee and tobacco related contaminants, contribute markedly to all stations. Specifically, Metformin (antidiabetic agent), the metabolite of the antiplatelet drug Clopidogrel (Clopidogrel COOH), Vigabatrin (pharmaceutical-used for epilepsy treatment), Theophylline (pharmaceutical-used for respiratory diseases), and Irbesartan (pharmaceutical-used to treat hypertension, heart failure, and diabetic kidney disease) were present in high levels at all sampling points with mean concentrations of 125.9 ng/L, 103.9 ng/L, 86.0 ng/L, 52.1 ng/L, and 45.5 ng/L, respectively (Table 24). Caffeine (coffee and tobacco related contaminants) was mostly abundant at station A1 (184 ng/L). All these compounds, with the exemption of Vigabatrin, tended to exhibit higher concentration levels at surface seawater layer compared to the near-bottom layer.

Table 24: Mean concentrations (ng/L) during the four seasonal and one additional sampling campaigns of the detected organic micropollutants

ANALYTES (ng/L)	Sampling Campaigns-Dates				
	21/10/2024	6/3/2025	29/5/2025	24/7/2025	2/10/2025
ANABASINE	8.9	8.6	17.4	3.8	68.2
ANATABINE	13.1	11.6	35.5	12.0	48.0
AMISULPRIDE	1.1	2.1	0.5	1.7	1.4
CAFFEINE	27.5	31.6	2.9	4.9	12.2
CLINDAMYCIN	2.2	0.4	0.7	0.6	-
CLOPIDOGREL	0.2	0.3	1.2	0.3	0.3
CLOPIDOGREL COOH	83.4	103.9	30.2	20.5	18.1
IRBESARTAN	1.1	45.5	3.3	5.8	6.9
LIDOCAINE	1.3	1.6	0.8	0.5	0.8
MEMANTINE	6.1	0.6	9.6	3.3	0.3
MEFENAMIC ACID	2.5	31.1	24.4	23.6	17.4
MINOXIDIL	5.2	2.1	-	-	-
NICOTINE	14.3	12.5	55.9	10.3	6.5
NORFLOXACIN	60.6	1.8	18.6	112.0	15.4
TELMISARTAN	5.0	14.6	1.5	1.6	0.8
THEOPHYLLINE	21.6	52.1	5.3	6.3	17.4
TRAMADOL	0.5	1.5	0.2	0.9	0.7
TRIMETHOPRIM	5.9	10.2	0.9	1.4	1.6
VENLAFAXINE	2.1	1.8	1.5	40.0	1.7
VIGABATRIN	80.0	86.0	29.0	157.2	8.2
AZITHROMYCIN	0.9	1.9	1.1	0.4	0.9

ANALYTES (ng/L)	Sampling Campaigns-Dates				
	21/10/2024	6/3/2025	29/5/2025	24/7/2025	2/10/2025
CARBAMAZEPINE	2.2	4.5	13.7	5.1	1.0
CETIRIZINE	0.9	1.1	6.0	9.9	24.5
CIMETIDINE	5.5	19.8	-	-	-
CITALOPRAM	2.9	4.9	5.7	7.4	1.8
CLARITHROMYCIN	1.1	9.2	0.1	0.8	-
LEVOFLOXACIN	3.1	3.5	-	-	0.6
METFORMIN	30.4	125.9	2.5	1.7	76.7
MIRTAZAPINE	0.2	0.4	0.7	0.3	0.3
NIFLUMIC ACID	1.3	1.5	0.5	0.3	0.2
O-DESMETHYL-TRAMADOL	0.2	0.6	0.1	0.5	0.5
O-DESMETHYL-VENLAFAXINE	0.7	2.4	0.4	2.4	5.5
OFLOXACIN	1.6	1.5	0.6	0.6	1.9
COTININE	45.3	39.3	10.3	10.1	41.9
QUETIAPINE	0.1	0.1	0.0	0.1	0.3
TORSEMIDE	0.8	0.5	0.2	8.1	1.8
BENZOYLECGONINE	0.2	0.6	0.1	0.1	0.5
CUMULATIVE CONCENTRATION LEVEL	**12.22**	**17.69**	**8.52**	**13.77**	**11.99**

6.2.2.3 Levels of organic micropollutants during spring sampling campaign

Figure 19 provides an overview of cumulative compound concentrations across all sampling stations, based on surface and near-bottom seawater samples during the third seasonal sampling campaign conducted in May 2025.

Stations A1 and A2, as in the previous two campaigns, were the most polluted sampling stations in terms of concentration of compounds detected, highlighting the strong influence from water circulation. The cumulative concentrations of stations A1 and A2 were 360.7 ng/L and 345.0 ng/L, respectively.

Stations A4 (near-bottom sample) and B1 exhibited slightly lower concentration levels (314.4 ng/L and 317.0 ng/L, respectively). At station A3, the surface and near-bottom seawater layers exhibited comparable levels of organic micropollutants (195.3 ng/L and 188.5 ng/L, respectively) mainly due to vertical mixing. In contrast, at station A4, the seawater surface layer displayed lower cumulative concentration than the corresponding near-bottom layer (260.1 ng/L and 314.4 ng/L, respectively). At station B1, the observed levels of organic compounds (317.0 ng/L) were higher than at stations B2 and B3 (264.7 ng/L and 227.4 ng/L, respectively), possibly due to a combination of the proximity to the Psyttalia Island and seawater circulation.

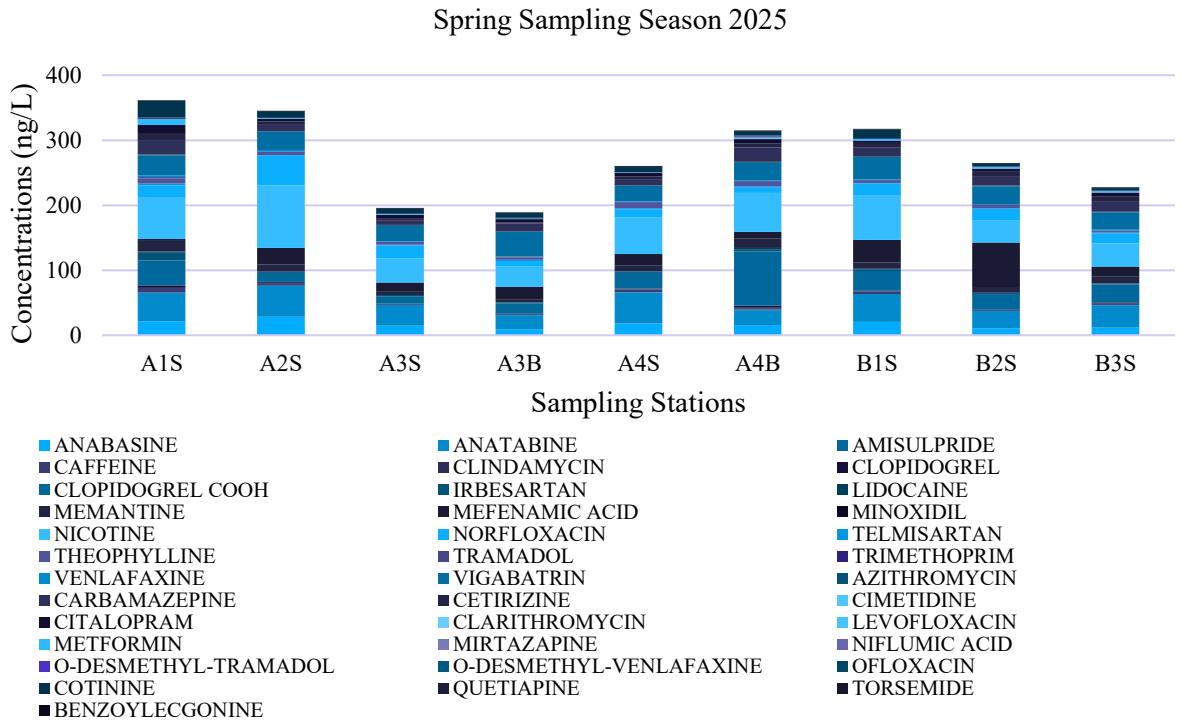

Figure 19: Cumulative levels of the target compounds displayed as a stacked bar plot at the different sampling stations during monitoring in spring period

