Field investigations and risk assessment
in La Vall d'Uixó (Castellón, Spain)

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Summary: The La Vall d'Uixó (Spain) pilot site has been selected by DEMEAU because it is a new Aquifer Storage Transfer and Recovery (ASTR) site consisting of two injection wells surrounded by farmer wells for irrigation in a water scarce area. Potential water source for this MAR site is the effluent of the local WWTP, which is a quite constant water source in terms of availability, but gives concerns in terms of water quality. The investigations carried out within DEMEAU supports the work previously done by the Water Recovery Project (2011 – 2014), coordinated by IGME (Instituto Geológico y Minero de España) and UJI (Universitat Jaume I). The Water Recovery Project consists of different implementation phases and aimed to establish an appropriate MAR scheme with reclaimed wastewater to counteract salinity ingress in the coastal aquifer. In the third phase of the project two injection wells have recharged 310,000 m^3 with water from the Belcaire River. To foster the implementation of the fourth and final phase of the Water Recovery Project, DEMEAU focused on the evaluation of the effluent of the local WWTP as source water for the ASTR system. This has been done by three sampling campaigns to analyse bulk chemistry, emerging pollutants and bioassays in native groundwater (six agricultural wells), Belcaire River (the current source water of the MAR scheme) and WWTP effluent (potential future source water). Risk assessment based on Australian MAR guidelines have been applied to evaluate risks related to the usage of WWTP effluent as source water. The Australian guidelines have been applied in two steps: entry level assessment and maximal risk assessment. Entry level assessment concluded that La Vall d'Uixó is suitable for a MAR scheme using reclaimed water, while maximal risk assessment identified hazards associated to reclaimed water as source water. As La Vall d'Uixó is an agricultural area of citrus crops, the use of reclaimed water for the injection in the MAR system must be compatible with the use of recovered water for irrigation. The risk assessment done in this report considered this end use of water, as there are no drinking water wells in the area. High risks have been identified for inorganic chemicals (conductivity, chloride and bicarbonate) and nutrients (nitrate). Risks associated to inorganics can be minimized by mixing effluent and Belcaire River water 1:1.

Bulk chemistry coincided mainly with the description carried out in Water Recovery project, identifying two main quality problems in native groundwater: (1) salinity ingress (2) high nitrate concentration due to the intensive agricultural practices in the area. Ion displacement pattern in groundwater samples clearly indicates on-going salinization and documents minor effects of the injected water on few wells only. Cl/Br ratios indicate additional sources of chloride apart from seawater. It seems plausible that the underlying Keuper formations (Triassic) contribute to salinity ingress and SO4 excess in groundwater to some extent.

Chlorides and nitrate are regulated by the implementation in Spain of the EU Water Framework Directive for the Castellón aquifer. The threshold value for nitrate is 200 mg/L, while the threshold

value for chloride is 650 mg/L. WWTP effluent has nitrate and chlorides below the threshold concentrations (60 mg/L and 140 mg/L respectively) and, therefore, the MAR with reclaimed water would suppose a reduction of groundwater pollution and a step towards a qualitative good status in the aquifer.

In total 63 organic micro pollutants have been analysed in groundwater, surface water and WWTP effluent. WWTP effluent shows elevated concentrations in almost all groups of organic micro pollutants compared to river- or groundwater. Only pesticides are found in higher concentrations in groundwater compared to the effluent. The Belcaire River shows the lowest concentrations for all groups of micro pollutants. It was shown that the Vall d'Uixó aquifer is contaminated by various organic micro pollutants and does not reflect a near natural aquifer condition. The aquifer chemistry in terms of organic micro pollutants reflects the usage of (untreated) effluent for direct irrigation over years. Elevated concentration of artificial sweeteners, analgesics, stimulants, caffeine metabolites and cocaine metabolites were found in WWTP samples taken during weekends compared to workday samples. In contrast, iopromide has been quantified in higher concentrations in the effluent of WWTP in work days than in the weekend, as this contrast media is used in hospitals for diagnostic tests normally carried out from Monday to Friday. These patterns of the effluent of WWTP during the week of weekend could be determinant for the selection of the working days as most suitable days to store treated waste water.

In order to link analysed chemical concentrations to the observed toxicity in the samples a procedure based on bioassay-specific relative potency (REP) factors was applied. REP factors are determined by the effect concentrations of the reference compound and of the test compound.

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 the usefulness of combined analyses of environmental samples. Effect-based methods could complement conventional chemical analysis in water quality monitoring as pre-screening techniques by (1) identifying toxic "hotspots" for further investigation, (2) assessing the effect of the entire mixture of compounds present in waters and therefore and (3) reduce uncertainty in safety evaluation.

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Table of contents

List of Tables

List of Figures

1   Introduction

In Vall d'Uixó near Castellón (Spain) a new Managed Aquifer Recharge (MAR) site based on Aquifer Storage Transfer and Recovery (ASTR) is currently under development. The framework of the work is the collaboration between the DEMEAU project, looking for demonstrating the feasibility of MAR to eliminate emerging micropollutants and the Water Recovery project, carried out in the area to promote the implementation of a new MAR site in the Mediterranean Spanish coast. DEMEAU has contributed to the Water Recovery project by doing additional sampling campaigns to assess the potential impact of the use of reclaimed water in the system. This report presents the results obtained in La Vall d'Uixó consisting mainly in the following investigations:

- Bulk chemistry: characterisation of source- and groundwater
- Assessment of emerging pollutants and their qualitative behaviour during MAR
- Entry level assessment (based on Australian Guidelines)
- Maximal risk assessment (based on Australian Guidelines)
- Bioassays of MAR samples

A risk assessment has been performed to evaluate the hazards of the injection of reclaimed water coming from the secondary effluent of the local Waste Water Treatment Plant (WWTP). To this end, Australian Guidelines (NRMMC-EPHC-NHMRC 2009) have been applied, following the entry level assessment and the maximal risk evaluation. Three sampling campaigns (2014 to 2015) have been carried out to characterise effluent, river- and groundwater. Bulk chemistry has been analysed in local laboratories, while emerging pollutants have been analysed in two specialised laboratories: University of Castellón (Spain), Göttingen University (Germany). Moreover, there has been an interdisciplinary collaboration with other partners of the DEMEAU project consortium related to assess water toxicity caused by emerging pollutants through bioassays. Samples from Vall d'Uixó were collected, shipped and analysed at BDS (The Netherlands) and EAWAG (Switzerland) using different bioassays. Bioassays have been carried out to assess water toxicity in different steps of the system (surface water, effluent of WWTP and groundwater). The bioassays results are also presented in this report and give information about the toxicity of water samples in the Castellón MAR site: injection water (surface water and reclaimed water) and the initial levels of toxicity in groundwater.

The essential idea of the Water Recovery project is to establish an appropriate implementing methodology for a pilot artificial recharge system with reclaimed wastewater to counteract salinity ingress and thereby contribute to the rehabilitation of coastal aquifers in the Castellón plain. The Water Recovery project has four phases. Phase I (September 2011-October 2012) focused on the selection of the pilot area, its hydrogeological characterisation, the design of the recharge system and establishment of the monitoring network and control program. Phase II (2013) included the construction of the injection wells and new piezometers to complement the existing monitoring network. Phase III (2013-2014) consisted in the injection of about 300,000 m^3 of surface water in the two injection wells. During this phase source water came from a reservoir, which accumulated freshwater in rainy season from the Belcaire River. Water Recovery project has currently finished the third phase. The fourth phase was planned to consist in the injection of effluent from the local Waste

Water Treatment Plant (WWTP) in La Vall d'Uixó (Figure 1-1). This last phase is currently in stand-by due to financial constraints.
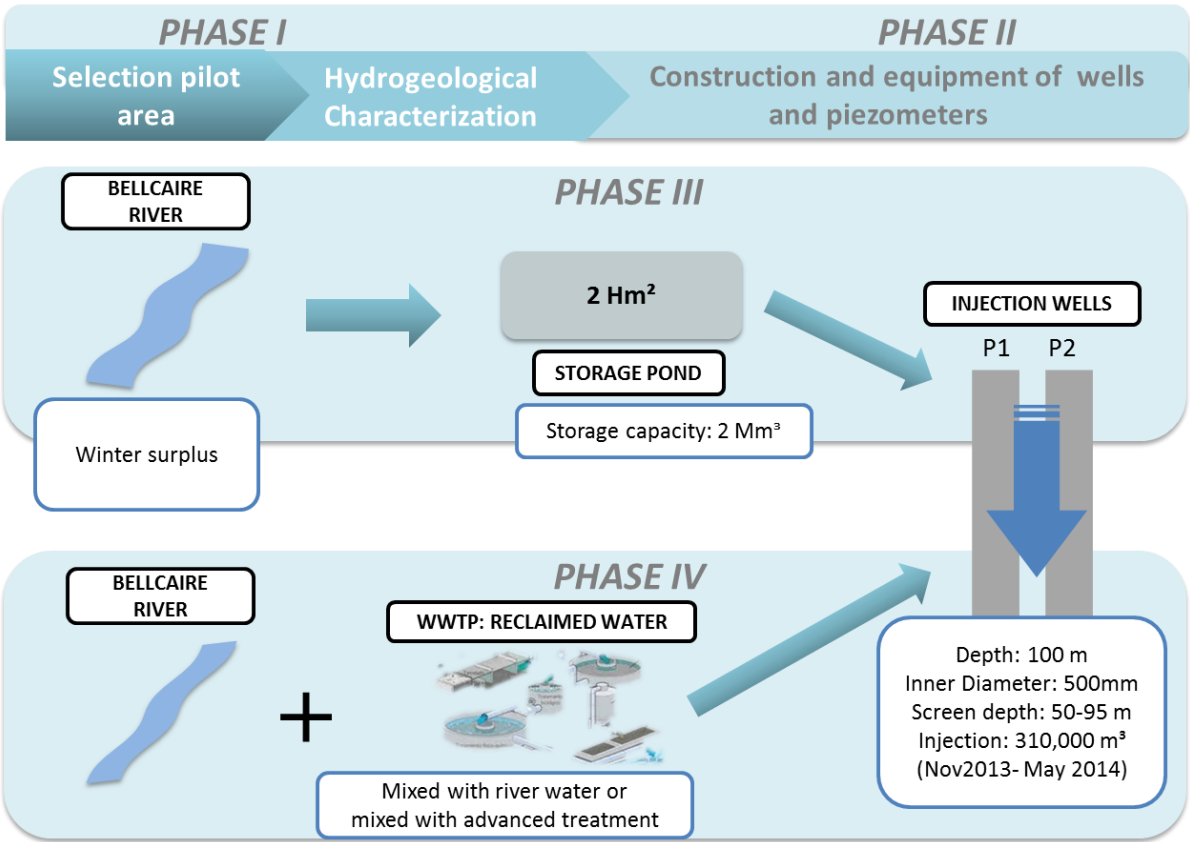
Figure 1-1:   The Water Recovery Project scheme

2 General site description

La Vall d'Uixó is a municipality of approximately 33,000 inhabitants and 67.1 Km^2 of surface, located on the coastline of the province of Castellón (administrative regional district of Spain). The coastal area has an intense agricultural and industrial activity and also supports the main urban centers that attract a growing number of tourists. Almost half of the water demand from these activities is satisfied by groundwater from coastal aquifers.

These aquifers have suffered problems of overexploitation and salinization caused by seawater intrusion. Seawater intrusion is a significant threat to the sustainability of coastal water resources. Therefore, the essential idea of the Water Recovery project was to establish an appropriate implementation methodology to conduct a pilot study of artificial recharge with reclaimed wastewater to combat saltwater intrusion and contribute to the recovery of coastal aquifers.

The study area is located in the natural region called Plana de Castellón (Spain), specifically in the area of La Vall d'Uixó - Rambleta. It consists of a floodplain roughly triangular in shape with an area of 464 km^2 and an altitude between 0 and 130 above sea level.
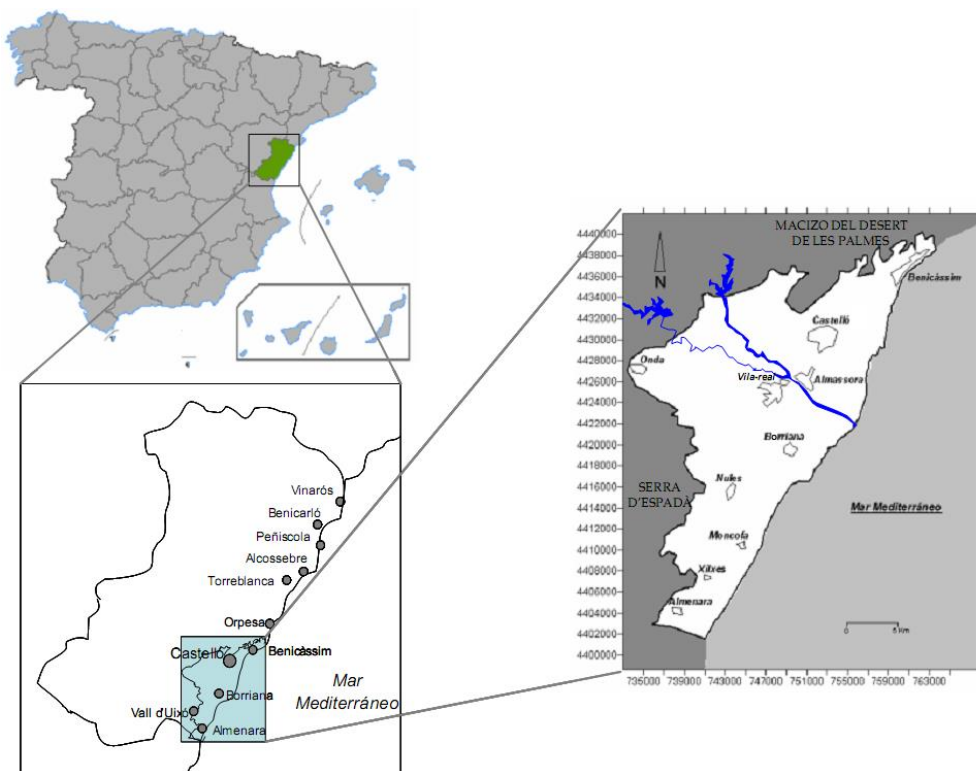
Figure 2-1:   Map of location of La Vall d'Uixó (Morell *et al.* 2012)

The historical development of salinity ingress in the aquifer is illustrated in Figure 2-2. Seawater intrusion is known to have occurred in this area since the 1960s, and so the area is characteristic for salinization in Spain. In order to mitigate the seawater intrusion, a variety of organizations and institutions have undertaken studies in this area, including the Júcar Hydrographic Confederation

(JHC), the Spanish Geological and Mining Institute (IGME), the Generalitat Valenciana (the Valencia Regional Government), the University Jaume I de Castelló and the Polytechnic University of Valencia. As a result, there is abundant hydrogeological, hydrogeochemical and hydrochemical data for the concerned groundwater body in this area.
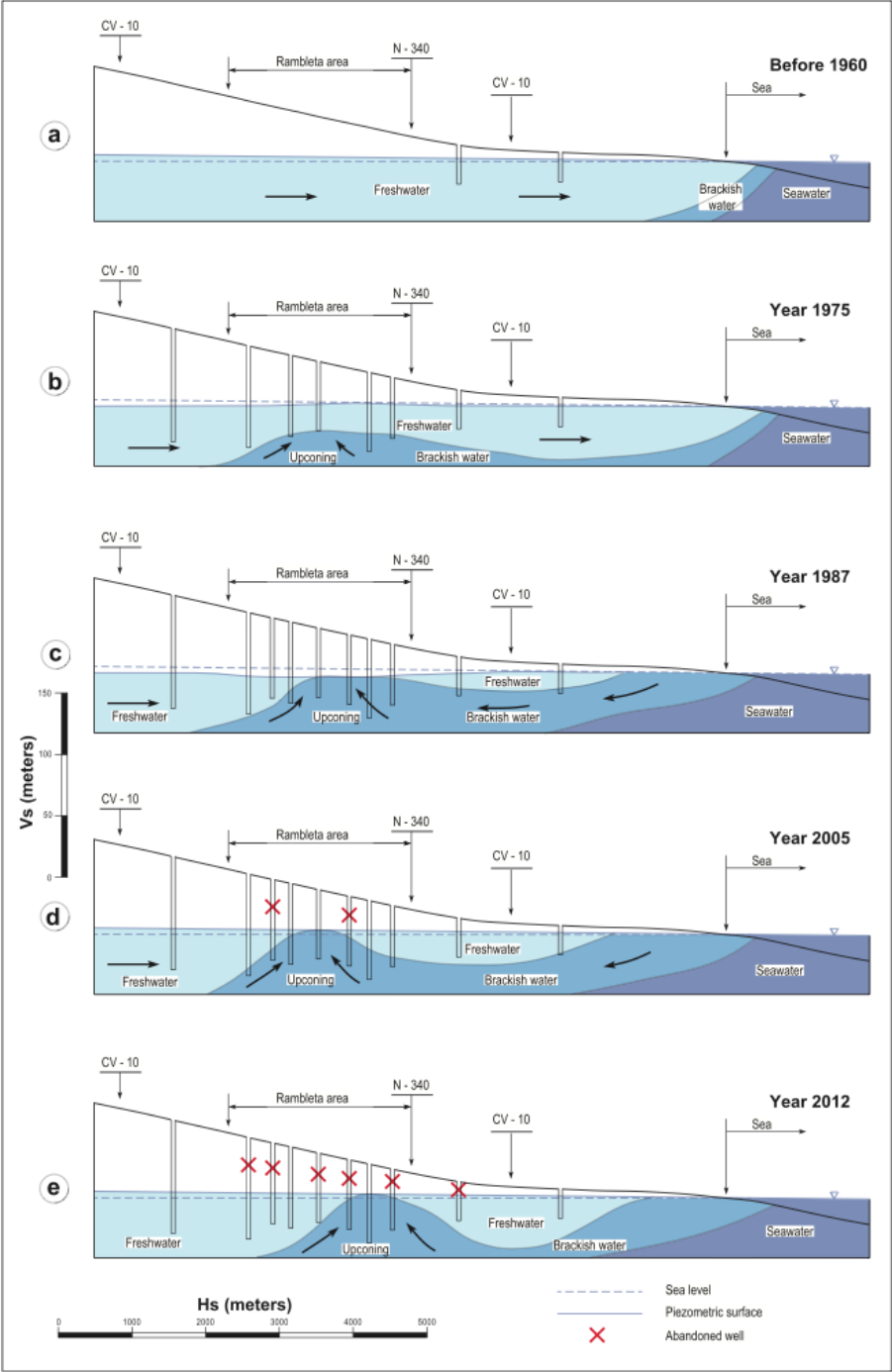
Figure 2-2: Sketch of historical development of salinity ingress in the La Vall d'Uixó aquifer (Morell *et al.* 2012)

The salinization process that affects the Rambleta area is peculiar, since it originates not only from the lateral advance of the so-called saline wedge, but also from the up-coning of saline water generated by the hydrodynamic effect of local concentrations of pumped groundwater abstractions. Considering these two origins of salinization is crucial in the design of the MAR system aiming at not only acting as a hydraulic barrier to repulse the saline front, but also exercising a hydrodynamic effect capable of reducing the saline up-coning.

