

# Fluorescence spectroscopy for wastewater monitoring

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DOI:

[10.1016/j.watres.2016.03.021](https://doi.org/10.1016/j.watres.2016.03.021)

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*Document Version*

Peer reviewed version

*Citation for published version (Harvard):*

Carstea, E, Bridgeman, J, Baker, A & Reynolds, DM 2016, 'Fluorescence spectroscopy for wastewater monitoring: a review', *Water Research*, vol. 95, pp. 205-219. <https://doi.org/10.1016/j.watres.2016.03.021>

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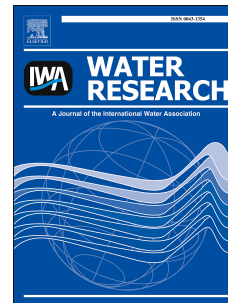
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# Accepted Manuscript

Fluorescence spectroscopy for wastewater monitoring: A review

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PII: S0043-1354(16)30148-8

DOI: [10.1016/j.watres.2016.03.021](https://doi.org/10.1016/j.watres.2016.03.021)

Reference: WR 11905

To appear in: *Water Research*

Received Date: 16 November 2015

Revised Date: 7 March 2016

Accepted Date: 8 March 2016

Please cite this article as: Carstea, E.M., Bridgeman, J., Baker, A., Reynolds, D.M., Fluorescence spectroscopy for wastewater monitoring: A review, *Water Research* (2016), doi: 10.1016/j.watres.2016.03.021.

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1                   **Fluorescence spectroscopy for wastewater**  
2                   **monitoring: a review**

3

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15

16   Abstract: Wastewater quality is usually assessed using  
17   physical, chemical and microbiological tests, which are not  
18   suitable for online monitoring, provide unreliable results, or use  
19   hazardous chemicals. Hence, there is an urgent need to find a  
20   rapid and effective method for the evaluation of water quality  
21   in natural and engineered systems and for providing an early  
22   warning of pollution events. Fluorescence spectroscopy has  
23   been shown to be a valuable technique to characterize and  
24   monitor wastewater in surface waters for tracking sources of  
25   pollution, and in treatment works for process control and  
26   optimization. This paper reviews the current progress in

27 applying fluorescence to assess wastewater quality. Studies  
28 have shown that, in general, wastewater presents higher  
29 fluorescence intensity compared to natural waters for the  
30 components associated with peak T (living and dead cellular  
31 material and their exudates) and peak C (microbially  
32 reprocessed organic matter). Furthermore, peak T fluorescence  
33 is significantly reduced after the biological treatment process  
34 and peak C is almost completely removed after the chlorination  
35 and reverse osmosis stages. Thus, simple fluorometers with  
36 appropriate wavelength selectivity, particularly for peaks T and  
37 C could be used for online monitoring in wastewater treatment  
38 works. This review also shows that care should be taken in any  
39 attempt to identify wastewater pollution sources due to  
40 potential overlapping fluorophores. Correlations between  
41 fluorescence intensity and water quality parameters such as  
42 biochemical oxygen demand (BOD) and total organic carbon  
43 (TOC) have been developed and dilution of samples, typically  
44 up to  $\times 10$ , has been shown to be useful to limit inner filter  
45 effect. It has been concluded that the following research gaps  
46 need to be filled: lack of studies on the on-line application of  
47 fluorescence spectroscopy in wastewater treatment works and  
48 lack of data processing tools suitable for rapid correction and  
49 extraction of data contained in fluorescence excitation-emission  
50 matrices (EEMs) for real-time studies.

51

52 Key words: fluorescence spectroscopy, wastewater, organic  
 53 matter, monitoring

54

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82

## 83 **1 Introduction**

84 Environmental monitoring is applied to determine the  
85 compliance with ambient and discharge standards and to  
86 identify areas with persistent issues for timely and effective  
87 remediation (Cahoon and Mallin 2013). Wastewater quality  
88 assessment is an essential part of environmental monitoring due  
89 to the high anthropogenic impact of treated and untreated  
90 discharges on water bodies (Suthar et al. 2010). There are two  
91 important aspects of wastewater quality monitoring: the first  
92 concerns the detection of pollution events for early warning and  
93 rapid remedial responses of water bodies, while the second  
94 aspect relates to wastewater treatment works where quality  
95 monitoring is required for process control and compliance with  
96 regulations at the effluent discharge point (Bourgeois et al.  
97 2001, Michael et al. 2015, Rehman et al. 2015).

98 The quality of wastewater is generally assessed using  
99 physical, chemical and microbiological tests. Among these  
100 techniques, reliance is often placed on biological oxygen  
101 demand (BOD), chemical oxygen demand (COD) and total  
102 organic carbon (TOC) (Bourgeois et al. 2001, Bridgeman et al.  
103 2013). However, these global parameters depend on expensive

104 or time-consuming methods, offering only snapshots of  
105 moments in time (Bourgeois et al. 2001, Chong et al. 2013,  
106 Yang et al. 2015a), which makes them unsuitable for online  
107 monitoring. Research conducted almost two decades ago  
108 (Ahmad and Reynolds 1995, Tartakovsky et al. 1996, Reynolds  
109 and Ahmad 1997, Ahmad and Reynolds 1999) has shown that  
110 fluorescence spectroscopy could be used for wastewater quality  
111 assessment as a tool for discharge detection in natural water  
112 systems and for process control in wastewater treatment plants  
113 (WwTPs). Fluorescence is the release of energy in the form of  
114 light when molecules or moieties, named fluorophores, are  
115 excited with a high-energy light source (Lakowicz 2006,  
116 Reynolds 2014). The technique has been suggested for its  
117 multiple advantages: it is fast, inexpensive, reagentless,  
118 requires little sample preparation, is highly sensitive and non-  
119 invasive (Reynolds 2003, Hudson et al. 2007, Cao et al. 2009,  
120 Henderson et al. 2009, Hambly et al. 2010, Murphy et al. 2011,  
121 Chong et al. 2013, Yang et al. 2015a). According to Reynolds  
122 (2002) fluorescence monitoring could provide rapid feedback,  
123 allowing dynamic, high spatial and temporal resolution studies.

124 In the past decades, more studies have proved the  
125 potential of fluorescence spectroscopy as a monitoring and  
126 detection tool in natural and engineered systems. This  
127 technique has been used successfully to characterize organic  
128 matter in seawater (Coble et al. 1990, Coble 1996, Conmy et al.

