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Doctoral School of Exact Sciences and their Applications

For the purpose of obtaining the grade of

Doctor at the University of Pau and Pays de l'Adour
&
the University of Basque Country

Subject area

Analytical chemistry and Environment

Achieved and defend by

Laura Huguenin

December 12th 2019

Rocky benthic communities' response to sewage discharges and associated micropollutants in the southeastern Bay of Biscay

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Jury

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À mes parents.
To my parents.

“*Nous réalisons que ce que nous accomplissons n’est qu’une goutte dans l’océan. Mais si cette goutte n’existait pas dans l’océan, elle manquerait.*"
(Mère Teresa)

“*Rien ne se perd, rien ne se crée, tout se transforme*”
(Antoine Lavoisier)

“STEP by STEP”

Research environment and financial supports

Within the multi-disciplinary University of Pau and Pays de l'Adour (UPPA), the Ministry recognized for the period 2016-2021 three research federations. Among those, the "Research Federation on Aquatic Environment and Resources (MIRA)" (FED 4155), created in 2011, aiming to study anthropogenic pressures and sustainability in the aquatic environment. The Joint Research Unit CNRS/UPPA (UMR 5254; i.e. the Institute of Analytical Sciences and Physico-Chemistry for Environment and Materials (IPREM)), is one of the seven units within the Federation.

Among research projects launched up to 2019, the MICROPOLIT project is supported by the MIRA federation. It was initiated in 2016 by Mathilde Monperrus (lecturer/associate professor from the IPREM laboratory). It was implemented with the goal of studying the state and the evolution of environmental quality along the Southern New-Aquitanian coast on 3 workshop areas (the Adour estuary, the rocky Basque coast and the Capbreton canyon). More precisely, it focused on micropollutants along this coast to improve knowledge about their source, reactivity and fate as well as their concentrations in organisms to assess their ecological/biological state. At its founding, the project set several "Actions", described below (**Fig. 1**). The research work presented in this thesis was carried out on the 'rocky Basque coast' workshop area and was an integral part of Action 2, which was a dual-track approach between biology and chemistry.

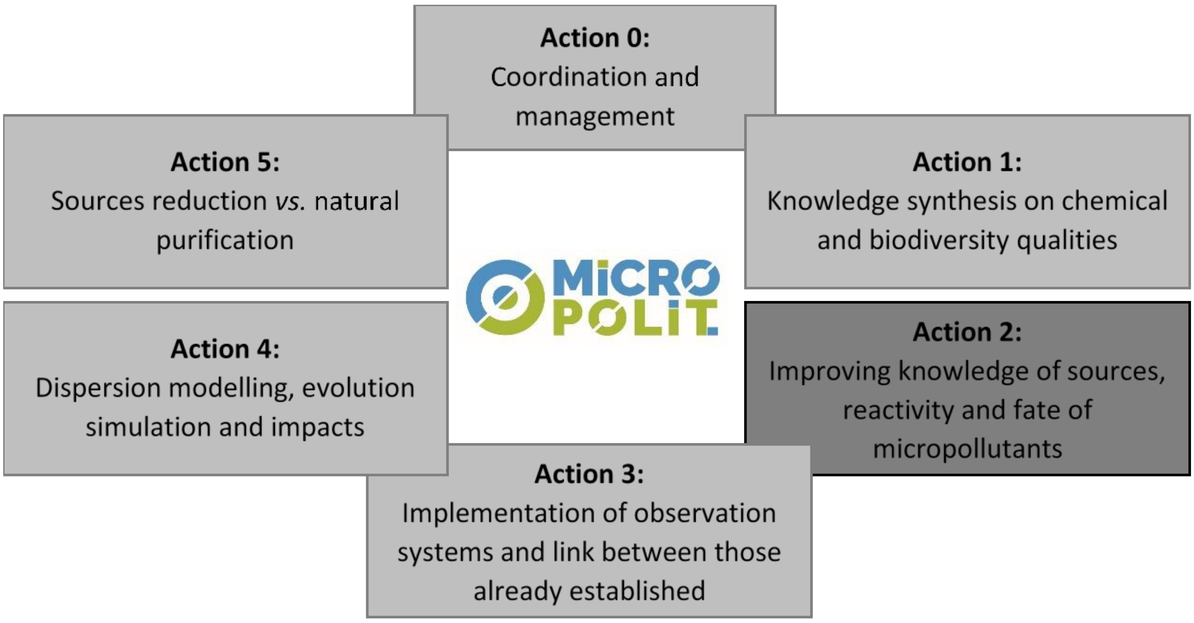

Fig. 1: The six actions around which the MICROPOLIT project is structured.

The MICROPOLIT project was co-financed by the European Union (**European Regional Development funds**) through the **New-Aquitaine Region** and the **Adour-Garonne Water Agency**. Seven financial scientific partners (IPREM, ECOBIOP, LMAP, SIAME, IFREMER, IMA, CMB) and two scientific contractor partners (LAPHY and RIVAGES PRO TECH) were also associated.

La Nouvelle-Aquitaine et l' Europe
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The present thesis under joint supervision (with the University of Pays and Pays de l'Adour and the University of Basque Country) thanks to the French Ministry of Education & Research, which provided a PhD Grant (Doctoral School ED 211, University of Pau and Pays Adour).

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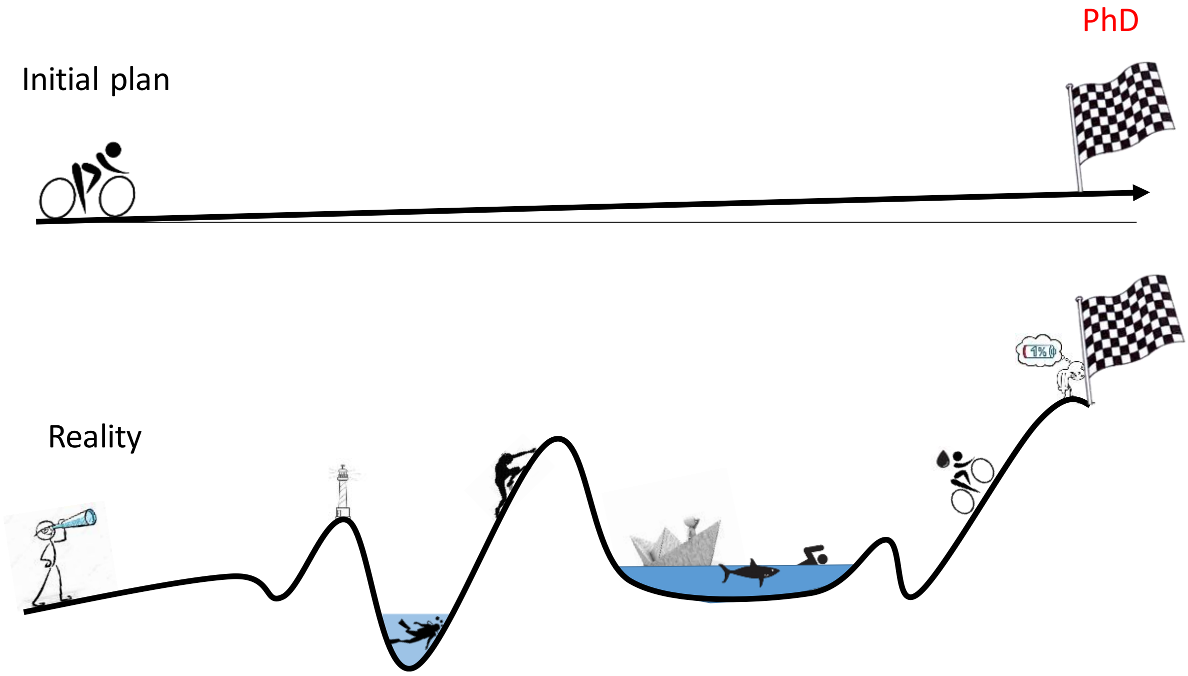

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Glossary

ACE: Angiotensin Converting Enzyme

ADBI: Celestolide

AFB: Agence Française pour la Biodiversité

Ag: Silver

AHMI: Phantolide

AHTN: Tonalide

AMBI: AZTI Marine Biotic Index

ANDRA: the National Agency for Radioactive Waste Management

ANOVA: ANalysis Of VAriance

ANSES: Agence Nationale de SEcurité Sanitaire de l'alimentation, de l'environnement et du travail

AP: Alkylphenol

As: Arsenic

ATII: Traseolide

BAF: BioAccumulation factor

BC: Benzylidene camphor

BC: Biotic Coefficient

BCF: BioConcentration Factor

BHC: Hexachlorocyclohexane

BMF: BioMagnification Factor

BRGM: Bureau de Recherches Géologiques et Minières

$CaCO_3$: Calcium carbonate

CCO: Cover Characteristic – Opportunistic species

Cd: Cadmium

CEC: Contaminants of emerging concern

CEDEF: CEntre de Documentation Économie-Finances

CEMP: Coordinated Environmental Monitoring Program

CESER: Conseil Economique, Social et Environnemental Régional

CMB: Centre de la Mer de Biarritz

CNRS: Centre National de la Recherche Scientifique

Cr: Chromium

Cu: Copper

DL: Detection limit

DOC: Dissolved Organic Carbon

EC: European Regulation

EEC: European Communities adopted Council Regulation

E1: Estrone

E2: 17-beta oestradiol

ECOBIOP: Ecologie COmportementale et bIOlogie des populations de Poissons

EE2: 17-alpha ethinylestradiol

e.g.: for example (from the Latin « *exempli gratia* »)

EG: Ecological Group

EHMC: Ethylhexyl methoxycinnam

EQS: Environmental Quality Standard

EQSD: the European Quality Standards Directive

ESG: Ecological Status Group

EU: EUropean, European Union

FEDER: Fonds Européen de DEveloppement Régional

GC-MS: Gas Chromatrograph coupled with Mass Spectrometer

GC-ICP-MS: Gas Chromatrograph coupled to an Inductively Coupled Plasma Mass Spectrometer

GEQ: Good Ecological Quality

GES: Good Ecological Status

HCl: Hydrochloric Acid

HHCB: Galaxolide

HHCB-lactone: Galaxolidone

HNO$_3$: Nitric Acid

ICP-MS: Inductively Coupled Plasma Mass Spectrometer

IFREMER: Institut Français de Recherche pour l'Exploitation de la MER

IE (or i.e.): Inhabitant Equivalent

i.e.: that is (from the Latin « *id est* »)

IHg: Inorganic mercury

IMA: Institut des Milieux Aquatiques

INEE: INstitut Ecologie et Environnement du CNRS

INRA: Institut National de la Recherche Agronomique

IPRA: Institut Pluridisciplinaire de Recherche Appliquée

IPREM: Institut des sciences analytiques et de physico-chimie pour l'environnement et les matériaux

LAPHY: Laboratoire d'Analyses de Prélèvements HYdrobiologiques

LC-MS-MS: Liquid Chromatograph-tandem Mass Spectrometer

LER: Laboratoire Environnement Ressources

LMAP: Laboratoire de Mathématiques et de leurs Applications

LIUPPA: Laboratoire d’Information de l’Univeristé de Pau et des Pays de l’Adour

MA: Musk Ambrette

MAC-EQS: Maximum Allowed filtered Concentration-Environmental Quality Standard

M-AMBI: Multivariate AZTI Marine Biotic Index

MBC: Methylbenzylidene Camphor

MDS: Multi-Dimensional Scaling

MEA: Millennium Ecosystem Assessment

MFG: Morphological Functional Groups

$MgSO_4$: Magnesium sulfate

MICROPOLIT: MICROPOllutants le long du LITtoral sud Aquitain

MIRA: Federation on Aquatic Environment and Resources

MK: Musk Ketone

MLWS: Mean Low Water Springs

MNHN: National Museum of Natural History

MM: Musk Moskene

MMHg: Monomethylmercury

Mo: Molybdenum

MSFD: Marine Strategy Framework Directive

MTR: Mean Taxonomic Richness

MX: Musk Xylene

$NaBEt_4$: Sodium tetraethylborate

NaCl: Sodium chloride

N: North

Ni: Nickel

nMDS: Non-metric Multi-Dimensional Scaling

NP: Nonylphenol

NPE01: Nonylphenol monoethoxilated

NPE02: Nonylphenol diethoxilathed

NSAIDS: Nonsteroidal anti-inflammatory drugs

NUMEA: NUtrition MEtabolisme Aquaculture

NW: North-West

OC: Octocrylene

OCP: Pesticide

OD-BAPA: Octyl-dimethyl-PABA

OS: Other Substance

OSPAR: OSlo-PARis convention

OTs: Organotins

PE (or p.e.): Population Equivalent

POC: Particulate Organic Carbon

ROCCH: Réseau d'Observation de la Contamination Chimique

PAH: Polycyclic Aromatic Hydrocarbon

PAMM: Action Plan for Marine environment

Pb: Lead

PCA: Principal Component Analysis

PCB: Polychlorinated biphenyl

PCDD: PolyChlorinated Dibenzo-p-Dioxins

PCDF: PolyChlorinated DibenzoFurans

PE: Population Equivalent

PET: Polyethylene Terephthalate

PERMANOVA: Permutational Multivariate Analysis of Variance

pH: Potentiel Hydrogène

PHs: Pharmaceuticals

PHS: Priority Hazardous Substance

PS: Priority Substance

QI: Quality Index

QL: Quantification Limit

QuEChERS: Quick, Easy, Cheap, Efficient, Rugged and Safe

SAGE: Water Development and Management Pan

Sb: Antimony

SDAGE: Water Development and Management Master Plan

SG: Sensitivity Group

SHOM: Service Hydrographique et Océanographique de la Marine

SIAME: Laboratoire des Sciences pour l'Ingénieur Appliquées à la Mécanique et au génie Electrique

SIMPER: SIMilarity PERcentage

SM: Suspended Matter

Sn: Tin

SPE: Solid Phase Extraction

TC: Total Carbon

TN: Total Nitrogen

TS: Taxa Sensitivity

UMR: Unité Mixte de Recherche

UPPA: University of Pau and Pays Adour

UV: Ultraviolet

V: Vanadium

VOC: Volatile Organic Compound

WFD: Water Framework Directive

WWTP: WasteWater Treatment Plant

4nOP: 4-nitro-O-phenylenediamine

4tOP: Para-tert-octylphenol

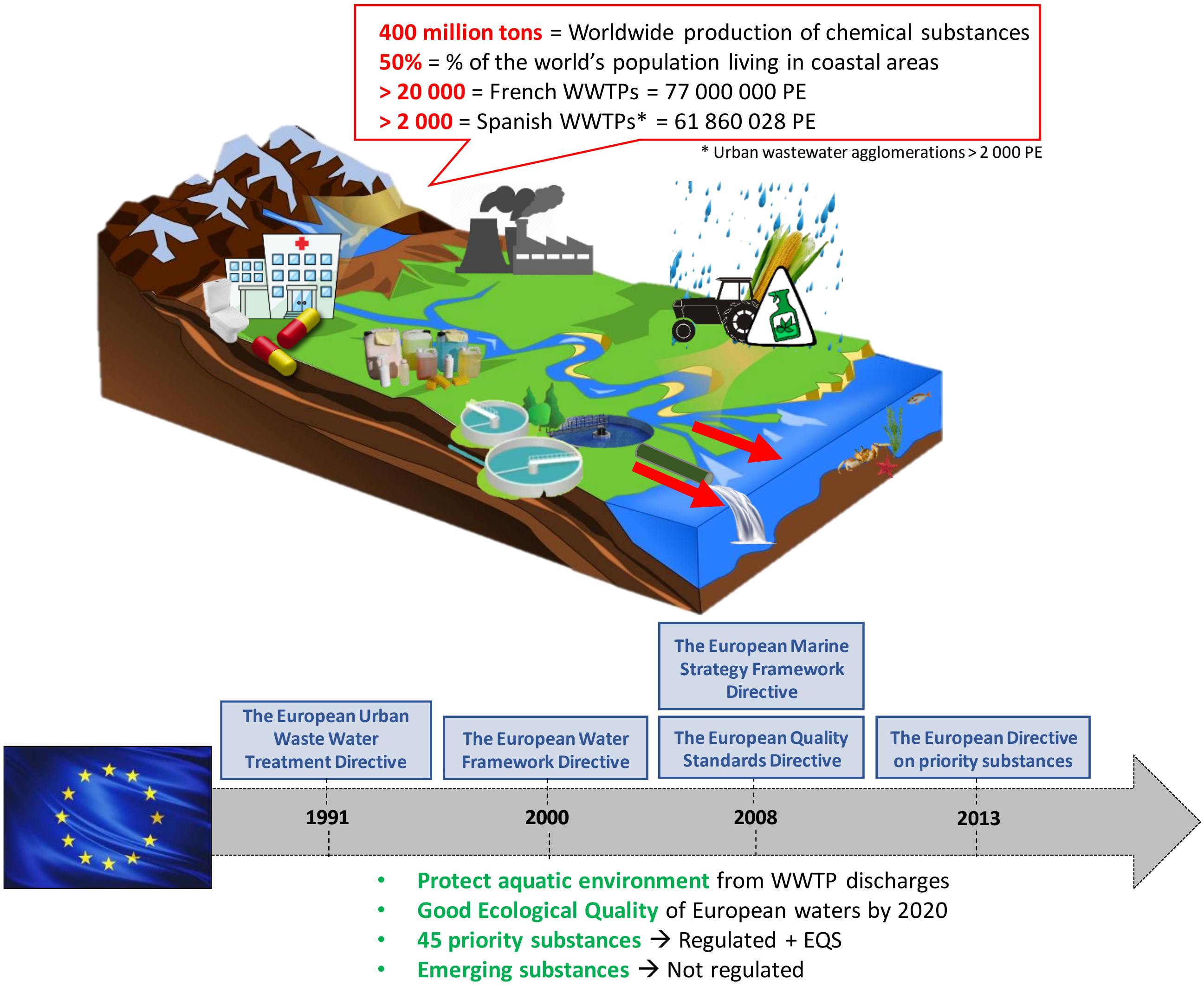
4,4’-DDD: 4,4’-Dichlorodiphenyldichloroethane

4,4’-DDE: 4,4’-Dichlorodiphenyldichloroethylene

4,4’-DDT: 4,4’-Dichlorodiphenyltrichloroethane

Chapter I - Introduction:

Environmental and Regulatory Frameworks



400 million tons = Worldwide production of chemical substances
50% = % of the world's population living in coastal areas
> 20 000 = French WWTPs = 77 000 000 PE
> 2 000 = Spanish WWTPs* = 61 860 028 PE

* Urban wastewater agglomerations > 2 000 PE

- **Protect aquatic environment** from WWTP discharges
- **Good Ecological Quality** of European waters by 2020
- **45 priority substances** → Regulated + EQS
- **Emerging substances** → Not regulated

Fig. 1: Graphical abstract of the Chapter I

Chapter structure:

1. Environmental Context and Associated Pressures
2. Regulatory Context
3. The Purpose of the Thesis Research

1. Environmental context and associated pressures

1.1 The rocky shore

Rocky shore habitats constitute one of the most common environments in coastal areas, i.e. more than 80% of the coastline worldwide (Coutinho et al., 2016; Emery and Kuhn, 1982; Granja, 2004). They may be composed by platforms, boulder fields, cobbles, mixed substrata, pools, cliffs and crevices which constitute a heterogeneous mosaic of habitats and microhabitats (Coutinho et al., 2016; Le Gal and Derrien-Courtel, 2015; Murray et al., 2006). This induce a high valuable habitat in terms of biodiversity and productivity which is used by many organisms for feeding, growth and reproduction (Coutinho et al., 2016).

The intertidal zone (or stage), located at the boundary between land and ocean, represents the area between the low tide and the high tide limits. It constitutes an Important part of the coastal ecosystem and provides many services in terms of primary productivity, fisheries and tourism (Seitz et al., 2013). This zone is mainly governed by tide cycles leading a zonation of this area (Murray et al., 2006). The three zones which constitute it are the supralittoral fringe, the midlittoral zone, itself divided into three parts (upper, middle and lower midlittoral zones) and the infralittoral fringe (**Fig. 2**). The supralittoral is seldom immerged explaining the low diversity living there (mainly orange-grey and black lichens) (Borja and Collins, 2004). It is mainly exposed to winds, sea sprays and sun. The only period during which it is underwater may be throughout high equinoctial spring tides. The midlittoral zone is alternatively immerged and emerged making it a more stable environment (Borja and Collins, 2004). The upper and middle midlittoral zones are both characterized by *Chthamalus stellatus* (barnacles) with a higher macrofauna diversity in the second one. It is also colonized by crustose (*Ralfsia verrucosa*) and caespitose (*Caulacanthus ustulatus*) macroalgae. The lower midlittoral zone shows wider diversity than the two others (including dominant algae as *Lithophyllum inscrustans* and *Ellisolandia elongata* and macrofauna such as molluscs, cnidarian, polychaetes, amphipods and isopods). By contrast, infralittoral fringe is only emerged during spring tides. *Gelidium corneum*, *Lithophyllum incrustans*, *Ellisolandia elongata* and *Patella aspera* make up the major characterized species (Borja and Collins, 2004).

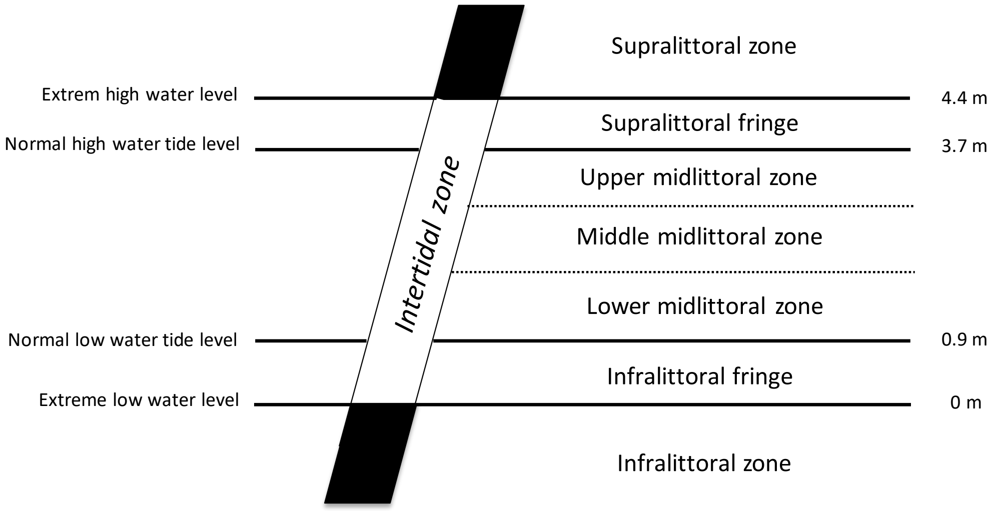


Fig. 2: Zonation scheme of the intertidal zone according to Borja and Collins (2004) (data from Ibanez and Iribar, 1979, following the terminology of Lewis, 1964).

In contrast, subtidal rocky areas are always submerged and are governed by pressure variations, currents, waves, oxygen layer, thermocline and sediment resuspension caused by wind (Falcão and Vale, 1998). These various features, associated to incident light attenuation, induce a vertical zonation of communities (Witman et al., 1993) (**Fig. 3**). Indeed, the intensity of light reaching the seabed directly induces the depth at which the subtidal zones begin (https://inpn.mnhn.fr). For example, "*in highly turbid conditions, the circalittoral zone may begin just below water level at mean low water springs (MLWS)*" (https://inpn.mnhn.fr). Shallow subtidal areas such as the infralittoral zone are dominated by large brown macroalgae (apart from the southern Bay of Biscay which is dominated by red algae) (Ojeda, 1989). They constitute a canopy divided in two sub-zones (upper and lower) and characterized by a total density of structuring macroalgae (L*aminaria digitata, Laminaria hyperborea, Laminaria ochroleuca, Saccharina latissima, Saccorhiza polyshides, Cystoseira baccata, Cystoseira tamariscifolia, Halydris siliquosa* and *Sargassum muticum*, depending of latitude and region), higher or lower than 3 individuals (feet) per m^2 respectively (de Casamajor et al., 2017; Le Gal and Derrien-Courtel, 2015). At deeper depths, where light and thus primary productivity become limiting for erected macroalgae (i.e. in the circalittoral zone), invertebrates progressively replace macroalgae (Britton-Simmons et al., 2009; https://inpn.mnhn.fr). This latter zone is divided into the upper circalittoral zone, characterized by an absence of structuring macroalgae and rather associated to foliose red algae (but not dominant) and the lower circalittoral zone where only encrusting macroalgae remain (Le Gal and Derrien-Courtel, 2015; https://inpn.mnhn.fr).

Along the Basque coast, studied locations were at 20 m depth. Only "red" and "orange" wavelengths were attenuated at this depth. Therefore, macroalgae were still present and benthic communities were as such more impacted by other factors as swell, climate conditions (e.g. rain and storms), river discharges, etc.

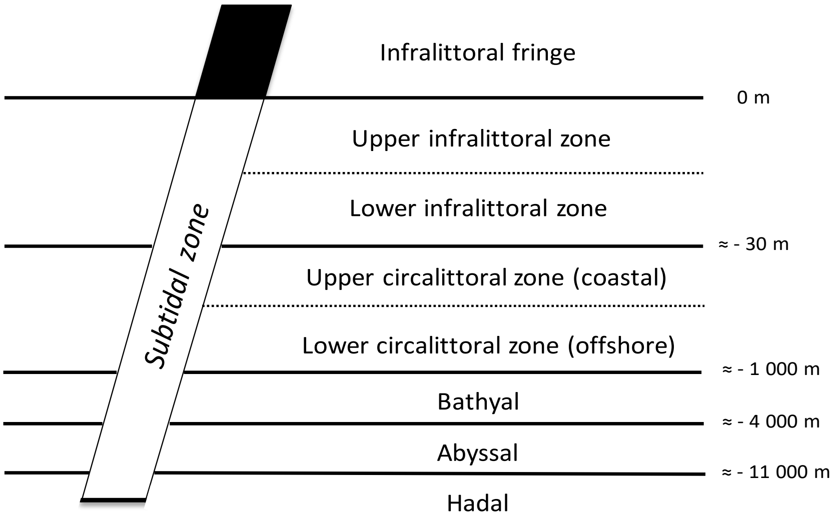


Fig. 3: Zonation scheme of the subtidal zone.

1.2 Pressures impacting these zones

Marine coastal ecosystems are governed by environmental and anthropogenic factors responsible for stressful physical conditions (Ghilardi et al., 2008). Indeed, they may be modified by many biotic and abiotic factors, such as biological interactions (e.g. settlement, recruitment, predation, and competition), physical actions (e.g. wave action/hydrodynamics, temperature gradients, tides, irradiance, salinity, topography, shore's slope, coastline's profile and coast orientation) and anthropogenic pressures (overexploitation, invasive species introduction, habitat fragmentation and destruction and direct or indirect introduction of chemicals) (Borja and Collins, 2004; Ghilardi et al., 2008; Macdonald et al., 2003; Rial et al., 2017; Vinagre, 2017).

Anthropogenic disturbances are partly due to the growing urban development (Becherucci et al., 2016; Crain et al., 2008; de-la-Ossa-Carretero et al., 2016). Indeed nowadays, half of the world's population lives in coastal areas (less than 60 km from the shoreline) especially for goods and services that provide marine ecosystems (Halpern et al., 2008; Le Gal and Derrien-Courtel, 2015). Disturbances caused by humans may come from a variety of sources such as industries, hospitals, agriculture, WWTP or septic tanks, mining, transport and waste disposal (**Fig. 4**) (European Environment Agency, 2018a). They may be punctual (e.g. accidental effluents which are easy to identify) or diffuse (less identifiable due to the geographical scope) (Berlioz-Barbier, 2015; Bernard, 2012; European Environment Agency, 2018a). They may be introduced directly into the environment through pipelines or indirectly by riverine inputs, surface runoff, atmospheric deposition, etc. (Rial et al., 2017). Marine coastal areas are thus constantly impacted by a mixture of disturbances and pollutants (Benali et al., 2017).

All factors that mediate marine coastal ecosystems are summarized in the below diagram **Fig. 5**.

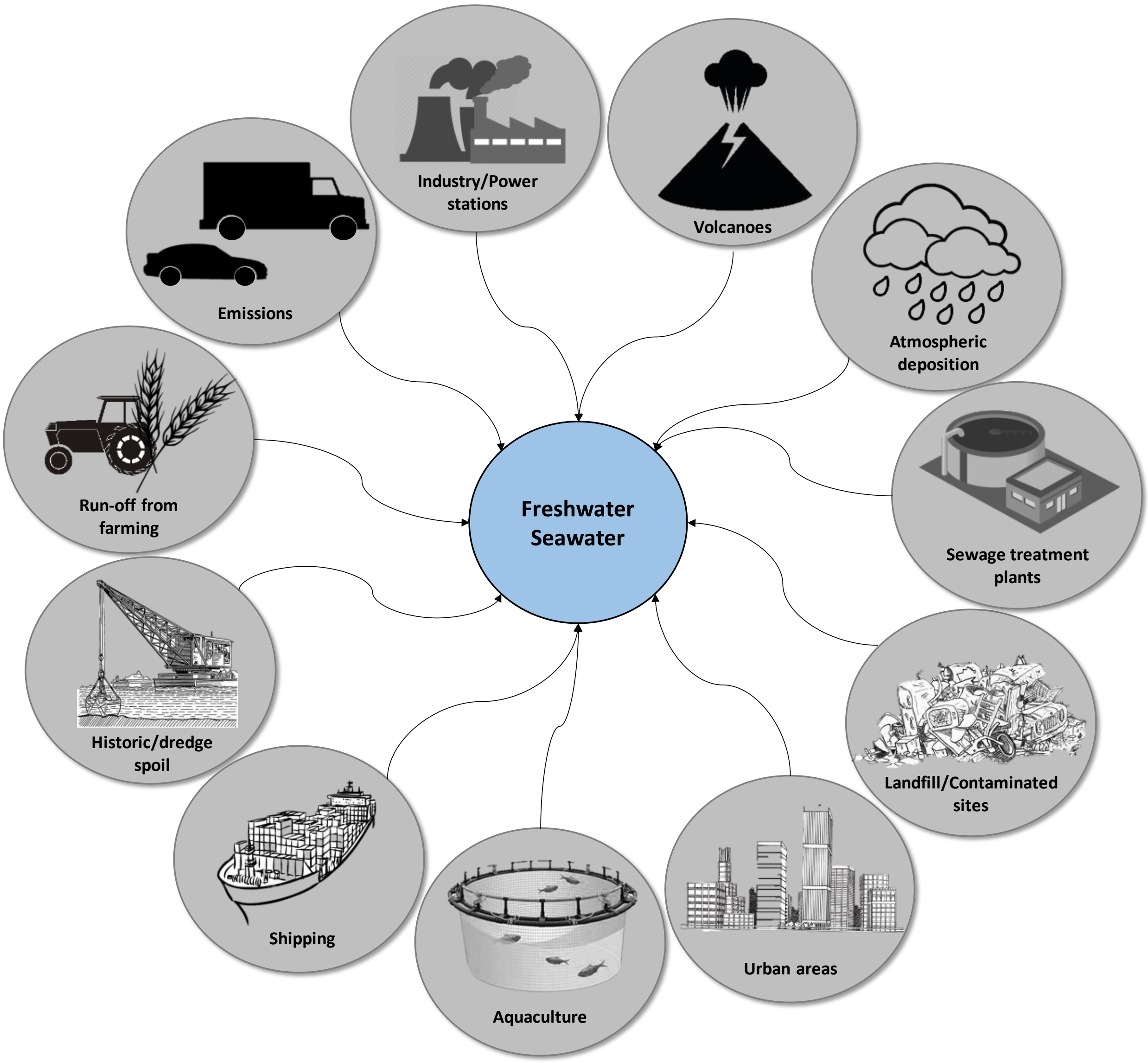
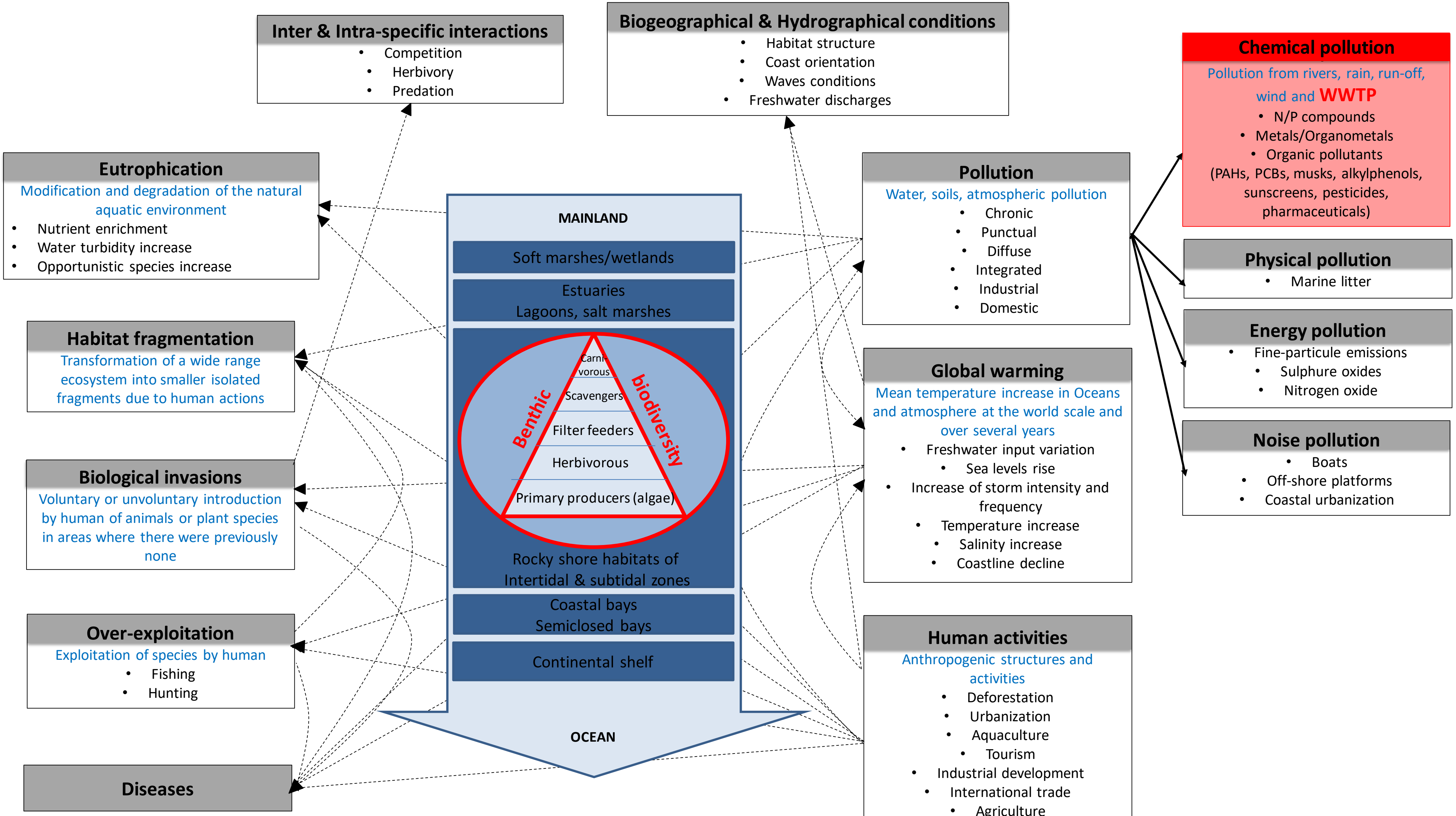


Fig. 4: Example of water pollution sources.



Inter & Intra-specific interactions
- Competition
- Herbivory
- Predation

Biogeographical & Hydrographical conditions
- Habitat structure
- Coast orientation
- Waves conditions
- Freshwater discharges

Chemical pollution

Pollution from rivers, rain, run-off, wind and **WWTP**
- N/P compounds
- Metals/Organometals
- Organic pollutants

(PAHs, PCBs, musks, alkylphenols, sunscreens, pesticides, pharmaceuticals)

Eutrophication

Modification and degradation of the natural aquatic environment
- Nutrient enrichment
- Water turbidity increase
- Opportunistic species increase

Pollution

Water, soils, atmospheric pollution
- Chronic
- Punctual
- Diffuse
- Integrated
- Industrial
- Domestic

Physical pollution
- Marine litter

Habitat fragmentation

Transformation of a wide range ecosystem into smaller isolated fragments due to human actions

Energy pollution
- Fine-particule emissions
- Sulphure oxides
- Nitrogen oxide

Global warming

Mean temperature increase in Oceans and atmosphere at the world scale and over several years
- Freshwater input variation
- Sea levels rise
- Increase of storm intensity and frequency
- Temperature increase
- Salinity increase
- Coastline decline

Noise pollution
- Boats
- Off-shore platforms
- Coastal urbanization

Biological invasions

Voluntary or unvoluntary introduction by human of animals or plant species in areas where there were previously none

Over-exploitation

Exploitation of species by human
- Fishing
- Hunting

Human activities

Anthropogenic structures and activities
- Deforestation
- Urbanization
- Aquaculture
- Tourism
- Industrial development
- International trade
- Agriculture
- Fishing
- Hunting

Diseases

Fig. 5: Pressures that may affect coastal zones.

1.2.1 Wastewater treatment plants (WWTPs)

To deal with coastal urban sprawl and thus to inputs of untreated urban or industrial wastewaters, many pipeline systems releasing via outfalls were built during the XXth century to reject urban sewage effluents into coastal areas (e.g. intertidal and shallow subtidal habitats) or further out to sea (Augier, 2014; Becherucci et al., 2018; Bernard, 2012; Borja and Collins, 2004; Cabral-Oliveira and Pardal, 2016; Cearreta et al., 2004; Chust et al., 2009; Koop and Hutchings, 1996; Le Treut, 2013a). In addition, since 1991, the European Union Regulations has imposed on all member states to treat urban wastewaters prior to reject them into riverbanks, lakes and seas (Barreales-Suárez et al., 2018; EEC, 1991). Wastewater treatment plants were thus built to reach required discharge standards (Von Sperling, 2007). In France in 2017, 21 631 WWTPs were reported for a total load of 77 000 000 equivalent habitants (purification capacity equal to 104 million inhabitant/population equivalent (i.e/IE or p.e./PE) (www.assainissement.developpement-durable.gouv.fr) and 2 063 urban wastewater agglomerations* of more than 2 000 were identified in Spain in 2014, for a total load of 61 860 028 p.e. (www.uwwtd.eu). Wastewater treatment plant discharges are still considered as the most-effective technique to get rid of sewages (coming from agricultural, industrial, domestic and municipal activities) (Islam and Tanaka, 2004; Little and Kitching, 1996) owing to the dilution rate of the ocean (Elías et al., 2005) and constitute thus a common source of disturbances, the oldest form of marine pollution (Fraschetti et al., 2006; Pearson and Rosenberg, 1978; Benali, 2017). In France, the Article 10 of June 22th 2007 (and thereafter the Article 8 of July 21th 2015 Decision; Decision, 2015), requires that all discharges occurring in the public maritime domain have to be located below the low tide level (Decision, 2007).

** The term agglomeration refers in the first place to a sufficiently concentrated area for urban wastewater to be collected and conducted to an urban wastewater treatment plant (Directive; 91/271/EEC; ec.europa.eu).*

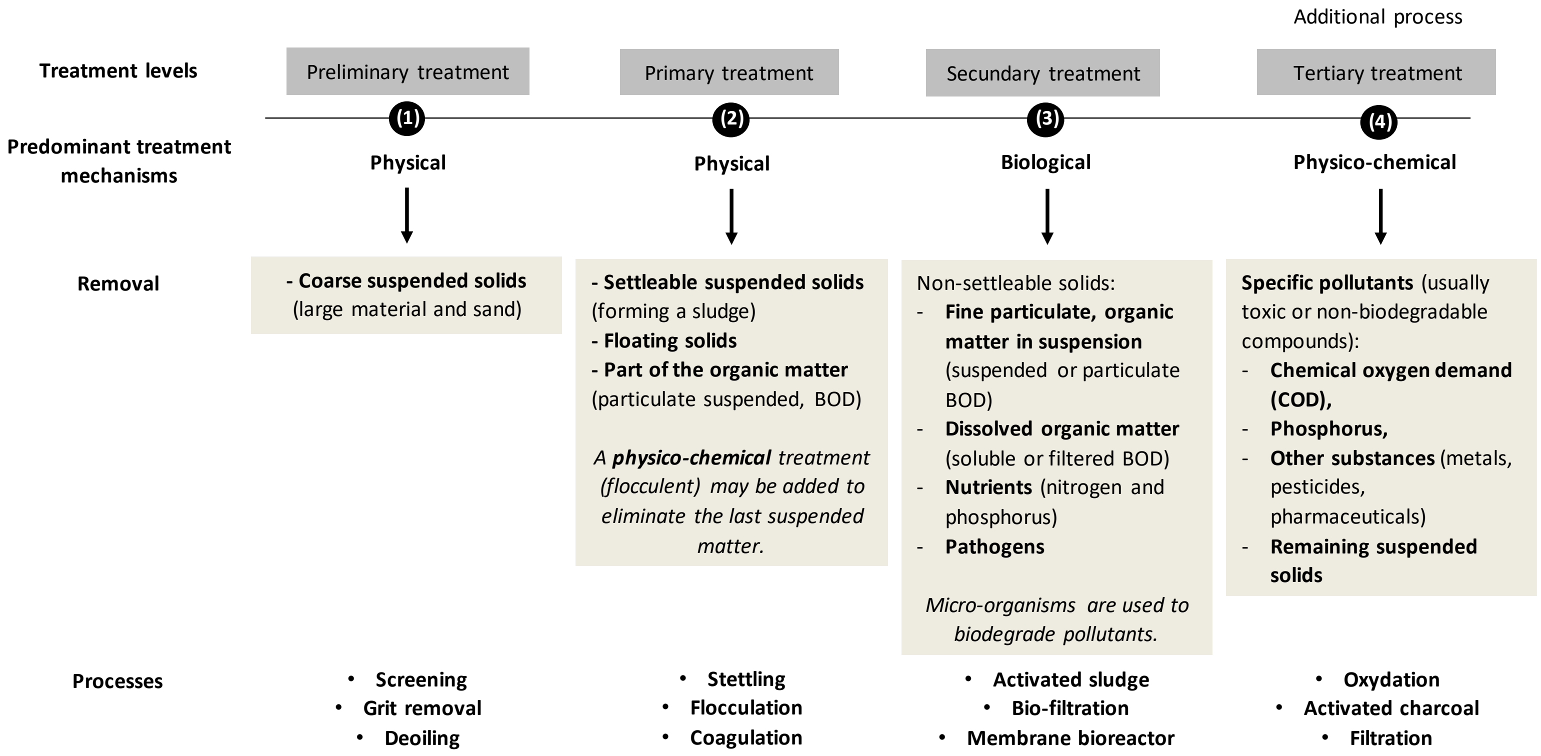
1.2.2 General functioning of Wastewater treatment plants (WWTPs)

Once rejected by private households or industries, effluents are brought to the WWTP by sewer systems. They may be either combined (rainwater, domestic wastewater and industrial waters are mixed into the same sewer) or separate (system or rainwater is separated from domestic and industrial sources).

All treatments carried out on wastewaters are characterized by predominant treatment mechanisms composed by unit operations and processes (Metcalf and Eddy, 1991; Von Sperling, 2007):

- Physical unit operations: dominance of physical forces (e.g. screening, mixing, flocculation, sedimentation, flotation, filtration),
- Chemical unit processes: contaminants removal or conversion due to the addition of chemical products or to chemical reactions (e.g. precipitation, adsorption, disinfection),
- Biological unit processes: contaminants removal as a result of biological process (e.g. carbonaceous, organic matter removal, nitrification, denitrification).

They are usually classified into several treatment levels (**Fig. 6**): (1) preliminary treatment, (2) primary treatment (physico-chemical), (3) secondary treatment (biological) and (4) tertiary treatment (rare in developing countries) (Berlioz-Barbier, 2015).



				Additional process
Treatment levels	Preliminary treatment	Primary treatment	Secundary treatment	Tertiary treatment
	(1)	(2)	(3)	(4)
Predominant treatment mechanisms	**Physical**	**Physical**	**Biological**	**Physico-chemical**
Removal	**- Coarse suspended solids** (large material and sand)	**- Settleable suspended solids** (forming a sludge) **- Floating solids** **- Part of the organic matter** (particulate suspended, BOD) *A **physico-chemical** treatment (flocculent) may be added to eliminate the last suspended matter.*	Non-settleable solids: - **Fine particulate, organic matter in suspension** (suspended or particulate BOD) - **Dissolved organic matter** (soluble or filtered BOD) - **Nutrients** (nitrogen and phosphorus) - **Pathogens** *Micro-organisms are used to biodegrade pollutants.*	**Specific pollutants** (usually toxic or non-biodegradable compounds): - **Chemical oxygen demand (COD),** - **Phosphorus,** - **Other substances** (metals, pesticides, pharmaceuticals) - **Remaining suspended solids**
Processes	• **Screening** • **Grit removal** • **Deoiling**	• **Stettling** • **Flocculation** • **Coagulation**	• **Activated sludge** • **Bio-filtration** • **Membrane bioreactor**	• **Oxydation** • **Activated charcoal** • **Filtration**

Fig. 6: Treatment levels and their characteristics usually employed within a WWTP (from Berlioz-Barbier, 2015; Von Sperling, 2007).

1.2.3 Micropollutants

The current worldwide production of chemical substances is estimated at 400 million tons compared to around 1 million tons in 1930 (CEDEF, 2006). In the 1970’s/1980’s, micropollutants were associated with industry and urban discharge (Briand et al., 2018) whereas since 1990’s, they are mainly linked to agricultural (i.e. pesticides) and daily consumer products (**Fig. 7**).

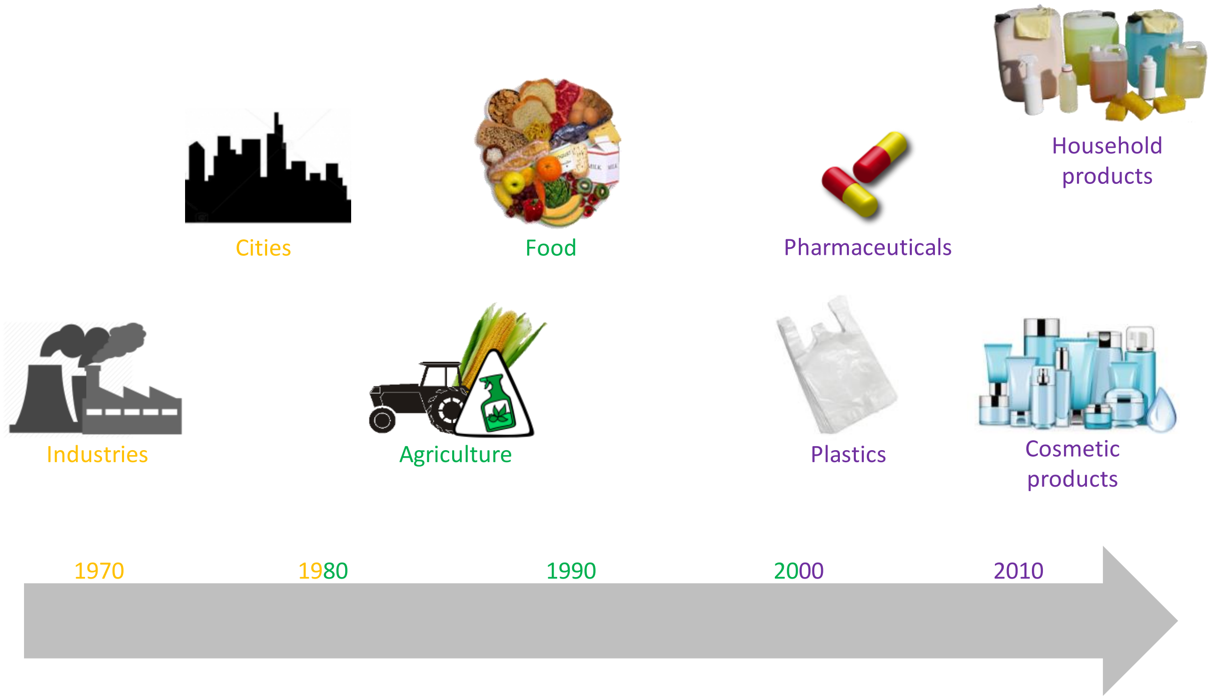


Fig. 7: Micropollutant sources since 1970 according to Briand et al. (2018) and Moilleron (2016).

Micropollutants are potentially toxic, natural or synthetic, inorganic or organic substances. They are persistent and bioaccumulative in the environment at low concentrations (in the range of ng/L to µg/L) (Sousa et al., 2019). Their introduction into the aquatic environment at any point of their life cycle and in different steps of the water cycle (European Environment Agency, 2018a; Le Treut, 2013b) is a result of continuous and/or uncontrolled release and their resistance to degradation (Cruzeiro et al., 2016; Radović et al., 2015). Indeed, many factors such as compound specificity and the treatment used, influence their efficient removal in WWTP, which were not originally designed to eliminate this type of pollutants (Sousa et al., 2019). This was confirmed by concentrations reported in the litterature in **Table 1**. These information originated from seventy-four publications, published between 1995 and 2018. The aim of this data base was not only to compare ranges of analyte concentrations reported in the literature with those found in the present study but also to know the maximum amount the studied analytes could reach in WWTP effluents. Plant size, treatment processes and analytical methods were not included as selection criteria because of the wide number of used technics and, sometimes, the paucity of information about them. In addition, only concentrations of each analyte were independently reported instead of concentrations of the whole analytical group due to the varying number of analytes considered into each group in studies. Generally, a large number of publications was achieved on metal, alkylphenol, musk and pharmaceutical analyses (**Table 1**). The interest of the scientific community in studying these specific molecules may be linked to their important probability of occurrence and great concentrations already detected in urban discharges which allow to ensure their detection despite the cost and the time these analyses required. By contrast, studies on PCBs and OCPs were scarce (Deblonde et al., 2011; Miège et al., 2009).

Table 1: Comparison of analyte concentrations (expressed as ng.L^{-1}) detected in treated effluents reported in the literature. Analytes were ordered in alphabetic order.

Substance families	Analytical Groups	Analytes	Range of concentrations (ng.L^{-1})	References
Priority substances				
Metal		Antimony (Sb)	/	/
Metal		Arsenic (As)	500 - 9 200	5
Metal		Cadmium (Cd)	20 - 170 000	4, 5, 8, 11, 43, 47, 48
Metal		Copper (Cu)	690 - 190 000	4, 5, 11, 43, 47, 48
Metal		Chromium (Cr)	400 - 5 600 000	4, 5
Metal		Lead (Pb)	40 - 160 000	4, 5, 8, 11
Metal		Molybdenum (Mo)	/	/
Metal		Nickel (Ni)	330 - 620 000	4, 5, 11, 43, 46, 47, 48
Metal		Silver (Ag)	600 - 12 200	5, 11
Metal		Tin (Sn)	<LOQ	11
Metal		Vanadium (V)	500 - 2 200	5
Metal		Mercury (Hg)	100 - 9 500	5, 8, 11
Organic	PAH	Acenaphthene	156 - 164	3, 71
Organic	PAH	Acenaphthylene	<DL - 336	3, 71
Organic	PAH	Anthracene	13 - 151	3, 32, 71
Organic	PAH	Benzo[a]anthracene	0.9 - 213	3, 32, 71
Organic	PAH	Benzo[a]pyrene	0.7 - 3.0	3, 32
Organic	PAH	Benzo[b]fluoranthene	1.5 - 4.2	3, 32
Organic	PAH	Benzo[g,h,i]perylene	0 - 2.3	3, 32
Organic	PAH	Benzo[k]fluoranthene	0.5 - 2.5	3, 32
Organic	PAH	Chrysene	0.7 - 285	3, 32, 71
Organic	PAH	Dibenzo[a,h]anthracene	0 - 3.3	3, 32
Organic	PAH	Fluoranthene	2.4 - 210	3, 32, 71
Organic	PAH	Fluorene	2.6 - 200	3, 32, 71
Organic	PAH	Indeno[1,2,3-cd]pyrene	0.8 - 3.3	3, 32
Organic	PAH	Naphthalene	101 - 3 490	3, 71
Organic	PAH	Phenanthrene	10.2 - 169	3, 32, 71
Organic	PAH	Pyrene	1.4 - 201	3, 32, 71
Organic	PCB	PCB 18	/	/
Organic	PCB	PCB 28+31	/	/
Organic	PCB	PCB 44	/	/
Organic	PCB	PCB 52	0	3
Organic	PCB	PCB 101	0	3
Organic	PCB	PCB 118	0	3
Organic	PCB	PCB 138	0	3
Organic	PCB	PCB 149	/	/
Organic	PCB	PCB 153	0	3
Organic	PCB	PCB 180	/	/
Organic	PCB	PCB 194	/	/
Organic	AP	NP	<30 - 37 000	3, 14, 17, 20, 21, 22, 27, 28, 32, 33, 37, 38, 43, 47, 48, 49
Organic	AP	NPEO1	6 - 47 700	3, 33, 71
Organic	AP	NPEO2	631 - 12 600	3, 71
Organic	AP	4tOP	2 - 1 700	14, 17, 18, 20, 21, 22, 28, 32, 33, 37, 38
Organic	AP	4nOP	<LQ - 74	33
Organic	OCP	Aldrin	ND - 0.048	51
Organic	OCP	Alpha BHC	0.630 - 3.55	51
Organic	OCP	Alpha Endosulfan	/	/
Organic	OCP	Beta BHC	0.168 - 1.44	51
Organic	OCP	Bêta Endosulfan	/	/
Organic	OCP	Delta BHC	/	/
Organic	OCP	Dieldrine	ND - 0.0250	51
Organic	OCP	Endosulfan Sulfate	/	/
Organic	OCP	Endrin	/	/
Organic	OCP	Endrin Aldehyde	/	/
Organic	OCP	Endrin Ketone	/	/
Organic	OCP	Gamma BHC	0.241 - 212	51, 71
Organic	OCP	Heptachlor	ND - 0.001	51
Organic	OCP	Heptachlor Epoxide	ND - 1.24	51
Organic	OCP	Methoxychlor	/	/
Organic	OCP	4,4'-DDD	ND	51
Organic	OCP	4,4'-DDE	0.028 - 0.161	51
Organic	OCP	4,4'-DDT	/	/

Table 1: (Continued)

Substance families	Analytical Groups	Analytes	Range of concentrations (ng.L^{-1})	References
Emerging substances				
Organic	Musk	ADBI	14 - 129	40, 41, 72
Organic	Musk	AHMI	5 - 13	72
Organic	Musk	AHTN	24 - 2 080	23, 28, 36, 40, 41, 42, 43, 45, 70, 72
Organic	Musk	ATII	8 - 203	40, 72
Organic	Musk	HHCB	10 - 7 030	23, 28, 36, 40, 41, 42, 45, 70, 72
Organic	Musk	HHCB-lactone	66 - 4 000	36, 45
Organic	Musk	MA	/	/
Organic	Musk	MK	13 - 177	41, 70, 72
Organic	Musk	MM	/	/
Organic	Musk	MX	1.4 - 16.1	70, 72
Organic	Sunscreen	Benzophenone 3	<79 - 230	13, 23
Organic	Sunscreen	EHMC	126 - 347	57
Organic	Sunscreen	OC	0 - <60	57, 73, 74
Organic	Sunscreen	OD-PABA	56	57
Organic	Sunscreen	3-BC	/	/
Organic	Sunscreen	4-MBC	43	57
Organic	Pharmaceutical (Pain killer)	Acetaminophen	3 - 6 000	6, 13, 15, 16, 28, 54, 62
Organic	Pharmaceutical (Glaucoma)	Acetazolamide	/	/
Organic	Pharmaceutical (Pain killer)	Acetylsalicylic acid	0.1 - 3 170	2, 6
Organic	Pharmaceutical (Antiarrhythmic)	Amiodarone	/	/
Organic	Pharmaceutical (Antibiotics)	Amoxicillin	4.7 - 66	7, 52, 64
Organic	Pharmaceutical (Antibiotics)	Ampicilline	ND - 498	53
Organic	Pharmaceutical (Antihypertensive)	Atenolol	2 - 7 600	7, 12, 13, 18, 23, 28, 32, 54, 64
Organic	Pharmaceutical (Antibiotics)	Azithromycin	<LOQ	11
Organic	Pharmaceutical (Psychotropic)	Caffeine	60 - 34 198.3	1, 2, 13, 15, 19, 23, 65
Organic	Pharmaceutical (Anticonvulsant)	Carbamazepine	1 - 7 570	2, 6, 12, 13, 18, 19, 23, 28, 32, 33, 50, 54, 55, 60, 61, 64
Organic	Pharmaceutical (Antibiotics)	Ciprofloxacin	51 - 5 600	1, 7, 9, 11, 19, 32, 33, 54
Organic	Pharmaceutical (Antibiotics)	Clarithromycin	18.1 - 536	7, 9, 11
Organic	Pharmaceutical (Anticancer)	Cyclophosphamide	20	6
Organic	Pharmaceutical (Anti-inflammatory)	Diclofenac	<1 - 2 830	2, 6, 10, 13, 16, 18, 19, 23, 25, 26, 28, 30, 31, 32, 33, 34, 35, 40, 53, 60, 63, 64, 66, 67
Organic	Pharmaceutical (Antibiotics)	Doxycycline	46	9
Organic	Pharmaceutical (Hormones)	E1	0.15 - 80	2, 17, 21, 31, 32, 40, 60, 63, 68
Organic	Pharmaceutical (Hormones)	E2	0.1 - 16	2, 13, 17, 21, 31, 33, 39, 60, 63, 68
Organic	Pharmaceutical (Hormones)	EE2	0.2 - 180	2, 6, 17, 21, 31, 33, 60, 63, 68
Organic	Pharmaceutical (Antibiotics)	Erythromycin A	1.3 - 2 840	9, 10, 11, 16, 18, 23, 28, 29, 32, 33, 35, 58, 63, 64, 66, 67
Organic	Pharmaceutical (Antibiotics)	Flumequine	257	19
Organic	Pharmaceutical (Glycemia)	Gemfibrozil	<2.5 - 5 240	2, 6, 13, 16, 19, 23, 26, 28, 40, 54
Organic	Pharmaceutical (Antihypertensive)	Hydrochlorothiazide	439.1 - 2 800	7, 54
Organic	Pharmaceutical (Antineoplastic)	Hydroxycarbamide	/	/
Organic	Pharmaceutical (Anti-inflammatory)	Ibuprofen	0.42 - 8 200	2, 6, 7, 10, 13, 16, 18, 19, 23, 25, 28, 29, 31, 32, 33, 35, 40, 54, 56, 60, 61, 63, 64, 66, 67
Organic	Pharmaceutical (Antibiotics)	Josamycin	/	/
Organic	Pharmaceutical (Pain killer)	Ketoprofen	<3 - 3 920	2, 6, 13, 16, 18, 19, 24, 28, 30, 32, 33, 40, 64
Organic	Pharmaceutical (Anxiolytics)	Lorazepam	<LQ - 23	32, 33
Organic	Pharmaceutical (Antihypertensive)	Losartan	/	/
Organic	Pharmaceutical (Antiarrhythmic)	Metoprolol	3 - 2 200	6, 12, 13, 16, 18, 23, 64
Organic	Pharmaceutical (Antibiotics)	Metronidazole	29 - 373	11, 18, 32, 64
Organic	Pharmaceutical (Pain killer)	Niflumic acid	/	/
Organic	Pharmaceutical (Anxiolytics)	Nordazepam	/	/
Organic	Pharmaceutical (Antibiotics)	Norfloxacin	29 - 364	9, 32, 52
Organic	Pharmaceutical (Antibiotics)	Ofloxacin	10 - 980	7, 9, 11, 32, 33, 52, 54, 63
Organic	Pharmaceutical (Anxiolytics)	Oxazepam	5 - 1 766	19, 32, 33, 64, 69
Organic	Pharmaceutical (Antibiotics)	Oxolinic acid	/	/
Organic	Pharmaceutical (Pain killer)	Phenazone	410	6
Organic	Pharmaceutical (Antibiotics)	Piperacillin	/	/
Organic	Pharmaceutical (Antibiotics)	Roxithromycine	18 - 155	9, 32, 33, 52
Organic	Pharmaceutical (Antibiotics)	Rifampicin	/	/
Organic	Pharmaceutical (Antibiotics)	Spiramycin	/	/
Organic	Pharmaceutical (Antibiotics)	Sulfadiazine	8 - 105	9, 19, 32
Organic	Pharmaceutical (Antibiotics)	Sulfamethazine	12 - 363	9, 52, 54
Organic	Pharmaceutical (Antibiotics)	Sulfamethoxazole	<3 - 10 800	1, 2, 7, 9, 13, 15, 16, 18, 19, 20, 23, 25, 32, 33, 50, 54, 59, 64
Organic	Pharmaceutical (Antibiotics)	Tetracycline	34 - 977	1, 9, 11, 33, 52
Organic	Pharmaceutical (Antibiotics)	Trimethoprim	9 - 3 050	1, 2, 11, 13, 15, 16, 18, 19, 23, 28, 30, 32, 35, 52, 54, 64, 66, 67
Organic	Pharmaceutical (Antibiotics)	Tylosine	/	/
Organic	Pharmaceutical (Contraceptif)	19-Norethindrone	/	/

1, Batt et al., 2006; **2**, Martín et al., 2012; **3**, Sánchez-Avila et al., 2009; **4**, Singh et al., 2004; **5**, Busetti et al., 2005; **6**, Ternes, 1998; **7**, Zuccato et al., 2005; **8**, Raach et al., 1999; **9**, Miao et al., 2004; **10**, Kay et al., 2017; **11**, Östman et al., 2017; **12**, Alder et al., 2010; **13**, Behera et al., 2011; **14**, Cespedes et al., 2008; **15**, Choi et al., 2008; **16**, Gracia-Lor et al., 2012; **17**, Janex-Habibi et al., 2009; **18**, Kasprzyk-Hordern et al., 2009; **19**, Loos et al., 2013; **20**, Martin Ruel et al., 2010; **21**, Nie et al., 2012; **22**, Pothitou and Voutsa, 2008; **23**, Santos et al., 2009; **24**, Singer et al., 2010; **25**, Stamatis and Konstantinou, 2013; **26**, Stamatis et al., 2010; **27**, Rosal et al., 2010; **28**, Terzić et al., 2008; **29**, Yu and Chu, 2009; **30**, Zhou et al., 2010; **31**, Zorita et al., 2009; **32**, Mailler et al., 2015; **33**, Mailler et al., 2016; **34**, Kuster et al., 2008; **35**, Roberts and Thomas, 2006; **36**, Horii et al., 2007; **37**, Snyder et al., 1999; **38**, Lee and Peart, 1995; **39**, Huang and Sedlak, 2001; **40**, Lishman et al., 2006; **41**, Chase et al., 2012; **42**, Clara et al., 2011; **43**, Clara et al., 2012; **44**, Simonich et al., 2002; **45**, Reiner et al., 2007; **46**, Fuchs et al., 2002; **47**, NOVANA, 2005; **48**, De Jong et al., 2005; **49**, Fahlenkamp et al., 2008; **50**, K'oreje et al., 2018; **51**, Man et al., 2018; **52**, Leung et al., 2012; **53**, Papageorgiou et al., 2016; **54**, Kostich et al., 2014; **55**, Fernández-López et al., 2016; **56**, Petrie et al., 2015; **57**, Tsui et al., 2014; **58**, Boleda et al., 2011; **59**, Subedi et al., 2015; **60**, Azzouz and Ballesteros, 2013; **61**, Vulliet et al., 2011; **62**, Lin et al., 2016; **63**, Gardner et al., 2012; **64**, Kasprzyk-Hordern et al., 2008; **65**, Baker and Kasprzyk-Hordern, 2013; **66**, Roberts and Thomas, 2006; **67**, Ashton et al., 2004; **68**, Koh et al., 2009; **69**, Baker and Kasprzyk-Hordern, 2011; **70**, Gatermann et al., 2002; **71**, Sánchez-Avila et al., 2011; **72**, Lee et al., 2003; **73**, Bueno et al., 2012 ; **74** , Rodil et al., 2012.

Once in the aquatic environment, they can cause various (biochemically and physiologically) harmful effects on organisms: endocrine disruption, behavioral changes, energy metabolism disturbances and genetic responses (Patisaul and Adewale, 2009; Vajda et al., 2011, 2008; Wilkinson et al., 2018). Therefore, over the past two decades, particular and increasing attention is paid to micropollutants due to their negative impacts on the environment (Carey and McNamara, 2015; Sousa et al., 2019).

Even though some substances are monitored and regulated (i.e. 45 priority substances through environmental quality standards) within European Directives (EC, 2013, 2000), many of them are still not regulated (i.e. contaminants of emerging concern, CECs; Hermes et al., 2018). They were thus identified as relevant environmental contaminants and became a major concern (Mezzelani et al., 2018). These two types of chemical substances belong to three main groups: **(1)** metals (e.g. Cadmium, Mercury, Nickel, Lead, Silver, Chromium, Zinc, etc.); **(2)** organometals (e.g. inorganic mercury, monomethylmercury, dibutyltin, tributyltin, organotins, etc.); and **(3)** organics (e.g. polycyclic aromatic hydrocarbons, polychlorinated biphenyls, alkylphenols, pesticides, pharmaceuticals).

(1) Metals may be naturally present in the environment (through dissolution of reservoir rocks for example) or introduced by human (through anthropogenic activities). Their concentration may widely vary due to physical-chemical conditions (e.g. temperature, salinity, pH, etc.) (Deycard et al., 2014).

(2) Organometals are generally compounds with at least one metal-carbon polarized bond (Cruz et al., 2017). They may be formed by arsenic, mercury, tin and lead, may occur naturally or associated to anthropogenic inputs (used in different industrial processes, as a component of antifouling paints, etc.) (Gadd, 1993; Hoch, 2001). Due to their biocidal properties and their wide use (e.g. worldwide production estimated at 50 000 tons only for organotins) they constitute an environmental threat, especially for aquatic ecosystems (Ayanda et al., 2012; Cruz et al., 2017; Deycard et al., 2014).

(3) Organics may be the result of natural sources and/or human activities. For example, PAHs (Polycyclic aromatic hydrocarbons) come from either natural sources or anthropogenic activities (partial oil burning, tarmac manufacture, etc.), from benzene cycle fusion (Borja and Collins, 2004). They are sparingly soluble in water explaining their adsorption and concentration on suspended matter, sediments or fish lipids. Among other organic substances, PCBs (Polychlorobyphenyls) are organochlorine aromatic compounds derived from biphenyl, so they constitute chlorine synthetic substances not naturally present in the environment. Some of them havebeen identified as priority hazardous substances by the WFD due to their low biodegradability. Alkylphenols are anionic surfactants present in soaps, paint, cosmetics, etc. No specific treatment is used in WWTP to eliminate these substances. As such, they are found in sediments and other soil types due to their highly lipophilic nature and persistence features. They were also identified as endocrine disruptors for human and animals (Bolong et al., 2009; Daughton and Ternes, 1999). Moreover, thousands tons of pharmaceuticals are also now widely used worldwide for human (3 000 molecules) and veterinary uses (300 molecules) to prevent, cure and treat diseases (Ali et al., 2018; Berlioz-Barbier, 2015; Puckowski et al., 2016). Even given the high dilution rate of the ocean, their concentrations in the marine environment may vary from few ng/L to hundreds of µg/L (Mezzelani et al., 2018). Pharmaceutical molecules can be distinguished in pharmacotherapeutic classes (Brandao et al., 2013; Fent et al., 2006) according to their medical function such as: (A) antibiotics, (B) steroid hormones, (C) antihypertensive drugs, (D) neuroactive drugs and (E) analgesic and anti-inflammatory drugs (Puckowski et al., 2016).

(A) Antibiotics refer to any (natural or synthetic) drug, agent or substance, that has toxic actions on microorganism growth (e.g. bacteria, fungi, protozoa) (Puckowski et al., 2016). They are widely used in human medicine (the third most frequently prescribed group of pharmaceuticals), veterinary medicine (more than 70% of all consumed pharmaceuticals) and in aquaculture around the world to treat microbial infectious diseases, with an annual estimation around hundreds of thousands of tons with a maximum in China (Binh et al., 2018; Kümmerer, 2009a; Liu et al., 2018; Puckowski et al., 2016). Once in the environment, several factors (e.g. physical-chemical properties, climatic conditions, pH, soil type) may influence the fate and effects of these substances (Puckowski et al., 2016; Sarmah et al., 2006). The consequence of their introduction (even at low concentrations) may be the formation of antibiotic resistant bacteria which could constitute a potential threat to environment and human health (Binh et al., 2018; Kümmerer, 2009b; Liu et al., 2018).

(B) Steroids are organic compounds having many functions, both in human and animal organisms and belonging to the lipid molecules family (Puckowski et al., 2016). They can be divided into three groups: cholesterol, bile salts and steroid hormones (a steroid that acts as a hormone) (Puckowski et al., 2016). The latter is itself divided into glucocorticoids, mineralocorticoids, androgens, estrogens and progestogens (Puckowski et al., 2016). In the case of estrogens, even if they are partially eliminated by WWTP (with still a significant level after treatment, ng.L^{-1}), the main source in aquatic environment are anyway the WWTP (Tan et al., 2007; Ternes et al., 1999). Once in the aquatic environment, they may have negative effects on the hormonal functions of humans and animals as the decrease of fertility or the emergence of problems in development and growth which may cause losses of habitats and biodiversity (Jauković et al., 2017; Liu et al., 2009; Naldi et al., 2016)..

(C) Antihypertensive drugs (calcium channel blockers, beta-blockers, angiotensin converting enzyme, ACE, inhibitors and angiotensin II receptor antagonists, sartans) are used in human medicine to lower or moderate the high blood pressure (Hanselin et al., 2011; Puckowski et al., 2016). Cardiovascular diseases constitute a current and growing problem around the world which are therefore accompanied by a high consumption of associated medications (Bayer et al., 2014; Godoy et al., 2015; Gu et al., 2012).

(D) Neuroactive drugs are a group of medications (anti-epileptics and antidepressants) that treat epilepsy, depression, eating disorders and personality disorders (Brooks et al., 2003). Their consumption has drastically increased (by 60%) over the past decade worldwide (Silva et al., 2015). The most common are paroxetine, carbamazepine, fluoxetine and sertraline (Puckowski et al., 2016). As above substances, they are discharged in aquatic environment even after their treatment by WWTP. Once in the aquatic environment, neuroactive compounds (e.g. antiepilectics, antidepressants) can alter and modulate nervous system functions of organisms and behavioral parameters (e.g. inhibition of reproduction and physiological development, stress responses, scototaxis, thigmotaxis, shoal cohesion, predator avoidance, feeding behaviour, locomotion of fish and invertebrates (e.g. swimming performance) and consequently, growth (Brandao et al., 2013; Puckowski et al., 2016).

(E) Anti-inflammatory drugs are nonsteroidal drugs (NSAIDS) including analgesics. They are used as painkillers in both human and veterinary medicines (Puckowski et al., 2016). “They are one of the most important groups of pharmaceuticals in the world” (Cleuvers, 2004) and their production is estimated at several kilotons annually (Cleuvers, 2004). The most common are ibuprofen, naproxen, diclofenac and ketoprofen (Kosjek et al., 2005).

1.3 Communities’ response

Even if treatment plants aim to remove coarse solids (i.e., primary treatment), organic matter (i.e., secondary treatment), and to ensure the reduction of nutrient (such as N and P) and bacteria to prevent eutrophication (i.e., tertiary treatment), they do not treat contaminants which may have toxic effects on aquatic organisms (Cabral-Oliveira and Pardal, 2016; Stark et al., 2016). Sewage discharges are thus responsible for nutrient and organic enrichment, increased sedimentation and turbidity, decreased salinity (Azzurro et al., 2010; Terlizzi et al., 2005) and contamination (by heavy metals, priority and emerging contaminants, fecal sterols and bacteria) (Costanzo et al., 2001; Millennium Ecosystem Assessment -MEA, 2005). Therefore, sewage discharges constitute an important stressor for marine communities in many intertidal and subtidal systems around the world (Andral et al., 2011; Arévalo et al., 2007; Becherucci et al., 2016; Borowitzka, 1972; Littler and Murray, 1975; Liu et al., 2007; O’Connor, 2013; Vinagre et al., 2016a). Depending on their type, source and level, sewage discharges may have direct or indirect effects (biological, chemical or physical) on the environment (Borja et al., 2011a; Del-Pilar-Ruso et al., 2010) which may varies from little or no impact to major changes (Pastorok and Bilyard, 1985).

In the marine environment, organisms can be thus used to give the fraction of the bioavailable environmental pollution (Gust et al., 2010), to monitor the level of sea water pollution (Borja and Collins, 2004; Claisse, 1991), evaluate its transfer (bioavailability and bioaccumulation) and inform of associated effects (Bergé and Vulliet, 2015). Indeed, they are known to have the ability to accumulate contaminants present in the water. Different terms are used to define the process about the fate of contaminants in different environment compartments (biological or physical): (1) Bioconcentration, (2) Bioaccumulation and (3) Biomagnification and may be calculated through three bio-uptake factors (Bodin, 2005; Casas, 2005; Mackay et al., 2018; Puckowski et al., 2016; Zenker et al., 2014):

(1) Bioconcentration is the accumulation of a dissolved substance by an aquatic organism with no dietary intake. It means that the concentration of test chemical substance in organism or tissue is higher than those in its environment (e.g. sediment or water). The bioconcentration factor (BCF) (in L.kg^{-1}) is the ratio between the concentration of the substance of interest in the biota sample and the concentration in the surrounding environment.

(2) Bioaccumulation is the accumulation of a substance, dissolved in water, by an aquatic organism with dietary intake (i.e. absorption through direct contact with water and food ingestion). The organism absorbs faster than it secretes a substance presents in its environment. The bioaccumulation factor (BAF) (in L.kg^{-1}) is the ratio between the concentration of a substance in the organism and the concentration of the substance in the surrounding medium. It highly depends on the compound bioavailability in the environment which may vary with the water physico-chemical features (pH, salinity, oxygen, etc.).

(3) Biomagnification is when the concentration of a test substance in a predator is higher than in its food, the predator's prey. This means that contaminant concentrations increase as it passes up the food chain through two or more trophic levels. The biomagnification factor (BMF) (in kg.g^{-1}) is the ratio of organism to diet concentrations (i.e. between the concentration of a substance in the predator and this same concentration in the prey).

Thanks to the improvement of analytical methodologies, especially on the detection of low concentrations, the chemical substances are increasingly detected in a variety of biological samples (Puckowski et al., 2016). But, until now, few studies have been undertaken to assess pharmaceuticals in wild biota leading to a knowledge gap in the extent and route of exposure these organisms encounter (Miller et al., 2018). Indeed, the database achieved to identify concentrations already reported in marine organisms support this information (**Table 2**). One hundred and forty-three publications, published between 1963 and 2019, were listed in the latter. As studies achieved on wastewaters, metals were the compounds identified in the highest concentrations even though no specific species was highlighted as the main accumulator of these compounds. By contrast, much less works were done on the study of pharmaceutical compounds in benthic organisms.

Table 2: Comparison of analyte concentrations (metals expressed as mg.kg^{-1} and organic compounds as ng.g^{-1} on a dry weight basis) detected in different marine organisms reported in the literature. Asterisk (*) indicates results expressed on a wet weight basis. Analytes were ordered in alphabetic order.

Substance families	Analytical Groups	Analytes	Species	Range of concentrations (ng.g^{-1})	References
Priority substances					
Metal		Antimony (Sb)		/	/
Metal		Arsenic (As)	Sea cucumbers	120 - 33 300	15, 19
			Other algae	180 - 1 441 000	15, 39, 58, 97
			Cystoseira spp.	4 200 - 131 00	39
			Ulva spp.	2 060 - 85 500	39, 50, 97
			Other mollusca	920 - 17 200	15, 97
			Mussels	2 600 - 58 400	78, 97, 107, 108, 132
			Sponges	320 - 1 090	139
Metal		Cadmium (Cd)	Sea cucumbers	40 - 128 930	4, 5, 6, 7, 8, 9, 11, 12, 13, 14, 15, 16, 17, 18, 19
			Other algae	<20 - 28 000	15, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 38, 39, 40, 44, 49, 56, 57, 97
			Cystoseira spp.	<20 - 2 340	38, 39, 40, 56
			Ulva spp.	0 - 179 600	38, 39, 42, 43, 44, 45, 46, 48, 50, 97
			Gelidium spp.	210 - 450	56
			Other mollusca	<80 - 299 000	15, 60, 61, 81, 97
			Limpets	23 - 78 300	62, 63, 64, 65, 66, 67, 69, 70, 71, 72, 73, 92, 96, 97
			Mussels	400 - <10 000	76, 78, 97, 107, 108, 132
			Sponges	40 - 79 900	81, 139, 141, 143
Metal		Chromium (Cr)	Sea cucumbers	<4 - 9 310	1, 15, 18, 19
			Other algae	<60 - 110 700	15, 24, 27, 28, 29, 31, 32, 33, 34, 35, 38, 39, 44, 49, 57
			Cystoseira spp.	<60 - 775 000	36, 38, 39
			Ulva spp.	<60 - 45 700	38, 39, 43, 44, 50
			Other mollusca	420 - 12 200	15, 60, 81
			Limpets	200 - 23 200	64, 68, 70, 71, 72, 73, 92
			Mussels	<500 - 24 000	76, 78, 107, 108, 132
			Sponges	2 800 - 12 300	81, 139
Metal		Copper (Cu)	Sea cucumbers	20 - 100 450	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 17, 18, 19
			Other algae	<30 - 302 000	15, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 38, 39, 44, 49, 56, 57, 97
			Cystoseira spp.	1 700 - 8 780	38, 39, 56
			Ulva spp.	1 820 - 750 000	38, 39, 42, 44, 48, 50, 97, 100
			Gelidium spp.	1 340 - 6 600	56
			Other mollusca	3 100 - 1 876 000	15, 60, 61, 81, 97
			Limpets	600 - 45 900	62, 64, 65, 66, 67, 68, 69, 70, 71, 72, 92, 96, 97
			Mussels	2 000 - 17 300	76, 78, 97, 107, 108, 132
			Sponges	350 - 299 300	81, 139, 140, 143
Metal		Lead (Pb)	Sea cucumbers	26 - 97 520	4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19
			Other algae	<100 - 250 000	15, 24, 25, 27, 29, 30, 31, 32, 33, 34, 35, 38, 39, 40, 44, 49, 56, 57
			Cystoseira spp.	0 - 28 600	36, 38, 39, 40, 56
			Ulva spp.	0 - 54 000	38, 39, 42, 43, 44, 45, 46, 48, 50
			Gelidium spp.	90 - 83	56
			Other mollusca	100 - 184 000	15, 60, 81
			Limpets	300 - 95 600	64, 65, 66, 67, 69, 70, 71, 73, 92, 96
			Mussels	370 - 25 000	76, 78, 107, 108, 132
			Sponges	<200 - 32 500	81, 140, 143
Metal		Molybdenum (Mo)	Other mollusca	200	81
			Mussels	100 - 1 000	76
			Sponges	200 - 1 200	81
Metal		Nickel (Ni)	Sea cucumbers	<130 - 35 500	1, 4, 8, 9, 12, 15
			Other algae	<100 - 70 600	15, 24, 25, 27, 28, 29, 31, 32, 33, 34, 35, 38, 56, 57
			Cystoseira spp.	<100 - 9 100	38, 56
			Ulva spp.	<100 - 225 800	38, 42, 46, 48, 50
			Gelidium spp.	4 450 - 15 050	56
			Other mollusca	700 - 48 400	15, 81
			Limpets	600 - 83 700	64, 65, 66, 67, 68, 70, 71, 72, 92, 96
			Mussels	800 - 17 000	76, 78, 107, 108, 132
			Sponges	1 260 - 9 130	81, 139
Metal		Silver (Ag)	Sea cucumbers	<70 - <250	15
			Other algae	<70 - 510	15
			Other mollusca	<140 - 24 100	15
			Mussels	100 - 300	76
Metal		Tin (Sn)		/	/
Metal		Vanadium (V)	Ulva spp.	6 970 - 9 240	50
			Mussels	1 970 - 8 000	76, 78
Metal		Mercury (Hg)	Sea cucumbers	540 - 445 690	15, 18
			Other algae	<5 - 10 200	15, 56
			Cystoseira spp.	<5 - 10	56
			Gelidium spp.	10	56
			Other mollusca	8 220 - 111 000	15
			Limpets	ND - 90	73
			Mussels	39 - 5 000	107, 108

Table 2: (continued)

Substance families	Analytical Groups	Analytes	Species	Range of concentrations (ng.g $^{-1}$)	References
Organic	PAH	Acenaphthene	Sea cucumbers	0.85 - 57.81	21
			Other mollusca	0 - 1.0	81, 115
			Limpets	12.40 - 43.47	135
			Mussels	0 - 287*	80, 106, 108, 111 112*, 113*, 114
			Sponges	1.0 - 4.8	80, 81
Organic	PAH	Acenaphthylene	Sea cucumbers	0.15 - 49.01	21
			Other mollusca	0 - 1.0	81, 115
			Limpets	15.06 - 440.2	135
			Mussels	<0.063* - 718.0*	80, 108 112*, 113*
			Sponges	0.1 - .8.3	80, 81
Organic	PAH	Anthracene	Sea cucumbers	0.28 - 39.95	21
			Other mollusca	3.0* - 47.9	22*, 81, 115
			Limpets	2.1* - 72.92	133*, 135
			Mussels	0 - 287.0*	79, 80, 87*, 91*, 106, 107, 108, 110, 111 112*, 113*, 114
			Sponges	3.0 - 141.0	22*, 80, 81
			Other marine organisms	0.98 - 7.15	22*, 79
Organic	PAH	Benzo[a]anthracene	Sea cucumbers	0.33 - 36.73	21, 22*
			Other mollusca	1.0* - 28.4	22*, 81, 115
			Limpets	0.02 - 80.50	135, 136
			Mussels	0 - 2214.0*	79, 80, 87*, 91*, 106, 107, 108, 110, 111 112*, 113*, 114
			Sponges	1.0* - 85.6	22*, 80, 81
			Other marine organisms	0.11 - 2.95	79
Organic	PAH	Benzo[a]pyrene	Sea cucumbers	0.02 - 58.0*	21, 22*
			Other algae	2.0 - 64.0	22*, 51, 59, 60
			Other mollusca	3.4 - 540.0	22*, 51, 81, 115
			Limpets	0.01 - 70.30	132, 51, 133*, 135, 136
			Mussels	00.019* - 3339.0*	51, 79, 80, 87*, 106, 107, 108, 111 112*, 113*, 114, 132
			Sponges	<0.01 - 89.8	22*, 51, 80, 81, 142
			Other marine organisms	0.5 - 3.34	79
Organic	PAH	Benzo[b]fluoranthene	Sea cucumbers	1.69 - 7.64	21
			Other algae	10.0*	22*
			Other mollusca	7.0* - 33.1	22*, 81
			Limpets	0.02 - 61.11	135, 136
			Mussels	0 - 242.0	79, 80, 87*, 106, 110, 111, 114
			Sponges	7.0* - 138.0	22*, 80, 81
			Other marine organisms	0.3 - 1.53	79
Organic	PAH	Benzo[g,h,i]perylene	Sea cucumbers	1.36 - 1.83	21
			Other mollusca	0 - 47.0*	22*, 81, 115
			Limpets	0.04 - 1.19	132, 136
			Mussels	0 - 659.0	79, 80, 87*, 106, 107, 108, 110, 111 112*, 113*, 114, 132
			Sponges	1.0 - 165.0*	22*, 80, 81
			Other marine organisms	1.02 - 2	79
Organic	PAH	Benzo[k]fluoranthene	Sea cucumbers	0.18 - 32.98	21
			Other mollusca	7.0* - 71.0*	22*, 81
			Limpets	0.01 - 1.03	132, 136
			Mussels	0 - 178.46	79, 80, 87*, 91*, 106, 110, 111, 114, 132
			Sponges	5.4 - 48.0	22*, 80, 81
			Other marine organisms	0.13 - 9.0*	22*, 79
Organic	PAH	Chrysene	Sea cucumbers	0.09 - 22.99	21, 22*
			Other algae	5.0*	22*
			Other mollusca	3.0* - 86.2	22*, 81, 115
			Limpets	0.07 - 791.7	133*, 135, 136
			Mussels	0 - 6372.0*	79, 80, 87*, 91*, 106, 107, 110, 111 112*, 113*, 114
			Sponges	2.5 - 546.0*	22*, 80, 81
			Other marine organisms	0.83 - 5.3	79
Organic	PAH	Dibenzo[a,h]anthracene	Sea cucumbers	0.08 - 0.059*	21, 22*
			Other algae	36.0*	22*
			Other mollusca	0 - 73.0*	22*, 81, 115
			Limpets	0.01 - 1512	135, 136
			Mussels	0 - 405.0*	79, 80, 106, 107, 108, 110, 111 112*, 113*, 114
			Sponges	1.0 - 449.0*	22*, 80, 81
Organic	PAH	Fluoranthene	Sea cucumbers	0.33 - 37.91	21, 22*
			Other algae	16.0*	22*
			Other mollusca	5.0* - 430.0*	22*, 81, 115
			Limpets	0.08 - 74.67	132, 133*, 135, 136
			Mussels	0 - 979.0	79, 80, 87*, 91*, 106, 107, 108, 110, 111 112*, 113*, 114, 132
			Sponges	0.26 - 121.8	22*, 80, 81, 142
			Other marine organisms	0.35 - 2.9	79
Organic	PAH	Fluorene	Sea cucumbers	0.5 - 28.89	21
			Other mollusca	0 - 22.0*	22*, 81, 115
			Limpets	0.03 - 15.78	133*, 135, 136
			Mussels	<0.014* - 115.0*	87*, 91*, 106, 108, 110, 111 112*, 113*, 114
			Sponges	8.8 - 28.9	81

Table 2: (continued)

Substance families	Analytical Groups	Analytes	Species	Range of concentrations (ng.g $^{-1}$)	References
Organic	PAH	Indeno[1,2,3-cd]pyrene	Sea cucumbers	2.75 - 3.1	21
			Other mollusca	0 - 13.0	81, 115
			Limpets	0.01 - 0.02	136
			Mussels	0 - 747.0	79, 80, 106, 107, 108, 110, 111 112*, 113*, 114
			Sponges	1 - 109.0	22*, 80, 81
			Other marine organisms	1.6	79
Organic	PAH	Naphthalene	Sea cucumbers	1.11 - 8.93	21
			Other mollusca	2.9 - 111.9	81, 115
			Limpets	308.4 - 451.0	135
			Mussels	<0.028* - 1286.0*	80, 106, 108, 110, 111, 112*, 113*
			Sponges	3.7 - 335	80, 81
Organic	PAH	Phenanthrene	Sea cucumbers	1.04 - 87.17	21, 22*
			Other mollusca	0 - 259.0*	22*, 81, 115
			Limpets	0.23 - 17.18	132, 133*, 135, 136
			Mussels	0 - 319*	79, 80, 87*, 91*, 106, 107, 108, 110, 111 112*, 113*, 114, 132
			Sponges	1 - 53.2	22*, 80, 81
			Other marine organisms	1.75 - 13.3	79
Organic	PAH	Pyrene	Sea cucumbers	0.30 - 73.23	21, 22*
			Other mollusca	3.0* - 58.3	22*, 81, 115
			Limpets	0.25 - 81.41	133*, 135, 136
			Mussels	0 - 309.0	79, 80, 87*, 106, 107, 108, 110, 111 112*, 113*, 114
			Sponges	3.0* - 127.1	22*, 80, 81
			Other marine organisms	0.90 - 3.40	79
Organic	PAH	Total PAHs	Other algae	30.0* - 4665.0	22*, 41, 51
			Cystoseira spp.	1.3 - 27.3	41
			Ulva spp.	1.0 - 56.4	41, 98
			Sea cucumbers	8.08 - 505.44	21, 22*
			Other mollusca	4.1* - 1135.0	22*, 51, 82*, 90
			Limpets	3.1* - 142925.0	51, 132, 133*, 134
			Mussels	14.6 - 101.76	51, 79, 106, 107, 108, 109, 110, 111, 112*, 115, 121, 132
			Sponges	4.74* - 769.0	22*, 51, 94*
			Other marine organisms	12.0* - 32.63	22*, 51, 79, 99*
Organic	PCB	PCB 18	Other algae	<0.1 - 0.31	23*, 47
			Sponges	0.11* - 367.0*	23*, 93
Organic	PCB	PCB 28+31	Other mollusca	2.25*	23*
Organic	PCB	PCB 44	Sea cucumbers	0.20* - 6.06*	23*
			Other algae	<0.07 - 0.3	47
			Other mollusca	0.35* - 1.14*	23*
			Sponges	0.12* - 258.0*	23*, 93
Organic	PCB	PCB 52	Sea cucumbers	0.39* - 19.1*	23*
			Other algae	0.11 - 2.16	23*, 47, 53
			Ulva spp.	0.52 - 7.53	53
			Other mollusca	0.09* - 3.16*	23*, 81
			Mussels	0.06* - 50.0	87*, 107, 108, 124*, 126*
			Sponges	0.21* - 1839.0*	23*, 81, 93
Organic	PCB	PCB 101	Sea cucumbers	0.11* - 45.0*	23*
			Other algae	<0.06 - 1.21	23*, 47, 53
			Ulva spp.	0.31 - 2.45	53
			Other mollusca	0.06* - 4.81*	23*, 81
			Mussels	0.08* - 136.0	87*, 108, 124*, 126*
			Sponges	0.13* - 1848.0*	23*, 81
			Other marine organisms	0.16* - 0.41*	23*
Organic	PCB	PCB 118	Sea cucumbers	0.05* - 591.0*	23*
			Other algae	<0.05 - 2.84	23*,47, 53
			Ulva spp.	0.2 - 0.55	53
			Other mollusca	0.07* - 4.5	23*, 81
			Mussels	0.15* - 78.0	87*, 107, 108, 124*, 126*
			Sponges	0.11* - 1278*	23*, 81, 93
			Other marine organisms	0.18* - 0.47*	23*
Organic	PCB	PCB 138	Sea cucumbers	0.09* - 22.9*	23*
			Other algae	0.06* - 0.55	23*,47
			Ulva spp.	2.6	53
			Other mollusca	0.06* - 7.24*	23*, 81
			Mussels	0.15* - 133.0	87*, 108, 124*, 126*
			Sponges	0.18* - 1281.0*	23*, 81, 93
			Other marine organisms	0.17* - 0.32*	23*

Table 2: (continued)

Substance families	Analytical Groups	Analytes	Species	Range of concentrations (ng.g $^{-1}$)	References
Organic	PCB	PCB 149	Sponges	22.92 - 610.85	93
Organic	PCB	PCB 153	Sea cucumbers	0.05* - 570*	23*
			Other algae	<0.10 - 0.68	23*,47
			Other mollusca	0.017* - 21.0*	23*, 81
			Mussels	0.2* - 176.0	87*, 107, 108, 124*, 126*
			Sponges	0.13* - 1281.0*	23*, 81, 93
			Other marine organisms	0.07* - 0.69*	23*
Organic	PCB	PCB 180	Sea cucumbers	0.03* - 0.74*	23*
			Other algae	<0.05 - 4.54	23*,47, 53
			Ulva spp.	3.1 - 12.23	53
			Other mollusca	0.02* - 5.01*	23*, 81
			Mussels	0.05* - 41.0	87*, 107, 108, 124*, 126*
			Sponges	0.15* - 1037.0*	23*, 81, 93
			Other marine organisms	0.03* - 0.50*	23*
Organic	PCB	PCB 194	Sponges	12.56 - 25.53	93
Organic	PCB	Total PCBs	Sea cucumbers	0.03* - 1279.0*	23*
			Other algae	0.39* - 20.0	23*,41, 55
			Cystoseira spp.	0.4 - 4.2	41
			Ulva spp.	0.1 - 25.0	41, 54
			Other mollusca	0.029* - 8836.0	23*,77, 82*, 116
			Limpets	0.064* - 39.0*	132*, 137, 138*
			Mussels	0.55* - 591.0	107, 108, 116, 124*, 126*, 132
			Other marine organisms	0.10* - 3.0*	23*
			Sponges	0.65* - 9740.0*	23*
Organic	AP	NP	Sea cucumbers	194.7 - 358.1	20, 101
			Ulva spp.	7.5 - 50.4	101
			Other mollusca	35.5 - 538.6	101
			Mussels	2.0 - 3.0	104, 105, 117, 118, 119, 120, 121, 122, 123
Organic	AP	NPEO1	Sea cucumbers	14.5 - 29.3	20
			Mussels	6.3 - 300.0	87*, 104, 105
Organic	AP	NPEO2	Sea cucumbers	1.83 - 3.87	20
Organic	AP	4nOP	Mussels	<1.7	117
Organic	AP	4tOP	Mussels	0.3 - 54.4	117, 118, 123
			Mussels	0 - 823.0	104, 105, 125
Organic	OCP	Aldrin	Other algae	Not detectable	37
			Cystoseira spp.	Not detectable	36
			Mussels	0.04 - 40.3*	110, 124*, 126*
Organic	OCP	Alpha BHC	Other algae	Not detectable	37
			Cystoseira spp.	Not detectable	36
			Mussels	3.0* - 47.6*	126*
Organic	OCP	Alpha Endosulfan		/	/
Organic	OCP	Beta BHC	Other algae	Not detectable	37
			Cystoseira spp.	Not detectable	36
			Mussels	0.02 - 102.2*	110, 126*
Organic	OCP	Bêta Endosulfan		/	/
Organic	OCP	Delta BHC	Other algae	12.2	37
			Cystoseira spp.	Not detectable	36
Organic	OCP	Dieldrine	Other algae	Not detectable	37
			Cystoseira spp.	Not detectable	36
			Mussels	0.066 - 58.7*	124*, 126*
Organic	OCP	Endosulfan Sulfate		/	/
Organic	OCP	Endrin	Other algae	Not detectable	37
			Cystoseira spp.	Not detectable	36
			Mussels	0.170 - 0.257	124*
Organic	OCP	Endrin Aldehyde	Mussels	0.01 - 4.5	110
Organic	OCP	Endrin Ketone		/	/
Organic	OCP	Gamma BHC	Other algae	27.6	37
			Cystoseira spp.	22.28	36
			Other mollusca	<0.028 - 232.0	77
			Mussels	0 - 33.6*	104, 108, 110, 124*, 126*
Organic	OCP	Heptachlor	Other algae	Not detectable	37
			Cystoseira spp.	Not detectable	36
			Mussels	0.02 - 21.6*	110, 126*
Organic	OCP	Heptachlor Epoxide	Mussels	0.03 - 83.9*	110, 126*
Organic	OCP	Methoxychlor		/	/
Organic	OCP	4,4'-DDD	Other algae	0.2 - 3.5	54
			Cystoseira spp.	0.1 - 3.2	54
			Ulva spp.	0.1 - 1.3	54
			Other mollusca	<0.018 - 77.0	77
			Mussels	0.08* - 29.0	87*, 124*, 126*, 127
Organic	OCP	4,4'-DDE	Other algae	0.1 - 1.8	54
			Cystoseira spp.	0.1 - 0.9	54
			Ulva spp.	0.1 - 4.2	54
			Other mollusca	0.7 - 483.0	77
			Limpets	<0.01* - 4.0*	132*, 138*
			Mussels	0.04* - 135.1*	87*, 124*, 126*, 127, 132
Organic	OCP	4,4'-DDT	Other algae	0.7 - 15.7	54
			Cystoseira spp.	1.0 - 16.4	54
			Ulva spp.	1.6 - 18.9	54
			Other mollusca	<0.013	77
			Limpets	2.0* - 7.0*	138*
			Mussels	0.09* - 629.8*	110, 124*, 126*, 127
Organic	OCP	Total pesticides	Other algae	0.4 - 2.8	41
			Cystoseira spp.	0.4 - 4.9	41
			Ulva spp.	0.2 - 1.7	41

Table 2: (continued)

Substance families	Analytical Groups	Analytes	Species	Range of concentrations (ng.g $^{-1}$)	References
Emerging substances					
Organic	Musk	ADBI	Mussels	Not detected - 14.5	128, 129
Organic	Musk	AHMI	Mussels	Not detected	129
Organic	Musk	AHTN	Mussels	6.98 - 31.7	128, 129
Organic	Musk	ATII	Mussels	Not detected	129
Organic	Musk	HHCB	Mussels	8.68 - 159.4	86, 128, 129
Organic	Musk	HHCB-lactone	Mussels	Not detected - 63.51	129
Organic	Musk	MA		/	/
Organic	Musk	MK	Mussels	Not - detected - <50.0	86, 128
Organic	Musk	MM	Mussels	10.5 - 15.2	128
Organic	Musk	MX	Mussels	Not detected - 18.4	128
Organic	Sunscreen	Benzophenone 3	Sea cucumbers	1.66 - 53.9	20
			Mussels	51.2 - 622.1	128
Organic	Sunscreen	EHMC	Mussels	<2.0 - 1765.0	74, 75, 86, 128
Organic	Sunscreen	OC	Mussels	2.0 - 7112.0	74, 86
Organic	Sunscreen	OD-PABA	Mussels	0 - 833.0	74, 86
Organic	Sunscreen	3-BC		/	/
Organic	Sunscreen	4-MBC	Mussels	74.6 - 88.3	128
Organic	Pharmaceutical (Pain killer)	Acetaminophen	Mussels	65.0 - 115.0	130
Organic	Pharmaceutical (Glaucoma)	Acetazolamide		/	/
Organic	Pharmaceutical (Pain killer)	Acetylsalicylic acid		/	/
Organic	Pharmaceutical (Antiarrhythmic)	Amiodarone		/	/
Organic	Pharmaceutical (Antibiotics)	Amoxicillin		/	/
Organic	Pharmaceutical (Antibiotics)	Ampicilline		/	/
Organic	Pharmaceutical (Antihypertensive)	Atenolol	Other algae	Not detected	52
			Other mollusca	<1.0* - 0.3*	83*
			Mussels	0 - 13.0	104
			Other marine organisms	1.3 - 8.1	95
Organic	Pharmaceutical (Antibiotics)	Azithromycin	Mussels	2.9	131
Organic	Pharmaceutical (Psychotropic)	Caffeine	Other algae	Not detected - 41.3	52
			Mussels	0 - 140.0	104, 105
			Other marine organisms	Not detected	95
Organic	Pharmaceutical (Anticonvulsant)	Carbamazepine	Other algae	Not detected - 1.7	52
			Other mollusca	1.3* - 5.3*	83*
			Mussels	<0.4 - 11.0	84*, 85, 130
			Other marine organisms	Not detected - 5.5	95
Organic	Pharmaceutical (Antibiotics)	Ciprofloxacin	Sea cucumbers	8	102
Organic	Pharmaceutical (Antibiotics)	Clarithromycin		/	/
Organic	Pharmaceutical (Anticancer)	Cyclophosphamide		/	/
Organic	Pharmaceutical (Anti-inflammatory)	Diclofenac	Mussels	ND - 0.24	85
Organic	Pharmaceutical (Antibiotics)	Doxycycline		/	/
Organic	Pharmaceutical (Hormones)	E1	Sea cucumbers	<LOD	20
Organic	Pharmaceutical (Hormones)	E2	Sea cucumbers	<LOD	20
Organic	Pharmaceutical (Hormones)	EE2	Sea cucumbers	<LOD	20
Organic	Pharmaceutical (Antibiotics)	Erythromycin A	Mussels	0 - 2.0	104
Organic	Pharmaceutical (Antibiotics)	Flumequine		/	/
Organic	Pharmaceutical (Glycemia)	Gemfibrozil		/	/
Organic	Pharmaceutical (Antihypertensive)	Hydrochlorothiazide		/	/
Organic	Pharmaceutical (Antineoplastic)	Hydroxycarbamide		/	/
Organic	Pharmaceutical (Anti-inflammatory)	Ibuprofen	Other algae	Not detected	52
			Other marine organisms	Not detected	95
Organic	Pharmaceutical (Antibiotics)	Josamycin		/	/
Organic	Pharmaceutical (Pain killer)	Ketoprofen		/	/
Organic	Pharmaceutical (Anxiolytics)	Lorazepam		/	/
Organic	Pharmaceutical (Antihypertensive)	Losartan		/	/
Organic	Pharmaceutical (Antiarrhythmic)	Metoprolol		/	/
Organic	Pharmaceutical (Antibiotics)	Metronidazole		/	/
Organic	Pharmaceutical (Pain killer)	Niflumic acid		/	/
Organic	Pharmaceutical (Anxiolytics)	Nordazepam		/	/
Organic	Pharmaceutical (Antibiotics)	Norfloxacin	Sea cucumbers	Not detected	102
Organic	Pharmaceutical (Anxiolytics)	Oxazepam		/	/
Organic	Pharmaceutical (Antibiotics)	Ofloxacin	Sea cucumbers	Not detected - 15.7	103
			Mussels	0 - 65.0	104, 105, 130
Organic	Pharmaceutical (Antibiotics)	Oxolinic acid		/	/
Organic	Pharmaceutical (Pain killer)	Phenazone		/	/
Organic	Pharmaceutical (Antibiotics)	Piperacillin		/	/
Organic	Pharmaceutical (Antibiotics)	Rifampicin		/	/
Organic	Pharmaceutical (Antibiotics)	Roxithromycine		/	/
Organic	Pharmaceutical (Antibiotics)	Spiramycin		/	/
Organic	Pharmaceutical (Antibiotics)	Sulfadiazine	Sea cucumbers	Not detected - 17.7	103
Organic	Pharmaceutical (Antibiotics)	Sulfamethazine	Sea cucumbers	11.6	102
			Mussels	0 - 430.0	104, 105
Organic	Pharmaceutical (Antibiotics)	Sulfamethoxazole	Sea cucumbers	5.6 - 11.0	102, 103
			Other algae	Not detected	52
			Other marine organisms	Not detected - 13.1	95
Organic	Pharmaceutical (Antibiotics)	Tetracycline		/	/
Organic	Pharmaceutical (Antibiotics)	Trimethoprim	Sea cucumbers	8.0 - 15.2	102, 103
			Other algae	Not detected	52
			Mussels	<0.87 - <4.0	85
			Other marine organisms	0.84 - 1.5	95
Organic	Pharmaceutical (Antibiotics)	Tylosine		/	/
Organic	Pharmaceutical (Contraceptif)	19-Norethindrone		/	/

Table 2: (continued)

1, Bechtel et al., 2013; **2**, Chang-Lee et al., 1989; **3**, Xing and Chia, 1997; **4**, Culha et al., 2016; **5**, Sicuro et al., 2012; **6**, Warnau et al., 2006; **7**, Medina et al., 2004; **8**, Denton et al., 2006a; **9**, Mageswaran and Balakrishnan, 1985; **10**, Matsumoto et al., 1964; **11**, Noël et al., 2011; **12**, Denton and Morrison, 2009; **13**, Jinadasa et al., 2014; **14**, González et al., 2004; **15**, Denton et al., 2009; **16**, Givianrad et al., 2014; **17**, Mohammadizadeh et al., 2016; **18**, Jinadasa et al., 2014; **19**, Sicuro et al., 2012; **20**, Martín et al., 2017; **21**, Khazaali et al., 2016; **22**, Denton et al., 2006b; **23**, Denton et al., 2006c; **24**, Miramand and Bentley, 1992; **25**, Preston et al., 1972; **26**, Fuge and James, 1974; **27**, Foster, 1976; **28**, Bryan and Hummerstone, 1977; **29**, Bryan and Uysal, 1978; **30**, Melhuus et al., 1978; **31**, Bryan, 1983; **32**, Bryan et al., 1983; **33**, Langston, 1986; **34**, Söderlund et al., 1988; **35**, Söderlund et al., 1988; **36**, Benfares et al., 2015; **37**, Lupsor et al., 2009; **38**, Topcuoglu et al., 2003; **39**, Al-Masri et al., 2003; **40**, Lozano et al., 2003; **41**, Pavoni et al., 2003; **42**, Ho, 1990; **43**, Kamala-Kannan et al., 2008; **44**, Laib and Leghouchi, 2012; **45**, Muse et al., 2006; **46**, Rybak et al., 2012; **47**, Montone et al., 2001; **48**, Żbikowski et al., 2006; **49**, Conti et al., 2015; **50**, Diop et al., 2016; **51**, Knutzen and Sortland, 1982; **52**, Ali et al., 2018; **53**, Fytianos et al., 1997; **54**, Amico et al., 1979; **55**, Maroli et al., 1993; **56**, Wallenstein et al., 2009; **57**, Denton et al., 1980; **58**, Dight and Gladstone, 1993; **59**, Mallet et al., 1963; **60**, Ahn et al., 2002; Boucart and Mallet, 1965; **61**, De Moreno et al., 1997; **62**, Noel-Lambot et al., 1980; **63**, Bergasa et al., 2007; **64**, Miramand and Bentley, 1992; **65**, Segar et al., 1971; **66**, Preston et al., 1972; **67**, Dutton et al., 1973; **68**, Navrot et al., 1974; **69**, Stenner and Nickless, 1975; **70**, Bryan et al., 1977; **71**, Bryan and Hummerstone, 1977; **72**, Lande, 1977; **73**, Storelli and Marcotrigiano, 2005; **74**, Bachelot et al., 2012; **75**, Fent et al., 2010; **76**, Brooks and Rumsby, 1965; **77**, Mondon et al., 2001; **78**, Diop et al., 2016; **79**, Soclo et al., 2008; **80**, Batista et al., 2013; **81**, Gentric et al., 2016; **82**, Tornero and d’Alcala, 2014; **83**, Klosterhaus et al., 2013; **84**, Bueno et al., 2013; **85**, McEneff et al., 2014; **86**, Groz et al., 2014; **87**, Sánchez-Avila et al., 2011; **88**, Boucart and Mallet, 1965; **89**, Mallet et al., 1963; **90**, Tolosa et al., 2005; **91**, Perugini et al., 2007; **92**, Ramelow, 1985; **93**, Perez et al., 2003; **94**, Webster et al., 2018; **95**, Ali et al., 2018; **96**, Shiber and Shatila, 1978; **97**, Klumpp and Peterson, 1979; **98**, DiSalvo et al., 1976; **99**, Sun et al., 2016; **100**, Ratkevicius et al., 2003; **101**, Xu et al., 2015; **102**, Zhu et al., 2018b; **103**, Zhu et al., 2018a; **104**, Dodder et al., 2014; **105**, Maruya et al., 2014; **106**, Barhoumi et al., 2016; **107**, Bodin et al., 2004; **108**, Rocher et al., 2006; **109**, Francioni et al., 2007; **110**, Toro et al., 2004; **111**, Waszak et al., 2019; **112**, Balcıoğlu, 2016; **113**, Balcioğlu et al., 2017; **114**, Mercogliano et al., 2016; **115**, Solaun et al., 2015; **116**, Carro et al., 2016; **117**, Salgueiro-González et al., 2016; **118**, Staniszewska et al., 2014; **119**, Li et al., 2008; **120**, Pojana et al., 2007; **121**, Isobe et al., 2007; **122**, Wang et al., 2007; **123**, Wenzel et al., 2004; **124**, Campillo et al., 2017; **125**, Cathum and Sabik, 2001; **126**, Khaled et al., 2004; **127**, Bayen et al., 2004; **128**, Castro et al., 2018; **129**, Cunha et al., 2015; **130**, Wille et al., 2011; **131**, Álvarez-Muñoz et al., 2015; **132**, Pérez et al., 2019; **133**, Koyama et al., 2004; **134**, Næs et al., 1998; **135**, Peña-Méndez et al., 1999, p.; **136**, Delgado et al., 1999; **137**, Tena and Montelongo, 1999; **138**, Bastürk et al., 1980; **139**, Rao et al., 2009; **140**, Cebrian et al., 2007; **141**, Bargagli et al., 1996; **142**, Sieben et al., 1983; **143** Negri et al., 2006.

In addition to processes previously described, irreversible negative effects may also be observed under pollution stress such as the alteration of benthic composition and abundance patterns (Guidetti et al., 2003; Nicolodi et al., 2009; Terlizzi et al., 2005, 2002). The consequences are diverse, for example, a biotic homogenization with a simplification of community structure (Amaral et al., 2018) through a decline in diversity (Borowitzka, 1972; Díez et al., 2010, 1999; Littler and Murray, 1975) and a decrease of pollution-sensitive species (e.g. perennial, stable benthic algae) (Scherner et al., 2013). In contrast, an increase of pollution/stress-tolerant opportunistic species (i.e., ephemeral algae) occurs due to their high reproductive capability, an increase of food availability (organic enrichment) and lower competition for space and food (Amaral et al., 2018; Cabral-Oliveira and Pardal, 2016; Dauer and Conner, 1980; Elías et al., 2006; Gorostiaga and Diez, 1996). A shift from algal-dominated assemblages to invertebrate-dominated assemblages may also happen (e.g. crustacean and bivalve filter-feeders) (Díez et al., 2012a; López-Gappa et al., 1993; Pinedo et al., 2007). Finally, contaminants released into the environment may also be accumulated in biological tissues or cause harmful effects such as endocrine disruption, behavioral changes, energy metabolism disturbances and genetic responses (Macdonald et al., 2003). Therefore, different responses may be observed depending on the type of analysis used and the response variables considered (Fraschetti et al., 2006). Fortunately, these anthropogenic impacts may be mitigated thanks to high dilution and mixing rates of coastal waters (Borja and Collins, 2004).

The study of environmental pollution through benthic assemblages (i.e., invertebrates and macroalgae) is considered as a powerful tool to assess environmental quality and has become of major importance due to several advantages. Indeed, benthic organisms may give precise information of deleterious effects of contaminants especially in assessing local effects (Belan, 2003; Borja et al., 2011a), they are mainly sedentary and have long lives, show marked responses to stress, play a critical role in cycling nutrients and materials, reflect both previous and present conditions to which communities have been exposed (Reish, 1987), are easy to sample even without using destructive sampling methods (Roberts et al., 1994) and have already been studied worldwide (Ar Gall and Le Duff, 2014; Becherucci et al., 2018; Borja and Dauer, 2008; de-la-Ossa-Carretero et al., 2016; Derrien-Courtel, 2010; Díez et al., 2012a; Le Gal and Derrien-Courtel, 2015; Zubikarai et al., 2014). Macroalgae are primary food chain producers and the dominant group on rocky shores (Amaral et al., 2018). Because of their sedentary nature and the sensitivity of their components, they are known to be accurate bioindicators (e.g., biochemical and physiological) of environmental changes (e.g. water quality of coastal waters for the WFD (Ar Gall et al., 2016; Borja et al., 2013a; Gorostiaga and Diez, 1996). Their assessment is fundamental because their modification can also alter the trophic structures of other communities (e.g. grazers, carnivorous, scavengers) (Airoldi et al., 2008; Scherner et al., 2013; Schramm, 1999; Viaroli et al., 2008). Macrofauna also must to be considered, as requested by the MSFD (2008/56/CE; EC, 2008). The use of mobile macrofauna as an indicator constitutes a "*snapshot in space and time*" because their community structure respond with short-term variability to environmental changes (Davidson et al., 2004; de Casamajor and Lalanne, 2016; Mieszkowska, 2015; Takada, 1999). Moreover, sessile species or slightly mobile species cannot redistribute themselves when faced with disturbances. They are thus highly sensitive and constitute the first biological compartment impacted by environmental stressors (Maughan, 2001; Mieszkowska, 2015; Murray et al., 2006; Roberts et al., 1998). So, dispersion patterns of sessile macrofauna constitute more precise descriptors of population dynamics (e.g. recruitment and mortality), community structure, individual performance (e.g. physiology, morphology and behavior changes) in response to environmental changes (Mieszkowska, 2015).

