

UPWATER

UNDERSTANDING GROUNDWATER POLLUTION TO PROTECT AND
ENHANCE WATER QUALITY

DELIVERABLE D4.4

REPORT ON THE VALIDATION IN THE BESÓS SITE



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LIST OF ABBREVIATIONS

Abbreviation	Definition
ANSTO	Australian Nuclear Science and Technology Organisation.
AU	Aarhus University.
BR	Barcelona Regional Agencia Metropolitana de Desenvolupament Urbanístic d'infrastructures SA.
BW	Bioelectrochemical Wetland
CEC	Contaminant of Emerging Concern
Cl-	Chloride
CPS	Ceramic passive sampler
CSIC	Agencia Estatal Consejo superior de Investigaciones Científicas.
CW	Constructed Wetlands
DGT	Diffusive gradients in thin films
DOC	Dissolved organic carbon
EC	Electrical conductivity
Eh	Redox potential
EYDAP	Athens Water Supply and Sewerage Company.
FC	Future City Foundation
FNCA	Fundación Nueva Cultura del Agua.
FRM	Floating root mat
HAdV	Human Adenovirus
HEV	Hepatitis E Virus
IDAEA	Institute of Environmental Assessment and Water Research.
INERIS	Institut National de L'Environnement Industriel et des Risques.
IWW	Rheinisch-Westfälisches Institut für Wasserforschung gemeinnützige GmbH.
NH ₄ ⁺	Ammonium
NoV GII	Norovirus Genogroup II
NTUA	National Technical University of Athens.
TARH	Terra, Ambiente e Recursos Hídricos Lda.
UB	Universitat de Barcelona.
UPWATER	Understanding groundwater Pollution to protect and enhance WATER quality.
UWA	University of Western Australia



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EXECUTIVE SUMMARY

This document describes the outcome of Task 4.4 within the UPWATER project (Understanding groundwater Pollution to protect and enhance WATER quality, Horizon Europe project No 101081807). It summarizes the activities undertaken to validate passive sampling tools—namely Ceramic Passive Samplers (CPS), Diffusive Gradients in Thin Films (DGTs), and Virus Passive Samplers (VPS)—developed in WP2. These samplers were deployed across selected monitoring wells in the Besòs river catchment during six monitoring campaigns conducted between 2023 and 2025. The goal was to assess the occurrence and spatial-temporal variability of emerging pollutants, metals, and viruses in groundwater. In parallel, the performance of a Nature-Based Solution (NBS) pilot plant—developed under Task 4.1—was optimized and evaluated. The system was operated under three hydraulic loading rates (HLRs: 200, 400, and 800 mm/day) and across two seasons (warm and cold) to assess its capacity to attenuate nutrients and emerging contaminants. Results revealed the presence of multiple emerging pollutants, metals, and viruses in the groundwater, with concentrations strongly influenced by factors such as proximity to wastewater treatment plant (WWTP) discharge points, distance from the river main channel, well depth, and potential leakage from sewer infrastructure. These observations suggest that treated wastewater effluents are likely a significant source of groundwater contamination in the study area. The NBS pilot consisting only of biochar achieved >80% removal of most abundant emerging pollutants at an HLR of 400 mm/day during the warm season. In colder conditions, similar attenuation levels were achieved only at a reduced HLR of 200 mm/day, highlighting the impact of seasonality and hydraulic loading on treatment performance. Passive samplers deployed within the NBS system for one month confirmed high pollutants removal efficiencies: over 80% for emerging pollutants, and substantial reductions in metals (60-80%) and viruses (2-3 log units). These findings demonstrate the potential of combining passive sampling technologies with NBS as a robust and sustainable strategy for monitoring and mitigating groundwater pollution by emerging contaminants, metals and viruses.



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1. MONITORING OF GROUNDWATER QUALITY

The sampling campaign strategy was followed according to the monitoring plan from Deliverable 2.1. Figure 1 shows the different sampling points in the Besòs case study. In every sampling point, two ceramic passive samplers (CPS) and two diffusive gradients in thin films (DGT) were installed. Grab samples were taken to enable comparison with passive sampling. A total of six sampling campaigns were carried out lasting from July 2023 to April 2025.

In addition, ten sampling campaigns with Virus passive samplers (VPS) were conducted between September 2023 and March 2024 in the wells *SAP-2b* at 3 m depth and *SAP-1* at 11m depth. Each VPS contained three membranes.

To evaluate the feasibility of coupling CPS with compound-specific isotope analysis (CSIA) for selected emerging pollutants, three additional targeted campaigns were conducted. These campaigns employed a modified CPS configuration, as detailed in Section 1.2, and the samplers were deployed in duplicate at one river location (downstream) and three groundwater wells (*SAP-2b*, *ADS6.1*, and *Well R*), following a transect aligned with the groundwater flow. The first of these campaigns coincided with the sixth deployment of standard CPS (February 2025), while the remaining two were conducted between March and June 2025. Large-volume grab samples were also collected from the same locations to compare isotopic signatures obtained via passive sampling and conventional methods.



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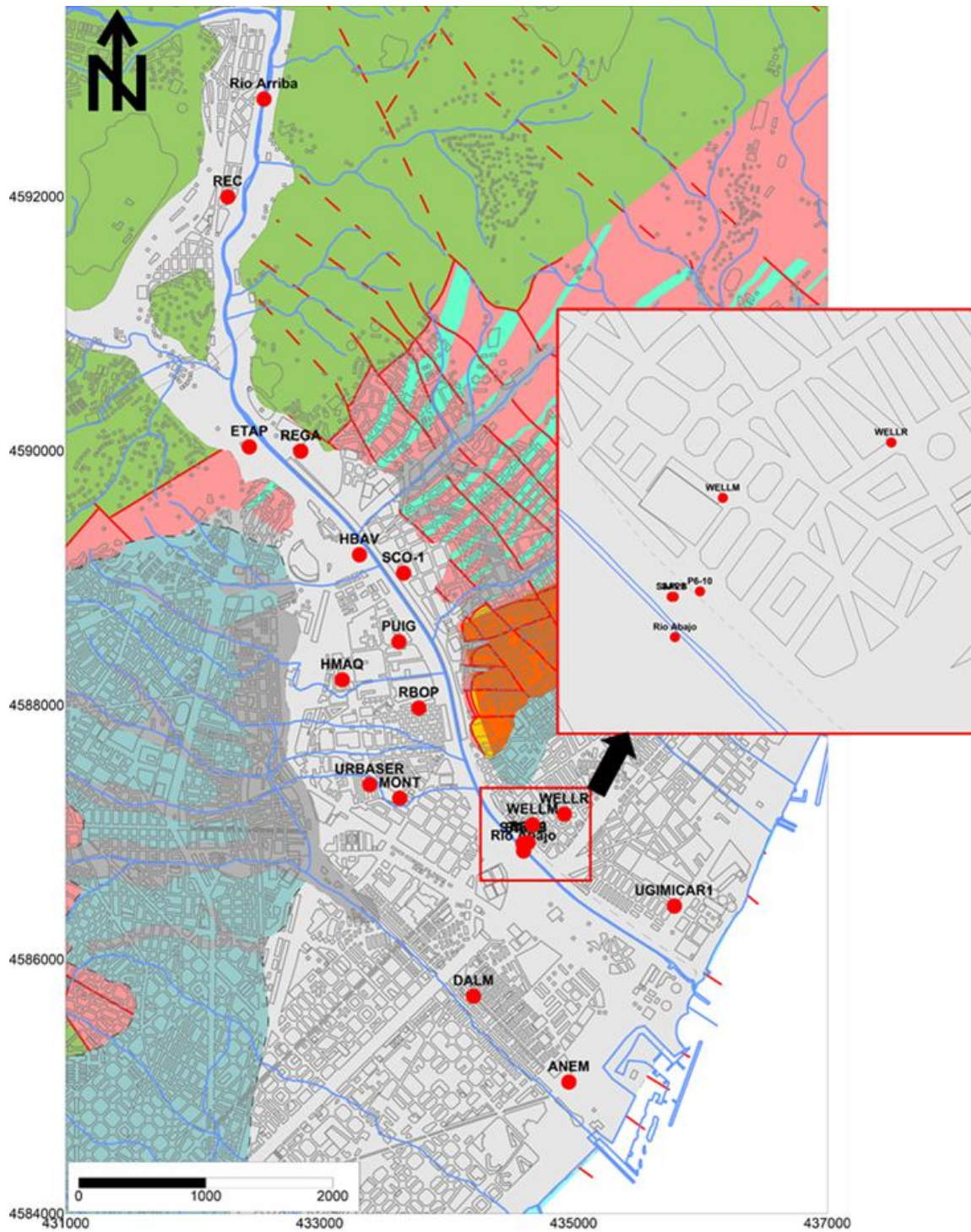


Figure 1. Map of the Besòs (Spain) GW sampling points (red).



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1.1 OCCURRENCE OF GENERAL WATER QUALITY PARAMETERS

The UPWATER sampling campaigns measured a large range of different water quality parameters, all of which were comprehensively specified in UPWATER deliverable 2.1 ‘Monitoring Plan’. The occurrence of some of the general water quality parameters in the six UPWATER sampling campaigns in the Besós is summarized in the following figures, that show electrical conductivity (EC), the redox potential (Eh) and the concentration of sulphate (SO_4^{2-}), nitrate (NO_3^-) and DOC. The EC and the Eh may be understood as indicators of pollution where a high EC and a low Eh indicate a higher level of pollution. Across the UPWATER sampling campaigns, *SCO-1* showed consistently the lowest EC of all sampling points (Figure 2). The upstream sampling points *ETAP*, *REGA*, *SCO-1* and *MONT* had a rather stable EC across all sampling campaigns (Figure 2). For the majority of the remaining sampling points, lower ECs were measured for UPWATER campaigns 3-6 (after May 2024), relative to campaigns 1 and 2 (before May 2024), which may have been caused by increased dilution of the contaminants due to the heavy rainfall in the period of May 2024, after a prolonged period of severe drought. Campaign 6 showed selected elevated EC for *RBOP*, *HMAQ* and *HBAV* (Figure 2). The EC generally reflected the changes in the concentration of chloride, except for at the well *UGUIMICAR* in the first campaign (Figure 2).

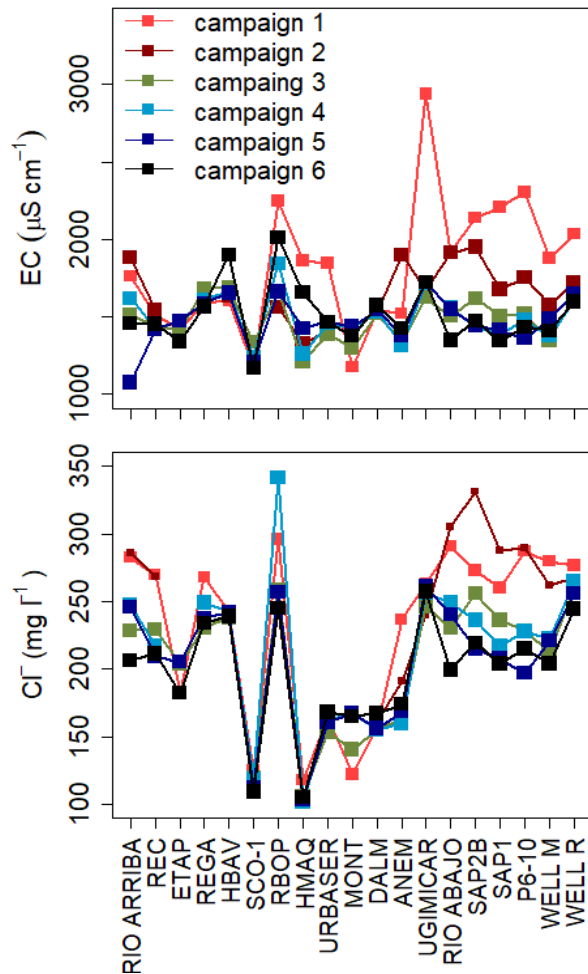


Figure 2. Variation of the electrical conductivity and concentration of chloride in the six UPWATER sampling campaigns.



