Distribution of antibiotics in water, sediments and biofilm in an urban river (Córdoba, Argentina, LA)

Valdés, M. Eugenia[1,2]; Santos, Lúcia H.M.L.M.[3,4]; Rodríguez Castro, M. Carolina[5]; Giorgi, Adonis[5]; Barceló, Damià[3,4,6];Rodríguez-Mozaz, Sara[3,4]; Amé, M.Valeria[1*]

[1]Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI- CONICET) and Dpto. Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Medina Allende esq. Haya de la Torre, Ciudad Universitaria, 5000 Córdoba, Argentina.

[2]Instituto de Ciencia y Tecnología de Alimentos Córdoba (ICYTAC-CONICET/UNC) and Dpto. de Química Orgánica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Av. Juan Filloy s/n, Ciudad Universitaria, 5000 Córdoba, Argentina.

[3]Catalan Institute for Water Research (ICRA), H_2O Building, Scientific and Technological Park of the University of Girona, Emili Grahit 101, 17003 Girona, Spain.

[4]Universitat de Girona, Girona, Spain.

[5]Instituto de Ecología y Desarrollo Sustentable (INEDES-CONICET)- Programa de Ecología de Protistas y Hongos, Dpto. de Ciencias Básicas, Universidad Nacional de Luján, Av. Constitución y Ruta Nacional N° 5, 6700, Buenos Aires, Argentina.

[6]Water and Soil Quality Research Group, Department of Environmental Chemistry, IDAEA-CSIC, Jordi Girona 18-26, 08034 Barcelona, Spain.

***Corresponding author:**

Dra. María Valeria Amé

Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI- CONICET) and Dpto. Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba. Ciudad Universitaria, 5000. Córdoba, Argentina.

e-mail: valeria.ame@unc.edu.ar

Tel.: 54-0351-5353851

Abstract

In this study, we evaluated the distribution of up to forty-three antibiotics and 4 metabolites residues in different environmental compartments of an urban river receiving both diffuse and point sources of pollution. This is the first study to assess the fate of different antibiotic families in water, biofilms and sediments under a real urban river scenario. Solid phase extraction, bead-beating disruption and pressurized liquid extraction were applied for sample preparation of water, biofilm and sediment respectively, followed by the quantification of target antibiotics by UPLC-ESI-MS/MS. Twelve antibiotics belonging to eight chemical families were detected in Suquía River samples (67% positive samples). Sites downstream the WWTP discharge were the most polluted ones. Concentrations of positive samples ranged 0.003-0.29 $\mu g\ L^{-1}$ in water (max. cephalexin), 2-652 $\mu g\ kg^{-1}_{d.w.}$ in biofilm (max. ciprofloxacin) and 2-34 $\mu g\ kg^{-1}_{d.w.}$ in sediment (max. ofloxacin). Fluoroquinolones, macrolides and trimethoprim were the most frequently detected antibiotics in the three compartments. However cephalexin was the prevalent antibiotic in water. Antibiotics exhibited preference for their accumulation from water into biofilms rather than in sediments (bioaccumulation factors > 1,000 $L\ kg^{-1}_{d.w.}$ in biofilms, while pseudo-partition coefficients in sediments < 1,000 $L\ kg^{-1}_{d.w.}$). Downstream the WWTP there was an association of antibiotics levels in biofilms with ash-free dry weight (indicative of heterotrophic communities). Cephalexin and clarithromycin in river water were found to pose high risk for the aquatic ecosystem, while ciprofloxacin presented high risk for development of antimicrobial resistance. This study contributes to the understanding of the fate and distribution of antibiotic pollution in urban rivers, reveals biofilm accumulation as an important environmental fate, and calls for attention to government authorities to manage identified highly risk antibiotics.

Antibiotics downstream urban WWTP and diffuse sources accumulate preferentially in river biofilms rather than sediments, posing environmental and resistance risk.

Keywords

Emerging pollutants; urban river system; bioaccumulation; sediment pseudo-partitioning coefficient; environmental risk.

1. Introduction

A particular group of contaminants of emerging concern that has received great attention in the last 20 years is that of antibiotics. Major concern around antibiotics use, occurrence in the environment and spread of antimicrobial resistance has become a worldwide health problem (Carvalho & Santos, 2016; UN, 2016). In Argentina (Latin America), there is a government strategy adopted since 2015 for the Antimicrobial Resistance Control, following the concept of "One Health" (BORA, 2015). This strategy is based on the control of antimicrobials commercialization, promotion of rational consumption and early detection and control of infections in hospitals and agricultural establishments. However, environmental monitoring campaigns are almost circumscribed to the research field and there are very few reports on antimicrobials levels and their fate in Latin America countries (Furley *et al*., 2018).

After human or animal consumption, antibiotics are partially metabolized, leading to the excretion of diverse active chemical compounds through urine and faeces. Depending on antibiotic family, between 10–90% of consumed antibiotics are excreted as parent compounds and further released with animal manure or sewage into wastewater treatment plants (WWTPs) (Kovalakova *et al*., 2020).WWTPs are considered an important source of antibiotics into receiving water bodies, as they are not designed to remove this type of compounds, as well as leaching of landfills, effluents from hospitals and industries of antibiotic production with poor decontamination methods (Manzetti & Ghisi, 2014).

Freshwater resources are particularly sensitive ecosystems susceptible of urban pollution, as they usually cross cities and are used for different purposes (water supply, irrigation, recreation, wastes depuration, etc.) receiving diffuse and punctual contamination. Urban usage of antimicrobials and poor or non-efficient treatment of the effluents generated has contributed to river pollution globally, where antimicrobials have been detected at

87 concentrations ranging pg L^{-1} to μg L^{-1} in surface water and μg kg^{-1} to mg kg^{-1} in
88 sediments (Danner *et al*., 2019; Peña-Guzmán *et al*., 2019). Sediments may act as
89 reservoirs for some antibiotic families such as tetracyclines and fluoroquinolones, since
90 these families tend to sorb onto particles (Zhou *et al*., 2011).
91 Among aquatic biota, river biofilms have been recognized as key components of aquatic
92 ecosystems in which important ecological processes such as carbon and nutrient cycling
93 occur. Fluvial biofilms (also named periphyton) are communities composed mainly by
94 bacteria, algae, archaea, fungi and protozoa embedded in an organic polymeric matrix
95 attached to submerged surfaces (Huerta *et al*., 2016; Pu *et al*., 2019). Biofilms have been
96 recognized as good indicators of water pollution because of their capacity to sorb
97 contaminants and their rapid development, widespread distribution and large biomass
98 (Sabater *et al*., 2007). Moreover, biofilms could also transfer pollutants to higher trophic
99 levels of riverine food webs within freshwater ecosystems (Danner *et al*., 2019).
100 Nevertheless, bioaccumulation of antibiotics in urban fluvial biofilms has not been
101 extensively reported, particularly in field studies under real scenarios.
102 The environmental science field has a key role in addressing the problem of antibiotics in
103 the aquatic environment (Manzetti & Ghisi, 2014). In Latin America (LA), it has been
104 stated that there is a need to increase control of emerging pollutants within different
105 components of the urban water cycle (Peña-Guzmán *et al*., 2019. Particularly in
106 Argentina, antibiotics have been quantified in river water receiving urban pollution
107 (Teglia *et al*., 2019), agricultural pollution, (Alonso *et al*., 2019) and in fish (Ondarza *et*
108 *al.,* 2019). In all these studies between 3 and 8 compounds were monitored
109 simultaneously in one river compartment. To the author´s knowledge, there are not yet
110 multi-residue field studies covering a wide variety of antibiotics and metabolites which
111 explore their environmental fate among different compartments of rivers. Considering

this, we conducted this study under two hypotheses: 1) antibiotic families will partition differentially from river water into biofilm and sediments; 2) antibiotic urban sources will include others than WWTP effluents, posing a risk to aquatic biota and antibiotic resistance proliferation.

