Pilot call 2013
WatEUr

-

Funded projects Final Presentations

-

Water JPI

FRAME

FRAME

A novel framework to assess and manage **contaminants of emerging concern** in **indirect potable reuse**

Kevin S. Jewell, Jörg E. Drewes, Christian Dietrich, Uwe Hübner, Stefano Polesello, Giuseppe Mascolo, Mario Carere, Kevin V. Thomas, Daniel Pierre, Wolfram Kloppmann, Marie Pettenati, Géraldine Pico-Colbeaux, Nina Hermes, Johann Müller, Sapia Murgolo, Sara Valsecchi, Axel Aurouet, Loïc Thomas, Aurore Hertout, Karina Petersen, Soňa Fajnorova, Meriam Muntau, Saer Samanipour, Ailbhe L. Macken, Thomas A. Ternes

Rationale

- Overexploitation of water resources calls for increased application of indirect potable reuse (IPR)
- Management strategies for IPR in the European context are currently lacking

Aims

- Develop new strategies to manage CECs and pathogens in IPR for drinking water augmentation
- Overall evaluation procedure for IPR

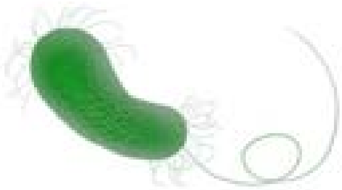
FRAME Handbook

Chapters:

- Regulation, guidelines – EU, USA, WHO, …
- Evaluation scheme – Indicator parameters
- Monitoring – Methods, examples
- Treatment barriers – Innovative solutions
- Decision support – Software

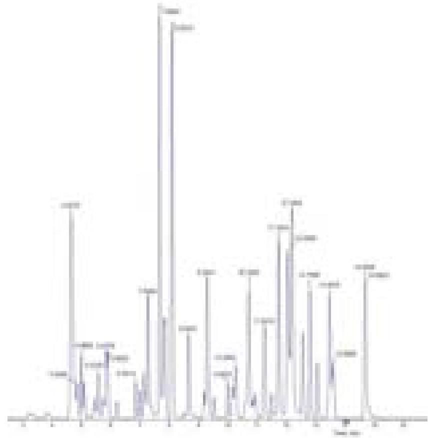
Analysis methods

- Biological contaminants
  - FIBs
    - Escherichia coli and total coliform bacteria – ISO 9308-2:2012
    - Pseudomonas aeruginosa – EN ISO 16266:2008
    - Enterococci – DIN EN ISO 7899-2
  - Antibiotic resistant bacteria, resistance genes
    - Ampicillin
    - Imipenem
    - Vancomycin
    - Erythromycin
    - Sulfamethoxazole

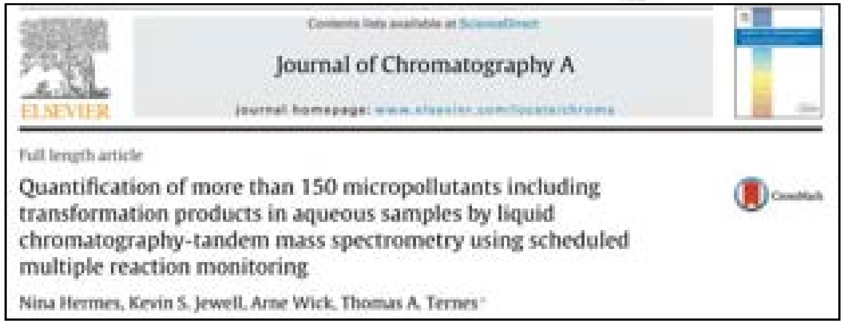

Wikimedia-commons


Hampton T. (2013)

Analysis methods



- Chemical contaminants
  - Target analysis methods
    - 166 CECs incl. 12 PFAS,
      70 transformation products
      (biological, ozone)
    - **Three** multi-residue methods
  - Non-target analysis



Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

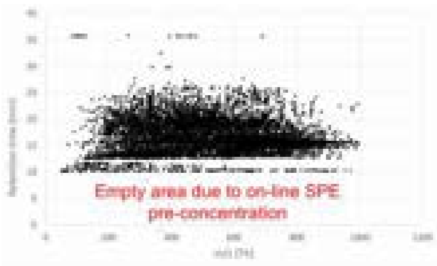
ELSEVIER

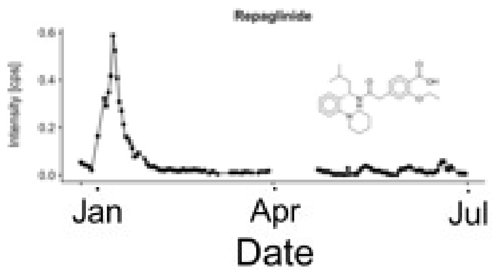
Full length article

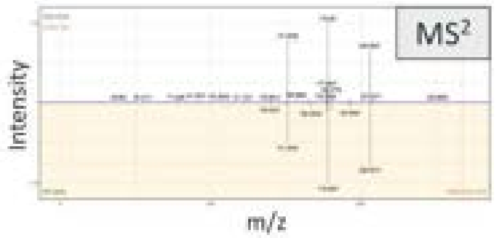
Quantification of more than 150 micropollutants including transformation products in aqueous samples by liquid chromatography-tandem mass spectrometry using scheduled multiple reaction monitoring

Nina Hermes, Kevin S. Jewell, Arne Wick, Thomas A. Ternes

Hermes et al. 2018



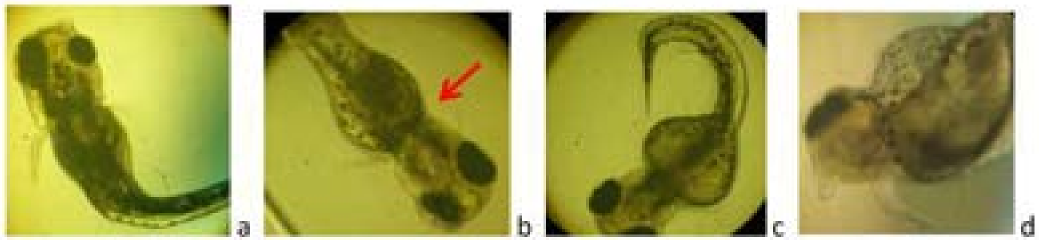




Analysis methods

- Chemical contaminants (cont.)
  - Effect-based monitoring *in vitro*
    - Ames test – mutagenic/carcinogenic activity
    - GeneBLAzer® – endocrine activity

  - Effect-based monitoring *in vivo*



Evaluation scheme

- Point of compliance:

P.O.C.

WWTP → Advanced treatment → Environ. buffer → Water works

Health-based

Performance-based

- Threshold limits
  - CECs
  - Pathogens
- Effect-based
  - Zebrafish embryo test (lethal)

Below threshold

- CEC removal: Comparative treatment process evaluation incl. TPs
- Treatment targets for pathogens
- Effect-based
  - Zebrafish embryo test (sub-lethal)
  - Receptor assays (*in vitro* effects)

Evaluation scheme

- Selection of CECs for health-based targets
  - Cat. A: Health-related compounds (e.g. GOW of UBA)
  - Cat. B: Health-related compounds WFD
  - Cat. C: Ozonation by-products

Example of Cat. B:

Name	Type or use	Regulatory values	IPR
Terbutryn	Biocide	0.065 µg/L (WFD)	< 65 ng/L
Isoproturon & …	Herbicide	0.1 µg/L (DWD 98/83/EC)	< 100 ng/L
PFOS	Industrial	0.65 ng/L (WFD)	< 0.65 ng/L
PFOA	Industrial	0.1 µg/L (proposal DWD revision)	< 100 ng/L
PFASs – Total	Industrial	0.5 µg/L (proposal DWD revision)	< 500 ng/L

Monitoring studies

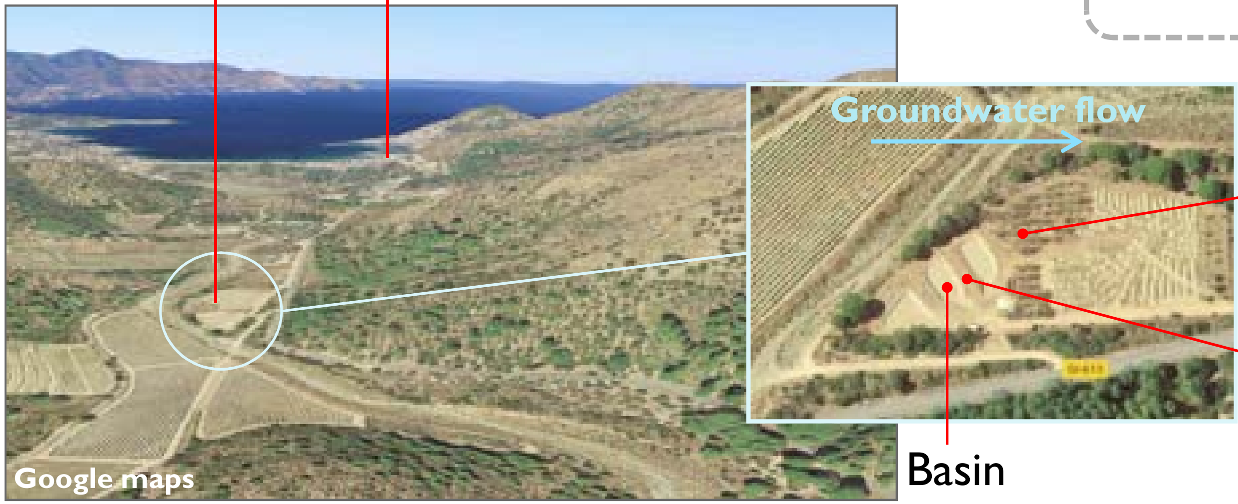
- El Port de la Selva, Spain – Indirect Potable Reuse groundwater augmentation, prevent sea-water intrusion

Infiltration basins

Town, WWTP

WWTP

Secondary effluent → UV → SAT



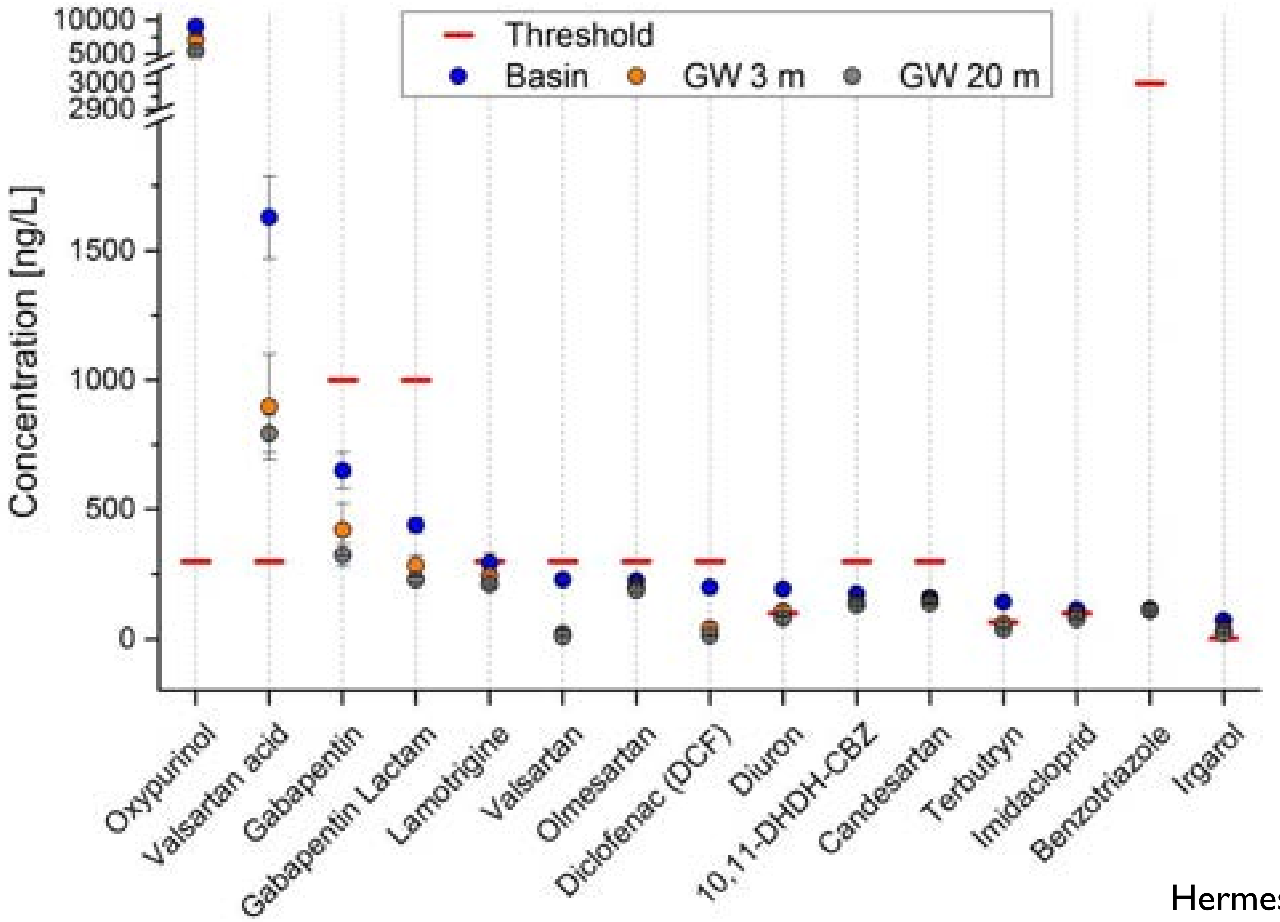
Sampling well 2 (20 m)

Sampling well 1 (3 m)

Basin

Monitoring studies

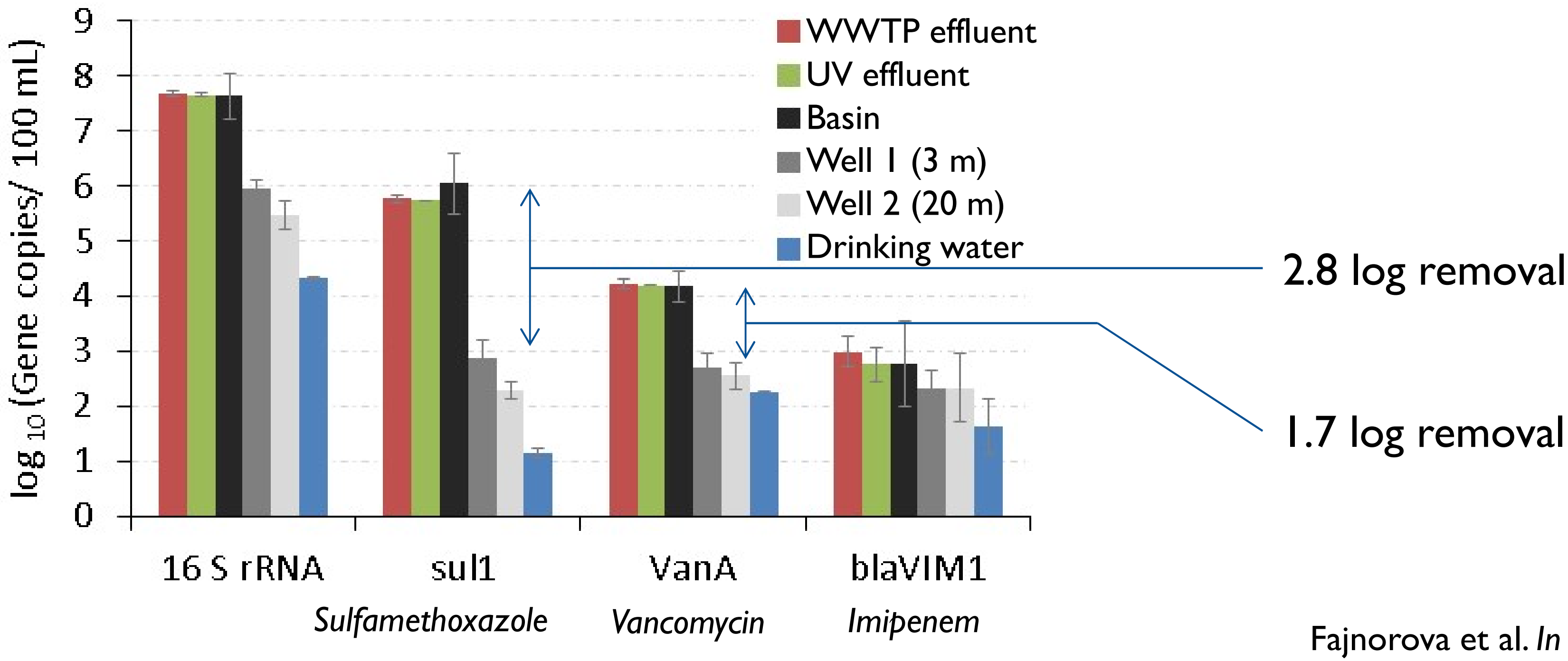
- CEC removal measurements



Hermes et al. *in prep.*

Monitoring studies

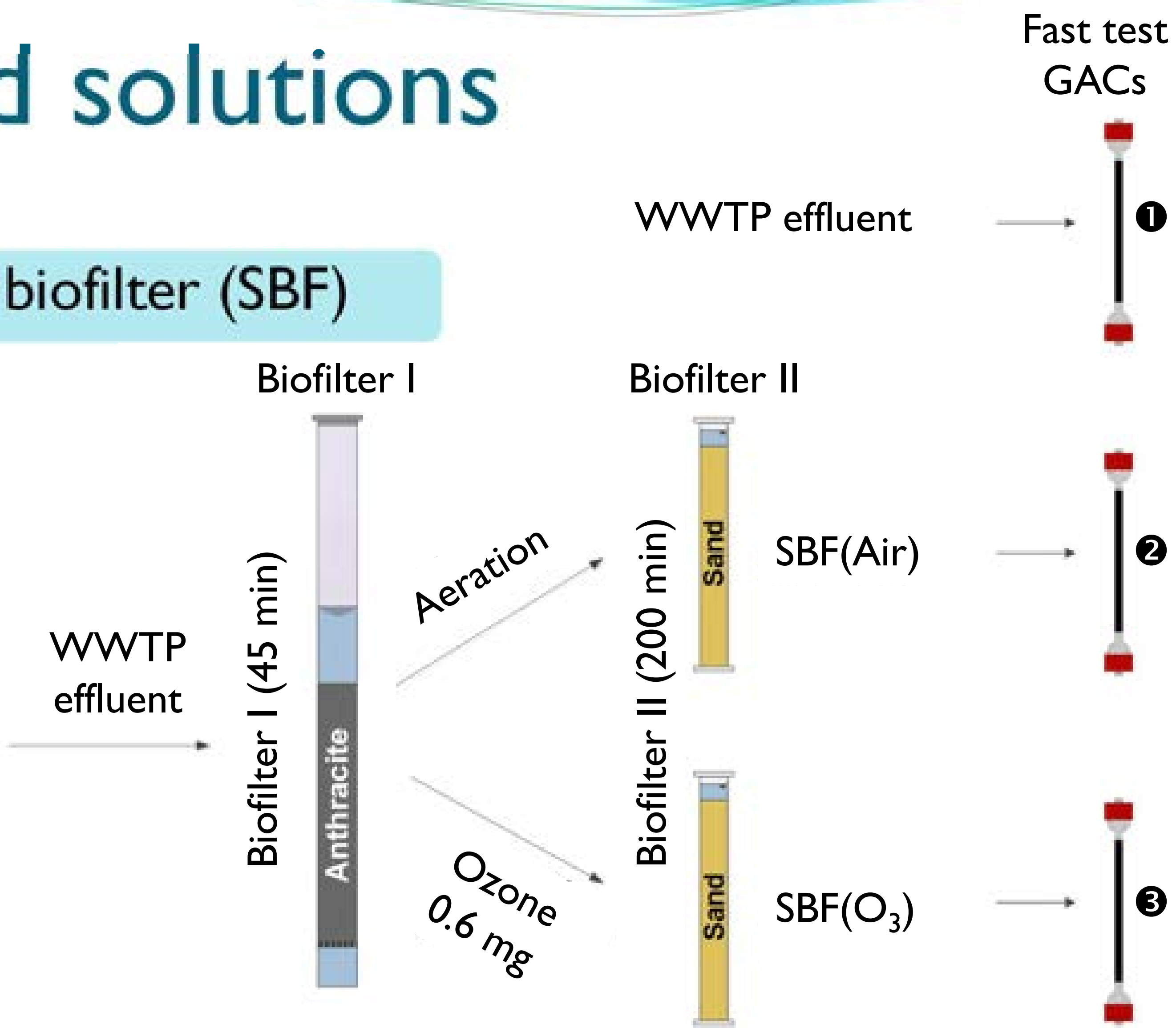
- Antibiotic resistance: removal of A.R. genes



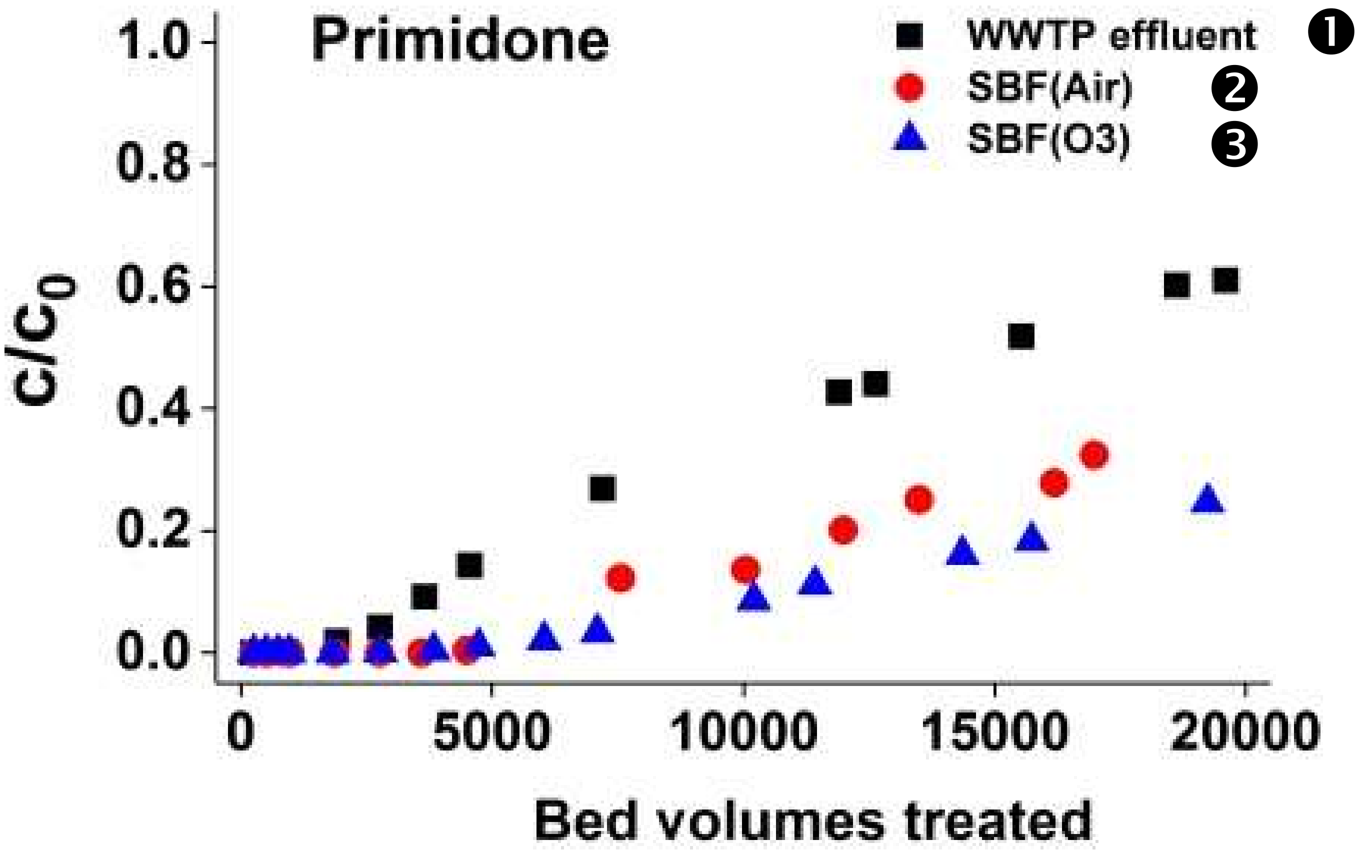
Fajnorova et al. *In prep.*

Advanced solutions

- Hybrid sequential biofilter (SBF)
  - Biofilter I (short)
  - Biofilter II (long)
  - Interstitial aeration or ozonation
  - Post-column GAC



Advanced solutions



Müller et al. *In prep.*

Conclusion, outlook

- Take home messages:
  - **Mitigation:** Multi-barrier systems (Ozone, Activated Carbon, Soil passage) are the most effective options[1]
  - **Evaluation/control:** A small range of indicator substances and pathogens, supplemented with effect-based measures
  - **Modelling/decision support:** Useful tool to assist process understanding and decision making
    - Link: [http://www.geo-hyd.net/install/Frame_DSS]

[1] cf. Muntau et al. *Sci. Tot. Environ.* 2017

Stakeholder engagement

- Input/consultation in the development of the evaluation strategy
  - Point of compliance & regulatory values

- Final workshop included presentations and discussion sessions with stakeholders from Europe and overseas
  - Panel discussions
  - Outputs fed into FRAME Handbook

Impact and knowledge output

- Handbook tailored to decision-makers
- Sensitive multi-residue analytical methods
- Impact on monitoring and evaluation strategies, EU policy implementation and future research
- CEC mitigation strategies, e.g. SMART concept

Collaboration, coordination, mobility, synergies

- Six exchange visits within the consortium
- Nine consortium or bilateral meetings at different institutes
- Links gained between all groups of the FRAME consortium, e.g. treatment design and CEC analysis
- Most institutes are continuing to work together

Future work

- Linking effect-based results with responsible contaminants
- Development of non-target strategies for contaminant prioritization
- Up-scaling of pilot-scale technical solutions, e.g. SMART system
- Further development of decision support tools and online resources

Acknowledgement

- Representatives at the field sites
- Partners, institutions
- www.frame-project.eu

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Bundesministerium für Bildung und Forschung

ONEMA

Office national de l'eau et des milieux aquatiques

The Research Council of Norway

MINISTERO DELL'ISTRUZIONE, DELL'UNIVERSITÀ E DELLA RICERCA

Last slide

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Laboratory
of **Viruses** Contaminants
of **Water** and **Food**

Emerging viruses in irrigation waters

Rusiñol M., Hundesa A., Fernández-Cassi X., Martínez-Puchol S., Timoneda N., Abril J.F., Bofill-Mas S., Girones R

The 2nd Water JPI Conference
Emerging pollutants in freshwater ecosystems
6-7th of June 2018, Helsinki

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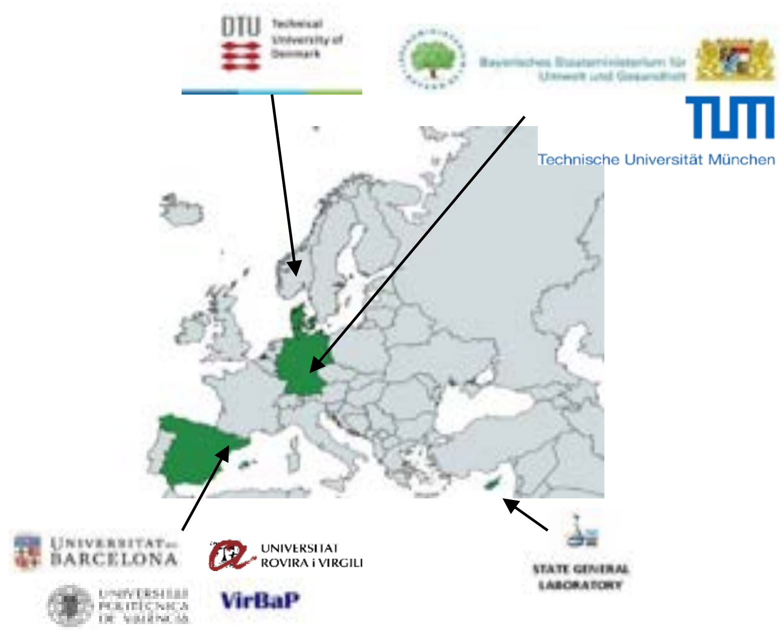
Metawater

New Metagenomics and molecular based tools for European scale identification and control of emergent microbial contaminants in irrigation water



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Do we have methods for quantification of viruses?

Journal of Microbiological Methods 134 (2017) 46–53

Contents lists available at ScienceDirect

Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmeth

Characterization of the efficiency and uncertainty of skimmed milk flocculation for the simultaneous concentration and quantification of water-borne viruses, bacteria and protozoa

Eloy Gonzales-Gustavson [a], Yexenia Cárdenas-Youngs [a], Miquel Calvo [a], Marcelle Figueira Marques da Silva [a], Ayalkibet Hundesa [a], Inmaculada Amorós [b], Yolanda Moreno [b], Laura Moreno-Mesonero [b], Rosa Rosell [c], Llilianne Ganges [c], Rosa Araujo [a], Rosina Girones [a,]*

[a] Department of Genetics, Microbiology and Statistics, Faculty of Biology, University of Barcelona, Av. Diagonal 643, 08028 Barcelona, Catalonia, Spain

- Evaluate the aplicability of SMF for the simultaneous concentration of viruses, bacteria and protozoa in water.
- Determine the efficacy of the SMF recovery
- Compare q(RT)PCR and infectivity assays for viruses
- Define variability and uncertainty values of the method to use them in QMRA studies.

Virus recovery by qPCR and Infectivity quantification

Virus	Method	% Recovery			
		Mean %	CI 95% of mean	min	max
HAdV	qPCR	66	53.5 - 78.5	32.2	86.7
	IFA	58.7	4.5 - 100	8.1	49.8
MS2	q(RT)PCR	23.9	19.6 - 28.1	13.8	36.8
	PFU	11.9	9 - 14.7	9.5	13.9
RoV	q(RT)PCR	28.2	25.6 - 30.7	16	37.1
	TCID50	26.1	17.1 - 35.1	43.5	83.7
BVDV	q(RT)PCR	14.7	10.8 - 18.7	12.9	15.8
	TCID50	0.7	0.4 -1.1	0.67	0.89

Bacteria and protozoa recovery with qPCR and IFA quantification

	Method	% Recovery			
		Mean %	CI 95% of mean	min	max
E. coli	qPCR	59.6	40.3 - 79	15.6	98.7
H. pylori	qPCR	30.2	24.4 - 36.1	20.8	41.5
A. castellanii	qPCR	20.5	14.9 - 26.1	13	32.1
G. lamblia	IFA	17.8	15 - 20.7	12.8	21.5
C. parvum	IFA	12.8	12.5 - 15.2	9.6	17.4

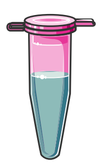
Protocol for the treatment of water samples for metagenomics

Acidified skimmed milk solution into preconditioned sample to flocculate viral particles

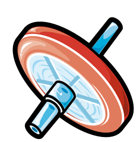
Stirring during 8h

Centrifugation of the sediment 8000 *xg*

Viral concentrate in 4mL



0,45 µm Filtration step



DNAse treatment

280 µl QIAGEN Nucleic acid Extraction

Eluted DNA/RNA in 60 µl

Eluted DNA/RNA

RT SuperScript III using primer A

Second strand synthesis with Sequenase 2.0

25 cycles PCR with primer B

Zymo concentration

QUBIT dsDNA quantification

Nextera XT

Illumina Mi-Seq

NGS and molecular techniques for the analysis of emerging bacteria, protozoa and viruses in irrigation water

One year sampling, 72 irrigation water samples + 12 sewage +12 secondary treated effluents

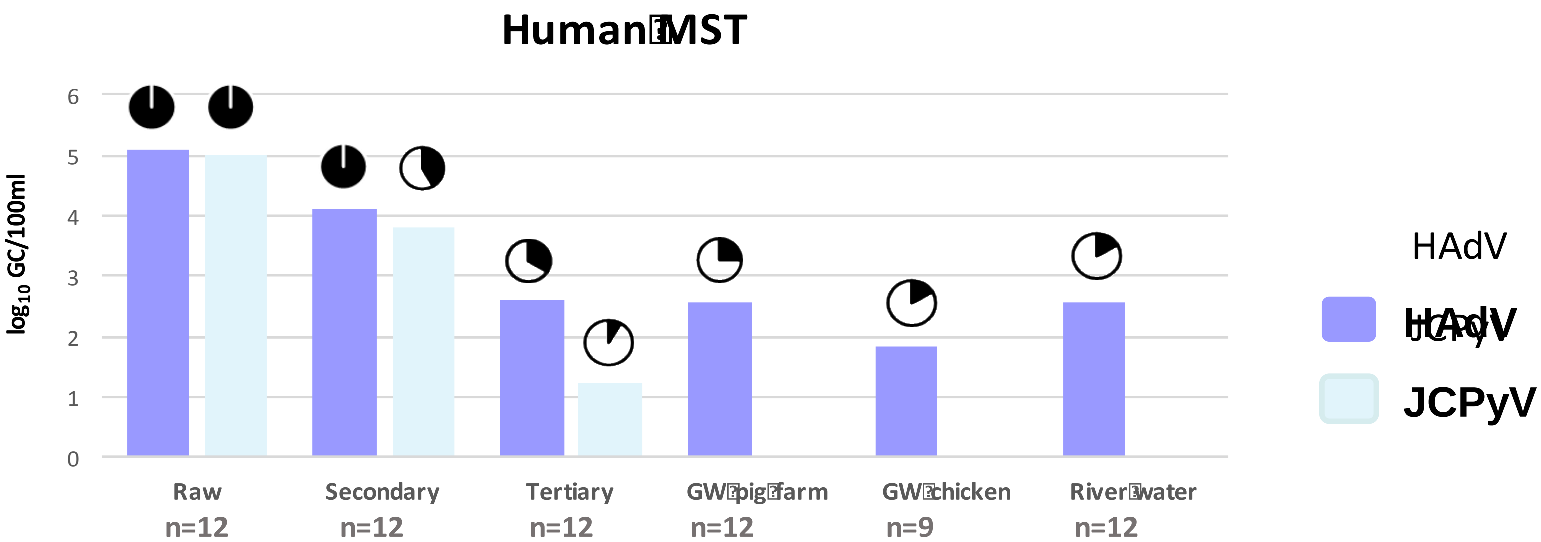
Conventional irrigation water sources

Drinking water tank (DW) and Sediment of the DW tank
Reservoir water
Reservoir water for orchard irrigation
Growndwater
River water

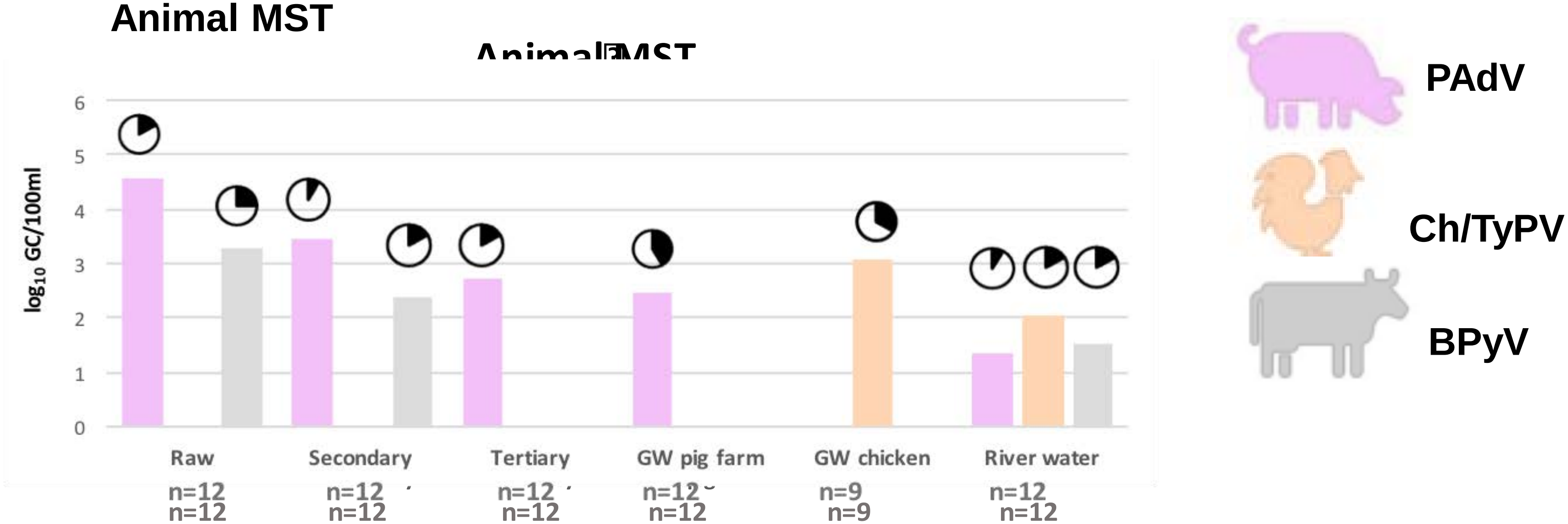
Wastewater and recycled water

Raw sewage
Secondary treated effluent (CAS)
Wetland effluent (tertiary treatment)
Wetland treated effluent (Filtration+Cl_2)

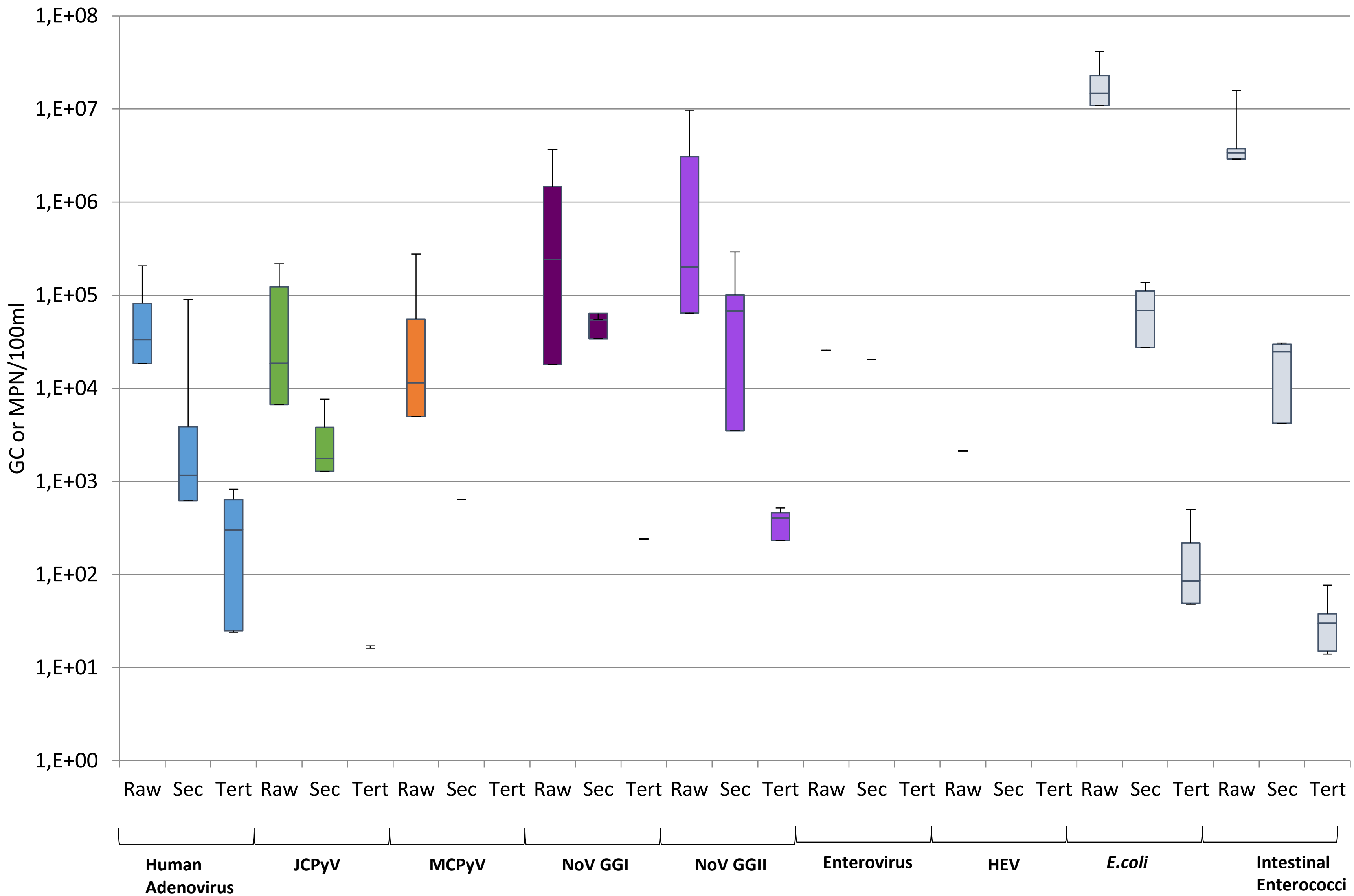
MST markers as irrigation water management tools



Animal MST



qPCR results in raw sewage, secondary and tertiary treated effluents (wetland)



Microbial removal efficiency in wastewater treatment plants and risk assessment studies

Quantitative Microbial Risk Assessment to estimate the health risk associated with the ingestion of lettuce irrigated with tertiary effluents from 2 WWTPs

WWTP	Virus	Observed	To reach 10^{-6} DALYs
1 UV, chlorin. Actiflo®	HAdV	2.5	5.6
	NoV GII	1.9	7
2 Wetlands	HAdV	2.8	5.1
	NoV GII	3.9	6.7

Mean of the best fit distributions of reductions in tertiary effluent by each virus in actual scenario and required reductions to reach suggestions of WHO (10^{-6} DALYs).

