water

MDPI

Article

Long-Term Study of Antibiotic Presence in Ebro River Basin (Spain): Identification of the Emission Sources

Samuel Moles [1,*], Sebastiano Gozzo [1], María P. Ormad [2], Rosa Mosteo [2], Jairo Gómez [3], Francisco Laborda [4] and Joanna Szpunar [1]

[1] Institute of Analytical Sciences and Physico-Chemistry for Environment and Materials (IPREM), Centre National de la Recherche Scientifique (CNRS), CEDEX 9, 64053 Pau, France; sebastiano.gozzo@univ-pau.fr (S.G.); joanna.szpunar@univ-pau.fr (J.S.)
[2] Water and Environmental Health Research Group, c/María de Luna 3, 50018 Zaragoza, Spain; mpormad@unizar.es (M.P.O.); mosteo@unizar.es (R.M.)
[3] Navarra de Infraestructuras Locales SA, av. Barañain 22, 31008 Pamplona, Spain; jgomez@nilsa.com
[4] Analytical Spectroscopy and Sensors Group, Science Faculty, Environmental Science Institute, University of Zaragoza, 50009 Zaragoza, Spain; flaborda@unizar.es
* Correspondence: sma@unizar.es

Abstract: Water monitoring is key to determining the presence of potentially hazardous substances related to urban activities and intensive farming. This research aimed to perform a long-term (four years) quantitative monitoring of selected antibiotics (azithromycin, enrofloxacin, trimethoprim and sulfadiazine) both in rivers and wastewaters belonging to the Ebro River basin (North of Spain). The target antibiotics were chosen on the basis of a preliminary multispecies screening. The analysis of the antibiotics was carried out by LC-MS/MS on wastewater-treatment plant (WWTP) effluent, effluents of a slaughterhouse and hospital, rivers downstream and upstream of these WWTPs, and rivers close to extensive farming areas. The ANOVA test was performed to study the significant differences between the points exposed to concrete emission sources and antibiotic concentration. The monitoring, carried out from 2018 to 2020, has been essential to illustrating the presence of the most abundant antibiotics that were detected in the Ebro River basin. Enrofloxacin has appeared in river waters in significant concentrations, especially near intensive farming, meanwhile azithromycin has been frequently detected in wastewaters.

Keywords: antibiotics; wastewater-treatment plants; Ebro River basin; hospital effluent; slaughterhouse effluent

Citation: Moles, S.; Gozzo, S.; Ormad, M.P.; Mosteo, R.; Gómez, J.; Laborda, F.; Szpunar, J. Long-Term Study of Antibiotic Presence in Ebro River Basin (Spain): Identification of the Emission Sources. *Water* **2022**, *14*, 1033. https://doi.org/10.3390/w14071033

Academic Editor: Chengyun Zhou

Received: 27 January 2022
Accepted: 23 March 2022
Published: 24 March 2022

1. Introduction

The presence of emerging pollutants such as antibiotics and their metabolites have been demonstrated in natural waters in recent years. It is a consequence of the improvement of the analytical methods, which allow the quantification of these substances at concentrations down to the ng/L level by using tandem mass spectrometry (HPLC/MS/MS) [1–4]. These compounds enter the water cycle when they are partially metabolized and excreted by humans and animals. According to the bibliography, more than a half of administered antibiotics, as well as their metabolites, are introduced into urban wastewater [5–7].

It is widely known that conventional treatments applied in WWTPs are not planned to eliminate antibiotics; however, some processes involved in the treatment, such as biological and adsorption processes, tend to significantly reduce their concentration [8]. Conventional treatments usually do not achieve removal performances superior to 50–80%, depending of the physicochemical characteristics of each antibiotic and the type of treatment [8–10]. As a result, antibiotics and their metabolites are inevitably emitted into receiving rivers. WWTP effluents are an important emission source to the environment [11,12]. On the other hand, it should be mentioned that not only WWTPs are related to antibiotic pollution in waters. In fact, according to the European Centre for Disease Prevention and Control, the European Food Safety Authority, and the European Medicines Agency, the majority of antibiotics in Europe are consumed by animals [13], despite the fact that the preventive use of antibiotics in groups of animals is not allowed in the EC Regulation N° 2019/6 [14]. The environmental impact of antibiotics is expected to be especially acute in areas of farming and indeed, intensive farming represents one of the main sources of antibiotic pollution and the spread of gene resistance, since animals contribute to the dissemination of antibiotic-resistant genes (ARGs) in surface waters through the stool excretion during the free grazing [7]. Thus, it is very important to determine the real emission sources of antibiotics in a specific area.

Antibiotic impact on the aquatic environment has been widely discussed during the last decade [11,15–18]. These antibiotics are found in wastewater surface waters, plants and animals [19–22], confirming their introduction and persistence in the environment and ecosystem.

Nevertheless, the most important issue related to antibiotics is the development of antibiotic resistance, which occurs when bacteria and other microorganisms evolve and are no longer sensitive to medicines, resulting in infections that are hard to treat and increasing death risk [11,23,24]. This problem is mainly due to the misuse of antibiotics, which are excessively applied for human and veterinary treatments [25–28]. Antibiotic resistance is a significant public-health threat nowadays, since the coronavirus disease (COVID-19) has additionally contributed to the use of antibiotics and their subsequent emission into surface waters [29,30].

Despite the introduction of the Spanish National Plan against Antibiotic Resistance in 2014, which contributed to the reduction of 48% in the overall consumption of antimicrobial agents for veterinary use from 2014 to 2018 [29], the presence of antibiotics continues to be reported in surface waters, groundwater, and treated and untreated waters [8,16,31–33].

The occurrence of a wide variety of antibiotics has been reported in the North of Spain for many years [32,33]. However, little research has been carried out for systematically monitoring the most frequently detected antibiotics in this specific area. The main objective of this research was to establish a comprehensive long-term study of antibiotic presence in surface waters and wastewaters, determining their emission sources, seasonal behavior, and comparing with other reports.

This paper examines the concentration of enrofloxacin, azithromycin, sulfadiazine, and trimethoprim in 17 rivers, which are located near urban areas and intensive farming, wastewaters including the effluents and affluent of three WWTPs, and also hospital and slaughterhouses effluents. Moreover, the results are compared with other monitoring programs carried out in Ebro basin river and Europe.

2. Materials and Methods

2.1. Study Area

The Ebro River basin is located in the northeast of Spain (Figure 1). The extension is 85,000 km^2 flowing out into the Mediterranean Sea, in the Province of Tarragona. In the Iberian peninsula, the Ebro ranks second in length after the Tajo River and second in discharge volume and drainage basin after the Duero River. It is the longest river entirely within Spain. The importance of studying this basin lies in the fact that it encompasses more than twenty urban areas, including large areas such as Pamplona, Zaragoza and Logroño. Moreover, one of the main economic activities of most of these areas is animal farming.

The area involved in this study includes 20 surface-water-sampling points corresponding to 17 rivers from the Ebro River basin (Spain), which are listed in Table 1 and shown in Figure 1. The selection criteria for surface-water-sampling points were: (i) their proximity to poultry- and pig-intensive farms (Figures 2 and 3) and their selection was carried out in collaboration with the Ebro Hydrographic Confederation; (ii) their proximity to WWTPs, taking a sample upstream from the WWTP discharge and another one downstream.

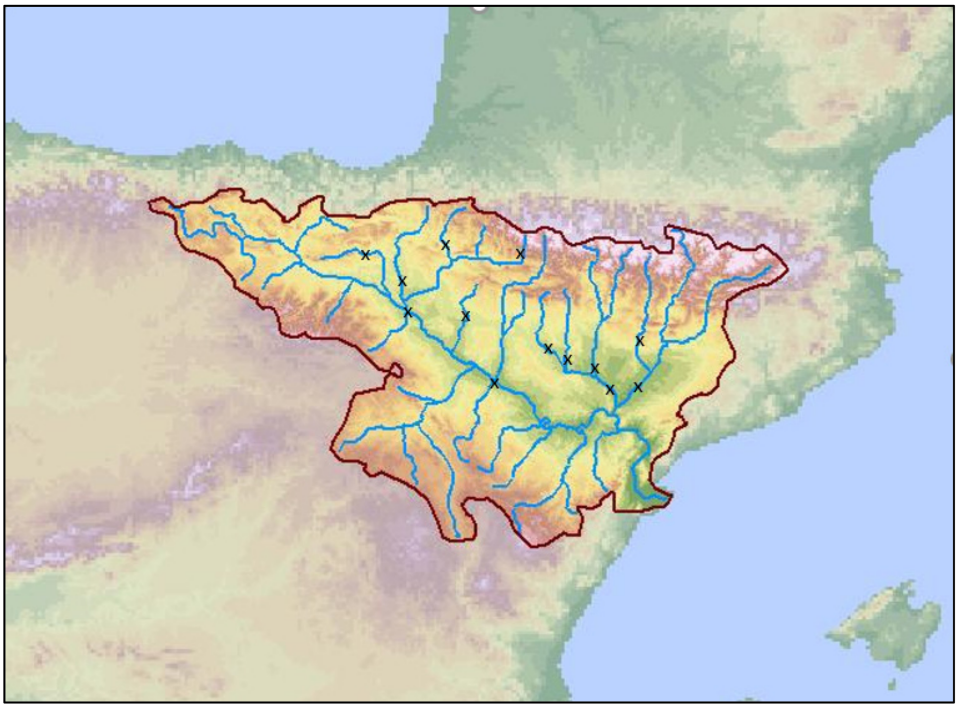


Figure 1. Surface-water-sampling points in the Ebro River basin (North of Spain).

