Field investigations in Sant Vicenç dels Horts (Barcelona, Spain): MAR effects on groundwater resources

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Title: Field investigations in Sant Vicenç dels Horts (Barcelona, Spain): MAR effects on groundwater resources

Summary: DEMEAU project has continued field investigations at the groundwater replenishment site in Sant Vicenç dels Horts. This report presents an interdisciplinary work including the quantification of emerging pollutants and the qualitative assessment of their behaviour in the MAR system, the application of the temperature as a tracer, leaching tests to assess the reactivity of the organic layer and the use of bioassays to determine the toxicity of infiltration water and groundwater.

The hydraulic characterisations by temperature breakthrough curves showed that this approach is feasible and contributes to an improved understand of the infiltration system. Through the application of temperature as a tracer it is possible to achieve a reasonable good understanding of spatial and temporal dynamics in MAR systems.

Three sampling campaigns have been carried out under different recharge conditions: i) full operation in July 2014, ii) dry conditions in January 2015 where the infiltration pond was dry and iii) mixed conditions in May 2015 where the pond was partially filled with water.A brief hydrochemical overview based on bulk water chemistry indicates organic carbon consumption along the flow path.

Leaching tests have been made with fresh compost and four year-old compost samples. The objective of the leaching tests was the evaluation of the long-term (purification) performance of the reactive organic layer installed at the bottom of the infiltration pond. The leaching tests showed that there are no evidences of additional organic carbon release after four years of operation. This finding is crucial for the interpretation of the bulk chemistry and the emerging pollutants, because it is very likely that no additional contaminant removal can be expected from the compost layer anymore.

In total 53 organic micropollutants have been measured during the sampling campaigns. The substances are divided in three groups: i) pesticides, ii) pharmaceuticals and iii) stimulants/sweeteners/corrosion inhibitors, including those identified by the DEMEAU project as target substances. The behaviour of organic micropollutants during subsurface passage was evaluated based on the hydraulic understanding of the recharge system. Most substances have been measured below LOQ and do not allow for removal approximation.

Field results are compared to results obtained from column experiments which were carried out previously within DEMEAU. The comparison between laboratory results and field results found similar removal trends of emerging pollutants. Removal was found to be higher under field conditions, which may be attributed to longer travel times.

The application of effect-based methods (bioassays) enabled to measure the combined effects of emerging pollutants. The broad range in vitro screening of the MAR water samples revealed the importance of endocrine - (particularly the activation of the ERα-, anti-AR, anti-PR receptors), oxidative stress (Nrf2-CALUX) and photosynthesis inhibition (Combined algae test) pathways, and showed

differences between the samples collected within two different time points (two sampling campaigns).

Despite the lack of toxicological data for a number of the selected target compounds and the lower relevance of the selected compounds for (eco)toxicological risk assessment, this study greatly demonstrate as well the usefulness of combined analyses of environmental samples. Sampling sites, water sources can this way cost-efficiently pre-screened and characterized for low/high risk even without extensive measurement of a priori selected target chemicals. Not to mention that the targeted chemical analysis might overlook certain chemicals exerting specific effects. Effect-based methods, therefore, could complement conventional chemical analysis in water quality monitoring as pre-screening techniques by (i) identifying toxic "hotspots" for further investigation, (ii) assessing the effect of the entire mixture of compounds present in waters and therefore, (iii) reduce uncertainty in safety evaluation.

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1 Introduction

This report presents the work carried out at a groundwater replenishment site in Sant Vicenç dels Horts (Spain). The following topics are covered:

- Bulk chemistry: characterisation of infiltration and groundwater
- Assessment of emerging pollutants and their behaviour during MAR
- Using temperature of groundwater as a tracer to determine arrival time of recharged water
- Leaching test to assess the reactivity of organic layer (laboratory test)
- Bioassays of MAR samples

DEMEAU project has also contributed in the knowledge of the fate of emerging pollutants with a column experiment simulating the MAR system of Sant Vicenç dels Horts (Hernández and Gibert, 2014; Schaffer *et al.* 2015). A comparison between field results (this report) and column experiment from Hernández and Gibert (2014) and Schaffer *et al.* 2015 can also be found in this report.

2 Materials and methods

2.1 MAR profile

The infiltration system of Sant Vicenç dels Horts (SVH) was constructed in 2007 and started its operation in 2008. The main objective of the system is to introduce additional freshwater into the aquifer to gain an average extra volume of one Mm^3/year. The operation consists in a direct intake of Llobregat river water two km upstream of the ponds. The catchment area is an intake channel that has to be reconstructed from time to time according to rainy periods that can destroy totally or partially the intake channel. Collected water circulates downstream by a concrete pipe of an inner diameter of 1000 mm. the system is controlled manually by CUADLL (Association of users of the aquifer) according to quality alerts and meteorological forecast.

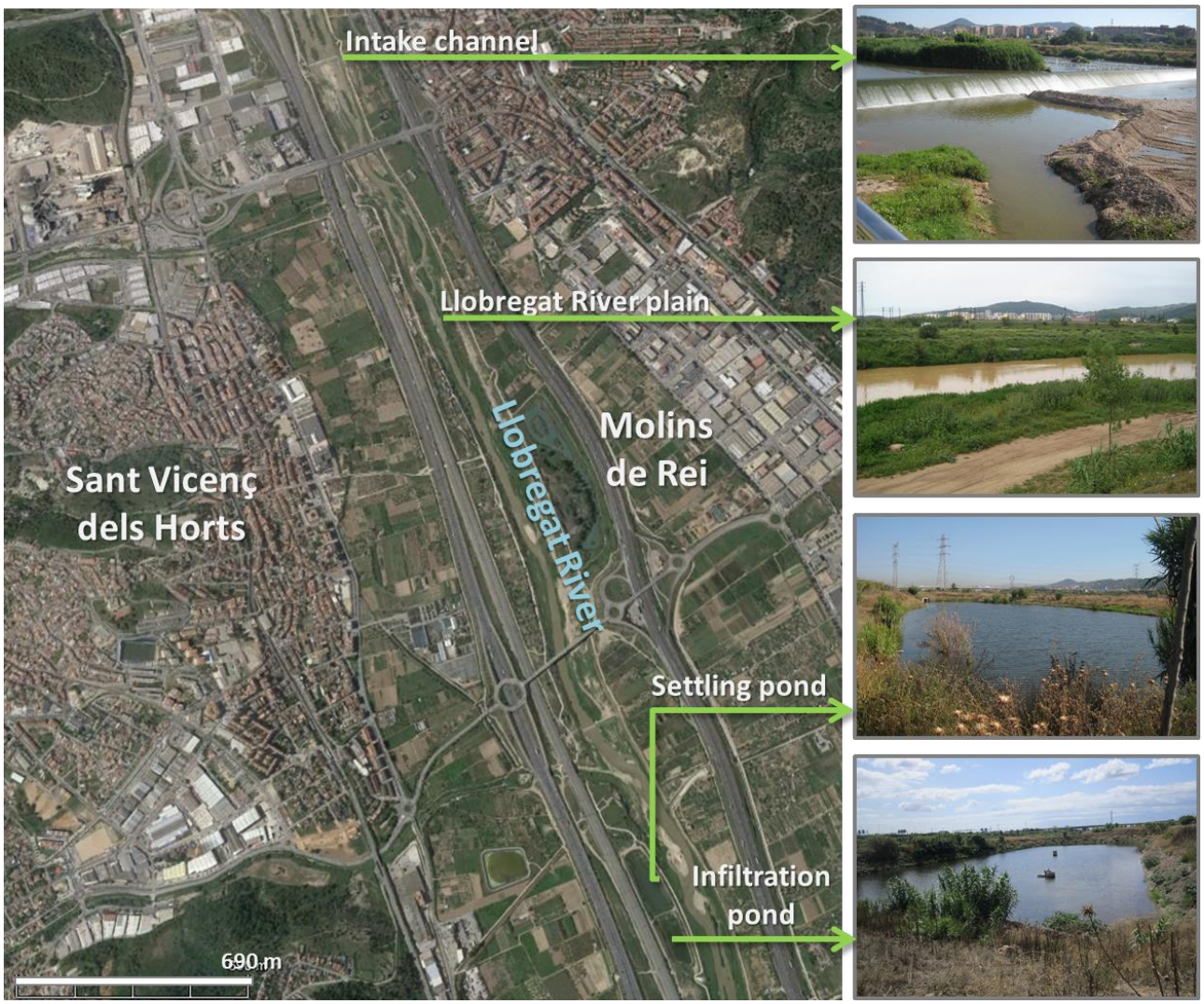


Figure 2-1: Overview of Llobregat area and SVH system location.

Water enters in the settling pond (6,000 m^2), with a residence time of about two or three days. Settling pond and infiltration pond are inter-connected by a concrete pipe of 1000 mm. The connection is instrumented with a flowmeter to quantify the volume of water introduced in the infiltration pond. Moreover, there is a datalogger installed in one of the concrete islands of the pond that gets automatic

data of water level and temperature of the infiltration water. Spreading surface of the infiltration pond is about 5,600 m^2. In 2011 the infiltration pond was enhanced with a reactive organic layer compost-made. The compost was 100% vegetal compost and it was mixed half-and-half with local sand and gravels. The main objective of the installation of the reactive layer was the increase of removal of emerging pollutants. Effectiveness of the reactive layer was tested at field scale in the Life+ ENSAT project (results available at http://www.life-ensat.eu/). The reactive layer has been also tested in DEMEAU in a simulation of the real system of SVH at laboratory scale, by a column experiment (results available at http://demeau-fp7.eu/ and Schaffer *et al.* 2015).

SVH site is very well-known from previous projects carried out there[1]. The observation network is very completed. An accurate selection of groundwater observations wells has been done for the network of DEMEAU sampling campaigns. "INF" represents water entering in the infiltration pond. BSV-1 represents native conditions of the aquifer, and the rest of the points (BSV-5, BSV-8.1, BSV-8.3, BSV-9, and BSV-10) have the influence of the infiltrated water in different proportion according to the depth and distance to the infiltration pond.

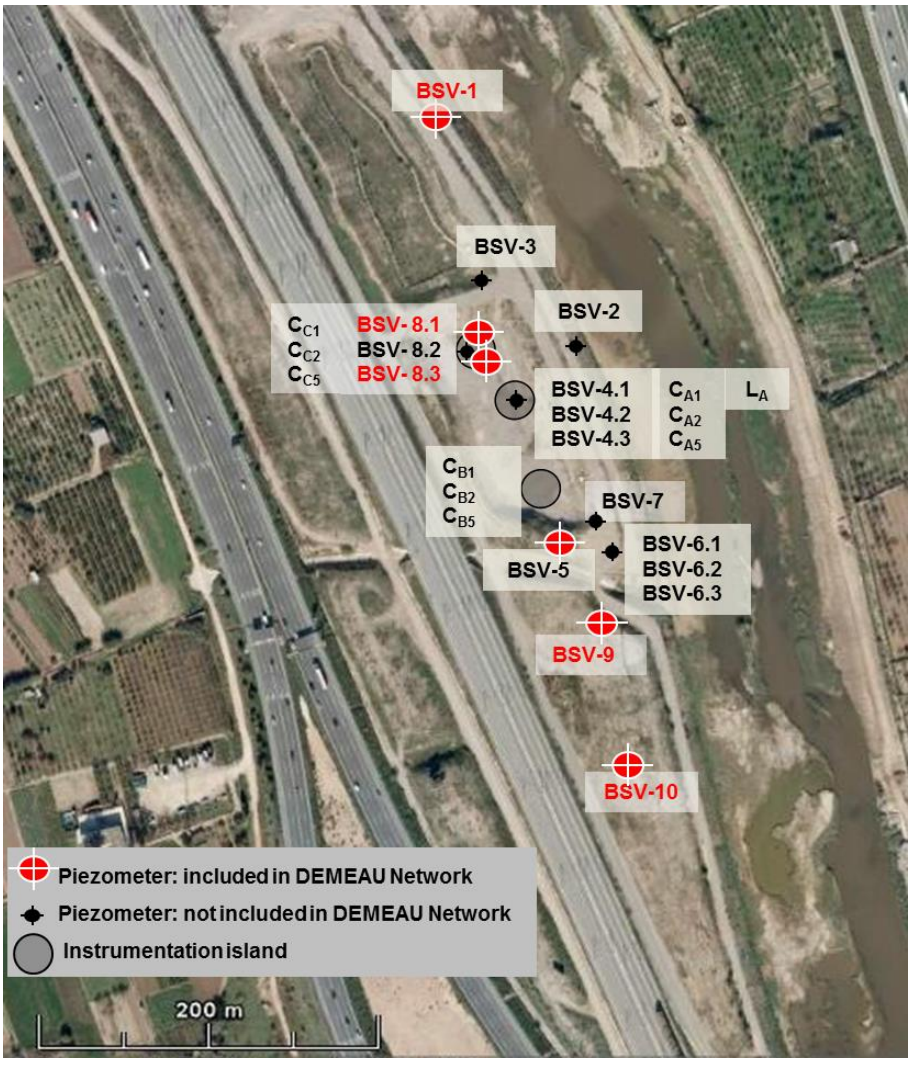


Figure 2-2: Aerial view of location of sampling points

[1] ENSAT project: http://www.life-ensat.eu/
PREPARED project: www.prepared-fp7.eu
GABARDINE project: http://cordis.europa.eu/publication/rcn/13034_en.html

Table 2-1 shows the location of the DEMEAU's sampling points and depths of filter screens. A profile with essential characteristics of the Sant Vicenç dels Horts groundwater replenishment site can be found in annex 1.