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		Distribution 6M	Reservoir 6M	Groundwater SP	Groundwater SU	Groundwater AU	Groundwater WI	River water SP	River water SU	River water AU	River water WI	Wetland (Reclaimed) SP	Wetland (Reclaimed) SU	Wetland (Reclaimed) AU	Wetland (Reclaimed) WI	Raw sewage SP	Raw sewage SU	Raw sewage AU	Raw sewage WI
Vertebrate	Anelloviridae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	Adenoviridae	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	3	1	0
	Caliciviridae	0	0	0	0	0	0	0	0	1	0	0	0	0	0	4	3	5	4
	Papillomaviridae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
	Picornaviridae	0	1	0	0	1	1	0	0	3	1	0	2	1	1	17	11	11	11
	Circoviridae	0	3	1	6	0	0	4	0	5	3	3	7	2	3	9	12	14	5
	Astroviridae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	4	8	4
	Hepeviridae	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Bacteria	Microviridae	2	6	5	7	2	1	12	7	8	8	7	9	9	9	41	25	36	46
	Podoviridae	6	12	12	15	2	0	25	6	7	6	13	11	4	3	14	86	33	53
	Myoviridae	10	25	19	33	11	8	31	17	16	21	23	25	7	4	49	103	51	144
	Siphoviridae	15	38	15	40	11	12	38	16	44	34	50	33	16	16	39	167	85	172
	Inoviridae	4	1	3	4	1	0	2	3	2	2	2	2	2	0	4	4	1	3
	Leviviridae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	2
	Picobirnaviridae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	6	5	8
Fungi	Alphaflexiviridae	0	0	0	0	1	0	0	0	0	0	0	0	0	0	2	6	2	1
	Partitiviridae	0	0	0	1	0	0	4	0	1	0	0	0	0	0	2	0	1	5
	Betaflexiviridae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	2
Plants	Secoviridae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	1	1
	Potyviridae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	2	0
	Closteroviridae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	3	2	1
	Tymoviridae	0	0	0	0	0	0	0	0	0	1	0	0	0	0	2	2	1	3
	Virgaviridae	0	4	0	3	1	1	1	1	14	11	0	4	8	8	15	16	14	9
	Tombusviridae	0	2	0	9	0	0	3	5	20	17	0	0	1	1	8	9	9	2
	Bromoviridae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
	Geminiviridae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
	Luteoviridae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Alga	Phycodnaviridae	2	11	4	9	1	1	11	5	2	7	4	4	2	1	0	3	2	0
Amoeba	Mimiviridae	0	9	1	3	0	0	4	2	1	1	1	0	1	0	0	1	0	0
Invertebrate	Dicistroviridae	0	0	0	1	0	0	0	0	5	2	0	3	3	2	3	4	2	1
Multiple host	Iridoviridae	0	8	1	1	0	0	2	0	4	4	8	4	0	3	0	0	1	1
	Nodaviridae	0	0	0	0	0	0	0	0	2	1	0	0	0	0	1	1	1	4
	Parvoviridae	0	9	2	2	0	0	4	0	4	3	2	2	1	7	11	20	20	17

Counts: 160, 120, 80, 40

Average of 1,2 million reads per sample

Main conclusions and recommendations

1. Concentration protocols for the efficient (low-cost) quantification of pathogens in water are useful for improving QMRA studies to facilitate evidence based decisions for water safety management.

Recommendation: There is a need of implementing highly advanced sewage treatments (such as MBR) or additional disinfection treatments to achieve acceptable microbial quality for water reuse in irrigation of fresh vegetables

2. Analysis of the virome using metagenomics in raw sewage has shown the presence important human pathogens including emerging strains of both RNA and DNA viruses

Recommendation: Implementation of metagenomic public health surveillance systems using NGS techniques in urban sewage as a valuable data base with information of the circulating viruses in the population.

Xavier Fernández Cassi
Ayalkibet Hundesa
Natàlia Timoneda
Aiora Areguita
Eloy Gonzales Gustavson
David Aguado
Sandra Martínez Puchol
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http://www.ub.edu/microbiologia_virology/en

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MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD

Laboratory
of **Viruses** Contaminants
of **Water** and **Food**

MOTREM

MOTREM

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Pedro Cano (BRUKER)

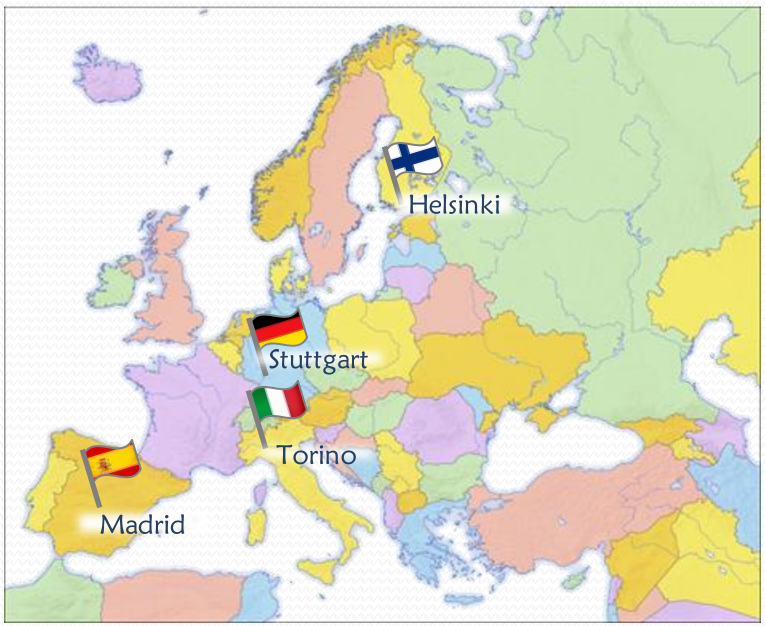
Water JPI
Pilot Call Final Meeting
4th of June 2018, Helsinki

MOTREM

www.motrem.eu

Integrated Processes for Monitoring and Treatment of Emerging Contaminants for Water Reuse

URJC – Universidad Rey Juan Carlos (Spain)
UST – Universität Stuttgart (Germany).
UH – University of Helsinki (Finland).
UNITO – Università di Torino (Italy).
FCC Aqualia S.A. (Spain).
Bruker Española, S.A. (Spain).



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UNIVERSITY OF HELSINKI
ACADEMY OF FINLAND
UNIVERSITÀ DEGLI STUDI DI TORINO
MINISTERO DELL'ISTRUZIONE, DELL'UNIVERSITÀ E DELLA RICERCA
FCC Aqualia
CDTI Centro para el Desarrollo Tecnológico Industrial
BRUKER
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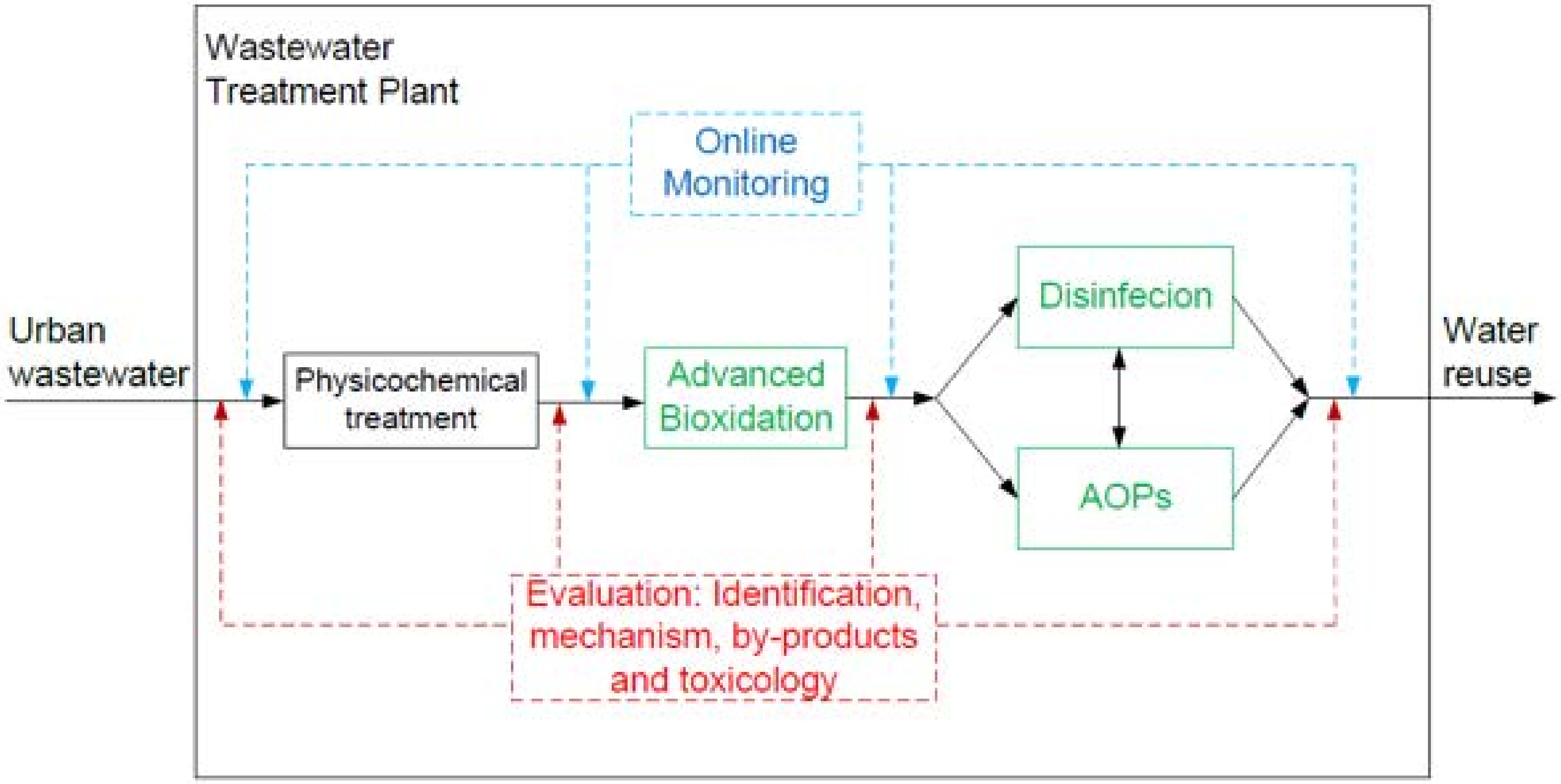
Integrated Processes for MOnitoring and TReatment of EMerging Contaminants for Water Reuse – Conceptual Diagram



MOTREM Work-Packages

WORK PACKAGES

Project execution is structured in 5 work packages:

WP1 Development of New Treatment Technologies (*URJC*)

WP2 Development of New Monitoring Technologies (*UST*)

WP3 Emerging Contaminants Evaluation (*UH*)

WP4 Dissemination and Exploitation of Project Outcome (*AQUALIA*)

WP5 Project Coordination and Management (*URJC*)

Scientific and technological results

Main topics:

a) **Optimised biotreatment processes** with enhanced efficiency in the removal of CECs based on the incorporation of specific microorganisms.

b) **Optimised disinfection technologies and AOPs** able to deal simultaneously with the inactivation of pathogenic microorganisms and CECs before water reuse or discharge to the environment.

c) **Optimised technologies for the monitoring** of the WWTP operation regarding the removal of CECs, including analytical procedures and measurements of integrative parameters.

d) **Identification of the most representative CECs** for the evaluation and monitoring of the efficiency of the water treatment processes, including its degradation mechanism and toxicology.

Scientific and technological progress

WP3 – Emerging Contaminants Evaluation

Selection of representative ECs

Representative micropollutants for monitoring in a municipal WWTP were selected. The final list included 10 target compounds (20 as an extended list) that were chosen based on:

- Current and forthcoming legislation,
- Frequency of occurrence in municipal WWTP,
- Expected concentration levels,
- Elimination potential in conventional and advanced treatment.
- Analytical feasibility

The short list comprises:

Chemical	CAS	Acronym	Reason
Atrazine	1912-24-9	**ATZ**	Target
Caffeine	58-08-2	**CFN**	Indicator
Carbamazepine	298-46-4	**CBZ**	Indicator/Target
Diclofenac	15307-79-6	**DCF**	Target
Estron	53-16-7	**EST**	Target
Ibuprofen	51146-56-6	**IBP**	Indicator
Simazine	122-34-9	**SMZ**	Target
Sucralose	56038-13-2	**SCL**	Indicator
Sulfamethoxazole	723-46-6	**SMX**	Target
Triclosan	3380-34-5	**TCS**	Indicator/Target

The extended list additionally comprises:

Chemical	CAS	Acronym
Metoprolol	51384-51-1	**MTP**
Iopamidol	60166-93-0	**IPM**
HHCB (Galaxolide)	1222-05-5	**HHCB**
HHCB-Lactone (Galaxolidone)	N/A	**HHCB-L**
DEET	134-62-3	**DEET**
Terbutryn	886-50-0	**TBT**
Bisphenol A	80-05-7	**BPA**
Tris-chloroethyl-phosphate (TCEP)	115-96-8	**TCEP**
Perfluorooctanic acid (PFOA)	335-67-1	**PFOA**
Acesulfame K	55589-62-3	**ACF**

LCT Standard Analytical Method

Complete analytical method for the rigorous determination of these substances based on:

- Standard extractions cartridges.
- Use of isotopically labelled internal standards.
- GC-MS/MS, LC-MS/MS, LC-TOF/MS analytical equipment.

LC

- Column: Waters Acquity UPLC HSS T3 1,8µm, 2,1x100mm
- Eluent A: 5% MeOH / H_2O + 0,1% Formic acid
- Eluent B: 100% MeOH + 0,1% Formic acid
- Flow: 0,2 ml/min
- Gradient, 22 min:
  - 1 min 100% A
  - 10 min 100%A -> 100% B
  - 8 min 100 % B
  - 3 min 100% A
- Injection volume: 20 µl

TOF

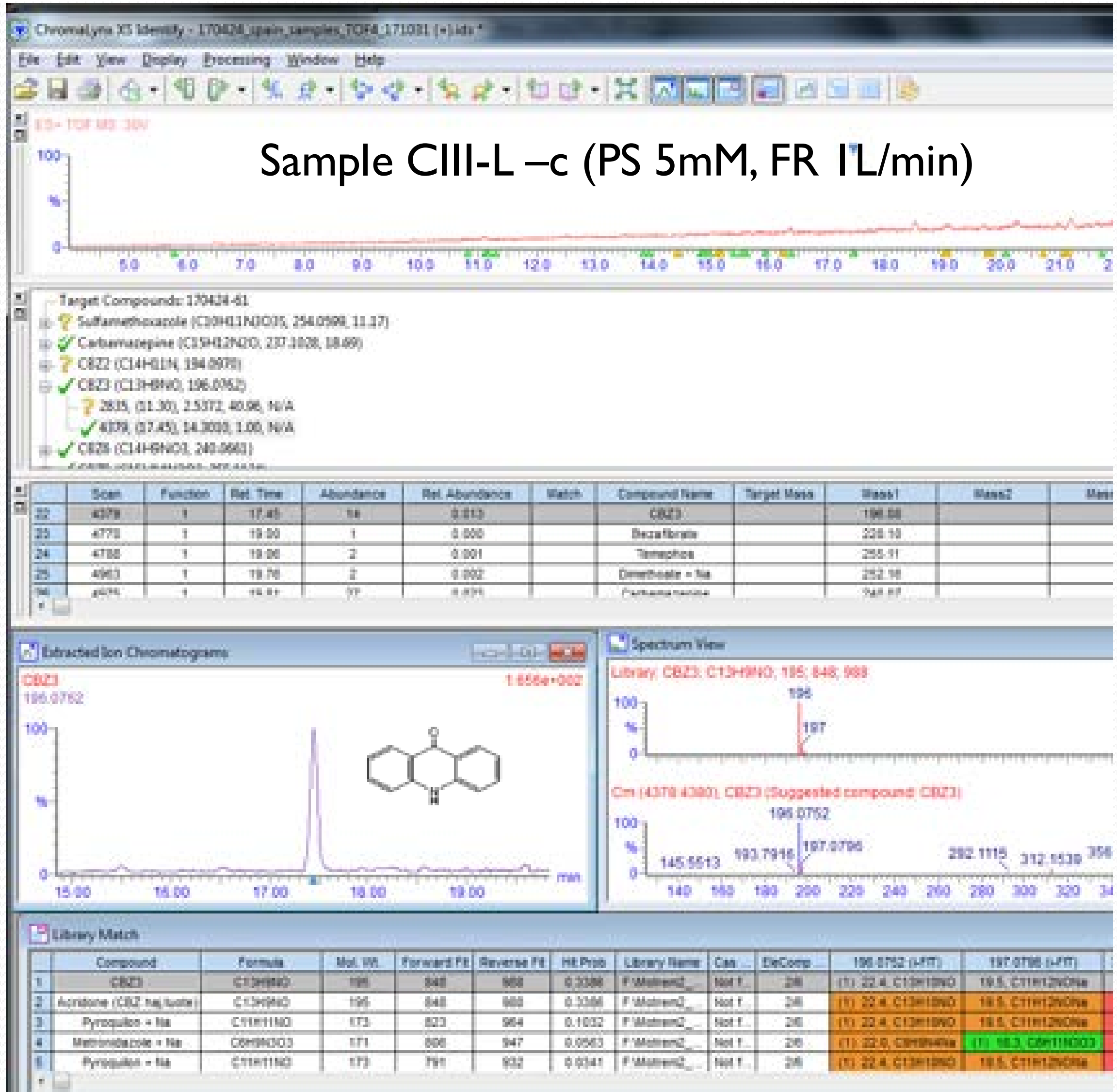
- Scan range: 60-1000 m/z
- 0,9 sec scan time, interscan delay 0,1 s
- Separate runs for ESI + and -
- ESI+: Caffeine, Simazine, Carbamazepine, Atrazine, Estrone
- ESI-: Sulfamethoxazole, Sucralose, Diclofenac, Triclosan, Ibuprofen

LCT Standard Analytical Method: Validation Data

	ESI(+)	ESI(-)	
	LOQ, ng/l	LOQ, ng/l	Linear range up to, ng/l
Caffeine	6.3		6000
Sulfamethoxazole	1.7	14	8000
Sucralose Na-adduct	17		4000
Simazine	0.3		100
Carbamazepine	1.3		4000
Atrazine	0.4		800
Estrone	4.4		800
Diclofenac	3.0		8000
Sucralose		48	8000
Diclofenac		2.6	8000
Ibuprofen		14	8000
Triclosan		8.5	200

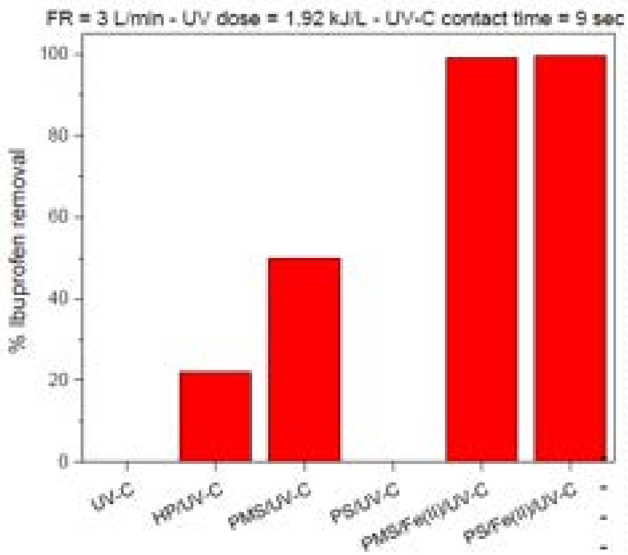
Post target LC-TOF

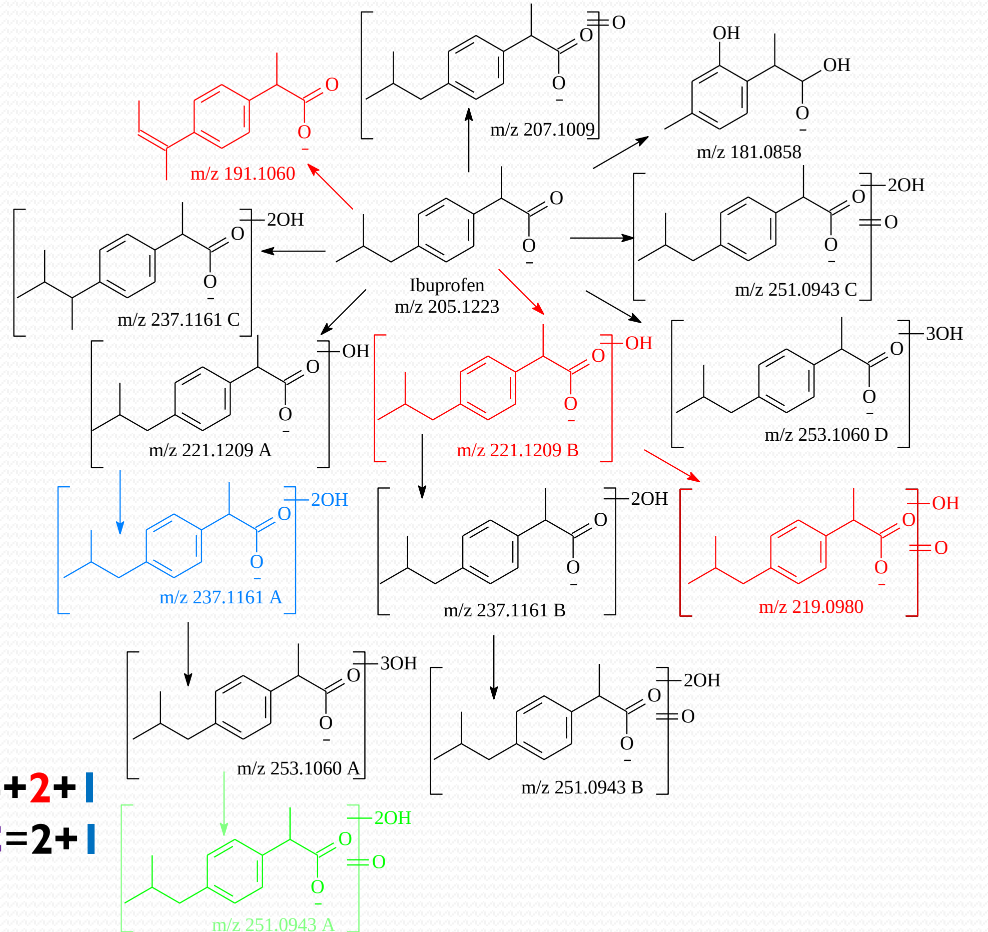
- A list of 30 possible transformation products of SMX, CBZ, and DCF was compiled using literature information
- These compounds were screened from the Spanish wastewater samples



Degradation intermediates

Example: the case of ibuprofen





TPs number vs treatments

HP/UV-C=9 PMS/UV-C=3+3 PS/UV-C= 4+2+1

PMS/Fe(II)/UV-C=3+2+1 PS/Fe(II)/UV-C=2+1

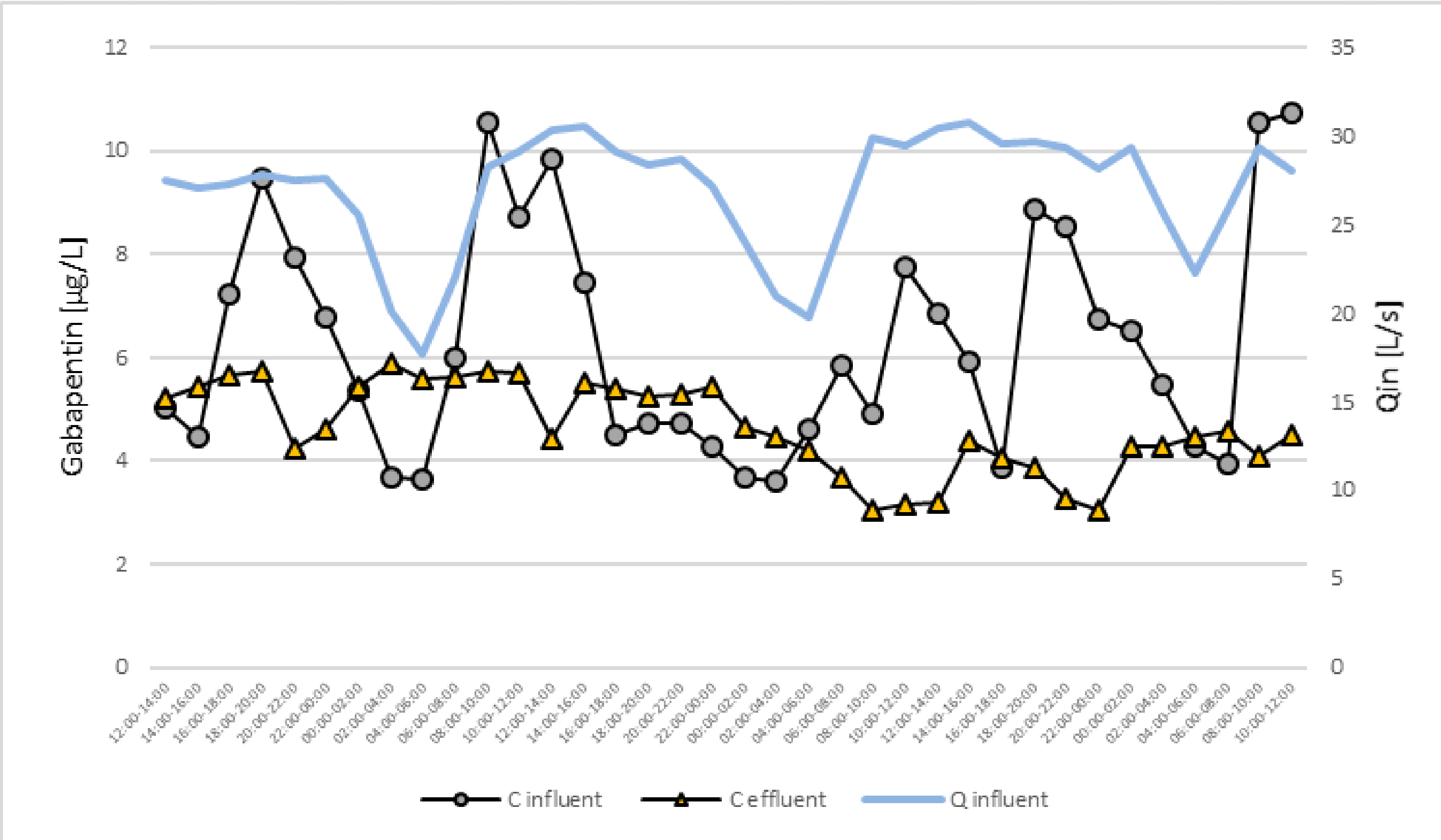
- ✓ 14 different TPs
- ✓ Some of them are common to several treatments.
- ✓ Other ones are specific to the treatment (green, blue)

Scientific and technological progress

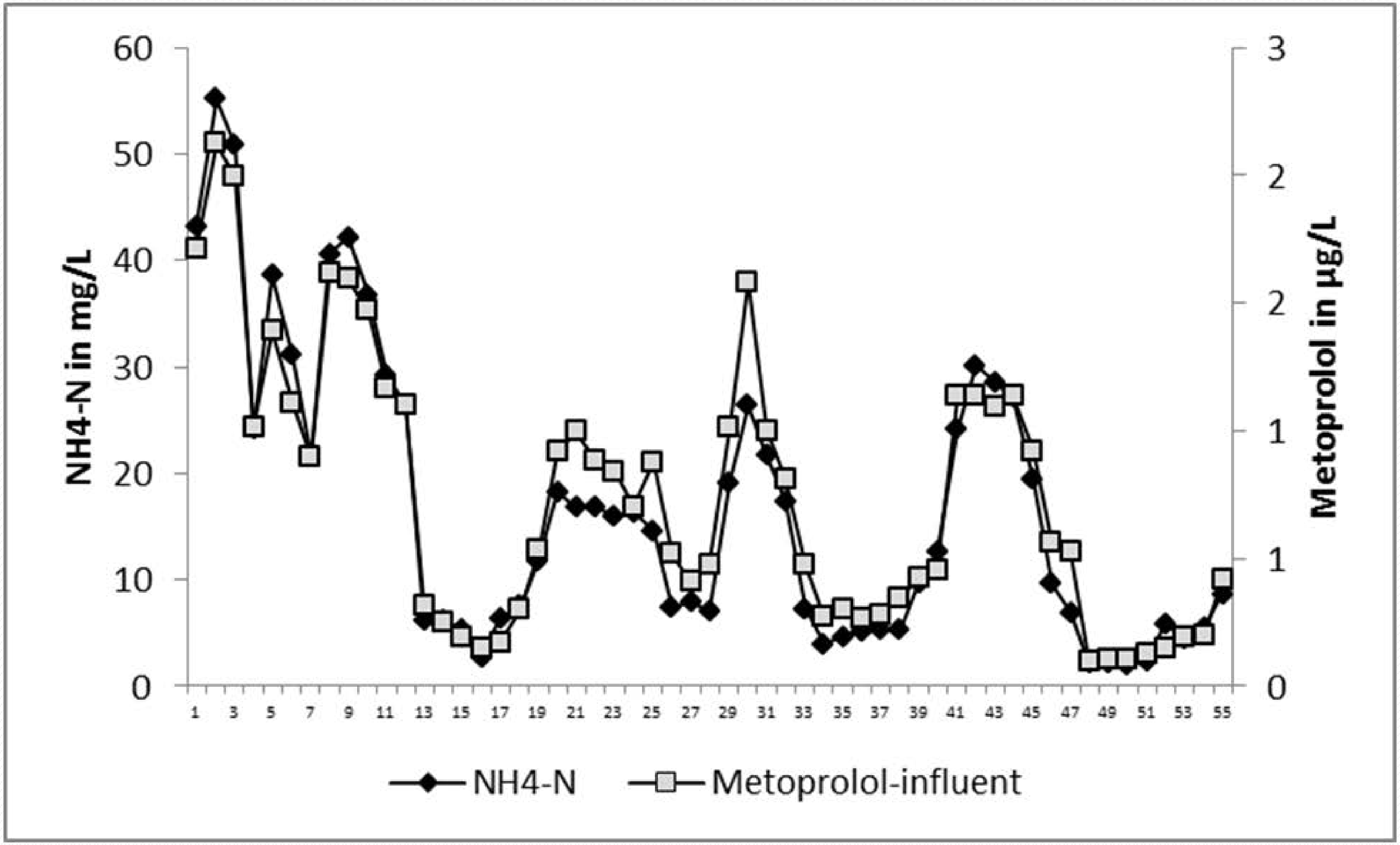
WP2 - Development of New Monitoring Technologies