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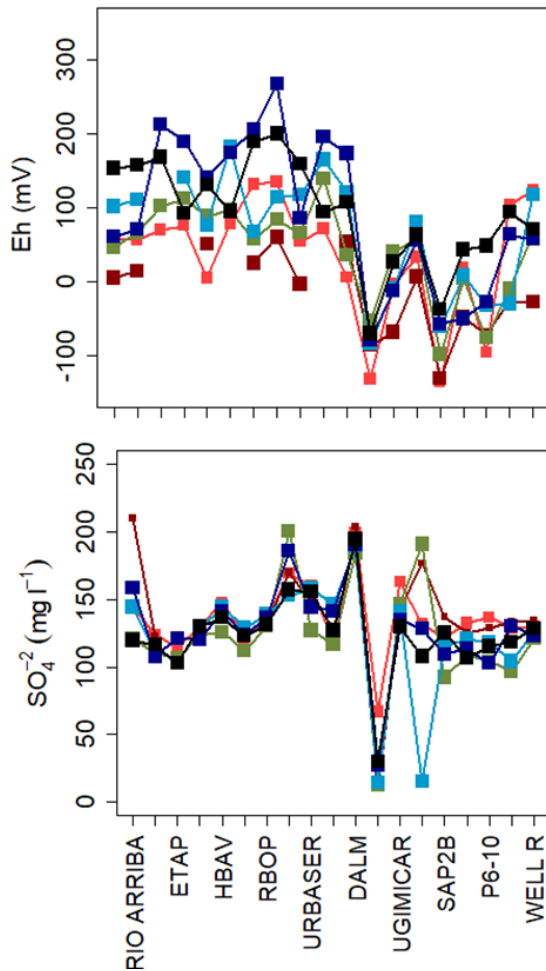


Figure 3. Variation of the redox potential and the concentration of sulphate in the six UPWATER sampling campaigns.

sulfate and nitrate concentrations, although a few sampling points, such as *SAP2B*, *SAP1*, *RIO ABAJO*, *RIO ARRIBA* showed consistent trends over time (Figure 5).

The variations in Eh and sulfate and nitrate concentrations across the UPWATER sampling points in the Besós were generally consistent throughout the sampling campaigns (Figures 3 and 4), with more variation observed for Eh, a more difficult parameter to measure (Appelo and Postma 2007). An exception is the sulfate concentration in *RIO ABAJO* where the fourth sampling campaign, as opposed to the remaining campaigns, showed a very low sulfate concentration (Figure 3). A low Eh was consistent with low sulfate and nitrate concentrations and vice versa (see points *HMAQ*, *DALM*, *ANEM*, *SAP2B*), in line with standard redox theory (e.g., Appelo and Postma 2007). In *ANEM*, *SAP2B* and *P6-10* the oxidation of nitrate to ammonium under anaerobic conditions, i.e., low Eh, is clearly visible (Figures 3, 4).

The Eh was higher in the upstream wells and lower in the downstream wells, as hypothesized. The lowest Eh were measured in the wells *ANEM*, *SAP2B* and *P6-10* (Figure 3), indicating a lack of dissolved oxygen and polluted GW. The severe pollution of *ANEM*, *SAP2B* and *P6-10* was also reflected in the elevated DOC load of these wells (Figure 5). For *ANEM* the likely cause for elevated DOC is sewer leakage. *SAP2B*, being in very close proximity to the river (Figure 1), is likely impacted by the river's high loads of DOC (*RIO ABAJO*; Figure 5). *P6-10* may likely receive a mix of the two. Low redox conditions can lead to the release of harmful chemicals that may negatively impact the GW ecosystem.

The concentration of DOC was generally low across sampling points (Figure 5). More variability between sampling campaigns was observed relative to the



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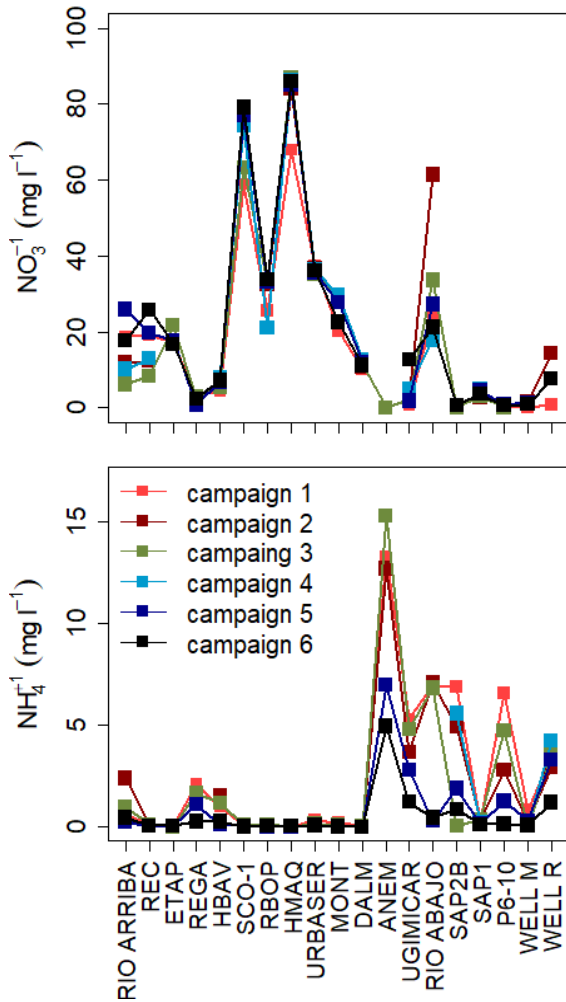


Figure 4. Variation of the concentration of nitrate and ammonium in UPWATER sampling campaigns. Concentrations shown as zero are below the detection limit. Ammonium samples from campaign 5, 6 and most of campaign 4 suffered a freezer failure and had to be omitted.

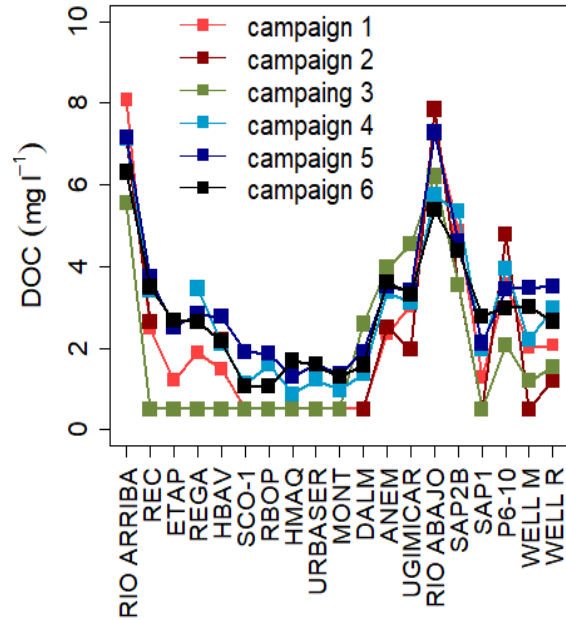


Figure 5. Variation of the concentration of DOC in the six UPWATER sampling campaigns. Concentrations shown as 0.5 are below the detection limit of 1 mg/L.



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1.2 ORGANIC POLLUTANTS

In the six sampling campaigns carried out in and around the river Besòs, CPS were deployed in 14 groundwater wells and 2 river locations, upstream and downstream. Grab sampling was carried out in parallel in the same locations, plus 2 wells which do not support the installation of CPS.

CPS were deployed in the field for a period of 14 days and were then brought to the lab. Extraction was carried out through three 10-minute cycles of sonication in 10 ml of methanol (MeOH), followed by centrifuging, evaporation and reconstitution in 0.4 ml of 50% water and 50% MeOH. Internal standards were added, and the extract was analyzed through targeted HPLC-MS/MS. Two similar methods were developed, and each sample was run twice in the positive and negative ionization mode.

Grab samples were filtered and collected in falcon tubes on the day on which CPS were installed. 20 ml of sample was evaporated with a Vacuum-assisted evaporator (VAE) (BÜCHI™ SyncorePlus, Switzerland), for 4 hours at a temperature of 55 °C. The bottom part of the evaporation tube was kept cooled at a temperature of 0 °C throughout the evaporation, where an approximate volume of 0.3 ml of concentrated water is collected at the end of the cycle. It was then brought to a volume of 0.5 ml with MeOH. Internal standards were added and the extract was centrifuged and then collected, to be analysed with the same HPLC-MS/MS methods used for the CPS.

CPS have to be calibrated for quantification purposes. Because each contaminant diffuses at a different rate, calibration experiments were carried out a priori (WP2) to determine said diffusion rates for a total of 60 contaminants of emerging concern (CEC). CEC are compounds of various origins and uses, including pesticides, pharmaceuticals, artificial sweeteners, stimulants, plasticizers, flame retardants and related transformation products.

Between the 2nd and the 3rd campaign, several challenges that were observed in the 1st campaign were tackled and solved. In particular, the sorbent was changed from 300 mg of OASIS HLB to a mixture of 300 mg of OASIS HLB and 100 mg of PoraPak Rxn RP, expanding the number of compounds that can be measured from 41 to 59. In particular, compounds with a higher polarity such as artificial sweeteners could be quantified from the 3rd campaign and onwards. Issues related to loose caps were solved, improving the CPS robustness and preventing them from losing sorbent in the following campaigns.

A comparison between the 2nd sampling campaign (March-April 2024) and the 3rd (May-June 2024) shows a significant reduction in the total concentration of contaminants detected, for 7 out of the 9 common sampling points between the 2 campaigns (Fig. 6). This could be due to the use of a different sorbent, with a higher uptake of more polar contaminants and therefore more accurate calibration. Alternatively, it could be linked to increased dilution of the contaminants due to the heavy rainfall in the period of May 2024 after a prolonged period of drought, a trend that is also reflected in the EC (Fig. 2).



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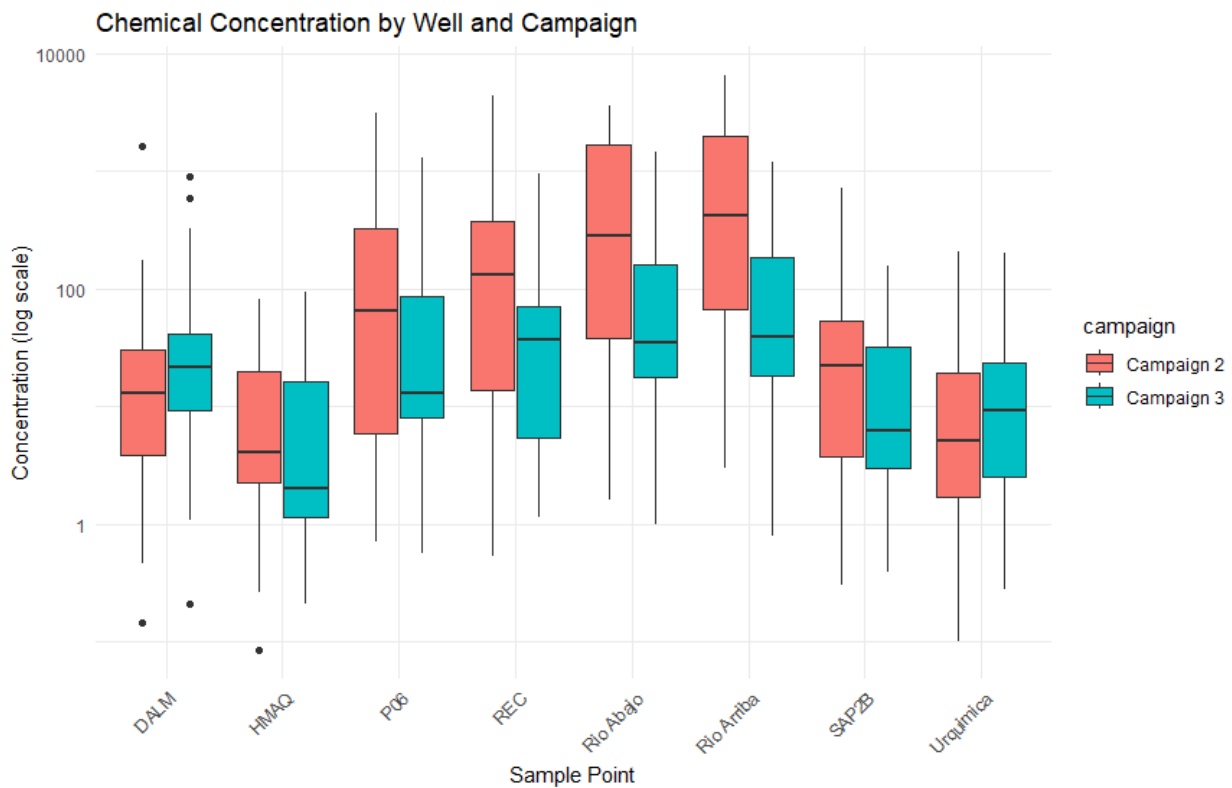


Figure 6. Boxplot with the distribution of the concentrations of 32 chemicals (2,4-Dichlorophenol, 2,4-Dichlorophenoxyacetic Acid, Acetaminophen, Atrazine, Bisphenol A, Bisphenol S, Caffeine, Carbamazepine, Cotinine, DEET, Desisopropylatrazine, Diclofenac, Diuron, Fipronil, Fipronil desulfinyl, Fipronil sulfide, Fipronil sulfone, Hexamethoxymethylamine, Imidacloprid, Isoproturon, MCPA, Mecoprop, Metoprolol, O-desmethylvenlafaxine, Simazine, Sulfamethoxazole, Tebuconazole, Tramadol, Trimethoprim, Valsartan, Valsartan acid and Venlafaxine) measured with CPSs in campaigns 2 and 3 (March-April and June 2024) in 7 groundwater wells and 2 river sampling locations. The y axis is expressed as the log of the contaminant concentration in water, ng/L. Artificial sweeteners (Acesulfame K, Cyclamate and Saccharine) are excluded.