2.1 MAR profile and hydrogeological characterisation of the pilot area

Along the coast of Valencia Community there are a number of coastal plains that accommodate a series of essentially detrital aquifers of great hydrogeological interest. They all feature an extremely flat morphology extending from sea level to maximum elevations of between 100 and 130 m. These geomorphological elements, known by the generic name of "planas" or plains, tend to be distributed longitudinally along the coastline. There is wide variability in their spatial development, both in area and the thickness of the deposits that they comprise. All are limited to the East by the Mediterranean Sea. The Castellón Plain, like other Mediterranean coastal plains, is a tectonic basin that formed during the Tertiary as a result of the Neogene distension. This distension determined the current structure of the entire coastal strip, generating a complex of horsts and grabens that continue beneath the sea. The fill of the basin, including the Castellón Plain aquifer, comprise Neogene and Quaternary sediments of polygenic, continental, littoral and mixed type, capped by a gently sloping glacis that extends to the present-day coastline.

The aquifer formation consists of layers and lenses of conglomerates, sands and gravels, intercalated and contained within a silty-clay series, making up a single Plioquaternary aquifer unit. Nevertheless, two formations are recognized: an upper one and a lower one. The upper one comprises conglomerates, sands and gravels with a significant proportion of fines. The lower one accommodates a higher proportion of clays. Permeability of the upper formation is quite high, while the lower one has much lower permeability. The series overlies either Mesozoic strata of variable permeability (which may form a second aquifer), or impermeable Tertiary or even other Mesozoic sediments.

The thickness of the detrital formation is highly variable, as a result of the morphology of the underlying substratum; it is generally thicker towards the coast. Most commonly it is between 50 and 200 m thick, with a mean of 70 m. The hydrodynamic parameters of the system are extraordinarily variable: transmissivity falls in the range of less than 500 m^2/day to 6,000 m^2/day, peaking near the coast. Storage coefficients are between 5 and 15 %, values characteristic of a free detrital aquifer. Specific flows oscillate between 1 and 20 L/s/m, though it is normally between 5 and 10 L/s/m; meanwhile hydraulic permeability varies from 30-50 to 100-120 m/day.

The area of Rambleta in La Vall d'Uixó is situated in the far southwest of the geographical district known as the Plana de Castellón. This occupies the southern part of Castellón province on the eastern seaboard of the Iberian Peninsula and comprises a more or less triangular alluvial plain covering 464 km^2, with elevations of between 0 and 130 m a.s.l. The hydrographical network consists of a single permanent watercourse - the River Mijares, which bisects the plain. All other surface

watercourses are by nature intermittent and sporadic. The administrative code for the identification of this groundwater body is MASub 080.127.
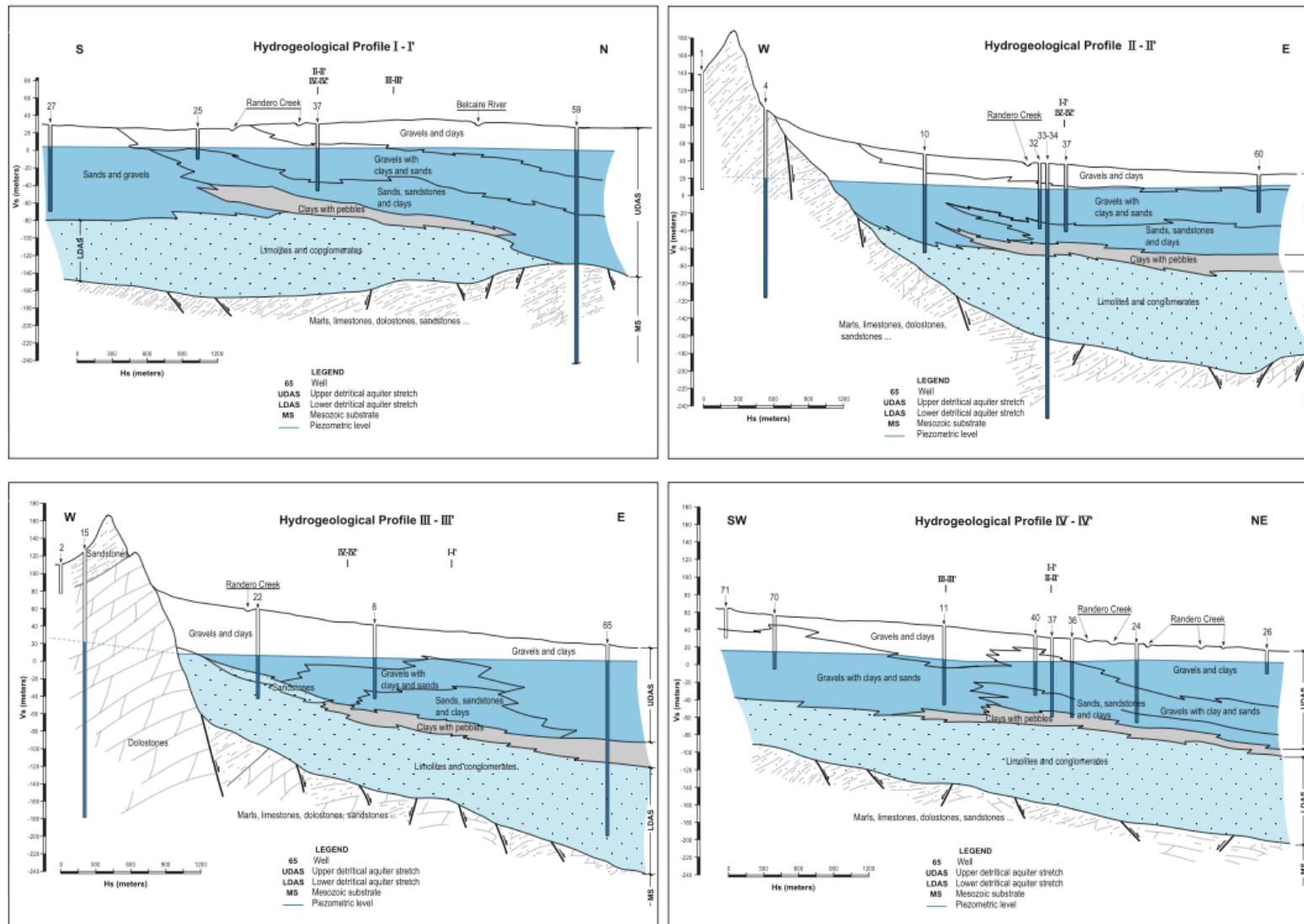
Figure 2-3: Hydrogeological cross-sections (Morell *et al.* 2012)

As a summary, the geologic section presents the following hydrogeological units:

- **Upper detritic aquifer (UDAS):** Sands and gravels (80-90 m). The aquifer exploited by the most of the wells.

- **Lower detritic aquifer (LDAS):** Siltstones, clays, sandstones and conglomerates (50-100 m).

- **Mesozoic substratum:** Triassic limestones, marls and dolomites (Muschelkalk facies), orthoquarzite sandstones (Buntsandstein facies), marl with gypsums (Keuper facies).

2.2 Role of groundwater in the pilot area

The whole district is underlined by the detrital Castellón Plain Aquifer. The area occupied by this hydrogeological system is economically vigorous, with thriving agriculture, ceramics and chemical industries coexisting. The agriculture is mainly citrus fruit cultivation; industry is dominated by the ceramics industry, which produces 85 % of Spain's total (Spain is the second largest world producer). There is also a significant chemical industry producing essential oils, and factories manufacturing chemical, organic and phyto-sanitary products and fertilizers.

These three economic activities are complemented by an agro-food industry and a well-developed service sector, the tourism. The economic scheme described needs he availability of groundwater resources, which are essential for sustaining these industries. Thus, the pumped abstractions were quantified nearly 201 Mm^3/y over the 1980s, of which 32 Mm^3/y was destined for urban water supply, 11 Mm^3/y for industrial usage, 158 Mm^3/y for irrigation and 0.44 Mm^3/y for livestock. Figure 2-4 shows typical pictures of the area, with the presence of citrus crops close to the wells.

Figure 2-4: Selection of pictures of the pilot area

2.3   Groundwater management and reclamation scheme: Water Recovery Project

Figure 2-5 represents the scheme of the MAR established along the Water Recovery Project. Two injection wells were constructed with the aim of injecting water coming from the storage dam. The storage dam can receive surplus water of the Belcaire River (Phase III) and reclaimed water from the WWTP of La Vall d'Uixó (Phase IV, not executed yet). There is a recharge pipe connecting the dam outflow with the injection wells. Figure 2-5 shows an aerial view of the MAR scheme.

Along the Phase III of Water Recovery project, the impact of surface water in the aquifer has been assessed (results available in Water Recovery project final report). The aim of the additional campaigns performed in DEMEAU project is to assess the impact of reclaimed water to/for groundwater. To this end, water quality of the secondary effluent of the WWTP has been considered as injection water. It is the most pessimistic scenario, taking into account that there could be an advanced treatment plant installed or a mix with river water.

Figure 2-5:   MAR scheme in La Vall d'Uixó

2.4 Monitoring network

Seven of the sampling points of Water Recovery project have been selected for the additional sampling campaigns carried out within the DEMEAU project. Because the objectives of both projects was to assess the feasibility of a new scheme using reclaimed water, WWTP effluent has been also included in the sampling campaigns, as well as storage dam water for further comparison. A total sum of 13 different types of sampling locations was sampled in the three campaigns. Table 2-1 summarises the main properties of the sampling points and Figure 2-6 shows their geographical distribution.

Table 2-1: Identification of the DEMEAU sampling points

Code	Type of water	Total Depth (m)	Coordinate (X) ETRS 89	Coordinate (Y) ETRS 89
CAS-01 EDAR	Effluent WWTP Working day sample	-	738565	4410127
CAS-02 EDAR	Effluent WWTP Working day sample	-	739120	4410826
CAS-03 EDAR	Effluent WWTP Weekend sample	-	741831	4411429
CAS-04 EDAR	Effluent WWTP Weekend sample	-	741873	4411860
CAS-05 BALSA	Surface Water Storage Dam	-	739584	4412176
CAS-06 SAN VICENTE	Groundwater Agricultural well	52	741067	4412526
CAS-07 LA PAZ	Groundwater Agricultural well	51	740485	4411955
CAS-08 RANDERO	Groundwater Agricultural well	100	740583	4412081
CAS-09 RAMBLETA 2	Groundwater Agricultural well	42	740047	4412030
CAS-10 PRIMITIVA	Groundwater Agricultural well	100	740235	4411950
CAS-11 GARROFERA	Groundwater Agricultural well	N.A.	740105	4411936
CAS-12 PIEZOMETRO 2	Groundwater Borehole near injection wells	59	738565	4410127

Code	Type of water	Total Depth (m)	Coordinate (X) ETRS 89	Coordinate (Y) ETRS 89
CAS-13 PIEZOMETRO 1	Groundwater Borehole near injection wells	60	739120	4410826
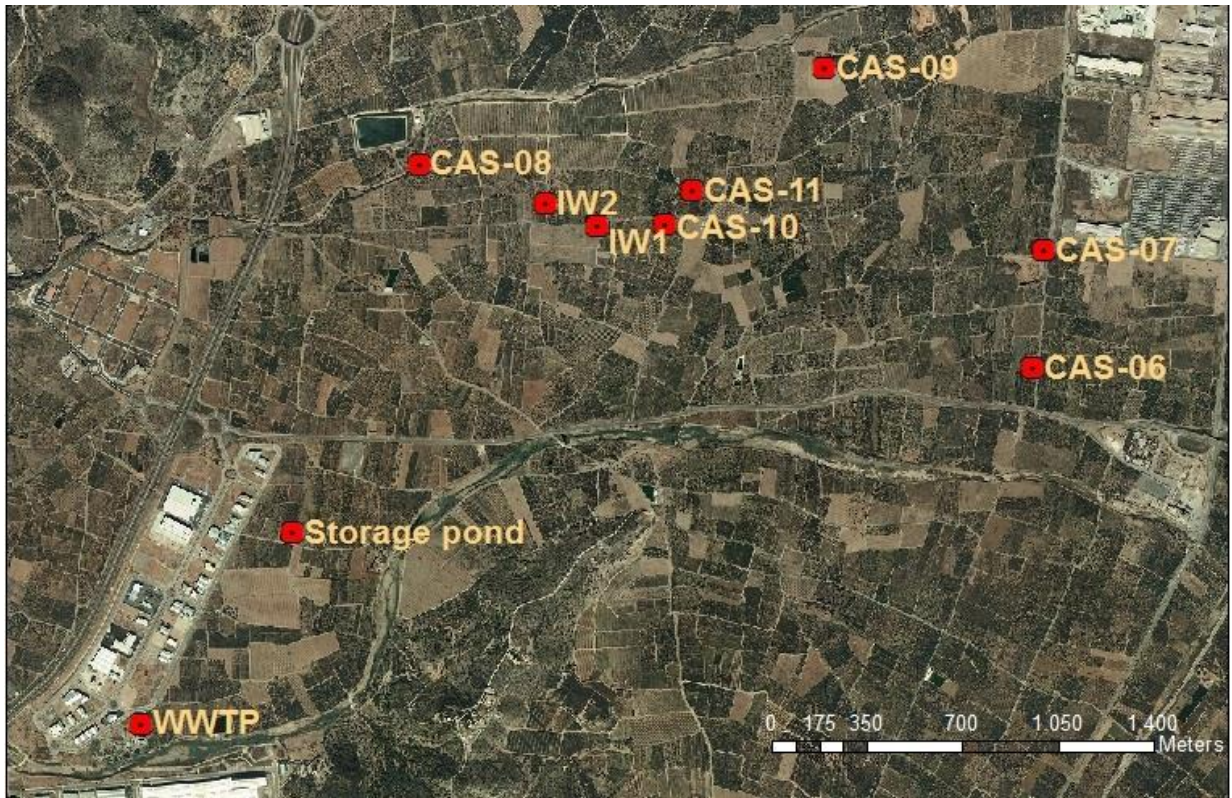
Figure 2-6: Aerial view of sampling points locations

3 Materials and Methods

3.1 Sampling campaigns

Sampling campaigns have been performed in La Vall d'Uixó three times along the last phase of DEMEAU project in 2014 and 2015. Each laboratory applied validated and certified analytical procedures. Taking advantage of the sampling campaign, samples for bioassays tests were collected. Bioassays tests were performed by BDS (The Netherlands) and Oekotoxzentrum (Switzerland) simultaneously (Table 3-1).

Table 3-1:   Summary of sampling campaigns and laboratories involved

Sampling campaign	Micropollutants laboratory	Bulk chemistry laboratory	Bioassays Survey
May 2014	University of Göttingen 250 mL (WWTP effluent; 500 mL groundwater). Glass amber bottles	Instituto Geológico y Minero de España IGME	YES (2 L frozen) Glass amber bottles
January 2015	University of Castellón (Jaume I, IUPA institute) 1L plastic bottle	Instituto Geológico y Minero de España IGME	NO
April 2015	University of Castellón (Jaume I, IUPA institute) 1L plastic bottle	Instituto Geológico y Minero de España IGME	YES (500 mL refrigerated) Glass amber bottles

3.2 Hydrochemistry

Hydrochemistry and calculated ion balance $\left(\text{ionbalance}(\%) = \sum \text{cations} - \sum \text{anions} / (\sum \text{cations} + \sum \text{anions} / 2) \times 100\right)$ are shown in annex 3. Only samples with ion balance ≤10% were used. All measured and calculated hydrochemical parameters are found in annex 3.

3.3 Organic micro pollutants analysis

The analysis of micropollutants has been done in two different laboratories, applying the same limit of quantification. DEMEAU's list of pharmaceuticals have been kept in both laboratories, and each of them have added additional emerging compounds and priority substances in the list (see annex 4). Samples were taken with bailers, submerged approximately five meters below groundwater level previously measured. Monitoring wells were not purged before sampling. Specific bottles types and volumes were used according laboratories requirements.

3.3.1 University of Göttingen (Geoscience Centre)

Samples from May 2014 where analysed by University of Göttingen. Samples were conserved at 4 °C during the transport and storage in Barcelona. Samples were shipped to Göttingen University in cold conditions. Once the bottles arrived to the laboratory, organic micropollutants 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 organic micropollutants 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).

3.3.2 University of Castellón (UIPA Institute)

Samples collected in January and April 2015 were analysed in Castellón in the IUPA Institute. Samples were conserved at 4ºC during the transport (few hours) and directly frozen in Castellón until the analysis. The analytical procedure is fully described in Boix *et al.* (2015) and consists in the direct injection of the sample after a simple centrifugation (2 mL of sample centrifuged at 12,000 rpm for 10 minutes) and then 100 µL of volume was injected into the chromatographic system (liquid chromatography–tandem mass spectrometry UHPLC-MS/MS). Pantoprazole, iopromide and bezafibrate were quantified by absolute areas. However, in most cases it was necessary to correct the matrix effect, for which isotopically labelled internal standards were used. The correction was performed using the labelled analyte itself when this was available (11 of 21 compounds). For other compounds (gemfibrozil, desethyl atrazine, atrazine desisopropil, phenazone, simazine, primidone, and metoprolol) a labelled analogue was used, whose election was a function of retention time and based on preliminary studies. Bulk chemistry was analysed in IGME Laboratory (Valencia) using standard methods.

3.4 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 water samples from two sampling campaigns conducted in May 2014 and April 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. Table 3-1 summarises the sampling conditions in La Vall d'Uixó. 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 (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).
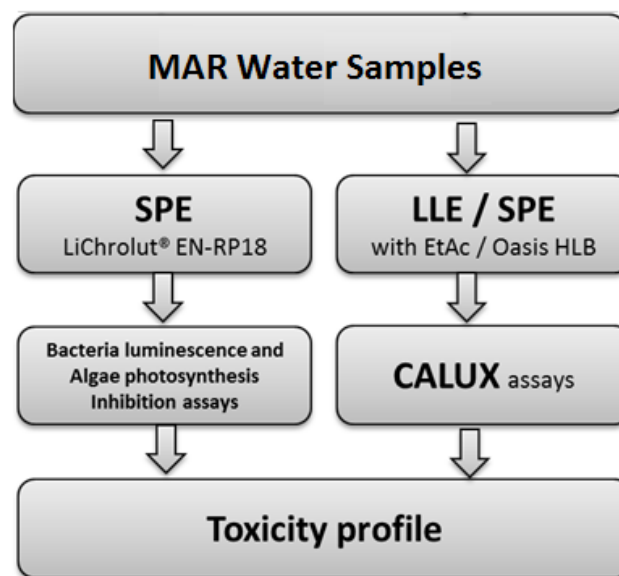
Figure 3-1:   Schematic study design of bio screening

Table 3-2 lists the sampling points in La Vall d'Uixó for the performance of bioassays. Additional information as the total depth of the wells or geographical coordinates can be found in Table 2-1, and aerial view for their location is shown in Figure 2-6.

Table 3-2: Selected sampling points for the bioassays

Code	Type of water
CAS-01- EDAR	Effluent WWTP (Working day sample)
CAS-05 - BALSA	Surface Water (Storage Dam)
CAS-06- SAN VICENTE	Groundwater (Agricultural well)
CAS-07- LA PAZ	Groundwater (Agricultural well)
CAS-08- RANDERO	Groundwater (Agricultural well)
CAS-09- RAMBLETA 2	Groundwater (Agricultural well)
CAS-11- GARROFERA	Groundwater (Agricultural well)
CAS-12- PIEZOMETRO 2	Groundwater (Borehole near injection wells)

3.4.1 Sample workup

Samples were transported to the partners (BDS, Amsterdam and Oecotox Centre – EAWAG, Dübendorf) for bioassay analyses either frozen (1st campaign done in May 2014) and refrigerated (2nd campaign done in April 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 1st 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 °C until analysis.

From the samples of the second campaign in April 2015 somewhat different volumes were worked up due to the various sample volume availability. 350 mL from the Castellón 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 1st campaign, extracts were dissolved in 100 μL of DMSO and stored at -18 °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 35 times (samples from the second campaign enriched samples were tested in the CALUX bioassays.

