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Occurrence, distribution and fate of pharmaceuticals as chemical markers of contamination from urban sources in the vulnerable area of the Ebro Delta (Spain)

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OUTLINE

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  - Sources and routes of entry of pharmaceuticals
  - INTEGRA-COAST project
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  - Extraction of water samples
  - Target pharmaceuticals in sediment samples
  - Extraction of sediment samples
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  - Occurrence of PhACs in water samples
  - Removal during wastewater treatment processes
  - Occurrence of PhACs in sediment
- **Conclusion**

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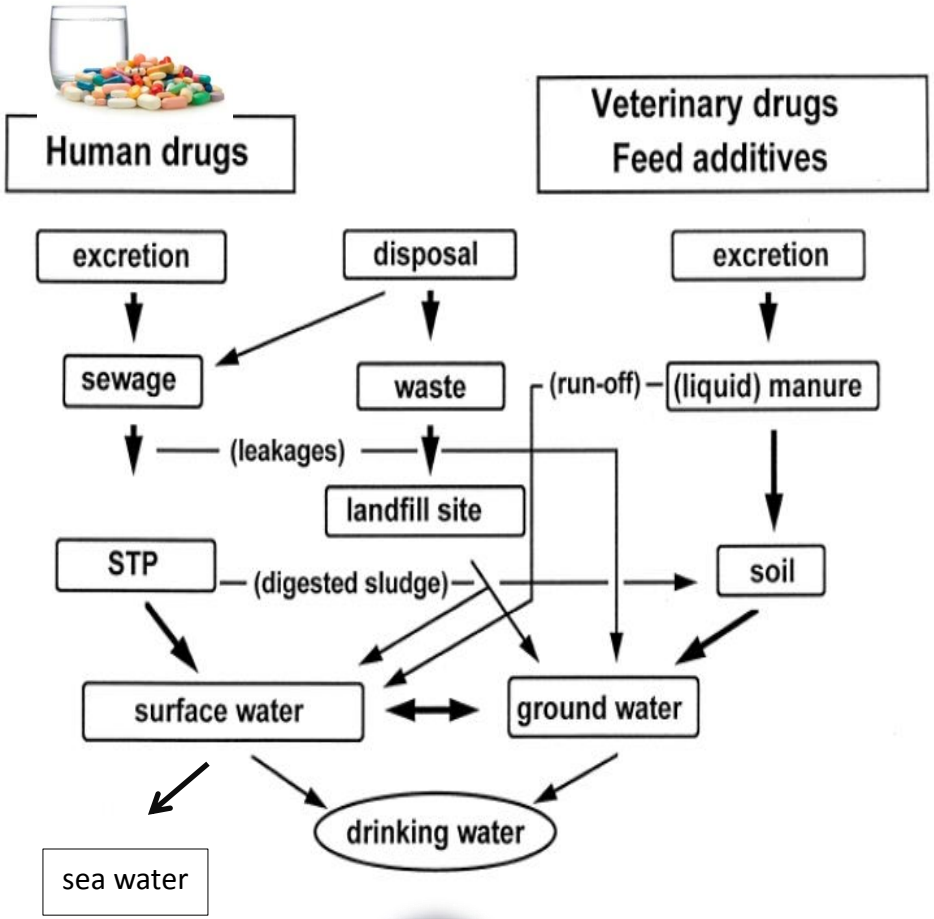
Sources and routes of entry of pharmaceuticals

PhACs are the contaminants of anthropogenic origin with the biggest input into the environment.



- After their consumption they **are discharged** into wastewaters.
- PhACs are **partially removed** during wastewater treatment processes, being discharged into receiving water bodies.
- **Changes in river flow** have one of the greatest effects on **water quality** (point source pollution is controlled by river flow).
- Up to now there have been a lot of studies on their occurrence in waste and surface waters but information about their presence in sea and coastal areas **is still sparse**.

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INTEGRA-COAST PROJECT

INTEGRA-COAST study microplastics, nanomaterials, **emerging organic contaminants** (pharmaceuticals and personal care products, new pesticides, perfluoroalkyl substances and siloxanes) **and marine biotoxins in a vulnerable coastal area.**

The main goal of **INTEGRA-COAST** is to perform an integrated study of the *fate, behaviour, and the river transport* of emerging pollutants, nanomaterials and microplastics in estuaries, wetlands and coastal waters and to identify specific organic contaminants used as chemical markers of wastewater pollution.

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OBJECTIVES

- **To assess the impact of WWTP discharges in coastal areas** and protected ecosystems, with special focus on the contamination by pharmaceuticals.
- **To study the fate and transport of a large number of multiple-class pharmaceuticals** in riverine and coastal ecosystems (e.g. occurrence in water and sediments).
- **To evaluate possible seasonal fluctuations** in the occurrence and behavior **of** these **contaminants** in these ecosystems.
- **Identify relevant pharmaceuticals as markers of urban pollution in coastal areas** with the objective to include them in risk assessment schemes and future monitoring programs.

Study area – the Ebro Delta

- wetland area of 320 km^2
- the third largest delta in the Mediterranean Sea with high biological productivity
- highly relevant for conservation
- has a typical Mediterranean climate (with rainfall concentrated in autumn and spring (200–300 mm) and intense summer drought (<50 mm))
- c.a. 13% of its total surface is composed of natural lagoons, bays and marshes
- agricultural is one of the main activities such as **rice** and **orchards**.

Three sampling campaigns:

- October-November 2015 **(autumn)**
- February-April 2016 **(winter)**
- June-July 2016 **(spring-summer)**

Total number of **213** samples: **87 waters** and **71 sediments**

Sampling: 29 “hot-spots” sites

- wastewater IN/OUT
- emissary
- canals
- Ebro river
- lagoons
- sea water (water/sediment)

Wastewater treatment plants (WWTP)	Edar Amposta (WWTP1)	Sant Carles de la Ràpita (WWTP2)
Served population:	19 805	14 262
Population equivalent (PE):	27.500	28.921
Flow (m^3/dan):	5.500	6.310
Type of the treatment:	Biological with activated sludge	Biological with elimination of N with tertiary treatment

Target pharmaceuticals in water samples

ANALYSIS

Analgesics/anti-inflammatories

Ketoprofen
Naproxen
Ibuprofen
Indomethacine
Acetaminophen
Salicylic acid
Diclofenac
Phenazone
Propylphenazone
Piroxicam
Tenoxicam
Meloxicam
Oxycodone
Codeine

Psychiatric drugs

Carbamazepine
2-Hydroxycarbamazepine
10,11-epoxycarbamzepine
Acridone
Sertraline
Citalopram
Venlafaxine
Olanzapine
Trazadone
Fluxotine
Norfluxotine
Paroxetine
Diazepam
Lorazepam
Alprazolam

Antibiotics

Erithromycin
Azithromycin
Clarithromycin
Tetracycline
Ofloxacin
Ciprofloxacin
Sulfamethoxazole
Trimethoprim
Metronidazole
Metronidazole-OH
Dimetridiazole
Ronidazole
Cefalexin