Over the last decades, large investigations and survey methods have been developed to study benthic communities of intertidal rocky shores (e.g. Huguenin et al., 2018; Le Hir and Hily, 2005; Vinagre et al., 2016b, 2016a; Wells et al., 2007; Zhao et al., 2016) in different contexts such as global climate change prospects (Barange, 2003; Thompson et al., 2002) or ecological status assessment of water bodies (e.g., WFD) (Borja et al., 2013a; Guinda et al., 2014). In addition, effects of sewage discharges have been studied on different environmental compartments (e.g. sediments, water body, trophic web, benthic and pelagic communities) (Bothner et al., 2002; Echavarri-Erasun et al., 2007; Mearns et al., 2015) and their impact on benthic communities have been widely documented in the intertidal zone (e.g. Becherucci et al., 2016; Bishop et al., 2002; Cabral-Oliveira et al., 2014; Cabral-Oliveira and Pardal, 2016; Díez et al., 2013; Guinda et al., 2014; Huguenin et al., 2019; Liu et al., 2007; O'Connor, 2013; Vinagre et al., 2016b). However, most studies are focused either on macroalgae or macrofauna assemblages independently (Anderlini and Wear, 1992; Cabral-Oliveira et al., 2014; Díez et al., 1999; Souza et al., 2013) and rarely together (Bishop et al., 2002; Echavarri-Erasun et al., 2007; Littler and Murray, 1975; López-Gappa and Tablado, 1990; O'Connor, 2013; Terlizzi et al., 2002; Vinagre et al., 2016a). Some research also described their impact on subtidal rocky and soft bottoms but, similar to the intertidal zone, they were often carried out on either macroalgae or macrofauna assemblages (de-la-Ossa-Carretero et al., 2016; Díez et al., 2014; Elías et al., 2005; Fraschetti et al., 2006; Souza et al., 2016, 2013; Stark et al., 2016) but rarely together, especially in rocky habitats (Terlizzi et al., 2002; Underwood, 1996; Vinagre et al., 2016a; Zubikarai et al., 2014).

2. Regulatory context

Ecosystems functioning and European waters are impacted by a wide range of human activities, which usually act at the same time (European Environment Agency, 2018a). Water policy and associated monitoring programs aim to ensure good water quality for both human needs and the environment (European Environment Agency, 2018a). Indeed, environment protection, user protection and the reduction of pollution, by means of effective and coherent water policy, is a major issue around the word. Major Directives, Conventions and French laws are summarized below in chronological order (**Fig. 8**). In addition, the two main European Directives (**Table 3**) implemented to assess, protect and manage the health of coastal and marine environments (Water Framework Directive - WFD and Marine Strategy Framework Directive - MSFD) and the European Directive about Urban Waste Water Treatment are developed thereafter.

Table 3: Summary of Water Framework Directive (WFD) and Marine Strategy Framework Directive (MSFD) features (inspired from CESER Nouvelle-Aquitaine, 2017).

	Water Framework Directive (WFD)	Marine Strategy Framework Directive (MSFD)
Creation	October 2000	June 2008
Due date	2015	2021
Study are	River basin districts	Marine sub-regions
Consultative body	Basin committee	Maritime coastline council
Planning document	SDAGE	PAMM

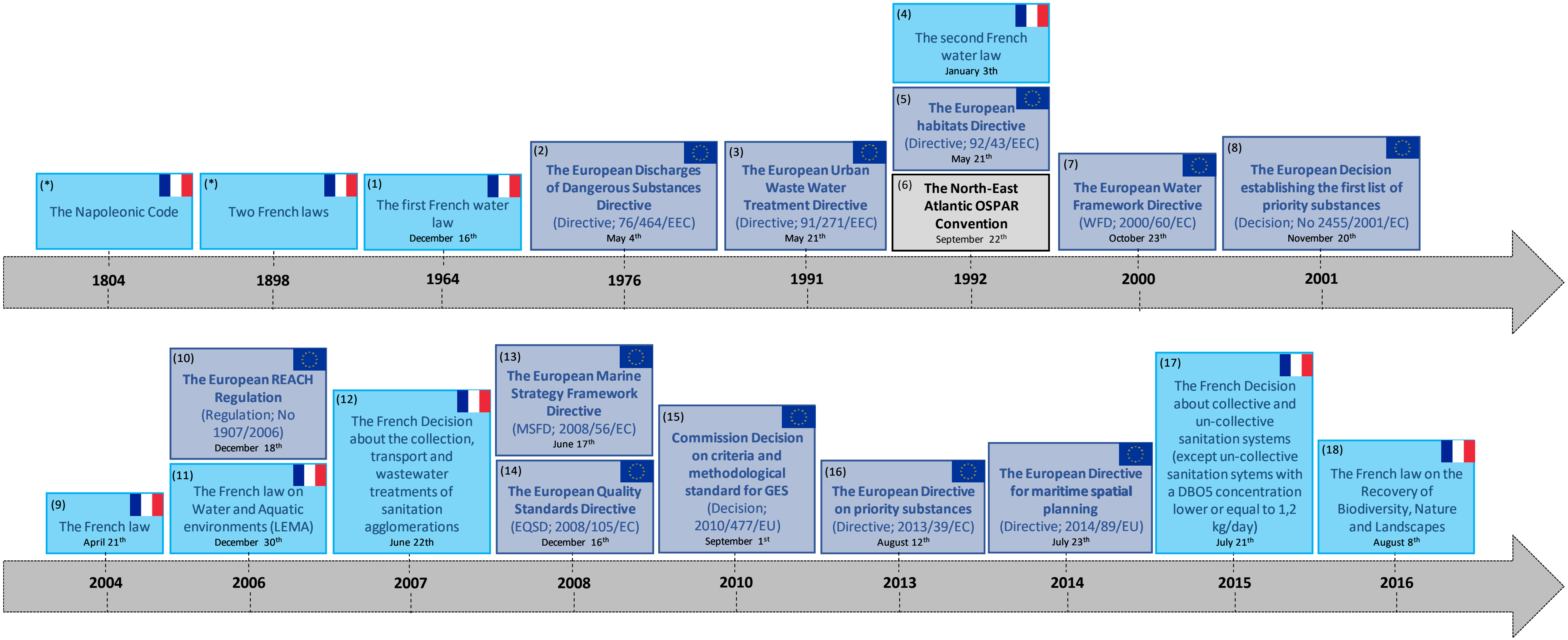
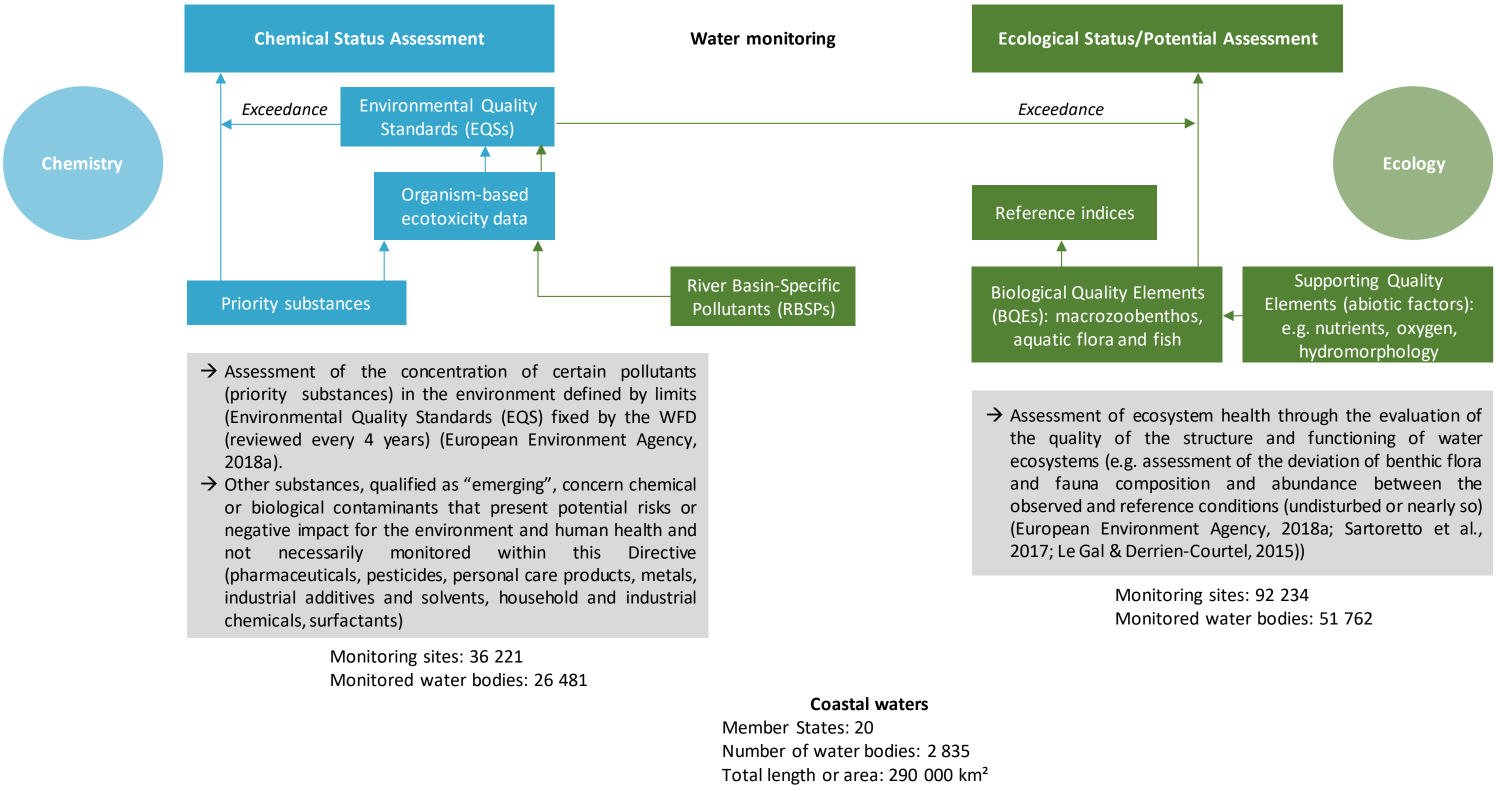


Fig. 8: Chronology of major Conventions, European Directives and French laws about water, aquatic environment and chemical substances impacting them. Details of each of those key dates are presented in Annex 1.

2.1 The Water Framework Directive (WFD; 2000/60/EC)

This European Directive was created in October 23th 2000 to standardize policies and implement a framework for the assessment, management, protection and improvement of the quality of water resources and aquatic environment at the European scale (EC, 2000; European Environment Agency, 2018a). Member States needed to evaluate (through the assessment of the chemical and ecological status of surface and groundwater (**Fig. 9**), take measures to improve (i.e. meeting certain standards for ecology, chemistry and quantity of waters) and reduce pressures on water resources (e.g. hydromorphological pressures which may cause damages on morphology and hydrology of water bodies) (European Environment Agency, 2018a). In France, the WFD was established at the fourteen river basin district scale (nine in France, gathered in six areas governed by the six water Agencies and five in French Overseas Territories). The district concerned by this study is the “Adour-Garonne”.

The GEQ of European surface waters (rivers, lakes, transitional waters - estuaries and coastal waters) and groundwater had to be then reached or maintained by Member States by 2020 (initially fixed in 2015). “Good” quality is considered as such when only slight changes are detected compared to those that would be expected under undisturbed conditions (i.e. under low human impact) (European Environment Agency, 2018a). Until now, around 40% of surface waters are in good ecological status or potential, and only 38% are in good chemical status (European Environment Agency, 2018a).



→ Assessment of the concentration of certain pollutants (priority substances) in the environment defined by limits (Environmental Quality Standards (EQS) fixed by the WFD (reviewed every 4 years) (European Environment Agency, 2018a).

→ Other substances, qualified as “emerging”, concern chemical or biological contaminants that present potential risks or negative impact for the environment and human health and not necessarily monitored within this Directive (pharmaceuticals, pesticides, personal care products, metals, industrial additives and solvents, household and industrial chemicals, surfactants)

Monitoring sites: 36 221
Monitored water bodies: 26 481

→ Assessment of ecosystem health through the evaluation of the quality of the structure and functioning of water ecosystems (e.g. assessment of the deviation of benthic flora and fauna composition and abundance between the observed and reference conditions (undisturbed or nearly so) (European Environment Agency, 2018a; Sartoretto et al., 2017; Le Gal & Derrien-Courtel, 2015))

Monitoring sites: 92 234
Monitored water bodies: 51 762

Coastal waters
Member States: 20
Number of water bodies: 2 835
Total length or area: 290 000 km²

Fig. 9: Existing approach to the assessment of chemical and ecological status under the WFD (according to European Environment Agency, 2018 and European Environment Agency, 2018b).

This Directive also established provisions for a list of priority substances. In 2001, the European Decision (Decision; No 2455/2001/EC) amended the latter Directive and established the first list of 33 priority substances or groups of substances, identified as action priorities at the Community level. Among them, some were identified as “priority hazardous substances” and others as “priority substances”. The aim was to stop or remove their discharge, emission and loss within 20 years (EC, 2001). In 2008, a successor of the WFD, the European Quality Standards Directive (EQSD; 2008/105/CE), amended previous Directives and fixed Environmental Quality Standards (EQS) for these 33 substances and 8 other pollutants (EC, 2008b). Finally, a second successor WFD, the European Directive on priority substances of 2013 (Directive; 2013/39/EC), modified it and added 12 additional priority substances (EC, 2013). In this context and with the aim of preventing and reducing water pollution, pollutant concentrations found in the environment are compared to an EQS (i.e. a concentration of a pollutant or a group of pollutants in water, sediment or biota that has not to be exceeded). These standards, established following a European methodology (Technical Guidance for Deriving EQS) and revised every four years, are used to assess the chemical status (**Fig. 9**). An extract of the last Directive with the whole priority substances list associated to EQS is available in **Annex 2**.

2.2 The European Marine Strategy Framework Directive (MSFD; 2008/56/EC)

The European Marine Strategy Framework Directive has been enacted in June **2008** (EC, 2008a). It constitutes an extension of the WFD to all marine ecosystems (at local to national to regional seas scales) (O’Connor, 2013). The aim is to achieve/maintain/gain a healthy and productive state (sustainably manage human activities at all scales), called the Good Ecological Quality (GEQ) (GES, GES, (Borja et al., 2013a), of the European marine waters by 2021. The MSFD proposed 11 environmental qualitative descriptors (as biological diversity, invasive species, eutrophication, etc.) to determine the environmental status (Borja et al., 2011a; Danovaro et al., 2016; Patrício et al., 2016) (**Table 4**).

Table 4: Qualitative descriptors needed to assess the environmental status, within the MSFD adapted from Borja et al., 2011a; The references indicate the reports published by each descriptor Task Group).

N°	Qualitative descriptors	Description	References	French organisations
1	**Biological diversity**	Biological diversity is maintained and the quality and occurrence of habitats and the distribution and abundance of species are in line with prevailing physiographic, geographic and climatic conditions	Cochrane et al. (2010)	MNHN / AFB
2	**Invasive species**	Non-indigenous species introduced by human activities are at levels that do not adversely alter the ecosystems	Olenin et al. (2010)	MNHN / AFB
3	**Exploited species**	Populations of exploited fish and shellfish are within safe biological limits, exhibiting a population age and size distribution indicative of a healthy stock	Piet et al. (2010)	IFREMER
4	**Food webs**	All elements of the marine food webs occur at normal abundance and diversity and levels capable of ensuring the long-term abundance of the species	Rogers et al. (2010)	CNRS – INEE
5	**Eutrophication**	Human-induced eutrophication is minimised, especially adverse effects	Ferreira et al. (2010)	IFREMER
6	**Seafloor integrity**	Seafloor integrity is at a level that ensures that the structure and functions of the ecosystems are safeguarded and benthic ecosystems are not adversely affected	Rice et al. (2010)	BRGM
7	**Hydrographical conditions**	Permanent alteration of hydrographical conditions does not adversely affect marine ecosystems		SHOM
8	**Contaminants in the environment**	Concentrations of contaminants are at levels not giving rise to pollution effects	Law et al. (2010)	IFREMER
9	**Contaminants in seafood**	Contaminants in fish and other seafood for human consumption do not exceed levels established by legislation or other standards	Swartenbroux et al. (2010)	ANSES
10	**Marine litter**	Properties and quantities of marine litter do not cause harm to the coastal and marine environment	Galgani et al. (2010)	IFREMER
11	**Introduction of energy**	Introduction of energy, including underwater noise, is at levels that do not adversely affect the marine environment	Tasker et al. (2010)	SHOM / IFREMER

This Directive incites European Member States to take suitable measures to manage human activities and decrease their impact. It is carried out by the development of Plans of Action of the Marine environment (PAMM) and is adapted to each marine sub-region to take into account their specificities (Patrício et al., 2016). In France, there are four marine sub-regions including the Bay of Biscay (**Fig. 10**). Assessment and monitoring programs carried out under the MSFD must meet several requirements such as: (i) the coordination of monitoring between EU Member States, (ii) compatibility of monitoring with the EU WFD, Habitats Directive (92/43/EEC; EEC, 1992), Birds Directive (2009/147/EC; EC, 2009), and international agreements, and (iii) the incorporation of physical, chemical and biological components in monitoring (Patrício et al., 2016).

However, this Directive emphasized significant inadequacies (Berg et al., 2015; Heiskanen et al., 2016; Queirós et al., 2016; Teixeira et al., 2014) in particular on Basque Country's biocenosis (southern sub-region of the Bay of Biscay) (Borja et al., 2011; Derrien-Courtel and Le Gal, 2011), on responses of biological indicators to various pressures and on the integration of fauna in assessment studies to better understand the environment functionality (Queirós et al., 2016; Teixeira et al., 2014). Furthermore, both European Directives (WFD and MSFD) (**Table 3**) concern overlapping common marine areas. Consequently, they have to take into account various common features (e.g. physical, chemical, geomorphologic) and then to elaborate adapted and consistent tools and documents.

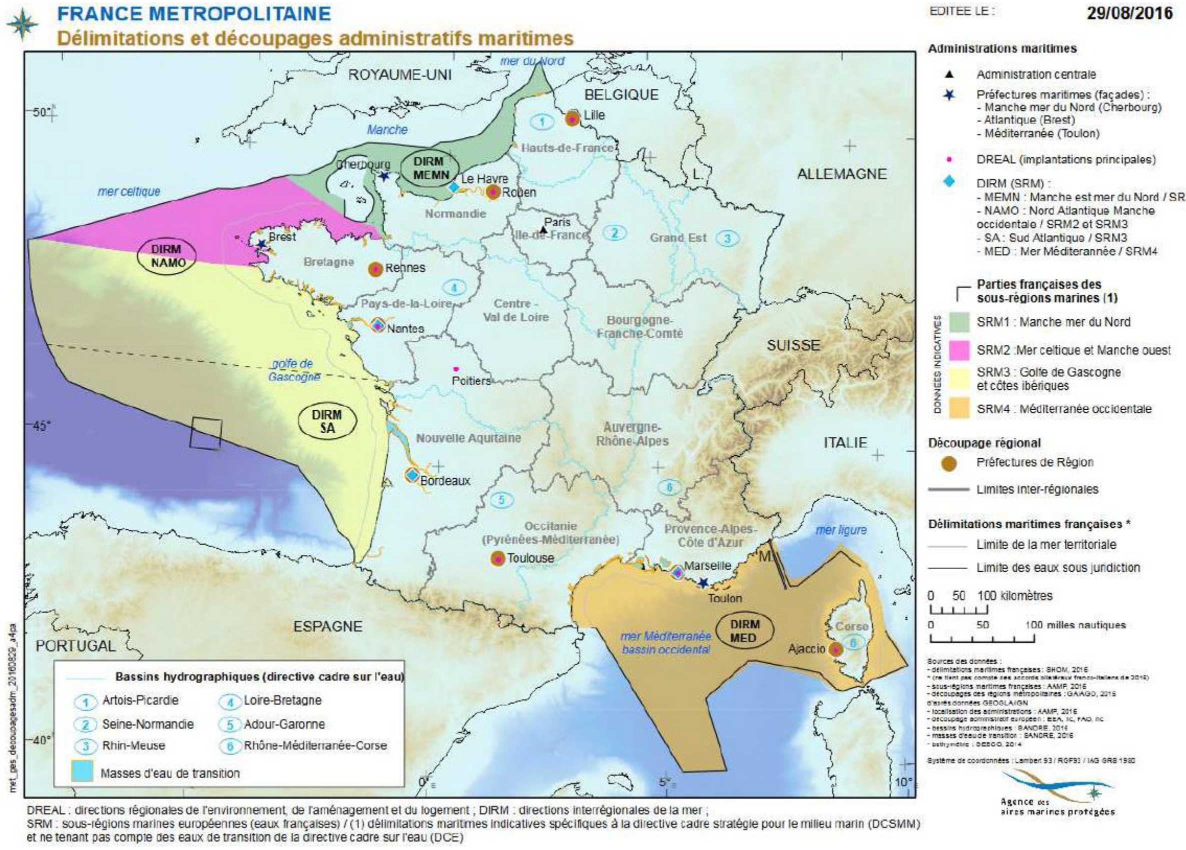


Fig. 10: The perimeter of the four French marine sub-regions of the MSFD

2.3 The European Directive for maritime spatial planning (2014/89/EU)

The European Directive of July 2014 has been enacted to establishe a framework for maritime spatial planning (i.e. of marine activities and integrated management of European coastal zones) (EC, 2014). Member States have thus to ensure the coordination of human activities and habits at sea to attain different goals such as ecological, economic and social. They have to develop by 2021 a maritime spatial planning which identify (current and future) spatial and temporal distribution of relevant activities and usages. This Directive was translated by all Member States at the National scale (e.g. in France, through one "Document Stratégique de Façade (DSF)" for each coastline which represent a tool for implementation). The establishment of this Directive has several advantages such as the reduction of conflicts between sectors, the creation of synergies between activities, the encouragement of investements, the increase of cross-boder cooperation between European countries and the proctection of the environment.

2.4 The European Urban Waste Water Treatment Directive (Directive; 91/271/EEC) and the 2007 and 2015 French Decisions

Sanitation aims to protect human health and the environment from hazards caused by rainwater and sewage discharges (www.assainissement.developpement-durable.gouv.fr). National regulations are now tightly supervised at the European scale, especially through the Urban Waste Water Treatment Directive from May 21th 1991 which set minimal European requirements for collective sanitation of household wastewaters. It imposed the collect and treatment of urban wastewaters of all Member states (of towns and cities with a population equivalent of more than 15 000 inhabitants) prior to their discharge into the environment. It also ensures that total quantities of toxic, persistent or bioaccumulative substances of WWTP sludge must be subject to authorization and progressively reduced (EEC, 1991). This Directive is transposed into the French law through the General Code of the Territorial Authorities and the Decision of June 22th 2007 concerning the collection, transport and wastewater treatments of city sanitation (Decision, 2007). It includes all technical prescriptions for sanitation systems (design, dimension, exploitation, purification performance, self-monitoring, control) and concerns all collective sanitations and wastewater treatment plants as well as all un-collective systems receiving a DBO5 concentration higher than 1.2 kg/day (www.assainissement.developpement-durable.gouv.fr). The Article 10 of the present Decision also requires that all discharges occurring in the public maritime domain must be located below the low tide level (Decision, 2007).This Decision was replaced by the one of July 21th 2015. For sanitation agglomerations with a DBO5 concentration lower than 600 kg/day, the project owner establishes a diagnosis of the sanitation system (with a frequency not exceeding 10 years) which aims to:

- Identify and localize all outfalls as well as overflow outfalls,
- Quantify frequency, annual discharges, pollutant flows discharged into the environment,
- Check connections conformity,
- Estimate quantity of clear parasite waters in the system and found their origins,
- Collect information about structural and functional conditions of the sanitation system,
- Identify rainwater management systems that limit rainwater in the collection system.

For those with a DBO5 concentration higher or equal to 600 kg/day, the project owner establishes and updates a continuous diagnosis of the sanitation system, which aims to:

- Know, continuously, the functioning and structural states of the sanitation system,
- Prevent and identify as soon as possible sanitation system dysfunctions,
- Follow and assess preventive or rectifier action efficiency,
- Manage the sanitation system to improve it continuously.

All WWTP self-monitoring features, conditions and performances are detailed in three Annexes of the present Decision (available in **Annexes 3**).

3. The purpose of the thesis research

In compliance with the European Union (EU) Directives (I.e. the WFD, the MSFD and the Habitats Directive), the good environmental status of European coastal and marine waters has to be achieved by 2021. For this purpose, monitoring programs were implemented and a certain number of priority substances are followed and regulated to be reduced or eliminated in order to achieve the good ecological and chemical status (Carey and McNamara, 2015). These Directives (especially MSFD) also emphasized significant deficiencies on how biological indicators respond to each anthropogenic pressure, in particular to WWTP and untreated urban discharges. For example, coastal areas generally combine a high biodiversity with a high anthropogenic pressure leading to include monitoring of micropollutants and their potential effects on the environment.

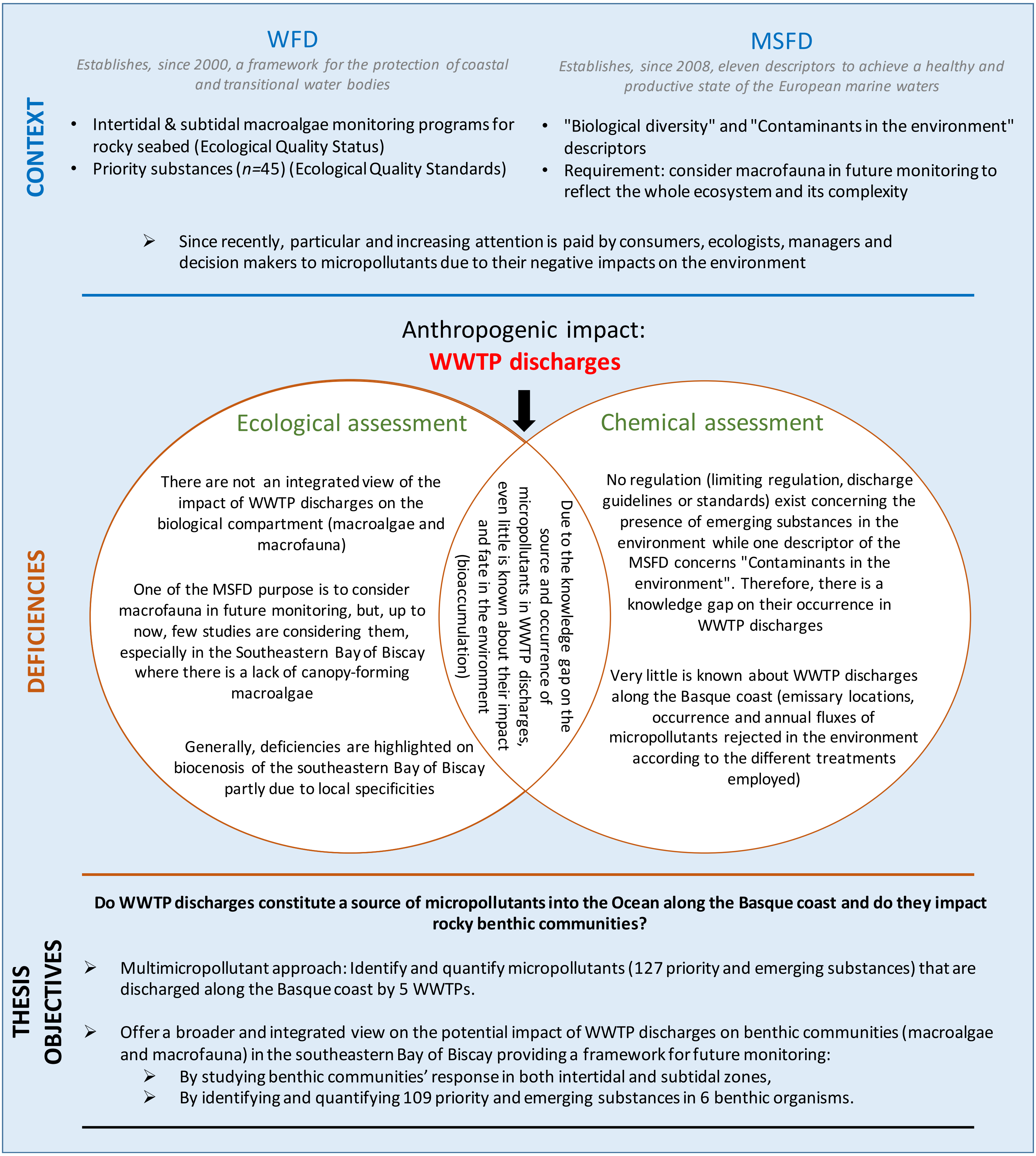
In this context, benthic communities are often used to assess marine pollution because they reflect both previous and present conditions to which communities have been exposed (Reish, 1987). Up to now, monitoring programs were mainly focused on benthic macroalgae, the dominant group on hard substrata (especially of rocky platforms) which is known to include accurate bioindicators of environmental changes (WFD; 2000/60/EC; EC, 2000). Even if macrofauna of soft bottoms and pelagic fauna are already integrated and monitored, the MSFD (2008/56/CE; EC, 2008) requested that macrofauna of rocky substrata also has to be considered because it may also respond with short-term variability to environmental changes and allow to better reflect the complexity of the whole ecosystem. This is also important for the assessment of the conservation status of habitats and species as requested by the European Habitats Directive (Directive; 92/43/EEC; EEC, 1992). Furthermore, this must be implemented at the biogeographical scale to understand and assess the ecological condition of the area including local specificities. For instance, inadequate knowledge of biocenosis were highlighted in the southeastern Bay of Biscay, especially due to particular environmental conditions inducing a lack of canopy-forming macroalgae (i.e. presence of small macroalgae specimens and few macrofauna organisms).

In addition to the ecological assessment and the chemical assessment of priority substances, there are other substances named emerging pollutants (household products, cosmetics, pharmaceuticals) which are continuously release into the environment. Indeed, most of them are not efficiently eliminated by WWTP, and even at low concentrations they may have toxic effects on aquatic organisms (e.g. endocrine disruption, behavioral changes, energy metabolism disturbances and genetic responses). Up to now, these latter substances were not considered as action priorities at the Community level and were thus not regulated within European Directives. That is why, it would be interesting to identify their occurrence in the environment to highlight the importance to follow them and maybe to include them in future monitoring.

In the present work, two main problematics are studied:

- Do WWTP discharges constitute a source of micropollutants along the Basque coast?
- How benthic communities (macrofauna and macroalgae) from rocky substrata respond to this pressure?

Therefore, the occurrence and concentrations of priority and emerging substances in WWTP discharges are firstly studied. Then, benthic communities are studied from a chemical point of view to know if these micropollutants are also detected in some benthic organisms and if the latter may constitute good bioaccumuators of micropollutants. Finally, the impact of this pressure on benthic organisms is also studied from an ecological point of view to know if benthic assemblages are impacted by WWTP discharges and if these organisms may constitute good bioindicators of this disturbance. This is achieved trough the analysis of community structure in both impacted and control intertidal and subtidal locations (**Fig. 11**). The originality of the present work is that both macrofauna and macroalgae are considered together. Indeed, up to now, most studies were focused either on macroalgae or macrofauna assemblages independently and rarely together especially in rocky habitats.



CONTEXT

WFD

Establishes, since 2000, a framework for the protection of coastal and transitional water bodies

- Intertidal & subtidal macroalgae monitoring programs for rocky seabed (Ecological Quality Status)
- Priority substances (*n*=45) (Ecological Quality Standards)

MSFD

Establishes, since 2008, eleven descriptors to achieve a healthy and productive state of the European marine waters

- "Biological diversity" and "Contaminants in the environment" descriptors
- Requirement: consider macrofauna in future monitoring to reflect the whole ecosystem and its complexity

➢ Since recently, particular and increasing attention is paid by consumers, ecologists, managers and decision makers to micropollutants due to their negative impacts on the environment

DEFICIENCIES

Anthropogenic impact:

WWTP discharges

Ecological assessment

There are not an integrated view of the impact of WWTP discharges on the biological compartment (macroalgae and macrofauna)

One of the MSFD purpose is to consider macrofauna in future monitoring, but, up to now, few studies are considering them, especially in the Southeastern Bay of Biscay where there is a lack of canopy-forming macroalgae

Generally, deficiencies are highlighted on biocenosis of the southeastern Bay of Biscay partly due to local specificities

Due to the knowledge gap on the source and occurrence of micropollutants in WWTP discharges, even little is known about their impact and fate in the environment (bioaccumulation)

Chemical assessment

No regulation (limiting regulation, discharge guidelines or standards) exist concerning the presence of emerging substances in the environment while one descriptor of the MSFD concerns "Contaminants in the environment". Therefore, there is a knowledge gap on their occurrence in WWTP discharges

Very little is known about WWTP discharges along the Basque coast (emissary locations, occurrence and annual fluxes of micropollutants rejected in the environment according to the different treatments employed)

THESIS OBJECTIVES

Do WWTP discharges constitute a source of micropollutants into the Ocean along the Basque coast and do they impact rocky benthic communities?

➢ Multimicropollutant approach: Identify and quantify micropollutants (127 priority and emerging substances) that are discharged along the Basque coast by 5 WWTPs.

➢ Offer a broader and integrated view on the potential impact of WWTP discharges on benthic communities (macroalgae and macrofauna) in the southeastern Bay of Biscay providing a framework for future monitoring:
  - ➢ By studying benthic communities' response in both intertidal and subtidal zones,
  - ➢ By identifying and quantifying 109 priority and emerging substances in 6 benthic organisms.

Fig. 11: Summary of deficiencies highlighted within the current context and of present thesis objectives

Thesis outline

The present manuscript is structured into 6 sections:

- A **first chapter - Introduction** (I) which presents the environmental and regulatory frameworks,
- A **second chapter - Methodology** (II) which presents the specific context of the thesis and means implemented to achieve the objectives,
- **3 chapters** (III, IV and V) articulated around 3 published/submitted articles (summarized below) and,
- A **final chapter** (VI) about the main findings highlighted in previous chapters, remarks, prospects and improvements.

In Chapter III, concentrations of priority and emerging micropollutants in wastewater discharges and in six benthic organisms were studied. A review was also included to highlight which concentrations were already reported in wastewater treatment plant effluents and benthic organisms.

Huguenin L., Deborde J., Lalanne Y., de Casamajor M-N., Gorostiaga J-M., Monperrus M. (-) **Release in coastal environment of priority and emerging pollutants from WWTP effluents and their contribution to the contamination of benthic organisms.**

In Chapter IV and V, response of benthic communities (macroalgae and macrofauna) to wastewater treatment plant discharges was studied in both intertidal and subtidal zones. The ecological quality of studied locations was also assessed using current European Directives indices.

Huguenin L, Lalanne Y., de Casamajor M-N., Gorostiaga J-M., Quintano E., Salerno M., Monperrus M. (2019) **Impact of wastewater treatment plant discharges on macroalgae and macrofauna assemblages of the intertidal rocky shore in the southeastern Bay of Biscay.** *Continental Shelf Research, 191, 34-49* (https://doi.org/10.1016/j.csr.2019.04.014).

Huguenin L, Lalanne Y., de Casamajor M-N., Gorostiaga J-M., Quintano E., Monperrus M. (-) **Do wastewater discharge drive rocky subtidal community shifts? A case study.** *Will be submitted to Marine Pollution Bulletin.*

In the Annex, another rocky habitat (boulder fields) was studied to assess the possibility to monitoring macrofauna communities in this type of habitat to meet European Directives requirements.

Huguenin L., Lalanne Y., Bru N., Lissardy M., D'Amico F., Monperrus M., de Casamajor M-N. (2018) **Identifying benthic macrofaunal assemblages and indicator taxa of intertidal boulder fields in the Bay of Biscay (northern Basque coast). A framework for future monitoring.** *Regional Studies in Marine Science, 20, 13-22* (http://doi.org/10.1016/j.rsma.2018.03.012).

Chapter II - Methodology:

Specific context of the thesis and means implemented to achieve the objectives

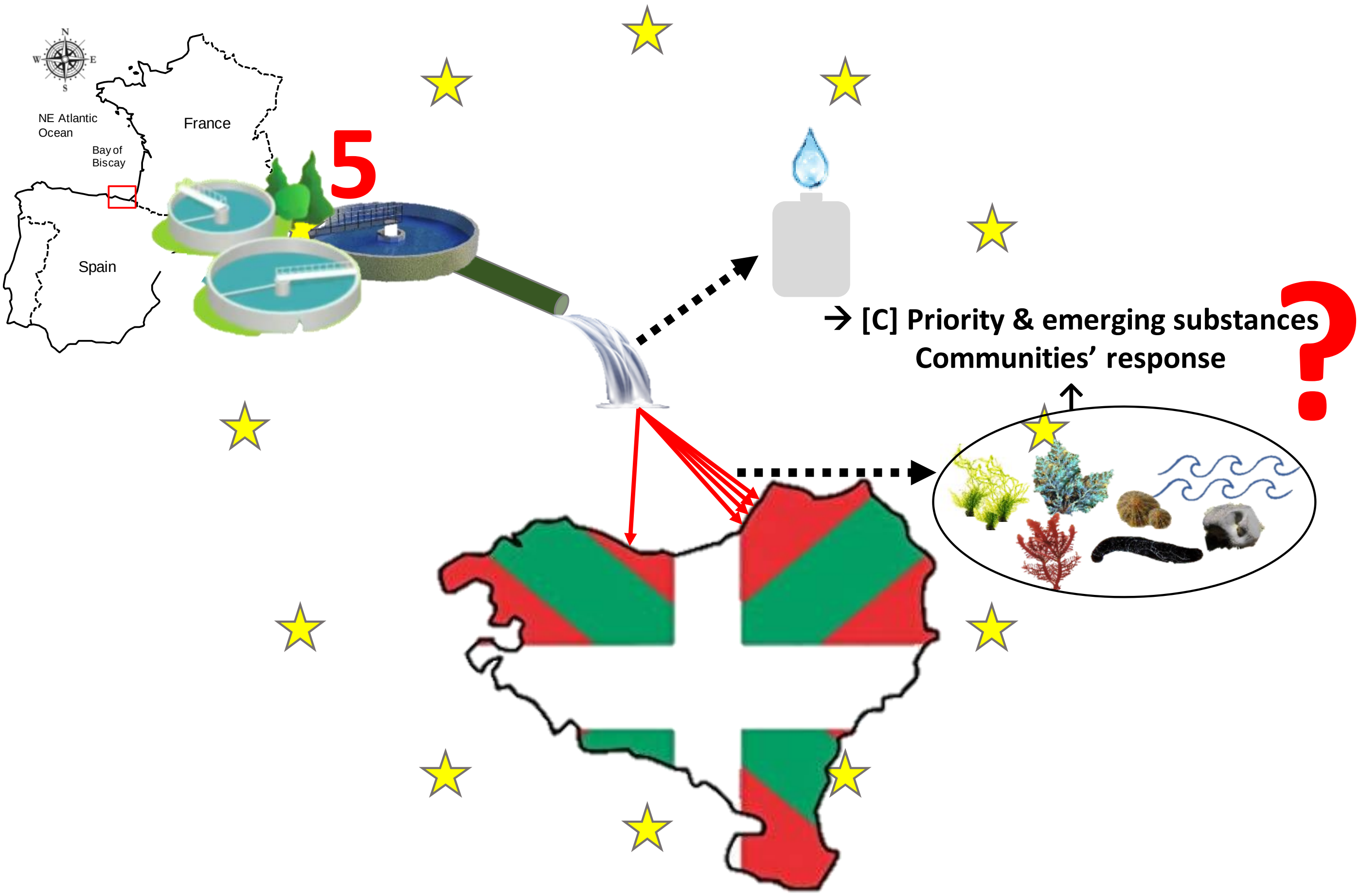


Fig. 1: Graphical abstract of the Chapter II

Chapter structure:

1. Study area: the Basque coast
2. Sampling processes and analytical methods

1. Study area: the Basque coast

1.1 Environmental context

The gulf Bay of Biscay is located in the northeastern Atlantic Ocean bathing their waters the coasts of the NW of France (Point Penmarc'h in Brittany is its northernmost limit) and northern Spain (Cape Ortegal in Galicia is its westernmost limit). At the southeastern area of the gulf the Aquitanian coast (the old region) occupies 270 km of the coastline (Le Treut, 2013). Further south, in the inner part of the bay and after a long sandy shoreline, is the Basque coast (**Fig. 2**). It is located between the Adour river (France) and Kobaron (Spain) over a length of 200 km (Borja and Collins, 2004). The French Basque coastline is about 50 km long whereas the Spanish Basque coastline is about 150 km long.

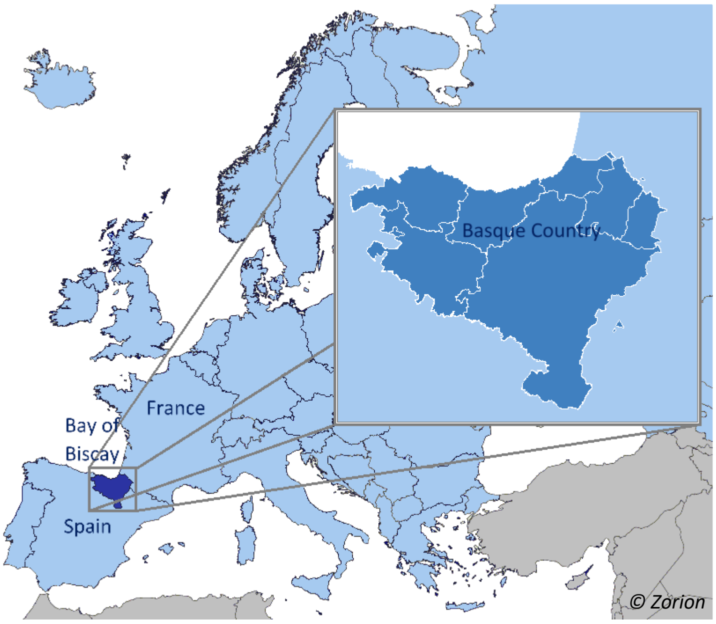


Fig. 2: Location of the Basque Country

1.1.1 The geomorphology

In this geographical area, the ocean meets the Pyrenees and the mountains plunge into the depths of the Bay of Biscay, resulting in a rocky and jagged coastline. The Basque coast is thus characterized by geomorphology organized alternatively by cliffs, rocky shores (platforms and boulder fields) and semi-enclosed bays with sandy beaches appearing in some places, namely next to small rivers and estuaries. In Spain, around 90% of the coastal zone is made up of rocky substrata (Borja and Collins, 2004). In France, only 30% of the coastline is rocky (Chust et al., 2009). One of the main characteristic of the Basque coast are sedimentary geological formations, named "flysch" and dating back over fifty million years. From Biarritz to Bilbao, this marine terrigenous sediment is constituted by a succession of hard sandstone layers (Calcium carbonate, $CaCO_3$) and softer "shale" layers (a mix of limestone and clay)

(Berger et al., 2015). Depending their orientation (perpendicular or parallel), they are subjected to more or less wave action and erosion (Berger et al., 2015).

1.1.2 Climatic conditions

Due to its orientation (N and NW) and the proximity to Pyrenees which are an obstacle for oceanic disturbances, the Basque coast is subject to moderate climatic conditions (sub-oceanic (MétéoFrance data in Peter-Borie et al., 2009) influenced by "*the Gulf Stream and the atmospheric westerlies*" (Borja and Collins, 2004). Even if its location, in the southeastern Bay of Biscay, protects it from strong disturbances and ensures a mild and wet climate (Peter-Borie et al., 2009), it is still exposed very energetic wave actions and N, NW dominant winds (Abadie et al., 2005; Bajjouk et al., 2015). Rainfall (with over 1 500 mm of precipitation) is unevenly distributed over the year and the annual mean temperatures are above 10°C (Borja and Collins, 2004). As such, there is high freshwater inputs to the ocean and river systems are usually perennial (Winckel et al., 2004).

1.1.3 Hydrography

The river system is relatively dense with 173 km of rivers spread out over the municipalities of the French Basque coast (Peter-Borie et al., 2009). The Basque coastline is thus crisscrossed by a set of estuaries, "*differentiated by the size of the basin and by other hydrological, morphological and dynamic features*" (Borja and Collins, 2004). They are typically shallow and filled with sandy-clay soils (Peter-Borie et al., 2009). From the north to the south, rivers in France are the: Adour (Bayonne), Uhabia (Bidart), La Nivelle (Saint-Jean-de-Luz), Untxin (Saint-Jean-de-Luz), Bidassoa (Hendaye), and in Spain: Oiartzun (Pasaia), Urumea (San Sebastian), Orioko Itsasadarra (Orio), Inurritza (Zarautz), Urola (Zumaia), Deba (Deba), Artibai Ibaia (Ondarroa), Lea Ibaia (Lekeitio), Oka (Urdaibai), Bakioko ibaia (Bakio), Burton (Plentzia), Nervion (Bilbao), Barbadun (Pobeña) (Augris et al., 2009; Borja and Collins, 2004).

1.1.4 Tidal Conditions

The south of the Bay of Biscay is characterized by mesotidal conditions, with a range between 1.85 and 3.85 meters (according to water depth), and semidiurnal tides with a period of approximately 12 hours (Augris et al., 2009; Borja and Collins, 2004). This means that there are two high and two low tides per day.

1.1.5 The Rocky Basque Coast

The rocky Basque coast, considered as marine protected area in compliance with the OSPAR convention (Natura 2000 site of the EEC; 1992 named "FR7200813 - Rocky Basque coast and offshore extension"), is part of the "Basque coast" water body (FRFC11) according to the WFD classification.

All features of the water body during the present study are detailed in **Annex 4**.

1.2 Wastewater treatment plants along the Basque coast

The coastal zone represents 12% of the total surface of the Basque country although it holds 60% of the population and 33% of industrial activities (Borja and Collins, 2004). Until the end of 20th century, the Basque coast was impacted by huge untreated urban or industrial wastewater input, due to the lack of wastewater treatment plants. This was aggravated by urban sprawl along the coast and summer overcrowding (Bernard, 2012; Borja and Collins, 2004; Cearreta et al., 2004; Chust et al., 2009; Le Treut, 2013a). To deal with this problem, many outfalls were built to reject urban sewage effluents off the coasts (Augier, 2014). Moreover, since 1991, European regulations were established to impose all member states to treat urban wastewater prior to its discharge into the environment (Barreales-Suárez et al., 2018; EEC, 1991). Since then, a number of WWTP were built to treat and reject urban effluents (e.g. more than 20 000 in France). Along the French Basque coast, six direct WWTP exist (from the north to the south): Biarritz (69 673 p.e.), Bidart (19 238 p.e.), Guéthary (10 000 p.e.), Saint-Jean-de-Luz (Erromardie) (55 000 p.e.), Urrugne (40 000 p.e.) and Hendaye (45 000 p.e.) (**Fig. 3**) (www.assainissement.developpement-durable.gouv.fr). The first two discharge over soft substrata, contrary to the four others which discharge over hard substrata, constituted mainly by rocky stable platforms. The context is similar along the Spanish Basque coast where more outfalls exist (from the East to the West): Irun-Hondarribia (116 581 p.e.), Donostia-San Sebastián (553 000 p.e.), Zarautz-Orio (38 500 p.e.), Getaria (4 440 p.e.), Zumaia (17 000 p.e), Deba (7 000 p.e.) and Mutriku (6 962 p.e.) on the Gipuzkoa coast, Ondarroa (27 500 p.e.), Lekeitio (20 854 p.e.), Ea (2 228 p.e.), Bermeo (41 000 p.e.), Bakio (8 645 p.e.) and Gorliz (31 399 p.e.) on the Bizkaia coast (**Fig. 3**) (uwwtd.eu). In addition to these WWTPs, a number of other WWTPs exist in the inner part of rivers or estuaries which may present, for some of them, much higher p.e.

In the present work, only four French WWTPs and one Spanish one were studied due to their discharges over hard substrata. General WWTP information and features were summarized in the below table (**Table 1**).

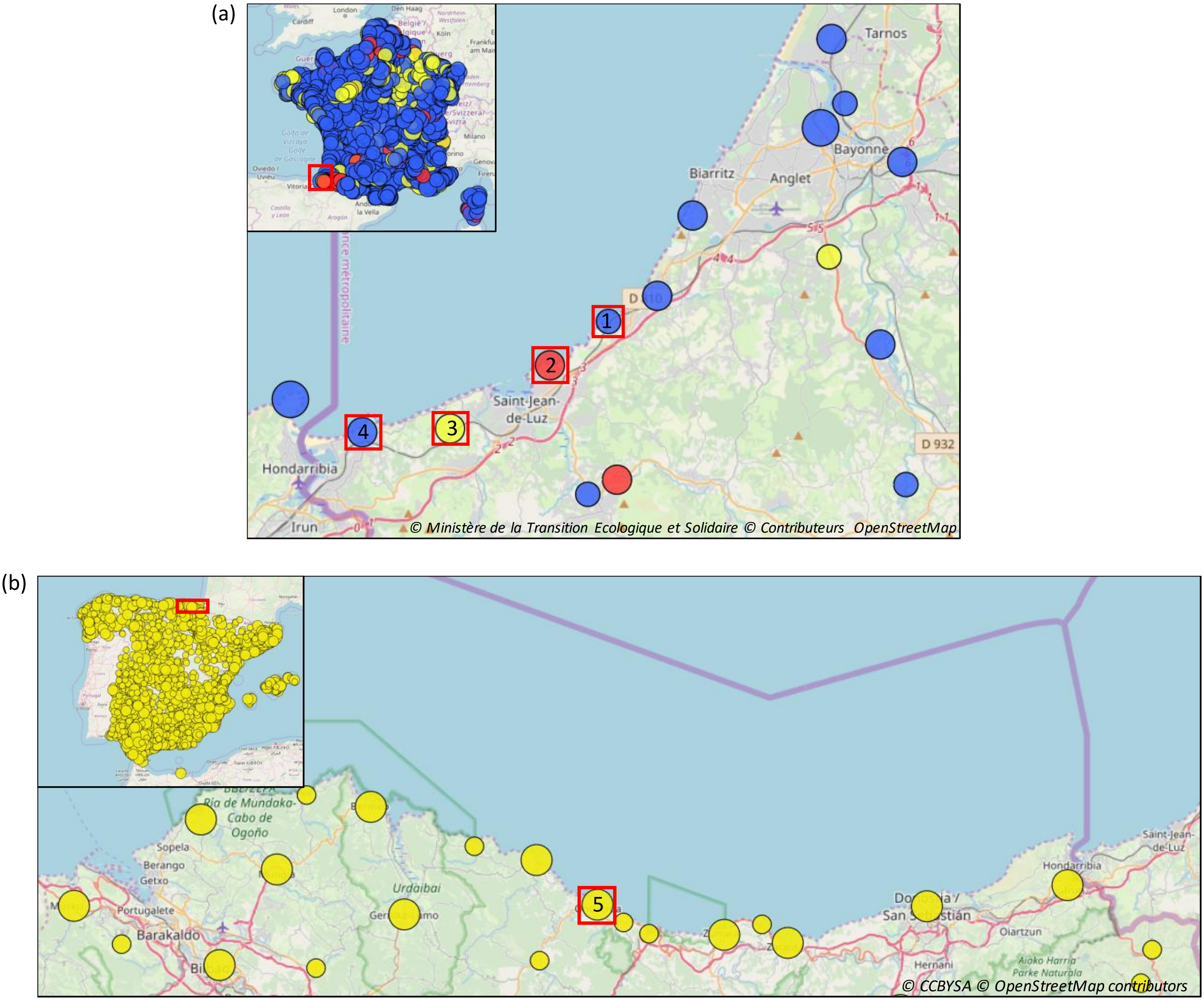


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Fig. 3: Wastewater treatment plants (WWTPs) in both French (a) and Spanish study areas (b). Significance codes and colors: Red boxes corresponds to WWTPs concerned by the present work. For French area, different size circles correspond urban wastewater agglomerations of less than 2 000 p.e., from 2 000 to 10 000, from 10 000 to 100 000, from 100 000 to 1 000 000 or more than 1 000 000 p.e. Blue circles correspond to WWTPs which are compliant in terms of equipment and performance, yellow circles to WWTPs compliant in terms of equipment but not of performance and red circle to non-compliant WWTPs (http://assainissement.developpement-durable.gouv.fr/); For Spanish area, different size circles correspond to urban wastewater agglomerations of 2 000, 10 000 or more than 10 000 population equivalent (p.e.) (https://uwwtd.eu/Spain/content/home-page).

Table 1: Features of French and Spanish studied WWTPs

City **Country**	**1** **France**	**2** **France**	**3** **France**	**4** **France**	**5** **Spain**
Manager	SUEZ	SUEZ	SUEZ	SUEZ	ACCIONA
WWTP coordinates	43° 25' 20.807" N 1° 37' 4.007" W	43° 24' 15.905" N 1° 39' 2.427" W	43° 22' 40.526" N 1° 42' 25.743" W	43° 22' 37.445" N 1° 45' 24.803" W	43° 19' 53.988" N 2° 25' 44.044" W
Population (INSEE) *(Bilan annuel sur le système d'assainissement année 2018)* *(Nacional de Estadística, 2018)* **(www.insee.fr, 2015)*	1 347	14 561	*9 674 6 447	17 006 1 243	8 397 1 247
In service date ; Rehabilitation *(Manuel autosurveillance du systeme d'assainissement, 2013)*	1995 ; 2004	1984 ; 2003	2009 ; 2014	1980 ; 1992 ; 2013	*Unknown*
Sewer system *(Manuel autosurveillance du systeme d'assainissement, 2013)*	**Separated**	**Combined**	**Separated**	**Combined**	*Unknown*
Size of the system (km ; % separated; % combined) *(Bilan annuel sur le système d'assainissement année 2018)*	97.7 % 2.3 %	87 73 % 27 %	87 99 % 1 %	106 1.1% 98.9%	*Unknown*
Eq/inhab. (equivalent inhabitant) *(Bilan annuel sur le système d'assainissement année 2018)* (www.acciona-agua.com, 2019)	**10 000**	**55 000**	**40 000**	**45 000**	**27 500**
Number of stormwater overflows *(Manuel autosurveillance du systeme d'assainissement, 2013)*	**0**	**19**	**8**	**17**	*Unknown*
Connected industries *(Manuel autosurveillance du systeme d'assainissement, 2013)* *(Gutierrez et al., 2019)*	**/**	**/**	**/**	**1 fish canning company** (BETIKO)	**4 fish canning companies** (Conservas Aguirreoa, Heisa, Marmar and Conservas Güenaga)
Main treatment *(Rapport de visite courante de l'Aturosurveillance MATEMA 64, 2017)* *(www.services.eaufrance.fr, 2018)*	**Activated sludge** **Membrane bio-reactor** **UV**	**Activated sludge** **extended aeration** **(low laod)**	**Biofiltration** **(rotating biological contactor)**	**Activated sludge** **extended aeration** **(medium load)**	**Biological reactor** **UV desinfection**
Nominal flow (m^3/day) (dry weather; rainy weather) *(Bilan annuel sur le système d'assainissement année 2018)* (www.acciona-agua.com, 2019)	1 600 2 000	8 500 10 450	7 000 21 600	7 200 /	**5 930**
Mean of total annual volumes at the entrance from 2012 to 2018 (m^3) **(Total annual volumes at the entrance in 2017; 2018)** *(Bilan annuel sur le système d'assainissement année 2018)*	410 652 (396 979; 418 468)	1 946 522 (1 880 120 ; 2 111 040)	1 809 849 (1 680 700; 1885 250)	1 611 333 (1 407 108; 1592 115)	*Unknown*
Mean of total annual volumes at the outlet from 2012 to 2018 (m^3) **(Total annual volumes at the outlet in 2017 ; 2018)** *(Bilan annuel sur le système d'assainissement année 2018)*	344 459 (325 539; 366 143)	1 856 498 (1 803 110; 2004 040)	1 794 944 (1 789 980; 1961 710)	1 516 737 (1 455 321; 1642 139)	*Unknown*
Mean of total annual overflows from 2012 to 2018 (m^3) **(Mean of total annual overflows in 2017 ; 2018)** *(Bilan annuel sur le système d'assainissement année 2018)*	39 303 (12 474; 29 853)	698 863 (730 329; 635 797)	143 836 (75 925; 102 716)	53 223 (23 527; 28 739)	*Unknown*
Water treatment steps *(Rapport de visite courante de l'Aturosurveillance MATEMA 64, 2017)* *(Manuel autosurveillance du systeme d'assainissement, 2013)* *(http://www.agglo-sudpaysbasque.fr, 2016)*	- **Pre-treatment**: 2 rotary sieves (1mm mesh) - Anoxic basin (400 m^3) - **Secondary/biological treatment:** Biosep™ basin (i.e. bio-reactor with immerged membranes associating a biological aerobic treatment (activated sludge) and a filtration with immerged membranes) (1 010 m^3) - **Tertiary treatment (in summer)**: UV reactor	- **Pre-treatment**: Screen (6 mm mesh) + grit chamber/grease removal (150 m^3) - **Secondary treatment:** 2 aeration basins (activated sludge) (2 x 1 582 m^3) + 2 clarifiers (i.e. decanters) (2 x 1 450 m^3)	- Stripping (i.e. degassing chamber) - **Pre-treatment**: 2 screens (6 mm mesh) + 2 Biolix™ grit chamber/grease removal (i.e. aerobic biological treatment for grease and oils removal) - **Primary/physico-chemical treatment**: Multiflo™ lamella settlers (i.e. clarifier produicing thick and highly concentrated mud) - **Secondary treatment**: Biostyr™ biofiltration (i.e. 4 aerated biological filters for simultaneous nitrification/denitrification	- **Pre-treatment**: Automatic screen (1 mm mesh) + aerated grit chamber/grease removal (100 m^3) - **Secondary/biological treatment:** Aeration basin (activated sludge) (2 300 m^3) + clarifier (i.e. decanter) (1 800 m^3)	- **Pre-treatment**: Sieve + sand/silt trapping – grease trapping in 2 lines - **Primary/physico-chemical treatment**: Lamellar settler - **Secondary/biological treatment:** Biological reactor, secondary settling in 2 units - **Tertiary treatment:** UV desinfection
Equipment compliant ; performance compliant *(http://assainissement.developpement-durable.gouv.fr/)*	Yes ; Yes	No ; /	Yes ; No	Yes ; Yes	*Unknown*
Emissary lenght (m) *(Manuel autosurveillance du systeme d'assainissement, 2013)*	240	800-1000 m	*Unknown*	500	*Unknown*
Receiving environment	**Subtidal zone**	**Intertidal zone**	**Subtidal zone**	**Intertidal zone**	**Intertidal zone**
Emissary depth (m)	1	/	3	/	/
Outfall coordinates	43° 25' 22.415" N 1° 37' 14.711" W	43° 24' 19.382" N 1° 39' 5.899" W	43° 23' 20.796" N 1° 42' 45.839" W	43° 23' 2.228" N 1° 45' 7.632" W	43° 19' 54.449" N 2° 25' 40.702" W

2. Sampling processes and analytical methods

Different methods were achieved according to matrices (wastewater, seawater, biota) and sampling locations (WWTP or offshore, intertidal or subtidal zones) (**Table 2**).

(1) **Wastewaters** from WWTP were sampled just before their release into the environment. The aim was to characterize the nature of each discharge by identifying and quantifying organic micropollutants, metals, organomercury and other major elements.

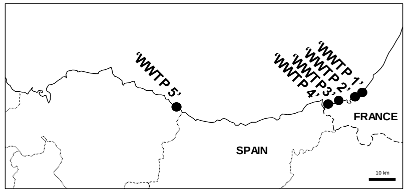
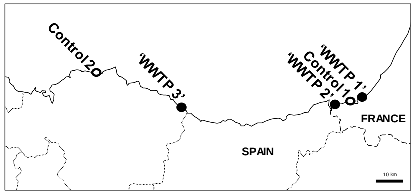
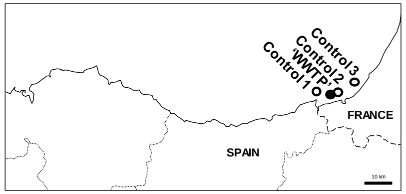
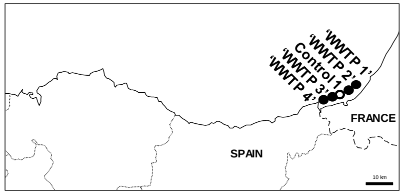
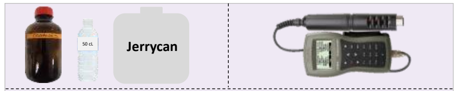
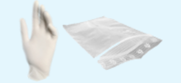
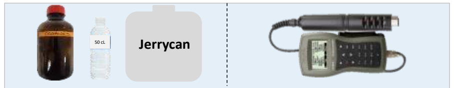
(2) and (4) **Benthic organisms** were sampled in the intertidal and subtidal zones in both impacted and control locations. The aim was to study the impact of discharges on benthic communities through the analysis of micropollutants concentration and assemblages structure.

(3) **Seawater** was sampled offshore in front of each sampling location. The aim was to characterize the receiving environment by doing physic-chemical measures and analysing major elements (**Annex 4**).

Zones, types of samples, locations, sampling frequencies, pre-analytical and analytical methods and objectives of each sampling type were summarized in the below diagram (**Table 2**).

Thereafter, chemical and statistical analysis were detailed in different sections through schematic layouts in order to facilitate understanding.

Table 2: Sampling processes and analytical methods used during the present study for each matrix. For confidentiality reasons, each sampling and WWTP were assigned to a code (not homogeneous between chapters).

Zones	Continental / Coastal	Intertidal	Subtidal	Off-shore
Samples	**Wastewater from wastewater treatment plant discharges**	**Benthic communities (macroalgae and macrofauna)**	**Benthic communities (macroalgae and macrofauna)**	**Sea water**
Problematics	Do WWTP discharges constitute a source of micropollutants into the Ocean along the Basque coast and do they impact rocky benthic communities?			
	Which micropollutants (and in what amount) are rejected into the Ocean through WWTPs?	Are rocky benthic communities affected by WWTP discharges? Are current WFD indices enough sensitive to study such a pressure? Could benthic communities constitute a good bioindicator/accumulator of such a pressure?		
Locations				
Periods	**2017** August **2018** May July December	**2017** March ↔ July **2018** March ↔ July	**2017** May ↔ June **2018** May ↔ June	**2017** **2018** March May July September October
Sampling	 Nb of operators: **2** Nb of sampling days: **11**	 **180** Quadrats 0.1m² • Upper midlittoral • Lower midlittoral Nb of operators: **3-4** Nb of sampling days: **17** (2017) + **12** (2018)	 **80** Quadrats 0.25m² • Upper infralittoral • Upper circalittoral Nb of operators + pilot + safety: **4** Nb of sampling days: **10** (2017) + **7** (2018) Nb of dives: **20** (2017) + **14** (2018)	 Nb of operators + pilot: **3** Nb of sampling days: **5**
Analyses	**Major elements:** → Particulate organic carbon (POC), Dissolved organic carbon (DOC), $\delta^{13}C$, $\delta^{15}N$, Nutrients (PO_4, NO_3, NO_2, $SiOH_4$, NH_4) **Organics (by GC-MS, LC-MS):** → **16** PAHs, **11** PCBs, **18** OCPs, **10** musks, **6** sunscreens, **6** APs, **48** pharmaceuticals **Metals (by ICP-MS):** → **11**: V, Cr, Ni, Cu, As, Mo, Ag, Cd, Sn, Sb, Pb **Organomercury compounds (by GC-ICP-MS):** MMHg, IHg **Physico-chemical measures:** → pH, O_2 saturation, conductivity, salinity, temperature	**Organics (by GC-MS, LC-MS):** → **16** PAHs, **11** PCBs, **18** OCPs, **10** musks, **6** sunscreens, **6** pharmaceuticals **Statistical analyses (using R® software, Excel v7®, PRIMER®):** → Assemblage structure → Diversity structure → Functional traits → Ecological quality index (WFD)	**Organics (by GC-MS, LC-MS):** → **16** PAHs, **11** PCBs, **18** OCPs, **10** musks, **6** sunscreens, **6** pharmaceuticals **Statistical analyses (using R® software, Excel v7®, PRIMER®):** → Assemblage structure → Diversity structure → Functional traits → Ecological quality indices: WFD + 2 other macrofauna indices	**Major elements:** → Particulate organic carbon (POC), Dissolved organic carbon (DOC), $\delta^{13}C$, $\delta^{15}N$, Nutrients (PO_4, NO_3, NO_2, $SiOH_4$, NH_4) **Physico-chemical measures:** → pH, O_2 saturation, conductivity, salinity, temperature
	See details in below figures and in Chapter III	**See details in Chapter IV**	**See details in Chapter V**	**See details in Chapter III**
Objectives	• Characterize each discharge • Study potential spatial and temporal variabilities • Study filtration effect on analyte concentrations • Estimate annual and daily flows	Study the impact of intertidal discharges on benthic commmunities: • Know which chemical substances are mainly detected in 3 benthic organisms • Analyze assemblage structures between impacted and control locations	Study the impact of subtidal discharges on benthic commmunities: • Know which chemical substances are mainly detected in 3 benthic organisms • Analyze assemblage structures between impacted and control locations	Characterize the recieving environment
	Study the impact of wastewater treatment plant discharges on benthic communities along the rocky Basque coast			

2.1 Chemical analyses

All sample preparations and analytical methods achieved and used during the present study to analyze micropollutant concentrations in wastewater samples are summarized in **Table 3**. In addition, those used to analyze biota samples are summarized in **Table 4**. Each method is detailed in the **Chapter III**. Moreover, a list of all analytes analyzed during this study with their features is available in **Annex 5**. In general, for:

Wastewater analysis: Specific containers (previously cleaned at the laboratory) were used to sample wastewaters for each chemical analysis. During sampling, bottles were rinsed three times with water sample before a final sample was collected. After filling them with wastewater, they were transported to the laboratory in an icebox. Then, a part of wastewater samples were filtered prior to extraction and analyses. After an extraction phase, analyses (except those for pharmaceuticals, only achieved on filtered samples) were carried out on the total fraction (dissolved + particulate) and on the filtered fraction. Nine analytical groups were selected, accounting for 127 individual substances (i.e. analytes). Six analytical methods have thus been used: one for metals (Ag, As, Cd, Cu, Cr, Mo, Ni, Pb, Sb, Sn, V), one for organomercury compounds (IHg and MMHg), one for the simultaneous analysis of PAHs (n=16), PCBs (n=11) and OCPs (n=18), one for the simultaneous analysis of musks (n=10) and sunscreens (n=6), one for APs (n=6) and one for pharmaceuticals (n=48).

Biological analysis: Similar size organisms were hand-collected by observers or scuba divers with gloves and stainless steel knifes at the outlet of each WWTP discharge (in 1-meter square zone around the outfall) and also at several control locations (i.e. without WWTP emissary and located more than 2.5 km from the impacted locations) in the intertidal and subtidal zones. Organisms were rinsed with sea water, pooled per location and placed in 23 x 15 m polyethylene sampling bags. They were then provided to the laboratory with a cold chain. Then, they were weighed and stored in a freezer (-80°C). They were lyophilized during 72h and weighed again. Dry samples were then crushed and homogenized using an agate mortar and ceramic scissors. After an extraction and a purification phases using QuEChERS biota samples were analyzed for organic micropollutants (priority and emerging ones, except APs) accounting for 109 individual substances.

Metals were analyzed by inductively coupled plasma mass spectrometer (ICP-MS), organometallics by gas chromatrograph (GC) coupled to an inductively coupled plasma mass spectrometer (ICP-MS), organics by gas chromatrograph-mass spectrometer (GC-MS) and pharmaceuticals by liquid chromatograph-tandem mass spectrometer (LC-MS-MS).

Table 3: Sampling methods and analytical methods employed according analytical groups analyzed in sampled WWTP discharges.

Substance families	**Metals** (V, Cr, Ni, Cu, As, Mo, Ag, Cd, Sn, Sb, Pb)	**Organomercury compounds** (MMHg, IHg)	**Organics** (PAHs, PCBs, pesticides, musks, sunscreens)	**Organics** (Pharmaceuticals)
Cleaning	- **HNO$_3$** 10% - **HCl** 10% - Dried in a heat chamber	- **HNO$_3$** 10% - **HCl** 10% - Dried in a heat chamber	- **Acetone** - Pyrolyzed 4 hours at 450°C	- **Acetone** - Pyrolyzed 4 hours at 450°C
Sampling	50cl polyethylene terephthalate (PET) bottle	50cl polyethylene terephthalate (PET) bottle	2L amber glass bottle	50cl polyethylene terephthalate (PET) bottle
Filtration	Polysulfone filtration system and PVDF filters (0.45 µm, 47 mm) Unfiltereted	Polysulfone filtration system and PVDF filters (0.45 µm, 47 mm) Unfiltereted	Glass filtration system and cellulose acetate membrane filters (0.45 µm, 47 mm) Unfiltereted	Glass filtration system and cellulose acetate membrane filters (0.45 µm, 47 mm)
Storage	HNO$_3$ Instra (1%) 4°C	HCl Ultrex (1%) 4°C	/	-20°C
Pre-concentration Derivation		Ethylation (NaBEt4 5%)	Solid phase extraction (PES: 1.5 mm long) NaCl 10g/L	Solid phase extraction (SPE: Cartridges (Oasis HLB 3cc))
Extraction		Extraction in Isooctane	Back extraction in Ethyl Acetate (EToAC)	Back extraction in MeOH/pure water (25/75)
Analytes	11	2	PAHs: 16 PCBs: 11 Musks: 10 Pesticides: 18 Sunscreens: 6 Alkylphenols: 5	48
Analysis	**ICP-MS**	**GC-ICP-MS**	**GC-MS**	**LC-MS-MS**

Table 4: Sampling methods and analytical methods employed according analytical groups analyzed in benthic organisms sampled at the outlet of each WWTP and in control locations.

Substance families	**Organics** (PAHs, PCBs, pesticides, musks, sunscreens)	**Organics** (Pharmaceuticals)
Sampling	Polythene bags -80°C	Polythene bags -80°C
Preparation	Lyophilization during 72h Crushing with an agate mortar and ceramic scissors	Lyophilization during 72h Crushing with an agate mortar and ceramic scissors
Storage	Glass vials -80°C	
Extraction	QuEChERS extraction (5982-7650) (4g MgSO4; 1g NaCl; 1g Na3Citrate; 0.5g Na2HCitrate)	QuEChERS extraction (5982-7650) (4g MgSO4; 1g NaCl; 1g Na3Citrate; 0.5g Na2HCitrate)
Purification	QuEChERS purification (55982-5158CH) (400 mg PSA, 400 mg C18EC, 1200 mg MgSO4) Evaporation with compressed air till 1 mL	Ethanol (MeOH)/pure water (95/5) Dry evaporation
Analytes	PAHs: 16 PCBs: 11 Musks: 10 Pesticides: 18 Sunscreens: 6	48
Analysis	**GC-MS**	**LC-MS-MS**

2.2 Statistical analyses

All statistical analyses achieved in this study are detailed below (**Table 5**). Further information concerning each of those analyses are available in the following sections (chapters or articles).

Table 5: Summary of data collected during this study and associated analyses (descriptive, statistical and ecological quality analyses).

Zones	**Continental / Coastal**	**Intertidal**		**Subtidal**		**Off-shore**
Samples	**Wastewater from wastewater treatment plant discharges**	**Benthic communities (macroalgae and macrofauna)**		**Benthic communities (macroalgae and macrofauna)**		**Sea water**
Type of data	Analyte concentrations per WWTP, per month and per filtered and unfiltered sample: **Quantitative continuous data**	Analyte concentrations per location and organism: **Quantitative continuous data**	Abundance of benthic organisms per location (impacted and controls): **Quantitative discrete data**	Analyte concentrations per location and organism: **Quantitative continuous data**	Abundance of benthic organisms per location (impacted and controls): **Quantitative discrete data**	Major elements concentrations and physico-chemical measures per off-shore location: **Quantitative continuous data**
Transformation	/	/	**Standardisation to analyze macrofauna and macroalgae simultaneously:** each couting value, for one taxon, was divided by the maximum reached by this taxon	/	/	/
Descriptive analyses	To visualize differences in total concentrations between sampling locations (WWTPs) and sampling campaigns (per analytical group): - **Balloonplot** (R® software; "*ggplot2*" package) To visualize differences in mean concentrations between sampling locations (WWTPs) and between unfiltered and filtered samples (per analytical group): - **Box plot** (R® software; "*ggplot2*" package)	To know which analytical group(s) are mainly detected in which organism: - **Principal component analysis** (PCA) (R® software; "*ade4*" package) To visualize differences in total analytical group concentrations between locations (per organism): - **Histograms** (Excel v7®)	To explore the structure of benthic assemblages among locations (impacted and control) and within locations (between sites) - **Non-metric multi-dimensional scaling** (nMDS) (R® software; "vegan" package) - **Hierarchical cluster analysis** (HCA) (R® software; "stats" package) To visualize differences in mean abundance, total and mean taxonomic richness between locations and sites: - **Histograms** (Excel v7®)	To know which analytical group(s) are mainly detected in which organism: - **Principal component analysis** (PCA) (R® software; "*ade4*" package) To visualize differences in total analytical group concentrations between locations (per organism): - **Histograms** (Excel v7®)	To visualize differences in benthic assemblages, morpho-functional and ecological groups and in phylum between impacted and control locations: - **Hierarchical cluster analysis** (HCA) (R® software; "stats" package)	To visualize differences in mean concentrations and physico-chemical measures between off-shore samples and WWTP discharges samples: - **Hierarchical cluster analysis** (HCA) (R® software; "stats" package)
Statistical tests	To test differences between sampling campaigns and locations: - ***two-way* ANOVA** with 2 fixed factors without interaction (R® software; "*car*" package) - **Tuckey HSD post hoc test** (R® software; "*stats*" package) To test differences between filtered and unfiltered samples (paired samples): - **Wilcoxon signed rank test** (R® software)	To test differences between sampling locations: - **Kruskall Wallis-test** (R® software; "*stats*" package) - **Pairwise permutational multivariate analysis of variance** (R® software; "*RVAideMemoire*" package) To identify the important contributors to differences: - **SIMilarity PERcentage** (SIMPER) analysis	To test variations of species composition and abundance: - **Permutational Multivariate Analysis of Variance** (PERMANOVA) (R® software; "*vegan*" package)	To test differences between sampling locations: - **Kruskall Wallis-test** (R® software; "*stats*" package)	To test variations of species composition and abundance: - **Permutational Multivariate Analysis of Variance** (PRIMER V. 6. PERMANOVA) To identify the important contributors to differences: - **SIMilarity PERcentage** (SIMPER) analysis	/
Ecological quality analyses	/	/	To assess the sensitivity of the Quality index to a such pressure: - **The "intertidal macroalgae" WFD protocol** (calculated for each location)	/	To study the ecological quality of studied locations based on macroalgae species: - **The Ecological Quality Status (EQS)** for the upper infralittoral To study the ecological quality of studied locations based on macrofauna species: - **The Biotic Coefficient (BC)** from the AMBI index - **The Taxa Sensitivity (TS)** from the INDEX-COR index	/

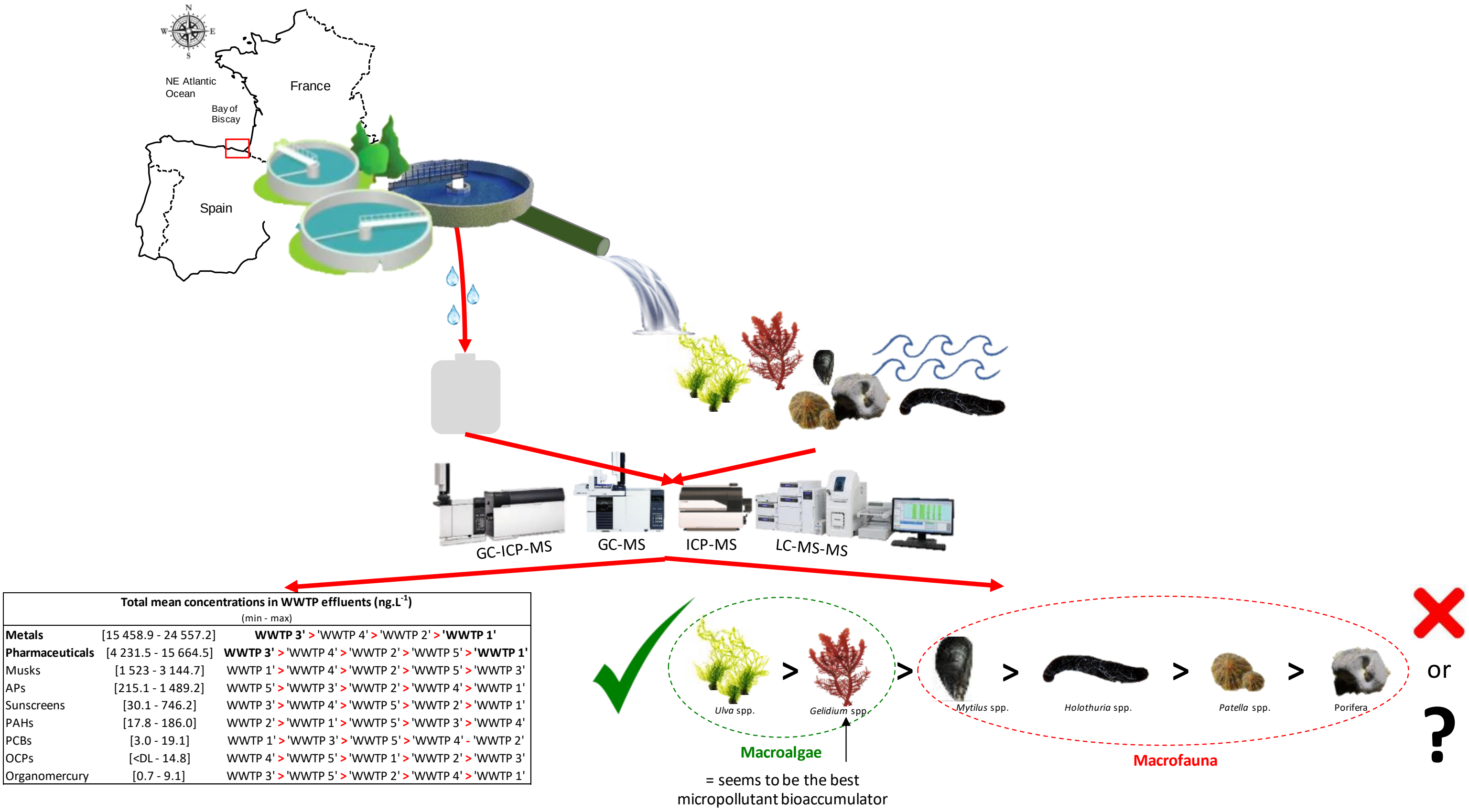
The following chapters (III to V) are structured according to the main parts identified in **Table 2** ("Continental/coastal" – purple column, "Intertidal" – green column and "Subtidal" – light blue column):

- The **Chapter III** deals with concentrations of priority and emerging micropollutants in wastewater discharges and in six benthic organisms,
- The **Chapter IV** deals with the response of benthic communities (macroalgae and macrofauna) to wastewater treatment plant discharges in the intertidal zone,
- The **Chapter V** deals with the response of benthic communities (macroalgae and macrofauna) to wastewater treatment plant discharges in the subtidal zone.

For **confidentiality** reasons, each sampling **location and WWTP** were **assigned to different codes** which are not homogeneous throughout the manuscript.

Chapter III:

Release in coastal environment of priority and emerging micropollutants from WWTP effluents and their contribution to the contamination of benthic organisms



Total mean concentrations in WWTP effluents (ng.L^{-1}) (min - max)		
Metals	[15 458.9 - 24 557.2]	**WWTP 3'** > 'WWTP 4' > 'WWTP 2' > **'WWTP 1'**
Pharmaceuticals	[4 231.5 - 15 664.5]	**WWTP 3'** > 'WWTP 4' > 'WWTP 2' > 'WWTP 5' > **'WWTP 1'**
Musks	[1 523 - 3 144.7]	WWTP 1' > 'WWTP 4' > 'WWTP 2' > 'WWTP 5' > 'WWTP 3'
APs	[215.1 - 1 489.2]	WWTP 5' > 'WWTP 3' > 'WWTP 2' > 'WWTP 4' > 'WWTP 1'
Sunscreens	[30.1 - 746.2]	WWTP 3' > 'WWTP 4' > 'WWTP 5' > 'WWTP 2' > 'WWTP 1'
PAHs	[17.8 - 186.0]	WWTP 2' > 'WWTP 1' > 'WWTP 5' > 'WWTP 3' > 'WWTP 4'
PCBs	[3.0 - 19.1]	WWTP 1' > 'WWTP 3' > 'WWTP 5' > 'WWTP 4' - 'WWTP 2'
OCPs	[<DL - 14.8]	WWTP 4' > 'WWTP 5' > 'WWTP 1' > 'WWTP 2' > 'WWTP 3'
Organomercury	[0.7 - 9.1]	WWTP 3' > 'WWTP 5' > 'WWTP 2' > 'WWTP 4' > 'WWTP 1'

Fig. 1: Graphical abstract of the Chapter III

Chapter structure:

- Huguenin L., Deborde J., Lalanne Y., de Casamajor M-N., Gorostiaga J-M., Monperrus M. (-) **Release in coastal environment of priority and emerging pollutants from WWTP effluents and their contribution to the contamination of benthic organisms.**

Release in coastal environment of priority and emerging micropollutants from WWTP effluents and their contribution to the contamination of benthic organisms

Over the last few decades, the occurrence of micropollutants in the aquatic environment has become an environmental issue of major concern throughout the world (Luo et al., 2014). They may be natural or synthetic and represent active minerals or organic substances potentially toxic, persistent and bioaccumulative in the environment in low concentrations (in the range of ng/L to µg/L). Their introduction into the aquatic environment at any point of their life cycle and in different steps of the water cycle (European Environment Agency, 2018a; Le Treut, 2013b) is a result of their continuous and/or uncontrolled release (e.g. via WWTPs) and their resistance to degradation (Cruzeiro et al., 2016; Radović et al., 2015). Indeed, WWTPs were not specifically designed to eliminate this type of pollutants (Cavalheiro et al., 2017; Sousa et al., 2019) and thus, a large range of micropollutants are found in WWTP discharges and then in the environment (Dimpe and Nomngongo, 2016; Loos et al., 2013; Mailler et al., 2016, 2015; Miege et al., 2009; Verlicchi et al., 2012).

Marine organisms are known to have the ability to accumulate contaminants present in the water (Arias et al., 2009). Even if biotic samples constitute complex matrices to analyze (demand extensive extraction and clean-up procedures), they can be used to give the fraction of the bioavailable environmental pollution (Gust et al., 2010), to monitor the level of sea water pollution (Borja et al., 2004; Claisse, 1991), evaluate its transfer (bioavailability and bioaccumulation) and inform of associated effects (Bergé and Vulliet, 2015).

Even if particular and increasing attention is paid by consumers, ecologists, managers and decision makers to micropollutants due to their negative impacts on the environment (Carlsson et al., 2006; Sousa et al., 2019) to date, only a small number of micropollutants are monitored and regulated within the framework of European Directives (EC, 2013, 2000) and no regulation (e.g. limiting regulation, discharge guidelines or standards) exists concerning the presence of emerging micropollutants in the environment (Bolong et al., 2009; Luo et al., 2014).

<u>Problematic:</u>

- ➔ Do WWTP discharges constitute a source of micropollutants in the Ocean along the Basque coast?
- ➔ Which micropollutants (and in what amount) are rejected into the Ocean through WWTPs?

This chapter/article deals with the study of the occurrence of 127 priority and emerging substances (metals, organomercury and organic compounds belonging to several analytical groups such as PAHs, PCBs, musks, sunscreens, alkylphenols and pharmaceuticals) in 5 WWTP effluents and of 109 substances in six specific benthic organisms (*Ulva* spp., *Gelidium* spp., Porifera, *Holothuria* spp., *Mytilus* spp. and *Patella* spp.) sampled close to emissaries in the southeastern Bay of Biscay.

Release in coastal environment of priority and emerging pollutants from WWTP effluents and their contribution to the contamination of benthic organisms

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Abstract

The aim of this study was to measure the concentrations of 127 priority and emerging substances in 5 wastewater treatment plant (WWTP) effluents and of 109 substances in 6 marine benthic organisms sampled on rocky substrata at the outlet of each emissary. The treatment plants in question, located in the southeastern Bay of Biscay, were mainly fed by municipal sources (from 10 000 to 78 217 inhabitant equivalents). Treatment processes were either activated sludge treatments associated or not to membrane filtration, or a biofiltration or a UV treatment. Even though activated sludge biological treatment and membrane filtration appeared as the most effective to remove suspended matter and associated adsorbed substances, a large amount of micropollutants were anyway released into the ocean. Indeed, among the analytes analyzed in effluents, a total of 11 metals (ranging from 13.2 to 4 884.7 ng.L^{-1}), 2 organomercury compounds (ranging from 0.2 to 2.7 ng.L^{-1}) and 98 organics (16 PAHs, 11 PCBs, 5 alkylphenols, 18 OCPs, 10 musks, 4 sunscreens and 34 pharmaceuticals ranging from 0.1 to 1 544.7 ng.L^{-1}) were detected and quantified. Spatial and temporal variabilities were associated to several factors such as rainfall, summer overcrowding, sewer system, plant capacity, treatment process and inefficiency of the current applied treatment. Among the organic substances analyzed in biota samples, a total of 51 analytes (9 PAHs, 6 PCBs, 1 OCP, 5 musks, 3 sunscreens and 27 pharmaceuticals with mean concentrations) ranging from 0.1 to 3 765.2 ng.g^{-1} were detected and quantified. Considering our results and biological and technical drawbacks of each taxa, *Gelidium* spp. was highlighted as the better bio-accumulator and -indicator species for this area. Finally, it seems important to consider the concentrations found in the present work with the aim to include in the future highlighted substances in the list because up to now no regulatory limits have been set for musks, pharmaceuticals, sunscreens and associated metabolite compounds.

Keywords: Micropollutants; Wastewater treatment plants; Sewage; Macrofauna; Macroalgae; Biota; WFD.

1. Introduction

Over the last few decades, the occurrence of micropollutants in the aquatic environment has become an environmental issue of major concern throughout the world (Luo et al., 2014). Indeed, the current worldwide production of chemical substances is estimated to 400 million tons while it has been around 1 million tons in 1930 (www.minefe.gouv.fr) and their presence in groundwaters, drinking waters, surface waters, plants and wastewaters was already proven by scientists (Janna, 2011). Micropollutants may be natural or synthetic and represent active minerals or organic substances potentially toxic, persistent and bioaccumulative in the environment in low concentrations (in the range of ng/L to µg/L) (Sousa et al., 2019). They may be pharmaceuticals, components of personal care products, steroid hormones, pesticides (OCPs), fragrances, sunscreen agents, insect repellents and many other emerging compounds (Luo et al., 2014; Trapido et al., 2014).

Their introduction into the aquatic environment at any point of their life cycle and in different steps of the water cycle (European Environment Agency, 2018a; Le Treut, 2013b) is a result of their continuous and/or uncontrolled release and their resistance to degradation (Cruzeiro et al., 2016; Radović et al., 2015). Many factors, such as compound specificity and the treatment employed in wastewater treatment plants (WWTP) influence their efficient remove. Generally, treatment plants allowed to fulfill European requirements, especially those from the Urban Waste Water Treatment Directive established in May 21th 1991. The latter fixed minimal European requirements for collective sanitation of household wastewaters and imposed on all Member states to collect and treat urban wastewaters (from human activities and industrial discharges) prior to their discharge into the environment (i.e. riverbanks, lakes and seas) (EEC, 1991). Even if, treatment plants are still considered as the most-effective technique to get rid of sewages owing to the dilution rate of the ocean (Elías et al., 2005; Islam and Tanaka, 2004; Little and Kitching, 1996)), they were not specifically designed to eliminate this type of pollutants (Cavalheiro et al., 2017; Sousa et al., 2019). Indeed, they only allow to remove coarse solids, organic matter, and to ensure the reduction of nutrient and bacteria to prevent eutrophication (Cabral-Oliveira and Pardal, 2016; Stark et al., 2016). Hydrophobic, volatile and biodegradable micropollutants may be also substantially removed but this is not the case of hydrophilic and refractory organic compounds (Clara et al., 2007; Loos et al., 2013; Mailler et al., 2015, 2014; Ruel et al., 2012). Consequently, a large range of emerging micropollutants are found in WWTP discharges and then in the environment (Dimpe and Nomngongo, 2016; Loos et al., 2013; Mailler et al., 2016, 2015; Miege et al., 2009; Verlicchi et al., 2012).

Once in the aquatic environment and depending on their type, source and level, sewage discharges may have direct or indirect effects (biological, chemical or physical) which may varies from little or no impact to major changes (Borja et al., 2011a; Del-Pilar-Ruso et al., 2010; Pastorok and Bilyard, 1985; Puente and Diaz, 2015). Consequences on benthic communities may be diverse such as a biotic homogenization (Amaral et al., 2018) through a decline in diversity (Borowitzka, 1972; Díez et al., 2010, 1999; Littler and Murray, 1975), a decline in pollution-sensitive species (Scherner et al., 2013) and an increase of pollution/stress-tolerant species (Amaral et al., 2018; Cabral-Oliveira and Pardal, 2016; Gorostiaga and Diez, 1996) which may occur on the intertidal zone (Huguenin et al., 2019) as well as on the subtidal zone (**see Chapter V**). In addition, sewage discharges can cause various (biochemically and physiologically) harmful effects on organisms: endocrine disruption, behavioral changes, energy metabolism disturbances, antibiotic resistance of microorganisms and genetic responses (Patisaul and Adewale, 2009; Vajda et al., 2011, 2008; Wilkinson et al., 2018). Indeed, marine organisms are known to have the ability to accumulate contaminants present in the water at different levels depending on feeding behaviors, trophic levels and habitats (Arias et al., 2009; de los Ríos et al., 2012). For example, macroalgae were already described as one of the most reliable organisms to study heavy metal concentration due to their rapid accumulation rate (Phillips, 1977). Marine sponges and bivalve molluscs such as mussels were also reported to accumulate these compounds and other emerging substances (De los Ríos et al., 2018, 2013; de los Ríos et al., 2012; Gentric et al., 2016) contrary to crustaceans that may have the ability to regulate them (Haynes and Johnson, 2000; Rainbow and Phillips, 1993). Therefore, marine organisms can be used to give the fraction of the bioavailable environmental pollution (Gust et al., 2010), monitoring the level of sea water pollution (Borja et al., 2004; Claisse, 1991), evaluate its transfer (bioavailability and bioaccumulation) and inform of associated effects (Bergé and Vulliet, 2015). Even if biotic samples constitute complex matrices to analyze (demand extensive extraction and clean-up procedures), the improvement of analytical methodologies, especially on the detection of low concentrations, allows a increasingly detection of chemical substances in different biological samples (Puckowski et al., 2016; Wille et al., 2011).

To date, only a small number of micropollutants are monitored and regulated within the framework of European Directives (EC, 2013, 2000) and no regulation (e.g. limiting regulation, discharge guidelines or standards) exists concerning the presence of emerging micropollutants in the environment (Bolong et al., 2009; Luo et al., 2014). Indeed, the WFD only established in 2000 provision for a list of priority substances which are continuously released into the environment and are resistant to degradation (e.g. polycyclic hydrocarbons - PAHs, alkylphenols - APs, organotins - OTs, volatile organic compounds - VOCs, OCPs and heavy metals; Belgiorno et al., 2007). In 2001, the European Decision (No 2455/2001/EC) amended the latter Directive and established the first list of 33 priority substances or groups of substances (priority or priority hazardous) identified as action priorities at the Community level with the aim to stop or remove their discharge, emission and loss within 20 years (EC, 2001).

Following the European Quality Standards Directive (the successor of the WFD, EQSD; 2008/105/CE) and the European Directive on priority substances (Directive; 2013/39/EC) Environmental Quality Standards (EQS) (EC, 2008b) and 12 additional priority substances were added (EC, 2013). Within this context and with the aim to prevent and reduce these substances, pollutant concentrations found in the environment are since compared to EQS (i.e. concentrations of pollutants or groups of pollutants in water, sediment or biota, that have not to be exceeded) to assess the chemical status. Moreover, the study of contaminant concentrations in the environment constitutes one of the 11 descriptors of the MSFD to assess the environmental status (MSFD; 2008/56/EC).

Even if particular and increasing attention is paid by consumers, ecologists, managers and decision makers to micropollutants due to their negative impacts on the environment (Carlsson et al., 2006; Sousa et al., 2019), few studies have been undertaken to assess emerging substances in WWTP discharges and in wild biota leading to a knowledge gap in the extent and route of exposure these organisms encounter (Cavalheiro et al., 2017; Miller et al., 2018). By contrast, a large number of studies already assessed metals in the latter matrices (Busetti et al., 2005; Clara et al., 2012; Culha et al., 2016; Givianrad et al., 2014; Mohammadizadeh et al., 2016; Östman et al., 2017; Singh et al., 2004) and micropollutants in marine sediments or in soft bottom communities (Azaroff et al., 2018; De los Ríos et al., 2016a; Hassan et al., 2018; Ma et al., 2017; Sun et al., 2016). This study therefore aims to evaluate the occurrence of 127 priority and emerging substances (metals, organomercury and organic compounds belonging to several analytical groups such as PAHs, PCBs, musks, sunscreens, alkylphenols and pharmaceuticals) in WWTP effluents and of 109 substances in six specific benthic organisms (*Ulva* spp., *Gelidium* spp., Porifera, *Holothuria* spp., *Mytilus* spp. and *Patella* spp.) sampled close to WWTP discharges in the southeastern Bay of Biscay.

2. Materials and Methods

2.1 Field data collection strategy

The study was conducted in the southeastern Bay of Biscay along the Basque coast. Two matrices were studied: effluents from 5 wastewater treatment plants (WWTP) and benthic organisms collected at the WWTP outlets in the intertidal and subtidal zones (**Fig. 2**). WWTPs included in this study were selected due to their outfalls were lying on rocky bottoms (**Fig. 2**). They received from ~1 600 to ~21 600 m^3/day (according to dry and rainy weathers) of raw waterwaters from the major neighboring municipalities and urban runoff for a population equivalent ranged from 10 000 to 78 217 inhabitants (**Table 1**). Treatment processes included pre-treatment and primary treatments (e.g. screening, gritting, oil/grease removal), secondary treatments (e.g. membrane bioreactor, activated sludge or biofiltration) and, for two of them, a tertiary treatment (UV treatment). General information and features of each WWTP were summarized in **Table 1**. In addition, weather conditions, flow rates, measured physico-chemical parameters and analyzed major elements were reported for each sampling campaign and WWTP in **SM 1**.

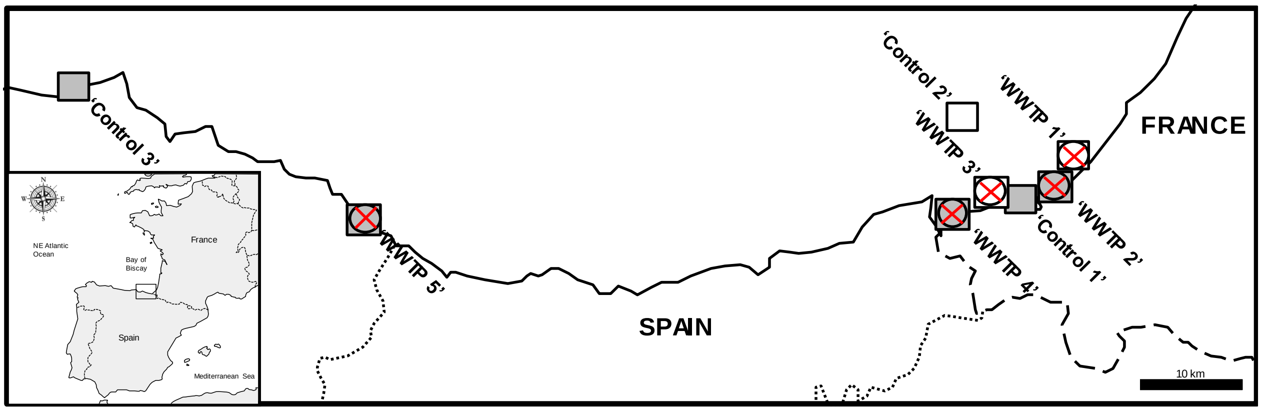


Fig. 2: Study area and sampling locations. Crossed circles correspond to WWTPs where wastewater samples were achieved. Squares correspond to locations where biota samples were collected. Those in grey are located in the intertidal zone and those in white in the subtidal zone.

Table 1: Summary of general WWTP features. All data (per WWTP and sampling month) are available in SM 1.

		'WWTP 1'	'WWTP 2'	'WWTP 3'	'WWTP 4'	'WWTP 5'
WWTP	**Sewer system** (% separated - % combined)	Separated *(97.7 - 2.3)*	Combined *(73 - 27)*	Separated *(99 - 1)*	Combined *(1.1 - 98.9)*	-
	Inhabitant equivalent	10 000	78 217	40 000	45 000	-
	Main treatment	Activated sludge Membrane reactor UV reactor (in summer)	Activated sludge extended aeration	Biofiltration	Activated sludge extended aeration	Biological reactor UV desinfection
	Emissary depth (m)	1	-	3	-	-
	Receiving environment	Subtidal zone	Intertidal zone	Subtidal zone	Intertidal zone	Intertidal zone
Flow rates	**Average of total volumes rejected between 2017 and 2018** (m^{3})	345 841	1 903 575	1 875 845	1 548 730	-
	Daily flow in entry (m^{3}.day^{-1}) *the 5 preceding days + the day after the sampling* (total - mean - min - max)	**25 605** **6 401** *(4 149 - 8 788)*	**147 150** **36 788** *(26 860 - 41 070)*	**120 360** **30 090** *(19 230 - 38 570)*	**90 923** **22 731** *(18 429 - 26 512)*	-
	Daily flow at the outlet (m^{3}.day^{-1}) *the 5 preceding days + the day after the sampling* (total - mean - min - max)	**21 498** **5 375** *(2 994 - 7 447)*	**138 500** **34 625** *(25 710 - 38 880)*	**127 380** **31 845** *(23 000 - 37 810)*	**93 440** **23 360** *(20 041 - 26 350)*	-
Physico-chemical measures/analyses	**pH** (mean - min - max)	**7.94** *(7.92 - 7.96)*	**7.08** *(6.82 - 7.60)*	**6.96** *(6.26 - 7.54)*	**7.49** *(7.20 - 7.94)*	**7.82** *(6.76 - 8.87)*
	Oxygen saturation (%) (mean - min - max)	**92.60** *(86.40 - 98.80)*	**61.85** *(47.50 - 84.10)*	**107.80** *(90.20 - 120.10)*	**22.85** *(12.00 - 32.40)*	**63.20** *(57.40 - 69.0)*
	Conductivity (mS.cm^{-1}) (mean - min - max)	**0.66** *(0.53 - 0.81)*	**0.92** *(0.54 - 1.67)*	**1.28** *(0.52 - 2.39)*	**1.54** *(0.77 - 2.19)*	**13.67** *(12.21 - 15.13)*
	Salinity (µg.L^{-1}) (mean - min - max)	**0.34** *(0.26 - 0.40)*	**0.44** *(0.26 - 0.79)*	**0.65** *(0.25 - 1.24)*	**0.78** *(0.38 - 1.12)*	**7.96** *(7.04 - 8.87)*
	Temperature (°C) (mean - min - max)	**19.87** *(16.85 - 22.50)*	**20.73** *(17.42 - 23.83)*	**20.33** *(17.12 - 23.08)*	**20.56** *(16.36 - 23.74)*	**17.80** *(16.59 - 19.00)*
	SM (mg.L^{-1}) (mean - min - max)	**1.14** *(0.49 - 3.00)*	**9.02** *(6.58 - 13.71)*	**29.12** *(17.95 - 46.38)*	**13.53** *(4.44 - 29.22)*	**11.38** *(4.94 - 17.20)*
	TC (%) (mean - min - max)	**38.28** *(5.27 - 65.23)*	**34.53** *(29.49 - 38.38)*	**32.43** *(30.95 - 33.47)*	**37.24** *(34.23 - 44.07)*	**15.17** *(12.26 - 18.50)*
	DOC (mg.L^{-1}) (mean - min - max)	**4.34** *(2.37 - 5.73)*	**6.83** *(5.90 - 7.84)*	**8.70** *(6.74 - 10.08)*	**5.51** *(5.01 - 5.84)*	**6.88** *(5.90 - 7.74)*
	POC (%) (mean - min - max)	**30.50** *(8.39 - 51.96)*	**36.83** *(31.79 - 38.93)*	**31.11** *(27.51 - 38.74)*	**34.24** *(33.29 - 36.40)*	**12.64** *(11.83 - 13.16)*

The wastewater sampling was conducted four times from August 2017 to December 2018 (August 2017, May, July and December 2018). They were collected using automatic 24-h sampling devices installed at the each WWTP which allowed to take a constant volume at variable time intervals after a certain volume of treated wastewater has passed the sampling point. Different containers were used according to envisaged analyses. For organic micropollutant analyses (PAHs, PCBs, OCPs, musks and APs), wastewaters were sampled with amber glass bottles. For pharmaceutical, metal and organomercury compound analyses, wastewaters were sampled with polyethylene terephthalate (PET) bottles. In addition to specific cleanings previously achieved in the laboratory (i.e. acetone for organics and HNO_3 10% and HCl 10% for metals and organomercury compounds), bottles were rinsed with sample three times before a final sample was collected. After filling them with wastewater, they were transported to the laboratory in an icebox.

Biota samples were collected two times (from March to July in 2017; the same in 2018) in the intertidal or subtidal zones. Six benthic organisms: *Ulva* spp., *Gelidium* spp. (mainly *G. corneum*), Porifera (mainly *Clathrina* spp. and *Pachymatisma areolata*), *Holothuria* spp. (mainly *H. tubulosa* and *H. forskalii*), *Mytilus* spp. (mainly *M. edulis* and *M. galloprovincialis*) and *Patella* spp. (mainly *P. depressa*, *P. vulgata* and *P. ulyssiponensis*), were chosen due to their presence in most of locations, their relative ease of sampling and their sufficient amount of matter. Some of them were anyway absent from some locations (intertidal *vs*. subtidal) or provided insufficient amount of matter (e.g. *Mytilus* spp.).

The primary producer *Ulva* spp., is a foliose non-corticated chlorophyte reported as opportunistic taxa along the Basque coast (de Casamajor et al., 2016). The terete corticated rhofophyte *Gelidium* spp. (*G. corneum*, *G. spinosum*, *G. pusillum*) usually grow in different environmental conditions. *G. corneum* form extensive subtidal stands at the Basque coast and it has been considered as characteristic species from the Basque coast. This species is being used to assess the ecological status of the whole water body, being considered as an indicator of good ecological status (de Casamajor and Lissardy, 2018). The *Holothuria* spp., a sea cucumber from the Echinodermata phylum, is a filter-feeding organism reported as a good sentinel for monitoring organic micropollutants due to its ability to take up these compounds through its gills and/or digestive tract (Hu et al., 2010; Jiang et al., 2015; Martín et al., 2017). The mussel, *Mytilus* spp., is a filter-feeding bibalve. It is widely used for environmental pollution monitoring in coastal waters (De los Ríos et al., 2016a; Eertman et al., 1995; Kasiotis et al., 2015). It is already known to bioaccumulate contaminants (Gielazyn et al., 2003) and to be tolerant to reduced salinity conditions (Wilson et al., 1998) as well as to a wide range of pollutants (Kasiotis et al., 2015). The gastropod, *Patella* spp., is a herbivorous mollusc already reported as a good biomonitor and as one of the sentinel organisms used to monitor marine environmental health (Goldberg, 1975; Storelli and Marcotrigiano, 2005). This may be partly due to its high aptitude to accumulate very low concentration of metals in seawater (Campanella et al., 2001; Conti et al., 2015, 2010). Finally, the Porifera phylum, is a water filter-feeder which was also already reported as metals and other emerging substances accumulator (Gentric et al., 2016).

Similar size organisms were hand-collected by observers or scuba divers with gloves and stainless steel knifes at the outlet of each WWTP discharge (in 1-meter square zone around the outfall) and also at several control locations (i.e. without WWTP discharge and located more than 2.5 km from the impacted locations) in the intertidal and subtidal zones ('Control 1', 'Control 2' and 'Control 3') (**Fig. 2**). Organisms were pooled by species and a minimum of 10 individuals for *Patella* spp., 50 for *Mytilus* spp., 2 for *Holothuria* spp. and enough algae to fill one 23 x 15 m sampling bag were sampled per location. Samples were rinsed with sea water and placed in sterile polyethylene sampling bags suitably labelled. They were then provided to the laboratory with a cold chain.

2.2 Sample preparations

Wastewater samples: Once to the laboratory, a part of wastewater samples were filtered prior to extraction and analyses using 0.45 µm/47 mm PVDF filters (for metal and organomercury analyses) and 0.45 µm/47 mm cellulose acetate membrane filters (for organic compound analyses). Nitric acid (HNO_3 Instra 1%) and Hydrochloric acid (HCl Ultrex 1%) were added to samples for metal and organomercury compound analyses, respectively. Bulk metal samples were digested at 85°C using a DigiPREP Jr block digestion system (SCP science, Canada) and according to the EPA 200.8 Method (Creed et al., 1994). Only filtered samples for organomercury compound analyses were derivatized using $NaBEt_4$ 5% and extracted using isooctane. Samples for organic compound analyses (PAHs, PCBs, OCPs, musks, sunscreens) were prepared in triplicate and were extracted using solid phase extraction (SPE) and Ethyl acetate. Samples for pharmaceutical analyses were firstly stored in a freezer at -20°C and were thereafter extracted using solid phase extraction (SPE) cartridges and 25/75 (v/v) methanol/pure water. Analyses (except those for pharmaceuticals, only achieved on filtered samples) were carried out on the total fraction (dissolved + particulate) and on the filtered fraction. Metals were not analyzed in 'WWTP 5' samples due to a too high salinity (between 7 to 8 µg/L) caused by the artisan production of canned tuna (Gutierrez et al., 2019).

Biological samples: Benthic organism samples were weighed and stored in a freezer (-80°C). They were lyophilized during 72h and weighed again. Dry samples were crushed and homogenized using an agate mortar and ceramic scissors. They were then placed in glass vials and kept at -20°C until organic analyses. Two grams (for organic substance analysis) and 200 mg samples (for pharmaceutical analysis) were extracted using QuEChERS extraction tubes containing 4 g of magnesium sulfate ($MgSO_4$), 1 g of sodium chloride (NaCl), 1 g of trisodium citrate (Na3Citrate) and 0.5 g of disodium citrate (Na2Citrate).

The purification stage was achieved using QuEChERS purification for organic analysis and 95/5 (v/v) ethanol/pure water for pharmaceuticals according to (Miossec et al., 2018; Saraiva et al., 2016).

2.3 Analytical methods

Abbreviations used throughout the article: Metals: (Ag) Silver, (As) Arsenic, (Cd) Cadmium, (Cr) Chromium, (Cu) Copper, (Mo) Molybdenum, (Ni) Nickel, (Pb) Lead, (Sb) Antimony, (Sn) Tin, (THg) Total mercury; (V) Vanadium; Organomercury compounds: (MMHg) monomethylmercury, (IHg) inorganic mercury; Organic compounds: (ADBI) Celestolide, (AHMI) Phantolide, (AHTN) Tonalide, (AP) Alkylphenol, (ATII) Traseolide, (BC) Benzylidene camphor, (BHC) Hexachlorocyclohexane, (E1) Estrone, (E2) 17-beta oestradiol, (EE2) 17-alpha ethinylestradiol, (EHMC) Ethylhexyl methoxycinnamate, (HHCB) Galaxolide, (HHCB-lactone) Galaxolidone, (PAH) Polycyclic aromatic hydrocarbon, (PCB) Polychlorinated biphenyl, (MA) Musk Ambrette, (MBC) Methylbenzylidene camphor, (MK) Musk Ketone, (MM) Musk Moskene, (MX) Musk Xylene, (NP) Nonylphenol, (NPE01) Nonylphenol monoethoxilated, (NPE02) Nonylphenol diethoxilathed, (OC) Octocrylene, (OCPs) Pesticides, (OD-BAPA) Octyl-dimethyl-PABA, (4,4’-DDD) 4,4’-Dichlorodiphenyldichloroethane, (4,4’-DDE) 4,4’-Dichlorodiphenyldichloroethylene, (4,4’-DDT) 4,4’-Dichlorodiphenyltrichloroethane, (4nOP) 4-nitro-O-phenylenediamine, (4tOP) Para-tert-octylphenol; Others: (SM) Suspended matter, (POC) Particulate organic carbon, (DOC) Dissolved organic matter.

Wastewater samples: Nine analytical groups were selected, accounting for 127 individual substances (i.e. analytes). Six analytical methods have been used: one for metals (Ag, As, Cd, Cu, Cr, Mo, Ni, Pb, Sb, Sn, V), one for organomercury compounds (IHg and MMHg), one for the simultaneous analysis of PAHs (n=16), PCBs (n=11) and OCPs (n=18), one for the simultaneous analysis of musks (n=10) and sunscreens (n=6), one for APs (n=6) and one for pharmaceuticals (n=48).

Biological samples: Contrary to wastewater samples, only organic micropollutants (priority and emerging ones, except APs) were analyzed in biota samples. Thus, 6 analytical groups were chosen (PAHs, PCBs, musks, OCPs, sunscreens and pharmaceuticals), accounting for 109 individual substances. The Porifera phylum and the two genus, *Ulva* spp. and *Gelidium* spp., were used to analyze the 6 analytical groups whereas the three others, *Holothuria* spp., *Mytilus* spp. and *Patella* spp., were only used to analyze pharmaceuticals.

Metal analysis: Metals were analyzed by inductively coupled plasma mass spectrometer (ICP-MS, Agilent 7500, Agilent Technologies, Waldbronn, Germany) according to the method described in Monperrus et al. (2005). Instrument control, data acquisition and data treatment were performed using Agilent Chemstation software.

Organomercury analysis: Organomercury compounds were analyzed by Gas Chromatrograph (GC; HP 6850) equipped with a capillary column and coupled to an Inductively Coupled Plasma Mass Spectrometer (ICP-MS) via a Silcosteel (Restek) transfer line. The analyses were achieved according to the method described in Monperrus et al. (2005). The THg concentrations were calculated from the concentrations of IHg and MMHg.

PAH, PCB, OCP, musk, sunscreen and AP analysis: Organic compounds were analyzed by 7890 Gas Chromatrograph coupled with 5975C Mass Spectrometer (GC-MS) with an Electron Ionization source using a Large Volume Injection (Agilent Technologies). Methods described in Salem et al. (2016) and Saraiva et al. (2016) were followed. Instrument control, data acquisition and data treatment were performed using Agilent Chemstation software.

Pharmaceutical analysis: Pharmaceuticals were analyzed by Liquid Chromatograph-tandem Mass Spectrometer (LC-MS-MS) using an Acquity UPLC system connected to a Xevo TQ MS (Triple quadrupole) with an electrospray interface (Waters). Analyses were achieved following the method described in Miossec et al. (2019). Instrument control, data acquisition and data treatment were performed using MassLynx software (Waters).

