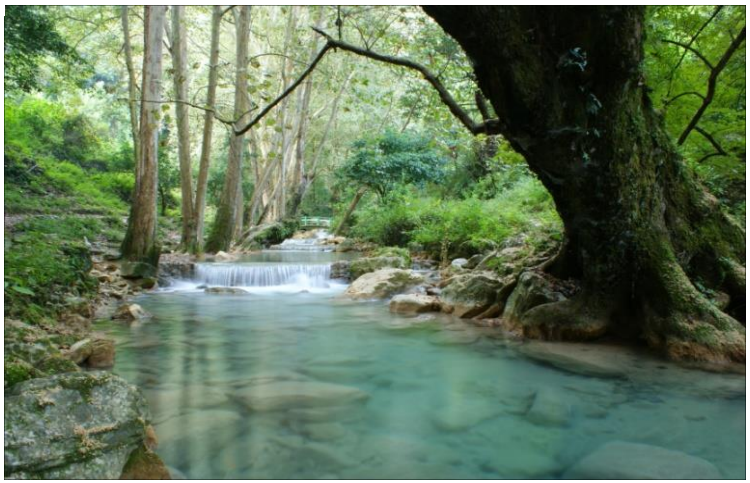
ICRA
Institut Català de Recerca de l'Aigua
Instituto Catalán de Investigación del Agua
Catalan Institute for Water Research

Contaminantes emergentes en el medioambiente. El caso de los compuestos farmacéuticos en el agua



Sara Rodriguez-Mozaz, Belinda Huerta, Meritxell Gros, Gianluigi Buttiglieri, Laura Ferrrando-Climent, Daniel Lucas, Sara Chamorro, Carles Cruz-Morato, Albert Serra-Compte, Jose Castaño, Teresa Vicent, Diana Alvarez, Antonio Marqués, Damià Barceló

Girona 16 Dec 2022

Contaminación ambiental. Contaminantes prioritarios

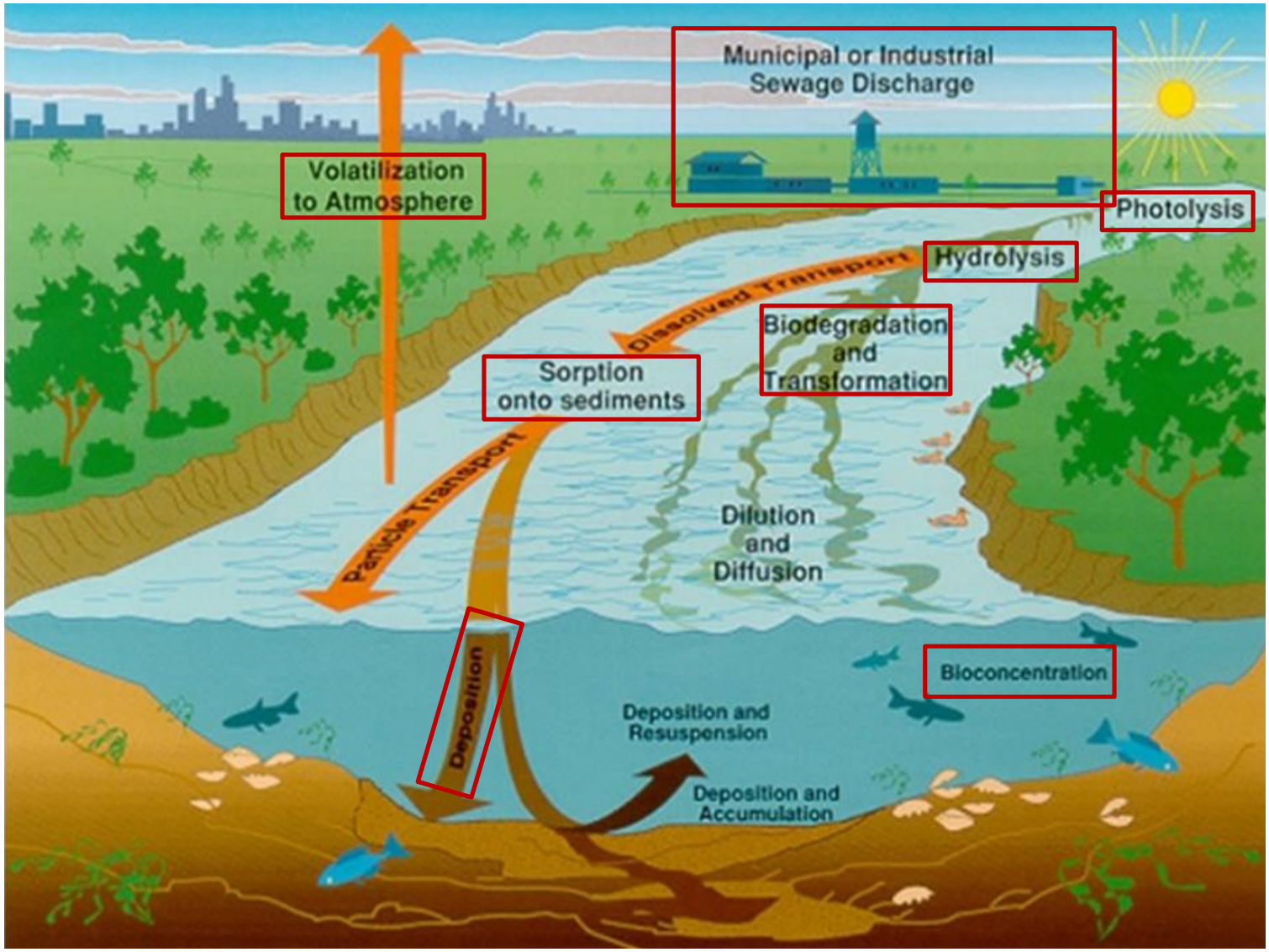
Contaminación ambiental. Contaminantes emergentes

"Synthetic or naturally occurring chemicals that are not commonly monitored in the environment bu[illegible]d cause known or su[illegible]

s)

*** Klaper, R. and Welch, L.** (2011) Emerging contaminant threats and the Great Lakes: Existing science, estimating relative risk and determining policies. *Alliance for the Great Lakes*.

Contaminantes químicos en el medioambiente

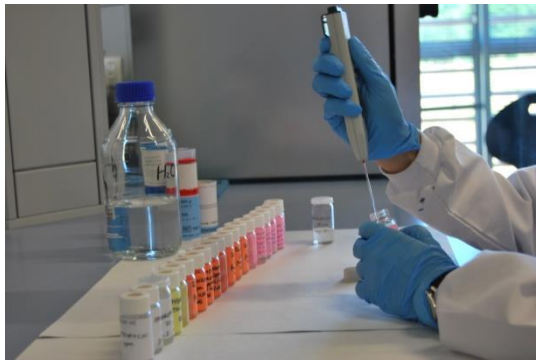


Contaminantes químicos en el medioambiente

- **Origen de Contaminantes Químicos**
  - Industrial
  - Agricultural
  - Urbano

- **Compatimentos Ambientales:**
  - **Agua** (continenteal y subterranea) and agua tratada (agua potable y residual)
  - **Sedimentos**, Suelos, Fangos
  - **Biota** (Peces, macroinvertebrados, biofilm)

- **Contaminantes Emergentes**
  - Fármacos y productos de higiene personal
  - Compuestos disruptores endocrinos (EDCs)
  - Drogas anti-cancer
  - Microplásticos, nanopartículas,
  - Genes de Resistencia



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Fármacos en el medioambiente

Human use

Excretion

Sewer

Overflow.

Treatment plant

Wastewater Treatment Plant WWTP

- Conventional Biological and phiscochemical treatment
- incomplete elimination of emerging contaminants
- **primary points of discharge of human drugs**

Continuous input into the environment: **"pseudo-persistent contaminants"**

Agricultural soils

Sludge

Surface water

Drinking water

Groundwater

Food chain

Fármacos en el medioambiente

Pharmaceuticals as a potential risk for the aquatic life

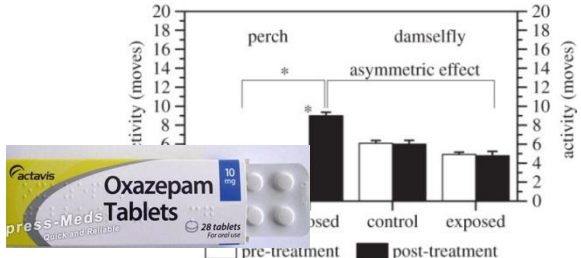
- ❑ Pseudo-persistent (continuously released)
- ❑ Highly consumed (increase foreseen)
- ❑ Biologically active. Potent at low conc
- ❑ Mixtures of compounds

→ Unexpected effects in non-target organisms

Feminisation of fish due to the presence of synthetic hormones in the rivers

Death of vultures due to the presence of diclofenac residues in cattle

Alterations in fish behaviour after exposure to psychoactive drug



Pharmaceuticals. Regulations

Water Framework Directive "Watch List" April 2022

-**Psychiatric Drugs**
Venlafaxine
O-desmethylvenlafaxine

- **Antibiotics**
Sulfamethoxazole
Trimethoprim
Clindamycin
Ofloxacin

Drinking Water Contaminant Candidate List 2016

- **Antibiotic**
  - Erythromycin
- **Quinoline antimalarials**

- **Synthetic hormones**
  - E1
  - E2
  - E3
  - 17α E2
  - EEME
  - Equilenin
  - Equilin

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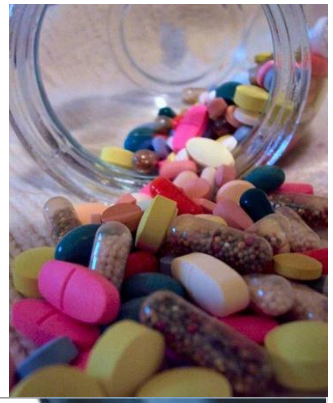
Análisis de Fármacos en muestras ambientales

In the UE around **3000** different PhACs are used in human medicine

↓

✓ Need to develop **multi-residue** analytical methods

✓ The development of such metodologies is closely tied to analytical capabilities ... Increased **sensitivity in mass spectrometry field**



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LIQUID CRHOMATOGRAPHY-TANDEM MASS SPECTROMETRY

✓ **Triple quadrupole (QqQ) instruments widely used (good sensitivity and selectivity in SRM mode).**

✓ **However, with QqQ equipments, qualitative information to support structural information is lost.**

✓ **Hybrid equipments (QTOF and QqLIT) are gaining more acceptance**

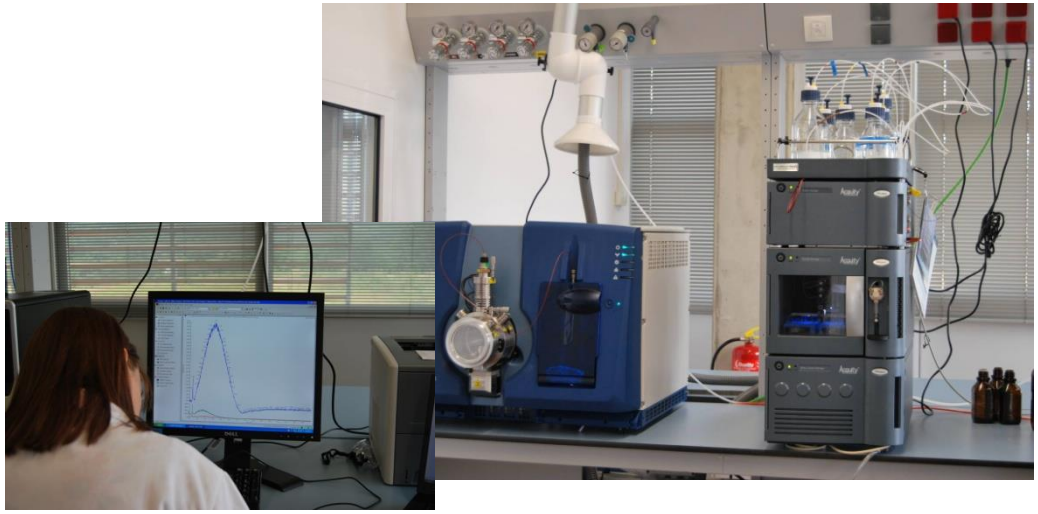
Equipamiento

Sample Pretreatment

- ✓ Acelerated Solvent Extraction (ASE)
- ✓ Automated Solid Phase Extraction: ASPEC
- ✓ Semipreparative Chromatography for sample fractionation and clean-up

