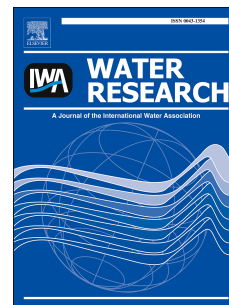# Accepted Manuscript

Fate of organic microcontaminants in wastewater treatment and river systems: An uncertainty assessment in view of sampling strategy, and compound consumption rate and degradability

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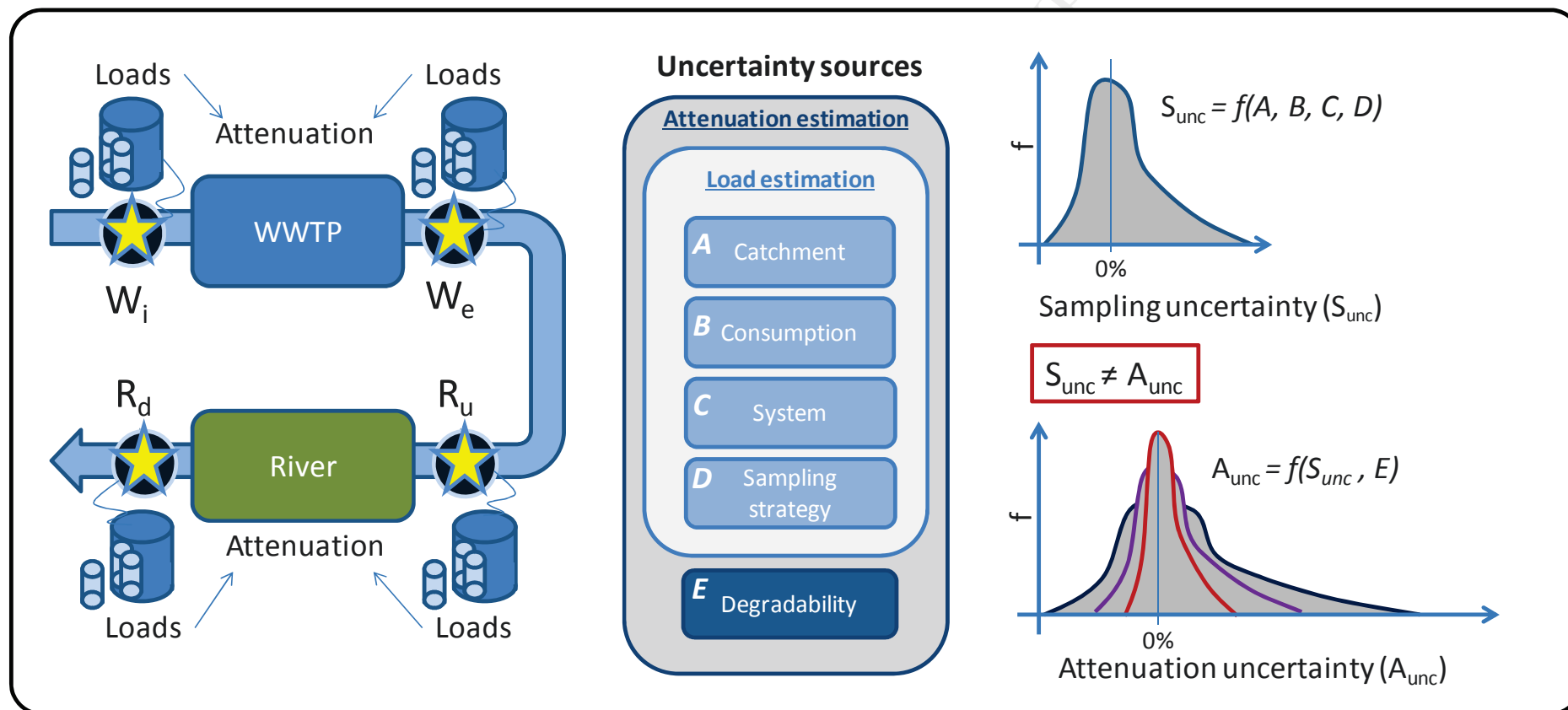
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Graphical Abstract

Fate of organic microcontaminants in wastewater treatment and river systems: an uncertainty assessment in view of sampling strategy, and compound consumption rate and degradability

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18 **Abstract**

19 The growing awareness of the relevance of organic microcontaminants on the environment

20 has led to a growing number of studies on attenuation of these compounds in wastewater

21 treatment plants (WWTP) and rivers. However, the effects of the sampling strategies

22 (frequency and duration of composite samples) on the attenuation estimates are largely

23 unknown. Our goal was to assess how frequency and duration of composite samples

24 influence uncertainty of the attenuation estimates in WWTPs and rivers. Furthermore, we

25 also assessed how compound consumption rate and degradability influence uncertainty. The

26 assessment was conducted through simulating the integrated wastewater system of

27 Puigcerdà (NE Iberian Peninsula) using a sewer pattern generator and a coupled model of

28 WWTP and river. Results showed that the sampling strategy is especially critical at the

29 influent of WWTP, particularly when dealing with compounds with a low consumption ($\leq$100

30 toilet flushes with compound day^{-1}), and less critical at the effluent of the WWTP and in the

31 river due to the mixing effects of the WWTP. For example, at the WWTP, when evaluating a

32 compound that is present in 50 pulses·d^{-1} using a sampling frequency of 15-min to collect a

33 24-h composite sample, the attenuation uncertainty can range from 94% (0% degradability)

34 to 9% (90% degradability). The estimation of attenuation in rivers is less critical than in

35 WWTPs, as the attenuation uncertainty was lower than 10% for all evaluated scenarios.

36 Interestingly, the errors in the estimates of attenuation are usually lower than those of loads

37 for most sampling strategies and compound characteristics (e.g. consumption and

38 degradability), although the opposite occurs for compounds with low consumption and

39 inappropriate sampling strategies at the WWTP. Hence, when designing a sampling

40 campaign, one should consider the influence of compounds' consumption and degradability

41 as well as the desired level of accuracy in attenuation estimations.

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45 **Highlights**

46   -   Sampling matters when investigating microcontaminants in WWTPs and rivers

47   -   Uncertainty in the loads and attenuation estimations is not the same

48   -   Short sampling intervals and longer sampling durations are needed

49   -   The WWTP influent is especially critical when designing sampling strategies

50   -   Degradability and desired attenuation accuracy should be defined upfront

51

52 **1. INTRODUCTION**

53 Many organic microcontaminants enter freshwater ecosystems mainly via point-source

54 discharges of wastewater treatment plants (WWTP) (Pal et al., 2010;Li, 2014). Although

55 concentrations of these microcontaminants are often below one microgram per litre, many

56 microcontaminants raise environmental and human health concerns and have become a key

57 environmental problem (Acuña et al., 2015; Schwarzenbach et al., 2006). Unfortunately, the

58 understanding of processes that control the occurrence and fate of these chemical

59 compounds remains incomplete (Antweiler et al., 2014), mainly because of the

60 predominance of lab-scale studies (Joss et al., 2006), the lack of integrated studies of both

61 WWTP and rivers (Petrie et al., 2015), and the diversity of sampling strategies used to assess

62 attenuation (Ort et al., 2010a, 2010b). Thus, many studies have been published reporting

63 attenuation (net balance between removal and release) rates in either WWTP or rivers, but

64 they often differ in the sampling strategy (i.e., sampling frequency and composite duration),

65 thus limiting the comparability of the obtained results. In fact, there are few examples of

66 studies estimating loads and attenuation in both WWTP and rivers in a comparable manner

67 (Alder et al., 2010; Aymerich et al., 2016).

