

UPWATER

UNDERSTANDING GROUNDWATER POLLUTION TO PROTECT AND
ENHANCE WATERQUALITY

DELIVERABLE D4.2

REPORT ON THE VALIDATION IN THE STENGAARDEN DUMPSITE



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LIST OF ABBREVIATIONS

Abbreviation	Definition
2,4-DCP	2,4-dichlorophenol
2,4-D	2,4-dichlorophenoxyacetic acid
2-CPP	2-(2-chlorophenoxy)propanoic acid
2-MPP	2-(2-methylphenoxy)propanoic acid
3-MeCat	3-methyl catechol
4-Cl-2Me-Ph	4-chloro- 2-methylphenol
4-ClCat	4-chlorocatechol
4-ClPh	4- chlorophenol
4-CPP	2-(4-chlorophenoxy)propanoic acid
AU	Aarhus University
BOD	Biochemical Oxygen Demand
BSTFA	N,O-bis(trimethylsilyl)trifluoroacetamide
CPS	Ceramic passive sampler
CSIA	Compound Specific Isotope Analysis
DCPP	Dichlorprop
DCPP-D6	Stable Isotope Labelled Dichlorprop
DGT	Diffusive gradients in thin-films passive sampler
EA-IRMS	Elemental Analyzer coupled with Isotope Ratio Mass Spectrometry
ESI	Electrospray ionisation
GAC	Granulated activated carbon
GC-IRMS	Gas Chromatography coupled with Isotope Ratio Mass Spectrometry
HPLC-MS/MS	High-Performance Liquid Chromatography coupled with Tandem Mass Spectrometry
HRT	Hydraulic retention time
IS	Internal standard
LOD	Limit of detection
LOQ	Limit of quantification
MBBR	Mooving Bed Biofilm Reactor
MCPP	Mecoprop
MCPP-D6	Stable Isotope Labelled Mecoprop
MCPP-sulf	Mecoprop sulfate



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MRM	Multi reacti
PCA	Principal component analysis
SPE	Solid-phase extraction
TMSH	Trimethylsulfonium hydroxide
UPWATER	Understanding groundwater Pollution to protect and enhance WATER quality.



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EXECUTIVE SUMMARY

This report describes the outcome of Task 4.2 within the UPWATER project (Understanding groundwater Pollution to protect and enhance WATER quality, Horizon Europe project No 101081807).

It presents the results of a three-year pilot-scale study on the biological treatment of phenoxy acid herbicides—specifically mecoprop (MCP), dichlorprop (DCPP), and their metabolites—in contaminated groundwater at the Stengaarden site in Denmark. The study assessed the performance of two parallel treatment trains, from now on referred to as Line B and Line C, each combining Moving Bed Biofilm Reactors (MBBRs) with biofilters. In addition, a two-year groundwater monitoring campaign was carried out on nearby wells to evaluate spatial and temporal contamination patterns, and passive sampling techniques were tested within the pilot system, including diffusive gradients in thin-films (DGT) samplers for trace metal analysis and ceramic passive sampler (CPS) for pesticide and metabolite monitoring.

- Overall, the pilot provided high removal rates and had low outflow concentrations.
- All analysed parent compounds and metabolites were removed.
- Winter operation was well possible as the pilot allowed both summer operation (with the water infiltrating from the surface) and winter operation (with the water being piped through the frozen layer and pumped directly into the subsurface rhizome). Operating limits were -23°C over several days or -10°C over several weeks as the pipes of the pilot froze, which might not be relevant for full scale operation.
- Space demand was affordable.
- Oxygen levels were the single most controlling parameter for contaminant removal
- A two-year monitoring campaign (2024–2025) confirmed that contamination by phenoxy herbicides and phenolic compounds remains spatially limited but persistent.
- CPS were successfully calibrated and developed for phenoxy herbicides and metabolites. Field deployment demonstrated linear uptake kinetics, high reproducibility ($R^2 = 0.92\text{--}0.98$), and strong agreement with grab samples.
- DGT deployment showed substantial metal attenuation in both treatment lines, particularly for Mn and Co, with Line B performing slightly better, indicating favourable redox conditions promoting metal sequestration.

In detail:

Line B consistently outperformed Line C, achieving average removal rates of approximately 80% for MCP and 73% for DCPP, probably because oxygen concentrations were slightly higher.

The biofilters acted as the main drivers of herbicide removal, while the MBBRs provided bulk degradation support and protected the system from silt and iron oxide dependent clogging (as planned).



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Among the operational parameters, the availability of oxygen showed the strongest correlation with the contaminant removal, whereas hydraulic retention time and seasonal temperature variations had only minor impacts.

Despite the high removal rates of parent compounds, persistent metabolites—including 4-chlorophenol and 4-chloropropiophenone (4-CPP)—exceeded drinking water levels in some samples of treated water. This highlights the need for further process optimization and possibly the integration of complementary treatment barriers to ensure complete removal of transformation products.

The findings confirm the potential of combined MBBR–biofilter systems as a viable strategy for groundwater remediation at pesticide-contaminated sites. The study provides valuable insights into the design, operation, and optimization of biological treatment systems that can support the EU's objectives for safeguarding groundwater quality and ensuring safe drinking water resources.



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1. HIGHLIGHTS

- Pilot-scale treatment of phenoxy acid herbicides (mecoprop, dichlorprop and their metabolites) in contaminated groundwater was successfully tested at the Stengaarden site (Denmark).
- Operations through normal Northern European winters (+5 to occasional -10 °C) is possible under maintaining removal.
- Two parallel MBBR–biofilter treatment lines (Line B and Line C) were compared over a two-year monitoring period.
- In this combination, the biofilter removed most of the herbicide residues, while the MBBR protected the biofilter from clogging with silts and iron oxides.
- Line B consistently outperformed Line C, with average removals of 80% (MCP) and 73% (DCPP).
- Oxygen availability correlated strongly with removal efficiency of the phenoxy herbicide residues, while hydraulic retention time and temperature had limited influence.
- Persistent metabolites (e.g., 4-chlorophenol, 4-CP) indicate that further process optimization or complementary barriers are needed.
- Results confirm the potential of combined biological systems for groundwater remediation at pesticide-contaminated sites.



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2. INTRODUCTION: THE PHENOXY ACID HERBICIDES MECOPROP AND DICHLORPROP IN GROUNDWATER

Mecoprop (MCP; 2-(4-chloro-2-methylphenoxy)propionic acid) and dichlorprop (2-(2,4-dichlorophenoxy)propionic acid, often reported as DCP) are widely used phenoxyalkanoic herbicides. In Denmark the compound has been produced in relatively small operations and production and transport wastes of these compounds have been dumped into municipal waste landfills in the last decades and because of their relatively high-water solubility and moderate persistence, both compounds are repeatedly detected in landfill leachates, wastewater-impacted waters and occasionally in groundwater at low $\mu\text{g}\cdot\text{L}^{-1}$ to $\text{ng}\cdot\text{L}^{-1}$ levels in national monitoring programmes (Mitra et al., 2024).

In Denmark between 1960 and 1990, residues of mecoprop and dichlorprop originating from production and transportation activities were deposited in small, locally available landfills. This practice resulted in numerous point sources distributed across the Danish landscape, where leachate and groundwater frequently exhibit concentrations exceeding $10\ \mu\text{g}/\text{L}$. This is also the case at Stengaarden dumpsite. In addition, these leachate waters commonly contain elevated levels of dissolved iron, predominantly in the form of Fe^{2+} . Upon oxidation, this iron forms Fe(III) oxides and hydroxides, which present significant operational challenges by precipitating and obstructing filtration units.

Leaching of MCP and DCP from landfills and contaminated sites into groundwater has been documented (Baun et al., 2003; Milosevic et al., 2013) as well as first tests to overcome these contaminations by MBBRs or biofiltration units (Escolà Casas et al., 2017; Nord et al., 2020). Both the parent compounds and their metabolites can persist due to heterogeneous geological settings and varying redox conditions, i.e., these compounds degrade even slower under oxygen-depleted conditions than under aerobic ones. Nevertheless, reductive dechlorination has been observed in iron-reducing environments (Milosevic et al., 2013). For example, in two Danish landfills, the transformation of DCP to 4-CPP and of MCP to 2-MPP has been reported, suggesting that high leachate concentrations create favourable conditions for dechlorination (Milosevic et al., 2013; Nord et al., 2020). To safeguard drinking water resources, advanced treatment technologies are required at pesticide point sources. Currently, the most commonly applied approaches for removing organic micropollutants, including MCP, rely on sorption to granular activated carbon (GAC) or degradation in biofilm-based systems such as managed soil or sand filters (Hedegaard et al., 2014; Betsholtz et al., 2021; Nord et al., 2020).

Under aerobic conditions, the degradation pathways of MCP and DCP are well established, initiating with the formation of 2,4-dichlorophenol and 4-chloro-2-methylphenol, respectively (Müller and Kohler, 2004; Buss et al., 2006). These processes are enantioselective, with a general preference for the (S)-enantiomer; however, variations in environmental conditions, such as nitrate-reducing or other redox settings, can shift enantiomeric preference (Escolà Casas et al., 2017; Frková et al., 2016; Williams et al., 2003). Under anaerobic or reductive conditions, an alternative degradation pathway involves dechlorination, yielding products such as 2-methylphenoxypropionic acid (2-MPP), 2-(2-chlorophenoxy)propanoic acid (2-CPP), and 2-(4-chlorophenoxy)propanoic acid (4-CPP) (Reitzel et al., 2004) (Table 1).

Stengaarden is in the vicinity of a major drinking water production site for the city of Copenhagen and is also polluting potential groundwater (drinking water) resources of the smaller city of Roskilde.



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It was thus the aim of this task to test whether:

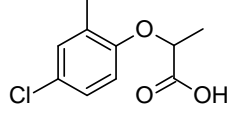
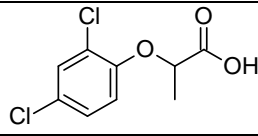
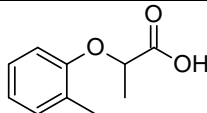
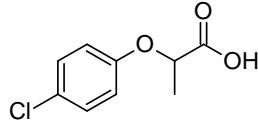
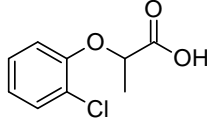
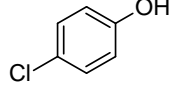
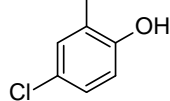
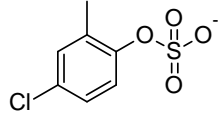
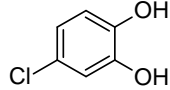
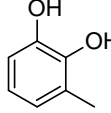
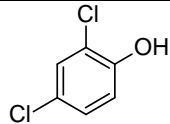
- biobased or nature-based technology is able to reduce pollutant concentrations.
- the combination MBBR/Biofilter operated in a stable way
 1. obtaining high removal rates also through winter conditions
 2. removing the parent compounds without producing elevated concentrations of metabolites.



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Table 1. Mecoprop, dichlorprop and their metabolites name, CAS No. and structure.

Compound Name	CAS No.	Structure
Mecoprop (MCPP)	93-65-2	
Dichlorprop (DCPP)	120-36-5	
2-(2-methyl phenoxy) propanoic acid (2-MPP) (Metabolite of MCPP)	943-45-3	
2-(4-chlorophenoxy) propanoic acid (4-CPP) (Metabolite of DCPP)	3307-39-9	
2-(2-chlorophenoxy) propanoic acid (2-CPP) (Metabolite of DCPP)	25140-86-7	
4-Chlorophenol (4-CIPh) (Metabolite of DCPP)	106-48-9	
4-chloro-o-cresol (4-CICr) Or 4-chloro-2-methyl-phenol (4-Cl-2MePh) (Metabolite of MCPP)	1570-64-5	
4-chloro-2-methylphenyl sulfate (MCPP-sulfate) (Metabolite of MCPP)	-	
4-chlorocatechol (4-CLcat) (Metabolite of MCPP and DCPP)	2138-22-9	
3-methylcatechol (3-MeCat) (Metabolite of MCPP)	488-17-5	
2,4-dichlorophenol (2,4-DCP) (Metabolite of DCPP)	120-83-2	



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3. EXPERIMENTAL SET-UP

The experimental system consisted of two independent trains, Line B and Line C, each with one MBBR (upstream) and a biofilter downstream (Figure 1).

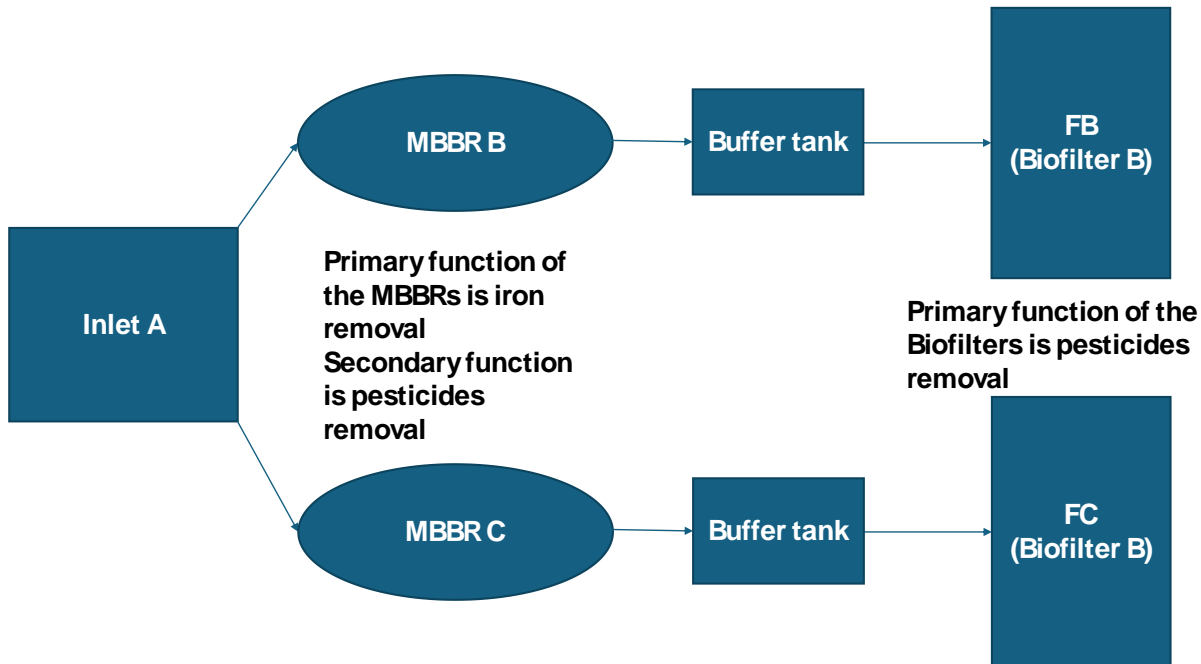


Figure 1. Schematics of the MBBR-biofilter trains, Line B and Line C, where the main functionality of the MBBRs reactors is the removal of iron and the main functionality of the biofilters is the pesticides removal.

