FEDERAL UNIVERSITY OF SANTA MARIA
SOIL DEPARTMENT
GRADUATE PROGRAM IN SOIL SCIENCE

Jocelina Paranhos Rosa de Vargas Brunet

SURFACE WATER CONTAMINATION IN SOUTHERN BRAZIL BY PHARMACEUTICAL COMPOUNDS: APPLICATION IN A HIGH ANTHROPIC PRESSURE CONTEXT

Poitiers, France

2021

Jocelina Paranhos Rosa de Vargas Brunet

SURFACE WATER CONTAMINATION IN SOUTHERN BRAZIL BY PHARMACEUTICAL COMPOUNDS: APPLICATION IN A HIGH ANTHROPIC PRESSURE CONTEXT

Thesis submitted to the Graduate Program in Soil Science at the Federal University of Santa Maria (UFSM, RS), as partial requirement for the degree of **Doctor in Soil Science**.

Supervisor: Dr. Danilo Rheinheimer dos Santos

Poitiers, France

2021

This study was financied in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) – Finance Code 001

Brunet, Jocelina
SURFACE WATER CONTAMINATION IN SOUTHERN BRAZIL BY PHARMACEUTICAL COMPOUNDS: APPLICATION IN A HIGH ANTHROPIC PRESSURE CONTEXT / Jocelina Brunet.- 2021.
226 p.; 30 cm

Orientador: Danilo Rheinheimer dos Santos
Coorientador: Jérôme Labanowski
Tese (doutorado) - Universidade Federal de Santa Maria, Centro de Ciências Rurais, Programa de Pós Graduação em Ciência do Solo, RS, 2021

1. Poluição da água. 2. Contaminação ambiental. 3. Produtos farmacêuticos na água. 4. Biofilmes. 5. POCIS. I. Rheinheimer dos Santos, Danilo II. Labanowski, Jérôme III. Título.

Sistema de geração automática de ficha catalográfica da UFSM. Dados fornecidos pelo autor(a). Sob supervisão da Direção da Divisão de Processos Técnicos da Biblioteca Central. Bibliotecária responsável Paula Schoenfeldt Patta CRB 10/1728.

Declaro, JOCELINA BRUNET, para os devidos fins e sob as penas da lei, que a pesquisa constante neste trabalho de conclusão de curso (Tese)foi por mim elaborada e que as informações necessárias objeto de consulta em literatura e outras fontes estão devidamente referenciadas. Declaro, ainda, que este trabalho ou parte dele não foi apresentado anteriormente para obtenção de ualquer outro grau acadêmico, estando ciente de que a inveracidade da presente declaração poderá resultar na anulação da titulação pela Universidade, entre outras consequências legais.

Jocelina Paranhos Rosa de Vargas Brunet

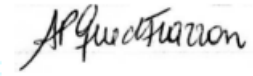
SURFACE WATER CONTAMINATION IN SOUTHERN BRAZIL BY PHARMACEUTICAL COMPOUNDS: APPLICATION IN A HIGH ANTHROPIC PRESSURE CONTEXT

Thesis submitted to the Graduate Program in Soil Science at the Federal University of Santa Maria (UFSM, RS), as partial requirement for the degree of **Doctor in Soil Science**.

Approved July 23, 2021:

Danilo Rheinheimer dos Santos, Dr. (UFSM)
(Presidente/Orientador)

Marília Camotti Bastos, Drª. (INRAe)



Ana Paula Guedes Frazzon, Drª. (UFRGS)

Tales Tiecher, Dr. (UFRGS)

Leslie Mondamert, Drª. (Université de Poitiers)

Poitiers, France

DEDICATÓRIA
DEDICACE

Dedico aos meus pais José e Isirenes e ao meu marido Julien pelo amor, atenção e compreensão. Além de todo o esforço desprendido para que este momento fosse alcançado.

ACKNOWLEDGMENTS
AGRADECIMENTOS
REMERCIEMENTS

A caminhada do aluno da pós-graduação torna-se muito mais do que uma busca pelo conhecimento ou pelo diploma, ela torna-se uma busca de autoaceitação, autoconhecimento, comprometimento e de frustação. Durante essa jornada de caminhos as vezes árduos e as vezes mais leves, nos deparamos com pessoas, alguns mesmo anjos, que cruzam nosso caminho para deixar a jornada mais leve, ou mais pesada.

Se você é uma mulher, acredite que essa jornada será ainda mais difícil.

Primeiramente eu quero agradecer a Deus por me permitir chegar até aqui, minha única fonte inesgotável e infinita de força e amor.

Faz-se primordial o agradecimento a educação pública de qualidade brasileira. A Universidade Federal de Santa Maria e ao Programa de Pós Graduação em Ciência do Solo, pela oportunidade de realizar tanto o mestrado quanto o doutorado de forma gratuita e sempre com bolsa de estudos.

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), pela concessão da bolsa de doutorado sanduíche (Projeto PVE/2014 - Processo: 400887/2014-2) e financiamento dos projetos.

A Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), pela concessão da bolsa de doutorado, inclusive no tempo em que permaneci no exterior com a bolsa nacional.

Ao meu orientador e grande incentivador, Danilo Rheinheimer dos Santos, que me acolheu desde o primeiro momento, acreditou na minha capacidade e me apoiou nessa jornada. Por ter compartilhado comigo tantos momentos. Obrigada pela oportunidade de receber 6 meses de bolsa no exterior, que foram o suficiente para mudar minha trajetória.

Agradeço a todo grupo de pesquisa durante essa jornada. Agradeço aos bolsistas do EM: Joana Becher, Claudenir Rocha e Marcieli que em algum momento participaram comigo e com o grupo nas minhas atividades, vocês foram essenciais na minha passada pelo laboratório. Aos bolsistas de IC: Marcelo Lima, Elisane Kralik, Robson, Jonathan, Luana, Sabrina, Pedro Bolzan, Diogo e Victor que em algum momento estiveram comigo ou com o grupo e participaram das atividades, permitindo o desenvolvimento delas. Aos colegas da pós-graduação: Rodrigo Knevitz e sua namorada Daniela, que em alguns momentos estiveram junto e ajudaram nas atividades, Roque Júnior e Gilmar Schaefer que também ajudaram nas atividades. A Deonice, Jaqueline, Andressa e André, por fazerem parte do grupo. A doutoranda Gracieli, por partilhar trabalhos e conhecimentos sobre nossa temática de trabalho.

A minha grande amiga que a pós-graduação me apresentou: Taciara Horst, ao qual esteve comigo desde o início compartilhando os medos, frustações, conhecimentos, risadas e bons momentos.

As minhas colegas de apartamento, Edicarla Trentin, Valéria Ortaça e Adrian Schu pela convivência, amizade, lealdade e por me proporcionarem tanto momentos alegres e de cumplicidade. Um agradecimento especial a Edi e a Val, ao qual moraram comigo desde o início e me permitiram viver tantos momentos agradáveis, obrigada por todos os bolos, sorvetes, momentos de risada e mesmo de lágrimas.

A minha amiga de vida e de trabalho, Marília Camotti Bastos. Sem a sua presença em minha vida, tudo teria sido muito mais difícil. Obrigada pelas boas risadas e momentos de trabalho, pela ajuda pessoal e intelectual. Você é um raio de sol na vida de quem te conhece.

A Alice, minha melhor amiga há 20 anos. Pela paciência e apoio sempre que necessário.

As minhas amigas/irmãs Ariana e Nathalia por me mostrarem que o mais importante são os laços afetivos, e não a distância.

A mon directeur de thèse, Jérôme Labanowski, pour m'avoir accueilli au sein du laboratoire de Poitiers, pour m'avoir aidé dans la rédaction de ma thèse et pour m'avoir toujours soutenu.

Aux filles qui ont rendu mes journées au laboratoire plus joyeuses et plus drôles : Olha Matviichuk et Hiba Zinc. A ma collègue de bureau, de gym et de recettes, Maha Al-Badany, personne sans qui ce travail n'existerait pas, merci de m'avoir aidé dans toutes les analyses et d'avoir été toujours disponible.

Tous ceux que j'ai rencontré sur le plate-forme Eaux : Quentin, Alexis, Bertrand, Leslie et notre chère Cristina, qui illumine nos journées.

A mon meilleur ami et mari, Julien Brunet, qui a toujours été à mes côtés, m'encourage à devenir une meilleure personne et me montre le vrai sens du mot "spécial". Merci de m'avoir appris à fabriquer des pastilles, d'avoir été patient avec moi et d'avoir toujours cru en nous.

A minha família, por todo apoio e em especial aos meus pais, por sempre me incentivarem a estudar.

A todas as mulheres na ciência, que assim como eu, tiveram que enfrentar julgamentos, difamações, receberam menos oportunidades que seus colegas homens mas mesmo assim venceram.

A todas as pessoas que conviveram e passaram por mim durante esses anos, que de alguma forma me ajudaram. Aos professores e aos colegas do PPGCS.

Enfim a todos ... Meu muito obrigada por tudo!

“O conhecimento nos faz responsáveis.”

(Che Guevara)

RESUMO

CONTAMINAÇÃO DAS ÁGUAS SUPERFICIAIS NO SUL DO BRASIL POR COMPOSTOS FARMACÊUTICOS: APLICAÇÃO EM UM CONTEXTO DE ALTA PRESSÃO ANTRÓPICA

AUTORA: Jocelina Paranhos Rosa de Vargas Brunet
ORIENTADOR: Danilo Rheinheimer dos Santos

As aglomerações urbanas de médio porte têm experimentado um enorme crescimento populacional nas últimas décadas. A ausência de um plano diretor e de planejamento ambiental e/ou a falta de controle da ocupação do espaço têm destruído os poucos espaços verdes e a proteção ambiental. A ocupação desordenada do espaço, a falta de coleta e o tratamento dos esgotos são padrões e não exceções. Além disso, outro problema enfrentado no Brasil é a deficiência na coleta e no tratamento dos resíduos hospitalares. Neste sentido, o presente projeto propõe o monitoramento da presença de compostos farmacêuticos na água e biofilmes nos cursos de água do Campus da Universidade Federal de Santa Maria. Entre as fontes de poluição estão: (a) o esgoto doméstico das casas e indústrias do bairro de Camobi, (b) o esgoto doméstico da Casa dos Estudantes da Universidade, (c) os efluentes do Hospital Universitário e (d) os efluentes do Hospital Veterinário. Desenvolvemos 4 estudos: um primeiro estudo preliminar para caracterizar as coletas de biofilmes, o segundo foi para monitorar o estado de contaminação do campus, o terceiro foi para realizar um experimento de resiliência para verificar a velocidade de descontaminação dos biofilmes, e o quarto foi para avaliar a capacidade de depuração de drogas de uma Estação de Tratamento de Esgoto (ETE). Este estudo mostra pela primeira vez o impacto de um campus universitário sobre o meio ambiente. A área do campus de Santa Maria está contaminada por diferentes tipos de produtos farmacêuticos, com concentrações de mais de 900 ng g^{-1} em biofilmes e mais de 800 ng g^{-1} em POCIS (Polar Organic Chemical Integrative Sampler). Os medicamentos anticancerígenos epirubicin, o estimulante cafeína e o antibiótico eritromycin apresentaram um risco médio e alto para a biota aquática. Esta experiência destaca como a resiliência de um biofilme fluvial é um fenômeno rápido, pois já é perceptível após 8 horas e leva a uma dissipação de dois terços dos compostos (>95% de dissipação) após 8 dias. O presente trabalho mostra que uma pequena ETE consegue eliminar uma parte significativa da carga poluente presente nas águas residuais domésticas, como mais de 60% da demanda química de oxigênio, concentração de sólidos solúveis e demanda bioquímica de oxigênio, e 45% do nitrogênio total, mas tem baixa eficiência na remoção de compostos farmacêuticos.

Palavras-chave: Poluição da água. Contaminação ambiental. Produtos farmacêuticos na água. Biofilmes. POCIS.

ABSTRACT

SURFACE WATER CONTAMINATION IN SOUTHERN BRAZIL BY PHARMACEUTICAL COMPOUNDS: APPLICATION IN A HIGH ANTHROPIC PRESSURE CONTEXT

AUTHOR: Jocelina Paranhos Rosa de Vargas Brunet
ADVISOR: Danilo Rheinheimer dos Santos

Medium-sized urban agglomerations have experienced huge population growth in recent decades. The absence of a master plan and environmental planning and / or the lack of control of space occupation have been destroying the few green spaces and environmental protection. The deodernized occupation of space, the lack of collection and the treatment of sewage are standards and not exception. In addition, another problem faced in Brazil is the deficiency in the collection and treatment of hospital waste. In this sense, the present project proposes the monitoring of the presence of pharmaceutical compounds in the water and biofilms in the water courses of the Campus of the Federal University of Santa Maria. Among the sources of pollution are: (a) the domestic sewage of houses and industries in the Camobi neighborhood, (b) the domestic sewage of the University Students' House, (c) effluents from the University Hospital and (d) effluents of the Veterinary Hospital. We developed 4 studies: a first preliminary study to characterize the biofilm collections, the second was to monitor the contamination status of the campus, the third was to perform a resilience experiment to verify the speed of decontamination of the biofilms, and the fourth was to evaluate the drug depuration capacity of a WWTP (Wastewater Treatment Plants). This study shows for the first time the impact of a university campus on the environment. The area of the Santa Maria campus appears to be contaminated by different types of pharmaceuticals, with concentrations of more than 900 ng g^{-1} in biofilms and more than 800 ng g^{-1} in POCIS (Polar Organic Chemical Integrative Sampler). The anticancer drugs epirubicin, the stimulant caffeine and the antibiotic erythromycin presented a medium and high risk for aquatic biota. This experiment highlights how the resilience of a river biofilm is a fast phenomenon since it is already noticeable after 8 hours and leads to a dissipation of two-thirds of the compounds (>95% dissipation) after 8 days. The present work shows that a small WWTP succeed to eliminate a significant part of the pollution load present in domestic wastewater, such as more than 60% of total solids, chemical oxygen demand and biochemical oxygen demand, and 45% of total nitrogen, but has low efficiency in removing pharmaceutical compounds.

Key Words: Water pollution. Environmental contamination. Pharmaceuticals in water. Biofilms. POCIS.

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

AC	Acre
AL	Alagoas
AM	Amazonas
ANA	Agência Nacional de Águas e Saneamento Básico
AP	Amapá
BA	Bahia
BOD_5	Biochemical Oxygen Demand
CE	Ceará
COD	Chemical Oxygen Demand,
CONAMA	Conselho Nacional do Meio Ambiente
CONSEMA	Conselho Estadual do Meio Ambiente
CW	Constructed Wetland
DF	Distrito Federal
ED	Endocrine Disruptors
EPS	Extracellular Polymeric Substance
ES	Espírito Santo
FDA	Factorial Statistical Analysis
GO	Goiás
HUSM	Hospital Universitário de Santa Maria
HV	Hospital Veterinário
MA	Maranhão
MBBR	Moving Bed Biofilm Reactors
MEC	Measured Environmental Concentration
MG	Minas Gerais
MS	Mato Grosso do Sul
MT	Mato Grosso
$N\text{-}NH_4^+$	Ammoniacal Nitrogen
$N\text{-}NO_3^-$	Nitrate Nitrogen
NSWP	National Solid Waste Policy

Org-N	Organic Nitrogen
PA	Pará
PB	Paraíba
PCP	Pre-Conditioning Plants
PE	Pernambuco
PhAC	Pharmaceutically Active Compounds
PI	Piauí
PNEC	Predicted no-Effect Concentration
POCIS	Polar Organic Chemical Integrative Sampler
PPCP	Pharmaceuticals and Personal Care Products
$P\text{-}PO_3^{4-}$	Orthophosphate
PR	Paraná
RJ	Rio de Janeiro
RN	Rio Grande do Norte
RO	Rondônia
RQ	Risk Quotient
RR	Roraima
RS	Rio Grande do Sul
Rs	Sampling rate
SC	Santa Catarina
SE	Sergipe
SP	São Paulo
SPS	Sewage Pumping Stations
STP	Sewage Treatment Plants
TKN	Total Kjeldahl nitrogen
TN	Total Nitrogen
TO	Tocantins
TS	Total Solids
TSS	Total Suspended Solids
UN	United Nations
UNICEF	United Nations Children's Fund
UR	University Restaurant

VPR	Veterinary Pharmaceutical Waste
WHO	World Health Organization
WWTP	Wastewater Treatment Plant
DOX	Doxorubicin
EPI	Epirubicin
DAU	Daunorubicin
IRI	Iritnotecan
MID	Midecamycin
SPI	Spiramycin
JOS	Josamycin
AZI	Azithromycin
SFX	Sulfamethoxazole
CLA	Clarithromycin
ERY	Erythromicin
ROX	Roxythromycin
DCF	Diclofenac
CBZ	Carbamazepine
Trans-CBZ	10,11-dihydro-10,11-transdihydroxycarbamazepine
Acrid	Acridone
Benz	2-[(2-chlorophenyl)-amino]-benzaldehyde
3OH-CBZ	3-hydroxycarbamazepine
OH-DCF	Hydroxydiclofenac

LISTE OF CONTENTS

1 GENERAL INTRODUCTION

More than half of the Brazilian population does not have access to sewage collection and treatment, a fact that contributes to pollution of the rivers that receive these effluents. One of the critical points of the pollution is the contamination of aquatic environments by pharmaceuticals. While the population discharges sewage directly into water bodies, there is no law dealing with the maximum allowed concentration of pharmaceuticals within the rivers.

Another problem is that many compounds cannot be removed efficiently by conventional treatment systems. The presence of these organic pollutants even in small concentrations can cause deleterious effects, leading to numerous effects in aquatic life and even in humans. Thus, the health monitoring of our springs becomes an important tool to know the current state of water bodies, evaluate the impact of domestic sewage discharges to the springs and generate information that can lead to the creation of new environmental laws.

However, one of the main limitations related to the monitoring of the chemical condition of seasons is related to the temporal representativeness of the sampling performed. Passive sampling has been used with the perspective of improving the spatial variability resulting from traditional sampling. Passive sampling consists of a device exposure that contains a receiving phase, with the capacity in the adsorption of certain chemical pollutant, for prolonged periods. This technique is less sensitive to environmental variations in the concentration of organic pollutants, thus providing more adequate information for the long-term monitoring of contaminants in an environmental compartment. Within this perspective, in recent years, epileptic biofilms have been studied as efficient environmental bioindicators, because they present functional clusters and polar and apolar sites that allow the accumulation and capture of different molecules, characteristics that make epileptic biofilms matrixes promising in systems for monitoring the occurrence of pharmaceuticals in water.

In this way, studies that include new types of sampling, that show the pollution status of our water bodies, and that evaluate the purifying capacity of the WWTPs (Wastewater Treatment Plants), are indispensable so that from this database new solutions can be suggested and considered.

2 LITERATURE REVIEW

2.1 WATER RESOURCE AND SEWAGE TREATMENT IN THE WORLD

The consumption of drinking water and basic sanitary services are considered by the United Nations (UN) as a human right. However, more than 663 million people in the world do not have access to any source of drinking water and about 2.4 billion people live without any type of basic sanitation in their regional infrastructure (UN, 2017).

According to the World Health Organization (WHO) and UNICEF, in 2015, only 68% of the world's population used improved sanitation facilities, with Sub-Saharan Africa and Southern Asia having only 30% and 47%, respectively. About 13% of the world's population lives without any form of sanitation and practice open defecation. In sub-Saharan Africa, water collection is a women's responsibility, with 72% of the water consumed in the region being collected by women. This activity ends up influencing the presence of women in school, where one in five girls does not attend school due to the need to ensure the provision of water in their homes for their families. In addition, the precarious conditions of school sanitation facilities often make access difficult for women, who naturally need a less precarious structure, than men (UN, 2017).

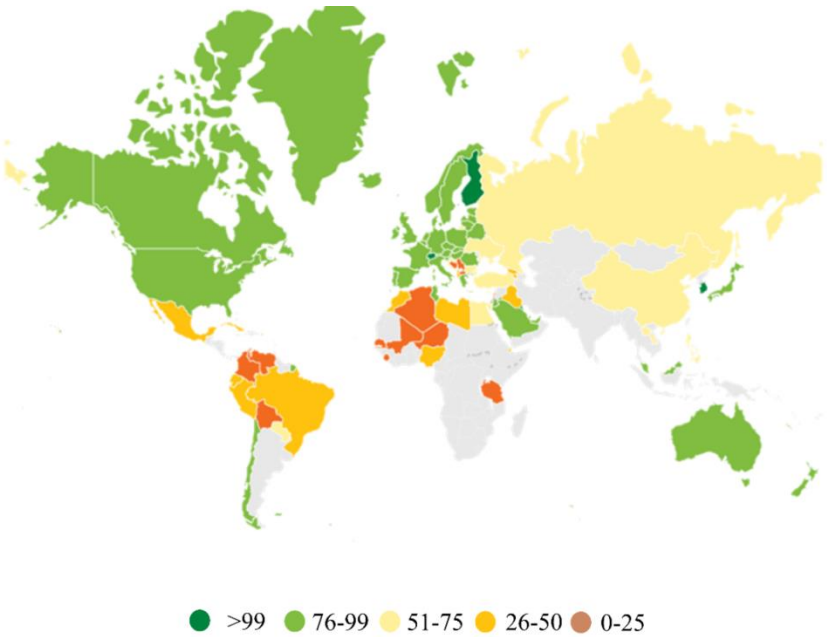
Associated with the lack of drinking water, problems related to the treatment of effluents from various regions of the world are also a cause of concern. More than 1 billion people in the world do not have access to toilets, which represents 15% of the world's population. The lack of sewage collection and treatment is responsible for 3.5 million people annual deaths (among these 1.5 million children) due to problems related to inadequate water supply. Thus, 10% of the diseases recorded around the world could be prevented if governments invested more in access to water, hygiene and sanitation (UN, 2017).

In addition to countries in precarious situations, in low- and middle-income countries about 842.000 people die every year due to drinking water without treatment, lack of sanitation and hygiene, 58% of all deaths being caused by diarrhea. The lack of sanitation is believed to be the main cause of about 280.000 of these deaths. Diarrhea, which could be avoided, claims more victims than AIDS, malaria and measles combined. It is the second largest cause of death among boys and girls between one month and five years of age in the world. Hence, the improvement of water quality, sanitation and hygiene could prevent the death of 361.000 children per year.

Analyzing this context, the situation of countries with poor urban populations represents a growing challenge for world development, since these agglomerations have increasingly become megacities where sewage treatment is precarious or non-existent and space for toilets and waste removal is rare. Inequalities in access to these human-rights practices are exacerbated when sewage collected from the richest households, rather than being sent to sewage treatment plants (STPs), is discharged into drains, waterways or landfills, leading to pollution in poorer residential areas. A large proportion of wastewater in developing countries is discharged partially treated or untreated directly into rivers, lakes or the ocean.

Almost all developed countries have achieved universal access, but sanitation coverage varies widely in developing countries (Figure 1). As with medical care, food and drinking water, access to sanitation is marked by the economic gap that separates the countries of the North from those of the South. Since 2000 the number of countries with less than 50 per cent of the population using a basic sanitation facility has declined only slightly, from 56 to 49 (WHO, 2018).

Literature Review – Figure 1 – Proportion of population using safely managed sanitation services in percentage



Source: (WHO/UNICEF, 2018).

Population growth in urban areas has been a key feature of population dynamics in all regions, with the most pronounced changes taking place in four regions where the urban population has more than doubled: sub-Saharan Africa (an increase in urban population of 169 %), Eastern Asia (136 %), South-eastern Asia (115 %) and Western Asia (109 %). In Eastern Asia, gains in access to drinking water and sanitation not only kept up with, but far exceeded, population growth. By contrast, sub-Saharan Africa registered a decline in water or sanitation coverage in urban areas in 14 out of 46 countries. In Latin America there are 490 million people who still do not know what it is to have access to safe sanitation (WHO/UNICEF, 2018).

In recent decades, another function has been associated with sanitation due to society's and governments' awareness of environmental degradation: the preservation of aquatic ecosystems. More and more restrictive regulations and advanced techniques allow in some cases the discharge of water of very good quality allowing the development of aquatic fauna and flora which had sometimes disappeared. However, these results are disparate on a global scale and some regions of the world still do not manage to ensure the protection of the health of the populations. In the Northern hemisphere, if sanitation aspects remain at the center of concerns, sanitation challenges are strongly oriented towards environmental protection. In the Southern hemisphere, public health remains the main driver of sanitation programs.

The lack of sanitation appears to be an indicator of poverty and the consequences of waterborne diseases associated with it are dramatic and diverse on a global scale. According to the UN, it would take $10 billion per year for ten to twenty years to win the sanitation battle, which is: - less than 1% of global military spending; one-third of the world's spending on bottled water; - the equivalent of what Europeans spend each year on ice cream.

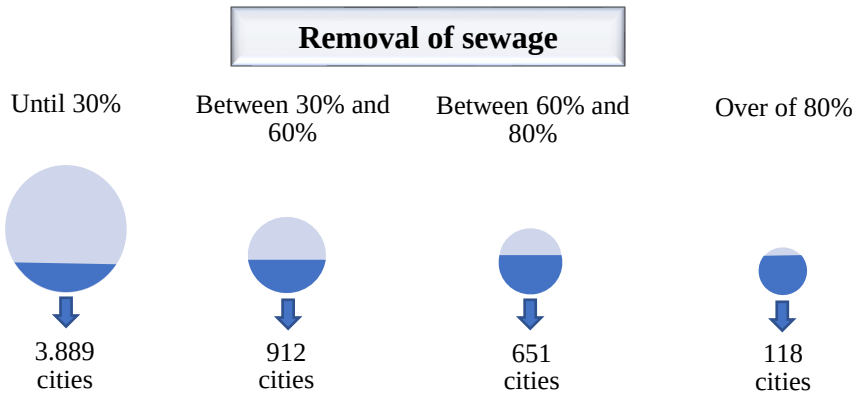
2.2 WASTEWATER TREATMENT

2.2.1 Brazil

Brazil is the fifth most populous country in the world, and the fifth in territorial terms, comprising a population of 213.253.198 distributed in 8.515.767.049 km^2 (IBGE, 2021). Associated with this continental dimension, there are a diversity in economic, social, cultural and climatic conditions. Some of these factors, especially related to demography and climate, may influence the sewerage system and the wastewater treatment processes to be adopted in each case. The Brazilian population is distributed in 5570 municipalities, of which 18.2% have their sewage collected and untreated, 43.5% collected and treated, 26.3% not collected and 12.0% have individual solutions (septic tank). Adding up the share of citizens who do not have treated sewage and those who do not, there are 45% of the population, or 93.6 million people (ANA, 2019). The Northeast and Southeast regions of the country are the most in need of investment. The evaluation is due to the low level of coverage, the high occurrence of intermittent or ephemeral rivers (case of the Northeast) and the large number of densely inhabited urban agglomerations (case of the Southeast) (ANA, 2019). Data shows that, even though most of the population has some type of sewage collection, even if individually, this waste is treated inadequately and inefficiently in most of the country. The national resolution that established conditions and standards for the discharge of sewage into nature (CONAMA 430, 2011) states that the treatment of waste must remove 60% of the organic load before direct discharge into clean water. However, most Brazilian cities (4.801 cities, totaling 129.5 million inhabitants) have sewage removal levels below the percentage defined by the government (ANA, 2019). The National Water Agency survey shows that 70% of the 5.570 municipalities have sewage treatment with a maximum efficiency of 30% (Figure 2). The rest of the waste that is not treated goes to the rivers.

At the other extreme, only 769 cities (14% of the total) have sewage organic removal rates higher than 60%, and the Southeast Region concentrates most of these cities. Only 31 of the 100 most populous municipalities have sewage organic removal above 60%. The states of São Paulo and Paraná, in addition to the Federal District, adequately treat more than half of all the sewage generated by their population. Approximately 9.1 thousand tons of sewage are generated per day throughout the country. The 106 municipalities with a population above 250 thousand inhabitants are responsible for 48% of the total (ANA, 2019).

Literature Review – Figure 2– Removal of sewage load in Brazil



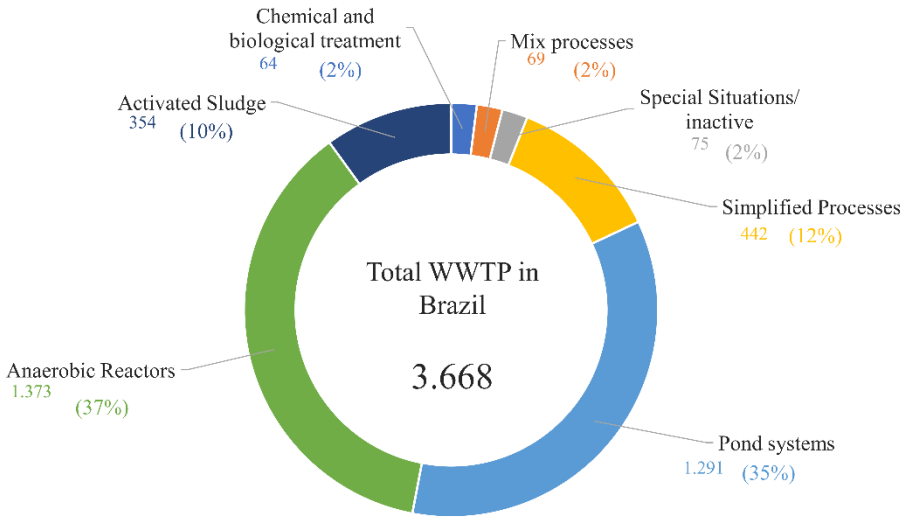
Source: (ANA, 2019).

Of the total hydric network evaluated in the current situation, about 4.5% (83.450 kilometers) are with a concentration of organic matter equivalent to the limits established for class 4, the worst degree in the classification made by ANA, significantly restricting the possibilities of use of these waters. The necessary investment to universalize the collection and treatment of sewage in Brazil by 2035 is R$150 billion reais, about $36 billion dollars.

More than 110.000 km of river stretches have their quality compromised due to excessive organic load, and in 83,450 km abstraction for public supply is no longer allowed due to pollution, and in 27.040 km abstraction can be done, but requires advanced treatment (ANA, 2019).

In Brazil, the main route of discharge of treated or untreated wastewater is in surface fresh waters. Stabilization ponds and anaerobic reactors play a decisive role in wastewater treatment in Brazil, being the two most widely used treatment configurations (Figure 3). Pond systems are systems that require a lot of land. However, in places where space is available, this type of wastewater treatment still plays an especially important role, as it is a simple, relatively inexpensive and effective method. The efficiency of the lagoons is intrinsically related to the climate. Warmer places favor the speed of the organisms' metabolism and photosynthesis (KAYOMBO et al., 2002). Therefore, this system is more effective in tropical countries than in cold countries. Anaerobic reactors have advantages over conventional treatments in that they are not very mechanized, require little maintenance, are easy to operate, and have low implementation and operational costs. This type of treatment is more feasible for countries with a predominantly warm climate, such as Brazil, which have higher temperatures during the year that are highly favorable to the biodegradation reactions that occur inside the reactors, allowing a more efficient degradation of organic matter (JENICEK et al., 2010).

Literature Review – Figure 3 – Total of WWTP, percentage, and representative number of each type of treatment used in Brazil



Source: (ANA, 2019).

The special situation consists of inactive or existing WWTPs whose received information could not be confirmed. Simplified processes consist of the simplified treatment processes, with little mechanization and very limited treatment efficiency. This group includes collective septic tank systems followed by anaerobic filters (323 units), collective septic tanks (66 units), and the PCP - Pre-Conditioning Plants (19 units). The PCP s seek to use the dilution and self-depuration capacity of the sea or high-flowing rivers. Although this capacity is practically unlimited, localized environmental degradation may occur in the region of the discharge. The Activated Sludge Systems are very complex and mechanized, and therefore require specialized maintenance and operation. These stations present high efficiency in the removal of organic matter from the treated effluent and can be implemented in much smaller areas than the lagoon systems. For this reason, they are appropriate to serve large populations and to be implemented in very dense areas. There are many stations with this technology (354 units, excluding those that are anaerobic reactor). Chemical and biological treatments include processes that make use of a combination of biological and chemical processes. The most used design in this group consists of the combination of anaerobic reactor followed by chemical treatment (flotation, decantation or filtration) with 24 units. The mix process consists of

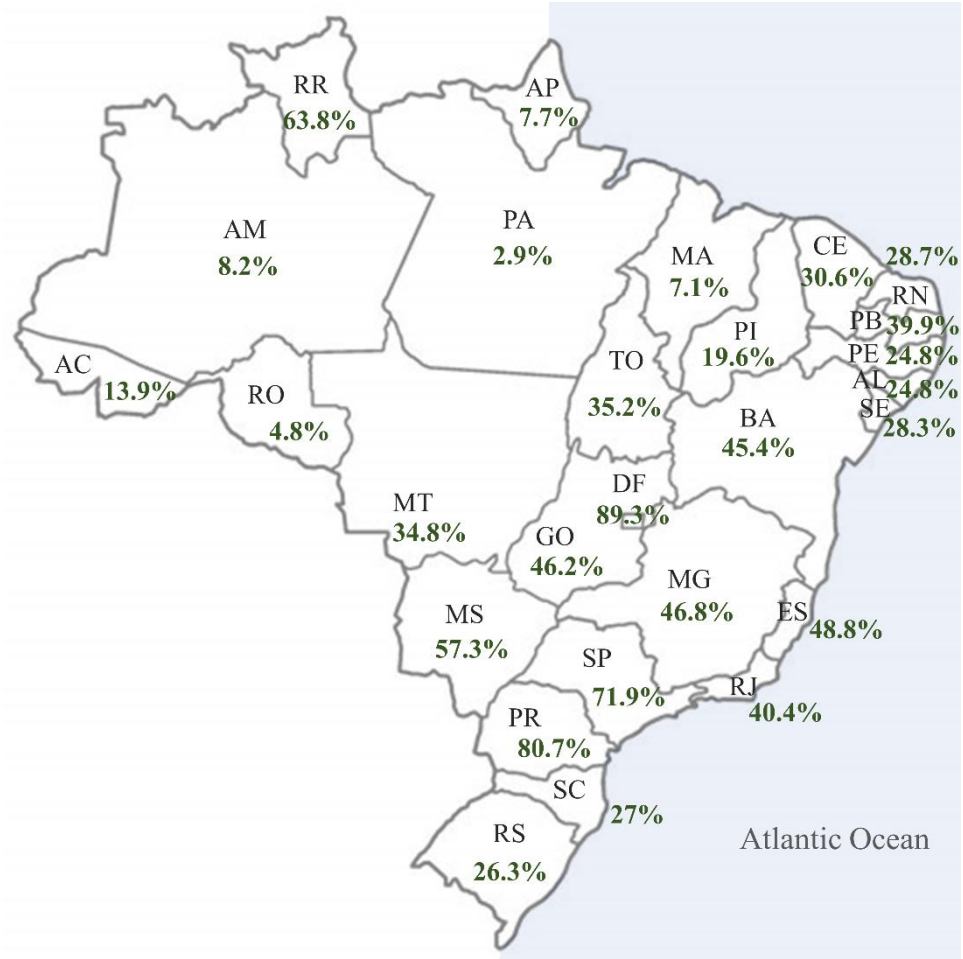
filtration systems and other less conventional treatment designs. In this set the most used systems are anaerobic filter followed by infiltration trench with 15 units, biological filters and decanter (Trickling Filter System) with 9 units, and septic tank followed by facultative lagoon with 7 units.

Due to its vast territorial dimensions, Brazil presents considerable regional diversities in economic and climatic conditions, what can influence the selection and adoption of wastewater treatment processes. Most of the population is in towns and cities that are situated within less than 1.000 km from the Atlantic coast. Within this area, the Northeast region has exceptional climatic conditions for the adoption of natural treatment systems, and temperature and sunlight decrease towards the South of Brazil, but still keeping favorable conditions for biological treatment processes. The inverse occurs in terms of economic conditions, with the South and Southeast regions showing better indicators, what is reflected in terms of the coverage of the sanitation infrastructure.

Regarding the distribution of the population according to size, from the 5.570 Brazilian municipalities, around 25% of them have populations lower than 5.000 inhabitants, about 70% have populations with less than 20.000 inhabitants and approximately 95% of the municipalities have populations lower than 100.000 inhabitants. Therefore, most municipalities in Brazil are small to medium-sized, and the selection of wastewater treatment process needs to take this into account.

Due to the continental size of Brazil combined with its territorial partition into states, different levels of sanitation are created in the country. The Federal District, Paraná and São Paulo are the Federation Units with the best indexes, higher than 70%. The states with the lowest indexes, below 10% are Amazonas, Amapá, Maranhão, Rondônia and Pará (Figure 4). Thus, while there are regions with first-world sanitation levels, there are cities where the rate is zero.

Literature Review – Figure 4 – Urban population provided with collective sewage treatment system



Source: (ANA, 2020).

Access to sanitation services in Brazil is not universalized, although this is constitutionally established (BRASIL, 1988). States in the Southeast have the best average coverage, while states in the North and Northeast have the worst deficits in access to sanitary sewage. The average municipal access in almost all Brazilian regions showed some degree of improvement (ANA, 2019), but representative deficits and regional discrepancies persist. The Brazilian coast (from the Northeast to the South) is the region with the highest demographic density, but the disparities among the regions are clear. Political investment is concentrated in areas of greater economic development but not necessarily in areas where there is more need. The northern region is the region with the lowest demographic density in the country and also the lowest rate of sewage treatment coverage. The investment in these basic sanitation processes has more to do with the return of votes for politicians than with the needs of the population. The model for the provision of basic sanitation services in Brazil has proven to be unsustainable

and harmful to the most vulnerable population. It is therefore important to develop alternative models of provision that are more efficient and less exclusive.

2.2.2 Rio Grande do Sul

The state of Rio Grande do Sul (RS) has an urban population of 9.512.434 inhabitants, distributed in 497 municipalities. The sanitary exhaustion of the state is distributed as follows: 28.17% of the sewage is collected and untreated, only 26.24% is collected and treated, 21.56% is not collected and untreated and 24.02% have individual solutions. In the state, a sewage load of 511.769 (kg Biological oxygen demand (BOD)/day) is generated, with a remainder of 331.036 (kg BOD/day).

In Brazil, the Resolution nº 357 of March 17, 2005, of the National Council of the Environment (CONAMA, 357), disposes about the classification of the hydric bodies and the environmental directives for its framing. However, discharges from point sources are governed by Resolution n°. 430 of May 17, 2011 (CONAMA 430) which provides for conditions, parameters, standards and guidelines for managing the discharge of effluents into receiving water bodies. The issue of a discharge authorization or consent by the public authorities depends on the carrying capacity of the receiving body: there should be no compromise of water quality or significant deleterious effect on the survival and reproduction of organisms.

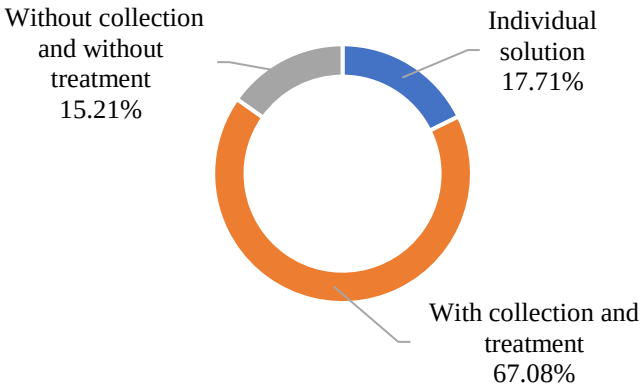
In the RS state scenario, the State Environmental Council (CONSEMA) instituted in 2017 the Resolution nº. 355, which stipulates the criteria and standards for the emission of liquid effluents for generating sources that discharge their effluents into surface waters. It also sets limit values for the release of chemical compounds such as heavy metals, oils, greases, persistent organic pollutants, as well as pH, temperature, phosphorus, and potassium values. Some values are more restrictive than those stipulated by Resolution 430/2011.

In Brazil, basic sanitation projects are the responsibility of the municipalities. The prefecture is responsible for developing the project that needs to be approved by the state to receive the money for the investment.

2.2.3 Santa Maria

Santa Maria is the 5th most populated municipality in the state of RS with an estimated population of 280.505 people (IBGE, 2018). The sanitary situation of Santa Maria city is represented in Figure 5.

Literature Review – Figure 5 – Sanitary situation of the city of Santa Maria



Source: (ATLAS SEWERAGE, 2017).

The Santa Maria sewage collection network was designed in 1972 to transport only sanitary sewage and must operate separated from the stormwater drainage network (separative network). The Santa Maria sewage network currently has 16 sewage pumping stations (SPSs), which transport the sewage generated at a lower point of the network to a higher point, so that it can be transported by gravity to the WWTP.

The WWTP is located in Vila Lorenzi where most of the sewage is treated. There are several mini WWTP scattered throughout the city with septic tank and anaerobic filter treatment. The WWTP, designed in 1972, uses an Activated Sludge system with prolonged aeration and treats an average of 260 L s^{-1} of sanitary sewage and efficiency of 76%. WWTP operates since November 1986 and currently meets the conditions of Operation License LO nº 5756/2011-DL of October 2011.

The treatment system consists of the following steps:

(i) Preliminary treatment: grating and desanding.

(ii) Primary treatment: biological reactor (activated sludge).

(iii) Secondary treatment: secondary decanter and recirculation.

(iv) Tertiary treatment: chemical coagulation with ferric chloride.

(v) Sludge treatment: dewatering by densification and drying beds.

The maximum design flow rate is 280 L s^{-1} in the operating module. The average daily flow of 260 L s^{-1} serves approximately 136.000 inhabitants. The treatment station was designed to achieve a 90% efficiency in the reduction of BOD (tested by BOD5) and suspended solids, with adaptation of the prolonged aeration variable for the improvement of the process. The dosage of coagulant in the raw sewage was adopted to favor the chemical precipitation of phosphates to maintain the controlled concentration within the treatment blocks and reduce the impacts on the system.

There are areas of the city where allotments have been implemented that do not have a sewage collection system and that, by determination of the environmental agencies, have implemented treatment systems composed of septic tanks and anaerobic filters. Individual sewage treatment systems (pits and drains) are used in areas not served by the collection network and in the districts. Due to the lack of maintenance and inspection of septic tanks and/or sinks, the effluents end up infiltrating the soil or being discharged into the bleeds. The maintenance of this type of system, which is basically the cleaning of the pit when it is saturated, is the responsibility of the owner of the property and there is no control by the Municipal Administration of the final destination of the effluent removed from the septic tanks. The lack of maintenance of these systems causes contamination of the soils and surface and underground waters, which can cause public health problems and degradation of the quality of water courses.

Camobi neighborhood, the most populated in the city and where the Federal University is located, had its works on the sewage collection network started in 2013, with a deadline of 2015, but only 13% of the services have been performed so far.

2.3 PHARMACEUTICAL CONSUMPTION AND WASTE GENERATION

The irrational use of drugs and the intense market pressure exerted, mainly, on health professionals, caused the demand for drugs to grow exponentially, and currently countless tons of drugs from different pharmaceutical classes are produced worldwide, which gives this segment great economic importance (SCHWEITZER; LU, 2018). Although global and national consumption data for all classes of drugs are not available, several studies point to excessive consumption of this type of product in Brazil (BRASIL, 2009; IBGE, 2018).

The irrational use of antibiotics can also be considered a public health problem in Brazil, since the consumption of this product has become so widespread in the country that the sale of this class of drugs is characterized as a sales leader in some regions and reached 40% of all pharmaceuticals sold nationally. This problem is aggravated by self-medication and the sale of over-the-counter drugs by pharmacies and drugstores (ANVISA, 2018; LOCATELLI; SODRÉ; JARDIM, 2011a).

This indiscriminate use has led the National Health Surveillance Agency to adopt more restrictive measures for the prescription and trade of these products, through the implementation of DRC 44/2010. This resolution, updated by DRC 20/2011 determined that antibiotics sold in pharmacies and drugstores in the country can only be delivered to the consumer through special control prescription in two copies, and since January 2013 can only be marketed through mandatory bookkeeping.

Brazilian's expenditure on pharmaceuticals is also very high. According to data from the Brazilian Institute of Geography and Statistics (IBGE, 2018), spending on pharmaceuticals consumes 48.6% of the average monthly expenditure on family health. According to the IMS Health Institute (IMS HEALTH, 2012), which monitors the global pharmaceutical market, the Brazilian retail pharmaceutical market grew 19% in 2011, reaching the mark of approximately R$ 38 billion in sales, with estimates of growth for the coming years. In 2018, pharmacies' revenues during the 12 months were 11.76% higher compared to the same period the previous year.

Additionally, it is estimated that the implementation of social programs for the low-income population, such as "Here is a Popular Pharmacy", and the breaking of patents in the coming years, will represent more and more a great opportunity for the entry of generics and similar products in the market, contributing to the increase in sales volume.

In addition to the excretion of Pharmaceutically Active Compounds (PhACs) in domestic sewage, high drug consumption can generate a secondary source of residues of these compounds: inadequate disposal of expired or remaining drugs. Although precise data on the amount of pharmaceuticals that lose their validity annually in Brazil are not available, it is estimated that this value can reach 34 thousand tons annually (ANVISA, 2018).

The improper disposal of unused medications directly in the sink, toilet or household waste is especially problematic, because as in this case the drug is not submitted to any metabolization process, there is the potential for a large amount of the active compound to reach the WWTP and the environment (BRAUND; PEAKE; SHIEFFELBIEN, 2009).

In Brazil, the National Solid Waste Policy (NSWP) was instituted, enacted by Law nº. 12305 of August 2010, which establishes that expired or remaining pharmaceuticals, as part of the Health Service Waste (HSW), should receive adequate treatment prior to their final disposal, according to the characteristics of each substance, by manufacturers and distributors. However, the current rules do not address the shared responsibility of each entity of the pharmaceutical chain and do not address the residues of household pharmaceuticals. Thus, the Decree 7404 of December 23, 2010, which regulates Law 12305 of August 2010, creates the Steering Committee for the Implementation of Reverse Logistics Systems, which establishes home use as a priority sector of action.

One of the concepts introduced by the NSWP is that reverse logistics is an instrument of economic and social development characterized by a set of actions, procedures and means aimed at enabling the collection and restitution of solid waste to the business sector, for reuse, in its cycle or in other production cycles, or other environmentally appropriate destination. According to the decree, consumers are obliged, whenever a selective collection system is established by the municipal plan for integrated solid waste management or when reverse logistics systems are instituted, to adequately and differently condition the solid waste generated and to adequately make available the reusable and recyclable solid waste for collection or return. Which may substantially contribute to the reduction of the generation of PhACs waste in the environment.

2.4 PHARMACEUTICALS IN THE ENVIRONMENT

In the last few decades, the definitive verification of the vulnerability of the environment has led to an in-depth discussion on the effect of various activities of anthropic origin, highlighting the need for changes that contribute to the condition of sustainability. However, as the discussions on the subject advance, so do the tools and routines of environmental monitoring, which makes new problems stand out, before being implemented measures for the definitive resolution of problems considered more classic (BOXALL, 2004; HEBERER, 2002).

A clear example of this situation is represented by the issue of domestic sewage, especially in developing countries, as Brazil. While collection, treatment and disposal programs are belatedly implemented, even though the polluting potential of this matrix and the treatment technology have been known for a long time, new polluting species are evidenced, most of which cannot be efficiently implemented. Consequently, treatment systems become obsolete before being established (COLAÇO; PERALTA-ZAMORA; GOMES, 2014).

Within this context, it is necessary to highlight the presence of organic micropollutants, which, even in small concentrations, of the order of ng L^{-1} may cause deleterious effects. Among the various substances that are characterized as organic micropollutants, are highlight the compounds with pharmacological activity, which can act as endocrine disruptors (ED), causing adverse effects on the reproductive system of humans and animals, selects bacterial resistance and also several effects that are under discussion (LOCATELLI; SODRÉ; JARDIM, 2011a; MARTINS et al., 2008b; PUSCEDDU et al., 2019).

Pharmaceuticals are essential tools for the maintenance and recovery of health, with an important participation in the history of humanity. Despite their fundamental contribution to the improvement in the quality of life, with evident reflexes in modern society, the consumption of pharmaceuticals may be related to environmental contamination problems. Dichotomy is associated with the metabolism of drugs in the body, the deficiencies presented by conventional domestic sewage treatment systems and the dynamics of pollutants in water bodies (BOXALL, 2004).

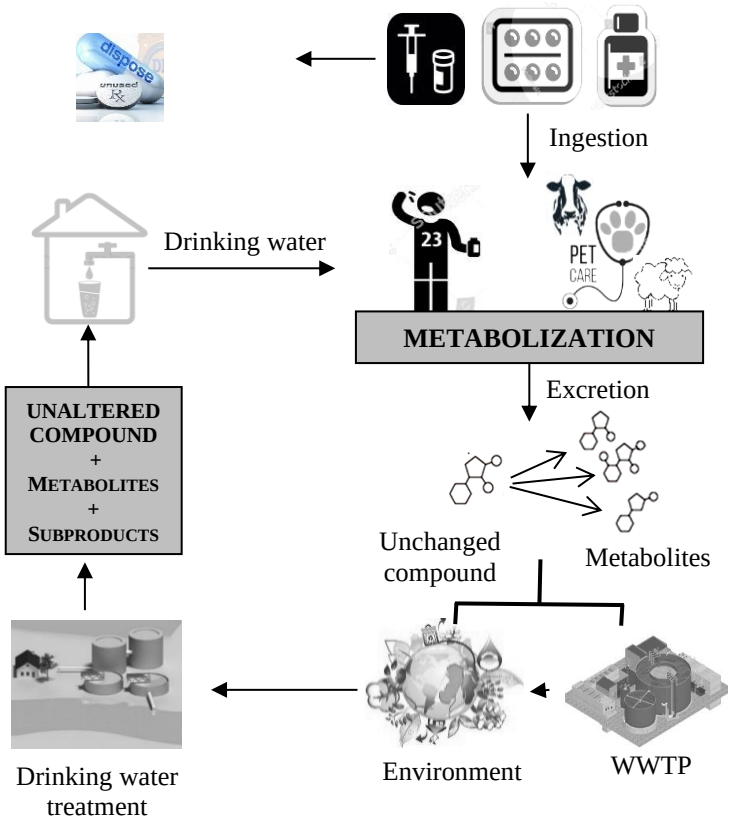
After administration, significant portions of the drug and/or their metabolites are excreted in domestic sewage, or directly into the environment through animal use (Figure 7). The main problem is related to the wide range and structural complexity of drug molecules that present resistance to conventional treatment applied to sewage, and then discarded in aquatic environments (AZUMA et al., 2016). In Brazil, the major concern is still focused on environmental and health damage caused by macropollutants; only 43.45% of all sewage generated in the country receives some type of treatment.

Some drugs, such as penicillin and acetylsalicylic acid (ASA) are practically completely removed through the usual technology. Thus, the lack of sewage treatment in Brazil may cause an additional problem related to the presence of drug residues in the environment: the continuous release of drugs in natural waters that would be easily removed by the conventional treatment system (ARSAND; KÜMMERER; MARTINS, 2013; LOCATELLI; SODRÉ; JARDIM, 2011a; MARTINS et al., 2008b; REIS et al., 2019).

Thus, legislative measures need to be in place to prevent and manage any possible risks that these compounds pose to aquatic systems, for both domestic effluents and pharmaceutical industry effluents, which are also sources of pollution for the aquatic environment (FREITAS et al., 2019). In places such as Australia, Canada, the USA and some European countries where preventative measures have been implemented, the regulations are stringent and mainly apply to controlled substances and cytotoxic drugs other than pharmaceuticals and still preclude the

release in sewage. However, most developing countries, including Brazil, do not have regulations pertaining to pharmaceutical traces as pollutants in aquatic systems.