Daily Fluctuations and HRT

Example: System WWTP – Q variable,
2h-mixed samples for 72 h

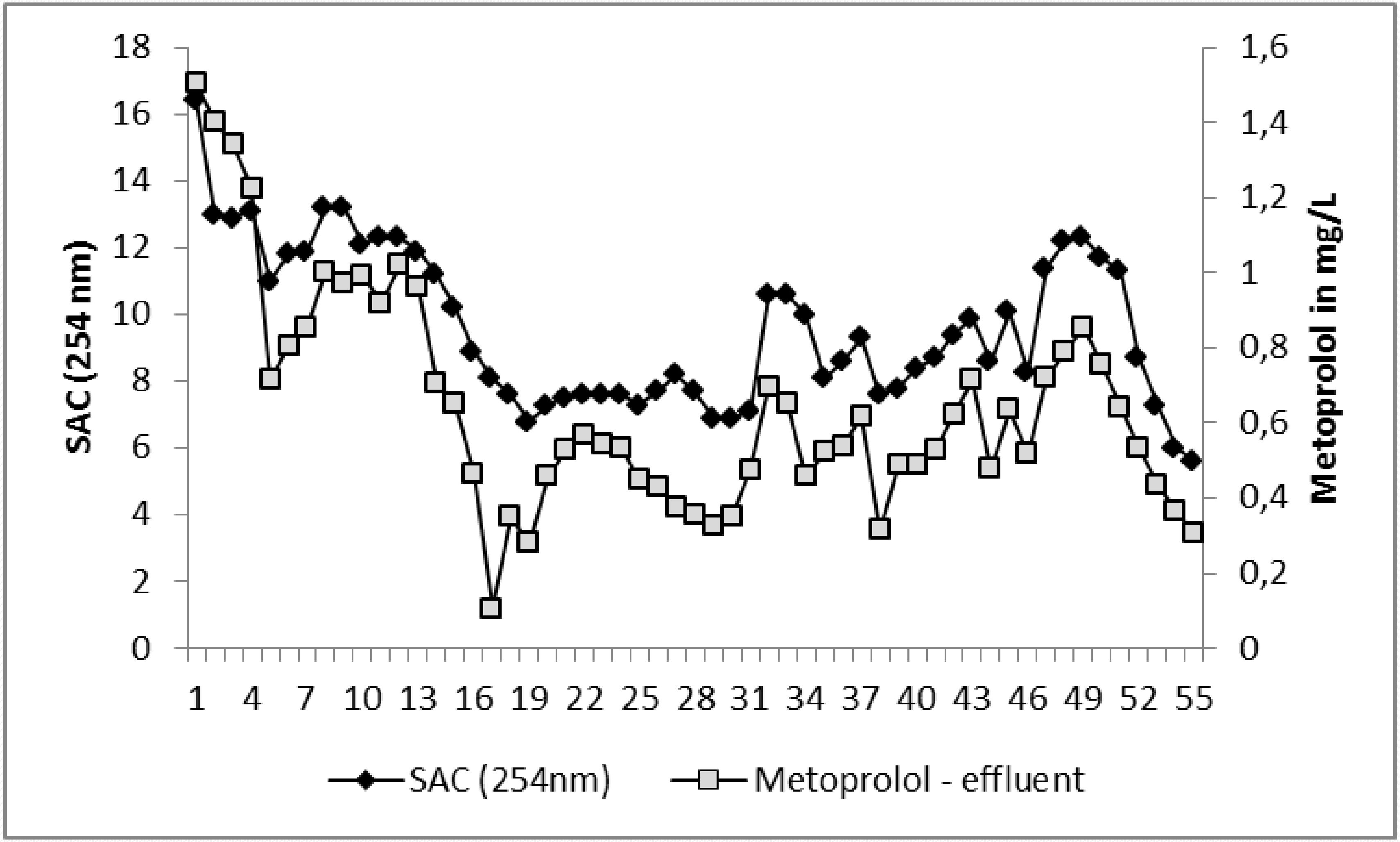


Correlation with conventional parameters

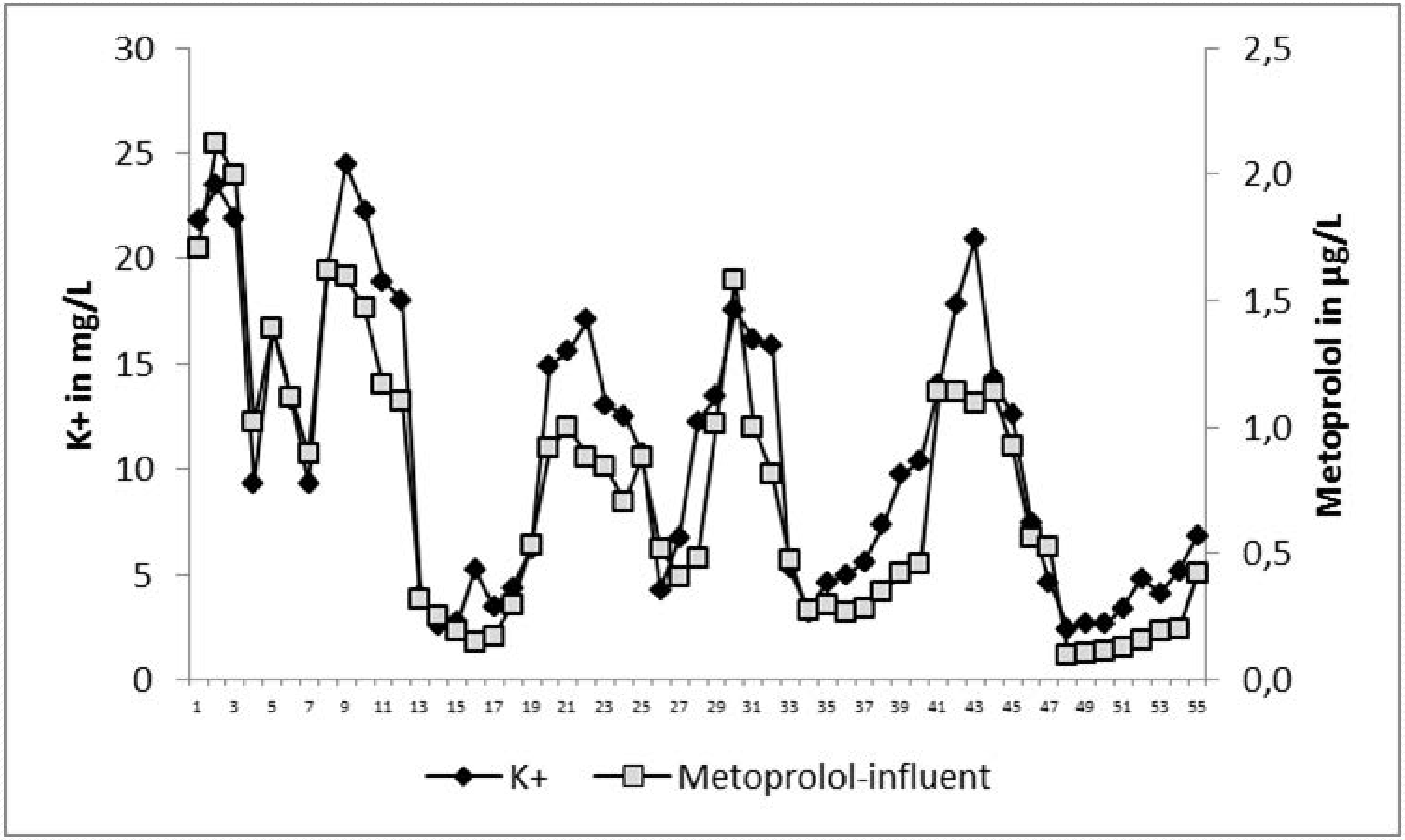


WaterJPI motrem

Correlation with conventional parameters



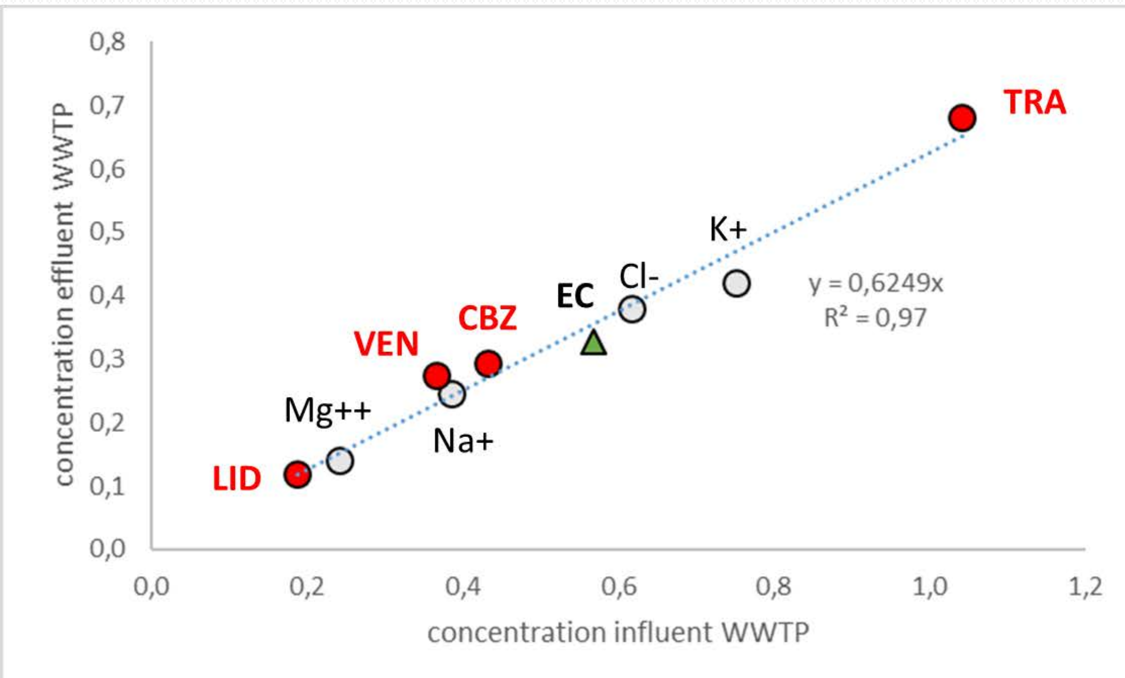
Correlation with conventional parameters



Correlation with conventional parameters

Verification of Sampling with conventional parameters

24h-mixed samples influent/effluent WWTP (E)



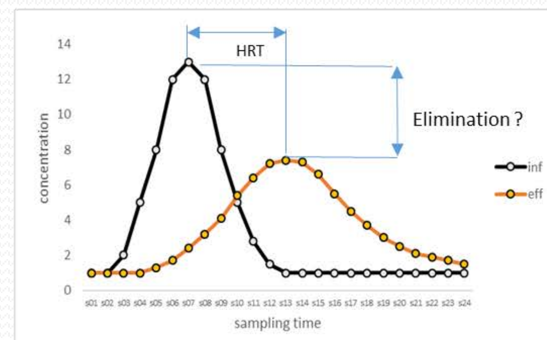
LID: Lidocaine; VEN: Venlafaxine, CBZ: Carbamazepine, TRA: Tramadol, EC: Electric Conductivity

Removal of micropollutants appr. 37 % ?

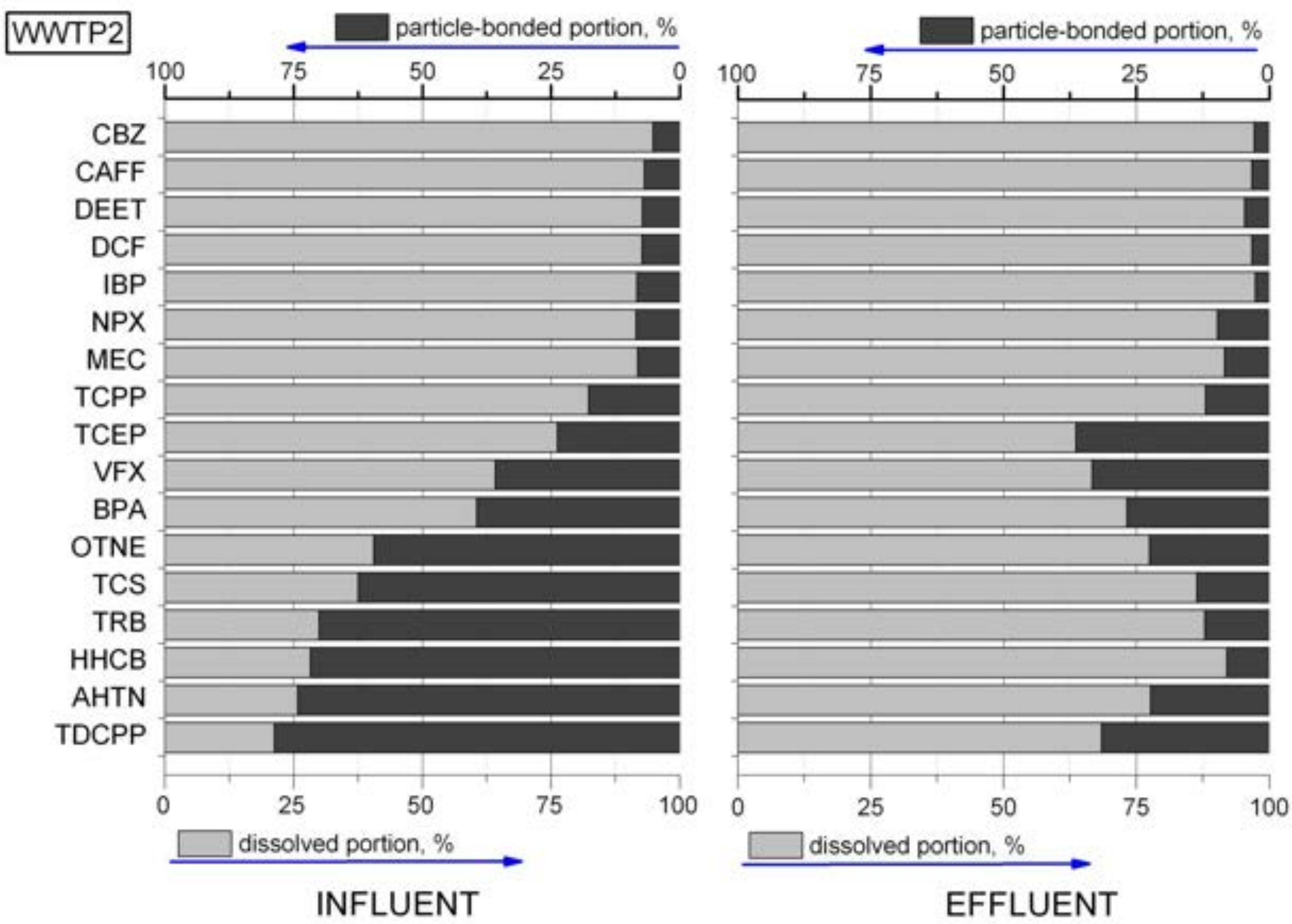
HRT not considered?

Dispersion?

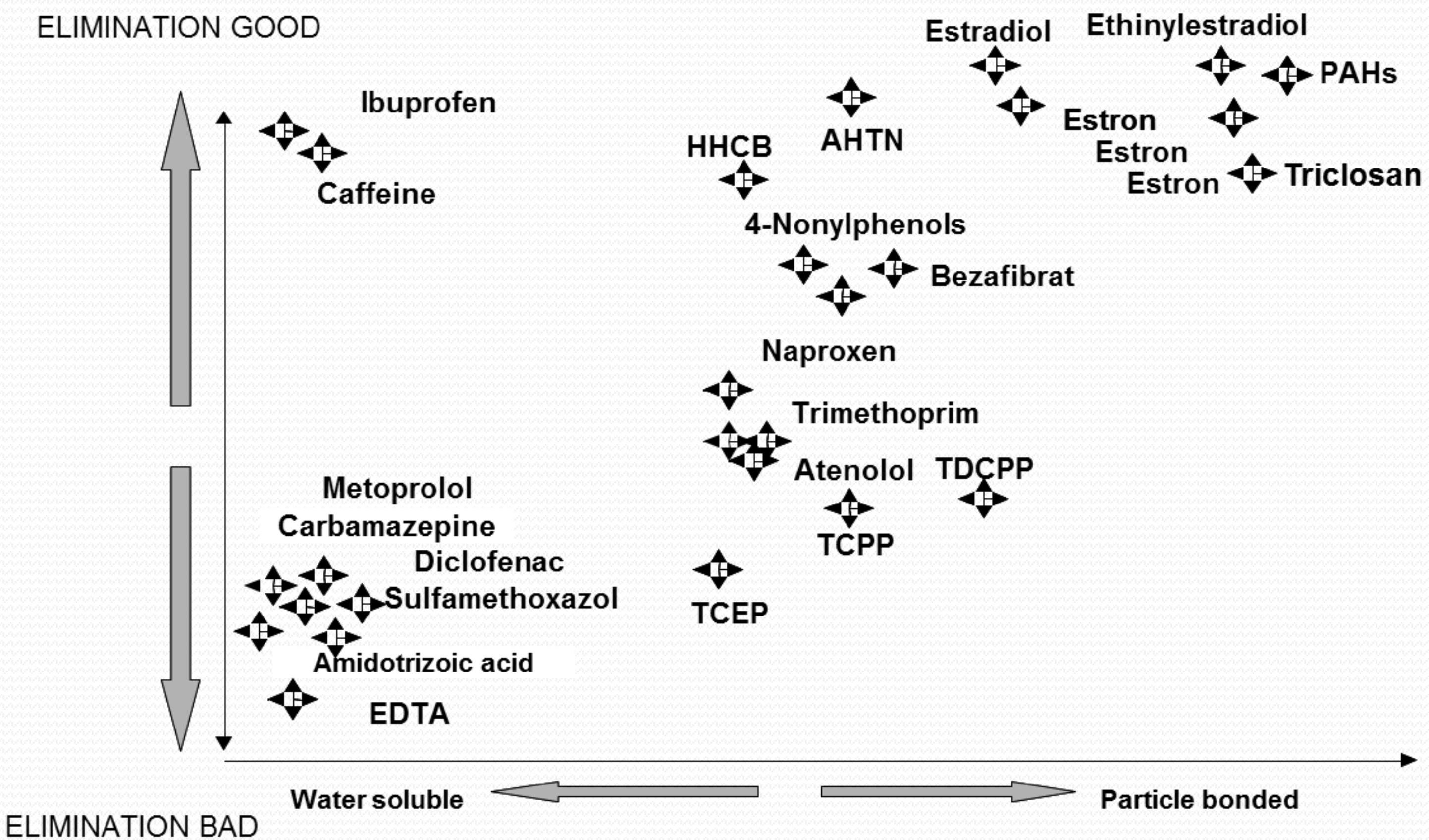
Foreign Water?



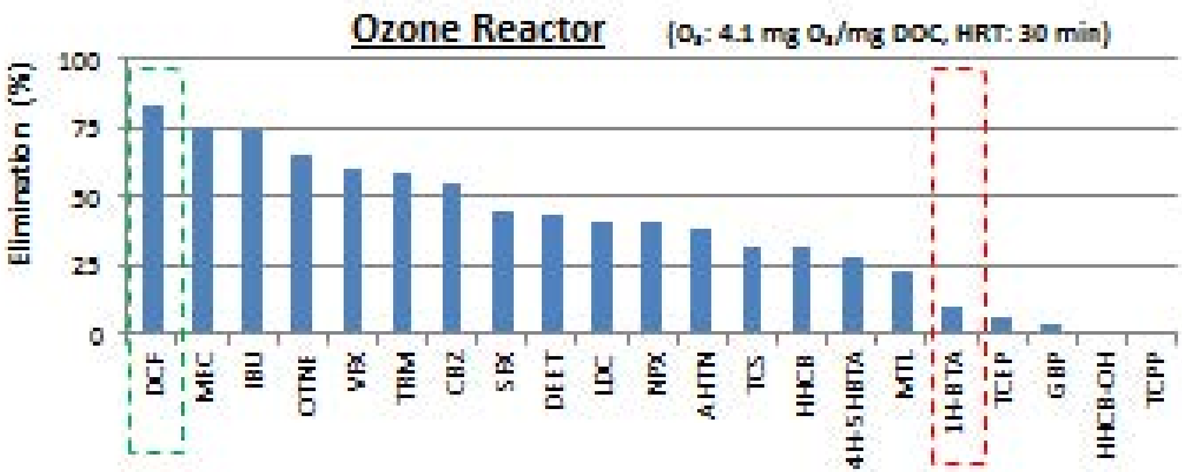
Dissolved vs Particle bonded



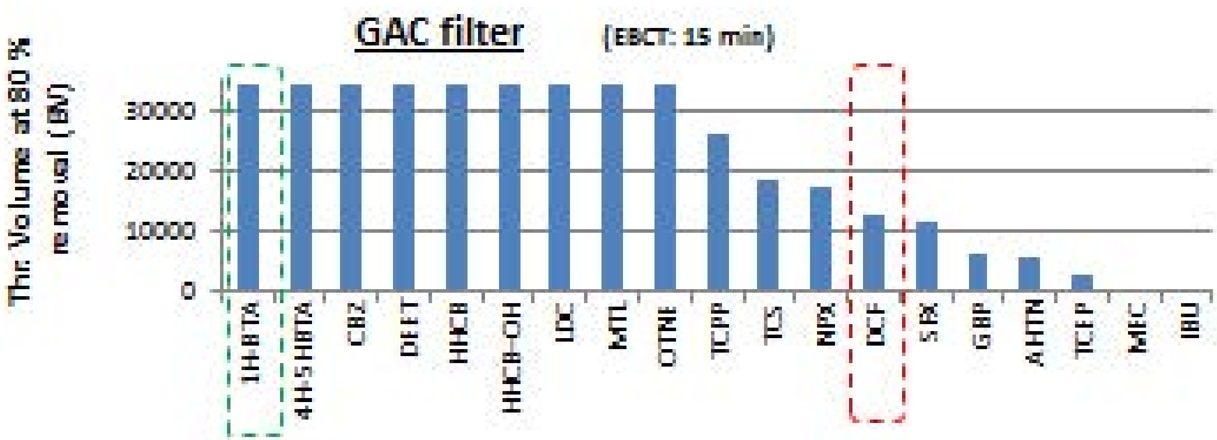
MICROPOLLUTANTS – ELIMINATION IN MUNICIPAL WASTEWATER TREATMENT



Selection of representative substances



Micropollutants are removed at different extent by each process based on their properties
➢Process-related micropollutants to be used for control



Selection of representative substances

Selection should based on

- Process to be monitored
- Chemico-physical properties of the substance
- Occurence and detection frequency (periodic/episodic)
- Source and entry path
- Substances should be representatives of a group with a similar behaviour

Scientific and technological progress

WP1 - Development of New Treatment Technologies

Efficient Biodegradation of ECs with ABOP

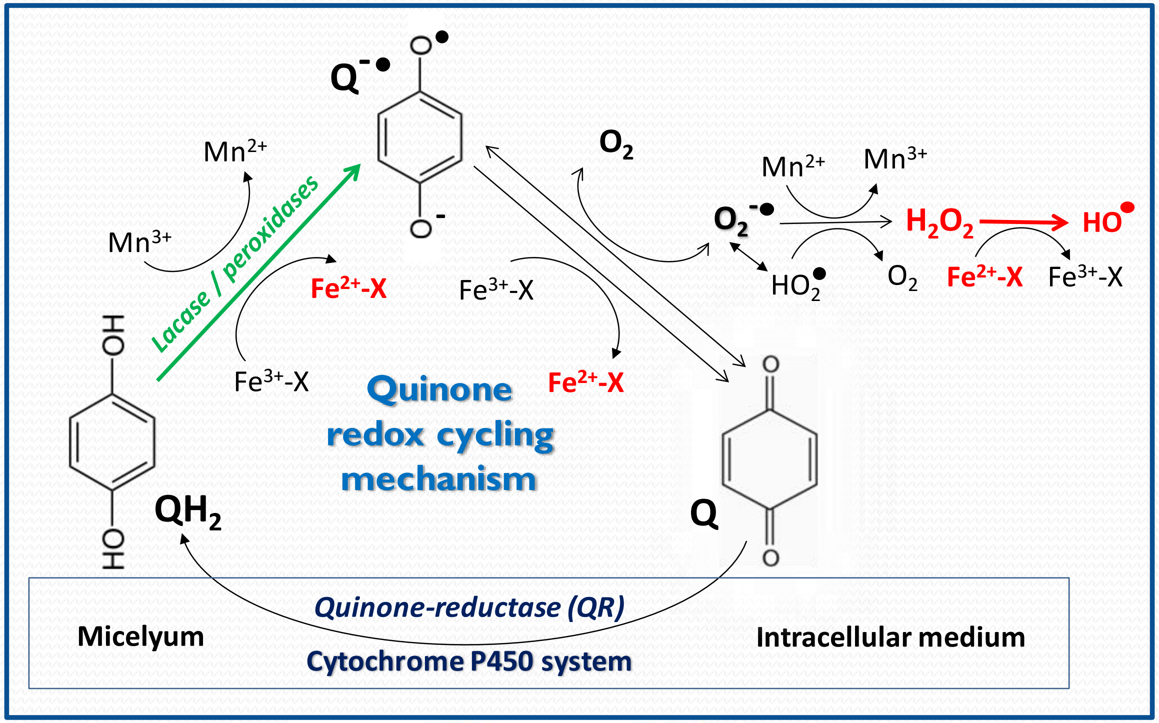
Trametes versicolor

Ganoderma lucidum

Advanced bio-oxidation process (ABOP) mediated by white-rot fungi

- Biological Fenton-like system
- Non-specific biodegradation system
- Preconditioning is not needed

Generation of oxidizing radicals by extracellular enzymes



Process catalysed by intracellular quinone reductase (cytochrome P450 system) and any of the ligninolytic enzymes of white-rot fungi (peroxidases and laccases)

Key points

- Quinone mediator
- Fe (II) and Mn (II) species

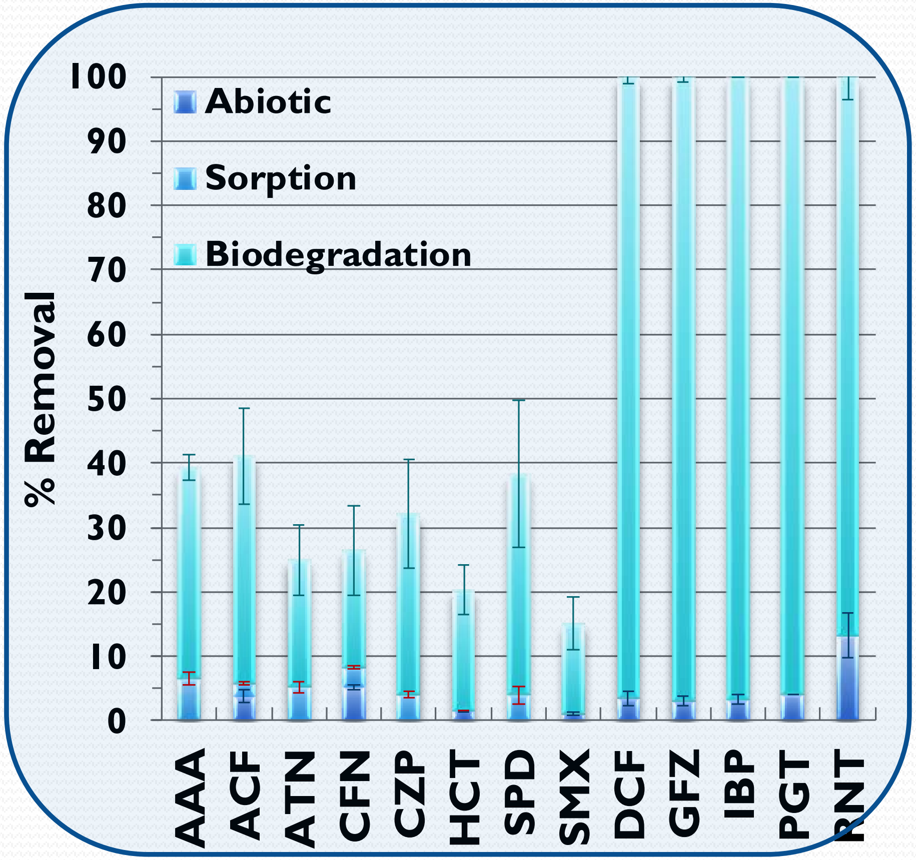
Supplementary substrates for AOBP

Water JPI

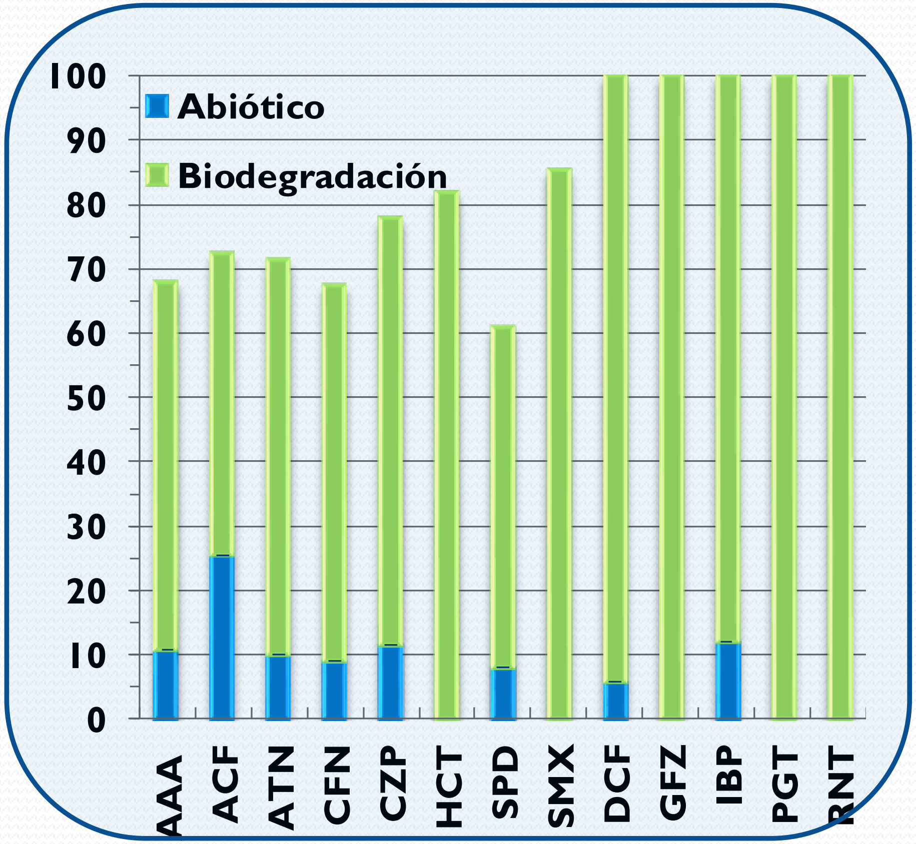
motrem

Efficient Biodegradation of ECs with ABOP

Bioassays in batch reactors



ONLY DEFINED MEDIUM



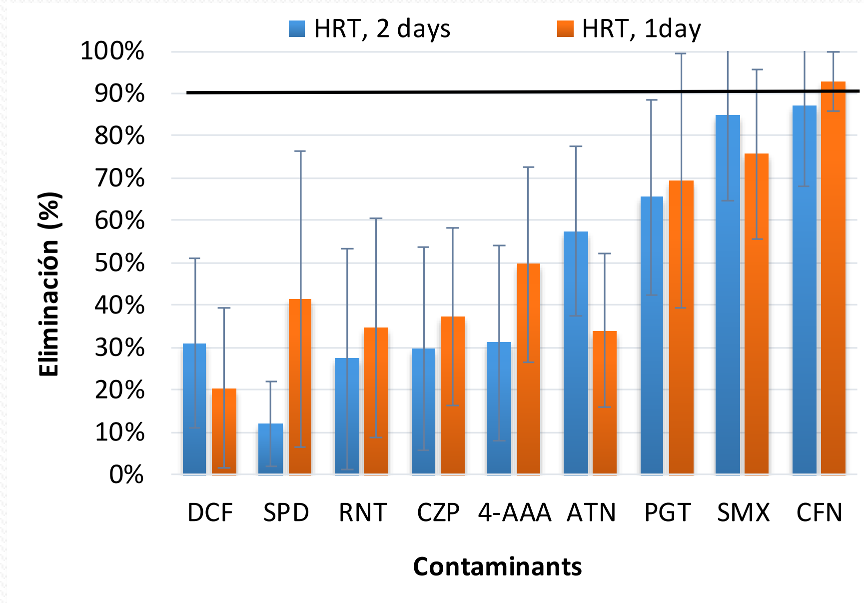
DEFINED MEDIUM +
SUPPLEMENTARY SUBSTRATES
FOR AOBP

Addition of Fe(III), Mn(II) and DMBQ activator

Efficient Biodegradation of ECs with ABOP

Fungal biodegradation in continuous Rotating Biological Contactors (RBCs)

Units	1 (5 discs each)
Total volume	24.5 L
Disc diameter	30 cm
Disc area	1.42 m^2
Disc submerged	40% (10 L)
Rotation speed	20 rpm
HRT	1-2 days
Temperature	26±2°C



C-TOC Reduction ≈ 80%

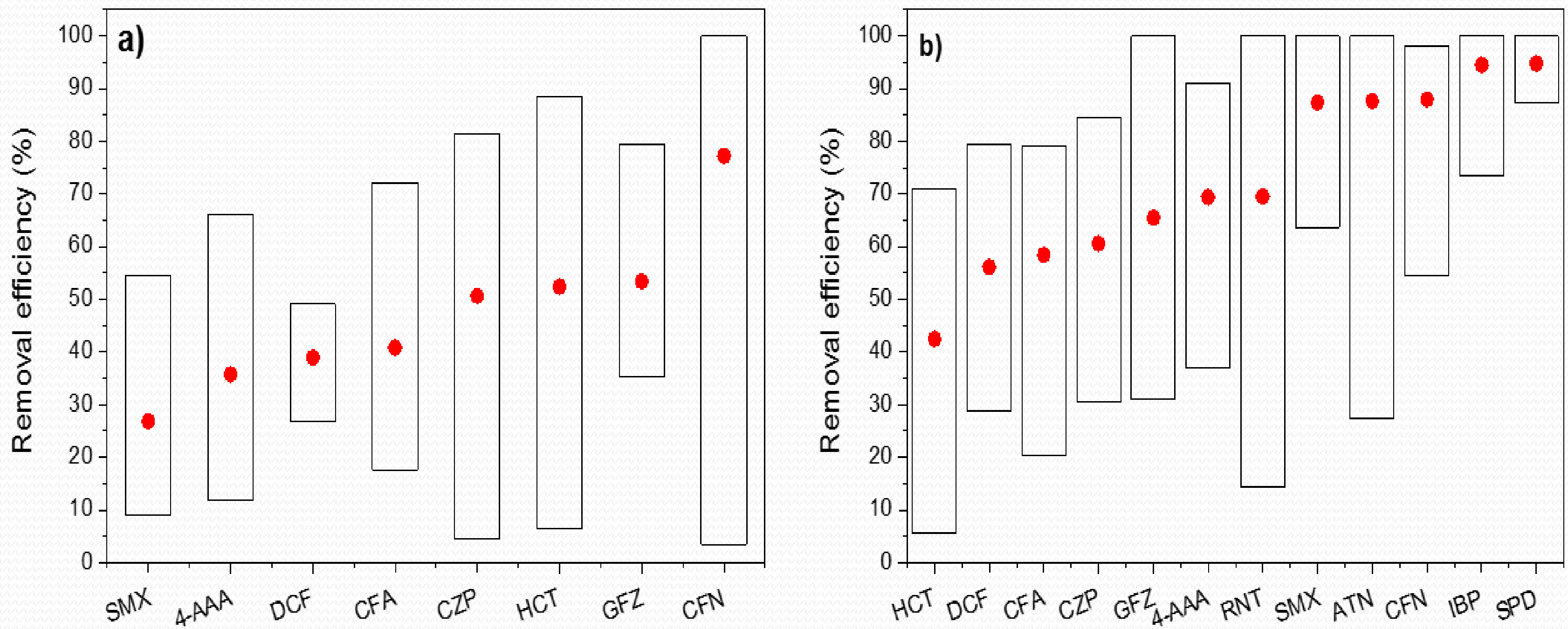
N-NH_4^+ Reduction ≈ 90-95%

Efficient Biodegradation of ECs with ABOP

CONTINUOUS TREATMENT USING ROTATING BIOLOGICAL CONTACTORS

A. Synthetic Urban WasteWater (SUWW), spiked 50 μg/L, 1d HRT

B. Real Urban WasteWater (RUWW) from DAF (URJC), spiked 50 μg/L, 1d HRT



Intervals of removal of pharmaceutical compounds for the treatment of a) SUWW and b) RUWW (red dot: average value)

Water JPI

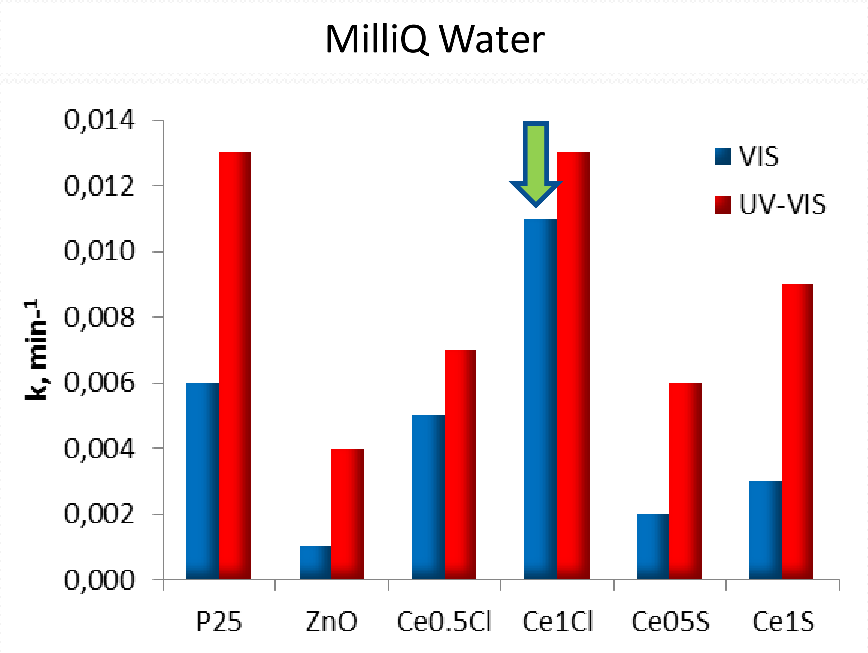
motrem

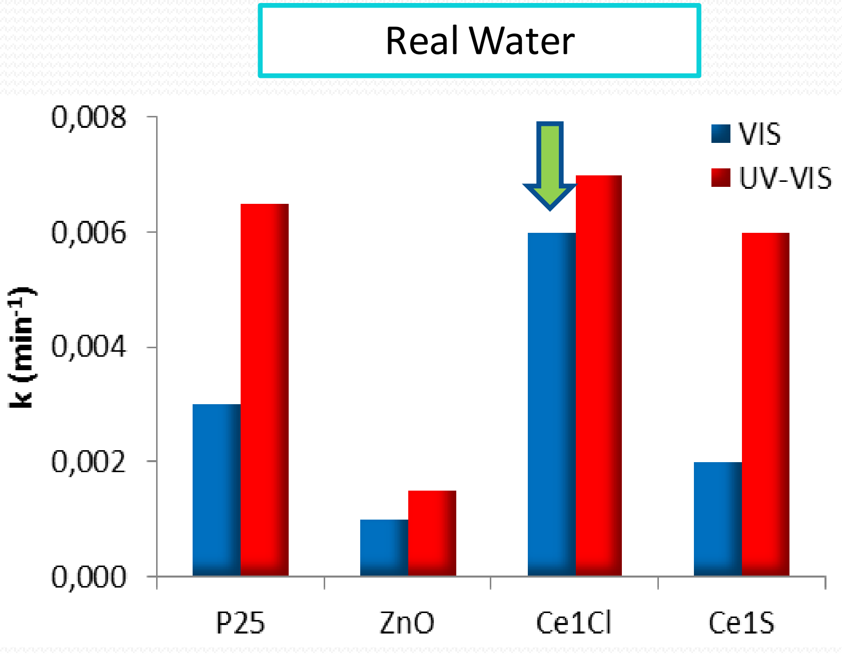
ZnO and Ce-ZnO photocatalytic materials

Synthesis

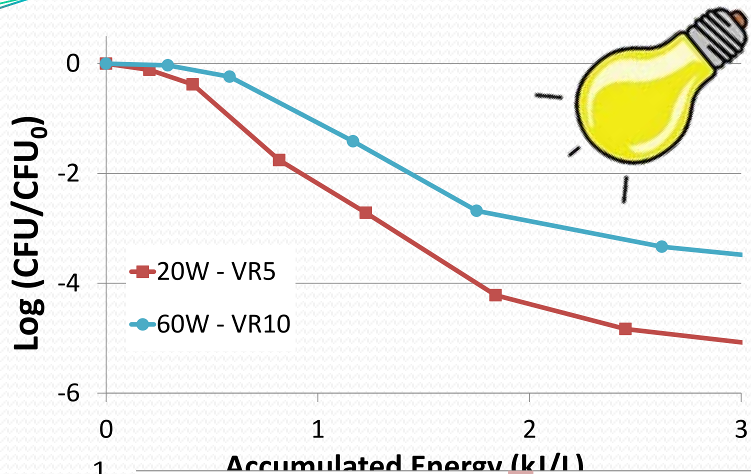
ZnO and Ce-ZnO synthesised via hydrothermal route from Zn acetate and $Ce(SO_4)_2$ or $CeCl_3$ at 0.5 % level (Ce05S) (Ce05Cl) and 1 % level (Ce1S) (Ce1Cl).