2.4 Statistical analyses

Wastewater samples: Mean and median concentrations, minimum and maximum, and percent occurrence of detected metals, organomercury compounds and organics (i.e. PAHs, PCBs, APs, OCPs, musks, sunscreens and pharmaceuticals) in wastewater were calculated for the whole sampling campaign and for each WWTP. An estimation of the total amount of analyzed analytes released into the Ocean by each plant was also calculated by multiplying the concentration of each analytes with the total volume rejected by each WWTP the day preceding the sampling. Differences in total and mean concentrations between WWTPs, sampling campaigns (per analytical group), and between unfiltered and filtered samples were described using Balloon and Box plots. A first Principal Component Analysis (PCA) was used to assess which metals characterized WWTP samples. Variations on concentrations between sampling campaigns and between WWTPs were studied by means of a two-way ANOVA with two fixed factors without interaction and a Tuckey HSD post hoc test. The usual assumptions (normality, homogeneity of variance, residuals) were verified. Variations on concentrations between filtered and unfiltered samples were tested using a Wilcoxon signed rank test for paired samples. Tests were achieved with an a priori chosen significant level of $\alpha = 0.05$.

Biological samples: Mean concentrations of detected organics (i.e. PAHs, PCBs, OCPs, musks, sunscreens and pharmaceuticals) in *Ulva* spp., *Gelidium* spp. and Porifera were calculated. The same was achieved for *Mytilus* spp., *Patella* spp. and *Holothuria* spp. but only for pharmaceutical compounds. A second PCA was performed in order to compare the micropollutant profiles detected in benthic organisms, i.e., the relative distribution of analytical group(s) detected in biota samples.

Tables and statistical analyses were performed with R® software and Excel v.7®.

3. Results

3.1 Micropollutant analysis in WWTP effluents

3.1.1 Occurrence

Mean concentrations of measured analytes in the five WWTP discharges are detailed in **Table 2**. Among the 127 analytes analyzed in this study, a total of 11 metals (ranging from 13.2 to 4 884.7 ng.L^{-1}), 2 organomercury compounds (ranging from 0.2 to 2.7 ng.L^{-1}) and 98 organics (ranging from 0.1 to 1 544.7 ng.L^{-1}) were detected in WWTP effluents. These organic compounds comprise of 16 PAHs, 11 PCBs, 5 alkylphenols, 18 OCPs, 10 musks, 4 sunscreens and 34 pharmaceuticals. The total mean concentrations, summated for all analytes per analytical group, amounted to (in descending order): 19 021.9 ng.L^{-1} for metals, 8 937.0 ng.L^{-1} for pharmaceuticals, 2 274.3 ng.L^{-1} for musks, 725.7 ng.L^{-1} for alkylphenols, 232.6 ng.L^{-1} for sunscreens, 62.2 ng.L^{-1} for PAHs, 7.2 ng.L^{-1} for OCPs, 6.8 ng.L^{-1} for PCBs and 2.9 ng.L^{-1} for organomercury compounds. All metals and the THg occurred in all plants and samples. Among the organic substance family, NP, HHCB, HHCB-lactone, AHTN, MK were the most frequently detected in discharges (occurrence= 100%). The same occurred for Hydrochlorothiazide, Oxazepam, Caffeine, Diclofenac, Ketoprofen, Carbamazepine, Atenolol, Losartan, Ciprofloxacin, Sulfamethoxazole, Ofloxacin, Clarithromycin and Metoprolol among pharmaceutical compounds. All of these major organic analytes had mean concentrations ranging from 70.5 to 1 544.7 ng.L^{-1}. Moreover, analytes recorded as having the highest mean concentrations in WWTP discharges (i.e. mean analyte concentration ≥ 25% of the total mean concentration of the analytical group, except for pharmaceuticals for which a limit of 10% was used, in descending order) were Vanadium (4 884.7 ng.L^{-1}), Chromium (4 710.3 ng.L^{-1}), Hydrochlorothiazide (1 544.7 ng.L^{-1}), HHCB (1 438.6 ng.L^{-1}), Oxazepam (1 421.6 ng.L^{-1}), Caffeine (1 224.2 ng.L^{-1}), Diclofenac (917.3 ng.L^{-1}), NP (573.6 ng.L^{-1}), HHCB-lactone (561.4 ng.L^{-1}), OC (175.6 ng.L^{-1}), Naphthalene (31.5 ng.L^{-1}), IHg (2.7 ng.L^{-1}) and PCB 138 (2.0 ng.L^{-1}).

To identify the proportion of micropollutants associated to the particulate/dissolved fraction, a part of wastewater samples was filtered (except samples used for pharmaceutical analysis which were all filtered). Filtered samples presented significant lower micropollutant concentrations than unfiltered samples (Wilcoxon, $p<0.05$) (**SM 2**). Proportions in the particulate phase varied according to the analytical group: from 0 to 97.5% for organomercury compounds, from 0 to 86.9% for organic substances and from 48.5 to 73.1% for metals. Therefore, a large part of micropollutants might be associated to the particulate phase of WWTP effluents depending on the MES concentration.

Table 2: Mean concentrations, median concentrations, minimum and maximum (Min, Max), and percent occurrence of detected priority and emerging substances (metals, organomercury compounds and organics expressed in ng.L^{-1}) in bulk wastewater samples from the five WWTPs. Total daily flux estimations (in mg.day^{-1}) were also calculated considering the four French WWTPs (thus, without 'WWTP 5'). Analyte mean concentrations were ordered from the highest to the lowest mean concentrations. Significance codes: Bold analytes are those found at the highest concentrations; Underlined analytes are those followed and regulated within European Directives; DL: Detection limit; QL: Quantification limit; '-': corresponds to molecules whose pre-analytical or analytical methods were not adapted to their quantification in that sample. In the flux estimation column, '-' means that the estimation was not possible for this molecule. All those concentrations and flux estimations per WWTP are available in SM 4.

			Concentrations (ng.L^{-1})					Total daily flux estimations (mg.day^{-1})			
Substance families	**Analytical Groups**	**Analytes**	**Mean**	**Median**	**Min**	**Max**	**Occurrence (%)**	**Mean**	**Median**	**Min**	**Max**
Priority substances											
Metal		**Vanadium (V)**	4884.7	4573.3	4053.3	7183.3	100	69231.8	70152.1	43881.7	92741.2
Metal		**Chromium (Cr)**	4710.3	4415.5	3749.0	7045.0	100	67561.7	68143.2	43988.7	89971.8
Metal		Copper (Cu)	2725.2	1837.7	1253.7	5735.7	100	41609.8	42230.9	21417.1	60560.2
Metal		Nickel (Ni)	2530.1	2107.5	1534.0	4476.0	100	36528.1	36829.2	26164.1	46289.8
Metal		Arsenic (As)	1748.4	1737.3	1353.8	2420.8	100	23899.7	24604.4	14498.4	31891.6
Metal		Antimony (Sb)	1155.8	1079.3	816.3	1893.3	100	16873.6	16487.0	8308.0	26212.3
Metal		Lead (Pb)	553.4	558.5	238.5	1243.5	100	9109.9	9104.4	4320.6	13910.0
Metal		Molybdenum (Mo)	463.3	316.0	53.0	1075.0	100	7567.7	7125.2	3481.6	12538.7
Metal		Tin (Sn)	220.0	201.0	134.0	481.0	100	3087.3	3047.7	2078.8	4174.7
Metal		Silver (Ag)	17.4	15.5	1.5	42.5	100	290.1	278.9	138.6	464.1
Metal		Cadmium (Cd)	13.2	10.3	2.3	26.3	100	193.5	188.9	98.0	298.2
	TOTAL		**19021.9**		**13189.2**	**31622.2**		**275953.1**		**168375.6**	**379052.8**
Organomercury compound		**IHg**	2.7	1.4	0.2	15.8	100.0	50.8	45.2	21.6	85.9
Organomercury compound		MMHg	0.2	0.1	<DL	1.7	94	3.8	1.6	0.8	9.0
	TOTAL		**2.9**		**0.2**	**17.5**		**54.6**		**22.4**	**94.8**
Organic	PAH	**Naphthalene**	31.5	10.0	<DL	147.7	50.0	979.3	979.3	942.3	1016.2
Organic	PAH	Indeno[1,2,3-cd]pyrene	5.1	<DL	<DL	44.1	23.8	82.4	-	-	330.2
Organic	PAH	Dibenzo[a,h]anthracene	3.8	<DL	<DL	36.0	23.8	57.4	-	-	230.4
Organic	PAH	Fluorene	3.8	<DL	<DL	50.5	47.6	23.3	9.9	-	74.4
Organic	PAH	Pyrene	3.0	<DL	<DL	12.8	38.1	64.2	72.0	-	114.2
Organic	PAH	Phenanthrene	2.8	<DL	<DL	23.2	42.9	19.2	6.3	-	64.5
Organic	PAH	Acenaphthene	2.2	0.4	<DL	21.2	52.4	45.4	12.1	-	157.6
Organic	PAH	Benzo[g,h,i]perylene	2.2	<DL	<DL	22.8	28.6	28.1	-	-	113.0
Organic	PAH	Benzo[b]fluoranthene	2.0	<DL	<DL	17.0	33.3	36.4	7.1	-	133.7
Organic	PAH	Benzo[a]anthracene	1.7	<DL	<DL	27.5	33.3	15.0	2.9	-	55.0
Organic	PAH	Anthracene	1.1	<DL	<DL	18.3	33.3	9.3	-	-	36.5
Organic	PAH	Benzo[a]pyrene	1.0	<DL	<DL	10.5	28.6	13.7	-	-	55.0
Organic	PAH	Benzo[k]fluoranthene	1.0	<DL	<DL	11.6	23.8	12.1	-	-	49.2
Organic	PAH	Chrysene	0.6	<DL	<DL	9.8	14.3	3.6	-	-	14.9
Organic	PAH	Fluoranthene	0.3	<DL	<DL	2.1	25.0	3.0	1.3	-	9.3
Organic	PAH	Acenaphthylene	0.2	<DL	<DL	1.2	14.3	1.2	-	-	4.8
	TOTAL		**62.2**		**<DL**	**456.3**		**1393.4**		**942.3**	**2459.0**
Organic	PCB	**PCB 138**	2.0	<DL	<DL	18.8	23.8	25.1	1.9	-	97.1
Organic	PCB	PCB 194	1.4	<DL	<DL	22.7	14.3	9.8	-	-	42.0
Organic	PCB	PCB 149	0.9	<DL	<DL	5.2	33.3	15.0	5.4	-	50.0
Organic	PCB	PCB 28+31	0.7	<DL	<DL	5.1	28.6	11.1	0.2	-	44.9
Organic	PCB	PCB 101	0.5	<DL	<DL	4.2	23.8	4.6	-	-	19.0
Organic	PCB	PCB 52	0.3	<DL	<DL	2.7	28.6	2.5	-	-	10.4
Organic	PCB	PCB 180	0.3	<DL	<DL	3.5	23.8	2.5	-	-	10.3
Organic	PCB	PCB 44	0.2	<DL	<DL	2.5	23.8	1.9	-	-	8.2
Organic	PCB	PCB 18	0.2	<DL	<DL	2.7	14.3	1.4	-	-	6.3
Organic	PCB	PCB 153	0.2	<DL	<DL	1.4	14.3	1.0	-	-	4.8
Organic	PCB	PCB 118	0.1	<DL	<DL	1.0	33.3	0.9	-	-	4.3
	TOTAL		**6.8**		**<DL**	**69.9**		**75.8**		-	**297.3**
Organic	AP	**NP**	573.6	656.8	19.0	1449.4	100	10231.2	8739.4	3312.0	19142.8
Organic	AP	NPEO1	78.0	5.0	<DL	390.5	50.0	353.9	353.9	338.3	369.5
Organic	AP	NPEO2	62.5	<DL	<DL	1006.3	17.6	83.1	-	-	261.7
Organic	AP	4tOP	10.4	<DL	<DL	104.8	23.5	112.5	74.6	-	330.4
Organic	AP	4nOP	1.2	<DL	<DL	<10.0	11.8	6.2	-	-	31.2
	TOTAL		**725.7**		**19.0**	**2961.0**		**10786.9**		**3650.3**	**20135.6**
Organic	OCP	Beta BHC	1.6	<DL	<DL	22.8	12.5	17.8	-	-	71.3
Organic	OCP	4,4'-DDE	1.4	<DL	<DL	10.2	23.8	18.4	-	-	76.0
Organic	OCP	Alpha BHC	0.4	<DL	<DL	3.6	12.5	2.7	-	-	10.9
Organic	OCP	Methoxychlor	0.4	<DL	<DL	2.0	18.2	2.1	-	-	6.3
Organic	OCP	Delta BHC	0.4	<DL	<DL	2.4	18.2	2.1	-	-	6.2
Organic	OCP	Endosulfan Sulfate	0.4	<DL	<DL	<2.0	18.2	2.1	-	-	6.2
Organic	OCP	Gamma BHC	0.4	<DL	<DL	<2.0	18.2	2.1	-	-	6.2
Organic	OCP	4,4'-DDT	0.3	<DL	<DL	<2.0	18.2	2.1	-	-	6.2
Organic	OCP	Bêta Endosulfan	0.3	<DL	<DL	2.6	12.5	2.0	-	-	8.1
Organic	OCP	Alpha Endosulfan	0.3	<DL	<DL	2.3	12.5	1.8	-	-	7.1
Organic	OCP	Heptachlor	0.2	<DL	<DL	<2.0	12.5	1.6	-	-	6.2
Organic	OCP	Aldrin	0.2	<DL	<DL	<2.0	9.5	1.2	-	-	6.2
Organic	OCP	Dieldrine	0.2	<DL	<DL	<2.0	9.5	1.2	-	-	6.2
Organic	OCP	Endrin Aldehyde	0.2	<DL	<DL	<2.0	9.5	1.2	-	-	6.2
Organic	OCP	Heptachlor Epoxide	0.2	<DL	<DL	<2.0	9.5	1.2	-	-	6.2
Organic	OCP	4,4'-DDD	0.2	<DL	<DL	<2.0	9.5	1.2	-	-	6.2
Organic	OCP	Endrin Ketone	0.2	<DL	<DL	<2.0	9.5	1.2	-	-	6.0
Organic	OCP	Endrin	0.1	<DL	<DL	<2.0	9.5	0.5	-	-	2.3
	TOTAL		**7.2**		**<DL**	**67.8**		**62.6**		-	**250.1**

Table 2: (Continued)

			Concentrations (ng.L^{-1})					Total daily flux estimations (mg.day^{-1})			
Substance families	**Analytical Groups**	**Analytes**	**Mean**	**Median**	**Min**	**Max**	**Occurrence (%)**	**Mean**	**Median**	**Min**	**Max**
Emerging substances											
Organic	Musk	**HHCB**	1438.6	1246.9	600.0	2943.7	100	18654.5	16795.1	9970.4	29213.8
Organic	Musk	**HHCB-lactone**	561.4	511.0	28.8	960.2	100	6410.6	6677.2	2926.3	9974.8
Organic	Musk	AHTN	196.9	196.5	24.5	347.4	100	2685.5	2470.6	1419.6	4549.5
Organic	Musk	MK	70.5	62.5	7.3	203.1	100	679.6	705.6	231.0	1133.7
Organic	Musk	ADBI	6.0	1.0	<DL	20.4	57.1	82.9	43.1	-	231.7
Organic	Musk	ATII	0.2	<DL	<DL	3.3	9.5	2.1	-	-	10.4
Organic	Musk	AHMI	0.2	<DL	<DL	1.5	19.0	3.1	0.5	-	12.1
Organic	Musk	MX	0.2	<DL	<DL	2.4	9.5	33.5	-	-	134.4
Organic	Musk	MA	0.1	<DL	<DL	<1.0	9.5	0.8	-	-	3.7
Organic	Musk	MM	0.1	<DL	<DL	<1.0	9.5	0.6	-	-	3.1
	TOTAL		**2274.3**		**<DL**	**4483.8**		**28553.1**		**14547.2**	**45267.3**
Organic	Sunscreen	**OC**	175.6	21.2	<DL	2334.0	94.7	3675.7	1109.4	38.4	12464.2
Organic	Sunscreen	Benzophenone 3	28.9	15.1	<DL	127.1	73.7	417.9	295.2	32.1	970.4
Organic	Sunscreen	4-MBC	27.4	<DL	<DL	178.5	42.1	247.5	65.9	-	965.0
Organic	Sunscreen	EHMC	0.7	0.1	<DL	3.4	57.9	8.0	6.7	-	18.9
Organic	Sunscreen	3-BC	<DL	<DL	<DL	<DL	0.0	87.7	-	-	350.7
Organic	Sunscreen	OD-PABA	<DL	<DL	<DL	<DL	0.0	-	-	-	-
	TOTAL		**232.6**		**<DL**	**2643.0**		**4436.7**		**70.5**	**14769.1**
Organic	Pharmaceutical (Antihypertensive)	**Hydrochlorothiazide**	1544.7	1425.8	504.7	3228.4	100	22733.2	19179.6	16001.7	36571.9
Organic	Pharmaceutical (Anxiolytics)	**Oxazepam**	1421.6	1446.4	139.6	2911.4	100	24384.7	23446.8	18301.6	32343.6
Organic	Pharmaceutical (Psychotropic)	**Caffeine**	1224.2	458.6	26.9	12360.5	100	23997.7	12658.9	4050.1	66623.0
Organic	Pharmaceutical (Anti-inflammatory)	**Diclofenac**	917.3	900.4	418.6	2436.2	100	13994.4	11413.2	8220.5	24930.7
Organic	Pharmaceutical (Pain killer)	Ketoprofen	454.8	227.6	40.5	2472.4	100	8702.4	6695.1	4831.7	16587.5
Organic	Pharmaceutical (Anticonvulsant)	Carbamazepine	383.8	350.7	17.9	1127.8	100	5759.8	5391.8	2402.8	9852.9
Organic	Pharmaceutical (Antihypertensive)	Atenolol	333.9	294.1	17.4	731.9	100	6568.9	6040.1	4193.1	10002.4
Organic	Pharmaceutical (Antihypertensive)	Losartan	292.4	201.7	16.0	1105.5	100	5362.2	4643.4	3151.0	9010.8
Organic	Pharmaceutical (Antibiotics)	Ciprofloxacin	289.6	221.7	36.2	853.0	100	4057.5	3414.7	1311.3	8089.0
Organic	Pharmaceutical (Anti-inflammatory)	Ibuprofen	250.6	83.4	<QL	1660.8	63.2	4773.3	3417.9	2299.9	9957.4
Organic	Pharmaceutical (Antibiotics)	Sulfamethoxazole	216.8	208.3	55.0	559.5	100	3738.0	3624.3	2392.0	5311.3
Organic	Pharmaceutical (Antibiotics)	Ofloxacin	203.4	124.6	53.7	915.5	100	2551.2	2174.7	1015.5	4839.8
Organic	Pharmaceutical (Antibiotics)	Azithromycin	201.2	105.0	<QL	750.2	89.5	2384.0	2238.3	662.2	4397.0
Organic	Pharmaceutical (Pain killer)	Niflumic acid	198.6	158.6	<QL	516.6	94.7	3675.9	3108.5	2368.0	6118.5
Organic	Pharmaceutical (Antibiotics)	Clarithromycin	118.9	88.2	3.6	351.1	100	1713.0	1865.1	870.0	2251.5
Organic	Pharmaceutical (Pain killer)	Acetaminophen	108.9	12.5	<QL	951.4	84.2	2090.2	1641.3	119.4	4958.9
Organic	Pharmaceutical (Glycemia)	Gemfibrozil	98.5	107.6	<QL	255.3	94.7	1817.4	1762.4	1098.1	2646.9
Organic	Pharmaceutical (Antiarrhythmic)	Metoprolol	87.0	67.0	8.3	417.8	100	1776.7	1249.5	726.0	3881.7
Organic	Pharmaceutical (Antibiotics)	Erythromycin A	77.9	34.3	<QL	438.3	94.7	1563.7	693.1	228.3	4640.2
Organic	Pharmaceutical (Antibiotics)	Roxithromycin	77.0	53.5	<QL	292.2	78.9	1452.4	1348.4	500.0	2612.9
Organic	Pharmaceutical (Glaucoma)	Acetazolamide	76.2	52.1	<QL	231.1	52.6	1497.4	1434.4	0.0	3121.0
Organic	Pharmaceutical (Antibiotics)	Trimethoprim	67.5	58.9	<QL	159.3	94.7	1139.0	1214.1	624.8	1503.0
Organic	Pharmaceutical (Antibiotics)	Metronidazole	61.3	67.1	<QL	134.7	94.7	1160.4	1100.6	701.3	1739.2
Organic	Pharmaceutical (Antibiotics)	Spiramycin	55.8	31.1	<QL	344.4	63.2	841.3	561.7	106.6	2135.2
Organic	Pharmaceutical (Antibiotics)	Norfloxacin	49.3	<DL	<QL	212.8	47.4	801.7	385.7	0.0	2435.4
Organic	Pharmaceutical (Anxiolytics)	Lorazepam	43.2	28.5	<QL	160.0	94.7	400.3	445.9	189.4	520.2
Organic	Pharmaceutical (Antibiotics)	Josamycin	22.5	13.6	<QL	83.2	78.9	387.6	328.5	210.4	683.0
Organic	Pharmaceutical (Anxiolytics)	Nordazepam	15.7	13.1	8.9	36.8	100	182.8	187.9	129.3	226.0
Organic	Pharmaceutical (Pain killer)	Phenazone	13.4	<DL	<QL	137.6	21.1	18.3	-	-	73.1
Organic	Pharmaceutical (Antibiotics)	Piperacillin	9.2	<DL	<QL	169.2	15.8	154.2	-	-	616.6
Organic	Pharmaceutical (Antibiotics)	Tetracycline	6.4	<DL	<QL	44.6	21.1	67.1	30.8	-	206.8
Organic	Pharmaceutical (Anticancer)	Cyclophosphamide	5.8	<DL	<QL	35.0	26.3	78.7	31.3	-	252.2
Organic	Pharmaceutical (Antibiotics)	Sulfadiazine	5.4	<DL	<QL	16.0	42.1	70.4	46.0	-	189.4
Organic	Pharmaceutical (Antibiotics)	Flumequine	4.1	<DL	<QL	57.8	10.5	81.7	35.4	-	256.1
Organic	Pharmaceutical (Antibiotics)	Ampicilline	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Antibiotics)	Doxycycline	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Antibiotics)	Oxolinic acid	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Antibiotics)	Rifampicin	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Antibiotics)	Sulfamethazine	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Antibiotics)	Tylosine	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Hormones)	E2	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Hormones)	EE2	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Hormones)	E1	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Contraceptif)	19-Norethindrone	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Antiarrhythmic)	Amiodarone	-	-	-	-	-	-	-	-	-
Organic	Pharmaceutical (Antibiotics)	Amoxicillin	-	-	-	-	-	-	-	-	-
Organic	Pharmaceutical (Pain killer)	Acetylsalicylic acid	-	-	-	-	-	-	-	-	-
Organic	Pharmaceutical (Antineoplastic)	Hydroxycarbamide	-	-	-	-	-	-	-	-	-
	TOTAL		**8937.0**		**1347.4**	**36158.3**		**149977.4**		**76705.1**	**279585.1**

3.1.2 Temporal variability

The present study was conducted on five WWTPs and at four periods during the two years (August 2017, May, July and December 2018). Statistical analyses conducted on the total concentration of each substance family showed significant differences between sampling months (except for organomercury compounds where no significant difference was detected) (ANOVA, $p<0.05$; **Fig. 3**; **SM 3**). August samples appeared as having the highest total concentrations compared to certain other sampling months (Tuckey HSD, $p<0.05$; **Fig. 3**; **SM 3**). For example, the metal total concentration was significantly higher in August samples than those of July and December (Tuckey HSD, $p<0.05$; **Fig. 3**; **SM 3**). The same occurred for organic and pharmaceutical total concentrations. August samples presented higher total concentrations than those of December and July, respectively (Tuckey HSD, $p<0.05$; **Fig. 3**; **SM 3**).

3.1.3 Spatial variability

Moreover, significant differences were identified between WWTPs considering total concentrations of metals and pharmaceuticals (ANOVA, $p<0.05$; **Fig. 3**; **SM 3**). Indeed, metal total concentrations from 'WWTP 3' samples were always significantly higher than those from other WWTPs (without considering 'WWTP 5' due to the too high salinity, Gutierrez et al., 2019) (Tuckey HSD, $p<0.05$; **Fig. 3**; **SM 3**). Even if the 11 analyzed metals were always detected in the four WWTP effluents (occurrence= 100%), 'WWTP 3' presented anyway the highest total mean concentration compared to the three others (24 557.2 ng.L^{-1} *vs*. 19 383.4 ng.L^{-1}, 16 687.9 ng.L^{-1} and 15 458.9 ng.L^{-1} for 'WWTP 4', 'WWTP 2' and 'WWTP 1', respectively) (**SM 4**). According to the PCA (**SM 5**), showing the metal distribution between the four WWTPs, Vanadium, Chromium, Copper and Nickel (positively correlated between them and characterized by strongly negative coordinates on the first axis) contributed to the definition of the first axis and to the position of the 'WWTP 3' which appeared to be correlated to this group of analytes. Indeed, their mean concentrations were at 5 708.3 and 5 678.3 ng.L^{-1} (for Vanadium and Chromium, respectively) and at 4 641.2 and 3 868.0 ng.L^{-1} (for Copper and Nickel, respectively) in 'WWTP 3' (**SM 4**). This contrasts with samples of others WWTPs where their mean concentrations varied from 4 339.5 to 5 105.8 ng.L^{-1} and from 3 881.8 to 5 239.5 ng.L^{-1} (for Vanadium and Chromium, respectively), from 1 589.9 to 2 797.7 ng.L^{-1} (for Copper) and from 2 034.8 to 2 211.5 ng.L^{-1} (for Nickel) (**SM 4**). To a lesser extent, Cadmium, Silver and Molybdenum influenced also differences between 'WWTP 3' samples and the others. By contrast, Antimony and Lead (positively correlated) appeared higher in 'WWTP 2' than the others (1 280.5 and 892.3 ng.L^{-1} *vs*. 1 090.3-1 136.0 and 281.3-532.8 ng.L^{-1} in other WWTPs) (**SM 4** and **5**). The same occurred for pharmaceutical compounds, but only between 'WWTP 3' and 'WWTP 1' samples (Tuckey HSD, $p<0.05$; **Fig. 3**; **SM 3**). Indeed, whatever the pharmaceutical family, concentrations were always higher in 'WWTP 3' samples than in 'WWTP 1' ones with differences ranging from 446 to 4 496 ng.L^{-1} (**SM 4**). The main mean concentration identified in 'WWTP 3' was due to the Caffeine analyte (a psychotropic compound, 4 420.5 ng.L^{-1}) (**SM 4**). Four other analytes were also identified with mean concentrations higher than 1 000 ng.L^{-1}: Hydrochlorothiazide (an antihypertensive), Oxazepam (an axiolytic), Ketoprofen (a painkiller) and Diclofenac (an anti-inflammatory). Among other analytical groups which did not present significant differences between WWTPs, musk compounds were highlighted in higher concentration in 'WWTP 1' (3 144.7 ng.L^{-1} *vs.* 2 630.2 ng.L^{-1}, 2 037.3 ng.L^{-1}, 1 947.1 ng.L^{-1} and 1 523.0 ng.L^{-1} in 'WWTP 4', 'WWTP 2', 'WWTP 5' and 'WWTP 3', respectively) (**SM 4**). APs were mainly found in 'WWTP 5' wastewaters with a total mean concentration equal to 1 489.2 ng.L^{-1} (**SM 4**) compared to other WWTPs where they ranged from 215.1 ng.L^{-1} (in 'WWTP 1') to 899.1 ng.L^{-1} (in 'WWTP 3'). Sunscreens presented a higher mean concentration in 'WWTP 3' samples (746.2 ng.L^{-1}) while they were found in much lower concentrations in other WWTPs (from 30.1 to 156.8 ng.L^{-1}). The same occurred with PAHs, mainly detected in 'WWTP 2' (186.0 ng.L^{-1}) compared to in other WWTPs (from 17.8 to 50.5 ng.L^{-1}). Other analytical groups, such as PCBs, OCPs, and organomercury compounds were identified with mean concentrations lower than 20 ng.L^{-1}.

According to total daily volumes rejected and measured by each French WWTP, daily flux estimations were calculated for each analytical group and analyte (**Table 2**; **SM 4**). Generally, the group rejected in highest mean quantity were metals (with a total mean daily flux estimation equal to 275 953.1 mg.day^{-1}) (**Table 2**). At the WWTP scale, this mean ranged from 11 923.1 (in 'WWTP 1') to 112 390.8 mg.day^{-1} (in 'WWTP 3') (**Fig. 4**; **SM 4**). The second main analytical group rejected by WWTP were pharmaceuticals (**Table 2**; **SM 4**). Their flux estimations were around two times lower than metals with a total mean daily flux equal to 149 977.4 mg.day^{-1} (**Table 2**). The minimum occurred in 'WWTP 1' (3 349.3 mg.day^{-1}) and the maximum in 'WWTP 3' (70 430.8 mg.day^{-1}) (**Fig. 4**; **SM 4**). Other analytical groups, such as musks, APs, Sunscreens and PAHs obtained much lower total flux estimations (between 1 393.4 to 28 553.1 mg.day^{-1}) (**Table 2**; **Fig. 4**). This was even more the case for PCBs, OCPs and organomercury compounds which presented total mean flux estimations between 54.6 to 75.8 mg.day^{-1} (**Table 2**; **Fig. 4**).

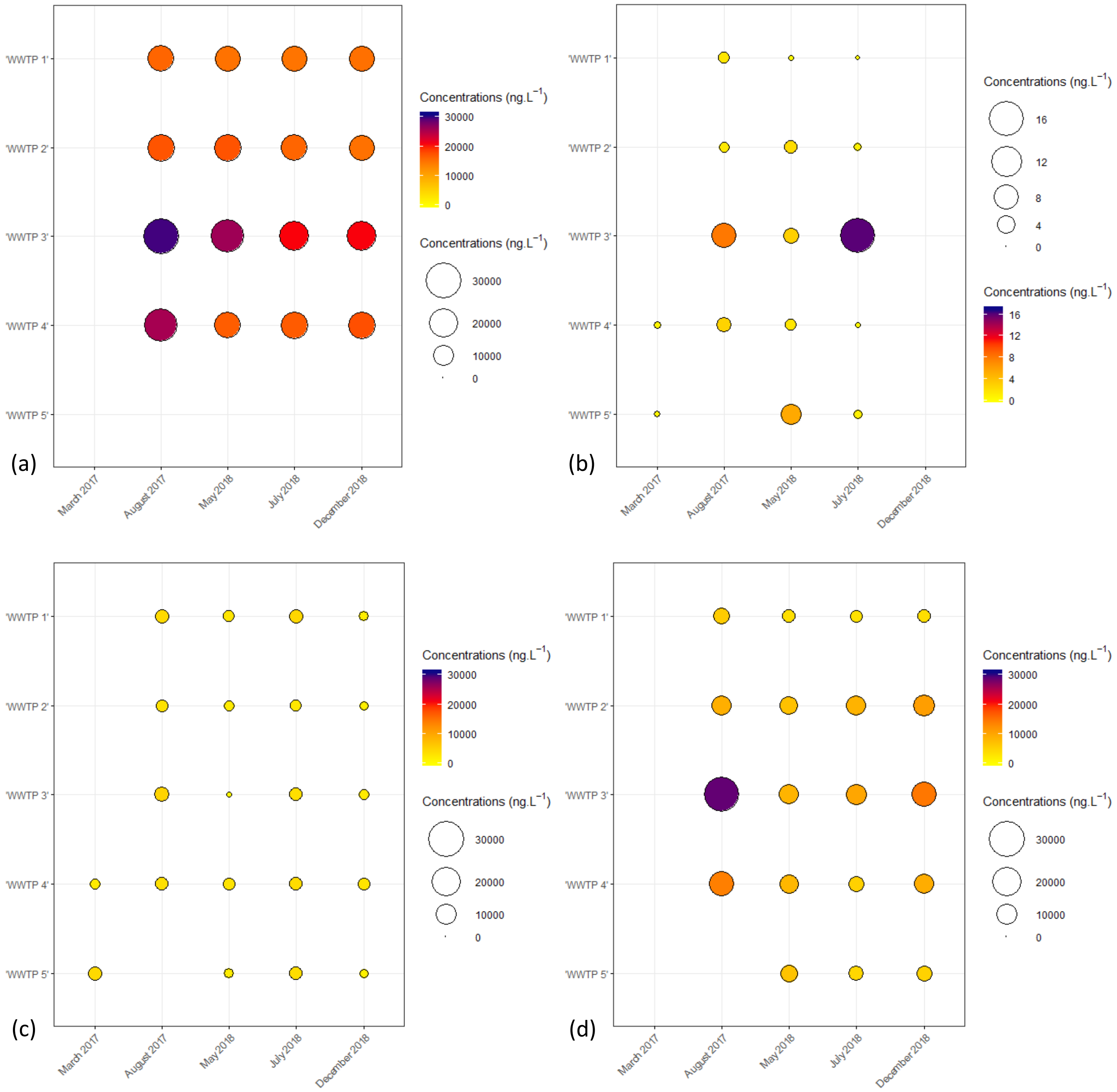


Fig. 3: Total concentrations of metals (a), organomercury compounds (b), organics (PAHs, PCBs, musks, sunscreens, OCPs, alkylphenols) (c) and pharmaceuticals (d) detected in wastewaters (bulk samples) per sampling campaign for each WWTP discharge.

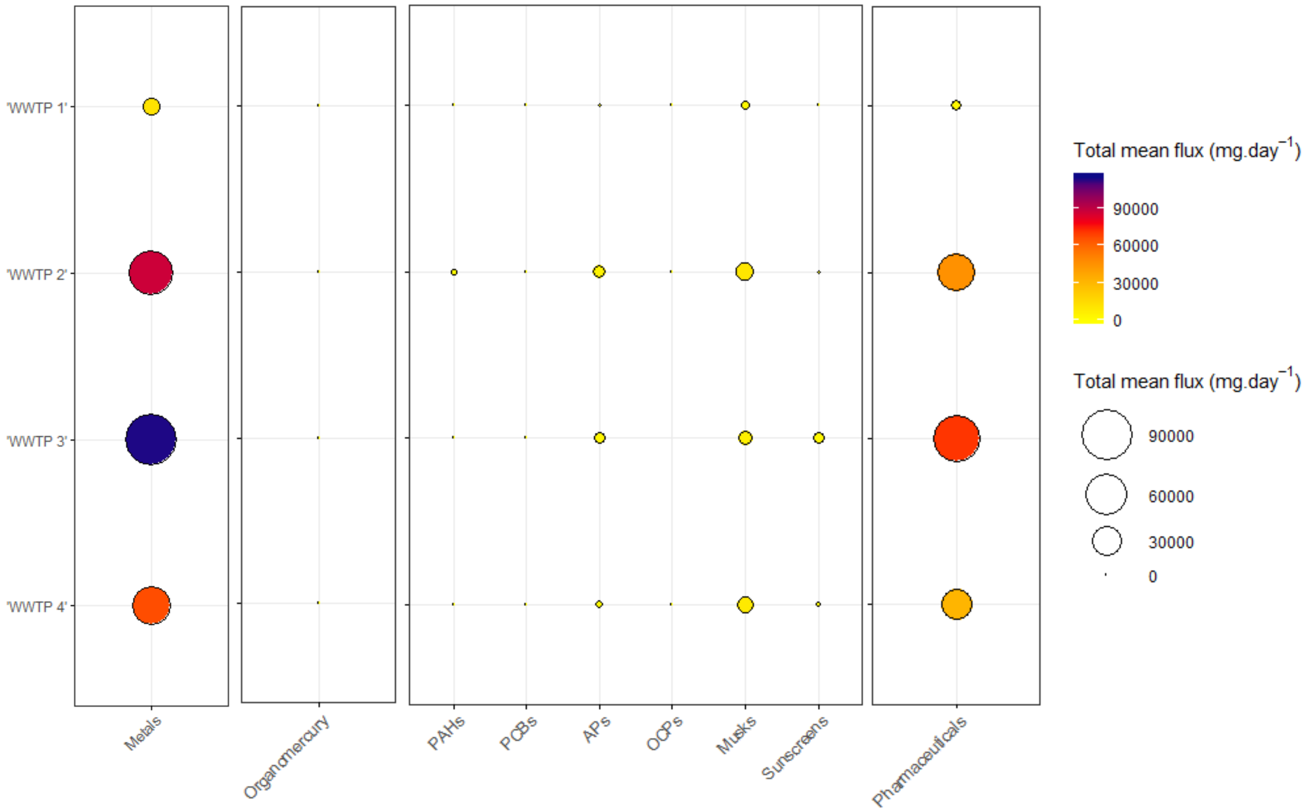


Fig. 4: Total mean daily flux estimations (mg.day^{-1}) per analytical group (metals, organomercury compounds, PAHs, PCBs, APs, OCPs, musks, sunscreens and pharmaceuticals) per WWTP.

3.2 Micropollutant analysis in benthic organisms

Concentrations of detected analytes in each benthic organism sampled proximate to WWTP outfalls (i.e. in impacted locations) and in control locations are detailed in **Tables 3** and **4**. Among the 109 organic substances analyzed in biota samples, a total of 51 analytes (9 PAHs, 6 PCBs, 1 OCP, 5 musks, 3 sunscreens and 27 pharmaceuticals with mean concentrations ranging from 0.1 to 3 765.2 ng.g^{-1}) were detected and quantified.

A PCA was performed to show the analytical group distribution between the three benthic organisms used for all analyses (i.e. *Ulva* spp., *Gelidium* spp. and Porifera) (**Fig. 5**). Pharmaceuticals and musks (positively correlated between them and characterized by negative coordinates on the first axis and positive coordinates on the second axis; **Fig. 5**) appeared to contribute to the position of *Ulva* spp.. Indeed, this alga presented the highest number of detected pharmaceuticals and the highest total mean concentrations of pharmaceuticals (235.8 ng.g^{-1} *vs.* 10.8 ng.g^{-1} in Porifera and 55.3 ng.g^{-1} in *Gelidium* spp.) and musk compounds (87.5 ng.g^{-1} *vs.* 78.5 ng.g^{-1} in Porifera and 4.4 ng.g^{-1} in *Gelidium* spp.) (**Tables 3**). Main analytes responsible for these high concentrations were Azithromycin (an antibiotic), Metoprolol (an antiarrhythmic), Oxazepam (an anxiolytic) and Ibuprofen (an anti-inflammatory) with mean concentrations ranging between 26.9 and 62.2 ng.g^{-1} and HHCB (49.3 ng.g^{-1}) and MA (26.9 ng.g^{-1}) (**Table 3**). At the location scale, total concentrations of these both analytical groups (i.e. pharmaceuticals and musks) were always higher in *Ulva* spp. sampled in impacted locations compared to control locations (from 35.7 to 582.5 ng.g^{-1} in impacted locations *vs*. 24.4 ng.g^{-1} in locations without outfall for pharmaceuticals and from 20.6 to 220.0 *vs*. 11.6 ng.g^{-1}, respectively for musk compounds) (**Tables 3**; **Fig. 5**). Among analytical groups detected in lower concentrations in this alga, the same occurred for PAHs (**Table 3**). Total concentrations of these three analytical groups were always higher in *Ulva* spp. sampled in 'WWTP 2' and often lower in those from 'WWTP 1' and to a lesser extent in those from 'WWTP 5'. By contrast, the reverse occurred for sunscreens, detected in higher concentrations in control locations (**Table 3**; **Fig. 5**). Moreover, PCBs and OCPs were found in higher concentrations in 'WWTP 5' (impacted) and 'Control 3' (control) than in 'WWTP 2' and 'WWTP 1' (impacted).

Even if sunscreens did not show strongly positive coordinates, they were anyway associated to *Gelidium* spp. (**Fig. 5**). The total mean concentration (equal to 1 890.6 ng.g^{-1}) was due to OC found up to 3 765.2 ng.g^{-1} while it was below 111 ng.g^{-1} in the two other taxa (**Table 3**). As for pharmaceuticals and musk compounds in *Ulva* spp., sunscreens were always found in higher concentrations in *Gelidium* spp. collected in impacted locations than those sampled in control locations (from 24.2 to 3 809 ng.g^{-1} in impacted locations *vs*. from <DL to 1.3 ng.g^{-1} in the others). The same occurred for musk compounds even if they were detected in lower concentrations. In both cases, these analytical groups were found in higher concentrations in *Gelidium* spp. collected in 'WWTP 4' compared to those collected in 'WWTP 3' (**Table 3**). By contrast, pharmaceuticals and PAHs, were found in higher concentration in algae collected at 'WWTP 3' than those from 'WWTP 4' and control locations ('Control 1' and 'Control 3'). Finally, PCBs were always found under the detection limit whatever the location (impacted or control).

Moreover, PAHs and PCBs (characterized by negative coordinates on both axes; **Fig. 5**) were mainly associated to Porifera. Indeed, PAHs and PCBs were found in higher concentrations in this organism than in others (PAHs: total maximum equal to 523.7 ng.g^{-1} in Porifera *vs*. 257.9 and 26.9 ng.g^{-1} in *Ulva* spp. and *Gelidium* spp., respectively; PCBs: 741.3 ng.g^{-1} *vs*. 67.5 ng.g^{-1} and <DL, respectively). The major PAHs and PCBs were Naphthalene (426.0 ng.g^{-1}), Dibenzo[a,h]anthracene (385.6 ng.g^{-1}) and PCB 28+31 (703.8 ng.g^{-1}) while other analytes had mean concentrations below 65 ng.g^{-1} (**Table 3**). Contrary to preceding analytical groups, PAHs and PCBs were mainly concentrated in Porifera sampled in control locations (**Fig. 5**). By contrast, even if musks, sunscreens and pharmaceuticals were detected in lower concentrations in this phylum, they were anyway identified in higher concentrations in Porifera

samples from ‘WWTP 3’ (the only one sampled impacted location) than in ‘Control 1’ and ‘Control 2’ (**Table 3**).

Finally, concentrations of PCBs and OCPs in *Ulva* spp., PAHs and pharmaceuticals in *Gelidium* spp. and PAHs in Porifera did not show clear concentration distinction between impacted and control locations.

Among the three other organisms (only analyzed for pharmaceuticals), *Mytilus* spp. presented the highest total mean concentration (41.1 ng.g^{-1}) compared to *Holothuria* spp. (7.1 ng.g^{-1}) and *Patella* spp. (35.2 ng.g^{-1}) (**Table 4**). However, these concentrations were from six to 33 times lower than those detected in *Ulva* spp. (235.8 ng.g^{-1}). These compounds anyway appeared in higher concentrations in organisms sampled in impacted locations than in those sampled in controls (except *Patella* spp. sampled in ‘WWTP 1’ which appeared lower concentrated than other impacted locations and the two controls). Indeed, *Mytilus* spp. from ‘WWTP 2’ appeared more concentrated than those from ‘Control 3’ (control). For *Patella* spp., the highest total concentration was found in ‘WWTP 5’ samples which were also more concentrated than those from ‘WWTP 2’ and ‘WWTP 4’ as well as those from two controls (‘Control 1’ and ‘Control 3’). But, ‘WWTP 1’ presented the lowest pharmaceutical concentrations. Concentrations of pharmaceuticals in *Holothuria* spp. appeared higher in ‘WWTP 3’ than in ‘WWTP 1’ and ‘Control 2’ (**Table 4**).

Table 3: Mean concentrations of detected priority and emerging substances ($ng.g^{-1}$) in *Ulva* spp., *Gelidium* spp. and Porifera sampled at the WWTP effluents and at control locations (far from point source of pollution; 'Control 1', 'Control 2', 'Control 3'). Analyte mean concentrations were ordered according to the wastewater table. Significance codes: Underlined analytes are those followed and regulated within European Directives; DL: Detection limit; QL: Quantification limit; '-': corresponds to molecules whose pre-analytical or analytical methods were not adapted to their quantification in that sample.

			Ulva spp.										*Gelidium* spp.										Porifera					
			'WWTP 1'		'WWTP 2'		'WWTP 5'		Mean		'Control 3'		'WWTP 3'		'WWTP 4'		Mean		'Control 1'		'Control 3'		'WWTP 3'		'Control 1'		'Control 2''	
Substance families	**Analytical Groups**	**Analytes**	**Average**	**SD**	**Average**	**SD**	**Average**	**SD**	**Average**	**SD**	**Average**	**SD**	**Average**	**SD**	**Average**	**SD**	**Average**	**SD**	**Average**	**SD**	**Average**	**SD**	**Average**	**SD**	**Average**	**SD**	**Average**	**SD**
Priority substances																												
Organic	PAH	Naphthalene	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	36.3	4.4	426.0	59.4	9.2	1.0
Organic	PAH	Indeno[1,2,3-cd]pyrene	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	PAH	Dibenzo[a,h]anthracene	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	385.6	116.9	<DL	-	<DL	-
Organic	PAH	Fluorene	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	PAH	Pyrene	8.6	0.8	10.7	2.4	8.7	0.3	9.3	1.2	8.2	0.8	<DL	-	0.3	0.1	0.1	0.2	<DL	-	<DL	-	4.5	5.0	<DL	-	2.7	0.3
Organic	PAH	Phenanthrene	<DL	-	48.0	8.4	<DL	-	16.0	27.7	30.3	1.1	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	11.7	5.3	<DL	-	5.0	0.9
Organic	PAH	Acenaphthene	64.2	7.2	69.3	22.4	79.4	4.9	71.0	7.7	52.5	11.1	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	PAH	Benzo[g,h,i]perylene	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	PAH	Benzo[b]fluoranthene	45.7	3.5	92.7	17.5	42.5	6.7	60.3	28.1	24.1	12.3	7.8	0.5	2.7	0.6	5.2	3.6	6.9	0.6	7.37	0.6	<DL	-	25.8	11.6	<DL	-
Organic	PAH	Benzo[a]anthracene	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	1.1	0.2
Organic	PAH	Anthracene	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	0.2	0.4
Organic	PAH	Benzo[a]pyrene	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	PAH	Benzo[k]fluoranthene	4.0	3.2	28.3	5.6	1.8	0.3	11.4	14.8	0.2	0.2	14.8	2.2	6.9	1.1	10.8	5.6	12.2	0.6	12.53	0.5	<DL	-	63.4	4.2	<DL	-
Organic	PAH	Chrysene	<DL	-	<DL	-	5.9	0.6	2.0	3.4	<DL	-	0.9	0.1	<DL	-	0.5	0.7	1.3	0.1	1.17	-	8.4	1.0	<DL	-	2.8	0.3
Organic	PAH	Fluoranthene	6.8	0.5	8.9	1.8	5.6	0.6	7.1	1.7	4.7	0.2	3.4	0.3	9.0	0.4	6.2	4.0	1.7	0.2	0.55	0.1	5.1	4.1	8.5	2.2	3.5	0.4
Organic	PAH	Acenaphtylene	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
			129.2	-	**257.9**	-	**143.9**	-	**177.0**	-	**119.9**	-	**26.9**	-	**18.9**	-	**22.9**	-	**22.0**	-	**21.6**	-	**451.6**	-	**523.7**	-	**24.6**	-
Organic	PCB	PCB 138	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	PCB	PCB 194	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	PCB	PCB 149	8.4	0.1	6.5	3.7	8.74	0.27	7.9	1.2	8.6	0.1	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	11.7	2.8	<DL	-
Organic	PCB	PCB 28+31	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	703.8	98.1	<DL	-
Organic	PCB	PCB 101	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	PCB	PCB 52	13.8	0.2	18.0	1.6	38.39	10.95	23.4	13.2	15.5	0.4	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	PCB	PCB 180	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	17.5	5.1	<DL	-
Organic	PCB	PCB 44	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	PCB	PCB 18	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	PCB	PCB 153	8.1	0.03	8.3	0.1	8.37	0.26	8.3	0.1	8.4	0.2	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	PCB	PCB 118	9.1	0.04	9.5	0.5	11.96	2.59	10.2	1.5	15.5	2.3	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	8.4	6.2	0.5	0.4
			39.5	-	**42.3**	-	**67.5**	-	**49.8**	-	**48.0**	-	**<DL**	-	**<DL**	-	**<DL**	-	**<DL**	-	**<DL**	-	**<DL**	-	**741.3**	-	**0.5**	-
Organic	OCP	Beta BHC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Organic	OCP	4,4'-DDE	13.1	0.1	<DL	-	19.5	3.2	10.9	9.9	13.78	0.03	-	-	-	-	-	-	-	-	-	-	<DL	-	<DL	-	<DL	-
Organic	OCP	Alpha BHC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Organic	OCP	Methoxychlor	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Organic	OCP	Delta BHC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Organic	OCP	Endosulfan Sulfate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Organic	OCP	Gamma BHC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Organic	OCP	4,4'-DDT	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Organic	OCP	Bêta Endosulfan	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Organic	OCP	Alpha Endosulfan	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Organic	OCP	Heptachlor	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Organic	OCP	Aldrin	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Organic	OCP	Dieldrine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Organic	OCP	Endrin Aldehyde	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Organic	OCP	Heptachlor Epoxide	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Organic	OCP	4,4'-DDD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	<DL	-	<DL	-	<DL	-
Organic	OCP	Endrin Ketone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Organic	OCP	Endrin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			13.1	-	**<DL**	-	**19.5**	-	**10.9**	-	**13.8**	-	**-**	-	**-**	-	**-**	-	**-**	-	**-**	-	**<DL**	-	**<DL**	-	**<DL**	-

			Ulva spp.										*Gelidium* spp.										Porifera					
			'WWTP 1'		'WWTP 2'		'WWTP 5'		Mean		'Control 3'		'WWTP 3'		'WWTP 4'		Mean		'Control 1'		'Control 3'		'WWTP 3'		'Control 1'		'Control 2''	
Substance families	**Analytical Groups**	**Analytes**	**Average**	**SD**	**Average**	**SD**	**Average**	**SD**	**Average**	**SD**	**Average**	**SD**	**Average**	**SD**	**Average**	**SD**	**Average**	**SD**	**Average**	**SD**	**Average**	**SD**	**Average**	**SD**	**Average**	**SD**	**Average**	**SD**
Emerging substances																												
Organic	Musk	HHCB	5.7	0.9	136.8	12.3	5.5	1.5	49.3	75.7	0.1	0.2	0.3	0.3	3.3	0.6	1.8	2.1	0.2	0.2	<DL	-	41.0	-	41.2	9.4	5.2	0.0
Organic	Musk	HHCB-lactone	-	-	-	-	-	-	-	-	-	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	-	-	-	-	-	-
Organic	Musk	AHTN	<DL	-	25.9	2.3	1.1	0.5	9.0	14.7	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Musk	MK	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Musk	ADBI	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Musk	ATII	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Musk	AHMI	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Musk	MX	0.4	0.1	6.6	0.2	<DL	-	2.3	3.7	0.5	0.2	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	37.5	-	<DL	-	6.0	0.0
Organic	Musk	MA	15.68	1.75	50.8	5.0	14.1	1.0	26.9	20.8	11.0	1.8	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Musk	MM	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	0.02	0.04	5.1	0.6	2.6	3.6	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
			21.8	-	**220.0**	-	**20.6**	-	**87.5**	-	**11.6**	-	**0.4**	-	**8.4**	-	**4.4**	-	**0.2**	-	**<DL**	-	**78.5**	-	**41.2**	-	**11.1**	-
Organic	Sunscreen	OC	4.9	0.1	25.2	6.4	41.8	16.9	24.0	18.5	110.8	23.6	16.1	6.7	3765.2	1837.1	1890.6	2651.0	<DL	-	1.3	1.3	<DL	-	52.6	6.0	<DL	-
Organic	Sunscreen	Benzophenone 3	43.2	3.8	17.0	3.7	7.2	0.5	22.5	18.6	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Sunscreen	4-MBC	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Sunscreen	EHMC	2.1	0.1	<DL	-	40.1	3.4	14.0	22.6	10.3	0.4	8.1	1.7	43.8	12.8			<DL	-	<DL	-	95.8	-	<DL	-	4.2	-
Organic	Sunscreen	3-BC	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Sunscreen	OD-PABA	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
			50.1	-	**42.2**	-	**89.1**	-	**60.5**	-	**121.1**	-	**24.2**	-	**3809.0**	-	**1890.6**	-	**<DL**	-	**1.3**	-	**95.8**	-	**52.6**	-	**4.2**	-
Organic	Pharmaceutical (Antihypertensive)	Hydrochlorothiazide	<DL	-	10.5	4.0	<DL	-	3.5	6.1	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	8.0	2.7	<DL	-	<DL	-
Organic	Pharmaceutical (Anxiolytics)	Oxazepam	<DL	-	96.8	3.1	<DL	-	32.3	55.9	<DL	-	<DL	-	9.1	0.7	4.5	6.4	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Psychotropic)	Caffeine	<DL	-	<DL	-	9.4	1.1	3.1	5.4	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	1.1	0.0	<DL	-	<DL	-
Organic	Pharmaceutical (Anti-inflammatory)	Diclofenac	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Organic	Pharmaceutical (Pain killer)	Ketoprofen	<DL	-	60.3	-	<DL	-	20.1	34.8	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Anticonvulsant)	Carbamazepine	<DL	-	8.8	0.9	<DL	-	2.9	5.1	<DL	-	<DL	-	3.5	-	1.7	2.5	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Antihypertensive)	Atenolol	<DL	-	38.4	0.2	2.3	0.01	13.6	21.5	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Antihypertensive)	Losartan	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Antibiotics)	Ciprofloxacin	<DL	-	6.6	3.6	<DL	-	2.2	3.8	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Anti-inflammatory)	Ibuprofen	12.8	-	67.8	-	<DL	-	26.9	36.0	<DL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Organic	Pharmaceutical (Antibiotics)	Sulfamethoxazole	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	-	-	-	-	-	-
Organic	Pharmaceutical (Antibiotics)	Ofloxacin	0.2	-	4.6	0.2	1.7	-	2.1	2.2	0.4	-	1.1	-	1.3	-	1.2	0.1	1.0	0.4	1.4	-	1.7	0.0	<DL	-	1.1	0.6
Organic	Pharmaceutical (Antibiotics)	Azithromycin	0.3	-	148.9	9.4	37.3	2.1	62.2	77.3	<DL	-	0.2	0.04	1.4	0.4	0.8	0.9	0.6	0.2	<DL	-	-	-	-	-	-	-
Organic	Pharmaceutical (Pain killer)	Niflumic acid	<DL	-	2.3	1.4	<DL	-	0.8	1.3	<DL	-	<DL	-	1.2	-	0.6	0.9	<DL	-	<DL	-	-	-	-	-	-	-
Organic	Pharmaceutical (Antibiotics)	Clarithromycine	<DL	-	1.9	0.1	<DL	-	0.6	1.1	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	-	-	-	-	-	-
Organic	Pharmaceutical (Pain killer)	Acetaminophen	1.0	0.2	<DL	-	1.3	0.2	0.8	0.7	0.1	0.002	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Glycemia)	Gemfibrozil	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Organic	Pharmaceutical (Antiarrhythmic)	Metoprolol	<DL	-	99.6	1.7	<DL	-	33.2	57.5	<DL	-	<DL	-	0.5	-	0.3	0.4	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Antibiotics)	Erythromycin A	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	-	-	-	-	-	-
Organic	Pharmaceutical (Antibiotics)	Roxithromycine	<DL	-	0.4	-	<DL	-	0.1	0.2	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	-	-	-	-	-	-
Organic	Pharmaceutical (Glaucoma)	Acetazolamide	<DL	-	<DL	-	0.5	0.3	0.2	0.3	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Antibiotics)	Trimethoprim	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Antibiotics)	Metronidazole	<DL	-	0.3	0.03	<DL	-	0.1	0.2	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Antibiotics)	Spiramycin	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	-	-	-	-	-	-
Organic	Pharmaceutical (Antibiotics)	Norfloxacin	<DL	-	5.2	0.9	10.7	-	5.3	5.3	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	-	-	-	-	-	-
Organic	Pharmaceutical (Anxiolytics)	Lorazepam	<DL	-	1.6	0.0	<DL	-	0.5	0.9	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Antibiotics)	Josamycin	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	-	-	-	-	-	-
Organic	Pharmaceutical (Anxiolytics)	Nordiazepam	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Pain killer)	Phenazone	<DL	-	11.8	-	7.2	-	6.3	5.9	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Antibiotics)	Piperacillin	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Antibiotics)	Tetracycline	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Anticancer)	Cyclophosphamide	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Antibiotics)	Sulfadiazine	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Antibiotics)	Flumequine	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Antibiotics)	Ampicilline	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	8.3	3.1	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Antibiotics)	Doxycycline	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	-	-	-	-	-	-
Organic	Pharmaceutical (Antibiotics)	Oxolinic acid	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Antibiotics)	Rifampicin	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	92.1	-	<DL	-	46.0	65.1	83.6	30.8	<DL	-	-	-	-	-	-	-
Organic	Pharmaceutical (Antibiotics)	Sulfamethazine	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Antibiotics)	Tylosine	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	-	-	-	-	-	-
Organic	Pharmaceutical (Hormones)	E2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Organic	Pharmaceutical (Hormones)	EE2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Organic	Pharmaceutical (Hormones)	E1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Organic	Pharmaceutical (Contraceptif)	19-Norethindrone	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Antiarrhythmic)	Amiodarone	21.3	2.0	16.6	0.7	<DL	-	19.0	3.3	24.0	1.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Organic	Pharmaceutical (Antibiotics)	Amoxicillin	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Organic	Pharmaceutical (Pain killer)	Acetylsalicylic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Organic	Pharmaceutical (Antineoplastic)	Hydroxycarbamide	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			35.7	-	**582.5**	-	**70.4**	-	**235.8**	-	**24.4**	-	**93.4**	-	**17.1**	-	**55.3**	-	**93.4**	-	**1.4**	-	**10.8**	-	**<DL**	-	**1.1**	-

Table 4: Mean concentrations of detected pharmaceutical compounds (emerging substances) (ng.g^{-1}) in *Holothuria* spp., *Mytilus* spp. and *Patella* spp. sampled at the WWTP effluents and at control locations (far from point source of pollution; 'Control 1', 'Control 2', 'Control 3'). Analyte mean concentrations were ordered according to the wastewater table. Significance codes: DL: Detection limit; QL: Quantification limit; '-': corresponds to molecules whose pre-analytical or analytical methods were not adapted to their quantification in that sample.

			Holothuria spp.								*Mytilus* spp.				*Patella* spp.													
			'WWTP 1'		'WWTP 3'		Mean		'Control 2'		'WWTP 2'		'Control 3'		'WWTP 1'		'WWTP 2'		'WWTP 4'		'WWTP 5'		Mean		'Control 1'		'Control 3'	
Substance families	**Analytical Groups**	**Analytes**	**Average**	**SD**	**Average**	**SD**	**Average**	**SD**	**Average**	**SD**	**Average**	**SD**	**Average**	**SD**	**Average**	**SD**	**Average**	**SD**	**Average**	**SD**	**Average**	**SD**	**Average**	**SD**	**Average**	**SD**	**Average**	**SD**
Emerging substances																												
Organic	Pharmaceutical (Antihypertensive)	Hydrochlorothiazide	<DL	-	<DL	-	<DL	-	<DL	-	-	-	-	-	<DL	-	0.9	0.1	<DL	-	1.7	-	0.7	0.8	<DL	-	<DL	-
Organic	Pharmaceutical (Anxiolytics)	Oxazepam	<DL	-	<DL	-	<DL	-	<DL	-	16.5	-	<DL	-	<DL	-	13.3	1.6	<DL	-	0.0	-	3.3	6.7	<DL	-	<DL	-
Organic	Pharmaceutical (Psychotropic)	Caffeine	<DL	-	5.2	0.1	2.6	3.7	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	46.6	0.7	11.6	23.3	<DL	-	<DL	-
Organic	Pharmaceutical (Anti-inflammatory)	Diclofenac	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Organic	Pharmaceutical (Pain killer)	Ketoprofen	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Organic	Pharmaceutical (Anticonvulsant)	Carbamazepine	<DL	-	<DL	-	<DL	-	<DL	-	2.2	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Antihypertensive)	Atenolol	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Antihypertensive)	Losartan	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Antibiotics)	Ciprofloxacin	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Anti-inflammatory)	Ibuprofen	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Organic	Pharmaceutical (Antibiotics)	Sulfamethoxazole	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Antibiotics)	Ofloxacin	0.8	0.8	1.0	-	0.9	0.1	<DL	-	2.5	-	<DL	-	<DL	-	4.0	-	<DL	-	<DL	-	1.0	2.0	<DL	-	<DL	-
Organic	Pharmaceutical (Antibiotics)	Azithromycin	1.6	1.8	1.8	0.6	1.7	0.1	1.1	1.4	3.1	-	1.1	0.6	0.3	0.1	14.0	1.5	7.7	1.8	10.3	3.1	8.1	5.8	1.8	0.4	0.7	0.3
Organic	Pharmaceutical (Pain killer)	Niflumic acid	2.2	2.2	1.3	0.7	1.7	0.7	1.0	0.1	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Antibiotics)	Clarithromycine	<DL	-	<DL	-	<DL	-	<DL	-	0.6	-	<DL	-	<DL	-	2.2	0.6	0.2	0.1	3.1	0.4	1.4	1.5	<DL	-	<DL	-
Organic	Pharmaceutical (Pain killer)	Acetaminophen	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	32.1	1.6	8.0	16.1	<DL	-	<DL	-
Organic	Pharmaceutical (Glycemia)	Gemfibrozil	-	-	-	-	-	-	-	-	-	-	-	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Antiarrhythmic)	Metoprolol	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	3.6	1.0	<DL	-	<DL	-	0.9	1.8	<DL	-	<DL	-
Organic	Pharmaceutical (Antibiotics)	Erythromycin A	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Antibiotics)	Roxithromycine	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Glaucoma)	Acetazolamide	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Antibiotics)	Trimethoprim	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Antibiotics)	Metronidazole	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Antibiotics)	Spiramycin	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	0.8	-	<DL	-	0.2	0.4	<DL	-	<DL	-
Organic	Pharmaceutical (Antibiotics)	Norfloxacin	<DL	-	<DL	-	<DL	-	<DL	-	16.0	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Anxiolytics)	Lorazepam	<DL	-	<DL	-	<DL	-	0.8	0.4	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Antibiotics)	Josamycin	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Anxiolytics)	Nordiazepam	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Pain killer)	Phenazone	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Antibiotics)	Piperacillin	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Antibiotics)	Tetracycline	<DL	-	<DL	-	<DL	-	1.0	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Anticancer)	Cyclophosphamide	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Antibiotics)	Sulfadiazine	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Antibiotics)	Flumequine	<DL	-	0.4	0.1	0.2	0.3	0.2	0.03	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Antibiotics)	Ampicilline	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Antibiotics)	Doxycycline	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Antibiotics)	Oxolinic acid	<DL	-	<DL	-	<DL	-	0.5	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Antibiotics)	Rifampicin	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Organic	Pharmaceutical (Antibiotics)	Sulfamethazine	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Antibiotics)	Tylosine	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Hormones)	E2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Organic	Pharmaceutical (Hormones)	EE2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Organic	Pharmaceutical (Hormones)	E1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Organic	Pharmaceutical (Contraceptif)	19-Norethindrone	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-	<DL	-
Organic	Pharmaceutical (Antiarrhythmic)	Amiodarone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Organic	Pharmaceutical (Antibiotics)	Amoxicillin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Organic	Pharmaceutical (Pain killer)	Acetylsalicylic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Organic	Pharmaceutical (Antineoplastic)	Hydroxycarbamide	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			4.7	-	**9.6**	-	**7.1**	-	**4.6**	-	**41.1**	-	**1.1**	-	**0.3**	-	**38.1**	-	**8.7**	-	**93.8**	-	**35.2**	-	**1.8**	-	**0.7**	-

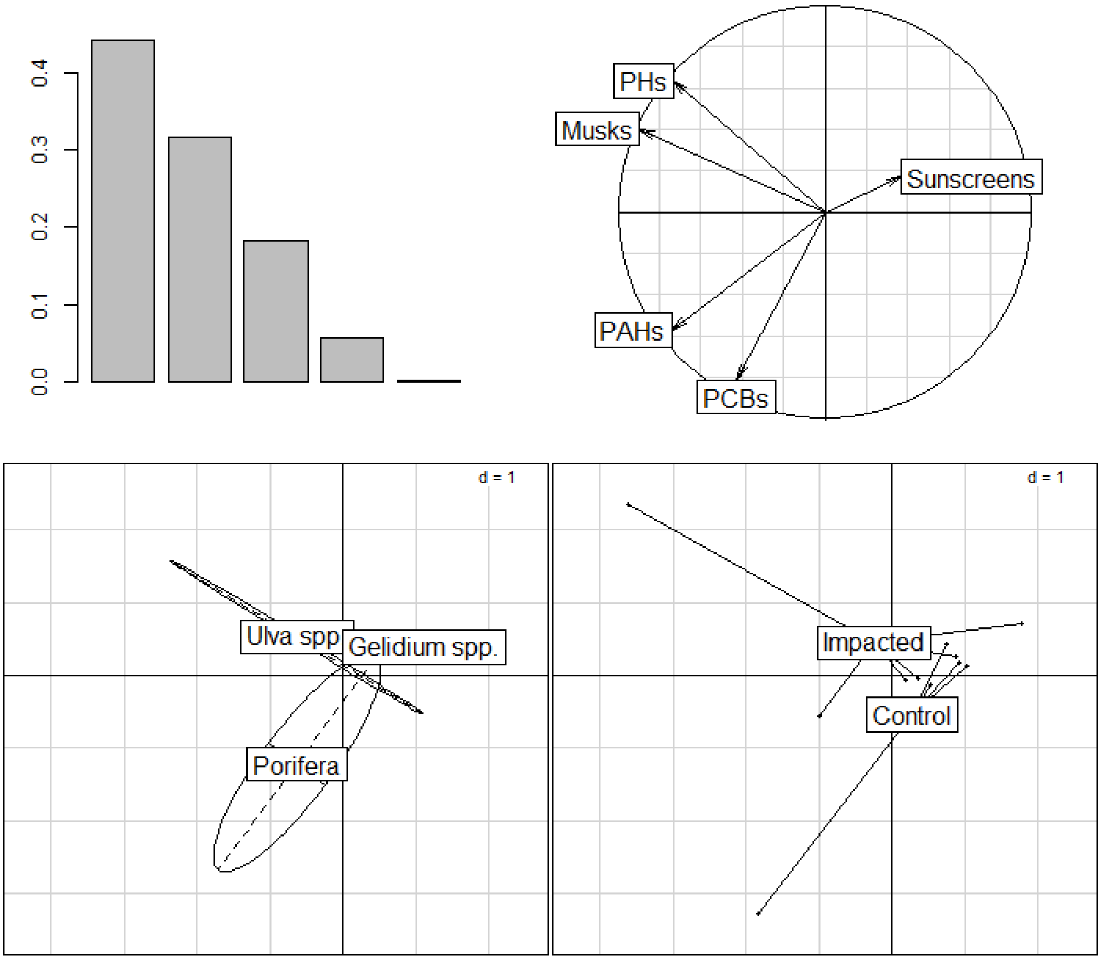


Fig. 5: Principal component analysis (PCA) showing analytical group distribution between marine organisms sampled in both impacted and control locations. PHs: Pharmaceuticals.

4. Discussion

4.1 Characterization of WWTP effluents

4.1.1 Occurrence of micropollutants in WWTP effluents

Generally, a large number of publications was achieved on metal, alkylphenol, musk and pharmaceutical analyses (**see Table 1 in Chapter I**). By contrast, studies on PCBs and OCPs were scarce as it was already highlighted by other authors (Deblonde et al., 2011; Miège et al., 2009). The interest of the scientific community in studying these specific molecules may be linked to their important probability of occurrence and great concentrations already detected in urban discharges which allow to ensure their detection despite the cost and the time these analyses required.

Metal compounds

Among analytes detected in this study, those having the highest mean concentrations in WWTP discharges were two metals, Vanadium and Chromium with mean concentrations near 5 000 ng.L^{-1}

(**Table 2**). Generally, these refractory metals are used in alloys of stainless steels to increase hardness of steel and impact resistance (Aygün et al., 2019). Their concentrations found in this study go against other studies which found these substances in lower concentrations than the other metal compounds such as Nickel, Copper and Arsenic (Busetti et al., 2005). For example, Vanadium was found in the present study in much higher concentrations than that analyzed in WWTP discharges located close to the city of Venice in Italy (4 884.7 ng.L^{-1} *vs*. 500-2 200 ng.L^{-1}) (Busetti et al., 2005). This could surprise in view of the higher nominal flow from urban and industrial wastes compared to those in the present study (100 000 m^{3}.day^{-1} *vs*. from 4 149 to 41 070 m^{3}.day^{-1}). By contrast, Chromium was found in similar concentrations to those found in this same study even if it was reported in much higher concentrations in another case in India (20 000- 370 000 ng.L^{-1}) (Singh et al., 2004) but where the nominal flow was between 5 to 80 million liters per day. In the present study, no metal was detected with concentrations exceeding legislation limits (EC, 2013) although two priority substances (Nickel and Lead) and two priority hazardous substances (Cadmium and Mercury) were identified.

Generally, heavy metals were already described in the literature as non-biodegradable substances and their removal from aqueous solutions as rather challenging (Rajasulochana and Preethy, 2016). Even if conventional technologies (e.g. flocculation/coagulation, precipitation, adsorption, activated charcoal, ion exchange resins and membrane filtration) are able to remove a great portion of metals from influents (Busetti et al., 2005), their performances encounter difficulties especially in case of very high concentrations (Rajasulochana and Preethy, 2016; Rezania et al., 2016). Indeed, in the present study, metals appeared as the most frequently detected (occurrence=100%) and concentrated analytical group (mean concentrations > 2 000 ng.L^{-1}) (**Table 2**). But, as stated by Busetti et al. (2005), variations in metal concentrations may be characteristic of household effluents and their high concentrations could be associated to the resuspension of pipe sediments deposited in the sewerage.

Pharmaceutical substances

Four pharmaceuticals also highly contributed to the high total mean concentration of pharmaceutical group (Hydrochlorothiazide, Oxazepam, Caffeine and Diclofenac) (**Table 2**). Their mean concentrations were ranged from 917.3 ng.L^{-1} to 1 544.7 ng.L^{-1} which corresponds to those found in the literature (**Table 2**; **Table 1 in Chapter I**). Indeed, Hydrochlorothiazide was found between 504.7 to 3 228.4 ng.L^{-1} in the present study (**Table 2**), around 439.1 ng.L^{-1} in another one achieved on nine urban Italian WWTPs (Zuccato et al., 2005) and around 2 800 ng.L^{-1} on 50 plants in US (Kostich et al., 2014). This diuretic compound, often associated to other anti-hypertensives for long-term treatments, is essentially eliminated from the plasma unchanged in the urine (with a half-life time of 6 to 15h) (http://www.vidal.fr). This could thus explain its high concentration in sewages. Theorically, once administrated, pharmaceuticals are metabolized to varying degrees and are excreted as the parent compound (in large part) or as metabolites (Deblonde et al., 2011; Lishman et al., 2006; Verlicchi et al., 2012; Zuccato et al., 2005). In fact, the parent drug is converted into a more polar metabolite on which glucuronic acid, sulphuric acid or acetic acid are then added to increase the ability to be excreted (Deblonde et al., 2011). But, in the case of Hydrochlorothiazide, this molecule is essentially eliminated unchanged and it is thus not surprising to find the original compound in urban discharges.

Oxazepam had a mean concentration equal to 1 421.6 ng.L^{-1} (with a range from 139.6 to 2 911.4 ng.L^{-1}) (**Table 2**). In a study where 90 WWTPs across Europe were analyzed, the maximum detected concentration was 1 766 ng.L^{-1} while the average was around 162 ng.L^{-1} (Loos et al., 2013). No information was available concerning the plant which present this maximum concentration. Therefore, it is only possible to confirm the possibility to find such concentrations in WWTP effluents. Furthermore, this substance, prescribed as anxiolytic (Seresta product), is described as being renally eliminated at 90% as glucuronide (inactive metabolite) (http://www.vidal.fr). Therefore, only a very small percentage of Oxazepam (parent compound) is finally found in the urine.

Caffeine was detected in this study in mean concentrations ranging from 26.9 to 12 360.5 ng.L^{-1} (with a median at 458.6 ng.L^{-1}) (**Table 2**). These concentrations are lower (but still included in the range) than those found in some other studies (Batt et al., 2006; Loos et al., 2013; Santos et al., 2009), especially those from (Baker and Kasprzyk-Hordern, 2013). They found concentration up to 34 198.3 ng.L^{-1} (with a median at 1 744.2 ng.L^{-1}) in samples from seven WWTPs in England (serving a population from 9 967 to 244 205). By contrast, these concentrations appeared much higher than those found in effluents from four WWTPs sited in Seville city (mean concentrations from 80 to 370 ng.L^{-1}) while their capacities were between 200 000 to 950 000 equivalent inhabitants which is 2 to 10 times higher than those of the present study (Martín et al., 2012). Nevertheless, Batt et al. (2006) which detected Caffeine from 190 to 9 900 ng.L^{-1} in effluent samples mentioned that these concentrations were similar to those previously reported in wastewater effluent which correspond also to those found in the present studies. In addition, the substance was reported as readily biodegradable (Gómez et al., 2007; Thomas and Foster, 2005) and with a very high removal rate (around 97%) with a final concentration in the effluent exceeding not 1 770 ng.L^{-1} compared to a mean at 56 630 ng.L^{-1} in the influent (Deblonde et al., 2011). Caffeine is supposed to be completely metabolized in the liver after its consumption (http://www.vidal.fr).

Finally, Diclofenac was identified with a mean concentration at 917.3 ng.L^{-1} (**Table 2**). This substance was already widely documented in WWTP effluents with concentrations ranging from 1.0 to 2 830 ng.L^{-1} (**see Table 1 in Chapter I**). It was described as less hydrophobic compounds compared to some others (triclosan estradiol, estrone, etc.) and highly biodegradable compounds under aerobic conditions but persistent in anoxic conditions (Verlicchi et al., 2012). When this substance, is administrated as anti-inflammatory, less than 1% is eliminated unchanged in the urine and the rest is eliminated through the biliary tract as glucuronide conjugate metabolites (http://www.vidal.fr). Therefore, 3 of the 4 pharmaceuticals detected in this study in high concentrations should not be present as parent compounds in discharges considering their elimination process by the human body. Indeed, they supposed to be almost completely or totally metabolized. This raises the question to the quantity consumed by local people or to the source of these compounds explaining such concentrations in urban discharges if it does not come from medical consumption and excretion. For example, this could suggest that Caffeine could be widely directly released into the water system even before their ingestion. Other sources should thus be investigated to explain and ideally mitigate the discharge into the sewer system (and finally in the aquatic environment) of such substances. This is to enhance by the fact that, up to now, no regulation exist for these compounds. Moreover, another question may arise about the quantity of discharged metabolites in the environment because they would certainly found in much higher concentrations than parent compounds.

Generally, the high concentration of pharmaceuticals in the effluents is align with most articles which generally identified these compounds in the µg/L range in such matrices (Deblonde et al., 2011; Zuccato et al., 2005). These high amounts may be explained by their high consumption by modern society and their continuous introduction in the aquatic environment through WWTP discharges considered as important sources of contamination (Bueno et al., 2012; Zuccato et al., 2005). France was also reported by the British Government as the third European country (on the 14 studied) consuming more drugs (Bueno et al., 2012; Richards, 2010). Mainly due to the great variability of their chemical and physical properties (i.e. solubility, volatility, adsorbability, absorbability, biodegradability, polarity and stability), pharmaceuticals were reported as not efficiently removed by common WWTPs (i.e. only partially eliminated during the secondary treatment) (Bueno et al., 2012; Verlicchi et al., 2012) which explains that they appeared as the second highest concentrated analytical group (after metals) in the present study (mean concentrations > 2 000 ng.L^{-1}) (**Table 2**). For all these reasons and in addition to their possible effects on wildlife and humans, pharmaceuticals are considered as "emerging contaminants" (Bueno et al., 2012).

Musk compounds

HHCB and HHCB-lactone (oxidation product of HHCB) were the two polycyclic musks identified in highest mean concentrations among other musk compounds (1 438.6 ng.L^{-1} and 561.4 ng.L^{-1}, respectively) (**Table 2**). Indeed, polycyclic musks are used in many consumer products (Reiner and

Kannan, 2006), their production and usage increased the last decade contrary to nitro musks (found in lower concentrations in this study) (Gatermann et al., 2002; Sommer, 2004) and 77% of them are estimated to go down the drain and then in WWTPs. As in the present study, HHCB was reported as predominant polycyclic musks in wastewater explained by its greater production and usage compared with AHTN for example (found in lower concentrations) (Horii et al., 2007; HERA, 2004). But, this finding runs counter to its removal efficiency identified between 72 and 98% due to its lipophilic nature inducing its adsorption to particles captured in sewage sludges (Artola-Garicano et al., 2003; Carballa et al., 2004; Horii et al., 2007; Simonich et al., 2002). Consequently, their concentrations were either much higher in influents than those found in effluents or their removal was not efficient as the removal highly depends on the type of treatment process (J. Reiner et al., 2007). Indeed, even if these concentrations correspond to those found in the literature, they widely varied between studies and WWTPs (from 10 to 7 030 ng.L^{-1} for HHCB and from 66 to 4 000 ng.L^{-1} for HHCB-lactone) (**see Table 1 in Chapter I**). Moreover, even though HHCB-lactone was reported as increasing following the treatment (by oxidation of HHCB during the activated sludge process), it was anyway identified in the present study in lower concentrations than HHCB which is consistent with other studies (Horii et al., 2007; J. Reiner et al., 2007). Indeed, influents were not identified as direct sources of HHCB-lactone; only the oxidation processes within treatment plants were reported as generating this compound, explaining thus their presence in effluents (Horii et al., 2007).

Generally, the musk group was identified as the third group having the highest mean concentrations (mean concentrations > 2 000 ng.L^{-1}) (**Table 2**). Originally, the natural musk substance was contained by the exocrine glands of the male musk deer (*Moschus moschiferus*) (Lee et al., 2003). Now endangered, the natural substance was substituted by synthetic musk fragrances (Lee et al., 2003). Polycyclic musks (HHCB and its HHCB-lactone metabolite, AHTN, AHMI, ADBI, ATII) and nitro musks (MX and MK) constitute nowadays fragrance components widely used in household and personal-care products such as detergents, soaps, softeners, shampoos, shaving foams, etc. (Chase et al., 2012; Lee et al., 2003). Their high consumption associated to their hydrophobic and lipophilic nature (i.e. low water solubility due to $\log K_{ow}$ ranging from 4.3 to 5.9), explain their high concentrations in WWTP discharges at the end of their lifecycle, their tendency to be bioconcentrated and their difficulty to be biodegraded (Lee et al., 2003; Lishman et al., 2006). Moreover, it was also described that, sometimes, their concentrations could be higher in effluents than in influents (Chase et al., 2012). This phenomenon was attributed to the back transformation of metabolites to parent compounds through biotic and/or abiotic activities during treatments (Biselli et al., 2004; Chase et al., 2012; Jjemba, 2008; Karnjanapiboonwong et al., 2011; J. Reiner et al., 2007; J. L. Reiner et al., 2007; Ternes et al., 1999) although the increasing concentration of HHCB-lactone metabolite throughout treatments was anyway described by other authors (J. Reiner et al., 2007; J. L. Reiner et al., 2007). Furthermore, their removal by sludge treatments (through adsorption) influenced by their features could explain their lower concentrations in effluents that the two previous analytical groups (Lee et al., 2003). But, as highlighted by Chase et al. (2012), their ability to be eliminated by plants is highly linked to the size and the processes used and the type and origin of wastewater. Up to now, no regulatory limits have been set for musks compounds.

Other main substances detected per analytical group

Nonylphenol (NP) was found with a mean concentration at 573.6 ng.L^{-1} and values ranging from 19.0 to 1 449.4 ng.L^{-1} (**Table 2**). Even if this range varied widely among plants, concentrations never exceeded the regulation limit fixed at 2 000 ng.L^{-1} (MAC-EQS; EC, 2013). This variability and these extreme values were already reported in the literature (from <30 to 37 000 ng.L^{-1}; **see Table 1 in Chapter I**) which was attributed to plant designs and their efficiencies (Ying et al., 2002). For example, a such range was also found in a unique study achieved in Michigan on four WWTPs (Snyder et al., 1999). NP is one metabolite (i.e. degradation product) of the Alkylphenol ethoxylate (APEs) which is widely used in surfactants in industrial and domestic products (e.g. detergents, OCP formulations, foaming agents, wetting agents, dispersants, emulsifiers, solubilizers) (Ying et al., 2002). They were identified as ubiquitous in the environment, resistant to biodegradation and common in wastewater effluents while conventional biological treatments should normally efficiently treat them (due to their hydrophobic nature) (Johnson and Sumpter, 2001; Navarro et al., 2009; Ying et al., 2002). This could thus explain their concentrations lower than those of previous analytes. In another study, the concentration of phenolic compounds (such as NP) was reported as higher in the effluent than in the influent (Nie et al., 2012). Indeed, they identified a notable increase after passing through the aerated grit chamber and suggested then that it would probably due to the peeling off of the grit due to agitation. Consequently, the presence of NP in WWTP discharges could be either due to inefficient treatments for its removal or to its release during the treatment process. Another AP substance was stated as priority substance (4tOP) but it never exceed the regulatory limit during this study (EC, 2013).

OC (a sunscreen) and Naphthalene (a PAH) were found as main analytes in their group but were detected in much lower concentrations than those previously described (i.e. from 31.5 to 175.6 ng.L^{-1}) (**Table 2**). Compared to concentrations found in the literature, OC was found slightly more concentrated (175.6 ng.L^{-1} vs. from 0 to <60 ng.L^{-1}; **see Table 1 in Chapter I**). But, it is important to note that this substance varied considerably between plants (from <DL to 2 334.0 ng.L^{-1}). Bueno et al. (2012) also reported such variations for this compound and mentioned that it would depend on the intensity of recreational activities and on season (Giokas et al., 2007). Moreover, OC was describe as one of the

most hydrophobic sunscreens (as well as ODBAPA, IAMC and EHMC) (Rodil et al., 2012). Consequently, in this same study, they did not found these compounds in wastewater samples likely because they are rapidly absorbed on the particulate matter, which could anyway explain that OC had a lower median in the present study (21.2 ng.L^{-1}). Up to now, no regulatory limits have been set for sunscreens.

PAHs were already described as very often picked up by activated sludge treatment which represents the treatment employed in three of the five studied WWTPs (those having also the highest inhabitant equivalents) (Deblonde et al., 2011). Naphthalene was found in lower concentrations than those reported in the literature (31.5 ng.L^{-1} vs. from 101 to 3 450 ng.L^{-1}; **see Table 1 in Chapter I**) and than the regulation limits fixed at 130 000 ng.L^{-1} for the MAC-EQS. Indeed, the maximum obtain in the present study (147.7 ng.L^{-1}) approximately corresponds to the minimum found in the literature (**Table 2**). The reported maximum corresponded to effluents from a WWTP located in a heavily industrialized area, receiving thus domestic as well as industrial raw wastewater, for approximately 30 000 m^{3}.day^{-1} (Sánchez-Avila et al., 2009), which corresponds to 3 times the maximum nominal flow reported in the present study (i.e. at 'WWTP 2'). Among other PAHs found in lower concentrations, 6 were stated as priory hazardous substances (Anthracene, Benzo[a]pyrene, Benzo[b]fluoranthene, Benzo[g, h, i]perylene, Benzo[k]fluoranthene and Indeno[1, 2, 3-cd]pyrene) and one as priority substances (Fluoranthene). Only Benzo[g,h,i]perylene presented a mean concentration exceeding the MAC-EQS (2.2 ng.L^{-1} *vs*. 0.82 ng.L^{-1}). The maximum was reached by 'WWTP 1' with a mean concentration up to 22.8 ng.L^{-1} (**SM 4**). In addition, the maximum of at least one WWTP ('WWTP 3' and 'WWTP 4', respectively) exceeded the MAC-EQS for Benzo[b]fluoranthene and Beta BHC (fixed at 17 ng.L^{-1} and 20 ng.L^{-1}, respectively) (**Table 2**; **SM 4**). But, even if their maximum reached or exceeded these limits, their means were anyway below the EQS.

Finally, PCB and OCP substances were found in very low concentrations (mean concentrations <2.0 g.L^{-1}) (**Table 2**). Due to their chlorine content which attribute them a low water solubility, they were already described as very often picked up by activated sludge treatment which represents the treatment employed in three of the five studied WWTPs (those having also the highest inhabitant equivalents) (Deblonde et al., 2011; Man et al., 2018; Pham and Proulx, 1997; Sánchez-Avila et al., 2009). Concentrations were less or equivalent to those found in the literature (**see Table 1 in Chapter I**) which also corroborates with findings of Sánchez-Avila et al. (2009) who did not found PCB even in effluents from a heavily industrialized area. Moreover, a decrease of the concentrations of most OCPs has also already been demonstrated throughout the treatment processes (Man et al., 2018). Nine OCPs were stated as priority hazardous substances (Alpha BHC, Alpha endosulfan, Beta BHC, Bêta endosulfan, Delta BHC, Endosulfan sulfate, Gamma BHC, Heptachlor, Heptachlore epoxide) and 6 other were only assigned to EQS but not classified in such a category (Aldrin, Dieldrine, Endrin, 4,4'-DDD, 4,4’-DDE and 4,4’-DDT) (**Table 2**). By contrast, up to now no regulation limit exist for PCB in surface waters.

4.1.2 Temporal variability

Significant differences were highlighted between sampling months (except for organomercury compounds), especially between August samples (with the highest total concentrations), and those from July and December (**Fig. 3**; **SM 3**). Even if some parameters (e.g. temperature, precipitation rate, solar radiation, pH) could influence or greatly affect the amount of certain substances in wastewater, their variations were not enough important to explain these differences (**Table 1**; **SM 1**). Indeed, pH was identified as affecting the removal efficiency of some pharmaceuticals, with a higher removal efficiency under acidic conditions (at pH 5). At this pH, compounds mainly in their hydrophobic form, are more readily adsorb during treatments (Deblonde et al., 2011; Verlicchi et al., 2012). But, in the present study, the pH only varied from 6.3 to 8.9 and the minimum occurred in August (**Table 1**; **SM 1**). Other studies also described lower efficiencies during colder seasons (Vieno et al., 2005). But, as previously, this runs counter to the present results which presented higher concentrations during summer. Therefore, differences were mainly associated to rainfall and flow rates (lower in August than in July), suspended matter (higher in August than in other sampling months) and summer overcrowding. Indeed, the population along the Basque coast considerably increases during summer. No data were found about population of studied cities but, for example, the population varied from 25 480 in winter to 110 000 in summer in Biarritz (a neighboring city) (https://ville.biarritz.fr). In addition, the rainfall was reported as lower in August than in July (99.2 mm *vs*. 124.9 mm during the 5 days preceding the sampling; **SM 1**). Effluents were thus more concentrated in August and more diluted in July. This is confirmed by the daily flow in entry which was also lower in August than in July. Finally, the concentrations of suspended matter supported also these results because they were much more concentrated in August than in other sampling months (20.79 mg.L^{-1} vs. from 9.17 to 12.08 mg.L^{-1}) (**SM 1**). As previously explained, micropollutants seemed to be mainly absorbed on the particulate matter due to their hydrophobic nature (differences between unfiltered and filtered samples) (**SM 2**). It is thus not surprising to find higher micropollutants concentrations the month where suspended matter values were higher (i.e. in August) (Lee et al., 2003; Lishman et al., 2006; Man et al., 2018; Pham and Proulx, 1997; Rodil et al., 2012; Sánchez-Avila et al., 2009; Stackelberg et al., 2007).

4.1.3 Spatial variability

Significant differences were identified between WWTPs (**Fig. 3**; **SM 3** and **4**). Metals were found in higher concentrations in ‘WWTP 3’ effluents than in other WWTPs and pharmaceuticals (especially Caffeine) were also found in higher concentrations in ‘WWTP 3’ effluents than in those of ‘WWTP 1’.

The same occurred for sunscreens and organomercury compounds (more concentrated at 'WWTP 3' compared to other WWTPs) even if differences between WWTPs were not significant. Even if 'WWTP 3' had a lower capacity than 'WWTP 4' and 'WWTP 2' (40 000 inhabitant equivalent *vs*. 45 000 and 78 217, respectively), it had a separate sewer system (at 99%) (**Table 1**). Effluents were thus not diluted by rainwater which could partly explain the higher concentrations detected in 'WWTP 3'. 'WWTP 1' also presented a separate system but, compared to 'WWTP 3', its capacity was 4 times lower than 'WWTP 3' (**Table 1**). In addition, 'WWTP 1' possessed a membrane filtration and a tertiary UV treatment processes operating mainly in summer (**Table 1**). These two treatment methods were identified as capable to remove suspended solids, organic compounds and inorganic contaminants such as heavy metals (Gunatilake, 2015) and as efficient for the removal of some few pharmaceuticals such as Ketoprofen and Diclofenac (Kim et al., 2009), respectively. Indeed, these compounds were detected in lower concentrations in 'WWTP 1' than in other WWTPs, especially than in 'WWTP 3' where the highest concentrations were found (**SM 4**). Moreover, 'WWTP 1', 'WWTP 2' and 'WWTP 4' presented an activated sludge process contrary to 'WWTP 3' (**Table 1**). This secondary biological treatment where microorganisms play a role of breaking down organic material with aeration and agitation and then settling solids in the solution, was supported by most of the research on heavy metals removal in biological system (Gunatilake, 2015). It was also identified as having the capacity to remove some pharmaceuticals such as Caffeine and Diclofenac with a removal rate > 80%, > 70%, respectively, Atenolol with a removal rate around 50% and sunscreens (> 86%) (Bueno et al., 2012; Wang and Wang, 2016). This is in line with results of the present study where these compounds were always found in lower concentrations in samples from WWTPs having such a treatment process (i.e. 'WWTP 1', 'WWTP 2' and 'WWTP 4') (**SM 4**). But, these compounds represent anyway the main analytes found in the effluents despite this treatment applied. By contrast, according to Deblonde et al. (2011), Man et al. (2018), Pham and Proulx (1997), Sánchez-Avila et al. (2009) and Stackelberg et al. (2007), Hydrochlorothiazide, PAHs, PCBs and OCPs were also supposed to be efficiently removed by this process due to their hydrophobic nature. This runs counter to the present results which identified the higher concentrations in plants having a sludge treatment process. The high mean values of suspended matter in 'WWTP 3' (29.12 mg.L^{-1} with a maximum at 46.38 mg.L^{-1} in August) compared to the other WWTPs (from 1.14 to 13.53 mg.L^{-1}) may be a sign of treatment process insufficiency (i.e. biofiltration) (**Table 1**). This could be due to sewerage plugging, which may be influenced by water inflows getting into the treatment network located before the plant. Indeed, this latter supposition could be confirmed by the flow rate which seemed to be influenced by heavy rainfalls while it was supposed to be separated at 99% (**SM 1**). These high suspended matter values associated to the high nutrient concentrations could thus explain the high concentrations of some micropollutants in the 'WWTP 3' effluents. Indeed, it has been found that a large amount of micropollutants were associated to the particulate phase (**SM 2**). This concerns especially hydrophobic substances which have a low solubility and thus a high affinity to organic matter (Campbell et al., 2006) such as several compounds found in this study: metals (24 557.2 ng.L^{-1} *vs*. from 15 458.9 to 19 383.4 ng.L^{-1}), organomercury compounds (9.1 ng.L^{-1} *vs*. from 0.7 to 2.3 ng.L^{-1}), considered as insoluble (https://pubchem.ncbi.nlm.nih.gov) and OC (701.4 ng.L^{-1} *vs*. from 9.3 to 78.1 ng.L^{-1}), described as the most hydrophobic sunscreens by Rodil et al. (2012) in 'WWTP 3' (**SM 4**). Furthermore, the high concentration of metals in 'WWTP 3' could finally be associated to the resuspension of pipe sediments deposited in the sewerage as it was already supposed in another study to explain high metal concentrations (Busetti et al., 2005). By contrast, the high suspended matter values found in 'WWTP 3' did not affect the other analytical groups (musks, APs, PAHs, PCBs and OCPs) which were in found higher concentrations at other WWTPs (**SM 4**). High pharmaceutical concentrations in 'WWTP 3' cannot be discussed with suspended matter values because samples were all filtered before analyses. But, looking at DOC values, those were in higher concentrations in 'WWTP 3' (8.70 mg.L^{-1} *vs*. from 4.34 to 6.88 mg.L^{-1}) (**Table 1**), therefore it is not surprising to find pharmaceuticals (analyzed only in the dissolved fraction) in higher concentrations in this WWTP. The reverse occurred in 'WWTP 1' where suspended matter, DOC values and pharmaceutical concentrations appeared as the lowest ones confirming that the membrane filtration technic was efficient to remove such particles and substances. It would be interesting to achieve further investigations on the number of connected hospitals and veterinary clinic in 'WWTP 3' and neighboring municipalities even though drug residues from hospitals could only represented 20% of the total drug load of the whole agglomeration (PILLS, 2012). Indeed, the wastewater origin is a fundamental parameter to take into account because some links were already observed between sources of wastewater and chemical pollutant concentrations (Deblonde et al., 2011). To date, there is not hospital in this area and only one nursing home and two veterinary clinics are present. These findings on the high micropollutant concentrations in 'WWTP 3' effluents (especially of metals and pharmaceuticals) could be thus the result of several factors: a separated sewer system in 'WWTP 3' contrary to the other WWTPs, a higher capacity (population equivalent) in comparison with another similar sewer system ('WWTP 1'), the absence of an activated sludge treatment and/or the inefficiency of the current applied biofiltration treatment (confirmed by high suspended matter and nutrient values) probably due to a malfunction of the sewer system before the WWTP. Considering the total volume rejected, the total amount of these analytical groups (metals, pharmaceuticals, sunscreens and organomercury) were still higher in 'WWTP 3' than in other WWTPs (**Fig. 4**; **Table 5** and **SM 4**).

Even if concentrations of other analytical groups were not identified as significantly different between WWTPs, 'WWTP 1' presented anyway higher concentrations of musks and PCBs than other WWTPs.

By contrast, PAHs appeared in higher concentrations in ‘WWTP 2’ effluents and OCPs as more concentrated in ‘WWTP 4’. But, once the total volume rejected considered and the daily flux estimated, ‘WWTP 1’ unloaded the lowest quantity of musks and PCBs than ‘WWTP 3’ (**Fig. 4**). Indeed, the concept of dilution (dependent to the plant capacity, the water flow and the number of inhabitants connected to the sewerage network) is important to consider because effluents can be more or less diluted and molecule concentrations may vary accordingly (Deblonde et al., 2011; Karthikeyan and Meyer, 2006). By contrast, flux estimations calculated for PAHs and OCPs, were always higher in ‘WWTP 2’ and ‘WWTP 4’, respectively (**Fig. 4**).

Finally, looking at the land use map (www.geoportail.fr), ‘WWTP 3’ and ‘WWTP 5’ areas were mainly occupied by agricultural zones or forests compared to those of ‘WWTP 1’ and ‘WWTP 4’, essentially occupied by urban zones. Furthermore, half of the ‘WWTP 2’ area was constituted by the urban tissue and half other by forests, grasslands and cropping systems. It would have been possible to image that municipalities presenting the bigger agricultural zones would present the highest pesticides concentrations. The same for other compounds such as musks, pharmaceuticals and sunscreens which would be supposed to be more concentrated in urban areas. But, results of the present work were not in line with this latter supposition. Indeed, ‘WWTP 4’ presented the highest pesticide concentrations (the municipality with the smaller agricultural area) and conversely, ‘WWTP 3’ exhibited the highest concentrations of pharmaceuticals and sunscreens while it constituted the municipality with the bigger agricultural zone.

4.2 Micropollutant concentrations in biota

4.2.1 Micropollutant concentrations in biota impacted by WWTP discharges

Contrary to studies achieved on wastewaters, much less works were done on the study of pharmaceutical compounds in benthic organisms (**see Table 2 in Chapter I**). In addition to the fact that analytical methods for quantifying these substances in such matrices are currently under development, this could be also explained by their rather high solubility in water and low lipophilicity contrary to metal, PAH, PCB, OCP, some musk and sunscreen compounds (Bueno et al., 2012; Carballa et al., 2004; Lee et al., 2003; Lishman et al., 2006; Man et al., 2018; Pham and Proulx, 1997; Rodil et al., 2012; Sánchez-Avila et al., 2009; Stackelberg et al., 2007). Indeed, pharmaceutical substances are less likely to accumulate in matrices such as sediments, sludges and biota. However, in the present study, pharmaceuticals were sometimes found in higher concentrations than other analytical groups which could run counter the latter supposition. In addition, metals and APs were not analyzed in this study. But, according to studies reported in the database, metals were the compounds identified in the highest concentrations in organisms even though no specific species was highlighted as the main accumulator of these compounds (**see Table 2 in Chapter I**).

Among analyzed compounds, pharmaceuticals and musks appeared in higher concentrations in *Ulva* spp. (**Fig. 5**; **Table 3**). Their concentrations were always higher in *Ulva* spp. sampled in the impacted locations (i.e. 'WWTP 2', 'WWTP 1' and 'WWTP 5') than in those sampled in the control location ('Control 3') (**Table 5**). Among other analytical groups detected in lower concentrations in this species, the same occurred for PAHs, found in higher concentrations in the impacted locations than in control (**Table 5**). Concentrations of these latter analytical groups were in line with daily flux estimations (of 'WWTP 2' and 'WWTP 1') (**Fig. 4**) because the highest concentrations were found in *Ulva* spp. sampled proximate to the WWTP having the highest daily flux estimation for corresponding substances ('WWTP 2' > 'WWTP 1') (**Table 5**). However, no comparison was possible between micropollutant concentrations found in biota sampled in 'WWTP 5' their daily associated flux due to the absence of information. Finally, compared to other organisms (*Gelidium* spp., *Patella* spp., *Mytilus* spp., *Holothuria* spp.) sampled in the same location ('WWTP 2', 'WWTP 1', 'Control 3'), *Ulva* spp. presented most of the time a higher total concentration of pharmaceuticals (except one time in 'WWTP 5' where *Patella* spp. presented a higher pharmaceutical concentration than *Ulva* spp.) (**Table 3**, **4** and **5**). For these reasons, *Ulva* spp. seemed to constitute a rather good bioaccumulator for musk, pharmaceutical and PAH compounds. Unfortunately, no bibliographic comparison was performed because lack of studies dealing with the bioaccumulation of these both analytical groups in this alga. In general manner, and according to its short life cycle and fast growing, it was anyway a little bit surprising to find such concentrations. Indeed, it constitutes an annual species, i.e. growing especially in spring and early summer when temperature and solar radiation increase drastically, thus during a short period (Cabioc'h et al., 2014). Therefore, its use as a sentinel bioaccumulator organism would be mainly focused to its favorable growth period. *Ulva* spp. is considered as opportunistic (i.e. sign of disturbances) (de Casamajor et al., 2016; Juanes et al., 2008), as tolerant to hypoxia and responding to nutrient enrichments (Anderson et al., 1996; Simboura and Zenetos, 2002). These features would facilitate its use in the evaluation of the impact of treated water discharges at organism level, in addition to the fact that its vegetative trait (foliose with all tissue photosynthetic) would favor the bioaccumulation of different compounds.

Sunscreens (especially OC) were mainly detected in *Gelidium* spp. (**Fig. 5**). The highest mean concentrations were found in the impacted locations ('WWTP 4' > 'WWTP 3') than in controls ('Control 3' > 'Control 1') (**Table 3** and **5**). Even if these results goes against daily flux estimations (those from 'WWTP 3' were higher than those from 'WWTP 4') (**Fig. 4**), this could anyway be explained by the location of the sampling area, located close to the big beach of 'WWTP 4'. Indeed, it is highly frequented zone during summer compared to 'WWTP 3' (located in the subtidal zone at the bottom of a cliff and far away from beaches). Among other substances found in lower concentrations in *Gelidium* spp., pharmaceuticals, musks and PAHs seemed anyway be align with concentrations found in the different WWTP discharges ('WWTP 4' and 'WWTP 3') (**Table 5**). Indeed, lowest concentrations were found in specimens collected at the outlet of the WWTP with the least flux estimation, and *vice versa*. In addition, *Gelidium* spp. was also identified as having a higher bioaccumulation capacity for pharmaceutical compounds (after *Ulva* spp.) than other organisms (*Patella* spp., *Holothuria* spp., Porifera) sampled in the same location ('WWTP 3', 'Control 1' and 'Control 3') (**Table 3** and **4**). By contrast, it seemed less likely to accumulate other compounds (musks, sunscreens and PAHs) than Porifera but the comparison was only possible in 'WWTP 3'. Finally, it was not surprising not find PCBs in *Gelidium* spp. because they were found in very low concentrations in WWTP discharges compared to previous compounds (from 9.3 to 25.3 mg.day^{-1} vs. from 55.4 to 70 430.8 mg.day^{-1}). Therefore, this suggest that *Gelidium* spp. could constitute a good bioaccumulator for these compounds in addition to the fact it constitute a perennial species. Particularly, *G. corneum* have been found in highest abundance in impacted locations and also as the main (together *Matacallophyllis laciniata*) responsible of dissimilarities between impacted and control subtidal locations (**see Chapter V**). Consequently, this species could constitute a good bioaccumulator as well as a good bioindicator from an ecological point of view.

Moreover, PAHs and PCBs were mainly found in Porifera (**Fig. 5**; **Table 3**). Contrary to analytical groups associated to preceding algae, they were found in higher concentrations in control locations than in the impacted one ('WWTP 3') even though Porifera from 'WWTP 3' presented anyway high PAHs concentrations (**Table 5**). By contrast, other analytical groups found in lower concentrations in Porifera (musks, sunscreens and pharmaceuticals) were anyway present in higher concentrations in the impacted location ('WWTP 3') than in controls ('Control 3' and 'Control 2'). But, compared to other organisms (*Gelidium* spp., *Holothuria* spp. and *Patella* spp.) sampled in same locations ('WWTP 3', 'Control 1' and 'Control 2'), Porifera appeared to less bioaccumulate pharmaceuticals. Only one impacted location was sampled for this species, consequently no firm conclusion could be drawn on the bioaccumulation potential of this species because no link was possible established with concentrations found in different WWTP discharges to confirm this. In the present study, the identification of this phylum was not made at the species level because it required additional competencies. But, it has been demonstrated that varying concentrations could be detected according to the Porifera species which are usually associated to symbiotic micro-organisms (for more than 40 % of their tissue) (Perez et al., 2002; Reiswig, 1981). Therefore, using Porifera as bioaccumulator seems interesting but rather difficult in view of its several technical drawbacks.

Among other sampled benthic organisms (*Mytilus* spp., *Patella* spp. and *Holothuria* spp.) which were only analyzed for pharmaceuticals, *Holothuria* spp. and *Patella* spp. appeared to follow WWTP flux estimations even though the detected concentrations were much lower than those found in the two previous algae (**Table 4** and **5**). Indeed, in a same location ('WWTP 1', 'WWTP 2', 'WWTP 3', 'WWTP 4', 'Control 1' and 'Control 3') these three species appeared to less bioaccumulate pharmaceuticals than *Gelidium* spp. and *Ulva* spp. (with one exception in 'WWTP 5' where *Patella* spp. presented a higher pharmaceutical concentration than *Ulva* spp.) (**Table 3** and **4**). Moreover, *Mytilus* spp. and *Patella* spp. seemed to similarly bioaccumulate pharmaceutical compounds (in 'WWTP 2') while *Holothuria* spp. appeared to more accumulate than *Patella* spp. (in 'WWTP 1'). However, it seemed anyway difficult to highlight such conclusions given the limited amount of sampled locations, especially for *Mytilus* spp. and *Holothuria* spp. even if they were already widely described as good bioaccumulators (**see Table 2 in Chapter I**). Indeed, looking at the literature, mussels appeared as the main bioaccumulator of micropollutants (musks, pharmaceuticals, PAHs, OCPs and sunscreens except PCBs) which confirmed that the scientific community was more interested in studying bioaccumulation of chemical substances in these organisms. But, in the present study, only pharmaceuticals were analyzed in mussels (i.e. *Mytilus* spp.) which appeared as the third organism accumulating the highest concentration of these compounds after *Ulva* spp. and *Gelidium* spp. Finally, the main drawback identified for this species was its absence (or the presence in too small individuals) in studied sites along the Basque coast. Therefore, this wild species seemed not to constitute a good bioaccumulator in this area.

A list of substances has been made to highlight common compounds in wastewaters and biota sampled proximate to outfalls (**Table 6**). A total of 14 priority substances (9 PAHs, 4 PCBs, 1 OCP) and 31 emerging substances (5 musks, 3 sunscreens, 23 pharmaceuticals) were highlighted. Among them, few were detected in all matrices (i.e. Chrysene, Fluoranthene, Pyrene, HHCB, EHMC and Ofloxacin). Moreover, substances detected in highest concentrations in wastewaters were not necessarily those detected in highest concentrations in biota samples.

4.2.2 Comparison with control locations

Even though organisms sampled in the impacted locations presented various concentrations of micropollutants, it has been noticed that, sometimes (for some organisms and analytical groups), control locations presented more concentrated organisms than those of impacted locations (**Table 3**, **4** and **5**). For example, this was the case for sunscreens, found in higher concentrations in *Ulva* spp. from 'Control 3' than in those from impacted locations ('WWTP 5', 'WWTP 1' and 'WWTP 2'). This high concentration could be related to the fact that this location constitutes an appreciated and frequented location by local people due to its remote location from populated areas (and thus from WWTP discharges). Furthermore, *Ulva* spp. from 'Control 3' presented also higher concentrations of PCBs and OCPs than those from 'WWTP 1' and 'WWTP 2' (**Table 5**). The same occurred for pharmaceuticals and PAHs in *Gelidium* spp. ('Control 1' > 'WWTP 4') and for PAHs and PCBs in Porifera ('Control 1' > 'WWTP 3') (**Table 5**). Consequently, this suggests that control locations were not as much un-impacted as supposed and were thus not totally free of pollution.

4.2.3 Micropollutant concentrations *vs*. regulatory limits

According to the regulatory limits, only concentrations of PAHs and PCBs have been compared to threshold values (i.e. in *Ulva* spp., *Gelidium* spp. and Porifera) because, up to now, no regulatory limits have been set for other substances. For OCPs, limits were already fixed but no comparison was possible in the present study because analyses were not adapted for the analysis of these substances in such matrices. Generally, as it is stated in the Directive, only Benzo[a]pyrene (a priority hazardous PAH) needs to be monitored for comparison with the biota EQS because it can be considered as a marker for other PAHs (Benzo[b]fluoranthene, Benzo[k]fluoranthene, Benzo[g,h,i]perylene and Indeno[1,2,3-cd]pyrene) (EC, 2013). But, in the present study, all Benzo[a]pyrene concentrations were below the detection limit whereas those of Benzo[k]fluoranthene and Benzo[b]fluoranthene were much higher (from 0.2 to 92.7 ng.g^{-1}) and thus often above the limit fixed at 5 ng.g^{-1} (**Table 3**). This was the case in *Ulva* spp., *Gelidium* spp. and Porifera in all sampled locations for Benzo[b]fluoranthene (except in *Gelidium* spp. from 'WWTP 4' and Porifera from 'WWTP 3' and 'Control 2') and in *Ulva* spp. from 'WWTP 2', *Gelidium* spp. from 'WWTP 3', 'WWTP 4', 'Control 1' and 'Control 3' and Porifera from 'Control 1' for Benzo[k]fluoranthene. By contrast, Fluoranthene, another PAH (identified as hazardous substance) having its own limit, was always found below the regulatory limit fixed at 30 ng.g^{-1}. Moreover, a regulatory limit was fixed at 0.0065 ng.g^{-1} for the sum of polychlorinated dibenzo-p-dioxins (PCDD), polychlorinated dibenzofurans (PCDF) and dioxin-like PCBs which were considered as priority hazardous substances. This limit was often exceeded except in *Gelidium* spp. in all locations (impacted and controls) and in Porifera from 'WWTP 3'.

Table 5: Summary of micropollutant concentrations detected in wastewater treatment plant discharges and in biota samples from control and impacted locations. A classification of locations was made according to concentrations found.

Analytical groups	Concentration ranges of samples from control locations	Concentration ranges of samples from impacted locations	Location classification according to found concentrations	Fit with flow estimations
Wastewater (ng.L^{-1})				
Metals		[24 557.2 - 15 458.9]	WWTP 3' > 'WWTP 4' > 'WWTP 2' > 'WWTP 1'	
Pharmaceuticals	-	[4 231.5 - 15 664.5]	WWTP 3' > 'WWTP 4' > 'WWTP 2' > 'WWTP 5' > 'WWTP 1'	-
Musks	-	[1 523 - 3 144.7]	WWTP 1' > 'WWTP 4' > 'WWTP 2' > 'WWTP 5' > 'WWTP 3'	-
Sunscreens	-	[30.1 - 746.2]	WWTP 3' > 'WWTP 4' > 'WWTP 5' > 'WWTP 2' > 'WWTP 1'	-
PAHs	-	[17.8 - 186.0]	WWTP 2' > 'WWTP 1' > 'WWTP 5' > 'WWTP 3' > 'WWTP 4'	-
PCBs	-	[3.0 - 19.1]	WWTP 1' > 'WWTP 3' > 'WWTP 5' > 'WWTP 4' - 'WWTP 2'	-
OCPs	-	[<DL - 14.8]	WWTP 4' > 'WWTP 5' > 'WWTP 1' > 'WWTP 2' > 'WWTP 3'	-
Organomercury	-	[0.7 - 9.1]	WWTP 3' > 'WWTP 5' > 'WWTP 2' > 'WWTP 4' > 'WWTP 1'	-
Wastewater daily flows (mg.day^{-1})				
Metals		[11 923.1 - 112 390.8]	'WWTP 3' > 'WWTP 2' > 'WWTP 4' > 'WWTP 1'	
Pharmaceuticals	-	[3 349.3 - 70 430.8]	'WWTP 3' > 'WWTP 2' > 'WWTP 4' > 'WWTP 1'	-
Musks	-	[2 608.7 - 10 441.2]	'WWTP 2' > 'WWTP 4' > 'WWTP 3' > 'WWTP 1'	-
Sunscreens	-	[115.8 - 3 543.9]	'WWTP 3' > 'WWTP 4' > 'WWTP 2' > 'WWTP 1'	-
PAHs	-	[55.4 - 1 135.9]	'WWTP 2' > 'WWTP 3' > 'WWTP 1' > 'WWTP 4'	-
PCBs	-	[9.3 - 25.3]	'WWTP 3' > 'WWTP 1' > 'WWTP 2' > 'WWTP 4'	-
OCPs	-	[<DL - 46.3]	'WWTP 4' > 'WWTP 2' > 'WWTP 1' > 'WWTP 3'	-
Organomercury		[0.7 - 40.7]	'WWTP 3' > 'WWTP 2' > 'WWTP 4' > 'WWTP 1'	-
***Ulva* spp. (ng.g^{-1})**				
Pharmaceuticals	24.4	[35.7 - 582.5]	'WWTP 2' > 'WWTP 5' > 'WWTP 1' > 'Control 3'	✓
Musks	11.6	[20.6 - 220]	'WWTP 2' > 'WWTP 1' > 'WWTP 5' > 'Control 3'	✓
Sunscreens	121.1	[50.1 - 89.1]	'Control 3' > 'WWTP 5' > 'WWTP 1' > 'WWTP 2'	*X*
PAHs	119.9	[129.2 - 257.9]	'WWTP 2' > 'WWTP 5' > 'WWTP 1' > 'Control 3'	✓
PCBs	48	[39.5 - 67.5]	'WWTP 5' > 'Control 3' > 'WWTP 2' > 'WWTP 1'	*X*
OCPs	13.8	[<LD - 19.5]	'WWTP 5' > 'Control 3' > 'WWTP 1' > 'WWTP 2'	*X*
***Gelidium* spp. (ng.g^{-1})**				
Pharmaceuticals	[1.4 - 93.4]	[17.1 - 93.4]	'WWTP 3' - 'Control 1' > 'WWTP 4' > 'Control 3'	✓
Musks	[<DL - 0.2]	[0.4 - 8.4]	'WWTP 4' > 'WWTP 3' > 'Control 1' > 'Control 3'	✓
Sunscreens	[<DL - 1.3]	[24.2 - 3 809]	'WWTP 4' > 'WWTP 3' > 'Control 3' > 'Control 1'	*X* (✓)*
PAHs	[21.6 - 22]	[18.9 - 26.9]	'WWTP 3' > 'Control 1' > 'Control 3' > 'WWTP 4'	✓
PCBs	<DL	<DL	-	✓
OCPs	-	-	-	-
Porifera (ng.g^{-1})				
Pharmaceuticals	[<DL - 1.1]	10.8	'WWTP 3' > 'Control 2' > 'Control 1'	-
Musks	[11.1 - 41.2]	78.5	'WWTP 3' > 'Control 1' > 'Control 2'	-
Sunscreens	[4.2 - 52.6]	95.8	'WWTP 3' > 'Control 1' > 'Control 2'	-
PAHs	[24.6 - 523.7]	451.6	'Control 1' > 'WWTP 3' > 'Control 2'	-
PCBs	[0.5 - 741.3]	<DL	'Control 1' > 'Control 2' > 'WWTP 3'	-
OCPs	<DL	<DL	-	-
***Mytilus* spp. (ng.g^{-1})**				
Pharmaceuticals	1.1	41.1	'WWTP 2' > 'Control 3'	-
***Patella* spp. (ng.g^{-1})**				
Pharmaceuticals	[0.7 - 1.8]	[0.3 - 93.8]	'WWTP 5' > 'WWTP 2' > 'WWTP 4' > 'Control 1' > 'Control 3' > 'WWTP 1'	✓
***Holothuria* spp. (ng.g^{-1})**				
Pharmaceuticals	4.6	[4.7 - 9.6]	'WWTP 3' > 'WWTP 1' > 'Control 2'	✓

** the high sunscreen concentrations were probably due to the proximity of Hendaye beach, highly frequented during summer, compared to 'WWTP 3' location located far away and in the subtidal zone.*

Table 6: Priority and emerging substances detected in wastewaters as well as in biota samples.

