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Occurrence and effects of antibiotics on the environment.
Presència i efectes dels antibiòtics en el medi ambient.

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June 2025

Universitat de Barcelona

B:KC Barcelona Knowledge Campus
Campus d'Excel·lència Internacional

Gràcies a la Maria pel suport constant durant tot el procés. També a les meves tutores, Marta i Marinella, per la seva guia i ajuda valuosa i a tot el grup *On Health* per l'oportunitat, per com m'han acollit i acompanyat durant aquests mesos. A la meva família i amigues, per ser sempre al meu costat i donar-me força quan més ho he necessitat. Finalment, a mi mateixa, per la constància i esforç al llarg de la carrera.

REPORT

IDENTIFICATION AND REFLECTION ON THE SUSTAINABLE DEVELOPMENT GOALS (SDG)

In the current study, water samples from various locations were analyzed to detect and quantify antibiotics, assess their ecotoxicological risk, and examine the influence of environmental factors, using both *target analysis* and *suspect screening* analysis to identify other compounds outside the target study. From this, a significant contribution is made in the Sustainable Development Goals (SDG), a set of global challenges, such as poverty, inequality or climate action, to achieve a sustainable future for all [1].

SDGs can be grouped into 5 major areas (the 5Ps): people, prosperity, planet, peace and partnership. In the first of these, the current study contributes to protecting public health by identifying the presence of emerging contaminants that favor antimicrobial resistance. Regarding prosperity, to address the current issue, the use of more efficient and sustainable technologies for water treatment is encouraged. On the other hand, impacts on aquatic biota and the persistence of antibiotics in the environment affect the third P: the planet. In addition, rigorous environmental monitoring is promoted to prevent future global health crises, thus contributing to a more just and peaceful society. Finally, for the last of these, the continuous study of this problem around the world enhances comparability and international scientific collaboration.

First, the results show that different antibiotics are detected in various types of water, with hazard quotients exceeding 10, indicating a significant ecotoxicological risk. These drugs contribute to the spread of antibiotic resistance genes (ARGs), a significant risk to both human and animal health. For this reason, this work emphasizes the need to reinforce environmental measures to promote health and welfare (SDG 3).

The sixth objective to improve water quality by reducing water pollution and minimizing its presence in the aquatic ecosystem is also considered in this research. Through the detection of antibiotics in different types of water, it has been observed that wastewater treatment plants (WWTPs) are important sources of contamination, pointing out the importance of improving removal technologies.

In addition, the antibiotics detected can persist in the environment, which underlines the need for a more responsible consumption of these drugs, thus emphasizing the environmental consequences of their excessive use (SDG 12). As for this objective, the presence of certain antibiotics in sea samples could have negative effects on the aquatic ecosystem, which demonstrates the need to apply measures to prevent and significantly reduce this contamination.

This study also indirectly influences other SDGs, such as 13 on climate action and 15 on earth life. Antibiotics not only reach the aquatic environment but can also cause global changes in terrestrial ecosystems. In the first of these, this research focuses on environmental persistence and the impact of these drugs. However, in the second SDGs, it may be related to infiltration into the soil through reused water, which can alter microbiota and ecosystems.

Finally, this project has been done externally at the *Institute of Environmental Assessment and Water Research (IDAEA*), a research center that forms part of the *Consell Superior d'Investigacions Cientifiques* (CSIC). This institute is actively working to strengthen its commitment to sustainability and environmental health, in line with the principles of the United Nations 2030 Agenda and its research linked to different SDGs.

CONTENTS

1. SUMMARY

Antibiotics are one of the most significant discoveries of the 20th century, but over the last few years, a new problem has emerged due to their excessive use and persistence in the environment. The presence of these drugs in ecosystems has led to the spread of antimicrobial resistance, prompting the World Health Organization to warn that by 2050, if nothing changes, 10 million deaths caused by bacterial infections could be reached. For this reason, this study analyzes the occurrence of antibiotics in aquatic environments to determine their risk in ecosystems and for antimicrobial resistance. Different samples from river, sea and around wastewater treatment plants (WWTPs) in Barcelona, Valencia (Spain), Paris (France) and Helsinki (Finland), areas of considerable interest due to their anthropogenic impact and different climate conditions, were examined to determine the occurrence of antibiotics by HPLC-HRMS, using either *target analysis* focused on 18 antibiotics and *suspect screening* for the identification of other drugs. The results obtained were used to perform a statistical analysis of environmental factors and to evaluate ecotoxicological risk using the Hazard Quotient. The most frequently detected antibiotics with the highest risk were sulfamethoxazole, metronidazole, azithromycin and tetracycline. On the other hand, the highest concentrations were found in samples near WWTPs, while sea samples have the lowest concentrations due to the dilution effect. Finally, this study shows that these drugs could represent a significant risk to aquatic ecosystems, as in several cases they exceed environmental risk thresholds, demonstrating the need for improved removal treatments for these substances and stricter environmental regulation.

Keywords: Antibiotic, Antimicrobial resistance, Water analysis, HPLC-HRMS, Ecotoxicological risk, Emerging contaminants, *Suspect screening*, Hazard quotient risk.

2. RESUM

Els antibiòtics són un dels descobriments més importants del segle XX, però en els darrers anys, una nova problemàtica ha sorgit a causa del seu ús excessiu i la seva persistència en el medi. L'arribada d'aquests fàrmacs als ecosistemes ha provocat la disseminació de la resistència als antimicrobians, fent que l'Organització Mundial de la Salut adverteixi que l'any 2050, si no es canvia res, es podrien arribar als 10 milions de morts provocades per infeccions bacterianes. Per aquest motiu, l'objecte d'estudi d'aquest treball ha estat analitzar la presència d'antibiòtics en el medi aquàtic, per tal de preveure el risc que tenen per l'ecosistema i per la resistència als antimicrobians. Diferents mostres de riu, de mar i al voltant de les plantes depuradores d'aigües residuals (EDARs) de Barcelona, València (Espanya), París (França) i Hèlsinki (Finlàndia), zones de gran interès pel seu impacte antropogènic i diferents pressions climatològiques, han estat examinades amb l'objectiu de detectar antibiòtics mitjançant HPLC-HRMS, mitjançant tant l'anàlisi diana per a 18 antibiòtics com la metodologia d'anàlisi de sospitosos per identificar altres substàncies. Els resultats obtinguts s'han utilitzat per fer una anàlisi estadística dels factors ambientals i per avaluar el risc ecotoxicològic mitjançant el quocient de risc. Els antibiòtics més detectats i amb major risc han estat el sulfametoxazol, el metronidazol, l'azitromicina i la tetraciclina. Per altra banda, les concentracions més elevades s'han trobat en mostres properes a EDARs, mentre que les mostres marines són les que s'han detectat amb concentracions inferiors a causa de l'efecte de dilució. Finalment, aquest estudi mostra que aquests fàrmacs podrien representar un risc significatiu per als ecosistemes aquàtics, ja que en diversos casos superen els llindars de risc ambientals, demostrant la necessitat de millorar els tractaments d'eliminació d'aquestes substàncies i una regulació ambiental més estricta.

Paraules clau: Antibiòtic, Resistència als antimicrobians, Anàlisi d'aigua, HPLC-HRMS, Risc ecotoxicològic, Contaminants emergents, Anàlisi de sospitosos, Quocient de risc.

3. INTRODUCTION

3.1. ANTIBIOTICS: ORIGIN, TYPES AND APPLICATIONS

Antibiotics are chemical compounds that help slow down the growth of microorganisms [2]. Although some advances in the treatment of infectious diseases had already been made before the discovery of penicillin, such as salvarsan for syphilis, these medicines were one of the great discoveries of the 20th century because they reduced deaths from infectious diseases. Since then, their use has increased worldwide in human and veterinary medicine [3]. They also play an important role in the livestock industry and agriculture by helping to control the spread of harmful microorganisms [4]. However, their use has developed other problems, such as resistance to them or their impact on the environment [3].

These substances can be classified according to different concepts such as antibacterial effect, mechanism of action, chemical structure, or spectrum of action [5]. In the first classification, substances are distinguished as bactericidal, which destroy bacteria (such as penicillin), or bacteriostatic, which inhibit reproduction (for example, tetracyclines) [6].

The second classification criterion is based on the mechanism of action, or how antibiotics interfere with bacteria's vital functions. This classification includes several main groups. First, some antibiotics inhibit cell wall synthesis, such as β-lactam antibiotics. On the other hand, some antibiotics prevent protein synthesis, with tetracyclines and macrolides as examples. Furthermore, some substances prevent nucleic acid synthesis, notably in quinolones. Additionally, some induce alterations in the cell membrane, among which are polymyxins. Finally, some inhibit the synthesis of essential metabolites, such as sulfonamides [7].

Thirdly, the term spectrum of action refers to the range of substances against which antibiotics are effective. Some are considered narrow-spectrum, which act against a limited number of species, and broad-spectrum, which are effective against various microorganisms. Typically, most narrow-spectrum agents are bactericidal, while broad-spectrum agents are bacteriostatic [8].

The last type is based on chemical structure, which allows antibiotics to be grouped into families with similar characteristics. In this, there are β-lactam antibiotics, aminoglycosides, macrolides, tetracyclines, and quinolones, among others. It is important to note that excessive or inadequate use of antibiotics can lead to bacterial resistance, which is a growing public health problem [9].

3.2. ANTIBIOTIC RESISTANCE

Antibiotic resistance (AR) is a genetic trait that different bacterial populations can acquire, allowing them to survive and grow in the presence of the drug. Moreover, this phenomenon worsens due to the excessive or improper use of antibiotics in humans and animals, as well as their accumulation in the environment [3]. In this way, antibiotic residues accumulated in the environment allow the spread of ARGs, which can alter microbial communities and be transferred through trophic chains [10].

During the last years, the scientific community has studied how bacterial communities acquire resistance in relation to the frequency of human activities. This means that a higher degree of resistance to antibiotics can be observed in areas with more anthropogenic impact [9]. Moreover, another way of promoting it is a lack of specific wastewater treatment for its elimination. This allows these drugs to encounter microorganisms in the environment, promoting horizontal gene transfer (HGT) of resistance [11].

Additionally, the presence of these substances in the environment can lead to other issues, such as delayed nitrite oxidation, metagenesis, and increased toxicity of various chemical combinations and their respective metabolites [12]. These adverse effects can pose a significant health risk to humans and other species [13]. In this way, the accumulation of antibiotics in the environment may alter the environmental microbiota [14].

This phenomenon has grown in importance to become one of the main threats to global public health [15], as can be seen in Figure 1 with the estimated number of bacterial infections due to antimicrobial resistance. The WHO, World Health Organization, and the EMA, European Medicines Agency, have developed surveillance methods to assess risk and prevent future health problems [16]. They

have established classifications such as AWaRe and Watch List that attempt to identify and categorize antibiotics with a high environmental risk and potential to develop antimicrobial resistance, as will be discussed below about the regulatory framework [17].

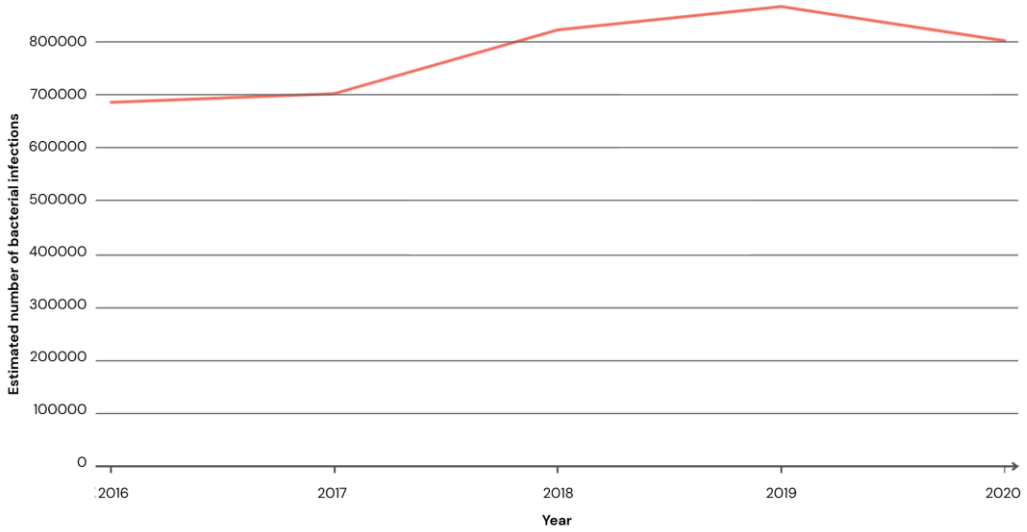

Figure 1. Representation of the estimated number of infections due to antibiotic-resistant bacteria in the European Union and the European Economic Area (EEA) [17].

3.3. Impact on the Environment

Antibiotics have revolutionized medicine by drastically reducing deaths from infectious diseases and improving overall public health. However, another current concern of antibiotic release is its environmental impact and the biota [3]. The released drugs can interfere with the ecological balance of microorganisms, as mentioned previously, causing various species to develop resistance [10]. These bacteria can promote resistance genes (ARGs) through two mechanisms: vertical and horizontal transfer. The first one involves the transmission of these genes in an inherited form. In contrast, horizontal transfer can occur between phylogenetically distant bacteria through the transformation (uptake of free DNA), transduction (via bacteriophages), or conjugation process (direct transfer via pilus) [18] (Figure 2).

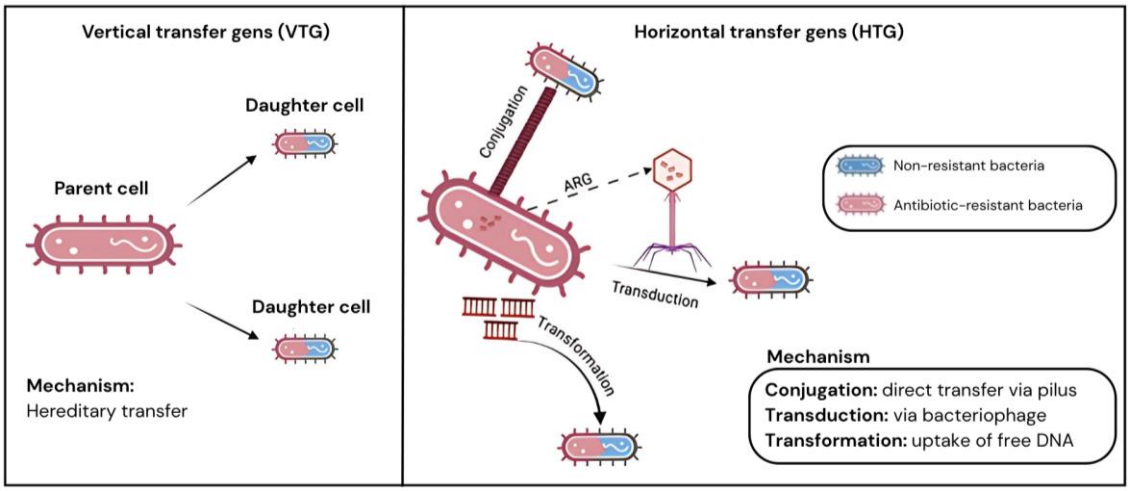

Figure 2. Mechanisms of ARGs transfer in bacteria.

On the other hand, there is also an impact on food chains where they can accumulate in species such as algae or fish. This occurrence can cause negative effects on their behavior and metabolism [19]. For example, in some algae, there may be an alteration in their photosynthesis due to tetracyclines and sulfonamides, or, in quinolones, DNA replication or protein synthesis is interrupted in cyanobacteria [20]. Moreover, this affects not only aquatic organisms, but also their food chain [21].

Human health is also threatened by this phenomenon because it impedes the successful treatment of infections caused by bacterial resistance. In the last years, mortality and healthcare costs have increased due to continuous research into effective antibiotics [22]. In other terms, it influences the treatment of infections that were previously easily treatable to become increasingly difficult to cure, extending the risk of serious complications and rising the use of hospital resources. According to the EMA, these costs can reach billions of euros annually in Europe, in addition to overloading hospitals [17]. This problem may lead, as warned by the WHO, to 10 million deaths by 2050 if preventive and effective measures are not taken urgently [23].

3.3.1. Sources of Pollution

To reduce the environmental impact of antibiotics, it is important to analyze where the routes of entry of these compounds into the environment are located (Figure 3). One of the main sources is found in the excretion of patients who have received pharmacologic treatment [24]: these drugs, when administered to humans and animals, as they are not fully metabolized, spread to WWTPs as partially active metabolites or in unmetabolized form [12] [3]. Some studies indicate that approximately 90% of the antibiotics consumed are excreted in their original form or as active metabolites, which increases their presence and persistence in the environment [25].

Also, the use of antibiotics in agriculture is one of the ways by which they reach the environment, because they are used in livestock farming not only to prevent and treat animal diseases but also as feed additives to promote the growth of animals. These compounds, once excreted, can contaminate the soil and surface water by using manure as fertilizer [13]. Beyond agriculture, sectors such as aquaculture and mariculture also habitually use antibiotics to prevent the spread of bacteria and treat infections in fish and other aquatic organisms [10]. Uncontrolled use of these drugs can lead to an increase in selective pressure, favoring the survival and proliferation of resistant bacteria and eliminating sensitive ones. This phenomenon contributes to the emergence and dissemination of antimicrobial resistance [26]. To prevent this, several specific laws and regulations have been established, such as Royal Decree 666/2023 on the responsible use of antibiotics in veterinary medicine in Spain [27].