Literature Review – Figure 6 – Possible drug routes in the environment



Source: (AUTHOR).

2.5 MONITORING OF RESIDUES OF PHARMACEUTICALLY ACTIVE COMPOUNDS IN THE ENVIRONMENT

The first investigations of PhACs residues in the environment involved clofibric acid, major metabolite of lipid regulators and female hormones. However, at this time interest was centered mainly on the presence of petroleum derivatives in natural water matrices (KÜMMERER, 2004; STUMPF et al., 1999a). In the 1980s, the impact of chemical pollutants on the environment was focused on so-called "priority pollutants", such as heavy metals, polycyclic aromatic hydrocarbons, chlorinated dioxins, furans, as well as pesticides and detergents, which were part of rigorous monitoring programs. Thus, the presence of PhACs generated little interest in the 1980s (DAUGHTON, 2010; KÜMMERER, 2004).

The concern with the presence and possible effects of pharmacological residues on the environment became intense in the mid-1990s. At this time, several extraction techniques were proposed, which, associated with chromatographic techniques of greater sensitivity, allowed the analysis of pollutants in trace and ultra-trace concentrations (DAUGHTON, 2010; PETROVIĆ; GONZALEZ; BARCELÓ, 2003). In the late 1990s, Halling-Sørensen et al.

(1998) published one of the first review articles on the occurrence, fate and effect of drugs in the environment, demonstrating the scarce knowledge at the time regarding pharmaceutical products for human treatment. Since 2000, several proposals for the treatment of water contaminated by micropollutants stand out, as well as analysis procedures oriented to the determination of multi-residues. Also, in this period some types of pharmacological residues begin to be considered priorities, highlighting in this group some natural and synthetic drugs and estrogens.

In a study conducted in South Africa, assessing the contamination of pharmaceutical waste in water bodies, Matongo et al. (2015) found high concentrations of ibuprofen (117 μg L^{-1}, 84.60 μg L^{-1} and 659 ng g^{-1}) in wastewater, surface water and sediment, respectively. Antibiotics were detected at generally low concentrations of <10 μg L^{-1} in surface water samples and up to 34.50 μg L^{-1} in wastewater. In addition, the authors also address the percentage removal efficiency of the studied group, which was 6.55 - 98.00% for antipyretics, 73.33 - 98.90% for antibiotics, 48.80% for the antiepileptic drug and 86.40% for caffeine. Evidencing that the treatment processes are not necessarily projects to remove these contaminants.

Addressing the idea that water contamination by veterinary pharmaceutical waste (VPRs) can occur in water resources, but also in tap water Charuaud et al. (2019) evaluated the occurrence of VPRs in water resources and tap water in Brittany, France. The analyzed compounds were quantified in 20% of tap water samples, with concentration below 40 ng L^{-1}.

In recent years, several studies have been carried out all over the world, and drugs from different pharmaceutical classes have been identified in different aquatic matrices. Such as water bodies that, receive effluents from Sewage Treatment Plants in natural waters that have not received effluents from STPs (where the presence of drug residues may indicate the discharge of untreated sewage) and even in drinking water (AUVINEN et al., 2017; KERMIA; FOUIAL-DJEBBAR; TRARI, 2016; PESSOA et al., 2014; REIS et al., 2019). However, it appears that regardless of the extraction system, detection adopted and polarity of the compounds, the capture of compounds with active sampling may present low frequency, regardless of the time sampled (BASTOS et al., 2018; FERNANDES et al., 2019).

Literature Review – Table 1– Studies in the world with the molecules selected for this work.

Compound	Concentration (ng L^{-1})	Matrix	City	Reference
Cafeine	6 – 10.234	River water	Diferrents sites in Lebanon	Mokh et al. (2017)
	102 – 5597	Influent wastewater	Volos, Greek	Papageorgiou et al. (2016)
Doxorubicin	< LOD	Wastewater	Bejing, China	Yin et al. (2010)
	4.5	Influent wastewater	Seville, Spain	Martins et al. (2011)
	< 0.26 (LOQ) –1.35	Sewer of the oncologic in-patient	Vienna, Austria	Mahnik et al. (2007)
	n.d	River water	Catalonia, Sapain	Franquet-Griell et al. (2017)
Epirobicin	n.d	River water / Influent e efflent wastewater	Seville, Spain	Martins et al. (2011)
	< 45 – 60	Hospital effluent	Barcelone, Spain	Gómez-Canela et al. (2014)
	n.d	River water	Catalonia, Sapain	Franquet-Griell et al. (2017)
Daunorubicin	n.d	River water	Catalonia, Sapain	Franquet-Griell et al. (2017)
Irinotecan	n.d	River water	Catalonia, Sapain	Franquet-Griell et al. (2017)
	n.d	River water / Influent e efflent wastewater	Seville, Spain	Martins et al. (2011)
	< 4 – 730	Hospital effluent	Barcelone, Spain	Gómez-Canela et al. (2014)
Spiramycin	n.d – 2.92	Water lake	Hebei, China	Li et al. (2012)
	8.4 – 68·3	River water	Italy	Zuccato et al. (2000)
Erythromycin	nd–17·	River water		Zuccato et al. (2000)
	>LOD – 28	Fountain	Diferrents sites in Lebanon	Mokh et al. (2017)
	>LOD – 2806	River water	Diferrents sites in Lebanon	Mokh et al. (2017)
	n.d	Laker	Diferrents sites in Lebanon	Mokh et al. (2017)
	n.d.–320	Influent wastewater	Volos, Greek	Papageorgiou et al. (2016)
Spiramycin	35000 ± 7000	Influent wastewater	Campania, Italy	Lofrano et al. (2018)
	500 ± 200	Influent wastewater	Campania, Italy	Lofrano et al. (2018)
	32000 ± 7000	Efflent wastewater	Campania, Italy	Lofrano et al. (2018)
	< 100	Efflent wastewater	Campania, Italy	Lofrano et al. (2018)
	118.70	Efflent wastewater	North of Italy	Castiglioni et al. (2008)
	137.80 / 126.70	Olona River site 2 /3	North of Italy	Castiglioni et al. (2008)
	459.50 / 176.70	Lambro River site 4 /5	North of Italy	Castiglioni et al. (2008)
	3.37/ 8.07/ 5.71	Po River site 6/7/8	North of Italy	Castiglioni et al. (2008)
Josamicyn	nd - 0.90	Water lake	Hebei, China	Li et al. (2012)
	>LOD	Fountain	Diferrents sites in Lebanon	Mokh al. (2017)
	n.d	River water	Diferrents sites in Lebanon	Mokh al. (2017)
	>LOD	Laker	Diferrents sites in Lebanon	Mokh al. (2017)
	>LOD – 16	Untreated wastewater	Visoko, Bosnia and Herzegovina	Terzić et al. (2008)

Azithromycin	6–1140	Untreated wastewater	Belgrade, Serbia	Terzić et al. (2008)

(Continuation)

	n.d.–507	Influent wastewater	Volos, Greek	Papageorgiou et al. (2016)
	n.d	Fountain	Diferrents sites in Lebanon	Mokh al. (2017)
	>MDL	River water	Diferrents sites in Lebanon	Mokh al. (2017)
Sulfamethoxazole	n.d	Laker	Diferrents sites in Lebanon	Mokh al. (2017)
	nd - 940	Water lake	Hebei, China	Li et al. (2012)
	nd - 7.86	Sediment	Hebei, China	Li et al. (2012)
	0.019–11.6	Sediment	Hebei, China	Li et al. (2012)
Clarythromycin	100.10	Efflent wastewater	North of Italy	Castiglioni et al. (2008)
	4.30 / 4.50 / 3.04	Po River site 6/7/8	North of Italy	Castiglioni et al. (2008)
	24–420	Untreated wastewater	Belgrade, Serbia	Terzić et al. (2008)
	nd – 121	Water lake	Hebei, China	Li et al. (2012)
	<MDL	Planorbidae	USA	Du et al. (2015)
	<MDL	Periphyton	USA	Du et al. (2015)
Erythromycin	n.d	Eau de surface	USA	Du et al. (2015)
	<LOD	Eau de mariculture	Chine	Xie et al. (2019)
	<LOD - 0,09	Sediment	Chine	Xie et al. (2019)
	<LOD - 5,5	Fish and shellfish	Chine	Xie et al. (2019)
	nd - 3.04	Sediment	Hebei, China	Li et al. (2012)
	Nd – 155	Water lake	Hebei, China	Li et al. (2012)
Roxithromycin	nd - 302	Sediment	Hebei, China	Li et al. (2012)
	>LOD — 0.050	Untreated wastewater	Visoko, Bosnia and Herzegovina	Terzić et al. (2008)
Diclofenac	50–4200	Untreated wastewater	Belgrade, Serbia	Terzić et al. (2008)

2.6 MONITORING OF RESIDUES OF PHARMACEUTICAL COMPOUNDS IN BRAZIL

Although the removal of pharmaceutical compounds is widely discussed internationally, in the national literature this issue has been gaining strength only in recent years. The first studies that investigated pharmacological residues in natural waters and WWTP effluents in Brazil were carried out at the end of the 90's, and the values found in studies carried out in Brazil that compare the concentration of PhACs in raw sewage with treated sewage are in the same order of magnitude of values observed in the literature in studies that employ similar treatment technologies.

Some studies carried out in Rio de Janeiro reveal concentrations of hormones in raw sewage with an average of 0.75 to 5 g per day (TERNES et al., 1999). Also, the low efficiency of sewage treatments was demonstrated by Stumpf et al. (1999) who evaluated the presence of several drugs and metabolites in raw sewage, the percentage of removal in treated sewage and the occurrence in river waters. All compounds present in the raw sewage were found in the treated sewage, with removal efficiency during the process from 12 to 90%. As a consequence of the incomplete removal of these residues, these compounds were found in river waters in average concentrations between 0.02 and 0.04 μg L^{-1}, reaching a maximum concentration of 0.5 μg L^{-1}.

The results of many studies that find considerable concentrations of drugs in treated sewers and even in rivers indicate that the studied rivers receive uncontrolled sewage levels from different sources and/or these compounds are not efficiently removed in the WWTP. In the state of São Paulo, Lopes et al. (2010) published a study that determined the estrogen concentration between the source of the Corrego Rico and after passing through the water treatment plant (WWTP) of the Jaboticabal Region (SP). The results showed the presence of estrogens in 22% of the samples. The 17β-estradiol was detected at the source of the Rico Stream, which demonstrates the contribution of agricultural activities to the dispersion of estrogens in the environment. This compound was also determined in the public water supply, in the contraction of 6.8 ng L^{-1}, which suggests the partial efficiency of the water treatment process for the removal of 17β-estradiol.

The assessment of contamination by drug residues using sediments as an indicator in the largest bay in Brazil, the Bay of All Saints (Salvador, Bahia), was conducted by (BERETTA et al., 2014). The researchers found concentrations of galaxolide (52.5 ng g^{-1}) and tonalide (27.9 ng g^{-1}), followed by caffeine (23.4 ng g^{-1}) and ibuprofen drugs (14.3 ng g^{-1}), atenolol (9.84 ng

g^{-1}), carbamazepine (4.81 ng g^{-1}), erythromycin (2.29 ng g^{-1}), diclofenac (1.06 ng g^{-1}) and diazepam (0.71 ng g^{-1}). The examined compounds were present in all sediment samples at parts-per-billion levels of dry sediment.

Not even so-called "preserved" or forest areas are free from environmental contamination. As shown by Veras et al. (2019), who analyzed the presence of anti-inflammatories in two points of the Beberibe River (Pernambuco), one in a preserved area, not presenting urbanization in its surroundings, the other in an area with intense urbanization. During six months of surface water collection, contamination by diclofenac and paracetamol was evaluated. The authors concluded that both points are polluted, even in low population density area. In addition, it was observed that the highest concentrations were detected during periods of drought.

Martins et al. (2008) determined the presence of ciprofloxacin in the effluent of the Santa Maria University Hospital, in Santa Maria - RS, one of the most used antibiotics in the health institution. The values for the "expected environmental concentration" were 5 to 20.000 times higher than those previously described, which may indicate that the environmental risk associated with the use of pharmaceutical products and emissions to the environment in developing countries may be higher than in developed countries.

In recent years, several compounds have been monitored, both for veterinary and human use: hormones, antibiotics, stimulants, among others, in tap water, as well as in rivers and sediments, showing widespread contamination in Brazilian waters (ARSAND et al., 2018; CALDAS et al., 2018; GHESTI PIVETTA; DO CARMO CAUDURO GASTALDINI, 2019; MONTAGNER et al., 2018; PUSCEDDU et al., 2019; REIS et al., 2019).

Although the patterns of release of these compounds and their presence in rivers located in regions with high population density are not fully known in all regions of the country, the studies performed demonstrate the incomplete removal by the sewage treatment system used and the release of these compounds in Brazilian surface waters. Thus, it is evident the need for evaluations on the impact of pharmaceutical compounds on the Brazilian ecosystem and the adoption of new methodologies to increase the removal of these micropollutants.

Literature Review – Table 2– Studies conducted in Brazil regarding the contamination of water bodies by pharmaceutical compounds.

Compoud	Matrix	Local	Reference
Carbamazepine, Diazepam	River waters	Curitiba, PR	Böger et al. (2018)
Estriol, 17α-ethinylestradiol, 17β-estradiol, estrone, progesterone, testosterone	River waters	Piracicaba, SP	Torres et al. (2013)
Cafeine	River waters	Curitiba, PR	Mizukawa et al. (2019)
Clofibric acid, ibruprofen, diclofenac, bezafibrate, naproxen, gemfibrozil, ketoprofen, fenofribic acid and indometacine, naproxen, tolfenamic acid, Acetylsalicylic acid	WWTP effluent	Rio de Janeiro, RJ	Stumpf et al. (1999)
17α-etinilestradiol, levonorgestrel, estrona, 17βestradiol, progesterona, 4-octilfenol, 4-nonilfenol, dietilftalato, di-n-butilftalato, bisfenol A), ibuprofeno, paracetamol, ácido acetilsalicílico , diclofenaco e cafeína)	River waters	Campinas, SP	Sodré et al. (2007)
Acetaminophen, acetylsalicylic acid, diclofenac, ibuprofen, caffeine, 17β-estradiol, estrone, progesterone, 17α-ethynylestradiol, levonorgestrel, diethylphthalate, dibutylphthalate, 4-octylphenol, 4-nonylphenol and bisphenol A	River waters	Campinas, SP	Montagner and Jardim, (2011)
Amoxicillin, ampicillin, cefalexin, sulfamethoxazole, tetracycline, norfloxacin, ciprofloxacin and trimethoprim	River waters	Belo Horizonte, MG	Locatelli et al. (2010)
17β-estradiol, 17α-etinilestradiol, nonilfenol, bisfenol A, sulfametoxazol, trimetoprima, bezafibrato, diclofenaco and miconazol	River waters	Fortaleza, CE	Brandt, (2012)
Estrone, 17β-estradiol , 17α-ethynylestradiol and 17β-estradiol 17-acetate	Influent and effluent WWTP	CE	Pessoa et al. (2014)
17 β-estradiol, 17 α-ethynylestradiol and 4nonylphenol	Surface water	Belo Horizonte, MG	Moreira et al. (2009)
Estrone, 17-α-ethinylestradion, 17-β-estradiol, bisphenol-A, and caffeine	Treated sewage	Curitiba, PR	Froehner et al. (2011)
Dexamethasone	Hospital wastewater (minimally treated)	Santa Maria, RS	Arsand et al. (2013)
Sulfamethoxazole and Trimethoprim and Their Metabolites	Hospital effluent (minimally treated)	Santa Maria, RS	Brenner et al. (2011)
Bromazepam, lorazepam, carbamazepine, clonazepam and Diazepam	Hospital effluent (minimally treated)	Santa Maria, RS	de Almeida et al. (2013)
Carbamazepine, diazepam and their metabolites	Hospital effluent (minimally treated)	Santa Maria, RS	de Almeida et al. (2015)
Nonylphenol ethoxylate and 4-nonylphenol	Hospital wastewater (minimally treated)	Santa Maria, RS	Henriques et al. (2012)
Ciprofloxacin	Hospital effluent (minimally treated)	Santa Maria, RS	Martins et al. (2008b)
Amoxicillin	Hospital wastewater (minimally treated)	Santa Maria, RS	Martins et al. (2009)
Sulfamethoxazole and trimethoprim	Hospital wastewater (minimally treated)	Santa Maria, RS	Martins et al. (2011)

Atorvastatin e simvastatim	Hospital effluent (minimally treated)	Santa Maria, RS	Martins et al. (2017)
Diclofenac and metabolites	Hospital effluent (minimally treated)	Santa Maria, RS	Minetto et al. (2012)
Epirubicin, Daunorubicin, Doxorubicin and Irinotecan	Hospital effluent (minimally treated)	Santa Maria, RS	Souza et al. (2018)
Ciprofloxacin	Hospital effluent (minimally treated)	Santa Maria, RS	Vasconcelos et al. (2009)
Atenolol, metoprolol and propranolol	Hospital wastewater (minimally treated)	Santa Maria, RS	Wilde et al. (2013)
Atenolol, metoprolol and propranolol	Hospital wastewater (minimally treated)	Santa Maria, RS	Wilde et al. (2012)
Diclofenac and naproxen	Surface water	Três Lagoas, MS	Américo-Pinheiro et al. (2017)
Piroxicam and atenolol	Urban stream	Três Lagoas, MS	Américo et al. (2015)
Diclofenaco, Ibuprofeno, Naproxeno, Paracetamol and piroxicam	Raw and treated sewage	Três Lagoas, MS	Américo et al. (2012)
Carbamazepine, Caffeine, and Atrazine	Water sources	Brasília, Brazil	Sodré and Cavalcanti (2018)
Diclofenac and paracetamol	Surface water	Recife, PE,	Veras et al. (2019)
Caffeine, paracetamol, atenolol, estrone and 17-β-estradiol	Surface water	São Paulo, SP	Campanha et al. (2015)
Oxytetracycline, doxycycline hyclate, hydrochloride salts of chlortetracycline, demeclocycline, dapsone, facetamide, sulfadimethoxin, sulfamerazine, methazine,sulphaquinoxaline, sulfathiazole, tylosin tartarate, troleandomycin, erythromycin, cephapirin sodium salt, ceftiofur, cefoperazone, benzylpenicillin sodium salt, oxacillin sodium salt hydrate, moxifloxacin, ofloxacin , amoxicillin tryhidrate, ampicillin, cefaclor, cefadroxila, cefalexin hydrate, cefazolin, clarithromycin, ciprofloxacin hydrochloride, norfloxacin, tetracycline hydrochloride, sulfamethoxazole, 4-epioxytetracycline, 4-epitetracycline, 4-epichlortetracycline hydrochloride, azithromycin dehydrate, roxithromycin, spiramycin, oleandomycin, tilmicosin, cefquinome sulphate salt, cloxacillin sodium salt hydrate, dicloxacillin sodium salt hydrate, nafcillin sodium salt, desacetylcephapirin and ampicillin-D5.	Surface and drinking water	Rio de Janeiro, RJ	Monteiro et al. (2017)
Estrone, 17β-estradiol, estriol, 17α-ethinylestradiol, diethylstilbestrol, levonorgestrel, mestranol, progesterone, testosterone, bisphenol A, triclosan, atrazine, caffeine, octylphenol and nonylphenol	Drinking and surface water	AM, RO, PA, MT, MS, GO, DF, TO, MA, PI, CE, RN, PB, PE, AL, SE, BA, ES, MG, RJ, SP, PR, SC and RS.	Machado et al. (2016)

Acetaminophen, atenolol, atorvastatin, benzoylecgonine, bromazepam, caffeine, carbamazepine, ciproterone, citalopram, clonazepam, clopidrogel, chlorpheniramine, chlorthalidone,vocaine, Diazepam, diclofenac, drospirenone, enalapril, haloperidol, ibuprofen, loratadine, losartan, midazolam, orfenadrine, paroxetine, propanolol, ranitidine, risperidone, rosuvastatin, Sildenafil, simvastatin, tadalafil, valsartan	Seawater	Santos, SP	Pereira et al. (2016)
Acetylsalicylic acid, salicylic acid, ketoprofen, naproxen, gemfibrozil, fenofibrate, estradiol, ethinylestradiol, estrone, 4-methylbenzylidene, camphor, octylmethoxycinnamate and caffeine	River waters	Curitiba, PR	Ide et al. (2017)
Estrone, 17β-estradiol, estriol, 17α-ethinylestradiol, bisphenol A ,4-n-octylphenol and 4-n-nonylphenol	Surface water	São Paulo, SP	Sodré et al. (2010)
17β-estradiol, estriol, 17α-ethinylestradiol, bisphenol A, 4-*n*-octylphenol and 4-*n*-nonylphenol	Riverns and drinking water	São Paulo, SP	Jardim et al. (2012)
Miconazole nitrate, nimesulide, methylparaben, mebendazoleb, glibenclamide, furosemide, gemfibrozil, eusolex 6300, diclofenac sodium, chlorpropamide, carbamazepine, caffeine, benzophenone, avobenzone and atenolol	Surface and drinking waters	Morro Redondo, RS	Caldas et al. (2013)
Acetaminophen, acetylsalicylic acid, amoxycillin, ampicillin, caffeine, cephalexin, ciprofloxacin, diclofenac, ibuprofen, norflaxacin, phenolphthalein, sulfamethoxazole, trimethoprim, triclosan, 17a-ethinylestradiol, 17b-estradiol, diethylstilbestrol, estriol, estrone, levonorgestrel, mestranol, progesterone, testosterone	Raw and treated wastewaters, surface, ground and drinking waters	São Paulo, SP	Montagner et al. (2019)
Phenazone, phenylbutazone, betamethasone, ranitidine, loratadine, cimetidine, clarithromycin, erythromycin, paroxetine, scopolamine, omeprazole, trimethoprim, atenolol, fenofibrate, prednisone, fluconazole, ampicillin, amoxicillin, atorvastatin, caffeine, danofloxacin, enoxacin, enrofloxacin, metformin, norfloxacin, ketoprofen, ibuprofen and gemfibrozil	Drinking water treatment plants	MG	Reis et al. (2019)
Mefenamic acid, atenolol, caffeine, cephalexin, ciprofloxacin, clindamycin, diclofenac, lidocaine, metoprolol, naproxen, paracetamol, propranolol, sotalol, sulfamethoxazole, trimethoprim, carbendazin, diuron and tricyclazole	WWTP effluent and surface water	Porto Alegre, RS	Arsand et al. (2018)
17 β-estradiol, estriol, estrone, ethisterone, megestrol acetate, diclofenac, ibuprofen and paracetamol	River Waters	Santa Maria, RS	Pivetta and Gastaldini, (2019)
Avobenzone, caffeine, glibenclamide, methylparaben, nimesulide, propylparaben, triclocarban and triclosan	Drinking and surface water	Rio Grande, RS	Caldas et al. (2018)
Estriol, 17β-estradiol and 17α-ethynylestradiol	Sediment	Santos, SP	Pusceddu et al. (2019)
Cholesterol, brassicasterol, ergosterol, epicoprostanol, coprostanol, cholestanol, campesterol, b-sitosterol, cholesterol, stigmasterol, and stigmastanol	Surface sediment	Manaus, AM	Melo et al. (2019)
Acetaminophen, acetylsalicylic acid, amoxycillin, ampicillin, caffeine, cephalexin, ciprofloxacin, diclofenac, ibuprofen, norflaxacin, phenolphthalein, sulfamethoxazole, trimethoprim, triclosan, 17a-ethinylestradiol, 17b-estradiol, diethylstilbestrol, estriol, estrone, levonorgestrel, mestranol, progesterone, testosterone	Raw and treated wastewaters, surface, ground and drinking waters	São Paulo, SP	Montagner et al. (2019)
Phenazone, phenylbutazone, betamethasone, ranitidine, loratadine, cimetidine, clarithromycin, erythromycin, paroxetine, scopolamine, omeprazole, trimethoprim, atenolol, fenofibrate,	Drinking water treatment plants	MG	Reis et al. (2019)

prednisone, fluconazole, ampicillin, amoxicillin, atorvastatin, caffeine, danofloxacin, enoxacin, enrofloxacin, metformin, norfloxacin, ketoprofen, ibuprofen and gemfibrozil			
Mefenamic acid, atenolol, caffeine, cephalexin, ciprofloxacin, clindamycin, diclofenac, lidocaine, metoprolol, naproxen, paracetamol, propranolol, sotalol, sulfamethoxazole, trimethoprim, carbendazin, diuron and tricyclazole	WWTP effluent and surface water	Porto Alegre, RS	Arsand et al. (2018)
17 β-estradiol, estriol, estrone, ethisterone, megestrol acetate, diclofenac, ibuprofen and paracetamol	River waters	Santa Maria, RS	Pivetta and Gastaldini, (2019)
Avobenzone, caffeine, glibenclamide, methylparaben, nimesulide, propylparaben, triclocarban and triclosan	Drinking and sufarce water	Rio Grande, RS	Caldas et al. (2018)
Estriol, 17β-estradiol and 17α-ethynylestradiol	Sediment	Santos, SP	Pusceddu et al. (2019)
Cholesterol, brassicasterol, ergosterol, epicoprostanol, coprostanol, cholestanol, campesterol, b-sitosterol, cholesterol, stigmasterol, and stigmastanol	Surface sediment	Manaus, AM	de Melo et al. (2019)

AM, Amazônas State. MG, Minas Gerais State. PR, Paraná State. RS, Rio Grande do Sul State. SP, São Paulo State. RO, Rondônia State. PA, Pará State. MT, Mato Grosso State. MS, Mato Grosso do Sul State. GO, Goiás State. DF, Distrito Federal. TO, Tocantins State. MA, Maranhão State. PI, Piauí State. CE, Ceará State. RN, Rio Grande do Norte State. PB, Paraíba State. PE, Pernambuco State. AL, Alagoas State. SE, Sergipe State. BA, Bahia State. ES, Espírito Santo State. RJ, Rio de Janeiro State. SC, Santa Catarina State.

2.7 REMOVAL OF PHARMACEUTICALLY ACTIVE COMPOUNDS BY THE SEWAGE TREATMENT SYSTEM IN BRAZIL

Sewage treatment is essential for maintaining health and conserving the environment. However, not all substances submitted to conventional treatment processes are completely removed and thrown into the receiving bodies. The removal efficiency at WWTP of most drugs is not fully understood. The studies that are carried out with this objective generally consider the removal of the standard compound. The metabolites, conjugated metabolites, and secondary products of the treatment process, which can also have environmental effects, are generally not evaluated.

The technologies most used in sewage treatment in Brazil are anaerobic reactors or the association of aerobic and anaerobic reactors, which present a lower energy consumption and operation, and the generation of a smaller amount of sludge when compared to the conventional activated sludge system. However, despite the numerous advantages of the combined system, this process is not able to completely remove the micropollutants present (CHARUAUD et al., 2019; MATONGO et al., 2015; REIS et al., 2019).

The physical-chemical characteristics of the drugs are directly related to their degradation or fate during the sewage treatment process. Polar compounds tend to remain in the liquid phase of sewage. Some compounds are easily eliminated by conventional sewage treatment and others have a low removal efficiency. Pessoa et al. (2014) analyzed the presence of natural and synthetic estrogens in WWTP of Fortaleza - Ceará. In raw sewage concentrations varied between 560 and 2570 ng L^{-1} with removal efficiencies between 19.7 and 100%, associated with changes in affluent load of the micropollutants analyzed. Colaço; Peralta-Zamora; Gomes, (2014) evaluated the removal of diclofenac in WWTP that operate by aerobic and anaerobic routes in the city of Curitiba – Paraná. The drug was determined in all samples of raw sewage and treated, with concentration ranging from 417 ng L^{-1} to 1505 ng L^{-1}. No difference was observed between the treatments used, and because of the low removal efficiency of diclofenac, it was found in the evaluated river water of one of the WWTPs.

Some compounds such as estrogens, conjugated estrogens, progestins and phytoestrogens can be found in natural waters in large quantities (up to approximately 400 ng L^{-1}) in regions with high population density. In other words, the levels of these compounds at the exit of the treatment plants are not controlled and due to low removal efficiency they end up being found in the rivers that receive the treated effluents from the treatment plant (KUSTER et al., 2009). The big problem with the lack of sewage treatment and/or the low recovery rate

of these compounds during the treatment of effluents is their persistence and mobility in the environment (WANG; WANG, 2016). Anthropogenic and seasonal aspects have a direct influence on the concentrations of drugs when released into watercourses. The major problem with these compounds in the water is that the drinking water treatment carried out in Brazil does not include additional steps such as exposure of the water to activated carbon and ozone (COLAÇO; PERALTA-ZAMORA; GOMES, 2014).

These data are quite alarming, since the lack of basic sanitation causes pollution of water resources and launches directly into the environment PhACs eliminated by urine and feces or disposed of in the sink and toilet. Fresh sewage disposal into water bodies probably represents the largest environmental source of drugs that are easily removed by conventional sewage treatment (KERMIA; FOUIAL-DJEBBAR; TRARI, 2016).

PhACs discharge patterns and their presence in rivers located in regions with high population density are not fully known in all regions of the country and for all classes of PhACs. The studies carried out demonstrate the incomplete removal by the sewage treatment system employed and the release of these compounds in Brazilian surface waters, thus highlighting the need for assessments on the impact of PhACs on the Brazilian ecosystem and the adoption of new methodologies to increase the removal of these micropollutants. Drugs that present high biodegradability or chemical instability, such as acetylsalicylic acid (AAS) and penicillins, show removal efficiency above 99% in conventional treatments used by WWTPs, and thus are hardly found in environmental matrices (HEBERER, 2002)

In Brazil the discharge of effluents is regulated by Resolution of the National Environmental Council n° 430 of 2011 (CONAMA 430) and currently there is no limit to the discharge of emerging contaminants such as pharmaceuticals in current legislation. However, despite the fact that there are no values established for the release of PhACs, in Article 8 the resolution states that the release of Persistent Organic Pollutants (POPs) is prohibited, a class in which drugs may be included, and in Article 18 it is verified that the effluent should not cause or have the potential to cause toxic effects to aquatic organisms in the receiving body, in accordance with the ecotoxicity criteria established by the competent environmental agency; which may set precedents for the inclusion of prohibited contaminants in the effluent or set maximum permitted limits.

The lack of access to basic sanitation and adequate treatment of sewage can contribute to the proliferation of numerous infectious and parasitic diseases, result in high environmental pollution, significantly increase medical and labor expenses and substantially devalue regions without access to this type of treatment.

Literature Review – Table 3– Brazilian laws dealing with the disposal of effluents.

Resolution CONAMA	Laws
n° 20/1986	Classification and framing of water bodies
n° 274/2000	Defines microbiological standards for waters intended for primary contact recreation (bathing standards).
n° 357/2005	Provides for the classification of water bodies and environmental guidelines for their classification, as well as establishes the conditions and standards for the discharge of effluents.
n° 370/2006	Extends the deadline for completion of the conditions and standards for the discharge of effluents, provided for in Article 44 of Resolution No. 357 of March 17, 2005.
n° 375/2006	Defines criteria and procedures for the agricultural use of sewage sludge generated in sewage treatment plants and their by-products, and makes other provisions.
n° 377/2006	Provides for simplified environmental licensing of sanitary sewage systems.
n° 380/2006	Corrects CONAMA Resolution Nº 375/2006 - Defines criteria and procedures for the agricultural use of sewage sludge generated in sewage treatment plants and their derived products, and makes other provisions.
n° 393/2007	Extends the deadline for complementing the conditions and standards for discharge of effluents, provided for in art. 44 of Resolution nº 357, of 17 March 2005, and in Art. 3 of Resolution nº 397, of 3 April 2008.
n° 396/2008	Provides for environmental classification and guidelines for groundwater classification and makes other provisions.
n° 397/2008	Provides for the classification of water bodies and environmental guidelines for their classification, as well as establishing the conditions and standards for the discharge of effluents.
n° 430/2011	Establishes parameters, standards and guidelines for the management of effluent discharge into receiving water bodies after treatment.

In developed countries, several technologies have been studied and applied to promote the most effective removal of micropollutants. However, these methodologies present high energy and operating costs, which may generate additional costs to the population through new tariffs. The high consumption of pharmaceuticals in Brazil and the lack of an adequate policy for the disposal of pharmaceuticals makes the problem of waste PhACs even more worrying.

The coverage of conventional depletion services, which is insufficient for the removal of most drug and metabolite residues, can take many decades to reach 100% of the population. The implementation of advanced treatment technologies for the removal of PhACs may take even longer, especially since it is an environmental problem that does not generate immediate impacts on the health of the population. Thus, all biota is susceptible to the effects of these micropollutants.

2.8 WATER SAMPLING STRATEGIES - POCIS

Monitoring the impact of human activities on the aquatic ecosystem and water quality has been developed mainly by two approaches: monitoring the physical condition of the habitat (water chemistry, hydrology and sediment quality) and by monitoring the significant components of ecological diversity, such as fish and macro invertebrates (LEAR and LEWIS, 2009). One of the main limitations related to the monitoring of the physical condition of the habitat is related to the temporal representativeness of the sampling performed, especially in watersheds.

The complexity of the aquatic system, together with the pollution sources, promotes differently the contribution of pharmaceuticals to surface waters. Consequently, it is possible that during a rainfall event or even in a certain period of time, there may be entry of greater or lesser intensity and concentration of pollutants. Thus, at the time of active sampling of surface waters it is not possible to know which stage of contamination is represented. Another factor is related to the physical-chemical diversity of the pharmaceutical products, to understand which matrices (water and/or sediments) should be sampled.

In the perspective of improving the spatial variability arising from traditional sampling, there is passive sampling. Passive sampling consists of exposing a device that contains a receptor phase, with recognized capacity to adsorb a particular chemical class of pollutant, for prolonged periods. This technique is less sensitive to environmental variations in the concentration of organic pollutants, thus providing more suitable information for long-term monitoring of contaminants in an environmental compartment.

To address these problems, Petty et al. (2002) developed and patented a passive integrative sampling device (Polar Organic Chemical Integrative Sampler-POCIS). This device includes a hydrophilic microporous membrane shell formed by a tube or membranes. A mixed sequestration media, which interacts with dissolved polar organic compounds. The sequestration media consists of a three-phase mixture of a hyper-crosslinked polystyrene-divinylbenzene resin, and a carbonaceous adsorbent dispersed in a size exclusion styrene-divinylbenzene copolymer.

The POCIS sampling is well-suited to monitoring hydrophilic compounds such as medical drugs, surfactants (nonylphenols), triazines and caffeine. POCIS is currently used to (qualitatively) screen for organic hydrophilic contaminants in aquatic environments. It is well adapted to sampling micropollutants in surface water. POCIS samplers present characteristics that open up possibilities for detecting compounds present at concentrations below the limits of

detection in grab samples. The process of accumulation in the POCIS is essentially adsorption on the internal solid phase after contaminants passively diffuse through the hydrophilic membrane. In order to assess the time-averaged ambient concentration of POCIS-available contaminants, the POCIS is exposed during the linear-phase. Furthermore, POCIS can be coupled with ecotoxicology tests to assess the potential toxicity of mixtures of organic micropollutants and through the sampling rate (Rs) we can convert the values into L. The sampling rate (Rs) is specific to each compound, and is therefore critical for calculating the ambient concentration.

2.9 EPILITHIC BIOFILMS AND PHARMACEUTICALS

2.9.1 Epilithic biofilms

Biofilms are communities of microbial cells of greater or lesser complexity, both eukaryotes (with predominance of bacteria) and prokaryotes (microalgae, such as diatoms or green algae and cyanobacteria, and fungi) (ZACHEUS et al., 2001), and protozoa (CORSARO et al., 2010) and viruses (TEUNIS et al., 2009). They prevail in most aquatic environments such as rivers, lakes, and oceans, adhered to an inert (i.e., rocks) or living (i.e., plants) surfaces. The study of ecology and biogeochemistry of epilithic biofilms has developed slowly over the years. Unlike laboratory-grown biofilms, naturally developed epilithic biofilms are continuously exposed to the dynamic flow of the water stream, constituting intrinsically complex and variable structures, as changes and processes occur in the aquatic ecosystem. In streams and rivers, biofilms are key sites of enzymatic activity, participating in the cycling of organic matter, ecosystem respiration and primary production, that is, they form the basis of the food chain (FINDLAY, 2010; ROMANÍ et al., 2008). Similarly, these biofilms adsorb, retain and transform organic substances and nutrients in the matrix, as well as accumulate substances that, in the water flow would be highly diluted, such as dissolved organic carbon or contaminants (FLEMMING; WINGENDER, 2010a)

Currently, epilithic biofilms are recognized as complex communities that harbor an immense degree of biodiversity in all domains of life, conducting ecosystem processes and biogeochemical cycles (BATTIN et al., 2016). These characteristics make them promising indicators of contamination by organic and inorganic compounds in rivers and streams, since they are the first organisms to interact with dissolved substances (nutrients, organic matter and toxic substances) (SABATER et al., 2007a). Thus, the abundance of different organisms in a community is the result of a cumulative response to the above conditions. Thus, the use of biological indicators provides a more comprehensive temporal aspect than chemical techniques and traditional hydrological monitoring (LEAR; LEWIS, 2009).

2.9.2 Physical morphology of biofilm

The physical morphology of biofilm depends on temperature, solvent composition, pH and ionic concentrations through osmotic pressure. This gives a physically based mechanism for biomass redistribution within the biofilm (RANZINGER et al., 2016). The physical

structure of the biofilm can be characterized using various parameters such as roughness, porosity, density, surface area, etc. Some of these parameters can greatly affect the mechanical strength of biofilm and, consequently, its degradation of the fixing surface (HORN; REIFF; MORGENROTH, 2003). The density and porosity are certainly the most influential and can vary from one biofilm to another and also through the thickness of the biofilm (LASPIDOU; ARAVAS, 2007). A balance between detachment and growth governs the structure of biofilm. (SANTOS et al., 2018).

Using observations of grown biofilm in an air transport bioreactor and simulations, Loosdrechter et al. (1995) concluded that heterogeneous porous structure occurs when a biofilm is strongly limited by diffusion and when a low shear stress is applied. From the moment the development of biofilm begins, it becomes heterogeneous and structurally complex. Biofilm will normally grow to an almost stable state, i.e., the thickness of the biofilm will tend to a constant average thickness generally less than 300 microns.

These form porous structures (up to 90% porosity) with high water content and densities very close to those of water (WAGNER et al., 2010). In a study on the morphology of biofilm, Desmond et al. (2018) observed an average thickness of 803 ± 70 μm for biofilms without any growth restriction. And in limiting conditions of P and N a respective thickness of 251 ± 374 18 μm and 214 ± 37 μm, both after 30 days of growth.

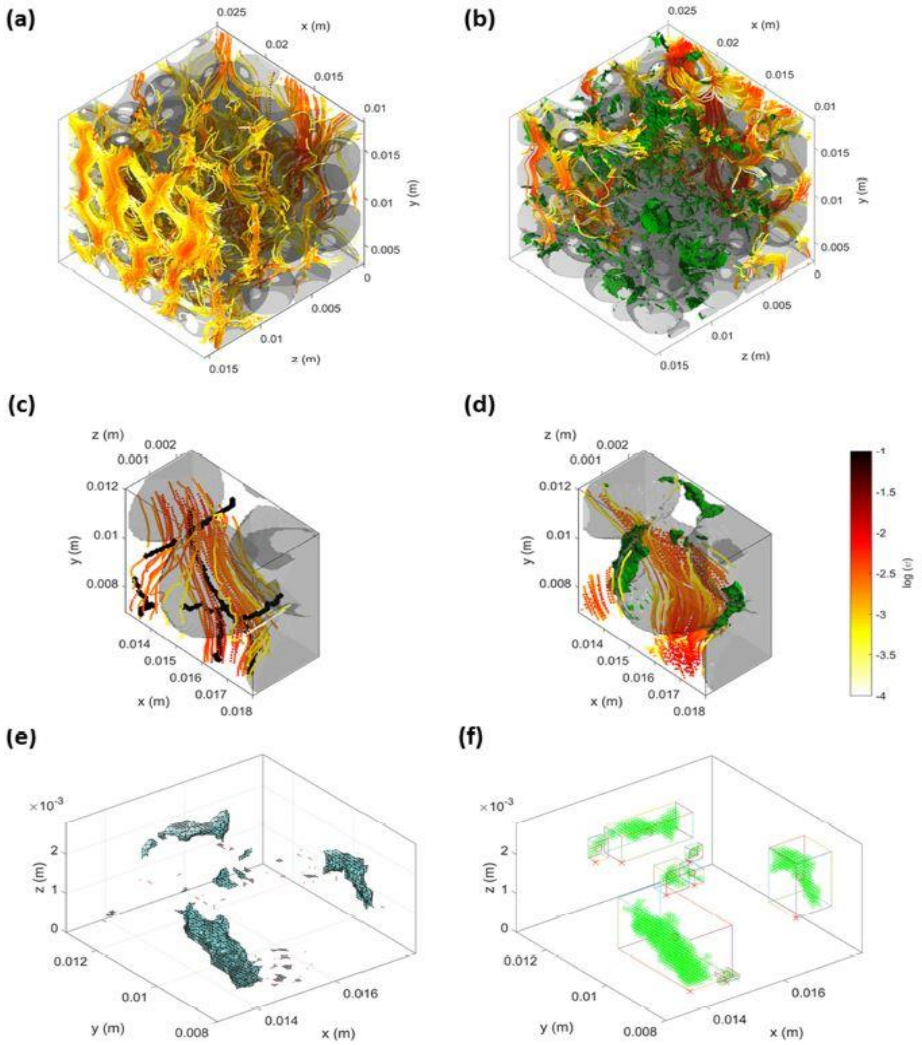
Thus, contrasting growth conditions produce biofilms with different physical structures, and often this can be strongly associated with different hydraulic resistances and nutrient supply growth constraints (DERLON et al., 2016). Heterogeneous biofilm morphologies were associated with different hydraulic strengths; biofilms formed under restricted conditions showed significantly lower hydraulic strength and biofilms formed under nutrient enriched conditions, although also heterogeneous, had higher hydraulic strength (DRESZER et al., 2014; VALLADARES LINARES et al., 2016).

In a study on the modeling of the biofilm structure, Laspidou et al. (2014) concluded that the higher density, close to the biofilm medium (100 – 180 μm), was a result of active biomass, EPS (Extracellular Polymeric Substance) and inert biomass with significant densities together. According to the authors, the top of the biofilm is composed of active biomass and EPS, while the bottom is composed of residual inert biomass. Higher composite density does not correspond to the same location as the active biomass has its higher density, but is somewhat deeper in biofilm, where the inert biomass had time to accumulate. The lower part of the biofilm has a moderate density, composed almost entirely of inert biomass.

Through modeling Zhang and Bishop (1994), found that the densities in the lower layers of biofilm were 5 to 10 times higher than in the upper layers; the proportion of live cells for total biomass decreased from 72 to 91% in the upper layers to 31 – 39% in the lower layers; the porosities of biofilms changed from 84 to 93% in the upper layers to 58-67% in the lower layers. In contrast, the radius average pore size of biofilms decreased from approximately 1.7 to 2.7 μm in the upper layers to 0.3 to 0.4 μm in the lower layers.

In a study on the development of biofilms in porous media, Carrel et al. (2018) recorded the initial central cell development (T = 0 h) and after 36 h of biofilm development. In Figure 8 it is possible to observe the growth of biofilm in the central pore area (in green) (a) and (b), the approximation of the structure (c) and (d) and the delimiting boxes from which the ratio of appearance of the samples was calculated (e) and (f).

Literature Review – Figure 7 – Three-dimensional particulate tracking (RTP-3D) and x-ray data from the central zone to the cleaned



Source: (CARREL et al., 2018).

(a) and colonized (b) porous medium. Solid surfaces (Nafion grains and biofilm) represent a Delaunay color triangulation (segmented X-ray data) (Nafion grains in ash and biofilm in green). (c) and (d) show a local amplification of a pore before and after biofilm colonization. Black lines in (c) represent the skeleton along which the pore rays were calculated. The color bar in (d) shows the magnitude scale of the velocity used for (a) - (d). The pore skeleton is not shown in (a), (b) and (d) for reasons of clarity. (e) shows the biofilm structures illustrated in (d). (f) shows the same corrections and bounding boxes from which the aspect ratio of the samples was calculated. Objects smaller than 10 voxels visible in (e) were removed in (f).

2.9.3 Biofilms as environmental indicator

Faced with limitations regarding temporal variability in the environment of aquatic ecosystems, European researchers have been studying biofilms for many years to evaluate the environmental quality of aquatic ecosystems (FLEMMING; WINGENDER, 2010a; FUCHS; HARITOPOULOU; WILHELMI, 1996; SABATER, 2017). These organisms are capable of bioaccumulating (BERLIOZ-BARBIER et al., 2014; RAMIREZ et al., 2009) and/or participating in the biomagnification process (RUHÍ et al., 2016). In addition, the accumulation of active molecules, such as antibiotics, can affect bacterial resistance (AUBERTHEAU et al., 2017; BASTOS et al., 2018). Many laboratory experiments are conducted with biofilms, but field level experiments are still scarce.

In biodegradation research, Writer et al. (2011) show that steroid hormones and alkylphenols can divide very quickly in the organic matter of biofilms. They can also contribute to the oxidation of organic material, changing the chemical profiles in the system. Organic compounds can also be retained in the biofilm community and undergo transformation processes (EDWARDS; KJELLERUP, 2013) and factors such as temperature, light, competing carbon substrates and oxygen concentrations can influence the ability of compounds to retain in biofilms.

More recently, epilithic biofilms have been used as a matrix for the adsorption of contaminant molecules. Laurent (2013) found concentrations of pharmaceutical products in biofilms about 1000 times higher than in river water. Huerta et al. (2016) demonstrated that epileptic biofilms can accumulate pharmaceutical products. The authors developed an analytical methodology for simultaneous analysis of pharmaceuticals and Endocrine Disruptor Compounds (EDs) in epileptic biofilms and assessed the persistence, distribution and bioaccumulation of these contaminants in biofilms affected by wastewater treatment plants. As a result of the study conducted by the Spanish research team, seven pharmaceutical products (diclofenac, diltiazem, gemfibrozil, verapamil, norverapamil, OH-CBZ and venlafaxine) and five EDs (ethyl paraben, methylparaben, propyl paraben) were detected in concentrations higher than 100 ng g^{-1}.

Aubertheau et al. (2017) found that wastewater treatment plants are one of the main sources of pharmaceutical waste in surface waters and that epilithic biofilms constitute an adequate adsorption matrix to assess the impacts of this activity on the surrounding aquatic environment. In all biofilms, the authors verified the presence of several compounds (5 to 11 of the 12 studied), which are among the most frequent pharmaceutical products occurring in

natural waters (carbamazepine, diclofenac, propranolol, sulfamethoxazole). The presence of several antibiotics at concentrations above 276 ng g^{-1} was also detected, which provides favorable conditions for the maintenance of antibiotic resistance.

In Brazil, the assessment of water contamination using epileptic biofilms as an adsorption matrix was performed by Bastos et al. (2018), Fernandes et al. (2019), Rheinheimer et al. (2020) and Vargas et al. (2021), in Rio Grande do Sul biofilms sample, demonstrating that biofilms have a high potential to adsorb agrochemicals, human and veterinary drugs in aquatic ecosystems. All authors confirmed the potential of using epilithic biofilms as a source of information for environmental pollution studies. The results showed that the concentrations of pesticides and pharmaceuticals varied according to the source of pollution (agricultural and urban) and the intensity of land use (more or less anthropized), and the most recent published work evaluated the ability of biofilms in WWTPs as accumulators and purifiers of pharmaceutical compounds.

Published works on natural biofilms are still recent (Table 4). Biofilms have been and still are much studied in the laboratory because the methodology for both sampling and extraction is still a challenge. We can see that the levels of contamination are variable in the countries presented, and this comparison is difficult because we know that several environmental factors influence the accumulation of compounds in biofilms. As such, biofilms can serve as an indicator of the state of environmental degradation, but it is worth remembering that it is not an indicator that measures aquatic pollution like passive samplers, for example the POCIS. Biofilms as well as other living organisms are indicators and not quantitative measures of pollution.

Literature Review – Table 4– Studies that evaluated the concentration of pharmaceuticals in natural epilithic biofilms.

Compound	Concentration (min - max)	Local	Reference
Triclosan	18 – 76.5	Spain	Huerta et al. (2016)
1-OH-IBU	n.d		
Ibuprofen	n.d		
Diclofenac	23.5 – 103.0		
Clarithromycin	n.d		
Sulfamethoxazole	n.d		
Diltiazem,	4.7 – 11.8		
Norverapamil	4.20 – 20.9		
Verapamil	11.1 – 21.7		
Furosemide	n.d		
Hydrochlorothiazide	n.d		
Bezafibrate	n.d		
Gemfibrozil	4.0 – 10.3		
Pravastatin	n.d		
Carbamazepine	n.d		
OH-CBZ	<LOQ – 1.8		
Citalopram	n.d		
Venlafaxine	6.5 – 43.7		
Carbamazepine	2.1 – 583.5	France	Aubertheau et al. (2017)
Levofloxacin + Ofloxacin	10.0 – 276.0		
Propranolol	20.6 – 965.0		
Ketoprofen	1.5 – 31.3		
Trimethoprim	1.2 – 10.4		
Diclofenac	4.0 – 190.3		
Sulfamethoxazole	1.3 – 20.1		
Bezafibrate	1.4 – 34.5		
Atenolol	2.8 – 54.2		
10,11-epoxy-carbamazepine	1.6 – 5.3		
Metronidazole	<LOQ		
Iohexol	1.9 – 25.6		
Carbamazepin	<LOD – 3.6	Brazil	Bastos et al. (2018)
Sucralose	< LOD – 48.2		
Doxorubicin	<LOQ – 3.1	Brazil	Vargas et al. (2021)
Epirubicin	<LOQ – 15.5		
Daunorubicin	<LOQ – 9.1		
Irinotecan	<LOQ – 15.6		
Midecamycin	<LOQ – 45.7		
Spiramycin	<LOQ – 19.6		
Josamycin	<LOQ – 7.8		
Azithromycin	2.0 – 20.0		
Sulfamethoxazole	<LOQ – 40.5		
Clarithromycin	1.2 – 17.1	Argentina	Valdés et al. (2021)
Erythromycin	<LOQ – 58.6		
Roxythromycin	<LOQ – 2.8		
Diclofenac	<LOQ – 5.3		
Carbamazepine	<LOQ – 12.4		
Norfloxacin	n.d. – 364.0		
Ofloxacin	n.d – 349.0		
Coprofloxacin	n.d. – 706.0		
Cinoxacin	n.d. –154.0		
Cephalexin	n.d. – 0.3		
Azitrhomicin	n.d. – 336.0		
Clarithromycin	n.d. – 334.0		
Doxycycline	n.d. – 34.0		
Clindamycin	n.d. – 0.02		

Sulfathiazole	n.d. – 3.8
Trimethoprim	n.d. – 7.9
Metronidazole	n.d. – 0.05

Concentrations in ng g^{-1}. N.d = not detected, LOQ = limit of quantitation, LOD= limit of detection.

