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1 **Pharmaceutical compounds removal efficiency by a small**
2 **constructed wetland located in south Brazil**

3

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25

26 **Abstract**
27

28 The fate of pharmaceuticals during the treatment of effluents is of major concern since they are not
29 completely degraded and because of their persistence and mobility in environment. Indeed, even at low
30 concentrations they represent a risk to aquatic life and human health. In this work, fourteen
31 pharmaceuticals were monitored in a constructed wetland wastewater treatment plants (WWTP) assessed
32 in both influent and effluent samples. The basic water quality parameters were evaluated and the removal
33 efficiency of pharmaceutical, potential for bioaccumulation and the impact of WWTP were assessed
34 using Polar Organic Chemical Integrative Sampler (POCIS) and biofilms. The pharmaceuticals
35 compounds were quantified by High Performance Liquid chromatography coupled to mass spectrometry.
36 The sampling campaign was carried out during winter (July/2018) and summer (January/2019). The
37 WWTP performed well regarding the removal of TSS, COD₅ and BOD; succeeded to eliminate a
38 significant part of the organic and inorganic pollution present in domestic wastewater but has low
39 efficiency regarding the removal of pharmaceutical compounds. Biofilms were shown to interact with
40 pharmaceuticals and were reported to play a role in their capture from water. The antibiotics were
41 reported to display a high risk for aquatic organisms.

42 Keywords: POCIS; Biofilms; Passive sampling; Wastewater; Water Pollution; Environmental risks.

43

1. Introduction

Contamination of water resources with pharmaceuticals compounds have raised concerns due to their extensive use and continuous discharge in to the aquatic environment. The administered dose of pharmaceuticals are rarely entirely decomposed by the body, thus significant portions of not metabolized drugs and/or their metabolites are excreted by feces and urine (Kakimoto and Onoda 2019) in domestic sewage. Numerous studies have confirmed that conventional wastewater treatment plants (WWTPs) are not always efficient to remove these compounds (Joss et al. 2005), leading to their widespread presence in all aquatic compartments (water, sediments, biota, biofilms) (Okuda et al. 2008; Chonova et al. 2016; Aubertheau et al. 2017; Sathishkumar et al. 2020). About 80% of countries in the world release their sewage water directly to the environment without any treatment (UNESCO 2017). Environmental concentrations depend on contamination sources (cities, agriculture, animal husbandry, industries, hospitals) and hydroclimatic conditions (temperature, rainfalls, drought, season) (Bussi et al. 2017; Bisognin et al. 2018). Thus, the vulnerability of the environment facing this pollution has resulted in profound discussions about the need to carry out actions that enable the maintenance of environmental sustainability.

Conventional advanced sewage treatment systems use different biological treatments (activated sludge, membrane bioreactor, upflow anaerobic reactors, trickling filters) in association with ternary treatment involving oxidation (ozonation or advanced oxidation process AOP) or adsorption (powder or micrograin fluidized activated carbon) for pharmaceuticals removal (Bisognin et al. 2018). However, such a WWTP design is expensive and the financing required for the construction of such infrastructure to cover sanitation services is not economically feasible in all countries. Thus, natural-based treatment systems such as constructed wetlands (CWs) or stabilization ponds have become an attractive option, especially in economically underdeveloped regions (Machado et al. 2017). Indeed, CW systems are widely used for the treatment of domestic, industrial and agricultural effluents worldwide because of low-cost of implementation, operation and maintenance (Stumpf et al. 1999; Suárez et al. 2008; Hyland et al. 2012; Fischer and Majewsky 2014; Bisognin et al. 2019). In CW-based wastewater treatment plant, organic pollutants can undergo mainly adsorption/settling with suspended particles or biological degradation (leading to transformation by-products) but they are rarely completely mineralized (Auvinen et al. 2017). There is often more than one mechanism responsible for the removal of a compound, this indicates the need of compound specific examination for removal mechanisms (Ilyas et al. 2020). The compounds (parent compounds, by-products) released from CWs still represent a risk - even at low concentrations (ng L^{-1}) - to aquatic life and human health (Ebele et al. 2017; Liu et al. 2017; Aubertheau et al. 2017; Ondarza et al. 2019) given their harmful effects as endocrine disruptors or bacterial resistance trigger (Klatte et al. 2017) and their persistence (Wang and Wang 2016).

In recent years, there has been increasing number of studies concerning the removal of pharmaceuticals in CWs (Matamoros et al. 2009; Verlicchi and Zambello 2014; Chen et al. 2016). Compared with tertiary treatment, the CWs are more commonly utilized as primary or secondary treatment for organics and nutrient (nitrogen and Phosphorus) removal around the world (Chen et al. 2016). Furthermore, it is worthwhile noticing that CW systems can offer removal efficiencies for many of the pharmaceuticals as good as conventional WWTPs. This has been attributed to the coexistence of

85 various microenvironments with different physico-chemical conditions in CW systems allowing for both
86 aerobic and anaerobic metabolic degradation pathways of pharmaceuticals, while the more homogeneous
87 conditions in WWTPs induce fewer degradation pathways (Ávila et al. 2010). The available evidence in
88 the literature and physicochem-ical properties of pharmaceuticals indicate that specific processes
89 areinvolved in the removal of a certain type of molecules in CWs (Ilyas and van Hullebusch 2020), and
90 these complexphysical, chemical, and biological processes may occur simul-taneously including
91 photodegradation, volatilization, adsorp-tion/sorption, plant uptake and accumulation, as well as bio-
92 degradation (aerobic and anaerobic), mainly depending on thedesign of the CWs (Zhang et al.2014).

93 Previous studies showed that the use of CWs as tertiary treatment systems resulted in a
94 comparable removal efficiency for some pharmaceuticals to advanced treatment systems. The results
95 show that the constructed wetland (61%) removes emerging contaminants significantly more efficiently
96 than the pond (51%), presumably due to the presence of plants (*Phragmites and Thypha*) as well as the
97 higher hydraulic residence time (HRT) in the CW (Matamoros and Salvadó 2012). It is possible to
98 observe removal of pharmaceuticals in the literature from insignificant (<10%) for carbamazepine
99 (Ternes et al. 2007; Carballa et al. 2007), Diclofenac (Hijosa-Valsero et al. 2016), to>90% for Ibuprofen
100 (Joss 2005), Naproxen (Zhang et al. 2014), Caféine (Camacho-Muñoz et al. 2012), Atenolol (Dordio et al.
101 2009). Also, removal efficiency of CWs for antibiotics show good performance (average value = over
102 50%), especially vertical flow constructed wetlands (VFCWs) (average value = 80.44%) (Liu et al. 2019).
103 But there are many factors determining the removal of specific classes of contaminants in WWTPs:
104 compound chemical properties, plant configuration, hydraulic retention time, operating conditions (i.e.
105 pH, temperature, etc.), presence of industrial wastewater, etc (Krzeminski et al. 2019). Therefore, there is
106 a need for technological solutions effective for various contaminants and under different operating
107 conditions.

108 In Brazil, the consequences of pharmaceutical residues spills to waters are just emerging issues.
109 Indeed, Brazil is the largest consumer of medicines in Latin America and the fifth-largest market in the
110 world (Interfarma 2018). However, since 2014 about 45% of the population are not connected to the
111 sewer network (Machado et al. 2017; ANA 2019) being responsible for the large number of direct
112 connections of clandestine sewerage to rivers in the country. For collected and treated sewages, CW
113 represent about only 0.36 % of WWTP facilities (Salgado and Araújo 2016). Nevertheless, most of them
114 are not regularly maintained and without supervision. Another aggravating factor is the lack of legislation
115 regarding pharmaceutical compounds to regulate effluent quality discharge. Countries such as the USA,
116 France, Spain and other developed countries have legislation that controls the concentration of the
117 pharmaceuticals in the environment (MEDDE, 2012), while in Brazil only parameters such as Total
118 Solids, Chemistry Oxygen Demand, Biochemical Oxygen Demand, Ammoniacal Nitrogen are
119 considered. However, Brazil is the country with the largest amount of fresh water in the world,
120 representing 12% of the total existing volume (Worldatlas 2018) and it is important to develop research to
121 identify, understand, alert and create public policies to prevent and combat the problems of pollution of
122 water bodies in the country. Furthermore, the conservation of subtropical and tropical biodiversity is an
123 international issue that needs to be assessed.

124 Limiting pollution by pharmaceutical compounds in Brazil is essential for human or
125 environmental health protection as well as the establishment of efficient and inexpensive treatment
126 systems to allow the collection and treatment of as much wastewater as possible. In this context, CW
127 appear to be a realistic and locally applicable solution. The present work aimed to evaluate the efficiency
128 of a small CW regarding the removal of several pharmaceutical compounds. Unremovable compounds
129 were measured from treated water using passive sampling devices and biofilms devices to estimate the
130 “mean” exposition of the receiving river over period in different seasons.

