



Towards the understanding of antibiotic occurrence and transport in groundwater: Findings from the Baix Fluvià alluvial aquifer (NE Catalonia, Spain)



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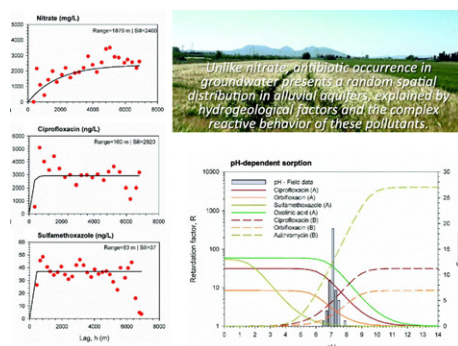
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HIGHLIGHTS

- Hydrogeological, chemical, and isotopic data assess antibiotic pollution in groundwater.
- Most frequent antibiotics are sulfamethoxazole and ciprofloxacin.
- A lack of spatial correlation exists for antibiotic occurrence, yet not for nitrate.
- Fate of antibiotics in groundwater not only depend on chemical properties but on hydrogeology.

GRAPHICAL ABSTRACT



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ABSTRACT

Antibiotics are an increasing focus of interest due to their high detection frequency in the environment. However, their presence in water bodies is not regulated by environmental policies. This field study investigates, for the first time, the occurrence, behavior and fate of a selection of 53 antibiotics, including up to 10 chemical groups, in an alluvial aquifer originated from manure application in an agricultural region using hydrogeological, hydrochemical and isotopic approaches. Up to 11 antibiotics were found in groundwater corresponding to 4 different chemical groups: fluoroquinolones, macrolides, quinolones and sulfonamides. In surface water, only 5 different antibiotics from 2 chemical groups: fluoroquinolones and sulfonamides, were quantified. The most frequent antibiotics were sulfamethoxazole and ciprofloxacin. Concentrations of antibiotics were in the order of ng/L, with maximum concentrations of 300 ng/L in groundwater. Hydrochemistry and isotopic data and geostatistics confirmed the spatial trend observed for nitrates, where nitrate concentrations tend to be higher in the margin areas of the study area, and lower concentrations are found nearby the river. On the other hand, no clear continuous spatial concentration trend of antibiotics was observed in the aquifer, supported by the short spatial correlation found in the variograms. This indicates that the physical-chemical properties and processes of each antibiotic (mainly, sorption and degradation), and other environmental issues, such as a patchy diffuse input and the manure antibiotic content itself, play an important role in their spatial distribution in groundwater. A discussion on the estimation of the antibiotic sorption parameter reveals the difficulties of describing such phenomena. Furthermore, retardation factors will extend over several orders of magnitude, which highly affects the movement of individual antibiotics within the aquifer. To summarize, this study points

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out the difficulties associated with antibiotic research in groundwater in order to define water resources quality management strategies and environmental regulations.

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1. Introduction

Diffuse pollution from agricultural and livestock activities and point source pollution from sewage treatment and industrial discharge are the principal sources of groundwater contamination (e.g., Hiscock, 2005). Nutrients are the most common contaminants associated with farming (particularly nitrogen and phosphorus), but slurry and manure are also a potential source of organic contaminants of emerging concern, such as veterinary pharmaceuticals including antibiotics. Antibiotics, as biologically active compounds, are an increasing focus of interest due to their high detection frequency in the environment and the growing bacterial resistance formation. According to the European Medicines Agency (ECDC et al., 2015), of the overall sales of antibiotics in Europe in 2014, tetracyclines (33.4%), penicillins (25.5%) and sulfonamides (11%) were the antimicrobial classes with the highest consumption in veterinary medicine, while β -lactams and penicillins (70.6%) and fluoroquinolones (11.6%) were the most used in humane medicine. A rough estimation comparing consumption data of antimicrobials for humans and animals indicates that about 70% of the total sales in Spain is for veterinary use (ECDC et al., 2015). Main applications of veterinary antibiotics are described by De Briyne et al. (2014).

Antibiotics may be discharged to the environment and reach the groundwater bodies by different pathways (Carvalho and Santos, 2016). Following administration to livestock, veterinary antibiotics may be released to the soil by spreading of slurry and manure to land and they are subject to reach the groundwater primarily through leaching (Blackwell et al., 2009). Besides, human antibiotics can enter water bodies through direct discharge of wastewater treatment plant (WWTP) effluents to surface water, because these compounds are barely removed in conventional WWTPs (Lapworth et al., 2012; Rodriguez-Mozaz et al., 2015). Thus, surface water - groundwater interaction is another important pathway of groundwater pharmaceutical pollution, since surface water usually contains higher concentrations of antibiotics than groundwater (Focazio et al., 2008; Li, 2014; Stuart et al., 2011). Therefore, the widespread and overuse of antibiotics in both human and veterinary medicine has led to a constant input of a vast array of chemicals into aquatic systems. Chronic exposure to antibiotic not only disturbs natural ecological processes, but it can also pose a risk to human health due to the appearance of multidrug resistant bacteria. In fact, an increase of antibiotic-resistance among microbes has already been observed in recent years (Jechalke et al., 2014; Servais and Passet, 2009).

Pharmaceuticals and, especially, antibiotics have been studied in detail in WWTP effluents (Martínez Bueno et al., 2012; Rodriguez-Mozaz et al., 2015) and surface water (López-Serna et al., 2012; Osorio et al., 2012; Pal et al., 2010), but less in groundwater. Some studies investigate the occurrence of emerging organic contaminants in groundwater (Fram and Belitz, 2011; Jurado et al., 2012; Lapworth et al., 2012; Lopez et al., 2015; Meffe and de Bustamante, 2014), but only a few of them focus on the presence of antibiotics and the processes that control their fate in the subsurface (Sarmah et al., 2006; García-Galán et al., 2010; Bartelt-Hunt et al., 2011; Frey et al., 2015). None of these investigations neither describe in detail the hydrogeological context of the study area, nor approach the occurrence of antibiotics in groundwater from the regional hydrogeological perspective, that are essential to address management strategies.

The occurrence of antibiotics in the subsoil depends on the physical-chemical properties and processes of each compound (e.g. solubility, sorption, degradation or transformations), as well as other environmental characteristics (e.g. vulnerability of the aquifer, groundwater

residence time, pH or redox conditions). Due to this complexity, the fate and transport of antibiotics in groundwater is still poorly understood. Nevertheless, batch tests complemented with soil column experiments have been performed under controlled laboratory conditions to investigate the transport and attenuation of a specific compound within a specific sediment or substrate. Even though column experiments provide good estimates for all relevant transport parameters, the obtained results are limited to the scale of the experiment and they are not directly transferrable to field scale scenarios (Banzhaf et al., 2012; Banzhaf and Hebig, 2016; Salvia et al., 2014; Siemens et al., 2010). These references, among so many other related papers, have shown the reactive behavior of antibiotics and, more importantly, their dependence on the environmental conditions.

Groundwater recharge poles, as end-members that act as pollutant sources, need to be determined so the role of hydrogeological processes is clearly understood and permits focusing on the geochemical processes that control the fate of pollutants. Therefore, understanding the hydrogeological system of a given site becomes a key factor to comprehend the dynamics of antibiotics in groundwater at a field scale. The application of classical hydrogeological field studies and methods, together with multi-isotopic data including the isotopes of the water, nitrate and sulfate molecules, allows determining the origin of groundwater and the geochemical processes that occur in aquifers (Otero et al., 2009; Vitòria et al., 2008; Sassine et al., 2015), as well as to contribute to the flow system knowledge.

The main goal of this study is to investigate the occurrence and behavior of a selection of antibiotics in groundwater coupling hydrochemical and isotopic approach to gain insight on 1) the distribution of antibiotics in water; 2) the hydrogeological and geochemical processes that determine their occurrence and migration in the subsurface in the light of state-of-the-art knowledge of their transport processes; and finally, 3) the representativeness of field data as indicators of the quality of a groundwater body. To this end, 53 antibiotics, including up to 10 chemical groups, are studied in the agricultural area of the Baix Fluvià (NE Catalonia, Spain).

In this sense, our objective looks forward to describe a field case study that frames the dynamics of antibiotic transport in groundwater at a regional scale in its specific hydrogeological setting, evaluating and discussing the detail in which their occurrence can be evaluated from field data. This is the reason why significant emphasis is given to hydrogeological data and its interpretation. We finally conduct this research with the aim to assess groundwater quality management plans, so actions can be engaged to develop appropriate measures and programs to cope with these pollutants of emerging concern, all the more when their presence in water bodies is not regulated by environmental policies yet.

1.1. Factors affecting transport and fate of antibiotics in groundwater

Antibiotic transport and fate in groundwater must be addressed from a set of assumptions; namely, 1) antibiotics are reactive compounds, therefore their migration will be retarded with respect to groundwater, 2) sorption processes that trigger retardation, as well as potential biodegradation, are highly compound-specific and site-specific; meaning that each antibiotic (or group of antibiotics) behaves differently and that the spatial variability of the soil and aquifer properties can also influence migration rates of each antibiotic along its flow path from the source, and 3) given their complex molecular structure, antibiotic sorption processes are not simple and they are still not completely characterized, so a wide range of coefficients is found for distinct

experimental settings and for distinct calculation/conceptual approaches. A short review follows with the aim to summarize the principal aspects of antibiotic transport processes and the reactive characteristics of the main antibiotic groups.

1.1.1. Processes involved in antibiotic reactive transport in aquifers

The transport and fate of organic contaminants in the subsurface and groundwater depend on both the physico-chemical characteristics of the compound, contaminant loading and environmental characteristics (Lapworth et al., 2012). The knowledge of physical-chemical properties of antibiotics, such as dissociation constants and partition coefficients, are essential for the interpretation of the processes that control their occurrence and persistence in the environment.

1.1.1.1. Sorption. One of the most important properties is the sorptive exchange of antibiotics between the water and solid phases, which is characterized by the distribution coefficient (K_d). Porous media have much larger solid-to-solution phase ratios than surface water. Then, even the transport of a chemical with small to moderate solid/water distribution ratios (K_d) is noticeably influenced by sorption processes (Schwarzenbach et al., 2003). However, the sorption coefficient K_d for organic compounds varies substantially depending on each soil type and its fraction of organic carbon (f_{oc}). Indeed, the sorption capacity by unit of organic carbon fraction is expressed by the soil organic carbon-water partition coefficient (K_{oc}), which strongly linearly correlates with the octanol/water partition coefficient (K_{ow}) and it determines the affinity of a chemical to adsorb on soils:

$$K_{oc} = \frac{K_d}{f_{oc}} \quad (1)$$

$$\log K_{oc} = A \cdot \log K_{ow} + B \quad (2)$$

being A and B compound class-specific empirical coefficients. Unfortunately, the $\log K_{ow}$ is not sufficient for the assessment of the sorption and distribution behavior of antibiotics because it strongly depends on the chemical structure and on the speciation (charge state) of the ionisable organic sorbate (bases, acids, ampholytes; Schaffer and Licha, 2015). Antibiotics are complex molecules as they can contain acidic and basic groups within their structure, and therefore, sorption or distribution between water and soil depends on the solution pH. Consequently, pH-dependent speciation of the sorbates within the water phase must be considered for ionizable compounds. Therefore, the estimation K_{oc} for compounds having several functional groups, such as zwitter ions whose net neutral fraction displays simultaneously positive and negative charge, is not a straightforward task. Experiments by Franco and Trapp (2008), successfully predicted the sorption of organic electrolytes with cationic dissociating groups (cations and amphoters) to organic carbon (K_{oc}) using the octanol-water partition coefficient of the neutral molecule and its dissociation constant (pK_a). Conversely, predictive equations for acids with $pK_a < 6$ were difficult to develop. They found that the sorption of anions is generally low (K_{oc} between 10 and 300 L/kg) which cannot be explained from the aforementioned two variables.

As mentioned, the calculation of K_{oc} values for ionisable compounds includes an extended concept considering the pH-dependent speciation of sorbates within the water phase. Then, the pH-dependent n -octanol/water distribution coefficient $\log D$, as an apparent $\log K_{ow}$ value, is introduced into Eq. (2) following Schaffer et al. (2012) formulation:

$$\log K_{oc} = A \cdot \log D + B \quad (3)$$

Values for the empirical parameters A and B are extensively published in the literature. In this study, the empirical descriptors for non-hydrophobic compounds values from Sabljic et al. (1995) of $A = 0.52$ and $B = 1.02$ are used. Nevertheless, these authors also determined

distinct A and B values for predominantly hydrophobic chemicals (0.81 and 0.10, respectively), indicating that the precision of estimates is lower for the more hydrophobic substances ($\log K_{ow}$ data from 4.0 to 7.5). The corresponding $\log D$ for acidic or basic compounds was calculated using literature values for $\log K_{ow}$ and for the dissociation constant pK_a , which accounts for the degree of ionization of the compound for every pH value:

$$\log D_{acid} = \log K_{ow} + \log (\Phi_n) = \log K_{ow} + \log \left(\frac{1}{1 + 10^{pH-pK_a}} \right) \quad (4)$$

$$\log D_{base} = \log K_{ow} + \log (\Phi_n) = \log K_{ow} + \log \left(\frac{1}{1 + 10^{pK_a-pH}} \right) \quad (5)$$

where Φ_n refers to the speciation of monovalent ionic compounds according to the Henderson-Hasselbalch equation (Franco and Trapp, 2008). Schaffer et al. (2012) discusses the limitations of Eq. (3), which apply particularly to compounds having several functional groups, as zwitter-ions, with more than one pK_a value. They also advise about the transferability and limitations of the K_{oc} concept as it only considers non-polar interactions and partitioning to the sediment total organic carbon content, disregarding additional processes as ion-exchange, cation bridging, surface complexation and hydrogen bonding. A summary of values from the compound-specific parameters involved in Eqs. (4) and (5) taken from references are reported in Supplementary Material 1 (SM1).

Nevertheless, such approximations to the K_{oc} value, and therefore to K_d , permit an estimation of the migration and fate in such reactive solutes in the subsurface. Sorption processes lead to a retardation of the sorbates, in our case antibiotics, compared to the groundwater flow and conservative solutes (i.e., non-sorbing species as, for instance, chloride or bromide). The retardation factor R , considering the assumption of linear sorption processes, is defined as (e.g., Freeze and Cherry, 1979, p. 404):

$$R = \left(1 + \frac{\rho}{\theta_e} K_d \right) \quad (6)$$

where ρ is the bulk density, θ_e is the effective porosity and K_d is the distribution coefficient.