Both lines received water from the contaminated site via a rapid sandfilter for de-ironing which was pumped into the inlet A tank.

Moving Bed Biofilm Reactors (MBBRs) provide high specific surface area carriers for aerobic biofilms and are widely used as compact biological reactors to treat municipal and industrial wastewaters and to target micropollutants. Bench/pilot studies show MBBRs can support co-metabolic and catabolic biodegradation of phenoxy herbicides (including mecoprop and other phenoxy-carbonic acid herbicides)—often after a period of acclimation or when a co-substrate is available (Escolà Casas et al., 2017; Nord et al., 2020).

Biofilters (packed-bed biological filters, sand, wood/cork, or biologically active carbon) are effective finishing reactors, combining sorption and extended biofilm contact times that help degrade residual parent compounds and their metabolites. Combining MBBR (bulk removal and transformation) with a downstream biofilter (polishing and metabolite transformation) is a logically coherent pilot approach for contaminated groundwater/leachate (Escolà Casas et al., 2017; Nord et al., 2020). For this study, we established a MBBR followed by a biofilter pilot scale plant. The MBBR reactor consisted of a 300 L cylindrical tank equipped with

an aeration system, a propeller and 20000 carriers. Carriers K5 type (AnoxKaldness, Lund, Sweden), with a surface area of $800 \text{ m}^2 / \text{m}^3$, were used.

The system was fed continuously with Stengaarden water with a hydraulic retention time (HRT) of 10 h. From a common inlet of contaminated water, two parallel treatment lines were established, referred to in the text as Line B and Line C, each comprising one MBBR unit and one biofilter (Figure 1).

A picture of the pilot is shown in Figure 2.



Figure 2. Stengaarden pilot plant

3.1. OPERATING STRATEGY AND START-UP

3.1.1. START-UP & ACCLIMATIZATION:

The pilot was inoculated with activated sludge obtained from a municipal WWTP of Roskilde (Denmark). The MBBR was started at a conservative hydraulic retention time (HRT 8-10 h) and 100 % of hydraulic saturation. For the biofilters, the media (sand) were inoculated with MBBR effluent for 3 months prior to full operational flow. The removal of the parent compounds and metabolites were monitored on a weekly basis during start-up and HRT were adjusted as needed.



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3.1.2. HYDRAULIC TESTING & OPTIMIZATION:

After stable start-up, the HRT on the MBBR was gradually decreased to establish the minimum HRT (to test how far the tank size can be decreased; similarly, the biofilter retention time was varied (8-24 h) to measure the removal for residual parents and metabolites.

3.2. SAMPLING STRATEGY

On weakly basis, duplicate water samples of inlet, MBBR B, MBBR C, Biofilter B and Biofilter C were collected in 40 ml glass vials and stored at -20 °C from summer 2023 until summer 2025. The samples were analysed batchwise approximately every 2 months.

3.3. ANALYSIS OF PHENOXY ACIDS

For the analysis, a Shimadzu Nexera X3 ultrahigh performance liquid chromatographic (UPLC) system (Shimadzu Corporation, Kyoto, Japan) coupled to a Sciex 7500 triple quadrupole mass spectrometer (Sciex, Framingham, MA, USA) was employed. The separation system was fitted with an Acquity UPLC BEH Shield RP18 column (1.7 µm, 2.1 × 30 mm) and a corresponding Acquity UPLC BEH Shield RP18 1.7 µm VanGuard pre-column (Waters, Milford, MA, USA), housed in the LC oven and maintained at 30 °C.

Chromatographic separation was performed under the following conditions: flow rate of 0.3 mL/min; eluent A: water with 0.1% formic acid; eluent B: methanol with 0.1% formic acid. The gradient program was as follows: 0.0–1.0 min, 0% B; 1.0–6.0 min, 100% B; 6.0–7.2 min, 100% B; 7.1–7.9 min, 0% B; and 8.0–10.0 min, 0% B.

Mass spectrometric detection was carried out using an electrospray ionization (ESI) source operated in negative mode, with data acquisition in multiple reaction monitoring (MRM). Table 9 of the Annex summarizes the optimized mass spectrometer parameters and the limits of detection (LOD) and quantification (LOQ) for each compound.

3.4. MONITORING OF GROUNDWATER WELLS

To support this study and have a comprehensive overview of the contamination around the site, 12 groundwater wells (Table 2) were sampled in two sampling campaigns (2024 and 2025). For each well, 1 L of ground water in duplicates was collected in glass bottles and stored at -20°C until analysis.



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Table 2. Wells for groundwater monitoring at Stengaarden dump site (GEUS, 2023)

Well n°	Co-ordinate EUREF89 format	Depth [m]
206.1841	681.166,48 , 6.164.476,67	54
206.1839	681.222,86 , 6.164.451,63	50
206.1838	681.285,12 , 6.164.428,3	58
206.1840	681.336,95 , 6.164.405,67	54
206.1238	681.394,24 , 6.164.379,38	49
206.1316	680.971,44 , 6.164.148,28	20
206.1843	681.218 , 6.164.207	55
206.1822	681.035 , 6.163.409	40
206.1817	681.270 , 6.163.916	28
206.2073	681.379,9 , 6.163.881,2	25
206.2163	681.323,86 , 6.163.980,49	26
206.2164	681.259,94 , 6.164.008,99	26

3.5 CALIBRATION AND DEPLOYMENT OF PASSIVE SAMPLERS

Innovative ceramic passive samplers (CPS) and diffusive gradients in thin films (DGT) have been tested on the Stengaarden pilot plant. For testing the CPS, a calibration with the target compounds naturally present in the ground water was necessary before the deployment test on the pilot plant.



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4. RESULTS

4.1. COMPOUNDS CONCENTRATION IN THE PILOT INLET

The influent concentrations of both MCPP, DCPP and their metabolites were variable over time (Figure 3 and Figure 4), with peaks and fluctuations reflecting realistic operating conditions with a non-constant loading. Despite this variability, the inlet concentrations remained within a comparable range throughout the experimental period, ensuring reliable evaluation of the pilot treatment study. Except for a few sampling events in 2024, inlet concentrations measured between 2023 and 2025 consistently exceeded 0.5 µg/L of the parent compounds and the sum of parent compounds and metabolites usually exceeded 1 µg/L. Additionally, the metabolites 4-Cl-2MePh and 2-MPP are contained in the inflow at considerable concentrations. This finding confirms that the Stengaarden site requires particular attention and targeted interventions in order to safeguard the quality of the underlying groundwater.

From August to November 2023 the inlet concentrations of MCPP, 2-MPP and 4-Cl-2MePh were relatively constant with an average of 0.9 µg/L, 0.18 µg/L and 0.14 µg/L respectively. The DCPP concentrations were 0.13 µg/L, while its metabolites were present in higher amounts, i.e. 1.6 µg/L of 4-ClPh and 1.2 µg/L of 4-CPP. These results indicate that biodegradation is ongoing in the soil/groundwater of the site as well as in the rapid sand filtration unit for de-ironing (especially for DCPP) which is according to the previous study on the same site (Nord et al., 2020).

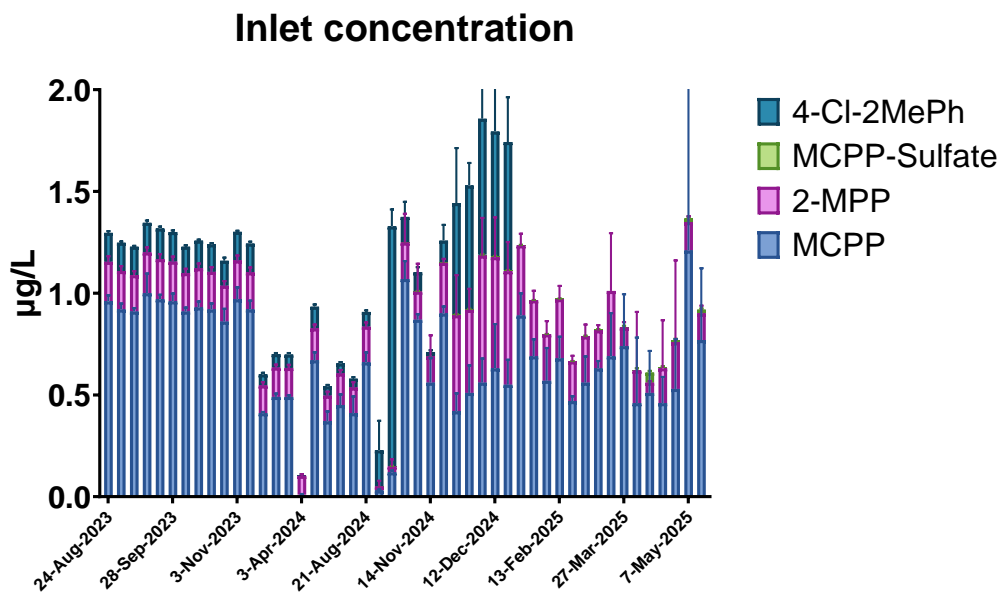


Figure 3. Concentration of MCPP and its metabolites 2-MPP, MCPP-sulfate and 4-chloro-2methylphenol found in the inlet water of the pilot plant. The metabolite 3-methylcatechol was analysed for but not detected during the inlet monitoring.

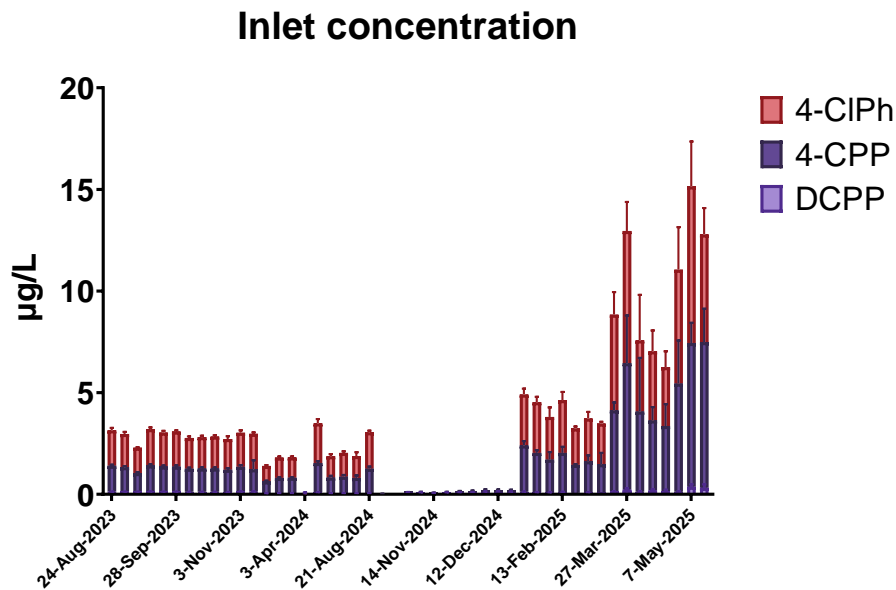


Figure 4. Concentration of DCP and its metabolites 4-chlorophenol and 4-CPP found in the water inlet of the pilot plant. The metabolites 2-CPP, 2,4-dichlorophenol and 4-chlorocatechol were analysed, but not detected during the inlet monitoring.

4.2. OPERATIONAL CHALLENGES

Pre-treatment

Usually, the pilot received the water after quick treatment through a fast sand filter (with the goal to reduce the iron loading). However, starting from November 2023 Region Sjælland installed additional anthracite filters, with the consequence of decreasing inflow concentrations to the upwater pilot; this filter was bypassed after some period.

From February 2024 to May 2025 the inflow concentrations of all compounds rose, in connection to the maintenance of the main plant.

Temperature extreme event: cold winter 2023

During the winter of 2023 exceptionally low temperatures were experienced in Denmark with temperatures of -20°C for two weeks, while normal winter temperatures are between +5 and -5°C. This led to the freezing of the pipe carrying water from the contaminated site to the pilot plant. The pilot plant was shut down for several weeks waiting for the pipe to thaw and for the pumping of contaminated water to resume after the pre-treatment through the first sand biofilter.

MBBRs feeding start-up

At the beginning of the plant start-up, the MBBRs were continuously fed with a glucose-based substrate in order to allow the microbial biomass to grow. The main challenges during this period were, on the one



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hand, that when glucose was supplied, the biomass preferentially consumed it instead of degrading the micropollutants, and on the other hand, that the strong biomass growth increased the oxygen demand, leading to oxygen depletion in the water entering the biofilter.