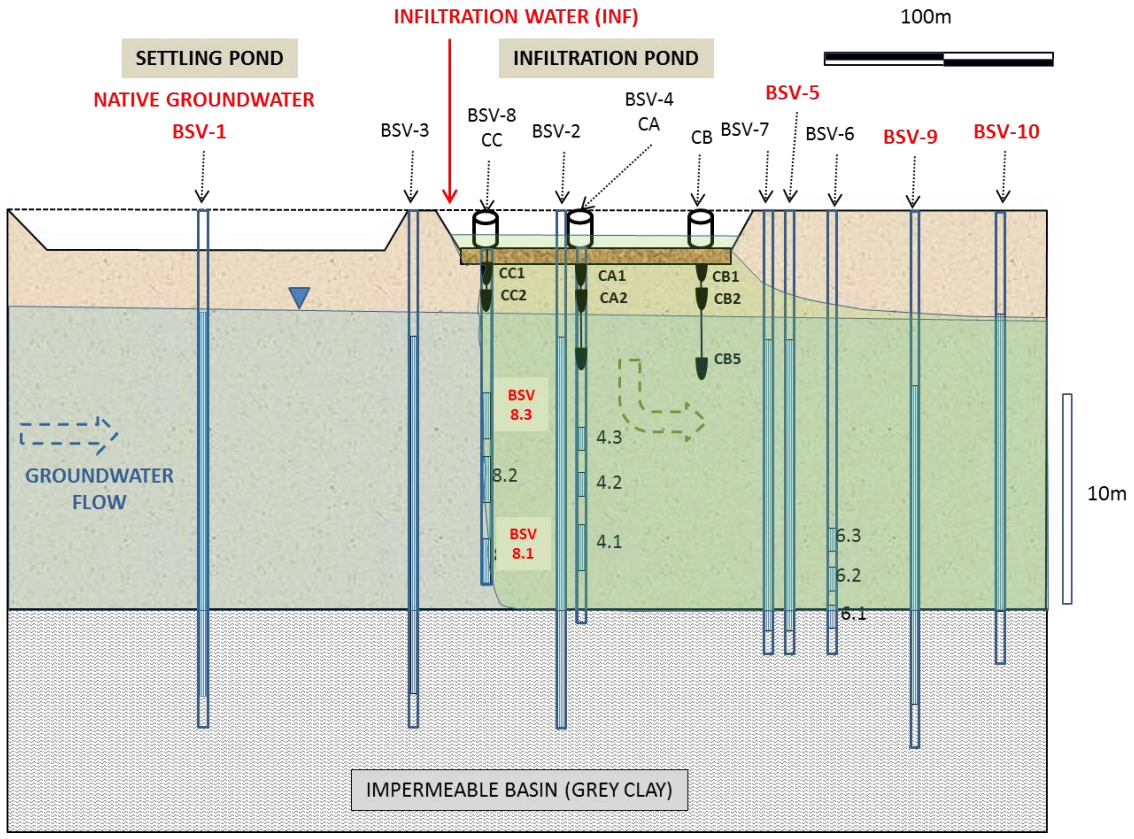


Figure 2-3: **Hydrogeological profile section at Sant Vicenç dels Horts (red coloured observation wells were sampled in this study)**

Table 2-1: **Type, depth, filter screen position and hydraulics of sampling points.**

Sampling points	Type	Total depth (m)	Filter screen depth (m below surface)	Approximate travel time or recharged water (days)	Proportion of infiltrated water (conservative tracer test)*
INF	River water			0	100%
BSV-1	Groundwater	24.5	6-24	Not influenced	0%
BSV-5	Groundwater	21.5	5-23	6	98%
BSV-8.1	Groundwater	16.0	13-15	N.A.	57%
BSV-6.2	Groundwater	19	17.5 – 18.5		
BSV 6.3	Groundwater	17	15.4-16.6		
BSV-8.3	Groundwater	10.0	7 - 9	4	88%
BSV-9	Groundwater	26.6	9.5 – 24.4	13	96%
BSV-10	Groundwater	22.5	6-20	17	98%

(*) this conservative tracer test was done in previous project ENSAT (2011) using chloride as a conservative tracer for the comparison between INF and BSV-1 (native groundwater)

2.2 Field sampling campaigns

Three sampling campaigns have been performed along the DEMEAU project: July 2014, January 2015 and May 2015. Different recharge conditions have been assessed, as the infiltration system was fully in operation in the first sampling campaign (July 2014), while in January 2015 the infiltration pond was dry. The last sampling campaign (May 2015) has been classified as "mixed conditions", as there was partial infiltration due to the excavation of an infiltration channel to assess the infiltration rate in the pond. Figure 2-4 shows the recharge conditions in each of the sampling campaign:

Figure 2-4: Pictures of the infiltration pond in the 3 sampling campaigns *Note: Left wet conditions July 2014; Middle dry conditions January 2015; Right mix conditions May 2015.*

The sampling points have been described in the MAR profile section of this document. Infiltration water was substituted by river water in the second sampling period in January 2015, as the system was in stand-by. Sampling bottles were provided by the laboratories. Groundwater samples were taken after a purge of one volume of the piezometers, using disposable 1L plastic bailers. Bottles and bottle caps were rinsed with sampled water. Samples were taken with gloves to avoid contamination. Plastic bottles for metal determination by ICP contained nitric acid for the direct acidification of the sample. No additional treatment was done (filtration, extraction, etc.) on site. Samples were directly analysed at the laboratory using standard methods. Table 2-2 lists the parameters analysed in the Laboratory of Aigües de Barcelona to assess the bulk chemistry.

Table 2-2: Summary of sampling campaigns and laboratories involved

Sampling campaign	Recharge conditions	Micropollutants laboratory	Bulk chemistry laboratory	Bioassays Survey
July 2014	Wet conditions (infiltration pond filled)	University of Göttingen 250 mL (WWTP effluent; 500 mL groundwater). Glass amber bottles	Aigües de Barcelona	YES (2 L frozen) Glass amber bottles
January 2015	Dry conditions (infiltration pond empty)	Kompetenzzentrum Wasser Berlin (KWB) 50 mL amber bottle	Aigües de Barcelona	NO
May 2015	Mixed conditions (infiltration channel in the middle of the infiltration pond)	Kompetenzzentrum Wasser Berlin (KWB) 50 mL amber bottle	Aigües de Barcelona	YES (500 mL refrigerated) Glass amber bottles

2.3 Temperature as a tracer

In this study a numerical approach is developed to investigate heat as a tracer for travel time evaluation during subsurface passage from the infiltration basin to the monitoring wells. Additional information on tracer studies related to MAR (Sprenger, 2015) can be found in the DEMEAU tool box (http://demeau-fp7.eu/toolbox). In this approach the numerical software VS2DH (Healy and Ronan, 1996) is used as a:

- pre-processor for setting up the model simulation
- numerical engine for computing flow and heat transport (VS2DHI 3.3) and solute transport (VS2DTI 3.3)

However, its pre-processor offers no advanced features, e.g. automatically changing model input parameters or performing batch runs, which is required for automatized model calibration. To overcome this drawback the programming language R (http://www.r-project.org) in conjunction with the user-friendly integrated development environment R-Studio (http://www.rstudio.org) is chosen for this study in order to perform:

- Data analysis: checking and visualising available monitoring data
- Data preparation: e.g. summarising of monitoring data (e.g. calculation of statistical parameters)
- Automatized numerical engine runs and result evaluation

2.3.1 Model structure

A two dimensional vertical cross section model was created. The model structure of the unsaturated zone, the aquifer and the filter screens of the observation wells (piezometers) are deduced from Figure 2-3 and implemented in the VS2DI model (Table 2-3).

Table 2-3: Temperature model set-up.

Model domain	Location Pond 1	Location Pond 2	Boundary conditions	Location observation wells
x=700 m z=35 m	x=140-315 m z=2 m	x=350-480 m z=2 m	X=0: constant head X=700: constant head	BSV3: x=330 m BSV2: x=404 m BSV4.1: x=406 m BSV5: x=515 m BSV6.3: x=525 m

The following boundary conditions are implemented in the numerical model:

- Specific flux boundary: infiltration from the infiltration pond to the unsaturated/saturated zone is calculated for each stress period by dividing the daily inflow rate through the infiltration pond surface area (5423.5 m^2) resulting in an average infiltration rate per unit area of 0.95 m/day. Note that this approach assumes that no water is neither lost through evapotranspiration nor stored in the pond, thus possibly overestimating the real infiltration rate,
- Constant head boundaries: upstream/downstream of MAR ponds.

Flow through the unsaturated zone is calculated based on the default values of the van Genuchten model (van Genuchten, 1980).

2.3.2 Calibration

The calibration period from 2/03/2009 to 11/04/2009 was subdivided into 41 stress periods, each one day long. The model is calibrated by fitting measured hydraulic heads and temperature to calculated values. During calibration the hydraulic conductivity was adjusted to achieve the best fit.

The calibrated heat transport model (VS2DHI) is then translated to a solute transport model (VS2DTI). The solute transport is used to approximate travel times and mixing proportions in MAR systems. In MAR systems the point of recharge (e.g. the infiltration pond) is assigned to species concentration C = 1, while the rest of the model domain is assigned to species concentration of C = 0. The resulting breakthrough curves for continuous infiltration are shown in Figure 2-5. The final hydraulic and thermal properties used for the calibrated model are shown in Table 2-4 and Table 2-5. Calibration results are shown in Figure 2-6 and Figure 2-7. Not all observation wells were equipped with data loggers and only observation wells with continuous measurements are used.

Figure 2-5: Log-log scale of an exemplary breakthrough curve (BTC) of an ideal tracer and calculation of minimum (t_{min}), dominant travel time (t_{mean}) and share of infiltrate (C_{max})

Table 2-4: Calibrated hydraulic parameters

Hydraulic conductivity (m/d)	Anisotropy (Kz/Kh)	Effective porosity (-)
850	1	0.35

Table 2-5: Calibrated thermal properties

Thermal conductivity (W/mK)	Heat capacity of sediment (J/m^3K)	Thermal dispersivity (m)*	Heat capacity of water (J/m^3K)
1.4 to 2.2	1×10^6	1	4.2×10^6

*thermal dispersivity is assumed analogues to solute dispersivity

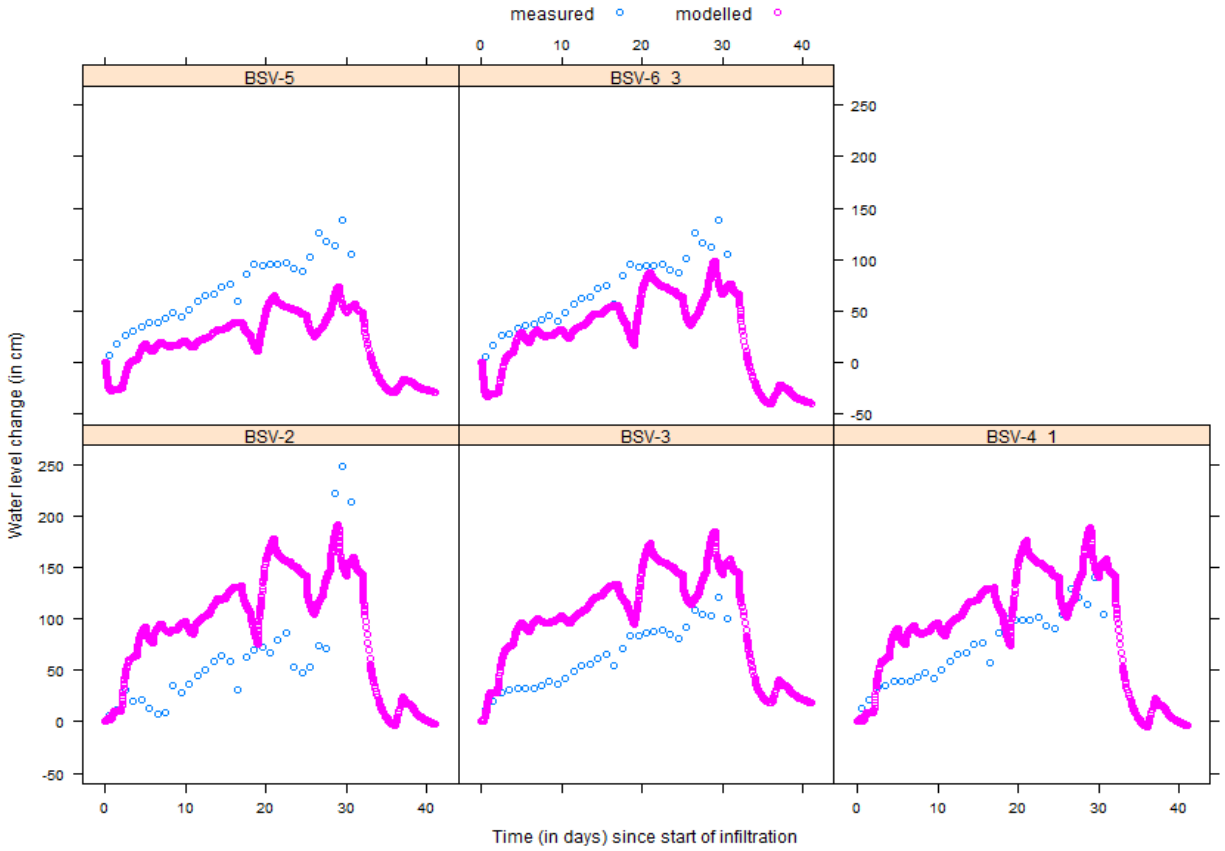


Figure 2-6: Measured (blue) vs. modelled (pink) hydraulic heads

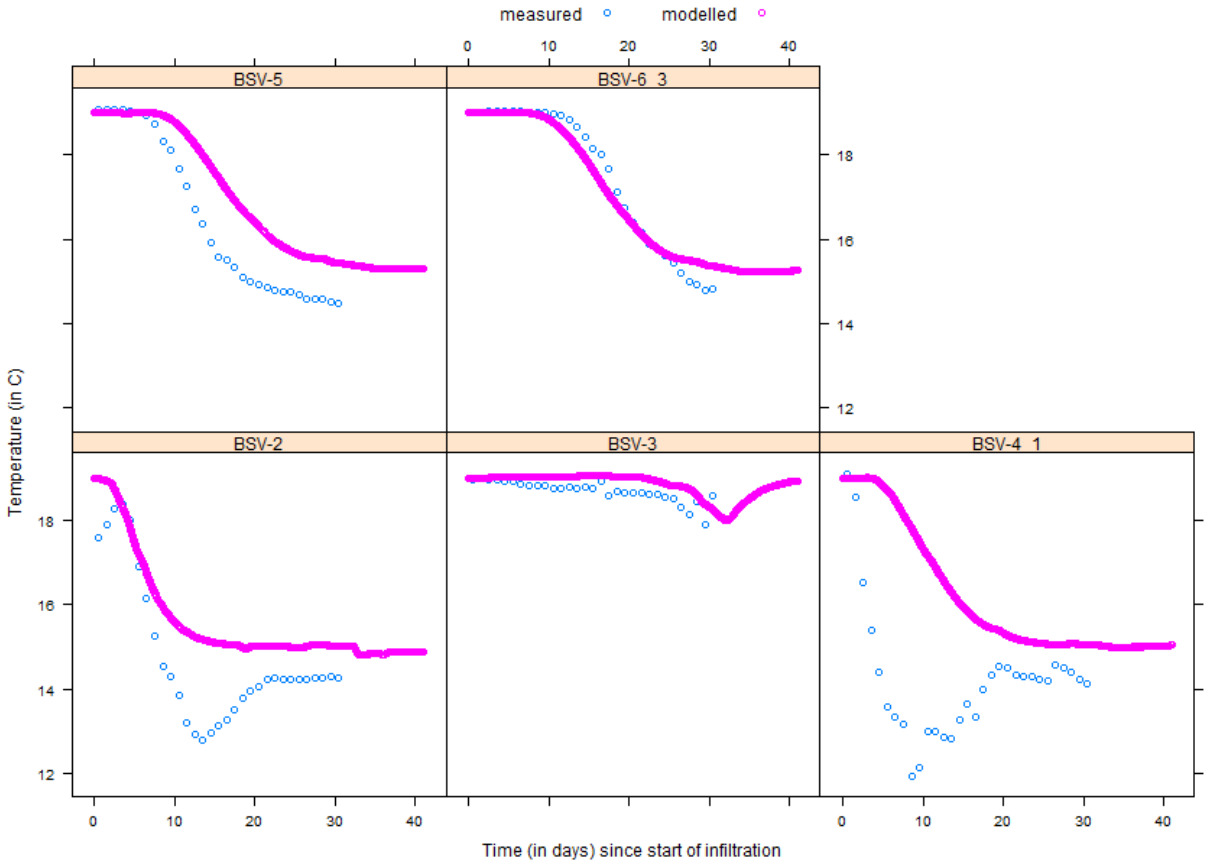


Figure 2-7: Measured (blue) vs. modelled (pink) temperature

A good fit was achieved for BSV5, BSV6.3 and BSV3. Other observation wells (BSV4.1 and BSV2) cannot be reproduced correctly, but show a similar trend of temperature variations. The resulting breakthrough curves for the conservative transport are then used to calculate the dominant travel time (t_{mean}).

2.4 Leaching test

The leaching experiment was designed to compare fresh compost and four year-old compost in terms of DOC release. The objective of this test was the evaluation of the long-term (purification) performance of the reactive layer after four years of operation under field conditions. The main indicator of the purification of the layer is the dissolved organic carbon (DOC) release. DOC was monitored during Life+ ENSAT project as a control parameter at laboratory and field scale. In brief, the enrichment of infiltrated groundwater in DOC creates an additional source of assimilable organic carbon for the microbiological community, increasing the biological activity and thus the removal potential of micropollutants present in recharge water.

Disturbed samples of the compost layer were collected in freshly excavated pits in the bottom of the infiltration ponds in 40 cm depth below surface (see Figure 2-8). Fresh vegetal compost was acquired from remainder stored compost in 2011 supplied by ECOMOIANES, the same supplier which provided the compost material for the reactive layer. The remaining compost in the collected material was carefully separated from sand and gravels to perform the leaching test.