The 6th sampling campaign in the river Besòs (February 2025) included an *intensive sampling* in 2 of the wells (HMAQ and Well M). The CPS were deployed for 14 days (11-25 February 2025) and grab samples were collected 7 times during this period (February 11, 12, 14, 18, 20, 24 and 25).

The goal was to compare the concentration of contaminants measured through CPS, which is an averaged value by nature, with the time-weighted average concentration of the contaminants as measured through grab sampling.

The results are presented in Figure 7, 8 and 9, for the contaminants that were present and detected with both methods. HMAQ showed significantly lower contamination than Well M.



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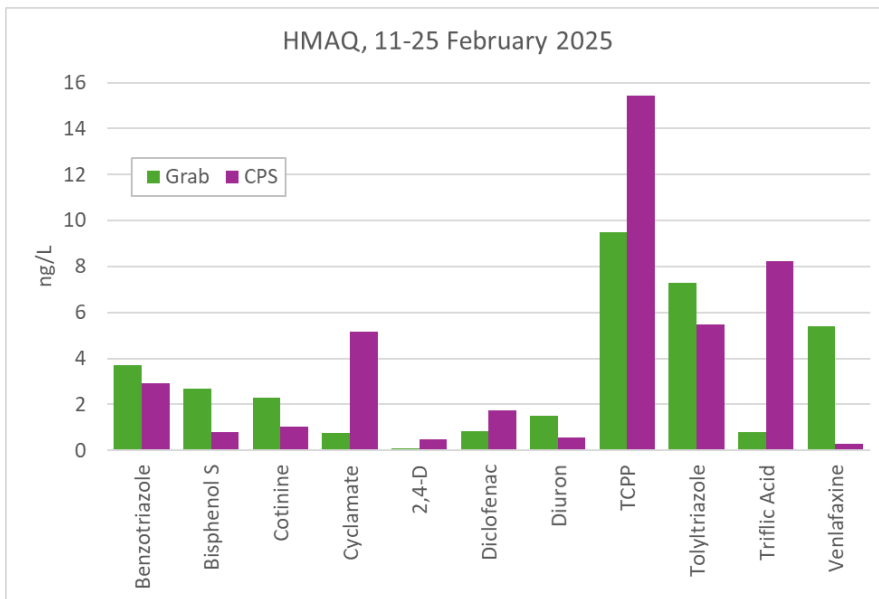


Figure 7. Average concentration of contaminants in well HMAQ in the period 11-25 Feb 2025 measured through CPS (purple) and grab sampling (green).

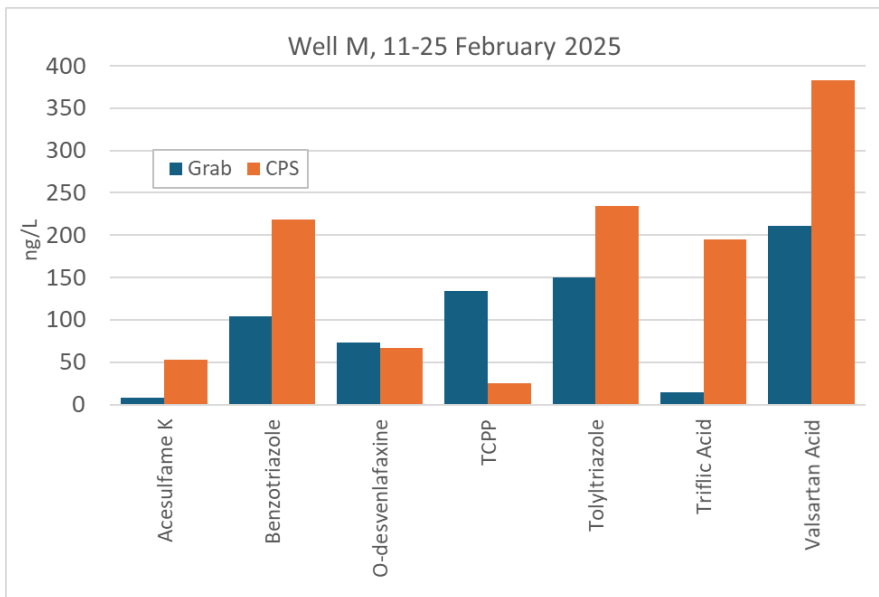


Figure 8. Average concentration of contaminants in Well M in the period 11-25 Feb 2025 measured through CPS (orange) and grab sampling (blue). The compounds shown are present in concentrations above 50 ng/L (ppt).



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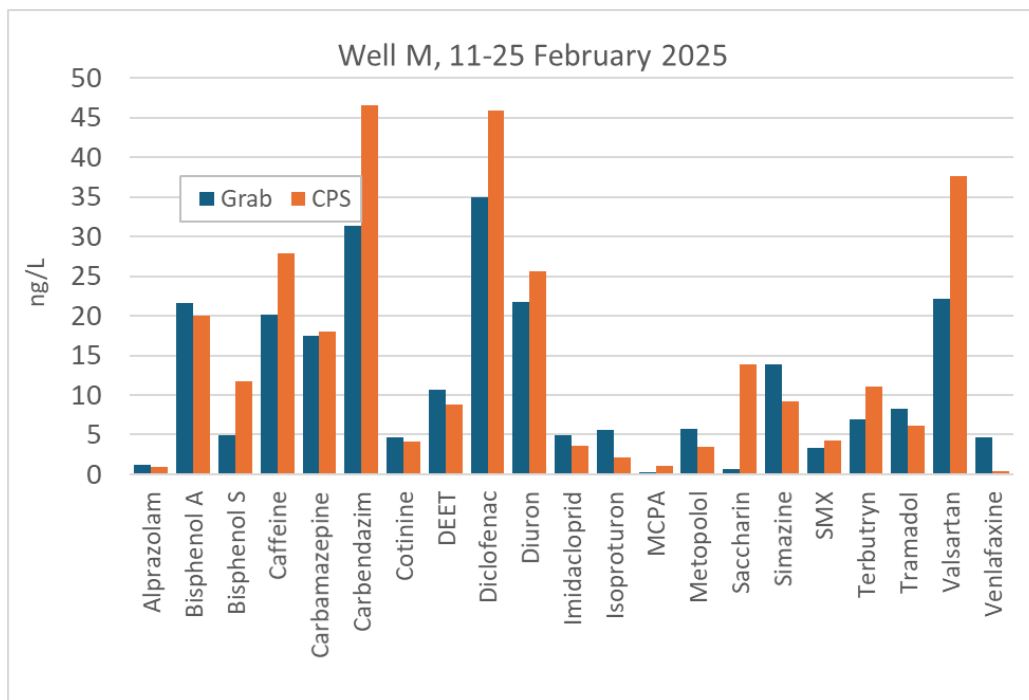


Figure 9. Average concentration of contaminants in Well M in the period 11-25 Feb 2025 measured through CPS (orange) and grab sampling (blue). The compounds shown are present in concentrations below 50 ng/L (ppt).

The results show a strong similarity in concentrations between grab and composite sampling (CPS); however, some differences were observed for certain compounds. These variations may be attributed to matrix interferences in grab samples or natural groundwater variability. Overall, the findings suggest that composite sampling provides more reliable values, which may better represent actual groundwater concentrations compared to grab sampling.

1.1.1. CPS-CSIA APPROACH

The approach for coupling CPS with CSIA was based on the configuration used in the 1st and 2nd UPWATER campaigns, with two key modifications: (1) the sorbent mass inside the cylindrical ceramic membrane was increased from 200-300 mg to 1 g of OASIS HLB; and (2) the deployment duration was extended from 2 weeks to 5 weeks.



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To support this new configuration, two specific calibration experiments were conducted to determine diffusion rates and assess potential isotopic fractionation for selected CECs. The CECs included in these experiments were: 1H-benzotriazole, 5-methyl-benzotriazole, diclofenac, lamotrigine, ibuprofen, carbamazepine, caffeine, 2-(4-chlorophenoxy)-propionic acid (4-CPP), dichlorprop, mecoprop, and 2,4-dichlorophenoxyacetic acid (2,4-D). Due to delays in CPS production and increased demand for groundwater and wetland monitoring, these calibration experiments were postponed until June 2025. The experiments lasted five weeks, and sample analysis is currently ongoing.

Emerging pollutants are extracted from the modified CPS using polypropylene cartridges with polyethylene frits in which the sorbent recovered from the CPS is introduced, dried and, finally, the organic contaminants are eluted.

The development of CSIA analytical methods (i.e., derivatization-GC-IRMS for carbon and nitrogen isotope analysis) for selected emerging pollutants (1H-benzotriazole, 5-methyl-benzotriazole, diclofenac, 4-CPP, dichlorprop, mecoprop, and bisphenol A) was initially described in Deliverable D2.4. These methods have since been further optimized and expanded to include additional CECs not originally planned, such as lamotrigine, ibuprofen, carbamazepine, caffeine, 2,4-D, paracetamol, and naproxen. Two derivatization techniques are currently available: methylation with trimethylsulfonium hydroxide (TMSH) and silylation with N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA). When combined with multiple elution steps using different solvents, these methods allow for the analysis of a broad range of emerging pollutants with varying polarities.

Large-volume grab samples (20 L) are processed using solid-phase extraction (SPE) with 1 g OASIS HLB cartridges. The SPE method, originally developed for small volumes (see Deliverable D2.2), has been scaled up and adapted for the expanded list of emerging pollutants. The large-volume SPE-derivatization-GC-IRMS methods have been validated across different water matrices (e.g., MilliQ water, distilled water, tap water, WWTP effluent), with sample volumes ranging from 1 to 10 L and emerging pollutant concentrations from 1 to 10 µg/L. The results showed no isotopic fractionation associated to extraction for most of the target emerging pollutants (Figure 10). For large volume extractions (up to 10 L) of the tested water matrices, high recoveries were obtained for target CECs using one cartridge filled with 500 mg or 1g of OASIS HLB. However, some tests performed with real groundwater samples from the Besòs case study resulted in lower recoveries than expected for some of the target CECs due to a breakthrough effect. To avoid this, we thus decided to change the approach for real samples. The final approach consists in the extraction of 4 times 5L-aliquots with 1g of OASIS HLB for each 20L-water sample.



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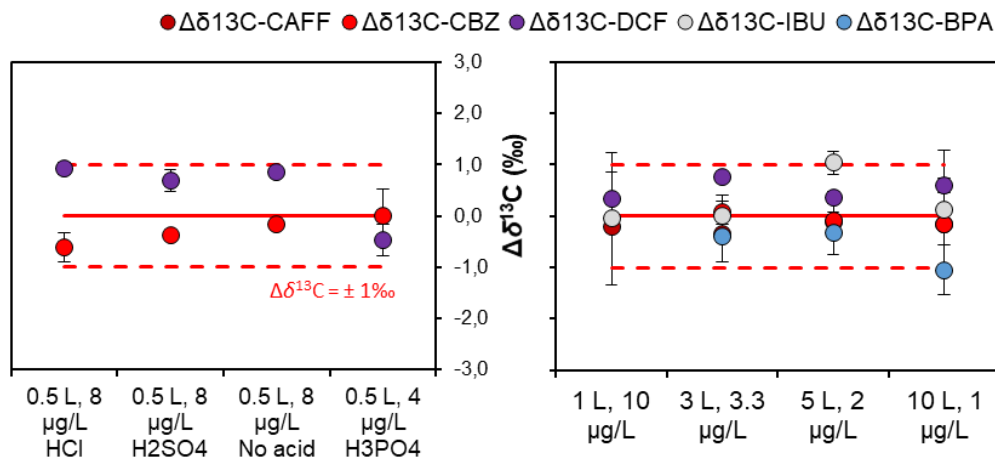


Figure 10. Extraction test spiked with selected emerging pollutants of known isotopic composition to assess potential carbon isotopic fractionation during extraction using Oasis HLB: Effect of water acidification during SPE-methylation-GC-IRMS (left), and effect of sample volume at a fixed load mass during SPE-silylation-GC-IRMS (right). Selected emerging pollutants: Caffeine (CAFF), Carbamazepine (CBZ), Diclofenac (DCF), Ibuprofen (IBU) and Bisphenol A (BPA).

The extracts from the CPS deployed for 5 weeks at the downstream river location (*RIO ABAJO*) and the three groundwater wells (*SAP2bis*, *ADS6.1*, *Well-R*) during the first additional CPS-CSIA campaign have already been analyzed by derivatization-GC-MS. As anticipated (see Deliverable D2.4), the low concentrations of emerging pollutants in Besòs groundwater resulted in insufficient peak amplitudes for GC-IRMS analysis. Another possible explanation is analyte breakthrough from the sorbent due to the extended deployment time. The ongoing calibration experiments are expected to provide further insight. It is also important to note that these three campaigns coincided with varying precipitation regimes, which may have influenced the results. Particularly, the wetter first (118 mm accumulated rainfall during the deployment period) and third (82 mm) campaigns may have resulted in dilution of the emerging pollutants. The second campaign, however, occurred during a very dry period (5 mm).

These findings suggest that, under the tested conditions, the CPS-CSIA approach may not be suitable for low-concentration samples of the Besòs groundwater, although results from the second and the third campaigns are not available yet and the ongoing calibration experiments are expected to clarify the method limitations and guide future improvements. Consequently, further efforts have been directed toward evaluating this methodology in the Besòs pilot plant, where higher emerging pollutant concentrations are expected (see Section 2.2).