2.10 ENVIRONMENTAL RISK

One way to assess the environmental risk that pollutants pose to aquatic microbiota is to use real-world bases to define the health effects of exposure of individuals to hazardous materials. A widely used tool for ecological risk assessment is the determination of the Risk Quotient (RQ) defined as: the product of the Predicted Environmental Concentrations (PEC), or Measured Environmental Concentrations (MEC), by the Predicted No-Effect Concentrations (PNEC) for a given organism or trophic levels. In Brazil researchers have employed the determination of the Risk Quotient (RQ) in ecological risk assessment to emerging contaminants (ALBUQUERQUE et al., 2016, SODRE et al., 2018). These authors point out the categories of emerging contaminants with the highest QR values being: hormones/endocrine disruptors (17α-ethinyl estradiol, 17β-estradiol, and estrone), pharmaceuticals (caffeine), antibiotics (trimethoprim and sulfamethoxazole), personal care and hygiene products (triclosan), insecticides (ethion and fipronil), and herbicides (atrazine and endosulfan). The occurrence of these compounds with QR values >1 indicate that their concentrations in the environment exceed the ecological safety threshold (PNEC), posing a threat to aquatic species (GONZALEZ et al., 2012).

Waterways, especially lotic ones, are responsible for connecting diverse biomes, crossing ecosystems with distinct biotic characteristics and transporting matter and energy along their course. In this transport, many emerging contaminants can also be dispersed without suffering degradation and/or change in concentration because they are persistent and can be transported over long distances from their generating source (Gavrilescu et al., 2015).

Attributing greater concern to the presence of emerging contaminants in water bodies, Brazil has 3,148 species of freshwater Neotropical fish, with 1,761 endemic species, representing one of the largest freshwater fish faunas in the world. Of these, 312 species are assessed as threatened (101 critically endangered, 112 endangered, and 99 vulnerable). Other groups of aquatic organisms such as amphibians (41 species) and invertebrates (37 species) are also threatened (SPADOTTO, 2009). Pollution, along of habitat loss, is indicated as the main causes of threat to the survival of species (ICMBio/MMA 2018). The adoption of environmental risk assessment may represent a methodological advance in the consideration of possible environmental problems associated with "stressors".

The assessment of environmental risks, is particularly important in countries where the lack of data and resources may limit the adoption of environmental risk assessment and management

3 REFERENCES

ALBUQUERQUE A. F. et al. Pesticides in Brazilian freshwaters: a critical review. **Environ. Sci.: Processes Impacts**, 18 (7): 779–787, 2016. DOI: 10.1039/c6em00268d.

AMÉRICO, J. et al. Fármacos em Uma Estação de Tratamento de Esgoto na Região Centro-Oeste do Brasil e os Riscos aos Recursos Hídricos. **Revista Brasileira de Recursos Hídricos**, v. 17, n. 3, p. 61–67, 2012.

AMÉRICO, J. H. P. et al. Monitoreo del anti-inflamatorio piroxicam y del anti-hipertensivo atenolol en un arroyo urbano en três lagoas, Brasil. **Interciencia (Caracas)**. v. 40, p. 8, 2015.

AMÉRICO-PINHEIRO, J. H. P. et al. Ocorrência de diclofenaco e naproxeno em água superficial no município de Três Lagoas (MS) e a influência da temperatura da água na detecção desses anti-inflamatórios. **Engenharia Sanitaria e Ambiental**, v. 22, n. 3, p. 429–435, maio 2017.

ANA. **Agência Nacional de Águas — Agência Nacional de Águas**. Capa. Disponível em: <https://www.ana.gov.br>. Acesso em: 29 jul. 2019.

ANVISA. **Antibióticos: uso indiscriminado deve ser controlado - Notícias - Anvisa**. Disponível em: <http://portal.anvisa.gov.br/noticias >. Acesso em: 29 jul. 2019.

ARSAND, D. R.; KÜMMERER, K.; MARTINS, A. F. Removal of dexamethasone from aqueous solution and hospital wastewater by electrocoagulation. **Science of The Total Environment**, v. 443, p. 351–357, jan. 2013.

ARSAND, J. B. et al. Wide-Scope Determination of Pharmaceuticals and Pesticides in Water Samples: Qualitative and Confirmatory Screening Method Using LC-qTOF-MS. **Water, Air, & Soil Pollution**, v. 229, n. 12, dez. 2018.

ATLAS SEWERAGE, 2017, A. Atlas Sewerage, 2017. p. 1, [s.d.].

AUBERTHEAU, E. et al. Impact of wastewater treatment plant discharge on the contamination of river biofilms by pharmaceuticals and antibiotic resistance. **Science of The Total Environment**, v. 579, p. 1387–1398, fev. 2017.

AUVINEN, H. et al. Removal of pharmaceuticals by a pilot aerated sub-surface flow constructed wetland treating municipal and hospital wastewater. **Ecological Engineering**, v. 100, p. 157–164, mar. 2017.

AZUMA, T. et al. Detection of pharmaceuticals and phytochemicals together with their metabolites in hospital effluents in Japan, and their contribution to sewage treatment plant influents. **Science of The Total Environment**, v. 548–549, p. 189–197, 1 abr. 2016.

BATTIN, T. J. et al. The ecology and biogeochemistry of stream biofilms. **Nature Reviews Microbiology**, v. 14, n. 4, p. 251–263, abr. 2016.

BERETTA, M. et al. Occurrence of pharmaceutical and personal care products (PPCPs) in marine sediments in the Todos os Santos Bay and the north coast of Salvador, Bahia, Brazil. **Journal of Soils and Sediments**, v. 14, n. 7, p. 1278–1286, 1 jul. 2014.

BERLIOZ-BARBIER, A. et al. Multi-residue analysis of emerging pollutants in benthic invertebrates by modified micro-quick-easy-cheap-efficient-rugged-safe extraction and nanoliquid chromatography–nanospray–tandem mass spectrometry analysis. **Journal of Chromatography A**, v. 1367, p. 16–32, 7 nov. 2014.

BÖGER, B. et al. Determination of carbamazepine and diazepam by SPE-HPLC-DAD in Belém River water, Curitiba-PR/Brazil. **Ambiente e Agua - An Interdisciplinary Journal of Applied Science**, v. 13, n. 2, p. 1, 29 mar. 2018.

BOXALL, A. B. A. The environmental side effects of medication. **EMBO Reports**, v. 5, n. 12, p. 1110–1116, dez. 2004.

BRANDT, E. M. F. Avaliação da remoção de fármacos e desreguladores endócrinos em sistemas simplificados de tratamento de esgoto (reatores uasb seguidos de pós-tratamento). p. 143, 2012. Dissertação (Mestrado em Saneamento, Meio Ambiente e Recursos Hídricos) - **Universidade Federal de Minas Gerais**, Belo Horizonte (MG), Brasil

BRASIL. Constituição da República Federativa do Brasil. p. 498, 1988.

BRAUND, R.; PEAKE, B. M.; SHIEFFELBIEN, L. Disposal practices for unused medications in New Zealand. **Environment International**, v. 35, n. 6, p. 952–955, 1 ago. 2009.

BRAZIL; Ministério da saúde; centro brasileiro de análise e planejamento. **Pesquisa nacional de demografia e saúde da criança e da mulher: PNDS 2006, dimensões do processo reprodutivo e da saúde da criança.** Brasília, DF: Ministério da Saúde : Centro Brasileiro de Análise e Planejamento, 2009.

BRENNER, C. G. B. et al. Determination of Sulfamethoxazole and Trimethoprim and Their Metabolites in Hospital Effluent. **CLEAN - Soil, Air, Water**, v. 39, n. 1, p. 28–34, jan. 2011.

CALDAS, S. et al. Occurrence of Pesticides and PPCPs in Surface and Drinking Water in Southern Brazil: Data on 4-Year Monitoring. **Journal of the Brazilian Chemical Society**, 2018.

CALDAS, S. S. et al. Determination of pharmaceuticals, personal care products, and pesticides in surface and treated waters: method development and survey. **Environmental Science and Pollution Research**, v. 20, n. 8, p. 5855–5863, ago. 2013.

CAMOTTI BASTOS, M. et al. Presence of Anthropogenic Markers in Water: A Case Study of the Guaporé River Watershed, Brazil. **CLEAN - Soil, Air, Water**, v. 46, n. 3, p. 1700019, mar. 2018.

CAMPANHA, M. B. et al. A 3-year study on occurrence of emerging contaminants in an urban stream of São Paulo State of Southeast Brazil. **Environmental Science and Pollution Research**, v. 22, n. 10, p. 7936–7947, maio 2015.

CARREL, M. et al. Biofilms in 3D porous media: Delineating the influence of the pore network geometry, flow and mass transfer on biofilm development. **Water Research**, v. 134, p. 280–291, maio 2018.
CASTIGLIONI, S. et al. Novel homologs of the multiple resistance regulator marA in antibiotic-contaminated environments. **Water Research**, v. 42, n. 16, p. 4271–4280, out. 2008.

CHARUAUD, L. et al. Veterinary pharmaceutical residues in water resources and tap water in an intensive husbandry area in France. **Science of The Total Environment**, v. 664, p. 605–615, 10 maio 2019.

COLAÇO, R.; PERALTA-ZAMORA, P. G.; GOMES, E. C. Poluição por resíduos contendo compostos farmaceuticamente ativos: aspectos ambientais, geração a partir dos esgotos domésticos e a situação do Brasil. p. 10, 2014.

CONAMA 430, M. P. F.-. **Resolução CONAMA nº 430, de 13 de maio de 2011 — 4ª Câmara - Meio Ambiente e Patrimônio Cultural**. Arquivo. Disponível em: <http://www.mpf.mp.br/atuacao-tematica/ccr4/dados-da-atuacao/projetos/qualidade-da-agua/legislacao/resolucoes/resolucao-conama-no-430-de-13-de-maio-de-2011>. Acesso em: 29 jul. 2019.

CORSARO, D. et al. Biodiversity of amoebae and amoeba-associated bacteria in water treatment plants. **International Journal of Hygiene and Environmental Health**, First Ph.D. student seminar on “Water & Health” organized within the “Cannes Water Symposium”, Cannes, July 2009. v. 213, n. 3, p. 158–166, 1 jun. 2010.

COYTE, K. Z. et al. Microbial competition in porous environments can select against rapid biofilm growth. **Proceedings of the National Academy of Sciences**, v. 114, n. 2, p. E161–E170, 10 jan. 2017.

DAUGHTON, C. G. Drugs and the Environment: Stewardship & Sustainability. p. 198, 2010.

DE ALMEIDA, C. A. A. et al. Determination of anti-anxiety and anti-epileptic drugs in hospital effluent and a preliminary risk assessment. **Chemosphere**, v. 93, n. 10, p. 2349–2355, nov. 2013.

DE ALMEIDA, C. A. A. et al. Determination of the psychoactive drugs carbamazepine and diazepam in hospital effluent and identification of their metabolites. **Environmental Science and Pollution Research**, v. 22, n. 21, p. 17192–17201, 1 nov. 2015.

DE MELO, M. G. et al. Sewage contamination of Amazon streams crossing Manaus (Brazil) by sterol biomarkers. **Environmental Pollution**, v. 244, p. 818–826, jan. 2019.

DERLON, N. et al. The composition and compression of biofilms developed on ultrafiltration membranes determine hydraulic biofilm resistance. **Water Research**, v. 102, p. 63–72, out. 2016.

DESMOND, P.; MORGENROTH, E.; DERLON, N. Physical structure determines compression of membrane biofilms during Gravity Driven Membrane (GDM) ultrafiltration. **Water Research**, v. 143, p. 539–549, out. 2018.

DRESZER, C. et al. Impact of biofilm accumulation on transmembrane and feed channel pressure drop: Effects of crossflow velocity, feed spacer and biodegradable nutrient. **Water Research**, v. 50, p. 200–211, mar. 2014.

EDWARDS, S. J.; KJELLERUP, B. V. Applications of biofilms in bioremediation and biotransformation of persistent organic pollutants, pharmaceuticals/personal care products, and heavy metals. **Applied Microbiology and Biotechnology**, v. 97, n. 23, p. 9909–9921, dez. 2013.

FERNANDES, G. et al. Indiscriminate use of glyphosate impregnates river epilithic biofilms in southern Brazil. **Science of The Total Environment**, v. 651, p. 1377–1387, fev. 2019.

FINDLAY, S. Stream microbial ecology. **Journal of the North American Benthological Society**, v. 29, n. 1, p. 170–181, 1 mar. 2010.

FLEMMING, H.-C.; WINGENDER, J. The biofilm matrix. **Nature Reviews Microbiology**, v. 8, n. 9, p. 623–633, set. 2010.

FRANQUET-GRIELL, H. et al. Determination of cytostatic drugs in Besòs River (NE Spain) and comparison with predicted environmental concentrations. **Environmental Science and Pollution Research**, v. 24, n. 7, p. 6492–6503, mar. 2017.

FREITAS, R. X. A. et al. Characterization of the Primary Sludge from Pharmaceutical Industry Effluents and Final Disposition. **Processes**, v. 7, n. 4, p. 231, 24 abr. 2019.

FROEHNER, S. et al. Removal Capacity of Caffeine, Hormones, and Bisphenol by Aerobic and Anaerobic Sewage Treatment. **Water, Air, & Soil Pollution**, v. 216, n. 1–4, p. 463–471, mar. 2011.

FUCHS, S.; HARITOPOULOU, T.; WILHELMI, M. Biofilms in freshwater ecosystems and their use as a pollutant monitor. **Water Science and Technology**, Water Quality International ’96 Part 4. v. 34, n. 7, p. 137–140, 1 jan. 1996.

GHESTI PIVETTA, G.; DO CARMO CAUDURO GASTALDINI, M. Presence of emerging contaminants in urban water bodies in southern Brazil. **Journal of Water and Health**, v. 17, n. 2, p. 329–337, abr. 2019.

GÓMEZ-CANELA, C. et al. Occurrence of cytostatic compounds in hospital effluents and wastewaters, determined by liquid chromatography coupled to high-resolution mass spectrometry. **Analytical and Bioanalytical Chemistry**, v. 406, n. 16, p. 3801–3814, jun. 2014.

GONÇALVES, P. B. D. HOSPITAL UNIVERSITÁRIO DE SANTA MARIA. p. 210, 2014.

GONZÁLEZ S. et al. Presence and biological effects of emerging contaminants in Llobregat River basin: a review. **Environmental Pollution**, 161: 83–92, 2012. DOI: 10.1016/j.envpol.2011.10.002.

HALLING-SØRENSEN, B. et al. Occurrence, fate and effects of pharmaceutical substances in the environment- A review. **Chemosphere**, v. 36, n. 2, p. 357–393, 1 jan. 1998.

HEBERER, T. Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data. **Toxicology Letters**, v. 131, n. 1, p. 5–17, 10 maio 2002.

HENRIQUES, D. M. et al. Nonylphenol polyethoxylate in hospital wastewater: A study of the subproducts of electrocoagulation. **Journal of Environmental Science and Health, Part A**, v. 47, n. 3, p. 497–505, fev. 2012.

HORN, H.; REIFF, H.; MORGENROTH, E. Simulation of growth and detachment in biofilm systems under defined hydrodynamic conditions. **Biotechnology and Bioengineering**, v. 81, n. 5, p. 607–617, 5 mar. 2003.

HUERTA, B. et al. Determination of a broad spectrum of pharmaceuticals and endocrine disruptors in biofilm from a waste water treatment plant-impacted river. **Science of The Total Environment**, v. 540, p. 241–249, jan. 2016.

IBGE, 2018. **IBGE | Portal do IBGE | IBGE**. Disponível em: <https://www.ibge.gov.br/>. Acesso em: 29 jul. 2019.

ICMBio/MMA. 2018. Livro Vermelho da Fauna Brasileira Ameaçada de Extinção. / -- 1ª ed. -- Brasília, DF. Disponível em: https://www.icmbio.gov.br/portal/images/stories/comunicacao/publicacoes/publicacoes-diversas/livro_vermelho_2018_vol1.pdf

IDE, A. H. et al. Occurrence of Pharmaceutical Products, Female Sex Hormones and Caffeine in a Subtropical Region in Brazil: Water. **CLEAN - Soil, Air, Water**, v. 45, n. 9, p. 1700334, set. 2017.

IMS HEALTH. **Institute**. Disponível em: <https://www.iqvia.com/institute>. Acesso em: 29 jul. 2019.

JENICEK, P. et al. Advantages of anaerobic digestion of sludge in microaerobic conditions. **Water Science and Technology**, v. 62, n. 2, p. 427–434, 1 jul. 2010.

KAYOMBO, S. et al. Diurnal cycles of variation of physical–chemical parameters in waste stabilization ponds. **Ecological Engineering**, v. 18, n. 3, p. 287–291, 1 jan. 2002.

KERMIA, A. E. B.; FOUIAL-DJEBBAR, D.; TRARI, M. Occurrence, fate and removal efficiencies of pharmaceuticals in wastewater treatment plants (WWTPs) discharging in the coastal environment of Algiers. **Comptes Rendus Chimie**, v. 19, n. 8, p. 963–970, ago. 2016.

KÜMMERER, K. Pharmaceuticals in the Environment — Scope of the Book and Introduction. In: KÜMMERER, K. (Ed.). . **Pharmaceuticals in the Environment: Sources, Fate, Effects and Risks**. Berlin, Heidelberg: Springer Berlin Heidelberg, 2004. p. 3–11.

KUSTER, M. et al. Analysis of phytoestrogens, progestogens and estrogens in environmental waters from Rio de Janeiro (Brazil). **Environment International**, v. 35, n. 7, p. 997–1003, 1 out. 2009.

LASPIDOU, C. S. et al. Material modeling of biofilm mechanical properties. **Mathematical Biosciences**, v. 251, p. 11–15, maio 2014.

LASPIDOU, C. S.; ARAVAS, N. Variation in the mechanical properties of a porous multi-phase biofilm under compression due to void closure. **Water Science and Technology**, v. 55, n. 8–9, p. 447–453, abr. 2007.

LAURENT, É. Évaluation de l'état de contamination des bassins versants par les résidus de médicaments : utilisation des biofilms épilithiques comme marqueur d'imprégnation du milieu. p. 224, 2013.

LEAR, G.; LEWIS, G. D. Impact of catchment land use on bacterial communities within stream biofilms. **Ecological Indicators**, v. 9, n. 5, p. 848–855, 1 set. 2009.

LI, W. et al. Occurrence of antibiotics in water, sediments, aquatic plants, and animals from Baiyangdian Lake in North China. **Chemosphere**, v. 89, n. 11, p. 1307–1315, nov. 2012.

LOCATELLI, M. A. F.; SODRÉ, F. F.; JARDIM, W. F. Determination of Antibiotics in Brazilian Surface Waters Using Liquid Chromatography–Electrospray Tandem Mass Spectrometry. **Archives of Environmental Contamination and Toxicology**, v. 60, n. 3, p. 385–393, abr. 2011.

LOFRANO, G. et al. Municipal wastewater spiramycin removal by conventional treatments and heterogeneous photocatalysis. **Science of The Total Environment**, v. 624, p. 461–469, maio 2018.

LOOSDRECHTER, M. C. M. et al. Biofilm structures. **BIOFILM STRUCTURES**, v. 32, n. 8, p. 9, 1995.

LOPES, L. G. et al. Estrogênios em águas naturais e tratadas da região de Jaboticabal - São Paulo. **Química Nova**, v. 33, n. 3, p. 639–643, 2010.

MACHADO, K. C. et al. A preliminary nationwide survey of the presence of emerging contaminants in drinking and source waters in Brazil. **Science of The Total Environment**, v. 572, p. 138–146, dez. 2016.

MAHNIK, S. N. et al. Fate of 5-fluorouracil, doxorubicin, epirubicin, and daunorubicin in hospital wastewater and their elimination by activated sludge and treatment in a membrane-bio-reactor system. **Chemosphere**, v. 66, n. 1, p. 30–37, jan. 2007.

MARTINS, A. F. et al. Photooxidation as Advanced Oxidation Treatment of Hospital Effluents. In: KÜMMERER, K. (Ed.). . **Pharmaceuticals in the Environment**. Berlin, Heidelberg: Springer Berlin Heidelberg, 2008a. p. 455–466.

MARTINS, A. F. et al. Concentration of Ciprofloxacin in Brazilian Hospital Effluent and Preliminary Risk Assessment: A Case Study. **CLEAN – Soil, Air, Water**, v. 36, n. 3, p. 264–269, mar. 2008b.

MARTINS, A. F. et al. A Study of Photocatalytic Processes Involving the Degradation of the Organic Load and Amoxicillin in Hospital Wastewater. **CLEAN - Soil, Air, Water**, v. 37, n. 4–5, p. 365–371, abr. 2009.

MARTINS, A. F. et al. Occurrence of the Antimicrobials Sulfamethoxazole and Trimethoprim in Hospital Effluent and Study of Their Degradation Products after Electrocoagulation. **CLEAN - Soil, Air, Water**, v. 39, n. 1, p. 21–27, jan. 2011.

MARTINS, A. F. et al. Determination of statin drugs in hospital effluent with dispersive liquid–liquid microextraction and quantification by liquid chromatography. **Journal of Environmental Science and Health, Part A**, v. 52, n. 10, p. 939–945, 24 ago. 2017.

MATONGO, S. et al. Pharmaceutical residues in water and sediment of Msunduzi River, KwaZulu-Natal, South Africa. **Chemosphere**, v. 134, p. 133–140, 1 set. 2015.

MINETTO, L. et al. Quantification of Diclofenac in Hospital Effluent and Identification of Metabolites and Degradation Products. **CLEAN - Soil, Air, Water**, v. 40, n. 9, p. 950–957, set. 2012.

MIZUKAWA, A. et al. Caffeine as a chemical tracer for contamination of urban rivers. **RBRH**, v. 24, n. 0, 2019.

MOKH, S. et al. Innovative SPE-LC-MS/MS technique for the assessment of 63 pharmaceuticals and the detection of antibiotic-resistant-bacteria: A case study natural water sources in Lebanon. **Science of The Total Environment**, v. 609, p. 830–841, dez. 2017.

MONTAGNER, C. et al. Ten Years-Snapshot of the Occurrence of Emerging Contaminants in Drinking, Surface and Ground Waters and Wastewaters from São Paulo State, Brazil. **Journal of the Brazilian Chemical Society**, 2018.

MONTEIRO, M. et al. Development and Validation of Liquid Chromatography-Tandem Mass Spectrometry Methods for Determination of Beta-Lactams, Macrolides, Fluoroquinolones, Sulfonamides and Tetracyclines in Surface and Drinking Water from Rio de Janeiro, Brazil. **Journal of the Brazilian Chemical Society**, 2017.

MOREIRA, D. S. et al. Occurrence of endocrine disrupting compounds in water sources of Belo Horizonte Metropolitan Area, Brazil. **Environmental Technology**, v. 30, n. 10, p. 1041–1049, set. 2009.

NADELL, C. D. et al. Flow environment and matrix structure interact to determine spatial competition in Pseudomonas aeruginosa biofilms. **eLife**, v. 6, p. e21855, 13 jan. 2017.

PAPAGEORGIOU, M.; KOSMA, C.; LAMBROPOULOU, D. Seasonal occurrence, removal, mass loading and environmental risk assessment of 55 pharmaceuticals and personal care products in a municipal wastewater treatment plant in Central Greece. **Science of The Total Environment**, v. 543, p. 547–569, fev. 2016.

PEREIRA, C. D. S. et al. Occurrence of pharmaceuticals and cocaine in a Brazilian coastal zone. **Science of The Total Environment**, v. 548–549, p. 148–154, abr. 2016.

PESSOA, G. P. et al. Occurrence and removal of estrogens in Brazilian wastewater treatment plants. **Science of The Total Environment**, v. 490, p. 288–295, ago. 2014.

PETTY J, HUCKINS J, ALVAREZ D. Device for sequestration and concentration of polar organic chemicals from water. U.S. Patent 6,478,961. **U.S. Patent and Trademark Office**, Washington, DC. 2002.

PETROVIĆ, M.; GONZALEZ, S.; BARCELÓ, D. Analysis and removal of emerging contaminants in wastewater and drinking water. **TrAC Trends in Analytical Chemistry**, v. 22, n. 10, p. 685–696, 1 nov. 2003.

PUSCEDDU, F. H. et al. Estrogen levels in surface sediments from a multi-impacted Brazilian estuarine system. **Marine Pollution Bulletin**, v. 142, p. 576–580, maio 2019.

RAMIREZ, A. J. et al. Occurrence of pharmaceuticals and personal care products in fish: Results of a national pilot study in the united states. **Environmental Toxicology and Chemistry**, v. 28, n. 12, p. 2587–2597, 2009.

RANZINGER, F. et al. Direct surface visualization of biofilms with high spin coordination clusters using Magnetic Resonance Imaging. **Acta Biomaterialia**, v. 31, p. 167–177, fev. 2016.

REIS, E. O. et al. Occurrence, removal and seasonal variation of pharmaceuticals in Brasilian drinking water treatment plants. **Environmental Pollution**, v. 250, p. 773–781, 1 jul. 2019.

ROMANÍ, A. M. et al. Relevance of Polymeric Matrix Enzymes During Biofilm Formation. **Microbial Ecology**, v. 56, n. 3, p. 427–436, 1 out. 2008.

RUHÍ, A. et al. Bioaccumulation and trophic magnification of pharmaceuticals and endocrine disruptors in a Mediterranean river food web. **Science of The Total Environment**, 5th Special Issue SCARCE: River Conservation under Multiple stressors: Integration of ecological status, pollution and hydrological variability. v. 540, p. 250–259, 1 jan. 2016.

SABATER, S. et al. Monitoring the effect of chemicals on biological communities. The biofilm as an interface. **Analytical and Bioanalytical Chemistry**, v. 387, n. 4, p. 1425–1434, 1 fev. 2007.

SABATER, S. Microbial Ecotoxicology: Looking to the Future. In: CRAVO-LAUREAU, C. et al. (Eds.). . **Microbial Ecotoxicology**. Cham: Springer International Publishing, 2017. p. 339–352.

SANTOS, A. L. S. DOS et al. What are the advantages of living in a community? A microbial biofilm perspective! **Memórias do Instituto Oswaldo Cruz**, v. 113, n. 9, 26 jul. 2018.

SCHWEITZER, S. O.; LU, Z. J. **Pharmaceutical Economics and Policy: Perspectives, Promises, and Problems**. [s.l.] Oxford University Press, 2018.

SIE, 2019. **UFSM em Números - UFSM em Números**. Disponível em: <https://portal.ufsm.br/ufsm-em-numeros/publico/index.html>. Acesso em: 29 jul. 2019.

SODRÉ, F. F. et al. Ocorrência de Interferentes Endócrinos e Produtos Farmacêuticos em Águas Superficiais da Região de Campinas (SP, Brasil). **Journal of the Brazilian Society of Ecotoxicology**, v. 2, n. 2, p. 187–196, 2007.

SODRÉ, F. F.; CAVALCANTI, C. M. P. Method Development for Assessing Carbamazepine, Caffeine, and Atrazine in Water Sources from the Brazilian Federal District Using UPLC-QTOF/MS. **International Journal of Analytical Chemistry**, v. 2018, p. 1–10, 11 nov. 2018.

SOUZA, D. M.; REICHERT, J. F.; MARTINS, A. F. A simultaneous determination of anti-cancer drugs in hospital effluent by DLLME HPLC-FLD, together with a risk assessment. **Chemosphere**, v. 201, p. 178–188, jun. 2018.

SPADOTTO, C.A. Avaliação de riscos ambientais do uso de defensivos agrícolas para a qualidade da água. **Horticultura Brasileira** v. 27, n. 2, p. S4060-S4070, ago. 2009.

STUMPF, M. et al. Polar drug residues in sewage and natural waters in the state of Rio de Janeiro, Brazil. **Science of The Total Environment**, v. 225, n. 1–2, p. 135–141, jan. 1999.

TERNES, T. A. et al. Behavior and occurrence of estrogens in municipal sewage treatment plants--I. Investigations in Germany, Canada and Brazil. **The Science of the Total Environment**, v. 225, n. 1–2, p. 81–90, 12 jan. 1999.

TERZIĆ, S. et al. Occurrence and fate of emerging wastewater contaminants in Western Balkan Region. **Science of The Total Environment**, v. 399, n. 1–3, p. 66–77, jul. 2008.

TEUNIS, P. F. M. et al. Characterization of drinking water treatment for virus risk assessment. **Water Research**, v. 43, n. 2, p. 395–404, 1 fev. 2009.

TORRES, N. H. et al. FÁRMACOS NO AMBIENTE – REVISÃO. **Revista de Estudos Ambientais**, v. 14, n. 4, p. 67–75, 17 dez. 2013.

HUSM, 2016. **Hospital Universitário de Santa Maria - HUSM - EBSERH**. Disponível em: <http://www2.ebserh.gov.br/web/husm-ufsm>. Acesso em: 29 jul. 2019.

UN. Disponível em: <https://nacoesunidas.org/acao/agua/>. Acesso em: 29 jul. 2019.

VALDÉS, M. E. et al. Distribution of antibiotics in water, sediments and biofilm in an urban river (Córdoba, Argentina, LA). **Environmental Pollution**, v. 269, p. 116133, jan. 2021.

VALLADARES LINARES, R. et al. Compaction and relaxation of biofilms. **Desalination and Water Treatment**, v. 57, n. 28, p. 12902–12914, 14 jun. 2016.

VARGAS, J. P. R. et al. Pharmaceutical compound removal efficiency by a small constructed wetland located in south Brazil. **Environmental Science and Pollution Research**, 16 fev. 2021.

VASCONCELOS, T. G. et al. Ciprofloxacin in hospital effluent: Degradation by ozone and photoprocesses. **Journal of Hazardous Materials**, v. 169, n. 1–3, p. 1154–1158, set. 2009.

VERAS, T. B. et al. Analysis of the presence of anti-inflammatories drugs in surface water: A case study in Beberibe river - PE, Brazil. **Chemosphere**, v. 222, p. 961–969, 1 maio 2019.

WAGNER, M. et al. Investigation of the mesoscale structure and volumetric features of biofilms using optical coherence tomography. **Biotechnology and Bioengineering**, v. 107, n. 5, p. 844–853, 1 dez. 2010.

WANG, J.; WANG, S. Removal of pharmaceuticals and personal care products (PPCPs) from wastewater: A review. **Journal of Environmental Management**, v. 182, p. 620–640, nov. 2016.

WHO. **World Health Organization - Report on Surveillance of antibiotic Consumption.** Disponível em: <https://www.who.int/medicines/areas/rational_use/who-amr-amc-report-20181109.pdf?ua=1>. Acesso em: 19 set. 2020.

WHO/UNICEF. **Progress on sanitation and drinking water.** World Health Organization ed. Acesso em: 19 set. 2020.

WILDE, M. L. et al. Oxidation–coagulation of β-blockers by K2FeVIO4 in hospital wastewater: Assessment of degradation products and biodegradability. **Science of The Total Environment**, v. 452–453, p. 137–147, maio 2013.

WILDE, M. L.; KÜMMERER, K.; MARTINS, A. F. Multivariate optimization of analytical methodology and a first attempt to an environmental risk assessment of β-blockers in hospital wastewater. **Journal of the Brazilian Chemical Society**, v. 23, n. 9, p. 1732–1740, set. 2012.

WRITER, J. H. et al. Biodegradation and Attenuation of Steroidal Hormones and Alkylphenols by Stream Biofilms and Sediments. **Environmental Science & Technology**, v. 45, n. 10, p. 4370–4376, 15 maio 2011.

YIN, J. et al. A Preliminary Study on the Occurrence of Cytostatic Drugs in Hospital Effluents in Beijing, China. **Bulletin of Environmental Contamination and Toxicology**, v. 84, n. 1, p. 39–45, jan. 2010.

ZACHEUS, O. M. et al. Soft deposits, the key site for microbial growth in drinking water distribution networks. **Water Research**, v. 35, n. 7, p. 1757–1765, 1 maio 2001.

ZHANG, T. C.; BISHOP, P. L. Density, porosity, and pore structure of biofilms. **Water Research**, v. 28, n. 11, p. 2267–2277, nov. 1994.

ZUCCATO, E. et al. Presence of therapeutic drugs in the environment. **The Lancet**, v. 355, n. 9217, p. 1789–1790, maio 2000.

4 HYPOTESES

More than half of the Brazilian population does not have access to sewage collection and treatment, thus contributing to great pollution of rivers. While collection, treatment and disposal programs are implemented late, new polluting species are evidenced, most of which cannot be efficiently removed by conventional treatment systems.

Within this context, the presence of organic micropollutants, which, even in low concentrations, can cause deleterious effects, causing innumerable effects on aquatic life and even on humans. The increasing concentration of antibiotics in the aquatic environment can lead to the development of bacterial resistance and even disrupt the growth and function of aquatic organisms, several effects that are not known.

In order to study the contamination of water resources, new adsorption matrices and/or new collections techniques have been sought. Within this perspective, in recent years, epilithic biofilms have been studied as efficient environmental bioindicators, because they present functional clusters and polar and apolar sites that allow the accumulation and capture of different molecules, characteristics that make the epilithic biofilms promising matrices s for monitoring the occurrence of pharmaceuticals in water. However, there are many gaps in knowledge to be filled within this theme, for example, the capacity of self-depuration and resilience of epileptic biofilms is not yet known. Thus, the hypotheses of the present study are that:

(a) The Federal Santa Maria University watershed targeted by the study is contaminated with various organic compounds.

(b) The University Hospital and the Veterinary Hospital are major polluters of water resources.

(c) The epilithic biofilms are bioindicators of pharmaceuticals and metabolites discharge in the environment.

(d) Passive sampling and biofilm devices make possible the identification of compounds that are not completely removed by the Effluent Treatment Station (WWTP).

(e) Epilithic biofilms exhibit rapid regeneration capacity and resilience after being exposed to conditions of extreme pollution.

(f) The university campus works as a micro-watershed, a representative micro-scale of contamination with different sources of pollution.

5 OBJECTIVES

5.1 GENERAL OBJECTIVE

This project aims to improve the knowledge on the use of epileptic biofilms to detect and quantify the accumulation of drugs in the environment present in the small university watershed of Lagoão do Ouro stream within the Federal University of Santa Maria, generating a map of distribution of drug residues in the university campus as well as providing the groundwork for future evaluations of the impact that sewage treatment plant will bring to water quality within the University. Also, to study the kinetics of resilience and decontamination of epilithic biofilms in natural ecosystems, and finally, to evaluate the efficiency of the Effluent Treatment Station in the removal of pharmaceutical compounds, using biofilms as an indicator matrix.

6 SPECIFIC OBJECTIVES

a. Detect, identify and quantify the residues of drugs and veterinary drugs in the watercourses of the central campus of UFSM.

b. Present the impact of sewage from the University Hospital, Veterinary Hospital and other buildings on water resources.

c. Check if biofilms are good indicators of system degradation/ water contamination.

d. Evaluate pharmaceuticals resilience (purification capacity) of epilithic river biofilms.

e. Evaluate the capacity of degradation of pharmaceutical compounds in WWTPs.

7 PROPOSED STUDIES

The present thesis consisted of three studies and an appendix with an additional study. Study 1 dealt with the quantification and identification of pharmaceuticals present along the small watershed of the Federal University of Santa Maria and Camobi neighborhood. Study 2 consisted of addressing the capacity and speed of resistance of biofilms, the self-depuration of pharmaceuticals, and the change in bacterial composition. Finally, Study 3 addressed the efficiency of a WWTP located at UFSM in removing pharmaceutical compounds from wastewater coming from the University's Student House. The appendix is an additional study to present supplementary data to the main studies. Characterization of the mineral fraction of the biofilm was performed and these results were presented in the appendices in a simplified form.

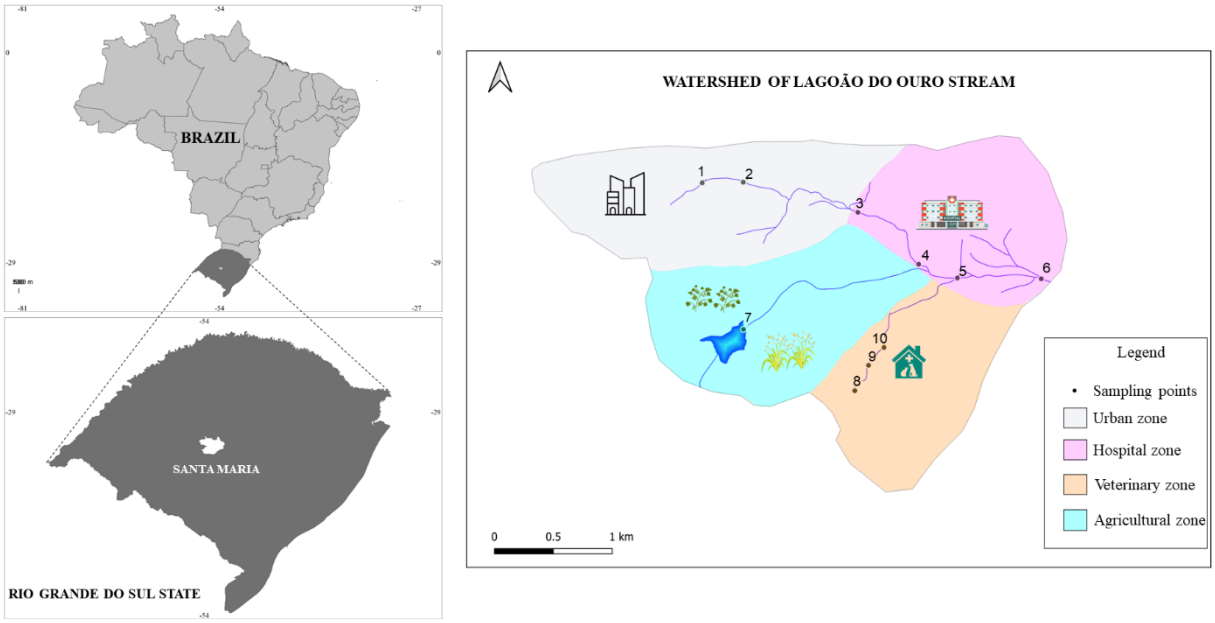
8 GENERAL MATERIAL AND METHODS

8.1 STUDY AREA

The studies were carried out in the Lagoão do Ouro watershed (coordinates 29° 45'S and 53° 43' W), located in the city of Santa Maria, State of Rio Grande do Sul, Brazil (Figure 1). The climate of the region is characterized as Cfa type, subtropical (Koppen classification), with average temperatures of 18.2°C, annual rainfall averages of 1.382 mm, and insolation of 2300 hours (COGO; ELTZ; CASSOL, 2006).

The study area is about 10 km^2 occupied by popular housing groups, high rise buildings, agricultural activities, and the buildings of the Federal University of Santa Maria (65% of the surface area). The relief is gently undulated, characterized by the presence of stretches and alluvial plains, with altimetric quotas that vary from 40 to 200 meters (CECONI et al., 2018). This area is of major importance to the development of the region as a source of water for human and animal consumption, irrigation, and recreation. However, the area is anthropized due to a high degree of urbanization combined with poor domestic wastewater collection and treatment systems. In the city of Santa Maria, only 49.3% of the population has access to wastewater collection services (ANA, 2019). However, one of the major sanitation problems in the city is the lack of connections to the wastewater treatment system, which means that the actual treatment rate is much lower. Wastewater is not collected by the municipality; it is probably discharged into the rainwater network or directly into the nearest water bodies.

Material and Methods – Figure 1– Map of the monitoring points of the small university watershed of the Lagoão do Ouro Stream in Santa Maria/RS.



In the upstream portion of the sub-watershed, which does not belong to the University, there is an intense process of urbanization, through the implementation of of low-income housing and high-standard buildings. In these areas several environmental problems involving open sewage, solid urban waste, animal husbandry, degradation of natural vegetation fragments, removal of riparian forest, among others end up causing pollution of the receiving waterways (BRUN et al., 2011).

8.2 SANTA MARIA FEDERAL UNIVERSITY AND ITS EXTENSIONS

The Federal University of Santa Maria (UFSM) has 28.974 thousand students, of which 2.203 are master students and 1.696 doctoral students 263 courses, 2.051 teachers and 2.693 administrative technicians (SIE, 2019). Most of UFSM's water supply comes from artesian wells (90%), which cost R$ 35.000.00 (about 9 thousand dollars) per month for the institution due to its maintenance and expenditure of electricity. At the Santa Maria campus, monthly water consumption is estimated at 100.000 m^3.

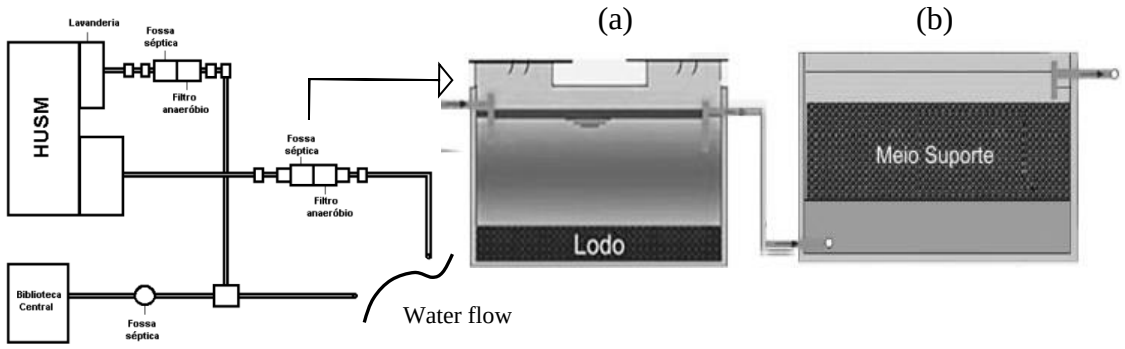
Most of the university buildings use the septic tank followed by a biological filter for subsequent discharge of their effluents into the water body as an effluent treatment system. Some more distant buildings, where it is not feasible to discharge the treated effluents into the

water body, have a septic tank system and a sink. As for student housing, those recently built also have a septic tank system with a biological filter and the discharge of its effluents is carried out in the Lagoão do Ouro stream. However, the oldest constructions have only a septic tank and the disposal of the treated effluents been connected to the rainwater drainage network. The university has two University Restaurants (UR); UR–1 has a septic tank + biological filter and a discharge into Lagoão do Ouro stream and UR–2 has a septic tank and sink. The University Veterinary Hospital has a septic tank and biological filter, with subsequent release into water body. The cleaning of the septic tank is done routinely in the University Restaurants, and in the in the main buildings occurs only when requested, in the case of clogging.

The university also has the University Hospital of Santa Maria (HUSM), which since its foundation in 1970, is a reference in health for the central region of RS. The HUSM works as a teaching hospital, with its attention focused on the development of teaching, research and assistance in health, with the objective of providing quality health care to the population of the municipality and the Santa Maria region, covering 42 municipalities with a population of 1.000.000 inhabitants. Currently, it has a structure of 59 offices and 361 hospital beds (HUSM, 2016).

In Rio Grande do Sul, the hospital is a reference in hematology/oncology care, providing outpatient care, adult and pediatric inpatient units, bone marrow transplant center and radiotherapy sector. In the High Complexity Assistance Unit in Oncology (Unacon), 24.852 patients are assisted per month (HUSM, 2016). The Antineoplastic Chemotherapy Center of the Pharmacy Service is part of this care network, where antineoplastic drugs are manipulated, which are essential for hematological and oncological treatment. Currently at the Antineoplastic Chemotherapy Center, about 430 patients are treated monthly and more than 1700 antineoplastic therapies are manipulated. This number has been gradually increasing as well as the number of patients seen. In 2011, for example, 18421 drugs were produced, followed by 19057 manipulations in 2012 and 21347 in 2013. The number of patients seen in 2011, 2012 and 2013 were 4565, 4944 and 5680, respectively (GONÇALVES, 2014). The effluents generated by HUSM, after going through a treatment system equipped with a septic tank and an anaerobic filter (Figure 6) are then drained into UFSM streams.

Literature Review – Figure 8 – Schematic representation of an effluent treatment system adopted by HUSM: (a) septic tank; (b) anaerobic tank



Source: ADAPTED FROM ALMEIDA ET AL. (2015).

8.3 SAMPLING POINTS

- **P1**: Downstream point of the city, 3rd order tributary of the Lagoão do Ouro Stream. The catchment area is 474 140.13 m^2. Width of the stream around 1 m and water depth of 20 to 30 cm. It has 10 meters of riparian forest, located within the green area, not yet occupied in the neighborhood, however there are buildings and occupations disordered above and below the point. Some species found: Samambaia (*Pteridium aquilinum* L.), Aroeira-vermelha (*Schinus terebinthifolius*), Angico (*Parapiptadenia rígida*), Coqueiro (*Cocos nucifera*) and many native shrub species.
- **P2**: Downstream of P1 at 765.95 m. Catchment area of 726 900,72 m^2. Point within the area occupied by the population, receives urban contribution. Strong smell of sewage, width of the river between the banks of 2 and 3 m, water depth around 40 cm. Presence of riparian forest around 1 m. Some species found: Natural grassland of Pampa Biome, Aroeira-vermelha (*Schinus terebinthifolius*), Aroeira-preta (*Myracrodruon urundeuva*), Mamona (*Ricinus communis*), Caraguatá (*Bromelia antiacantha*), Capim-elefante (*Pennisetum purpureum*) e Capim-annoni (*Eragrostis plana Nees*).
- **P3**: First point inside the university, upstream of University Hospital, bordered by buildings. Area of capture of 204 161.28 m^2. Distant 758.19 m from P2. Upstream from hospital and gas station. Riparian forest present in the first three meters on the riverbank. Width of the river between the banks around 7 m, water depth greater than 1 m (difficult to measure). Some species found: Pitangueira (*Eugenia uniflora*), Pata-de-vaca (*Bauhinia forficata*), Mamona (*Ricinus communis*), Capim-elefante (*Pennisetum purpureum*), Angico (*Parapiptadenia rígida*), Goiabeira (*Psidium guajava*) and Amoreira (*Rubus brasiliensis*).

- **P4**: Downstream point to the University Hospital. Area of catchment area 112.570.90 m^2. Distant 364.60 m from P3. Riparian forest around 5 m. Absence of buildings and constructions around, presence of garbage and foam. Width of the river from 4 to 5 m, water depth from 10 to 20 cm. Some species found: Cana-de-açúcar (*Saccharum officinarum*), Pinus (*Pinus elliottii*) e Aroeira-vermelha (*Schinus terebinthifolius*).
- **P5**: First point of the exutory. 146 725,85 m^2 of catchment area. Distant 81.9 m from P4, riparian forest of 5 m. Width of 1.20 m of margin, water depth of 10 cm to 15 cm and presence of garbage. Some species found: Pitangueira (*Eugenia uniflora*), Timbaúva (*Enterolobium contortisiliquum* (Vell.) Morong), Angico (*Parapiptadenia rígida*), Ipê-roxo (Handroanthus impetiginosus), Camboatá (Cupania vernalis), Bushy Peach Tree (Prunus myrtifolia (L.) Urb.) and grasses
- **P6**: Point of confluence of the 4 rivers. Situated inside Pinus Forest (*Pinus elliottii*), end point inside the University. Catchment area of 477 559.67 m^2. Distant 677.42 m from P5. Edge width from 1 to 2 m, water blade from 20 to 80 cm. Some species found: Ipê-roxo (*Handroanthus impetiginosus*), Camboatá (*Cupania vernalis*), Pessegueiro-do-mato (*Prunus myrtifolia* (L.) Urb.) and Pitangueira (*Eugenia uniflora*).
- **P7**: Water tank point. Agricultural area, weir used by the agricultural sectors for the culture of Rice (*Oryza sativa*) and in the horticulture sector for irrigation and other agricultural activities. Catchment area of 641 868,60 m^2. Distant 326.58 m from P6.
- **P8**: Starting point of the source of the third river, allocated within the experimental area of the Department of Veterinary Medicine, downstream to the University Veterinary Hospital. Catchment area of 43 874, 75 m^2. Distant 609.72 m from P7. Area covered by pasture, presence of herds of cattle, horses and goats.
- **P9**: Point still allocated within the experimental area of the Department of Veterinary Medicine, downstream to the University Veterinary Hospital. Catchment area of 260 303,11 m^2. Distant 494,55 m from P8. Area covered by pasture, presence of herds of cattle, horses and goats. Area destined for quarantine of treated animals.
- **P10**: Point located within small woodland, in the experimental area of the Department of Veterinary Medicine. Receives influence from the herd present within the experimental areas. Catchment area of 286 956.90 m^2. Distant 632.35 m from P9. Some species found: Pinus (*Pinus elliottii*), Eucalipto (*Eucalyptus globulus Labill*), Caraguatá (*Bromelia antiacantha*) e Capim-annoni (*Eragrostis plana* Nees).

Material and Methods – Figure 2 – Digital images of the catchment areas monitored

Source: (AUTHOR).

8.4 STUDIED COMPOUNDS

Four anticancer drugs, eight antibiotics (the whole 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 (MARTINS et al., 2011b) as well as the four anti-cancer compounds (SOUZA; REICHERT; MARTINS, 2018b). These compounds are known to be highly reactive represents a risk to human health and the environment.

Material and Methods – Table 1– Pharmaceutical compounds studied grouped according to their therapeutical class.

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[c]	3.20	$C_{33}H_{38}N_4O_6$
Midecamycin	Antibiotic	6.90[d]	-	$C_{41}H_{67}NO_{15}$
Spiramycin		7.88 [a]	1.87	$C_{43}H_{74}N_2O_{14}$
Josamycin		7.10[e]	-	$C_{42}H_{69}NO_{15}$
Azithromycin		8.74 [a]	4.02	$C_{38}H_{72}N_2O_{12}$
Sulfamethoxazole		5.70[a]	0.89	$C_{10}H_{11}N_3O_3S$
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[b]	0.05	$C_{41}H_{76}N_2O_{15}$
Diclofenac	Anti-inflammatory	4.15 [a]	4.51	$C_{14}H_{11}Cl_2NO_2$
Carbamazepine	Anti-epileptic	13.90 [a]	2.45	$C_{15}H_{12}N_2O$

For each substance, chemical dissociation constant (pk_a), octanol-water partition coefficient ($\log K_{ow}$) and molecular formula.[a] pubchem; [b] (CHEN et al., 2016); [c] (ANILANMERT et al., 2006); [d] drugbank.ca ; [e] drugfuture.com; *phenol.

Material and Methods – Table 2– PNEC (Predicted no-Effect Concentration) and Rs (Sampling rate) values used for each molecule in studies 1 and 3.

Compound	**PNEC (ng L^{-1})**	**Reference**	**Rs**	**Reference**
Doxorubicin	100	Martín et al. (2014)	0.713213	Calculated
Epirubicin	355	Souza et al. (2018)	0.0003244	Calculated
Daunorubicin	0,49	Souza et al. (2018)		
Irinotecan	23	Souza et al. (2018)		
Spiramycin	1100	Tell et al. (2019)		
Josamycin	40000	Li et al. (2014)		
Azithromycin	150	Lin et al. (2008)	0.27	Alavarez et l. (2004)
Sulfamethoxazole	900	Komori et al. (2013)	0.22	Zhang et al. (2008)
Clarithromycin	52	Komori et al. (2013)	0.668	MacLeod et al. (2007)
Erythromycin	40	Lin et al. (2008)	0.183	MacLeod et al. (2007)
Roxythromycin	150	Lin et al. (2008)	0.134	MacLeod et al. (2007)
Diclofenac	10000	Komori et al. (2013)	0.166	MacLeod et al. (2007)
Carbamazepine	250	Komori et al. (2013)	0.6	Li et al. (2010)

Source: (AUTHOR).