Amidst the third seasonal campaign, tobacco related contaminants, antibiotics, drugs for reduction of heart disease, stroke risk and epilepsy treatment, and nonsteroidal anti-inflammatory drugs (NSAIDs) had significant contribution on the cumulative concentration to all stations. Notably, Nicotine, Anatabine, and Anabasine (tobacco related contaminants), Vigabatrin (pharmaceutical-used for epilepsy treatment), and the metabolite of the antiplatelet drug Clopidogrel (Clopidogrel COOH), with mean concentrations of 55.9 ng/L, 35.5 ng/L, 17.4 ng/L, 29.0 ng/L, and 30.2 ng/L, respectively (Table 24), contributed similarly across all sampling points. Norfloxacin (antibiotic) exhibited the highest concentration at station A2 (45.9 ng/L), while at the remaining stations the average concentration was approximately 14 ng/L. At stations B1 and B2, the recorded concentrations of Mefenamic acid (nonsteroidal anti-inflammatory drugs-NSAIDs) were 34.4 ng/L and 70.0 ng/L, respectively.

Moreover, at station A4, Clopidogrel COOH and Carbamazepine (pharmaceutical-used for epilepsy treatment and neuropathic pain) exhibited higher concentrations in the near-bottom seawater layer compared to the surface layer leading to the higher cumulative concentration in sample A4B.

6.2.2.4 Levels of organic micropollutants during summer sampling campaign

Figure 20 illustrates cumulative concentrations of the detected compounds in each sampling point obtained during the fourth seasonal monitoring campaign taken place in July 2025. Station A1 presented once again the highest cumulative concentration of organic micropollutants, with a value of 800.9 ng/L. During the summer campaign, the cumulative concentration recorded at station A2 was approximately half of that observed at station A1 (445.9 ng/L).

Station B2 exhibited the lowest concentration levels (247.0 ng/L) among all stations. In station A3, the seawater surface layer had lower concentration of organic micropollutants than the near-bottom layer (308.1 ng/L and 400.3 ng/L, respectively) mainly due to the dilution effect and the proximity of the later sampling point to the discharge pipeline. In contrast, during this sampling campaign, at station A4, only a near-bottom sample was collected, with cumulative concentration of 464.4 ng/L, which was higher than that measured in the near-bottom layer at station A3. At station B3 the observed levels of organic compounds in the seawater surface (575.7 ng/L) were considerably higher than in near-bottom layer (382.7 ng/L). Cimetidine (pharmaceutical-used for heartburn and peptic ulcers treatment) was not detected at any station, with measured concentrations remaining below the LOD.

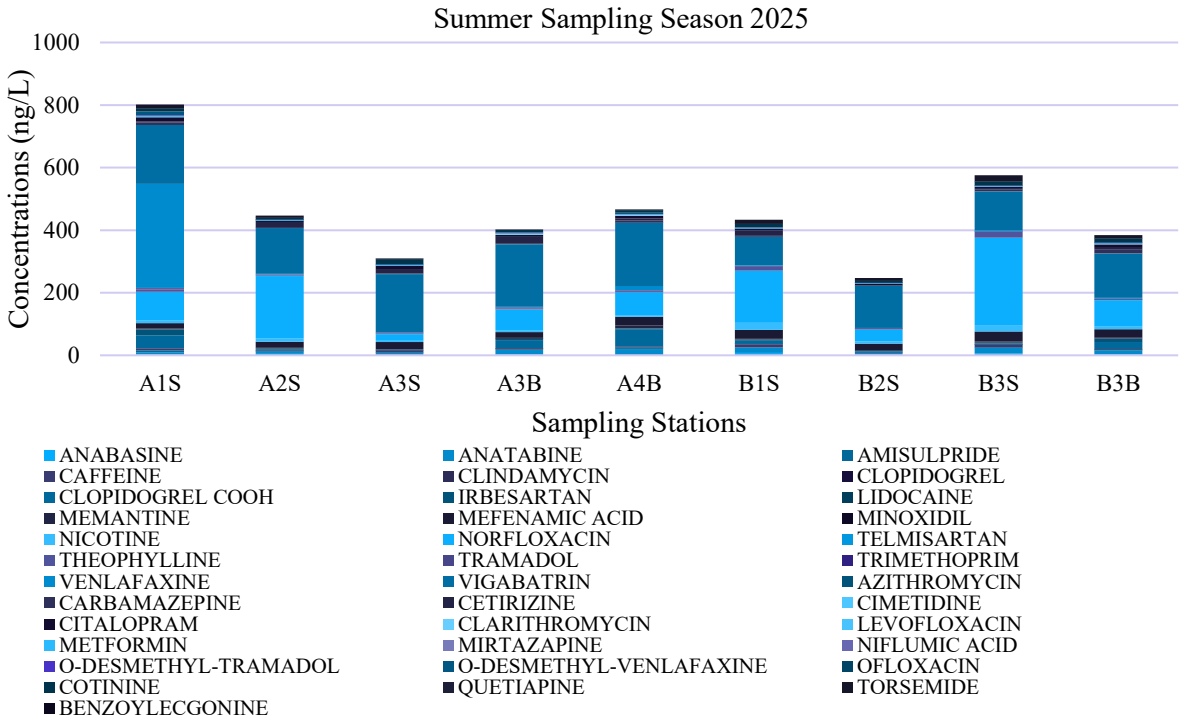

Figure 20: Cumulative levels of the target compounds displayed as a stacked bar plot at the different sampling stations during monitoring in summer period.

Among the organic micropollutants that were detected in each sampling point, antibiotics and drugs for epilepsy treatment, contributed significantly to the cumulative concentration levels. Specifically, Vigabatrin (pharmaceutical-used for epilepsy treatment), and Norfloxacin (antibiotic) with mean concentrations of 157.2 ng/L and 112.0 ng/L, respectively (Table 24), were present at elevated concentrations at all sampling point. Additionally, Venlafaxine (antidepressant medication) was most abundant at station A1 (332.9 ng/L) while the average concentration in the remaining stations was approximately 3.4 ng/L. Contribution of Norfloxacin in surface layer of station B3 was considerably higher than in the remaining sampling points (concentration of 280.0 ng/L) explaining the high cumulative concentration of the aforementioned sample.

