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<b>Brief Description</b>	The present report provides the findings of the selected organic micropollutants concentrations through the exploratory sampling campaign and the developed HYDRO1-6 technologies in water, sludge, and compost.		
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## EXECUTIVE SUMMARY

The present report summarises and evaluates the concentrations of selected organic micropollutants (OMP) in the water streams of HYDROs 1,2,3,4,5, and 6 as well as in sludge and compost from HYDRO1. It should be mentioned that the analysis of the pathogens and the inorganic micropollutants (heavy metals) in the water/wastewater streams of all HYDROs are presented in detail in Deliverable 5.1 (Pilot Assessment Report) along with their operational results. The analysis of organic and inorganic micropollutants in soil are crops are reported in Deliverable 4.6 (Report on food safety issues and pest control).

A first preliminary sampling campaign in various sites in the Greek islands was performed in 2019 to select the target compounds to monitor through the extensive sampling campaigns in the HYDRO sites in 2021, 2022, and 2023. Several analytical methods were applied for the analysis of the water samples in this preliminary campaign and most of the compounds detected belonged to pharmaceutically active compounds (PhACs) and endocrine-disrupting compounds (EDCs). Therefore, these were the selected groups of organic micropollutants (OMPs), a total of 76 compounds, to monitor in the subsequent sampling campaigns of the HYDRO technologies.

Regarding **HYDRO1** in **Lesvos Island** (Greece), two intensive sampling campaigns were performed (fall 2021 and summer 2022) along the municipal wastewater treatment train, which consisted in an Upflow Anaerobic Sludge Blanket (UASB) coupled to Constructed Wetlands (CW) and a UV disinfection. The UASB removed partially the OMP inlet concentrations (on average 50%), in particular acetaminophen and caffeine, both predominant compounds in the influent wastewater (IW). With the subsequent treatment by CW circa 70% of the target compounds were eliminated in both fall and summer sampling campaigns. Finally, an increase of the OMP concentration was observed after tertiary treatment (based on UV), which was attributed to long storage times of treated water in the tanks before its application as irrigation water in HYDRO2. Moreover, up to 22 OMPs were detected in the sludge produced in the UASB system of HYDRO1, whereas 15 contaminants were detected in the composted sludge, though at low concentrations. Analgesics and anti-inflammatories were found at levels between 8 ng/g (acetaminophen) and 104 ng/g (diclofenac) in the sludge but were not detected in the compost samples.

Two sampling campaigns were performed as well in the **intensified wetlands** (4 parallel vertical sub-surface flow CWs: three electroactive and one intensified), installed in parallel to HYDRO1. During the 1<sup>st</sup> sampling campaign (29/03/22-02/04/22) the removals were high for all OMP (>67%), except 5-chlorobenzotriazole (CBTR) in the hybrid electroactive pilot (49%). Higher removals were observed in the saturated electroactive pilot. In the second sampling campaign (06/03/23-11/03/23) the number of pulses was increased to 150, which resulted in an improvement in the removal of benzotriazoles in all four pilots.

**HYDRO2** (Lesvos Island, Greece) was used as an agroforestry system, to grow crops (including lettuce, oregano, and lavender, as reported in the Deliverable 4.6\_ Report on food safety issues and pest control) irrigated with two different types of treated wastewater: T1 (UASB-CW-UV) and T2 (UASB-UV, excluding wetlands). Periodic monitoring of selected OMP in both irrigation waters was performed from 14/08/2021 to 25/01/2023 and less than 50,000 ng/L was measured. The compounds at the highest concentrations, up to 8,900 ng/L, in T1 and T2 irrigation waters were valsartan, irbesartan, metoprolol acid, salicylic acid, and 2-OH-ibuprofen which were also at high concentrations in raw wastewater and not efficiently removed by the UASB-CW-UV system.

In rainwater collector systems **HYDRO3** and **HYDRO4 (Mykonos Island)** for lavender and oregano irrigation, some PhACs (acetaminophen, ketoprofen diclofenac, 1-OH-ibuprofen, naproxen, clarithromycin, ciprofloxacin, MTPA, venlafaxine, O-Desmethyl-venlafaxine, N-desmethyl-venlafaxine, pravastatin, and irbesartan) as well as few EDCs (methylparaben, propylparaben, and caffeine) were detected in both sites at very low concentrations in at least one sampling campaign.



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In the **HYDRO5 (Tinos Island)**, the mangrove seawater desalination system, only caffeine, BPA, benzotriazole, and five pharmaceuticals (diclofenac, ibuprofen 1 and 2-OH-ibuprofen, ofloxacin) were detected after the desalination process. The highest observed concentration corresponded to 2-OH-IBU (198 ng/L). In **HYDRO6 (Tinos Island)**, 14 pharmaceuticals and 3 EDCs (BPA, methylparaben and caffeine) were detected in at least one of the sampling points of the eco-tourist with wastewater reclamation system and rainwater harvesting technologies. Highest concentrations were detected for ibuprofen (rainwater) and benzotriazole (reedbed effluent).

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## ABBREVIATIONS

<b>1-OH-IBU</b>	<b>1-Hydroxy-ibuprofen</b>
<b>2-OH-IBU</b>	<b>2-Hydroxy-ibuprofen</b>
<b>5TTR</b>	<b>5-methyl-1H-benzotriazole</b>
<b>AEW</b>	<b>saturated aerated wetland</b>
<b>BP</b>	<b>Bisphenol</b>
<b>BTR</b>	<b>Benzotriazole</b>
<b>CBTR</b>	<b>5-chlorobenzotriazole</b>
<b>CBX-IBU</b>	<b>Carboxy-ibuprofen</b>
<b>CW</b>	<b>Constructed wetlands</b>
<b>DCF</b>	<b>Diclofenac</b>
<b>EWV</b>	<b>Effluent wastewater</b>
<b>EDCs</b>	<b>Endocrine-disrupting compounds</b>
<b>ESI</b>	<b>Electrospray ionization source</b>
<b>GS</b>	<b>Gas source</b>
<b>IWW</b>	<b>Influent wastewater</b>
<b>H.T.</b>	<b>Heating Tank</b>
<b>HRT</b>	<b>Hydraulic retention time</b>
<b>HS-SPME-GC-MS/MS</b>	<b>Headspace-solid phase microextraction coupled with gas chromatography tandem mass spectrometry</b>
<b>KFN</b>	<b>Ketoprofen</b>
<b>LOQ</b>	<b>Limit of quantification</b>
<b>N-Des-VLF</b>	<b>N-Desmethyl-venlafaxine</b>
<b>NI</b>	<b>Negative ionization</b>
<b>NPX</b>	<b>Naproxen</b>
<b>O-Des-VLF</b>	<b>O-desmethyl-venlafaxine</b>
<b>OH-BTH</b>	<b>2-hydroxy-benzothiazole</b>
<b>OLR</b>	<b>Organic load rate</b>
<b>PDMS</b>	<b>Polydimethylsiloxane</b>
<b>PhACs</b>	<b>Pharmaceutically Active Compounds</b>
<b>PI</b>	<b>Positive Ionization</b>
<b>SRM</b>	<b>Selected reaction monitoring</b>
<b>TCS</b>	<b>Triclosan</b>
<b>TST</b>	<b>Target scan time</b>
<b>SAT</b>	<b>Saturated</b>
<b>UASB</b>	<b>Upflow Anaerobic Sludge Blanked</b>
<b>UNSAT</b>	<b>Unsaturated</b>
<b>UV</b>	<b>Ultra-violet</b>
<b>VSSF</b>	<b>Vertical subsurface flow</b>
<b>XTR</b>	<b>Xylylriazole</b>



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## 1. INTRODUCTION

The aim of the HYDROUSA European Project is to find new possible sources of freshwater, considering multiple water sources: wastewater, rainwater, and seawater. Wastewater is reclaimed in HYDRO1 through an Upflow Anaerobic Sludge Blanked (UASB) coupled to a wetland system (CW) and a UV tertiary treatment and the regenerated water is used for crops irrigation in HYDRO2. Rainwater is harvested from the seasonal rains in HYDRO3 and HYDRO4 whereas seawater is desalinated through a mangrove forest in HYDRO5. Finally, in HYDRO6 several principles of water treatment/harvesting, and reuse are demonstrated at a local level.

The Catalan Institute for Water Research (ICRA-CERCA) is in charge of the assessment of emerging organic pollutants in the water sources as well as in the reclaimed water after the dedicated treatment of most HYDRO1. The University of Aegean and NTUA analyzed selected organic micropollutants in the intensified wetlands of HYDRO1. The results obtained after analysing target pollutants in the different scenarios (HYDROs) are reported in this deliverable.

## 2. MATERIALS AND METHODS

### 2.1. Exploratory Sampling Campaign

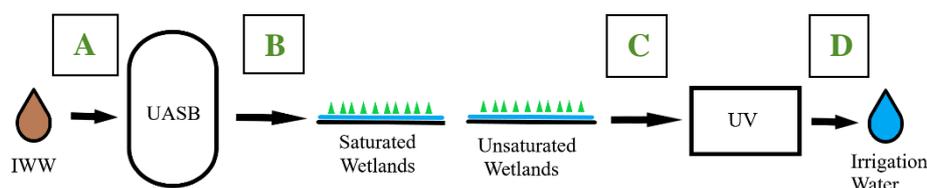
An exploratory sampling campaign was performed from 10 to 20 of September 2019. Grab samples of wastewater (i.e., the influent wastewater of both HYDRO1 and the Antissa conventional wastewater treatment plant, Lesvos Island), river water (Lesvos Island), and sea water (Tinos Island, influent water of HYDRO 5) were taken to obtain a first screening and an overall characterization of the organic micropollutants that can be detected on the HYDROUSA Greek islands. Pharmaceuticals (PhACs), endocrine-disrupting compounds (EDCs), pesticides, selected EU 2015 watch list compounds (Commission Implementing Decision (EU) 2015/495), triclosan and analogues were analyzed in those samples. Therefore, the detailed methodologies listed in section 2.8 were applied and the classes of contaminants that were non-detected (or at very low concentrations) in the collected samples were discarded in the monitoring campaigns of the HYDROs (2021-2023). To be mentioned that in 2019 the HYDROs were not built yet.

### 2.2. HYDRO1

#### Full scale demonstration site:

- Description of the site

The aim of the HYDRO1 demonstration site is to demonstrate the possibility to treat wastewater produced by a touristic site (high fluctuation in sewage production due to seasonality of touristic activities) and produce an effluent suitable for reuse in irrigation under strict Greek water quality standards (JMD, 2011). The demonstration site for this HYDRO1 system is next to the Wastewater Treatment Plant (WWTP) of Antissa (design population of 1,086 PE), in the island of Lesvos, Greece. The proposed HYDRO1 system treats a flow from 10 up to 100 m<sup>3</sup>/d. Pre-treatment system consisted of an inlet chamber, a fine screening unit, and a grease and grit removal unit. After the pre-treatments, HYDRO1 includes an Upflow Anaerobic Sludge Blanket (UASB) treatment, followed by a Constructed Wetlands (CWs) system, and tertiary treatment (ultraviolet irradiation lamp). The scheme of the HYDRO is shown in Figure 2.1.



**Figure 2.1: Sampling design of the HYDRO1 with the sampling points A (influent wastewater), B (UASB effluent), C (Wetlands effluent), and D (tertiary treatment effluent).**

More into details, HYDRO1 comprises as first treatment two UASBs reactors that can operate simultaneously or individually, depending on the actual inlet flow rate to be treated. The UASB outlet is released into a constructed wetlands (CW) system, composed of a hybrid combination of a 1<sup>st</sup> vertical subsurface flow (VSSF) CWs and a 2<sup>nd</sup> stage unsaturated intermittent load VF CW. Recirculation and by-pass chambers allow to test up to 6 different configurations, investigating the best scheme for Greek and other Mediterranean conditions (e.g., different water quality standards for TN). The last treatment step of HYDRO1 is UV disinfection, before the use of reclaimed wastewater for crops irrigation in HYDRO2.



- **Sampling points:**

The selected organic micropollutants were tracked through the process of HYDRO1 wastewater reclamation (UASB-CW-UV system). Two sampling campaigns were performed, the first one in fall 2021 (18<sup>th</sup> to 23<sup>rd</sup> of September), with lower tourist activity and the second one in summer 2022 (6<sup>th</sup> to 11<sup>th</sup> of June), with higher tourist activity. In both cases, 24h composite samples were taken from the HYDRO1 for three consecutive days considering the hydraulic retention time (HRT). Those points include the inlet source water (urban influent wastewater), the outlet of the UASB reactor, the outlet of the wetland system, and the effluent of the UV-tertiary treatment to be further used as irrigation waters (respectively the points A, B, C, and D in Figure 2.1. The flows treated by HYDRO1 in fall 2021 and summer 2022 were  $65.3 \pm 3.7$  and  $82 \pm 7.9$  m<sup>3</sup>/d, respectively. Specific details of the technology are reported in the annex “HYDRO1” tab “a) operational UASB-CW”. The samples were frozen at -20°C, sent to Spain (ICRA partner) in dry ice, and stored at -20°C until analysis. Moreover, grab sludge and compost samples were collected in February and March 2023, and kept at -20°C, during shipment to Spain (ICRA partner) in dry ice and in the freezer until analysis.