3.4.2 Method for combined Algae Assay methodology

The Combined Algae Assay on the green algae *Pseudokirchneriella subcapitata* was conducted as described earlier (Escher *et al.* 2008a). The photosynthesis inhibition was measured by means of effective quantum yield (after 2 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.

3.4.3 Method for 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).

3.4.4 Method for CALIX 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. The 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, van der Linden *et al.* 2008).

3.4.5 Methodology for data analysis

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

For 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.

3.4.5.2 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 3-3 summarises the ecotoxicological effects detected by the in vitro bioassays performed.

Table 3-3: 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
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

Toxic pathway	Pertinent in vitro bioassay	Possible adverse health/ecotoxicological effects
(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

3.4.5.3 Quality controls

All samples were tested in the bioassays together with the

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

Neither the procedure blank nor the bioassay solvent blank (data not illustrated) 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 – mostly 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.

4 Results

4.1 Hydrochemistry

Native groundwater is characterized by elevated salinity caused by anthropogenic induced salinity ingression that has affected the area for decades as a consequence of overexploitation of the aquifer. Annex 3 shows all hydrochemical results obtained from the three sampling campaigns plus an additional campaign that was carried out in 2012 in the framework of the Water Recovery project. Native groundwater hydrochemistry has been previously described in the reports of the Water Recovery Project and in publications (Morell *et al.,* 2014; García *et al.* 2014). According to information reported in these studies native groundwater has a chloride facies with chloride concentrations between 200 and 750 mg/L, originating from the mixing of fresh water and seawater. However, the facies are also sulphate type, with sulphate concentrations of 250 - 700 mg/L, which is much higher that would be expected if the sulphate is coming only from seawater. Magnesium concentrations are also very elevated, between 100 and 230 mg/L. Another notable hydrochemical feature of groundwater in this sector is the presence of very high nitrate concentrations between 190 and 520 mg/L, caused from the intensive agricultural activity.

Ion exchange is a common process during re-freshening or salinization of coastal aquifers (Appelo and Postma 2005). The displacement chromatography under re-freshening conditions, when fresh water flushes a salty or brackish water aquifer, follows:

$$\tfrac{1}{2}Ca^{2+} + Na\text{-}X \rightarrow \tfrac{1}{2}Ca\text{-}X2 + Na^{+} \qquad \text{(eq. 1)}$$

Where Ca^{2+} is transferred from the water to the binding sites of the exchanger and Na^{+} is dissolved in return $NaHCO_3$ water type results. When seawater intrudes in a fresh water aquifer ion exchange process can be described as:

$$Na^{+} + \tfrac{1}{2}Ca\text{-}X2 \rightarrow Na\text{-}X + \tfrac{1}{2}Ca^{2+} \qquad \text{(eq. 2)}$$

Where Na is taken up by the binding sites of the exchanger and Ca is released to the water in return. The water type changes here from NaCl to $CaCl_2$. These ion exchange processes are illustrated by displaying the alkaline elements (Na + K) substracted by the binding partner chloride against the earth alkaline elements (Ca + Mg) substracted by sulphate and bicarbonate (Figure 4-1). Samples are plotted in units of meq/l. Plausible samples must plot on the 1:1 line and ground water which is not altered by ion exchange would plot in the centre (± 5 meq/L), because the alkaline cations (Na + K) would be balanced by halogen anions (Cl) and the earth alkaline cations (Ca + Mg) would be balanced by sulphate and bicarbonate. If ion exchange takes place according to eq. 1 (re-freshening conditions) sodium would be in excess and Ca would be depleted. Samples displaying a re-freshening effect lie in the lower right corner of **Figure 4-1**. Samples that underwent ion exchange due to salinization (eq.2) of the aquifer are plotting in the upper left corner.
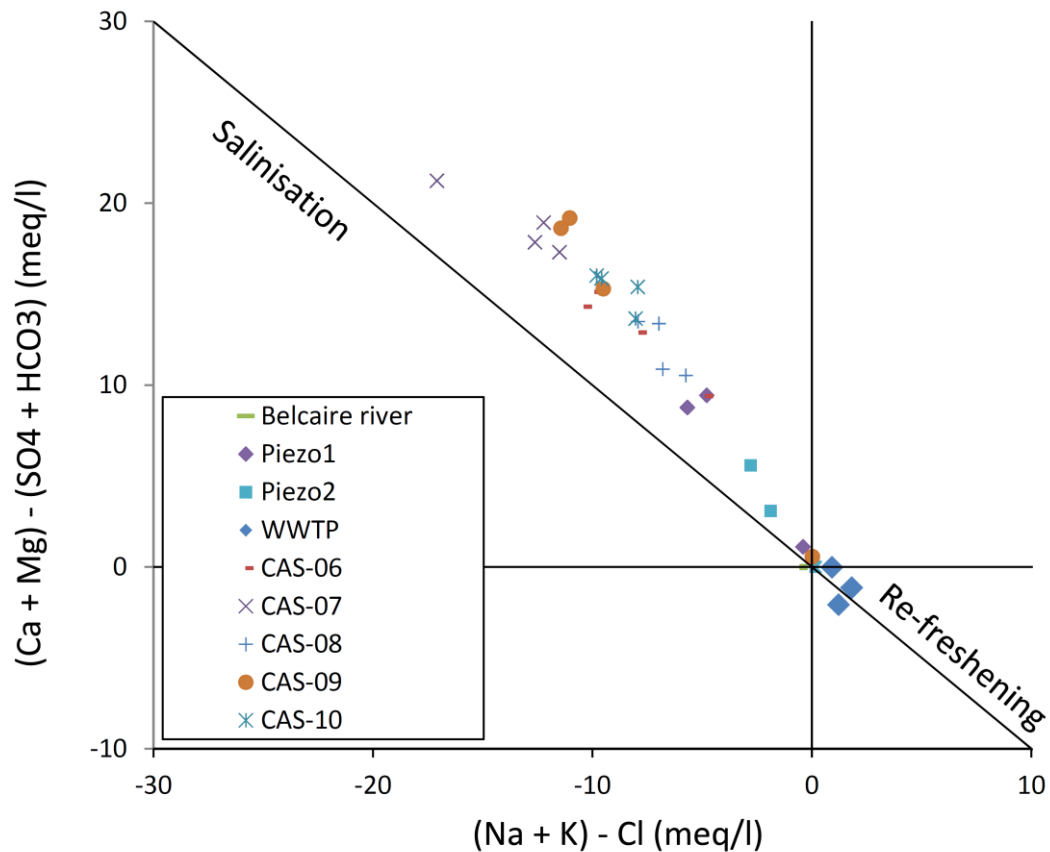
Figure 4-1: Ion exchange due to salinity ingress

Ion exchange due to salinization can be observed in most of the wells. The WWTP and Belcaire River samples, as expected, do not indicate ion exchange. In CAS-07 salinization is pronounced as it plots in the very upper left corner of. CAS-09 is also under the influence of on-going salinization, but the sample taken in April 2015 shows a fresh water signature as it plots in the centre. CAS-08 and CAS-10 are deep wells (~ 100 m total depth) and also influenced by on-going salinization. Figure 4-1 clearly indicates on-going salinization and documents minor effects of the injected water on CAS-09 in April 2015 only.

The Cl/Br mass ratio can be used as a tracer of groundwater flow since both ions are chemically inert except in the presence of high amounts of organic matter (Davis et al., 1998). Standard mean ocean water (SMOW) carries a Cl/Br weight ratio of ~288 (Cl_{SMOW} = 19 352 mg/l, Br_{SMOW} = 67.3 mg/l). The Cl/Br ratio in seawater does not change during evaporation until halite starts to precipitate (Alcala and Custodio, 2008). Bromide is less compatible in precipitating halite and enriches disproportionately high compared to chloride in the residual water. The resulting halite is therefore depleted in bromide (rCl/Br ~ 9000), and water which dissolves halite carries high Cl/Br ratios between 1000 - 10 000, while rainfall at coastal areas carries the Cl/Br mass ratio similar to the sea (Davis et al., 1998). The measured Cl/Br mass ratios show a wide range from low ratios ~ 200 to ratios around the standard mean seawater (SMOW) ~300. Most of the samples from the coastal

aquifer in Castellón plot above the SMOW Cl/Br mass ratio with increasing Cl concentration (Figure 4-2).
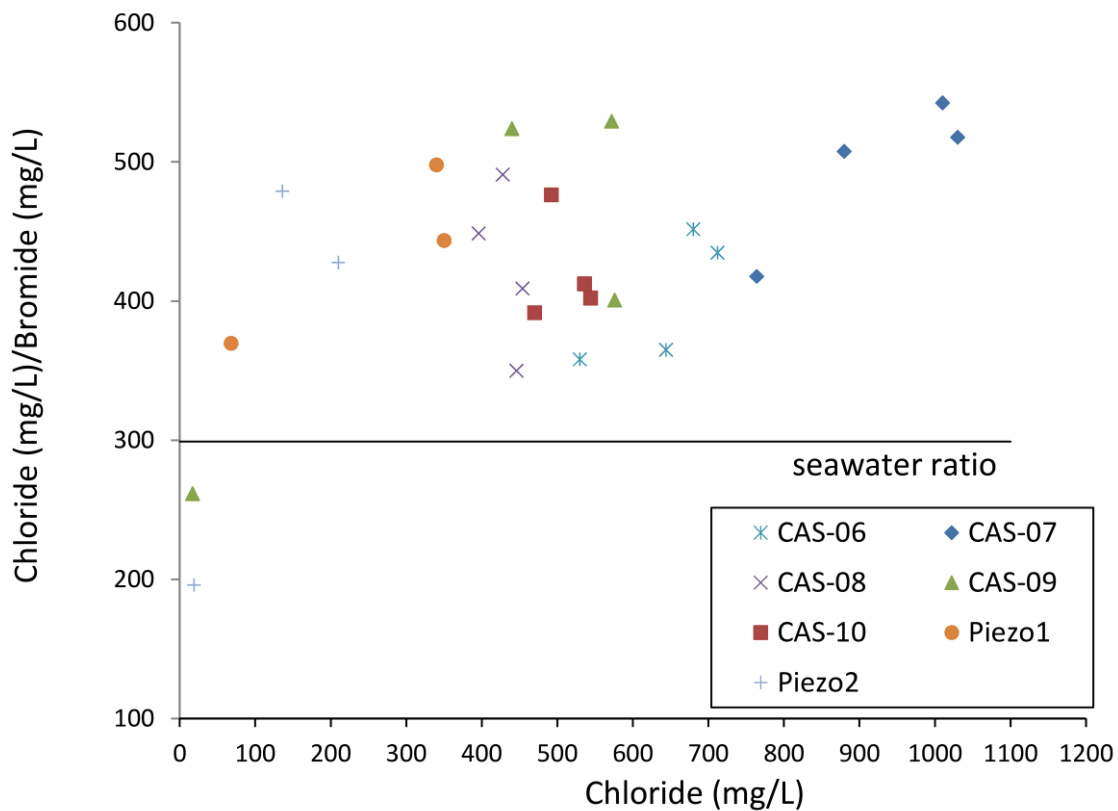
Figure 4-2:   Cl/Br weight ratio against Cl (mg/L)

As already indicated by the ion displacement diagram (Figure 4-1) only CAS-09 sampled in April 2015 is influenced by recharge water, because of Cl/Br weight ratio <300 and Cl concentration <200 mg/L. The fact that most other groundwater samples plot above the SMOW ratio indicates additional sources of chloride apart from seawater. Considering the local geology with underlying Keuper formations (Triassic), it is possible that these low lying formations contribute to salinity ingress to some extent. However, since Keuper signature is unknown it is not possible to clearly attribute the elevated Cl/Br ratios to deep groundwater circulation. Moreover, other Cl sources may contribute, e.g. waste water infiltration. Waste water typically carries Cl/Br mass ratios up to 900 (Katz et al. 2011)) and may have contributed to elevated Cl/Br ratios. Bromide measurements from the Belcaire River are not available, but it seems plausible that the river carries Cl/Br weight ratios below that of SMOW. The spring of the Belcaire River lies in the Alfondeguilla Mountains where the rain is most likely depleted in chloride, resulting in Cl/Br ratios below SMOW.

The redox conditions in native groundwater are oxic to suboxic, because N occurs only in the form of nitrate. Other redox sensitive compounds (e.g. Fe) do not occur in elevated concentrations (average 0.5 mg/L). As a summary, Figure 4-3 shows the Piper diagrams (Piper 1944) for the five selected wells

representing native groundwater. Presentation in the diagram bases on the equivalent concentrations of the main cations and anions in groundwater
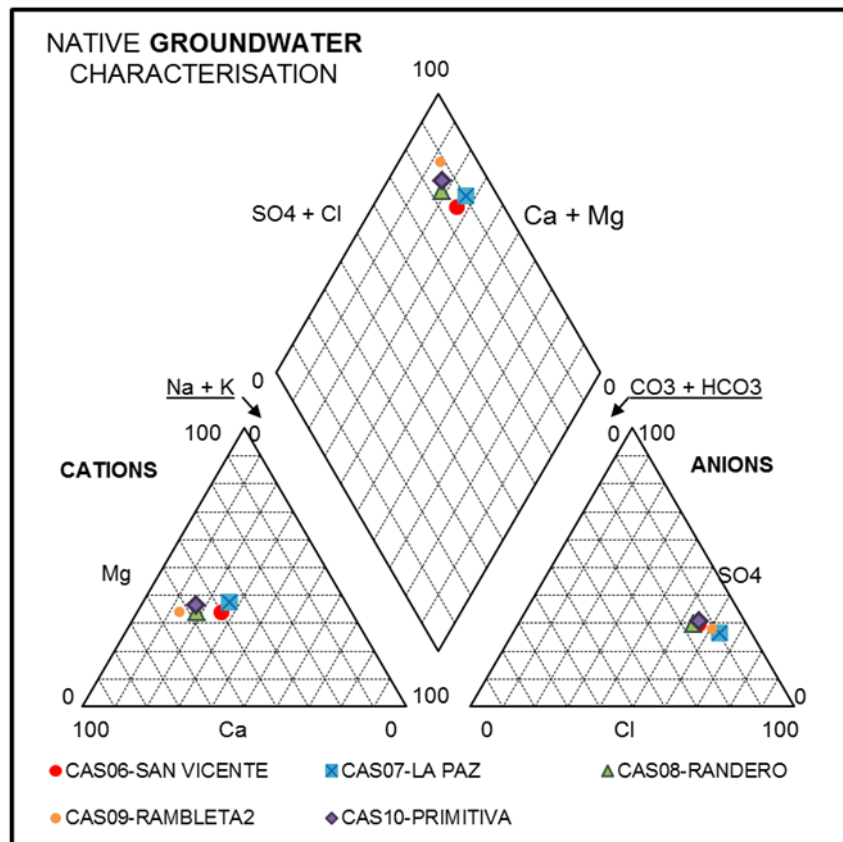
Figure 4-3:   Piper diagram of native groundwater (average values)

4.2   Bioassays - 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 5. The activities had been classified according to the activity significance Table 4-1. The obtained activity profiles of the MAR samples (left part in Figure 4-4) 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, see right part in Figure 4-4). Trigger values for the other endpoints are currently being established.

Table 4-1:   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

Bioassay	Trigger value	Unit
GR-CALUX	30	ng Dexamethasone-Eq / L
PPARγ-CALUX	20	ng Rosiglitazone-Eq / L
Nrf2-CALUX	10	µg Curcumin-Eq / L
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.

Complete results are listed in annex 5. Figure 4-4 summarises activity profile of the tested MAR water samples from the La Vall d'Uixó sampling site collected at two time points: 06/2014 (Campaign I) and 04/2015 (Campaign II) 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. 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 at two sampling campaigns.
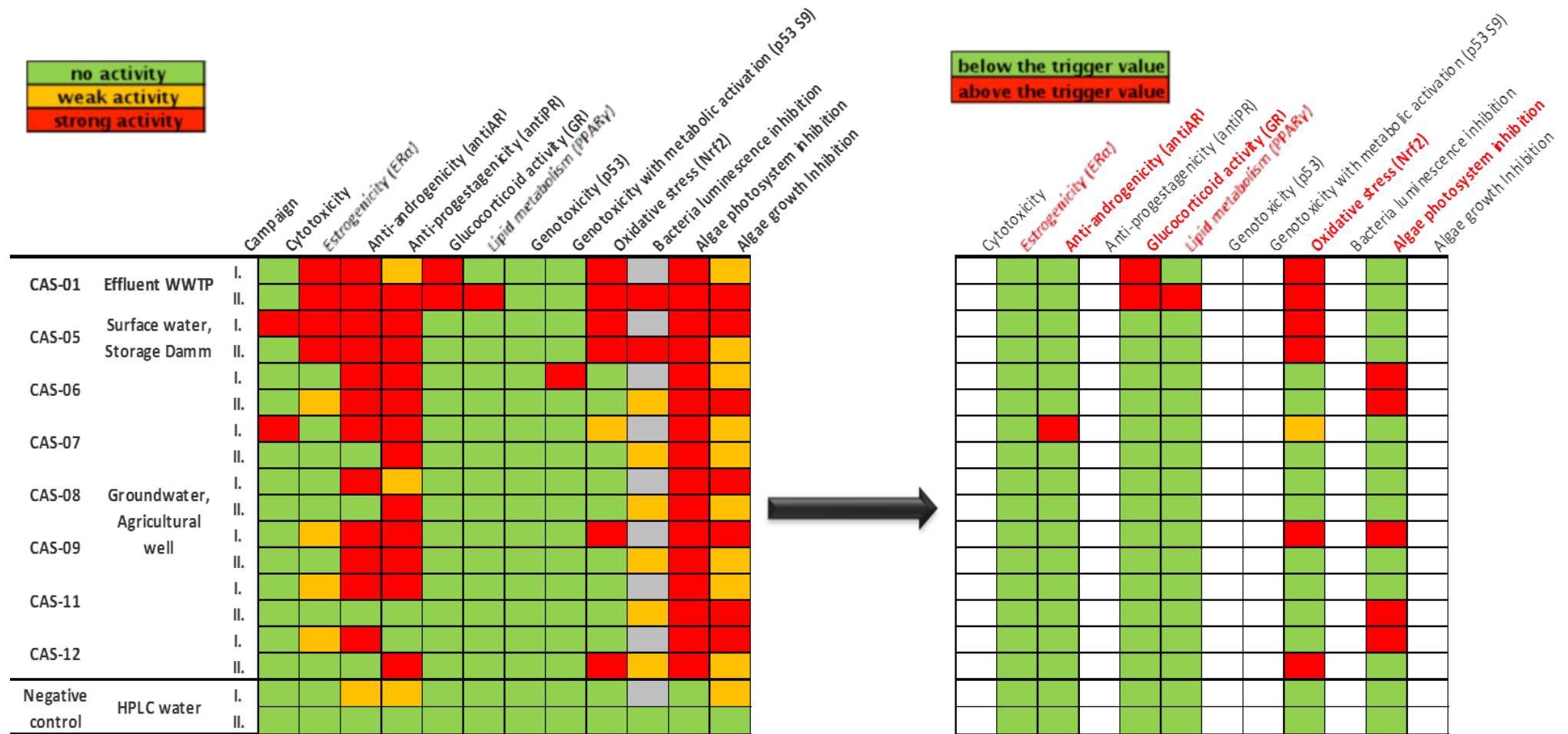
Figure 4-4: Activity profile determined in the bioassays (colours in right matrix: green = activity below trigger value, yellow = coinciding with trigger value, red = activity above trigger value).

