

1 **Occurrence and removal of micropollutants in full-scale aerobic,**
2 **anaerobic and facultative wastewater treatment plants in Brazil**

3 *Oladapo Komolafe^{a*}, Wojciech Mrozik^b, Jan Dolfing^c, Kishor Acharya^b, Lucas Vassalle^d,*
4 *Cesar R. Mota^d, Russell Davenport^b*

5 ^aGFL Environmental Inc. Greater Toronto Area, L5T 2L2, Ontario, Canada

6 ^bSchool of Engineering, Newcastle University, NE1 7RU, Newcastle Upon Tyne, UK

7 ^cDepartment of Mechanical and Construction Engineering, Northumbria University, NE1
8 8QH, Newcastle Upon Tyne, UK

9 ^dDepartamento de Engenharia Sanitária e Ambiental, Federal University of Minas Gerais
10 (UFMG), Belo Horizonte, Brazil

11 *Corresponding author. Email: okomolafe@gflenv.com

12 **Abstract**

13 This study aims to evaluate micropollutant occurrence and removal in a low-middle income
14 country (LMIC) by investigating the occurrence of 28 chemicals from different classes
15 (triclosan, 15 polycyclic aromatic hydrocarbons (PAHs), 4 estrogens and 8 polybrominated
16 diphenyl ether (PBDE) congeners) in three technologically diverse full-scale Brazilian
17 wastewater treatment plants (WWTPs). These chemicals were detected at concentrations
18 similar to those reported in other low-middle income countries (LMICs) and high-income
19 countries (HICs) (0.1 – 49 µg/L) indicating their widespread use globally and the need for
20 more studies in LMICs that are typically characterised by relatively inadequate wastewater
21 treatment barriers. Among the three different WWTPs investigated for removal of these
22 chemicals, the least energy intensive system, waste stabilization ponds (WSPs), was the most
23 effective (95-99%) compared to the activated sludge (79 – 94%), and Up-flow sludge blanket
24 reactor (UASB) with trickling filters system (89 - 95%). These results highlight the potential

25 of WSPs for micropollutant removal- especially in warm climates. However, the effluent from
26 all three WWTP could pose a risk to aquatic organisms when discharged into the receiving
27 waters as the effluent concentrations of triclosan, some estrogens, PAHs and BDE 209 were
28 above European environmental quality standards (EQS) or predicted no effect concentration
29 (PNEC values), indicating that receiving water bodies could benefit from further treatment. In
30 combination, these results help to further understand prevailing concentrations of
31 micropollutants globally and fate in current wastewater treatment systems.

32 **Keywords:** Micropollutants Occurrence; Wastewater Treatment; Micropollutant Removal;
33 Surface Water Protection; Low-middle Income Countries.

34 **1 Introduction**

35 Over the past few decades, the occurrence of micropollutants in the aquatic environment has
36 instigated several studies globally on their environmental concentrations, effects, fate and
37 removal from the environment (Luo et al., 2014). Despite the many reports on the occurrence
38 of these chemicals around the world, there are limited studies in wastewater and receiving
39 waters of low-middle income countries (LMICs). A review of the peer-review and grey
40 literature by the UBA (German Environmental Agency- Umweltbundesamt) showed that out
41 of 1,016 reviewed publications on pharmaceuticals in the environment, Africa, GRULAC
42 (Group of Latin American and Caribbean States) and Asia only accounted for 2%, 5%, and
43 14% respectively, while developed countries in Europe and America accounted for 72% (aus
44 der Beek et al., 2015; aus der Beek et al., 2016). The UBA also reported a high correlation
45 between the number of studies and detection of pharmaceuticals in the environment as more
46 than 100 different chemicals were detected in Europe and USA compared to approximately 30
47 chemicals detected in Africa and GRULAC (aus der Beek et al., 2015). Although the total
48 number of reviewed publications was updated to 1,519 in 2019, the publication quota between
49 LMICs and HICs was not further updated (Eike et al., 2019). This issue of inadequate studies

50 in LMICs is of urgent concern as the risk is as great or even greater in LMICs since
51 manufacturing there is as high as in high income countries (HICs) and there are less stringent
52 regulatory and wastewater treatment barriers to protect wildlife and humans from exposure
53 (Weiss et al., 2016). Such countries suffer from the double jeopardy of risks from modern and
54 more traditional environmental hazards such as waterborne infectious diseases (Lvovsky,
55 2001). Such risks are difficult to quantify when there is little understanding of the extent of
56 micropollutant contamination in LMICs.

57 The occurrence of steroidal hormones (such as estrogens- E1, E2, E3 and EE2), personal care
58 products (such as triclosan) and industrial chemicals (such as polyaromatic hydrocarbons
59 PAHs; and polybrominated diphenyl ethers PBDEs) in raw wastewater and treated effluents
60 around the world has been reported in ng/L or µg/L levels (Luo et al., 2014; Wang et al.,
61 2013). At these concentration levels, the adverse effects of estrogens (Combalbert and
62 Hernandez-Raquet, 2010), triclosan (Singer et al., 2002), PAHs (Jones et al., 2012) and
63 PBDEs (Cristale et al., 2013) on aquatic organisms have been reported, thereby instigating
64 research efforts on appropriate technologies to remove such pollutants to harmless levels
65 before their release into water bodies. Furthermore, these chemicals have been listed as
66 contaminants of concern due to their adverse effects (EU, 2013; UKTAG, 2013)

67 This study uses Brazil as an example from which one could extrapolate for LMICs that are
68 mostly characterized by inadequate wastewater treatment infrastructures. There are some
69 studies on the occurrence and removal of estrogens in full-scale Brazilian WWTPs (Aquino et
70 al., 2013; da Cunha et al., 2017; Froehner et al., 2010; Pessoa et al., 2014), and one report on
71 the concentration levels of triclosan in surface water in Brazil (Santos et al., 2016). To our
72 knowledge, there has been no report on the levels of PAHs and PBDEs in full-scale Brazilian
73 WWTPs and water bodies. The relative shortage of studies on micropollutants in Brazil and
74 other LMICs might be due to the difficulties and high costs associated with chemical analysis

75 (Pessoa et al., 2014). Therefore, one of the objectives of this study was to investigate the
76 occurrence and removal of different chemical classes, including four estrogens (natural
77 hormones and a synthetic hormone), triclosan (personal care products), fifteen PAHs
78 (industrial chemical), and eight PBDE congeners (flame retardant in household products) in
79 different types of full-scale Brazilian wastewater treatment plants.

80 As far as wastewater treatment technologies are concerned, activated sludge is the world's
81 most widely used secondary treatment process, especially in HICs and it has been shown to be
82 one of the better technologies for the oxidative removal of micropollutants. Hence, most of
83 the worldwide studies on micropollutants removal refer to this system (Luo et al., 2014).