### Pilot intensified wetlands

- **Description of the site**

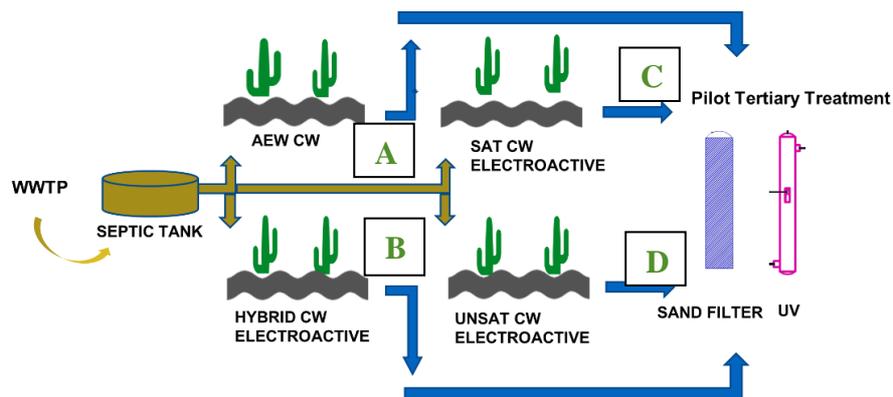
As part of the HYDROUSA project, four pilot systems (Figure 2.2) have been put into operation and are part of HYDRO1 (Antissa, Lesvos). The pilot system consisted of 4 parallel CWs (three electroactive and one intensified) operating as vertical sub-surface flow CW (VSSF CW) with a total surface of 1 m<sup>2</sup> and height of 1 m. The construction material is PVC, and the technical characteristics of each pilot are presented in Figure 2.2. Three of them were filled with carbon electroconductive biocompatible material (METfilter), initially colonized by electroactive *Geobacter* bacteria and the fourth one was filled with gravel, while an air pump was installed to provide aeration bottom-up. Each wetland was planted with aquatic plants *Scirpus lacustris*. Regarding the electroconductive CWs, three different schemes were tested in terms of saturation; the first one was fully saturated, the second was fully unsaturated, and the third one was practically a two-stage system, unsaturated on top half – saturated on bottom half. It is noted that aerated CW was also saturated, and the aeration was intermittent so that anoxic conditions could occur. The pilot system operated for almost two years (April 2021 – March 2023), while the maximum applied OLR was kept constant for a long time to assess long-time performance. The conditions prevailing in each of the above wetlands are as follows:

- Unsaturated (UNSAT): Unsaturated vertical flow wetland (METfilter).
- Saturated (SAT): Saturated wetland (METfilter).
- Hybrid: A combination of unsaturated and saturated wetland (METfilter).
- Aerated (AEW): Saturated aerated wetland.

**Table 2.1: Technical characteristics of the pilot systems**

Tanks	Dimensions	Porosity	V (m <sup>3</sup> )	V water (m <sup>3</sup> )	A (m <sup>2</sup> )
AEW	1.10 x 0.90 x 0.80 m	0.35	0.8	0.3	1.0
SAT	1.10 x 0.90 x 0.80 m	0.5	0.8	0.4	1.0
HYBRID (UNSAT)	1.10 x 0.90 x 0.40 m	0.5	0.4	0.2	1.0
HYBRID (SAT)	1.10 x 0.90 x 0.40 m	0.5	0.4	0.2	1.0
UNSAT	1.10 x 0.90 x 0.70 m	0.5	0.7	0.3	1.0

The feeding system of the pilot wetlands consists of a two-stage septic tank, which is fed with pre-treated wastewater from the Wastewater Treatment Plant of Antissa. The effluent from the two-stage septic tank is discharged into a third compartment, where the pilot feeding pump is located. Feeding of the systems is carried out in 150 pulses per day. Different number of pulses have been tested: 8, 73, 100 and 150. The pilot systems treat 1 m<sup>3</sup>/d and aim to test the possibility to reduce the areal footprint of CWs with innovative solutions, i.e., aerated and bioelectrified CWs (Deliverable 3.2\_ Design of the constructed wetland).



**Figure 2.2: MET constructed wetlands; electroconductive saturated, aerated, two-stage and unsaturated (from left to the right) with the sampling sites A, B, C, and D.**

- **Sampling points:**

Two sampling campaigns were carried out in the pilot CWs, and the total number of samples was 50. The total duration of each sampling campaign was 5 days, 25 samples were collected (5 inlets and 20 effluents of the pilot CWs). It should be mentioned that the collection of the wastewater samples was done by considering the hydraulic retention time in each sub-system of HYDRO1. The inlet was collected from the 3<sup>rd</sup> compartment of the septic tank and the pilot effluents from the effluent tanks of each (Figure 2.1). The 1<sup>st</sup> and 2<sup>nd</sup> sampling campaign were carried out between 29/03/22-02/04/22 and 06/03/23-11/03/23, respectively, and a key factor separating the two sampling periods was the number of pulses in the systems, with 73 and 150 pulses, respectively. The organic load rate (OLR) of 1<sup>st</sup> and 2<sup>nd</sup> sampling campaign was 146±83 and 143±20 gCOD m<sup>-2</sup> d<sup>-1</sup>, respectively.

## 2.3. HYDRO2

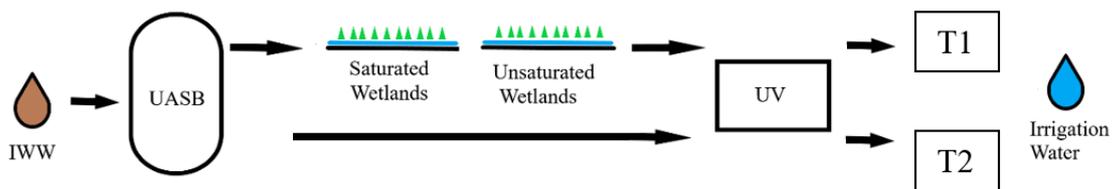
- **Site description**

HYDRO2 demonstration site is located on Antissa, Lesvos Island (Greece) and next to HYDRO 1. In HYDRO2, land is used as an agroforestry system, which combines the distinct functions of an ecosystem and serves as a point of attraction on the island for citizens and other farmers. The site is divided into three fields main plots. Field 1 corresponds to the main area with about 0.75 ha, and it is in the north-east of HYDRO1. Then, Field 2 is a small part of HYDRO1 with about 0.05 ha close to the wastewater treatment installations of HYDRO 1, and Field 3 comprises an additional 0.2 ha on the other side of the road of the HYDRO1. Field 1 and 3 are irrigated with treated wastewater from HYDRO1 (T1), except a small part that is irrigated with tap water to compare

different conditions of plant growth. Water is applied on the fields by a drip irrigation system, which was designed to address properly each crop category's water needs (trees, bushes, herbs, etc.). The irrigation of the trees is conducted with online drip emitters, while the herbs and the bushes are irrigated through inline drip emitters. Field 1 was the selected plot for the characterization of HYDRO2 irrigation water in terms of OMPs because it included the chosen crops to be evaluated in terms of organic micropollutants (i.e., lettuce, oregano, lavender), and to be irrigated with both HYDRO1 treated water and tap water.

An extra field close to the tertiary treatment of HYDRO1 was added in summer 2021, specifically for this Deliverable 5.9, planted, and irrigated by a direct bypass from UASB of HYDRO1 as Figure 2.3 depicts and it is referred in this deliverable as "partially treated water" (T2).

To be strongly emphasized that T2 water had a much longer tank retention time (a few days to 1.5 months, depending on the season) than T1 water before its use for crops irrigation (and sampling). Most likely degradation and/or sorption of emerging pollutants occurred in the tank itself, as well as water evaporation, before T2 water was used for irrigation. Consequently, water characterisation is not meant here to compare treatment technologies schemes but to relate micropollutants occurrence in water with occurrence in soils and crops irrigated by the treated water, as provided in the corresponding Deliverable D4.6 (Report on food safety issues and pest control).



**Figure 2.3: Difference between the T1 (full treatment) route and the T2 (alternative without CW) route**

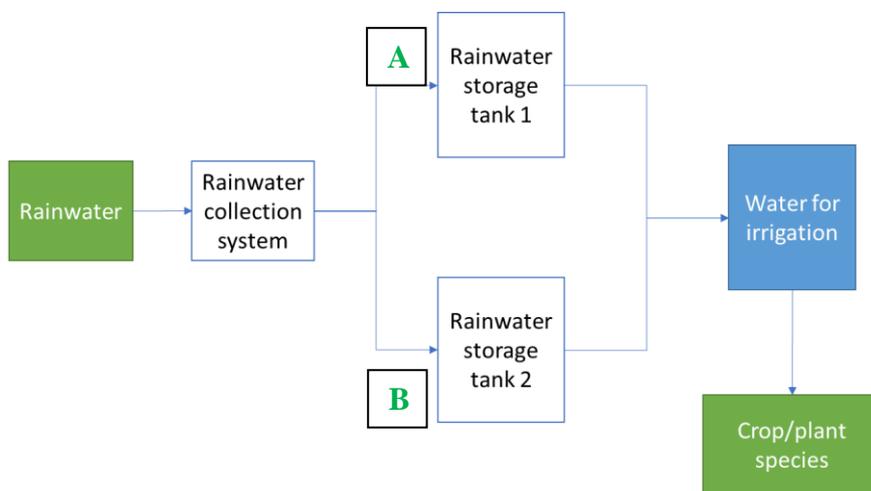
- **Sampling points:**

Irrigation waters were sampled between 10/8/2021 to 25/01/2023 for conventional treatment (T1) and from 14/08/2021 to 25/01/2023 for alternative treatment (T2) at least once per month. Detailed information about the dates is reported in the annex "HYDRO2", tab from "a" to "d". In total, 32 irrigation samples for each of the considered treated water (T1 and T2) were collected for their characterization. Samples were frozen at -20 °C, sent to Spain (ICRA partner) in dry ice, and stored at -20°C until analysis. Samples were analyzed in 4 (for T1) and 3 (for T2) analytical batches as indicated in the annex "HYDRO2".

## 2.4. HYDRO3

- **Site description**

HYDRO3 demonstration site is located on Ano Mera, Mykonos Island (Greece). The rainwater harvesting system consists of a 280m<sup>2</sup> shallow, sub-surface, rainwater collection system developed to collect rainwater by draining and then used to irrigate 0.4ha of agricultural area. Rainwater collection tanks (Figure 2.4) are located at the South-West end of the field, and they are linked to irrigation pipes with control valves that are extended on the South end along the field from West to East. In HYDRO3, oregano is cultivated by drip irrigation using the harvested stored rainwater stored in the collection tanks. The collected samples were frozen at -20 °C, sent to Spain (ICRA partner) in dry ice and stored at -20°C until analysis.



**Figure 2.4: Schematic illustration of HYDRO3: remote and innovative rainwater harvesting system for irrigation with the sampled points: A and B (grab samples).**

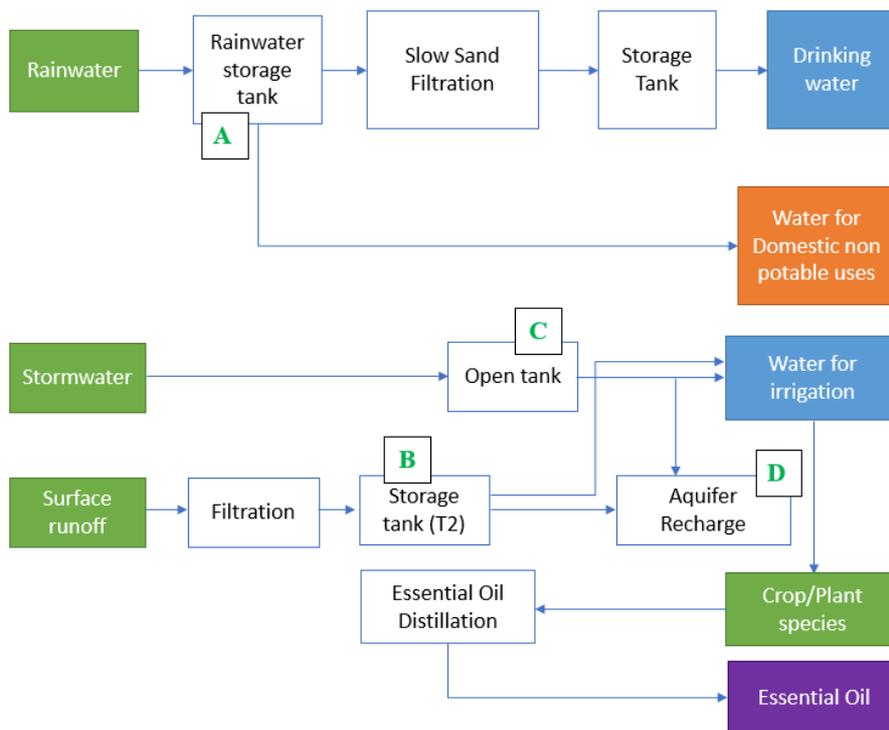
- **Sampling points:**

Tank 1 (A) and Tank 2 (B) were sampled on 14/02/2023 (grab samples). Tank 1 was sampled again on 06/03/2023.