68 In the case of rivers, the most commonly used sampling strategies for the estimation of

69 attenuation are "mass balances" and "Lagrangian". The mass balance strategy implies the

70 calculation of microcontaminant loads at different points along a stretch of river, normally

71 using 24 h flow- or time-proportional composite samples (e.g., Alder et al., 2010; Kunkel and

72 Radke, 2012). The Lagrangian strategy involves tracking and sampling a water parcel as it

73 moves downstream, which it can be used either with natural river concentrations (e.g.,

74 Aymerich et al., 2016; Barber et al., 2013) or after artificially increasing river concentrations

5

75   with a Dirac pulse (e.g., Kunkel and Radke, 2011; Writer et al., 2013). There are some recent

76   studies on the effects of sampling strategy on the uncertainty of attenuation estimates (e.g.,

77   Antweiler et al., 2014), as well as some studies suggesting improved numerical methods to

78   estimate attenuation (e.g., Riml et al., 2013; Aymerich et al., 2016).

79   In the case of WWTPs, the most commonly used sampling strategy is mass balance,

80   calculating loads of microcontaminants by taking one or multiple subsequent 24-hour

81   composite samples from WWTP influent and effluent. Normally, the start of the effluent

82   composite sample is delayed by a multiple of the hydraulic residence time. Such composite

83   samples are composed of individual samples taken at a predefined frequency. This sampling

84   strategy is equivalent to the previously described mass balance strategy for rivers, with the

85   only difference being that in rivers the composite samples between the 2 sites along a river

86   stretch are either not delayed or delayed one time for the hydraulic travel time. Additionally,

87   similar to those proposed for rivers, recent studies have explored the effects of sampling

88   strategy on the reliability of loads and attenuation rate estimates in WWTPs. For example,

89   Ort et al. (2010a, 2010b) demonstrated that sampling mode (volume- or flow-proportional)

90   and frequency matters in the estimation of loads at the influent of a WWTP. As for the

91   estimation of attenuation, several authors have highlighted the importance of considering

92   residence time distributions (Majewsky et al., 2011) applying time-shifted mass balancing

93   approaches (Rodayan et al., 2014), or applying longer sampling durations (Ternes and Joss,

94   2006; Majewsky et al., 2013). Although Ort et al. (2010a) proved that sampling strategy

95   influences the load estimates at a WWTP influent, it is not yet clear how the sampling

96   strategy influences the load estimates at the effluent of a WWTP and, most importantly, the

97   calculation of attenuation rates. Overall, none of the studies assessing the effects of

98   sampling strategies considered both engineered and natural systems (i.e., a WWTP and a

99   river stretch).

100   Given this background, our goal was to assess how different sampling strategies influence

101   the estimates of loads and attenuation rates in WWTPs and their receiving rivers.

102   Specifically, we were interested in assessing the effect of different sampling frequencies and

103   durations on estimated attenuation rates of organic microcontaminants. Other investigated

104   properties include compound characteristics (i.e., degradability) and served population (i.e.,

105   size and consumption rates). We expected higher uncertainty i) for small population size

106   and/or consumption rates, ii) microcontaminants with low degradability and iii) sampling

107   strategies in the WWTP influent because of high temporal concentration/load variability at

108   this point. The assessment was conducted by simulating a real case study of the integrated

109   wastewater system of Puigcerdà (NE Iberian Peninsula).

110

2. METHODS

Given our objectives, we built and calibrated a model for the Puigcerdà integrated system (catchment, sewer, WWTP and river). Then, we estimated attenuation rates in the WWTP and in the river for microcontaminants with different degradability and consumption rates under several scenarios of population size and sampling strategies. To assess the effects of microcontaminants consumption rates and population size, we generated realistic patterns of compounds in the study site. For the assessment of sampling strategies, we used different scenarios differing in frequency and duration of composite samples.

2.1. Study site - Puigcerdà

The experimental study is the integrated system formed by the catchment of Puigcerdà (population equivalent to 16,000 inhabitants) drained by the sewer system towards the influent of the local WWTP and a reach of 4,550 m of the Segre River (Ebro basin, NE Iberian Peninsula) just after the discharge of the WWTP effluent. The sewer is a combined gravity system and has a length of 1.5 km. The WWTP treats an average flow rate of 6,200 m^3 d^{-1} in a conventional active sludge system composed of a secondary treatment (total volume of 4860 m^3) and secondary settler (1103 m^3). The average total hydraulic residence time (HRT) between the influent of the WWTP (*Wi*) and the effluent of the WWTP (*We*) is of 21 h. The Segre River drains an area of 287 km^2 with a rain-snow fed flow regime. The average flow of the river before the WWTP is approximately 26,400 m^3 d^{-1}, where the WWTP effluent accounts for approximately 23% of the river flow. *Ru* (river upstream) corresponds to a sample location 500 m downstream the river after the WWTP discharge, and *Rd* (river downstream) corresponds to a location 4500 m downstream after the WWTP discharge. The HRT of the system between *Ru* and *Rd* is 5.3 hours. Lateral water inputs along the river result

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134 in a dilution of 55% in *Rd* compared to *Ru*. The experimental study was modelled and

135 calibrated to describe hydraulics and the attenuation of microcontaminants. It consists of

136 two main submodels, a catchment-sewer model (section 2.1.1) and a WWTP-river coupled

137 model (section 2.1.2).

138 **2.1.1 Catchment-Sewer model**

139 The sewage patter generator SPG (SPG, 2013) based on Ort et al.(2005) was used to model

140 the Puigcerdà catchment to generate realistic flows and concentrations at the inlet of the

141 WWTP (W_i). The model was calibrated against measured flows and ammonia concentrations

142 (Aymerich et al. 2016) by adjusting parameters related to catchment characteristics, water

143 consumption from households (110 L inhabitants^{-1} d^{-1}), groundwater infiltration (1200 L s^{-1}),

144 pulse mass (150 mg),and total number of pulses (e.g., toilet flushes containing the substance

145 of interest) per person per day (assuming 5 toilet flushes per inhabitant per day). Industrial

146 activity is negligible in the catchment. The number of expected pulses per day is an average

147 and the effective number is generated with a none-homogenous Poisson process, following

148 the diurnal pattern. The temporal resolution of simulated flows and load patterns was of 2

149 minutes. Values for concentration and flow between the two-minute time steps are

150 interpolated linearly (for sampling, see 2.2.1).