6.2.2.5 Levels of organic micropollutants during 2024 and 2025 autumn sampling campaign

An additional sampling campaign conducted in October 2025 aiming at the enhancement of the database and the production of a comparative analysis concerning the concentration levels of micropollutants between October 2024 and October 2025 sampling periods. Amidst this supplementary sampling campaign, samples were collected from stations A1, A3, B1, and B2. Figure 21 summarizes the cumulative contribution of the detected compounds to total concentration at each sampling station during the two sampling campaigns. Focusing on the campaign in October 2025, the highest cumulative concentration was observed at station A3, reaching 468.1 ng/L. In contrast to all other sampling campaigns, station A1 exhibited the lowest cumulative concentration among the monitored stations, with a total concentration of 297.9 ng/L. Comparable cumulative levels were recorded at stations B1 and B2, with values of 398.3 ng/L and 373.2 ng/L, respectively. Several compounds, such as Clindamycin (antibiotic), Minoxidil (pharmaceutical-used for high blood pressure treatment and pattern hair loss), Cimetidine (used for heartburn and peptic ulcers treatment), and Clarithromycin (antibiotic), were not detected at any station, with measured concentrations remaining below the LOD.

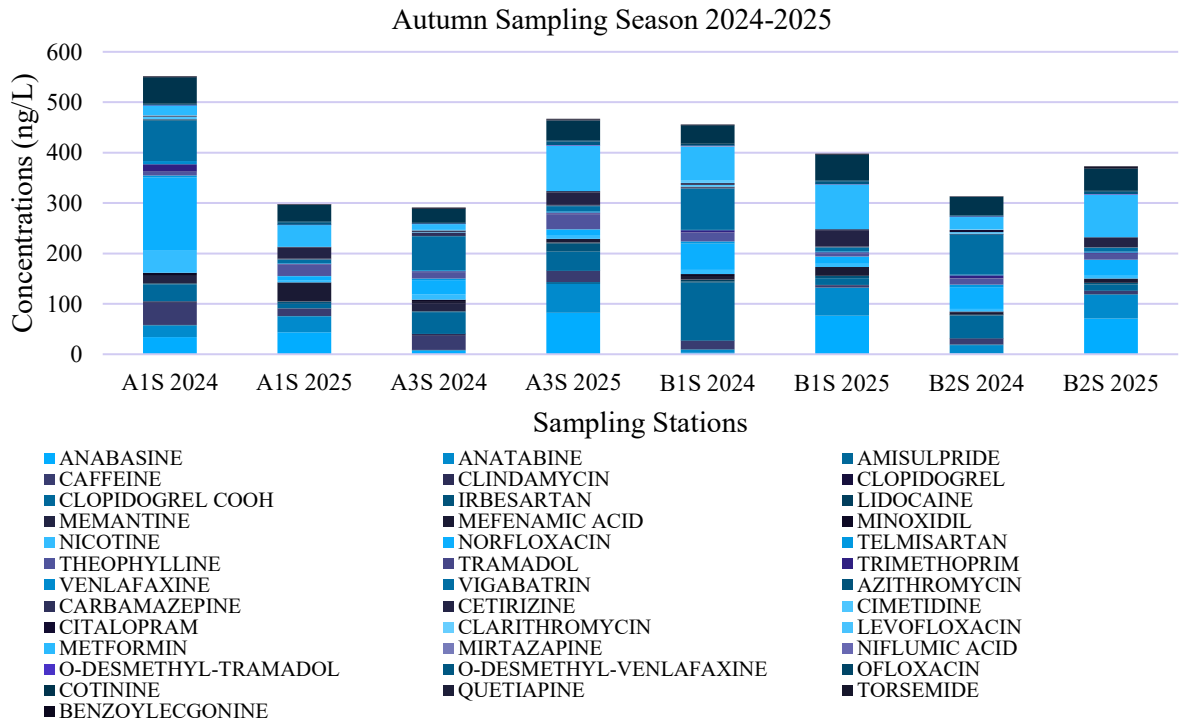

Figure 21: Cumulative levels of the target compounds displayed as a stacked bar plot at the different sampling stations during monitoring in October 2024 and 2025.

Concerning compound distribution during the additional seasonal sampling campaign a diverse range of organic micropollutants contributed to the cumulative concentration of each station. Among these, tobacco related contaminants, antibiotics, and antidiabetic drugs, contribute markedly to all stations. Specifically, Anabasine, Anatabine, and Cotinine (tobacco related contaminants), and Metformin (antidiabetic agent), were detected at elevated levels at all sampling points with mean concentrations of 68.2 ng/L, 48.0 ng/L, 41.9 ng/L, and 76.7 ng/L, respectively (Table 24). In addition, Norfloxacin (antibiotic) exhibited high concentration (29.9

ng/L) at station B2, whereas Mefenamic acid (nonsteroidal anti-inflammatory drugs-NSAIDs) was mostly abundant at station A1 (37.5 ng/L).

The comparative evaluation of the data obtained during the two monitoring campaigns clearly illustrates that both the cumulative concentration levels and the relative contribution of each micropollutant varied between these two years. As previously noted, the cumulative concentration in station A1 in 2025 was significantly lower than recorded in 2024 (552.5 ng/L), whereas at station A3 the total concentration in 2025 was considerably higher than in 2024 (293.1 ng/L). In contrast, in stations B1 (456.3 ng/L and 398.3 ng/L, respectively for 2024 and 2025) and B2 (316.2 ng/L and 373.2 ng/L, respectively for 2024 and 2025) the differences in cumulative concentrations between the two campaigns were negligible.

The distribution of organic micropollutants varied significantly between 2024 and 2025. During the first seasonal sampling campaign Anabasine and Anatabine (tobacco related contaminants) were present at lower concentrations (mean concentrations of approximately 11 ng/L) compared to those measured in 2025. Norfloxacin (antibiotic), Cotinine (tobacco related contaminants), and Metformin (antidiabetic agent) were detected at high concentrations during both campaigns contributing to the overall cumulative concentration. However, in 2024, the concentration of Norfloxacin was significantly higher (66.8 ng/L) than in 2025, Cotinine occurred at comparable levels in both campaigns, and Metformin exhibited lower concentrations in 2024 (30.0 ng/L) than in 2025.

6.2.2.6 Spatial and temporal distribution of organic micropollutants

A combined spatial and seasonal assessment of the four seasonal monitoring campaigns revealed station-specific differences as well as compound-dependent seasonal variability. Seasonal trends are further illustrated in Figure 17 to Figure 21, while mean seasonal concentrations are summarized in Table 24.

Evaluation of the seasonal datasets demonstrated that elevated cumulative concentrations in all sampling points were observed during winter period (second sampling campaign). Concentration levels decreased markedly during the spring period (third sampling campaign), whereas cumulative concentration recorded during autumn (first sampling campaign) and summer period (fourth sampling campaign) were comparable.

Stations A1 and A2 located in vicinity to Psyttalia Island exhibited consistently higher cumulative concentrations compared to stations close to the discharge points. This spatial pattern was observed across all four seasonal campaigns, however, its magnitude varied seasonally, with more pronounced differences during the winter period (second seasonal campaign). Among these two stations, higher concentrations were consistently recorded at station A1 located closer to the island, reflecting the effect of the seawater circulation pattern. The variability observed at the stations close to the discharge points may indicate a combined influence of station location and seasonality and reflect seasonal consumption patterns in combination with environmental and hydrodynamic factors.