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 Castellón samples, the use of the effluent of WWTP as injection water could suppose some adverse effects listed below:

- Glucocorticoid activity
- Lipid metabolism
- Oxidative stress
- Algae metabolism inhibition

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.
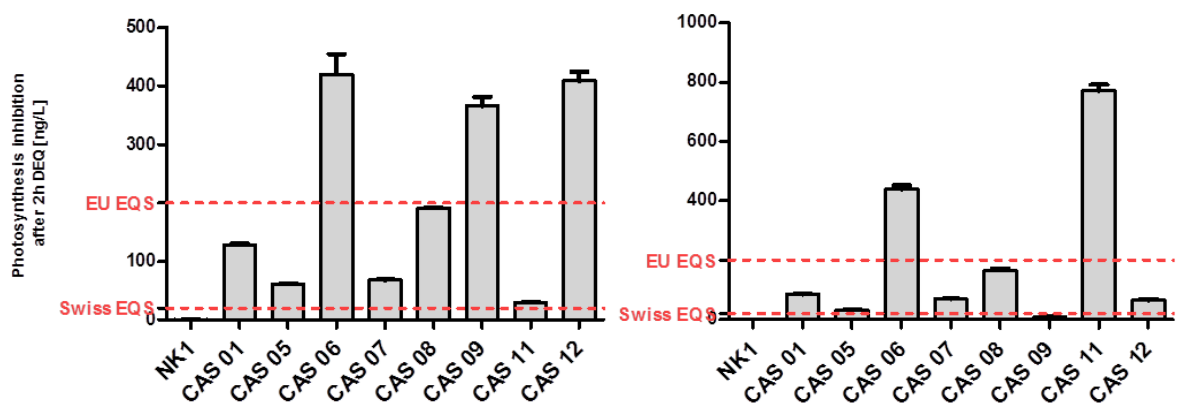
Figure 4-5:   Photosynthesis inhibition (after 2 hours of exposure) of the MAR samples from Castellón – 1st sampling campaign (left) and 2nd sampling campaign (right) - expressed as ng Diuron Eq./L water. NK refers to negative control (HPLC water) went through on extraction and bioanalysis just as the samples.
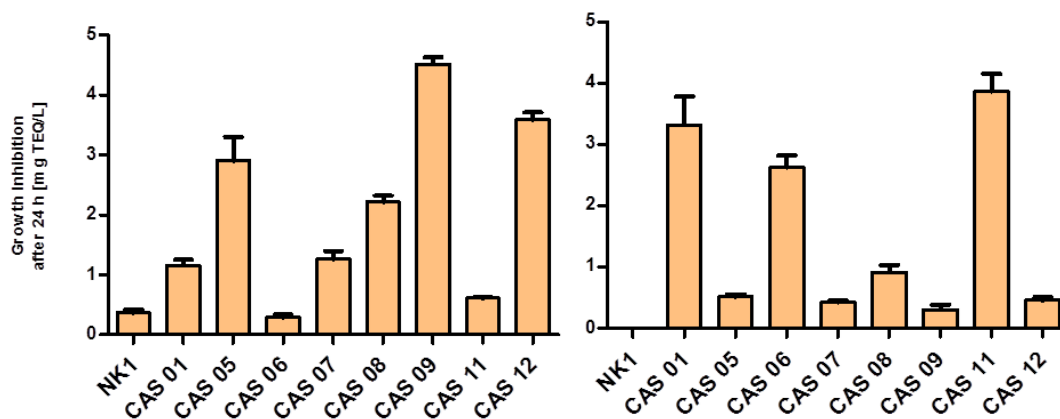
Figure 4-6: Growth inhibition (after 24 hours of exposure) of the MAR samples from Castellón – 1st sampling campaign (left) and 2nd sampling campaign (right) - expressed as ng Diuron Eq./L water. NK refers to negative control (HPLC water) went through on extraction and bioanalysis just as the samples.
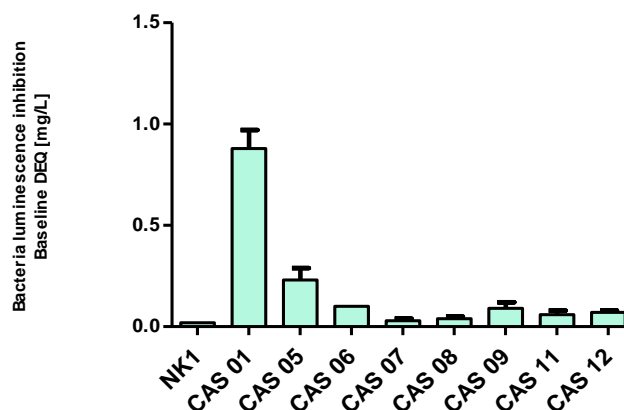
(*) Campaign expressed as ng Diuron Eq./L water

Figure 4-7: Bacteria luminescence inhibition of the MAR samples from Castellón – 2nd sampling. NK refers to negative control (HPLC water) went through on extraction and bioanalysis just as the samples.

Figure 4-5 and Figure 4-6 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. Regarding Figure 4-7, it shows the inhibition of bacteria luminescence. Effluent of WWTP (code CAS 01) shows a high response in the inhibition of bacteria luminescence, while the rest of samples have a similar response, corresponding to the control values (NK1).

During these investigation the MAR water samples from Castellón were also chemically characterized and analysed for a set of target compounds including the 12 DEMEAU compounds: pharmaceuticals, pesticides, sweeteners, transformation products and various other metabolites, cocaine and nicotine for instance. In order to link analysed chemical concentrations to the observed toxicity in the samples the following exercise was conducted:

- Assay-specific relative potency (REP) factors, which express the estimated toxicity of a test compound compared to the reference compound for a certain mode-of-action (i.e. in a certain bioassay), were gathered for the chemically analyzed compounds or tested in the bioassays (DEMEAU compounds, Table 4-2). REP factors are determined by the effect concentrations (ECx) of the reference compound and of the test compound: REP factor test comp = EC(x) reference compound/ EC(x) test compound. Available REP factors for the chemically analyzed compounds are presented in annex 5.
- Chemical concentrations were then transformed into toxicity equivalent concentrations with the aid of the available REP factors.
- To calculate the theoretically expected toxic activities for the chemically analyzed compounds (in terms of equivalent concentrations), the concentration of each compound was multiplied with its REP factor. The expected total biological activity of the sample extract was calculated by summation of the activities calculated for the individual compounds in the mixture.
- Finally, recoveries were determined by comparing the actually measured activity of the sample extracts to the calculated total activity of the chemically analyzed compounds (annex 5 Table A-0-10:).

The comparison of chemical and toxicological analyses of the samples taking into account the 12 "DEMEAU compounds" is shown in Table 4-2. This information has to be carefully interpreted due to the following facts:

- Toxicological information was only available for 18 out of the 63 target analysed compounds and limited therefore the prediction of the expected activities.
- The selection of the target analysed compounds do not seem to overlap with compound classes that are known to be present in water samples and exert adverse (ecosystem) health effects.
- Pharmaceuticals were included in the target compound list that have (almost) no activity (based on in-house screening data of a large set of compounds and compound classes at BDS), whereas other compounds often found in environmental waters with relatively high hormonal potency, were not included in the target list, such as personal care products, hormones and hormone-like compounds, chlorinated organic solvents and chemicals.
- A number of pesticides were, however, included in the target analysis and could also be accounted for a greater part of the measured activities in the combined algae assay (sample CAS05 and CAS11 for instance).
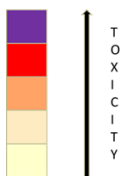
Compound	CAS number	DR	PXR	PPARa	PPARg	LXR	ERa	ERa-anti	ERb	AR	AR-anti	PR	PR-anti	GR	GR-anti	RAR	p53	p53 S9	Nrf2	Hif1a	TCF	AP1	NFkB	ESRE	p21	Cytotox10%	Cytotox50%
E-Carbamazapine	36507-30-9	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5	>4	>4	>5	>5	>5	>5	>5	>5	>5	>5	>5
Gemfibrozil	25812-30-0	>5	>5	-5.5	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5	>4	>4	>5	>5	>5	>5	>5	>5	>5	>5	>5
Iopromide	73334-07-3	>4	>4	>4	>4	>4	>4	>4	>4	>4	>4	>4	>4	>4	>4	>4	>3	>3	>4	>4	>4	>4	>4	>4	>4	>4	>4
Primidone	125-33-7	>4	>4	>4	>4	>4	>4	>4	>4	>4	>4	>4	>4	>4	>4	>4	>3	>3	>4	>4	>4	>4	>4	>4	>4	>4	>4
Trimethoprim	738-70-5	-4	>4	>4	>4	>4	>4	>4	>4	>4	-4.3	>4	>4	>4	>4	>4	>3	>3	>4	>4	>4	>4	>4	>4	>4	>4	>4
Metoprolol	37350-58-6																										
Bezafibrate	41859-67-0																										
Phenazone	60-80-0	>4	>4	>4	>4	>4	-4.1	>4	>4	>4	>4	>4	>4	>4	>4	>4	-3.4	>3	>4	>4	>4	>4	>4	>4	>4	>4	>4
Diclofenac	15307-79-6	>4	-4.7	>4	-4.6	>4	>4	>4	>4	>4	-4.9	>4	-4.5	>4	-4.2	>4	-3.6	>3	>4	>4	>4	>4	>4	>4	>4	>4	>4
Benzotriazole	95-14-7	>4	>4	>4	>4	>4	>4	>4	>4	>4	>4	>4	>4	>4	>4	>4	>3	>3	>4	>4	>4	>4	>4	>4	>4	>4	>4
Sulfamethoxazole	723-46-6	>4	>4	>4	>4	>4	>4	>4	>4	>4	>4	>4	>4	>4	-4	>4	>3	>3	>4	>4	>4	>4	>4	>4	>4	>4	>4
Carbamazepine	298-46-4	>4	>4	>4	>4	>4	>4	>4	>4	>4	-4.7	>4	-4.5	>4	>4	>4	>3.4	>3.4	>3.4	>4	>3.4	>3.4	>3.4	>3.4	>3.4	-4	-3.4

Not active
Not measured

TOXICITY

Table 4-2: Toxicity profile of the 12 "DEMEAU compounds" evaluated in Castellón. NOTE: Values represent logarithmic PC10 (agonistic assays) and PC20 (antagonistic assays) concentrations. PC10 or PC20 refers to a response level induced by a test chemical equals to 10% or 20% of that induced by a maximally inducing concentration of the positive control (PC). NOTE: Metoprolol and Bezafibrate were not tested due to technical issues (i.e. lack of authentic standard and dissolving problem).

4.3 Risk assessment of the use of WWTP effluent as source water

4.3.1 Stage 1 Entry level risk assessment

MAR guidelines (NRMMC-EPHC-NHMRC, 2009) establish an initial checklist to assess the viability of a potential new MAR site. The key factors for entry-level viability assessment for managed aquifer recharge projects are: demand, source, aquifer, detention storage and management capability. The template for the viability assessment addresses these factors. If the answer to all of the questions given in the table is "Yes", proponents then proceed to determine the degree of difficulty. Table 4-3 shows the viability assessment for La Vall d'Uixó MAR site. The second part of the entry-level assessment is intended to inform stakeholders about the degree of difficulty of the proposed project. The template proposed by Australian guidelines suggests 14 questions related to information needs, and they will determine the scope of further investigations. Following table corresponds to the Part 2 completed for the present case study.

Table 4-3: La Vall d'Uixó entry level assessment part 1 - viability

Attribute	La Vall d'Uixó answer
1. Intended water use	
• Is there an ongoing local demand or clearly defined environmental benefit for recovered water that is compatible with local water management plans?	✓ **Yes.** Economic activity in La Vall d'Uixó is mainly based in citrus crops. There is a local demand of fresh groundwater
2. Source water availability and right of access	
• Is adequate source water available and is harvesting this volume compatible with catchment water management plans?	✓ **Yes.** The storage dam has been constructed to store surplus water in rainy season (volume depending on rainfall annual patterns). WWTP effluent is also potential recharge water (9,000 m^3/day).
3. Hydrogeological assessment	
• Is there at least one aquifer at the proposed manager aquifer recharge site capable of storing additional water?	✓ **Yes**. The quaternary aquifer located in Castellón plain (code MASub 080.127) has storage capacity.
• Is the project compatible with groundwater management plans?	✓ **Yes**. Injection of water in the aquifer is intended to replenish local groundwater resources and improve groundwater quality.
4. Space for water capture and treatment	
• Is there sufficient land available for capture and treatment of the water?	✓ **Yes**. The accumulation dam has 2 Mm^3 of storage capacity. Pre-treatment need has been not yet assessed and is not yet constructed.
5. Capability to design, construct and operate	

Attribute	La Vall d'Uixó answer
• Is there a capability to design, construct and operate a managed aquifer recharge project?	✓ **Yes**. University of Castellón (UJI) and National Geological Survey (IGME) has experience in local hydrogeology, as well as hydrochemistry. IGME has participated actively in the design and construction of several MAR projects on national level. ACUAMED (public national company) is expected to manage the aquifer recharge project or to open a public tender for its operation and maintenance.

Table 4-4: La Vall d'Uixó entry level assessment part 2 – degree of difficulty assessment

NOTE: source water corresponds to the WWTP effluent (secondary treatment)

Question from the Australian Guideline	La Vall d'Uixó answers	Investigations required
1. Source water quality with respect to groundwater environmental values		
• Does source water meet the water quality requirements for the environmental value of ambient groundwater?	**No.** Environmental values of groundwater good quality status are not totally fulfilled by the effluent of WWTP[1]. Require Stage 2 investigations to assess risks. ✓ Yes. Nitrate < 200 mg/L ✓ Yes. Chloride < 650 mg/L ✓ Yes. Sulphate < 525 mg/L ✗ No. Total pesticides > 0.5 µg/L ✗ No. Individual pesticides > 0.1 µg/L ? Not reported: Selenium Good status Se < 0.0207 mg/L	Additional campaigns of pesticides: punctual campaigns reported contradictory values. Quantification of selenium concentration.
2. Source water quality with respect to recovered water end use environmental values		
• Does source water meet the water quality requirements for the environmental values of the intended end use of the water on recovery?	**No.** Water quality standards for water reclamation in Spain are regulated by RD1620/2007. Specifically for final use of direct injection (use 5.2 defined in Spanish regulation RD1620/2007): ✗ Yes. Nematodes eggs < 1 egg/10L ✗ Yes. TSS < 10 mg/L ✗ Yes. Turbidity < 2 NTU ✗ No. Total nitrogen > 10 mg N/L ✗ No. Nitrate > 25 mg NO_3/L ✗ Yes. E. coli > 0 UFC/100 mL	Evaluation of the impact of high concentration of nitrate in the aquifer. Comparison with native groundwater Evaluation of the removal of E. coli along soil aquifer treatment.
3. Source water quality with respect to clogging		

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[1] Based on criteria for the chemical status classification in Castellon Plain Groundwater mass.
See annex 1 for further information.

Question from the Australian Guideline	La Vall d'Uixó answers	Investigations required
• Does source water have low quality; for example: TSS > 10 mg/L; TOC > 10 mg/L; Total nitrogen > 10 mg/L; and is the soil or aquifer free of macropores?	**Low water quality: Yes.** ✓ Yes. TOC > 10 mg/L ✓ Yes. Total nitrogen < 10 mg N/L ✗ No. TSS < 10 mg/L **Presence of macropores: unknown**	Evaluation of the impact of high concentration of nitrate in the aquifer. Comparison with native groundwater. Evaluation of impact of high TOC in the aquifer and potential natural biodegradation.

4. Groundwater quality with respect to recovered water end use environmental values

• Does ambient groundwater meet the water quality requirements for the environmental values of intended end uses of water on recovery?	**Yes:** Target aquifer is use for agriculture. Farmers use ambient groundwater for direct irrigation without any pre-treatment. Note: salinized wells are abandoned.	None

5. Groundwater and drinking water quality

• Is either drinking water supply, or protection of aquatic ecosystems with high conservation or ecological values, an environmental value of the target aquifer?	**No**. Castellón Plain aquifer is not intended to produce drinking water. There are no aquatic ecosystems directly related to groundwater due to higher seasonal fluctuations of groundwater level.	None

6. Groundwater salinity and recovery efficiency

• Does the salinity of native groundwater exceed either of the following: (a) 10,000 mg/L; (b) the salinity criterion for uses of recovered water?	**Yes (b)**. Some groundwater wells have been abandoned due to salinity values above crops requirements (1,100 µS/cm is the tolerance value, while 3,200 µS/cm causes 50% of yield)[2]	Analysis of the impact of aquifer recharge in the reduction of salinity (already done in Phase III Water Recovery Project). The numerical model developed for conservative transport could be used for the simulation and assessment of WWTP effluent injection

7. Reactions between source water and aquifer

• Is redox status, pH, temperature, nutrient status and ionic strength of groundwater similar to that of source water?	**No**. Different water quality has the potential of change chemical equilibrium, especially redox status (WWTP effluent is in contact with the atmosphere, while groundwater is anoxic.	Geochemical evaluation

8. Proximity of nearest existing groundwater users, connected ecosystems and property boundaries

• Are there other groundwater users, groundwater connected ecosystems or a property boundary within 100-1.000m of the MAR site?	**No**. Castellón Plain aquifer is not intended to produce drinking water. There are no aquatic ecosystems directly related to groundwater.	None

9. Aquifer capacity and groundwater levels

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[2] Irrigation Water Quality Standards and Salinity Management Strategies, Texas Agicultural Extension Service, The Texas A&M University System, 1996.
Cited in: http://www.fcca.es/static_media/file_uploads/Salinidad_del_agua_de_riego1.pdf

Question from the Australian Guideline	La Vall d'Uixó answers	Investigations required
• Is the aquifer: (a) confined and not artesian?; (b) unconfined, with a water table deeper than 4 m in rural areas or 8 m in urban areas?	**Yes (b)**. Unconfined and with water table around 40 – 50 meters below surface level.	None

10. Protection of water quality in unconfined aquifers

• If the aquifer unconfined, with an intended use of recovered water being drinking water supplies?	**No**	None

11. Fractured rock, karstic or reactive aquifers

• Is the aquifer composed of fractured rock or karstic media, or known to contain reactive minerals?	**No**. there is no evidences of reactive minerals in the aquifer. Phase III of Water Recovery Project (injection of surface water) has not revealed any undesired mobilization.	None

12. Similarity to successful project

• Has another project in the same aquifer with similar source water been operating successfully for at least 12 months?	**No**. this will be the first controlled experience. Nevertheless pharmaceuticals have been identified in groundwater, meaning that uncontrolled irrigation with WWTP effluent has been done.	None

13. Management capability

• Does the proponent have experience with operating managed aquifer recharge sites with the same or higher degree of difficulty, or with water treatment or water supply operations involving a structured approach to water quality risk management?	**Yes**. IGME has experience in previous MAR experiences in Spain. UJI has experience in groundwater hydrochemistry and hydrogeological studies.	None

14. Planning and related requirements

• Does the proposed project require development approval? Is it in built up area; built on public, flood-prone or steep land; or close to a property boundary? Does it contain open water storage or engineering structures; or is it likely to cause public health or safety issues, nuisance from noise, dust, odour or insects, or adverse environmental impacts?	**No**. The most costly infrastructure is already constructed (accumulation dam and injection wells). Additional pipes for WWTP and dam connection will be necessary, as well as potential pre-treatment and control points for reclaimed water monitoring.	None

4.3.2   Maximal risk assessment

Key hazards and acceptance criteria given by Australian guidelines have been applied. In this section 12 key hazards have been evaluated using a semi-quantitative risk assessment, for human health and environmental endpoints. Table 4-5 classifies the risk in low (green), uncertain (orange) and high (red) levels.