84 However, these systems are energy intensive - they account for up to 1 % of United
85 Kingdom's (UK) electricity use and yet are ineffective in reducing micropollutants to
86 environmentally safe levels (Gardner et al., 2012). In LMICs like Brazil, wastewater
87 treatment technologies with relatively lower energy requirements and operational costs such
88 as waste stabilization ponds (WSPs) and up-flow sludge blanket reactors (UASBs) are often
89 used (Bressani-Ribeiro et al., 2019; de Lemos Chernicharo et al.; Pessoa et al., 2014). As the
90 water industry needs to move away from unsustainable energy-costly treatment systems to
91 more sustainable low energy systems, studying the Brazilian systems could benchmark such
92 technologies against each other since they are likely to be part of the future wastewater
93 treatment technologies in temperate climates too (Lester et al., 2009; Li et al., 2015).

94 (Froehner et al., 2010) and (Pessoa et al., 2014) have previously investigated the removal of
95 estrogens in three Brazilian WWTPs utilizing different technologies – activated sludge,
96 UASB and WSP. However, in their work, they only reported the total removal achieved by
97 the plant without considering the individual contribution of the different treatment stages. To
98 our knowledge, there have been no similar studies for other chemical classes and specifically
99 for triclosan (PCP), PAHs (industrial chemical) and PBDEs (flame retardant). Therefore, the
100 second objective of this study was to investigate the fate and removal of four classes of

101 micropollutants with different physico-chemical properties (estrogens, triclosan, PAHs and
102 PBDEs) in wastewater treatment plants utilizing different technologies (conventional
103 activated sludge and low energy systems- UASB and WSP); with emphasis on removal
104 achieved after primary and secondary treatment individually. Comparing the effectiveness of
105 these low- and high-energy technologies in removing different groups of pollutants will
106 provide insights on contrasting removal systems and potentially help to implement
107 appropriate technologies for the removal of specific contaminants. The removal mechanisms
108 of the selected chemicals in these different treatment systems was investigated in an ensuing
109 study.

110 **2 Material and methods**

111 **2.1 Materials**

112 A standard solution of 15 mixed priority PAHs (including naphthalene, acenaphthylene,
113 acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz(a)anthracene,
114 chrysene, benzo(b)fluoranthene, chrysene, indeno(1,2,3-cd)pyrene, dibenz(a,h)anthracene and
115 benzo(ghi)perylene at 2 mg/ml in dichloromethane) and triclosan (100 mg) were purchased
116 from Sigma, (UK). A standard solution mix of PBDEs (> 98% purity) containing eight
117 primary congeners including BDE 28, BDE 47, BDE 99, BDE 100, BDE 153, BDE 154, BDE
118 183 and BDE 209 was obtained from Accustandard Inc via Kinesis (UK). The concentration
119 of the congeners was 2.5 µg/ml in isooctane, except BDE 209 which was present at 25 µg/ml.
120 Surrogate standards including isotope labelled ¹³C₁₂-Triclosan (50 µg/ml in methanol), PCB
121 209 (10 µg/ml in heptane) and 4PC-BDE-208 (50 µg/ml in toluene) and a mix of deuterated
122 PAHs (including acenaphthene-d₁₀, phenanthrene-d₁₀, chrysene-d₁₂ and perylene-d₁₂ at 2
123 mg/ml in dichloromethane) were purchased from Wellington Laboratories (via Greyhound
124 Chromatography, UK), Sigma Aldrich (UK) and Accustandard (via Kinesis UK) respectively,
125 with purities higher than 98%. Estrogen standards E1, E2, E3 and EE2 (>98% purity) were

126 purchased from Sigma Aldrich (UK). Deuterated labelled internal standards of estrogens (E1-
127 d₄, E2-d₄, E3-d₂ and EE2-d₄) were purchased from C/D/N Isotopes (QMX Laboratories, UK)
128 (>98% purity). Derivatization reagent BSTFA with 1% TCMS was also purchased from
129 Sigma Aldrich (UK).

130 Stock solutions were prepared by dissolving the reference and surrogate standards in
131 methanol or acetone. Stock solutions were used during extraction and instrumental analysis of
132 the target compounds as detailed in Supporting Information S2. Working solutions were then
133 prepared by diluting the stock solutions in acetone or methanol and dichloromethane or
134 ethylacetate for sample fortification and instrumental analysis respectively. All solutions were
135 stored at 4 °C and allowed to reach room temperature for 15 mins before use. Ultra-trace
136 grade of methanol, acetone, MTBE, dichloromethane and isopropanol were obtained from
137 Casa Lab or Biosan (Brazil). Cartridges used for solid phase extraction were Isolute C18
138 (1000mg, 6 ml) and Isolute C18 (500mg, 6 ml) were purchased from Biotage (UK), while
139 Oasis HLB (200mg, 6ml) was purchased from Waters (UK). Glass microfiber filters were
140 purchased from Sartorius (MGB filters, 0.7mm thick, 1.0 µm particle retention).

141 **2.2 Wastewater treatment plants and sampling**

142 Three WWTPs near Belo Horizonte (Brazil) comprising different treatment technologies were
143 selected in this study. More details on these technologies are summarized in *Table 1* and
144 Supporting Information (S1). These three WWTPs were selected because, they serve large
145 populations that are therefore likely to contain the compounds of interest and represent the
146 main technologies used for wastewater treatment in Brazil- from the expensive energy-
147 intensive activated sludge system to the cheaper less energy-intensive UASB and WSP
148 systems. Sampling was carried out in the summer season in Brazil (December 2016 –
149 February 2017). As summer in Brazil is usually wet, sampling was usually done after 2 days
150 of dry weather when possible. Grab water samples were collected from different points in the

151 WWTPs (*Figure 1*) and were analysed separately. Influent and effluent water samples were
152 collected after preliminary and secondary treatment processes respectively for all WWTPs.
153 Primary effluents were sampled after the primary sedimentation tank, UASB reactors and the
154 anaerobic pond for WWTP A, WWTP B, and WWTP C respectively. Emphasis was placed
155 on removal of micropollutants in the biological processes. Grab sludge samples were also
156 collected in all WWTPs (activated sludge from WWTP A, UASB sludge from WWTP B, and
157 facultative pond sludge/sediment from WWTP C).

158 In WWTP B, the influent was distributed into twenty-one UASB reactors operated in parallel.
159 Therefore, sludge samples were collected from three of the reactors (one at the beginning,
160 middle and end of the train) and mixed together for a better representation of the plant. Grab
161 water and sludge samples were collected in cleaned and disinfected (with 1% Virkron for 24
162 hours, followed by several rinse cycles with distilled water) 5 L high-density polyethylene
163 (HDPE) containers; analysis of the empty rinsed containers showed no contamination with
164 any of the target compounds. Samples were stored at 4 °C upon arrival to the laboratory and
165 micropollutant extraction was carried out within 24 hours.