4.3. COMPOUND CONCENTRATION IN PILOT OUTLET

In the start-up phase from August to October 2023, the outlet concentrations in both trains varied between 0.20–0.85 µg/L for MCPP and between 0.09–0.11 for DCP, with outlet B generally achieving lower values than outlet C, suggesting more consistent removal performance in Line B (Figure 5, Figure 6, Figure 7 and Figure 8).

During the extreme cold and maintenance activities of the main plant (rapid sandfilter, anthracite filter and GAC filter of Region Sjælland) (November 2023 and February 2024), outlet concentrations showed irregular patterns. Outlet B exhibited the lowest concentrations of the period on several occasions (as low as 0.21 µg/L for MCPP), whereas Outlet C frequently remained higher (0.71–0.85 µg/L for MCPP), indicating possible reduced biological activity. DCP fluctuations were limited and within a range of 0.03–0.13 µg/L in both the outlets.

From February 2024, the effluent concentrations displayed a progressive stabilization. In the first quarter of 2024, Outlet B frequently maintained values in the range of 0.20–0.40 µg/L for MCPP, while Outlet C was more variable, ranging from 0.23–0.78 µg/L. This suggests that the recovery of full biological activity was not immediate and that differences in reactor dynamics influenced the outlet water quality (Figure 5 and Figure 6).

To overcome oxygen depletion in the biofilters the substrate amendment was stopped, and additional aeration was amended to the buffer tanks between the MBBR and the biofilters in May 2024. After the upgrade of the aeration system the MCPP concentration of both outlets started to slowly decrease from April to August 2024 (Figure 5 and Figure 6). During late summer and autumn 2024, the lowest outflow concentrations were detected documenting well-functioning systems with outlet C reaching minimum values of 0.10–0.19 µg/L for MCPP.

From January to May 2025, we started to modulate the HRT of the biofilters from 11 to 26 h. As a consequence, the effluent concentrations started to oscillate, with Outlet B typically showing 0.18–0.48 µg/L MCPP while MCPP concentrations in Outlet C ranged between 0.14–0.41 µg/L. Outlet B generally maintained concentrations close to 0.1 µg/L for MCPP and 0.50 µg/L considering MCPP plus its metabolites, indicating an overall effective treatment. However, occasional higher values highlight sensitivity to load or process fluctuations. In Table 10 of the Annex data about HRT (h), temperature (°C) and O₂ (%air saturation) of the outlet of the two monitored biofilters are presented.

A separate discussion is required for DCP. Due to the high loading of its metabolites, 4-CIPh and 4-CPP, in the inlet water, especially in spring 2025 with the sum reaching 12 µg/L, the pilot treatment plant did not achieve concentrations compliant with the drinking water limits of 0.1 µg/L.



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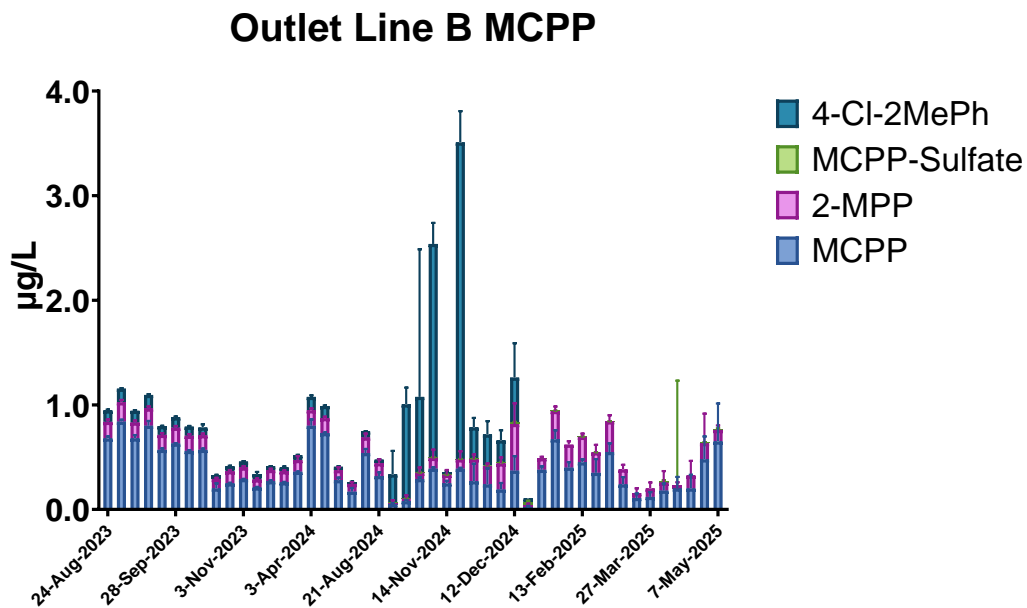


Figure 5. Concentration of MCPP and its metabolites MCPP-sulfate and 4-chloro-2methylphenol found in the water outlet B of the pilot plant. The metabolite 3-methylcatechol was not detected during the monitoring.

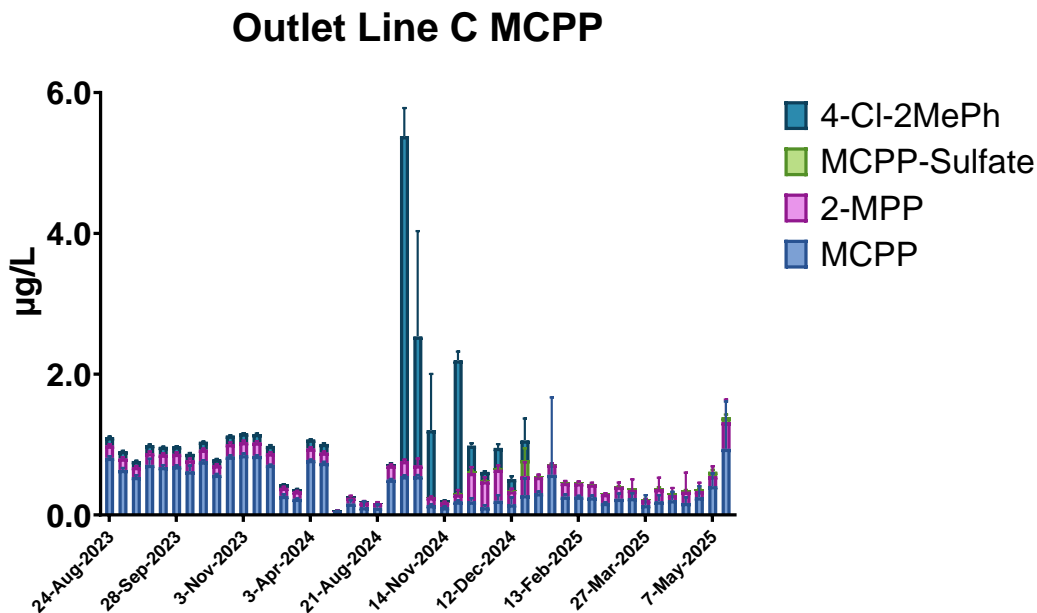


Figure 6. Concentration of MCPP and its metabolites MCPP-sulfate and 4-chloro-2methylphenol found in the water outlet V of the pilot plant. The metabolite 3-methylcatechol was not detected during the monitoring.



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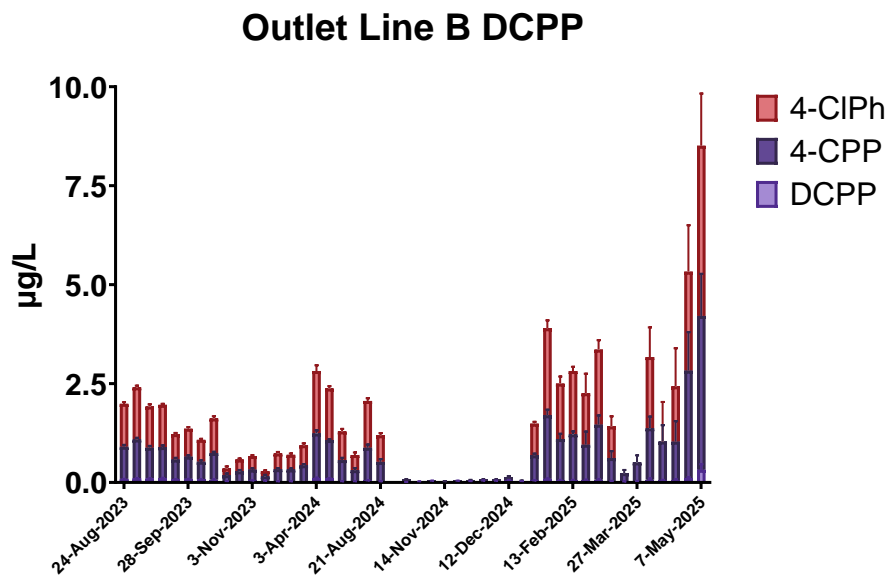


Figure 7. Concentration of DCP and its metabolites 4-chlorophenol and 4-CPP found in the water outlet B of the pilot plant. The metabolites 2-CPP, 2,4-dichlorophenol and 4-chlorocatechol were not detected during the monitoring.

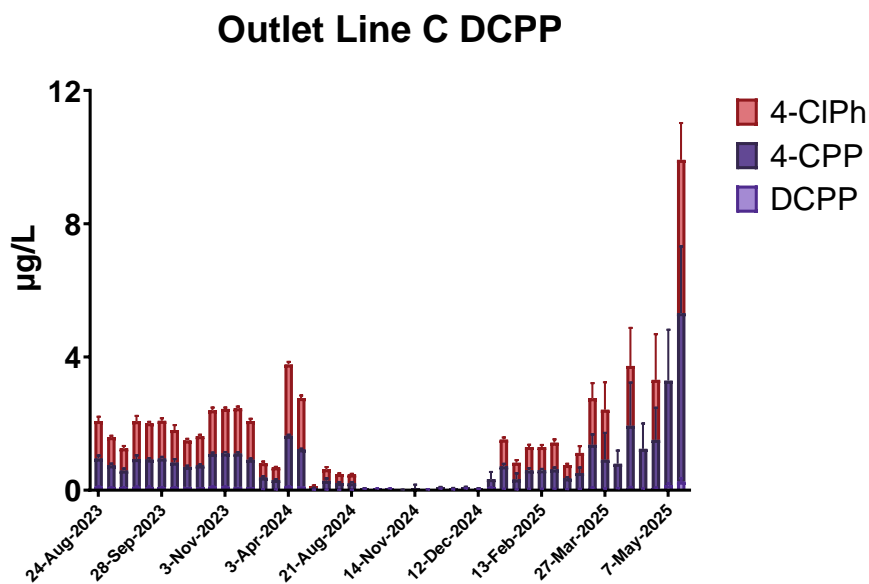


Figure 8. Concentration of DCP and its metabolites 4-chlorophenol and 4-CPP found in the water outlet C of the pilot plant. The metabolites 2-CPP, 2,4-dichlorophenol and 4-chlorocatechol were not detected during the monitoring.



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Among all the monitored metabolites, MCPP-sulfate was the only compound detected in the outlet but not detected in the inlet. However, it appeared intermittently in both outlets, with concentrations ranging from 0.001 to 0.230 µg/L. Its presence in the outlets was first observed in October 2024 and it was mainly produced in Line C. This compound is not new for this site in Denmark, since was previously described by Escolà Casas et al. (2017). The presence of MCPP-sulfate is further discussed in paragraph 3.3.2.

Overall, the data demonstrate that Outlet B consistently achieved lower or comparable concentrations relative to Outlet C, particularly after the maintenance of the main plant in 2024. Seasonal factors (temperature effects on biological activity) and operational disruptions (maintenance stops) appear to have significantly influenced treatment efficiency, especially in Outlet C, which exhibited greater variability. Samples for the analysis of the microbial community on the biofilms composition have been collected, and the analysis is currently in progress. The forthcoming results will contribute to a better understanding of the microbial dynamics within the system and will help explain potential functional differences observed between reactors B and C.

4.4. CONTAMINANT REMOVAL

The removal efficiency of MCPP and DCPP in Lines B and C exhibited strong temporal variability (Figure 9 and Figure 10). In general, Line B showed moderate but more stable removal with respect line C, with values typically ranging from 20–96% for MCPP and from 7–89% for DCPP. Several peaks of higher performance were observed (e.g., 78–96% for MCPP and 66–86% for DCPP) between October and December 2024, and again in March 2025), corresponding to periods of stable operation conditions. At the beginning of the activities on the pilot (August 2023) the removal was generally below 50% because the biomass on the MBBR carriers as well the biomass on the biofilters was not fully grown and adapted. From January to May 2025 we changed the HRT on the biofilters from 11 to 26 h (Table 10 of the ANNEX for the data about HRT, temperature and air saturation). For this reason, in the rest of the chapter, only the results on stable operation conditions are presented.