131

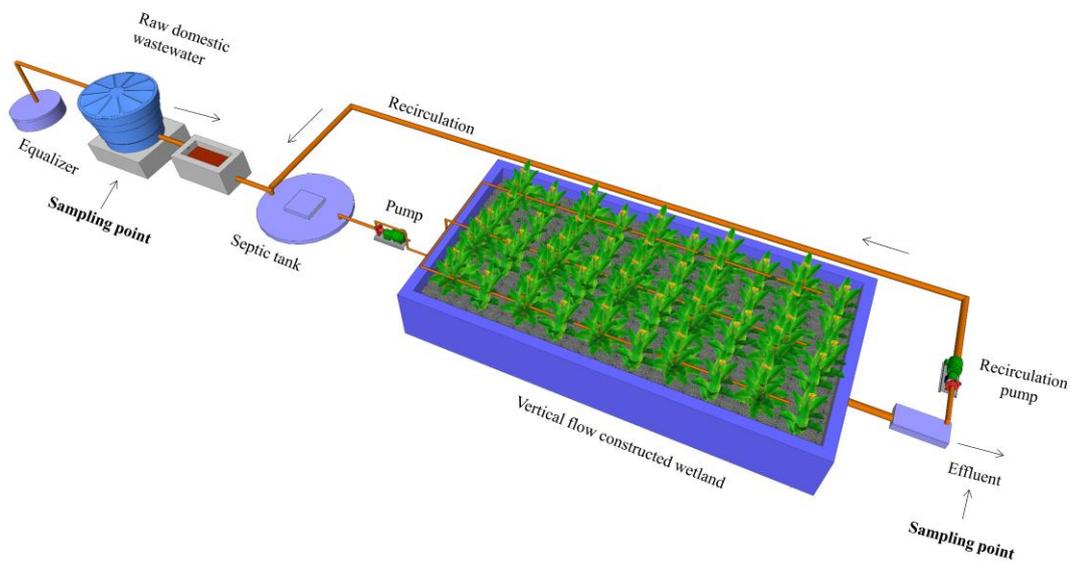
132 2. Material and Methods

133

134 2.1 Constructed wetland

135

136 Experiments were performed at the CWs wastewater treatment plant of the Federal University of
137 Santa Maria, in southern Brazil (under subtropical climate; latitude: -29.7175 ; longitude: -53.7132); This
138 CW was installed in 2015 to collect and to treat sewage of student residences (10 students). The
139 installation involves a septic tank (working volume = 4.7 m^3) operating as primary treatment followed by
140 a Vertical subsurface Flow Constructed wetland (VF-CW) (Figure 1). The VF-CW was 7.0 m long, 3.5 m



141 wide (surface area = 24.5 m^2), and 0.75 m deep, with gravel (19 mm and 25 mm) as bed media.

142

143 **Fig. 1** Typical organization of WWTP involving vertical flow constructed wetland (adapted from:
144 Decezaro et al., 2019)

145

146 The CW was designed for a capacity of 1500 L d^{-1} of raw sewage, with an average input flow of
147 1100 L d^{-1} . This small unit work with a recirculation rate of 90% of VF-CW effluent to the septic tank
148 (ST) inlet line. On average, the ST and VF-CW had an inlet flow rate of 2100 L d^{-1} . Under these

149 conditions, the VF-CW had a hydraulic application rate of 85 mm d⁻¹, operating with an intermittent
 150 supply of 12 pulses per day, with an interval of two hours per pulse.

151 VF-CW was planted with *Heliconia psittacorum* L.f. at a density of 7.7 m² seedlings (3
 152 individuals per seedling) in October 2015. However, as the plants could not withstand the winter, in
 153 September 2016 a pruning was performed to remove the dead plants from the VF-CW surface. From
 154 October 2016, *Canna generalis* L. and *Canna indica* L. were planted and growth at a density of 2.3 m²
 155 seedlings .

156

157

158 2.2 Sampling

159

160 Efficiency of the CW was assessed from the quality of discharge water. Sampling campaigns
 161 were performed in the influent and effluent every fortnight from 6:30 a.m. in the period from May 2018
 162 to January 2019 (Figure 2). The regulatory parameters were analyzed according to standard methods for
 163 the examination of water and wastewater quality (APHA 2017): Total Suspended Solids (TSS), Total
 164 Solids (TS), Chemistry Oxygen Demand (COD), Biochemical Oxygen Demand (BOD₅), N-NH₄⁺
 165 (Ammoniacal Nitrogen), Org-N (Organic Nitrogen), N-NO₃⁻ (Nitrate Nitrogen), TN (Total Nitrogen),
 166 TKN (Org-N + N-NH₄⁺) and P-PO₃⁴⁻ (Orthophosphate). Water samples were collected in clean glass
 167 bottles and rapidly analyzed.

SAMPLING PERIOD		Wastewater influent	Wastewater effluent	POCIS	Biofilm
May		 [1x]	 [1x]		
June		 [2x]	 [2x]		
July		 [2x]	 [2x]		
August	2018	 [2x]	 [2x]		
September		 [2x]	 [2x]		
October		 [2x]	 [2x]		
November		 [2x]	 [2x]		
December		 [2x]	 [2x]		
January	2019	 [1x]	 [1x]		
Total Sampled		16	16	2	2

168

169

170 **Fig. 2** Samplings period from May to December 2018 and January 2019

171 Other sampling approaches were specifically designed to determine the removal efficiency of
 172 pharmaceutical compounds. Thus, passive sampling devices (i.e., Polar Organic Chemical Integrative
 173 Sampler: POCIS) and epilithic biofilm were exposed to the influent and effluent to provide a broader
 174 view of the composition and quantity of the pharmaceuticals released by the CW. The samples of POCIS
 175 and biofilm were performed in July 2018 and January 2019 to evaluate the effect of the season (winter

176 and summer, respectively). Seasonal variations in the removal efficiency have already been observed,
 177 with higher efficiencies in the warm season compared with the cold season due to higher biodegradation
 178 and photodegradation in summer (Ilyas et al. 2020).

179 POCIS were man-made in the laboratory with stainless steel washers of $102 \times 54 \times 3.0$ mm in
 180 external diameter, internal diameter and thickness, respectively. The sequestering agent for
 181 pharmaceuticals was constituted by 200 mg of OASIS[®] HLB resin at 30 μ m particle diameter, with
 182 hydrophilic/lipophilic characteristics. A SUPOR[®] Polyethersulfone filter membrane, with pores of 0.1 μ m
 183 and 90 mm in diameter were used to maintain the resin into the stainless steel washers. The POCIS was
 184 exposed during 15 days in the raw or treated water pipes. After this period, the POCIS were collected,
 185 wrapped in aluminum foils, placed in plastic bags in a thermal box with ice and transported to the
 186 laboratory. In the laboratory, the adsorbent material was transferred to cartridges with the aid of ultrapure
 187 water jets, vacuum dried and under N₂ flow, with subsequent weighing and storage at -80°C.

188 For epilithic biofilm devices, basaltic rocks of known size were autoclaved prior to be
 189 submerged in galvanized steel cages during 45 days on sites before their sampling. This formation period
 190 guarantees to have a mature biofilm for samplings (Mercier et al. 2013). Biofilms were then manually
 191 scrap from rock with a nylon bristle brushes and 0.5 L of deionized water (Aubertheau et al. 2017). The
 192 resulting biofilm suspensions were transferred to individual bottles of high-density polystyrene to be
 193 frozen in ultra-freezer at -80°C. Subsequently, the samples were lyophilized (lyophilizer LS3000-
 194 TERRONI), homogenized in agate mortar to obtain a representative sample. All the dry biofilm recovered
 195 was weighted and the values were converted to the specific surface area of the stones inside each cage.
 196 The values are available in the “Online Resource”.

197

198 2.3 Compounds and analytical procedures

199

200 Four anticancer, eight antibiotics (all macrolides class, except sulfamethoxazole) and two
 201 “classical” pharmaceuticals (carbamazepine and diclofenac) were determined (Table 1). In Brazil,
 202 antibiotics are among the most widely used medicines, especially those belonging to the group of
 203 penicilines and macrolides (WHO 2018). In addition, previous studies lead in the university watershed
 204 highlighted the presence of sulfamethoxazole and (Martins et al. 2011) as well as the four anti-cancer
 205 compounds (Souza et al. 2018). These compounds are known to be highly reactive represents a risk to
 206 human health and the environment.

207 **Table 1** - Pharmaceutical compounds studied grouped according to their therapeutical class. For each
 208 substance, chemical, dissociation constant (pK_a), octanol-water partition coefficient ($\log K_{ow}$) and
 209 molecular formula.