1.1.1.2. Biodegradation. Besides sorption, other processes like the role of microorganisms in degradation are recognized as removal pathways of organic molecules in the environment (Gothwal and Shashidhar, 2015). Biodegradability of antibiotics has been poor for most of the performed laboratory studies (Kümmerer, 2009a, 2009b), and there are only very few studies that investigate biodegradation of pharmaceuticals in groundwater. However, biodegradation is expected to be limited since aerobic conditions in the subsurface are restricted, and microbial diversity and activity are low compared to other environments. Moreover, the redox conditions and mixtures of organic contaminants in certain groundwater systems may preserve these contaminants for longer than would be expected under aerobic conditions, leading to incomplete or null degradation of antibiotics. Veterinary antibiotics were found to persist in groundwater below poorly sealed lagoons due to the anaerobic groundwater conditions (Watanabe et al., 2010). In addition, it was observed that the contrasting redox conditions below the lagoons (anaerobic conditions) and the manure sites (aerobic) may also strongly influence the potential of degradation, and therefore, their leaching towards the water table.

The molecular structure and physico-chemical characteristics of the antibiotics also determine the biodegradability of the compound. For instance, sulfamethoxazole underwent less biodegradation compared to other pharmaceuticals in microcosm studies (Benotti and Brownawell, 2009; Grossberger et al., 2014). Another study showed that no reduction for ciprofloxacin, and only a 5% reduction for ofloxacin were observed in a batch test (Kümmerer et al., 2000).

1.1.1.3. Hydrochemical conditions. Groundwater hydrochemistry is paramount on the behavior of antibiotics in groundwater. Chen et al. (2011) studied the effect of ionic strength and pH on the retention and transport of sulfamethoxazole and ciprofloxacin in saturated porous media and showed that sulfamethoxazole was more mobile in groundwater than ciprofloxacin.

It becomes evident from this theoretical background that the reactive component of antibiotic mass transport process will play a very relevant part in their occurrence in groundwater. In this sense, we emphasize sorption processes and, in particular, considering the retardation factor. It provides a quantitative idea of each compound migration rate with respect to groundwater flow, despite the uncertainty resulting from the wide range of all involved coefficients as a function of the soil/aquifer characteristics. Biodegradation, yet difficult to quantify from field data, is another process of high concern to determine the fate of these pollutants in the subsurface.

1.1.2. Characteristics of the behavior of the main antibiotic groups in groundwater

The main hydrogeochemical characteristics of antibiotic groups investigated in the study area are herein summarized as a framework of their occurrence in groundwater:

- **Fluoroquinolones:** Antibiotics of this group strongly absorb, and tend to accumulate in soil and sediments. Fluoroquinolones have several potentially ionisable functional groups, which will depend on the physico-chemical properties of the solvent. Moreover, the antibacterial activity of these compounds is pH-dependent. Within environmental pH ranges, fluoroquinolones exist mostly as zwitterions which favour their hydrophobicity (Vazquez-Roig et al., 2012). A study from Drillia et al. (2005) found that ofloxacin was more strongly absorbed in soil than other pharmaceuticals. Enrofloxacin is the most lipophilic compound within the antibiotics detected in this study, with a K_{OW} of 12.59 (SM1). Ciprofloxacin, enrofloxacin, norfloxacin and ofloxacin have high K_d values and they rapidly move from water to soil/sediments, mainly because of their lipophilicity. According to a classification of pesticide mobility in soil, these fluoroquinolones can be considered as immobile (Tolls, 2001). Nonetheless, these antibiotics have very high solubility coefficients in water (SM1). As a reference, ciprofloxacin is the most widely prescribed fluoroquinolones in the world, and orbifloxacin one of the most important veterinary-used fluoroquinolones (Picó and Andreu, 2007), yet not many studies have detected fluoroquinolones in groundwater samples (Barnes et al., 2004; Focazio et al., 2008; Leal et al., 2013).
- **Quinolones:** Quinolones are polar, mostly amphoteric and exhibit poor water solubility at pH 6–8. Quinolones are likely to persist in the environment and they are considered as immobile in soils (Boxall et al., 2002). Several studies detected quinolones at concentrations in the lower hundreds of ng/L in river water (Carvalho and Santos, 2016; and references herein). López-Serna et al. (2010) also reported the occurrence of quinolones in drinking water in Barcelona (Spain) at similar concentrations than in this study.
- **Tetracyclines:** they are the most frequently used antibiotics in veterinary medicine. Tetracyclines have three pK_a values, although at pH between 7 and 8 they are negatively charged in water and are expected to be sorbed to sediment. They tend to form stable complexes and as a result, they are more likely to remain on the topsoil or bind to suspended organic matter. Therefore, their presence in surface or groundwater is not prevalent, and they have just been detected in a few studies (Boxall et al., 2002).
- **Macrolides:** high concentrations of macrolides in aqueous environment are not expected due to their low water solubility and sorption capacity (high values of K_{OW}) as shown in SM1. However, these antibiotics persist in the environment and in water as a consequence of their considerable and continuous consumption in both veterinary and human medicine (Carvalho and Santos, 2016).

- **Sulfonamides:** they are one of the oldest antibiotic groups that are still currently in use and the most extensively studied group. Occurrence of sulfonamides in groundwater has been reported in several studies (Sui et al., 2015; and references herein), and it is important to highlight the high frequency of detection of these antibiotics in water, in particular sulfamethoxazole. In fact, sulfonamides is the most frequently reported group of pharmaceuticals in groundwater in Catalonia (Jurado et al., 2012), and sulfamethoxazole was among the most prevalent antimicrobial contaminant detected in a nationwide groundwater survey conducted by the U.S. Geological Survey (Underwood et al., 2011). The widespread occurrence of sulfonamides in groundwater might be a result of their weak sorption to soil, low biodegradability and fast migration (Strauss et al., 2011), which makes these compounds likely to leach to groundwater bodies.
- **Lincosamides:** even though lincosamides are generally used in veterinary medicine, they are not commonly detected in aqueous matrices, neither in this study case, due to their limited solubility in water and moderately high sorption potential.
- **Nitroimidazoles:** the presence of nitroimidazoles in water has been analyzed and reported in several studies, even though their consumption was prohibited for use in animal husbandry in the European Union (Council Regulation EEC, No 2377/90). Metronidazole is a weak base and moderately lipophilic, and it is the most studied antibiotic of this class. This compound and its metabolite metronidazole-OH were analyzed in this study; however, they were not detected in any of the sampling points.

1.2. Hydrogeological setting and characteristics of the study site

The study site (near 60 km²) is in a fluvio-deltaic area located in the Alt Empordà region (NE Catalonia, Spain; Fig. 1). Mean annual rainfall in Sant Pere Pescador is 566.4 mm (2007–2015; www.ruralcat.net). The Fluvià River drains 1125 km² flowing into the Mediterranean Sea, and it has an average discharge of 3.7 ± 0.2 m³/s in the Esponellà gauging station, few kilometers upstream from the study area (ACA, 2017). The region surrounding the lower reaches of the Fluvià River, also known as Baix Fluvià, constitute an alluvial plain formed by sediments of the Fluvià and nearby the Muga rivers during the Pleistocene and Holocene (ICG, 1996). The Quaternary fluvio-deltaic infilling of the Fluvià River plain takes place in the Empordà neogen tectonic graben, which in its southern limit is characterized by neogene normal faults and paleogene fold-and-thrust structures that allow the outcropping of Mesozoic limestone formations. The displacement of these allochthonous units was enhanced by the Upper Triassic (Keuper) gypsum and clay layers.

The hydrogeological connection between the Fluvià River and the aquifer takes place across the upper sedimentary sequence, which consists of a 10–15 m thick unit of coarse materials (gravel and sand). This unit becomes thinner to the east as it is replaced by finer materials, sand and silt, which may still include some gravel lenses. The bottom of this unconfined aquifer is constituted by a level of silt and loam belonging to a lower sedimentary sequence that acts as an aquitard. However, it also overlays deeper coarse detritic layers to the west, which belong to the lower sedimentary sequence, and they are both in hydrological connection (Mas-Pla et al., 1999). The hydrogeological system of the Baix Fluvià is influenced by the stream-aquifer relationship. In this sense, the unconfined alluvial aquifer is mainly recharged by river infiltration, local precipitation and, in less extent, by irrigation returns in summer. Geological boundaries with Neogene sedimentary formations are essentially considered as flow boundaries as they contribute to the recharge of the alluvial formation through mountain front deposits as Pleistocene alluvial fans and terraces. Some additional recharge from regional upward flows through the main fault and thrust-belt zones is also expected. Natural aquifer discharge takes place towards the Fluvià River, except when summer irrigation drawdown does not capture stream discharge, and to the Mediterranean Sea.

Irrigation rates, estimated in 4.9 hm³/year, stand as a main pressure on the aquifer water resources, since the dominant land use is agricultural.

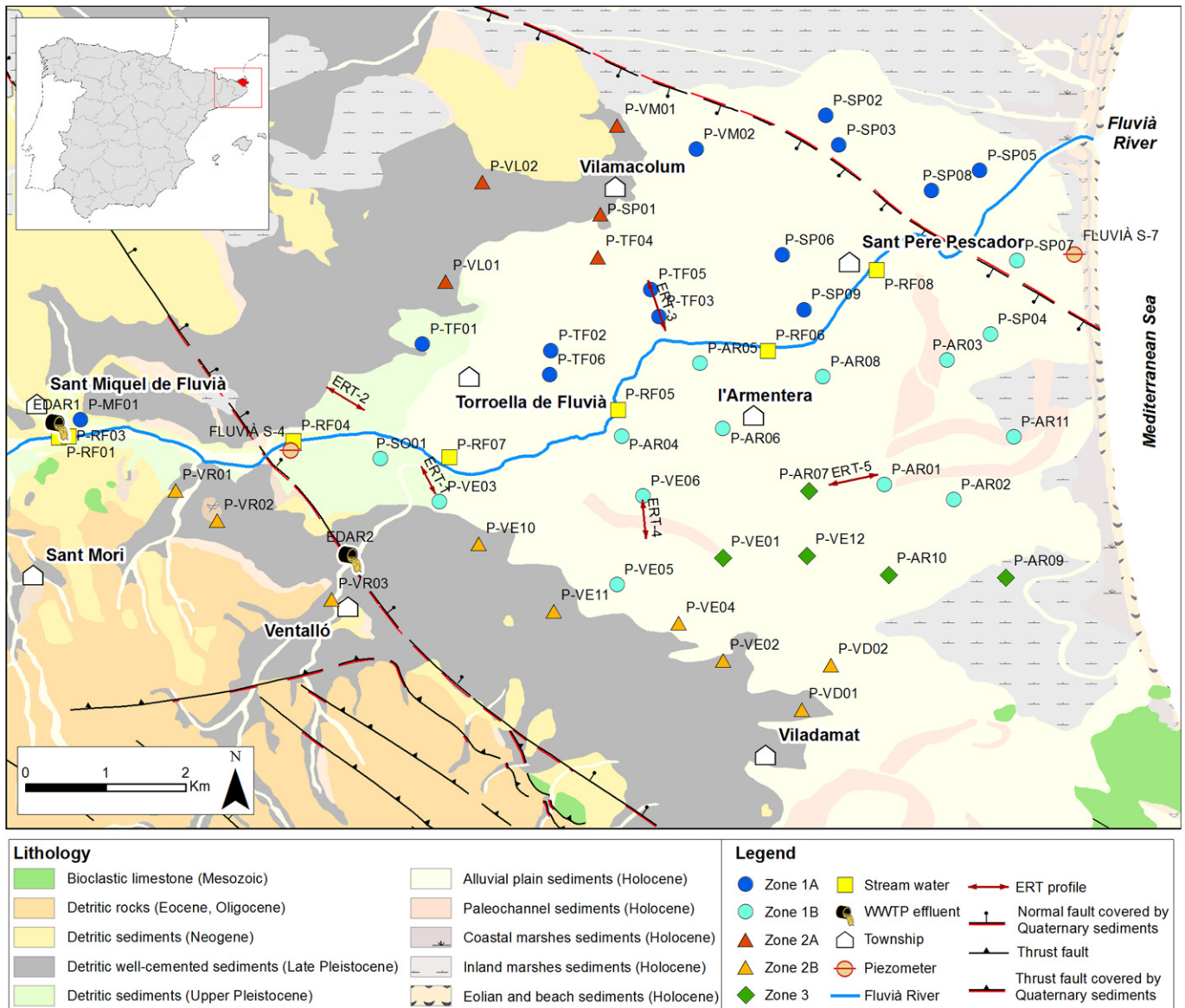


Fig. 1. Map of the geographical and geological setting of the study area, with the location of the sampling points classified in different zones, ERT profiles and piezometers. Geological formations are mapped according to the main lithology in the area (geological cartography simplified from ICCG, 1996).

Up to 61.3% (3212 ha) of the agricultural land (5239 ha) is irrigated using groundwater supply, being apple orchards (958 ha), corn (849 ha), barley (324 ha), wheat (251 ha) and alfalfa (197 ha) the largest water consumers (DARP, 2014). Furrow irrigation is used for corn fields, while drip irrigation is used in orchards. Poultry and pig farming are the main livestock farming industry in the area, with 254,290 and 59,940 head of livestock, respectively (SIR, 2014). Pig farming is the principal nitrogen producer with 412 tons of N/year, which are used a source of nitrogen for cereal crops (as corn, barley and wheat). Nevertheless, the Catalan Water Agency has detected that groundwater quality in the Baix Fluvià aquifer is affected by agricultural practices that include the use of nitrogen fertilizers and pesticides (ACA, 2013). In fact, 6 out of 7 municipalities (78.8% of the area) of the study area are declared as vulnerable zones to nitrate pollution from agricultural sources by the Catalan Parliament (Acord GOV/13/2015). The total population of this area is 6124 inhabitants which rely on groundwater resources for their supply. Wastewater from these municipalities is treated in 2 small waste water treatment plants (WWTP) located in Sant Miquel de Fluvià and Ventalló. These two WWTP represent a 26.7% of the area population, and they dump their effluents, one, to the Fluvià River and, the other, in a small drain

where it usually infiltrates to the subsurface. The sewage system from the two largest towns in the region (Sant Pere Pescador and l'Armentera, 49.6% of the area population) is connected to a bigger wastewater treatment plant located outside the study area. Isolated farms and houses accumulate their wastewater in cesspits and septic tanks, which later infiltrate to the aquifer.

Three distinct zones have been distinguished as hydrogeological references in all plots: zones 1A and 1B refers to the alluvial aquifer in its left (north) and right (south) banks of the Fluvià River, respectively; zones 2A and 2B denote the margins of the alluvial formations in the left and right banks of the Fluvià River, respectively; and zone 3 includes a few wells with distinct hydrochemical features in the distant parts of the right margin alluvial aquifer.