8.5 ANALYTICAL PROCEDURES

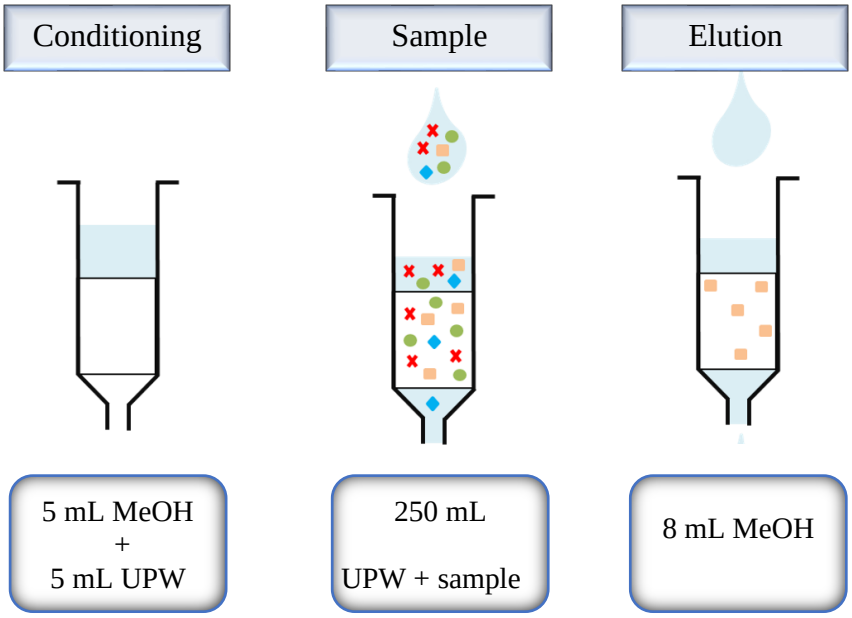
Biofilm extraction procedure was performed using the method described by Aubertheau et al. (2017). Five hundred milligrams of biofilms were extracted by extraction liquid at high pressured (ASETM 350, Thermo Fisher Scientific Inc, Waltham, USA) at 80 °C using methanol / water (1/2; v/v) as the extraction solvent (Table 2). 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).

Material and Methods – Table 3– Parameters used for pharmaceuticals extractions of biofilms using pressurized liquid extraction.

ASE 350 (*Thermo Fischer Scientific*)	
Temperature	80 ºC
Pressure	100 bars
Static cycles number	4
Cycle time	5 min
Flush volume	60% loaded volume
Solvents	Methanol/ Water
Volume ratio	½
Cells type	Cell inox 10 mL
Sample mass	500 mg dry biofilm
Dispersive phase	Diatomaceous Earth

The extracts of biofilm and POCIS were purified by solid phase extraction (Autotrace TM 150, Thermo Scientific, 125 Waltham, USA) using Oasis® HLB cartridges (6cc, 200 mg of sorbent; Waters, Milford, USA) with methanol as eluent (Figure 4).

Material and Methods – Figure 3– Solid Phase Extraction purification procedures



(UPW - ultrapure water)

Source: (AUTHOR).

Purified extracts were concentrated by evaporation under gentle N_2 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-8060 Shimadzu).

Material and Methods – Table 4 – Chromatographic method.

Chromatographic conditions	
Column	Waters, UPLC® Acquity C18 1.7 µm, 2.1x100 mm
Injection volume	5 µL
Composition of the mobile phase	Solvent 1 = Ultrapure water + 0.1% Formic acid Solvent 2 = ACN + 0.1% Formic acid
Gradient	Solvent 2: 10% at 1 min, ramp 60% at 2 min, ramp 70% at 6 min, ramp 85% at 7 min, then resume initial conditions 10% at 9 min. Break 1 min at 10%, stop at 10 min
Oven temperature	25 °C
Flow rate	0.40 mL/min
Initial pressure	630 bars
Spectrometric conditions	
Ionization mode	ESI (+)
Temperature of the DL	250 °C
Source temperature	400 °C
Collision gas flow rate (Ar)	3 L/min
Acquisition mode	MRM (Multiple Reaction Monitoring)

8.6 VALIDATION OF THE LINEARITY

Linearity is the ability to obtain results for a given analytical method within a certain range of results directly proportional to the quantity of the substance analysed in the sample. Linearity is achieved in two steps:

Step 1 *: determination of the linear calibration function*

First. we have to look for the equation: $y=b_0+b_1u$.

With :

b_1 : slope

b_0 : ordinate at the origin

n : number of repetitions (at least n=5)

p : number of calibration standards (at least p=5)

N : Total number of measurements N=n*p

$$b_1 = \frac{[\sum_i^p \sum_j^n (u_{ij}-\bar{\bar{u}})(y_{ij}-\bar{\bar{y}})]}{\sum_{i=1}^p \sum_{j=1}^n (u_{ij}-\bar{\bar{u}})^2} \text{ where } \bar{\bar{u}} = \frac{\sum_i \sum_j u_{ij}}{N} \text{ et } \bar{\bar{y}} = \frac{\sum_i \sum_j y_{ij}}{N} \quad \text{(equation 1)}$$

Then. the residual variance (S^2(res)) can be calculated:

$$S^2_{rés}=\frac{\sum_i \sum_j (y_i-\check{y}(u_i))^2}{N-2}$$ (equation 2)

$\check{y}$: is the response predicted by the model for the standard solution.

Therefore, by combining the residual variance. The results of the standard deviations of the slope (S(b1)) and the y-axis at origin (S(b0)) are determined according to the following formulas:

$$S(b1) = \sqrt{\frac{S^2_{rés}}{\sum_{i=1}^{p}\sum_{j=1}^{n}(u_{ij}-\bar{\bar{u}})^2}}$$ et $$S(b0) = \sqrt{S^2_{rés}\left(\frac{1}{N}+\frac{\bar{\bar{u}}^2}{\sum_{i=1}^{p}\sum_{j=1}^{n}(u_{ij}-\bar{\bar{u}})^2}\right)}$$ (equations 3 and 4)

Step 2 : *Fisher Linearity assessment and Fisher test*

The evaluation of linearity depends on the sums of the squares of the deviations due:

- to the linear regression ($SCE_l(y)$)
- to a non-linearity model error ($SCE_{nl}(y)$)
- to an experimental error ($SCE_e(y)$)

After obtaining all the sums of the squares of the deviations, the Fisher test was performed to verify whether the regression model is considered acceptable or not. If the calculated variance is greater than the critical variance under the given conditions, then the linearity is checked. The Fisher test also verifies whether the non-linearity variance is less than or equal to the critical variance, in which case the model error is negligible. If these two conditions are verified, the selected calibration domain is then validated. The table 4 summarizes the formulas used and their conditions. By applying the linear regression model to the 5 previous linear regression lines, a new average linear regression equation was determined which will then be used for method validation.

Material and Methods – Table 5 **–** Formula used to verify whether the regression model is considered acceptable or not.

Requirements	Degree of freedom	Sum of the squares of the differences	Variance	F de Fisher	F of Ficher at a=1%	Conclusion
Regression	1	$SCE_l(y)= \frac{[\sum_i^p \sum_j^n (u_{ij}-\bar{u})(y_{ij}-\bar{y})]^2}{\sum_{i=1}^p \sum_{j=1}^n (u_{ij}-\bar{u})^2}$	$s^2_l(y)= SCE_l(y)$	$F_l= \frac{s_l^2(y)}{s_e^2(y)}$	$F_{crit\text{-}linear}=F_{1\text{-}a.1.N\text{-}P}$	If $F_l > F_{crit\text{-}linear.}$ Linear calibration
Non-linearity model error of the model	p-2	$SCE_{nl}(y)= SCE(y)-SCE_l(y)-SCE_e(y)$	$s^2_{nl}(y)= \frac{SCEnl(y)}{p-2}$	$F_{nl}= \frac{s_{nl}^2(y)}{s_e^2(y)}$	$F_{crit\text{-}nonlinear}=F_{1\text{-}a.1.N\text{-}P}$	If F_{nl} £ $F_{crit\text{-}nonlinear.}$ Calibration range validated
Experimental error	N-p	$SCE_e(y)=\sum_i^p \sum_j^{n_i} (y_{ij}-\bar{y})^2$	$s^2_e(y)= \frac{SCEe(y)}{N-p}$			
Total variation	N-1	$SCE(y)= \sum_{i=1} \sum_{j=1} (y_{ij}-\bar{\bar{y}})^2$				

8.7 REPEATABILITY AND COCHRAN'S TEST

The repeatability is the minimum value of the accuracy of a method through samples analysed several times "under identical operating conditions" and "in a short time interval". The Cochran's test is used to demonstrate repeatability stability and to detect outliers over the measured application range. The Cochran's test is calculated by:

Calculation of the variance S_i^2 of each standard and their sum ($S = \sum s_i^2$)
Determination of the highest variance (S_{max}^2)
Calculation of ratio : $T_f = \frac{s_{max}^2}{S}$
Read the limit value from Cochran's tables for p standards with a 1% risk of error
If Tf ≤ critical values read in Cochran's table, then the repeatability of the method is constant throughout the calibration domain.

Internal Reproducibility

Internal reproducibility represents the precision of the method when the repetitions are made by several operators and/or at important time intervals regarding the method. For this, calculations were performed on a single sample by carrying out measurements for p = 10 days at a rate of n = 2 repetitions per day.
The internal repeatability is verified by two steps:

- Cochran C test is performed at α = 1 % to verify if the repeatability is constant within the application domain
- The ISO 5725 formulas are applied in order to calculate the repeatability (r95%) and reproducibility (R95%) limits. If the repeatability limit (r95%) is less than the reproducibility limit (R95%), the internal repeatability is acceptable

$r_{95\%} = 2{,}83 * S_r \; et \; R_{95\%} = 2{,}83 * S_R$

$r_{95\%}$: is the maximum risk difference of 5% which separates two results, obtained under repeatability conditions (same operator, same day, etc)

$R_{95\%}$: is the maximum difference at risk of 5% which separates two results, obtained under reproducibility conditions (different operator, different day, etc.)

Where

$$S_R = \sqrt{S_L^2 + S_r^2}$$

$$S_L^2 = \frac{(p-1)\left(\frac{SCE_L}{p-1} - S_r^2\right)}{N'} \; and \; S_r^2 = \frac{SCE_r}{N-p}$$

$$N' = N - \frac{\sum_i^p n^2}{N}$$

$$SCE_r = \sum_i^p \sum_j^{n_i} (X_{ij} - \bar{X}_i)^2$$

$$SCE_L = \sum_i^p n_i (\bar{X}_i - \bar{\bar{X}}_i)^2$$

With

S_R: Standard deviation of reproducibility

N : Total number of measurements (N=n*p)

p : Days number (at least p=10)

n : Repetition number (at least n=2)

N' : Corrected average number of repetitions

$\bar{X}_i$: Results dispersion with p modalities

$\bar{\bar{X}}_i$: General average

8.8 APPLICATION TO THE PRESENT WORK

The results of the method validation procedure show that the calibration curves are linear and that the calibration domains are validated for all pesticide molecules included in the study. Regarding repeatability, all coefficients for determining regression lines are acceptable and constant over the entire range of application.

8.9 LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ)

Several methods exist to calculate the LOD and LOQ. The method used for this work is the “blank” method. According to the XP T 90-210 (1999), another method can be used to determine the LOD and LOQ, when the analytical procedure provides a graphic recording (chromatography), then LOD and LOQ are estimated from the background noise. In this case, the LOD and LOQ are calculated by determining the maximum amplitude hmax of the signal over a distance equal to 20 times the width at mid-height of the peak corresponding to the substance to be searched:

The limits of quantification in biofilms are given in table 6:

LOD=3*hmax*R and LOQ=10*hmax*R

(R: quantity/signal response fator, hmax: maximum signal amplitude)

Material and Methods – Table 6 – Transition ions and confirmation of the compounds analyzed.

Compounds	Optimisation LCMSMS Shimadzu			
	Transition quantification (CE)	Transition confirmation 1 (CE)	Transition confirmation 2 (CE)	Retention time in minutes
Doxorubicin	544.20>397 (-14)	544.20>361.10 (-26)	544.20>379.15 (-20)	2.991
Epirubicin	544.20>397.05 (-13)	544.20>361.10 (-26)	544.20>379.15 (-20)	3.70
Daunorubicin	528.29>363.10 (-15)	528.29>381.10 (-10)	-	3.061
Irinotecan	587.30>124.30 (-34)	587.30>167.30	-	2.956
Erythromycin	734.45>158.30 (-29)	734.45>576.30 (-18)	734.45>116.20 (-42)	3.060
Spiramycin	844.45>109.25 (-42)	844.45>174.25 (-35)	844.45>83.25 (-52)	3.181
Josamycin	828.50>109.25 (-43)	828.50>174.30 (-33)	828.50>229.30 (-30)	3.309
Clarithromycin	748.45>158.30 (-29)	748.45>590.35 (-19)	748.45>116.25 (-41)	3.266
Roxithromycin	838.30>158.25 (-33)	838.30>680.30 (-21)	838.30>116.20 (-45)	3.183
Azithromycin	749.50>158.30 (-35)	749.50>116.30 (-43)	749.50>591.30 (-27)	3.190
Midecamycin	814.50>174.30 (-32)	814.50>109.25 (-44)	814.50>201.30 (-29)	3.208
Sulfamethoxazole	254.20>92.25 (-25)	254.20>156.20 (-15)	254.20>108.15 (-23)	3.107
Diclofenac	296.15>214.10 (-34)	296.15>215.15 (-19)	296.15>250.10 (-12)	4.199
Carbamazepine	237.00>194.20 (-19)	237.00>193.20 (-33)	237.00>192.20 (-22)	3.257

Material and Methods – Table 7 – Result of the validation of the compounds analyzed and the limits of quantification and detection.

Compound	Validation Norme XP T90-210			
	Mode ESI	Linearity Repeatability	LOD µg/L	LOQ µg/L
Doxorubicin	H+	Validated	0.035	0.115
Epirubicin	H+	Validated	0.081	0.270
Daunorubicin	H+	Validated	0.034	0.114
Irinotecan	H+	Validated	0.015	0.048
Erythromycin	H+	Validated	0.006	0.021
Spiramycin	H+	Validated	1.838	6.127
Josamycin	H+	Validated	0.128	0.428
Clarithromycin	H+	Validated	0.100	0.332
Roxithromycin	H+	Validated	0.026	0.088
Azithromycin	H+	Validated	0.095	0.316
Midecamycin	H+	Validated	0.043	0.145
Sulfamethoxazole	H+	Validated	0.034	0.115
Diclofenac Na	H+	Validated	0.081	0.271
Carbamazepine	H+	Validated	0.037	0.123

Material and Methods – Table 8 – Method validation of pharmaceutical compounds LC-MS/MS (general list and compounds covered only in study 3).

Compounds	**Linearity**		**Repeatability**	**Reproducibility**	
	Regression model	**Calibration domain**	p = 8 n = 5 α = 1 % Critical value = 0.463	p = 10 n = 2 α = 1 % Critical value = 0.718	p = 10 n = 2 α = 5 %
CBZ	Acceptable (R^2 = 0.998 ± 0.002)	Validated (0.0112 – 0.1125 mg L^{-1})	Acceptable (C = 0.310)	Constant Repeatability (C = 0.625)	Acceptable $r_{95\%}$=0.0020< $R_{95\%}$=0.1770
3OH-CBZ	Acceptable (R^2 = 0.998 ± 0.001)	Validated (0.0076 – 0.076 mg L^{-1})	Acceptable (C = 0.293)	Constant Repeatability (C = 0.213)	Acceptable $r_{95\%}$=0.0390< $R_{95\%}$=0.0490
CBZ-epox	Acceptable (R^2 = 0.996 ± 0 .004)	Validated (0.0088 – 0.0885 mg L^{-1})	Acceptable (C = 0.259)	Constant Repeatability (C = 0.367)	Acceptable $r_{95\%}$=0.0003< $R_{95\%}$=0.0930
Trans-CBZ	Acceptable (R^2 = 0.998 ± 0.003)	Validated (0.01 – 0.1 mg L^{-1})	Acceptable (C = 0.291)	Constant Repeatability (C = 0.360)	Acceptable $r_{95\%}$=0.0003< $R_{95\%}$=0.1090
Dibenz	Acceptable (R^2 = 0.991 ± 0.006)	Validated (0.0117 – 0.1173 mg L^{-1})	Acceptable (C = 0.392)	Constant Repeatability (C = 0.715)	Acceptable $r_{95\%}$=0.0080< $R_{95\%}$=0.1640
Acrid	Acceptable (R^2 = 0.997 ± 0.001)	Validated (0.0119 – 0.1192 mg L^{-1})	Acceptable (C = 0.265)	Constant Repeatability (C = 0.372)	Acceptable $r_{95\%}$=0.0010< $R_{95\%}$=0.1160
DCF	Acceptable (R^2 = 0.994 ± 0.003)	Validated (0.0117 – 0.1174 mg L^{-1})	Acceptable (C = 0.405)	Constant Repeatability (C = 0.688)	Acceptable $r_{95\%}$=0.0008< $R_{95\%}$=0.146
OH-DCF	Acceptable (R^2 = 0.9995 ± 0.0003)	Validated (0.0202 – 0.2018 mg L^{-1})	Acceptable (C = 0.403)	Constant Repeatability (C = 0.553)	Acceptable $r_{95\%}$=0.0010< $R_{95\%}$=0.1000
Benz	Acceptable (R^2 = 0.998 ± 0.001)	Validated (0.01 – 0.1 mg L^{-1})	Acceptable (C = 0.259)	Constant Repeatability (C = 0.311)	Acceptable $r_{95\%}$=0.0020< $R_{95\%}$=0.1200
Levofloxacin	Acceptable (R^2 = 0.983 ± 0.005)	Validated (0.0010– 0.0746 mg L^{-1})	Acceptable (C = 0.387)	Acceptable (C = 0.634)	Acceptable $r_{95\%}$=0.0079< $R_{95\%}$=0.0169
Ciprofloxacin	Acceptable (R^2 = 0.996 ± 0.004)	Validated (0.0010– 0.0749 mg L^{-1})	Acceptable (C = 0.436)	Constant Repeatability (C = 0.243)	Acceptable $r_{95\%}$=0.0240< $R_{95\%}$=0.0404

Azithromycin	Acceptable (R^2 = 0.995 ± 0.004)	Validated (0.0001– 0.0100 mg L^{-1})	Acceptable (C = 0.451)	Constant Repeatability (C = 0.347)	Acceptable $r_{95\%}$=0.0010< $R_{95\%}$=0.0013
Clarithromycin	Acceptable (R^2 = 0.997 ± 0.002)	Validated (0.0009– 0.0996 mg L^{-1})	Acceptable (C = 0.329)	Constant Repeatability (C = 0.406)	Acceptable $r_{95\%}$=0.1825< $R_{95\%}$=0.3178
Sulfamethoxazole	Acceptable (R^2 = 0.997 ± 0.002)	Validated (0.0010– 0.1009 mg L^{-1})	Acceptable (C = 0.499)	Constant Repeatability (C = 0.262)	Acceptable $r_{95\%}$=0.0020< $R_{95\%}$=0.0030
Trimetroprin	Acceptable (R^2 = 0.998 ± 0.001)	Validated (0.0010– 0.0743 mg L^{-1})	Acceptable (C = 0.459)	Constant Repeatability (C = 0.410)	Acceptable $r_{95\%}$=0.0088< $R_{95\%}$=0.0089
Amoxicillin	Acceptable (R^2 = 0.998 ± 0.001)	Validated (0.0105– 0.1047 mg L^{-1})	Acceptable (C = 0.282)	Constant Repeatability (C = 0.213)	Acceptable $r_{95\%}$=0.0390< $R_{95\%}$=0.0490
Carbamazepine	Acceptable (R^2 = 0.998 ± 0.002)	Validated (0.0112 – 0.1125 mg L^{-1})	Acceptable (C = 0.310)	Constant Repeatability (C = 0.625)	Acceptable $r_{95\%}$=0.0020< $R_{95\%}$=0.1770
Diclofenac	Acceptable (R^2 = 0.994 ± 0.003)	Validated (0.0117 – 0.1174 mg L^{-1})	Acceptable (C = 0.405)	Constant Repeatability (C = 0.688)	Acceptable $r_{95\%}$=0.0008< $R_{95\%}$=0.1460
Atenolol	Acceptable (R^2 = 0.998 ± 0.001)	Validated (0.0010– 0.0923 mg L^{-1})	Acceptable (C = 0.429)	Constant Repeatability (C = 0.244)	Acceptable $r_{95\%}$=0.0080< $R_{95\%}$=0.0098
Bezafibrate	Acceptable (R^2 = 0.989 ± 0.005)	Validated (0.0010– 0.1000 mg L^{-1})	Acceptable (C = 0.501)	Constant Repeatability (C = 0.432)	Acceptable $r_{95\%}$=0.0038< $R_{95\%}$=0.0067
Iohexol	Acceptable (R^2 = 0.997 ± 0.003)	Validated (0.0010– 0.0995 mg L^{-1})	Acceptable (C = 0.445)	Constant Repeatability (C = 0.550)	Acceptable $r_{95\%}$=0.0046< $R_{95\%}$=0.0108
Daunorubicin	Acceptable (R^2 = 0.976 ± 0.023)	Validated (0.0001– 0.0100 mg L^{-1})	Acceptable (C = 0.468)	Constant Repeatability (C = 0.435)	Acceptable $r_{95\%}$=0.0021< $R_{95\%}$=0.0078
Irinotecan	Acceptable (R^2 = 0.975 ± 0.025)	Validated (0.0100– 0.1000 mg L^{-1})	Acceptable (C = 0.502)	Constant Repeatability (C = 0.431)	Acceptable $r_{95\%}$=0.0022< $R_{95\%}$=0.0033

8.10 COMPLEMENTARY ANALYSES TO CHARACTERIZE BIOFILMS

8.10.1 Infrared

A Thermo Nicolet 6700 FT-IR Spectrometer was used for infrared analysis. The spectra were obtained in the region of 4000 to 400 cm^{-1}, with a resolution of 4 cm^{-1} and 100 scans. The samples were obtained from the diffuse reflectance spectrum, converted to absorbance by the Kubelka-Munk method, from the direct analysis of dried and freeze-dried samples at room temperature prepared in kiln-dried KBr tablets. The analyses were performed in the HydrASA laboratory, which is part of the "Institut de Chimie des Miliex et Matériaux" of the Université de Poitiers - France.

8.10.2 X – Ray

The analysis in the form of disoriented powder was performed from samples dried at room temperature and determined in X– ray diffractometer, at angles of 2 to 35° 2θ. The sample in oriented deposit was submitted to treatment for removal of organic matter with H_2O_2 5% at a temperature of 40 °C and with replacement of the frequent solution of H_2O_2 until the end of the effervescence. The sample was dispersed with NaCl 1 mol L^{-1}; sonification and stirring for 16 hours, then washed with distilled water. Saturation was done with $CaCl_2$ at a concentration of 1 mol L^{-1} at room temperature, and dispersion was done by sonification and stirring for 16 hours. Then, the biofilm was washed with distilled water until a negative result for the silver nitrate. Then, 20 mg of biofilm was diluted in 1 mL of distilled water and deposited in a glass layer (0.2 to 0.4 mm thick; 2.5 x 2.5 cm in length and width) so that the solution is evenly distributed on the glass surface. The samples were diffracted in the angular amplitude of 2 to 35° 2θ, with a reading interval of 0.03°, with a reading time of 1 s at each point treatments at room temperature and under ambient relative humidity and after solvency with ethylene glycol. The analyses were carried out in the HydrASA laboratory, which is part of the "Institut de Chimie des Miliex et Matériaux" of the Université de Poitiers - France.

STUDY 1 - OCCURRENCE AND RISK OF PHARMACEUTICALS IN ENVIRONMENTAL COMPARTMENTS IN SOUTHERN BRAZIL

1 INTRODUCTION

In Brazil, large population densities associated with the lack of infrastructure for collection, treatment, and final disposal of domestic sewage result in the release of these effluents directly into ecosystems, causing damage to the qualities of water, sediments, and local living resources, with serious consequences for human health (ARAÚJO et al., 2021). Population growth with accelerated economic development has promoted an increasing entry of organic pollutants into water bodies, such as pharmaceuticals compounds. Brazil is the largest consumer of medicines in Latin America and the fifth-largest consumer market in the world (INTERFARMA, 2018a) but the country has a precarious sewage collection and treatment network. About 45% of the Brazilian population is not connected to the sewage network (ANA, 2019b; MACHADO et al., 2017a) being responsible for a large number of direct connections of clandestine sewage to the country's rivers. This situation is more, or less, serious when it comes to the different regions of the country. The capital Brasília, the states of Paraná and São Paulo are the Federation Units which have the best sewage treatment indexes, higher than 70%. In the state of Rio Grande do Sul this rate is 26.24% (ANA, 2019). Brazil invests much less per year than it would need to reach the goal of universal sanitation (foreseen for 2033). At the current pace, the universalization of sanitation will still take many decades to become a reality, not expected before 2060. Meanwhile, today, 4 million Brazilians still do not have a toilet in their homes and the 100 largest cities in Brazil discharge around 80.500 m^3 of untreated sewage daily into rivers and seas. This has a direct impact on nature, especially for life in rivers and oceans. When discharged into rivers without treatment, sewage alters the natural composition of ecosystem, causing harm to the human beings who live there, and to the aquatic fauna and flora.

Wastewater has been highlighted as the main route of entry of pharmaceuticals into surface waters (HANSEN et al., 2016). Domestic wastewater collects the pharmaceutical residues - either as compounds or unchanged metabolites - excreted after ingestion in urine and feces (FALÅS et al., 2012a). Hospital wastewater has also been highlighted as an important sources for pharmaceuticals (VERLICCHI et al., 2012a). Due to their intrinsic bioactive properties, pharmaceuticals are recognized as being capable of causing potential effects on

aquatic organisms; even those at the first level of the trophic web, such as biofilms for example (DAR; BHAT, 2020a). Then, environmental risk assessment studies are recommended to consider the potential effect of pharmaceuticals at their exposure levels (SANTOS et al., 2013).

The presence of pharmaceuticals and their metabolites in the environment are points of serious concern for researchers and policymakers. Numerous studies have pointed out the occurrence and fate of pharmaceuticals in the environment mainly in developed countries (AUBERTHEAU et al., 2017; BAI et al., 2018; CHAUMET et al., 2019; HANAMOTO et al., 2018; JAFFRÉZIC et al., 2017). However, only a limited number of studies can be found regarding the status of pharmaceutical contamination in emerging countries. In contrast, the reported environmental concentration of pharmaceuticals is higher in developing countries than in developed countries (REHMAN et al., 2015), suggesting greater toxicity risks. Moreover, studies on identifying and monitoring organic contamination of effluents through different types of sampling could help prevent potentially adverse effects on both public health and environmental quality standards. Assessing the risk that these products pose to aquatic microbiota, which is the basis of the trophic web, can provide important results for river health status responses. Most studies conducted in Brazilian aquatic systems have focused on active contamination of water (CHAVES et al., 2020; LOCATELLI; SODRÉ; JARDIM, 2011b; MARTINS et al., 2011c; MONTAGNER; JARDIM, 2011; PIVETTA; GASTALDINI, 2019), but rarely use complementary techniques.

Thus, detailed and targeted investigations are needed to investigate the sources, pathways, and fate of these drugs and their interaction with the aquatic microbiota throughout the environment. These baseline studies provide valuable information to propose remediation techniques for each environmental scenario, risk and impact assessment of the presence of these pollutants in the environment. Moreover, highly sensitive, selective, and robust multi-residue analytical methods are needed to detect pharmaceuticals in various environmental matrices. A detailed investigation of the health status of Brazilian rivers needs to be conducted to know the current level of contamination as well as to create a database and possible solutions.

Therefore, the objective of this study was to evaluate the risk and variability of the contamination of waterways within the central campus of the Federal University of Santa Maria. The university structure has several sources of urban, hospital, and even agricultural contamination, thus becoming a small-scale territory suitable to study different pollution sources and their evolution. To this end, we determined the presence of 14 pharmaceutical products and their respective risks along the small university watershed. Samplings were

performed in two seasons (winter and summer) through passive sampling with Polar Organic Chemical Integrative Sampler (POCIS) and epilithic biofilms.

2 MATERIAL AND METHODS

2.1 SAMPLING POINTS

The study was carried out in the central campus area of the Federal University of Santa Maria, as described previously in the general material and methods. The choice of sampling points was based on the type of pollution present near the river banks and/or anthropic activities that could result in contamination and reduction in water quality:

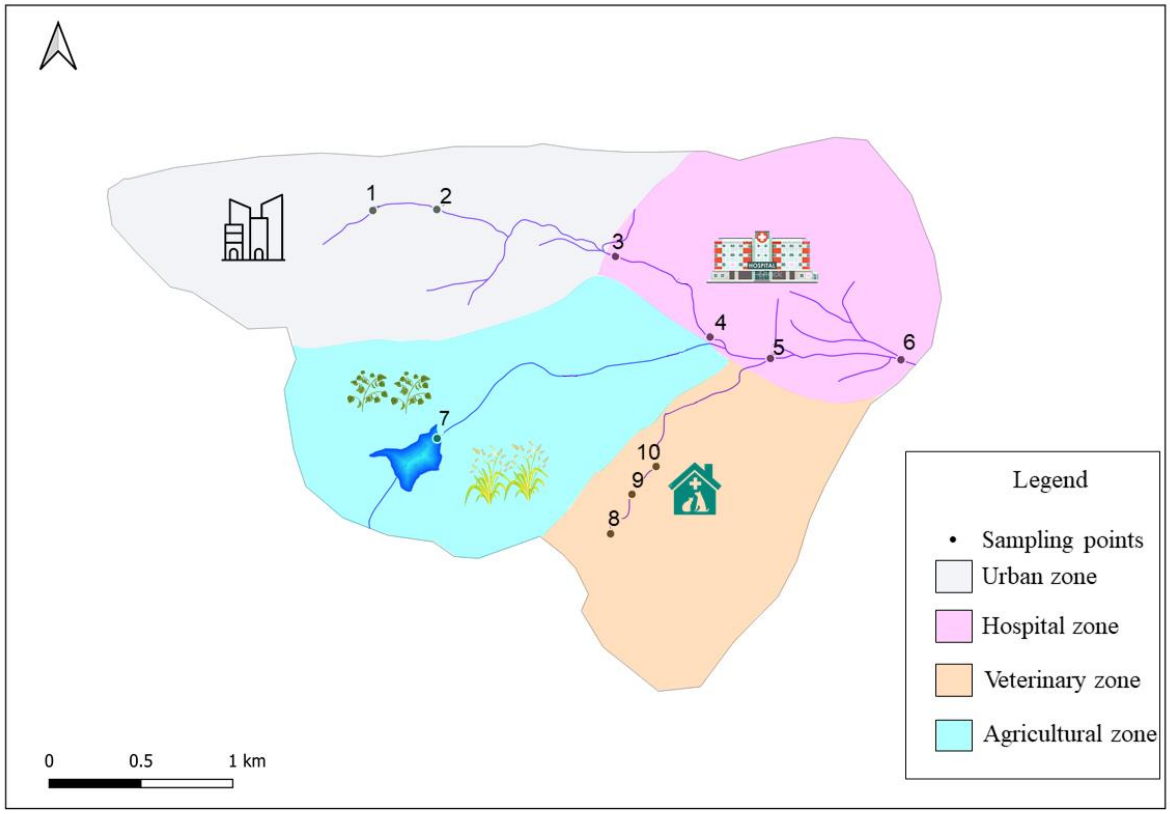
- Sampling points P1 and P2 were designated as Urban Zone. The samples from collection point P2 are exposed to the influence of an area mostly occupied by houses and that drains many clandestine domestic effluents.

- Sampling points P3, P4, P5, and P6 were designated as the Hospital Zone. Point P3 was used as the upstream of the Santa Maria University Hospital (SMUH), point P4 is positioned downstream of the SMUH effluent treatment plant outlet, and the other points were incorporated into this zone by the influence of the hospital discharge. This hospital is one of the largest hospitals in the region with 309 beds and water demand of around 150 m^3 day^{-1}.

- Sampling point P7 was denominated Agricultural Zone. Point P7 is located at the weir in the experimental area of the agrarian courses of UFSM. Water from the weir is used for crop irrigation, mainly rice (*Oryza sativa* sp.) and soybean (*Glycine max*).

- Sampling points P10, P11 and P12 were named Veterinary Zone. Point P10 is upstream of the Veterinary Hospital (VH) and the following points are downstream of the VH, passing through the experimental area of the veterinary course.

Study 1 – Figure 1 – Sampling points in the studied basin and separation of the zones according to the pollution source



Source: (AUTHOR).

2.2 BIOFILMS SAMPLING

In each sampling point, three metallic structures of galvanized material of dimension 70 x 50 cm were allocated, with basalt stones in its interior (Figure 2). Before the implantation of the cages, the stones were washed with deionized water to avoid contamination prior to sampling. Sampling was performed in July and December 2018.

Study 1 – Figure 2– Metallic structure with rocks, allocated within the fluvial course

Source: (AUTHOR).

Sampling consisted of manual collection and brushing of material adhered to fragments of submerged rocks, with the aid of nylon bristle brushes and 0.5 L of deionized water (AUBERTHEAU et al., 2017). Then, the extracted material was then packed in glass containers, stored in thermal boxes with ice (4°C) and transported to the laboratory.

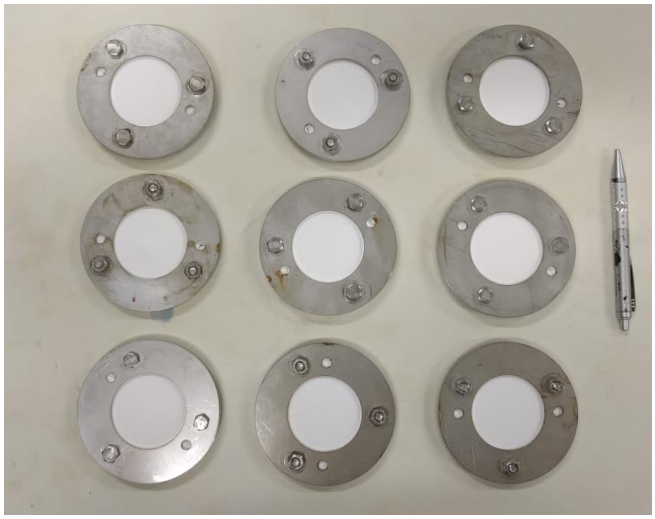
In the laboratory, the biofilms sampled from the glass flasks were transferred to individual high-density polystyrene flasks to be frozen in deep-freezers at -80ºC. Subsequently, the samples were lyophilized (lyophilizer LS3000-TERRONI), homogenized in an agate mortar to obtain a representative sample for chemical and physical analysis.

2.3 POCIS SAMPLING

Together with biofilm samples (in July and December 2018) sampling by Polar Organic Chemical Integrative Sampler (POCIS) was performed to monitor the presence of drugs at the studied points. Thirty-two monitoring devices (POCIS) were assembled and positioned at the points for 15 days. The washers were made of 316 stainless steel with the following dimensions: 102 × 54 × 3.0 mm of external diameter, internal and thickness; containing five 7 mm holes, two in the central axis at 6 mm from the internal edge and three in the shape of an equilateral triangle with 67.5 mm of side (Figure 3). The SUPOR® Polyethersulfone filter membrane was used, with pores of 0.1 µm and 90 mm in diameter. The sequestering medium of the active

ingredients in the water was constituted by 200 mg of OASIS® HLB resin with 30 µm particle diameter, with hydrophilic/lipophilic characteristics.

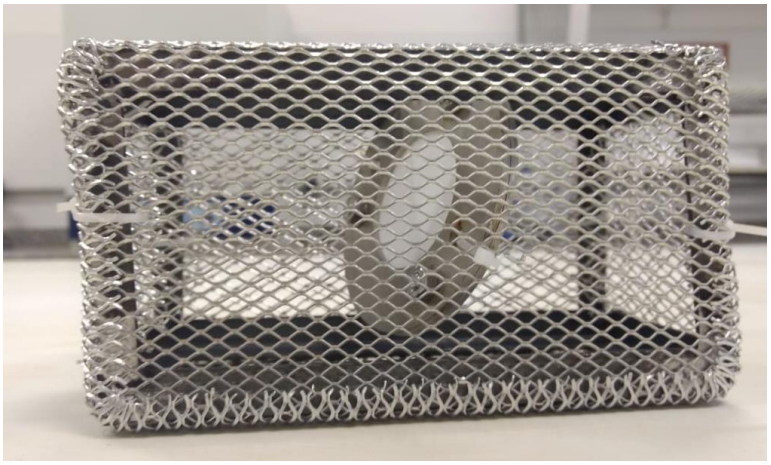
Study 1 – Figure 3– Ready devices, with washer, membrane and adsorbent



Source: (AUTHOR).

These devices were installed at the sampling points between July 16 and 31, 2018, fixed to be submerged during the sampling period and protected by an iron box with aluminum mesh, in order to allow water flow and act as a physical barrier against branches and stones (Figure 4). After 15 days of exposure in the field, the POCIS were collected, cleaned, wrapped in aluminum sheets, placed in plastic bags in a thermal box with ice and transported to the laboratory. In the laboratory the resin was recovered from inside the membranes, placed in cartridges, dried in N_2 flow and stored in the freezer until the time of analysis.

Study 1 – Figure 4– POCIS system ready to be displayed in the aquatic environment



Source: (AUTHOR).

2.4 ENVIRONMENTAL RISK EVALUATION

The Risk Quotient (RQ) 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:

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

The RQ were calculated from concentration measured in 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.

2.5 STATISTICS

The data obtained during the "multi-compartment" campaign were statistically processed in order to highlight inter-compartment and inter-site variability, particularly between rivers and between upstream and downstream exposure. Discriminant factorial statistical analysis (FDA) was chosen for its ability to study the link between a qualitative variable to be explained and quantitative variables called explanatory variables. It allows to highlight the characteristics that best separate the different groups of a given population. The data were obtained using the software Xlstat® (Addinsoft 2018).

3 RESULTS AND DISCUSSION

3.1 DISCRIMINATION OF THE CONTAMINATION ZONES

The study of river contamination was performed by a discriminant factorial statistical analysis (FDA), comparing the concentration of the pharmaceuticals in POCIS and biofilm samplings at each sampling site. Different colors of the points represent the different zones, and the barycenters are represented in yellow.

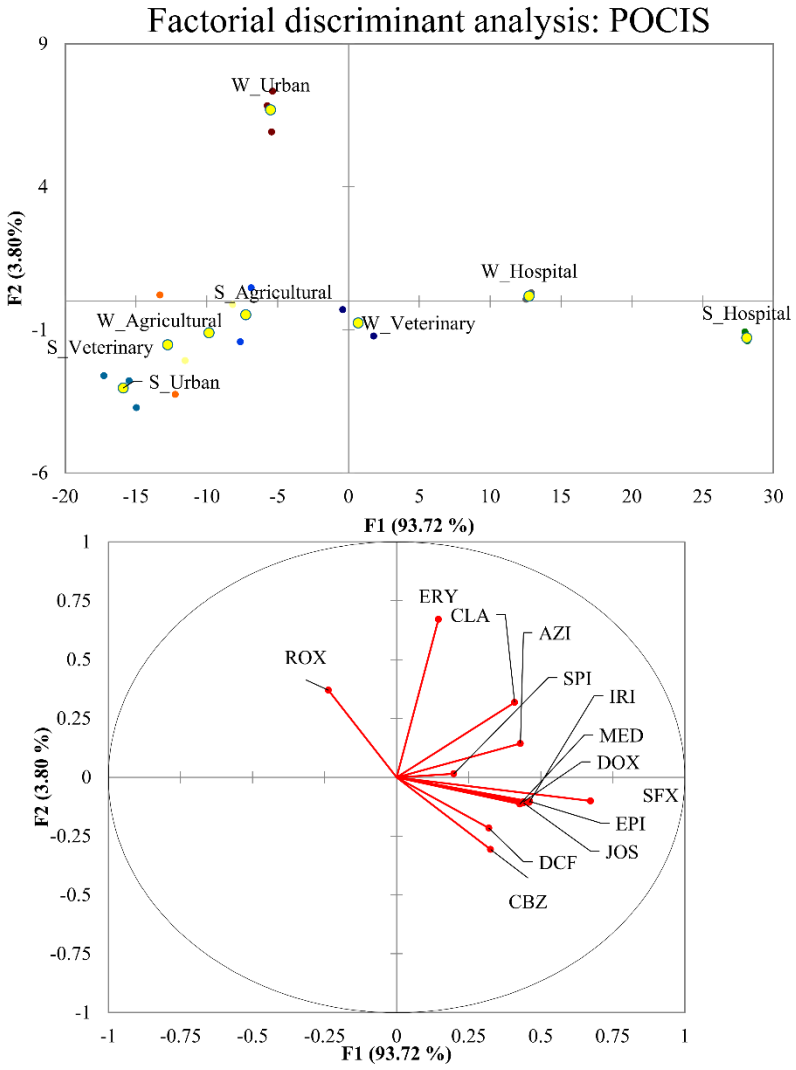
The FDA obtained with pharmaceutical concentration of POCIS (Figure 6) samples explained 97.52% of the data by the two projection axes, with the first axis with more inertia than the second. In general, the zones are quite close. In the spatial representation of the FDA, the barycenter obtained by the pharmaceuticals concentrations quantified each collection site

through the POCIS is represented by axe F1, except for the samples from the urban area where the winter collection is represented by axe F2. Similarities in the contamination of the agricultural and veterinary zones in the winter and summer seasons are present in this analysis. The molecules from the zones represented by axe F1, which are mainly sampling areas from agricultural and veterinary zones, had very similar contamination, with no predominance of compounds that could separate these zones. The hospital zone proved to be the most distinct zone, especially in the samples taken in the summer. This zone is more separated from the others due to the presence of a larger quantity of molecules and with high concentrations, as is the case of antibiotics. In general, in the hospital zone it is possible to verify that there are compounds in concentrations that can serve as tracers of the hospital pollution source. The pollution of the other points is more similar, but the agricultural points are different from the urban points. In winter, the pollution of the hospital points is still different from the other points. On the other hand, the urban and agricultural veterinary pollution is better differentiated than in summer.

Biofilms pharmaceuticals concentration were also analyzed by DFA (Figure 5) and presented 84.73% of the data represented by the first two components, with the first component explaining 71% of the data variance. The same data trend obtained for POCIS samples was also seen in the biofilms, with almost all samples represented by the axe F1 except both seasons urban samples. The agricultural and veterinary zones were considered similar and the concentration of pharmaceuticals obtained in the summer samples of the hospital was inversely correlated with the winter samples. The urban zone in winter is positively correlated with SFX and ROX contents, whereas in summer this correlation is more linked to DCF. Most compounds are correlated with the hospital in summer, agriculture and veterinary in winter.

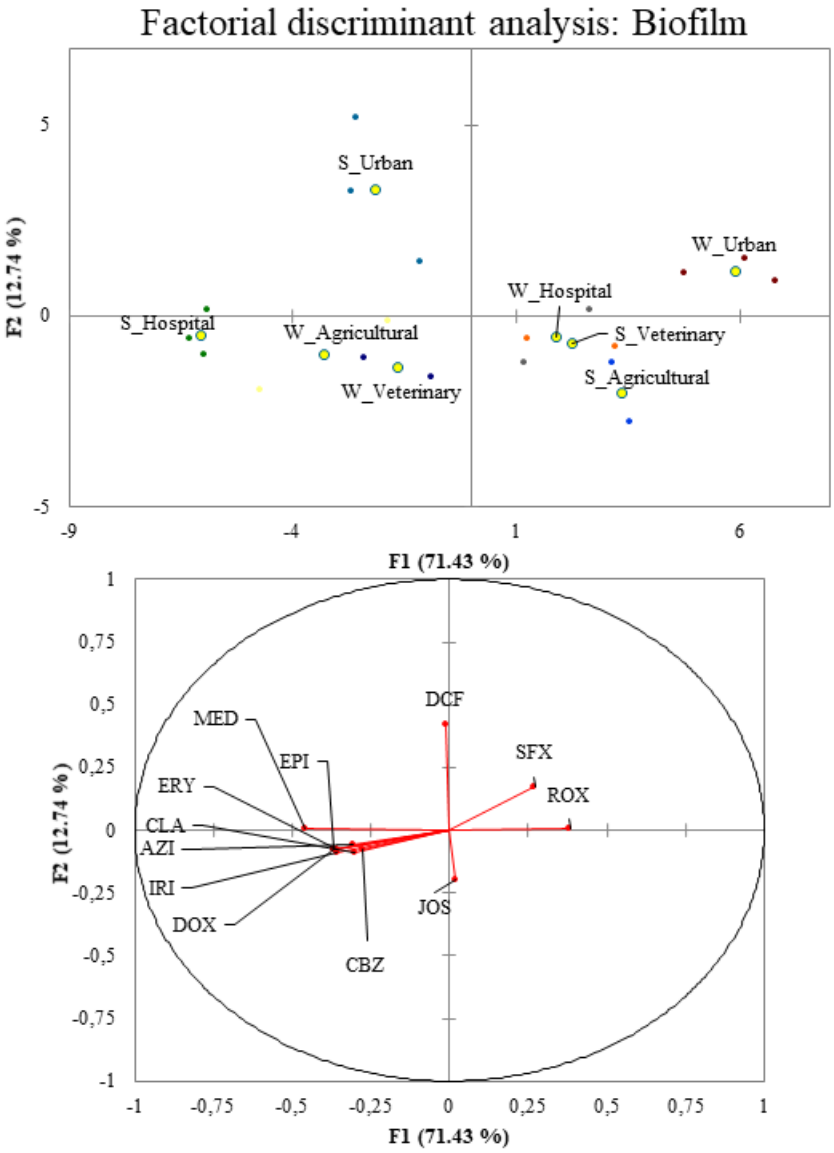
The results show the most marked differences between the zones, being most marked in the POCIS. Moreover, the points are clustered near the barycenters for the POCIS, but less so for biofilms, thus showing more variability. This could be explained by the natural heterogeneity of this compartment, as well as by the phenomena of sorption/desorption, loss and natural production of the microbial community that allow a constant renewal of biofilms compared to the POCIS.

Study 1 – Figure 5– Factor discriminant analysis of the POCIS samples in winter and summer. The letter S represents summer collection, and the letter W represents winter collection. ROX = roxithromycin, ERY = erythromycin, CLA= clarithromycin, AZI = azithromycin, SPI = spiramycin, IRI = irinotecan, MED = medicamycin, DOX = doxorubicin, SFX= sulfamethoxazole, EPI = epirubicin, JOS = josamycin, DCF = diclofenac and CBZ = carbamazepine



Source: (AUTHOR).

Study 1 – Figure 6– Factor discriminant analysis of the Biofilm samples in winter and summer. The letter S represents summer collection, and the letter W represents winter collection. ROX = roxithromycin, ERY = erythromycin, CLA= clarithromycin, AZI = azithromycin, SPI = spiramycin, IRI = irinotecan, MED = medicamycin, DOX = doxorubicin, SFX= sulfamethoxazole, EPI = epirubicin, JOS = josamycin, DCF = diclofenac and CBZ = carbamazepine



Source: (AUTHOR).

3.2 CONTAMINATION OF THE WATERSHED BY PHARMACEUTICAL COMPOUNDS

The concentration of the compounds found at each monitored point in the catchment are presented in Figures 11 and 12 (SM). About 86%, or 12 of the 14 compounds evaluated, were detected in the biofilm and POCIS samples in summer. In this season the only compounds not

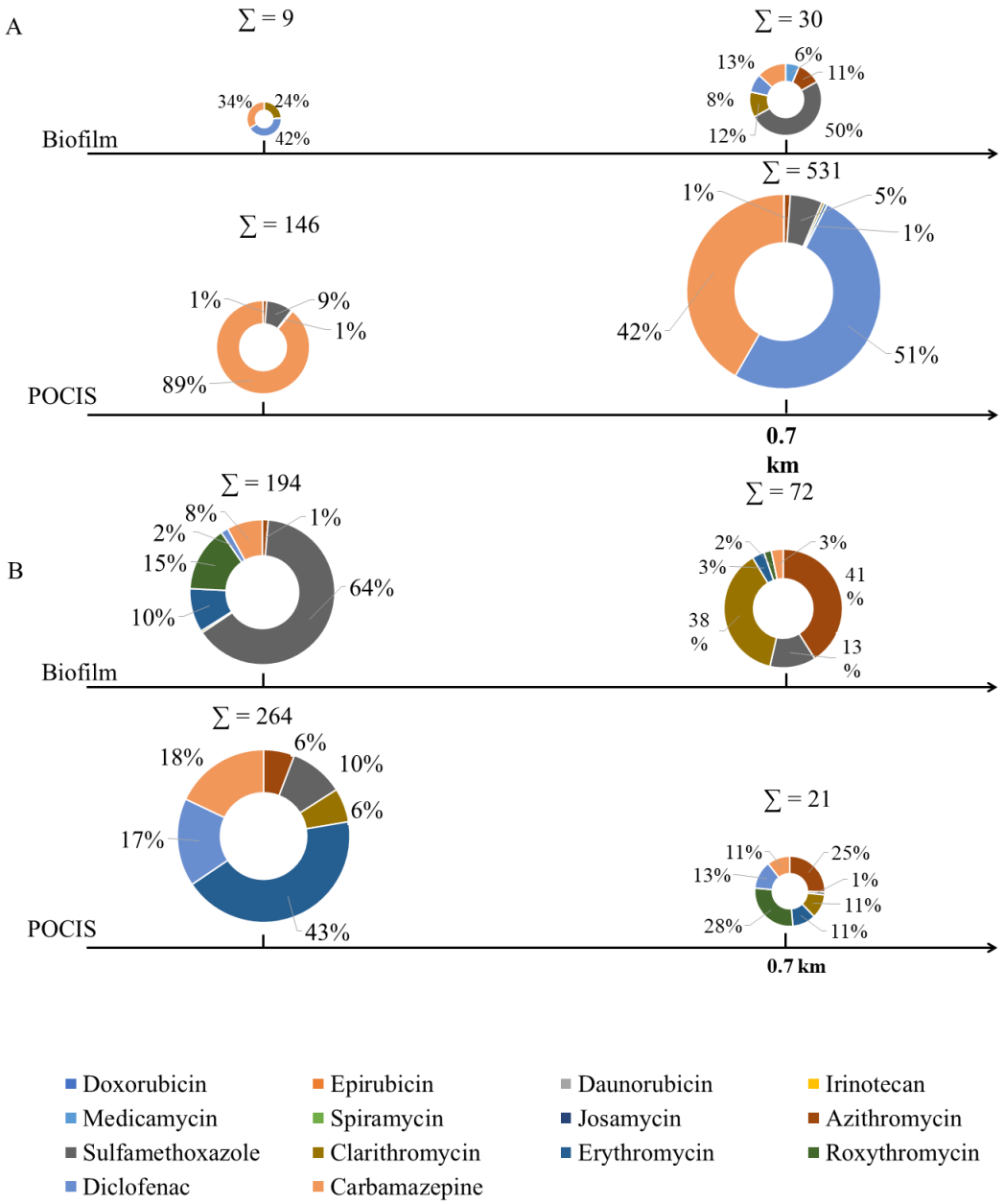
found at any point were DAU and SPI in both matrices. In winter the percentage of detected compounds drops to 64% in both POCIS and biofilms. The compound DAU was the only compound not found in any method and any season sampled. To better discuss the data, we separated them by zone according to the contaminant sources found.