Compound	Classe	pK_a	* $\log K_{ow}$	Molecular formula
Doxorubicin	Anticancer	7.34 ^a *	1.27	C ₂₇ H ₂₉ NO ₁₁
Epirubicin		9.17 ^a *	1.41	C ₂₇ H ₂₉ NO ₁₁
Daunorubicin		7.85 ^a	0.77	C ₂₇ H ₂₉ NO ₁₀
Irinotecan		8.57 ^d	3.20	C ₃₃ H ₃₈ N ₄ O ₆
Midecamycin	Antibiotic	6.90 ^e	-	C ₄₁ H ₆₇ NO ₁₅

Spiramycin		7.88 ^a	1.87	C ₄₃ H ₇₄ N ₂ O ₁₄
Josamycin		7.10 ^f	-	C ₄₂ H ₆₉ NO ₁₅
Azithromycin		8.74 ^a	4.02	C ₃₈ H ₇₂ N ₂ O ₁₂
Sulfamethoxazole		5.70 ^a	0.89	C ₁₀ H ₁₁ N ₃ O ₃ S
Clarithromycin		8.99 ^a	3.16	C ₃₈ H ₆₉ NO ₁₃
Erythromycin		8.88 ^a	3.06	C ₃₇ H ₆₇ NO ₁₃
Roxythromycin		9.20 ^c	0.05	C ₄₁ H ₇₆ N ₂ O ₁₅
Diclofenac	Anti-inflammatory	4.15 ^a	4.51	C ₁₄ H ₁₁ Cl ₂ NO ₂
Carbamazepine	Anti-epileptic	13.90 ^a	2.45	C ₁₅ H ₁₂ N ₂ O

^a pubchem; ^c (Chen et al. 2016); ^d (Anilanmert et al. 2006); ^e drugbank.ca ; ^f drugfuture.com; *phenol

210
211

212 Biofilm extraction procedure was performed using the method described by Aubertheu et al.,
213 (2017). Five hundred milligrams of dry weight biofilms were extracted by extraction liquid at high
214 pressured (ASETM 350, Thermo Fisher Scientific Inc, Waltham, USA) at 80 °C using methanol / water
215 (1/2; v/v) as the extraction solvent. The final extracts were evaporated under mild nitrogen steam for
216 posterior restitution to 500 µL with a mixture of methanol / water (10/90; v/v). The extracts of biofilm
217 and POCIS were purified by solid phase extraction (Autotrace™ 150, Thermo Scientific, 125 Waltham,
218 USA) using Oasis® HLB cartridges (6cc, 200 mg of sorbent; Waters, Milford, USA) using methanol as
219 eluent.

220 Purified extracts were concentrated by evaporation under gentle N₂ flow. The recovered liquid
221 was then filtered one last time with the aid of medium filters Mini-Uni prep™ (PVDF Filter Media with
222 polypropylene Housing, pore 0.45µm, Durapore®, Millipore, Billerica, USA). The compounds were
223 analyzed by rapid high-performance liquid chromatography coupled to tandem mass spectrometry
224 (LCMS/MS-8050 Shimadzu). The HPLC column used in the method was a Waters, UPLC® Acquity C18
225 1.7µm, 2.1 x 100mm. Two mobile phase solvent systems were used: ultrapure water + 0.1% Formic acid
226 and acetonitrile solution contains 0.1 % (v/v) formic acid. The column temperature was kept at 25°C. The
227 sample injection volume employed was 5 µL. The MS and other parameters of the chromatographic
228 conditions are available in the “Online Resource”.

229

230 2.4 Environmental risk evaluation

231

232 The Risk Quotient (RQ_i) were evaluated using the method of the quotient between the maximum
233 Measured Environmental Concentration (MEC) and the Predicted no-effect Concentration (PNEC), as
234 demonstrated in the equation below:

$$235 \quad RQ = \frac{MEC}{PNEC} \quad (1)$$

236 The RQ were calculated from concentration measured by POCIS (expressed in ng L⁻¹ with
237 sampling rate (Rs) correction). The RQ were calculated for the molecules where they had Rs, MEC and
238 PNEC values available in the literature.

239

3. Results and discussion

3.1 Conventional water quality parameters

CW performance results are shown in Table 2. The concentrations of TSS (Total Suspended Solids), TS (Total Solids), COD (Chemistry Oxygen Demand), BOD₅ (Biochemical Oxygen Demand), TKN (Org-N + N-NH₄⁺), N-NH₄⁺ (Ammoniacal Nitrogen), Org-N (Organic Nitrogen), N-NO₃⁻ (Nitrate Nitrogen), TN (Total Nitrogen) and P-PO₃⁴⁻ (Orthophosphate) were evaluated in the inflow, ST (septic tank), influent and effluent of the CW.

For ST, the efficiency removal of TSS was 9%, relatively low when compared to the typical expected value of 30% commented by Jordão and Pessoa (2014). In addition, the effluent from TS presented a higher mean concentration of DBO₅ than the affluent, causing a negative efficiency (-5%) in the removal of this parameter. The removal of NH₄⁺-N was also negative by the ST. Septic tank is most commonly used for pre-treatment of domestic wastewater in on-site applications. The basic function of the septic tank is to separate sludge, effluent and scum layer of the domestic water. It removes a portion of settleable solids by retention and organic matter by partial anaerobic digester. Septic tanks remove 60 to 80% of non soluble material in domestic wastewater (Adhikari and Lohani 2019). The actual performance of the tank depends on the ambient condition, retention time inhabitation in the influent. This ST performance can be associated with maintenance problems, with high accumulation and drag of sludge in the effluent of the unit. The TS also showed low efficiency in the removal of COD, removing only 4% of this parameter.

However, regarding the removal of NO₃⁻-N from the effluent of the recirculated VF-CW, the ST presented a good performance, with efficiency of 88%. According to Al-Zreiqat et al. (2018), ST can provide ideal conditions for the denitrification process, with good availability of organic matter and formation of anoxic environments. The COD/TN ratio of 9.6 determined in this study would allow a removal of approximately 99% of the NO₃-N (Sant'Anna Jr 2011; Al-Zreiqat et al. 2018).

Table 2 – Concentration and loading of TSS (Total Suspended Solids), TS (Total Solids), COD (Chemistry Oxygen Demand), BOD₅ (Biochemical Oxygen Demand), TKN (Org-N + N-NH₄⁺), N-NH₄⁺ (Ammoniacal Nitrogen), Org-N (Organic Nitrogen), N-NO₃⁻ (Nitrate Nitrogen), TN (Total Nitrogen) and P-PO₃⁴⁻ (Orthophosphate) in the wetland studied, during the monitoring period from May 2018 to January 2019

Parameter	Inflow (raw sewage)	ST influent	VF-CW influent	VF-CW effluent	Outflow
			mg L ⁻¹		
TSS	1192 ± 1046	674 ± 560	610 ± 507	90 ± 40	90 ± 40
TS	1756 ± 1142	1210 ± 620	1035 ± 469	595 ± 74	595 ± 74
COD	1253 ± 741	744 ± 421	716 ± 334	171 ± 92	172 ± 92
BOD ₅	612 ± 144	398 ± 67	419 ± 244	155 ± 36	119 ± 23
TKN	132 ± 49	83 ± 28	77 ± 26	27 ± 9	27 ± 9
NH ₄ ⁺ -N	73 ± 22	47 ± 14	51 ± 25	16 ± 6	17 ± 6
Org-N	63 ± 48	38 ± 27	26 ± 18	10 ± 7	10 ± 7

NO ₃ ⁻ -N	2 ± 2	8 ± 4	1 ± 1	16 ± 8	16 ± 8
TN	137 ± 49	93 ± 28	78 ± 26	43 ± 12	43 ± 12
PO ₄ ³⁻ -P	12 ± 2	12 ± 2	10 ± 2	10 ± 2	10 ± 2
			L d ⁻¹		
Flow	1100	2100	2100	2100	1000
			g m ⁻² d ⁻¹		
TSS	-	-	52 ± 43	8 ± 3	-
TS	-	-	89 ± 40	51 ± 6	-
COD	-	-	61 ± 29	15 ± 8	-
BOD ₅	-	-	36 ± 21	13 ± 3	-
TKN	-	-	7 ± 2	2 ± 1	-
NH ₄ ⁺ -N	-	-	4 ± 2	1 ± 0.5	-
Org-N	-	-	2 ± 2	1 ± 0.6	-
NO ₃ ⁻ -N	-	-	0.1 ± 0.1	1 ± 0.7	-
TN	-	-	7 ± 2	4 ± 1	-
PO ₄ ³⁻ -P	-	-	1 ± 0.2	1 ± 0.2	-