		Wastewaters	Biota samples					
			Ulva spp.	*Gelidium* spp.	Porifera	*Holothuria* spp.	*Mytilus* spp.	*Patella* spp.
	Piority substances							
PAHs	**Chrysene**	✓	✓	✓	✓	-	-	-
	Fluoranthene	✓	✓	✓	✓	-	-	-
	Pyrene	✓	✓	✓	✓	-	-	-
	Naphthalene	✓			✓	-	-	-
	Benzo[k]fluoranthene	✓	✓	✓		-	-	-
	Phenanthrene	✓	✓		✓	-	-	-
	Benzo[b]fluoranthene	✓	✓	✓		-	-	-
	Acenaphthene	✓	✓			-	-	-
	Dibenzo[a,h]anthracene	✓			✓	-	-	-
PCBs	PCB 52	✓	✓			-	-	-
	PCB 118	✓	✓			-	-	-
	PCB 149	✓	✓			-	-	-
	PCB 153	✓	✓			-	-	-
OCP	4,4’-DDE	✓	✓			-	-	-
	Emerging substances							
Musks	**HHCB**	✓	✓	✓	✓	-	-	-
	AHTN	✓	✓			-	-	-
	MA	✓	✓			-	-	-
	MX	✓	✓		✓	-	-	-
	MM	✓		✓		-	-	-
Sunscreens	**OC**	✓	✓	✓		-	-	-
	EHMC	✓	✓	✓	✓	-	-	-
	Benzophenone 3	✓	✓			-	-	-
Pharmaceuticals	**Ofloxacin**	✓	✓	✓	✓	✓	✓	✓
	Azithromycin	✓	✓	✓		✓	✓	✓
	Caffeine	✓	✓		✓	✓		✓
	Oxazepam	✓	✓	✓			✓	✓
	Carbamazepine	✓	✓	✓			✓	
	Hydrochlorothiazide	✓	✓		✓			✓
	Metoprolol	✓	✓	✓				✓
	Niflumic acid	✓	✓	✓		✓		
	Norfloxacin	✓	✓				✓	
	Acetazolamide	✓	✓					✓
	Clarithromycin	✓	✓					✓
	Acetaminophen	✓	✓					
	Atenolol	✓	✓					
	Ciprofloxacin	✓	✓					
	Ibuprofen	✓	✓					
	Ketoprofen	✓	✓					
	Lorazepam	✓	✓					
	Metronidazole	✓	✓					
	Phenazone	✓	✓					
	Roxithromycin	✓	✓					
	Rifampicin	✓		✓				
	Flumequin	✓				✓		
	Spiramycin	✓						✓

5. Conclusion

This paper provided important data on the occurrences and levels of priority and emerging micropollutants in effluents of 5 wastewater treatment plants and in 6 rocky benthic organisms (*Ulva* spp., *Gelidium* spp., Porifera, *Holothuria* spp., *Mytilus* spp. and *Patella* spp.) sampled at the WWTP outfalls in the southeastern Bay of Biscay. These latter concentrations were compared to those found in organisms from control locations (without WWTP discharges and away from impacted locations). Among the 127 analytes analyzed in wastewater effluents, a total of 11 metals, 2 organomercury compounds and 98 organics (16 PAHs, 11 PCBs, 5 alkylphenols, 18 OCPs, 10 musks, 4 sunscreens and 34 pharmaceuticals) were detected and quantified. Spatial and temporal variabilities were mainly associated to rainfall (and thus the flow rate), summer overcrowding, sewer system (separated or combined), plant capacity, treatment process and inefficiency of the current applied treatment. Activated sludge biological treatment and membrane filtration appeared as the most effective to remove suspended matter and adsorbed substances. But, despite the treatments applied, effluents from WWTPs still rejected a large number and amount of priority and emerging pollutants into the ocean. WWTPs are thus among the main pathway responsible for pollution of coastal surface waters. This was confirmed by the fact that among the 109 organic substances analyzed in biota samples, a total of 51 analytes (9 PAHs, 6 PCBs, 1 OCP, 5 musks, 3 sunscreens and 27 pharmaceuticals) were detected and quantified. *Gelidium* spp., *Ulva* spp. and to a lesser extent, *Patella* spp. and *Holothuria* spp. appeared as reflecting well the micropollutant concentrations discharged by WWTPs. Considering the biological and technical drawbacks of each species, the macroalgae *Ulva* spp. and *Gelidium* spp. were highlighted as the best bio-accumulators and -indicators for this area.

Furthermore, it would be interesting to make further researches to identify the potential sources of highlighted substances with the aim to mitigate their continuous release. In addition, further experimental analyses should be made to deeply study the effect of each treatment process on removal efficiency of each analytical group and substance (De los Ríos et al., 2016). The same should also be made to confirm the bioaccumulation capacity of previous species (according to their life cycle or ecological groups) and study the potential adverse effects of the main released substances and chronic effects on both species of macroalgae. As the potential better bioaccumulators are primary producers, it seemed also interesting to make further researches on the study of the biomagnification process because contaminant concentrations would be expected to increase as it passes up the food chain. Finally, although there is a legislation regulating the presence of some metals, PAHs, APs, PCBs, pesticides in surface waters and biota, musks, pharmaceuticals, sunscreens, and all metabolite compounds are still not regulated. Therefore, it seems important to consider the latter substances and results found in the present work with the aim to include them in the survey list in the future.

Highlights:

- **111** priority and emerging micropollutants were detected and quantified in **WWTP effluents**.
- **Spatial** and **temporal variabilities** were associated to rainfall, summer overcrowding, sewer system, plant capacity, treatment process and inefficiency of the current applied treatment.
- **Activated sludge biological treatment** and **membrane filtration** appeared as the most effective to remove suspended matter and thus adsorbed substances.
- **51** organic priority and emerging micropollutants were detected and quantified in **biota samples**.
- ***Ulva spp.*** and ***Gelidium* spp.** were highlighted as the best bio-accumulators and –indicators organisms for this area.

Prospects & improvements:

Sources, treatments and release into the environment

- **Identify potential sources** to suggest source control options
- Make further researches on **medical facility treatment processes**
- Make **experimental analyses and analyses on influents** to study and confirm **treatment process efficiencies**
- Analyze major **metabolites** whose parent compounds are supposed to be completely (or almost) metabolized during the transport or within the human body
- Study the **dilution effect** once these substances are rejected into the Ocean

Bioaccumulation and impact

- Make **experimental analyses** to confirm the **bioaccumulation capacity** of selected species
- Study the potential **adverse effects** (including mixture/chronic effects)
- Study the **biomagnification process**
- **Identify** some species **at the species level** (e.g. Porifera via the morpho-anatomical approach)

Monitoring

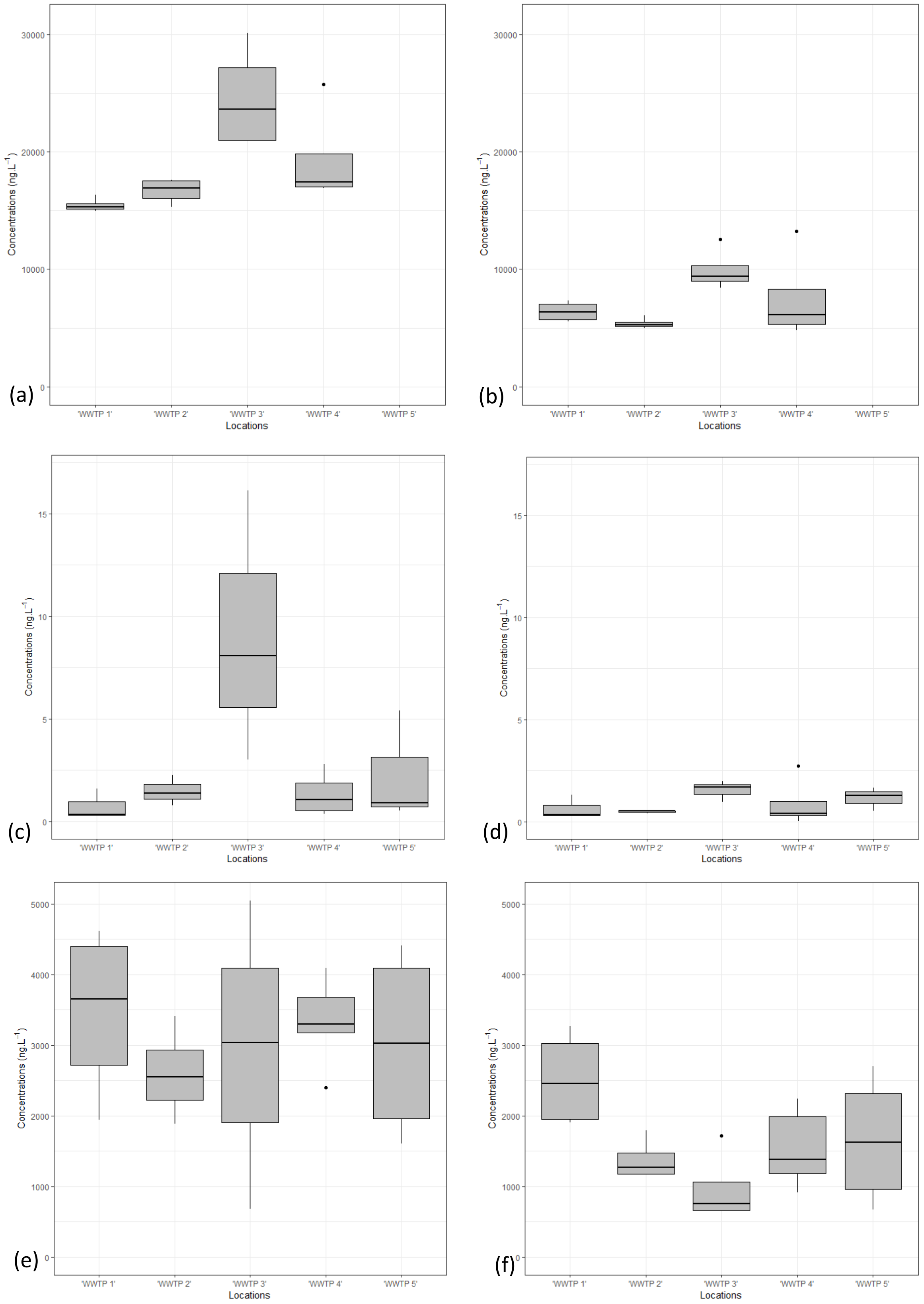
- Reflect upon how **routinely implemented** these analyses
- Highlight substances that could be integrated in **regulatory lists**

Supplementary materials (SM)

SM 1: Weather conditions, flow rates, physico-chemical parameters and major elements analyzed at each sampling campaign and WWTP (Unpublished data).

		August 2017					May 2018					
		'WWTP 1'	'WWTP 2'	'WWTP 3'	'WWTP 4'	Mean	'WWTP 1'	'WWTP 2'	'WWTP 3'	'WWTP 4'	'WWTP 5'	Mean
Weather	**Weather** *the 5 preceding days*	Rainy since several days + Storms					Rainfalls + Storms					
	Rainfall (mm) *the 5 preceding days + the day after the sampling* (total - mean - min - max)	36	49.8	11	2.4	**99.2** **24.8** *(2.4 - 49.8)*	29	42.8	37	35.8	-	**144.6** **36.15** *(29 - 42.8)*
Flow rates	**Daily flow in entry** ($m^3.day^{-1}$) *the 5 preceding days + the day after the sampling* (total - mean - min - max)	8 788	41 070	26 690	18 429	**94 977** **23 744** *(8 788 - 41 070)*	5 542	38 500	38 570	25 936	-	**108 548** **27 137** *(5 541 - 38 570)*
	Daily flow at the outlet ($m^3.day^{-1}$) *the 5 preceding days + the day after the sampling* (total - mean - min - max)	6 881	38 880	30 090	20 041	**95 892** **23 973** *(6 881 - 38 880)*	4 176	36 930	37 810	26 350	-	**105 266** **26 317** *(4 176 - 37 810)*
Physico-chemical measures/analyses	**pH** (mean - min - max)	-	6.8	6.3	7.2	**6.8** *(6.3 - 7.2)*	-	-	-	-	6.8	**6.76**
	Oxygen saturation (%) (mean - min - max)	-	47.5	120.1	12.0	**59.9** *(12.0 - 120.1)*	98.8	84.1	106.4	27.8	69.0	**77.2** *(27.8 - 106.4)*
	Conductivity ($mS.cm^{-1}$) (mean - min - max)	-	0.54	1.16	2.19	**1.30** *(0.54 - 2.19)*	0.53	0.74	1.04	1.64	12.21	**3.23** *(0.53 - 12.21)*
	Salinity ($\mu g.L^{-1}$) (mean - min - max)	-	0.26	0.57	1.12	**0.65** *(0.26 - 1.12)*	0.26	0.36	0.52	0.83	7.04	**1.80** *(0.26 - 7.04)*
	Temperature (°C) (mean - min - max)	-	23.83	23.08	23.67	**23.53** *(23.08 - 23.83)*	20.25	18.30	18.28	18.46	19.00	**18.82** *(18.28 - 20.25)*
	SM ($mg.L^{-1}$) (mean - min - max)	0.97	6.58	46.38	29.22	**20.79** *(0.97 - 46.38)*	1.20	13.71	17.95	12.20	12.00	**11.41** *(1.20 - 17.95)*
	TC (%) (mean - min - max)	29.31	29.49	32.40	36.31	**31.88** *(29.31 - 36.31)*	5.27	35.33	30.95	34.50	14.75	**24.16** *(5.27 - 35.33)*
	DOC ($mg.L^{-1}$) (mean - min - max)	2.37	6.22	6.74	5.54	**5.22** *(2.37 - 6.74)*	4.36	5.90	8.11	5.65	5.90	**5.98** *(4.36 - 8.11)*
	POC (%) (mean - min - max)	23.92	38.32	29.40	36.40	**32.01** *(23.92 - 38.32)*	8.39	31.79	27.51	33.29	12.92	**22.78** *(8.39 - 33.29)*
Nutrients	**$\sum PO_4^{3-}$** ($\mu mol.L^{-1}$) (mean - min - max)	21.16	12.66	117.38	68.86	**55.02** *(12.66 - 117.38)*	338.40	24.65	17.70	58.24	3.43	**88.48** *(3.43 - 338.40)*
	NO_3^- ($\mu mol.L^{-1}$) (mean - min - max)	223.32	10.99	473.00	0.19	**176.88** *(0.19 - 473.00)*	3.21	29.00	572.00	11.80	118.17	**146.84** *(3.21 - 572.00)*
	NO_2^- ($\mu mol.L^{-1}$) (mean - min - max)	24.75	3.03	43.20	0.30	**17.82** *(0.30 - 43.20)*	0.30	7.86	40.75	4.79	0.15	**10.77** *(0.15 - 40.75)*
	$Si(OH)_4$ ($\mu mol.L^{-1}$) (mean - min - max)	144.04	90.93	122.05	120.54	**119.39** *(90.93 - 144.04)*	101.75	68.62	96.91	92.20	103.28	**92.55** *(68.62 - 103.28)*
	NH_4^+ ($\mu mol.L^{-1}$) (mean - min - max)	42.90	86.74	399.62	264.57	**198.46** *(42.90 - 399.62)*	28.85	50.32	70.32	33.87	1.69	**37.01** *(1.69 - 70.32)*

		July 2018						December 2018					
		'WWTP 1'	'WWTP 2'	'WWTP 3'	'WWTP 4'	'WWTP 5'	Mean	'WWTP 1'	'WWTP 2'	'WWTP 3'	'WWTP 4'	'WWTP 5'	Mean
Weather	**Weather** *the 5 preceding days*	Warm weather						Good weather + Light rain					
	Rainfall (mm) *the 5 preceding days + the day after the sampling* (total - mean - min - max)	1	59.5	32	32.4	-	**124.9** **31.2** *(1 - 59.5)*	3	15.6	10.8	14.6	-	**44** **11** *(3 - 15.6)*
Flow rates	**Daily flow in entry** ($m^3.day^{-1}$) *the 5 preceding days + the day after the sampling* (total - mean - min - max)	7 126	40 720	35 870	26 512	-	**110 228** **27 557** *(7 126 - 40 720)*	4 149	26 860	19 230	20 046	-	**70 285** **17 571** *(4 149 - 26 860)*
	Daily flow at the outlet ($m^3.day^{-1}$) *the 5 preceding days + the day after the sampling* (total - mean - min - max)	7 447	36 980	36 480	26 167	-	**107 074** **26 769** *(7 447 - 36 980)*	2 994	25 710	23 000	20 882	-	**72 586** **18 147** *(2 994 - 25 710)*
Physico-chemical measures/analyses	**pH** (mean - min - max)	7.9	6.8	7.1	7.3		**7.3** *(6.8 - 7.9)*	8.0	7.6	7.5	7.9	8.9	**8.0** *(7.5 - 8.9)*
	Oxygen saturation (%) (mean - min - max)	-	62.6	114.5	32.4	-	**69.8** *(32.4 - 114.5)*	86.4	53.2	90.2	19.2	57.4	**61.3** *(19.2 - 90.2)*
	Conductivity ($mS.cm^{-1}$) (mean - min - max)	0.64	0.74	0.52	0.77	-	**0.67** *(0.52 - 0.77)*	0.81	1.67	2.39	1.55	15.13	**4.31** *(0.81 - 15.13)*
	Salinity ($\mu g.L^{-1}$) (mean - min - max)	0.36	0.36	0.25	0.38	-	**0.34** *(0.25 - 0.38)*	0.40	0.79	1.24	0.79	8.87	**2.42** *(0.40 - 8.87)*
	Temperature (°C) (mean - min - max)	22.50	23.36	22.83	23.74	-	**23.11** *(22.50 - 23.74)*	16.85	17.42	17.12	16.36	16.59	**16.87** *(16.36 - 17.42)*
	SM ($mg.L^{-1}$) (mean - min - max)	3.00	6.79	26.70	4.44	4.94	**9.17** *(3.00 - 26.70)*	0.49	9.00	25.44	8.27	17.20	**12.08** *(0.49 - 25.44)*
	TC (%) (mean - min - max)	53.32	34.94	33.47	44.07	12.26	**35.61** *(12.26 - 53.32)*	65.23	38.38	32.92	34.23	18.50	**37.85** *(18.50 - 65.23)*
	DOC ($mg.L^{-1}$) (mean - min - max)	5.73	7.84	9.88	5.01	7.74	**7.24** *(5.01 - 9.88)*	4.89	7.36	10.08	5.84	7.02	**7.04** *(4.89 - 10.08)*
	POC (%) (mean - min - max)	51.96	38.28	38.74	33.56	11.83	**34.87** *(11.83 - 51.96)*	37.73	38.93	28.79	33.73	13.16	**30.47** *(13.16 - 38.93)*
Nutrients	**$\sum PO_4^{3-}$** ($\mu mol.L^{-1}$) (mean - min - max)	138.50	51.94	31.10	64.14	338.40	**124.82** *(31.10 - 338.40)*	-	7.35	13.66	62.76	5.42	**22.30** *(5.42 - 62.76)*
	NO_3^- ($\mu mol.L^{-1}$) (mean - min - max)	15.80	89.70	1363.00	90.20	103.68	**332.48** *(15.80 - 1 363.00)*	-	8.60	750.00	0.89	416.80	**294.07** *(0.89 - 750.00)*
	NO_2^- ($\mu mol.L^{-1}$) (mean - min - max)	0.62	9.55	79.60	10.34	0.27	**20.08** *(0.27 - 79.60)*	-	5.65	40.00	2.12	1.24	**12.25** *(1.24 - 40.00)*
	$Si(OH)_4$ ($\mu mol.L^{-1}$) (mean - min - max)	144.14	157.25	153.81	96.19	165.68	**143.41** *(96.19 - 165.68)*	114.12	141.69	110.97	115.01	116.27	**119.61** *(110.97 - 141.69)*
	NH_4^+ ($\mu mol.L^{-1}$) (mean - min - max)	80.74	168.15	222.65	140.91	1.88	**122.87** *(1.88 - 222.65)*	39.94	192.66	190.45	192.41	2.13	**123.52** *(2.13 - 192.66)*



SM 2: Comparison of mean concentrations between unfiltered (a, c, e) and filtered samples (b, d, f) per WWTP (‘WWTP 1’, ‘WWTP 2’, ‘WWTP 3’, ‘WWTP 4’, ‘WWTP 5’) and analytical group: metals (a, b), organomercury compounds (c, d) and organics (PAHs, PCBs, musks, sunscreens, OCPs, alkylphenols) (e, f).

SM 3: Summary of ANOVA (a, b, c, d) and pairwise post hoc results (e, f, g, h, i, j, k, l) testing for effects of seasonality and location (WWTPs) on metal (a, e, i), organomercury (b, f, j), organic (PAHs, PCBs, musks, sunscreens, OCPs, alkylphenols) (c, g, k) and pharmaceutical (d, h, l) concentrations detected in wastewater discharges (bulk samples).

ANOVA results

(a)

Factors	Df	Sum Sq	Mean Sq	F value	Pr(>F)	Significance
Months	3	68.82	22.94	4.16	0.04	*
Locations	3	195.65	65.22	11.83	0.002	**
Residuals	9	49.61	5.51			

(b)

Factors	Df	Sum Sq	Mean Sq	F value	Pr(>F)	Significance
Months	2	13.74	6.87	0.53	0.62	
Locations	3	138.39	46.13	3.53	0.09	.
Residuals	6	78.51	13.09			

(c)

Factors	Df	Sum Sq	Mean Sq	F value	Pr(>F)	Significance
Months	4	1.47E+07	3.66E+06	4.77	0.02	*
Locations	4	1.84E+06	4.59E+05	0.60	0.67	
Residuals	12	9.22E+06	7.68E+05			

(d)

Factors	Df	Sum Sq	Mean Sq	F value	Pr(>F)	Significance
Months	3	1.75E+08	5.83E+07	3.94	0.04	*
Locations	4	2.76E+08	6.91E+07	4.67	0.02	*
Residuals	11	1.63E+08	1.48E+07			

Pairwise post hoc results (seasonality)

(e)

Months	August 2017	May 2018	July 2018
August 2017	-	-	-
May 2018	0.23	-	-
July 2018	**0.05**	0.76	-
December 2018	**0.06**	0.78	1.00

(f)

Months	March 2017	August 2017	May 2018	July 2018
March 2017				
August 2017				
May 2018		*Not investigated*		
July 2018				
December 2018				

(g)

Months	March 2017	August 2017	May 2018	July 2018
March 2017	-	-	-	-
August 2017	0.88	-	-	-
May 2018	0.56	0.06	-	-
July 2018	0.97	0.99	0.09	-
December 2018	0.49	**0.04**	1.00	0.07

(h)

Months	August 2017	May 2018	July 2018
August 2017	-	-	-
May 2018	0.06	-	-
July 2018	**0.04**	1.00	-
December 2018	0.18	0.86	0.77

Pairwise post hoc results (location)

(i)

Locations	'WWTP 1'	'WWTP 2'	'WWTP 3'
'WWTP 1'	-	-	-
'WWTP 2'	0.88	-	-
'WWTP 3'	**0.002**	**0.005**	-
'WWTP 4'	0.15	0.41	**0.05**

(j)

Locations	'WWTP 1'	'WWTP 2'	'WWTP 3'	'WWTP 4'
'WWTP 1'				
'WWTP 2'				
'WWTP 3'		*Not investigated*		
'WWTP 4'				
'WWTP 5'				

(k)

Locations	'WWTP 1'	'WWTP 2'	'WWTP 3'	'WWTP 4'
'WWTP 1'				
'WWTP 2'				
'WWTP 3'		*Not investigated*		
'WWTP 4'				
'WWTP 5'				

(l)

Locations	'WWTP 1'	'WWTP 2'	'WWTP 3'	'WWTP 4'
'WWTP 1'	-	-	-	-
'WWTP 2'	0.43	-	-	-
'WWTP 3'	**0.01**	0.18	-	-
'WWTP 4'	0.39	1.00	0.20	-
'WWTP 5'	0.81	0.98	0.10	0.97

SM 4: Mean concentrations, median concentrations, minimum and maximum (Min, Max), and percent occurrence of detected priority and emerging substances (metals, organomercury compounds and organics expressed in ng.L^{-1}) in bulk wastewater samples. Daily flux estimations (in mg.day^{-1}) were also calculated. Analyte mean concentrations were ordered from the highest to the lowest mean concentrations. Significance codes: Underlined analytes are those followed and regulated within European Directives; DL: Detection limit; QL: Quantification limit; '-': corresponds to molecules whose pre-analytical or analytical methods were not adapted to their quantification in that sample. In flux estimation column, '-' means that the estimation was not possible for this molecule.

'WWTP 1'

			Concentrations (ng.L^{-1})					**Daily flux estimations (mg.day^{-1})**			
Substance families	**Analytical Groups**	**Analytes**	**Mean**	**Median**	**Min**	**Max**	**Occurrence (%)**	**Mean**	**Median**	**Min**	**Max**
Priority substances											
Metal		Vanadium (V)	4339.5	4388.8	4053.3	4527.3	100	3332.2	3022.7	1805.1	5478.0
Metal		Chromium (Cr)	3881.8	3901.5	3749.0	3975.0	100	2952.4	2827.8	1617.8	4536.3
Metal		Nickel (Ni)	2034.8	1946.5	1740.0	2506.0	100	1609.2	1558.3	769.1	2551.1
Metal		Arsenic (As)	1888.5	1863.8	1763.8	2062.8	100	1478.8	1350.7	717.8	2495.9
Metal		Copper (Cu)	1589.9	1586.7	1253.7	1932.7	100	1207.5	1099.8	662.9	1967.5
Metal		Antimony (Sb)	1116.5	1022.8	948.3	1472.3	100	894.0	845.8	385.9	1498.8
Metal		Lead (Pb)	281.3	244.0	238.5	398.5	100	225.3	197.4	100.7	405.7
Metal		Tin (Sn)	160.5	156.5	134.0	195.0	100	127.4	128.3	54.5	198.5
Metal		Molybdenum (Mo)	151.5	147.0	53.0	259.0	100	83.3	86.9	54.0	105.4
Metal		Cadmium (Cd)	9.8	5.3	2.3	26.3	100	8.5	2.6	1.9	26.7
Metal		Silver (Ag)	5.0	5.5	1.5	7.5	100	4.5	4.8	0.6	7.9
	TOTAL		**15458.9**		**13937.2**	**17362.2**		**11923.1**		**6170.4**	**19271.7**
Organomercury compound		IHg	0.7	0.3	0.2	1.6	100	0.7	0.3	0.1	1.6
Organomercury compound		MMHg	0.1	0.1	0.0	0.1	100	0.04	0.05	0.03	0.1
	TOTAL		**0.7**		**0.3**	**1.6**		**0.7**		**0.1**	**1.6**
Organic	PAH	Indeno[1,2,3-cd]pyrene	11.0	<DL	<DL	44.1	25.0	13.4	-	-	53.4
Organic	PAH	Dibenzo[a,h]anthracene	9.0	<DL	<DL	36.0	25.0	10.9	-	-	43.5
Organic	PAH	Benzo[a]anthracene	6.9	<DL	<DL	27.5	25.0	8.3	-	-	33.3
Organic	PAH	Benzo[g,h,i]perylene	5.7	<DL	<DL	22.8	25.0	6.9	-	-	27.6
Organic	PAH	Anthracene	4.6	<DL	<DL	18.3	25.0	5.5	-	-	22.1
Organic	PAH	Benzo[k]fluoranthene	2.9	<DL	<DL	11.6	25.0	3.5	-	-	14.1
Organic	PAH	Benzo[a]pyrene	2.6	<DL	<DL	10.5	25.0	3.2	-	-	12.7
Organic	PAH	Benzo[b]fluoranthene	2.5	<DL	<DL	10.0	25.0	3.0	-	-	12.1
Organic	PAH	Chrysene	2.4	<DL	<DL	9.8	25.0	3.0	-	-	11.8
Organic	PAH	Phenanthrene	1.2	<DL	<DL	4.7	25.0	1.4	-	-	5.7
Organic	PAH	Fluorene	0.9	<DL	<DL	3.6	25.0	1.1	-	-	4.4
Organic	PAH	Acenaphthene	0.5	<DL	<DL	1.8	25.0	0.6	-	-	2.2
Organic	PAH	Acenaphthylene	0.3	<DL	<DL	1.2	25.0	0.5	-	-	1.4
Organic	PAH	Naphthalene	<DL	<DL	<DL	<DL	0.0	3.3	3.3	-	6.5
Organic	PAH	Fluoranthene	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	PAH	Pyrene	<DL	<DL	<DL	<DL	0.0	-	-	-	-
	TOTAL		**50.5**		**<DL**	**202.0**		**64.5**		-	**251.0**
Organic	PCB	PCB 194	5.7	<DL	<DL	22.7	25.0	6.9	-	-	27.5
Organic	PCB	PCB 138	5.6	1.9	<DL	18.8	50.0	6.6	1.9	-	22.8
Organic	PCB	PCB 149	2.0	1.5	<DL	4.9	50.0	2.1	1.8	-	5.0
Organic	PCB	PCB 28+31	1.3	<DL	<DL	5.1	25.0	1.6	-	-	6.2
Organic	PCB	PCB 101	1.1	<DL	<DL	4.2	25.0	1.3	-	-	5.1
Organic	PCB	PCB 180	0.9	<DL	<DL	3.5	25.0	1.1	-	-	4.3
Organic	PCB	PCB 18	0.7	<DL	<DL	2.7	25.0	0.8	-	-	3.2
Organic	PCB	PCB 52	0.7	<DL	<DL	2.7	25.0	0.8	-	-	3.2
Organic	PCB	PCB 44	0.6	<DL	<DL	2.5	25.0	0.8	-	-	3.1
Organic	PCB	PCB 153	0.3	<DL	<DL	1.4	25.0	0.4	-	-	1.7
Organic	PCB	PCB 118	0.2	<DL	<DL	1.0	25.0	0.3	-	-	1.2
	TOTAL		**19.1**		**<DL**	**69.6**		**22.7**		-	**83.3**
Organic	AP	NP	215.1	83.9	19.0	673.9	100	254.9	150.3	32.9	686.0
Organic	AP	4tOP	<DL	<DL	<DL	<DL	0.0	2.1	-	-	8.4
Organic	AP	4nOP	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	AP	NPEO1	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	AP	NPEO2	<DL	<DL	<DL	<DL	0.0	-	-	-	-
	TOTAL		**215.1**		**19.0**	**673.9**		**257.0**		**32.9**	**694.4**
Organic	OCP	4,4'-DDE	3.1	1.3	<DL	9.8	50.0	3.3	1.6	-	10.0
Organic	OCP	Aldrin	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	OCP	Alpha BHC	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	OCP	Beta BHC	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	OCP	Delta BHC	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	OCP	Dieldrine	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	OCP	Alpha Endosulfan	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	OCP	Bêta Endosulfan	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	OCP	Endosulfan Sulfate	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	OCP	Endrin	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	OCP	Endrin Aldehyde	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	OCP	Endrin Ketone	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	OCP	Gamma BHC	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	OCP	Heptachlor	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	OCP	Heptachlor Epoxide	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	OCP	Methoxychlor	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	OCP	4,4'-DDD	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	OCP	4,4'-DDT	<DL	<DL	<DL	<DL	0.0	-	-	-	-
	TOTAL		**3.1**		**<DL**	**9.8**		**3.3**		-	**10.0**

‘WWTP 1’

			Concentrations (ng.L^{-1})					Daily flux estimations (mg.day^{-1})			
Substance families	**Analytical Groups**	**Analytes**	**Mean**	**Median**	**Min**	**Max**	**Occurrence (%)**	**Mean**	**Median**	**Min**	**Max**
Emerging substances											
Organic	Musk	HHCB	2064.3	2093.6	1126.1	2943.7	100	1621.8	1462.8	-	3561.8
Organic	Musk	HHCB-lactone	774.9	818.9	501.8	960.2	100	562.5	531.9	24.5	1161.8
Organic	Musk	AHTN	257.9	253.5	196.5	328.1	100	361.9	294.0	80.0	779.6
Organic	Musk	MK	38.4	40.5	17.1	55.4	100	22.6	15.0	-	60.4
Organic	Musk	ADBI	8.7	7.2	<DL	20.4	50.0	4.3	-	-	17.4
Organic	Musk	AHMI	0.6	0.4	<DL	1.5	50.0	2.5	0.5	-	9.0
Organic	Musk	MA	<DL	<DL	<DL	<DL	0.0	0.2	-	-	0.6
Organic	Musk	ATII	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	Musk	MX	<DL	<DL	<DL	<DL	0.0	32.8	-	-	131.3
Organic	Musk	MM	<DL	<DL	<DL	<DL	0.0	-	-	-	-
	TOTAL		**3144.7**		**1841.4**	**4309.2**		**2608.7**		**104.4**	**5722.0**
Organic	Sunscreen	4-MBC	13.3	<DL	<DL	53.0	25.0	17.6	3.1	-	64.2
Organic	Sunscreen	OC	9.3	10.4	1.4	15.0	100	5.9	4.0	0.4	15.2
Organic	Sunscreen	Benzophenone 3	7.2	6.9	<DL	15.1	50.0	4.6	-	-	18.3
Organic	Sunscreen	EHMC	0.3	0.1	<DL	1.0	75.0	0.05	-	-	0.2
Organic	Sunscreen	3-BC	<DL	<DL	<DL	<DL	0.0	87.7	-	-	350.7
Organic	Sunscreen	OD-PABA	<DL	<DL	<DL	<DL	0.0	-	-	-	-
	TOTAL		**30.1**		**1.4**	**84.1**		**115.8**		**0.4**	**448.5**
Organic	Pharmaceutical (Antihypertensive)	Hydrochlorothiazide	1076.0	1014.5	849.2	1425.8	100	834.6	730.5	426.1	1451.4
Organic	Pharmaceutical (Anxiolytics)	Oxazepam	1056.0	1087.4	622.3	1427.0	100	780.7	634.4	401.2	1452.7
Organic	Pharmaceutical (Anti-inflammatory)	Diclofenac	556.6	446.9	432.3	900.4	100	457.7	369.2	175.9	916.6
Organic	Pharmaceutical (Anticonvulsant)	Carbamazepine	535.1	449.9	263.8	976.8	100	469.0	387.2	107.4	994.4
Organic	Pharmaceutical (Antibiotics)	Sulfamethoxazole	125.6	97.9	59.2	247.4	100	118.3	73.9	26.2	299.4
Organic	Pharmaceutical (Pain killer)	Ketoprofen	124.9	129.9	40.5	199.1	100	103.3	96.3	17.9	202.7
Organic	Pharmaceutical (Pain killer)	Niflumic acid	119.0	108.5	64.7	194.4	100	102.9	93.7	26.3	197.9
Organic	Pharmaceutical (Psychotropic)	Caffeine	104.9	90.1	76.3	163.3	100	72.6	79.9	38.3	92.4
Organic	Pharmaceutical (Antibiotics)	Ciprofloxacin	98.1	87.1	36.2	182.1	100	71.8	86.7	14.7	99.2
Organic	Pharmaceutical (Antibiotics)	Ofloxacin	96.8	91.6	53.7	150.2	100	74.2	60.0	23.8	152.9
Organic	Pharmaceutical (Anti-inflammatory)	Ibuprofen	59.8	77.9	<QL	83.4	75.0	52.7	54.9	-	100.9
Organic	Pharmaceutical (Antihypertensive)	Atenolol	30.4	31.9	17.4	40.4	100	21.7	17.1	14.2	38.5
Organic	Pharmaceutical (Antibiotics)	Spiramycin	29.1	25.5	<QL	65.5	50.0	19.6	13.3	-	51.8
Organic	Pharmaceutical (Antiarrhythmic)	Metoprolol	26.0	24.3	8.3	46.9	100	23.3	21.1	3.4	47.8
Organic	Pharmaceutical (Antihypertensive)	Losartan	25.0	26.7	16.0	30.5	100	18.9	14.1	10.6	36.9
Organic	Pharmaceutical (Antibiotics)	Norfloxacin	24.6	<DL	<QL	98.6	25.0	10.9	-	-	43.6
Organic	Pharmaceutical (Antibiotics)	Roxithromycin	20.0	12.0	<QL	56.1	75.0	10.4	9.4	-	22.8
Organic	Pharmaceutical (Antibiotics)	Azithromycin	17.9	3.6	<QL	64.5	50.0	17.2	1.5	-	65.7
Organic	Pharmaceutical (Glycemia)	Gemfibrozil	17.8	12.0	<QL	47.4	75.0	17.6	6.6	-	57.3
Organic	Pharmaceutical (Anxiolytics)	Lorazepam	16.5	22.0	<QL	22.1	75.0	14.7	16.1	-	26.6
Organic	Pharmaceutical (Antibiotics)	Clarithromycin	13.1	13.5	3.6	21.7	100	11.9	9.9	1.5	26.2
Organic	Pharmaceutical (Antibiotics)	Metronidazole	11.7	11.0	<QL	24.8	75.0	7.7	6.8	-	17.0
Organic	Pharmaceutical (Anxiolytics)	Nordazepam	10.7	11.1	8.9	11.8	100	8.5	8.6	3.6	13.1
Organic	Pharmaceutical (Antibiotics)	Erythromycin A	10.3	8.3	<QL	24.5	75.0	9.2	3.6	-	29.7
Organic	Pharmaceutical (Pain killer)	Acetaminophen	8.1	7.7	<QL	17.0	50.0	6.4	3.4	-	18.7
Organic	Pharmaceutical (Antibiotics)	Josamycin	6.8	8.2	<QL	10.6	75.0	5.8	5.9	-	11.4
Organic	Pharmaceutical (Antibiotics)	Tetracycline	5.1	0.0	<QL	20.2	25.0	2.2	-	-	8.9
Organic	Pharmaceutical (Antibiotics)	Trimethoprim	2.8	3.4	<QL	4.6	75.0	2.3	2.6	-	4.1
Organic	Pharmaceutical (Antibiotics)	Sulfadiazine	2.6	0.0	<QL	10.2	25.0	3.1	-	-	12.4
Organic	Pharmaceutical (Antibiotics)	Ampicilline	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Antibiotics)	Doxycycline	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Antibiotics)	Flumequine	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Antibiotics)	Oxolinic acid	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Antibiotics)	Piperacillin	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Antibiotics)	Rifampicin	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Antibiotics)	Sulfamethazine	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Antibiotics)	Tylosine	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Hormones)	E2	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Hormones)	EE2	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Hormones)	E1	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Pain killer)	Phenazone	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Contraceptif)	19-Norethindrone	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Glaucoma)	Acetazolamide	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Anticancer)	Cyclophosphamide	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Antiarrhythmic)	Amiodarone	-	-	-	-	-	-	-	-	-
Organic	Pharmaceutical (Antibiotics)	Amoxicillin	-	-	-	-	-	-	-	-	-
Organic	Pharmaceutical (Pain killer)	Acetylsalicylic acid	-	-	-	-	-	-	-	-	-
Organic	Pharmaceutical (Antineoplastic)	Hydroxycarbamide	-	-	-	-	-	-	-	-	-
	TOTAL		**4231.5**		**2552.6**	**6567.1**		**3349.3**		**1291.0**	**6492.8**

‘WWTP 2’

			Concentrations (ng.L^{-1})					Daily flux estimations (mg.day^{-1})			
Substance families	**Analytical Groups**	**Analytes**	**Mean**	**Median**	**Min**	**Max**	**Occurrence (%)**	**Mean**	**Median**	**Min**	**Max**
Priority substances											
Metal		Vanadium (V)	4385.3	4344.3	4181.3	4671.3	100	22524.5	22809.6	14676.2	29802.6
Metal		Chromium (Cr)	4041.8	3996.0	3787.0	4388.0	100	20699.1	20401.5	13997.9	27995.4
Metal		Nickel (Ni)	2006.0	1970.0	1855.0	2229.0	100	10158.4	10908.6	6981.4	11834.9
Metal		Copper (Cu)	1872.2	1718.7	1582.7	2468.7	100	9685.9	9349.6	5948.3	14096.1
Metal		Arsenic (As)	1680.0	1692.3	1452.8	1882.8	100	8647.3	9287.7	5099.2	10914.6
Metal		Antimony (Sb)	1280.5	1206.3	816.3	1893.3	100	6959.7	6447.4	2865.0	12078.9
Metal		Lead (Pb)	892.3	805.5	714.5	1243.5	100	4642.7	4481.2	2507.9	7100.4
Metal		Molybdenum (Mo)	328.0	316.0	207.0	473.0	100	1716.6	1587.0	991.5	2700.8
Metal		Tin (Sn)	176.5	164.5	143.0	234.0	100	889.4	935.8	565.1	1120.9
Metal		Silver (Ag)	16.0	15.5	14.5	18.5	100	82.1	84.2	54.4	105.6
Metal		Cadmium (Cd)	9.5	9.3	8.3	11.3	100	48.4	48.5	32.5	64.2
	TOTAL		**16687.9**		**14762.2**	**19513.2**		**86054.0**		**53719.3**	**117814.5**
Organomercury compound		IHg	1.4	1.4	0.7	2.2	100	8.2	8.6	3.5	12.4
Organomercury compound		MMHg	0.1	0.1	0.0	0.1	100	0.3	0.3	0.2	0.4
	TOTAL		**1.5**		**0.8**	**2.3**		**8.5**		**3.7**	**12.9**
Organic	PAH	Naphthalene	147.7	147.7	147.7	147.7	100	942.3	942.3	942.3	942.3
Organic	PAH	Pyrene	9.3	12.2	<DL	12.8	75.0	43.3	51.6	-	70.0
Organic	PAH	Indeno[1,2,3-cd]pyrene	8.0	<DL	<DL	32.2	25.0	38.5	-	-	154.0
Organic	PAH	Dibenzo[a,h]anthracene	5.7	<DL	<DL	22.8	25.0	27.4	-	-	109.4
Organic	PAH	Acenaphthene	5.7	0.8	<DL	21.2	50.0	35.7	3.7	-	135.3
Organic	PAH	Benzo[g,h,i]perylene	2.7	<DL	<DL	11.0	25.0	13.1	-	-	52.5
Organic	PAH	Fluorene	2.1	0.6	<DL	7.4	50.0	13.1	2.7	-	47.2
Organic	PAH	Benzo[a]pyrene	1.1	<DL	<DL	4.5	25.0	5.3	-	-	21.4
Organic	PAH	Phenanthrene	1.0	<DL	<DL	3.8	25.0	4.6	-	-	18.4
Organic	PAH	Benzo[k]fluoranthene	0.8	<DL	<DL	3.3	25.0	3.9	-	-	15.7
Organic	PAH	Benzo[b]fluoranthene	0.7	<DL	<DL	2.8	25.0	3.4	-	-	13.4
Organic	PAH	Anthracene	0.4	<DL	<DL	1.7	25.0	2.1	-	-	8.2
Organic	PAH	Benzo[a]anthracene	0.4	<DL	<DL	1.5	25.0	1.8	-	-	7.3
Organic	PAH	Fluoranthene	0.3	0.4	<DL	0.5	66.7	1.4	1.3	-	2.8
Organic	PAH	Acenaphthylene	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	PAH	Chrysene	<DL	<DL	<DL	<DL	0.0	-	-	-	-
	TOTAL		**186.0**		**147.7**	**273.1**		**1135.9**		**942.3**	**1598.0**
Organic	PCB	PCB 149	1.7	0.8	<DL	5.2	50.0	10.1	3.6	-	33.2
Organic	PCB	PCB 28+31	1.3	0.0	<DL	5.0	50.0	8.1	0.2	-	31.9
Organic	PCB	PCB 52	0.1	<DL	<DL	0.3	25.0	0.4	-	-	1.6
Organic	PCB	PCB 18	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	PCB	PCB 44	<DL	<DL	<DL	<DL	25.0	-	-	-	-
Organic	PCB	PCB 101	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	PCB	PCB 118	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	PCB	PCB 153	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	PCB	PCB 138	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	PCB	PCB 180	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	PCB	PCB 194	<DL	<DL	<DL	<DL	0.0	-	-	-	-
	TOTAL		**3.0**		**<DL**	**10.5**		**18.6**		**-**	**66.7**
Organic	AP	NP	821.8	821.8	194.2	1449.4	100	4964.5	4964.5	681.8	9247.2
Organic	AP	4tOP	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	AP	4nOP	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	AP	NPEO1	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	AP	NPEO2	<DL	<DL	<DL	<DL	0.0	-	-	-	-
	TOTAL		**821.8**		**194.2**	**1449.4**		**4964.5**		**681.8**	**9247.2**
Organic	OCP	4,4'-DDE	2.6	<DL	<DL	10.2	25.0	16.3	-	-	65.1
Organic	OCP	Aldrin	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	OCP	Alpha BHC	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	OCP	Beta BHC	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	OCP	Delta BHC	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	OCP	Dieldrine	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	OCP	Alpha Endosulfan	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	OCP	Bêta Endosulfan	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	OCP	Endosulfan Sulfate	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	OCP	Endrin	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	OCP	Endrin Aldehyde	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	OCP	Endrin Ketone	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	OCP	Gamma BHC	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	OCP	Heptachlor	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	OCP	Heptachlor Epoxide	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	OCP	Methoxychlor	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	OCP	4,4'-DDD	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	OCP	4,4'-DDT	<DL	<DL	<DL	<DL	0.0	-	-	-	-
	TOTAL		**2.6**		**<DL**	**10.2**		**16.3**	**-**	**-**	**65.1**

‘WWTP 2’

			Concentrations (ng.L^{-1})					Daily flux estimations (mg.day^{-1})			
Substance families	**Analytical Groups**	**Analytes**	**Mean**	**Median**	**Min**	**Max**	**Occurrence (%)**	**Mean**	**Median**	**Min**	**Max**
Emerging substances											
Organic	Musk	HHCB	1309.9	1222.1	984.4	1811.0	100	6706.0	7347.0	3455.1	8674.9
Organic	Musk	HHCB-lactone	485.2	468.3	435.4	568.9	100	2489.1	2589.9	1528.3	3248.3
Organic	Musk	AHTN	186.5	167.8	147.5	262.9	100	973.2	917.7	556.5	1500.9
Organic	Musk	MK	49.7	56.9	18.7	66.2	100	240.6	232.8	119.0	378.0
Organic	Musk	ADBI	6.0	4.5	<DL	15.0	50.0	32.2	21.8	-	85.4
Organic	Musk	AHMI	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	Musk	MA	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	Musk	ATII	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	Musk	MX	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	Musk	MM	<DL	<DL	<DL	<DL	0.0	-	-	-	-
	TOTAL		**2037.3**		**1585.9**	**2723.9**		**10441.2**		**5659.0**	**13887.5**
Organic	Sunscreen	Benzophenone 3	51.2	38.9	<DL	127.1	75.0	248.3	192.3	-	608.7
Organic	Sunscreen	OC	18.8	19.6	10.8	25.3	100	99.9	100.8	37.9	160.0
Organic	Sunscreen	EHMC	0.5	0.4	<DL	1.3	75.0	2.2	2.2	-	4.4
Organic	Sunscreen	3-BC	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	Sunscreen	4-MBC	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	Sunscreen	OD-PABA	<DL	<DL	<DL	<DL	0.0	-	-	-	-
	TOTAL		**70.6**		**10.8**	**153.6**		**350.4**		**37.9**	**773.1**
Organic	Pharmaceutical (Antihypertensive)	Hydrochlorothiazide	2194.8	1929.9	1691.1	3228.4	100	10635.6	10493.9	9250.9	12303.7
Organic	Pharmaceutical (Anxiolytics)	Oxazepam	2160.9	2201.3	1791.5	2449.2	100	10887.2	10185.7	8596.7	14580.7
Organic	Pharmaceutical (Anti-inflammatory)	Diclofenac	1020.9	1062.5	788.1	1170.6	100	5202.8	4840.9	3661.3	7468.3
Organic	Pharmaceutical (Antihypertensive)	Atenolol	480.5	486.0	237.0	712.9	100	2476.5	2002.4	1353.2	4548.2
Organic	Pharmaceutical (Pain killer)	Niflumic acid	335.8	305.4	261.2	471.2	100	1641.4	1572.7	1437.8	1982.4
Organic	Pharmaceutical (Anticonvulsant)	Carbamazepine	331.2	334.8	197.1	458.1	100	1778.8	1750.2	691.7	2923.0
Organic	Pharmaceutical (Pain killer)	Ketoprofen	318.5	321.5	155.6	475.3	100	1640.8	1582.8	860.9	2536.9
Organic	Pharmaceutical (Antibiotics)	Azithromycin	225.4	217.2	75.6	391.6	100	1104.1	1078.4	362.2	1897.3
Organic	Pharmaceutical (Antibiotics)	Sulfamethoxazole	222.6	216.8	191.7	265.4	100	1127.8	1247.0	731.1	1286.1
Organic	Pharmaceutical (Antihypertensive)	Losartan	204.4	196.6	130.8	293.8	100	1019.9	980.0	712.4	1407.2
Organic	Pharmaceutical (Antibiotics)	Ciprofloxacin	189.6	180.5	123.1	274.1	100	955.5	833.6	589.5	1565.2
Organic	Pharmaceutical (Psychotropic)	Caffeine	165.9	85.5	26.9	465.8	100	690.6	499.4	128.8	1634.8
Organic	Pharmaceutical (Antibiotics)	Ofloxacin	139.4	120.8	96.4	219.6	100	734.7	673.3	338.2	1253.9
Organic	Pharmaceutical (Antibiotics)	Clarithromycin	133.7	136.0	88.2	174.6	100	695.5	811.6	309.5	849.5
Organic	Pharmaceutical (Antiarrhythmic)	Metoprolol	126.9	108.7	86.3	203.6	100	676.2	525.7	354.1	1299.0
Organic	Pharmaceutical (Glycemia)	Gemfibrozil	126.1	121.2	63.6	198.3	100	612.5	568.3	363.4	950.1
Organic	Pharmaceutical (Glaucoma)	Acetazolamide	118.9	122.2	<QL	231.1	75.0	537.8	522.2	-	1107.1
Organic	Pharmaceutical (Antibiotics)	Roxithromycin	109.8	107.2	36.7	188.2	100	515.1	480.4	234.1	865.6
Organic	Pharmaceutical (Antibiotics)	Erythromycin A	88.6	46.3	34.3	227.5	100	513.1	240.3	120.4	1451.2
Organic	Pharmaceutical (Antibiotics)	Metronidazole	73.4	72.5	59.7	88.9	100	371.8	403.4	235.4	444.9
Organic	Pharmaceutical (Antibiotics)	Trimethoprim	73.2	67.2	45.3	112.9	100	344.3	345.8	289.1	396.3
Organic	Pharmaceutical (Antibiotics)	Spiramycin	62.0	40.6	<QL	166.9	50.0	276.1	259.3	-	585.6
Organic	Pharmaceutical (Anti-inflammatory)	Ibuprofen	43.0	0.0	<QL	172.1	25.0	151.0	-	-	604.2
Organic	Pharmaceutical (Antibiotics)	Norfloxacin	31.1	29.4	<QL	65.7	50.0	162.6	157.4	-	335.7
Organic	Pharmaceutical (Anxiolytics)	Lorazepam	27.7	29.9	13.6	37.5	100	147.1	179.5	47.6	181.6
Organic	Pharmaceutical (Antibiotics)	Josamycin	22.8	13.6	5.5	58.5	100	95.7	71.4	35.0	205.2
Organic	Pharmaceutical (Anxiolytics)	Nordazepam	13.3	13.2	10.1	16.7	100	66.6	69.5	47.1	80.1
Organic	Pharmaceutical (Pain killer)	Acetaminophen	11.6	10.3	5.0	20.9	100	64.2	53.2	17.4	133.1
Organic	Pharmaceutical (Antibiotics)	Sulfadiazine	3.0	<DL	<QL	11.9	25.0	14.2	-	-	56.9
Organic	Pharmaceutical (Antibiotics)	Piperacillin	0.8	<DL	<QL	3.3	25.0	2.9	-	-	11.7
Organic	Pharmaceutical (Antibiotics)	Ampicilline	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Antibiotics)	Doxycycline	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Antibiotics)	Flumequine	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Antibiotics)	Oxolinic acid	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Antibiotics)	Rifampicin	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Antibiotics)	Sulfamethazine	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Antibiotics)	Tetracycline	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Antibiotics)	Tylosine	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Hormones)	E2	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Hormones)	EE2	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Hormones)	E1	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Pain killer)	Phenazone	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Contraceptif)	19-Norethindrone	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Anticancer)	Cyclophosphamide	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Antiarrhythmic)	Amiodarone	-	-	-	-	-	-	-	-	-
Organic	Pharmaceutical (Antibiotics)	Amoxicillin	-	-	-	-	-	-	-	-	-
Organic	Pharmaceutical (Pain killer)	Acetylsalicylic acid	-	-	-	-	-	-	-	-	-
Organic	Pharmaceutical (Antineoplastic)	Hydroxycarbamide	-	-	-	-	-	-	-	-	-
	TOTAL		**9055.8**		**6214.2**	**12854.5**		**45142.5**		**30767.8**	**64945.4**

‘WWTP 3’

			Concentrations (ng.L^{-1})					Daily flux estimations (mg.day^{-1})			
Substance families	Analytical Groups	Analytes	Mean	Median	Min	Max	Occurrence (%)	Mean	Median	Min	Max
Priority substances											
Metal		Vanadium (V)	5708.3	5449.3	4751.3	7183.3	100	25973.6	25897.1	16255.8	35844.4
Metal		Chromium (Cr)	5678.3	5612.5	4443.0	7045.0	100	26050.3	26485.5	15787.4	35443.0
Metal		Copper (Cu)	4641.2	4971.7	2885.7	5735.7	100	21753.8	22630.2	9118.7	32635.9
Metal		Nickel (Ni)	3868.0	3861.0	3274.0	4476.0	100	17249.1	16832.0	12997.1	22335.2
Metal		Arsenic (As)	1896.3	1785.8	1592.8	2420.8	100	8550.2	8544.1	5033.1	12079.5
Metal		Antimony (Sb)	1090.3	1040.8	828.3	1451.3	100	5037.3	5044.1	2819.5	7241.7
Metal		Molybdenum (Mo)	858.5	927.5	504.0	1075.0	100	3987.1	3905.4	2021.0	6116.8
Metal		Lead (Pb)	532.8	558.5	337.5	676.5	100	2505.9	2553.9	1066.5	3849.3
Metal		Tin (Sn)	239.5	237.5	207.0	276.0	100	1078.1	1140.5	654.1	1377.2
Metal		Silver (Ag)	25.0	25.0	17.5	32.5	100	115.6	122.5	55.3	162.2
Metal		Cadmium (Cd)	19.3	20.8	12.3	23.3	100	89.6	96.6	38.7	126.6
	TOTAL		**24557.2**		**18853.2**	**30395.2**		**112390.8**		**65847.2**	**157211.9**
Organomercury compound		IHg	8.4	6.4	2.9	15.8	100	37.4	32.0	16.8	63.5
Organomercury compound		MMHg	0.7	0.3	0.1	1.7	100	3.3	1.1	0.5	8.3
	TOTAL		**9.1**		**3.0**	**17.5**		**40.7**		**17.2**	**71.8**
Organic	PAH	Indeno[1,2,3-cd]pyrene	7.5	<DL	<DL	29.8	50.0	29.9	-	-	119.7
Organic	PAH	Pyrene	5.3	5.3	<DL	10.3	75.0	19.5	20.4	-	37.1
Organic	PAH	Benzo[b]fluoranthene	5.1	1.8	<DL	17.0	75.0	24.7	7.1	-	84.8
Organic	PAH	Dibenzo[a,h]anthracene	4.6	<DL	<DL	18.5	50.0	18.6	-	-	74.3
Organic	PAH	Phenanthrene	2.6	2.0	<DL	6.2	75.0	9.4	6.3	-	25.1
Organic	PAH	Fluorene	2.2	2.1	<DL	4.5	75.0	7.7	7.2	-	16.6
Organic	PAH	Acenaphthene	2.0	1.9	<DL	4.4	75.0	7.2	6.9	-	15.1
Organic	PAH	Benzo[g,h,i]perylene	1.9	<DL	<DL	7.4	50.0	7.4	-	-	29.8
Organic	PAH	Benzo[a]pyrene	1.1	<DL	<DL	4.5	50.0	4.5	-	-	17.9
Organic	PAH	Benzo[a]anthracene	1.1	0.9	<DL	2.6	75.0	4.1	2.9	-	10.5
Organic	PAH	Benzo[k]fluoranthene	1.0	<DL	<DL	4.1	50.0	4.1	-	-	16.4
Organic	PAH	Anthracene	0.1	<DL	<DL	0.5	50.0	0.5	-	-	2.2
Organic	PAH	Naphthalene	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	PAH	Fluoranthene	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	PAH	Acenaphthylene	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	PAH	Chrysene	<DL	<DL	<DL	<DL	0.0	-	-	-	-
	TOTAL		**34.5**		**<DL**	**110.0**		**137.7**		**-**	**449.3**
Organic	PCB	PCB 138	4.4	<DL	<DL	17.8	50.0	17.8	-	-	71.3
Organic	PCB	PCB 101	0.7	<DL	<DL	2.7	50.0	2.7	-	-	10.7
Organic	PCB	PCB 149	0.5	<DL	<DL	2.2	50.0	2.2	-	-	8.7
Organic	PCB	PCB 28+31	0.2	<DL	<DL	0.8	50.0	0.8	-	-	3.0
Organic	PCB	PCB 180	0.2	<DL	<DL	0.7	50.0	0.7	-	-	2.9
Organic	PCB	PCB 52	0.2	<DL	<DL	0.6	50.0	0.6	-	-	2.5
Organic	PCB	PCB 44	0.1	<DL	<DL	0.5	50.0	0.5	-	-	2.0
Organic	PCB	PCB 18	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	PCB	PCB 118	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	PCB	PCB 153	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	PCB	PCB 194	<DL	<DL	<DL	<DL	0.0	-	-	-	-
	TOTAL		**6.3**		**<DL**	**25.3**		**25.3**	**-**	**-**	**101.3**
Organic	AP	NP	815.9	689.2	656.8	1101.8	100	3436.6	2633.9	2177.8	5498.0
Organic	AP	NPEO1	67.8	67.8	67.8	67.8	100	338.3	338.3	338.3	338.3
Organic	AP	NPEO2	15.4	<DL	<DL	46.2	33.3	76.8	-	-	230.5
Organic	AP	4tOP	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	AP	4nOP	<DL	<DL	<DL	<DL	0.0	-	-	-	-
	TOTAL		**899.1**		**724.6**	**1215.8**		**3851.7**		**2516.1**	**6066.8**
Organic	OCP	Aldrin	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	OCP	Alpha BHC	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	OCP	Beta BHC	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	OCP	Delta BHC	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	OCP	Dieldrine	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	OCP	Alpha Endosulfan	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	OCP	Bêta Endosulfan	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	OCP	Endosulfan Sulfate	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	OCP	Endrin	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	OCP	Endrin Aldehyde	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	OCP	Endrin Ketone	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	OCP	Gamma BHC	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	OCP	Heptachlor	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	OCP	Heptachlor Epoxide	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	OCP	Methoxychlor	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	OCP	4,4'-DDD	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	OCP	4,4'-DDE	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	OCP	4,4'-DDT	<DL	<DL	<DL	<DL	0.0	-	-	-	-
	TOTAL		**<DL**		**<DL**	**<DL**		-	-	-	-

'WWTP 3'

			Concentrations (ng.L^{-1})					Daily flux estimations (mg.day^{-1})			
Substance families	**Analytical Groups**	**Analytes**	**Mean**	**Median**	**Min**	**Max**	**Occurrence (%)**	**Mean**	**Median**	**Min**	**Max**
Emerging substances											
Organic	Musk	HHCB	1017.3	845.2	600.0	1778.7	100	4354.6	3793.2	2699.6	7132.6
Organic	Musk	HHCB-lactone	331.3	418.9	28.8	458.8	100	1369.3	1530.1	164.1	2252.9
Organic	Musk	AHTN	113.2	140.4	24.5	147.5	100	471.4	508.6	139.5	728.9
Organic	Musk	MK	56.8	63.0	7.3	94.2	100	219.7	230.0	41.3	377.6
Organic	Musk	ADBI	4.3	4.1	<DL	9.1	50.0	20.8	18.2	-	46.8
Organic	Musk	AHMI	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	Musk	MA	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	Musk	ATII	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	Musk	MX	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	Musk	MM	<DL	<DL	<DL	<DL	0.0	-	-	-	-
	TOTAL		**1523.0**		**660.6**	**2488.3**		**6435.9**		**3044.4**	**10538.8**
Organic	Sunscreen	OC	701.4	235.7	<DL	2334.0	75.0	3367.0	910.7	-	11646.7
Organic	Sunscreen	Benzophenone 3	25.5	25.1	6.4	45.5	100	98.8	101.9	32.1	159.2
Organic	Sunscreen	4-MBC	17.9	2.0	<DL	67.5	50.0	72.6	9.8	-	270.7
Organic	Sunscreen	EHMC	1.4	1.2	<DL	3.3	75.0	5.5	4.5	-	13.1
Organic	Sunscreen	3-BC	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	Sunscreen	OD-PABA	<DL	<DL	<DL	<DL	0.0	-	-	-	-
	TOTAL		**746.2**		**6.4**	**2450.3**		**3543.9**		**32.1**	**12089.6**
Organic	Pharmaceutical (Psychotropic)	Caffeine	4420.5	2431.4	458.6	12360.5	100	20701.9	9645.0	1838.8	61679.0
Organic	Pharmaceutical (Antihypertensive)	Hydrochlorothiazide	1679.3	1425.1	798.3	3068.6	100	7486.4	5045.3	4542.6	15312.4
Organic	Pharmaceutical (Anxiolytics)	Oxazepam	1493.2	1581.0	1073.8	1737.0	100	6431.0	6435.9	5488.9	7363.4
Organic	Pharmaceutical (Pain killer)	Ketoprofen	1331.2	1118.7	615.0	2472.4	100	5946.5	3974.7	3499.5	12337.3
Organic	Pharmaceutical (Anti-inflammatory)	Diclofenac	1113.7	1064.5	510.6	1815.5	100	4927.3	3872.3	2905.1	9059.3
Organic	Pharmaceutical (Anti-inflammatory)	Ibuprofen	929.1	828.0	399.8	1660.8	100	4053.3	2976.5	1972.6	8287.3
Organic	Pharmaceutical (Antihypertensive)	Losartan	657.9	583.6	359.0	1105.5	100	2944.4	2272.7	1715.8	5516.5
Organic	Pharmaceutical (Antihypertensive)	Atenolol	656.8	664.5	566.4	731.9	100	2917.1	3078.9	1936.3	3574.3
Organic	Pharmaceutical (Pain killer)	Acetaminophen	482.7	476.9	25.5	951.4	100	1986.5	1548.3	102.1	4747.5
Organic	Pharmaceutical (Antibiotics)	Sulfamethoxazole	385.2	349.6	282.1	559.5	100	1650.1	1541.6	1273.7	2243.6
Organic	Pharmaceutical (Anticonvulsant)	Carbamazepine	293.2	256.7	164.7	494.9	100	1355.5	1216.0	520.5	2469.7
Organic	Pharmaceutical (Antibiotics)	Ciprofloxacin	272.2	260.3	103.9	464.4	100	1355.3	1225.1	328.5	2642.3
Organic	Pharmaceutical (Pain killer)	Niflumic acid	233.4	163.7	135.1	471.1	100	1074.4	722.8	501.1	2350.8
Organic	Pharmaceutical (Antibiotics)	Clarithromycin	181.2	205.8	69.7	243.7	100	747.5	808.1	396.4	977.3
Organic	Pharmaceutical (Antiarrhythmic)	Metoprolol	174.7	105.6	69.7	417.8	100	823.8	495.0	220.3	2084.8
Organic	Pharmaceutical (Antibiotics)	Ofloxacin	162.4	153.2	79.2	264.2	100	793.4	710.1	250.2	1503.3
Organic	Pharmaceutical (Antibiotics)	Roxithromycin	153.9	134.9	53.5	292.2	100	658.2	604.9	214.7	1208.5
Organic	Pharmaceutical (Glaucoma)	Acetazolamide	142.3	175.9	<QL	217.5	75.0	633.0	647.1	-	1237.8
Organic	Pharmaceutical (Antibiotics)	Erythromycin A	141.3	54.3	18.0	438.3	100	689.7	257.3	57.0	2187.2
Organic	Pharmaceutical (Glycemia)	Gemfibrozil	140.6	124.8	107.6	205.1	100	622.4	652.7	361.9	822.3
Organic	Pharmaceutical (Antibiotics)	Azithromycin	136.3	84.4	46.0	330.5	100	536.1	457.7	184.6	1044.4
Organic	Pharmaceutical (Antibiotics)	Spiramycin	108.1	33.4	21.4	344.4	100	380.5	163.6	106.6	1088.4
Organic	Pharmaceutical (Antibiotics)	Metronidazole	105.5	98.2	91.0	134.7	100	479.9	412.8	327.8	766.3
Organic	Pharmaceutical (Antibiotics)	Trimethoprim	99.2	88.9	65.6	153.4	100	440.5	469.9	207.2	615.0
Organic	Pharmaceutical (Antibiotics)	Norfloxacin	71.3	36.2	<QL	212.8	50.0	375.4	145.3	-	1211.0
Organic	Pharmaceutical (Antibiotics)	Josamycin	47.5	40.7	25.3	83.2	100	195.3	194.8	128.8	262.9
Organic	Pharmaceutical (Anxiolytics)	Lorazepam	24.3	23.3	20.7	29.9	100	108.2	111.4	68.9	140.8
Organic	Pharmaceutical (Anxiolytics)	Nordazepam	11.5	10.6	9.3	15.4	100	50.2	51.9	35.0	61.8
Organic	Pharmaceutical (Anticancer)	Cyclophosphamide	8.2	7.8	<QL	17.0	50.0	39.8	31.3	-	96.8
Organic	Pharmaceutical (Antibiotics)	Sulfadiazine	7.2	6.3	<QL	16.0	50.0	25.3	25.2	-	50.7
Organic	Pharmaceutical (Antibiotics)	Piperacillin	0.6	0.0	<QL	2.4	25.0	1.9	-	-	7.5
Organic	Pharmaceutical (Antibiotics)	Ampicilline	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Antibiotics)	Doxycycline	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Antibiotics)	Flumequine	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Antibiotics)	Oxolinic acid	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Antibiotics)	Rifampicin	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Antibiotics)	Sulfamethazine	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Antibiotics)	Tetracycline	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Antibiotics)	Tylosine	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Hormones)	E2	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Hormones)	EE2	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Hormones)	E1	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Pain killer)	Phenazone	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Contraceptif)	19-Norethindrone	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Antiarrhythmic)	Amiodarone	-	-	-	-	-	-	-	-	-
Organic	Pharmaceutical (Antibiotics)	Amoxicillin	-	-	-	-	-	-	-	-	-
Organic	Pharmaceutical (Pain killer)	Acetylsalicylic acid	-	-	-	-	-	-	-	-	-
Organic	Pharmaceutical (Antineoplastic)	Hydroxycarbamide	-	-	-	-	-	-	-	-	-
	TOTAL		**15664.5**		**6169.7**	**31312.0**		**70430.8**		**29184.8**	**152950.1**

‘WWTP 4’

			Concentrations (ng.L^{-1})					Daily flux estimations (mg.day^{-1})			
Substance families	Analytical Groups	Analytes	Mean	Median	Min	Max	Occurrence (%)	Mean	Median	Min	Max
Priority substances											
Metal		Chromium (Cr)	5239.5	4815.5	4474.0	6853.0	100	17859.9	18428.5	12585.7	21997.1
Metal		Vanadium (V)	5105.8	4626.8	4135.3	7034.3	100	17401.5	18422.6	11144.5	21616.3
Metal		Copper (Cu)	2797.7	2759.7	1610.7	4060.7	100	8962.6	9151.2	5687.3	11860.8
Metal		Nickel (Ni)	2211.5	2107.5	1534.0	3097.0	100	7511.5	7530.3	5416.6	9568.6
Metal		Arsenic (As)	1528.8	1430.3	1353.8	1900.8	100	5223.4	5421.8	3648.4	6401.6
Metal		Antimony (Sb)	1136.0	1174.8	830.3	1364.3	100	3982.5	4149.8	2237.5	5392.9
Metal		Molybdenum (Mo)	515.3	441.5	154.0	1024.0	100	1780.6	1545.9	415.0	3615.7
Metal		Lead (Pb)	507.5	532.5	239.5	725.5	100	1736.0	1871.8	645.5	2554.7
Metal		Tin (Sn)	303.5	273.0	187.0	481.0	100	992.3	843.1	805.1	1478.1
Metal		Silver (Ag)	23.8	21.0	10.5	42.5	100	87.9	67.4	28.3	188.4
Metal		Cadmium (Cd)	14.3	11.8	7.3	26.3	100	47.0	41.2	24.9	80.7
	TOTAL		**19383.4**		**14536.2**	**26609.2**		**65585.2**		**42638.6**	**84754.7**
Organomercury compound		IHg	1.3	1.1	0.4	2.7	100	4.5	4.3	1.2	8.4
Organomercury compound		MMHg	0.04	0.03	0.01	0.1	100	0.1	0.1	0.03	0.2
	TOTAL		**1.3**		**0.4**	**2.8**		**4.7**		**1.3**	**8.6**
Organic	PAH	Naphthalene	10.8	10.8	<DL	21.6	50.0	33.7	33.7	-	67.4
Organic	PAH	Benzo[b]fluoranthene	1.7	<DL	<DL	7.6	40.0	5.3	-	-	23.4
Organic	PAH	Phenanthrene	1.2	<DL	<DL	4.9	40.0	3.8	-	-	15.3
Organic	PAH	Acenaphthene	0.7	0.4	<DL	1.9	60.0	1.9	1.6	-	5.1
Organic	PAH	Fluoranthene	0.5	<DL	<DL	2.1	25.0	1.6	-	-	6.5
Organic	PAH	Pyrene	0.5	<DL	<DL	2.3	20.0	1.4	-	-	7.1
Organic	PAH	Fluorene	0.4	<DL	<DL	2.0	40.0	1.3	-	-	6.3
Organic	PAH	Anthracene	0.4	<DL	<DL	1.2	40.0	1.1	-	-	3.9
Organic	PAH	Benzo[a]anthracene	0.2	<DL	<DL	1.2	20.0	0.8	-	-	3.9
Organic	PAH	Acenaphthylene	0.2	<DL	<DL	1.1	25.0	0.7	-	-	3.4
Organic	PAH	Benzo[a]pyrene	0.2	<DL	<DL	<1.0	40.0	0.7	-	-	3.1
Organic	PAH	Chrysene	0.2	<DL	<DL	<1.0	20.0	0.6	-	-	3.1
Organic	PAH	Benzo[k]fluoranthene	0.2	<DL	<DL	<1.0	20.0	0.6	-	-	3.1
Organic	PAH	Indeno[1,2,3-cd]pyrene	0.2	<DL	<DL	<1.0	20.0	0.6	-	-	3.1
Organic	PAH	Dibenzo[a,h]anthracene	0.2	<DL	<DL	<1.0	20.0	0.6	-	-	3.1
Organic	PAH	Benzo[g,h,i]perylene	0.2	<DL	<DL	<1.0	20.0	0.6	-	-	3.1
	TOTAL		**17.8**		**<DL**	**51.9**		**55.4**		**-**	**160.7**
Organic	PCB	PCB 194	0.9	<DL	<DL	4.7	20.0	2.9	-	-	14.6
Organic	PCB	PCB 28+31	0.2	<DL	<DL	1.2	20.0	0.7	-	-	3.7
Organic	PCB	PCB 180	0.2	<DL	<DL	<1.0	40.0	0.7	-	-	3.1
Organic	PCB	PCB 18	0.2	<DL	<DL	<1.0	20.0	0.6	-	-	3.1
Organic	PCB	PCB 52	0.2	<DL	<DL	<1.0	20.0	0.6	-	-	3.1
Organic	PCB	PCB 44	0.2	<DL	<DL	<1.0	20.0	0.6	-	-	3.1
Organic	PCB	PCB 101	0.2	<DL	<DL	<1.0	20.0	0.6	-	-	3.1
Organic	PCB	PCB 149	0.2	<DL	<DL	<1.0	20.0	0.6	-	-	3.1
Organic	PCB	PCB 118	0.2	<DL	<DL	<1.0	20.0	0.6	-	-	3.1
Organic	PCB	PCB 153	0.2	<DL	<DL	<1.0	20.0	0.6	-	-	3.1
Organic	PCB	PCB 138	0.2	<DL	<DL	<1.0	20.0	0.6	-	-	3.1
	TOTAL		**3.0**		**<DL**	**14.8**		**9.3**		**-**	**46.1**
Organic	AP	NP	506.4	254.0	118.8	1207.8	100	1575.3	990.8	419.5	3711.6
Organic	AP	4tOP	32.7	23.9	<DL	104.8	60.0	110.4	74.6	-	322.1
Organic	AP	NPEO1	5.0	5.0	<DL	<10.0	50.0	15.6	15.6	-	31.2
Organic	AP	4nOP	2.0	<DL	<DL	<10.0	20.0	6.2	-	-	31.2
Organic	AP	NPEO2	2.0	<DL	<DL	<10.0	20.0	6.2	-	-	31.2
	TOTAL		**548.2**		**118.8**	**1342.3**		**1713.8**		**419.5**	**4127.2**
Organic	OCP	Beta BHC	5.7	<DL	<DL	22.8	25.0	17.8	-	-	71.3
Organic	OCP	Alpha BHC	0.9	<DL	<DL	3.5	25.0	2.7	-	-	10.9
Organic	OCP	4,4'-DDE	0.7	<DL	<DL	3.5	20.0	2.2	-	-	10.9
Organic	OCP	Bêta Endosulfan	0.7	<DL	<DL	2.6	25.0	2.0	-	-	8.1
Organic	OCP	Methoxychlor	0.7	<DL	<DL	2.0	33.3	2.1	-	-	6.3
Organic	OCP	Delta BHC	0.7	<DL	<DL	<2.0	33.3	2.1	-	-	6.2
Organic	OCP	Endosulfan Sulfate	0.7	<DL	<DL	<2.0	33.3	2.1	-	-	6.2
Organic	OCP	Gamma BHC	0.7	<DL	<DL	<2.0	33.3	2.1	-	-	6.2
Organic	OCP	4,4'-DDT	0.7	<DL	<DL	<2.0	33.3	2.1	-	-	6.2
Organic	OCP	Alpha Endosulfan	0.6	<DL	<DL	2.3	25.0	1.8	-	-	7.1
Organic	OCP	Heptachlor	0.5	<DL	<DL	<2.0	25.0	1.6	-	-	6.2
Organic	OCP	Aldrin	0.4	<DL	<DL	<2.0	20.0	1.2	-	-	6.2
Organic	OCP	Dieldrine	0.4	<DL	<DL	<2.0	20.0	1.2	-	-	6.2
Organic	OCP	Heptachlor Epoxide	0.4	<DL	<DL	<2.0	20.0	1.2	-	-	6.2
Organic	OCP	4,4'-DDD	0.4	<DL	<DL	<2.0	20.0	1.2	-	-	6.2
Organic	OCP	Endrin Aldehyde	0.4	<DL	<DL	<2.0	20.0	1.2	-	-	6.2
Organic	OCP	Endrin Ketone	0.4	<DL	<DL	1.9	20.0	1.2	-	-	6.0
Organic	OCP	Endrin	0.1	<DL	<DL	0.7	20.0	0.5	-	-	2.3
	TOTAL		**14.8**		**<DL**	**58.4**		**46.3**		**-**	**185.0**

‘WWTP 4’

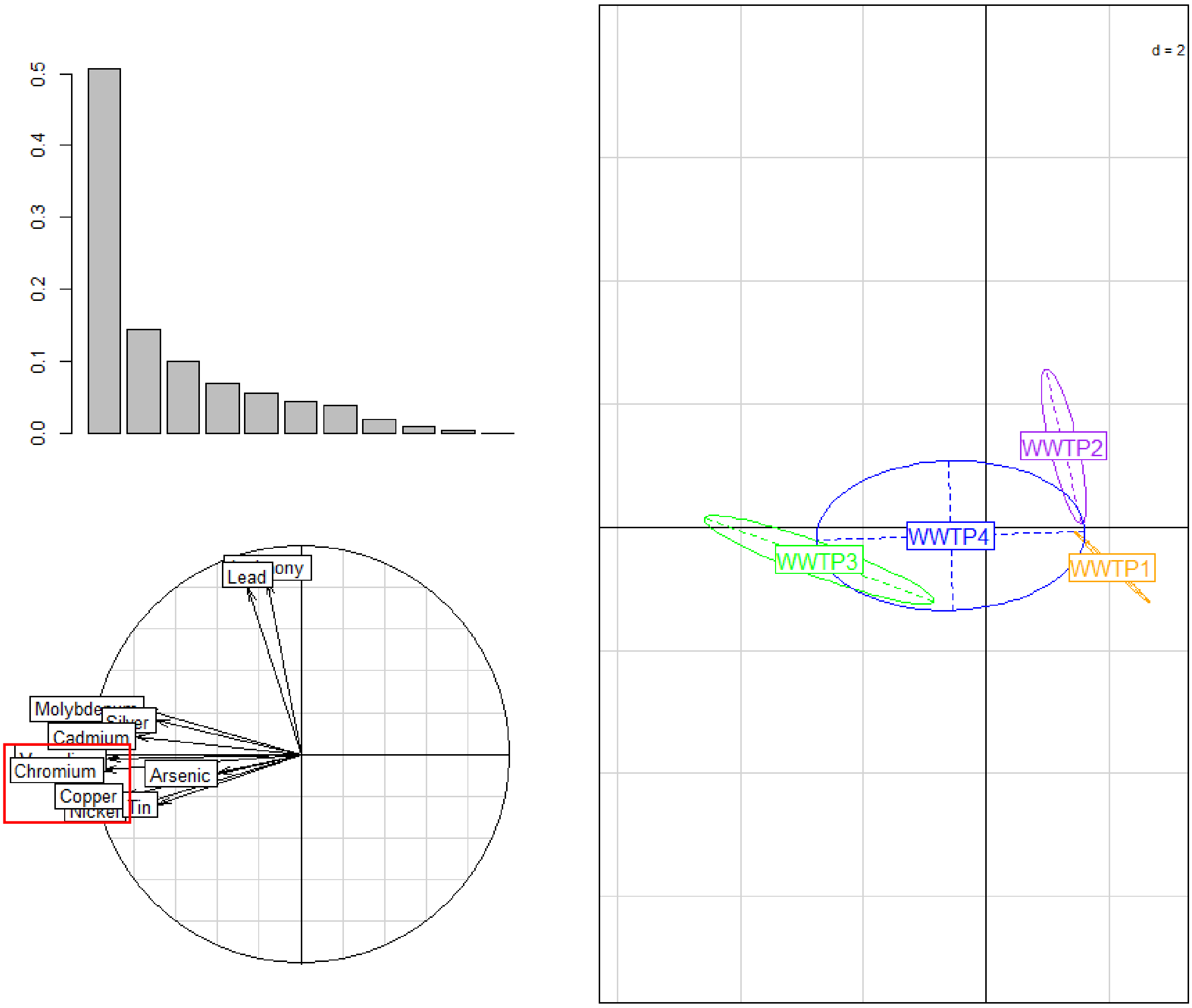
			Concentrations (ng.L^{-1})					Daily flux estimations (mg.day^{-1})			
Substance families	**Analytical Groups**	**Analytes**	**Mean**	**Median**	**Min**	**Max**	**Occurrence (%)**	**Mean**	**Median**	**Min**	**Max**
Emerging substances											
Organic	Musk	HHCB	1735.9	1555.5	1241.7	2788.0	100	5972.1	4192.2	3815.7	9844.5
Organic	Musk	HHCB-lactone	575.5	573.6	387.5	746.9	100	1989.7	2025.4	1209.4	3311.8
Organic	Musk	AHTN	253.8	238.8	219.5	347.4	100	879.0	750.3	643.6	1540.2
Organic	Musk	MK	57.3	71.7	23.0	84.5	100	196.6	227.8	70.7	317.7
Organic	Musk	ADBI	6.3	1.0	<DL	18.5	60.0	25.5	3.1	-	82.1
Organic	Musk	ATII	0.7	<DL	<DL	3.3	20.0	2.1	-	-	10.4
Organic	Musk	AHMI	0.2	<DL	<DL	<1.0	20.0	0.6	-	-	3.1
Organic	Musk	MA	0.2	<DL	<DL	<1.0	20.0	0.6	-	-	3.1
Organic	Musk	MX	0.2	<DL	<DL	<1.0	20.0	0.6	-	-	3.1
Organic	Musk	MM	0.2	<DL	<DL	<1.0	20.0	0.6	-	-	3.1
	TOTAL		**2630.2**		**1871.7**	**3992.6**		**9067.4**		**5739.4**	**15119.1**
Organic	Sunscreen	OC	78.1	44.2	15.2	209.0	100	202.8	93.9	-	642.3
Organic	Sunscreen	4-MBC	57.2	25.2	<DL	178.5	75.0	157.3	53.1	-	630.2
Organic	Sunscreen	Benzophenone 3	21.4	16.6	<DL	52.2	75.0	66.2	1.0	-	184.3
Organic	Sunscreen	EHMC	0.1	<DL	<DL	0.3	25.0	0.2	-	-	1.1
Organic	Sunscreen	3-BC	<DL	<DL	<DL	<DL	0.0	-	-	-	-
Organic	Sunscreen	OD-PABA	<DL	<DL	<DL	<DL	0.0	-	-	-	-
	TOTAL		**156.8**		**15.2**	**440.0**		**426.6**		**-**	**1457.9**
Organic	Pharmaceutical (Anxiolytics)	Oxazepam	1913.2	1830.5	1080.4	2911.4	100	6285.8	6190.8	3814.8	8946.9
Organic	Pharmaceutical (Antihypertensive)	Hydrochlorothiazide	1176.6	879.8	504.7	2442.0	100	3776.6	2909.9	1782.2	7504.4
Organic	Pharmaceutical (Anti-inflammatory)	Diclofenac	1035.3	643.3	418.6	2436.2	100	3406.6	2330.8	1478.2	7486.5
Organic	Pharmaceutical (Psychotropic)	Caffeine	769.5	814.7	461.0	987.7	100	2532.6	2434.7	2044.2	3216.9
Organic	Pharmaceutical (Anticonvulsant)	Carbamazepine	632.5	502.7	396.9	1127.8	100	2156.5	2038.4	1083.2	3465.8
Organic	Pharmaceutical (Antibiotics)	Ciprofloxacin	455.4	430.6	107.2	853.0	100	1674.9	1269.3	378.7	3782.3
Organic	Pharmaceutical (Antihypertensive)	Losartan	416.0	397.5	201.7	667.2	100	1378.9	1376.6	712.3	2050.3
Organic	Pharmaceutical (Antihypertensive)	Atenolol	331.2	328.8	251.9	415.3	100	1153.6	941.8	889.4	1841.4
Organic	Pharmaceutical (Pain killer)	Ketoprofen	293.7	284.2	128.4	478.1	100	1011.7	1041.3	453.4	1510.7
Organic	Pharmaceutical (Antibiotics)	Ofloxacin	264.7	254.7	114.2	435.2	100	948.8	731.2	403.2	1929.7
Organic	Pharmaceutical (Pain killer)	Niflumic acid	253.9	187.9	123.2	516.6	100	857.2	719.3	402.8	1587.4
Organic	Pharmaceutical (Antibiotics)	Sulfamethoxazole	244.1	232.8	117.5	393.2	100	841.7	761.8	361.0	1482.2
Organic	Pharmaceutical (Antibiotics)	Azithromycin	232.0	189.9	32.7	515.6	100	726.6	700.7	115.3	1389.7
Organic	Pharmaceutical (Glycemia)	Gemfibrozil	167.2	152.8	108.1	255.3	100	564.9	534.8	372.8	817.2
Organic	Pharmaceutical (Anti-inflammatory)	Ibuprofen	158.2	116.8	85.1	314.0	100	516.3	386.5	327.3	965.1
Organic	Pharmaceutical (Antibiotics)	Erythromycin A	107.0	46.4	18.9	316.4	100	351.7	191.9	50.9	972.2
Organic	Pharmaceutical (Antibiotics)	Trimethoprim	105.9	114.0	36.4	159.3	100	352.0	395.8	128.5	487.7
Organic	Pharmaceutical (Antibiotics)	Metronidazole	86.3	91.5	44.9	117.2	100	301.1	277.7	138.0	510.9
Organic	Pharmaceutical (Glaucoma)	Acetazolamide	84.4	81.3	<QL	175.1	75.0	326.6	265.1	-	776.2
Organic	Pharmaceutical (Antibiotics)	Roxithromycin	81.9	60.7	14.5	191.5	100	268.7	253.7	51.2	516.0
Organic	Pharmaceutical (Antibiotics)	Clarithromycin	80.2	63.5	46.1	147.9	100	258.1	235.6	162.7	398.5
Organic	Pharmaceutical (Antiarrhythmic)	Metoprolol	77.1	60.0	42.0	146.5	100	253.4	207.7	148.2	450.1
Organic	Pharmaceutical (Antibiotics)	Norfloxacin	59.4	23.5	<QL	190.6	50.0	252.8	83.0	-	845.0
Organic	Pharmaceutical (Antibiotics)	Spiramycin	57.9	39.8	<QL	151.9	75.0	165.1	125.5	-	409.4
Organic	Pharmaceutical (Antibiotics)	Piperacillin	42.3	0.0	<QL	169.2	25.0	149.3	-	-	597.4
Organic	Pharmaceutical (Anxiolytics)	Lorazepam	37.9	34.5	27.0	55.7	100	130.4	138.8	72.8	171.2
Organic	Pharmaceutical (Antibiotics)	Josamycin	29.9	15.6	12.7	75.5	100	90.8	56.5	46.6	203.5
Organic	Pharmaceutical (Antibiotics)	Flumequine	19.5	10.0	<QL	57.8	50.0	81.7	35.4	-	256.1
Organic	Pharmaceutical (Anxiolytics)	Nordazepam	17.0	16.1	13.1	22.7	100	57.6	57.9	43.6	71.0
Organic	Pharmaceutical (Antibiotics)	Tetracycline	15.5	8.7	<QL	44.6	50.0	64.9	30.8	-	197.8
Organic	Pharmaceutical (Pain killer)	Acetaminophen	8.8	9.5	<QL	16.3	75.0	33.1	36.4	-	59.6
Organic	Pharmaceutical (Anticancer)	Cyclophosphamide	8.8	0.0	<QL	35.0	25.0	38.8	-	-	155.3
Organic	Pharmaceutical (Antibiotics)	Sulfadiazine	6.9	5.9	<QL	15.7	50.0	27.7	20.8	-	69.4
Organic	Pharmaceutical (Pain killer)	Phenazone	5.9	0.0	<QL	23.8	25.0	18.3	-	-	73.1
Organic	Pharmaceutical (Antibiotics)	Ampicilline	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Antibiotics)	Doxycycline	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Antibiotics)	Oxolinic acid	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Antibiotics)	Rifampicin	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Antibiotics)	Sulfamethazine	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Antibiotics)	Tylosine	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Hormones)	E2	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Hormones)	EE2	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Hormones)	E1	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Contraceptif)	19-Norethindrone	<QL	<QL	<QL	<QL	0.0	-	-	-	-
Organic	Pharmaceutical (Antiarrhythmic)	Amiodarone	-	-	-	-	-	-	-	-	-
Organic	Pharmaceutical (Antibiotics)	Amoxicillin	-	-	-	-	-	-	-	-	-
Organic	Pharmaceutical (Pain killer)	Acetylsalicylic acid	-	-	-	-	-	-	-	-	-
Organic	Pharmaceutical (Antineoplastic)	Hydroxycarbamide	-	-	-	-	-	-	-	-	-
	TOTAL		**9276.0**		**4387.1**	**16861.3**		**31054.8**		**15461.4**	**55196.8**

‘WWTP 5’

			Concentrations (ng.L^{-1})				
Substance families	**Analytical Groups**	**Analytes**	**Mean**	**Median**	**Min**	**Max**	**Occurrence (%)**
Priority substances							
Metal		Vanadium (V)	-	-	-	-	-
Metal		Chromium (Cr)	-	-	-	-	-
Metal		Nickel (Ni)	-	-	-	-	-
Metal		Copper (Cu)	-	-	-	-	-
Metal		Arsenic (As)	-	-	-	-	-
Metal		Molybdenum (Mo)	-	-	-	-	-
Metal		Silver (Ag)	-	-	-	-	-
Metal		Cadmium (Cd)	-	-	-	-	-
Metal		Tin (Sn)	-	-	-	-	-
Metal		Antimony (Sb)	-	-	-	-	-
Metal		Lead (Pb)	-	-	-	-	-
	TOTAL		**-**	**-**	**-**	**-**	**-**
Organomercury compound		IHg	2.2	0.8	0.5	5.2	100
Organomercury compound		MMHg	0.1	0.1	0.0	0.2	66.7
	TOTAL		**2.3**		**0.5**	**5.4**	
Organic	PAH	Naphthalene	19.9	19.9	19.9	19.9	100
Organic	PAH	Fluorene	14.2	3.1	<DL	50.5	75.0
Organic	PAH	Phenanthrene	8.5	5.4	<DL	23.2	75.0
Organic	PAH	Acenaphthene	2.4	2.3	<DL	5.0	75.0
Organic	PAH	Benzo[g,h,i]perylene	0.9	0.5	<DL	2.5	50.0
Organic	PAH	Fluoranthene	0.5	0.0	<DL	1.4	33.3
Organic	PAH	Pyrene	0.4	0.0	<DL	1.6	25.0
Organic	PAH	Anthracene	0.3	0.0	<DL	<1.0	50.0
Organic	PAH	Benzo[a]anthracene	0.3	0.1	<DL	<1.0	50.0
Organic	PAH	Acenaphthylene	0.3	0.0	<DL	1.0	25.0
Organic	PAH	Chrysene	0.2	0.0	<DL	<1.0	25.0
Organic	PAH	Benzo[b]fluoranthene	0.2	0.0	<DL	<1.0	25.0
Organic	PAH	Benzo[k]fluoranthene	0.2	0.0	<DL	<1.0	25.0
Organic	PAH	Benzo[a]pyrene	0.2	0.0	<DL	<1.0	25.0
Organic	PAH	Indeno[1,2,3-cd]pyrene	0.2	0.0	<DL	<1.0	25.0
Organic	PAH	Dibenzo[a,h]anthracene	0.2	0.0	<DL	<1.0	25.0
	TOTAL		**49.1**		**19.9**	**112.4**	
Organic	PCB	PCB 101	0.7	0.5	<DL	1.7	50.0
Organic	PCB	PCB 28+31	0.6	0.0	<DL	2.3	25.0
Organic	PCB	PCB 52	0.3	0.1	<DL	<1.0	50.0
Organic	PCB	PCB 18	0.2	0.0	<DL	<1.0	25.0
Organic	PCB	PCB 44	0.2	0.0	<DL	<1.0	25.0
Organic	PCB	PCB 149	0.2	0.0	<DL	<1.0	25.0
Organic	PCB	PCB 118	0.2	0.0	<DL	<1.0	25.0
Organic	PCB	PCB 153	0.2	0.0	<DL	<1.0	25.0
Organic	PCB	PCB 138	0.2	0.0	<DL	<1.0	25.0
Organic	PCB	PCB 180	0.2	0.0	<DL	<1.0	25.0
Organic	PCB	PCB 194	0.2	0.0	<DL	<1.0	25.0
	TOTAL		**3.5**		**<DL**	**12.1**	
Organic	AP	NP	755.7	789.7	39.4	1438.1	100
Organic	AP	NPEO1	390.5	390.5	390.5	390.5	100
Organic	AP	NPEO2	335.4	0.0	<DL	1006.3	33.3
Organic	AP	4tOP	4.2	0.0	<DL	12.6	33.3
Organic	AP	4nOP	3.3	0.0	<DL	<10.0	33.3
	TOTAL		**1489.2**		**429.9**	**2857.4**	
Organic	OCP	Delta BHC	1.2	1.2	<DL	2.4	50.0
Organic	OCP	Alpha BHC	1.2	0.0	<DL	3.6	33.3
Organic	OCP	Endosulfan Sulfate	1.0	1.0	<DL	<2.0	50.0
Organic	OCP	Gamma BHC	1.0	1.0	<DL	<2.0	50.0
Organic	OCP	Methoxychlor	1.0	1.0	<DL	<2.0	50.0
Organic	OCP	4,4'-DDT	0.9	0.9	<DL	1.7	50.0
Organic	OCP	Alpha Endosulfan	0.7	0.0	<DL	2.2	33.3
Organic	OCP	Bêta Endosulfan	0.7	0.0	<DL	<2.0	33.3
Organic	OCP	Beta BHC	0.7	0.0	<DL	<2.0	33.3
Organic	OCP	Heptachlor	0.7	0.0	<DL	<2.0	33.3
Organic	OCP	4,4'-DDE	0.6	0.0	<DL	2.2	25.0
Organic	OCP	Aldrin	0.5	0.0	<DL	<2.0	25.0
Organic	OCP	Dieldrine	0.5	0.0	<DL	<2.0	25.0
Organic	OCP	Endrin	0.5	0.0	<DL	<2.0	25.0
Organic	OCP	Heptachlor Epoxide	0.5	0.0	<DL	<2.0	25.0
Organic	OCP	4,4'-DDD	0.5	0.0	<DL	<2.0	25.0
Organic	OCP	Endrin Aldehyde	0.5	0.0	<DL	<2.0	25.0
Organic	OCP	Endrin Ketone	0.5	0.0	<DL	<2.0	25.0
	TOTAL		**13.0**		**<DL**	**36.9**	

‘WWTP 5’

			Concentrations (ng.L^{-1})				
Substance families	**Analytical Groups**	**Analytes**	**Mean**	**Median**	**Min**	**Max**	**Occurrence (%)**
Emerging substances							
Organic	Musk	HHCB	991.6	1000.0	719.3	1246.9	100
Organic	Musk	HHCB-lactone	636.4	631.7	511.0	771.2	100
Organic	Musk	AHTN	159.2	148.4	114.8	225.2	100
Organic	Musk	MK	153.7	173.9	63.8	203.1	100
Organic	Musk	ADBI	4.7	4.3	<DL	10.4	75.0
Organic	Musk	MX	0.6	0.0	<DL	2.4	25.0
Organic	Musk	AHMI	0.3	0.0	<DL	<1.0	25.0
Organic	Musk	MA	0.3	0.0	<DL	<1.0	25.0
Organic	Musk	ATII	0.3	0.0	<DL	<1.0	25.0
Organic	Musk	MM	0.3	0.0	<DL	<1.0	25.0
	TOTAL		**1947.1**		**1408.9**	**2462.8**	
Organic	Sunscreen	4-MBC	55.9	16.8	<DL	150.9	66.7
Organic	Sunscreen	Benzophenone 3	42.6	36.6	<DL	91.4	66.7
Organic	Sunscreen	OC	35.3	43.9	11.6	50.4	100
Organic	Sunscreen	EHMC	1.1	0.0	<DL	3.4	33.3
Organic	Sunscreen	3-BC	<DL	<DL	<DL	<DL	0.0
Organic	Sunscreen	OD-PABA	<DL	<DL	<DL	<DL	0.0
	TOTAL		**135.0**		**11.6**	**296.0**	
Organic	Pharmaceutical (Antihypertensive)	Hydrochlorothiazide	1614.0	1543.6	1420.7	1877.6	100
Organic	Pharmaceutical (Anti-inflammatory)	Diclofenac	841.1	1044.1	431.8	1047.3	100
Organic	Pharmaceutical (Antibiotics)	Ciprofloxacin	480.4	392.3	230.9	818.0	100
Organic	Pharmaceutical (Psychotropic)	Caffeine	472.0	356.1	196.1	863.9	100
Organic	Pharmaceutical (Antibiotics)	Azithromycin	458.5	520.6	104.6	750.2	100
Organic	Pharmaceutical (Antibiotics)	Ofloxacin	404.1	176.2	120.6	915.5	100
Organic	Pharmaceutical (Antibiotics)	Clarithromycin	208.8	226.3	49.1	351.1	100
Organic	Pharmaceutical (Anxiolytics)	Oxazepam	172.5	165.2	139.6	212.8	100
Organic	Pharmaceutical (Anxiolytics)	Lorazepam	131.8	137.9	97.7	160.0	100
Organic	Pharmaceutical (Pain killer)	Ketoprofen	123.0	143.9	80.4	144.7	100
Organic	Pharmaceutical (Antihypertensive)	Atenolol	116.4	116.4	114.8	117.9	100
Organic	Pharmaceutical (Antihypertensive)	Losartan	114.4	128.7	50.8	163.7	100
Organic	Pharmaceutical (Pain killer)	Phenazone	77.0	51.2	42.3	137.6	100
Organic	Pharmaceutical (Antibiotics)	Sulfamethoxazole	69.8	73.8	55.0	80.4	100
Organic	Pharmaceutical (Antibiotics)	Norfloxacin	63.6	68.5	<QL	122.4	66.7
Organic	Pharmaceutical (Antibiotics)	Trimethoprim	52.7	54.9	44.2	58.9	100
Organic	Pharmaceutical (Anticonvulsant)	Carbamazepine	41.3	41.2	17.9	64.9	100
Organic	Pharmaceutical (Antibiotics)	Erythromycin A	30.6	24.3	13.3	54.3	100
Organic	Pharmaceutical (Anxiolytics)	Nordazepam	29.6	30.2	21.8	36.8	100
Organic	Pharmaceutical (Glaucoma)	Acetazolamide	21.5	0.0	<QL	64.6	33.3
Organic	Pharmaceutical (Glycemia)	Gemfibrozil	21.4	21.6	3.4	39.0	100
Organic	Pharmaceutical (Antibiotics)	Metronidazole	18.8	19.3	10.7	26.3	100
Organic	Pharmaceutical (Anticancer)	Cyclophosphamide	14.1	15.7	<QL	26.6	66.7
Organic	Pharmaceutical (Antibiotics)	Tetracycline	13.3	0.0	<QL	40.0	33.3
Organic	Pharmaceutical (Antiarrhythmic)	Metoprolol	11.5	11.7	10.9	12.0	100
Organic	Pharmaceutical (Antibiotics)	Spiramycin	10.5	0.0	<QL	31.4	33.3
Organic	Pharmaceutical (Pain killer)	Acetaminophen	8.1	10.2	3.6	10.5	100
Organic	Pharmaceutical (Antibiotics)	Sulfadiazine	8.1	11.2	<QL	13.1	66.7
Organic	Pharmaceutical (Pain killer)	Niflumic acid	1.7	1.5	<QL	3.7	66.7
Organic	Pharmaceutical (Antibiotics)	Ampicilline	<QL	<QL	<QL	<QL	0.0
Organic	Pharmaceutical (Antibiotics)	Doxycycline	<QL	<QL	<QL	<QL	0.0
Organic	Pharmaceutical (Antibiotics)	Flumequine	<QL	<QL	<QL	<QL	0.0
Organic	Pharmaceutical (Antibiotics)	Josamycin	<QL	<QL	<QL	<QL	0.0
Organic	Pharmaceutical (Antibiotics)	Oxolinic acid	<QL	<QL	<QL	<QL	0.0
Organic	Pharmaceutical (Antibiotics)	Piperacillin	<QL	<QL	<QL	<QL	0.0
Organic	Pharmaceutical (Antibiotics)	Rifampicin	<QL	<QL	<QL	<QL	0.0
Organic	Pharmaceutical (Antibiotics)	Roxithromycin	<QL	<QL	<QL	<QL	0.0
Organic	Pharmaceutical (Antibiotics)	Sulfamethazine	<QL	<QL	<QL	<QL	0.0
Organic	Pharmaceutical (Antibiotics)	Tylosine	<QL	<QL	<QL	<QL	0.0
Organic	Pharmaceutical (Hormones)	E2	<QL	<QL	<QL	<QL	0.0
Organic	Pharmaceutical (Hormones)	EE2	<QL	<QL	<QL	<QL	0.0
Organic	Pharmaceutical (Hormones)	E1	<QL	<QL	<QL	<QL	0.0
Organic	Pharmaceutical (Anti-inflammatory)	Ibuprofen	<QL	<QL	<QL	<QL	0.0
Organic	Pharmaceutical (Contraceptif)	19-Norethindrone	<QL	<QL	<QL	<QL	0.0
Organic	Pharmaceutical (Antineoplastic)	Hydroxycarbamide	-	-	-	-	-
Organic	Pharmaceutical (Antiarrhythmic)	Amiodarone	-	-	-	-	-
Organic	Pharmaceutical (Antibiotics)	Amoxicillin	-	-	-	-	-
Organic	Pharmaceutical (Pain killer)	Acetylsalicylic acid	-	-	-	-	-
	TOTAL		**5630.7**		**3260.1**	**8245.3**	



SM 5: Principal component analysis (PCA) showing the metal distribution between the four sampled WWTPs (‘WWTP 1’, ‘WWTP 2’, ‘WWTP 3’ and ‘WWTP 4’).

Chapter IV:

Benthic communities' response to WWTP discharges in the intertidal zone

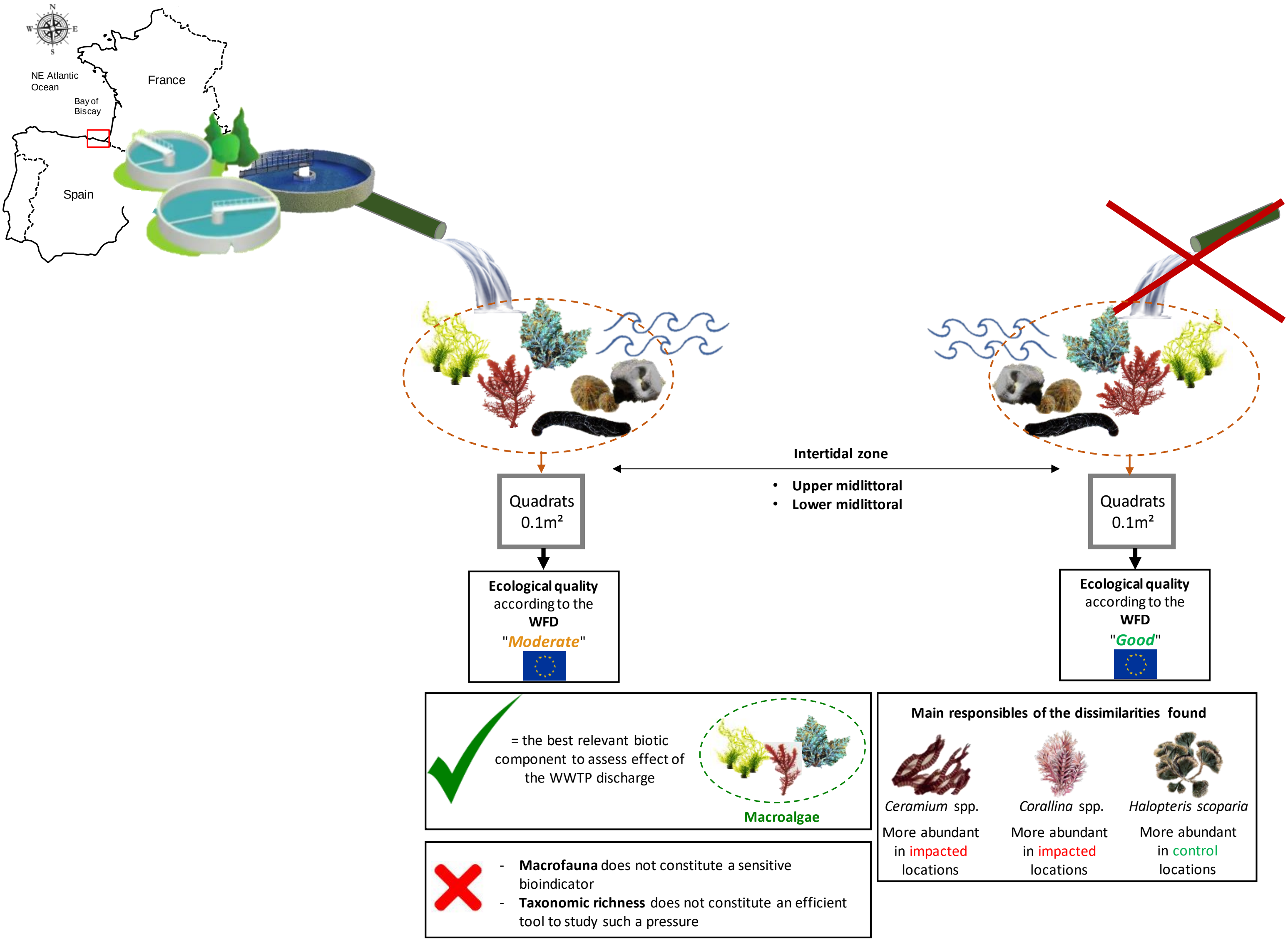


Fig. 1': Graphical abstract of the Chapter IV

Chapter structure:

- **Huguenin L.**, Lalanne Y, de Casamajor MN., Gorostiaga J-M., Quintano E., Salerno M., Monperrus M. (**2019**). "*Impact of sewage discharges on macroalgae and macrofauna assemblages of the intertidal rocky shores in the southeastern Bay of Biscay*". ***Continental Shelf Research***, 181, 34-49 (https://doi.org/10.1016/j.csr.2019.04.014).

Benthic communities’ response to WWTP discharges in the intertidal zone

Rocky shore habitats constitute one of the most common environments in coastal areas (Coutinho et al., 2016) but the intertidal zone is very vulnerable to anthropogenic pressures (Becherucci et al., 2016; Crain et al., 2008). Among those, sewage discharges constitute an important stressor for marine communities and may have diverse consequences (e.g. biotic homogenization, shift from algal-dominated assemblages to invertebrate-dominated assemblages) (Arévalo et al., 2007; Becherucci et al., 2016; Borowitzka, 1972; Littler and Murray, 1975; Liu et al., 2007; O’Connor, 2013; Vinagre et al., 2016a).

The study of environmental pollution through biotic diversity analyses has become of major importance because it gives precise information of the deleterious effects of contaminants (Borja et al., 2011a). Indeed, benthic communities are often used to assess marine pollutions because they reflect both previous and present conditions to which communities have been exposed (Reish, 1987). Because of their sedentary nature of macroalgae and the sensitivity of their components, they are known to be an accurate bioindicator of environmental changes (e.g. water quality of coastal waters for the WFD (Ar Gall et al., 2016; Borja et al., 2013a; Gorostiaga and Diez, 1996). In addition, macrofauna (fixed macrofauna as a snapshot indicator and mobile one as a precise descriptor of population dynamics, community structure, individual performance in response to environmental changes) has also to be considered, as it is requested by the Marine Strategy Framework Directive (MSFD; 2008/56/CE; EC, 2008). However, up to now, most studies are focused either on the survey of macroalgae or macrofauna assemblages independently and rarely together.

Problematic:

- ➔ Are intertidal rocky benthic communities affected by WWTP discharges?
- ➔ Are current WFD indices enough sensitive to study such a pressure?

This chapter/article deals with the study of the potential impact of WWTP discharges on intertidal rocky benthic assemblages (macroalgae and macrofauna) in the southeastern Bay of Biscay by comparing control and impacted locations and sites within locations (i.e. different distances from the outfalls). The general hypothesis is that if WWTP treatments are efficient, structural parameters of communities and results based on the WFD monitoring between impacted and control locations should be similar. The interest in studying both benthic fauna and flora is also discussed in this context.

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Impact of wastewater treatment plant discharges on macroalgae and macrofauna assemblages of the intertidal rocky shore in the southeastern Bay of Biscay

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ABSTRACT

Rocky intertidal habitats are particularly vulnerable to anthropogenic pressures especially in areas with high urban concentrations such as southeastern Bay of Biscay. This research aims to establish an assessment of the potential impact of sewage discharges on intertidal rocky benthic assemblages on macroalgae and on macrofauna as required by the European Directives (Water Framework Directive -WFD and Marine Strategy Framework Directive -MSFD). The assemblages were sampled at five locations according to a control-impact design. A moderate detectable effect of discharges was highlighted on the assemblage structure by means of multivariate analyses but this was less evident using other biological and ecological metrics. Results would also suggest that benthic macroalgae constitute for the study area the best relevant biotic component to assess the effect of this pressure on the intertidal rocky platform habitats. Changes in the relative abundance of *Ceramium* spp., *Corallina* spp. and *Halopteris scoparia* were mainly responsible of the dissimilarities found. Finally, a pseudo-ecological quality ratio, based on the current WFD metrics, was also calculated for each site within locations (i.e. each distance from the outfall) to assess its sensitivity to this type of pressure. Results were conformed with those of the WFD monitoring because the un- or less-impacted sites were ranked as *"Good"* contrary to the others ranked as *"Moderate"*. Thus, this work provides additional information for the MSFD and bridges deficiencies emphasized by Directives on the response of biological indicators to various pressures and the biocenosis of southeastern Bay of Biscay.

1. Introduction

Rocky shore habitats constitute one of the most common environments in coastal areas (Coutinho et al., 2016). The intertidal zone is a very important part of the coastal ecosystem providing many services in terms of primary productivity, fisheries and tourism (Seitz et al., 2013). These areas are governed by particular environmental factors (e.g., hydrodynamics, tides, salinity and temperature gradients) (Ghilardi et al., 2008) but these coastal habitats are very vulnerable to anthropogenic pressures (e.g. waste waters, urban runoff, spilled chemicals, overexploitation, invasive species introduction, habitat fragmentation and destruction) (Becherucci et al., 2016; Crain et al., 2008).