The influence of the prevailing seawater circulation pattern is highlighted by the higher cumulative concentrations consistently observed at stations A1 and A2 which were located closer to the island, compared to stations A3 and A4 situated near the Psyttalia WWTP discharge pipelines. The differences observed between stations A3 and A4 may be attributed to variations in the volume of effluent discharged through each pipeline during the sampling

period along with the dilution effect. Moreover, stations A3 and A4 exhibited cumulative concentrations comparable to those measured at more distant stations (B1, B2, B3), with differences being more pronounced during the winter period.

Seasonal fluctuations were particularly evident at southern stations (stations near discharge points), whereas the influence of seasonality was less pronounced at stations located on the western side of Psyttalia Island. Indeed, stations B1, B2, and B3 exhibited relatively stable cumulative concentration levels throughout the annual monitoring period. This observation may be associated with mixing processes and dilution effect in the area. Overall, the spatial and temporal patterns suggest that proximity to the discharge point, seawater circulation, dilution effect, seasonal environmental conditions, and usage patterns influence the distribution of organic compounds in the marine environment.

The analysis of individual organic compound concentrations across the sampling points and four seasonal monitoring campaigns revealed substantial compound-specific variability. Several compounds exhibited pronounced seasonal fluctuations, whereas others remained relatively stable throughout the year across all stations. For example, Irbesartan (pharmaceutical-used to treat hypertension, heart failure, and diabetic kidney disease), Clarithromycin (antibiotic), and Metformin (antidiabetic agent) showed consistently higher concentrations at all sampling stations during the second seasonal campaign, while Vigabatrin (pharmaceutical-used for epilepsy treatment) showed markedly elevated concentrations at all sampling stations during the fourth seasonal campaign. Therefore, the observed spatial variability was primarily driven by seasonal factors rather than station-specific influences.

In contrast, for other compounds, seasonal variability differed markedly between stations. For example, Venlafaxine (antidepressant medication) and Azithromycin (antibiotic) exhibited significant seasonal variability at station A1 which was affected by the water circulation, whereas concentrations at the remaining stations remained comparatively stable throughout the year.

Additionally, a subset of compounds displayed limited variability regardless of season or station. For instance, Clopidogrel (antiplatelet medication), Amisulpride (antipsychotic, antidepressant, antiemetic), and Quetiapine (antipsychotic medication) exhibited limited variation across both seasons and sampling stations. However, the concentrations of most of the organic compounds did not exhibit a consistent pattern. For example, concentration of Anabasine, Anatabine, Cotinine, Caffeine (coffee and tobacco related contaminants), Mefenamic acid (nonsteroidal anti-inflammatory drugs-NSAIDs), and Norfloxacin (antibiotic) did not follow any trend across either stations or seasons. To conclude, these findings demonstrate that seasonal behavior is compound-specific and for selected compounds, influenced by station location.

6.3 Preliminary Interpretation

The observed spatial and seasonal variability of organic compound concentrations provides preliminary insights into the processes governing their distribution in the studied marine environment. Elevated concentrations at stations located closer to the Psyttalia WWTP discharge points (southern stations) may reflect the influence of treated effluent, in combination with dilution, dispersion, and seawater circulation. Comparable concentration levels between surface and near-bottom layers indicate enhanced vertical mixing.

Seasonal variations observed for selected compounds might be potentially related to seasonal consumption patterns, changes in hydrodynamic conditions, and compound physicochemical properties, such as persistence, solubility, and degradation potential influenced by seasonal environmental changes. Therefore, the limited variability observed for several compounds across both seasons and stations might be attributed to a combination of relatively steady input through WWTP effluent and of their physicochemical properties. In addition, notwithstanding that the detected concentrations of the micropollutants were generally low, their widespread occurrence underscores their persistence in the marine ecosystem. Although interannual differences were observed between the October 2024 and October 2025 campaigns, extended monitoring would be required to confirm longer-term trends

Overall, the findings provide valuable baseline information and support the need for continuous spatial monitoring in coastal environments in order to better understand the behavior of organic micropollutants in marine environments influenced by wastewater discharges.

6.4 Ecotoxicological effects of marine seawaters: exposure studies

6.4.1 Experimental parameters and Seawater characterization

For the ecotoxicity studies, CNR performed *in vivo* experiments on mussels (*Mytilus galloprovincialis)*, and *in vitro* experiments on coelomocytes from sea urchins (*Paracentrotus lividus)* both collected in the Gulf of Naples. The main purpose of these studies is to investigate the ecotoxicological effects of Greek seawater samples on the immune cells isolated from the selected marine invertebrates and to find a possible correlation between these effects and the presence of pollutant pharmaceuticals in the seawater. Three standardized assays were selected: Lysosome Membrane Stability (LMS) assay as parameter for stress, Phagocytic assay as parameter for functional immunity and Micronucleus Cytome assay as parameter for genotoxicity.

For the *in vivo* experiments, Neapolitan mussels were exposed for 2 hours to filtered Greek seawater (3 animals/0.5 L). Following exposure, live haemocytes were isolated from haemolymph of the animals, collected with saline anticoagulant solution, and subjected to immunotoxicity assays. Control experiments, consisting of mussels exposed to Neapolitan filtered seawater (hereinafter local seawater), were conducted in parallel.

For the *in vitro* experiments, live coelomocytes were isolated from coelomic fluid of Neapolitan Sea urchins and directly exposed for 30 minutes to 100 µL of filtered Greek seawater. Following incubation, immunotoxicity assays were performed, with control experiments conducted in parallel using cells exposed to local filtered seawater.

Five marine campaigns were conducted and organised by EYDAP in collaboration with CNR, as previously described. EYDAP collected seawater and marine fauna from selected sampling points, namely A1, A3, B1, and B2 (see Figure 16). Mussels were collected only in areas with a hard substrate (e.g., at site B1). Specifically, mussels from B1 and seawater from sites A1, B1, and B2 were collected during the spring campaign; seawater from A1, B1, and B2 during the summer campaign and mussels from B1 and seawater from A1, A3, B1, and B2 during the autumn campaigns. The immunoecotoxicity assays performed on mussels are described in D6.1. The present section focuses only on the immunoecotoxicological effects of the seawater samples. Chemical analyses of the seawater samples collected during the annual monitoring

campaigns has been conducted as shown in the previous paragraphs. Moreover, a sample of seawater collected in the Gulf of Naples was also analysed, and the detected organic micropollutants are presented in Table 25.