To treat asthma
Salbutamol

Histimine H2 receptor antagonists

Loratadine
Desloratadine
Ranitidine
Famotidine
Cimetidine

Cholesterol lowering agents

Atrovastatine
Pravastatine
Mevastatine

Antihelmintics

Albendazole
Thiabendazole
Levamisole

B-Blocking agents

Atenolol
Sotalol
Metoprolol
Propranolol
Carzalol
Nadalol

Antihypertensives

Amlodipine
Losartan
Ibersartan
Valsartan

Diuretic

Hydroclorothiazide
Furosemide
Torasemide

Lipid regulators and cholesterol lowering drugs

Bezafibrate
Gemfibrozil
Pravastatin
Fluvastatin
Atrovastatin

X-ray contrast agents
Iopromide

Anticoagulant
Warfarin

Prostatic hyperplasia
Tamsulosin

Antiplatelet agent
Clopidogrel

Sedation and muscle relaxation
Xylizine

Tranquilizer
Azaperone
Azaperol

Synthetic glucocoticoid
Dexamethasone

Calcium channel blockers
Diltiazem
Verapamil
Norverapamil

Antidiabetic
Glibenclamide

Total=81 PhACs

Extraction of water samples

ANALYSIS

SAMPLE

PRE-TREATMENT

FILTRATION
0.7 µm/ 0.45 µm

500 mL sea water
100 mL river water and canals
50 mL effluent
25 mL influent

Addition of
0.1% Na_2EDTA

Surrogate standards

EXTRACTION &

PRE-CONCENTRATION

SPE
OASIS HLB®

Elution with
6 mL MeOH

Evaporation to dryness
1mL MeOH/H_2O (10:90, v/v)

Addition of 34
isotopically
labelled standards

HPLC-ESI-MS/MS (QqLIT)

Gros et al., Journal of Chromatography A, **1248 (2012) 104-121**

HPLC-MS/MS

LC colum
Purospher ® RP-18 (125x2mm) (5 µm)

Quantification of target compounds:

- **MRM mode: two transitions**
  [M+H]+ > 2 Products in PI
  [M-H]- > 2 Products in NI

liquid chromatography coupled to tandem mass spectrometry

Target pharmaceuticals in sediment samples

ANALYSIS

Analgesics/anti-inflammatories Acetaminophen Diclophenac Ibuprofen Indomethacine Ketoprofen Naproxen Mefenamic acid	**Lipid regulators and cholesterol lowering drugs** Bezafibrate Fenofibrate Gemfibrozil Mevastatin Pravastatin Atrovastatin	**Macrolide antibiotics** Erytromicin Roxithromycin Clarithromicin Josamycin Tylosin A	**B-blockers** Atenolol Sotalol Metoprolol Timolol Nadalol Pindolol
Histimine H2 receptor antagonists Ranitidine Famotidine Cimetidine	**Psychiatric drugs** Diazepam Lorazepam Carbamazepine	**Diuretic** Hydrohlorothiazide Furosemide	**Antihypertensive** Nifuroxazide Enalapril
	Sulfonamid antibiotics Sulfamethazine	**B-agonists** Clenbuterol Salbutamol	**Antidiabetic** Glibenclamide
Other antibiotics Trimethoprim Chloramphenicol Metronidazole	**Phenazone type of drugs** Phenazone	**Barbiturates** Butalbital	Total=43 PhACs 

Extraction of solid samples

Sample pre-treatment

freeze-drying (-40 ºC, 0.044 bar)
store at -20 ºC

Sample extraction

PLE - ASE 300 Dionex

pressurized liquid extraction

- 1g of sediment sample;
- T=100ºC
- methanol/water, 1/2 v/v
- 3 static cycles (5 min)
- total flush volume 100% of cell
- 60s of nitrogen purge

PLE extract (~22 mL) is diluted in 500 mL of HPLC water + 15mL Na_2EDTA

SPE – extraction and clean-up

SPE OASIS HLB®

Elution with 8mL MeOH
Reconsituted with 1mL MeOH/H_2O (25:75, v/v)

Addition of 34 isotopically labelled standards

HPLC-MS/MS (QqLIT)

liquid chromatography coupled to tandem mass spectrometry

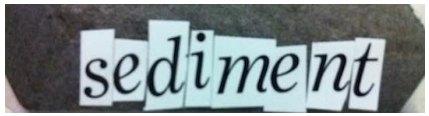
***Jelić et al., Talanta 80* (2009) 363-371**

Quality parameters obtained during analysis, recoveries (%) and method detection limits (MDL)

ANALYSIS

WATER

Recovery, % (n=3), ± RSD	Sea	Lagoons	Canal	Effluent	Influent
Analgesics/ant-inflammatories	59-114%	50-120%	56-146%	57-112%	59-110%
Lipid regulators and cholesterol lowering statin drugs	58-101%	60-96%	42-117%	70-99%	88-103%
Psychiatric drugs	63-114%	51-124%	41-147%	61-113%	55-115%
Histamine H1 and H2 receptor antagonist	62-124%	52-80%	40-95%	87-119%	50-112%
β-Blocking agents	71-126%	60-90%	57-120%	56-88%	57-109%
Duretic	85-101%	68-113%	53-91%	55-98%	63-76%
Antidiabetic	87%	104%	97%	103%	112%
Antihypertensives	73-102%	60-90%	41-101%	51-85%	51-85%
Antiplstelet agent	91%	95%	86%	90%	60%
Prostatic hyperplasia	110%	50-97%	100%	112%	56%
To treat asthma	72%	82%	73%	117%	108%
Anticoagulant	82%	123%	90%	76%	62%
X-ray contrast agent	102%	52-85%	100%	94%	70%
Anti helmintics	55-120%	50-110%	50-115%	74-98%	60-112%
Synthetic glucocorticoid	80%	60-118%	82%	74%	65%
Sedation and muscle relaxation	85%	123%	88%	83%	87%
Tranquilizer	90-92%	52-85%	93-100%	92-102%	76-92%
Antibiotics	63-121%	57-128%	53-116%	66-116%	61-120%
Calcium channel blocker	86-120%	60-127%	64-96%	83-115%	69-74%
MDL (ng/L)	0.01-7.2	0.01-9	0.03-15.2	0.2-26	0.2-50



Sediment	Recovery (%)
Analgesics/antiinflammatories	60-118%
Phenayone type drugs	87-110%
Lipid regulators and cholesterol lowering statin drugs	77-115%
Psychiatric drugs	80-105%
Histamine H1 and H2 receptor antagonist	87-104%
Macrolide antibiotics	68-95%
Sulfonamid antibiotics	59-100%
Other antibioptics	75-93%
β-blockers	90-114%
β-agonist	84-111%
Diuretic	71-84%
Antidiabetic	89-116%
MDL (ng/g)	0.01-3.20