129 2004, Drozdowska 2007), freshwater (Baker 2001, McKnight  
130 et al. 2001, Spencer et al. 2007b, Carstea et al. 2009) or  
131 estuarine water (Huguet et al. 2009). Also, it has been used to  
132 monitor riverine organic matter and diesel pollution (Downing  
133 et al. 2009, Carstea et al. 2010), evaluate drinking water  
134 treatment processes (Bieroza et al. 2009, Cumberland et al.  
135 2012, Shutova et al. 2014) or detect pesticides (Ferretto et al.  
136 2014). Fluorescence spectroscopy has been used to assess the  
137 quality of raw sewage and effluents (Baker 2001, Boving et al.  
138 2004, Pfeiffer et al. 2008), industrial (Santos et al. 2001,  
139 Borisover et al. 2011, Li et al. 2015), or farm (Baker 2002b,  
140 Old et al. 2012) discharges into natural systems. Moreover,  
141 recent studies on short and long-term fluorescence monitoring  
142 along the WwTPs process train have been undertaken, to  
143 determine the potential of the technique for treatment processes  
144 control (for example, (Murphy et al. 2011, Bridgeman et al.  
145 2013, Cohen et al. 2014, Ou et al. 2014, Singh et al. 2015).  
146 Although considerable work has been done so far in this field,  
147 there are still issues with regard to the “matrix effects”, as  
148 reviewed by Henderson et al. (2009), or with fouling (Reynolds  
149 2002) that must be overcome to allow application of the  
150 technique in WwTPs.

151 Other reviews proved the potential of applying  
152 fluorescence spectroscopy to water quality monitoring (Hudson  
153 et al. 2007, Henderson et al. 2009, Fellman et al. 2010, Ishii



154 and Boyer 2012, Yang et al. 2015b). However, none of them  
155 focused only on wastewater, which requires a specific  
156 discussion due to its complexity in composition and impact on  
157 the environment. Moreover, a growing number of studies are  
158 published each year on the application of fluorescence  
159 spectroscopy to wastewater quality evaluation, proving its  
160 scientific and industrial importance. In this paper, we review  
161 the current progress in applying fluorescence spectroscopy to  
162 assess wastewater quality. The technique's capabilities as a  
163 detection and early warning tool of pollution with treated or  
164 raw wastewater from different sources are discussed. Also, its  
165 potential for process control in WwTPs is presented.

166

## 167 **2 Fluorescence assessment of wastewater components**

### 168 **2.1 Organic matter fluorescence assessment**

169 The most common methods of recording fluorescence  
170 spectra for wastewater are excitation – emission matrices  
171 (EEM) and synchronous fluorescence spectra (SFS). EEMs  
172 represent fluorescence contour maps, which comprise a series  
173 of repeated emission scans recorded in a range of excitation  
174 wavelengths (Coble 1996). SFS are obtained by scanning  
175 simultaneously both excitation and emission monochromators  
176 at a fixed wavelength interval between them (Patra and Mishra  
177 2002, Reynolds 2003). For many years, since the mid-1970s,  
178 SFS were preferred as a multidimensional technique for the

179 analysis of complex solutions, because it provided better peak  
180 resolution, compared to emission spectra, and faster recording  
181 time than EEMs (Ryder 2005). However, the improvement of  
182 instrumentation allowed researchers to obtain fast, high-  
183 resolution EEM collection, which increased the method  
184 popularity in the research community. In addition, EEMs offer  
185 varied possibilities of data interpretation, from simple peak-  
186 picking and Fluorescence Regional Integration to the more  
187 complex Parallel Factor Analysis (PARAFAC) and Self-  
188 Organizing Maps. Among these methods, peak-picking and  
189 PARAFAC are the most popular in the research community  
190 and therefore only these two methods will be discussed in the  
191 following sections.

192         The peak-picking method is a very simple tool to identify  
193 components based on their maximum intensity and  
194 corresponding excitation and emission wavelength pairs (Coble  
195 1996). An example of peak-picking analysis is shown in Figure  
196 1 (a). According to Goldman et al. (2012), peak-picking is a  
197 viable analysis technique and can be employed for the  
198 development and use of a real-time tool and may be related to  
199 custom sensors available today. However, its applicability may  
200 be limited due to peak shifts, possible overlapping and  
201 interferences between peaks (Yang et al. 2015b). Moreover, it  
202 may lead to misleading observations by associating each peak

203 with a specific fluorophore, when two excitation wavelengths  
204 are seen at fluorescent components (Fig. 1).

205 PARAFAC is a mathematical tri-linear model that  
206 deconvolutes EEMs into chemically meaningful components  
207 (Fig. 1b). It separates the contribution of different fluorophores  
208 without additional assumptions about their excitation and  
209 emission spectra (Cohen et al. 2014). A thorough description of  
210 PARAFAC method and components in wastewater is given by  
211 Yang et al. (2015b). PARAFAC has become common practice  
212 in water quality studies, over the past 10 years (Murphy et al.  
213 2014). Yang et al. (2015b) proposed that PARAFAC be  
214 developed into a surrogate method for conventional water  
215 quality parameters, treatability of organic matter (OM) and  
216 performance of treatment processes. Yu et al. (2014) suggested  
217 that the PARAFAC tool, the EEMizer, developed by Bro and  
218 Vidal (2011), could be implemented to monitor on-line the  
219 WwTPs performance. The studies of Yu et al. (2015a) implied  
220 that PARAFAC is able to identify contamination events and  
221 can be used for early warning, but the component that indicates  
222 contamination must be spectrally different from the existing  
223 components, without major spectral overlap, which may  
224 undermine the online monitoring strategy. Similarly, Murphy et  
225 al. (2011) showed that at times PARAFAC had difficulties  
226 distinguishing between components, returning hybridized  
227 spectra. Also, in a comparison between chromatographic

228 fluorescence fingerprints and EEM-PARAFAC, Li et al. (2014)  
229 showed that the latter method could not reflect the variety of  
230 organic matter species with similar fluorescence, but different  
231 physico-chemical properties. In addition, PARAFAC is  
232 currently applied only as post-processing technique, making it  
233 unsuitable for continuous monitoring. Also, there is no  
234 consensus regarding the optimum model in terms of sample  
235 size and variability (Yu et al. 2015a).

236 All these techniques have been employed successfully to  
237 analyse OM from various natural to engineered sources. A  
238 thorough review on OM fluorescence is provided by Hudson et  
239 al. (2007) and Fellman et al. (2010). Crude sewage is a  
240 combination of domestic waste, industrial discharges, surface  
241 runoff and storm flow. Its composition varies depending on the  
242 age and type of sewerage, time of day, weather conditions and  
243 type of incoming sewer (Ahmad and Reynolds 1995, Hudson et  
244 al. 2007). Ellis (2004) showed that the general organic  
245 composition of wastewater is 50 % proteins, 14 %  
246 carbohydrates, 10 % fats and oils and trace amounts of priority  
247 pollutants and surfactants, which are present in detergents,  
248 soaps, shampoo and similar consumer products. More recently,  
249 Huang et al. (2010) found that fibres, proteins and sugars are  
250 the largest groups of OM in wastewaters, accounting for 20.64  
251 %, 12.38 % and 10.65 %, respectively, of the total TOC.  
252 According to the researchers, food related substances are the

253 main source of OM in wastewaters (Huang et al. 2010). Using  
254 gas chromatography/mass spectrometry, Huang et al. (2010)  
255 detected 90 compounds from the groups of alkyls and aromatic  
256 hydrocarbons, alkenes, alcohols, organic acids, ketones,  
257 phenols, nitrogenous compounds, ethers, amines and esters. In  
258 addition, they found lipids, volatile fatty acids, humic acids,  
259 DNA + RNA, tannic acids and linear alkylbenzene sulfonates.  
260 Within the organic composition, there are numerous  
261 overlapping fluorophores that contribute to the EEMs (Aiken  
262 2014). Due to the difficulty of assigning specific fluorophores  
263 to the peaks identified in EEMs, the fluorescence of wastewater  
264 will be discussed as two regions based on the classification  
265 provided by Li et al. (2014): the region  $E_m < 380$  nm is  
266 associated with fluorophores containing a limited number of  
267 aromatic rings and the indole moiety of free tryptophan whilst  
268 the region  $> 380$  nm is associated with polycyclic aromatic  
269 fluorophores.