Leaching test consisted in mixing 40 gr of selected compost (sample 1, sample 2 and fresh compost) with 400 mL of river water in three beakers to obtain a ratio 1:10 solid-liquid[2]. A fourth beaker was filled only with 400 mL of river water as a control experiment. Beakers were gentle removed with a spatula to favour the solid/liquid contact at the beginning of the experiment. After 2 hours of contact, 100 mL supernatant was collected using a plastic syringe and filtered (pore size 1.2 µm). This procedure was done repeated for all beakers. The supernatant samples were analysed in the laboratory of Aigües de Barcelona for DOC.

[2] Ratio 1:10 is recommended for this type of testing, according to UNE-EN 12457-4

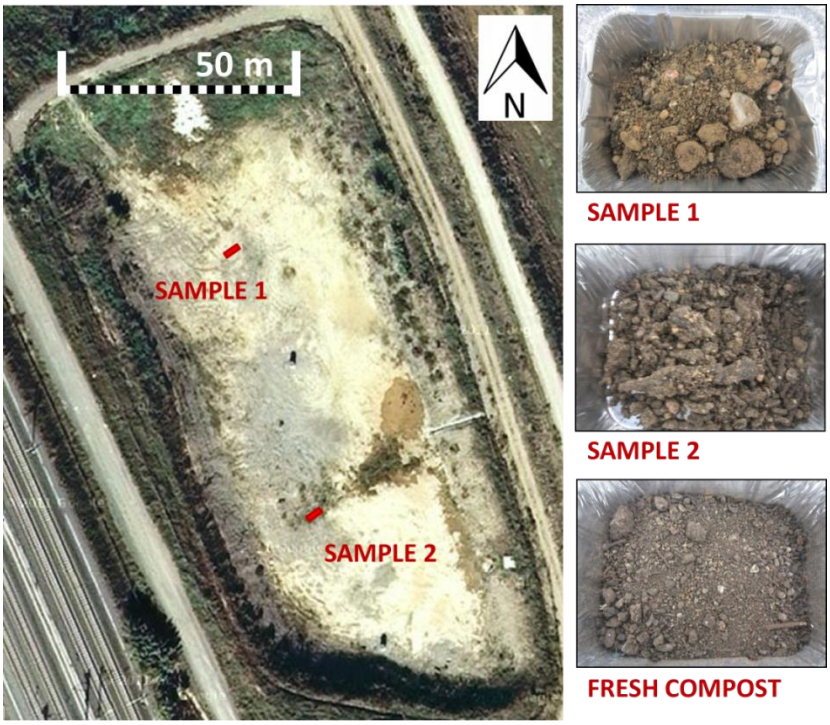


Figure 2-8: **Location of the excavated pits for compost collection and pictures.**

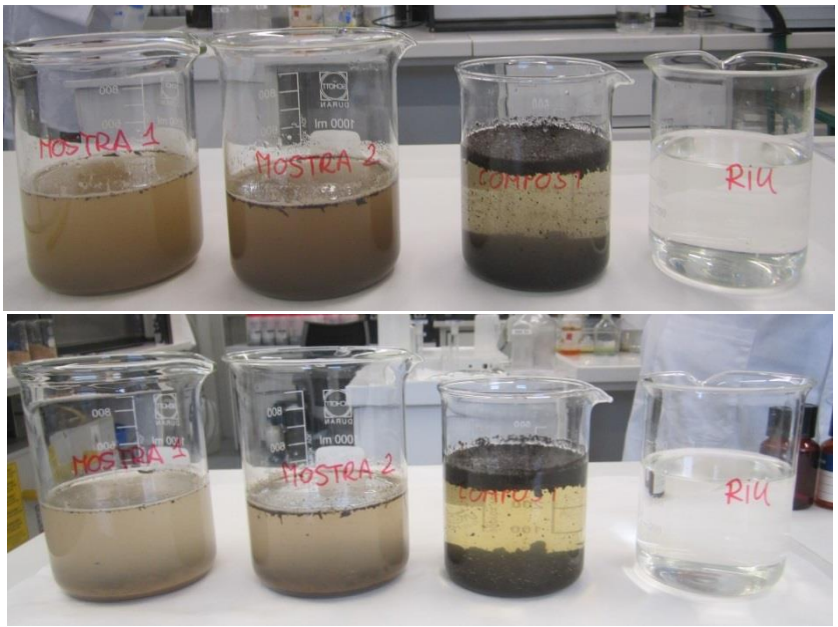


Figure 2-9: **Leaching test performance,** *Above, in the initial shake gently; below after two hours*

2.5 Emerging pollutants

At each sampling campaigns a different set of micropollutants was analysed. An overview of substances which were analysed for each campaign can be found in annex2. Laboratory analyses of the emerging pollutants have been carried out by Göttingen University (GU) and Berliner Wasserbetriebe (BWB) in Germany.

2.5.1 Berliner Wasserbetriebe (BWB)

The BWB laboratory uses German standard methods for the examination of water, waste water and sludge according to DIN 38407-F36 (Determination of selected active substances of plant protection

products and other organic substances in water) using a high performance liquid chromatography and mass spectrometric detection (HPLC-MS/MS or -HRMS) after direct injection. List of analysed parameters and limit of quantification for each substance is found in annex 3.

2.5.2 Geoscience centre of Göttingen University (GU)

Emerging pollutants were extracted by using the stacked-cartridges approach for solid phase extraction (SPE) similar to Nödler et al. (2013). In brief, the OASIS HLB (6 mL, 500 mg) and the OASIS WAX (6 mL, 150 mg; both from Waters) were connected for the extraction procedure with the HLB being first in contact with the sample. ACE was extracted by the WAX sorbent whereas all other compounds were extracted by the HLB sorbent material. After the extraction process, the cartridges were stored at −18 °C until analysis, which had been proved to be most suitable regarding analyte stability and recovery (Hillebrand *et al.,* 2013). Prior to analysis the emerging pollutants were eluted as described earlier (Nödler et al., 2010; Nödler *et al.,* 2013). The sample extracts were analysed by high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC/MS MS). Organic compounds were analysed as described by Nödler *et al.* (2010).

2.6 Bioassays

Bioassays allow the identification of the observed biological effects caused by environmental chemicals and the mixtures that contain them. Recent technological developments have provided powerful quantitative in vitro bioassays to effectively measure a wide range of major classes of toxicants (i.e. acutely toxic compounds, endocrine disrupting substances and genotoxic agents) in the water cycle. As part of the DEMEAU project, scientists recently developed the CALUX cell panel, a type of bioassay panel with the ability to run in an efficient and automated way (Van der Linden et al., 2008). In order to show the potential of these integrated techniques in the field of MAR, collaboration was done between La Vall d'Uixó test site and the laboratories developing and testing these techniques.

MAR samples from two sampling campaigns - conducted in July 2014 and May 2015 - were subjected to sample preparation (i.e. extraction) and screening with selected bioassays to characterize their toxicity profile and investigate the impact of micropollutants present in these water samples. Table 2-2 summarises the sampling conditions in Sant Vicenç dels Horts. The aim of this duplicated experiment was to compare results obtained in the same season to assess the replicability of the bioassays. Techniques applied are listed below:

- **CALUX®-panel** consisting of 9 assays (covering toxic endpoints found to be relevant for water quality benchmarking indicated by the toxicity profiling of the DEMEAU compounds and other case studies (see references van der Linden, 2014; Leusch *et al.* 2014 and Escher *et al.* 2014).
- **Combined algae assay** assessing both photosystem II-inhibition and effects on algae growth
- **Bacteria luminescence inhibition** evaluating acute toxicity of the samples.

Prior to the bioassay analyses samples were concentrated by various extraction methods allowing for enriched pollutant concentrations in the extracts and thereby enabling their better detection in the bioassays. It also limits the impact of the matrix components and metals, which are partially separated during the extraction (Macova *et al.* 2010).

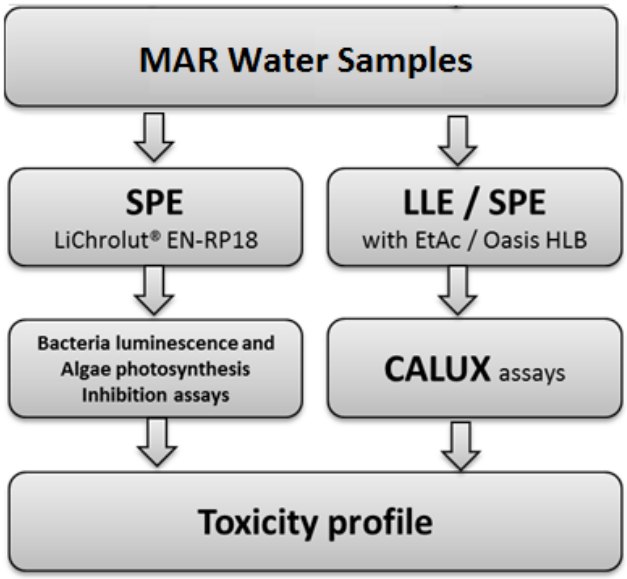


Figure 2-10: Schematic study design of bio screening.

Table 2-6 lists the sampling points Sant Vicenç dels Horts for the performance of bioassays. Additional information as the total depth of the wells of geographical coordinates can be found in Table 2-1, and aerial view for their location is shown in Figure 2-2.

Table 2-6: Sampling points selected for the bioassays.

Code	Type of water
INF	Surface Water
BSV-1	Groundwater
BSV-5	
BSV-8.1	
BSV-8.3	
BSV-9	
BSV-10	

2.6.1 Sample workup

Samples were transported to the partners (BDS, Amsterdam and Ecotox Centre – EAWAG, Dübendorf) for bioassay analyses either frozen (1st campaign done in July 2014) and refrigerated (2nd campaign done in May 2015) and subjected to extraction as soon as possible.

Prior to the combined algae and bacteria luminescence inhibition (Ecotox Centre-EAWAG, Dübendorf) the sample enrichment was done by solid phase extraction (SPE), which allows for increased pollutant concentrations in the extracts and thereby enables a better detection in the bioassays. Briefly, 500 mL was

enriched 500 times using LiChrolut® EN-RP18 cartridges (Merck, Germany) after filtration and pH adjustment (pH=3) of the samples. For each SPE a blank is prepared and treated in the same way as the samples, including filtration and pH adjustment. The volume of the SPE blank (ultrapure water) corresponded to the highest sample volume (i.e. 500 mL). Extracts were then stored in 1 ml of a solvent mixture (~50% ethanol, ~50% acetone and methanol) at −20 °C until analysis following the method described by Escher *et al.* (2008b).

Prior to CALUX analysis (BDS, Amsterdam) samples of the first sampling campaign (06/2014) were liquid-liquid extracted (LLE) following the in-house standard operation protocol (SOP) of BDS (p-BDS-053). Briefly, from each sample 250 mL was extracted three times with ethyl acetate (200, 50 and 50 mL). All three ethyl acetate fractions were collected, combined and evaporated under a gentle stream of nitrogen till almost dryness and taken up in a final volume of 100 µL of dimethyl sulfoxide (DMSO). DMSO is a suitable solvent for the CALUX screening. All extracts were stored at -18 ^{o}C until analysis.

From the samples of the second campaign (05/2015) somewhat different volumes were worked up due to the various sample volume availability. 600 mL from the Sant Vicenç dels Horts samples were extracted by SPE using Oasis HLB cartridges. During the time between the two sampling campaigns BDS modified his in-house extraction method and stepped over from LLE to SPE with Oasis HLB cartridges. The two methods were fully compared and evaluated and resulted in no changes in extraction efficiency. Similarly to the sample handling in the first campaign, extracts were dissolved in 100 µL of DMSO and stored at -18 ^{o}C until analysis.

Taking into account all the sample manipulation steps (concentration during extraction and then dilution in the bioassay) during the analysis, 25 times (samples from the first campaign) and 60 times (samples from the second campaign enriched samples were tested in the CALUX bioassays.

2.6.2 Combined Algae Assay

The Combined Algae Assay on the green algae *Pseudokirchneriella subcapitata* was conducted as described by Escher *et al. (*2008a). The photosynthesis inhibition was measured by means of effective quantum yield (after two h of exposure) and the inhibition of the algae growth by means of absorbance at 685 nm (after 24 h of exposure). The herbicide diuron served as the reference substance and ethanol as the solvent control (30 and 80 µl/well, respectively with a setup of 8wells/plate). The reference substance in duplicate and the extracts of the water samples in triplicate were tested in a 1:2 dilution series, with the highest concentration of diuron being 3 × 10−7 M (69.9 µg l−1, in ethanol). Maximum enrichment factors of the water samples in the assay were 133 times. The toxicity of the water samples was expressed as diuron-equivalent concentrations (DEQs) for the endpoint “inhibition of Photosystem II” and toxic equivalent concentrations (TEQs, virtual baseline toxicant) for growth inhibition.

2.6.3 Bacteria luminescence inhibition assay

The inhibition of the luminescence of the bacterium *Aliivibrio fischeri* (bacteria luminescence inhibition assay) is a commonly used bioassay for screening of surface waters to detect non-specific effects of toxicants. The extracts were added in microtiter plate wells, a geometric dilutions series in ethanol was done and the solvent left to evaporate to dryness. The residues were redissolved in a NaCl buffer solution and added to the reconstituted freeze-dried bacteria (Dr Lange, Düsseldorf, Germany) in another microtiter plate. The bacteria luminescence output was measured prior to addition of sample and after 30 min

incubation and the inhibition of bioluminescence was reported as toxic equivalent concentrations for baseline toxicity (baseline-TEQ) (Escher *et al.*, 2008b).

2.6.4 CALUX reporter assays

All CALUX reporter assays used for this screening are stable cell lines based on the human osteosarcoma U2OS cells with a luciferase gene under the transcriptional control of responsive elements for activated hormone receptors. These cell lines allow sensitive and specific measurements of hormone receptor action by complex mixtures of compounds. In short, cells were seeded in 384-well plates and cultured for 24 h, after which they were exposed to a dilution series of 13 dilutions with 0.5 log unit increments of the compound or extract in DMSO (final concentration in the well was 1 %). Along with the test samples, a concentration series of a reference compound was included on the same well plate. After 24 h of exposure cells were lysed, and luciferase activity was quantified using a luminometer (Berthold Technologies, Bad Wildbad, Germany) that adds substrate to each well and subsequently measures luminescence for 1 s per well. Only dilutions that were negative in the cytotoxicity test were used for quantification of the response (Pieterse *et al.* 2015 and van der Linder *et al.* 2008).

2.6.5 Data analysis

2.6.5.1 Hormone assays, PPARγ-CALUX assays, bacteria luminescence inhibition assay and combined algae assay

For these assays (showing and S-shaped dose-response curves), the measured activity is expressed as being equivalent to a reference compound concentration in the sample, which is determined by interpolating the response of the extract into the concentration-response curve of the reference compound (generally at 50 % effect level) and further back-calculation taking all previous dilution and concentration factors into account. Equivalent concentrations are expressed ng or µg reference compound-Eq/L water.

2.6.5.2 P53 (+/-S9)-CALUX and Nrf2-CALUX

For these assays (showing other type of dose-response relationship, i.e. no S-shaped curve) induction factors (IF) were calculated by dividing the level of response (relative light units [RLU]) in the assay by the average RLU level of the solvent control wells (DMSO only). Samples were considered to be positive in the assays when the response of at least one concentration showed an increase of at least 50% (i.e., a 1.5-fold induction compared to the negative control). This effect level of the sample was then interpolated from the reference dose-response curve and back-calculated taking all previous dilution and concentration factors into account. Equivalent concentrations are expressed ng or µg reference compound-Eq/L water. Table 2-7 summarises the ecotoxicological effects detected by the in vitro bioassays performed.