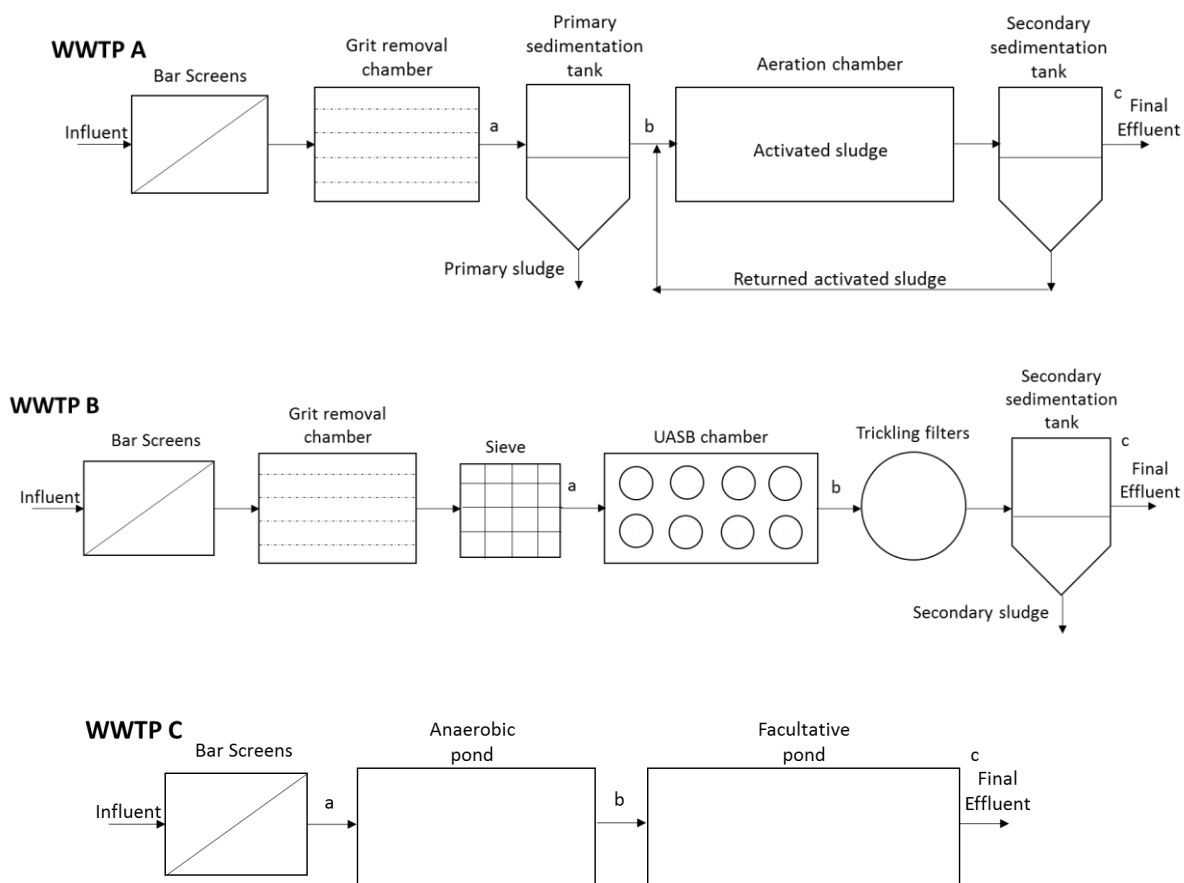


Figure 1. Schematic diagram of the three treatment process streams studied at Brazilian WWTPs. The sampling points for influent, primary effluent and secondary effluent collection is indicated by a, b and c respectively

167

168

169 2.3 Wet chemistry analysis for the WWTPs

170 Suspended solids (SS) and chemical oxygen demand (COD) were measured in the three

171 WWTPs, which were important in assessing the general performance of the WWTPs. The

172 total suspended solids (TSS) and volatile suspended solids (VSS) content of the liquid and

173 solid samples were measured according to the Standard Method 2540B, while COD was

174 measured according to Standard Method 5220D (Clesceri et al., 2005).

175 **2.4 Analysing the micropollutants in wastewater**

176 Sample extraction was carried out by solid phase extraction (SPE) and analysed by GC-MS
177 for triclosan and PAHs, LC-MS/MS for estrogens and GC-ECD for PBDEs (Komolafe et al.,
178 2019). To extract these compounds in the aqueous phase of the samples, 500 mL of filtered
179 (Sartorius MGB filters, 0.7mm thick, 1.0 μm particle retention) wastewater was processed for
180 triclosan and PAHs, 150 – 250 mL for estrogens, and 50 – 100 ml for PBDEs to measure their
181 concentrations in the aqueous phase only. For all these compounds, except estrogens,
182 combined aqueous and particulate matter (PM) phases were analysed by using 50 mL of
183 unfiltered influent and 200 mL of unfiltered effluent passed through the SPE cartridge for
184 triclosan and PAHs (Sánchez-Avila et al., 2009). 20 mL and 50 mL of unfiltered influent and
185 effluent respectively were used for extraction of total PBDEs (i.e. particulate and aqueous
186 phases). Details on the extraction protocol for the chemicals are given in the Supporting
187 Information (S2). SPE cartridges were stored at -20 °C in Brazil, then transported in boxes
188 with cooling packs to the UK, where elution and instrumental analysis was performed. Details
189 of the instrumental analysis are given in the Supporting Information (S3).