Mass Spectrometry Unity:

- ✓ Ultraperformance Liquid Chromatography (UPLC) coupled to AB SCIEX QTRAP® 5500 system
- ✓ GC-MS-MS GC: Trace GC Ultra (SSL - PTV) coupled to QqQ: TSQ Quantum
- ✓ On-line SPE system coupled to UHPLC - TSQ Vantage™ triple quadrupole MS
- ✓ UHPLC Turboflow/EquanMax-UPLC-LTQ Orbitrap Velos



Análisis de Fármacos en muestras ambientales

Sample pre-treatment

Filtration

Addition Na_2EDTA

Only antibiotics: pH adjustment To 3

Addition of surrogate standards

81 PhACs and 53 Antibiotics analysis by UPLC-MS-MS (5500QTRAP)

Automated SPE

Oasis HLB®

50mL hospital wastewater

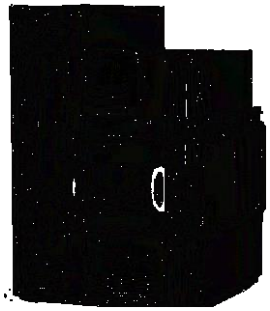
Elution: 6mL MeOH

Instrumental analysis

UPLC-ESI-MS/MS

PhACs: ESI(+) and ESI (-)

Antibiotics: ESI(+)



Gros et al. J of Chromat. 2012; Gros et al. J of Chromat. 2013

Análisis de Fármacos en muestras ambientales

Compuestos de Interes

Analgesics and anti-inflammatories	**Macrolide antibiotics**	**Fluoroquinolone antibiotics**	**Sulfonamide and other antibiotics**	**B-blockers**
Ketoprofen	Erithromycin	Ofloxacin	Sulfamethoxazole	Atenolol
Naproxen	Azithromycin	Ciprofloxacin	Sulfamethazine	Sotalol
Ibuprofen	Roxithromycin	Enrofloxacin	Sulfadiazine	Metoprolol
Indomethacine	Clarithromycin	Enoxacin	Trimethoprim	Propranolol
Diclofenac	Tylosine	Danofloxacin	Chloramfenicol	Timolol
Mefenamic acid	Josamycin	Norfloxacin	Metronidazole	Betaxolol
Salicylic acid	Spiramycin		Nifuroxazide	Carazolol
Codeina	Tilmicosin			Pindolol
Acetaminophen				Nadolol
Phenazone type analgesics	**Tetracycline antibiotics**	**Lipid regulators**	**Histamine H_1 and H_2 receptor antagonists**	**Agents to treat hypertension**
Propiphenazone	Tetracycline	Clofibric acid	Famotidine	Enalapril
Phenazone	Doxicycline	Gemfibrozil	Ranitidine	Hydroclorothiazide
Phenylbutazone	Oxytetracicline	Bezafibrate	Cimetidine	Lisinopril
	Chlortetracycline	Fenofibrate	Loratadine	
Psychiatric drugs	**Barbiturates**	**Cholesterol lowering agents**	**B-agonists and Diuretic**	**Antidiabetic and to treat cancer**
Fluoxetine				
Paroxetine	Butalbital	Atorvastatine	Salbutamol	
Diazepam	Pentobarbital	Pravastatine	Clenbuterol	Glibenclamide
Lorazepam	Phenobarbital	Mevastatine	Furosemide	Tamoxifen
Carbamazepine				

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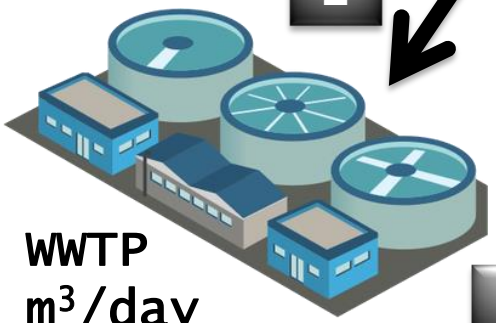
Antibióticos en un sistema urbano

1

Hospital
200 beds
1500 m^3/day

GIRONA Town
North-East SPAIN
115.000 inhabitants

2



Urban WWTP
45000 m^3/day

3

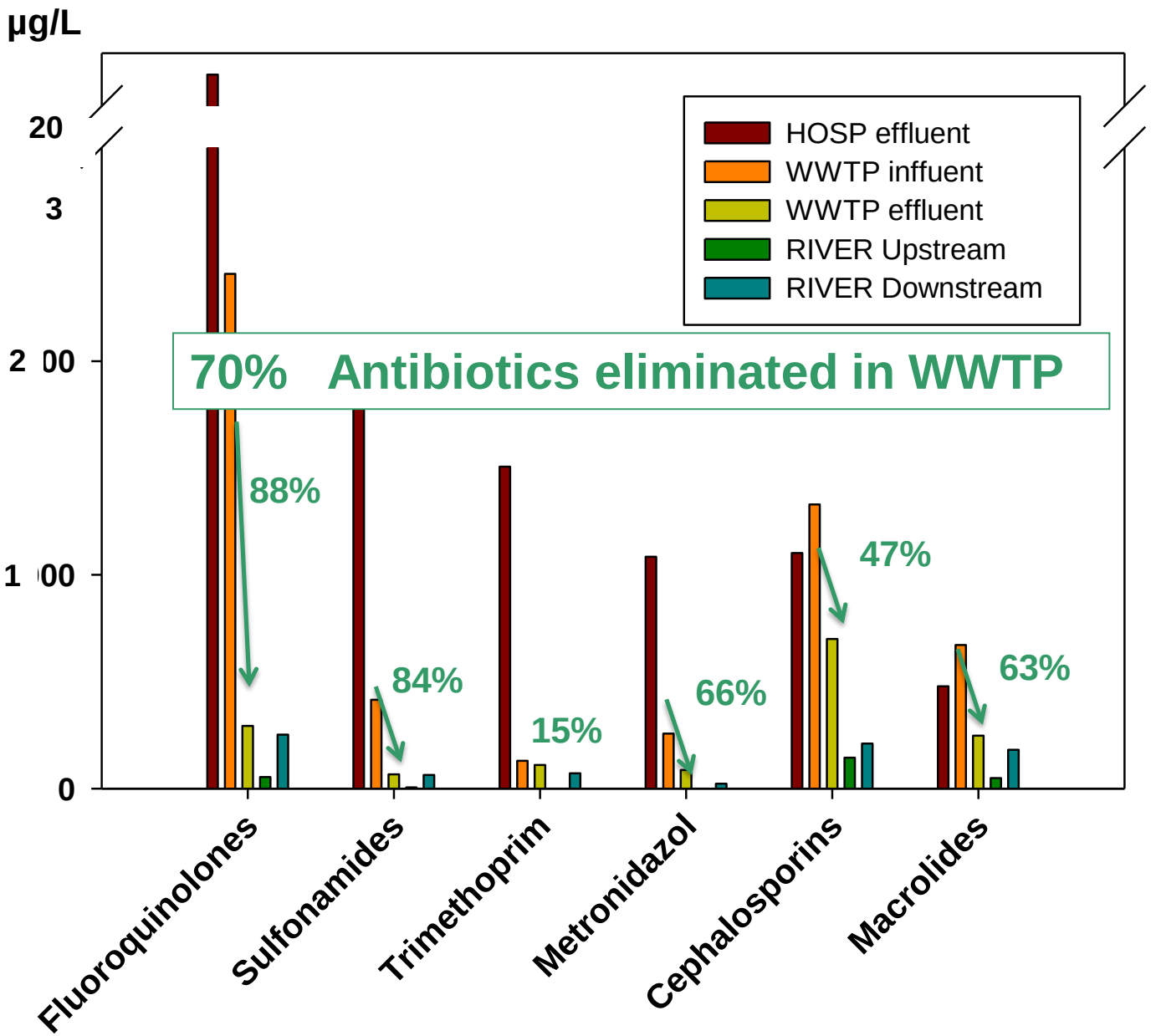
4 Upstream

River TER

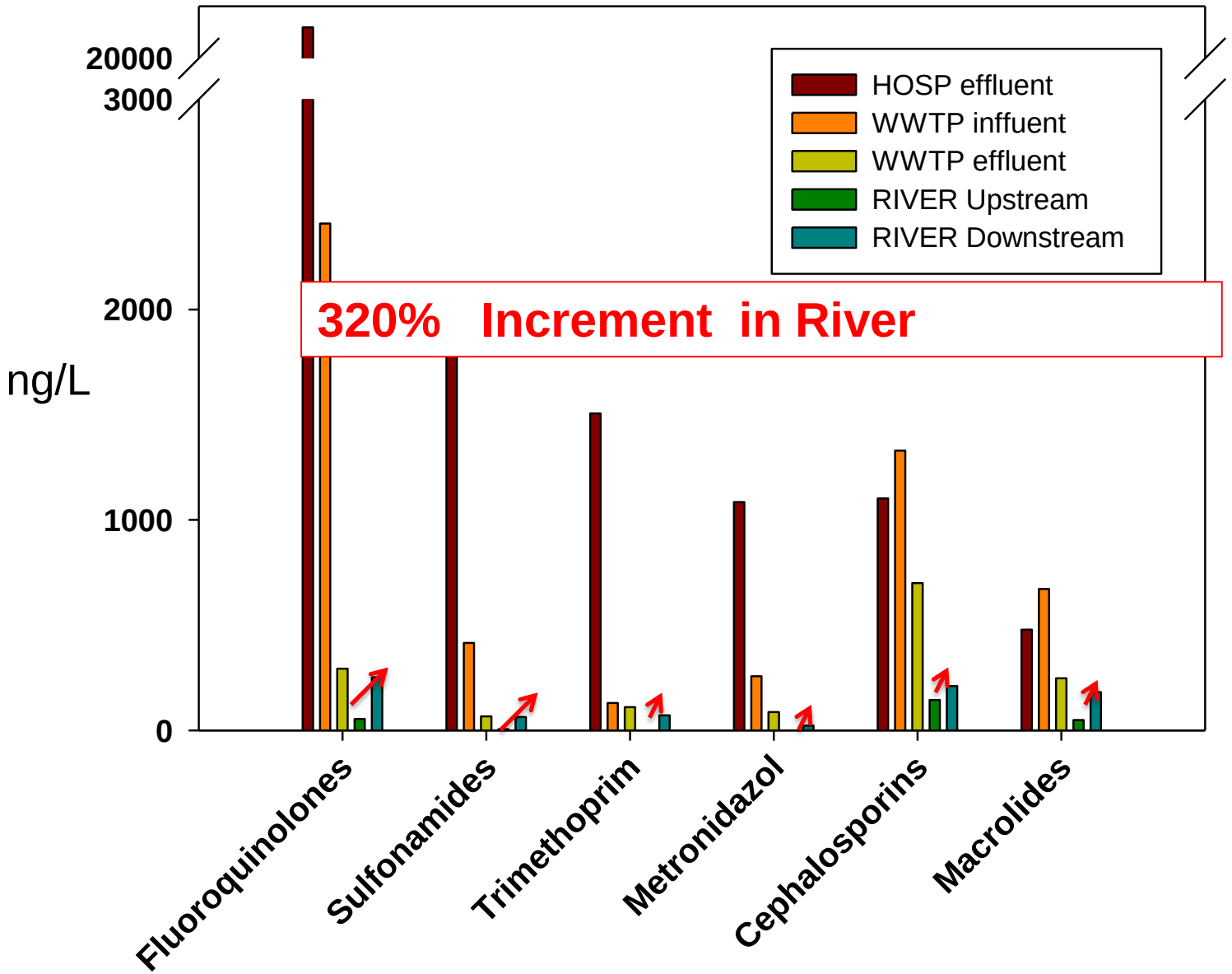
5 Downstream

Sampling Points

Eliminación de Farmacos de las Aguas Residuales



Eliminación de Farmacos de las Aguas Residuales



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Physico-Chemical treatments

- Advanced oxidation processes (AOPs)
  - Ozonation
  - Photolysis
  - Fenton related reactions…

Good removal values

High economic investment
Toxic transformation products

- Filtration technologies
  - Nanofiltration
  - Ultrafiltration
  - Reverse osmosis

Good removal values

Hazardous residues
(concentrates or brines)

- Adsorption to activated carbon

Good removal values

High economic investment

Biological treatment

- Membrane bioreactors (MBRs)

- Artificial wetlands

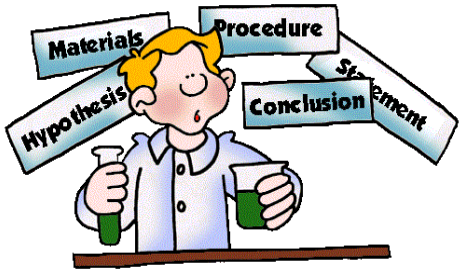
- Fungal treatment

Good removal values

Membrane fouling
High economic investment

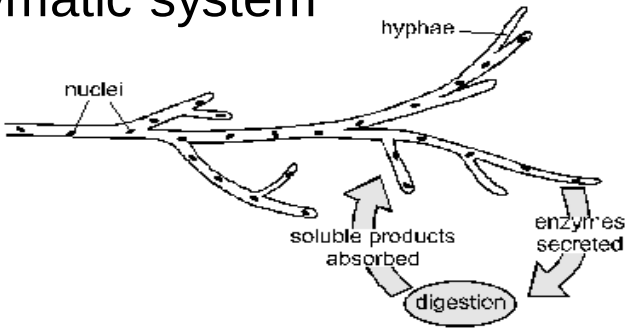
Good removal values
Low energy requirements

High land use
High retention time



White-Rot Fungi, why?