2. Methodology

The sampling campaign during May–July 2015 consisted of the collection of 47 groundwater samples from private wells mainly used for irrigation and farming. Well depths were between 12 and 20 m. Moreover, 7 samples along the Fluvià River and 2 samples from the existing

WWTP effluents were collected (Fig. 1). Geophysical exploration (5 cross-sections) was conducted during the sampling period.

Physico-chemical parameters include electrical conductivity (EC), pH, redox potential (Eh), dissolved oxygen (DO), and temperature. Hydrochemical analysis consists of major hydrochemical components, total nitrogen (TN) and total organic carbon (TOC), and the concentration of a selection of 53 human and veterinary antibiotics including 10 chemical groups. Stable isotopes of the water, nitrate and sulfate molecules were also analyzed, and tritium, were analyzed for hydrogeochemical completeness.

Additional river samples were taken in the following year (October 2015 – September 2016) at a monthly basis to determine a mean value of its hydrochemical and isotopic parameters. Rainfall samples were also collected at a meteorological station located in Verges, located about 5 km south from the study area, for hydrochemical, water stable isotopes and tritium data.

Supplementary Material 2 (SM2) provides a description of the sampling methodology and analytical procedures used in hydrochemical, isotopic and antibiotic analyses. Geophysical, and statistical methods, including geostatistics, are also reported. Potentiometric data were obtained from Water Catalan Agency records (ACA, 2017).

3. Results

3.1. Geophysics and geology of the alluvial aquifers

Electrical tomography cross-sections reveal a continuous alluvial plain that varies in thickness and lithology from the westernmost parts of the fluvio-deltaic formation to the coast. A Quaternary sedimentary formation formed by unconsolidated detritic sediments ($T_1 + T_2$ in Fig. 2), overlays the low resistivity of the Neogene (Pliocene) formation (NP in Fig. 2) in those cross-sections located in the central part of the aquifer. Neogene materials constitute the lower limit of the alluvial plain, and they are considered as a no-flow boundary in the conceptual hydrogeological scheme. The Quaternary formation presents two distinct sedimentary units of higher and medium resistivity values (namely, T_1 and T_2 in Fig. 2), attributed to the Fluvià River sedimentary sequences controlled by the sea-level fluctuations (ICGC, 1996; ICGC/ACA, 2014). In the inland areas, they are mainly constituted by clean gravels and sand layers represented by high resistivity units (ERT1 and ERT2). In the central area (ERT3 and ERT4), medium resistivity values suggest a major proportion of sand and fine sediments, especially in the upper unit (T_1). Lithological differences between T_1 and T_2 are pointed out by ERT5: the lower layer (T_2) presents a higher resistivity indicating the occurrence of imbedded coarser sediments bodies, while the upper layer (T_1) presents a major proportion of fine sediments (lower resistivity) given by a prograding sedimentary unit towards a coastal environment. Transition between T_1 and T_2 in the eastern part may include a fine sediment layer that converts T_2 in a leaky aquifer. Older alluvial fan deposits and fluvial terraces (Pleistocene, named both as Q_p in Fig. 2) are interbedded between the fluvial sediments and the Neogene basement. These deposits are formed by coarse, unstructured sedimentary materials, which locally present a high degree of cementation.

The thickness of the fluvial-deltaic deposits varies from 15 to 20 m in the inland areas, up to 40–45 m near the coastline. Most of the sampling wells were drilled in this fluvial formation exploiting levels T_1 and T_2 , indistinctively, as they present a neat hydraulic connection in most of the study area. Well depths rarely attain >20–25 m.

3.2. Potentiometric data

Potentiometric maps of the Fluvià alluvial aquifer point out the stream influence in the western part of the alluvial plain where recharge takes place along the year. Under natural, non-influenced by pumping conditions, the Fluvià River acts as a gaining stream in its eastern

reaches. Nevertheless, stream capture during the irrigation season may reverse this behavior turning the river as a continuous losing stream in all its length across the alluvial. Non-influenced hydraulic gradients in the winter season are usually between 0.001 and 0.002, which may even be lower in summer. Approximate hydraulic conductivity values of the alluvial aquifer plain are of 25 to 75 m/d, and porosity between 0.05 and 0.10 (Mas-Pla et al., 1999). Continuous monitoring of hydraulic head levels since 2000 by the Catalan Water Agency (ACA, 2017) indicates that annual differences between maximum and minimum head values in three piezometers located in the Baix Fluvià alluvial aquifer were between 0.26 and 1.80 m, with a mean of 0.98 ± 0.06 m. More importantly, recharge in winter and springtime is able to retrieve hydraulic heads back to their maximum level, indicating a complete recovery of the exploited groundwater resources (see data at ACA, 2017).

From a pollution perspective, potentiometric data suggested that infiltrating rainfall and groundwater fluxes from the stream and the aquifer boundaries efficiently contributed to the recharge of the Baix Fluvià unconfined aquifer. Therefore, an effective leaching of those pollutants originated from fertilization (nitrate, antibiotics) is expected from most of the arable land, as well as a contribution (or dilution depending on the compound) from the stream and boundary fluxes, which are also paramount to understand pollutant occurrence.

3.3. Hydrochemistry

Supplementary material 3 (SM3) presents the values of the relevant hydrochemical and isotopic parameters. Groundwater samples were classified in different zones (Fig. 1) according to their location in the alluvial plain (whether from the right or left river bank, or the surrounding margins), their lithology, and their hydrochemical properties. Hydrochemical results showed that surface water-groundwater interaction has a relevant influence in the aquifer flow system. Piper diagram shows that groundwater samples from the alluvial aquifer (zones 1A and 1B) are closely related to surface water chemistry, plus the effect of infiltrating rainfall and, at a minor extent, some contribution from the flow originated in the alluvial margins (zones 2A and 2B; Fig. 3). Those samples in zones 2A and 2B show a large variability, especially in their sulfate content, due to the mixing with alluvial water affected by stream recharge. Two other wells (P-AR11, P-SP05) show some contribution of seawater intrusion whether from the Mediterranean Sea or the salty water of the lower reach of the Fluvià River (Mas-Pla et al., 1999). Samples in the alluvial plain located on both sides of the Fluvià River (Zone 1A and 1B) show a sulfate content similar than that of stream water, around 250 mg/L (Fig. 4). Sulfate content decreases in distance from the river as it mixes with rainfall recharge and the lateral inflows from the alluvial aquifer boundaries. Sulfate is herein taken as a tracer since the Fluvià River drains Tertiary (Eocene) evaporitic rocks (gypsum) in the headwaters of the catchment, and it presents an average concentration of 222 ± 12 mg/L at sampling location P-RF03 (Sant Miquel de Fluvià). It is worth mentioning the occurrence of samples with even larger sulfate content ($SO_4 > 300$ mg/L) with a Ca/ SO_4 ratio of ≈ 0.50 , expressed in mg/L, which approaches the mass ratio of 0.42, indicating that calcium origin is mainly due to gypsum dissolution rather than calcite (Fig. 4).

Furthermore, some wells in the south-eastern part of the study area (Zone 3) showed a singular behavior. Their larger sulfate content (mean concentration of 341 ± 34 mg/L) is attributed to an upward flow from the Mesozoic units that constitute the aquifer basement. Mesozoic units are highly affected by thrust faults that involve the Upper Triassic evaporitic layers, mainly constituted by gypsum and clay. The contribution of the Upper Triassic (Keuper) gypsum dissolution to the alluvial aquifer sulfate content is later on validated using a multi-isotopic approach.

Nitrate in groundwater samples, expressed as NO_3 concentration, ranged from 8 to 187 mg/L, with a mean value of 42.8 ± 7.2 mg/L, and 20% of the wells had nitrate concentrations higher than the drinking

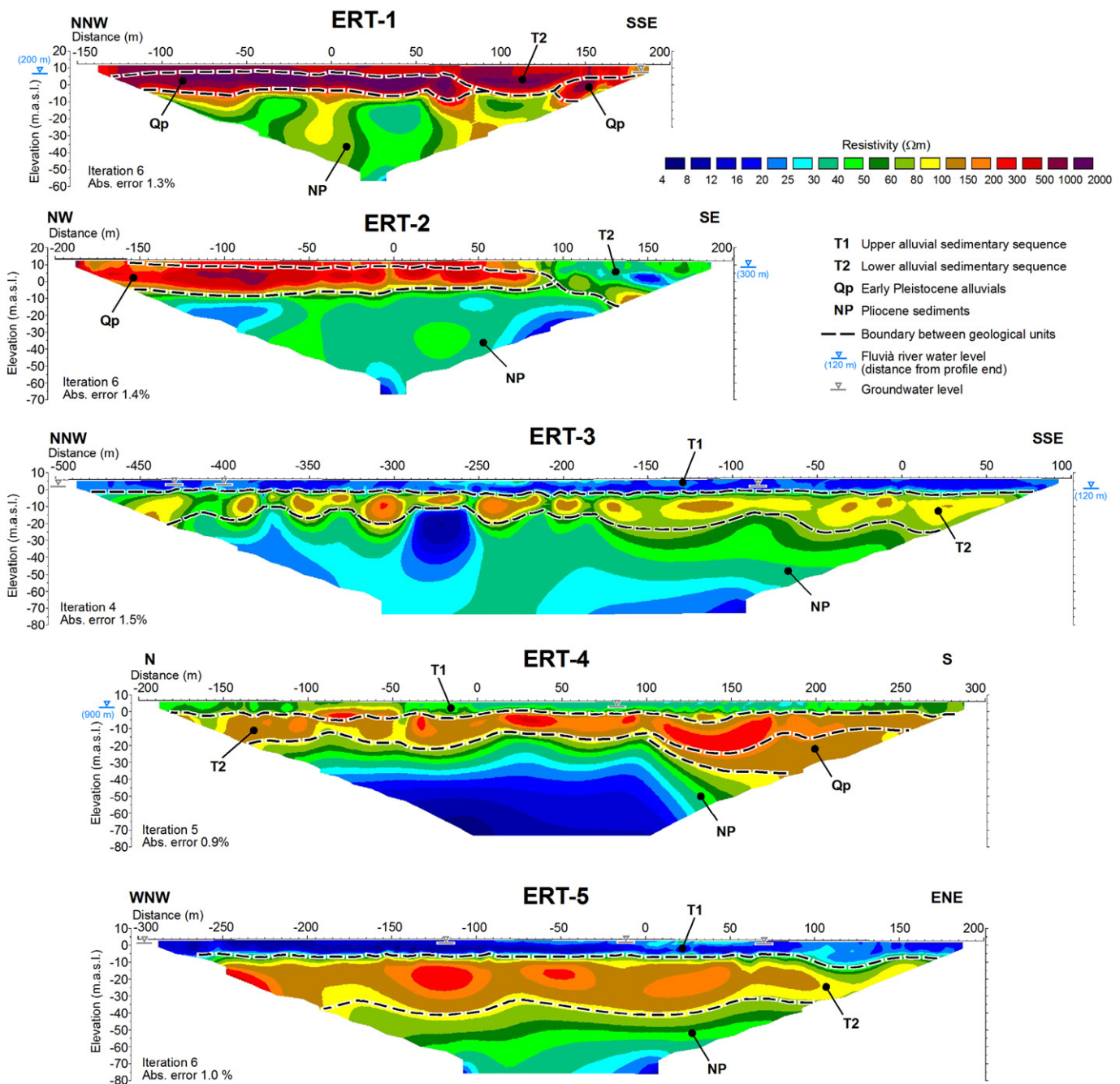


Fig. 2. Electrical Resistivity Profiles (ERT) performed in the Baix Fluvià alluvial aquifer.

water threshold of 50 mg/L (Table SM2). Lower concentrations of nitrate were observed nearby the Fluvià river (Zones 1A and 1B with concentrations lower than 20 mg/L) suggesting a dilution effect by stream water (mean nitrate concentration was 7.3 ± 0.4 mg/L). Contrarily, samples located in the margins had larger nitrate contents (Zone 2A and 2B with concentrations around 90 mg/L). Given a similar land-use type, such high nitrate values are potentially related to the fact that rainfall recharge dominates in these areas causing a continuous input of nitrogen from fertilization practices unaffected by the stream dilution effect.

Dissolved oxygen (DO) has a mean value of 5.6 ± 0.3 mg/L, and Eh average is 117 ± 10 mV. Redox potential values lay in a range tending to reduced conditions that may enhance denitrification. Large DO values suggest some relevant inputs of infiltrating water from the vadose zone.

3.4. Environmental isotopes

Values of water stable isotopes ($\delta^{18}\text{O}_{\text{H}_2\text{O}}$ and $\delta^2\text{H}_{\text{H}_2\text{O}}$) for groundwater samples ranged from -6.4% to -5.5% , with a mean value of $-6.0 \pm 0.03\%$ for $\delta^{18}\text{O}_{\text{H}_2\text{O}}$, and from -39.3% to -35.0% , with a mean value of $-37.7 \pm 0.17\%$ for $\delta^2\text{H}_{\text{H}_2\text{O}}$ (Fig. 5). Values of $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ for surface water samples along the Fluvià River in the 2015 campaign were less variable, with a mean value of $-5.90 \pm 0.04\%$ and ranging from -6.1% to -5.8% , and for $\delta^2\text{H}_{\text{H}_2\text{O}}$ the mean value is $-37.09 \pm 0.22\%$, ranging between -37.8% to -36.3% . In order to compare surface water stable isotopes with those of groundwater, mean values from the monthly dataset were used to obtain an integrated value for the Fluvià River discharge. Mean monthly values for $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ and $\delta^2\text{H}_{\text{H}_2\text{O}}$ were $-6.3 \pm 0.02\%$ and $-38.6 \pm 0.12\%$, respectively. Considering these mean values, surface water stable isotopes show lighter values than