190 **2.5 Quality assurance and statistical analysis**

191 Instrumental limits of detection (IDL) were established as the lowest analyte concentration
192 that gave a signal to noise ratio of three ($s/n = 3$) upon the injection of standard solutions. The
193 method detection limit (MDL) was determined according to EPA method 1984 (USEPA,
194 1995) (Table S3), and method quantification limit (MQL) was set at three times the MDL. All
195 samples were fortified with labelled internal standards- $^{13}\text{C}_{12}$ - triclosan, $^{13}\text{C}_{12}$ - methyl
196 triclosan, acenaphthene- d_{10} , phenanthrene- d_{10} , chrysene- d_{12} , E1- d_4 , E2- d_4 , E3- d_2 , EE2- d_4 and
197 perylene- d_{12} (except for PBDE analysis where unlabelled standards PCB 209 and 4PC-BDE-
198 208 was used) for possible correction of matrix effect and losses during sample extraction.
199 Five levels of concentration for each analyte was used for the calibration curve, and linearity

200 was observed when the correlation coefficient was > 0.99 . Method accuracy was evaluated
201 by performing recovery experiments in blanks (deionized water, $n = 3$) and matrix samples
202 (final effluent, $n = 3$) at different fortification levels (data not shown). The repeatability of the
203 method was determined by the relative standard deviation (% RSD) from the recovery
204 experiments in the fortified blank and matrix sample (Nácher-Mestre *et al.*, 2010; USEPA,
205 2012). We compared the concentrations of these chemicals to European environmental quality
206 standards and benchmarks as Brazilian ones does not exist. Also, the Brazilian standards will
207 most likely be based on these European standards when they are created. For example,
208 Environmental Risk Assessment for pharmaceuticals in Belo Horizonte, Brazil was performed
209 according to methods proposed by the European Medicines Agency (Neves and Mol, 2019)
210

211 **3 Results and discussion**

212 **3.1 Characteristics and performance of the treatment plants**

213 In terms of total suspended solids (TSS) removal, WWTP A (activated sludge) was least
214 effective, removing only 48 % as compared to 76 % and 75 % achieved by WWTP B (UASB)
215 and WWTP C (WSP) respectively (*Table 1*). COD removal was also lowest in WWTP A with
216 42 % recorded when compared to 83 % and 80 % achieved by WWTP B and WWTP C
217 respectively. Although the TSS and COD removal rates provided by the plant operator for
218 WWTP A was 80 % and 82 % respectively. As our sampling was not taken around the same
219 time as the plant operator and the system HRT was not taken into account, this discrepancy in
220 TSS and COD measurement in WWTP A might be due to some temporal diurnal variation
221 that was captured by the operators sampling.

222

223 Table 1. Characteristics of the selected WWTPs, including the concentrations of suspended solids and chemical oxygen demand across the
 224 different treatment stages

WWTP	Population (millions)	Treatment type (secondary)	TSS (mg/L)				VSS (mg/L)				COD (mg/L)			
			Sludge	Influent	P Eff	S. Eff	Sludge	Influent	P. Eff	S. Eff	Sludge	Influent	P. Eff	S. Eff
A	1.6	Activated sludge	4,290 (210)	196 (25)	105 (12)	102 (12)	3,750 (160)	161 (16)	86 (7)	65 (4)	1,832 (79)	312 (12)	265 (12)	180 (9)
B	1.1	UASB + Trickling filters	22,400 (790)	102 (9)	64 (3)	24 (2)	17,640 (920)	100 (9)	55 (4)	24 (1)	997 (11)	252 (12)	112 (9)	43 (1)
C	0.015	WSP (1 AP, 1 FP)	75 (6)	208 (18)	121 (9)	52 (4)	73 (8)	103 (7)	109 (3)	50 (3)	111* (6)	456 (31)	91 (6)	90 (4)

- 225 - $n = 3$ for COD measurements, total suspended solids (TSS) and volatile suspended solids (VSS) measurements
 226 - *Inf* = Raw wastewater, *P. Eff* = primary effluent, *S. Eff* = secondary effluent, *AP* = anaerobic pond, *FP* = facultative pond
 227 - * Sludge for WWTP C is wastewater taken from the top end of the facultative pond
 228 - Values in parentheses are standard deviations

229

230 **3.2 Assessing the fate and removal of micropollutants in the different** 231 **WWTPs**

232 **3.2.1 Fate and extent of triclosan removal**

233 The total (aqueous and particulate matter) concentration of triclosan in raw influent was 49.2
234 $\mu\text{g/L}$, 67.1 $\mu\text{g/L}$ and 17.8 $\mu\text{g/L}$ in WWTP A, WWTP B and WWTP C respectively (**Error!**
235 **Not a valid bookmark self-reference.**). The total concentration of triclosan in the final
236 effluent was 1.5 $\mu\text{g/L}$, 3.6 $\mu\text{g/L}$ and 0.9 $\mu\text{g/L}$ for WWTP A, WWTP B, and WWTP C
237 respectively- indicating a removal of 97 %, 95% and 95% respectively. These concentrations
238 are similar to reported levels of triclosan in unfiltered municipal wastewater around the world
239 (**Error! Reference source not found.** S3) (Kumar et al., 2010; Lozano et al., 2013). The
240 concentration of triclosan in the aqueous phase of the WWTP A reduced from 1.3 $\mu\text{g/L}$ to 0.6
241 $\mu\text{g/L}$ with primary treatment and secondary treatment accounting for 53 % and 47% of the
242 removal respectively (**Error! Not a valid bookmark self-reference.**). Lozano *et al.* reported
243 75 % triclosan removal from the aqueous phase after primary treatment in an activated sludge
244 WWTP (Lozano et al., 2013), while another study reported up to 40 % triclosan removal
245 (Thomas and Foster, 2005). Removal of triclosan in this plant was most likely due to
246 partitioning and settling out with sludge in the primary clarifier and biodegradation during
247 secondary treatment (Lozano et al., 2013). In WWTP B, the concentration of triclosan in the
248 aqueous phase reduced from 1.3 $\mu\text{g/L}$ to 0.8 $\mu\text{g/L}$ (60% removal). This removal was solely
249 achieved after UASB treatment as no further removal was observed after trickling filters
250 treatment. In WWTP C, the concentration of triclosan decreased from 1.4 $\mu\text{g/L}$ to 0.4 $\mu\text{g/L}$
251 with stage 1 secondary treatment (anaerobic pond) and stage 2 secondary treatment
252 (facultative pond) responsible for 54% and 19% removal respectively.

253 Table 2. Concentration of triclosan in the influent, primary effluent and secondary effluent of
 254 three different WWTPs in Brazil

WWTP	Influent (µg/L)		Primary effluent (µg/L)		Secondary effluent (µg/L)		Removal (%)	
	Total	Aqueous	Total*	Aqueous	Total	Aqueous	Total removal	Aqueous removal
A	49.18 (7.42)	1.30 (0.22)	n.d	0.89 (0.04)	1.49 (0.01)	0.55 (0.02)	97	58
B	67.05 (0.72)	1.26 (0.09)	n.d	0.76 (0.12)	3.57 (1.35)	0.78 (0.05)	95	39
C	17.80 (2.00)	1.42 (0.04)	n.d	0.66 (0.03)	0.92 (0.08)	0.39 (0.02)	95	73

255 -Mean concentration presented with standard deviation in bracket, n.d represents not determined
 256 -As these are single grab samples done in triplicate, temporary variation in overall removal cannot be
 257 reported.