## 270 **2.2 Region $E_m < 380$ nm**

271 Based on the peak-picking method, fluorescence in this  
272 region is represented by peak T ( $\lambda_{excitation} / \lambda_{emission} \sim 225$  ( $\sim 280$ ) /  
273  $\sim 350$  nm) and peak B ( $\lambda_{excitation} / \lambda_{emission} \sim 225$  ( $\sim 280$ ) /  $\sim 305$   
274 nm) (Fig. 1a). Peaks T and B have been observed in all studies  
275 that used the peak-picking method for EEM processing,  
276 irrespective of the wastewater source (Table SM1). These  
277 peaks have been associated with living and dead cellular

278 material and their exudates and indicate microbial activity  
279 (Bridgeman et al. 2013) and material derived from  
280 anthropogenic activities (Yu et al. 2014). In PARAFAC, the  
281 region  $Em < 380$  nm is generally identified as components with  
282 2 excitation wavelengths and 1 emission wavelength (Fig. 1b)  
283 in the same wavelength ranges as peaks T and B in the peak-  
284 picking method. These components are identified in both  
285 municipal and industrial wastewater samples; however, the  
286 component similar to peak T is more common in wastewater  
287 compared to other components in this region (Table SM2).

288 By examining the list of wastewater organic components  
289 (Dignac et al. 2000, Huang et al. 2010, Navalon et al. 2011),  
290 and the literature review of Aiken (2014), Stedmon and Cory  
291 (2014) and Baker et al. (2014), the following components were  
292 considered as contributors to the fluorescence in the region  $Em$   
293  $< 380$  nm: phenols (for example cresols), indoles, mono and  
294 polyaromatic hydrocarbons, DNA, aromatic amino acids  
295 (phenylalanine, tyrosine), degradation products of lignin (lignin  
296 phenols, vanillic acid, syringic acid etc.). These compounds are  
297 derived from domestic waste, chemical, pharmaceutical,  
298 plastic, petrochemical, paper, leather or textile industries (del  
299 Olmo et al. 1996, Pokhrel and Viraraghavan 2004, He et al.  
300 2007, Tchaikovskaya et al. 2007, Tertuliani et al. 2008). The  
301 potential contributing fluorophores to this region are presented  
302 in Table 1.

303

304 **2.3 Region Em > 380 nm**

305 The peak-picking method classifies this region as  
306 follows: Peak A ( $\lambda_{excitation} / \lambda_{emission} \sim 225 / 400 - 500 \text{ nm}$ ), peak C  
307 ( $\lambda_{excitation} / \lambda_{emission} 300 - 350 / 400 - 500 \text{ nm}$ ) and peak M  
308 ( $\lambda_{excitation} / \lambda_{emission} 310 - 320 / 380 - 420 \text{ nm}$ ) (Fig. 1a). All  
309 studies done so far on wastewater OM have identified peak C  
310 and most studies found peak A (Table 1); however, peak M  
311 was analysed only by Yu et al. (2014) at municipal wastewater.  
312 Most of the studies that employed PARAFAC for EEM  
313 analysis identified a maximum of 4 components associated and  
314 microbially and terrestrially derived DOM (example of two  
315 components in Fig 1b). However, Ishii and Boyer (2012) have  
316 identified the PARAFAC components common in natural and  
317 engineered water systems: Component 1 similar to peak A with  
318 excitation in the region  $< 230 - 260 \text{ nm}$  and emission between  
319  $400$  and  $500 \text{ nm}$ ; Component 2 similar to peaks A + C found in  
320 excitation region  $< 240 - 275$  ( $339 - 420 \text{ nm}$ ) and emission  
321 within  $434 - 520 \text{ nm}$ ; and Component 3 similar to peak A + M  
322 appearing in the excitation domain  $< 240 - 260 \text{ nm}$  ( $295 - 380$   
323  $\text{nm}$ ) and within the  $374 - 450 \text{ nm}$  emission range. According to  
324 Ishii and Boyer (2012), component 1 is found mostly in OM  
325 sources dominated by terrestrial precursor material. Component  
326 2 was defined as reduced quinone-like and was identified in  
327 OM from a wide variety of aquatic systems, including those

328 dominated by terrestrial and microbial inputs. While,  
329 component 3 fluorophores were defined as oxidised quinone-  
330 like and were similar to those with terrestrial and marine  
331 precursors. Component 1 has not been reported in wastewater  
332 studies, but components 2 and 3 were seen at studies made on  
333 municipal and industrial wastewater (Table SM2). Additional  
334 components were observed in wastewater (Table SM2), but  
335 they vary depending on source.

336 As shown in Table 1, there are several fluorophores that  
337 could contribute to the fluorescence of region  $E_m > 380$  nm:  
338 lignins, PAHs, flavonoids, humic acids, quinones, aromatic  
339 ketones, fluorescent whitening agents (FWAs),  
340 pharmaceutically active compounds (Dignac et al. 2000, Huang  
341 et al. 2010, Aiken 2014, Baker et al. 2014, Stedmon and Cory  
342 2014). Among these components, FWAs have been proposed  
343 as an indicator of human faecal contamination (Assaad et al.  
344 2014), sewer misconnections (Chandler and Lerner 2015) and  
345 presence of landfill leachates (Graham et al. 2015). FWAs are  
346 highly soluble and poorly biodegraded, and therefore likely to  
347 pass through biological treatment in WwTPs (Kramer et al.  
348 1996, Poiger et al. 1998, Assaad et al. 2014). Research has  
349 shown that these components can be detected with handheld  
350 fluorometers, which enhances the capability for in situ water  
351 monitoring (Hartel et al. 2007). Nevertheless, issues with  
352 detecting FWAs in waters have been reported: the fluorescence



353 of other peak C fluorophores overlap the peaks of FWAs, these  
354 components are easily photodegraded and DOM hinders the  
355 reaction of FWAs (Kramer et al. 1996, Baker 2002a, Hartel et  
356 al. 2007, Assaad et al. 2014). Solutions to overcome  
357 fluorescence overlap have been proposed, yet the other issues  
358 identified may limit the method's applicability in detecting  
359 sewage. The following solutions have been proposed: a) to use  
360 the photodegradation rate to separate FWAs from organic  
361 matter (Hartel et al. (2007)); b) to take into account the  
362 differences in shape of the photodecay curve between FWAs  
363 and natural organic matter (Cao et al. (2009)); c) to use a  
364 baseline correction method to compare the differences in  
365 fluorescence intensity of FWA, between the regions  $320\text{ nm} -$   
366  $345\text{ nm}$  and  $345\text{ nm} - 360\text{ nm}$ , with the same values for the  
367 water samples (Takahashi and Kawamura (2006)); and d) to  
368 apply three-way analysis of EEMs assisted by second-order  
369 chemometric analyses (Gholami et al. 2015). Discrimination  
370 between humic substances and FWAs was achieved by Boving  
371 et al. (2004), who analysed FWAs in solution with humic acid  
372 and tannic acid. FWAs were recorded at  $344\text{ nm}$  and  $422\text{ nm}$   
373 emission wavelength, and  $250\text{ nm}$  excitation wavelength. The  
374 authors found that the second peak of the FWAs was separated  
375 from humic acids by 22 nm, but there was a 4 nm separation  
376 from tannic acid. Therefore, the  $\lambda_{excitation} / \lambda_{emission} = 250 / 422$

377 *nm* peak could be used for FWAs detection without  
378 interference from humic acid.