Table 25. Concentrations (ng/L) of the detected compounds in the marine area surrounding the Gulf of Naples in October 2025

20/10/2025			
ANALYTES	**Gulf of Naples**	***LOQ***	***LOD***
ANABASINE	52.4	0.87	0.26
ANATABINE	39.7	0.86	0.26
AMISULPRIDE	0.694	0.1	0.032
CAFFEINE	5.54	1.8	0.53
CLINDAMYCIN	<LOD	0.74	0.25
CLOPIDOGREL	0.167	0.16	0.054
CLOPIDOGREL COOH	13.2	5	1.7
IRBESARTAN	2.71	0.1	0.033
LIDOCAINE	0.409	0.28	0.092
MEMANTINE	0.243	0.091	0.03
MEFENAMIC ACID	2.34	0.5	0.17
MINOXIDIL	<LOD	0.18	0.062
NICOTINE	6.16	0.65	0.22
NORFLOXACIN	18.4	0.26	0.088
TELMISARTAN	0.817	0.2	0.067
THEOPHYLLINE	14.1	0.18	0.059
TRAMADOL	0.470	0.05	0.016
TRIMETHOPRIM	1.79	0.26	0.088
VENLAFAXINE	1.91	0.1	0.033
VIGABATRIN	11.7	0.73	0.24
AZITHROMYCIN	<LOQ	0.71	0.24
CARBAMAZEPINE	1.33	0.006	0.002
CETIRIZINE	13.2	1	0.33
CIMETIDINE	<LOD	1.2	0.4
CITALOPRAM	1.66	0.17	0.055
CLARITHROMYCIN	<LOD	0.1	0.035
LEVOFLOXACIN	0.912	0.04	0.013
METFORMIN	17.0	0.25	0.083
MIRTAZAPINE	0.347	0.2	0.066
NIFLUMIC ACID	<LOQ	0.21	0.071
O-DESMETHYL-TRAMADOL	0.915	0.077	0.026
O-DESMETHYL-VENLAFAXINE	3.05	0.056	0.019
OFLOXACIN	1.80	0.038	0.013
COTININE	45.5	0.41	0.14
QUETIAPINE	0.307	0.024	0.008
TORSEMIDE	2.24	0.059	0.02
BENZOYLECGONINE	1.47	0.054	0.018
CUMULATIVE CONCENTRATION LEVEL	**8.47**	-	-

6.4.2 Ecotoxicological results

The seawaters were analyzed individually by sampling site and season. However, since no significant seasonal variations in the observed biological effects were detected, data are presented as the average across seasons, while maintaining distinction by site. Figure 22 illustrates the effects of seawaters from four sites near WWTPs on mussels and sea urchins collected from the Gulf of Naples. All seawater samples induced measurable cellular stress, more pronounced in mussel cells than in sea urchin cells, with no substantial differences among the Greek sampling sites.

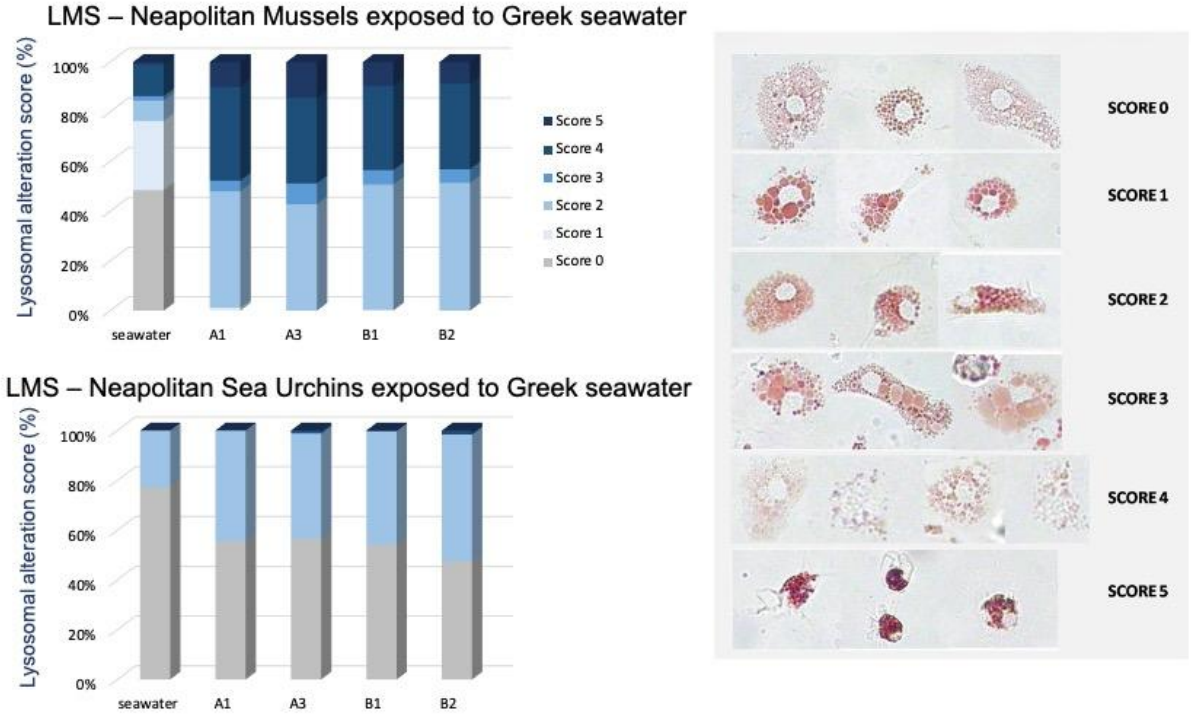

Figure 22: Lysosomal membrane stability measured on mussels and sea urchins collected in Gulf of Naples after exposure to Greek seawaters. Lysosomal alterations are classified into six scores representing increasing levels of cellular stress and lysosomal dysfunction: Score 0, no effect with dye retained in intact lysosomes; Score 1, lysosomal enlargement without leakage; Score 2, initial membrane destabilization with dye release into the cytosol; Score 3, enlargement with leakage; Score 4, enlarged lysosomes unable to retain the dye; Score 5, severe damage with widespread cytosolic dye accumulation and cell rounding. "Seawater" indicates the local seawater.

Differences became more evident when comparing the effects of Greek seawaters with those induced by local seawater (Figure 23). Figure 23 shows the ability of cells isolated from mussels and sea urchins exposed to the different water samples to phagocytose bacteria. This observes that this immune function increases or decreases depending on exposure to waters from different sampling sites. This modulation of phagocytic capacity indicates a potential disruption of immunological homeostasis. No relevant genotoxic effects were observed after exposure of mussels' cells to the seawaters collected in Greece, at any of the sampling sites (Figure 24).