151 **2.1.2 WWTP-river coupled model**

152 The model is the one developed in Aymerich et al. (2016), which is based on ordinary

153 differential equations and includes a first-order attenuation kinetic on top of a hydraulic

154 model for both the WWTP and the river. The WWTP hydraulics were modelled with a

155 Combined Stirred Tank Reactor (CSTR) approach, with one biological reactor and one

156 secondary clarifier. The hydraulics in the river were modelled following a Lagrangian

9

157 approach with 5 river sections and including lateral flows. Both hydraulic models were

158 constructed and calibrated using a tracer test (see Supporting Information). At the WWTP,

159 inputs to the model were the dynamic flow and the influent concentrations of the

160 pharmaceuticals at the *Wi* (obtained from the catchment/sewer model). Simulations were

161 run to obtain WWTP effluent concentration profiles at the *We* and, in turn, concentrations at

162 *Ru* and *Rd*. This modelling approach was built in SIMBA® (version 6, IFAK, Germany).

2.2. Numerical methods to quantify attenuation rates

164 In total, we ran 12 simulations to cover all possible combinations of number of pulses per

165 day at all households and target compound degradability (Table 1). All the simulations were

166 run for 400 days at 2-min time resolutions. For each simulation, we applied the 8

167 combinations of sampling frequencies and composite durations using a flow-proportional

168 sampling model. Composite durations corresponded to approximately 1 & 4 times the HRT

169 of the WWTP and to 2 & 4 times the HRT of the river (Table 1). The total number of

170 combinations evaluated was 96, as a result of 4 (number of pulses) x 4 (frequency) x 2

171 (composite duration) x 3 (degradability). Then, samples were composited using a script that

172 is part of SPG (SPG, 2013).

2.2.1 Calculation of sampling errors at the different sampling points

174 For each of the composite samples generated at the different sampling points (*Wi*, *We*, *Ru*,

175 and *Rd*) we calculated the associated sampling error between the "true" average

176 concentration and the "estimated" average concentration for the sampling strategy

177 evaluated. For a given sampling strategy (i.e. sampling frequency and composite duration)

178 the "estimated" average concentration was calculated following eq. 1, according to Ort et al.

179 (2010a). The "true" concentration was calculated using the same approach as before but

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180 with the highest possible frequency allowed by the simulation (i.e., 2 minutes). This "true"

181 concentration would be equivalent to the case of using a continuous sampling strategy.

182 $$\bar{C}_x = \frac{\sum_i c_{xi} \cdot v_{xi}}{\sum_i v_{xi}}$$

183 $i = t, t + t_{int}, t + 2t_{int}+, t + 3t_{int}, \dots, +t_{cdur}$ (eq 1)

184 Where x is the sampling location (*Wi*, *We*, *Ru* and *Rd*), i is the time step, c_{xi} is the

185 instantaneous concentration at the sampling interval applied, v_{xi} is the volume of a flow-

186 proportional discrete sample, V_x is the total wastewater volume of the composite sample,

187 t_{int} is the sampling interval and t_{cdur} is the composite duration applied. Then, the relative

188 sampling error for the load at each site was calculated with eq. 2:

189 $relative\ sampling\ error_x = \frac{\bar{C}_{true_x} - \bar{C}_{flow-prop_x}}{\bar{C}_{true_x}}$ (eq2)

190 **2.2.2 Attenuation error calculation**

191 The approach to calculate the attenuation error associated with each sampling strategy

192 consisted of taking one sample pair at the WWTP (*Wi* and *We*) and one sample pair at the

193 river (*Ru* and *Rd*). The starting times of samples taken at *We* and *Rd* were delayed by 1 time

194 for the HRT of the evaluated subsystems (time-shifted mass balancing approach, as defined

195 in Rodayan et al., 2014). The attenuation for each sample pair was calculated as the

196 proportion of load removed between the two sampling points (*Wi* and *We*, *Ru* and *Rd*),

197 where loads (L) were calculated as the product of the average concentration ($\bar{C}_x$) and the

198 wastewater volume (V_x) obtained for each of the composite samples. Then, loads calculated

199 for each sample pair were used to calculate the "true" attenuation (A_{true}) (eq 3), "estimated"

200   attenuation ($A_{flow-prop}$) (eq 4) and the relative attenuation error between the true and the

201   estimated through flow-proportional sampling (eq 5):

202    $$A_{true} = \frac{L_{true_{in}} - L_{true_{out}}}{L_{true_{in}}}$$ (eq3)

203    $$A_{flow-prop} = \frac{L_{flow-prop_{in}} - L_{flow-prop_{out}}}{L_{flow-prop_{in}}}$$ (eq 4)

204    $$relative\ attenuation\ error = \frac{A_{true} - A_{flow-prop}}{A_{true}}$$ (eq5)

205   In order to obtain a distribution in sampling and attenuation errors, the procedure explained

206   in the previous paragraph was repeated 100 times; hence 100 sample pairs taken randomly

207   at the WWTP (*Wi* and *We*) and 100 pairs at the river (*Ru* and *Rd*). The result is a distribution

208   of the load and attenuation errors for both the WWTP and river. To characterize the load

209   and attenuation error, we used two measures: bias and uncertainty. Bias is calculated as the

210   difference between the true value and the central value of our estimates (expressed as the

211   median of the error distribution). Uncertainty is the dispersion of the error distribution of

212   our estimates (expressed as the 90-interquartile range of the error distribution, calculated

213   from the difference between the 95th and 5th percentiles).

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215 # 3. RESULTS

216 ## 3.1. Temporal and spatial concentration patterns

217 The 24-hour temporal and spatial concentration patterns are shown in Figure 1 (shown as

218 relative to the maximum value of each time-series), including the influent of the WWTP (Wi),

219 the effluent of the WWTP (We) and the river (Rd). For the studied system, if only a few such

220 wastewater pulses are expected over the course of a day (e.g., 50 pulses day^{-1}), the observed

221 pattern at Wi is intermittent with large short-term fluctuations. Thus, in these cases, it is

222 evident that a very high sampling frequency would be necessary to capture these pulses.

223 When increasing the number of wastewater pulses (e.g., 1000 pulses day^{-1}), a smoother

224 pattern with more systematic diurnal variation is observed, which is very similar to the

225 typical ammonia pattern observed at the influent of a WWTP (e.g., a compound that would

226 be present in the majority of toilet flushes). At We, we can see that the mixing processes

227 occurring in the WWTP smooth the patterns observed at the influent of the WWTP between

228 the different wastewater pulses (e.g., 50 and 1000 pulses day^{-1}), and thus in such cases a

229 similar sampling frequency would be valid for both compounds. At the river (Ru), the

230 difference in the concentrations between the different wastewater pulses also becomes

231 negligible and similarly as observed in We, where relative concentration variations are

232 slightly increased due to the flow variations occurring in the WWTP. The profile at Rd (results

233 not shown), is the same as for Ru due to the plug-flow behaviour of the river but with a time-

234 shift of 5.3 hours.