In addition, another important source of contamination can be the pharmaceutical industries, which can release antibiotic residues during the manufacturing process or due to accidental leaks. Hospitals and health care centers also contribute to this because they use large quantities of these drugs that can end up in wastewater. Despite the application of various purification processes, some of these contaminants persist and end up reaching aquatic ecosystems [28].

Finally, other sources that should also be considered are the inadequate disposal of drugs at the household level, as well as the transport of pharmaceutical waste into groundwater or surface water through leachates generated in landfills [29].

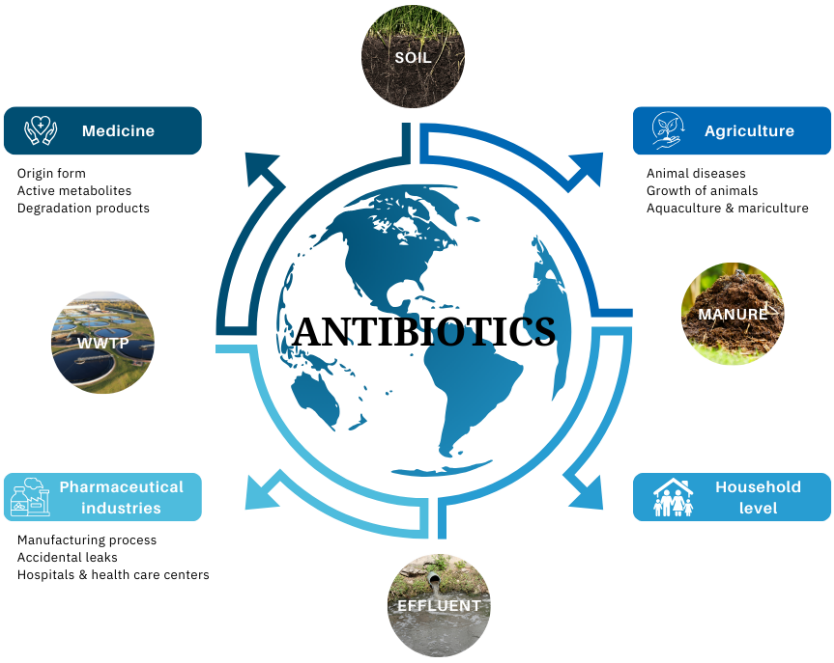

Figure 3. Sources of pollution of antibiotics.

3.3.2. Degradation and Persistence in the Environment

When antibiotics are already in the environment, it is necessary to evaluate how they persist and their degradation rate. For example, penicillins are one of the cases that degrade easily, while macrolides and tetracyclines are more persistent. This fact causes them to accumulate in higher concentrations. Therefore, this may also influence their study, because compounds that persist longer in the ecosystem will be more detectable, such as quinolones, sulfonamides, and diaminopyrimidines [30].

Table 1. Estimated environmental half-lives of antibiotic classes in soil, water, and sediments.

Antibiotic class	***Half-life*** [33]		
	Soil	**Water**	**Sediments or other**
Penicillins	<1 day to a few days	<1 day to a few days	Rapidly degraded
Macrolides	Days to weeks (>30 days)	3 to 27 days	Weeks to months
Tetracyclines	30 to 180 days	Weeks to months	Up to 180 days (highly persistent)
Quinolones	21 to 100 days	Days to months	Up to 100 days
Sulfonamides	4 to 30 days	Days to weeks	Days to weeks
Diaminopyrimidines	Weeks	Weeks	Weeks
Lincosamides	Days to weeks	Days to weeks	Days to weeks
Nitroimidazoles	Days to weeks	Days to weeks	Days to weeks
Glycopeptides	Days to weeks	Days to weeks	Days to weeks
Aminoglycosides	Days to weeks	Days to weeks	Days to weeks

Table 1 presents approximate values for the half-life of different classes of antibiotics, obtained from the cited literature. The real persistence of these compounds in the environment, introduced continuously into the environment, depends on several factors, which will be discussed in subsequent sections.

3.3.2.1. Mechanisms of antibiotic degradation

Two main mechanisms of antibiotic degradation can be distinguished: biotic and abiotic. In the first case, the transformation of drugs occurs by the action of microorganisms, such as bacteria or algae, according to their degradation capacity [31]. Depending on the sensibility of the species and the type of antibiotic involved, this mechanism may vary and involves the chemical alteration of substances through enzymatic action, converting them into less active compounds [32]. In contrast, abiotic degradation occurs through various physical and chemical processes, which include photodegradation, hydrolysis, redox reactions, sorption in soils, volatilization, and thermolysis [25]. These mechanisms, particularly photodegradation and hydrolysis, depend on the physicochemical properties of the substances and the environmental conditions, which makes their knowledge essential to address environmental impact [3]. In these mechanisms, antibiotics can be transformed into degradation products that are equally or more persistent and toxic than the original compounds. Therefore, these substances must also be considered when evaluating toxicity and persistence [33].

3.3.2.2. Factors impacting antibiotic persistence and degradation

In addition to this study, several factors influence the environmental distribution of contaminants. This distribution depends in part on the physicochemical properties of the pharmaceuticals under study, such as molecular structure, size, shape, solubility, or hydrophobicity. The properties of the compounds determine their tendency to accumulate in different environments, such as water, soil, or sediments, and this determines their behavior [2]. For example, several studies have shown that antibiotics with high lipophilicity and bioavailability can pass through cells by free diffusion or active transport and induce adverse effects [34].

Sorption, one of the physicochemical properties, can be estimated using hydrophobicity and the sorption coefficient. Depending on these values, the reversible adsorption exchange between water and sediment can be studied. Specifically, hydrophobicity is expressed by the Octanol-Water Partition Coefficient (K_{ow}) and the Octanol-Water Distribution Coefficient (D_{ow}). Compounds with a

logarithm Octanol-Water Partition Coefficient ($\log(K_{ow})$) lower than 2.5 have low sorption potential, while those between 2.5 and 4 have moderate potential. Only compounds with a $\log(K_{ow})$ greater than 4 have high sorption potential and tend to associate with the organic phase, such as sediments, rather than the aqueous phase. [10].

The polarity of antibiotics is another property that influences their distribution in the environment, especially in processes such as sorption. Highly polar compounds have a greater affinity for aqueous media, meaning they are hydrophilic. In contrast, low-polar or hydrophobic compounds tend to be more soluble in organic phases. Polar compounds are highly soluble in water because they can interact with water molecules, favoring their presence in this phase. The opposite occurs with non-polar compounds, which are poorly soluble in water and tend to move to the organic phase (e.g., solids and sediments) [35].

Considering these aspects, the water-solids Distribution Coefficient (K_d) is relevant because it is an indicator of the ability of the composts to be absorbed in solids or sediments and captures all the above factors. When a compound is polar and soluble, they have a small K_d and a greater tendency to be in the aqueous phase. In the opposite case, hydrophobic and poorly soluble substances have high K_d and more affinity for the organic phase [36].

In addition to physicochemical properties, environmental conditions are a key factor in the degradation and persistence of antibiotics. Parameters such as temperature, pH, presence of organic matter, amount of dissolved oxygen, microbial activity, among others, determine the speed and efficiency with which these compounds degrade or persist in the environment [37]. In the case of temperatures, high values accelerate both chemical reactions and microbial activity, a fact that enhances the degradation of antibiotics. The stability of these compounds can also vary depending on the environmental pH, causing their elimination to be faster in acidic or basic media [38]. For example, sulfonamides are substances with ionizable groups, and these are strongly affected by environmental pH [10]. The presence of organic matter can contribute to the absorption of antibiotics, facilitating their persistence and, sometimes, their transformation[39].

As for dissolved oxygen, high concentrations may favor the aerobic degradation of antibiotics, an oxidation that occurs in the presence of oxygen due to the activity of aerobic microorganisms [40]. Conductivity should also be considered, because high values indicate an increased presence of salts and ions, which can influence solubility, microbial activity, and reactivity [41]. Another factor to be considered is the redox potential, which, if high, can facilitate the chemical degradation of antibiotics by creating oxidizing conditions [40]. Precipitation, on the other hand, can dilute the concentrations of these substances in surface waters and change the environmental conditions, modifying degradation processes [41]. Finally, the class of antibiotic involved also contributes to understanding its behavior in the environment because its intrinsic properties determine its stability, mobility, and persistence [42].

3.3.3. Ecotoxicological Risk Assessment

To estimate the risk that a substance presents to organisms and ecosystems, an ecotoxicological assessment is performed. In this, considering all the toxicity and environmental exposure data, possible adverse effects can be prevented, and future environmental management can be improved [31]. The ecotoxicological risk of antibiotics is evaluated by several indicators that allow estimating the potential for toxicity in the environment, such as HQ, PNEC, CE_{50}, bioaccumulation factors, among others. In the case of the Hazard Quotient (HQ), it relates the measured environmental concentration to the Predicted No Effect Concentration (PNEC) to estimate the ecological risk of antibiotics. PNEC is the maximum concentration below which no adverse effects on organisms are expected [34]. On the other hand, another indicator is the CE_{50}, the effective concentration of an antibiotic that in 50% of the population tested produces an effect during a given time. In contrast, the bioaccumulation factor measures the capacity of organisms to accumulate certain substances [10]. Other important indicators are the NOEC (No Observed Effect Concentration), which is the maximum concentration at which no adverse effect is observed [43]; the LOEC (Lowest Observed Effect Concentration), which corresponds to the minimum concentration at which adverse effects are observed in exposed organisms; and the LC_{50}, the Lethal Concentration that causes the death of 50% of the tested organisms [20]. The latter are common in ecotoxicological studies and provide the basis for calculating the PNEC [44]. In summary, the use of all these indicators provides important information on the potential risk associated with the presence of antibiotics in aquatic ecosystems and is a fundamental tool for future environmental management [45].

3.4. Regulatory Framework and Knowledge Limitations

3.4.1. Currently Environmental Legislation

Due to the increasing potential risk of antibiotics to the environment, regulation of their presence has also been involved, especially in the European Union [46]. First, the Water Framework Directive (WFD) was introduced to establish the basis for guaranteeing the quality and protection of surface and groundwater. This directive highlights the need for water treatment to monitor emerging pollutants [47]. In addition, the European Commission periodically updates the watch lists containing priority or potentially harmful substances to assess their impact and establish possible restrictions [48]. On the other hand, in veterinary medicines, a new regulation has been approved by the European Union that reinforces the control to prevent antimicrobial resistance and ensure a more responsible use [49].

At the state level, Spain has implemented a National Plan against Antimicrobial Resistance (PRAN) with specific regulations governing the sustainable use of these compounds in livestock farming. Specifically, this decree mandates the reporting of pre-registration and treatment of antibiotics in animals from January 2025 [50]. In Catalonia, several initiatives have emerged to strengthen surveillance and control, especially in food safety and livestock waste management [51].

Since June 2023, certain antibiotics, for tonsillitis or cystitis, have been available in France without prescription [52]. However, it has implemented national plans against antimicrobial resistance, such as Ecoantibo Plan, which includes restrictions for veterinary use [53]. In contrast, Finland had a national strategy in which it reduced the consumption of these in human medicine through strict monitoring programs [54]. In the environmental field, it is one of the countries that stands out in applying the principle of active monitoring in surface waters [55]. All the countries studied are part of the Joint European Action against Antimicrobial Resistance (JAMRAI), an environmental monitoring plan [56].

3.4.2. Key Research Priorities

Although all the current regulations and legislation are in force, there are certain limitations to be considered to correctly assess the risk and suggest improvements for the future [57]. Currently, WWTPs do not use specific processes to eliminate antibiotics and their metabolites [58]. Moreover, these compounds do not have stable limits of residues in the environment, and current regulations do not consider the possible interactions, generation of metabolites, or degradation products, which can also influence the environment. It has become evident that the threat comes not only from the persistence of antibiotics, but also from their metabolites and degradation products, which can reach rivers, aquifers, and other ecosystems [59]. In this way, their importance as contaminants is remarkable and, therefore, their toxicity must be evaluated, because at present, it is little known [58].

In addition, other aspects need to be addressed, such as the long-term effects and the transfer of resistance from the environment, assessing the dynamics and the real impact on human and animal health [60]. Furthermore, to do so, there is a need for more ecotoxicological and environmental monitoring data [58].

Consequently, in this emerging risk, it is necessary to carry out studies on the combined effects to improve the monitoring systems [61], establish specific regulatory limits [45] and develop more efficient treatment technologies to reduce the presence of antibiotics and their derivatives in the environment, minimizing their impact and the risk of resistance generation [62].

4. OBJECTIVES

The main objective of this study was to determine the occurrence of antibiotics in surface water samples. For this reason, seawater and riverine samples from Barcelona, Valencia (Spain), Paris (France) and Helsinki (Finland) were analyzed to quantify the concentration of 18 antibiotics. Therefore, the objectives were (1) to determine the presence of antibiotics in different water samples; (2) to quantify the concentration through *target analysis* and to identify tentative antibiotics using *suspect screening*; and (3) to evaluate whether these levels may pose a potential risk to the environment.

5. MATERIAL AND METHODS

5.1. CHEMICALS AND REAGENTS

The standard antibiotics used were of high purity and were obtained from Sigma-Aldrich (St. Louis, MO, EE. UU.). Further information about the compound can be found in Appendix 1, Tables 1-1 and 1-2. The antibiotic mix was prepared by taking 50 µL of each 1000 ppm pure standard, introducing them into a 10 mL volumetric flask and adding 9.1 mL of methanol to achieve a final concentration of 5 ppm. In the same way, the mix of isotopically labeled antibiotics was prepared to perform the internal standard monitoring and normalization. On the other hand, the standards of the calibration were prepared by adding the volume determined in each one and were made up to volume with methanol and HPLC water (1:1). All the solutions were kept at -20ºC to guarantee their stability and avoid degradation. The solvents used, methanol, water, and acetonitrile, were obtained from LiChrosolv (Darmstadt, Germany), and their purity was HPLC grade. In contrast, the hydrochloric acid used in sample pretreatment was purchased from PanReac AppliChem (Murcia, Spain) and EDTA from Sigma-Aldrich (St. Louis, MO, EE. UU.), both with a purity above 95%.

5.2. SAMPLING AND STUDY AREAS

The water samples analyzed came from different points in Barcelona, Valencia (Spain), Paris (France) and Helsinki (Finland), including both river and seawater in the case of the Catalan city. Among the 100 samples, 66 belong to river samples, 17 to effluent WWTPs, 9 to influent WWTPs and 8 to sea samples (Figure 4). For each sampling point, two-liter bottles were used, from which 200 mL were transferred for sea samples and, for the river samples, volumes varying between 25 and 100 mL, according to the origin of the sample (WWTPs effluent, influent or river surface water). These were kept in the freezer until preparation for analysis to avoid degradation of the antibiotics present.

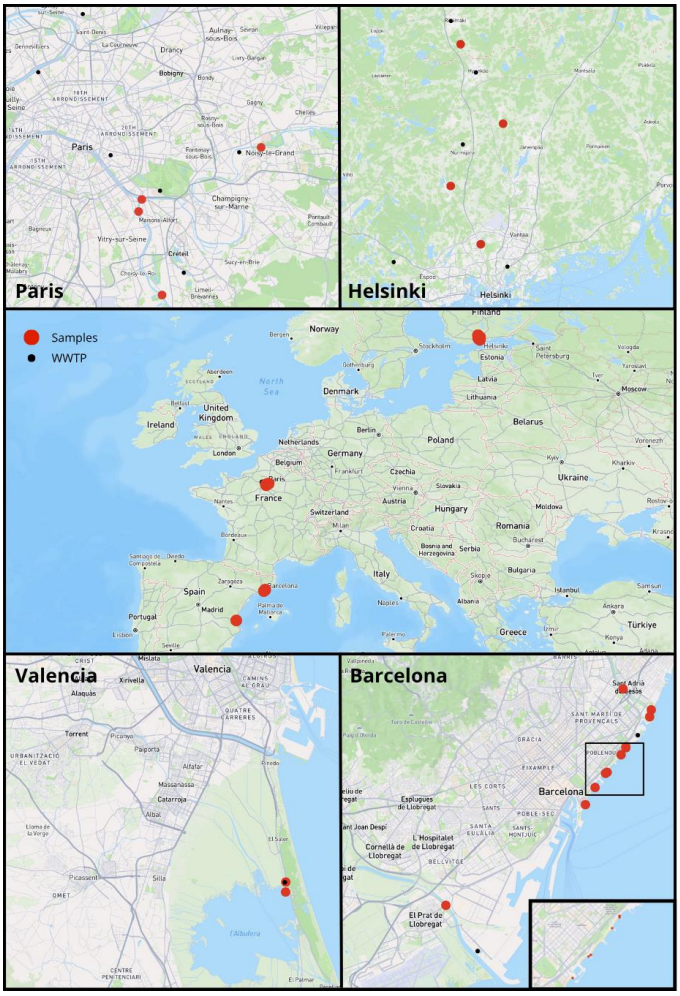

Figure 4. Study area of the samples collected in Barcelona, Valencia (Spain), Paris (France) and Helsinki (Finland), and the corresponding WWTPs in the study zone.