2. Materials and Methods

2.1 Study site and monitoring campaigns

The Suquía River is an urbanized important river in the province of Córdoba, Argentina (LA) which crosses the second most populated city in the country, Córdoba (1,330,023 inhabitants). Description of the study site is found in supplementary material (SM). Five sampling sites along Suquía River were selected, S1 to S5 following river direction (Fig. S1), considering previous reports on the water quality of the basin and observation of different sources of pollution along the urban area of Córdoba city (Wunderlin *et al*., 2001; Valdés *et al*., 2014). Surface water, surface sediment and natural river biofilm samples were taken in two monitoring campaigns during 2016: wet season (May 2016-particularly rainy year, Table S1) and dry season (October 2016). Amber glass bottles were used to collect water samples (n = 2) 20 cm below the river surface. Composite surface sediment samples were taken with a shovel (n = 2). Between 45 and 60 days before each monitoring campaign, *in situ* biofilm colonization was performed placing artificial samplers in each site according to Giorgi´s laboratory group experience (SM, Fig. S2).This period of time is considered adequate for the development of a community of periphyton similar to the natural one (Corcoll *et al*., 2012; Vilches *et al.*, 2013). The day of sampling, artificial samplers were removed from the riverbed and glasses colonized by biofilm were separated carefully from blocks, placing them in plastic

136 containers with river water for transportation. All samples were ice refrigerated and
137 covered from light for transportation to the laboratory.

138 *2.2 Chemicals and materials*

139 Antibiotic therapeutic families analysed in this study were: fluoroquinolones, quinolones,
140 penicillins, cephalosporins, macrolides, tetracyclins, lincosamides, sulfonamides,
141 dihydrofolate reductase inhibitors and nitroimidazoles. Analytical antibiotic standards,
142 isotopically labelled standards, chemicals and materials used for the analysis of
143 antibiotics in water, biofilm and sediments are described in SM.

144 *2.3 Physico-chemical and microbiological parameters*

145 Temperature, pH and conductivity of water were measured *in situ* (WTW, Multiline F/Set
146 3).Water velocity (m s^{-1}) was measured by observing the rate of travel of a float (Gordon*et*
147 *al*., 2004). Water alkalinity, dissolved oxygen, suspended and dissolved solids, inorganic
148 nitrogen species (nitrates, nitrites and ammonia), phosphates, chlorides, turbidity, total
149 mesophyll aerobic bacteria (TMAB) and total coliforms were measured at the laboratory,
150 following APHA (2005). Water parameters results were integrated in a water quality
151 index (WQI) for aquatic biota protection (Pesce & Wunderlin, 2000, SM). Biofilm
152 biomass and chlorophyll-a were measured at the laboratory and the autotrophic index (AI)
153 calculated, according to Vilches *et al*. (2013; AI = ash-free dry weight
154 (AFDW)/chlorophyll-a, SM). Sediment texture (sand, silt and clay percentage) and pH
155 were determined according to the Soil Science Society of America methodology (Klute,
156 1986). Organic matter was measured as organic carbon content (OC, %) by wet
157 combustion (Walkley & Black, 1934).

158 *2.4 Antibiotics analysis in river samples*

159 *2.4.1 Water extraction*

160 Two hundred and fifty millilitres of river water were filtered within 24 h (0.45 µm), 25
161 ng surrogate standard (sulfadimethoxine-d_6) were added to each sample and they were
162 stored at 4 °C until solid phase extraction (SPE), according to Gros *et al*.(2013) (SM).

163 *2.4.2. Biofilm extraction*

164 Biofilms were scratched from glass surfaces of each site next day after arrival to
165 laboratory, by using a soft bristle brush and tap water free from chlorine. Composite
166 samples were prepared in 2 mL-plastic tubes (n = 2), freeze-dried and kept at -80°C until
167 extraction, following Santos *et al*. (2019) (SM).

168 *2.4.3. Sediment extraction*

169 Sediment samples were freeze-dried upon arrival to laboratory. Dried sediments were
170 sieved through 125 µm (Table S4, SM) and the finest fraction extracted by pressurized
171 liquid extraction following Gros *et al*. (2019), with minor modifications (SM).

172 *2.4.4. Instrumental analysis and quantification*

173 Chromatographic separation and detection of antibiotics were carried out by UPLC-ESI-
174 MS/MS according to Gros *et al*. (2013) (SM). Internal standard quantification was
175 performed by using isotopically labelled antibiotics for each family (Gros *et al*., 2013).
176 Matrix matched calibration curves were performed: water (control site sample–S1),
177 biofilm (control site sample–S1) and two composite sediment samples with different
178 organic carbon content of less polluted sites (sediment type 1: 0.3-0.8 % OC and sediment
179 type 2: 2.3-5.2 % OC).The four matrices were also used in recovery experiments (SM).

180 *2.5. Biofilm bioaccumulation factors and sediment pseudo-partitioning coefficients*

181 The term "bioaccumulation" in this study refers to the concentration of antibiotics found
182 within the biofilm, both inside the cells and in the matrix surrounding them, as a result of
183 active biological uptake or passive sorption (Huerta *et al*., 2016). Field-derived
184 bioaccumulation factors (BAF) for a particular antibiotic (AB) were calculated

185 considering the average biofilm concentration (µg $kg^{-1}_{d.w.}$) and water concentration (µg
186 L^{-1}) measured at the same sampling site and season (equation 1). Only antibiotics
187 quantified both in water and biofilm samples simultaneously were considered. When a
188 concentration value was "b.q.l.: below quantification limit", half the method
189 quantification limit was considered. It is worth mentioning that concentration of AB in
190 water samples represent the concentration at the day of sampling while biofilm samples
191 integrate their exposure history from 46 and 68 days in dry and wet season, respectively.
192 As a result, BAFs should be considered as a tendency of antibiotic bioaccumulation
193 (Huerta *et al*., 2016).

194 $$BAF\ \left(\frac{L}{kg_{d.w.}}\right) = \frac{biofilm\ AB\ concentration\ \left(\frac{\mu g}{kg_{d.w.}}\right)}{water\ AB\ concentration\ \left(\frac{\mu g}{L}\right)} \quad (1)$$

195 Pseudo-partitioning coefficients (P-PC) were calculated for ABs quantified both in water
196 and sediment samples, following equation 2 (Kim & Carlson 2007).These authors
197 indicated that since the river and sediment are not at equilibrium, P-PC cannot be regarded
198 as a true partitioning coefficient. However, calculated P-PC can be a valuable indicator
199 of the sorption characteristics of individual compounds.

200 $$P-PC\ \left(\frac{L}{kg_{d.w.}}\right) = \frac{sediment\ AB\ concentration\ \left(\frac{\mu g}{kg_{d.w.}}\right)}{water\ AB\ concentration\ \left(\frac{\mu g}{L}\right)} \quad (2)$$

201 *2.6. Environmental risk assessment (ERA)*

202 The environmental risk of antibiotics in Suquía River water was assessed by estimation
203 of the risk quotient (RQ), according to equation 3:

204 $$RQ\ = \frac{MEC}{PNEC} \quad (3)$$

205 where "MEC" is the "Measured Environmental Concentration" of an antibiotic in river
206 water (maximum concentration of an AB in this study, considering a worst case scenario)

and "PNEC" is the "Predicted No Effect Concentration", based on the approach by Tell *et al*. (2019). In this approach, the lower of two PNECs is considered, either environmental PNECs compiled by Tell *et al*. (2019), by reviewing ecotoxicity data ($PNEC_{environmental}$), or PNECs based on minimum inhibitory concentrations ($PNEC_{resistance\ selection}$), reported in Bengtsson-Palme & Larsson (2016). This ERA aims to be protective with ecological resources, and also to lower the pressure for the evolution, selection and maintenance of antimicrobial resistance in the environment (Tell *et al*., 2019).