Table 2-7: In vitro bioassay panel used for the characterisation of the activity profile of the MAR samples received from two sampling campaigns

Toxic pathway	Pertinent in vitro bioassay	Possible adverse health/ecotoxicological effects
Cell viability	Cytotox-CALUX	General (non-specific) toxicity

Toxic pathway	Pertinent in vitro bioassay	Possible adverse health/ecotoxicological effects
Hormone mediated mode of action (MoA)	ERα-CALUX, (anti)AR-CALUX, (anti)PR-CALUX, GR-CALUX	Tumor development, Birth defects, (Sexual) developmental disorders
Lipid metabolism	PPARγ-CALUX	Obesity and inflammatory diseases
Reactive MoA	P53-CALUX, P53 S9-CALUX	Tumor development
(Oxidative) stress response	Nrf2-CALUX	Inflammation, sensitisation and neurodegenerative diseases
Inhibition of the luminescence of the bacterium	Bacteria luminescence inhibition assay	General (non-specific) toxicity
Inhibition of the photosystem II	Combined algae assay	Photosynthesis inhibition linked to reduced algae/plant survival and growth

3 Results

3.1 Temperature as a tracer

The first step in understanding a MAR site is the hydraulic characterisation. This can be done in various ways and tracer tests are one way (Sprenger, 2015). Without a proper understanding of the temporal and spatial dynamics of subsurface flow the interpretation of contaminant transport is very challenging.

Modelling results of temperature transport are shown in Table 3-1. Compared to travel time estimations based on Darcy´s law the modeled travel times show large differences. In general Darcy´s law estimation overestimate the travel time compared to modelled values based on transport BTC´s.

Table 3-1: Dominant travel time and share of infiltrate for observation wells.

Monitoring well ID	Calibrated dominant travel time (d)	Dilution ratio
BSV-3	11.5	1
BSV-6.2	9.5	1
BSV-2	5.8	1
BSV-5	8.9	1
BSV-4.1	3	1

The resulting BTC is shown exemplary for the observation well BSV-6.2 in Figure 3-1. The red curve is the previously calibrated temperature BTC (see Figure 2-7 for calibration results). It starts from the initial temperature (19 °C) and decreases as the colder (15 °C) infiltrate reaches the observation well. The black curve describes the BTC of the artificially introduced conservative species which is used for the travel time calculation. The mean thermal travel time is about two times longer than the mean conservative travel time.

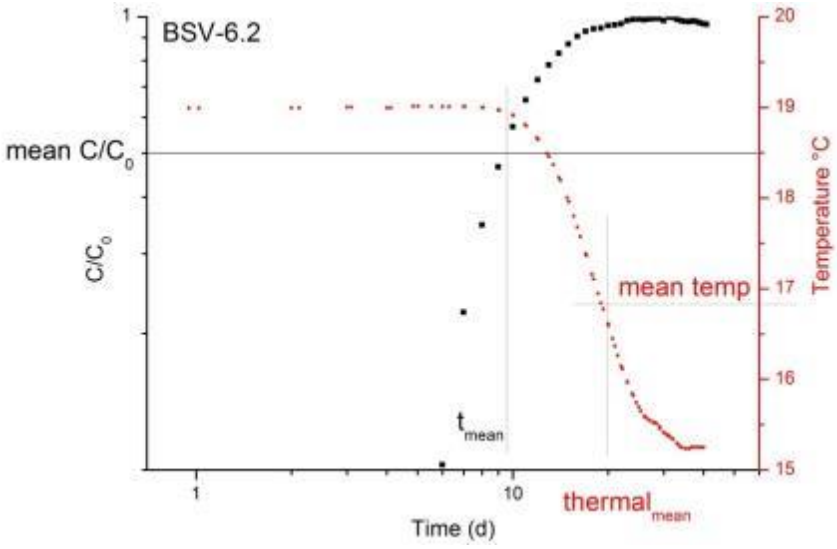


Figure 3-1: **Resulting breakthrough curves of conservative transport (black curve) and retarded temperature (red curve) shown for the observation well BSV-6.2**

3.2 Hydrochemistry

The complete results of all bulk chemistry parameter are shown in annex 2. **Fehler! Verweisquelle konnte nicht gefunden werden.** shows Piper diagrams (Piper 1944) for data sampled during dry conditions (left) and wet recharge conditions (right). Water chemistry in the observation wells is more similar to source water during recharge (wet conditions) than under dry conditions.

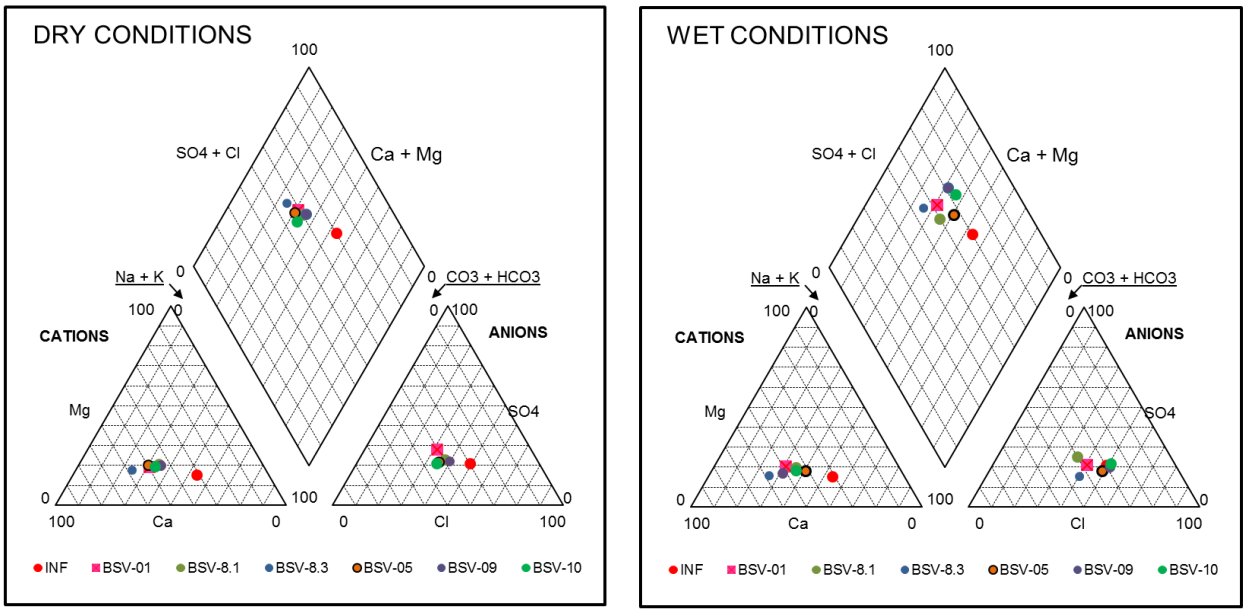


Figure 3-2: **Piper diagram of dry and wet conditions,** NOTE: infiltration values under dry conditions represents Llobregat River quality (there are no significant changes between Llobregat water and infiltration water)

Figure 3-3 shows slightly higher values of Ammonium and Total Organic Carbon (TOC) in source water (River), while the concentration of both parameters are found in decreased concentrations in the groundwater. Ammonium is not detected in the observations wells (below detection limit) and average concentration of TOC is mostly below 3 mg/L in the observation wells. The decrease of TOC values from

BSV-8.3 to BSV-8.1, measured during wet conditions, is indicative of organic carbon consumption along the flow path.

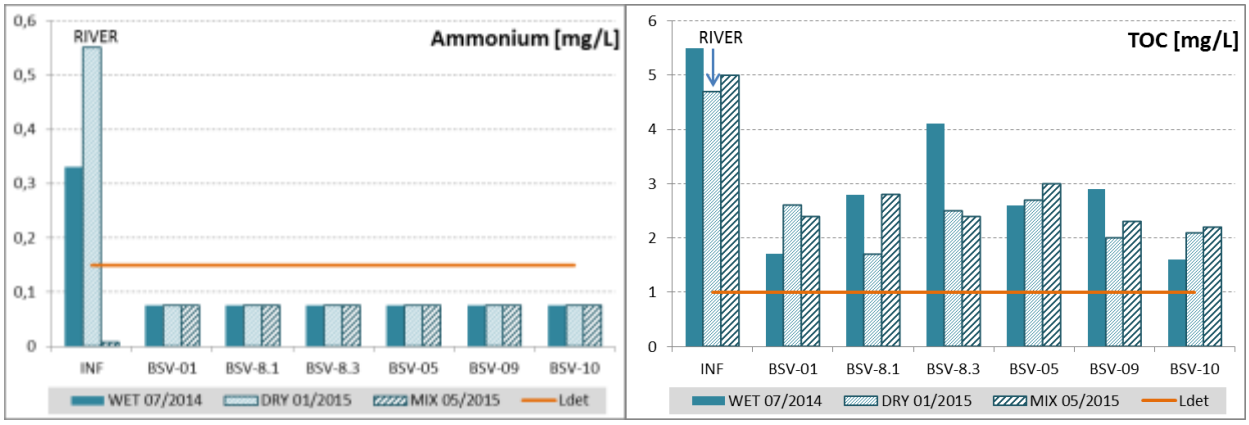


Figure 3-3: Ammonium and TOC concentrations in SVH sampling campaigns NOTE: "Ldet"= Limit of detection

Iron and manganese are found in elevated concentrations in some observation wells (BSV-8.3 and BSV-9) compared to source water concentration (Figure 3-4). This may be explained by dissolution of Fe-/Mn-bearing minerals from the aquifer material. This dissolution may be triggered by recharge periods since high peaks of both metals occur only during wet and mixed conditions, while during dry conditions the concentration of iron and manganese is lowered.

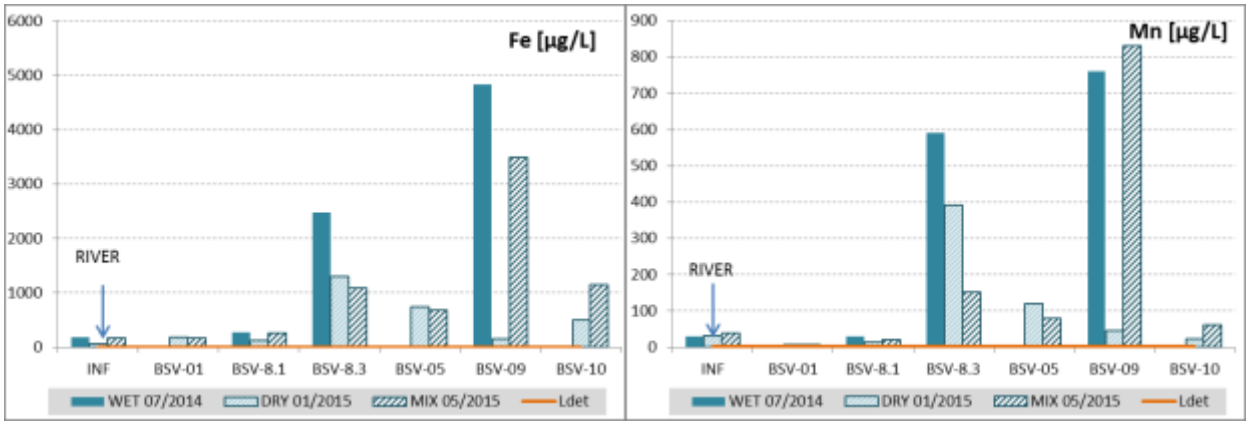


Figure 3-4: Iron and manganese concentrations in SVH sampling campaigns NOTE: "Ldet"= Limit of detection.

3.3 Emerging pollutants

3.3.1 Source water and native groundwater

Dry condition and BSV-01 samples are used to calculate the native background concentration. From the 53 analysed parameters 5 compounds have been detected equal or above limit of quantification (LOQ).

Averaged concentration of emerging pollutants in native groundwater and source water are shown in Figure 3-5.

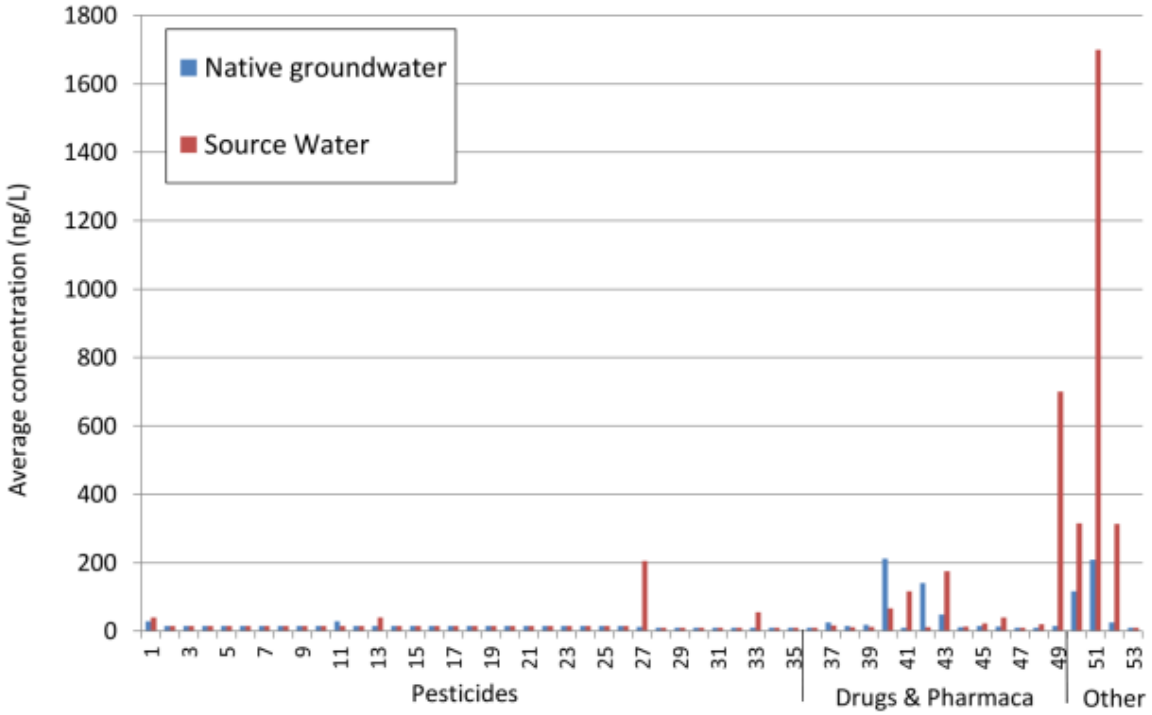


Figure 3-5: **Comparison of emerging pollutants in source water and native groundwater (numbers correspond to compounds as shown in annex 3).**

Out of 35 analysed pesticides 4 compounds (PBSM, Diuron, FAA, MCPA) have been detected equal or above LOQ in source water samples (measured in pond- or river water samples). FAA and MCPA show the highest concentration with 220 and 100 ng/L, respectively.

From the group of drugs and pharmaceuticals most compounds have been detected equal or above LOQ. Out of 14 measured drugs and pharmaceuticals 6 compounds have been detected equal or above LOQ in source water samples. Dihydroxydihydrocarbamazepine and Gabapentine show the highest concentration of 175 ng/L and 700 ng/L in average, respectively. Gabapentin is used to treat some types of seizures and for post-herpetic neuralgia (nerve pain caused by shingles).

In the group of stimulants/sweeteners/corrosion inhibitors, acesulfame is present in high concentration of about 1700 ng/L in average. Acesulfame is a calorie-free sugar substitute (artificial sweetener). In the European Union, it is known under the E number (additive code) E950. This compound is not metabolized by the body. It passes through the gastrointestinal tract unchanged.