In Barcelona, samples were collected at the following points: Besòs River, Forum Beach, Llevant Beach, Nova Mar Bella Beach, Mar Bella Beach, Bogatell Beach, Nova Icària Beach, Somorrostro Beach, Sant Miquel Beach, Llobregat River (Figure 4). At these points, water was collected, and various parameters were also measured, such as temperature, pH, conductivity, and pressure, among others. These environmental variables were collected in situ using a Pro Plus multiparameter sonde (YSI, Yellow Springs, OH, USA) (Appendix 2, Table 2-1). In the other sampling points, water samples provided by various collaborators of the project were only available, and no direct collections were carried out there, except for the parameters of the samples collected in France, which are provided in Appendix 2, Table 2-2.

The Catalan city is characterized by a high population density, which may increase the anthropogenic impact on the environment. In 2023, Barcelona was estimated to have approximately 1.7 million inhabitants [63]. In addition, the constant influx of tourism may further increase the human influence on the environment. These characteristics make this city an interesting site to analyze the potential of high population density to influence the presence of antibiotics. On the other hand, it should be noted that, near the sampling zone, two WWTPs are located, in the Besòs and Llobregat rivers. The first uses several processes such as the activated sludge system, nitrification-denitrification processes, advanced biofiltration and deodorization systems. This plant is highly important because it covers more than half of the metropolitan area of Barcelona [64]. In the second case, this plant integrates some of the processes used in the Besòs treatment plant, combining them with ultrafiltration and reverse osmosis, which allows the production of high-quality water. Their importance lies in their high reuse in different fields such as agriculture, industry, and environment [65].

In the city of Valencia, influent and effluent areas of WWTPs were sampled. This one has a population of approximately 850.000 inhabitants (2025) [66] and the objective is to evaluate the anthropogenic impact. In addition, its importance lies in the possibility of analyzing the water quality around the treatment plants. Specifically, the sampling area includes WWTPs where processes such as nitrification-denitrification [67], phosphorus removal, advanced biofiltration and other treatments of interest [68].

Sampling in Paris was carried out at different points along the Seine River and the Marne River, as well as at various locations at the inlet and outlet of WWTPs (Figure 4) by ANSES. Paris, which has a population of about 2.2 million inhabitants (2024) [69], is an interesting site not only to assess the relationship with anthropogenic impact, but also to evaluate whether wastewater treatment is effective for the different antibiotics studied. In this sampling area, a wide variety of WWTPs processes are used to remove phosphorus, among other advanced treatment processes [70].

Finally, in Helsinki (Finland), the samples collected from different points are located within the catchment area of the Vantaanjoki, a river course that flows through the most densely populated region of the metropolitan district of southern Finland (about 252 thousand inhabitants (2025) [71]). In addition, in this area, several WWTPs are concentrated (Figure 4), which makes it possible to analyze the influence of these on the water quality of the river. It should be noted that Finnish WWTPs commonly use chemical phosphorus removal processes, nitrification-denitrification, and activated sludge, which can have a significant impact on both water quality and biota [72].

In summary, 100 water samples were analyzed from all sampling points, including river and sea areas, in the four locations mentioned: Barcelona, Valencia, Paris and Helsinki. This set is of great interest because it allows us to compare the presence of antibiotics in regions with different hydrological characteristics, weathering effects and anthropogenic pressure.

5.3. Sample Preparation and Pre-treatment

Sample preparation was performed to ensure proper analysis based on the methodology described in the literature [73], with minor modifications. First, 10 µL of an internal standard solution was added to correct possible matrix effects and instrumental errors. Next, samples were filtered through 0.7 µm filters, and chlorohydric acid (45%) was added drop by drop to achieve an acidic solution (pH 2.5). Afterwards, a 0.1 M EDTA (in water) solution was incorporated at a final concentration of 0.1% to minimize macrolide's chelation. Then, the samples were agitated vigorously in a vortex and solid phase extraction (SPE) was carried out with Oasis HLB cartridges (200mg, 6 mL) at a flow rate of 1 mL/min in a manifold. The procedure for SPE includes the first conditioning with 2 x 2 mL of methanol, followed by 2 x 2 mL of HPLC water, both under gravity conditions. Subsequently, the samples were loaded using a vacuum system by pumping. Then, the cartridges were further dried under a vacuum system for 30 minutes to allow complete drying. Immediately thereafter, the cartridges were eluted by using 2 x 2 mL of methanol under gravity conditions. The extracts obtained were evaporated

under a nitrogen stream and then transferred to HPLC vials and further evaporated to dryness. Finally, the samples were reconstituted in 1 mL of a solution of methanol : water HPLC (1:1), vortexed, and kept at -20ºC until analysis.

5.4. HPLC-HRMS ANALYSIS

The analysis of the samples was carried out by high-performance liquid chromatography coupled to high-resolution mass spectrometry (HPLC-HRMS). The equipment used consists of a liquid chromatograph Aquity LC system (Waters®, Milford, MA, USA) equipped with a C_{18} analytical column (Hibar® HR 50-21 Purospher® STAR RP-18 end-capped column, Merck KGaA, Darmstadt, Germany) (3 µm, 2 × 125 mm). Mobile phases (A) Acetonitrile and (B) HPLC water with 0.1% formic acid were used at a flow rate of 0.2 mL/min. The study was performed using an initial elution gradient of 10% A, increasing to 99% in 8 min, maintained at 10 min, and returning to initial conditions after 12 minutes. Finally, the conditions were maintained for one more minute. The chromatographic system was coupled to a Q-Exactive mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA), which was equipped with an electrospray ionization source (ESI) operating in positive mode. The data was acquired in full scan mode at a resolution of 70,000 full width at half maximum (FWHM) from 100 to 1000 m/z and, in parallel, data-dependent scanning of the most intense ions at a resolution of 30,000 FWHM. The entire system was controlled by Xcalibur 4.0 software. Table 2 summarizes all the experimental conditions.

Table 2. Operational conditions for HPLC-HRMS analysis.

	Condition	Value
1	Chromatographic column	C18 analytical column (Hibar® HR 50-21 Purospher® STAR RP-18 end-capped column) (3 µm, 2 × 125 mm)
2	Mobile phase	A: Acetonitrile; B: H_2O + Formic acid 0.1%
3	Flow rate	0.2 mL/min
4	Samples temperature	15 ºC
5	Detection mode	Full Scan
6	Injection volume	10 µL
7	Ionization source	ESI Positive
8	Gradient	10%-99%
9	Mass range	100.0-1000.0 m/z

5.5. DATA PROCESSING

The data obtained from HPLC-HRMS analyses were processed with Xcalibur Quan Browser software (Thermo Fisher Scientific, San Jose, CA, USA). Initially, the antibiotics and their respective isotopically labeled analytes were identified using their molecular masses, with an error in exact mass within ±2.5 ppm, and specific fragmentation patterns. These were determined by comparing the masses obtained with the known masses of the antibiotics. In this way, the spectra acquired were analyzed to detect the peaks of each antibiotic, allowing their identification. The data of interest were the areas of the corresponding peaks and the concentration of each sample. Then, to quantify the antibiotics in each sample, the calibration was elaborated. Specifically, the ratio between the concentration of the sample and that of the internal standard was calculated. In addition, the ratio between the area of the corresponding peak and the area of the isotopically labeled antibiotic peak was also calculated. This ratio among areas and concentrations was used to build the calibration curve and allowed the quantification of each compound in the analyzed samples.

This type of analysis is known as "*target screening*", where specific compounds, already known, are searched for and quantified in the analyzed samples. Using reference standards, fragmentation patterns and known retention times, an analysis with a very high identification reliability can be achieved. On the other hand, if one is looking for non-targeted compounds in the samples, but these have been pre-treated and the compounds separated in a chromatographic system is known as "*suspect screening*". Then, based on lists of suspicious substances in databases and predictions, a *suspect screening* could be performed [74]. There are different levels of confirmation within the *suspect screening*, based on Schymanski's levels, that are shown in Figure 5 [75].

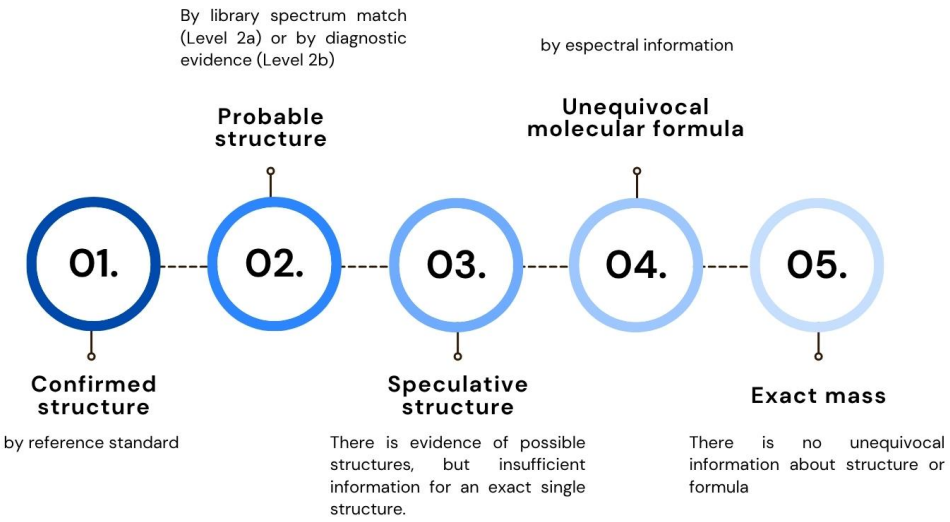

Figure 5. Confirmation levels for compound identification according to Schymanski et al. [75].

In this case, the samples were analyzed using Compound Discoverer software version 3.3 SP1 from Thermo Fisher Scientific, to detect antibiotics that were not part of the set initially studied. This software allows for performance analysis of the data obtained by HPLC-HRMS, identifying the compounds from two online databases, ChemSpider for structural information and MzCloud for the mass spectral data. In addition, the *List S6 ITNANTIBIOTIC on NORMAN Suspect List Exchange* was added to detect compounds identified as antibiotics and their main transformation products (TPs).

According to the analysis described, a first and second level of confirmation is obtained with the target and suspect, respectively.

5.6. Antibiotics Risk Assessment

The bioaccumulation of antibiotics and their potential effect on the environment can be estimated using the HQ, values which allow the evaluation of the potential ecological risk associated with their presence in the aquatic environment [31] [76]. The HQ is calculated as the ratio between the measured environmental concentration of the studied antibiotics and their respective PNEC, obtained from the NORMAN Ecotoxicology Database [77].

$$HQ = \frac{Ca}{PNEC} \quad (1)$$

where Ca is the environmental concentration of the antibiotics.

If the HQ values are below 0.1, no adverse effect is expected, in other words, the risk is insignificant. If the values are included between 0.1 and 1, the risk is low, but there is a possibility of negative impacts. There is a moderate risk if HQs values are between 1 and 10. And, if the HQs are greater than 10, high risk and a significant possibility of adverse effects are predicted [78]. In this way, the estimation of the HQ for each antibiotic and each sampling point was carried out to determine whether its presence may represent a potential ecological risk for aquatic organisms.

6. Detection and Quantification of Antibiotics

The detection and quantification of antibiotics is important for understanding water quality and the potential negative environmental effects. As previously mentioned, this study has evaluated 18 antibiotics by *target analysis* in various samples, classified as river water, seawater and samples collected around WWTPs.

Table 3 presents the percentage of detection determined for each antibiotic, which has been calculated considering the samples where compounds are detected among the total number of samples. The results obtained show that, in river samples, the antibiotic presence is moderated, although they have been detected in most of the samples analyzed. This phenomenon may be due to human activity and spillage from WWTPs. In contrast, in the sea samples, only two antibiotics have been detected, attributed to the higher degree of dilution of the environment (Table 3). This factor causes concentration to be reduced to values below the detection limits of the analytical method used, either due to their absence or because of their presence at really low concentrations [79].

In addition, Table 3 also includes the maximum, minimum and mean concentrations of the substances analyzed, with azithromycin, tilmicosin and sulfamethoxazole highlighting their maximum concentrations.

Certain antibiotics are present in samples collected near WWTPs. Although a reduction in detection frequency between influent and effluent is sometimes observed, it is unclear whether this is generalized in this study. For example, in azithromycin, the detection rate in the influent (11%) is much lower than in the effluent (53%) (Table 3). These could be explained due to the degradation of azithromycin-glucuronide that occurs during WWTPs treatment and then detected as azithromycin in the effluent but not in the influent [58].

Of all the samples analyzed, the most frequently detected antibiotics were sulfamethoxazole (32%), metronidazole (21%) and, to a minor effect, azithromycin and tetracycline (16%). These results can be justified by their clinical use, their persistence in the environment and the low elimination ratio in WWTPs [79]. Compared with previous studies carried out in Valencia and Barcelona, the authors detected different antibiotics than the ones observed in this work. For example, a study carried out in the Barcelona metropolitan area showed the presence of ciprofloxacin, ofloxacin, azithromycin, and others in urban wastewater [80]. Similarly, another study from Valencia and Ebro found azithromycin, clarithromycin, among other waste and surface waters [81].

Table 3. Detection frequency and concentration levels of antibiotics in rivers, sea and samples collected near WWTPs.

Antibiotic classification	Antibiotic	Detected frequency [%]					Concentration [µg/L]		
		River samples	Sea samples	WWTPs Samples		Total samples	Maximum	Minimum	Mean
				Influent	Effluent				
Macrolides	Azithromycin	9.09	-	11.11	52.94	16.00	119.10	1.01	14.17
	Clarithromycin	9.09	-	-	-	6.00	8.38	7.37	7.80
	Erythromycin	3.03	-	-	-	2.00	0.75	0.67	0.71
	Roxithromycin	9.09	-	-	-	6.00	3.48	1.33	2.31
	Tilmicosin	9.09	-	-	-	6.00	62.24	4.08	27.71
Fluoroquinolones	Ciprofloxacin	7.58	-	-	-	5.00	34.28	10.93	16.74
	Norfloxacin	6.06	-	-	-	4.00	29.43	5.00	11.88
	Ofloxacin	3.03	-	-	-	2.00	23.29	6.81	15.05
Lincosamides	Clindamycin	10.61	-	-	5.88	8.00	40.40	0.76	19.90
	Lincomycin	1.52	-	-	-	1.00	33.46	33.46	33.46
Nitroimidazoles	Dimetridazole	7.58	-	-	-	5.00	4.64	0.54	1.96
	Metronidazole	19.70	-	-	47.05	21.00	17.73	6.09	9.26
	Hydroxymetronidazole	1.52	-	-	-	1.00	0.79	0.79	0.79
Sulfonamides	Sulfamethazine	6.06	-	-	-	4.00	3.03	0.76	1.64
	Sulfamethoxazole	13.64	88.00	100.00	41.17	32.00	69.65	0.55	6.26
	Sulfapyridine	18.18	-	-	-	12.00	4.45	0.62	2.08
Tetracyclines	Tetracycline	12.12	100.00	-	-	16.00	12.57	0.50	2.58
Diaminopyrimidine	Trimethoprim	9.09	-	-	-	6.00	7.57	2.12	4.82
Total number of samples		66	8	9	17	100			

Concerning the quantification, the heat map in Figure 6 shows the visualization of the results obtained (also summarized in Appendix 3, Tables 3-1 and 3-2). Due to the large dispersion of values, it has been normalized using a logarithmic scale. In this way, it is possible to identify which antibiotics have been detected at higher concentrations and, also, which are the locations where more types of antibiotics have been found.

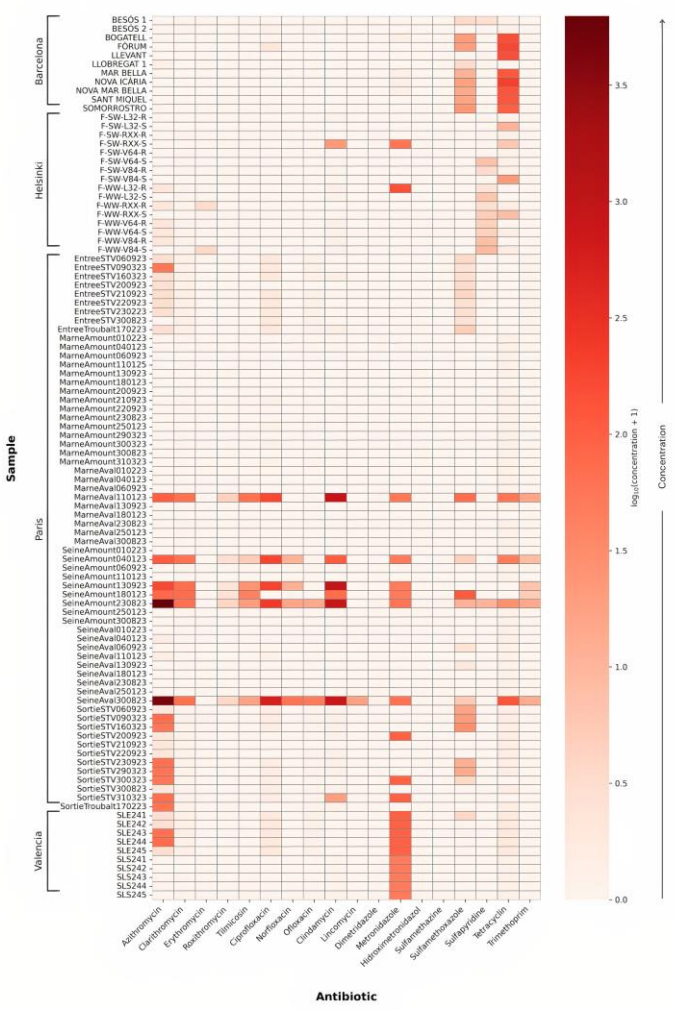

Figure 6. Distribution of antibiotic concentrations detected in water samples, represented on a logarithmic scale.