379 As shown above, there are several fluorophores that  
380 contribute to the < 380 nm > Em regions, but the list is not  
381 exhaustive. More studies are needed to identify new fluorescent  
382 components and especially those specific to source with the  
383 highest contribution to EEMs. Since the regions exhibit the  
384 fluorescence of xenobiotic compounds, both can be used for  
385 wastewater quality assessment. In particular, peaks T and C,  
386 and the PARAFAC analogous components, are present in all  
387 wastewater studies (Tables SM1 and SM2) and may be applied  
388 to the control of wastewater treatment processes. However, it  
389 may be difficult to identify the source and type of sewage  
390 pollution in receiving water bodies. In this sense, Baker et al.  
391 (2014) advise caution and stress the importance of using a good  
392 sampling framework combined with an appropriate  
393 multivariate analysis of data for successful investigation of  
394 water pollution.

395

### 396 **3 Correlation of the fluorescence peaks with BOD, COD** 397 **and TOC**

398 In order to assess the capability of fluorescence  
399 spectroscopy to act as a monitoring tool it is important to  
400 consider the correlations between fluorescence peaks and BOD,  
401 COD and TOC, commonly used indicators of OM

402 concentration in natural waters and wastewater. As reviewed by  
403 Bourgeois et al. (2001) and (Jouanneau et al. 2014), BOD is a  
404 desirable measurement in treatment processes, it presents  
405 several disadvantages, which make this technique unsuitable  
406 for on-line monitoring and process control: it is slow to yield  
407 information, it is labour intensive, toxic substances affect  
408 bacteria, it may not reflect conditions in the treatment  
409 processes, it is insensitive and imprecise at low concentrations  
410 and has an uncertainty of 15-20% in the results. COD takes less  
411 time to give a result than BOD (2-4 h) and is not affected by  
412 toxic substances. However, it is still not suitable for on-line  
413 monitoring and process control due to the measuring time and  
414 because it requires hazardous chemicals. Also, COD is able to  
415 discriminate between biodegradable and biologically inert  
416 organic matter only in conjunction with BOD and not on its  
417 own (Bourgeois et al. 2001, Chen et al. 2014). TOC is very  
418 fast, as triplicates can be analyzed in minutes. However, it  
419 cannot differentiate between biodegradable and  
420 nonbiodegradable OM (Orhon et al. 2009). Also, conflicting  
421 results have been reported between different techniques of  
422 measuring TOC (Bourgeois et al. 2001).

423 Correlation between fluorescence and standard  
424 parameters revealed that peaks T and C relate to BOD, COD  
425 and TOC, as reviewed by (Henderson et al. 2009). Slightly  
426 better correlation with BOD is seen at peak T compared to peak

427 C. An exception to the above observation is found at the study  
428 of Wang et al. (2007) who obtained better correlation with the  
429 PARAFAC component exhibiting fluorescence in the peak C  
430 region, compared to the peak T component (Table 2). They  
431 observed the best correlation with BOD at the component  
432 similar to peak M (0.73). The researchers concluded that this  
433 component contributed the most to BOD for wastewater-  
434 impacted lakes. Nevertheless, these results highlight the  
435 complexity of the source and that there are potentially several  
436 fluorophores, which display fluorescence in the peak T/C  
437 regions. It also shows that both regions could contribute to  
438 BOD. The difference in correlation coefficients could also be  
439 determined by the low sample sizes in some studies, which  
440 might under or overestimate the relationship between  
441 fluorescence and BOD, COD and TOC (Table 2). Another  
442 cause of the difference could be the method used for data  
443 processing, as PARAFAC offers better separation of  
444 overlapping components compared to peak-picking.

445 Based on the correlation between BOD and peak T  
446 fluorescence, Hur and Kong (2008) tried to estimate, using SFS  
447 and first derivative spectra, the concentration of BOD of  
448 samples from urban rivers affected by treated sewage. They  
449 found that the relative fluorescence intensity, at 283 *nm* to 245  
450 *nm* from SFS, is the optimum estimation index as it has the best  
451 positive correlation with BOD values (0.91). It has been

452 reported that the multiple regression method, using the light  
453 scattering intensity at 633 nm or turbidity, greatly enhances the  
454 correlation between measured and predicted BOD values. Hur  
455 and Kong (2008) also observed that filtered samples presented  
456 enhanced correlation; however, Bridgeman et al. (2013)  
457 reported slightly higher correlation coefficient between BOD  
458 and fluorescence at unfiltered samples compared to filtered  
459 with 0.45 or 0.2 µm. These differences could be site specific  
460 and may depend on the sizes of OM components.

461 As reviewed by Baker et al. (2014), the correlation  
462 between BOD and peak T fluorescence suggests a direct link  
463 with microbiological activity in this region of fluorescence,  
464 although the source of peak T fluorescence is generally  
465 unknown. It was also implied that handheld instruments could  
466 be used in the future to investigate the temporal variability of  
467 BOD (Baker et al. 2014). Due to the relation with  
468 microbiological activity, peak T fluorescence was suggested as  
469 indicator of the presence / absence faecal coliforms (Sorensen  
470 et al. 2015, Sorensen et al. 2016). Pfeiffer et al. (2008) obtained  
471 excellent correlation (0.90 – 0.95) with faecal coliforms on  
472 samples from a wastewater polluted river and (Tedetti et al.  
473 2012) found a good correlation (0.78) between the PARAFAC  
474 component and Escherichia Coli + enterococci on wastewater  
475 impacted coastal water samples. More recently, (Baker et al.  
476 2015) obtained a log correlation of 0.74 between fluorescence

477 and E. Coli measurements. These findings are encouraging, but  
478 more work should be done to explore the link between  
479 fluorescent components and faecal coliforms and its potential  
480 use in on-line monitoring applications. In a comparison with  
481 flow cytometer measurements, peak T intensity correlated with  
482 an increase of total live and dead bacteria numbers (Bridgeman  
483 et al. 2015). The researchers found that four bacteria isolated  
484 from a potable water tap sample showed different responses in  
485 the fluorescence signal, although the intensity of peak T  
486 fluorescence did not correlate with the bacteria counts.  
487 Nevertheless, peak T fluorescence could be used to assess the  
488 microbiological activity in a water system.