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The VF-CW showed average removal efficiencies > 40% for all analyzed parameters except for NO₃⁻ N e PO₄³⁻P, suggesting problem of clogging in the CW. Clogging is generally caused by non-degraded particles/solids accumulation within bed media pores, and/or excessive loading of organic matter and suspended solid. Also, for N as nitrification occurs in CW, this causes an increase in the nitrate level in the final effluent. The difference in NO₃⁻ N concentrations between inflow and outflow indicates a denitrification throughout the recirculating part of the system, apparently due to nitrification in the VF-CW and subsequent denitrification in the recirculation tank (Al-Zreiqat et al. 2018). Most of the TN entering the system is in the form of ammonium, with a mean concentration of 51 mg L⁻¹. The major processes responsible for phosphorus removal in CWs are adsorption on the substrate media, chemical precipitation, and assimilation into microbial and plant biomass (Ilyas and Masih 2018). Usually, the removal of PO₄³⁻-P is not investigated by VF-CW in the studies (Ilyas and Masih 2018). However, previous studies suggest that in all types of CWs, the removal of total phosphorus (TP) varied between 40 and 60% with removed load ranging between 45 and 75 g P m⁻² year⁻¹ depending on CW types and inflow loading (Vymazal 2007). In our study the the system was not efficient in removing P, removal of phosphorous in constructed wetlands is limited by the capacity of the media to plant uptake, adsorption by solid materials and precipitate the incoming P (Arias and Brix 2005), thus formation of clogging may result in reduced treatment performance (Žibienė et al. 2015). Vertical-flow systems, where wastewater is fed intermittently, may not be as effective because the oxygenation of the bed may cause desorption and subsequent release of phosphorus. However, materials which are commonly used for sub-surface flow CWs, i.e., washed gravel or crushed rock, usually provide very low ca-pacity for sorption and precipitation. (Vymazal 2007). According to Sun et al. (2003) the removal of phosphorus in a VF-CW was attributed to the adsorption of phosphorus on substrate media, uptake by the reeds, and chemical precipitations. The most efficient and cost-effective solution for CWs is the use of a filter media with high adsorption capacity and high content of the cations that are able to precipitate phosphorus (Rittmann et al.

297 2011). The media texture and grain size distribution should also be considered to increase the surface area
298 and consequently the adsorption sites, which will help to provide adequate hydraulic conductivity and
299 reduce the risk of clogging (García et al. 2010).

300 In the international literature, there are contradictions regarding the efficiency of nitrogen and
301 phosphorus removal by VF-CW systems. Trein et al. (2015) found high N and P removal efficiency
302 (maximum values of 94 and 93%, respectively), however, certain VF-CW station do not exceed 66 and
303 15%, respectively. Greenway (2005) encounter very low removal efficiencies for phosphorus, while had
304 good nitrogen removal values. Vymazal (2007) states that in single-stage constructed wetlands is
305 expected to reach lower removal efficiencies for nitrogen, compared to hybrid systems (vertical +
306 horizontal flow).

307 VF-CW showed removal efficiencies for TSS, COD, BOD₅, TKN, Org-N, and TN of 83, 76, 63,
308 65, 62 and 45%, respectively. The highly oxidizing conditions provided by intermittent feeding and
309 passage of the effluent through a porous medium make VF-CWs highly efficient systems for removing
310 carbon organic matter and suspended solids (Dotro et al. 2017), and can be optimized with the
311 recirculation strategy. (Zhao et al. 2004; Wu et al. 2014). Usually, removal efficiencies in VF-CW are in
312 the 48-99% DBO₅, 44-95% DQO, and 52-99% TSS ranges (Stefanakis and Tsihrintzis 2009). The
313 application of a 60% recirculation rate in the treatment of domestic sewage in VF-CW, filling with gravel
314 and sand, has the capacity to increase on average 4% of the efficiency of COD and TSS removal
315 compared to its operation without recirculation, with values of 85% and 76%, respectively (Foladori et al.
316 2013).

317 These values are in agreement with data reported for VF-CW systems treating domestic sewage
318 (between 30-99% of removal) estimated by Stefanakis et al. (2016). In a literature review Ilyas and Masih
319 (2017) found BOD, COD_t and SST removal values of 90%, >65% and >85%, respectively in studies with
320 VF-CW. Good removal values are expected with the recirculation system, the concept behind is to
321 increase the aerobic microbial activity through the intense interaction between pollutants and micro-
322 organism without significant alterations in the system operation. The effluent recirculation (ER) has been
323 proposed by many researchers as an operational modification to improve the effluent quality of CWs
324 (Ilyas and Masih 2017; Sharma et al. 2018). Anyway, Rossmann et al. (2013, 2012), Costa et al. (2015)
325 and Machado et al. (2017), in Brazilian conditions, showed that CWs applying gravel can achieve good
326 results for the removal of reviewed contaminants but particularly appear mostly effective for COD and
327 BOD₅.

328 The pollutants removal depends on the operational strategies used, such as recirculation.
329 Foladori et al. (2013) demonstrated the effectiveness of recirculated and/or aerated vertical subsurface
330 flow constructed wetlands (VSSF) systems operated under high loadings by the increase of the removed
331 loads of COD, TKN and TN. This strategy has been proposed by many researchers as an operational
332 modification to improve the effluent quality of CWs (Ilyas and Masih 2017). CW are originally designed
333 for the removal of organic matter (i.e., BOD, COD) and suspended solids - in accordance with the
334 minimum discharge standard (Yang et al. 2017). Machado et al. (2017) showed that a modified CW (SSF-
335 CWs - subsurface flow) achieved the highest traditional pollutants removal percentages. However, these

336 systems are particularly effective in regions with warmer climate, as well as in regions with high light
 337 radiation to enhance plant growth (Devault et al. 2020).

338

339

340 3.2 Compliance with regulations

341

342 Discharge of wastewater, treated or untreated, are known to alter the quality of receiving
 343 environment (Ashfaq et al. 2017; López et al. 2019). In Brazil, wastewater discharges are governed by
 344 Resolution No. 430 of May 17, 2011 (CONAMA, 2011) and n° 355/2017 (CONSEMA) which provides
 345 for conditions, parameters, standards, and guidelines for managing the discharge into receiving water
 346 bodies (Table 2). Only four traditional parameters were considered for the Brazilian legislation. Although
 347 the water would be suitable for release into the water bodies there is no legislation concerning
 348 pharmaceutical compounds. Therefore, the increase in the concentration of emerging compounds has
 349 caused several countries such as the USA, France, and other European countries to incorporate analysis of
 350 these compounds in sewage treatment networks in order to control their impact on the waters receiving
 351 these effluents (Tran et al. 2018).

352

353 **Table 3** - Maximum permissible values of effluent releases according to Brazilian legislation (CONAMA
 354 2011; CONSEMA 2017). Concentrations and the removal efficiency of some parameters analyzed in the
 355 studied treatment station.

Parameter	Maximum permissible value		Effluent system TS/VF-CW	
	CONAMA 430/2011	CONSEMA 355/2017	Concentration mg L ⁻¹	Removal rate %
pH	5 ≤ x ≤ 9	6 ≤ x ≤ 9	7.2	-
DBO _{5,20} (mg L ⁻¹)	≤ 120 mg L ⁻¹ ou 60%	≤ 120 mg L ⁻¹ (Q* < 100 m ³ d ⁻¹)	119.0	75
DQO _t (mg L ⁻¹)	-	≤ 330 mg L ⁻¹ (Q* < 100 m ³ d ⁻¹)	172.0	86
Sólidos (mg L ⁻¹)	SSd ≤ 1,0 mL L ⁻¹	SSd ≤ 1,0 mL L ⁻¹	0.3	96
	-	SST ≤ 140 mg L ⁻¹	90.0	92
N-NH ₄ ⁺ (mg L ⁻¹)	≤ 20	≤ 20 (Q* < 100 m ³ d ⁻¹)	17.0	77

* SSd= Sedimentable solids

356

357

358 Although monitored CW effluents present a high risk of eutrophication, wastewater can be
 359 discharged into water bodies because it is within the standards allowed by Brazilian law. The CW may

360 still represent a risk of eutrophication due to the amount P and N release. The increase N and P in waters
361 may lead to algal blooms, which in turn may disturb ecosystems. Algal blooms can be harmful when they
362 lead to hypoxia or release for toxins, and secondary effects such as reduction in light penetration and
363 dissolved oxygen, losses of submersed grasses and associated habitat, and potentially fish kills (Glibert
364 2017). Eutrophication may thus result in changes in ecosystems and reduced biodiversity, it may also lead
365 to reduced natural resources of fish and algal toxins may lead to toxicity problems in ecosystems and for
366 humans (Blaas and Kroeze 2016). The undesirable symptoms of eutrophication in rivers occur primarily
367 at low flows when abundant light levels and high water temperatures promote rapid algal growth
368 (Mainstone and Parr 2002). Further, within-river phosphorus cycling and flux attenuation involves a
369 complex interaction between sediments, aquatic plants and biofilms and the water column (Jarvie et al.
370 2006). The reviews of Machado et al. (2017) highlights that in each type of CW certain chemical,
371 physical and biological transformation mechanisms favor the elimination of some contaminants rather
372 than others. Despite legally satisfying performance to treat main parameters (C, N) with an efficiency
373 superior to 70%, the wastewater treatment plant still represents a continuous input source of pollution to
374 receiving watercourse. Shifts in nutrient loadings can enhance eutrophication processes and have a direct
375 impact on aquatic organisms (Freixa et al. 2020). Previous studies reported an increase in bacterial
376 abundance in rivers after receiving WWTP discharge (Vivas et al. 2017).