In particular, the samples from the Seine River (France), especially “SeineAval300823” and “SeineAmount”, are the ones with the highest variability of antibiotics and the highest concentration. Also, the “MarneAval110123” sample shows a great diversity of antibiotics, with especially high concentrations of clarithromycin and erythromycin. In contrast, Catalan locations present the lowest concentration compared to the other regions. This fact may be due to the geographical distribution and the type of water, because the first ones are surface river samples near WWTPs, while those from Barcelona come from sea sampling sites.

Considering all the results, macrolides, azithromycin, clarithromycin and tilmicosin have significant concentrations in several locations. On the other hand, fluoroquinolones, ciprofloxacin and norfloxacin, were quantified at more specific points at high concentrations. As for the nitroimidazoles, especially metronidazole, they have a very significant presence in the samples from Valencian and in small proportions in specific samples from Paris and Helsinki. In the case of sulfamethoxazole, it has been quantified in a generalized distribution, especially in the samples from the Seine River (France) and some from the Marne River (France). Finally, tetracyclines and diaminopyrimidines have a punctual presence in river samples, with the first ones standing out for their significant amounts in seawater. A review of the literature has reported that concentrations can reach µg/L in urban surface waters and, especially, in effluent water from WWTPs [82]. In the case of the samples from France and Valencia, some antibiotics were found in high concentrations, which is consistent with previous studies. In these, it should be noted that the areas around the WWTPs are hot spots of contamination, as was expected [83]. In contrast, concentrations are considerably lower in sea samples or samples far from

discharge points, below 1 µg/L [84]. These results reinforce the idea that WWTPs and their discharge points are the main source of pollution in proximity, while in surface or less impacted waters, concentrations are much lower (Appendix 3, Table 3-1).

To better visualize the dispersion of quantified antibiotics, bar charts were made, grouped according to the type of water sample, normalized on a logarithmic scale (Figure 7).

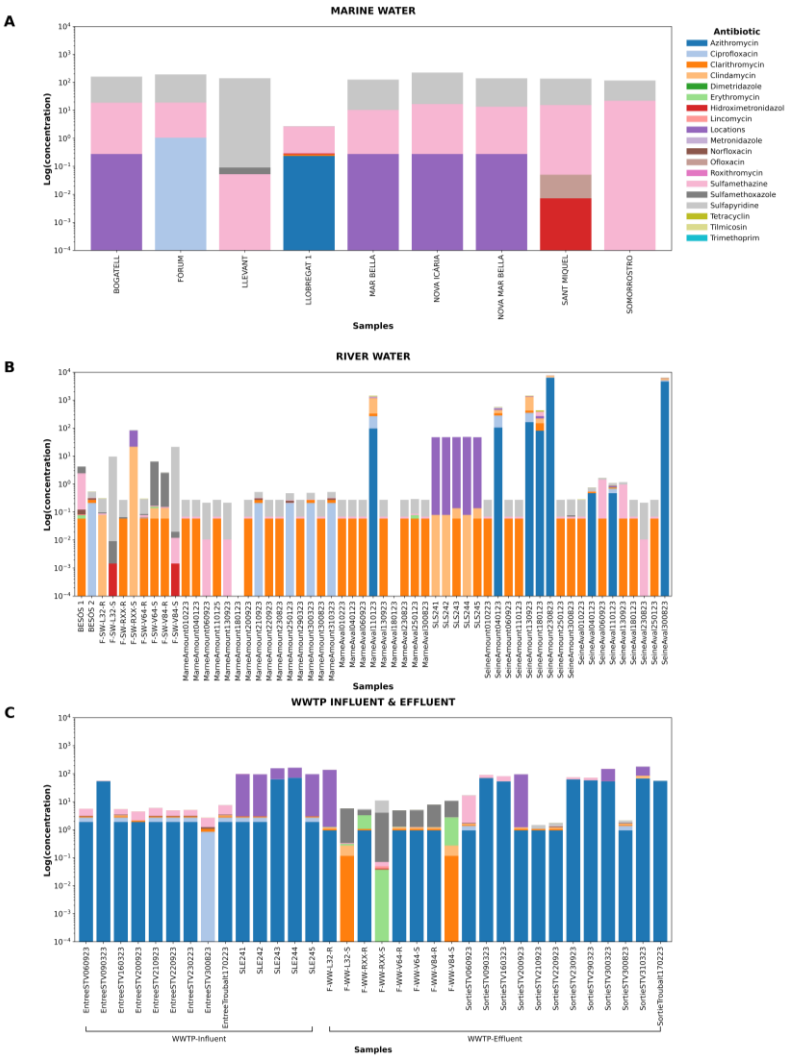

Figure 7. Antibiotic concentrations detected in sea (A), river (B) and near WWTPs (C) water samples, represented on a logarithmic scale.

In the case of seawater samples (Figure 7A), tetracyclines, sulfamethoxazole and metronidazole are mostly observed. In contrast, river water samples from Paris have the highest concentration peaks, while those of other locations have lower quantities and less variety (Figure 7B). The presence of metronidazole should be noted in the Valencian samples. Finally, in the case of the samples near WWTPs, a notable presence of azithromycin, metronidazole and, to a lesser extent, sulfamethoxazole and sulfapyridine can be observed (Figure 7C).

Comparing the samples from the influent and the effluent of Paris' WWTPs, the results suggest that the current water treatment systems do not completely eliminate these compounds because the concentrations are slightly higher in the effluent. Moreover, this can also be explained by the transformation of these compounds into active metabolites or glucuronides. The substances that have been excreted are found at the inlet of the treatment plant, in the form of glucuronide or active metabolite, because they are metabolized in their more soluble form to facilitate their excretion [85], and this cannot be detected by the *target analysis*. However, in WWTPs, the bond between the antibiotic and the glucuronide acid can be broken, which justifies the fact that a greater quantity of these compounds is quantified in the effluent [58].

The most concentrated antibiotics found are azithromycin, tilmicosin, clindamycin, sulfamethoxazole, lincomycin and ciprofloxacin. In the case of the first three, this may be due to their high persistence in the environment. Note that their log(K_{ow}) is between 2.2 and

4 (Appendix 4, Table 4-1). This value indicates that these compounds are moderately hydrophobic and, therefore, have a high tendency to be in the organic phase. The fact that they are mostly retained in sediment or soil does not mean that they cannot be found in the aqueous phase under certain environmental conditions. Moreover, in literature, it can be found that their persistence also depends on their dynamic equilibrium between the aqueous and organic phases. Therefore, their presence in the environment will depend on this balance between sorption, desorption, external supply and the intrinsic persistence of each compound [86].

On the other hand, sulfamethoxazole, lincomycin and ciprofloxacin have much smaller log(K_{ow}) values (Appendix 4, Table 4-1), which means that they are much less hydrophobic compounds with a high solubility in water. Due to these values, they have a higher mobility in aquatic systems and lower affinity for the organic phase, which explains their greater detection in water samples. In contrast, these compounds have a long half-life in aquatic systems (Table 1), considering that metronidazole and sulfamethoxazole pertain to the nitroimidazoles and sulfonamides family of antibiotics, respectively. Therefore, their environmental persistence is more related to their resistance to degradation than to sorption processes [87].

It is important to note that in the current EU Watch list, the following antibiotics are included: clindamycin and sulfamethoxazole [48]. Azithromycin and ciprofloxacin are part of the 2022 Watch list [88], reaffirming the importance of environmental studies for monitoring and control of emerging contaminants. In addition, these two are included in the WHO Watch list (AWaRe classification) for antimicrobial resistance surveillance [89].

The diversity of antibiotic quantification may also be due to the different use and consumption depending on the locality. Figure 8 shows the pattern of antibiotic consumption at the national level, according to the AWaRe classification [17]. This graph illustrates how Spain is the country that consumes the least amount of antibiotics compared to the other two countries studied.

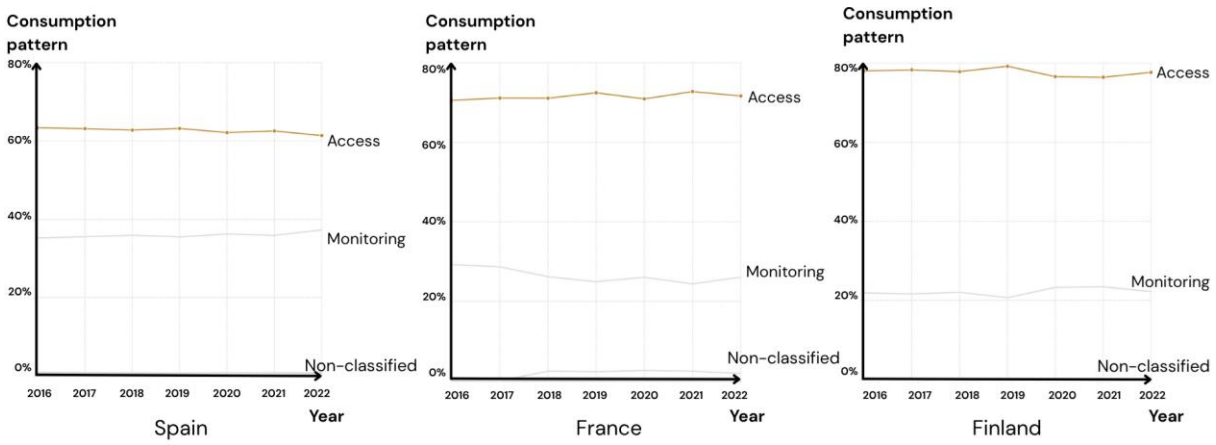

Figure 8. Antibiotic consumption pattern at the national level (relative consumption by AWaRe classification)[17].

These trends may also be an influential factor in the detection of these compounds, but it is necessary to consider all the factors mentioned to interpret the observed differences. For example, the amount of precipitation that occurred during the sampling. In the case of Barcelona, the sampling was carried out after two weeks of continuous rainfall, which could have reduced the concentration of the antibiotics detected. In the case of French samples, the values are listed in the environmental conditions measured (Appendix 2, Table 2-2) where less rainfall episodes took place during the sampling period. In contrast, in Finland, the concentrations in surface waters tend to be lower than in, for example, France, because of the dilution factor due to the rain episodes which are typical of this geographical sampling area.

Finally, different external factors must be taken into consideration that can influence the results obtained. In this study, the antibiotics mentioned have been analyzed and their detection depends on their stability in the environment [45]. However, the ARGs have not been evaluated and could be evidence of resistance in the ecosystem, more resistant than the antibiotics themselves [90].

7. STATISTICAL ANALYSIS

Once the results obtained have been studied, a statistical analysis has been made to see if there are groupings between samples or if there are parameters that influence the presence of antibiotics.

7.1. PRINCIPAL COMPONENT ANALYSIS (PCA) OF ANTIBIOTIC PROFILES

First, PCA on antibiotic concentrations has been developed to identify patterns of variation and possible clustering between samples. In particular, it allows for the visualization of the overall structure of the data set by reducing its dimensionality but preserving most of the variability.

In Figure 9, PCA has been performed on samples in different water types (river, sea, WWTPs-Influent, WWTPs-Effluent). The combination of both graphs allows a much clearer interpretation of the relationship between the samples and the variables. In the case of clustering, it helps to understand which antibiotics have the most influence on how they are distributed according to the type of water.

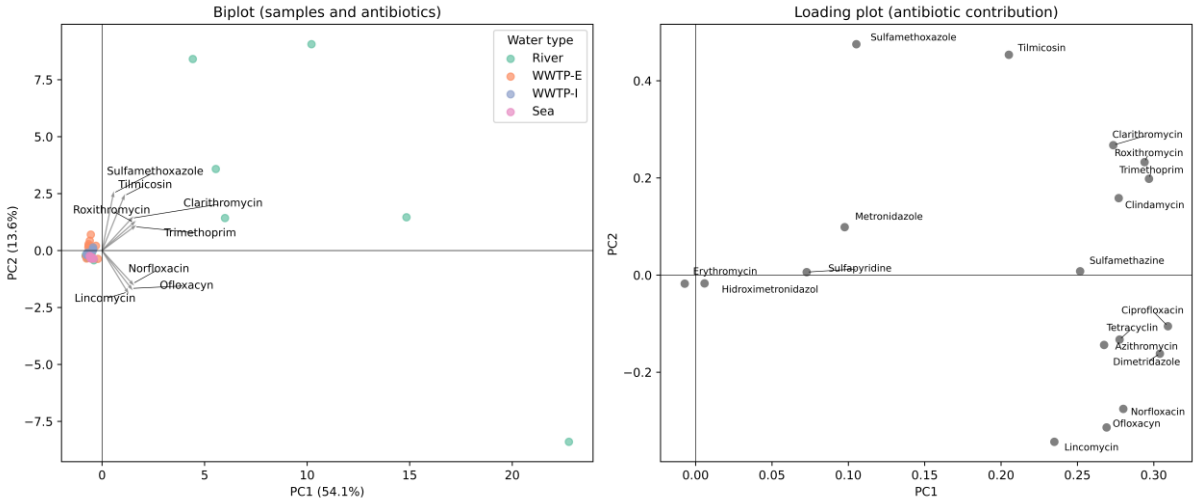

Figure 9. PCA of water samples, score plot (left) and loading plot (right).

In the left graph, the samples are plotted on the first two principal components, which explain around 70% of the variability as a whole. In this graph, a clear separation of the river samples from the others is evident, especially along the first component (PC1). This separation reveals that these samples have a different concentration profile from the others. In addition, the contribution of antibiotics and their directionality has been added. Thus, it can be observed that sulfamethoxazole, clarithromycin and trimethoprim are some of those that favor this differentiation, because they have vectors oriented to river samples.

The graph on the right shows the loading plot, which represents all the variables (antibiotics) in a function of their loadings in PC1 and PC2. The compounds that are more to the right of PC1, are the ones that contribute more to its differentiation according to the type of water, because the river samples are more displaced to this component. Thus, the results indicate that the river samples are strongly influenced by clarithromycin, tilmicosin, sulfamethoxazole and sulfamethazine, among others. It should be noted that antibiotics such as erythromycin, hydroxymetronidazole and metronidazole are very low or close to zero loads, and this explains that they have little influence on these two main components.

7.2. HIERARCHICAL CLUSTERING BASED ON COMBINED DATA

One of the ways to see natural groupings of samples according to their overall similarity is by means of a hierarchical cluster. In this diagram, the antibiotic concentrations and their respective environmental factors are considered without reducing their dimensionality. Samples and clusters are colored according to their water type on the x-axis and according to their differentiation, respectively.

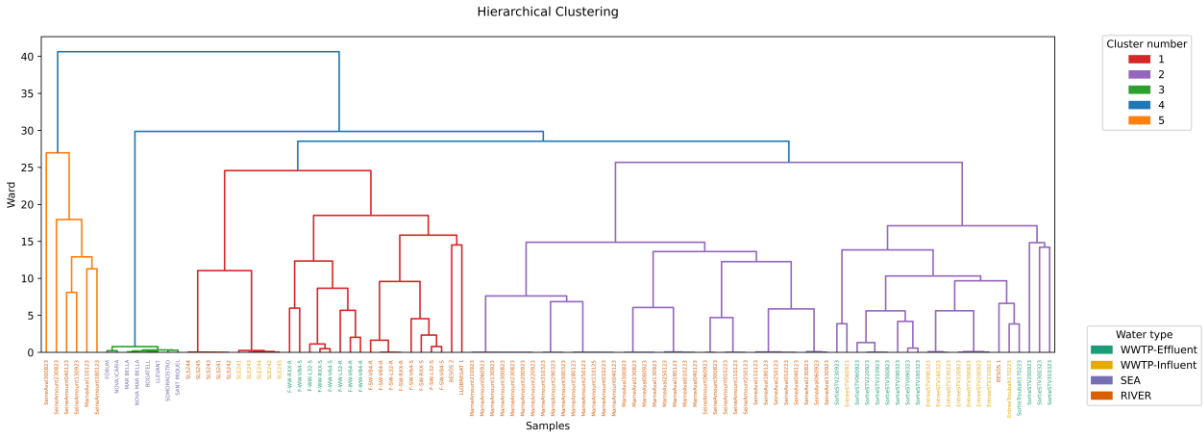

Figure 10. Hierarchical clustering using Ward's method of water samples, colored by water type and clustering.

The orange cluster (number 4 in Figure 10) contains all the samples from Paris with antibiotic concentration much higher than the others. In particular, there are "SeineAval", "SeineAmount" and "MarneAval". The green one (num. 5), on the other hand, includes all the seawater samples, because they have a matrix and concentrations that are very different from the others. Then, in the red cluster (num. 2), the samples from Valencia and Finland, both river water and wastewater effluent, are grouped. In addition, there are also two river samples from Barcelona, which show that they have similar characteristics. Finally, in the purple cluster, all the remaining river samples from Paris have been combined. Next to them, the corresponding influent and effluent samples of the Parisian rivers, which have a similar distribution of concentrations, are grouped in the same cluster. In addition, the remaining Barcelona River sample is also located in this one, a fact that could reflect similar distributions of similar properties. This distribution of the samples may give an idea of the similarity of the matrices, their antibiotic concentration and environmental conditions.