Table 4-5:   Maximal risk assessment for La Vall d'Uixó

MAR hazards			Human endpoint - agricultural threshold values for citrus irrigation	Environmental endpoint-aquifer WFD targets
1. Pathogens – present in high levels			H	L
2. Inorganic chemicals		Electric conductivity	H	L
		pH	L	L
		Sodium	L	L
		Chloride	H	L
		Boron	L	L
		Bicarbonate	H	L
3. Salinity and sodicity			H	L
4. Nutrients		Nitrate	H	L
		Total Nitrogen	H	L
		Total phosphorous	U	U
		Organic Carbon	U	U
5. Organic chemicals		Pesticides	H	H
		Pharmaceuticals and others	H	H
6. Turbidity and particulates			U	U
7. Radionuclides			L	L
8. Pressure, flow rates, volumes and groundwater levels			L	L
9. Contaminant migration in fractured rocks and karstic aquifers			NA	NA
10. Aquifer dissolution and stability of well and aquitard – pumping wells observed to be stable after 30 years			U	U
11. Aquifer and groundwater – dependent ecosystems			NA	NA
12. Energy and greenhouse gas considerations			L	L

L = Low risk; U = Uncertain risk; H = High risk; NA = Not applicable

4.3.2.1 Pathogens

Little information is available on the microbial impact of Castellón well injection systems on the environment. This section focuses on the impact of pathogens that affect humans only. Main source of pathogens in the Castellón Plain aquifer would be the injection of WWTP effluent without disinfection pretreatment. RD1620/2007 identifies Intestinal nematodes eggs (maximum 1 egg/10 L) and *E. coli* (maximum 0 UFC/100 mL). According to the controls done in the effluent of WWTP, only E. *coli* has been detected with an average of 19,700 UFC/100 mL and a maximum of 31,000 UFC/100 mL (see weekly results in Figure 4-8). Intestinal nematodes eggs is also analysed in routine analysis, with no positive samples.
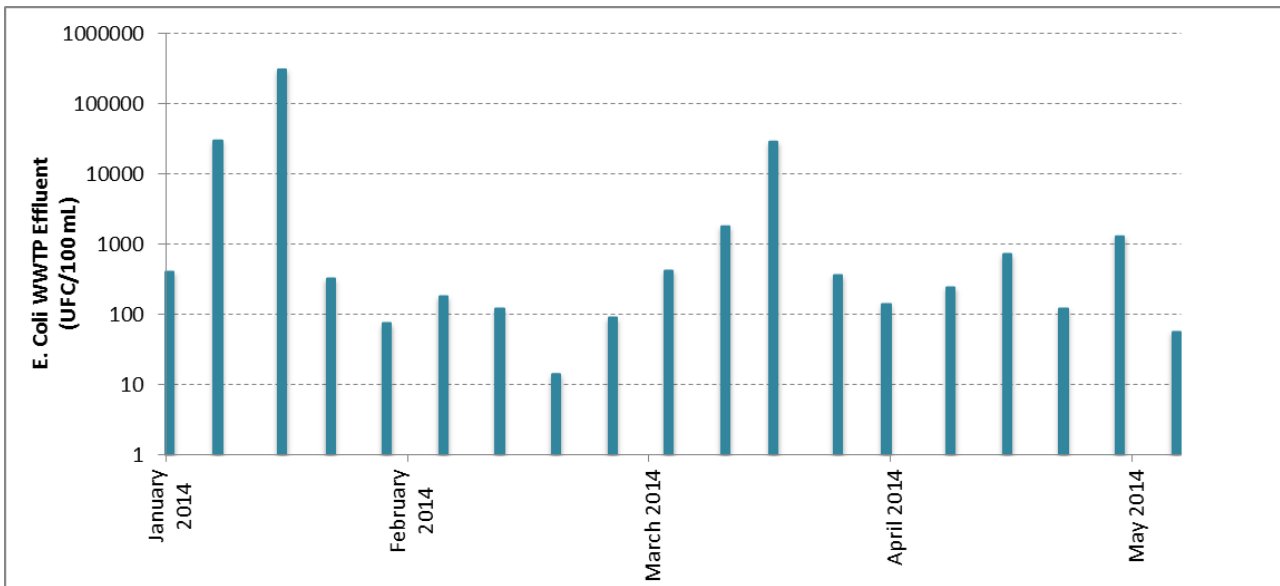
Figure 4-8:   E. coli concentration in WWTP Effluent (2014)

E. coli survival has been included in multiple studies to evaluate its decay rate in groundwater along MAR. Banning *et al.* (2005) determined that *E. coli* was dramatically reduced in no sterilized microcosms compared to the sterilized ones, suggesting that indigenous microorganisms play an important role in the die-off of *E.coli* in the environment. Same conclusion was achieved by RECLAIM WATER[3] project in their results of pathogens decay studies carried out in Adelaide (Australia). They found a decay rate of *E. coli* in non-filtered groundwater of -10 log·day^{-1}, while the decay rate in filtered groundwater[4] was -5 log·day^{-1} (Tandoi *et al.* 2012).

Table 4-6 summarises maximum inactivation times reported in literature. Considering the high levels of *E. coli* found in WWTP effluent, this MAR hazard has been considered as high risk, despite there are no evidences at test site of the potential elimination or/and reduction of pathogens concentration.

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[3] http://cordis.europa.eu/result/rcn/46853_en.html

[4] Achieved by passing the groundwater sample twice thorough sterile 0.2 μm nitrocellulose filters.

Table 4-6:   Minimum die-off of *E. coli*, in days observed during MAR

Parameter	days	Reference	Comments
T_{90}[5]	3	NRMMC-EPHC-NHMRC, 2009	In situ Australian aerobic and anoxic aquifers (20ºC)
T_{90}	0.1	Tandoi *et al.* (2012)	Diffusion chamber experiments performed in Adelaide (Australia)
T_{90}	1	Gordon and Toze (2003)	Aerobic conditions. Study on pathogen decay in groundwater not necessarily relating to specific MAR schemes. Same result at 18ºC and 25ºC
T_{90}	3	Toze et al (2002)	Aerobic conditions. Study undertaken as part of specific MAR scheme (22ºC)
T_{90}	1	Toze and Hanna (2002)	Aerobic conditions. Study undertaken as part of specific MAR scheme (27ºC)

A preliminary calculation has been done to estimate the residence time in the aquifer. Australian Guidelines suggest the "Dual-well system" for ASTR systems considering equal abstraction and injection rates. At this stage of pre-evaluation in La Vall d'Uixó site this is unpredictable, as the pumping regime of each well depends on the abstraction strategy, energy costs, alternative source of water availability, crops seasonality and rainfall regime among others.

Regarding the distance between injection and recovery locations, travel time has been calculated under the less favourable condition, taking into account the closest wells: CAS08 (located at 550 meters of injection well 1) and CAS11 (located at 445 meters of injection well nº2). Following formula has been applied:

$$t_{min} = \frac{\pi D n_e L^2}{3Q}$$

Where,

t_{min} = minimum travel time

L = distance between injection and recovery wells (m)

D= aquifer thickness (m)

n_e = porosity of the aquifer

Q = rate of steady-state pumping (in and out) (m^3/d)

Assuming steady-state conditions and equal abstraction and injection rates (D = 85-100 m; n_e = 0.02-0.08; Q=8640 m^3/d) and minimum distance from injection well to the nearest farmer well (L=445 m) the estimated travel time distribution is shown in Figure 4-9.

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[5] T_{90} = The time taken for 90% die-off
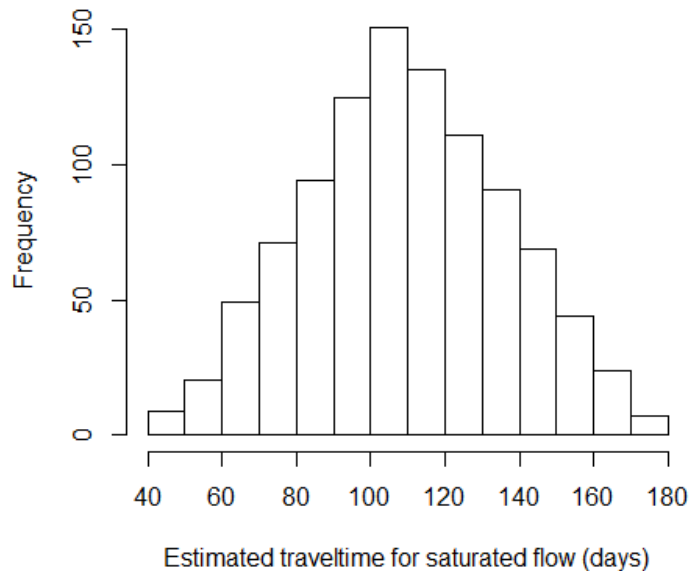
Figure 4-9: Estimated travel time distribution from injection well to nearest farmer well

Travel time was calculated by a very simple stochastic approach in order to account for hydrogeological variations, fluctuations in pathogen concentration and resulting variations in pathogen removal during subsurface passage. Minimum travel time is about 40 d assuming a low removal rate of T90 = 6d accounting only for die-off, it is reasonable to conclude that most of pathogens injected in the aquifer will not reach the extraction point of groundwater.

 Anyway, some preventive measures can be applied to reduce the risk of pathogenic hazards and achieve performance targets can be classified in source control, process control and end point control:

- Source control: removing pathogens using technical pre-treatment processes.
- Process control: Enlarge residence time in the aquifer before recovery.
- End-point control: reducing exposure through preventive measures on-site, e.g. providing individual protection equipment (gloves and masks) to the farmers during irrigation with recovered water.

The concept of tolerable risk is central to the management of enteric pathogens via MAR. Australian guidelines adopt a tolerable risk of 10^{-6} disability adjusted life years (DALYs) per person and year, which is the same value that WHO adopted in 2006. Pathogen decay should be assessed at each specific site where reliance is placed on the aquifer for pathogen inactivation. An in situ assessment of pathogen survival is described in Pavelic et al. (1998). If residual risk of pathogens remains unacceptable, then additional forms of disinfection should be considered along with their consequences, e.g. trihalomethanes risks.

4.3.2.2 Inorganic chemicals

This section is applicable to the major ions (calcium, magnesium, sodium, potassium, chloride, sulphate, bicarbonate, bromide and fluoride), metals (aluminium, cadmium. chromium, cooper, iron, manganese,

nickel, lead, strontium and zinc), metalloids (arsenic, boron and silicon) and gases (hydrogen, sulphide and methane).

Despite monitoring programme along DEMEAU sampling included a large list of inorganic chemicals (see annex 3 of the document), these key inorganic compounds for citrus irrigation and environmental standards of WFD have been selected. Figure 4-10 and Table 4-7 show mean values measured in WWTP effluent, native groundwater and Belcaire River and the comparison to citrus irrigation recommendations.

Regarding irrigation requirements, salinity has been determined as the limiting factor for local groundwater and WWTP effluent direct utilisation. In fact, historical aquifer salinization has been the driver for the execution of the Water Recovery project in La Vall d'Uixó area, as it is the most common problem for the agricultural areas near the coast. A table published in 1976 about agricultural water quality criteria for irrigation is nowadays applied in La Vall d'Uixó as a guideline for water classification and fertilisation plans according to the water suitability for crops irrigation (Ayers and Westcott 1976). Table 4-7 shows the citrus irrigation recommendations in La Vall d'Uixó compared to mean values found in native groundwater, Belcaire river water and WWTP effluent. Red coloured values indicate concentrations above citrus irrigation recommendations. Electrical conductivity, chloride, nitrate and bicarbonate are the critical parameters exceeding irrigation recommendations. Specifically four WWTP effluent components (electric conductivity, chloride, nitrate and bicarbonate) have high risk of pollute groundwater if their initial concentrations are not reduced along aquifer passage. All these compounds have been identified with high risk in the summary table of MAR hazards (Table 4-7). If source water for the MAR scheme is a 1:1 blend of WWTP effluent with Belcaire River these critical substances can be reduced to nitrate and bicarbonate only. As there is a specific section for the risk evaluation of nutrients, including nitrate, it has been included separately in the following section.

Table 4-7:   Inorganic quality standards for citrus irrigation

Parameter	Native GW	Belcaire River	WWTP effluent	1:1 Blend of WWTP effluent with Belcaire water	Citrus irrigation recommendations
Electric conductivity [µS/cm]	*3225*	310	*1329*	820	1100 (a)
pH-value	7.4	6.7	7.5	7	6.5 – 8.4 (b)
Sodium [SAR index]	2.0	0.6	2.4	1.5	3 (b)
Chloride [mg/L]	*581*	19.0	*143.25*	81	142 (b)
Boron [mg/L]	*1.0*	-	0.16	-	0.7 (b)
Nitrate [mg/L]	*287*	0.0	*61*	*30*	5 (b)
Bicarbonate HCO_3- [mg/L]	*229*	*110.0*	*353*	*231.5*	91.5 (b)

NOTE: Red cursive values indicate values above citrus irrigation recommendations

    (a)   http://www.fcca.es/static_media/file_uploads/Salinidad_del_agua_de_riego1.pdf

    (b)   FAO recommendations: Ayres and Westcott (1976)

Regarding the environmental fate of WWTP effluent as recharge water, there is the list of compounds established as control indicators for the good status of groundwater bodies in Spain. The quality standards are specific for each aquifer. Table 4-8 lists the quality standards for inorganic substances in the Castellón aquifer plain. Concentrations above the standards are marked in red colour. Only nitrate concentration in native groundwater exceeds the good quality status standards. None of the inorganic parameters in WWTP effluent is above quality standards for the aquifer, so in this case, risk of MAR using this water has been classified as low in Table 4-5.

Table 4-8:   Inorganic quality standards for WFD accomplishment in Castellón plain aquifer
(NOTE: Red cursive values indicate values above threshold values established in the Castellón plain aquifer)

Parameter	Native GW	Belcaire River	WWTP effluent	Threshold values stabilised (transposition of WFD in the Castellón plain aquifer)
Nitrate [mg/L]	*288*	0	61.5	< 200
Chloride [mg/L]	581.1	19.0	143.25	< 650
Sulphate [mg/L]	387.0	44.0	189.25	< 525
Selenium [mg/L]	N.A.	N.A.	N.A.	< 0.0207
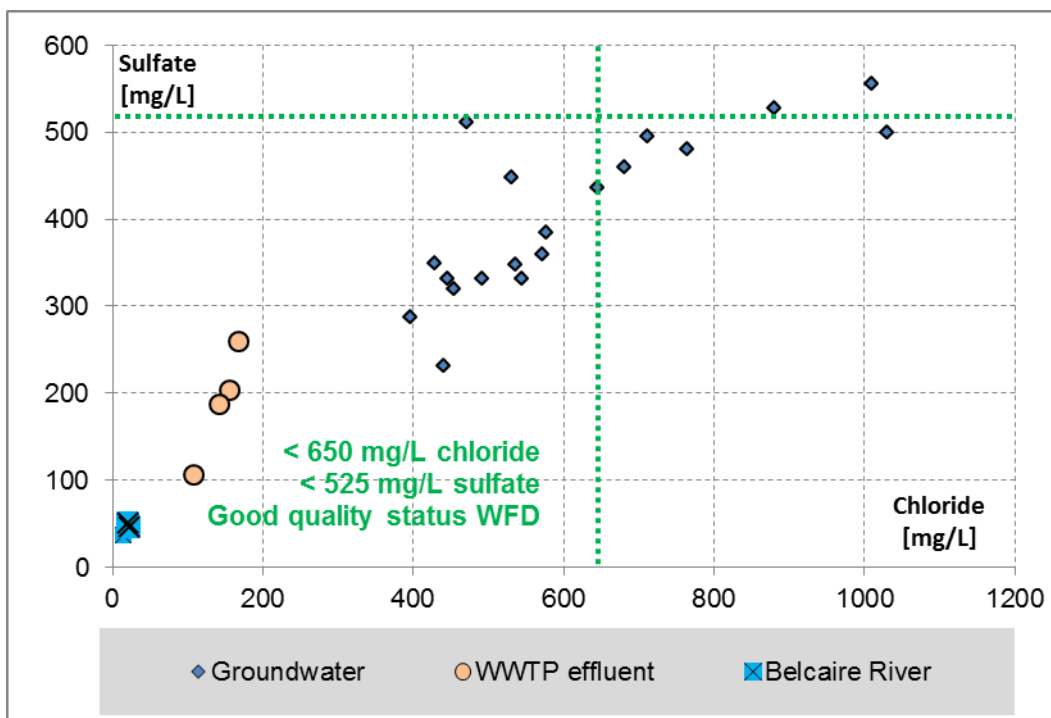
Figure 4-10:   Sulphate and chloride in recharge water compared to Environmental standards WFD in Castellón plain aquifer

4.3.2.3   Salinity and sodicity

The mixing of recharge water and ambient groundwater in MAR will cause the salinity of recovered water to differ from that of the recharge water. In general, the salinity of ambient groundwater within aquifer targeted for MAR should be similar to or higher than the source water. Therefore, native groundwater will represent an additional source of salinity (and sodicity) in recovered water. Levels reported of salinity in La Vall d'Uixó groundwater wells are around 3,000 – 4,000 µS/cm. Due to the dilution effect, high salinity values are not expected in recovered water. In fact, phase III of Water Recovery project demonstrated a quick response of the aquifer in the reduction of groundwater salinity.

4.3.2.4   Nutrients: nitrogen, phosphorous and organic carbon

Nitrogen and phosphorous are identified as key environmental hazards in the Australian guidelines. This is due to their potential to cause nutrient imbalance in irrigation water, soil eutrophication, and toxic effects on terrestrial biota. While subsurface storage is likely to reduce nutrient concentration, the overall nutrient balance of the recovered water still needs to be considered in relation to its beneficial use. The dominant nitrogen species in recycled water are organic nitrogen, ammonium and nitrate.

Figure 4-11 represents values of nitrate in WWTP effluent compared to groundwater nitrate concentrations. Direct injection of WWTP effluent would represent the introduction in the aquifer of nitrate levels below the standard value assigned for nitrate in the Castellón plain aquifer (200 mg/L).

Risk related to nitrate has been classified as high risk in Table 4-5 for the agricultural endpoint. In contrast, total organic carbon (TOC) in WWTP effluent is much higher than present in the aquifer (17 mg/L in the WWTP effluent versus 1 mg/L in groundwater). The risk of injecting WWTP effluent has been classified as high, as the consequences of high TOC in WWTP effluent.
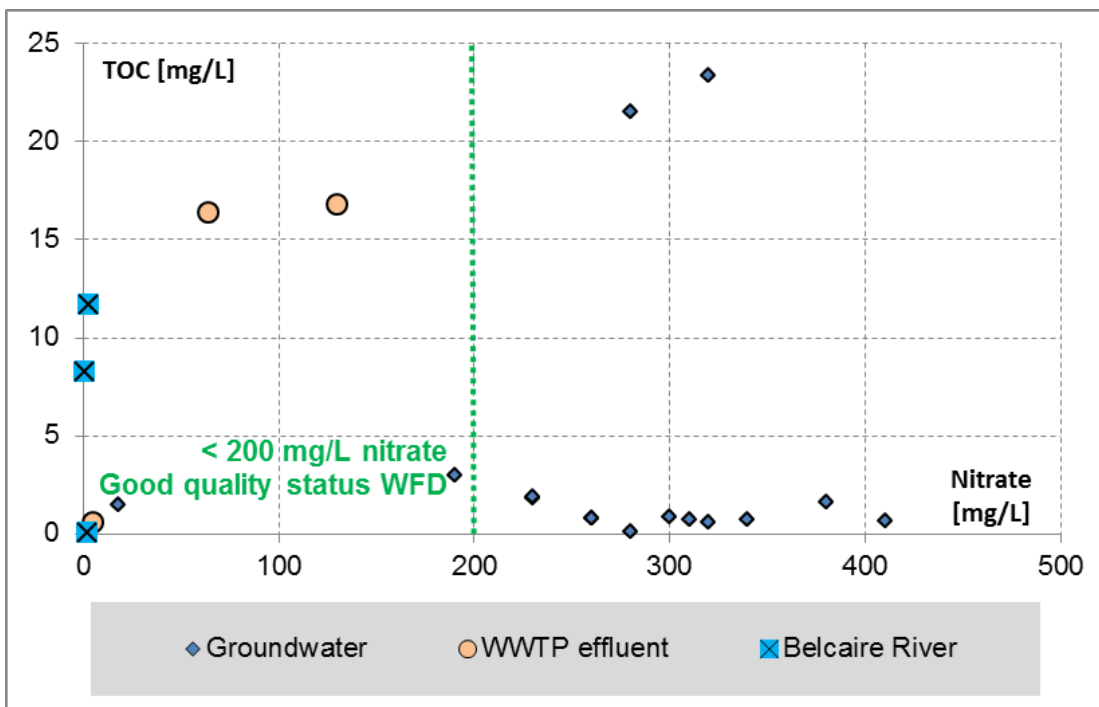
Figure 4-11:   TOC and nitrate in recharge water compared groundwater in La Vall d'Uixó

Regarding the risk assessment of nitrogen for crops irrigation, it has been classified as high risk, as the quality standards for agriculture establishes a maximum of 5 mg/L of nitrogen, while in WWTP effluent the average concentration of nitrate is 61 mg/L and ammonium concentration mean value is 17 mg/L.