235 **3.2. Effects of sampling strategies on the estimation of loads and**

236 **attenuation rates in the WWTP**

237 The effects of sampling strategies on the load and attenuation estimations at the WWTP for

238 a conservative compound (i.e., 0% degradability) are shown in Figure 2, which shows the

239 effects of different sampling frequencies (5 to 60 min) and duration of composite samples

240 (24 to 96 h) for different wastewater pulses (from 50 to 10,000 pulses d^{-1}). The results for all

241 the compounds are all the combinations evaluated are summarized in Tables SI1-6

242 (Supporting Information). The results show that the sampling frequency is especially critical

243 at *Wi* in cases with a small number of toilet flushes of a compound (≤100 pulses day^{-1}),

244 where sampling errors (90-interquartile range of the obtained error distribution) can range

245 from 171% (50 pulses day^{-1} and 60-min sampling frequency) to 14% (100 pulses day^{-1} and 5-

246 min sampling frequency) (Figure 2A). Errors at *We* are far lower (<1%) than those at *Wi*, with

247 almost no influence from the sampling frequency and number of pulses (Figure 2B), owing to

248 the mixing effects within the WWTP.

249 The effects of sampling strategies on attenuation estimates are shown in Figure 2C, where

250 we can see that the uncertainty is of similar magnitude to the uncertainty of loads at *Wi* and

251 *We* (Figure 2A-B). For example, for 5-min sampling frequency and 50 pulses d^{-1}, sampling

252 errors in the load estimations in *Wi* range from -11% to 9% (5thand 95thpercentile), which are

253 similar to corresponding attenuation estimation errors ranging from -20% to 16%. With

254 decreasing sampling frequencies, we can see that the underestimation exceeds -200% in

255 some cases. In particular, we see that the distribution of errors in the attenuation

256 estimations is not as symmetric as those that occur at the effluent and influent of the

257 WWTP; instead, the distribution is skewed towards negative values, which means that we

258 would tend to underestimate attenuation. Negative skewness in attenuation error

259 distributions originates due to mathematical issues when differences in the magnitudes and

260 distributions of sampling errors at the different sampling location are obtained. For example,

261 for an overestimation of Wi sampling error of 75% or 50% (and We sampling error of

262 practically 0%), an overestimation of attenuation error of 43% and 33% would be obtained;

263 while for an underestimation of the Wi sampling error of 50% and 75%, an underestimation

264 of attenuation error of -100% and -300% is obtained. Finally, results show that the effects of

265 duration of composite samples are is lower than those of frequency, as the gains of

266 increasing the frequency far exceed those of increasing the duration (Figure 2D-F). However,

267 increasing the composite durations under low wastewater pulses can be used as a strategy

268 to reduce errors in those cases when the sampling frequency cannot be further reduced,

269 such as in the case of 50 pulses day^{-1} and 15-min sampling frequency, where the uncertainty

270 can be decreased from 94% to 32%. However, in that case, the uncertainty would still be too

271 high (32%), and a higher sampling frequency (e.g., continuous sampling or sampling

272 frequency lower than 5-min) or a longer composite duration would thus be required.

273 **3.3. Effects of sampling strategies on the estimation of loads and**

274 **attenuation rates in the river**

275 The effects of sampling strategies on the estimation of sampling and attenuation rates in the

276 river are shown in Figure 2 and summarized in Tables SI7-12. For the sampling frequencies

277 evaluated (15 to 240 min), uncertainty associated with the sampling and attenuation

278 estimations always remains lower than 10%. However, when decreasing the sampling

279 frequency from 15 to 240 min, uncertainty increases (from 1 to 10%) but to a lower extent

280 than that obtained at the influent of the WWTP (Figure 3). Overall, the distribution width of

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281 the errors depends on both sampling frequency and the number of wastewater pulses,

282 although similar errors were observed for *Ru* and *Rd* sampling locations due to the plug-flow

283 behaviour of the river hydraulics. Similar to observations at the WWTP, when estimating

284 attenuation (Figure 3C), precision decreases but the bias disappears. The increase of

285 composite duration also shows some benefits for reducing errors but only in cases when low

286 sampling frequencies are applied (i.e., 240-min), especially for lower composite durations

287 (12-h). When increasing the sampling duration to 24-h (approximately 4 times the hydraulic

288 residence time of the studied river section), the uncertainty in all cases remains lower than

289 2%.

290 **3.4. Effects of compound degradability on the estimation of loads and**

291 **attenuation rates**

292 The effects of different degradability levels (0%, 50%, and 90% attenuation from *Wi* to *We*

293 and *Ru* to *Rd*) for a given sampling strategy are shown in Figure 4. At the influent of the

294 WWTP (Figure 4A), we observe the same sampling errors for the different degradability

295 levels. At the effluent of the WWTP (Figure 4B), sampling errors increase when degradability

296 increases, but always remain lower than 0.5%. When propagating those errors to the

297 attenuation calculations (Figure 4C), the uncertainty decreases with increasing degradability.

298 This is a consequence of higher signal to noise ratios at higher degradabilities, which in fact

299 result in higher differences which are not so affected by the errors in the estimates of We or

300 Wi. Again, we also observe attenuation error distributions skewed to negative values for the

301 different degradability cases. In particular, for the same sampling strategy but different

302 degradability rates, for the most critical case (50 pulses d^{-1}), uncertainty in attenuation

303 estimations can change from -62% to 32% for a compound which attenuates 0% and from -

304   6% to 3% for a compound which attenuates 90%. At the river (Figure 4D-E), we observe

305   trends in sampling error distributions similar to those observed at the effluent of the WWTP

306   (Figure 3B), but slightly increased due to the different sampling frequencies (15-min at the

307   WWTP and 240-min at the River) and increased concentration variations due to the flow

308   variations occurring in the WWTP (Figure 1). Again, when propagating those errors in

309   attenuation calculations, we observe that the lowest uncertainties occur with high

310   degradability levels, even the highest sampling errors are applied in the calculations for the

311   latter. With regards to attenuation error distributions, we see that symmetric distributions

312   are obtained due to similar magnitude and distributions in sampling errors obtained at the

313   different sampling locations (Ru and Rd).