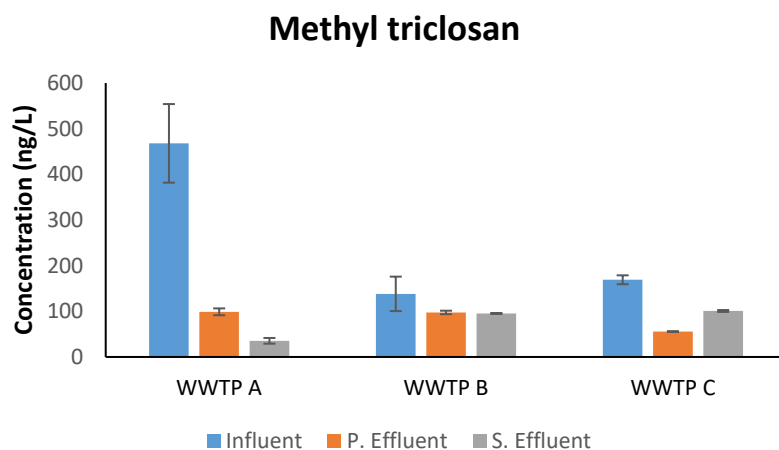
258 -Total represents triclosan concentration in the aqueous and particulate matter (total removal for %
 259 removal in both phases) while Aqueous represents triclosan concentration in the aqueous phase alone
 260 (aqueous removal for % removal in only the aqueous phase).
 261

262 About 92 – 98 % and 59 – 78 % of triclosan was present in the particulate phase of the
 263 influent and effluent samples respectively from the three WWTPs (Figure S1). Some
 264 previous studies reported about 75 – 80 % and 10 – 20 % of triclosan in influent and effluent
 265 respectively (Lozano et al., 2013; Thomas and Foster, 2005). However, this partitioning
 266 might vary with different suspended solids concentrations especially in effluent samples
 267 (Thomas and Foster, 2005). Furthermore, this association with suspended solids was expected
 268 due to the high Log K_{ow} value (4.8) of triclosan (Kantiani *et al.*, 2008). The relatively higher
 269 removal rates observed in the total liquid phase than in the aqueous phase might have been
 270 due to the high adsorption potential of triclosan, which promoted their adsorption to sludge
 271 across the treatment stream.

272 Triclosan effluent concentrations in the WWTPs (0.9 – 3.5 µg/L in the total phase, and 0.4 –
 273 0.8 µg/L in the aqueous phase) exceeded the annual mean recommended standard of 100
 274 ng/L (0.1 µg/L) for triclosan in freshwater and saltwater (UKTAG, 2013). The effluent
 275 concentration in these WWTPs was similar to reported levels in activated sludge WWTPs in

276 Canada (Lee et al., 2003), Australia (Kookana et al., 2011), USA (Kumar et al., 2010),
277 Germany (Bester, 2005) and UK (Sabaliunas et al., 2003) (Table S3). Hence, the
278 concentration of triclosan is as high in low-middle income countries (LMICs) as in high-
279 income countries (HICs). No previous study has reported the levels of triclosan in effluents of
280 full-scale UASB or WSP WWTPs. However, exceeding the recommended safety standard
281 for triclosan indicates that WWTPs around the world (even with different treatment
282 technologies) are ineffective in removing triclosan in their current format.

283 The concentration of methyl triclosan (a known biotransformation product of triclosan) was
284 also monitored in the three WWTPs. Methyl triclosan was detected in influents, and this
285 might be due to biotransformation of triclosan in the sewage network during transportation to
286 the WWTPs (*Figure 2*). The concentration of methyl triclosan ranged from 138 – 468 ng/L in
287 the three WWTPs. This was not surprising as methyl triclosan formation under aerobic and
288 anoxic conditions has been reported, with the latter being relatively slower (Bester, 2005;
289 Chen et al., 2011). Removal of methyl triclosan was observed in all three WWTPs (up to
290 85 % in WWTP A). However, the concentration of methyl triclosan increased by 45% after
291 the facultative pond treatment in WWTP C. This suggests that methylation occurred in the
292 facultative pond that operates under aerobic and anoxic conditions. However, this might also
293 be due to the grab samples not equating with the hydraulic load (retention times) of the
294 systems.



295

296 Figure 2. Concentration of methyl triclosan in the aqueous phase of influent, primary effluent
 297 and secondary effluent samples of the three WWTPs. P and S represents primary and secondary
 298 respectively. n = 3, standard deviation presented as error bars

299

300 3.2.2 Fate and extent of PAH removal

301 The total (aqueous and particulate matter) concentration of PAHs (sum of 15 individual
 302 PAHs) in raw influent was 10.2 µg/L, 20.2 µg/L and 27.3 µg/L in WWTP A, WWTP B and
 303 WWTP C respectively (*Table 3*). The total concentration of PAHs in the final effluent was
 304 1.1 µg/L, 1.6 µg/L and 1.1 µg/L for WWTP A, WWTP B, and WWTP C respectively. All
 305 individual PAHs except indeno(1,2,3-cd)pyrene, dibenz(a,h)anthracene
 306 and benzo(ghi)perylene were detected in all samples (*Table S5*, *Table S6*, *Table S7*).

307 Low molecular weight (LMW) PAHs were the most abundant group of PAHs, followed by
 308 middle molecular weight (MMW) and high molecular weight PAHs in both influent and
 309 effluent samples. MMW PAHs have previously been reported to be the most abundant PAHs
 310 in raw wastewater, while LMW PAHs were the most abundant in the effluent (Busetti et al.,
 311 2006), although this distribution mostly depends on the source of the PAHs entering the
 312 WWTP. The total concentration of PAHs in influents of the studied WWTPs detected in this
 313 study were similar to those reported in Spain (Sánchez-Avila et al., 2009) and Greece

314 (Manoli and Samara, 2008) , but two and ten times lower than those detected in China (Qi et
 315 al., 2013) and Italy (Busetti et al., 2006; Fatone et al., 2011) respectively (Table S8).
 316 Naphthalene (Naph), phenanthrene (Phen), and benzo(b)fluoranthene (BbF) were the most
 317 abundant LMW, MMW and HMW PAHs respectively detected in the three WWTPs, which
 318 is in agreement with some other studies (Manoli and Samara, 2008; Wang et al., 2013).
 319 Furthermore, aqueous concentrations of PAHs in the WWTPs were similar to those generally
 320 reported around the world (Cao et al., 2005; Qi et al., 2013; Yao et al., 2012), but five to ten
 321 times lower than levels reported by Wang *et al.*, in China (Wang et al., 2013) (Table S9).
 322 This shows that concentration of PAHs in both HICs and LMICs are similar.