Phosphorous has not been monitored in the project. Only total phosphorous concentration was analysed in the WWTP effluent in a punctual sample in 2012 that was quantified 3.7 mg/L. The risk assessment of phosphorous in La Vall d'Uixó has thus been classified as unknown (uncertain risk).

4.3.2.5   Organic chemicals

Determining the presence of organic chemicals in WWTP effluent and carrying out the associated risk assessment can be difficult, due to intermittent loadings. This study has taken advantage of the outputs generated in DEMEAU project. Six sampling campaigns have been performed during 2014 – 2015 in the effluent of the WWTP Vall d'Uixó. Three sampling campaigns were carried out in weekends (Sunday afternoon) and three sampling campaigns were done in weekdays (Monday or Wednesday morning). All results can be found in the annex 4.

During maximal risk assessment the maximum concentrations of organic micro pollutants is compared to native groundwater assuming no removal at all during subsurface passage. This shows the maximum risks if no preventive measures or natural attenuation occurs.

The maximal concentration measured in WWTP effluent, storage pond (Belcaire River) and groundwater shows large differences (

Figure 4-12). WWTP effluent shows elevated concentrations in almost all groups of organic micro pollutants compared to storage pond or groundwater. Only pesticides are found in higher concentrations in groundwater compared to the effluent. The Belcaire River shows the lowest concentrations for all groups of micro pollutants. The Vall d'Uixó aquifer is contaminated by various organic micro pollutants and does not reflect a near natural aquifer condition. The aquifer chemistry reflects the usage of effluent for irrigation over years.
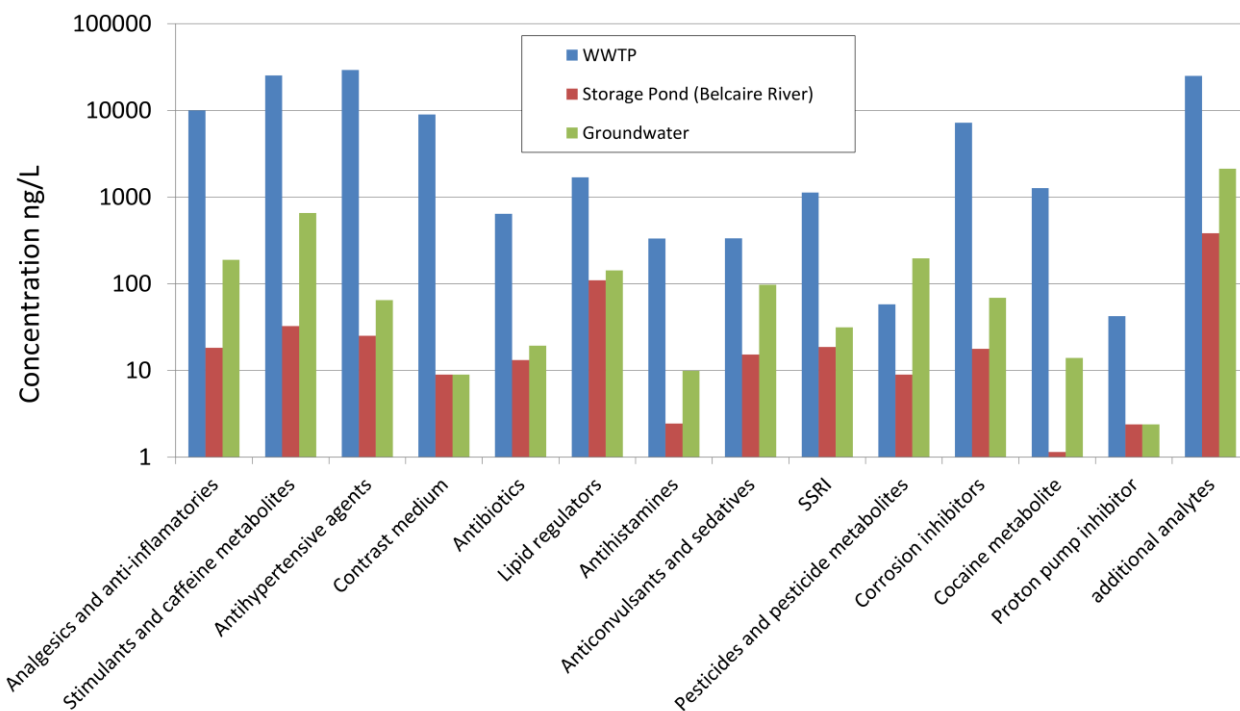
Figure 4-12: Maximal sum concentration found in WWTP effluent, storage dam and groundwater for the different groups of organic compounds (SSRI = selective serotonin and norepinephrine re-uptake inhibitors).

WFD application in Castellón plain aquifer indicates a maximum of 0.1 µg/L of individual concentration of active substances in pesticides and a maximum of 0.5 µg/L of total pesticides (CHJ, 2014). The analysis included seven pesticides (atrazine, DEA, DIA, diuron, isoproturon, mecoprop and metazchlor) as well as two herbicides (simazine and terbuthylazine, shown in additional analytes). The most abundant substance was terbuthylazine, with a maximum concentration of 155 ng/L. Nevertheless, maximal risk associated to pesticides is classified high, due the loading of pesticides in effluent. For all other groups risks associated to OMP´s are considered high.

Figure 4-13 shows the mean concentration of types of chemical compounds in the effluent of the WWTP La Vall d'Uixó. Number in brackets in the legend means the number of different compounds considered in in the study. Mean values have been calculated separating samples of working days and samples of weekend (Sunday afternoon). Some classes of compounds as artificial sweeteners (cyclamate and acesufame), stimulants, caffeine metabolites and cocaine metabolites (benzoylecgonine) were found in high concentration in the weekend compared to concentration along the weekdays. Contrast media (iopromide) was found more abundant in weekdays than in the weekend samples. This phenomenon highlights the importance of population habits in the compounds found in the WWTP effluent. As sampling campaigns performed were limited due to analytic costs, these results can be used as first estimation values and not as reference values.
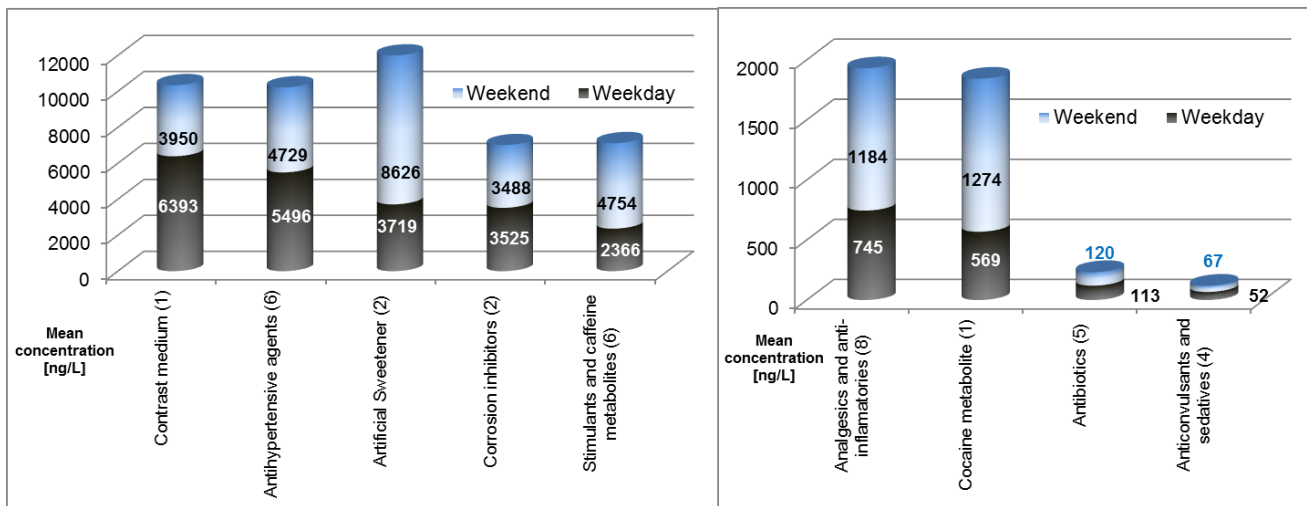
Figure 4-13: Mean concentrations of organic compounds in WWTP effluent (Number in brackets correspond to total number of compounds for each class)
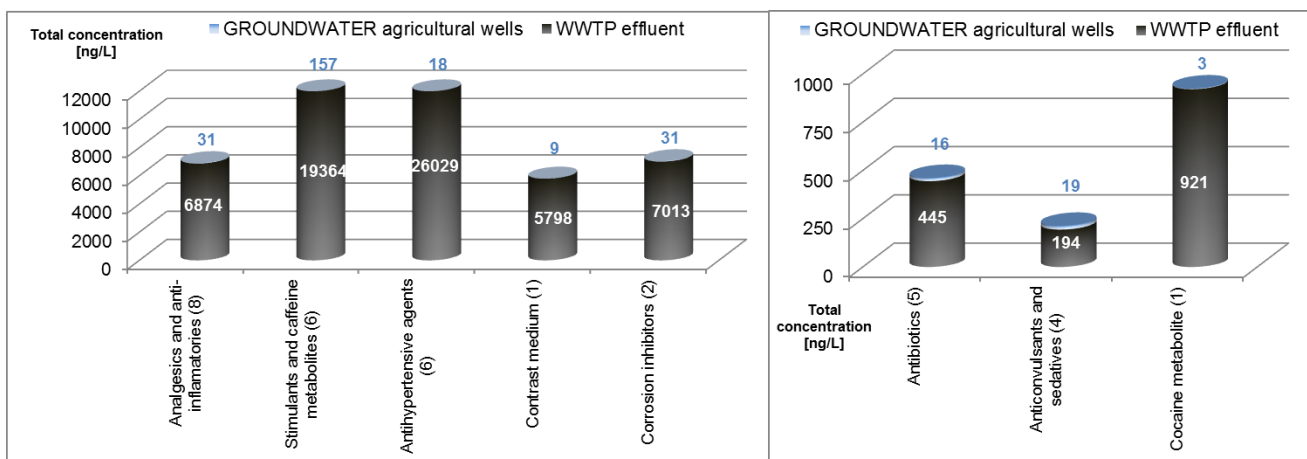
Figure 4-14: Pharmaceuticals and drugs in WWTP effluent and native groundwater (Number in brackets correspond to total number of compounds for each class)
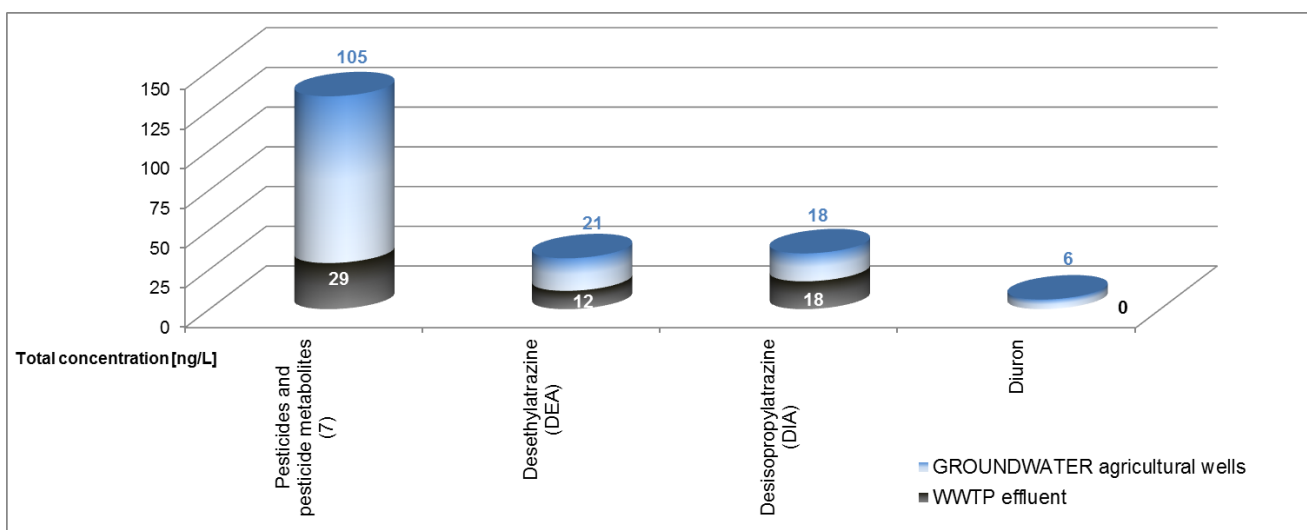
Figure 4-15: Pesticides in WWTP effluent and native groundwater (Number in brackets correspond to total number of compounds for each class)

Figure 4-14 and Figure 4-15 represents total concentration of drugs and pesticides found in WWTP effluent and in native groundwater. There is a clear relation in the type of pollutant and the source of water. As expected, antibiotics or analgesics are present in the effluent of WWTP.

Regarding undesirable substances as pharmaceuticals, contrast media, artificial sweeteners and illicit drugs metabolites found in WWTP effluent, there are no trigger values already established to determine level of risk. Individual quantification of contaminants had been complemented with the toxicity assessment of water samples. Results of toxicity of WWTP effluent of Vall d'Uixó are presented in this report in the bioassays section.

Due to the toxic character of the water, and according to the prevention principle, the risk assessment of organic pollution had been classified as "high" in Table 4-5.

Once established the threshold values in the aquifer or in the recovery water in irrigation wells, preventive measures to reduce the amount of organic pharmaceuticals and undesirable substances in recharge water can be:

- **Controlling source of pollutants:** avoid the inflow from hospitals and nursing homes to canalisation. This is an emerging trend of separating concentrate effluents containing pharmaceuticals. Finally, the effluent of the WWTP municipal plant will contain less concentration of these undesirable compounds.
- **Installing pre-treatment:** install a technical pre-treatment at the WWTP (e.g. advanced oxidation processes) to reduce the input of micro pollutants in the environment.
- **Ensuring enough residence time in soil-aquifer:** study the fate of degradable compounds and ensure sufficient residence time in the aquifer for removal below threshold values (residual risk assessment).

4.3.2.6 Turbidity and particulates

Turbidity has not been monitored in the project. Only turbidity in the WWTP effluent in a punctual sample in 2012 was determined and quantified 1.77 NTU. The risk assessment of turbidity and particulates in La Vall d'Uixó has been classified as unknown (uncertain risk) and requires further investigations.

4.3.2.7 Radionuclides

Radionuclides are radioactive isotopes or unstable forms of elements. There are no evidences of radium or radon in the groundwater of the area. Conglomerates, sands and clays constitute the Castellón plain aquifer. Sedimentary deposits are usually not related to radioactive activity. Volcanic and metamorphic rocks are correlated to potential release of radionuclides in groundwater, but they are not present in the study area. Therefore, this risk is considered to be low.

4.3.2.8 Pressure, flow rates, volumes and groundwater levels

Excessive pressure can cause failure of poorly completed injection or other wells, allowing water to escape to other aquifer or the ground surface. Castellón plain aquifer is a free aquifer, so aquitard rupture cannot happen. During phase III of Water Recovery Project, 100 L/s have been injected in the aquifer, without any negative consequence in the injection well nor in the farmers' recovery wells. The risk evaluation for these aspects is considered to be low..

4.3.2.9 Contaminant migration in fractured rocks and karstic aquifers

The intended MAR scheme is located in Castellón plain aquifer which is neither karstic nor fractured aquifer. Hence, this category does not apply to the Castellón plain aquifer.

4.3.2.10 Aquifer dissolution and stability of well and aquitard

Recharge water may react with the aquifer matrix material, resulting in dissolution of mineral or reduction in the aquifers bulk volume or strength. To assess this point a hydrochemical reactive numerical model should be carried out. PRHEEQC software is recommended to perform the numerical model, as it can include dissolution and precipitation equations, as well as reactive transport. This evaluation is out of the scope of this work, so the risk assessment of the hydrochemical stability has been classified as unknown (uncertain risk).

4.3.2.11 Aquifer and groundwater-dependent ecosystems

Castellón plain aquifer has no related wetlands, streams, lakes, estuaries or other aquatic ecosystems in La Vall d'Uixó. Neither indigenous microorganisms nor stygofauna have been clearly identified in the aquifer. This section does not apply to La Vall d'Uixó MAR scheme.

4.3.2.12 Energy and greenhouse gas considerations

Decisions to establish a MAR project need to take into account energy requirements in relation to alternative supply systems. In that sense, a local project of injection of WWTP effluent to improve groundwater levels and reduce salinity in the aquifer is more energy saving than the installation of a desalination of a reverse osmosis plant to reclaim WWTP effluent. Moreover, the most costly infrastructures (accumulation dam and injection wells) have been already constructed, so future investment will be focused on pre-treatment, operation, and maintenance and monitoring of the MAR scheme.

4.3.3 Residual risk assessment

Table 4-5 summarises the maximal risk assessment carried out assuming the injection of effluent from the WWTP directly into the Castellón plain aquifer. This is an important step to identify main hazards for the environment and the end use of reclaimed water: irrigation of citrus in La Vall d'Uixó. According to the Australian Guidelines, next step in risk assessment development will be to refill the table with the valuation of risk (High or Low) after the inclusion of some improvements in the MAR scheme.

5   Recommendations for future studies

Some information gaps have been identified during the risk assessment for the MAR scheme in Castellón. Specifically the following aspects should be analysed in future studies before using the WWTP effluent as source water:

- **Pathogens**: residence time within the aquifer has been calculated theoretically. Preferential flow paths or high abstraction pumping rates have been not considered in the preliminary assessment in this report. It is recommended to assess the hydraulic performance by tracer tests under real conditions.

- **Hydrogeochemical changes in the aquifer:** changes in redox state and pH value can cause variations in precipitation-dissolution reactions. A mineralogical analysis is recommended to know if there are arsenic bearing minerals that could possibly mobilise.

- **Physical clogging:** During the study there were no data of turbidity values and suspended solids in WWTP effluent. This is a topic very well studied in ASR and ASTR schemes that should be addressed in future studies.

The aim of Water Recovery Project was to check the feasibility of reclaimed water as source water for MAR. Phase IV of the project was intended to test effluent and to design additional pre-treatment or mixing ratios of river water with treated effluent. Unfortunately, phase IV of the Water Recovery Project has been cancelled due to lack of funding. Hopefully this work will help in future to support the implementation of the MAR reuse scheme.