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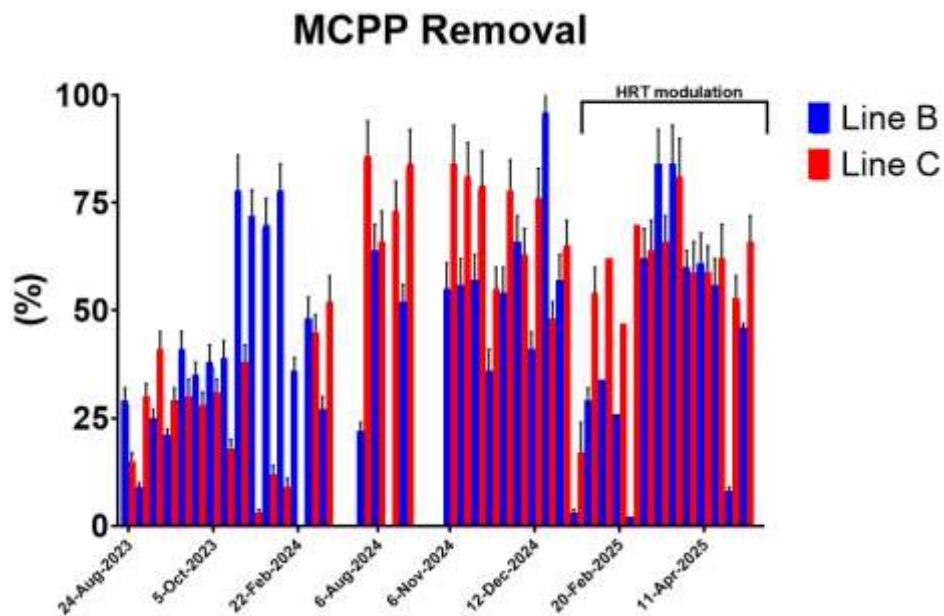


Figure 9. MCPP removal (%) during the entire pilot plant monitoring (2023-2025)

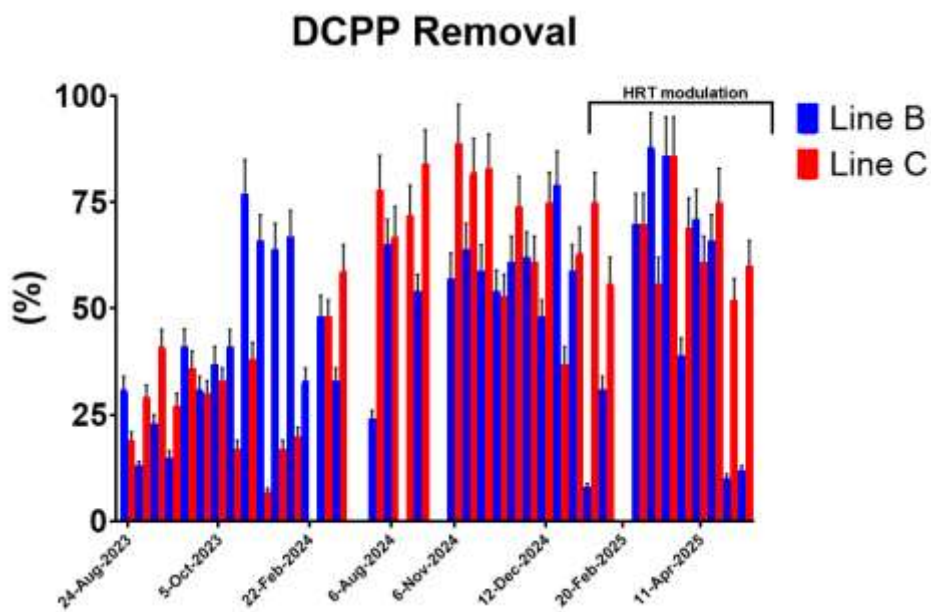


Figure 10. DCPP removal (%) during the entire pilot plant monitoring (2023-2025)



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4.4.1. REMOVAL OF COMPOUNDS AND THEIR METABOLITES DURING STABLE OPERATION CONDITION

The optimal operational conditions, under which the highest removal efficiencies were achieved, occurred during the monitoring periods of October–November 2024 and March 2025, corresponding to a period where the aeration system was upgraded (October 2024) and the HRT of the biofilters were higher (March 2025) (see Table 10 of Annex 1 for HRT, temperature and air saturation data over the time period). For this reason, in the rest of the paragraph the results presented are on these selected periods.

The overall removal of MCPP and DCPD are shown in Table 3 and differ substantially between the two treatment lines. Line B consistently achieved higher parent removal efficiencies compared to Line C, with mean values of $78 \pm 3\%$ for MCPP and $73 \pm 4\%$ for DCPD. In contrast, Line C showed only $51 \pm 4\%$ and $47 \pm 6\%$ removal for MCPP and DCPD, respectively.

Process-level analysis highlights the different contributions of the treatment steps. In Line B, the biofilter was the dominant removal stage, accounting for $59 \pm 5\%$ (MCPD) and $49 \pm 5\%$ (DCPD), while the MBBR contributed more modestly ($23 \pm 4\%$ and $22 \pm 4\%$). Conversely, in Line C the MBBR played a comparatively greater role, with $38 \pm 6\%$ (MCPD) and $42 \pm 5\%$ (DCPD) removal, whereas the biofilter performance was consistently poor ($10 \pm 6\%$ and $11 \pm 6\%$) (Figure 11 and Figure 12).

Although Lines B and C are nominally identical, the systematically poorer performance of Line C can be plausibly attributed to a combination of biological, hydraulic and operational factors. Primary hypotheses include: (1) distinct microbial communities established in the MBBR and/or biofilter of Line C that are less competent at degrading MCPD/DCPD (different community composition, lower abundance of key degraders, or reduced functional gene content); (2) development of anoxic/anaerobic zones in the biofilter of Line C (oxygen limitations or redox stratification) that inhibit aerobic biodegradation pathways; (3) biofilter clogging or channelling causing reduced effective contact time and lower biodegradation.

Table 3. Parent removal (%) under stable and optimal operation conditions and contribution (%) of MBBR reactor and biofilter along Lines B and C. The data were selected based on the best period (best removal), corresponding to a period when the aeration system was upgraded (October 2024) and the HRT of the biofilters were higher (March 2025) (see Table 10 Annex 1 for HRT, temperature and air saturation data over the time period).

Parent removal		Line B	Line C
MCPD	Removal (%)	78 ± 3	51 ± 4
	MBBR	23 ± 4	38 ± 6
	Biofilter	59 ± 5	10 ± 6
DCPD	Removal (%)	73 ± 4	47 ± 6
	MBBR	22 ± 4	42 ± 5
	Biofilter	49 ± 5	11 ± 6



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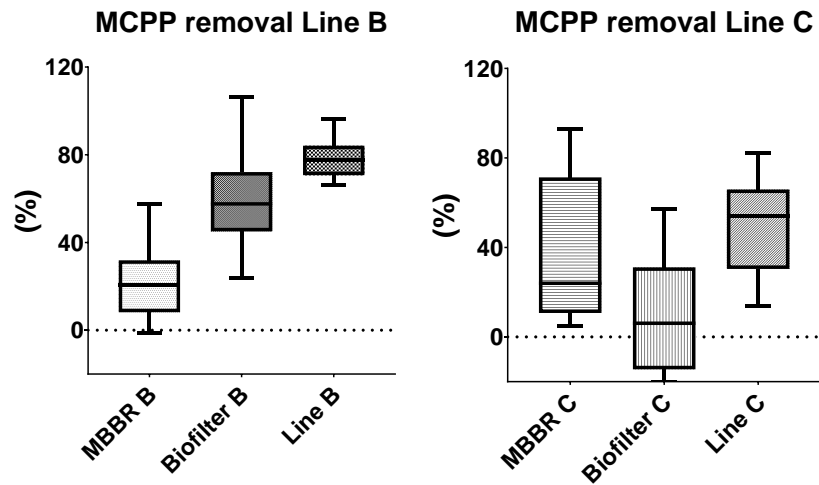


Figure 11. Graphical representation of the MCPP removal (%) in Line B and Line C, showing the contributions of MBBR reactors, biofilters, and the total removal under stable operation conditions. The data in the boxplot are shown using Tukey's method (whiskers represent the 0-95 percentile).

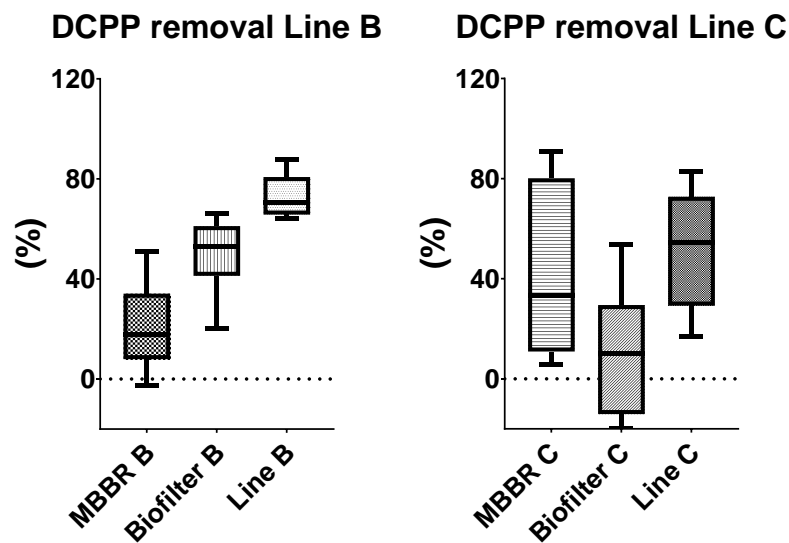


Figure 12. Graphical representation of the DCPD removal (%) in Line B and Line C, showing the contributions of MBBR reactors, biofilters, and the total removal under stable operation conditions. The data in the boxplot are shown using Tukey's method (whiskers represent the 0-95 percentile).



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These findings suggest that the biofilter in Line B was the main driver of overall contaminant reduction, providing robust removal for both compounds. In contrast, the biofilter in Line C appeared to be less effective, limiting the overall removal capacity of that line despite the relatively good performance of its MBBR unit.

Overall, the results indicate a more balanced and efficient treatment in Line B, whereas Line C relied heavily on the MBBR, with limited additional benefit from the biofilter. This asymmetry underscores the importance of MBBRs and biofilters functionality in ensuring stable and high removal of parent compounds.

Looking at the outlet concentrations of MCPP and DCPP (Table 4, Table 5, Figure 11 and Figure 12), the effluent quality of the two parallel treatment lines demonstrates consistent differences despite their nominally identical configuration. Line B outperformed Line C across nearly all target compounds, achieving lower average concentrations and narrower ranges.

For the parent compounds (MCPP, DCPP), Line B exhibited superior removal, with outflows of 0.22 µg/L (MCPP) and 0.06 µg/L (DCPP), compared to 0.40 µg/L and 0.08 µg/L in Line C. These differences are consistent with the previously reported higher parent removal efficiencies in Line B, mainly attributable to the biofilter contribution. For MCPP’s metabolites, both lines showed low effluent concentrations. MCPP-sulfate was below 0.02 µg/L in both cases, while 2-MPP remained at 0.14–0.17 µg/L, indicating that transformation intermediates are effectively attenuated.

For DCPP metabolites (4-CPP, 4-CIPh), both lines demonstrated limited removal, with effluent concentrations above 0.6 µg/L and 1.0 µg/L, respectively. These findings suggest that such chlorophenolic compounds are more persistent under the current treatment conditions, and that further process optimization or additional treatment barriers may be required to ensure robust elimination.

Overall, Line B consistently achieved lower effluent concentrations than Line C, reinforcing the conclusion that the reduced performance of Line C is linked to a suboptimal biofilter function (likely due to microbial

Table 4. Outflow (effluent) concentrations of MCPP and its metabolites of the pilot plant under stable and optimal operation conditions. The data were selected based on the best period (best removal), corresponding to a period when the aeration system was upgraded (October 2024) and the HRT of the biofilters were higher (March 2025) (see Table 10 Annex 1 for HRT, temperature and air saturation data over the time period).

Compound	Outflow (µg/L) Line B	Outflow (µg/L) Line C
MCPP	0.22 (0.05-0.6)	0.40 (0.05-0.9)
2-MPP	0.14	0.17
MCPP-sulfate	0.02	<0.01
4-Cl-2MePh	0.38	0.47



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Table 5. Outflow (effluent) concentrations of DCPP and its metabolites of the pilot plant under stable operation conditions.

Compound	Outflow (µg/L) Line B	Outflow (µg/L) Line C
DCPP	0.06 (<0.03-0.15)	0.08 (<0.03-0.32)
4-CPP	0.65	0.71
4-CIPh	1.03	1.10

community differences, oxygen limitations, or hydraulic effects). At the same time, the persistence of chlorinated phenols in both lines highlights the inherent limitations of the biological treatment process for this compound class.

The overall removal efficiencies observed in this study are generally consistent with ranges reported in the literature for similar biological treatment systems (Nord et al., 2020, Escolà Casas et al., 2017), though notable differences exist between the two lines.

4.4.2. DEPENDENCIES OF MCPP REMOVAL ON HRT, TEMPERATURE, AND DISSOLVED OXYGEN

To determine whether the observed pesticide removal was affected by the air saturation (oxygen level), variations in temperature, or differences in HRT, a series of systematic experiments were carried out on the biofilters. Each variable was independently assessed under controlled conditions to isolate its specific impact. The following sections present the results of these experiments, providing insights into the factors governing the removal process.

Figure 13 and Figure 14 illustrate the *Pearson* correlation matrices obtained from the statistical analysis. The numbers displayed in the matrix cells correspond to the *Pearson correlation coefficients* (r), expressing the strength and direction of the linear relationships among the examined parameters. The statistical significance of these correlations (p -values) is discussed in the accompanying text. The results on the Line B (Figure 13) indicate a strong positive correlation between the removal of MCPP and the air saturation, with a p -value of 0.05. In comparison, although some positive correlation was observed with temperature and hydraulic retention time, these relationships were not statistically significant, suggesting a limited influence of these factors under the conditions tested.

For Line C, a weak positive correlation was observed between the removal of MCPP and air saturation, although this was not statistically significant. No correlation was found with HRT, while a slightly negative correlation was noted with temperature (Figure 14). The results on DCPP show similar correlations and are presented in Annex 1.