7.3. Distribution of Antibiotic Concentration by Water Type

To evaluate if some factors influence more than others, such as the type of water or the season of the year of sampling, an ANOVA was performed, which allows to know if these factors are statistically significant. First, a population ANOVA was made, but due to a lack of sample diversity, these results were not significant. For this reason, a statistical analysis was realized according to the type of matrix, whether they are seawater, river water or influent/effluent from WWTPs (Figure 11).

In Figure 11, all the comparative box plots are distributed, showing how the concentration of each antibiotic varies according to the type of water. In addition, below each one its p-value, which, in case its value is lower than 0.05, is statistically significant and is marked in red. Thus, the antibiotics with significant results are erythromycin, metronidazole and sulfapyridine. In these, it is observed that the distribution of antibiotics is generally found in wastewater, both in influent and effluent.

As for the other antibiotics, their concentrations in seawater are much lower than in rivers and wastewater, due to environmental dilution [41]. For river water, there is a great variety depending on the specific antibiotic.

The combined study for PCA, clustering and boxplots allows to analyze the observed groupings and provides a solid basis to discuss the source and persistence of antibiotics in different aquatic environments.

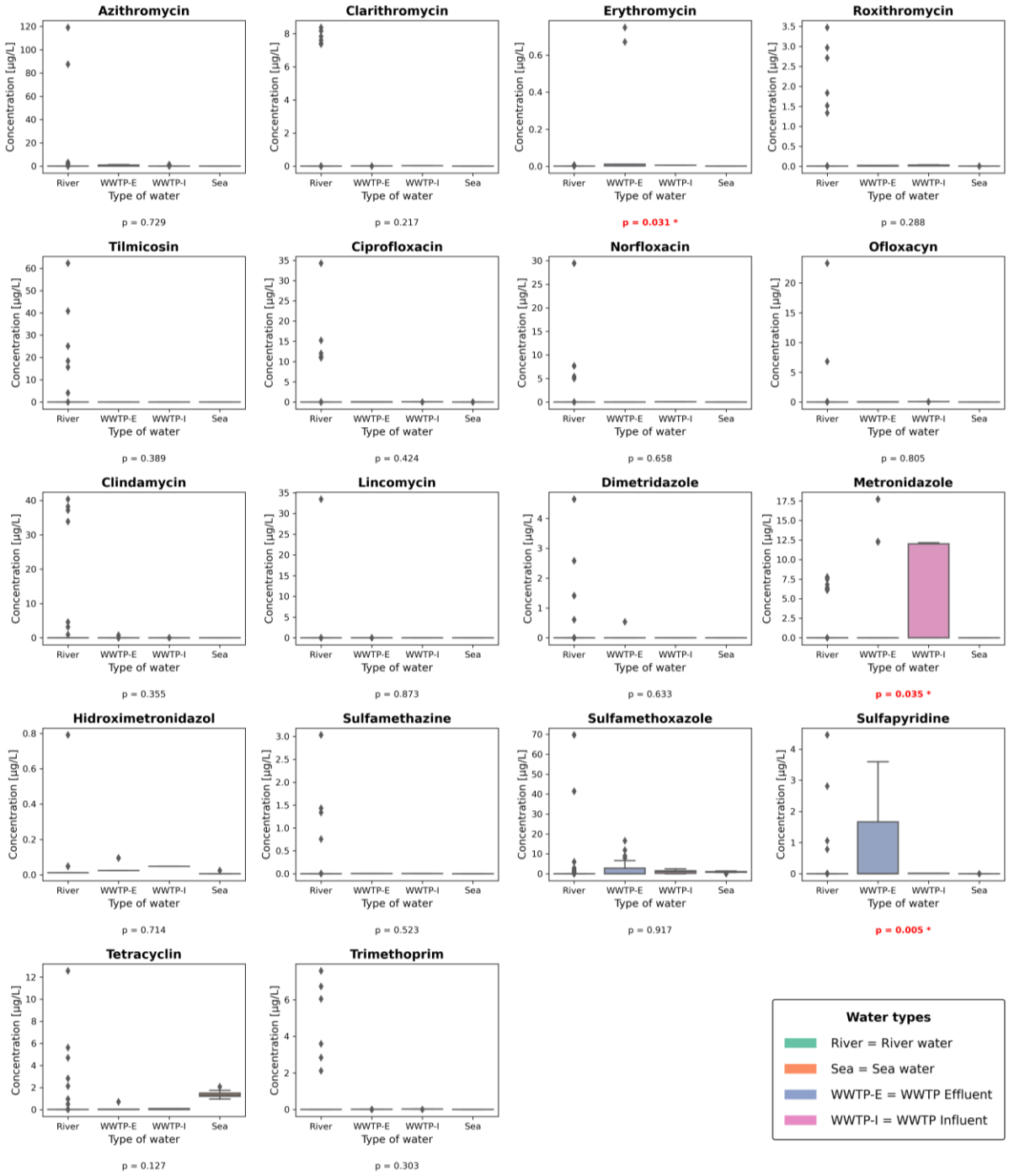

Figure 11. Boxplots showing antibiotic concentrations across different water types with ANOVA p-values (p-values in red* are statistically significant).

8. SUSPECT SCREENING

The *suspect screening* was performed to identify more antibiotics than the ones analyzed by *target analysis*. As has been explained in the methods section, the data from HRMS was filtered by Compound Discoverer 3.3. Due to the variety of parameters of the samples (such as location, sample type, etc.), the data have been grouped according to the type of water, to compare the results in similar matrices. Therefore, there are three defined groups: river water samples, seawater samples and samples collected near WWTPs.

The procedure followed was, first, the first filtering of compounds in the software used, choosing only correct and relevant peaks (as in Figure 12). Then, from all selected substances, duplicates are eliminated and the blank and the mobile phase area are subtracted from the sample area to determine if they are present in them. Finally, all the compounds obtained are classified according to whether they are antibiotic or not, using the *List S6 ITNANTIBIOTIC on NORMAN Suspect List Exchange* as an aid. In this way, the graphs show two levels of classification: the distribution of antibiotics in the samples and, second, the categorization of these substances according to their class.

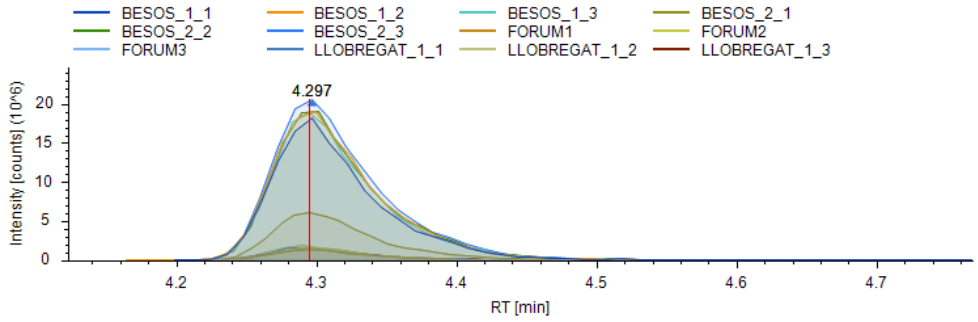

Figure 12. Example chromatogram of sulfamethazine detected in Barcelona water samples from the *suspect screening* analysis.

In the river water samples, approximately 3% of antibiotics have been tentatively identified, in addition to those already studied. The most relevant groups are macrolides, quinolones and sulfonamides, which are characteristic of their high use and persistence in the aquatic ecosystem. Also, the presence of the group "Others" can be observed, which corresponds to those substances that do not have a specific classification (Figure 13). The continuous input from different sources of contamination and the variety of environmental degradation could explain the greater diversity of types of compounds detected [45].

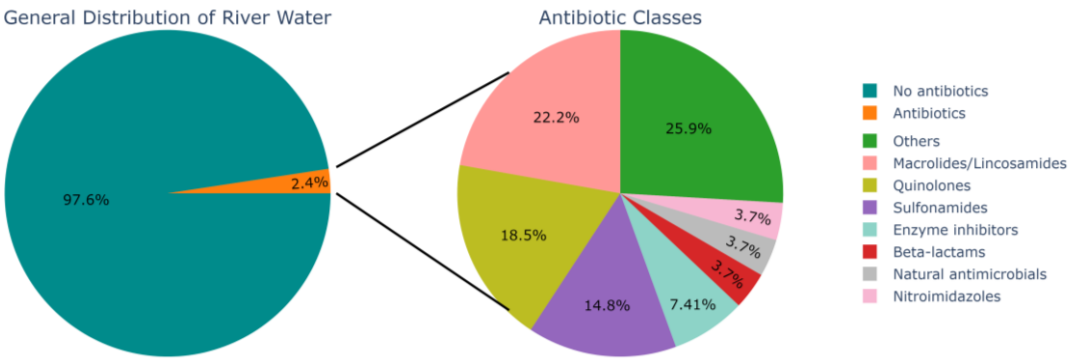

Figure 13. Distribution of tentatively identified antibiotics and their classes in river water samples.

In the case of seawater, the percentage of antibiotics tentatively identified is the same as in river water (3%), but they differ in their classification and distribution. In this, it can be observed that half of those detected are classified as antibiotics (Figure 14), which demonstrates the importance of implementing effective control and monitoring measures to reduce their presence in the environment [58]. In this type of sample, the entry of contaminants is constant and, therefore, there is more diversity of compounds.

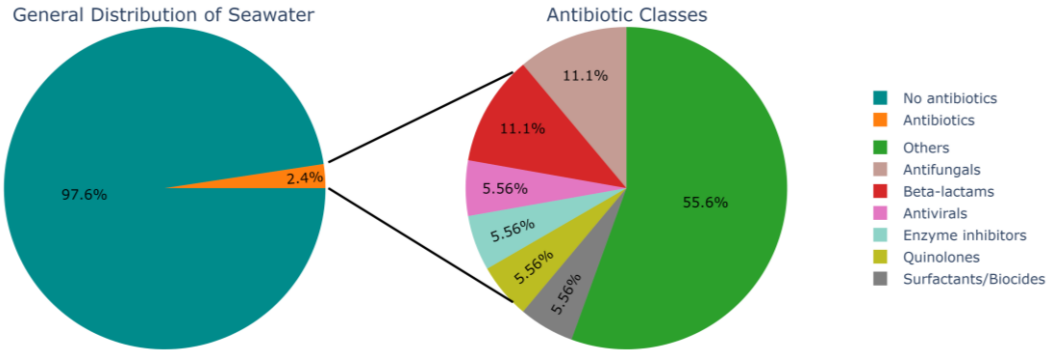

Figure 14. Distribution of tentatively identified antibiotics and their classes in seawater samples.

On the other hand, samples collected near WWTPs have been separated into those located in the influent (Figure 15) or effluent (Figure 16). In both cases, a predominance of unclassified compounds can be observed, which may signify the presence of emerging contaminants [58].

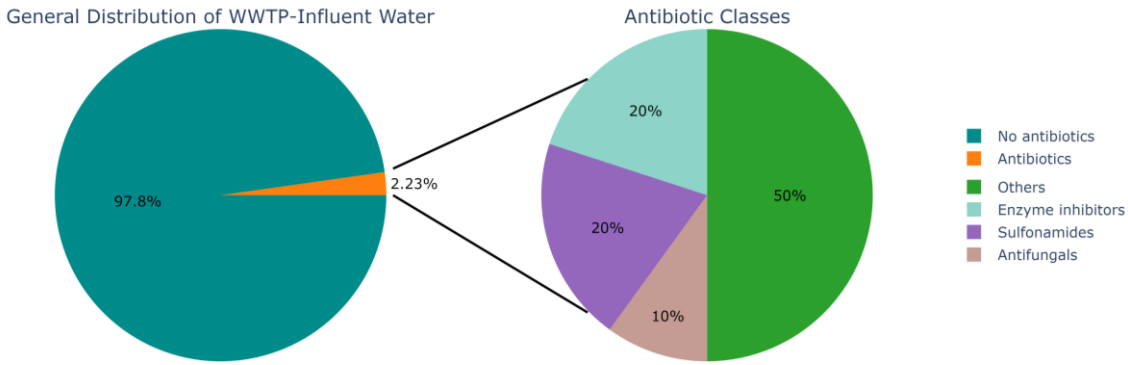

Figure 15. Distribution of tentatively identified antibiotics and their classes in samples near influent WWTPs.

In the effluent, there is a similar amount of antibiotics tentatively identified as in the influent (about 2%), but a diversity of antibiotic classes (Figure 16). As has been explained, the wastewater treatments break the glucuronide-antibiotic bonds, which makes these compounds more detectable [58]. For this reason, for the future, it is important to note all the research that is focused on the development of more effective treatments to prevent these contaminants from reaching the aquatic ecosystem and thus reduce the spread of antimicrobial resistance [45].

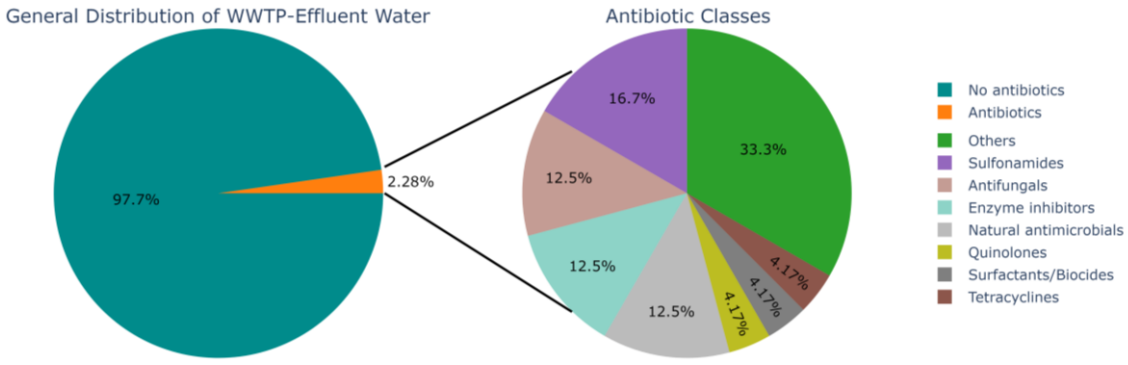

Figure 16. Distribution of tentatively identified antibiotics and their classes in samples near the effluent WWTPs.

In summary, although the *suspect screening* study allows the tentative identification of different groups of chemicals, a small percentage of all of these are antibacterials. The results suggest that there may be a risk of contamination in the aquatic environment where these will end up [58]. It is important to note that this study only reports the presence of antibiotics and not their concentration. However, to see the real risk and its impact, it would be necessary to carry out a *target analysis* to quantify the concentrations [74]. Therefore, it is recommended to reinforce the elimination treatments, to intensify the monitoring of these compounds, and to study all their forms and transformations in the environment [58].

9. ENVIRONMENTAL RISK ASSESSMENT

To evaluate if the quantified antibiotics could pose a risk for the environment, it is possible to evaluate their ecotoxicological risk to determine if measures should be taken. As explained in the Materials & Methods section, the HQ is calculated by dividing the ambient concentration detected by the corresponding PNEC, depending on the type of water [76]. These values are available in Appendix 4, Table 4-1 and 4-2.

First, the risk percentage corresponds to the ratio of samples with an HQ greater than 10 to the total number of samples with a minimal risk (HQ greater than 0.1). By comparing this percentage with the detection rate, it is possible to know if an antibiotic that is very present in the environment can hurt the ecosystem (Table 4) [91].

Table 4. Detection and risk frequency of antibiotics analyzed by *target analysis* in rivers, sea and samples collected near WWTPs.

Antibiotic	**Detection** [%]				**Risk** [%]*			
	Sea water	**River water**	**WWTP Influent**	**WWTP Effluent**	**Sea water**	**River water**	**WWTP Influent**	**WWTP Effluent**
Azithromycin	-	9.09	29.42	55.56	-	42.86	31.25	55.56
Clarithromycin	-	9.09	-	-	-	46.15	-	-
Erythromycin	-	3.03	-	-	-	-	-	-
Roxithromycin	-	9.09	-	-	-	-	-	-
Tilmicosin	-	9.09	-	-	-	83.33	-	-
Ciprofloxacin	-	7.58	-	-	-	45.45	-	-
Norfloxacin	-	6.06	-	-	-	100.00	-	-
Ofloxacin	-	3.03	-	-	-	100.00	-	-
Clindamycin	-	10.61	-	11.11	-	53.85	-	33.33
Lincomycin	-	1.52	-	-	-	100.00	-	-
Dimetridazole	-	7.58	-	-	-	-	-	-
Metronidazole	-	19.70	-	88.89	-	100.00	-	100.00
Hydroxymetronidazole	-	1.52	-	-	-	-	-	-
Sulfamethazine	-	6.06	-	-	-	-	-	-
Sulfamethoxazole	88.00	13.64	82.35	22.22	100.00	22.22	35.71	-
Sulfapyridine	-	18.18	-	-	-	-	-	-
Tetracycline	100.00	12.12	-	-	100.00	8.62	-	-
Trimethoprim	-	9.09	-	-	-	50.00	-	-

*This percentage is calculated by dividing the number of samples having an HQ >10 by the total number of samples having an HQ, i.e., greater than 0.1.

This table shows that the antibiotics detected in all seawaters present a high risk to the environment. In the case of river samples, except for tetracycline, all the antibiotics detected have a very high level of risk for the aquatic ecosystem, highlighting the urgent need for monitoring and mitigation measures to prevent further environmental impact.

Concerning the samples located near WWTPs, it can be observed that those in the effluent present higher risk values than those in the influent. For example, all effluent samples with metronidazole have a very high risk of having adverse effects on the ecosystem. This event underlines the need to improve their elimination during water treatment to reduce their presence in the natural environment.