3.3.2 Fate of micropollutants during MAR

Four categories have been established to classify its fate during subsurface passage for each emerging pollutant:

- **Compound in source water below LOQ:** only compounds which are detected ≥ LOQ in source water samples are subject of removal approximation.

- **Compound not removed:** removal < 10% of concentration measured in source water (C_0)

- **Removal approximation:** low removal = 10-50% of C_0, high removal > 50 % of C_0
- **Increase during MAR:** apparent increase of concentration during MAR

The raw data from the laboratories can be found in annex 3 of this document. Three examples of removal behaviour are shown in the following. A good example of removal during MAR is shown in Figure 3-6. Only wet and mixed conditions samples are shown for groundwater monitoring wells downstream of the infiltration pond. Source water sampled in the pond or in the river and the not influenced monitoring well (BSV-01) upstream of the pond are shown by all available samples.

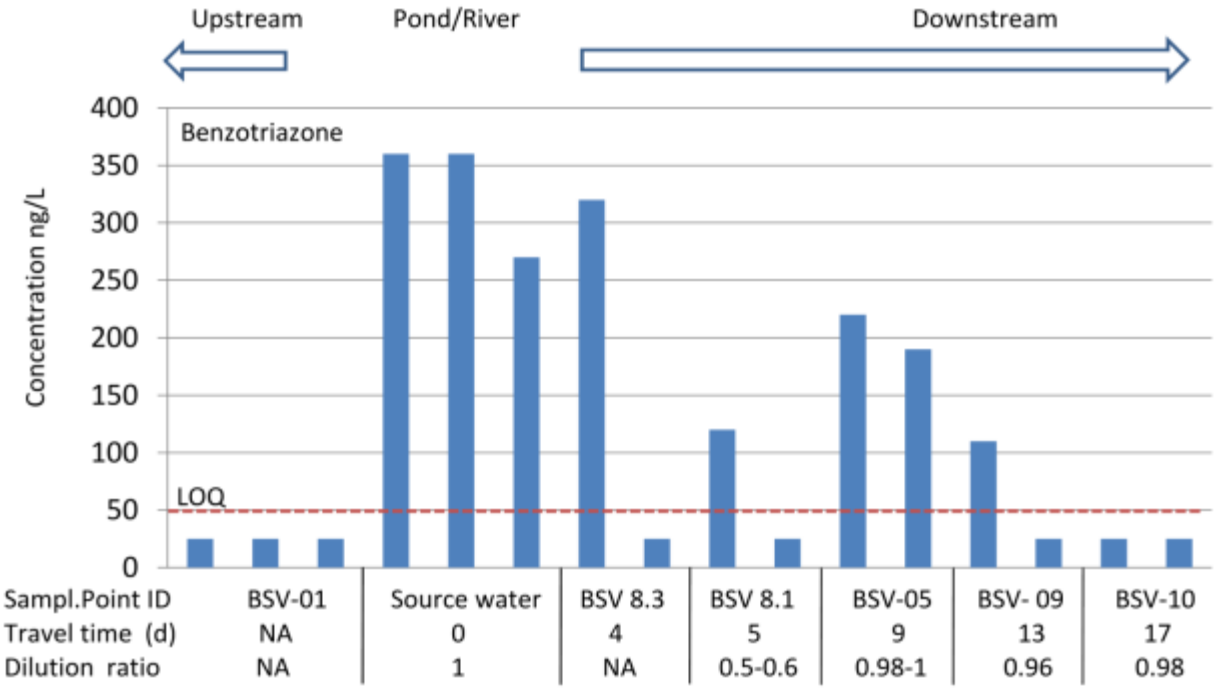


Figure 3-6: Fate of Benzotriazole at Sant Vicenç dels Horts (NA = not applicable, LOQ = limit of quantification).

Benzotriazole in native groundwater is below detection limit (see BSV-01). Source water concentration may reach up to 360 ng/L. After approximately 4 days of travel time in the subsurface concentration in BSV8.3 is decreased to 320 ng/L. This attenuation can be attributed to removal. The dilution factor is not determined for BSV8.3 but can be assumed according to filter screen position and modal distance from infiltration pond to be 1. Further downstream in BSV8.1 large proportion of attenuation must be attributed to dilution. In BSV05 dilution is again minimal and the removal of Benzotriazole to 220 ng/L can be observed. This trend continues in BSV09 until in BSV10 native background concentration is measured. Total removal measured in BSV-10 is therefore calculated with 92%.

Another example of removal is shown in Figure 3-7. Iopromide is measured in source water with 80-90 ng/L and in all groundwater samples below LOQ even after few days of travel time.

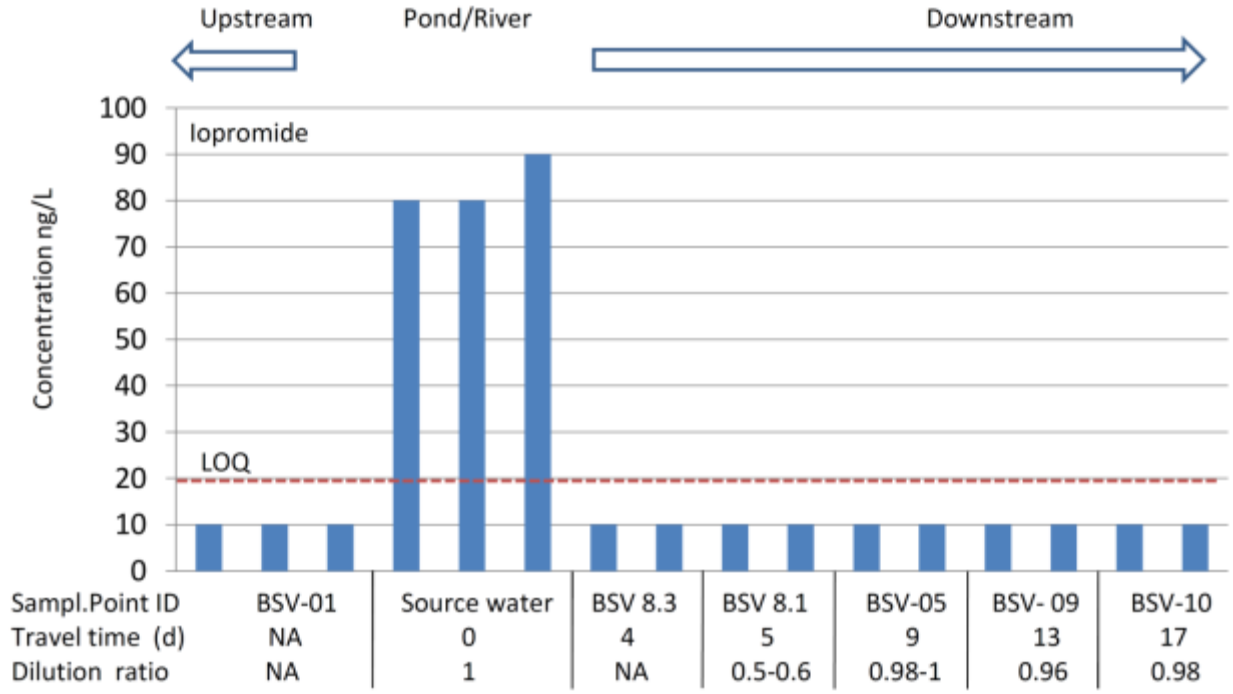


Figure 3-7: **Fate of Iopromide at Sant Vicenç dels Horts (NA = not applicable, LOQ = limit of quantification).**

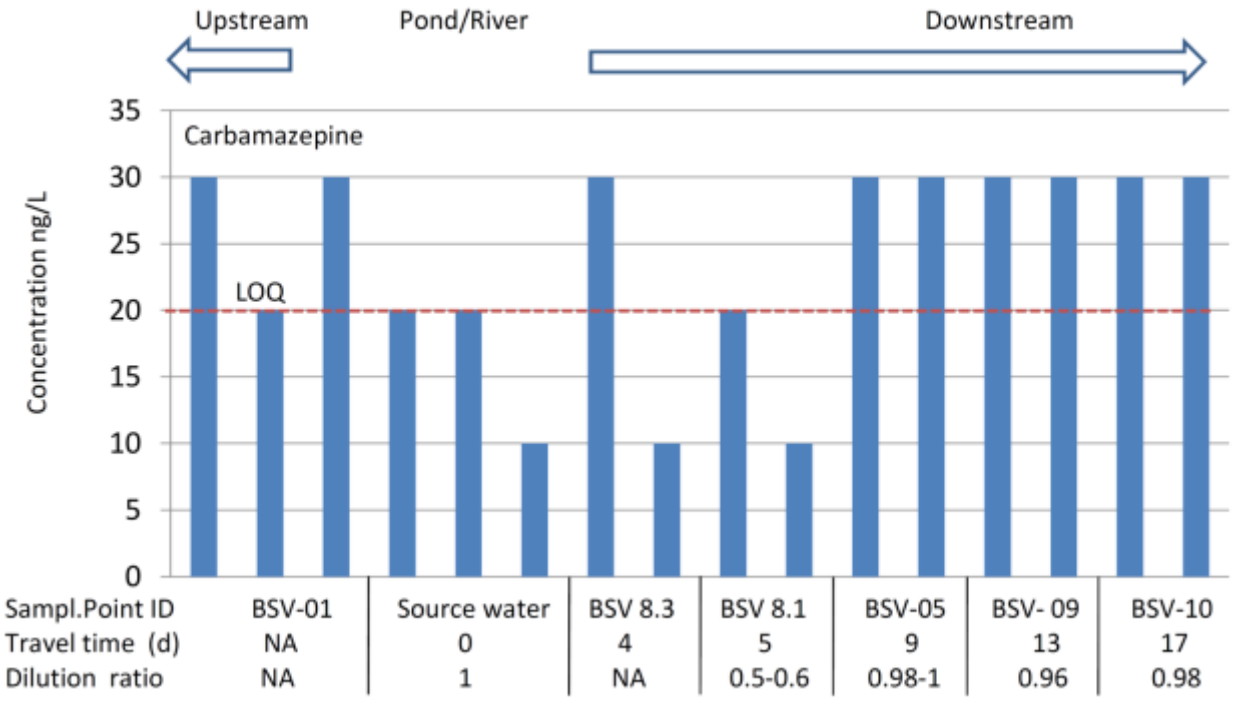


Figure 3-8: **Carbamazepine at Sant Vicenç dels Horts (NA = not applicable, LOQ = limit of quantification).**

In native groundwater Carbamazepine occurs in concentration of 20-30 ng/L (see BSV-01). Source water concentrations are at or below LOQ (20 ng/L). The fate of carbamazepine is difficult to assess, because concentrations are closely around LOQ. It seems that source water concentrations are below native groundwater. Therefore, infiltration of source water should result in concentrations below that of native groundwater. This effect is observed only in BSV8.3 and BSV8.1, but not in observation wells further

downstream. Carbamazepine is characterized by strong retardation (R=1.7 – 2.3) and long half-life times (t_{50}=125-233 d). Due to the rather strong substance specific retardation and high stability it seems plausible that Carbamazepine is retarded compared to advective groundwater flow. The measured concentrations may reflect therefore artefacts from recharge periods some time ago, where concentration in source water was higher.

Removal (R) is calculated by $R_{abs} = C_{SW} - C_{BSV10}$ where C stands for average concentration in SW = source water and BSV-10, when average $C_{SW} \geq LOQ$. Measurements below LOQ are calculated to $LOQ/2$, and do not allow for removal evaluation. Substance specific results for all measured pesticides are show in Table 3-2.

Table 3-2: Fate of pesticides during MAR at Sant Vicenç dels Horts.

Compound	Concentration below LOQ or not measured	Not removed	Percentage removal*	Increasing / Accumulating in MAR (MAR conc. > infiltration water
PBSM			25	
Alachlor	X			
Atrazine	X			
Boscalid	X			
Bromacil	X			
Chlorfenvinphos	X			
Chloridazon	X			
Chlortoluron	X			
Desethylatrazine	X			
Desethylterbutylazine	X			
Desisopropylatrazine				X
2,6-Dichloro Benzamide	X			
Diuron			62	
Ethofumesate	X			
Isoproturon	X			
Lenacil	X			
Metalaxyl	X			
Metamitron	X			
Metazachlor	X			
Chloridazon-Methyl-Desphenyl	X			
Metolachlor	X			
Metribuzin	X			
Quinoxyphen	X			
Simazine	X			
Terbuthylazine	X			
Quinmerac	X			
FAA			95	
2,4,5-Trichlorophenoxyacetic acid (2,4,5-T)	X			
2,4-Dichlorophenoxyacetic acid (2,4-D)	X			
Bentazon	X			
Bromoxynil	X			

Compound	Concentration below LOQ or not measured	Not removed	Percentage removal*	Increasing / Accumulating in MAR (MAR conc. > infiltration water
Dichlorprop	X			
2-methyl-4-chlorophenoxyaceticacid (MCPA)			82	
Mecoprop	X			
Chlofibric acid	X			

*removal after approx. 17 days of subsurface travel time measured in BSV10 under wet/mixed conditions, dilution ratio of 1

Table 3-3: Fate of pharmaceuticals during MAR at Sant Vicenç dels Horts.

Compound	Concentration below LOQ or not measured	Not removed	Percentage removal**	Increasing / Accumulating in MAR (MAR conc. > infiltration water
Phenazone (*)	X			
Carbamazepine (*)				X
Metoprolol (*)	X			
Phenylethylmalonamide	X			
Diclofenac (*)			85	
Iopromide (*)			91	
Ibuprofen (*)				X
Dihydroxydihydrocarbamazepine			71	
Primidone (*)	X			
Trimethoprim (*)	X			
Sulfamethoxazole (*)			75	
Bezafibrate (*)	X			
N-Acetyl-sulfamethoxazole			50	
Gabapentine			97	

*DEMEAU listed compound; **Removal after approx. 17 days of subsurface travel time measured in BSV10 under wet/mixed conditions, dilution ratio of 1

Table 3-4: Fate of other substances during MAR at Sant Vicenç dels Horts.

Compound	Concentration below LOQ or not measured	Not removed	Percentage removal**	Increasing / Accumulating in MAR (MAR conc. > infiltration water
Coffeine			84	
Acesulfame			77	
Benzotriazole*			92	
Phenylsulfonylsarcosin	X			

*DEMEAU listed compound; **Removal after approx. 17 days of subsurface travel time measured in BSV10 under wet/mixed conditions, dilution ratio of 1

3.3.3 Column vs field results

The observations under field conditions are then compared to observations made under lab conditions in column studies. Table 3-5 shows the comparison between observed removal in Sant Vicenç dels Horts and in soil column experiments. A detailed description of the methodology and results of the soil experiments can be found in the project deliverable D12.3 (Hernandez and Gibert (2014)) and in Schaffer *et al. (*2015).

Table 3-5: Qualitative comparison of behaviour in field site and column experiments for DEMEAU compounds

Compound	Soil column results (Schaffer et al. 2015)*	Field site results**
Benzotriazole	Slight removal	High removal
Diclofenac	Slight removal	High removal
Sulfamethoxazole	High removal	High removal
Carbamazepine	Not removed	difficult to assess

*after approx. 7.5 days of travel time in column; **after approx. 17 days of subsurface travel time measured in BSV10

There is a clear correlation in the trends observed at laboratory scale and at field scale. Trimethoprim, iopromide, bezafibrate and benzotriazole show elimination evidences in MAR system, while diclofenac, primidone and carbamazepine concentrations remain almost constant in both experiments. Due to the limited number of samples from the field site and the changing conditions in the MAR system (wet, dry and mix) it is not possible go further with the quantification of the removal at field scale.

3.4 Leaching test

The supernatant samples from the reactive layer installed in the field show a DOC similar to river water, whereas the fresh compost provides DOC almost 8 times more than the river water (**Table 3-6**). Analyses were performed in duplicated in the lab to assure the replicability of the experiment.