314   To better understand the effect of degradability on attenuation uncertainty Figure 5 shows

315   the relationship between sampling uncertainty and attenuation uncertainty for different

316   degradability levels (0%, 50%, 90%), and for numbers of wastewater pulses (from 50 to

317   10,000 pulses d^{-1}). In both the WWTP (Figure 5A) and the river (Figure 5B), we observe that

318   sampling errors in the load estimations and attenuation estimations show a linear

319   relationship. In particular, the results show that 0% degradability is the most critical case, as

320   any error in the estimate of Wi is directly translated in errors in attenuation (e.g., an error of

321   40% in Wi equals 40% in attenuation); except for low number of wastewater pulses (< 100

322   pulses d^{-1}) and inappropriate sampling frequencies where attenuation errors are increase up

323   to a factor of 1.8 to 2.5 (respect to sampling errors). Instead, higher degradabilities imply

324   that errors in the estimate of Wi are minimized when estimating attenuation (e.g., an error

325   of 40% in Wi equals 5%). Note that the number of pulses do not apparently influence these

326   relationships between the errors in loads at Wi and attenuation. In the river, similarly to

327   what described for the WWTP, increases in degradability also imply that the transmission of

328   errors from load estimates to attenuation is minimized. However, this minimization is not as

329   pronounced as for the WWTP, as the slope between load and attenuation errors for a

330   degradability of 90%, is 0.1 at the WWTP and 0.25 at the river.

4. DISCUSSION

332   Based on the results from this study, we can conclude that sampling strategy matters, as

333   errors in loads and attenuation change considerably as a function of sampling strategy.

334   Regarding bias and uncertainty, the most important factor is the number of pulses

335   (combination of served population and compound consumption rate), followed by the

336   frequency, then the composite duration, and finally the compound degradability.

337   *Number of pulses (served population and consumption rate).* We confirmed our initial

338   hypothesis that the sampling strategy has a significant influence on the estimation of organic

339   microcontaminant attenuation in WWTPs and rivers when the number of pulses is low (<

340   100 pulses day^{-1}). In particular, the effect of the number of pulses is especially critical at the

341   influent of the WWTP (where the highest short-term variations occur), and to a lower extent

342   at the effluent of the WWTP and at the river when short-term variations are reduced due to

343   mixing processes in the WWTP. Hence, we must increase our sampling effort when working

344   in i) small municipalities, ii) medium-large municipalities with compounds with a very low or

345   low consumption rate, or iii) large catchments or catchments where pumping activity in the

346   sewer system generates patterns that dominate the fluctuation in the influent of a WWTP

347   (rather small number of pump events than number of toilet flushes). Hence, the calculation

348   of the number of pulses would be the first step when designing a sampling campaign.

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349   Specifically, a similar effort should be devoted to rivers with a low dilution capacity, where

350   other factors should also be accounted for, such as the impact of WWTP flow variations

351   (Antweiler et al., 2014).

352   *Sampling frequency.* We confirmed the hypothesis that it is important to sample at

353   sufficiently high frequencies at the influent of the WWTP to obtain attenuation estimates

354   with low uncertainty. Proper guidance on sampling at the influent of WWTPs is provided in

355   Ort et al. (2010a, 2010b), but more information is needed when sampling for integrated

356   studies (Petrie et al., 2015). In this study, the results showed that the frequency of sampling

357   in the effluent of the WWTP (and in the river) can be reduced (as compared to the influent

358   of the WWTP), as uncertainty is much lower due to the mixing effects occurring in the

359   WWTP. However, for inappropriate sampling frequencies ($\geq$ 30-min) and low number of

360   pulses ($\leq$ 100 pulses day^{-1}), attenuation uncertainty can be greater than the one obtained in

361   the loads estimation, with attenuation error distributions skewed to negative values. Thus,

362   when designing a sampling strategy, we should be aware that sampling strategies that

363   appear to be sufficiently accurate for loads representation are not always appropriate for

364   attenuation estimations. In the river, we recommend following the mass balance approach

365   (same approach applied for WWTPs) in integrated studies (WWTP and river) and when

366   evaluating the influence of the discharge of the WWTP on the river. In the river, the

367   frequency of the sampling should be sufficiently high to capture concentrations variability

368   governed due to the flow variations at the WWTP effluent (Antweiler et al., 2014). In fact,

369   the importance of sampling frequency has been underestimated in river studies on the

370   attenuation of microcontaminants, as it has been rarely reported in studies on

371   microcontaminant attenuation (e.g., Alder et al., 2010).

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372 *Composite and sampling duration*. We evaluated the influence of composite duration and

373 concluded that longer composite durations decrease uncertainty. In the case of WWTPs,

374 given equivalent costs in modifying frequency and duration of composite samples, it is

375 preferable to increase frequency, as errors in magnitude and dispersion decrease to a major

376 extent when frequency is increased. The common practice in WWTPs and in rivers (for the

377 mass balancing approach) is to set composite duration to at least 1 times the HRT of the

378 system and adjust the sampling frequency. However, in this study, we see that 1 times the

379 HRT sometimes is not enough to ensure low bias and uncertainty in attenuation estimations

380 (e.g., very low number of pulses); thus, for the estimation of attenuation, it is further

381 recommended to sample along consecutive days. This can be accomplished by increasing the

382 duration of the composite samples or by compositing daily samples over several consecutive

383 days, as suggested for WWTPs in Majewsky et al. (2011; 2013) and in Ternes and Joss (2006).

384 Note that a 96-h composite sample as presented in this study would mean taking 4

385 consecutive 24-h composite samples; one sample of 96-h would be subject to degradation

386 inside the sampling bottle. In the case of rivers, a composite duration of at least 24-h is

387 advisable, as the mechanisms behind attenuation experience 24 hour changes due to

388 temperature and irradiation cycles (Schwientek et al., 2016). In our study, the attenuation

389 uncertainty of a 12-h composite sample doubled that of a 24-h cycle, thus stressing the need

390 to cover at least one full diel cycle. It is worth noting that another factor influencing the

391 estimation of attenuation (which has not been evaluated in this study) is the delay between

392 the start of sampling influent-effluent (or upstream-downstream). In this study we have

393 assumed a fixed delay of 1 time the HRT; future work will be conducted to evaluate

394 variations in such delay.

395   *Compound degradability*. We have demonstrated that the lowest uncertainties in

396   attenuation estimates are observed for compounds with high degradability levels and the

397   highest uncertainties for compounds with low degradability. The propagation of uncertainty

398   in the calculation of attenuation using influent-effluent (or upstream and downstream) loads

399   is dependent on the degradability of the compound. For compounds with high degradability

400   (e.g., 90%), the uncertainty in influent or upstream loads is much higher than the uncertainty

401   in attenuation estimations. For compounds with low degradability (e.g., 0%), the uncertainty

402   in the estimation of attenuation of similar magnitude than the uncertainty in the influent or

403   upstream loads. In fact, the same relationship between degradability and uncertainty can be

404   observed in the review from Luo et al. (2014); in there, compounds that degrade more than

405   90% show a deviation between 0 and 8%, and compounds that degrade less than 50% show

406   a larger deviation between 13 and 34% (see Figure SI2). In agreement with the results

407   obtained in this study, one of the explanations of this deviation could be the compounds'

408   degradability and the use of different (and in some cases not optimal) sampling strategies in

409   the reviewed studies, as well as combined with the fact of not addressing sampling

410   uncertainty (Ort et al., 2010b). Hence, when designing a sampling campaign, one should

411   consider the compounds' consumption and theoretical degradability as well as the desired

412   level of accuracy in attenuation estimations, as compounds with lower degradability require

413   higher sampling frequencies. As it is expected that studies will not only focus on one

414   compound, the sampling strategy should be designed for the compound with the most

415   important combination of number of pulses and non-degradability case.

