



**HAL**  
open science

## **Pesticide bioaccumulation in epilithic biofilms as a biomarker of agricultural activities in a representative watershed**

Danilo Rheinheimer dos Santos, José Augusto Monteiro de Castro Lima, Jocelina Paranhos Rosa de Vargas, Marilia Camotti Bastos, Maria Alice Santanna dos Santos, Leslie Mondamert, Jérôme Labanowski

### ► To cite this version:

Danilo Rheinheimer dos Santos, José Augusto Monteiro de Castro Lima, Jocelina Paranhos Rosa de Vargas, Marilia Camotti Bastos, Maria Alice Santanna dos Santos, et al.. Pesticide bioaccumulation in epilithic biofilms as a biomarker of agricultural activities in a representative watershed. *Environmental Monitoring and Assessment*, 2020, 192 (6), 10.1007/s10661-020-08264-8 . hal-03089856

**HAL Id: hal-03089856**

**<https://hal.science/hal-03089856v1>**

Submitted on 28 Dec 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Title:**

2 Pesticide bioaccumulation in epilithic biofilms as a biomarker of agricultural activities in a  
3 representative watershed

4 **Journal name:**

5 Environmental Monitoring and Assessment

6

7 **Author names:**

8 Danilo Rheinheimer dos Santos, José Augusto Monteiro de Castro Lima, Jocelina Paranhos Rosa de  
9 Vargas, Marilia Camotti Bastos, Maria Alice Santanna dos Santos, Leslie Mondamert, Jérôme  
10 Labanowski

11

12 **Corresponding author:**

13 Jocelina Paranhos Rosa de Vargas, Universidade Federal de Santa Maria. Av. Roraima nº 1000.  
14 Cidade Universitária, Bairro Camobi. Centro de Ciências Rurais, Prédio 42, Departamento de Solos,  
15 Santa Maria – Rio Grande do Sul, Brazil. CEP: 97105-900. Tel: +55 28 999 29 83 09 E-mail:  
16 jocelinaparanhos@mail.ufsm.br

17

18 Pesticide Bioaccumulation in Epilithic Biofilms as a Biomarker of Agricultural Activities in a Representative  
19 Watershed

20

21 **Abstract**

22 Brazil is one of the largest consumers of pesticides in the world. The high rainfall rate and inadequate soil use  
23 and management promote the transfer of these compounds to the aquatic system. The aim of this study was to  
24 identify and quantify pesticides present in epilithic biofilms in order to evaluate the effectiveness of this matrix  
25 as a bioindicator able to discriminate areas and periods with different inputs of pesticides. Among the 25  
26 pesticides analyzed in the biofilms, 20 compounds were detected. The epilithic biofilms picked up pesticides  
27 independent of their polarities, even in the period of lower use. The frequency and median concentration of five  
28 herbicides (2,4-D, atrazine, desethyl-atrazine, simazine, nicosulfuron), three fungicides (carbendazim,  
29 epoxiconazole, tebuconazole), and one insecticide (imidacloprid) were highest in biofilms sampled in summer  
30 crops during the growing period. Biofilms collected in the upper region of the catchment, where genetically  
31 modified soybean and corn cultivated in a no-tillage system prevail, the highest frequency and median  
32 concentration of three herbicides (2,4-D, thifensulfuron, isoproturon), four fungicides (carbendazim,  
33 epoxiconazole, tebuconazole, metconazole), and one insecticide (imidacloprid) were observed. Despite the  
34 excessive amounts of pesticides used in the catchment, the median values of all pesticides in the epilithic biofilm  
35 were considered low. The lower diversity and concentration of pesticides observed in the autumn/winter season  
36 is representative of lower use of pesticides, barriers to pesticide transfer from soil to water, and the biofilm's  
37 resilience capacity to decompose pesticides.

38

39 **Key-words:** contamination; soil use management; soil erosion; runoff; riparian forest.

40

41

42

## 43 **Introduction**

44

45 As elsewhere in the world, the model of Brazilian agriculture is based on the production and export of  
46 major crops such as soybean, corn, cotton, citrus, and coffee (Graminha et al. 2008). The availability of  
47 genetically modified seeds, advanced agricultural machinery, intensive usage of fertilizers and pesticides, and  
48 management of no-till farming led to the consolidation of this agricultural model. A problem affecting most of  
49 the producing countries is the increase in pesticide use, resulting in an increase in the augmentation of pesticide  
50 use per area of cropland from 1.5 kg ha<sup>-1</sup> in 1990 to 2.7 kg ha<sup>-1</sup> in 2016 (FAO, 2018). Based on these values, it is  
51 assumed that a significant part of the pesticides applied to the cultures (active compounds and metabolites) are  
52 not achieving their primary targets or are not being metabolized by plants.

53 Nowadays, environmental contamination with pesticides has received great scientific and societal  
54 attention due to the major risk posed to the natural environment and human health (Özkara et al. 2016; Tang et  
55 al. 2018). The occurrence of various contaminants in the surface water is correlated with agricultural activities,  
56 exhibiting the conflict between productivity and contamination (Bastos et al. 2018; Fernandes et al. 2019; Kaiser  
57 et al. 2015; Zafar et al. 2016). Pesticide residues can be transferred to water sources by soil erosion, surface  
58 runoff, and percolation. These losses are related to low-quality no-tillage management (Tiecher et al. 2017a), the  
59 withdrawal of the terraces, and a deficient supply of straw, leading to high loss rates of water, soil (Deuschle et  
60 al. 2019), and nutrients (Zafar et al. 2016).

61 The transfer of pesticides to aquatic ecosystems occurs mainly in diffuse form, that is, most of it is  
62 originated by the transport system, in this case, mainly rainwater transport carrying contaminated soil particles;  
63 therefore, pesticide residues are divided between the aqueous phase and adsorbent matrix eroded from the soil  
64 (Vryzas et al. 2018), depending on their physicochemical properties (aqueous solubility  $S_w$ , octanol-water  
65 partition coefficient  $K_{ow}$ , vapor pressure  $P$ , Henry's law constant  $H$ , octanol-air partition coefficient  $K_{OA}$ , octanol  
66 solubility  $S_o$  (Shen and Wania 2005). In water, these compounds are present in low concentrations ( $\mu\text{g L}^{-1}$  to ng  
67  $\text{L}^{-1}$ ), making it difficult for the monitoring process to perform active sampling. Once in the aquatic ecosystem,  
68 the relations between the pesticides found in soluble form and those adsorbed to a given matrix can change in the  
69 face of new geochemical and biological conditions in the environment. An example are the epilithic biofilms,  
70 which can interact with pesticides by adsorbing soluble compounds or incorporating contaminated sediments in  
71 its extracellular polymeric matrix (Battin et al. 2016; Lawrence et al. 2001).

72           The epilithic biofilms are composed of an inorganic matrix (weathering products with sediment  
73 attached), a diversity of autotrophic and heterotrophic microorganisms (bacteria, algae, fungi, and microfauna),  
74 and detritus exogenous or produced in situ (Flemming and Wingender 2010). Biofilms are of fundamental  
75 ecological importance in the aquatic ecosystem because their water/substrate interface is an autotrophic primary  
76 producer, an organic matter decomposer, and an adsorption/desorption reactor (Feckler et al. 2015). Biofilms  
77 have a high physicochemical reactivity due to the great diversity of surface functional groups, such as amine,  
78 amide, carboxyl, and phenolic in organic matrixes provided by organic constituents, as well as a permanent  
79 charge and surface hydroxyl sites in clay minerals and oxides in the inorganic colloid matrix provided by the  
80 inorganic constituents (Essington 2005; Fernandes et al. 2019). Proia et al. (2013) have shown that  
81 microorganisms in biofilms have a short life cycle and there is a strong interaction between the microbiota. Due  
82 to these characteristics, biofilms can be used as a powerful ecological indicator (Edwards and Kjellerup 2013;  
83 Sabater et al. 2007) because the availability of diverse sorption sites and their likely saturation contribute to  
84 multiple kinetics of bioaccumulation, being consecutive or stationary (Zhang et al. 2018).

85           In areas of intense agriculture, aquatic ecosystems and food webs are exposed to pesticides that alter  
86 species abundance and diversity, causing negative effects even at low concentrations (Beketov et al. 2013).  
87 When exposed to this type of contamination, the photosynthetic activity of biofilms can decrease, and inhibition  
88 of growth and changes in community structure can be observed (Kim et al. 2016; Pesce et al. 2012). For  
89 example, Bricheux et al. (2013) showed that by exposing biofilms to glyphosate and diuron, the growth of the  
90 autotrophic community was inhibited, and the bacterial communities adapted by changing their composition. In  
91 addition, areas with water collection for drinking water distribution may be contaminated, presenting a risk to the  
92 population if the treatment is inefficient for the removal of these organic compounds from the water (Beketov et  
93 al. 2013). Previous studies in the Guaporé catchment showed that bioaccumulation in epilithic biofilms could be  
94 used as biomarkers of anthropic contamination by glyphosate and its metabolite AMPA (Fernandes et al. 2019)  
95 and by carbamazepine and sucralose (Bastos et al. 2018). This study aimed at identifying and quantifying  
96 pesticides present in epilithic biofilms in order to evaluate the effectiveness of this matrix as a bioindicator  
97 capable of discriminating areas and periods with different inputs of pesticides.