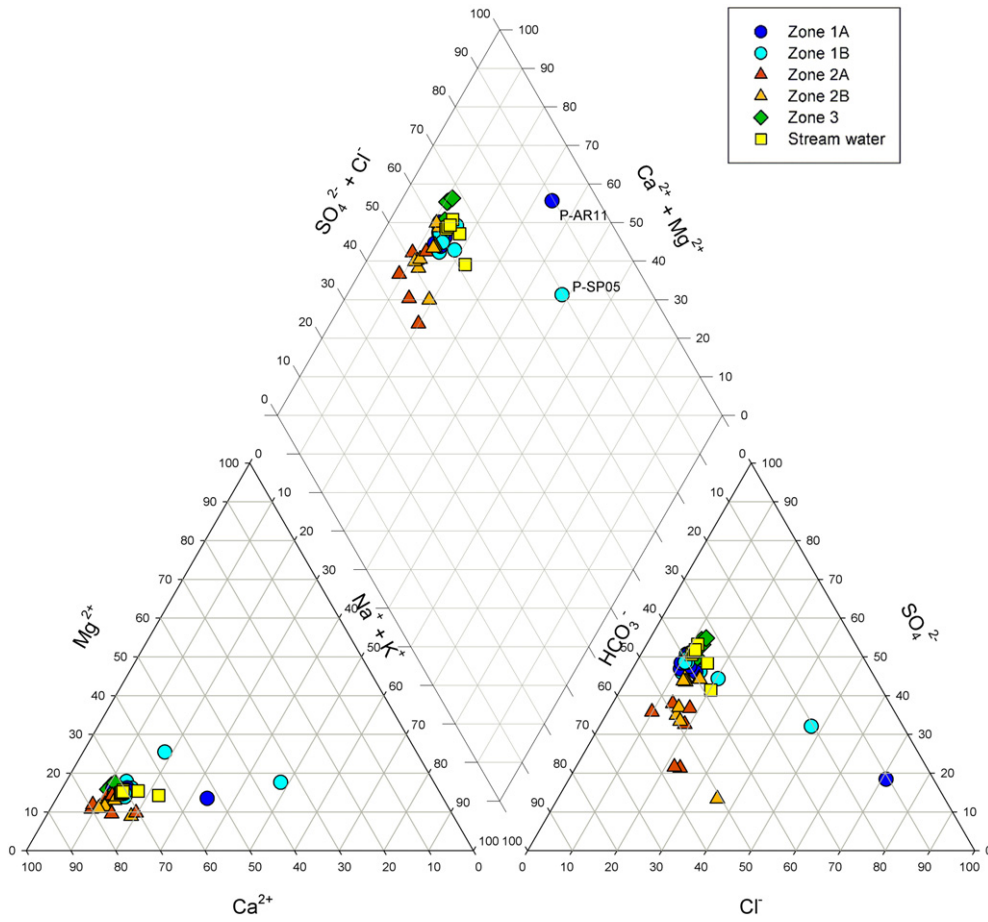


Fig. 3. Piper plot of the groundwater samples (according to the distinct zones in which the aquifer has been herein divided) and surface water from the Fluvia River.

groundwater, since groundwater recharge is a mixture of rainfall recharge, which takes place at low altitudes in the alluvial plain, and surface water exchange, which comes from higher altitudes (highest basin elevation around 900 m a.s.l.). Indeed, the mean isotopic content for rainfall at the Verges station, at 14 m a.s.l., was of $\delta^{18}\text{O}_{\text{H}_2\text{O}} = -5.8 \pm 0.8\text{‰}$ and $\delta^2\text{H}_{\text{H}_2\text{O}} = -37.9 \pm 5.7\text{‰}$ based on 13 samples from monthly accumulated rainfall from April 2015 to May 2016. This value differs from another more complete series of 35 rainfall isotopic data

($\delta^{18}\text{O}_{\text{H}_2\text{O}} = -5.6\text{‰}$, $\delta^2\text{H}_{\text{H}_2\text{O}} = -34.7\text{‰}$; Vilanova et al., 2008) sampled only 3 km away from Verges from 1997 to 2001.

According to the isotopic altitudinal line defined in the Pre-Pyrenees region (Brusi et al., 2011), north from the study area, stream water corresponds to a mean recharge altitude of 450 m, which is consistent with the Fluvia River basin hypsometric curve. Samples from the aquifer margins, which are expected to recharge only from local rainfall, show an

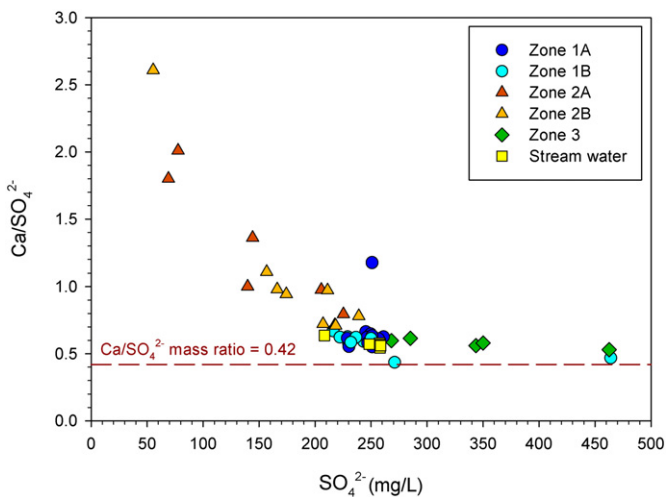


Fig. 4. Bivariate relationship of sulfates and calcium/sulfate ratio for groundwater and surface water samples. The dashed line represents the $\text{Ca}/\text{SO}_4^{2-}$ mass ratio.

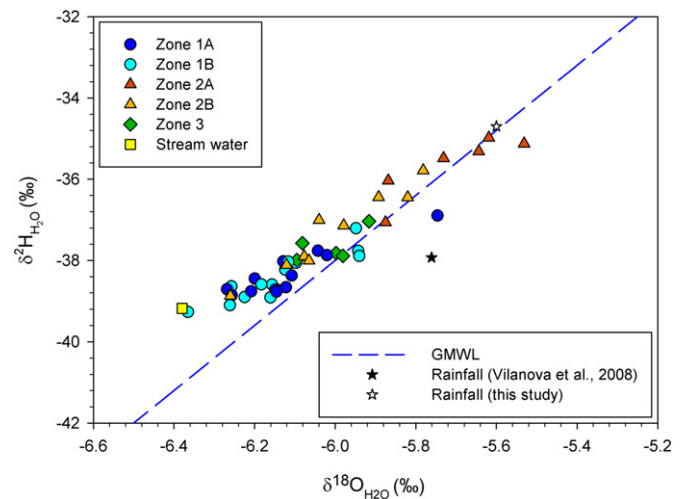


Fig. 5. Water stable isotopes ($\delta^{18}\text{O}_{\text{H}_2\text{O}}$ and $\delta^2\text{H}_{\text{H}_2\text{O}}$, ‰ vs. V-SMOW) diagram for groundwater samples and mean value for surface water. The GMWL is plotted in a dashed line and values for rainfall from Verges weather station and from Vilanova et al. (2008) are represented as a white and black star, respectively.

isotopic composition consistent with the altitudinal range of the surrounding hills between 50 and 100 m. Local rainfall isotopic data represent sea-level altitude. Consequently, samples from the central part of the alluvial aquifer can then be understood as a mixing between stream water and local rainfall recharges at different proportions. Moreover, d -excess values show low dispersion, with a mean value of $10.56 \pm 0.08\text{‰}$, suggesting that no other sources or processes but mixing between these two end-members are necessary to explain the hydrogeological dynamics (Fig. 4). Zone 3 samples, partially recharged by an upward flow from the basement, are not distinguished as water stable isotopes. Although estimate mixing percentages is not the point of this study, this conceptual model based on hydrochemical and isotopic data sets up a framework, also in agreement with potentiometric data, where to discuss the fate and migration of antibiotics in the Baix Fluvià aquifer.

Nitrate isotopes in groundwater ranged between 3.7‰ and 13.3‰ for $\delta^{15}\text{N}_{\text{NO}_3}$, with a mean value of $9.2 \pm 0.29\text{‰}$, and between 1.7‰ and 9.5‰ for $\delta^{18}\text{O}_{\text{NO}_3}$ with a mean value of $5.1 \pm 0.28\text{‰}$. Most samples are originally in agreement with isotopic values from manure or sewage water, and from organic nitrogen from the soil as well (Fig. 6A), yet some minor mixing percentages with nitrate from chemical fertilizers, as those used in orchards, also occur. In this particular case, oxygen isotopic values ($\delta^{18}\text{O}_{\text{NO}_3}$, which are not affected by fractionation associated

to volatilization processes) higher than 15‰ are attributed to denitrification. During denitrification, as nitrate concentration decreases, residual nitrate becomes enriched in heavy isotopes with an isotope enrichment $\varepsilon_{\text{N}}/\varepsilon_{\text{O}}$ ratio that ranges between 1.3 (Fukada et al., 2003) and 2.1 (Böttcher, 1990). In this case, we link denitrification to the presence of organic matter in the aquifer matrix, as stated by Puig et al. (2016) in a similar alluvial aquifer located nearby.

Nitrate isotopic values in surface water samples ranged from 10.9 to 13.3‰ for $\delta^{15}\text{N}_{\text{NO}_3}$ and from 5 to 6.9‰ for $\delta^{18}\text{O}_{\text{NO}_3}$. These values are in agreement with isotopic values from sewage, and could be explained by the dumping of wastewater treatment plant effluents along the Fluvià River watercourse. Fig. 6B shows $\delta^{15}\text{N}_{\text{NO}_3}$ vs. $\ln(\text{NO}_3)$, revealing the influence of two end-members on the nitrogen isotopic signature of groundwater: surface water with low nitrate concentrations and $\delta^{15}\text{N}_{\text{NO}_3}$ between 11‰ and 14‰; and a second pole with lower $\delta^{15}\text{N}_{\text{NO}_3}$ values between 6 and 8‰, laying in the lower range similar to pig manure isotopic values (Vitória, 2004). Wells located nearby the river are more influenced by surface-water inputs, while wells in the margin areas of the study area presented $\delta^{15}\text{N}_{\text{NO}_3}$ similar to manure fertilizers, which is consistent with the hydrochemical results. The large dispersion of the values along a potential mixing line between these two poles points out that geochemical processes affecting nitrogen evolution in the subsurface, as denitrification in some samples, are also relevant. Denitrification processes, however, are complex in the aquifer due to the distinct original isotopic values of the different applied manures and the evidence of distinct fractionation stages (i.e., some samples show larger denitrification levels than others; Fig. 6A). Therefore, nitrate occurrence and spatial distribution is not solely explained by the groundwater flow pattern and its effects on solute migration, being natural attenuation processes also fundamental to describe nitrate occurrence.

In the case of sulfate isotopes, groundwater values varied from 6.1 and 15.6‰ for $\delta^{18}\text{O}_{\text{SO}_4}$, and from 5.5 to 18.6‰ for $\delta^{34}\text{S}_{\text{SO}_4}$. Values of $\delta^{18}\text{O}_{\text{SO}_4}$ in surface water varied from 12.1 and 17.3‰ (mean value equal to $13.3 \pm 0.68\text{‰}$) and from 17.1‰ to 18.1‰ (mean value of $17.7 \pm 0.12\text{‰}$) for $\delta^{34}\text{S}_{\text{SO}_4}$ (Fig. 7A and B). The use of sulfate isotopes has been paramount to differentiate the distinct groundwater sources that recharge the alluvial aquifer. For instance, it became clear that most of the samples from the alluvial plain (zones 1A and 1B) show similar isotopic values than surface water. Moreover, margin samples corresponding to Zones 2A and 2B, as well as those from Zone 3, clearly differed from those of the alluvial plain based on sulfate isotopes, tending to lower isotopic ratios: samples from Zones 2A and 2B tended to 5‰ and low sulfate content, whereas Zone 3 tended to 10‰ and even higher sulfate concentrations (Fig. 7A).

The joint use of sulfur and oxygen isotopes of the sulfate molecule allows a further interpretation of these groundwater samples once the average isotopic ranges of the main sulfate sources were plotted in Fig. 7B. Geological sulfate origin can derive from Tertiary (Eocene) evaporitic rocks, which outcrop in the headwaters of the Fluvià River basin, and the Triassic (Keuper) evaporitic layers involved in the thrusts faults that appear in the subsurface in the southern area of the alluvial aquifer. It can be observed that most of the surface water and alluvial plain samples are related to the Tertiary gypsum, with a mean value $\delta^{34}\text{S}_{\text{SO}_4} = 21.4 \pm 0.4\text{‰}$, and $\delta^{18}\text{O}_{\text{SO}_4} = 12.6 \pm 0.9\text{‰}$ (Utrilla et al., 1992), introduced to groundwater by the effect of stream recharge from the Fluvià River. Conversely, samples from Zone 3 lay closer to the Keuper isotopic region ($\delta^{34}\text{S}_{\text{SO}_4} = 13.7\text{‰}$, and $\delta^{18}\text{O}_{\text{SO}_4} = 11.7\text{‰}$; Utrilla et al., 1992), indicating that upward vertical flows from the Mesozoic basement lying below the quaternary and Neogene deposits also play an important role as inputs to the alluvial aquifer water balance. Present seawater appears to have no influence, as reflected by the geochemical and water stable isotope data, despite the salty stream water infiltration during the irrigation periods. This points out that, at a regional level, stream recharge is mainly significant at the westernmost reaches of the stream. Low isotopic values found in samples of zones 2a and 2B are in

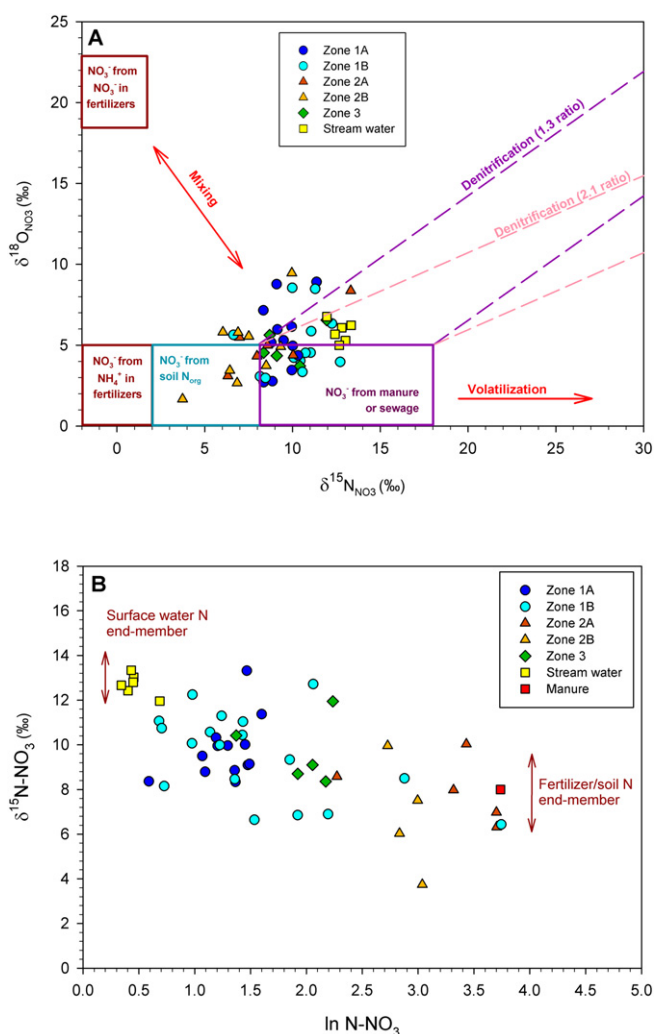


Fig. 6. A) Nitrate isotopes ($\delta^{15}\text{N}_{\text{NO}_3}$ and $\delta^{18}\text{O}_{\text{NO}_3}$) diagram. Boxes represent the isotopic composition of nitrate from different sources (Vitória et al., 2008, and references therein) Dashed lines represent the denitrification ratios using the extreme values from literature that ranges between 1.3 (Fukada et al., 2003) and 2.1 (Böttcher, 1990). B) $\delta^{15}\text{N}_{\text{NO}_3}$ vs. nitrate concentrations diagram with the ranges of the surface water and organic nitrogen end-member.