5. CONCLUSIONS

In this study, we assessed the effects of the number of pulses (size of served population and consumption rate), sampling frequency, composite duration and compound degradability on the bias and uncertainty of the attenuation rates of microcontaminants in WWTP and rivers. We conclude that sampling strategy matters, as errors in loads and attenuation rates change considerably as a function of sampling strategy. Regarding bias and uncertainty, the most important factor is the combination of served population and consumption rate (number of pulses), followed by the frequency, then the composite duration, and finally the compound degradability. Overall, we conclude the following:

1. We must increase our sampling effort when dealing with low number of pulses, that is, in small municipalities or with compounds with a low consumption rate.

2. We must focus our sampling efforts at the WWTP influent, as the errors in the load estimations there are higher than at the effluent or at the river for a given sampling effort.

3. Given equivalent costs in modifying frequency and duration of composite samples, it is preferable to increase frequency, as errors in magnitude and dispersion decrease to a major extent with increasing frequency. In cases where we cannot further reduce sampling frequency, a good option would be to composite several 1-day composite samples.

4. When designing the sampling strategy, we must first consider population size and compound characteristics and use, as well as the maximum acceptable

438   error in the attenuation rates. As it is expected that studies will not only focus

439   on one compound, the sampling strategy should be designed for the

440   compound with the most important combination of number of pulses and

441   non-degradability case.

442 **6. ACKNOWLEDGEMENTS**

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TABLES

Table 1. Summary of the combinations of number of pulses, degradability, sampling frequencies and composite durations evaluated in this study.

FIGURES

Figure 1. Illustrative example of the variations of concentrations (scaled on the maximum value) at different sampling locations for the system under study, for 50 and 1000 pulses d^{-1}. Wi = influent of the WWTP; We = effluent of the WWTP; Rd = Downstream river 4500 m after the WWTP discharge.

Figure 2. Loads and attenuation errors at the WWTP for a flow-based sampling mode and different wastewater pulses (from 50 to 10 000 pulses d^{-1}) and sampling frequencies (from 5 to 60 min). A-C: composite duration of 24-h. D-F: composite duration of 96-h. The results presented as boxplots stands for the 5-percentile, 25-percentile, 50-percentile, and 95-percentile.

Figure 3. Loads and attenuation errors at the river for a flow-based sampling mode and different wastewater pulses (from 50 to 10 000 p d^{-1}) and sampling frequencies (from 15 to 240 min). A-C: composite duration of 12-h. D-F: composite duration of 24-h. The results presented as boxplots stands for the 5-percentile, 25-percentile, 50-percentile, and 95-percentile.

Figure 4. Load and attenuation errors for compounds at degradability rates of 0%, 50% and 90%. At the WWTP for a composite duration of 24-h and a sampling frequency of 15-min (A-

553   C) and at the river for a composite duration of 24-h and a sampling frequency of 240-min (D-

554   F).

555   **Figure 5.** Relationships between *Wi* sampling and attenuation errors in the WWTP (A) and *Ru*

556   sampling and attenuation errors in the river (B) at different degradability levels. All number

557   of pulses, composite frequencies and durations evaluated in the study are plotted. Red

558   symbols show the sampling strategy presented in Figure 4. Uncertainty is expressed as the

559   90-interquartile range of the error distributions obtained. Note that only load and

560   attenuation errors lower than 100% are presented.

Table 1. Summary of the combinations of number of pulses, degradability, sampling frequencies and composite durations evaluated in this study

	WWTP	River
Number of pulses (pulses · day^{-1})	50,100, 1000, 10000	
Degradability (%)	0, 50, 90	
Sampling frequencies (min)	5, 15, 30, 60	15, 60, 120, 240
Composite durations (h)	24, 96	12, 24