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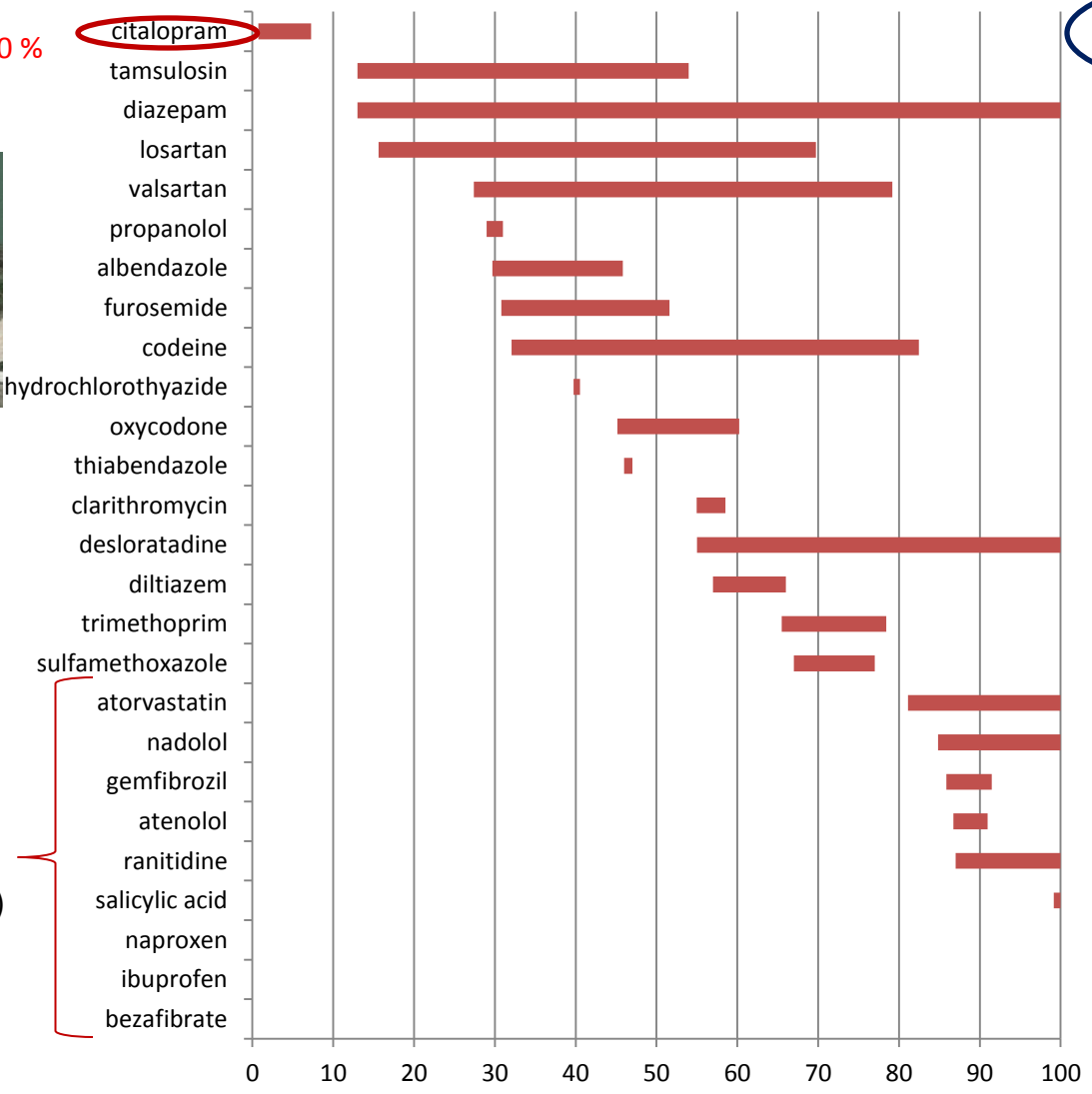
Elimination efficiency, %

RESULTS

Less then < 10 % removal

>80%
(good removal)



1st Campaing

Low to moderate removal rates were obtained for most compounds, except for **analgesics** and **anti-inflammatories** (>80%).

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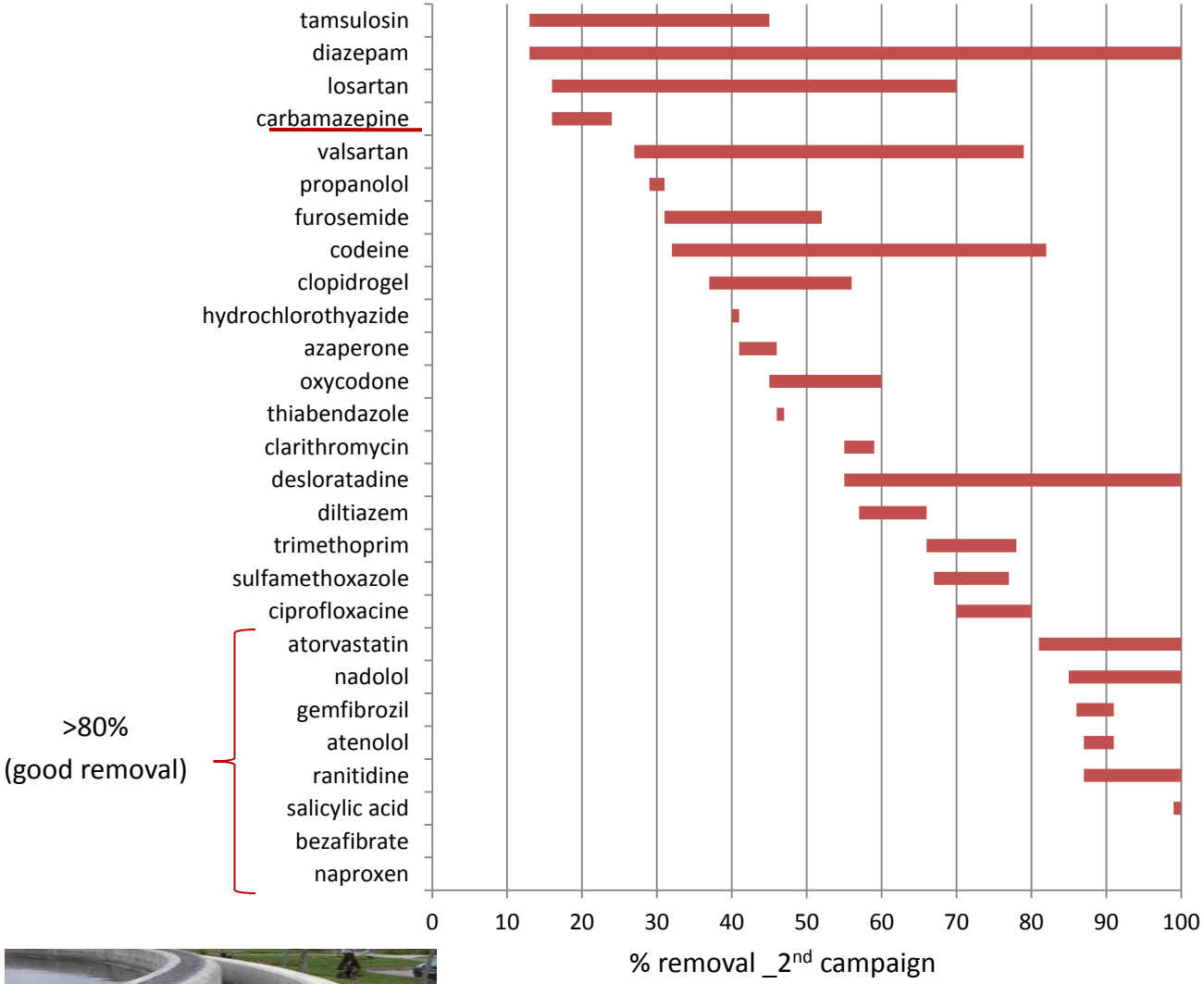
Elimination efficiency, %

RESULTS



2nd Campaing

The pharmaceuticals belonging to the same therapeutic groups show the similar removal for both sampling campaing.