377

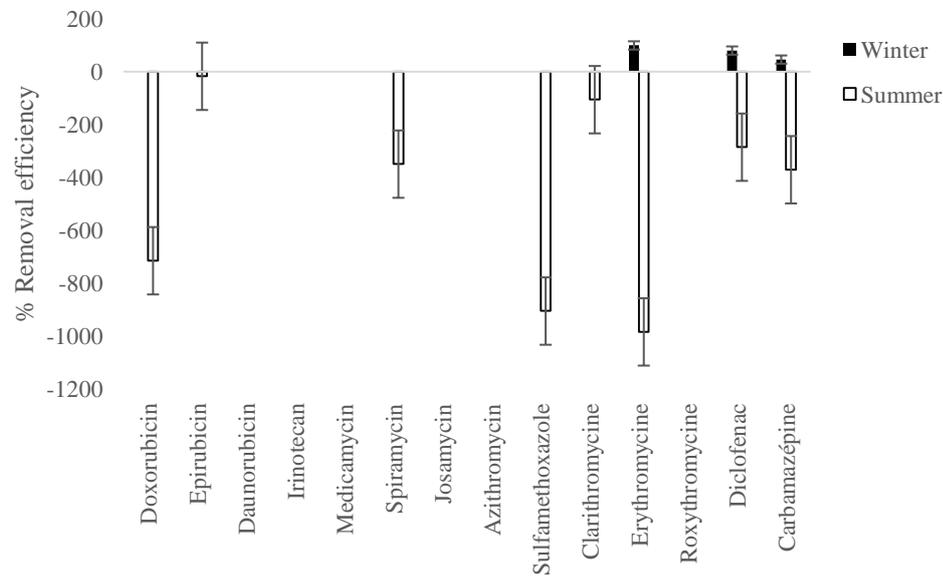
378 3.3 Efficiency of pharmaceuticals compounds removal

379

380 The survey realized by passive sampling (POCIS) highlight positive removal rates in winter and
381 negative rates in summer (Figure 4). Furthermore, more pharmaceutical compounds were detected in
382 summer compared to winter, suggesting that the concentrations of some compounds were influenced by
383 the chemical usage, degradation, temperature seasonal differences in treatment efficiency or
384 pharmaceuticals consumption (Li et al. 2018). The analysis of seasonal changes of pharmaceutical and
385 personal care products (PPCPs) concentrations in the WWTPs suggested that the concentrations of some
386 PPCPs were influenced by the chemical usage, degradation and temperature. Only three compounds were
387 found in winter (erythromycin, diclofenac and carbamazepine) with removal efficiencies of 99, 80 and 46
388 %, respectively. In the summer season, seven compounds (1 anticancer, 3 antibiotics, and the two
389 classical pharmaceuticals) were observed with negative removal efficiency. The effluent from the CW
390 system released large quantities of these pharmaceuticals. The POCIS device can be selective and hence
391 not capture certain compounds depending of their polarity and charge. Thus, the azithromycin, for
392 example, was not detected in any of our samples, probably due to their high octanol-water partition
393 coefficient (eg. $k_{ow} = 4.02$), which ensures greater retention of these compounds in apolar matrices and
394 limits their accumulation in POCIS. Even for the capture of less apolar compounds ($\log K_{ow} > 3.7$), there
395 is a strong influence of the POCIS exposure time in the field. Further, affecting the availability of the
396 concentration in solution, the diffusion between the two phases (from the membranes with a porosity of
397 $0.1 \mu\text{m}$ to the adsorbent - Ibrahim et al. 2013). It is also known that in-situ exposure conditions (water
398 flow rates, temperature, pH, incrustations, etc.) affect the capture rates of more polar compounds (Harman
399 et al. 2011). Temperature of wastewater in the Santa-Maria CW are between 12 to 19°C (mean of 17°C)

400 and between 21 to 27°C (mean of 24°C) in winter and in summer, respectively. Thus, the difference of
401 temperature is too small to explain the difference of removal yield.

402
403



404

405

406 **Fig 4** Removal efficiency of the pharmaceuticals compounds studied in POCIS sampled in winter 2018
407 and summer 2019

408

409 Negative removal values for clarithromycin, erythromycin and azythromycin in hospital
410 wastewater were also observed by Lucas et al. (2016). Other authors also found negative removal for the
411 antibiotics erythromycin, azythromycin and clarithromycin (Gao et al. 2012; Verlicchi et al. 2012b; Gros
412 et al. 2014; Rodriguez-Mozaz et al. 2015). The negative removal can be attributed to some particular
413 processes that take place during wastewater treatment. For example, clarithromycin is excreted under
414 conjugated chemical forms (Verlicchi et al. 2012a; Kumar et al. 2012; Jelic et al. 2015) but can be further
415 deconjugated by some enzymes present in wastewater bioreactor reverting them to their original form
416 (Celiz et al. 2009). Also, analyte behavior such as adsorption to particles may be altered by changing
417 physicochemical parameters during the treatment process, thus influencing the removal efficiency (Zhang
418 et al. 2015). In Beijing (China) WWTP, Li et al. (2013) stated that macrolides (including spiramycin and
419 erythromycin), were persistent during conventional treatment. Only after ultrafiltration coupled to
420 ozonation, all tar-get antibiotics were effectively reduced (from 85% up to 99.9%) decreasing their
421 environmental risk.

422 The negative removal efficiency of sulfamethoxazole in WWTPs also may be caused by the
423 presence of metabolites in the influents, which can subsequently be transformed to their parent
424 compounds during biological treatment (Zhang et al. 2015). For example, N₄-acetylsulfamethoxazole
425 usually accounts for more than 50% of an administered dose in human excretion and can occur in WWTP
426 influents at concentrations of 2.5–3.5 times higher than concentrations of the parent compound (Gobel et
427 al. 2007). N₄-acetylsulfamethoxazole can also deconjugate into sulfamethoxazole during waste

428 wattertreatment (Göbel et al. 2005), leading to an underestimation of removal efficiency for
429 sulfamethoxazole if this metabolite is not considered. This might be a reason for the highly negative
430 elimination rates.

431 The compound carbamazepine also had negative removal in other studies (Kahl et al. 2017). The
432 increase in concentration during biological treatment is attributed to the biotransformation of its
433 transformation products (Petrie et al. 2018), as well as the UV irradiation might has the ability to convert
434 its hydroxyl transformation products to the parent compound (Miao et al. 2005). Moreover, compounds
435 such as carbamazepine present low biodegradability (Kahl et al. 2017).

436 In our study, the CW was not efficient in eliminating anticancer compounds (doxorubicin and
437 epirubicin): Field studies on the elimination of these compounds in WWTPs are lacking in the literature,
438 the development of alternative or improvement of current processes is needed, aiming at the degradation
439 of these compounds s in wastewater. In a literature review Zhang et al. (2013) reported only partial
440 removal by conventional activated sludge of anticancer drugs. Among these, Ruel et al. (2011) detected
441 six (i.e. cytarabine, doxorubicin, etoposide, gemcitabine, ifosfamide and vinorelbine) of 14 most
442 frequently-used anticancer drugs at concentrations up to 15 ng/L in influent and effluent of a traditional
443 activated sludge; they were showing insignificant degradation during the wastewater treatment. In general,
444 Biological processes do not achieve high removal efficiencies for these compounds, since many of them
445 are poorly biodegradable and have a high metabolic activity (Ferre-Aracil et al. 2016). However, some studies
446 indicate that the removal of anticancer drugs can be better with enzymatic degradation and ozonation
447 (Castellet-Rovira et al. 2018; Kelbert et al. 2020; Ferre-Aracil et al. 2016). A study by Somensi et al. (2012)
448 demonstrated that O₃ oxidation of doxorubicin was a pH-dependent process with a second-order rate
449 constant at neutral pH of 0.3373/min.

450
451 Muter et al. (2017), evaluating the removal efficiency of pharmaceutical compounds in WWTP
452 as a function of time, observed diclofenac positive removal during the first 2 h and desorption after 7
453 days. The variants showed increased concentrations of diclofenac after 7 days, irrespectively of its
454 removal during the previous stage. The content of diclofenac increased by almost 2.9 times after the
455 primary mechanical treatment of wastewater and there was a further 10-fold increase after the secondary
456 biological treatment stage in comparison with the initial level in untreated wastewater (Reinholds et al.
457 2017). The negative removal yield of diclofenac at a real urban WWTP was reported also by González-
458 Pérez et al. (2017). This effect could happen due to the destruction of glucuronide or sulfate conjugates of
459 diclofenac (Zorita et al. 2009). The bacteria (predominantly *Escherichia coli*) can secrete the β -
460 glucuronidase enzyme which can deconjugate already metabolized PPCs during the wastewater
461 treatment process and consequently release the parent compound (Suárez et al. 2008). Furthermore, other
462 mechanisms (like abiotic hydrolysis) can also occur.