98

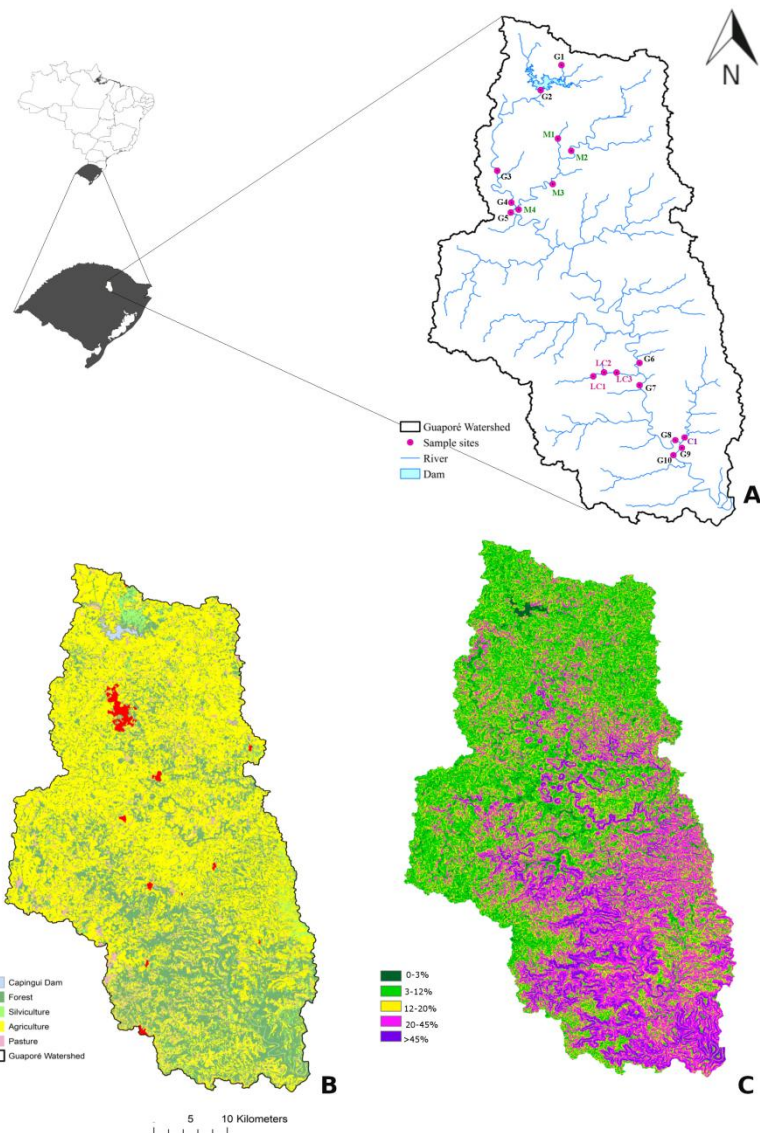
99 **Materials and Methods**

100

101 Catchment description

102

103            Located in the state of Rio Grande do Sul, southern Brazil, the Guaporé River catchment drains an area  
104 of 2,490 km<sup>2</sup> and is 153 km long (Lima et al. 2020). Classified as Cfa in the Köppen system, the climate is  
105 characterized by an average annual temperature of 17.9 °C and an average annual minimum and maximum  
106 temperatures of 12.6 and 24.7 °C, respectively. In this catchment, previous studies revealed that rain events have  
107 high erosion potential (8,800 MJ mm ha<sup>-1</sup> h<sup>-1</sup> year<sup>-1</sup>) (Didoné et al. 2014) with greater occurrence from July  
108 through November (INMET 2019). Sediment production in 2012 amounted to 390.2 Mg km<sup>-2</sup> year<sup>-1</sup>, and the  
109 average was estimated at 140 mg km<sup>-2</sup> year<sup>-1</sup> from 2000 to 2010, with a runoff coefficient of up to 31% (Didoné  
110 et al. 2014). The land use in this area is dominated by cropland (60.0%), natural forest (30.1%), pastureland  
111 (5.6%), forestry (3.1%), urban areas (0.6%), and water bodies (0.4%) (Fig. 1) characteristic of rural areas with  
112 high production in southern Brazil. In the upper third of the catchment, the relief is nearly level to gently sloping  
113 and predominated by Ferralsols; the no-tillage system has been adopted and has predominated for two decades.



114

115 **Fig 1** - Characteristics of area and location of the sampling sites in the Guaporé River and its  
 116 tributaries:(a) location of the sampling sites in the Guaporé watershed, (b) Guaporé watershed land use and (c)  
 117 Guaporé watershed slopes. (RS – Brazil).

118 Besides soybean (*Glycine max* L. Merr.) cultivation, livestock, swine, and poultry are also produced in  
 119 the catchment in spring/summer. The animals reside in marginal noncultivated areas, generally along the  
 120 watercourses. The pesticides used are characterized by high doses of herbicides and fungicides applied to  
 121 soybean production, including 2,4-D for plants resistant to glyphosate. The annual production density of this  
 122 region is 161.6 mg year<sup>-1</sup> km<sup>-2</sup> of soybean; 41.5 and 148.2 mg year<sup>-1</sup> km<sup>-2</sup> of corn and corn silage respectively;  
 123 and 0.3 and 1.3 mg year<sup>-1</sup> km<sup>-2</sup> of tobacco and yerba mate, respectively. In the other two-thirds (middle and  
 124 lower parts) of the catchment, land use is more diverse and conventional soil management predominates in  
 125 shallow soils with a rocky surface (Luvisols, Acrisols, and Regosols). The cultivated surface is imbricated in

126 landscape and the family farmers have less than 10 ha. The annual production density of this region is 38.1 mg  
127 year<sup>-1</sup> km<sup>-2</sup> of soybean; 53.4 and 233.1 mg year<sup>-1</sup> km<sup>-2</sup> of corn and corn silage, respectively; 3.5 and 34.1 mg  
128 year<sup>-1</sup> km<sup>-2</sup> of tobacco and yerba mate, respectively (IBGE, 2019).

129

130 Sampling sites

131

132 A total of 18 sampling sites were selected, which were located in Guaporé River (G1, G2, G3, G4, G5,  
133 G6, G7, G8, G9, and G10), in the tributary of the Marau Stream (M1, M2, M3, and M4), Lajeado Carazinho  
134 Stream Tributary (LC1, LC2, and LC3) and the tributary of the Carazinho Stream (C). Descriptions of each site  
135 and locations are shown in Table 1 and Figure 1, respectively.

136



Sample sites	Upper Region									
	G1	G2	G3	G4	G5	M1	M2	M3	M4	
Catchment area (km <sup>2</sup> )	1.3	123	201	267	542	13.4	165	227	256	
Distance (km)	0	6	23	32	35	-	0	10	23	
	<i>Land use (%)</i>									
Forest	100	28	24	23	22	22	20	21	22	
Agriculture	0	64	69	71	70	72	74	72	72	
Width riparian forest	>800	15-60	15	15	15	15	15	15	15	
	<i>Slope class (%)</i>									
0-3%	5	10	9	8	7	6	7	6	6	
3.1-12%	49	60	59	58	57	56	60	57	56	
12.1-20%	35	22	23	23	24	25	23	24	24	
20.1-45%	12	8	9	10	11	13	9	12	13	
> 45%	0	0	0	0	1	0	0	1	1	
	<b>Middle/Lower Region</b>									
Sample sites	<b>G6</b>	<b>G7</b>	<b>G8</b>	<b>G9</b>	<b>G10</b>	<b>LC1</b>	<b>LC2</b>	<b>LC3</b>	<b>C</b>	
Catchment area (km <sup>2</sup> )	1442	1505	1697	1850	1853	30	3.2	39	144	
Distance (km)	85	90	106	108	110	0	-	3.2	0	
	<i>Land use (%)</i>									
Forest	27	28	31	32	32	50	61	52	46	
Agriculture	67	66	62	61	61	42	32	40	50	
Width riparian forest	15	>60	>100	>100	>100	>100	15	>100	>100	
	<i>Slope class (%)</i>									
0-3%	6	5	5	5	5	4	1	3	2	
3.1-12%	47	45	42	41	41	34	10	28	23	
12.1-20%	24	24	23	23	23	24	20	22	27	
20.1-45%	21	22	24	26	26	29	53	34	43	
> 45%	3	4	5	5	5	10	17	14	5	