2.7. Statistical analysis

Infostat Software Package (2018) was used for statistical analyses, with significance level $\alpha = 0.05$ (Di Rienzo *et al*., 2018). Differences in antibiotic concentrations between sites and seasons were assessed using linear mixed models, followed by LSD Fisher comparison test. The association between measured variables in each matrix (antibiotic concentrations and physico-chemical descriptors) and sites was performed by principal component analysis (PCA) of standardized values at each season(multivariate analysis). The following physico-chemical descriptors were included in PCA: WQI (water), AFDW and chlorophyll-a (biofilm), pH and organic carbon content (sediment).

3. Results and discussion

3.1 Physico-chemical and microbiological parameters

The entrance of Suquía River into Cordoba city (S2-S5 compared to S1) was evidenced by the increase in conductivity and bacteria counts (Table 1). An even worse scenario was evidenced downstream WWTP (S4 and S5), with depletion of dissolved oxygen concentration and bacteria counts notably increasing its number by 2 orders of magnitude (Table 1).Water quality index showed a continuous decrease along and downstream Córdoba city (S1: good quality> S2≈S3: medium quality> S4≈S5: bad quality, not

232 suitable for healthy living organisms), with lower values in dry season (Table 1). This
233 pollution gradient has been reported for more than 20 years in Suquía River, pointing out
234 the bad quality of water downstream the WWTP and lack of river depuration capacity to
235 recover the good quality upstream the city (Pesce & Wunderlin, 2000, Merlo *et al*., 2011;
236 Amé & Pesce, 2015).The structure of biofilm changed from autotrophic communities,
237 predominating in sites upstream the WWTP (AI < 200), to heterotrophic communities,
238 downstream the WWTP (AI > 200) (Table 1). Sediment samples varied in composition
239 (organic carbon content and texture) according to seasons and sites (Table 1). However,
240 differences were more evident between seasons, which could be related to river flow and
241 its influence on turbulence and riverbed composition. Organic carbon content was highly
242 variable showing higher content during wet season for most sampling sites. Overall,
243 water, biofilm and sediment parameters allowed differentiating between sites upstream
244 and downstream the WWTP, the last ones with a worse quality scenario. This worse
245 scenario was more pronounced during the dry season months.

246 *3.2 Antibiotics in river samples*

247 *3.2.1 Antibiotics concentration and spatio-temporal distribution*

248 Out of forty-three antibiotics and 4 metabolites monitored in river water, 36 of them were
249 analysed in biofilm and 31 in sediments. To the author´s knowledge, this is the first study
250 to assess the environmental fate of a large number of antibiotics in 3 compartments of a
251 river, including biofilm. Method performance results can be found in SM (Tables S2a,
252 S2b). Method detection limits were in the range 0.2-31 ng L^{-1} in water, 0.3-28 µg $kg^{-1}_{d.w.}$
253 in biofilm and 0.1-6 µg $kg^{-1}_{d.w.}$ in sediments. Recoveries varied between 21-156% for the
254 three matrices. These validation results are in accordance with previous analytical
255 methodologies for the same matrices (Jelic *et al*., 2009, Gros *et al*., 2013, Huerta *et al*.,
256 2016, Santos *et al*., 2019, Gros *et al*., 2019).

257 Taking into account the total of samples analysed, 12 out of 47 compounds (norfloxacin,
258 ofloxacin, ciprofloxacin, cinoxacin, cephalexin, azithromycin, clarithromycin,
259 doxycycline, clindamycin, sulfathiazole, trimethoprim and metronidazole) were detected
260 in 67% of samples (Table 2). Complete table of all the compounds analysed in all samples
261 with statistical differences is in Table S3(SM). Water and biofilm were the compartments
262 with higher frequency of antibiotics detection, both 80% of positive samples, while
263 sediments presented 40% of positive detection. Antibiotics in water were in the range
264 0.003-0.29 $\mu g\ L^{-1}$ (Table 2) and they belong to 6 families: fluoroquinolones,
265 cephalosporins, macrolides, lincosamides, dihydrofolate reductase inhibitors and
266 nitroimidazoles (Fig. 1 and Fig. S4-SM). Three of these antibiotic families were the ones
267 present in biofilm: fluoroquinolones, macrolides and dihydrofolate reductase inhibitors,
268 with concentrations in the range 2-652 $\mu g\ kg^{-1}_{d.w.}$. The same three families were detected
269 in sediments, with the addition of two more: tetracyclins and sulfonamides, however in a
270 lower concentration range, 2-34 $\mu g\ kg^{-1}_{d.w.}$. Quinolones and penicillins were not detected
271 in any sample, as neither were metabolites. Absence of quinolones could be related to
272 veterinary uses of these antibiotics (not relevant in the area of study). No detection of
273 penicillins has been attributed to their chemical instability (Rodríguez-Mozaz *et al*.,
274 2015b). Sulfonamide metabolites absence could be related to the absence of parent
275 compounds, while metronidazole-OH not being detected could be associated to river
276 dilution, since it has been reported in WWTP effluents (Gros *et al*., 2013).
277 Considering spatial distribution of contaminants, no antibiotic residues were detected in
278 control site S1 (El Diquecito-La Calera). This is a relevant result as the water intake pipe
279 of the city drinking water facility is very close. On the contrary, some antibiotic residues
280 were detected in all sampling sites corresponding to Córdoba city in both seasons. The
281 number and concentration of compounds detected increased downstream the river, with

a remarkable difference between samples upstream (S2, S3), which receive diffuse urban runoff and probable clandestine discharge of non-treated sewage in the pluvial drainage system, and downstream the WWTP(S4, S5), point source of pollution (Table 2, Fig. 1). This pollution gradient seen in antibiotic concentrations is in accordance with physico-chemical and microbiological water results (Table 1).The highest antibiotic concentration in water was 0.29 µg L^{-1} cephalexin in S4, while maximum level in biofilm was 652 µg $kg^{-1}_{d.w.}$ of ciprofloxacin and 34 µg $kg^{-1}_{d.w.}$of ofloxacinin sediments, both in S5.

Regarding temporal variation, antibiotics frequency of detection in water was similar in wet *vs.* dry season (Table 2, Fig. 1). However, in biofilm and sediments higher frequencies and concentrations were detected during the wet season (Table 2, Fig. 1).

In a previous study, where 2 antibiotics were measured in river water, Valdés *et al*. (2014) reported a range of n.d.-0.036 µg L^{-1} ciprofloxacin in water of Suquía River in sites near and downstream the ones monitored in this study. Concentrations in the same order (n.d.-0.078 µg L^{-1}) but higher frequency of detection were currently found, which could be explained by monitoring sites closer to WWTP and a worse present situation 4 years later. This is reaffirmed by the current presence of clarithromycin (n.d.-0.145 µg L^{-1}), not detected before (Valdés *et al*., 2014). Clarithromycin was quantified in a higher flow river of Córdoba province, the Ctalamochita River, at 0.008 µg L^{-1} (Bertrand *et al*., 2019).The Suquía River has been suffering a serious environmental quality degradation as a result of different anthropogenic uses and Córdoba WWTP impact, which has increased its deterioration (Pesce & Wunderlin, 2000, Wunderlin *et al*., 2001, Merlo *et al*., 2011, Amé & Pesce, 2015). This quality deterioration is also highlighted by antibiotic concentrations, originating from diffuse and point urban sources of pollution along Suquía River.

Antibiotics presence in aquatic ecosystems receiving urban pollution has been extensively documented worldwide, with focus on water samples and less frequently including

sediments (Tamtam *et al*., 2008; Kümmerer, 2009; Carvalho & Santos, 2016; Sousa *et al*., 2018; Kovalakova *et al*., 2020). However, there are few reports in Latin America (Peña-Guzmán *et al*., 2019). Even fewer studies have included fluvial biofilms.