489

#### 490 **4 Fluorescence detection of wastewater pollution**

491 Fluorescence spectroscopy has shown its capabilities as a  
492 real-time assessment tool for wastewater quality due to its  
493 advantages and correlation with standard parameters. This  
494 technique could be very effective in detecting raw wastewater  
495 contamination in water bodies. Also, the impact of wastewater  
496 effluents on natural waters could be evaluated, since effluent  
497 organic matter has different composition and characteristics  
498 from naturally occurring OM (Wang et al. 2015). Therefore it  
499 is important to look at the different types of wastewater for  
500 particular characteristics that may facilitate identification in the  
501 receiving water bodies.

502

503 **4.1 Sources of wastewater**

504 Studies published so far on fluorescence spectroscopy  
505 have focused on domestic, farm and industrial wastewater,  
506 which includes textile, pulp mill, coke or brewery industries.  
507 More studies are needed on wastewater from oil refineries,  
508 metal processing, fermentation factories, pharmaceutical  
509 industry, chemical plants, meatpacking and processing etc.

510

511 **4.1.1 Domestic wastewater**

512 Wastewater is the flow of water used by a community  
513 and includes household wastes, commercial and industrial  
514 waste stream flows, and stormwater (Drinan and Spellman  
515 2012). Domestic wastewater contains the solid and liquid  
516 discharges of humans and animals, contributing with millions  
517 of bacteria, virus, and non-pathogenic and pathogenic  
518 organisms. It may also contain sanitary products, cleaners and  
519 detergents, trash, garbage and any other substances that are  
520 poured or flushed into the sewer system (Drinan and Spellman  
521 2012). Public treatment facilities may also collect industrial  
522 effluents and thus chemicals, dyes, acids, alkalies, grit or  
523 detergents can be found in municipal wastewater (Drinan and  
524 Spellman 2012). Stormwater runoff, if collected by WwTPs,  
525 may bring into the system large amounts of sand, gravel, road-  
526 salt and other grit (Drinan and Spellman 2012).

527 As discussed in the previous sections, there are numerous  
528 compounds that may contribute to the fluorescence peaks.  
529 Generally, fluorescence spectra of untreated and treated  
530 domestic wastewater are characterized by intense peaks in the  
531 region  $E_m < 380$  nm, especially peak T, associated with high  
532 microbial abundance, and by significantly lower intensity peaks  
533 A and C fluorescence (Baker 2001, Hudson et al. 2007, Hur  
534 and Cho 2012, Bridgeman et al. 2013). In some studies, the  
535 fluorescence spectra of effluents showed a higher prevalence of  
536 peaks A and C, compared to peaks T and B (Ghervase et al.  
537 2010a, Riopel et al. 2014). Among peaks, T and C seem to be  
538 present at most municipal wastewater samples (Tables SM1  
539 and SM2) and may serve as indicators of wastewater  
540 contamination. Peak B is rarely analysed at wastewater EEMs  
541 due to the potential interferences from scattering; however, this  
542 fraction could indicate the proximity of the measurement point  
543 to the discharge point or freshness of the contamination.  
544 According to Pfeiffer et al. (2008), the fluorescence of both  
545 peak T and peak B decreases in intensity with increasing  
546 distance from the release point, but peak B is completely  
547 removed at longer distances, due to dilution or breakdown of  
548 the organic fraction. For peak B removal, seasonal shifts should  
549 also be taken into account as rainfall could contribute to  
550 dilution, sunlight irradiation could cause photodegradation or  
551 increase microbial uptake during summer (Meng et al. 2013).



552 From the myriad of fluorophores, FWAs may display  
553 distinctive features in the EEMs for municipal wastewater  
554 samples (Bridgeman et al. 2013). However, this fraction is not  
555 specific to domestic wastewater, as it has been detected at  
556 paper mill effluents (Baker 2002a, Ciputra et al. 2010,  
557 Bassandeh et al. 2013) or landfill leachates (Graham et al.  
558 2015). Therefore, peaks T and C seem to be the best tools of  
559 monitoring domestic wastewater quality.

560 In addition to fluorescence intensity increase, it has been  
561 shown that discharge of domestic sewage may change the  
562 properties of OM from the receiving water bodies. For  
563 example, Xue et al. (2011) found that sewage effluents change  
564 the capacity of OM to form disinfection by-products and  
565 decrease its sensitivity to UV light. Also, changes in  
566 aromaticity and hydrophobicity of OM have been reported.  
567 These OM characteristics have been assessed after discharge,  
568 using the emission wavelength of peak C. In two studies  
569 undertaken by Goldman et al. (2012) on OM wastewater  
570 effluent and by Ghervase et al. (2010b) on untreated sewage  
571 discharge, it was found that the fluorescence signal of the two  
572 types of samples presented lower peak C emission wavelength,  
573 indicating lower aromaticity compared to natural OM. While,  
574 Spencer et al. (2007a) reported higher aromaticity of the OM  
575 from an estuarine sample with anthropogenic impact from  
576 domestic wastewater effluents, compared to the estuarine OM.

577 Goldman et al. (2012) found that the mixture of effluent and  
578 river waters produce midrange values and, therefore, a potential  
579 increase in aromaticity with distance from discharge could be  
580 expected. In marine environments, fluorescence measurements  
581 on wastewater discharges showed great complexity of the  
582 mixing properties. Petrenko et al. (1997) observed 4 layers in  
583 the seawater column, 2 layers being affected by sewage  
584 representing the “old” and “new” plume waters and 2 layers  
585 unaffected by effluent. According to the researchers, the release  
586 of wastewater increased 2 fold to the concentration of  
587 ammonium, silicate and phosphate in sewage affected plumes  
588 and could stimulate the growth of phytoplankton. Baker and  
589 Inverarity (2004) also found an increase in nitrate and  
590 phosphate concentrations downstream of discharge into urban  
591 rivers.

592

#### 593 ***4.1.2 Animal wastewater***

594 Animal wastes represent an important source of water  
595 pollution, through the release of untreated wastewater or  
596 surface runoff from farms. This type of wastewater produces  
597 BOD values that are 1 to 3 times higher than sewage BOD  
598 (Baker 2002b). Most meat processing units treat the wastewater  
599 prior to release, however animal wastewater varies temporally  
600 in composition, requiring continuous monitoring for effective  
601 detection and removal of pollutants. Relatively few studies

602 have looked at the potential of using fluorescence spectroscopy  
603 to monitor the quality of animal wastewater. However, data  
604 gathered so far can help define particular characteristics of  
605 animal wastewater OM. The fluorescence of animal wastewater  
606 is generally dominated by the region  $E_m < 380$  nm. In  
607 particular, peak T fluorescence seems to be common to all  
608 samples, as it has been detected at farmyard runoff (Old et al.  
609 2012), pig and cattle slurry, silage liquor, sheep barn waste  
610 (Baker 2002b), poultry processing unit (Ghervase et al. 2010b)  
611 and cattle slaughter house (Louvet et al. 2013). The researchers  
612 also observed a low peak C fluorescence relative to peak T.  
613 Baker (2002b) calculated the ratio between the fluorescence  
614 intensity of these two peaks and found that peak T intensity  
615 was 2 to 25 times higher than that of peak C, the highest ratio  
616 being obtained for silage liquor, while the lowest was seen at  
617 the sheep barn waste. A similar peak T/C ratio was obtained by  
618 Old et al. (2012) at farmyard runoff samples. The ratio of peaks  
619 T and C fluorescence intensity shows that farm waste pollution  
620 events could leave a signature in river waters (Baker 2002b)  
621 and confirm the potential of using fluorescence as a low cost  
622 and rapid technique for tracing animal derived pollutants (Old  
623 et al. 2012). Interestingly, pig and cattle slurry presented peak  
624 B fluorescence at a similar intensity to that of peak T. Peak B  
625 was also detected at poultry wastewater (Ghervase et al.  
626 2010b), having even higher fluorescence than that of peak T.