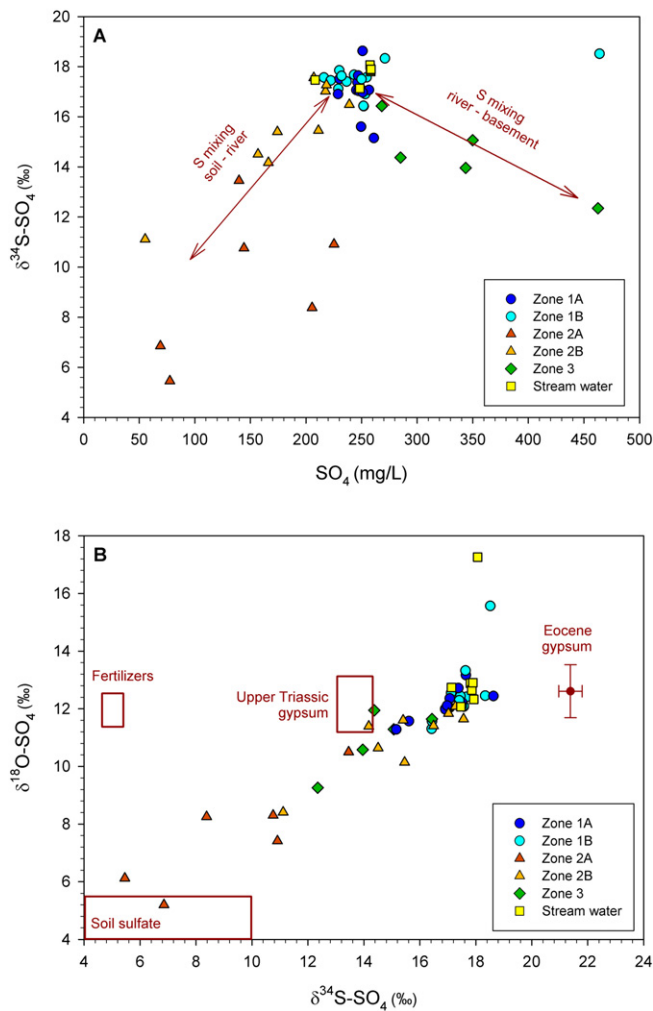


Fig. 7. A) $\delta^{34}\text{S-SO}_4$ vs. sulfate concentrations diagram indicating samples influenced by sulfate mixing between soil and river, and between river and the basement of the aquifer; B) Sulfate isotopes ($\delta^{18}\text{O-SO}_4$ and $\delta^{34}\text{S-SO}_4$) diagram. Reference values for soil and fertilizer sulfate, Upper Triassic gypsum and Eocene gypsum are presented by Otero et al. (2009) and Utrilla et al. (1992), respectively.

agreement with soil sulfate and, to lesser extent, with pig manure isotopic values (Otero et al., 2009), indicating that these zones do not receive any other groundwater inputs than rainwater infiltration; while others, especially in zone 2B, are influenced by the contribution of Eocene gypsum sulfate.

Tritium values in groundwater ranged between 0.03 and 5.3 TU, with a mean value of 3.9 ± 0.1 TU (Table SM3). Usual values (80% of the samples) lay between 3.5 and 4.5 TU, which can be considered as recent rainfall or stream recharge, in agreement with the monthly rainfall mean tritium content from the Verges station samples: 4.8 ± 0.6 TU (2016–2017), with a median of 4.15 TU. Two values lower than or close to 3.0 TU are located in the northern margin (P-VL02), close to the ocean (P-SP05), and in one of the deepest wells (>20 m; P-SO01). Those values larger than 4.5 TU are primarily located in the southern margin (P-VE02, P-VD01) and in the alluvial plain (P-SP05). The small amplitude of the samples within the alluvial plain does not offer any clue of specific groundwater pathways. It can be noticed that samples from Zone 3 have a lower tritium content between 3.0 and 4.0 TU, while the rest of the alluvial plain (Zones 1A and 1B) are predominantly between 4.0 and 5.0 TU. This lower tritium content of samples from Zone 3 could be interpreted as a recharge contribution of groundwater from the underlying aquifer, as indicated by sulfate isotopes (Fig. 7B); yet the tritium content of such vertical recharge is unknown to appraise the mixing proportions.

3.5. Antibiotic distribution and quantification

Antibiotics occurrence presents a distinct distribution in each of the water bodies (Supplementary material 4; SM4). For instance, more compounds were detected in groundwater samples (11 antibiotics; $n = 47$), than in WWTP effluents (9 antibiotics; $n = 2$) and surface water (5 antibiotics; $n = 7$), as shown in Fig. 8. Such a different number of chemicals can be attributed to their occurrence in the environment; assuming that the number of compounds solely depends on the input composition and the fate of each chemical in the media. Nevertheless, from a cautious perspective, it might also be associated to the number of samples analyzed for each water body in the sense that a larger number of groundwater samples is more probable to reflect a greater variability. Even though most antibiotics were generally found in each water type (stream, aquifer, WWTP effluent), some chemical groups such as quinolones were only detected in groundwater.

Looking at the different water compartments and considering the most commonly detected antibiotics in the Baix Fluvià aquifer, boxplots considering non-detected values as 2/3 the value of the detection limit (LOD) (Fig. 9A) show that median of the total antibiotic concentrations were much higher in WWTP effluents (1375 ng/L), than in surface water (204 ng/L) and groundwater (49.5 ng/L). Higher total concentrations in river water than in groundwater indicate the relevance of surface water antibiotic inputs, as for instance WWTP effluents, along the whole basin as relevant sources of both human and veterinary antibiotics. This observation is also documented by other studies (e.g., Focazio et al., 2008). Higher total antibiotic concentrations found in WWTP effluents are just of local relevance as they only represent a small amount of recharge at a regional scale. Their direct impact to groundwater is limited to the drain that collects Ventalló's WWTP effluents. Moreover, the median of the values for the seven most detected antibiotics in groundwater (i.e., those with a frequency of detection above 10%) considering non-detected samples are close to or below 1 ng/L, except for sulfamethoxazole and ciprofloxacin. This plot reveals the actual state of the aquifer, with low concentrations of these selected antibiotics, and stands as a reference of the possibility of finding a specific concentration.

Conversely, as shown in Fig. 9B where non detected samples are not considered, the median of the total antibiotic concentrations in the water compartments are similar to those estimated considering all samples. However, the boxplots in Fig. 9B show the concentration range of these seven most detected antibiotics in groundwater, excluding nondetected values, being representative of the magnitude of the pollution in those wells where those compounds were detected.: fluoroquinolones (ciprofloxacin, enrofloxacin and norfloxacin) are found at concentrations above 10 ng/L, while others are below this concentration (Fig. 8).

Out of the 53 antibiotics analyzed, 11 antibiotics found in groundwater correspond to four different chemical groups: fluoroquinolones (ciprofloxacin, danofloxacin, enrofloxacin, norfloxacin, ofloxacin and orbifloxacin), macrolides (azithromycin), quinolones (flumequine, oxolinic acid, pipemidic acid), and sulfonamides (sulfamethoxazole). The most frequent chemical groups were fluoroquinolones and sulfonamides. In particular, fluoroquinolones are the most abundant chemicals detected in the Baix Fluvià aquifer. Several antibiotics such as ciprofloxacin, enrofloxacin, norfloxacin, ofloxacin and orbifloxacin were found both in groundwater and stream water, as well as in WWTP effluents, with relatively high mean concentrations (between 20 and 100 ng/L) despite their highly sorption coefficients. This is attributed to the fact that they are among the most important antibiotics used in human and veterinary medicine and therefore, there is a relatively high input load into the environment. Moreover, the facts that fluoroquinolones have pH-dependent speciation dictating the mechanism of sorption, they degrade slowly in soils, and have high solubility coefficients might also explain their occurrence in the Baix Fluvià aquifer. Ciprofloxacin was observed in 45% of the groundwater samples, with

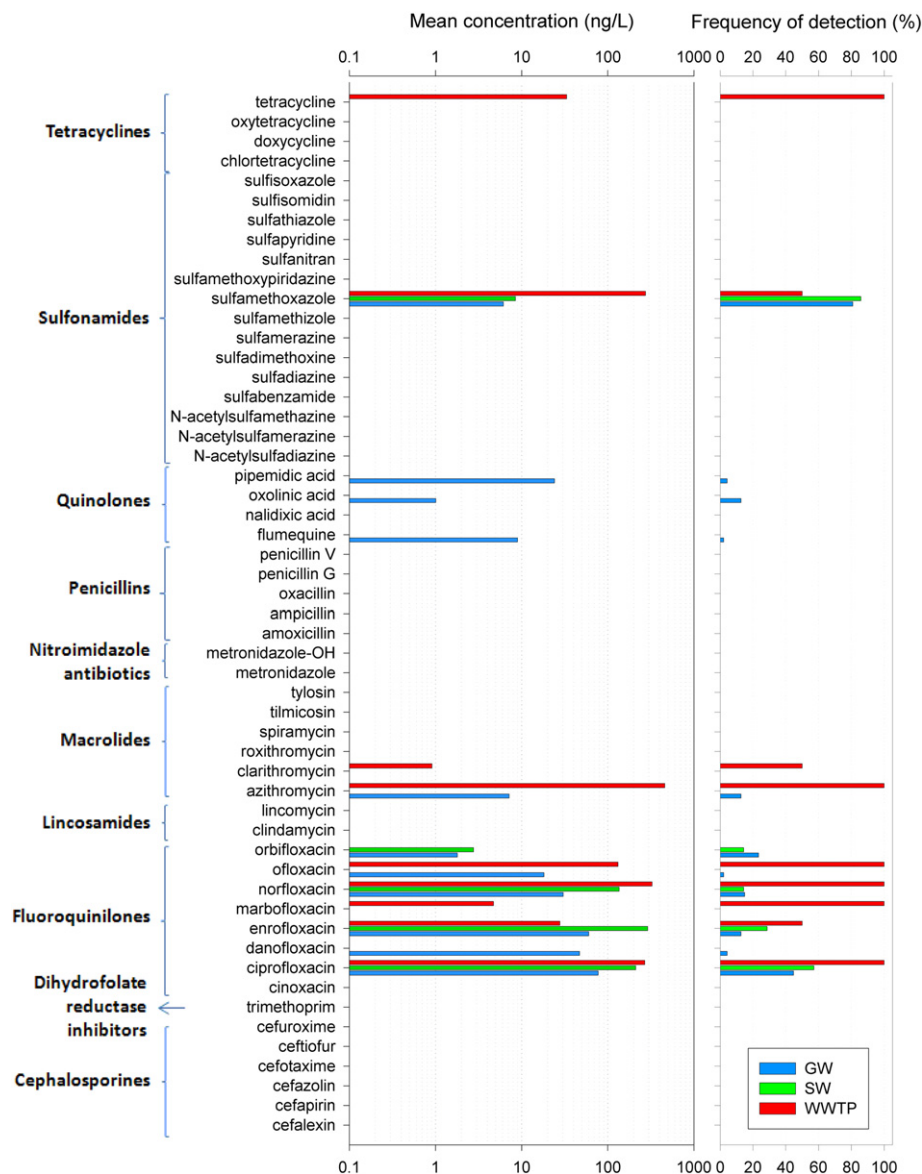


Fig. 8. List of the antibiotic families and antibiotics analyzed in this study. Mean concentrations and frequency of detection for groundwater (blue), surface water (green) and WWTP effluents (red) are represented in the bar diagram.

mean concentration of 77.2 ng/L, and highest concentration of 298.3 ng/L. Orbifloxacin was the third most frequent detected antibiotic (23.4%) with a mean concentration of 1.8 ng/L, and a maximum concentration of 5.2 ng/L; yet not many studies have detected fluoroquinolones in groundwater samples (Barnes et al., 2004; Focazio et al., 2008; Leal et al., 2013).

In this study, macrolides such as azithromycin (only in groundwater with a 15% frequency of detection) and clarithromycin (only in WWTP effluent) were detected in this study and their occurrence in groundwater was also reported by previous studies (López-Serna et al., 2013). Quinolones, and in particular oxolinic acid, pipemidic acid and flumequine, were the compounds exclusively detected in groundwater at concentrations below the limit of quantification up to 28.6 ng/L. Sulfamethoxazole was detected in 81% of the groundwater samples with mean concentration of 6.1 ng/L and a highest concentration of 28.6 ng/L.

In surface water samples, only five different antibiotics were quantified from two chemical groups: fluoroquinolones (ciprofloxacin, enrofloxacin, norfloxacin and orbifloxacin) and sulfonamides (sulfamethoxazole). The most detected compounds were the same as in groundwater: sulfamethoxazole and ciprofloxacin (mean

concentrations of 8.5 ng/L and 211.8 ng/L, and frequency of detection equal to 85.7% and 57.1%, respectively). Enrofloxacin was also quantified in two samples with relatively high mean concentrations of 290 ng/L. Among the 9 antibiotics detected in wastewater treatment plant effluents, ciprofloxacin, norfloxacin, azithromycin and sulfamethoxazole presented concentrations above 200 ng/L. Antibiotics found in effluents from WWTP, discharging to rivers or infiltrating into the subsurface along its way to the main stream, are indicative of the antibiotics coming from human sources. In this sense, tetracycline was only detected in WWTP effluents, but neither in surface water nor in groundwater although both of them also get inputs from urban sources.