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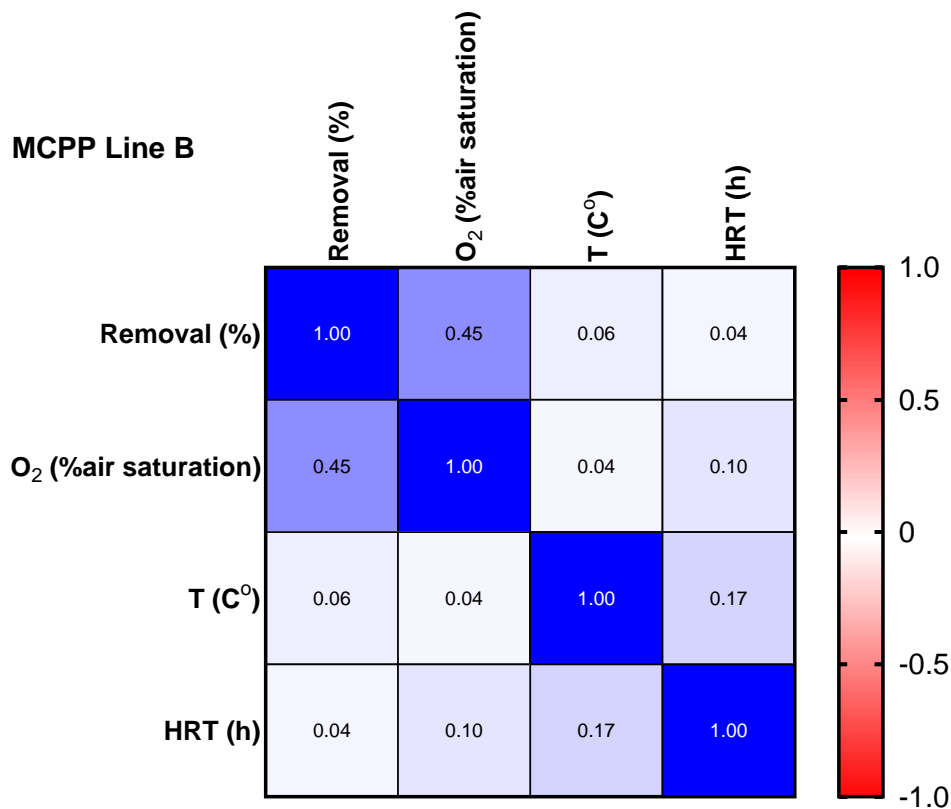


Figure 13. Pearson correlation matrix for Line B showing the relationships between the analyzed variables (Removal, air saturation, water temperature and hydraulic retention time) of MCCP, with colour intensity indicating the strength of correlation (blue = positive, red = negative).

The principal component analysis PCA loadings plot for Line B and Line C (Figure 15) confirmed that Removal (%) and O₂ are closely associated and drive variability along Principal Component 2 (PC2), whereas HRT and Temperature cluster together and contribute mainly to Principal Component 1 (PC1). PC1 explains 71.48% of the cumulative variance and represents the main gradient in operational conditions (HRT and temperature), while PC2 accounts for an additional 21.31%, bringing the total explained variance to 92.79%, and primarily reflects the influence of oxygen availability on removal efficiency. This separation highlights distinct patterns, where oxygen availability relates more directly to removal efficiency, while operational conditions (HRT and T) represent another axis of variation (and they have no effect on the removal). These results are consistent with previously published studies, which have shown that herbicides such as MCCP and DCCP are degraded more effectively under aerobic conditions (Levi et al., 2014, Hedegaard et al., 2014). Another difference between Line B and Line C was observed on the production of the MCCP-sulfate, as shown before on the outlet results (Section 3.2). Figure 16 and Figure 17 show the correlation matrix for MCCP-sulfate in Line B and Line C.



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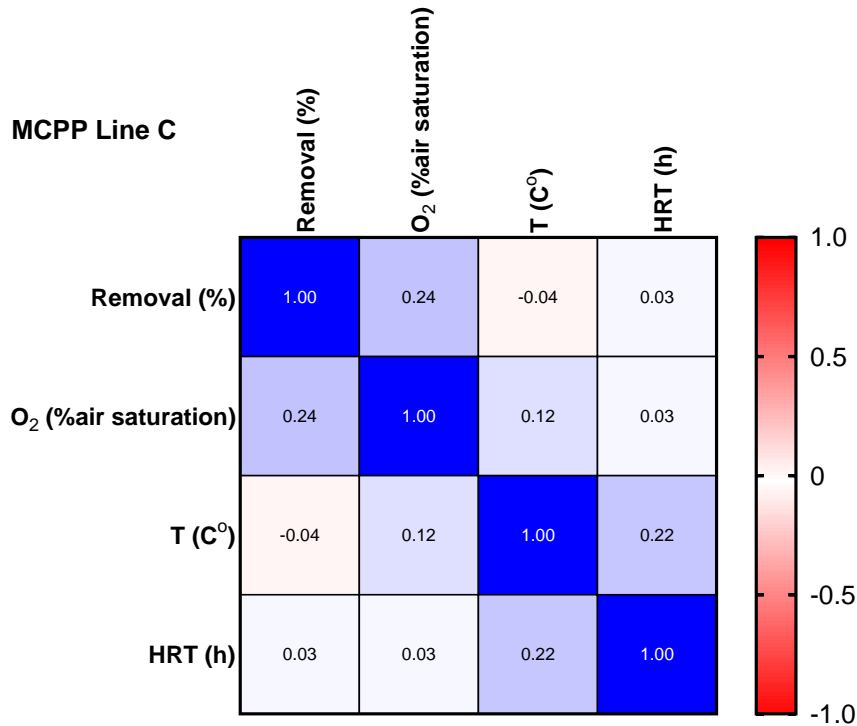


Figure 14. Pearson correlation matrix for Line C showing the relationships between the analyzed variables (Removal, air saturation, water temperature and hydraulic retention time) of MCPP, with colour intensity indicating the strength of correlation (blue = positive, red = negative).

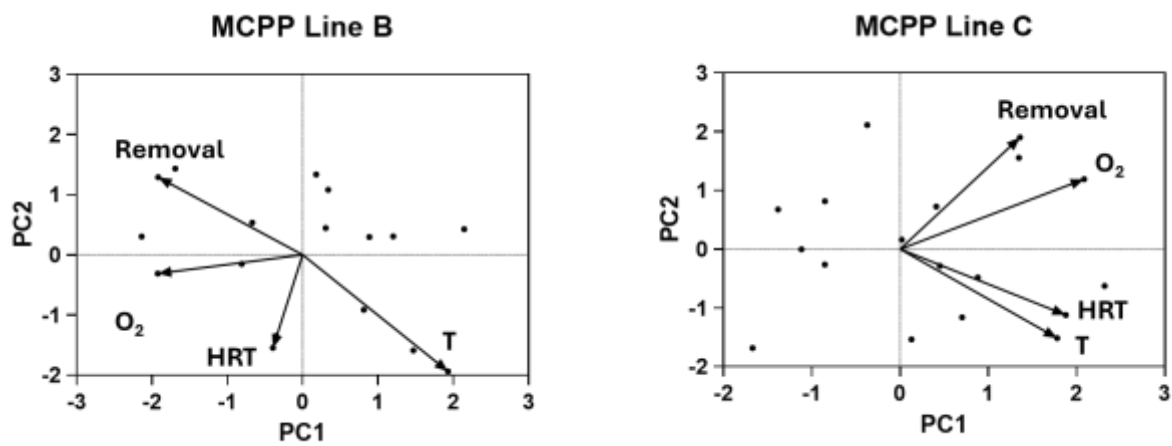


Figure 15. PCA Score and loadings plot showing how the variables Removal (%), O₂ (air saturation), HRT (h), and Temperature (°C) contribute to the principal components (PC) in Lines B and C.



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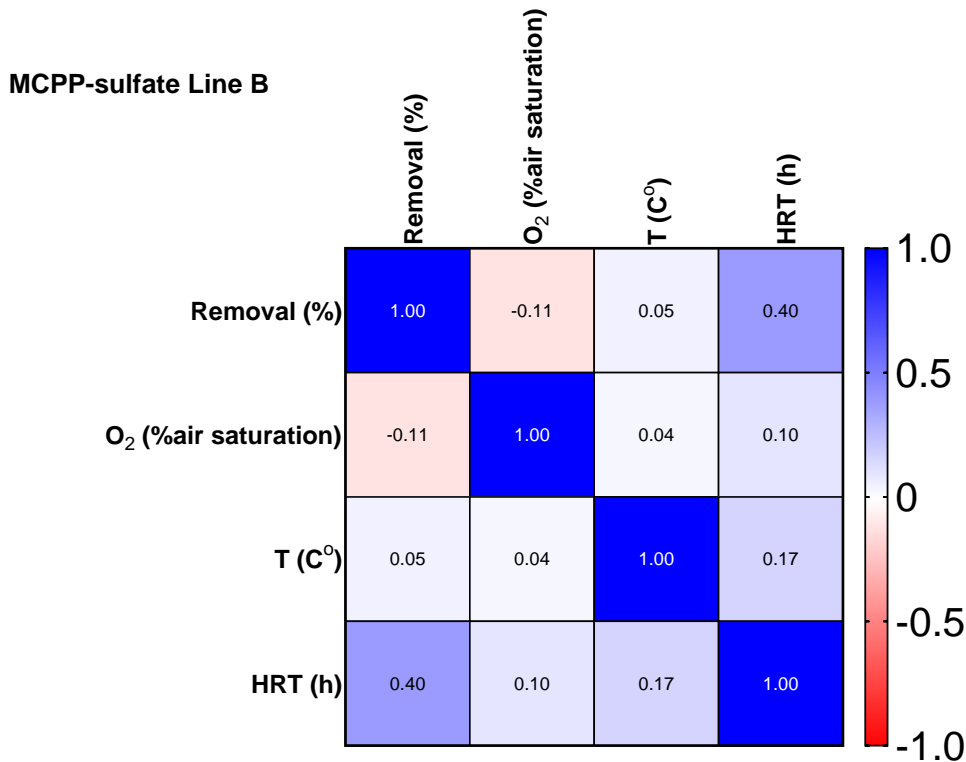


Figure 16. Pearson correlation matrix for Line B showing the relationships between the analyzed variables (Removal, air saturation, water temperature and hydraulic retention time) of MCPPP-sulfate, with colour intensity indicating the strength of correlation (blue = positive, red = negative).

The results indicate that, unlike the parent compound MCPPP, which degrades more effectively under aerobic conditions, the metabolite MCPPP-sulfate removal is positively correlated with HRT in Line B and with T in Line C, showing an opposite pattern relative to the parent compound. MCPPP-sulfate is possibly formed in higher concentrations in Line C which is the line with less overall parent compound removal. This could suggest that the metabolites accumulate into the biofilter when the parent is slowly degraded. Furthermore, the removal of the metabolites is positively related to HRT, suggesting that the suboptimal removal in biofilter C could be related to some HRT issues (like water stagnation, which was observed on top of the Biofilter B while less so on Biofilter C several times during the pilot plant monitoring). Alternatively, the presence of MCPPP-sulfate may be related to a metabolization product of fungi (Escolà Casas et al., 2017).



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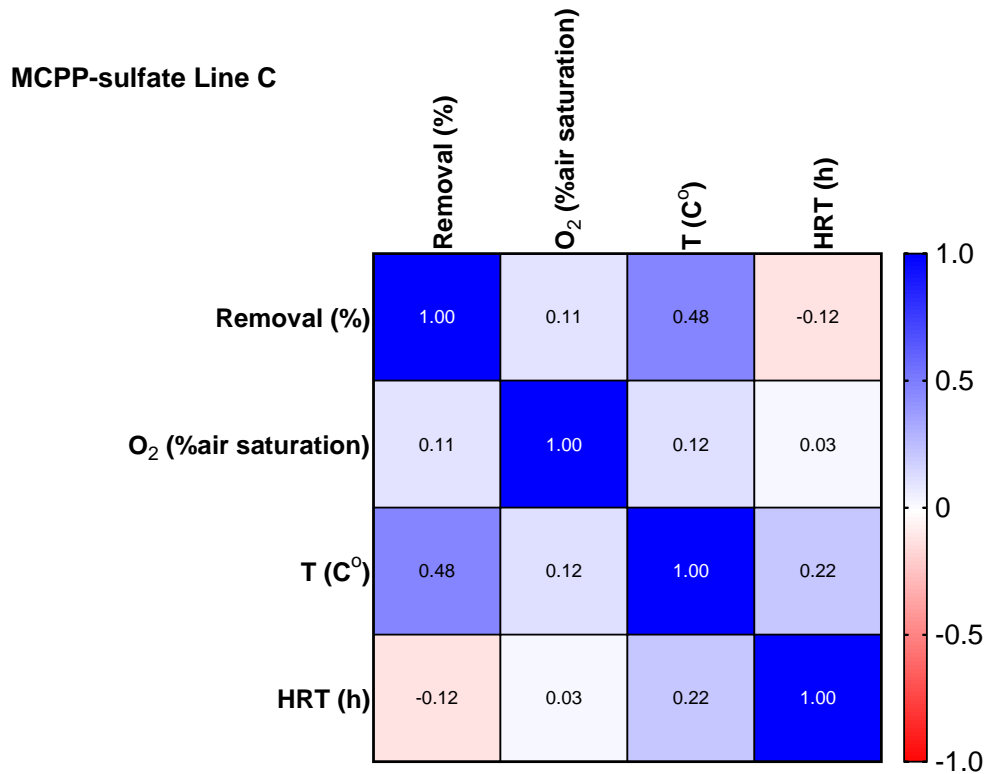


Figure 17. Pearson correlation matrix for Line C showing the relationships between the analyzed variables (Removal, air saturation, water temperature and hydraulic retention time) of MCPP-sulfate, with colour intensity indicating the strength of correlation (blue = positive, red = negative).