138 **Table 1** - Catchment area, land use and slope class at the different sampling sites in the Guaporé

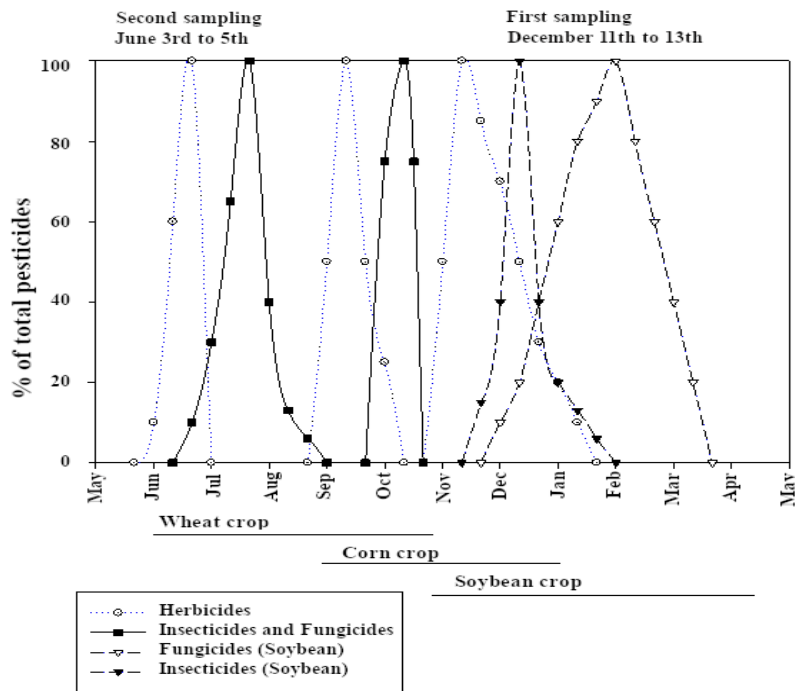
139 River and its tributaries.

140

141 Biofilm sampling

142

143 The first biofilm sampling campaign was conducted between 11 and 13 December 2014 (spring/summer  
144 season), two days after a high precipitation event 89 mm (INMET 2019). By this time, the soybean crop was in  
145 V3 stage, the corn was in R1 stage, and the first leaves of flue-cured tobacco were harvested. The second biofilm  
146 sampling campaign was performed between 3 and 5 June 2015 (autumn/winter season); it registered 4 mm of  
147 precipitation during the preceding week. During this period, less than one-fifth of the total agricultural surface  
148 was cultivated with wheat, barley, and winter grassland forage. The rate of pesticide application was low  
149 compared to that of the spring/summer season (Fig. 2, data obtained from farmers).



150

151 **Fig 2** – Relationship between the period of the year and the intensity of pesticide applied in wheat, corn and soy  
 152 crops in the Guaporé watershed.

153

154 At each collection site, epilithic biofilms were obtained from submerged rocks with the aid of  
 155 toothbrushes. The rock adhered material was washed with deionized water and recuperated in glass jars. The  
 156 detailed sampling procedure has been published by Bastos et al. (2018).

157 The pesticides were selected according to the Brazilian registered agricultural compounds and with field  
 158 investigations. Subsequently, a second selection was made according to the operational and methodological  
 159 capacity of the laboratories involved.

160 The selected molecules and their physicochemical properties are presented in the appendix (Table A1).  
 161 Sampling points were selected in order to represent two macro-regions in the river basin, north and south  
 162 regions, which are contrasting in terms of relief, land use, and agricultural management aspects.

163

164 Analytical procedure

165 In the laboratory, glass jars with biofilms were transferred to individual high-density polyethylene jars  
 166 and frozen at -80 °C for subsequent lyophilization. After being freeze-dried, samples were homogenized in an  
 167 agate mortar to obtain a representative sample for analysis. The biofilm extraction procedure was performed  
 168 using an adapted method from Jelić et al. (2009) that is well described by Aubertheau et al. (2017). Five hundred

169 milligrams of biofilm were extracted by accelerated solvent extraction equipment (ASE<sup>TM</sup> 350, Thermo Fisher  
170 Scientific Inc., Waltham, USA) at 80 °C using methanol/water (1/2, v/v) as the extraction solvent. The extracts  
171 were purified by solid-phase extraction (Autotrace<sup>TM</sup> 150, Thermo Scientific, 125 Waltham, USA) using Oasis®  
172 HLB cartridges (6 cc, 200 mg of sorbent, Waters, Milford, USA) with methanol as eluent. The extracts obtained  
173 by the Solid Phase Extraction (SPE) system were evaporated under N<sub>2</sub> flow for restitution to 500 µl with a  
174 methanol/water mixture (10/90, v/v). The recovered liquid was then filtered one last time using Mini-Uniprep<sup>TM</sup>  
175 filters (PVDF Filter Media with polypropylene housing, pore size 0.45 µm, Durapore®, Millipore, Billerica,  
176 USA). Each biofilm sample was analyzed twice with triplicate measurements.

177           The identification and quantification of the molecules were performed by ultra-high performance liquid  
178 chromatography (Thermo Fisher Scientific, Waltham, USA), coupled to a time-of-flight mass spectrometry  
179 (Impact II<sup>TM</sup> model, Bruker Daltonics, Billerica, USA) using the method Pesticide Screener (developed by  
180 Thermo Fisher Scientific, Waltham, USA). For each extract, there were three injections of 20 µL in positive and  
181 negative ionization mode. Data processing was performed using Compass Data Analysis 4.2 and Target Analysis  
182 software (Bruker, Billerica, USA).

183           The concentration of pesticides in biofilms was determined by adjusting the linear model used between  
184 the concentration of the standards and the peak area of each injection for each compound detected. Validation of  
185 the method was followed by the French norm NF XP T90-210 carried out through linearity, repeatability, and  
186 Cochran's test (more details on the methodology available in Lima et al. 2020). The limits of detection (LD)  
187 ranged from 0.5 to 1 ng/g. The LD and limit of quantification LQ were estimated from the background noise. In  
188 this case, the LD and LQ were calculated by determining the maximum amplitude of the signal over a distance  
189 equal to 20 times the width at mid-height of the peak corresponding to the compound searched, and these values  
190 were used in the statistical analysis. A total of 25 compounds were analyzed, namely: 2,4-D, atrazine, boscalid,  
191 carbendazim, chlorotoluron, chlorpyrifos-ethyl, cypermethrin, deltamethrin, desethyl-atrazine, dicamba,  
192 epoxiconazole, imidacloprid, iodosulfuron-methyl-sodium, isoproturon, MCPA, mesotrione, mesosulfuron-  
193 methyl, metconazole, nicosulfuron, prochloraz, prosulfuron, prothioconazole, simazine, tebuconazole, and  
194 thifensulfuron-methyl.

195           The methodology description for method validation and the physico-chemical properties of the  
196 pesticides studied are available in the appendixes of this work (A1, A2 and A3).

197

198 Statistical treatment

199

200           The Kolmogorov-Smirnov and Lilliefors tests were used to assess whether the concentrations of  
201 pesticides exhibited a normal distribution. Because the results did not show normal distribution, the median was  
202 used as a measure of position, and the upper and lower quartiles, interquartile range, and maximum and  
203 minimum values were used as dispersion measures. The hypothesis that the epilithic biofilms could discriminate  
204 spatial and temporal flux of pesticides from soil to aquatic ecosystems was statistically evaluated by the Mann-  
205 Whitney *U* nonparametric test. The STATISTICA 7.0 program was used to carry out statistical procedures.

206

## 207 **Results and Discussion**

208

### 209 Occurrence and concentration

210

211           Only five pesticides (cypermethrin, deltamethrin, dicamba, iodosulfuron-methyl, and MCPA) were not  
212 detected in the epilithic biofilms sampled in the Guaporé River and its tributaries. The absence of these  
213 compounds in biofilms is not proof that these compounds are not being used in agricultural fields and does not  
214 refute the possibility that the functional groups of biofilms may not have affinity with pesticides. However, the  
215 fieldwork carried out by our team demonstrates that, in this region, the use of pyrethroid insecticides is being  
216 replaced by systemic compounds, in particular imidacloprid and other neonicotinoid pesticides. In southern  
217 Brazil, pyrethroid is still used in urban sprayings and in fruit production. In addition, the herbicide dicamba is  
218 used in combination with genetically modified soybean varieties (Xtend®) that have not yet been cultivated in  
219 Brazil. Some farmers in the Guaporé catchment use these pesticides for controlling plants resistant to glyphosate.  
220 The last two non-detected herbicides were recommended for dicotyledonous control in corn and cereal  
221 production, but they are rarely used this catchment (data obtained from farmers).