627 Ghervase et al. (2010b) suggested using the ratio of peak T and  
628 peak B to detect poultry wastewater pollution in rivers.  
629 However, this ratio applicability could be limited only to  
630 certain types of animal wastewaters.

631 Cattle slaughterhouse wastewater may contain albumin  
632 and haemoglobin that would contribute to the  $E_m < 380$  nm  
633 fluorescence region (Louvet et al. 2013). Also, bovine serum  
634 albumin may contribute to the fluorescence region of  $E_m > 380$   
635 nm. Louvet et al. (2013) found another fluorescence peak that  
636 could belong to metalloporphyrins ( $\lambda_{excitation} / \lambda_{emission} = 400 -$   
637  $440$  nm /  $450 - 510$  nm). These components are attributed to red  
638 blood, which is a major pollutant in slaughterhouse wastewater.  
639 Again, the ratio of peaks T and C fluorescence intensity was  
640 found to be an effective indicator of biodegradation of  
641 slaughter house wastewater (Louvet et al. 2013). Nevertheless,  
642 the composition of animal derived pollutants is highly variable  
643 in time and depends on the animal species, physiological state  
644 and diet (Baker 2002b, Louvet et al. 2013). Therefore, more  
645 studies are needed to better understand the properties of OM  
646 from animal derived wastewater and set clear characteristics for  
647 enhanced detection of pollution events.

648

#### 649 ***4.1.3 Industrial sources of wastewater***

650 Industrial wastewater is primarily derived from the  
651 manufacturing and processing of chemicals, textiles, wood,

652 pulp mill or paper. The composition of effluents varies  
653 depending on the raw materials used, the type of process and  
654 the efficiency of material removal (Sánchez Rojas and Bosch  
655 Ojeda 2005). Studies on continuous monitoring and evaluation  
656 of industrial wastewater using fluorescence spectroscopy are  
657 scarce, limiting identification of particular features of  
658 wastewater fluorescence spectra. Few studies focussed on  
659 wastewater from petrochemical, chemical and biochemical  
660 industry (Borisover et al. 2011), brewery (Janhom et al. 2009,  
661 Janhom et al. 2011), textile (Li et al. 2015), pulp mill and paper  
662 processing (Baker 2002a, Ciputra et al. 2010, Cawley et al.  
663 2012, Bassandeh et al. 2013) computer components  
664 manufacturing (Cohen et al. 2014) and coke industry (Ou et al.  
665 2014). In one short-term monitoring study, Yang et al. (2015a)  
666 analysed and compared the fluorescence spectra of samples  
667 from the effluents of 57 facilities belonging to 12 industrial  
668 categories (non-alcoholic drinks, electronic devices, food,  
669 leather and fur, meat, organic chemicals, pulp and paper,  
670 petrochemical, resin and plastic, steel, steam-power and textile  
671 dyeing) aiming to evaluate the potential of fluorescence  
672 spectroscopy to identify wastewater sources. The researchers  
673 were able to characterise and differentiate industrial effluents  
674 using cluster analysis, EEM-PARAFAC and FT-IR.  
675 Components from both < 380 nm > regions were observed, but  
676 no component dominated over all samples. For instance, the

677 peak T component presented the highest fluorescence intensity  
678 at leather and fur wastewater, while peak C components  
679 dominated the EEMs of food wastewater samples. Therefore,  
680 Yang et al. (2015a) concluded that, without additional analyses  
681 it may be difficult to identify an industrial source with  
682 fluorescence spectroscopy. However, Borisover et al. (2011)  
683 observed a bathochromic shift of the peak T component  
684 induced by polarity and composition of local environment.  
685 They studied samples collected from rivers impacted by  
686 industrial effluents of oil refineries, petroleum and chemical  
687 and biochemical plants. The researchers recommended using  
688 this component as fluorescent tracer of non-specific industrial  
689 pollution.

690 Studies that evaluated wastewater samples from  
691 particular industries have identified specific fluorophores. For  
692 example, at pulp mill wastewater effluents, Cawley et al.  
693 (2012) found a component that was attributed to liginosulfonic  
694 acid or to a mixture of fluorophores from the many lignin  
695 degradation products. However, the authors highlighted that  
696 this component may exhibit different emission maxima  
697 depending on variations in the actual chemical moieties present  
698 in each sample. A similar component was found by Bassandeh  
699 et al. (2013) at samples collected from the biologically treated  
700 effluent of a newsprint mill and the authors attributed it to  
701 lignins or chemicals involved in the paper making process.

702 Cawley et al. (2012) and Bassandeh et al. (2013) both  
703 identified distinctive PARAFAC peaks for the lignin derived  
704 components. However, Santos et al. (2001) observed very  
705 intense peaks and additional shoulders at the peak C for  
706 samples collected from rivers downstream of pulp mill effluent  
707 discharge. Also, compared to samples upstream, the researchers  
708 detected an additional peak at  $\lambda_{excitation} / \lambda_{emission} \sim 290 / \sim 340 \text{ nm}$ ,  
709 which coincides with the peak T fluorescence. Baker (2002a)  
710 suggested that peak T fluorescence results from the lignin and  
711 sugars produced by the pulping process, which are likely to be  
712 rich in aromatic proteins. This component correlated with TOC  
713 ( $r=0.62$ ,  $N=18$ ), indicating that peak T fluorescence was a  
714 significant contributor to the TOC at paper mill effluents, as  
715 this correlation was not seen at the river samples. In addition to  
716 lignin derived components, Baker (2002a) identified a peak  
717 associated with FWAs, which are commonly used in papers.  
718 The differences in results, found by these studies, could be  
719 attributed to variations in chemical moieties or to the fact that  
720 Cawley et al. (2012) and Bassandeh et al. (2013) used  
721 PARAFAC for data processing to provide better separation  
722 between lignin and other peak T or peak C fluorophores.

723 A distinctive feature was also detected at textile industry  
724 effluents by Li et al. (2015), who found a triple excitation  
725 component with emission wavelength at  $460 \text{ nm}$ . They  
726 considered this feature as specific to textile-derived

727 components, because most fluorophores in region  $E_m > 380$   
728 nm present dual excitation peaks at emission wavelength  
729 between 400 and 500 nm. The triple excitation peaks were  
730 associated with 1-amino-2-naphthol structure, based on a  
731 spectral comparison with the standard solution and were  
732 suggested to be used as specific indicators in textile effluents.  
733 Li et al. (2015) also found that for peak T fluorescence there  
734 were much more species with varying emission wavelengths,  
735 which could relate to azo dyes as these substances emit similar  
736 fluorescence in this region.