## 2.5. HYDRO4

- **Site description**

HYDRO4 demonstration site is located on a small village of Ano Mera, Mykonos Island (Greece) and the agricultural area to be cultivated is about 0.2 ha. This site is situated in the premises of a house with little to almost no slope. The HYDRO4 (Figure 2.5) system is based on collection of rainwater from rooftops, surface runoffs and stormwater through a bioswale system. The water collected is stored into tanks and also in the aquifer, recharging it. Rainwater harvesting, storage, and recovery is developed during winter months to be reused during the summer. Part of the water is used for domestic, non-potable use (rainwater from rooftops) and another part for agricultural irrigation. In HYDRO4 lavender is cultivated and drip irrigated with rainwater/surface runoff stored in the aquifer and the tanks.



**Figure 2.5: Schematic illustration of HYDRO4: rainwater harvesting and treatment system to produce irrigation and potable water and for aquifer recharge with the sampled points: A, B, C, and D.**

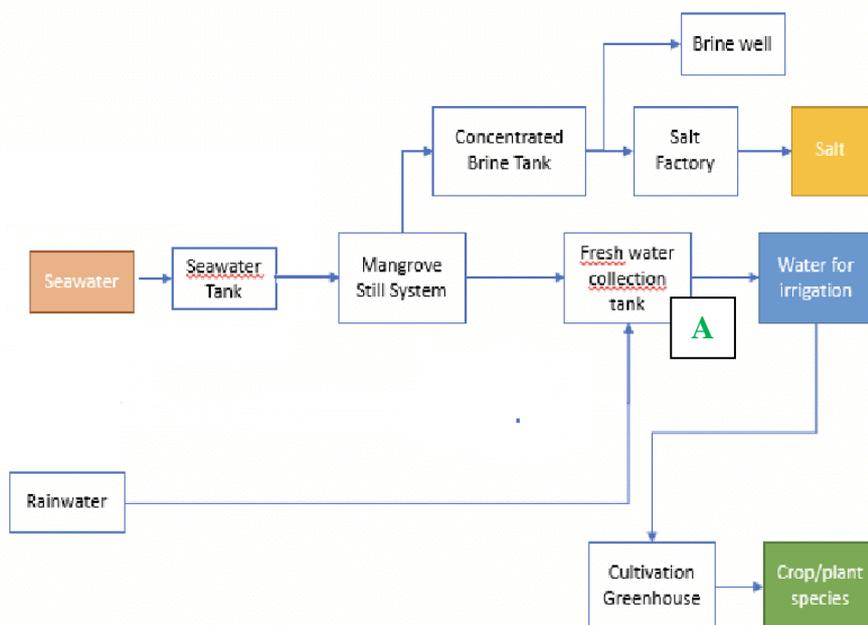
- **Sampling points**

Samples from 4 tanks were collected: Rainwater from rooftops (A); rainwater from surface runoffs (B); stormwater collected through the bioswale system (C), and aquifer recharge tank (D) (grab samples). The samples were collected on 14/02/2023 and 06/03/2023. The samples were frozen at -20 °C, sent to Spain (ICRA partner) in dry ice, and stored at -20°C until analysis.

## 2.6. HYDRO5

- **Site description**

HYDRO5 demonstration site, is located in Tinos Island (Greece). The agricultural demo site is located on the water desalination facilities of the municipality of Agios Focas. HYDRO5 (Figure 2.6) is a classical greenhouse attached to a Mangrove-still desalination system, which provides desalinated water. The greenhouse occupies an area of about 200 m<sup>2</sup>. The water is collected in storage tanks that are connected to a drip irrigation system used to irrigate the plants.



**Figure 2.6: Schematic illustration of HYDRO5: Mangrove Still Desalination System and Saltwater Evaporation Greenhouse with the sampled point A.**

- **Sampling points**

Freshwater from the collection tank was sampled twice (grab samples). The first sample was collected on 14/02/2023, and the second was collected on 20/02/2023. The samples were frozen at -20 °C, sent to Spain (ICRA partner) in dry ice and stored at -20°C until analysis.

## HYDRO6

- **Site description**

HYDRO6 demonstration site is located on Tinos Ecolodge (Potamia, Akeratos), one of the main points of attraction in Tinos Island (Greece). This place attracts tourists who would like to spend time embracing nature and living the experience of a nature-based society. and, in total, it has a cultivated area of about 0.15 ha. The HYDRO6 (Figure 2.7) demonstration system is a mixture of different systems, including rainwater harvesting, wastewater treatment reuse and surface water use, which allows the irrigation of a variety of plants based on the different water quality. Harvested rainwater is used to irrigate edible plants, while reclaimed water are

used to irrigate herbal and medicinal plants. The cultiva HYDRO6 are connected with a network of drip irrigation system (Deliverable HYDROUSA D22). The samples were kept at -20 °C, sent to Spain (ICRA partner) in dry ice, and stored at -20°C until analysis.

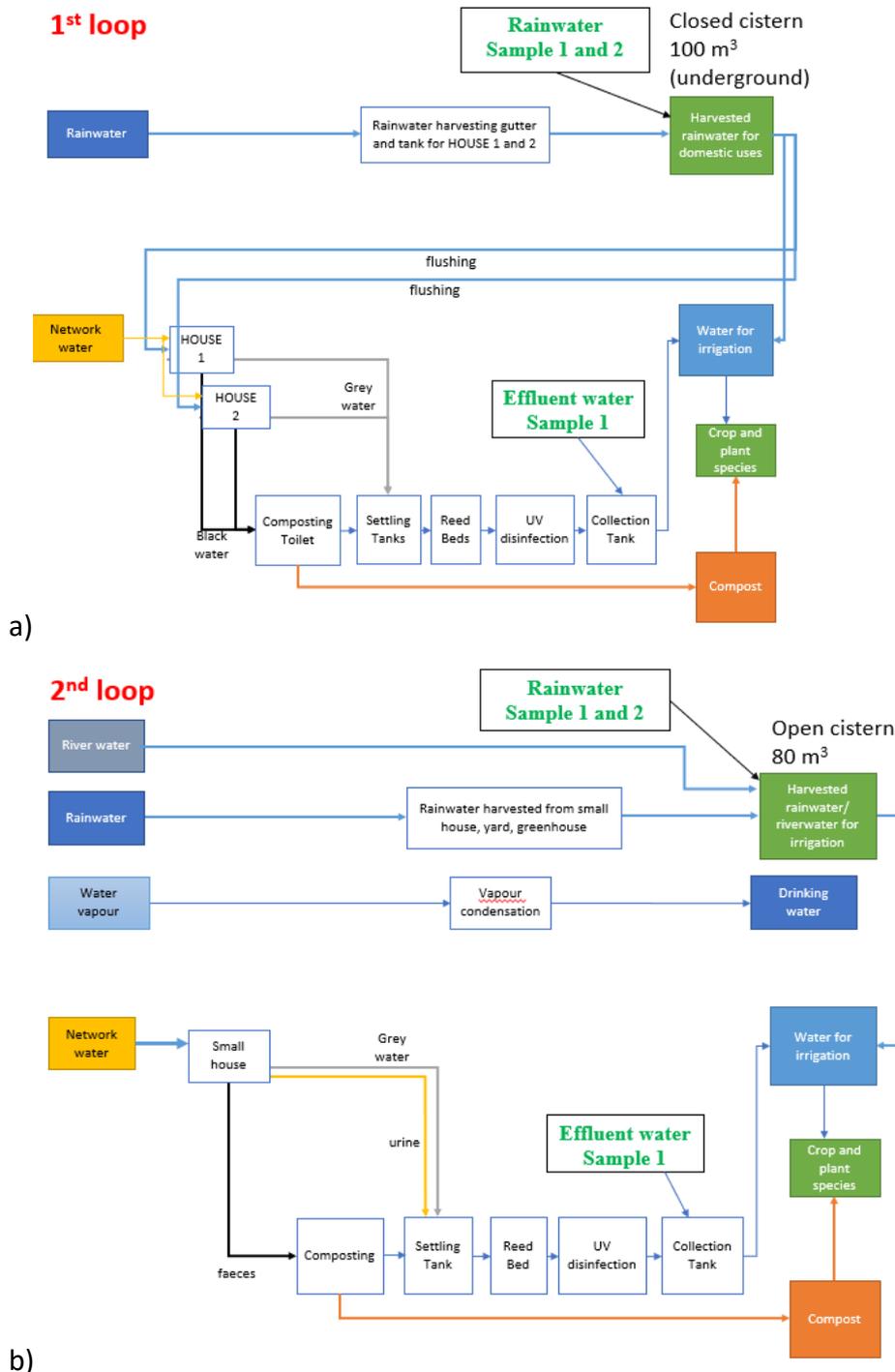


Figure 2.7: Schematic illustration of HYDRO6: Tinos Ecolodge - Water loops in an ecotourist facility. Moving towards water, energy, and food self-sufficiency: a) first loop; b) second loop.



- **Sampling points:**

The rainwater cistern of both 1<sup>st</sup> and 2<sup>nd</sup> loops as well as the effluent water of both applied reedbed systems were sampled on the 13/03/2023 (grab samples). The samples were frozen at -20 °C, sent to Spain (ICRA partner) in dry ice and stored at -20°C until analysis.

## 2.7. Analytical Methodologies

### 2.7.1. Pharmaceutical Active Compounds (PhACs)

A total of 66 pharmaceuticals, including 11 human metabolites, belonging to 13 therapeutic groups were studied. The list of selected pharmaceuticals can be found in the annex "HYDRO1" and belong to different therapeutical families, i.e., analgesics and anti-inflammatories, anthelmintics, anti-asthmatic drugs, antibiotics, antihypertensives; anti-prostatic hyperplasia drugs,  $\beta$ -blockers, calcium channel blockers, diuretics, histamine H<sub>2</sub> receptor antagonists, lipid regulators, psychiatric drugs; and x-ray contrast agents.

#### 2.7.1.1 Water

Water samples were vacuum filtered through 1.0  $\mu$ m glass fiber membrane filters (Whatman) followed by 0.45  $\mu$ m PVDF membrane filters (Merck Millipore Ltd.). After filtration, an adequate volume of a 0.1 M EDTA solution was added to 25 mL of influent wastewater and UASB effluent; 50 mL of coupled wetlands effluent wastewater, 50 mL of irrigation water to achieve a final concentration of 0.1% ( $\text{g}_{\text{solute}}/\text{g}_{\text{solution}}$ ). Afterwards samples were pre-concentrated using solid phase extraction following a protocol adapted from Gros et al. (2012). Oasis HLB cartridges (200 mg, 6 mL) were conditioned with 6 mL of methanol followed by 6 mL of water MS grade, then the sample was loaded in the cartridges using a vacuum manifold system at a flow rate of 1 mL/min. After that, the cartridges were washed with 6 mL of water MS grade and dried under vacuum for 15 min to remove the excess of water. Finally, analytes were eluted with 6 mL of methanol. Then, 50  $\mu$ L of a 500 ng/ $\mu$ L standard mixture containing all the isotopically labelled compounds were added in the extract. Lastly, they were evaporated until dryness under a gentle nitrogen flow and reconstituted in a mixture of methanol/water (2:8, v/v).

Analysis of PhACs in the water samples extracts was performed following the methodology by Castaño-Trias et al. (2023) and using a Waters Acquity Ultra-Performance™ liquid chromatography system equipped with two binary pumps systems (Milford, MA, USA) and coupled to a 5500 QTRAP hybrid triple quadrupole-linear ion trap mass spectrometer (Applied Biosystems, Foster City, CA, USA) with a turbo ion spray source. The sample volume injected was set at 5  $\mu$ L for positive (PI) and negative (NI) ionization modes. For positive ionized compounds the chromatographic method was adapted from Gros et al. (2013) with an Acquity HSS T3 column (50 mm  $\times$  2.1 mm i.d., 1.8  $\mu$ m particle size, Waters Corporation). Acetonitrile and 0.1% formic acid were selected as mobile phases at a flow of 0.5 mL/min. The method for the negative ionized compounds consisted in an adaptation of (Gros et al., 2012) with an Acquity BEH C18 column (50 mm  $\times$  2.1 mm i.d., 1.7  $\mu$ m particle size). The mobile phases were acetonitrile and an aqueous solution of 5mM ammonium acetate/ammonia at pH 8 at a flow of 0.6 mL/min.