222

223           All monitored sites in the spring/summer season present biofilms impregnated by three herbicides (2,4-  
224 D, atrazine, and simazine), and one metabolite (desethyl-atrazine), three fungicides (carbendazim, tebuconazole,  
225 and epoxiconazole), and two insecticides (imidacloprid and chlorpyrifos-ethyl). In addition, another seven  
226 herbicides (25 to 81% frequency) and four fungicides (6 to 69% frequency) were detected in biofilms occurring  
227 in both the Guaporé River and in three of its main tributaries. In autumn/winter season, only two pesticides were  
228 found at 100% of the sites monitored (2,4-D and carbendazim), but all 20 compounds were quantified in biofilms  
with frequency ranging from 22% (thifensulfuron-methyl) to 83% (atrazine and tebuconazole, Table 2). Some

229 studies have demonstrated that the highest levels of pesticides in surface water occur as pulses in response to late  
 230 spring and early summer rainfall events (Pascual Aguilar et al. 2017; Pérez et al. 2017; Rabiet et al. 2010); the  
 231 same was observed in biofilms in the present study.

232 The frequency of pesticide detection in biofilms is consistent with their consumption in Brazil and  
 233 consequently, with use in the Guaporé catchment. Over the last few years, Brazilian farmers used  $5.8 \times 10^7$  Mg  
 234 of 2,4-D,  $2.5 \times 10^7$  Mg of atrazine,  $9.4 \times 10^6$  Mg of imidacloprid, and  $6.5 \times 10^6$  Mg of chlorpyrifos-ethyl  
 235 (IBAMA 2018). The three most frequently detected fungicides in biofilms are also the most widely used in  
 236 Brazil (tebuconazole, carbendazim, and epoxiconazole, with 4.5, 3.7, and  $0.8 \times 10^6$  mg, respectively).

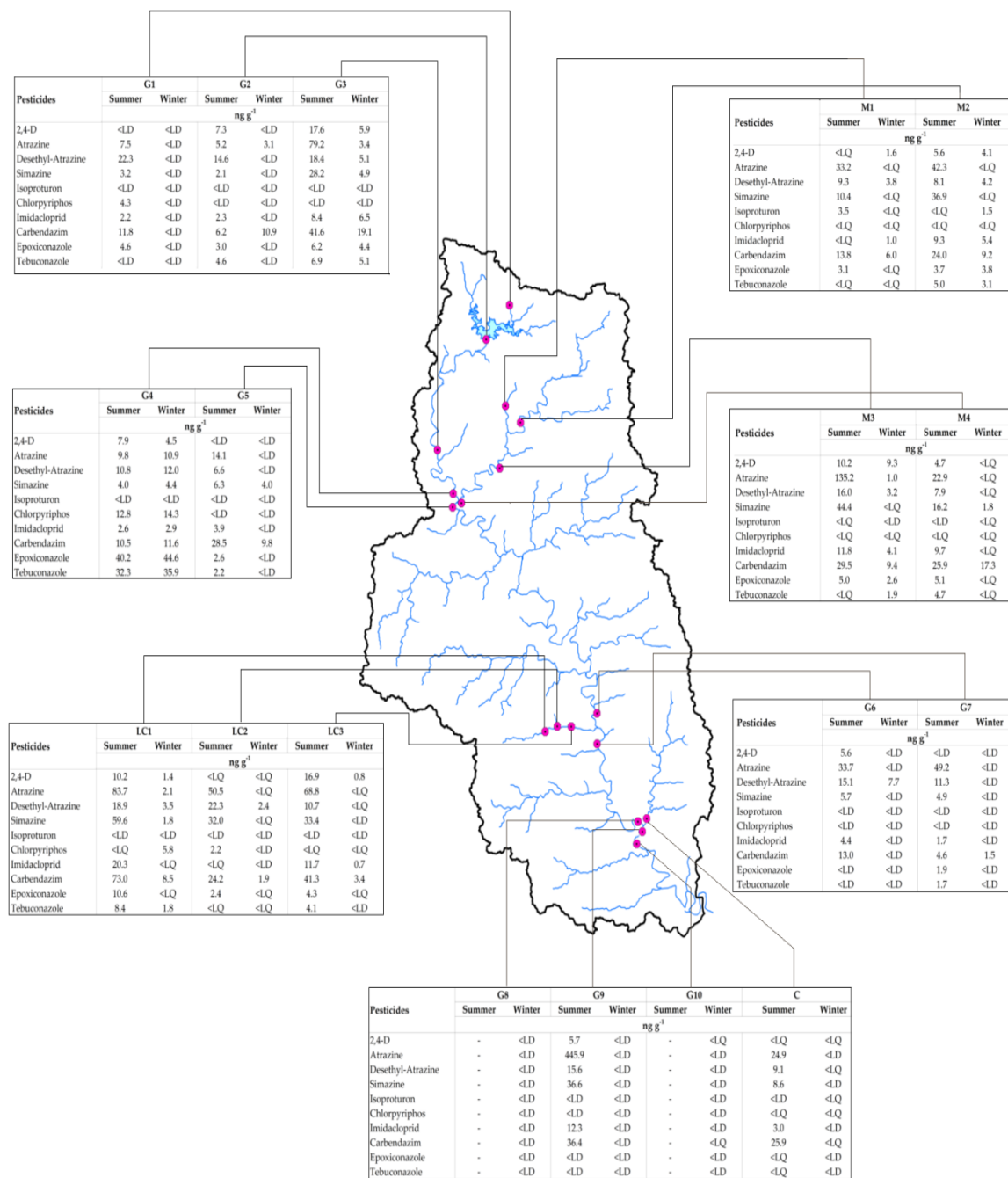
Pesticide	11 to 13 December			3 to 5 June		
	Frequency (%)	IQR (ng g <sup>-1</sup> )	IQR <sup>1</sup> (%)	Frequency (%)	IQR (ng g <sup>-1</sup> )	IQR (%)
<b>Herbicides</b>						
2,4-d	100	9.1	162	100	1.7	348
Atrazine	100	60.3	159	83	0.8	159
Desethyl-atrazine	100	8.7	67	78	3.5	698
Simazine	100	30.7	231	72	1.9	374
Nicosulfuron	81	0	0	39	0.5	0
Mesosulfuron-methyl	50	0.5	200	28	0.5	0
Chlorotoluron	50	0.5	200	28	0.5	0
Prosulfuron	44	0.5	0	39	0.5	0
Mesotrione	38	0.5	0	61	0.5	100
Thifensulfuron-methyl	25	0.4	0	33	0.5	0
Isoproturon	31	0.5	0	22	0.1	0
Dicamba	0	0	0	0	0	0
Iodosulfuron-methyl	0	0	0	0	0	0
MCPA	0	0	0	0	0	0
<b>Fungicides</b>						
Carbendazim	100	22.6	90	100	9.6	205
Epoxiconazole	100	3.0	89	78	0.7	132
Tebuconazole	100	4.4	229	83	1.5	299
Metconazole	69	0.5	100	56	0.5	100
Prochloraz	56	0.5	100	50	0.5	200
Prothioconazole	56	0.5	100	56	0.5	100
Boscalid	6	0	0	33	0.5	0
<b>Insecticides</b>						
Imidacloprid	100	17.0	272	67	3.0	292
Chlorpyrifos-ethyl	100	0	0	78	0.1	25
Cypermethrin	0	0	0	0	0	0
Deltamethrin	0	0	0	0	0	0

237 <sup>1</sup>IQR (%) = IQR (ng/g) / media \* 100

238 Table 2 – Frequency of pesticide detection (%) and inter-quartile interval (median and %) in epilithic biofilms  
 239 sampled in Guaporé River and its tributaries, in December (spring/summer) and June (autumn/hiver)  
 240 season.

241

242           The maximum concentrations of herbicides, in  $\text{ng g}^{-1}$  of biofilms, were 17.6, 445.9, 22.3, and 59.6 for  
243 2,4-D, atrazine, desethyl-atrazine, and simazine, respectively. The highest concentrations of insecticides were  
244 20.3 and 14.3 in  $\text{ng g}^{-1}$  of biofilms for imidacloprid and chlorpyrifos-ethyl, respectively; and finally, the highest  
245 concentrations of fungicides in biofilms were 73.0, 44.6, and 35.9  $\text{ng g}^{-1}$  of biofilms for carbendazim,  
246 epoxiconazole, and tebuconazole, respectively (Fig. 3). Fifty per cent of the epilithic biofilms sampled had  
247 median concentrations varying, in  $\text{ng g}^{-1}$  of biofilms, between 0.5 and 6.0 (2,4-D), 0.5 and 34.0 (atrazine), 0.5  
248 and 12.0 (desethyl-atrazine), 0.5 and 10.0 (simazine), 0.5 and 5.0 (imidacloprid), 3.4 to 24.0 (carbendazim), and  
249 0.5 and 4.0 (epoxiconazole and tebuconazole). Usually, the highest concentration of pesticides in biofilms is in  
250 accordance with the highest frequency of detection. High concentrations and loads can also be linked to  
251 catchment and climate conditions, where peaks in concentrations are observed in the summer, which is usually a  
252 rainy period with conditions known to be linked to high pollutant mobilization (Zhang et al. 2016).