Among those pressures, sewage discharges are responsible for nutrient enrichment, turbidity, increased sedimentation, decreased salinity (Azzurro et al., 2010; Terlizzi et al., 2005) and contamination (by heavy metals, priority and emerging contaminants) (Costanzo et al., 2001; Millennium Ecosystem Assessment (MEA), 2005). In this regard, the European Urban Waste Water Treatment Directive (91/271/EEC) was adopted to protect the water environment from harmful effects of wastewater discharges (urban and industrial). It constitutes a prerequisite for the achievement of the objectives within the Water Framework Directive (WFD; 2000/60/EC; EC, 2000) which aims to attain "good ecological status" of all water bodies by 2020. This obliges politicians to make additional efforts to increase connections between a

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given population and wastewater systems and to improve the running of sewage treatment plants. Monitoring networks also have to be implemented by scientists and environmental managers to understand benthic communities' response and to distinguish changes caused by anthropogenic impacts from natural variability (Veríssimo et al., 2013). Indeed, sewage discharges constitute an important stressor for marine communities in many intertidal systems around the world (Arévalo et al., 2007; Becherucci et al., 2016; Borowitzka, 1972; Littler and Murray, 1975; Liu et al., 2007; O'Connor, 2013; Vinagre et al., 2016a). Depending on their type, source and level, they may have direct or indirect effects on the environment (Borja et al., 2011). Some studies highlight negative effects such as the alteration of benthic composition and abundance patterns (Guidetti et al., 2003; Terlizzi et al., 2005, 2002). The consequences may be diverse: a biotic homogenization (Amaral et al., 2018) with a simplification of community structure through a decrease in macroalgae species richness and abundance (Borowitzka, 1972; Díez et al., 1999; Littler and Murray, 1975), a decrease of pollution-sensitive species (Scherner et al., 2013), an increase of pollution-tolerant opportunistic species abundance due to their high reproductive capacity (Amaral et al., 2018) and a shift from algal-dominated assemblages to invertebrate-dominated assemblages (Díez et al., 2012). Contaminants released into the environment may also thereafter be accumulated in biological tissues or cause harmful effects such as endocrine disruption, behavioral changes, energy metabolism disturbances and genetic responses (Macdonald et al., 2003). However, other studies did not find an effect of this stressor on species richness of rocky shores (Archambault et al., 2001; O'Connor, 2013).

Over the last decades, large investigations and survey methods have been developed to study benthic communities of intertidal rocky shores (e.g. Huguenin et al., 2018; Le Hir and Hily, 2005; Vinagre et al., 2016b, 2016a; Wells et al., 2007; Zhao et al., 2016) in different contexts such as global climate change prospects (Barange, 2003; Thompson et al., 2002) or ecological status assessment of water bodies (e.g., WFD) (Borja et al., 2013; Guinda et al., 2014). In addition, the study of environmental pollution through biotic diversity analyses has become of major importance because it gives precise information of the deleterious effects of contaminants (Borja et al., 2011). In this context, and as described by Echavarri-Erasun et al. (2007), effects of sewage discharges have already been studied on different environmental compartments (e.g. sediments, water body, trophic web, benthic and pelagic communities). Benthic communities are often used to assess marine pollution because they reflect both previous and present conditions to which communities have been exposed (Reish, 1987).

Macroalgae constitute the primary food chain producers and the dominant group on rocky shore bottoms (Amaral et al., 2018). Because of their sedentary nature and the sensitivity of their components, they are known to be an accurate bioindicator (e.g., biochemical and physiological) of environmental changes (e.g. water quality of coastal waters for the WFD (Ar Gall et al., 2016; Borja et al., 2013; Gorostiaga and Diez, 1996). Their assessment is fundamental because their modification can also alter the trophic structures of other communities (e.g. grazers, carnivorous, scavengers) (Airoldi et al., 2008; Scherner et al., 2013; Schramm, 1999; Viaroli et al., 2008). Macrofauna has also to be considered, as it is requested by the Marine Strategy Framework Directive (MSFD; 2008/56/CE; EC, 2008). The use of mobile macrofauna as an indicator constitutes a "*snapshot in space and time*" because their community structure respond with short-term variability to environmental changes (Casamajor (de) and Lalanne, 2016; Davidson et al., 2004; Mieszkowska, 2015; Takada, 1999). Moreover, sessile species or slightly mobile species cannot redistribute themselves when faced with disturbances. They are then highly sensitive and constitute the first biological compartment impacted by environmental stressors (Maughan, 2001; Mieszkowska, 2015; Murray et al., 2006; Roberts et al., 1998). So, dispersion patterns of sessile macrofauna constitute more precise descriptors of population dynamics (e.g. recruitment and mortality), community structure, individual performance (e.g. physiology, morphology and behavior changes) in response to environmental changes (Mieszkowska, 2015). However, most studies are focused either on the survey of macroalgae or macrofauna assemblages independently (Anderlini and Wear, 1992; Cabral-Oliveira et al., 2014; Díez et al., 1999; Souza et al., 2013) and rarely together (Bishop et al., 2002; Echavarri-Erasun et al., 2007; Littler and Murray, 1975; López Gappa and Tablado, 1990; O'Connor, 2013; Terlizzi et al., 2002; Vinagre et al., 2016a).

The Basque coast ("Bay of Biscay" subregion) displays a set of environmental specificities: mesotidal conditions, with a magnitude between 1.85 and 3.85 m (Augris et al., 2009), energetic waves (Abadie et al., 2005), freshwater inputs caused by rainfall and a dense river system (Winckel et al., 2004), N-NW dominant winds, a specific coast orientation and geomorphology (cliffs, rocky platforms, boulder fields and semi-enclosed bays with sandy beaches) (Borja and Collins, 2004). In the western Basque coast (Spanish side), around 90% of the shore is constituted by rocky substrata (Borja and Collins, 2004) whereas in the eastern (French side) it is only 30% (Chust et al., 2009). All those parameters make this region a heritage area (Augris et al., 2009; Casamajor (de) and Lalanne, 2016) and justify the presence of specific communities in these remarkable habitats (Borja et al., 2004). Thus, rocky platforms constitute a habitat of European Community importance (High energy littoral rock; EUNIS A1-1). But, over the last decades, the French Basque coast has been subjected to urban sprawl and massive summer overcrowding (Le Treut, 2013) which explains the large number of WWTP (Wastewater Treatment Plant) outfalls along the coast.

Studies are scarce and local and are carried out only on the Spanish coastal area on macroalgae (Díez et al., 2013) and macrofauna (Bustamante et al., 2012) independently. This study therefore aims to offer a broader and integrated view on the potential impact of these discharges on intertidal rocky benthic assemblages (macroalgae and macrofauna) in the southeastern Bay of Biscay by comparing control and impacted locations and sites within locations (i.e. different distances from the outfalls). The general hypothesis is that if WWTP treatments are efficient, structural parameters of communities and results based on the WFD monitoring between impacted and control locations should be similar. This work also provides a framework for future monitoring allowing an assessment of benthic communities' changes related to WWTP mitigation measures. The interest in studying both benthic fauna and flora is also discussed in this context.

2. Methodology

2.1. Choice of the sampling design

To evaluate the impact of WWTP discharges, a control-impact design was chosen due to the absence of previous data of benthic assemblages in the impacted locations (before-after design) and models based on data characterizing the study area under reference conditions. This design is widely used to study an impact, a perturbation or a stressor on the environment (Murray et al., 2006) and allows temporal variation to be integrated. Impacted locations (with direct discharges from WWTP) and control locations (natural conditions) were thus chosen. Control locations were selected by expert judgment that is to say with similar features to WWTP locations: wave exposure (N-NW) and slight to moderate slope (< 30°).

2.2. Study area and sampling locations

The study was conducted in the southeastern Bay of Biscay. The field sampling campaign took place on intertidal rocky platforms in French and Spanish coastal areas of the Basque coast. The sampling was carried out during spring tide periods and in a relatively short period, from March 2nd to July 27th, 2017 (the same as used within the WFD). A total of five locations were selected (Fig. 1). Three locations were

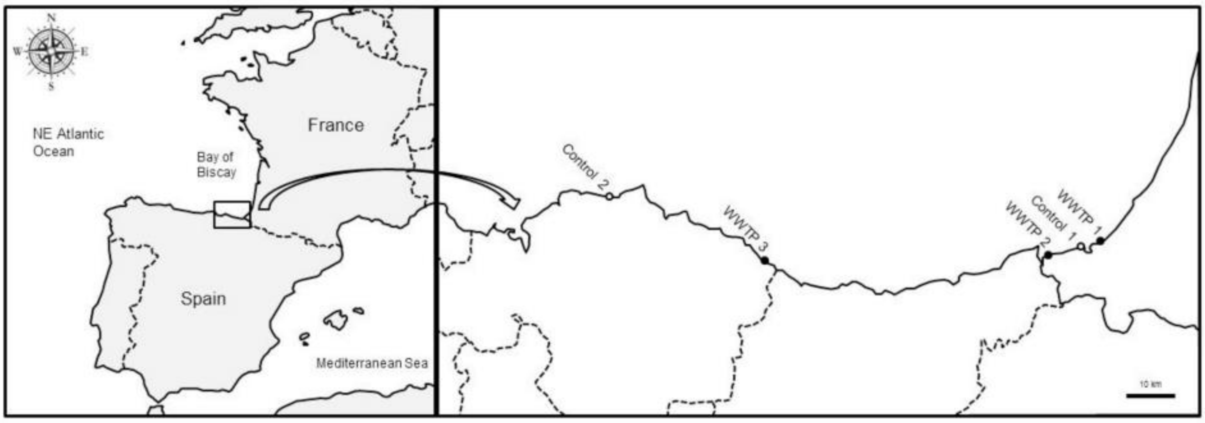

Fig. 1. Study area and locations: 'Control 1' (France) and 'Control 2' (Spain) (white points) and 'WWTP 1' (France), 'WWTP 2' (France) and 'WWTP 3' (Spain) constitute the impacted locations (black points).

Table 1
General WWTP features.

	'WWTP 1'	'WWTP 2'	'WWTP 3'
Location	France	France	Spain
Population equivalent (PE)	78 217	45 000	27 500
Nominal flow (m^3/day)	10 450	7350	5930
Outfall location	Intertidal zone	Intertidal zone	Intertidal zone

potentially impacted by Wastewater Treatment Plants (WWTP): 'WWTP 1' and 'WWTP 2' in France and 'WWTP 3' in Spain. General information of each WWTP were summarized in (Table 1). Two locations were considered as control: 'Control 1' in France and 'Control 2' in Spain.

2.3. Field data collection strategy

Each location was 200 m long and was represented by three sites in order to explore the spatial variability in a lower spatial scale. The selection of sites was done by means of a random stratified sampling design (i.e. sites were placed 100 m from each other and within them, the sampling was done randomly). Within impacted locations, sites corresponded to three distances from the outfall. They were all positioned on one side of the outfall, the first one being to a maximum of 10 m from the discharge. Sites within controls were established along the location maintaining the mentioned distances (Fig. 2). Within each site, two midlittoral zones were separately sampled: upper and lower midlittoral zones, characterized by algal-dominated communities described in the WFD "*Corallina* spp. & *Caulacanthus ustulatus*" and "*Halopteris scoparia & Gelidium* spp." (Ar Gall et al., 2016). In each site, a set of six randomly selected surfaces (33 × 33 cm quadrats) were positioned on comparable substrata (stable substrate and continuous bedrock) avoiding special microhabitats (crevices and pools) and separated by at least 1 m. The random sampling design ensures independence of errors and allows samples to be considered as replicates (Murray et al., 2006). In each quadrat, the percentage cover of macroalgae and sessile macrofauna (e.g. hexacorallia, mussels, barnacles, ascidiacea, etc.) was visually estimated and the abundance of mobile or slightly mobile macroafauna (e.g. gasteropods, crustaceans or limpets) was counted. This size quadrat is the same as those used for the WFD sampling and allows for direct comparison with others studies (Ar Gall et al., 2016; Casamajor (de) et al., 2016; Huguenin et al., 2018). Most organisms were identified *in situ* at species level to limit the sampling impact. When identification was impossible in the field (especially for small species), specimens were taken to the laboratory for further identification by taxonomic specialists. Due to the complex taxonomy of certain taxa, some organisms were identified at genus level (Huguenin et al., 2018).

2.4. Statistical analyses

The variation on the species composition and abundance (community structure) was studied by means of PERMANOVA analysis (Permutational multivariate analysis of variance using distance matrices; with 999 permutations) with pairwise post hoc tests (Anderson, 2001) using the standardized data set with an *a priori* chosen significant level of α = 0.05. For each midlittoral zone, statistical analyses were

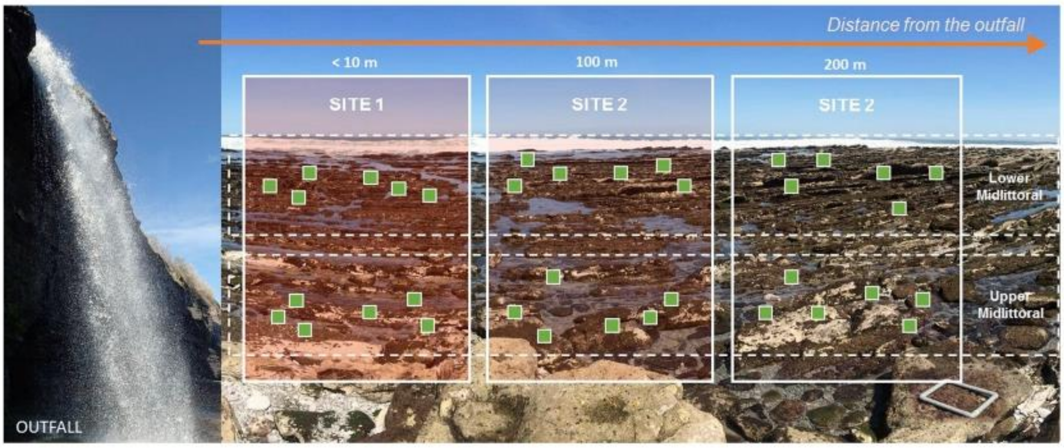

Fig. 2. Schematic layout of the sites in each location and midlittoral zone.

carried out separately for both areas (French and Spanish Basque coast), as a consequence of differences in the geomorphology (abrasion platforms *vs.* sloped platforms, respectively) and hydrodynamics (higher in the Spanish side). 'WWTP' within the French area were also studied separately as they have different features (Table 1). Therefore, each of the three 'WWTP' was compared with their corresponding 'Control'. Two factors were considered: (i) location (fixed, 2 levels) and (ii) sites (fixed and nested in location, 3 levels, representing increasing distances from the outfall in the case of impacted locations) with 6 random replicate samples. To avoid problems with unidentified species, analyses were conducted on aggregated data containing mixed taxonomic levels (species, genus, family, class). Data were standardized (e.g. each counting value, for one taxon, was divided by the maximum reached by this taxon in order to avoid differences in sampling units (percentage *vs.* abundance).

In order to explore the structure of benthic assemblages among locations (impacted and control) and within each location (different sites), a non-metric multi-dimensional scaling (nMDS) (after a distance matrix calculation) and a cluster analysis, based on Bray-Curtis dissimilarity, were conducted. These tools are useful in benthic marine community studies as they define the relative (dis)similarity between samples in multidimensional space (in two or more dimensional plots according to a number of reduced dimensions *k* defined by a degree of stress). nMDS does not use the absolute abundances of species in communities, but rather their rank distances (Clarke and Warwick, 2001; Murray et al., 2006). To identify the important contributors to differences among assemblages, the SIMilarity PERcentage (SIMPER) analysis was used (Oksanen et al., 2013). It enables the identification of taxa which contribute (according to their abundance) to the dissimilarity between locations and sites within each midlittoral zone.

Apart from the community structure, the mean abundance and the total taxonomic richness were calculated for each ecological group (macroalgae, mobile and sessile macrofauna). The mean taxonomic richness (MTR) was also calculated for each sample for macrofauna, for macroalgae and for characteristic and opportunistic taxa, in order to calculate the characteristic/opportunistic MTR ratio. For the MTR of macrofauna, species were assigned to one of five Ecological Groups (EG I–V) according to their responses to natural and man-induced changes in water quality: the higher the group, the higher the tolerance to pollution (Borja et al., 2000). Macroalgae species were classified as characteristic (per shore level) or opportunistic algae (signal of increasing eutrophication) according to existing lists achieved by Ar Gall et al. (2016) for French locations and Juanes et al. (2008) for Spanish ones. The spatial variability of the mean taxonomic richness was studied by means of PERMANOVA analysis (Permutation analysis of variance; with 999 permutations) with pairwise post hoc tests (Anderson, 2001) using raw data considering the two factors and the design mentioned above.

The graphs and statistical analyses were undertaken using Excel v7® and R® software.

2.5. Ecological quality

The quality index, achieved using the "intertidal macroalgae" WFD protocol (Casamajor (de) et al., 2016), was calculated for each location to assess its sensitivity to the pressure (Table 2). The WFD protocol was based on the Spanish CFR index (Guinda et al., 2008) and it was firstly adapted to Brittany by Ar Gall and Le Duff (2007). Then, it was adapted to the Basque coast by Casamajor (de) et al., (2010) due to a greater number of warm water species, the absence of large fucoids and a lower number of algal belt on the Basque coast. It constitutes a simplified version of the CCO index (Cover Characteristic - Opportunistic species; Ar Gall et al., 2016). The final rating of the index used in this study (on 1 point) was based on the sum of three subindices: (i) the global cover of macroalgae communities [C] (rated on 0.40 points), (ii) the number of characteristic species [N] (rated on 0.30 points) and (iii) the cover of opportunistic species [O] (rated on 0.30 points) (Casamajor (de) et al., 2016). This quality index was also calculated for each distance from the outfall of impacted location. It was called "pseudo-index" because it was calculated on only 12 quadrats (6 per midlittoral zone) randomly sampled during the campaign, as opposed to 18 (9 per midlittoral zone) in the WFD protocol.

Table 2
Ecological quality according to the CFR index.

Score	Ecological quality
0.80–1	Very good
0.60–0.79	Good
0.40–0.59	Moderate
0.20–0.39	Poor
0–0.19	Bad

3. Results

3.1. Effects of WWTP discharges on the structure of intertidal rocky benthic assemblages

Taking into account the whole study area, benthic assemblages differed in relation to coastal stretch (French or Spanish) and locations (WWTP and control) for both midlittoral zones (Fig. 3; Supplementary materials 1).

The analyses showed significant differences between each WWTP and their respective control (PERMANOVA, $p < 0.05$; Table 3). Furthermore, the analyses also detected significant variability at a lower scale (sites) in all three cases and for both midlittoral zones (Table 3). Post hoc pairwise comparisons revealed significant differences between almost of all the distances from the outfall within the French impacted locations ('WWTP 1' and 'WWTP 2') in both midlittoral zones (Table 4). In contrast, no such obvious differences were found in the control location ('Control 1') (only differences between sites at the upper level). In the Spanish area the variability among sites was slightly higher (at both midlittoral zones) in the control ('Control 2') than the impacted one ('WWTP 3') (Table 4). Differences at the site level were also supported by nMDS and dendrograms which also showed clear distinctions between them (Supplementary materials 2; Fig. 3).

3.2. Identification of contributors to differences in assemblages structure

SIMPER analyses identified, per each midlittoral zone, taxa responsible for differences between impacted and control locations and between sites within each location. Very few macrofauna species/taxa appeared in the analyses, consequently only macroalgae taxa identified as significant contributors were listed in Supplementary materials 3.

In both areas and midlittoral zones, the global dissimilarity between impacted and control locations varied from 42% to 71.14% (Table 5). The highest dissimilarity was obtained between 'WWTP 2' vs. 'Control 1' with 53.81% in the upper zone and 71.14% in the lower zone. The lowest occurred between 'WWTP 3' vs. 'Control 2' with 42% and 52.54%, respectively. At a lower scale, the highest global dissimilarity always appeared between S1 vs. S3 (the furthest sites) within impacted locations. Within control locations, a global dissimilarity between sites was also observed but higher values were either between S1 vs. S2 or between S2 vs. S3.

In the French area, among species/taxa identified in the upper midlittoral as significant contributors to the dissimilarity between impacted and control locations, only *Ceramium* spp. had a contribution higher than 10%, being more abundant in 'WWTP 1' than in 'Control 1' (2.07 vs. 0.38) (Supplementary materials 3). Furthermore, species such as *Laurencia obtusa* and *Osmundea pinnatifida* were more abundant in 'Control 1' than in 'WWTP 1' and 'WWTP 2', despite their contribution was lower than 10%. Actually, *Osmundea pinnatifida* was not present in

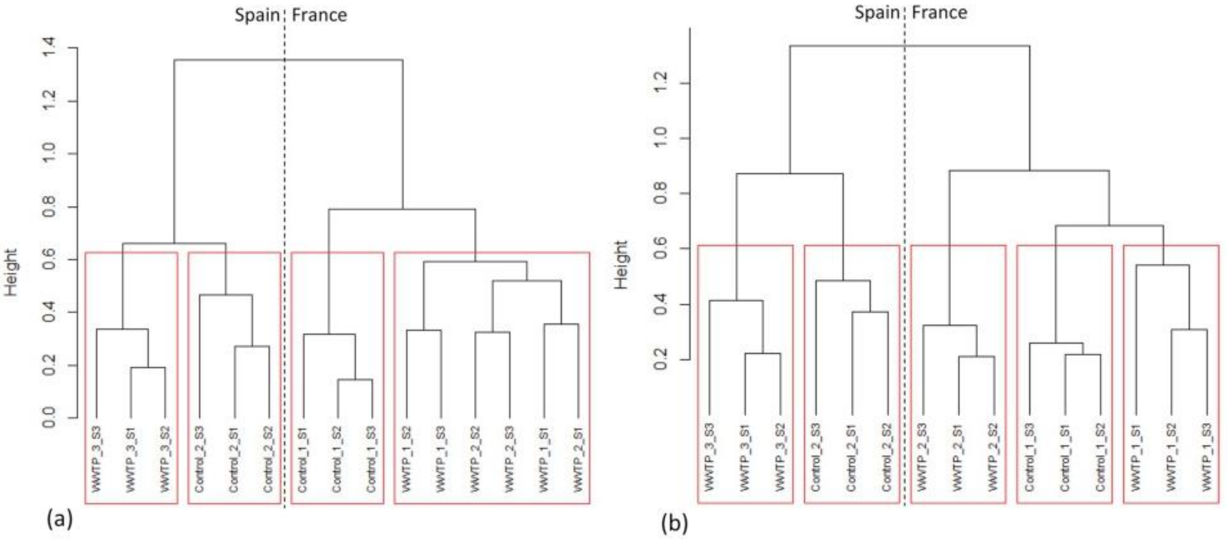

Fig. 3. Cluster analysis dendrograms computed on benthic taxon assemblages (macroalgae and macrofauna) in the upper midlittoral zone (Corallina spp. belt) (a) and the lower midlittoral zone (Halopteris scoparia belt) (b) of French and Spanish impacted ('WWTP 1', 'WWTP 2', 'WWTP 3') and control locations ('Control 1', 'Control 2'). Red boxes around the branches corresponded to a group of similar locations/sites.

'WWTP 1'. In the lower midlittoral zone, the significant contributors (> 10%) to the dissimilarity between impacted and control locations were *Halopteris scoparia* and *Ceramium* spp. (Supplementary materials 3). The former was more abundant in 'Control 1' than in 'WWTP1' (2.62 vs. 1.07) and 'WWTP 2' (2.62 vs. 0.15), whereas the latter showed higher values in 'WWTP 1' comparing to 'Control 1' (2.40 vs. 0.22). With a contribution below 10%, it should be highlighted the absent and the lower abundance of *Cystoseira tamariscifolia* in WWTP 1 and WWTP 2 (average abundance of 0.20), respectively, comparing to Control 1 (average abundance of 0.78). In the Spanish area, only *Corallina* spp. showed a contribution higher than 10% in the upper midlittoral zone (Supplementary materials 3). The abundance of this species was higher in 'WWTP 3' than in 'Control 2' (4.56 vs. 3.06). Furthermore, with a contribution below 10%, *Halopteris scoparia* showed slightly higher abundance in 'Control 2' comparing to 'WWTP 3' (0.50 vs. 0.03). Regarding the lower midlittoral zone, *Chondria coerulescens* was the species that contributed most (9.63%) to the dissimilarity between 'Control 2' and 'WWTP 3', being more abundant in the former location (1.44 vs. 0.03).

Focusing on the upper midlittoral zone and within French 'WWTP' locations, *Caulacanthus ustulatus* was a significant contributor (> 10%) in 'WWTP1' (Supplementary materials 3). The abundance of this species was higher in Site 1 (close to the outfall), decreasing towards Site 3. Within 'WWTP 2', *Ceramium* spp., *Corallina* spp. and *Laurencia obtusa* showed contributions higher than 10% (Supplementary materials 3). The former two species showed higher abundances in Site 1, whereas the latter, absent in Site 1, increased from Site 2 to Site 3 (0.39–1.67). Within the French control location ('Control 1'), four significant contributors were detected: *Halopteris scoparia*, *Caulacanthus ustulatus*, *Enteromorpha* spp. and *Codium adharens* (Supplementary materials 3). It is remarkable the higher abundance of *Halopteris scoparia* in Site 3 (1.33) comparing to Site 1 (absent), two distant sites according to the cluster (Fig. 3). It is also noticeable the higher abundance of *Caulacanthus ustulatus* and the lower abundance of *Enteromorpha* spp. in Site 2 comparing to Site 1.

Regarding the lower midlittoral zone, four species had a contribution higher than 10% within 'WWTP 1' (Supplementary materials 3). Among them, it should be highlighted the increasing abundance of *Halopeteris scoparia* from Site 1 to Site 3 (away from the outfall). By contrast, *Ceramium* spp showed higher abundance (3.08) in Site 1. Within 'WWTP 2', there was no contributor higher than 10%, but the abundances of *Cystoseira tamariscifolia* and *Halopteris scoparia* were higher in Site 3 (0.56 and 0.28, respectively) (Supplementary materials 3). However, it should be noticed that *Cystoseira tamariscifolia* was also present, with a very low abundance, in Site 1 (the closest from the outfall), whereas it was not detected in Site 2. Within the French control ('Control 1') and for the same tidal level no significant contributor was detected. Looking at sites comparisons, the abundance of *Enteromorpha* spp. decreased from Site 1 to Site 3 (from 0.38 to 0.05), whereas *Gelidium* spp. showed higher abundance in Site 3 comparing to Site 2 (Supplementary materials 3).

Regarding the upper midlittoral zone within Spanish 'WWTP 3', *Lithophyllum incrustans* was pointed as a significant contributor with lower abundance in Site 3 comparing to Site 1 (1.25 vs. 2.17) (Supplementary materials 3). By contrast, in the Spanish control location ('Control 2') three species with contributions higher than 10% were detected: *Chondria coerulescens*, *Corallina* spp. and *Halopteris scoparia* (Supplementary materials 3). The former two showed lower abundances in Site 3 comparing to Site 2, whereas the values of *Halopteris scoparia* were higher in Site 3 than in Site 1. In relation to the lower midlittoral zone within 'WWTP3', three significant contributors were recorded. Whilst *Codium adhaerens* increased from Site 1 to site 3, *Ceramium* spp. and *Corallina* spp. were higher in Site 1 (Supplementary materials 3). By contrast, within 'Control 2', four species had contribution higher than 10%. *Halopteris scoparia* showed the higher abundance in Site 2 (1.83), *Corallina* spp. decreased from Site 1 to Site 3 and *Cladostephus spongiosus* and *Codium adhaerens* were more abundant in Site 3 (Supplementary materials 3).

3.3. Effects of WWTP discharges on the diversity of intertidal rocky benthic assemblages

Eighty-eight species/taxa were identified during the field campaigns: 59 macroalgae (38 Rhodophyta, 12 Ochrophyta and 9 Chlorophyta), 7 sessile and 22 mobile macrofauna (Table 6).

Mean taxonomic richness (MTR) per location, site and midlittoral zone showed a clear distinction between macroalgae and macrofauna taxa (Figs. 4 and 5). Generally, macrofauna MTR were associated to low values and high standard deviations with higher values in Spanish part than in French part (Fig. 4, Supplementary materials 4). Regarding fauna MTR and within French part, univariate PERMANOVA analyses did not found significant differences between 'WWTP 1' and 'Control 1' for both midlittoral zones (Fig. 4, Supplementary materials 5). By contrast, 'WWTP 2' showed significantly lower MTR values than

Table 3
Summary of PERMANOVA results testing for effects of presence of sewage discharges on benthic assemblages between impacted and control locations (‘WWTP 1’/‘Control 1’ (a), ‘WWTP 2’/‘Control 1’ (b), ‘WWTP 3’/‘Control 2’ (c)) in both midlittoral zones.

(a)						
'WWTP 1'/ 'Control 1'	Df	MeanSqs	F.Model	R2	Pr (> F)	Significance
Upper midlittoral zone						
Locations	1	2.93519	27.8767	0.19025	1.00E-04	***
Locations/Sites	4	0.59622	5.6626	0.15458	1.00E-04	***
Residuals	96	0.10529	0.65517			
Lower midlittoral zone						
Locations	1	2.3794	13.3604	0.11347	1.00E-04	***
Locations/Sites	4	0.50673	2.8453	0.09666	1.00E-04	***
Residuals	93	0.17809	0.78986			
(b)						
'WWTP 2'/ 'Control 1'	Df	MeanSqs	F.Model	R2	Pr (> F)	Significance
Upper midlittoral zone						
Locations	1	4.1775	34.2	0.19055	1.00E-04	***
Locations/Sites	4	0.7719	6.319	0.14084	1.00E-04	***
Residuals	120	0.1221	0.66861			
Lower midlittoral zone						
Locations	1	4.6721	23.5977	0.15924	1.00E-04	***
Locations/Sites	4	0.3758	1.8983	0.05124	0.0011	**
Residuals	117	0.198	0.78952			
(c)						
'WWTP 3'/ 'Control 2'	Df	MeanSqs	F.Model	R2	Pr (> F)	Significance
Upper midlittoral zone						
Locations	1	1.05628	8.1187	0.12313	1.00E-04	***
Locations/Sites	4	0.31941	2.4551	0.14893	2.00E-04	***
Residuals	48	0.1301	0.72795			
Lower midlittoral zone						
Locations	1	2.41452	17.8223	0.22095	1.00E-04	***
Locations/Sites	4	0.50256	3.7096	0.18396	1.00E-04	***
Residuals	48	0.13548	0.59509			

Table 4
Summary of pairwise post hoc results testing for effects of presence of sewage discharges on benthic assemblages between sites within each location (‘WWTP 1’ (a), ‘WWTP 2’ (b), ‘WWTP 3’ (c), ‘Control 1’ (d), ‘Control 2’ (e)) in both midlittoral zones.

		Site 1	Site 2
(a) Upper midlittoral zone		WWTP 1	
WWTP 1	Site 2	**0.0015**	–
	Site 3	**0.0015**	**0.004**
Lower midlittoral zone		WWTP 1	
WWTP 1	Site 2	**0.0015**	–
	Site 3	**0.0015**	**0.017**
(b) Upper midlittoral zone		WWTP 2	
WWTP 2	Site 2	**0.001**	-
	Site 3	**0.001**	**0.001**
Lower midlittoral zone		WWTP 2	
WWTP 2	Site 2	0.479	–
	Site 3	**0.006**	**0.019**
(c) Upper midlittoral zone		WWTP 3	
WWTP 3	Site 2	0.284	–
	Site 3	**0.042**	0.112
Lower midlittoral zone		WWTP 3	
WWTP 3	Site 2	0.327	–
	Site 3	**0.003**	**0.01**
(d) Upper midlittoral zone		Control 1	
Control 1	Site 2	**0.0015**	–
	Site 3	**0.0015**	0.2967
Lower midlittoral zone		Control 1	
Control 1	Site 2	0.21	–
	Site 3	0.21	0.27
(e) Upper midlittoral zone		Control 2	
Control 2	Site 2	0.094	–
	Site 3	**0.015**	**0.033**
Lower midlittoral zone		Control 2	
Control 2	Site 2	**0.002**	–
	Site 3	**0.002**	**0.002**

‘Control 1’ (Fig. 4, Supplementary materials 5). Within the Spanish part, significant differences were found between ‘WWTP 3’ and ‘Control 2’ for both midlittoral zones and also at the scale of site (Fig. 4, Supplementary materials 5). Comparing the ecological groups, EG1 showed higher MTR values than EG2 and EG3 in most cases (Fig. 4).

In relation to macroalgae MTR, no difference was found between impacted and control in both countries except between ‘WWTP 3’ and ‘Control 2’ in the upper zone (Fig. 5; Supplementary materials 6). Within impacted locations, the only significant difference between sites occurred within ‘WWTP 1’ in the lower zone (Site 1 < Site 2 < Site 3) (PERMANOVA; p-value < 0.05). There were also significant differences within control locations in the lower zone (i.e. between Site 1 and 3 in ‘Control 1’ and between Site 2 and 3 in ‘Control 2’) (Supplementary materials 6).

Focusing on the ratio characteristic/opportunistic MTR, there were significant differences between impacted and control locations in both countries and midlittoral zones (except in the upper zone between ‘WWTP 3’ and ‘Control 2’) (PERMANOVA; p-value < 0.05; Supplementary materials 7). The ratio was always lower in impacted locations than in control with higher opportunistic MTR in impacted locations (except between ‘WWTP 2’ and ‘Control 1’ in the lower zone). This was not so obvious regarding the characteristic MTR. Indeed, it was higher in the control only between ‘Control 1’ and ‘WWTP 1’ in both midlittoral zones. At the site scale within impacted locations, there were only significant differences within ‘WWTP 2’ (i.e. between Site 1 and 3 in both zones and between Site 1 and 2 in the upper zone). In all three cases, the ratio was always lower in Site 1. The characteristic MTR was higher in furthest sites from the outfall (Sites 2 and 3) contrary to the opportunistic MTR which was higher in Site 1 in the upper zone. Within control locations, only ‘Control 1’ in the upper zone presented significant differences between sites (i.e. Site 1 significantly differed from Site 2 and Site 3).

3.4. Ecological quality

The quality index was calculated per location for controls and a pseudo-index was calculated per distance from the outfall (i.e. site) for impacted locations (Table 7). In France, sites from all locations were ranked as *“Good”* except the closest site from the outfall in ‘WWTP 1’. In Spain, all final scores were ranked as *“Moderate”* in the impacted location and as *“Good”* in the control one.

Table 5
Summary of global dissimilarities between 2 groups from SIMPER analyses (i.e. between impacted and control locations and between sites within each location) for both midlittoral zones (upper and lower).

Global dissimilarity (%)			
		Upper midlittoral zone	Lower midlittoral zone
	WWTP 1' vs. 'Control 1'	**51.12**	**63.58**
	WWTP 2' vs. 'Control 1'	**53.81**	**71.14**
'WWTP 1'	S1 vs. S2	43.62	52.00
	S2 vs. S3	39.45	41.41
	S1 vs. S3	52.74	56.96
'WWTP 2'	S1 vs. S2	49.57	56.88
	S2 vs. S3	49.17	55.08
	S1 vs. S3	52.93	57.36
'Control 1'	S1 vs. S2	42.41	56.27
	S2 vs. S3	40.95	61.04
	S1 vs. S3	41.89	57.44
	WWTP 3' vs. 'Control 2'	**42.00**	**52.54**
'WWTP 3'	S1 vs. S2	31.16	33.44
	S2 vs. S3	33.08	50.08
	S1 vs. S3	36.56	51.28
'Control 2'	S1 vs. S2	32.65	30.99
	S2 vs. S3	40.26	46.28
	S1 vs. S3	37.47	45.78

4. Discussion

The present study aimed to assess the effects of wastewater treatment plant (WWTP) discharges on rocky benthic intertidal assemblages (macroalgae and macroinvertebrates) of the French and Spanish Basque coast (southeastern Bay of Biscay). The results from this research show significant differences in the composition and abundances of taxa, including those sensitive to pollution, between the three studied WWTP and their respective controls for both midlittoral zones (upper and lower). Significant differences in the composition and abundance of assemblages were also found at a lower spatial scale (sites corresponding to the three distances from the outfalls). Regarding mean taxonomic richness, no evident differences were found, especially for macrofauna.

When detecting impacts due to pollution in the marine environment, the study of benthic communities provides several advantages, among which the bioindicator nature of some species (opportunists vs. sensitives) should be highlighted (Díez et al., 2009). In the present study, three macroalgae taxa (*Ceramium* spp., *Corallina* spp. and *Halopteris scoparia*) were identified as significant contributors (Ct (%) > 10) to the dissimilarity between the three WWTP and their respective controls. *Ceramium* spp., a corticated filamentous red alga that includes diverse opportunistic species tolerant to pollution (Díez et al., 1999; Juanes et al., 2008), showed higher abundance in WWTP locations for both midlittoral zones. In the upper midlittoral zone, *Corallina* spp. showed high abundances in both controls and WWTP locations being one of the most frequent macrophyte forming a distinctive belt in the intertidal zone at the southeastern Bay of Biscay (Gorostiaga et al., 2004). Nevertheless, its abundance was higher in WWTP comparing to the controls. This genus is considered as characteristic and is formed by articulated calcareous algae which show certain tolerance to moderated polluted environments (Díez et al., 1999, 2009; Gorostiaga et al., 2004; Mangialajo et al., 2008; Pellizzari et al., 2017). *Halopteris scoparia,* with a terete corticated thallus and considered as a characteristic species, was more abundant in the lower midlittoral of control locations. This species has been already reported in locations with good environmental conditions (Arévalo et al., 2007; Díez et al., 2012, 1999). Therefore, its lower abundance or even its absence (in Spanish WWTP location) could suggest an effect of discharges. Nevertheless, considering this species was also present in French WWTP locations, the impact of the discharges might not be considered as elevated.

Apart from these high contributors, other species appeared to be responsible for the difference between WWTP and control locations. For instance, in the upper midlittoral zone of French area, *Laurencia obtusa* and *Osmundea pinnatifida* were more abundant in the control location than in 'WWTP 1' and 'WWTP 2'. In fact, *Osmundea pinnatifida* was not present in 'WWTP 1'. These rhodophytes are typical intertidal species related to clean waters (Díez et al., 2009). In the lower midlittoral zone of the same area, the leathery species, *Cystoseira tamariscifolia,* also showed higher abundances in the control location comparing to 'WWTP 1' and 'WWTP 2'. It is well known the sensitiveness of the species of the genus *Cystoseira* to anthropogenic impact, and they are thus considered as indicators for good water quality in the European Directive (Duarte et al., 2018; García-Fernández and Bárbara, 2016; Valdazo et al., 2017). However, similar to that described for *Halopteris scoparia* above, *Laurencia obtusa* and *Cystoseira tamariscifolia* were also present (with lower abundances) in WWTP locations and, therefore, the potential impact of WWTP discharges might be considered as moderate.

WWTP and control locations also presented high variability in terms of taxa composition and abundance at the site scale (i.e. distance from the outfall) for both midlittoral zones and for French and Spanish areas. Within French WWTP locations, among the significant contributors (Ct (%) > 10), it should be highlighted the increase in the abundance of the sensitive species (*Halopteris scoparia)* and the decrease of *Caulacanthus ustulathus* from Site 1 (closest to the outfall) to Site 3 (furthest to the outfall) in 'WWTP 1'. The latter red macroalga was described as a more abundant species close the outfall in a study carried out in an inlet on the Basque coast (Díez et al., 2013). In 'WWTP 2', the abundance of *Ceramium* spp. and *Corallina* spp. decreased towards Site 3, whereas *Laurencia obtusa* increased. In this location *Cystoseira tamariscifolia* and *Halopteris scoparia* showed their highest values in Site 3 (the furthest one from the outfall). Within the Spanish WWTP location, similar trends were detected with some sensitive species being more abundant in Site 3 and opportunistic species in Site 1. For instance, *Chondria coerulescens,* a species related to high levels of sedimentation and tolerant to moderate pollution levels (Gorostiaga et al., 2004) decreased towards Site 3. Taking into account these trends of bioindicator macroalgae within WWTP locations, it might be deduced a gradient of the effect of the outfall on benthic intertidal assemblages. However, looking at Sites 1 and 3 within control locations, separated 200 m but in the absence of any gradient, results were somewhat similar. In this regard, sensitive species, such as *Cystoseira tamariscifolia* or *Halopteris scoparia,* dominated in Site 3, whereas opportunistic taxa, such as *Ceramium* spp and *Enteromorpha* spp., were more abundant in Site 1. Chlorophytes like the genus *Enteromorpha* are also common in non-polluted areas and their higher presence could be explained by the

Table 6

List of species/taxa identified into quadrats in control locations ('Control 1' and 'Control 2') and in impacted locations ('WWTP 1', 'WWTP 2' and 'WWTP 3'). Mean abundances (ind./0.1 m^2) and total mean taxonomic richness for each location are shown. Macroalgae were classed into taxonomic groups (red, brown and green) and functional groups (characteristic, opportunistic) according to Ar Ar Gall et al. (2016)or French locations and Juanes et al. (2008) for Spanish ones. Macroalgae were assigned to one of two Ecological Status Groups (ESG) according to morphological and functional characteristics (Ar Gall et al., 2016; Gaspar et al., 2012; Neto et al., 2012; Orfanidis et al., 2011, 2001; Vinagre et al., 2016a). ESG I corresponded to late successional or perennial to annual taxa and ESG II to opportunistics or annual taxa. Macrofauna species were assigned to one of five Ecological Groups (EG I–V) according to their responses to natural and man-induced changes in water quality: the higher the group, the higher the tolerance to pollution (Borja et al., 2000). Significance codes: M: Macroalgae; ESG: Ecological Status Groups for macroalgae species; EG: Ecological groups for macrofauna; L = Lower midlittoral zone; U = Upper midlittoral zone. Sampling fluctuations were described by their standard deviation (SD).

Species/Taxa	Ecological group	Phylum	Locations									
			France					Spain				ESG/EG
			'Control 1'	'WWTP 1'	'WWTP 2'	Characteristic	Opportunistic	'Control 2'	'WWTP 3'	Characteristic	Opportunistic	
Acrosorium spp.	M	Rhodophyta	**0.11** *(SD = 0.31)*	**0.02** *(SD = 0.13)*	**0.07** *(SD = 0.30)*			–	–			II
Ahnfeltiopsis devoniensis	M	Rhodophyta	–	–	**0.06** *(SD = 0.31)*			–	–			II
Antithamnionella sp.	M	Rhodophyta	–	–	–			**0.03** *(SD = 0.17)*	**0.07** *(SD = 0.26)*			
Antithamnion	M	Rhodophyta	–	–	–			–	**0.03** *(SD = 0.17)*			
Asparagopsis/Falkenbergia	M	Rhodophyta	**0.33** *(SD = 0.57)*	**0.80** *(SD = 1.07)*	**1.21** *(SD = 1.28)*	✓ (L)		**0.58** *(SD = 0.50)*	**0.54** *(SD = 0.67)*			II
Bonnemaisonia hamifera	M	Rhodophyta	**0.01** *(SD = 0.17)*	–	**0.05** *(SD = 0.25)*			–	–			
Caulacanthus ustulatus	M	Rhodophyta	**0.55** *(SD = 0.81)*	**0.58** *(SD = 1,08)*	**0.29** *(SD = 0.64)*	✓ (L + U)		–	**0.06** *(SD = 0.23)*	✓ (L + U)		I
Ceramium spp.	M	Rhodophyta	**0.28** *(SD = 0.57)*	**2.23** *(SD = 0.95)*	**0.75** *(SD = 0.90)*		✓ (L + U)	**2.31** *(SD = 0.58)*	**2.42** *(SD = 1.20)*		✓ (L + U)	II
Champia parvula	M	Rhodophyta	**0.06** *(SD = 0.26)*	–	**0.03** *(SD = 0.17)*			**0.11** *(SD = 0.40)*	**0.06** *(SD = 0.23)*			II
Chondracanthus acicularis	M	Rhodophyta	**0.88** *(SD = 0.85)*	**0.55** *(SD = 0.81)*	**1.23** *(SD = 0.93)*	✓ (U)		**0.06** *(SD = 0.33)*	–			II
Chondria coerulescens	M	Rhodophyta	**0.33** *(SD = 0.59)*	**0.60** *(SD = 0.67)*	**1.00** *(SD = 0.96)*	✓ (L + U)		**1.19** *(SD = 0.75)*	**0.01** *(SD = 0.12)*			II
Chylocladia verticillata	M	Rhodophyta	**0.04** *(SD = 0.19)*	–	–			–	–			
Corallina spp.	M	Rhodophyta	**1.81** *(SD = 0.98)*	**2.30** *(SD = 0.77)*	**2.11** *(SD = 1.40)*	✓ (L + U)		**3.14** *(SD = 1.25)*	**4.17** *(SD = 0.95)*	✓ (L + U)		I
Gastroclonium reflexum	M	Rhodophyta	**0.02** *(SD = 0.14)*	**0.05** *(SD = 0.22)*	**0.20** *(SD = 0.49)*			**0.17** *(SD = 0.38)*	**0.39** *(SD = 0.49)*			II
Gelidium spp.	M	Rhodophyta	**0.28** *(SD = 0.62)*	**0.12** *(SD = 0.32)*	**1.19** *(SD = 1.60)*	✓ (L)		**0.47** *(SD = 0.61)*	**0.68** *(SD = 0.69)*	✓ (L + U)		I
Gigartina spp.	M	Rhodophyta	**0.01** *(SD = 0.12)*	**0.07** *(SD = 0.25)*	**0.01** *(SD = 0.10)*			–	–	✓ (L + U)		II
Gymnogongrus spp.	M	Rhodophyta	**0.09** *(SD = 0.36)*	**0.07** *(SD = 0.25)*	**0.22** *(SD = 0.48)*			–	–			I
Halopitys incurva	M	Rhodophyta	**0.12** *(SD = 0.42)*	–	–			**0.14** *(SD = 0.42)*	–			
Cryptopleura ramosa	M	Rhodophyta	**0.01** *(SD = 0.08)*	–	–			–	–			
Halurus equisetifolius	M	Rhodophyta	**0.37** *(SD = 0.65)*	**0.15** *(SD = 0.36)*	**0.10** *(SD = 0.36)*	✓ (L)		**0.06** *(SD = 0.23)*	–			II
Hypnea musciformis	M	Rhodophyta	**0.52** *(SD = 0.97)*	**0.95** *(SD = 1.05)*	**0.10** *(SD = 0.30)*			–	–			II
Hypoglossum hypoglossoides	M	Rhodophyta	**0.03** *(SD = 0.17)*	**0.03** *(SD = 0.18)*	**0.07** *(SD = 0.30)*			–	–			I

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Table 6 (*continued*)

Species/Taxa	Ecological group	Phylum	Locations									
			France					Spain				ESG/EG
			'Control 1'	'WWTP 1'	'WWTP 2'	Characteristic	Opportunistic	'Control 2'	'WWTP 3'	Characteristic	Opportunistic	
Jania rubens	M	Rhodophyta	**0.17** *(SD = 0.48)*	**0.25** *(SD = 0.51)*	**0.03** *(SD = 0.17)*	✓ (L)		**0.56** *(SD = 0.77)*	**0.10** *(SD = 0.30)*			I
Laurencia obtusa	M	Rhodophyta	**0.92** *(SD = 1.15)*	**0.67** *(SD = 1)*	**0.69** *(SD = 0.83)*			–	**0.21** *(SD = 0.44)*	✓ (L + U)		II
Lithophyllum incrustans	M	Rhodophyta	**0.70** *(SD = 0.87)*	**1.05** *(SD = 0.75)*	**1.18** *(SD = 0.77)*	✓ (L + U)		**2.17** *(SD = 0.70)*	**1.64** *(SD = 0.77)*			I
Mastocarpus/Petricelis	M	Rhodophyta	**0.22** *(SD = 0.45)*	**0.07** *(SD = 0.25)*	**0.19** *(SD = 0.54)*			–	**0.01** *(SD = 0.12)*			I
Mesophyllum lichenoides	M	Rhodophyta	**0.05** *(SD = 0.25)*	**0.18** *(SD = 0.50)*	**0.58** *(SD = 0.84)*			–	–			
Nitophyllum punctatum	M	Rhodophyta	**0.13** *(SD = 0.38)*	**0.02** *(SD = 0.13)*	**0.29** *(SD = 0.53)*			**0.03** *(SD = 0.17)*	**0.03** *(SD = 0.17)*			II
Ophidocladus spp.	M	Rhodophyta	**0.01** *(SD = 0.12)*	–	–			–	**0.03** *(SD = 0.17)*			
Osmundea pinnatifida	M	Rhodophyta	**0.32** *(SD = 0.67)*	–	**0.03** *(SD = 0.17)*			–	**0.14** *(SD = 0.39)*			II
Peyssonnelia atropurpurea	M	Rhodophyta	**0.01** *(SD = 0.08)*	–	**0.02** *(SD = 0.14)*			–	**0.03***(SD = 0.17)*			I
Phymatolithon lenormandii	M	Rhodophyta	**0.35** *(SD = 0.60)*	**0.03** *(SD = 0.18)*	**0.26** *(SD = 0.50)*	✓ (U)		**0.03** *(SD = 0.17)*	**0.14** *(SD = 0.51)*			
Plocamium cartilagineum	M	Rhodophyta	**0.16** *(SD = 0.47)*	**0.05** *(SD = 0.22)*	**0.70** *(SD = 0.96)*			–	–			I
Porphyra spp.	M	Rhodophyta	–	–	–			–	**0.01** *(SD = 0.12)*			II
Rhodymenia pseudopalmata	M	Rhodophyta	**0.04** *(SD = 0.20)*	–	**0.03** *(SD = 0.21)*			–	–			
Scinaia furcellata	M	Rhodophyta	**0.01** *(SD = 0.08)*	–	–			–	–			I
Tenarea tortuosa	M	Rhodophyta	–	–	**0.02** *(SD = 0.14)*			–	–	✓ (L + U)		
Vertebrata fruticulosa	M	Rhodophyta	–	–	–		✓ (L + U)	**0.03** *(SD = 0.17)*	–			II
Cladostephus spongiosus	M	Ochrophyta	–	–	–			**0.56** *(SD = 0.88)*	–	✓ (L + U)		I
Colpomenia peregrina	M	Ochrophyta	**0.36** *(SD = 0.48)*	**0.22** *(SD = 0.42)*	**0.33** *(SD = 0.55)*	✓ (L + U)		**0.03** *(SD = 0.17)*	**0.58** *(SD = 0.50)*			II
Cutleria adspersa	M	Ochrophyta	–	–	**0.11** *(SD = 0.34)*			**0.06** *(SD = 0.33)*	**0.29** *(SD = 0.46)*			
Cutleria multifida	M	Ochrophyta	–	–	**0.01** *(SD = 0.10)*			–	–			
Cystoseira tamariscifolia	M	Ochrophyta	**0.37** *(SD = 0.89)*	–	**0.10** *(SD = 0.39)*			–	–			I
Dictyota dichotoma	M	Ochrophyta	**0.05** *(SD = 0.22)*	**0.02** *(SD = 0.13)*	**0.23** *(SD = 0.49)*	✓ (L)		–	–			II
Ectocarpales/Ectocarpus	M	Ochrophyta	**0.01** *(SD = 12)*	–	**0.01** *(SD = 0.10)*		✓ (L + U)	**0.14** *(SD = 0.35)*	**0.22** *(SD = 0.42)*		✓ (L + U)	II
Ralfsia verrucosa	M	Ochrophyta	–	–	–			**0.03** *(SD = 0.17)*	**0.07** *(SD = 0.26)*			I
Scytosiphon lomentaria	M	Ochrophyta	–	–	–			–	**0.08** *(SD = 0.28)*			
Halopteris scoparia	M	Ochrophyta	**1.41** *(SD = 1.66)*	**0.58** *(SD = 1)*	**0.08** *(SD = 0.39)*	✓ (L)		**0.69** *(SD = 0.86)*	**0.01** *(SD = 0.12)*	✓ (L + U)		II

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Table 6 (*continued*)

Species/Taxa	Ecological group	Phylum	Locations									
			France					Spain				ESG/EG
			'Control 1'	'WWTP 1'	'WWTP 2'	Characteristic	Opportunistic	'Control 2'	'WWTP 3'	Characteristic	Opportunistic	
Taonia sp.	M	Ochrophyta	**0.04** *(SD = 0.20)*	**0.03** *(SD = 0.18)*	**0.23** *(SD = 0.49)*			**0.03** *(SD = 0.17)*	**0.15** *(SD = 0.36)*			
Zonardinia typus	M	Ochrophyta	–	–	**0.05** *(SD = 0.29)*			–	–			
Chaetomorpha spp.	M	Chlorophyta	–	–	**0.01** *(SD = 0.10)*			–	**0.13** *(SD = 0.33)*		✓ (L + U)	
Cladophora spp.	M	Chlorophyta	**0.01** *(SD = 0.12)*	–	**0.04** *(SD = 0.19)*			–	**0.01** *(SD = 0.12)*		✓ (L + U)	**II**
Codium adaerens	M	Chlorophyta	**0.33** *(SD = 0.86)*	**0.32** *(SD = 0.75)*	**0.30** *(SD = 0.76)*	✓ (L)		**0.42** *(SD = 0.77)*	**0.57** *(SD = 0.85)*			**II (spp.)**
Codium decorticatum	M	Chlorophyta	–	–	–			–	**0.08** *(SD = 0.28)*			**II (spp.)**
Codium fragile	M	Chlorophyta	–	**0.02** *(SD = 0.13)*	–			**0.17** *(SD = 0.38)*	**0.08** *(SD = 0.28)*			**II (spp.)**
Derbesia tenuissima	M	Chlorophyta	–	–	**0.01** *(SD = 0.10)*			–	–			
Enteromorpha spp.	M	Chlorophyta	**0.64** *(SD = 0.83)*	**0.67** *(SD = 0.68)*	**0.48** *(SD = 0.73)*		✓ (L + U)	**0.53** *(SD = 0.61)*	**0.33** *(SD = 0.47)*		✓ (L + U)	**II**
Pterosiphonia spp.	M	Chlorophyta	**0.33** *(SD = 0.78)*	**0.02** *(SD = 0.13)*	**0.12** *(SD = 0.43)*			**0.03** *(SD = 0.17)*	–			**II (*P. complanata*)**
Ulva spp.	M	Chlorophyta	**1.55** *(SD = 1)*	**1.47** *(SD = 0.60)*	**1.11** *(SD = 0.78)*		✓ (L + U)	**0.28** *(SD = 0.51)*	**0.39** *(SD = 0.52)*		✓ (L + U)	**II**
Macroalgae mean taxonomic richness			**8.79** *(SD = 3.19)*	**8.38** *(SD = 2.39)*	**9.90** *(SD = 3.22)*			**7.97** *(SD = 1.58)*	**7.88** *(SD = 2.46)*			
Balanus sp.	SM	Crustacea	–	**0.05** *(SD = 0.39)*	–			**1.72** *(SD = 3.58)*	**0.53** *(SD = 1.14)*			**I**
Chthamalus spp.	SM	Crustacea	**0.02** *(SD = 0.25)*	–	–			**1.33** *(SD = 1.82)*	**1.04** *(SD = 1.44)*			**I**
Mytilus spp.	SM	Mollusca	**0.02** *(SD = 0.25)*	**0.30** *(SD = 0.91)*	**0.22** *(SD = 0.79)*			**1.53** *(SD = 2.67)*	**0.92** *(SD = 2.11)*			**III**
Rocellaria dubia	SM	Mollusca	–	–	**0.03** *(SD = 0.21)*			–	–			**I**
Serpula sp.	SM	Annelida	–	–	**0.02** *(SD = 0.19)*			–	–			**I**
Spirobranchus spp.	SM	Annelida	–	–	–			–	**0.04** *(SD = 0.35)*			**II**
Porifera	SM	Porifera	–	–	**0.28** (SD = 1.56)			–	–			
Sessile macrofauna mean taxonomic richness			**0.01** *(SD = 0.12)*	**0.12** *(SD = 0.32)*	**0.16** *(SD = 0.39)*			**1.06** *(SD = 1.24)*	**0.79** *(SD = 0.85)*			
Acanthochitona spp.	MM	Mollusca	–	**0.02** *(SD = 0.13)*	**0.01** *(SD = 0.10)*			–	–			**I**
Actinia equina	MM	Cnidaria	**0.01** *(SD = 0.08)*	–	–			–	–			**I**
Actinothoe sphyrodeta	MM	Cnidaria	–	–	–			**0.03** *(SD = 0.17)*	–			
Anemonia viridis	MM	Cnidaria	**0.01** *(SD = 0.12)*	–	–			–	–			

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Table 6 (*continued*)

Species/Taxa	Ecological group	Phylum	Locations									
			France					Spain				ESG/EG
			'Control 1'	'WWTP 1'	'WWTP 2'	Characteristic	Opportunistic	'Control 2'	'WWTP 3'	Characteristic	Opportunistic	
Annelida	MM	Annelida	–	–	**0.02** *(SD = 0.14)*			–	–			
Bittium reticulatum	MM	Mollusca	**0.02** *(SD = 0.19)*	–	**0.07** *(SD = 0.43)*			–	–			**I**
Cerithium spp.	MM	Mollusca	**0.02** *(SD = 0.19)*	–	–			–	–			**II**
Chiton spp.	MM	Mollusca	**0.01** *(SD = 0.08)*	–	**0.01** *(SD = 0.10)*			–	–			**II**
Diodora gibberula	MM	Mollusca	–	–	**0.01** *(SD = 0.10)*			–	–			
Eulalia viridis	MM	Annelida	–	**0.07** *(SD = 0.25)*	**0.03** *(SD = 0.17)*			**0.11** *(SD = 0.40)*	**0.90** *(SD = 2.46)*			**II**
Melarhaphe neritoides	MM	Mollusca	–	–	**0.14** *(SD = 1.44)*			–	–			**II**
Ocenebra edwardsii	MM	Mollusca	**0.04** *(SD = 0.22)*	–	**0.03** *(SD = 0.17)*			–	–			**II (sp.)**
Pachygrapsus marmoratus	MM	Crustacea	**0.01** *(SD = 0.12)*	–	–			–	–			**II**
Paguridae spp.	MM	Crustacea	**0.13** *(SD = 0.58)*	**0.02** *(SD = 0.13)*	**0.01** *(SD = 0.10)*			–	–			**II (*Pagurus* sp.)**
Paracentrotus lividus	MM	Echinodermata	–	–	–			–	**0.10** *(SD = 0.30)*			**I**
Patella spp.	MM	Mollusca	**0.12** *(SD = 0.68)*	**0.12** *(SD = 0.67)*	**0.03** *(SD = 0.21)*			**3.08** *(SD = 4.44)*	**3.81** *(SD = 3.81)*			**I**
Porcellana platycheles	MM	Crustacea	–	–	–			–	**0.21** *(SD = 1.10)*			**I**
Steromphala cineraria	MM	Mollusca	**0.02** *(SD = 0.19)*	–	–			–	–			**I**
Steromphala pennanti	MM	Mollusca	**0.45** *(SD = 1.32)*	**0.03** *(SD = 0.18)*	**0.03** *(SD = 0.17)*			–	–			**I**
Steromphala umbillicalis	MM	Mollusca	**0.16** *(SD = 0.62)*	–	**0.02** *(SD = 0.14)*			–	–			**I**
Stramonita haemastoma	MM	Mollusca	–	–	**0.01** *(SD = 0.10)*			–	–			
Tritia incrassata	MM	Mollusca	–	–	**0.03** *(SD = 0.21)*			–	–			**II**
Mobile macrofauna mean taxonomic richness			**0.52** *(SD = 0.88)*	**0.18** *(SD = 0.50)*	**0.25** *(SD = 0.55)*			**0.67** *(SD = 0.63)*	**1.15** *(SD = 0.82)*			
Total mean taxonomic richness			**9.29** *(SD = 3.26)*	**8.68** *(SD = 2.46)*	**10.31** *(SD = 3.26)*			**9.69** *(SD = 2.29)*	**9.82** *(SD = 2.98)*			

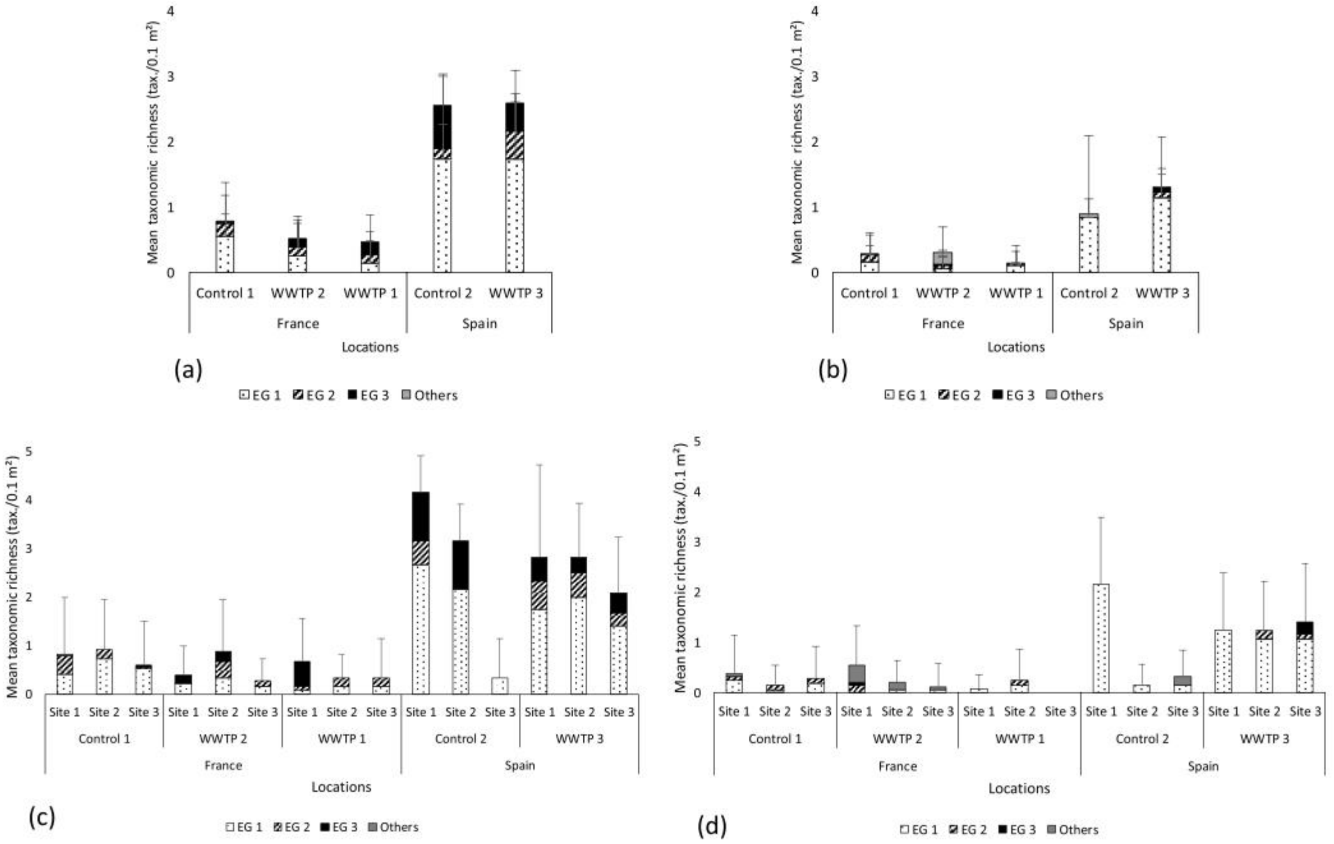

Fig. 4. Mean taxonomic richness of macrofauna in the upper (a, c) and lower midlittoral zones (b, d) for each impacted and control locations and site (i.e. each distance) within locations. Macrofauna species/taxa were. classed into ecological groups (EG1 in white with black points, EG2 hatched, EG3 in black and others in grey).

effect of other factors such as sediments accumulation (Littler et al., 1983) or grazing pressure (Hay, 1981). In relation to this taxa composition and abundance approach, it should be highlighted that some species were aggregated for the analysis at the genus level. This fact might have supposed a decrease of the bioindicator nature of some species. For example, two species from the same genus may have different sensitivity (e.g. *Gelidium pusillum* less sensitive to pollution than other species from this same genus such as *Gelidium corneum*) (Díez et al., 1999).

An environmental stress such as eutrophication or anthropogenic disturbances can result in a loss of richness (Amaral et al., 2018; Simboura and Zenetos, 2002). Therefore, the mean taxonomic richness (MTR) was assessed to detect changes caused by WWTP discharges because this metric could be also used as a criterion of ecological quality (Amaral et al., 2018; Simboura and Zenetos, 2002; Wells et al., 2007). However, using the macrofauna MTR, no detectable effect of WWTP discharges was highlighted due to very low values (< 1 in France and < 5 in Spain) and high variability compared to macroalgae (Figs. 4 and 5) for which rocky platforms constitute a suitable habitat for their colonization (Guinda et al., 2014). Macrofauna settlement was not as favorable because the lack of canopy-forming macroalgae (Díez et al., 2014), the uniform geomorphology, the high exposure to a strong hydrodynamic regime (Abadie et al., 2005) and the competitive advantage of the macroalgae in the lower levels of the intertidal zone (especially in the case of the caespitose vegetation). Therefore, it was only possible to highlight general trends, such as a higher macrofauna MTR in the Spanish side and in the upper midlittoral zone. Furthermore, results of macrofauna patterns would be probably quite different if outfalls were located in an intertidal boulder field providing hiding places for high macrofauna diversity (Bernard, 2012; Huguenin et al., 2018).

Macroalgae MTR appeared not to be really affected by discharges at the location scale. Indeed, no difference was highlighted between impacted and control locations (except in the upper zone in the Spanish side). However, the ratio between characteristic and opportunistic taxa was significantly affected in the three WWTP locations at both levels (one exception was the upper level of 'WWTP 3'). Between sites within impacted locations (i.e. between the three distances from the outfall), the only one significant MTR increase (from Site 1 to 3) was in 'WWTP 1' in the lower zone.

Thus, similarly to other works (Simboura and Zenetos, 2002; Vinagre et al., 2016a), our results show the difficulty to make accurate predictions of the effect of WWTP discharges on the MTR (especially on macrofauna). By contrast, multivariate analysis appeared as more appropriate because it allows to integrate all benthic assemblages (i.e. species composition and abundance of macroalgae as well as macrofauna). This may also be explained by the fact that the MTR does not consider the relative abundance of the species neither other relevant traits of the taxa (life cycle and morphology). Thus, only strong impacts could potentially influence the MTR. Some authors had already mentioned that such metrics are not universally relevant to study the effect of this type of disturbance (Harper and Hawksworth, 1994; Magurran, 2004) and that they could be often affected by sampling effort (Clarke and Warwick, 2001). Average cover parameter could be thus probably more useful to detect impacts.

In this study, macroalgae and macrofauna communities were considered to assess potential effects of wastewater discharges as recommended by some studies (Archambault et al., 2001; Bishop et al.,

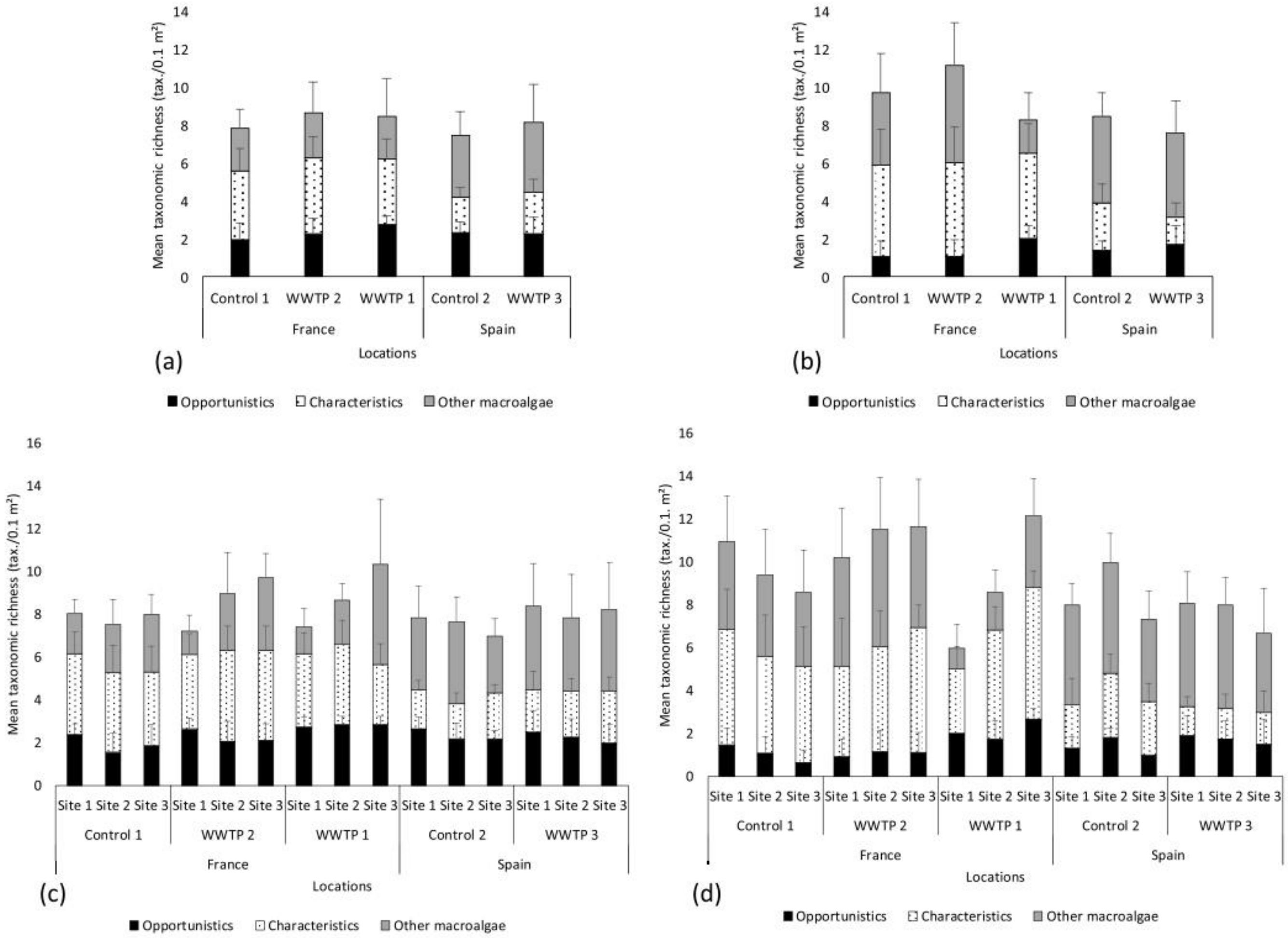

Fig. 5. Mean taxonomic richness of macroalgae of each impacted and control locations (a, b) and of each site (i.e. each distance) within locations (c, d) classed into functional groups (opportunistics in black, characteristics in white with black points and others in grey) according to Ar Gall et al., 2016 for French locations and Juanes et al. (2008) for Spanish ones for (a and c) upper and (b and d) lower midlittoral zones.

2002; Underwood, 1996) and to fulfill European Directives requirements (WFD and MSFD). Indeed, these communities are playing a key role in water quality for the conservation status and functional aspects of the environment (Casamajor (de) et al., 2016). Vinagre et al. (2016a) even suggest that macrofauna might be considered as an indicator of disturbance in intertidal rocky shores as good as the macroalgae.

Using the pseudo-quality index, all sites within 'WWTP 2' were ranked as "*Good*", while all sites within the Spanish location 'WWTP 3' were ranked as "*Moderate*". In 'WWTP 1', only the proximate site from the outfall was ranked as "*Moderate*", while site 2 and 3 were ranked as "*Good*". A study achieved in compliance with the WFD along the French Basque coast (Casamajor (de) et al., 2016) ranked two other locations (considered as not impacted and representative of the whole water body) as "*Good*" (with values between 0.706 and 0.732). This is entirely in line with indices calculated on 'Control 1' and sites away from the outfall on impacted locations, which seems to be less impacted and have a better ecological quality. Moreover, in Spain, the WWTP location was moderately impacted whatever the distance from the outfall. But, it is important to note that the ratio was calculated according to the list initially established for the French Basque coast (Ar Gall et al., 2016; Casamajor (de) et al., 2010). A Spanish list was anyway defined by Juanes et al. (2008) for the calculation of WFD metrics, but the number of opportunistic and characteristic species was much lower than the French one. Thus, scores assigned to each metric would have been not really significant and the ecological quality would have been underestimated. If we had wanted to calculate the Spanish CFR index (Guinda et al., 2008) with our data, this would not have been possible due to the differences of sampling designs (transects *vs.* random

Table 7
Metrics calculated using the Water Framework Directive (WFD) protocol for each control location and each distance of impacted locations.

	Max. points	'Control 1'	'WWTP 1'			'WWTP 2'			'Control 2'	'WWTP 3'		
			Site 1	Site 2	Site 3	Site 1	Site 2	Site 3		Site 1	Site 2	Site 3
Global cover of macroalgae [C]	0.4	0.306	0.306	0.306	0.306	0.306	0.306	0.306	0.306	0.306	0.306	0.306
Occurrence of characteristic species [N]	0.3	0.15	0.075	0.25	0.2	0.15	0.2	0.25	0.2	0.15	0.1	0.1
Total cover of opportunistic species [O]	0.3	0.2	0.075	0.1	0.15	0.2	0.15	0.2	0.15	0.125	0.15	0.1
Final score	1	0.656	0.456	0.656	0.656	0.656	0.656	0.756	0.656	0.581	0.556	0.506
Ecological quality		**Good**	**Moderate**	**Good**	**Good**	**Good**	**Good**	**Good**	**Good**	**Moderate**	**Moderate**	**Moderate**

quadrats). In addition, despite the fact that the Basque coast has only two algal belts, it seemed preferable to stratify the protocol according to these two belts (quadrats in each belt) rather than to perform a transect covering the two belts.

5. Conclusion

The present work established the assessment of the potential impact of WWTP discharges on intertidal rocky benthic assemblages in the southeastern Bay of Biscay. Even if the importance to consider both communities was proved, it suggests that benthic macroalgae constitute the best relevant organisms to assess the effect of this pressure on the intertidal rocky platform habitat in the study area. The results from the present study do not evidence a clear impact of the WWTP discharges on the rocky benthic intertidal assemblages. Taking into account the presence of some sensitive taxa in WWTP locations and that a Good ecological status has been ranked in French WWTP locations, only the existence of a moderate impact associated with discharges could be concluded. In the Spanish side the ecological quality ratio offered lower values than those expected for the control and impacted locations. The use of the complementary metric "mean taxonomic richness" was not helpful to discriminate the potential impacts due to the absence of clear trends. Finally, multivariate analyses appeared thus to be more efficient than other biological and ecological metrics although certain difficulties emerge when discriminating between changes associated to natural variability and those caused by anthropic activity. For this reason, it is necessary to deepen on the bioindicator character of the different macroalgae. These results will enable several MSFD descriptors to be supported, such as "Biodiversity", "Non-indigenous species", "Eutrophication", "Sea-floor integrity" and "Contaminants" whilst also bridging deficiencies emphasized by Directives on the response of biological indicators to various pressures and the biocenosis of the southeastern Bay of Biscay.

Declarations of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.csr.2019.04.014.

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Highlights:

- **Detectable effects** of discharges were highlighted on **assemblage structure**,
- **Macroalgae** constituted a **relevant biotic component** to study impact of WWTP discharges compared to macrofauna,
- The **EQR ratio** based on the current WFD metrics was **sensitive to the WWTP pressure**.

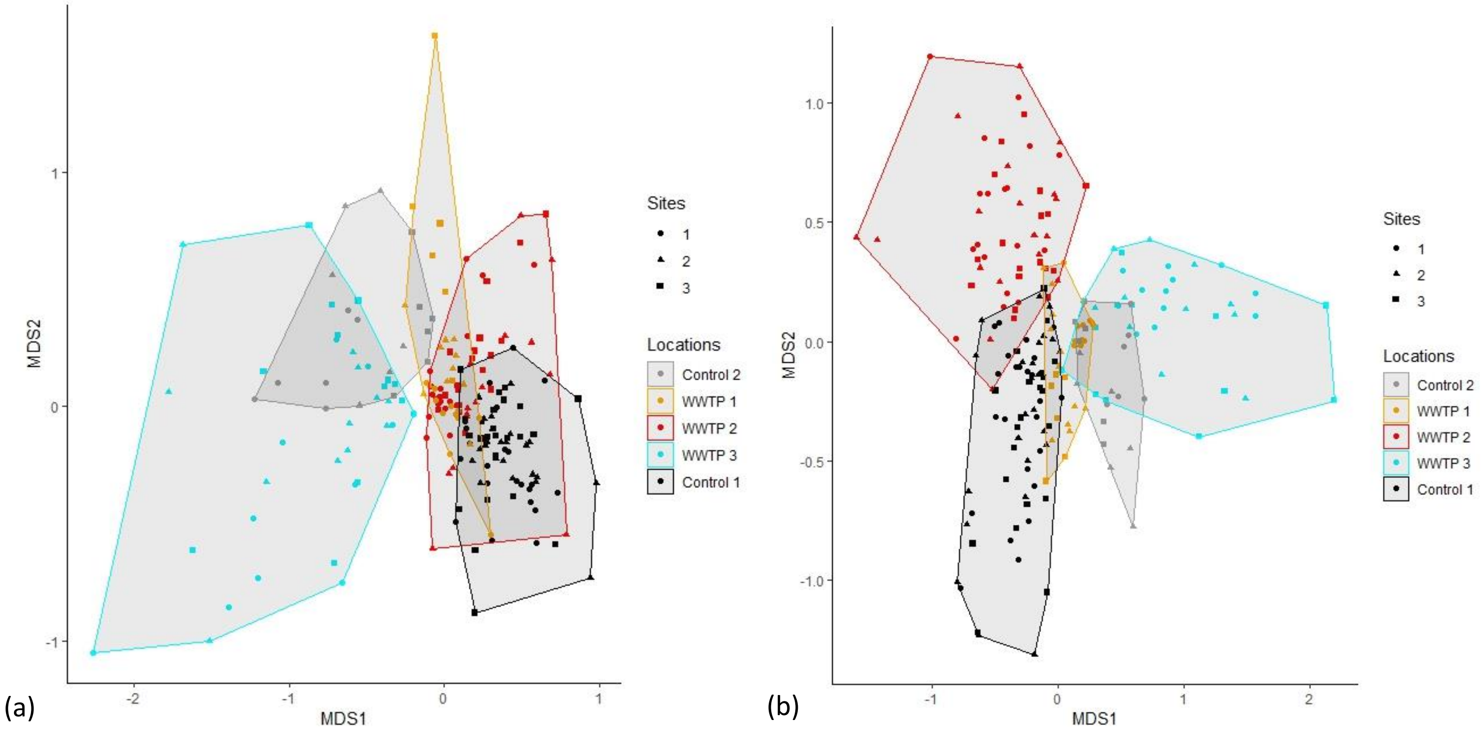
Main contributors responsible for differences between impacted and control locations. Purple species are those identified as opportunistics and grey ones as characteristic of the studied area within the WFD (de Casamajor and Lissardy, 2018). Species in parenthesis are those identified with a low contribution (Ct < 10%) or not significant.

	Impacted locations/sites	Control loc. or less impacted sites
Upper midlittoral zone	- ***Ceramium* spp.** Fr. - *Caulacanthus ustulatus* - *Corallina* spp. Sp. - *Lithophyllum incrustans*	Fr. - ***Laurencia obtusa*** - (*Osmundea pinnatifida*) Sp. - (*Halopteris scoparia*)
Lower midlittoral zone	Fr. - ***Ceramium* spp.** Sp. - *Corallina* spp.	- *Halopteris scoparia* Fr. - (*Cystoseira tamariscifolia*) - *Chondria coerulescens* Sp. - *Codium adhaerens*

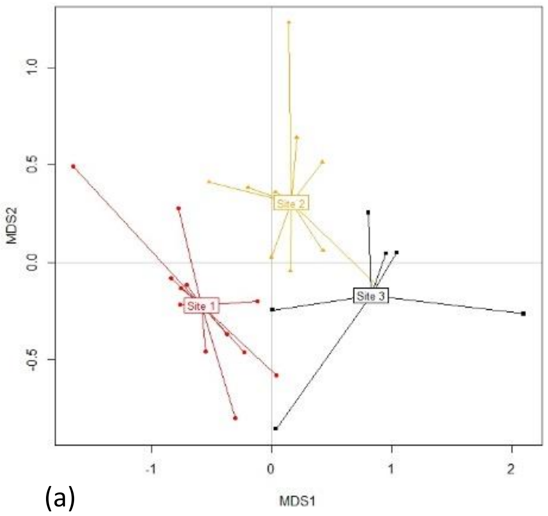
Prospects & improvements:

- Reflect upon another sampling method for **macrofauna**
- Explore the interest of studying macrofauna in **boulder field habitat**
- Reflect upon **how to integrate main contributors** (mainly present in impacted or control locations) **in WFD monitoring** in addition to those defined as opportunistic and characteristic

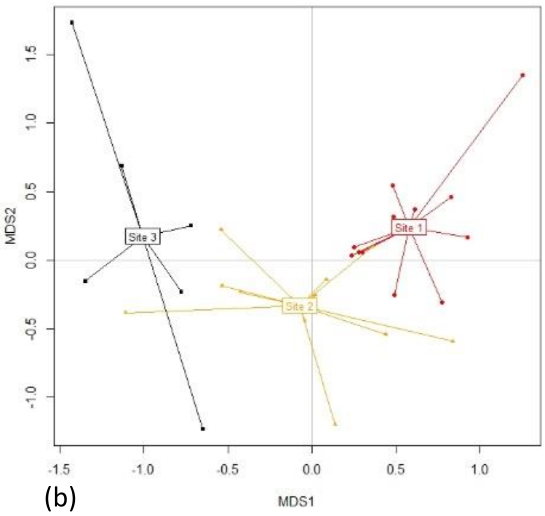
Supplementary materials (SM)



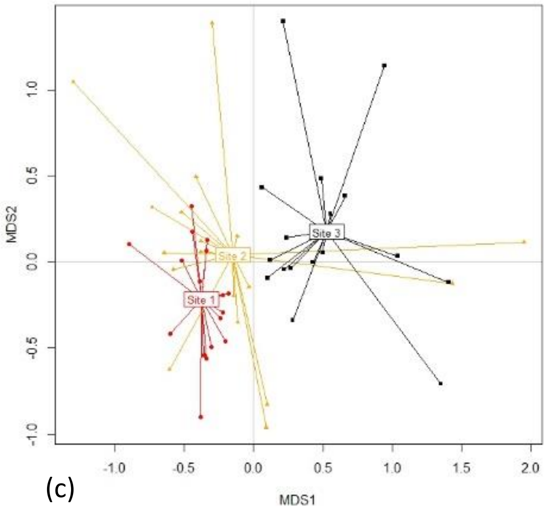
SM 1: Non-metric multi-dimensional scaling plots (nMDS) computed on benthic taxon assemblages (macroalgae and macrofauna) in the upper midlittoral zone (Corallina spp. belt) (a) and in the lower midlittoral zone (Halopteris scoparia belt) (b) of French and Spanish impacted ('WWTP 1', 'WWTP 2', 'WWTP 3') and control locations ('Control 1', 'Control 2') and at varying distances to the outfall (site 1: circles; site 2: triangles; sites 3: squares).


MDS1
MDS2
Site 1
Site 2
Site 3
-1
0
1
2
-0.5
0.0
0.5
1.0

(a)


MDS1
MDS2
Site 1
Site 2
Site 3
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5

(b)


MDS1
MDS2
Site 1
Site 2
Site 3
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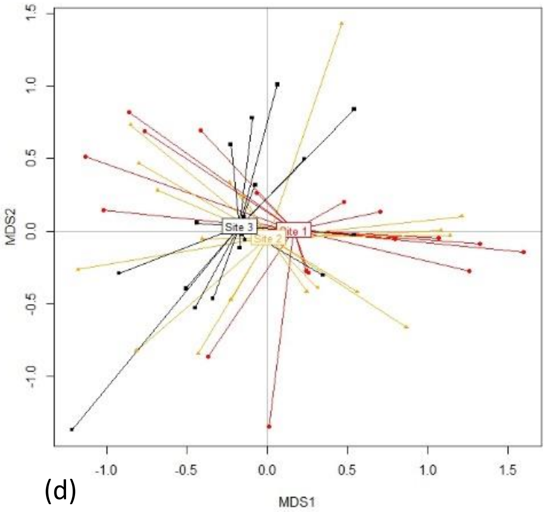
0.0
0.5
1.0
1.5
2.0

(c)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MDS2
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Site 2
Site 3
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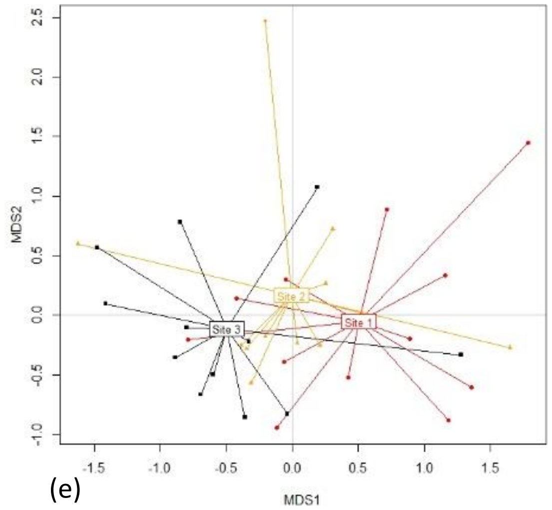
0.0
0.5
1.0
1.5

(d)


MDS1
MDS2
Site 1
Site 2
Site 3
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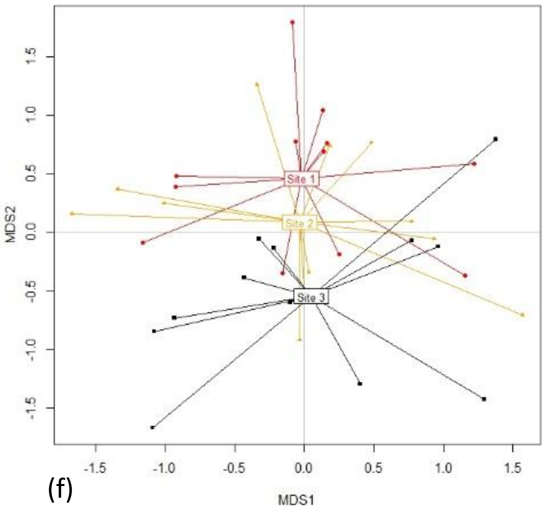
1.0
1.5
2.0
2.5

(e)


MDS1
MDS2
Site 1
Site 2
Site 3
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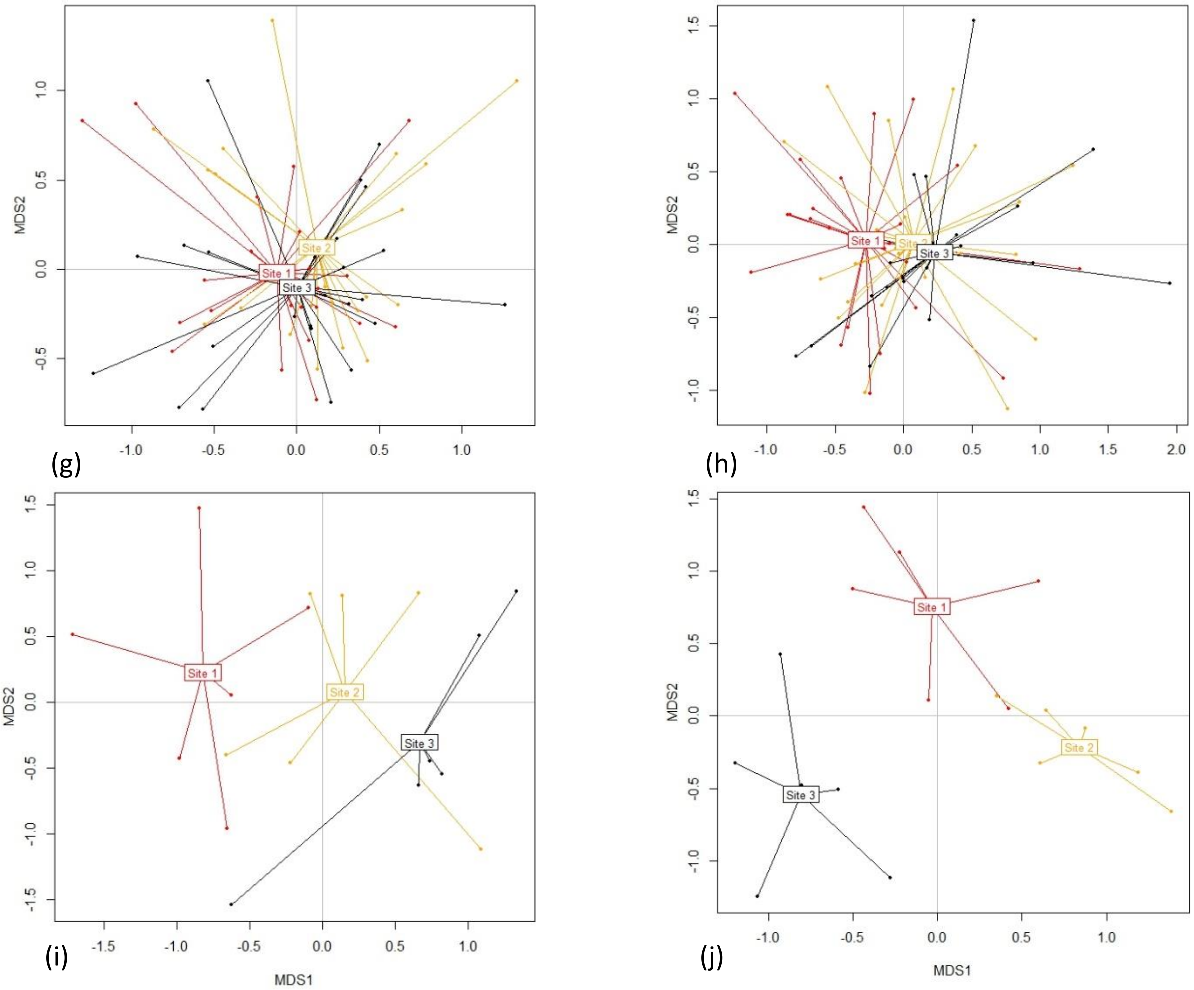
0.5
1.0
1.5

(f)



SM 2: Non-metric multi-dimensional scaling plots (nMDS) computed on benthic taxon assemblages (macroalgae and macrofauna) of impacted locations ('WWTP 1' (a, b), 'WWTP 2' (c, d), 'WWTP 3' (e, f)) and control locations ('Control 1' (g,h), 'Control 2' (i,j)) according to distances (site 1: red circles; site 2: orange triangles; sites 3: black squares) and midlittoral zones (upper (a, c, e, g, i) and lower (b, d, f, h, j)).

SM 3: List of significant species/taxa (contributors with p-value <0.05) identified by SIMPER analysis explaining the dissimilarity between impacted and control locations and between sites within each location in the upper (a) and lower midlittoral zones (b). Significance codes: "av": (average) corresponds to the mean class of the taxa; Bold percentages on the top left correspond to the global dissimilarity between the 2 groups; "Ct(%)": corresponds to the total contribution of each species/taxa to the dissimilarity; Species with a contribution higher than 10 % are above dotted lines.

(a)

WWTP 1' vs. 'Control 1'

51.12 %	**av Control 1**	**av WWTP 1**	**Ct (%)**
Ceramium spp.	0.38	**2.07**	**12.46**
Laurencia obtusa	1.54	1.07	9.66
Caulacanthus ustulatus	1.03	1.17	8.34
Colpomenia peregrina	0.71	0.27	4.23
Osmundea pinnatifida	0.61	0.00	4.21
Hypnea musciformis	0.04	0.60	4.08
Phymatolithon lenormandii	0.51	0.03	3.63
Mastocarpus	0.38	0.10	2.87
Mesophyllum lichenoides	0.00	0.27	1.70
Gigartina spp.	0.00	0.03	0.19

WWTP 2' vs. 'Control 1'

53.81 %	**av Control 1**	**av WWTP 2**	**Ct (%)**
Laurencia obtusa	1.54	0.69	9.09
Lithophyllum incrustans	0.19	1.31	7.70
Caulacanthus ustulatus	1.03	0.50	6.48
Enteromorpha spp.	1.10	0.78	6.45
Asparagopsis spp.	0.13	0.72	4.75
Plocamium cartilagineum	0.01	0.70	4.58
Colpomenia peregrina	0.71	0.26	4.22
Osmundea pinnatifida	0.61	0.06	4.10
Phymatolithon lenormandii	0.51	0.31	3.61
Chondria coerulescens	0.00	0.56	3.57
Gastroclonium reflexum	0.00	0.41	2.69
Mastocarpus	0.38	0.02	2.58
Hypoglossum woodwardii	0.00	0.09	0.68
Peyssonnelia atropurpurea	0.00	0.04	0.24

WWTP 3' vs. 'Control 2'

42.00 %	**av Control 2**	**av WWTP 3**	**Ct (%)**
Corallina spp.	3.06	**4.56**	**15.01**
Chondria coerulescens	0.94	0.00	8.05
Gelidium spp.	0.50	1.06	6.09
Halopteris scoparia	0.50	0.03	4.51
Ectocarpales/Ectocarpus	0.28	0.36	3.81
Cutleria adspersa	0.00	0.28	2.24
Codium fragile	0.22	0.03	2.20
Jania rubens	0.11	0.14	1.79
Antithamnionella sp.	0.06	0.14	1.44
Chaetomorpha spp.	0.00	0.17	1.33
Ralfsia verrucosa	0.06	0.11	1.27
Halurus equisetifolius	0.11	0.00	0.96
Cladostephus spongiosus	0.11	0.00	0.83
Scytosiphon lomentaria	0.00	0.11	0.82
Codium decorticatum	0.00	0.08	0.65
Pterothamnion	0.00	0.08	0.65

'WWTP 1'

Site 1 vs. Site 2

43.62 %	**av S1**	**av S2**	**Ct (%)**
Laurencia obtusa	0.17	**1.92**	**14.32**
Caulacanthus ustulatus	**2.00**	0.75	**13.49**
Codium adhaerens	1.17	0.00	9.49
Lithophyllum incrustans	0.58	1.50	7.29
Colpomenia peregrina	0.17	0.50	3.87

Site 2 vs. Site 3

39.45 %	**av S2**	**av S3**	**Ct (%)**
Mesophyllum lichenoides	0.08	1.00	7.74
Ulva spp.	1.67	1.00	5.57
Jania rubens	0.00	0.67	5.36

Site 1 vs. Site 3

52.74 %	**av S1**	**av S3**	**Ct (%)**
Caulacanthus ustulatus	**2.00**	0.33	**11.64**
Chondracanthus acicularis	1.25	0.00	8.29
Hypnea musciformis	0.00	1.17	7.38
Lithophyllum incrustans	0.58	1.50	7.21
Corallina spp.	3.17	2.17	7.00
Mesophyllum lichenoides	0.08	1.00	6.12
Ulva spp.	1.75	1.00	4.96
Jania rubens	0.00	0.67	4.25
Chondria coerulescens	0.00	0.67	4.16
Gastroclonium reflexum	0.08	0.33	2.22
Phymatolithon lenormandii	0.00	0.17	1.24
Gigartina spp.	0.00	0.17	0.94
Hypoglossum woodwardii	0.00	0.17	0.94

'WWTP 2'

Site 1 vs. Site 2

49.57 %	**av S1**	**av S2**	**Ct (%)**
Corallina spp.	**3.83**	2.72	**11.64**
Ceramium spp.	**1.89**	0.56	**10.22**
Plocamium cartilagineum	0.28	1.28	9.49
Chondracanthus acicularis	1.06	1.94	8.43
Caulacanthus ustulatus	0.28	1.17	8.02
Enteromorpha spp.	1.28	0.56	7.16
Ulva spp.	1.22	1.67	6.71
Lithophyllum incrustans	0.94	1.50	5.90
Gastroclonium reflexum	0.50	0.50	4.98
Hypoglossum woodwardii	0.22	0.06	1.95
Nitophyllum punctatum	0.00	0.17	1.18
Peyssonnelia atropurpurea	0.00	0.11	0.84
Ectocarpales/Ectocarpus	0.00	0.06	0.37
Cutleria multifida	0.00	0.06	0.33

Site 2 vs. Site 3

49.17 %	**av S2**	**av S3**	**Ct (%)**
Laurencia obtusa	0.39	1.67	9.05
Codium adhaerens	0.44	1.17	7.76
Chondria coerulescens	0.33	1.28	7.73
Caulacanthus ustulatus	1.17	0.06	7.50

Site 1 vs. Site 3

52.93 %	**av S1**	**av S3**	**Ct (%)**
Corallina spp.	**3.83**	2.56	**12.48**
Laurencia obtusa	0.00	**1.67**	**11.25**
Chondria coerulescens	0.06	1.28	8.51
Codium adhaerens	0.00	1.17	7.90
Ceramium spp.	1.89	0.89	7.65
Enteromorpha spp.	1.28	0.50	7.20
Lithophyllum incrustans	0.94	1.50	5.35
Tenarea tortuosa	0.00	0.06	0.44
Taonia sp.	0.00	0.06	0.43
Mastocarpus	0.00	0.06	0.33
Halopteris scoparia	0.00	0.06	0.32

'WWTP 3'

Site 1 vs. Site 2

31.16 %	**av S1**	**av S2**	**Ct (%)**

Site 2 vs. Site 3

33.08 %	**av S2**	**av S3**	**Ct (%)**
Codium adhaerens	0.33	0.83	8.35
Asparagopsis spp.	0.08	0.25	2.84

Site 1 vs. Site 3

36.56 %	**av S1**	**av S3**	**Ct (%)**
Lithophyllum incrustans	**2.17**	1.25	**10.10**
Colpomenia peregrina	0.83	0.25	6.54
Caulacanthus ustulatus	0.25	0.08	2.76

'Control 1'

Site 1 vs. Site 2

42.41 %	**av S1**	**av S2**	**Ct (%)**
Caulacanthus ustulatus	0.46	**1.70**	**11.31**
Enteromorpha spp.	**1.83**	0.61	**11.04**
Chondracanthus acicularis	1.71	1.00	8.75

Site 2 vs. Site 3

37.47 %	**av S1**	**av S3**	**Ct (%)**
Halopteris scoparia	0.00	**1.33**	**13.84**
Gelidium spp.	0.83	0.17	7.74
Ectocarpales/Ectocarpus	0.67	0.00	7.02

Site 1 vs. Site 3

40.95 %	**av S2**	**av S3**	**Ct (%)**
Codium adhaerens	0.52	**1.36**	**11.54**

'Control 2'

Site 1 vs. Site 2

32.65 %	**av S1**	**av S2**	**Ct (%)**
Ectocarpales/Ectocarpus	0.67	0.17	6.88

Site 2 vs. Site 3

40.26 %	**av S2**	**av S3**	**Ct (%)**
Corallina spp.	**3.67**	2.33	**14.52**
Halopteris scoparia	0.17	**1.33**	**11.44**
Chondria coerulescens	**1.33**	0.67	**10.84**
Enteromorpha spp.	0.67	0.67	8.76
Jania rubens	0.33	0.00	3.34
Colpomenia peregrina	0.17	0.00	1.74

Site 1 vs. Site 3

37.47 %	**av S1**	**av S3**	**Ct (%)**
Halopteris scoparia	0.00	**1.33**	**13.84**
Gelidium spp.	0.83	0.17	7.74
Ectocarpales/Ectocarpus	0.67	0.00	7.02

(b)

WWTP 1' vs. 'Control 1'			
63.58 %	**av Control 1**	**av WWTP 1**	**Ct (%)**
Ceramium spp.	0.22	**2.40**	**12.78**
Halopteris scoparia	**2.62**	1.07	**11.70**
Hypnea musciformis	1.04	1.30	7.02
Ulva spp.	0.87	1.37	4.28
Halurus equisetifolius	0.80	0.30	3.92
Cystoseira tamariscifolia	0.78	0.00	3.91
Pterosiphonia sp.	0.72	0.03	3.71
Gigartina spp.	0.04	0.10	0.66

WWTP 2' vs. 'Control 1'			
71.14 %	**av Control 1**	**av WWTP 2**	**Ct (%)**
Halopteris scoparia	**2.62**	0.15	**11.11**
Gelidium spp.	0.61	2.35	9.31
Asparagopsis spp.	0.58	1.70	6.45
Chondria coerulescens	0.68	1.44	4.82
Mesophyllum lichenoides	0.13	1.07	4.78
Chondracanthus acicularis	0.58	1.06	4.33
Cystoseira tamariscifolia	0.78	0.20	3.50
Plocamium cartilagineum	0.30	0.70	3.37
Halurus equisetifolius	0.80	0.20	3.28
Pterosiphonia sp.	0.72	0.24	3.26
Laurencia obtusa	0.33	0.69	3.05
Nitophyllum punctatum	0.29	0.52	2.51
Gymnogongrus spp.	0.19	0.44	2.33
Taonia sp.	0.09	0.44	1.98
Dictyota dichotoma	0.04	0.46	1.93
Mastocarpus	0.06	0.37	1.70
Acrosorium spp.	0.20	0.13	1.19
Caulacanthus ustulatus	0.12	0.07	0.84
Cladophora spp.	0.03	0.07	0.52
Ahnfeltiopsis devoniensis	0.00	0.13	0.50
Bonnemaisonia hamifera	0.03	0.09	0.49
Rhodymenia pseudopalmata	0.07	0.06	0.48
Zanardinia typus	0.00	0.09	0.35

WWTP 3' vs. 'Control 2'			
52.54 %	**av Control 2**	**av WWTP 3**	**Ct (%)**
Chondria coerulescens	1.44	0.03	9.63
Cladostephus spongiosus	1.00	0.00	6.75
Jania rubens	1.00	0.06	6.37
Codium adhaerens	0.56	0.64	6.10
Colpomenia peregrina	0.00	0.64	4.15
Pterothamnion	0.00	0.47	3.01
Gastroclonium reflexum	0.06	0.47	2.93
Cutleria adspersa	0.11	0.31	2.51
Champia parvula	0.22	0.03	1.63
Codium fragile	0.11	0.14	1.44
Ectocarpales/Ectocarpus	0.00	0.08	0.50
Codium decorticatum	0.00	0.08	0.47
Vertebrata fruticulosa	0.06	0.00	0.39

'WWTP 1'

Site 1 vs. Site 2			
52.00 %	**av S1**	**av S2**	**Ct (%)**
Asparagopsis spp.	0.25	**1.75**	**13.27**
Halopteris scoparia	0.00	**1.75**	**13.21**
Hypnea musciformis	0.42	**1.67**	**10.97**
Ceramium spp.	**3.08**	1.83	**10.19**
Lithophyllum incrustans	0.58	1.33	8.12
Ulva spp.	1.58	1.17	6.04
Colpomenia peregrina	0.42	0.00	3.28
Gelidium spp.	0.08	0.25	2.17
Phymatolithon lenormandii	0.00	0.08	0.57
Mastocarpus	0.00	0.08	0.55

Site 2 vs. Site 3			
41.41 %	**av S2**	**av S3**	**Ct (%)**
Jania rubens	0.33	1.17	7.22
Enteromorpha spp.	0.25	1.00	7.04
Plocamium cartilagineum	0.00	0.50	3.74
Mesophyllum lichenoides	0.00	0.50	3.55
Taonia sp.	0.00	0.33	2.35

Site 1 vs. Site 3			
56.96 %	**av S1**	**av S3**	**Ct (%)**
Hypnea musciformis	0.42	**2.33**	**11.93**
Halopteris scoparia	0.00	**1.83**	**11.35**
Jania rubens	0.00	1.17	7.18
Corallina spp.	2.33	1.33	6.23
Enteromorpha spp.	0.00	1.00	6.06
Halurus equisetifolius	0.00	0.83	5.21
Plocamium cartilagineum	0.00	0.50	3.17
Mesophyllum lichenoides	0.00	0.50	2.97
Gigartina spp.	0.00	0.33	1.97
Taonia sp.	0.00	0.33	1.97
Acrosorium spp.	0.00	0.17	1.00
Dictyota dichotoma	0.00	0.17	1.00
Hypoglossum woodwardii	0.00	0.17	1.01
Nitophyllum punctatum	0.00	0.17	1.00

'WWTP 2'

Site 1 vs. Site 2			
56.88 %	**av S1**	**av S2**	**Ct (%)**
Lithophyllum incrustans	1.06	1.17	5.37
Gymnogongrus spp.	0.78	0.44	3.57
Zanardinia typus	0.00	0.28	1.26

Site 2 vs. Site 3			
55.08 %	**av S2**	**av S3**	**Ct (%)**
Taonia sp.	0.28	0.61	3.57
Cystoseira tamariscifolia	0.00	0.56	2.91
Cutleria adspersa	0.39	0.22	2.27
Zanardinia typus	0.28	0.00	1.29

Site 1 vs. Site 3			
57.36 %	**av S1**	**av S3**	**Ct (%)**
Mesophyllum lichenoides	1.61	0.44	6.94
Gymnogongrus spp.	0.78	0.11	4.09
Colpomenia peregrina	0.39	0.61	3.37
Cystoseira tamariscifolia	0.06	0.56	2.94
Halopteris scoparia	0.17	0.28	2.10
Codium adhaerens	0.00	0.17	0.95

'WWTP 3'

Site 1 vs. Site 2			
33.44 %	**av S1**	**av S2**	**Ct (%)**

Site 2 vs. Site 3			
50.08 %	**av S2**	**av S3**	**Ct (%)**
Codium adhaerens	0.25	**1.58**	**10.52**
Asparagopsis spp.	1.42	0.42	8.46
Phymatolithon lenormandii	0.00	0.67	6.56
Colpomenia peregrina	0.83	0.25	5.31
Taonia sp.	0.50	0.00	3.98
Mastocarpus	0.08	0.00	0.74
Chondria coerulescens	0.08	0.00	0.68

Site 1 vs. Site 3			
51.28 %	**av S1**	**av S3**	**Ct (%)**
Ceramium spp.	**3.50**	1.75	**16.74**
Corallina spp.	**4.25**	3.17	**12.03**
Codium adhaerens	0.08	**1.58**	**10.49**
Phymatolithon lenormandii	0.00	0.67	6.17
Colpomenia peregrina	0.83	0.25	5.05
Peyssonnelia atropurpurea	0.17	0.00	1.12
Champia parvula	0.08	0.00	0.66
Ralfsia verrucosa	0.08	0.00	0.52

'Control 1'

Site 1 vs. Site 2			
56.27 %	**av S1**	**av S2**	**Ct (%)**
Ceramium spp.	0.25	0.38	3.05
Enteromorpha spp.	0.38	0.08	2.09

Site 2 vs. Site 3			
61.04 %	**av S2**	**av S3**	**Ct (%)**
Gelidium spp.	0.58	0.90	5.22
Jania rubens	0.38	0.67	4.13

Site 1 vs. Site 3			
57.44 %	**av S1**	**av S3**	**Ct (%)**
Enteromorpha spp.	0.38	0.05	2.07
Chylocladia verticillata	0.17	0.05	1.05

'Control 2'

Site 1 vs. Site 2			
30.99 %	**av S1**	**av S2**	**Ct (%)**
Halopteris scoparia	0.17	**1.83**	**17.33**

Site 2 vs. Site 3			
46.28 %	**av S2**	**av S3**	**Ct (%)**
Corallina spp.	**3.67**	1.67	**14.78**
Codium adhaerens	0.00	**1.67**	**11.92**
Halopteris scoparia	1.83	0.67	8.91
Enteromorpha spp.	0.83	0.00	6.04
Lithophyllum incrustans	1.50	2.33	5.94
Asparagopsis spp.	1.00	0.33	4.80
Vertebrata fruticulosa	0.17	0.00	1.30
Gastroclonium reflexum	0.17	0.00	1.22

Site 1 vs. Site 3			
45.78 %	**av S1**	**av S3**	**Ct (%)**
Corallina spp.	**4.33**	1.67	**21.12**
Codium adhaerens	0.00	**1.67**	**12.86**
Cladostephus spongiosus	0.33	**1.83**	**12.35**
Chondria coerulescens	1.33	1.67	6.04
Asparagopsis spp.	1.00	0.33	5.18
Champia parvula	0.67	0.00	5.15

SM 4: Mean taxonomic richness per ecological group at varying distances from the outfall (sites 1 to 3) for each location and midlittoral zone.

	Locations				
	France			Spain	
	Control 1	WWTP 1'	WWTP 2'	Control 2	WWTP 3'
Macroalgae					
Upper					
Site 1	**8.04** *(SD=3.20)*	**7.42** *(SD=1.38)*	**7.22** *(SD=1.22)*	**7.83** *(SD=1.47)*	**8.42** *(SD=2.64)*
Site 2	**7.52** *(SD=2.59)*	**8.67** *(SD=0.89)*	**9.00** *(SD=2.28)*	**7.67** *(SD=0.82)*	**7.83** *(SD=2.89)*
Site 3	**8.00** *(SD=2.22)*	**10.33** *(SD=3.50)*	**9.72** *(SD=1.87)*	**7.00** *(SD=0.89)*	**8.25** *(SD=2.73)*
Lower					
Site 1	**10.96** *(SD=3.80)*	**6.00** *(SD=1.60)*	**10.22** *(SD=4.52)*	**8.00** *(SD=1.90)*	**8.08** *(SD=1.68)*
Site 2	**9.42** *(SD=3.67)*	**8.58** *(SD=1.38)*	**11.56** *(SD=3.40)*	**10.00** *(SD=1.10)*	**8.00** *(SD=1.76)*
Site 3	**8.57** *(SD=3.78)*	**12.17** *(SD=1.17)*	**11.67** *(SD=2.91)*	**7.33** *(SD=1.51)*	**6.67** *(SD=2.87)*
Fixed Macroafauna					
Upper					
Site 1	-	**0.5** *(SD=0.52)*	**0.17** *(SD=0.38)*	**2.67** *(SD=0.82)*	**1.25** *(SD=1.06)*
Site 2	-	**0.08** *(SD=0.29)*	**0.28** *(SD=0.46)*	**2.17** *(SD=0.75)*	**1.25** *(SD=0.87)*
Site 3	**0.08** *(SD=0.28)*	-	**0.06** *(SD=0.24)*	**0.17** *(SD=0.41)*	**0.92** *(SD=0.90)*
Lower					
Site 1	-	-	**0.28** *(SD=0.57)*	**1.33** *(SD=1.03)*	**0.50** *(SD=0.67)*
Site 2	-	-	**0.17** *(SD=0.38)*	-	**0.33** *(SD=0.49)*
Site 3	-	-	-	-	**0.5** *(SD=0.67)*
Mobile Macroafauna					
Upper					
Site 1	**0.83** *(SD=1.17)*	**0.17** *(SD=0.58)*	**0.22** *(SD=0.43)*	**1.50** *(SD=0.55)*	**1.58** *(SD=1.08)*
Site 2	**0.91** *(SD=1.04)*	**0.25** *(SD=0.45)*	**0.61** *(SD=0.92)*	**1.00** *(SD=0.00)*	**1.58** *(SD=0.67)*
Site 3	**0.52** *(SD=0.87)*	**0.33** *(SD=0.82)*	**0.22** *(SD=0.43)*	***0.17*** *(SD=0.41)*	**1.17** *(SD=0.58)*
Lower					
Site 1	**0.38** *(SD=0.77)*	**0.08** *(SD=0.29)*	**0.28** *(SD=0.46)*	**0.83** *(SD=0.41)*	**0.75** *(SD=0.75)*
Site 2	**0.17** *(SD=0.38)*	**0.25** *(SD=0.62)*	**0.06** *(SD=0.24)*	**0.17** *(SD=0.41)*	**0.92** *(SD=0.79)*
Site 3	**0.29** *(SD=0.64)*	-	**0.11** *(SD=0.47)*	**0.33** *(SD=0.52)*	**0.92** *(SD=0.67)*
Total					
Upper					
Site 1	**8.88** *(SD=2.03)*	**8.08** *(SD=2.07)*	**7.61** *(SD=1.42)*	**12.00** *(SD=2.00)*	**11.25** *(SD=3.65)*
Site 2	**8.43** *(SD=2.84)*	**9.00** *(SD=0.95)*	**9.89** *(SD=2.61)*	**10.83** *(SD=1.33)*	**10.67** *(SD=3.37)*
Site 3	**8.6** *(SD=2.47)*	**10.67** *(SD=3.44)*	**10.00** *(SD=2.00)*	**7.33** *(SD=1.51)*	**10.33** *(SD=2.87)*
Lower					
Site 1	**11.33** *(SD=3.67)*	**6.08** *(SD=11.62)*	**10.78** *(SD=4.63)*	**10.17** *(SD=2.32)*	**9.33** *(SD=1.44)*
Site 2	**9.58** *(SD=3.68)*	**8.83** *(SD=1.40)*	**11.78** *(SD=3.26)*	**10.17** *(SD=1.17)*	**9.25** *(SD=1.82)*
Site 3	**8.86** *(SD=3.92)*	**12.17** *(SD=1.17)*	**11.78** *(SD=3.04)*	**7.67** *(SD=1.37)*	**8.08** *(SD=3.42)*

SM 5: Summary of PERMANOVA (a) and pairwise post hoc results (b) testing for effects of presence of sewage discharges on the mean taxonomic richness of macrofauna.