Apart from of the scientific work carried out, it was noticed that it was a fluent collaboration between technicians and local farmers. This is a result of a strong interest of farmers for having an improvement on water quantity and quality. An important recommendation for following studies is to maintain this good cooperation and present information in an open manner.

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ANNEX 1 Environmental quality standards for groundwater bodies in Castellón

Table A-0-1: Environmental quality standards for groundwater bodies in Castellón

	Nitrate [mg/L]	Pesticides [µg/L]	Chloride [mg/L]	Sulphate [mg/L]	Selenium [mg/L]
Chemical status indicator (*)	< 200	Individual concentration of active substances < 0.1 µg/L Total pesticides < 0.5 µg/L	< 650	< 525	0.0207
WWTP Effluent 10/02/2012 [a]	46.9	Chlorphirifos = 0.6 µg/L Molinate = 2.6 µg/L Terbutilazine = 0.26 µg/L Total pesticides = 3.96 µg/L	156	204	Not reported
WWTP Effluent 20/05/2014	130 [b]	Pesticides < < 0.1 µg/L [c] Total pesticides < 0.5 µg/L [c]	168 [b]	260 [b]	Not reported
WWTP Effluent 20/01/2015	5 [b]	Pesticides < < 0.1 µg/L [d] Total pesticides < 0.5 µg/L [d]	142 [b]	187 [b]	Not reported
WWTP Effluent 15/04/2015	64 [b]	Pesticides < < 0.1 µg/L [d] Total pesticides < 0.5 µg/L [d]	107 [b]	106 [b]	Not reported

(a) Aklabs Laboratory. Source: Water Recovery Project report. Internal report.
(b) IGME Laboratory: Source: sampling campaign DEMEAU (this report)
(c) Göttingen University Laboratory: Source: sampling campaign DEMEAU (this report)
(d) University of Castellón (UJI): Source: sampling campaign DEMEAU (this report)
(*) Source: CHJ, 2014 (Environmental objectives for the accomplishment of WFD):

Table A-0-2: Threshold values established in the adaptation of WFD in Spain (original table):

Código masa de agua	Nombre masa de agua	Area (km²)	Horizonte cuantitativo	Horizonte químico	Horizonte global	Art. DMA	Causa incumplimiento	Indicador estado cuantitativo	Indicador estado químico	Requerimiento adicional por zona protegida
080.127	Plana de Castellón	495,03	2027	OMR	OMR	4(5)	Cuantitativo (Test balance hídrico e intrusión) y Químico (Nitratos y valores umbral)	Test global	NO3 ≤ 200 mg/L. Sustancias activas de plaguicidas individualmente < 0,1 µg/L y suma de todos < 0,5 µg/L Cloruros < 650 mg/L, Sulfatos < 525 mg/L y Selenio < 0,0207 mg/L.	Zona Captación abastecimiento Perimetros protección aguas minerales y termales Zona vulnerable Zona Húmeda Red Natura

ANNEX 2 MAR profile

Table A-0-3:   MAR profile at La Vall d'Uixó

MAR component	Attribute	Description		
General information	Country	Spain		
	City	Vall d'Uixó		
	Site name	Aquifer Recharge system of la Vall d'Uixó		
	Operator name	AQUAMED		
	Type of MAR (e.g. Well injection and recovery, Aquifer transfer and recovery, bank filtration etc.)	Injection wells		
	Year commenced	2012		
	Current status	Experimental – pilot scale		
	Map coordinates	40.018/ -0.15		
	Operational scale (m^3/a)	Pilot		
	Objective	Irrigation		
Capture zone	Influent source (Type of water used for recharge)	River water (Reclaimed water in future)		
Pre-treatment	Source water treatment before recharge	Storage pond		
Recharge	No of recharge facilities	2 injection wells		
	Hydraulic loading rate (m^3/m^2 d)	100 L/s each well		
	Recharged volume (m^3/a)	Puntual		
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)	1.4×10^{-3} - 3.5×10^{-4}	
		Lithology of target aquifer	porous	
		Range of thickness of unsaturated zone (m)	Between 10 – 50	
		Thickness of target aquifer (m)	100 – 500 m	
Recovery	Distance of recovery wells from point of recharge (m)	Unknown (private wells for irrigation)		
	Recovered volume (m^3/a)	Unknown (private wells for irrigation)		
	Recovered infiltrate (%) Average percentage of recovered infiltrate (in case of bank filtration share of bank-filtrate in abstraction wells)	More than 20 wells in the area		
	No of recovery facilities (e.g. no. of wells, drains)			
Post-treatment	Water treatment after recovery	None		
End-use	Final use of water recharged by the facility	Agriculture, Irrigation of citric crops (orange and clementine)		

ANNEX 3 Bulk chemistry

Table A-0-4: List of chemical parameters analysed in sampling campaigns

Parameters		Unit
General parameters	Temperature	ºC
	Turbidity	NTU
	pH	Units of pH
	Electrical Conductivity	µS/cm
	COD	mg O_2/L
	BOD_5	mg O_2/L
	TOC	mg C/L
	Total hardness	mg HCO_3/L
Major compounds	Nitrate	mg/L
	Total nitrogen	mg/L
	Ammonium	mg/L
	Chloride	mg/L
	Total Phosphorous	mg/L
	Sulphate	mg/L
Metals	Sodium	mg/L
	Potassium	mg/L
	SiO_2	mg/L
	Zinc	mg/L
	Lead	mg/L
	Chromium (VI)	mg/L
	Calcium	mg/L
	Magnesium	mg/L
	Arsenic	mg/L
	Bromine	mg/L
	Aluminum	µg/L
	Barium	µg/L
	Strontium	µg/L
	Manganese	µg/L
	Total Chromium	µg/L
	Iron	µg/L
Aggregated indexes	Ion balance	%
	SAR index	meq

Table A-0-5:   Bulk chemistry of groundwater samples

	RANDERO 04/2012	RANDERO 05/2014	RANDERO 01/2015	RANDERO 04/2015	RAMBLETA 2 04/2012	RAMBLETA 2 05/2014	RAMBLETA 2 01/2015	RAMBLETA 2 04/2015	PRIMITIVA 04/2012	PRIMITIVA 05/2014	PRIMITIVA 01/2015	PRIMITIVA 04/2015	LA PAZ 04/2012	LA PAZ 05/2014	LA PAZ 01/2015	LA PAZ 04/2015	SAN VICENTE 04/2012	SAN VICENTE 05/2014	SAN VICENTE 01/2015	SAN VICENTE 04/2015
	CAS-08	CAS-08	CAS-08	CAS-08	CAS-09	CAS-09	CAS-09	CAS-09	CAS-10	CAS-10	CAS-10	CAS-10	CAS-07	CAS-07	CAS-07	CAS-07	CAS-06	CAS-06	CAS-06	CAS-06
Turbidity [NTU]																				
pH	7.33	7.24	7.08	7.17	7.62	7.64	7.23	8.12	7.43	7.2	7.13	7.2	7.42	7.66	7.5	7.17	7.57	7.35	7.28	7.48
E. Conductivity	2660	280	2770	2420	2300	341	334	454	3360	393	309	272	4340	393	437	454	3090	346	378	374
Suspended																				
COD [mgO2/L]																				
BOD5[mgO2/L]																				
TOC [mgC/L]			0.71	0.02		0.6	1.53	1.43		0.78	23.3	0.52		0.64	21.4	0.75		1.73	2.89	1.82
Nitrate [mg/L]	270	310	260	280	300	410	380	18	400	300	320	320	360	340	280	260	300	230	190	230
Total nitrogen																				
Ammonium		0	0	0		0	0	0		0	0	0		0	0	0		0	0	0
Chloride [mg/L]	454	446	428	396	440	576	572	17	470	544	536	492	1030	764	880	101	530	644	680	712
Total		0	0	0		0	0	0.32		0.13	0	0		0.13	0	0		0.45	0	0.49
Sulphate [mg/L]	320	332	350	288	232	384	360	46	512	332	348	332	500	480	528	556	448	436	460	496
Lead [µg/L]		0.02	1.14	1.93		0.91	1.57	2.11		1.04	1.56	1.79		1.73	180	28.6		10.7	0.04	6.33
Zinc [mg/L]		6.34	23.3	8.26		12.5	64.8	12.3		13.2	13.1	11.9		15.9	259	18.6		24.5	22.5	25.1
Aluminium		5.3	10.6	15.6		13	12.8	39.6		21.4	9.17	17.3		17.2	350	608		10.7	9.54	69.6
Barium (µg/L)		44.6	40.8	48.9		68.3	63.9	68.3		51.4	44.5	46.7		31.8	100	67.2		50.3	42.8	51.9
Br [mg/L]	0.001	1.27	0.87	0.88	0.0008	1.43	1.08	0.06	0.0012	1.35	1.3	1.03	0.0019	1.82	1.73	1.86	0.0014	1.76	1.50	1.63
Sodium [mg/L]	107	125	118	122	65	118	107	11	120	125	125	132	273	200	304	371	226	187	199	277

	RANDERO 04/2012	RANDERO 05/2014	RANDERO 01/2015	RANDERO 04/2015	RAMBLETA 2 04/2012	RAMBLETA 2 05/2014	RAMBLETA 2 01/2015	RAMBLETA 2 04/2015	PRIMITIVA 04/2012	PRIMITIVA 05/2014	PRIMITIVA 01/2015	PRIMITIVA 04/2015	LA PAZ 04/2012	LA PAZ 05/2014	LA PAZ 01/2015	LA PAZ 04/2015	SAN VICENTE 04/2012	SAN VICENTE 05/2014	SAN VICENTE 01/2015	SAN VICENTE 04/2015
	CAS-08	CAS-08	CAS-08	CAS-08	CAS-09	CAS-09	CAS-09	CAS-09	CAS-10	CAS-10	CAS-10	CAS-10	CAS-07	CAS-07	CAS-07	CAS-07	CAS-06	CAS-06	CAS-06	CAS-06
K [mg/L]	9	7	6	5	3	3	2	1	4	4	4	4	4	9	4	5	11	6	6	6
SiO2 [mg/L]	13.3	14.5	15	15	10.1	12.8	13.1	7.8	14.7	15.6	16.4	15.9	13.9	15.2	16.5	16	13.6	24.7	28.3	27.4
Sr [µg/L]	855	109	103	982	624	124	110	230	928	120	113	108	732	755	146	156	817	144	137	141
Mn [µg/L]		4.35	13.3	5.38		3.72	4.98	6.96		1	1	1		1	263	27.2		595	557	564
Total Chromium	0.1				0.39				0.31				0.31				0.68			
Chromium (VI)																				
Total hardness	178	248	223	243	157	128	189	203	187	236	231	246	176	271	248	253	231	293	320	320
Calcium [mg/L]	268	300	256	228	290	336	368	56	300	326	308	272	316	304	332	358	240	324	320	300
Magnesium	118	114	110	111	100	152	132	25	172	128	140	134	228	208	192	204	128	156	160	164
Arsenic [mg/L]	0.00014				0.0002				0.0001				0.0002				0.0008			
Fe (µg/L)		91.2	392	263		194	147	120		30	59.7	132		30	389	567		64.4	373	377
Calculated values																				
Ion balance [%]	4.47	4.81	-0.36	1.07	3.78	4.53	3.21	5.74	2.98	4.35	3.51	1.52	-3.48	-0.62	3.13	5.09	-0.86	4.12	2.34	3.25
SAR index	1.4	1.6	1.6	1.7	0.8	1.3	1.2	0.3	1.4	1.5	1.5	1.6	2.9	2.2	3.3	3.9	2.9	2.1	2.3	3.2

Table A-0-6: Bulk chemistry of WWTP effluent, Belcaire River and groundwater monitoring Piezo 1+2 wells.

	WWTP Historical 02/02/2012	WWTP DEMEAU May 2014	WWTP DEMEAU Jan 2015	WWTP DEMEAU April 2015	average	Belcaire River DEMEAU May 2014	Belcaire River DEMEAU Jan 2015	Belcaire River DEMEAU April 2015	Average	1:1 blend	Piezo 1 DEMEAU May 2014	Piezo 1 DEMEAU Jan 2015	Piezo 1 DEMEAU April 2015	Piezo 2 DEMEAU May 2014	Piezo 2 DEMEAU Jan 2015	Piezo 2 DEMEAU April 2015
Turbidity [NTU]	1.77				1.77											
pH	7.64	7.36	7.59	7.27	7.465	8.04	7.8	4.29	6.7	7.09	7.43	7.11	7.08	7.45	7.39	7.17
E. Conductivity [µS/cm]	1408	1432	1454	1023	1329	232	352	347	310.3	819.79	583	1970	2100	327	893	1245
Suspended Solids [mg/L]	7.2				7.2											
COD [mgO2/L]	72				72											
BOD5[mgO2/L]	16				16											
TOC [mgC/L]		16.78	0.57	16.4	11.26	8.28	0.03	11.7	6.7	8.95	2.52	0.025	0.7	3.21	0.025	1.32
Nitrate [mg/L]	46.9	130	5	64	61.48	0	-	0	0.0	30.74	27	180	220	4	68	140
Total nitrogen [mg/L]	46				46											
Ammonium [mg/L]	34			0	17			0.44	0.4	8.72	0	0	0	0	0	0
Chloride [mg/L]	156	168	142	107	143.3	14	21	22	19.0	81.13	68	340	350	19	136	210
Total Phosporous[mg/L]	3.7				3.7						0	0	0	0	0	0.16
Sulphate [mg/L]	204	260	187	106	189.3	37	50	45	44.0	116.63	85	252	282	45	121	162
Lead [µg/L]	0.27				0.27						0.38	0.02	1.18	0.23	0.2	0.72
Zinc [mg/L]	2.42			12.6	7.51						6.28	10.7	10.4	5.15	6.31	5.06
Aluminium (µg/L)				13.6	13.6			370	370.0	191.80	8.24	13.7	18.8	10.1	7.37	8.41
Barium (µg/L)				11.8	11.8			63.2	63.2	37.50	41.4	95.3	109	38.5	79.3	115
Br [mg/L]	0.16				0.16						0.18	0.683	0.789	0.1	0.284	0.491
Sodium [mg/L]	95	130	120	111	114	12	14	19	15.0	64.50	35	90	117	16	45	72
K [mg/L]											3	6	7	2	4	5

	WWTP Historical 02/02/2012	DEMEAU May 2014	DEMEAU Jan 2015	DEMEAU April 2015	average	Belcaire River DEMEAU May 2014	DEMEAU Jan 2015	DEMEAU April 2015	Average	1:1 blend	Piezo 1 DEMEAU May 2014	DEMEAU Jan 2015	DEMEAU April 2015	Piezo 2 DEMEAU May 2014	DEMEAU Jan 2015	DEMEAU April 2015
SiO2 [mg/L]											10.7	14	13.8	9.9	11.8	12.6
Sr [µg/L]											298	869	894	170	411	590
Mn [µg/L]											60.8	92.3	185	31.9	81.7	40.4
Total Chromium [µg/L]	25															
Chromium (VI) [mg/L]	0.003															
Total hardness [mg HCO3/L]	439	272	400	304	353.8	74	118	138	110.0	231.88	156	203	233	119	134	149
Calcium [mg/L]	102	120	92	75	97.25	11	28	30	23.0	60.13	61	204	220	35	92	128
Magnessium [mg/L]	45	47	46	28	41.5	17	19	17	17.7	29.58	29	87	99	14	39	61
Arsenic[mg/L]																
Fe (µg/L)		156	188	114	152.7	151	127		139.0	145.83	9651	13747	32068	6627	13703	15348
Calculated																
Ion balance [%]	-24.91	-7.44	-6.7	-3.3	-	29.4	0.11	-32.5	-	-	3.99	0.87	4.66	2.99	1.1	3.78
SAR index					2.4				0.6							

ANNEX 4 Organic micro pollutants

Table A-0-7: Results of organic pollutants in the WWTP effluent

Application	Compound	LOQ [ng/l]	05.06.2014 Work. DAY CAS 01	05.06.2014 Work. DAY CAS 02	20.01.2015 Work. DAY CAS 01	15.04.2015 Work. DAY CAS 01	08.06.2014 Weekend CAS 03	08.06.2014 Weekend CAS 04	18.01.2015 Weekend CAS 03	12.04.2015 Weekend CAS 03
Analgesics and anti-inflamatories	Diclofenac*	10	965	1026	538	769	1084	1128	408	802
	Ibuprofen*	18	na	na	9	-	na	na	9	-
	Naproxen	24	35	54	na	na	95	103	na	na
	Paracetamol	19	8.5	8.5	na	na	8.5	8.5	na	na
	Phenazone*	10	567	614	612	563	468	452	145	607
	Acetaminophen	19	na	na	106	42	na	na	44	36
	Benzoylecgonine	12	na	na	226	898	na	na	585	197
	4-Acetaminoantipyrine (Metamizole metabolite)	15	3018	3386	na	na	6095	7132	na	na
Stimulants and caffeine metabolites	Caffeine	22	1895	2002	na	na	2525	3062	na	na
	Paraxanthine	16	1383	1380	na	na	2247	2306	na	na
	Theobromine	26	1796	1882	na	na	3132	3212	na	na
	Theophylline	17	468	489	na	na	447	582	na	na
	1-Methylxanthine	105	2380	2452	na	na	4901	5381	na	na
	3-Methylxanthine	140	5888	6379	na	na	10413	10856	na	na
Antihypertensive agents	Atenolol	18	48	45	na	na	49	49	na	na
	Metoprolol*	21	10.5	10.5	36	30	10.5	10.5	26	21
	Sotalol	24	12	12	na	na	12	12	na	na

Application	Compound	LOQ [ng/l]	05.06.2014 Work. DAY CAS 01	05.06.2014 Work. DAY CAS 02	20.01.2015 Work. DAY CAS 01	15.04.2015 Work. DAY CAS 01	08.06.2014 Weekend CAS 03	08.06.2014 Weekend CAS 04	18.01.2015 Weekend CAS 03	12.04.2015 Weekend CAS 03
	Valsartan	15	24632	25523	23172	23350	23945	25168	13509	24283
	Losartan	10	1144	1164	na	na	1098	1141	na	na
	Irbesartan	10	2550	2527	1547	1147	2434	2351	1123	1246
Contrast medium	Iopromide*	90	8995	8942	1244	6734	4794	4694	2362	8616
Antibiotics	Clarithromycin	38	17	17	na	na	17	17	na	na
	Erythromycin	22	11	11	na	na	11	11	na	na
	Roxithromycin	48	24	24	na	na	24	24	na	na
	Sulfamethoxazole*	13	301	303	140	153	342	328	115	158
	Trimethoprim*	13	241	248	103	145	197	185	81	102
Lipid regulators	Bezafibrate*	18	9	9	1219	1095	9	9	780	1270
	Clofibric acid	17	8.5	8.5	na	na	8.5	8.5	na	na
	Gemfibrozil*	10	5.0	5.0	327.0	416	19	17	359	322
Antihistamines	Cetirizine	11	308	327	na	na	285	299	na	na
	Loratadine	14	7	7	na	na	7	7	na	na
Anticonvulsants and sedatives	Carbamazepine*	11	148	137	84	73	124	128	97	86
	Diazepam	7.0	27	30	na	na	24	25	na	na
	Primidone*	14	39	44	151	37	28	23	57	28
	Tetrazepam	13	6.5	6.5	na	na	6.5	6.5	na	na
SSRI (selective serotonin and norepinephrine reuptake inhibitors)	Citalopram	16	83	86	na	na	70	72	na	na
	Fluoxetine	80	40	40	na	na	40	40	na	na
	Sertraline	80	40	40	na	na	40	40	na	na
	Venlafaxine	5.0	na	na	962	831	na	na	619	801