Compound dependent MS parameters (declustering potential, collision energy, and collision cell exit potential) were optimized for each compound. All transitions were recorded by using the Scheduled MRMTM algorithm. In PI mode, target scan time (TST) was set at 0.25 s, with an SRM detection window of 20 s, whereas for NI



mode, TST was set at 0.25 s with an SRM detection window of 30 s. In both cases, the resolution at the first quadrupole (Q1) was set at unit, and at the third quadrupole (Q3), it was set at low and the pause between mass ranges was 5 ms. The settings for source-dependent parameters were set-up as follows. For compounds analyzed under PI the parameters were: curtain gas, 30 V; Nitrogen collision gas medium; source temperature of 650 °C; ion spray voltage at 5500 V; ion source gases GS1 and GS2 set at 60 and 50 V, respectively. For compounds analyzed under NI, such parameters were: curtain gas, 30 V; Nitrogen collision gas medium; source temperature of 650 °C; ion spray voltage at -3500 V; ion source gases GS1 and GS2 set at 60 and 70. The entrance potential was set at 10. All data were acquired and processed using Analyst 1.5.1 software.

Quantification of analytes was performed by SRM by monitoring two mass transitions between the precursor ion and the most abundant fragment ions for each compound. The one at higher intensity was used for quantification purposes, while the second one was used for confirmation of the compound identification.

### 2.8.1.2 Sludge/Compost

Solid samples were freeze-dried in aluminum trays for 3 days and further kept at -20°C until their processing and analysis. Freeze-dried samples were sieved in a 2 mm sieve. Samples were extracted according to Gros et al. (2019). Briefly, 1 g of freeze-dried solid was sequentially mixed with 5 mL of buffer McIlvaine (pH=7) and 5 mL of Methanol, vortexing after each addition. The samples were placed in an ultrasound bath for one cycle of 15 minutes, and later centrifuged at 10,000 rpm for 5 minutes. The supernatant was collected, and the extraction was repeated twice. The 3 fractions were combined, mixed with 7.5 ml of EDTA 0.1N solution and diluted to 200 ml. The solutions were filtered through 1.0 µm glass fiber membrane filters (Whatman) followed by 0.45 µm PVDF membrane filters (Merck Millipore Ltd.) and finally purified by SPE. Oasis Accell™ Plus QMA (500 mg, 6 mL) cartridges in tandem with Oasis HLB (200 mg, 6 mL) were conditioned with Methanol and HPLC water, and then the samples were loaded on the cartridges. Afterwards, the Oasis Accell™ Plus QMA cartridges were discarded, and the Oasis HLB ones were washed with HPLC water, and dried under vacuum for 30 minutes. The cartridges were eluted with 6 mL of Methanol, and the extracts were spiked with a 50 µL of a 1000 ng/µL mix solution of isotopically labelled standards. The solvent was evaporated, and the sample was reconstituted in a mixture of methanol/HPLC water (1:1, v/v).

The instrumental analysis of pharmaceuticals was performed with a Waters Acquity Ultra-Performance™ liquid chromatography system coupled with a 5500 QTRAP hybrid triple quadrupole-linear ion trap mass spectrometer (Applied Biosystems, Foster City, CA, USA) with a turbo Ion Spray source. The instrumental conditions for the chromatographic separation and detection as previously described in section 2.8.1.1 were applied.

Quantification of analytes was performed by SRM by monitoring two mass transitions between the precursor ion and the most abundant fragment ions for each compound. The one at higher intensity was used for quantification purposes, while the second one was used for confirmation of the compound identification.



## 2.7.2. Endocrine-disrupting compounds (EDCs)

A total of 10 endocrine-disrupting compounds (EDCs) belonging to 4 families were studied: flame retardants (TBEP, TCEP), plasticizers (BPA, BPS), preservatives (methylparaben, propylparaben), triazoles (1H-benzotriazole), hormones (estrone, ethinyl estradiol), and one stimulant (caffeine).

### 2.8.2.1 Water

The extracts of the water samples in methanol/water 2:8 obtained for the analysis of pharmaceuticals (see Section 2.8.1.1) were used for the determination of EDCs.

The exploratory campaign samples were analyzed in an Equan MAX™ liquid chromatography system coupled to a TSQ Vantage mass spectrometer (all from Thermo Fisher Scientific), equipped with an electrospray ionization source (ESI). Analysis was performed following the method by Becker et al. (2017) where the chromatographic separation was carried out using a Kinetex 1.7u C18 (50 × 2.1 mm) column and methanol (A) and HPLC grade water (B) were used as the mobile phases. For the negative ion mode 20 µL of sample extract were injected in the system and the gradient elution was as follows; initial conditions: 20% A during the first minute, increase to 50% A between minute 1 and 2.75 and arriving to 100% A at 2.75–6.50 min; 100% A was maintained for 1.5 min; returning to 20% A in 1.5 min and maintained for 1 min. The flow rate was 0.4 mL/min and total run time was 10.5 min. For the positive ion mode, 10 µL of the sample extract were injected in the system using the same mobile phase as in the negative ion mode. The gradient was: 10% A during 1 min, from 1 to 2.75 min increase to 100% A and maintained for 2.75 min, returning to initial conditions in 1 min and then maintained for 1 min. The flow rate was 0.3 mL/min and the total run time was 7.5 min. Two selected reaction monitoring (MRM) transitions were selected for each compound.

For the sampling campaigns of 2021, 2022 and 2023, analysis was performed in a Waters Acquity Ultra-Performance™ liquid chromatography system equipped with two binary pumps systems (Milford, MA, USA), coupled to a 5500 QTRAP hybrid triple quadrupole-linear ion trap mass spectrometer (Applied Biosystems, Foster City, CA, USA) with a turbo Ion Spray source. Two selected reaction monitoring (MRM) transitions were selected for each compound. All data were acquired and processed using Analyst 1.5.1 software. The analysis of EDCs was performed using the method by Turull et al., 2023. Chromatographic separation was achieved using a Kinetex Biphenyl column (50 x 2.1 mm i.d.; 2.6 µm particle size) equipped with a pre-column. For positive ionization mode (PI), the mobile phase consisted in methanol (A) and water MS grade (B), while for negative ionization mode (NI) methanol (A) and water MS grade pH 9 (adjusted with ammonia) (B) was used. For both ionization modes, the flow rate was 0.4 mL/min, and the elution gradient was as follows: 0-5 min, 20-100% A; 5-6 min, 100% A; 6-6.5 min, return to initial conditions; 6.5-8 min, equilibration of the column. The column temperature was set at 40 °C and an injection volume of 5 µL was used. All data were acquired and processed using Analyst 1.5.1 software.

In both methodologies, quantification of analytes was performed by SRM by monitoring two mass transitions between the precursor ion and the most abundant fragment ions for each compound. The one at higher intensity was used for quantification purposes, while the second one was used for confirmation of the compound identification.



### 2.8.2.2 Sludge/Compost

The extracts of the sludge samples in methanol/water 2:8 obtained for the analysis of pharmaceuticals (see Section 2.8.1.2) were used for the analysis of EDCs. A UHPLC-MS/MS methodology for the analysis of EDCs was applied according to Turull et al. (2023) as described in section 2.8.2.1. Quantification of analytes was performed by SRM by monitoring two mass transitions between the precursor ion and the most abundant fragment ions for each compound. The one at higher intensity was used for quantification purposes, while the second one was used for confirmation of the compound identification.

### 2.8.3 Endocrine disrupting chemicals and non-steroidal anti-inflammatory drugs method applied in the analysis of intensified wetlands

The target micropollutants were: three endocrine disrupting chemicals, namely nonylphenol (NP), triclosan (TCS) and bisphenol A (BPA) and four non-steroidal anti-inflammatory drugs, namely ibuprofen (IBU), naproxen (NPX), diclofenac (DCF) and ketoprofen (KFN). The target compounds were analyzed according to the method developed by Samaras et al. (2011). Briefly, the method includes: (1) filtration of the samples, (2) acidification (pH = 2.5), (3) addition of the surrogates (meclofenamic acid sodium salt (MCF) for NSAIDs and deuterated bisphenol A (BPA-d16) for EDCs), (4) solid phase extraction, (5) evaporation of the elutions to dryness by purging them with nitrogen, (6) derivatization using 10  $\mu$ L pyridine and 50  $\mu$ L bis(trimethylsilyl)trifluoroacetamide (BSTFA) +1% trimethylchlorosilane (TMCS) at a bathing device of 70°C for 20 min, and (7) injection of 1  $\mu$ L into GC-MS. The gas chromatograph (GC) was a 7890A, and the mass selective detector (MSD) was a 5975C from Agilent Technologies, while the software used was Agilent ChemStation. This analysis was performed by the University of the Aegean.

### 2.8.4 Benzotriazoles (BTR) method applied in the analysis of intensified wetlands

An aliquot of 100 mL was collected and filtered through membrane filters (0.45  $\mu$ m; Whatman, Germany), acidified at pH 3.0  $\pm$  0.1 with few drops of HCl and stored at 4 °C. The analysis of the liquid samples included SPE, and it was based on the method developed by Asimakopoulos et al. (2013). For the chromatographic analysis, the mobile phase consisted of MilliQ grade water (acidified with 0.1% v/v formic acid) and ACN. Gradient elution was carried out as follows: 25% ACN to 75% ACN in 15 min, hold for 9 min and then decrease to 25% ACN in 1 min. Before each run, equilibration was performed for 10 min with 25% ACN. Each run had a total duration of 35 min and a flow rate of 0.5 mL min<sup>-1</sup> (Mazioti et al., 2015). The model of High-Performance Liquid Chromatography (HPLC) was Shimatzu (Japan) LC20-AD with an SPD-M20A diode array detector DAD (using signal at 254 nm) and a SIL-20AC auto sampler. The model of column was Zorbax SB-C18 4.6 mm 150 mm (5 mL connected with a pre-column Zorbax SB-C18 (Agilent, USA) and they were heated at 35 °C with a CTO-20AC column oven (Shimatzu-Japan).

For the analysis of micropollutants, standards of XTR and CBTR were purchased by Sigma-Aldrich (USA), BTR was supplied by Merck (Germany), 5TTR by Acros Organics (Belgium) and OHBTH by Alfa Aesar (USA). Methanol (MeOH-MS grade) and acetonitrile (ACN, HPLC grade) were purchased by Merck (Germany) and Fisher (USA), respectively. Stock solutions (1000  $\mu$ g/mL) of all target analytes were prepared in MeOH and were stored at -18 °C. Strata-X (33u Polymeric Reversed Phase, 200 mg/6 mL) were used for the solid phase extraction (SPE) and they were supplied by Phenomenex (USA). Ultrapure water was prepared using a MilliQ/Milliro Milli-pore system (USA). This analysis was performed by the University of the Aegean.



### 2.8.5 Pesticides

Eleven pesticides and two transformation products belonging to four different families were selected for analysis in water samples, namely phenylurea (diuron, isoproturon, and the diuron transformation products 1-(3,4-Dichlorophenyl) urea (DCPU) and 1-(3,4-Dichlorophenyl)-3-methylurea (DCPMU); organophosphates (dichlorvos, chlorpyrifos and chlorfenvinphos); organochlorides (alachlor and trifluralin); triazines (atrazine, terbuthylazine and simazine); and irgarol. A customized method was applied for the analysis of pesticides in water samples based on the following paper (Gervais et al., 2008) using SPE followed by UHPLC-MS/MS analysis. In brief, Strata-X cartridges (200 mg, 6 mL) were conditioned with 6 mL methanol and 6 mL water. Then, 250 mL of water sample, previously filtered by 0.45  $\mu\text{m}$  PVDF membrane filters, were loaded onto the cartridge at a flow rate of 1 mL/min. After that, the cartridges were washed with 6 mL of water, dried under vacuum for 5 min and eluted with 4 + 4 mL of methanol. The extracts were dried to dryness under a gentle stream of nitrogen and finally reconstituted with 0.5 mL of methanol:water (20:80, v/v).

Instrumental analysis was performed on a Waters Acquity Ultra-Performance™ liquid chromatography system, equipped with two binary pumps systems (Milford, MA, USA). Chromatographic separation was achieved using an Acquity HSS T<sub>3</sub> column (50 x 2.1 mm; 1.8  $\mu\text{m}$  particle size). The mobile phase consisted of acetonitrile (A) and water with 0.1% formic acid (B). The flow rate was 0.6 mL/min and the elution gradient was as follows: 0-5 min, 20-100% A; 5-5.1 min, return to initial conditions; 5.1-6 min, equilibration of the column. The injection volume was 10  $\mu\text{L}$ . The UHPLC instrument was coupled to a 5500 QTRAP hybrid triple quadrupole-linear ion trap mass spectrometer (Applied Biosystems, Foster City, CA, USA) with a turbo Ion Spray source. Pesticides were quantified in the samples using the isotopic dilution method.

Quantification of analytes was performed by SRM by monitoring two mass transitions between the precursor ion and the most abundant fragment ions for each compound. The one at higher intensity was used for quantification purposes, while the second one was used for confirmation of the compound identification.