253

254 **Fig 3** – Spatial and temporal pesticides concentration in biofilms sampled in the Guaporé catchment

255

256 Pesticides are from punctual and non-punctual sources and are transported by runoff from agricultural  
 257 and urban areas, discharge from reservoirs and aquifers, and atmospheric deposition (Pascual Aguilar et al.  
 258 2017). They are transported from farmland toward water bodies in various forms including dissolved, sorbed on  
 259 dissolved organic carbon, and sorbed on suspended or colloidal particles. Pesticides in each phase have different  
 260 means of transportation and mobility. In particular, sorption on dissolved organic carbon can effectively increase

261 the mobility of some pesticides (e.g., atrazine and 2,4-D, Li et al. 2005; Tang et al. 2012). The physicochemical  
262 properties of pesticides such as water solubility, octanol-water partition coefficient, dissociation (pKa), and  
263 Henry's law constant are also determining factors of leaching potential. However, in the case of preferential  
264 flow, pesticides can be rapidly transferred to groundwater irrespective of their physicochemical properties.  
265 Vryzas (2018) reported higher pesticide concentrations in shallow groundwater from experimental boreholes  
266 compared to the concentrations in the adjacent deep groundwater (drinking or irrigation water wells). Mixed  
267 leaching mechanisms ("chromatographic" and preferential flow) have been reported by the authors to be  
268 involved in the contamination of adjacent aquifers with different water residence times.

269 Epilithic biofilms can adsorb pesticides by specific adsorption (inner-sphere surface complexation), by  
270 nonspecific mechanisms (physical adsorption), and by hydrophobic interaction (Barrett and McBride 2007;  
271 Kleber et al. 2007; Lawrence et al. 2001; Späth et al. 1998). Additionally, microorganisms and  
272 macroinvertebrates can uptake and accumulate pesticides, since they are in constant interaction with biofilms and  
273 are sometimes used as a source of nourishment (Rhea et al. 2006). The observed high diversity and frequency of  
274 detection of pesticides in the Guaporé catchment showed that biofilms can be used as a powerful ecological  
275 indicator of pesticide exposure, as has been proposed by Edwards and Kjellerup (2013).

276

277 Spatial sources of pesticide contamination (soil use and management)

278

279 The frequency and the median concentration of some pesticides in epilithic biofilms allow  
280 discrimination of the upper and the middle/lower regions of the Guaporé catchment (Table 3). In the upper  
281 region, by comparison with other catchment places, biofilms were more impregnated by three herbicides (2,4-D,  
282 thifensulfuron-methyl, isoproturon), four fungicides (carbendazim, epoxiconazole, metconazole, tebuconazole),  
283 and one insecticide (imidacloprid). In addition, the interquartile range for the atrazine and simazine in the upper  
284 region was lower than that found in the middle/lower region. The upper region has a plane relief terrain with low  
285 slopes and almost 100% of the agricultural surface is cultivated on a no-tillage system with soybean and corn  
286 genetically modified. Moreover, high hydraulic connectivity between cultivated fields and watercourses was  
287 observed. In contrast, although agriculture is performed in a more declivous area and mainly under conventional  
288 tillage, the cultivated areas are imbricated in the landscape and the riparian forest is very large in the  
289 middle/lower region of the Guaporé watershed in comparison to the upper region (Table 1).

290



Pesticides	Median spring/summer	Median autumn/winter	Median upper	Median middle/lower
	(ng g <sup>-1</sup> )	(ng g <sup>-1</sup> )	(ng g <sup>-1</sup> )	(ng g <sup>-1</sup> )
<b>Herbicides</b>				
2,4-D	5.6	0.5	4.6	0.5
Atrazine	38.0	0.5	7.5	1.3
Desethyl-atrazine	13.0	0.5	7.3	5.6
Simazine	13.3	0.5	4.2	1.2
Mesotrione	0.0	0.5	0.5	0.0
Mesosulfuron- methyl	0.3	0.0	0.3	0.0
Nicosulfuron	0.5	0.0	0.5	0.5
Prosulfuron	0.0	0.0	0.0	0.0
Thifensulfuron- methyl	0.0	0.0	0.3	0.0
Isoproturon	0.0	0.0	0.3	0.0
Chlorotoluron	0.3	0.0	0.0	0.0
<b>Fungicides</b>				
Carbendazim	25.0	4.7	12.7	4.0
Epoxiconazole	3.4	0.5	3.4	0.5
Metconazole	0.5	0.5	0.5	0.0
Tebuconazole	1.9	0.5	2.6	0.5
Prothioconazole	0.5	0.5	0.5	0.3
Boscalid	0.0	0.0	0.0	0.0
Prochloraz	0.5	0.3	0.5	0.0
<b>Insecticides</b>				
Imidacloprid	4.1	0.5	3.4	0.5
Chlorpyrifos-ethyl	0.5	0.5	0.5	0.5

291 **Table 3** – Medians concentration of pesticides in epilithic biofilms sampled in Guaporé River and its tributaries,  
292 according to their sampling period (spring/summer and autumn/hiver) seasons and location (upper and  
293 middle/lower).

294 The southern region of Brazil presents a subtropical humid climate with an average annual rainfall  
295 between 1,550 and 1,700 mm, but it may even reach 2,500 mm (INMET 2019). The rain has very high erosion  
296 potential (Didoné et al. 2014). Even several decades after the adoption of a no-tillage system, agricultural areas  
297 are still the main source of sediments to rivers in the Guaporé catchment (Tiecher et al. 2017a), as well as in  
298 other watersheds in the south of Brazil (Tiecher et al. 2018; Tiecher et al. 2017b). In Brazil, the no-tillage system  
299 is usually a non-optimal soil management system because farmers removed the terraces, and there is an  
300 insufficient amount of straw in the surface of the soil. High rates of water loss, sediment (Deuschle et al., 2019),  
301 and nutrients (Zafar et al. 2016) have been observed in the Guaporé catchment. In the no-tillage system, the

302 pesticides added do not reach the soil surface because of the interception by the mulch of crop residues (Aslam et  
303 al. 2018), decreasing the reactivity with functional groups of inorganic and organic colloids. Moreover, due to  
304 regularity and frequent rainfall occurring in the catchment, there is a constant pesticide wash-off the superficial  
305 residues. In fact, under these conditions, it is not possible to produce crops without transferring sediment and  
306 agrochemicals to the superficial water resources. There is no doubt that certain amounts of the pesticides used in  
307 crops are transferred to water courses. The amount and frequency of pesticides reaching surface water reserves  
308 depends on the quantity added, the distribution of crops in the landscape, and the presence of physical-  
309 mechanical and/or natural barriers to the transfer of sediment and water by erosion and runoff.

310           Direct runoff and erosion of soil particles are the major pathways responsible for the transportation of  
311 pesticides to surface water. Rates of soil erosion, irrigation, precipitation, and half-life are the major factors that  
312 govern the transportation of pesticides to the surface water (Aravinna et al. 2017). The compounds with low  $K_{ow}$   
313 and  $K_{oc}$ , and high mobility were in more variable concentrations in biofilms sampled in spring/summer season  
314 (simazine, carbendazim, and imidacloprid). However, pesticides with moderate adsorption capacity in colloids  
315 ( $K_{ow}$  3.0 to 5.0, Lewis et al. 2016) were also found in high concentrations in biofilms (tebuconazole,  
316 epoxiconazole, and chlorpyrifos-ethyl). Pesticides are transferred from soil to watercourse, mostly in soluble  
317 form by runoff from cultivated fields, but also adsorbed by inorganic and organic colloids in eroded sediment.  
318 The transport of pesticides through preferential water flow to macropores to tile drainage plays an important role  
319 in the rapid transport of pesticides to surface waters (Tang et al. 2012; Vymazal and Březinová 2015). When  
320 these pesticides enter the water bodies, desorption from colloids occurs, releasing them to the aqueous phase  
321 (Aravinna et al. 2017). The water flow velocity also plays an important role in the accumulation of these  
322 compounds in biofilms. Chaumet et al. (2019a) showed higher accumulation of diuron at lower flow velocity  
323 compared to the higher flow for raw biofilms.