Table 3-6: DOC release from leaching tests

Samples code	Sample type	Dissolved Organic Carbon (DOC) mg/L
RIVER - A	River water	5.0
RIVER - B		5.1
LAYER - 1A	Sample 1 (4 year compost layer)	4.8
LAYER - 1B		4.9
LAYER - 2A	Sample 2 (4 year compost layer)	4.9
LAYER - 2B		4.9
Compost - A*	Fresh compost layer	39
Compost - B*		39

* These samples were previously diluted to calculate the TOC. Samples A and B are duplicated analysis.

The similarities between river water and samples 1 and 2 indicate no effect of additional DOC release of the 4-year old compost layer. This is a valuable data for operation and maintenance tasks of the infiltration pond in terms of replacement of the reactive layer once the release of DOC is exhausted.

3.5 Bioassays: toxicity assessment

3.5.1 Quality controls

All samples were tested in the bioassays together with the

- procedure blank,
- bioassay solvent blank (DMSO, EtOH),
- and corresponding reference compound of the assay.

Neither the procedure blank nor the bioassay solvent blank (data not shown) showed activity in the assays. The corresponding reference compound showed in each assay the maximum response in agreement with the historical positive control/reference compound data.
The limit of detection (LOD) - denoting the minimum amount of activity reliably detected – greatly depends on the amount of sample extracted, the concentration factor achieved during sample preparation, and the dilution factor required when testing an extract dissolved in a solvent (e.g. DMSO or ethanol) in the bioassay. Assay LOD and LOQ (limit of quantification, which is triple LOD) values are clearly indicated in the results tables (annex 4).

3.5.2 Measured activities and toxicity profiles

The activity of the tested extract was expressed as reference compound-equivalent concentration per sample unit and summarized in annex 4. The activities were then classified according to the activity significance (

Figure 3-9).

The obtained activity profiles of the MAR samples (left part in

Figure 3-9) were then evaluated and modified according to the available preliminary Algae test EQS (environmental quality standard proposals) and CALUX trigger values (van der Oost et al. 2015) (right part in

Figure 3-9). Trigger values for the other endpoints, are currently being established. Preliminary trigger values currently available for bioassays are shown in Table 3-7.

Table 3-7: Currently available preliminary trigger values for ecosystem health (van der Oost et al. 2015)

Bioassay	Trigger value	Unit
ERα-CALUX	1	ng 17β-Estradiol-Eq / L
Anti-AR-CALUX	40	µg Flutamide-Eq / L
GR-CALUX	30	ng Dexamethasone-Eq / L
PPARγ-CALUX	20	ng Rosiglitazone-Eq / L
Nrf2-CALUX	10	µg Curcumin-Eq / L

Bioassay	Trigger value	Unit
Combined Algae Test (Photosystem II Inhibition)*	20 (EQS proposal CH), 200 (EQS EU)	ng Diuron-Eq / L

(*)For the “high/low risk evaluation” of the measured activities in the combined algae assay the trigger value based on the EU EQS proposal was used and not based on the Swiss value.

Figure 3-9 summarises activity profile of the tested MAR water samples from Sant Vicenç dels Horts sampling site collected at two time points: I) July 2014 (wet conditions) and II) May 2015 (mixed conditions) in the in vitro bioassay panel (on the left). Detected activities are classified following the criteria showed on the upper part of the figure. The activity profile was then modified (on the right) considering available, preliminary trigger values (for estrogenic, anti-androgenic, glucocorticoid activity, oxidative stress and lipid metabolism). Samples that showed lower activity than the pertinent trigger value became “green” in the table on the right indicating low risk despite of the measured (quantifiable) activity.

The application of effect-based methods (bioassays) enabled to measure the combined effects of emerging pollutants (see results presented in
Figure 3-9). The broad range *in vitro* screening of the MAR water samples revealed the importance of ENDOCRINE - (particularly the activation of the ERα-, anti-AR, anti-PR receptors), OXIDATIVE STRESS (Nrf2-CALUX) and PHOTOSYNTHESIS INHIBITION (Combined algae test) pathways, and showed differences between the samples collected within two different time points (two sampling campaigns). The application of trigger values (thresholds) demonstrated the possibility for estimation of potential environmental risks with *in vitro* bioassay responses.

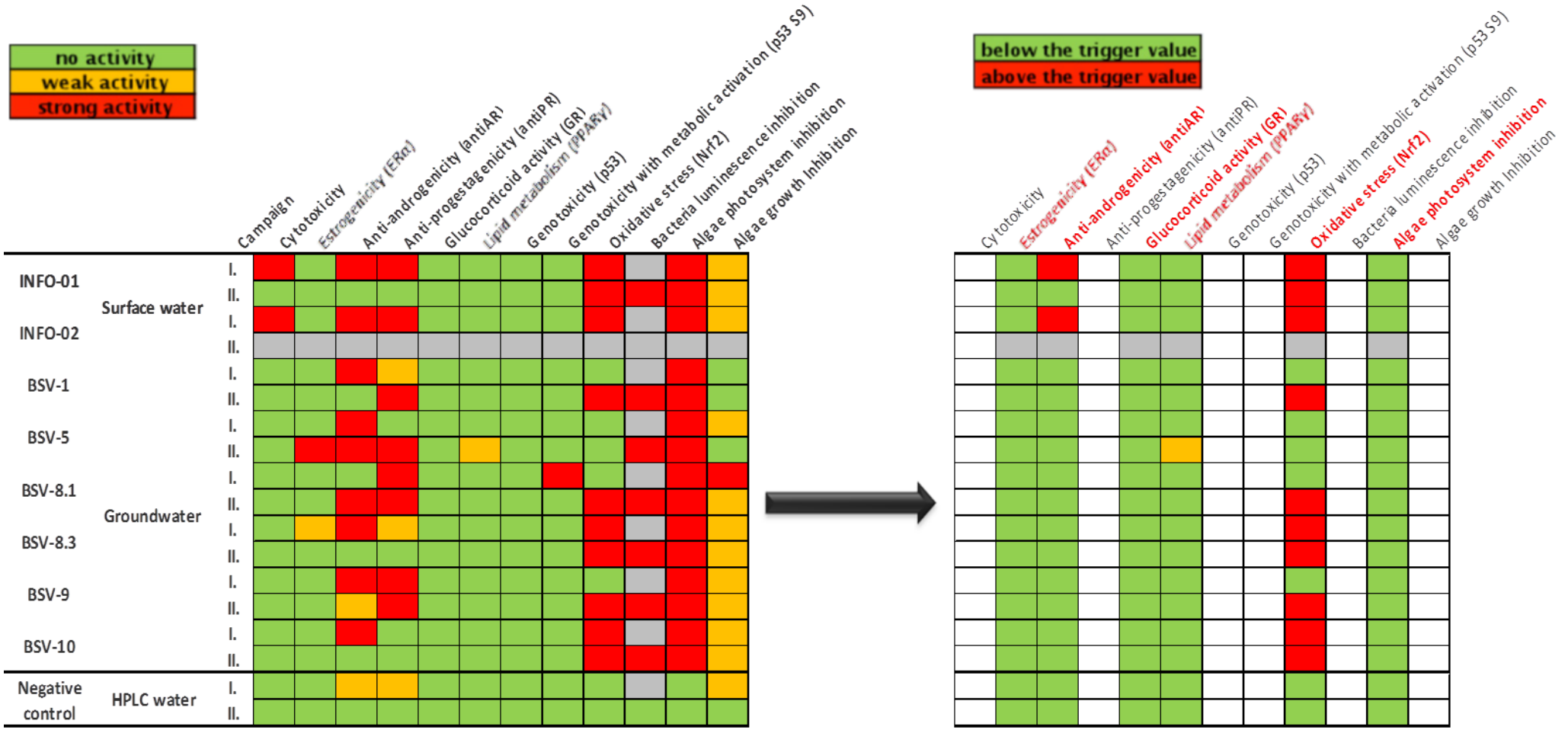


Figure 3-9: **Activity profile determined in the bioassays (colours in right matrix: green = activity below trigger value, yellow = coinciding with trigger value, red = activity above trigger value, I =** July 2014 sampling (wet conditions); II = May 2015 sampling (mixed conditions)**).**

Activities that fell under the defined trigger value of the certain bioassay are considered as low risk and suggested no need for further in-depth investigation (effect-directed analysis [EDA] or chemical analysis) to identify the source of the activity, the responsible compound(s). On the contrary, activities above the pertinent trigger values suggest the need for further investigations and imply the possibility of adverse (ecological) health effects. In the case of the Sant Vicenç dels Horts samples, the infiltration water (Llobregat River raw water) could suppose some adverse effects listed below:

- Anti-androgenic activity
- Oxidative stress

This approach – screening samples first with bioassays, followed by low/high risk evaluation with trigger values and chemical analysis if reasonable/justifiable is favoured by WA4 (Bioassays team in DEMEAU project). In this study applied trigger values are preliminary values, thus it is recommend to consider this exercise as an exemplification for the application of such threshold values and discriminating therefore between low and high risk sites.

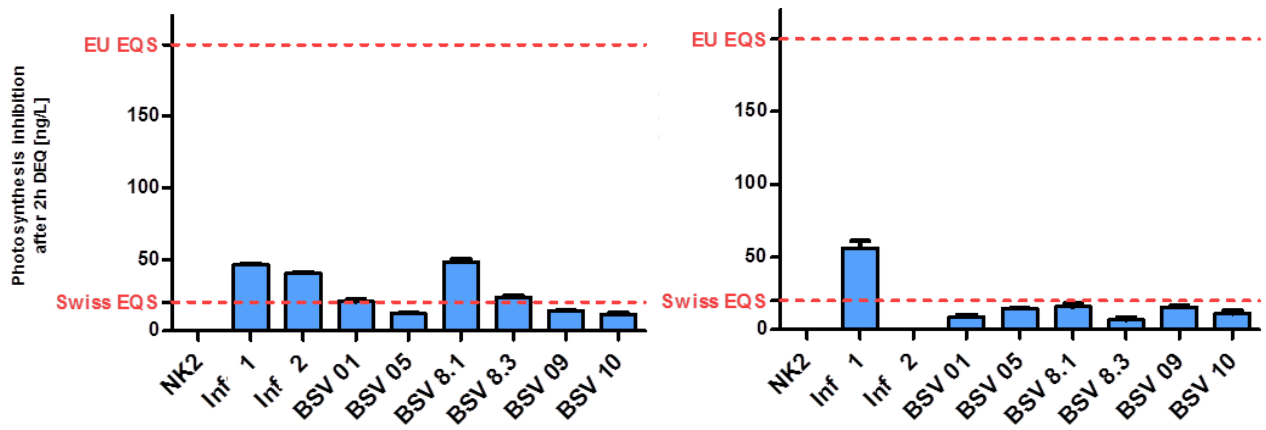


Figure 3-10: **Photosynthesis (after 2 hours of exposure) of the MAR samples from SVH – 1st sampling campaign (left) and 2nd sampling campaign (right) - expressed as ng Diuron Eq./L water. NK refers to negative control (HPLC water) went through on extraction and bioanalysis just as the samples.**

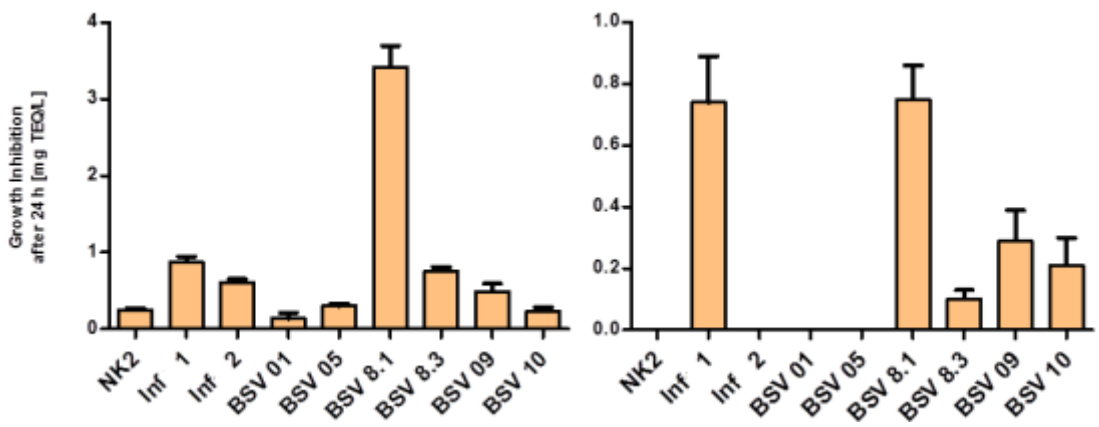
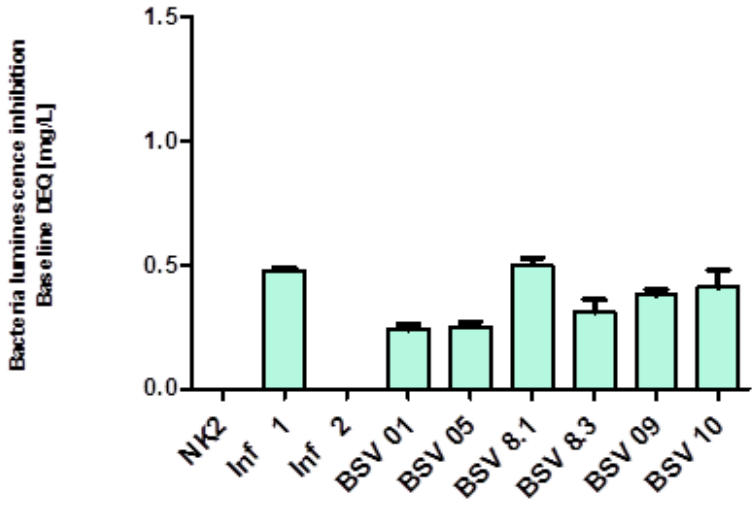


Figure 3-11: **Growth (after 24 hours of exposure) inhibition of the MAR samples from SVH – 1st sampling campaign (left) and 2nd sampling campaign (right) - expressed as ng Diuron Eq./L water. NK refers to negative control (HPLC water) went through on extraction and bioanalysis just as the samples.**

Figure 3-10, Figure 3-11 and represent the response of water samples to the inhibition of photosynthesis and the growth inhibition. The comparison between the two sampling campaigns performed as a duplicated of the experiments is presented. There are no evidences of the same responses in same samples. There are no clear conclusions.



(*) Campaign expressed as ng Diuron Eq./L water

Figure 3-12: Bacteria luminescence inhibition of the MAR samples from SVH – 2nd sampling. NK refers to negative control (HPLC water) went through on extraction and bioanalysis just as the samples.

Regarding **Fehler! Verweisquelle konnte nicht gefunden werden.**, it shows the inhibition of bacteria luminescence, with similar response in all the samples. Trigger values (see Table 3-7) are far above measured activity.

4 Recommendations for future studies

Operators of MAR system in Sant Vicenç dels Horts are now evaluating the possibility of replacing the vegetal compost layer, as there are no evidences of reactivity after four years of operation. Moreover, the infiltration rate decreased from $1 m^3/m^2/d$ to 0.15 $m^3/m^2/d$. This reduction of the infiltration rate has resulted in the temporal inactivity of the MAR system. Future operational practice of the infiltration pond should also include regular maintenance and the DEMEAU project contributed with cost analysis for different maintenance strategies (see report D51.1 available at http://demeau-fp7.eu).

Despite there are hot topics in the research of MAR as the behaviour of emerging pollutants or the dependence of removal rates of hydrogeochemical conditions of the system. Also there is only basic knowledge about the hydraulics of the MAR systems that are still unknown. Elemental knowledge about preferential pathways, residence time or differentiation of infiltration areas of the infiltration pond is an essential request to go deeper in the understanding of the behaviour of emerging pollutants. Continuous temperature measurements are recommended to allow detailed assessment of hydraulic dynamics of the MAR scheme and improved understanding of contaminant behaviour.