### 2.8.6 EU 2015/495 Watch List compounds

Twelve compounds included in the Watch List of the Commission Implementing Decision (EU) 2015/495, namely imidacloprid, thiacloprid, thiamethoxam, clothianidin, acetamiprid, methiocarb, azithromycin, erythromycin, clarithromycin, estrone, estriol, and ethinylestradiol, were analyzed in water samples (except for sea water, which is not compatible with the methodology and instrument) using the on-line SPE-UHPLC-MS/MS methodology described in Gusmaroli et al., 2019. Briefly, water samples were filtered with glass and PVDF membrane filters and then transferred into 10-mL amber SPE vials and spiked with an appropriate volume of a working standard mixture containing all the isotopically labelled compounds in order to obtain an IS concentration of 50 ng/L. Online preconcentration was carried out in an Equan MAX™ fully automated system consisting of a PAL autosampler and two pumps: a loading pump and an eluting pump, both from Thermo Fisher Scientific. The injection volume was set at 2 mL and two LC columns were used, one for analyte preconcentration and the other for chromatographic separation. Sample preconcentration was done in a Hypersil GOLD aQ (20 x 2.1 mm, 12  $\mu\text{m}$  particle size, Thermo Fisher Scientific) column. The sample was loaded at a flow rate of 1750  $\mu\text{L}/\text{min}$  and then the column was washed and conditioned with methanol and water during the chromatographic run. In the positive ionization mode, chromatographic separation was achieved using a Kinetex Biphenyl column (100 x 2.1 mm; 1.7  $\mu\text{m}$  particle size, Phenomenex), and the mobile phase consisted of methanol (A) and water with 0.1% formic acid (B) at a flow rate of 0.4 mL/min. The elution gradient was the following: 0-1.75 min, 60%A; 1.75-1.88 min, 60-75%; 1.88-4.00 min, 75-100%; 4.00-5.75 min, 100%; 5.75-8.00 min, return to initial conditions and equilibration of the column. In the negative ionization mode, a Kinetex EVO C<sub>18</sub> column (100 x 2.1 mm; 1.7  $\mu\text{m}$  particle size, Phenomenex) was used and the mobile phase consisted of a mixture of methanol (A) and acetonitrile (B), for the organic phase, and water with ammonium fluoride 1 mM (C) for the aqueous phase. The elution gradient was as follows: 0-2.0 min, 15%A



and 15%B; 2.0-4.0 min, 15%-100%B; 4.0-5.5 min, 100%B; 5.5-6.5 min, return to initial conditions; 6.5-10.5 min, equilibration of the column. The detection was performed using a TSQ Vantage mass spectrometer (Thermo Fisher Scientific), equipped with an electrospray ionization source (ESI).

Quantification of analytes was performed by SRM by monitoring two mass transitions between the precursor ion and the most abundant fragment ions for each compound. The one at higher intensity was used for quantification purposes, while the second one was used for confirmation of the compound identification.

### 2.8.7 Triclosan and its transformation products

Triclosan and its transformation products (methyl-triclosan and 2,4-dichlorophenol) were analyzed in water samples using the on-line HS-SPME-GC-MS/MS methodology described in Santos et al. (2019). Briefly, 10 mL of water sample were placed in a 20 mL amber headspace vial, where 3.5 g sodium hydrochloride and 0.1 g di-sodium hydrogen phosphate dehydrate were previously weighted. Then, 100  $\mu$ L of a 10  $\mu$ g/L aqueous mixture of isotopically labeled compounds (TCS-d3 and methyl-triclosan-d3) were added as internal standards. Finally, 100  $\mu$ L of acetic anhydride (derivatization reagent) were added and the vials were sealed with aluminum caps furnished with silicone/PTFE septa. The compounds were extracted using a 100  $\mu$ m polydimethylsiloxane (PDMS) fiber from Supelco. The following conditions were applied: samples were equilibrated in an incubator at 90  $^{\circ}$ C, with continuous agitation, and then the fiber was exposed to the headspace for 15 min. Once finished the exposure step, the absorbent was transferred to the GC-MS/MS injector. Desorption of the compounds from the fiber was made at 280  $^{\circ}$ C in the splitless mode. Triclosan and its transformation products were analyzed in a GC coupled to a triple quadrupole mass spectrometer (Thermo Fisher Scientific) under the following optimized conditions: the separation was carried out on a ZB1701 capillary column (30 m  $\times$  0.25 mm i.d.  $\times$  0.25  $\mu$ m film thickness) from Phenomenex and helium was used as carrier gas at a flow rate of 1.0 mL/min. The GC oven program was the follow: 2 min at 60  $^{\circ}$ C, then increasing by 30  $^{\circ}$ C/min up to 200  $^{\circ}$ C and held for 2 min. Then increasing by 40  $^{\circ}$ C/min up to 280  $^{\circ}$ C min and held for 4 min. The mass spectrometer detector was operated with an electron ionization source (EI) with a voltage of 70 eV and source temperature at 250  $^{\circ}$ C. Compounds were analyzed in SRM mode, and their identification was based on the selected ion fragments ratio and their retention time.

Quantification of analytes was performed by SRM by monitoring two mass transitions between the precursor ion and the most abundant fragment ions for each compound. The one at higher intensity was used for quantification purposes, while the second one was used for confirmation of the compound identification.

## 3. RESULTS AND DISCUSSION

### 3.1 Exploratory Sampling Campaign

All the samples collected in the 2019 exploratory sampling campaign were analyzed using the methodologies described in section 2.8 for pharmaceuticals, endocrine-disrupting compounds, pesticides, EU 2015 watch list (2015/495) compounds, and triclosan (and its metabolites).

Thirty-four out of 66 pharmaceutical compounds of different therapeutic classes were found at least once in the raw influent wastewater samples (Antissa WWTP, Lesvos Island) of the exploratory sampling campaign. The highest concentrations in wastewater were found for analgesic and anti-inflammatories (with values as high as 305,607 ng/L for acetaminophen) followed by the antihypertensives and psychiatric drugs (with values as high as 13,233 ng/L for O-desmethyl-venlafaxine, annex "Exploratory Campaign", tab "PhACs"). Some other pharmaceuticals were also detected in the river Vulgaris (Lesvos Island) but at lower concentration: irbesartan, salicylic acid, ketoprofen, hydrochlorothiazide, iopromide, O-desmethyl-venlafaxine, and azithromycin. In the sea (Tinos Island), the concentrations were even lower: all compounds detected at concentrations lower than 20 ng/L except for ketoprofen at 125 ng/L.

Five EDCs out of the 10 analyzed were detected in this sampling campaign at different levels in the three considered matrices (Annex "Exploratory Campaign", tab "EDCs"): estrone, methylparaben, BP A, BPS and benzotriazole-1H. Estrone was found (average concentration of 318 ng/L) in wastewater but not in river and sea water, same trend was observed for BPS. The maximum concentrations in each matrix were the following: 523.6 ng/L (estrone in IWW), 103 ng/L (BPA in river) and 71 ng/L (caffeine in sea water).

Triclosan and its transformation product 2,4-dichlorophenol, were both detected in wastewater samples at low concentration (up to a maximum of 47 and 20 ng/L respectively), not detected in the river, and barely found in sea samples at 2 ng/L (Annex "Exploratory Campaign", tab "TCS"). The only detected compounds out of the 13 analyzed pesticides (Annex "Exploratory campaign", tab "pesticides") were atrazine (4 ng/L) and terbuthylazine (382 ng/L), and only in sea water. As to the EU watch list (2015/495) compounds (Annex "Exploratory campaign", tab "WL2015"), 5 compounds (out of the 12 analyzed) were detected: thiacloprid, clarithromycin, azithromycin, estrone, and estradiol. Three out of these 5 compounds were also analyzed using the PhACs and EDCs analytical methodologies previously mentioned.

Due to the low observed concentrations for pesticides, triclosan (and metabolite) were discarded as target analytes in the project campaigns in 2021, 2022, and 2023. Regarding the EU watch list (2015/49) compounds, since 3 of the 5 detected compounds are already included in the PhACs and EDCs methodologies, only these two latter 2 methodologies were used in the further monitoring campaigns of 2021, 2022, and 2023.

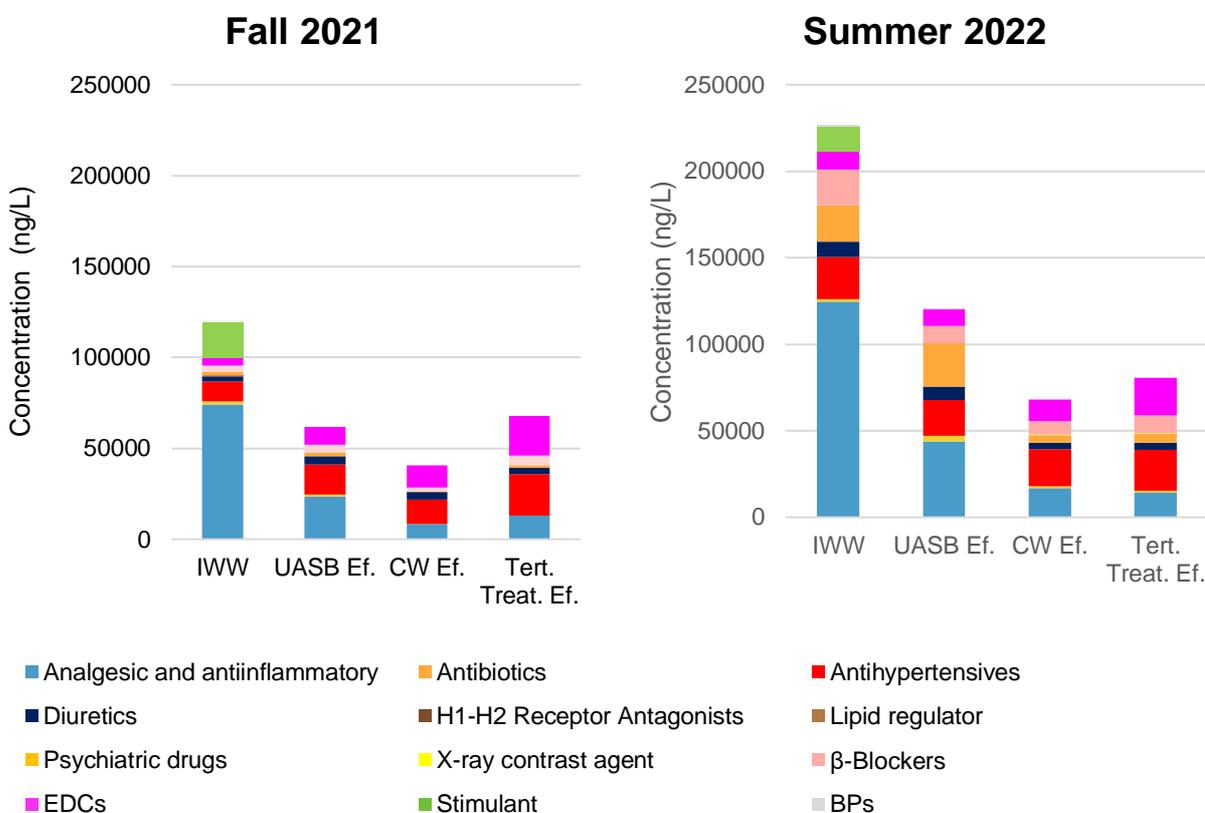


## 3.2 HYDRO1

### 3.2.1-Characterization of influent wastewater

The wastewater influent of HYDRO1 was sampled in two different seasons, i.e., fall 2021 and summer 2022. A similar pattern in terms of pharmaceutical active compounds (PhACs) presence was observed in both seasons with high presence of analgesics and anti-inflammatory compounds followed by antihypertensives and  $\beta$ -blockers. Nevertheless, the total PhAC + EDCs concentration was higher in summer than in fall (226,349 and 187,744 ng/L, respectively) as it is shown in Figure 3.1. Considering only PhACs, higher differences were observed (95,533 and 202,727 ng/L in fall and in summer, respectively). Most compounds were at higher concentration in summer, for example acetaminophen at 85,440 and 63,200 ng/L in fall and summer, respectively. Some exceptions were observed: clarithromycin and gemfibrozil had higher concentrations in fall. Clarithromycin is prescribed to treat respiratory diseases and it was previously pointed as a pharmaceutical showing high seasonality occurrence differences through different countries in Europe (Rodriguez-Mozaz et. al., 2020). In HYDRO1 influent, this compound was found at a higher level in fall than in summer (929 ng/L and below LOQ, respectively). The higher prescription of gemfibrozil as lipid regulator in the cold season (Tang et al., 2019) is in line with its detection in influent wastewater at higher concentrations in fall (up to 377 ng/L) than in summer (<LOQ).