737 As shown in section 2.2 and Table 1, peak B fluorescence  
738 could represent phenol-like matter, hydrocarbons or cresols as  
739 found by Ou et al. (2014) at coke wastewater samples. In  
740 addition to peak B and peak C fluorophores, Ou et al. (2014)  
741 identified a component associated with heterocyclic  
742 components and polycyclic aromatic hydrocarbons (PAHs),  
743 such as fluoranthene or naphthol. PAHs were also detected by  
744 Cohen et al. (2014) at samples collected from a WwTPs that  
745 receives 50% of its crude wastewater from a computer  
746 component factory. Based on spectral similarities, Cohen et al.  
747 (2014) suggested that this component contains a pyrene-like  
748 moiety.

749 While for textile, pulp mill or coke wastewater,  
750 distinctive components have been identified, brewery  
751 wastewater has been shown to contain only the typical peaks T,



752 A and C (Janhom et al. 2009, Janhom et al. 2011), generated by  
753 the cleaning and washing of raw materials. They also showed  
754 that the fluorescence of brewery wastewater samples belonged  
755 primarily to hydrophobic acids and hydrophilic bases OM  
756 fractions.

757

#### 758 **4.2 Wastewater tracking in aquatic systems**

759 Discrimination between sources using fluorescence  
760 spectroscopy may be challenging since domestic wastewater  
761 can be mixed with industrial effluents and agricultural runoffs  
762 (Andersen et al. 2014). Industrial wastewater could also contain  
763 domestic discharges from the toilets and kitchens within  
764 factories (Reynolds and Ahmad 1995). Moreover, organic  
765 pollutants like optical brighteners, PAHs or lignins have  
766 widespread application and thus can be found in any type of  
767 wastewater.

768 In particular for industrial wastewater it may be more  
769 difficult to separate sources due to the varied composition of  
770 the solution. The release of industrial effluents in water bodies  
771 may lead to the production of fluorescent fractions formed of a  
772 mixture of proteinaceous and non-proteinaceous substances,  
773 which generates a bathchromic shift in the typical peak T  
774 fluorescence emission wavelength. According to Borisover et  
775 al. (2011) this component may be used as a tracer of non-  
776 specific industrial pollution. However, various industrial

777 wastewaters produce high quantities of particular fluorophores  
778 like PAHs or heterocyclic compounds, differentiating them  
779 from domestic wastewater. As shown by Cohen et al. (2014)  
780 the pyrene-like components separated the wastewater with 50%  
781 industrial input from the more domestic wastewater sources.  
782 Also, the devices, developed by Tedetti et al. (2013) and Puiu  
783 et al. (2015), that separate PAHs from other peak T  
784 fluorophores, hold great promise in detecting both domestic  
785 and industrial sources of pollution. Additionally, chemical  
786 separation can be undertaken by the use of time resolved laser  
787 induced fluorescence, which is capable to identify components  
788 based on their lifetimes. PAHs have a relatively long  
789 fluorescence lifetimes and great quantum efficiency, which  
790 help at distinguishing PAHs from the OM background  
791 (McGowin 2005).

792         However, the question remains as to how to differentiate  
793 between wastewater from domestic, animal farms and industry  
794 sources, which are characterized by intense  $E_m < 380$  nm  
795 region. Domestic wastewater contains PAHs (Huang et al.  
796 2010), which have a distinctive fluorescence signal; however,  
797 the quantities could be too low in comparison to other  
798 fluorophores and therefore the fluorescence of PAHs could be  
799 exceeded by other compounds.

800         Component distinction can also be undertaken by  
801 PARAFAC, which may be able to separate overlapping

802 components or identify specific pollutant indicators (Cohen et  
803 al. 2014, Yang et al. 2015b). However, in case of low  
804 concentrated pollutants, such as detergents, peak picking has  
805 been shown to be more effective than PARAFAC (Mostofa et  
806 al. 2010). Therefore, a combination of these techniques could  
807 better provide a thorough view of the sample composition and  
808 OM interaction with pollutants. Fluorescence spectroscopy  
809 could be used as an early warning system in case of accidental  
810 pollution and could serve as a quick method in initial  
811 identification of the source of wastewater, before more  
812 complex and expensive analyses would be employed.

813

## 814 **5 Control of wastewater treatment processes using** 815 **fluorescence spectroscopy**

816 Two decades ago, the studies of Reynolds and Ahmad  
817 (1995) and Tartakovsky et al. (1996) demonstrated the potential  
818 of using fluorescence spectroscopy for both off- and on-line  
819 monitoring in wastewater treatment. Recent studies have  
820 suggested that this technique could be applied to process  
821 control and optimization (Bridgeman et al. 2013). With  
822 increasingly stringent regulation it will be more difficult to  
823 control treatment efficiency with current techniques, (BOD,  
824 COD and TOC), which are expensive, time-consuming and  
825 unreliable (Bridgeman et al. 2013, Rehman et al. 2015). More  
826 pressure is put on WwTPs when other environmental

827 implications, such as energy and chemical consumption or  
828 greenhouse gases emissions are considered (Wang et al. 2015).

829 Fluorescence spectroscopy offers a robust technique available  
830 for a rapid and low cost estimation of effluent quality.

831 However, studies on fluorescence monitoring of WwTPs  
832 processes are scarce and only one long-term study at 5  
833 municipal WwTPs has been achieved (Cohen et al. 2014).

834 Also, only one real-time monitoring study has been published  
835 on two recycled water systems (Singh et al. 2015). According  
836 to Reynolds (2002), WwTPs are hostile environments, making  
837 continuous and dynamic monitoring of wastewater quality  
838 difficult due to problems associated with fouling. This would  
839 require regular cleaning, which is time consuming. In addition,  
840 the fluorescence signal could be affected by pH, IFE,  
841 temperature and metal ions, requiring subsequent corrections.

842 However, recent development of devices, already on market,  
843 show great promise since they convert the on-line peak T  
844 fluorescence signal into BOD equivalent values, using an  
845 internal calibration factor or a multispectral approach  
846 (ChelseaInstruments 2015, ModernWater 2015,  
847 ZAPSTechnologies 2015). This type of instruments could  
848 provide an immediate estimation of changes in wastewater  
849 quality, displaying capabilities of effective process control.

850

## 851 **5.1 Monitoring of fluorescent OM**

852 Fluorescence real-time monitoring of wastewater quality  
853 is difficult to implement due to multiple potential factors that  
854 may interfere with the signal. The only real-time monitoring  
855 study was undertaken by ([Galinha et al. 2011a](#)) on a pilot scale  
856 membrane bioreactor system to predict performance  
857 parameters. EEMs were recorded for 10 months and processed  
858 with multivariate techniques. They concluded that although  
859 fluorescence was able to describe total COD for influent and  
860 effluent, it could not accurately predict other performance  
861 parameters and hence, fluorescence cannot totally replace  
862 conventional monitoring of membrane bioreactors ([Galinha et  
863 al. 2011a](#)). Nevertheless, real-time monitoring studies at full-  
864 scale WwTPs should be undertaken in order to assess the  
865 feasibility of the method and the issues that can arise from its  
866 implementation. The studies done on the monitoring of surface  
867 waters identified major issues and offered solutions, which  
868 could be used to build a strategy for wastewater on-line  
869 monitoring. The issues reported so far include: biofilm  
870 formation, temperature, turbidity, inner filter effect, calibration  
871 procedure, presence of quenching elements. Most of these  
872 problems are thoroughly reviewed by [Henderson et al. \(2009\)](#).  
873 Therefore, only the recent studies will be discussed. Before the  
874 study of [Carstea et al. \(2010\)](#) no long-term, real-time  
875 monitoring experiments were done due to fouling issues.