324 Temporal use of pesticides (application – transfer to watercourse – decomposition – resilience)

325

326           All 20 pesticides detected were detected in epilithic biofilms in both seasons. However, the  
327 concentrations of five herbicides (2,4-D, atrazine, desethyl-atrazine, simazine, and nicosulfuron), three  
328 fungicides (carbendazim, epoxiconazole, and tebuconazole), and one insecticide (imidacloprid) were higher in  
329 the spring/summer than in the autumn/winter season (Table 3, statistical analysis is available in Table A4. These  
330 results were coherent with the phase of development of soybean, corn, and tobacco plants and the period of  
331 pesticide application. The maximum herbicide consumption occurs from October to December, the period in

332 which they are used in pre-sowing and post-emergence of genetically modified soybean and corn (> 90% of  
333 herbicides consumption in the Guaporé catchment). Also, although in smaller quantities, herbicides are used in  
334 pre-transplantation and during the development of tobacco during the spring/summer season. The fungicides and  
335 insecticides were used more intensely between December and February, mainly in soybean and tobacco  
336 production (Fig. 2).

337         After February, pesticide application is reduced drastically in the Guaporé catchment. The tobacco  
338 harvest ends in mid-February and many farmers sow corn or silage production, but without the use of fungicide  
339 and insecticides. The soybean harvest takes place from mid-March until the end of April. More than 90% of the  
340 agricultural area remains fallow after the harvest of soybean and maize. The sowing of winter crops, wheat,  
341 colza, and barley starts in June, after the second sampling of epilithic biofilms. The median concentrations of  
342 atrazine, carbendazim, and imidacloprid, for example, were drastically reduced between the first  
343 (spring/summer) and the second (autumn/winter) sampling: 38.0 to 0.5, 25.0 to 4.7, and 4.1 to 0.5 ng g<sup>-1</sup> of  
344 biofilms, respectively (Table 3, statistical analysis is available in appendix Table A5). The concentration of  
345 many pesticides in biofilms sampled in autumn/winter was lower than the LD (between 0.5 and 1 ng g<sup>-1</sup>). The  
346 capacity of the epilithic biofilms to biodegrade pesticides is evident and pointed out in various studies (Feckler et  
347 al. 2015; Sabater et al. 2007; Staley et al. 2015) that attribute pesticide removal by biofilms to biodegradation,  
348 not to sorption. Carles et al. (2019), through a study of exposure of biofilms to glyphosate in mesocosm, were  
349 able to define that the biological decomposition of the glyphosate pesticide was performed in approximately 13  
350 days, in the case of low concentration of this pesticide in water (< 10 µg L<sup>-1</sup>).

351         The Guaporé River and its tributaries can be classified as turbulent systems. The area presents a well-  
352 preserved riparian forest, especially in the middle/lower region of the catchment (Table 1). Villeneuve et al.  
353 (2010) showed that in turbulent mesocosms, the biofilms were more diversified in comparison to static  
354 environments. Additionally, the relation between the autochthonous primary production and the heterotrophic  
355 organisms in biofilms is modified with direct exposure of rivers to the sun which may alter the  
356 autotrophic/heterotrophic proportion in biofilms (Feckler et al. 2015). Furthermore, the average temperature of  
357 the water is also adequate to optimal microbiota development in the winter season (17.9 and 22.5 °C in  
358 autumn/winter and spring/summer samplings, respectively).

359         However, the soil is a reservoir of pesticides, and it can transfer the compounds to the surface and  
360 groundwater ecosystems (Sassine et al. 2017) slowly and for a long time. For example, in Brazil, the use of the  
361 herbicide triazines is authorized (2.5 x 10<sup>4</sup> mg of atrazine consumed in 2017, the fifth most used pesticide), and

362 the persistence in biofilms in the autumn/winter season is reasonable. The storage and remobilization from soil  
363 and the continuing legal applications guarantee the flux of pesticides from higher to lower landscape position,  
364 and the water can be a constant pesticide source to the biofilms. The carbendazim and imidacloprid persistence  
365 in biofilms sampled in July, especially in the upper region of the Guaporé catchment, are probably being used for  
366 the control of end-cycle maladies (*Cercospora kikuchii* and *Septoria glycines*) and sucking insect pests in  
367 soybeans. These pesticides are widely used until a few days before the soybean harvest.

368 Tien et al. (2013) evaluated the maximum pesticide removal capacity in river biofilms and showed that  
369 natural river biofilms from different seasons were able to remove different pesticides with rates ranging from  
370 99.6 to 41.2%. The authors observed different dissipation rates and degradation capacities in biofilms from  
371 different seasons which might be due to the colonization of microbial species with different pesticide  
372 degradation capacity within river biofilms and might differ with the variation of pesticide concentration in river  
373 water associated with the lower use of pesticides in different seasons.

374

375 Biofilms as a monitoring tool at a global scale

376

377 The use of biofilms as an environmental indicator can be carried out all over the world due to their  
378 development in all aquatic ecosystems as part of the trophic network (Aubertreau et al. 2017; Bastos et al. 2018;  
379 Fernandes et al. 2019; Huerta et al. 2016). In this study, it was possible to verify that biofilms were able to  
380 represent the anthropic pressure near the collection points, even with the use of different pesticides and times of  
381 application of agricultural crops, biofilms were able to capture pesticide compounds even in rivers located in  
382 areas with low anthropic activity and/or with low concentration of these compounds. In addition, pollution from  
383 different soil management systems can be identified through sampling and analysis of biofilms; thus, biofilms  
384 can be used as tools to evaluate the health and water quality of water bodies. Furthermore, pesticides are  
385 ubiquitous in aquatic environments and constantly interact with aquatic organisms, including those that makeup  
386 biofilms at fluctuating concentrations (Chaumet et al. 2019a). The main advantage over point sampling (water  
387 sampling) is its capacity to accumulate compounds in an integrative way because of the heterogeneity of biofilm  
388 composition, the availability of diverse sorption sites, and their probable saturation may contribute to multiple  
389 kinetics of bioaccumulation, being consecutive or staking (Zhang et al. 2018). Chaumet et al. (2019b) studying  
390 diuron bioaccumulation in biofilms have demonstrated a phenomenon of continuous diffusion, but it was not  
391 linearly correlated with bioaccumulation, highlighting the complex capture mechanisms operating within the

392 biofilm matrix. The study of biofilm contamination becomes important as a basis for better exposure assessment  
393 of biofilm feeding organisms and as useful for determining the risk of trophic transfer of pesticides along the  
394 aquatic food chain. Besides, biofilms develop naturally on several surfaces, such as rock surfaces in riverbeds,  
395 not having costs for implementation, like other passive samplers that are expensive and must be installed at  
396 sampling points.

397

## 398 **Conclusions**

399

400 The epilithic biofilms sampled in the Guaporé River watershed bioaccumulated the active principles of  
401 10 herbicides (2,4-D, atrazine, chlorotoluron, simazine, nicosulfuron, mesosulfuron-methyl, mesotrione,  
402 prosulfuron, isoproturon, thifensulfuron-methyl), 7 fungicides (boscalid, carbendazim, epoxiconazole,  
403 tebuconazole, metconazole, prochloraz, prothioconazole), 2 insecticides (chlorpyrifos and imidacloprid), and 1  
404 metabolite (desethyl-atrazine).

405 The use of epilithic biofilms as integrative matrix is effective in discriminating the spatial and temporal  
406 sources of contamination independent of the physicochemical characteristics of the compounds. Additionally, by  
407 employing the detection frequency and median concentrations of pesticides in biofilms, it was possible to  
408 estimate in which of the two main cultures (soybean or maize) the pesticides were employed.

409 The concentrations of the main pesticides monitored were sharply reduced in the epilithic biofilms  
410 sampled some months after the application in the summer cultures, showing the high capacity of resilience and  
411 detoxification.