Contrary to the general observed trend in the PhACs occurrence (concentrations in summer much higher than in fall), the stimulant caffeine, BPs, and other EDCs were detected at higher concentration in fall compared to summer: 92,210 and 23,622 ng/L of total EDCs concentration in fall and summer sampling campaigns, respectively. In the case of caffeine, the difference is even more extreme: mean of 68,362 ng/L in fall and of 19,776 ng/L in summer. This difference can be attributed to the overlap of the summer campaign to the period of vacations in Greece and, therefore, less consumption of coffee (Papageorgiou et al., 2016). BPS mean concentration in fall was 8,556 ng/L, one order of magnitude higher than in summer (769 ng/L). Conversely, BPA was not detected in fall while it was up to 225 ng/L in summer. In-detail concentrations are reported in the annex "HYDRO1" (tab "b" and "c").



**Figure 3.1: Total concentration of pharmaceuticals and endocrine disrupting compounds, classified per class, at each sampled point of the treatment train in HYDRO1: influent wastewater (IWW), UASB effluent (UASB Ef.), coupled wetlands effluent (CW Ef.), and tertiary treatment effluent (Tert. Treat. Ef.). The concentrations in fall 2021 are presented on the left, and those in summer 2022 on the right.**

### 3.2.2- UASB treatment

The efficiency of the UASB-CW-UV system (Figure 3.1) in removing target micropollutants was evaluated at each of the treatment steps. The Upflow Anaerobic Sludge Blanket (UASB) was the first treatment applied, and it achieved a similar decrease of PhACs concentrations in both seasons (46% and 43% in fall and summer, respectively), despite the decrease in HRT ( $15.1 \pm 1.3$  and  $12.2 \pm 1.8$  h in fall 2021 and summer 2022, respectively). These values can be attributed to the efficient removal of the compound at the highest concentration; acetaminophen (88% and 87% removal in fall and summer, respectively). However, for the rest of pharmaceuticals only a 14% removal was observed in summer whereas a 36% increase in the overall levels was observed in fall. The antihypertensives irbesartan, valsartan, and losartan, in fact, even increased their concentration in fall: 82%, 51%, and 70%, respectively. Slight differences between the sampling time and the technology HRT might lead to that apparent increase of concentration after the treatment. Nevertheless, a real increase in PhACs concentration may indeed sometimes happen, due to the cleavage of the conjugates and/or re-transformation of metabolites back to the parent compound by the microbial community present



in the wastewater and in the bioreactor as observed for these 3 antihypertensive also by Jelic et al. (2015) in an anaerobic pressurized sewer system. Similar PhACs removals by UASB have been described in the literature: e.g., diclofenac is reported to be barely removed (less than 14%) in the investigated system (UASB of HYDRO1) which come in agreement with what is reported by (Queiroz et al., 2012). Likewise, ibuprofen was poorly removed (less than 10%) by the HYDRO1 UASB as well as by the UASB system studied by Arias et al. (2018). The UASB was neither efficient in the removal of BPs nor of the rest of analyzed EDCs, and even an increase of 1H-benzotriazole in both seasons was observed: from not detected up to 12,494 ng/L (mean concentration) in fall and from 4,131 ng/L to 9,810 ng/L, (mean concentration) in summer. In contrast, caffeine was removed at high rate both in summer (100%) and fall (89%). Despite the efficient removals of caffeine and acetaminophen, the UASB does not achieve a successful removal of the rest of target contaminants. The UASB treatment was designed as the 1<sup>st</sup> wastewater treatment step in HYDRO1 for achieving partial organic matter and suspended solids removal (i.e., as primary treatment) and biogas production.

### 3.2.3- Constructed Wetlands

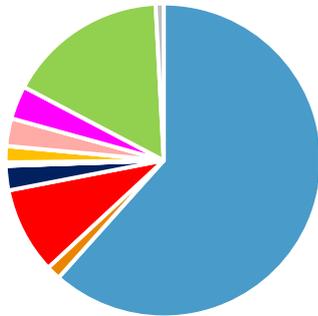
The observed removal of PhACs + EDCs after the wetlands system (compared to the HYDRO1 influent (IWW) concentration) did not differ between the seasons: 76% and 74% in fall and summer, respectively, even though the CW inlet total concentrations were higher in summer (118,835 ng/L) than in fall (86,115 ng/L). At the outlet of the CW, only 16 pharmaceutical compounds were found at concentrations higher than 1,000 ng/L in at least one of the samples (Annex "HYDRO1", tabs "b" and "c"). A very high removal for acetaminophen (one of the compounds at the highest concentration in the CW inlet) was achieved (98 and 99% in fall and summer, respectively). On the other hand, in the case of valsartan, only 13% removal in summer (and negative removal of 16% in fall) was observed, being the compound found at the highest concentrations in the outlet of the CW in both seasons (10,387 and 15,142 ng/L fall and in summer respectively). Removal of BPs was higher in summer (70%) than in fall (31%) whereas for the rest of EDCs a better removal was observed in fall than in summer (with also negative removal observed for some compounds).

### 3.2.4-Tertiary treatment:

The UV treatment is applied for disinfection of treated water. Nonetheless, different studies in literature report an impact of UV on organic microcontaminants. For example, Collado et al. (2014) reported UV removals up to 61% (for meloxicam). In contrast with the literature, the total concentration of OMP (PhACs + EDCs) in HYDRO1 increased from the inlet to the outlet of the UV system from 44,250 ng/L to 86,019 ng/L in fall and from 59,025 ng/L to 80,105 ng/L in summer (Annex "HYDRO1", tabs "b" and "c"). For instance, caffeine concentration was five times higher after UV treatment. This increase could be likely explained by evaporation of water stored in the tank connected to the tertiary treatment (treated water was stored there, before its use for irrigation for periods of time between few hours up to few days, depending on the season) together with a possible cleavage of the conjugates and or re-transformation of metabolites by the microbial community.

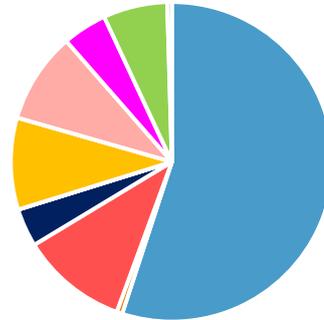
Comparing both seasons, the overall HYDRO1 removal of PhACs + EDCs was higher than 50% (54% in fall and 65% in summer, with higher initial concentrations). EDCs and antihypertensives were the compounds with higher contribution to the total load of HYDRO1 effluents in both seasons (Figure 3.2). Antihypertensives loads in IWW were higher in summer compared to fall (24,357 and 10,719 ng/L, respectively) while in the UV outlet their concentrations were similar (23,747 and 22,820 µg/L, respectively). In the case of EDCs, a similar effect is observed for the compound 1H-Benzotriazole.

**Wastewater influent  
Fall 2021**



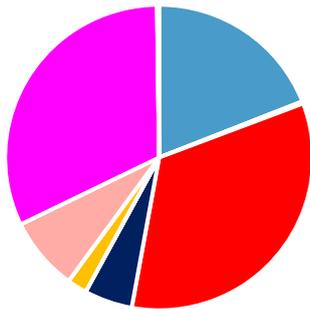
Total Concentration: 187,744 ng/L

**Wastewater influent  
Summer 2022**



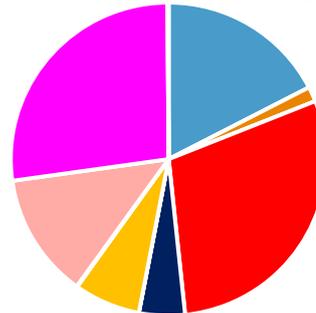
Total Concentration 226,349 ng/L

**Tertiary treatment effluent  
Fall 2021**



Total Concentration: 86,019 ng/L

**Tertiary treatment effluent  
Summer 2022**



Total Concentration: 80,105 ng/L

- |                                  |                              |                     |
|----------------------------------|------------------------------|---------------------|
| ■ Analgesic and antiinflammatory | ■ Antibiotics                | ■ Antihypertensives |
| ■ Diuretics                      | ■ H1-H2 Receptor Antagonists | ■ Lipid regulator   |
| ■ Psychiatric drugs              | ■ X-ray contrast agent       | ■ β-Blockers        |
| ■ EDCs                           | ■ Stimulant                  | ■ BPs               |

**Figure 3.2: Relative concentrations of each therapeutic class in influent wastewater and the final effluents of HYDRO1, in fall 2021 (on the left side) and in summer 2022 (on the right side).**

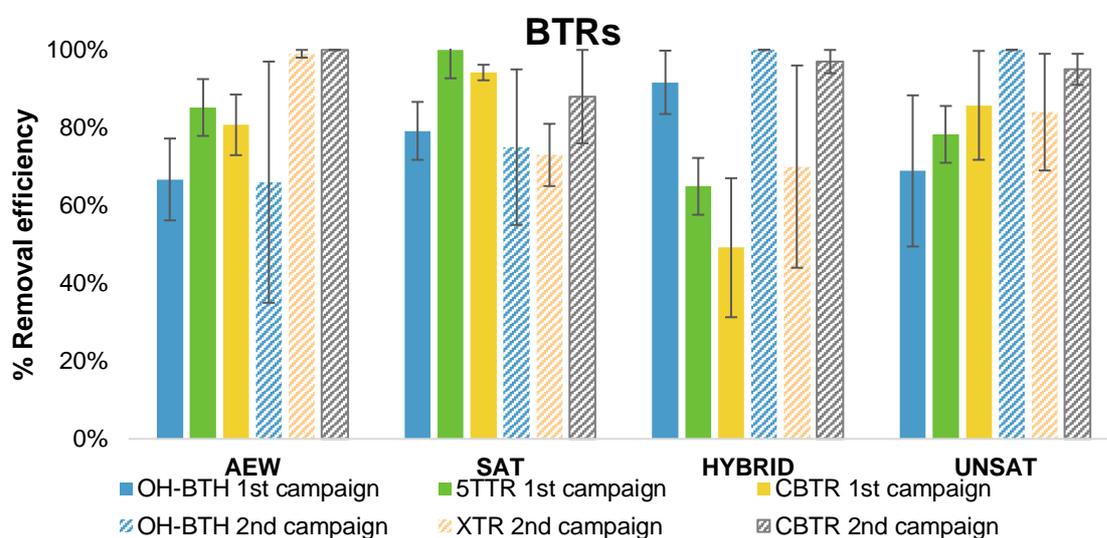
### 3.2.5-Intensified wetlands

#### *Benzotriazoles*

The main removal mechanisms for micropollutants in CWs are adsorption, plant uptake, phytodegradation, and biodegradation (Overton et al., 2023). At the 1<sup>st</sup> sampling campaign, the removals were significantly high for all benzotriazoles (>67%), except for CBTR in the hybrid electroactive pilot (49%). As shown in Figure 3.3, higher removals were observed in the saturated electroactive pilot. The higher standard deviation of the unsaturated pilots (HYBRID and UNSAT) is possibly due to the poor solid retention, affected by the percolation

velocity and the granulometry. Previous studies have shown that biodegradation is considered the dominant mechanism affecting the removal of these compounds (Mazioti et al., 2017; Gatidou et al., 2019; Kora et al., 2020). On the other hand, limited information is available on the role of adsorption on their removal (Yu et al., 2022; Zhang et al., 2021). Carbon-based materials have relatively large surface areas and a great number of adsorption sites, characteristics that could enhance the removal of target compounds due to adsorption in these systems.

Increasing the number of pulses to 150 (2<sup>nd</sup> sampling campaign) improved the performance in terms of BTR removals in all four pilots (Figure 3.3). The poor percolation time seems to have affected the performance of the pilots for the BTRs. The removals were greater than 66%, with the highest observed for CBTR (88-100%). The micropollutant BTR and xylylriazole (XTR) were not detected in either sampling campaign. Also, XTR was not detected in the 1<sup>st</sup> sampling campaign as well as 5-methyl-1H-benzothiazole (5TTR) in the 2<sup>nd</sup> sampling campaign, therefore it was impossible to compare the performance of the pilots in relation to the number of pulses for the above substances.



**Figure 3.3: Removal of benzotriazoles (BTRs) in intensified CWs.**

### **Endocrine disrupting chemicals and non-steroidal anti-inflammatory drugs**

The target micropollutants were: three endocrine disrupting chemicals, namely nonylphenol (NP), triclosan (TCS), and bisphenol A (BPA) and four non-steroidal anti-inflammatory drugs, namely ibuprofen (IBU), naproxen (NPX), diclofenac (DCF), and ketoprofen (KFN). Since the pilot wetlands are subsurface vertical flow wetlands, photodegradation is not expected and the main removal mechanisms are biodegradation, sorption, and plant uptake. The influent concentrations between the two sampling events are quite different for most of the compounds (NP, BPA, IBU, DCF, KFN) causing a possible impact on the removal efficiency.