In addition, to visualize the ecotoxicological risk at each sampling point, a heatmap has been made (Figure 17). First, in French river samples, it can be observed that “MarneAval” and those of the Seine River are very important sources of pollution and with a very high risk, which might have adverse effects on the ecosystem. Moreover, the samples near WWTPs, the influent samples have a rather moderate or low risk, while a high risk characterizes those of the effluent. As mentioned in previous sections, in the WWTPs, the glucuronide bonds can be broken and, consequently, increase the amount of antibiotics present in the environment [85]. Therefore, this could be one of the reasons why a higher risk could be obtained in the effluent. Other reasons could be the incorrect removal, insufficient treatment of all metabolites and degradation products, among others [92].

In Finland samples, a moderate risk can be observed for sulfapyridine and tetracycline in samples near WWTPs and some river samples. In contrast, in the case of specific samples with clindamycin, tetracycline and metronidazole, there is a high probability of the adverse effects being noticed.

The presence of metronidazole is high in samples coming from Valencia, where a very significant risk has been found in both river and near WWTPs samples. In contrast, in the Barcelona sea samples, the antibiotics sulfamethoxazole and tetracycline show a high probability of having negative effects on the environment.

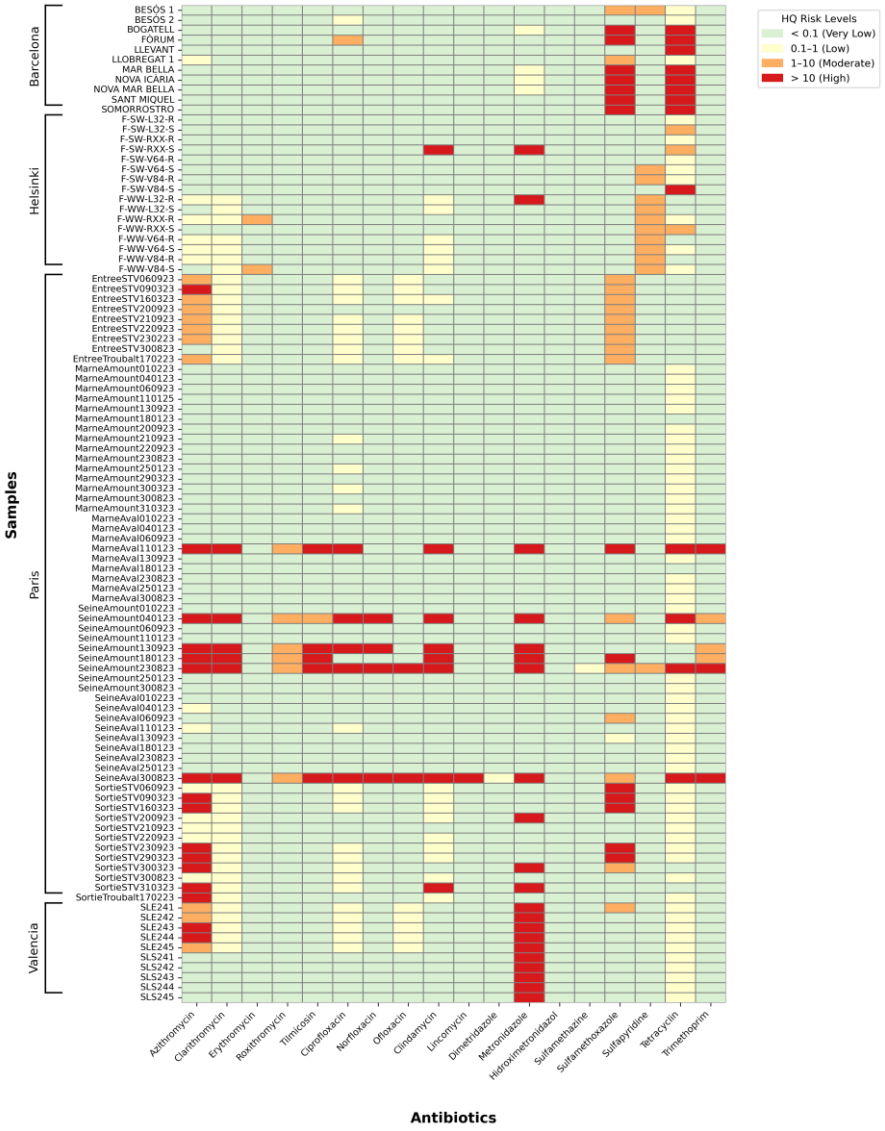

Figure 17. Heatmap of HQ values for antibiotics detected in water samples.

According to the literature, the results obtained are consistent with previous studies. For example, antibiotics such as sulfamethoxazole, ciprofloxacin and metronidazole have an HQ often greater than 1 in river water and WWTPs samples [93]. Other studies have detected azithromycin and metronidazole in WWTPs effluents from Spain, Finland, Portugal, among others, with a detailed ecological risk assessment based on the risk quotients, demonstrating the associated environmental hazard [83].

Considering the results obtained, both concentrations and their associated risk, the possible effects on aquatic ecosystems should be evaluated. For example, macrolides can inhibit bacterial growth, enhance bioaccumulation of aquatic organisms and have toxic effects on fish and invertebrates [60]. In contrast, fluoroquinolones are highly toxic to bacteria and algae, can bioaccumulate in fish and decrease the efficacy of bacteria in WWTPs [94]. In the case of lincosamides, they can alter the aquatic microbial composition [45]. Moreover, genotoxic and disruptive effects can occur in the presence of nitroimidazoles [95], and, in the case of sulfonamides, they can alter nitrogen processes [31]. Tetracyclines are toxic to algae, bacteria and aquatic invertebrates [96] and trimethoprim

enhances toxicity when combined with sulfonamides [4]. Finally, all of them can contribute to the selection and propagation of ARGs, a risk to public health [45].

In summary, the results highlight the importance of assessing the associated risk in different aquatic environments, especially in anthropologically influenced areas and near WWTPs [58]. These studies make it possible to identify the critical contamination points and the substances to be targeted.

10. CONCLUSIONS

In this study, a total of 18 antibiotics were analyzed in 100 water samples from Barcelona and Valencia (Spain), Helsinki (Finland) and Paris (France). In these, the most detected antibiotics were sulfamethoxazole, metronidazole, azithromycin and tetracycline. Depending on the sample, concentration can vary significantly. In the case of sea samples, the values are lower than in the other, which can be explained by the effect of dilution. However, in rivers and near WWTPs samples, the concentrations and ecological risk are higher. Moreover, some effluent WWTPs samples have higher concentrations than in the influent, possibly due to glucuronic transformation processes or insufficient elimination.

Statistical analyses have revealed that the samples studied exhibit groupings based on their antibiotic profile or type of water, providing important information for future studies.

As for the *suspect screening*, other antibiotics were tentatively identified in the samples, in addition to those already studied by the *target analysis*. These results reinforce the concern about the entry of these emerging contaminants into the ecosystem and their respective negative effects.

HQ were calculated to support the analysis performed and, in several samples, a significant ecological risk was found for some antibiotics, such as metronidazole, sulfamethoxazole, tetracycline and azithromycin.

The conclusions extracted from this research show that wastewater treatment technologies are not sufficient for the removal of antibiotics and their TPs. Therefore, it is necessary to improve them and implement continuous monitoring and more effective regulation to reduce their environmental impact and the spread of antimicrobial resistance. In summary, it is important to continue this study considering the variability of environmental factors and their combined effect, resistance genes and long-term ecotoxicological impacts, to protect aquatic ecosystems and ensure good environmental management.

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12. ACRONYMS

ANOVA	Analysis of Variance
ARGs	Antibiotic Resistance Genes
AWaRe	Access, Watch, and Reserve classification
CSIC	*Consejo Superior de Investigaciones Científicas*
EMA	European Medicines Agency
FWHM	Full Width at Half Maximum
HQ	Hazard Quotient
HPLC	High-performance Liquid Chromatography
HPLC-HRMS	High-Performance Liquid Chromatography – High-Resolution Mass Spectrometry
IDAEA	Institute of Environmental Assessment and Water Research
K_d	Distribution Coefficient
$Log(K_{ow})$	Logarithm Octanol-Water Partition Coefficient
PCA	Principal Component Analysis
PNEC	Predicted No Effect Concentration
SDG	Sustainable Development Goals
SPE	Solid Phase Extraction
TPs	Transformation Products
WHO	World Health Organization
WWTPs	Wastewater Treatment Plants

APPENDICES

APPENDIX 1: LIST OF ANTIBIOTICS

Table 1-1. Selected antibiotics and their internal standards with molecular formulas and weights.

Antibiotic	Molecular formula	Molecular height [g/mol] *[97]*	Antibiotic internal standard	Internal standard formula	Molecular weight [g/mol] *[97]*
Azithromycin	$C_{38}H_{72}N_2O_{12}$	748.51	Azithromycin-d_3	$C_{38}H_{69}D_3N_2O_{12}$	751.53
Ciprofloxacin	$C_{17}H_{18}FN_3O_3$	331.13	Ciprofloxacin-d_8	$C_{17}H_{10}D_8FN_3O_3$	339.18
Clarithromycin	$C_{38}H_{69}NO_{13}$	747.48	Clarithromycin-d_3	$C_{38}H_{66}D_3NO_{13}$	750.50
Clindamycin	$C_{18}H_{33}ClN_2O_5S$	424.18	Clindamycin-^{13}C-d_3	$C_{18}{}^{13}CH_{30}D_3ClN_2O_5S$	444.20
Dimetridazole	$C_5H_7N_3O_2$	141.05	Dimetridazole-d_3	$C_5H_4D_3N_3O_2$	144.07
Erythromycin	$C_{37}H_{67}NO_{13}$	733.46	Erythromycin-d_3	$C_{37}H_{64}D_3NO_{13}$	736.48
Hydroxymetronidazole	$C_6H_9N_3O_4$	187.06	Hydroxymetronidazole-d_4	$C_6H_5D_4N_3O_4$	191.08
Lincomycin	$C_{18}H_{34}N_2O_6S$	406.21	Lincomycin-d_3	$C_{18}H_{31}D_3N_2O_6S$	409.23
Metronidazole	$C_6H_9N_3O_3$	171.06	Metronidazole-d_4	$C_6H_5D_4N_3O_3$	175.09
Norfloxacin	$C_{16}H_{18}FN_3O_3$	319.13	Norfloxacin-d_8	$C_{16}H_{10}D_8FN_3O_3$	327.18
Ofloxacin	$C_{18}H_{20}FN_3O_4$	361.14	Ofloxacin-d_8	$C_{18}H_{12}D_8FN_3O_4$	369.19
Roxithromycin	$C_{41}H_{76}N_2O_{15}$	836.52	Roxithromycin-d_7	$C_{41}H_{69}D_7N_2O_{15}$	843.57
Sulfamethazine	$C_{12}H_{14}N_4O_2S$	278.08	Sulfamethazine-d_4	$C_{12}H_{10}D_4N_4O_2S$	282.11
Sulfamethoxazole	$C_{10}H_{11}N_3O_3S$	253.05	Sulfamethoxazole-d_4	$C_{10}H_7D_4N_3O_3S$	257.08
Sulfapyridine	$C_{11}H_{11}N_3O_2S$	249.06	Sulfapyridine-d_4	$C_{11}H_7D_4N_3O_2S$	253.08
Tetracycline	$C_{22}H_{24}N_2O_8$	444.15	Tetracyclin-d_6	$C_{22}H_{18}D_6N_2O_8$	450.19
Tilmicosin	$C_{46}H_{80}N_2O_{13}$	868.57	Tilmicosin-d_3	$C_{46}H_{77}D_3N_2O_{13}$	871.58
Trimethoprim	$C_{14}H_{18}N_4O_3$	290.14	Trimethoprim-d_3	$C_{38}H_{69}D_3N_2O_{12}$	293.16

Table 1-2. Selected antibiotics with their antibiotic family and main use.

Antibiotic family [98]	**Antibiotic**	**Main use** [98]
Macrolides	Azithromycin	Respiratory bacterial infections
	Clarithromycin	Bacterial infections
	Erythromycin	Bacterial infections
	Roxithromycin	Respiratory and skin bacterial infections
	Tilmicosin	Infections in veterinary medicine
Fluoroquinolones	Ciprofloxacin	Serious bacterial infections caused by susceptible microorganisms
	Norfloxacin	Urinary tract infections
	Ofloxacin	Bacterial infections caused by susceptible microorganisms
Lincosamides	Clindamycin	Serious bacterial infections caused by susceptible microorganisms
	Lincomycin	Serious bacterial infections caused by susceptible microorganisms
Nitroimidazoles	Dimetridazole	Infections in veterinary medicine
	Metronidazole	Infections caused by sensitive anaerobic bacteria and protozoa
	Hydroxymetronidazole	Active metabolite of metronidazole
Sulfonamides	Sulfamethazine	Infections in veterinary medicine
	Sulfamethoxazole	Bacterial infections in combination with trimethoprim
	Sulfapyridine	Inflammatory bowel disease and bacterial infections
Tetracyclines	Tetracycline	Sensitive bacterial infections
Diaminopyrimidine	Trimethoprim	Urinary tract infections caused by sensitive bacteria

APPENDIX 2: SAMPLING LOCATIONS AND WATER PARAMETERS

Table 2-1. Sampling locations and water parameters from seawater samples taken from Barcelona sampling area (Spain).

Location	Type of water	Coordinates [Lat, Long]	Temperature [ºC]	Pressure [mmHg]	DO [%]	DO [mg/L]	SPC [µS/cm]	C [µS/cm]	pH	ORP [mV]
Besós 1	River (non-mouth)	41°25'46.7"N 2°12'54.4"E	12.0	750.9	101.0	10.85	1068	803	7.13	-82.7
Besós 2	River (mouth)	41°25'12.5"N 2°13'55.8"E	14.9	751.1	116.3	10.27	34735	28029	7.00	-64.3
Fòrum	Sea (coast)	41°25'01.1"N 2°13'52.6"E	13.0	751.5	102.6	9.22	37505	28946	7.24	-88.6
Llevant	Sea (coast)	41°24'12.0"N 2°13'00.5"E	12.9	751.6	109.2	10.09	34400	26417	7.20	-77.8
Nova Mar Bella	Sea (coast)	41°24'10.3"N 2°13'00.6"E	12.9	751.5	102.3	8.36	614439	47192	7.27	-83.6
Mar Bella	Sea (coast)	41°23'58.7"N 2°12'50.0"E	13.0	751.6	111.2	9.07	61537	47387	7.26	-84.0
Bogatell	Sea (coast)	41°23'30.1"N 2°12'19.4"E	13.0	751.5	124.7	10.81	47923	36962	7.23	-76.1
Nova Icària	Sea (coast)	41°23'28.5"N 2°12'16.2"E	13.0	751.4	125.8	10.59	54421	41988	7.26	-72.7
Somorrostro	Sea (coast)	41°23'05.2"N 2°11'53.2"E	13.5	751.2	171.2	14.56	49811	38916	7.30	-65.5
Sant Miquel	Sea (coast)	41°22'36.9"N 2°11'32.2"E	13.5	751.2	128.4	10.36	61207	47802	7.33	-75.4
Llobregat 1	River (non-mouth)	41°19'52.0"N 2°06'27.4"E	12.3	751.0	130.8	13.95	1223	926	7.48	-85.1

Table 2-2. Sampling locations and water parameters from surface river and influent and effluent WWTPs samples taken from Paris (France).