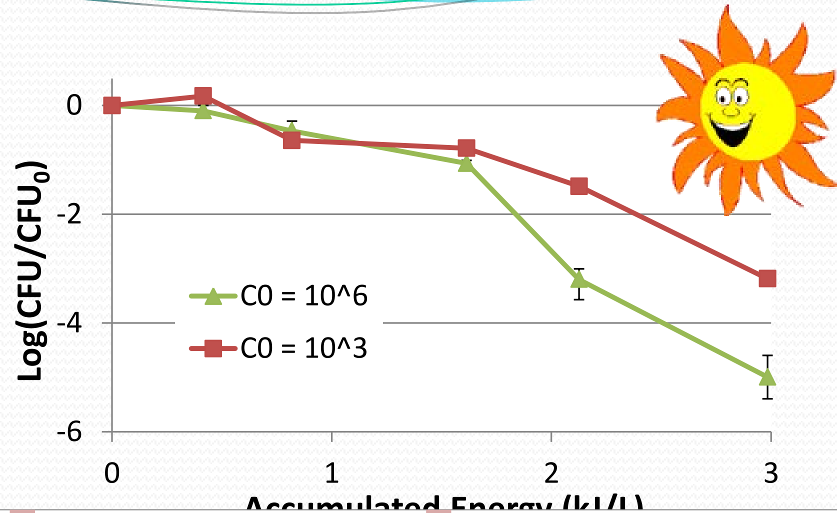
Removal of Acesulfame K removal

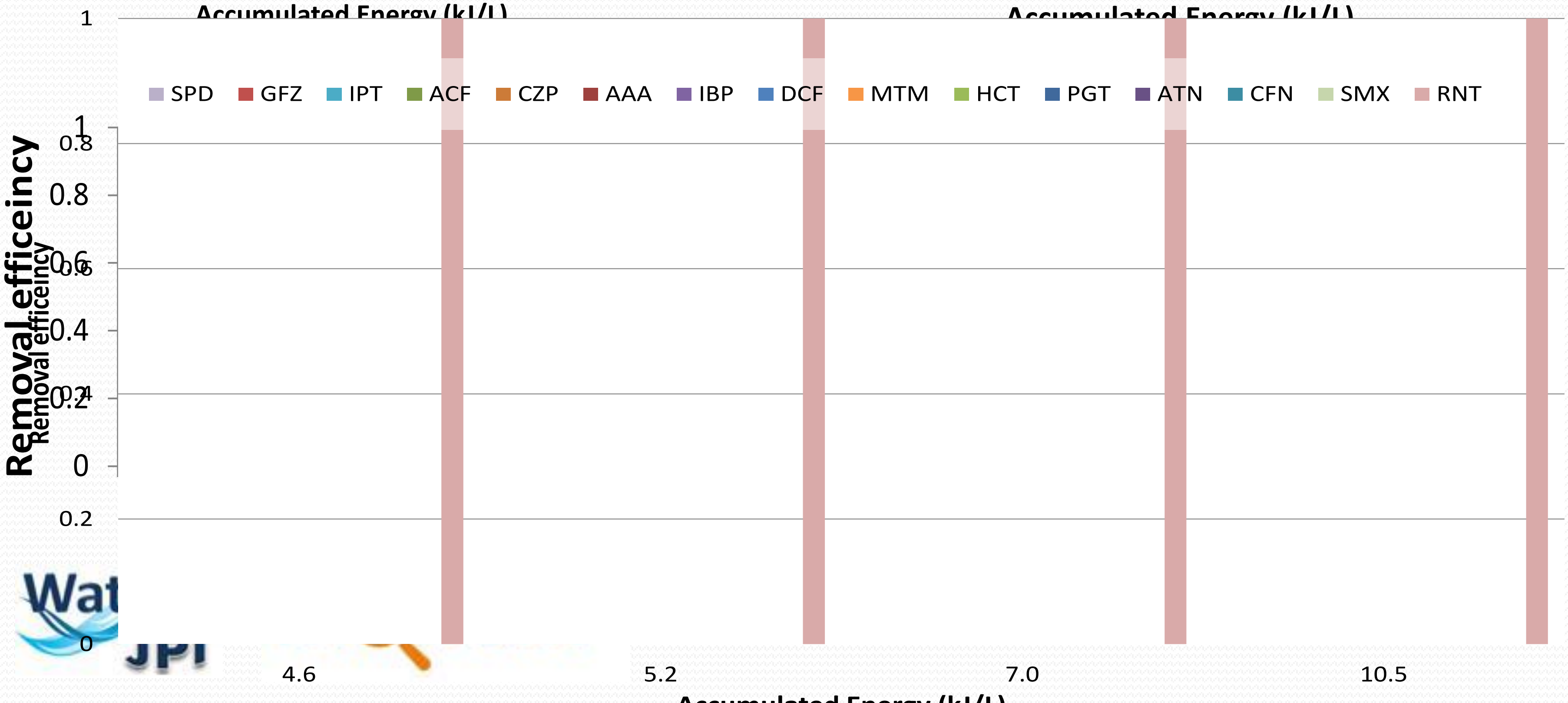




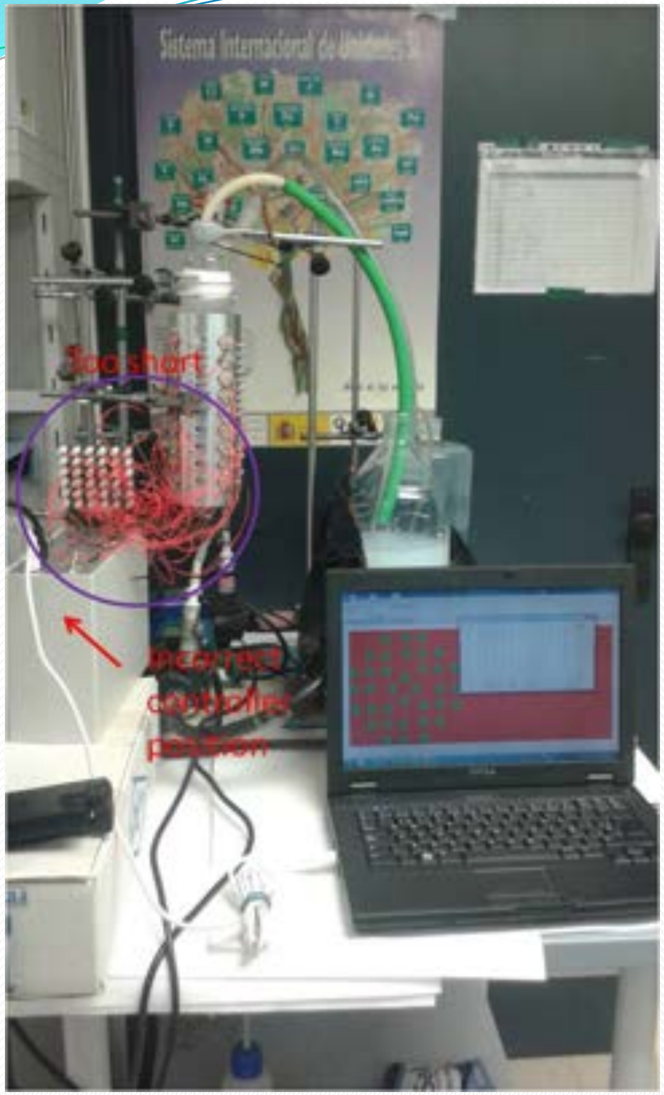
Efficient Removal of ECs during Photochemical Disinfection

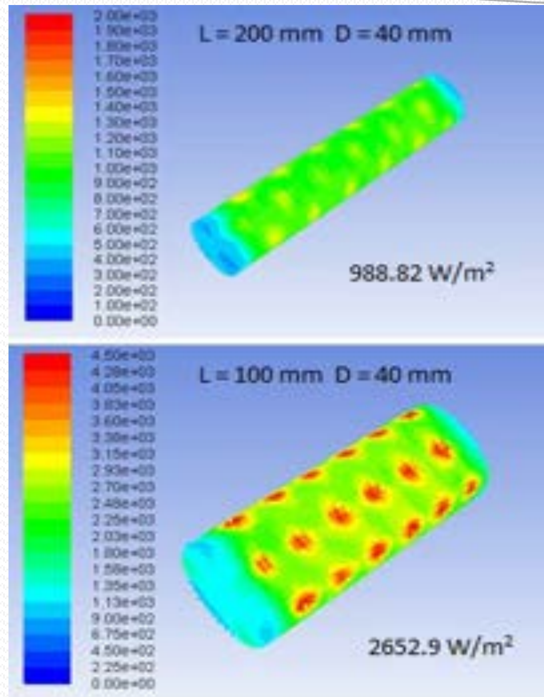


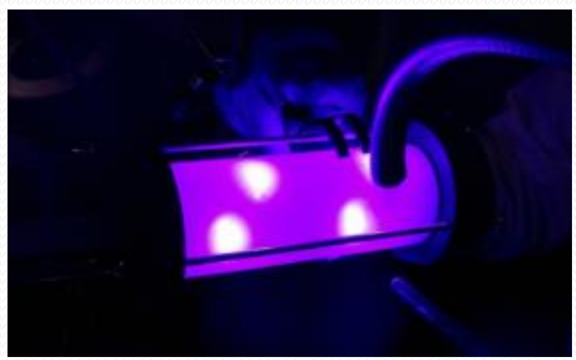




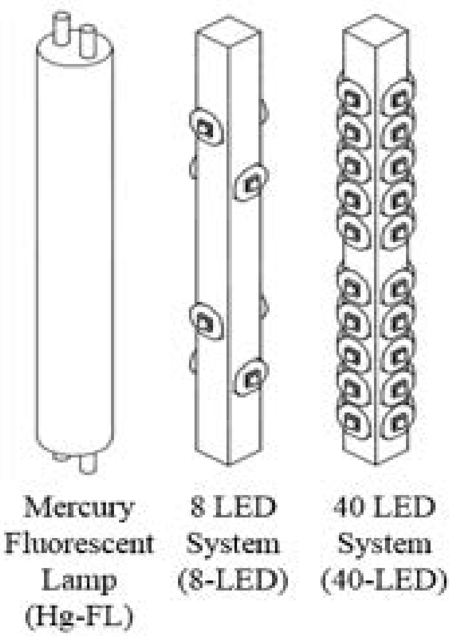
Advance Photoreactors for Disinfection & CEC Removal

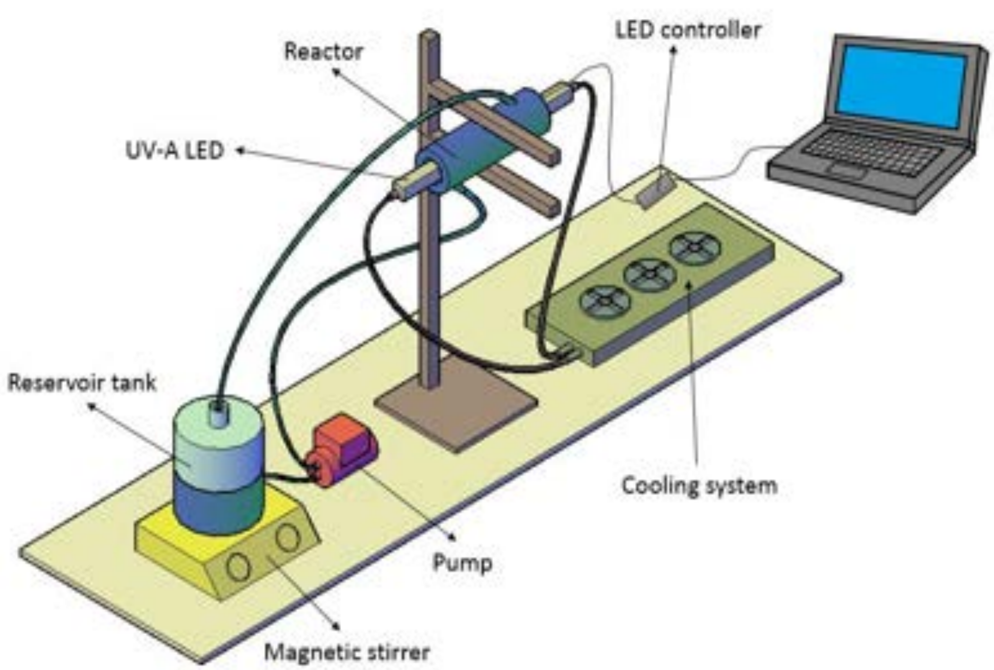




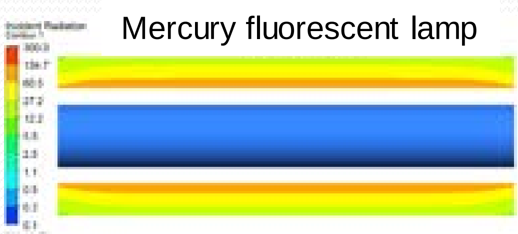


Advance Photoreactors for Disinfection & CEC Removal

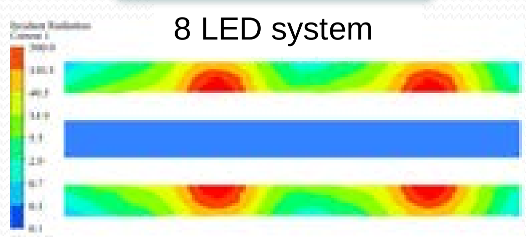




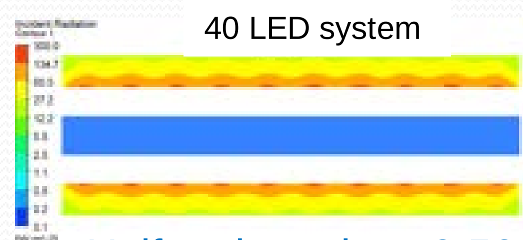
ANSYS Fluent



Uniformity Index : 0.68



Uniformity Index : 0.38

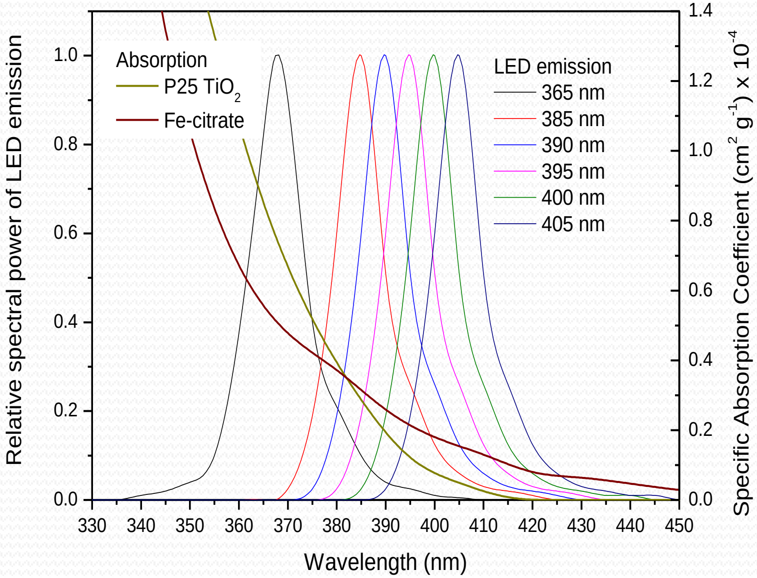


Uniformity Index : 0.56

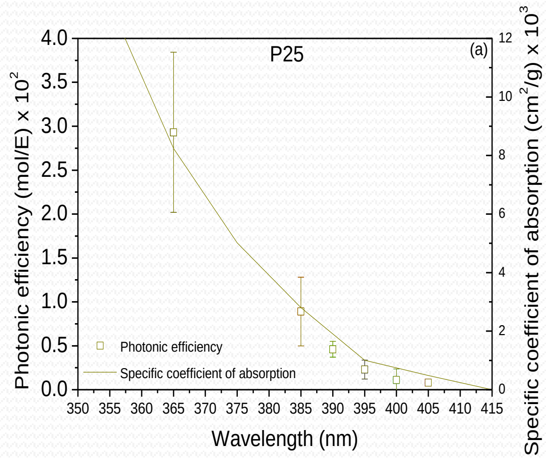
LED optimization

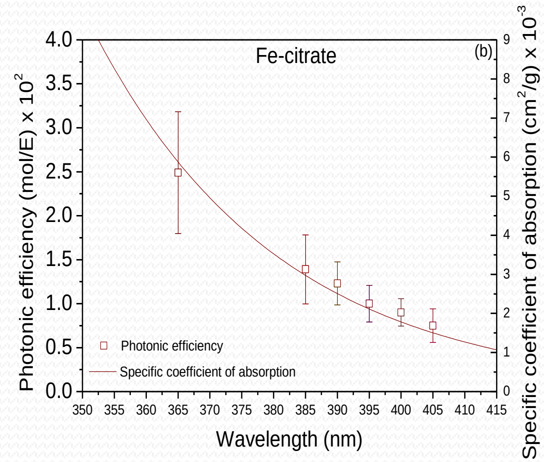
Water JPI motrem

Advance Photoreactors for Disinfection & CEC Removal

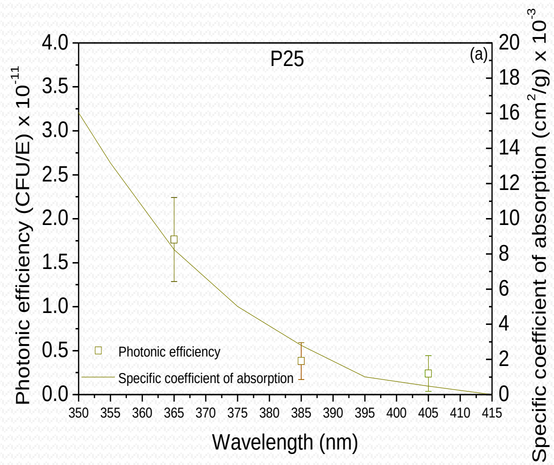


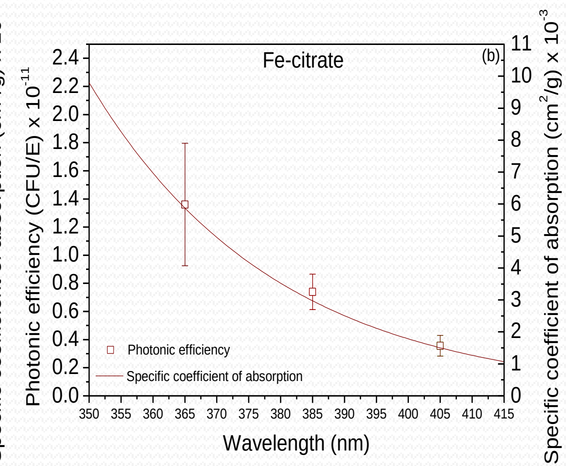
CHEMICAL OXIDATION





BACTERIAL INACTIVATION

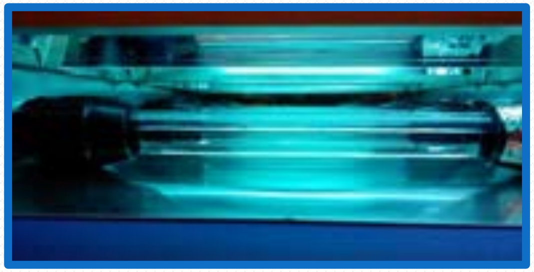




WP1: Task 1.3. Disinfection & Removal of emerging contaminants

Fixed-Bed Reactor: Catalytic Foams

Disinfection & CEC Removal: UV-C Pilot Plant



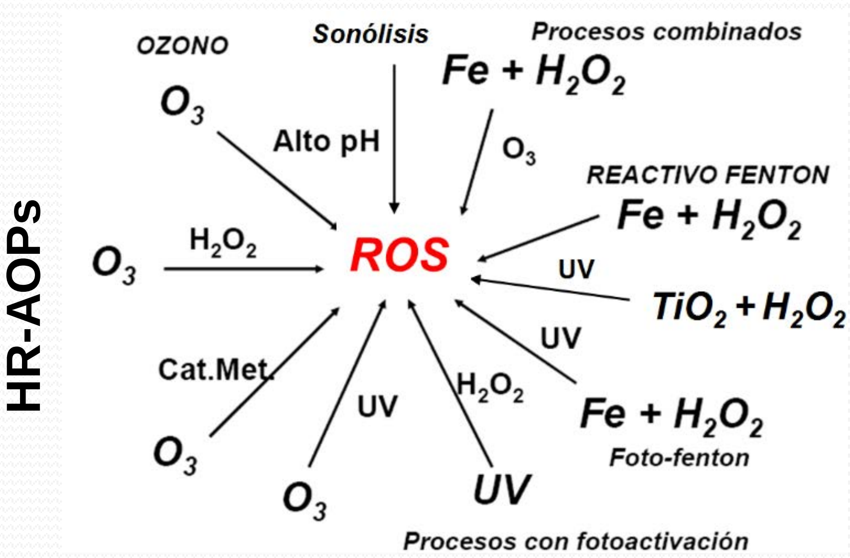
Treatments:

- UV-C
- PMS/UV-C
- PS/UV-C
- PMS/Fe(II)/UV-C
- PS/Fe(II)/UV-C
- H_2O_2/UV-C

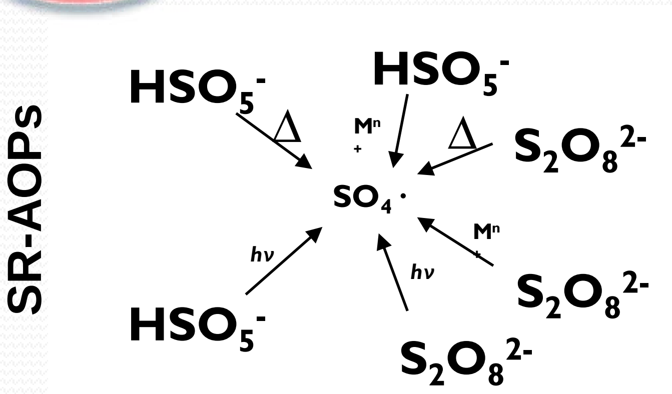
Maximum operational conditions

- 4 UV-C lamps (380 W; λ = 254 nm)
- 4 serial quartz pipes
- Illuminated volumen = 2.84 L
- Flowrate = 1 – 54 L/min
- Maximum contact time = 2.84 minutes

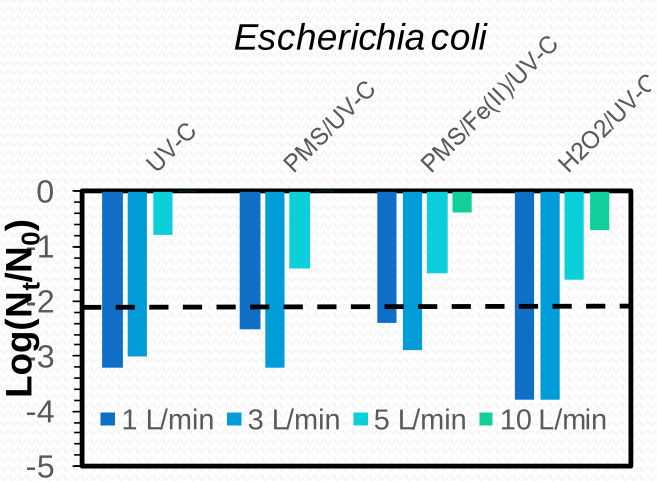
Advanced Oxidation Processes

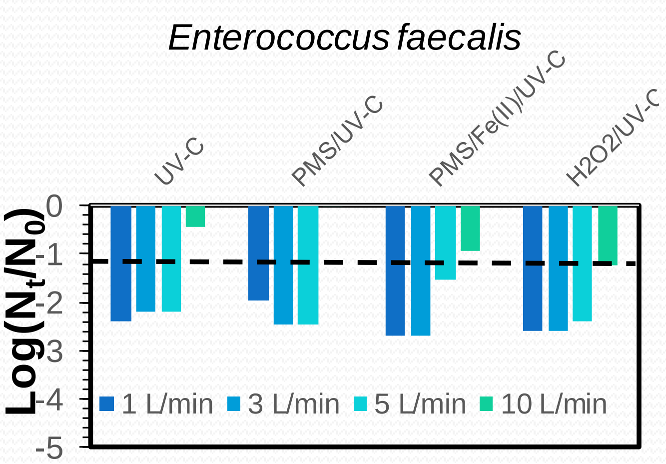


Oxidation Potential (V)								
F_2	$\cdot OH$	$SO_4\cdot$	O at.	O_3	H_2O_2	MnO_4^{2-}	Cl_2	ClO_2
3.03	2.80	2.60	2.42	2.07	1.76	1.67	1.36	1.15



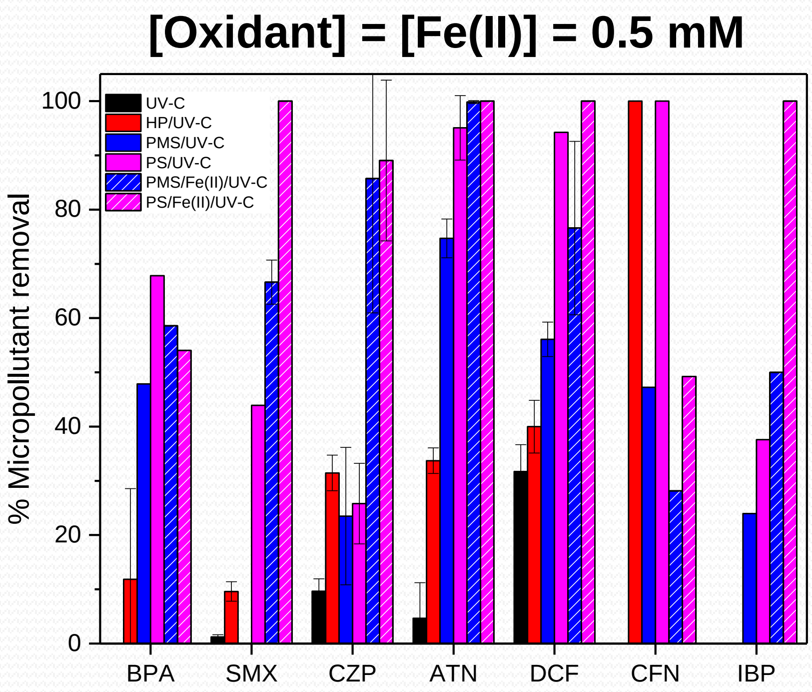
Disinfection & CEC Removal: UV-C Pilot Plant + Real WW

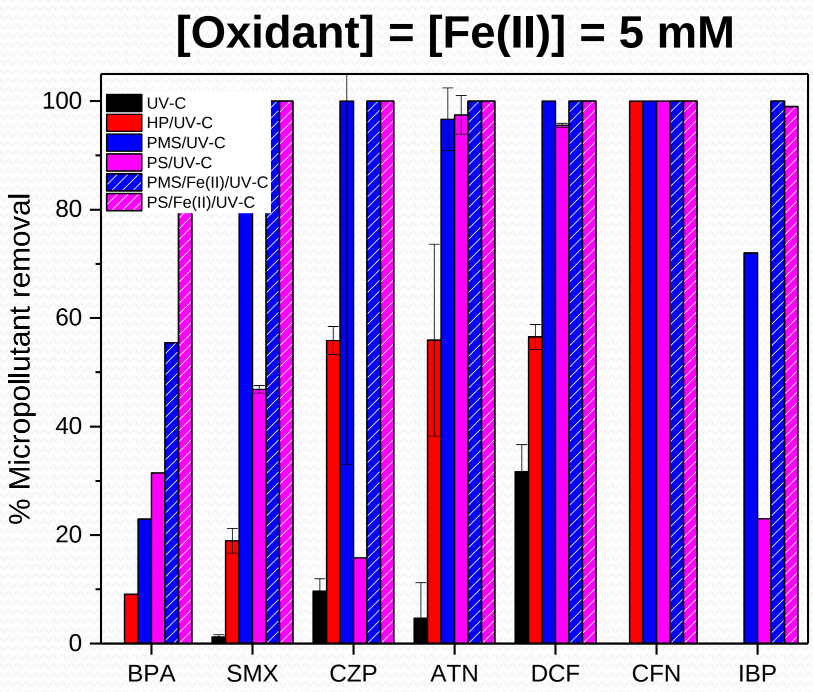




Disinfection

Disinfection & CEC Removal: UV-C Pilot Plant + Real WW





CEC Removal

Carbamazepine (t_R=21.82 min; 42.8 ± 36.9 µg/L)

Number of TPs vs treatments:

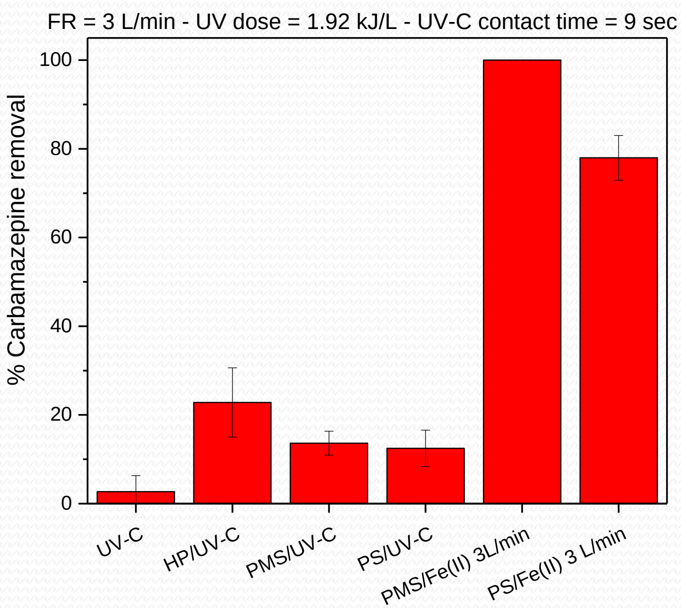
HP/UV-C=5

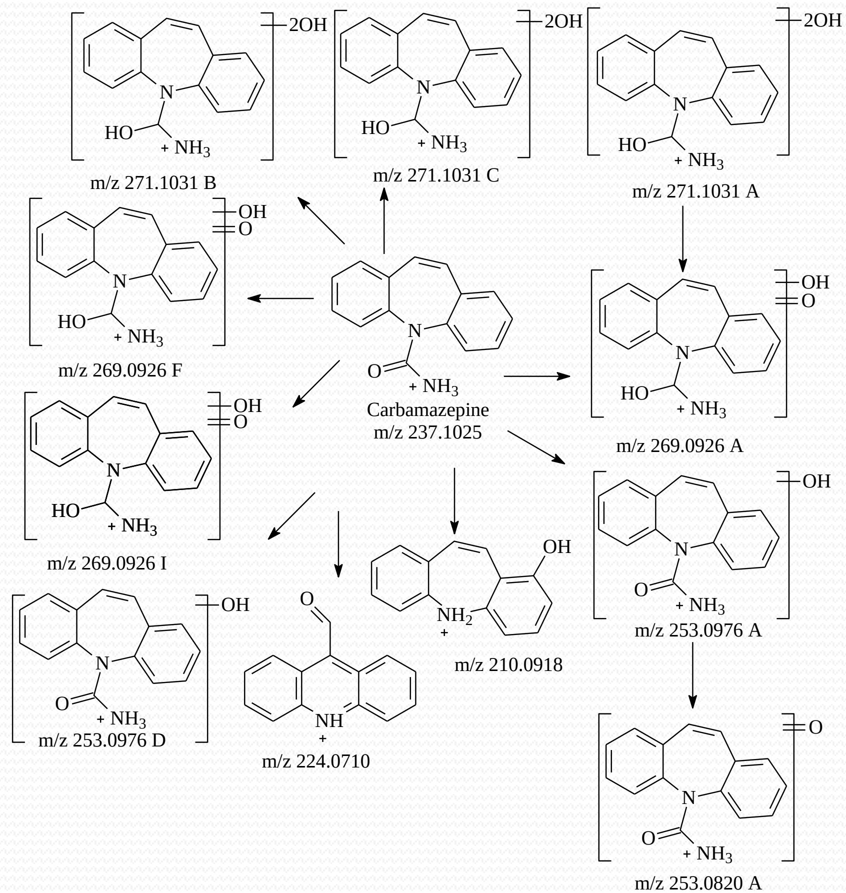
PMS/UV-C=2

PS/UV-C=5+2

PMS/Fe(II)/UV-C=3

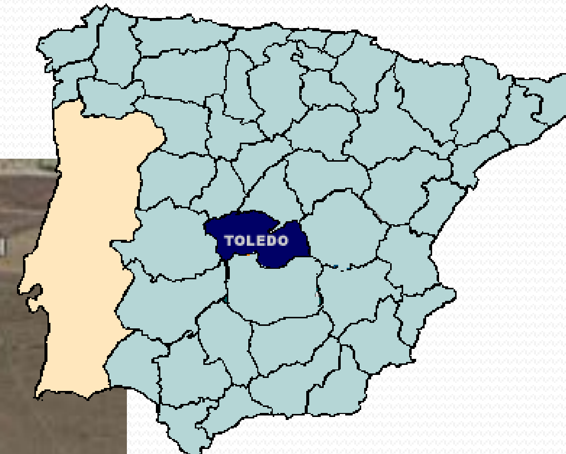
PS/Fe(II)/UV-C=2+1+2





Disinfection & CEC Removal: UV-C Full Scale Plant WWTP

Full Scale UV-C Reactor: 2 campaigns



- 36,000 m^3/day
- 270,000 PE
- Biotreatment: Activated sludge
- UV tertiary treatment

Disinfection & CEC Removal: UV-C Full Scale Plant WWTP

Estiviel WWTP

Location: Toledo

Design flow: 36,000 m^3/day

Biological process: Activated sludge

Tertiary treatment: 270 m^3/day (irrigation and internal industrial use)

Coagulation/Flocculation – Sedimentation – Microfiltration (discs) – UV

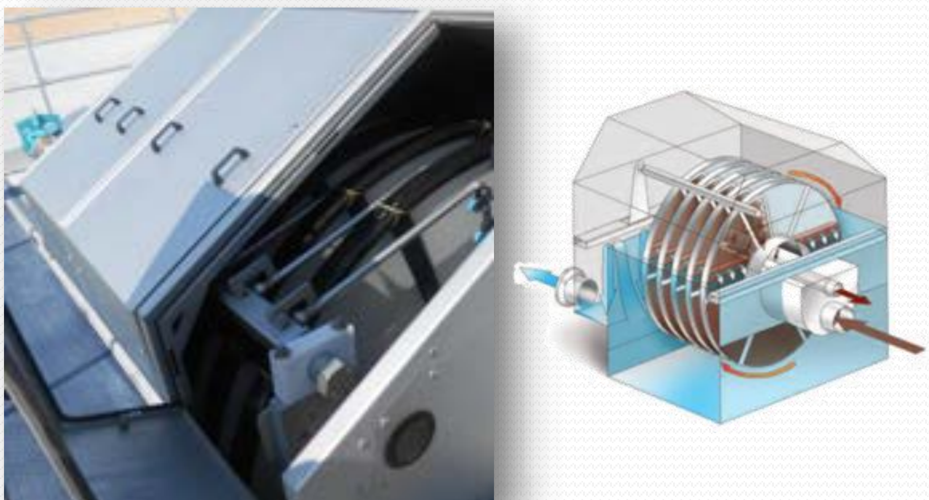
Population equivalent: 270,000 PE

Influent: Urban WW

Effluent discharge: Tajo River

Influent:

COD = 820 mg/l
BOD_5 = 450 mg/l
SS = 490 mg/l

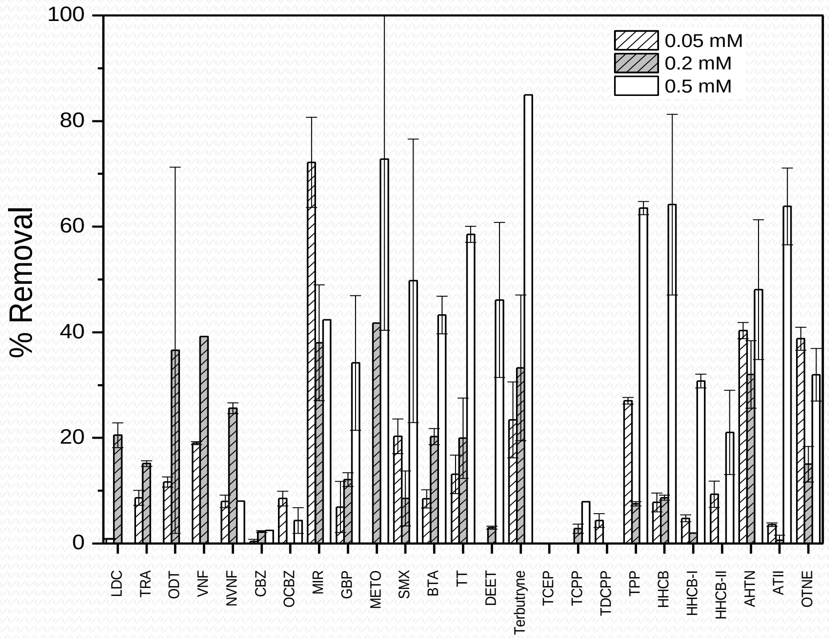


Microfiltration (8 discs) (10 µm)

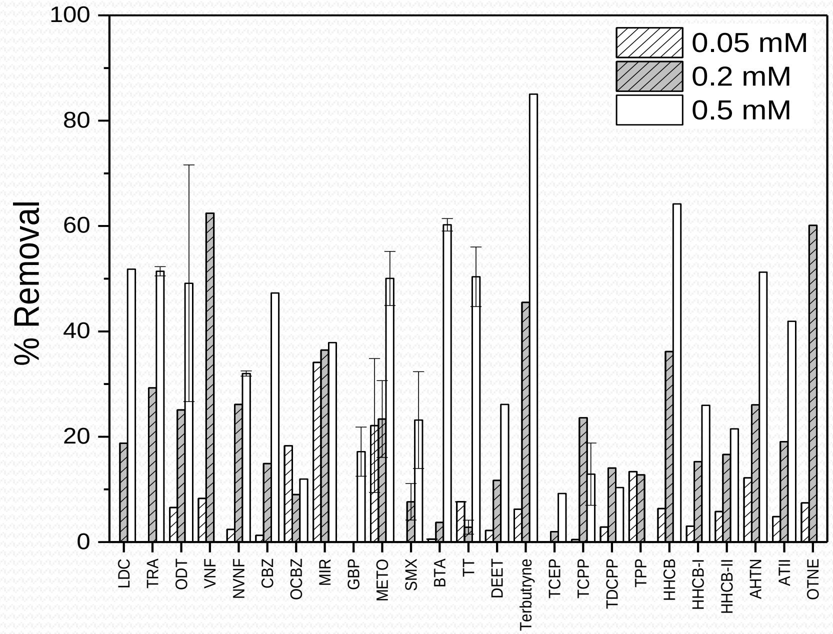
UV reactor (16 lamps)