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As an alternative approach, large-volume grab samples (20 L) collected from the same locations where CPS were deployed were extracted and analyzed using derivatization-GC-MS. Preliminary results showed promising options for subsequent CSIA of certain emerging pollutants, particularly diclofenac. However, further optimization is needed to address matrix effects and ensure reliable isotopic measurements before analysis by GC-IRMS. As an example of such limitation, Figure 11 presents a methylation-GC-MS chromatogram from an extract obtained via SPE of one of the large-volume grab samples from Besòs groundwater. To improve performance, the SPE protocol was adjusted to involve four separate 5 L extractions, followed by merging of the eluates. Current efforts also focus on testing different chromatographic columns and developing cleanup procedures to isolate target compounds and minimize matrix interferences.

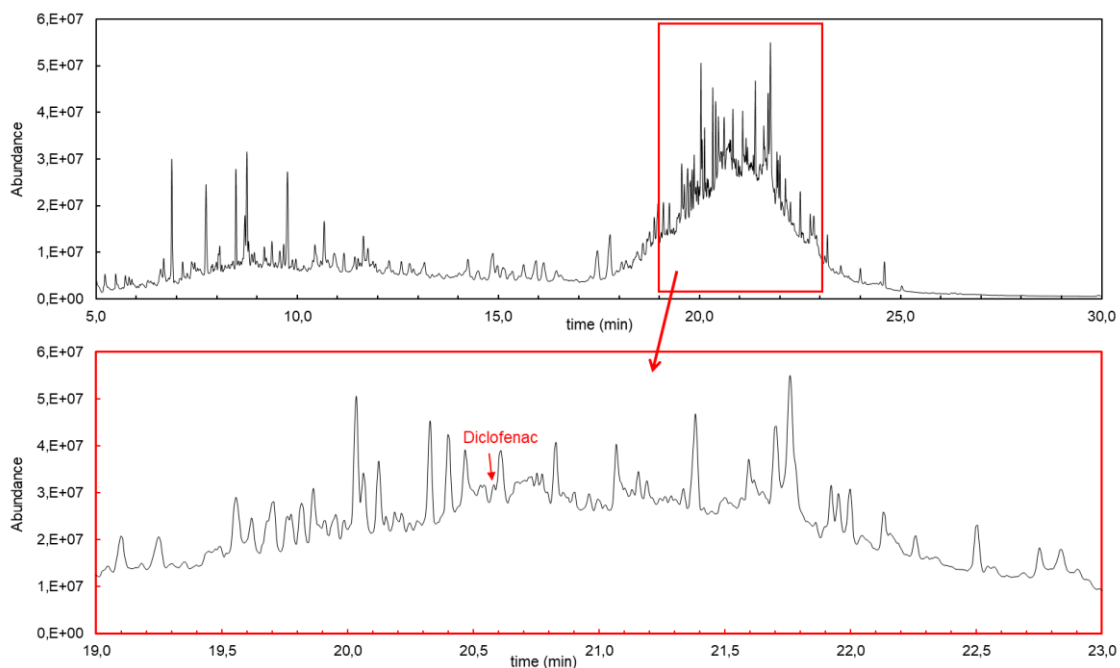


Figure 11. Chromatogram of the extract obtained from a groundwater sample collected from well SAP2B in October 2024, following large-volume SPE and methylation-GC-MS analysis. A zoomed-in view highlights the retention time window where diclofenac is detected.



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1.2. METALS

1.2.1. GRAB SAMPLING VS PASSIVE SAMPLING

In the sixth sampling campaign an intense comparison of metal concentrations obtained through grab samples and DGTs took place in the wells HMAQ (upstream) and WELL M (downstream, Fig. 1) in the Besòs. Grab samples were taken every 2-3 days during a 2-week period, whereas the DGT were deployed during the entire 14 days, sampling continuously. *Importantly, DGT only sample bioavailable metal while grab samples measure the total metal concentration.*

The results reveal generally much lower bioavailable metal concentrations (DGT) than total metal concentrations (grab sampling, Figure 12), as expected, and point towards an overestimation of the risk for metal exposure and adverse effects when relying on grab sample results, only. In addition, contrasting patterns of attenuation and enrichment can be seen between DGT and grab samples (Figure 12). In grab samples, elements such as Cr, Zn, and Al were substantially reduced at WELL M, with Cr dropping to undetectable levels and Zn and Al decreasing by about one third. Boron remained stable across sites. However, several trace metals increased downstream: Cu, Ni, and Co rose by 167%, 580%, and

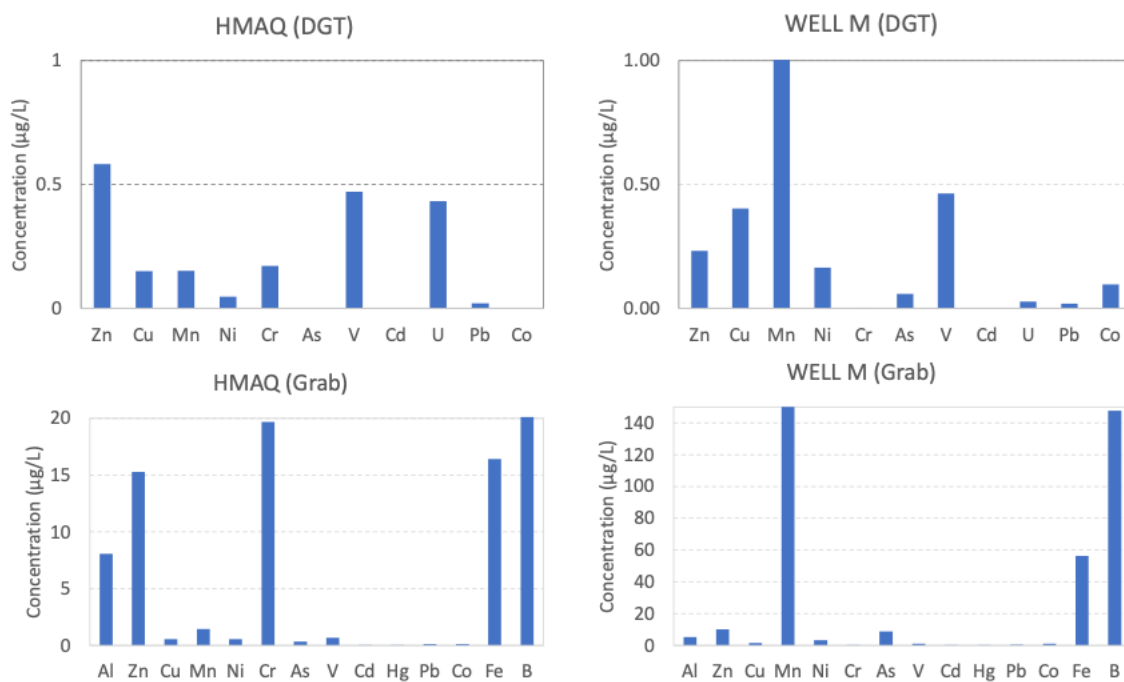


Figure 12. Concentrations of trace metals according to Grab vs. DGT sampling methods at HMAQ and Well M sampling points.



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800%, respectively, while Mn showed an exceptional increase from 1 to 435 µg/L. Arsenic, Pb, and Cd also followed this trend, with concentrations rising several-fold.

DGT results confirm similar trends but highlight stronger attenuation for certain metals. Zn, Cr, and U were markedly reduced at WELL M, with decreases of 60%, 100%, and >90%, respectively. Conversely, Cu and Ni increased by 167% and 242%, while Mn rose from 0.15 to 114.5 µg/L. As and Co, absent at HMAQ, were detected at WELL M. Overall, both methods indicate attenuation for Zn and Cr but mobilization of Mn, Cu, Ni, Co, As, and Pb. These findings underline the complexity of geochemical processes, with DGTs providing a clearer picture of metal bioavailability and stronger evidence of mobilization dynamics.

1.2.2. METALS MONITORING IN GROUNDWATER BY PASSIVE SAMPLING

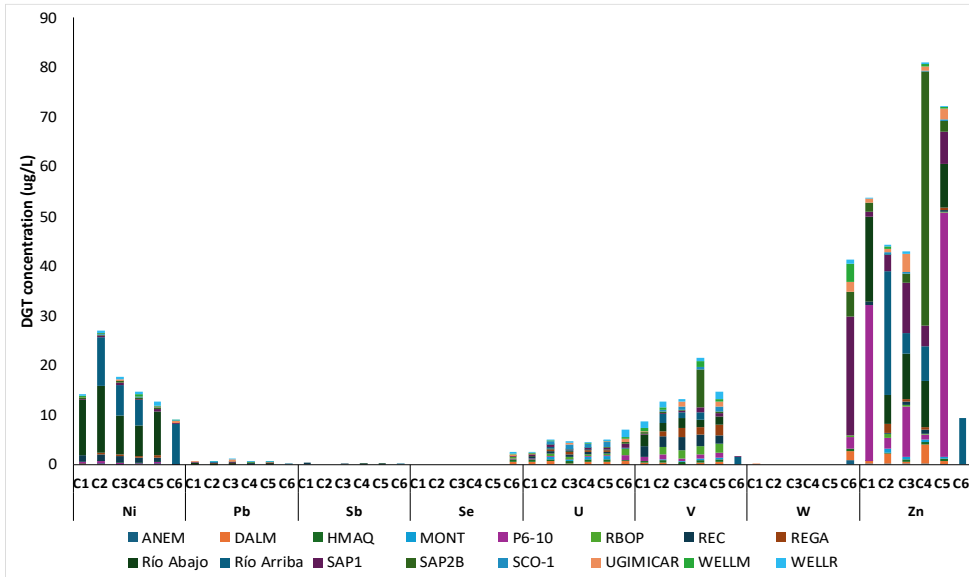
Several metals exhibited consistent accumulation or evidence of continuous input across the 6 UPWATER campaigns (Figure 13). Cobalt (Co) showed a clear upward trend, peaking at DALM and with elevated levels also detected at SAP1, SAP2B, Río Abajo, and REC, suggesting both widespread and localized inputs. Copper (Cu) demonstrated a widespread increase, with pronounced peaks at C2 and C6. Elevated concentrations were distributed across REC, REGA, WELLM, WELLR, Río Abajo, and Río Arriba, indicating diffuse contamination and high mobility of Cu in the system. Nickel (Ni) followed a strong upward trajectory across nearly all sites, with pronounced peaks at REC and downstream locations, consistent with either dispersion from upstream sources or multiple inputs along the river. Uranium (U) showed a steady rise, particularly at UGIMICAR, DALM, MONT, and HMAQ, pointing to localized enrichment. Similarly, Vanadium (V) increased throughout the monitoring period, with a distinct peak at SAP2B during C5, and persistently high levels across REC, REGA, and the broader riverine zone.



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a)



b)

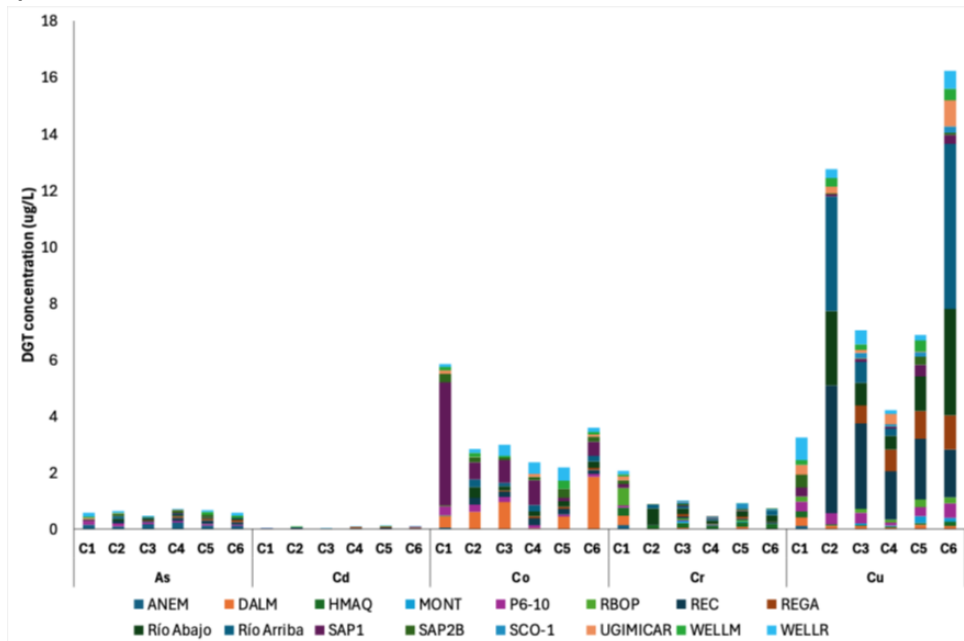


Figure 13. a) DGT concentration of Pb, Sb, Se, U, V, W and Zn by sampling campaign and b) DGT concentration of Mn per sampling campaign.