323 Table 3. Total concentration of PAHs in the influent, primary effluent and secondary effluent
 324 of three different WWTPs in Brazil

WWTP	Influent (µg/L)		Primary effluent (µg/L)		Secondary effluent (µg/L)		Removal (%)	
	Total	Aqueous	Total*	Aqueous	Total	Aqueous	Total removal	Aqueous removal
A	10.20	0.55	n.d	0.58	1.07	0.48	89.5	12.6
B	20.19	0.70	n.d	0.80	1.57	0.47	92.2	32.5
C	27.30	12.11	n.d	0.88	1.07	0.43	96.1	64.4

325 -The concentration presented is the sum of 15 PAHs. Individual PAH concentrations with standard
 326 deviations is given in the supplementary information

327 - As these are single grab samples done in triplicate, temporary variation in overall removal cannot be
 328 reported..

329 - Total represents PAH concentration in the aqueous and particulate matter (total removal for %
 330 removal in both phases) while Aqueous represents triclosan concentration in the aqueous phase alone
 331 (aqueous removal for % removal in only the aqueous phase).
 332
 333

334 Furthermore, the total effluent concentration from the WWTPs of those PAHs falling under
 335 the regulation of the EU WFD (naphthalene-Naph, anthracene- Anth, fluoranthene- Flt,
 336 benzo(b)fluoranthene- BbF, benzo(a)pyrene BaP) were below the mean allowable EQS
 337 concentrations for inland surface waters for fours PAHs, Naph, Anth, Flt, BaP (MAC-EQS
 338 values of 130, 000 ng/L, 100 ng/L, 120 ng/L, and 270 ng/L respectively), but were higher for
 339 BbF (17 ng/L) (EU, 2013). The measured concentrations were compared to EU benchmarks
 340 since a Brazil benchmark does not exist.

341 In terms of PAHs removal, WWTP C (WSP) performed better with 96 % removal (64% in
342 aqueous phase), compared to 92 % (33 % in aqueous phase) in WWTP B (UASB + trickling
343 filters), and 90 % (13 % in aqueous phase) in WWTP A (activated sludge) (*Table 3*).
344 Although, an increment in the concentration of some PAHs (aqueous phase) after UASB
345 treatment in WWTP B, and anaerobic pond treatment in WWTP C was observed. This was
346 perhaps due to their residual concentrations already present in these treatment stages before
347 sampling. The removal of PAHs from the studied WWTPs was most likely mainly via
348 sorption onto solids in the settling tanks and reactors, with volatilization and biodegradation
349 contributing less (Lei et al., 2007; Liu et al., 2011). Volatilization has been reported to
350 contribute to only about 1 – 2 % removal of PAHs in conventional wastewater treatment
351 plants (Manoli and Samara, 2008). The higher removal observed in the total phase of the
352 WWTPs suggests that removal was principally through sorption. To further explain this,
353 about 90 – 97 % of LMW, MMW and HMW PAHs were present in the particulate phase of
354 the influent samples from the three WWTPs, while 60 – 70 % of LMW to HMW PAHs
355 partitioned onto the particulate matter in effluent samples (Figure S3). This is expected due to
356 the high Log K_{ow} values of the PAHs that promotes their sorption onto solids (Sánchez-Avila
357 et al., 2009).

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361 3.2.3 Fate and extent of estrogen removal

362 Only E1 and E3 were detected in these WWTPs, while E2 and EE2 were below the method
363 detection limit (0.5 ng/L) (*Table 4*). E3 was the most abundant estrogen in the influent of all
364 the WWTPs, with an aqueous phase concentration of 1234 ng/L, 1548 ng/L and 625 ng/L in
365 WWTP A, WWTP B and WWTP C respectively. E3 has been reported to be the most
366 abundant estrogen in wastewater around the world (Coleman et al., 2010; Kim et al., 2007) as
367 they are excreted the most by humans (Ternes and Joss, 2007). Furthermore, the E3
368 concentrations reported in this study were similar to globally recorded concentrations (Gabet-
369 Giraud et al., 2010; Kim et al., 2007; Liu et al., 2015; Luo et al., 2014)

370 The aqueous concentration of E1 in WWTP A, WWTP B and WWTP C was 78 ng/L, 107
371 ng/L and 51 ng/L respectively (*Table 4*). These results differ from the reported occurrence
372 and concentrations of estrogens (E1, E2 and EE2) by Pessoa et al. (Pessoa et al., 2014) in
373 Brazilian WWTPs with identical technologies and process configurations to those reported in
374 this study. These authors detected E1, E2 and EE2 in influent samples but did not measure E3
375 concentrations. They suggested that E1 was more frequently detected in influents due to
376 partial degradation of E2 to E1, and de-conjugation of E1 conjugated compounds (E1
377 sulfonide or glucuronide) in sewer systems. This assertion was further supported by E1
378 concentrations in the influent that were five times higher than E2. Therefore, the relatively
379 low levels of E1 in this study, and the reported E2 degradation might be responsible for non-
380 detection of E2 in the examined WWTPs. Furthermore, some studies reported a five to ten
381 times higher concentration of E1 in influents from Brazilian wastewater plants (Froehner et
382 al., 2010; Pessoa et al., 2014) in comparison with our results. However, Queiroz *et al.* could
383 not detect E2 and EE2 above the LODs (9.3 and 12.4 ng/L respectively) from influent
384 samples from one of the same WWTPs (WWTP A) used in this study (Queiroz et al., 2012),
385 while de Castro *et al.* reported low concentrations of E1 (de Castro et al., 2018). Floripes et

386 al., detected E3 and EE2 in residential condominiums but not in the wastewater influent from
 387 WWTP A and WWTP B (Floripes et al., 2018). They suggested adsorption to sediments or
 388 biodegradation in the sewage pipe during transport. Coleman *et al.*, also reported that E2 and
 389 EE2 concentrations in WWTP influent in Australia was below their method detection limits
 390 (1 and 0.1 ng/L respectively) (Coleman et al., 2010). The E1 influent concentrations in this
 391 study are similar to those reported around the world (Gabet-Giraud et al., 2010; Salgado et
 392 al., 2010) (**Error! Reference source not found.**). E1 is considered as one of the most
 393 environmentally important estrogens because of its more frequent detection at higher
 394 concentrations compared to the other estrogens, and can be used to measure estrogenicity in
 395 the environment despite its lower potency compared to E2 (Coleman et al., 2010).