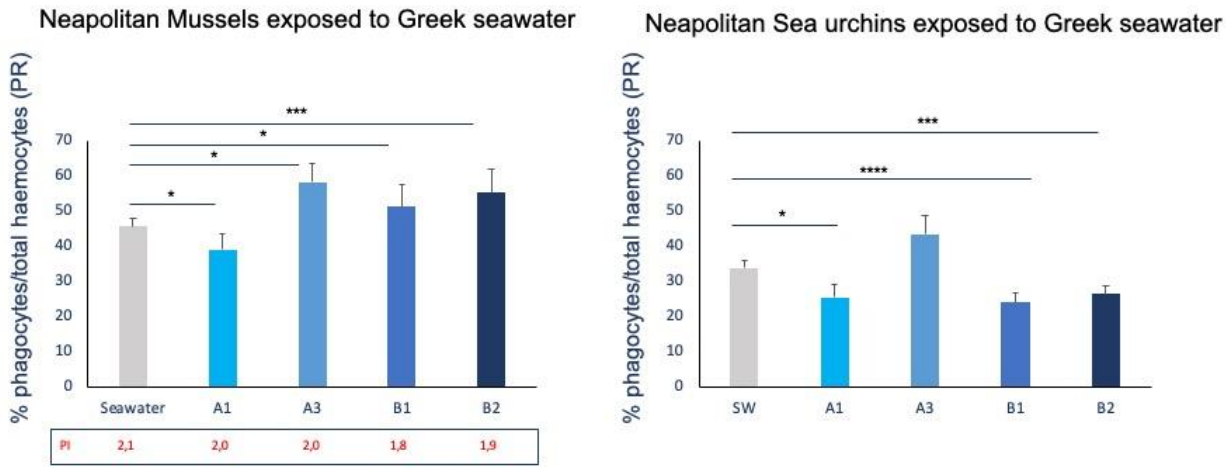

Figure 23: *Immunotoxic effect of Greek seawaters measured in terms of phagocytic capacity of Neapolitan mussels (left) and on Neapolitan sea urchins (right) collected in the Gulf of Naples in Autumn. PI: Phagocytic Index - Number of bacteria ingested per cell. PR: Phagocytic Rate - Number of cells that have phagocytosed the bacteria relative to the total number of phagocytic cells. For the data obtained using sea urchins, the PI value could not be calculated due to technical issues related to the excessively small size of the cells. "Seawater" indicates the local seawater.*

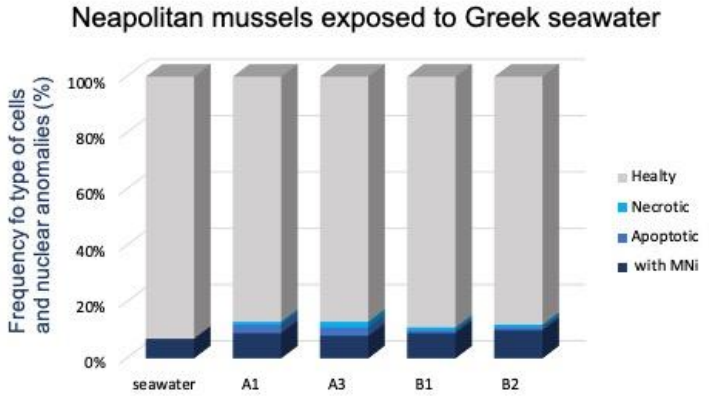

Figure 24: Genotoxic effect of Greek seawaters collected during marine monitoring campaign on Neapolitan mussels. The genotoxic effect on Neapolitan Sea Urchin could not be calculated due to technical issues related to the excessively small size of the cells. "Seawater" indicates the local seawater.

6.4.3 Interpretation of ecotoxicological results

Exposure of mussels and sea urchins collected in Naples to seawater samples from Greece resulted in signs of stress and modulation of phagocytic capacity when compared to the effects induced by local seawater. However, these effects cannot be directly attributed to the presence of pharmaceuticals in the seawaters apparently for three main different reasons:

- ✓ The chemical analysis of the seawaters has showed a very low concentration of pharmaceuticals, on the order of ng/L, a concentration one million times lower than the

therapeutic concentration of 1 mg/L used to observe *in vitro* effects of selected pharmaceuticals, as described in D6.1. Moreover, only three of these selected pharmaceuticals (Clopidrogrel COOH, Carbamazepine and Metformin) have been effectively measured in the Greek seawaters during the marine environmental campaigns.

- ✓ The seawater from Naples used as control in the ecotoxicological assay is very similar to all the Greek seawaters in terms of pharmaceutical concentrations (see Table 24 and Table 25).

- ✓ Lack of correlation: No clear correlation was observed between cumulative pharmaceutical concentrations and the magnitude of stress or phagocytic effects at the different sampling sites (see Figure 19, Figure 20 and Table 24).

Furthermore, the observed differences cannot be ascribed to biotic factors, since all seawater samples were filtered before exposure to remove microorganisms but not dissolved chemical components. It is therefore plausible that variations in unidentified physicochemical parameters such as pH, ionic strength, or salinity or the presence of non-pharmaceutical contaminants may underlie the observed differences in cellular responses.

7 Cross-Campaign Environmental Insights

The integrated analysis across ENVIROMED's three monitoring campaigns conducted by MITERA Hospital (clinical wastewater), EYDAP/Psyttalia WWTP (municipal treatment), and marine Saronic Gulf stations reveals a clear pollution continuum from hospital sources through WWTP treatment to receiving waters, with DCF and CBZ as persistent tracers of pharmaceutical loading. MITERA's 56-sample campaign (Dec 2024-Oct 2025) identified extreme episodic peaks exceeding 100,000 ng/L DCF and 134,000 ng/L MTP, confirming hospitals as high-intensity point sources with marked summer seasonality. EYDAP/Psyttalia's 94 samples demonstrated limited WWTP removal efficiency (CBZ: -3%, DCF: 8%, 1H-BTR: -55%), with effluent concentrations remaining elevated (mean 994 ng/L DCF, 336 ng/L CBZ), highlighting conventional treatment inadequacy.

Partner contributions enriched this analysis: MITERA established hospital seasonality patterns; EYDAP quantified WWTP performance gaps; NKUA/TraMS provided LC/GC-HRMS/MS gold-standard validation (92% DCF agreement with WSA); CNR ecotoxicity studies exposed Neapolitan mussels (Mytilus galloprovincialis) and sea urchins (Paracentrotus lividus) to Greek marine samples, revealing physiological stress and immunosuppression despite pharmaceutical levels at harmless ng/L (<< 1 mg/L toxic threshold, D6.1). CNR concluded no direct compound-ecotoxicity correlation due to dilution from µg/L WWTP effluent to ng/L marine concentrations, suggesting other stressors dominate marine biological impacts.

Marine stations A1/A2 (nearest Psyttalia) showed highest cumulative loads (552-2218 ng/L) driven by circulation patterns. Key insight: Seasonal hospital peaks persist through WWTP, but marine ecotoxicity indicates complex stressor interactions beyond pharmaceuticals alone, requiring integrated chemical-biological monitoring frameworks.

8 Decision Support Framework for Designing Pharmaceutical Monitoring Campaigns in Wastewater Systems

This section presents a decision support framework that summarises the key operational steps required to design and implement monitoring campaigns for pharmaceutical micropollutants in wastewater systems. It builds on the monitoring activities carried out within the ENVIROMED project, which combined structured sampling campaigns with complementary analytical approaches across different stages of the urban water cycle. The framework summarises practical experience gained during the project and highlights how analytical methods and predictive information tools may support monitoring design and interpretation.

It is intended to support wastewater utilities, environmental authorities, and research organisations in establishing structured monitoring programmes for pharmaceutical emissions. Monitoring strategies can combine conventional laboratory-based chemical analyses with emerging field-deployable analytical tools, allowing both robust quantification and improved temporal resolution of pharmaceutical occurrence in wastewater systems.

Figure 25 summarises the main steps involved in designing and implementing pharmaceutical monitoring campaigns, which are described in more detail below.

Figure 25: Monitoring workflow for designing pharmaceutical monitoring campaigns.

Step 1: Define monitoring objectives

The first step is to clearly define the objectives of the monitoring activity. These may include identifying sources of pharmaceutical emissions, evaluating the removal efficiency of wastewater treatment processes, assessing environmental exposure in receiving water bodies, or supporting regulatory monitoring obligations.

Clearly defined objectives help determine the scope of the monitoring campaign, including the selection of monitoring sites, compounds of interest, sampling frequency, and analytical methods.