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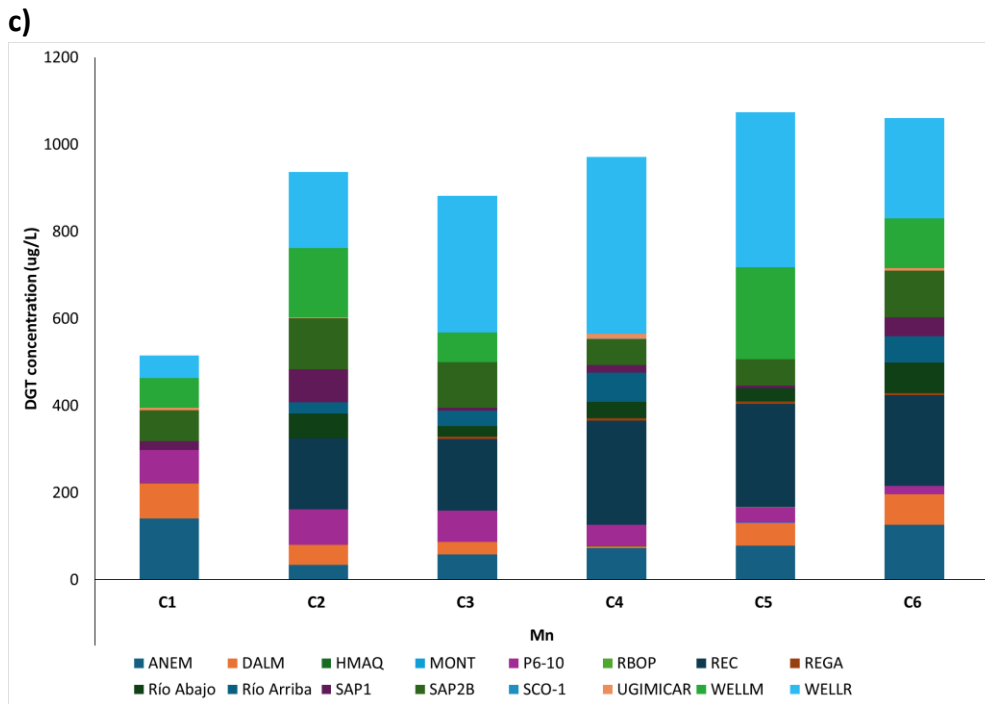


Figure 14c. DGT metal concentrations per sampling campaign for metals below 25 µg/L.

Other metals showed stable or declining concentrations. Arsenic (As) decreased slightly from C1 to C6 but remained moderately elevated at ANEM, REC, and REGA, reflecting distinct geological or anthropogenic sources. Chromium (Cr) displayed an initial decline followed by a minor rebound, with maximum concentrations at SAP sites, DALM, and Río Abajo—locations linked to anthropogenic pressures. Lead (Pb) showed only minor fluctuations, with localized peaks at SCO-1 and REGA, but an overall downward trend. Cadmium (Cd) remained very low and stable, detected mainly at REC and DALM, suggesting highly localized contamination. Antimony (Sb) stayed close to zero throughout, with only slight increases at REC. Zinc (Zn) was highly variable, lacking a clear temporal pattern but showing localized maxima at SAP2B and P6-10. Manganese (Mn) was also unstable, with strong peaks between C4 and C6, especially at WELLR, indicating localized or event-driven fluctuations. Metals Not Detected. Selenium (Se) and Tungsten (W) were consistently undetectable across all sites and campaigns, suggesting negligible inputs or concentrations below detection limits.

Spatial patterns revealed clear hotspots and gradients across the study area. Cobalt peaked sharply at DALM, marking a potential accumulation zone, while also being elevated at SAP1, SAP2B, Río Abajo, and REC. Copper was widely distributed, with particularly high levels at REC, REGA, WELLM, WELLR, and the riverine zone, confirming diffuse sources. Nickel concentrations pointed to REC as a primary input source, with downstream transport evident. Uranium was spatially elevated at UGIMICAR, DALM, MONT, and HMAQ, highlighting localized inputs or geological factors. Vanadium peaked dramatically at SAP2B, though levels remained high in REC, REGA, and riverine zones.



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In contrast, metals with stable or decreasing trends presented localized contamination signatures. Arsenic showed distinct sources in the north (REC/REGA) and south (ANEM). Chromium was elevated at anthropogenically influenced sites, especially in the south. Lead hotspots were confined to SCO-1 and REGA. Cadmium was detected only at REC and DALM. Zinc and Manganese exhibited localized peaks at SAP2B, P6-10, and WELLR, suggesting site-specific contamination or mobilization processes.

The combined spatial–temporal assessment highlights several key findings. Metals such as Ni and Cr showed downstream accumulation, consistent with riverine transport. Clear contamination hotspots were identified at DALM (Co), SAP2B (V, Zn), REC/DALM (Cd), and SCO-1/REGA (Pb). The southern cluster (SAP2B, WELLR, P6-10, SAP1) emerged as a concentrated contamination zone, hosting multiple elevated metals. In contrast, sites such as MONT, SCO-1, HMAQ, and RBOP maintained consistently low values, suggesting either minimal contamination inputs or effective dispersion capacity.

In terms of total accumulation, WELLR (1551) and REC (1048) stood out as the dominant hotspots, followed by WELLM, SAP2B, P6-10, ANEM, and DALM. This indicates heterogeneous contamination patterns, with both northern (REC) and southern (wells and SAPs) clusters acting as accumulation zones. Metals like Copper showed diffuse contamination across the system, while Cadmium remained highly localized.

Overall, trace element analysis revealed complex but distinct patterns of contamination. Post-2023 campaigns showed increasing concentrations of most metals, with Manganese and Zinc dominating in intensity. The results highlight the coexistence of diffuse and localized inputs, underscoring the need for targeted monitoring and management strategies. Particular attention should be directed to hotspot areas in both northern and southern clusters, where multiple metals converge, posing a heightened risk of ecological and human exposure.

1.3. VIRUSES

A total of ten sampling campaigns were conducted between September 2023 and May 2024. In total, 54 membrane samples were collected from piezometer SAP-2b (upper screened interval; VPS installed at 3 m depth) and 60 samples from piezometer SAP-1 (intermediate screened interval; VPS installed at 11 m depth). Each Virus Passive Sampler (VPS), containing three membrane samples, was deployed for a period of 10 days. Upon retrieval, viral nucleic acids (NA) were extracted from nitrocellulose (NC) and nylon (NY) membranes, NC and NY following the protocol described by Mejías-Molina et al., 2024. Briefly, membranes were carefully introduced inside Power Bead Tubes (glass 0.1 mm Qiagen) and 700 µl of glycine (0.25 N, pH 9.5) were added. After 30 s at 4 m/s of bead-beating using a Fast-prep homogenizer, the tube was centrifuged at 14.000xg for 1 min and 280 µl were used in the NA extraction using the Viral RNA Mini Kit (Qiagen) and the Qiacube Automatic system (Qiagen) into a final volume of 70 µl. NA extraction was performed as described above into a final volume of 70 µl. Specific real-time q(RT)PCR assays were used to quantify 8 viral pathogens (HAdV, EV, NoV GI and GII, RoV, IAV, SARS-CoV-2, HEV), one viral process control (PMMoV) and two fecal indicators (ChrAssphage and *E.coli*) following the specifications previously described (Da Silva et al., 2007; García-Aljaro et al., 2017; Hernroth et al., 2002; Hoehne and Schreier, 2006; Jothikumar et al., 2006; Kageyama et al., 2003; Khan et al., 2007; Loisy et al., 2005; Rusiñol et al., 2020a; Svraka et al., 2007; Zeng et al., 2008).



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Quantitative PCR (qPCR) assays targeting *Pepper Mild Mottle Virus* (PMMoV) and *Human Adenovirus* (HAdV) were systematically performed on all collected samples to assess the presence of human fecal contamination. In addition, reverse transcription qPCR (RT-qPCR) assays targeting *Enteroviruses* (EV), *Hepatitis E Virus* (HEV), *Influenza A Virus* (IAV), *Norovirus* genogroups GI and GII (NoV GI, NoV GII), *Rotavirus A* (RoV-A), and *SARS-CoV-2* were conducted on samples obtained during the colder months (October, December, and January), corresponding to periods of increased incidence of gastrointestinal and respiratory viral infections in Catalonia (SIVIC, 2024).

Cumulative precipitation data for each 10-day sampling period were retrieved from the Automatic Weather Stations (AWS) of the Meteorological Service of Catalonia (XEMA, Meteocat). Groundwater temperature, pH, and electrical conductivity were recorded during the first sampling campaign only, in accordance with previous findings indicating minimal seasonal variability in the aquifer's hydrochemical parameters (Jurado et al., 2019). This study was conducted before the drought; however, as we show, the electrical conductivity (EC) was indeed affected by the drought (Fig. 2).

In total, 54 membrane samples from piezometer SAP-2b and 60 from SAP-1 were analysed. Detection of pathogens and human fecal markers was confirmed at both wells, which differ in sampling depth (3 m for SAP-2b and 11 m for SAP-1). Pathogen detection results were considered valid only when PMMoV was simultaneously detected in the corresponding sample. Figure 14 summarizes virus detection and the reported accumulated precipitation (in millimeters) during the 7-month campaign (September 2023 to May 2024). No correlation was observed between precipitation (mm) and virus detection ($p > 0.3$). Nearly 50 % of all groundwater samples analyzed presented human fecal pollution using the HAdV qPCR assay (with mean concentrations between $1.23E+02$ and $3.66E+03$ GC per membrane). EV and NoV GI and GII were occasionally detected. EV were consistently detected during December and January 2024 at both sampling depths and using both VPS-NY and -NC. Norovirus GIs were detected in the VPS from October at both wells, but only in SAP-2b did the VPS-NY adsorb enough NoV GII to be detected and quantifiable.

For groundwater virus surveillance, both NY and NC demonstrated similar virus capture pattern and are proposed to be included in combination in the VPS. The maximum adsorption capacity of NC and NY materials has only been studied for SARS-CoV-2 in wastewater. This capacity to attach to the materials depends on the size, isoelectric point, and abundance of the virus. With the typical pH range of 6 to 8.5 in groundwater, viruses carry a negative charge, rendering them prone to adsorption onto positively charged soils. It has been reported that sand and gravel aquifers, such as the Besós River aquifer under study, are particularly susceptible to retain viral contamination compared to another aquifer types (Jurado et al., 2019). Consequently, it is plausible to expect that the adsorption of viral particles in groundwater onto membranes would be enhanced by the presence of suspended solids, which in turn adhere to VPS materials.



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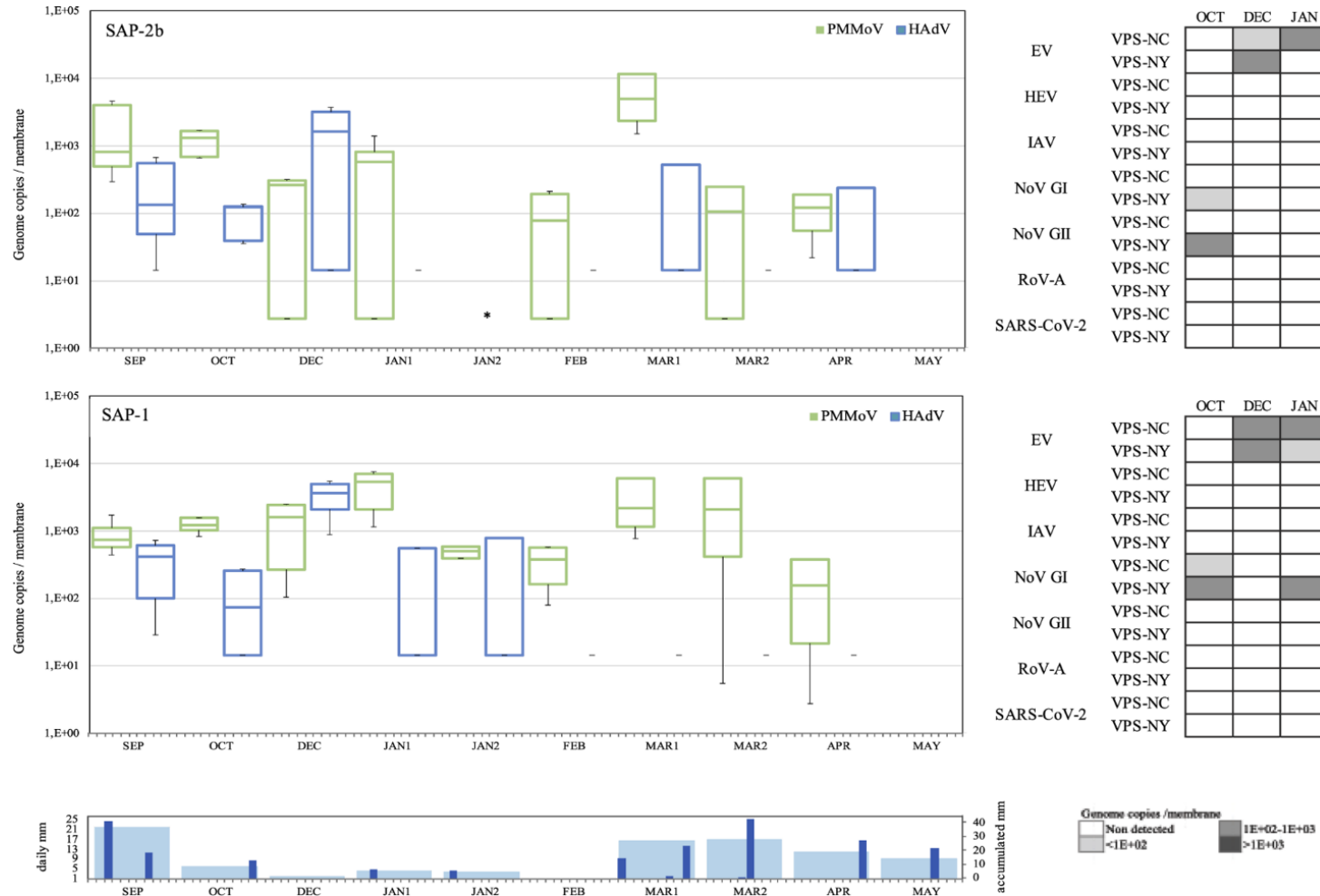


Figure 15. Boxplots and heatmaps representing virus concentrations in genome copies per membrane (GC/mbr) detected during the surveillance program in SAP-2b (top boxplots) and SAP-1 (bottom boxplots). Each boxplot chart includes the results of 3 NC and 3 NY membranes. *SAP-2b was not monitored in JAN2. The bar chart below the boxplots, indicates accumulated precipitation (mm) over each sampling event.