396 Table 4. Concentration of E1 and E3 in the influent, primary effluent and secondary effluent
 397 of the three studied Brazilian WWTP

Compound	E1 (ng/L)				E3 (ng/L)			
	Influent	P. Effluent	S. Effluent	% Removal	Influent	P. Effluent	S. Effluent	% Removal
WWTP A	77.8 (3.3)	65.3 (5.4)	16.0 (2.0)	79.4	1233.7 (17.1)	1068.7 (51.8)	64.3 (9.1)	94.8
WWTP B	106.5 (6.6)	151.8 (10.2)	11.9 (1.4)	88.9	1547.9 (148.8)	1254.5 (641.3)	12.5 (1.0)	99.2
WWTP C	50.5 (5.4)	130.2 (29.4)	0.1 (0.08)	99.7	625.1 (26.4)	3814 (559.8)	13.0 (1.7)	97.9

398 -P and S represents primary and secondary effluent; standard deviation in bracket

399 - As these are single grab samples done in triplicate, temporary variation in overall removal cannot be
 400 reported.

401 -Concentrations of E2 and EE2 was below the MDL, hence result not shown

402

403 The mean removal of E1 from the aqueous phase was 79 %, 89 % and 99 % in WWTP A,
 404 WWTP B and WWTP C respectively. Whereas, E3 removal was 95 %, 99 % and 98 % for
 405 WWTP A, WWTP B and WWTP C respectively. In WWTP A, most removal was achieved
 406 after secondary treatment (62% for E1 and 81 % for E3), and the removal rates achieved were
 407 similar those reported previously (Nie et al., 2012; Pessoa et al., 2014; Salgado et al., 2010).
 408 However, it is the final effluent concentration, not percentage removal and how it compares
 409 to the PNEC values that are important for determining risk to the environment.

410 In WWTP B, secondary treatment (trickling filters) accounted for 90 % and 80 % of E1 and
411 E3 removal respectively (*Table 4*). However, an initial increase in E1 concentration was
412 observed after UASB treatment- perhaps due the residual E1 concentration already present in
413 the reactor that our grab sampling did not account for. Pessoa *et al.* (Pessoa et al., 2014)
414 reported E1 removal below their detection limits (35.4 ng/L) in a WWTP utilizing UASB
415 technology equipped with chlorine post-treatment (that may have also contributed to E1
416 removal). Froehner *et al.*, also reported E1 removal below their detection limits (values not
417 reported) from a WWTP using UASB treatment with an attached dissolved air flotation
418 reactor (Froehner et al., 2010). Hence, the efficiency of the UASB system alone could not be
419 determined from these previous studies. In this study, it was the trickling filter post-UASB
420 treatment that removed the majority of the E1 and E3. Trickling filter systems have been
421 reported to possess a similar E1 removal capacity as activated sludge system, with an
422 achieved removal of 90 – 95 % (Salgado et al., 2010). Although some studies have shown
423 they are ineffective (Liu et al., 2015) .There is no report of E3 removal by full-scale UASB
424 systems in literature, but up to 90 % removal has been reported in trickling filter systems
425 (Gabet-Giraud et al., 2010). Also, pilot-scale UASB reactors in Brazil have also been
426 reported to be ineffective in removal of estrogens (Brandt et al., 2013).

427 In WWTP C, secondary treatment accounted for 99 % and 98 % of E1 and E3 removal
428 respectively (*Table 4*). However, an initial increase in E1 and E3 was observed after primary
429 treatment (anaerobic pond)- perhaps due the residual E1 concentration already present in the
430 reactor that our grab sampling did not account for. Although deconjugation of E1 and E3
431 conjugated compounds might also have contributed to this (Pessoa et al., 2014), and a similar
432 occurrence of increased E3 concentration after waste stabilization pond treatment was
433 reported in another study (Coleman et al., 2010). The high removal rate achieved by WWTP
434 C was consistent with those reported by Froehner *et al.* by WSPs (Froehner et al., 2010), but

435 was higher than the 34-82 % reported by Coleman *et al.*, (Coleman *et al.*, 2010) and 31 –
436 62 % reported by Pessoa *et al.*, (Pessoa *et al.*, 2014). The 98 % E3 removal achieved by the
437 WWTP was also higher than the 26 – 36 % reported in another study (Coleman *et al.*, 2010).
438 The relatively poor removal reported by Pessoa *et al.*, and Coleman *et al.*, might be due the
439 different system configuration (absence of an anaerobic pond and presence of a maturation
440 pond). The photo-oxidizing effect of WSPs may have contributed to high estrogen removal
441 in WWTP C. This is due to increased photo-oxidative potential of the ponds because of the
442 high oxygen concentrations and presence of humic substances (Davies-Colley *et al.*, 1999).
443 Furthermore, Coleman *et al.*, reported complete photo-degradation of E1 and E3 under UV
444 light in their studies (Coleman *et al.*, 2010).

445 The effluent concentration of E1 ranged from 0.1 ng/L for WWTP C to 16 ng/L for WWTP
446 A. These concentrations were similar to those reported around the world (Liu *et al.*, 2015;
447 Luo *et al.*, 2014), but were ten times lower than those reported by other studies in Brazil
448 (Pessoa *et al.*, 2014). The concentration of E3 in effluent also ranged from 13 ng/L for
449 WWTP C to 64 ng/L for WWTP A. These concentration were similar to those reported in
450 France (Gabet-Giraud *et al.*, 2010), but over ten times higher than those reported in Korea
451 (Behera *et al.*, 2011), China (Nie *et al.*, 2012), and two to five times lower than reported
452 levels in another study in Brazil (Coleman *et al.*, 2010) (Table S8). Caldwell *et al.* estimated
453 the predicted no effect concentration (PNEC) values in aquatic organisms for E1 and E3 to be
454 6 ng/l and 60 ng/L respectively (Caldwell *et al.*, 2012). Only the effluent from WWTP C was
455 below the PNEC value for E1 in this study. For E3, the effluent from WWTP B and WWTP
456 C were below the PNEC value, while effluent from WWTP A exceeded this level.

457 **3.2.4 Fate and extent of PBDE removal**

458 BDE 47 and BDE 209 are the most abundant PBDE congeners often detected in wastewater
 459 and rivers (Kim et al., 2013). In this study, the total (aqueous and particulate matter)
 460 concentration of BDE 47 ranged from 0 – 44 ng/L in influent samples from the three
 461 WWTPs, while the concentration BDE 209 was between 103.2 ng/L to 251.2 ng/L (*Table 5*).
 462 Aqueous phase concentrations of BDE 47 and BDE 209 was between 2.6 – 12.8 ng/L and
 463 13.2 – 136.9 ng/L respectively (*Table 5*). Other PBDE congeners were also detected in the
 464 WWTPs except BDE 28 (*Table S10*, *Table S11*, *Table S12*). The total PBDE concentration
 465 (Σ PBDE) in the influent of the three WWTPs was between 193.8 – 269.6 ng/L, and these
 466 determined concentrations are similar to reported levels around the world (Clarke et al., 2010;
 467 Kim et al., 2013; Xiang et al., 2014) (*Table S13*). To our knowledge, this is the first time that
 468 PBDEs have been reported in Brazilian wastewater treatment plants.