463 The efficiency of the pharmaceutical removal depends also on the dominant mechanism (aerobic
464 or anaerobic biodegradation, photo-degradation or adsorption and settling). Also, the different CW
465 designs influenced both the range of compounds removed as well as the removal efficiency in the
466 respective season (Zhang et al. 2015). In VF-CW the aerobic biodegradation is responsible for the

467 removal of pharmaceuticals among other dominant processes (e.g., sedimentation, adsorption, and plant
 468 uptake (Ilyas and van Hullebusch 2020).

469

470 In contrast, the CW systems providing favorable substrates for biofilms have the high potential
 471 for removal of compounds by biodegradation, especially in the cold season. However, during summer,
 472 the removal of exclusively aerobically degradable compounds seems to be limited by the high oxygen
 473 consumption in the pond with floating macrophytes (Rühmland et al. 2015).

474

475 3.4 Removal of pharmaceuticals by CW biofilms

476

477 In order to evaluate the role of the microorganism living in the CW, the capture of
 478 pharmaceuticals by biofilms was evaluated.

479 **Table 4** - Amount of biofilm accumulated in the sewage effluent and corresponding bioconcentration
 480 factor (BCF).

Compound	Biofilm (ng g ⁻¹)		BCF (L g ⁻¹) sewage effluent	
	Summer	Winter	Summer	Winter
Doxorubicin	<LOQ	<LOQ	-	-
Epirubicin	<LOQ	<LOQ	-	-
Daunorubicin	<LOQ	<LOQ	-	-
Irinotecan	<LOQ	<LOQ	-	-
Medicamycin	<LOQ	<LOQ	-	-
Spiramycin	<LOQ	<LOQ	-	-
Josamycin	<LOQ	<LOQ	-	-
Azithromycin	2.0	3.6	-	-
Sulfamethoxazole	8.5	<LOQ	0	51.7
Clarithromycin	3.7	2.7	2.2	0
Erythromycin	3.6	0.8	0.6	380
Roxythromycin	<LOQ	0.5	-	-
Diclofenac	1.5	<LOQ	-	-
Carbamazepine	7.2	<LOQ	1.8	0.6

481

482 Table 3 presents the amount of pharmaceuticals accumulated in the sewage biofilm and
 483 corresponding BCF. The results show that there is a potential to retain certain pharmaceutical compounds
 484 within the sewage biofilms. The accumulation and degradation of compounds are affected by many
 485 factors such as chemical characteristics of the molecule, the concentrations of organic carbon, the
 486 microbial community structures that can cause the different scenarios of the bioaccumulation and
 487 degradation (Chen et al. 2020). The extracellular enzymes that are secreted by the cells to serve as an
 488 external digestion system also interact with polysaccharide and accumulate in the biofilm (Flemming et
 489 al. 2016). Protein-like substances can provide active sites, such as carboxyl, amine, hydroxyl groups, and
 490 hydrophobic regions for organic micropollutants absorption (Zhang et al. 2018). The antibiotic
 491 sulfamethoxazole is the main compound accumulated by epilithic summer biofilms. Kim and Carlson
 492 (2007) have found 1.9 ng g⁻¹ of sulfamethoxazole in sediments in the United States, in our study we found

493 8.5 ng g⁻¹. The results of a study in six Italian WWTPs (Castiglioni et al. 2006) indicated high inputs of
494 antibiotics (including sulfamethoxazole) in rivers. The notable fixation of antibiotics on environmental
495 particles may be explained by surface complexation/sorption reactions (Gu and Karthikeyan 2005).
496 Therefore, six compounds were fixed by the biofilms in summer (azithromycin, sulfamethoxazole,
497 clarithromycin, erythromycin, diclofenac and carbamazepine) and four in winter (azithromycin,
498 clarithromycin, erythromycin and roxythromycin). Some of these compounds (e.g., azithromycin,
499 clarithromycin, erythromycin and diclofenac) present high octanol-water partition coefficient (log K_{ow})
500 values ranging from 3.0 to 4.5 (Table 1) whereas the other (e.g., sulfamethoxazole and roxythromycin)
501 present log K_{ow} values below one (i.e., log K_{ow} indicates the hydrophilic character of a molecule, higher is
502 the value higher is the hydrophobicity). These findings, which indicate the chemical properties of
503 pharmaceuticals (pKa, log K_{ow}), are not the determining factors for the fixation of these compounds by
504 biofilms. The presence of “the classical” pharmaceuticals (i.e., carbamazepine, diclofenac and
505 sulfamethoxazole) could be attributed to their large distribution and resistance to degradation (Aubertreau
506 et al. 2016).

507 The BCF factor indicate chemical behavior in aquatic organisms. In our study, the results
508 indicate low BCF levels (summer) for the compounds clarithromycin, erythromycin and carbamazepine,
509 ranging from 0.6 to 2.2 (Table 3). In winter there was a higher BCF to for sulfamethoxazole and
510 erythromycin 51 and 380 L g⁻¹, respectively. Sulfamethoxazole and sulfamethazine, are the most
511 abundant sulfonamides detected in the environment (Cheong et al. 2020), and have been detected at
512 relatively high concentrations in sewage treatment plant effluent, surface waters and ground water in
513 diverse world regions (Pei et al. 2006; Pruden et al. 2006; Hsu et al. 2014).

514 Despite the good efficiency of the WWTPs in removing the erythromycin from the effluents, this
515 compound was found accumulated in fishin habiting downstream rivers of WWTPs (Liu et al. 2015). Liu
516 et al. (2014) determined the BCF value of erythromycin in fish, and that the animals can metabolize this
517 substance (BCF= 72.2). In the microbial community, the aromatic protein fraction of extracellular
518 polymeric substances (EPS) displays a steady affinity for erythromycin regardless of either the origin of
519 type of bacterial aggregate treated at the wastewater plant (flocs, granules, biofilms) or EPS location in
520 the bacterial aggregate (Métivier et al. 2013), factor that may have favored the bioaccumulation of these
521 compounds in this study. A bioaccumulation study of the antiepileptic drug carbamazepine found
522 bioaccumulation factors of 2.2 and 12.6 in algae and crustaceans, respectively (Vernouillet et al., 2010).
523 In our study, the BCF of carbamazepine was calculated to be 1.8 in summer and 0.6 in winter. All these
524 findings suggest that these chemicals are partly removed by accumulation in the biofilm matrix.

525 Organic compounds adsorption and biodegradation by biofilms have been studied for many
526 years (Flemming and Ridgway 2009; Flemming and Wingender 2010; Dalahmeh et al. 2019; Dar and
527 Bhat 2020). It is evident that an additional step with the presence of capturing biofilms could be added
528 within the sewage treatment station of Santa Maria. The use of biofilms as new technology to the
529 treatment of wastewater has been tested to understand the removal efficiency of pharmaceuticals and their
530 dependence on biological treatment step and design for the WWTP (Falás et al. 2012). The Moving Bed
531 Biofilm Reactors (MBBR) is a successful example of technology that uses attached growth biofilms on
532 carriers to the treatment of industrial and municipal wastewater (Kermani et al. 2008). This technology is

533 considered a new approach in biological sewage treatment for the preservation of water and is gaining
534 momentum worldwide. The advantages of biofilm systems over activated sludge systems are that they are
535 more compact, thus the treatment capital costs are reduced (Rusten et al. 1998). It has been reported that
536 the magnitude of the WWTP input can decrease downstream in the river thanks to the transformation and
537 removal of nutrients and pollutants, in part, by fluvial biofilms (Acuña et al. 2019). Natural biofilms from
538 rivers are able to bind drugs, and this may limit the effect of such chemical substances in natural water.

539 The plants of WWTP contributed to an average increase in hydraulic detention time. Increasing
540 the contact time of wastewater with the vegetative tissue and biofilms can contribute to filtration
541 processes, which may be a beneficial aspect in VF-CWs allows greater contact time of wastewater with
542 microorganisms in wetland treatment (Decezaró et al. 2018). Moreover, plant roots can promote
543 microbial biodegradation of pollutants (Shehzadi et al. 2016; Noszczyńska and Piotrowska-Seget 2018).
544 Microbial biodegradation can be promoted by a nonspecific increase in microbial metabolic activity in the
545 area surrounding roots (Singer et al. 2003). In a study analyzing the microbial biofilm populations
546 residing on the plant roots immersed in wastewater of an ecological WWTP Balcom et al. (2016) found
547 greater heterogeneity and higher relative abundances of xenobiotic metabolism genes in the root biofilm.
548 According to the studies conducted by Hijosa-Valsero et al. (2016) macrophytes can take up PPCPs
549 through their roots. In the above mentioned study, ibuprofen, salicylic acid, caffeine, methyl
550 dihydrojasmonate, galaxolide and tonalide were present on the root surface with a predominance of
551 galaxolide and caffeine in all the planted systems. Naproxen, ibuprofen, salicylic acid, methyl
552 dihydrojasmonate, galaxolide and tonalide were uptaken by the roots. Log K_{ow} is an important descriptor
553 to understand the behaviour of emerging pollutants in environmental matrices. In general, adsorption
554 processes are more common for organic compounds with $\log K_{ow} > 4$; on the contrary, when the
555 substances have a $\log K_{ow}$ value in the range 1–4, they rather are taken up by plants or remain in the
556 dissolved phase (Briggs et al. 1982). But less hydrophobic substances can also be detected, and maybe it
557 was due to the fact that these compounds were present at high concentrations in wastewater. However,
558 compounds such as galaxolid and tonalide even with high K_{ow} values, can be easily adsorbed into the root
559 exodermis, which would facilitate absorption (Hijosa-Valsero et al. 2016). Thus, the authors claim that
560 biodegradation pathways are therefore suggested for most of the studied PPCPs in the assessed CWs.