No clear continuous spatial concentration trend of antibiotics has been observed in the aquifer. The relative proportion of antibiotics, shown in the pie charts at Fig. 10, indicates the dominance of sulfamethoxazole, enrofloxacin and ciprofloxacin in groundwater samples, which are not regularly distributed across the aquifer. Similarly, surface water samples along the Fluvià River show an alternation of these three chemicals as the most usual components, without any

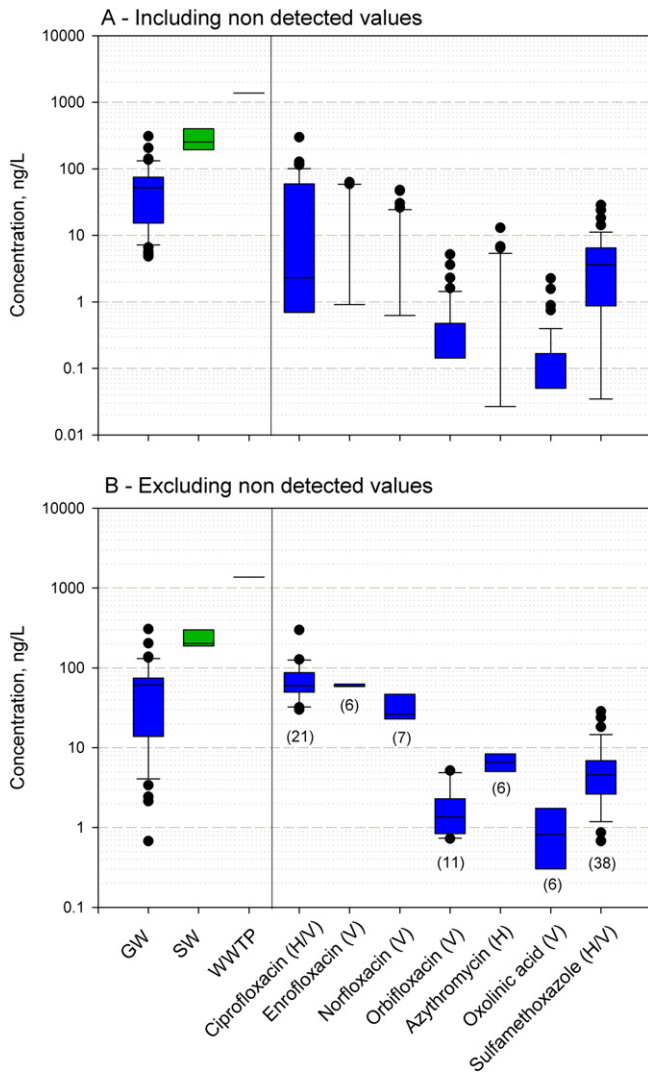


Fig. 9. Boxplots showing antibiotic concentration for groundwater (GW, $n = 47$), surface water (SW, $n = 7$) and waste water treatment plant effluents (WWTP; $n = 2$) on the left. On the right side, boxplots with the seven most commonly found antibiotics in groundwater and their respective use in human (H) and/or veterinary (V) medicine. Plot 9A represents all the data, including samples that showed a nondetected value. In this case, a 2/3 of the detection limit was assigned as potential concentration. Plot 9B considers only those samples with concentrations above the detection limit; numbers in parenthesis indicate the number of samples where the specific antibiotic was detected.

pattern of continuity along the stream. In this sense, the two main origins of antibiotic pollution, i.e., pig manure used as fertilizer and stream recharge, do not imprint an antibiotic distribution that reflect some kind of continuity neither in the aquifer nor in the stream. Moreover, no significant correlation (p -value > 0.05) and no clear relationship between nitrate pollution and presence of antibiotics has been observed (Fig. 11). While high nitrate concentrations were found in the agricultural areas in the margin areas of the study area, and low concentrations were mostly detected in wells nearby the river, no clear spatial pattern could be drawn for antibiotics. Similar results were obtained in previous studies on the occurrence of nitrates and sulfonamides in Catalonia (García-Galán et al., 2010).

3.6. Geostatistics

Variograms were performed to investigate and compare the spatial variability of electrical conductivity, nitrates, sum of antibiotics,

ciprofloxacin and sulfamethoxazole in groundwater samples (Fig. 12). Comparable variograms were observed for conductivity and nitrates. Correlation lengths, indicated the distance at which data are no longer correlated and given by the range values, were of 1450 m and 1870 m, respectively. Geostatistics confirmed that the overall groundwater solute content, represented by its electrical conductivity, shows some spatial continuity in agreement with the hydrogeological flow system. Moreover, nitrate content show a similar range of spatial correlation the EC despite the patchy fertilization practices in the area (see land uses in Fig. 10).

On the other hand, the ranges of the variograms for the sum of antibiotics and for the two most detected antibiotics, ciprofloxacin and sulfamethoxazole, are much smaller (from 83 to 165 m). The variograms for antibiotics indicate a very small spatial correlation (which could be almost understood as a pure nugget effect meaning absence of spatial correlation) across the alluvial aquifer, as already suggested by Fig. 12.

3.7. Reactive transport of antibiotics in groundwater

As stated in the introduction, the reactive nature of antibiotics will play a determinant role on their occurrence, spatial distribution, and finally to their impact in groundwater resources quality. Based on published physico-chemical parameters (Table SM1) and taking alluvial aquifer values consistent with the Baix Fluvià dominant lithology, such as $f_{OC} = 1$ mg/g (0.1%); $\rho = 1.8$ g/cm³; and $\theta_e = 0.1$, the sorption behavior of these pollutants can be estimated considering their hydrophobicity and their ionisable character by the inclusion of the pH-dependent n -octanol/water distribution coefficient ($\log D$, in Eq. (3)).

Retardation factors substantially vary among these antibiotics, considering the potential dual (anionic/cationic) behavior of some of them (Fig. 13), and the Baix Fluvià pH range (i.e., $6.5 < \text{pH} < 8.0$; mean field $\text{pH} 7.13 \pm 0.04$). Sulfamethoxazole, for instance, present almost no retardation for the field site usual pH values (6.5–8.0), indicating that its transport will be as fast as that of groundwater. For the field pH range, orbifloxacin present R-values between 1.5 and 3.0, corresponding the maximum to high pH and anionic nature, and the minimum to low pH and cationic behavior. Similarly, ciprofloxacin R-values vary between 3 and 20 for the field pH. However, other antibiotics show larger retardation values varying with pH and with the antibiotic ionic behavior. Even larger R-values are estimated for oxolinic acid between 50 at pH 6.5, and 15 at pH 8.0, and for azithromycin between 7 and 245 for the same pH boundaries. It is worth recalling that the retardation factor for azithromycin, being a hydrophobic compound, has been estimated using the adequate A and B values in Eq. (3) of 0.81 and 0.10, respectively.

Antibiotic transport is thus highly restricted by their physico-chemical properties as well as those of the environment; that is, organic matter content, groundwater pH, and ionic strength (not considered in the sorption equation, yet experimentally proved to influence the antibiotic transport velocity in groundwater; Chen et al., 2011; Wegst-Uhrich et al., 2014). Consequently, given an initial input concentration of antibiotics in the manure, the antibiotic content in groundwater will be determined by the magnitude of the sorption processes, the flow velocity field, and the distance from the fertilized crop to the monitoring well. At high values of R within a common flow field, antibiotic contribution from certain large distances to a monitoring well might be very small, even negligible depending on the magnitude of the source. Estimations of these contributions are evaluated in Appendix A, which presents the percentage of initial concentration (i.e., C/C_0 as percentage) under a natural hydraulic gradient scenario (refer to Mas-Pla et al., 1999, for potentiometric maps), as a function of the retarded reactive solute velocity, $v_s = v_w / R$, where v_w refers to the average pore water velocity (Fig. A2).

For instance, the estimated contribution of a reactive solute input source located 50 m from a monitoring well along a single flow line for a solute velocity (v_s) of 0.20 m/d will be 80.3% of the initial

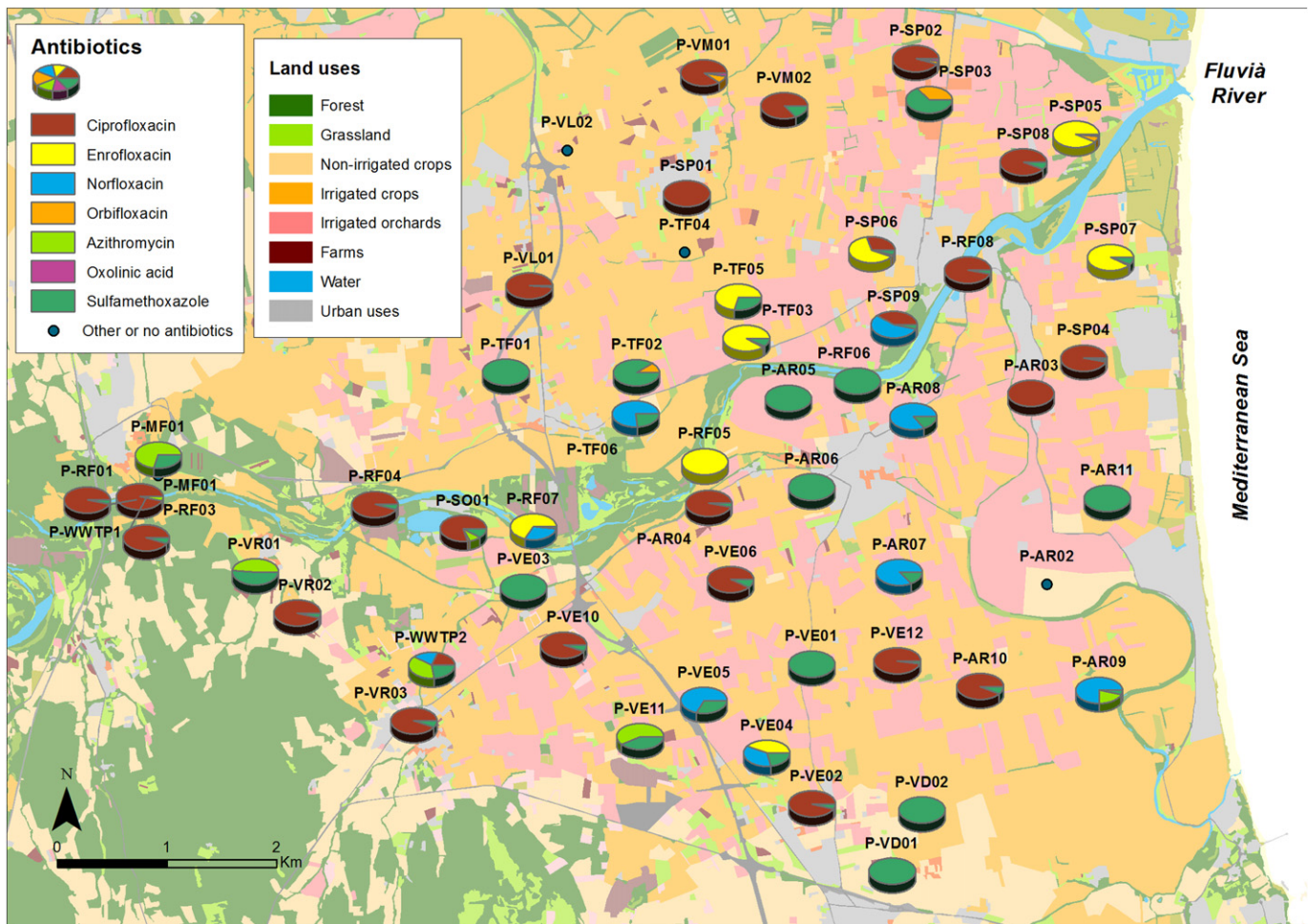


Fig. 10. Pie charts showing the mass-proportion of the most frequent antibiotics in the Baix Fluvià alluvial aquifer for groundwater, surface water (codes starting with P-RF##), and WWTP effluents (P-WWTP#) samples. Land use map is shown in the background.

concentration after one year, and of 99.4% after two years. For a chemical $v_s = 0.05$ m/d, the contribution along this flow line starting at 50 m will be of 0.9% of the initial concentration after one year, and of 24.0% after two years (Fig. A2). Lower percentages will obviously occur as distance and retardation factor increase. For a $v_w = 1$ m/d, consistent with the Baix Fluvià flow field magnitude, the first example will represent a solute with a retardation factor equal to 5; and the second, a retardation factor of 20.

Nevertheless, sampling in a monitoring well supposes the mixing of all flow lines reaching the well, and therefore a dilution of the solute mass is expected provided from the flow lines originated near the well, with those arriving from further positions. Fig. A3 simulates the average concentration, as C/C_0 in percentage, that will be recorded in a time span of 5 years at a monitoring well that intercepts polluted flow lines originated from 125 and 250 m under natural conditions (refer to Fig. A1 for explanation), for distinct v/R ratios, according to the assumptions stated in the appendix. In these examples, for instance, a monitoring well that captures groundwater flow lines from a distance of 250 m up-gradient, will present an antibiotic concentration of a 28.9% with $v_s = 0.2$ m/d, and of 7.3% with $v_s = 0.05$ m/d after one year of the manure application; and of 58.3% and 14.4% after two years, respectively.

4. Discussion

Exhaustive field data on antibiotic distribution in the Baix Fluvià alluvial aquifer indicate a strong variability in both occurrence of

antibiotics and magnitude of the total antibiotic concentration (Fig. 10). In order to frame these results, a conceptual hydrogeological model that explains such variable distribution of these reactive pollutants and their environmental relevance must be discussed in terms of the subsurface flow field, the input characteristics, and the transport processes that finally determine their fate.