469 Table 5. Concentration of BDE congeners in the influent, primary effluent and secondary
 470 effluent of the WWTPs

Compound	Influent (ng/L)		P. Effluent (ng/L)		S. Effluent (ng/L)		Removal (%)	
	Total	Aqueous	Total	Aqueous	Total	Aqueous	% Total removal	% Aqueous removal
WWTP A								
BDE 47	44.0 (6.0)	9.3 (0.1)	<MDL	13.4 (2.3)	3.5 (0.9)	1.5 (0.2)	92.0	62.4
BDE 209	103.2 (22.2)	13.2 (0.2)	<MDL	12.9 (0.8)	<MDL	<MDL	100.0	100.0
Σ PBDE	193.8	26.4	<MDL	33	11.0	3.6	98.1	86.4
WWTP B								
BDE 47	41.9 (3.1)	12.8 (1.3)	<MDL	20 (2.0)	4.7 (1.4)	4.1 (0.4)	88.8	68.0
BDE 209	143.0 (21.8)	45.3 (7.0)	<MDL	10.0 (1.2)	12.1 (0.1)	<MDL	91.5	100.0
Σ PBDE	222.4	83.6	<MDL	64.8	32.1	14.8	85.6	82.3
WWTP C								
BDE 47	<MDL	2.6 (0.3)	<MDL	9.8 (2.8)	3.9 (0.7)	2.9 (0.5)	-	0.0
BDE 209	251.2 (20.4)	136.9	<MDL	<MDL	<MDL	<MDL	100.0	100.0
Σ PBDE	269.6	148.0	<MDL	14.3	13.8	8.8	94.9	94.1

471 -P. and S. represents primary and secondary respectively; MDL represents method detection limit; standard
 472 deviation in bracket. - As these are single grab samples done in triplicate, temporary variation in overall
 473 removal cannot be reported.

474 In terms of PBDEs removal, WWTP A (activated sludge) achieved 98 % removal (73% in
475 aqueous phase), compared to 95 % (94 % in aqueous phase) in WWTP C (WSP), and 86 %
476 (43 % in aqueous phase) in WWTP B (UASB + trickling filters) (Table 5). Primary treatment
477 accounted for 90 % and 22 % PBDE removal in WWTP B and WWTP C respectively, while
478 an increase in concentration was observed in WWTP A. Additionally, an increment in the
479 aqueous concentration of some PBDE congeners (BDE 47, 100 in WWTP A, BDE 47, 100,
480 183 in WWTP B, and BDE 47 in WWTP C) after primary treatment was observed. This
481 might be as a result of gradual release of the particulate matter-bound compounds in the
482 influent samples into the dissolved/aqueous phase. However, this might also be due to the
483 accumulation of these compounds in the primary treatment process before grab-sampling.
484 Hence, these post-primary treatment concentrations may not have been representative of the
485 levels in the raw influent. Some other authors have reported increased level of BDE 47, and
486 BDE100 after primary treatment (Clarke et al., 2010) and secondary treatment (Daso et al.,
487 2012) in conventional wastewater treatment plants.

488 The total concentration of PBDE in the effluent of the three studied WWTPs was between 11
489 – 32 ng/L (\sum PBDE EU WFD congeners = 10 - 13 ng/L). These concentrations are an order
490 of magnitude lower than the EU WFD MAC-EQS and are similar to reported levels in
491 Canada (Kim et al., 2013), Australia (Clarke et al., 2010) and China (Deng et al., 2015; Xiang
492 et al., 2014) (Table S12). BDE 209 has been reported to dominate the total concentrations of
493 PBDEs founds in wastewater around the world (Cristale and Lacorte, 2015; Peng et al.,
494 2009). This was also the case in Brazilian wastewaters in this study as BDE 209 accounted
495 for 53 %, 64 % and 93 % of total PBDE concentration in the influent of WWTP A, WWTP B
496 and WWTP C respectively. BDE 209 was not detected in the effluent of WWTP A and
497 WWTP C, but was found in the effluent of WWTP B at a concentration that will pose risk on
498 aquatic organisms – estimated Risk Quotient of 2.5 according to Cristale *et al.* (Cristale et al.,

499 2013). Due to the high Log K_{ow} of PBDEs, sorption onto solids and subsequent removal
500 during primary and secondary treatment is expected (Sánchez-Avila et al., 2009). However,
501 biodegradation can also play a major role in their removal. Biodegradation of PBDEs can
502 occur under both aerobic and anaerobic conditions (Xia, 2013).

503

504

505 **4 Conclusion**

506 This study has investigated the occurrence of twenty-eight chemicals from four classes
507 (biocide- triclosan, industrial chemical- 15 PAHs, steroidal hormones- 4 estrogens, and flame
508 retardants-8 PBDEs) for the first time in Brazil (except for estrogens). The results indicated
509 that the concentrations of triclosan, PAHs, estrogens and PBDEs were similar in both LMICs
510 and HICs, suggesting that these chemicals are in widespread use globally. The comparable
511 use and concentrations of these chemicals in HICs and LMICs further indicates that the
512 aqueous environment in Brazil and other LMICs are at a greater overall risk due to
513 insufficient wastewater treatment infrastructure. Hence, the measured environmental
514 concentrations of these chemicals contribute to a wider understanding of the micropollutant
515 contamination in LMICs, which will be useful in quantifying the risks they pose on a global
516 scale.

517 The study also investigated the fate of such pollutants in three types of WWTP that differ in
518 their energy requirements and types of processes including; energy-intensive aerobic systems
519 (activated sludge), low-energy anaerobic-aerobic systems (UASBs and trickling filters) and
520 passive-energy facultative systems (waste stabilization ponds). In most cases, and for all the
521 chemical classes, the least energy intensive and cheapest WSP system (WSP) outperformed
522 (89 – 99 % removal) or matched the high energy activated sludge system (WWTP A) (79 –
523 94 % removal), therefore showing its potential for micropollutant removal. However, despite
524 the removal achieved by these treatment systems, the concentration of triclosan and estrogens
525 were above their PNEC value, therefore the effluent might pose risk to aquatic organisms
526 when discharged. Additionally, the three types of WWTP did not show any bias towards a
527 certain class of pollutant.

528 Furthermore, the partitioning of the chemicals onto the suspended solids (specifically PAHs,
529 triclosan and PBDEs) indicates that sorption plays an important role in their removal in these
530 systems. However, the extent of biodegradation and sorption of these chemicals needs to be
531 investigated to provide key knowledge required to design better removal systems.

532

533 **Conflict of interest**

534 The authors declare no potential conflict of interest.

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