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Figure 14 also presents the detection of human viral pathogens in groundwater samples collected during the colder months. The analysis focused on key enteric viruses, including Enterovirus, Norovirus GI, and Norovirus GII, which were occasionally detected across the samples. The intermittent presence of these viruses indicates a potential health risk, particularly depending on the intended use of the water, such as for drinking, irrigation, or recreational activities. These results underscore the importance of regular monitoring and the implementation of appropriate water management strategies to safeguard public health.

2. PERFORMANCE OF NBS

2.1. DESCRIPTION OF THE NBS AND SAMPLING STRATEGY

A detailed description of the construction and dimensions of the four NBS was provided in Deliverable D4.1. In brief, four hybrid NBS combining root mat and biochar-based constructed wetland (CW) technologies were assessed (PB: 100% biochar-based CW, P1: 75% biochar + 25% root mat; P2: 50% biochar + 50% root mat, P3: 25% biochar + 75% root mat (Figure 15). Each hybrid NBS had a surface area of 12 m² and a water depth of 0.3 m.

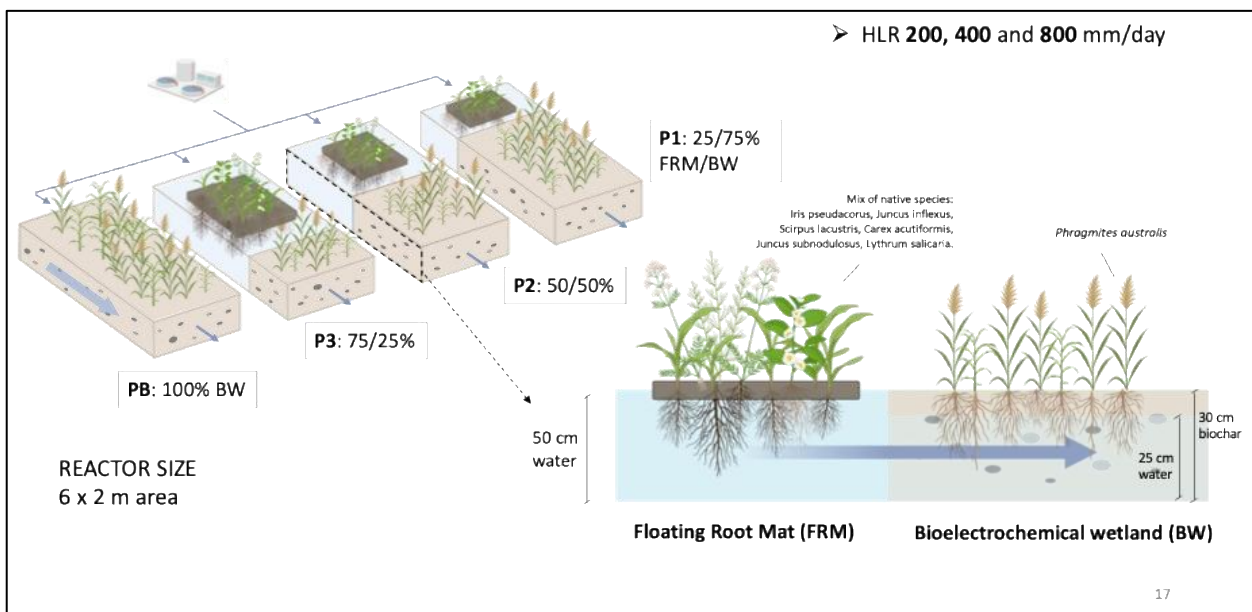


Figure 16. Diagram of the four hybrid NBS configurations tested, illustrating water flow paths and system dimensions.

Figure 16 shows the four pilot units, with the combinations of root mat technology and biochar-based CWs, during both warm and cold seasons. Different operational conditions were tested, with hydraulic loading rates (HLRs) of 200, 400, and 800 mm/d under both seasonal scenarios, which correspond to hydraulic retention times (HRTs) of approximately 4, 2, and 1 days, respectively.



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Figure 17. Photos of the pilot plant showing the four different treatment units during the warm and cold season.

Sampling campaigns were conducted to evaluate the performance of the four hybrid NBS units under different HLRs. Each HLR was assessed over a period of at least four weeks, preceded by a two-week equilibration period to allow system stabilization before sampling began.

Water samples were collected at both the inlet and outlet of each hybrid NBS unit. Sampling was performed twice per week, resulting in a total of eight sampling days per HLR.

The campaigns were carried out during two distinct seasonal periods: Warm season: July to September 2024 and cold season: January to March 2025.

This approach allowed for the evaluation of seasonal variability in treatment performance under varying operational conditions.

During the cold-season sampling period, additional large-volume water samples were collected for CSIA. Sampling focused on two NBS: the biochar-only system (PB) and the hybrid system with 25% root mat coverage (P1). In addition to sampling at the inlet and outlet of each NBS unit, intermediate points were also included: three in PB and two in P1. For each HLR tested (200 and 400 mm/d), 10-L water samples were collected weekly over a two-week period.

2.2. OPTIMIZATION

2.2.1. GENERAL WATER QUALITY PARAMETERS

Figure 17 shows the occurrence of general water quality parameters during both the warm and cold seasons. The four treatment reactors exhibited comparable removal efficiencies for ammonia, COD, phosphate, and turbidity, with reductions ranging from 70% to 80%. However, during the warm season, performance decreased significantly across all systems when operated at a high HLR of 800 mm/day, resulting in lower attenuation of these parameters. In the cold season, treatment efficiency remained relatively stable across most systems. Notably, the PB unit—operating solely with biochar—achieved the highest ammonia removal among all treatments, indicating that biochar may be particularly effective for ammonia attenuation under low-temperature conditions.



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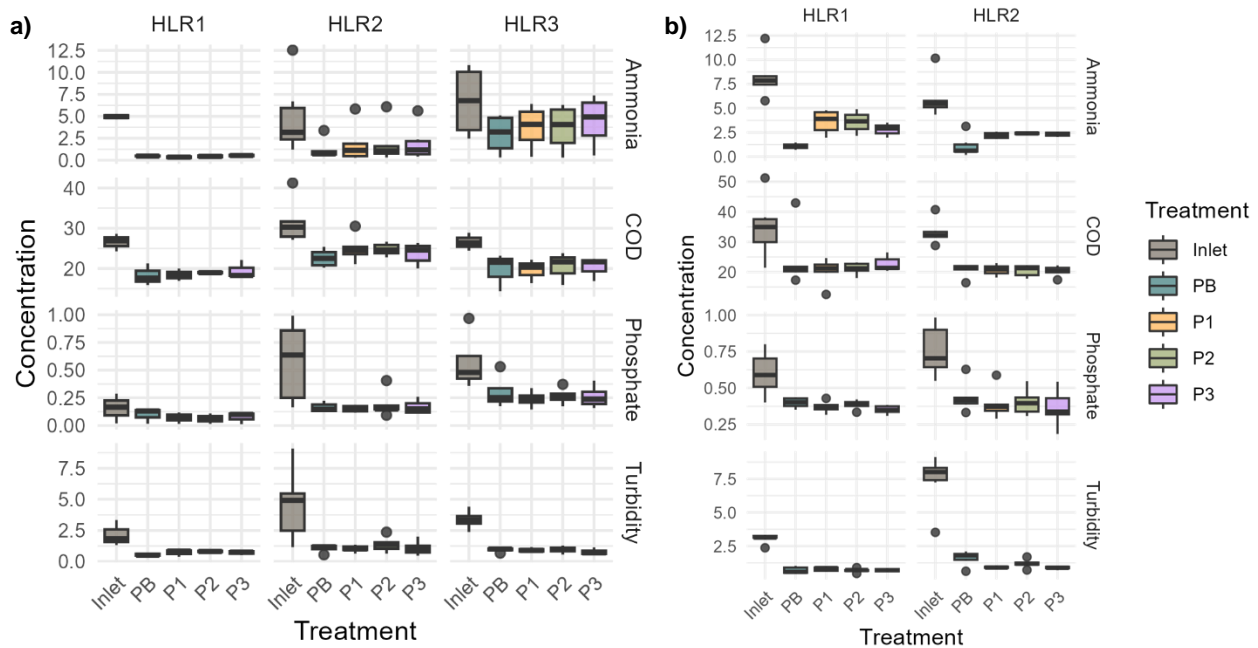


Figure 18. Performance of the four NBS units in reducing ammonia, COD, phosphate, and turbidity across different HLRs during a) warm and b) cold seasons.

2.2.2. ORGANIC POLLUTANTS

The analysis of emerging pollutants in the effluent of the Moncada i Reixac WWTP revealed that the most abundant compounds during the warm season were benzotriazoles, gemfibrozil, diclofenac, and venlafaxine. In contrast, during the cold season a larger number of compounds exceeded 1 µg/L, highlighting a seasonal increase in concentrations (Figure 18). The higher average concentrations observed in the cold season may be attributed to reduced treatment efficiency at lower temperatures or to increased consumption of these pharmaceuticals during that period, as previously reported in other studies. The observed pollutant levels in the WWTP effluent are consistent with earlier research conducted at the same WWTP (Matamoros et al, 2017).



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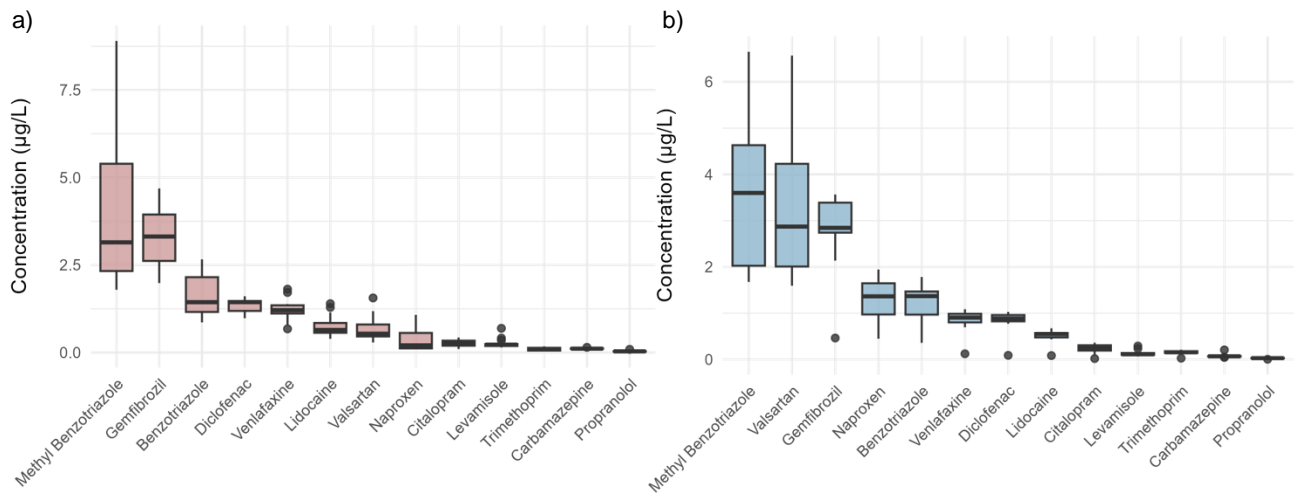


Figure 19. Occurrence of Emerging Pollutants in the Effluent of the Montcada i Reixac WWTP During the (a) Warm Season and (b) Cold Season.