Figure 1. Illustrative example of the variations of concentrations (scaled on the maximum value) at different sampling locations for the system under study, for 50 and 1000 pulses d^{-1}. *Wi* = influent of the WWTP; *We* = effluent of the WWTP; *Rd* = Downstream river 4500 m after the WWTP discharge.
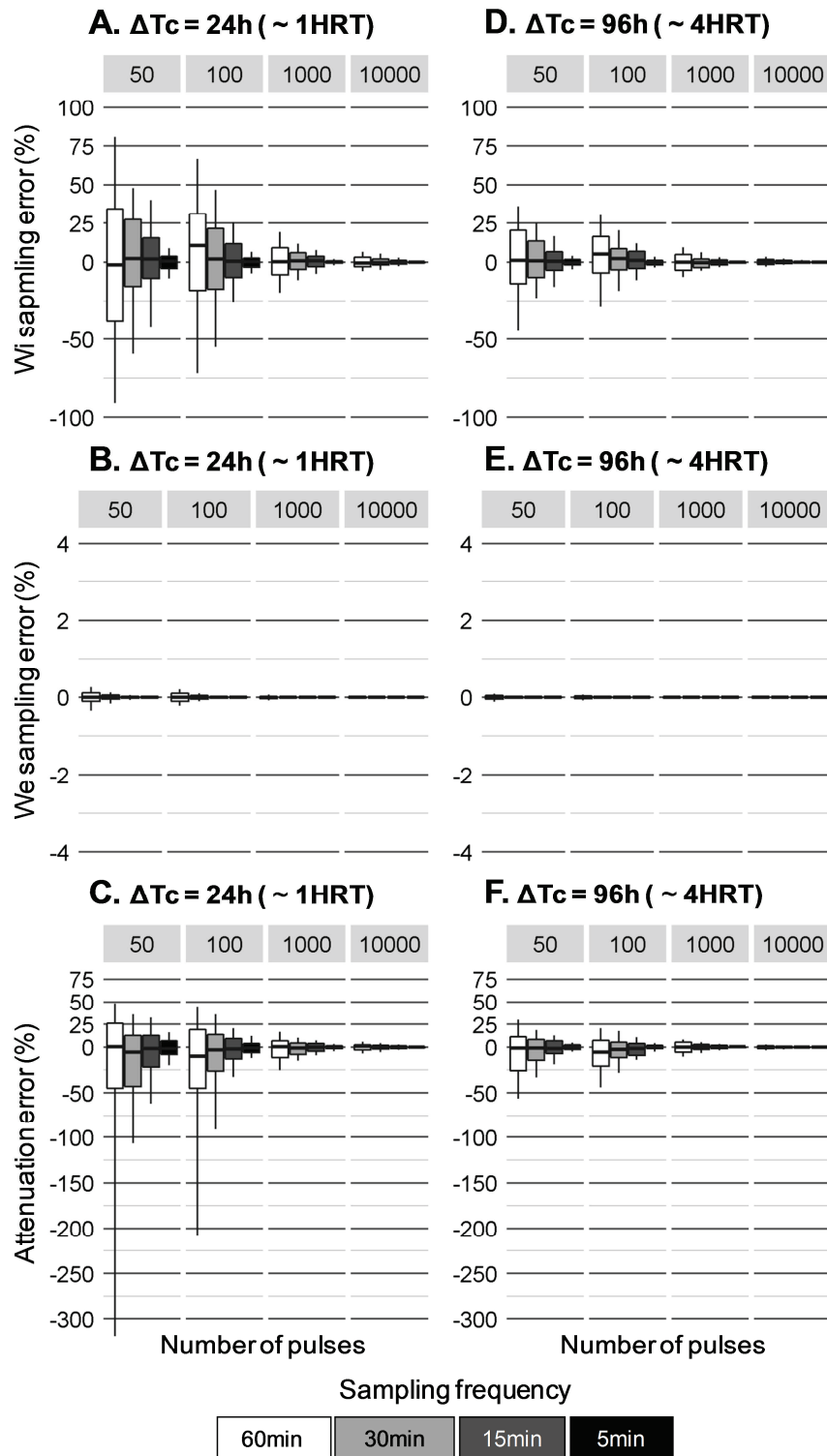
Figure 2. Loads and attenuation errors at the WWTP for a flow-based sampling mode and different wastewater pulses (from 50 to 10 000 pulses d^{-1}) and sampling frequencies (from 5 to 60 min). A-C: composite duration of 24-h. D-F: composite duration of 96-h. The results presented as boxplots stands for the 5-percentile, 25-percentile, 50-percentile, and 95-percentile.
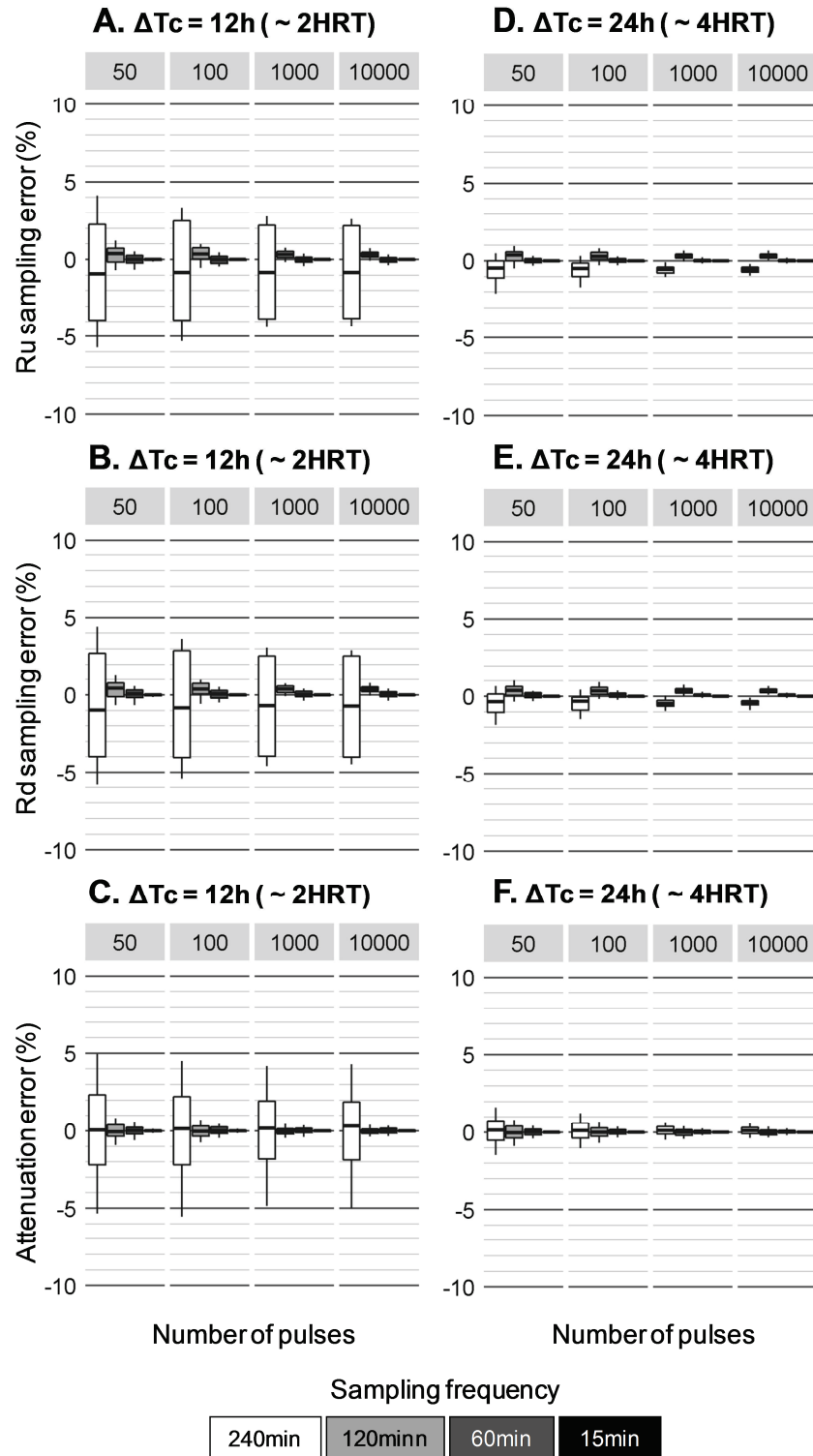
Figure 3. Loads and attenuation errors at the river for a flow-based sampling mode and different wastewater pulses (from 50 to 10 000 p d^{-1}) and sampling frequencies (from 15 to 240 min). A-C: composite duration of 12-h. D-F: composite duration of 24-h. The results presented as boxplots stands for the 5-percentile, 25-percentile, 50-percentile, and 95-percentile.
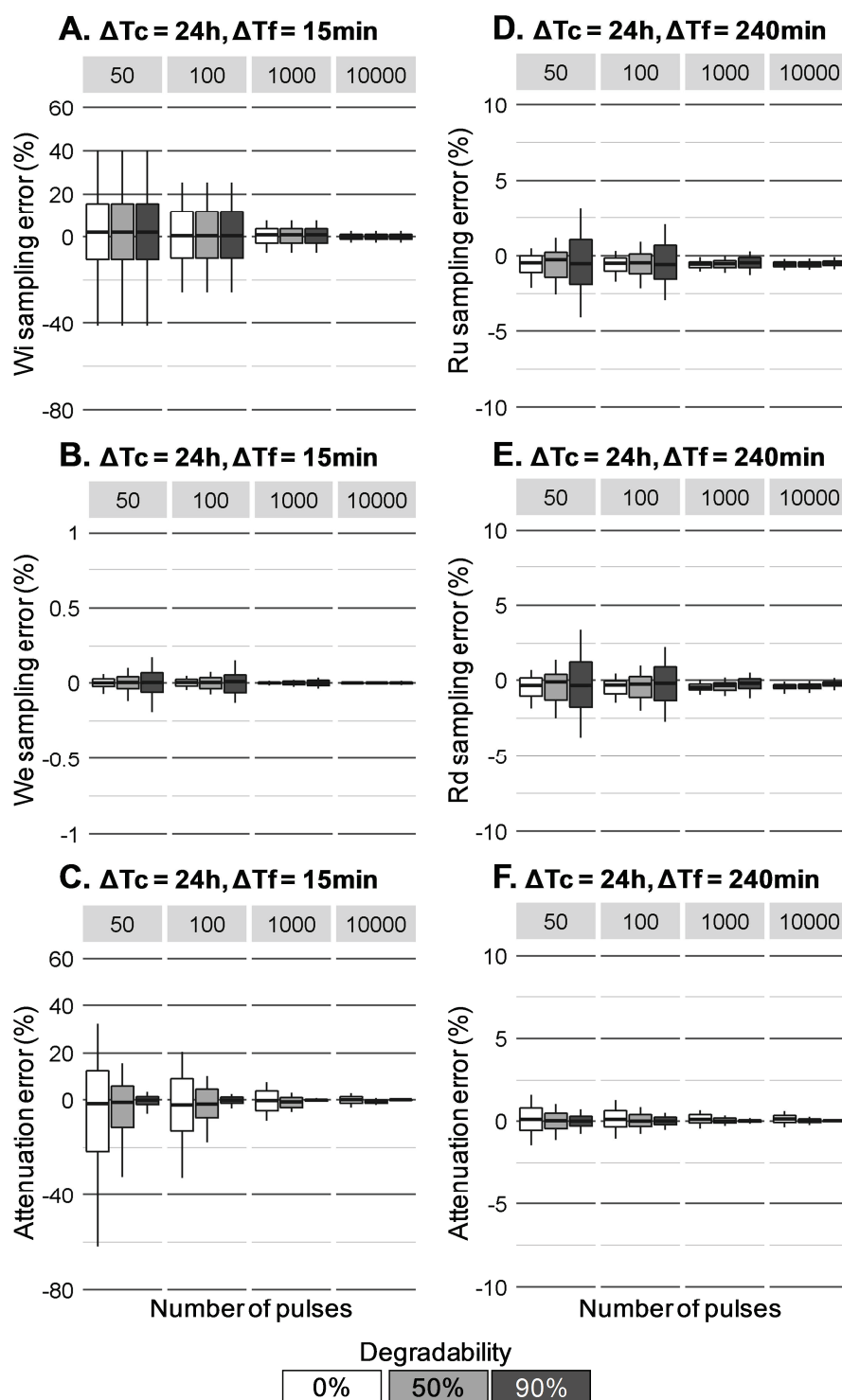