Application	Compound	LOQ [ng/l]	05.06.2014 Work. DAY CAS 01	05.06.2014 Work. DAY CAS 02	20.01.2015 Work. DAY CAS 01	15.04.2015 Work. DAY CAS 01	08.06.2014 Weekend CAS 03	08.06.2014 Weekend CAS 04	18.01.2015 Weekend CAS 03	12.04.2015 Weekend CAS 03
Pesticides and pesticide metabolites	Atrazine	7.0	3.5	3.5	na		3.5	3.5	na	na
	Desethylatrazine (DEA)	8.5	4.3	4.3	11	-	4.3	4.3	12	-
	Desisopropylatrazine (DIA)	28	14	14	16	-	14	14	19	-
	Diuron	17	8.5	8.5	na		8.5	8.5	na	na
	Isoproturon	15	7.5	7.5	na		7.5	7.5	na	na
	Mecoprop	6.0	3.0	3.0	na		3.0	3.0	na	na
	Metazachlor	9.0	4.5	4.5	na		4.5	4.5	na	na
Corrosion inhibitors	1H-Benzotriazole	24	2152	2222	na		2056	2163	na	na
	Tolyltriazole	25	4729	4996	na		4857	4875	na	na
Cocaine metabolite	Benzoylecgonine	12	550	588	na		1274	1274	na	na
Proton pump inhibitor	Pantoprazole	24	26	34	12	12	32	42	12	12
Antipsychotic	Haloperidol	20	10	10	na	na	10	10	na	na
Breast cancer therapy	Tamoxifen	30	15	15	na	na	15	15	na	na
Nicotine metabolite	Cotinine	15	7.5	7.5	na	na	7.5	7.5	na	na
Herbicide	Terbuthylazine	10	150	155	15	23	122	125	9	70
	Simazine	-	na	na	2.5	1.1	na	na	2	2.1
Aten./Metopr. Metabolite	Atenololic acid	20	10	10	na	na	10	10	na	na
SMX-TP	Desamino-SMX	7.5	3.75	3.75	na	na	3.75	3.75	na	na
	4-Nitro-SMX	5.0	2.5	2.5	na	na	2.5	2.5	na	na
Metamizole metabolite	4-Acetaminoantipyrine	15	3018	3386	na	na	6095	7132	na	na
(Val-)Sartan TP	Valsartan acid	10	256	283	na	na	306	297	na	na
Artif. Sweetener	Acesulfame	5.0	6910	6758	na	na	15476	15735	na	na

Application	Compound	LOQ [ng/l]	05.06.2014 Work. DAY CAS 01	05.06.2014 Work. DAY CAS 02	20.01.2015 Work. DAY CAS 01	15.04.2015 Work. DAY CAS 01	08.06.2014 Weekend CAS 03	08.06.2014 Weekend CAS 04	18.01.2015 Weekend CAS 03	12.04.2015 Weekend CAS 03
	Cyclamate	5.0	598	611	na	na	1651	1643	na	na
Biocide	Irgarol	5.0	2.5	2.5	na	na	2.5	2.5	na	na

na = not analysed; *DEMEAU listed compound

ANNEX 5 Bioassays

Table A-0-8: Activities (ng or µg reference compound equivalent concentration/L water sample) detected in the in vitro bioassays for the MAR water samples from the CASTELLÓN sampling site collected at two time points: 06/2014 (Campaign I) and 04/2015 (Campaign II)

Sample name	Campaign	Cytotox CALUX -	ERα CALUX ng 17ß-estradiol eq./L water	antiAR CALUX ng Flutamide eq./L water	antiPR CALUX ng Ru486 eq./L water	GR CALUX ng Dexamethasone eq./L water	PPARg2 CALUX ng Rosiglitazone eq./L water	p53 CALUX µg Actinomycin D eq./L water	p53 S9 CALUX* µg Cyclophosphamide eq./L water	Nrf2 CALUX µg Curcumine eq./L water	Bacteria luminescence inhibition mg baseline toxicity eq./L water	PSII inhibition** ng Diuron eq./L water	Growth inhibition*** mg baseline toxicity eq./L water
CAS-01 EDAR	I.	-	0.1	10600	<LOQ (0.09)	109.3	LOD (<11.2)	LOD (<0.04)	LOD (<2400)	47	na****	128.8	1.16
	II.	-	3.8	10000	3.4	260.0	450.0	LOD (<0.01)	LOD (<715)	130	0.88	85.0	3.32
CAS-05 BALSA	I.	+	0.1	15700	1.1	< LOD (0.9)	LOD (<11.2)	LOD (<0.04)	LOD (<2400)	25	na	61.8	2.90
	II.	-	1.2	21000	7.0	< LOD (7.6)	LOD (<76)	LOD (<0.01)	LOD (<715)	67	0.23	31.7	0.52
CAS-06 SAN VICENTE	I.	-	LOD (<0.01)	7000	0.2	< LOD (0.9)	LOD (<11.2)	LOD (<0.04)	59500	LOD (<17)	na	420.4	0.29
	II.	-	<LOQ (0.11)	32000	5.3	< LOD (7.6)	LOD (<76)	LOD (<0.01)	LOD (<715)	LOD (<22)	0.10	439.5	2.63
CAS-07 LA PAZ	I.	+	LOD (<0.01)	44400	0.9	< LOD (0.9)	LOD (<11.2)	LOD (<0.04)	LOD (<2400)	<LOQ (13)	na	68.4	1.26
	II.	-	LOD (<0.06)	LOD (<2500)	1.5	< LOD (7.6)	LOD (<76)	LOD (<0.01)	LOD (<715)	LOD (<22)	0.03	69.6	0.43
CAS-08 RANDERO	I.	-	LOD (<0.01)	6500	<LOQ (0.07)	< LOD (0.9)	LOD (<11.2)	LOD (<0.04)	LOD (<2400)	LOD (<17.0)	na	191.5	2.22
	II.	-	LOD (<0.06)	LOD (<2500)	2.9	< LOD (7.6)	LOD (<76)	LOD (<0.01)	LOD (<715)	LOD (<22)	0.04	166.0	0.92
CAS-09 RAMBLETA 2	I.	-	<LOQ (0.01)	13800	0.2	< LOD (0.9)	LOD (<11.2)	LOD (<0.04)	LOD (<2400)	35.4	na	366.5	4.52
	II.	-	LOD (<0.06)	11000	3.0	< LOD (7.6)	LOD (<76)	LOD (<0.01)	LOD (<715)	LOD (<22)	0.09	7.9	0.31
CAS-11 GARROFERA	I.	-	< LOQ (0.02)	4607	0.4	< LOD (0.9)	LOD (<11.2)	LOD (<0.04)	LOD (<2400)	LOD (<17.0)	na	30.7	0.61
	II.	-	LOD (<0.06)	LOD (<2500)	LOD (<0.03)	< LOD (7.6)	LOD (<76)	LOD (<0.01)	LOD (<715)	LOD (<22)	0.06	772.7	3.87
CAS-12 PIEZOMETRO 2	I.	-	<LOQ (0.01)	5200	LOD (<0.03)	< LOD (0.9)	LOD (<11.2)	LOD (<0.04)	LOD (<2400)	LOD (<17.0)	na	408.8	3.59
	II.	-	LOD (<0.06)	LOD (<2500)	2.3	< LOD (7.6)	LOD (<76)	LOD (<0.01)	LOD (<715)	27	0.07	65.5	0.46
Negative Control	I.	-	LOD (<0.01)	< LOQ (242)	0.08	< LOD (0.9)	LOD (<11.2)	LOD (<0.04)	LOD (<2400)	LOD (<17)	na	0.8	0.25
	II.	-	LOD (<0.06)	LOD (<2500)	LOD (<0.03)	< LOD (7.6)	LOD (<76)	LOD (<0.01)	LOD (<715)	LOD (<22)	0.02	0.0	0.00

* Sample extracts were also measured in the p53 CALUX assay with metabolic activation using the S9 enzyme mix
** Photosynthesis inhibition was measured 2 hours after exposure
*** Growth inhibition was measured 24 hours after exposure
**** Not analysed

Table A-0-9: Overview of the individual REP factors available for the target compounds analysed in the MAR water samples from Castellón. REP factors are calculated by the following equation: test comp= EC(x) Reference compound/ EC(x) Test compound

		ERα-CALUX		antiAR-CALUX		antiPR-CALUX		GR-CALUX		PPARγ-CALUX		p53-CALUX		p53 S9-CALUX		Nrf2-CALUX		PS II Inhibition	
		PC10 (M)	REP	PC10 (M)	REP	PC10 (M)	REP	PC10 (M)	REP	PC10 (M)	REP	PC10 (M)	REP	PC10 (M)	REP	PC10 (M)	REP	EC50 (M)	REP
Reference compound		3.2E-12	1.0E+00	3.2E-07	1.0E+00	1.0E-10	1.0E+00	3.2E-10	1.0E+00	1.0E-08	1.0E+00	1.3E-09	1.0E+00	na	na	6.3E-06	1.0E+00	1.8E-08	1.0E+00
DEMEAU	Diclofenac	-	-	1.3E-05	-	3.2E-05	3.2E-06	-	-	2.5E-05	4.0E-04	2.5E-04	5.1E-06	-	-	-	-	na	na
	Phenazone	7.9E-05	4.0E-08	-	-	-	-	-	-	-	-	4.0E-04	3.2E-06	-	-	-	-	na	na
	Metoprolol	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na
	Iopromide	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	na	na
	Sulfamethoxazole	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	na	na
	Trimethoprim	-	-	5.0E-05	6.3E-03	-	-	-	-	-	-	-	-	-	-	-	-	na	na
	Bezafibrate	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na
	Gemfibrozil	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	na	na
	Carbamezapine	-	-	2.0E-05	1.6E-02	3.2E-05	3.2E-06	-	-	-	-	-	-	-	-	-	-	na	na
	Primidone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	na	na
OTHERS	Diuron	-	-	2.5E-06	1.3E-01	-	-	-	-	-	-	7.9E-05	1.6E-05	-	-	-	-	1.8E-08	1.0E+00
	Atrazine	3.2E-05	1.0E-07	3.2E-05	1.0E-02	-	-	-	-	-	-	-	-	-	-	-	-	2.3E-07	8.0E-02
	Simazine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.8E-07	6.6E-02
	Irgarol – Cerbutryn	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.6E-08	1.2E+00
	Tamoxifen	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Isoproturon	-	-	-	-	-	-	-	-	-	-	3.2E-04	4.1E-06	-	-	-	-	1.2E-07	1.5E-01
	Mecoprop	-	-	-	-	-	-	-	-	1.0E-04	1.0E-04	1.0E-03	1.3E-06	-	-	-	-	-	-
	Terbuthylazine	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	5.2E-08	3.5E-01

"na": not available / not analysed

"-": not detected

Table A-0-10:   A and B. Overview of the theoretically expected and actually measured activities (ng or µg reference compound equivalent concentrations / L water) of the tested MAR water samples from Castellón. Expected activities are calculated based on the REP factors of the individual compounds and their actual concentration in the water samples. The magnitude of activity that could be explained by the chemically measured compounds are indicated as explained activity (%).

A		ERα-CALUX			antiAR-CALUX			antiPR-CALUX			GR-CALUX			PPARγ-CALUX		
		Expected	Measured	Explained	Expected	Measured	Explained	Expected	Measured	Explained	Expected	Measured	Explained	Expected	Measured	Explained
		ng EEQ/L water		%	ng Flu-Eq/L water		%	ng Ru486-Eq/L water		%	ng Dex.-Eq/L water		%	ng Rosigl.-Eq/L water		%
CAS-01 EDAR	I.	2.3E-05	1.3E-01	0.02%	2.9E+01	1.1E+04	0.27%	3.5E-03	1.6E+00	0.21%	0.0E+00	1.1E+02	0.0%	3.8E-01	LOD (<11.2)	-
	II.	2.2E-05	3.8E+00	0.0006%	2.1E+01	1.0E+04	0.21%	2.7E-03	LOD (<0.06)	-	0.0E+00	2.6E+02	0.0%	3.1E-01	4.5E+02	0.07%
CAS-05 BALSA	I.	1.1E-07	1.3E-01	0.00008%	1.6E-01	1.6E+04	0.0010%	6.6E-06	1.0E+00	0.0006%	0.0E+00	< LOD (0.9)	-	4.6E-04	LOD (<11.2)	-
	II.	4.0E-08	1.2E+00	0.000003%	2.2E-01	2.1E+04	0.0011%	4.1E-05	-	-	0.0E+00	< LOD (7.6)	-	4.0E-04	LOD (<76)	-
CAS-06 SAN VICENTE	I.	1.2E-06	LOD (<0.01)	-	1.2E+00	7.0E+03	0.017%	1.5E-04	<LOQ(0.03)	0.52%	0.0E+00	< LOD (0.9)	-	8.4E-03	LOD (<11.2)	-
	II.	9.5E-07	1.1E-01	0%	3.2E-01	3.2E+04	0.0010%	6.0E-05	1.5E+00	0.004%	0.0E+00	< LOD (7.6)	-	4.0E-04	LOD (<76)	-
CAS-07 LA PAZ	I.	1.1E-07	LOD (<0.01)	-	6.2E-01	4.4E+04	0.0014%	6.0E-05	LOD (<0.02)	-	0.0E+00	< LOD (0.9)	-	7.2E-03	LOD (<11.2)	-
	II.	2.8E-08	LOD (<0.06)	-	5.0E-02	LOD (<2500)	-	6.6E-06	3.40	0.0002%	0.0E+00	< LOD (7.6)	-	4.0E-04	LOD (<76)	-
CAS-08 RANDERO	I.	1.1E-07	LOD (<0.01)	-	7.3E-01	6.5E+03	0.011%	7.9E-05	1.0E-01	0.08%	0.0E+00	< LOD (0.9)	-	9.6E-03	LOD (<11.2)	-
	II.	4.0E-08	LOD (<0.06)	-	5.0E-02	LOD (<2500)	-	6.6E-06	2.3E+00	0.0003%	0.0E+00	< LOD (7.6)	-	4.0E-04	LOD (<76)	-
CAS-09 RAMBLETA 2	I.	7.2E-07	1.0E-02	0.007%	1.4E+00	1.4E+04	0.010%	5.1E-05	6.0E-02	0.08%	0.0E+00	< LOD (0.9)	-	7.5E-03	LOD (<11.2)	-
	II.	4.0E-08	LOD (<0.06)	-	5.0E-02	1.1E+04	0.0005%	6.6E-06	LOD (<0.14)	-	0.0E+00	< LOD (7.6)	-	4.0E-04	LOD (<76)	-
CAS-11 GARROFERA	I.	7.2E-07	2.0E-02	0.004%	9.4E-01	4.6E+03	0.02%	6.6E-06	2.2E-01	0.003%	0.0E+00	< LOD (0.9)	-	4.6E-04	LOD (<11.2)	-
	II.	4.0E-08	LOD (<0.06)	-	5.0E-02	LOD (<2500)	-	6.6E-06	1.4E+00	0.0005%	0.0E+00	< LOD (7.6)	-	4.0E-04	LOD (<76)	-
CAS-12 PIEZOMETRO 2	I.	1.1E-07	1.0E-02	0.001%	1.6E-01	5.2E+03	0.003%	6.6E-06	LOD (<0.02)	-	0.0E+00	< LOD (0.9)	-	9.1E-04	LOD (<11.2)	-
	II.	7.9E-08	LOD (<0.06)	-	5.0E-02	LOD (<2500)	-	6.6E-06	LOD (<0.06)	-	0.0E+00	< LOD (7.6)	-	4.0E-04	LOD (<76)	-

"-" not to be calculated

B		p53-CALUX			p53 S9-CALUX			Nrf2-CALUX			Bacteria luminescence inhibition			PSII Inhibition		
		Expected	Measured	Explained*	Expected	Measured	Explained	Expected	Measured	Explained	Expected	Measured	Explained	Expected	Measured	Explained
		µg Act D-Eq/L water		%	µg Cychlop.-Eq/L water		%	µg Curcumin-Eq/L water		%	mg TEQ/L water		%	ng Diuron.-Eq/L water		%
CAS-01 EDAR	I.	6.8E-03	LOD (<0.04)	-	0.0E+00	LOD (<2400)	-	0.0E+00	4.7E+01	0	-	-	-	6.1E+01	1.3E+02	47%
	II.	5.7E-03	LOD (<0.01)	-	0.0E+00	LOD (<715)	-	0.0E+00	1.3E+02	0%	na	8.8E-01	-	0.0E+00	8.5E+01	0%
CAS-05 BALSA	I.	2.7E-05	LOD (<0.04)	-	0.0E+00	LOD (<2400)	-	0.0E+00	25.00	0%	-	-	-	2.8E+01	6.2E+01	45%
	II.	8.3E-06	LOD (<0.01)	-	0.0E+00	LOD (<715)	-	0.0E+00	67.00	0%	na	2.3E-01	-	1.5E+01	3.2E+01	48%
CAS-06 SAN VICENTE	I.	2.2E-04	LOD (<0.04)	-	0.0E+00	6.0E+04	0%	0.0E+00	LOD (<17)	-	-	-	-	7.9E+00	4.2E+02	2%
	II.	8.2E-05	LOD (<0.01)	-	0.0E+00	LOD (<715)	-	0.0E+00	LOD (<22)	-	na	1.0E-01	-	3.8E+00	4.4E+02	1%
CAS-07 LA PAZ	I.	1.1E-04	LOD (<0.04)	-	0.0E+00	LOD (<2400)	-	0.0E+00	<LOQ (13)	0%	-	-	-	8.9E+00	6.8E+01	13%
	II.	8.3E-06	LOD (<0.01)	-	0.0E+00	LOD (<715)	-	0.0E+00	LOD (<22)	-	na	0.03	-	2.1E+00	69.65	3%
CAS-08 RANDERO	I.	1.4E-04	LOD (<0.04)	-	0.0E+00	LOD (<2400)	-	0.0E+00	LOD (<17.0)	-	-	-	-	2.0E+00	1.9E+02	1%
	II.	8.3E-06	LOD (<0.01)	-	0.0E+00	LOD (<715)	-	0.0E+00	LOD (<22)	-	na	3.9E-02	-	9.0E-01	1.7E+02	1%
CAS-09 RAMBLETA 2	I.	2.8E-04	LOD (<0.04)	-	0.0E+00	LOD (<2400)	-	0.0E+00	35.40	0%	-	-	-	1.4E+01	3.7E+02	4%
	II.	8.3E-06	LOD (<0.01)	-	0.0E+00	LOD (<715)	-	0.0E+00	LOD (<22)	-	na	9.3E-02	-	1.5E+01	7.9E+00	19%
CAS-11 GARROFERA	I.	1.3E-04	LOD (<0.04)	-	0.0E+00	LOD (<2400)	-	0.0E+00	LOD (<17.0)	-	-	-	-	1.6E+01	3.1E+01	52%
	II.	8.3E-06	LOD (<0.01)	-	0.0E+00	LOD (<715)	-	0.0E+00	LOD (<22)	-	na	6.4E-02	-	3.1E+00	7.7E+00	0%
CAS-12 PIEZOMETRO 2	I.	4.4E-05	LOD (<0.04)	-	0.0E+00	LOD (<2400)	-	0.0E+00	LOD (<17.0)	-	-	-	-	4.1E+00	4.1E+02	1%
	II.	1.2E-05	LOD (<0.01)	-	0.0E+00	LOD (<715)	-	0.0E+00	27.00	0%	na	7.2E-02	-	1.3E+00	6.6E+01	2%

"-" not to be calculated

*Expected activities here are way below the detection limit (LOD) of the assay; therefore explained activity share is not to be calculated