Table 1. List of surface-water-sampling points: locations and pressures [34].

River	Location	Sampling Point	Sub-Basin	Livestock Pressure	WWTP Pressure
Segre River	Torres de Segre	01_ASE	Segre	High	Null
Noguera Ribagorzana River	Corbins	02_ASE	Segre	High	Null
Clamor Amarga River	Zaidín	03_ASE	Cinca	High	High
Cinca River	Fraga	04_ASE	Cinca	High	Null
Alcanadre River	Sariñena	05_ASE	Alcanadre	High	Null
Flumen River	Albalatillo	06_ASE	Alcanadre	High	Null
Gállego River	San Mateo de Gállego	07_ASE	Gallego	Low	Null
Arba de Ríquel River	Ejea de los Caballeros	08_ASE	Ebro	High	Low
Aragon Subordan River	Javierregay	09_ASE	Aragón	Low	Null
Aragon River	Caparroso	10_ASE	Aragón	High	Null
Irantzu River	Estella	11_ASE	Ega	Medium	Null
Arakil River	Irañeta	12_ASE	Arga	High	Null
Queiles River	Novallas	13_ASE	Queiles	High	High
Alhama River	Alfaro	14_ASE	Alhama	High	Null
Ega River	Estella	15_ASE	Ega	Low	Medium
Ega River	Downstream Estella	16_ASE	Ega	Low	High
Ega River	Upstream Pamplona	17_ASE	Arga	Null	Low
Arga River	Downstream Pamplona	18_ASE	Arga	Null	High
Ebro River	Upstream Tudela	19_ASE	Ebro	Low	Low
Ebro River	Downstream Tudela	20_ASE	Ebro	Low	Medium

ASE: assay-sampling Ebro River basin.

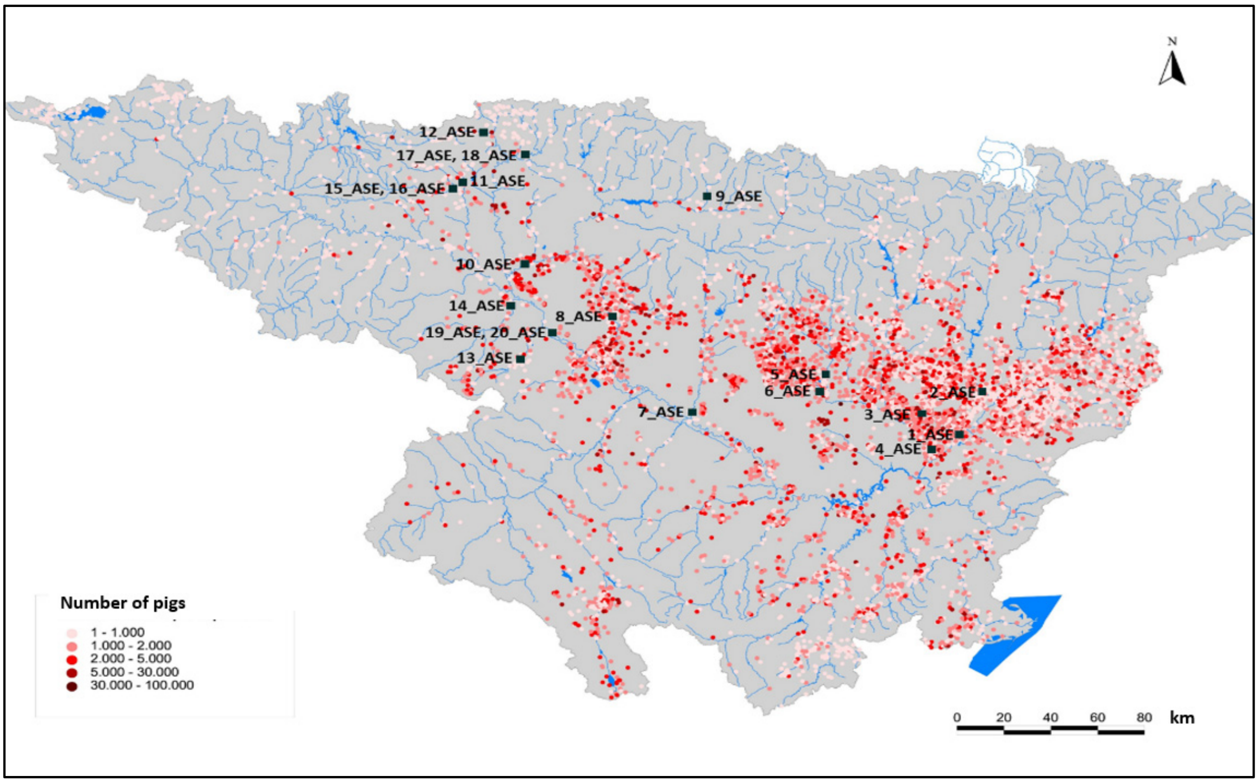


Figure 2. Distribution of number of pigs per farm in the Ebro River basin and sampling points (Source: Ebro Hydrographic Confederation, 2016).

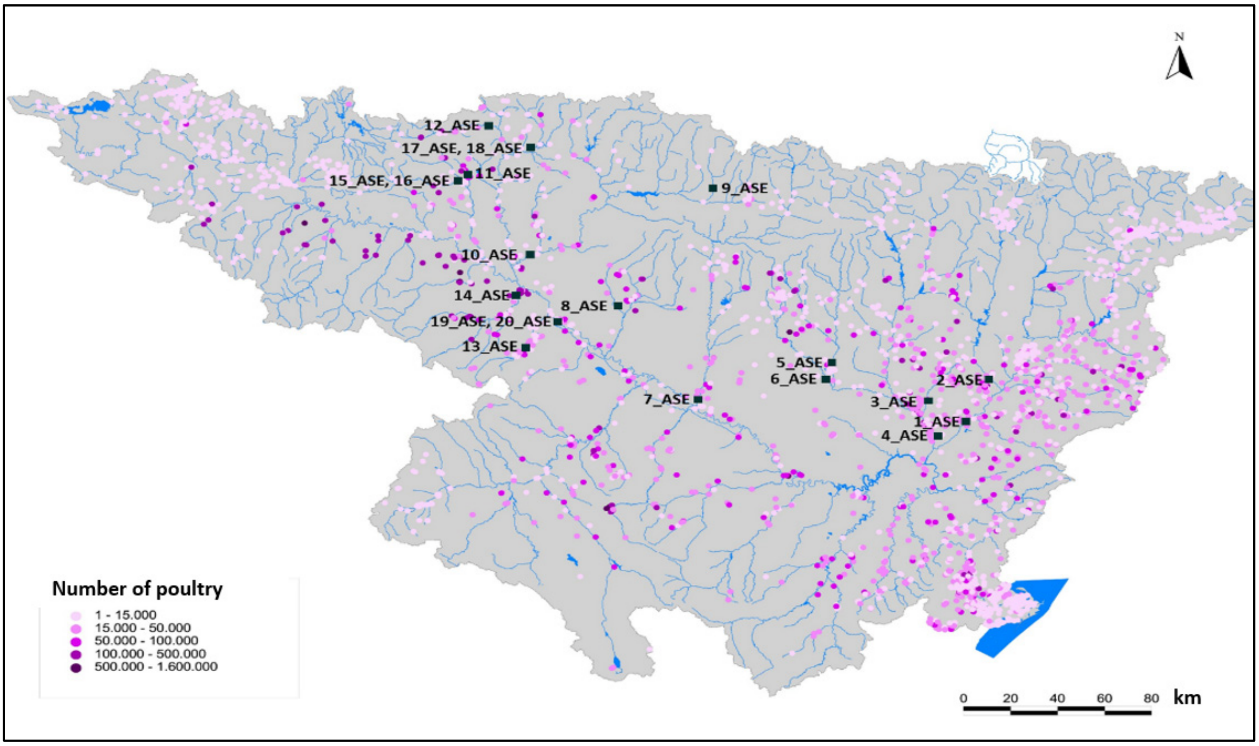


Figure 3. Distribution of number of poultry per farm in the Ebro River basin and sampling points (Source: Ebro Hydrographic Confederation, 2016).