Location	**Date**	**Type of water**	**Coordinates** [Lat, Long]	**Temperature** [ºC]	**Pluviometry 24h** [mm]	**pH**
Marne Amount	04/01/2023	River (non-mouth)	48°51'11.74"N, 2°32'34.58"E	n.m.	0	n.m.
Marne Aval	04/01/2023	River (non-mouth)	48°48'57.56"N, 2°24'47.84"E	n.m.	0	n.m.
Seine Amount	04/01/2023	River (non-mouth)	48°44'51.22"N, 2°26'6.90"E	n.m.	0	n.m.
Seine Aval	04/01/2023	River (non-mouth)	48°48'26.24"N, 2°24'35.17"E	n.m.	0	n.m.
Marne Amount	11/01/2023	River (non-mouth)	48°51'11.74"N, 2°32'34.58"E	n.m.	4.2	n.m.
Marne Aval	11/01/2023	River (non-mouth)	48°48'57.56"N, 2°24'47.84"E	n.m.	4.2	n.m.
Seine Amount	11/01/2023	River (non-mouth)	48°44'51.22"N, 2°26'6.90"E	n.m.	4.2	n.m.
Seine Aval	11/01/2023	River (non-mouth)	48°48'26.24"N, 2°24'35.17"E	n.m.	4.2	n.m.
Marne Amount	18/01/2023	River (non-mouth)	48°51'11.74"N, 2°32'34.58"E	7.3	0.6	8.12
Marne Aval	18/01/2023	River (non-mouth)	48°48'57.56"N, 2°24'47.84"E	7.7	0.6	8.10
Seine Amount	18/01/2023	River (non-mouth)	48°44'51.22"N, 2°26'6.90"E	7.6	0.6	7.69
Seine Aval	18/01/2023	River (non-mouth)	48°48'26.24"N, 2°24'35.17"E	8	0.6	7.64
Marne Amount	25/01/2023	River (non-mouth)	48°51'11.74"N, 2°32'34.58"E	5.0	0.8	8.20
Marne Aval	25/01/2023	River (non-mouth)	48°48'57.56"N, 2°24'47.84"E	4.2	0.8	8.20
Seine Amount	25/01/2023	River (non-mouth)	48°44'51.22"N, 2°26'6.90"E	5.8	0.8	8.16
Seine Aval	25/01/2023	River (non-mouth)	48°48'26.24"N, 2°24'35.17"E	5.9	0.8	8.10
Marne Amount	18/01/2023	River (non-mouth)	48°51'11.74"N, 2°32'34.58"E	6.6	0.4	7.57
Marne Aval	18/01/2023	River (non-mouth)	48°48'57.56"N, 2°24'47.84"E	6.2	0.4	8.27
Seine Amount	18/01/2023	River (non-mouth)	48°44'51.22"N, 2°26'6.90"E	6.8	0.4	8.17
Seine Aval	18/01/2023	River (non-mouth)	48°48'26.24"N, 2°24'35.17"E	7.2	0.4	8.08
Sortie STV	06/09/2023	WWTP-Effluent	48°52'27.2"N, 2°40'21.6"E	n.m.	0.0	n.m.
Sortie STV	09/03/2023	WWTP-Effluent	48°52'27.2"N, 2°40'21.6"E	n.m.	17.2	n.m.
Sortie STV	16/03/2023	WWTP-Effluent	48°52'27.2"N, 2°40'21.6"E	n.m.	9.4	n.m.
Sortie STV	21/09/2023	WWTP-Effluent	48°52'27.2"N, 2°40'21.6"E	n.m.	0.0	n.m.
Sortie STV	22/09/2023	WWTP-Effluent	48°52'27.2"N, 2°40'21.6"E	n.m.	0.0	n.m.
Sortie STV	23/09/23	WWTP-Effluent	48°52'27.2"N, 2°40'21.6"E	n.m.	0.6	n.m.
Sortie STV	29/03/2023	WWTP-Effluent	48°52'27.2"N, 2°40'21.6"E	n.m.	0.0	n.m.
Sortie STV	30/08/2023	WWTP-Effluent	48°52'27.2"N, 2°40'21.6"E	n.m.	0.0	n.m.
Entrée STV	06/09/2//023	WWTP-Influent	48°52'27.2"N, 2°40'21.6"E	n.m.	0.0	n.m.
Entrée STV	09/03/2023	WWTP-Influent	48°52'27.2"N, 2°40'21.6"E	n.m.	17.2	n.m.
Entrée STV	16/03/2023	WWTP-Influent	48°52'27.2"N, 2°40'21.6"E	n.m.	9.4	n.m.
Entrée STV	20/09/2023	WWTP-Influent	48°52'27.2"N, 2°40'21.6"E	n.m.	0.0	n.m.
Entrée STV	21/09/2023	WWTP-Influent	48°52'27.2"N, 2°40'21.6"E	n.m.	0.0	n.m.
Entrée STV	22/09/23	WWTP-Influent	48°52'27.2"N, 2°40'21.6"E	n.m.	0.0	n.m.
Entrée STV	23/02/2023	WWTP-Influent	48°52'27.2"N, 2°40'21.6"E	n.m.	0.6	n.m.
Entrée STV	30/08/2023	WWTP-Influent	48°52'27.2"N, 2°40'21.6"E	n.m.	0.0	n.m.

*n.m. not mesured

APPENDIX 3: QUANTITATIVE RESULTS

Table 3-1. Detailed concentrations (µg/L) of the analyzed antibiotics in each water sample.

SAMPLES	**Sample Concentration** [µg/L]																	
	Azithromycin	*Clarithromycin*	*Erythromycin*	*Roxithromycin*	*Tilmicosin*	*Ciprofloxacin*	*Norfloxacin*	*Ofloxacin*	*Clindamycin*	*Lincomycin*	*Dimetridazole*	*Metronidazole*	*Hydroxymetronidazole*	*Sulfamethazine*	*Sulfamethoxazole*	*Sulfapyridine*	*Tetracyclin*	*Trimethoprim*
F-SW-L32-R	ND	ND	ND	ND	ND	ND	ND	ND	<LOQ	ND	<LOQ	ND	ND	ND	<LOQ	<LOQ	<LOQ	<LOQ
F-SW-RXX-R	ND	<LOQ	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<LOQ	<LOQ	ND
F-SW-V64-R	ND	<LOQ	ND	<LOQ	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<LOQ	<LOQ	<LOQ	<LOQ
F-SW-V84-R	ND	<LOQ	ND	ND	ND	ND	ND	ND	<LOQ	<LOQ	ND	ND	ND	ND	<LOQ	1.0585	<LOQ	<LOQ
F-WW-L32-R	<LOQ	<LOQ	<LOQ	<LOQ	ND	ND	ND	ND	<LOQ	ND	ND	17.7311	ND	ND	<LOQ	0.6225	ND	<LOQ
F-WW-RXX-R	<LOQ	<LOQ	0.6711	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.8319	<LOQ	ND
F-WW-V64-R	<LOQ	<LOQ	<LOQ	<LOQ	ND	ND	ND	ND	<LOQ	ND	ND	ND	ND	ND	<LOQ	1.6673	ND	<LOQ
F-WW-V84-R	<LOQ	<LOQ	ND	ND	ND	ND	ND	ND	<LOQ	ND	0.5363	ND	ND	ND	<LOQ	3.0646	ND	<LOQ
MarneAmount010223	ND	<LOQ	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<LOQ	ND	<LOQ	ND
MarneAmount040123	ND	<LOQ	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<LOQ	ND	<LOQ	ND
MarneAmount060923	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<LOQ	ND	<LOQ	ND
MarneAmount110125	ND	<LOQ	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<LOQ	ND	<LOQ	ND
MarneAmount130923	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<LOQ	ND	<LOQ	ND
MarneAmount180123	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MarneAmount200923	ND	<LOQ	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<LOQ	ND	<LOQ	ND

MarneAmount210923	ND	<LOQ	ND	ND	ND	<LOQ	ND	<LOQ	ND	ND	ND	ND	ND	ND	<LOQ	ND	<LOQ	ND
MarneAmount220923	ND	<LOQ	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<LOQ	ND	<LOQ	ND
MarneAmount230823	ND	<LOQ	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<LOQ	ND	<LOQ	ND
MarneAmount250123	ND	ND	ND	ND	ND	<LOQ	ND	<LOQ	ND	<LOQ	ND	ND	ND	ND	<LOQ	ND	<LOQ	ND
MarneAmount290323	ND	<LOQ	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<LOQ	ND	<LOQ	ND
MarneAmount300323	ND	<LOQ	ND	ND	ND	<LOQ	ND	ND	ND	ND	ND	ND	ND	ND	<LOQ	ND	<LOQ	ND
MarneAmount300823	ND	<LOQ	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<LOQ	ND	<LOQ	ND
MarneAmount310323	ND	<LOQ	ND	ND	ND	<LOQ	ND	<LOQ	ND	ND	ND	ND	ND	ND	<LOQ	ND	<LOQ	ND
MarneAval010223	ND	<LOQ	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<LOQ	ND	<LOQ	ND
MarneAval040123	ND	<LOQ	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<LOQ	ND	<LOQ	ND
MarneAval060923	ND	<LOQ	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<LOQ	ND	<LOQ	ND
MarneAval110123	1.8394	7.4083	<LOQ	3.4761	62.2395	10.9275	ND	<LOQ	37.1772	ND	1.4111	6.7944	<LOQ	<LOQ	41.3834	ND	5.6228	7.5746
MarneAval130923	ND	<LOQ	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<LOQ	ND	<LOQ	ND
MarneAval180123	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MarneAval230823	ND	<LOQ	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<LOQ	ND	<LOQ	ND
MarneAval250123	ND	<LOQ	<LOQ	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<LOQ	ND	<LOQ	ND
MarneAval300823	ND	<LOQ	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<LOQ	ND	<LOQ	ND
SeineAmount010223	ND	<LOQ	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<LOQ	<LOQ	ND	<LOQ	ND
SeineAmount040123	1.9831	7.3738	<LOQ	1.8339	4.0841	11.2479	5.0024	<LOQ	4.6303	<LOQ	0.6066	6.3206	ND	1.3374	2.1845	<LOQ	4.7004	3.5951
SeineAmount060923	ND	<LOQ	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<LOQ	ND	<LOQ	ND
SeineAmount110123	ND	<LOQ	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<LOQ	ND	<LOQ	ND
SeineAmount130923	3.0977	8.3777	<LOQ	1.3322	25.0848	12.0345	5.4195	<LOQ	40.4045	<LOQ	<LOQ	6.3919	ND	<LOQ	<LOQ	ND	ND	2.8327
SeineAmount180123	1.5282	8.1759	<LOQ	1.5140	40.7782	ND	ND	ND	3.1650	<LOQ	<LOQ	6.1434	ND	0.7558	69.6497	<LOQ	ND	2.1219
SeineAmount230823	119.0977	7.6110	<LOQ	2.9717	18.3751	15.2142	7.6825	6.8120	38.2576	<LOQ	2.5804	7.4957	<LOQ	3.0316	5.9993	4.4533	2.8301	6.7267
SeineAmount250123	ND	<LOQ	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<LOQ	ND	<LOQ	ND
SeineAmount300823	ND	<LOQ	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<LOQ	<LOQ	<LOQ	ND

SeineAval010223	ND	<LOQ	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<LOQ	ND	<LOQ	<LOQ
SeineAval040123	<LOQ	<LOQ	ND	ND	ND	ND	ND	ND	ND	ND	<LOQ	ND	ND	ND	<LOQ	ND	<LOQ	<LOQ
SeineAval060923	ND	<LOQ	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.8367	ND	<LOQ	<LOQ
SeineAval110123	<LOQ	<LOQ	<LOQ	<LOQ	ND	<LOQ	ND	<LOQ	ND	ND	<LOQ	<LOQ	ND	ND	<LOQ	ND	<LOQ	<LOQ
SeineAval130923	ND	<LOQ	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.5450	ND	<LOQ	ND
SeineAval180123	ND	<LOQ	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<LOQ	ND	<LOQ	ND
SeineAval230823	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<LOQ	ND	<LOQ	ND
SeineAval250123	ND	<LOQ	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<LOQ	ND	<LOQ	ND
SeineAval300823	87.5741	7.8316	<LOQ	2.7127	15.6826	34.2839	29.4321	23.2856	33.8861	33.4578	4.6416	7.7961	<LOQ	1.4331	2.8197	ND	12.5657	6.0443
SortieSTV060923	<LOQ	<LOQ	ND	<LOQ	ND	<LOQ	ND	<LOQ	<LOQ	ND	ND	ND	ND	ND	8.9733	ND	<LOQ	<LOQ
SortieSTV090323	1.3347	<LOQ	<LOQ	<LOQ	ND	<LOQ	ND	<LOQ	<LOQ	ND	ND	ND	ND	ND	11.8056	ND	<LOQ	<LOQ
SortieSTV160323	1.0098	<LOQ	<LOQ	ND	ND	<LOQ	ND	<LOQ	<LOQ	ND	ND	ND	ND	ND	16.5496	ND	<LOQ	<LOQ
SortieSTV210923	<LOQ	<LOQ	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<LOQ	ND
SortieSTV220923	<LOQ	<LOQ	ND	<LOQ	ND	ND	ND	<LOQ	<LOQ	ND	ND	ND	ND	ND	<LOQ	ND	<LOQ	<LOQ
SortieSTV230923	1.1933	<LOQ	ND	<LOQ	ND	<LOQ	ND	<LOQ	<LOQ	ND	ND	ND	ND	ND	6.6485	ND	<LOQ	<LOQ
SortieSTV290323	1.1018	<LOQ	ND	ND	ND	<LOQ	ND	<LOQ	<LOQ	ND	ND	ND	ND	ND	7.9413	ND	<LOQ	<LOQ
SortieSTV300823	<LOQ	<LOQ	ND	ND	ND	<LOQ	ND	<LOQ	<LOQ	ND	ND	ND	ND	ND	ND	ND	<LOQ	<LOQ
BESÓS 1	ND	<LOQ	<LOQ	ND	ND	ND	ND	<LOQ	ND	<LOQ	<LOQ	ND	<LOQ	ND	1.3830	0.7816	<LOQ	<LOQ
BESÓS 2	ND	<LOQ	ND	ND	ND	<LOQ	ND	<LOQ	ND	<LOQ	<LOQ	ND	ND	ND	<LOQ	ND	<LOQ	<LOQ
BOGATELL	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<LOQ	ND	ND	1.1204	ND	1.4150	ND
F-SW-L32-S	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<LOQ	ND	ND	<LOQ	0.9567	ND
F-SW-RXX-S	ND	ND	ND	ND	ND	ND	ND	ND	0.9525	ND	ND	7.6985	<LOQ	ND	ND	<LOQ	0.5015	ND
F-SW-V64-S	ND	<LOQ	<LOQ	ND	ND	ND	ND	ND	<LOQ	ND	ND	ND	<LOQ	ND	<LOQ	2.8133	<LOQ	<LOQ
F-SW-V84-S	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<LOQ	ND	<LOQ	<LOQ	2.1337	ND
F-WW-L32-S	ND	<LOQ	<LOQ	ND	ND	ND	ND	ND	<LOQ	ND	ND	ND	<LOQ	ND	<LOQ	2.5045	ND	<LOQ
F-WW-RXX-S	ND	ND	<LOQ	ND	ND	ND	ND	ND	ND	<LOQ	ND	ND	<LOQ	ND	<LOQ	1.8380	0.7023	<LOQ

F-WW-V64-S	<LOQ	<LOQ	ND	<LOQ	ND	ND	ND	ND	<LOQ	ND	ND	ND	<LOQ	ND	<LOQ	1.6700	<LOQ	<LOQ
F-WW-V84-S	ND	<LOQ	0.7495	ND	ND	ND	ND	ND	<LOQ	ND	ND	ND	<LOQ	ND	<LOQ	3.5967	<LOQ	<LOQ
FÒRUM	ND	ND	ND	ND	ND	<LOQ	ND	ND	ND	ND	ND	ND	ND	ND	1.0830	ND	1.7420	ND
LLEVANT	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<LOQ	<LOQ	1.4108	ND
LLOBREGAT 1	<LOQ	<LOQ	ND	ND	<LOQ	ND	ND	ND	ND	ND	ND	ND	0.7907	ND	1.4074	<LOQ	<LOQ	ND
MAR BELLA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<LOQ	ND	ND	0.6082	ND	1.1571	ND
NOVA ICÀRIA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<LOQ	ND	ND	0.9977	ND	2.0740	ND
NOVA MAR BELLA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<LOQ	ND	ND	0.7963	ND	1.2668	ND
SOMORROSTRO	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.3310	ND	0.9550	ND
SANT MIQUEL	ND	ND	ND	<LOQ	ND	ND	ND	ND	ND	ND	ND	ND	<LOQ	ND	0.9255	ND	1.2151	ND
EntreeSTV060923	<LOQ	<LOQ	ND	ND	ND	<LOQ	ND	<LOQ	ND	ND	ND	ND	ND	ND	1.4702	ND	ND	<LOQ
EntreeSTV090323	1.0200	<LOQ	ND	<LOQ	ND	<LOQ	ND	<LOQ	ND	ND	ND	ND	ND	ND	0.8949	ND	ND	<LOQ
EntreeSTV160323	<LOQ	<LOQ	ND	<LOQ	ND	<LOQ	ND	<LOQ	<LOQ	ND	ND	ND	ND	ND	1.1683	ND	ND	<LOQ
EntreeSTV200923	<LOQ	<LOQ	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.4019	ND	ND	<LOQ
EntreeSTV210923	<LOQ	<LOQ	ND	ND	ND	<LOQ	ND	<LOQ	ND	ND	ND	ND	ND	ND	1.6924	<LOQ	ND	<LOQ
EntreeSTV220923	<LOQ	<LOQ	ND	ND	ND	<LOQ	ND	<LOQ	ND	ND	ND	ND	ND	ND	1.0601	ND	ND	<LOQ
EntreeSTV230223	<LOQ	<LOQ	ND	<LOQ	ND	<LOQ	ND	<LOQ	ND	ND	ND	ND	ND	ND	1.1512	ND	ND	<LOQ
EntreeSTV300823	ND	<LOQ	ND	ND	ND	<LOQ	ND	<LOQ	ND	ND	ND	ND	ND	ND	0.8607	ND	ND	<LOQ
EntreeTroubalt170223	<LOQ	<LOQ	ND	<LOQ	ND	<LOQ	ND	<LOQ	<LOQ	ND	ND	ND	ND	ND	2.3987	<LOQ	ND	<LOQ
SLE241	<LOQ	<LOQ	ND	ND	ND	<LOQ	ND	<LOQ	ND	ND	ND	12.1714	ND	ND	1.6693	ND	<LOQ	ND
SLE242	<LOQ	<LOQ	ND	ND	ND	<LOQ	ND	<LOQ	ND	ND	ND	12.0325	ND	ND	<LOQ	ND	<LOQ	<LOQ
SLE243	1.1965	<LOQ	ND	ND	ND	<LOQ	ND	<LOQ	ND	ND	ND	12.0341	ND	ND	<LOQ	<LOQ	<LOQ	<LOQ
SLE244	1.3469	<LOQ	ND	ND	ND	<LOQ	ND	<LOQ	ND	ND	ND	12.0746	ND	ND	<LOQ	<LOQ	<LOQ	<LOQ
SLE245	<LOQ	<LOQ	ND	ND	ND	<LOQ	ND	<LOQ	ND	ND	ND	12.1352	ND	ND	<LOQ	<LOQ	<LOQ	<LOQ
SLS241	ND	ND	ND	ND	ND	ND	ND	ND	<LOQ	ND	ND	6.1198	ND	ND	<LOQ	ND	<LOQ	ND
SLS242	ND	ND	ND	ND	ND	ND	ND	ND	<LOQ	ND	ND	6.1349	ND	ND	<LOQ	ND	<LOQ	ND

SLS243	ND	<LOQ	ND	ND	ND	ND	ND	ND	<LOQ	ND	ND	6.1680	ND	ND	<LOQ	ND	<LOQ	ND
SLS244	ND	ND	ND	ND	ND	ND	ND	ND	<LOQ	ND	ND	6.2475	ND	ND	<LOQ	ND	<LOQ	ND
SLS245	ND	<LOQ	ND	ND	ND	ND	ND	<LOQ	<LOQ	ND	ND	6.0857	ND	ND	<LOQ	ND	<LOQ	ND
SortieSTV200923	<LOQ	<LOQ	ND	<LOQ	ND	ND	ND	ND	<LOQ	ND	ND	12.2810	ND	ND	<LOQ	<LOQ	<LOQ	ND
SortieSTV300323	1.0126	<LOQ	ND	<LOQ	ND	<LOQ	ND	<LOQ	ND	ND	ND	12.3129	ND	ND	1.5269	<LOQ	ND	<LOQ
SortieSTV310323	1.2818	<LOQ	ND	<LOQ	ND	<LOQ	ND	<LOQ	0.7624	ND	ND	12.2596	ND	ND	<LOQ	<LOQ	ND	<LOQ
SortieTroubalt170223	1.0552	<LOQ	ND	<LOQ	ND	ND	ND	<LOQ	<LOQ	ND	ND	ND	ND	ND	<LOQ	ND	<LOQ	<LOQ

Table 3-2. Calculated limits of detection (LOD) and quantification (LOQ) for each antibiotic in each water matrix.