Figures 19 and 20 illustrate the attenuation of identified emerging pollutants across the four tested hybrid NBS configurations. The pollutants detected at the highest concentrations in the WWTP effluent—such as benzotriazoles and valsartan—also exhibited the greatest reductions, with removal efficiencies reaching up to 80–90%. The results indicate that contaminant attenuation is dependent not only on the chemical compound but also on the treatment and the HLR. At lower HLRs, pollutant removal was generally higher. The configuration consisting solely of biochar proved to be the most effective, achieving average removal efficiencies of up to 78% during the summer at an HLR of 200 mm/day. In contrast, the hybrid systems that included root mats showed reduced removal performance (Table 1). Furthermore, the decline in performance appeared to be correlated with the proportion of root mat included in the system. Specifically, the configuration with 25% root mat coverage (P1) demonstrated higher pollutant attenuation than the one with 75% root mat coverage (P3).



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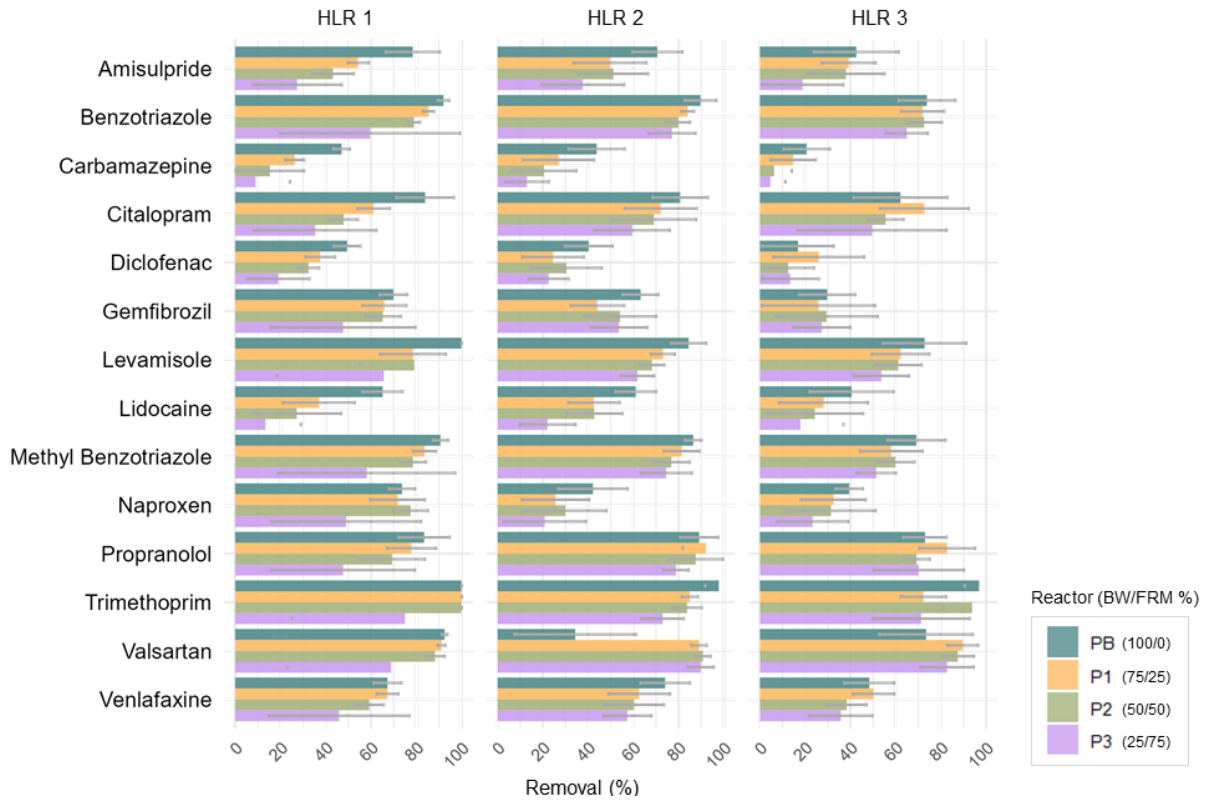


Figure 20. Reduction (%) of Emerging Pollutants in the Four Assessed NBS Treatments During the Warm Season at HLR1 (200 mm/day), HLR2 (400 mm/day), and HLR3 (800 mm/day).

During the cold season, the performance of all assessed hybrid NBS decreased. The PB reactor showed an average reduction of 62% at an HLR of 200 mm/day and 51% at 400 mm/day. The other configurations showed average reductions below 50%, even at the lower HLR (Table 1).



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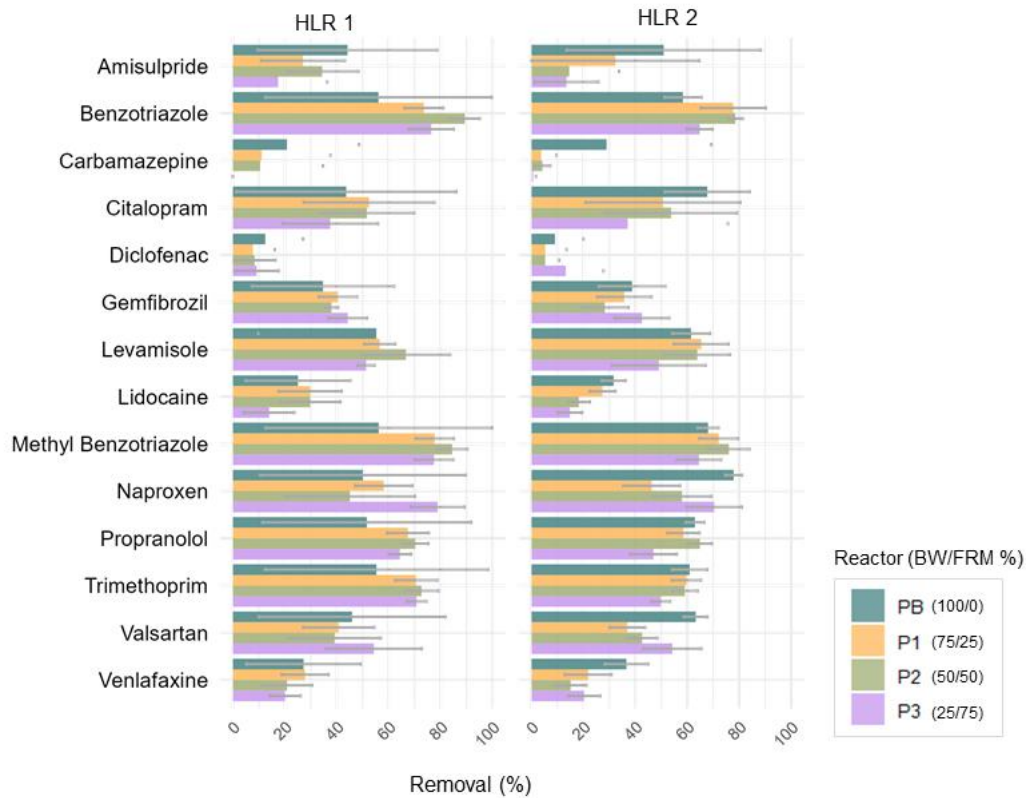


Figure 21. Reduction (%) of Emerging Pollutants in the 4 assessed NBS Treatments in the Cold Season at HLR1 (200 mm/day) and HLR2 (400 mm/day).

Table 1. Average percentage reduction of emerging pollutants under all tested conditions. The first value refers to the warm season, and the second to the cold season.

(FRM/BW %)	HLR 1	HLR 2	HLR 3
	200 mm/day	400 mm/day	800 mm/day
PB (0/100)	78±18 / 62±25	70±22 / 51±24	55± 25 / -
P1 (25/75)	67±22 / 46±25	62±25 / 42±26	52±27 / -
P2 (50/50)	62±26 / 47±28	61±25 / 42±28	49±29 / -
P3 (75/25)	59±29 / 44±29	55±26 / 39±25	42±28 / -



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The results on the removal of emerging pollutants indicated that the biochar-based CW was the only system capable of achieving an average reduction of over 70% in both seasons at a hydraulic loading rate of 200 mm/d, and over 50% at a hydraulic loading rate of 400 mm/d. It is worth noting that these reductions were calculated as percentage removals, not based on mass loading. When considering pollutant removal based on mass loads—which account for the concentration of each compound—the reduction exceeded 80% and 70% at a HLR of 400 mm/d in the warm and cold seasons, respectively. This is because the compounds present at the highest concentrations at the inlet—such as benzotriazoles and valsartan—were also those most effectively removed (>90% in the PB reactor) (see Table 2). In contrast, conventional removal efficiency calculations do not consider compound-specific concentrations.

Table 2. Mass balance reduction of emerging pollutants during warm and cold season (in percentage).

(FRM/BW %)	HLR 1	HLR 2	HLR 3 800 mm/day
	200 mm/day	400 mm/day	
PB (0/100)	81 / 78	78 / 56	54 / -
P1 (25/75)	72 / 53	71 / 46	59 / -
P2 (50/50)	65 / 54	66 / 48	51 / -
P3 (75/25)	70 / 56	60 / 51	48 / -

Water samples for CSIA (10 L) were extracted by SPE with 1 g OASIS HLB cartridges, following the procedure described in section 1.2. For samples collected under the 200 mm/d HLR, one cartridge was used per sample. In contrast, samples from the 400 mm/d HLR were processed by extracting two 5 L aliquots per cartridge. All extracts were analyzed by derivatization-GC-MS using both methylation and silylation methods. Preliminary results showed promising options for subsequent CSIA of certain emerging pollutants, particularly diclofenac, for which concentrations in the final extract are high enough for CSIA (up to 30 mg/L). However, as observed with the groundwater samples (Figure 11), further optimization of the analytical protocol is ongoing to ensure accurate and reliable isotopic measurements prior to GC-IRMS analysis.

2.3. NBS MONITORING BY PASSIVE SAMPLING

2.3.1. CPS

The CPS were deployed in the inlet and outlet of the NBS in May 2025 for a period of one month (Figure 21). After this time, they were analyzed following the protocols established in WP2. The emerging pollutants identified were consistent with those detected during the grab sampling campaigns conducted during the NBS optimization phase operated at a HLR of 200 mm/d (HRT of 3-4 days).



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Figure 22. Picture of the deployment of CPS in the NBS.

The results indicate that pollutant removal efficiency was comparable to that observed in grab sampling. Specifically, the average reduction of emerging pollutants in the PB reactor was 81%, closely matching the 78% obtained during the optimization studies (Figure 22). Similarly, the biochar-based CW proved to be the most effective, with pollutant attenuation decreasing in proportion to the reduction in biochar content within the reactor.

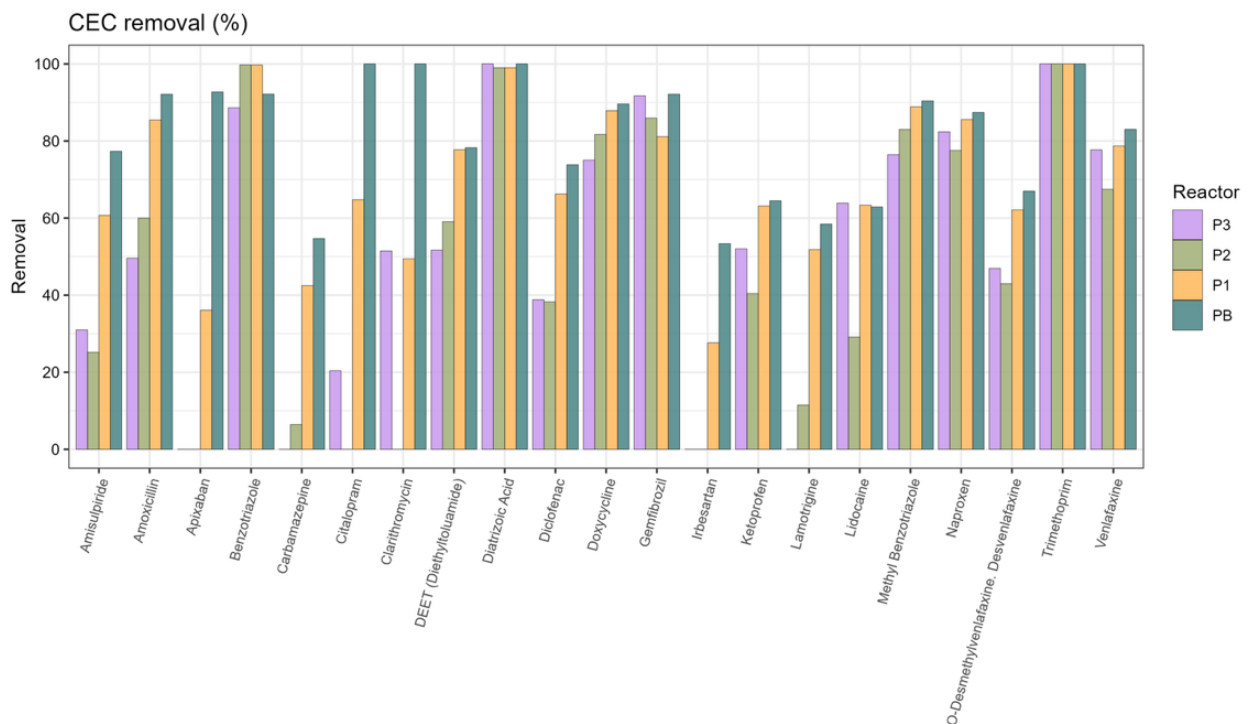


Figure 23. Attenuation of emerging pollutants based on CPS deployed for one month in the NBS operated at a hydraulic loading rate of 200 mm/d.