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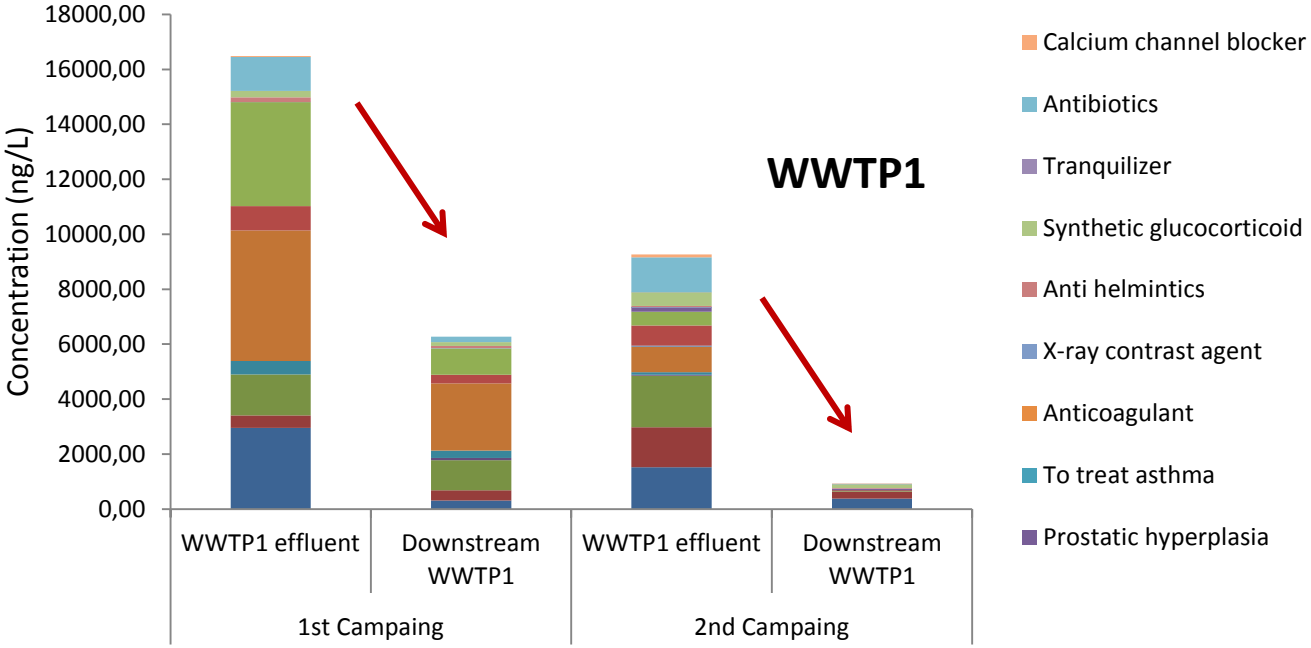
Concentration of pharmaceuticals in WWTP effluents and in receiving natural water bodies

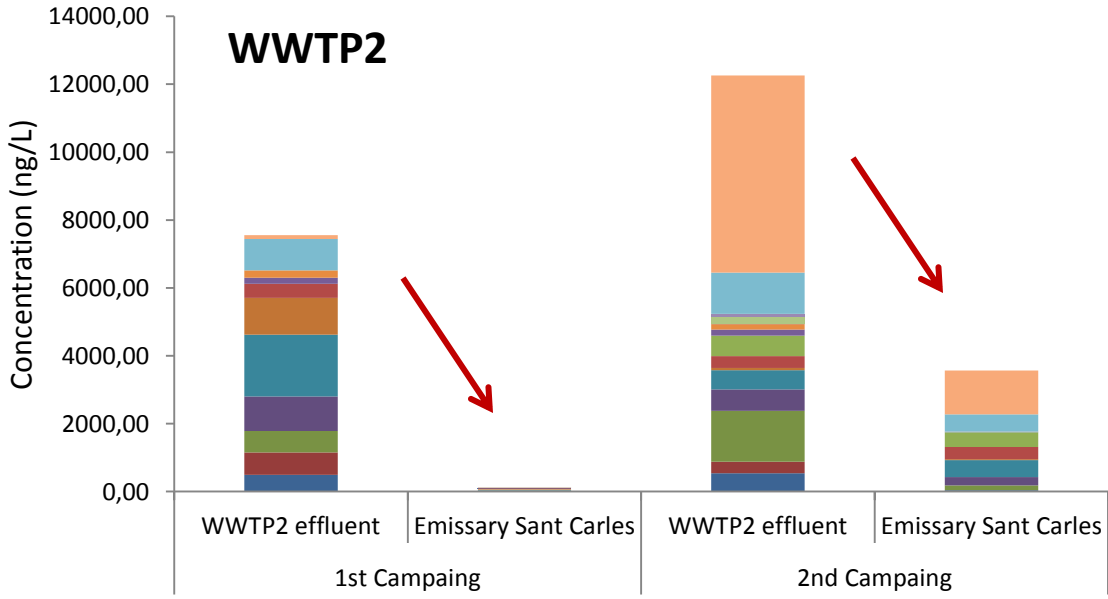


Wastewater effluents are main source of pharmaceuticals in river waters

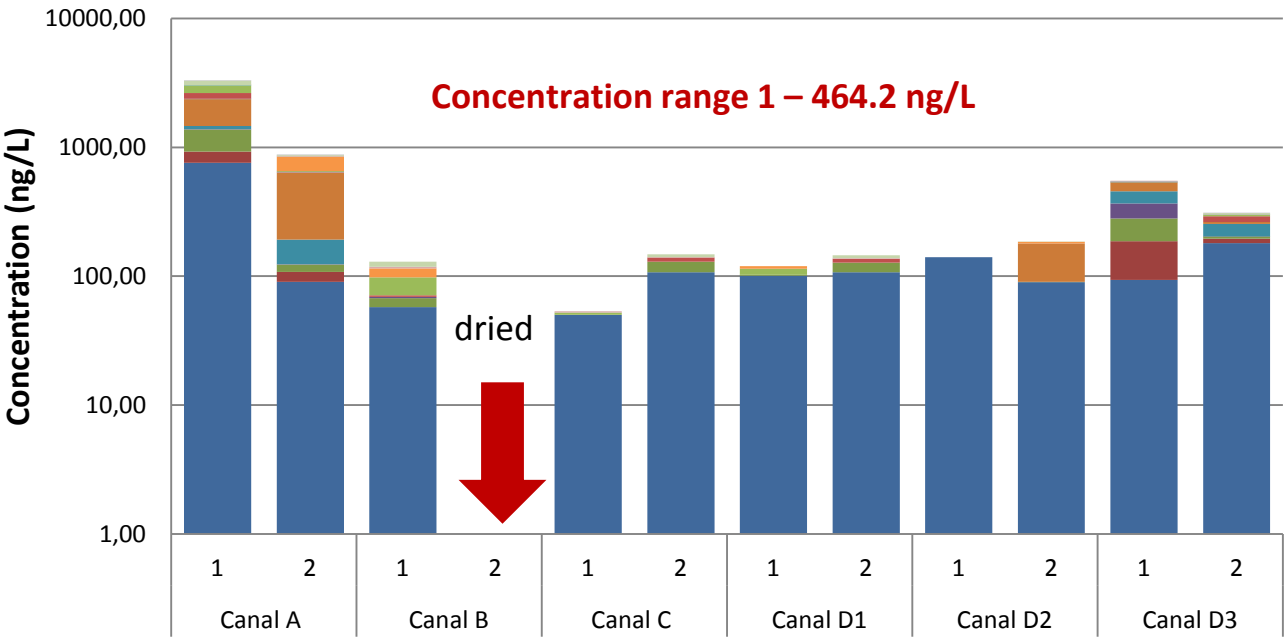
↓

Important - Dilution factor

the natural water bodies receiving effluents discharge **has the same chemical profile** as wastewater effluents, detected in µg/L range.