- ✓ Powerful and unspecific degradative enzymatic system
- ✓ Extracellular digestive enzymes
- ✓ High tolerance to toxic compounds

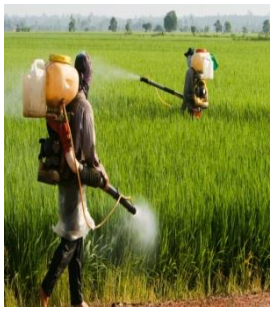


Widely studied in bioremediation since 80's

PAHs

Ammunition wastes

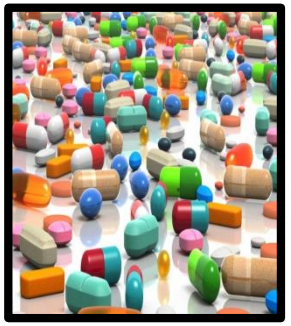
Pesticides



PCBs

Textile dyes

PhACs



Tratamiento alternativos y/o avanzados de aguas residuales

Trametes Versicolor

Ligninolytic fungi (white-rot fungi)

Biodegradation of a broad range of xenobiotics and recalcitrant contaminants

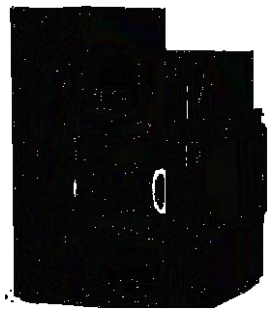
Target Analysis

Removal of 81 PhACs by UPLC-MS-MS

Instrumental analysis

UPLC-ESI-MS/MS

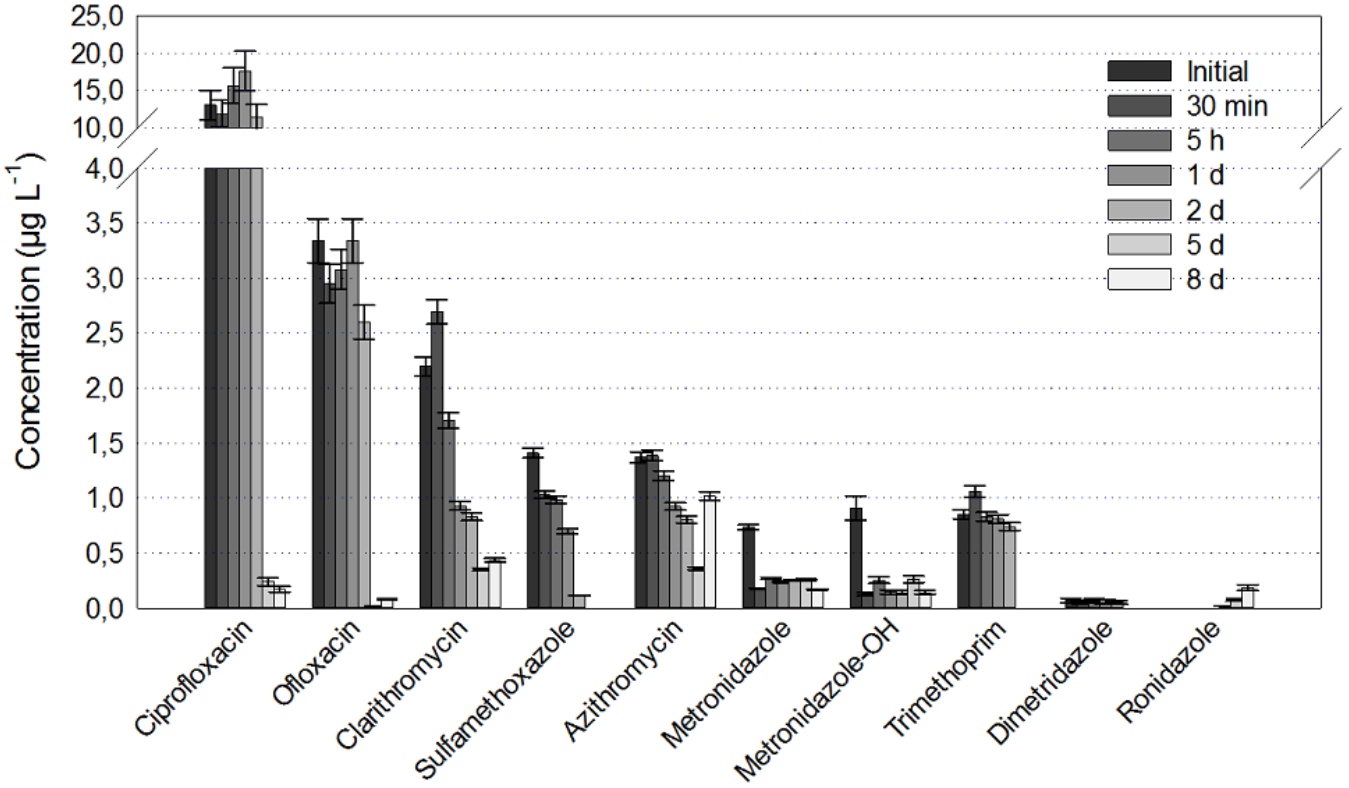
ESI (+) and ESI (-)



Tratamiento alternativos y/o avanzados de aguas residuales

Trametes Versicolor

- Hospital batch bioreactor
- Non-Sterile treatment
- Antibiotics (53 compounds)

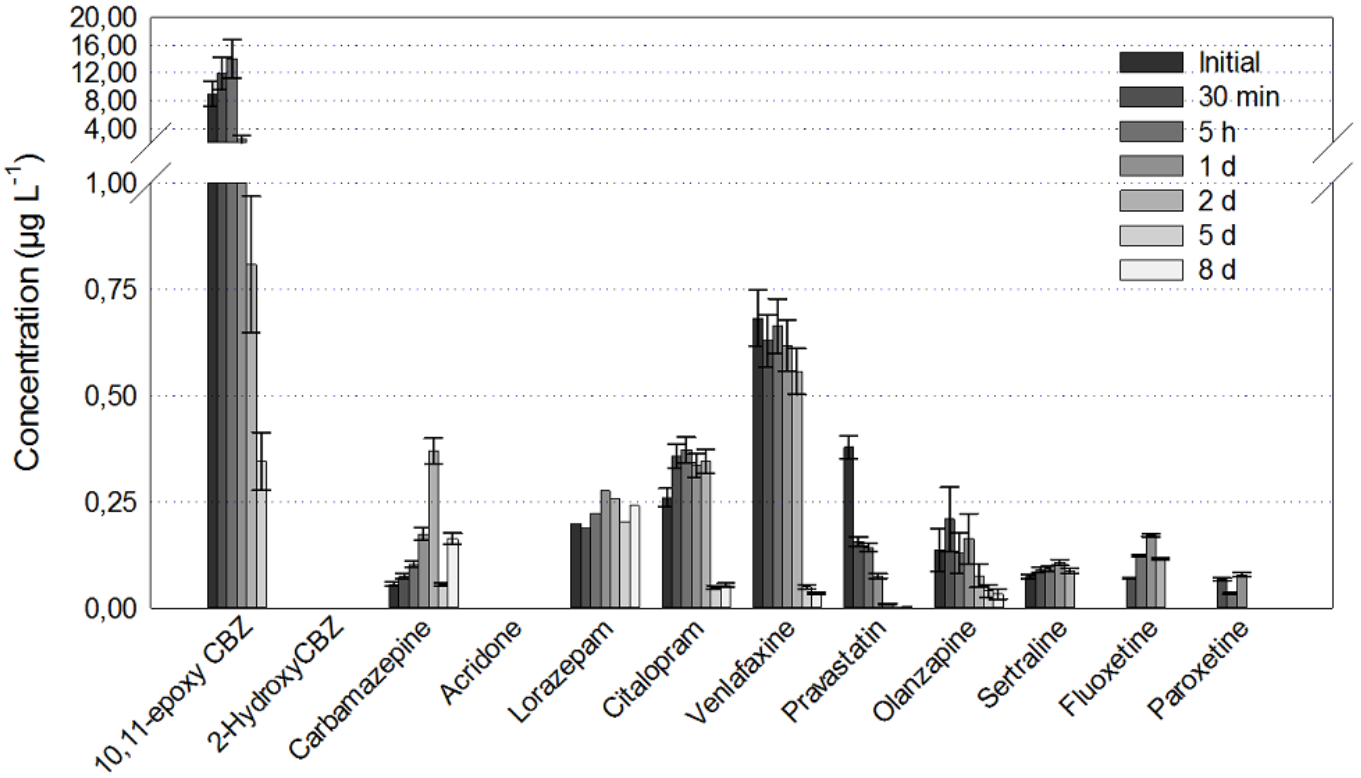


Cruz-Morato et al., STOTEN, 2014

Tratamiento alternativos y/o avanzados de aguas residuales

Trametes Versicolor

- Hospital batch bioreactor
- Non-Sterile treatment
- Psychiatric Drugs (out of 81 PhAcs)



Cruz-Morato et al., STOTEN, 2014

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Productos de degradación de fármacos

Dissapearance of the parent compound does not necessarily mean that the treatment was succesful!

Pharmaceuticals

Metabolites

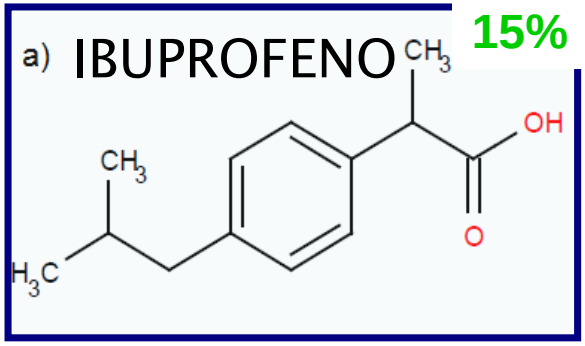
Transformation products (TPs)

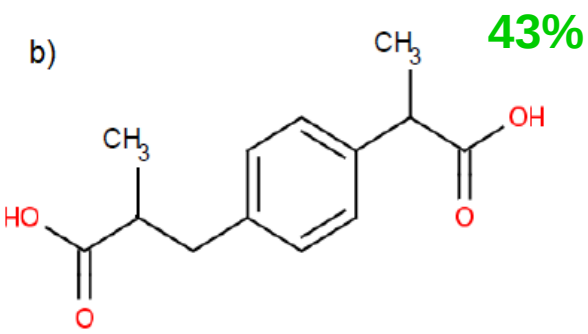
- biodegradation and oxidation in water/wastewater treatment processes
- photodegradation in surface water

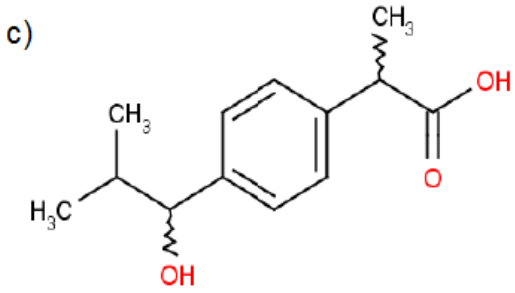
TPs and metabolites can be more persistent, bioaccumulative and/or toxic than their parent compounds