412

## 413 **References**

- 414 Aravinna, P., Priyantha, N., Pitawala, A., & Yatigammana, S. K. (2017). Use pattern of pesticides and their  
415 predicted mobility into shallow groundwater and surface water bodies of paddy lands in Mahaweli river  
416 basin in Sri Lanka. *Journal of Environmental Science and Health, Part B*, 52(1), 37–47.  
417 <https://doi.org/10.1080/03601234.2016.1229445>
- 418 Aslam, S., Iqbal, A., Lafolie, F., Recous, S., Benoit, P., & Garnier, P. (2018). Mulch of plant residues at the soil  
419 surface impact the leaching and persistence of pesticides: A modelling study from soil columns. *Journal*  
420 *of Contaminant Hydrology*, 214, 54–64. <https://doi.org/10.1016/j.jconhyd.2018.05.008>

421 Aubertheu, E., Stalder, T., Mondamert, L., Ploy, M.-C., Dagot, C., & Labanowski, J. (2017). Impact of  
422 wastewater treatment plant discharge on the contamination of river biofilms by pharmaceuticals and  
423 antibiotic resistance. *Science of The Total Environment*, 579, 1387–1398.  
424 <https://doi.org/10.1016/j.scitotenv.2016.11.136>

425 Barrett, K. A., & McBride, M. B. (2007). Phosphate and glyphosate mobility in soil columns amended with  
426 roundup: *Soil Science*, 172(1), 17–26. <https://doi.org/10.1097/01.ss.0000240549.44551.3d>

427 Bastos, M. C., Rheinheimer dos Santos, D., Monteiro de Castro Lima, J. A., le Guet, T., Santanna dos Santos, M.  
428 A., Zanella, R., et al. (2018). Presence of Anthropogenic Markers in Water: A Case Study of the  
429 Guaporé River Watershed, Brazil. *CLEAN - Soil, Air, Water*, 46(3), 1700019.  
430 <https://doi.org/10.1002/clen.201700019>

431 Battin, T. J., Besemer, K., Bengtsson, M. M., Romani, A. M., & Packmann, A. I. (2016). The ecology and  
432 biogeochemistry of stream biofilms. *Nature Reviews Microbiology*, 14(4), 251–263.  
433 <https://doi.org/10.1038/nrmicro.2016.15>

434 Beketov, M. A., Kefford, B. J., Schafer, R. B., & Liess, M. (2013). Pesticides reduce regional biodiversity of  
435 stream invertebrates. *Proceedings of the National Academy of Sciences*, 110(27), 11039–11043.  
436 <https://doi.org/10.1073/pnas.1305618110>

437 Bricheux, G., Morin, L., Le Moal, G., Coffe, G., Balestrino, D., Charbonnel, N., et al. (2013). Pyrosequencing  
438 assessment of prokaryotic and eukaryotic diversity in biofilm communities from a French river.  
439 *MicrobiologyOpen*, 2(3), 402–414. <https://doi.org/10.1002/mbo3.80>

440 Carles, L., Gardon, H., Joseph, L., Sanchís, J., Farré, M., Artigas, J., 2019. Meta-analysis of glyphosate  
441 contamination in surface waters and dissipation by biofilms. *Environment International* 124, 284–293.  
442 <https://doi.org/10.1016/j.envint.2018.12.064>

443 Chaumet, B., Morin, S., Boutry, S., & Mazzella, N. (2019a). Diuron sorption isotherms in freshwater biofilms.  
444 *Science of The Total Environment*, 651, 1219–1225. <https://doi.org/10.1016/j.scitotenv.2018.09.286>

445 Chaumet, B., Morin, S., Hourtané, O., Artigas, J., Delest, B., Eon, M., & Mazzella, N. (2019b). Flow conditions  
446 influence diuron toxicokinetics and toxicodynamics in freshwater biofilms. *Science of The Total  
447 Environment*, 652, 1242–1251. <https://doi.org/10.1016/j.scitotenv.2018.10.265>

448 Deuschle, D., Minella, J. P. G., Hörbe, T. de A. N., Londero, A. L., & Schneider, F. J. A. (2019a). Erosion and  
449 hydrological response in no-tillage subjected to crop rotation intensification in southern Brazil.  
450 *Geoderma*, 340, 157–163. <https://doi.org/10.1016/j.geoderma.2019.01.010>

451 Didoné, E. J., Minella, J. P. G., Reichert, J. M., Merten, G. H., Dalbianco, L., de Barros, C. A. P., & Ramon, R.  
452 (2014). Impact of no-tillage agricultural systems on sediment yield in two large catchments in Southern  
453 Brazil. *Journal of Soils and Sediments*, 14(7), 1287–1297. <https://doi.org/10.1007/s11368-013-0844-6>

454 Edwards, S. J., & Kjellerup, B. V. (2013). Applications of biofilms in bioremediation and biotransformation of  
455 persistent organic pollutants, pharmaceuticals/personal care products, and heavy metals. *Applied*  
456 *Microbiology and Biotechnology*, 97(23), 9909–9921. <https://doi.org/10.1007/s00253-013-5216-z>

457 Essington, M. E. (2005). *Soil and water chemistry: an integrative approach*. Boca Raton: CRC Press.  
458 [http://public.eblib.com/choice/publicfullrecord.aspx?p=198638\\_0](http://public.eblib.com/choice/publicfullrecord.aspx?p=198638_0). Accessed 12 August 2019

459 Feckler, Kahlert, M., & Bundschuh, M. (2015). Impacts of Contaminants on the Ecological Role of Lotic  
460 Biofilms | SpringerLink. <https://link.springer.com/article/10.1007/s00128-015-1642-1>. Accessed 5  
461 August 2019

462 Fernandes, G., Aparicio, V. C., Bastos, M. C., De Gerónimo, E., Labanowski, J., Prestes, O. D., et al. (2019).  
463 Indiscriminate use of glyphosate impregnates river epilithic biofilms in southern Brazil. *Science of The*  
464 *Total Environment*, 651, 1377–1387. <https://doi.org/10.1016/j.scitotenv.2018.09.292>

465 Food and Agriculture Organization of the United Nations, (2018). FAO [WWW Document]. Pesticides - Use per  
466 area of cropland. URL <http://www.fao.org/faostat/en/#data/EP/visualize> (accessed 10.19.19).

467 Flemming, H.-C., & Wingender, J. (2010). The biofilm matrix. *Nature Reviews Microbiology*, 8(9), 623–633.  
468 <https://doi.org/10.1038/nrmicro2415>

469 Graminha, E. B. N., Gonçalves, A. Z. L., Pirola, R. D. P. B., Balsalobre, M. A. A., Da Silva, R., & Gomes, E.  
470 (2008). Enzyme production by solid-state fermentation: Application to animal nutrition. *Animal Feed*  
471 *Science and Technology*, 144(1–2), 1–22. <https://doi.org/10.1016/j.anifeedsci.2007.09.029>

472 Huerta, B., Rodriguez-Mozaz, S., Nannou, C., Nakis, L., Ruhí, A., Acuña, V., et al. (2016). Determination of a  
473 broad spectrum of pharmaceuticals and endocrine disruptors in biofilm from a waste water treatment  
474 plant-impacted river. *Science of The Total Environment*, 540, 241–249.  
475 <https://doi.org/10.1016/j.scitotenv.2015.05.049>

476 IBGE | Portal do IBGE | IBGE. (n.d.). <https://ibge.gov.br/>. Accessed 29 February 2020

477 IBAMA - Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis.  
478 <https://www.mma.gov.br/assuntos-internacionais/item/11586-ibama-instituto-brasileiro-do-meio-ambiente-e-dos-recursos-naturais-renovaveis>. Accessed 29 February 2020

479

480 INMET, Banco de Dados Meteorológicos para Ensino e Pesquisa. Instituto Nacional de Meteorologia (2015).  
481 [WWW Document]. URL <http://www.inmet.gov.br/projetos/rede/pesquisa/> (accessed 10.20.19).

482 INMET, Banco de Dados Meteorológicos para Ensino e Pesquisa. Instituto Nacional de Meteorologia (2019).  
483 [WWW Document]. URL <http://www.inmet.gov.br/projetos/rede/pesquisa/> (accessed 10.20.19).

484 Jelić, A., Petrović, M., & Barceló, D. (2009). Multi-residue method for trace level determination of  
485 pharmaceuticals in solid samples using pressurized liquid extraction followed by liquid  
486 chromatography/quadrupole-linear ion trap mass spectrometry. *Talanta*, *80*(1), 363–371.  
487 <https://doi.org/10.1016/j.talanta.2009.06.077>

488 Kaiser, D. R., Sequinato, L., Reinert, D. J., Reichert, J. M., Rheinheimer, D. S., & Dalbianco, L. (2015). High  
489 Nitrogen Fertilization of Tobacco Crop in Headwater Watershed Contaminates Subsurface and Well  
490 Waters with Nitrate. *Journal of Chemistry*, *2015*, 1–11. <https://doi.org/10.1155/2015/375092>

491 Kim, S. T., Fauvelle, V., Morin, S., & Mazzella, N. (2016). Improving Toxicity Assessment of Pesticide  
492 Mixtures: The Use of Polar Passive Sampling Devices Extracts in Microalgae Toxicity Tests. *Frontiers*  
493 *in Microbiology*, *7*. <https://doi.org/10.3389/fmicb.2016.01388>

494 Kleber, M., Sollins, P., & Sutton, R. (2007). A conceptual model of organo-mineral interactions in soils: self-  
495 assembly of organic molecular fragments into zonal structures on mineral surfaces. *Biogeochemistry*,  
496 *85*(1), 9–24. <https://doi.org/10.1007/s10533-007-9103-5>