Disinfection & CEC Removal: UV-C Full Scale Plant WWTP

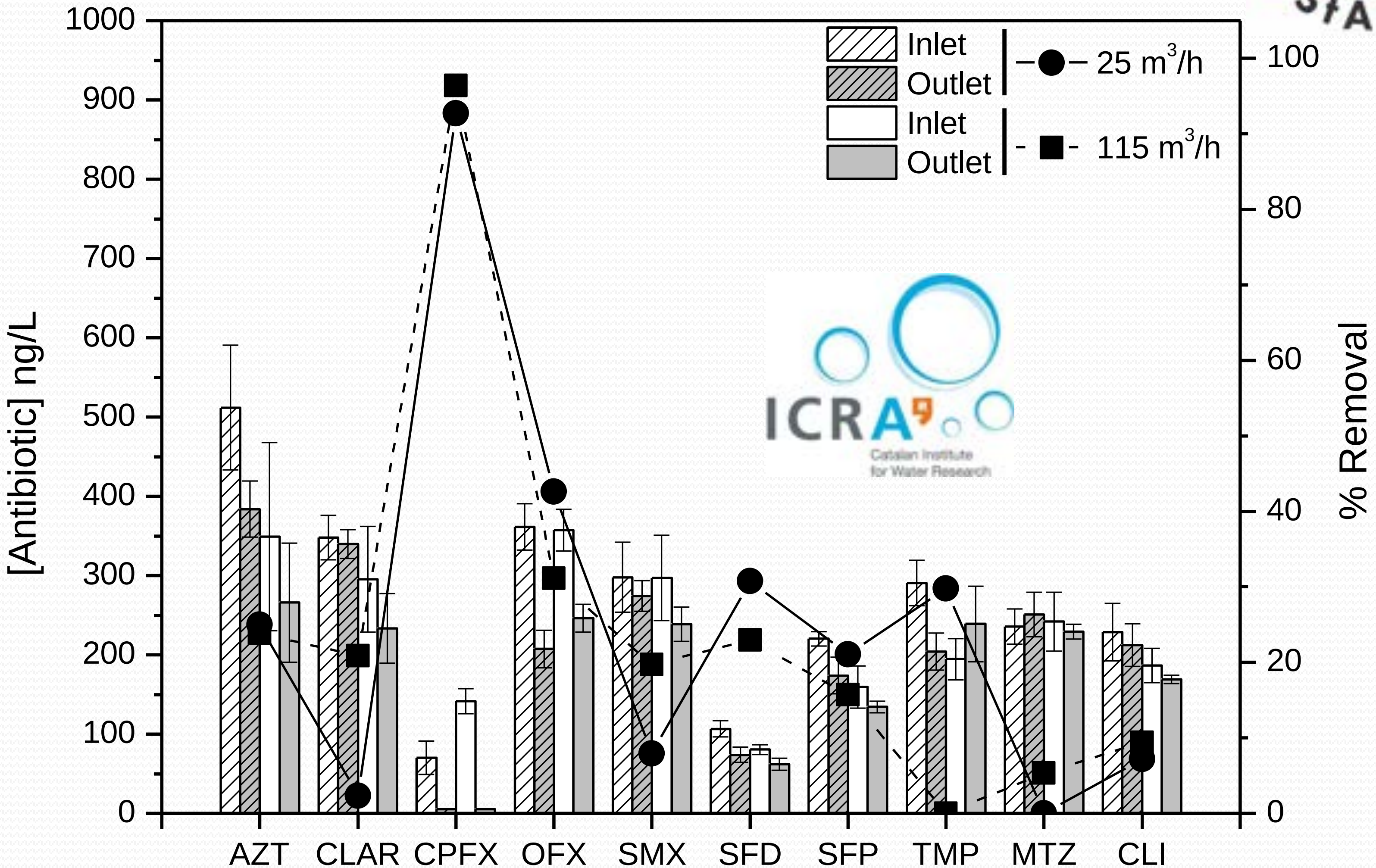
PMS/Fe(II)/UV-C treatment



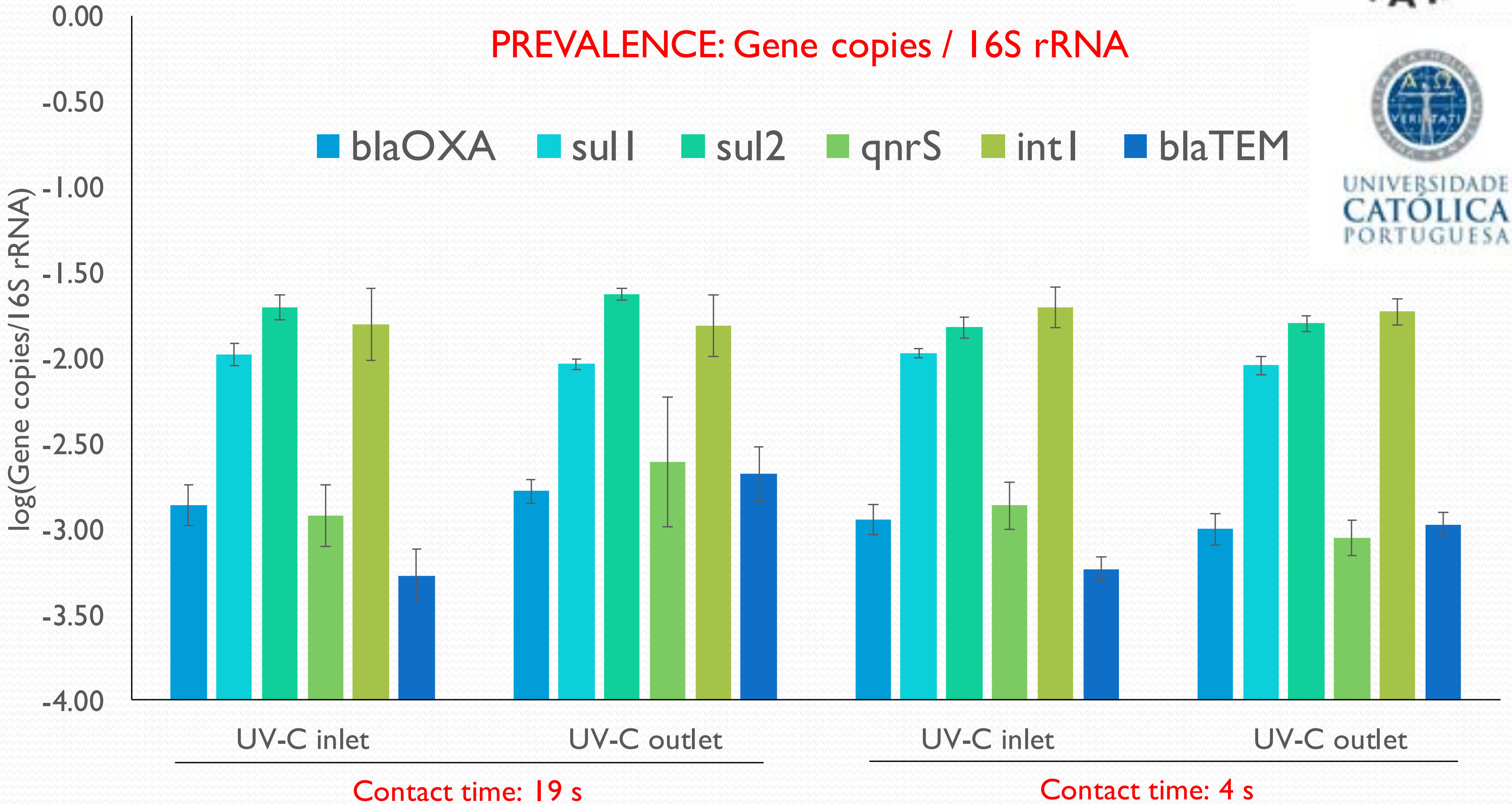
PS/Fe(II)/UV-C treatment



Removal of antibiotics



Removal of antibiotic resistance genes



Preliminary Economic Evaluation

Table 2
Economical estimation of operating cost of proposed oxidation treatments in the tertiary step.

UV-C contact time (s)	[Reagents]	UV-C**			H_2O_2/UV-C			PMS/UV-C			PS/UV-C		
		€/m³	% Removal	€/m³·order	€/m³	% Removal	€/m³·order	€/m³	% Removal	€/m³·order	€/m³	% Removal	€/m³·order
18	0.05				0.017	18	0.189	0.072	20	0.727	0.022	4	1.24
18	0.2				0.023	26	0.179	0.243	29	1.65	0.045	11	0.919
18	0.5	0.012	13	0.200	0.035	55	0.102	0.585	48	2.03	0.090	10	2.00
7	0.5	0.004	8	0.120	0.026	31	0.164	0.576	25	4.71	0.081	5	3.32
4	0.5	0.003	4	0.153	0.025	14	0.365	0.574	12	10.6	0.079	3	6.05

*H_2O_2, PMS and PS.

**No reagents required.

Most demanding operating conditions: highest UV-C contact time and reagents dosages.

Collaboration, coordination and synergies

Collaboration, with complementary key roles in:

- Treatment technologies (URJC).
- Monitoring and control of WWTP (UST).
- Analytical methodologies (UH).
- Mechanistic and toxicological studies (UNITO).
- Full-scale applications (AQUALIA).
- Analytical equipment (BRUKER).

Synergistic collaborations among them, beyond their individual work in the project.

Coordination and organization of the project: all the milestone and deliverables have been successfully and effectively completed without unexpected issues. The number of project meetings was sufficient, as a fluid communication was always kept.

Vast amount of samples from URJC and AQUALIA to UST, UH and UNITO

Identified problems or specific risks
The multidisciplinary work is somewhat missing, and should be considerable strengthen. This will be especially important in the future success of this project as several deliveries in the last part of the project will depend on strong collaborations at a multidisciplinary approach. I did not identified specific risks.

RECOMMENDATIONS
How to improve project scientifically?
As mention above, collaboration through a multidisciplinary approach will be crucial to improve scientifically. This said, the quality of the published papers holds a very high quality and reports important findings. No specific recommendations. Indicate if there is more cooperation with other JPI projects. If not present perhaps it can be more intensified.
How to advance the impact of the project?
There already seems to be a good impact from this project as several high quality papers has been published. However, to advance further the impact even a broader and larger number of possible stakeholders should actively be involved in the project (e.g. through spin-off projects). More mobility. I see nothing on costs of new technologies.

Collaboration between projects

Collaboration between MOTREM and STARE to explote the synergistic expertise developed in both projects.

Estiviel WWTP (**AQUALIA**, Toledo, Spain) + **URJC**

Antibiotics (**ICRA**) + Antibiotic Resistences (**UCP**)

Mobility

Consortium meetings, conferences, workshops, training courses and other events attended: 14 mobility actions + 80 conferences.

- Macarena San Martín (**UST**) and María José Martín de Vidales (**URJC**) at the facilities of **AQUALIA** in Estiviel WWTP (Toledo, Spain) for extensive on-week monitoring campaign of the plant in order to have a background of the plant behaviour before implementation of new technologies.
- Riikka-Juulia Lepistö (**UH**) did a short stay in **URJC** labs in order to implement the required sample treatment and analytical methodologies
- Irene Fiore (**UNITO**) did a 3-month research stay in **URJC** focused on the development and testing of new photocatalytic materials.
- 3 **URJC** researchers (Jorge Rodríguez, Carmen García, Victoria Romeral) and one researcher from **ICRA** (Saulo Varela) to **AQUALIA** Estiviel WWTP for 2 weeks.
- Jorge Rodríguez (**URJC**) did a short stay in **ICRA** to assist on the analysis of the huge amount of samples collected from the WWTP.

Water JPI motrem

Infrastructures

URJC: Rotating **biological contactors**, Materials synthesis and characterization equipment (XRD, XRF, SEM, TEM, DR-UV-Vis, ICP-OES), **UV photochemical reactors**, **Pilot WWTP** Technological Support Centre at URJC, **Water analytical laboratory** (LAGUA) of URJC: Physicochemical & Microbiological analysis.

UST: **WWTP** for Education and Research (LFKW, ISWA), municipal **WWTP** of Herbolzheim (Germany). Analytical equipment DOC, TOC, metals, etc and **GC-MS, LC-MS-MS and ICP-MS**.

UH: Waters LCT Premier XE **LC-TOF-MS**, Waters GCT Premier **GC-TOF-MS**, Shimadzu QP2010 **Ultra GC-MS**.

UNITO: **ICP-AES, GC-QTOF-MS, HPLC-MS** (LTQ- Orbitrap, QqQ and QTrap analysers), TOC, HPLC UV-vis and fluorescence detectors, an ion chromatograph and a **Microtox** device.

AQUALIA: Real scale **WWTP** facilities were provided by AQUALIA (Mérida, Tortosa, Benquerencia and Estiviel WWTPs). The full-scale experiments on a UV-C reactor (16 UV-C lamps, WEDECO ELR-30-1; 330 W) and flow rates of 114, 75 and 28 m3/h, (4 – 18 s of UV contact time).

BRUKER: Applications Development Laboratory located in Madrid, **UHPLC–ESI–Q-TOF**, Bruker Maxis; **UHPLC–ESI–IT**, Bruker AmaZon Ion Trap; **UHPLC(OLE)–ESI–TQ**, Bruker EVOQ; and **GC-MS-MS** Triple Quad, Bruker SCION.

Water JPI motrem

Stakeholder/industry engagement

WP4 Dissemination and Exploitation of Project Outcome

Task 4.1 Public and industrial engagement

Objective: To disseminate project results and techniques as widely as possible to scientists, general stakeholders, end-users and public.

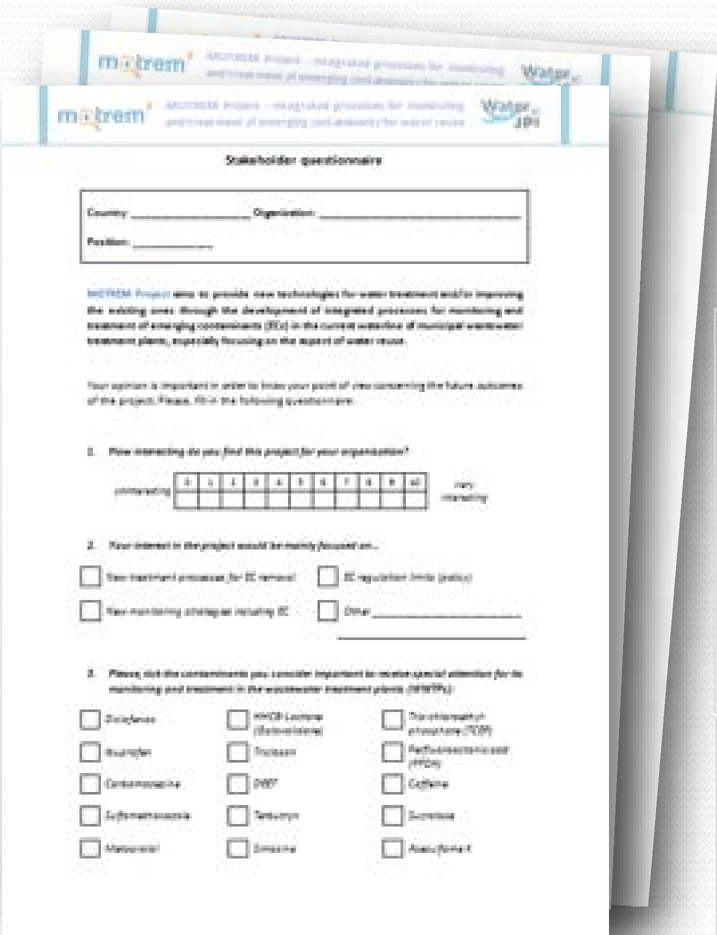
- Stakeholders identification (target market, regulatory authorities, environmental agencies, etc.) to transfer the knowledge developed by MOTREM consortium. **Questionnaires** from stakeholders
- Spread and distribution of knowledge: Publications and Dissemination activities
- Social networks and Website.
- Open international workshop: **23-24 November 2017** (speakers from universities, companies, research centers). Invitation to funding agencies and regulatory agents.
- Training Workshop. On **Friday 24th Nov** at **BRUKER**

Task 4.3 Prospecting plan

- Preliminary study of existing technologies in market

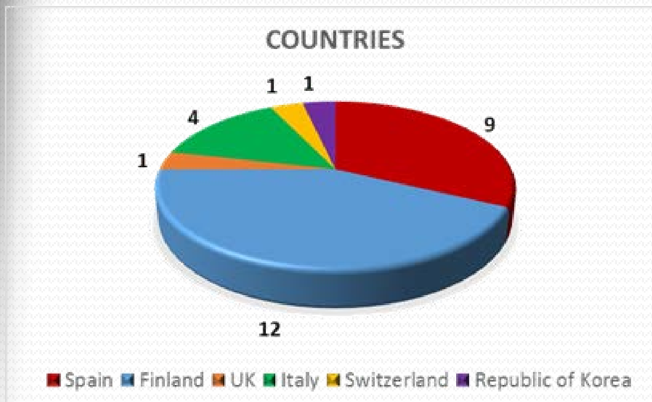
Stakeholder/industry engagement

Questionnaire for stakeholders



- Interest in MOTREM project
  - Treatment, Monitoring, Regulation limits (policy)
- Most important ECs
- National/local legislation
  - Groundwater, Drinking water, Surface water

Etc.…



✓ 6 countries

✓ 9 universities

✓ 5 research centres

✓ 6 private enterprises

✓ 4 public entities

Stakeholder/industry engagement

FCC Aqualia, S.A. is the water management parent company of FCC, one of the largest European services groups. **Aqualia** is the first water management company in Spain, third largest private water company in Europe and sixth in the world, according to the latest ranking by the specialist publication, Global Water Intelligence, and serves 22.5 million users.

4 municipal WWTPs operated by AQUALIA:

- Mérida

- Tortosa

- Benquerencia
- **Estiviel**

Stakeholder/industry engagement

ESTIVIEL WWTP (TOLEDO)

Location: Toledo **Population equivalent:** 270,000 PE **Design flow:** 36,000 m^3/day

Influent: Urban WW **Biological process:** Activated sludge **Effluent discharge:** Tajo River

Tertiary treatment: 270 m^3/day (irrigation / internal industrial use) → Coagulation/Flocculation – Sedimentation – Microfiltration (discs) – UV

Stakeholder/industry engagement

BRUKER
MADRID Lab

Applications Development Laboratory
Chemical & Applied Markets

Provided with most of the last MS technologies from Bruker.

GC/MS/MS Scion™ Triple Quad (2 units)
LC/MS/MS Amazon™ SL Ion Trap (1 unit)
LC/MS/MS QTOF Impact™ II (1 unit)
LC/MS/MS EVOQ™ Triple Quad (2 units)

Water JPI

motrem

Stakeholder/industry engagement

Other companies:

- **DeNora Industries**, a company with several research centres throughout the world and devoted to the development of adsorbent materials to be exploited in water treatment.

- **IRIS**, a micro-enterprise which has a patent application on an innovative AOP device. It develops plasma technology applications to liquid/solid waste treatment, aimed to improve technical and economic efficiency of small scale - on site treatments enabling no waste / zero carbon footprint processes.

- **SMAT**, a large company dedicated to the management of the water cycle. SMAT manages the Integrated Water Service production and distribution of drinking water and waste water in the province of Turin.

Impact and Knowledge Output

Technical impacts:

- New **analytical methods** and protocols for MP.
- Reliable **indicator and surrogate parameters** for MP removal.
- Full scale WWTP tests of **technologies for MP removal** with cost analysis.

Societal impacts:

- Improvement in the wastewater treatment processes
- Assessment of the impact in recipient water bodies.
- Relevant information to set the basis of regulation of the discharges of CECs.
- Wastewater processes may also offer business opportunities to companies.

Impact and Knowledge Output

Local impacts:

- **Estiviel WWTP in Toledo**: influence the **WWTP staff** and the **local authorities** who were not aware of this environmental problem before the project.
- Public and private clients belonging to the third-party funding agencies of the **ISWA**, accept the necessity of adapted monitoring strategies and consider the requirements on appropriate sampling and monitoring strategies. Spillovers of the project tasks are passed on to **stakeholders at the executive level of German Federal states**, where UST is involved in WWTP monitoring programs.

Impact and Knowledge Output

Academic impacts:

- **Lectures** in master courses of the academic partners of MOTREM: UST German lectures for "**Environmental protection technology**", English lectures of the international programs "**Air Quality Control, Solid Waste and Waste Water Process Engineering (WASTE, UST)**" and "**Water Resources Engineering and Management (WAREM, UST)**".
- **More than 40 graduate and post-graduate** students have developed their bachelor, master and PhD thesis in the framework of MOTREM project activities. Here, the results of the project have already reached a large group of international and multidisciplinary working **future engineers**.

Impact and Knowledge Output

- **Publications**
  - International
    - Peer-reviewed journals JCR: **41**
      (**25** published + **10** submitted + **6** in preparation)
    - Communications in conferences: **67**
  - National
    - Communications in conferences: **12**
      (Spain, Germany, Finland, Italy)
- **Dissemination & Popularization**: **10**
  (1 Article + 5 conferences + 4 media appearances)

Events were **administration representatives** or **general public** were present

FuturEnviro, Nov 2015

Impact and Knowledge Output

Website: http://motrem.eu

Facebook : https://www.facebook.com/motremproject

Twitter: @MotremProject

Continuation of work in the future

The **complementary expertise** of the partners of MOTREM project has been successfully demonstrated and exploited during the project, as the joint publications and collaborative activities have proven.

The synergistic profiles on different areas such as:

- Treatment technologies (URJC),
- Monitoring and control of WWTP (UST),
- Analytical methodologies (UH),
- Mechanistic and toxicological studies (UNITO) and
- Full-scale applications (AQUALIA)
- Partners from other Water JPI funded projects.

Will be definitively exploited in the future in the form of **new project proposals and collaborations**.

Water JPI motrem

Continuation of work in the future

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- Partners from other Water JPI funded projects.

Will be definitively exploited in the future in the form of **new project proposals and collaborations**.

Looking for funding….

motrem A Water JPI Project

Thanks!

MOTREM

Javier Marugán (URJC, coord.),
Bertram Kuch (UST),
Jukka Pellinen (UH),
Paola Calza (UNITO),
Frank Rogalla (AQUALIA),
Pedro Cano (BRUKER)

Water JPI
Pilot Call Final Meeting
4th of June 2018, Helsinki

PROMOTE

PROMOTE

PROtecting water resources from MObile Trace chEmicals

Urs Berger, Hans Peter H. Arp, Hervé Gallard, Thomas P. Knepper, Michael Neumann, José Benito Quintana, Pim de Voogt, Thorsten Reemtsma

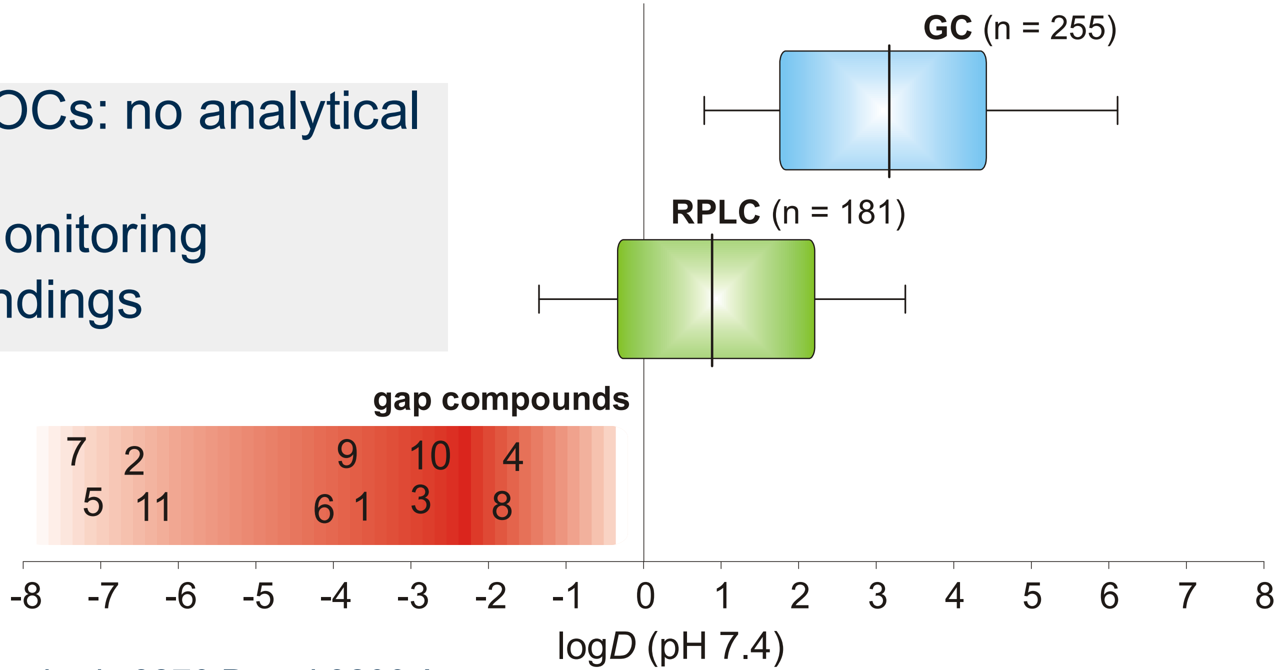
Water JPI final evaluation meeting, Helsinki, June 4, 2018

HELMHOLTZ CENTRE FOR ENVIRONMENTAL RESEARCH – UFZ

Background: Persistent and mobile organic chemicals (PMOCs)

Polarity (logD_{ow}) of analytes covered by GC- or RPLC-MS analysis

For PMOCs: no analytical method
→ no monitoring
→ no findings



GC-MS: EPA methods 8270 D and 8290 A

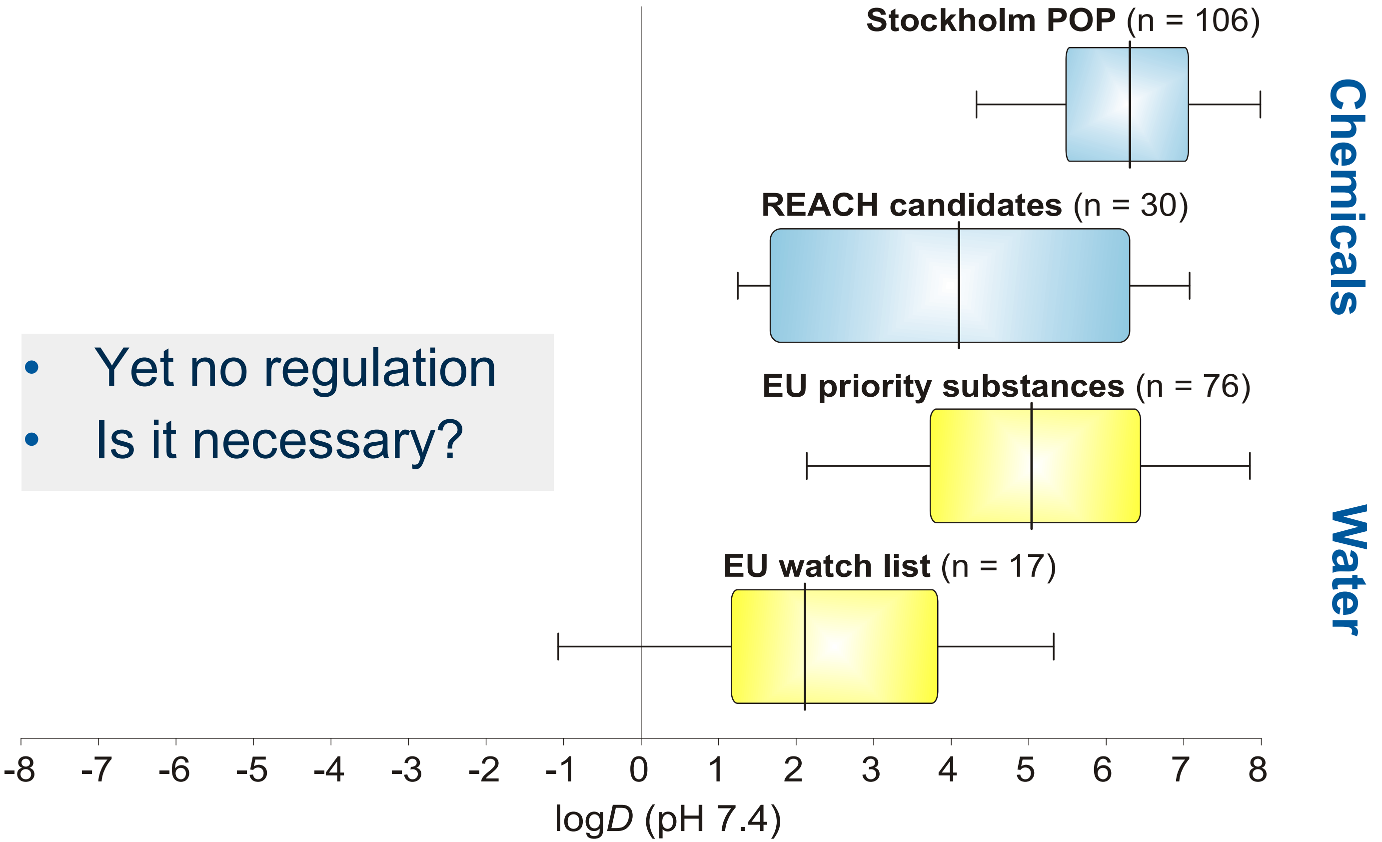
LC-MS: Schymanski et al. (2014) Environ. Sci. Technol. 48, 1811-1818

1: Aminomethylphosphonic acid (AMPA), 2: Paraquat, 3: Cyanuric acid, 4: DMS, 5: Diquat, 6: 5-Fluorouracil, 7: Glyphosate, 8: Melamine, 9: Metformin, 10: Perfluoroacetic acid, 11: EDTA

Reemtsma et al. (2016) Environ. Sci. Technol. 50, 10308

Background: Persistent and mobile organic chemicals (PMOCs)

- Yet no regulation
- Is it necessary?



REACH candidates of SVHC, REACH, Article 57, d-f

Priority substances according to Water Framework Directive (WFD)

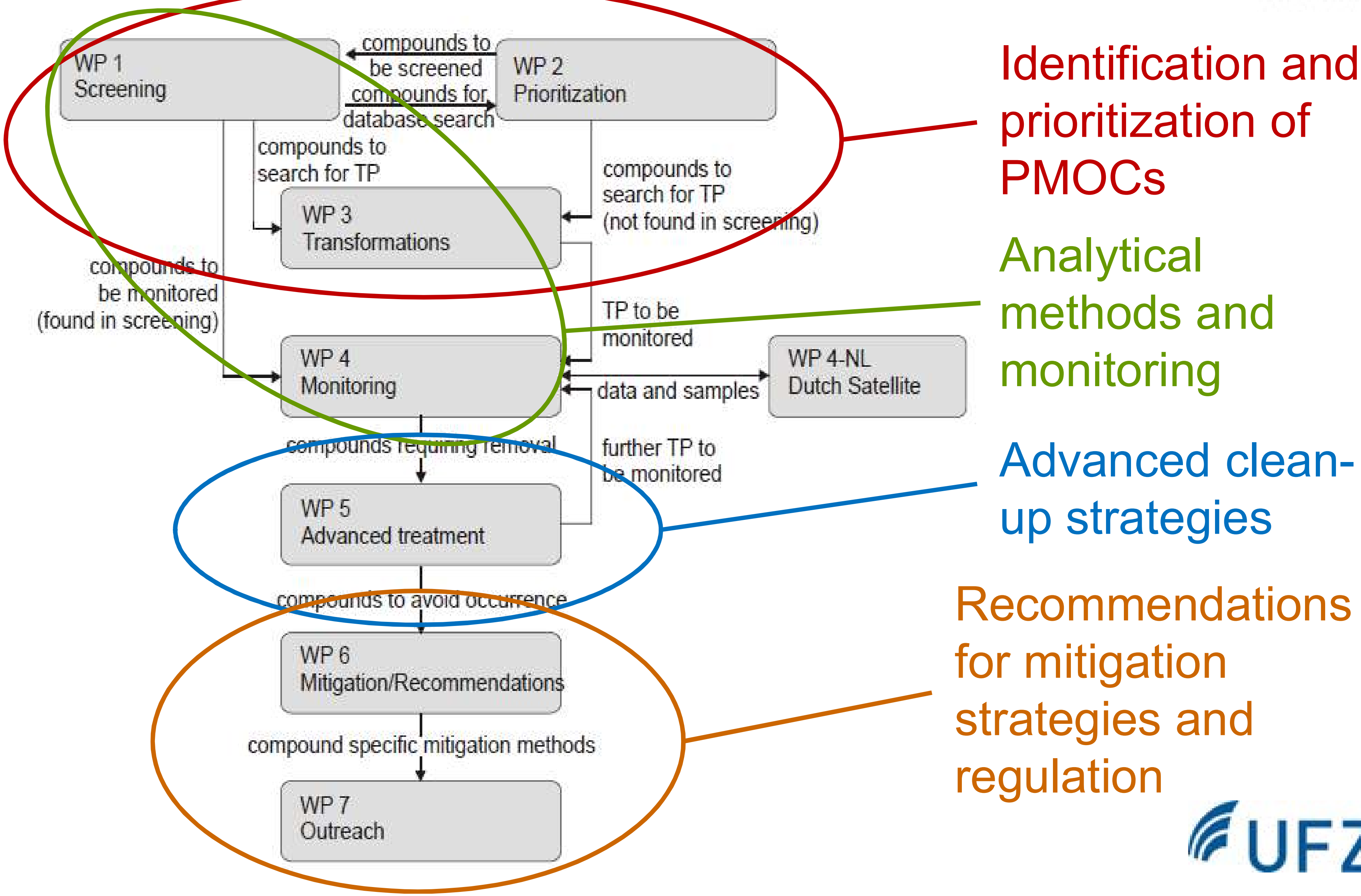
Watch list of the WFD

Reemtsma et al. (2016) Environ. Sci. Technol. 50, 10308

Overarching aim

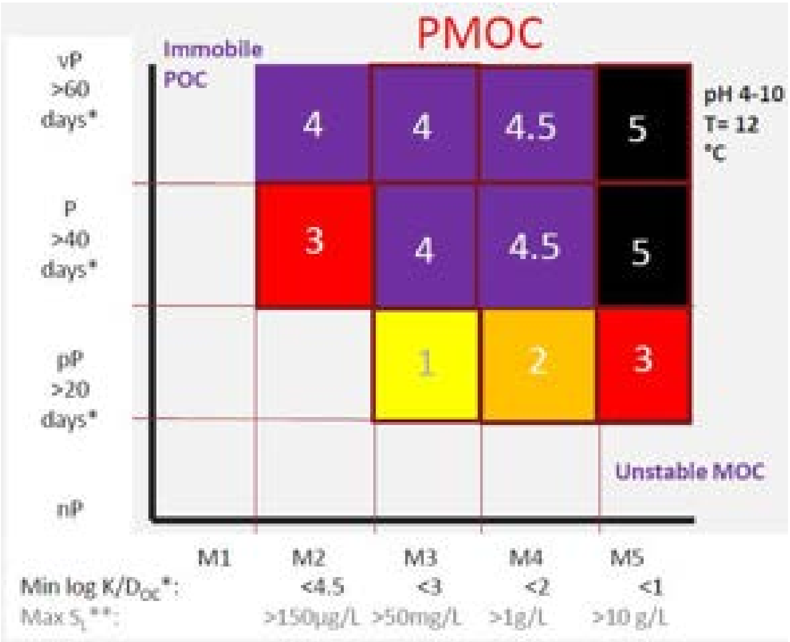
- PROMOTE aimed at answering the question whether there is a need as well as the potential for regulatory protection of drinking water resources with respect to PMOCs
- PROMOTE linked European chemicals policy (REACH) with water policy (e.g. WFD)

PROMOTE work flow



Results: Identification and prioritization of PMOCs by modeling

(H.P.H. Arp, M. Neumann, U. Berger, T. Reemtsma)



Estimating P and M

Arp et al. (2017) Environ. Sci. Process Impacts, 19, 939-955

Use descriptors

Marketing volumes

Ranking emission potential

Schulze et al. (2018) Sci. Total Environ., 625, 1122-1128

→ Ranked list of 1100 suspected PMOCs

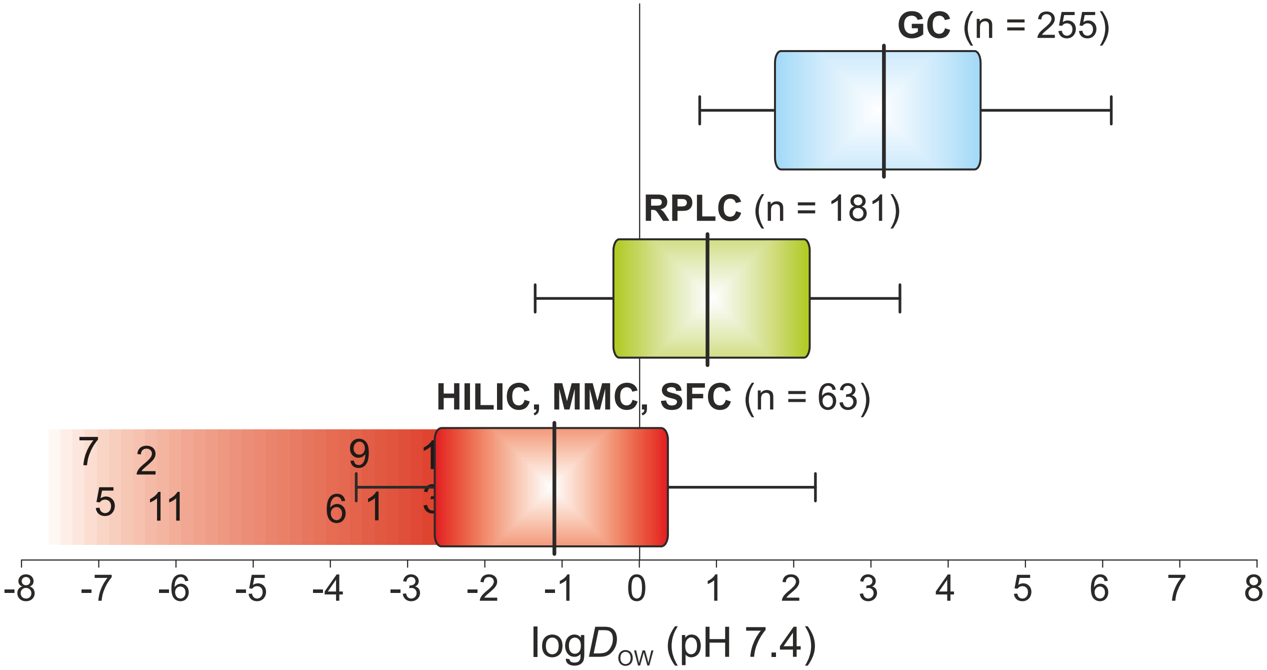
→ 70 compounds chosen for monitoring

Results: Analytical methods

(J.B. Quintana, T. Knepper, U. Berger, T. Reemtsma)

"Novel" separation methods (chromatography) and detection (MS)