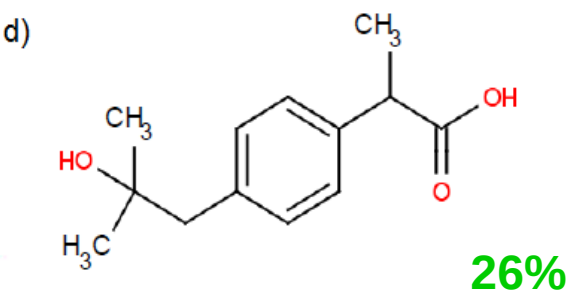
Emerging Environmental Concern

Degradacion de Ibuprofeno









Model Compound:

- Present in Wastewater treatment plants
- Biodegradable in aerobic conditions
- Low toxicity

(a) ibuprofen (anti-inflammatory drug)
(b) carboxyl ibuprofen (IBU-CBX)
(c) 1-hydroxyl-ibuprofen (IBU-1OH)
(d) 2-hydroxyl-ibuprofen (IBU-2OH)

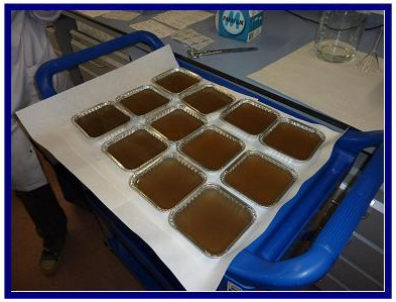
Degradacion de Ibuprofeno

Activated Sludge Batch Experiments IBUPROFEN

Biomass of sludge from a WWTP (35,000 m^3 d^{-1}, 175,000 I.E.) spiked with Ibuprofen at different concentrations. Controled aerobic conditions. Short term study (hours/days):

Conditions to optimize:

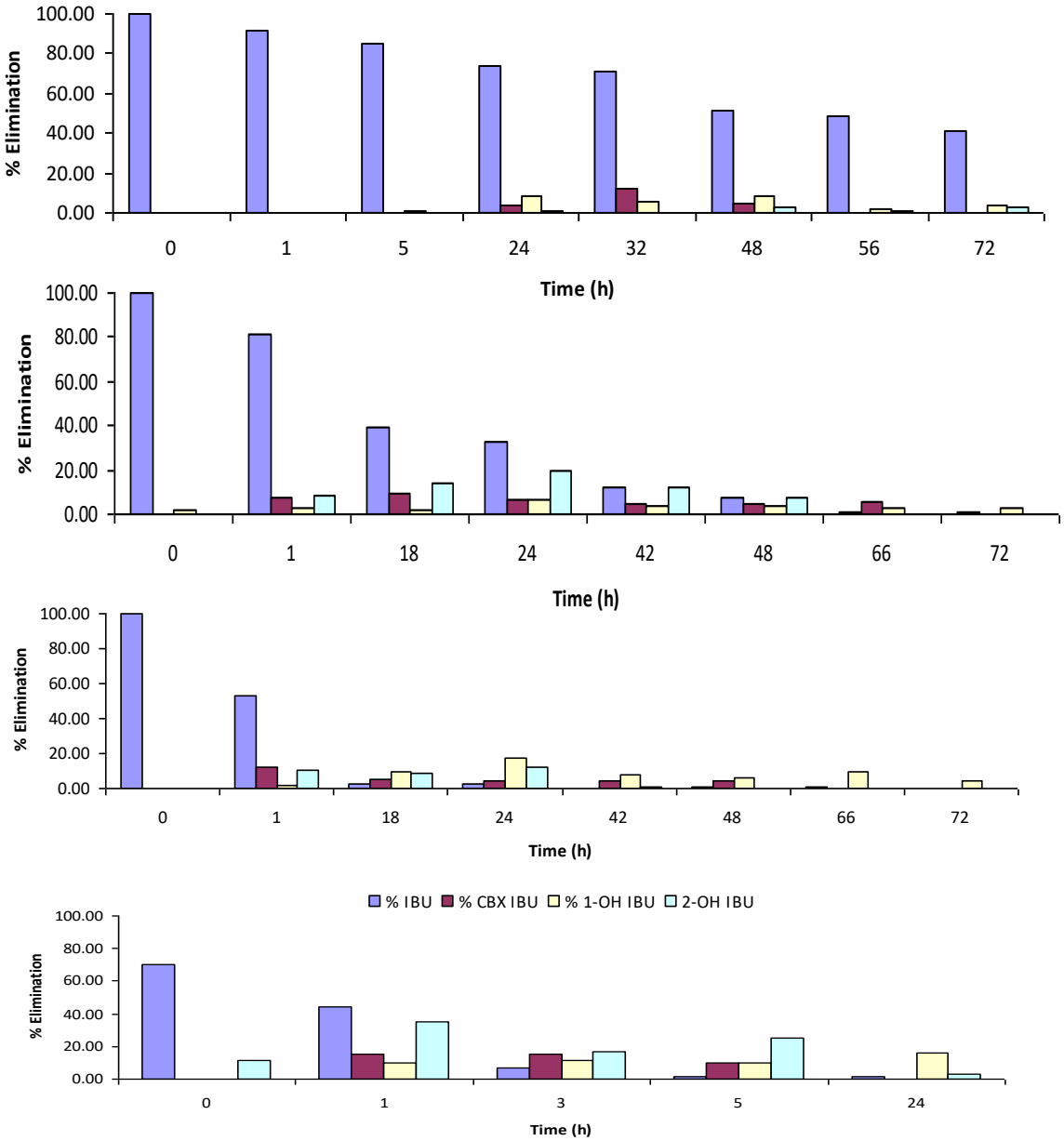
- 10, 100, 200, 1000 $\mu g_{IBU}/L$
- 50, 100, 200, 1000 mg_{TSS}/L



Degradacion de Ibuprofeno

Activated Sludge Batch Experiments IBUPROFEN

- → Follow up of degradation profiles of ibuprofeno
- → Study of the **generation** of different **Metabolites**
- ✓ **Suspended solids**



Ferrando-Climent et al. STOTEN 2012

Productos de degradación de fármacos

- Study of **Ibuprofen** as a Model Compound
- Field Study. Monitoring of **Ibuprofen** and metabolites in a WWTP and the receiving River Water

Compound	*November 2011 (µg/L)*			*December 2011 (µg/L)*			*January 2012 (µg/L)*		
	WWTP Influent	*WWTP Effluent*	*River*	*WWTP Influent*	*WWTP Effluent*	*River*	*WWTP Influent*	*WWTP Effluent*	*River*
IBU	12.65	0.48	0.29	8.04	1.90	0.75	7.00	1.48	0.15
1-OH IBU	2.55	0.11	bloq	5.00	0.92	0.45	5.78	0.81	0.56
2-OH IBU	1.21	0.39	bloq	93.65	3.15	3.00	93.98	5.56	1.46
CBX IBU	bloq	bloq	bloq	19.88	5.37	3.95	18.38	9.40	1.99

Ferrando-Climent et al. Comprehensive study of ibuprofen and its metabolites in activated sludge batch experiments and aquatic environment. The Science of Total Environment.438, 404-413 (2012)

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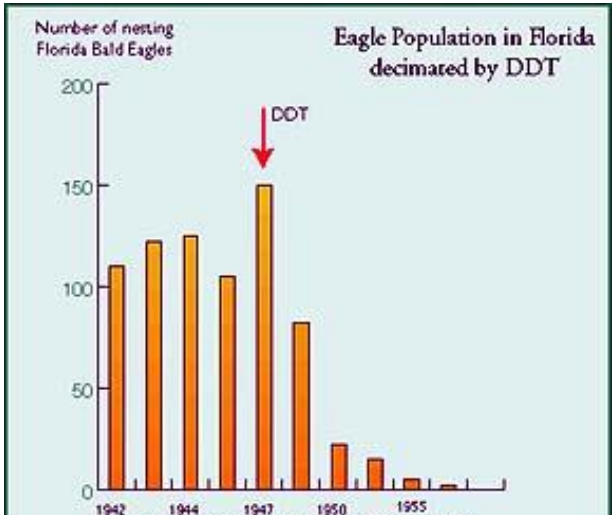
Harmful effects

- Extremely potent, even at very low concentrations
- Potential to be biologically active in wildlife
- Exposure to multiple compounds, which could increased risk of adverse effects

Antibiotic resistance

Alterations in reproduction



Decline of wildlife populations

Impacto Ambiental

Bioaccumulation

Impact of Chemical contaminants in aquatic life evaluated by:

- Classical approach: (eco)toxicological parameters
- Other approaches: **bioaccumulation** in aquatic organisms

plants (terrestrial environment)





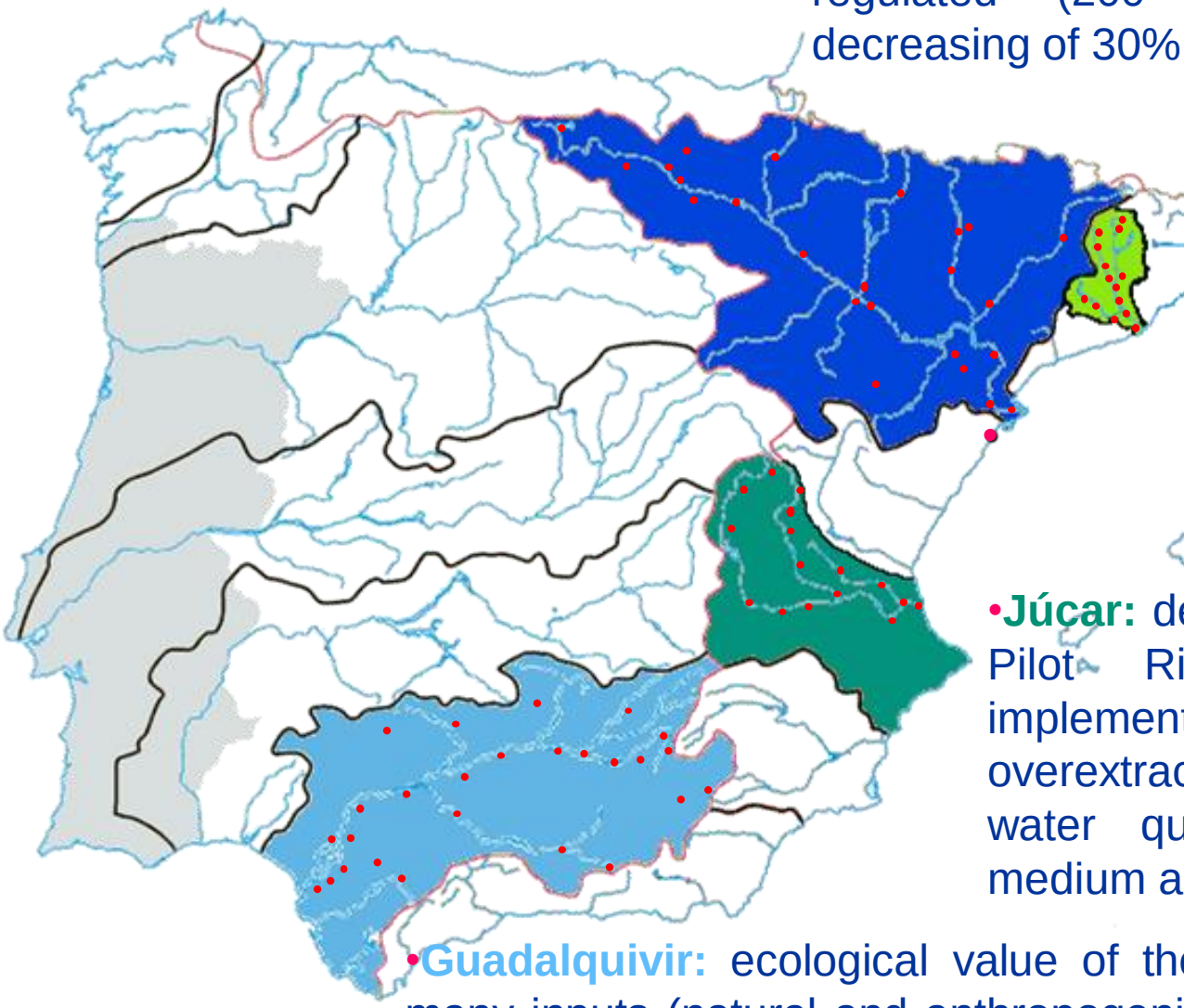
Need of development of appropriate methodologies for:

- Multiple compounds
- Multiple Biological matrices

Analytical challenges:

- Low concentration
- Broad variety of physicochemical properties
- Highly complex matrix: lipids, proteins, pigments, etc.