Surface-water-sampling points were also characterized by the livestock and WWTP pressure associated with their location, which were determined by the Ebro Hydrographic Confederation, the organism that manages water quality in Ebro River basin. They characterized the rivers according to punctual and diffuse sources of pollution in four levels: null, low, medium and high [34].

Complementarily, this study monitored the affluent and effluent of three WWTPs, as well as one hospital and three slaughterhouse effluents twice a year over the period of 2018–2021. The characteristics of the studied WWTPs are given in Table 2. In summary, a total of 30 sampling points was examined. Out of these, 2/3 corresponded to surface waters and 1/3 to wastewaters.

Table 2. Main characteristics of studied WWTPs.

WWTP	Equivalent Inhabitants	Inlet Flow (m^3/day)
WWTP1	695.232	129.600
WWTP2	82.500	22.150
WWTP3	51.336	7.500

2.2. Antibiotic Selection

The first selection criterion was a revision of the literature, determining which antibiotics show the most significant sales and use in Spain. It should be mentioned that several previous studies have been carried out on the surface waters of the Ebro River basin related to monitoring selected emerging pollutants, such as microplastics [35] or pharmaceuticals [32,36], and sulfonamide residues [37]. Several authors have also studied the presence of pharmaceuticals in wastewater-treatment plants located in the Ebro River [32], including some antibiotics [32,36]. According to the most recent studies, trimethoprims, macrolides, sulfonamides and fluoroquinolones are four of the most detected antibiotic groups in Spanish and European rivers and wastewaters [11]. The literature reports the concentrations of antibiotics up to μg/L for: sulfonamides [32], trimethoprim [38,39] fluoroquinolones [26] and macrolides [32,36,40], which all represent a potentially significant risk for the environment. The European Medicines Agency (EMA) annually publishes a report on the sales and use of veterinary antibiotics within the framework of the European Surveillance Survey of the Consumption of Veterinary Antimicrobial Medicines (ESVAC). According to the last ESVAC report, sales of tetracycline, penicillin, and sulfonamides represented almost 70% of all antibiotics sold in Europe [41].

The first step to establish a target antibiotic for quantitative analysis is a qualitative screening, which was carried out in the spring of 2018. Its results were grouped by antibiotic families, due to the great variety of antibiotics detected. As revealed in Figure 4, fluoroquinolones were the most detected species with enrofloxacin present in 70% of the samples. The second group of antibiotics that was more frequently detected is the family of sulfonamides (present in 30% of the samples). Sulfadiazine was detected in more than 70% of the samples. Finally, trimethoprim and azithromycin were present in 60% and 55% of the samples, respectively. As a result of the screening data, sulfadiazine (sulfonamide), enrofloxacin (fluoroquinolone), trimethoprim (trimethoprim) and azithromycin (macrolide) were selected as target antibiotics for quantitative analysis. Table 3 shows the group and CAS numbers as well as physicochemical properties of the target antibiotics, (acid-dissociation constant (pKa), molecular weight and molecular structure).

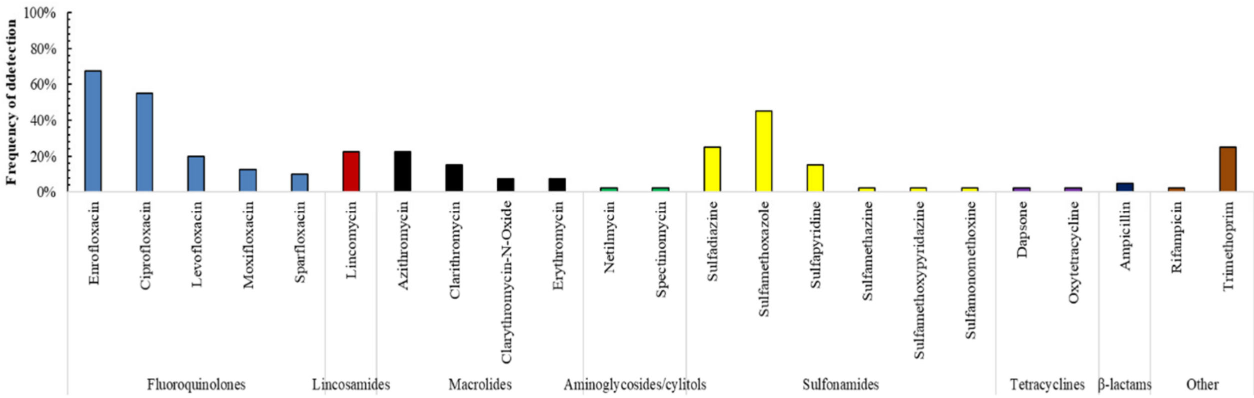
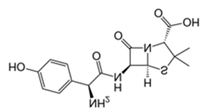
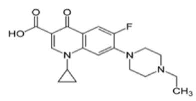
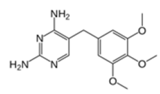
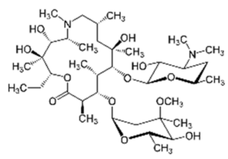


Figure 4. Frequency of occurrence of most detected antibiotics in the screening (2018).

Table 3. Antibiotics characteristics.

Group	Antibiotic	CAS	Molecular Weight (g/mol)	Molecular Structure
Sulfonamide	Sulfadiazine	26787-78-0	365.4	
Trimethoprim	Trimethoprim	93106-60-6	359.4	
Fluoroquinolone	Enrofloxacin	738-70-5	290.3	
Macrolide	Azithromycin	83905-01-5	749.0	

2.3. Sampling, Conditioning and Conservation Procedure

The main difficulties surrounding environmental aqueous samples are the lack of representativeness and repeatability of the matrix and the integrity of the sample. Consequently, in this research, samples were taken in spring and autumn for 4 years (2018–2021) and a storage procedure was developed to guarantee their integrity. According to the methodology USEPA1694 [42], 2 L of samples were taken in amber glass bottles to avoid possible UV degradation of the antibiotic. Moreover, bottles were filled to overflowing to minimize the presence of oxygen in the sample, which could also degrade the antibiotics. Samples were filtered in two stages, at first to avoid larger solids, by using glass fiber filters of Øp = 1.6 µm and, then using GVS nylon filters with a smaller pore size Øp = 0.45 µm [43,44]. Immediately after the sampling, the samples were placed in an ice-cold refrigerator. Subsequently, samples were refrigerated at 4 °C.

2.4. Antibiotic Quantification

The analytes were preconcentrated by solid-phase extraction. OASIS HLB, waters were conditioned with 32 mL of MeOH and 12 mL of water (pH 2 + 0.5). A 250 mL sample volume was loaded, and the retained species were eluted with MeOH (25 mL). The antibiotic concentration was determined by liquid chromatography coupled to a tandem mass spectrometer (LC-MS/MS). Samples were diluted to 1:1 with 0.1% (*w*/*v*) formic acid/methanol/acetonitrile (0.8/0.1/0.1 *w*/*v*), prior to LC-MS/MS analysis. Chromatographic separations were carried out using an Ultimate 3000 RSLC system (Thermo Fisher Scientific, Lyon, France). A 2.6 µm column Accucore C18 (100 × 2.1 mm) was used for the analysis. The mobile phases were (A) 0.4% formic acid and 5 mM ammonium formate and (B) 1: 1 (*v*/*v*) MeOH/ACN. A 20 µL sample aliquot was injected. The detection was performed by a QExactive Plus mass spectrometer (Thermo Fisher Scientific, Lyon, France). Resolution was 70,000. Operation was chosen in positive-ion selective monitoring. Samples were examined in triplicate. The limits of quantification and detection of selected antibiotics were: 2.0 ng/L for azithromycin, 1.2 ng/L for enrofloxacin, 0.8 ng/L for sulfadiazine and trimethoprim, and the limits of quantification were: 6.5 ng/L for azithromycin, 3.7 ng/L for enrofloxacin, 2.5 ng/L for sulfadiazine and trimethoprim.

2.5. Statistical Analysis

To complement this study, we studied the frequency of detection of the selected antibiotics, the average concentration among the 6 campaigns, the quartile values, the quartile differences of the average concentrations, and the mean and median for each antibiotic in surface-water-, WWTP- and wastewater-sampling points. The data were treated with Microsoft Excel, using this software to perform the ANOVA test between the points exposed to concrete emission sources and antibiotic concentration (p-value < 0.05 for significant differences, [45]). Complementarilyy, Tukey's honestly significant-difference test (Turkey's HSD) was used to determine significant differences between the concentration of selected antibiotics.

3. Results

3.1. Antibiotics Presence in Surface Waters

The overall results obtained for the concentrations of the target antibiotics in all surface-water-sampling points (2018–2021) are shown in Figure 5a. For a more detailed interpretation of the results, the data were processed by grouping the surface-water-sampling points into the different sub-basins that form the Ebro River basin (Figure 5b). It should be noticed that the boxplots of antibiotic concentrations have been elaborated by the concentration results shown in Tables S1–S4 from the different sampling campaigns. Unusual values are not represented in the boxplot graphs.