In Argentina, between 0.246-7.7 µg $L^{-1}$of fluoroquinolones and ionophore antibiotics have been reported in livestock and poultry wastewater samples and streams receiving direct runoff from animal production (Alcaraz *et al*., 2016, Teglia *et al*., 2019, Alonso *et al*., 2019). More recently, Mastrángelo *et al*. (2020) reported sulfametoxazole > ciprofloxacin > clarithromycin > metronidazole > ofloxacin > trimethoprim in decreasing order of concentration (range 0.072-0.326 µg L^{-1}) in water samples of Reconquista and Luján Rivers (Buenos Aires, Argentina) receiving urban effluents, very similar to the present study, with exception of sulfamethoxazole.

Suquía River water concentrations were in the same range compared to Latin America countries and in the lower-middle range compared to other continents´rivers (mostly Europe and Asia) (Table 2). Noticeably, Locatelli *et al*. (2011) also found cephalexin as the highest concentration antibiotic in urban and sewage impacted rivers in São Paulo, Brazil. Gros *et al*. (2013) and Rodríguez-Mozaz *et al*. (2015b), using the same analytical methodology, reported up to nearly 0.200 µg L^{-1} of very similar antibiotics in Ter River(Catalonia, Spain), receiving WWTP effluents of Girona city (hospital and municipal wastewater), similar situation than Suquía River, even though with less population. The WWTP of Cordoba city is a secondary treatment plant, comparable to most worldwide WWTPs. However, it has been working un-properly because of increased population and lack of facility maintenance (when overcapacity occurs, urban sewage is by-passed without treatment). Therefore, present results should be compared to rivers receiving poorly treated urban effluents. Yet, it has been thoroughly reported that common WWTPs are not prepared to remove pharmaceuticals and, even working

efficiently, antibiotics pass through the system facilities reaching aquatic ecosystems, as recently reported in a study comparing antibiotics in WWTP effluents of 7 European countries (Rodriguez-Mozaz *et al*., 2020).

Regarding the accumulation of antibiotics in fluvial biofilm, to the author´s best knowledge, only one report is available in Latin America Rivers and very few in the world (Table 2). In agreement with Suquía River biofilms, Mastrángelo *et al*. (2020) reported ciprofloxacin as the highest antibiotic concentration (179 µg $kg^{-1}_{d.w.}$) and also the presence of clarithromycin and azithromycin in fluvial biofilms receiving urban effluents. When comparing to other continents, present concentrations were in the same range than the Vienne River in the central part of France (up to 276 µg $kg^{-1}_{d.w.}$Aubertheau *et al*., 2017) or even higher than reported values in Spain: Ebro, Llobregat, Júcar and Guadalquivir Rivers (Rodríguez-Mozaz *et al*., 2015a) and the River Segre (Huerta *et al*., 2016) (Table 2). The high levels detected in Suquía River biofilms highlight their considerable exposure to urban microcontaminants and support the idea of these communities as suitable bioindicators of environmental pollution (Rodríguez-Mozaz *et al*., 2015a, Aubertheau *et al*., 2017, Pu *et al*., 2019).

The antibiotics detected in biofilms have different chemical properties, *e.g*. ionization state and K_{ow} or log D (pH = 8) (Table S6, SM). Although the accumulation of positively charged compounds in biofilm would be favoured, due to negative charges present on its surface, some authors also reported the bioaccumulation of neutral and negative ionizable compounds (Huerta *et al*. 2016, Aubertheau *et al*., 2017). This shows that chemical properties of pharmaceuticals are not the determining factors for the accumulation of contaminants by biofilms. Biofilm characteristics, such as biomass density, porosity and composition of extracellular polymeric substances (EPS), have been found to influence the sorption and intra-biofilm diffusion of contaminants (Sheng *et al*., 2010; Torresi *et*

357 *al*., 2017; Zhang *et al*., 2018). Indeed, different mechanisms are involved in the
358 accumulation of organic contaminants by biofilms that might include three steps:
359 diffusion of contaminants in the biofilm surrounding, their adsorption to EPS and their
360 diffusion inside the biofilm (Chaumet *et al*., 2019). In river samples, it is difficult to know
361 which mechanism is controlling the process; however, we hypothesize EPS might have
362 an important contribution. Heterotrophic communities predominant in sites downstream
363 WWTP presented a visible gelatinous matrix, darker color and more vertical development
364 compared to communities upstream the WWTP. In addition, it is widely known that EPS
365 works as a protection mechanism, acting as a buffer between organisms in the biofilm
366 and solutes in the water column. For example, microbial cells of WWTPs activated sludge
367 and biofilms exposed to toxic substances (as metals) produced more EPS to protect
368 themselves (Sheng *et al*., 2010). Therefore, biofilm communities downstream the
369 WWTP, which are exposed to continuous stress, are expected to produce more EPS as a
370 mechanism of protection.
371 Finally, antibiotics in the sediments of Suquía River were in the same or lower range than
372 reported values worldwide (Table 2). To the author's knowledge, antibiotic levels for
373 Latin America river sediments have not been reported to date.
374 Besides fluoroquinolones, macrolides and trimethoprim, a tetracycline (doxycycline) and
375 a sulfonamide (sulfathiazole) were detected in sediments. Tetracyclins were expected,
376 since they are known for binding more preferentially to suspended solids and sediment
377 than sulfonamides (Wilkinson *et al*., 2017). However, sulfathiazole presence could be
378 related to a past contamination (not detected in water samples at day of sampling).
379 Adsorption to suspended solid material is suggested to aid in the transportation of such
380 compounds in the aquatic environment (Wilkinson *et al*., 2017), which could explain the
381 higher levels found in S5 compared to S4.

382 Finding higher concentration of antibiotics in the wet season in S5 sediments could be
383 related to higher organic matter content (Table 1). These sediments might also have
384 increased the AFDW in S5 wet season, since rainstorms are known to remove sediments
385 and they could have attached to fluvial biofilms (Giorgi & Feijoó, 2010).

386 *3.2.2 Antibiotics distribution, bioaccumulation factors and sediment pseudo-*
387 *partitioning coefficient*

388 Antibiotics presence in each environmental compartment varied according to families.
389 Metronidazole, cephalexinand clindamycin were only detected in river water;
390 doxycyclineand sulfathiazoleonly in sediments and cinoxacin only in biofilms. On the
391 other hand, trimethoprim, ofloxacin (fluoroquinolones) and clarithromycin (macrolides)
392 were detected in the 3 matrices. It is worth mentioning that ciprofloxacin was not analysed
393 in sediments because of low recoveries, but it was present in water and biofilms. While
394 azithromycin was only present in biofilm and sediments, which might be explained by
395 photodegradation in surface waters (Tong *et al*., 2011).

396 Bioaccumulation factors in biofilms ranged 66-12258 L $kg_{d.w.}^{-1}$(Table 3). The lowest BAF
397 corresponded to trimethoprim and the highest to ciprofloxacin. Bioaccumulation is
398 considered significant when BAFs >1,000 L $kg_{d.w.}^{-1}$ (Rodríguez-Mozaz *et al*., 2015a). In
399 this sense, there is a bioaccumulation potential for the 3 fluoroquinolones and the
400 macrolide clarithromycin (Table 3). Similar results were reported by Rodríguez-Mozaz
401 *et al*. (2015a) for azithromycin in periphyton of Spanish rivers, where it achieved values
402 up to 1,638 L kg^{-1}. Antibiotics not detected in water but quantified in biofilms (*i.e.*
403 cinoxacin and azithromycin) might also have a bioaccumulation potential although BAFs
404 could not be calculated in this study.

405 Sediment pseudo-partition coefficients ranged 4-831 L $kg_{d.w.}^{-1}$ for ofloxacin,
406 clarithromycin and trimethoprim (Table 3). Since P-PC were less than 1,000 L $kg_{d.w.}^{-1}$,

these antibiotics had low tendency to partition to river sediments. Azithromycin, doxycycline and sulfathiazole were quantified in sediments but not in water samples, therefore P-CPs could not be calculated. These results are in agreement with previous reports which mentioned that sorption of most pharmaceuticals is a minor natural attenuation pathway in freshwater and marine ecosystems (Čelić *et al*., 2019).