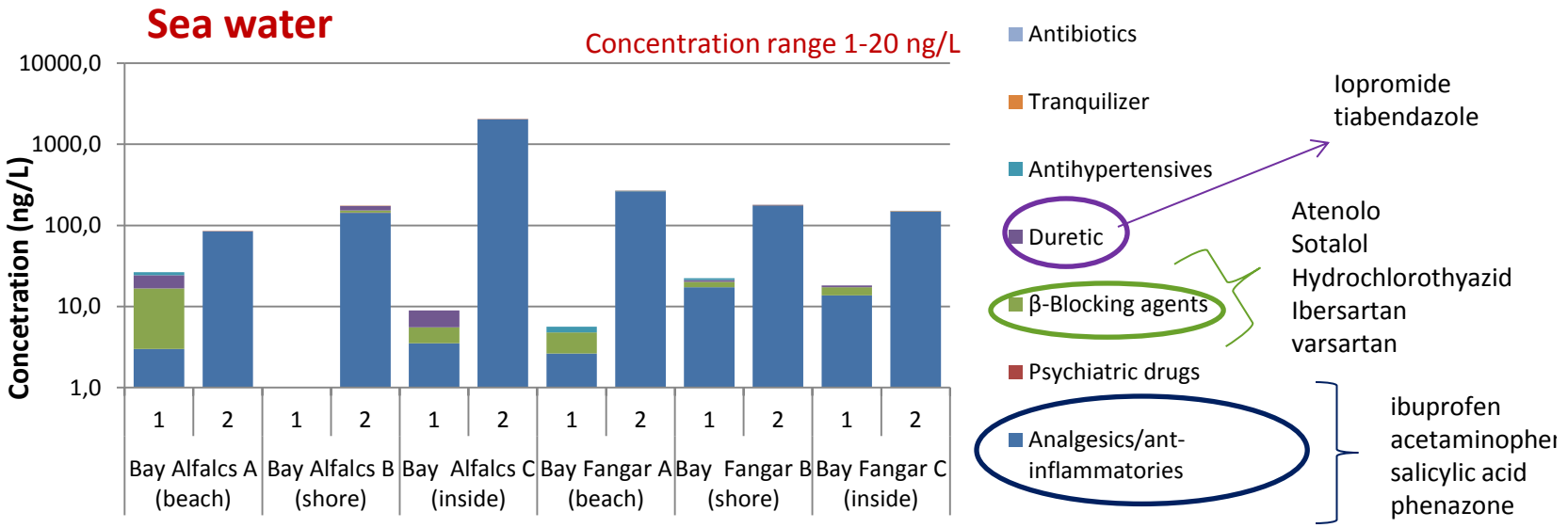
Canals

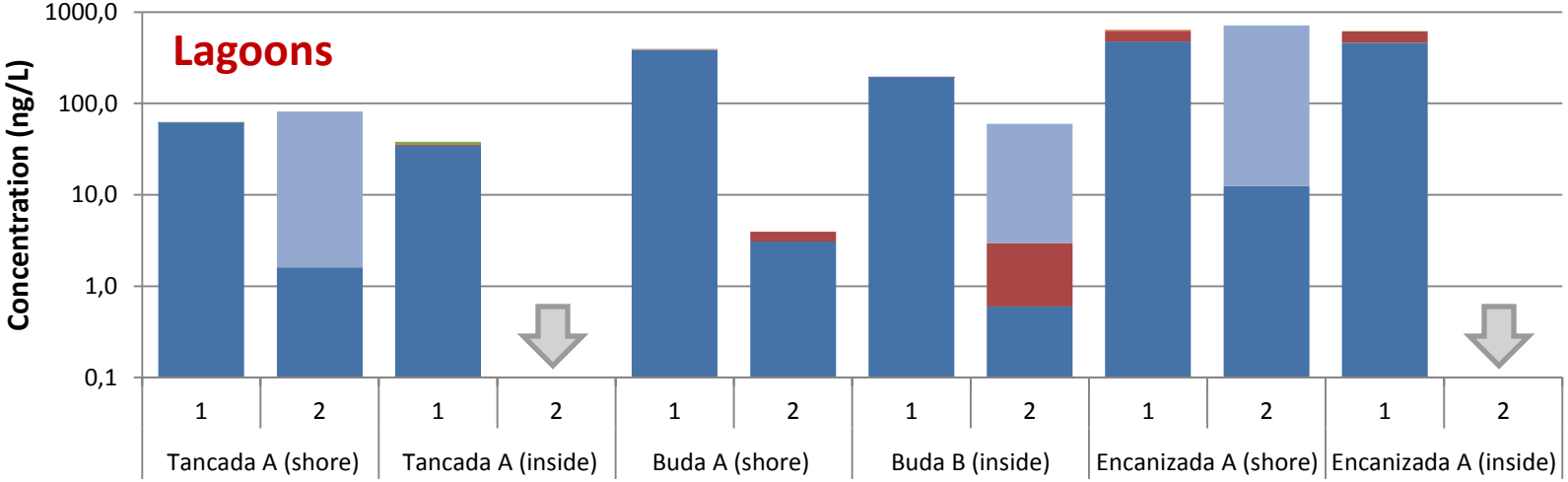


Canals are used to assist in the growing of agricultural crops, thus water quality requirements are essential. Water from this canals could be significant route of crop contamination.

- Analgesics/ant-inflammatories
- Psychiatric drugs
- β-Blocking agents
- Antidiabetic
- Antiplstelet agent
- To treat asthma
- Synthetic glucocorticoid
- Antibiotics
- Lipid regulators and cholesterol lowering statin drugs
- Histamine H1 and H2 receptor antagonist
- Duretic
- Antihypertensives
- Prostatic hyperplasia
- Anti helmintics
- Tranquilizer
- Calcium channel blocker