In order to complement the statistical analysis of this research, Tables S1–S4 list quantitative antibiotic-concentration results obtained during the 6 sampling campaigns that were carried out. Figure S1 shows the river flows of the six sampling campaigns. According to Figure 5a, enrofloxacin and sulfadiazine were frequently detected in concentrations from 20–180 ng/L in the surface-water-sampling points.

Tables S4–S6 present the ANOVA and Turkey's HSD test results in high-livestock-pressure sampling points. Significant differences (p-value < 0.05) were found between the enrofloxacin concentration and the rest of antibiotic concentrations at points that represent high-livestock-pressure sampling points. This result, coupled to the high concentration detected of this veterinary-use antibiotic, in comparison with the rest of the selected antibiotics, points to the fact that the fluoroquinolone was present in higher concentration than the rest of the antibiotics in rivers near intensive-farming areas.

In addition, the sampling point ASE_19 can be considered as a reference point, because it is the only one that presents low wastewater pressure and low livestock pressure. As a result, the ANOVA test was used for the concentration of the different drugs at this point and others with medium or high livestock pressure. Significant differences (p-value < 0.05) were found for sulfadiazine concentration. The levels of drugs at this point were lower than the rest of the points in this study, except for enrofloxacin in the spring of 2019, which presented an unusual concentration (Table S2).

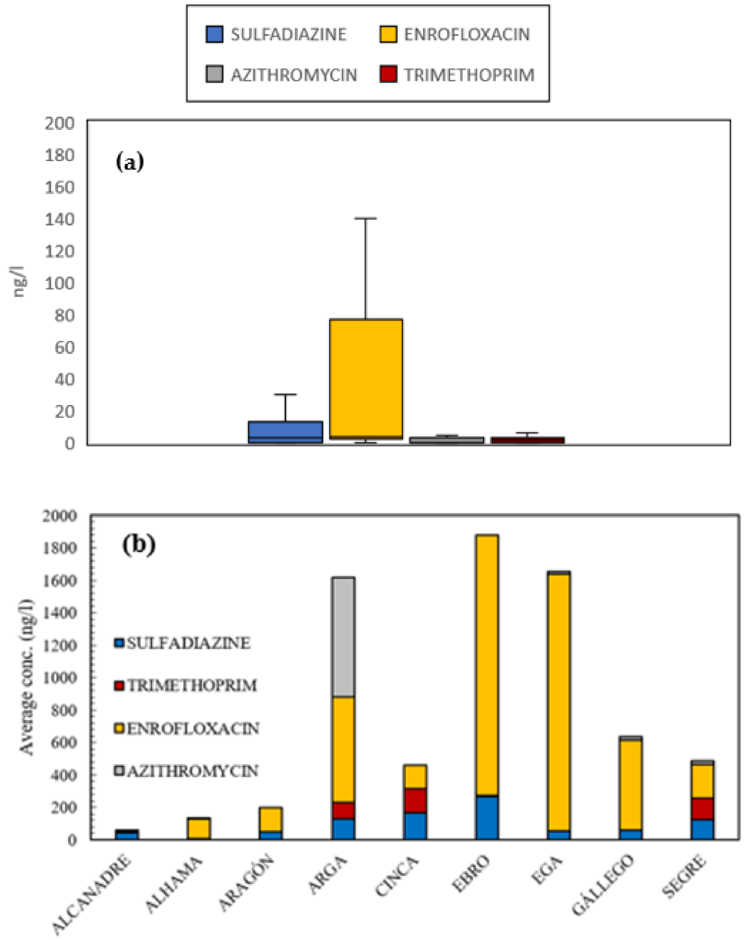


Figure 5. (**a**) Boxplots of selected antibiotics and (**b**) average concentration (ng/L) of target antibiotics among all surface-water-sampling points in the Ebro River basin area (2018–2021).

ANOVA and Turkey's HSD test results in high-WWTP-pressure sampling points showed that a significant difference between the points exposed to this kind of pressure and the concentration of individual antibiotics in rivers does not exist. However, as revealed by Figure 5b, the areas that present the highest total concentrations of antibiotics are Arga, Ebro and Ega, which present a medium-high WWTP pressure.

In the Alcanadre River sub-basin, 46 ng/L average concentration of azithromycin appeared in Flumen River (Table S3). Moreover, the presence of enrofloxacin, trimethoprim and amoxicillin was also detected in concentrations close to the quantification limit; this might be associated with the presence of pig farms and low-flow rivers. In terms of detection frequency, sulfadiazine appeared in 40% of the samples, enrofloxacin in 20% of the surface-water-sampling points; these antibiotics can be associated with the presence of pig farms. In fact, trimethoprim was present only in 10% of the samples, and azithromycin was not detected in this area.

The Aragón River sub-basin presented an average concentration of 147 ng/L of enrofloxacin. Downstream, as it passes through the town of Caparroso, the Aragón River area has a significant presence of pigs, poultry and rabbit farms and, as a result, 40% of the total samples contained fluoroquinolone (Table S2) and 30% of the surface-water-sampling points were polluted by sulfadiazine (Table S1).

Regarding the Arga River sub-basin, the four target antibiotics were detected. The presence of sulfadiazine and enrofloxacin was detected in the concentration range of 100 to 130 ng/L (Tables S1 and S2). However, azithromycin and enrofloxacin appeared in average concentrations of up to 739 ng/L. It should be noted that this region is marked by the presence of an urban area (Pamplona). Concerning the detection frequency of target antibiotics, sulfadiazine and trimethoprim were found in 55% of the samples. Moreover, enrofloxacin was present in more than 50% of the river samples. These results might suggest that urban areas show a greater variety of antibiotics.

In the Cinca River sub-basin, where there is a notable presence of pig and poultry farms, average concentrations close to 150 ng/L of enrofloxacin, sulfadiazine and trimethoprim were detected. Enrofloxacin and sulfadiazine appeared in 60% of the samples, while trimethoprim was detected only in 10% of them. This behavior confirms that enrofloxacin mainly appears in rivers where diffuse pollution from intensive farming of pig and poultry and agriculture-activity occurs.

In the Ebro sub-basin, a high average concentration of enrofloxacin (1604 ng/L) was detected. It should be noted that the detection frequency of this fluoroquinolone antibiotic was about 75%. Sulfadiazine presented the average concentration of 270 ng/L; it appeared in 40% of the samples. It is interesting that all the target antibiotics appeared in this sub-basin, which is very close to urban areas such as Logroño or Zaragoza, so it is marked by both urban areas and intensive farming, in which pig farms predominate. Consequently, these results point to the fact that a greater number of antibiotics were detected near urban areas. Moreover, this trend could also suggest that enrofloxacin and sulfadiazine can be associated with farming. Enrofloxacin was also detected in the French rivers Seine, Marne and Oise, presenting a maximum concentration of 100 ng/L [46]. The presence of 249 ng/L of this substance was also reported in the Polish rivers Gościcina and Reda, which are also associated with livestock pressure [26]. This antibiotic was also detected in the Mondego River (Portugal), in the Lllobregat River (Spain) and in the Ebro River (Spain), presenting concentrations of 76–178 ng/L [47].

In the Gállego sub-basin, sulfadiazine and trimethoprim presented average concentrations of up to 60 ng/L. On the other hand, enrofloxacin significantly exceeded 700 ng/L. This behavior can be attributed to the fact that the sampling point (at San Mateo de Gállego) is located downstream of several pig farms, as well as receiving the contribution of other rivers that discharge upstream, in areas where there is also an important farming presence (Huesca).

Regarding the sub-basin of the Ega River, enrofloxacin once again presented high average concentrations, exceeding 1600 ng/L which can be linked to poultry and pig farming predominating in this area. Sulfadiazine was also detected at an average level close to 60 ng/L.

In the Alhama River sub-basin, an average concentration of 125 ng/L of enrofloxacin appeared, which can be associated with the presence, in this case, of poultry farming. There are a smaller number of poultry farms in this area than in others such as Segre or Ebro; however, in the areas where poultry farming predominates, antibiotic concentration is lower than in rivers that are located near pig farming. This behavior might suggest that pig-intensive farming presents a higher antibiotic load than poultry farming.

Finally, in the Segre River sub-basin, which is subject to high farming pressure due to the presence of a large number of pig farms, all the studied antibiotics appeared. Enrofloxacin was again the antibiotic that presented the highest average concentration (205 ng/L) and detection frequency (80%). This behavior confirms that the vast majority of rivers near pig farms tend to be polluted by enrofloxacin. In fact, sulfadiazine chronicity was around 40% and trimethoprim was detected in 30% of the samples, but their average concentrations were relatively low: 121 ng/L and 140 ng/L, respectively. Furthermore, azithromycin appeared only in 10% of the samples. This decrease could confirm that the macrolide is only present near large urban areas in the Ebro River basin. However, other authors report concentrations of this macrolide antibiotic up to 1000 ng/L in rivers of Spain and France [48,49].