Considering results in this study, antibiotics partition preferentially from water to biofilms rather than sediments, which confirms biofilms as excellent proxy of antibiotic pollution, in this case of fluoroquinolones, macrolides and trimethoprim. Yet, some antibiotic families are only found in water (a cephalosporin, nitroimidazole and lincosamide) and others in sediments (a tetracycline and a sulfonamide), which suggests either they degrade into metabolites or transformation products not evaluated in this study or they show different physico-chemical properties which condition their environmental fate.

3.3 Multivariate analysis (PCA)

The biplot of principal component 1 and 2 explained 93% of the variability of data, both in wet and dry seasons (Fig. S5, SM). An association of better river water quality (WQI) and organic carbon content in sediment with autotrophic communities (chlorophyll-a) in biofilm of sites upstream WWTP (even though with low diffuse antibiotic pollution) was observed from PCA results. This trend was opposite to worst quality sites downstream WWTP (point source), associated to antibiotic concentrations in all matrices and biofilm biomass (AFDW). This last result would confirm that point sources of high pollution *e.g*. city effluents poorly treated, are associated with changes in biofilm communities (shift to prevalence of heterotrophic communities) and higher accumulation of antibiotics in biofilms and sediments. This is in agreement with Aubertheau *et al*. (2017), who reported modifications in bacterial communities of the Vienne River exposed to WWTP effluents,

431 together with higher levels of pharmaceuticals biofilm accumulation and presence of
432 Class 1 resistance integrons.

433 *3.4 Environmental risk assessment*

434 Following commonly used risk ranking criterion, only trimethoprim presented a low risk
435 value (RQ <0.1) (Table 4). Norfloxacin, ofloxacin, clindamycin and metronidazole had
436 moderate risk (RQs 0.14-0.41). While ciprofloxacin, cephalexin and clarithromycin
437 presented high risk (RQ > 1), either for resistance selection (ciprofloxacin) or the
438 ecosystem (cephalexin and clarithromycin). Accordingly, preliminary studies indicated
439 the presence of extended spectrum β-lactamases genes in water samples of Suquía River
440 downstream the WWTP (Valdés *et al.*, 2019).

441 Following the same ERA approach, Rodríguez-Mozaz *et al*. (2020) reported cephalexin,
442 ciprofloxacin and azithromycin (same family as clarithromycin) as antibiotics posing a
443 moderate environmental risk in water bodies of Portugal, Spain, Cyprus and Germany.
444 They also proposed these 3 antibiotics as markers of antibiotic pollution for widespread
445 temporal and geographical characterization of environmental water or WWTP effluents.
446 Finally, it is clear from the risk assessment, as well as previously mentioned reports, the
447 urgent need of urban effluent treatment improvements in order to reduce antibiotic inputs
448 into freshwater ecosystems. Biofilms appear as communities continuously exposed to
449 antibiotics which are shifting as a consequence of point pollution sources and
450 accumulating them in high amounts. However, there are yet questions to answer as
451 regards mechanisms of bioaccumulation in field changing scenarios and EPS role in this
452 process. Lastly, sediments are a minor antibiotic environmental fate but yet not less
453 important when considering specific families such as tetracyclins and sulfonamides.

454

455 **4. Conclusion**

The presence of antibiotics along the Suquía River (Argentina) during dry and wet seasons points out wastewater treatment plant discharges as the most important source of these compounds. Urban runoff also contributed to the levels of antibiotics in the river upstream WWTP. Antibiotics exhibited preference for their accumulation from water column in biofilms rather than in sediments. An association of antibiotics levels in biofilms was found with AFDW. The most prevalent antibiotic families in the three environmental compartments (water, biofilm and sediments) were fluoroquinolones, macrolides and trimethoprim. However cephalexin was the prevalent antibiotic in water. High environmental risk was found for cephalexin and clarithromycin while ciprofloxacin may pose high risk for resistance selection. Biofilms are pointed out as excellent bioindicators of antibiotics pollution and the high levels and risk found call for attention to possible effects on these communities (and higher trophic levels) and selection of antibiotic resistance.

Acknowledgments

This study has been co-financed by the European Union through the European Regional Development Fund (FEDER), by the Agencia Nacional de Promoción Científica y Técnica (FONCyT/PICT-2015-01784) and the International Atomic Energy Agency (CRP: D52039, CN:18849). It has also been partly supported by the Generalitat de Catalunya (Consolidated Research Group: Catalan Institute for Water Research 2014 SGR 291). Lúcia H.M.L.M. Santos thanks the Juan de la Cierva program (IJCI-2017-32747) and Sara Rodríguez-Mozaz thanks the Ramon y Cajal program (RYC-2014-16707) from the Spanish State Research Agency of the Spanish Ministry of Science, Innovation and Universities (AEI-MCIU). ICRA researchers thank funding from CERCA program.Argentinean authors would also like to thank CONICET (Consejo Nacional de

Investigaciones Científicas y Técnicas-Argentina) and Sci-Hub for useful access to knowledge.

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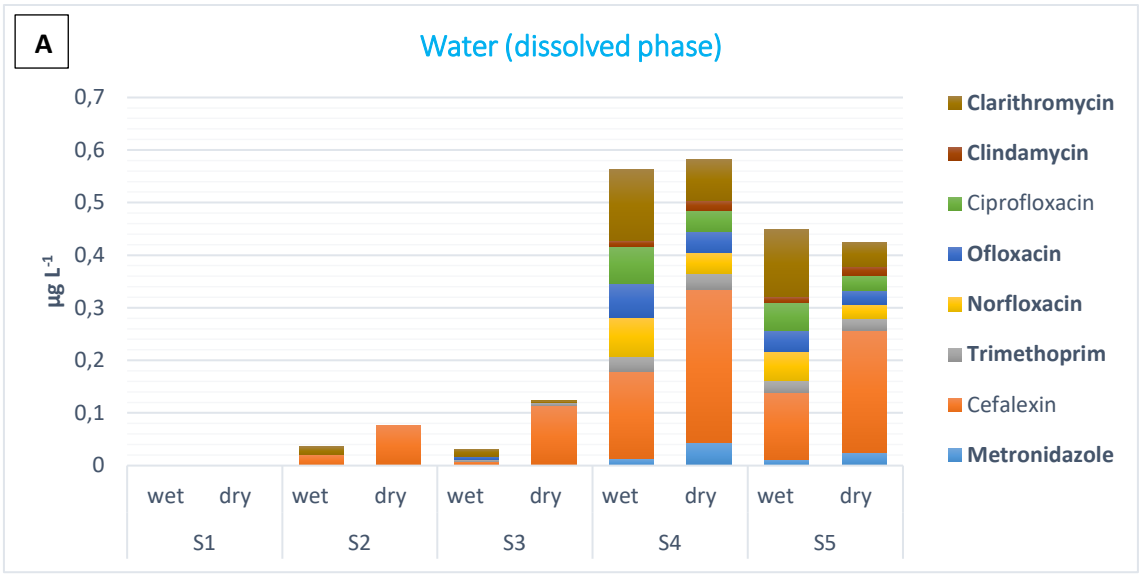
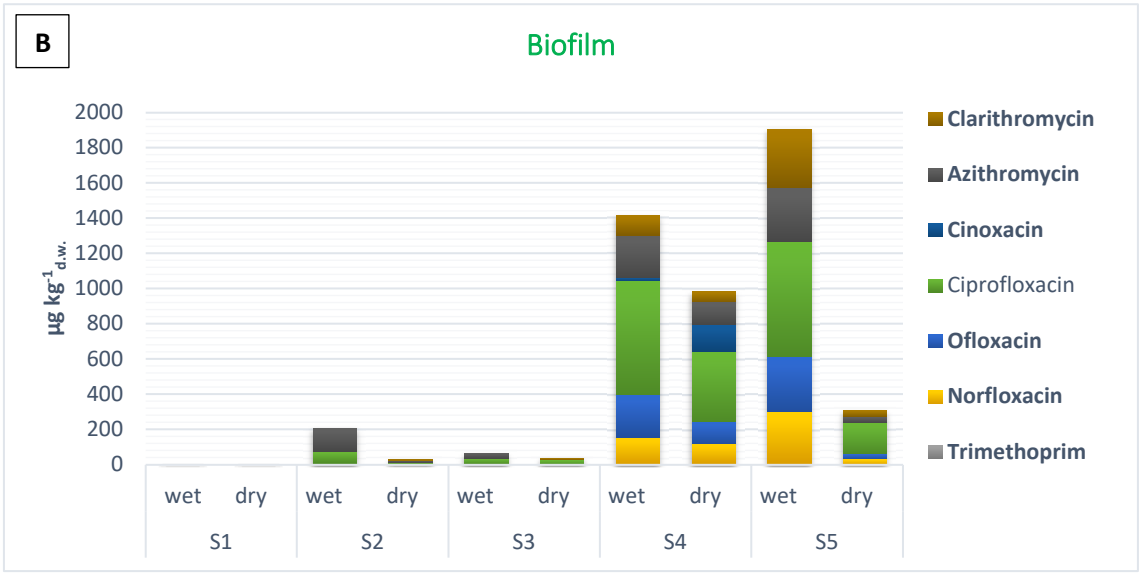
670
671 **Figure Captions:**
672 **Figure1**. Antibiotic concentrations in each matrix. A: water (µg L^{-1}); B: biofilm (µg $kg^{-1}_{d.w.}$); C: sediment (µg $kg^{-1}_{d.w.}$). Antibiotics in bold were analysed in the 3 matrices