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In conclusion, the results from the deployment of passive samplers for monitoring organic pollutants demonstrate that this approach can be effectively used to assess the performance of water treatment technologies—significantly reducing the need for conducting hundreds of individual analyses.

In parallel, to assess the feasibility of the CPS-CSIA approach, modified CPS were also deployed in two NBS units. These samplers, containing 1 g of OASIS HLB each, were deployed in duplicate at the inlet (Figure 17), at the outlet and one intermediate point of PB, as well as at the outlet and one intermediate point of P1. The deployment period was extended to 5 weeks, and two consecutive campaigns were performed (May and June 2025). Large-volume grab samples (10 L) were also collected from the same sampling points for comparison. Both the modified CPS and the large-volume water samples were extracted as described in section 1.2. Analysis of these samples is currently ongoing.

2.3.2. DGT

The DGTs were deployed in the Montcada i Reixac WWTP setup at both the inlet and outlet of the NBS in May 2025 for a duration of 7 days (Figure 23). Afterwards, they were analyzed according to the protocols established in WP2. The metals detected at the inlet are: Co, Cr, Cu, Mn, Ni, Pb, U, V, As, Sb and Zn, as shown in Figure 24.



Figure 24. Picture showing the deployment of DGTs (white) and VPS (orange) in the NBS.

The results indicate that, unlike grab sampling, the DGT measurements revealed a substantial reduction in metal concentrations between the inlet and outlet of the NBS. In grab sampling, only the PB configuration showed significant decreases in a few cases (e.g., ~25% for Cr and ~50% for Zn), whereas the DGTs consistently evidenced a more pronounced attenuation of metals across the different systems (Figure 25).



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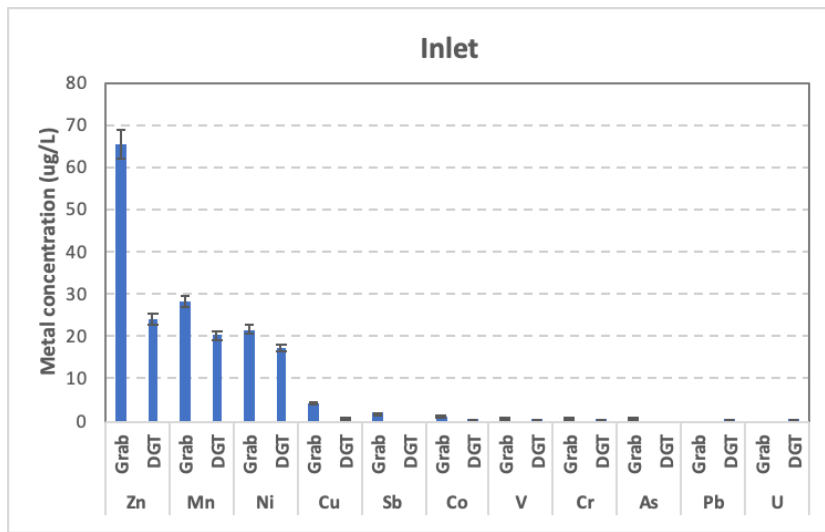


Figure 25. Occurrence of metals in the Effluent of the Montcada i Reixac WWTP During the Warm Season.

This suggests that NBS are an effective strategy in reducing metals bioavailability. The biochar-based CW proved to be the most effective, with metal attenuation decreasing in proportion to the reduction in biochar content within the reactor. Reduction values close to 80% and 60% were obtained for metals such as Co and Zn, respectively.

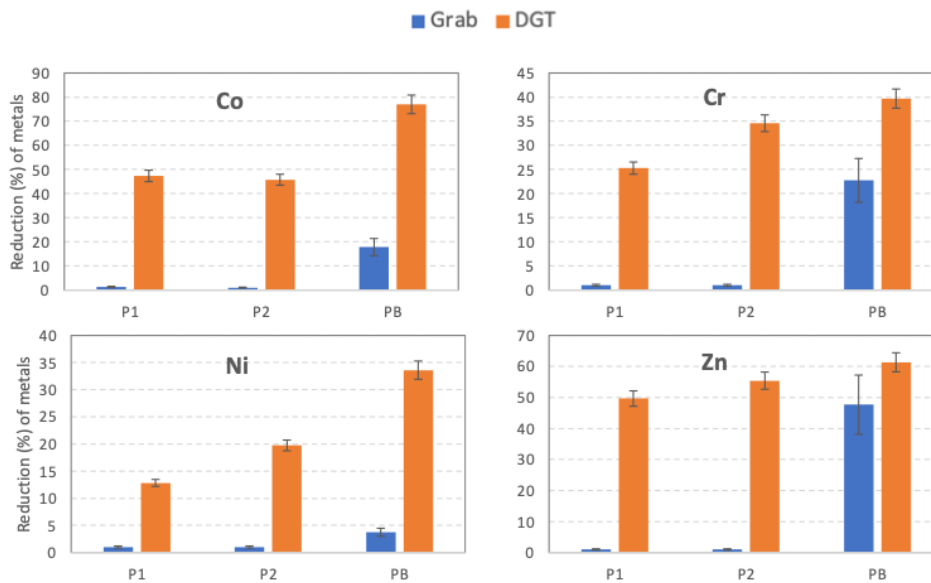


Figure 26. Attenuation of metals based on DGTs deployed for one month in the NBS operated at a hydraulic loading rate of 200 mm/d.



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In conclusion, the findings from the use of passive samplers DGT-type to monitor trace metals show that this method is an effective tool for evaluating the performance of water treatment technologies, while greatly minimizing the need for numerous individual analyses.

2.3.3. VPS

To evaluate the capacity of the pilot-scale NBS systems to retain pathogenic viruses, we conducted a comparative analysis using VPS and traditional grab sampling in April 2025 when the system was operated at a HLR of 200 mm/d and HRT of 2 days (Figure 26).

Virus detection was performed using digital PCR targeting three human pathogenic viruses:

- Human Adenovirus (HA_dV): A robust marker of human fecal contamination due to its high environmental persistence and human specificity.
- Norovirus Genogroup II (NoV GII): A highly prevalent enteric pathogen responsible for acute gastroenteritis, typically found in higher concentrations in wastewater than HA_dV due to its abundant excretion in feces.
- Hepatitis E Virus (HEV): A pathogen causing acute hepatitis, with zoonotic reservoirs including domestic pigs and wild boars, and of emerging public health concern in Europe.

The detection results (Figure 26) are presented as a heatmap showing the number of positive detections out of five replicates for each virus, sampling method, and NBS configuration.

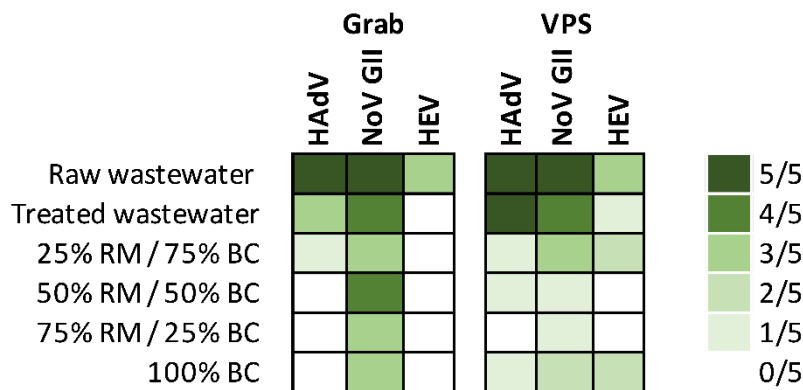


Figure 27. Positive detections of viruses in the 5 sampling campaigns, at the raw wastewater from the inlet of the WWTP, at the WWTP effluent (after the secondary treatment with activated sludge) and after the different NBS configurations.



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In raw wastewater, both grab sampling and VPS yielded high detection frequencies for HAdV and NoV GII (5/5), with slightly lower values for HEV (3/5 with grab, 3/5 with VPS), confirming the presence of these viruses at the inlet of the system by both conventional (grab) sampling and virus concentration method, and by the VPS time-integrated sampling and concentration approach.

However, clear differences emerged in treated wastewater: HAdV was detected consistently at low frequencies in the VPS samples across the treatments but not in the grab samples, suggesting that VPS may offer improved sensitivity for persistent viral indicators like HAdV under low-concentration conditions. In other words, VPS outperformed grab sampling in terms of virus sensitivity. After all the NBS combinations, VPS enabled the detection of at least one of the tested pathogens, with especially notable differences for NoV GII at 50% RM / 50% BC (3/5 vs. 1/5) or 100% BC in which grab sampling and ultrafiltration resulted in the detection of NoV GII genome copies in 3 out of 5 sampling events, whereas VPS allowed the detection of HAdV (1/5), NoV GII (2/5) and HEV (2/5).

A progressive reduction in virus detection was observed with increasing proportions of biochar, particularly for HAdV and NoV GII. Intermediate treatments (e.g., 50% RM / 50% BC, 75% RM / 25% BC) showed a more consistent reduction in viral signals, especially when compared to raw wastewater.

With the available data and over the 5 sampling events, **no single NBS configuration emerged as a clearly best-performing system**, but overall, combinations containing between 50% and 75% RM and 25% to 50% BC showed the most reliable reduction in viral presence.

This performance is particularly relevant considering that the concentrations of pathogenic viruses at the outlet of the wastewater treatment plant (WWTP), before NBS treatment, typically range between 10^3 and 10^5 genome copies per milliliter (gc/mL).

These results underscore the potential of NBS systems to contribute to the control of viral contaminants in treated wastewater, especially when using mixed RM/BC substrates, and emphasize the added value of VPS as a sensitive, non-invasive monitoring tool.

3. STAKEHOLDERS ENGAGEMENT

An essential part of the project's success has been the proactive engagement of key stakeholders throughout the implementation of the pilot plant in Besòs case study (Spain). To this end, a dedicated inauguration workshop was organized to mark the official launch of the pilot activities and to promote transparent communication between project partners, local authorities, industry representatives, and other relevant actors. This workshop served as a platform to present the preliminary results of pilot operations, showcase the technology being implemented, and initiate a dialogue around the potential replication and scale-up of the solutions.

The inauguration event was instrumental in building trust, raising awareness, and fostering long-term collaboration among stakeholders. It also laid the groundwork for continued engagement in the upcoming activities of WP6, where stakeholder input and participation will be crucial for the co-development and validation of policy recommendations and exploitation strategies. Figure 27 shows images from the inauguration workshop held in Spain, where participants had the opportunity to interact with the project team, discuss findings from the pilot site, and express interest in future collaboration.



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Figure 28. Pictures of the inauguration of the Besòs pilot plant in Montcada i Reixac WWTP.



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4. CONCLUSIONS

- The deployment of Ceramic Passive Samplers (CPS), Diffusive Gradients in Thin Films (DGTs), and Virus Passive Samplers (VPS) in groundwater successfully validated their applicability for long-term groundwater monitoring of emerging pollutants, metals, and viruses.
- The six monitoring campaigns confirmed the widespread occurrence of contaminants in the Besòs river catchment, with spatial and temporal patterns strongly linked to wastewater discharges, river connectivity, well depth, and sewer leakage.
- Evidence indicates that treated wastewater effluents are a major contributor to groundwater contamination, emphasizing the vulnerability of aquifers in urbanized river basins.
- The biochar-based Nature-Based Solution (NBS) pilot demonstrated high efficiency in removing emerging pollutants (>80%), with performance modulated by both hydraulic loading rate (HLR) and temperature.
- Passive samplers integrated within the NBS confirmed removal efficiencies for pollutants, metals, and viruses, supporting their use as reliable complementary tools to conventional grab sampling.
- Overall, the combined application of passive sampling technologies and NBS represents a promising and sustainable strategy for mitigating groundwater pollution by multiple contaminant classes.

5. RECOMMENDATIONS

- **Monitoring framework:** Incorporate passive sampling tools into routine groundwater monitoring programs to provide cost-effective, integrative, and reliable assessments of contaminant dynamics.
- **NBS optimization:** Further optimize biochar-based NBS systems under varying hydraulic and seasonal conditions, with special focus on maintaining high performance during cold periods.
- **Source control:** Strengthen management of wastewater treatment plant discharges and urban sewer infrastructure to reduce pollutant loads reaching groundwater.
- **Scaling up:** Explore the feasibility of scaling up biochar-based NBS units for full-scale application in groundwater protection strategies.
- **Integration:** Promote the combined use of passive sampling and NBS technologies within EU water management policies as a sustainable and transferable solution to address emerging pollutants, metals, and viruses in groundwater.
- **Further research:** Investigate long-term performance, regeneration, and cost-effectiveness of passive samplers and biochar filters to ensure their applicability across diverse hydrogeological contexts.



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