3.2.1 Urban zone

These points are located in the urban area of Camobi, in the part exposed to irregular constructions and receiving diffuse urban runoff and probable clandestine discharge of untreated wastewater into the storm sewer system. These points are characterized by the presence of antibiotic compounds, carbamazepine and diclofenac in both biofilm and POCIS (Figure 7). These compounds confirm the mainly urban pressure on the environment at this location. No tracers of hospital activities, including anticancer compounds, were found. Thus, the results highlight concentrations ranging from 2 (CLA) (summer minimum) to 125 ng g^{-1} (SFX) (winter maximum) in biofilms and from 0.15 to 130 ng g^{-1} (summer minimum and maximum, respectively) in POCIS. Valdés et al. (2021) working with river biofilms in Argentina found similar concentrations for CLA (n.d. – 10 ng g^{-1}) in urban areas upstream of the treatment plant. It is possible to verify the effect of seasonality on the data as the concentration and variety of pharmaceuticals increase considerably in the winter season. In biofilms the only antibiotic detected in summer was CLA at a concentration of 2.26 ng g^{-1}. In winter, five antibiotic compounds were found: AZI, SFX, CLA, ERY and ROX, in concentrations ranging from 0.9 (CLA) to 125 (SFX) ng g^{-1}. In POCIS, the same antimicrobial compounds were found in both winter and summer (AZI, SFX, CLA, ERY), but at 2 to 100 times higher concentrations in winter.

The different occurrences of antibiotics between the two sampling seasons can be explained by the higher consumption of this pharmaceutical class in winter (LI; ZHANG, 2011). Furthermore, some researchers have found that seasonal fluctuations of antibiotics mass flows were very high between winter and summer. Antibiotic mass flows in winter may be two times higher than that in summer (MCARDELL et al., 2003). In addition, the biodegradation and photo-degradation of antibiotics may be higher in summer than in winter, due to the higher activity of microorganisms and the strong sunlight in summer (KARTHIKEYAN; MEYER, 2006).

Study 1 – Figure 7– Representation of the sum of the concentrations (ng g^{-1}) of compounds found in biofilms and POCIS at points 1 and 2 (Urban zone) of the university catchment. Seasons of the year represented by the letters (A) - summer and (B) - winter



Source: (AUTHOR).

3.2.2 Hospital zone - Close to hospital

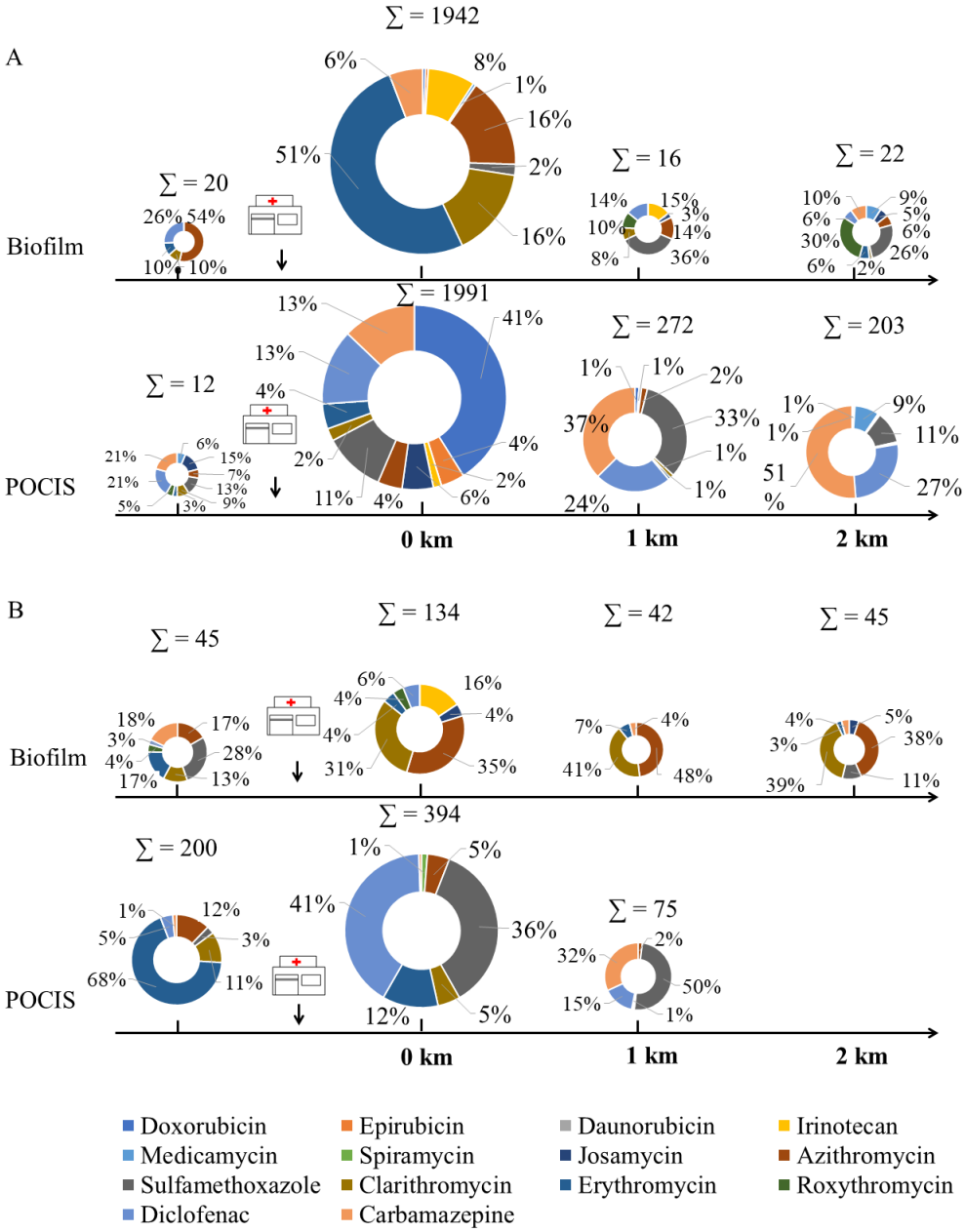
In figure 8, the one site upstream and three sites downstream of the hospital were monitored. The number and concentration of detected compounds increased downstream of the river, with a notable difference between the samples upstream, and downstream of HUSM.

Four anti-cancer compounds, considered to be tracers of hospital activities, were analyzed. The results show their presence (in high concentrations) exclusively downstream of the discharge point of the university hospital and in no other tributaries of the river. In summer, these compounds are found in smaller quantities also in the points further downstream. The concentrations at the effluent discharge point are 159.32 ng g^{-1} EPI in the biofilm and 818.40 ng g^{-1} DOX in the POCIS. Anticancer drugs are also regularly found in hospital wastewater and surface water samples from various countries (SANTANA-VIERA et al., 2019). DOX is an anticancer drug widely used for the treatment of various cancers and can be eliminated through urine (3-10%) and infections (40-50%) (GÓMEZ-CANELA et al., 2014b). DOX has an environmental concern, eco-genotoxicity studies have shown that this compound damages the DNA of Ceriodaphnia dubiacells Richard at a concentration of 0.05 μg L^{-1} (PARRELLA et al., 2015). Due to the potential risks, DOX is also one of the substances included in the list of emerging substances made by NORMAN (Network of reference laboratories, research centers, and related organizations for monitoring emerging environmental substances) (KELBERT et al., 2020a). All studied anti-cancer are suspected of carcinogenicity and mutagenicity, which are hazardous to the aquatic environment, and environmentally persistent. They are suspected of being respiratory sensitizers and having reproductive toxicity; and, irinotecan, as being a skin sensitizer (ECHA, 2016).

In addition to the concentration of the drugs increasing downstream of the hospital, the number and varieties of these drugs also increase. At the upstream point, only four compounds were found in the biofilms sampled in summer, after the hospital discharge this value increased to nine. For summer POCIS at the upstream point nine compounds were measured (all with concentrations less than 2.5 ng g^{-1}), and at the downstream point 10 compounds were found, with a minimum concentration of 28.5 ng g^{-1} (irinotecan - an anticancer). This same trend was found for winter biofilms, with a lower sum concentration of compounds at the point just after the effluent discharge. However, for the POCIS higher summations were found at the second point downstream, 1 km after HUSM. This shows that even though the contribution from the hospital is large, we cannot disregard the urban contribution within this zone, contributing large amounts of organic compounds.

It can be observed that the concentration of the sum of the pharmaceuticals is about 100 (biofilm) and 10 (POCIS) times higher downstream of the hospital compared to the more distant monitored sites. Although the hospital treats its waste in septic and anaerobic tanks before discharging it into the river, this treatment step appears to be ineffective in removing these compounds. Thus, the concentration and presence of these compounds increases immediately downstream of the hospital and then decreases through dilution. Furthermore, the presence of pollutants at the point upstream of the hospital shows that the water was already contaminated, probably due to the presence of little or untreated wastewater from the city and the university.

Study 1 – Figure 8 – Representation of the sum of the concentrations of compounds (ng g^{-1}) found in biofilms and POCIS in points 3, 4, 5 and 6 (Hospital zone) of the Vacaí-Mirim river basin. Seasons of the year represented by the letters (A) – summer and (B) – winter. * The last point the sampler was lost in a rainfall event



Source: (AUTHOR).

The present results suggest that the hospital is a major source of certain pharmaceuticals entering municipal wastewater, and associated water quality parameters are impacted. Hospitals, where a diverse range of compounds are used, are critical sources for pharmaceuticals entering municipal distribution networks. In addition, specific drug classes

(and individual compounds) are expected to result in higher hospital contributions, based on prescribing practices (e.g., hospital-specific substances) and national formularies (NIEMI et al., 2020). The hospital contribution related to the burden of an antibiotic was reported as 36% up to 94% in the UK and Germany, respectively (BEIER et al., 2011; HELWIG et al., 2013). Here we see the 85% contribution of the antibiotic class in hospital contamination in biofilms. In POCIS, on the other hand, this contribution drops to 26%.

In general, the mean concentrations followed the trend: erythromycin ≫ clarithromycin > azithromycin. For all compounds, higher concentrations were observed in the HUSM discharge compared to the other sampled points. PAULUS et al. (2019) reported that hospital wastewater contained approximately 25% more pharmaceutical compounds than community wastewater, indicating high use of these compounds within the hospital. Hospitals with specialized multi-professional wards (e.g. oncology, geriatrics, psychiatry, maternity, pediatrics, etc.) may produce wastewater with a much wider range of pharmaceuticals, as a larger number of different drugs will be routinely used (NIEMI et al., 2020). HUSM discharge concentrations were compared to literature values from medium-sized hospitals. AZI and CLA concentrations ranged respectively: from 0 - 4.5 and 0.10 - 0.87 ng L^{-1} (our study, converted values), 56 - 115 and 61- 96 ng L^{-1} (RODRIGUEZ-MOZAZ et al., 2015a), 0 - 179 and 4 - 24 ng L^{-1} (AZUMA et al., 2019) for hospitals with 400 and 480 beds, respectively. This indicates that the values found in our study with a 309-bed capacity hospital are slightly below those found in the literature with a slightly larger capacity.

Far from hospital influence

The two most downstream points of the hospital area also correspond to the outlet of the micro-catchment with the confluence of several tributaries of the monitored river. The pollution found here shows the presence of residues from the different sources of pollution.

In the biofilms sampled in summer, the concentration of the anticancer drug IRI is still perceptible at the first point (2.3 ng g^{-1}) but is no longer found at the last point. The presence of antibiotics and DCF is observed in both points and CBZ only in the last point. In the summer POCIS we found the presence of 2 anticancer drugs in the second point downstream of the hospital (DOX and IRI), and the persistence of DOX in the last point. Similarly, the concentration of SFX is the highest among the antibiotics found at the last two points (89.9 and 22.7 ng g^{-1}) outfall 1 and 2, respectively. Contamination by the anthropogenic markers CBZ and DCF is also observed at concentrations ranging from 58.7 to 104.2 ng g^{-1} at the last point.

Working with the contamination of French rivers with pharmaceutical molecules, Vystavna et al. (2017) found higher concentrations of CBZ in the Jaller River, concentrations in POCIS ranged from 100 to 600 ng g^{-1}, values much higher than found in this study.

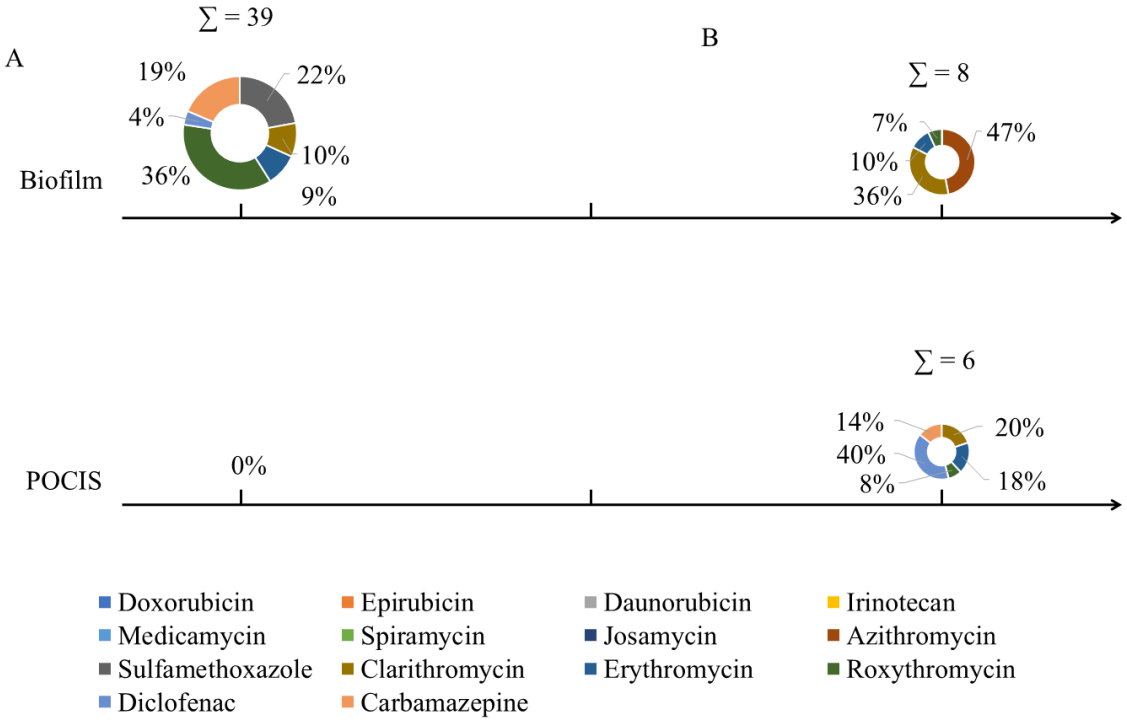
In winter there is no presence of anticancer compounds in the matrices studied. The concentrations of antibiotics increased compared to summer, a trend observed in the other collection points regarding seasonality. In the biofilms the highest concentrations of antibiotics were of AZI (20 and 17 ng g^{-1}) in both collection points. For the POCIS, of the antibiotics even higher concentrations were found for SFX (141.3 and 37.5 ng g^{-1}) in exutory 1 and 2, respectively. High concentrations were found of DCF in the exutory 1 (162.07 ng g^{-1}).

3.2.3 Agricultural zone

This part of studied area is mainly influenced by university crop-livestock interaction, fish farming, and public botanical garden. None of the compounds investigated were found in the POCIS during the summer period (Figure 9). However, in winter 3 antibiotics (CLA, ERY, ROX), and two tracers of human presence, CBZ and DCF were found at low concentrations (0.8 ng CBZ g^{-1}) to 2.2 ng DCF g^{-1}). The maximum antibiotic concentration was for CLA with 2.2 ng g^{-1}. In contrast, all antibiotics were found to be present in biofilms in both seasons, even the compounds DCF and CBZ were found only in summer.

The constant presence of students, professors, and visitors in the botanical garden; the circulation of cattle, even with direct access to the water courses; and especially the fish farming activity is transferring various pharmaceuticals residues into the environment. Zheng et al. (2012) studying the impact of aquaculture activities and the occurrence of antibiotics in rivers in China, found that concentrations of ERY and SFX near aquaculture activities were higher, suggesting that greater intensity of aquaculture activities could contribute to increased levels of antibiotics in the environment. The presence of other pharmaceuticals, other than antibiotics, is regularly reported in rural areas with low population density (NEBOT et al., 2015), proving that effluents can produce quantifiable levels of human pharmaceutical in the natural aquatic environment.

Study 1 – Figure 9 – Representation of the sum of the concentrations of compounds (ng g^{-1}) found in biofilms and POCIS in point 7 (Agricultural zone) of the monitored catchment. Seasons of the year represented by the letters (A)



– summer and (B) – winter

Source: (AUTHOR).

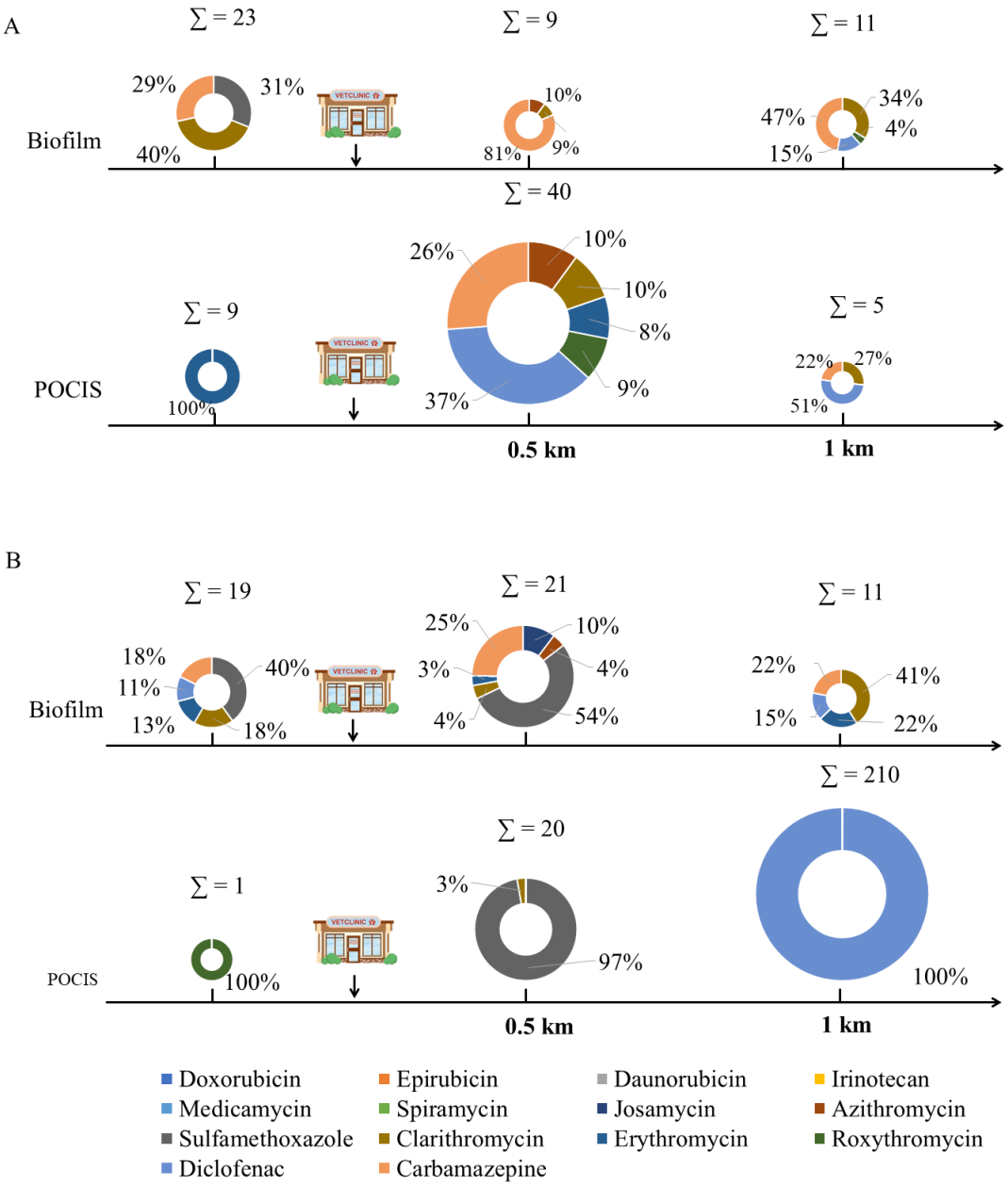
3.2.4 Veterinary zone

The University Veterinary Hospital (UVH) of Santa Maria is a small clinic that treats different types of animals. The biofilms monitored at all sites contain different pharmaceutical compounds. In summer, the biofilm sampled downstream of UHV contains two antibiotics with low concentrations (0.8 and 0.7 ng g^{-1} of AZI and CLA, respectively) and the high concentration of CBZ (7.1 ng g^{-1}) (Figure 10). However, the measurements made by POCIS show a greater number of compounds downstream of the UHV: 6 pharmaceuticals compounds (4 antibiotics and 2 anthropogenic markers) were found. The antibiotics varied in concentration from 3.3 to 4 ng g^{-1}, while the concentration of DCF reached 14.7 ng g^{-1}.

In winter, a greater number of compounds is observed - with the most marked presence of antibiotics - in the epilithic biofilms sampled downstream of the HV. Higher concentrations of SFX were found with values of 11.2 and 40.5 ng g^{-1} at 0.5 and 1.0 km downstream of the UVH. This compound, SFX, is used for both humans and animals, leading to the release of

these compounds into surface water from both agricultural and urban areas (JIANG et al., 2011). Investigating the contamination of surface water by antibiotics (CHEN; ZHOU, 2014) found that the sulfonamide group had the highest concentrations. Moreover, sulfonamides have a high potential to resist degradation and are hydrophilic enough to be transported in the aquatic environment for a long distance. These properties may contribute to their high detection frequencies and concentrations in the aquatic environment. In the POCIS in winter, fewer compounds appeared than in summer. After the UVH only 2 antibiotics were found: SFX (19.7 ng g^{-1}) and CLA (0.5 ng g^{-1}). Although these concentrations are not remarkably high, the constant input of these compounds, especially at a known point source of pollution, can cause damage to the aquatic biota. Antibiotics for human use are continuously released into aquatic environments via point-source wastewater, whereas those for veterinary and agricultural use enter by non-point sources, mainly after rainfall events.

Study 1 – Figure 10 – Representation of the sum of the concentrations of compounds (ng g^{-1}) found in biofilms and POCIS in points 8, 9 and 10 (Hospital veterinary zone). Seasons of the year represented by the letters (A) – summer and (B) – winter



Source : (AUTHOR).

3.2.5 Preferential location of the pollution

For POCIS and biofilm, the selectivity of the molecules is different.

In general, we can observe that the matrices present different selectivity for the compounds type. In the urban zone monitored during the summer season, the diversity and amounts of pharmaceutical residues bioaccumulated by epilithic biofilms are less than by POCIS. The POCIS shows a greater capacity to adsorb CBZ and DCF from water in the river and is very sensitive of the climate season. While POCIS was great adsorbent of ERY, epilithic biofilms bioaccumulated higher concentrations of SFX. Recent works dealing with biofilms pointed out that the accumulation of organic pollutants inside biofilms is governed by the affinity between biofilm structural and these compounds (DING et al., 2015; MÉTIVIER et al., 2013a). Thus, Huerta al. (2016) and Aubertheau et al. (2017) have explained low accumulation of CBZ, for example, in their samples by the low affinity of CBZ for the biofilm despite the large distribution of this compound in the considered river. This finding indicates that the small presence of CBZ in the biofilms represents a significant presence of CBZ in the corresponding site evidenced by the high values found in POCIS. In a study with POCIS and biofilms in a southern Brazilian watershed, BASTOS et al. (2018) found higher CBZ concentrations in POCIS. The authors point out that biotransformation of contaminants by biofilms could explain the reasons that POCIS are sometimes more capable to capture CBZ and others compounds.

We can observe a different affinity between the two absorption matrices, most likely due to the physicochemical characteristics of the compounds as their octanol-water partition coefficient. Compounds like IRI have a high octanol-water partition coefficient (Kow 3.20), which ensures higher retention of these compounds in non-polar matrices, reducing their concentration in solution and consequently decreasing their exposure to POCIS. In our results, we can observe that in the hospital monitored zone, the compound found in higher concentration in the POCIS (DOX) has its lowest octanol-water partition coefficient (Kow 1.27) while the compound most found in the biofilm (IRI), as already mentioned, has a higher coefficient.

However, in less contaminated zone, Veterinary and Agricultural, in general we observed that the biofilm matrix bioaccumulates more compounds than by POCIS sampler. showing itself to be less selective and broader for capturing organic compounds from the aquatic environment. Biofilm shows broad selectivity for antibiotics and the anthropic markers. Biofilms are pointed out as excellent bioindicators of antibiotics pollution and the high levels and risk found call for attention to possible effects on these communities (and higher trophic levels) and selection of antibiotic resistance (VALDÉS et al., 2021).

We can see that the antibiotic CLA is present in 100% of the epilithic biofilms monitored (80% in POCIS sampler) at all sites in both seasons. The permanent antibiotics impregnated in the biofilm can modify the diversity and functionality of bacterial community. It has already been verified that bacterial mortality increased in biofilms from the most polluted site, and significantly correlated with concentrations of quinolones and sulfonamides (PROIA et al., 2013). The CLA, for example, is protein synthesis inhibitor, which may limit the number of enzymes (e.g., peptidase and phosphatase) that each cell can produce. Nevertheless, biofilms grown under certain levels of antibiotics concentration are expected to develop a bacterial community more tolerant to the direct stressors (antibiotic), which can become an even bigger problem.

In summary, our study showed that the POCIS proved to be able at capturing more apolar molecules, such as ERY, DCF and CBZ. Even though it is expected that these types of compounds do not accumulate well in the reservoir due to their high octanol-water partition coefficient (Kow > 3), this does not ensure better retention of these compounds in polar matrices. Although the use of passive samplers, POCIS and biofilm, are promising in improving the detection system of contaminants in river waters, there are knowledge gaps that need to be overcome, such as identifying the processes that occur within the biofilm community, standardizing the sampling rates (Rs) in each study, adequacy of this type of sampling in areas with high organic matter loading, among others. To make the monitoring more efficient in detecting the drugs, as well as for comparative purposes in each study and between studies.

3.3 ENVIRONMENTAL RISK ASSESSMENT

To assess environmental risk (RQ) induced by pharmaceuticals residues pollution, RQ values were calculated for the summer and winter samples (Figure 11). Values lower than 0.1 are considered minimal risk, between 0.1 and 1 the risk is medium and greater than 1 is considered high risk. The median risks of the compounds are very similar, most compounds showed minimal risk (< 0.1) in both seasons. However, for 4 compounds in the summer median risk values were found: DOX, CAF, EPI and ERY. In winter, the same compounds except for DOX also presented a medium risk. High risk was found for EPI (anticancer) and CAF in summer. In winter high risk was found for ERY (antibiotic) and CAF.

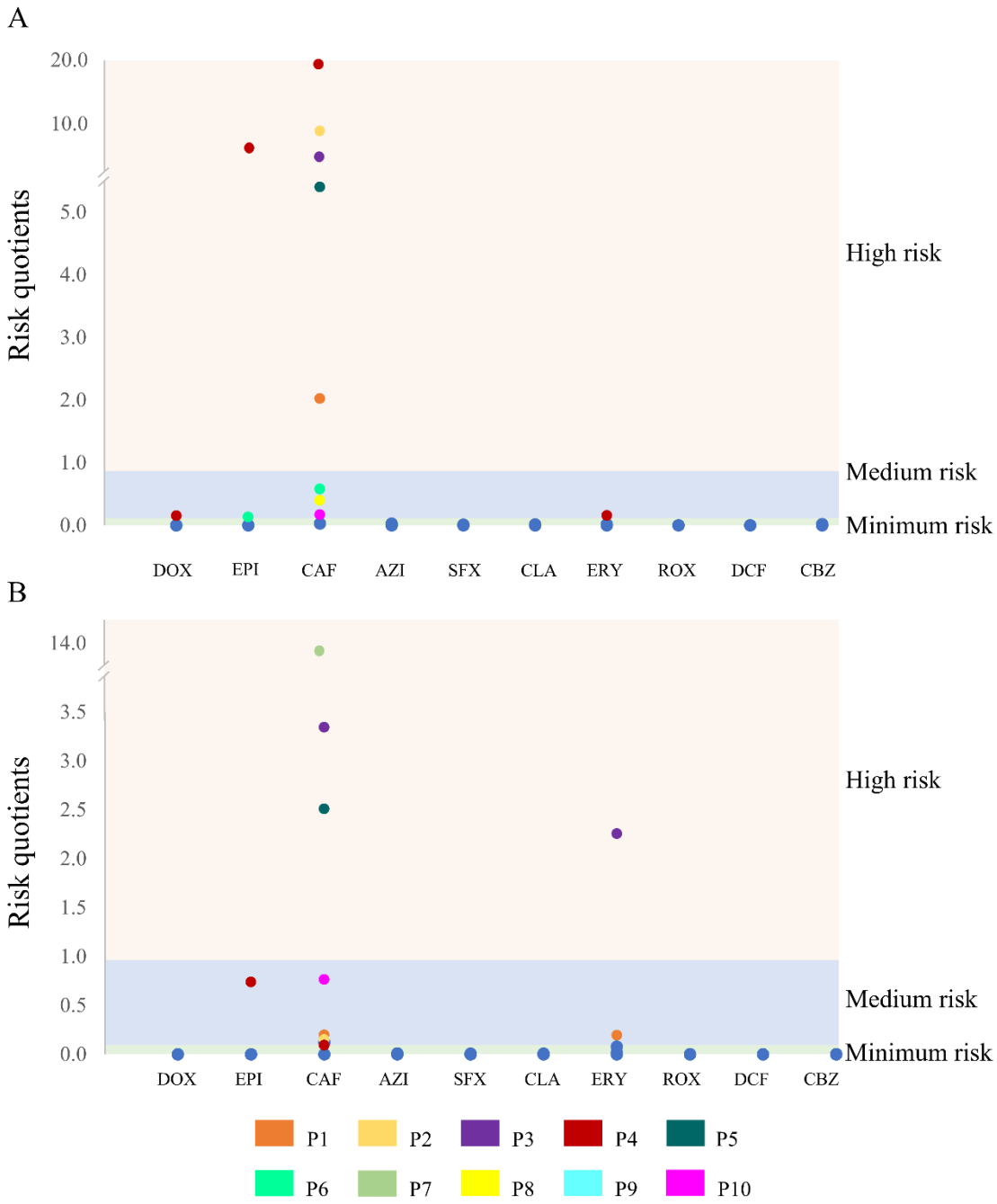
High risks for caffeine have already been found in other work in surface waters in southern Brazil (PETEFFI et al., 2019). These results highlight the anthropogenic influence on the health and quality of the environment since the most vulnerable spots are the most urbanized

ones. Caffeine is a marker for the disposal of untreated sewage and considering the high loads of untreated domestic sewage in addition to the often inefficient Brazilian sanitation system (LÓPEZ-DOVAL et al., 2017). The results obtained in this study indicate contamination of Santa Maria's rivers and reservoirs by domestic sewage and that such contamination presents a high risk to aquatic biota. Under long-term exposure, even low concentrations of caffeine and can trigger adverse effects targeting the physiology and behavior of aquatic species (DI LORENZO et al., 2019). The effect of chronic exposure to sub-lethal concentrations of caffeine is known to increase activity and alertness, disrupt sleep patterns, and drop learning and memory attitudes in invertebrates (MARANHO et al., 2015).

In Spain, DOX and IRI were quantified in wastewater at concentrations that posed insignificant and high environmental risks, respectively (OLALLA et al., 2018). Medium risk levels for DOX and low-risk levels for EPI and IRI were found in the effluent from a WWTP in Spain (MARTÍN et al., 2014). In the case of IRI and DOX, the risk can be attributed to their persistence and toxicity since neither of them bioaccumulates.

Souza et al. (2018) working directly with SMUH effluent, found high risks for both DOX and PPE. These authors found much higher risk values working directly with the hospital effluent, which shows that although there was a dilution effect in the river, even after its disposal the effluent remains a strong source of contamination. These ecotoxicological risks should be taken as an indication of a potential hazard to the environment. In the inventory compiled by the European Chemicals Agency, DOX and EPI are suspected of carcinogenicity and mutagenicity, which are hazardous to the aquatic environment, and environmentally persistent.

Study 1 – Figure 11 – Risk quotients of pharmaceuticals at the points sampled in summer (A) and winter (B). DOX = doxorubicin, EPI = epirubicin, CAF = caffeine, AZI = azithromycin, SFX= sulfamethoxazole, CLA= clarithromycin, ERY = erythromycin, ROX = roxithromycin, DCF = diclofenac and CBZ = carbamazepine



Source: (AUTHOR).

The individual concentrations of most of the compounds studied pose low environmental risks. However, the potential negative effects on aquatic ecosystems should draw our attention because of their continued long-term discharge. Several studies point out that the

effects of the toxic mixture of chemicals are higher than the individual effect (GEIGER; HORNEK-GAUSTERER; SAÇAN, 2016a). Therefore, we should be cautious when evaluating the concentrations and risks that pharmaceutical compounds contribute to the environment. Furthermore, even low concentrations of pharmaceuticals could be potentially risky for the environment because they are typically designed to cause biological effects at low doses.

Anticancer drugs could constitute a threat for aquatic matrices, especially the one which receives effluents from WWTPs. The occurrence of widely used anti-cancer in waters can cause both acute and chronic toxicity, as well as have a detrimental impact on the genetic material in a variety of organisms (JURECZKO; KALKA, 2020). Studies have shown that pharmaceuticals may act as ecological disruptors even at low levels, altering biofilm structure and some aspects of ecosystem function, and potentially influencing important biogeochemical processes in streams (STOVER; JUDD, 2021). As well as continuous exposure to drugs in urban streams may select for sub-populations of highly resistant bacteria that maintain community function in response to urban contaminants (ROSI et al., 2018).

4 CONCLUSION

The results of this study show that there is a high variability of pharmaceutical pollution on a micro-catchment scale, and that locally concentrations are amplified by exposure to a source and enriched by compounds that may be specific. Further away from the source of exposure, dilution or other inputs contribute to a reduction in concentrations, but low levels may remain. The study also shows that the season has a significant impact on pollution and can modify the diversity and quantity of compounds found. Our observations show that a catchment area is a mosaic of areas of anthropogenic pressure arranged with each other and influenced by each other.

This study also shows for the first time the impact of a university campus on the environment. The area of the Santa Maria campus appears to be contaminated by different types of pharmaceuticals, with concentrations of more than 900 ng g^{-1} in biofilms and more than 800 ng g^{-1} in POCIS. We can see that antibiotic contamination is strongly present and that HUSM is the largest pollutant source in the watershed. However, despite the hospital's high drug loads, these are diluted a few kilometers after the hospital effluent is discharged. Thus, the adoption of the university campus as a model watershed becomes efficient to distinguish the sources of contamination.

Our study demonstrates success in applying passive sensors to monitor water quality. Both biofilms and POCIS showed to be effective captors but with different specificity between the compounds. In POCIS there is a tendency to accumulate in large concentrations and the biofilm shows a tendency to accumulate less, but with a greater diversity of compounds.

This presence of drug residues represents an environmental and health risk. The anticancer drugs epirubicin, the stimulant caffeine and the antibiotic erythromicin presented a medium and high risk for aquatic biota. The anticancer doxorubicin presents a medium risk. Further studies on the effects of these pharmaceuticals on other aquatic organisms and their presence in water sources are needed to evaluate possible future measures.

5 REFERENCES

ALVAREZ, D. A. et al. Development of a Passive, in Situ, Integrative Sampler for Hydrophilic Organic Contaminants in Aquatic Environments. **Environmental Toxicology and Chemistry**, v. 23, n. 7, p. 1640, 2004.

ANA. **Saneamento — Agência Nacional de Águas**. Capa. Disponível em: <https://www.ana.gov.br/aguas-no-brasil/saiba-quem-regula/saneamento>. Acesso em: 4 jun. 2020.

ANILANMERT, B. et al. Potentiometric determination of the dissociation constants of epirubicin HCl and irinotecan HCl. **Mendeleev Communications**, v. 16, n. 2, p. 97–98, jan. 2006.

ARAÚJO, M. P. et al. Fecal sterols as sewage contamination indicators in Brazilian mangroves. **Marine Pollution Bulletin**, v. 165, p. 112149, 1 abr. 2021.

AUBERTHEAU, E. et al. Impact of wastewater treatment plant discharge on the contamination of river biofilms by pharmaceuticals and antibiotic resistance. **Science of The Total Environment**, v. 579, p. 1387–1398, fev. 2017a.

AZUMA, T. et al. Environmental fate of pharmaceutical compounds and antimicrobial-resistant bacteria in hospital effluents, and contributions to pollutant loads in the surface waters in Japan. **Science of The Total Environment**, v. 657, p. 476–484, mar. 2019.

BAI, X. et al. Occurrence, distribution, and seasonality of emerging contaminants in urban watersheds. **Chemosphere**, v. 200, p. 133–142, 1 jun. 2018.

BASTOS, M. C. et al. Presence of Anthropogenic Markers in Water: A Case Study of the Guaporé River Watershed, Brazil. **CLEAN - Soil, Air, Water**, v. 46, n. 3, p. 1700019, mar. 2018.

BEIER, S. et al. Full scale membrane bioreactor treatment of hospital wastewater as forerunner for hot-spot wastewater treatment solutions in high density urban areas. **Water Science and Technology**, v. 63, n. 1, p. 66–71, 1 jan. 2011.

BRUN, F. G. K. et al. Impactos ambientais no entorno da sanga “Lagoão do Ouro” – bairro Camobi, Santa Maria (RS) Environmental impacts in around the “Lagoão do Ouro” stream – Camobi neighborhood, Santa Maria (RS). **AMBIÊNCIA**, v. 7, n. 3, p. 551–573, 16 dez. 2011.

CECONI, D. E. et al. Composição florística e potencial de regeneração da mata ciliar remanescente de um tributário do Vacacaí-Mirim, Santa Maria - RS. **Ciência Florestal**, v. 28, p. 1546–1558, dez. 2018.

CHAUMET, B. et al. Diuron sorption isotherms in freshwater biofilms. **Science of The Total Environment**, v. 651, p. 1219–1225, fev. 2019.

CHAVES, M. DE J. S. et al. Pharmaceuticals and personal care products in a Brazilian wetland of international importance: Occurrence and environmental risk assessment. **Science of The Total Environment**, v. 734, p. 139374, 10 set. 2020.

CHEN, K.; ZHOU, J. L. Occurrence and behavior of antibiotics in water and sediments from the Huangpu River, Shanghai, China. **Chemosphere**, v. 95, p. 604–612, jan. 2014.
CHEN, Y. et al. Occurrence, removal and environmental risk assessment of pharmaceuticals and personal care products in rural wastewater treatment wetlands. **Science of The Total Environment**, v. 566–567, p. 1660–1669, out. 2016.

COGO, C. M.; ELTZ, F. L. F.; CASSOL, E. A. Erosividade das chuvas em Santa Maria, RS, determinada pelo índice EI30. v. 14, p. 9, 2006.

DAR, S. A.; BHAT, R. A. Aquatic Pollution Stress and Role of Biofilms as Environment Cleanup Technology. In: QADRI, H. et al. (Eds.). . **Fresh Water Pollution Dynamics and Remediation**. Singapore: Springer Singapore, 2020. p. 293–318.

DI LORENZO, T. et al. The role of freshwater copepods in the environmental risk assessment of caffeine and propranolol mixtures in the surface water bodies of Spain. **Chemosphere**, v. 220, p. 227–236, 1 abr. 2019.

DING, Z. et al. Role of extracellular polymeric substances (EPS) production in bioaggregation: application to wastewater treatment. **Applied Microbiology and Biotechnology**, v. 99, n. 23, p. 9883–9905, 1 dez. 2015.

ECHA. **ECHA - European Chemicals Agency**. Disponível em: <https://echa.europa.eu/information-on-chemicals/annex-iii-inventory>. Acesso em: 14 jun. 2021.

FALÅS, P. et al. Occurrence and reduction of pharmaceuticals in the water phase at Swedish wastewater treatment plants. **Water Science and Technology**, v. 66, n. 4, p. 783–791, 1 ago. 2012.

GEIGER, E.; HORNEK-GAUSTERER, R.; SAÇAN, M. T. Single and mixture toxicity of pharmaceuticals and chlorophenols to freshwater algae Chlorella vulgaris. **Ecotoxicology and Environmental Safety**, v. 129, p. 189–198, jul. 2016.

GÓMEZ-CANELA, C. et al. Occurrence of cytostatic compounds in hospital effluents and wastewaters, determined by liquid chromatography coupled to high-resolution mass spectrometry. **Analytical and Bioanalytical Chemistry**, v. 406, n. 16, p. 3801–3814, jun. 2014.

HANAMOTO, S. et al. Source estimation of pharmaceuticals based on catchment population and in-stream attenuation in Yodo River watershed, Japan. **Science of The Total Environment**, v. 615, p. 964–971, 15 fev. 2018.

HANSEN, K. M. S. et al. Ozonation for source treatment of pharmaceuticals in hospital wastewater – Ozone lifetime and required ozone dose. **Chemical Engineering Journal**, v. 290, p. 507–514, 15 abr. 2016.

HELWIG, K. et al. Micropollutant Point Sources in the Built Environment: Identification and Monitoring of Priority Pharmaceutical Substances in Hospital Effluents. **Journal of Environmental & Analytical Toxicology**, v. 3, n. 4, 1 jan. 2013.

INTERFARMA. **Guia 2018 : Dados do setor**. Disponível em: <https://www.interfarma.org.br/guia/guia-2018/dados_do_setor#ranking_das_industrias>. Acesso em: 15 mar. 2020.

JAFFRÉZIC, A. et al. Veterinary pharmaceutical contamination in mixed land use watersheds: from agricultural headwater to water monitoring watershed. **Science of The Total Environment**, v. 609, p. 992–1000, 31 dez. 2017.

JIANG, L. et al. Occurrence, distribution and seasonal variation of antibiotics in the Huangpu River, Shanghai, China. **Chemosphere**, v. 82, n. 6, p. 822–828, fev. 2011.

JURECZKO, M.; KALKA, J. Cytostatic pharmaceuticals as water contaminants. **European Journal of Pharmacology**, v. 866, p. 172816, 5 jan. 2020.

KARTHIKEYAN, K. G.; MEYER, M. T. Occurrence of antibiotics in wastewater treatment facilities in Wisconsin, USA. **Science of The Total Environment**, v. 361, n. 1–3, p. 196–207, maio 2006.

KELBERT, M. et al. Laccase as an efficacious approach to remove anticancer drugs: A study of doxorubicin degradation, kinetic parameters, and toxicity assessment. **Journal of Hazardous Materials**, p. 124520, nov. 2020.

KOMORI, K. et al. Occurrence of selected pharmaceuticals in river water in Japan and assessment of their environmental risk. **Environmental Monitoring and Assessment**, v. 185, n. 6, p. 4529–4536, jun. 2013.

LI, H.; HELM, P. A.; METCALFE, C. D. Sampling in the Great Lakes for pharmaceuticals, personal care products, and endocrine-disrupting substances using the passive polar organic chemical integrative sampler. **Environmental Toxicology and Chemistry**, v. 29, n. 4, p. 751–762, abr. 2010.

LI, B.; ZHANG, T. Mass flows and removal of antibiotics in two municipal wastewater treatment plants. **Chemosphere**, v. 83, n. 9, p. 1284–1289, 1 maio 2011.

LI, N. et al. Occurrence, seasonal variation and risk assessment of antibiotics in the reservoirs in North China. **Chemosphere**, v. 111, p. 327–335, 1 set. 2014.

LIN, A. Y.-C.; YU, T.-H.; LIN, C.-F. Pharmaceutical contamination in residential, industrial, and agricultural waste streams: Risk to aqueous environments in Taiwan. p. 11, 2008.

LOCATELLI, M. A. F.; SODRÉ, F. F.; JARDIM, W. F. Determination of Antibiotics in Brazilian Surface Waters Using Liquid Chromatography–Electrospray Tandem Mass Spectrometry. **Archives of Environmental Contamination and Toxicology**, v. 60, n. 3, p. 385–393, 1 abr. 2011.

LÓPEZ-DOVAL, J. C. et al. Nutrients, emerging pollutants and pesticides in a tropical urban reservoir: Spatial distributions and risk assessment. **Science of The Total Environment**, v. 575, p. 1307–1324, jan. 2017.

MACHADO, A. I. et al. Overview of the state of the art of constructed wetlands for decentralized wastewater management in Brazil. **Journal of Environmental Management**, v. 187, p. 560–570, fev. 2017.

MACLEOD, S.; MCCLURE, E.; WONG, C. Laboratory calibration and field deployment of the Polar Organic Chemical Integrative Sampler for pharmaceuticals and personal care products in wastewater and surface water. **Environmental Toxicology and Chemistry**, v. preprint, n. 2007, p. 1, 2007.

MARANHO, L. A. et al. A Candidate Short-Term Toxicity Test Using Ampelisca brevicornis to Assess Sublethal Responses to Pharmaceuticals Bound to Marine Sediments. **Archives of Environmental Contamination and Toxicology**, v. 68, n. 2, p. 237–258, 1 fev. 2015.

MARTÍN, J. et al. Occurrence and Ecotoxicological Risk Assessment of 14 Cytostatic Drugs in Wastewater. **Water, Air, & Soil Pollution**, v. 225, n. 3, p. 1896, 16 fev. 2014.

MARTINS, A. F. et al. Occurrence of the Antimicrobials Sulfamethoxazole and Trimethoprim in Hospital Effluent and Study of Their Degradation Products after Electrocoagulation. **CLEAN - Soil, Air, Water**, v. 39, n. 1, p. 21–27, jan. 2011.
MCARDELL, C. S. et al. Occurrence and Fate of Macrolide Antibiotics in Wastewater Treatment Plants and in the Glatt Valley Watershed, Switzerland. **Environmental Science & Technology**, v. 37, n. 24, p. 5479–5486, 1 dez. 2003.

MÉTIVIER, R. et al. Interaction of erythromycin ethylsuccinate and acetaminophen with protein fraction of extracellular polymeric substances (EPS) from various bacterial aggregates. **Environmental Science and Pollution Research**, v. 20, n. 10, p. 7275–7285, out. 2013.

MONTAGNER, C. C.; JARDIM, W. F. Spatial and seasonal variations of pharmaceuticals and endocrine disruptors in the Atibaia River, São Paulo State (Brazil). **Journal of the Brazilian Chemical Society**, v. 22, p. 1452–1462, ago. 2011.

NEBOT, C. et al. Introduction of human pharmaceuticals from wastewater treatment plants into the aquatic environment: a rural perspective. **Environmental Science and Pollution Research**, v. 22, n. 14, p. 10559–10568, 1 jul. 2015.

NIEMI, L. et al. Assessing hospital impact on pharmaceutical levels in a rural ‘source-to-sink’ water system. **Science of The Total Environment**, v. 737, p. 139618, out. 2020.

OLALLA, A. et al. A case study to identify priority cytostatic contaminants in hospital effluents. **Chemosphere**, v. 190, p. 417–430, 1 jan. 2018.

PARRELLA, A. et al. Eco-genotoxicity of six anticancer drugs using comet assay in daphnids. **Journal of Hazardous Materials**, v. 286, p. 573–580, abr. 2015.

PAULUS, G. K. et al. The impact of on-site hospital wastewater treatment on the downstream communal wastewater system in terms of antibiotics and antibiotic resistance genes. **International Journal of Hygiene and Environmental Health**, v. 222, n. 4, p. 635–644, maio 2019.

PETEFFI, G. P. et al. Ecotoxicological risk assessment due to the presence of bisphenol A and caffeine in surface waters in the Sinos River Basin - Rio Grande do Sul - Brazil. **Brazilian Journal of Biology**, v. 79, n. 4, p. 712–712, nov. 2019.

PIVETTA, G.; GASTALDINI, M. Presence of emerging contaminants in urban water bodies in southern Brazil. **Journal of Water and Health**, v. 17, n. 2, p. 329–337, 5 fev. 2019.

PROIA, L. et al. Response of biofilm bacterial communities to antibiotic pollutants in a Mediterranean river. **Chemosphere**, v. 92, n. 9, p. 1126–1135, 1 ago. 2013.

REHMAN, M. S. U. et al. Global risk of pharmaceutical contamination from highly populated developing countries. **Chemosphere**, v. 138, p. 1045–1055, 1 nov. 2015.

RODRIGUEZ-MOZAZ, S. et al. Occurrence of antibiotics and antibiotic resistance genes in hospital and urban wastewaters and their impact on the receiving river. **Water Research**, v. 69, p. 234–242, fev. 2015.

ROSI, E. J. et al. Urban stream microbial communities show resistance to pharmaceutical exposure. **Ecosphere**, v. 9, n. 1, p. e02041, 2018.
SANTANA-VIERA, S. et al. Simultaneous and systematic analysis of cytostatic drugs in wastewater samples by ultra-high performance liquid chromatography tandem mass spectrometry. **Journal of Chromatography B**, v. 1110–1111, p. 124–132, mar. 2019.

SANTOS, L. H. M. L. M. et al. Contribution of hospital effluents to the load of pharmaceuticals in urban wastewaters: Identification of ecologically relevant pharmaceuticals. **Science of The Total Environment**, v. 461–462, p. 302–316, set. 2013.

SOUZA, D. M.; REICHERT, J. F.; MARTINS, A. F. A simultaneous determination of anti-cancer drugs in hospital effluent by DLLME HPLC-FLD, together with a risk assessment. **Chemosphere**, v. 201, p. 178–188, jun. 2018a.

STOVER, E. M.; JUDD, K. E. Low-level pharmaceuticals alter stream biofilm structure and function. **Chemistry and Ecology**, v. 0, n. 0, p. 1–17, 6 maio 2021.

TELL, J. et al. Science-based Targets for Antibiotics in Receiving Waters from Pharmaceutical Manufacturing Operations. **Integrated Environmental Assessment and Management**, v. 15, n. 3, p. 312–319, 2019.

VALDÉS, M. E. et al. Distribution of antibiotics in water, sediments and biofilm in an urban river (Córdoba, Argentina, LA). **Environmental Pollution**, v. 269, p. 116133, jan. 2021.

VERLICCHI, P. et al. Hospital effluent: Investigation of the concentrations and distribution of pharmaceuticals and environmental risk assessment. **Science of The Total Environment**, v. 430, p. 109–118, jul. 2012.

VYSTAVNA, Y. et al. Removal efficiency of pharmaceuticals in a full scale constructed wetland in East Ukraine. **Ecological Engineering**, v. 108, p. 50–58, nov. 2017.