674 (ciprofloxacin was not analysed in sediments, doxycycline was not analysed in biofilm
675 and cephalexin was not analysed in biofilm nor in sediments).
676
677 **Table Captions:**
678 **Table 1**. Physico-chemical parameters measured in river water, biofilms and sediment
679 during wet and dry seasons. S1: El Diquecito-La Calera; S2: Campo La Rivera
680 neighbourhood; S3: Post-ringway; S4: 6 km downstream WWTP; S5: 10 km downstream
681 WWTP.
682 **Table 1 Footnote:**
683 *TMAB (CFU mL^{-1}): total mesophyll aerobic bacteria, reported in colony forming
684 unit per mL of river water. **Total coliforms are expressed as the most probable number
685 of coliforms in 100 mL if river water. ***AFDW (mg m^{-2}): ash free dry weight, expressed
686 as mg of organic matter content per m^2 of biofilm surface. (1)n.a.: not analysed (problems
687 in recovery of biofilm artificial samplers). (2)n.d.: not detected (below method limit of
688 detection).(3)AI>200, because chlorophyll-a value in S4 wet season was below method
689 detection limit (APHA, 2005).
690
691 **Table 2.**Antibiotic concentrations in river water-W (µg L^{-1}), biofilm-B and sediment-S
692 (µg kg^{-1} dry weight, d.w.) samples, during wet and dry seasons. S1: El Diquecito-La
693 Calera; S2: Campo La Rivera neighbourhood; S3: Post-ringway; S4: 6 km downstream
694 WWTP; S5: 10 km downstream WWTP. Values are expressed as mean ± standard
695 deviation (SD).
696 **Table 2 Footnote:**
697 (1)Frequency of detection is calculated for each antibiotic in each matrix at each season,
698 as the percentage of total samples with antibiotic concentration > MDL (n = 10).

699 [(2)]References for Latin America reported values (supplementary material-SM).
700 [(3)]References for world reported values (SM). [(4)]n.d.: not detected (below method limit of
701 detection, SM);[(5)]b.q.l: below method limit of quantification (SM).[(6)]n.a.: not analysed.
702
703 **Table 3.** Bioaccumulation factors (BAF, L $kg^{-1}_{d.w.}$) and pseudo-partition coefficient (P-
704 PC, L $kg^{-1}_{d.w}$) of antibiotics in biofilms of sites 3, 4 and 5 of Suquía River.
705 **Table 3 Footnote:**
706 [(1)]n.d.: not detected (below method limit of detection).[(2)]n.a.: not analysed.
707
708 **Table 4.**Risk of antibiotics in Suquía river water to select for resistant bacteria or to the
709 aquatic ecosystem, based on risk quotients (RQ). Values in bold correspond to the lowest
710 of the two PNECs and in the RQ column, to high environmental risk values.
711 **Table 4 Footnote:**
712 [(1)]$PNEC_{resistance\ selection}$: predicted no effect concentration for resistance selection taken
713 from Bengtsson-Palme &Larsson (2016).[(2)]$PNEC_{environmental}$: environmental predicted no
714 effect concentration, reported by Tell *et al*. (2019). [(3)]MEC: measured environmental
715 concentration, in this study the maximum concentration of antibiotic in Suquía River
716 water samples (Table 2). [(4)]RQ: risk quotient, calculated as the ratio of MEC and the lower
717 of the two PNECs (Tell *et al*., 2019). $RQ \leq 0.1$: low risk (green); $0.1 < RQ \leq 1$: moderate
718 risk (yellow); $RQ > 1$: highrisk (orange).
719

Figure 1.


A
Water (dissolved phase)
µg L-1
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
wet dry wet dry wet dry wet dry wet dry
S1 S2 S3 S4 S5
Clarithromycin
Clindamycin
Ciprofloxacin
Ofloxacin
Norfloxacin
Trimethoprim
Cefalexin
Metronidazole


B
Biofilm
µg kg-1 d.w.
2000
1800
1600
1400
1200
1000
800
600
400
200
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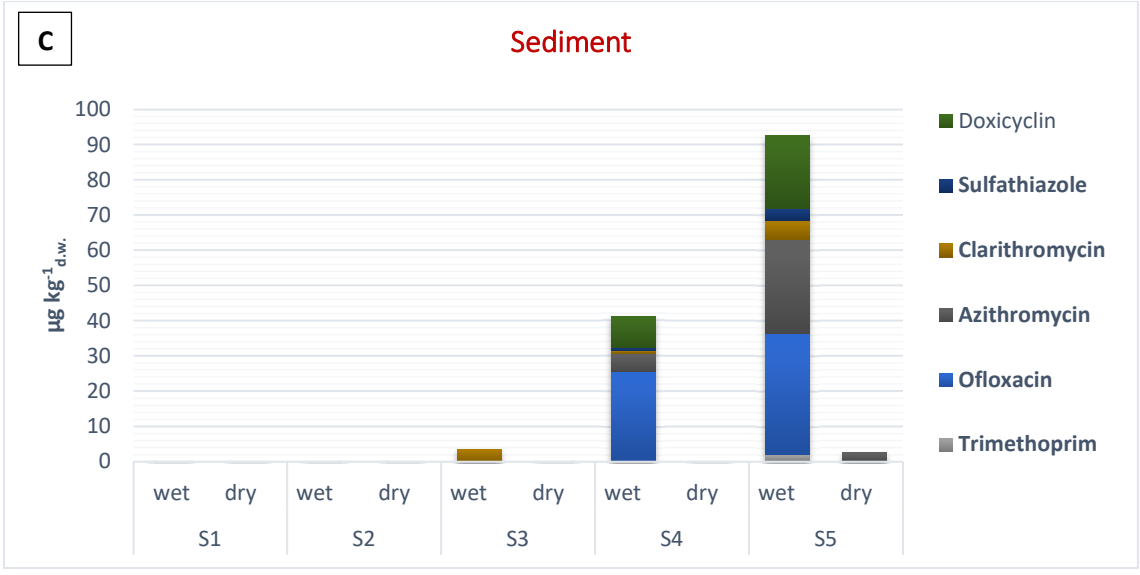
wet dry wet dry wet dry wet dry wet dry
S1 S2 S3 S4 S5
Clarithromycin
Azithromycin
Cinoxacin
Ciprofloxacin
Ofloxacin
Norfloxacin
Trimethoprim