Regarding emerging pollutants and MAR systems, future research should be focus in the identification of the hazards in infiltration water. Lot of studies and publications have listed and quantified the emerging substances present in Llobregat River and infiltration water (pharmaceuticals, pesticides, hormones, personal care products, detergents). In this sense, bioassays are a powerful tool to determine the toxic effects of the cocktail of substances present in infiltration water and could help to determine which of them are principally responsible of the undesired effects in organisms.

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ANNEX 1 MAR profile

Table A-0-1: MAR profile at Sant Vicenç dels Horts.

MAR component	Attribute		Description
General information	Country		Spain
	City		Barcelona
	Site name		Sant Vicenç dels Horts infiltration system
	Operator name		Agència Catalana de l'Aigua
	Type of MAR (e.g. Well injection and recovery, Aquifer transfer and recovery, bank filtration etc.)		Infiltration Ponds
	Year commenced		2008
	Current status		Full operation
	Map coordinates		41.39/ 2.02
	Operational scale (m^3/a)		~ 1.8
	Objective		Environmental
Capture zone	Influent source (Type of water used for recharge)		River water (Reclaimed water in future)
Pre-treatment	Source water treatment before recharge		Settling basin
Recharge	No of recharge facilities		2 ponds (1 recharge, 1 settling)
	Hydraulic loading rate (m^3/m^2 d)		~ 1
	Recharged volume (m^3/a)		~ 1.8
Sub-surface	Residence time (d) of recharged water in the sub-surface until recovery		unknown
	Aquifer properties	Range of hydraulic conductivity representative for the target aquifer (m/s)	1×10^{-2}
		Lithology of target aquifer	porous
		Range of thickness of unsaturated zone (m)	Few m
		Thickness of target aquifer (m)	Up to 10
Recovery	Distance of recovery wells from point of recharge (m)		-
	Recovered volume (m^3/a)		-
	Recovered infiltrate (%) Average percentage of recovered infiltrate (in case of bank filtration share of bank-filtrate in abstraction wells)		-
	No of recovery facilities (e.g. no. of wells, drains)		-
Post-treatment	Water treatment after recovery		-
End-use	Final use of water recharged by the facility		Environmental (aquifer is used for agriculture, drinking water, industry)

ANNEX 2 Bulk chemistry

Table A-0-2: List of parameters and detection methods of bulk chemistry.

Parameters	Parameters	Units of determination	Methodology	Limit of detection (LDet)
General parameters	pH	Units of pH	Portable probe	4 - 14
	Electrical Conductivity	µS/cm	Portable probe	15-15000
	TOC	mg C/L	UV-VIS spectrophotometer	> 1
	Total hardness	mg HCO_3/L	Potentiometric titration	--
Major compounds	Nitrate	mg/L	Ionic chromatography	>0.5
	Ammonium	mg/L	Colorimetric (method indophenol)	>0.15
	Chloride	mg/L	Volumetric titration	>30
	Total Phosphorous	mg/L	UV-VIS spectrophotometer	>0.1
	Sulphate	mg/L	Ionic chromatography	>5
Metals	Sodium	mg/L	Spectroscopy inductively coupled plasma (ICP / AES)	>5
	Potassium	mg/L		>5
	Calcium	mg/L		>5
	Magnesium	mg/L		>2
	Aluminium	µg/L		>25
	Manganese	µg/L		>2
	Iron	µg/L		>5

Table A-0-3: Analytical results of bulk chemistry for the infiltration basin (INF) and monitoring well BSV-01

	INF1	INF2	INF			BSV-01		
	July 2014	July 2014	July 2014	January 2015	May 2015	July 2014	January 2015	May 2015
NO2-(mg/L)	< 0.03	< 0.03	0.015	0.31	0.25	0.015	0.015	0.015
NO3- (mg/L)	0.713	0.616	0.6645	19.2	5.53	6.2	17.5	12.3
Ni (µg/L)	12	5	8.5	7	8	2.5	2.5	2.5
Ammonium (mg/L)	0.43	0.23	0.33	0.55	0.0075	0.075	0.075	0.075
Cl (mg/L)	239	227	233	275	283	233	150	232
EC. (20°C) µS/cm	1141	1131	1136	1525	1426	1371	1145	1488
TOC (mg/L)	5.7	5.3	5.5	4.7	5	1.7	2.6	2.4
pH	8.1	8.2	8.15	8.4	8.3	7.4	7.7	7.6
SO42-(mg/L)	139	126	132.5	205	158	159	178	200
Fe (µg/L)	227	154	190.5	62	158	9	176	166
Mn (µg/L)	40	18	29	31	38	1	6.4	7
Al (µg/L)	122	99	110.5	55	226	12.5	258	204
Na (µg/L)	-	-	-	156	-	-	89	-
HCO_3^-(mg/L)	-	-	-	275	239	-	327	367
CO_3^{-2} (mg/L)	-	-	-	11.5	7.1	-	0	0
P (µg/L)	111	74	92.5	146	184	44	10	25
K(mg/L)	26	26	26	32	33	16	20	23
Ca (mg/L)	81	75	78	133	101	121	122	151
Mg (mg/L)	24	23	23.5	45	31	30	28	38

Table A-0-4: Analytical results of bulk chemistry for BSV-8.1 & BSV-8.3

	BSV8.1	BSV8.1	BSV8.1	BSV8.3	BSV8.3	BSV8.3
	July 2014	January 2015	May 2015	July 2014	January 2015	May 2015
NO2-(mg/L)	0.041	0.015	0.073	0.015	0.015	0.015
NO3- (mg/L)	12.7	11.2	21.4	2.75	8.93	19.3
Ni (µg/L)	5	5	2.5	8	9	2.5
Ammonium (mg/L)	0.075	0.075	0.075	0.075	0.075	0.075
Cl (mg/L)	214	199	198	226	168	201
EC. (20°C) µS/cm	1448	1333	1475	1248	1211	1470
TOC (mg/L)	2.8	1.7	2.8	4.1	2.5	2.4
pH	7.6	7.6	7.7	7.8	8	7.9
SO42-(mg/L)	209	164	212	113	142	200
Fe (µg/L)	276	121	256	2472	1297	1079
Mn (µg/L)	29	12	19	590	390	150
Al (µg/L)	133	172	266	1547	1602	1615
Na (µg/L)	-	117	-	-	101	-
HCO_3^-(mg/L)	-	362	422	-	362	423
CO_3^{-2} (mg/L)	-	0	0	-	0	0
P (µg/L)	71	23	59	441	376	165
K(mg/L)	25	18	22	25	23	26
Ca (mg/L)	127	132	150	196	204	184
Mg (mg/L)	34	37	41	32	37	43

Table A-0-5: Analytical results of bulk chemistry for BSV-05, BSV-09 & BSV10.

	BSV05	BSV05	BSV05	BSV09	BSV09	BSV09	BSV10	BSV10	BSV10
	July 2014	January 2015	May 2015	July 2014	January 2015	May 2015	July 2014	January 2015	May 2015
NO2-(mg/L)	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015
NO3- (mg/L)	3.43	11.3	12.8	4.42	9.78	17	5.63	13.9	13.1
Ni (µg/L)	2.5	5	2.5	8	6	2.5	6	2.5	2.5
Ammonium (mg/L)	0.075	0.075	0.075	0.075	0.075	0.075	0.075	0.075	0.075
Cl (mg/L)	237	169	279	254	204	206	269	168	252
EC. (20°C) µS/cm	1287	1205	1478	1402	1295	1363	1473	1215	1500
TOC (mg/L)	2.6	2.7	3	2.9	2	2.3	1.6	2.1	2.2
pH	7.6	8	7.9	7.8	7.6	8	7.6	7.7	7.9
SO42-(mg/L)	120	141	169	132	152	177	156	137	176
Fe (µg/L)	13	738	676	4832	150	3478	10	498	1140
Mn (µg/L)	1	120	78	760	45	830	1	23	61
Al (µg/L)	104	377	367	802	69	1835	12.5	374	1086
Na (µg/L)	-	106	-	-	119	-	-	110	-
HCO_3^-(mg/L)	-	351	277	-	333	344	-	365	347
CO_3^{-2} (mg/L)	-	0	0	-	0	0	-	0	0
P (µg/L)	50	189	152	178	22	368	10	31	63
K(mg/L)	27	27	31	26	25	27	23	24	26
Ca (mg/L)	95	150	132	171	128	311	119	133	148
Mg (mg/L)	25	37	36	34	35	56	29	33	38

ANNEX 3 Emerging pollutants

Table A-0-6: Overview of micropollutants for each sampling campaign.

Name	Chemical formula	Molecular Weight (gr/mol)	Substance class	07/2014-WET	01/2015 - DRY	05/2015-MIX
Alachlor	$C_{14}H_{20}ClNO_2$	269.78	Herbicide		X	
Atrazine	$C_8H_{14}ClN_5$	215.68	Herbicide		X	X
Boscalid	$C_{18}H_{12}Cl_2N_2O$	343.2	Pesticide/ fungicide		X	X
Bromacil	$C_9H_{13}BrN_2O_2$	261.11	Pesticide/ herbicide		X	X
Chlorfenvinphos	$C_{12}H_{14}Cl_3O_4P$	359.56	Insecticide		X	
Chloridazon	$C_{10}H_8ClN_3O$	22.66	Herbicide		X	X
Chlortoluron	$C_{10}H_{13}ClN_2O$	212.68	Herbicide		X	X
Desethylatrazine	$C_6H_{10}ClN_5$	187.63	Herbicide		X	X
Desethylterbutylazine	$C_7H_{12}ClN_5$	201.65	Herbicide		X	X
Desisopropylatrazin	$C_5H_8ClN_5$	173.6	Herbicide		X	X
2,6-Dichloro Benzamide	$Cl_2C_6H_3CONH_2$	190.03	Fungicide		X	X
Diuron	$C_9H_{10}Cl_2N_2O$	233.1	Herbicide		X	X
Ethofumesate	$C_{13}H_{18}O_5S$	286.34	Herbicide		X	X
Isoproturon	$C_{12}H_{18}N_2O$	206.28	Pesticide/ herbicide		X	X
Lenacil	$C_{13}H_{18}N_2O_2$	234.3	Herbicide		X	
Metalaxyl	$C_{15}H_{21}NO_4$	27933	Fungicide		X	
Metamitron	$C_{10}H_{10}N_4O$	202.22	Herbicide		X	X
Metazachlor	$C_{14}H_{16}ClN_3O$	277.75	Herbicide		X	X
Chloridazon-Methyl-Desphenyl	$C_5H_6ClN_3O$	159.57	Pesticide		X	X
Metolachlor	$C_{15}H_{22}ClNO_2$	283.80	Herbicide		X	X
Metribuzin	$C_8H_{14}N_4OS$	214.28	Pesticide/ fungicide		X	X
Quinoxyphen	$C_{15}H_8Cl_2FNO$	308.13	Fungicide		X	
Simazine	$C_7H_{12}ClN_5$	201.66	Herbicide		X	X
Terbuthylazine	$C_9H_{16}ClN_5$	229.71	Herbicide		X	X
Quinmerac	$C_{11}H_8ClNO_2$	221.64	Herbicide		X	X
2,4,5-Trichlorophenoxyacetic acid (2,4,5-T)	$C_8H_3Cl_3O_3$	255.48	Pesticide		X	
2,4-Dichlorophenoxyacetic acid (2,4-D)	$C_8H_6Cl_2O_3$	221.04	Herbicide		X	X
Bentazon	$C_{10}H_{12}N_2O_3S$	240.28	Herbicide		X	X
Bromoxynil	$C_7H_3Br_2NO$	276.92	Herbicide		X	
Dichlorprop	$C_9H_8Cl_2O_3$	235.06	Herbicide		X	X
MCPA	$C_9H_9ClO_3$	200.62	Herbicide		X	X
Mecoprop	$C_{10}H_{11}ClO_3$	214.65	Herbicide	X	X	X
Chlofibric acid	$C_{10}H_{11}ClO_3$	214.645	Herbicide		X	X
Phenazone (*)	$C_{11}H_{12}N_2O$	188.22	Analgesic	X	X	X
Carbamazepine (*)	$C_{15}H_{12}N_2O$	236.27	Anticonvulsant	X	X	X

Name	Chemical formula	Molecular Weight (gr/mol)	Substance class	07/2014-WET	01/2015 - DRY	05/2015-MIX
Metoprolol (*)	$C_{15}H_{25}NO_3$	267.36	Blocker	X	X	X
Diclofenac (*)	$C_{14}H_{11}NCl_2O_2$	296.15	Analgesic	X	X	X
Iopromide (*)	$C_{18}H_{24}I_3N_3O_8$	791.11	Contrast medium	X	X	X
Ibuprofen (*)	$C_{13}H_{18}O_2$	206.29	Antiinflammatory			X
Dihydroxydihydrocarbamazepine	$C_{15}H_{14}N_2O_2$	254.28	Metabolite of Carbamazepine		X	X
Primidone (*)	$C_{12}H_{14}N_2O_2$	218.25	Anticonvulsant	X	X	X
Phenylethylmalonamide	$C_{11}H_{14}N_2O_2$	206,24	Metabolite of Primidone		X	X
Trimethoprim (*)	$C_{14}H_{18}N_4O_3$	290.32	Antibiotic	X	X	
Sulfamethoxazole (*)	$C_{10}H_{11}N_3O_3S$	253.27	Antibiotic	X	X	X
4-formylaminoantipyrin (FAA)	$C_{12}H_{13}N_3O_2$	231.25	Antiinflamatory		X	X
Bezafibrate (*)	$C_{19}H_{20}ClNO_4$	361.82	Fibrate drug	X	X	X
N-Acetyl-sulfamethoxazole	$C_{12}H_{13}N_3O_4S$	295.31	Antibiotic		X	X
Gabapentine	C=9	171.23	Analgesic		X	X
Phenylethylmalonamide	$C_{11}H_{14}N_2O_2$	206.24	Anticolvulsant		X	X
Caffeine	$C_8H_{10}N_4O_2$	194,19	Stimulant		X	X
Acesulfame	$C_4H_4KNO_4S$	201,24	Artificial sweetener		X	X
Benzotriazole (*)	$C_6H_5N_3$	119,13	Corrosion Inhibitor	X	X	X
Phenylsulfonylsarcosin	$C_{11}H_{14}N_2O_2$	206,24	Metabolite of corrosion inhibitor		X	X

(*) indicates DEMEAU listed compound

Table A-0-7: Analytical results of pesticides.