Antibiotic	**LOD** [µg/L]				**LOQ** [µg/L]			
	Seawater	**River water**	**WWTP Influent**	**WWTP Effluent**	**Seawater**	**River water**	**WWTP Influent**	**WWTP Effluent**
Azithromycin	0.0011	0.0023	0.0090	0.0045	0.0045	0.0090	0.0360	0.0180
Clarithromycin	0.0009	0.0018	0.0070	0.0035	0.0035	0.0070	0.0280	0.0140
Erythromycin	0.0007	0.0014	0.0057	0.0029	0.0029	0.0057	0.0229	0.0115
Roxithromycin	0.0011	0.0022	0.0087	0.0043	0.0043	0.0087	0.0347	0.0173
Tilmicosin	0.0013	0.0026	0.0105	0.0052	0.0052	0.0105	0.0419	0.0209
Ciprofloxacin	0.0017	0.0034	0.0135	0.0068	0.0068	0.0135	0.0541	0.0271
Norfloxacin	0.0051	0.0103	0.0410	0.0205	0.0205	0.0410	0.1640	0.0820
Ofloxacin	0.0025	0.0049	0.0197	0.0098	0.0098	0.0197	0.0787	0.0393
Clindamycin	0.0004	0.0009	0.0035	0.0017	0.0017	0.0035	0.0139	0.0069
Lincomycin	0.0011	0.0021	0.0085	0.0043	0.0043	0.0085	0.0341	0.0171
Dimetridazole	0.0003	0.0005	0.0021	0.0010	0.0010	0.0021	0.0083	0.0041
Metronidazole	0.0009	0.0018	0.0072	0.0036	0.0036	0.0072	0.0288	0.0144
Hydroxymetronidazole	0.0059	0.0118	0.0473	0.0237	0.0237	0.0473	0.1893	0.0947
Sulfamethazine	0.0006	0.0013	0.0050	0.0025	0.0025	0.0050	0.0200	0.0100
Sulfamethoxazole	0.0008	0.0016	0.0063	0.0032	0.0032	0.0063	0.0253	0.0127
Sulfapyridine	0.0004	0.0009	0.0035	0.0018	0.0018	0.0035	0.0141	0.0071
Tetracycline	0.0026	0.0051	0.0205	0.0103	0.0103	0.0205	0.0821	0.0411
Trimethoprim	0.0008	0.0016	0.0065	0.0032	0.0032	0.0065	0.0259	0.0129

APPENDIX 4: ENVIRONMENTAL INFORMATION ON THE ANALYZED ANTIBIOTICS

Table 4-1. Summary of predicted no-effect concentrations (PNECs) and octanol-water partition coefficients for the selected antibiotics.

Antibiotic	PNEC [µg/L]		Log(K_{ow}) [97]
	Seawater	River water	
Azithromycin	0.0019	0.0190	4.0
Clarithromycin	0.0130	0.1200	3.2
Erythromycin	0.0500	0.3000	2.7
Roxithromycin	0.1000	1.0000	3.1
Tilmicosin	0.1000	1.0000	3.6
Ciprofloxacin	0.0064	0.0640	-1.1
Norfloxacin	0.0500	0.5000	-1.0
Ofloxacin	0.0500	0.5000	-0.4
Clindamycin	0.0044	0.0440	2.2
Lincomycin	0.2000	2.0000	0.2
Dimetridazole	2.9500	29.5000	0.1
Metronidazole	0.0130	0.1300	0.0
Hydroxymetronidazole	3.2800	32.8000	-1.3
Sulfamethazine	3.0000	30.0000	0.3
Sulfamethoxazole	0.0600	0.6000	0.9
Sulfapyridine	0.0460	0.4600	0.0
Tetracycline	0.0100	0.1000	-2.0
Trimethoprim	10.0000	0.5000	0.9

Table 4-2. Detailed Hazard Quotient (HQ) of the analyzed antibiotics in each water sample, with color coding indicating risk levels (green, yellow, orange, red – from lowest to highest).

SAMPLES	Hazard Quotient																	
	Azithromycin	*Clarithromycin*	*Erythromycin*	*Roxithromycin*	*Tilmicosin*	*Ciprofloxacin*	*Norfloxacin*	*Ofloxacin*	*Clindamycin*	*Lincomycin*	*Dimetridazole*	*Metronidazole*	*Hydroxymetronidazole*	*Sulfamethazine*	*Sulfamethoxazole*	*Sulfapyridine*	*Tetracyclin*	*Trimethoprim*
F-SW-L32-R	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.21	0.01
F-SW-RXX-R	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.21	0.00
F-SW-V64-R	0.00	0.06	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.21	0.01
F-SW-V84-R	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.01	2.30	0.21	0.01
F-WW-L32-R	0.95	0.12	0.04	0.02	0.00	0.00	0.00	0.00	0.16	0.00	0.00	136.39	0.00	0.00	0.02	1.35	0.00	0.03
F-WW-RXX-R	0.95	0.12	2.24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.81	0.41	0.00
F-WW-V64-R	0.95	0.12	0.04	0.02	0.00	0.00	0.00	0.00	0.16	0.00	0.00	0.00	0.00	0.00	0.02	3.62	0.00	0.03
F-WW-V84-R	0.95	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.16	0.00	0.02	0.00	0.00	0.00	0.02	6.66	0.00	0.03
MarneAmount010223	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.21	0.00
MarneAmount040123	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.21	0.00
MarneAmount060923	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.21	0.00
MarneAmount110125	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.21	0.00
MarneAmount130923	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.21	0.00
MarneAmount180123	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
MarneAmount200923	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.21	0.00
MarneAmount210923	0.00	0.06	0.00	0.00	0.00	0.21	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.21	0.00
MarneAmount220923	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.21	0.00
MarneAmount230823	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.21	0.00

MarneAmount250123	0.00	0.00	0.00	0.00	0.00	0.21	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.21	0.00
MarneAmount290323	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.21	0.00
MarneAmount300323	0.00	0.06	0.00	0.00	0.00	0.21	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.21	0.00
MarneAmount300823	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.21	0.00
MarneAmount310323	0.00	0.06	0.00	0.00	0.00	0.21	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.21	0.00
MarneAval010223	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.21	0.00
MarneAval040123	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.21	0.00
MarneAval060923	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.21	0.00
MarneAval110123	96.81	61.74	0.02	3.48	62.24	170.74	0.00	0.04	844.94	0.00	0.05	52.26	0.00	0.00	68.97	0.00	56.23	15.15
MarneAval130923	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.21	0.00
MarneAval180123	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
MarneAval230823	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.21	0.00
MarneAval250123	0.00	0.06	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.21	0.00
MarneAval300823	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.21	0.00
SeineAmount010223	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.21	0.00
SeineAmount040123	104.37	61.45	0.02	1.83	4.08	175.75	10.00	0.04	105.23	0.00	0.02	48.62	0.00	0.04	3.64	0.01	47.00	7.19
SeineAmount060923	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.21	0.00
SeineAmount110123	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.21	0.00
SeineAmount130923	163.04	69.81	0.02	1.33	25.08	188.04	10.84	0.04	918.28	0.00	0.00	49.17	0.00	0.00	0.01	0.00	0.00	5.67
SeineAmount180123	80.43	68.13	0.02	1.51	40.78	0.00	0.00	0.00	71.93	0.00	0.00	47.26	0.00	0.03	116.08	0.01	0.00	4.24
SeineAmount230823	6268.30	63.42	0.02	2.97	18.38	237.72	15.37	13.62	869.49	0.00	0.09	57.66	0.00	0.10	10.00	9.68	28.30	13.45
SeineAmount250123	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.21	0.00
SeineAmount300823	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.21	0.00
SeineAval010223	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.21	0.01
SeineAval040123	0.47	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.21	0.01
SeineAval060923	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.39	0.00	0.21	0.01

SeineAval110123	0.47	0.06	0.02	0.01	0.00	0.21	0.00	0.04	0.00	0.00	0.00	0.06	0.00	0.00	0.01	0.00	0.21	0.01
SeineAval130923	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.91	0.00	0.21	0.00
SeineAval180123	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.21	0.00
SeineAval230823	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.21	0.00
SeineAval250123	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.21	0.00
SeineAval300823	4609.16	65.26	0.02	2.71	15.68	535.69	58.86	46.57	770.14	16.73	0.16	59.97	0.00	0.05	4.70	0.00	125.66	12.09
SortieSTV060923	0.95	0.12	0.00	0.02	0.00	0.42	0.00	0.08	0.16	0.00	0.00	0.00	0.00	0.00	14.96	0.00	0.41	0.03
SortieSTV090323	70.25	0.12	0.04	0.02	0.00	0.42	0.00	0.08	0.16	0.00	0.00	0.00	0.00	0.00	19.68	0.00	0.41	0.03
SortieSTV160323	53.15	0.12	0.04	0.00	0.00	0.42	0.00	0.08	0.16	0.00	0.00	0.00	0.00	0.00	27.58	0.00	0.41	0.03
SortieSTV210923	0.95	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.41	0.00
SortieSTV220923	0.95	0.12	0.00	0.02	0.00	0.00	0.00	0.08	0.16	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.41	0.03
SortieSTV230923	62.81	0.12	0.00	0.02	0.00	0.42	0.00	0.08	0.16	0.00	0.00	0.00	0.00	0.00	11.08	0.00	0.41	0.03
SortieSTV290323	57.99	0.12	0.00	0.00	0.00	0.42	0.00	0.08	0.16	0.00	0.00	0.00	0.00	0.00	13.24	0.00	0.41	0.03
SortieSTV300823	0.95	0.12	0.00	0.00	0.00	0.42	0.00	0.08	0.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.41	0.03
BESÓS 1	0.00	0.06	0.02	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	2.31	1.70	0.21	0.01
BESÓS 2	0.00	0.06	0.00	0.00	0.00	0.21	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.21	0.01
BOGATELL	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.28	0.00	0.00	18.67	0.00	141.50	0.00
F-SW-L32-S	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	9.57	0.00
F-SW-RXX-S	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	21.65	0.00	0.00	59.22	0.00	0.00	0.00	0.01	5.02	0.00
F-SW-V64-S	0.00	0.06	0.02	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.01	6.12	0.21	0.01
F-SW-V84-S	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	21.34	0.00
F-WW-L32-S	0.00	0.12	0.04	0.00	0.00	0.00	0.00	0.00	0.16	0.00	0.00	0.00	0.00	0.00	0.02	5.44	0.00	0.03
F-WW-RXX-S	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.02	4.00	7.02	0.03
F-WW-V64-S	0.95	0.12	0.00	0.02	0.00	0.00	0.00	0.00	0.16	0.00	0.00	0.00	0.00	0.00	0.02	3.63	0.41	0.03
F-WW-V84-S	0.00	0.12	2.50	0.00	0.00	0.00	0.00	0.00	0.16	0.00	0.00	0.00	0.00	0.00	0.02	7.82	0.41	0.03
FÒRUM	0.00	0.00	0.00	0.00	0.00	1.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	18.05	0.00	174.20	0.00

LLEVANT	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.04	141.08	0.00
LLOBREGAT 1	0.24	0.03	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	2.35	0.00	0.10	0.00
MAR BELLA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.28	0.00	0.00	10.14	0.00	115.71	0.00
NOVA ICÀRIA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.28	0.00	0.00	16.63	0.00	207.40	0.00
NOVA MAR BELLA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.28	0.00	0.00	13.27	0.00	126.68	0.00
SOMORROSTRO	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	22.18	0.00	95.50	0.00
SANT MIQUEL	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	15.42	0.00	121.51	0.00
EntreeSTV060923	1.89	0.23	0.00	0.00	0.00	0.85	0.00	0.16	0.00	0.00	0.00	0.00	0.00	0.00	2.45	0.00	0.00	0.05
EntreeSTV090323	53.68	0.23	0.00	0.03	0.00	0.85	0.00	0.16	0.00	0.00	0.00	0.00	0.00	0.00	1.49	0.00	0.00	0.05
EntreeSTV160323	1.89	0.23	0.00	0.03	0.00	0.85	0.00	0.16	0.32	0.00	0.00	0.00	0.00	0.00	1.95	0.00	0.00	0.05
EntreeSTV200923	1.89	0.23	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.34	0.00	0.00	0.05
EntreeSTV210923	1.89	0.23	0.00	0.00	0.00	0.85	0.00	0.16	0.00	0.00	0.00	0.00	0.00	0.00	2.82	0.03	0.00	0.05
EntreeSTV220923	1.89	0.23	0.00	0.00	0.00	0.85	0.00	0.16	0.00	0.00	0.00	0.00	0.00	0.00	1.77	0.00	0.00	0.05
EntreeSTV230223	1.89	0.23	0.00	0.03	0.00	0.85	0.00	0.16	0.00	0.00	0.00	0.00	0.00	0.00	1.92	0.00	0.00	0.05
EntreeSTV300823	0.00	0.23	0.00	0.00	0.00	0.85	0.00	0.16	0.00	0.00	0.00	0.00	0.00	0.00	1.43	0.00	0.00	0.05
EntreeTroubalt170223	1.89	0.23	0.00	0.03	0.00	0.85	0.00	0.16	0.32	0.00	0.00	0.00	0.00	0.00	4.00	0.03	0.00	0.05
SLE241	1.89	0.23	0.00	0.00	0.00	0.85	0.00	0.16	0.00	0.00	0.00	93.63	0.00	0.00	2.78	0.00	0.82	0.00
SLE242	1.89	0.23	0.00	0.00	0.00	0.85	0.00	0.16	0.00	0.00	0.00	92.56	0.00	0.00	0.04	0.00	0.82	0.05
SLE243	62.98	0.23	0.00	0.00	0.00	0.85	0.00	0.16	0.00	0.00	0.00	92.57	0.00	0.00	0.04	0.03	0.82	0.05
SLE244	70.89	0.23	0.00	0.00	0.00	0.85	0.00	0.16	0.00	0.00	0.00	92.88	0.00	0.00	0.04	0.03	0.82	0.05
SLE245	1.89	0.23	0.00	0.00	0.00	0.85	0.00	0.16	0.00	0.00	0.00	93.35	0.00	0.00	0.04	0.03	0.82	0.05
SLS241	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.00	47.08	0.00	0.00	0.01	0.00	0.21	0.00
SLS242	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.00	47.19	0.00	0.00	0.01	0.00	0.21	0.00
SLS243	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.00	47.45	0.00	0.00	0.01	0.00	0.21	0.00
SLS244	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.00	48.06	0.00	0.00	0.01	0.00	0.21	0.00
SLS245	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.04	0.08	0.00	0.00	46.81	0.00	0.00	0.01	0.00	0.21	0.00

SortieSTV200923	0.95	0.12	0.00	0.02	0.00	0.00	0.00	0.00	0.16	0.00	0.00	94.47	0.00	0.00	0.02	0.02	0.41	0.00
SortieSTV300323	53.29	0.12	0.00	0.02	0.00	0.42	0.00	0.08	0.00	0.00	0.00	94.71	0.00	0.00	2.54	0.02	0.00	0.03
SortieSTV310323	67.46	0.12	0.00	0.02	0.00	0.42	0.00	0.08	17.33	0.00	0.00	94.30	0.00	0.00	0.02	0.02	0.00	0.03
SortieTroubalt170223	55.54	0.12	0.00	0.02	0.00	0.00	0.00	0.08	0.16	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.41	0.03