Step 2: Select representative monitoring locations

Monitoring locations should represent key stages of the urban water cycle, as appropriate, in order to capture the pathways through which pharmaceutical compounds enter wastewater systems, are treated within wastewater treatment plants, and are ultimately discharged to receiving environments.

Typical monitoring points may include:

- Upstream emission sources, such as hospital or clinical facility wastewater
- Wastewater treatment plant influent and effluent streams, to assess treatment performance

- Receiving environments, including rivers, coastal waters, or marine areas affected by treated wastewater discharges

This multi-stage monitoring approach enables improved understanding of the lifecycle and environmental distribution of pharmaceutical residues across the water system.

Step 3: Identify target compounds

Target pharmaceutical compounds should be selected based on their environmental relevance, frequency of occurrence in wastewater systems, persistence in aquatic environments, potential regulatory interest, and other available knowledge sources. Predictive information sources providing estimates of ecotoxicity and/or bioaccumulation potential, such as the G.AI.A platform developed within ENVIROMED, may also provide supporting information when identifying substances that may warrant further monitoring.

Indicatively, compounds investigated in the ENVIROMED monitoring campaigns included diclofenac, carbamazepine, metoprolol, hydrochlorothiazide, and 1H-Benzotriazole, which are widely reported in wastewater monitoring studies and environmental assessments due to their persistence and frequent detection in aquatic environments. These compounds are also among those relevant to the implementation of the Directive (EU) 2024/3019 concerning urban wastewater treatment (recast), under which wastewater treatment plant (WWTP) operators are required to monitor selected micropollutants to support the implementation of advanced treatment processes aimed at their removal.

Step 4: Establish a harmonised monitoring protocol

A monitoring protocol should be defined to ensure consistency and comparability of results across sites and sampling periods, as appropriate for the monitoring objectives and local conditions.

Key elements may include:

- sampling frequency and timing
- sample handling and storage procedures
- data management, traceability, and quality assurance

Structured monitoring protocols are essential to ensure that datasets produced at different locations or time periods can be reliably compared and interpreted.

Step 5: Perform analytical measurements

Monitoring of pharmaceutical micropollutants can rely on a combination of laboratory-based chemical analyses and, where feasible, field-deployable analytical tools. Laboratory analytical techniques provide accurate identification and quantification of pharmaceutical compounds and remain essential for confirmatory analysis and regulatory monitoring.

In parallel, emerging automated or in-situ sensing technologies may enable faster analytical turnaround and more frequent measurements directly at relevant points of the wastewater system. Such tools, including the Wastewater Spectroscopic Analyser (WSA) developed within ENVIROMED, can generate near-real-time or high-frequency monitoring data, improving

temporal coverage and supporting the identification of short-term fluctuations or episodic emission events that may not be captured through periodic sampling alone. When used alongside laboratory analyses, these technologies may support screening activities, trend evaluation, and targeted sampling strategies.

Step 6: Analyse monitoring data

Monitoring results should be analysed to identify concentration trends, detect potential pollution hotspots, and evaluate the effectiveness of wastewater treatment processes in removing pharmaceutical residues. Temporal and spatial comparisons may provide insights into how pharmaceutical compounds behave within wastewater systems and receiving environments.

Where relevant, measured concentrations may also be compared with available environmental quality standards, predicted no-effect concentrations (PNECs), or other risk-based benchmarks to support environmental risk evaluation. Where available, predictive information on ecotoxicological properties may also complement measured data and support interpretation of monitoring results.

Step 7: Translate monitoring results into decision support

Finally, monitoring outcomes should be translated into actionable information that can support operational and regulatory decision-making and guide future monitoring activities.

Monitoring data may support:

- optimisation of wastewater treatment processes
- environmental risk assessments
- targeted monitoring programmes
- improved understanding of emission dynamics within the urban water cycle

By combining structured monitoring protocols, robust analytical methods, and complementary field monitoring technologies, monitoring programmes can provide more comprehensive information on pharmaceutical emissions and support improved management of pharmaceutical pollution in aquatic environments. Complementary information sources may further support environmental assessment by helping identify substances that may warrant additional investigation.

As an illustrative example, a wastewater utility aiming to evaluate pharmaceutical emissions could apply this framework by defining monitoring objectives related to treatment performance, selecting monitoring points at the influent and effluent of a wastewater treatment plant, identifying commonly reported pharmaceutical residues, and establishing a sampling protocol. Laboratory analyses would provide accurate compound quantification, while field-deployable analytical tools could complement periodic sampling by providing higher-frequency measurements and supporting the identification of short-term concentration variations. The resulting monitoring data may then support assessment of treatment efficiency and inform environmental monitoring strategies. Where relevant, monitoring results may also

be interpreted alongside available ecotoxicological information or predictive hazard data to support broader environmental assessment.

Such integrated monitoring approaches can contribute to evidence-based implementation of European environmental policy frameworks addressing micropollutants in wastewater, including the Directive (EU) 2024/3019, the Water Framework Directive, and related monitoring and risk assessment initiatives.

The framework is intended to support stakeholders when planning monitoring activities in wastewater systems by summarising practical lessons learned during the ENVIROMED project. It does not represent a prescriptive protocol but rather highlights key considerations that can be adapted to specific monitoring contexts. In this way, the framework may also support wastewater utilities and environmental authorities seeking to design monitoring programmes tailored to their local conditions and priority substances.

9 Conclusion

Deliverable D7.1 documents the demonstration and validation activities performed with the WSA at hospital (MITERA) and municipal (EYDAP/Psytallia WWTP) sites. WP7 successfully validated the ENVIROMED WSA at TRL 6–7, achieving 100% field operational success (7/7 consecutive sampling cycles), 91–92% quantitative agreement for DCF concentrations compared to LC/GC–HRMS/MS laboratory benchmarks, and exceptional SPE enrichment factors ranging from 97× to 19,875× across diverse wastewater matrices.

Partner monitoring campaigns generated a dataset of 190 samples, confirming pharmaceutical hotspots in hospital discharges (>100,000 ng/L), the limited removal efficiency of WWTP processes, and characteristic marine dispersion trends in post-treatment effluents.

In parallel, CNR conducted complementary ecotoxicological investigations using marine invertebrates to examine the biological effects of low-level pharmaceutical exposure. These studies revealed cellular stress responses and immune modulation even at ng/L exposure levels, underlining the potential ecological implications of persistent pharmaceutical residues despite their low concentrations.

The quantitative alignment between WSA field data and laboratory measurements demonstrates the instrument's analytical robustness. For example, DCF concentrations measured *in situ* by the WSA at hospital outlets (mean 21.8 µg/L) deviated by less than 10% from those obtained by confirmatory HRMS analyses on grab samples (mean 20.1 µg/L), validating both analytical linearity (R^2 = 0.96) and operational reproducibility across all campaigns. Similarly, at municipal sites, WSA trends accurately mirrored downstream concentration profiles measured in treated effluents, confirming its ability to track dynamic pollutant fluctuations in real time.