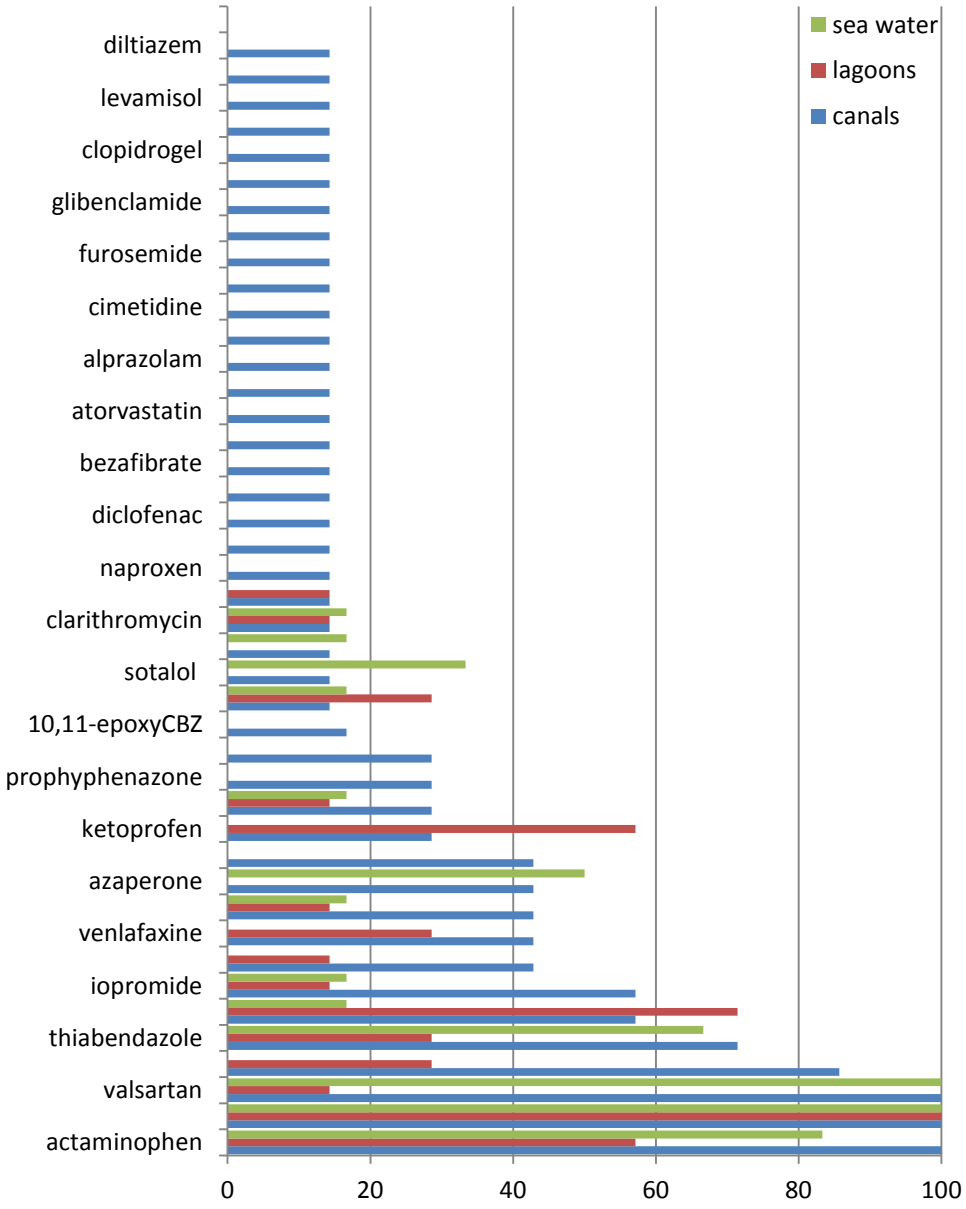
Sea water and lagoons





Salycilic acid the most abundant compound with max conc. **252.8** ng/L

Frequency of detection in water samples



- acetaminophen
- ibuprofen
- salicylic acid
- phenazone

→ **Analgesics/anti-inflammatories**

- valsartan
- irbersartan

→ **Antihypertensives**

- thiabendazole → **Antihelmintics**
- iopromide → **X-ray contrast agents**
- atenolol → **β-blocking agents**
- clarithromycin → **Antibiotics**

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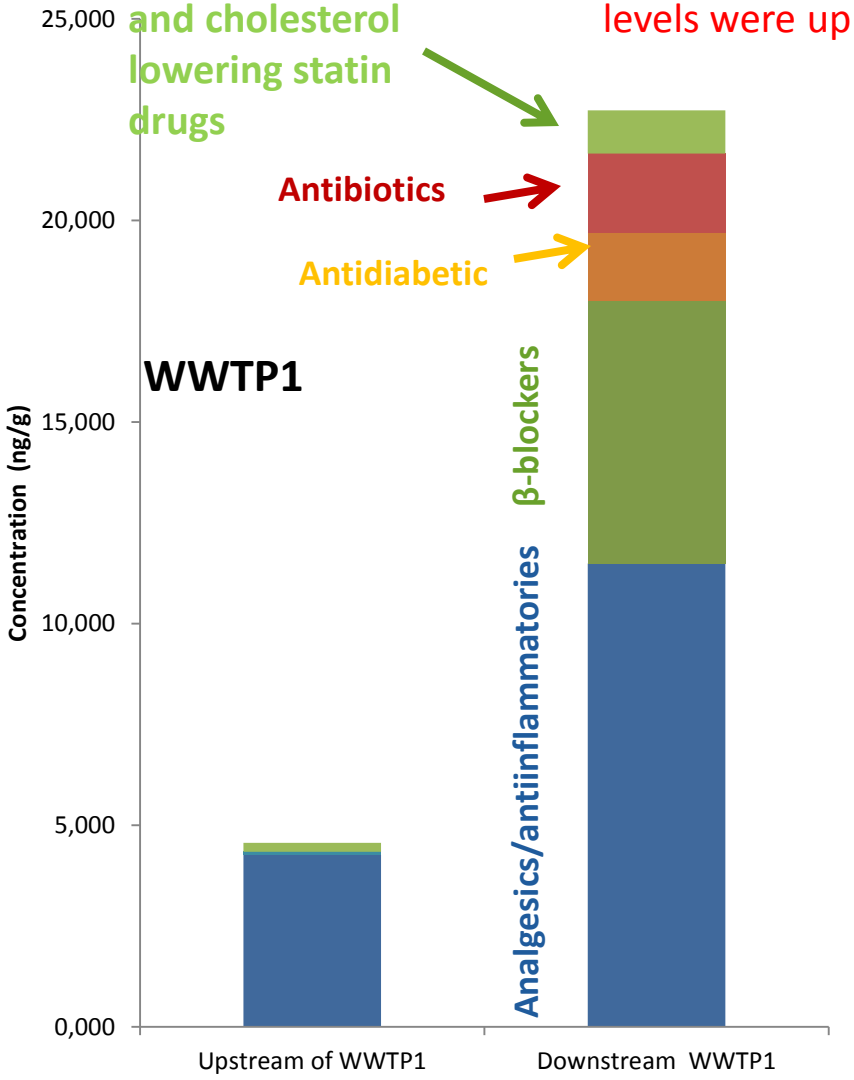
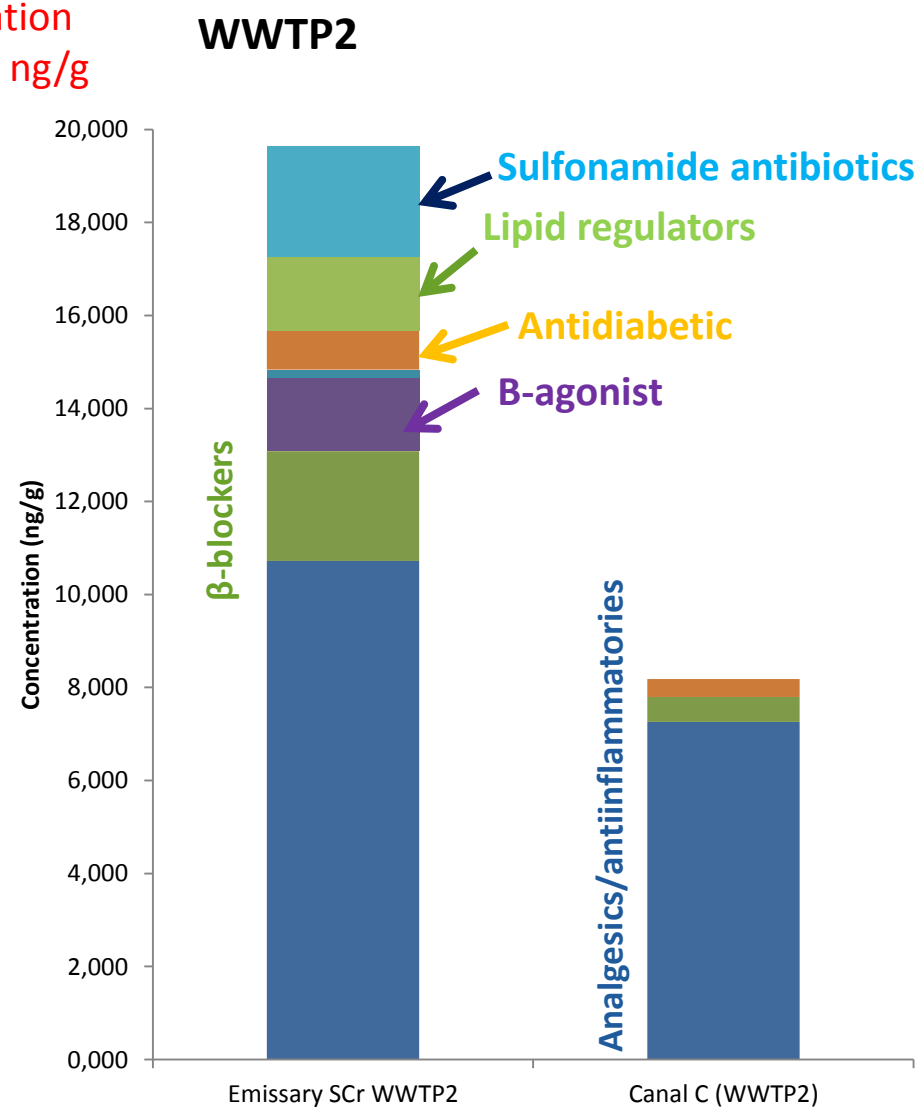
Sediment samples with influence of WWTPs discharges