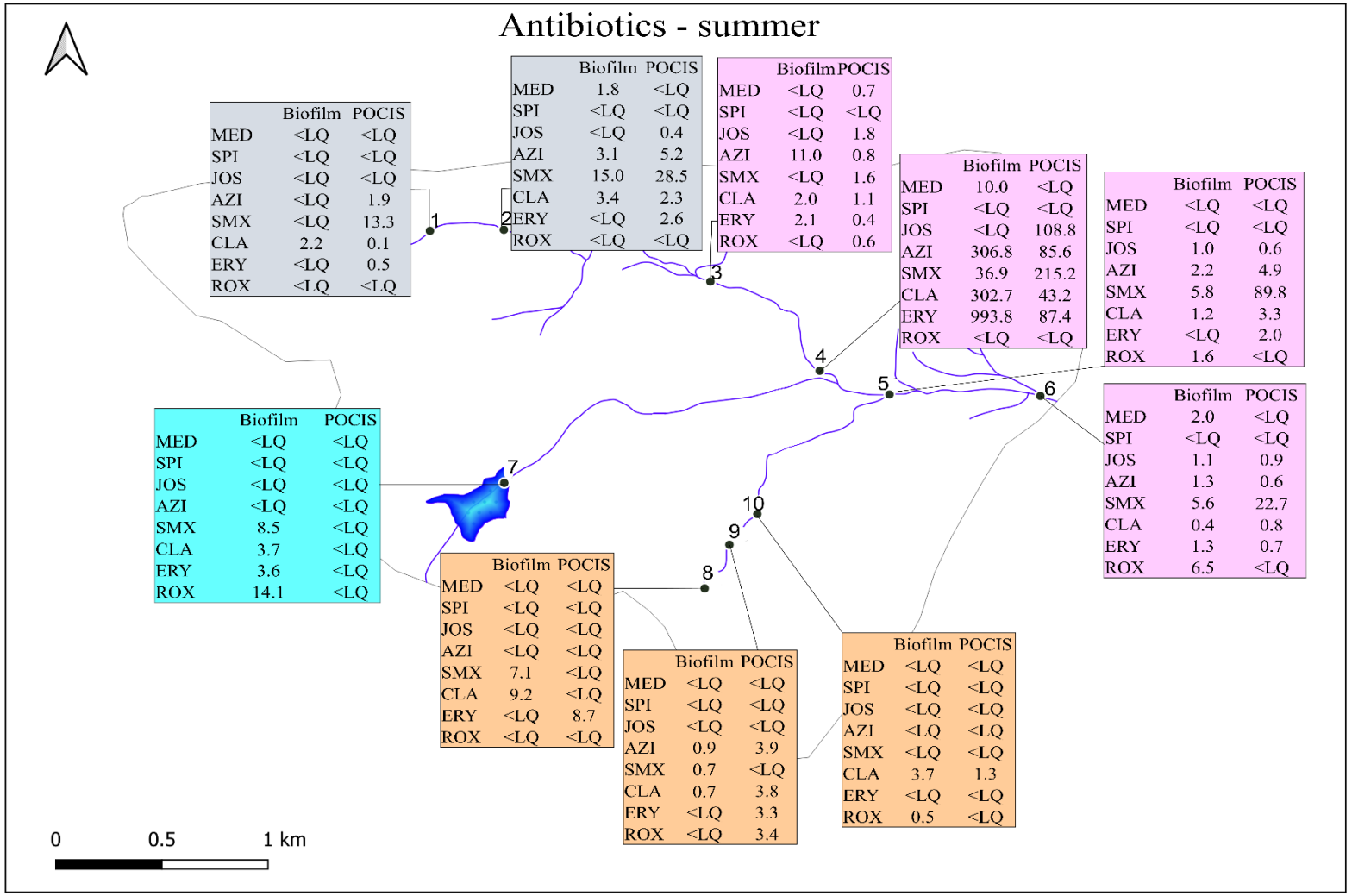
WHO. **World Health Organization - Report on Surveillance of antibiotic Consumption.** Disponível em: <https://www.who.int/medicines/areas/rational_use/who-amr-amc-report-20181109.pdf?ua=1>. Acesso em: 19 set. 2020.

ZHANG, Z.; HIBBERD, A.; ZHOU, J. L. Analysis of emerging contaminants in sewage effluent and river water: Comparison between spot and passive sampling. **Analytica Chimica Acta**, v. 607, n. 1, p. 37–44, jan. 2008.

ZHENG, Q. et al. Occurrence and distribution of antibiotics in the Beibu Gulf, China: Impacts of river discharge and aquaculture activities. **Marine Environmental Research**, v. 78, p. 26–33, jul. 2012.

6 SUPPLEMENTARY MATERIAL

Study 1 (SM) – Figure 1 – Antibiotic concentration map in ng g^{-1} present in biofilms and POCIS in the studied basin in summer.



	Biofilm	POCIS
MED	<LQ	<LQ
SPI	<LQ	<LQ
JOS	<LQ	<LQ
AZI	<LQ	1.9
SMX	<LQ	13.3
CLA	2.2	0.1
ERY	<LQ	0.5
ROX	<LQ	<LQ

	Biofilm	POCIS
MED	1.8	<LQ
SPI	<LQ	<LQ
JOS	<LQ	0.4
AZI	3.1	5.2
SMX	15.0	28.5
CLA	3.4	2.3
ERY	<LQ	2.6
ROX	<LQ	<LQ

	Biofilm	POCIS
MED	<LQ	0.7
SPI	<LQ	<LQ
JOS	<LQ	1.8
AZI	11.0	0.8
SMX	<LQ	1.6
CLA	2.0	1.1
ERY	2.1	0.4
ROX	<LQ	0.6

	Biofilm	POCIS
MED	10.0	<LQ
SPI	<LQ	<LQ
JOS	<LQ	108.8
AZI	306.8	85.6
SMX	36.9	215.2
CLA	302.7	43.2
ERY	993.8	87.4
ROX	<LQ	<LQ

	Biofilm	POCIS
MED	<LQ	<LQ
SPI	<LQ	<LQ
JOS	1.0	0.6
AZI	2.2	4.9
SMX	5.8	89.8
CLA	1.2	3.3
ERY	<LQ	2.0
ROX	1.6	<LQ

	Biofilm	POCIS
MED	2.0	<LQ
SPI	<LQ	<LQ
JOS	1.1	0.9
AZI	1.3	0.6
SMX	5.6	22.7
CLA	0.4	0.8
ERY	1.3	0.7
ROX	6.5	<LQ

	Biofilm	POCIS
MED	<LQ	<LQ
SPI	<LQ	<LQ
JOS	<LQ	<LQ
AZI	<LQ	<LQ
SMX	8.5	<LQ
CLA	3.7	<LQ
ERY	3.6	<LQ
ROX	14.1	<LQ

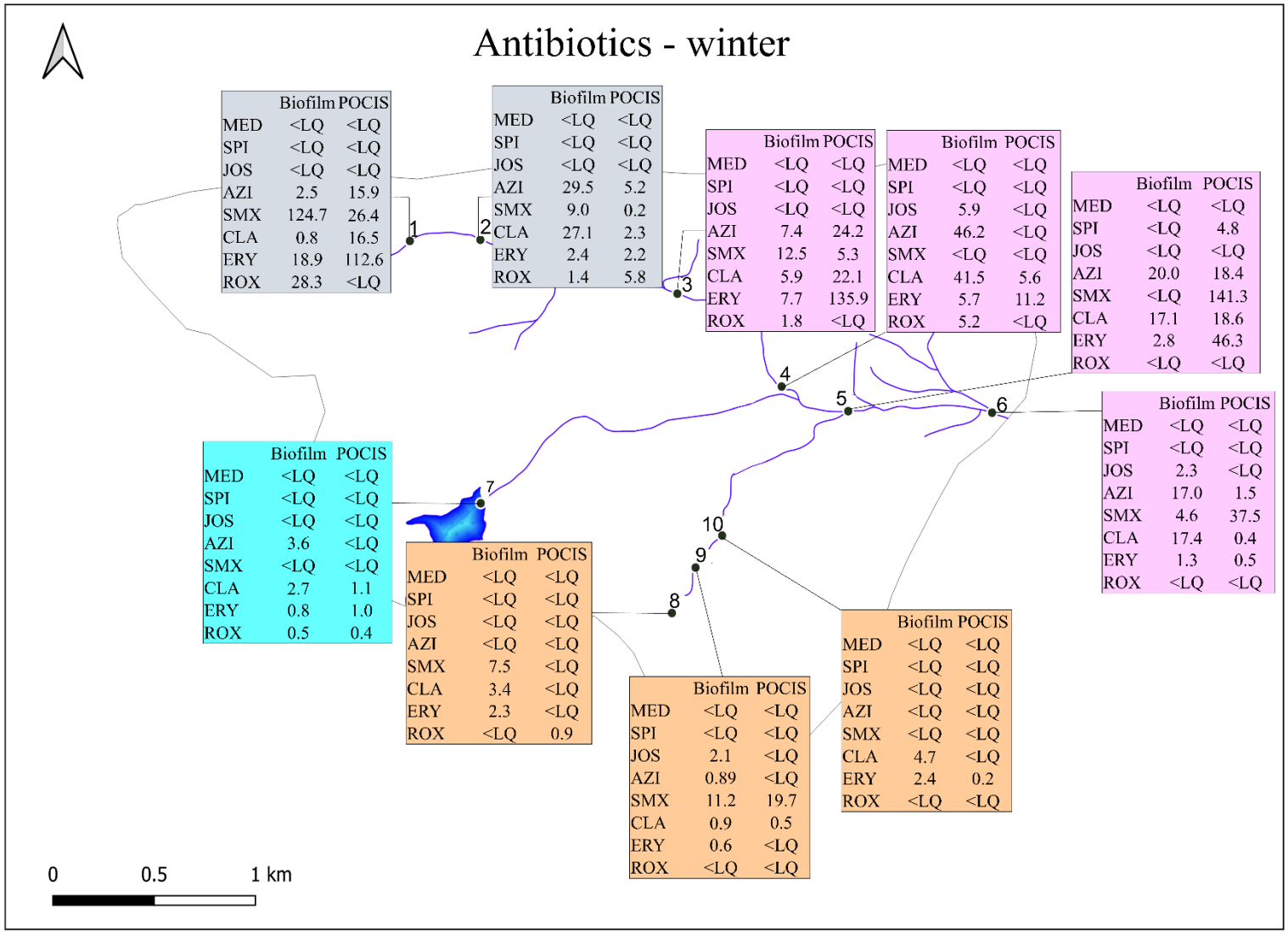
	Biofilm	POCIS
MED	<LQ	<LQ
SPI	<LQ	<LQ
JOS	<LQ	<LQ
AZI	<LQ	<LQ
SMX	7.1	<LQ
CLA	9.2	<LQ
ERY	<LQ	8.7
ROX	<LQ	<LQ

	Biofilm	POCIS
MED	<LQ	<LQ
SPI	<LQ	<LQ
JOS	<LQ	<LQ
AZI	0.9	3.9
SMX	0.7	<LQ
CLA	0.7	3.8
ERY	<LQ	3.3
ROX	<LQ	3.4

	Biofilm	POCIS
MED	<LQ	<LQ
SPI	<LQ	<LQ
JOS	<LQ	<LQ
AZI	<LQ	<LQ
SMX	<LQ	<LQ
CLA	3.7	1.3
ERY	<LQ	<LQ
ROX	0.5	<LQ

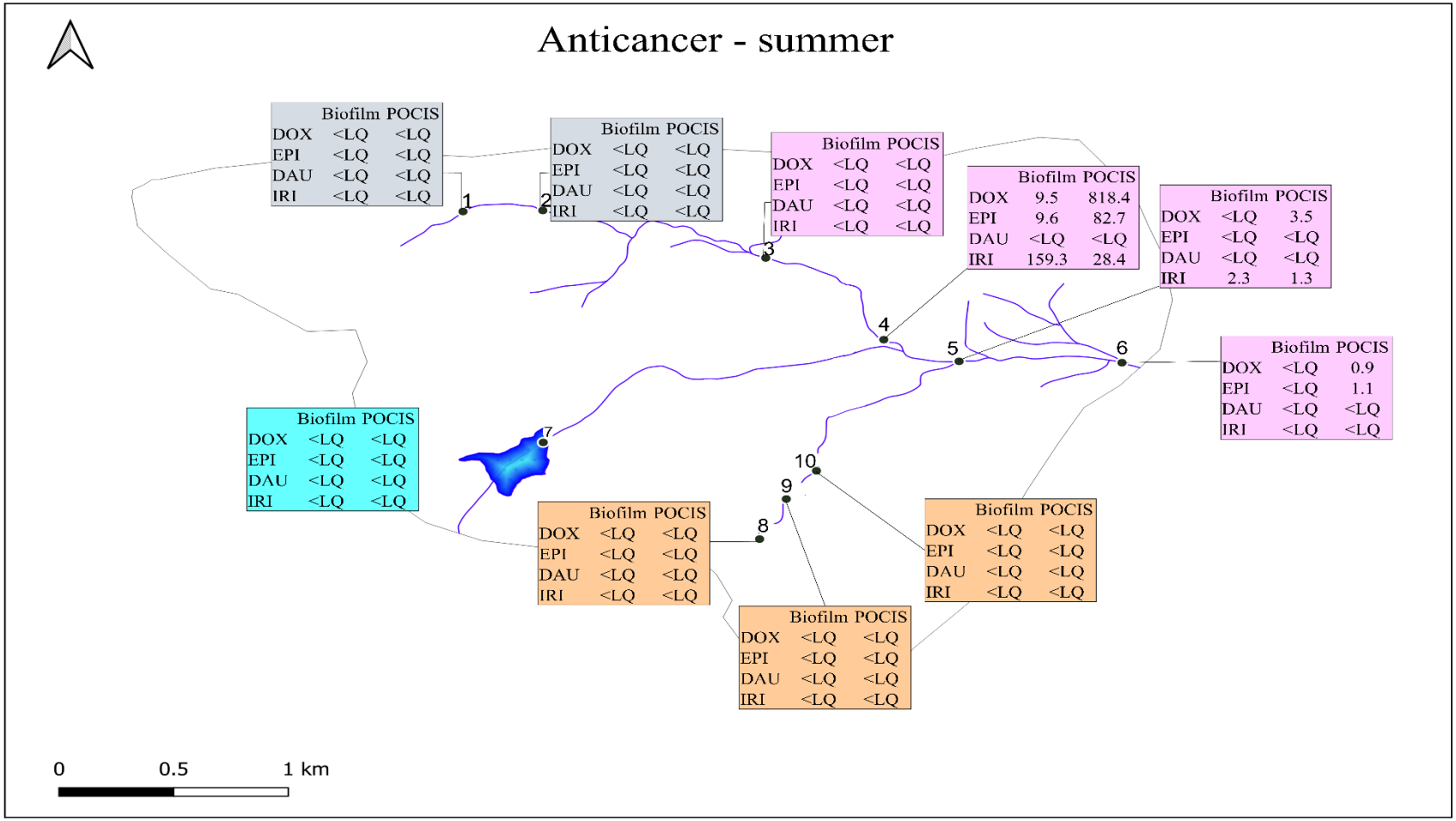
Source: (AUTHOR).

Study 1 (SM) – Figure 2 – Antibiotic concentration map in ng g^{-1} present in biofilms and POCIS in the studied basin in winter.



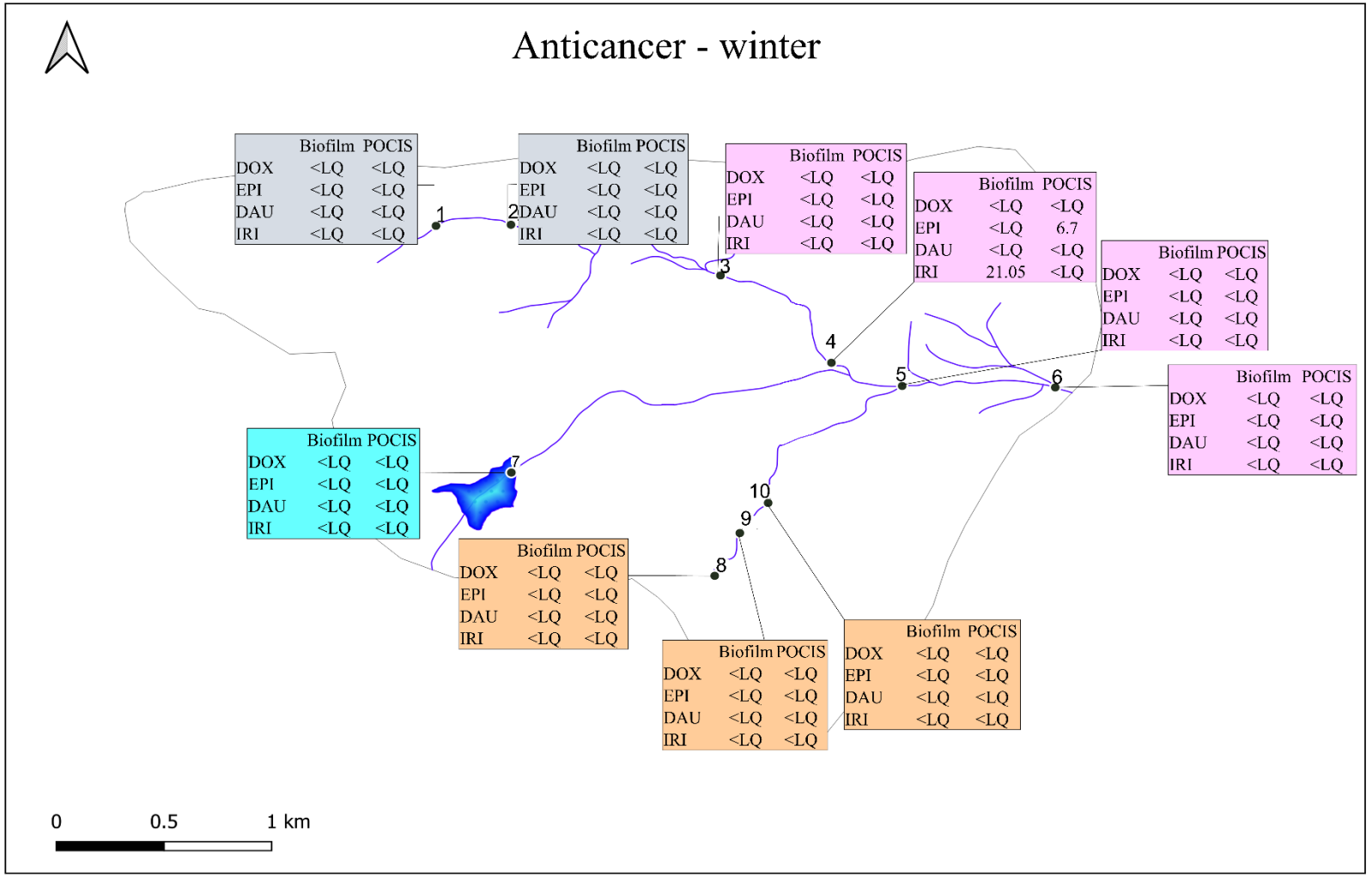
Source: (AUTHOR).

Study 1 (SM) – Figure 3 – Anticancer concentration map in ng g^{-1} present in biofilms and POCIS in the studied basin in summer.



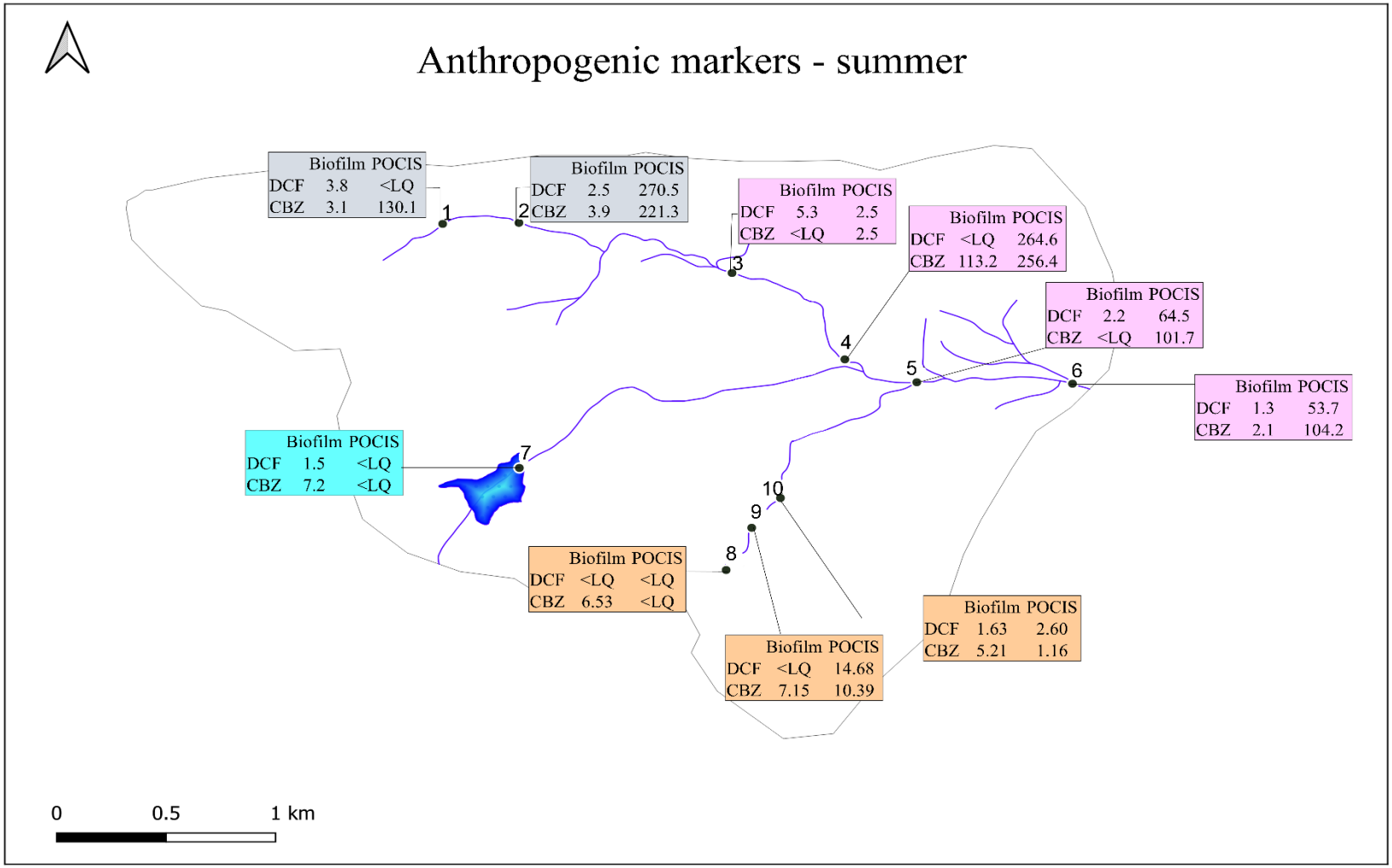
Source: (AUTHOR).

Study 1 (SM) – Figure 4 – Anticancer concentration map in ng g^{-1} present in biofilms and POCIS in the studied basin in winter.



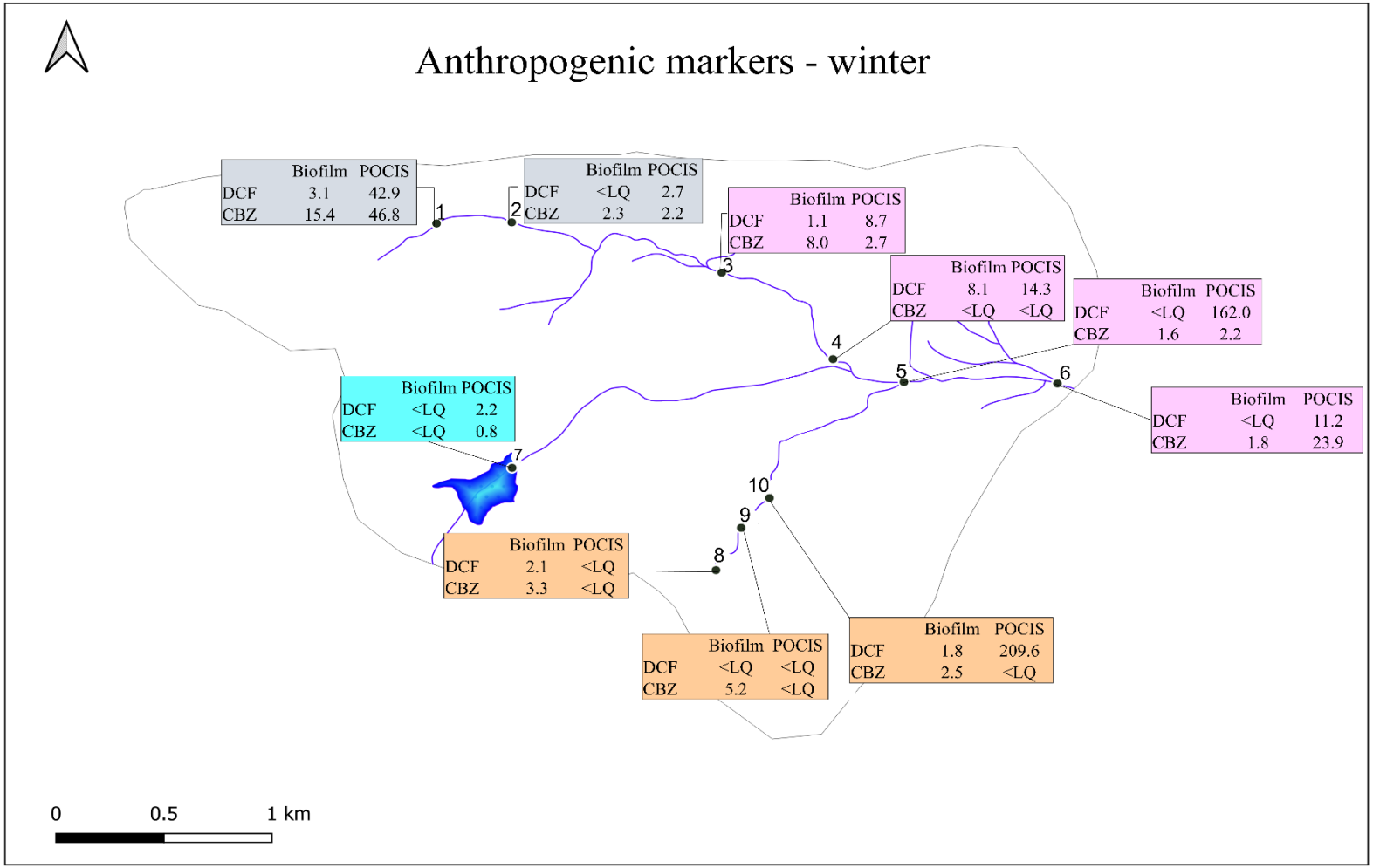
Source: (AUTHOR).

Study 1 (SM) – Figure 5– Anthropogenic markers concentration map in ng g^{-1} present in biofilms and POCIS in the studied basin in summer.



Source: (AUTHOR).

Study 1 (SM) – Figure 6 – Anthropogenic markers concentration map in ng g^{-1} present in biofilms and POCIS in the studied basin in winter.



Source: (AUTHOR).

STUDY 2 – THE FAST AND FURIOUS RESILIENCE OF PHARMACEUTICALS POLLUTION WITHIN RIVER BIOFILM

PROLOGUE

As concluded in the previous study, biofilms proved to be effective in capturing drug molecules in the university basin. We showed the impact of the different activities in the basin on the water contamination, highlighting the influence of the pollutant sources on the contamination load. Thus, it has been suggested that stream biofilms can also be used to monitor the effects of molecules of pharmaceuticals (MP) on river ecosystems (SABATER et al., 2007b). Specifically, the concept of Pollution-Induced Community Tolerance (PICT) can offer insights into whether the presence of MP modulates community responses to chemical stress. Since it is likely that different species within a community will exhibit different extents of tolerance to MPs, it is conceivable that, upon MP exposure, tolerant species within the community are favored, while sensitive ones disappear (TLILI et al., 2017).

Several studies have demonstrated that stream biofilms can remove MPs from the aquatic environment by acting as a natural sink (AUBERTHEAU et al., 2017; LAWRENCE et al., 2005; SCHORER; EISELE, 1997). However, there is rarely any differentiation between possible removal mechanisms. On the one hand, MPs can passively sorb to the EPS or cell surfaces within the biofilms. On the other hand, active biological processes, such as bioaccumulation or biotransformation, can remove compounds from the water phase. However, it must be noted that only biotransformation leads to permanent removal of organic compounds, while the other processes are potentially reversible and re-release of MPs into the water might occur.

Most of the studies available in the literature are done at the laboratory level, and field level experiments are difficult to control and manipulate. Thus, knowledge on the fate of complex mixtures of polar organic MPs in stream biofilms is still very limited. In this study, we aimed to develop a coherent methodological approach to investigate the behavior of MPs at environmentally relevant concentrations in biofilms, and how the microbial community behaves when there is a change in drug concentrations. We used the biofilm resilience experiment to investigate the biotransformation potential of natural flow biofilms collected upstream and downstream of HUSM (Hospital Universitário de Santa Maria) discharges. With this work, we provide a first set of valuable data and interesting first insights into how drug concentration in biofilms varies as a function of environmental loading.

1 INTRODUCTION

The beneficial effects of pharmaceutical substances on human or animal health and economic welfare (especially in agriculture) are widely acknowledged (OECD, 2020). However, occurrence of many of these substances and their residues in surface water, groundwater and drinking water is also increasingly pointed out worldwide (BEEK et al., 2016). Indeed, pharmaceutical compounds are continuously released into the environment either due to the discharge of treated, partially treated and untreated sewages into streams, or due to the use of veterinary pharmaceuticals by agriculture or aquaculture and their discharge directly from animals or through runoff and infiltration from soils (GOMES et al., 2020). Most of these substances remain bioactive even after release and may cause unintentional harmful impacts to non-target organisms. They produce a pharmacological response at low doses which makes them of environmental concern even at low concentrations. A growing body of evidence suggests that pharmaceutical substances may lead to significant changes in rivers, threatening wildlife (fish, frogs, insect, larvae, etc) (AZEVEDO et al., 2019; MENON et al., 2020; SIDORKIEWICZ et al., 2017) and even organisms that provide essential ecosystem services, such as the microbial communities colonizing the surface of rocks and sediments: biofilms. These cities of microbes are encased in extracellular polymeric substance that can adsorb pollutants (FLEMMING et al., 2016a). Concentrations of pharmaceuticals in the biofilm can range from a few ng g^{-1} to several µg g^{-1} (AUBERTHEAU et al., 2017; BASTOS et al., 2018a; VARGAS et al., 2021) and are therefore sufficient to induce effects. Moreover, many pharmaceutical compounds are toxic to environmental microorganisms because of their mechanism of action or their therapeutic properties (antimicrobial, antifungal, etc). However, the biofilms - and the whole river ecosystem - have capacities/processes to limit the toxic effects of these compounds on their own community. Thus, natural attenuation is the effect of naturally occurring physical (adsorption/desorption, dilution), chemical (hydrolysis, photolysis, redox transformation, complexation) and biological (biodegradation) processes that reduce the load, concentration, mobility or toxicity of pollutants in the biofilm community. These processes are all individually known but their combination/association and their respective importance are much less known, especially in rivers biofilms.

Thus, there is a gap in knowledge about the resilience of pharmaceutical substances in biofilms. How and how fast can these cities of microbes decontaminate? The present work succeeds in determining the in-situ dissipation capacity of several pharmaceuticals in a river

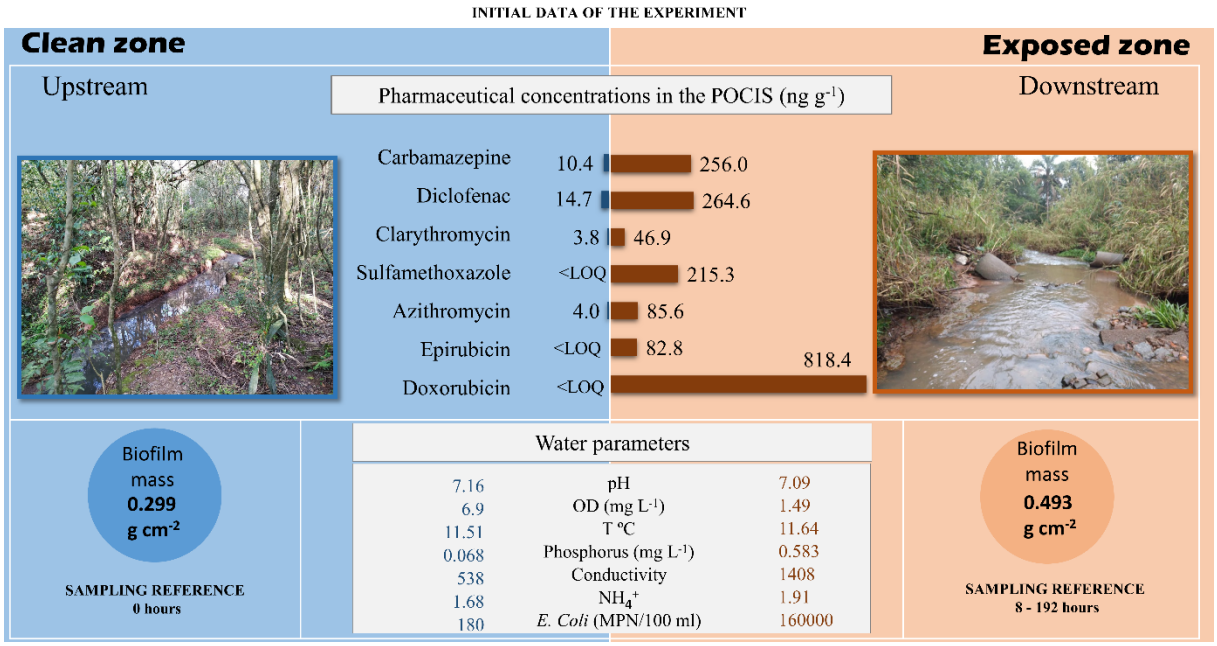
biofilm. For this purpose, we designed a simple and original experiment to “trigger” resilience. We moved a biofilm (with its adhesion support) from exposed aera (downstream a wastewater discharge point) to a few meters upstream in a clean area. In this way, the biofilm undergoes the same environmental characteristics, except for exposure to pharmaceuticals and other wastewater components. By sampling and analyzing the biofilm over a period of nine days, we were able to monitor the progress of pharmaceuticals dissipation and the evolution of the microbial communities.

2 MATERIAL AND METHODS

2.1 SAMPLE SITE

This sampling site receives the effluent from the southern wing of University Hospital (HUSM). The HUSM has a capacity of 309 patients and a water demand of around 150 m^3 day^{-1} (MARTINS et al., 2011c). The effluent first passes through the septic tank, with a volumetric capacity of 38.4 m^3. Subsequently, the waste is conveyed through piping located in the upper part of the pit, by gravity, to the lower part of the anaerobic filter. The anaerobic filter, with a capacity of 7.56 m^3, has a partition of pre-molded slabs, with holes of 3 cm and, as the volume increases, the effluent goes through the holes reaching a layer of gravel. The upper part of the filter has a chute that conducts the supernatant to the outside of the box, ending the treatment. The output flow in the anaerobic filter is of the order of 191 m^3 day^{-1}. After treatment, the effluent is discharged into the stream located inside the University campus, our collection point.

Study 2 – Figure 1– Characteristics and images of the monitored points. Concentrations of the pharmaceutical compounds and water quality parameters measured before the start of the experiment. Pharmaceutical compounds were analyzed using the Polar Organic Chemical Integrative Sampler (POCIS). Final mass of biofilm recovered at the end of the resilience experiment at each sampled point. MPN (Most Probable Number)



Source: (AUTHOR).

2.2 SAMPLING PROCEEDS

The study of the resilience capacity of epilithic biofilms was performed with the aid of 13 galvanized steel cages. Inside each cage, 16 basaltic rocks sized 10 x 20 x 5 cm (length, width, and height), were placed at the downstream point of the UH. The rocks were immersed in the watercourse for 40 days, time enough for biofilm development (MERCIER et al., 2013). After 40 days, half of the rocks were allocated to the upstream point of the UH (point with lower pollution load), and the other half remained at the downstream point, the latter being the control samples. From the reallocation of the cages, simultaneous collections were made at the upstream and downstream points in times 0, 8, 24, 48, 96, 144, and 192 hours, totaling 9 days of consecutive collections.

Biofilms were then manually brushed from rocks with 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 an ultra-freezer at -80ºC. Subsequently, the samples were lyophilized (LS3000-TERRONI freeze dryer), homogenized in an 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. Further details on extraction and the method of analysis have been specified in the chapter "general material and methods".

2.3 BACTERIA DIVERSITY ANALYSIS

2.3.1 DNA extraction

Total DNA from biofilms samples was extracted using E.Z.N.A. Stool DNA Kit. Omega Bio-Tek, according to the manufacturer's instructions. The DNA concentration was determined using the Qubit, and its quality was verified using the NanoDrop ND-1000 (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

2.3.2 Library preparation and 16s rna sequencing

To characterize the bacterial community, the fragments of V4 region of the 16S rRNA gene were amplified using the primers 515F and 806R and further sequenced using Illumina Miseq platform according to standard protocols. PCR assays were performed with the Platinum Taq DNA Polymerase High Fidelity kit (Invitrogen, Carlsbad, CA, USA), in a volume of 25 µL containing 1× High Fidelity PCR buffer, 2 U of Platinum Taq DNA Polymerase (InvitrogenTM), 1.5 mM $MgSO_4$, 0.2 mM dNTP Mix, 0.2 µM of each primer and approximately 50 ng of DNA template and ultrapure water to complete one volume. Amplification was carried out in a BioRad MyCycler Thermocycler (BioRad, USA) according to the following program: initial denaturation at 94°C for 3 min. followed by 30 cycles of 94°C for 30 sec, 55ºC for 30 sec, 72Cº for 30 sec and a final cycle at 72°C for 5 min.

After purifying PCR amplicons using Agencount AMPure Beads (Beckman Coulter), Amplicons were purified using Agencourt AMPure XP beads following manufacturer instructions. Purified products were again quantified and checked in Qubit Fluorometric Quantitation. Indexes were added to DNA libraries following the manufacturer instructions (Illumina Inc., San Diego, CA). Sequencing was conducted on platform Illumina MiSeq with a v2 500 kit, which generates paired-end reads of 250 bp. Sequences have been submitted and published to the NCBI database under accession number PRJNA641680.

2.4 BACTERIAL COMMUNITY ANALYSIS

Bioinformatics analysis was performed on 16S rRNA amplicons (BOLYEN et al., 2018). The raw sequence data from each sequencing run were quality filtered, denoised and chimera filtered using the q2-dada2 plugin with DADA2 pipeline (CALLAHAN et al., 2016). Using parameters to filter read sequences that have low quality, 5' nucleotide bases were trimmed from reverse and forward read sequences. Reads were truncated after that the read length filtering was applied using as parameter a score less than or equal to 11.

The nucleotide overlap used was to discard reads that are shorter than 250bp length. Following the steps to do the analysis, the consensus method was used to detect and remove chimeras in samples individually. The q2-phylogeny plugin was applied to construct the phylogeny. This is a plugin that uses the fasttree2 extension, but before this step the OTU'S must be aligned (PRICE; DEHAL; ARKIN, 2010). For this, the alignment was made using the q2-alignment plugin (KATOH, 2005).

Assignment of taxonomy to OTUs was made using the Naive Bayes via q2-feature classifier, and the features were collapsed using the q2-taxa plugin to collapse at Phylum and Genus level (BOKULICH et al., 2018). For the reference sequences, the Naive Bayes classifier was trained using Greengenes 13_8 99% OTUs reference.

The data (phylogeny, feature table, and taxonomy classification) were imported from QIIME2 to R v4.0.5 environment for graphic visualization using Phyloseq v1.34.0 R packages, Microbiome v1.12 and qiime2R v0.99.5 (MCMURDIE; HOLMES, 2013; XIA; CHEN; SUN, 2018). The compositional data, created using the data that were imported, was used for taxa bar plot composition visualization of the most abundant phylum and genus using plot composition function from Microbiome package.

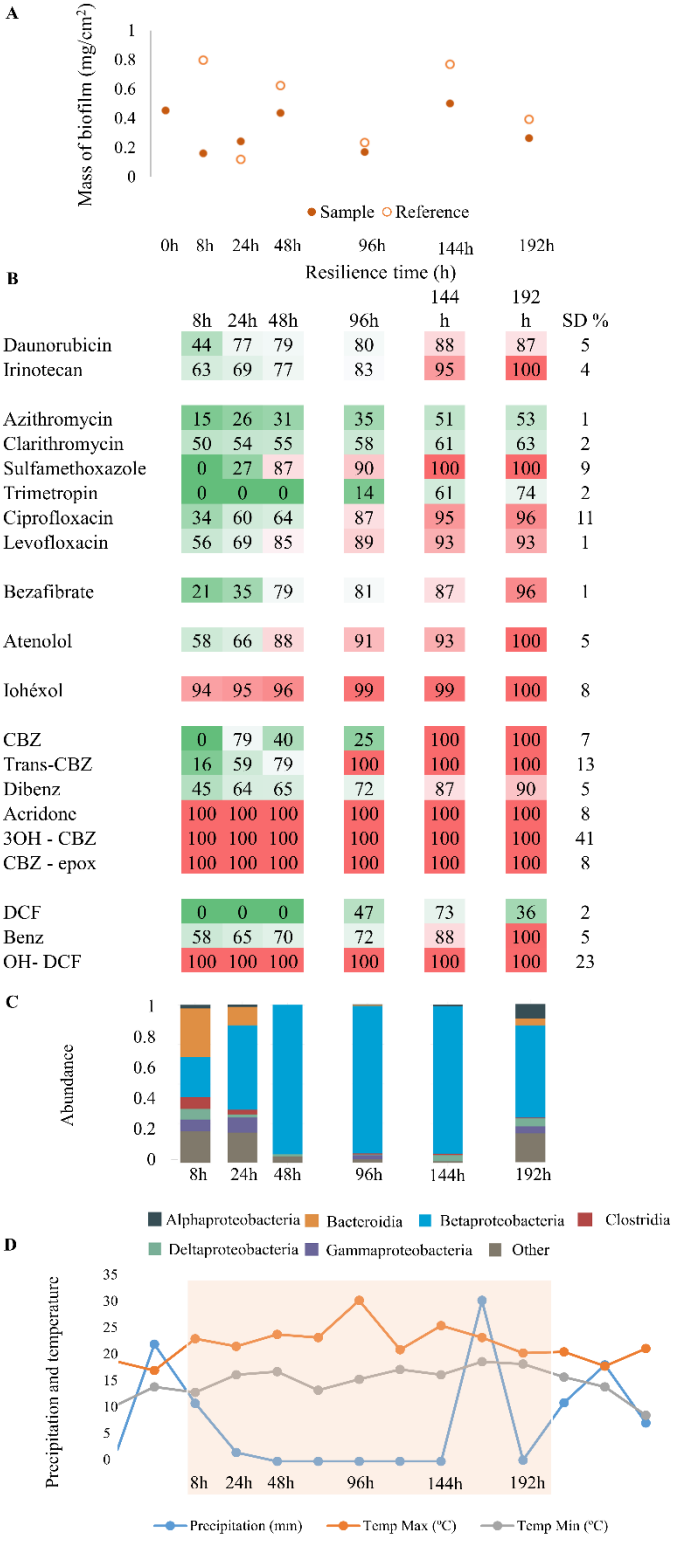
3 RESULTS AND DISCUSSION

Thus, we show that biofilm resilience is a 'fast and furious' process than previously thought. Rapid changes take place in the biofilm as soon as the exposure to the pollution is stopped. In particular, the biofilm loses about 2/3 of its initial mass within a few hours, whereas its mass should have even doubled if it had remained exposed to the discharge (see day 1 Figure 2A). Therefore, biofilm communities that are more exposed to contamination (to continuous stress), are expected to produce more EPS as a protective mechanism (VALDÉS et al., 2021).

The analysis of the pollutants shows that the loss of “biofilm matter” (probably by detachment) leads to a very high dissipation for many compounds (Figure 2B): 63% for IRI, 50% CLA, 56% LEV, 58% ATL, 94% IOX, 77% CBZ, 100% acrids and 58% benz after 8 hours. In constrat, the microbial diversity within the biofilm is only slightly affected by this matter loss (Fig. 1C). Seeding dispersal can be internally triggered, e.g. by enzyme-mediated breakdown of the biofilm matrix, production of surfactants which loosen cells from the biofilm, or externally triggered, e.g. changes in nutrient availability (DAVEY; CAIAZZA; O’TOOLE, 2003). After 24 hours of resilience, the mass of the biofilm starts to increase again suggesting a reformation of the biofilm, probably via bacterial growth and production of extracellular matrix (Figure 2A). Some experimental studies showed that cell dispersal rates can be promoted by environmental factors like nutrient depletion (BOLES; HORSWILL, 2008). Integration of nutrient or other stress aspects probably allows the cells in a population for a coordinate response to environmental challenges, optimized with respect to efficiency under the actual habitat conditions (BESTER; EDWARDS; WOLFAARDT, 2009). It has been shown experimentally that most cells produced in a biofilm will eventually detach and enter the aqueous phase. The evolution of microbial diversity shows that proteobacteria become the dominant group from this point on. Proteobacteria are often described as one of the main groups of bacteria in river biofilms (BRÜMMER,; FEHR; WAGNER-DÖBLER, 2000; CARLES; ARTIGAS, 2020; ZHANG et al., 2019). The results show that the dissipation still increase slightly during this frst day for all compounds. After only 48 hours of resilience, the dissipation already reaches more than 75% for 12 compounds: DAU, IRI, SFX, LEV, BEZ, ATL, IOX, trans-CBZ, with 4 compounds dissipating at a rate of 100% from day 1: Acrid, 3OH-CBZ, CBZ-epox and OH-DCF). Moreover, the biofilm underwent a "furious" modification of its population since proteobacteria became practically the only phylum populating the biofilm. After 96 hours of resilience, the dissipation has still increased (8 compounds ≥ 80% of which 2 ≥ 90%) (we are no more including with the 4 compounds already mentioned above, with 100 resilience after the first day). But the biofilm exhibis a new significant loss of matter: approximately 2.5 times the mass at 24h (also observed in the reference biofilm). This loss is probably caused by an excessive expansion of the biofilm due to the high air temperature which stimulated the microbial activity of bacteria, and then the breakdown of the biofilm. After 144 hours of resilience, the mass of the biofilm increased (x3) again to reach the value it had at 48 hours and the dissipation exceeded 51% for all compounds studied (except DCF). After 192h, 7 compounds have reached a 100% resilience rate (IRI, SFX, ATL, IOX, CBZ, trans-CBZ and benz). In a study of the constant degradation kinetics of various micropollutants in biofilms, it

was observed that for the compound ATL, for example, the only degradation pathway was biodegradation, reaching 100% removal of the compound at 96h. For this same compound we achieved 91% degradation at 96h (DESIANTE; MINAS; FENNER, 2021). Indicators of bacteroids are beginning to reappear in the population, but the evolution of the biofilm mass also shows that a new breakdown episode (about 50% loss of mass) was caused by the storm event (30 mm) 24 hours before (Figure 2D).

Study 2 – Figure 2– Evolution and change in biofilm samples during the sampling period (May 14-22, 2019). (**A**) mass of biofilm recovered each sampling day, (**B**) percentage of resilience or decontamination of each compost studied, (**C**) Class level bacterial community composition in river biofilm samples(**D**) precipitation, maximum and minimum temperature occurring in Santa Maria- Brazil



Source: (AUTHOR).

Thus, over the 8 days of resilience, a loss of all pharmaceutical substances was observed with the exception of four compounds: AZI, CLA, TMT and DCF, whose fluctuating or negative percentages show their fixation rather than their dissipation at the clean site (as also illustrated by the passive sampler monitoring data over the period of the experiment). The behaviour of the other compounds is rich in information and shows that the resilience of pharmaceutical substances involves different physical, physico-chemical or biological processes, some with a greater contribution than others. In particular, our results point out that the loss of matter - either by mechanical breakage during storm event (i.e. increasing the flow rate) or by dispersion and self-release (ie. when the biofilm needs/seeks to expand) (EMERENINI et al., 2015) - results in the loss of pharmaceuticals associated with the biofilm fragments. Likewise, the gain of matter - when the biofilm grows and produces extracellularly matrix and new cells - results in a decrease of the compound concentrations in the biofilm by physical dilution effect. In addition, some work suggests that this loss of biomass due to molting leads to a redistribution of cell density within the biofilm (JIANG et al., 2009) and may generate competition between different types of biomass (DUDDU et al., 2009).This resilience by “loss or gain” is a mechanism that is all the more important as it occurs in calm or turbulent hydrodynamic conditions.

Resilience is probably also related to the desorption of pharmaceuticals substances from the biofilm matrix back into the water - once the biofilm is no longer exposed to the pollution. This desorption is dependent on the affinity between the compound and the biofilm matrix and its reversibility, as well as other important factors (such as sorbent composition and water pH) that influence the functional groups of the adsorbent and the properties of the pharmaceuticals (KHANDAY et al., 2017). Not many studies have been done about the mechanisms and kinetics of pollutant desorption from river biofilms, but pharmaceuticals are known to be mainly bound by weak interactions. (e.g. van der Walls, H-bonding, or cation exchange and to a lesser extent hydrophobic interaction) which presages possibilities of desorption. Furthermore, the 3 compounds that presented the lowest resilience values (AZI, CLA and DCF) have log Kow > 3, indicating a high sorption potential on the biofilm matrix. However, desorption may be limited in the case of biofilms compared to what is known for other environmental surfaces (rocks, wood debris). Indeed, the biofilm matrix is like a "hydrogel" which implies that the compounds fixed inside must first diffuse to the exchange zones before being desorbed. This diffusion step adds a kinetic limitation to desorption. Thus, studies on the diffusion of organic contaminants in pure culture revealed a 100- to 10,000-fold reduction of diffusivity in biofilms as compared to free water (TORRESI et al., 2017; VOGT et al., 2000). Nevertheless, thicker

biofilms, which generally have a lower biomass density and significantly higher porosity than thin biofilms, can provide a higher available specific surface area and thus more desorption sites (TORRESI et al., 2017).

Our results also show that certain physicochemical dissipation processes play no or only a small role in the resilience of biofilms. Thus, several "specific" transformation products were considered during the experiment. In particular, the contribution of photolysis to resilience was studied. Phototransformation is generally considered to be the main dissipation pathway of DCF in aquatic environments (BOREEN; ARNOLD; MCNEILL, 2003). Thus, several works report that rapid photodegradation of this compound if conditions are favourable (~0.5 to 10h) - (POIRIER-LARABIE; SEGURA; GAGNON, 2016). However, we did not observe the formation of Benz, one of the most stable photolysis products of DCF (nb. but not the main product - (ERIKSSON; SVANFELT; KRONBERG, 2010), during the days of temperature increase while this period also corresponds to a high dissipation of DCF. Nevertheless, the evolution of DCF must be treated with caution because its presence/variability upstream disturbs the monitoring. Similarly, data on the evolution of Dibenz, a thermal degradation product of CBZ (DITTMANN et al., 2020), suggest that weather conditions do not have a direct influence on resilience. Indeed, we did not observe formation of this product when the air temperature reached 31°C at 96h (Figure 2D). However, several studies showed that weather factors affect water turbidity, hydraulic regime, season of the year, light intensity, and consequently the formation of transformation products in the aquatic environment (CRANE; WATTS; BOUCARD, 2006; KUNKEL; RADKE, 2012).

The limited presence of degradation products suggests a minimal role of biodegradation in the resilience of pharmaceutical substances (nb. within 8 days in our experiment). Thus, monitoring of acrid, 3OH-CBZ, CBZ-epox (microbial degradation products of CBZ (SHIRAISHI et al., 2001) and OH-Diclofenac (microbial degradation products of DCF) (LONAPPAN et al., 2016) did not show the production of any of these by-products in the biofilm during the entire resilience period. These by-products were found in the initial biofilm but were completely dissipated after 8 hours of resilience. Therefore, these by-products are not formed or are removed from the biofilm very quickly - which is unlikely given the diffusion constraints described above. Furthermore, several studies have shown that CBZ is recalcitrant to biodegradation (DALAHMEH; ALZIQ; AHRENS, 2019a; DESIANTE; MINAS; FENNER, 2021). Thus, the presence, and then near-omnipresence, of proteobacteria in the biofilm does not favor biodegradation. However, many studies highlight the ability of alpha- or gamma-proteobacteria to degrade compounds such as DCF, sulfonamides and CBZ by co-metabolism

(REIS et al., 2018). However, other works considered that these same compounds are not easily biodegradable (VERLICCHI; AL AUKIDY; ZAMBELLO, 2012). In any case, the accumulation of these compounds in biofilms and the increased contact time with the bacteria residing in these biofilms may allow the microorganisms to adapt and mineralize these pollutants (WICKE; BÖCKELMANN; REEMTSMA, 2007).