NP was the only compound that was successfully removed in all the pilot systems at both sampling campaigns, which could be attributed to its easy biodegradation both at aerobic and anaerobic environment, while sorption onto particulate matter could also be important due to its hydrophobic nature. TCS was removed more than 80% in the aerated and unsaturated pilot wetlands at both sampling campaigns, which is also in line with other researchers (Ávila, Matamoros, et al., 2014; Ávila, Nivala, et al., 2014), indicating that aerobic biodegradation could be an important removal mechanism. As can be seen in the Annex "HYDRO1" (tab "d"), the effluent concentration of TCS in the saturated wetland was higher than the influent maybe due to



desorption that may have occurred after 1 year of operation, since TCS is a hydrophobic compound. However, the hybrid wetland also seemed promising at the second campaign. Aerobic conditions seem to enhance the removal of BPA through aerobic biodegradation, since the average removal efficiencies were 79% ( $\pm 5\%$ ) in aerated and 75% ( $\pm 13\%$ ) in unsaturated wetland, respectively. The enhanced removal of BPA in unsaturated and aerated vertical flow wetlands has also been reported by other researchers (Ávila, Matamoros, et al., 2014; Ávila, Nivala, et al., 2014). The high fluctuations that were recorded in the first campaign need further investigation.

IBU is readily biodegraded under aerobic conditions with average removal efficiencies more than 70% in both aerated and unsaturated system in the second campaign and 69% in unsaturated system at the first campaign, which is in accordance with other researchers (Ávila, Nivala, et al., 2014; Kahl et al., 2017). The low removal efficiency of the aerated system at the first campaign was not expected and needs further investigation, while the hybrid system also seemed effective at the second campaign. This combination (aerobic-anaerobic) was proved effective also in the literature (Ávila, Matamoros, et al., 2014). NPX was moderately removed by the aerated and unsaturated wetland since the average removal efficiencies that were recorded at the second sampling period were  $67\% \pm 14\%$  and  $59\% \pm 18\%$ , respectively, while the performance was lower at the first period which was not expected. Complete removal was noticed by the saturated wetland and moderate removal by the hybrid system ( $64\% \pm 12\%$ ) at the second campaign which indicates that biodegradation of NPX could be high in anaerobic conditions (Monsalvo et al., 2014). During the second sampling campaign, both aerated and unsaturated wetland systems exhibited moderate to high efficacy in removing DCF, with removal rates of  $63\% \pm 15\%$  and  $82\% \pm 8\%$ , respectively. However, there were significant fluctuations in the removal rates during the first sampling campaign. A moderate removal of DCF (approximately 50%) was recorded in an unsaturated vertical flow wetland by Nowrotek et al. (2016) but the initial concentration was much higher (5 mg/L). In the study of Ávila et al. (2014) the removal of DCF ranged from 54-70% under aerobic environment in unsaturated and aerated vertical flow wetlands, depending on the bed and similar results obtained also by Kahl et al. (2017). In contrast to these findings, poor removal was observed for DCF in an aerated VF system by Auvinen et al. (2017). Even though high redox conditions are favorable for the removal of DCF, other mechanisms like reductive dehalogenation could be the predominant removal mechanism in anaerobic conditions (Ávila et al., 2015). This could be an explanation for the enhanced performance of hybrid wetland towards DCF ( $78 \pm 16\%$  at the second campaign). As far as the elimination of KFN is concerned, it was very low at the first campaign and moderate, ranging from 40%-63%, at the second for all the tested systems.

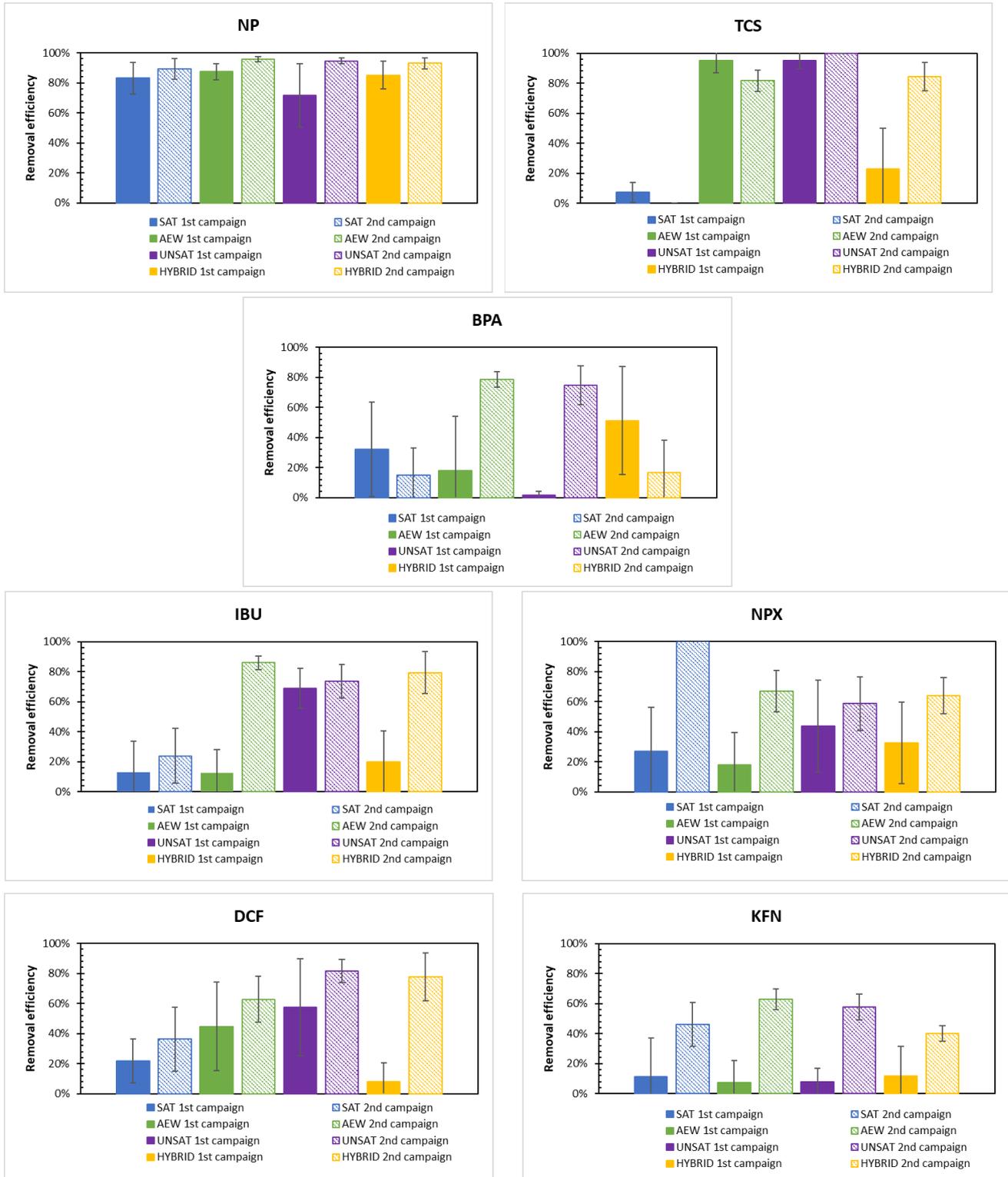
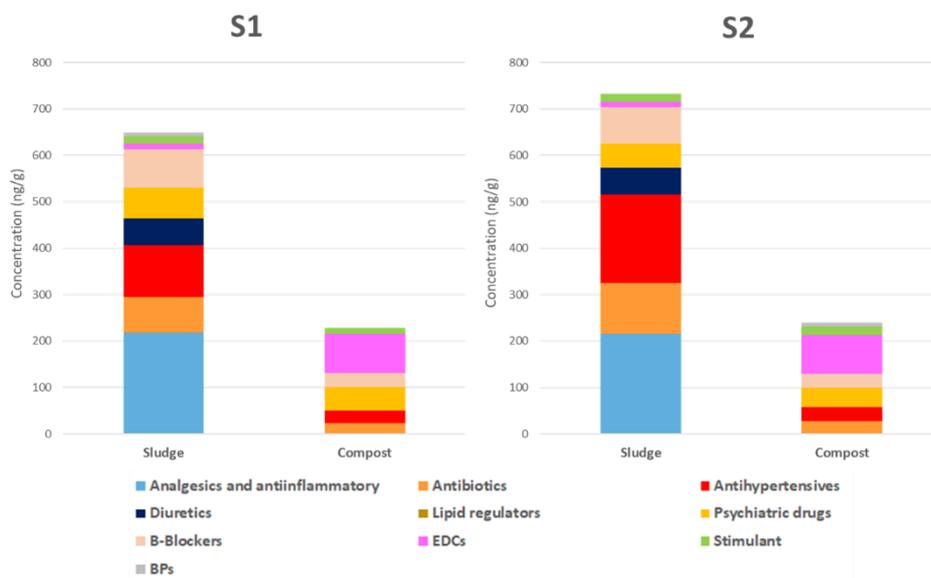


Figure 3.4: The performance of the four pilot intensified wetland systems in Lesvos Island in terms of the average removal efficiency of the target micropollutants for the two sampling campaigns.

### 3.2.6-Sludge and compost

A total of 36 and 37 compounds were analyzed in the sludge and compost samples, respectively during two sampling campaigns: S1 (February 2023) and S2 (March 2023). The recoveries were in a range of 20 - 128% for the sludge, and 20 – 119% for the compost, while the detection limits varied between 0.3-17 ng/g (sludge) and 0.1-16 ng/g. The list of analytes, their concentrations and the corresponding analytical parameters can be found in the annex "HYDRO1", tabs "e" and "f".

The sludge exhibited the occurrence of 21 (S1) and 22 (S2) analytes, while less compounds were detected in the compost (14 and 15 in S1 and S2, respectively). Moreover, a lower total OMP concentration was observed in the compost at both samplings (Figure 3.5). The analgesics and anti-inflammatories, which accounted for 33% and 29% of the total OMP mass in the sludge (in S1 and S2, respectively), were not detected in the compost samples. Diclofenac and ibuprofen, both detected in the sludge, are susceptible to biodegradation during composting processes, and removals of up to 99.9% had been previously reported (Butkovsky et al. 2019). As a particular case, methylparaben (EDC) showed higher concentrations in the compost samples at both campaigns. Other classes of OMP such as antibiotics, antihypertensives, and  $\beta$ -blockers were also detected at higher concentrations in the sludge than in the compost, with greater levels in S2 (March). The pattern of pharmaceuticals detected in the sludge is in line with the results found along HYDRO1 (section 3.2.1-4). The reduction in the levels of some compounds such as atenolol or irbesartan in the compost, which have shown some persistence throughout the wastewater treatment, is noteworthy. The observed decrease in the concentrations of these compounds in the composted sludge suggests the degradation of residual OMP, thus contributing to reducing the risk of soil contamination, when these materials are applied for fertigation of agricultural soils.

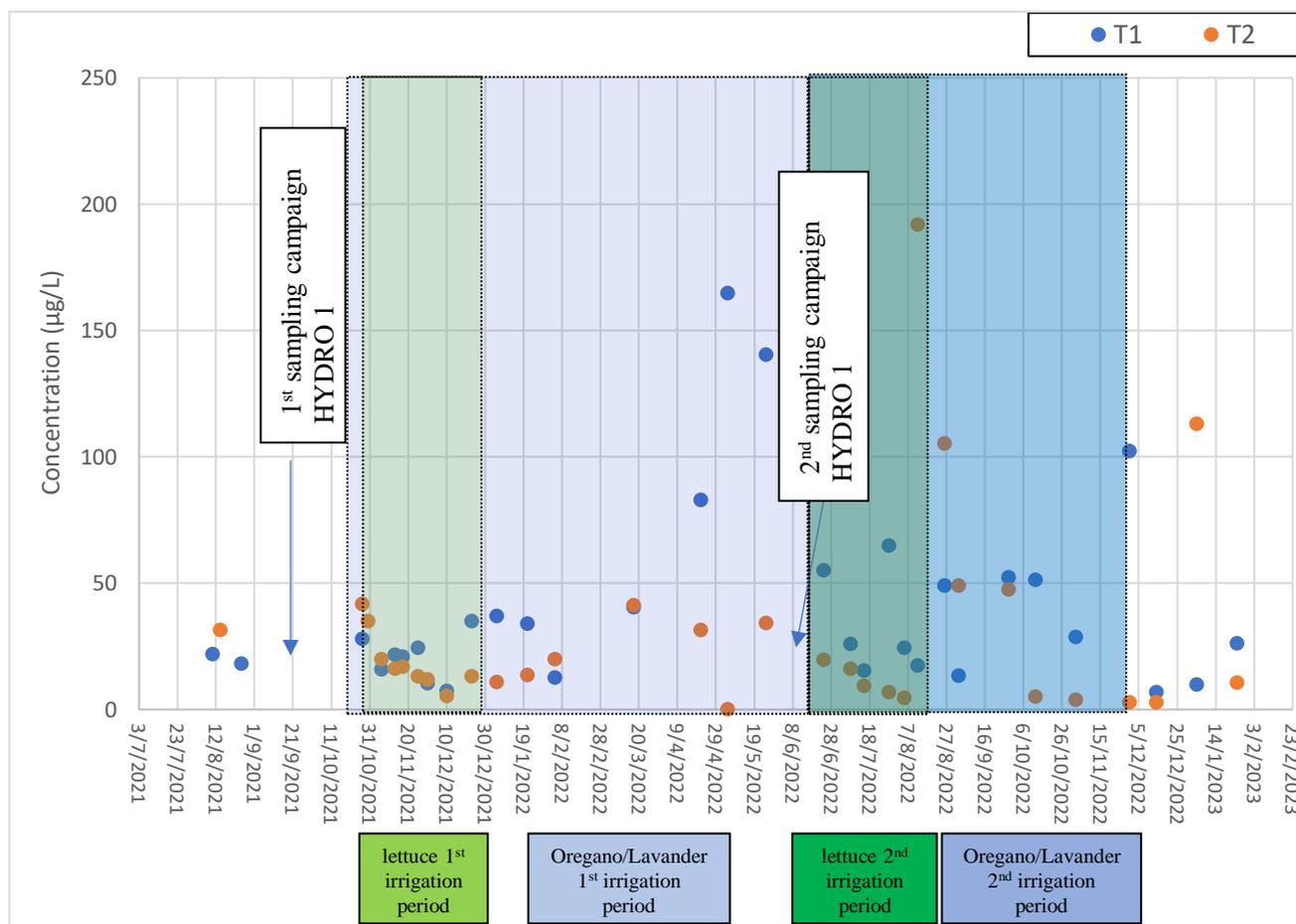


**Figure 3.5: Total concentrations of pharmaceuticals (PhACs) and endocrine disrupting compounds (EDCs) in the sludge and compost samples in the first (S1) and second (S2) campaign.**