Regarding the river-flow effect, despite the existence of a significant difference between the average flows on rainier days and a consequent dilution of the species (Figure S1), the concentration of the selected antibiotics remained quite similar in drier and rainier seasons. A relevant fluctuation of the levels of drugs in river water between the sampling campaigns was observed. This could be due to the different flows that have been observed during these campaigns, which are listed in Figure S1. Although the ANOVA test confirms that there are not significant differences between the antibiotic levels and river flow, the antibiotics enrofloxacin and sulfadiazine tended to present higher concentrations in the rainier seasons. The data showed an increase in the levels of these antibiotics in autumn of 2020, which could be due to the initial stage of the pandemic of COVID-19, when the use of antibiotics and their subsequent emission into surface waters was augmented, as other authors suggest [29,30].

3.2. Antibiotics Presence in Wastewaters

Tables S8 and S9 present the ANOVA and Turkey's HSD test of antibiotic concentrations in wastewater-sampling points. Significant differences (p-value < 0.05) were found between the azithromycin concentration and the rest of the selected antibiotics This result, coupled to the high detected concentration of this macrolide antibiotic, in comparison with the rest of the selected antibiotic, points to the fact that azithromycin was present in higher concentration than the other antibiotics in WWTPs.

Concerning the average concentration results obtained for antibiotics in wastewater, which are shown in Figure 6a, the macrolide azithromycin presented the highest average levels. Regarding Figure 6b, the presence of this antibiotic was especially high in the WWT1, which is the one that presented the highest number of equivalent inhabitants, where the average azithromycin concentration exceeded 5000 ng/L. Other authors have reported the presence of this macrolide antibiotic in WWTPs in the range 20–2800 ng/L [11,50,51]. The total average concentrations of all the studied antibiotics reached 8000 and 5000 ng/L in the affluent and in the effluent, respectively.

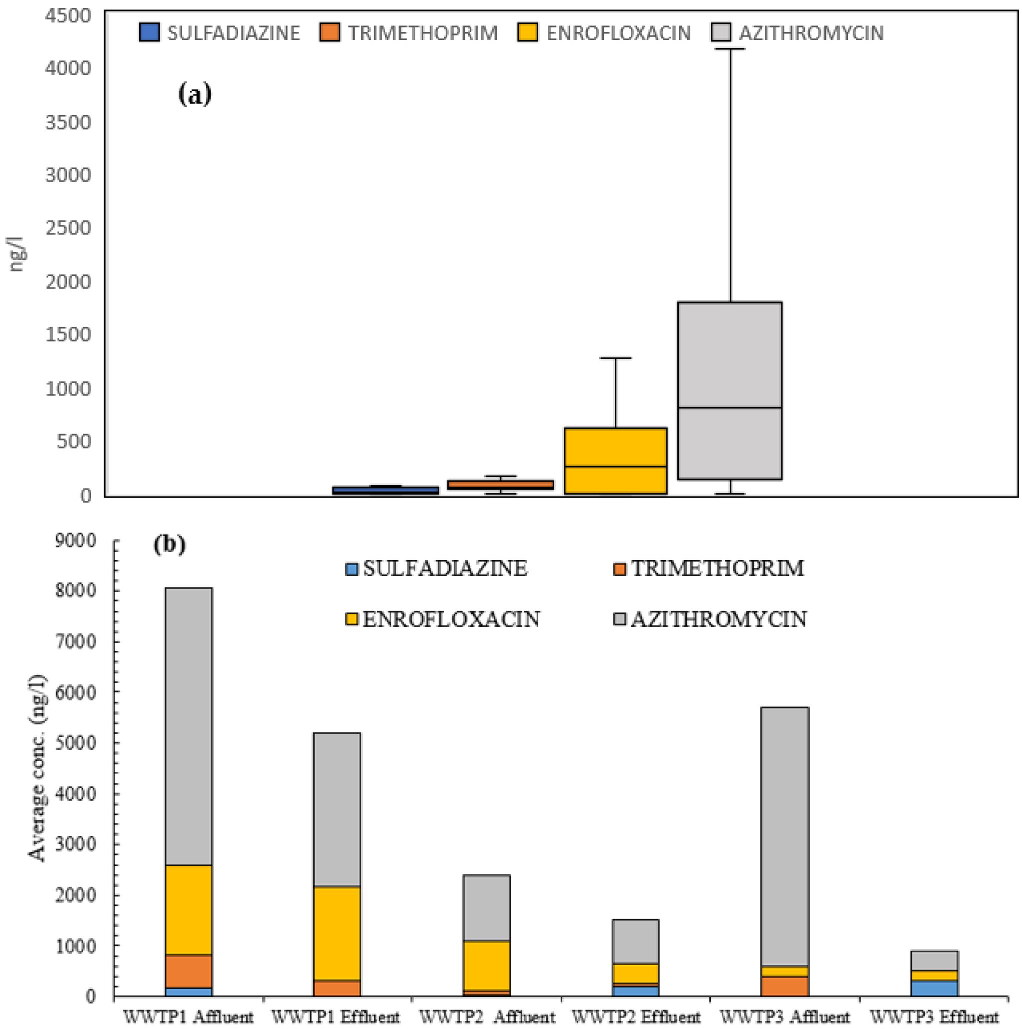


Figure 6. (**a**) Boxplots of selected antibiotics and (**b**) the average concentration of the target antibiotics in selected WWTPs (2018–2021) located in the Ebro River basin area.

According to Figure 6a, after azithromycin, enrofloxacin appeared in high concentration in the studied WWTPs, presenting average levels of 1300 ng/L. Enrofloxacin has been detected in 15 WWTPs of Croatia at a similar concentration [52]. Additionally, this antibiotic has also been found both in Slovakia in several WWTPs effluents [53] and in five WWTPs located in the Spanish territory [54]. The maximum level of azithromycin was reported at the entrance of WWTP1 and was up to 21,000 ng/L. These results are significantly superior to the ones reported in literature for other WWTPs located in Ebro River basin ten years before [32,55,56]. This increase might point to an incipient consumption of antibiotics, which is consistent with the reports published by the European Medicines Agency [41].

Sulfadiazine was also detected in the WWTP samples but in lower concentrations, reaching 300 ng/L. Comparing these outcomes with the literature, other authors detected the presence of this antibiotic in concentrations up to 846 ng/L in affluent and effluents from the Volos WWTP (Greece) [57,58]. The presence of sulfadiazine has also been evidenced in 22 treatment plants in Spain, with a concentration range of 49–1240 ng/L and 8–286 ng/L in in the affluents and effluents, respectively, which are similar to the concentrations found in this study [37].

The presence of trimethoprim in WWTPs was especially widespread in this study, appearing in the entirety of the effluents and showing an average concentration near 400 ng/L. However, concentrations of this substance up to 1866 ng/L have been reported in several WWTPs in Greece [59].

As revealed in Figure 6b, azithromycin was also present in smaller urban areas, such as WWTP3. The average concentration of this antibiotic in WWTP3 was higher than 5000 ng/L. Other authors have reported the same concentration in studies on wastewater quality [60,61]. This behavior is probably associated with the fact that both sampling points are subject to high urban and industrial pressure.

The results of the average antibiotic concentrations for the slaughterhouse and hospital effluents are shown in Figure 7. According to Figure 7a, azithromycin, again, was the antibiotic that presented the highest average concentration (2000 ng/L), especially in the hospital effluent, reaching 5000 ng/L (Figure 7b). It should be noted that this antibiotic was used to treat symptoms of COVID-19 in 2020 and 2021 [28]. In addition, the trimethoprim level was also relatively high in the hospital effluent (>1500 ng/L). These results confirmed that the presence of azithromycin and trimethoprim is commonly due to human medicine, whereas to a lesser extent, they could also be found in poultry and rabbit slaughterhouses. In the literature, azithromycin presence in European hospital effluents varies in the range 1–10 μg/L [54]. The sulfonamide antibiotic sulfadiazine was found in the hospital effluent in low concentration up to 80 ng/L. Sulfadiazine concentrations reported in the literature for hospital effluents in Valencia, Spain range from 9–137 ng/L [3]. According to the literature, trimethoprim has been detected in hospital effluents, reaching concentrations up to 1800 ng/L [50]. However, in our study, this antibiotic appeared only in the hospital effluent at a significant concentration (1368 ng/L) and in the poultry slaughterhouse at a concentration of 390 ng/L.

Compared to the rest of the studied slaughterhouses, only the duck slaughterhouse, where concentrations exceeding 1500 ng/L were detected, presented significant concentrations of azithromycin. Regarding enrofloxacin and sulfadiazine, the highest average concentrations were observed in the rabbit slaughterhouse (970 and 1835 ng/L, respectively).