Environmental Impact



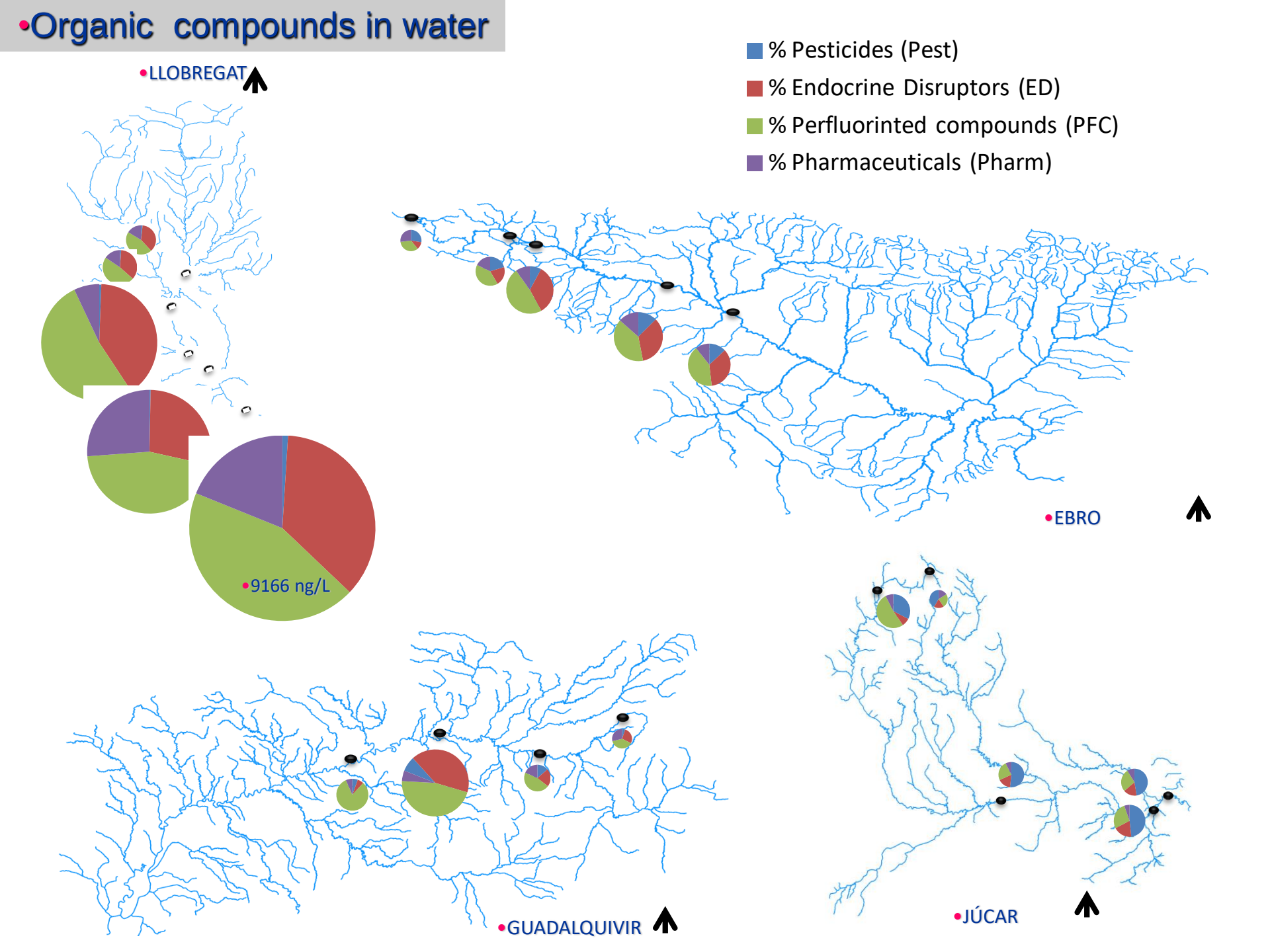
•**Ebro:** intensive agricultural activity, largely regulated (200 dams and channels), decreasing of 30% of the mean annual flow

•**Llobregat:** Heavily managed in its lower course, Barcelona's major drinking water resources, extensive urban and industrial waste water discharges

•**Júcar:** designated as a European Pilot River Basin for the implementation of the WFD, overextraction of groundwater, water quality problems in the medium and lower parts

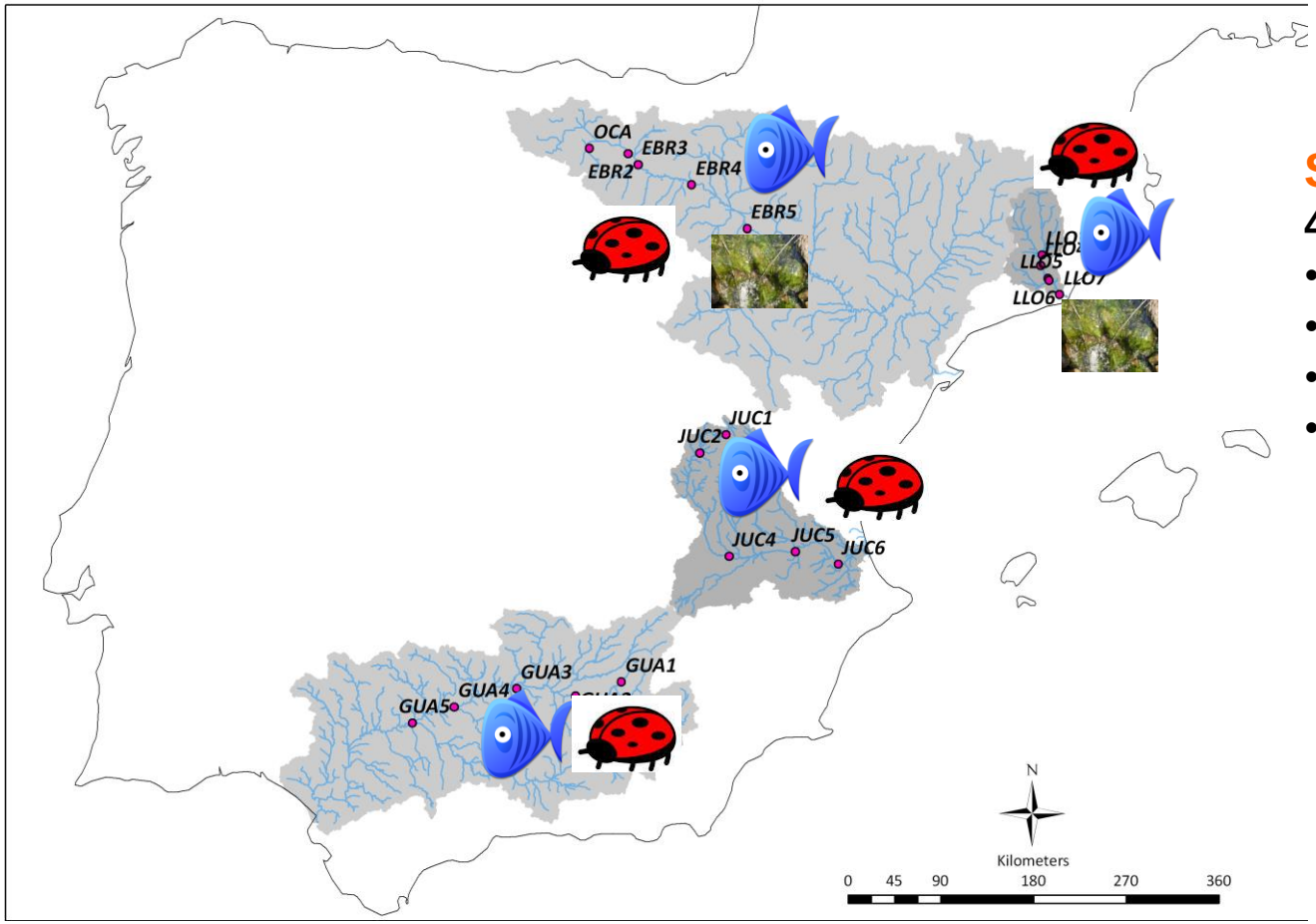
•**Guadalquivir:** ecological value of the Doñana National Park, many inputs (natural and anthropogenic origin), navigable up as far as Seville (serious environmental problem)

•Organic compounds in water



Environmental Impact

Analytical methods for study of bioaccumulation of Pharm & EDCs in biofilm, macroinvertebrates, mussel, and fish



Sampling Campaign

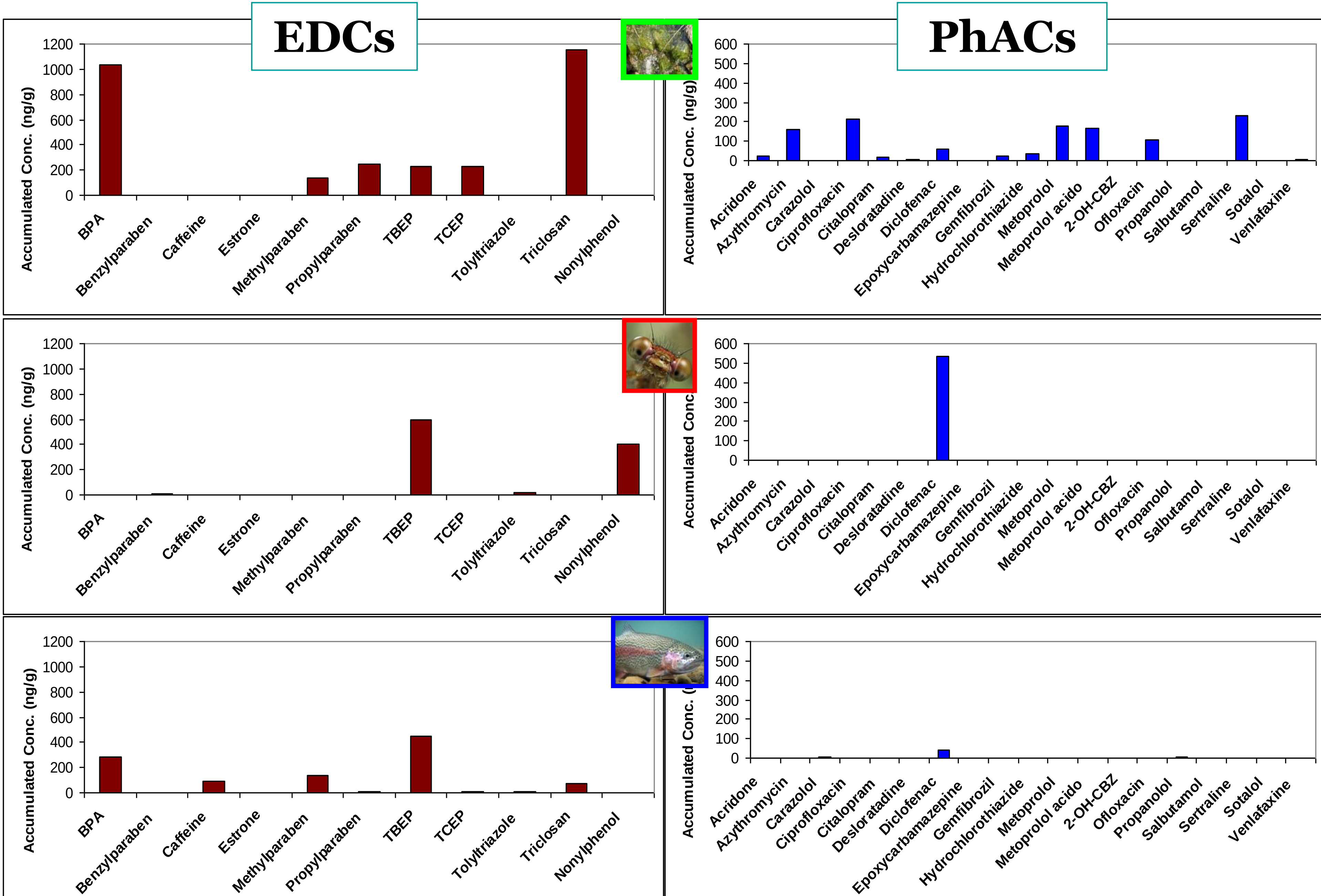
4 Mediterranean Rivers:

- **Llobregat**
- **Ebro**
- **Jucar**
- **Guadalquivir**

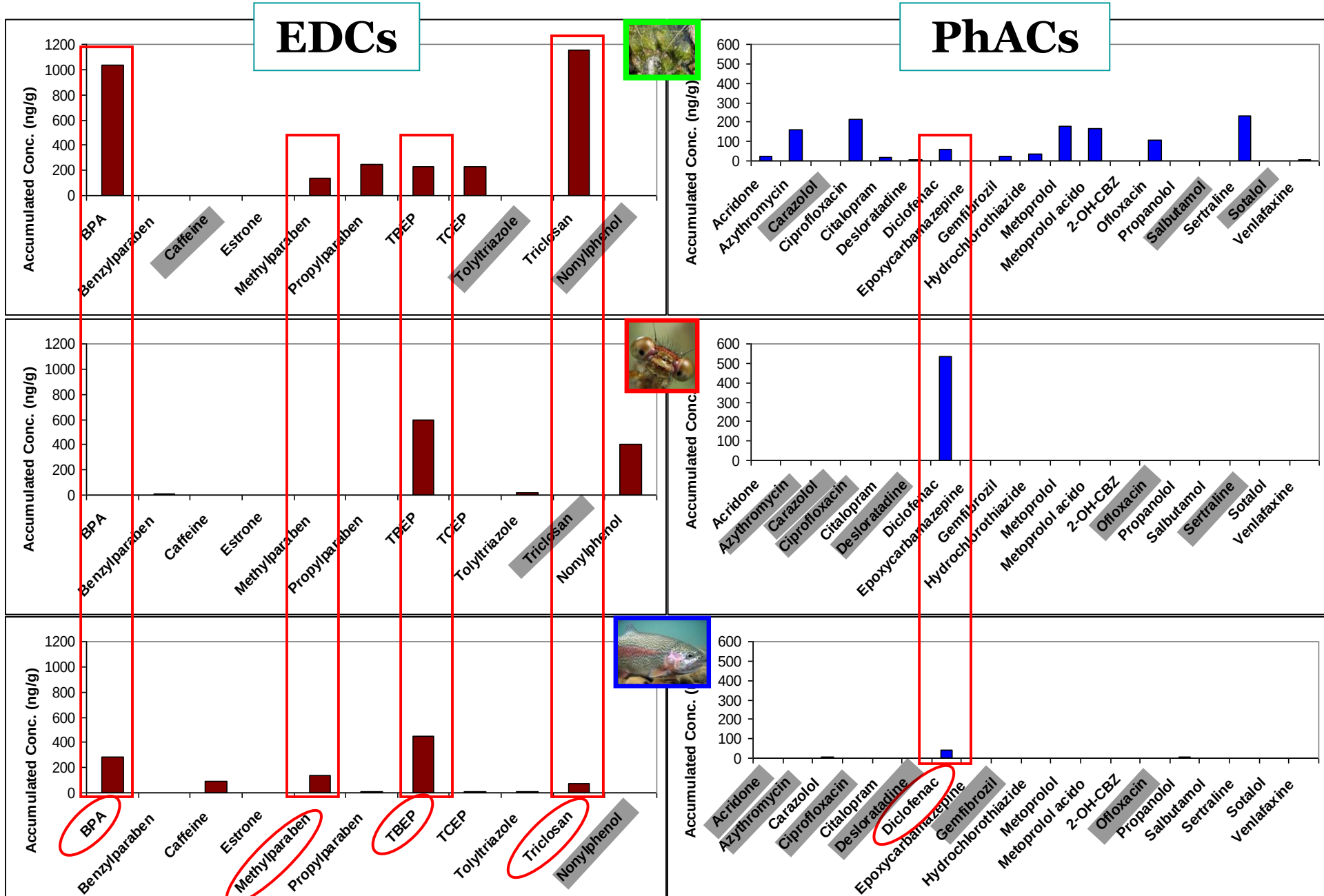
***Huerta et al*. Analysis of Multi-Class Pharmaceuticals in Fish Tissues by Ultra-Performance Liquid Chromatography Mass Spectrometry. *J. Chrom*. 1288, pp. 63-72 (2013)**

***Jakimska et al*. Development of a liquid chromatography–tandem mass spectrometry procedure for determination of endocrine disrupting compounds in fish from Mediterranean rivers. *J. Chrom*. 1306, 44-58 (2013)**