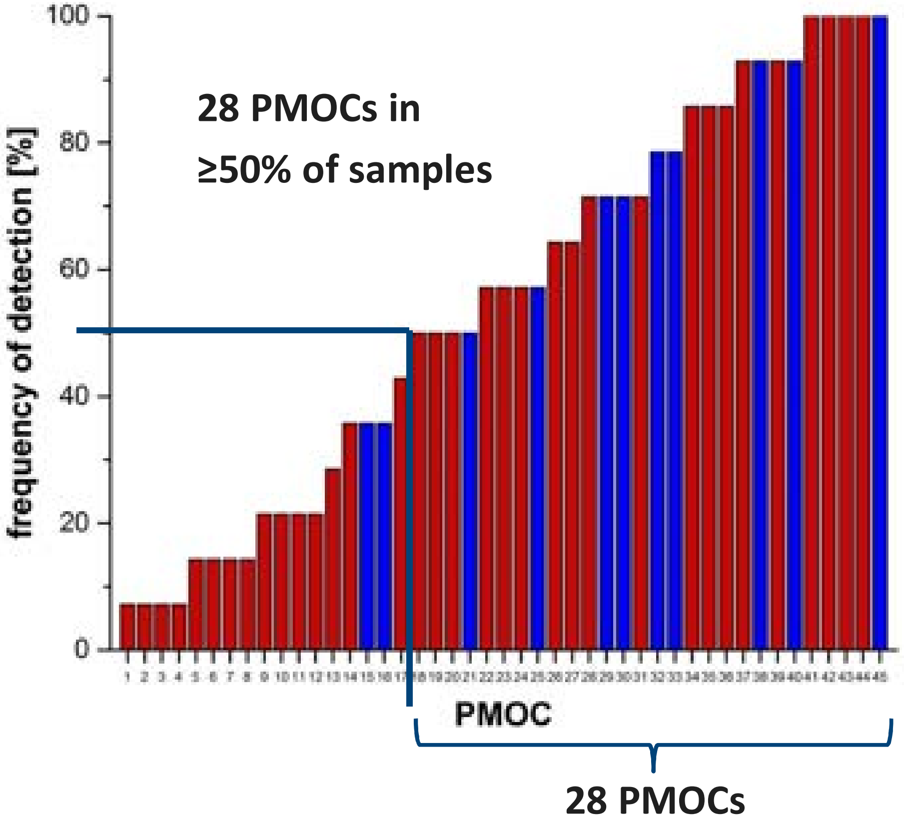
- Supercritical Fluid Chromatography (**SFC**)-qTOF-HRMS
- Hydrophilic Interaction Liquid Chromatography (**HILIC**)-MS/MS
- Mixed Mode Liquid Chromatography (**MMLC**)-qTOF-HRMS



Results: Environmental monitoring

(U. Berger, J.B. Quintana, T. Knepper, P. de Voogt, T. Reemtsma)

14 water samples (surface water, groundwater, bank filtrate, from drinking water treatment steps) from 5 European countries analyzed



A total of 45 (of 70 analyzed) PMOCs detected

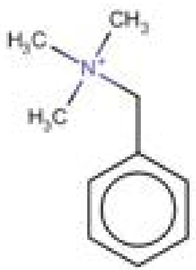
- Some PMOCs frequently detected, others in single samples
- Detection of „**known**" as well as „**novel**" PMOCs

Results: Environmental monitoring

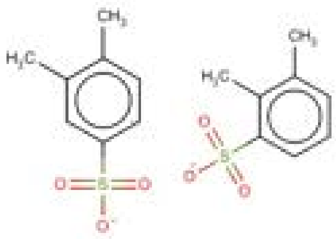
(U. Berger, J.B. Quintana, T. Knepper, P. de Voogt, T. Reemtsma)

Examples of „**novel**“ PMOCs

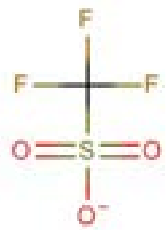
Benzyltrimethyl ammonium



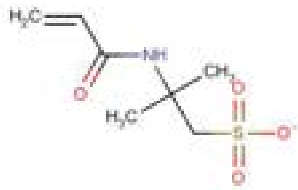
Dimethylbenzene sulfonic acid



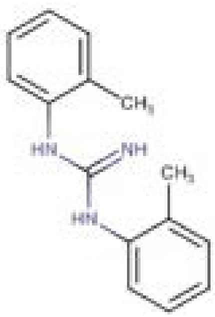
Trifluoro and Cl/Br methanesulfonic acids



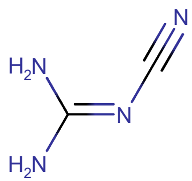
2-Acrylamido-2-methylpropane sulfonic acid



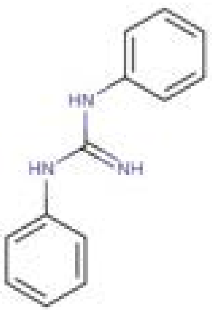
1,3-Di-*o*-tolylguanidine



Cyanoguanidine



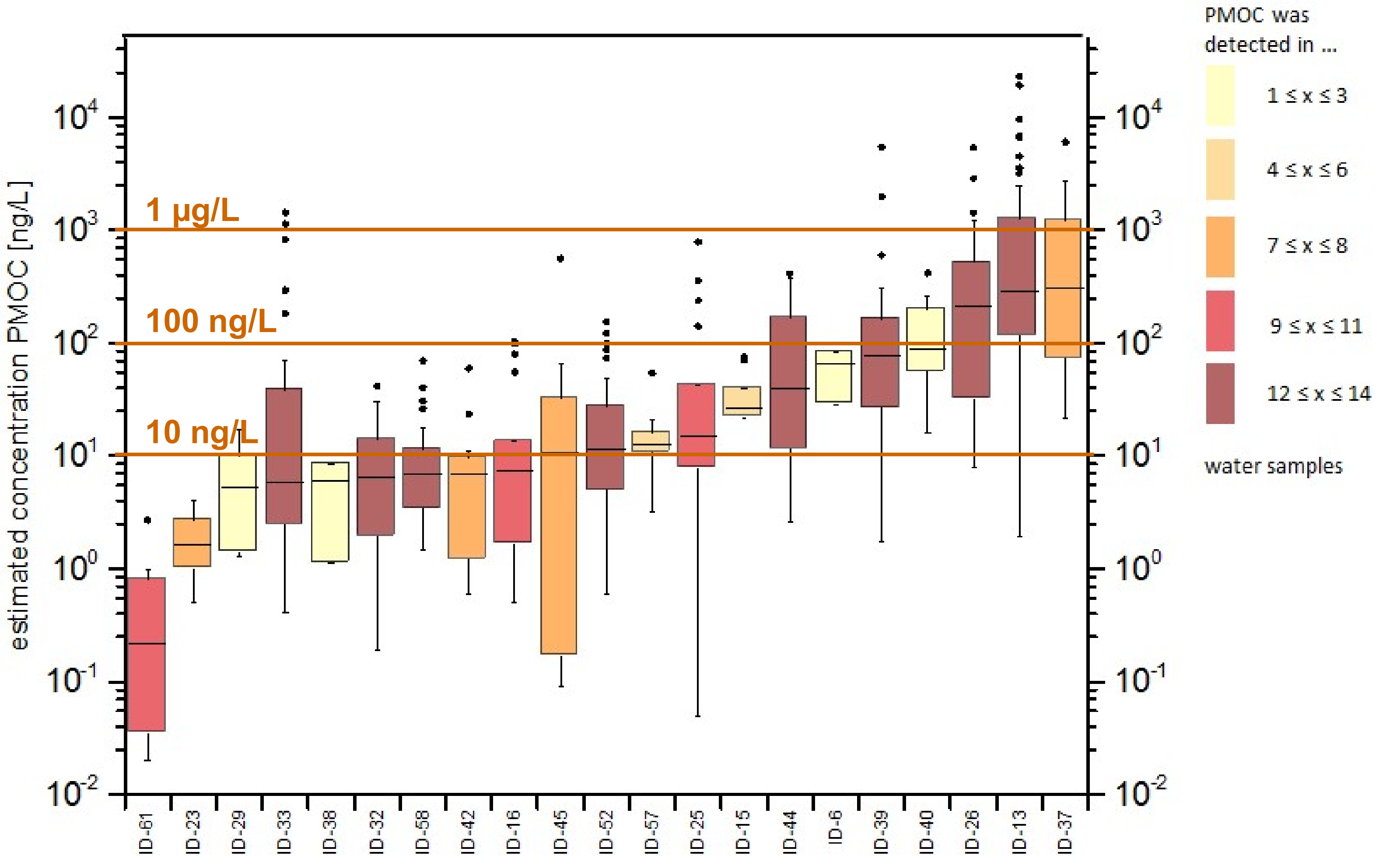
1,3-Diphenylguanidine



- Processing agents in
  - polymerization
  - vulcanization
  - production of resins
- Tires and rubber
- Disinfectants
- Washing and cleaning agents
- Textile industrie
- Water treatment
- Fertilizer

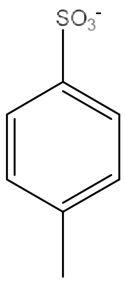
Results: Environmental monitoring

(U. Berger, J.B. Quintana, T. Knepper, P. de Voogt, T. Reemtsma)

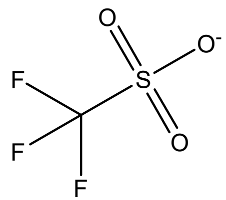


Results: Advanced drinking water treatment (H. Gallard, J.B. Quintana)

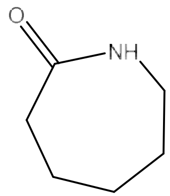
Examples of PMOCs that are only poorly or not at all removed by ozonation and activated carbon treatments



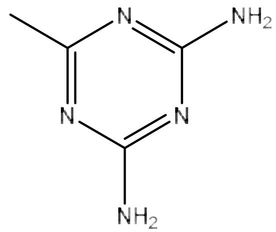
p-Toluenesulfonate



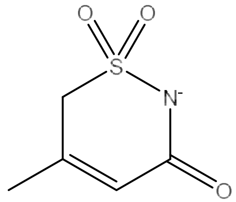
Trifluoromethanesulfonate



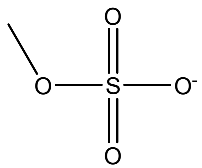
ε-Caprolactam



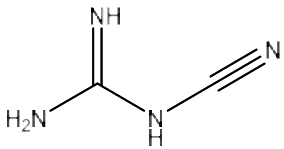
Acetoguanamine



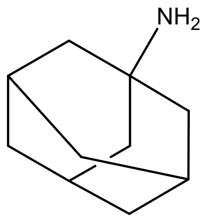
Acesulfame



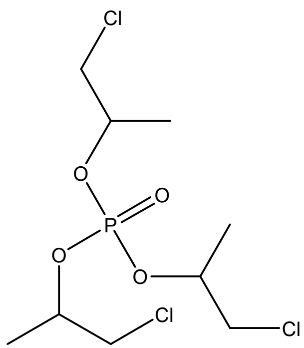
Methylsulfate



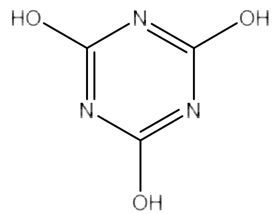
Cyanoguanidine



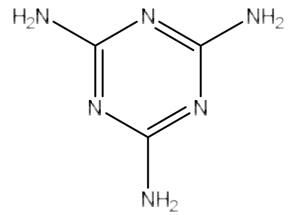
Adamantan-1-amine



TCPP



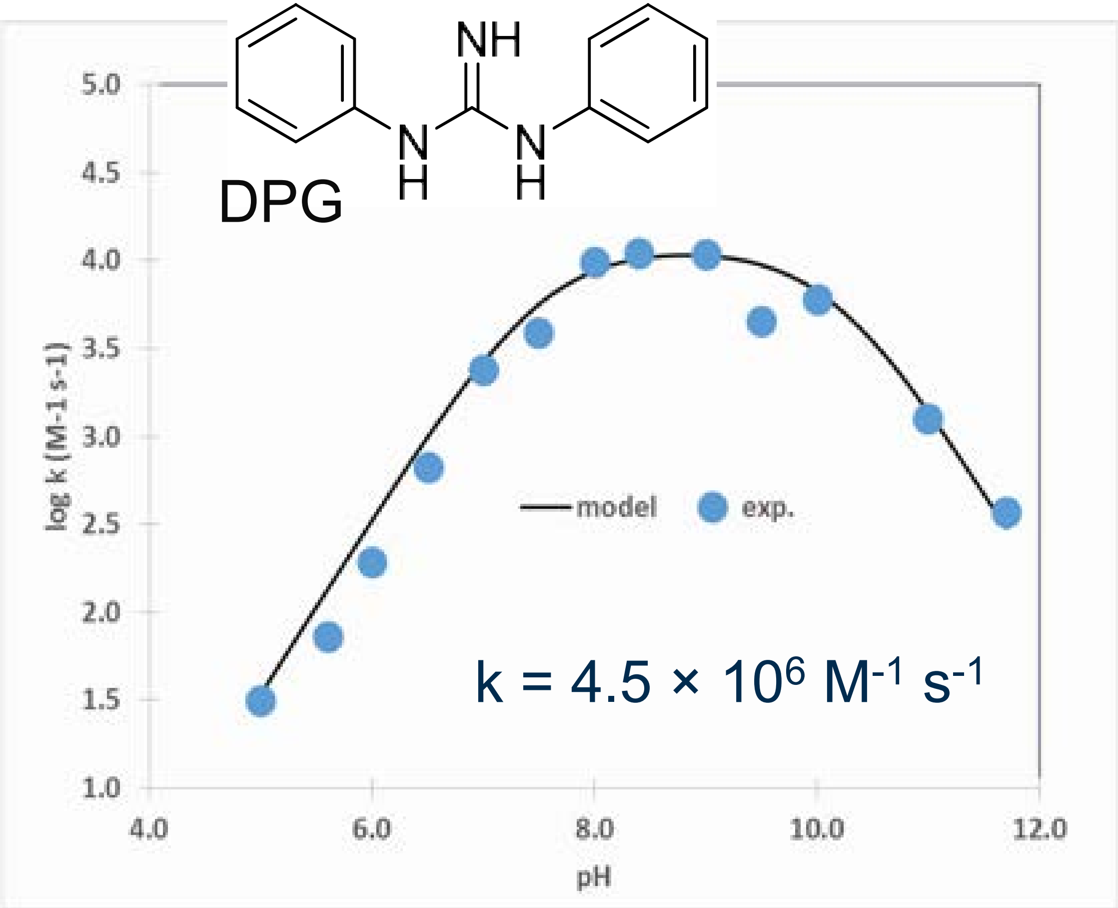
Cyanuric acid



Melamine

Results: Advanced drinking water treatment (H. Gallard, J.B. Quintana)

Other options? → Transformation with chlorine



Very fast reaction with chlorine

$t_{1/2}$ <10 s (1 mg Cl_2/L, pH >7)

BUT

A suite of chlorinated TPs formed that are more persistent and more toxic than DPG!

Kinetic model

HOCl + DPG → Products k

$HOCl = ClO^- + H^+$ Ka_{HOCl}

$DPG^+ = DPG + H^+$ Ka_G

Results: Advanced drinking water treatment (H. Gallard, J.B. Quintana)

Other options? → High pressure membrane processes

- **Nanofiltration (NF) as a polishing treatment after conventional treatments**
  - Efficiency depends mainly on the molecular weight (MW) and MW cut-off of the membranes (e.g. NF90 or NF270)
  - Removal rates between 10-20% (small molecules <200-220 g mol^{-1}) and 90% (largest molecules) for NF270 membrane

- **Reverse Osmosis (RO) instead of conventional treatments**
  - Removal rates usually >80% (e.g. 86% for Caprolactam at 8 bars with BW30LE membrane)
  - High removal rates for ionic (even small) compounds (repulsion)
  - Lower removal rates for neutral small compounds (diffusion)
  - Practicability?

Collaboration

- Highly collaborative environment, strong interactions between WPs and between partners (each WP involved several partners)

  Examples

  - Analytical method transfer
  - PMOC identification and prioritization
  - Sampling campaigns (associated partners)
  - “Transformation” and “Advanced Treatment” WPs
  - Currently ongoing: Recommendations for mitigation

Coordination, mobility

- **Coordination** and organization was efficient, based on the active participation of all partners and associated partners supporting the consortium
- **Mobility**: 5 mobility actions performed
  - Method transfer
  - Emission model
  - Regulation (PMOCs as SVHC)
  - Identification of TPs
  - Advanced water treatment methods

Stakeholder engagement

- Important stakeholders were directly involved as partners (German Environment Agency) or associated partners (drinking water suppliers) → prerequisite for accomplishment of objectives
- Europe-wide dissemination workshop for stakeholders

Persistent and Mobile Organic Chemicals in the Water Cycle:

Linking science, technology and regulation to protect drinking water quality

23 – 24 November 2017, in Leipzig, Germany

Contributions from (amongst others)

- European Commission DG Environment
- ECHA
- European Chemical Industry
- National environmental protection agencies
- EU CIS Working Group on Groundwater

Impact and knowledge output

- Scientific papers: 10 published/10 manuscripts
- Feature article in ES&T chosen as third runner up of ES&T's best Feature Article of the year 2016
- Project results feed into PMT/vPvM regulatory efforts
- Prioritized PMOC list and analytical methods published → way forward is mapped

Continuation of the work in the future

- Two partners and stakeholder have applied for a follow-up project in Germany (BMBF)
- Two partners collaborate on PMT/vPvM regulatory issues
- Two partners and stakeholders prepare a follow-up proposal for INTERREG
- Several single-partner follow-up activities ongoing

Conclusions from PROMOTE

- Number of PMOCs in European water cycles much larger than anticipated
- Analytical gap narrowed, but still many PMOCs not covered by current screening methods
- Water quality monitoring should direct more effort towards PMOCs to ensure drinking water quality
- European chemicals' legislation should consider mobility
- TPs tend to be more mobile than parent chemicals and need to be considered

Acknowledgements

Funding

Water Challenges for a Changing World
Joint Program Initiative (Water JPI)
Pilot Call 2013

- German Federal Ministry for Education and Research (02WU1347A/B)
- Research Council of Norway (241358/E50)
- French Office National de l'Eau et des Milieux Aquatiques (project PROMOTE)
- Spanish Ministry of Economy and Competitiveness (JPIW2013-117)

GEFÖRDERT VOM
Bundesministerium für Bildung und Forschung

Water JPI

Forskningsrådet

MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD

ONEMA
Office national de l'eau et des milieux aquatiques

Thank you for your attention!

UFZ

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FACULTY OF BIOTECHNOLOGY
PORTO

Stopping Antibiotic Resistance Evolution

Final meeting

Helsinki, 4th June 2018

Célia M. Manaia
ESB-UCP

Water JPI

StARE

StARE

Consortium
11 partners – 7 countries

Norwegian School of Veterinary Science

University of Helsinki

TECHNISCHE UNIVERSITÄT DRESDEN

Technische Universität Dresden

NUI Maynooth

NUI MAYNOOTH

Ollscoil na hÉireann Má Nuad

Escola Superior de Biotecnologia – Universidade Católica Portuguesa

KIT

Karlsruher Institut für Technologie

Karlsruhe Institute of Technology (KIT), IFG

Aquantec

Aquantec GmbH

University of Aveiro

CSIC

Agencia Estatal Consejo Superior de Investigaciones Cientificas

ICRA

Institut Català de Recerca de l'Aigua

Catalan Institute for Water Research (ICRA)

University of Cyprus

nireas

Nireas International Water Research Center, University of Cyprus

Consortium

Microbiology * Molecular Biology * Bioinformatics
Analytical Chemistry * Wastewater Treatment Engineering

UCP
Célia Manaia
Ivone Vaz-Moreira
Jaqueline Rocha
Carlos Narciso-Rocha
Pompeyo Ferro

ICRA
Sara Rodriguez-Mozaz
Marta Llorca
Saulo Varela

CSIC
Jose Luis Martinez
Felipe Lira
Javier Tamames

Nireas-IWRC, UCY
Despo Fatta-Kassinos
Irene Michael-Kordatou
Lida Ioannou
Stella Michael

NSVS
Henning Sorum
Kristin O'Sullivan

UAVR
Isabel Henriques
Marta tacão

TUD
Thomas Berendonk
Damiano Caccace

KIT-IFG
Thomas Schwartz
Thomas Jäeger

NUIM
Fiona Walsh

UHel
Marko Virta
Katariina Pärnänen

AQUANTEC
Christian Elpers

NORMAN
Network of reference laboratories, research centres and related organisations for monitoring of emerging environmental substances
Valeria Dúlio
Jaroslav Slobodnik

Center for Microbial Ecology Michigan State University, US
Robert Stedtfeld
James Tiedje

GLOBAL ACTION PLAN ON ANTIMICROBIAL RESISTANCE

World Health Organization

3/5

Objective 1: Improve awareness and understanding of antimicrobial resistance through effective communication, education and training

Objective 2: Strengthen the knowledge and evidence base through surveillance and research

Objective 3: Reduce the incidence of infection through effective sanitation, hygiene and infection prevention measures

StARE Overview

Scientific and technological results

Collaboration, coordination, mobility, synergies and infrastructures

Stakeholder engagement

Impact and knowledge output

Continuation of the work in the future

StARE Overview

Scientific and technological results

Collaboration, coordination, mobility, synergies and infrastructures

Stakeholder engagement

Impact and knowledge output

Continuation of the work in the future

Objectives

1. Antibiotics and Resistance in European Wastewater

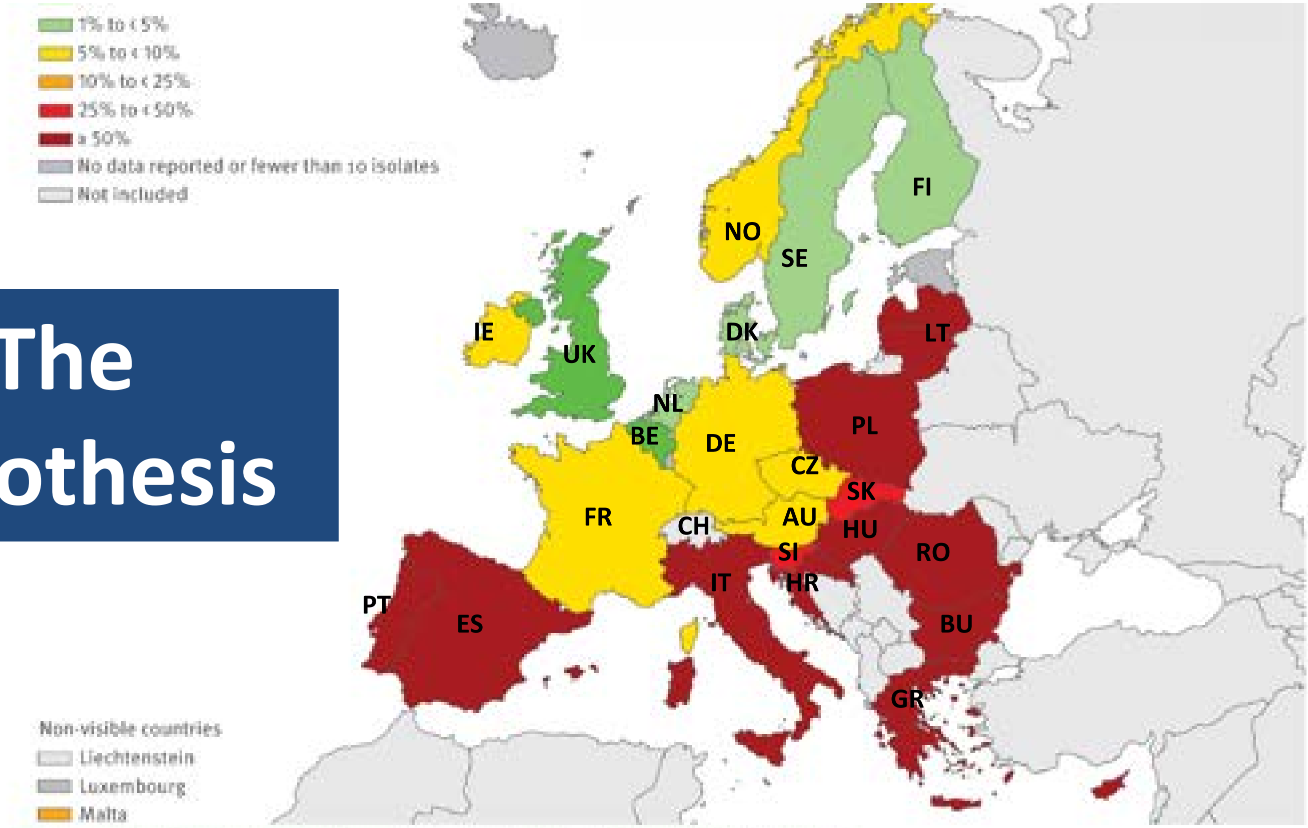
- **Implement harmonized** (advanced) **protocols to measure A & ARG**
- **Launch a public database on A&ARB&ARG**

2. Wastewater treatment and removal of A&ARB&ARG

- **Improved A&ARB&ARG mitigation in UWTP**
  - Cost-effective wastewater treatment
  - Minimal impact on ARG dissemination, by selection or horizontal gene transfer

Antibiotic resistance in European wastewaters

ANTIBIOTIC RESISTANCE IN CLINICAL ISOLATES IN EUROPE…



Objective 1

The hypothesis

Surveillance of antimicrobial resistance in Europe (2013-2016)

Except for carbapenem resistance, large inter-country variations were noted for all antimicrobial groups under regular surveillance, with generally **higher resistance percentages reported from the southern and eastern** parts of Europe than from northern.

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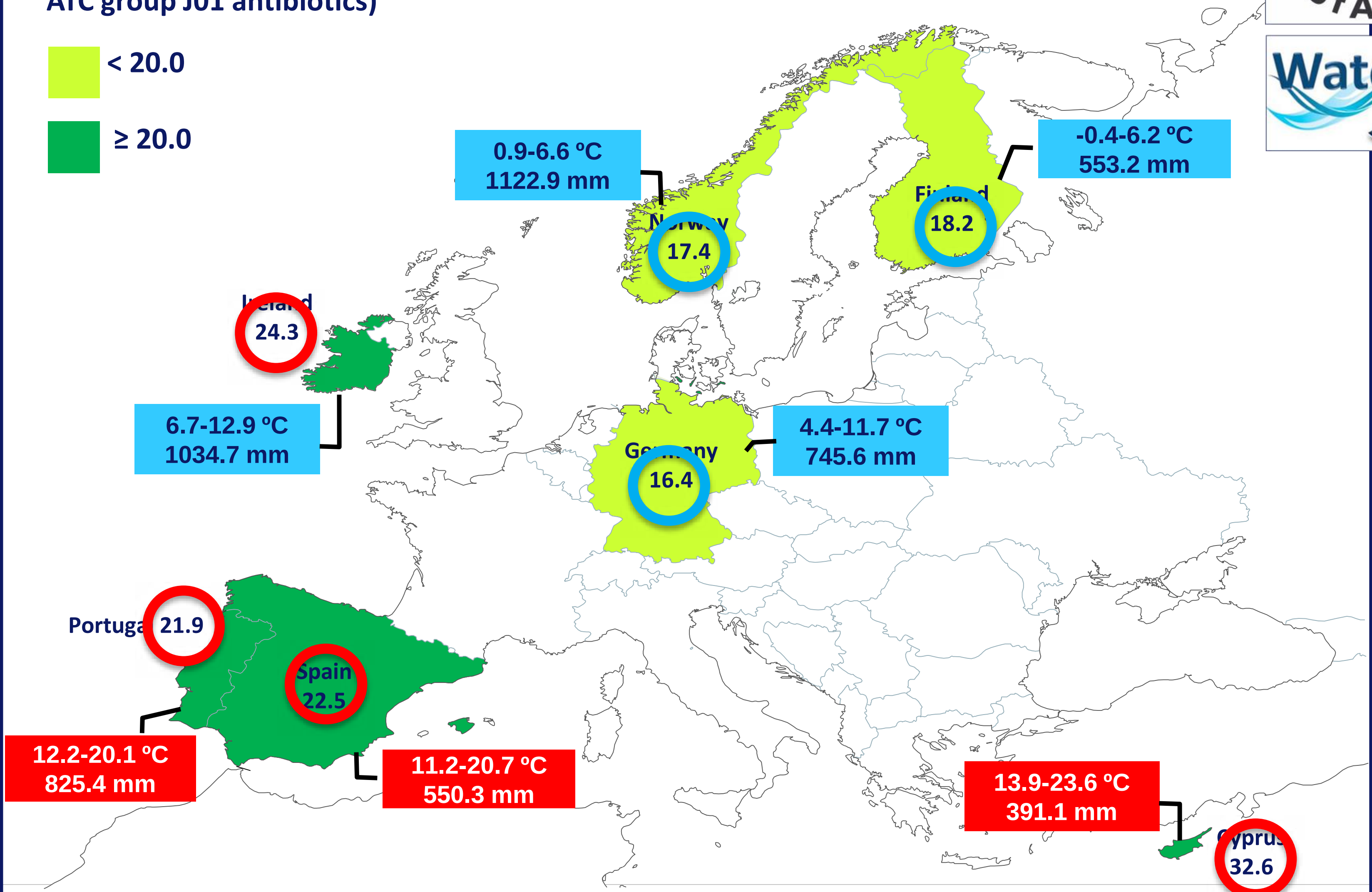
Antibiotic resistance in European wastewaters

DDD *per* 1000 inhabitants and *per* day (systemic use ATC group J01 antibiotics)

< 20.0

≥ 20.0



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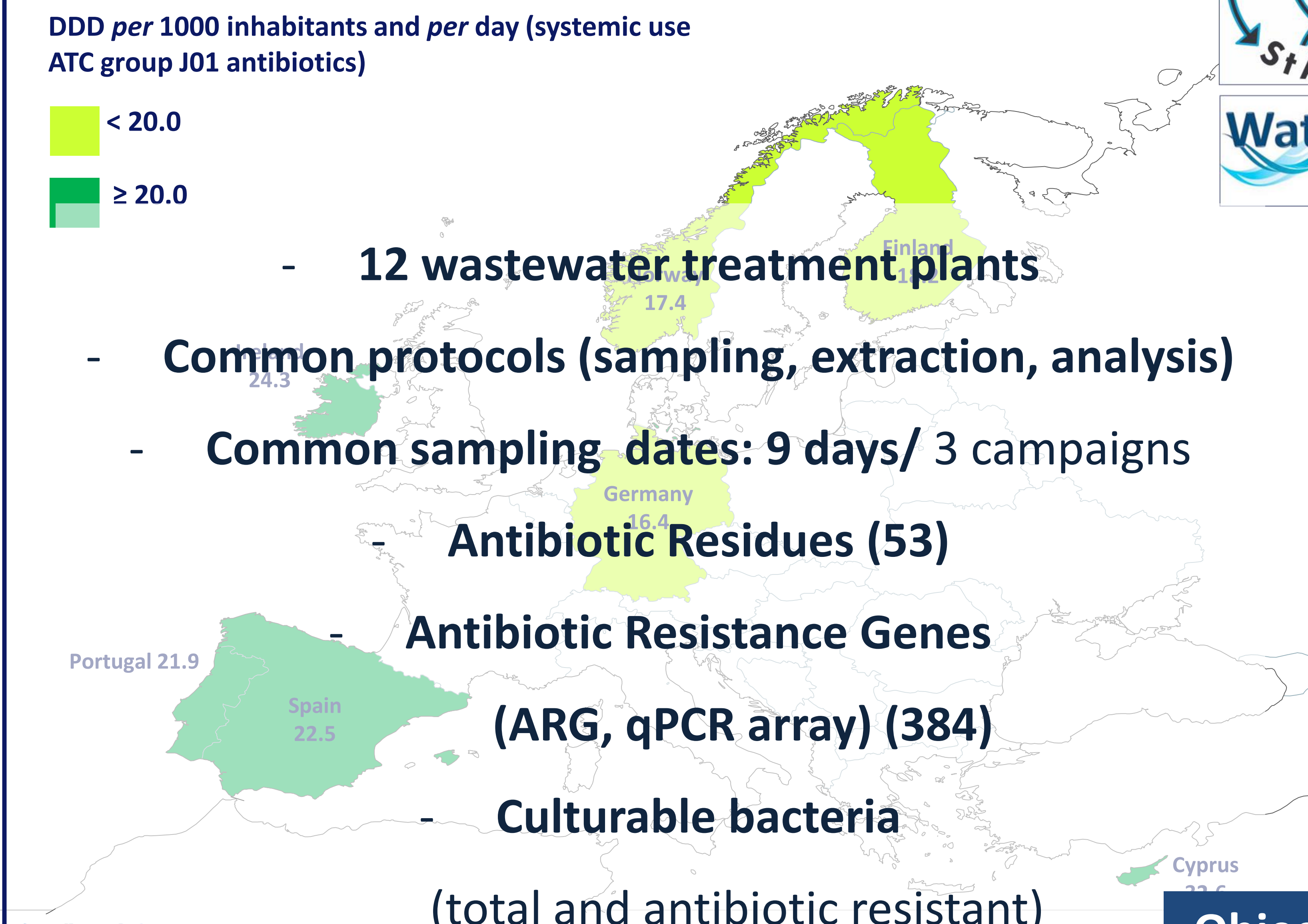
Approach



- **12 wastewater treatment plants**
- **Common protocols (sampling, extraction, analysis)**
- **Common sampling dates: 9 days/** 3 campaigns
- **Antibiotic Residues (53)**
- **Antibiotic Resistance Genes (ARG, qPCR array) (384)**
- **Culturable bacteria** (total and antibiotic resistant)

Objective 1

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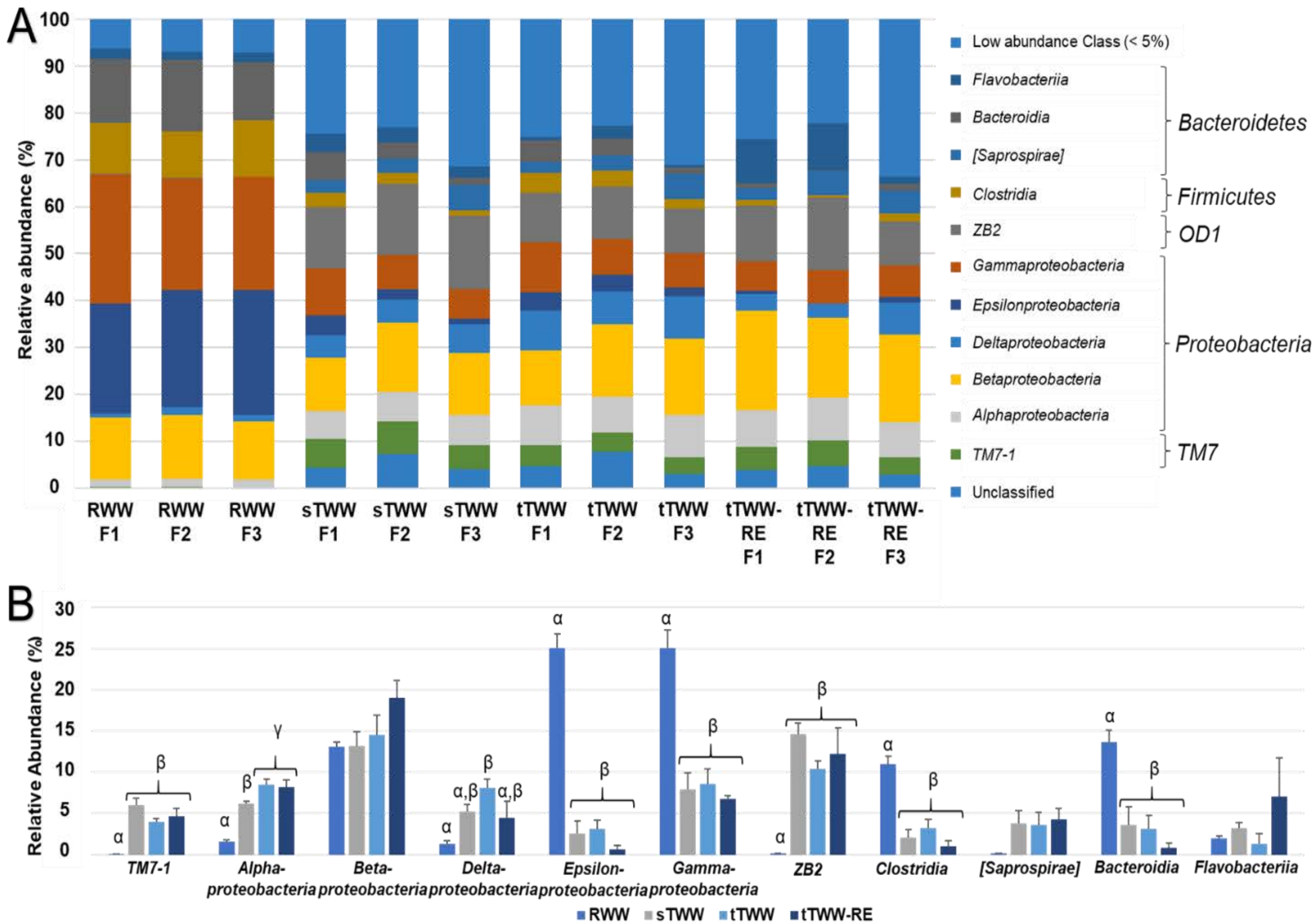
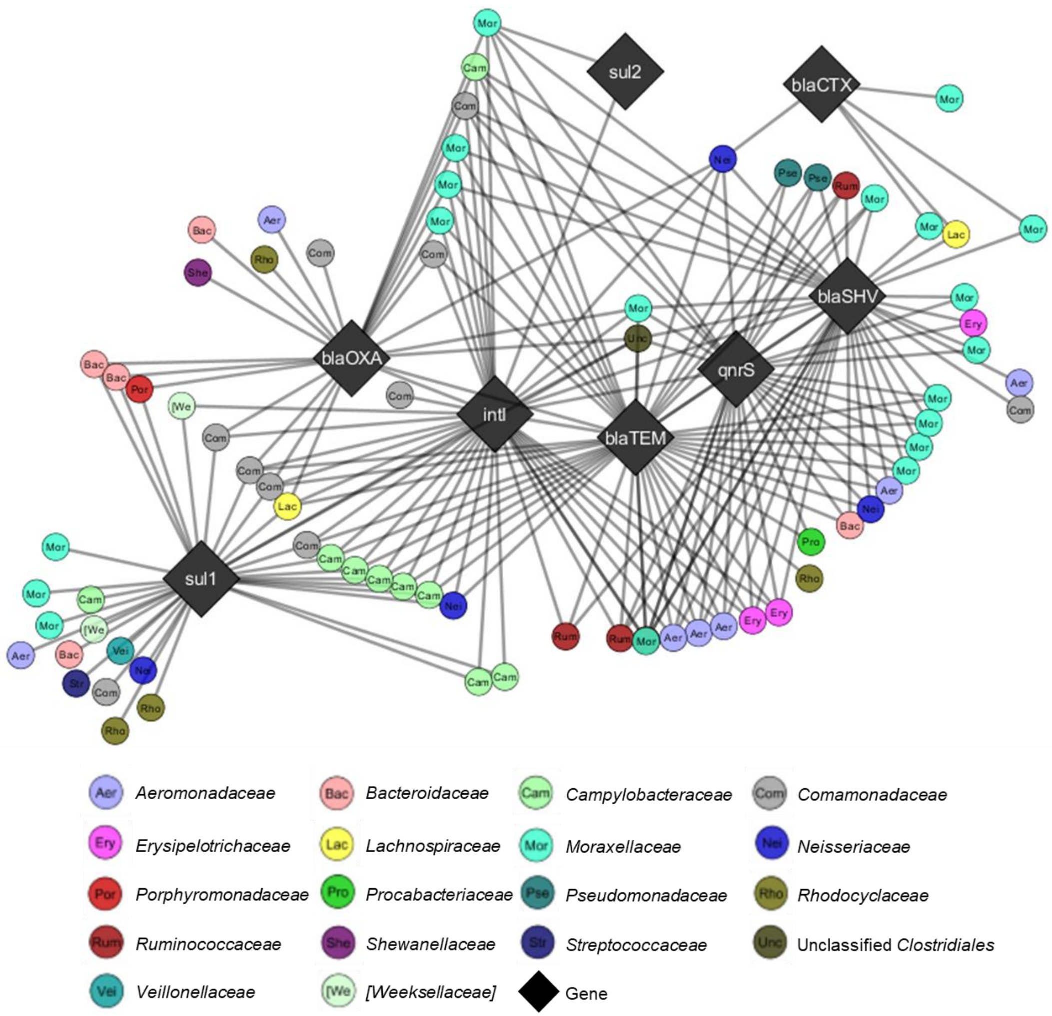
Microbiome dynamics during treatment