4.1. Hydrogeological processes that determine antibiotic occurrence

Determining the hydrogeological dynamics is paramount to avoid uncertainties about the interpretation of antibiotic occurrence in groundwater. This is the reason why special emphasis is put into identifying the geological features of the aquifer and the hydrological sources that contributes to its recharge and to its antibiotic content. In that way, hydrological factors can be clearly distinguished from chemical processes (sorption) and, consequently, the causes of the antibiotic spatial variability determined. In summary, the Baix Fluvià system presents the following hydrological dynamics:

- The influence of the Fluvià River as a recharge boundary in its upper-stream reaches. Given the higher antibiotic content of the stream water due to wastewater treatment plants discharges along its basin (Fig. 9), it is expected that stream flow will contribute to the antibiotic content in the wells nearby the river.
- Rainfall recharge, as the main term of the aquifer water budget, and irrigation returns will infiltrate manure applied as fertilizer

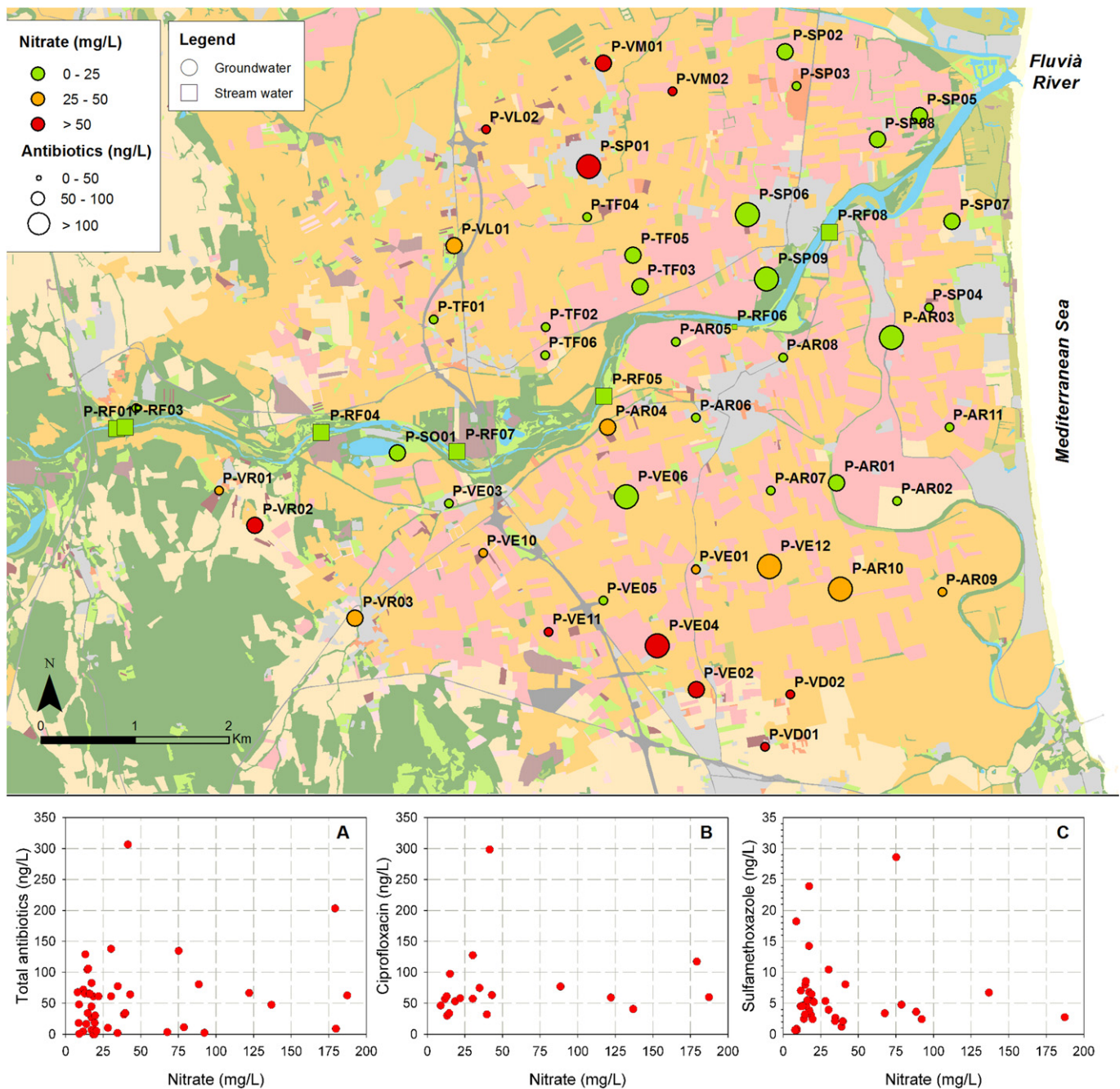


Fig. 11. Map of nitrate concentrations (indicated by the color of the symbol) vs. total antibiotic concentration (size of the symbol) for groundwater (circles) and surface water monitoring (squares) points. Land use map is shown in the background (see legend in Fig. 9). At the bottom of the map, bivariate correlations between nitrate concentration and total antibiotics (A), ciprofloxacin (B) and sulfamethoxazole (C) are shown.

on the crops, causing the entrance of pollutants into the vadose zone and, with time, to the aquifer. A partially saturated zone thickness between 1 and 4 m from the coast to the inland areas, respectively, will temporally retain pollutants, and progressively lead them to groundwater by gravity flow following rainfall events.

- c) Recharge from the aquifer margins as well as from its basement, as proved by sulfate isotopes (Fig. 7), should not present any antibiotic concentration since the surrounding hill belts are mainly forested with no arable land. Furthermore, regional large-scale flow systems, such as those identified in the alluvial aquifer as noticed in Zone 3, are not likely to contain any antibiotic either.

Therefore, based on the hydrogeological conceptual model, a spatial trend of antibiotic distribution consistent with potential pollution

sources should be expected. Nevertheless, neither their total concentration nor the occurrence of each antibiotic follows such hypothetical pattern (Figs. 10 and 11). While variograms for EC and nitrate point out some spatial correlation, they clearly indicate that such correlation does not exist for antibiotics (Fig. 12). In other words, the spatial distribution of antibiotics appears random compared with the overall hydrochemical composition, as indicated by the EC, and even than the nitrate content derived from fertilization practices. Being fertilization inputs using animal manure the main origin for nitrate and antibiotics in groundwater, the distinct correlation lengths between nitrate and antibiotics points out that antibiotic fate in the aquifer is affected by the influence of other processes unrelated to the hydrogeological dynamics. It must be recognized that a natural uninfluenced flow field only persists during non-irrigation periods, whereas it will be intensively perturbed

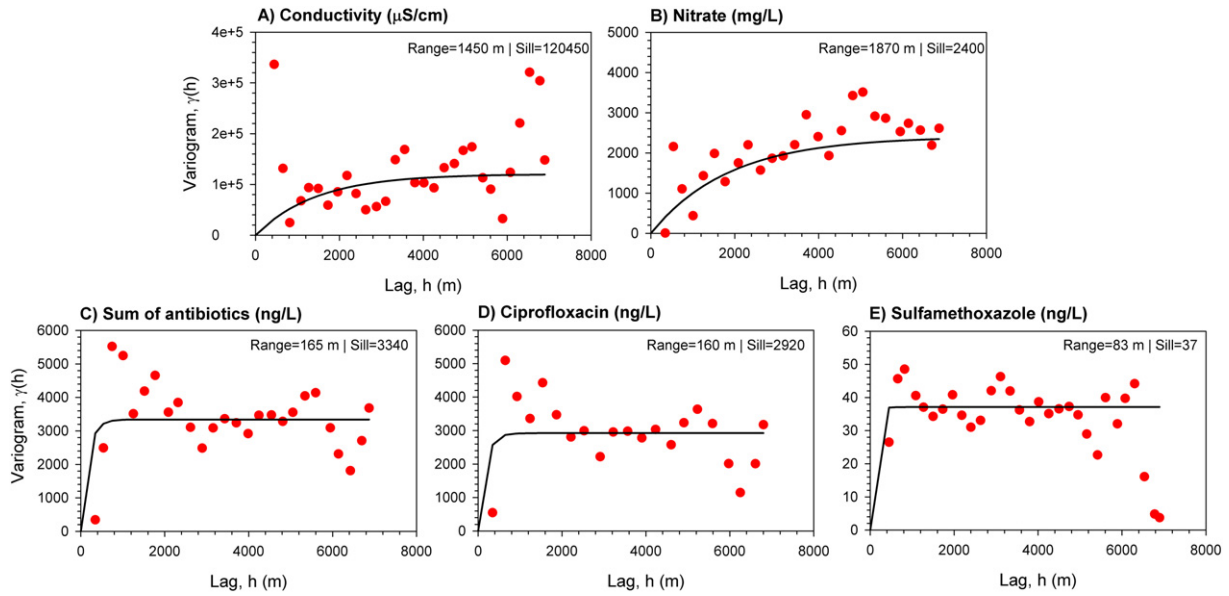


Fig. 12. Variograms for conductivity (A), nitrate (B), sum of antibiotics (C), ciprofloxacin (D) and sulfamethoxazole (E) with their ranges and sill parameters.

from mid-May to mid-September when most of the groundwater withdrawal for irrigation takes place. Such seasonal effect induces some mixing effects on the antibiotic concentration and blur the influence of the stream and aquifer boundaries flows on the pollutant concentration. Even though the sampling campaign in this study was conducted just before and at the beginning of the irrigation season, it is expected that such mixing effects persist from previous years. Therefore, hydrogeological factors related to the flow field alteration due to groundwater withdrawal can be envisaged as responsible for a large variability of the antibiotic spatial distribution.

The detailed knowledge of the hydrogeological dynamics performed in this study is then necessary to discard unproved hypothesis about the flow field characteristics and its influence on the antibiotic occurrence. For instance, field, hydrochemical and isotopic data prove that stream water, with a high antibiotic content, recharges the alluvial aquifer along the up-stream, westernmost reach; nevertheless, not all the wells nearby the river show high concentrations of antibiotics. Indeed, there is small agreement between the dominant antibiotic content in

the river reaches and the concentration of the nearby wells in the western segment of the Fluvià River. Moreover, an inverse relationship in the downstream segments is neither noticed, that is, aquifer antibiotic content is not correlated with the antibiotic stream composition in the gaining reaches. In fact, groundwater contribution is not expected to be relevant according to the low hydraulic gradients, for that reason fluvial transport is more likely to control surface water antibiotic concentrations. Nevertheless, as stream recharge occurs, other explanations must be considered to understand the observed low antibiotic concentrations in groundwater.

4.2. Sources and geochemical processes that determine antibiotic occurrence

Before considering the chemical processes that control antibiotic migration in aquifers (which have been described in Section 3.7), the characteristics of animal manure and slurry, as the main input sources, must be addressed. Even though it is commonly assumed that agricultural activities produce a diffuse pollution input, this concept should be reviewed when dealing with these organic contaminants as it may turn to be a misleading assumption. Land-use maps show that crops where organic fertilizers (manure and slurry) are applied and orchards, where only chemical fertilizers are spread, are heterogeneously distributed across the alluvial plain (Fig. 10). Indeed, nitrate isotopes (Fig. 5) report a combination of both types of fertilizers. Consequently, the idea of diffuse pollution regarding antibiotics must be reviewed in an area where land-use present a patchy pattern, as these pollutants would only infiltrate from specific land uses. In the case of the Fluvià River alluvial plain, these crops occupy a 55% of the total area, acting as antibiotic pollution sources. This scatter distribution of antibiotic sources becomes another factor that finally enhances the observed spatial variability of pollutants in groundwater. Such discontinuous, uneven application of manure and chemical fertilizers designs a different source distribution for nitrate and antibiotics. Nitrate will present a widespread diffuse input, while antibiotics will only appear in specific crops (mainly irrigated corn fields). In consequence, no relationship between nitrate and antibiotics concentrations should be expected at a regional scale (Fig. 11), leaving aside the fact that the former behaves as a conservative solute, and the latter, reactive. Additionally, the effect of the two WWTP

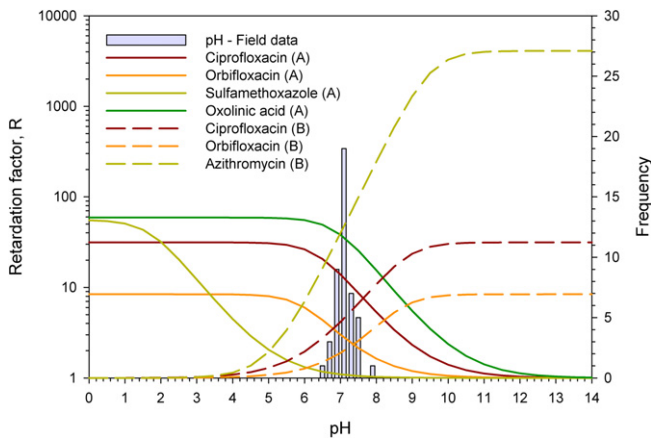


Fig. 13. Retardation factors for the most common antibiotics detected in the Baix Fluvià aquifer depending on their behavior as acids (A) or bases (B) as a function of pH, and the histogram of the pH values measured in the Baix Fluvià aquifer.

and occasionally the presence of cesspits in the area may also act as point sources of antibiotics related to human uses.

Still related to the organic fertilizer characteristics, the actual antibiotic content and the concentration of each chemical are troublesome to determine. Even if each farmer reported the type and amount of antibiotics used in the farm, their final concentration in the manure would still be uncertain. Moreover, excreted urine is stored together with other litter and farmyard cleaning water in reservoirs for several months until manure is applied on the crops, usually twice a year (February–March and September–October). Few research has been conducted on the antibiotic content of fresh manure (Wallace and Aga, 2016), neither on the degradation processes that these compounds will undergo along months of storage. Moreover, considering the fact that each farmer treats livestock with a different antibiotic cocktail and that storage conditions can be extremely variable among farms and between seasons (winter versus summer), the antibiotic content and load applied as manure stand as an uncertain parameter. Analysis of specific antibiotics in manure obtained at local storage ponds result in concentrations levels of 5×10^3 ng/L of ciprofloxacin and 10^4 ng/L of enrofloxacin in the liquid phase, and of 3×10^3 to 6×10^3 ng/g of them in the solid phase. Therefore, antibiotic concentrations found in the Baix Fluvià aquifer are one or two orders of magnitude lower than original results (Fig. 9). This comparison is provided here just for reference and must be taken with extreme caution, yet it provides an idea of the ratio between the concentration at the source and that of the polluted resource. Moreover, transport, transformation and degradation processes that occur in the vadose zone, also affect the final mixture of antibiotics reaching the water table. In this sense, considering the vadose zone hydrology and the efficiency on antibiotic transport of the distinct soil types in the region may help to describe the magnitude of the final input, yet this would constitute a tough and costly monitoring effort. Therefore, from a managing perspective, understanding antibiotic fate in aquifers faces a large uncertainty about the input source (manure) characteristics and contents.

Retardation factors are then key parameters to understand and predict antibiotic transport in groundwater. Each compound behaves differently, and this behavior is primarily conditioned by pH as well as other soil characteristics. For these reasons, R-values calculated for antibiotics in this study are just predictions, and must be handled with care since the sorption processes are complex and depend on many influencing factors, especially on the molecular structure and physical-chemical properties of each antibiotic, as well as on the environmental (soil) properties. The case of azithromycin illustrates the complexity of estimating an approximate R-value as its large $\log K_{ow}$ forces to include distinct A and B parameter values (Eq. (3)) adequate for hydrophobic chemicals. Because of its large $\log K_{ow}$, these estimates present a larger uncertainty in their precision (Sabljic et al., 1995) which it additionally adds more unpredictability on the transport modelling attempts.

As a result, retardation factors for the most frequently found antibiotics in this study present large values, covering several orders of magnitude in the field pH range. This result is of the uttermost relevance when considered altogether with the hydrogeological features of this system as it affects the travel time of each antibiotic, whether in the vadose layer or later in the aquifer. In particular, groundwater average pore velocity in coastal alluvial formations is of a few hundreds of meters per year, considering the above mentioned hydrological parameters (Section 3.2). Antibiotic pore velocities are then inversely related to the retardation factor, so their migration is reduced to units or tens of meters per year. This estimation is based on the advective component alone, yet the overall antibiotic mass transport, even considering dispersion, would remain in the same order of magnitude.