C
Sediment
µg kg-1 d.w.
100
90
80
70
60
50
40
30
20
10
0
wet dry wet dry wet dry wet dry wet dry
S1 S2 S3 S4 S5
Doxicyclin
Sulfathiazole
Clarithromycin
Azithromycin
Ofloxacin
Trimethoprim

724 **Table 1.**

	parameter (unit)	season	S1	S2	S3	S4	S5
water	Water velocity (m s^{-1})	wet	0.7	1.0	0.7	1.0	1.2
		dry	0.7	1.4	0.8	0.9	2.2
	Temperature (ºC)	wet	14.1	13.4	13.8	15.1	15.4
		dry	18.2	18.4	18.8	19.6	19.9
	pH	wet	7.58	7.92	8.02	7.76	5.98
		dry	8.91	7.64	7.45	7.59	7.67
	Conductivity (µS cm^{-1})	wet	203	623	620	754	761
		dry	272	566	531	673	628
	Dissolved oxygen (mgL^{-1})	wet	6.8	8.2	7.4	5.0	6.0
		dry	9.4	6.8	7.6	3.1	2.5
	TMAB* (CFU mL^{-1})	wet	520	3,900	4,500	250,000	200,000
		dry	140	410,000	560,000	590,000	1,200,000
	Total coliforms** (MPN 100 mL^{-1})	wet	240	9,300	9,300	930,000	430,000
		dry	93	230,000	930,000	930,000	2,300,000
	Water quality index (WQI)	**wet**	**89**	**68**	**65.5**	**49**	**49.5**
		dry	**75**	**50**	**54**	**41**	**39**
biofilm	AFDW*** (mg m^{-2})	wet	3,200 (679)	3,620 (877)	n.a.(1)	7,920 (1810)	20,024 (16,175)
		dry	2,680 (396)	2,840 (735)	n.a.	12,280 (57)	4,720 (849)
	Chlorophyll-a (mg m^{-2})	wet	55 (29)	51 (56)	42 (16)	n.d.(2)	1.02 (0.04)
		dry	76 (34)	108 (71)	n.a.	10 (13)	16 (13)
	Autotrophic index (AI)	**wet**	**58**	**71**	**n.a.**	**>200(3)**	**19,632**
		dry	**35**	**26**	**n.a.**	**1,193**	**298**
sediment	pH	wet	7.3 (0.1)	6.1 (0.2)	6.8 (0.2)	7.0 (0.1)	7.0 (0.3)
		dry	6.78 (0.03)	6.4 (0.1)	7.0 (0.2)	7.5 (0.2)	7.2 (0.1)
	Organic carbon content (%)	wet	3.1^{b} (0.3)	3.2^{b}(0.5)	5.2^{c}	0.3^{a}	2.5^{b}(0.5)
		dry	2.29^{b}(0.04)	0.5^{a}(0.1)	0.8^{a} (0.1)	0.35^{a} (0.04)	0.5^{a}(0.1)
	Sand (%)	wet	69	40	43	50	39
	Silt (%)		31	57	57	37	61
	Clay (%)		0	3	0	13	0
	Texture		sandy loam	loam	loam	loam	loam
	Sand (%)	dry	67	67	60	73	77
	Silt (%)		33	14	21	20	23
	Clay (%)		0	19	19	7	0
	Texture		sandy loam	sandy loam	sandy loam	sandy loam	loamy sand

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726

727 **Table 2.**

Family	Comp.	Matrix	S1		S2				S3				S4				S5				Detection frequency[1] (%)		Range	Reported values in Latin America[2]	Reported values in the world[3]
			wet mean	dry mean	wet mean	wet SD	dry mean	dry SD	wet mean	wet SD	dry mean	dry SD	wet mean	wet SD	dry mean	dry SD	wet mean	wet SD	dry mean	dry SD	wet	dry	Min. – max.		
Fluoroquinolones	Norfloxacin	W	n.d.[4]	n.d.	n.d.		n.d.		n.d.		n.d.		0.075	0.007	0.039	0.008	0.055	0.008	0.0274	0.0001	40	40	n.d. – 0.080	0.0041–1.744 (a)	0.004–6.06 (h)
		B	n.d.	n.d.	n.d.		n.d.		n.d.		n.d.		154	40	117	40	297	94	33	4	40	40	n.d. – 364		
		S	n.d.	n.d.	n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.	n.d.	n.d.		16.68–19591 (i)
	Ofloxacin	W	n.d.	n.d.	n.d.		n.d.		0.0062	0.0001	n.d.		0.064	0.006	0.040	0.008	0.041	0.002	0.027	0.002	60	40	n.d. –0.069	0.084–1.78 (b)	0.105–17.7 (j)
		B	n.d.	n.d.	8	3	n.d.		b.q.l.[5]		n.d.		246	53	123	61	313	51	28.80	0.02	80	40	n.d– 349	n.d. (e)	105.9–276* (k)
		S	n.d.	n.d.	n.d.		n.d.		n.d.		n.d.		26	7	n.d.		34	7	n.d.		40	n.d.	n.d. – 39		10–5808 (l)
	Ciprofloxacin	W	n.d.	n.d.	n.d.		n.d.		n.d.		n.d.		0.07	0.01	0.041	0.007	0.052	0.001	0.028	0.003	40	40	n.d. –0.078	0.0041–7.7 (c)	0.023–14.331 (m)
		B	n.d.	n.d.	69		b.q.l.		33	16	28	3	644	80	400	159	652	76	178	6	80	80	n.d. – 706	179 (e)	211 (n)
		S	n.a.[6]	n.a.	n.a.		n.a.		n.a.		n.a.		n.a.		n.a.		n.a.		n.a.		n.a.	n.a.	n.a.		<10–7812 (o)
	Cinoxacin	W	n.d.	n.d.	n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.	n.d.	n.d.		0.5086 (p)
		B	n.d.	n.d.	n.d.		n.d.		n.d.		n.d.		16	5	154		n.d.		n.d.		20	20	n.d. –154		
		S	n.d.	n.d.	n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.	n.d.	n.d.		
Cephalosporins	Cephalexin	W	n.d.	n.d.	0.0213	0.0006	0.077	0.008	0.01	0.01	0.11	0.04	0.17	0.05	0.29	0.01	0.127	0.009	0.233	0.004	70	80	n.d. – 0.299	0.133–2.422 (d)	0.027–0.868 (q)
		B	n.d.	n.d.	n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.	n.d.	n.d.		
		S	n.d.	n.d.	n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.	n.d.	n.d.		
Macrolides	Azi	W	n.d.	n.d.	n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.	n.d.	n.d.	n.d.(e)	0.027–16.633 (r)