561 3.5 River water quality

562

563 Studies of acute toxicity of pharmaceutical products in organisms of different trophic levels
564 reveal that the most impactful classes of compounds are antidepressants, antibiotics, antipsychotics,
565 cardiovascular drugs, antineoplastics, as well as natural and synthetic hormones, due to their recalcitrant
566 properties (Farré et al. 2008). In our work, of the four compounds found, three are antibiotics, revealing
567 the great impact has been bringing due to its high consumption, and consequently its constant presence in
568 the environment. Moreover, harmful effects have been reported, such as aquatic toxicity, genotoxicity,
569 endocrine disturbance in wild animals, feminization of male fish (Nash et al. 2004; Gross-Sorokin et al.
570 2006). There are also risks associated with the occurrence or the development of bacterial resistance to
571 antibiotics (Bastos et al. 2018a) and the serious consequences on public health.

572 Regardless of their very small concentration (e.g. ng L^{-1} to $\mu\text{g L}^{-1}$) in water bodies, the
573 continuous discharge of pharmaceuticals compounds can have negative impacts on human health as well
574 as aquatic and terrestrial life (Vymazal 2007; Vystavna et al. 2017). Thus, of the four anti-cancer
575 compounds, we detected three in the effluent of the treatment station in summer biofilms. However when
576 released in the creek, they are diluted and only one (Irinotecan) was impregnated the epilithic biofilms in
577 very low concentration (seven times lower - Table 4). Of the eight antibiotics monitored, six were found
578 in the effluent biofilms and five on watercourse biofilms, downstream. The medicamycin antibiotic had a
579 concentration of 45.78 ng g^{-1} in the effluent biofilms was no longer found in the downstream creek. All
580 other antibiotics, the concentrations in epilithic biofilms decreased downstream compared to the effluent,
581 exception of the Sulfamethoxazole. The DCF compound was not found in the effluent; it already
582 appeared in watercourse - upstream and downstream. The CBZ was present in biofilms both upstream
583 (concentration of 7.23 ng g^{-1}) and in the effluent (12.44 ng g^{-1}) but was not found downstream.

584 In general, biofilm collected in winter was less contaminated than biofilm collected in summer.
585 There was a small increase in concentrations downstream compared to upstream. Of the six antibiotics
586 found in the tributary (spiramycin, josamycin, azithromycin, sulfamethoxazole, clarithromycin and
587 erythromycin), two appear downstream in lower concentrations (azithromycin and erythromycin), one
588 with higher concentration (clarythromycin), and roxythromycin did not appear in the effluent but had a
589 concentration of 1.92 ng g^{-1} downstream. We believe it is an input from the watercourse itself since in
590 upstream the concentration of 1.88 ng g^{-1} was already found. The CBZ compound had a slightly higher
591 concentration in biofilms the effluent than downstream biofilms.

592 For the effluent water analyzed by the POCIS, we found higher concentrations if compared to
593 the biofilms and of the 14 compounds analyzed, only four were not found in our analyses (irinotecan,
594 josamycin, azythromicin and roxythromycin). Even though the effluent is heavily contaminated, the
595 pharmaceutical's concentrations are diluted along the creek, and CW has no significant impact on water
596 quality. However, sulfamethoxazole, diclifenac and carbamazepine compounds present a considerable
597 increase in their concentration from upstream to downstream. Compounds like carbamazepine even with
598 its concentration increasing, it is still much smaller than in some works that found concentrations in the
599 order of $\mu\text{g g}^{-1}$ of this downstream compound of WWTP (Zhang and Geißen 2010).

600 For all the compounds analyzed, there was no significant impact of the effluent having its
601 concentrations diluted along the creek, and some compounds already had an input from the watercourse
602 itself even upstream of the treatment plant.

603 In general, each sampling device (POCIS and epilithic biofilm) has its specificity and the
604 methods are complementary. The concentration capacity of compounds in POCIS (in terms of grams of
605 compound per gram of matrix) is higher, considering that in POCIS the maximum concentrations of 863
606 ng g^{-1} (ie. doxorubin in summer) was reported, while in biofilm the maximum concentration was 58.61 ng
607 g^{-1} (erythromycin, winter). For the POCIS, the winter sampling detected the highest concentrations,
608 already the biofilm worked as a similar capturing matrix in the two seasons. The POCIS showed great
609 capacity in capturing drug compounds, since the effluent is more contaminated than along the creek
610 (upstream and downstream) and that the values of pharmaceuticals found in the effluent are much higher
611 than those measured in the creek.

612 For some compounds, the epilithic biofilm has a higher capacity to accumulate than POCIS. This
613 ability is explained by the diversity of organic compounds present in the biofilm, which have a high
614 capacity for adsorption of pharmaceuticals (Bastos et al. 2018b). However, a progressive saturation
615 occurs with exposition time and may contribute to multiple kinetics of bioaccumulation, being
616 consecutive or staking (Zhang et al. 2018). The present results even show that the biofilm sampling
617 device has broad selectivity. In addition to dissolved compounds, suspended solids can be trapped by
618 biofilms and incorporated into the matrix, including biodegradable material that can be used as a source
619 of nutrients (Flemming et al. 2016).

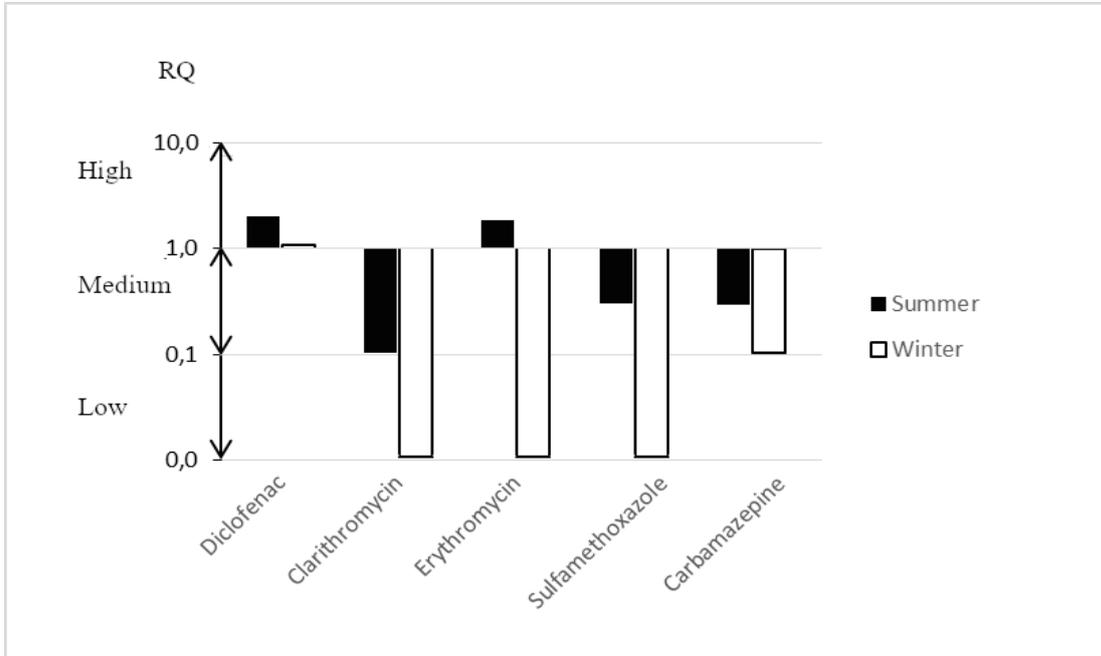
620 **Table 5** - Concentration of pharmaceuticals in biofilms and POCIS in winter 2018 and summer
621 2018/2019.
622