Rocha et al., 2018., Env. International, in press

Objective 2

ARGs associated with human- and animal related bacteria



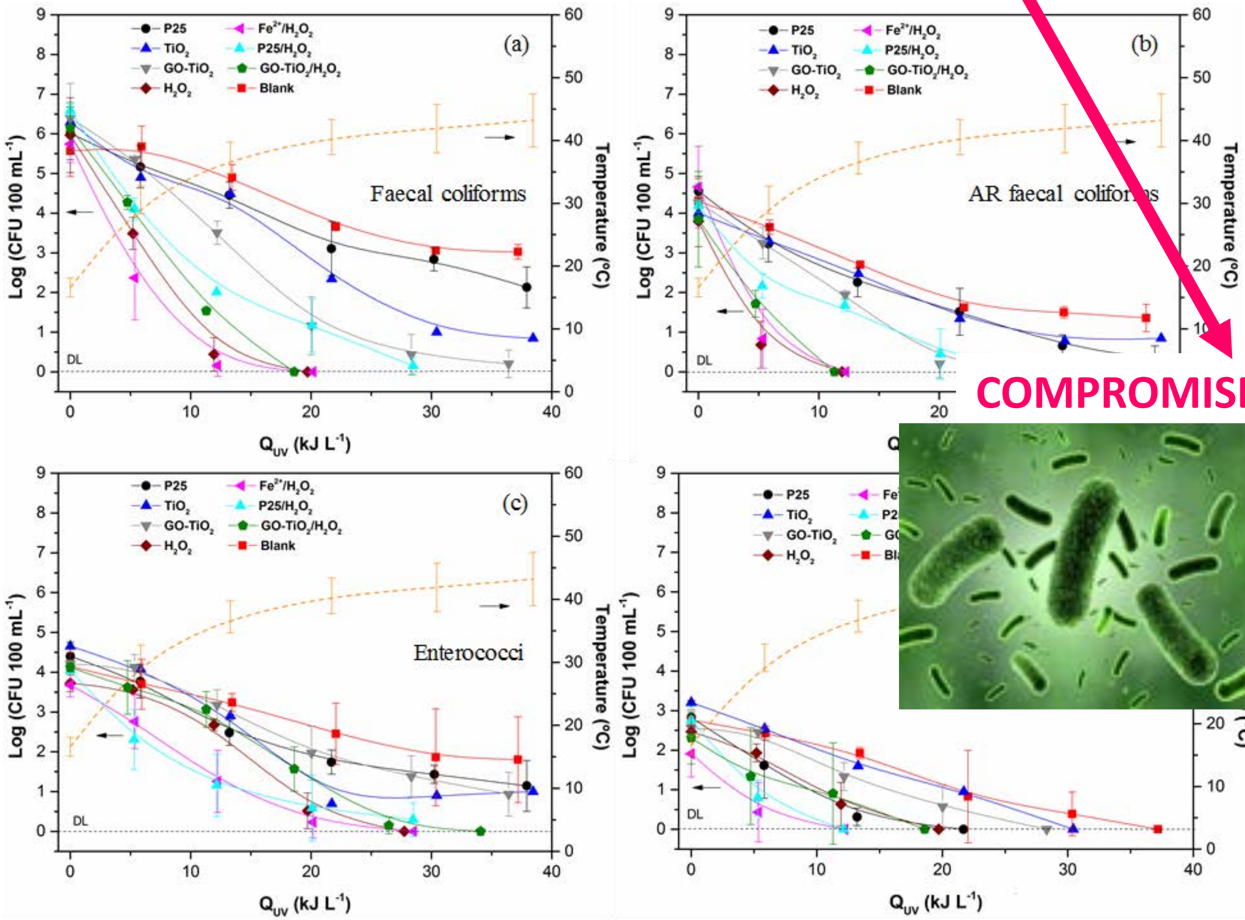
Rocha et al., 2018., Env. International, in press

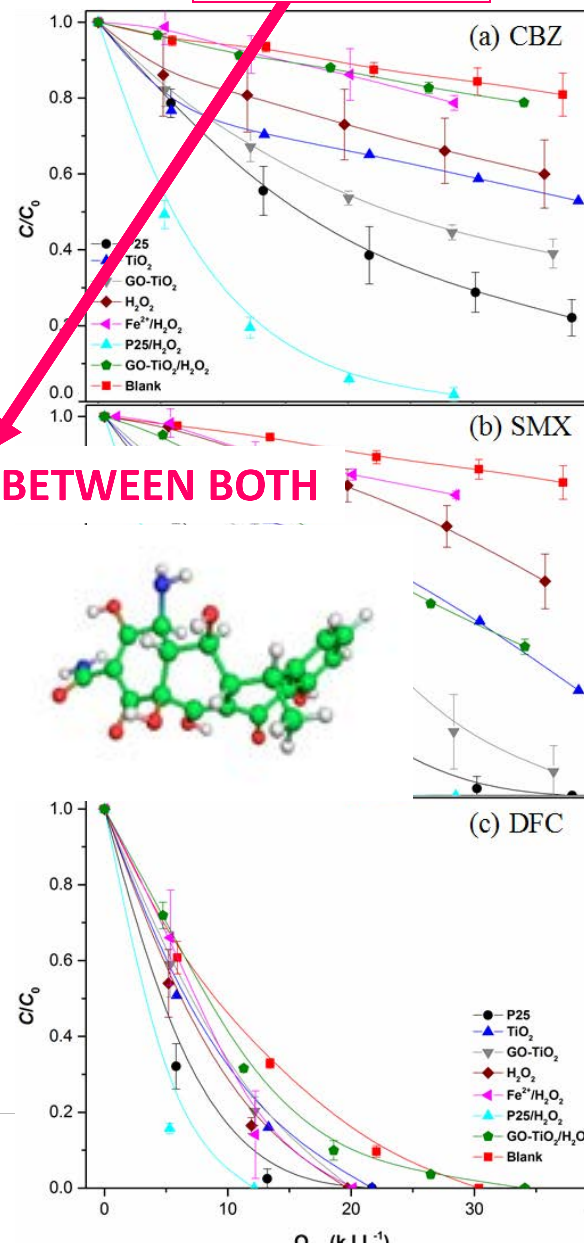
Objective 2

ADVANCED DISINFECTION PROCESSES

Microbiological

Chemical





COMPROMISE BETWEEN BOTH

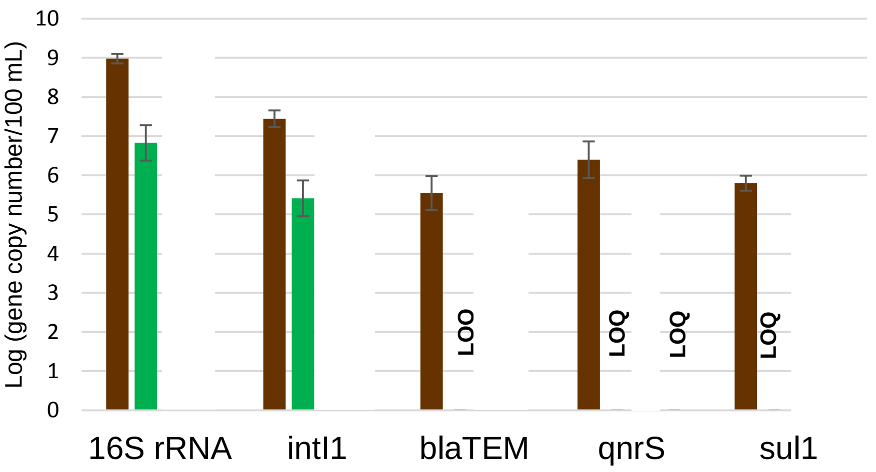
Objective 2

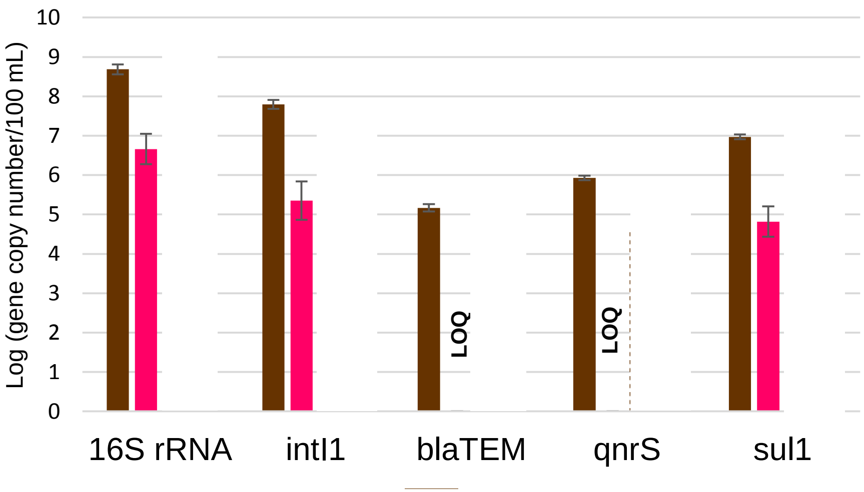
Moreira et al. 2018, Water Research, 135:195-206

ADVANCED DISINFECTION PROCESSES

Self-replication

Ozonation **UV**





Untreated control

Treated

Sousa et al, J Hazard Mater. 2017 5;323(Pt A):434-441.

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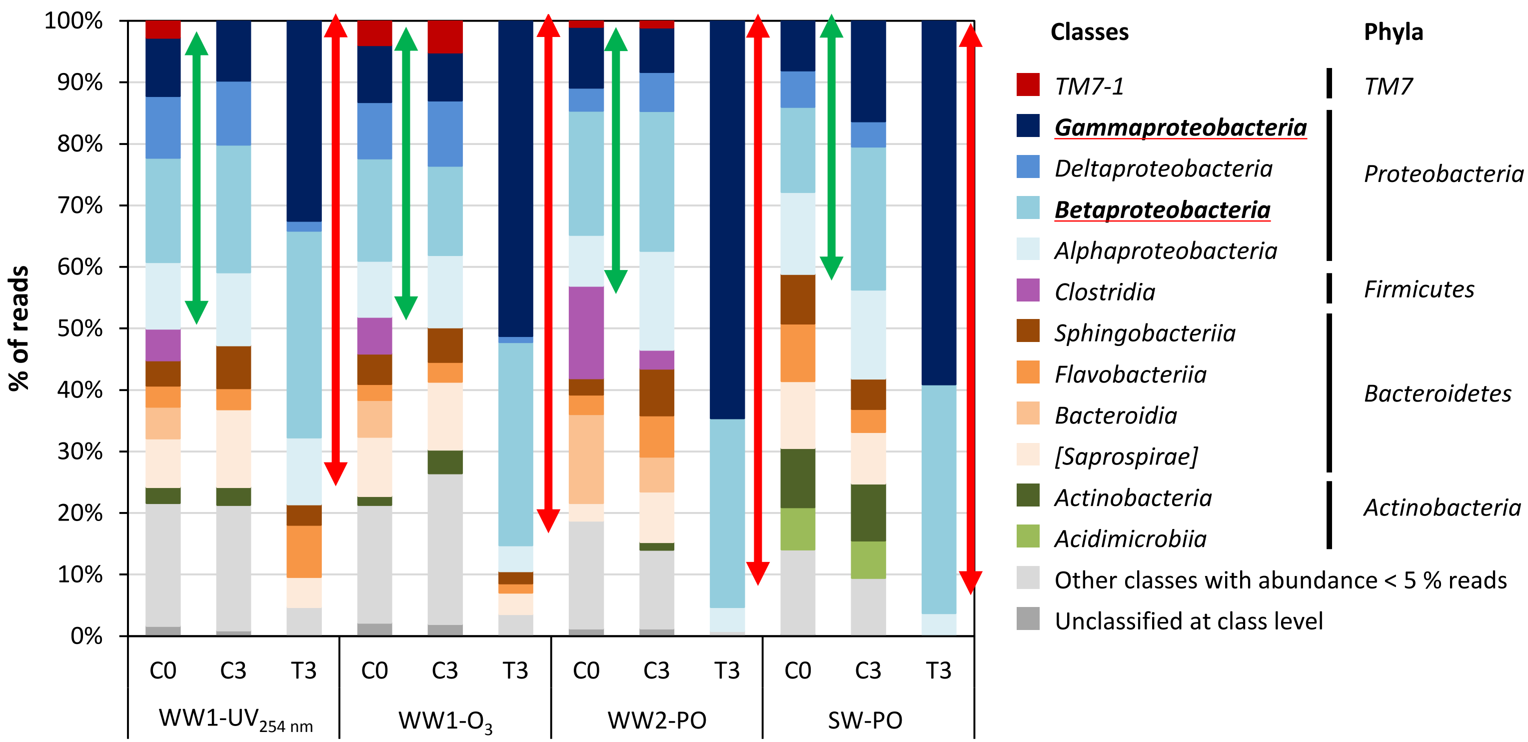
Objective 2

SELF-REPLICATION

…is not identical for all bacterial groups…



Most fitted groups include those able to acquire AR?

Objective 2

OTECHNOLOGY

Becerra-Castro et al., Sci Total Environ. 2016 ;573:313-323.

StARE Overview

Scientific and technological results

Collaboration, coordination, mobility, synergies and infrastructures

Stakeholder engagement

Impact and knowledge output

Continuation of the work in the future

Collaboration, coordination and mobility

9 Mobility actions – 12 months

12 Publications ≥ 2 StARE partners

A&ARG

AR Evolution

AR Removal

Norwegian School of Veterinary Science

UNIVERSITY OF HELSINKI

University of Helsinki

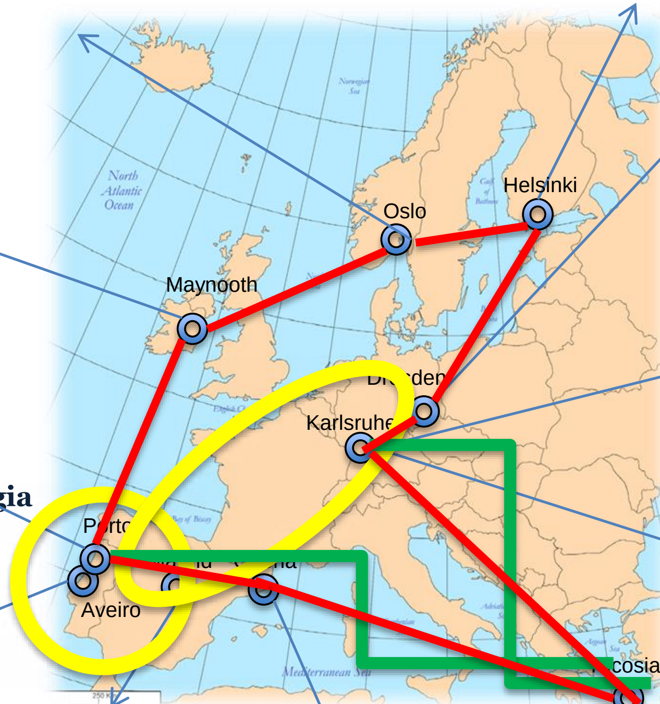
TECHNISCHE UNIVERSITÄT DRESDEN

Technische Universität Dresden

NUI Maynooth

NUI MAYNOOTH

Ollscoil na hÉireann Má Nuad



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University of Cyprus

nireas

Nireas International Water Research Center, University of Cyprus

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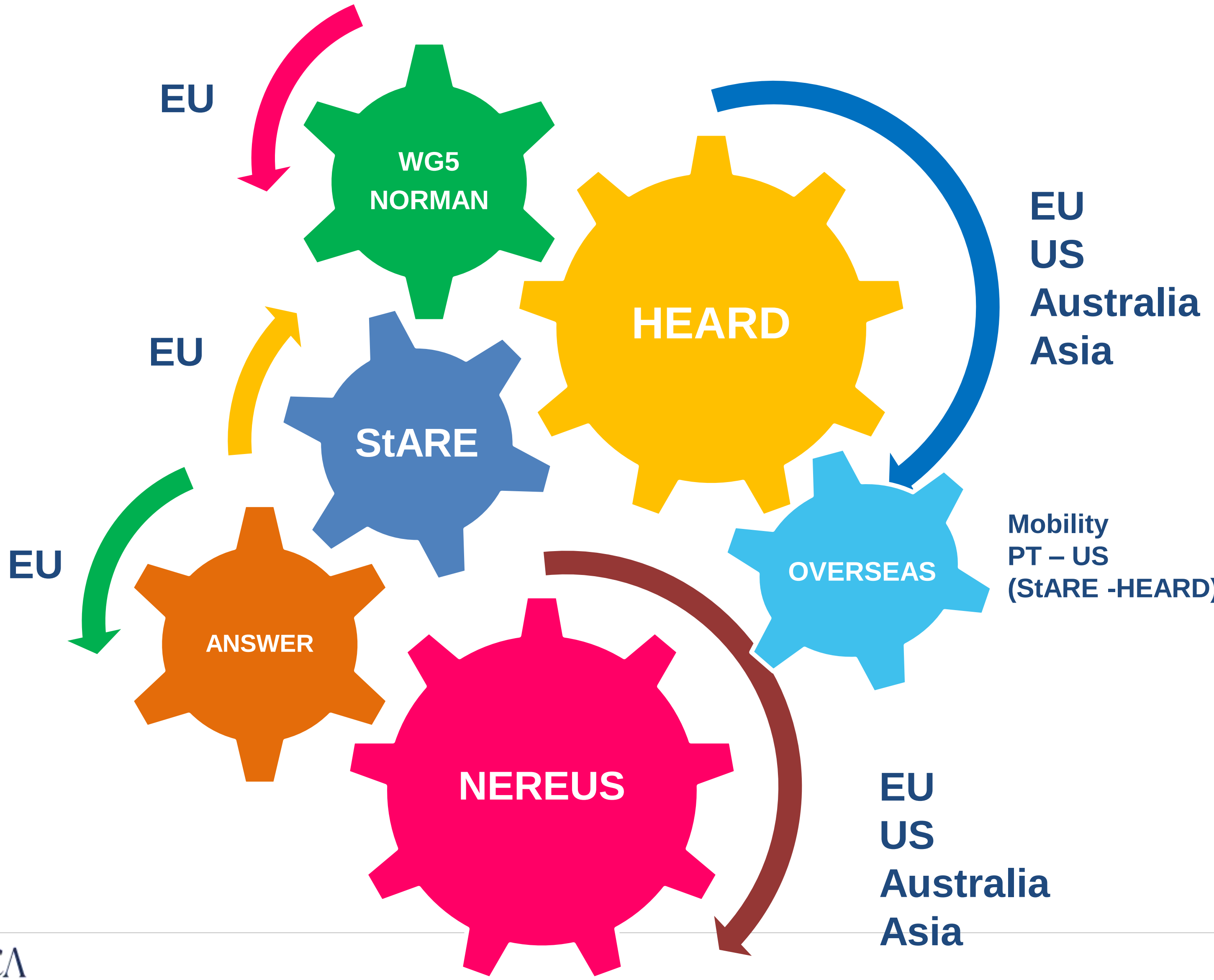
PORTO

External collaborations

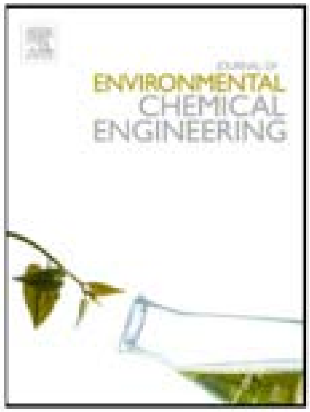


External collaborations

Accepted Manuscript

Title: Inter-laboratory calibration of quantitative analyses of antibiotic resistance genes

Authors: Jaqueline Rocha, Damiano Cacace, Ioannis Kampouris, Hélène Guilloteau, Thomas Jäger, Roberto B.M. Marano, Popi Karaolia, Célia M. Manaia, Christophe Merlin, Despo Fatta-Kassinos, Eddie Cytryn, Thomas U. Berendonk, Thomas Schwartz



WG5
NORMAN

Environmental Science & Technology

Feature

Cite This: Environ. Sci. Technol. 2017, 51, 13061-13069

pubs.acs.org/est

Toward a Comprehensive Strategy to Mitigate Dissemination of Environmental Sources of Antibiotic Resistance

Peter J. Vikesland,[*,†,‡] Amy Pruden,[†,‡] Pedro J. J. Alvarez,[§] Diana Aga,[||] Helmut Bürgmann,[⊥] Xiang-dong Li,[#] Celia M. Manaia,[▽] Indumathi Nambi,[○] Krista Wigginton,[◆] Tong Zhang,[¶] and Yong-Guan Zhu[⊗]

HEARD

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PORTO

External collaborations

COST Action ES1403

New and emerging challenges and opportunities in wastewater reuse

Cefotaxime-resistant fecal coliform survey

Eddie Cytryn et al.

NEREUS

Global cefotaxime-resistant fecal coliform survey participants

Canada
USA
Portugal
Spain
UK
France
Germany
Italy
Switzerland
Austria
Slovenia
Croatia
Estonia
Poland
Norway
Cyprus
Israel
India
China
Australia
South Africa
South Korea

Countries	Groups	WWTPs	Campaigns	Seasons
22	36	54	5	2

nereus
COST ACTION
ES1403

StARE Overview

Scientific and technological results

Collaboration, coordination, mobility, synergies and infrastructures

Stakeholder engagement

Impact and knowledge output

Continuation of the work in the future

Stakeholder engagement

Professionals (water management; public health, other): personal communication, sharing of research data

National **Workshop** in Portugal (15 April 2016)
~200 attendees
National **Workshop** in Cyprus (2 March 2017)

>20 invited keynote or **plenary talks** in (inter)national meetings attended by policy making and water management entites

General public: Media communication (e.g. TV, radio)

Summary

Scientific and technological results

Collaboration, coordination, mobility, synergies and infrastructures

Stakeholder engagement

Impact and knowledge output

Continuation of the work in the future

Impact and knowledge output

Training

> 12 students enrolled (10 PhD students)

Scientific impact

> 40 publications in international peer-reviewed journals

> 60 communications in international conferences

Stakeholders and community

> 40 popularization events (18 articles; 13 conferences, 10 live media, etc)

Technology transfer

Collaboration with two water treatment SMEs

Impact and knowledge output

First European integrated wastewater surveillance

Important drivers for resistance => improvement of wastewater treatment

Training

> 12 students enrolled (10 PhD students)

Scientific impact

> 40 publications in international peer-reviewed journals

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Stakeholders and community

> 40 popularization events (18 articles; 13 conferences, 10 live media, etc)

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Collaboration with two water treatment SMEs

StARE Overview

Scientific and technological results

Collaboration, coordination, mobility, synergies and infrastructures

Stakeholder engagement

Impact and knowledge output

Continuation of the work in the future

Continuation of the work in the future

- ARGs database open data

- Results dissemination in generalist journals and stakeholder- and public-oriented events

- New projects
  - Demonstration/implementation projects with companies (approved; applications in preparation – National funds, Life, Interreg, JPIAMR, Marie Curie, etc)

Thank you!

TRACE

Tracking and Assessing the Risk from Antibiotic Resistance Genes using Chip Technology in Surface Water Ecosystems (TRACE)

Water JPI

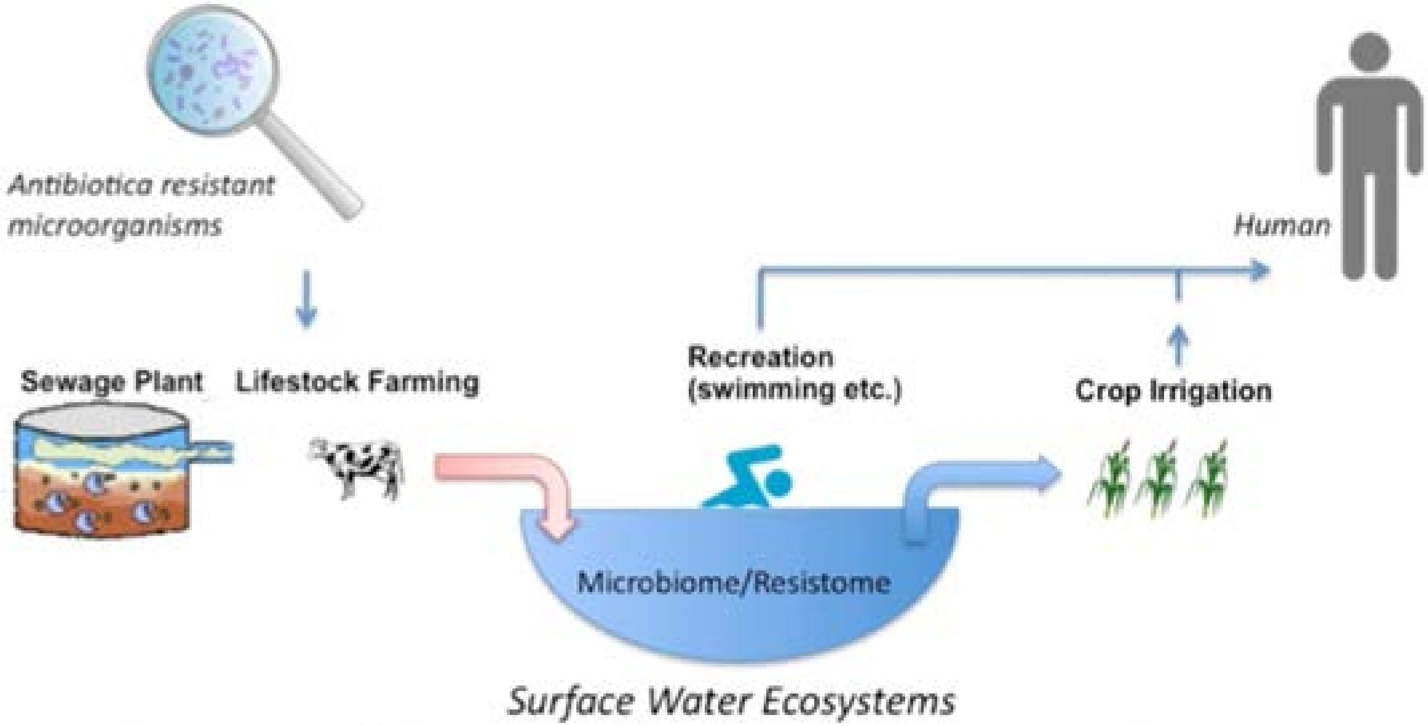
TRACE

Wolfgang Fritzsche

Final Evaluation Meeting of the Water JPI Pilot Call Projects
"Impact to Science and to Society"
Marina Congress Centre, Helsinki, Finland
June 4th 2018

Focus of TRACE

- Understand the sources and behaviour of antibiotic resistance in natural surface waters and infection routes
- Development of a novel detection technologies as (1) a on-site fast assay as well as (2) a chip-based solution to detect a panel of antibiotic resistance genes (ARG) for waterborne microorganisms, allowing time- and cost-efficient evaluation of AR patterns and the associated risk for human health



Partners

UCD DUBLIN

- Risk assessment

LEIBNIZ-INSTITUT für PHOTONISCHE TECHNOLOGIEN
ipht jena

- DNA chip design & test
- Chip optimization

UNIVERSIDADE NOVA DE LISBOA

- Probe design & optimization
- Lab tests

Food Analytik & Consulting

- Identification of marker organisms and sequences
- one-target assay

SAPIENZA UNIVERSITÀ DI ROMA

- ARB isolation and characterization

ICRA

- Antibiotic pollution
- ARGs quantification/screening
- Selection of target ARGs

Scientific and technological results -
Characterization of impacted sites

5 study sites:

- Rivers
  - Saale (GE)
  - Arrone (IT)
  - Tiber (IT)
  - Ter (SP)

- Other
  - Luetsche lake (GE)
  - Ostia Beach (IT)

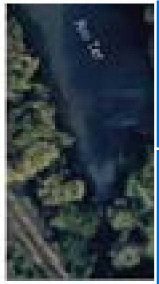
Scientific and technological results - Identification of biomarker genes

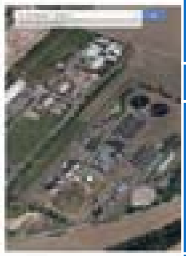
- Selected Genes

Gene	Product	Resistance to / Mode of action	Number of Variants	Target variant
qnrS	Pentapeptide repeat family, which protects DNA gyrase from inhibition by quinolones	Fluoroquinolones / DNA replication	5	qnrS1
bla_{TEM}	Class A ß-lactamases that hydrolyse penicillin and related antibiotics	Beta-lactam antibiotics / Cell wall synthesis	Many	bla_{TEM-1}
intI1	Class 1 integron integrase	Gene capture	None	Anthropogenic pollution
uidA	ß-glucuronidase	Hydrolysis of glucoronides	E. coli (≈97%) Shigella Salmonella	Fecal contamination of water

ARGs

Scientific and technological results -
Characterization of impacted sites

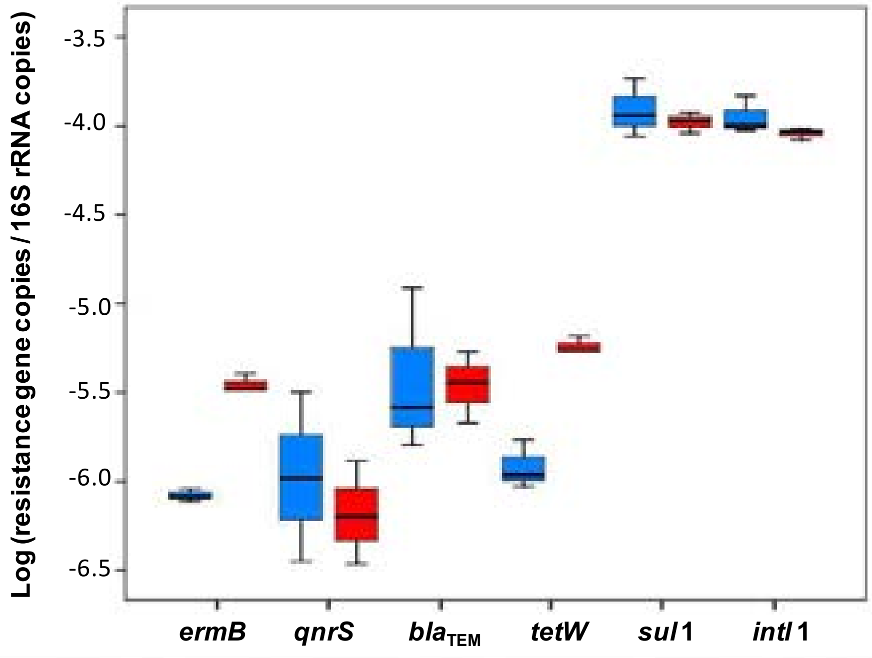




System	Location	Date	Analyses[1]
Ter	Girona (Spain)	07/10/2015	AB, ARB, ARG
		15/04/2016	ARB, ARG, MTG
		12/07/2016	ARB
		13/12/2016	ARB
Saale	Jena (Germany)	30/07/2015	AB, ARG
		13/10/2015	
		11/01/2016	
		14/03/2016	
		09/05/2016	
		20/07/2016	
Lago Luetsche		03/06/2015	AB, ARG
Tíber	Roma (Italy)	07/09/2016	AB, ARG
		12/12/2016	
		25/04/2017	
		12/07/2017	
Arrone		24/08/2016	AB, ARG
		05/12/2016	
		18/04/2017	
		18/07/2017	

Scientific and technological results –
Quantification of ARGs

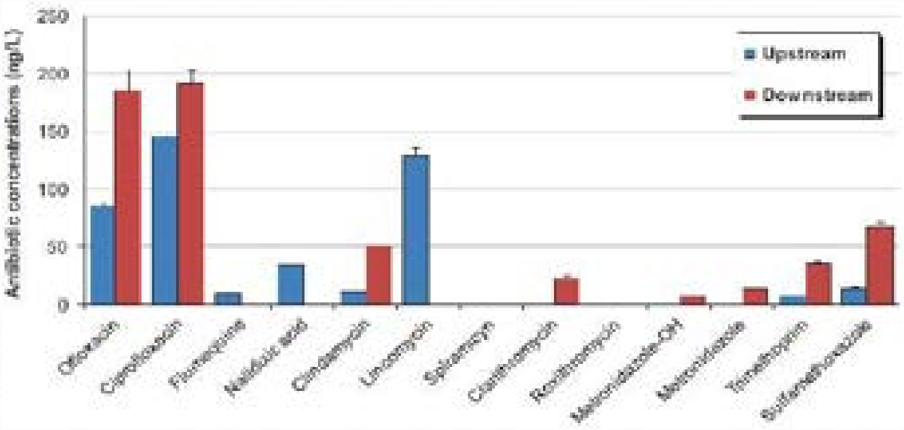
Relative concentration of ARGs in water samples from impacted (red) and non-impacted (blue) sites at the Luetsche Lake.



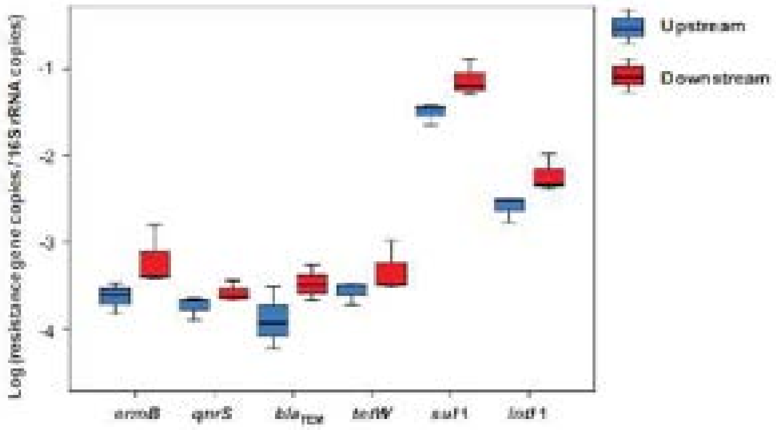
Scientific and technological results – Quantification of ARGs

- Antibiotic pollution and abundance of ARGs in river Ter

Antibiotic exposition in river Ter samples collected upstream and downstream sites

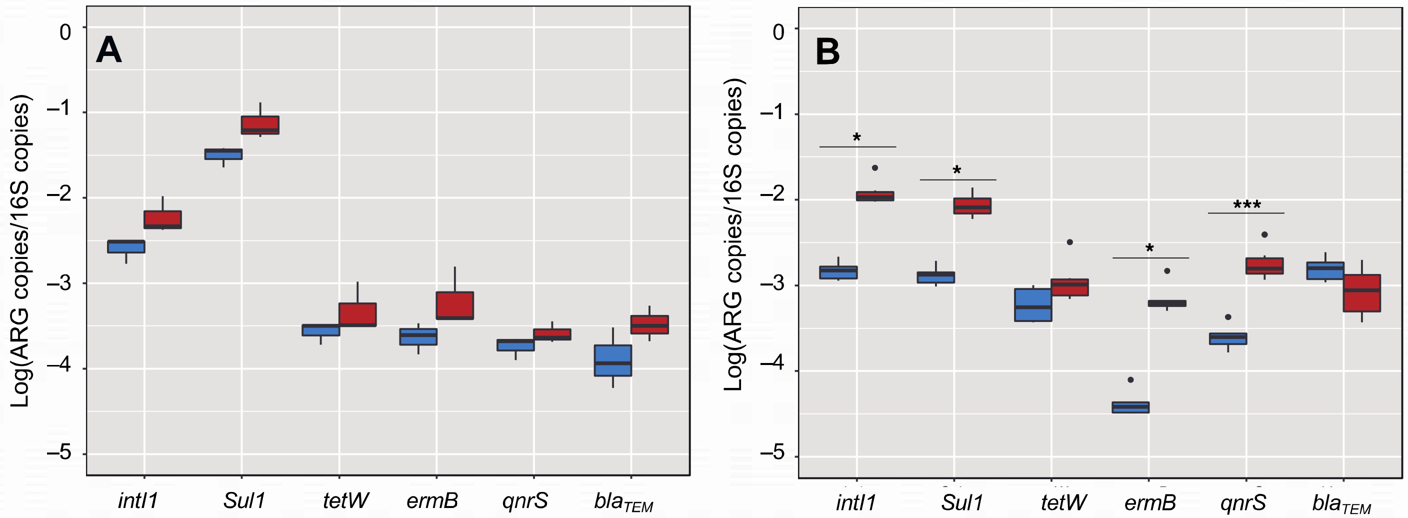


Relative concentration of ARGs in river Ter



Scientific and technological results –
Quantification of ARGs

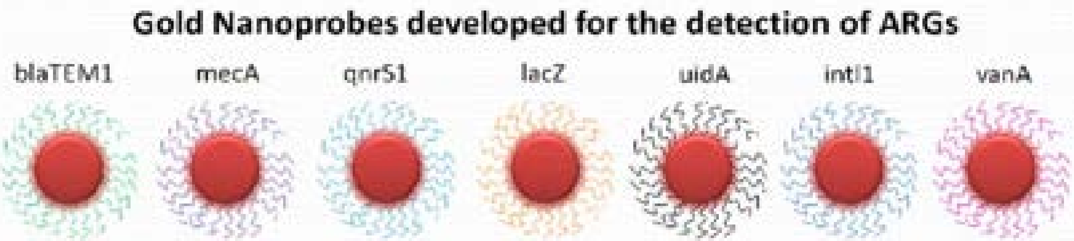
Normalized concentration of target ARGs in water samples from river Ter collected upstream (blue) and downstream (red) the WWTP effluent discharge point in October 2015 (A) and April 2016 (B).