(a)

'WWTP 1'/'Control 1'	Df	Mean Sq	F Value	Pr(>F)	Significance
Upper midlittoral zone					
Locations	1	0.02778	0.0943	0.6104	
Locations/Sites	4	0.47222	1.6038	0.1538	
Residuals	30	0.29444			
Lower midlittoral zone					
Locations	1	0.25	1.8	0.2118	
Locations/Sites	4	0.083333	0.6	0.3047	
Residuals	30	0.138889			

'WWTP 2'/'Control 1'	Df	Mean Sq	F Value	Pr(>F)	Significance
Upper midlittoral zone					
Locations	1	0.69444	5	0.005994	**
Locations/Sites	4	0.36111	2.6	0.092907	.
Residuals	30	0.13889			
Lower midlittoral zone					
Locations	1	1.77778	4.4444	0.03097	*
Locations/Sites	4	0.11111	0.2778	0.82817	
Residuals	30	0.4			

'WWTP 3'/'Control 2'	Df	Mean Sq	F Value	Pr(>F)	Significance
Upper midlittoral zone					
Locations	1	9	14.464	0.000999	***
Locations/Sites	4	13.5556	21.786	0.000999	***
Residuals	30	0.6222			
Lower midlittoral zone					
Locations	1	7.1111	9.0141	0.00999	**
Locations/Sites	4	3.8056	4.8239	0.005994	**
Residuals	30	0.7889			

(b)

Upper midlittoral zone		WWTP 3	
		Site 1	Site 2
WWTP 3	Site 2	0.258	-
	Site 3	0.084	0.258
Lower midlittoral zone		WWTP 3	
		Site 1	Site 2
WWTP 3	Site 2	1	-
	Site 3	1	1

Upper midlittoral zone		Control 2	
		Site 1	Site 2
Control 2	Site 2	0.098	-
	Site 3	**0.009**	**0.009**
Lower midlittoral zone		Control 2	
		Site 1	Site 2
Control 2	Site 2	**0.048**	-
	Site 3	**0.048**	1

SM 6: Summary of PERMANOVA (a) and pairwise post hoc results (b) testing for effects of presence of sewage discharges on the mean taxonomic richness of macroalgae.

(a)

'WWTP 1'/'Control 1'	Df	Mean Sq	F Value	Pr(>F)	Significance
Upper midlittoral zone					
Locations	1	3.3611	1.0485	0.32567	
Locations/Sites	4	9.5278	2.9723	0.05295	.
Residuals	30	3.2056			
Lower midlittoral zone					
Locations	1	0.111	0.0342	0.834166	
Locations/Sites	4	42.889	13.219	0.000999	***
Residuals	30	3.244			

'WWTP 2'/'Control 1'	Df	Mean Sq	F Value	Pr(>F)	Significance
Upper midlittoral zone					
Locations	1	0.1111	0.0613	0.7542	
Locations/Sites	4	3.3611	1.8558	0.1279	
Residuals	30	1.8111			
Lower midlittoral zone					
Locations	1	23.3611	2.8127	0.10989	
Locations/Sites	4	20.0556	2.4147	0.09391	.
Residuals	30	8.3056			

'WWTP 3'/'Control 2'	Df	Mean Sq	F Value	Pr(>F)	Significance
Upper midlittoral zone					
Locations	1	28.4444	5.8581	0.02098	*
Locations/Sites	4	3.1111	0.6407	0.55345	
Residuals	30	4.8556			
Lower midlittoral zone					
Locations	1	1.3611	0.5606	0.45055	
Locations/Sites	4	6.6389	2.7346	0.04695	*
Residuals	30	2.4278			

(b)

Lower midlittoral zone		WWTP 1	
		Site 1	Site 2
WWTP 1	Site 2	**0.006**	-
	Site 3	**0.012**	**0.022**

Lower midlittoral zone		Control 1	
		Site 1	Site 2
Control 1	Site 2	0.509	-
	Site 3	**0.036**	0.192

Lower midlittoral zone		WWTP 3	
		Site 1	Site 2
WWTP 3	Site 2	0.99	-
	Site 3	0.7	0.7

Lower midlittoral zone		Control 2	
		Site 1	Site 2
Control 2	Site 2	0.102	-
	Site 3	0.633	**0.006**

SM 7: Summary of PERMANOVA (a) and pairwise post hoc results (b) testing for effects of presence of sewage discharges on the ratio between characteristic and opportunistic macroalgae mean taxonomic richness.

(a)

'WWTP 1'/'Control 1'	Df	Mean Sq	F Value	Pr(>F)	Significance
Upper midlittoral zone					
Locations	1	20.5008	49.6617	0.000999	***
Locations/Sites	4	2.6744	6.4785	0.00999	**
Residuals	30	0.4128			
Lower midlittoral zone					
Locations	1	19.2623	11.6524	0.001998	**
Locations/Sites	4	2.2832	1.3812	0.252747	
Residuals	30	1.6531			

'WWTP 2'/'Control 1'	Df	Mean Sq	F Value	Pr(>F)	Significance
Upper midlittoral zone					
Locations	1	9.679	24.3857	0.000999	***
Locations/Sites	4	2.9614	7.4611	0.005994	**
Residuals	30	0.3969			
Lower midlittoral zone					
Locations	1	14.9082	5.4595	0.03397	*
Locations/Sites	4	7.909	2.8963	0.03996	*
Residuals	30	2.7307			

'WWTP 3'/'Control 2'	Df	Mean Sq	F Value	Pr(>F)	Significance
Upper midlittoral zone					
Locations	1	0.07716	0.4318	0.592408	
Locations/Sites	4	0.61883	3.4629	0.008991	**
Residuals	30	0.1787			
Lower midlittoral zone					
Locations	1	19.8767	40.5131	0.000999	***
Locations/Sites	4	0.7274	1.4827	0.24975	
Residuals	30	0.4906			

(b)

Upper midlittoral zone		WWTP 1	
		Site 1	Site 2
WWTP 1	Site 2	0.066	-
	Site 3	0.811	0.081

Upper midlittoral zone		Control 1	
		Site 1	Site 2
Control 1	Site 2	**0.045**	-
	Site 3	**0.045**	0.691

Upper midlittoral zone		WWTP 2	
		Site 1	Site 2
WWTP 2	Site 2	**0.021**	-
	Site 3	**0.006**	0.39
Lower midlittoral zone		WWTP 2	
		Site 1	Site 2
WWTP 2	Site 2	0.266	-
	Site 3	**0.036**	0.177

Upper midlittoral zone		Control 1	
		Site 1	Site 2
Control 1	Site 2	**0.045**	-
	Site 3	**0.045**	0.691
Lower midlittoral zone		Control 1	
		Site 1	Site 2
Control 1	Site 2	0.53	-
	Site 3	0.57	0.53

Upper midlittoral zone		WWTP 3	
		Site 1	Site 2
WWTP 3	Site 2	0.69	-
	Site 3	0.13	0.13

Upper midlittoral zone		Control 2	
		Site 1	Site 2
Control 2	Site 2	0.71	-
	Site 3	0.41	0.59

Chapter V:

Benthic communities’ response to WWTP discharges in the subtidal zone

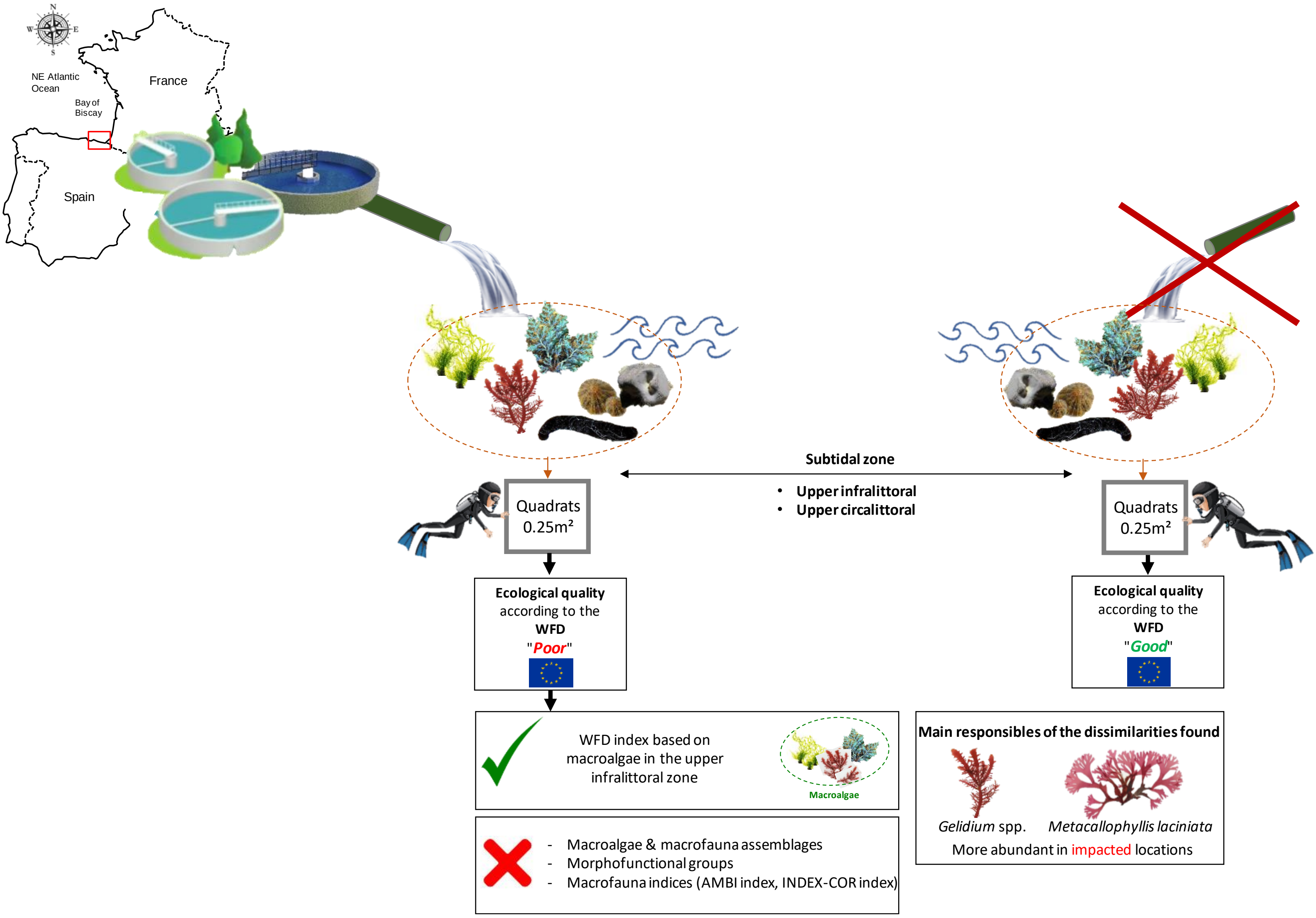


Fig. 1: Graphical abstract of the Chapter V

Chapter structure:

- **Huguenin L.**, Lalanne Y, de Casamajor MN., Gorostiaga J-M., Quintano E., Monperrus M. (**2019**). “*Does wastewater discharge drive rocky subtidal community shifts? A case study*. ***Will be submitted to Marine Pollution Bulletin.***

Benthic communities' response to WWTP discharges in the subtidal zone

Coastal habitats and marine environments are under great anthropogenic pressures (e.g. waste waters, urban runoff, spilled chemicals, overexploitation, invasive species introduction, habitat fragmentation and destruction) partly due to the urban expansion (Becherucci et al., 2016; Crain et al., 2008; de-la-Ossa-Carretero et al., 2016). In addition to environmental factors governing these areas, sewage discharges constitute a common source of disturbances (Fraschetti et al., 2006; Pearson and Rosenberg, 1978). Effluents may have direct or indirect effects (biological, chemical or physical) on the environment (Borja et al., 2011a; Del-Pilar-Ruso et al., 2010) and irreversible negative effects may occurred (e.g. alteration of benthic composition and abundance patterns) (Guidetti et al., 2003; Nicolodi et al., 2009; Terlizzi et al., 2005, 2002).

The study of benthic assemblages (i.e., macroalgae and invertebrates) presents several advantages (e.g. organisms are mainly sedentary and long-lived, reflect both previous and present conditions to which communities have been exposed, are easy to sample even without using destructive sampling methods, etc.) and is considered as a powerful tool to assess environmental quality. It has thus become of major importance by providing accurate information on deleterious effects of contaminants especially in assessing local effects (Belan, 2003; Borja et al., 2011a).

In this context and over the last decades, effects of sewage discharges have been studied on different environmental compartments (e.g. sediments, water body, trophic web, benthic and pelagic communities) and their impact on benthic communities have been widely documented in the intertidal zone. Some studies also described their impact on subtidal rocky and soft bottoms but they were often achieved either on macroalgae or macrofauna assemblages but rarely together especially in rocky habitats.

Problematic:

- ➔ Are subtidal rocky benthic communities affected by WWTP discharges?
- ➔ Are current WFD indices enough sensitive to study such a pressure?

This chapter/article deals with the study of the potential impact of WWTP discharges on subtidal rocky benthic assemblages in the southeastern Bay of Biscay in compliance with the European Directives. This was achieved by comparing control and impacted locations (in the immediate vicinity of the outfall) using the same sampling strategy at two different habitats defined in the Directive for the French Basque coast. Composition, abundance and functional traits of both macroalgae and macrofauna were then studied.

Does wastewater discharge drive rocky subtidal community shifts? A case study

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Abstract

This study aims to assess the potential impact of a wastewater treatment plant discharge on subtidal rocky benthic assemblages in the Southeastern Bay of Biscay in compliance with the European Directives. Results showed that only the EQS index based on macroalgae highlighted a clear effect of the discharge in the upper infralittoral zone. By contrast, no significant effect was detected using macrofauna indices and multivariate analyses based on assemblages, functional traits and ecological groups of both macroalgae and macrofauna in the two subtidal zones. Dissimilarities between impacted and control locations were also mainly due to the higher abundance of *Gelidium corneum* (upper infralittoral) and *Metacallophyllis laciniata* (circalittoral). Finally, this work provides a framework for future monitoring allowing an assessment of benthic communities' changes related to WWTP mitigation measures and suggests to reflect on others way of integrating macrofauna for an efficient impact evaluation.

Keywords: Macroalgae; Macrofauna; Sewage; Ecological Quality Status; WFD; MSFD.

1. Introduction

Coastal habitats and marine environments are under great anthropogenic pressures (e.g. waste waters, urban runoff, spilled chemicals, overexploitation, invasive species introduction, habitat fragmentation and destruction) partly due to the urban expansion (Becherucci et al., 2016; Crain et al., 2008; de-la-Ossa-Carretero et al., 2016). Nowadays, half of the world's population lives in these areas especially benefiting from goods and services provided by coastal and marine ecosystems (Halpern et al., 2008). In addition to environmental factors governing these areas (e.g., hydrodynamics, tides, salinity and temperature gradients) (Ghilardi et al., 2008), sewage discharges constitute a common source of disturbances (Fraschetti et al., 2006; Pearson and Rosenberg, 1978). Indeed, wastewater treatment plant discharges are still considered as the most-effective way to get rid of sewages owing to the dilution rate of the ocean (Elías et al., 2005). Sewages may come from agricultural, industrial, domestic and municipal activities (Islam and Tanaka, 2004; Little and Kitching, 1996) and after physical, chemical and biological treatments the effluents are released via outfalls onto coastal areas (e.g. intertidal and shallow subtidal habitats) or offshore at deeper waters (Becherucci et al., 2018; Cabral-Oliveira and Pardal, 2016; Koop and Hutchings, 1996). Several pressures are associated to wastewater discharges such as organic and nutrient enrichment, water turbidity, increased sedimentation, decreased salinity and contamination (by heavy metals, priority and emerging organic pollutants, faecal sterols and bacteria) (Azzurro et al., 2010; Costanzo et al., 2001; Millennium Ecosystem Assessment-MEA, 2005; Moon et al., 2008; Terlizzi et al., 2005).

Depending on their type, source and level, effluents may have direct or indirect effects (biological, chemical or physical) on the environment (Borja et al., 2011a; Del-Pilar-Ruso et al., 2010) which may vary from little or no impact to major changes (Pastorok and Bilyard, 1985). Under pollution stress, irreversible negative effects are produced such as the alteration of benthic composition and abundance patterns (Guidetti et al., 2003; Nicolodi et al., 2009; Terlizzi et al., 2005, 2002). The consequences may be diverse such as a biotic homogenization with a simplification of community structure (Amaral et al., 2018) through a decline in diversity (Borowitzka, 1972; Díez et al., 2010, 1999; Littler and Murray, 1975) and those pollution-sensitive species (e.g. perennial algae) (Scherner et al., 2013). In counterpart, pollution/stress-tolerant opportunistic species (i.e., ephemeral algae) proliferate due to their higher growth and reproductive rates in nutrient-enriched water bodies as well as lower competition for space and nutrients (Amaral et al., 2018; Cabral-Oliveira and Pardal, 2016; Dauer and Conner, 1980; Elías et al., 2006; Gorostiaga and Diez, 1996). Finally, a shift from algal-dominated assemblages to invertebrate-dominated assemblages may occur (e.g. crustacean and bivalve filter-feeders) (Díez et al., 2012a; López-Gappa et al., 1993; Pinedo et al., 2007). Therefore, different responses may be observed depending on the type of analysis used and the response variables considered (Fraschetti et al., 2006).

In this regard, the European Urban Waste Water Treatment Directive (91/271/EEC) was adopted to protect the water environment from harmful effects of wastewater discharges (urban and industrial). This Directive was the prelude to the further development of the Water Framework Directive (WFD; 2000/60/EC; EC, 2000) which aims to attain "Good Ecological Status" of all water bodies by 2020 based on both physicochemical and biological elements (Borja et al., 2009). This obliges politicians and regional water authorities to make additional efforts to increase connections between a given population and wastewater systems and to improve the efficiency of sewage treatment plants. Monitoring networks have also to be established by scientists to understand benthic communities' response distinguishing changes from natural variability (Veríssimo et al., 2013) and environmental managers may evaluate the effectiveness of the implemented measures.

The study of benthic assemblages (i.e., macroalgae and invertebrates) is considered as a powerful tool to assess environmental quality and has become of major importance by providing accurate information on deleterious effects of contaminants especially in assessing local effects (Belan, 2003; Borja et al., 2011a). Their study has several advantages: benthic organisms are mainly sedentary and long-lived, sensitive to stress, play a critical role in cycling nutrients and materials, reflect both previous and present conditions to which communities have been exposed (Reish, 1987), are easy to sample even without using destructive sampling methods (Roberts et al., 1994) and have already been studied worldwide (Ar Gall and Le Duff, 2014; Becherucci et al., 2018; Borja and Dauer, 2008; Castric-Fey, 2001; de-la-Ossa-Carretero et al., 2016; Derrien-Courtel, 2008, 2010; Derrien-Courtel et al., 2013; Díez et al., 2012a; Le Gal and Derrien-Courtel, 2015; Zubikarai et al., 2014).

In this context and over the last decades, effects of sewage discharges have been studied on different environmental compartments (e.g. sediments, water body, trophic web, benthic and pelagic communities) (Bothner et al., 2002; Echavarri-Erasun et al., 2007; Mearns et al., 2015) and their impact on benthic communities have been widely documented in the intertidal zone (e.g. Becherucci et al., 2016; Bishop et al., 2002; Cabral-Oliveira et al., 2014; Cabral-Oliveira and Pardal, 2016; Díez et al., 2013; Guinda et al., 2014; Huguenin et al., 2019; Liu et al., 2007; O'Connor, 2013; Vinagre et al., 2016b). Some studies also described their impact on subtidal rocky and soft bottoms but they were often achieved either on macroalgae or macrofauna assemblages (de-la-Ossa-Carretero et al., 2016; Díez et al., 2014; Elías et al., 2005; Fraschetti et al., 2006; Souza et al., 2016, 2013; Stark et al., 2016) but rarely together especially in rocky habitats (Terlizzi et al., 2002; Underwood, 1996; Vinagre et al., 2016a; Zubikarai et al., 2014).

The French rocky Basque coast is part of the water body named “FRFC11 – Basque coast” in compliance with the WFD. Since 2008, this water body has been classified as being in a “Good Ecological Status”, considering especially macroalgae as biological quality element in the intertidal and subtidal zones (http://envlit.ifremer.fr). But, over the last decades, this area has been subjected to urban sprawl and massive summer overcrowding (Cearreta et al., 2004; Chust et al., 2009; Le Treut, 2013a) which explains the large number of Wastewater Treatment Plant (WWTP) outfalls along the coast. At the French Basque coast, no study has been performed to assess effects of shallow WWTP discharges on rocky benthic communities assemblages in contrast to the Spanish area where another study has already been achieved (Díez et al., 2014). Therefore, this study aims to offer a broader and integrated view on the potential impact of these discharges on subtidal rocky benthic assemblages (composition, abundance and functional traits of macroalgae and macrofauna) in the southeastern Bay of Biscay. This was achieved by comparing control and impacted locations (in the immediate vicinity of the outfall) using the same sampling strategy at two different habitats defined in the Directive for the French Basque coast. Furthermore, the general hypothesis is that if WWTP treatments are efficient, structural parameters of communities and ecological quality indices between impacted and control locations should be similar.

2. Materials and Methods

2.1 Study area

The study was conducted in the southeastern Bay of Biscay. The rocky Basque coast, considered as marine protected area in compliance with the OSPAR convention (Natura 2000 site of Council Directive 92/43/EEC on the Conservation of natural habitats and wild fauna and flora named “FR7200813 - Rocky Basque coast and offshore extension”), is part of the “Basque coast” water body (FRFC11) according to the WFD classification. Within the “Bay of Biscay” marine subregion, the southern part displays a set of environmental specificities: mesotidal conditions, with a magnitude between 1.85 and 3.85 m (Augris et al., 2009), energetic waves (Abadie et al., 2005b), freshwater inputs caused by rainfall and a dense river system (Winckel et al., 2004), N-NW dominant winds**,** a specific coast orientation and heterogeneous geomorphology (cliffs, rocky platforms, boulder fields and semi-enclosed bays with sandy beaches) (Borja and Collins, 2004). In the eastern Basque coast (French side), around 30% of the shore is constituted by rocky substrata (Chust et al., 2009). All those parameters make this region a heritage area (Augris et al., 2009; de Casamajor and Lalanne, 2016) which is justified by the presence of specific communities in these remarkable habitats (Borja et al., 2004).

2.2 Field data collection strategy

The field sampling campaign was carried out in spring 2017 on subtidal rocky platforms in the French Basque coast. Four locations were selected along a coastal stretch of 11 km (**Fig. 2**) according a control-impact design: one considered as impacted by a shallow Wastewater Treatment Plant effluent (**I**) and three coastal controls (**C1-**Abbadia, **C2-**Socoa, **C3-**Alçyons) representing reference natural conditions (under very low anthropogenic pressures, i.e. far from main source of disturbances) and having similar features selected by expert judgment (e.g. comparable wave exposure, depth and substratum). The three controls constitute those monitored regularly within the context of applying the WFD and are those supposed be representative of the whole "Basque coast" water body (Derrien-Courtel and Le Gal, 2014a, 2014b; data are available in the Quadrige[2] database http://envlit.ifremer.fr). The impacted and control locations were sampled in two algal belts (the upper infralittoral: **I**U_p, **C1**U_p, **C2**U_p, **C3**U_p and the circalittoral: **I**C_i, **C1**C_i, **C2**C_i, **C3**C_i) defined within the WFD (**Table 1**) (Le Gal and Derrien-Courtel, 2015).

The impacted location (**I**) was placed in the proximity of the outfall of WWTP, carrying out this plant physical-chemical and biological treatment for a population equivalent of 40.000 inhabitants (**Table 2**). According to the assessment system of Le Gal and Derrien-Courtel (2015), the level pressure of the WWTP effluent was estimated as high based on the type of pollution (i.e. urban), the distance from the source of pollution (i.e. <50m), the magnitude of pollution (i.e. from 10 000 to 150 000 inhabitant equivalent) and the water mixing (i.e. high water mixing).

Table 1: Algal belts definition within the WFD for the study area (de Casamajor and Lissardy, 2018).

Stage	Algal belt	Definition	WFD code
Infralittoral	Upper Infralittoral	*Cystoseira* spp. ≥ 3 ind.m²	N2
Circalittoral	Upper Circalittoral	Absence of structuring macroalgae (*Cystoseira* spp.) Presence of erected algae	N4

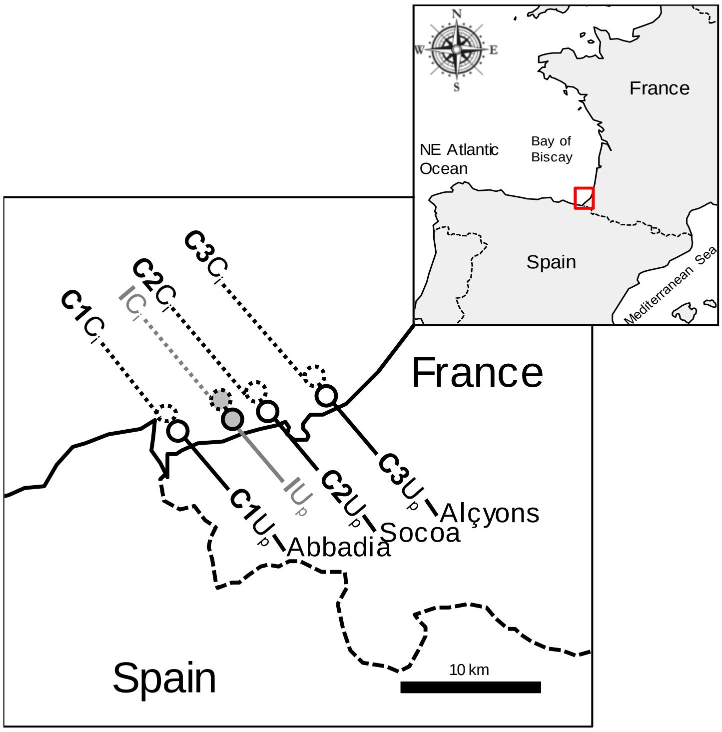

Fig. 2: Study area and locations: controls in the upper infralittoral C1U$_p$, C2U$_p$, C3U$_p$ (white points), controls in the circalittoral C1C$_i$, C2C$_i$, C3C$_i$ (white dotted points), the impacted location in the upper infralittoral 'IU$_p$' (grey point) and in the circalittoral 'IC$_i$' (grey dotted point). C1, C2 and C3 corresponded to WFD locations: Abbadia, Socoa and Alçyons, respectively.

Table 2: General WWTP features (www.insee.fr; SUEZ, 2018)

	'WWTP'
Racorded municipalities	2
Combined population	≈16 100
Inhabitant equivalent	40 000
Sewer system	Separated
Emissary depth (m)	3
Nominal flow (m^3/day)	7 000 (dry) / 21 600 (rainy weather)
Main treatment	Biofiltration
Pre-treatment	Fine screening, sand and grease removal
Primary treatment	Physico-chemical lamella settlers
Secondary treatment	Biological treatment (biofiltration)

Each algal belt was independently sampled by divers at 3 m and 20/30 m depth, respectively. Within each of them, a set of 10 randomly selected replicates (0.25 m^2 quadrats as those used within the WFD) were positioned on comparable substrata (stable substrate and continuous bedrock) avoiding special microhabitats (crevices) and separated by at least 1 m. The percentage cover of macroalgae and sessile macrofauna was visually estimated and the abundance of mobile or slightly mobile macrofauna was counted.

Most organisms were identified *in situ* as close as possible to the species level to limit the sampling impact. When identification was impossible in the field (especially for small species), specimens were taken to the laboratory for further identification by taxonomic specialists. Due to the complex taxonomy of certain taxa, some organisms were identified at genus level.

2.3 Data treatment and statistical analyses

The analyses of taxa (macroalgae and macrofauna) composition and abundance were conducted on aggregated data containing mixed taxonomic levels (species, genus, family, class) in order to keep all taxonomic information. Data of macroalgae species were also aggregated into morphological functional groups (MFG) as defined in Díez et al., 2010 (i.e. articulated calcareous, crustose calcareous, crustose non-calcareous, foliose non-corticated, foliose slightly corticated, polysiphonated, foliose highly corticated, terete corticated, terete slightly corticated, filamentous and leathery). According to Orfanidis et al. (2011), each macroalgae taxa was also assigned to an Ecological Status Groups based on trait combinations in relative terms of species morphology, physiology, life strategy and distribution: ESG I: late-successional or perennial to annual taxa (IA: thick perennial; IB: thick plastic; IC: shade-adapted plastic); ESG II: opportunist or annual taxa (IIA: fleshy opportunistic; IIB: filamentous or sheet-like, opportunistic).

Macrofauna species were aggregated into phylum and ecological groups according to two different classifications. One was based on five Ecological Groups (Borja et al., 2000): EG I: species very sensitive to organic enrichment and present under unpolluted conditions; EG II: species indifferent to enrichment, always present in low densities with non-significant variations with time; EG III: species tolerant to excess organic matter enrichment, occurring under normal conditions but stimulated by organic enrichment; EG IV: second-order opportunistic species; EG V: first-order opportunistic species. The second classification was based on the four Sensitivity Groups defined by Sartoretto et al. (2017) (SG I: taxa indifferent to organic matter and sediment input; SG II: opportunistic taxa; SG III: tolerant taxa; SG IV: sensitive taxa) completed by expert judgements.

Each algal belt (the upper infralittoral and the circalittoral) and biological element (macroalgae and macrofauna) were studied separately. The variation on taxa composition and abundance (community structure) between impacted and control locations was studied by means of PERMANOVA analysis (Permutational multivariate analysis of variance; with 9999 permutations) (Anderson et al., 2008) with an *a priori* chosen significant level of $\alpha = 0.05$. Two factors were considered: (i) Control *vs.* Impacted (CsI: 2 levels, fixed) and (ii) Location (L: 4 levels, random and nested in CsI) with n= 10. This analysis was based on the Bray-Curtis similarity matrix calculated from untransformed data. Dendrograms showing hierarchical clustering of locations were achieved on benthic assemblages, on morpho-functional and ecological groups and on phylum to visualize differences between impacted and control locations. The SIMilarity PERcentage (SIMPER) analysis was used to identify taxa that contribute most to differences between locations (Impacted *vs*. Controls) (Clarke, 1993; Oksanen et al., 2013). Taxa with a contribution higher than 1% were presented in results.

The graphs and statistical analyses were undertaken using Excel v7®, the PRIMER V. 6. PERMANOVA package (Anderson et al., 2008; Clarke and Gorley, 2006) and R® software.

2.4 Ecological quality

2.4.1 Ecological quality based on macroalgae species

In order to study the ecological quality of studied locations based on macroalgae species, the Ecological Quality Status (EQS) for the upper infralittoral was assigned to each control location (**C1**U$_p$, **C2**U$_p$, **C3**U$_p$) and the impacted location (**I**U$_p$) (**Table 3**). This was achieved according to Le Gal and Derrien-Courtel (2015) and de Casamajor and Lissardy (2018) who adapted the CFR index (Guinda et al., 2014) for the Basque coast. The EQS was assigned after the calculation of a Quality Index (QI) based on four metrics assigned to a score whose the sum was converted to 100 points (see details in de Casamajor and Lissardy, 2018 and Le Gal and Derrien-Courtel, 2015): **Metric 1** (30 point grading scale) corresponding to the depth extension (in metres) of both algal belts (upper infra- and upper circalittoral) based on the presence/absence and abundance of structuring algae (*Cystoseira* spp.) (**Table 1**), **Metric 2** (20 point grading scale) corresponding to the density of structuring species (i.e. *Cystoseira* spp.), **Metric 3** divided in three sub-notes reduced to 20 point grading scale, **Metric 3a** (20 point grading scale) corresponding to the number of characteristic species, **Metric 3b** (20 point grading scale) corresponding to the density of opportunistic species, **Metric 3c** (1 bonus point) if sensitive perennial macroalgae (*Gelidium corneum* and *Padina pavonica*) are present in the entire surveyed site and **Metric 4** (20 point grading scale) corresponding to the total number of taxa. Then, the QI of each location was divided by a reference QI (i.e. 74.8 defined using 3 reference sites according to Le Gal and Derrien-Courtel, 2015) which provided the Ecological Quality Ratio (EQR), finally associated to one of the EQS classes (**Table 3**).

Table 3: Ecological Quality Ratio (EQR) associated to Ecological Quality Status class (EQS) (de Casamajor and Lissardy, 2018; Le Gal and Derrien-Courtel, 2015).

EQR	EQS class
[0.85-1]	High
[0.65-0.85]	Goog
[0.45-0.65]	Moderate
[0.25-0.45]	Poor
<0.25	Bad

2.4.2 Ecological quality based on macrofauna species

No index is validated by European Directives for the assignment of the rocky shore ecological quality based on macrofauna. Thus, two metrics from existing indices were calculated: (i) the Biotic Coefficient (BC) from the AMBI index designed to establish the ecological quality of soft-bottom benthos within European estuarine and coastal environments (Borja et al., 2000) and (ii) the Taxa Sensitivity (TS) from the INDEX-COR index designed to evaluate and monitor the conservation state of coralligenous assemblages along the French Mediterranean coast (Sartoretto et al., 2017). Their computations are both achieved using a formula based on macrofauna species grouped according their level of sensitivity to organic matter and sediment input (EG and SG respectively; **Supplementary material 1**).

3. Results

A total of 74 macroalgae (48 Rhodophyta, 19 Ochrophyta and 7 Chlorophyta) and 89 macrofauna taxa were identified (**Table 4**). The whole species list with their assignments (i.e. phylum, MFG, ESG, EG and SG) according to their subtidal location is available in **Supplementary material 1**.

Benthic communities' response differed according to biological elements (macroalgae or macrofauna) and algal belts (the upper infralittoral and the circalittoral).

Table 4: Total taxonomic richness and taxonomic richness per Ecological groups (ESG for macroalgae and EG for macrofauna), depth boundary of the upper infralittoral zone and results of ecological quality indices calculated for both macroalgae and macrofauna per locations (impacted 'I' and controls 'C') and algal belts (the upper infralittoral zone, U.I. and the circalittoral zone, Ci.).

		I		C1		C2		C3	
		IU_p	IC_i	$C1U_p$	$C1C_i$	$C2U_p$	$C2C_i$	$C3U_p$	$C3C_i$
				Macroalgae					
Taxonomic richness	**74**	**26**	**23**	**38**	**19**	**35**	**20**	**38**	**24**
Number of species classified as ESG I	**17**	**8**	**3**	**14**	**5**	**10**	**4**	**13**	**4**
Number of species classified as ESG II	**39**	**18**	**19**	**21**	**13**	**22**	**14**	**22**	**16**
Number of characteristic species		**16**	**-**	**21**	**-**	**20**	**-**	**19**	**-**
Depth boundary (m)		**5**	**-**	**22.2**	**-**	**19**	**-**	**15.5**	**-**
Ecological Quality Ratio (EQR)		**0.27**	**-**	**0.7**	**-**	**0.77**	**-**	**0.83**	**-**
Ecological Quality Status class (EQS)		**Poor**	**-**	**Good**	**-**	**Good**	**-**	**Good**	**-**
				Macrofauna					
Taxonomic richness	**89**	**10**	**22**	**10**	**21**	**9**	**13**	**4**	**14**
Number of species classified as EG I	**18**	**4**	**8**	**3**	**6**	**4**	**4**	**2**	**7**
Number of species classified as EG II	**10**	**4**	**4**	**2**	**3**	**3**	**2**	**1**	**4**
Number of species classified as EG III	**2**	**0**	**1**	**0**	**2**	**0**	**0**	**1**	**1**
Biotic coefficient (BC)		**0.65**	**0.83**	**1.41**	**0.95**	**1**	**0.43**	**0.88**	**0.97**
Pollution classification from BC		**Unpolluted**	**Unpolluted**	**Slightly polluted**	**Unpolluted**	**Unpolluted**	**Unpolluted**	**Unpolluted**	**Unpolluted**
Benthic community health from BC		**Impoverished**	**Impoverished**	**Unbalanced**	**Impoverished**	**Impoverished**	**Impoverished**	**Impoverished**	**Impoverished**
Taxa sensitivity (TS)		**0.47**	**0.65**	**0.37**	**0.51**	**0.34**	**0.81**	**0.29**	**0.49**

3.1 Effects of WWTP discharge on macroalgae taxa composition and abundance

In the upper infralittoral and circalittoral zones, the analyses did not showed significant differences between the impacted location and controls according to macroalgae assemblages, MFG and ESG (PERMANOVA, p>0.05; **Table 5**; cluster analyses in **Supplementary materials 2** and **3**).

In the upper infralittoral, among taxa identified as significant contributors to the dissimilarity, only *Gelidium corneum* had a contribution higher than 10% (20.84%). It was described as more abundant in the impacted location (**Table 6**). *Mesophyllum lichenoides* (Ct (%) < 10) was absent from controls ($C1U_p$, $C2U_p$, $C3U_p$). *Corallina* spp., *Plocamium cartilagineum* and *Nithophyllum punctatum* present in both locations, were significantly more abundant in the impacted one. Among other macroalgae taxa (*p-value* > 0.05; Ct % >1), some were less abundant (*Lithophyllum incrustans* and *Jania rubens*) or absent (*Cystoseira baccata, Halopithys incurva and Halopteris scoparia*) from the impacted location compared to controls. By contrast, *Gymnogongrus griffithsiae* was identified as more abundant in the impacted location. In addition, two MFG and one ESG were identified as significant contributors to this dissimilarity with higher abundances in the impacted location: terete corticated (Ct % > 10), foliose heavily corticated (Ct % < 10) and ESG IIA (Ct % > 10), respectively (**Supplementary materials 4**).

In the circalittoral, *Metacallophyllis laciniata* was the main significant contributor (30.73%) with a higher abundance in the impacted location (60.90 vs. 6.43) (**Table 6**). Remaining significant contributors (i.e. *Drachiella spectabilis*, *Pterosiphonia complanata*, *Calliblepharis ciliata* and *Halymenia latifolia*) were also all more abundant in the impacted location than in controls. Among other macroalgae taxa (*p-value* > 0.05; Ct % >1), *Heterosiphonia plumosa*, *Rhodymenia pseudopalmata*, *Dictyopteris polypodioides, Halopteris filicina* and *Lithophyllum incrustans*, were much more abundant in controls than in the impacted location. By contrast, the reverse occurred for *Phyllophora crispa.* Finally, two MFG (foliose heavily corticated, Ct % > 10 and foliose non corticated, Ct % < 10) were identified as significant contributors to the dissimilarity with higher abundances in the impacted location while no ESG was highlighted in this algal belt (**Supplementary materials 4**).

Table 5: Summary of PERMANOVA results computed on macroalgae assemblages (a, d), morpho-functional groups (MFG) (b, e) and Ecological Status Groups (ESG) (c, f) in the upper infralittoral (a, b, c) and circalittoral zones (d, e, f).

Upper infralittoral

Assemblages

(a)

Source	Df	SS	MeanSqs	Pseudo-F	P-value
CsI	1	13402	13402	1.40	0.25
Location(CsI)	2	19183	9591.5	4.49	0.0001
Residuals	36	76922	2136.7		
Total	39	1.10E+05			

MFG

(b)

Source	Df	SS	MeanSqs	Pseudo-F	P-value
CsI	1	4917.8	4917.8	1.15	0.25
Location(CsI)	2	8531	4265.5	3.26	0.001
Residuals	36	47166	1310.2		
Total	39	60615			

ESG

(c)

Source	Df	SS	MeanSqs	Pseudo-F	P-value
CsI	1	5068.5	5068.5	1.51	0.50
Location(CsI)	2	6700	3350	3.93	0.0001
Residuals	36	30722	853.4		
Total	39	42491			

Circalittoral

(d)

Source	Df	SS	MeanSqs	Pseudo-F	P-value
CsI	1	19960	19960	2.57	0.24
Location(CsI)	2	15543	7771.7	3.84	0.0001
Residuals	36	72848	2023.5		
Total	39	1.08E+05			

(e)

Source	Df	SS	MeanSqs	Pseudo-F	P-value
CsI	1	12116	12116	3.03	0.25
Location(CsI)	2	7993.5	3996.7	2.66	0.005
Residuals	36	54081	1502.3		
Total	39	74191			

(f)

Source	Df	SS	MeanSqs	Pseudo-F	P-value
CsI	1	2045.2	2045.2	0.44	1.00
Location(CsI)	2	9351.2	4675.6	4.55	0.0004
Residuals	36	37003	1027.9		
Total	39	48400			

Table 6: Taxa identified by SIMPER analyses as contributors (Ct > 1%) to the dissimilarity between impacted and control locations computed on macroalgae assemblages in the upper infralittoral (a) and circalittoral zones (b).

(a)

IU$_p$ vs. Controls

	78.28%	av 'IU$_p$'	av 'Controls'	Ct (%)	Cum. Ct (%)	P-value	Significance
Upper infralittoral zone	*Gelidium corneum*	47.30	8.70	20.84	20.84	0.001	***
	Pterosiphonia complanata	14.40	12.33	8.53	29.37	0.260	
	Corallina spp.	19.40	7.13	8.36	37.73	0.027	*
	Lithophyllum incrustans	11.40	19.17	8.24	45.97	0.972	
	Encrusting brown algae	0.00	14.33	6.07	52.04	0.990	
	Plocamium cartilagineum	12.70	3.80	5.13	57.17	0.003	**
	Jania rubens	0.60	10.00	4.48	61.65	0.976	
	Ulva spp.	3.70	6.53	3.48	65.13	0.945	
	Acrosorium ciliolatum	2.50	5.40	2.91	68.04	0.934	
	Mesophyllum lichenoides	5.70	0.00	2.72	70.76	0.001	***
	Gymnogongrus griffithsiae	5.10	0.50	2.67	73.43	0.191	
	Drachiella spectabilis	4.70	2.83	2.62	76.05	0.243	
	Cystoseira baccata	0.00	4.87	2.47	78.52	0.965	
	Halopithys incurva	0.00	4.70	2.29	80.81	0.984	
	Rhodymenia pseudopalmata	3.50	1.53	2.21	83.02	0.083	.
	Halurus equisetifolius	1.80	3.67	1.86	84.88	0.964	
	Xiphosiphonia pennata	1.60	1.33	1.26	86.14	0.481	
	Halopteris scoparia	0.00	2.30	1.21	87.35	0.988	
	Ceramium spp.	1.40	1.60	1.19	88.54	0.578	
	Codium spp.	1.20	1.03	1.08	89.62	0.429	
	Nithophyllum punctatum	2.30	0.07	1.04	90.66	0.001	***
	Dictyota dichotoma	1.50	1.03	1.04	91.70	0.468	

(b)

IC$_i$ vs. Controls

	78.31%	**av 'IC$_i$'**	**av 'Controls'**	**Ct (%)**	**Cum. Ct (%)**	**P-value**	**Significance**
Circalittoral zone	*Metacallophyllis laciniata*	60.90	6.43	30.73	30.73	0.001	***
	Heterosiphonia plumosa	3.90	20.83	9.37	40.10	0.979	
	Drachiella spectabilis	15.40	4.93	7.61	47.71	0.015	*
	Rhodymenia pseudopalmata	8.40	12.77	6.85	54.56	0.996	
	Pterosiphonia complanata	12.40	2.97	6.57	61.13	0.020	*
	Dictyopteris polypodioides	1.60	9.83	4.94	66.07	1.000	
	Dictyota dichotoma	5.30	6.53	4.58	70.65	0.877	
	Peyssonnelia sp.	5.80	8.10	4.26	74.91	1.000	
	Halopteris filicina	0.50	8.13	3.93	78.84	1.000	
	Phyllophora crispa	7.10	2.47	3.82	82.66	0.095	.
	Acrosorium ciliolatum	3.00	5.60	3.47	86.13	0.998	
	Lithophyllum incrustans	2.60	6.73	3.46	89.59	1.000	
	Calliblepharis ciliata	4.20	1.57	2.55	92.14	0.039	*
	Halymenia latifolia	2.10	0.03	1.14	93.28	0.001	***
	Carpomitra costata	0.00	1.70	1.01	94.29	0.997	

3.2 Effects of WWTP discharge on macrofauna taxa composition and abundance

As for macroalgae taxa, no significant difference was observed using macrofauna assemblages, phylum and ecological distinction between impacted and control locations in both algal belts (PERMANOVA, $p>0.05$; **Table 7**). This was supported also by dendrograms showing the impacted location in a group including controls whatever the algal belt (**Supplementary materials 5** and **6**).

Based on SIMPER analysis, global dissimilarities between impacted and control locations were again quite similar between both algal belts (96.32% vs. 94.42%) but they were higher than those for macroalgae (**Table 8**). All significant contributors highlighted in this analysis were also always more abundant in the impacted location.

In the upper infralittoral, three taxa were highlighted as significant contributors: two with high contribution (i.e. *Botryllus schlosseri* and *Tritia reticulata* with 18.79% and 15.52%, respectively) and one with low contribution (i.e. Actiniaria with 5.88%) (**Table 8**). *Botryllus schlosseri* and Actiniaria were also absent from controls. All other macrofauna taxa (*p-value* > 0.05; Ct % >1), presented very low average abundances in both types of location.

In the circalittoral, more significant contributors were highlighted for dissimilarities (i.e. *Obelia* sp., Crustose bryozoa, *Caryophyllia smithii*, *Tritia incrassata* and *Echinaster sepositus*) although with minor contributions (Ct % < 10) (**Table 8**). Only *Obelia* sp. was absent from controls. As in the upper infralittoral, all other macrofauna taxa (*p-value* > 0.05; Ct % >1), presented very low average abundances in both types of location.

Table 7: Summary of PERMANOVA results computed on macrofauna assemblages (a, e), phylum (b, f) and Ecological Groups (EG) according to Borja et al. (2000) (c, g) and Sensitivity Groups (SG) according to Sartoretto et al. (2017) (d, h) in the upper infralittoral (a, b, c, d) and circalittoral zones (e, f, g, h).

Upper infralittoral

Assemblages

(a)

Source	Df	SS	MeanSqs	Pseudo-F	P-value
CsI	1	8691.8	8691.8	0.82	0.50
Location(CsI)	2	21297	10649	2.73	0.001
Residuals	36	1.40E+05	3898.4		
Total	39	1.70E+05			

Phylum

(b)

Source	Df	SS	MeanSqs	Pseudo-F	P-value
CsI	1	3717	3717	0.71	0.75
Location(CsI)	2	10424	5212.1	1.58	0.12
Residuals	36	1.18E+05	3290.6		
Total	39	1.33E+05			

EG

(c)

Source	Df	SS	MeanSqs	Pseudo-F	P-value
CsI	1	1139.9	1139.9	0.23	1.00
Location(CsI)	2	10009	5004.7	1.55	0.16
Residuals	36	1.16E+05	3222		
Total	39	1.27E+05			

SG

(d)

Source	Df	SS	MeanSqs	Pseudo-F	P-value
CsI	1	11249	11249	1.69	0.50
Location(CsI)	2	13280	6640	2.16	0.05
Residuals	36	1.11E+05	3071		
Total	39	1.35E+05			

Circalittoral

(e)

Source	Df	SS	MeanSqs	Pseudo-F	P-value
CsI	1	10524	10524	0.85254	0.6745
CsI(Location)	4	49378	12344	3.4216	0.0001
Residuals	54	1.95E+05	3607.8		
Total	59	2.55E+05			

(f)

Source	Df	SS	MeanSqs	Pseudo-F	P-value
CsI	1	6844	6844	0.62387	0.8325
CsI(Location)	4	43881	10970	4.5989	0.0001
Residuals	54	1.29E+05	2385.4		
Total	59	1.80E+05			

(g)

Source	Df	SS	MeanSqs	Pseudo-F	P-value
CsI	1	6926.8	6926.8	0.66	0.74
Location(CsI)	2	21124	10562	4.11	0.0003
Residuals	36	92562	2571.2		
Total	39	1.21E+05			

(h)

Source	Df	SS	MeanSqs	Pseudo-F	P-value
CsI	1	4204.3	4204.3	0.41	0.75
Location(CsI)	2	20514	10257	4.04	0.0002
Residuals	36	91405	2539		
Total	39	1.16E+05			

Table 8: Taxa identified by SIMPER analyses as contributors (Ct >1%) to the dissimilarity between impacted and control locations computed on macrofauna assemblages in the upper infralittoral (a) and circalittoral zones (d).

(a)

Upper infralittoral zone

IU$_p$ vs. Controls

96.32%	av 'IU$_p$'	av 'Controls'	Ct (%)	Cum. Ct (%)	P-value	Significance
Botryllus schlosseri	2.43	0.00	18.79	18.79	0.023	*
Tritia reticulata	1.29	0.29	15.52	34.31	0.049	*
Cerithium spp.	0.14	2.54	15.24	49.55	0.999	
Tritia incrassata	0.00	0.54	7.83	57.38	0.934	
Gibbula ardens	0.29	0.21	5.99	63.37	0.252	
Actiniaria	0.29	0.00	5.88	69.25	0.004	**
Calliostoma zizyphinum	0.29	0.08	4.39	73.64	0.236	
Spirobranchus spp.	0.71	0.00	4.11	77.75	0.090	.
Aglaophenia sp.	0.57	0.00	3.28	81.03	0.090	.
Sycon sp.	0.14	0.04	3.14	84.17	0.059	.
Paracentrotus lividus	0.00	0.38	2.75	86.92	0.952	
Anemonia viridis	0.00	0.17	2.28	89.20	0.921	
Demospongiae	0.00	0.21	2.28	91.48	0.972	
Actinothoe sphyrodeta	0.29	0.00	1.65	93.13	0.090	.
Holothuria tubulosa	0.00	0.08	1.31	94.44	0.894	
Rocellaria dubia	0.00	0.13	1.15	95.59	0.916	

(b)

Circalittoral zone

IC$_i$ vs. Controls

94.42%	av 'IC$_i$'	av 'Controls'	Ct (%)	Cum. Ct (%)	P-value	Significance
Sycon sp.	0.10	1.67	9.01	9.01	0.819	
Obelia sp.	1.30	0.00	8.27	17.28	0.013	*
Sertularella spp.	0.00	3.70	7.82	25.09	0.678	
Crustose bryozoa	0.60	0.30	6.44	31.53	0.035	*
Hydrozoa	0.00	1.19	5.55	37.08	0.897	
Polychaeta	0.10	1.48	5.38	42.45	0.622	
Rocellaria dubia	0.40	0.52	5.04	47.49	0.164	
Serpulidae	0.60	0.19	4.49	51.98	0.253	
Aglaophenia sp.	0.30	0.63	4.11	56.10	0.477	
Corynactis viridis	0.00	1.00	3.94	60.04	0.847	
Caryophyllia smithii	0.40	0.04	3.47	63.50	0.046	*
Sabella discifera	0.40	0.00	3.10	66.60	0.060	.
Tritia incrassata	0.30	0.04	2.79	69.39	0.039	*
Cliona celata	0.20	0.37	2.49	71.88	0.290	
Gymnangium montagui	0.00	0.19	2.44	74.31	0.725	
Crutose Ascidiacea	0.00	0.37	2.29	76.60	0.817	
Cerithium spp.	0.00	0.26	2.10	78.70	0.596	
Phoronis	0.00	1.11	1.99	80.70	0.331	
Echinaster sepositus	0.20	0.04	1.65	82.34	0.041	*
Parazoanthus axinellae	0.20	0.00	1.49	83.83	0.199	
Demospongiae	0.10	0.22	1.39	85.21	0.423	
Halichondria sp.	0.20	0.04	1.37	86.59	0.053	.
Eunicella verrucosa	0.20	0.00	1.26	87.85	0.221	
Aplysina spp.	0.10	0.04	1.21	89.06	0.362	
Holothuria tubulosa	0.10	0.07	1.19	90.26	0.374	

3.3 Ecological quality

In the upper infralittoral, response of indices differed from both biological elements (**Table 4**). Based on macroalgae species, all control locations were ranked as “*Good*” contrary to the impacted location which was ranked as “*Poor*”. Metrics mainly responsible for this rating were the depth boundary of the algal belt (Metric 1) (between 15.5 to 22.2 m depth for controls *vs.* 5 m depth for the impacted location), the number of characteristic species (Metric 3a) (between 19 to 21 in controls *vs.* 16 in the impacted location) and the total number of taxa (Metric 4) (between 35 to 38 in controls *vs.* 26 in the impacted location) (**Table 4; Fig. 3**). To a lesser extent, the density of structuring species (i.e. *Cystoseira* spp.) (Metric 2) and the density of opportunistic species (Metric 3b) influenced also this rating.

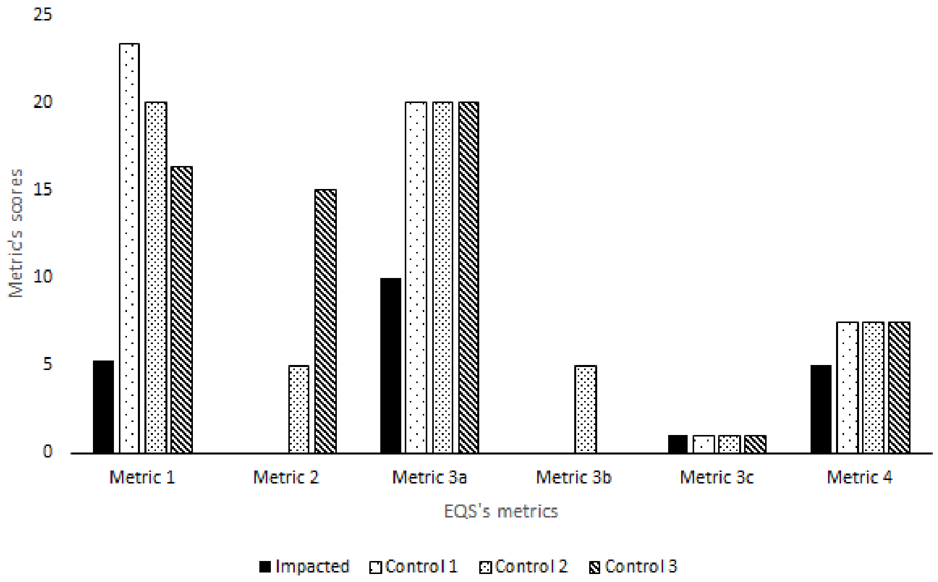

Fig. 3: Metric’s scores used for the calculation of the Quality Index (QI) and the assignment of the Ecological Quality Status (EQS) for each studied locations (impacted and controls). Metric 1: depth boundary of the algal belt, Metric 2: density of structuring species, Metric 3a: number of characteristic species, Metric 3b: density of opportunistic species, Metric 3c: sensitive perennial macroalgae and Metric 4: total number of taxa.

By contrast, the two metrics based on macrofauna species assigned better scores to the impacted location than to controls. According to the BC, the lowest value was found in the impacted location, which classes it as unpolluted location with impoverished community health. All controls presented higher values but were also assigned to the same status except ‘Control 1’ considered as a slightly polluted location with an unbalanced community health. The TS value was higher in the impacted location which highlighted also a higher sensitivity.

In the circalittoral, only macrofauna indices could be calculated. According to the BC, all locations (impacted and controls) were assigned to unpolluted location with impoverished community health. The TS values did not discriminate the impacted location from controls which presented variable values in comparison with the impacted one.

4. **Discussion**

The present study aimed to assess potential effects of a WWTP outfall on rocky benthic subtidal assemblages by comparing communities near the outfall with those from locations considered as "Control" and as representative of the "Basque coast" water body. Both biological elements (macroalgae and macrofauna) were considered to fulfill European Directives requirements (WFD and MSFD) playing a key role in water quality for the conservation status and functional aspects of the environment (de Casamajor et al., 2016).

Using multivariate analyses, no significant difference in the composition and abundances of macroalgae assemblages, morpho-functional groups (MFG) and ecological status groups (ESG) was highlighted between the impacted location and controls for both algal belts (the upper infralittoral and circalittoral zones). The same occurred with macrofauna assemblages, phylum and ecological groups (EG and SG). Therefore, using these parameters independently, no significant impact of WWTP on benthic organisms could be highlighted. Furthermore, significant differences among control locations highlighted strong natural variability along the studied area which could explain no significant differences detected between control and impacted locations. This goes against another work aiming to study the impact of a such pressure in the intertidal zone (Huguenin et al., 2019). This is probably due to a higher dilution factor in subtidal area. In the present study, macroalgae ESG were established according to Orfanidis et al. (2011) and expert judgement. This classification was chosen because it is widely used in other works and is one of the most precise classifications. It is important to note that some authors identified different sensitivity (i.e. ESG ranks) for a same species (Ar Gall and Le Duff, 2014; Gaspar et al., 2012; Neto et al., 2012; Vinagre et al., 2016a).

When detecting pollution impacts in the marine environment, the study of benthic communities provides several advantages, among which the bioindicator nature of some species (opportunists vs. sensitives) should be highlighted (Díez et al., 2009). Based on macroalgae from the upper infralittoral zone, only *Gelidium corneum* (formerly *G. sesquipedale*), a terete corticated Rhodophyta, was identified as significant high contributors (Ct % > 10) to the dissimilarity between the impacted location and controls, with a higher abundance in the impacted one. This macrophyte, together with *Cystoseira baccata,* are the main canopy-forming algae at the Basque coast (de Casamajor and Lissardy, 2018; Gorostiaga et al., 2004b) which is subjected to strong hydrodynamics. Indeed, the Basque coast is exposed to the most energetic waves (Abadie et al., 2005b) and the tolerance of *G. corneum* to wave action was demonstrated by Díez et al. (2003) who showed an increase of its abundance with wave action. This contrasts with colder northern French regions where kelp forests (essentially *Laminaria digitata* and *L. hyperborea*) are dominant in the subtidal zone (Ar Gall et al., 2016; Le Gal and Derrien-Courtel, 2015; Ramos et al., 2014). Under the implementation of the WFD, *Gelidium corneum* is thus used to assess the ecological status of the whole water body, being considered it as an indicator of Good Ecological Status (de Casamajor and Lissardy, 2018). Indeed, *Gelidium corneum* was already well described as sensitive to environmental disturbances such as increased sedimentation (Díez et al., 2003), irradiance (Quintano et al., 2019), sea surface temperature (Díez et al., 2012b; Muguerza et al., 2017) and reduced nutrient availability (Díez et al., 2012b). Even if *Gelidium corneum* and *Cystoseira baccata* were already described as dominant species along the Cantabria coast (South of the Bay of Biscay, N Spain ; Guinda et al., 2012), they have been considered as representative of the last successional stages after pollution abatement and as the most complex ones (Gorostiaga et al., 2004a) although the brown *Cystoseria* spp. seem to be somewhat more sensitive than *Gelidium corneum* (Borja et al., 2013b; Díez et al., 2003). *Gelidium corneum* was classified as ESG I by many authors (i.e. as late-successional or perennial to annual taxa; Gaspar et al., 2017; Neto et al., 2012; Vinagre et al., 2016a), but only Orfanidis et al. (2011) classified this phylum as ESG IIA (i.e. fleshy opportunistic). Recently, the authors of the present work have found in the same study area that *Gelidium corneum* could grow in the lower intertidal both in the impacted location by a wastewater treatment plant discharge as well in the control location. In the case of the impacted location, *Gelidium corneum* was dominant, with large and vigorous fronds (dark red pigmentation). In the control location, *Gelidium corneum* shared the space with *Cystoseira tamariscifolia* (Huguenin et al., 2019). This finding may indicate that low inputs of nutrients (especially nitrogen) can promote the growth of *Gelidium corneum*. Thus, this external nitrogen supply would favor the synthesis of phycobiliproteins in *Gelidium corneum* fronds, their growth rate as well as a higher photoprotection under strong irradiance and depletion of natural nutrients in the environment (Quintano et al., 2017). A similar situation could occur in the present study, where a greater abundance of *Gelidium corneum* was recorded in the infralittoral zone of the impacted location, probably favored by the fertilization of the water column coming from the outfall. It is assumed that this fertilization would be light since no significant differences (PERMANOVA analysis) were found in the infralittoral vegetation between the impacted station and the controls. In conditions of strong-moderate pollution, *Gelidium corneum* stands disappear as it has been reported for the coastal environment close to the Bilbao metropolitan area (Gorostiaga and Diez, 1996). The higher abundance of calcareous algae (*Mesophyllum lichenoides* and *Corallina* spp.) around the outfall in this algal belt could corroborate a nutrient enrichment. Co-dominance of both species in the low intertidal zone has been reported in the vicinity of the discharge of wastewater treatment plants (Díez et al., 2013). These calcareous algae show a wide range of tolerance to pollution and thus growing in moderately polluted environments as well as in less or not impacted locations (Díez et al., 2003; Huguenin et al., 2019). *Plocamium cartilagineum* and *Nithophyllum punctatum* were slightly more abundant in the impacted location *N. punctatum*, was associated to ESG II (i.e. opportunist or annual taxa) contrary to *Corallina* spp. and *P. cartilagineum* classed in ESG I (i.e. late-successional or perennial to annual taxa) (Gaspar et al., 2012; Neto et al., 2012; Orfanidis et al., 2011). This contrast with another study which described *P. cartilagineum* as being more associated to good quality environments whereas it was absent from moderately or highly impacted locations (Díez et al., 2012a). This species was anyway described as the most common macrophytes in subtidal zone in the Basque coast (Gorostiaga et al., 2004b) and also as a typical epiphyte of *Gelidium* spp. which was identified as the highest contributor in this study with higher concentrations in the impacted location (Quintano et al., 2015). Conversely, *N. punctatum* and *Corallina* spp. were described as being rather tolerant to moderately polluted environments (Díez et al., 2003, 1999; Gorostiaga et al., 2004a). One species from the genus *Corallina* spp., was even described as becoming dominant as pollution increases certainly favored by moderate nutrient increments (Díez et al., 1999). Among other macroalgae taxa identified in the upper infralittoral zone, *Lithophyllum incrustans, Jania rubens, Cystoseira baccata, Halopithys incurva and Halopteris scoparia* were less abundant or absent from the impacted location while they were supposed to be well represented in all locations of the studied area. Indeed, they were reported as biogeographical characteristic species of the Basque coast within the WFD (de Casamajor and Lissardy, 2018) but they were also described as exhibiting higher cover values at controls or in locations with a high quality level (Díez et al., 2013, 2012a). Moreover, the sensitiveness of the genus *Cystoseira* spp. to anthropogenic pollution (e.g. urban or wastewater discharges) and to natural stresses (e.g. wave action) is already well known (Díez et al., 2003; Duarte et al., 2018; García-Fernández and Bárbara, 2016; Gros, 1978; Hoffman et al., 1988; Pinedo et al., 2007; Valdazo et al., 2017). Species from this genus (*C. baccata* and *C. tamariscifolia*) are thus currently used as indicators of good water quality within the European Directive (de Casamajor et al., 2017). Among these species, only *Halopteris scoparia* was classified as opportunistic taxa in the Mediterranean Sea by Orfanidis et al. (2011) which contrast with the Basque coast, where this species is usually considered as characteristic (de Casamajor and Lissardy, 2018). Conversely, *Gymnogongrus griffithsiae* was identified as being more abundant in the impacted location. Indeed, this species was classified as opportunistic (ESG II; Orfanidis et al., 2011) and was identified with a higher average cover in locations from moderate to bad quality levels (Díez et al., 2012a). In addition, the two MFG (i.e. terete corticated and foliose heavily corticated) identified as significant contributors were more abundant in the impacted location. This runs counter to other studies which rather identified these complex morphology algae in reference locations (Díez et al., 2014; Rubal et al., 2011; Wells et al., 2007). By contrast, the ESG IIA, also identified as high contributor in the upper infralittoral algal belt and more abundant in the impacted location, corresponds to opportunistic species which were already usually found in polluted areas. Thus, consistent with the finding that *G. corneum* has a subnitrophile character and that other tolerant/sensitive species are found with greater abundance in the impacted/controls (respectively), some impact of the effluent on the upper infralittoral vegetation could be highlighted. This could thus be in line with the pressure level estimation, considered as high (according to the assessment system of Le Gal and Derrien-Courtel (2015)) and the EQS ranking the impacted location as "*Poor*". By contrast, all controls were ranked as "*Good*". This ranking was especially due to differences between impacted and control locations in the algal belt depth boundaries (whose definition was based on the density of structuring species, i.e. *Cystoseira* spp., described as sensitive), the number of characteristic species and the total number of taxa. By contrast, the last two ones were not described as sensitive metrics to a such pressure in intertidal zone of the same biogeographic area (Huguenin et al., 2019). These results reflect the interest to use a multimetric approach, such as the WFD macroalgae indicator (Derrien-Courtel and Le Gal, 2014b), and also the importance to consider structural engineering species of the habitat (e.g. C. *baccata*) (de Casamajor et al., 2019). Indeed, long-lived species forming canopies are the first to react to early disturbances. In addition, it plays a fundamental ecological role in conservation of ecosystem (García-Fernández and Bárbara, 2016).

Neither in the macrofauna of the upper infralittoral zone, significant differences between impacted and controls were found. It was notorious that the colonial ascidian *Botryllus schlosseri* was recorded only in the impacted location. This species has been associated to SG II (opportunistic taxa) (Sartoretto et al., 2017) and also to EG I (very sensitive species) groups (Borja et al., 2000). The gastropod mollusk *Tritia reticulata* showed a slightly higher abundance in the impacted location. This species was associated to EG II group (indifferent species to enrichment) (Borja et al., 2000) whereas it was not classified by Sartoretto et al. (2017). Consistent with the results obtained on the vegetation in the present study it seems that both species of fauna presented some tolerance to organic enrichment. On the other hand, ecological quality indices (Biotic Coefficient from the AMBI index and Taxa Sensitivity from the INDEX-COR index) seemed not to be sensitive to the outfall pressure. The impacted location had a better score than controls (INDEX-COR index) and most of locations were ranked as unpolluted with impoverished benthic community health (Biotic Coefficient). Results would be probably quite different if the outfall would be located in a boulder field habitat which provides different niches to house a varied fauna (Huguenin et al., 2018). These results run counter to the one obtained from macroalgae species evaluation which ranked the impacted location as "*Poor*" whereas the two macrofauna indices classified it as "unpolluted" and more sensitive than controls. Those results confirmed that the use of macrofaunal indicators must be improved with further investigation to be considered in European Directives.

Based on macroalgae from the circalittoral zone, *Metacallophyllis laciniata* (formerly *Callophyllis laciniata*), a foliose heavily corticated alga, was identified as the most significant contributor to the dissimilarity between the impacted location and controls with a higher abundance in the impacted one. As *Gelidium corneum,* it was reported as frequent in Spanish Basque coast (Gorostiaga et al., 2004b), although in the last two deccades it has become less frequent. It was classified as ESG II (i.e. opportunistic or annual taxa; Gaspar et al., 2012; Neto et al., 2012) and was already identified as being more abundant at slightly polluted habitats (Díez et al., 2003). Among other significant contributors more abundant in the impacted location, only *Calliblepharis ciliata* was classed in ESG I (i.e. late-successional or perennial to annual taxa) whereas the remain species (*Drachiella spectabilis*, *Halymenia latifolia* and *Pterosiphonia complanata*) were classed in ESG II (i.e. opportunist or annual taxa). Even if *Drachiella spectabilis* was not reported in previous studies as indicator of pollution, the related species, *Drachiella minuta,* was especially found in moderately polluted sites along the Basque coast (Díez et al., 2003). By contrast, *Pterosiphonia complanata* was mainly identified in good quality environments or slightly polluted habitats (Díez et al., 2012a, 2003) but was anyway described as rather adapted to pollution (Díez et al., 2003). *Rhodymenia pseudopalmata*, *Heterosiphonia plumosa*, *Lithophyllum incrustans*, *Dictyopteris polypodioides* and *Halopteris filicina* although were not significant contributors to location differences, were less abundant or absent at the impacted location. In addition to the fact that they were identified as characteristic species of the Basque coast (de Casamajor and Lissardy, 2018), *Rhodymenia pseudopalmata* and *Lithophyllum incrustans* were already associated to unpolluted habitats (Díez et al., 2012a, 2003). Conversely, *Phyllophora crispa* exhibited higher abundances in the impacted location. Indeed, it was classified as opportunistic species (ESG II; Orfanidis et al., 2011) but nevertheless it was identified as characterisitic species from the Basque coast (de Casamajor and Lissardy, 2018). Moreover, the two MFG identified as significant contributors (i.e. foliose heavily corticated and foliose non-corticated) were described as morphologically- complex and simple form algae, respectively (Díez et al., 2014). Complex species were rather identified in reference locations contrary to simple form ones mainly found in disturbed areas. Therefore, the identification of these contributors could suggest a persistent impact of the discharge in the circalittoral zone despite the distance to the emissary and the depth. Moreover, results suggest that it might be interesting to integrate the circalittoral zone in the WFD monitoring as, up to now, it is not considered to assess the ecological quality of the water body. This would allow to calculate the EQR (associated to the EQS) and thus to know if it supports or not previous conclusions.

Based on macrofauna from the circalittoral zone, no high contributor (Ct % > 10) of macrofauna assemblages was highlighted. Minor contributors such as *Obelia* sp., crustose bryozoa, *Caryophyllia smithii, Tritia incrassata* and *Echinaster sepositus* were more conspicuous around the effluent. Strengthening identification at the specific level would better assess the taxa sensitivity. However, considering most of them are suspended matter feeders, it is possible to assume that their sensitivity is low even interpretation of those results must be relativized considering variability of macrofauna in terms of presence/absence and abundance in this kind of habitat. Furthermore, as in the upper infralittoral, metrics from both macrofauna ecological quality indices did not appear as efficient to detect the effluent pressure although they used different sensitivity classification and formulas. But, it is important to note that these both indices (BC and TS) were establish for soft-bottom and coralligenous assemblages along the French Mediterranean coast, respectively. Moreover, only 45 percent of sampled species could have been associated to an ecological group using the classification of Borja et al. (2000) and 69.7 percent using the one of Sartoretto et al. (2017). Results could be different if sensitivity of more species were assigned especially for the first classification (Borja et al., 2000). In this study, macrofauna did not appear to be a sensitive indicator to such disturbance in these algal belts as in similar work achieved in the intertidal zone (Huguenin et al., 2019). This contrast with other studies achieved on other coastal ecosystems (Borja et al., 2000; Marques, 2009; O'Connor, 2013; Sartoretto et al., 2017; Vinagre et al., 2016a). Studying macroalgae and macrofauna using the same sampling strategy may be not suitable to this subtidal rocky biogeographic area. Nevertheless, the integration of macrofauna in addition to macroalgae is anyway important because it would allow to better reflect the complexity of the ecosystem (Van Hoey et al., 2010). The further challenge could be addressed to complete the sensitivity categories of macrofauna species to such disturbances and to reflect on another way of sampling macrofauna in these rocky habitats to meet European requirements. Indeed, to date, no validated index exists within European Directive to assess the ecological quality status of rocky habitats integrating macrofauna species. But, it is important to note that works are in progress concerning the intertidal zone (to improve the CCO index) and the subtidal zone (to improve the QISubMac).

5. **Conclusion**

The present work established the assessment of the potential impact of a WWTP discharge on subtidal rocky benthic assemblages in the southeastern Bay of Biscay. Both macroalgae and macrofauna were studied as required by European Directives to assess the response of biological indicators to various pressures. As few other studies, it was assessed the structural variation of the two biological elements coming from the same set of samples considering WFD protocol. Response to the WWTP disturbance differed between macroalgae and macrofauna. Descriptors and ecological indices based on macrofauna did not captured changes in structure between control and impacted locations. Consequently, macrofauna seemed not to be a sensitive indicator to such disturbance in the rocky habitat studied. By contrast, these findings suggested that descriptors based on the Ecological Quality Status (EQS) currently used within the WFD (in the upper infralittoral) appeared to be the more relevant tools to assess this disturbance. Indeed, it ranked the impacted location as "*Poor*" whereas all controls were ranked as "*Good*" which confirms the robustness of the WFD macroalgae indicator through the multimetric approach. According to species sensitivity to pollution, the impact of the discharge was also highlighted in both algal belts. To meet European Directives requirements, it seems thus important to delve into other ways of integrating or evaluating macrofauna to assess more efficiently their response to such pressure. Finally, this work provides a framework for future monitoring allowing an assessment of benthic communities' changes related to WWTP mitigation measures.

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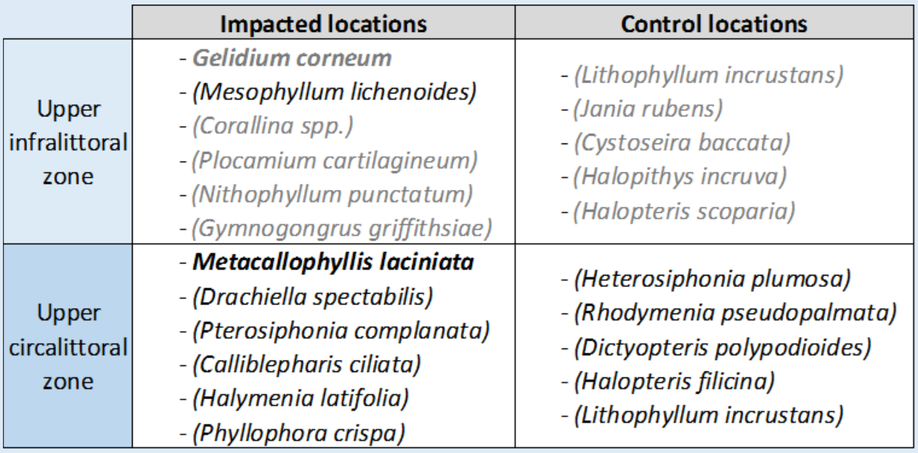
Conflicts of interest: none.

Highlights:

- **Detectable effects** of discharges highlighted **using EQS index** based on **macroalgae**
- **No** significant **effect** using **functional diversity** of both macroalgae and macrofauna

Main contributors responsible for differences between impacted and control locations. Grey species as those identified as characteristic of the studied area within the WFD (de Casamajor and Lissardy, 2018). Species in parenthesis are those identified with a low contribution (Ct < 10%) or not significant.



	Impacted locations	Control locations
Upper infralittoral zone	- ***Gelidium corneum*** - *(Mesophyllum lichenoides)* - *(Corallina spp.)* - *(Plocamium cartilagineum)* - *(Nithophyllum punctatum)* - *(Gymnogongrus griffithsiae)*	- *(Lithophyllum incrustans)* - *(Jania rubens)* - *(Cystoseira baccata)* - *(Halopithys incruva)* - *(Halopteris scoparia)*
Upper circalittoral zone	- ***Metacallophyllis laciniata*** - *(Drachiella spectabilis)* - *(Pterosiphonia complanata)* - *(Calliblepharis ciliata)* - *(Halymenia latifolia)* - *(Phyllophora crispa)*	- *(Heterosiphonia plumosa)* - *(Rhodymenia pseudopalmata)* - *(Dictyopteris polypodioides)* - *(Halopteris filicina)* - *(Lithophyllum incrustans)*

Prospects & improvements:

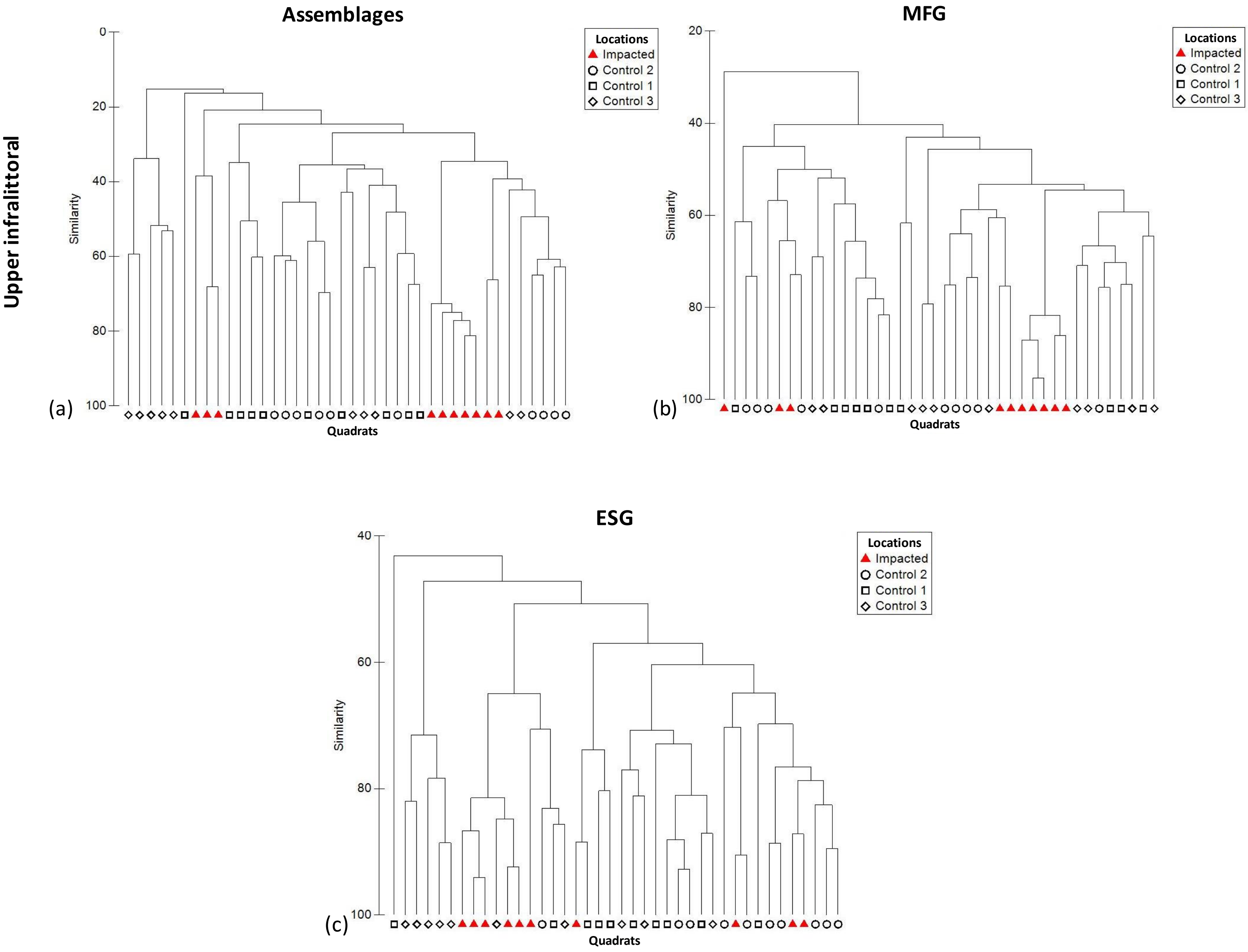
- As for the intertidal zone, reflect upon **how to include fauna** in monitoring in such a habitat (already in progress since 2014 in order to improve the current WFD metrics according to the REBENT)
- Reflect upon how to **integrate the circalittoral zone (N4)** in WFD monitoring and/or adapt current metrics to be able to assess the ecological status of the whole water body based on the N2, N3 and (already in progress since 2014 in order to improve the current WFD metrics according to the REBENT)
- Explore the idea to **fixe** different **depths** *a priori* (already achieve within the WFD)
- Make further sampling to study the **dilution effect** by doing quadrats at different distances from the outfall and at the same depth and compare results with chemical analyses achieved on seawater samples
- Make further sampling in other biogeographical regions of the Atlantic/Channel coastal areas

Supplementary materials

Supplementary material 1: List of macroalgae and macrofauna identified in each location (impacted and control) and algal belt (the upper infralittoral zone, U.I. and the circalittoral zone, Ci.) ('+' means that the species was found in the corresponding location). Taxa were classed into phylum. Macroalgae were assigned to one of the Morpho-Functional Groups (MFG) (Díez et al., 2010) and Ecological Status Groups: ESG I: late-successional or perennial to annual taxa (IA: thick perennial; IB: thick plastic; IC: shade-adapted plastic); ESG II: opportunist or annual taxa (IIA: fleshy opportunistic; IIB: filamentous or sheet-like, opportunistic) according to Orfanidis et al. (2011) and completed by Gaspar et al. (2012), Neto et al. (2012), Vinagre et al. (2016a). Macrofauna were aggregated into ecological groups according to Borja et al. (2000) (i.e. EG I: species very sensitive to organic enrichment and present under unpolluted conditions; EG II: species indifferent to enrichment, always present in low densities with non-significant variations with time; EG III: species tolerant to excess organic matter enrichment, occurring under normal conditions but stimulated by organic enrichment; EG IV: second-order opportunistic species; EG V: first-order opportunistic species) and Sartoretto et al. (2017) completed by expert judgements (SG I: taxa indifferent to organic matter and sediment input; SG II: opportunistic taxa; SG III: tolerant taxa; SG IV: sensitive taxa).

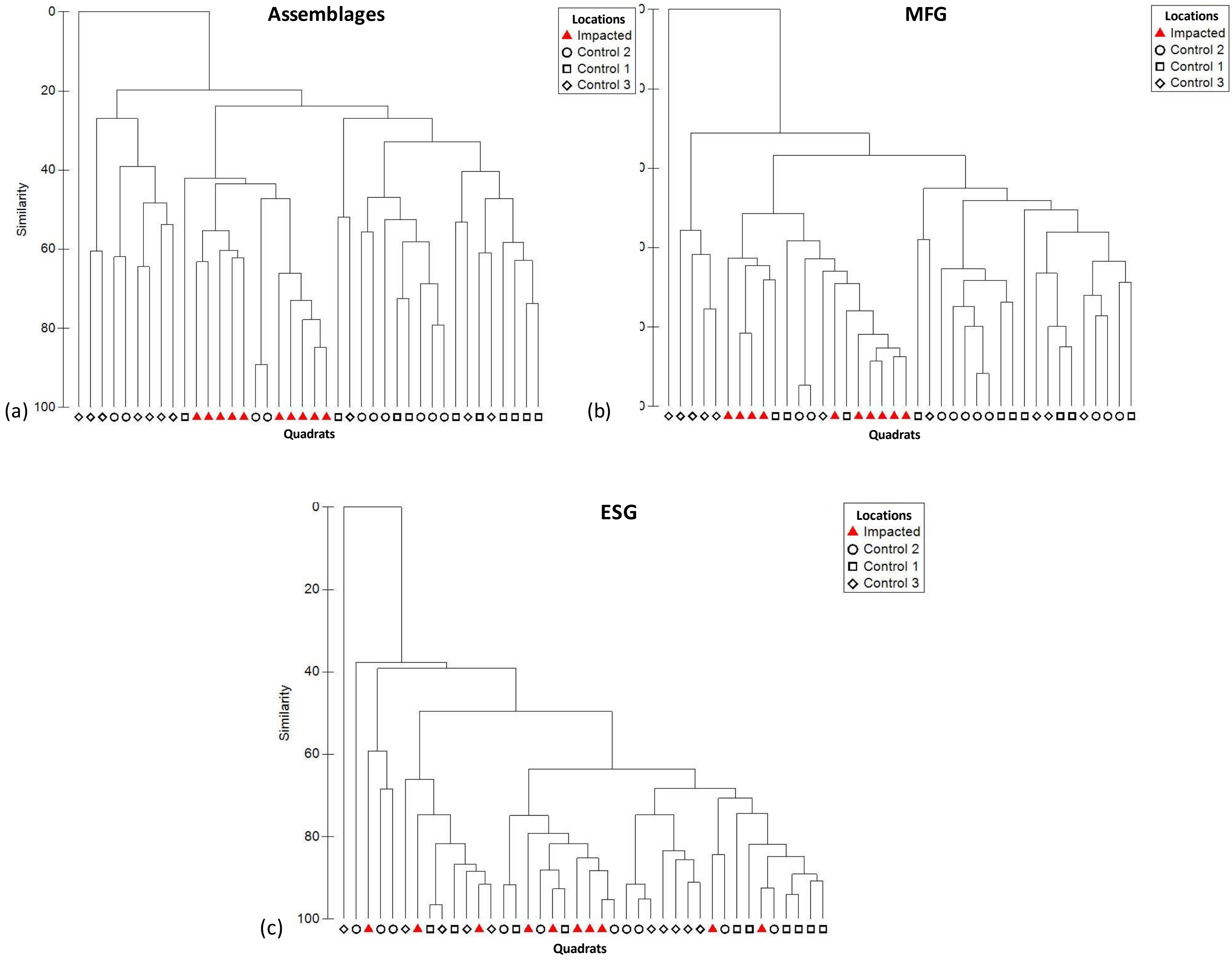
				I		**C1**		**C2**		**C3**	
Species/Taxa	Phylum	MFG	ESG	IU$_p$	IC$_i$	**C1**U$_p$	**C1**C$_i$	**C2**U$_p$	**C2**C$_i$	**C3**U$_p$	**C3**C$_i$
		Macroalgae									
Acrosorium ciliolatum	Rhodophyta	Foliose non corticated	IIA	+	+	+	+	+	+	+	+
Bonnemaisonia asparagoides	Rhodophyta	Terete slightly corticated				+		+	+	+	+
Calliblepharis ciliata	Rhodophyta	Foliose heavily corticated	II		+		+		+		+
Ceramium spp.	Rhodophyta	Polysiphonated	IIB	+	+	+		+		+	+
Chondracanthus acicularis	Rhodophyta	Terete corticated	IIA	+		+		+			
Chondria coerulescens	Rhodophyta	Terete slightly corticated	IIA			+		+			
Chrysimenia ventricosa	Rhodophyta	Terete slightly corticated	IIA		+						+
Corallina spp.	Rhodophyta	Articulated calcareous	IC	+		+		+		+	
Crustose red algae	Rhodophyta	Crustose non calcarous				+		+			+
Dasysiphonia sp.	Rhodophyta	Polysiphonated	II	+	+		+				
Drachiella spectabilis	Rhodophyta	Foliose non corticated	IIA	+	+	+	+	+	+	+	
Gelidium corneum	Rhodophyta	Terete corticated	I	+		+	+	+		+	+
Gracilaria gracilis	Rhodophyta	Terete corticated	IIA								+
Gracilaria sp.	Rhodophyta	Terete corticated	IIA							+	
Gymnogongrus crenulatus	Rhodophyta	Foliose heavily corticated	II							+	
Gymnogongrus griffithsia	Rhodophyta	Foliose heavily corticated	II	+		+		+		+	
Halopithys incurva	Rhodophyta	Terete corticated	IB			+		+		+	
Halurus equisetifolius	Rhodophyta	Foliose slightly corticated	IIB	+		+		+		+	
Halymenia latifolia	Rhodophyta	Foliose slightly corticated	IIA		+						+
Heterosiphonia plumosa	Rhodophyta	Polysiphonated	II		+		+		+		+
Hypnea musciformis	Rhodophyta	Terete slightly corticated	IIA							+	
Hypoglossum hypoglossoides	Rhodophyta	Foliose non corticated	IIA	+			+	+		+	
Jania rubens	Rhodophyta	Articulated calcareous	IC	+		+		+		+	
Lithophyllum incrustans	Rhodophyta	Crustose calcareous	IC	+	+	+	+	+	+	+	+
Mastocarpus/Petroselis	Rhodophyta	Foliose heavily corticated	I							+	
Mesophyllum lichenoides	Rhodophyta	Crustose calcareous	IC	+			+		+		
Metacallophyllis laciniata	Rhodophyta	Foliose heavily corticated	II	+	+	+	+		+	+	
Nitophyllum punctatum	Rhodophyta	Foliose non corticated	IIA	+				+	+		+
Peyssonnelia sp.	Rhodophyta	Crustose non calcarous	IC		+	+	+		+	+	+
Phyllophora crispa	Rhodophyta	Foliose heavily corticated	IIA		+		+	+	+	+	+
Phymatolithon lenormandii	Rhodophyta	Crustose calcareous	I			+		+		+	
Plocamium cartilagineum	Rhodophyta	Terete slightly corticated	IB	+	+	+	+	+	+	+	+
Polyneura bonnemaisonii	Rhodophyta	Foliose non corticated								+	
Polysiphonia spp.	Rhodophyta	Polysiphonated	IIB		+	+		+		+	
Rhodothamniella floridula	Rhodophyta	Filamentous	IIB					+			
Rhodymenia pseudopalmata	Rhodophyta	Foliose heavily corticated	IIA	+	+	+	+	+	+		+
Scinaia furcellata	Rhodophyta	Terete slightly corticated	I	+		+					
Sphaerococcus coronopifolius	Rhodophyta	Terete slightly corticated	I			+		+		+	
Sphondylothamnion multifidum	Rhodophyta	Filamentous	IIB			+		+		+	
Carpomitra costata	Ochrophyta	Terete slightly corticated							+		
Cladostephus spongiosus	Ochrophyta	Terete corticated	I			+					
Colpomenia peregrina	Ochrophyta	Foliose non corticated	IIA	+							
Crustose brown algae	Ochrophyta	Crustose non calcarous			+	+	+	+		+	+
Cystoseira baccata	Ochrophyta	Leathery	IB			+		+		+	
Cystoseira tamariscifolia	Ochrophyta	Leathery	IA	+		+		+			
Desmarestia ligulata	Ochrophyta	Foliose slightly corticated	II			+			+	+	
Dictyopteris polypodioides	Ochrophyta	Foliose slightly corticated	IIA	+	+	+	+		+	+	+
Dictyota dichotoma	Ochrophyta	Foliose slightly corticated	IIA	+	+	+	+	+	+	+	+
Ectocarpales	Ochrophyta	Filamentous	IIB		+			+			
Halopteris filicina	Ochrophyta	Terete slightly corticated	IIA		+		+		+		+
Halopteris scoparia	Ochrophyta	Terete corticated	IIA			+		+		+	
Hincksia spp.	Ochrophyta	Filamentous	IIB				+				
Padina pavonica	Ochrophyta	Foliose slightly corticated	IB							+	
Spatoglossum solieri	Ochrophyta	Foliose slightly corticated									+
Taonia atomaria	Ochrophyta	Foliose slightly corticated	IB			+				+	
Zanardinia typus	Ochrophyta	Crustose non calcarous	II			+		+	+		
Bryopsis plumosa	Chlorophyta	Filamentous	IIB								+
Cladophora spp.	Chlorophyta	Filamentous	IIB		+	+		+		+	+
Codium spp.	Chlorophyta	Crustose non calcarous	IIB	+		+		+		+	
Pterosiphonia complanata	Chlorophyta	Terete slightly corticated	IIB	+	+	+		+	+	+	+
Ulva spp.	Chlorophyta	Foliose non corticated	II	+	+	+				+	
Xiphosiphonia pennata	Chlorophyta	Polysiphonated	II	+		+		+		+	

					I		**C1**		**C2**		**C3**	
Species/Taxa	Phylum	MFG	EG	SG	**I**U$_p$	IC$_i$	**C1**U$_p$	**C1**C$_i$	**C2**U$_p$	**C2**C$_i$	**C3**U$_p$	**C3**C$_i$
		Macrofauna										
Polychaeta	Annelida	-				+		+		+		
Sabella discifera	Annelida	-	I	II		+						
Salmacina dysteri	Annelida	-		II				+				
Serpulidae	Annelida	-	I			+	+	+				
Spirobranchus spp.	Annelida	-	II		+							
Porcellana platycheles	Arthropoda	-	I				+					
Crustose bryozoa	Bryozoa	-	II			+		+				+
Botryllus schlosseri	Chordata	-	I	II	+							
Crutose Ascidiacea	Chordata	-	III					+				+
Diplosoma spongiforme	Chordata	-		III								+
Parablennius pilicornis	Chordata	-				+						
Actiniaria	Cnidaria	-	II		+							
Actinothoe sphyrodeta	Cnidaria	-		I	+			+				
Aglaophenia sp.	Cnidaria	-		II	+	+		+				
Aiptasia mutabilis	Cnidaria	-	II	II		+			+			
Alcyonium coralloides	Cnidaria	-	I	III								
Anemonia viridis	Cnidaria	-		I			+		+			
Balanophyllia regia	Cnidaria	-		III								
Caryophyllia smithii	Cnidaria	-	I	II		+						+
Corynactis viridis	Cnidaria	-	I	II			+	+		+		
Eunicella verrucosa	Cnidaria	-	I	IV		+						
Gymnangium montagui	Cnidaria	-		III						+		
Hydrozoa	Cnidaria	-	I					+				+
Nemertesia antennina	Cnidaria	-		III				+		+		
Obelia sp.	Cnidaria	-	II	II		+						
Parazoanthus axinellae	Cnidaria	-		III		+						
Sagartia troglodytes	Cnidaria	-	I									+
Sertularella spp.	Cnidaria	-	II	II				+				+
Echinaster sepositus	Echinodermata	-	I	III		+		+				
Holothuria forskali	Echinodermata	-	I	II						+		
Holothuria tubulosa	Echinodermata	-	I	II		+			+	+	+	+
Paracentrotus lividus	Echinodermata	-		III			+					
Aplysia spp.	Mollusca	-	I									
Berthellina edwardsii	Mollusca	-		III								
Calliostoma zizyphinum	Mollusca	-	I	II	+				+			+
Cerithium spp.	Mollusca	-	II	I	+		+		+	+		+
Diaphorodoris alba	Mollusca	-		III						+		
Discodoris rosi	Mollusca	-		IV								
Dondice banyulensis	Mollusca	-										
Doriopsilla areolata	Mollusca	-		III								
Edmundsella pedata	Mollusca	-		IV								
Facelina auriculata	Mollusca	-		IV								
Felimare cantabrica	Mollusca	-		III		+						
Felimare tricolor	Mollusca	-		III						+		
Felimida krohni	Mollusca	-		IV		+						
Felimida purpurea	Mollusca	-		IV								
Gibbula ardens	Mollusca	-	I		+						+	
Octopus vulgaris	Mollusca	-								+		
Peltodoris atromaculata	Mollusca	-		III			+					
Polycera spp.	Mollusca	-		III				+				
Rocellaria dubia	Mollusca	-	I	II		+		+	+			+
Tritia incrassata	Mollusca	-	II	I		+			+	+	+	
Tritia reticulata	Mollusca	-	II		+		+					+
Phoronis	Phoronida	-	II					+				
Aplysina spp.	Porifera	-		IV		+				+		
Axinella damicornis	Porifera	-		III				+				
Axinella sp.	Porifera	-		III			+					
Cliona celata	Porifera	-	III	I		+		+			+	
Crustose porifera	Porifera	-										
Demospongiae	Porifera	-				+	+	+	+			
Grantia compressa	Porifera	-										+
Halichondria sp.	Porifera	-		II		+		+				
Leucosolenia sp	Porifera	-										
Myxilla sp.	Porifera	-		II				+				
Pachymatisma johnstoni	Porifera	-		III								
Sycon sp.	Porifera	-	I	II	+	+		+	+	+		+



Supplementary material 2: Cluster analysis dendrograms computed on macroalgae assemblages (a), morpho-functional groups (MFG) (b) and Ecological Status Groups (ESG) (c) in the upper infralittoral zone.

Circalittoral



Supplementary material 3: Cluster analysis dendrograms computed on macroalgae assemblages (a), morpho-functional groups (MFG) (b) and Ecological Status Groups (ESG) (c) in the upper circalittoral zone.

Supplementary material 4: MFG (a, c) and ESG (b, d) identified by SIMPER analyses as significant contributors (p-value <0.05 %) to the dissimilarity between impacted and control locations computed on the upper infralittoral (a, b) and circalittoral zones (c, d).

Upper infralittoral

MFG

(a)

IU_p vs. Controls

57.42%	**av 'IU_p'**	**av 'Controls'**	**Ct (%)**
Terete corticated	48.10	16.50	25.34
Foliose heavily corticated	8.90	3.40	6.53

ESG

(b)

IU_p vs. Controls

47.04%	**av 'IU_p'**	**av 'Controls'**	**Ct (%)**
IIA	65.90	24.13	45.13

Circalittoral

(c)

IC_i vs. Controls

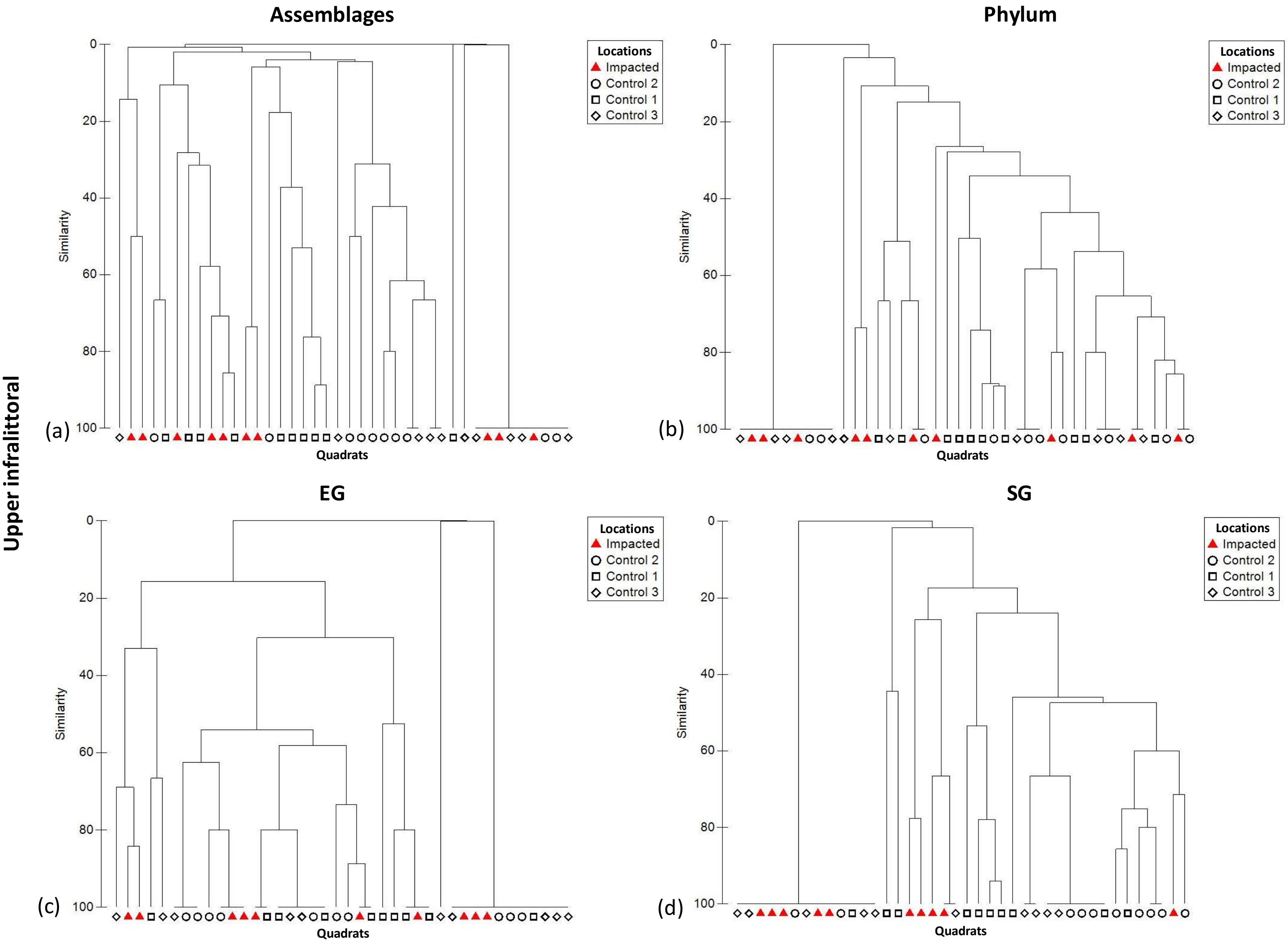
62.26%	**av 'IC_i'**	**av 'Controls'**	**Ct (%)**
Foliose heavily corticated	80.60	24.14	39.53
Foliose non corticated	18.60	8.90	9.73

(d)

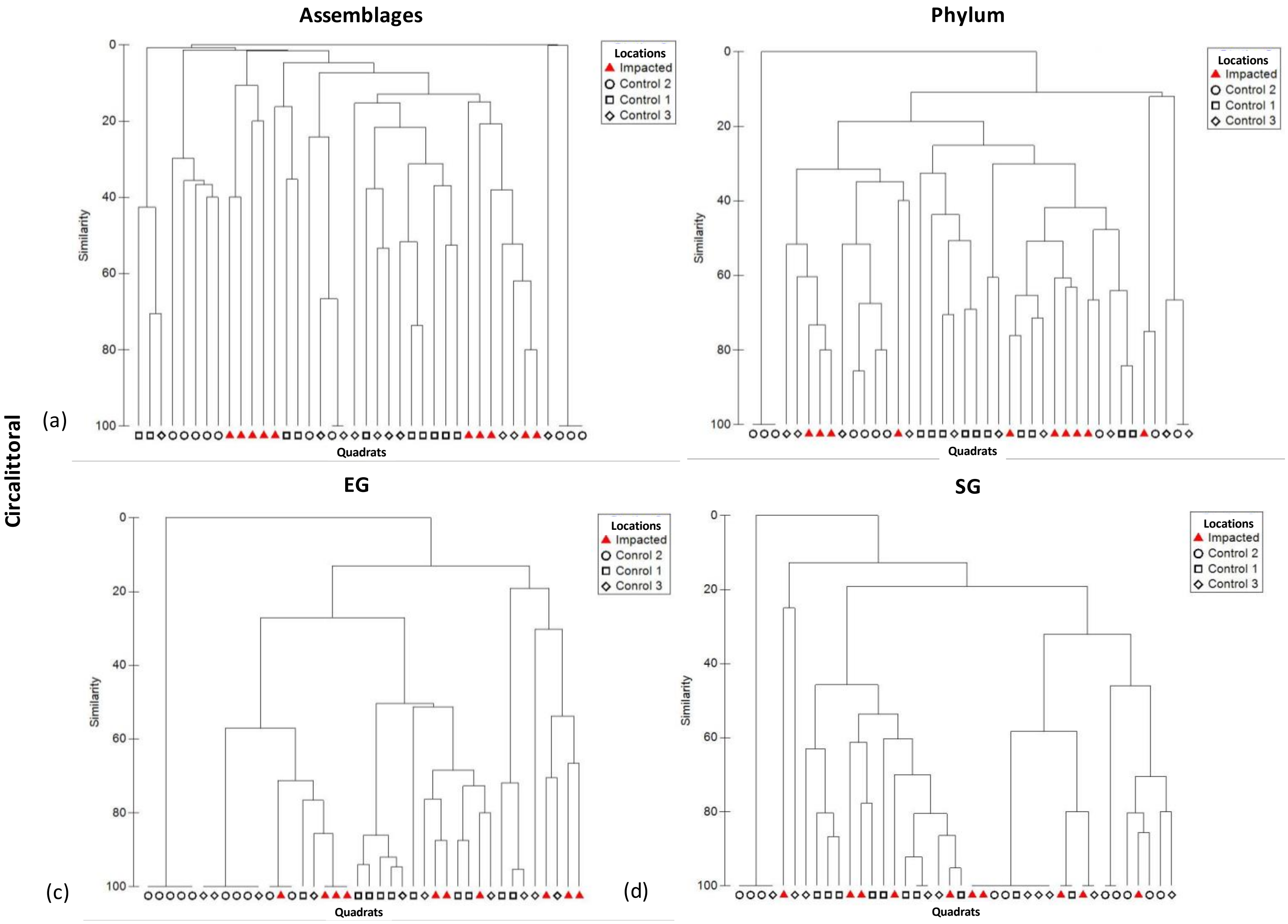
IC_i vs. Controls

46.74%	**av 'IC_i'**	**av 'Controls'**	**Ct (%)**

No significant contributor



Supplementary material 5: Cluster analysis dendrograms computed on macrofauna assemblages (a), phylum (b), Ecological Groups (EG) according to Borja et al. (2000) (c) and Sensitivity Groups (SG) according to Sartoretto et al. (2017) (d) in the upper infralittoral zone.



Supplementary material 6: Cluster analysis dendrograms computed on macrofauna assemblages (a), phylum (b), Ecological Groups (EG) according to Borja et al. (2000) (c) and Sensitivity Groups (SG) according to Sartoretto et al. (2017) (d) in the circalittoral zone.

Chapter VI:

General discussion - Conclusion

As results were already discussed within each chapter/article, this last chapter highlights remarks that have raised during this study and also lay out several prospects and/or recommendations. This study allowed to have a first insight of the potential impact of WWTPs on coastal environment, and more specifically on benthic communities of the southeastern Bay of Biscay along the Basque coast. Therefore, a number of points could be improved, confirmed or thorough therewith to reach firm conclusions. This chapter is structured according to the problematics posed at the beginning of the study. Remarks, prospects and/or recommendations listed in **Tables 1** and **2** are thus detailed within each below parts. This final section deals with the general issue of the present work and the usefulness of this research to improve knowledge on the good ecological status of coastal waters and the conservation status of habitats for the maintenance of biodiversity.

1. Which micropollutants (and in what amount) are rejected into the Ocean through WWTPs?

This study highlighted the main substances released into the environment through WWTP discharges along the Basque coast. But, only WWTPs rejecting into the Ocean on rocky substratum were studied. Therefore, even though this allowed to have a first insight of the occurrence and concentrations of micropollutants in wastewater effluents, this cannot be generalized to the whole Basque coast. Indeed, two other WWTPs exist along the coast and are rejecting on sandy substrata (one in Biarritz with 69 673 PE and one in Bidart with 19 238 PE). Four other exist along the Adour river (two in Bayonne with 112 715 and 53 118 PE and one Lahonce with 2 516 PE and Urt with 2 221 PE) which are connected to the Ocean by an estuary located in the extrem northern Basque coast (without counting other WWTPs rejecting in the upper reaches) (www.assainissement.developpement-durable.gouv.fr). It would be thus interesting to do other sampling for chemical analyses in these several WWTPs with the aim to have a more accurate idea of the ‘WWTP’ pressure impacting this coastal area. In addition to experimental analyses (to study treatment process efficiency on one substance or on a group of substances considering reactivity and mixture effects) and to analyses that could be achieved on influents as well as on effluents (to calculate removal rates), this would also allow to confirm highlighted assumptions concerning treatment process efficiency (which constitutes another problematic). Apart from analytical constraints these analyses may involve (time of filtration, matrix effects, etc.), all these additional analyses (experimental and others) could anyway provide guidance for the future in the treatment process implementation.

Table 1: Summary of main findings and remarks highlighted at the outcome of the present study. In tables showing main contributors, purple algae are those identified as opportunistic species and grey ones as characteristic within the WFD (de Casamajor et al., 2016; de Casamajor and Lissardy, 2018).