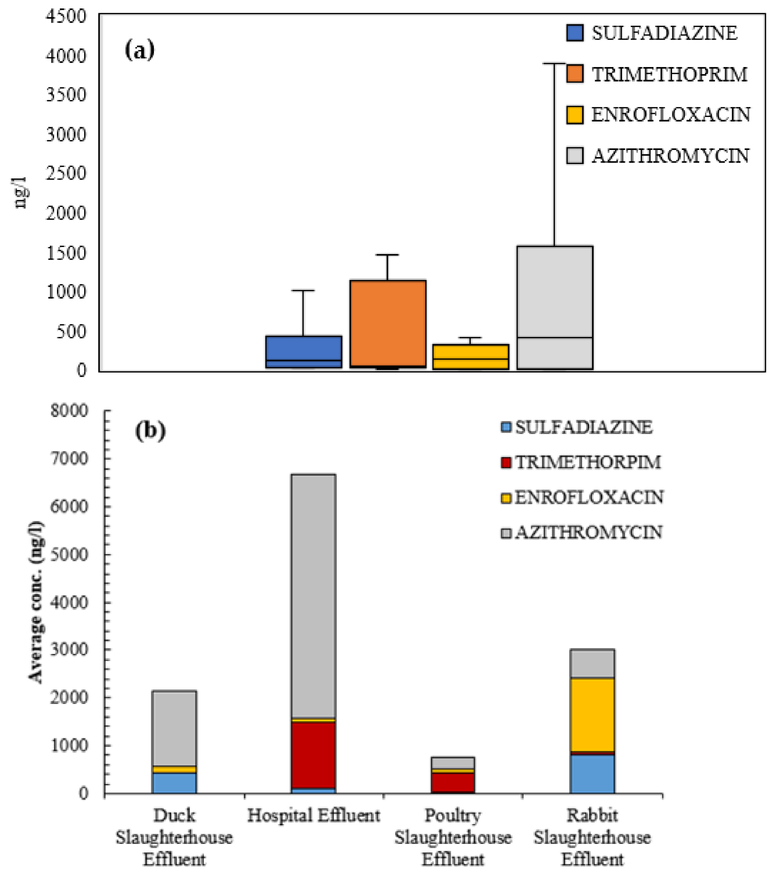


Figure 7. (**a**) Boxplots and (**b**) average concentration (ng/L) of target antibiotics in a hospital and three slaughterhouse effluents.

4. Conclusions

This research work presents a long-term study of the presence of antibiotics among surface waters and wastewaters in the Ebro basin (northeast of Spain) for four years (2018–2021). The choice of the target antibiotics was made based on a multispecies screening campaign carried out in the spring of 2018 and supported by the information on the sales and use of veterinary antimicrobials in Spain. Despite the European and national measures taken to restrict the use of antibiotics and exposure to these substances [14,60,61], the collected data demonstrated that:

- Enrofloxacin and sulfadiazine were present in almost all surface-water control points, which denotes high, direct exposure to these substances, especially in areas that are close to intensive farming. In fact, this fluoroquinolone antibiotic appears at very high concentrations in rivers of the Ebro basin near intensive farming, such as the Segre, Gallego or Cinca Rivers. Significant differences were found between the areas exposed to high livestock pressure and the concentration of enrofloxacin.
- Azithromycin was detected at very high concentrations in WWTPs. Complementarily, trimethoprim and enrofloxacin were detected in wastewaters of the Ebro River basin, especially in areas near large urban cores (>100,000 equivalent inhabitants).
- According to previous studies carried out in Ebro River basin in 2012 and 2010 [32,47], another important finding of this research is an increasing quantitative presence of antibiotics. Consequently, comprehensive studies of antibiotic assessment in Spanish rivers, wastewater, tap water, seawater and groundwater should be continued in order to establish water-quality standards for legislative guidance.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w14071033/s1, This section contains the concrete values of the selected antibiotics in the sampling network, the statistical analysis performed (one-way ANOVA test) and the flow control of the rivers among the 6 sampling campaings. Table S1. Sulfadiazine quantitative concentration results in surface control points (ng/L). D = detected (LOD < D < LOQ), n/d = not detected (<LOD). Table S2. Enrofloxacin quantitative concentration results (ng/L) in surface control points (ng/L). D = detected (LOD < D < LOQ), n/d = not detected (<LOD). Table S3. Azithromycin quantitative concentration results (ng/L) in surface control points (ng/L). D = detected (LOD < D < LOQ), n/d = not detected (<LOD). Table S4. Trimethoprim quantitative concentration results (ng/L) in surface control points. D = detected (LOD < D < LOQ), n/d = not detected (<LOD). Figure S1. River flow during the six sampling campaigns 2018–2021. Table S5. One-way ANOVA test of antibiotics concentration in high-livestock-pressure points Table S6. Tukey's honestly significant difference test results in high-livestock pressure points. Table S7. One-way ANOVA test of sulfadiazine concentration between the reference point ASE19 and the points exposed to high and medium livestock pressure. Table S8. One-way ANOVA test of antibiotics concentration in WWTPs. Table S9. Tukey's honestly significant difference test results in WWTPs.

Author Contributions: Conceptualization, R.M.; Data curation, S.M.; Formal analysis, S.G.; Investigation, J.G.; Methodology, M.P.O. and J.S.; Project administration, F.L.; Supervision, M.P.O., R.M. and J.S.; Writing—original draft, S.M.; Writing—review & editing, J.G. and J.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by EFA 183/16/OUTBIOTICS.

Conflicts of Interest: We have no conflict of interest to declare.

References

1. Conde-Cid, M.; Álvarez-Esmorís, C.; Paradelo-Núñez, R.; Nóvoa-Muñoz, J.C.; Arias-Estévez, M.; Álvarez-Rodríguez, E.; Fernández-Sanjurjo, M.J.; Núñez-Delgado, A. Occurrence of tetracyclines and sulfonamides in manures, agricultural soils and crops from different areas in Galicia (NW Spain). *J. Clean. Prod.* **2018**, *197*, 491–500. [CrossRef]
2. Mirzaei, R.; Yunesian, M.; Nasseri, S.; Gholami, M.; Jalilzadeh, E.; Shoeibi, S.; Bidshahi, H.S.; Mesdaghinia, A. An optimized SPE-LC-MS/MS method for antibiotics residue analysis in ground, surface and treated water samples by response surface methodology- central composite design. *J. Environ. Health Sci. Eng.* **2017**, *15*, 21. [CrossRef] [PubMed]
3. Mendoza, A.; Aceña, J.; Pérez, S.; de Alda, M.L.; Barceló, D.; Gil, A.; Valcárcel, Y. Pharmaceuticals and iodinated contrast media in a hospital wastewater: A case study to analyse their presence and characterise their environmental risk and hazard. *Environ. Res.* **2015**, *140*, 225–241. [CrossRef] [PubMed]
4. Díaz-Bao, M.; Barreiro, R.; Miranda, J.M.; Cepeda, A.; Regal, P. Fast HPLC-MS/MS Method for Determining Penicillin Antibiotics in Infant Formulas Using Molecularly Imprinted Solid-Phase Extraction. *J. Anal. Methods Chem.* **2015**, *2015*, 959675. [CrossRef]
5. Alygizakis, N.; Gago-Ferrero, P.; Borova, V.L.; Pavlidou, A.; Hatzianestis, I.; Thomaidis, N.S. Occurrence and spatial distribution of 158 pharmaceuticals, drugs of abuse and related metabolites in offshore seawater. *Sci. Total Environ.* **2016**, *541*, 1097–1105. [CrossRef]
6. Gracia-Lor, E.; Rousis, N.I.; Zuccato, E.; Bade, R.; Lomba, J.A.B.; Castrignanò, E.; Causanilles, A.; Hernández, F.; Kasprzyk-Hordern, B.; Kinyua, J.; et al. Estimation of caffeine intake from analysis of caffeine metabolites in wastewater. *Sci. Total Environ.* **2017**, *609*, 1582–1588. [CrossRef] [PubMed]
7. Gothwal, R.; Shashidhar, T. Antibiotic Pollution in the Environment: A Review. *CLEAN–Soil Air Water* **2014**, *43*, 479–489. [CrossRef]
8. Moles, S.; Mosteo, R.; Gómez, J.; Szpunar, J.; Gozzo, S.; Castillo, J.R.; Ormad, M.P. Towards the Removal of Antibiotics Detected in Wastewaters in the POCTEFA Territory: Occurrence and TiO2 Photocatalytic Pilot-Scale Plant Performance. *Water* **2020**, *12*, 1453. [CrossRef]
9. Abegglen, C.; Joss, A.; McArdell, C.S.; Fink, G.; Schlüsener, M.P.; Ternes, T.A.; Siegrist, H. The fate of selected micropollutants in a single-house MBR. *Water Res.* **2009**, *43*, 2036–2046. [CrossRef]
10. Massé, D.I.; Saady, N.M.C.; Gilbert, Y. Potential of Biological Processes to Eliminate Antibiotics in Livestock Manure: An Overview. *Animals* **2014**, *4*, 146–163. [CrossRef]
11. Rodriguez-Mozaz, S.; Vaz-Moreira, I.; Della Giustina, S.V.; Llorca, M.; Barceló, D.; Schubert, S.; Berendonk, T.U.; Michael-Kordatou, I.; Fatta-Kassinos, D.; Martinez, J.L.; et al. Antibiotic residues in final effluents of European wastewater treatment plants and their impact on the aquatic environment. *Environ. Int.* **2020**, *140*, 105733. [CrossRef]
12. Barbosa, M.O.; Ribeiro, A.R.; Ratola, N.; Hain, E.; Homem, V.; Pereira, M.F.; Blaney, L.; Silva, A. Spatial and seasonal occurrence of micropollutants in four Portuguese rivers and a case study for fluorescence excitation-emission matrices. *Sci. Total Environ.* **2018**, *644*, 1128–1140. [CrossRef] [PubMed]