### 3.3 HYDRO2

The final effluents of the HYDRO1 treatment system (T1) were monitored periodically and compared with effluents of the alternative treatment consisting in the same system without the wetlands step (T2). This continuous sampling allows direct comparison of the levels of OMP in the water used for the irrigation of crops in HYDRO2. To be highlighted, as mentioned in section 2.3, that T2 water had a much longer retention time than T1 water (a few days to 1.5 months, depending on the season) in the final tank before its use for crops irrigation (and sampling). Most likely degradation and/or sorption of emerging pollutants occurred in the tank itself as well as water evaporation before T2 water use for irrigation. Consequently, water characterization is not meant here to compare treatment technologies schemes but to relate micropollutants occurrence in irrigation water with occurrence in soils and crops, as reported in the corresponding Deliverable 4.6 (Report on food safety issues and pest control).

Individual concentrations of PhACs and EDCs in the irrigation waters along the monitoring time are shown in Annex "HYDRO2". Figure 3.6 depicts the total concentrations of the OMP across the sampled periods.



**Figure 3.6: Total concentrations of pharmaceuticals (PhACs) and endocrine disrupting chemicals (EDCs) in the irrigation water obtained after the treatment including wetlands (T1) and the treatment excluding wetlands (T2). Different tank retention times were applied for T1 and T2 water, before their use for irrigation, and sampling.**

Despite the similar final concentrations in the finished water after the 2 treatments (mean concentration of daily sums of OMPs in T1 was 39,501 ng/L and in T2 was 41,713 ng/L) (see annex "HYDRO2"), different patterns



in terms of OMP concentrations were observed; some compounds were found at higher concentration levels in T1 irrigation waters compared to T2 irrigation waters: e.g. irbesartan (3,917 to 1,261 ng/L), valsartan (5,851 to 4,768 ng/L), clindamycin (4,804 to 3,445 ng/L), and diclofenac (1,475 to 684 ng/L). On the other hand, some compounds have lower mean concentrations in the waters of the outlet of conventional treatment (T1) than in the alternative treatment (T2), as it is the case of 2-OH-Ibuprofen (not detected and at 5,475 ng/L in T1 and T2, respectively) and ketoprofen (288 to 1,119 ng/L in T1 and T2, respectively). In the case of EDCs, no major differences were observed between T1 and T2 irrigation waters. Further studies in risk assessment are required to study the differences of impact in the environment and usages of the reclaimed water through the two systems. Then, the best route could be determined in terms of implementation costs and removal of the OMPs systems. Then, the best route could be determined in terms of implementation costs and removal of the OMPs.

### 3.4 HYDRO3

In the first sampling campaign performed in the HYDRO 3 rainwater tanks, 13 PhACs were detected, with only four exhibiting concentrations above the quantification limit. In the second sampling campaign, a lower occurrence of PhACs was found (seven compounds were detected), but 5 were at quantifiable concentrations. Full data about pharmaceutical occurrence is reported in annex "HYDRO 3" (tabs "a" and "b"). Overall, a higher total concentration was detected in the second campaign, with a notable presence of analgesics and anti-inflammatories. These substances, known from their moderate to high adsorption capacity and low half-lives in soils, have been widely reported as soil pollutants, suggesting that their occurrence in the tanks could be linked to the transfer from the soil. As for the EDCs, only the stimulant agent caffeine was detected, in tank 2, in the first campaign and three of them in the second sampling campaign: 2 ng/L for caffeine, 46 ng/L for propylparaben, and 1,351 ng/L for BPA. Although the detected concentration is high, the presence of BPA in the water is not unexpected, as bisphenols are substances commonly found in agricultural areas, and they exhibit moderate to high mobility in the soil (Kodesova et al., 2023; Xu et al., 2021). Full data about EDCs occurrence is reported in annex "HYDRO 3-6" (tab "a" and "b").

### 3.5 HYDRO4

For HYDRO4 demo site the four sampling points were the rainwater storage tank (sampling point A), the storage tanks "T2" (sampling point B), the stormwater open tank (sampling point C), and the aquifer recharge (sampling point D). Full data is reported in the annex "HYDRO 4" (tab "d"). In the first sampling campaign, 17 pharmaceuticals out of 55 were detected in A, B, or C, though most of them were below LOQ. The total PhACs concentration was low and corresponding to 57, 91, and 132 ng/L for A, B, and C, respectively. In the second sampling campaign a similar trend was observed, with 6, 10, and 20 detected compounds for sampling point A, B, and C, respectively, and in many cases below LOQ or at low concentration. The total concentrations were higher, 167 ng/L, 89 ng/L, 123 ng/L in sampling points A, B, and C, respectively. Higher levels were registered for the open tank (C), which was not unexpected since it collects urban stormwater from the upstream residential area.

As for the EDCs, only 4 analytes were detected in A, B, or C, all of them above their quantification limits: methylparaben, propylparaben, benzotriazole, and caffeine (Annex "HYDRO 3-6", tab "c"). Full data is reported in the annex "HYDRO 4", tab "d". Methylparaben was only detected in the first campaign, while



propylparaben was detected in both the first and the second campaigns. Due to their extensive use as preservatives (in food, cosmetics, pharmaceuticals, etc.), parabens are frequently reported as relevant pollutants in residential areas (Zhao et al., 2022). Benzotriazole was detected in tank B during both campaigns, and it was also found in tank A during the second campaign. Its occurrence can be associated with its use as a corrosion inhibitor in a wide variety of materials (i.e., rooftops materials).

Finally, also in the tank for aquifer recharge (sampling point D), only 7 PhACs were detected in the 1<sup>st</sup> sampling campaign and 12 in the 2<sup>nd</sup>. A lower overall occurrence was found in this tank when compared to those found in B and C. The highest concentration was 79 ng/L of irbesartan in the first sampling campaign and 1,262 ng/L of sulfamethoxazole in the second, the latter being a mobile substance in soil (Boy-Roura et al., 2018). No EDCs were detected in none of the two sampling campaigns for this sampling point.

### 3.6 HYDRO5

In the samples of freshwater produced from seawater in HYDRO5 (Mangrove Still System), only caffeine and six PhACs were detected in the first sampling campaign in most cases at trace concentration (annex "HYDRO 3-6", tab "e"), whereas bisphenol A (38 ng/L), benzotriazole (11 ng/L), and 4 pharmaceuticals were found in the second campaign (annex "HYDRO 3-6", tab "f"). Regarding the PhACs, analgesics and anti-inflammatories comprised most of the detections, with 2-OH-IBU showing the highest concentration (198 ng/L) and diclofenac having quantifiable concentrations in both campaigns. This class of PhACs has been extensively detected in surface waters, including seawater (Hernández-Tenorio et al., 2022). As for the EDCs, the limited concentrations measured in the second campaign could possibly be related to the use of plastic and metal materials in the HYDRO5 system.

### 3.7 HYDRO6

Fourteen pharmaceuticals and 3 EDCs (BPA, methylparaben, and caffeine) were detected HYDRO6 demosite samples (rainwater samples and effluent water of the reedbed system-greywater and urine treatment). Among the rainwater samples, some PhACs (mainly analgesics and anti-inflammatory drugs), and EDCs (mainly trace levels of caffeine and bisphenols) were observed in one of the rainwater cistern samples. As to the effluent water, the total PhACs and EDCs concentration was similar (782 ng/L and 821 ng/L in the 1<sup>st</sup> and 2<sup>nd</sup> loop, respectively). The 1<sup>st</sup> loop effluent water had a higher EDC content (539 ng/L, due mainly to benzotriazole with 492 ng/L). On the other hand, the 2<sup>nd</sup> loop effluent water had higher concentration of PhACs (690 ng/L) with the largest contribution made by ibuprofen (212 ng/L). Full data is reported in the annex "HYDRO 3-6", tab "g". As has been previously discussed for the other HYDROs, analgesics and anti-inflammatories have been reported widely in many water sources, including tap water. Though their presence in rainwater and their input into the 1<sup>st</sup> and 2<sup>nd</sup> loops is not fully clear, it is possible that activities such as tank cleaning with tap water, or specific actions such as mixing with other types of water (i.e., river water) could have influenced the levels found. On the other hand, the collection of rainwater through the roofs is expected to lead to benzotriazole levels (anticorrosion material), as discussed for HYDRO4. More confirmation would be needed in this context.



## 4. CONCLUSIONS

The increasing need for freshwater in the global change scenario has prompted the search of alternative freshwater sources such as wastewater (after appropriate treatment), rainwater harvesting, and desalination, among others. HYDROUSA studied the application of different water harvesting and wastewater reclamation systems in its 6 demonstration sites (HYDROs) in three Greek Islands. The fate of organic micropollutants (OMP) is considered in the present Deliverable due to their potential risks for the environment and human health. It must be noted that the analysis involved 293 samples, collected from the six HYDROs, (211, 72, 5, and 5 for water, soil, sludge, and compost, respectively). The results regarding pathogens along with other operational parameters are provided in Deliverable 5.1 \_Pilot Assessment Report.

In 2019, a preliminary sampling campaign was conducted in various sites from Greek islands. Representative pharmaceuticals active compounds (PhACs) and endocrine-disrupting compounds (EDCs) were prioritized and selected for the extensive sampling campaigns in the HYDROs from 2021 to 2023.

The HYDRO1 system was applied for wastewater reclamation (Lesvos Island, Greece) and two intensive sampling campaigns were performed in fall 2021 and summer 2022. Differences among the seasons in terms of contaminants and specific compound removal rates were observed but, globally, the total OMP removal of the coupled UASB-CW system of HYDROUSA HYDRO1 was high and similar in both seasons: 76% and 74% in fall and summer, respectively. Up to 22 and 15 OMPs were detected in the UASB sludge and in the compost, respectively, with a significant lower total OMP in the compost. Two sampling campaigns were also performed in the 4 parallel vertical sub-surface flow (intensified CWs), installed in parallel to HYDRO1. High removal rates, in particular for the saturated electroactive pilot, were observed during the 1<sup>st</sup> campaign, and at higher number of pulses (150) in the 2<sup>nd</sup> campaign.

HYDRO2 is an agroforestry system, irrigated with reclaimed wastewater. Along the irrigation period, the profile of analytes varied importantly with higher levels in summer and in line with the concentrations and removal rates observed in HYDRO1.

Overall, the results exhibited the presence of a variety of OMP in HYDRO1 and HYDRO2, with analgesics and anti-inflammatory drugs as the most detected classes. The results observed for water, sludge, and compost are favorable evidence for future applications in agriculture like the ones applied in HYDROUSA. However, the presence of residual contaminants points out the need to assess the potential risks associated with these contaminants in soils and crops, as addressed in Deliverable 4.6 (Report on food safety issues and pest control).

The OMP were searched also in the rainwater collector systems from HYDRO 3 and HYDRO4 (Mykonos Island), with few analgesics and anti-inflammatories and EDCs detected at trace concentrations. Similarly, few OMPs were detected in the mangrove still seawater desalination systems of HYDRO5 (Tinos Island). Finally, in the eco-tourist resort of HYDRO6 (Tinos Island), 14 pharmaceuticals and 3 EDCs were detected at least once in the wastewater reedbed reclamation system, fewer than in HYDRO1.



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