4.5. WELL MONITORING

According to the results of the 2024 and 2025 groundwater monitoring campaigns (Table 6 and Table 7), the overall contamination pattern remains largely consistent across the investigated wells. The highest concentrations of phenoxy herbicides and phenolic compounds were observed in wells 206.1838, 206.1839, and 206.1841, while well 206.1843- previously showing elevated levels in 2024- was not sampled in 2025.

The dominant compounds continue to be Mecoprop, 4-Chloro phenol, and the chlorophenoxy acids 2-CPP and 4-CPP, confirming the persistence of phenoxy herbicide contamination in the area.

In summary, wells 206.1838 and 206.1839 remain the most impacted by the contamination, while 206.1841 shows increasing trends that warrant closer monitoring since these wells are 500 m far from the landfill. The remaining wells display stable or decreasing concentrations, indicating that contamination may be spatially limited or that natural attenuation processes are occurring. Overall, the monitoring results indicate



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some minor upward trends in contaminant concentrations in wells situated directly above the landfill (206.1817, 206.2073, 206.2163, and 206.2164), but no substantial increase or major breakthrough was detected over the monitoring period. Continued surveillance of these wells is recommended to confirm whether the observed concentrations reflect residual contamination or ongoing leaching processes.

Table 6. Well monitoring results (µg/L) from the 2024 campaign

Well	sampling date	Mecoprop	Dichloroprop	2,4-Dichlorophenol	2-(2-methylphenoxy)propionic acid (2-MMP)	4-Chlorophenol	2-(2-chlorophenoxy)propionic acids (2-PP)	2-(4-chlorophenoxy)propionic acids (4-CPP)	4-chloro-2-methylphenol	Mecoprop sulphate (MCPSP_sulf)	3-methylcatechol (3-MeCat)	4-chlorocatechol (4-CLCat)
206.1841	07-05-2024	1.22	0.42	0.44	0.25	0.55	0.28	0.38	1.26	<0.005	<0.005	<0.005
206.1839	07-05-2024	2.33	0.27	0.32	0.28	4.50	2.22	2.83	2.46	<0.005	<0.005	<0.005
206.1838	07-05-2024	3.52	0.02	< LOQ	0.15	11.90	5.53	6.96	3.88	<0.005	<0.005	<0.005
206.1840	07-05-2024	0.39	0.12	0.13	0.08	0.89	0.46	0.61	0.38	<0.005	<0.005	<0.005
206.1238	07-05-2024	<0.00025	<0.0004	<0.005	< LOD	< LOD	< LOD	< LOD	< LOD	<0.005	<0.005	<0.005
206.1316	out of service	-	-	-	-	-	-	-	-	<0.005	<0.005	<0.005
206.1843	03-05-2024	1.93	0.27	0.34	0.24	6.86	3.27	4.18	1.80	<0.005	<0.005	<0.005
206.1822	out of service	-	-	-	-	-	-	-	-	<0.005	<0.005	<0.005
206.1817	07-05-2024	0.55	<0.0004	0.06	0.28	1.86	0.92	1.21	0.56	<0.005	<0.005	<0.005
206.2073	07-05-2024	0.06	<0.0004	<0.005	< LOQ	0.12	0.06	0.08	< LOQ	<0.005	<0.005	<0.005
206.2163	07-05-2024	0.54	0.07	0.07	0.16	1.77	0.87	1.15	0.54	<0.005	<0.005	<0.005
206.2164	07-05-2024	1.00	0.15	0.17	0.24	3.19	1.62	2.10	1.00	<0.005	<0.005	<0.005



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Table 7. Wells monitoring results (µg/L) from the 2025 campaign

Well	samp ling date	Mecop rop	Dichlop rop	2,4- Dichl oro phen ol	2- (2meth yl pheno xy) propa noic acid (2- MMP)	4- Chl oro ph en ol	2-(2- chloroph enoxy) propionic acids (2-CPP)	2-(4- chloroph enoxy) propioni c acids (4-CPP)	4 chloro 2 methyl phenol	Mecop rop sulpha te (MCP _sulf)	3- methyl catech ol (3- MeCat)	4-chloro catechol (4- CLCat)
206.1 841	03- 07- 2025	1.04	0.17	0.18	0.24	4.1 9	2.33	0.07	2.13	<0.005	<0.005	<0.005
206.1 839	03- 07- 2025	2.40	0.31	0.33	0.11	4.0 6	2.20	0.08	2.28	<0.005	<0.005	<0.005
206.1 838	03- 07- 2025	2.74	0.02	<0.0 05	0.06	7.8 9	4.29	0.12	6.69	<0.005	<0.005	<0.005
206.1 840	03- 07- 2025	0.07	0.02	0.07	<0.005	<0. 005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
206.1 238	03- 07- 2025	<0.000 25	<0.0004	<0.0 05	<0.005	<0. 005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
206.1 316	out of servic e	-	-	-	-	-	-	-	-	-	-	-
206.1 843	not sampl ed	-	-	-	-	-	-	-	-	-	-	-
206.1 822	out of servic e	-	-	-	-	-	-	-	-	-	-	-
206.1 817	03- 07- 2025	0.40	0.40	0.09	0.15	1.2 5	0.72	0.01	0.90	>0.005	<0.005	<0.005
206.2 073	03- 07- 2025	<0.000 25	<0.0004	<0.0 05	<0.005	<0. 005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
206.2 163	03- 07- 2025	0.84	0.19	<0.0 05	0.23	2.6 4	1.46	< LOD	0.04	<0.005	<0.005	<0.005
206.2 164	03- 07- 2025	1.18	1.18	0.21	0.20	2.5 5	1.41	0.03	0.88	<0.005	<0.005	<0.005



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4.6. PASSIVE SAMPLERS

4.6.1. CPS CALIBRATION AND DEPLOYMENT

The calibration of CPS was carried out in naturally contaminated landfill leachate water to determine uptake kinetics, diffusion coefficients, and compound-specific sampling rates (Table 8). Calibration experiments demonstrated a linear accumulation of all target chlorophenoxy pesticides and metabolites over a 19-day period, confirming that the system operated within the kinetic uptake phase. The relationship between accumulated mass and deployment time showed high reproducibility ($R^2 = 0.92\text{--}0.98$), indicating predictable diffusion-controlled behavior. Compound-specific diffusivities ranged from 0.48×10^{-6} to $4.21 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$, while sampling rates (R_s) varied between 1.17 and 2.47 mL day^{-1} (Table 8).

Field deployment of the CPS was conducted at the Stengaarden NBS pilot treatment plant to evaluate sampler performance under real environmental conditions. Six CPS units were deployed for seven days—two in the inlet tank (untreated water) and four in the outlets of the two biofilters—concurrently with grab sampling for comparison. The CPS effectively accumulated all target analytes, yielding results statistically equivalent to those obtained from grab samples ($p > 0.05$) (Figure 18). For low-concentration compounds such as Dichlorprop and 2-MPP, the CPS demonstrated improved detection sensitivity, enabling quantification where grab samples were below the analytical LOQ. These findings confirm that CPS provide reliable time-integrated measurements, capable of capturing trace-level variations and complementing conventional grab sampling approaches in long-term groundwater and leachate monitoring programs.

Table 8. Calibration parameters for the compounds found in leachate water. k is the CPS uptake constant, R^2 is the coefficient of determination, diffusivity (D_e) and sample rate (R_s) obtained with eq. published by Vrana et al., 2025 and Chen et al., 2018.

Compound	k [ng/d]	R^2	D_e [$10^{-6} \text{ cm}^2/\text{s}$]	R_s [mL/d]
Mecoprop (MCP)	1.224	0.98	4.21	2.47
Dichlorprop (DCPP)	0.144	0.98	0.48	1.60
2-(2 methyl phenoxy) propanoic acid (2-MPP)	0.264	0.98	0.89	1.68
4-chlorophenol (4-CIPh)	1.104	0.93	3.76	1.17
Chlorophenoxy propionic acid (CPP)	0.936	0.92	3.16	1.77
4-chloro-o-cresol (4-CICr)	0.936	0.94	3.19	1.40



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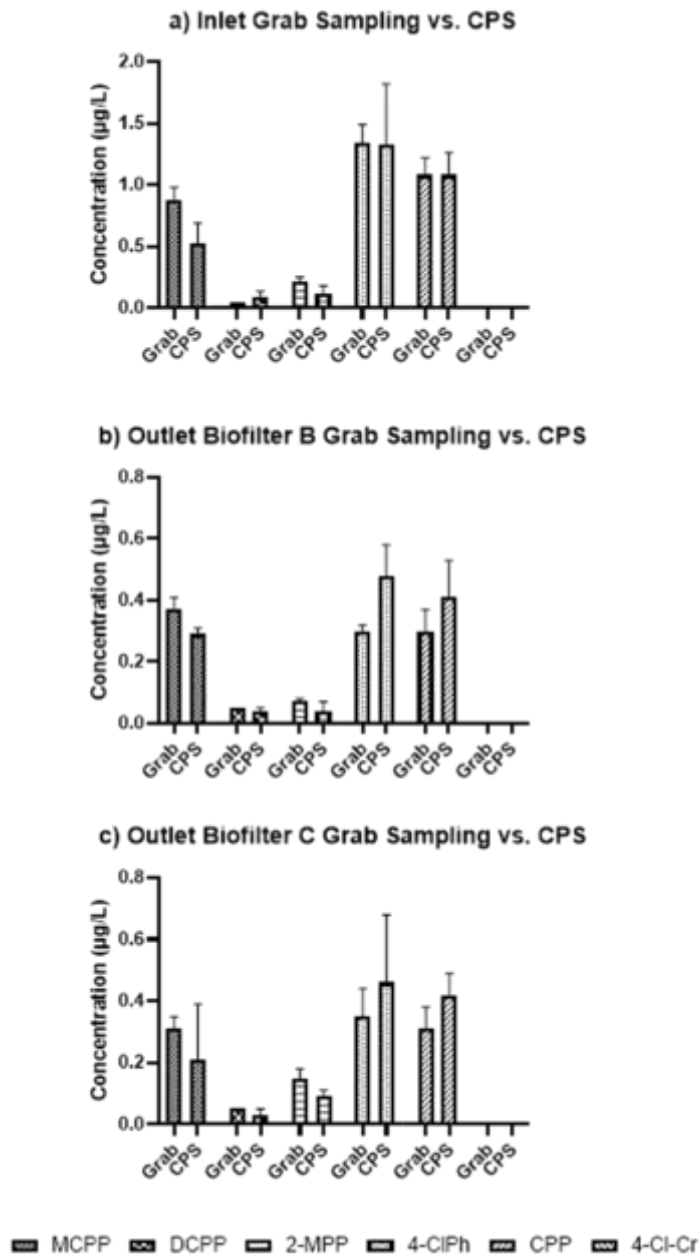


Figure 18. Comparison of the concentration of compounds of interest between grab sampling and CPS deployed in the pilot treatment plant of Stengaarden between 17/01/2025 and 23/01/2025: a) tank of the untreated water (inlet); b) and c) outlet of the 2 biofilter (B and C) placed at the end of treatment pilot plant. N grab sampling = 4, N CPS= 2.



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4.6.2. DGT DEPLOYMENT

The DGTs were deployed in the inflow tank and in the two outflow tanks of the MBBRs. Inflow and outlet display high Mn concentrations, while Co, Cr, Ni, and Zn exhibit moderate levels (Figure 19 and Figure 20).

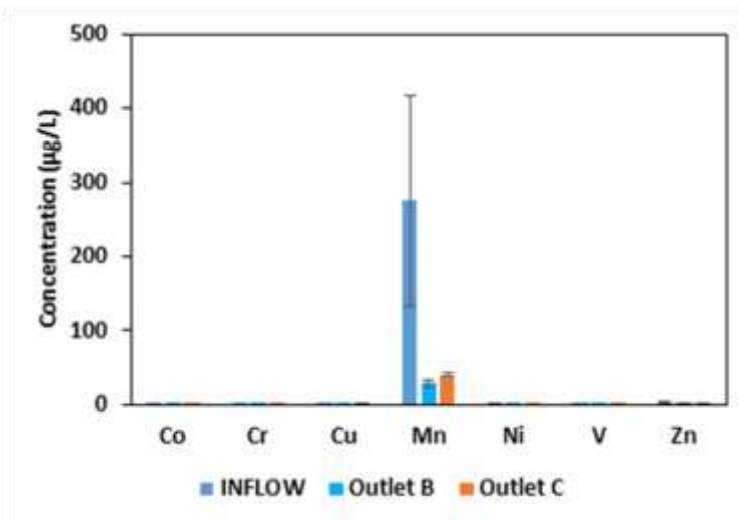


Figure 19. Concentration of Mn in the MBBR in and outlet water, as measured by DGT.

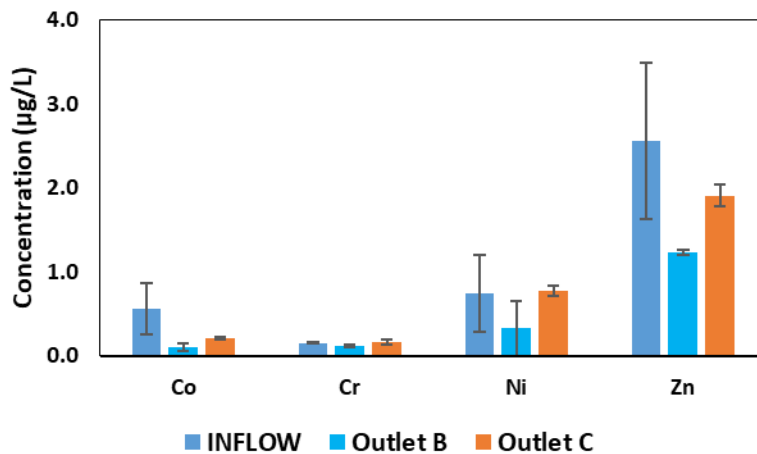


Figure 20. Concentration of Co, Cr, Ni, and Zn in the MBBR in and outlet water, as measured by DGT.