Although the current validation focused on wastewater matrices, the demonstrated stability, sensitivity, and enrichment capacity of the WSA indicate promising potential for future deployment in marine and coastal surveillance. With targeted hardware and calibration improvements (matrix interference from co-dissolved organic compounds, optimized microfluidic cell configuration for enhanced analyte interaction, increased system portability, mitigation of laser beam dispersion and air adsorption effects in the optical path, improved laser stability, refined operational conditions for customized SPE enrichment, temperature-compensated optical alignment), the WSA could quantify low ng/L pharmaceutical residues directly in seawater, complementing laboratory reference methods and enabling continuous environmental quality assessment.

Overall, the results confirm that field-deployable spectroscopic sensors such as the WSA represent a transformative step toward UWWTD (2024/3019) compliance, supporting the transition from periodic laboratory monitoring to continuous, cost-efficient (up to 95% savings), and data-driven surveillance of the entire urban water cycle.

References

1. Cappelli, F., Longoni, O., Rigato, J., Rusconi, M., Sala, A., Fochi, I., Palumbo, M. T., Polesello, S., Roscioli, C., Salerno, F., Stefani, F., Bettinetti, R., & Valsecchi, S. (2022). Suspect screening of wastewaters to trace anti-COVID-19 drugs: Potential adverse effects on aquatic environment. *Science of The Total Environment*, *824*, 153756. https://doi.org/10.1016/j.scitotenv.2022.153756
2. Choi, S., Yoom, H., Son, H., Seo, C., Kim, K., Lee, Y., & Kim, Y. M. (2022). Removal efficiency of organic micropollutants in successive wastewater treatment steps in a full-scale wastewater treatment plant: bench-scale application of tertiary treatment processes to improve removal of organic micropollutants persisting after secondary treatment. *Chemosphere*, *288*, 132629. https://doi.org/10.1016/j.chemosphere.2021.132629
3. Fontanals, N., Pocurull, E., Montes, R., González-Mariño, I., Santana-Viera, S., Miró, M., Rico, A., Rodríguez-Mozaz, S., Borrull, F., Benito Quintana, J., & Marcé, R. M. (2023). A new analytical method to determine trace level concentrations of pharmaceuticals in influent wastewater: A tool to monitor human use patterns. *Microchemical Journal*, *193*, 109131. https://doi.org/10.1016/j.microc.2023.109131
4. Jaén-Gil, A., Buttiglieri, G., Benito, A., Gonzalez-Olmos, R., Barceló, D., & Rodríguez-Mozaz, S. (2019). Metoprolol and metoprolol acid degradation in UV/H2O2 treated wastewaters: An integrated screening approach for the identification of hazardous transformation products. *Journal of hazardous materials*, *380*, 120851. https://doi.org/10.1016/j.jhazmat.2019.120851
5. Kosma, C. I., Lambropoulou, D. A., & Albanis, T. A. (2014). Investigation of PPCPs in wastewater treatment plants in Greece: occurrence, removal and environmental risk assessment. *Science of the total environment*, *466*, 421-438. https://doi.org/10.1016/j.scitotenv.2013.07.044
6. Krakkó, D., Licul-Kucera, V., Záray, G., & Mihucz, V. G. (2019). Single-run ultra-high performance liquid chromatography for quantitative determination of ultra-traces of ten popular active pharmaceutical ingredients by quadrupole time-of-flight mass spectrometry after offline preconcentration by solid phase extraction from drinking and river waters as well as treated wastewater. *Microchemical Journal*, *148*, 108-119. https://doi.org/10.1016/j.microc.2019.04.047
7. Loos, R., Carvalho, R., António, D. C., Comero, S., Locoro, G., Tavazzi, S., Paracchini, B., Ghiani, M., Lettieri, T., Blaha, L., Jarosova, B., Voorspoels, S., Servaes, K., Haglund, P., Fick, J., Lindberg, R. H., Schwesig, D., & Gawlik, B. M. (2013). EU-wide monitoring survey on emerging polar organic contaminants in wastewater treatment plant effluents. *Water research*, *47*(17), 6475-6487. https://doi.org/10.1016/j.watres.2013.08.024
8. Mailler, R., Gasperi, J., Coquet, Y., Derome, C., Bulet´e, A., Vulliet, E., Bressy, A., Varrault, G., Chebbo, G., & Rocher, V. (2016). Removal of emerging micropollutants from wastewater by activated carbon adsorption: experimental study of different activated carbons and factors influencing the adsorption of micropollutants in wastewater. *Journal of environmental chemical engineering*, *4*(1), 1102-1109. https://doi.org/10.1016/j.jece.2016.01.018
9. Mantovani, M., Rossi, S., Ficara, E., Collina, E., Marazzi, F., Lasagni, M., & Mezzanotte, V. (2024). Removal of pharmaceutical compounds from the liquid phase of anaerobic sludge in a pilot-scale high-rate algae-bacteria pond. *Science of the Total Environment*, *908*, 167881. https://doi.org/10.1016/j.scitotenv.2023.167881

10. Morosini, C., Marsoni, M., Torretta, V., Conti, F., Ragazzi, M., Rada, E. C., & Cioca, G. (2017). Factors affecting spatial and temporal concentration variability of pharmaceuticals: Comparison between two WWTPs. *Sustainability*, *9*(8), 1466. https://doi.org/10.3390/su9081466
11. Park, J., Kim, C., Hong, Y., Lee, W., Chung, H., Jeong, D. H., & Kim, H. (2020). Distribution and removal of pharmaceuticals in liquid and solid phases in the unit processes of sewage treatment plants. *International journal of environmental research and public health*, *17*(3), 687. https://doi.org/10.3390/ijerph17030687
12. Petromelidou, S., Evgenidou, E., Tziouvalekas, M., & Lambropoulou, D. A. (2024). Unravelling psychoactive substances and their metabolites and transformation products: High-Resolution Mass Spectrometry approaches for comprehensive target and suspect screening in wastewater. *Science of The Total Environment*, *931*, 172867. https://doi.org/10.1016/j.scitotenv.2024.172867
13. Rubirola, A., Llorca, M., Rodriguez-Mozaz, S., Casas, N., Rodriguez-Roda, I., Barceló, D., & Buttiglieri, G. (2014). Characterization of metoprolol biodegradation and its transformation products generated in activated sludge batch experiments and in full scale WWTPs. *Water research*, *63*, 21-32. https://doi.org/10.1016/j.watres.2014.05.031
14. Skalska-Tuomi, K., Kaijanen, L., Monteagudo, J. M., & Mänttäri, M. (2025). Efficient removal of pharmaceuticals from wastewater: Comparative study of three advanced oxidation processes. *Journal of environmental management*, *375*, 124276. https://doi.org/10.1016/j.jenvman.2025.124276
15. Stasinakis, A. S., Thomaidis, N. S., Arvaniti, O. S., Asimakopoulos, A. G., Samaras, V. G., Ajibola, A., Mamais, D., & Lekkas, T. D. (2013). Contribution of primary and secondary treatment on the removal of benzothiazoles, benzotriazoles, endocrine disruptors, pharmaceuticals and perfluorinated compounds in a sewage treatment plant. *Science of the Total Environment*, *463*, 1067-1075. https://doi.org/10.1016/j.scitotenv.2013.06.087
16. Vieno, N., & Sillanpää, M. (2014). Fate of diclofenac in municipal wastewater treatment plant-A review. *Environment international*, *69*, 28-39. https://doi.org/10.1016/j.envint.2014.03.021