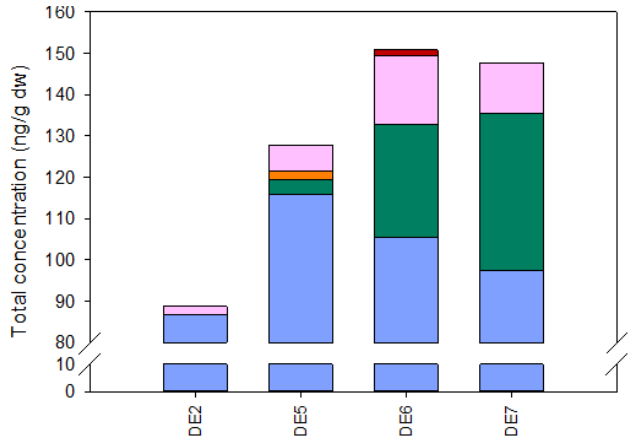
Environmental Impact



Environmental Impact



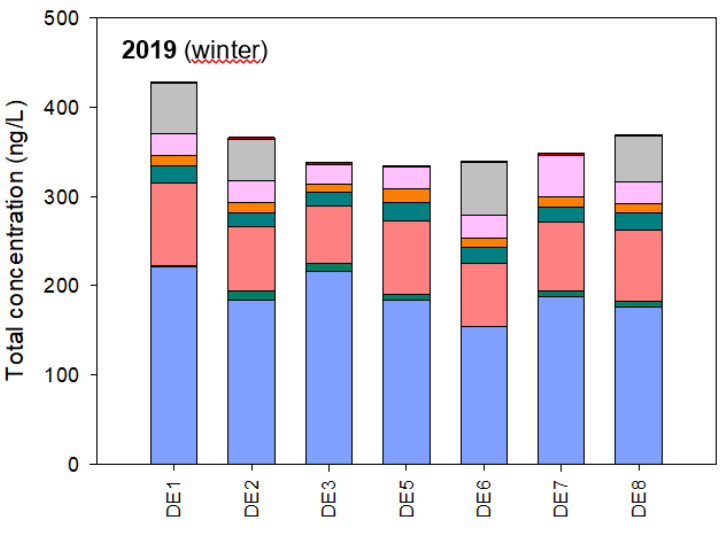
Pharmaceuticals in Ebro Delta

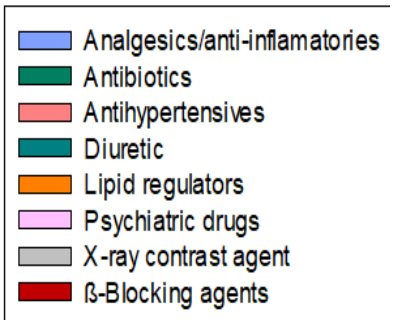


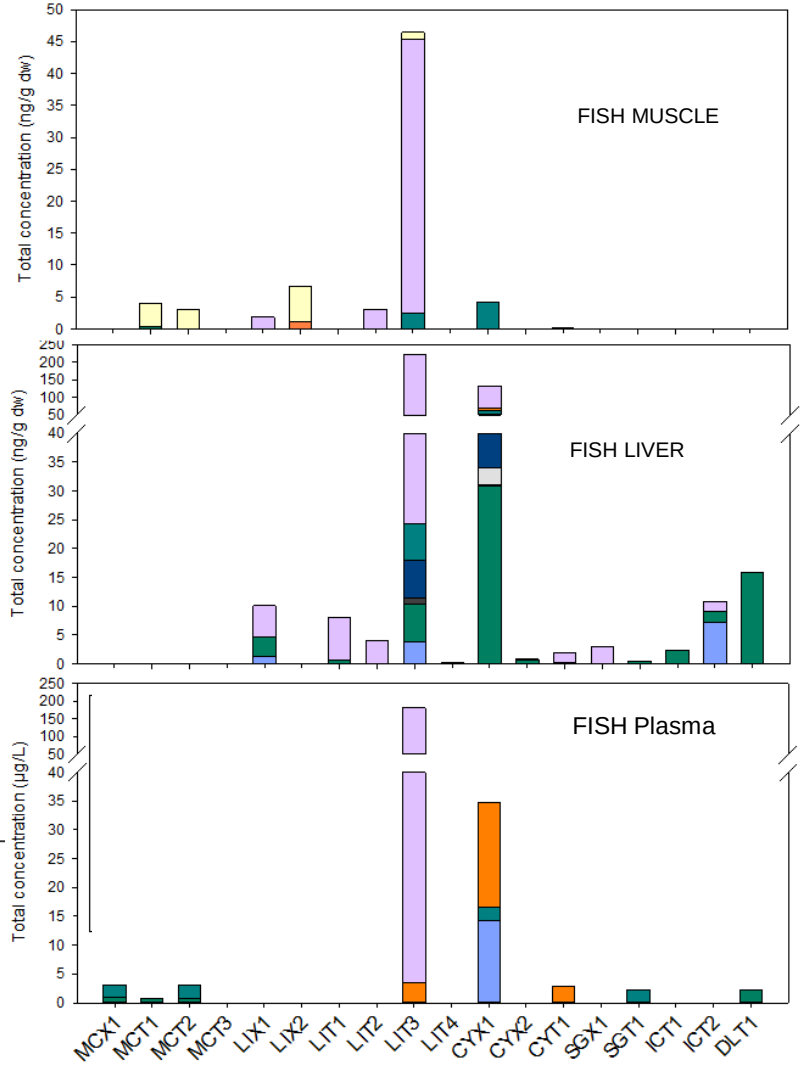
Biofilm

Fish

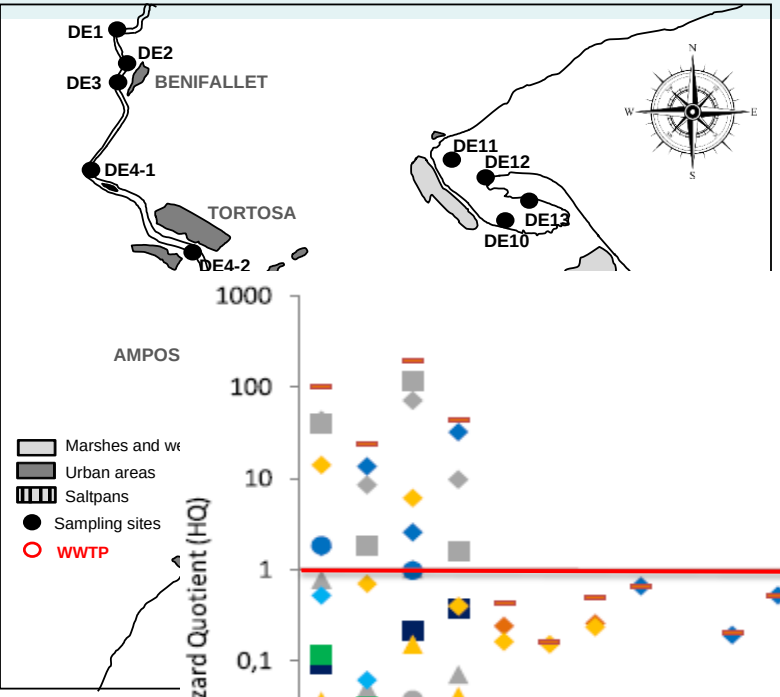
Water





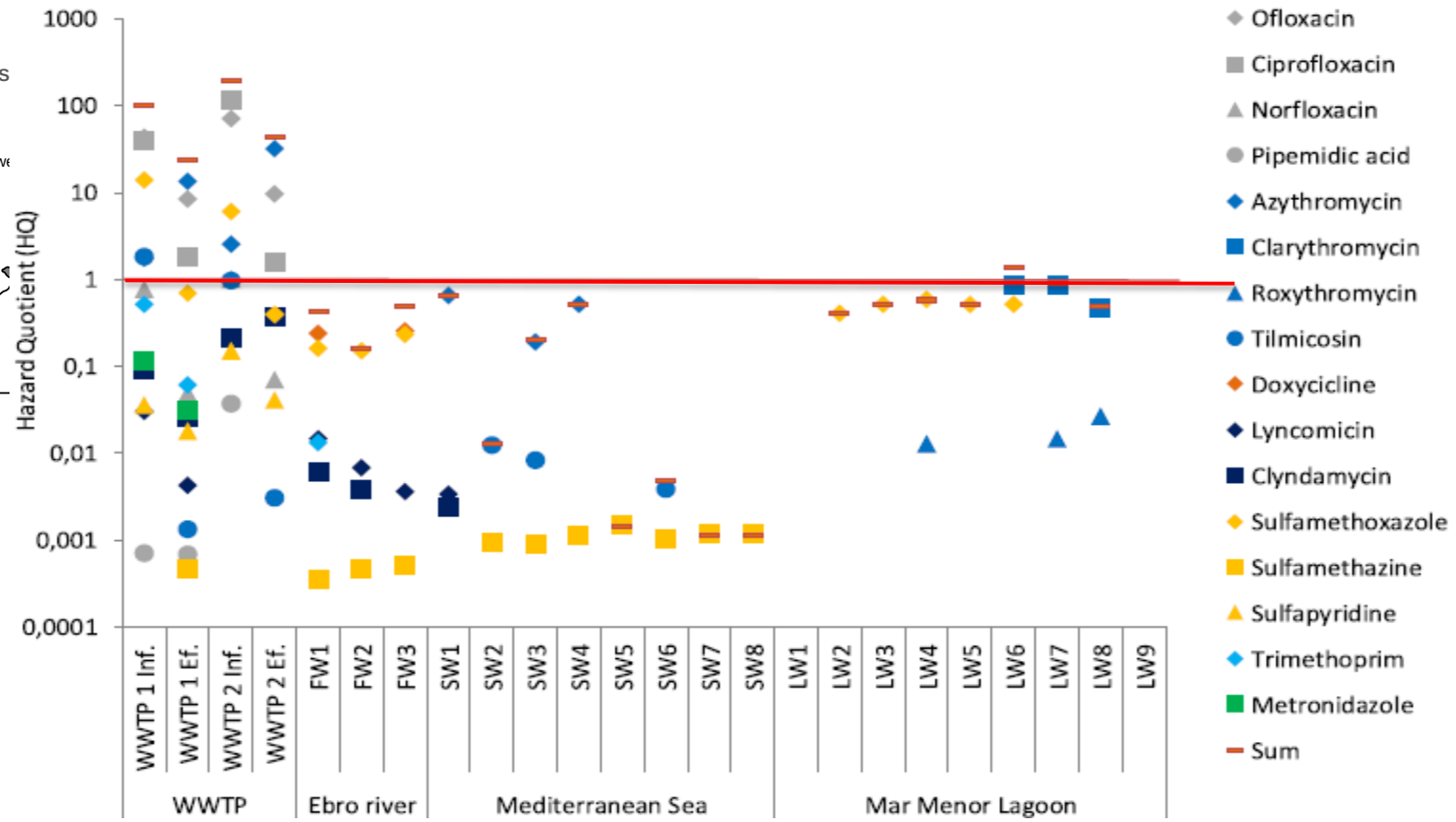


Impacto Ambiental



Environmental Risk Assessment for antibiotics in Ebro Delta

Antibiotic concentration Water



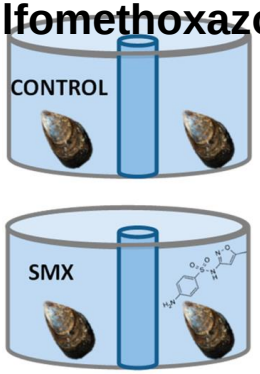
omote

Serra-Compte et al. Env Poll 2021

Impacto Ambiental

Objective: To evaluate the impact of antibiotics exposure on aquatic organisms under controlled experimental conditions

Marine mussels
Antibiotic: sulfomethoxazol

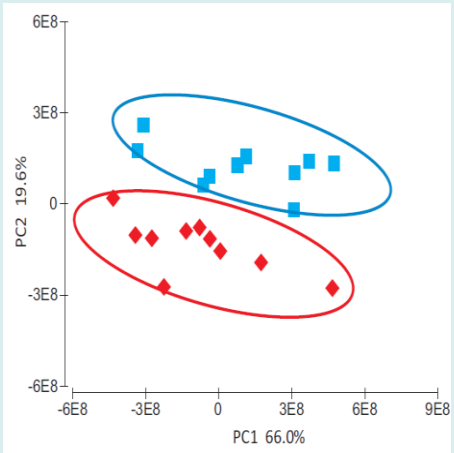


Water samples
Mussels **hemolymph**
Mussels **soft tissue**
Mussels **gastrointestinal tract**

1. **Bioaccumulation of antibiotic**
2. **Antimicrobial resistance**
   Antibiotic resistance genes
   Microbial community composition
3. **Ecotoxicological effects**
   Enzymatic activities
   Metabolome alterations

(ECO)METABOLOMICS

High Resolution Mass Spec



Metabolite name	Compound class
Aspartate	Amino acid
Valine	Amino acid
Phenylalanine	Amino acid
Tryptophan	Amino acid
Guanosine	Nucleotide
Inosine	Nucleotide
Benzoate	Carboxylic acid

Metabolite alteration

Metabolic pathways alterations

OUTLINE

1. FARMACOS EN EL MEDIOAMBIENTE
2. ANALISIS DE FARMACOS EN EL MEDIOAMBIENTE
3. ESTRATEGIAS DE ELIMINACION DE AGUAS RESIDUALES
4. TRATAMIENTO ALTERNATIVO DE AGUAS RESIDUALES
5. PRODUCTOS DE TRANSFORMACIÓN DE FARMACOS
6. IMPACTO AMBIENTAL
7. **IMPACTO EN LA SALUD HUMANA**

Impacto en la salud humana

Bioacumulación en peces y moluscos en medio marino

Environment

- 17α-ethinylestradiol (EE2)
- β-estradiol
- Macrolide antibiotics (Erythromycin, Clarithromycin, Azythromyin)
- Other antibiotics: Ciprofloxacine

→ ✓ Watch List for monitoring of water

Food safety

→ ✓Maximum Residues Limits (MRL)

✓Authorised
(Antibiotics and antiparasitic agents)

× Prohibited
(Chloramphenicol, dimetridazole, ronidazole, etc.).

Lack of regulation for many of these compounds

Bioacumulación en peces y moluscos en medio marino

Goals

1) To monitor the occurrence and levels of PhACs and EDCs in ecologically relevant species of seafood from potential contaminated areas in Europe.

2) To propose a preliminary list of compounds that can be prioritized based on their concentration, occurrence and frequency of detection.

3) To compare total contamination due to PhACs and EDCs in seafood from the hotspots areas

✓**ECsafeSEAFOOD** project aims to asses food safety issues related to priority contaminants present in seafood as a result of environmental contamination and evaluate their impact on public health.

Impacto en la salud humana

Bioacumulación en peces y moluscos en medio marino

•Macroalgae
(20 organisms)

Saccharina latissima

Laminaria digitata

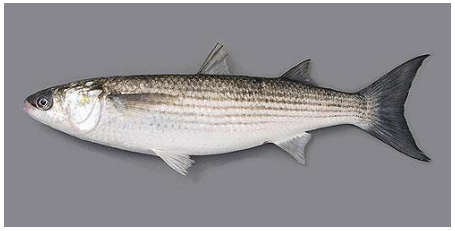
•Bivalve
(50 organisms)

Mytilus spp

Chamalea gallina

Crassostrea gigas

•Fish
(25 organisms)



Liza aurata

Platichthys flesus

➢**Pool => Grinded => homogenized => freeze-dried => kept at -20ºC until its analysis.**

Impacto en la salud humana

Bioacumulación en peces y moluscos en medio marino

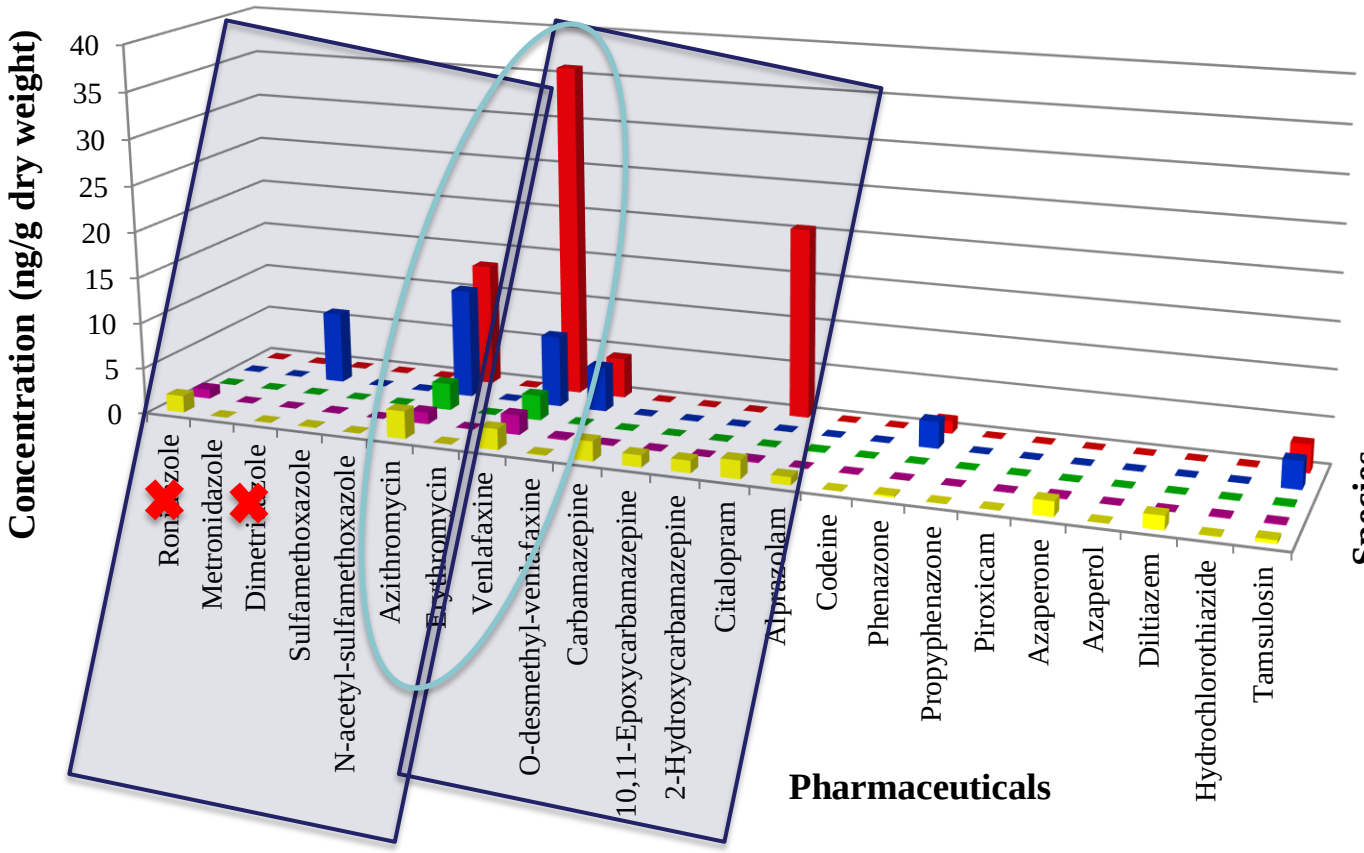
Sampling sites

Hot spots

➢100 organisms were collected at each sampling site and a pool was made by homogenizing the tissues. The composite sample was freeze dried and kept at -20ºC until its analysis.