Zones	Continental / Coastal	Intertidal		Subtidal
Samples	Wastewater from wastewater treatment pant discharges	Benthic communities (macroalgae and macrofauna)		Benthic communities (macroalgae and macrofauna)
Problematics	Do WWTP discharges constitute a source of micropollutants into the Ocean along the Basque coast and do they impact rocky benthic communities?			
	Which micropollutants (and in what amount) are rejected into the Ocean through WWTPs?	Are intertidal rocky benthic communities affected by WWTP discharges? Are current WFD indices enough sensitive to study such a pressure?	Could benthic communities constitute a good bioindicator/accumulator of such a pressure?	Are subtidal rocky benthic communities affected by WWTP discharges? Are current WFD indices enough sensitive to study such a pressure?
Main findings	*Concentrations of micropollutants* Substance families: **Metals > Pharmaceuticals > Musks > APs > Sunscreens > PAHs > OCPs > PCBs > Organomercury compounds** → From 0.7 to 24 557.2 ng.L^{-1} (Total mean concentrations) Main substances per analytical group: **Vanadium > Chromium > Hydrochlorothiazide > HHCB > Oxazepam > Caffeine > Diclofenac > NP > HHCB-lactone > OC > Naphthalene > IHg > PCB 138** Temporal variability (Metals & organic substances): **[C]August > July - December** → ↘ precipitations + ↗ pop. Spatial variability (Metals & pharmaceuticals): **[C]Urrugne > Hendaye - Erromardie - Ondarroa > Guéthary** → Urrugne ∋ separated sewer system + ↗ PE + Biofiltration ∌ activated sludge treatment → Hendaye ≈ Erromardie ≈ Ondarroa → Guéthary ∋ ↘ PE + activated sludge treatment + membrane filtration + UV treatment	*Communities' response* Biological and ecological metrics: - **Assemblages (macroalgae + macrofauna) using multivariate analyses** = efficient to discriminate the potential WWTP impacts - **Macroalgae and macrofauna MTR** (separately) = no helpful to discriminate the potential WWTP impacts - **The "macroalgae" WFD quality index** = sensitive to such a pressure - Impacted locations = "Moderate" - Control locations = "Good" Main contributors	*Bioaccumulation of micropollutants* Biological elements: - **Macroalgae** = good bioaccumulators, especially *Gelidium* spp. - **Macrofauna** = present several technical and biological drawbacks Bioconcentration ability (especially for pharmaceuticals): → ***Ulva* spp. > *Gelidium* spp. > *Mytilus* spp. > *Holothuria* spp. - *Patella* spp. - Porifera**	*Communities' response* Biological and ecological metrics: - **Macroalgae and macrofauna assemblages, functional traits and ecological groups using multivariate analyses** (separately) = present no significant effect of such a WWTP pressure - **The "macroalgae" WFD quality index** = sensitive to such a pressure - Impacted locations = "Poor" - Control locations = "Good" - **The two quality indices based on macrofauna =** present no significant effect of such a WWTP pressure Main contributors
Strengths	- **Quantitative** and **precise results** - **Undebatable** results - A **data base** rather **easy to** statistically **analyze** and/or **interpret** - Sampling and analyses **require** only **1 person** (a technicien or an engineer)	- Takes into account **all species** - Species **reflect** both **previous** and **present** local **conditions** to which they have been exposed - Existence of a **standardized protocol** (the **WFD index**) which provides quantitative and qualitative results - **Non-destructive**	- **Quantitative** and **precise results** - **Undebatable** results - **Analyses require** only **1 person** (a technicien or an engineer) - **Reflects conditions** to which species were **previously exposed**	- Takes into account **all species** - Species **reflect** both **previous** and **present** local **conditions** to which they have been exposed - Existence of a **standardized protocol** (the **WFD index**) which provides quantitative and qualitative results - **Non-destructive**
Weaknesses	- We **find what we are looking for** (not more) - **Not representative** of the general situation → provides only a **snapshot** of the situation at the instant (+/- 24h) of sampling - Results may **widely varied** according to WWTP and season - Analytical analyses are **expensive** and **time consuming** - Analytical analyses may be **difficult according to the matrix** - Requires **technical abilities**	- Communities' modifications may take time and communities may thus **not reflect the reality at a given moment** - Field sampling dependant on **field** and **weather conditions** (e.g. habitat heterogeneity, wave, wind and tidal conditions) - Sampling requires at least **2 persons** - The WFD index does not included yet **macrofauna** in the EQR calculation - Apart from the WFD index, **various methods exist** to analyze the data which makes **results debatable and difficult to compare** - Requires **biological abilities**	- We **find what we are looking for** (not more) - **Sampling requires** from **2** persons to **4 persons** - According to the matrix and the analytical group, **no validated method exist** - **Results debatable and difficult to analyze** (low number of samples, missing values) - Analytical analyses are **expensive** and **time consuming** - **Destructive** - Requires **biological** and **technical abilities**	- Communities' modifications may take time and communities may thus **not reflect the reality at a given moment** - Field sampling dependant on **field** and **weather conditions** (e.g. turbidity, habitat heterogeneity, wave, wind and tide conditions) - Sampling **requires human** (at least 4 persons), **financial** and **material ressources** (a boat and dive devices) - The **WFD index** does not included yet **macrofauna** and **N4** in the EQR calculation - Apart from the WFD index, **various methods exist** to analyze the data which makes **results debatable and difficult to compare** - Requires **biological** and **technical abilities**
Remarks	- Only WWTPs rejecting on **rocky substratum (platforms)** were studied along the Basque coast - **Metabolites** were not tanken into account - **Pharmaceuticals** were only analyzed on the dissolved phase	- **Difficulties** were encountered for monitoring **macrofauna** → seemed not to appear as a suitable bioindicator in such a habitat - **Difficulties** were sometimes encountered to implement the **sampling design** in locations where habitat was **heterogeneous** (i.e. pools, different distances from the outfall) - A **pseudo WFD index** (with only 12 quadrats) was calculated to assess the ecological quality at the site scale - It is possible to note that species mainly present in **impacted locations** were **not all** identified as **opportunistic** - It is important to note that: species defined as **characteristic** were **not necessarely sensitive**	- Some species were not identified at **the species level** - Reported concentrations were either expressed on a **dry wiegth** basis or on a **wet weight** basis → difficulties when comparing - Unfortunately, **metal** and **AP** concentrations from wastewaters were not compared to those in biota samples	- ***Gelidium* spp.** exhibited a higher abundance in the impacted locations while it was considered as an indicator of the good ecological status within the WFD and was found in unpolluted habitats in Spain (Díez, 2003) - Some **remarks** have been raised **about the WFD protocol:** • The **EQR** was calculated **only according to the** upper infralittoral (**N2**) while it is normally calculated by averaging EQRs of the N2 and the lower infralittoral (N3) because the N3 was not found this year • It has **not** been **possible** to calculate the **EQR for the** upper circalittoral (**N4**) because up to now no metric has been set for this zone - A problem might arise in the case of the impact **comparison between different WWTP discharges**: at a same depth, the distance from the outfall might not be similar (i.e. different bathymetrie and topography). Similarly, controls should also have the same topography

Main contributors

	Impacted locations/sites	Control loc. or less impacted sites
Upper midlittoral zone	- *Ceramium* spp. Fr. - *Caulacanthus ustulatus* - *Corallina* spp. Sp. - *Lithophyllumincrustans*	Fr. - *Laurencia obtusa* - *(Osmundea pinnatifida)* Sp. - *(Halopteris scoparia)*
Lower midlittoral zone	Fr. - *Ceramium* spp. Sp. - *Corallina* spp.	Fr. - *Halopteris scoparia* - *(Cystoseira tamariscifolia)* Sp. - *Chondria coerulescens* - *Codium adhaerens*

Main contributors

	Impacted locations	Control locations
Upper infralittoral zone	- *Gelidium corneum* - *(Mesophyllum lichenoides)* - *(Corallina spp.)* - *(Plocamium cartilagineum)* - *(Nithophyllum punctatum)* - *(Gymnogongrus griffithsiae)*	- *(Lithophyllum incrustans)* - *(Jania rubens)* - *(Cystoseira baccata)* - *(Halopithys incruva)* - *(Halopteris scoparia)*
Upper circalittoral zone	- *Metacallophyllis laciniata* - *(Drachiella spectabilis)* - *(Pterosiphonia complanata)* - *(Calliblepharis ciliata)* - *(Halymenia latifolia)* - *(Phyllophora crispa)*	- *(Heterosiphonia plumosa)* - *(Rhodymenia pseudopalmata)* - *(Dictyopteris polypodioides)* - *(Halopteris filicina)* - *(Lithophyllum incrustans)*

Table 2: Summary of prospects and improvements identified at the outcome of the present study.

Zones	Continental / Coastal	Intertidal		Subtidal
Samples	**Wastewater from wastewater treatment pant discharges**	**Benthic communities (macroalgae and macrofauna)**		**Benthic communities (macroalgae and macrofauna)**
Prospects & improvements	- **Identify potential sources** of highlighted substances to suggest source control options - Make further researches on **medical facility treatment processes** (hospital, nursing homes, veterinary clinics, etc.) in the light of the results obtained - Make **experimental analyses** to study the effect of **treatment processes** on removal efficiency of substances - Make the analyses on wastewater from **influents** to calculate the **removal rate** of each analytical group and analyte and **confirm treatment process efficiencies** highlighted in this study → in this case, be careful to the duration of the analyses (especially for filtration) and to the matrix effect - Analyze in influents and effluents major **metabolites** (according to the bibliography) whose parent compounds are supposed to be completely (or almost) metabolized within the human body or during the transport towards WWTPs - Think on how **routinely implemented** these analyses? - Highlight substances that could be integrated in **regulatory lists** - Study the **dilution effect** once these substances are rejected into the Ocean through WWTP outfalls by doing the sampling at different distances from the outfall → in this case, be careful to the high sample salinity which could pose some analytical problems	- For **macrofauna**, think about: • **Another habitat** (e.g. boulder fieds; cf. Article in Annex) if emissaries are located on platform habitat • **Another sampling method** (i.e. microfauna with submarine vacuum cleaner) • **Another way to monitor or analyze** them (make a list of species with high ecological interest; cf. Article in Annex) - As a list of rather **sensitive taxa** may be achieved according to the above table, think about **how integrate these taxa in WFD monitoring** in addition to those defined as characteristic	- Make **experimental analyses** to: • Confirm the **bioaccumulation capacity** of selected species • Study the potential **adverse effects** (including mixture/chronic effects) of released substances on these species - Study the **biomagnification process** - Highlight substances that could be integrated in **regulatory lists** for biota - Think on how **routinely implemented** these analyses?	- As for the intertidal zone, think about **how include fauna** in such a habitat - About the **WFD**: • Think about how **integrate the circalittoral zone (N4)** in monitoring and/or adapt current metrics to be able to assess the ecological status of the whole water body based on the N2, N3 and N4 • It would be maybe preferable to **fixe** different **depths** (which will be the **same at each sampling campaign**) and after note the corresponding algal belt instead of to do the reverse. This would allow to have a **balanced sampling** for **statistical analyses** and thus to easily analyze the temporal variability even an algal belt is missing one year - Make further sampling to study the **dilution effect** by doing quadrats at different distances from the outfall and at the same depth and compare results with chemical analyses achieved on seawater samples → this could allow to focus the sampling on a smaller area

The analytes selected in the present work were the most common studied in the literature. But, nowadays, micropollutants are not routinely assessed in WWTP influents or effluents and analytical groups are often separately studied or with a small set of analytes especially due to the high costs and the time required for analyses (Busetti et al., 2005). This explains that analytical methods for some groups are still currently under development. Among the 127 analyzed analytes, 111 were detected and quantified in wastewater samples but it seems important to keep in mind that only investigated analytes were detected. Therefore, other substances could also be found and maybe even in higher concentrations. In addition, it has been noted that some pharmaceutical compounds detected in high concentrations were supposed to be completely or partially metabolized by human body in addition to transformation occurring during the transport to the treatment plant or during the treatment process (Deblonde et al., 2011; Lishman et al., 2006; Zuccato et al., 2005). This raises the question to the quantity of metabolites rejected into the environment in addition to the parent compounds. As metabolite concentrations could be found in much higher concentrations, it would be interesting to analyze them in wastewaters because they could have a lower, the same or a higher impact than parent compounds on the aquatic environment (without even considering mixture effects). The transformation level is also important to consider because parent compounds could be underestimated if transformation occurs before or during the treatment process (Lishman et al., 2006). Finally, some pharmaceutical compounds were identified as rather hydrophobic while the analyses were achieved only on the dissolved phase. Their concentrations may have thus been underestimated. Consequently, even though pharmaceutical concentrations were detected in higher concentrations than other analytical groups, if metabolites and compounds associated to the particulate phase were taken into account, their concentrations would maybe be even higher. In addition, even if additional researches could be achieved to know the dilution effect of the Ocean (by doing several samples at different distance from the outfalls), it would be interesting to do further ecotoxicological researches on these main micropollutant to know the fate and the reactivity of parents compounds and metabolites and their potential environmental effects once they are rejected in the environment. Indeed, several factors such as photodegradation, geochemical gradient, suspended matter interactions could affect their transformation and their effects on the environment.

In parallel, further researches could be made on micropollutant sources. Indeed, treatment processes could be the best they can be, if sources are not controlled and regulated, the discharge problem will be persistent because there will always be a time lag between reality, legislation, monitoring and restrictions. Therefore, the better alternative to the constant improvement of treatment processes would be to identify the potential sources of the main released substances to suggest source control options and mitigating. For example, as pharmaceutical compounds appeared as one of the more concentrated analytical group, further researches could be achieved on medical facility treatment processes (hospital, nursing homes, veterinary clinics). The Decision n° 2008-DC-0095 which fixes the technical regulations about the removal of effluents and radionuclides contaminated wastes, have already set two types of liquid wastes management which mainly come from hospitalized patient toilets (Decision, 2008). The first one concerned effluents containing radionuclides with short reactive periods (less than 100 days) and the second one those containing radionuclides with long reactive periods (more than 100 days). In the first case, wastewaters are stocked into a tank with the aim to decrease their reactivity. Then, according to their physico-chemical nature, there are either transferred towards the sewer system or added to chemical solvent wastes. In the second case, they are conditioned, stocked and retrieved by the National Agency for Radioactive Waste Management (ANDRA) (Decision, 2008). Consequently, all other pharmaceutical compounds seemed not to be treated before their release into the sewer system. It would be thus interesting to do some analyses on their discharges to know if hospitals constitute a main source of pharmaceuticals into the water cycle. If so, it would maybe be interesting to reflect on potential pre-treatments that could be added before their release in the sewer system.

Finally, the aim of the present research and of suggested additional researches would be to highlight compounds with the highest environmental risks (taking into account resilience capacity of the environment) and/or with low removal rates which would allow to complete regulatory list (EQS) and set up monitoring. But, up to now, the most common method for measuring micropollutant concentrations is spot sampling followed by chemical analyses which are expensive and time consuming. Therefore, it seems important to reflect on alternative quantitative or qualitative analyses that could be routinely implemented (e.g. passive samplers) (Mills et al., 2011). They must be based on a compromise between the number and the accuracy of analyzed data and the feasibility in terms of costs and time while being as representative as possible of the reality.

2. Are intertidal rocky benthic communities affected by WWTP discharges? Are current WFD indices enough sensitive to study such a pressure?

Even if macroalgae were identified as a relevant biotic component to study impact of WWTP discharges, macrofauna appeared as not sensitive to this pressure mainly because it does not appear as suitable bioindicator in such a habitat (i.e. rocky platforms). Indeed, this habitat was not favorable to macrofauna settlement due to the lack of hiding places and of canopy-forming macroalgae. For example, macrofauna mean taxonomic richness was associated in this study to low values and high standard deviations. Therefore, it seems difficult to include macrofauna communities monitoring in this habitat even though its consideration constitutes one of the MSFD requirement. Indeed, the importance to consider this biological element, in addition to macroalgae, has already been highlighted (Vinagre et al., 2016a). Macrofauna is playing a key role in water quality for the conservation status and functional aspects on the environment (de Casamajor et al., 2016) and, its simultaneous monitoring with macroalgae allows to better reflecting the complexity of the ecosystem (Van Hoey et al., 2010). We should thus reflect on another methodology to monitor macrofauna. Different possibilities might be suggested:

- Focus only on taxa identified as having a high ecological interest or identified as good bioindicators,
- Monitor microfauna in addition or instead of macrofauna (using submarine vacuum cleaner),
- Focus on fixed macrofauna,
- Focus on soft sediment macrofaune (if emissary is located on those sediments).

This latter suggestion could be maybe a good alternative to sampling the entire local macrofauna biodiversity. Indeed, contrary to mobile macrofauna which often constitutes a snapshot in space and time due to its mobile capability (Davidson et al., 2004; Takada, 1999), study fixed/sessile macrofauna could constitute a more precise descriptor of recruitment and mortality in response to environmental changes because it cannot redistribute themselves (Chapman et al., 2009). In the context of studying chronical impact of WWTP discharges or of assessing the ecological status of water bodies over longer period, this possibility could be thus suitable. Additional selection criteria could also be made among the fixed taxa (e.g. sensitive species, species with high ecological interest, species at a specific level in the food chain).

Even though WWTP outfalls of the present study were located on rocky platforms, another habitat appeared interesting to be considered in the context of European Directives. The habitat in question is boulder fields, considered as a community interest habitat according to Habitat Directive (EEC, 1992; 92/43/CEE) (https://inpn.mnhn.fr). Indeed, this habitat, constituted by rock, gravel and soft sediment, may contain a high diversity (Le Hir and Hily, 2005). Even if boulders may regularly be overturned by waves affecting algae and invertebrates settlement (Bernard, 2012; Sousa, 1979), they provide anyway more hiding places for macrofauna contrary to rocky platforms. This was confirmed by brief analyses achieved on unpublished data collected in 2016 on three intertidal locations along the Basque coast (Guéthary, Saint-Jean-de-Luz and Socoa). This aim was to compare macrofauna diversity between both rocky habitats (i.e. platforms and boulder fields). The same sampling design as the one employed on boulder fields in Huguenin et al. (2018) was used. In addition, quadrats were also achieved on the upper side of platforms. 462 quadrats were performed and allowed to identify a total of 126 species/taxa: 39 macroalgae, 18 fixed macrofauna and 69 mobile macrofauna. Diagrams in **Annex 7** allowed to visually compare the mean taxonomic richness between locations and between both rocky habitats. These analyses showed that the mean taxonomic richness of both fixed and mobile macrofauna (**Tables 1** and **2**) was always significantly higher in boulder fields than in platforms and that no significant difference was detected between locations presenting the same rocky habitat. Even though further analyses should be made to complete these first findings, this constitutes a first element confirming the interest to study macrofauna in boulder field habitat instead of on rocky platforms. Nevertheless, it is important to note that the current WFD monitoring (intertidal and subtidal macroalgae protocols) is applied solely on rocky platforms. Therefore, if the interest of monitoring macrofauna in such a habitat is confirmed, in-depth reflection should be made to try to include this approach within the current WFD monitoring and to link it to the ‘WWTP’ pressure if some emissaries are located in this habitat.

Finally, the current WFD protocol which considers only macroalgae and which is applied on locations far away from any disturbances to assess the ecological status of the whole water body, appeared sensitive to detect the impact of the ‘WWTP’ pressure. Therefore, to have a global view of the ecological quality, it could be interesting to not only consider locations supposed as non-impacted but also to consider quality of coastal zones. They could be either impacted by anthropogenic pressures or supposed as rather non-impacted. Indeed, it is anyway important to note the difficulty to find pristine controls, especially along the Basque coast where a number rivers, WWTPs and bays exist. Variabilities within these locations could confirm this and be assigned to anthropogenic and/or natural impacts.

3. Are subtidal rocky benthic communities affected by WWTP discharges? Are current WFD indices enough sensitive to study such a pressure?

In the present study, macrofauna did not seem to appear as a pertinent bioindicator in this rocky habitat. But, it is important to keep in mind that all organisms were not necessarily sampled (e.g. organisms < 5 mm) and all were not identified at the species level (e.g. Porifera, etc.).

Concerning the current WFD protocol (applied only on macroalgae), several remarks have been raised. As the aim of this Directive is to assess the ecological status of the water body, consider the circalittoral (N4) in the EQR calculation would be interesting (even if this zone is already monitor since the sixties). Indeed, no metric has been yet established for this latter zone but it is currently under consideration with the aim to do a retro calculation up to 2014. This would allow to consider this additional zone it in future EQR calculations and thus to have a global view of the ecological quality of the water body. In addition, the same remark was made when a zone was exceptionally not found one year. This was, for example, the case in 2017 where the lower infralittoral zone (N3) was not found. In this case, the EQR calculation was only based on one zone which appears a little bit restrictive when the objective is to consider the whole water body. The problem of 'missing zone' mainly occurs because the sampling is essentially based on algal belt definition. Of course, this approach is really important to be able to do spatial and temporal comparisons. Indeed, this cannot be made if sampling was achieved in different algal belts (communities would inevitably be different). But, using this approach, the design may be unbalanced between years in case of missing data. Statistically, this poses some problems because some multivariate analyses and statistical tests, such as analysis of variances (ANOVA, parametric multivariate statistical test) or permutational multivariate analysis of variance (PERMANOVA, non-parametric multivariate statistical test) are not robust for unbalanced designs (Anderson, 2014). To deal with this, it would be better to fixed *a priori* different depths that will be sample each year by taking care (thanks to preceding campaigns) to have at least one depth in each algal belt even if the main risk by doing this could be to sample several times the same algal belt. Ideally, the best would be to sample, year by year, same depths in same algal belts, to be as accurate as possible. In this case, even if one algal belt is not found one year, the design would anyway be balanced because all depths would have been sampled and analyses may thus be done. In the case of the present study, which had the aim to compare the ecological status of locations currently followed within the WFD with the one of locations impacted by WWTP emissaries, it would have been better to have fixed depths (which was already proposed by Derrien-Courtel, 2008). This would have allowed to compare more depths in the present study. Indeed, some algal belts may not be found due to a too narrow algal belt to be defined maybe influenced by the presence of the wastewater discharge. For example, it may be difficult to find some algal belts close to the emissary especially because they are based on presence/absence and abundance of *Cystoseira* spp. which is a sensitive alga. Moreover, this could also be due to the presence of sediment leading to a truncated lower limit of the N2 (upper infralittoral) and/or the absence of the N3 (lower infralittoral) (Derrien-Courtel and Le Gal, 2014a). Finally, it is important to note that, in the case where the study of WWTP discharges on benthic communities continues, and where other subtidal emissary are added to the study, another problem might arise and should be considered: at a same distance from the emissary, depths between the different locations might be different (according to the location topography).

Following this study, it has been noted that the red alga, *Gelidium* spp. (mainly *G. corneum*) exhibited a higher abundance and larger and more vigorous fronds (dark red pigmentation) in the impacted locations. This was especially the case in the subtidal zone where this species was identified as the main contributor of dissimilarities between impacted and control locations with higher abundances in the impacted ones. The same was observed in the intertidal zone (especially in 'WWTP 4' location) even if this species was not identified as significantly more abundant in the impacted zones. This was a little surprising in view of the fact that this species, described as a dominant foundation species in the south-eastern Bay of Biscay (Quintano et al., 2019), was identified as indicator good ecological status within the WFD (de Casamajor and Lissardy, 2018) and as essentially found in unpolluted habitats in Spain (Díez et al., 2003). The sensitivity of this algae to irradiance was studied by Quintano et al. (2019). The authors highlighted that light conditions may play a role in the increase or decline of *G. corneum* because the stress response of this alga increased at higher irradiance levels. In the context of WWTP discharges, *Gelidium* spp. is probably more positively impacted by other factors related to sewage discharges: nutrient enrichment, turbidity, increased sedimentation, decreased salinity (Azzurro et al., 2010; Terlizzi et al., 2005). For example, *G. corneum* was already reported as increasing in sites with extra loads of nutrient and turbidity (Díez et al., 2012b). Another study which investigated the effects of several factor interaction (such as temperature, photosynthetic irradiance, UV radiation, nutrient availability) on the acclimation capacity of this alga, also highlighted a positive effect of nitrogen supply on its photosynthesis performance (Miguel-Vijandi et al., 2010). Consequently, even though this species was described as indicator of good ecological status, it would seemed that it would rather be a sign of disturbance in this area.

Finally, as suggested for chemical analyses, further quadrats positioned at different distances from the outfall (at the same depth) could be achieved to study the dilution effect (mainly linked to high energetic hydrodynamic conditions in this area) on assemblage structure and thus to know if assemblages follow the dilution gradient. Thus, if the study of the impact of WWTP discharge on benthic communities routinely and over longer periods is implemented, this would allow to delineate a smaller sampling area around the outfall.

4. Could benthic communities constitute good bio-accumulator/indicator of such a pressure?

Even though species selected for the bioaccumulation analysis were chosen due to their presence in most of locations, their relatively ease of sampling and their sufficient amount of matter, some difficulties were anyway encountered during the sampling. Indeed, it was rather difficult to find organisms providing sufficient amount of matter and being present in most of location even if some were widely reported as good bioaccumulator (e.g. mussels). For example, some organisms were either too small (e.g. mussels) or too sensitive to be collected at the outlet of emissaries while they presented sufficient amount of matter (e.g. *Cystoseira tamariscifolia* in the intertidal zone). We are aware that the number of samples was limited for this study but we have faced to field reality and constraints. That is why, this study allowed to identify species that could be interesting to be followed in such a context including technical constraints. The best bioindicators appeared to be *Ulva* spp. and *Gelidium spp.* which were also identified as a good indicator of WWTP disturbance according to the ecological approach. Further experimental analyses would be necessary to confirm its bioaccumulation capacity and explain its presence essentially proximate to WWTP discharges. The experiments should be made by taking into account mixture and chronic effects of different substances that were identified during the present study. Finally, as main bioacculumators were primary producers (*Ulva* spp. and *Gelidium* spp.), additional researches would be also interesting to do to explore potential biomagnification process up the food chain.

As some substances (i.e. metals and APs) were not analyzed in biota samples (due to a lack of time and the absence of validated analytical method), it would be interesting to do further efforts to analyses the missing substances in this matrix or develop analytical methods particularly because these substances were identified in high concentrations in WWTP effluents (especially for metals). Therefore, they would have great chances to be also detected in the organisms collected close the outfalls.

In general manner, and as for wastewater analyses, it appeared that analyses were expensive and time consuming. Consequently, it is important to consider these parameters in addition to the fact this approach is destructive, if this approach is kept in the future to monitor the WWTP discharge effects on coastal environment. Moreover, these first findings associated to those of additional suggested analyses, could allow to complete regulatory list establishing limits that have not to be exceeded in this matrix. It seems also important to perform the analyses on wet weight basis instead of on dry weigh basis to be able to do precise comparison with current regulatory limits but, in this case, analytical methods should certainly be reviewed, optimized and validated.

5. Do WWTP discharges constitute a source of micropollutants into the Ocean along the Basque coast and do they impact rocky benthic communities?

This study provided, for the first time in this area, a first insight of the occurrences and concentrations of priority and emerging substances in WWTP discharges and their potential impact on rocky benthic communities in the southeastern Bay of Biscay. Results have highlighted:

- Main released substances (or group of substances) into the Ocean by local WWTPs,
- Species identified as good bioindicators and/or bioaccumulators of the 'WWTP' pressure in this coastal area and,
- Assessment tools (validated and already commonly used) that appeared as enough sensitive and thus useful to assess the ecological impact of such a pressure on coastal biodiversity.

The two approaches followed during this study (chemical and ecological ones) presented each, some strengths and weaknesses (**Table 1**) but they appeared to be anyway complementary even though the link between both may be sometimes difficult to be made (due to technical constraints and difficulties to distinguish natural variability to anthropogenic impacts). The ecological approach highlighted the potential impact of the 'WWTP' pressure on all benthic communities and identified some species considered as indicator of polluted or unpolluted environment. By contrast, the chemical approach provided quantitative data (concentrations) on a specific number of analytes from wastewater samples and from a restricted selection of species. In both cases, it has been seen that results of one of both approaches could allow to confirm findings of the other one or help its implementation. Therefore, this work provides a framework for future monitoring and highlights ways that should be deepen explored in order to confirm present results and suggestions. The objective in the future will be thus to reflect upon how to implement such analyses in monitoring (adapted and applicable to the whole Atlantic coast) while reflecting as much as possible the reality (communities and habitat health status), by being simple to apply and easily understood, relevant in the context (fulfilling the regulatory requirements) and acceptable in terms of costs and time.

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ANNEXES

Annex captions:

Annex 1: Details of each key dates presented in the chronology of major Conventions, European Directives and French laws about water, aquatic environment and chemical substances impacting them.

(*)
- First French water regulations (Barataud, 2014)
- Organize ownership and usage of the water resource (Barataud, 2014)
- Aim to meet public health objectives (Barataud, 2014)

(1)
- Establishes (with the second French water law) a framework for an integrated water management per water catchment
- Creates the 6 Water supply Agencies which have the principal mission of redistributing aid from the feed collected from all industries and individuals impacting water resources (Barataud, 2014)
- Aims to decrease pollution from all sources and protect water resources and aquatic environment

(2)
- **Beginning of chemical pollutants regulation in water**
- Establishes a general regulation for the elimination of certain dangerous substances
- 2 lists containing 150 substances discharged into the aquatic environment:

 * **List I**: Dangerous substances according to their toxicity, persistence and bio-accumulation
 * **List II**: Less hazardous
(Briand et al., 2018; EEC, 1976)

(3)
- Establishes the first bases of sewage treatment by fixing types of treatment and deadlines to protect aquatic environment from wastewater discharges (Briand et al., 2018)
- Imposes on all Member states:

 * to collect and treat urban wastewaters prior to reject them into the environment
 * to ensure that total quantities of toxic, persistent or bioaccumulative substances of WWTP sludge have to be subject to authorization and progressively reduced (EEC, 1991)

(4)
- First time that attention to the environment itself and the notion of ecosystems appeared (Barataud, 2014)
- Introduces the notion of point-source of pollution (Barataud, 2014)
- Establishes new water management tools in order to define guidelines and objectives to attain the GEQ:

 * the SDAGE, a management plan at the catchment areas scale
 * the SAGE, the Water Development and Management Plan at the catchment area unit scale (EC, 2000)

(5)
- Promotes the maintenance of biodiversity the conservation of natural habitats and wild fauna and flora (EEC, 1992)
- Establishes the EU wide Natura 2000 ecological network of protected areas (EEC, 1992)
- Protects in various ways 200 habitat types and 1 000 animal and plant species (EEC, 1992)
- Supports the European Red Lists of Threatened Species elaborated by the IUCN (EEC, 1992)

(6)
- Concluded on behalf of the EU (formerly called European Community) (Convention, 1992)
- From the merged between the Oslo Convention (1972) and the Paris Convention (1974)
- Initiated to protect and monitor the marine environment from pollution and adverse effects of human activities in the North-East Atlantic (Convention, 1992)
- 5 thematic strategies (Biodiversity and Ecosystems, Eutrophication, Hazardous substances, Oil and Gas offshore industries and Radioactive substances)
- Highlights worrying substances for marine environment according to their persistence, bioaccumulative and toxic features (Convention, 1992)
- Lists 28 substances or groups of substances (with a further 264 compounds) as contaminants of possible concern (Miller, 2018).

(7)
- Standardizes policies and implements a framework for the assessment, management, protection and improvement of the quality of water resources and aquatic environment at the European scale (EC, 2000; European Environment Agency, 2018a)
- Good Ecological Quality of European surface waters and groundwater achieved by 2020
- Established at the catchment areas scale
- Assessment of status of surface and groundwater achieved through:
  * Ecological status
  * Chemical status
- **Establishes provision for a list of Priority Substances** (Annex X of the Directive)

(8)
- Amends the WFD (EC, 2001)
- **Establishes the First list of priority substances (33** among those 11 priority substances) (http://ec.europa.eu)
- Aims to stop or remove their discharge, emission and loss within 20 years (EC, 2001)

(9)
- Translates the WFD at the National scale

(10)
- Concerns the Registration, Evaluation, Authorisation and Restriction of Chemicals
- Regulates the assessment of their impacts on human health and the environment
- Imposes to industries to identify risks that marked and manufactured substances may have

(EC, 2006; European Environment Agency, 2018b)

Annex 1: (continued)

(11)
- Strengthens regulatory tools for a better implementation of the WFD and the achievement of its requirements (https://www.eaufrance.fr)
- First time that the notion of non-point of pollution appeared in a French law (Barataud, 2014)
- Creates the French National Agency for Water and Aquatic Environments (ONEMA) which accompanies the implementation of public water policy in France (Barataud, 2014)

(12)
- Transposes the European Directive of May 21th 1991 into the French law
- Includes all technical prescriptions for sanitation systems (design, dimension, exploitation, purification performance, self-monitoring, control)
- Concerns all collective sanitations and wastewater treatment plants as well as all un-collective systems receiving a DBO5 concentration higher than 1.2 kg/day

(13)
- **Constitutes an extension of the WFD to all marine ecosystems** (O'Connor, 2013)
- Aims to achieve or maintain the Good Ecological Quality (GEQ) of the European marine waters by 2021 (EC, 2008a)
- Proposes 11 environmental qualitative descriptors (Danovaro, 2016; Patricio, 2016; Borja, 2011)
- Established at the marine sub-regions scale

(14)
- Amends previous Directives including the WFD (EC, 2008b)
- Establishes the list of 33 priority substances in Annex II as Annex X of the WFD (EC, 2008b)
- **Fixes Environmental Quality Standards (EQS) for these substances and 8 other pollutants**
- The proposal of 2011, amends the WFD and the EQSD and adds 15 additional priority substances (including 6 priority hazardous substances) (http://ec.europa.eu)

(15)
Presents:
- General conditions of application of the criteria for GES
- Criteria for GES relevant to the descriptors of Annex I to Directive 2008/56/EC (EU, 2010)

(16)
- Modifies the WFD and the EQSD and adds **12 additional priority substances (for a total of 45)**

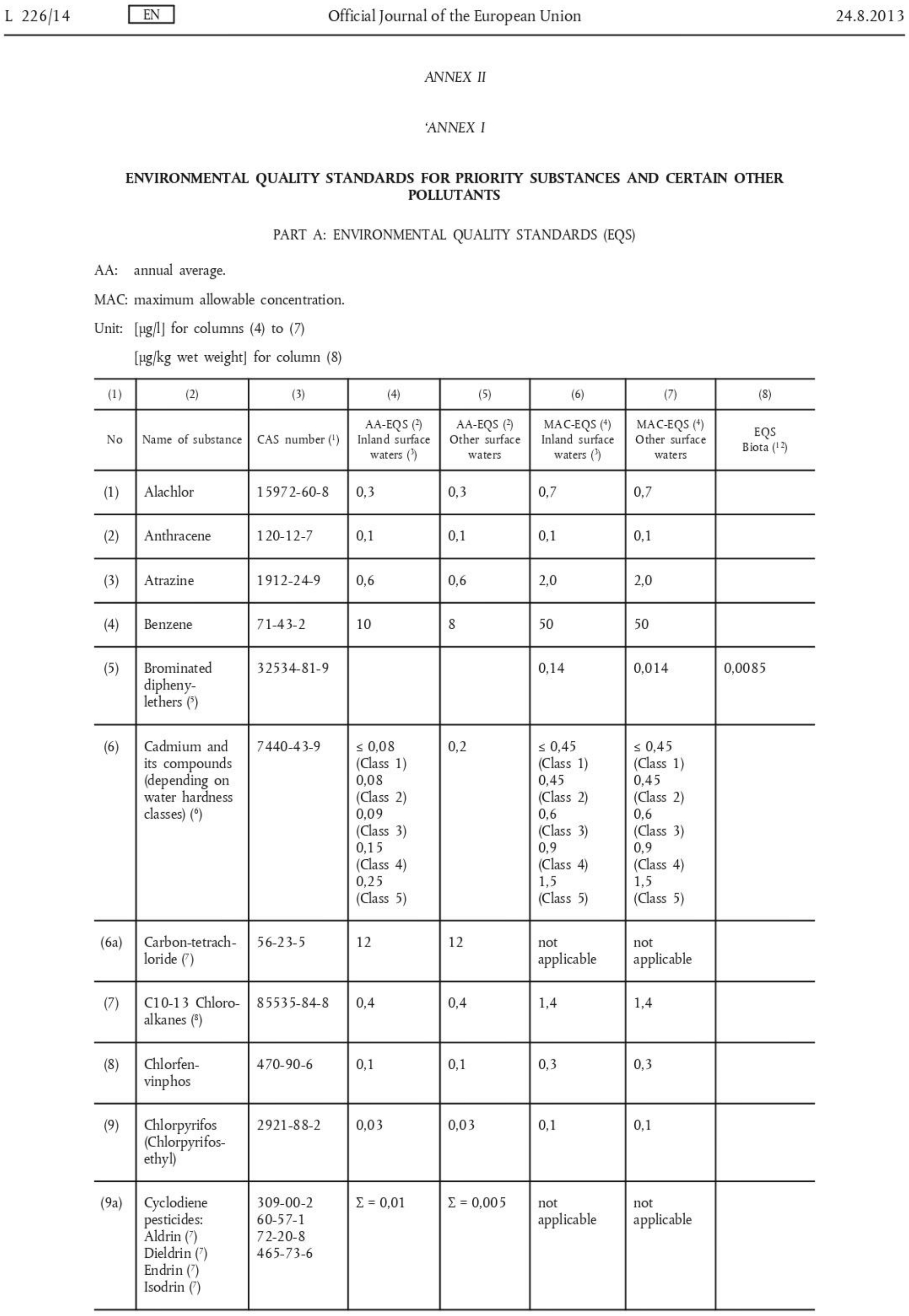
(EU, 2013; www.oreau.eu)

(17)
- Replaces the French Decision of June 22th 2007
- Main modifications:
  - * Introduces prescriptions about micropollutants monitoring in wastewater treatment plant discharges
  - * Regular monitoring by communities of their sanitation systems to ensure management over longer periods

(18)
- Creates the French Agency for Biodiversity (**AFB**) regrouping:
  - * the ONEMA,
  - * the Technical Workshop for Natural Areas (ATEN)
  - * the National Parks of France (PNF)
  - * the Agency for Protected Marine Areas (AAMP)

(www.gouvernement.fr)

Annex 2: Extract of the Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy Text with EEA relevance.



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ANNEX II

'ANNEX I

ENVIRONMENTAL QUALITY STANDARDS FOR PRIORITY SUBSTANCES AND CERTAIN OTHER POLLUTANTS

PART A: ENVIRONMENTAL QUALITY STANDARDS (EQS)

AA: annual average.

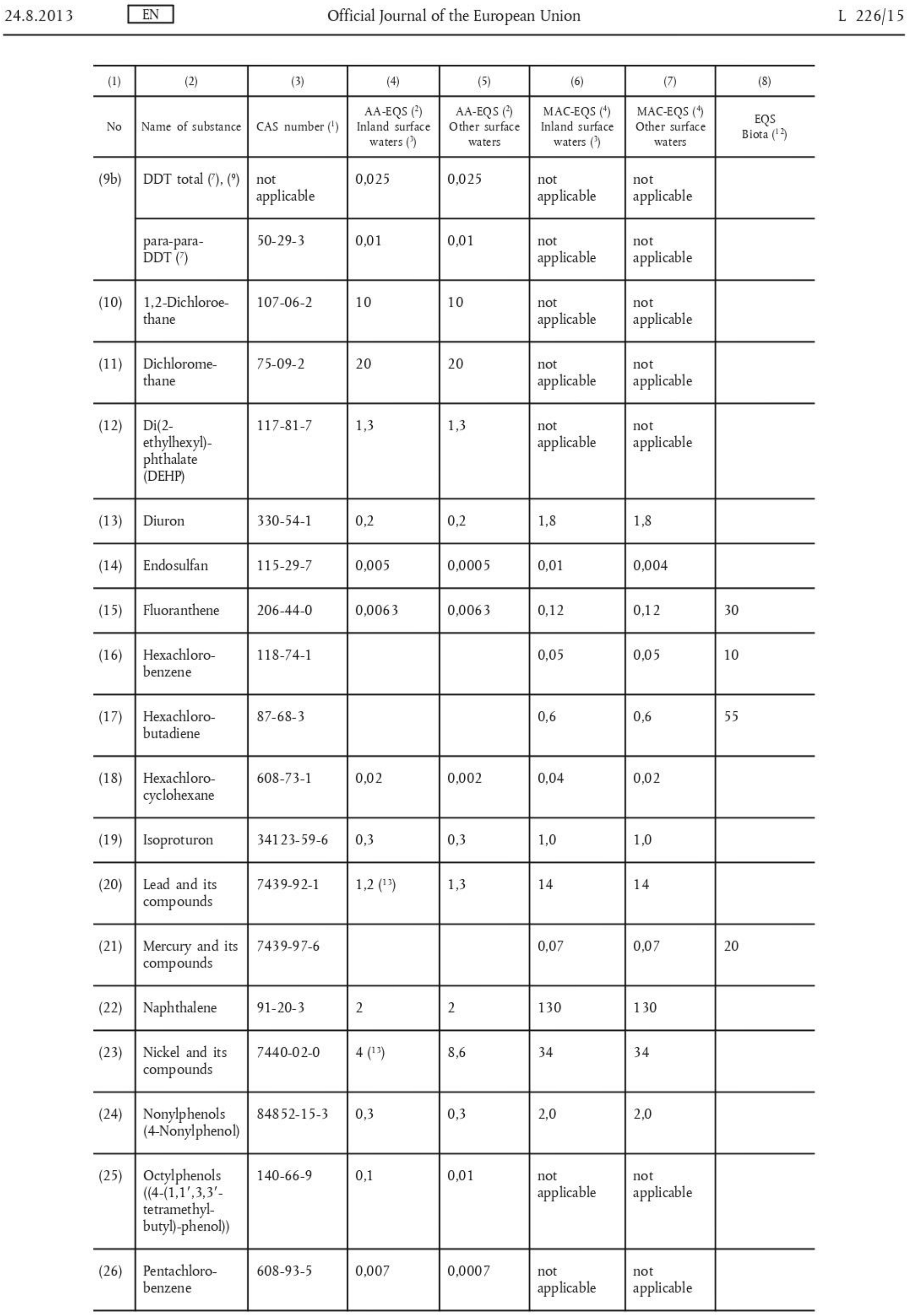
MAC: maximum allowable concentration.

Unit: [µg/l] for columns (4) to (7)

[µg/kg wet weight] for column (8)

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
No	Name of substance	CAS number (1)	AA-EQS (2) Inland surface waters (3)	AA-EQS (2) Other surface waters	MAC-EQS (4) Inland surface waters (3)	MAC-EQS (4) Other surface waters	EQS Biota (12)
(1)	Alachlor	15972-60-8	0,3	0,3	0,7	0,7	
(2)	Anthracene	120-12-7	0,1	0,1	0,1	0,1	
(3)	Atrazine	1912-24-9	0,6	0,6	2,0	2,0	
(4)	Benzene	71-43-2	10	8	50	50	
(5)	Brominated diphenylethers (5)	32534-81-9			0,14	0,014	0,0085
(6)	Cadmium and its compounds (depending on water hardness classes) (6)	7440-43-9	≤ 0,08 (Class 1) 0,08 (Class 2) 0,09 (Class 3) 0,15 (Class 4) 0,25 (Class 5)	0,2	≤ 0,45 (Class 1) 0,45 (Class 2) 0,6 (Class 3) 0,9 (Class 4) 1,5 (Class 5)	≤ 0,45 (Class 1) 0,45 (Class 2) 0,6 (Class 3) 0,9 (Class 4) 1,5 (Class 5)	
(6a)	Carbon-tetrachloride (7)	56-23-5	12	12	not applicable	not applicable	
(7)	C10-13 Chloroalkanes (8)	85535-84-8	0,4	0,4	1,4	1,4	
(8)	Chlorfenvinphos	470-90-6	0,1	0,1	0,3	0,3	
(9)	Chlorpyrifos (Chlorpyrifos-ethyl)	2921-88-2	0,03	0,03	0,1	0,1	
(9a)	Cyclodiene pesticides: Aldrin (7) Dieldrin (7) Endrin (7) Isodrin (7)	309-00-2 60-57-1 72-20-8 465-73-6	Σ = 0,01	Σ = 0,005	not applicable	not applicable	

Annex 2: (continued)



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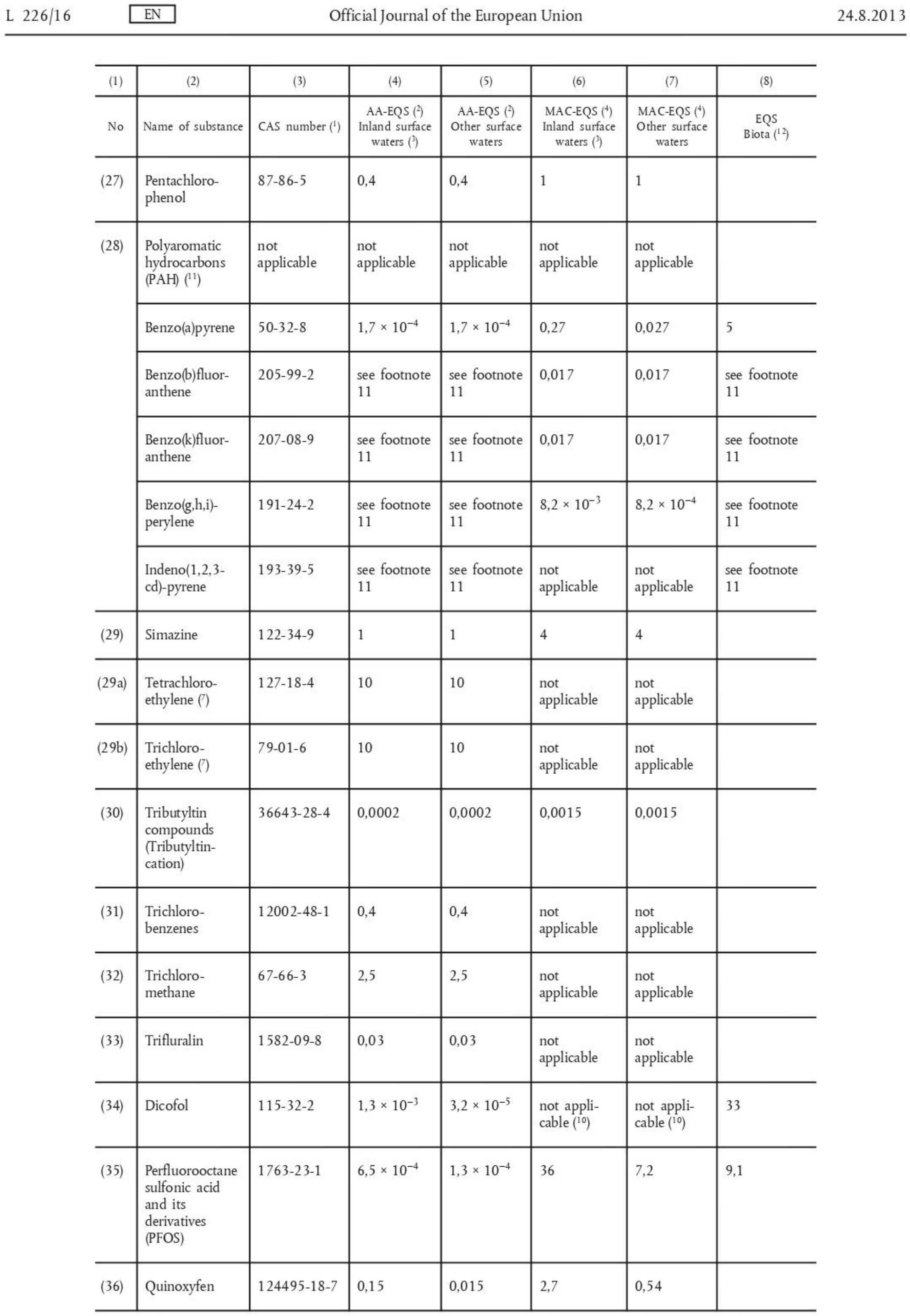
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
No	Name of substance	CAS number (1)	AA-EQS (2) Inland surface waters (3)	AA-EQS (2) Other surface waters	MAC-EQS (4) Inland surface waters (3)	MAC-EQS (4) Other surface waters	EQS Biota (12)
(9b)	DDT total (7), (9)	not applicable	0,025	0,025	not applicable	not applicable	
	para-para-DDT (7)	50-29-3	0,01	0,01	not applicable	not applicable	
(10)	1,2-Dichloroethane	107-06-2	10	10	not applicable	not applicable	
(11)	Dichloromethane	75-09-2	20	20	not applicable	not applicable	
(12)	Di(2-ethylhexyl)-phthalate (DEHP)	117-81-7	1,3	1,3	not applicable	not applicable	
(13)	Diuron	330-54-1	0,2	0,2	1,8	1,8	
(14)	Endosulfan	115-29-7	0,005	0,0005	0,01	0,004	
(15)	Fluoranthene	206-44-0	0,0063	0,0063	0,12	0,12	30
(16)	Hexachlorobenzene	118-74-1			0,05	0,05	10
(17)	Hexachlorobutadiene	87-68-3			0,6	0,6	55
(18)	Hexachlorocyclohexane	608-73-1	0,02	0,002	0,04	0,02	
(19)	Isoproturon	34123-59-6	0,3	0,3	1,0	1,0	
(20)	Lead and its compounds	7439-92-1	1,2 (13)	1,3	14	14	
(21)	Mercury and its compounds	7439-97-6			0,07	0,07	20
(22)	Naphthalene	91-20-3	2	2	130	130	
(23)	Nickel and its compounds	7440-02-0	4 (13)	8,6	34	34	
(24)	Nonylphenols (4-Nonylphenol)	84852-15-3	0,3	0,3	2,0	2,0	
(25)	Octylphenols ((4-(1,1′,3,3′-tetramethylbutyl)-phenol))	140-66-9	0,1	0,01	not applicable	not applicable	
(26)	Pentachlorobenzene	608-93-5	0,007	0,0007	not applicable	not applicable	

Annex 2: (continued)



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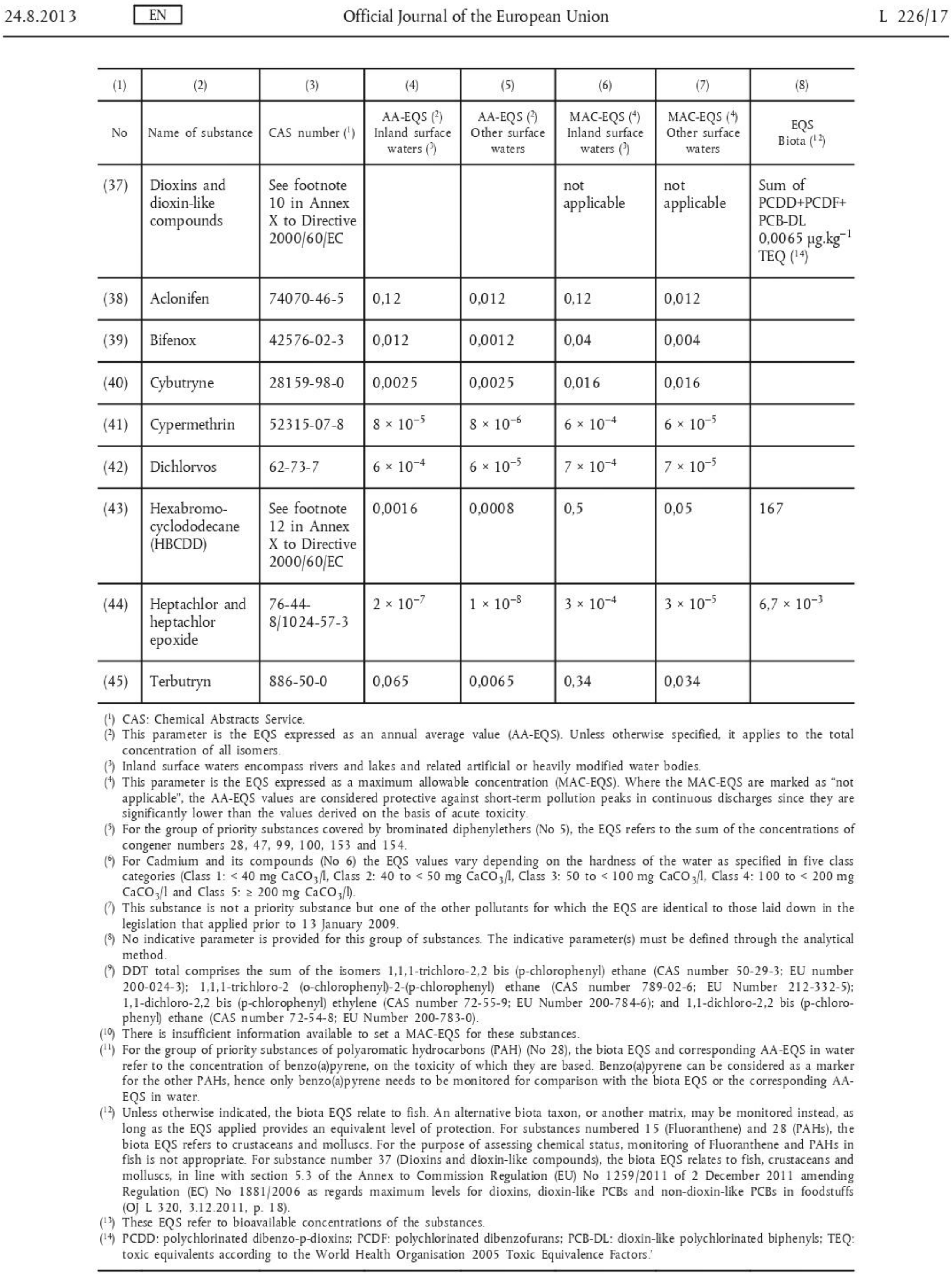
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
No	Name of substance	CAS number (1)	AA-EQS (2) Inland surface waters (3)	AA-EQS (2) Other surface waters	MAC-EQS (4) Inland surface waters (3)	MAC-EQS (4) Other surface waters	EQS Biota (12)
(27)	Pentachlorophenol	87-86-5	0,4	0,4	1	1	
(28)	Polyaromatic hydrocarbons (PAH) (11)	not applicable	not applicable	not applicable	not applicable	not applicable	
	Benzo(a)pyrene	50-32-8	$1,7 \times 10^{-4}$	$1,7 \times 10^{-4}$	0,27	0,027	5
	Benzo(b)fluoranthene	205-99-2	see footnote 11	see footnote 11	0,017	0,017	see footnote 11
	Benzo(k)fluoranthene	207-08-9	see footnote 11	see footnote 11	0,017	0,017	see footnote 11
	Benzo(g,h,i)-perylene	191-24-2	see footnote 11	see footnote 11	$8,2 \times 10^{-3}$	$8,2 \times 10^{-4}$	see footnote 11
	Indeno(1,2,3-cd)-pyrene	193-39-5	see footnote 11	see footnote 11	not applicable	not applicable	see footnote 11
(29)	Simazine	122-34-9	1	1	4	4	
(29a)	Tetrachloroethylene (7)	127-18-4	10	10	not applicable	not applicable	
(29b)	Trichloroethylene (7)	79-01-6	10	10	not applicable	not applicable	
(30)	Tributyltin compounds (Tributyltin-cation)	36643-28-4	0,0002	0,0002	0,0015	0,0015	
(31)	Trichlorobenzenes	12002-48-1	0,4	0,4	not applicable	not applicable	
(32)	Trichloromethane	67-66-3	2,5	2,5	not applicable	not applicable	
(33)	Trifluralin	1582-09-8	0,03	0,03	not applicable	not applicable	
(34)	Dicofol	115-32-2	$1,3 \times 10^{-3}$	$3,2 \times 10^{-5}$	not applicable (10)	not applicable (10)	33
(35)	Perfluorooctane sulfonic acid and its derivatives (PFOS)	1763-23-1	$6,5 \times 10^{-4}$	$1,3 \times 10^{-4}$	36	7,2	9,1
(36)	Quinoxyfen	124495-18-7	0,15	0,015	2,7	0,54	

Annex 2: (continued)



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(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
No	Name of substance	CAS number (¹)	AA-EQS (²) Inland surface waters (³)	AA-EQS (²) Other surface waters	MAC-EQS (⁴) Inland surface waters (³)	MAC-EQS (⁴) Other surface waters	EQS Biota (¹²)
(37)	Dioxins and dioxin-like compounds	See footnote 10 in Annex X to Directive 2000/60/EC			not applicable	not applicable	Sum of PCDD+PCDF+PCB-DL 0,0065 μg.kg^{-1} TEQ (¹⁴)
(38)	Aclonifen	74070-46-5	0,12	0,012	0,12	0,012	
(39)	Bifenox	42576-02-3	0,012	0,0012	0,04	0,004	
(40)	Cybutryne	28159-98-0	0,0025	0,0025	0,016	0,016	
(41)	Cypermethrin	52315-07-8	8×10^{-5}	8×10^{-6}	6×10^{-4}	6×10^{-5}	
(42)	Dichlorvos	62-73-7	6×10^{-4}	6×10^{-5}	7×10^{-4}	7×10^{-5}	
(43)	Hexabromo-cyclododecane (HBCDD)	See footnote 12 in Annex X to Directive 2000/60/EC	0,0016	0,0008	0,5	0,05	167
(44)	Heptachlor and heptachlor epoxide	76-44-8/1024-57-3	2×10^{-7}	1×10^{-8}	3×10^{-4}	3×10^{-5}	$6{,}7 \times 10^{-3}$
(45)	Terbutryn	886-50-0	0,065	0,0065	0,34	0,034	

(¹) CAS: Chemical Abstracts Service.

(²) This parameter is the EQS expressed as an annual average value (AA-EQS). Unless otherwise specified, it applies to the total concentration of all isomers.

(³) Inland surface waters encompass rivers and lakes and related artificial or heavily modified water bodies.

(⁴) This parameter is the EQS expressed as a maximum allowable concentration (MAC-EQS). Where the MAC-EQS are marked as "not applicable", the AA-EQS values are considered protective against short-term pollution peaks in continuous discharges since they are significantly lower than the values derived on the basis of acute toxicity.

(⁵) For the group of priority substances covered by brominated diphenylethers (No 5), the EQS refers to the sum of the concentrations of congener numbers 28, 47, 99, 100, 153 and 154.

(⁶) For Cadmium and its compounds (No 6) the EQS values vary depending on the hardness of the water as specified in five class categories (Class 1: < 40 mg $CaCO_3$/l, Class 2: 40 to < 50 mg $CaCO_3$/l, Class 3: 50 to < 100 mg $CaCO_3$/l, Class 4: 100 to < 200 mg $CaCO_3$/l and Class 5: ≥ 200 mg $CaCO_3$/l).

(⁷) This substance is not a priority substance but one of the other pollutants for which the EQS are identical to those laid down in the legislation that applied prior to 13 January 2009.

(⁸) No indicative parameter is provided for this group of substances. The indicative parameter(s) must be defined through the analytical method.

(⁹) DDT total comprises the sum of the isomers 1,1,1-trichloro-2,2 bis (p-chlorophenyl) ethane (CAS number 50-29-3; EU number 200-024-3); 1,1,1-trichloro-2 (o-chlorophenyl)-2-(p-chlorophenyl) ethane (CAS number 789-02-6; EU Number 212-332-5); 1,1-dichloro-2,2 bis (p-chlorophenyl) ethylene (CAS number 72-55-9; EU Number 200-784-6); and 1,1-dichloro-2,2 bis (p-chlorophenyl) ethane (CAS number 72-54-8; EU Number 200-783-0).

(¹⁰) There is insufficient information available to set a MAC-EQS for these substances.

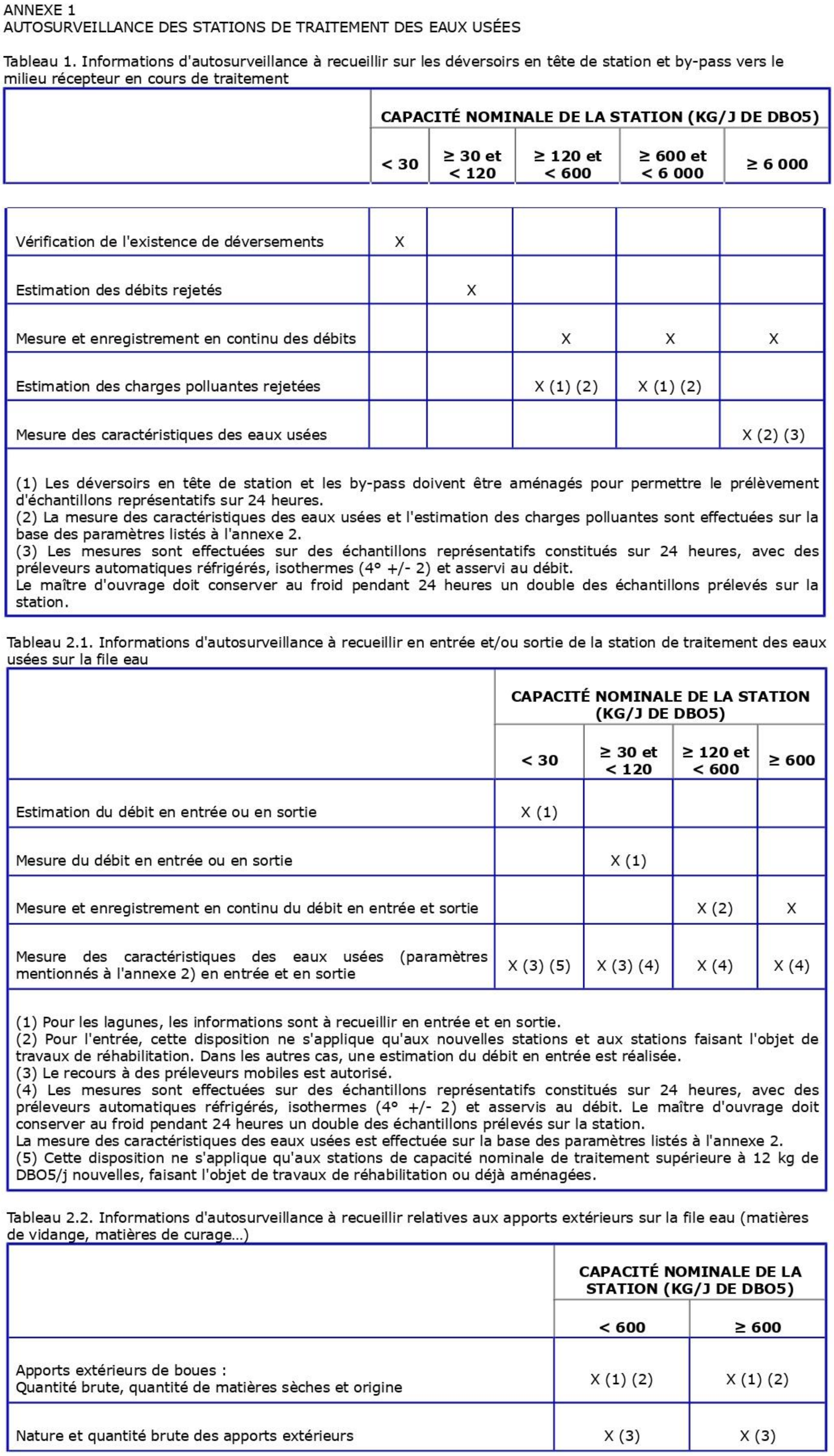
(¹¹) For the group of priority substances of polyaromatic hydrocarbons (PAH) (No 28), the biota EQS and corresponding AA-EQS in water refer to the concentration of benzo(a)pyrene, on the toxicity of which they are based. Benzo(a)pyrene can be considered as a marker for the other PAHs, hence only benzo(a)pyrene needs to be monitored for comparison with the biota EQS or the corresponding AA-EQS in water.

(¹²) Unless otherwise indicated, the biota EQS relate to fish. An alternative biota taxon, or another matrix, may be monitored instead, as long as the EQS applied provides an equivalent level of protection. For substances numbered 15 (Fluoranthene) and 28 (PAHs), the biota EQS refers to crustaceans and molluscs. For the purpose of assessing chemical status, monitoring of Fluoranthene and PAHs in fish is not appropriate. For substance number 37 (Dioxins and dioxin-like compounds), the biota EQS relates to fish, crustaceans and molluscs, in line with section 5.3 of the Annex to Commission Regulation (EU) No 1259/2011 of 2 December 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for dioxins, dioxin-like PCBs and non-dioxin-like PCBs in foodstuffs (OJ L 320, 3.12.2011, p. 18).

(¹³) These EQS refer to bioavailable concentrations of the substances.

(¹⁴) PCDD: polychlorinated dibenzo-p-dioxins; PCDF: polychlorinated dibenzofurans; PCB-DL: dioxin-like polychlorinated biphenyls; TEQ: toxic equivalents according to the World Health Organisation 2005 Toxic Equivalence Factors.'

Annex 3: Annex extracts of July 21th 2015 Decision about collective and un-collective sanitation systems (except un-collective sanitation systems with a DBO5 concentration lower or equal to 1,2 kg/day).



ANNEXE 1
AUTOSURVEILLANCE DES STATIONS DE TRAITEMENT DES EAUX USÉES

Tableau 1. Informations d'autosurveillance à recueillir sur les déversoirs en tête de station et by-pass vers le milieu récepteur en cours de traitement

	CAPACITÉ NOMINALE DE LA STATION (KG/J DE DBO5)				
	< 30	**≥ 30 et < 120**	**≥ 120 et < 600**	**≥ 600 et < 6 000**	**≥ 6 000**
Vérification de l'existence de déversements	X				
Estimation des débits rejetés		X			
Mesure et enregistrement en continu des débits			X	X	X
Estimation des charges polluantes rejetées			X (1) (2)	X (1) (2)	
Mesure des caractéristiques des eaux usées					X (2) (3)

(1) Les déversoirs en tête de station et les by-pass doivent être aménagés pour permettre le prélèvement d'échantillons représentatifs sur 24 heures.
(2) La mesure des caractéristiques des eaux usées et l'estimation des charges polluantes sont effectuées sur la base des paramètres listés à l'annexe 2.
(3) Les mesures sont effectuées sur des échantillons représentatifs constitués sur 24 heures, avec des préleveurs automatiques réfrigérés, isothermes (4° +/- 2) et asservi au débit.
Le maître d'ouvrage doit conserver au froid pendant 24 heures un double des échantillons prélevés sur la station.

Tableau 2.1. Informations d'autosurveillance à recueillir en entrée et/ou sortie de la station de traitement des eaux usées sur la file eau

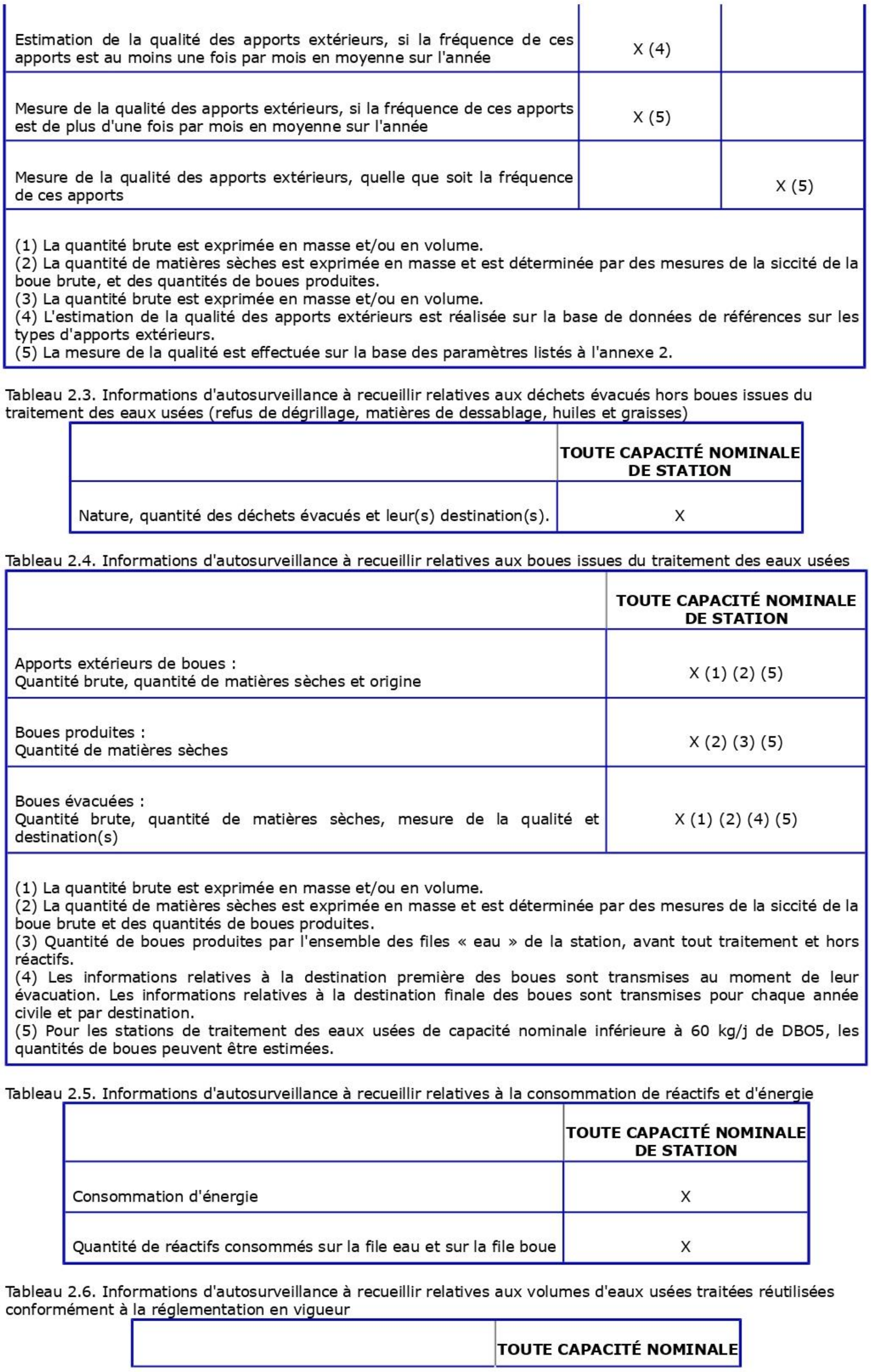
	CAPACITÉ NOMINALE DE LA STATION (KG/J DE DBO5)			
	< 30	**≥ 30 et < 120**	**≥ 120 et < 600**	**≥ 600**
Estimation du débit en entrée ou en sortie	X (1)			
Mesure du débit en entrée ou en sortie		X (1)		
Mesure et enregistrement en continu du débit en entrée et sortie			X (2)	X
Mesure des caractéristiques des eaux usées (paramètres mentionnés à l'annexe 2) en entrée et en sortie	X (3) (5)	X (3) (4)	X (4)	X (4)

(1) Pour les lagunes, les informations sont à recueillir en entrée et en sortie.
(2) Pour l'entrée, cette disposition ne s'applique qu'aux nouvelles stations et aux stations faisant l'objet de travaux de réhabilitation. Dans les autres cas, une estimation du débit en entrée est réalisée.
(3) Le recours à des préleveurs mobiles est autorisé.
(4) Les mesures sont effectuées sur des échantillons représentatifs constitués sur 24 heures, avec des préleveurs automatiques réfrigérés, isothermes (4° +/- 2) et asservis au débit. Le maître d'ouvrage doit conserver au froid pendant 24 heures un double des échantillons prélevés sur la station.
La mesure des caractéristiques des eaux usées est effectuée sur la base des paramètres listés à l'annexe 2.
(5) Cette disposition ne s'applique qu'aux stations de capacité nominale de traitement supérieure à 12 kg de DBO5/j nouvelles, faisant l'objet de travaux de réhabilitation ou déjà aménagées.

Tableau 2.2. Informations d'autosurveillance à recueillir relatives aux apports extérieurs sur la file eau (matières de vidange, matières de curage…)

	CAPACITÉ NOMINALE DE LA STATION (KG/J DE DBO5)	
	< 600	**≥ 600**
Apports extérieurs de boues : Quantité brute, quantité de matières sèches et origine	X (1) (2)	X (1) (2)
Nature et quantité brute des apports extérieurs	X (3)	X (3)

Annex 3: (continued)



Estimation de la qualité des apports extérieurs, si la fréquence de ces apports est au moins une fois par mois en moyenne sur l'année	X (4)	
Mesure de la qualité des apports extérieurs, si la fréquence de ces apports est de plus d'une fois par mois en moyenne sur l'année	X (5)	
Mesure de la qualité des apports extérieurs, quelle que soit la fréquence de ces apports		X (5)

(1) La quantité brute est exprimée en masse et/ou en volume.
(2) La quantité de matières sèches est exprimée en masse et est déterminée par des mesures de la siccité de la boue brute, et des quantités de boues produites.
(3) La quantité brute est exprimée en masse et/ou en volume.
(4) L'estimation de la qualité des apports extérieurs est réalisée sur la base de données de références sur les types d'apports extérieurs.
(5) La mesure de la qualité est effectuée sur la base des paramètres listés à l'annexe 2.

Tableau 2.3. Informations d'autosurveillance à recueillir relatives aux déchets évacués hors boues issues du traitement des eaux usées (refus de dégrillage, matières de dessablage, huiles et graisses)

	TOUTE CAPACITÉ NOMINALE DE STATION
Nature, quantité des déchets évacués et leur(s) destination(s).	X

Tableau 2.4. Informations d'autosurveillance à recueillir relatives aux boues issues du traitement des eaux usées

	TOUTE CAPACITÉ NOMINALE DE STATION
Apports extérieurs de boues : Quantité brute, quantité de matières sèches et origine	X (1) (2) (5)
Boues produites : Quantité de matières sèches	X (2) (3) (5)
Boues évacuées : Quantité brute, quantité de matières sèches, mesure de la qualité et destination(s)	X (1) (2) (4) (5)

(1) La quantité brute est exprimée en masse et/ou en volume.
(2) La quantité de matières sèches est exprimée en masse et est déterminée par des mesures de la siccité de la boue brute et des quantités de boues produites.
(3) Quantité de boues produites par l'ensemble des files « eau » de la station, avant tout traitement et hors réactifs.
(4) Les informations relatives à la destination première des boues sont transmises au moment de leur évacuation. Les informations relatives à la destination finale des boues sont transmises pour chaque année civile et par destination.
(5) Pour les stations de traitement des eaux usées de capacité nominale inférieure à 60 kg/j de DBO5, les quantités de boues peuvent être estimées.

Tableau 2.5. Informations d'autosurveillance à recueillir relatives à la consommation de réactifs et d'énergie

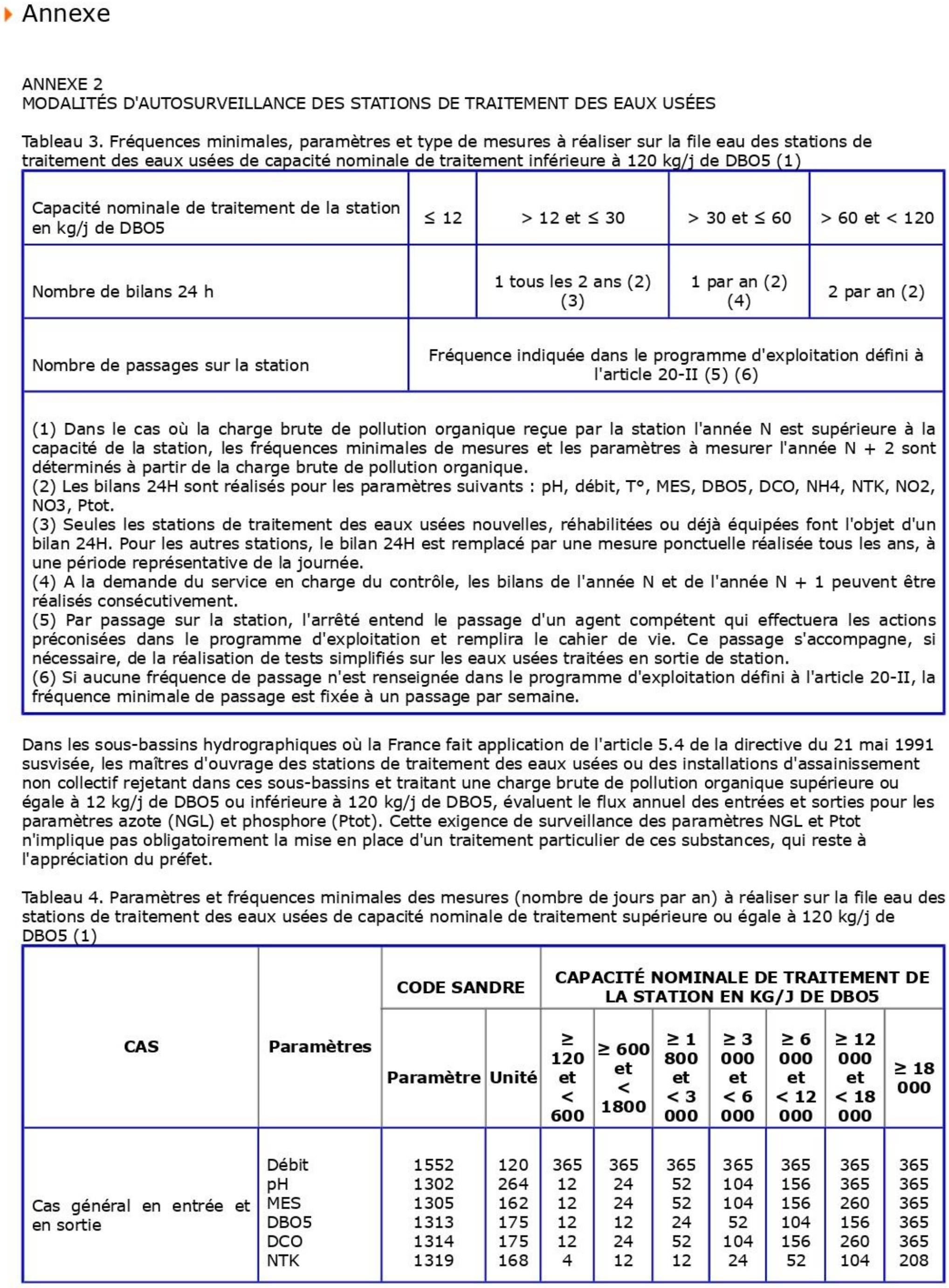
	TOUTE CAPACITÉ NOMINALE DE STATION
Consommation d'énergie	X
Quantité de réactifs consommés sur la file eau et sur la file boue	X

Tableau 2.6. Informations d'autosurveillance à recueillir relatives aux volumes d'eaux usées traitées réutilisées conformément à la réglementation en vigueur

	TOUTE CAPACITÉ NOMINALE

Annex 3: (continued)

	DE STATION
Volume d'eaux usées traitées réutilisées	X
Destination des eaux usées traitées réutilisées	X



Annexe

ANNEXE 2
MODALITÉS D'AUTOSURVEILLANCE DES STATIONS DE TRAITEMENT DES EAUX USÉES

Tableau 3. Fréquences minimales, paramètres et type de mesures à réaliser sur la file eau des stations de traitement des eaux usées de capacité nominale de traitement inférieure à 120 kg/j de DBO5 (1)

Capacité nominale de traitement de la station en kg/j de DBO5	≤ 12	> 12 et ≤ 30	> 30 et ≤ 60	> 60 et < 120
Nombre de bilans 24 h		1 tous les 2 ans (2) (3)	1 par an (2) (4)	2 par an (2)
Nombre de passages sur la station	Fréquence indiquée dans le programme d'exploitation défini à l'article 20-II (5) (6)			

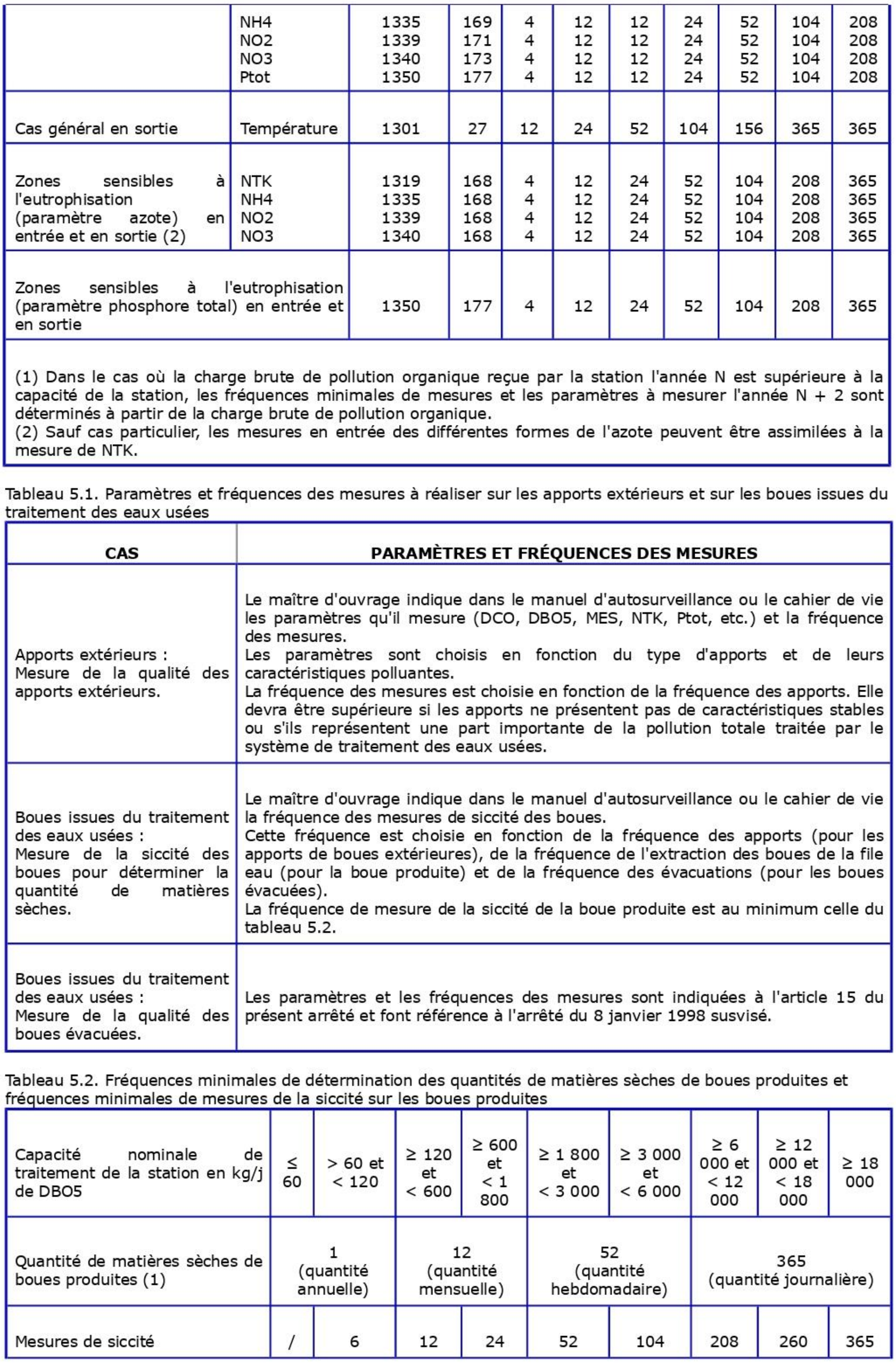
(1) Dans le cas où la charge brute de pollution organique reçue par la station l'année N est supérieure à la capacité de la station, les fréquences minimales de mesures et les paramètres à mesurer l'année N + 2 sont déterminés à partir de la charge brute de pollution organique.
(2) Les bilans 24H sont réalisés pour les paramètres suivants : pH, débit, T°, MES, DBO5, DCO, NH4, NTK, NO2, NO3, Ptot.
(3) Seules les stations de traitement des eaux usées nouvelles, réhabilitées ou déjà équipées font l'objet d'un bilan 24H. Pour les autres stations, le bilan 24H est remplacé par une mesure ponctuelle réalisée tous les ans, à une période représentative de la journée.
(4) A la demande du service en charge du contrôle, les bilans de l'année N et de l'année N + 1 peuvent être réalisés consécutivement.
(5) Par passage sur la station, l'arrêté entend le passage d'un agent compétent qui effectuera les actions préconisées dans le programme d'exploitation et remplira le cahier de vie. Ce passage s'accompagne, si nécessaire, de la réalisation de tests simplifiés sur les eaux usées traitées en sortie de station.
(6) Si aucune fréquence de passage n'est renseignée dans le programme d'exploitation défini à l'article 20-II, la fréquence minimale de passage est fixée à un passage par semaine.

Dans les sous-bassins hydrographiques où la France fait application de l'article 5.4 de la directive du 21 mai 1991 susvisée, les maîtres d'ouvrage des stations de traitement des eaux usées ou des installations d'assainissement non collectif rejetant dans ces sous-bassins et traitant une charge brute de pollution organique supérieure ou égale à 12 kg/j de DBO5 ou inférieure à 120 kg/j de DBO5, évaluent le flux annuel des entrées et sorties pour les paramètres azote (NGL) et phosphore (Ptot). Cette exigence de surveillance des paramètres NGL et Ptot n'implique pas obligatoirement la mise en place d'un traitement particulier de ces substances, qui reste à l'appréciation du préfet.

Tableau 4. Paramètres et fréquences minimales des mesures (nombre de jours par an) à réaliser sur la file eau des stations de traitement des eaux usées de capacité nominale de traitement supérieure ou égale à 120 kg/j de DBO5 (1)

CAS	**Paramètres**	**CODE SANDRE**		**CAPACITÉ NOMINALE DE TRAITEMENT DE LA STATION EN KG/J DE DBO5**						
		Paramètre	**Unité**	**≥ 120 et < 600**	**≥ 600 et < 1800**	**≥ 1 800 et < 3 000**	**≥ 3 000 et < 6 000**	**≥ 6 000 et < 12 000**	**≥ 12 000 et < 18 000**	**≥ 18 000**
Cas général en entrée et en sortie	Débit	1552	120	365	365	365	365	365	365	365
	pH	1302	264	12	24	52	104	156	365	365
	MES	1305	162	12	24	52	104	156	260	365
	DBO5	1313	175	12	12	24	52	104	156	365
	DCO	1314	175	12	24	52	104	156	260	365
	NTK	1319	168	4	12	12	24	52	104	208

Annex 3: (continued)



	NH4 NO2 NO3 Ptot	1335 1339 1340 1350	169 171 173 177	4 4 4 4	12 12 12 12	12 12 12 12	24 24 24 24	52 52 52 52	104 104 104 104	208 208 208 208
Cas général en sortie	Température	1301	27	12	24	52	104	156	365	365
Zones sensibles à l'eutrophisation (paramètre azote) en entrée et en sortie (2)	NTK NH4 NO2 NO3	1319 1335 1339 1340	168 168 168 168	4 4 4 4	12 12 12 12	24 24 24 24	52 52 52 52	104 104 104 104	208 208 208 208	365 365 365 365
Zones sensibles à l'eutrophisation (paramètre phosphore total) en entrée et en sortie		1350	177	4	12	24	52	104	208	365

(1) Dans le cas où la charge brute de pollution organique reçue par la station l'année N est supérieure à la capacité de la station, les fréquences minimales de mesures et les paramètres à mesurer l'année N + 2 sont déterminés à partir de la charge brute de pollution organique.
(2) Sauf cas particulier, les mesures en entrée des différentes formes de l'azote peuvent être assimilées à la mesure de NTK.

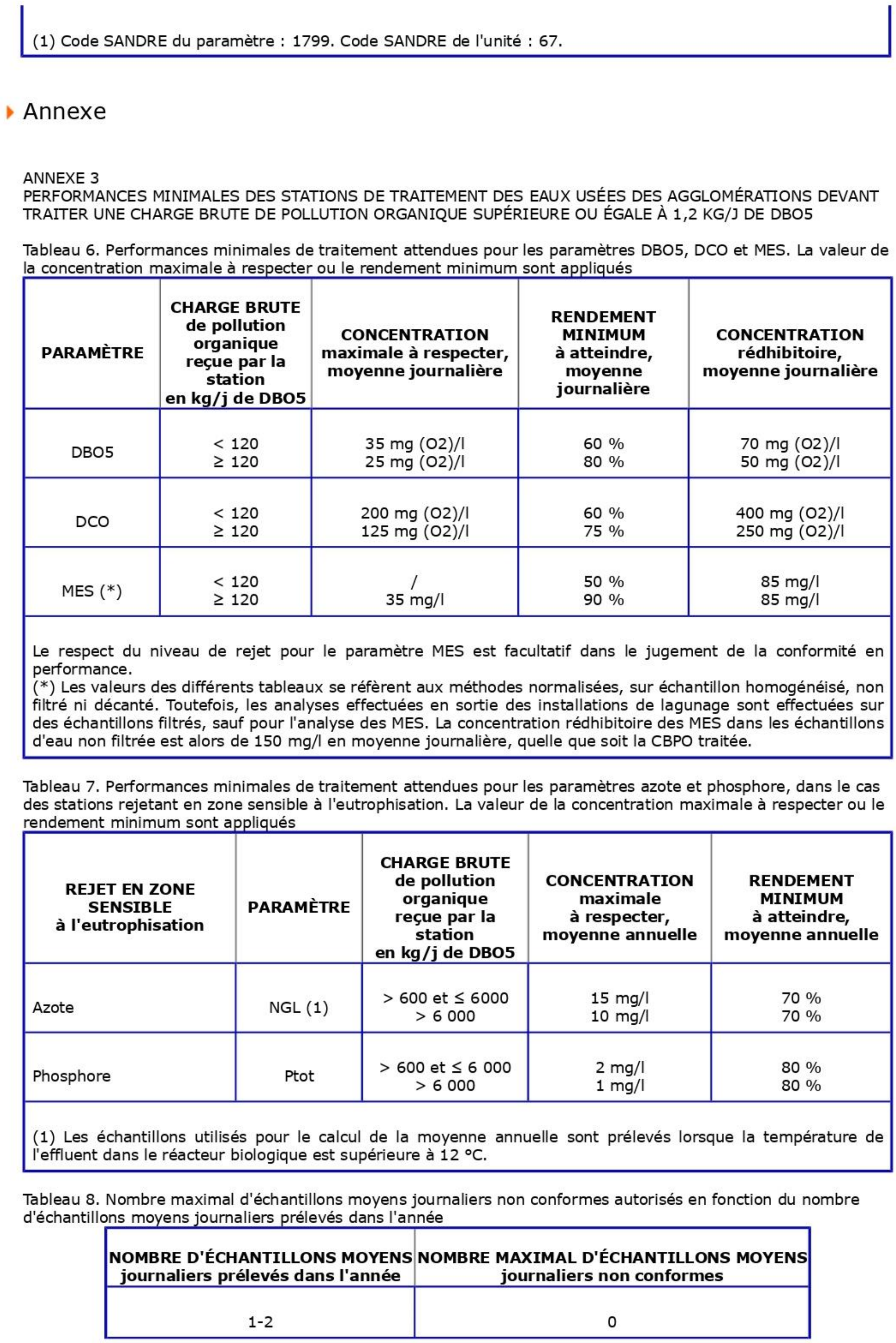
Tableau 5.1. Paramètres et fréquences des mesures à réaliser sur les apports extérieurs et sur les boues issues du traitement des eaux usées

CAS	PARAMÈTRES ET FRÉQUENCES DES MESURES
Apports extérieurs : Mesure de la qualité des apports extérieurs.	Le maître d'ouvrage indique dans le manuel d'autosurveillance ou le cahier de vie les paramètres qu'il mesure (DCO, DBO5, MES, NTK, Ptot, etc.) et la fréquence des mesures. Les paramètres sont choisis en fonction du type d'apports et de leurs caractéristiques polluantes. La fréquence des mesures est choisie en fonction de la fréquence des apports. Elle devra être supérieure si les apports ne présentent pas de caractéristiques stables ou s'ils représentent une part importante de la pollution totale traitée par le système de traitement des eaux usées.
Boues issues du traitement des eaux usées : Mesure de la siccité des boues pour déterminer la quantité de matières sèches.	Le maître d'ouvrage indique dans le manuel d'autosurveillance ou le cahier de vie la fréquence des mesures de siccité des boues. Cette fréquence est choisie en fonction de la fréquence des apports (pour les apports de boues extérieures), de la fréquence de l'extraction des boues de la file eau (pour la boue produite) et de la fréquence des évacuations (pour les boues évacuées). La fréquence de mesure de la siccité de la boue produite est au minimum celle du tableau 5.2.
Boues issues du traitement des eaux usées : Mesure de la qualité des boues évacuées.	Les paramètres et les fréquences des mesures sont indiquées à l'article 15 du présent arrêté et font référence à l'arrêté du 8 janvier 1998 susvisé.

Tableau 5.2. Fréquences minimales de détermination des quantités de matières sèches de boues produites et fréquences minimales de mesures de la siccité sur les boues produites

Capacité nominale de traitement de la station en kg/j de DBO5	≤ 60	> 60 et < 120	≥ 120 et < 600	≥ 600 et < 1 800	≥ 1 800 et < 3 000	≥ 3 000 et < 6 000	≥ 6 000 et < 12 000	≥ 12 000 et < 18 000	≥ 18 000
Quantité de matières sèches de boues produites (1)	1 (quantité annuelle)		12 (quantité mensuelle)		52 (quantité hebdomadaire)		365 (quantité journalière)		
Mesures de siccité	/	6	12	24	52	104	208	260	365

Annex 3: (continued)



(1) Code SANDRE du paramètre : 1799. Code SANDRE de l'unité : 67.

Annexe

ANNEXE 3
PERFORMANCES MINIMALES DES STATIONS DE TRAITEMENT DES EAUX USÉES DES AGGLOMÉRATIONS DEVANT TRAITER UNE CHARGE BRUTE DE POLLUTION ORGANIQUE SUPÉRIEURE OU ÉGALE À 1,2 KG/J DE DBO5

Tableau 6. Performances minimales de traitement attendues pour les paramètres DBO5, DCO et MES. La valeur de la concentration maximale à respecter ou le rendement minimum sont appliqués

PARAMÈTRE	CHARGE BRUTE de pollution organique reçue par la station en kg/j de DBO5	CONCENTRATION maximale à respecter, moyenne journalière	RENDEMENT MINIMUM à atteindre, moyenne journalière	CONCENTRATION rédhibitoire, moyenne journalière
DBO5	< 120 ≥ 120	35 mg (O2)/l 25 mg (O2)/l	60 % 80 %	70 mg (O2)/l 50 mg (O2)/l
DCO	< 120 ≥ 120	200 mg (O2)/l 125 mg (O2)/l	60 % 75 %	400 mg (O2)/l 250 mg (O2)/l
MES (*)	< 120 ≥ 120	/ 35 mg/l	50 % 90 %	85 mg/l 85 mg/l

Le respect du niveau de rejet pour le paramètre MES est facultatif dans le jugement de la conformité en performance.
(*) Les valeurs des différents tableaux se réfèrent aux méthodes normalisées, sur échantillon homogénéisé, non filtré ni décanté. Toutefois, les analyses effectuées en sortie des installations de lagunage sont effectuées sur des échantillons filtrés, sauf pour l'analyse des MES. La concentration rédhibitoire des MES dans les échantillons d'eau non filtrée est alors de 150 mg/l en moyenne journalière, quelle que soit la CBPO traitée.

Tableau 7. Performances minimales de traitement attendues pour les paramètres azote et phosphore, dans le cas des stations rejetant en zone sensible à l'eutrophisation. La valeur de la concentration maximale à respecter ou le rendement minimum sont appliqués

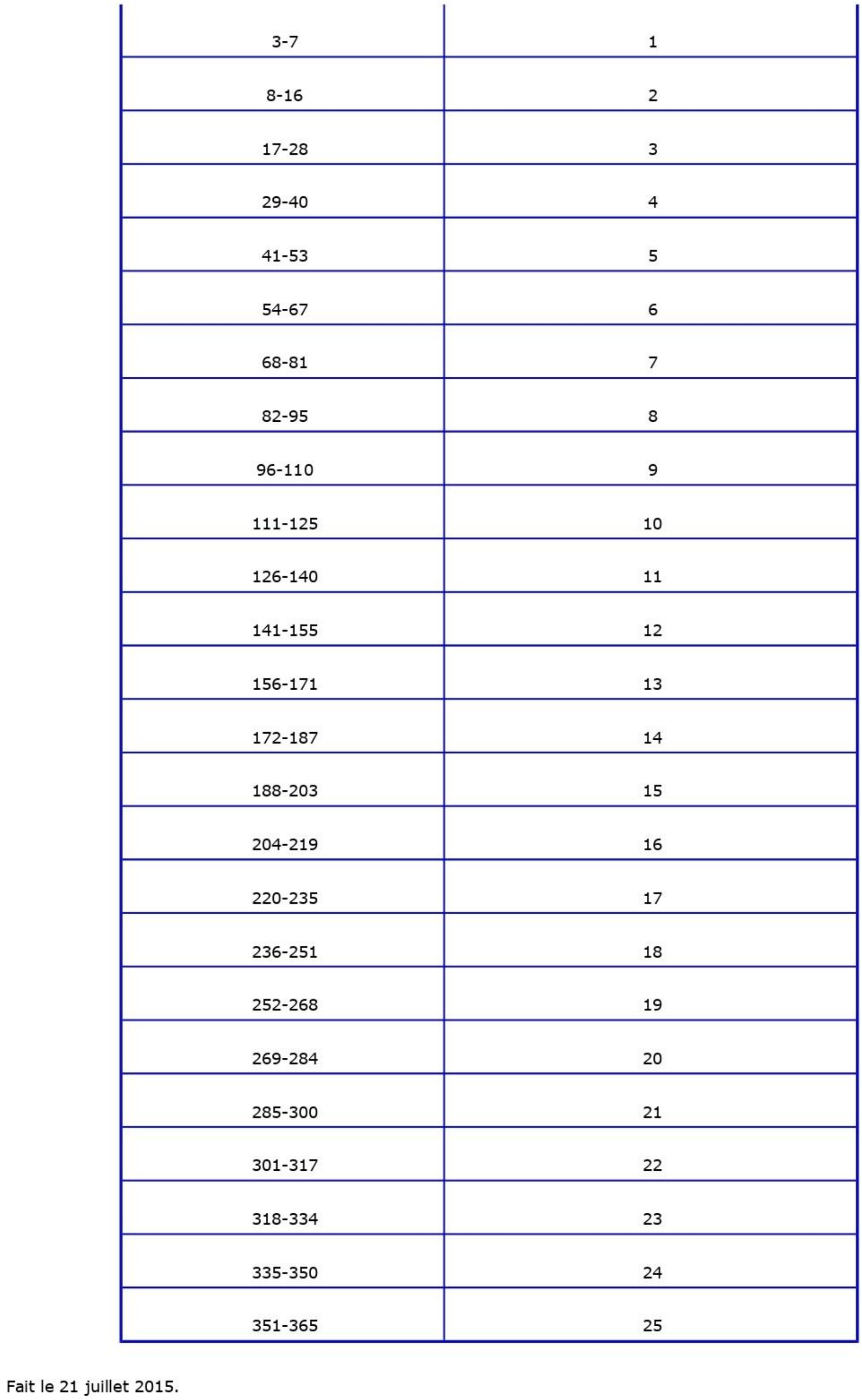
REJET EN ZONE SENSIBLE à l'eutrophisation	PARAMÈTRE	CHARGE BRUTE de pollution organique reçue par la station en kg/j de DBO5	CONCENTRATION maximale à respecter, moyenne annuelle	RENDEMENT MINIMUM à atteindre, moyenne annuelle
Azote	NGL (1)	> 600 et ≤ 6000 > 6 000	15 mg/l 10 mg/l	70 % 70 %
Phosphore	Ptot	> 600 et ≤ 6 000 > 6 000	2 mg/l 1 mg/l	80 % 80 %

(1) Les échantillons utilisés pour le calcul de la moyenne annuelle sont prélevés lorsque la température de l'effluent dans le réacteur biologique est supérieure à 12 °C.

Tableau 8. Nombre maximal d'échantillons moyens journaliers non conformes autorisés en fonction du nombre d'échantillons moyens journaliers prélevés dans l'année

NOMBRE D'ÉCHANTILLONS MOYENS journaliers prélevés dans l'année	NOMBRE MAXIMAL D'ÉCHANTILLONS MOYENS journaliers non conformes
1-2	0

Annex 3: (continued)



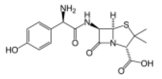
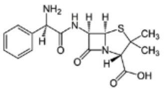
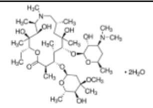
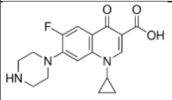
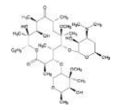
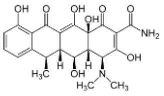
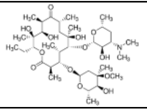
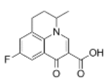
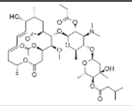
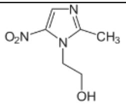
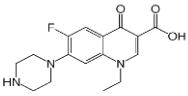
3-7	1
8-16	2
17-28	3
29-40	4
41-53	5
54-67	6
68-81	7
82-95	8
96-110	9
111-125	10
126-140	11
141-155	12
156-171	13
172-187	14
188-203	15
204-219	16
220-235	17
236-251	18
252-268	19
269-284	20
285-300	21
301-317	22
318-334	23
335-350	24
351-365	25

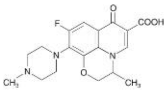
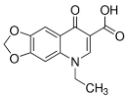
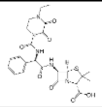
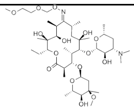
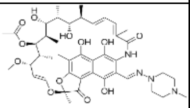
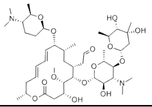
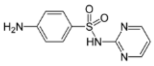
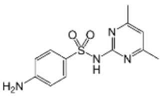
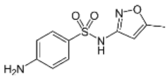
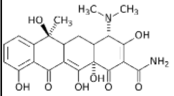
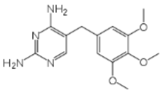
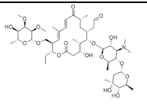
Fait le 21 juillet 2015.

Annex 4: Physico-chemical measures and analyses achieved on marine water samples collected off shore in front of studied locations (Unpublished data; Deborde J. (2019) "*MICROPOLIT Report – Dynamique des sels nutritifs et de la matière organique dans le système fluvio-estuarien de l'Adour/Golfe de Gascogne*").

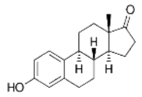
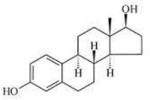
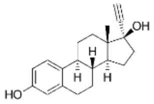
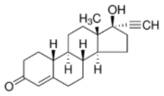
Months	March 2018					May 2018					July 2018					August 2018					November 2018				
Locations	**Location 5**	**Location 4**	**Location 3**	**Location 2**	**Location 1**	**Location 5**	**Location 4**	**Location 3**	**Location 2**	**Location 1**	**Location 5**	**Location 4**	**Location 3**	**Location 2**	**Location 1**	**Location 5**	**Location 4**	**Location 3**	**Location 2**	**Location 1**	**Location 5**	**Location 4**	**Location 3**	**Location 2**	**Location 1**
Sampling time (am)	10:40	10:20	09:57	09:40	09:25	10:20	10:07	09:57	09:45	09:28	10:28	10:11	09:56	09:44	09:28	10:05	09:50	09:35	09:26	09:11	11:07	10:52	10:39	10:23	10:07
Sampling depth (m)	-	-	-	-	-	8.0	13.0	10.0	12.0	6.0	8.0	16.0	26.0	13.0	12.0	7.0	15.0	25.0	11.0	12.0	5	14	23	9	9.6
pH	8.18	8.16	8.17	8.17	8.17	8.25	8.25	8.26	8.27	8.25	8.35	8.38	8.38	8.36	8.36	8.35	8.36	8.34	8.36	8.36	8.14	8.16	8.16	8.17	8.15
Oxygen saturation (%)	100.9	101.3	99.2	99.3	100.3	99.5	98.7	100.5	102.9	100.1	104.3	102.1	104.3	104.3	106	98.9	97.1	99.2	99.7	101.5	104.1	107.0	104.5	106.1	108.7
Conductivity (mS.cm^{-1})	54.3	55.0	55.1	54.4	54.6	45.9	45.3	45.9	46.9	46.5	40.0	39.8	40.2	39.6	40.6	52.97	53.66	53.30	53.46	53.62	62.42	62.21	61.68	62.00	61.91
Salinity (µg.L^{-1})	35.9	36.4	36.5	35.9	36.1	29.8	29.9	29.8	30.6	30.3	25.59	25.41	25.71	25.21	26.02	34.99	35.49	35.32	35.35	35.48	42.12	41.96	41.73	41.83	41.75
Temperature (°C)	12.4	12.4	12.3	12.3	12.3	15.1	15.2	15.1	15.3	15.5	22.25	22.13	22.21	22.01	22.24	22.82	22.8	22.6	22.7	22.9	16.49	16.61	16.63	16.75	16.76
∑ PO$_4^{3-}$ (µmol.L^{-1})	0.5	0.2	0.2	0.2	0.3	0.2	0.2	0.1	0.1	0.2	0.2	0.2	0.3	0.4	0.3	0.1	0.2	0.1	0.1	0.1	0.2	0.1	0.1	0.4	0.2
NO$_3^-$ (µmol.L^{-1})	4.1	4.1	4.1	3.4	2.7	2.3	3.4	3.5	3.1	0.9	4.4	5.8	6.9	6.2	9.4	0.9	0.8	1.7	0.5	0.1	2.0	2.6	3.4	2.9	3.7
NO$_2^-$ (µmol.L^{-1})	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.1	0.0	0.0	0.0	0.0	0.0	0.2	0.2	0.2	0.2	0.2
Si(OH)$_4$ (µmol.L^{-1})	3.6	2.0	2.1	4.0	3.5	4.0	4.6	4.9	2.9	3.5	3.5	3.2	3.2	3.2	2.0	0.2	0.5	1.6	1.0	0.4	2.2	2.2	2.9	2.7	2.8
NH$_4^+$ (µmol.L^{-1})	0.8	0.5	0.6	0.9	0.6	0.7	1.0	0.4	0.2	0.1	0.5	0.6	0.7	1.0	0.5	0.3	0.2	0.2	0.4	0.4	0.4	0.3	0.3	0.4	0.8
∑N$_{inorganic}$	5.1	4.8	4.9	4.5	3.5	3.2	4.6	4.0	3.4	1.1	5.0	6.6	7.7	7.4	10.1	1.2	1.1	1.9	0.9	0.6	2.6	3.0	3.9	3.5	4.6
N/P	9.5	21.9	24.9	20.2	13.3	13.0	24.8	30.3	25.7	7.3	22.2	41.7	27.8	20.3	33.5	16.4	5.7	25.5	14.1	4.3	13.1	21.2	27.1	8.0	25.9
Si/P	6.7	8.9	10.7	18.0	13.5	16.4	24.6	36.9	21.5	22.7	15.3	20.0	11.6	8.6	6.8	3.3	2.5	21.5	15.4	3.0	11.2	15.8	19.9	6.1	15.7
Si/N	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
DOC (mg.L^{-1})	0.8	0.7	1.0	0.8	0.9	1.1	1.1	1.2	1.0	1.1	1.4	1.5	1.6	1.6	1.7	1.3	1.3	1.3	1.2	1.3	0.9	1.0	1.1	1.1	1.2
TN (mg.L^{-1})	0.1	0.1	0.1	0.0	0.3	0.2	0.2	0.1	0.1	0.0	0.3	0.3	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.2	0.3	0.2	0.1	0.2
DOP* (-PO4)	0.2	0.1	0.4	0.5	0.2	0.1	0.2	0.2	0.1	0.1	0.2	0.1	0.2	0.3	0.1	0.2	0.2	0.2	0.2	0.2	-	-	-	-	-
DON (TN-nut)	7.0	6.4	6.4	7.0	7.6	3.8	7.6	5.1	8.9	8.9	13.4	10.8	15.3	8.3	8.3	5.1	4.5	5.1	4.5	4.5	-	-	-	-	-
DIP/DOP	2.8	1.6	0.6	0.4	1.4	3.0	1.0	0.7	1.2	1.9	1.1	3.0	1.8	1.2	2.8	0.5	1.2	0.5	0.4	0.8	-	-	-	-	-
DIN/DON	0.7	0.8	0.8	0.6	0.5	0.8	0.6	0.8	0.4	0.1	0.4	0.6	0.5	0.9	1.2	0.2	0.2	0.4	0.2	0.1	-	-	-	-	-
Chlorophyl α (µg.L^{-1} or µg.g^{-1})	1.2	1.2	1.1	1.1	1.4	0.6	0.6	0.8	1.0	0.8	3.0	4.4	4.7	4.2	4.7	1.8	0.8	1.2	1.1	0.4	0.4	0.6	1.0	0.7	0.4
Phéo (µg.L^{-1} or µg.g^{-1})	0.3	0.3	0.4	0.4	0.2	0.2	0.4	0.2	0.5	0.5	1.8	1.0	0.9	0.7	2.5	0.8	0.3	0.3	0.4	0.6	0.6	1.2	0.0	0.6	0.3
SM (mg.L^{-1})	9.5	9.3	8.3	8.2	12.9	6.6	6.7	6.9	6.9	9.6	12.5	12.6	12.7	12.2	12.6	10.8	9.7	9.4	10.5	10.7	11.3	10.8	11.4	9.8	10.8
POP* (µmol.g^{-1})	3.5	3.8	3.9	4.2	2.5	6.9	7.6	7.4	8.5	8.6	25.8	19.8	17.4	19.1	24.2	8.2	12.3	16.7	17.4	11.2	-	-	-	-	-
PON (µmol.g^{-1})	53.6	36.7	61.1	51.8	32.8	83.3	82.1	67.4	67.5	48.8	37.3	151.4	80.0	157.2	134.4	47.3	30.7	45.0	40.5	39.7	-	-	-	-	-
POC (%)	0.7	0.6	0.6	0.9	0.7	1.6	1.2	1.4	1.8	1.1	5.8	5.3	4.4	5.1	6.7	2.5	2.1	1.9	1.8	1.8	1.0	1.3	1.2	0.9	1.0
TC (%)	2.2	1.4	1.1	1.0	0.6	>3	2.7	1.9	3.4	3.3	6.0	5.4	4.5	5.3	7.1	3.2	2.4	2.2	2.0	2.0	1.3	1.6	1.5	1.3	1.4
C/N	5.5	5.1	4.9	5.6	4.7	5.5	5.2	5.3	5.2	4.9	5.5	5.6	3.5	5.5	5.4	7.0	7.0	6.6	6.6	6.8	-	-	-	-	-
δ^{13}C	-24.4	-24.7	-23.4	-24.8	-24.3	-22.8	-22.1	-22.5	-22.1	-22.4	-19.3	-19.3	-19.4	-19.2	-18.9	-21.3	-20.5	-21.0	-20.8	-20.8	-	-	-	-	-
δ^{15}N	1.2	0.6	0.4	1.0	-1.8	4.2	3.8	2.8	5.8	6.3	6.0	3.2	4.4	5.8	3.7	6.2	6.1	6.2	6.3	6.6	-	-	-	-	-

Annex 5: Physico-chemical structures and features of all micropollutants analyzed during the present study. Significance codes: AA-EQS: Annual Average Ecological Quality Standards, MAC-EQS: Maximum Allowed Concentrations, EQS (Biota): Biota Ecological Quality Standards, PHS: Priority Hazardous Substances; PS: Priority Substances; OS: Other substances considered as hazardous but not priority (Directive 2013/39/EU) and TEQ: Toxic Equivalency.