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Outlet B and C display lower Mn, Co, Ni and Zn compared to inflow samples. Overall, the data reveal variable metal distributions, reflecting differences between inflow and MBBR samples, and highlight the heterogeneity of metal behaviour within the system.

All possible trace elements were evaluated using DGT passive samplers to determine labile metal concentrations in Outlet B and C. The results revealed marked differences in metal behaviour between both systems. Manganese (Mn) showed the highest apparent removal, with mean reductions of 91.6% in MBBR B and 88.5% in MBBR C, reflecting strong immobilization or decreased lability (Figure 19). Cobalt (Co) also exhibited substantial decreases, particularly in MBBR B (85.8%), while reactor C showed slightly lower reductions (71.7%). Nickel (Ni) and zinc (Zn) presented moderate declines, with removals of 67.1% and 58.6% in MBBR B, and 21.8% and 35.7% in MBBR C, respectively. In contrast, chromium (Cr) displayed minimal, suggesting potential remobilization or differing speciation dynamics (Figure 20). Overall, the DGT-derived labile metal data reveal a more effective attenuation of trace metals in MBBR B, likely associated with more favourable conditions promoting metal sequestration or decreased DGT-labile fractions.

4.7. COMPOUND SPECIFIC STABLE ISOTOPE ANALYSIS

Compound-specific isotope analysis (CSIA) provides direct evidence of contaminant degradation and transformation pathways, independently of concentration levels or the detection of transformation products—even when such products are unknown (Hunkeler et al., 2008). Despite its potential, the application of CSIA to contaminants of emerging concern at field scale remains limited. This is primarily due to challenges associated with low analyte concentrations and high polarity, which hinder detection, as well as matrix interferences and chromatographic resolution issues that compromise measurement precision (Elsner & Imfeld, 2016; Blessing & Baran, 2022; Hofstetter et al., 2024).

To overcome these limitations, the UPWATER project developed CSIA methods for selected phenoxyalkanoic herbicides and their transformation products, among others. As detailed in Deliverable D2.4, a method involving derivatization with trimethylsulfonium hydroxide (TMSH) followed by carbon isotope analysis via gas chromatography–isotope ratio mass spectrometry (GC-IRMS) was successfully established for mecoprop (MCP), dichlorprop (DCPP), and 2-(4-chlorophenoxy)-propionic acid (4-CP). This method has since been optimized to include additional compounds such as 4-chloro-2-methylphenol (4-Cl-2MePh) and 2,4-dichlorophenoxyacetic acid (2,4-D). Two derivatization techniques are currently available: methylation with TMSH and silylation with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA).

Given the low concentrations of target compounds in environmental waters, extraction and preconcentration steps are required prior to CSIA. A solid-phase extraction (SPE) method was developed and adapted from Spahr et al. (2013) and Milosevic et al. (2013) for coupling with CSIA. This method employs divinylbenzene N-vinylpyrrolidone copolymer cartridges (Oasis HLB, Waters), which are pre-rinsed with ethyl acetate, conditioned with methanol, and rinsed with Milli-Q water adjusted to pH 2. Water samples, also adjusted to pH 2, are loaded at approximately 5 mL/min. After rinsing and drying under vacuum, elution is performed using ethyl acetate and dichloromethane. The eluates are evaporated to dryness, reconstituted, derivatized, and analysed for concentration and isotopic composition.



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The SPE-CSIA procedure was evaluated for extraction efficiency and potential isotope fractionation using water samples spiked with phenoxy acid standards of known carbon isotope ratios. For instance, Figure 21 presents carbon isotope results from tests assessing the effects of pH adjustment (Figure 21A) and sample volume (Figure 21B). Due to the acidic nature of phenoxy acids (pKa 3–4), acidification to pH 2 enhanced analyte retention and minimized isotope fractionation (Figure 21A). Furthermore, up to 10 L of bottled water spiked at 1 µg/L were successfully extracted without isotopic fractionation (Figure 21B).

Carbon isotope values are expressed in per mil (‰) using the delta notation (δ) relative to the Vienna Pee Dee Belemnite standard:

$$\delta \text{ (in ‰)} = (R/R_{\text{std}} - 1) \quad (1)$$

where R and Rstd represent the isotope ratios (i.e., $^{13}\text{C}/^{12}\text{C}$) of the sample and the standard, respectively.

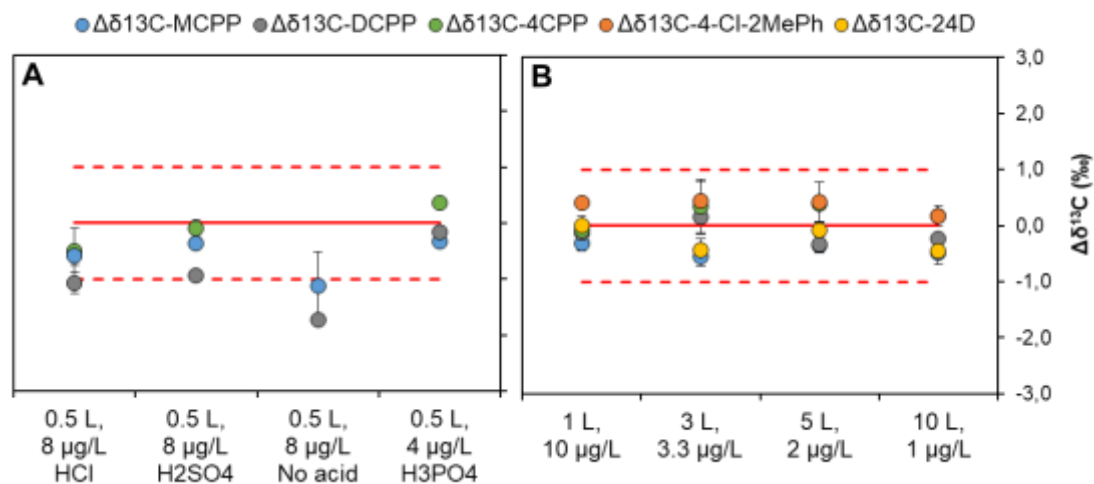


Figure 21. SPE-CSIA extraction tests with phenoxy acid standards to assess carbon isotope fractionation. (A) Effect of water acidification during SPE-methylation-GC-IRMS; (B) Effect of sample volume during SPE-silylation-GC-IRMS. Accuracy ($\Delta\delta^{13}\text{C}$) is reported as the deviation of $\delta^{13}\text{C}$ values from Elemental Analyzer (EA)-IRMS measurements of the standards. The red line indicates $\Delta\delta^{13}\text{C} = 0$ ‰, with dashed lines representing ± 1 ‰ uncertainty. Error bars reflect propagated uncertainty from replicate measurements.

Following successful lab-scale validation across different water matrices, the method was tested on water from the pilot inlet at the Stengaarden site. Although up to 6 L were extracted, due to the current phenoxy acid levels (see Section 4.1), concentrations of phenoxy acids in the extracts remained below CSIA detection. Additionally, trace compounds such as benzenesulfonamide and phthalates—likely originating from co-pollution from the dumpsite, pilot materials and sampling systems—were co-enriched, impairing chromatographic separation (Figure 22). Although further improvements could have been tested, such as optimizing elution or establishing clean-up procedures, the current phenoxy acid levels, the limitations of shipping large volumes of water and the project's timeline constraints led to the decision not to pursue further large volume SPE-CSIA applications at this site.



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Finally, the feasibility of coupling CSIA with CPS is currently under evaluation. However, the low concentrations of phenoxy acids in the pilot inlet—significantly lower than anticipated, due to the addition of a sandfilter to the current treatment process—pose substantial challenges. Based on CPS calibration data (Section 4.6.1) and current CSIA detection limits, reliable measurements at even 1 µg/L would require deploying more than 10–15 CPS units per sampling point over 30 days. Consequently, CPS-CSIA was not implemented for monitoring at Stengaarden.

Nevertheless, specific CPS-CSIA calibration experiments were recently conducted using a modified CPS design to determine compound-specific sampling rates and assess potential isotope fractionation for selected compounds. Key modifications included: (1) increasing the sorbent mass from 200 mg to 1 g of Oasis HLB within the ceramic membrane, and (2) extending deployment duration from 19 to 35 days. Target analytes included DCPP, 4-CPP, 4-Cl-2MePh, and 2,4-D, among others. Extraction from the modified CPS involved transferring the recovered sorbent into polypropylene cartridges with polyethylene frits, followed by drying and elution of the organic contaminants.

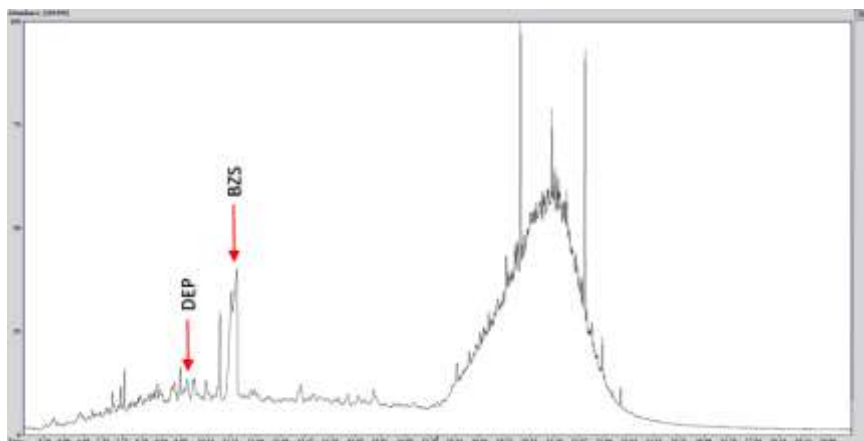


Figure 22. Methylation-GC-MS chromatogram of extract from 6 L of pilot inlet water (SPE with 500 mg Oasis HLB). Phenoxy acids (0.1–0.6 µg/L) were below detection limits. Peaks of benzenesulfonamide (BZS) and methylated phthalates (e.g., diethyl phthalate, DEP) were observed.

Due to delays in CPS production and prioritization of other project tasks, the calibration experiments had to be postponed until summer 2025. Figure 23 shows a picture of the two performed experiments, one spiked at 20 µg/L and another at 4 µg/L, with the former conducted under static renewal calibration (i.e., a closed system with a renewal of the contaminated water at certain interval times). In both cases, tanks were maintained at laboratory temperature and covered with aluminium foil to prevent photodegradation and algal development. Duplicate and quadruplicate CPS samples were collected from the experiments at 20 µg/L and at 4 µg/L, respectively, on days 0, 7, 14, 21, 28 and 35. Water samples for concentration and isotope analyses were collected at time 0 and at the final time for the experiment at 4 µg/L, and at time 0 and at each water renewal event for the experiment at 20 µg/L.



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Figure 23. Experiments for CPS-CSIA calibration and validation.

Although HPLC-MS/MS results have not been provided yet for these experiments, preliminary outlines were obtained by using derivatization-GC-MS measurements. A linear accumulation of all target analytes was observed over the 35-day period for both experiments, confirming that the system operated within the kinetic uptake phase with a predictable diffusion-controlled behaviour. For the experiment at 20 µg/L, in which the extracts of two modified CPS units are combined for each sampling time, the relationship between accumulated mass and deployment time showed high reproducibility ($R^2 = 0.92\text{--}0.99$). Preliminary compound-specific sampling rates (R_s) for this experiment varied between 2.0 and 3.5 mL/day for DCP, 4-CPP and 2,4-D. These values are slightly higher than those obtained with the normal CPS configuration (Table 8), although still too low for some analytes for robust application into the Stengaarden pilot at the current concentration levels.

Regarding isotopic results of these experiments, it is noteworthy that, independently of the accumulated mass, the CPS-CSIA method gave insignificant or reproducible method-induced isotope fractionation during the 35-day deployment period. For example, Figure 24 shows how the $\delta^{13}\text{C}$ values for 4-CPP from both water and CPS remained within ± 0.5 ‰ of the signature of the spiked standard throughout the exposure time. These results validate the use of CPS coupled to CSIA for measuring compound specific isotopic composition of the target phenoxyacids, although further work is required for optimizing the approach to increase sampling rates for allowing its application into the Stengaarden site at the current concentration levels.



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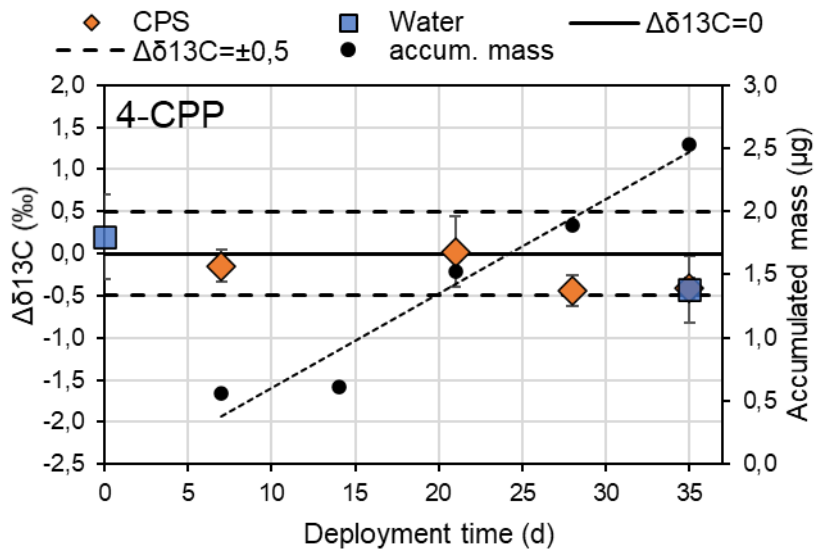


Figure 24. Changes over deployment time of the deviation of carbon isotope composition of 4-CCP in water and CPS samples ($\Delta\delta^{13}\text{C}$, ‰) from the EA-IRMS measurements of the standard used for spiking the experiment at 20 $\mu\text{g/L}$. The solid line indicates $\Delta\delta^{13}\text{C} = 0$ ‰, with dashed lines representing $\pm 0,5$ ‰ uncertainty. Error bars reflect propagated uncertainty from replicate measurements. Analyte mass (μg) accumulated in the two CPS units for each sampling time is also shown (preliminary data from derivatization-GC-MS measurements).