		B	n.d.	n.d.	131		8	4	29	15	n.d.		242	68	127	30	304	46	34	9	80	60	n.d. – 336	29 (e)	58.5 (n)
		S	n.d.	n.d.	n.d.		n.d.		n.d.		n.d.		5	3	n.d.		27	9	b.q.l.		40	20	n.d. – 33		315 (s)
	Clarithromycin	W	n.d.	n.d.	0.0147	0.0005	n.d.		0.0135	0.0003	0.003	0.001	0.14	0.01	0.08	0.01	0.13	0.01	0.05	0.01	80	60	n.d. – 0.145	0.063–0.216 (f)	0.00027–2.403 (t)
		B	n.d.	n.d.	n.d.		5.9	0.9	n.d.		10	3	110	18	57	12	331	4	34	16	40	80	n.d. – 334	38 (e)	n.d. (u)
		S	n.d.	n.d.	n.d.		n.d.		3.6	0.5	n.d.		b.q.l.		n.d.		6	4	n.d.		60	n.d.	n.d. – 8.3		6.80–9.93 (s)
Tetracyclins	Doxycycline	W	n.d.	n.d.	n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.	n.d.	n.d.	74 (f)	0.00624–0.40(v)
		B	n.a.	n.a.	n.a.		n.a.		n.a.		n.a.		n.a.		n.a.		n.a.		n.a.		n.a.	n.a.	n.a.		
		S	n.d.	n.d.	n.d.		n.d.		n.d.		n.d.		9	8	n.d.		21	19	n.d.		40	n.d.	n.d. – 34		6.04–2248 (w)
Lincosamides	Clindamycin	W	n.d.	n.d.	n.d.		n.d.		n.d.		n.d.		0.011	0.001	0.020	0.005	0.0109	0.0001	0.017	0.002	40	40	n.d. – 0.023	0.008 (f)	0.0015–0.01017 (x)
		B	n.d.	n.d.	n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.	n.d.	n.d.		
		S	n.d.	n.d.	n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.	n.d.	n.d.		
Sulfonamide	Sulfathiazole	W	n.d.	n.d.	n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.	n.d.	n.d.	0.039 (f)	0.04–3.971 (y)
		B	n.d.	n.d.	n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.	n.d.	n.d.		
		S	n.d.	n.d.	n.d.		n.d.		n.d.		n.d.		b.q.l.		n.d.		3.3	0.7	n.d.		40	n.d.	n.d. – 3.8		51.7 (s)
Dihydrofolate red. inhib.	Trimethoprim	W	n.d.	n.d.	n.d.		n.d.		b.q.l.		0.006	0.003	0.03	0.01	0.030	0.001	0.02246	0.00004	0.022	0.002	60	60	n.d. – 0.035	0.0056–3.58 (g)	0.0021–11.383 (z)
		B	n.d.	n.d.	n.d.		n.d.		n.d.		n.d.		b.q.l.		b.q.l.		5	4	b.q.l.		40	40	n.d. – 7.9		10.4 (aa)
		S	n.d.	n.d.	n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		2.0	0.9	n.d.		20	n.d.	n.d. – 2.6		2.34–3502 (bb)
Nitroimidazoles	Metronid	W	n.d.	n.d.	n.d.		n.d.		n.d.		n.d.		0.013	0.003	0.04	0.01	0.0114	0.0004	0.024	0.001	40	40	n.d. – 0.051	0.200 (e)	0.0016–4.02 (cc)
		B	n.d.	n.d.	n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.	n.d.	n.d.		n.d. (aa)

		S	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.			

728

729 **Table 3.**

Family	Compound	BAF (L $kg_{d.w.}^{-1}$) wet season S3	BAF (L $kg_{d.w.}^{-1}$) wet season S4	BAF (L $kg_{d.w.}^{-1}$) wet season S5	BAF (L $kg_{d.w.}^{-1}$) dry season S3	BAF (L $kg_{d.w.}^{-1}$) dry season S4	BAF (L $kg_{d.w.}^{-1}$) dry season S5	P-PC (L $kg_{d.w.}^{-1}$) wet season S3	P-PC (L $kg_{d.w.}^{-1}$) wet season S4	P-PC (L $kg_{d.w.}^{-1}$) wet season S5
Fluoroquinolones	norfloxacin	n.d.(1)	2063	5412	n.d.	3002	1187	n.d.	n.d.	n.d.
	ofloxacin	2143	3834	7570	n.d.	3080	1068	n.d.	398	831
	ciprofloxacin	n.d.	8255	12258	n.d.	8254	5839	n.a.(2)	n.a.	n.a.
Macrolides	clarithromycin	n.d.	808	2584	3670	739	729	264	4	44
Dihyd. red. inhibitors	trimethoprim	n.d.	70	234	n.d.	66	92	n.d.	n.d.	88

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731 **Table 4.**

Family	Compound	$PNEC_{resistance\ selection}$(1) (µg L^{-1})	$PNEC_{environmental}$(2) (µg L^{-1})	MEC(3) (µg L^{-1})	RQ(4) (MEC/PNEC)
Fluoroquinolones	norfloxacin	**0.5**	120	0.08	0.16
	ofloxacin	**0.5**	10	0.069	0.14
	ciprofloxacin	**0.064**	0.57	0.078	**1.22**
Cephalosporins	**cephalexin**	4	**0.08**	0.299	**3.74**
Macrolides	**clarithromycin**	0.25	**0.08**	0.145	**1.81**
Lincosamides	clindamycin	1	**0.1**	0.023	0.23
Dihyd. red. inhibitors	trimethoprim	**0.5**	100	0.035	0.07
Nitroimidazoles	metronidazole	**0.125**	N/A	0.051	0.41

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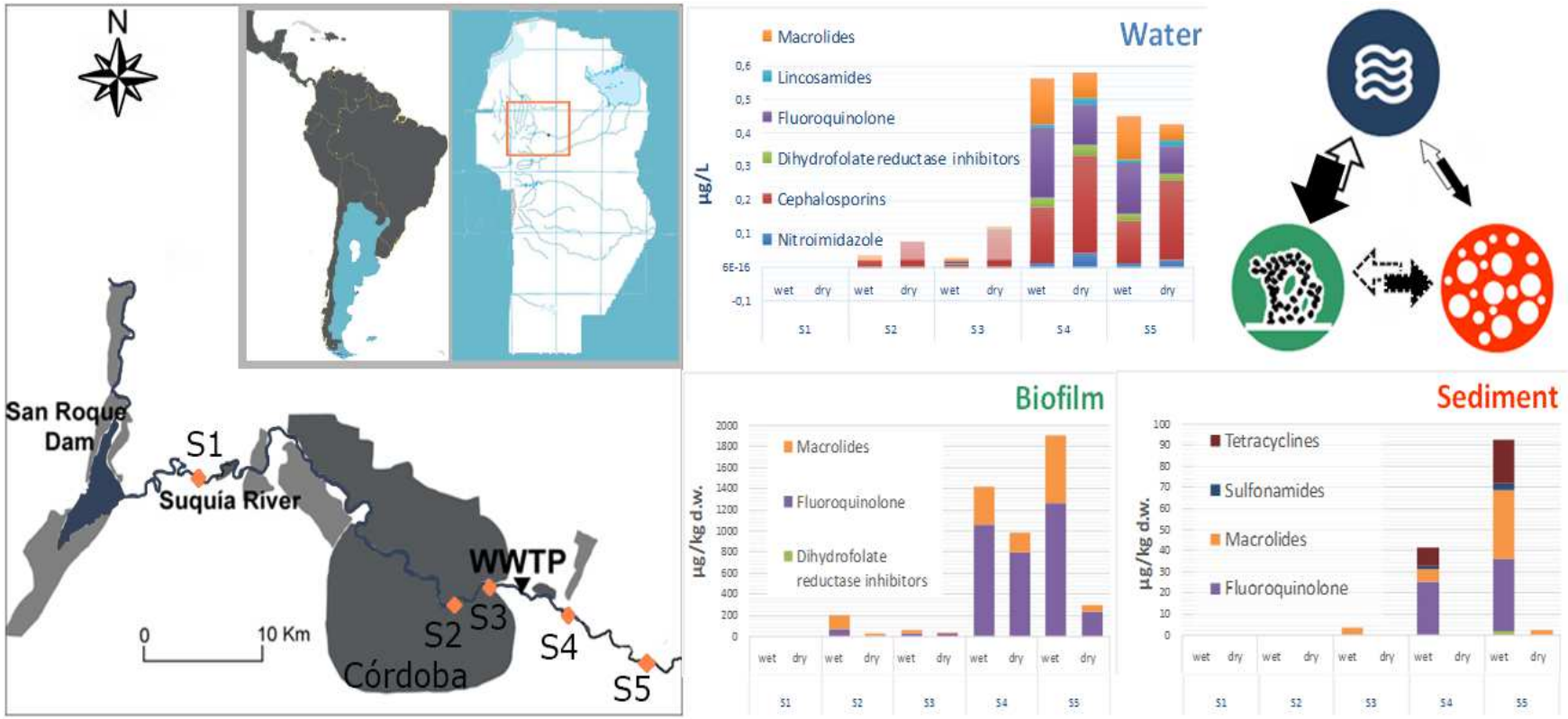
734 Graphical Abstract



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