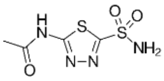
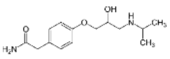
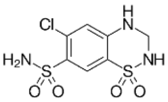
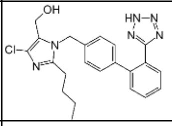
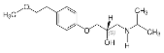
Antibiotics							
Name	**Formula**	**Structure**	**Prescription**	**ATC Code** **CAS number** **SANDRE Code**	**Molecular weight ($g.mol^{-1}$)**	**Molecular safety** (www.pubchem.ncbi.nlm.nih.gov)	**Water solubility ($mg.L^{-1}$) (Temperature °C)**
Amoxicillin	$C_{16}H_{19}N_3O_5S$		used to treat many different types of infection caused by bacteria, such as tonsillitis, bronchitis, pneumonia, gonorrhea, and infections of the ear, nose, throat, skin, or urinary tract	J01CA04 26787-78-0 6719	365.40	Health Hazard Irritant	3 430 (25)
Ampicillin	$C_{16}H_{19}N_3O_4S$		used to prevent and treat a number of bacterial infections, such as respiratory tract infections, urinary tract infections, meningitis, salmonellosis, and endocarditis	J01CA01 69-53-4 6759	349.40	Health Hazard Irritant	10 100 (21)
Azithromycin	$C_{38}H_{72}N_2O_{12}$		used to treat certain bacterial infections, such as bronchitis; pneumonia; sexually transmitted diseases (STD); and infections of the ears, lungs, sinuses, skin, throat, and reproductive organs	J01FA10 83905-01-5 7817	749.00	Irritant Health Hazard Environmental Hazard	2.37 (25)
Ciprofloxacin	$C_{17}H_{18}FN_3O_3$		used to treat a number of bacterial infections (bone and joint infections, intra abdominal infections, certain type of infectious diarrhea, respiratory tract infections, skin infections, typhoid fever, and urinary tract infections, among others)	J01MA02 85721-33-1 6540	331.34	Irritant Health Hazard Environmental Hazard	30 000 (20)
Clarithromycin	$C_{38}H_{69}NO_{13}$		used to treat many types of infections affecting the skin, ears, sinuses, lungs, and other parts of the body, including Mycobacterium avium complex (MAC) infection, a type of lung infection that often affects people with human immunodeficiency virus (HIV)	J01FA09 81103-11-9 6537	748.00	Irritant	1.693 (25)
Doxycycline	$C_{22}H_{24}N_2O_8$		used to treat infections caused by bacteria, including pneumonia and other respiratory tract infections; certain infections of the skin or eye; infections of the lymphatic, intestinal, genital, and urinary systems; and certain other infections that are spread by ticks, lice, mites, infected animals ...	J01AA02 564-25-0 6791	444.40	Health Hazard Irritant	50 000
Erythromycin (A)	$C_{37}H_{67}NO_{13}$		used to treat certain infections caused by bacteria, such as infections of the respiratory tract, including bronchitis, pneumonia, Legionnaires' disease, and pertussis; diphtheria…	J01FA01 114-07-8 6522	733.90	Health Hazard Irritant	2 000
Flumequine	$C_{14}H_{12}FNO_3$		used in the *treatment* of: Poultry: Coli bacillosis, enteritis, Salmonellosis. Calves: Diarrhoea, Salmonellosis, Colibacillosis& respiratory diseases caused by sensitive bacteria	J01MB07 42835-25-6 5635	261.25	Health Hazard Irritant	2 170 (25)
Josamycin	$C_{42}H_{69}NO_{15}$		used for Bacterial infections, Microbial infections and other conditions	J01FA07 16846-24-5 -	828.00	Irritant	
Metronidazol	$C_6H_9N_3O_3$		used to treat a wide variety of infections	J01XD01 443-48-1 6731	171.15	Health Hazard Irritant	11 000 (25)
Norfloxacine	$C_{16}H_{18}FN_3O_3$		used to treat different bacterial infections of the prostate or urinary tract (bladder and kidneys)	J01MA06 70458-96-7 6761	319.33	Corrosive Irritant	250 (25)

Ofloxacin	$C_{18}H_{20}FN_3O_4$		used to treat bacterial infections of the skin, lungs, prostate, or urinary tract (bladder and kidneys)	J01MA01 82419-36-1 6533	361.40	Health Hazard Irritant	10 800 (25)
Oxolinic acid	$C_{13}H_{11}NO_5$		used orally in the *treatment* of urinary tract infections caused by susceptible gram-negative organisms	J01MB05 14698-29-4 -	261.23	Irritant	1 910
Piperacillin	$C_{23}H_{27}N_5O_7S$		used to treat pneumonia and skin, gynecological, and abdominal (stomach area) infections caused by bacteria	J01CR05 66258-76-2 -	517.60	Health Hazard Irritant	119
Roxithromycin	$C_{41}H_{76}N_2O_{15}$		used to treat various infections caused by bacteria such as: acute pharyngitis; tonsillitis; sinusitis; acute bronchitis; pneumonia…	J01FA06 80214-83-1 -	837.00	Irritant	0.0189 (25)
Rifampicin	$C_{43}H_{58}N_4O_{12}$		used to prevent and *treat* tuberculosis and other infections	J04AB02 13292-46-1 -	822.90	Irritant	1 400 (25)
Spiramycin	$C_{43}H_{74}N_2O_{14}$		used to treat infections of the lung, skin, and mouth and toxoplasmosis during pregnancy and congenital toxoplasmosis	J01RA04 24916-50-5 6526	843.10	Irritant Health Hazard Environmental Hazard	196
Sulfadiazine	$C_{10}H_{10}N_4O_2S$		used to treat infections such as urinary tract infections, toxoplasmosis, and others	J01EC02 68-35-9 6758	250.28	Irritant Health Hazard Environmental Hazard	77 (25)
Sulfamethazine	$C_{12}H_{14}N_4O_2S$		used to treat rheumatoid arthritis in children and adults who have *used* other arthritis medicines without successful *treatment* of symptoms	J01EB03 57-68-1 6525	278.33	Health Hazard Irritant	1 500 (29)
Sulfamethoxazole	$C_{10}H_{11}N_3O_3S$		used to treat a wide variety of bacterial infections (such as middle ear, urine, respiratory, and intestinal infections). It is also *used* to prevent and *treat* a certain type of pneumonia (pneumocystis-type)	J01EC01 723-46-6 5356	253.28	Health Hazard Irritant	610 (37)
Tetracyclin	$C_{22}H_{24}N_2O_8$		used to treat many different bacterial infections of the skin, intestines, respiratory tract, urinary tract, genitals, lymph nodes, and other body systems	S01AA09 60-54-8 6750	444.40	Irritant	231 (25)
Trimethoprim	$C_{14}H_{18}N_4O_3$		used to treat bladder or kidney infections, or ear infections caused by certain bacteria	J01EA01 738-70-5 5357	290.32	Irritant Health Hazard Environmental Hazard	400 (25)
Tylosine	$C_{46}H_{77}NO_{17}$		used in veterinary medicine to *treat* felines, canines and livestock. However, the drug is only *used* as an antibiotic in the *treatment* of infections in livestock	QJ01FA90 738-70-5 6523	916.10	Health Hazard Irritant	211

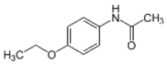
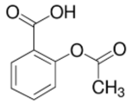
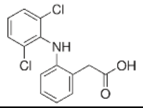
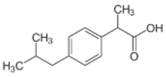
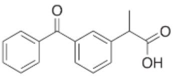
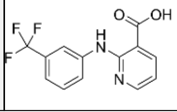
Steroïdes hormones

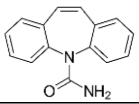
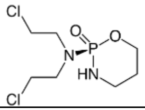
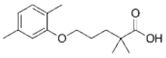
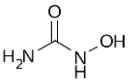
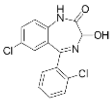
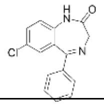
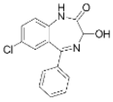
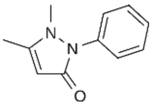
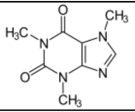
Name	Formula	Structure	Prescription	ATC Code CAS number SANDRE Code	Molecular weight ($g.mol^{-1}$)	Molecular safety (www.pubchem.ncbi.nlm.nih.gov)	Water solubility ($mg.L^{-1}$) (Temperature °C)
Estrone (E1) (Natural)	$C_{18}H_{22}O_2$		Regulation of metabolism; control of the sexual development; Keep homeostasis	G03CA07 53-16-7 5396	270.40	Health Hazard Irritant	760 (20)
17β-estradiol (βE2) (Natural)	$C_{18}H_{24}O_2$		Regulation of metabolism; control of the sexual development; Keep homeostasis	G03CA03 50-28-2 5397	272.40	Health & Environmental Hazards	3.6 (27)
17α-ethilnylestradiol (EE2) (Synthetic)	$C_{20}H_{24}O_2$		Regulation of metabolism; control of the sexual development; Keep homeostasis	G03CA01 57-63-6 2629	296.40	Irritant Health Hazard Environmental Hazard	11.3 (27)
(19-) Norethindrone	$C_{20}H_{26}O_2$		Regulation of metabolism; control of the sexual development; Keep homeostasis	? 68-22-4 5400	298.40	Health Hazard Irritant	7.04 (25)

Antihypertensive drugs

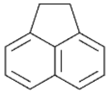
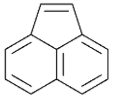
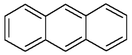
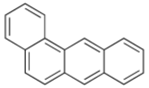
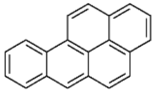
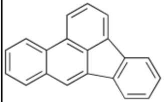
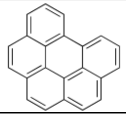
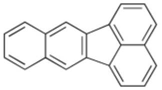
Name	Formula	Structure	Prescription	ATC Code CAS number SANDRE Code	Molecular weight ($g.mol^{-1}$)	Molecular safety (www.pubchem.ncbi.nlm.nih.gov)	Water solubility ($mg.L^{-1}$) (Temperature °C)
Acetazolamide	$C_4H_6N_4O_3S_2$		used to prevent and reduce the symptoms of altitude sickness. This medication can decrease headache, tiredness, nausea, dizziness, and shortness of breath	S01EC01 59-66-5 7136	222.30	Health Hazard Irritant	980 (30)
Atenolol	$C_{14}H_{22}N_2O_3$		Beta-blocker. Inhibit the hormone adrenalin and the neurotransmitter noradrenalin	C07AB03 29122-68-7 5361	266.34	Health Hazard Irritant	13 300 (25)
Hydrochlorothiazide	$C_7H_8ClN_3O_4S_2$		used to treat high blood pressure (hypertension). *Hydrochlorothiazide* is also *used to treat* fluid retention (edema) in people with congestive heart failure	C03AA03 58-93-5 6746	297.70	Health Hazard Irritant	722 (25)
Losartan	$C_{22}H_{23}ClN_6O$		used to treat high blood pressure and reduce the risk of stroke in certain people with heart disease	C09CA01 114798-26-4 -	422.90	Health Hazard Irritant	<1 000
Metoprolol	$C_{15}H_{25}NO_3$		Beta-blocker. Inhibit the hormone adrenalin and the neurotransmitter noradrenalin	C07AB02 37350-58-6 5362	267.36	Irritant	

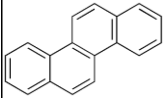
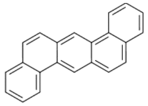
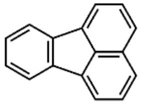
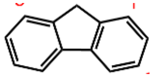
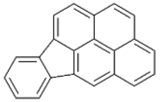
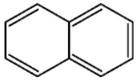
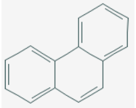
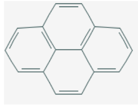
Inflammatory drugs

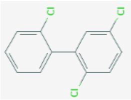
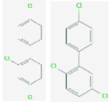
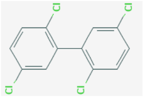
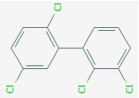
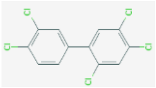
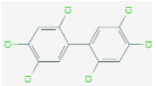
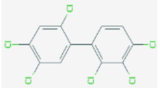
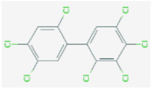
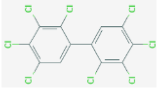
Name	Formula	Structure	Prescription	ATC Code CAS number SANDRE Code	Molecular weight ($g.mol^{-1}$)	Molecular safety (www.pubchem.ncbi.nlm.nih.gov)	Water solubility ($mg.L^{-1}$) (Temperature °C)
Acetaminophen/ Paracetamol	$C_8H_9NO_2$		used to treat many conditions such as headache, muscle aches, arthritis, backache, toothaches, colds and fevers.	N02BE01 103-90-2 5354	151.16	Irritant	14 000 (25)
Acetylsalycylic acid/Aspirin	$C_9H_8O_4$		used to treat gout	B01AC06 50-78-2 6735	180.16	Irritant	10 000
Diclofenac	$C_{14}H_{11}Cl_2NO_2$		used to treat mild to moderate pain, or signs and symptoms of osteoarthritis or rheumatoid arthritis	M01AB05 15307-86-5 5349	296.10	Acute toxic Irritant	2.37 (25)
Ibuprofen	$C_{13}H_{18}O_2$		used to relieve pain from various conditions such as headache, dental pain, menstrual cramps, muscle aches, or arthritis	M01AE01 15687-27-1 5350	206.28	Health Hazard Irritant	21 (25)
Ketoprofen	$C_{16}H_{14}O_3$		used to treat pain or inflammation caused by arthritis	M02AA10 22071-15-4 5353	254.28	Acute toxic Irritant	51 (22)
Niflumic acid	$C_{13}H_9F_3N_2O_2$		used in the *treatment* of rheumatoid arthritis	M02AA17 4394-00-7 6870	282.22	Irritant	19 (25)

Other							
Name	**Formula**	**Structure**	**Prescription**	**ATC Code** **CAS number** **SANDRE Code**	**Molecular weight (g.mol^{-1})**	**Molecular safety** (www.pubchem.ncbi.nlm.nih.gov)	**Water solubility (mg.L^{-1}) (Temperature °C)**
Carbamazepine	$C_{15}H_{12}N_2O$		used as antiepileptic	N03AF01 298-46-4 5296	236.27	Health Hazard Irritant	18 (25)
Cyclophosphamide	$C_7H_{15}Cl_2N_2O_2P$		used for the treatment of several types of cancers and often in combination with other drugs to treat breast cancer, leukemia and ovarian cancer	L01AA01 50-18-0 6733	261.08	Acute toxic Irritant Health Hazard Corrosive	10-50 (25)
Gemfibrozil	$C_{15}H_{22}O_3$		Regulation of triglycerides andcholesterol in blood. Used with diet changes (restriction of cholesterol and fat intake) to reduce the amount of cholesterol and triglycerides (other fatty substances) in the blood in certain people with very high triglycerides	C10AB04 25812-30-0 -	250.33	Irritant Health Hazard Environmental Hazard	10 000
Hydroxycarbamide = Hydroxyurea	$CH_4N_2O_2$		used primarily for the *treatment* of myeloproliferative diseases, which has an inherent risk of transforming to acute myeloid leukemia	L01XX05 127-07-1 6705	76.06	Irritant	1 000 000 (25)
Lorazepam	$C_{15}H_{10}Cl_2N_2O_2$		used to treat anxiety	N05BA06 846-49-1 5374	321.20	Health Hazard	80
Nordazepam	$C_{15}H_{11}ClN_2O$		used primarily in the *treatment* of anxiety disorders	N05BA16 1088-11-5 -	270.71	Irritant	179.00
Oxazepam	$C_{15}H_{11}ClN_2O_2$		used to treat anxiety and also acute alcohol withdrawal	N05BA04 604-75-1 5375	286.71	Health Hazard	20 (22)
Phenazone	$C_{11}H_{12}N_2O$		used for Fever, Ear pain due to infection and other conditions	N02BB01 60-80-0	188.23	Irritant	51 900 (25)
Caffeine	$C_8H_{10}N_4O_2$		used to treat breathing problems in premature infants and to improve mental alertness, but it has many other uses	N06BC01 58-08-2 6519	194.19	Irritant	21 600 (25)

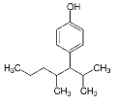
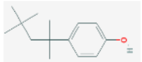
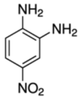
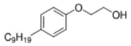
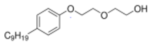
PAHs (Polycyclic aromatic hydrocarbons)

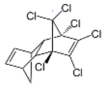
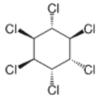
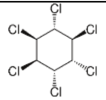
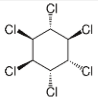
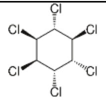
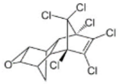
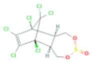
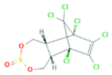
Name	Formula	Structure	Origin/Uses	CAS number SANDRE Code	Molecular weight ($g.mol^{-1}$)	Molecular safety (www.pubchem.ncbi.nlm.nih.gov)	Water solubility ($mg.L^{-1}$) (Temperature °C)	AA-EQS Surface waters ($\mu g.L^{-1}$) (EC, 2013)	MAC-EQS Surface waters ($\mu g.L^{-1}$) (EC, 2013)	EQS-Biota ($\mu g.kg^{-1}$; wet weight) (EC, 2013)	Identified as PHS/PS/OS (EC, 2013)
Acenaphthene	$C_{12}H_{10}$		Uses in the manufacture of pigments, dyes, plastics, pesticides and pharmaceuticals (Abdel-Shafy, 2016)	83-32-9 1453	154.21	Irritant Environmental Hazard	3.90 (25)	-	-	-	-
Acenaphthylene	$C_{12}H_8$			307-07-3 1622	152.19	Acute toxic Irritant	3.93 (25)	-	-	-	-
Anthracene	$C_{14}H_{10}$		Uses as diluent for wood preservatives and for manufacture of dyes and pigments (Abdel-Shafy, 2016)	120-12-7 1458	178.23	Health Hazard Irritant	0.6 (25-salt water)	**0.10**	**0.10**	-	**PHS**
Benzo[a]anthracene	$C_{18}H_{12}$		Organic product	56-55-3 1082	228.30	Health Hazard Environmental Hazard	0.0094 (25)	-	-	-	-
Benzo[a]pyrene	$C_{20}H_{12}$		Used in biological research (buffer manufacturing, analyses, toxicology)	50-32-8 1115	252.30	Irritant Health Hazard Environmental Hazard	0.0062 (25)	**1.7×10^{-4}**	**0.027**	**5.0**	**PHS**
Benzo[b]fluoranthene	$C_{20}H_{12}$		From incomplete hydrocarbon and coal combustion In oil refining, coal coking, vehicle traffic	205-99-2 1116	252.30	Health Hazard Environmental Hazard	0.0015	**1.7×10^{-4}**	**0.017**	**5.0**	**PHS**
Benzo[g,h,i]perylene	$C_{22}H_{12}$		From fuel combustion (car exhausts, oil refining, coal distillation, wood, oil and coal combustion)	191-24-2 1118	276.30	Environmental Hazard	0.00026 (25)	**1.7×10^{-4}**	**$8.2\text{-}10^{-4}$**	**5.0**	**PHS**
Benzo[k]fluoranthene	$C_{20}H_{12}$		Fossil fuel Use in biological research From incomplete hydrocarbon and coal combustion	207-08-9 1117	252.30	Health Hazard Environmental Hazard	0.00076 (25)	**1.7×10^{-4}**	**0.017**	**5.0**	**PHS**

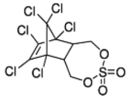
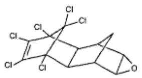
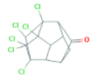
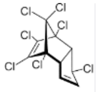
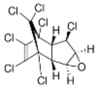
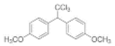
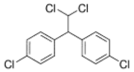
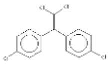
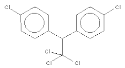
Chrysene	$C_{18}H_{12}$		Organic product manufacturing (coal, fate and oil distillation) Waste incinerator, natural gas household appliances, home healting (wood combustion)	218-01-9 1476	228.30	Health Hazard Environmental Hazard	0.002 (25)	-	-	-	-
Dibenzo[a,h]anthracen	$C_{22}H_{14}$		Present in fossil fuels, engine exhausts of diesel cars Also present in cigarette smoke, engine exhausts of petrol cars, smoke from coal boiler and tar	53-70-3 1621	278.30	Health Hazard Environmental Hazard	0.000627 (25)	-	-	-	-
Fluoranthene	$C_{16}H_{10}$		Uses for manufacture of agrochemicals, dyes and pharmaceuticals (Abdel-Shafy, 2016)	206-44-0 1191	202.25	Irritant Environmental Hazard	0.120 (24- seawater)	**0.0063**	**0.12**	**30.00**	**PS**
Fluorene	$C_{13}H_{10}$		Uses for manufacture of pharmaceuticals, pigments, dyes, pesticides and thermoset plastic (Abdel-Shafy, 2016)	86-73-7 1623	166.22	Irritant Environmental Hazard	1.69 (25)	-	-	-	-
Indeno[1,2,3-cd]pyrene	$C_{22}H_{12}$		From incomplete wood, coal, fuel combustion, wood burning-oven, waste incinerators, industrial and cigarette smokes. Present in bituminous coal, naturally present in fossile fuel, raw oil, shale oil, some tree leafs and tabacco, breeding ground, horse manure. From forest fires and volcanic eruption	193-39-5 1204	276.30	Health Hazard	0.062 (20)	**1.7×10^{-4}**	-	**5.0**	**PHS**
Naphtalene	$C_{10}H_8$		Used in organic manufacturing: dye, tar plasticizer, solvant, insecticide, mite repellent	91-20-3 1517	128.17	Irritant Health Hazard Environmental Hazard	31 (25)	**2.00**	**130.00**	-	**PS**
Phenanthrene	$C_{14}H_{10}$		Uses for manufacture of resins and pesticides (Abdel-Shafy, 2016)	85-01-8 1524	178.23	Irritant Environmental Hazard	1.10 (25)	-	-	-	-
Pyrene	$C_{16}H_{10}$		Uses for manufacture of pigments (Abdel-Shafy, 2016)	129-00-0 1537	202.25	Irritant Environmental Hazard	0.135 (25)	-	-	-	-

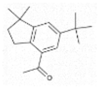
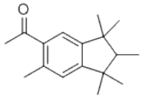
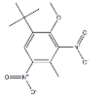
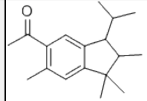
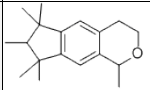
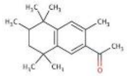
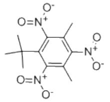
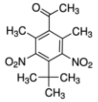
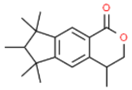
PCBs (Polychlorobiphenyles)											
Name	**Formula**	**Structure**	**Origin/Uses**	**CAS number SANDRE Code**	**Molecular weight (g.mol^{-1})**	**Molecular safety** (www.pubchem.ncbi.nlm.nih.gov)	**Water solubility (mg.L^{-1}) (Temperature °C)**	**AA-EQS Surface waters (µg.L^{-1})** (EC, 2013)	**MAC-EQS Surface waters (µg.L^{-1})** (EC, 2013)	**EQS-Biota (µg.kg^{-1}; wet weight)** (EC, 2013)	**Identified as PHS/PS/OS** (EC, 2013)
2,2',5-Trichlorobiphenyl (PCB 18)	$C_{12}H_7Cl_3$		Used as dielectrical insulator (from askarel class) in transformers and condensers, in microwaves, paintings and adhesives	37680-65-2 3164	257.50	Health Hazard Environmental Hazard		-	-	-	-
2,4',4-Trichlorobiphenyl + 2,4',5-Trichlorobiphenyl (PCB 28+31)	$C_{12}H_7Cl_3$		"	7012-37-5 + 16606-02-3 1239 + 1886 6965	257.5 + 257.5	Health Hazard Environmental Hazard		-	-	-	-
2,2',5,5'-Tetrachlorobiphenyl (PCB 52)	$C_{12}H_6Cl_4$		"	35693-99-3 1241	292.00	Health Hazard Environmental Hazard		-	-	-	-
2,2',3,5'-Tetrachlorobiphenyl (PCB 44)	$C_{12}H_6Cl_4$		"	41464-39-5 1628	292.00	Health Hazard Environmental Hazard		-	-	-	-
2,2',4,5,5'-Pentachlorobiphenyl (PCB 101)	$C_{12}H_5Cl_5$		"	37680-73-2 1242	326.40	Health Hazard Environmental Hazard		-	-	-	-
2,2',3,4',5',6-Hexachlorobiphenyl (PCB 149)	$C_{12}H_4Cl_6$		"		360.90	Health Hazard Environmental Hazard		-	-	-	-
2,3',4,4',5-Pentachlorobiphenyl (PCB 118)	$C_{12}H_5Cl_5$		"	31508-00-6 1243	326.40	Health Hazard Environmental Hazard		-	-	**0.0065** (Included in the sum with other PCDD+PCDF+**PCB-DL**) **TEQ**	**PHS**
2,2',4,4',5,5'-Hexachlorobiphenyl (PCB 153)	$C_{12}H_4Cl_6$		"	35065-27-1 1245	360.90	Health Hazard Environmental Hazard		-	-	-	-
2,2',3,4,4',5'-Hexachlorobiphenyl (PCB 138)	$C_{12}H_4Cl_6$		"	35065-28-2 1244	360.90	Health Hazard Environmental Hazard		-	-	-	-
2,2',3,4,4',5,5'-Heptachlorobiphenyl (PCB 180)	$C_{12}H_3Cl_7$		"	35065-29-3 1246	395.30	Health Hazard Environmental Hazard		-	-	-	-
2,2',3,3',4,4',5,5'-Octachlorobiphenyl (PCB 194)	$C_{12}H_2Cl_8$		"	35694-08-7 1625	429.80	Health Hazard Environmental Hazard		-	-	-	-

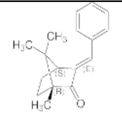
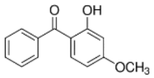
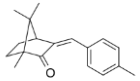
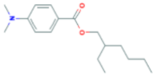
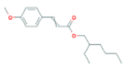
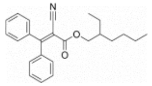
Alkylphenols

Name	Formula	Structure	Origin/Uses	CAS number SANDRE Code	Molecular weight (g.mol^{-1})	Molecular safety (www.pubchem.ncbi.nlm.nih.gov)	Water solubility (mg.L^{-1}) (Temperature °C)	AA-EQS Surface waters (µg.L^{-1}) (EC, 2013)	MAC-EQS Surface waters (µg.L^{-1}) (EC, 2013)	EQS-Biota (µg.kg^{-1}; wet weight) (EC, 2013)	Identified as PHS/PS/OS (EC, 2013)
Nonylphenols (NP)	$C_{15}H_{24}O$		Used as wetting agent, dispersant, emulsifier, defoaming agent,. Used in detergent, paintings, paper manufacturing, cosmetic, cleaning products, plastics, rubber	25154-52-3 1957	220.35	Environmental Hazard Irritant Health Hazard Corrosive	7 (25)	**0.30**	**2.00**	-	**PHS**
Para-tert-octylphenol (4tOP)	$C_{14}H_{22}O$		Polymers and detergents manufacturing	140-66-9 1959	206.32	Environmental Hazard Irritant Corrosive	5.113 (25)	**0.01**	-	-	**PS**
4-nitro-O-phenylenediamine (4nOP)	$C_6H_7N_3O_2$		Dye	99-56-9 -	153.14	Irritant	1300 (25)	-	-	-	-
Nonylphenol monoethoxilated (NPEO1)			Dispersive agent	9016-45-9 (for whole Ethoxylate nonylphenol family)							
Nonylphenol diethoxilathed (NPEO2)	$C_{19}H_{32}O_3$		Dispersive agent	9016-45-9 (for whole Ethoxylate nonylphenol family)	308.46						

Pesticides											
Name	**Formula**	**Structure**	**Origin/Uses**	**CAS number SANDRE Code**	**Molecular weight ($g.mol^{-1}$)**	**Molecular safety** (www.pubchem.ncbi.nlm.nih.gov)	**Water solubility ($mg.L^{-1}$) (Temperature °C)**	**AA-EQS Surface waters ($\mu g.L^{-1}$)** (EC, 2013)	**MAC-EQS Surface waters ($\mu g.L^{-1}$)** (EC, 2013)	**EQS-Biota ($\mu g.kg^{-1}$; wet weight)** (EC, 2013)	**Identified as PHS/PS/OS** (EC, 2013)
Aldrin	$C_{12}H_8Cl_6$		Insecticide	309-00-2 1103	364.90	Acute toxic Health Hazard Environmental Hazard	0.027 (27)	**∑(Aldrin, Dieldrine, Endrin, Isodrine)= 0.005**	-	-	**OS**
Alpha-Hexachlorocyclohexane (Alpha BHC)	$C_6H_6Cl_6$		Insecticide	319-84-6 1200	290.83	Environmental Hazard Irritant Health Hazard Acute toxic	7.3 (25)	**0.002**	**0.02**	-	**PHS**
Beta-Hexachlorocyclohexane (Beta BHC)	$C_6H_6Cl_6$		Insecticide	319-85-7 1201	290.83	Environmental Hazard Irritant Health Hazard Acute toxic	7.3 (25)	**0.002**	**0.02**	-	**PHS**
Delta-Hexachlorocyclohexane (Delta BHC)	$C_6H_6Cl_6$		Insecticide	319-86-8 1202	290.83	Environmental Hazard Irritant Health Hazard Acute toxic	7.3 (25)	**0.002**	**0.02**	-	**PHS**
Lindane/Gamma-Hexachlorocyclohexane (Gamma BHC)	$C_6H_6Cl_6$		Insecticide	58-89-9 1203	290.80	Environmental Hazard Irritant Health Hazard Acute toxic	7.3 (25)	**0.002**	**0.02**	-	**PHS**
Dieldrine	$C_{12}H_8Cl_6O$		Organochlorine Insecticide	60-57-1	380.93	Acute toxic Health Hazard Environmental Hazard	0.195 (25)	**∑(Aldrin, Dieldrine, Endrin, Isodrine)= 0.005**	-	-	**OS**
Alpha Endosulfan	$C_6H_6Cl_6O_3S$		Insecticide	959-98-8 1178	406.90			**0.0005** (Endosulfan)	**0.004** (Endosulfan)	-	**PHS** (Endosulfan)
Bêta Endosulfan	$C_9H_6Cl_6O_3S$		Insecticide and acaricide	33213-65-9 1179	406.90	Environmental Hazard Irritant Acute toxic		**0.0005** (Endosulfan)	**0.004** (Endosulfan)	-	**PHS** (Endosulfan)

Endosulfan Sulfate	$C_9H_6Cl_6O_4S$		Pesticide	1031-07-8 1742	422.90	Acute toxic Environmental Hazard	**0.48 (20)**	**0.0005**	**0.0040**	-	**PHS**
Endrin	$C_{12}H_8Cl_6O$		Insecticide	72-20-8 1181	380.90	Acute toxic Environmental Hazard	**0.195 (25)**	**∑(Aldrin, Dieldrine, Endrin, Isodrine)= 0.005**	-	-	**OS**
Endrin Aldehyde	$C_{12}H_8Cl_6O$			7421-93-4	380.90	Irritant		-	-	-	-
Endrin Ketone	$C_{12}H_8Cl_6O$		Insecticide	53494-70-5 5485	380.90	Acute toxic		-	-	-	-
Heptachlor	$C_{10}H_5Cl_7$		Insecticide	76-44-8 1197	373.30	Acute toxic Health Hazard Environmental Hazard	0.18 (25)	**$1.8 x 10^{-8}$**	**$3 x 10^{-5}$**	**$6.7 x 10^{-3}$**	**PHS**
Heptachlor Epoxide	$C_{10}H_5Cl_7O$		Insecticide	1024-57-3 1748	389.30	Acute toxic Health Hazard Environmental Hazard		**$1.8 x 10^{-8}$**	**$3 x 10^{-5}$**	**$6.7 x 10^{-3}$**	**PHS**
Methoxychlor	$C_{16}H_{15}Cl_3O_2$		Insecticide	72-43-5 1511	345.60	Environmental Hazard Irritant Health Hazard	0.1 (25)	-	-	-	-
4,4'-Dichlorodiphenyldichloroethane (4,4'-DDD)	$C_{14}H_{10}Cl_4$		Pesticide	72-54-8 1144	320.00	Environmental Hazard Irritant Health Hazard Acute toxic	0.09 (25)	**0.025** = Total DDT = ∑(4,4'-DDT + 2,4'-DDT + 4,4'-DDE + 4,4'-DDD)	-	-	**OS**
4,4'-Dichlorodiphenyldichloroethylene (4,4'-DDE)	$C_{14}H_8Cl_4$		Insecticide, degradation product of DDT	72-55-9 1146	318.00	Acute toxic Irritant Environmental Hazard	0.04 (25)	**0.025** = Total DDT = ∑(4,4'-DDT + 2,4'-DDT + 4,4'-DDE + 4,4'-DDD)	-	-	**OS**
Para-para-Dichlorodiphenyltrichloroethane (4,4'-DDT)	$C_{14}H_9Cl_5$		Insecticide, formely one of the most widely used	50-29-3 1148	354.50	Acute toxic Health Hazard Environmental Hazard	0.0055 (25)	**0.01**	-	-	**OS**

Musks											
Name	**Formula**	**Structure**	**Origin/Uses**	**CAS number SANDRE Code**	**Molecular weight (g.mol^{-1})**	**Molecular safety** (www.pubchem.ncbi.nlm.nih.gov)	**Water solubility (mg.L^{-1}) (Temperature °C)**	**AA-EQS Surface waters (µg.L^{-1})** (EC, 2013)	**MAC-EQS Surface waters (µg.L^{-1})** (EC, 2013)	**EQS-Biota (µg.kg^{-1}; wet weight)** (EC, 2013)	**Identified as PHS/PS/OS** (EC, 2013)
Celestolide (ADBI)	$C_{17}H_{24}O$		Used for fragrance compositions and perfumes with long-lasting fragrance	13171-00-1 -	244.37		3.29 (24)	-	-	-	-
Phantolide (AHMI)	$C_{17}H_{24}O$		A component of Musk fragrances	15323-35-0 -	244.37	Environmental Hazard Irritant		-	-	-	-
Musk Ambrette (MA)	$C_{12}H_{16}N_2O_5$			83-66-9 6688	268.27	Irritant	2.41 (25)	-	-	-	-
Traseolide (ATII)	$C_{18}H_{26}O$		A component of Musk fragrances	68140-48-7 6680	258.40	Irritant	0.539 (20)	-	-	-	-
Galaxolide (HHCB)	$C_{18}H_{26}O$		Used as a fragrance ingredient in perfumes, soaps, cosmetics and detergents	1222-05-5 6618	258.40	Irritant Environmental Hazard	1.65-1.99 (25)	-	-	-	-
Tonalide (AHTN)	$C_{18}H_{26}O$		Aromatic musk compound	21145-77-7 7881	258.40	Irritant Environmental Hazard	1.25	-	-	-	-
Musk Xylene (MX)	$C_{12}H_{15}N_3O_6$		used in fragrances and soap to mimic natural musk	81-15-2 6342	297.26	Explosive Health Hazard Environmental Hazard	0.49 (25)	-	-	-	-
Musk Moskene (MM)	$C_{14}H_{18}N_2O_4$				278.30			-	-	-	-
Musk Ketone (MK)	$C_{14}H_{18}N_2O_5$			81-14-1 6687	294.30	Health Hazard Environmental Hazard	0.46 (25)	-	-	-	-
Galaxolidone (HHCB-lactone)	$C_{18}H_{24}O_2$				272.40			-	-	-	-

Sunscreens											
Name	**Formula**	**Structure**	**Origin/Uses**	**CAS number SANDRE Code**	**Molecular weight ($g.mol^{-1}$)**	**Molecular safety** (www.pubchem.ncbi.nlm.nih.gov)	**Water solubility ($mg.L^{-1}$) (Temperature °C)**	**AA-EQS Surface waters ($\mu g.L^{-1}$)** (EC, 2013)	**MAC-EQS Surface waters ($\mu g.L^{-1}$)** (EC, 2013)	**EQS-Biota ($\mu g.kg^{-1}$; wet weight)** (EC, 2013)	**Identified as PHS/PS/OS** (EC, 2013)
3-Benzylidene camphor (3-BC)	$C_{17}H_{20}O$		Used in cosmetics as a component (UV filter) of sunscreens	15087-24-8 -	240.34	Health Hazard		-	-	-	-
Oxybenzone (Benzophenone 3)	$C_{14}H_{12}O_3$		UV filter	131-57-7 ⍰	228.24	Environmental Hazard Irritant	3.7 (25)	-	-	-	-
4-Methylbenzylidene camphor (4-MBC)/Enzacamene	$C_{18}H_{22}O$		UV filter	36861-47-9	254.40	Environmental Hazard Health Hazar		-	-	-	-
Octyl-dimethyl-PABA (OD-PABA)	$C_{17}H_{27}NO_2$		UV filter	21245-02-3	277.40	Irritant	0.54 (25)	-	-	-	-
Ethylhexyl methoxycinnamate (EHMC)/ Octinoxate	$C_{18}H_{26}O_3$		UV filter	5466-77-3 7816	290.40		0.2 (20)	-	-	-	-
Octocrylene (OC)	$C_{24}H_{27}NO_2$		Solvent for solid sunscreens. UV absorber for plastics and paints	6197-30-4 6686	361.50			-	-	-	-

Metals/Organometals											
Name	**Electronic shell/Formula**	**Picture** (©www.lenntech.com)	**Origin/Uses**	**CAS number SANDRE Code**	**Molecular weight ($g.mol^{-1}$)**	**Molecular safety** (www.pubchem.ncbi.nlm.nih.gov)	**Water solubility ($mg.L^{-1}$) (Temperature °C)**	**AA-EQS Surface waters ($\mu g.L^{-1}$)** (EC, 2013)	**MAC-EQS Surface waters ($\mu g.L^{-1}$)** (EC, 2013)	**EQS-Biota ($\mu g.kg^{-1}$; wet weight)** (EC, 2013)	**Identified as PHS/PS/OS** (EC, 2013)
Antimony (Sb)	[Kr] $4d^{10}5s^25p^3$		Sometimes naturally present in the environment. but also obtained from the ores stibnite (Sb2S3) and valentinite (Sb2O3)	7440-36-0 1376	121.76	Environmental Hazard Irritant Healt Hazard	Insoluble	-	-	-	-
Arsenic (As)	[Ar] $3d^{10}$ $4s^2$ $4p^3$		One of the most toxic compound. Naturally present in small quantity in soil and minerals. Also producted by copper. lead and zinc industries.	7440-38-2 1369	74.92	Environmental Hazard Acute toxic	Insoluble	-	-	-	-
Cadmium (Cd)	[Kr] $4d^{10}5s^2$		Naturally present in the environment and released in rivers through stone abrasion and in the air through forest fires and volcanos.	7440-43-9 1388	112.41	Environmental Hazard Flammable Health Hazard Acute toxic	Insoluble	**0.20**	**≤0.45 (classe 1)** **0.45 (classe 2)** **0.6 (classe 3)** **0.9 (classe 4)** **1.5 (classe 5)**	-	**PHS**
Chromium (Cr)	[Ar] $3d^5$ $4s^1$		Used in alloys such as stainless steel. in chrome plating and in metal ceramics. Mined as chromite (FeCr2O4) ore.	7440-47-3 1389	52.00	Environmental Hazard Irritant Healt Hazard	Insoluble	-	-	-	-
Copper (Cu)	[Ar] $3d^{10}4s^1$		Naturally present in the environment (wind-blown dust. decaying vegetation. forest fires and sea spray) and realsed by human activies. Mainly used in the industries and agriculture (used for electrical equipment. construction (roofing. plumbing). industrial machinery (heat exchangers and alloys)).	7440-50-8	63.55	Environmental Hazard Irritant Accute toxic	Insoluble	-	-	-	-
Lead (Pb)	[Xe] $4f^{14}5d^{10}6s^26p^2$		Rarelly naturally present in the environment. Used in car batteries (constituent of the lead-acid battery). uased as dye for ceramic glazes and in computer and TV glasses.	7439-92-1 3358	207.00	Healt Hazard	Insoluble	**1.30**	**14.00**	-	**PS**
Molybdenum (Mo)	[Kr] $4d^55s^1$		Valuable alloying agent. Used to the hardenability and toughness of quenched and tempered steels and used in alloys. eletrodes and catalysts.	7439-98-7 1395	96.00	Flammable Health Hazard	Insoluble	-	-	-	-
Nickel (Ni)	[Ar] $3d^84s^2$		Mainly used in the preparation of alloys or locked in the mlanet's iron-nickel molten core.	7440-02-0 1386	58.69	Irritant Healt Hazard	Insoluble	**8.60**	**34.00**	-	**PS**
Silver (Ag)	[Kr] $4d^{10}5s^1$		Used as a precious metal. used in photography and in the electrical industry (paintings. camputer keyboards). Present also naturally in the environment as crystals or compact masses.	7440-22-4 1368	107.87	Environmental Hazard	Insoluble	-	-	-	-
Tin (Sn)	[Kr] $4d^{10}5s^25p^2$		Used for can coating. as solder for joining pipes or electric circuits. Low natural concentrations in the envrionment.	7440-31-5 1380	118.71	Irritant	Insoluble	-	-	-	-
Vanadium (V)	[Ar] $3d^34s^2$		Always found bound in nature. Used as ferrovanadium or as a steel additive. Mixed also with Aluminium for titanium alliages. Found in the environment (in algae. invertebrates. fishes).	7440-62-2	50.94		Insoluble	-	-	-	-

Annex 6: Huguenin L., Lalanne Y., Bru N., Lissardy M., D'Amico F., Monperrus M., de Casamajor MN. (2018). "*Identifying macrofaunal assemblages and indicator taxa of intertidal boulder fields in the south of the Bay of Biscay (northern Basque coast). A framework for future monitoring*". *Regional Studies in Marine Science*, 20, 13-22 (http://doi.org/10.1016/j.rsma.2018.03.012).

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Identifying benthic macrofaunal assemblages and indicator taxa of intertidal boulder fields in the south of the Bay of Biscay (northern Basque coast). A framework for future monitoring

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ABSTRACT

The French southern marine subregion of the Bay of Biscay presents local environmental features explaining the presence of specific communities in remarkable habitats. The aim of the study is, for the first time, to assess benthic macrofaunal assemblages and to identify indicator species of each assemblage within intertidal boulder field habitats in this marine subregion.

Mobile boulders were sampled with a stratified random sampling design in upper and lower midlittoral zones in March–June 2015. Sessile (in percentage cover) and mobile macrofauna (in abundance) communities were identified and counted within 0.1 m^2 quadrats. Ecological function and precision between the two groups were found dissimilar thus species richness, abundance, macrofauna distribution and indicator species/taxa of sessile and mobile macrofauna were analyzed separately. 78 species/taxa of macrofauna were recorded. A restricted list of 12 singletons (8 for mobile and 4 for sessile macrofauna) and 33 combinations of species/taxa were identified as significant indicators of each assemblage. Species ecological features (food webs, signs of disturbance, alien species, biogeographical range limit) were also considered as additional selection criteria. Therefore, species with a high ecological interest but not considered statistically as valid indicators were also highlighted.

Thus, this work constitutes a framework for future monitoring of the Basque intertidal rocky shore and could be used as an alternative to sampling the entire biodiversity. It also meets the MSFD requirements (e.g. take into account marine subregion specificities; integrate the metric "macrofauna" and supports several descriptors as the D1 "Biodiversity" and D6 "Seafloor integrity").

1. Introduction

Over the last decades, investigations and monitoring of biological communities, used as bio-indicators, have been carried out worldwide to assess continental and marine ecosystems. Those were undertaken in different contexts either in the prospect of a global climatic change (Barange, 2003; Thompson et al., 2002) or to evaluate ecological status of water bodies (e.g., European Water Framework Directory (WFD)) (European Commission, 2000; Borja et al., 2013; Guinda et al., 2008). Indeed, ecological indicators are useful to monitor environmental changes and assess ecological management and conservation (Cairns et al., 1993; Legendre and Legendre, 1998; Marques et al., 2009; Siddig et al., 2016). They provide information to understand the environment and its ecological status while highlighting changes in the environment by giving, for example, early warning signals (Cairns et al., 1993). The identification of indicator species is commonplace in ecology and biogeography because they add ecological meaning to studied sites and their use is an alternative to sampling the entire biodiversity (Legendre and Legendre, 2012).

Nowadays, there is a large number of survey methods used to study the intertidal benthic environment. Various tools have been

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developed to analyze rocky shore communities data (e.g., Borja et al., 2013; Borja and Dauer, 2008; Clarke, 1993; Dauer, 1993; Panayotidis et al., 2004; Rombouts et al., 2013). Macroalgal communities, which are one of the dominant groups of hard substrata, are often used as bioindicators (for instance to assess the water quality of coastal waters for the WFD (Ar Gall et al., 2016; Ar Gall and Le Duff, 2014; Ballesteros et al., 2007; Díez et al., 2012; Orfanidis et al., 2001). More recently, the European Marine Strategy Framework Directive (European Commission, 2008, MSFD) has proposed a set of indicators (e.g. characteristic and opportunistic species) in order to assess the environmental status of marine habitats (Teixeira et al., 2016). But, assessment and monitoring programs must be achieved and adapted to the level of marine subregions while integrating and supporting the monitoring requirements imposed by other EU legislation, such as the Habitats Directive (92/43/EEC), Birds Directive (2009/147/EC) and international agreements (Patrício et al., 2016). Moreover, the MSFD emphasized significant deficiencies (Berg et al., 2015; Heiskanen et al., 2016; Queirós et al., 2016; Teixeira et al., 2014), in particular from the Basque Country (southern Bay of Biscay subregion) (Borja et al., 2011).

A great number of studies (including studies carried out on the national scale) highlighted the bathymetric zonation of benthic communities (species richness and diversity patterns) in the intertidal systems (e.g., Ballesteros, 1992; Bellan and Bellan-Santini, 1972; Bellan-Santini, 1966; Bouchet and Tardy, 1976; Fischer-Piette and Duperier, 1960; Folin, 1903; Hondt, d'; Murray et al., 2006; Ortea, 1979; Thompson et al., 2002; Underwood, 1981; Van Den Hoek and Donze, 1966). The relevance of studying benthic macrofauna in intertidal boulder fields is widely documented throughout the world (Archambault and Bourget, 1996; Grayson and Chapman, 2004; Hiscock et al., 2005; Kuklinski et al., 2006; Sousa, 1979). Within this habitat, macroalgal communities are ephemeral due to boulder instability. Sessile macrofauna are distributed in a patchwork of successional stages (Sousa, 1979). Most French studies on macrofauna are conducted in Brittany (Bernard, 2012; Guillaumont et al., 2009; Le Hir and Hily, 2005). Surprisingly, few studies have been undertaken in the northern Basque coast (France) while high hydrodynamic pressure may occasionally disrupt the bathymetric zonation of benthic communities. Since 2008, solely macroalgae surveys have been carried out to assess the ecological status on platforms within the WFD (Casamajor (de) et al., 2016). A macrofauna inventory is currently conducted on the Basque coast but without considering the habitat type such as platforms and boulders (Castège et al., 2014). By contrast, many studies have been carried out on platforms and other habitats in the southern Basque coast (Spain) (Aguirrezabalaga et al., 1986; Aroca et al., 1984; Artica, 1978; Borja et al., 2004; Elosegui, 1985; Elosegui et al., 1987; Gorostiaga et al., 2004).

The marine subregion "Bay of Biscay" shows environmental specificities. In France, thirty percent of this coastal zone is composed by rocky substrata (Chust et al., 2009). The northern Basque coast constitutes a smaller rocky area (around 15 km long; Chust et al., 2009) in comparison with the Brittany's coastline or the southern Basque coast (around 135 km long; Borja and Collins, 2004). Within the intertidal zone, taxa live in heterogeneous habitats listed in the Habitats Directive Annex I as boulder fields, platforms and rock pools (EUR 27, July 2007, European Commission). This zone, with considerable spatial and temporal variations of environmental components (temperature, salinity), is especially sensitive because impacts are concentrated. Communities are thus subject to environmental conditions which create a stressful habitat. It is due to mesotidal conditions, with a magnitude between 1.85 and 3.85 m (Augris et al., 2009), energetic waves (Abadie et al., 2005; Bajjouk et al., 2015), freshwater input caused by rainfall and a dense river system (Winckel et al., 2004) and coast orientation (exposition to swell, slope). Urban sprawl and summer overcrowding also explain the increased anthropogenic pressures on the coastline (Cearreta et al., 2004; Chust et al., 2009; Le Treut, 2013). All those parameters in this region, considered as a heritage area (Augris et al., 2009; Casamajor (de) and Lalanne, 2016), justify the presence of specific communities in these remarkable habitats (Borja et al., 2004).

The aim of the study is therefore: (i) to describe macrofauna assemblages of the boulder field habitat that is poorly known in the southern marine subregion of the Bay of Biscay, (ii) to identify, for the first time, indicator species/taxa of this habitat which could be used to detect time changes and as an alternative to an exhaustive sampling of biodiversity and (iii) to provide a framework for future monitoring. Lastly, this study bridges the data gap emphasized by the MSFD on rocky-shore macrofauna communities notably in this specific area.

2. Material and methods

2.1. Sampling site

The field sampling campaign took place between March 19th and June 5th, 2015 in Guéthary (43° 25′ 36.475″ N–1° 36′ 58.445″ W) (Fig. 1) in the south west of France. This site is included in the Natura 2000 area named "rocky Basque coast and offshore extension" (Special Area of Conservation) and is also a Natural Zone of Ecological, Faunal and Floral Interest (ZNIEFF). Sampling was carried out in the intertidal rocky shore. It is composed of three habitats of European Community importance: "High energy littoral rock" (EUNIS A1-1), "Rockpools and permanent pools" (EUNIS 1170–8) and "Boulder fields" (EUNIS H5-37). Only the last one, composed of irregular boulders of various sizes piled up on each other or on the bedrock, has been sampled in this study.

2.2. Field data collection strategy

A two-level stratified random sampling design was used to confirm if community structure in this particular ecosystem is similar to other geographical regions (Le Hir and Hily, 2005). Each month, mobile boulders (size less than 0.1 m^2) were sampled in upper and lower midlittoral zones established on algal-dominated communities described in the WFD survey ("*Corallina spp.* & *Caulacanthus ustulatus*" and "*Halopteris scoparia & Gelidium spp.*"; Ar Gall et al., 2016). Samples were taken only at low wave conditions and during great tide range periods (coefficients greater than 80). We used 33 × 33 cm quadrats, the same size as those used for the WFD sampling (Ar Gall et al., 2016; Bernard, 2012). Both mobile and sessile macrofauna were identified in abundance and percentage cover respectively on top and underneath boulders (Chapman et al., 2009). Most organisms were identified *in situ* to species level to limit the sampling impact. When identification was impossible in the field (especially small species), specimens were taken to the laboratory or sent to taxonomic specialists. Due to the complex taxonomy of certain taxa, some organisms were identified to genus because it was impossible to collect all unidentified specimens. Two hundred sixty-five random quadrats (265), representing about 29 m^2 and spread over 16 sampling days, were sampled. Three experienced observers were present during each sampling day to achieve a sufficient number of quadrats. No observer effect could be detected (*Wilcoxon matched-pairs signed-ranks test* and *Fisher test*: $p > 0.05$).

Fig. 1. Study site location (43° 25′ 36.475″N–1° 36′58.445″W) and area (white line). The dotted line represents the midlittoral zone boundary.

2.3. Data treatment

The database was divided into two matrices corresponding to each biological group (sessile and mobile macrofauna assemblages) because: (i) the data were heterogeneous (abundance –vs.– percentage) between each group and (ii) the ecological function and the precision were dissimilar between the two groups (Chapman and Underwood, 2009).

The graphs and statistical analyses were undertaken using Excel v7® and R® software. Prior to analyses, the abundance matrix (mobile macrofauna) was Hellinger-transformed to reduce the influence of rare species abundance values as suggested in Legendre and Gallagher (2001).

2.3.1. Diversity and communities structure

Mean abundance and mean taxonomic richness were calculated per quadrat in each algal belt. Sampling fluctuations around mean abundances were described by their standard deviation. The Kruskal–Wallis test and multiple comparison tests, with pairwise comparisons appropriately adjusted (''kruskalmc''), were performed using ''pgirmess'' package to determine which groups were different (Giraudoux and Giraudoux, 2016).

Three multivariate analyses were performed to compare the community structure between algal belts. The first two were computed separately for each database (mobile macrofauna, sessile macrofauna). (i) A Principal Component Analysis (PCA) was performed on the sessile macrofauna database containing quantitative variables. (ii) A Correspondence Analysis (CA) was computed on mobile macrofauna data represented through a contingency table (taxa abundance). (iii) Then, a last PCA was performed on a new matrix objectively composed of new ''synthetic'' and independent factorial coordinates coming from the first two analyses. Only the latest PCA is presented below. One-way ANOVA was then performed on factorial coordinates to test if there was significant variation in means among upper and lower zones.

2.3.2. Indicator species/taxa

To identify single species/taxa or combinations considered as indicators of upper and lower zones, statistical analyses were performed with R® software using the ''indicspecies'' package (De Cáceres et al., 2010). Two independent Indicator Species Analyses (ISA) were conducted to identify sessile and mobile macrofauna taxa significantly associated with upper and lower midlittoral zones. These analyses assessed the strength and statistical significance of the relationship between species occurrence/abundance and groups of sites (De Cáceres et al., 2015). Prior to analysis, the percentage cover matrix (sessile macrofauna) was converted into presence/absence and the abundance matrix (mobile macrofauna) was Hellinger-transformed as described previously. Then, to highlight single and/or combination species considered as indicators, a new matrix with a maximum of a triple combination species was created using the ''combinespecies'' function. The significance of the association between species pattern and each midlittoral zone was assessed with a permutation test ($n = 999$) using the ''multipatt'' function. The Indicator Value index (IndVal) was used to select significant species (single species as well as two or three species combinations) by ordering them through the two components' values (A: the specificity or uniqueness to particular zone; B: fidelity or frequency of occurrence within a particular zone) (detailed in Dufrene and Legendre, 1997). Significant single and combination species with a low indicator value were discarded using thresholds for A (≥ 0.6) and B (≥ 0.2) in order to have a restricted list of characteristic species/taxa.

3. Results and discussion

3.1. Diversity assessment

To avoid problems with unidentified species, analyses were conducted on aggregated data containing mixed taxonomic levels (species, genus, family, class). Seventy-eight macrofauna species/taxa were identified, 59 considered as mobile macrofauna and 19 as sessile macrofauna (Fig. 2). Among the 59 mobile macrofauna taxa, 56 were found in the lower zone and 30 in the upper zone. Some of them were identified in the lower zone as well as in the upper zone and some appeared to be more represented (with mean values above 0.3 ind. 0.1 m^{-2}): *Clibanarius erythropus, Steromphala spp., Porcellana platycheles, Cerithium spp., Xantho spp., Patella spp. and Pachygrapsus marmoratus*. For sessile macrofauna, 18 taxa were identified in each zone. As for mobile macrofauna, some appear more abundant: *Spirorbinae, Balanomorpha, Spirobranchus spp., Sertularella sp.* and *Chthamalus spp.* Few studies about diversity have been carried out locally that is the reason why it is difficult to make comparisons with other regions, the environmental and geographical contexts being different (e.g. topography,

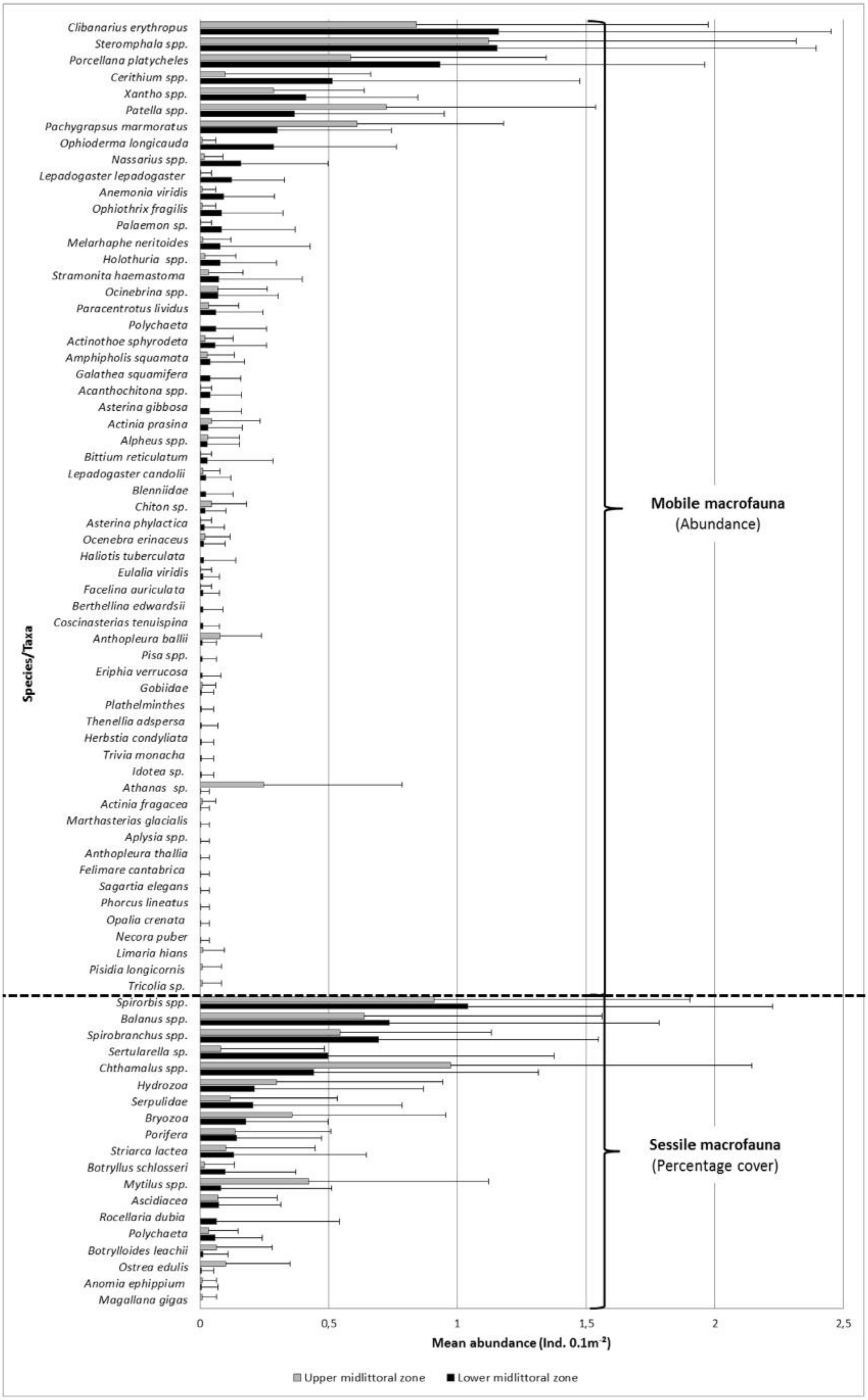

Fig. 2. List of species/taxa (mobile and fixed macrofauna) identified into quadrats in Guéthary in 2015 for lower (black) and upper zones (gray). Mean abundances were log-transformed.

tides, climate conditions). The only published study, undertaken on the same location, used a relatively different sampling design (16 m^2 stational quadrats on heterogeneous habitats including mobile, non-mobile boulders and platforms) (Castège et al., 2014). The use of smaller quadrats appears to be more appropriate to characterize biodiversity in a homogeneous habitat (here mobile boulders) as demonstrated by previous studies (Ar Gall et al., 2016; Bernard, 2012).

Sessile and mobile macrofauna assemblages were analyzed separately because of their different precision and ecological function. Indeed, species in the two assemblages could have quite different patterns of dispersion (Chapman et al., 2009). The mobile macrofauna living on and under boulders may move in response to changing environmental conditions. By contrast, the sessile macrofauna living in the same habitat cannot redistribute themselves. Dispersion patterns of sessile macrofauna are then more precise descriptors of recruitment and mortality in response to environmental changes. Hexacorallia, considered as relative mobile species by Chapman et al. (2009), *Patella spp.* and *Haliotis tuberculate*, slightly mobile species, were included with mobile macrofauna.

3.2. Communities structure

The first axis of the multivariate analysis based on factorial coordinates coming from PCA (sessile macrofauna) and CA (mobile macrofauna) confirms a significant stratification of the composition of communities between lower and upper zones (*one-way anova*; $p < 2.2e{-}16^{***}$) (Fig. 3). The total taxonomic richness and the mean taxonomic richness of mobile macrofauna (Fig. 4) are significantly higher in the lower midlittoral zone (*Kruskalmc*; $p < 0.05$). This bathymetric zonation has been already widely described in previous studies (Ballesteros, 1992; Bellan and Bellan-Santini, 1972; Bellan-Santini, 1966; Glémarec, 1973; Murray et al., 2006; Thompson et al., 2002; Underwood, 1981). But, contrary to other areas as Brittany, this zonation is not evident. This may be explained by the low tidal range, the topography (e.g. slope) and the extreme hydrodynamic features preventing a clear identification of the boundary between these two zones which are smaller than in the North of France (Abadie et al., 2005; Borja et al., 2004). No difference has been highlighted in mean taxonomic richness for sessile macrofauna communities between both zones (*Kruskalmc*; $p > 0.05$) and mean richness of mobile macrofauna has been always significantly higher than sessile macrofauna (*Kruskalmc*; $p < 0.05$). This may be due to the level of identification, which is not sufficiently precise for sessile macrofauna. *In situ* identification and sampling (e.g., *Porifera*, *Hydrozoa*, *Bryozoa* and *Ascidiacea*) were often difficult or impossible (time, cost, environmental conservation).

3.3. Indicator species/taxa identification

Indicator species analyses highlighted both a high statistical significance of singletons (individual species/taxa) and combinations (co-occurring species/taxa) (Table 1). Ecological parameters also provide some understanding in the functional aspect of coastal biocenosis. In this context, the following additional selection-criteria should then be considered (Table 2): opportunistic, characteristic or alien species, ZNIEFF determinant species, food webs, biogeographic range limit, life cycles and species sensitive to disturbances (Casamajor (de) and Lalanne, 2016; Díez et al., 2012).

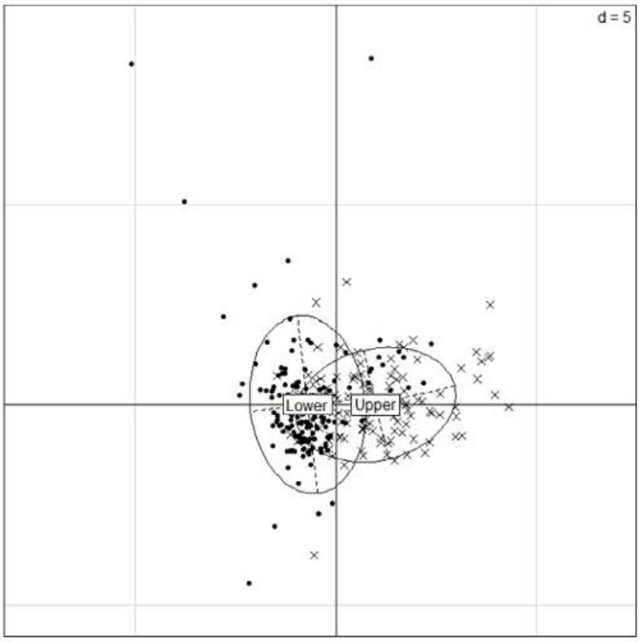

Fig. 3. Principal component analysis (PCA) (F1 × F2 plan) computed on factorial coordinates coming from the PCA (fixed macrofauna) and CA (mobile macrofauna) according to lower (black points) and upper midlittoral zones (gray crosses) in Guéthary.

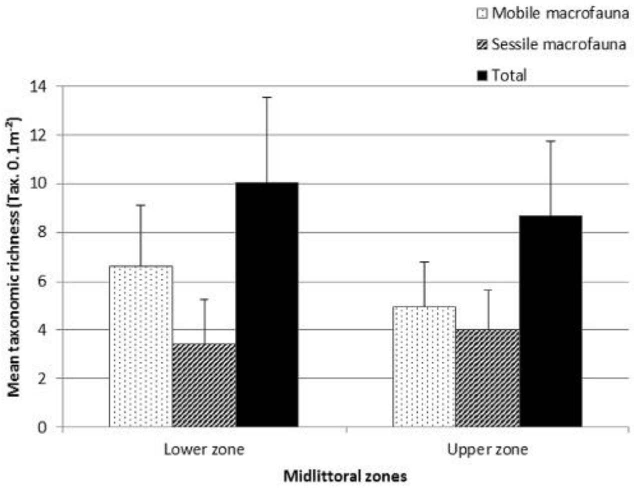

Fig. 4. Mean taxonomic richness of mobile macrofauna (point cloud), fixed macrofauna (hatching) all biological groups (black) identified into quadrats (0.1 m^2) of each midlittoral zones in Guéthary.

3.3.1. Mobile macrofauna indicator species

Among the 59 mobile macrofauna species/taxa recorded in this study, 9 were identified as valid significant characteristic species, with 3 common to both algal belts (Table 1).

In the lower zone, there are 6 singletons (*P. platycheles*, *C. erythropus*, *Ophioderma longicauda*, *Xantho spp.*, *Nassarius spp.* and *Lepadogaster lepadogaster*) and another taxa identified in combinations (*Steromphala spp.*). The number of combinations appears to be higher than singletons (20 double and triple combinations). *L. lepadogaster* and *O. longicauda* are indicators of the lower midlittoral zone because they occurred almost exclusively in quadrats belonging to this algal belt ($A = 0.98$ and 0.97 respectively), although not all quadrats belonging to this algal belt include these species ($B = 0.26$ and 0.41 respectively). *P. platycheles* may

Table 1
Results of Indicators Species Analysis and ecological features for valid single taxa and taxa combinations for fixed (a) and mobile macrofauna (b) (p-value <0.05; $A \geq 0.6$ and $B \geq 0.2$). $\underline{A}$ is a measure of specificity (uniqueness to upper or lower midlittoral zones) based on abundance values whereas $\underline{B}$ is a measure of fidelity (frequency of occurrence within a midlittoral zone) computed on presence data. ZNIEFF list (Natural Zone of Ecological, Faunal and Floral Interest; Casamajor (de) et al., 2013): zone of floristic, macrofaunal and ecological value; Sign of disturbance: disturbance-sensitive taxa to organic matter, sediments or habitat stability (as required by the WFD; Díez et al., 2012); EG: Ecological Group from the AMBI list (AZTI Marine Biotic Index; Borja et al., 2000).

		Midlittoral zones	A	B	Stat	Significance	Food web	Alien species	ZNIEFF list	Sign of disturbance	EG (AMBI list)
	Mobile macrofauna										
Singletons	*P. platycheles*	**Lower**	0.72	0.81	0.76	***	Omnivorous				
	C. erythropus	**Lower**	0.69	0.70	0.70	***	Omnivorous			✓	
	O. longicauda	**Lower**	0.97	0.41	0.63	***	Scavenger			✓	
	Xantho spp.	**Lower**	0.63	0.65	0.64	*	Omnivorous				I
	Nassarius spp.	**Lower**	0.90	0.21	0.43	***	Scavenger				II
	L. lepadogaster	**Lower**	0.98	0.26	0.50	***	Carnivorous				
	P. marmoratus	Upper	0.64	0.83	0.73	***	Omnivorous	✓	✓		II
	Patella spp.	Upper	0.66	0.73	0.70	***	Herbivorous				I
Combinations	*O. longicauda + C. erythropus*	**Lower**	0.99	0.34	0.58	***	Scavenger + Omnivorous				
	O. longicauda + P. platycheles	**Lower**	1.00	0.33	0.57	***	Scavenger + Omnivorous				
	O. longicauda + C. erythropus + P. platycheles	**Lower**	1.00	0.30	0.55	***	Scavenger + Omnivorous				
	Steromphala spp. *+ O. longicauda*	**Lower**	0.99	0.28	0.53	***	Herbivorous + Scavenger				
	Steromphala spp. + O. longicauda + C. erythropus	**Lower**	0.99	0.26	0.51	***	Herbivorous + Scavenger + Omnivorous				
	C. erythropus + P. platycheles	**Lower**	0.80	0.61	0.70	***	Omnivorous				
	Steromphala spp. + P. platycheles	**Lower**	0.66	0.57	0.62	**	Herbivorous + Omnivorous				
	Steromphala spp. + C. erythropus + P. platycheles	**Lower**	0.78	0.48	0.61	***	Herbivorous + Omnivorous				
	P. platycheles + Xantho spp.	**Lower**	0.68	0.55	0.61	**	Omnivorous				
	C. erythropus + P. platycheles + Xantho spp.	**Lower**	0.78	0.45	0.59	***	Omnivorous				
	C. erythropus + Xantho spp.	**Lower**	0.71	0.48	0.59	***	Omnivorous				
	Steromphala spp. + C. erythropus + Xantho spp.	**Lower**	0.68	0.37	0.50	*	Herbivorous + Omnivorous				
	Steromphala spp. + C. erythropus	**Lower**	0.68	0.56	0.62	**	Herbivorous + Omnivorous				
	O. longicauda + Xantho spp.	**Lower**	1.00	0.26	0.51	***	Scavenger + Omnivorous				
	Steromphala spp. + O. longicauda + P. platycheles	**Lower**	1.00	0.23	0.48	***	Herbivorous + Scavenger + Omnivorous				
	O. longicauda + C. erythropus + Xantho spp.	**Lower**	1.00	0.23	0.48	***	Scavenger + Omnivorous				
	O. longicauda + P. platycheles + Xantho spp.	**Lower**	1.00	0.23	0.48	***	Scavenger + Omnivorous				
	Steromphala spp. + L. lepadogaster	**Lower**	0.97	0.21	0.46	***	Herbivorous + Carnivorous				
	L. lepadogaster + P. platycheles	**Lower**	0.97	0.21	0.45	***	Carnivorous + Omnivorous				
	L. lepadogaster + C. erythropus	**Lower**	1.00	0.20	0.45	***	Carnivorous + Omnivorous				
	P. marmoratus + Patella spp.	Upper	0.72	0.66	0.69	***	Omnivorous + Herbivorous				
	Steromphala spp. + P. marmoratus	Upper	0.66	0.69	0.67	***	Herbivorous + Omnivorous				
	Steromphala spp. + Patella spp.	Upper	0.69	0.60	0.64	***	Herbivorous				
	Steromphala spp. + P. marmoratus + Patella spp.	Upper	0.74	0.56	0.64	***	Herbivorous + Omnivorous				
	P. marmoratus + C. erythropus + Patella spp.	Upper	0.66	0.36	0.48	**	Omnivorous + Herbivorous				
	P. marmoratus + Patella spp. + Xantho spp.	Upper	0.62	0.32	0.44	**	Omnivorous + Herbivorous				

(continued on next page)

be considered as characteristic of this belt because it occurred in most quadrats ($B = 0.81$) at that level and it was largely restricted to it ($A = 0.72$).

In the upper zone, 5 species/taxa are identified (Table 1): 2 singletons (*P. marmoratus* and *Patella spp.*) and 3 other taxa in combinations (*Steromphala spp.*, *C. erythropus* and *Xantho spp.*).

The Decapods identified in the results (Table 1) are omnivorous unlike the Mollusca *Patella spp.* and *Steromphala spp.* which are herbivorous. In Brittany, herbivorous species are favored as indicators because their presence/absence and abundance are directly related to the habitat ecological status (Hily and Grall, 2003). In the southern Bay of Biscay, habitats dominated by large brown algae are lacking, that is why we did not consider only the herbivores. So, the scavengers *Nassarius spp.* and *O. longicauda* and the carnivores *L. lepadogaster* (Pisces) were also considered as indicator species.

Finally, the Crustacea, biomonitors of metal pollution (Adamo (d') et al., 2008), and *P. marmoratus*, a remarkable species with strong heritage interest (ZNIEFF) (Derrien-Courtel, 2010), could also represent characteristic taxa.

Instead of just considering single species, the indicator value of species combinations has to be explored (Dufrene and Legendre, 1997). Indeed, two or three species found together may contain more ecological information than a single one (De Cáceres, 2013). For example, the combination of *O. longicauda* + *P. platycheles* + *Xantho spp.* occurs exclusively in the lower midlittoral zone ($A = 1$) but frequency of occurrence of this combination

Table 1 *(continued)*

	Sessile macrofauna									
Singletons	*Spirobranchus spp.*	**Lower**	0.64	0.81	0.72	*	Planktivorous			II
	Sertularella sp.	**Lower**	0.92	0.22	0.45	***	Planktivorous			I
	Chthamalus spp.	Upper	0.73	0.64	0.69	***	Planktivorous			I
	Mytilus spp.	Upper	0.89	0.31	0.53	***	Planktivorous	✓	✓	III
Combinations	*Spirobranchus spp.* + *Spirorbis spp.*	**Lower**	0.64	0.74	0.69	*	Planktivorous			
	Sertularella sp. + *Spirorbis spp.*	**Lower**	0.91	0.21	0.43	***	Planktivorous			
	Chthamalus spp. + *Spirorbis spp.*	Upper	0.72	0.55	0.63	***	Planktivorous			
	Chthamalus spp. + *Spirobranchus spp.*	Upper	0.69	0.46	0.56	***	Planktivorous			
	Chthamalus spp. + *Spirobranchus spp.* + *Spirorbis spp.*	Upper	0.68	0.44	0.55	***	Planktivorous			
	Chthamalus spp. + *Mytilus spp.*	Upper	0.96	0.23	0.47	***	Planktivorous			
	Mytilus spp. + *Spirorbis spp.*	Upper	0.89	0.23	0.46	***	Planktivorous			

Significance codes: Lower: Lower midlittoral zone; Upper: Upper midlittoral zone.

* $p < 0.05$.

** $p < 0.01$.

*** $p < 0.001$.

Table 2
Species with high ecological interest and sampled during this study but not considered statistically as valid indicators. Significance codes: Lower: Lower midlittoral zone; Upper: Upper midlittoral zone; ZNIEFF list (Natural Zone of Ecological, Faunal and Floral Interest; Casamajor (de) et al., 2013): zone of floristic, macrofaunal and ecological value; Sign of disturbance: disturbance-sensitive taxa to organic matter, sediments or habitat stability (as required by the WFD; Díez et al., 2012).

Taxa groups (Non indicators)	Sampled in	Biogeographic range limit	ZNIEFF list	Sign of disturbance	Food web
Hydrozoa					
Sertularella mediterranea	Lower	✓ North limit	✓		Planktivorous
Mollusca					
Opalia crenata	Lower	✓ North limit			Herbivorous
Stramonita haemastoma	Lower	✓ North limit			Carnivorous
Crustacea					
Eriphia verrucosa	Upper		✓		Carnivorous
Galathea squamifera	Lower		✓	✓	Carnivorous
Herbstia condyliata	Lower	✓ North limit			Omnivorous
Echinodermata					
Holothuria tubulosa	Lower			✓	Scavenger
Ophiothrix fragilis	Lower			✓	Scavenger
Paracentrotus lividus	Lower		✓	✓	Omnivorous
Pisces					
Parablennius pilicornis	Lower	✓ North limit			Omnivorous
Parablennius incognitus	Lower	✓ North limit			Omnivorous

is lower ($B = 0.4$) (Table 1). Two taxa such as *Steromphala spp.* and *C. erythropus* appear in the lower as well as in the upper zone. *Steromphala spp.* are highlighted as indicators in both zones in some combinations. Therefore, it is not possible to select this taxon as single valid indicator. *C. erythropus* is a singleton which indicates the lower midlittoral zone. In combination with other taxa, it also indicates the upper midlittoral zone. The distinction between the midlittoral zones could be clearer if the identification was achieved at the specific level. Moreover, a more precise taxonomic level will also be needed for other taxa such as *Patella sp.* The genus *Patella spp.*, sensitive to sewage pollution (Espinosa et al., 2007), contains four different species (*P. depressa, P. rustica, P. ulyssiponensis, P. vulgata*), including one with a north range limit (*P. rustica*) (Ibanez et al., 1986). A more precise identification would then permit to focus on species of higher interest. The use of mobile macrofauna as indicators is interesting because their small-scale pattern movements positively influence biodiversity (Davidson et al., 2004). Valid statistical indicators are numerous (singletons and combinations) and their ecological properties are various. Monitoring mobile macrofauna is also often a *"snapshot in space and time because they respond to environmental changes by short-terms variability of their community structure as dispersal, movements and migrations"* (Davidson et al., 2004; Takada, 1999).

3.3.2. Sessile macrofauna indicator species

Among the 19 sessile macrofauna taxa identified during the study, 7 taxa are listed as significant indicators. There are 11 singletons or combinations (Table 1). In the lower zone, there are 2 single taxa (*Spirobranchus spp.* and *Sertularella sp.*) and 2 combinations composed of *Spirobranchus spp.* with Spirobinae and *Sertularella sp.* with Spirobinae. The upper midlittoral zone was indicated by 2 singletons *Chthamalus spp.* and *Mytilus spp.*, 4 double combinations and 1 triple combination.

Sessile species or slightly mobile species are highly sensitive and constitute the first biological compartment impacted by any environmental changes such as sewage input, sedimentary processes or climate modifications (Maughan, 2001; Mieszkowska, 2015; Murray et al., 2006; Roberts et al., 1998). They may modify community structure and population dynamics of communities (Mieszkowska, 2015). For example, *Mytilus spp.*, *Balanomorpha* and *Chthamalus spp.* are disturbance indicators like filter feeders, deriving benefits from organic matter enrichment (Díez et al., 2012). *Mytilus spp.* is also a taxon listed in the ZNIEFF inventory. As mentioned previously, these species therefore represent better indicators than mobile macrofauna communities which may easily escape disturbances in their habitat through the modification of their distribution (Casamajor (de) and Lalanne, 2016), for example in "*higher latitudes where sea and air temperatures are cooler*" (Mieszkowska, 2015).

3.3.3. Ecological interest of non valid statistically indicator species/taxa (according to "indicspecies" package)

The Indicator Species Analysis method revealed valid single and combination indicator taxa for the lower and upper midlittoral zones. But some species with values lower than defined thresholds may present ecological interest. Therefore, they also constitute interesting species/taxa and have to be taken into account to monitor boulder fields (Table 2). For example, no valid indicator species are in the biogeographical range limit. However, some species sampled in this study are within northern range limit of geographical distribution, namely *Sertularella mediterranea* (Hydrozoa) (Altuna, 2006), *Opalia crenata* and *Stramonita haemastoma* (Mollusca), *Herbstia condyliata* (Crustacea), or Pisces such as *Parablennius pilicornis* and *Parablennius incognitus* (Casamajor (de) and Lalanne, 2016). It would be worthwhile considering all these additional species because in the context of global change they are directly impacted by rising sea levels and shallow water temperature increases (Le Treut, 2013). They could then disappear, migrate from the south to the north (when they are in the northern limit), or be impacted by an drastic modification of their population (Casamajor (de) and Lalanne, 2016). Some of these species are reported as remarkable (ZNIEFF) and also form good indicators of disturbance such as *Eriphia verrucosa*, *Galathea squamifera* (Crustacea), *Holothuria spp.*, *Ophiothrix fragilis*, *Paracentrotus lividus* (Echinodermata). For example, abundance of Echinodermata is strongly linked to organic matter (Burger, 2006).

This study is an initial overview of the benthic macrofauna diversity and bridges the data gap underlined by MSFD in the South of the Bay of Biscay. Moreover, these results argue in favor of two main descriptors of this Directive: "biological diversity" ("state" descriptor) and "sea-floor integrity" ("pressure" and "impact" descriptors). This study also constitutes a framework for future monitoring. After several years of use, it could improve the knowledge on community responses to natural changes and ascertain if ecosystem function and structure are maintained throughout the years and remain undisturbed.

4. Conclusion

This study describes the benthic macrofauna diversity (78 identified taxa) in rocky intertidal boulder fields habitat in the south of the Bay of Biscay. This work confirms the clear mobile macrofauna stratification between lower and upper midlittoral zones for the northern Basque coast as it is already well known in Brittany and in the NW of Spain. The present work's originality lies in the way it separately explores sessile and mobile macrofauna because they may provide different and/or complementary information. For the first time in this marine subregion, singletons (12) as well as combinations (33) of indicator species were identified. Other species of ecological interest (11) were also highlighted in this work. The identification of indicators is an alternative to sampling the entire local biodiversity and provides a framework for future monitoring of the Basque intertidal rocky shore. This work also is a highly topical subject in the context of global change and increases the knowledge about rocky biocenosis in the south of the Bay of Biscay, poorly assessed in this marine subregion up to now. Different MSFD descriptors, as diversity and sea-floor integrity, are also supported in this study providing information on algal belts functionality.

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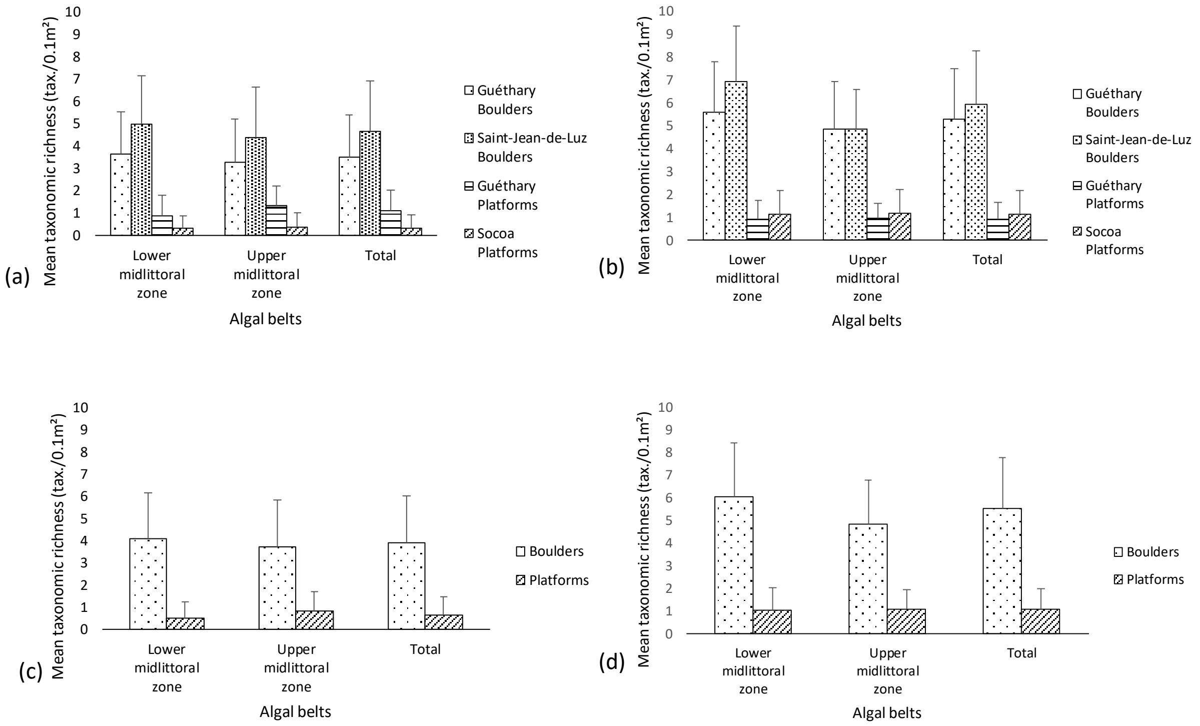
Annex 7: Mean taxonomic richness of fixed (a, c) and mobile macrofauna (b, d) identified in boulder fields (in Guéthary and Saint-Jean-de-Luz) and on platforms (Guéthary and Socoa) in each algal belt (upper and lower midlittoral zones).



Annex 8: Curriculum vitae

Doctorante en Biologie Marine et Chimie de l'environnement (Diplômée en Décembre 2019) :
Spécialisée dans l'étude de la diversité benthique (intertidale et subtidale) des substrats rocheux et dans la caractérisation des incidences des rejets urbains sur les écosystèmes côtiers

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PARCOURS UNIVERSITAIRE

- **2012** – **Licence 1** d'Océanographie – Marseille (**FR**)
- **2013** – **Licence 2** d'Océanographie – Marseille (**FR**)
- **2014** – **Bachelor's degree** In Oceanography – Norwich (**GB**)
- **2015** – **Master 1** Dynamique des Ecosystèmes Aquatiques – Anglet (**FR**)
- **2016** – **Master 2** Dynamique des Ecosystèmes Aquatiques – Anglet (**FR**)
- **2019** – **Doctorat (double diplôme)** Biologie/Ecologie Chimie – Anglet (**FR**) – Bilbao (**SP**)

EXPERIENCES PROFESSIONNELLES

2016-2019 : IPREM LCABIE (UMR CNRS / UPPA 5254) – 'Benthic Marine Research Group' (UPV/EHU)
Doctorat en double diplôme – Anglet (FR)/Bilbao (SP) – 3 ans
Etude de l'impact des rejets de stations d'épuration sur les communautés benthiques des zones intertidales et subtidales dans le sud-est du Golfe de Gascogne (France et Espagne). Double approche : (i) analyse de la diversité et de la structure des communautés benthiques, (ii) analyse des micropollutants dans les rejets de stations d'épuration et dans des organismes benthiques (bioaccumulation).

2017 : Station Marine de Roscoff (FR2424, UPMC (EI 937) /CNRS)) – Techniques appliquées à la plongée scientifiques en écologie côtière (UPMC 4UM54)
Formation de plongée scientifique professionnelle – Roscoff (FR) – 10 jours
Réalisation de divers travaux scientifiques : relevés de diversité et mise en place de systèmes de mesures *in situ*, mesures biométriques, divers types d'échantillonnage, relevés photographiques.

2016 : LMAP (Laboratoire de Mathématiques et de leurs Applications de Pau - UMR CNRS 5142) et LER-AR-AN (IFREMER)
Stage – Anglet (FR) - 6 mois
Etude de la biodiversité benthique des platiers rocheux et des champs de blocs du sud-est du Golfe de Gascogne. Mise en place d'un protocole d'échantillonnage pour caractériser la biodiversité des côtes rocheuses en accord avec les Directives Européennes (DCE, DCSMM). Mise en place de nouveaux outils de surveillance et d'aide à la décision. Comparaisons inter- et intra-sites et analyse des effets de l'effort d'échantillonnage en fonction de l'habitat. Identification d'espèces indicatrices de l'habitat.

2015 : LER-AR-AN (IFREMER)
Stage – Anglet (FR) - 6 mois
Etude des communautés benthiques (macrofaune et macroalgues) dans l'habitat "champs de blocs" à l'aide d'un protocole d'échantillonnage standardisé et adapté à la Côte Basque. Réalisation d'une base de données et d'un collection d'espèces et de photos pour améliorer la connaissance de la biodiversité de la Côte Basque.

2012-2013 :
STARESO (STAtion de REcherche Sous-marines et Océanographiques - ULg Belgique) *– Stage – Calvi, Corse (FR) - 1 mois*
MIO (Mediterranean Institute of Oceanography - UMR CNRS 7294 - IRD 235 - UM AMU 110) *– Stage – Marseille (FR) - 1 mois*
IESC (Institut d'Etudes Scientifiques de Cargèse - UMS CNRS 820) *– Stage – Cargèse, Corse (FR) - 1 mois*

LOISIRS : Plongée sous-marine (Niv. 4, photographie), équitation et volley-ball en compétition, course, natation, surf/paddle, VTT, randonnée.

COMPETENCES

Organisationnelles :

- Gestion administrative et technique de projets et de missions
- Management d'équipe

Techniques :

- **Plongée professionnelle :**
  - **CAH II B** : aptitude à réaliser des interventions subaquatiques à l'air et au Nitrox entre 0 et 50m
  - **Expérience** : réalisation de plongées au niveau de rejets de stations d'épuration, à l'intérieur de ports et de brises lames, sous forts courants et de nuit
  - **Matériels spécifiques** : combinaison étanche, manutention d'objets encombrants
- **Stratégies d'échantillonnage et expertise de terrain :**
  - **Suivis DCE** : paramètres "macroalgues subtidales" et "macroalgues intertidales" pour la masse d'eau FRFC11 « Côte Basque »
  - **Dénombrements** : quadrats, transects, points
  - **Identification *in situ* et en laboratoire :** Diversité benthique (macrofaune et macroalgues au genre et à l'espèce) des substrats rocheux.
  - **Prélèvements** : aspirateur sous-marin/suçeuse, panier de brossage, prélèvements biologiques, rosette, bouteilles hyperbares
  - **Relevés** : sonde multiparamètres, GPS, mesures biométriques, techniques de marquage, RAS
  - **Installation et relevage d'appareils de mesure** : chambres benthiques
  - **Prise de vue** : photographie, vidéo
- **Analyses :**
  - **Statistiques descriptives uni- et multivariées sous R® et PRIMER®**
  - **Cartographie – SIG :** QGIS®
  - **Chimie analytique** : métaux, organométaux, organiques (HAP, PCB, alkylphenols, muscs, substances solaires, pharmaceutiques) dans les matrices "Eau" et "Biote". Utilisation de lyophilisateur (Cryotec), Digiprep, four à pyrolyse et d'azote liquide
  - **Dosage de micropolluants** par GC-MS, ICP-MS, GC-ICP-MS, LC-MS/MS

Autres :

- **Actions de transfert** : aux scientifiques (3 articles, 6 conférences), aux élus et grand public.

Annex 9: Personal bibliography

Articles published in international peer-reviewed journals

Huguenin L., Lalanne Y, de Casamajor MN., Gorostiaga J-M., Quintano E., Salerno M., Monperrus M. (**2019**). "*Impact of sewage discharges on macroalgae and macrofauna assemblages of the intertidal rocky shores in the southeastern Bay of Biscay*". ***Continental Shelf Research***, 181, 34-49 (https://doi.org/10.1016/j.csr.2019.04.014).

de Casamajor MN., Lalanne Y, Derrien-Courtel S., Gorostiaga J-M., Le Gal A., **Huguenin L.**, Quintano E., Lissardy M. (**2019**). "*Cystoseira baccata* meadows along the French Basque coast (Bay of Biscay) as a reference for the implementation of the Water Framework and Marine Strategy EU directives". ***Continental Shelf Research***, *182*, 12-21 (https://doi.org/10.1016/j.csr.2019.05.017).

Huguenin L., Lalanne Y., Bru N., Lissardy M., D'Amico F., Monperrus M., de Casamajor MN. (**2018**). "*Identifying macrofaunal assemblages and indicator taxa of intertidal boulder fields in the south of the Bay of Biscay (northern Basque coast). A framework for future monitoring*". ***Regional Studies in Marine Science***, 20, 13-22 (http://doi.org/10.1016/j.rsma.2018.03.012).

Oral communications and posters presented during international and national conferences and seminars

Huguenin L., de Casamajor MN., Lalanne Y., Gorostiaga JM., Monperrus M. (**2018**). "*Intertidal communities' response to sewage discharges and associated micropollutants along the French and the Spanish rocky Basque coast*". **International conference** (**Oral communication**): ISOBAY 16 - XVIth International Symposium of Oceanography of the Bay of Biscay. June 5-7th 2018, Anglet, France.

de Casamajor MN., Lalanne Y., Sartoretto S., **Huguenin L.**, Mourguiart B., Bru N., Lissardy M. (**2018**). "*Why not monitoring circalittoral reef habitat of the south of the Bay of Biscay with INDEX-COR approach?*". **International conference** (**Poster**): ISOBAY 16 - XVIth International Symposium of Oceanography of the Bay of Biscay. June 5-7th 2018, Anglet, France.

de Casamajor MN., Lalanne Y., **Huguenin L.**, Derrien-Courtel S., Gorostiaga JM, Le Gal A., Quintano E., Lissardy M. (**2018**). *"Characterization of Cystoseira baccata rocky subtidal stands along the French Basque coast (Bay of Biscay)"*. **International conference** (**Oral communication**): ISOBAY 16 - XVIth International Symposium of Oceanography of the Bay of Biscay. June 5-7th 2018, Anglet, France.

Bisch A., **Huguenin L.**, Miossec C., de Casamajor MN., Lalanne Y., Gorostiaga JM., Monperrus M. (**2018**). "*Accumulation of priority and emerging pollutants in benthic macrofauna from the rocky coast of the Bay of Biscay*". **International conference** (**Poster**): ISOBAY 16 - XVIth International Symposium of Oceanography of the Bay of Biscay. June 5-7th 2018, Anglet, France.

Salerno M., **Huguenin L.**, de Casamajor MN., Lalanne Y., Gorostiaga JM., Bisch A., Monperrus M. (**2018**). "*Assessing impact of sewage outfalls on intertidal rocky shores along the Basque coast (southeastern Bay of Biscay): assemblage structure analysis of benthic communities*". **International conference** (**Poster**): ISOBAY 16 - XVIth International Symposium of Oceanography of the Bay of Biscay. June 5-7th 2018, Anglet, France.

Mourguiart B., Lalanne Y., **Huguenin L.**, Bru N., De Casamajor MN. (**2018**). *"Assessment of circalittoral reef benthic assemblages along a distance gradient to the coast in N2000 Basque coast (southern Bay of Biscay)".* **International conference** (**Poster**): ISOBAY 16 - XVIth International Symposium of Oceanography of the Bay of Biscay. June 5-7th 2018, Anglet, France.

Huguenin L., De Casamajor MN. (**2018**). *"Etat et évolution de la qualité du milieu littoral sud Aquitain – Impact des effluents urbains sur les communautés benthiques de la côte Basque rocheuse intertidale et subtidale".* **National seminar** (**Oral communication**): NATURALG – Development of indicators "MacroALGue" for NATURA 2000 and harmonization with WFD, MSFD and REBENT surveys - Initiated by the AFB, CNRS and MNHN. March 26-28th 2018, Concarneau, France.

Huguenin L., de Casamajor MN., Lalanne Y., Gorostiaga J-M., Monperrus M. (**2017**). *"Impact of urban effluents on benthic communities along the Basque coast".* **International conference** (**Poster**): COAST Bordeaux 2017 - Evolution systémique et de la biodiversité des environnements côtiers et littoraux sous la pression du changement climatique, des facteurs naturels et anthropiques locaux. November 7-10th 2017, Bordeaux, France (http://archimer.ifremer.fr/doc/00406/51742/)

Lalanne Y., **Huguenin L.**, de Casamajor MN. (**2017**). *"How determining a small set of indicator species useful in environmental monitoring? A case study of intertidal boulder fields macrofauna (Northern Basque coast) using the R package "indicspecies"".* **National conference** (**Poster**): 6èmes rencontres 'R'. June 28-30th 2017, Anglet, France (http://archimer.ifremer.fr/doc/00391/50216/)

Huguenin L., de Casamajor MN., Lalanne Y., Gorostiaga J-M., Monperrus M. (**2017**). *"Impact of urban effluents on subtidal and intertidal rocky biocenosis along the Basque coast"*. **National conference** (**Poster**): Carhamb'ar 2017. March 14-16th 2017, Brest, France (http://archimer.ifremer.fr/doc/00382/49332/)

Caill-Milly N., De Casamajor MN, Bru N., D'Amico F., Lalanne Y., **Huguenin L.**, Kermorvant C., Sanchez F., Lissardy M., Abadie S., Maron P., Maillet G., Regard V., Pigot T., Labrousse JM. (**2016**). *"Développement d'outils méthodologiques pour l'évaluation de biocénoses marines et de ressources halieutiques d'intérêt régional en vue de leur conservation ou de leur valorisation durable"*. **Seminar** (**Oral communication**): Rencontres de la Fédération MIRA (Fédération de recherche sur les milieux et les ressources aquatiques). December 8th 2016, Anglet, France.

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Huguenin L., Arnoux F., Beauvivre M. (**2016**). *"How two different studies carried out on French Basque coast could be complementary in the context of climate change?".* **International conference** (**Poster**): Littoral 2016 - The changing littoral. Anticipation and adaptation to climate change - 13th conference of the traditional biennial international event of the Coastal & Marine Union (EUCC). October 25-29th 2016, Biarritz, France.

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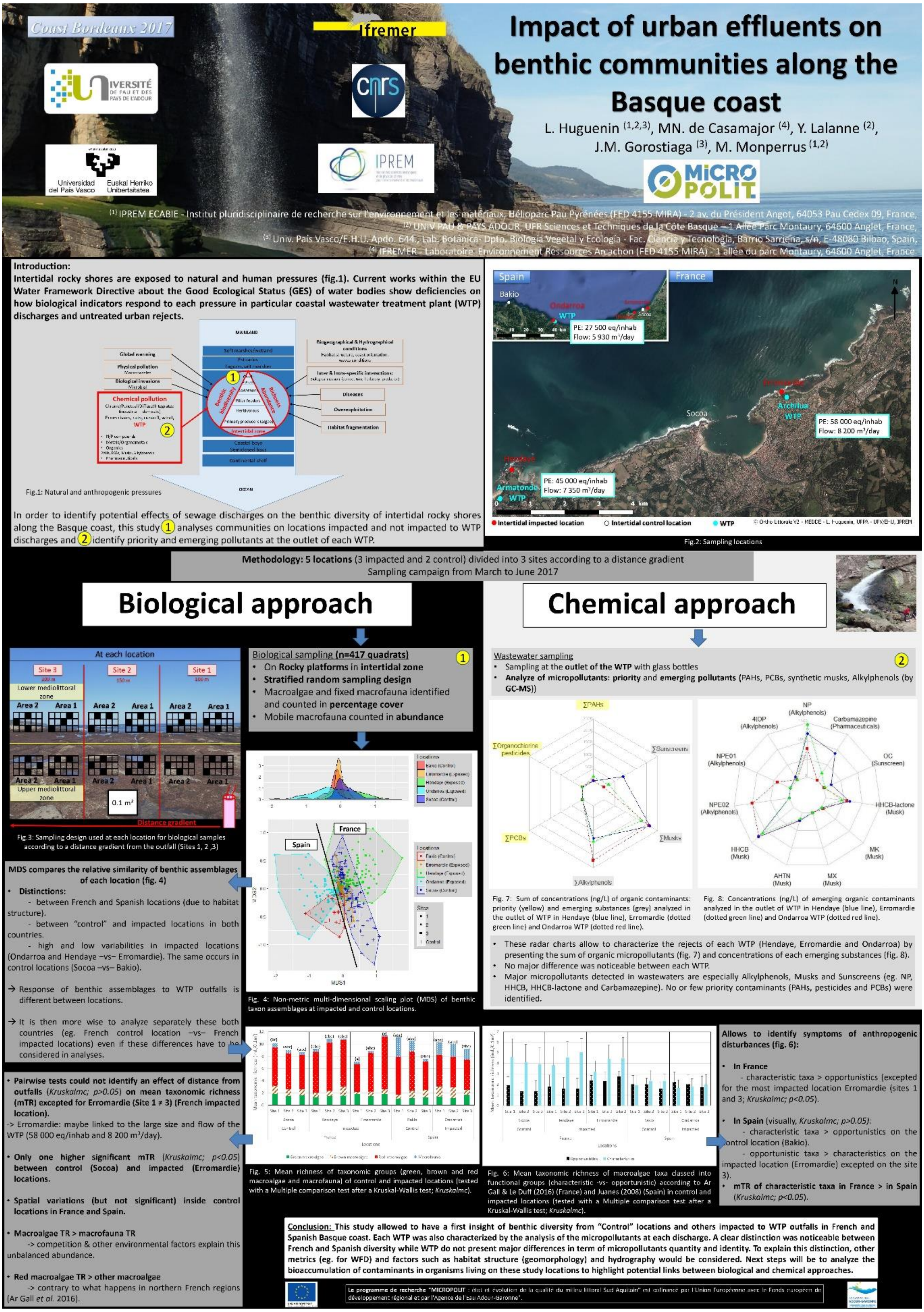
Annex 10: Posters

Huguenin L., de Casamajor MN., Lalanne Y., Gorostiaga J-M., Monperrus M. (**2017**). *"Impact of urban effluents on benthic communities along the Basque coast".* **International conference** (**Poster**): COAST Bordeaux 2017 - Evolution systémique et de la biodiversité des environnements côtiers et littoraux sous la pression du changement climatique, des facteurs naturels et anthropiques locaux. November 7-10th 2017, Bordeaux, France (http://archimer.ifremer.fr/doc/00406/51742/)



Coast Bordeaux 2017

Impact of urban effluents on benthic communities along the Basque coast

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Introduction:

Intertidal rocky shores are exposed to natural and human pressures (fig.1). Current works within the EU Water Framework Directive about the Good Ecological Status (GES) of water bodies show deficiencies on how biological indicators respond to each pressure in particular coastal wastewater treatment plant (WTP) discharges and untreated urban rejects.

Fig.1: Natural and anthropogenic pressures

In order to identify potential effects of sewage discharges on the benthic diversity of intertidal rocky shores along the Basque coast, this study ① analyses communities on locations impacted and not impacted to WTP discharges and ② identify priority and emerging pollutants at the outlet of each WTP.

Fig.2: Sampling locations

Methodology: 5 locations (3 impacted and 2 control) divided into 3 sites according to a distance gradient
Sampling campaign from March to June 2017

Biological approach

Fig.3: Sampling design used at each location for biological samples according to a distance gradient from the outfall (Sites 1, 2 ,3)

Biological sampling **(n=417 quadrats)** ①
- On **Rocky platforms** in **intertidal zone**
- **Stratified random sampling design**
- Macroalgae and fixed macrofauna identified and counted in **percentage cover**
- Mobile macrofauna counted in **abundance**

Fig. 4: Non-metric multi-dimensional scaling plot (MDS) of benthic taxon assemblages at impacted and control locations.

MDS compares the relative similarity of benthic assemblages of each location (fig. 4)

- **Distinctions:**
  - between French and Spanish locations (due to habitat structure).
  - between "control" and impacted locations in both countries.
  - high and low variabilities in impacted locations (Ondarroa and Hendaye –vs– Erromardie). The same occurs in control locations (Socoa –vs– Bakio).

→ Response of benthic assemblages to WTP outfalls is different between locations.

→ It is then more wise to analyze separately these both countries (eg. French control location –vs– French impacted locations) even if these differences have to be considered in analyses.

- **Pairwise tests could not identify an effect of distance from outfalls** (*Kruskalmc; p>0.05*) **on mean taxonomic richness (mTR) excepted for Erromardie (Site 1 ≠ 3) (French impacted location).**

-> Erromardie: maybe linked to the large size and flow of the WTP (58 000 eq/inhab and 8 200 m³/day).

- **Only one higher significant mTR** (*Kruskalmc; p<0.05*) **between control (Socoa) and impacted (Erromardie) locations.**
- **Spatial variations (but not significant) inside control locations in France and Spain.**
- **Macroalgae TR > macrofauna TR**
  -> competition & other environmental factors explain this unbalanced abundance.
- **Red macroalgae TR > other macroalgae**
  -> contrary to what happens in northern French regions (Ar Gall *et al.* 2016).

Fig. 5: Mean richness of taxonomic groups (green, brown and red macroalgae and macrofauna) of control and impacted locations (tested with a Multiple comparison test after a Kruskal-Wallis test; *Kruskalmc*).

Chemical approach

Wastewater sampling ②
- Sampling at the **outlet of the WTP** with glass bottles
- **Analyze of micropollutants: priority** and **emerging pollutants** (PAHs, PCBs, synthetic musks, Alkylphenols (by **GC-MS**))

Fig. 7: Sum of concentrations (ng/L) of organic contaminants: priority (yellow) and emerging substances (grey) analyzed in the outlet of WTP in Hendaye (blue line), Erromardie (dotted green line) and Ondarroa WTP (dotted red line).

Fig. 8: Concentrations (ng/L) of emerging organic contaminants analyzed in the outlet of WTP in Hendaye (blue line), Erromardie (dotted green line) and Ondarroa WTP (dotted red line).

- These radar charts allow to characterize the rejects of each WTP (Hendaye, Erromardie and Ondarroa) by presenting the sum of organic micropollutants (fig. 7) and concentrations of each emerging substances (fig. 8).
- No major difference was noticeable between each WTP.
- Major micropollutants detected in wastewaters are especially Alkylphenols, Musks and Sunscreens (eg. NP, HHCB, HHCB-lactone and Carbamazepine). No or few priority contaminants (PAHs, pesticides and PCBs) were identified.

Fig. 6: Mean taxonomic richness of macroalgae taxa classed into functional groups (characteristic -vs- opportunistic) according to Ar Gall & Le Duff (2016) (France) and Juanes (2008) (Spain) in control and impacted locations (tested with a Multiple comparison test after a Kruskal-Wallis test; *Kruskalmc*).

Allows to identify symptoms of anthropogenic disturbances (fig. 6):

- **In France**
  - characteristic taxa > opportunistics (excepted for the most impacted location Erromardie (sites 1 and 3; *Kruskalmc; p<0.05*).
- **In Spain** (visually, *Kruskalmc; p>0.05):*
  - characteristic taxa > opportunistics on the control location (Bakio).
  - opportunistic taxa > characteristics on the impacted location (Erromardie) excepted on the site 3).
- **mTR of characteristic taxa in France > in Spain** (*Kruskalmc; p<0.05*).

Conclusion: This study allowed to have a first insight of benthic diversity from "Control" locations and others impacted to WTP outfalls in French and Spanish Basque coast. Each WTP was also characterized by the analysis of the micropollutants at each discharge. A clear distinction was noticeable between French and Spanish diversity while WTP do not present major differences in term of micropollutants quantity and identity. To explain this distinction, other metrics (eg. for WFD) and factors such as habitat structure (geomorphology) and hydrography would be considered. Next steps will be to analyze the bioaccumulation of contaminants in organisms living on these study locations to highlight potential links between biological and chemical approaches.

Le programme de recherche "MICROPOLIT : état et évolution de la qualité du milieu littoral Sud Aquitain" est cofinancé par l'Union Européenne avec le Fonds européen de développement régional et par l'Agence de l'Eau Adour-Garonne".

Huguenin L., de Casamajor MN., Lalanne Y., Gorostiaga J-M., Monperrus M. (**2017**). *“Impact of urban effluents on subtidal and intertidal rocky biocenosis along the Basque coast”*. **National conference** (**Poster**): Carhamb’ar 2017. March 14-16th 2017, Brest, France (http://archimer.ifremer.fr/doc/00382/49332/)

Impact of urban effluents on subtidal and intertidal rocky biocenosis along the Basque coast

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Context:

In compliance with the Water Framework Directive (WFD), the good ecological status assessment for Channel-Atlantic coastal water bodies is currently carried out in the intertidal and subtidal zones using a biological indicator based on macroalgae on the rocky coasts. The second step of this Directive (2016-2021) deals with the workings of the marine ecosystem. Therefore, macrofauna has also to be taken into account in the both zones.

Current works about the WFD indicator of pressure-impact interactions show deficiencies on how the biological indicators respond to each pressure regarding magnitude and type. Therefore, the implementation of a monitoring program on the southern rocky coast is essential to understand and assess the proper ecological condition of the whole marine subregion.

Objectives:

This PhD project aims to study the impact of urban effluent on benthic communities (macrofauna and macroalgae). This work will be achieved in cooperation with the UPV/EHU. The objectives are then to define relevant indicators at the scale of the south of the bay of Biscay rocky shore.

Methodology: Field studies from March to June 2017/2018/2019


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Fig.1: Water Treatment Plant (WTP)


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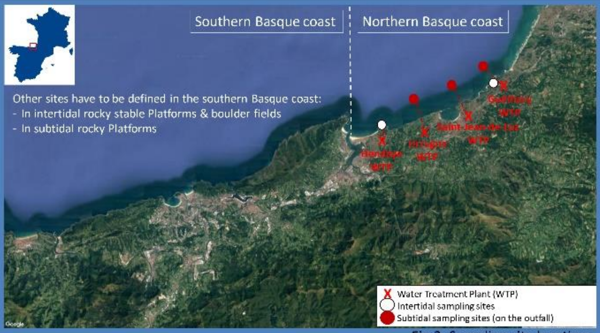
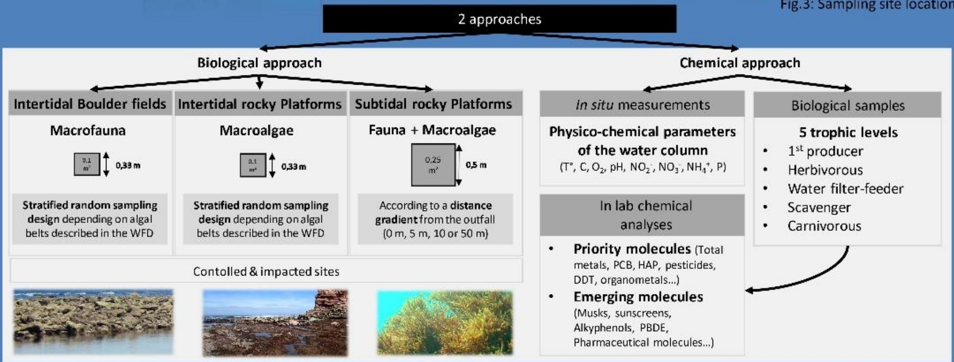
Fig.2: Subtidal outfall



Other sites have to be defined in the southern Basque coast:
- In intertidal rocky stable Platforms & boulder fields
- In subtidal rocky Platforms

X Water Treatment Plant (WTP)
○ Intertidal sampling sites
● Subtidal sampling sites (on the outfall)

Fig.3: Sampling site locations



2 approaches

Biological approach

Intertidal Boulder fields	Intertidal rocky Platforms	Subtidal rocky Platforms
Macrofauna	Macroalgae	Fauna + Macroalgae
Stratified random sampling design depending on algal belts described in the WFD	**Stratified random sampling design** depending on algal belts described in the WFD	According to a **distance gradient** from the outfall (0 m, 5 m, 10 or 50 m)

Contolled & impacted sites

Chemical approach

In situ measurements

Physico-chemical parameters of the water column
($T°$, C, O_2, pH, NO_2^-, NO_3^-, NH_4^+, P)

In lab chemical analyses

- **Priority molecules** (Total metals, PCB, HAP, pesticides, DDT, organometals…)
- **Emerging molecules** (Musks, sunscreens, Alkyphenols, PBDE, Pharmaceutical molecules…)

Biological samples

5 trophic levels

- 1st producer
- Herbivorous
- Water filter-feeder
- Scavenger
- Carnivorous

Perspectives:

- Improve knowledge about the impact of urban effluents on benthic communities living along the rocky Basque coast,
- Evaluate the anthropogenic pressure versus global change,
- Collect information and support the several Marine Strategy Framework Directive (MSFD) descriptors as the D1 “Biodiversity”, D2 “Non-indigenous species”, D5 “Eutrophication”, D6 “Sea-floor integrity” and D8 “Contaminants”.

Abstract:

The occurrence of micropollutants in the aquatic environment has become an environmental issue of major concern throughout the world because they may be potentially toxic, persistent and bioaccumulative in the environment even at low concentration levels. As wastewater treatment plants (WWTPs) are not specifically designed to eliminate this type of pollutants, a large range of micropollutants are found in wastewater effluents and then in the environment. This study, a dual-track approach between chemistry and biology, aims to provide a first insight of the occurrences and concentrations of priority and emerging substances in WWTP discharges and their potential impact on rocky benthic communities in the southeastern Bay of Biscay. These complementary approaches allow to study the benthic communities' response in both intertidal and subtidal zones and to quantify micropollutants in benthic organisms as well as in WWTP effluents. The results highlight that WWTP discharges constitute a source of micropollutants in coastal areas (especially metal, pharmaceutical and musk compounds) and that a number of substances are also found in benthic organisms but differences are identified between them. In general, *Ulva* spp. and *Gelidium* spp. are highlighted as the better bio-accumulator for this area. Results of the study of benthic communities' response suggest that benthic macroalgae constitute the best relevant biotic component to assess the effect of such a pressure in this area. Changes in the relative abundance of *Ceramium* spp., *Corallina* spp. and *Halopteris scoparia* (in the intertidal zone) and of *Gelidium corneum* and *Metacallophyllis laciniata* (in the subtidal zone) appear mainly responsible of the dissimilarities found between impacted and control locations. By contrast, no significant effect is detected using macrofauna assemblages and quality indices based on this biological element. The current 'macroalgae' WDF index, contributing to assess the ecological quality status of the water body, appears to be sensitive to such a pressure because it highlights a clear effect of discharges in the intertidal and subtidal zones. Finally, those results provide a framework for future monitoring allowing an assessment of benthic communities' changes related to WWTP discharges and highlight the importance to reflect upon another method to integrate macrofauna in future monitoring for an efficient impact evaluation.

Keywords: Wastewater treatment plants; Impacts; Macrofauna; Macroalgae; Bioindicators; Ecological Quality Status; WFD; MSFD.

Résumé :

La présence des micropolluants dans les milieux aquatiques est devenue un problème environnemental majeur dans le monde entier car ils peuvent être potentiellement toxiques, persistants et bioaccumulables même à de très faibles concentrations. Comme les stations d'épuration (STEUs) ne sont pas spécifiquement conçues pour éliminer ce type de polluants, un grand nombre d'entre eux se retrouvent dans les effluents d'eau usée puis dans l'environnement. Cette étude, qui repose sur une double approche chimique et biologique, vise à donner un premier aperçu de l'occurrence et des concentrations des substances prioritaires et émergentes dans les rejets de STEU et de leur potentiel impact sur les communautés benthiques des substrats rocheux du sud-est du Golfe de Gascogne. Ces approches complémentaires permettent d'étudier la réponse des communautés benthiques dans les zones intertidales et subtidales et de quantifier les micropolluants dans différents organismes benthiques et effluents de STEU. Les résultats montrent que les rejets de STEU constituent une source de micropolluants dans les zones côtières (en particulier les métaux, les pharmaceutiques et les muscs) et qu'un certain nombre d'entre eux sont également retrouvés dans les organismes benthiques bien que des différences soient détectées entre les différents taxa. De manière générale, les *Ulva* spp. et le *Gelidium* spp. sont identifiés comme étant les meilleurs bio-accumulateurs pour cette zone biogéographique. Les résultats concernant l'étude de la réponse des communautés benthiques mettent en évidence que les macroalgues constituent l'élément biologique le plus sensible et pertinent pour évaluer l'effet d'une telle pression dans cette région. Les variations d'abondance de *Ceramium* spp., *Corallina* spp. et d'*Halopteris scoparia* (dans la zone intertidale) et de *Gelidium corneum* et *Metacallophyllis laciniata* (dans la zone subtidale) contribuent significativement aux dissimilarités entre zones impactées et contrôles. En revanche, aucun effet significatif n'a été identifié en utilisant les assemblages de macrofaune et les indices de qualité écologique se basant sur cet élément biologique. L'indice DCE 'macroalgues' actuel, qui contribue à évaluer l'état écologique de la masse d'eau, semble être sensible à cette pression car il met en évidence un impact clair des rejets de STEU autant en zone intertidale que subtidale. Enfin, ces résultats fournissent des perspectives pour les suivis futurs pour évaluer les changements des communautés benthiques liés aux rejets de STEU et soulignent l'importance de réfléchir à la manière dont intégrer la macrofauna dans les suivis futurs afin de réaliser une évaluation la plus efficiente possible.

Mots-clefs: Stations d'épuration; Impacts, Macrofaune; Macroalgues; Bioindicateurs; Statut de qualité écologique ; DCE ; DCSMM.