Lipid regulators and cholesterol lowering statin drugs

The total concentration levels were up to 20 ng/g

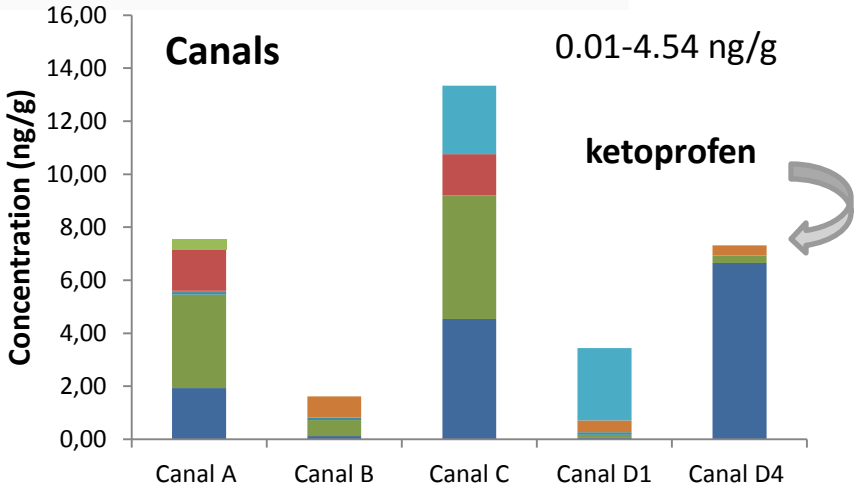


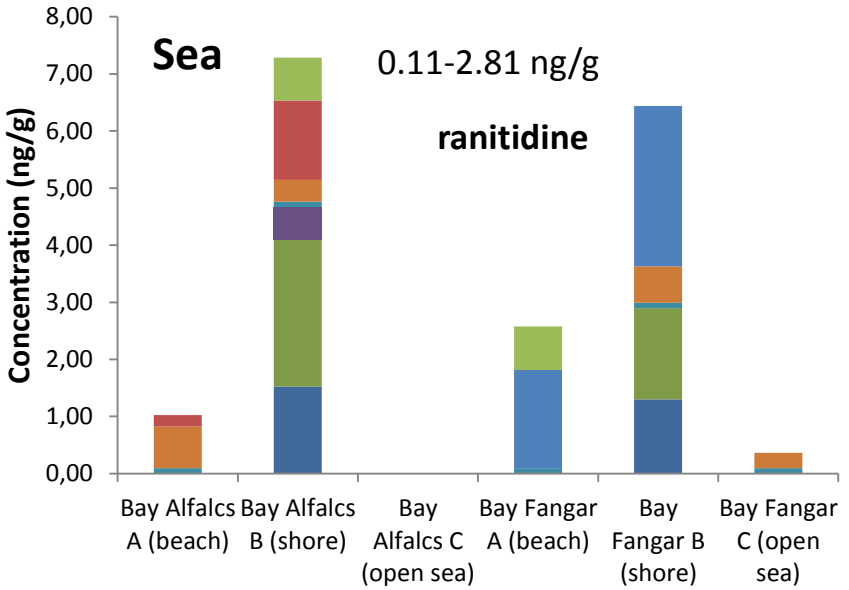
Concentration range
0.09 -7.08 ng/g

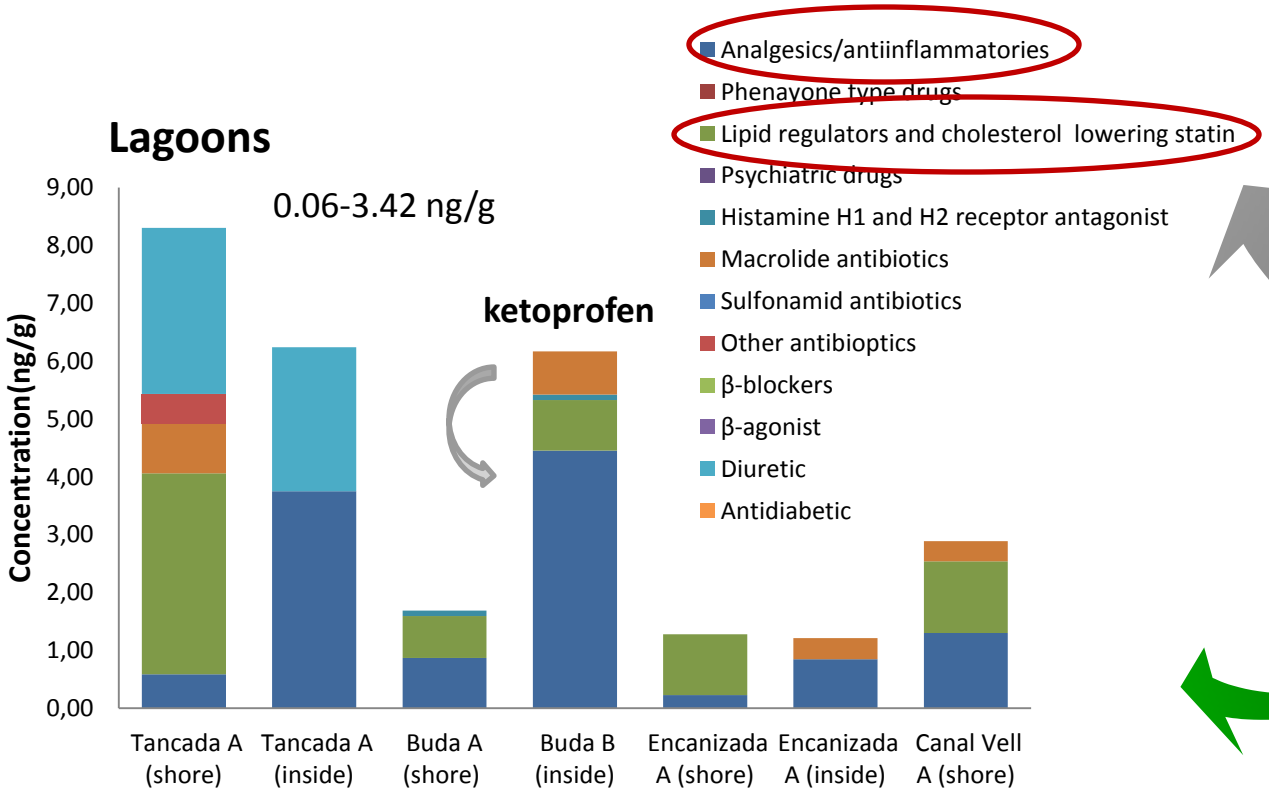


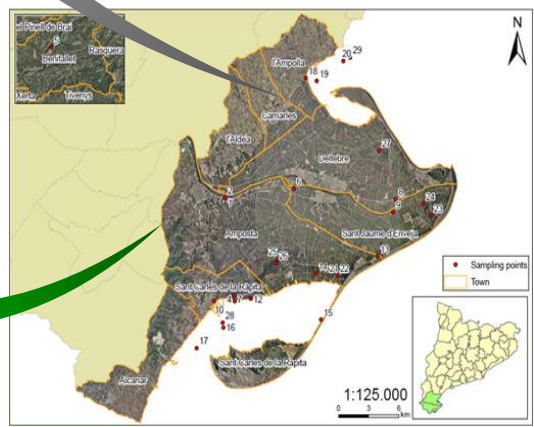
Concentration range
0.01 -5.98 ng/g

Sediment samples

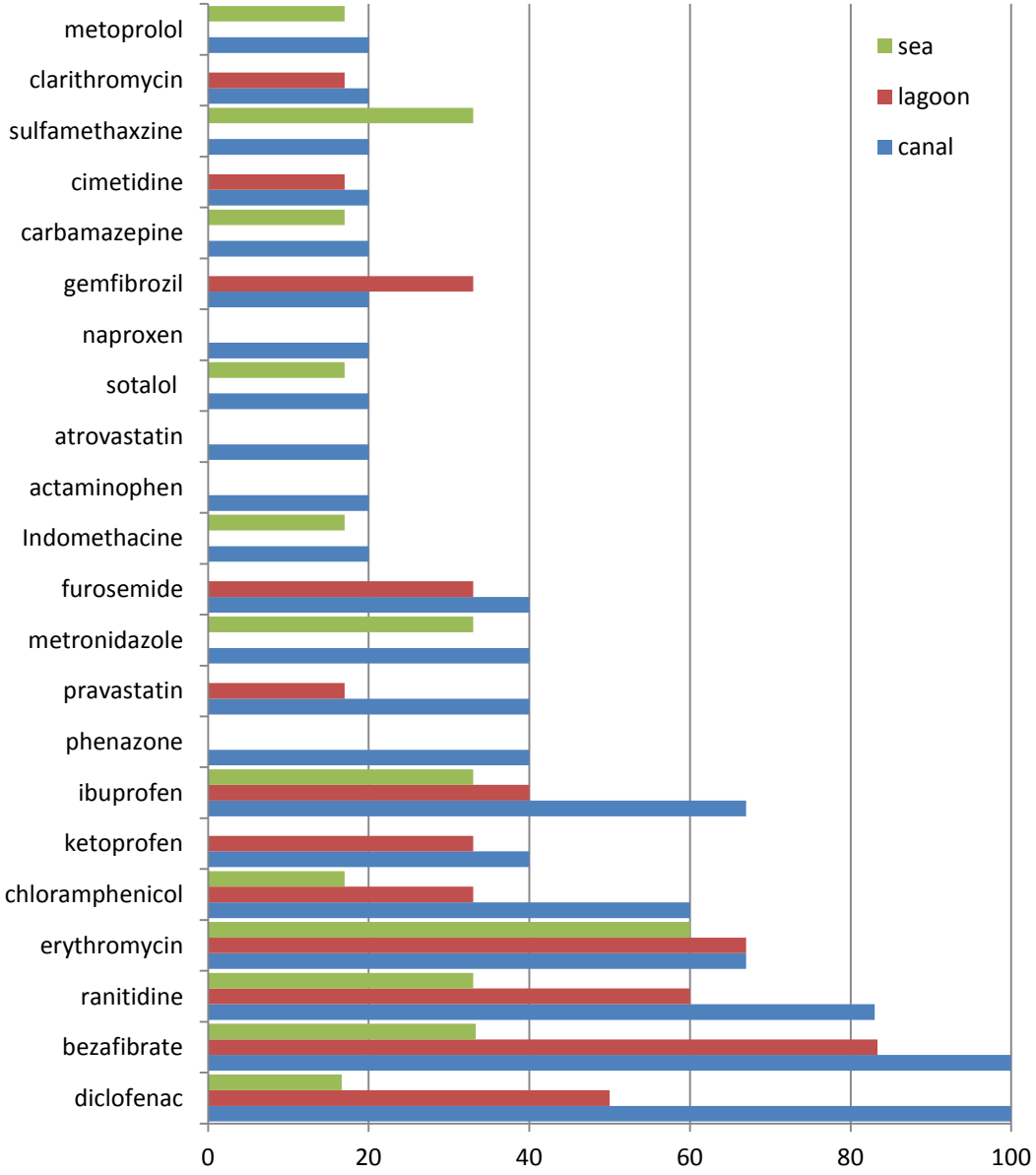








Frequency of detection in sediment samples



✓diclofenac
✓bezafibrate
✓ibuprofen

erythromycin
chloramphenicol

•Histamine H2 receptor antagonists
ranitidine

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CONCLUSION

o Levels of PhACs detected in waste, river, canals, lagoons and sea water indicate that they **are widespread pollutants** along the Ebro Delta.

o Wastewater treatment plants proven to be an important source of pollution for water bodies, as well as for sediment.

o The compounds reaching **the sea water** coming from WWTP discharges were from the several therapeutical groups: **analgesics/anti-inflammatories** (such as acetaminophen, ibuprofen, salicylic acid, phenazone), **β-blocking agents** (atenolol, sotalol, ibersartan, valsartan) and **diuretics drugs** (iopromide, thiabendazole).

o The compounds most widely detected in **sediments** were: diclofenac, bezafibrate, ranitidine, ibuprofen, erythromycin, chloramphenicol.

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ACKNOWLEDGMENTS

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Thank you!

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