497 Lawrence, J. R., Kopf, G., Headley, J. V., & Neu, T. R. (2001). Sorption and metabolism of selected herbicides  
498 in river biofilm communities. *Canadian Journal of Microbiology*, *47*(7), 634–641.  
499 <https://doi.org/10.1139/cjm-47-7-634>

500 Lewis, K. A., Tzilivakis, J., Warner, D. J., & Green, A. (2016). An international database for pesticide risk  
501 assessments and management. *Human and Ecological Risk Assessment: An International Journal*,  
502 *22*(4), 1050–1064. <https://doi.org/10.1080/10807039.2015.1133242>

503 Li, K., Xing, B., & Torello, W. A. (2005). Effect of organic fertilizers derived dissolved organic matter on  
504 pesticide sorption and leaching. *Environmental pollution (Barking, Essex : 1987)*, *134*(2), 187–194.  
505 <https://doi.org/10.1016/j.envpol.2004.08.011>

506 Lima, J. A. M. C., Labanowski, J., Bastos, M. C., Zanella, R., Prestes, O. D., de Vargas, J. P. R., et al. (2020).  
507 “Modern agriculture” transfers many pesticides to watercourses: a case study of a representative rural  
508 catchment of southern Brazil. *Environmental Science and Pollution Research*.  
509 <https://doi.org/10.1007/s11356-019-06550-8>



510 Özkara, A., Akyıl, D., & Konuk, M. (2016). Pesticides, Environmental Pollution, and Health. *Environmental*  
511 *Health Risk - Hazardous Factors to Living Species*. <https://doi.org/10.5772/63094>

512 Pascual Aguilar, J. A., Andreu, V., Campo, J., Picó, Y., & Masiá, A. (2017). Pesticide occurrence in the waters  
513 of Júcar River, Spain from different farming landscapes. *Science of The Total Environment*, 607–608,  
514 752–760. <https://doi.org/10.1016/j.scitotenv.2017.06.176>

515 Pérez, D. J., Okada, E., De Gerónimo, E., Menone, M. L., Aparicio, V. C., & Costa, J. L. (2017). Spatial and  
516 temporal trends and flow dynamics of glyphosate and other pesticides within an agricultural watershed  
517 in Argentina: Spatial-temporal and flow dynamics of pesticides in a basin. *Environmental Toxicology*  
518 *and Chemistry*, 36(12), 3206–3216. <https://doi.org/10.1002/etc.3897>

519 Pesce, S., Bouchez, A., & Montuelle, B. (2012). Effects of Organic Herbicides on Phototrophic Microbial  
520 Communities in Freshwater Ecosystems. In D. M. Whitacre (Ed.), *Reviews of Environmental*  
521 *Contamination and Toxicology* (Vol. 214, pp. 87–124). New York, NY: Springer New York.  
522 [https://doi.org/10.1007/978-1-4614-0668-6\\_5](https://doi.org/10.1007/978-1-4614-0668-6_5)

523 Proia, L., Lupini, G., Osorio, V., Pérez, S., Barceló, D., Schwartz, T., et al. (2013). Response of biofilm bacterial  
524 communities to antibiotic pollutants in a Mediterranean river. *Chemosphere*, 92(9), 1126–1135.  
525 <https://doi.org/10.1016/j.chemosphere.2013.01.063>

526 Rhea, D. T., Harper, D. D., Farag, A. M., & Brumbaugh, W. G. (2006). Biomonitoring in the Boulder River  
527 Watershed, Montana, USA: Metal Concentrations in Biofilm and Macroinvertebrates, and Relations  
528 with Macroinvertebrate Assemblage. *Environmental Monitoring and Assessment*, 115(1–3), 381–393.  
529 <https://doi.org/10.1007/s10661-006-7086-7>

530 Sabater, S., Guasch, H., Ricart, M., Romani, A., Vidal, G., Klünder, C., & Schmitt-Jansen, M. (2007).  
531 Monitoring the effect of chemicals on biological communities. The biofilm as an interface. *Analytical*  
532 *and Bioanalytical Chemistry*, 387(4), 1425–1434. <https://doi.org/10.1007/s00216-006-1051-8>

533 Sassine, L., Le, C. G. L. S., Khaska, M., Verdoux, P., Meffre, P., Benfodda, Z., & Roig, B. (2017). Spatial  
534 distribution of triazine residues in a shallow alluvial aquifer linked to groundwater residence time.  
535 *Environmental Science and Pollution Research International*, 24(8), 6878–6888.  
536 <https://doi.org/10.1007/s11356-016-7224-x>

537 Späth, R., Flemming, H.-C., & Wuertz, S. (1998). Sorption properties of biofilms. *Water Science and*  
538 *Technology*, 37(4–5), 207–210. <https://doi.org/10.2166/wst.1998.0623>

539 Staley, Z. R., Harwood, V. J., & Rohr, J. R. (2015). A synthesis of the effects of pesticides on microbial  
540 persistence in aquatic ecosystems. *Critical Reviews in Toxicology*, 45(10), 813–836.  
541 <https://doi.org/10.3109/10408444.2015.1065471>

542 Tang, G., Yao, J., Zhang, X., Lu, N., & Zhu, K. Y. (2018). Comparison of gene expression profiles in the aquatic  
543 midge (*Chironomus tentans*) larvae exposed to two major agricultural pesticides. *Chemosphere*, 194,  
544 745–754. <https://doi.org/10.1016/j.chemosphere.2017.12.040>

545 Tiecher, T., Caner, L., Minella, J. P. G., Pellegrini, A., Capoane, V., Rasche, J. W. A., et al. (2017a). Tracing  
546 sediment sources in two paired agricultural catchments with different riparian forest and wetland  
547 proportion in southern Brazil. *Geoderma*, 285, 225–239.  
548 <https://doi.org/10.1016/j.geoderma.2016.10.008>

549 Tiecher, T., Minella, J. P. G., Caner, L., Evrard, O., Zafar, M., Capoane, V., et al. (2017b). Quantifying land use  
550 contributions to suspended sediment in a large cultivated catchment of Southern Brazil (Guaporé River,  
551 Rio Grande do Sul). *Agriculture, Ecosystems & Environment*, 237, 95–108.  
552 <https://doi.org/10.1016/j.agee.2016.12.004>

553 Tiecher, T., Minella, J. P. G., Evrard, O., Caner, L., Merten, G. H., Capoane, V., et al. (2018). Fingerprinting  
554 sediment sources in a large agricultural catchment under no-tillage in Southern Brazil (Conceição  
555 River). *Land Degradation & Development*, 29(4), 939–951. <https://doi.org/10.1002/ldr.2917>

556 Tien, C.-J., Lin, M.-C., Chiu, W.-H., & Chen, C. S. (2013). Biodegradation of carbamate pesticides by natural  
557 river biofilms in different seasons and their effects on biofilm community structure. *Environmental*  
558 *Pollution*, 179, 95–104. <https://doi.org/10.1016/j.envpol.2013.04.009>

559 Villeneuve, A., Montuelle, B., & Bouchez, A. (2010). Influence of slight differences in environmental conditions  
560 (light, hydrodynamics) on the structure and function of periphyton. *Aquatic Sciences*, 72(1), 33–44.  
561 <https://doi.org/10.1007/s00027-009-0108-0>

562 Vymazal, J., & Březinová, T. (2015). The use of constructed wetlands for removal of pesticides from agricultural  
563 runoff and drainage: A review. *Environment International*, 75, 11–20.  
564 <https://doi.org/10.1016/j.envint.2014.10.026>

565 Vryzas, Z. (2018). Pesticide fate in soil-sediment-water environment in relation to contamination preventing  
566 actions. *Current Opinion in Environmental Science & Health*, 4, 5–9.  
567 <https://doi.org/10.1016/j.coesh.2018.03.001>

568 Zafar, M., Tiecher, T., de Castro Lima, J. A. M., Schaefer, G. L., Santanna, M. A., & dos Santos, D. R. (2016).  
569 Phosphorus seasonal sorption-desorption kinetics in suspended sediment in response to land use and  
570 management in the Guaporé catchment, Southern Brazil. *Environmental Monitoring and Assessment*,  
571 188(11), 643. <https://doi.org/10.1007/s10661-016-5650-3>

572 Zhang, W., Tang, X.-Y., Xian, Q.-S., Weisbrod, N., Yang, J. E., & Wang, H.-L. (2016). A field study of colloid  
573 transport in surface and subsurface flows. *Journal of Hydrology*, 542, 101–114.  
574 <https://doi.org/10.1016/j.jhydrol.2016.08.056>

575 Zhang, L., Dong, D., Hua, X., & Guo, Z. (2018). Inhibitory effects of extracellular polymeric substances on  
576 ofloxacin sorption by natural biofilms. *Science of The Total Environment*, 625, 178–184.  
577 <https://doi.org/10.1016/j.scitotenv.2017.12.271>

578

579