Figure 4. Load and attenuation errors for compounds at degradability rates of 0%, 50% and 90%. At the WWTP for a composite duration of 24-h and a sampling frequency of 15-min (A-C) and at the river for a composite duration of 24-h and a sampling frequency of 240-min (D-F).
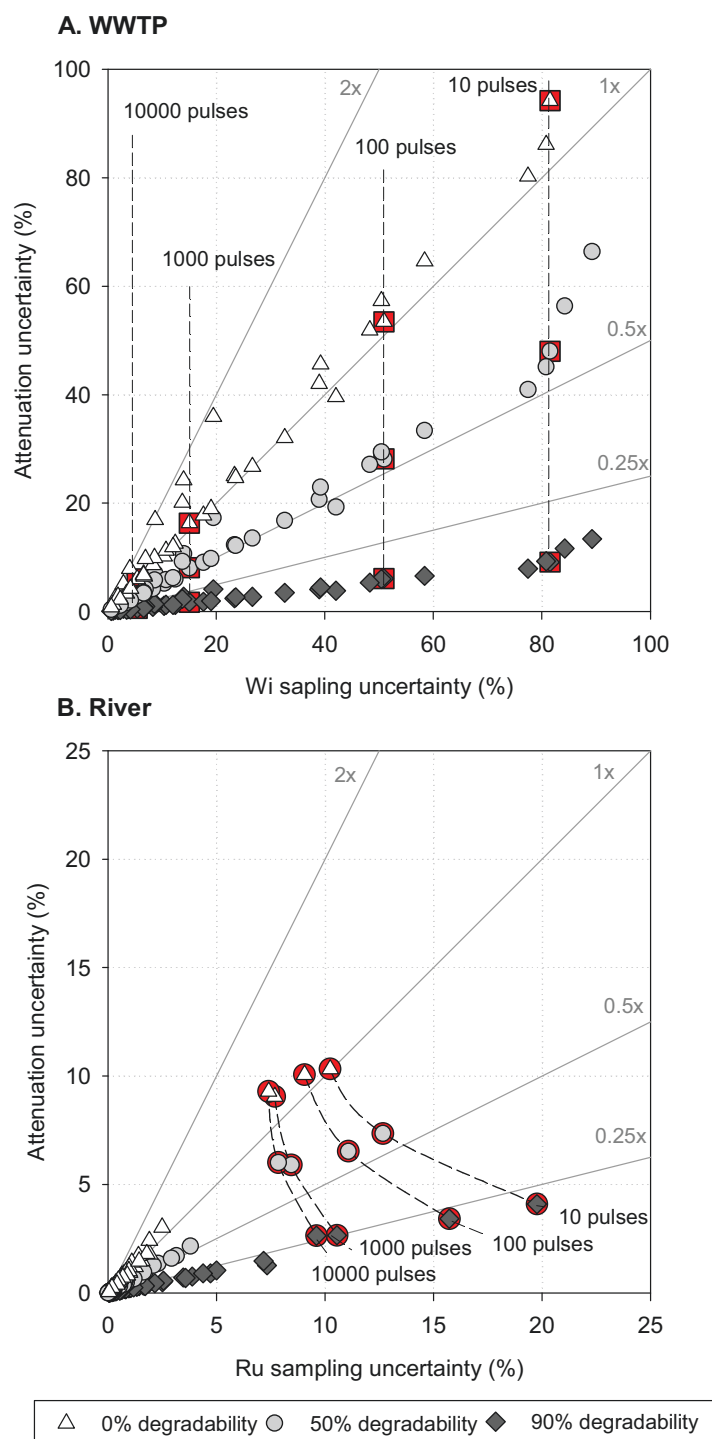
Figure 5. Relationships between *Wi* sampling and attenuation errors in the WWTP (A) and *Ru* sampling and attenuation errors in the river (B) at different degradability levels. All number of pulses, composite frequencies and durations evaluated in the study are plotted. Red symbols show the sampling strategy presented in Figure 4. Uncertainty is expressed as the 90-interquartile range of the error distributions obtained. Note that only load and attenuation errors lower than 100% are presented.

Highlights

- Sampling matters when investigating microcontaminants in WWTPs and rivers

- Uncertainty in the loads and attenuation estimations is not the same

- Short sampling intervals and longer sampling durations are needed

- The WWTP influent is especially critical when designing sampling strategies

- Degradability and desired attenuation accuracy should be defined upfront