Impacto en la salud humana

Bioacumulación en peces y moluscos en medio marino



- 4 PhACs macroalgae
- 10 PhACs fish
- 16 PhACs bivalve

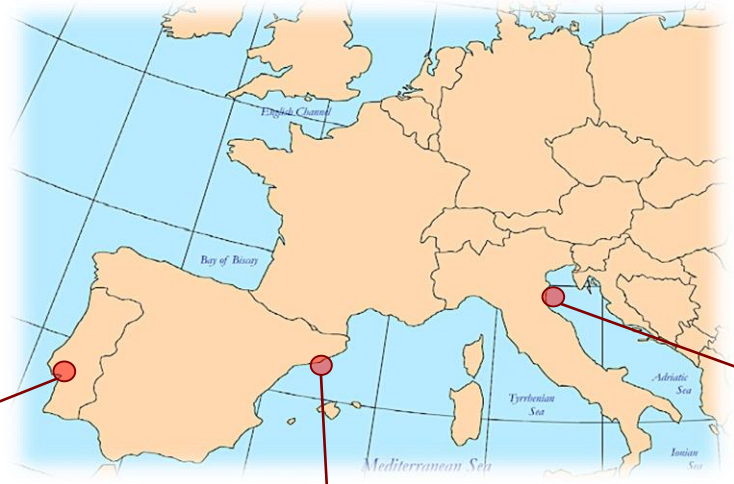
➢ Azithromycin and velanfaxine present in all analysed samples

Alvarez-Muñoz et al. Environmental Research 2015

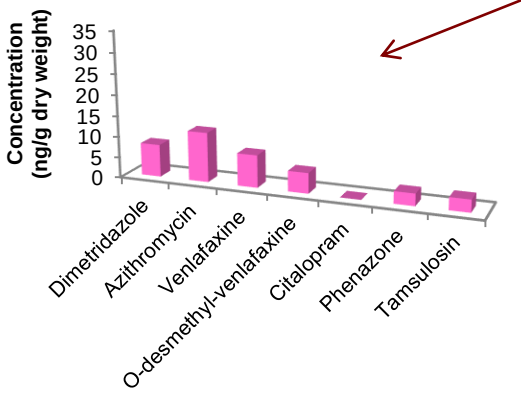
Impacto en la salud humana

Bioacumulación en peces y moluscos en medio marino

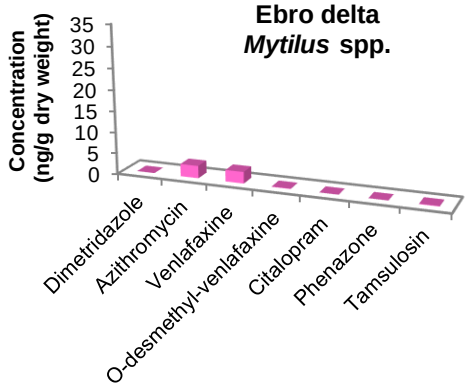
Analysis of pharmaceuticals in mussel from different European polluted areas (Hot spots)



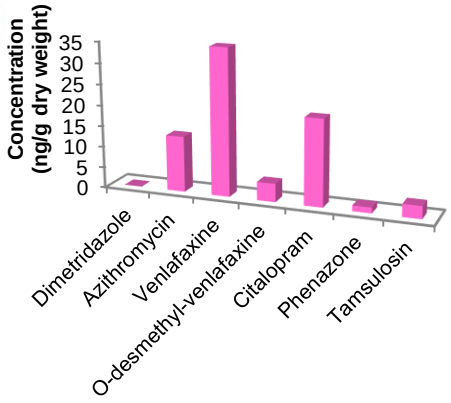
Tagus estuary
***Mytilus* spp.**



Ebro delta
***Mytilus* spp.**



Po delta
***Mytilus* spp.**



Alvarez-Muñoz et al. Environmental Research 2015

Impacto en la salud humana

Regenerated Water

Human Health Risk Assessment (HHRA)

Crops Irrigation




Acceptable Daily Intake (ADI)*
µg/kg bw/day

Pollutants Bioaccumulation in edible crops

Calculated human dietary exposure
µg/kg bw/day

** ECHA; EFSA; FAO; WHO;*

Impacto en la salud humana

Regenerated Water

Contaminated natural water Bodies

Crops Irrigation

Aquatic Organisms

Pollutants Bioaccumulation in VEGETABLES

Calculated human dietary exposure
µg/kg bw/day

Pollutants Bioaccumulation in SEAFOOD

Acceptable Daily Intake (ADI)*
µg/kg bw/day

Human Health Risk Assessment (HHRA)

** ECHA; EFSA; FAO; WHO;*

Acknowledgements

Integrating nature-based water ReUse strategies with advanced Monitoring of the Presence and impact of MicroPollutants and MicroPlastics. Spanish State Research Agency, **REUSEMP3**

Demonstration of water loops with innovative regenerative business models for the Mediterranean region" EU 2018 H2020. **HYDROUSA**

Microplastics and micro-pollutants on the Mediterranean coast: Toxicity and impact on environment and human health" Spanish State Research Agency, AEI. **PLASMED**

NATIONAL GEOGRAPHIC
Art: Oliver Uberti. Photo: Rebecca Hale

GRACIAS POR SU ATENCIÓN!

Catalan Institute for Water Research

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Institució CERCA
Centres de Recerca de Catalunya

WHAT IS ICRA?

- A **multidisciplinary water research centre** created on October 26, 2006 by the Catalan Regional Government within the framework of the Research Centres Programme (CERCA)

- A Foundation whose trustees are:

the Catalan Regional Government's Department of Economy and Knowledge

the University of Girona (UdG)

the Catalan Water Agency (ACA)

MAIN RESEARCH OBJECTIVES

- Focus on **Water Scarcity** issues including **Water Quality and Quantity** and impacts on ecosystems.

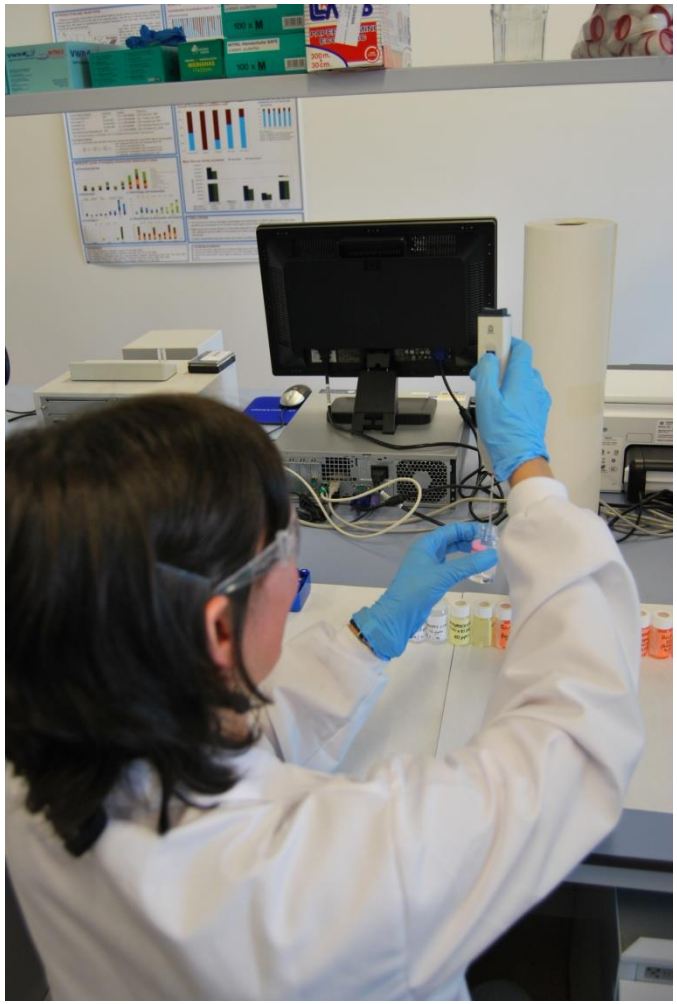
- Focus on **Water technologies** to improve **Water Management under Scarcity.**

- **Risk-based management of Water Resources** in the Mediterranean.

- **Train** highly specialised **researchers and technicians.**

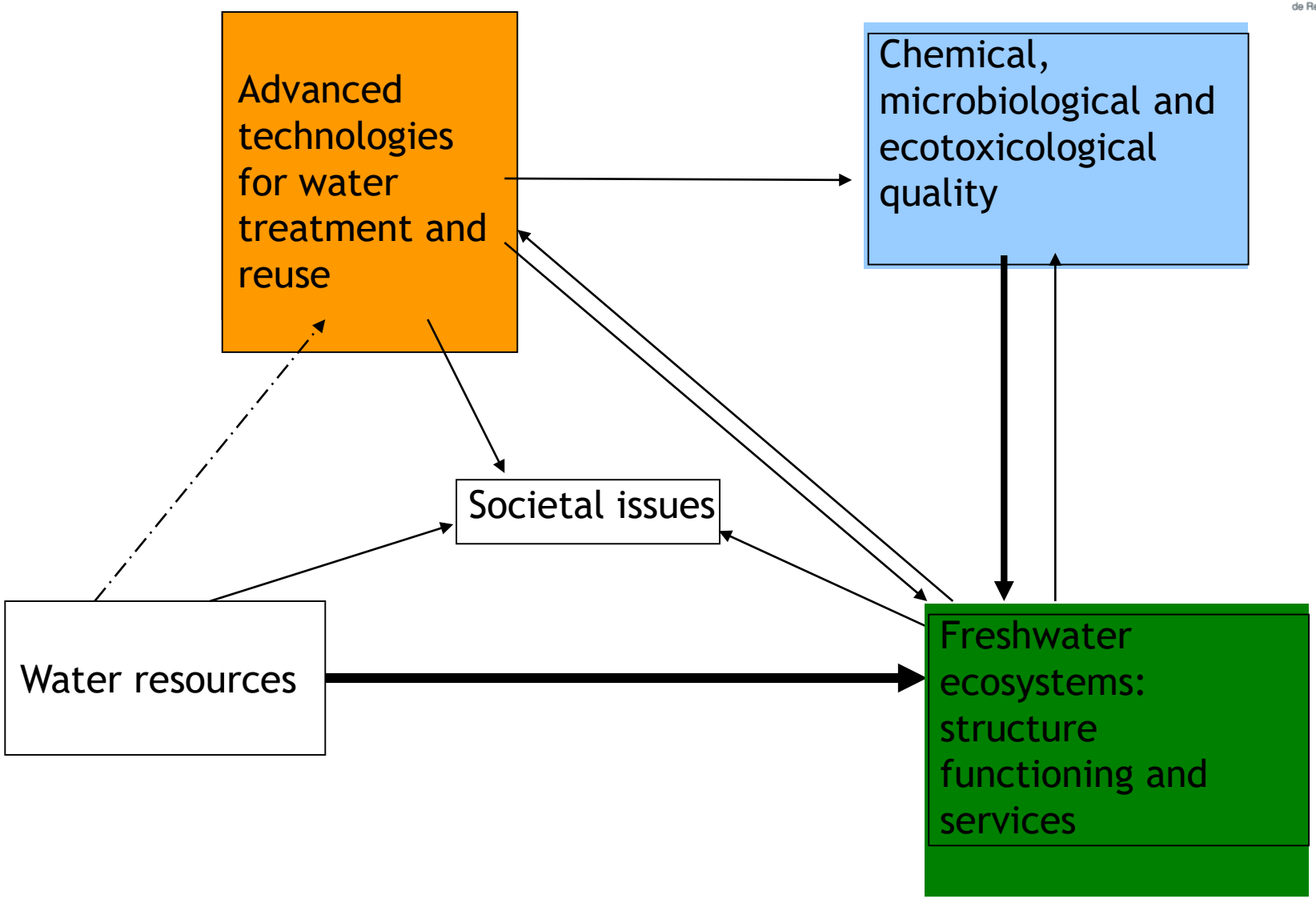
- **Organise national and international scientific meetings** and provide specialised services and products to the scientific community.

Who is (and could be) at ICRA?



✓**Researchers background: chemists, chemical engineers, ecologists, hydrologists, microbiologists, biotechnologists, environmental chemists, civil engineers, environmental engineers, laboratory technicians, economists, and other related areas**

MULTIDISCIPLINAR AND TRANSVERSAL APPROACH



Research Structure

AI. RESOURCES AND ECOSYSTEMS	AI1 Hydrological processes
	AI2 Lacustrine and reservoir systems
	AI3 Fluvial systems
	AI4 Modelling of ecosystems and basins
AII. WATER QUALITY	AII1 Chemical Contamination of Water Bodies
	AII2 Pollutants in wastewater
	AII3 Microbiologic quality and diversity
	AII4 Ecotoxicological response of the biota to pollutants
AIII. TECHNOLOGIES AND EVALUATION	AIII1 Purification and distribution
	AIII2 Treatment/reuse of waste water
	AIII3 Modelling and management systems
	AIII4 Unit operations

ICRA's facilities

✓20 laboratories fully equipped with latest-generation instrumentation to provide technical and methodological advice to researchers

ICRA's facilities

✓Scientific & Technical Services divided into 4 units: Chemical Analysis, Mass Spectrometry, Biological and Molecular Techniques and Microscopy

SCT's technicians with a Gas Chromatograph

What facilities are useful for?

- ✓Use to ICRA's researchers
- ✓Participation in R&D&i Projects
- ✓Specialized chemical and biological analyses