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

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Abstract

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

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

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 sustems 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 Phosporus) 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

various microenvironments with different physico-chemical conditions in CW systems allowing for both aerobic and anaerobic metabolic degradation pathways of pharmaceuticals, while the more homogeneous conditions in WWTPs induce fewer degradation pathways (Ávila et al. 2010). The available evidence in the literature and physicochemical properties of pharmaceuticals indicate that specific processes are involved in the removal of a certain type of molecules in CWs (Ilyas and van Hullebusch 2020), and these complex physical, chemical, and biological processes may occur simultaneously including photodegradation, volatilization, adsorption/sorption, plant uptake and accumulation, as well as biodegradation (aerobic and anaerobic), mainly depending on the design of the CWs (Zhang et al. 2014).

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

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

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

2. Material and Methods

2.1 Constructed wetland

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

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

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

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

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

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

2.2 Sampling

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

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

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

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

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

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

2.3 Compounds and analytical procedures

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

Table 1 - Pharmaceutical compounds studied grouped according to their therapeutical class. For each substance, chemical, dissociation constant (pK_a), octanol-water partition coefficient ($\log K_{ow}$) and 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 (Anilamert et al. 2006); ^e drugbank.ca ; ^f drugfuture.com; *phenol

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

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

2.4 Environmental risk evaluation

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

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

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

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	-

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 capacity 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.

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

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

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

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

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

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

3.2 Compliance with regulations

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

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

Parameter	Maximum permissible value		Effluent system TS/VF-CW	
	CONAMA 430/2011	CONSEMA 355/2017	Concentration	Removal rate
			mg L ⁻¹	%
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

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

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

3.3 Efficiency of pharmaceuticals compounds removal

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

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

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

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

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

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

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

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

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

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

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

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

3.4 Removal of pharmaceuticals by CW biofilms

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

Table 4 - Amount of biofilm accumulated in the sewage effluent and corresponding bioconcentration 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

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

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

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

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

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

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

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

3.5 River water quality

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

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

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

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

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

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

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

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

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

3.6 Environmental risk of wetland effluent

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

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

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

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

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

4. Conclusion

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

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

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

5. Declarations

Ethics approval and consent to participate: Not applicable.

Consent for publication: Not applicable.

Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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

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Authors' contributions:

Jocelina Paranhos Rosa de Vargas, Danilo Rheinheimer and Delmira Wolff participated in the conception and design of the work. Material preparation, data collection and analysis were performed by Jocelina Paranhos Rosa de Vargas, Marília Camotti Bastos, Jérôme Labanowski, Maha Al Badany and Rolando González. The first draft of the manuscript was written by Jocelina Paranhos Rosa de Vargas. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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