No	Compound	LOQ [ng/l]	INF1 - 07/2014 -WET	INF2 - 07/2014-WET	INF - 01/2015 - DRY	INF-05/2015-MIX	BSV-01 - 07/2014-WET	BSV-01 - 01/2015 - DRY	BSV-01-05/2015-MIX	BSV-8.1 - 07/2014-WET	BSV-8.1 - 01/2015 - DRY	BSV-8.1-05/2015-MIX	BSV-8.3 - 07/2014-WET	BSV-8.3 - 01/2015 - DRY	BSV-8.3-05/2015-MIX	BSV-05 - 07/2014-WET	BSV-05 - 01/2015 - DRY	BSV-05-05/2015-MIX	BSV-09 - 07/2014-WET	BSV-09 - 01/2015 - DRY	BSV-09-05/2015-MIX	BSV-10 - 07/2014-WET	BSV-10 - 01/2015 - DRY	BSV-10-05/2015-MIX
1	PBSM	30			30	50		15	30		40	15		30	30		15	30		30	70		40	30
2	Alachlor	30			15	n.a.		15	n.a.		15	n.a.		15	n.a.		15	n.a.		15	n.a.		15	n.a.
3	Atrazine	30			15	15		15	15		15	15		15	15		15	15		15	15		15	15
4	Boscalid	30			15	15		15	15		15	15		15	15		15	15		15	15		15	15
5	Bromacil	30			15	15		15	15		15	15		15	15		15	15		15	15		15	15
6	Chlorfenvinphos	30			15	n.a.		15	n.a.		15	n.a.		15	n.a.		15	n.a.		15	n.a.		15	n.a.
7	Chloridazon	30			15	15		15	15		15	15		15	15		15	15		15	15		15	15
8	Chlortoluron	30			15	15		15	15		15	15		15	15		15	15		15	15		15	15
9	Desethylatrazin	30			15	15		15	15		15	15		15	15		15	15		15	15		15	15
10	Desethylterbutylazin	30			15	15		15	15		15	15		15	15		15	15		15	15		15	15
11	Desisopropylatrazin	30			15	15		15	30		40	15		30	30		15	30		30	70		40	30
12	2,6-Dichlorbenzamid	30			15	15		15	15		15	15		15	15		15	15		15	15		15	15
13	Diuron	30			30	50		15	15		15	15		15	15		15	15		15	15		15	15
14	Ethofumesate	30			15	15		15	15		15	15		15	15		15	15		15	15		15	15
15	Isoproturon	30			15	15		15	15		15	15		15	15		15	15		15	15		15	15
16	Lenacil	30			15	n.a.		15	n.a.		15	n.a.		15	n.a.		15	n.a.		15	n.a.		15	n.a.
17	Metalaxyl	30			15	n.a.		15	n.a.		15	n.a.		15	n.a.		15	n.a.		15	n.a.		15	n.a.
18	Metamitron	30			15	15		15	15		15	15		15	15		15	15		15	15		15	15
19	Metazachlor	30			15	15		15	15		15	15		15	15		15	15		15	15		15	15

No	Compound	LOQ [ng/l]	INF1 - 07/2014 -WET	INF2 - 07/2014-WET	INF - 01/2015 - DRY	INF-05/2015-MIX	BSV-01 - 07/2014-WET	BSV-01 - 01/2015 - DRY	BSV-01-05/2015-MIX	BSV-8.1 - 07/2014-WET	BSV-8.1 - 01/2015 - DRY	BSV-8.1-05/2015-MIX	BSV-8.3 - 07/2014-WET	BSV-8.3 - 01/2015 - DRY	BSV-8.3-05/2015-MIX	BSV-05 - 07/2014-WET	BSV-05 - 01/2015 - DRY	BSV-05-05/2015-MIX	BSV-09 - 07/2014-WET	BSV-09 - 01/2015 - DRY	BSV-09-05/2015-MIX	BSV-10 - 07/2014-WET	BSV-10 - 01/2015 - DRY	BSV-10-05/2015-MIX
20	Methyldesphenylchloridazon	30			15	15		15	15		15	15		15	15		15	15		15	15		15	15
21	Metolachlor	30			15	15		15	15		15	15		15	15		15	15		15	15		15	15
22	Metribuzin	30			15	15		15	15		15	15		15	15		15	15		15	15		15	15
23	Quinoxyphen	30			15	n.a.		15	n.a.		15	n.a.		15	n.a.		15	n.a.		15	n.a.		15	n.a.
24	Simazine	30			15	15		15	15		15	15		15	15		15	15		15	15		15	15
25	Terbuthylazine	30			15	15		15	15		15	15		15	15		15	15		15	15		15	15
26	Quinmerac	30			15	15		15	15		15	15		15	15		15	15		15	15		15	15
27	FAA	20			220	190		10	20		10	10		10	10		10	70		10	10		10	10
28	2,4,5-T	20			10	n.a.		10	n.a.		10	n.a.		10	n.a.		10	n.a.			n.a.		10	n.a.
29	2,4-D	20			10	10		10	10		10	10		10	10		10	10		10	10		10	10
30	Bentazon	20			10	10		10	10		10	10		10	10		10	10		10	10		10	10
31	Bromoxynil	20			10	n.a.		10	n.a.		10	n.a.		10	n.a.		10	n.a.			n.a.		10	n.a.
32	Dichlorprop	20			10	10		10	10		10	10		10	10		10	10		10	10		10	10
33	MCPA	20			10	100		10	10		10	10		10	10		10	10		10	10		10	10
34	Mecoprop	20	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
35	Chlofibric acid	20			10	10		10	10		10	10		10	10		10	10		10	10		10	10

Table A-0-8: Analytical results of pharmaceuticals & other substances

No	Compound	LOQ [ng/l]	INF1 - 07/2014 -WET	INF2 - 07/2014-WET	INF - 01/2015 - DRY	INF-05/2015-MIX	BSV-01 - 07/2014-WET	BSV-01 - 01/2015 - DRY	BSV-01-05/2015-MIX	BSV-8.1 - 07/2014-WET	BSV-8.1 - 01/2015 - DRY	BSV-8.1-05/2015-MIX	BSV-8.3 - 07/2014-WET	BSV-8.3 - 01/2015 - DRY	BSV-8.3-05/2015-MIX	BSV-05 - 07/2014-WET	BSV-05 - 01/2015 - DRY	BSV-05-05/2015-MIX	BSV-09 - 07/2014-WET	BSV-09 - 01/2015 - DRY	BSV-09-05/2015-MIX	BSV-10 - 07/2014-WET	BSV-10 - 01/2015 - DRY	BSV-10-05/2015-MIX
36	Phenazone	20	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
37	Carbamazepine	20	30	20	30	20	20	10	30	10	20	10	30	30	30	30	30	30	30	20	30	20	20	10
38	Metoprolol	20	10	10	10	10	15	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	15	10
39	Phenylethylmalonamide	20		10	10		15	10		10		10		10		10		10		10	10		15	10
40	Diclofenac	20	10	970	10	10	180	10	10	10	10	10	10	10	10	10	10	10	10	970	10	10	180	10
41	Iopromide	20	10	10	10	80	180	90	10	10	10	10	10	10	10	10	10	10	10	10	10	80	180	90
42	Ibuprofen	50		n.a.	50		n.a.	11		50		50		50		50		50		n.a.	50		n.a.	11
43	Dihydroxydihydrocarbamazepine	30		40	70		170	180		15		15		130		100		50		40	70		170	180
44	Primidone	20	10	10	20	10	10	20	10	20	10	20	10	20	10	20	10	20	10	10	20	10	10	20
45	Trimethoprim	30	15	15	n.a.	15	30	n.a.	15	n.a.	15	n.a.	15	n.a.	15	n.a.	15	n.a.	15	15	n.a.	15	30	n.a.
46	Sulfamethoxazole	20	10	20	10	20	50	50	10	10	10	10	10	10	10	20	10	20	10	20	10	20	50	50
47	Bezafibrate	20	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
48	N-Acetyl-sulfamethoxazole	20		10	10		30	10		10		10		10		10		10		10	10		30	10
49	Gabapentine	30		15	15		750	650		15		15		300		100		15		15	15		750	650
50	Caffeine	10		190	150		420	210		150		50		320		50		50		190	150		420	210
51	Acesulfame			290	120		2000	1400		150		210		770		1100		390		290	120		2000	1400
52	Benzotriazole	50	25	25	25	360	310	270	320	25	120	25	220	190	110	25	25	25	25	25	25	360	310	270
53	Phenylsulfonylsarcosin	20		10	10		10	10		10		10		10		10		10		10	10		10	10
36	Phenazone	20	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10

No	Compound	LOQ [ng/l]	INF1 - 07/2014 -WET	INF2 - 07/2014-WET	INF - 01/2015 - DRY	INF-05/2015-MIX	BSV-01 - 07/2014-WET	BSV-01 - 01/2015 - DRY	BSV-01-05/2015-MIX	BSV-8.1 - 07/2014-WET	BSV-8.1 - 01/2015 - DRY	BSV-8.1-05/2015-MIX	BSV-8.3 - 07/2014-WET	BSV-8.3 - 01/2015 - DRY	BSV-8.3-05/2015-MIX	BSV-05 - 07/2014-WET	BSV-05 - 01/2015 - DRY	BSV-05-05/2015-MIX	BSV-09 - 07/2014-WET	BSV-09 - 01/2015 - DRY	BSV-09-05/2015-MIX	BSV-10 - 07/2014-WET	BSV-10 - 01/2015 - DRY	BSV-10-05/2015-MIX
37	Carbamazepine	20	30	20	30	20	20	10	30	10	20	10	30	30	30	30	30	30	30	20	30	20	20	10
38	Metoprolol	20	10	10	10	10	15	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	15	10
39	Phenylethylmalonamide	20		10	10		15	10		10		10		10		10		10		10	10		15	10
40	Diclofenac	20	10	970	10	10	180	10	10	10	10	10	10	10	10	10	10	10	10	970	10	10	180	10
41	Iopromide	20	10	10	10	80	180	90	10	10	10	10	10	10	10	10	10	10	10	10	10	80	180	90
42	Ibuprofen	50		n.a.	50		n.a.	11		50		50		50		50		50		n.a.	50		n.a.	11
43	Dihydroxydihydrocarbamazepine	30		40	70		170	180		15		15		130		100		50		40	70		170	180
44	Primidone	20	10	10	20	10	10	20	10	20	10	20	10	20	10	20	10	20	10	10	20	10	10	20
45	Trimethoprim	30	15	15	n.a.	15	30	n.a.	15	n.a.	15	n.a.	15	n.a.	15	n.a.	15	n.a.	15	15	n.a.	15	30	n.a.
46	Sulfamethoxazole	20	10	20	10	20	50	50	10	10	10	10	10	10	10	20	10	20	10	20	10	20	50	50
47	Bezafibrate	20	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
48	N-Acetyl-sulfamethoxazole	20		10	10		30	10		10		10		10		10		10		10	10		30	10
49	Gabapentine	30		15	15		750	650		15		15		300		100		15		15	15		750	650
50	Caffeine	10		190	150		420	210		150		50		320		50		50		190	150		420	210
51	Acesulfame			290	120		2000	1400		150		210		770		1100		390		290	120		2000	1400
52	Benzotriazole	50	25	25	25	360	310	270	320	25	120	25	220	190	110	25	25	25	25	25	25	360	310	270
53	Phenylsulfonylsarcosin	20		10	10		10	10		10		10		10		10		10		10	10		10	10

ANNEX 4 Bioassays

Table A-0-9: Activities (ng or µg reference compound equivalent concentration/L water sample) detected in the in vitro bioassays for the MAR water samples from the SVH sampling site collected at two time points: 07/2014 (Campaign I) and 05/2015 (Campaign II)

												Combined algae assay	
		Cytotox CALUX	Erα CALUX	antiAR CALUX	antiPR CALUX	GR CALUX	PPARg2 CALUX	p53 CALUX	p53 S9 CALUX	Nrf2 CALUX	Microtox assay	PSII inhibition 2 h	Growth inhibition
Sample name	Campaign	-	ng 17ß-estradiol eq./L water	ng Flutamide eq./L water	ng Ru486 eq./L water	ng Dexamethasone eq./L water	ng Rosiglitazone eq./L water	µg Actinomycin D eq./L water	µg Cyclophosphamide eq./L water	µg Curcumine eq./L water	Baseline TEQ mg/L water	ng Diuron eq./L water	mg baseline toxicity eq./L water
INF01	**I.**	+	LOD (<0.03)	85300	1.65	< LOD (0.9)	LOD (<33.7)	LOD (<0.04)	LOD (<2400)	165	-	46.5	0.88
	II.	-	LOD (<0.04)	LOD (<1500)	LOD (<0.06)	< LOD (4.5)	LOD (<45)	LOD (<0.01)	LOD (<290)	40	0.48	56.0	0.74
INF02	**I.**	+	LOD (<0.03)	74700	1.03	< LOD (0.9)	LOD (<33.7)	LOD (<0.04)	LOD (<2400)	57	-	40.5	0.61
	II.	-	-	-	-	-	-	-	-	-	-	-	-
BSV-1	**I.**	-	LOD (<0.01)	6900	<LOQ (0.03)	< LOD (0.9)	LOD (<11.2)	LOD (<0.04)	LOD (<2400)	LOD (<17)		20.6	0.14
	II.	-	LOD (<0.04)	LOD (<1500)	1.5	< LOD (4.5)	LOD (<45)	LOD (<0.01)	LOD (<290)	44	0.24	8.7	0.00
BSV-5	**I.**	-	LOD (<0.01)	2500	LOD (<0.02)	< LOD (0.9)	LOD (<11.2)	LOD (<0.04)	LOD (<2400)	LOD (<17)	-	12.4	0.30
	II.	-	0.23	25000	3.4	< LOD (4.5)	<LOQ (110)	LOD (<0.01)	LOD (<290)	LOD (<15)	0.25	14.5	0.00
BSV-8.1	**I.**	-	LOD (<0.01)	LOD (<1500)	0.10	< LOD (0.9)	LOD (<11.2)	LOD (<0.04)	22700	LOD (<17)	-	48.5	3.42
	II.	-	LOD (<0.04)	4900	2.3	< LOD (4.5)	LOD (<45)	LOD (<0.01)	LOD (<290)	83	0.50	15.8	0.75
BSV-8.3	**I.**	-	< LOQ (0.01)	6200	0.06	< LOD (0.9)	LOD (<11.2)	LOD (<0.04)	LOD (<2400)	19	-	23.8	0.76
	II.	-	LOD (<0.04)	LOD (<1500)	LOD (<0.14)	< LOD (4.5)	LOD (<45)	LOD (<0.01)	LOD (<290)	39	0.31	6.9	0.10
BSV-9	**I.**	-	LOD (<0.01)	8900	0.22	< LOD (0.9)	LOD (<11.2)	LOD (<0.04)	LOD (<2400)	LOD (<17)	-	14.2	0.49
	II.	-	LOD (<0.04)	<LOQ(2600)	1.4	< LOD (4.5)	LOD (<45)	LOD (<0.01)	LOD (<290)	50	0.38	15.6	0.29

												Combined algae assay	
		Cytotox CALUX	Erα CALUX	antiAR CALUX	antiPR CALUX	GR CALUX	PPARg2 CALUX	p53 CALUX	p53 S9 CALUX	Nrf2 CALUX	Microtox assay	PSII inhibition 2 h	Growth inhibition
Sample name	Campaign	-	ng 17ß-estradiol eq./L water	ng Flutamide eq./L water	ng Ru486 eq./L water	ng Dexamethasone eq./L water	ng Rosiglitazone eq./L water	µg Actinomycin D eq./L water	µg Cyclophosphamide eq./L water	µg Curcumine eq./L water	Baseline TEQ mg/L water	ng Diuron eq./L water	mg baseline toxicity eq./L water
BSV-10	I.	-	LOD (<0.01)	3400	LOD (<0.02)	< LOD (0.9)	LOD (<11.2)	LOD (<0.04)	LOD (<2400)	44	-	11.9	0.23
	II.	-	LOD (<0.04)	LOD (<1500)	LOD (<0.06)	< LOD (4.5)	LOD (<45)	LOD (<0.01)	LOD (<290)	39	0.41	11.0	0.21
Negative control	I.	-	LOD (<0.03)	< LOQ (242)	0.08	< LOD (0.9)	LOD (<11.2)	LOD (<0.04)	LOD (<2400)	LOD (<17)	-	0.6	0.37
	II.	-	LOD (<0.06)	LOD (<2500)	LOD (<0.03)	< LOD (7.6)	LOD (<76)	LOD (<0.01)	LOD (<290)	LOD (<15)	0.0	0.0	0.00

***Samples extracts were also measured in the p53 CALUX assay with metabolitic activation by adding 59 enzyme mix**

****Photosynthesis inhibition was measured 2 hours after exposure**

***** Growth inhibition was measured 24 hours after exposure**

****** Not analysed**