Low biodegradation rates also stand as an additional reason to explain the widespread occurrence of sulfamethoxazole and ciprofloxacin in groundwater, in addition to its high usage in both human and veterinary medicine, and despite their retardation factors at pH 7 of 13.37

and 6.18, respectively. However, some studies reported different results, and literature is sometimes inconsistent, presumably because of its unknown metabolic fate and the complexity of these processes.

4.3. Interpretation of antibiotic data and their representativeness

Such estimations are herein described to point out the influence of geochemical processes (basically, sorption) on the migration of reactive chemicals in the aquifer. Given the high retardation factor values for antibiotics (Fig. 13), it should be expected that field results reflect the inputs on the nearby capture zone after many years of agricultural activity. Therefore, monitoring wells are only representative of the antibiotic inputs mainly occurring in their vicinity. It must also be recognized that, 1) measured concentrations only indicate the dissolved mass of pollutant, recognizing that a proportional mass (defined by K_d) is sorbed on the aquifer sediments, and 2) measured concentrations represent a mixing between those flow lines initiated close to the well, that contribute with a high concentration of contaminants, and those originated at larger distances with lower concentrations due to travel time and sorption processes. Under the effect of depression cones, the patchy nature of the input sources adds an additional term of complexity to determine the relationship between the observed antibiotic concentration and the location and features of the source areas. It should be finally recalled that adsorbed antibiotic mass on the aquifer sediments might come into dissolution again through desorption processes when antibiotic groundwater concentrations decrease due to dilution following intense recharge events, or in the absence of further, continuous manure applications.

According to this simple numerical analysis, the heterogeneous application of manure in the area and the well depth (which determines the set of intercepted flow lines) are hydrogeological aspects that also contribute to resolve the observed variability of antibiotic concentrations in the Baix Fluvià aquifer. Therefore, we conclude that measured antibiotic concentrations cannot be easily interpolated to unsampled areas to understand the occurrence of antibiotics (and extensively, of all other reactive pollutants applied in sparse diffuse applications) in an aquifer. Many factors (hydrogeological, agronomic, hydrogeochemical, etc.) suggest that each measurement is mostly representative of the idiosyncrasy of each well.

5. Conclusions

After a few decades of intensive use of antibiotics in livestock husbandry and fertilization practices, the Baix Fluvià aquifer presents a highly variable occurrence of antibiotics in its groundwater resources based on an intensive field survey and laborious laboratory analyses including 53 compounds. These results indicate the qualitative state of this aquifer respect to these emergent contaminants; yet many factors difficult building up a comprehensive, conceptual model that predicts their migration and fate within the aquifer.

Field hydrogeological, hydrochemical and isotope data are herein used to improve the understanding of processes that control transport, fate and persistence of antibiotics in aquifers from agricultural origin. Even though antibiotic concentrations in groundwater are of the order of ng/L, these results reveal that several compounds are widely detected in the aquifer, specially sulfamethoxazole and ciprofloxacin. Their occurrence is also high in stream water. In this scenario, a hydrogeological dynamics controlled by rainfall recharge and the stream-aquifer interaction governs the migration of the antibiotics introduced as diffuse pollution by fertilization practices.

This study on groundwater antibiotic occurrence in the Baix Fluvià unconfined alluvial aquifer provides one of the most detailed datasets presented so far. Based on a detailed knowledge of the hydrogeological dynamics of the aquifer, which is crucial to distinguish hydrological factors from pollutant chemical behavior, antibiotic spatial distribution has been proved to show a large variability, showing no spatial correlation

among the sampling wells. As discussed, such variability is attributed to several factors, being the most relevant ones the reactive nature of these pollutants, the patchy pattern of their input from agricultural practices, and the changing antibiotic concentration of the many organic fertilizers (mainly pig manure or slurry) applied on the crops. Flow field alteration due to the groundwater variable withdrawal regimes of many wells also contributes to a larger dispersion of pollutants within the aquifer.

Under these circumstances, assessing groundwater quality of this simple alluvial aquifer, and by extension of other hydrogeological systems, becomes a difficult task; for instance, the determination of a sampling network that will provide a reliable idea of the occurrence, magnitude and spatial distribution of antibiotics to be used for groundwater quality assessment and the planning of priority actions. Independently whether management actions focus on individual pollutants from a watch-list (Marsland and Roy, 2016) or on the total antibiotic concentration, the Baix Fluvià dataset shows that a large uncertainty must be expected.

A brief research on the antibiotic sorption capacity modelling has pointed out two interesting facts. On the one hand, the difficulties of predicting the main coefficients involved in the sorption equations, when even the calculation of some of the involved chemical parameters still remain unsolved as, for instance, the K_{oc} of zwitter-ionic compounds (Schaffer et al., 2012; Schaffer and Licha, 2015). On the second, even using approximate estimations, retardation factors will vary over distinct orders of magnitude, causing some of these pollutants to be rather immobile compared to groundwater flow. Therefore, antibiotic transport modelling at a regional scale using conventional approaches will most probably be unsuccessful, as actually shown by the spatial variability of the antibiotic type and their concentrations along the Baix Fluvià aquifer.

To summarize, this dataset evidences the hydrochemical difficulties to build up water resources quality management strategies and environmental regulations in aquifers affected by antibiotic pollution.

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Appendix A. Influence of retardation on the transport of antibiotics towards a monitoring well

Monitoring wells, whether partially or fully penetrating, provide information about the averaged concentration of all flow lines that reach

the screened interval of the well. The source area of a monitoring well is defined as the area from where well water originates as recharge. The entire area can be the source of a pollutant if fertilizers are applied all over it; or only partially if fertilized crops are irregularly distributed on the surface. A geometric approximation provides a simplistic but useful estimate of the upgradient linear extent of the monitoring well source area (Harter et al., 2002; Fig. A1).

Solute transport along every flow line is described by the one-dimensional advection-dispersion equation including sorption processes:

$$D \frac{\partial^2 C}{\partial x^2} - v_w \frac{\partial C}{\partial x} = \frac{\partial C}{\partial t} + \frac{p \partial S}{n \partial t} \quad (A1)$$

where C is the solute concentration in groundwater; D is the hydrodynamic dispersion coefficient defined as $D = \alpha v_w + D^*$, being α the longitudinal dispersivity, v_w the average pore water velocity and D^* the molecular diffusion coefficient; and S is the solute mass sorbed per unit mass of sediment. Using a linear isotherm; i.e., $S = K_d C$, where K_d is the distribution coefficient, the general transport equation (Eq. (A1)) transforms to:

$$D \frac{\partial^2 C}{\partial x^2} - v_w \frac{\partial C}{\partial x} = R \frac{\partial C}{\partial t} \quad (A2)$$

where R is the retardation factor described by Eq. (6). The value of R equals 1 for a conservative solute, and is larger than 1 for a reactive solute, as antibiotics. Algebraically working on the above equation and using the expression for D , neglecting the magnitude of D^* in front of αv_w , it results on:

$$\frac{\alpha v_w}{R} \frac{\partial^2 C}{\partial x^2} - \frac{v_w}{R} \frac{\partial C}{\partial x} = \frac{\partial C}{\partial t} \quad (A3)$$

being the quotient v_w/R the average pore water (advective) velocity for a reactive solute (v_s). This one dimensional equation has several analytical solutions, as that by Ogata and Banks (1961), under the boundary conditions: $C(0,t) = C_0$, and $C(\infty,t) = 0$ for $t \geq 0$, and initial condition, $C(x,0) = 0$ for $x \geq 0$. Ogata and Banks analytical solution $C(x,t)$ for a reactive solute is expressed by:

$$C(x,t) = \frac{C_0}{2} \left[\operatorname{erfc} \left(\frac{x - \frac{v_w}{R} t}{2 \sqrt{\alpha \frac{v_w}{R} t}} \right) + \exp \left(\frac{x}{\alpha} \right) \operatorname{erfc} \left(\frac{x + \frac{v_w}{R} t}{2 \sqrt{\alpha \frac{v_w}{R} t}} \right) \right] \quad (A4)$$

being C_0 , the initial concentration at the recharge area once the infiltrating solute reaches the water table and incorporates into the groundwater flow. This expression is herein used to estimate the percentage of the ratio C/C_0 for a given aquifer length, and for values of the quotient v_w/R . This ratio represents the average pore velocity of a reactive solute, and it includes information of the groundwater flow, as given by Darcy's law, and the sorption properties of the solute (as given in Eq. (A4)). Therefore, using a dispersivity value of 5 m, consistent with the length of this numerical exercise (Gelhar et al., 1992), a plot of the ratio C/C_0 variation with distance for values of $1 \geq v_w/R \geq 0.005$ is presented in Fig. A2. The time span covered by this figure is one and two years after a continuous and constant infiltration of an antibiotic (C_0) was applied in an aquifer with no previous antibiotic occurrence. For the Baix Fluvià alluvial aquifer, an average pore water velocity of 0.5 to 1 m/d is consistent with the hydrogeological setting. So, taking the highest value ($v_w = 1$ m/d), the ordinate axis equals to R^{-1} , and it represents the range $1 \leq R \leq 200$.

Additionally, the concentration in the monitoring well can be estimated as the integration of all the solute mass transported along all flow lines intercepted by the screened interval. That is:

$$\frac{C_{\text{well}}(t)}{C_0} = \frac{1}{N} \int_0^L \frac{C(x,t)}{C_0} dx \quad (\text{A5})$$

where $C(x,t)$ is estimated using Eq. (A2), L represents the length of the source area, and N are the number of flow lines that originate in the source area and are intercepted by the monitoring well. As an example intended to support the discussion about antibiotic variable occurrence on the Baix Fluvià alluvial aquifer, the percentage of C / C_0 expected at the monitoring well, $C_{\text{well}}(t)$, is plotted in Fig. A3 for distinct values of v_w / R and lengths of $L = 125$ and $L = 250$ m.

Analytical expressions of $C_{\text{well}}(t)$ under non-uniform flow field, as converging radial flow to a well, have been proposed by many authors (e.g., Welty and Gelhar, 1994; see discussion in Rumynin, 2011), and these are suited to analyze the effect of arriving different flow lines under an induced flow field by a pumping well.

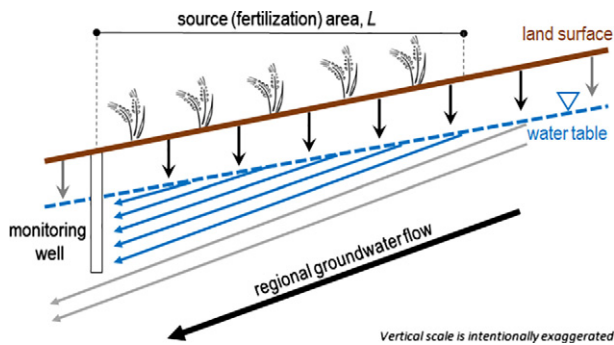


Fig. A1. Scheme of the upgradient flowlines sampled in a monitoring well under natural flow conditions (after Harter et al., 2002). Gray arrows represent infiltration and flow paths whose contributions will not reach the monitoring well.

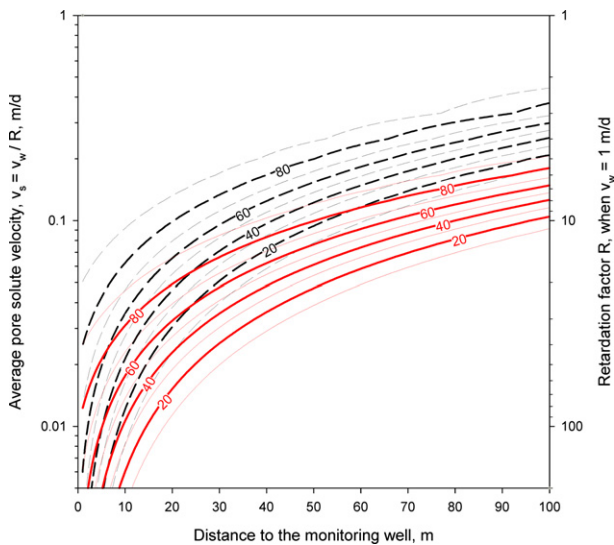


Fig. A2. Relative concentration (C/C_0 , in percentage) at time 1 year (black lines) and 2 year (red lines) after the input as a function of distance and average pore solute velocity (v_s), and the retardation factor (R). For the case when pore water velocity (v_w) equals 1 m/d, the retardation factor is invers of the average pore solute velocity ($v_s = 1/R$). Longitudinal dispersivity, 5 m.

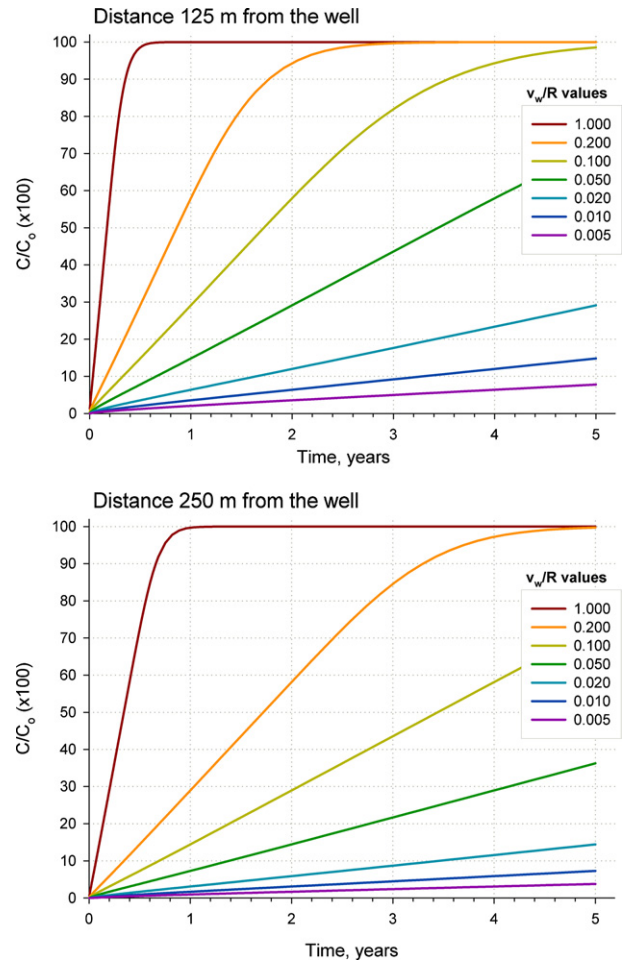


Fig. A3. Well-depth averaged breakthrough curves, i.e., relative concentration (C/C_0 , in percentage) as a function of time, for distinct values of average pore solute velocity ($v_s = v_w/R$), at two distances from the sampling well (125 and 250 m) under natural flow gradient (see Fig. A1).

Appendix B. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.scitotenv.2017.09.012>.

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