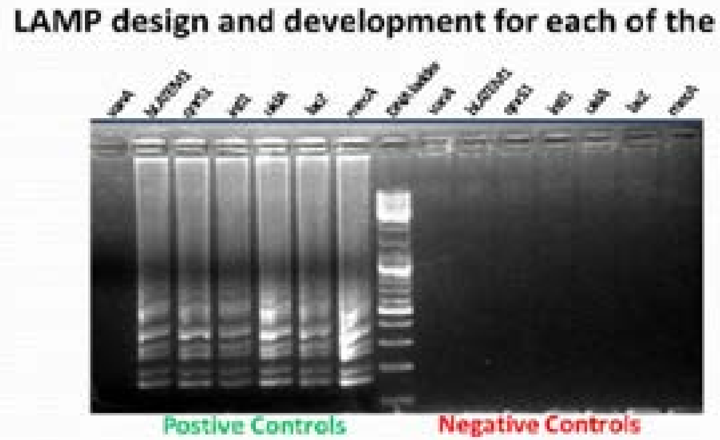


Scientific and technological results –

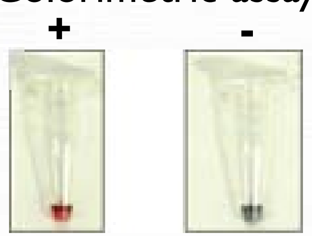
On-site detection technology development – A: Fast Assay

- Simple semi-quantitative assay for point-of-need: gold nanoprobes-based colormetric assay for marker pathogen and ARG identification





Colorimetric assay

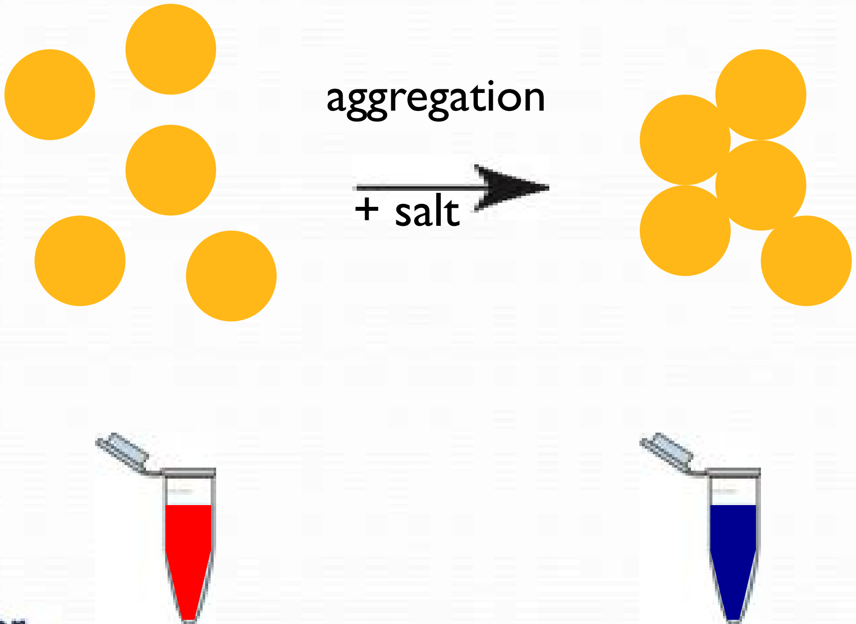


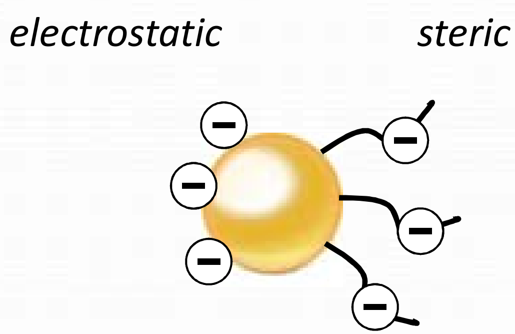
Localized Surface Plasmon Resonance (LSPR)

composition

size

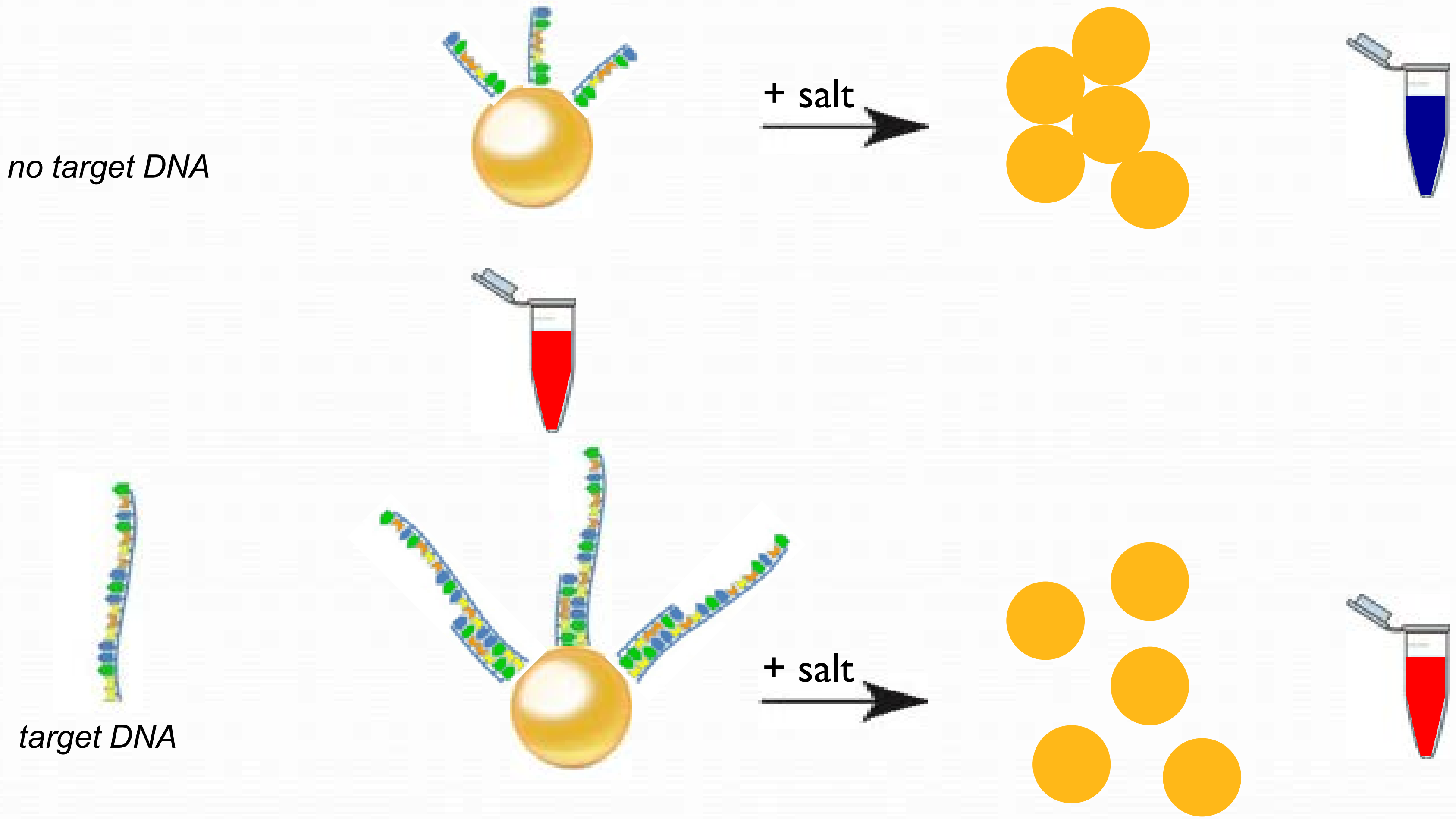
geometry





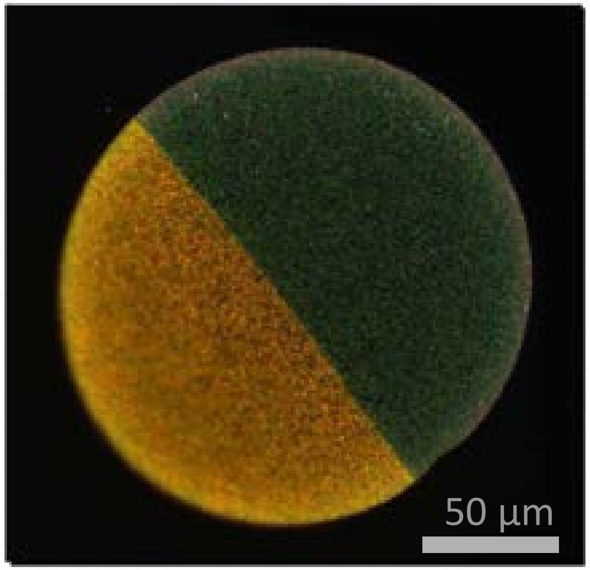
stabilization

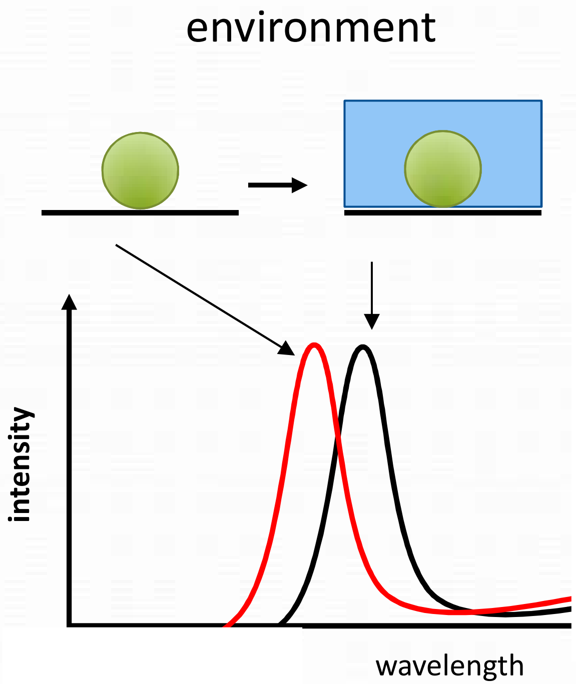
DNA sensing by LSPR



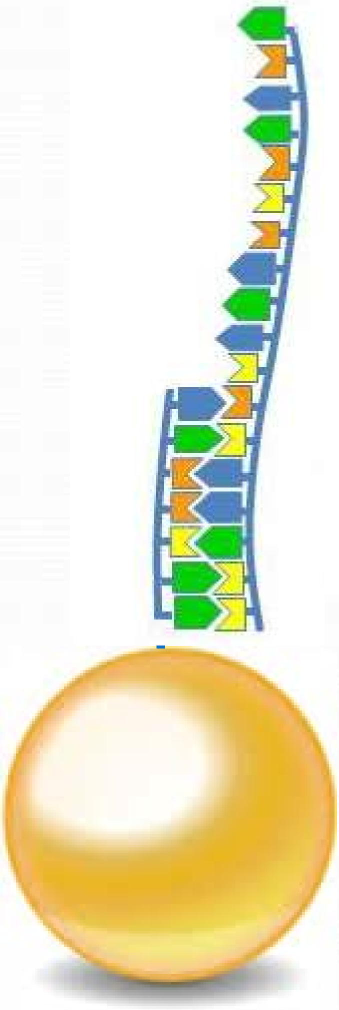
Scientific and technological results –

On-site detection technology development – B: Microarray





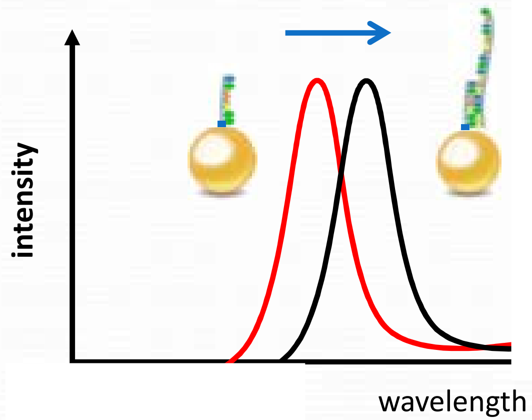
DNA sensing by LSPR



probe DNA
(surface attached):
capture DNA

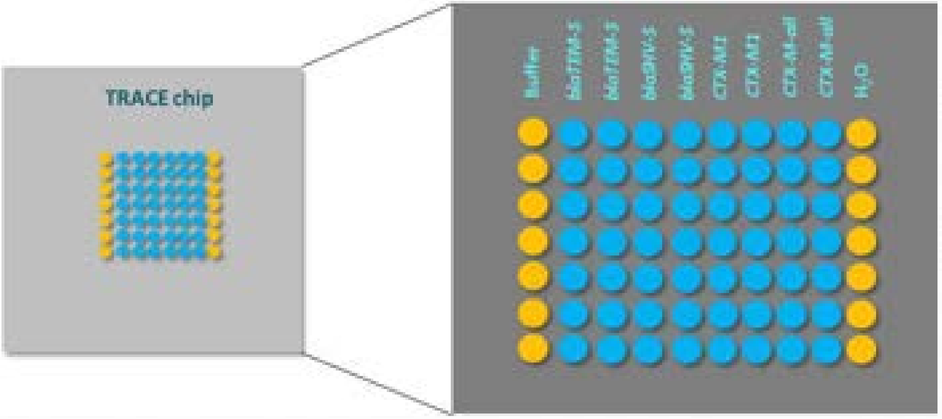
target (analyte) DNA

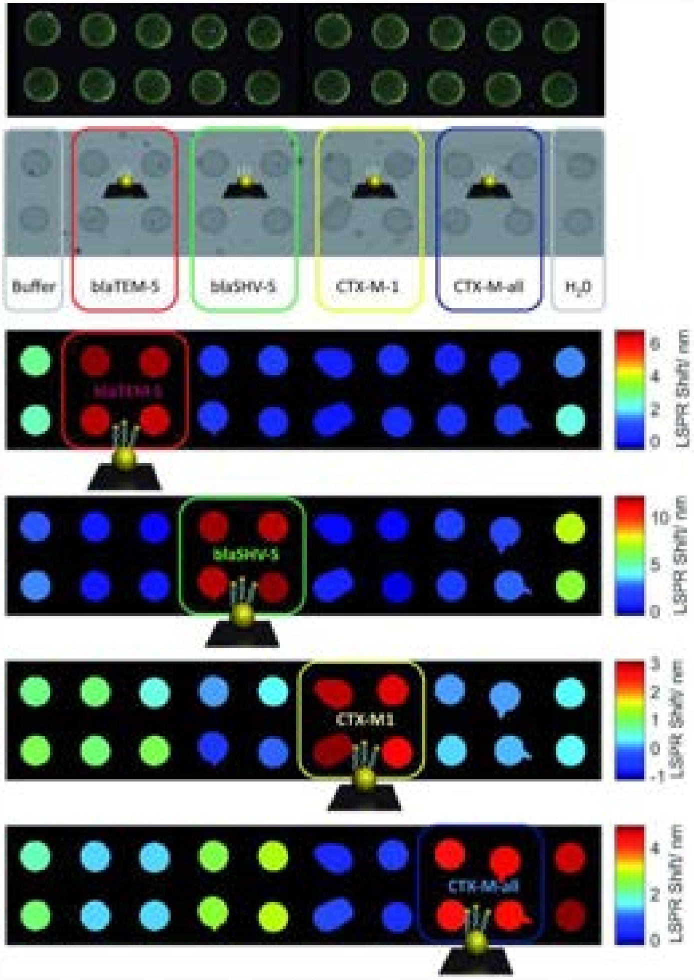
signal:
wavelenght shift



Scientific and technological results –
On-site detection technology development - B

- Microarray chip for 4 ARGs



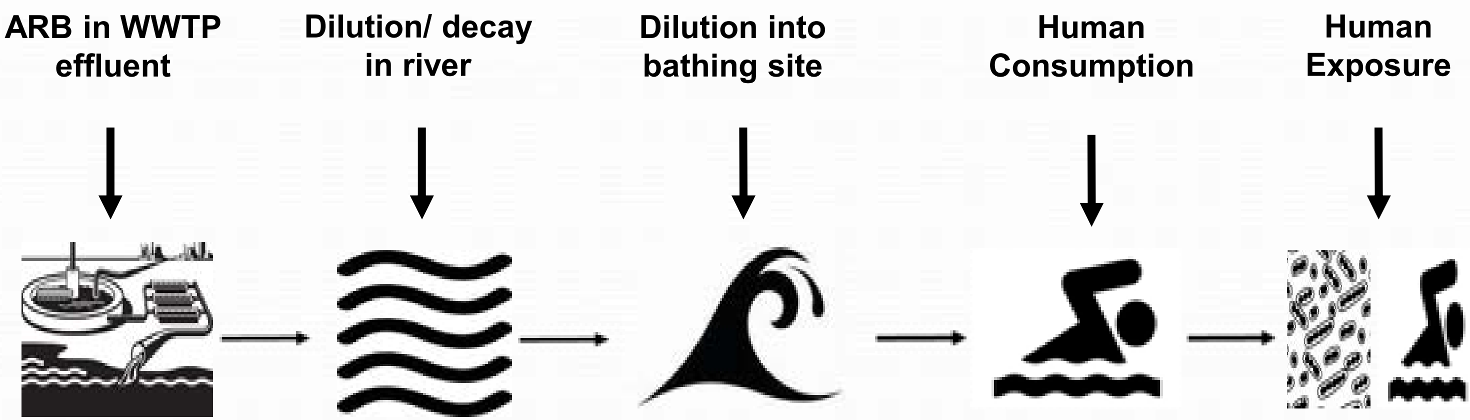


Scientific and technological results

Human Health Risk Assessment Model

Risk models for the prediction of environmental behaviour of antibiotic-resistant microorganisms in surface waters

- Drinking water model
- Recreational water model
- Irrigations model



Collaboration, coordination, mobility – Interactions in Consortium

- FFCT and ICRA developed a straight collaboration during this period for the evaluation of the ARGs selected by the consortium
- Sample sharing, e.g. FOOD provides samples from Germany to ICRA and to IPHT
- Ongoing site characterization for risk analysis with close collaboration between UCD, ICRA, Uniroma
- All the necessary FASTA files for sequence alignment in order to develop LAMP primers and probes sequences were provided by ICRA to FFCT
- DNA templates and biological samples were provided by ICRA to FFCT for LAMP amplification and posterior detection with Au-nanoprobes.
- Exchange of PhD students between Uniroma and ICRA was done on May 2017. Particularly, one student from Uniroma carried out a short stay (1-month) to learn the basics of qPCR technique for the quantification of ARGs in samples collected at the Italian systems (Ostia beach, rivers Tiber and Arrone).
- IPHT and FOOD are in direct contact related to practical protocols and PCR samples to validation.
- ICRA and UNL provide samples for validation by FOOD/IPHT.
- IPHT hold strong cooperation with all partners and coordinated the work as well as dissemination: home-page, joint poster presentation, etc.
- Four joint meetings were organized (ICRA, Uniroma, UCD and IPHT) with the participation of all project partners and external attendees. The project was finished with a workshop with internal as well as external presentations.

→ Strong interactions in consortium, both scientific as coordinative, synergies

Collaboration, coordination and mobility - Consortium Meetings

N°	Date	Location	Attending partners	Purpose
1	15/06/2015	Girona	All	**Kick-Off meeting**: Coordination of project activities and research plan, Sampling schedule of model ecosystems Coordination issues, Agreement on firsts deliverables, creation of project website.
2	26/06/2016	Rome	All	**Mid-Term meeting**: Pre discussion, - Milestones activities and project delay Coordination issues, Disse conferences, stakeholder engagement, etc.) 
3	29-30/06/2017	Dublin	All	**Last-Term meeting**: Presentat stakeholders, General discussion, delays, Coordination issues, Diss publications, conferences, stakeh 
4	7-8/12/2017	Jena	All	**Final meeting**: Presentation of final results to partners and stakeholders, pending tasks and problems unsolved, coordination issues, publication and dissemination strategy.

Collaboration, coordination and mobility - Conferences and Workshops, dissemination

- Participation on
  - 19 conferences and workshops, national and international
- Home page: http://jpi-trace.eu/
- Linkedin group: https://www.linkedin.com/groups/8385238
- Local actions adressing the general public

Stakeholder engagement

- The UCD-team has **6 monthly project meetings** and has a project steering group with relevant stakeholders included. This includes representatives from the Irish EPA and Irish water (water utility company in Ireland responsible for all water services including water treatment and distribution).

- **TRACE workshop 2017 (Dublin):** A workshop event focusing AR was organised by UCD project partners at the University College Dublin on June 29th 2017. The event was attended by stakeholders (Irish water, HSE), companies (H2Ozone, Quest Utility Services, Humanist Times), general public, steering committee (EPA, King's College London, Brunel University), researchers (University Limerick, NUI Maynooth, NUI Galway, Ulster University, Dublin Institute of Technology, Limerick Institute of Technology iCRAG, Trinity College Dublin) and TRACE project partners (58 participants).

- **TRACE workshop 2018 (Jena):** A workshop event was organized by IPHT on 7th December 2017. This event was organized from the project coordinator with participation of researcher (project partners as well as clinical and federal research institutes) and public administrations (e.g. ,Thuringian water reservoir administration).

Impact and knowledge output

- 1. O'Flaherty, E., C.M. Borrego, J.L. Balcázar and E. Cummins (2017) Human exposure assessment to antibiotic-resistant Escherichia coli through drinking water. Sci. Total Environ. Vol. 616-617, pp.1356-1364. doi.org/10.1016/j.scitotenv.2017.10.180
- 2. Subirats, J., X. Triadó-Margarit, L. Mandaric, V. Acuña, J.L. Balcázar, S. Sabater and C.M. Borrego (2017) Wastewater pollution differently affects the antibiotic resistance gene pool and biofilm bacterial communities across streambed compartments. Molecular Ecology, 26: 5567–5581.
- 3. Lekunberri I., M. Villagrasa, J.L. Balcázar and C.M. Borrego (2017) Contribution of bacteriophage and plasmid DNA to the mobilization of antibiotic resistance genes in a river receiving treated wastewater discharges. Sci. Total Environ. 601–602: 206-209. DOI: 10.1016/j.scitotenv.2017.05.174.
- 4. Lekunberri, I., J.L. Balcázar and C.M. Borrego (2017) Detection and quantification of the plasmid-mediated mcr-1 gene conferring colistin resistance in wastewater. Internat. J. Antimicrob. Agents. 50(6): 734–736. DOI: 10.1016/j.ijantimicag.2017.08.018.
- 5. Subirats, J., E. Royo, J.L. Balcázar and C.M. Borrego (2017) Real-time PCR assays for the detection and quantification of carbapenemase genes (blaKPC, blaNDM and blaOXA-48) in environmental samples. Environ. Sci. Pollut. Res. 24:6710–6714. DOI: 10.1007/s11356-017-8426-6.
- 6. Lekunberri, I., J. Subirats, C.M. Borrego and J.L. Balcázar (2017) Exploring the contribution of bacteriophages to antibiotic resistance. Environ. Pollut. 220(Pt B):981–984. DOI: 10.1016/j.envpol.2016.11.059
- 7. Subirats, J., A. Sànchez-Melsió, C.M. Borrego, J. L. Balcázar and P. Simonet (2016) Metagenomic analysis reveals that bacteriophages are reservoirs of antibiotic resistance genes. Internat. J. Antimicrobial Agents 48: 163-167. DOI: 10.1016/j.ijantimicag.2016.04.028.
- 8. O'Flaherty E and Cummins E. (2017a). Antibiotic resistance in surface water ecosystems: presence in the aquatic environment, prevention strategies and risk assessment. Human and Ecological Risk Assessment Vol. 23, No. 2, pp. 299–322. doi.org/10.1080/10807039.2016.1247254
- 9. Li, G., D. Zopf, G. Schmidl, W. Fritzsche, and O. Stranik. "Concentric Dot-Ring Metal Nanostructures Prepared by Colloidal Lithography." Applied Physics Letters 109, no. 16 (2016): 163101.
- 10. Thiele, Matthias, Andrea Knauer, Daniell Malsch, Andrea Csaki, Thomas Henkel, J. Michael Kohler, and Wolfgang Fritzsche. "Combination of Microfluidic High-Throughput Production and Parameter Screening for Efficient Shaping of Gold Nanocubes Using Dean-Flow Mixing." Lab on a Chip 17, no. 8 (2017): 1487-95.
- 11. Kosman, Joanna, Jacqueline Jatschka, Andrea Csaki, Wolfgang Fritzsche, Bernard Juskowiak, and Ondrej Stranik. "A New Strategy for Silver Deposition on Au Nanoparticles with the Use of Peroxidase-Mimicking Dnazyme Monitored Via a Localized Surface Plasmon Resonance Technique." Sensors 17, no. 4 (2017): 849.

Under review:

- O'Flaherty E., Membré JM. and Cummins E. (2018a) Meta-analysis of the reduction of sensitive and antibiotic resistant Escherichia coli as a result of low and medium pressure UV lamps
- O'Flaherty E., Solimini A. Pantanella F. and Cummins E. (2018b). Human exposure assessment to antibiotic resistant Escherichia coli through the irrigation of lettuce.

Manuscripts in preparation

- 1. Lekunberri, I., M. Villagrasa, J.L. Balcázar, B. Giese, J. Müller and C.M. Borrego. Seasonal variations of ARGs in the Saale River (Germany).
- 2. Lekunberri, I., G. Venutto, J. Subirats, A. Solimini, J.L. Balcázar and C. M. Borrego. Antibiotic resistance in two Italian rivers impacted by wastewater treatment plant discharges.
- 3. Ferreira, C.F., A.S. Matias, C. Roma-Rodrigues, J. Subirats, I. Lekunberri, J.L. Balcázar, C.M. Borrego and P.V. Baptista. Au-nanoprobes coupled to isothermal amplification for screening antibiotic resistance genes in surface waters.
- 4. O'Flaherty E., Solimini A. Pantanella F. and Cummins E. (2018c). Human exposure assessment to antibiotic resistant Escherichia coli through recreational water.
- 5. Zopf, D., Pittner A., Dathe A., Grosse N., Csáki A., Fritzsche W., Stranik O. Pathogen identification and detection by plasmonic microarray
- 6. Solimini, Venuto, Gagliardi, Schippa, De Giusti, Pantanella. Quantification of antibiotic resistant E. coli in two river systems of Central Italy

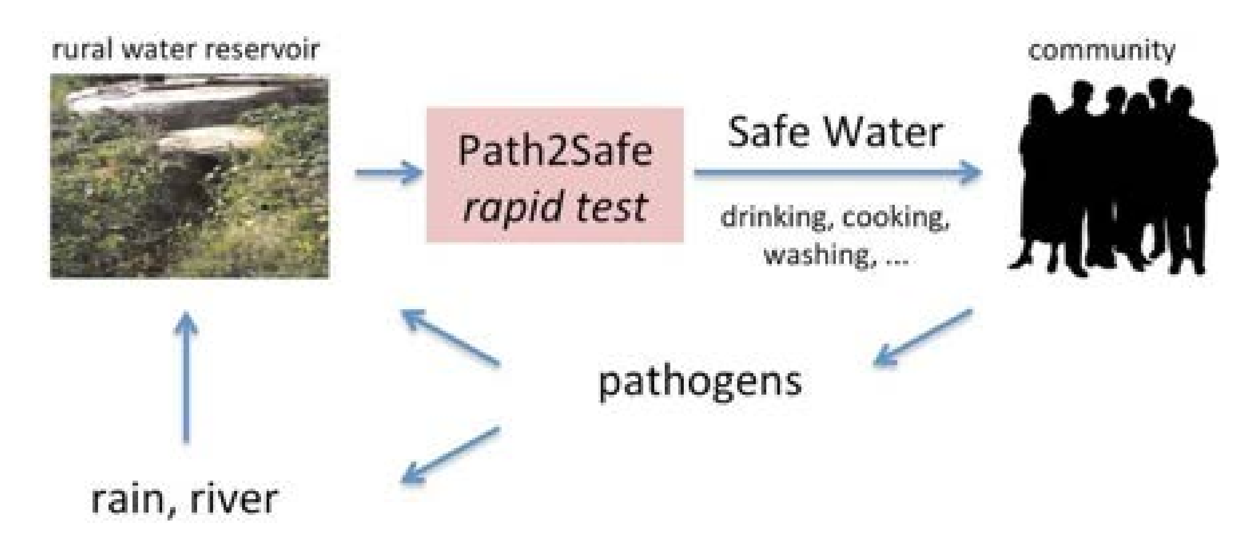
Impact and knowledge output

- Better knowledge on the impact of wastewater discharges on water systems (rivers Ter and Saale). Antibiotic pollution in both rivers and the impact of the anthropogenic pollution on the river resistome and mobilome were evaluated.
- Effect of wastewater discharges on the concentration of virulent and antibiotic-resistant *E. coli* in rivers Ter, Tiber and Arrone → risk to human health → elaboration of appropriate risk assessment models.
- The Meta-analysis model can be used to improve accuracy of risk assessment models investigating the effect of UV treatment on AR or AS *E. coli*. In particular risk assessment models examining the human exposure to AR *E. coli* through drinking water or examining the impact of a WWTP located near a recreational site could use this data to predict AR or AS *E. coli* concentrations found after UV treatment. The Drinking water model can provide guidelines to water management entities on the best water treatment combinations to use to provide the lowest human exposure to AR *E. coli*. The results can help set acceptable levels of AR *E. coli* in source water for DWTP. This study shows how research and risk assessment can help improve water regulations. The Irrigation model could help set local guidelines for producers on maximum permissible contamination levels in irrigation water. The results provide recommendations on the most suitable post-harvest treatment to use to reduce the human exposure to AR *E. coli*. The Recreational model also provides information on the possible human exposure levels to AR *E. coli* through recreational water.
- The nanoprobe based systems still require optimization but may be used for fast screening of the relevant ARGs in water beds. LAMP is a robust, yet simpler, alternative to PCR based approaches and the Au-nanoprobes potentiate specificity of amplification analysis. The system might be of use for the fast screening at point of need without the need for cumbersome equipment. Possible translation to a SME is under evaluation.
- The (nano)technological development of a plasmonic-array chip platform for multiplex molecular detection with optical readout allows for an effective discrimination of several targets in one assay, surpassing limitations of established analytical approaches in that field.

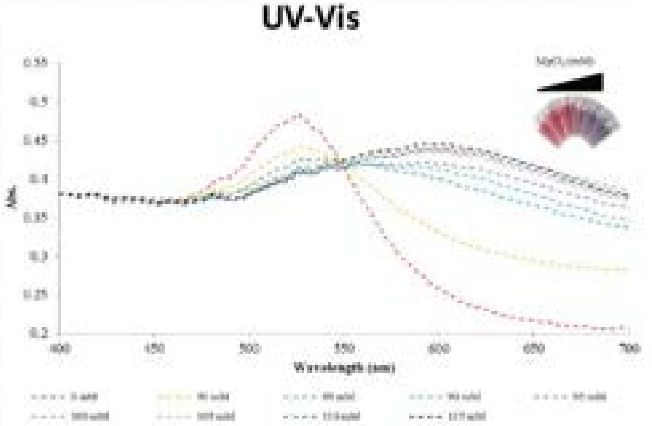
Continuation of the work in the future

- Application "A sustainable strategy for the self-management of water safety in low resource settings using a rapid pathogen screening - **Path2Safe**" submitted in the research funding initiative PRIMA (partnership for research and innovation in the Mediterranean area) in March 2018.

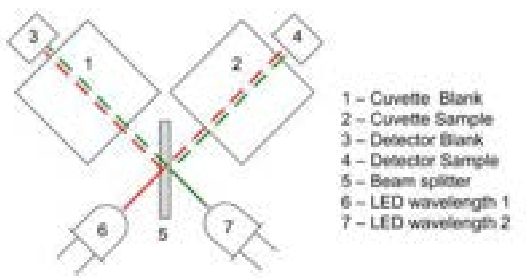
A sustainable strategy for the self-management of water safety in low resource settings using a rapid pathogen screening - Path2Safe

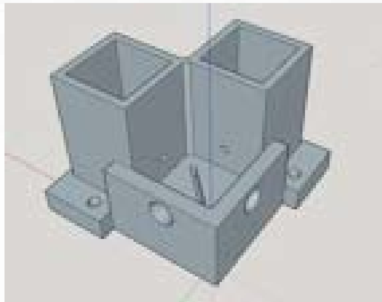


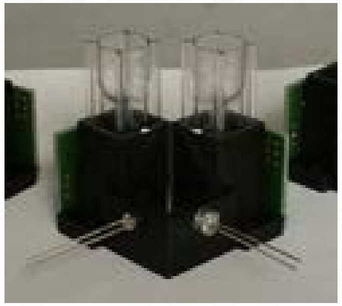
A sustainable strategy for the self-management of water safety in low resource settings using a rapid pathogen screening - Path2Safe



Pedro Baptista, Lisbon







Matthias Urban

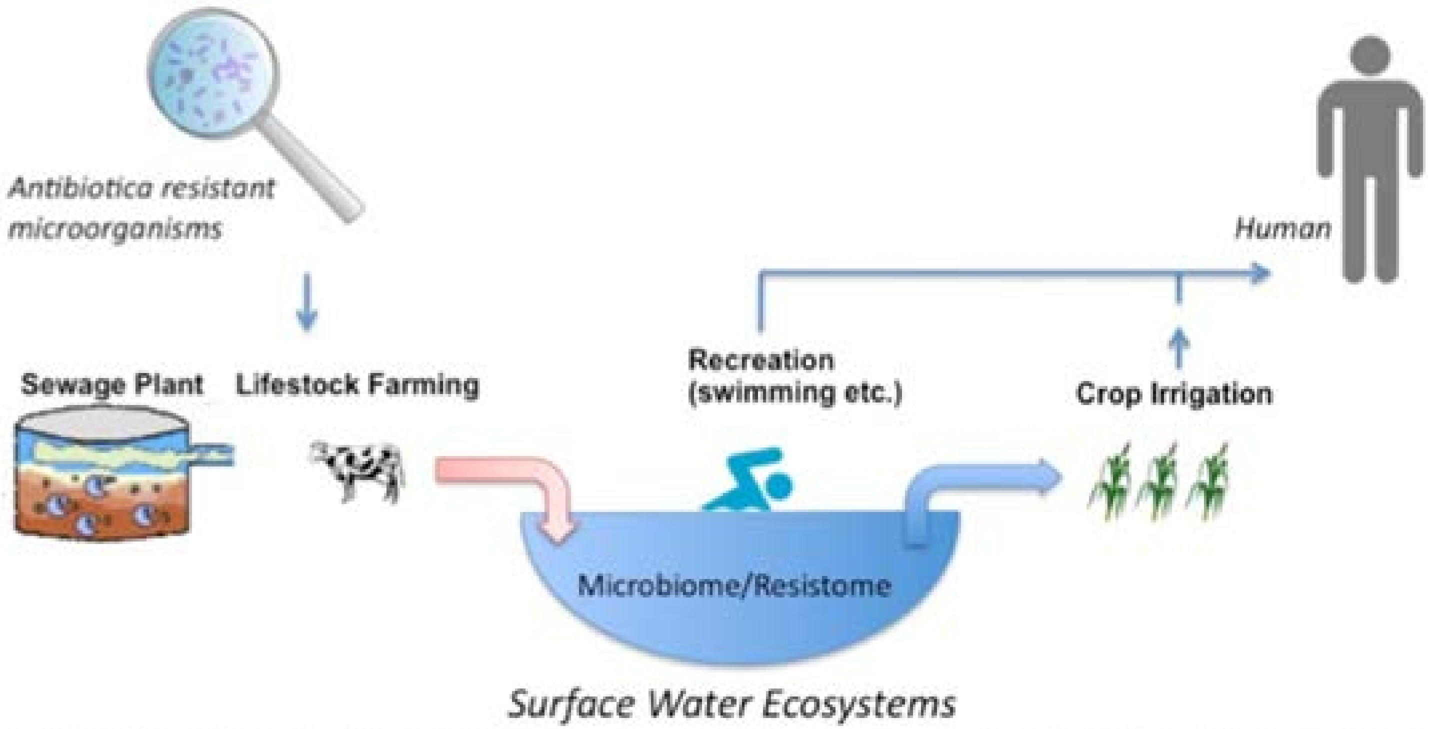
Continuation of the work in the future

- Application "A sustainable strategy for the self-management of water safety in low resource settings using a rapid pathogen screening - **Path2Safe**" submitted in the research funding initiative PRIMA (partnership for research and innovation in the Mediterranean area) in March 2018.

- An international project "One Platform- Multiple biomarker detection of Rheumatoid Arthritis - **RA-detect"** between the partners from Lisbon and Jena on antibody-based multiplex detection (with the microarray analyser of TRACE) is running.

- DAAD proposal "**Plasmonic nanoarray for detection of prostate cancer biomarkers**" between UNL and IPHT is starting at 2018.

- Additionally, several bilateral communication channels between the project partners were established und continued in future, as the joint participation conferences and meetings.


Antibiotica resistant microorganisms
Sewage Plant
Lifestock Farming
Recreation (swimming etc.)
Crop Irrigation
Human
Microbiome/Resistome
Surface Water Ecosystems