623

Molecules	Biofilm (ng g ⁻¹)					POCIS (ng g ⁻¹)				
	Upstream 1 (P10)	Upstream2 (P14)	Upstream3 (P3)	Effluent	Downstream (P6)	Upstream 1 (P10)	Upstream2 (P14)	Upstream3 (P3)	Effluent	Downstream (P6)
<i>Summer</i>										
Doxorubicin	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	863.15	3.52
Epirubicin	<LOQ	<LOQ	<LOQ	15.56	<LOQ	<LOQ	<LOQ	<LOQ	124.28	<LOQ
Daunorubicin	<LOQ	<LOQ	<LOQ	9.16	<LOQ	<LOQ	<LOQ	<LOQ	215.21	<LOQ
Irinotecan	<LOQ	<LOQ	<LOQ	15.65	2.35	<LOQ	<LOQ	<LOQ	<LOQ	1.36
Medicamycin	<LOQ	<LOQ	<LOQ	45.78	<LOQ	<LOQ	<LOQ	0.79	220.93	<LOQ
Spiramycin	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	64.38	<LOQ
Josamycin	<LOQ	0.88	<LOQ	7.89	0.49	<LOQ	<LOQ	1.8	<LOQ	0.69
Azithromycin	2.04	5.23	11.05	11.05	2.23	<LOQ	<LOQ	0.82	<LOQ	4.94
Clarithromycine	3.75	3.51	2.05	7.04	1.26	<LOQ	0.41	1.1	160.69	3.12
Erythromycine	3.61	0.97	2.16	5.88	<LOQ	<LOQ	0.75	0.41	138.7	2.06
Roxythromycine	<LOQ	<LOQ	<LOQ	2.87	1.64	<LOQ	<LOQ	0.63	<LOQ	<LOQ
Sulfamethoxazol	8.59	4.38	<LOQ	<LOQ	5.88	<LOQ	<LOQ	1.64	231.81	89.83
DCF	1.54	1.37	5.37	<LOQ	2.27	<LOQ	7.49	2.58	346.81	64.52
Carbamazépine	7.23	4.41	<LOQ	12.44	<LOQ	<LOQ	8.74	2.55	307.05	101.78
<i>Winter</i>										
	Upstream 1 (P10)	Upstream2 (P14)	Upstream3 (P3)	Effluent	Downstream (P6)	Upstream 1 (P10)	Upstream2 (P14)	Upstream3 (P3)	Effluent	Downstream (P6)
Doxorubicin	<LOQ	3.1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Epirubicin	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Daunorubicin	<LOQ	3.04	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Irinotecan	<LOQ	2.79	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.04
Medicamycin	<LOQ	2.38	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Spiramycin	<LOQ	<LOQ	<LOQ	19.66	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	4.87
Josamycin	<LOQ	<LOQ	<LOQ	1.06	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Azithromycin	3.66	7.23	7.47	29.72	20.02	<LOQ	2.89	24.25	<LOQ	18.46
Clarithromycine	2.78	5.65	5.91	2.63	17.19	1.12	2.43	22.11	<LOQ	18.61
Erythromycine	0.82	1.88	7.75	58.61	2.88	1.04	2.26	135.9	2.25	46.35
Roxythromycine	0.54	<LOQ	1.88	<LOQ	1.92	0.45	<LOQ	<LOQ	<LOQ	<LOQ
Sulfamethoxazol	<LOQ	40.56	12.53	11.34	<LOQ	<LOQ	18.21	5.37	3.85	141.38
DCF	<LOQ	<LOQ	1.16	<LOQ	<LOQ	2.25	2.87	8.77	134.47	162.07
Carbamazepine	<LOQ	2.57	8.07	2.33	1.63	0.82	4.41	2.77	179.92	2.23

625 3.6 Environmental risk of wetland effluent
626

627 Potential environmental risks of pharmaceutical compounds were evaluated using the method of
628 the risk quotient. RQ values were calculated for WWTP effluent sampling in summer and in winter
629 (Figure 5).



630

631 **Fig. 5** Risk quotients of pharmaceuticals in WWTP effluent. (The indices were calculated only for
632 compounds found in this work that had sampling rates (Rs) available in the literature)

633

634 Discharges from WWTPs have been identified as the primary point sources of pharmaceuticals
635 into the aquatic environment, which may pose potential risks to the aquatic organisms (Leung et al. 2013).
636 In the summer sampling, five compounds detected in WWTP effluent represent a potential
637 ecotoxicological risk. A high risk is estimated for erythromycin (2.34) and diclofenac (2.87), and a
638 medium risk is estimated for the sulfamethoxazole (0.27), clarithromycin (0.16) and carbamazepine
639 (0.39). Furthermore, it is worth noting that erythromycin and diclofenac have shown a low removal
640 efficiency in our CW and others WWTPs. The low removal efficiency of WWTPs could be an important
641 reason for the presence of antibiotics in the environment (Zhang et al. 2015). High risk quotients for the
642 erythromycin antibiotic in WWTP effluent were also found by Li et al. (2013b), Yuan et al. (2015) and
643 Ben et al. (2018). Although the concentrations of antibiotics in effluents may be diluted, when discharged
644 into the surrounding water, due to their continuous release, their potential to resist degradation, and their
645 ability to absorb to soil or sediment, these pseudo-persistent compounds may be widely present at the
646 surrounding water near discharge point from WWTP and have a big possibility of causing the
647 development and spread of antibiotic resistance gene (Pei et al. 2006). Even if in our study, medium risks
648 were found for sulfamethoxazole and clarythromycin, previous study conducted in France showed that
649 these compounds required prioritized attention because of their high ecological risk to aquatic
650 environments (Besse and Garric 2008).

651 In the winter period, only two compounds could represent a risk: diclofenac which is classified
652 as high risk with a value of 1.04, and carbamazepine which is classified as medium risk. Consequently,
653 the season and the functioning of the CW have a significant role on the “type” of environmental risk,
654 since the risk induced by antibiotics seems to be reduced in winter. The anti-inflammatory agent
655 diclofenac was previously identified as one of the main risk drivers in environmental mixtures (Busch et
656 al. 2016; Beckers et al. 2018) and has been associated with growth inhibition in daphnia and cell
657 multiplication in algae (Mijangos et al. 2018). The antibiotics clarithromycin, erythromycin and
658 sulfamethoxazole presented low risk in the winter period. Even though the environmental risks were
659 estimated to be low for these antibiotics, the potential negative effects on aquatic ecosystems should call
660 our attention because of their continuous discharge in the long term.

661 Individual concentrations of compounds represent low environmental risks. However, the
662 mixture toxicity of these pharmaceuticals and the long-term environmental impact and ecological risks
663 were not considered here, which warrants future investigations. Geiger et al. (2016) concluded that toxic
664 mixture effects of all tested chemicals are higher than the individual effect of each mixture component.
665 Further research should be conducted to investigate the impact of these compounds on various trophic
666 levels and environmental compartments.

667 **4. Conclusion**

668

669 The present work shows that a small VF-CW, under subtropical hydroclimatic conditions,
670 succeed to eliminate a significant part of the pollution load present in domestic wastewater, such as more
671 than 60% of TSS COD and BOD₅, and 45% of TN, although it was not efficient to eliminate P. But has
672 low efficiency in removing pharmaceutical compounds. All compounds found in summer had negative
673 removal efficiency (doxorubicin, epirubicin, spiramycin, Sulfametoxazole, clarithromycin, erythromycin,
674 diclofenaco and carbamazepine). However, all compounds found in winter had positive removal
675 efficiency (erythromycin, diclofenaco and carbamazepine). Furthermore, more pharmaceutical
676 compounds were detected in summer compared to winter Results highlight increase of some
677 pharmaceutical compound concentrations in water after treatment. This finding will be due to the
678 reactivation of metabolite by hydrolysis or microbiological activities during the treatment in the CW.
679 Indeed, biofilms shown to interact with pharmaceuticals and especially play a role in their capture from
680 water.

681 The removal efficiency was shown to be better during the winter period, where fewer
682 compounds were found, but with positive removal efficiency. In the summer period, most compounds had
683 negative removal efficiency. The risk assessment indicates, however, that the water quality could pose
684 high risks to aquatic organisms. The development of treatment trains that are more suited for the removal
685 of pharmaceuticals by upgrading existing WWTPs or designing new ones are important areas of research
686 and development. Some strategies can be employed to reduce the discharge of drugs into the
687 environment. Efforts to restrict the sale of controlled medicines such as antibiotics, antidepressants, etc.
688 should be made in countries like Brazil. As well as encouraging the use of more degradable compounds
689 with comparable therapeutic effects (green pharmacy), and other strategies focus on improving
690 wastewater treatment technologies and their scale of adoption (Ilyas et al. 2020).

691 Despite these limits, CW offer an interesting solution for limiting pollution of watercourses. The
692 dissemination of such technology in Brazil would benefit the poor Brazilian sanitation coverage,
693 especially for low-density regions with sensitive environments, in which advanced levels of wastewater
694 treatment are required but large investments are not feasible.

695

696 **5. Declarations**

697

698 **Ethics approval and consent to participate:** Not applicable.

699 **Consent for publication:** Not applicable.

700 **Availability of data and materials:** The datasets used and/or analysed during the current study are

701 available from the corresponding author on reasonable request.

702 **Competing interests:** The authors declare that they have no competing interests.

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705 **Authors' contributions:**

706 Jocelina Paranhos Rosa de Vargas, Danilo Rheinheimer and Delmira Wolff participated in the conception
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708 Paranhos Rosa de Vargas, Marília Camotti Bastos, Jérôme Labanowski, Maha Al Badany and Rolando
709 González. The first draft of the manuscript was written by Jocelina Paranhos Rosa de Vargas. All authors
710 commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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712 **6 . Bibliography**

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