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5. CONCLUSION

This study demonstrates that combined biological treatment using Moving Bed Biofilm Reactors (MBBRs) and biofilters can achieve significant removal of phenoxy acid herbicides, particularly mecoprop (MCP) and dichlorprop (DCPP), under realistic groundwater contamination conditions.

The results highlight that:

- 80% removal for both parent compounds and their metabolites by biofiltration units is feasible.
- Winter operation under normal winter conditions is possible anticipating a small decline in removal.
- Seasonal temperature changes influence the performance of the biofilters.
- Aerobic conditions, ensured by sufficient oxygen supply, are critical to maximize the degradation efficiency.
- Biofilters play a decisive role in the attenuation of parent compounds, while MBBRs contribute primarily controlling particle (especially iron oxides) loading to the biofilters.
- Certain metabolites, especially chlorinated phenols, remain persistent and require further optimization or additional treatment steps.
- Groundwater monitoring campaign (2024–2025) confirmed that contamination by phenoxy herbicides and phenolic compounds persists in specific wells close to the landfill, while concentrations remain stable or decreasing in surrounding wells, suggesting that pollution is spatially limited and that natural attenuation processes may be occurring.
- CPS provided reliable, time-integrated measurements of phenoxy herbicides and their metabolites, showing good agreement with grab samples and enhanced sensitivity for low-concentration analytes.
- DGT samplers allowed the assessment of labile metal fractions in pilot effluents, revealing marked differences between treatment lines. Reactor B demonstrated higher attenuation of trace metals (notably Mn, Co, Ni, and Zn) compared to Reactor C, indicating more favorable conditions for metal sequestration and reduced lability.

Overall, the findings confirm that MBBR–biofilter treatment can provide a viable remediation strategy for pesticide-contaminated groundwater. Depending on starting conditions and required targets further development might be needed.



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ANNEX 1 – SUPPLEMENTARY MATERIAL

Table 9. Mass-spectrometry conditions for the identification and quantification of compounds of interest.

Compound Name	MRM [Da]	RT (min)	CE (V)	CXP (V)	R ²	LOD (ng/L)	LOQ (ng/L)
Mecoprop (MCP)	213.0575 → 141.0106	6.14	-21	-10	0.9997	0.10	0.25
	215.0252 → 143.0009		-23	-9	0.9997	-	-
Dichlorprop (DCP)	233.008 → 160.9598	6.20	-20	-15	0.9988	0.10	0.40
	234.9946 → 162.9516		-20	-15	0.9990	-	-
2-(2-methyl phenoxy) propanoic acid (2-MPP)	179.058 → 107.0609	5.65	-24	-7	0.9956	0.8	2.50
	179.058 → 71.0104		-15	-5	0.9980	-	-
2-(2-chlorophenoxy) propanoic acid (2-CP)	200.0372 → 128.0133	5.71	-27	-9	0.9878	0.20	0.70
	200.0372 → 127.0158		-24	-7	0.9873	-	-
2-(4-chlorophenoxy) propanoic acid (4-CP)	199.0136 → 126.9972	5.56	-20	-11	0.9965	0.20	0.70
	201.001 → 129.000		-17	-15	0.9955	-	-
4-Chlorophenol (4-ClPh)	126.9762 → 90.9991	5.73	-23	-8	0.9867	4.50	6.00
	129.001 → 90.9991		-23	-15	0.9829	-	-
Mecoprop sulfate (MCP-sulf)	220.9986 → 141.0086	6.26	-27	-11	0.9963	0.10	0.40
	222.9858 → 142.9913		-27	-11	0.9926	-	-
Mecoprop-D ₆	219.0658 → 147.0606	6.14	-15	-15	-	-	-
	219.000 → 71.000		-21	-10	-	-	-
Dichlorprop-D ₆	239.000 → 164.000	6.20	-20	-15	-	-	-
	241.000 → 166.000		-20	-15	-	-	-



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Table 10. Changes in Hydraulic retention time (h), O₂ (% air saturation) and T (°C) recorded on the day of the sampling during the modulation of the HRT. * data not available because the inlet hose of the biofilters was frozen. ** data not available because the pumps were not working properly.

Date	Biofilter B		Biofilter C	
	HRT (h)	O ₂ (% air saturation)	O ₂ (% air saturation)	T °C
21-11-2024	17.9	21.0	58.0	11.8
28-11-2024	17.9	22.6	10.4	11.8
05-12-2024	14.1	21.0	17.0	10.4
12-12-2024	12.3	32.0	47.0	11.4
18-12-2024	11.3	45.3	29.0	12.0
23-01-2025	23.0	37.0	26.0	7.4
30-01-2025	17.9	15.0	21.1	10.2
06-02-2025	18.7	16.5	18.4	11.2
13-02-2025	n.d.*	24.0	30.0	4.6
20-02-2025	n.d.*	30.0	19.0	4.7
27-02-2025	n.d.*	33.3	18.5	10.8
05-03-2025	n.d.*	71.0	47.0	10.9
19-03-2025	26.3	24.0	24.0	10.4
27-03-2025	16.9	57.0	59.9	13.0
04-04-2025	26.0	55.0	60.0	18.5
11-04-2025	n.d.**	13.1	23.1	14.8
25-04-2025	23.0	32.2	19.5	17.2
30-04-2025	17.7	23.5	16.1	20.4
07-05-2025	18.2	16.7	33.3	18.5
14-05-2025	18.5	n.d.	22.1	20.4



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DCPP Line B

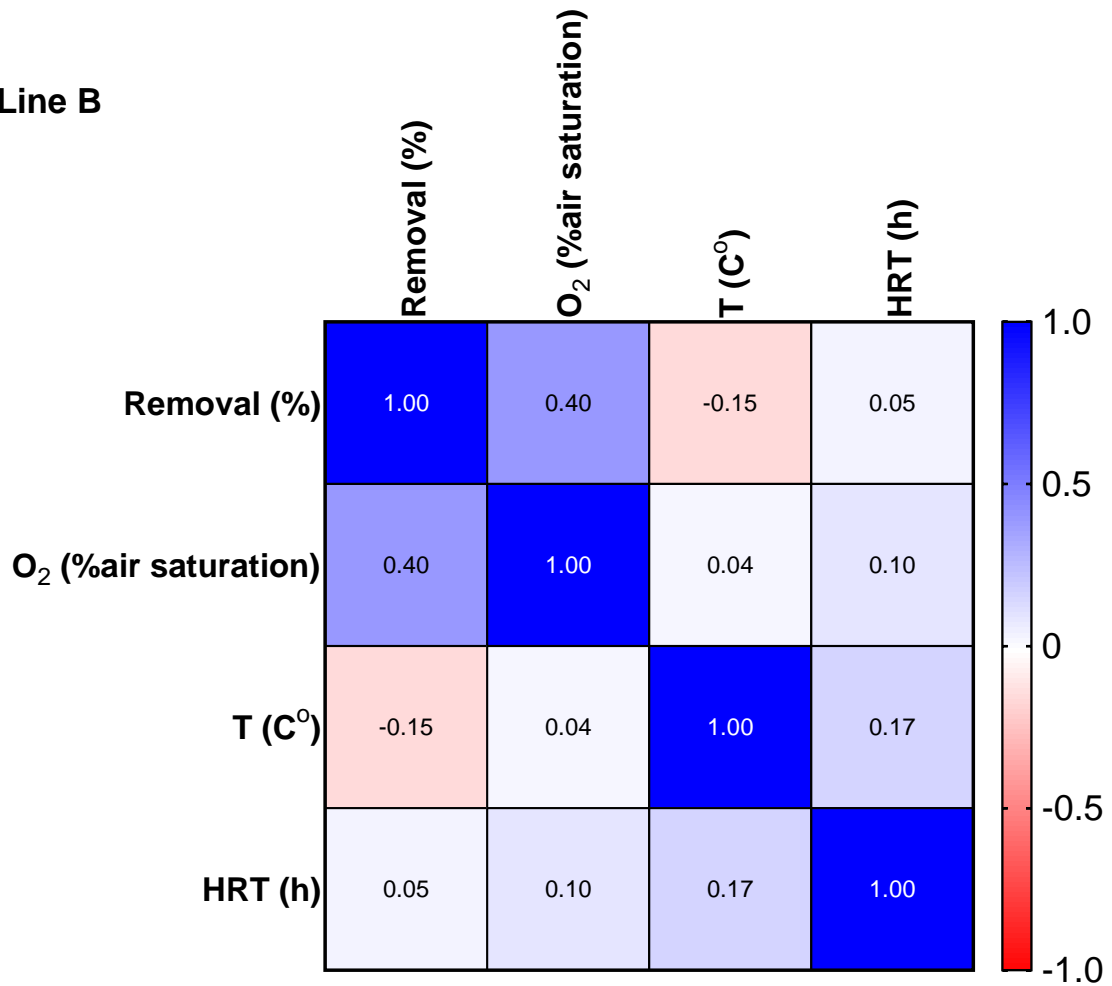


Figure 25. Pearson correlation matrix for line B showing the relationships between the analyzed variables (Removal, air saturation, water temperature and hydraulic retention time) of DCPP, with colour intensity indicating the strength of correlation (blue = positive, red = negative).



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DCPP Line C

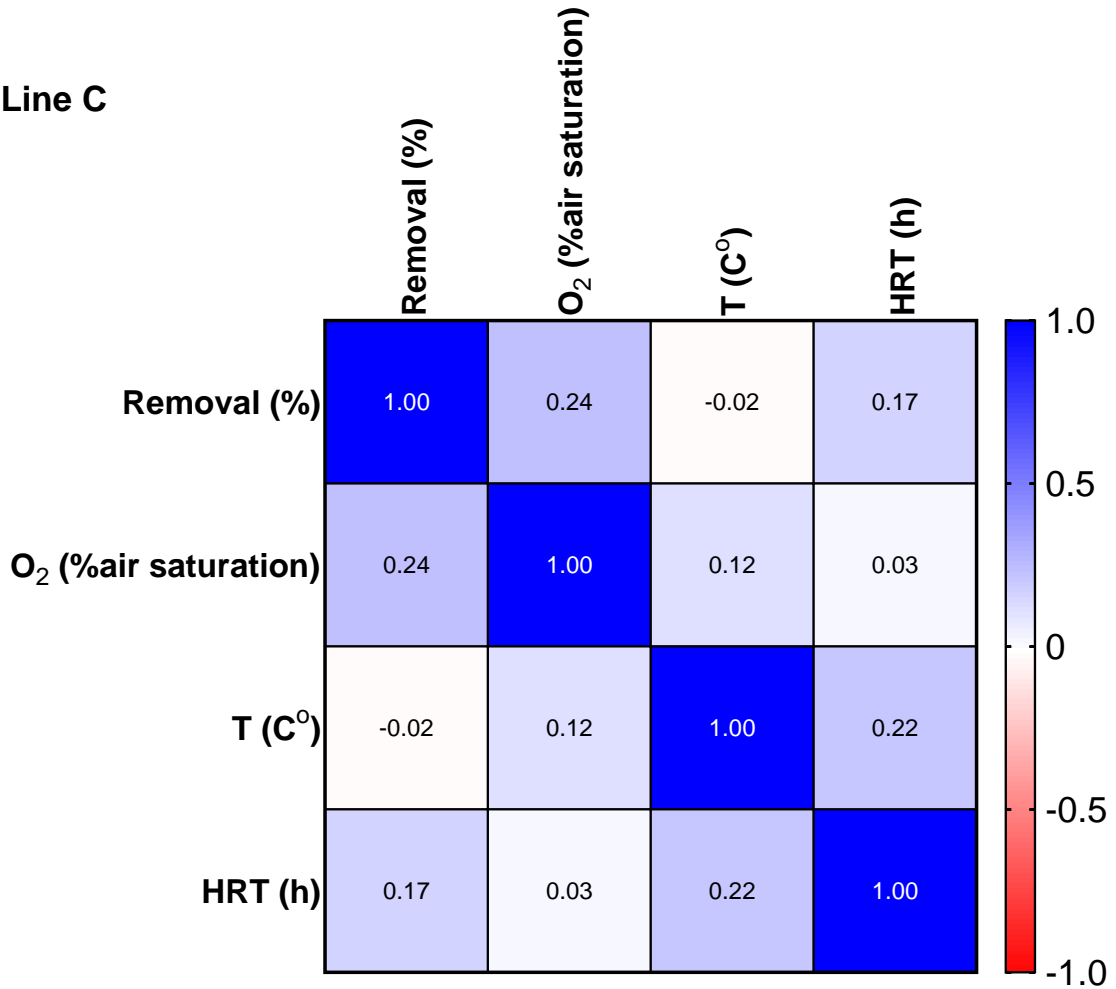


Figure 26. Pearson correlation matrix for Line C showing the relationships between the analyzed variables (Removal, air saturation, water temperature and hydraulic retention time) of DCPP, with colour intensity indicating the strength of correlation (blue = positive, red = negative).



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