In general, all these processes affect the dissipation of pharmaceuticals in the biofilm. But their effectiveness is also conditioned by the location of the compound in this microbial city. Spatially, a location at or near the surface layer is crucial for exchange processes involving hydrodynamic considerations like desorption. While a location near the bacteria maximizes the chances of biodegradation. Finally, a location near the adhesive backing avoids tearing or peeling effects. It has been shown that in the biofilm structure, the most active biomass detachment as well as the weakest cohesion points are located at the biofilm surface (DUDDU; CHOPP; MORAN, 2009). In any case, the accumulation of these compounds in biofilms and the increased contact time with the bacteria residing in these biofilms may allow the microorganisms to adapt and mineralize these pollutants (WICKE; BÖCKELMANN; REEMTSMA, 2007). For these reasons, the evolution of a biofilm can be critical to retaining and removing contaminants from systems. However, if these biofilms are detached, they can become a source of contaminants.

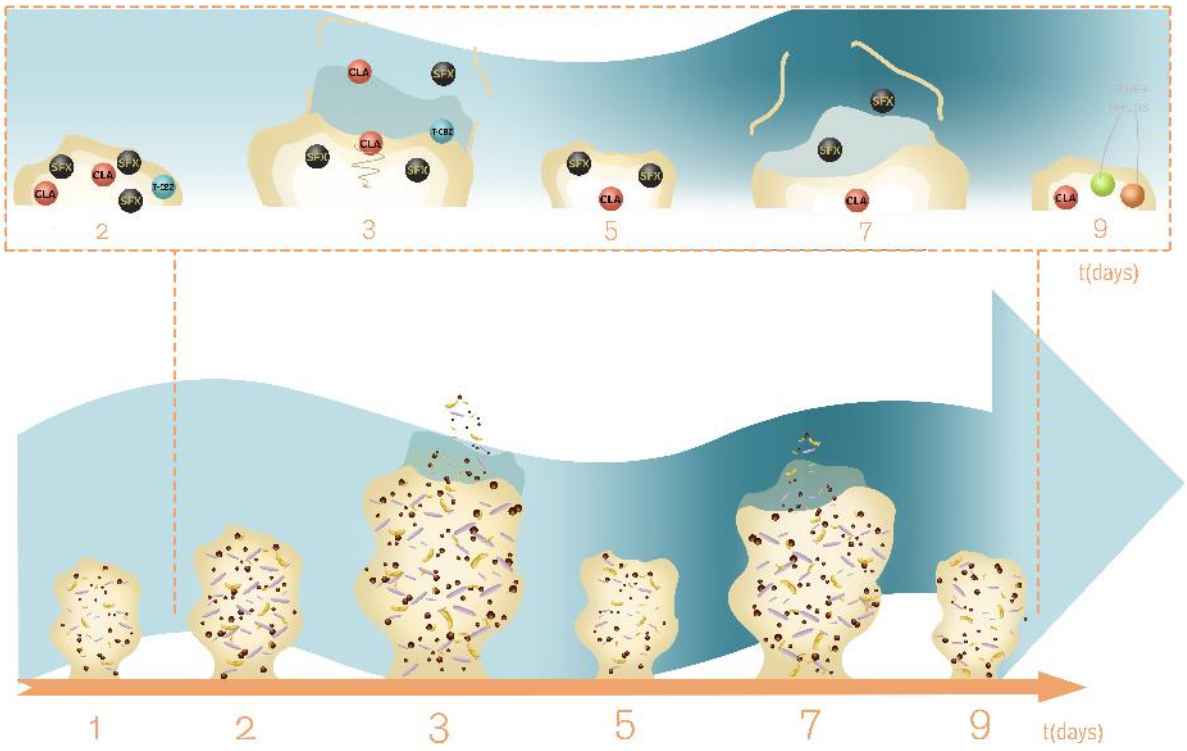
Furthermore, sorption of organic molecules by biofilm appears to occur in two phases: an initial rapid process to an equilibrium concentration and a second slower phase of sorption (WICKE; BÖCKELMANN; REEMTSMA, 2007). The biphasic sorption may reflect the heterogeneity of microbial biofilms with areas that are more easily accessible and others that are more rigid and therefore less easily accessible. Thus, older layers (presumably located at or near the core) and newer layers (typically at the periphery) may not have bound the same compounds, as the water composition (i.e. pollution) may have changed between periods of matrix development. In addition, this sorption occurs differently in the layers of the biofilm. Thus, detachment is more likely to remove recently localized compounds at the periphery of the biofilm than older compounds located in the core. Furthermore, some work suggests that this loss of biomass due to shedding does not lead to a decrease in colony size, but to a decrease in local cell density in the biofilm, with a lower biomass density in the inner cores than in the outer edges (JIANG et al., 2009). This reduction in biomass in the inner layer may be related to the substrate penetration limit, which generates competition between different types of biomass. This internal competition within the community itself leads to a distinct evolution at each biofilm (DUDDU; CHOPP; MORAN, 2009). Thus, the sorption of molecules by the

biofilm community can still be positively influenced by the reduction in particle size, i.e., greater specific surface area, in the suspended biomass (KHUNJAR; LOVE, 2011). Thus, in finer biofilms (≤50 μm), micropollutant transport could therefore be limited by the high biomass density and reduced porosity (TORRESI et al., 2017). In a study using NMR techniques, the authors found evidence for faster diffusion of glycerol into the biofilm pores and significantly slower diffusion through the EPS network (VOGT; FLEMMING; VEEMAN, 2000).

Torresi et al. (2017) evaluated the influence of thickness and distribution coefficient Kd,eq (L g^{-1}) of antibiotics in biofilms and observed that the macrolide group showed significantly higher values of distribution coefficient (Kd,eq - high sorption potential) for thicker biofilms. Therefore, an exponential increase of Kd,eq with biofilm thickness, being related to the increased porosity of the biofilm and, consequently, to the larger surface area accessible for sorption. Thus, the transport of molecules in the biofilm will depend on the characteristics of the molecules and the biofilm. Molecules with high sorption potential in thick biofilms suggest slower diffusive transport within the biofilm, as previously observed for hydrophobic organic molecules in sediment and soil (WU; GSCHWEND, 1988). The two macrolide antibiotics studied here (CLA and AZT) show low resilience over the time studied. This may be due to their large molecular weight and high sorption potential in biofilms, which decreases their mobility in the biofilm layers. And due to the protonation of the tertiary amino group, a strong ionic interaction of macrolides with the negatively charged biomass surface can be expected. Unlike macrolides, SMX is a negatively charged molecule at neutral pH, which would not facilitate its sorption into biofilms, a result observed in our data.

In general, all these processes affect the dissipation of pharmaceuticals in the biofilm. Thus, detachment is more likely to remove recently localized compounds at the periphery of the biofilm than older compounds located at the core. We try to exemplify these mechanisms in a simplified way within the biofilm community through Figure 3, where we report according to our results what could be happening with biofilm mass losses and concentration of the compounds.

Study 2 – Figure 3– Schematic of biofilm formation and detachment and its interaction with the studied compounds in the sampling period. The numbers represent the sampling days. The balls represent the studied compounds, clarithromycin (CLA), sulfamethoxazole (SFX) and Trans-CBZ (T-CBZ). The loss of biofilm mass due to precipitation and consequent increased river flow is represented by the darker blue color on day 7. The representation of the loss and diffusion of the compounds is represented in the top image, based on our results



Source: (AUTHOR).

4 CONCLUSION

This very simple experiment highlight how the resilience of a biofilm is a fast phenomenon since it is already noticeable after 8 hours and leads to a dissipation of two thirds of the compounds (>95% dissipation) after 8 days, and also a furious phenomenon since the microbial communities change drastically. In the short term, the biofilm activates more the physical and chemical processes of the environment to remove pollutants than its innate biological capacity to (bio)degrade. This confirms that biofilms are not just simple arrangements of living cells but are complex structures/interface that interact with their environment. However, as any living being their activity benefits or suffers from hydroclimatic events. Our highlight of the speed of resilience is of interest for emerging approaches using biofilms as environmental indicators of pollution. Our results clearly indicate that the response

of microorganisms to changes in the environment is fast but takes hours to days. Therefore, all studies that want to use biofilms should take this speed – ie. this time lag - into account in their assessment.

5 REFERENCES

AUBERTHEAU, E. et al. Impact of wastewater treatment plant discharge on the contamination of river biofilms by pharmaceuticals and antibiotic resistance. **Science of The Total Environment**, v. 579, p. 1387–1398, fev. 2017.

AZEVEDO, F. C. R. et al. Toxicological effects of ciprofloxacin and chlorhexidine on growth and chlorophyll a synthesis of freshwater cyanobacteria. **Brazilian Journal of Pharmaceutical Sciences**, v. 55, p. e17661, 2019.

BASTOS, M. C. et al. Presence of Anthropogenic Markers in Water: A Case Study of the Guaporé River Watershed, Brazil. **CLEAN - Soil, Air, Water**, v. 46, n. 3, p. 1700019, mar. 2018.

BEEK, T. et al. Pharmaceuticals in the environment-Global occurrences and perspectives: Pharmaceuticals in the global environment. **Environmental Toxicology and Chemistry**, v. 35, n. 4, p. 823–835, abr. 2016.

BESTER, E.; EDWARDS, E. A.; WOLFAARDT, G. M. Planktonic cell yield is linked to biofilm development. **Canadian Journal of Microbiology**, v. 55, n. 10, p. 1195–1206, out. 2009.

BOKULICH, N. A. et al. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. **Microbiome**, v. 6, n. 1, p. 90, dez. 2018.

BOLES, B. R.; HORSWILL, A. R. agr-Mediated Dispersal of Staphylococcus aureus Biofilms. **PLoS Pathogens**, v. 4, n. 4, p. e1000052, 25 abr. 2008.

BOLYEN, E. et al. An Introduction to Applied Bioinformatics: a free, open, and interactive text. **Journal of Open Source Education**, v. 1, n. 5, p. 27, 2 out. 2018.

BOREEN, A. L.; ARNOLD, W. A.; MCNEILL, K. Photodegradation of pharmaceuticals in the aquatic environment: A review. **Aquatic Sciences**, v. 65, n. 4, p. 320–341, 2003.

BRÜMMER, I. H. M.; FEHR, W.; WAGNER-DÖBLER, I. Biofilm Community Structure in Polluted Rivers: Abundance of Dominant Phylogenetic Groups over a Complete Annual Cycle. **Applied and Environmental Microbiology**, v. 66, n. 7, p. 3078–3082, 1 jul. 2000.

CALLAHAN, B. J. et al. DADA2: High-resolution sample inference from Illumina amplicon data. **Nature Methods**, v. 13, n. 7, p. 581–583, jul. 2016.

CARLES, L.; ARTIGAS, J. Interaction between glyphosate and dissolved phosphorus on bacterial and eukaryotic communities from river biofilms. **Science of The Total Environment**, v. 719, p. 137463, jun. 2020.

CRANE, M.; WATTS, C.; BOUCARD, T. Chronic aquatic environmental risks from exposure to human pharmaceuticals. **Science of The Total Environment**, v. 367, n. 1, p. 23–41, 15 ago. 2006.

DALAHMEH, S. S.; ALZIQ, N.; AHRENS, L. Potential of biochar filters for onsite wastewater treatment: Effects of active and inactive biofilms on adsorption of per- and polyfluoroalkyl substances in laboratory column experiments. **Environmental Pollution**, v. 247, p. 155–164, abr. 2019.

DAVEY, M. E.; CAIAZZA, N. C.; O'TOOLE, G. A. Rhamnolipid Surfactant Production Affects Biofilm Architecture in Pseudomonas aeruginosa PAO1. **Journal of Bacteriology**, v. 185, n. 3, p. 1027–1036, 1 fev. 2003.

DESIANTE, W. L.; MINAS, N. S.; FENNER, K. Micropollutant biotransformation and bioaccumulation in natural stream biofilms. **Water Research**, v. 193, p. 116846, abr. 2021.

DITTMANN, D. et al. Specific adsorption sites and conditions derived by thermal decomposition of activated carbons and adsorbed carbamazepine. **Scientific Reports**, v. 10, n. 1, p. 6695, dez. 2020.

DUDDU, R.; CHOPP, D. L.; MORAN, B. A two-dimensional continuum model of biofilm growth incorporating fluid flow and shear stress based detachment. **Biotechnology and Bioengineering**, v. 103, n. 1, p. 92–104, 1 maio 2009.

EMERENINI, B. O. et al. A Mathematical Model of Quorum Sensing Induced Biofilm Detachment. **PLOS ONE**, v. 10, n. 7, p. e0132385, 21 jul. 2015.

ERIKSSON, J.; SVANFELT, J.; KRONBERG, L. A Photochemical Study of Diclofenac and Its Major Transformation Products. **Photochemistry and Photobiology**, v. 86, n. 3, p. 528–532, 2010.

FLEMMING, H.-C. et al. Biofilms: an emergent form of bacterial life. **Nature Reviews Microbiology**, v. 14, n. 9, p. 563–575, set. 2016.

GOMES, I. B. et al. Emerging contaminants affect the microbiome of water systems—strategies for their mitigation. **npj Clean Water**, v. 3, n. 1, p. 39, dez. 2020.

JIANG, F. et al. A biofilm model for prediction of pollutant transformation in sewers. **Water Research**, v. 43, n. 13, p. 3187–3198, jul. 2009.

KATOH, K. MAFFT version 5: improvement in accuracy of multiple sequence alignment. **Nucleic Acids Research**, v. 33, n. 2, p. 511–518, 19 jan. 2005.

KHANDAY, W. A. et al. Mesoporous zeolite–activated carbon composite from oil palm ash as an effective adsorbent for methylene blue. **Journal of the Taiwan Institute of Chemical Engineers**, v. 70, p. 32–41, jan. 2017.

KHUNJAR, W. O.; LOVE, N. G. Sorption of carbamazepine, 17α-ethinylestradiol, iopromide and trimethoprim to biomass involves interactions with exocellular polymeric substances. **Chemosphere**, v. 82, n. 6, p. 917–922, fev. 2011.

KUNKEL, U.; RADKE, M. Fate of pharmaceuticals in rivers: Deriving a benchmark dataset at favorable attenuation conditions. **Water Research**, v. 46, n. 17, p. 5551–5565, 1 nov. 2012.

LAWRENCE, J. R. et al. Effects of selected pharmaceuticals on riverine biofilm communities. **Canadian Journal of Microbiology**, 2005.

LONAPPAN, L. et al. Diclofenac and its transformation products: Environmental occurrence and toxicity - A review. **Environment International**, v. 96, p. 127–138, nov. 2016.

MARTINS, A. F. et al. Occurrence of the Antimicrobials Sulfamethoxazole and Trimethoprim in Hospital Effluent and Study of Their Degradation Products after Electrocoagulation. **CLEAN - Soil, Air, Water**, v. 39, n. 1, p. 21–27, jan. 2011.

MCMURDIE, P. J.; HOLMES, S. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. **PLoS ONE**, v. 8, n. 4, p. e61217–e61217, 22 abr. 2013.

MENON, N. G. et al. Review on Occurrence and Toxicity of Pharmaceutical Contamination in Southeast Asia. In: KUMAR, M.; SNOW, D. D.; HONDA, R. (Eds.). . **Emerging Issues in the Water Environment during Anthropocene**. Springer Transactions in Civil and Environmental Engineering. Singapore: Springer Singapore, 2020. p. 63–91.

MERCIER, A. et al. Biofilm formation vs. PCB adsorption on granular activated carbon in PCB-contaminated aquatic sediment. **Journal of Soils and Sediments**, v. 13, n. 4, p. 793–800, abr. 2013.

OECD. **PHARMACEUTICAL RESIDUES IN FRESHWATER: hazards and policy responses.** S.l.: IWA (INTL WATER ASSN), 2020.

POIRIER-LARABIE, S.; SEGURA, P. A.; GAGNON, C. Degradation of the pharmaceuticals diclofenac and sulfamethoxazole and their transformation products under controlled environmental conditions. **Science of The Total Environment**, v. 557–558, p. 257–267, 1 jul. 2016.

PRICE, M. N.; DEHAL, P. S.; ARKIN, A. P. FastTree 2 – Approximately Maximum-Likelihood Trees for Large Alignments. **PLoS ONE**, v. 5, n. 3, p. e9490, 10 mar. 2010.

REIS, P. J. M. et al. Insights on sulfamethoxazole bio-transformation by environmental Proteobacteria isolates. **Journal of Hazardous Materials**, v. 358, p. 310–318, set. 2018.

SABATER, S. et al. Monitoring the effect of chemicals on biological communities. The biofilm as an interface. **Analytical and Bioanalytical Chemistry**, v. 387, n. 4, p. 1425–1434, 6 fev. 2007.

SCHORER, M.; EISELE, M. Accumulation of inorganic and organic pollutants by biofilms in the aquatic environment. **Water, Air, and Soil Pollution**, v. 99, n. 1, p. 651–659, 1 out. 1997.

SHIRAISHI, Y. et al. A Novel Desulfurization Process for Fuel Oils Based on the Formation and Subsequent Precipitation of S-Alkylsulfonium Salts. 5. Denitrogenation Reactivity of Basic and Neutral Nitrogen Compounds. **Industrial & Engineering Chemistry Research**, v. 40, n. 22, p. 4919–4924, 1 out. 2001.

SIDORKIEWICZ, I. et al. Endocrine-disrupting chemicals—Mechanisms of action on male reproductive system. **Toxicology and Industrial Health**, v. 33, n. 7, p. 601–609, jul. 2017.

TLILI, A. et al. Micropollutant-induced tolerance of in situ periphyton: Establishing causality in wastewater-impacted streams. **Water Research**, v. 111, p. 185–194, 15 mar. 2017.

TORRESI, E. et al. Diffusion and sorption of organic micropollutants in biofilms with varying thicknesses. **Water Research**, v. 123, p. 388–400, 15 out. 2017.

VALDÉS, M. E. et al. Distribution of antibiotics in water, sediments and biofilm in an urban river (Córdoba, Argentina, LA). **Environmental Pollution**, v. 269, p. 116133, jan. 2021.

VARGAS, J. P. R. et al. Pharmaceutical compound removal efficiency by a small constructed wetland located in south Brazil. **Environmental Science and Pollution Research**, 16 fev. 2021.

VERLICCHI, P.; AL AUKIDY, M.; ZAMBELLO, E. Occurrence of pharmaceutical compounds in urban wastewater: Removal, mass load and environmental risk after a secondary treatment—A review. **Science of The Total Environment**, v. 429, p. 123–155, jul. 2012.

VOGT, M.; FLEMMING, H.-C.; VEEMAN, W. S. Diffusion in Pseudomonas aeruginosa biofilms: a pulsed field gradient NMR study. **Journal of Biotechnology**, v. 77, n. 1, p. 137–146, jan. 2000.

WICKE, D.; BÖCKELMANN, U.; REEMTSMA, T. Experimental and modeling approach to study sorption of dissolved hydrophobic organic contaminants to microbial biofilms. **Water Research**, v. 41, n. 10, p. 2202–2210, maio 2007.

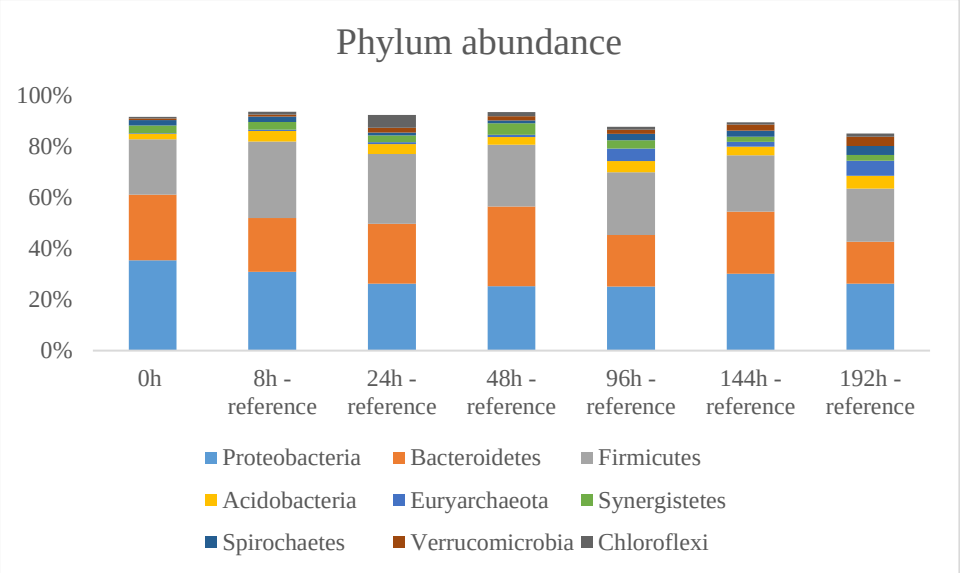
WU, S.-C.; GSCHWEND, P. M. Numerical modeling of sorption kinetics of organic compounds to soil and sediment particles. **Water Resources Research**, v. 24, n. 8, p. 1373–1383, ago. 1988.

XIA, Y.; CHEN, D.-G.; SUN, J. **Statistical Analysis of Microbiome Data with R**. 1st ed. 2018 ed. Singapore: Springer Singapore : Imprint: Springer, 2018.

ZHANG, N. et al. Fate and effects of triclosan in subtropical river biofilms. **Aquatic Toxicology**, v. 212, p. 11–19, jul. 2019.

6 SUPPLEMENTARY MATERIAL

Study 2 (SM) – Figure 1 – Bacterial composition at phylum level in biofilm samples. Downstream samples



Source: (AUTHOR).

STUDY 3- PHARMACEUTICAL COMPOUNDS REMOVAL EFFICIENCY BY A SMALL CONSTRUCTED WETLAND LOCATED IN SOUTH BRAZIL

PROLOGUE

Since the university campus presents several pollutant sources and those biofilms are capable of both accumulating drugs and detoxifying from them in a short period of time, the fourth study of this thesis was developed to evaluate the potential of a treatment plant in removing drugs evaluated by biofilms and POCIS.

As the problem of lack of wastewater treatment is a major barrier in Brazil, the search for feasible technologies and solutions becomes indispensable. Wastewater treatment plant (WWTP) effluents can be one of the sources of pharmaceutical and personal care products into streams, rivers, and lakes. Thus, the pharmaceuticals removal efficiencies of WWTPs vary to a great extent and negative removal efficiencies have also been observed in some studies. However, sampling and studies are often done from active sampling. To our knowledge there are no studies in Brazil that have performed passive sampling to evaluate the efficiency of a WWTP. Grab sampling does not capture short or long-term concentration variation since it takes an instantaneous picture of the concentration of the contaminants in the water at the time of sampling.

The behavior of pharmaceuticals during wastewater treatment is only partially understood and must therefore be further considered in order to control their release into the environment and avoid any potential adverse effects on aquatic ecosystems. A better understanding of the occurrence and removal of pharmaceuticals to control their release into the environment and avoid any potential adverse effects on aquatic ecosystems can only be achieved on the basis of reliable empirical data being inherently connected to well-defined sampling strategies.

This paper is available at: https://doi.org/10.1007/s11356-021-12845-6

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; 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) (AUBERTHEAU et al., 2017c; CHONOVA et al., 2016; OKUDA et al., 2008; 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) (BISOGNIN; WOLFF; CARISSIMI, 2018; BUSSI et al., 2017). 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; WOLFF; CARISSIMI, 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., 2017b). 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 (BISOGNIN et al., 2019; FISCHER; MAJEWSKY, 2014; HYLAND et al., 2012; STUMPF et al., 1999b; SUÁREZ et al., 2008). 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; MASIH; VAN HULLEBUSCH, 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; ABOU-ELWAFA ABDALLAH; HARRAD, 2017; LIU et al., 2017) given their harmful effects as endocrine disruptors or bacterial resistance trigger (KLATTE; SCHAEFER; HEMPEL, 2017) and their persistence (WANG; WANG, 2016).

In recent years, there has been increasing number of studies concerning the removal of pharmaceuticals in CWs (CHEN et al., 2016; MATAMOROS; HIJOSA; BAYONA, 2009; VERLICCHI; ZAMBELLO, 2014). 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; 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 bio-degradation (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 Thypa) as well as the higher hydraulic residence time (HRT) in the CW (MATAMOROS; SALVADÓ, 2012). It is possible to observe removal of pharmaceuticals in the literature from insignificant (<10%) for carbamazepine (CARBALLA et al., 2007; TERNES et al., 2007), Diclofenac (HIJOSA-VALSERO et al., 2016), to>90% for Ibuprofen (JOSS et al., 2005), Naproxen (ZHANG et al., 2014), Cafeíne (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, 2018b). However, since 2014 about 45% of the population are not connected to the sewer network (ANA, 2019c; MACHADO et al., 2017b) 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; 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.

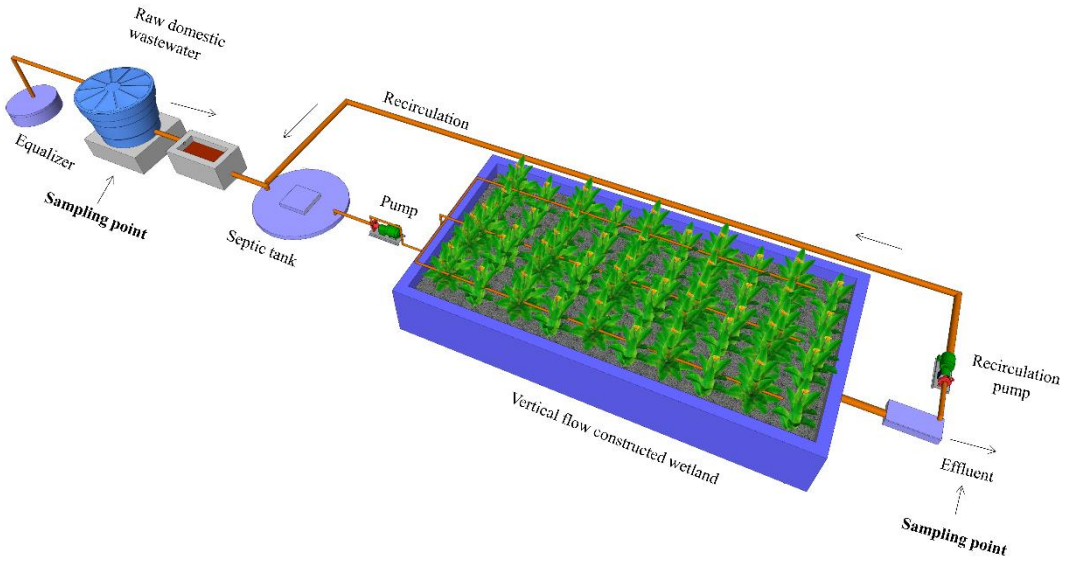
Limiting pollution by pharmaceutical compounds in Brazil is essential for human or environmental health protection as well as the establishment of efficient and inexpensive treatment systems to allow the collection and treatment of as much wastewater as possible. In this context, CW appear to be a realistic 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.

Study 3 – Figure 1 –Typical scheme of WWTP involving vertical flow constructed wetland



Source: (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 m^2 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 m^2 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), Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD_5), $N\text{-}NH_4^+$ (Ammoniacal Nitrogen), Org-N (Organic Nitrogen), $N\text{-}NO_3^-$ (Nitrate Nitrogen), TN (Total Nitrogen), TKN (Total Kjeldahl nitrogen = Org-N + $N\text{-}NH_4^+$) and $P\text{-}PO_3^{4-}$ (Orthophosphate). Water samples were collected in clean glass bottles and rapidly analyzed.

Study 3 – Figure 2– Samplings period from May to December 2018 and January 2019

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

.

Source: (AUTHOR).

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; MASIH; VAN HULLEBUSCH, 2020).

POCIS were man-made in the laboratory with stainless steel washers of 102 × 54 × 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 µm particle diameter, with hydrophilic/lipophilic characteristics. A SUPOR® Polyethersulfone filter membrane, with pores of 0.1 µm 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 N_2 flow, with subsequent weighing and storage at -80ºC. Formation of a biofilm layer on POCIS membranes may affect the transfer of the pharmaceuticals toward the resin. No specific measurement was performed but the appearance of the membranes has been checked.

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 -80ºC. Subsequently, the samples were lyophilized (LS3000-TERRONI freeze dryer), 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.

2.3 ENVIRONMENTAL RISK EVALUATION

The Risk Quotient (RQ) 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:

$$RQ = \frac{MEC}{PNEC}$$

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 (Chemical Oxygen Demand), BOD_5 (Biochemical Oxygen Demand), TKN (Org-N + $N\text{-}NH_4^+$), $N\text{-}NH_4^+$ (Ammoniacal Nitrogen), Org-N (Organic Nitrogen), $N\text{-}NO_3^-$ (Nitrate Nitrogen), TN (Total Nitrogen) and $P\text{-}PO_3^{4-}$ (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%. In addition, the effluent from TS presented a higher mean concentration of BOD5 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; LOHANI, 2019). The actual performance of the tank depends on the ambient condition, retention time inhabitance 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 NO_3^--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 NO_3^--N (AL-ZREIQAT et al., 2018; SANT'ANNA JR, 2011).

Study 3 – Table 1 – Concentration and loading of TSS (Total Suspended Solids), TS (Total Solids), COD (Chemistry Oxygen Demand), BOD_5 (Biochemical Oxygen Demand), TKN (Org-N + $N\text{-}NH_4^+$), $N\text{-}NH_4^+$ (Ammoniacal Nitrogen), Org-N (Organic Nitrogen), $N\text{-}NO_3^-$ (Nitrate Nitrogen), TN (Total Nitrogen) and $P\text{-}PO_3^{4-}$ (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 NO_3^--N e PO_4^{3-}-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 NO_3^--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; MASIH, 2018). Usually, the removal of PO_4^{3-}-P is not investigated by VF-CW in the studies (ILYAS; 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 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; 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 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. (WU et al., 2014; ZHAO; SUN; ALLEN, 2004). Usually, removal efficiencies in VF-CW are in the 48-99% DBO5, 44-95% DQO, and 52-99% TSS ranges (STEFANAKIS; 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; RUABEN; ORTIGARA, 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, CODt 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; MASIH, 2017; SHARMA et al., 2018). Anyway, Rossmann et al. (2012, 2013), Costa et al. (2015) and Machado et al. (2017b), 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 BOD_5.

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; 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; REINHARD; GIN, 2018).

Study 3 – Table 2 – 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 mg L^{-1}**	**Removal rate %**
pH	$5 \leq x \leq 9$	$6 \leq x \leq 9$	7.2	-
BOD_5 (mg L^{-1})	≤ 120 mg L^{-1} ou 60%	≤ 120 mg L^{-1} (Q*<100 m^3 d^{-1})	119.0	75
COD_t (mg L^{-1})	-	≤ 330 mg L^{-1} (Q*<100 m^3 d^{-1})	172.0	86
Sólids (mg L^{-1})	SSd $\leq 1{,}0$ mg L^{-1}	SSd $\leq 1{,}0$ mg L^{-1}	0.3	96
	-	TSS ≤ 140 mg L^{-1}	90.0	92
$N\text{-}NH_4^+$ (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

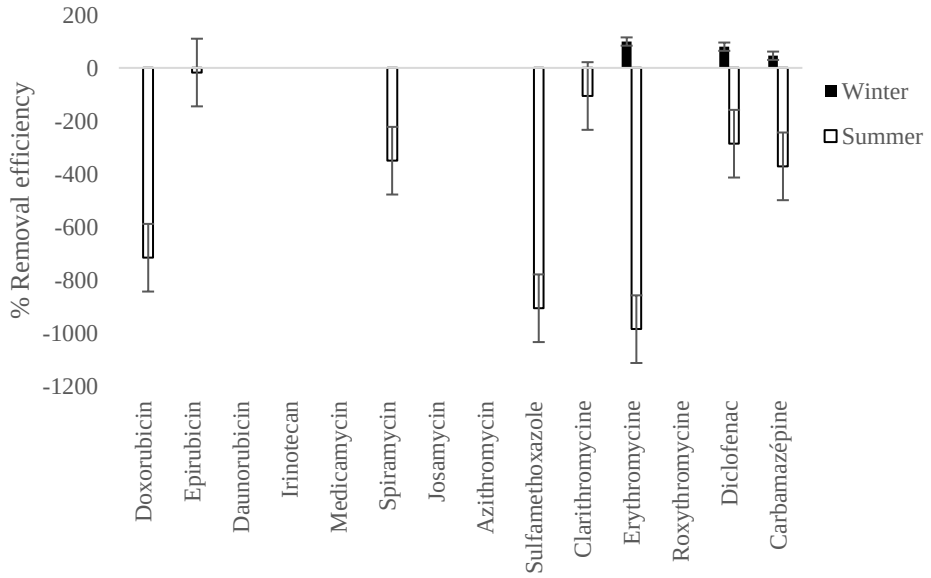
increase N and P in waters may lead to algal blooms, which in turn may disturb ecosystems. Algal blooms can be harmful when they lead to hypoxia or release for toxins, and secondary effects such as reduction in light penetration and dissolved oxygen, losses of submersed grasses and associated habitat, 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; 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; 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; NEAL; WITHERS, 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 3). 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.

Study 3 – Figure 3– Removal efficiency of the pharmaceutical compounds studied in POCIS sampled in winter 2018 and summer 2019



Source: (AUTHOR).

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 erytrhomycin, azytrhomycin and clarithromycin (GAO; MUNIR; XAGORARAKI, 2012; GROS et al., 2014; RODRIGUEZ-MOZAZ et al., 2015b; VERLICCHI; AL AUKIDY; ZAMBELLO, 2012). 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 (JELIC et al., 2015; KUMAR et al., 2012; VERLICCHI et al., 2012b) but can be further deconjugated by some enzymes present in wastewater bioreactor reverting them to their original form (CELIZ; TSO; AGA, 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 target 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, N_4-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 wastewater treatment (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; YANG; METCALFE, 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^{-1} 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., 2020b). A study by Somensi et al. (2012) demonstrated that O_3 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; MÅRTENSSON; MATHIASSON, 2009). The bacteria (predominantly Escherichia coli) can secrete the β-glucuronidase enzyme which can deconjugate already metabolized PPCPs 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; 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.

Study 3 – Table 3 –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; XIE; WEN, 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., 2016b). 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 sulfametoxazole 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 sulfametoxazole) in rivers. The notable fixation of antibiotics on environmental particles may be explained by surface complexation/sorption reactions (GU; 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 Kow) values ranging from 3.0 to 4.5 whereas the other (e.g., sulfametoxazole and roxythromycin) present log K_{ow} values below one (i.e., log Kow 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 (AUBERTHEAU et al. 2017).

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 waters in diverse regions world (HSU et al., 2014; PEI et al., 2006; PRUDEN et al., 2006).

Despite the good efficiency of the WWTPs in removing the erythromycin from the effluents, this compound was found accumulated in fish in 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., 2013b), 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 (DALAHMEH; ALZIQ; AHRENS, 2019b; DAR; BHAT, 2020b; FLEMMING; RIDGWAY, 2009; FLEMMING; WINGENDER, 2010b; TIJHUIS; VAN LOOSDRECHT;

HEIJNEN, 1994). 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., 2012b). 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 (DECEZARO et al., 2018). Moreover, plant roots can promote microbial biodegradation of pollutants (NOSZCZYŃSKA; PIOTROWSKA-SEGET, 2018; SHEHZADI et al., 2016). Microbial biodegradation can be promoted by a nonspecific increase in microbial metabolic activity in the area surrounding roots (SINGER; CROWLEY; THOMPSON, 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; BROMILOW; EVANS, 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 (GROSS-SOROKIN; ROAST; BRIGHTY, 2006; NASH et al., 2004). There are also risks associated with the occurrence or the development of bacterial resistance to antibiotics (BASTOS et al., 2018c) and the serious consequences on public health.

Regardless of their very small concentration (e.g. ng L^{-1} to µg 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., 2017b). 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 diclofenac compound was not found in the effluent; it already appeared in watercourse - upstream and downstream. The carbamazepine 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 carbamazepine 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 µg g^{-1} of this downstream compound of WWTP (ZHANG; 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., 2016b).

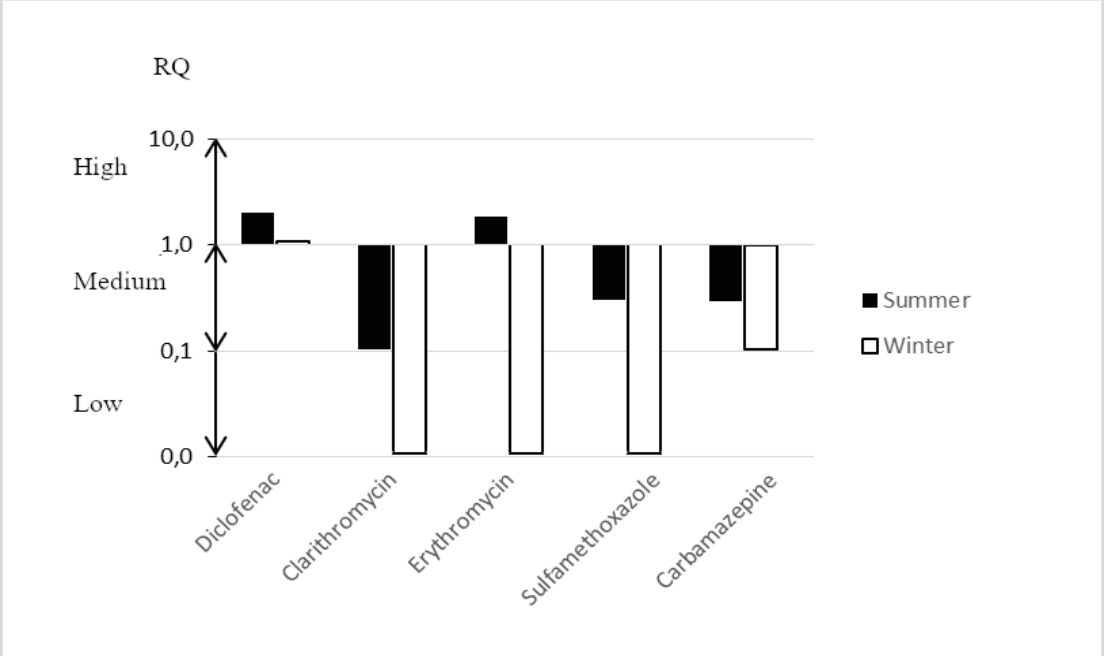
Study 3 – Table 4 – Concentration of pharmaceuticals in biofilms and POCIS in winter 2018 and summer 2018/2019.

Molecules	Biofilm (ng g^{-1})					POCIS (ng g^{-1})				
	Upstream 1	Upstream2	Upstream3	Effluent	Downstream	Upstream 1	Upstream2	Upstream3	Effluent	Downstream
					Summer					
Doxorubicin	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	863.1	3.5
Epirubicin	<LOQ	<LOQ	<LOQ	15.5	<LOQ	<LOQ	<LOQ	<LOQ	124.2	<LOQ
Daunorubicin	<LOQ	<LOQ	<LOQ	9.1	<LOQ	<LOQ	<LOQ	<LOQ	215.2	<LOQ
Irinotecan	<LOQ	<LOQ	<LOQ	15.6	2.3	<LOQ	<LOQ	<LOQ	<LOQ	1.3
Medicamycin	<LOQ	<LOQ	<LOQ	45.7	<LOQ	<LOQ	<LOQ	0.7	220.9	<LOQ
Spiramycin	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	64.3	<LOQ
Josamycin	<LOQ	0.9	<LOQ	7.8	0.4	<LOQ	<LOQ	1.8	<LOQ	0.6
Azithromycin	2.0	5.2	11.0	11.0	2.2	<LOQ	<LOQ	0.8	<LOQ	4.9
Clarithromycine	3.7	3.5	2.0	7.0	1.2	<LOQ	0.4	1.1	160.6	3.1
Erythromycine	3.6	0.9	2.1	5.8	<LOQ	<LOQ	0.7	0.4	138.7	2.0
Roxythromycine	<LOQ	<LOQ	<LOQ	2.8	1.6	<LOQ	<LOQ	0.6	<LOQ	<LOQ
Sulfamethoxazole	8.5	4.3	<LOQ	<LOQ	5.8	<LOQ	<LOQ	1.6	231.8	89.8
Diclofenac	1.5	1.3	5.3	<LOQ	2.2	<LOQ	7.4	2.5	346.8	64.5
Carbamazepine	7.2	4.4	<LOQ	12.4	<LOQ	<LOQ	8.7	2.5	307.0	101.7
					Winter					
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	3.0	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Irinotecan	<LOQ	2.7	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.0
Medicamycin	<LOQ	2.3	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Spiramycin	<LOQ	<LOQ	<LOQ	19.6	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	4.8
Josamycin	<LOQ	<LOQ	<LOQ	1.0	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Azithromycin	3.6	7.2	7.4	29.7	20.0	<LOQ	2.8	24.2	<LOQ	18.4
Clarithromycine	2.7	5.6	5.9	2.6	17.1	1.1	2.4	22.1	<LOQ	18.6
Erythromycine	0.8	1.8	7.7	58.6	2.8	1.0	2.2	135.9	2.2	46.3
Roxythromycine	0.5	<LOQ	1.8	<LOQ	1.9	0.4	<LOQ	<LOQ	<LOQ	<LOQ
Sulfamethoxazole	<LOQ	40.5	12.5	11.3	<LOQ	<LOQ	18.2	5.3	3.8	141.3
Diclofenac	<LOQ	<LOQ	1.1	<LOQ	<LOQ	2.2	2.8	8.7	134.4	162.0
Carbamazepine	<LOQ	2.5	8.0	2.3	1.6	0.8	4.4	2.7	179.9	2.2

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 4).

Study 3 – Figure 4 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)



Source: (AUTHOR).

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 erytrhomycin 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; 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 (BECKERS et al., 2018; BUSCH et al., 2016) 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 BOD_5, 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 may be due to the reactivation of metabolite by hydrolysis or microbiological activities during the treatment in the CW. Indeed, biofilms shown to interaction 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; MASIH; VAN HULLEBUSCH, 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 REFERENCES

ACUÑA, V. et al. Nutrient attenuation dynamics in effluent dominated watercourses. **Water Research**, v. 160, p. 330–338, set. 2019.

ADHIKARI, J. R.; LOHANI, S. P. Design, installation, operation and experimentation of septic tank – UASB wastewater treatment system. **Renewable Energy**, v. 143, p. 1406–1415, dez. 2019.

AL-ZREIQAT, I. et al. Influence of septic tank attached growth media on total nitrogen removal in a recirculating vertical flow constructed wetland for treatment of domestic wastewater. **Ecological Engineering**, v. 118, p. 171–178, ago. 2018.

ANA. **Saneamento — Agência Nacional de Águas**. Capa. Disponível em: <https://www.ana.gov.br/aguas-no-brasil/saiba-quem-regula/saneamento>. Acesso em: 4 jun. 2020.

APHA. Standard Methods For the Examination of Water and Wastewater, 23nd edition. **Standard Methods For the Examination of Water and Wastewater, 23nd edition**, 2017.

ARIAS, C. A.; BRIX, H. Phosphorus removal in constructed wetlands: can suitable alternative media be identified? **Water Science and Technology**, v. 51, n. 9, p. 267–273, 1 maio 2005.

ASHFAQ, M. et al. Ecological risk assessment of pharmaceuticals in the receiving environment of pharmaceutical wastewater in Pakistan. **Ecotoxicology and Environmental Safety**, v. 136, p. 31–39, fev. 2017.

AUBERTHEAU, E. et al. Impact of wastewater treatment plant discharge on the contamination of river biofilms by pharmaceuticals and antibiotic resistance. **Science of The Total Environment**, v. 579, p. 1387–1398, fev. 2017.

AUVINEN, H. et al. Removal of pharmaceuticals by a pilot aerated sub-surface flow constructed wetland treating municipal and hospital wastewater. **Ecological Engineering**, v. 100, p. 157–164, mar. 2017.

ÁVILA, C. et al. Capacity of a horizontal subsurface flow constructed wetland system for the removal of emerging pollutants: An injection experiment. **Chemosphere**, v. 81, n. 9, p. 1137–1142, nov. 2010.

BALCOM, I. N. et al. Metagenomic analysis of an ecological wastewater treatment plant's microbial communities and their potential to metabolize pharmaceuticals. **F1000Research**, v. 5, p. 1881, 28 jul. 2016.

BASTOS, M. C. et al. Presence of Anthropogenic Markers in Water: A Case Study of the Guaporé River Watershed, Brazil. **CLEAN - Soil, Air, Water**, v. 46, n. 3, p. 1700019, mar. 2018a.

BASTOS, M. C. et al. Antibiotics and microbial resistance in Brazilian soils under manure application. **Land Degradation & Development**, v. 29, n. 8, p. 2472–2484, ago. 2018b.

BECKERS, L.-M. et al. Characterization and risk assessment of seasonal and weather dynamics in organic pollutant mixtures from discharge of a separate sewer system. **Water Research**, v. 135, p. 122–133, maio 2018.

BEN, W. et al. Occurrence, removal and risk of organic micropollutants in wastewater treatment plants across China: Comparison of wastewater treatment processes. **Water Research**, v. 130, p. 38–46, mar. 2018.

BESSE, J.-P.; GARRIC, J. Human pharmaceuticals in surface waters. **Toxicology Letters**, v. 176, n. 2, p. 104–123, jan. 2008.

BISOGNIN, R. P. et al. Occurrence and fate of pharmaceuticals in effluent and sludge from a wastewater treatment plant in Brazil. **Environmental Technology**, p. 1–12, 17 dez. 2019.

BISOGNIN, R. P.; WOLFF, D. B.; CARISSIMI, E. Revisão sobre fármacos no ambiente. **Revista DAE**, v. 66, n. 210, p. 78–95, 2018.

BLAAS, H.; KROEZE, C. Excessive nitrogen and phosphorus in European rivers: 2000–2050. **Ecological Indicators**, v. 67, p. 328–337, ago. 2016.

BRIGGS, G. G.; BROMILOW, R. H.; EVANS, A. A. Relationships between lipophilicity and root uptake and translocation of non-ionised chemicals by barley. **Pesticide Science**, v. 13, n. 5, p. 495–504, out. 1982.

BUSCH, W. et al. Micropollutants in European rivers: A mode of action survey to support the development of effect-based tools for water monitoring: Micropollutants in European rivers: a mode-of-action. **Environmental Toxicology and Chemistry**, v. 35, n. 8, p. 1887–1899, ago. 2016.

BUSSI, G. et al. Dynamic response of land use and river nutrient concentration to long-term climatic changes. **Science of The Total Environment**, v. 590–591, p. 818–831, jul. 2017.

CAMACHO-MUÑOZ, D. et al. Effectiveness of Conventional and Low-Cost Wastewater Treatments in the Removal of Pharmaceutically Active Compounds. **Water, Air, & Soil Pollution**, v. 223, n. 5, p. 2611–2621, jun. 2012.

CARBALLA, M. et al. Fate of pharmaceutical and personal care products (PPCPs) during anaerobic digestion of sewage sludge. **Water Research**, v. 41, n. 10, p. 2139–2150, maio 2007.

CASTELLET-ROVIRA, F. et al. Stropharia rugosoannulata and Gymnopilus luteofolius : Promising fungal species for pharmaceutical biodegradation in contaminated water. **Journal of Environmental Management**, v. 207, p. 396–404, fev. 2018.

CASTIGLIONI, S. et al. Removal of Pharmaceuticals in Sewage Treatment Plants in Italy. **Environmental Science & Technology**, v. 40, n. 1, p. 357–363, jan. 2006.

CELIZ, M. D.; TSO, J.; AGA, D. S. Pharmaceutical metabolites in the environment: analytical challenges and ecological risks. **Environmental Toxicology and Chemistry**, v. 28, n. 12, p. 2473, 2009.

CHEN, S.; XIE, J.; WEN, Z. Removal of pharmaceutical and personal care products (PPCPs) from waterbody using a revolving algal biofilm (RAB) reactor. **Journal of Hazardous Materials**, p. 124284, out. 2020.

CHEN, Y. et al. Occurrence, removal and environmental risk assessment of pharmaceuticals and personal care products in rural wastewater treatment wetlands. **Science of The Total Environment**, v. 566–567, p. 1660–1669, out. 2016.

CHEONG, M. S. et al. Influence of Sulfonamide Contamination Derived from Veterinary Antibiotics on Plant Growth and Development. **Antibiotics**, v. 9, n. 8, p. 456, 28 jul. 2020.

CHONOVA, T. et al. Separate treatment of hospital and urban wastewaters: A real scale comparison of effluents and their effect on microbial communities. **Science of The Total Environment**, v. 542, p. 965–975, jan. 2016.

CONAMA. **Resolução CONAMA nº 430, de 13 de maio de 2011 — 4ª Câmara - Meio Ambiente e Patrimônio Cultural**. Disponível em: <http://www.mpf.mp.br/atuacao-tematica/ccr4/dados-da-atuacao/projetos/qualidade-da-agua/legislacao/resolucoes/resolucao-conama-no-430-de-13-de-maio-de-2011/view>. Acesso em: 4 jun. 2020.

CONSEMA. **Resolução CONSEMA Nº 355 DE 13/07/2017 - Estadual - Rio Grande do Sul - LegisWeb**. Disponível em: <https://www.legisweb.com.br/legislacao/?id=346397>. Acesso em: 4 jun. 2020.

DA COSTA, J. F. et al. Role of vegetation (Typha latifolia) on nutrient removal in a horizontal subsurface-flow constructed wetland treating UASB reactor–trickling filter effluent. **Water Science and Technology**, v. 71, n. 7, p. 1004–1010, 1 abr. 2015.

DALAHMEH, S. S.; ALZIQ, N.; AHRENS, L. Potential of biochar filters for onsite wastewater treatment: Effects of active and inactive biofilms on adsorption of per- and polyfluoroalkyl substances in laboratory column experiments. **Environmental Pollution**, v. 247, p. 155–164, abr. 2019.

DAR, S. A.; BHAT, R. A. Aquatic Pollution Stress and Role of Biofilms as Environment Cleanup Technology. In: QADRI, H. et al. (Eds.). . **Fresh Water Pollution Dynamics and Remediation**. Singapore: Springer Singapore, 2020. p. 293–318.

DECEZARO, S. T. et al. Vertical flow constructed wetland planted with *Heliconia psittacorum* used as decentralized post-treatment of anaerobic effluent in Southern Brazil. **Journal of Environmental Science and Health, Part A**, v. 53, n. 13, p. 1131–1138, 10 nov. 2018.

DECEZARO, S. T. et al. Influence of hydraulic loading rate and recirculation on oxygen transfer in a vertical flow constructed wetland. **Science of The Total Environment**, v. 668, p. 988–995, jun. 2019.

DEVAULT, D. A. et al. Removal efficiency of emerging micropollutants in biofilter wastewater treatment plants in tropical areas. **Environmental Science and Pollution Research**, 26 out. 2020.

DORDIO, A. et al. Atenolol removal in microcosm constructed wetlands. **International Journal of Environmental Analytical Chemistry**, v. 89, n. 8–12, p. 835–848, 15 jul. 2009.

DOTRO, G. et al. Treatment Wetlands. **Water Intelligence Online**, v. 16, p. 9781780408774, 2017.

EBELE, A. J.; ABOU-ELWAFA ABDALLAH, M.; HARRAD, S. Pharmaceuticals and personal care products (PPCPs) in the freshwater aquatic environment. **Emerging Contaminants**, v. 3, n. 1, p. 1–16, mar. 2017.

FALÅS, P. et al. Occurrence and reduction of pharmaceuticals in the water phase at Swedish wastewater treatment plants. **Water Science and Technology**, v. 66, n. 4, p. 783–791, 1 ago. 2012.