13. European Centre for Disease Prevention and Control; European Food Safety Authority; European Medicines Agency. Antimicrobial Consumption and Resistance in Bacteria from Humans and Animals: Third Joint Inter-Agency Report on Integrated Analysis of Antimicrobial Agent Consumption and Occurrence of Antimicrobial Resistance in Bacteria from Humans and Food-Producing Animals in the EU/EEA: JIACRA III 2016–2018. European Medicines Agency. 2021. Available online: https://data.europa.eu/doi/10.2900/056892 (accessed on 26 January 2022).
14. European Parliament and the Council of the European Union. Regulation (EU) 2019/6 of the European Parliament and of the Council of 11 December 2018 on veterinary medicinal products and repealing Directive 2001/82/EC. *Off. J. Eur. Union* **2019**, *L4*, 43–167.
15. European Medicines Agency. Sales of Veterinary Antimicrobial Agents in 31 European Countries in 2017; Trends from 2010 to 2017; European Medicines Agency: UK. Available online: https://www.ema.europa.eu/en/documents/report/sales-veterinary-antimicrobial-agents-31-european-countries-2017_en.pdf (accessed on 26 January 2022).
16. García-Galán, M.J.; Díaz-Cruz, M.S.; Barceló, D. Determination of 19 sulfonamides in environmental water samples by automated on-line solid-phase extraction-liquid chromatography-tandem mass spectrometry (SPE-LC-MS/MS). *Talanta* **2010**, *81*, 355–366. [CrossRef]
17. Chelliapan, S.; Wilby, T.; Sallis, P.J. Performance of an up-flow anaerobic stage reactor (UASR) in the treatment of pharmaceutical wastewater containing macrolide antibiotics. *Water Res.* **2006**, *40*, 507–516. [CrossRef]
18. Reverté-Villarroya, S.; Borrull, F.; Pocurull, E.; Marcé-Recasens, R.M. Determination of antibiotic compounds in water by solid-phase extraction–high-performance liquid chromatography–(electrospray) mass spectrometry. *J. Chromatogr. A* **2003**, *1010*, 225–232. [CrossRef]
19. Duarte-Davidson, R.; Jones, K.C. Screening the environmental fate of organic contaminants in sewage sludge applied to agricultural soils: II. The potential for transfers to plants and grazing animals. *Sci. Total Environ.* **1996**, *185*, 59–70. [CrossRef]
20. Eggen, T.; Asp, T.N.; Grave, K.; Hormazabal, V. Uptake and translocation of metformin, ciprofloxacin and narasin in forage- and crop plants. *Chemosphere* **2011**, *85*, 26–33. [CrossRef]
21. Hu, X.; Zhou, Q.; Luo, Y. Occurrence and source analysis of typical veterinary antibiotics in manure, soil, vegetables and groundwater from organic vegetable bases, northern China. *Environ. Pollut.* **2010**, *158*, 2992–2998. [CrossRef]
22. Li, W.; Shi, Y.; Gao, L.; Liu, J.; Cai, Y. Occurrence of antibiotics in water, sediments, aquatic plants, and animals from Baiyangdian Lake in North China. *Chemosphere* **2012**, *89*, 1307–1315. [CrossRef] [PubMed]
23. Matsubara, M.E.; Helwig, K.; Hunter, C.; Roberts, J.; Subtil, E.L.; Coelho, L.H.G. Amoxicillin removal by pre-denitrification membrane bioreactor (A/O-MBR): Performance evaluation, degradation by-products, and antibiotic resistant bacteria. *Ecotoxicol. Environ. Saf.* **2020**, *192*, 110258. [CrossRef]
24. Moreira, N.; Narciso-Da-Rocha, C.; Polo-López, M.I.; Pastrana-Martínez, L.M.; Faria, J.L.; Manaia, C.M.; Fernández-Ibáñez, P.; Nunes, O.; Silva, A.M. Solar treatment (H_2O_2, TiO_2-P25 and GO-TiO_2 photocatalysis, photo-Fenton) of organic micropollutants, human pathogen indicators, antibiotic resistant bacteria and related genes in urban wastewater. *Water Res.* **2018**, *135*, 195–206. [CrossRef] [PubMed]
25. Tian, L.; Khalil, S.; Bayen, S. Effect of thermal treatments on the degradation of antibiotic residues in food. *Crit. Rev. Food Sci. Nutr.* **2017**, *57*, 3760–3770. [CrossRef] [PubMed]
26. Wagil, M.; Kumirska, J.; Stolte, S.; Puckowski, A.; Maszkowska, J.; Stepnowski, P.; Białk-Bielińska, A. Development of sensitive and reliable LC-MS/MS methods for the determination of three fluoroquinolones in water and fish tissue samples and preliminary environmental risk assessment of their presence in two rivers in northern Poland. *Sci. Total Environ.* **2014**, *493*, 1006–1013. [CrossRef] [PubMed]
27. Nieuwlaat, R.; Mbuagbaw, L.; Mertz, D.; Burrows, L.L.; Bowdish, D.M.E.; Moja, L.; Wright, G.D.; Schünemann, H.J. Coronavirus Disease 2019 and Antimicrobial Resistance: Parallel and Interacting Health Emergencies. *Clin. Infect. Dis.* **2021**, *72*, 1657–1659. [CrossRef]
28. Lagier, J.-C.; Million, M.; Gautret, P.; Colson, P.; Cortaredona, S.; Giraud-Gatineau, A.; Honoré, S.; Gaubert, J.-Y.; Fournier, P.-E.; Tissot-Dupont, H.; et al. Outcomes of 3,737 COVID-19 patients treated with hydroxychloroquine/azithromycin and other regimens in Marseille, France: A retrospective analysis. *Travel Med. Infect. Dis.* **2020**, *36*, 101791. [CrossRef]
29. Madero, C.M. Primer año del plan estrategico y de acción para reducir el riesgo de selección y diseminación de resistencia a los antibióticos. *Albeitar Public. Vet. Indep.* **2016**, *194*, 4–6.
30. Aliste, M.; Garrido, I.; Flores, P.; Hellín, P.; Vela, N.; Navarro, S.; Fenoll, J. Reclamation of agro-wastewater polluted with thirteen pesticides by solar photocatalysis to reuse in irrigation of greenhouse lettuce grown. *J. Environ. Manag.* **2020**, *266*, 110565. [CrossRef]
31. López-Serna, R.; Jurado, A.; Vázquez-Suñé, E.; Carrera, J.; Petrović, M.; Barceló, D. Occurrence of 95 pharmaceuticals and transformation products in urban groundwaters underlying the metropolis of Barcelona, Spain. *Environ. Pollut.* **2013**, *174*, 305–315. [CrossRef]
32. Gros, M.; Petrovic, M.; Ginebreda, A.; Barceló, D. Sources, Occurrence, and Environmental Risk Assessment of Pharmaceuticals in the Ebro River Basin. *Ebro River Basin* **2010**, *13*, 209–237. [CrossRef]
33. López-Serna, R.; Petrovic, M.; Barceló, D. Development of a fast instrumental method for the analysis of pharmaceuticals in environmental and wastewaters based on ultra high performance liquid chromatography (UHPLC)–tandem mass spectrometry (MS/MS). *Chemosphere* **2011**, *85*, 1390–1399. [CrossRef] [PubMed]