FARRÉ, M. LA et al. Fate and toxicity of emerging pollutants, their metabolites and transformation products in the aquatic environment. **TrAC Trends in Analytical Chemistry**, v. 27, n. 11, p. 991–1007, dez. 2008.

FERRE-ARACIL, J. et al. Ozonation of hospital raw wastewaters for cytostatic compounds removal. Kinetic modelling and economic assessment of the process. **Science of The Total Environment**, v. 556, p. 70–79, jun. 2016.

FISCHER, K.; MAJEWSKY, M. Cometabolic degradation of organic wastewater micropollutants by activated sludge and sludge-inherent microorganisms. **Applied Microbiology and Biotechnology**, v. 98, n. 15, p. 6583–6597, ago. 2014.

FLEMMING, H.-C. et al. Biofilms: an emergent form of bacterial life. **Nature Reviews Microbiology**, v. 14, n. 9, p. 563–575, set. 2016.

FLEMMING, H.-C.; RIDGWAY, H. Biofilm Control: Conventional and Alternative Approaches. In: FLEMMING, H.-C. et al. (Eds.). . **Marine and Industrial Biofouling**. Springer Series on Biofilms. Berlin, Heidelberg: Springer Berlin Heidelberg, 2009. v. 4p. 103–117.

FLEMMING, H.-C.; WINGENDER, J. The biofilm matrix. **Nature Reviews Microbiology**, v. 8, n. 9, p. 623–633, set. 2010.

FOLADORI, P.; RUABEN, J.; ORTIGARA, A. R. C. Recirculation or artificial aeration in vertical flow constructed wetlands: A comparative study for treating high load wastewater. **Bioresource Technology**, v. 149, p. 398–405, dez. 2013.

FREIXA, A. et al. River biofilms adapted to anthropogenic disturbances are more resistant to WWTP inputs. **FEMS Microbiology Ecology**, v. 96, n. 9, p. fiaa152, 1 set. 2020.

GAO, P.; MUNIR, M.; XAGORARAKI, I. Correlation of tetracycline and sulfonamide antibiotics with corresponding resistance genes and resistant bacteria in a conventional municipal wastewater treatment plant. **Science of The Total Environment**, v. 421–422, p. 173–183, abr. 2012.

GARCÍA, J. et al. Contaminant Removal Processes in Subsurface-Flow Constructed Wetlands: A Review. **Critical Reviews in Environmental Science and Technology**, v. 40, n. 7, p. 561–661, 30 jun. 2010.

GEIGER, E.; HORNEK-GAUSTERER, R.; SAÇAN, M. T. Single and mixture toxicity of pharmaceuticals and chlorophenols to freshwater algae Chlorella vulgaris. **Ecotoxicology and Environmental Safety**, v. 129, p. 189–198, jul. 2016.

GLIBERT, P. M. Eutrophication, harmful algae and biodiversity — Challenging paradigms in a world of complex nutrient changes. **Marine Pollution Bulletin**, v. 124, n. 2, p. 591–606, nov. 2017.

GÖBEL, A. et al. Occurrence and Sorption Behavior of Sulfonamides, Macrolides, and Trimethoprim in Activated Sludge Treatment. **Environmental Science & Technology**, v. 39, n. 11, p. 3981–3989, jun. 2005.

GOBEL, A. et al. Fate of sulfonamides, macrolides, and trimethoprim in different wastewater treatment technologies. **Science of The Total Environment**, v. 372, n. 2–3, p. 361–371, 1 jan. 2007.

GONZÁLEZ-PÉREZ, D. M.; PÉREZ, J. I.; GÓMEZ, M. A. Behaviour of the main nonsteroidal anti-inflammatory drugs in a membrane bioreactor treating urban wastewater at high hydraulic- and sludge-retention time. **Journal of Hazardous Materials**, v. 336, p. 128–138, ago. 2017.

GREENWAY, M. The role of constructed wetlands in secondary effluent treatment and water reuse in subtropical and arid Australia. **Ecological Engineering**, v. 25, n. 5, p. 501–509, dez. 2005.

GROS, M. et al. Biodegradation of the X-ray contrast agent iopromide and the fluoroquinolone antibiotic ofloxacin by the white rot fungus Trametes versicolor in hospital wastewaters and identification of degradation products. **Water Research**, v. 60, p. 228–241, set. 2014.

GROSS-SOROKIN, M. Y.; ROAST, S. D.; BRIGHTY, G. C. Assessment of Feminization of Male Fish in English Rivers by the Environment Agency of England and Wales. **Environmental Health Perspectives**, v. 114, n. Suppl 1, p. 147–151, abr. 2006.

GU, C.; KARTHIKEYAN, K. G. Interaction of Tetracycline with Aluminum and Iron Hydrous Oxides. **Environmental Science & Technology**, v. 39, n. 8, p. 2660–2667, abr. 2005.

HARMAN, C. et al. Field comparison of passive sampling and biological approaches for measuring exposure to PAH and alkylphenols from offshore produced water discharges. **Marine Pollution Bulletin**, v. 63, n. 5–12, p. 141–148, 2011.

HIJOSA-VALSERO, M. et al. Behaviour of pharmaceuticals and personal care products in constructed wetland compartments: Influent, effluent, pore water, substrate and plant roots. **Chemosphere**, v. 145, p. 508–517, fev. 2016.

HSU, J.-T. et al. Prevalence of sulfonamide-resistant bacteria, resistance genes and integron-associated horizontal gene transfer in natural water bodies and soils adjacent to a swine feedlot in northern Taiwan. **Journal of Hazardous Materials**, v. 277, p. 34–43, jul. 2014.

HYLAND, K. C. et al. Sorption of ionized and neutral emerging trace organic compounds onto activated sludge from different wastewater treatment configurations. **Water Research**, v. 46, n. 6, p. 1958–1968, abr. 2012.

IBRAHIM, I.; TOGOLA, A.; GONZALEZ, C. Polar organic chemical integrative sampler (POCIS) uptake rates for 17 polar pesticides and degradation products: laboratory calibration. **Environmental Science and Pollution Research**, v. 20, n. 6, p. 3679–3687, jun. 2013.

ILYAS, H.; MASIH, I. The performance of the intensified constructed wetlands for organic matter and nitrogen removal: A review. **Journal of Environmental Management**, v. 198, p. 372–383, ago. 2017.

ILYAS, H.; MASIH, I. The effects of different aeration strategies on the performance of constructed wetlands for phosphorus removal. **Environmental Science and Pollution Research**, v. 25, n. 6, p. 5318–5335, fev. 2018.

ILYAS, H.; MASIH, I.; VAN HULLEBUSCH, E. D. Pharmaceuticals' removal by constructed wetlands: a critical evaluation and meta-analysis on performance, risk reduction, and role of physicochemical properties on removal mechanisms. **Journal of Water and Health**, v. 18, n. 3, p. 253–291, 1 jun. 2020.

ILYAS, H.; VAN HULLEBUSCH, E. D. Performance comparison of different types of constructed wetlands for the removal of pharmaceuticals and their transformation products: a review. **Environmental Science and Pollution Research**, v. 27, n. 13, p. 14342–14364, maio 2020.

INTERFARMA. **Guia 2018 : Dados do setor**. Disponível em: <https://www.interfarma.org.br/guia/guia-2018/dados_do_setor#ranking_das_industrias>. Acesso em: 15 mar. 2020.

JARVIE, H. P.; NEAL, C.; WITHERS, P. J. A. Sewage-effluent phosphorus: A greater risk to river eutrophication than agricultural phosphorus? **Science of The Total Environment**, v. 360, n. 1–3, p. 246–253, maio 2006.

JELIC, A. et al. Nano-occurrence and removal of PCBs within the Europe's largest petrochemical MBR system. **Water Research**, v. 83, p. 329–336, out. 2015.

JOSS, A. et al. Removal of pharmaceuticals and fragrances in biological wastewater treatment. **Water Research**, v. 39, n. 14, p. 3139–3152, set. 2005.

KAHL, S. et al. Effect of design and operational conditions on the performance of subsurface flow treatment wetlands: Emerging organic contaminants as indicators. **Water Research**, v. 125, p. 490–500, nov. 2017.

KAKIMOTO, T.; ONODA, Y. Fate of Pharmaceuticals in Composting Process. In: FUNAMIZU, N. (Ed.). . **Resource-Oriented Agro-sanitation Systems**. Tokyo: Springer Japan, 2019. p. 79–96.

KELBERT, M. et al. Laccase as an efficacious approach to remove anticancer drugs: A study of doxorubicin degradation, kinetic parameters, and toxicity assessment. **Journal of Hazardous Materials**, p. 124520, nov. 2020.

KERMANI, M. et al. Application of Moving Bed Biofilm Process for Biological Organics and Nutrients from Municipal Wastewater. **American Journal of Environmental Sciences**, v. 4, n. 6, p. 675–682, 1 jun. 2008.

KIM, S.-C.; CARLSON, K. Temporal and spatial trends in the occurrence of human and veterinary antibiotics in aqueous and river sediment matrices. **Environmental Science & Technology**, v. 41, n. 1, p. 50–57, 1 jan. 2007.

KLATTE, S.; SCHAEFER, H.-C.; HEMPEL, M. Pharmaceuticals in the environment – A short review on options to minimize the exposure of humans, animals and ecosystems. **Sustainable Chemistry and Pharmacy**, v. 5, p. 61–66, jun. 2017.

KRZEMINSKI, P. et al. Performance of secondary wastewater treatment methods for the removal of contaminants of emerging concern implicated in crop uptake and antibiotic resistance spread: A review. **Science of The Total Environment**, v. 648, p. 1052–1081, jan. 2019.

KUMAR, V. et al. De-conjugation behavior of conjugated estrogens in the raw sewage, activated sludge and river water. **Journal of Hazardous Materials**, v. 227–228, p. 49–54, ago. 2012.

LEUNG, H. W. et al. Pharmaceuticals in Tap Water: Human Health Risk Assessment and Proposed Monitoring Framework in China. **Environmental Health Perspectives**, v. 121, n. 7, p. 839–846, jul. 2013.

LI, W. et al. Occurrence, distribution and potential affecting factors of antibiotics in sewage sludge of wastewater treatment plants in China. **Science of The Total Environment**, v. 445–446, p. 306–313, fev. 2013a.

LI, W. et al. Occurrence and removal of antibiotics in a municipal wastewater reclamation plant in Beijing, China. **Chemosphere**, v. 92, n. 4, p. 435–444, jul. 2013b.

LI, W.-L. et al. An evaluation on the intra-day dynamics, seasonal variations and removal of selected pharmaceuticals and personal care products from urban wastewater treatment plants. **Science of The Total Environment**, v. 640–641, p. 1139–1147, nov. 2018.

LIU, H.-Q. et al. Spatial distribution and removal performance of pharmaceuticals in municipal wastewater treatment plants in China. **Science of The Total Environment**, v. 586, p. 1162–1169, maio 2017.

LIU, J. et al. Tissue distribution, bioconcentration, metabolism, and effects of erythromycin in crucian carp (Carassius auratus). **Science of The Total Environment**, v. 490, p. 914–920, ago. 2014.

LIU, J. et al. Occurrence, bioaccumulation and risk assessment of lipophilic pharmaceutically active compounds in the downstream rivers of sewage treatment plants. **Science of The Total Environment**, v. 511, p. 54–62, abr. 2015.

LIU, X. et al. A review on removing antibiotics and antibiotic resistance genes from wastewater by constructed wetlands: Performance and microbial response. **Environmental Pollution**, v. 254, p. 112996, nov. 2019.

LÓPEZ, A. et al. How does urban wastewater treatment affect the microbial quality of treated wastewater? **Process Safety and Environmental Protection**, v. 130, p. 22–30, out. 2019.

LUCAS, D. et al. Fungal treatment for the removal of antibiotics and antibiotic resistance genes in veterinary hospital wastewater. **Chemosphere**, v. 152, p. 301–308, jun. 2016.

MACHADO, A. I. et al. Overview of the state of the art of constructed wetlands for decentralized wastewater management in Brazil. **Journal of Environmental Management**, v. 187, p. 560–570, fev. 2017.

MAINSTONE, C. P.; PARR, W. Phosphorus in rivers — ecology and management. **Science of The Total Environment**, v. 282–283, p. 25–47, jan. 2002.

MATAMOROS, V.; HIJOSA, M.; BAYONA, J. M. Assessment of the pharmaceutical active compounds removal in wastewater treatment systems at enantiomeric level. Ibuprofen and naproxen. **Chemosphere**, v. 75, n. 2, p. 200–205, abr. 2009.

MATAMOROS, V.; SALVADÓ, V. Evaluation of the seasonal performance of a water reclamation pond-constructed wetland system for removing emerging contaminants. **Chemosphere**, v. 86, n. 2, p. 111–117, jan. 2012.

MERCIER, A. et al. Biofilm formation vs. PCB adsorption on granular activated carbon in PCB-contaminated aquatic sediment. **Journal of Soils and Sediments**, v. 13, n. 4, p. 793–800, abr. 2013.

MÉTIVIER, R. et al. Interaction of erythromycin ethylsuccinate and acetaminophen with protein fraction of extracellular polymeric substances (EPS) from various bacterial aggregates. **Environmental Science and Pollution Research**, v. 20, n. 10, p. 7275–7285, out. 2013.

MIAO, X.-S.; YANG, J.-J.; METCALFE, C. D. Carbamazepine and Its Metabolites in Wastewater and in Biosolids in a Municipal Wastewater Treatment Plant. **Environmental Science & Technology**, v. 39, n. 19, p. 7469–7475, out. 2005.

MIJANGOS, L. et al. Occurrence of emerging pollutants in estuaries of the Basque Country: Analysis of sources and distribution, and assessment of the environmental risk. **Water Research**, v. 147, p. 152–163, dez. 2018.

MUTER, O. et al. Removal of pharmaceuticals from municipal wastewaters at laboratory scale by treatment with activated sludge and biostimulation. **Science of The Total Environment**, v. 584–585, p. 402–413, abr. 2017.

NASH, J. P. et al. Long-Term Exposure to Environmental Concentrations of the Pharmaceutical Ethynylestradiol Causes Reproductive Failure in Fish. **Environmental Health Perspectives**, v. 112, n. 17, p. 1725–1733, dez. 2004.

NOSZCZYŃSKA, M.; PIOTROWSKA-SEGET, Z. Bisphenols: Application, occurrence, safety, and biodegradation mediated by bacterial communities in wastewater treatment plants and rivers. **Chemosphere**, v. 201, p. 214–223, jun. 2018.

OKUDA, T. et al. Removal efficiency of 66 pharmaceuticals during wastewater treatment process in Japan. **Water Science and Technology**, v. 57, n. 1, p. 65–71, 1 jan. 2008.

PEI, R. et al. Effect of River Landscape on the sediment concentrations of antibiotics and corresponding antibiotic resistance genes (ARG). **Water Research**, v. 40, n. 12, p. 2427–2435, jul. 2006.

PETRIE, B. et al. Biotic phase micropollutant distribution in horizontal sub-surface flow constructed wetlands. **Science of The Total Environment**, v. 630, p. 648–657, jul. 2018.

PRUDEN, A. et al. Antibiotic Resistance Genes as Emerging Contaminants: Studies in Northern Colorado. **Environmental Science & Technology**, v. 40, n. 23, p. 7445–7450, dez. 2006.

REINHOLDS, I. et al. Determination of pharmaceutical residues and assessment of their removal efficiency at the Daugavgriva municipal wastewater treatment plant in Riga, Latvia. **Water Science and Technology**, v. 75, n. 2, p. 387–396, 23 jan. 2017.

RITTMANN, B. E. et al. Capturing the lost phosphorus. **Chemosphere**, v. 84, n. 6, p. 846–853, ago. 2011.

RODRIGUEZ-MOZAZ, S. et al. Occurrence of antibiotics and antibiotic resistance genes in hospital and urban wastewaters and their impact on the receiving river. **Water Research**, v. 69, p. 234–242, fev. 2015.

ROSSMANN, M. et al. Performance of constructed wetlands in the treatment of aerated coffee processing wastewater: Removal of nutrients and phenolic compounds. **Ecological Engineering**, v. 49, p. 264–269, dez. 2012.

ROSSMANN, M. et al. Effect of influent aeration on removal of organic matter from coffee processing wastewater in constructed wetlands. **Journal of Environmental Management**, v. 128, p. 912–919, out. 2013.

RUEL, S. M. et al. On-site evaluation of the removal of 100 micro-pollutants through advanced wastewater treatment processes for reuse applications. **Water Science and Technology**, v. 63, n. 11, p. 2486–2497, 1 jun. 2011.

RÜHMLAND, S. et al. Fate of pharmaceuticals in a subsurface flow constructed wetland and two ponds. **Ecological Engineering**, v. 80, p. 125–139, jul. 2015.

RUSTEN, B. et al. The innovative moving bed biofilm reactor/solids contact reaeration process for secondary treatment of municipal wastewater. **Water Environment Research**, v. 70, n. 5, p. 1083–1089, jul. 1998.

SALGADO, S. R. T.; ARAÚJO, A. L. C. Levantamento da tipologia das estações de tratamento de esgoto oriundas do Programa de Aceleração do Crescimento e a previsão do impacto no índice de tratamento de esgoto no estado do Espírito Santo. **Engenharia Sanitaria e Ambiental**, v. 22, n. 2, p. 293–301, 21 nov. 2016.

SANT'ANNA JR, G. L. Tratamento biológico de efluentes: fundamentos e aplicações. **Engenharia Sanitaria e Ambiental**, v. 16, n. 2, p. IV–IV, jun. 2011.

SATHISHKUMAR, P. et al. Occurrence, interactive effects and ecological risk of diclofenac in environmental compartments and biota - a review. **Science of The Total Environment**, v. 698, p. 134057, jan. 2020.

SHARMA, P. K. et al. Treatment efficiency of vertical flow constructed wetland systems operated under different recirculation rates. **Ecological Engineering**, v. 120, p. 474–480, set. 2018.

SHEHZADI, M. et al. Ecology of bacterial endophytes associated with wetland plants growing in textile effluent for pollutant-degradation and plant growth-promotion potentials. **Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology**, v. 150, n. 6, p. 1261–1270, 1 nov. 2016.

SINGER, A. C.; CROWLEY, D. E.; THOMPSON, I. P. Secondary plant metabolites in phytoremediation and biotransformation. **Trends in Biotechnology**, v. 21, n. 3, p. 123–130, mar. 2003.

SOMENSI, C. A. et al. A comparison between ozonolysis and sonolysis/ozonolysis treatments for the degradation of the cytostatic drugs methotrexate and doxorubicin: Kinetic and efficiency approaches. **Journal of Environmental Science and Health, Part A**, v. 47, n. 11, p. 1543–1550, set. 2012.

STEFANAKIS, A. I. et al. Performance of pilot-scale horizontal subsurface flow constructed wetlands treating groundwater contaminated with phenols and petroleum derivatives. **Ecological Engineering**, v. 95, p. 514–526, out. 2016.

STEFANAKIS, A. I.; TSIHRINTZIS, V. A. Effect of outlet water level raising and effluent recirculation on removal efficiency of pilot-scale, horizontal subsurface flow constructed wetlands. **Desalination**, v. 248, n. 1–3, p. 961–976, nov. 2009.

STUMPF, M. et al. Polar drug residues in sewage and natural waters in the state of Rio de Janeiro, Brazil. **Science of The Total Environment**, v. 225, n. 1–2, p. 135–141, jan. 1999.

SUÁREZ, S. et al. How are pharmaceutical and personal care products (PPCPs) removed from urban wastewaters? **Reviews in Environmental Science and Bio/Technology**, v. 7, n. 2, p. 125–138, jun. 2008.

SUN, G. et al. Effect of effluent recirculation on the performance of a reed bed system treating agricultural wastewater. **Process Biochemistry**, v. 39, n. 3, p. 351–357, nov. 2003.

TERNES, T. A. et al. Irrigation of treated wastewater in Braunschweig, Germany: An option to remove pharmaceuticals and musk fragrances. **Chemosphere**, v. 66, n. 5, p. 894–904, jan. 2007.

TIJHUIS, L.; VAN LOOSDRECHT, M. C. M.; HEIJNEN, J. J. Formation and growth of heterotrophic aerobic biofilms on small suspended particles in airlift reactors. **Biotechnology and Bioengineering**, v. 44, n. 5, p. 595–608, 20 ago. 1994.

TRAN, N. H.; REINHARD, M.; GIN, K. Y.-H. Occurrence and fate of emerging contaminants in municipal wastewater treatment plants from different geographical regions-a review. **Water Research**, v. 133, p. 182–207, abr. 2018.

TREIN, C. M. et al. Tratamento descentralizado de esgotos de empreendimentos comercial e residencial empregando a ecotecnologia dos wetlandsconstruídos. **Ambiente Construído**, v. 15, n. 4, p. 351–367, dez. 2015.

UNESCO. **Wastewater, The Untapped Resource | United Nations Educational, Scientific and Cultural Organization**. Disponível em: <http://www.unesco.org/new/en/natural-sciences/environment/water/wwap/wwdr/2017-wastewater-the-untapped-resource/>. Acesso em: 26 ago. 2020.

VERLICCHI, P. et al. Hospital effluent: Investigation of the concentrations and distribution of pharmaceuticals and environmental risk assessment. **Science of The Total Environment**, v. 430, p. 109–118, jul. 2012.

VERLICCHI, P.; AL AUKIDY, M.; ZAMBELLO, E. Occurrence of pharmaceutical compounds in urban wastewater: Removal, mass load and environmental risk after a secondary treatment—A review. **Science of The Total Environment**, v. 429, p. 123–155, jul. 2012.

VERLICCHI, P.; ZAMBELLO, E. How efficient are constructed wetlands in removing pharmaceuticals from untreated and treated urban wastewaters? A review. **Science of The Total Environment**, v. 470–471, p. 1281–1306, fev. 2014.

VERNOUILLET, G. et al. Toxic effects and bioaccumulation of carbamazepine evaluated by biomarkers measured in organisms of different trophic levels. **Chemosphere**, v. 80, n. 9, p. 1062–1068, ago. 2010.

VIVAS, Z. et al. Changes in bacterioplankton density and viability in the Tordera river due to the input of effluents from waste water treatment plants. **Limnetica**, n. 36, p. 461–475, 25 out. 2017.

VYMAZAL, J. Removal of nutrients in various types of constructed wetlands. **Science of The Total Environment**, v. 380, n. 1–3, p. 48–65, jul. 2007.

VYSTAVNA, Y. et al. Removal efficiency of pharmaceuticals in a full scale constructed wetland in East Ukraine. **Ecological Engineering**, v. 108, p. 50–58, nov. 2017.

WANG, J.; WANG, S. Removal of pharmaceuticals and personal care products (PPCPs) from wastewater: A review. **Journal of Environmental Management**, v. 182, p. 620–640, nov. 2016.

WORLDATLAS. **Which Country Has the Most Fresh Water?** Disponível em: <https://www.worldatlas.com/articles/countries-with-the-most-freshwater-resources.html>. Acesso em: 15 mar. 2020.

WU, S. et al. Development of constructed wetlands in performance intensifications for wastewater treatment: A nitrogen and organic matter targeted review. **Water Research**, v. 57, p. 40–55, jun. 2014.

YANG, Y. et al. Occurrences and removal of pharmaceuticals and personal care products (PPCPs) in drinking water and water/sewage treatment plants: A review. **Science of The Total Environment**, v. 596–597, p. 303–320, out. 2017.

YUAN, X. et al. Distribution, mass load and environmental impact of multiple-class pharmaceuticals in conventional and upgraded municipal wastewater treatment plants in East China. **Environmental Science: Processes & Impacts**, v. 17, n. 3, p. 596–605, 2015.

ZHANG, D. et al. Removal of pharmaceuticals and personal care products in aquatic plant-based systems: A review. **Environmental Pollution**, v. 184, p. 620–639, jan. 2014.

ZHANG, H. et al. Occurrence, seasonal variation and removal efficiency of antibiotics and their metabolites in wastewater treatment plants, Jiulongjiang River Basin, South China. **Environmental Science: Processes & Impacts**, v. 17, n. 1, p. 225–234, 2015.

ZHANG, J. et al. Removal of cytostatic drugs from aquatic environment: A review. **Science of The Total Environment**, v. 445–446, p. 281–298, fev. 2013.

ZHANG, L. et al. Inhibitory effects of extracellular polymeric substances on ofloxacin sorption by natural biofilms. **Science of The Total Environment**, v. 625, p. 178–184, jun. 2018.

ZHANG, Y.; GEISSEN, S.-U. Prediction of carbamazepine in sewage treatment plant effluents and its implications for control strategies of pharmaceutical aquatic contamination. Chemosphere, v. 80, n. 11, p. 1345–1352, set. 2010.

ZHAO, Y.; SUN, G.; ALLEN, S. Purification capacity of a highly loaded laboratory scale tidal flow reed bed system with effluent recirculation. Science of The Total Environment, v. 330, n. 1–3, p. 1–8, 1 set. 2004.

ŽIBIENĖ, G. et al. Phosphorus removal in a vertical flow constructed wetland using dolomite powder and chippings as filter media. Journal of Water Security, v. 1, n. 1, p. 46–52, 22 dez. 2015.

ZORITA, S.; MÅRTENSSON, L.; MATHIASSON, L. Occurrence and removal of pharmaceuticals in a municipal sewage treatment system in the south of Sweden. Science of The Total Environment, v. 407, n. 8, p. 2760–2770, abr. 2009.

9 GENERAL CONCLUSION

The interest on the evaluation of pharmaceuticals compounds presence in the environment has increased in recent years. However, the knowledge on the possible consequences from pharmaceuticals compounds exposure to the intrinsic microbiome is still limited. The exposure to pharmaceuticals, even if at trace levels, may alter the microbial diversity and their function in the ecosystem. The colonizing microorganisms are responsible for depuration and nutrient removal in rivers, and for the nutrient cycle and plant interactions in the soil. The information available on the effects of pharmaceuticals on microbiome is scarce. In most of the works, the knowledge is limited to a few range of compounds and to biofilms formed only by one species of bacteria isolated. A reduction on the pharmaceuticals by acting on the source is impossible considering the societal dependence on these compounds. Therefore, the development of efficient strategies to remove pharmaceuticals is of utmost importance to impair putative risks for the public health.

The need of more complete information on the effects of pharmaceuticals compounds for microbiological communities is rising. This information will be crucial for the correct prioritization of pharmaceuticals removal in WWTPs and, consequently, to improve the strategies used for treatments. This study demonstrates that some compounds have significant effect in microbial behavior, being pharmaceuticals, particularly antibiotics, carbamazepine and diclofenac, pharmaceuticals typically studied in terms of effects in microbiomes of water systems. It seems unquestionable that antibiotics and other antimicrobial agents have a significant role on the spread of antibiotic resistance. However, the available literature is not so conclusive about the effects of these compounds on biofilms development and behavior. It is important not to disregard that the effects of pharmaceuticals may depend on different factors: concentration, nutrient availability, hydrodynamics, time of exposure, etc. The variability of experimental conditions, combined with the presence of mixtures as well as the lack of information about the effects of non-pharmaceutical constitute the main challenges to be overcome.

Through this study we can conclude that anthropic actions have impacted the aquatic environment and that the lack of sewage treatment is an aggravating factor for the concentration of drugs found both in the water and in the biofilms. The internal activities of the university are contributing to the contamination of the stream that crosses the university. For the first time the speed of biofilm resilience was presented, and it was found that the microbiological community

evolves rapidly. Leading to changes of drug concentrations in just a few hours and change of bacterial community in a few days. Some compounds studied here present a risk to aquatic life, again highlighting the need for research in this area.

Although the WWTP evaluated in this study presents limits in the removal of pharmaceuticals, this technology offers an interesting solution to limit the pollution of waterways. 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. For small scales we can use simple solutions that are not too expensive and that allow us to limit waste. Perhaps the campus should be the place to put the natural solutions that exist into practice. The university should be the model, the place to be able to do these experiments and show both the students and the (scientific) community that there can be a solution for water quality. According to our study this would be a viable solution and one that could raise awareness for environmental treatment and education. If the incentive to improve the environmental quality starts in the universities, we can reach larger scales, with more adapted treatments and a more conscious society.

As long as wastewater treatment remains a matter of political interest, only to win votes, and not a primary issue for the well-being of the population and environmental preservation, the lack of investment will be faced and the standardization of the sewage treatment network will be increasingly delayed. The moment there is a financial return behind the wastewater treatment, this reality will change. Just as a curiosity, research has been going on to study the formation of struvite (a phosphate mineral) from human urine. Since human urine alone contributes to 90% of phosphorous (P) load to municipal wastewater, retrieval of nutrients in the form of struvite from urine has gained much attention as it can act as an excellent slow-release fertilizer for plant growth. So, when this type of recovery is more profitable than the expense of sewage collection and treatment there will be more investment in this area.

10 CONTRIBUTION OF THE THESIS TO THE FUTURE

The development of this thesis work is a milestone in the evaluation of the contamination of the entire university stream by pharmaceuticals residues, considering that it is an extremely important theme given the fact that the interned structures of the university are major contributors to the contamination of resources and that there were no studies that measured this impact yet. The development of this study is also important, because there is planning to implement the channeling of the entire sewage load of the neighborhood where the university is located and also of the university. With the database obtained, it will be possible to evaluate the impact that the collection and treatment of waste will bring on the environment, since there is this preliminary study for future comparison. Based on this work, we will have information on environmental contamination, pollution load contributed by the University, local community and the first database based on a new analysis matrix for water quality.

11 PUBLICATION ARISING FROM THIS THESIS

Until the defense of this thesis, one article has been published and all the others have been submitted to international scientific journals.

Vargas, J.P.R., Bastos, M.C., Al Badany, M., Gonzalez, R., Wolff, D., Santos, D.R.D., Labanowski, J., 2021. Pharmaceutical compound removal efficiency by a small constructed wetland located in south Brazil. Environ Sci Pollut Res. https://doi.org/10.1007/s11356-021-12845-6 **(Appendix 2)**.

APPENDIX 1 - CHARACTERIZATION OF EPILITHIC BIOFILMS

1 INTRODUCTION

In aquatic environments, microbial cells grow in association with rock surfaces, leading to the formation of epilithic biofilms. Biofilms adsorb and transform organic substances and nutrients in the matrix, as well as accumulate substances that, in the water flow, would be highly diluted, such as dissolved organic carbon, metals, and contaminants (FLEMMING; WINGENDER, 2010). They can show early signs of changes in the environment, with alterations in their structure and function (SABATER et al., 2007).

In nature, the variability of the aquatic environment can exert control over the ecological processes of biofilms (LAURENT, 2013) and vary their composition in space. Thus, the first factor affecting the mineral constitution of epilithic biofilms is the origin of the rocks, as they differ in their mineralogical composition, texture and structure. The mineralogical composition deals with the presence of essential minerals and also determines the chemical composition of the rock, as the sum of the chemical composition of the minerals that compose it (SOURCES, 2015). The mineral constitution of biofilms can also be affected by the degree of weathering of rocks, since rocks, which have minerals as their constituent units, are the fundamental basis on which the weathering process will develop (SOURCES, 2015). Depending on their origin, rocks may present different porosities and dissolution capacities. These factors characterize different permeabilities and adhesion possibilities of epilithic biofilms, according to the fragility of the rocks (BATTIN et al., 2016).

As biofilms are included in most of the biological elements involved in the transfer of energy and matter in the river, changes in the composition of these communities may indicate temporary or irreversible changes in the aquatic environment such as, for example, decreased diversity and the formation of biological communities composed of species tolerant to the new conditions. In addition, the overall effect on river biodiversity can be evidenced (SABATER et al., 2016) as well as interference in contaminant adsorption processes. In this sense, to complement the main study of this work, we conducted this secondary study with the objective of making a rapid characterization of the chemical composition of the mineral fraction of epilithic biofilms used as pollution markers. The methodologies used were previously described in the general material and methods.

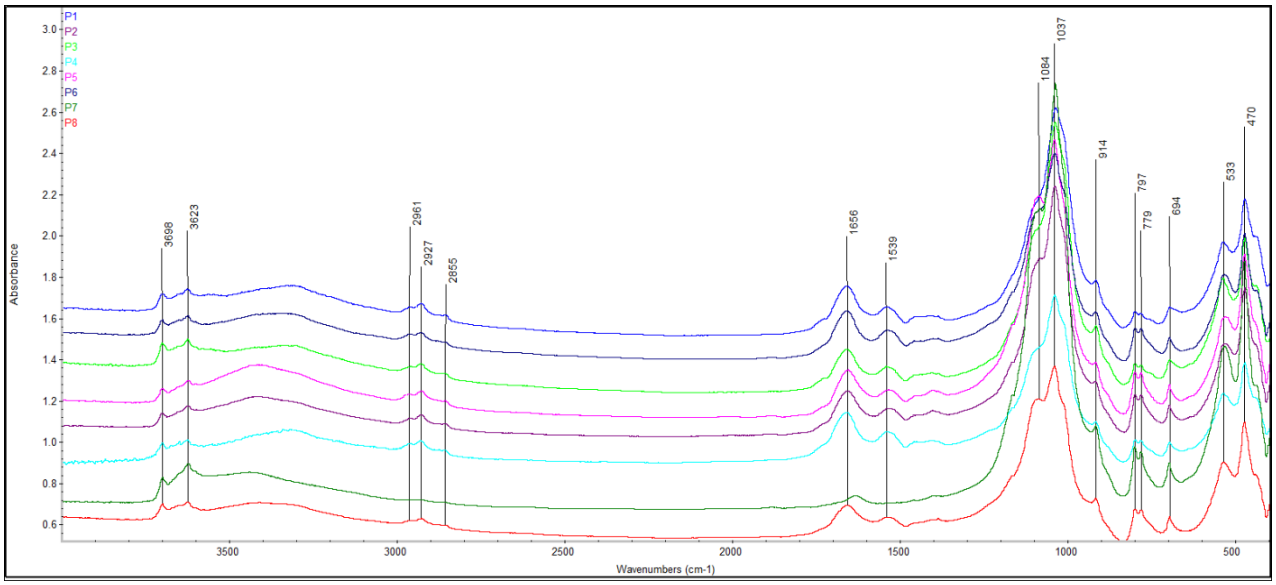
2 RESULTS AND DISCUSSION

2.1 INFRARED

Fifteen main spectral characteristics were identified and grouped according to the main classes of biochromoles (Figure 1 and 2). The spectra extracted indicate mainly the presence of polysaccharides, 1300 to 900 cm^{-1}, as well as compounds containing N, from 1700 to 1500 cm-1 (SILVERSTEIN; WEBSTER; KIEMLE, 2015).

Samples do not present great difference among themselves, but that in general the polysaccharides are identified by the bands around 1030 and 1005 cm^{-1}, which correspond to C-O-C elongation vibrations, typical of ethers, a group of organic molecules that is part of the polysaccharide structure (Figure 1 and 2). Acyclic and cyclic ethers and related compounds such as acetals and cetais present a typical infrared absorption in the region of 1300-900 cm^{-1} (BARBOSA, 2007). On the other hand, the 1410 cm^{-1} band corresponds to hydroxyl groups (OH) connected to the - CH_2 grouping of polysaccharides.

APPENDIX – Figure 1– Infrared spectra obtained in the winter collection of biofilm



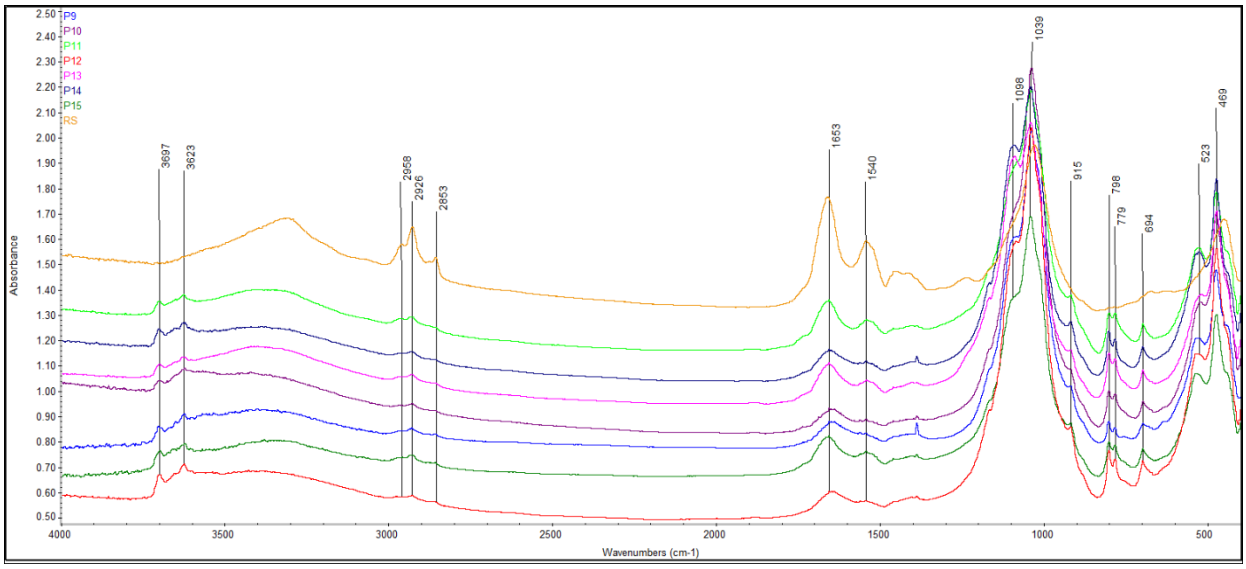
Source: (AUTHOR).

The 1656 and 15391 cm^{-1} bands are allocated to primary amides : amide I and amide II, respectively. Primary amides have a carbonyl group (C=O) attached to a NH_2 group and may also have alkyl or aryl groups attached to the N atom. In all amides, the spectra in IV have a band referring to the stretching of the C=O bond, which is known as the I amide band. In the amide spectra that present one or two atoms of hydrogen bonded to nitrogen are also observed stretching and angular deformation bands of N-H connections. The stretching bands of the C-

N connection are less important from the point of view of identification of this class of compounds.

The spectra also reveal bands typically associated with the portion of aliphatic hydrocarbons constituting fatty acids and lipids (symmetrical and asymmetrical stretches of CH_3 and CH_2 in the region of 2980 to 2800 cm^{-1}), at low absorbance intensities. Lipids are insoluble organic compounds found in biological tissues, which form the basic structure of all biological membranes. In general, the positions of the bands relative to these stretches vary little, and methyl groups present asymmetrical stretching absorptions around 2975-2950 cm^{-1}, which can be easily distinguished from the asymmetrical stretching of CH_2 that appears around 2930-2920 cm^{-1} (BARBOSA, 2007).

APPENDIX – Figure 2– Infrared spectra obtained in the winter collection of biofilm



Source: (AUTHOR).

The spectra of the biofilm samples are very similar, both in form and in absorbance intensity, indicating that the technique is not discriminant or that there is not much variation in the composition of the epilithic biofilms in qualitative terms. For polysaccharides, the bands are very similar, both in fall and spring and also between collection points. For proteins and lipids, the bands are relatively larger in autumn and for practically all samples. According to D'Abzac et al. (2010) the interference between the bands does not allow quantifying the quantities of biochemical compounds present in biofilms, but the IR spectra reveal an overview of the functional groups present.

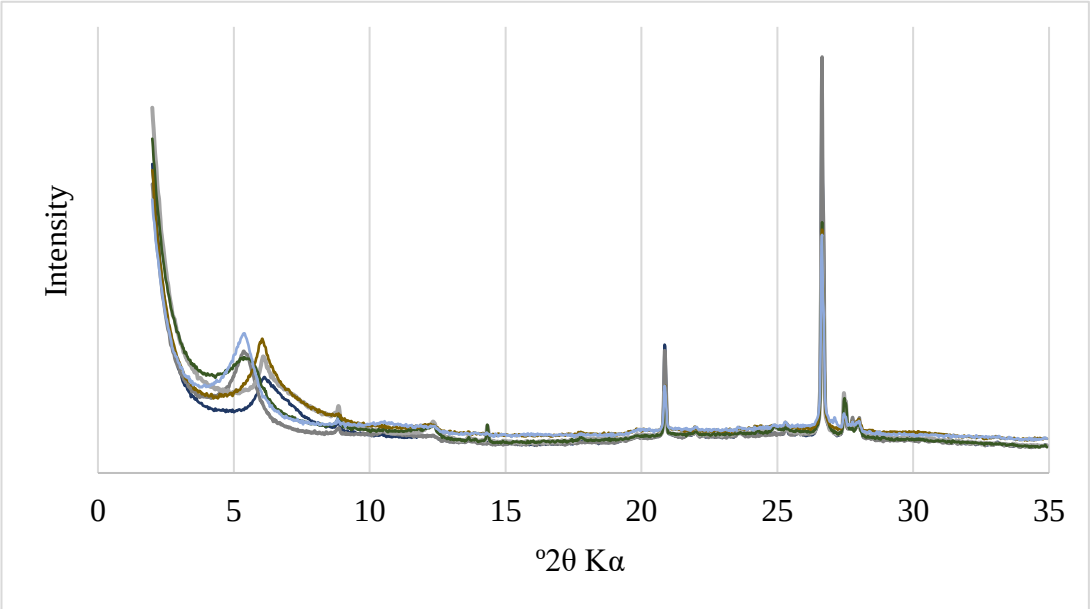
2.2 X- RAY

The X-ray diffraction (XRD) is the most widely used tool for studying minerals, particularly those present in the clay fraction. It produces detailed information about the crystallographic structure of the samples, which can be used to identify the phases present (RESENDE et al., 2011). Thus, this technique was also applied to the epilithic biofilms of points 1, 3 and 6 in order to obtain information about the presence of minerals in epilithic biofilms in natural environments.

The result of XRD analysis is presented in the form of a graph, the diffractograms, whose variables are the angle 2θ (diffraction angle of the minerals, which represent the spacing between the atomic planes of the minerals d: the fingerprint of the minerals) versus the intensity

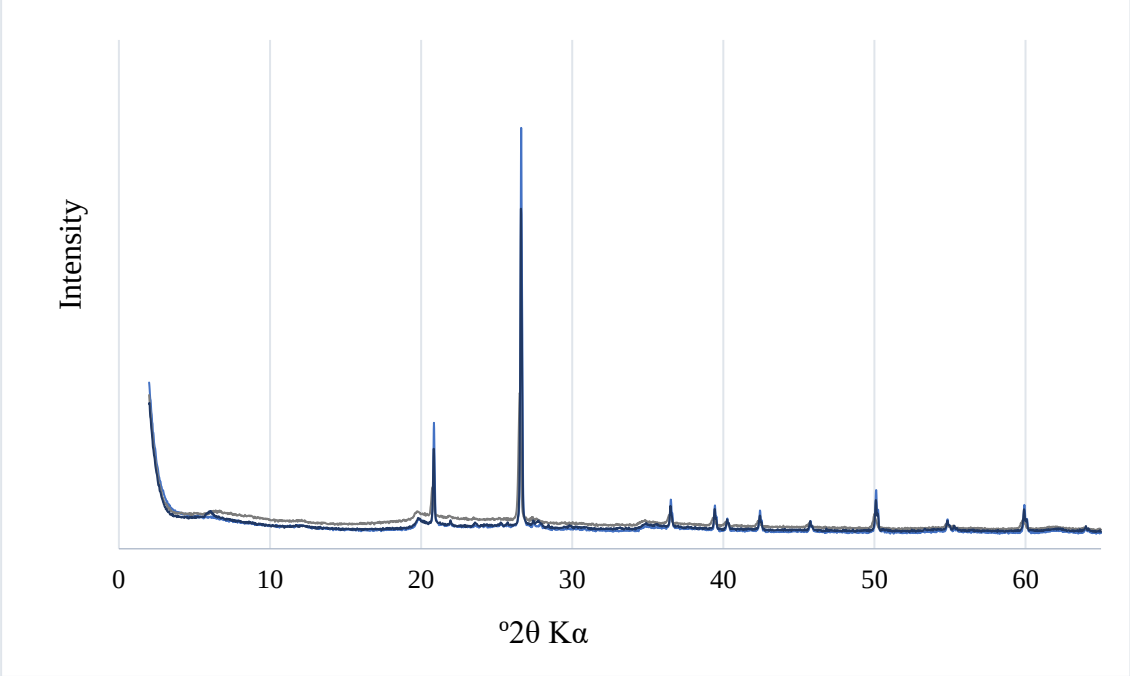
of the diffracted peaks (vertical axis, where also appears the emitting source, CuK α with λ = 0.15418 nm). The heights of the peaks are proportional to the intensities of the diffraction effects (RESENDE et al., 2011). Thus, Figure 3 displays the diffractogram obtained from the mineralogy analysis of the biofilm in the form of disoriented powder, whereas Figure 4 demonstrates the diffractogram obtained from the mineralogy analysis of the biofilm in the form of oriented sheet deposit and after Ethylene Grycol (EG).

APPENDIX – Figure 3 – X-ray diffractogram of the mineralogical analysis of the biofilm in the form of a disoriented powder



Source: (AUTHOR).

APPENDIX – Figure 4– X-ray diffractogram of the mineralogical analysis of biofilm in the form of oriented sheet deposit



Source: (AUTHOR).

The powder XRD shows a significant presence of quartz in these samples (peaks at 3.34 Å mainly). In these samples there are also clay minerals but in low proportion. There is kaolinite (7.17Å), there is a 2:1 mineral (certainly smectite) at 14 Å. The sample which contains the most clay minerals and in particular the 2:1 is the sample 6, because the peak at 14 Å is more intense. The oriented samples indicate presence of quartz: peaks 4.25 Å and 3.34 presence of kaolinite in small quantity: peak at 7.15-7.2 Å . Mineral 2:1 whose peak in AD is at 14.4 Å and which moves to 16.43 Å in EG: smectite or interstratified illite smectite rich: the proportion of this mineral is important. Mineral 2:1 illite with peak at 10 Å which does not move in EG.

The particularity of these samples is the high proportion of 2:1 smectite-rich minerals (illite smectite interlayers), that despite not being common in Santa Maria soil these samples are biofilms from the University River – stream Lagoão do Ouro, where the points are located in flooded areas, being very young soils that have not suffered from leaching phenomena. Thus, the contribution comes mainly from the flooded soils in the riverbed.

The identification of minerals by XRD is justified by the large percentage of mineral material present in epilithic biofilms. Anyway, the diffractogram results show that the presence of minerals in the epilithic biofilm are due to the presence of rock minerals, through weathering promoted by microorganisms, from sediments transported to the river, from the low weathering

that occurs in the soil and biofilms in the water or even the neoformation of minerals in epilithic biofilms, by the influence of microorganisms (bioformation).

Pires and Lacerda (2015) when analyzing mangrove biofilms, found that the general mineralogy for sediments and biofilms revealed the presence of quartz, micas, feldspar, mica-vermiculite and amphibole. The mineralogy of the clay minerals revealed the presence of kaolinite, illite, smectite, chlorite, and irregular mixed layers of illite-chlorite and illite-smectite. Thus, for the epilithic biofilms in this study, longer analysis times of the samples on oriented slide could also provide diffractograms with 2:1 clay minerals peaks.

3 CONCLUSION

The XRD revealed the presence of quartz, kaolinite, smectite and/or stratified illite in the biofilms. The spectra infrared extracted indicate mainly the presence of polysaccharides, as well as compounds containing N. The infrared also reveals bands typically associated with the portion of aliphatic hydrocarbons constituting fatty acids, lipids and aromatic structures that can interact and adsorb other molecules. Although environmental biofilm systems have been studied for several decades, there are still research gaps to be filled due to the typically heterogeneous composition of this matrix, from a spatial point of view.

4 REFERENCES

BARBOSA, L. C. de A. **Espectroscopia no infravermelho na caracterização de compostos orgânicos**. Viçosa: Editora UFV, 2007. 189 p.

BATTIN, T. J. et al. The ecology and biogeochemistry of stream biofilms. **Nature Reviews Microbiology**, v. 14, n. 4, p. 251–263, abr. 2016.

D'ABZAC, P. et al. Effects of extraction procedures on metal binding properties of extracellular polymeric substances (EPS) from anaerobic granular sludges. **Colloids and Surfaces B: Biointerfaces**, v. 80, n. 2, p. 161–168, 15 out. 2010.

FLEMMING, H.-C.; WINGENDER, J. The biofilm matrix. **Nature Reviews Microbiology**, v. 8, n. 9, p. 623–633, set. 2010.

LAURENT, E. **Evaluation de l'état de contamination des bassins versants par les résidus de médicaments**: Utilisation des biofilms épilithiques comme marqueur d'imprégnation du milieu. 2013. 221f. Tese (Doutorado em Chimie et Microbiologie de l'eau) - Université de Poitiers, Poitiers, 2013

PIRES, L. C.; LACERDA, L. D. de. Intemperismo de minerais associados a biofilmes de sedimentos de manguezais. **Arquivos de Ciências do Mar**, Fortaleza, v. 48, n. 1, p. 62-68, 2015

RESENDE, M.; CURI, N.; KER, J. C.; REZENDE, S. B. de. **Mineralogia de Solos Brasileiros**. Interpretação e Classificação. 2 ed. Lavras, MG: Editora UFLA, 2011. 206 p.

SABATER, S. et al. Monitoring the effect of chemicals on biological communities. The biofilm as an interface. **Analytical and Bioanalytical Chemistry**, v. 387, n. 4, p. 1425–1434, 6 fev. 2007.

SABATER, S. et al. Shared effects of organic microcontaminants and environmental stressors on biofilms and invertebrates in impaired rivers. **Environmental Pollution**, v. 210, p. 303–314, mar. 2016.

SILVERSTEIN, R. M.; WEBSTER, F. X.; KIEMLE, D. J. **Identificação espectrométrica de compostos orgânicos**.7. ed. Rio de Janeiro: LTC, 2006. 530 p

APPENDIX 2

Environmental Science and Pollution Research
https://doi.org/10.1007/s11356-021-12845-6

RESEARCH ARTICLE

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

Jocelina Paranhos Rosa De Vargas[1,2] · Marília Camotti Bastos[2] · Maha Al Badany[2] · Rolando Gonzalez[3] · Delmira Wolff[3] · Danilo Rheinheimer Dos Santos[1] · Jérôme Labanowski[2]

Received: 9 November 2020 / Accepted: 3 February 2021

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 pharmaceutical 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, COD, and BOD_5 and 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

Introduction

Contamination of water resources with pharmaceutical compounds has raised concerns due to their extensive use and continuous discharge into the aquatic environment. The administered dose of pharmaceuticals is rarely entirely decomposed by the body; thus, significant portions of unmetabolized 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

Responsible Editor: Alexandros Stefanakis

✉ Jocelina Paranhos Rosa De Vargas
jocelinavargas@hotmail.com

Marília Camotti Bastos
marilia.camotti-bastos@unilim.fr

Maha Al Badany
maha.al.badany@univ-poitiers.fr

Rolando Gonzalez
rolando.ufsm@gmail.com

Delmira Wolff
delmirawolff@hotmail.com

Danilo Rheinheimer Dos Santos
danilo.rheinheimer@pq.cnpq.br

Jérôme Labanowski
jerome.labanowski@univ-poitiers.fr

[1] Centro de Ciências Rurais, Departamento de Solos, Universidade Federal de Santa Maria, Av. Roraima nº 1000, Cidade Universitária, Bairro Camobi, Santa Maria, Rio Grande do Sul 97105-900, Brazil

[2] Institut de Chimie des Milieux et Matériaux de Poitiers, Université de Poitiers, IC2MP, Poitiers, France

[3] Departamento de Engenharia Ambiental, Centro de Tecnologia, Universidade Federal de Santa Maria, Av. Roraima nº 1000, Cidade Universitária, Bairro Camobi, Santa Maria 97105-900, Rio Grande do Sul, Brazil

Published online: 16 February 2021