34. Solaun, O.; Franco, J.; Borja, A.; Menchaca, I.; Otaola, J.; Manzanos, A. *Análisis de Presiones e Impactos*; Confederación Hidrográfica del Ebro: Zaragoza, Spain, 2015; pp. 175–187. Available online: https://www.chebro.es/.../6e31da48-b276-71b0-ec39-d1e50de17939?t=1617716661315 (accessed on 26 January 2022).
35. Simon-Sánchez, L.; Grelaud, M.; Garcia-Orellana, J.; Ziveri, P. River Deltas as hotspots of microplastic accumulation: The case study of the Ebro River (NW Mediterranean). *Sci. Total Environ.* **2019**, *687*, 1186–1196. [CrossRef] [PubMed]
36. da Silva, B.F.; Jelic, A.; López-Serna, R.; Mozeto, A.A.; Petrovic, M.; Barceló, D. Occurrence and distribution of pharmaceuticals in surface water, suspended solids and sediments of the Ebro river basin, Spain. *Chemosphere* **2011**, *85*, 1331–1339. [CrossRef] [PubMed]
37. García-Galán, M.J.; Blanco, S.G.; Roldán, R.L.; Díaz-Cruz, S.; Barceló, D. Ecotoxicity evaluation and removal of sulfonamides and their acetylated metabolites during conventional wastewater treatment. *Sci. Total Environ.* **2012**, *437*, 403–412. [CrossRef] [PubMed]
38. Tamtam, F.; Mercier, F.; Le Bot, B.; Eurin, J.; Dinh, Q.T.; Clément, M.; Chevreuil, M. Occurrence and fate of antibiotics in the Seine River in various hydrological conditions. *Sci. Total Environ.* **2008**, *393*, 84–95. [CrossRef]
39. Al Aukidy, M.; Verlicchi, P.; Jelic, A.; Petrovic, M.; Barcelò, D. Monitoring release of pharmaceutical compounds: Occurrence and environmental risk assessment of two WWTP effluents and their receiving bodies in the Po Valley, Italy. *Sci. Total Environ.* **2012**, *438*, 15–25. [CrossRef]
40. Dan, A.; Zhang, X.; Dai, Y.; Chen, C.; Yang, Y. Occurrence and removal of quinolone, tetracycline, and macrolide antibiotics from urban wastewater in constructed wetlands. *J. Clean. Prod.* **2020**, *252*, 119677. [CrossRef]
41. European Medicines Agency. Sales of Veterinary Antimicrobial Agents in 30 European Countries in 2015. Seventh ESVAC Report. Seventh ESVAC Rep. 2017. Available online: http://www.ema.europa.eu/docs/en_GB/document_library/Report/2017/10/WC500236750.pdf%0Ahttps://bi.ema.europa.eu/analyticsSOAP/saw.dll?PortalPages (accessed on 17 November 2021).
42. USEPA. Method 1694: Pharmaceuticals and Personal Care Products in Water, Soil, Sediment, and Biosolids by HPLC/MS/MS. EPA Method. 2007. Available online: https://www.epa.gov/sites/production/files/2015-10/documents/method_1694_2007.pdf (accessed on 26 January 2022).
43. Santos, L.H.M.L.M.; Gros, M.; Rodriguez-Mozaz, S.; Delerue-Matos, C.; Pena, A.; Barceló, D.; Montenegro, M.C.B.S.M. Contribution of hospital effluents to the load of pharmaceuticals in urban wastewaters: Identification of ecologically relevant pharmaceuticals. *Sci. Total Environ.* **2013**, *461–462*, 302–316. [CrossRef]
44. Vergeynst, L.; Haeck, A.; De Wispelaere, P.; Van Langenhove, H.; Demeestere, K. Multi-residue analysis of pharmaceuticals in wastewater by liquid chromatography–magnetic sector mass spectrometry: Method quality assessment and application in a Belgian case study. *Chemosphere* **2015**, *119*, S2–S8. [CrossRef]
45. Pujar, P.M.; Kenchannavar, H.H.; Kulkarni, R.; Kulkarni, U.P. Real-time water quality monitoring through Internet of Things and ANOVA-based analysis: A case study on river Krishna. *Appl. Water Sci.* **2020**, *10*, 22. [CrossRef]
46. Danner, M.C.; Robertson, A.; Behrends, V.; Reiss, J. Antibiotic pollution in surface fresh waters: Occurrence and effects. *Sci. Total Environ.* **2019**, *664*, 793–804. [CrossRef] [PubMed]
47. Serna, R.L.; Petrovic, M.; Barceló, D. Occurrence and distribution of multi-class pharmaceuticals and their active metabolites and transformation products in the Ebro River basin (NE Spain). *Sci. Total Environ.* **2012**, *440*, 280–289. [CrossRef]
48. Milaković, M.; Vestergaard, G.; Plaza, J.J.G.; Petrić, I.; Simatovic, A.; Senta, I.; Kublik, S.; Schloter, M.; Smalla, K.; Udiković-Kolić, N. Pollution from azithromycin-manufacturing promotes macrolide-resistance gene propagation and induces spatial and seasonal bacterial community shifts in receiving river sediments. *Environ. Int.* **2019**, *123*, 501–511. [CrossRef]
49. Gonzalez, I.; Muga, I.; Rogríguez, J.; Blanco, M. Contaminantes emergentes en aguas residuales urbanas y efluentes hospi-talarios. *Tecnoaqua* **2018**, *29*, 42–54.
50. Verlicchi, P.; Al Aukidy, M.; Galletti, A.; Petrovic, M.; Barceló, D. Hospital effluent: Investigation of the concentrations and distribution of pharmaceuticals and environmental risk assessment. *Sci. Total Environ.* **2012**, *430*, 109–118. [CrossRef] [PubMed]
51. Osorio, V.; Marcé, R.; Pérez, S.; Ginebreda, A.; Cortina, J.L.; Barceló, D. Occurrence and modeling of pharmaceuticals on a sewage-impacted Mediterranean river and their dynamics under different hydrological conditions. *Sci. Total Environ.* **2012**, *440*, 3–13. [CrossRef] [PubMed]
52. Senta, I.; Terzic, S.; Ahel, M. Occurrence and fate of dissolved and particulate antimicrobials in municipal wastewater treatment. *Water Res.* **2013**, *47*, 705–714. [CrossRef] [PubMed]
53. Birošová, L.; Mackuľak, T.; Bodik, I.; Ryba, J.; Škubák, J.; Grabic, R. Pilot study of seasonal occurrence and distribution of antibiotics and drug resistant bacteria in wastewater treatment plants in Slovakia. *Sci. Total Environ.* **2014**, *490*, 440–444. [CrossRef] [PubMed]
54. Garcia-Galan, M.J.; Diaz-Cruz, M.S.; Barcelo, D. Occurrence of sulfonamide residues along the Ebro river basin: Removal in wastewater treatment plants and environmental impact assessment. *Environ. Int.* **2011**, *37*, 462–473. [CrossRef]
55. Golovko, O.; Kumar, V.; Fedorova, G.; Randak, T.; Grabic, R. Seasonal changes in antibiotics, antidepressants/psychiatric drugs, antihistamines and lipid regulators in a wastewater treatment plant. *Chemosphere* **2014**, *111*, 418–426. [CrossRef]
56. Berges, J.; Moles, S.; Ormad, M.P.; Mosteo, R.; Gómez, J. Antibiotics removal from aquatic environments: Adsorption of enrofloxacin, trimethoprim, sulfadiazine, and amoxicillin on vegetal powdered activated carbon. *Environ. Sci. Pollut. Res.* **2020**, *28*, 8442–8452. [CrossRef]

57. Guillossou, R.; Le Roux, J.; Mailler, R.; Pereira-Derome, C.S.; Varrault, G.; Bressy, A.; Vulliet, E.; Morlay, C.; Nauleau, F.; Rocher, V.; et al. Influence of dissolved organic matter on the removal of 12 organic micropollutants from wastewater effluent by powdered activated carbon adsorption. *Water Res.* **2020**, *172*, 115487. [CrossRef]
58. Guillossou, R.; Le Roux, J.; Mailler, R.; Vulliet, E.; Morlay, C.; Nauleau, F.; Gasperi, J.; Rocher, V. Organic micropollutants in a large wastewater treatment plant: What are the benefits of an advanced treatment by activated carbon adsorption in comparison to conventional treatment? *Chemosphere* **2019**, *218*, 1050–1060. [CrossRef] [PubMed]
59. Viana, P.; Meisel, L.; Lopes, A.; de Jesus, R.; Sarmento, G.; Duarte, S.; Sepodes, B.; Fernandes, A.; dos Santos, M.; Almeida, A.; et al. Identification of Antibiotics in Surface-Groundwater. A Tool towards the Ecopharmacovigilance Approach: A Portuguese Case-Study. *Antibiotics* **2021**, *10*, 888. [CrossRef]
60. Directive 2013/11/EU of the European Parliament and of the Council. In *Fundamental Texts on European Private Law*; Hart Publishing: Oxford, UK, 2016; Volume 2013, pp. 1–17. [CrossRef]
61. Unión Europea. Directiva (UE) 2020/2184 del Parlamento Europeo y del Consejo de 16 de diciembre de 2020 relativa a la calidad de las aguas destinadas al consumo humano (versión refundida). *D Unión Eur.* **2020**, *53*, 1–62.