876 Carstea et al. (2010) showed that over a period of 11 days of  
877 continuous EEM recordings on an urban river, biofilm  
878 formation on the water extraction system had no influence on  
879 the fluorescence signal. However, higher rates of biofilm  
880 formation are expected in wastewater, compared to surface  
881 water, due to the large quantities of extracellular polymeric  
882 substances that enhance cell adhesion to solid surfaces  
883 (Tsuneda et al. 2003).

884       Regarding temperature, Chen et al. (2015) tested a newly  
885 developed, portable laser induced fluorescence system, for its  
886 monitoring capabilities, on estuarine water and found that  
887 temperature changes affected the fluorescence results.  
888 Yamashita et al. (2015) and Khamis et al. (2015) also reported  
889 the impact of temperature on the fluorescence of OM, at  
890 monitoring studies on open ocean and urban river. Carstea et al.  
891 (2014) have shown that peak T fluorescence suffers more  
892 thermal quenching at samples with higher urban anthropogenic  
893 impact compared to natural sources. Therefore, temperature  
894 could have a major impact on OM fluorescence from  
895 wastewater. However, a temperature-compensating tool has  
896 been proposed and tested by Watras et al. (2011). Khamis et al.  
897 (2015) also proposed a compensating tool for turbidity, which  
898 can have a great impact on the fluorescence signal when large  
899 particles are present. It is yet to be tested on wastewater  
900 samples.

901 The inner filter effect (IFE) is another major issue at  
902 wastewater samples. The IFE is the apparent decrease in the  
903 emitted fluorescence intensity or a distortion of the band-shape  
904 resulting from the absorption of the excited and emitted  
905 radiation (Henderson et al. 2009). Kothawala et al. (2013)  
906 found that the best correction tool for the IFE is the absorbance  
907 based approach, proposed by Lakowicz (2006). This approach  
908 can be applied to samples with absorbance values of up to 1.5  
909  $\text{cm}^{-1}$ ; at samples above this value a dilution of 2x is  
910 recommended (Kothawala et al. 2013). However, the study of  
911 Kothawala et al. (2013) was undertaken on lake water samples  
912 and it is not known if these rules apply to wastewater  
913 monitoring. As seen in Tables SM1 and SM2, for the  
914 wastewater evaluation studies there are two preferred methods  
915 for reducing the IFE: dilution and post-measurement  
916 mathematical correction. A dilution factor of 10 was used in  
917 some studies, while in others the samples were diluted until a  
918 specific absorbance value was achieved. Most studies report the  
919 absorbance values at wavelengths within the excitation region  
920 of peak T. In specific studies, no dilution was used to analyse  
921 samples as this procedure is not applied to on-line  
922 measurements (for example, (Baker and Inverarity 2004,  
923 Louvet et al. 2013, Li et al. 2014). However, IFE could be a  
924 serious issue for monitoring studies, as this factor might lead to  
925 an underestimation of the degree of pollution and poor

926 prediction of BOD, COD or TOC. In this case, dilutions to a  
927 certain absorbance value ( $< 0.05 \text{ cm}^{-1}$ , as used in most studies,  
928 Tables SM1 and SM2) or post-measurement IFE correction are  
929 recommended. However, other solutions should be found to  
930 counteract IFE, as the use of UV absorbance measurements, in  
931 addition to fluorescence spectroscopy, reduces the practicality  
932 of the method for on-line monitoring.

933 In addition, Yamashita et al. (2015) proposed  
934 fluorescence sensors calibration for dark blanks and/or  
935 sensitivity. Solutions of L-tryptophan (Tedetti et al. 2013,  
936 Khamis et al. 2015, Sorensen et al. 2015) and quinine sulphate  
937 (Conmy et al. 2004, Chen et al. 2015, Yamashita et al. 2015)  
938 are generally used as calibration standards for the two  
939 fluorescence regions. However, Khamis et al. (2015) mention  
940 that uncalibrated systems may be used if qualitative data is  
941 needed.

942 Finally regarding the presence of quenching components,  
943 Wang et al. (2014) have proved that the presence of humic-like  
944 components could reduce the fluorescence of peak T in effluent  
945 organic matter. However, even more complex interactions  
946 could occur in wastewater samples. Galinha et al. (2011b)  
947 found that the addition of bovine serum albumin to domestic  
948 wastewater samples determined a decrease with 31-58 % of  
949 peak T fluorescence. They concluded that the complexity of  
950 interferences on the fluorescence signal might not allow the



951 simple and direct quantitative measurement of specific  
952 fluorophores in complex biological systems, such as  
953 wastewater. Also, in a study aiming to identify the contribution  
954 of extracellular polymeric substances to dye removal, Wei et al.  
955 (2015) showed that methylene blue has a substantial quenching  
956 effect on peaks T and C fluorescence. Several studies (Baker  
957 2001, 2002b, Spencer et al. 2007a, Xue et al. 2011) have  
958 stressed that, although peak T is dominant in fluorescence  
959 spectra of wastewater, it is very likely that sewage generates  
960 high quantities of other components, which may significantly  
961 impact peak T fluorescence. Nevertheless, a study conducted  
962 by Zhou et al. (2015) on a drinking water source contaminated  
963 with domestic wastewater, showed that all peaks were sensitive  
964 to pollutant concentration, especially peak T, which could be  
965 used as an early warning tool for contamination. Moreover,  
966 Goldman et al. (2012) were able to predict the percentage of  
967 municipal wastewater in rivers with 80 % confidence, by the  
968 use of multivariate linear regression and the fluorescence of  
969 both peak T and peak C. They recommended applying this  
970 model to develop in situ instruments, inform monitoring  
971 progress and develop additional water quality indicators. Also,  
972 Hur and Cho (2012) recommended the use of absorbance  
973 values at 220 nm and 254 nm, and PARAFAC components  
974 similar to peaks T and C, as estimation indices for BOD and  
975 COD in wastewater effluent contaminated river.

976

977 **5.2 Monitoring of treatment processes with fluorescence**  
978 **spectroscopy**

979 Typical wastewater treatment begins with a series of  
980 physical operations (pre-treatment and primary treatment), such  
981 as screening and sedimentation to remove the floating and  
982 settleable solids. These steps are followed by biological  
983 processes, which are used to convert the finely divided and  
984 dissolved OM from wastewater into flocculant settleable  
985 biological solids (Tchobanoglous et al. 1991). Biological  
986 processes include the suspended growth activated sludge  
987 process, anaerobic/anoxic/oxic, sequencing batch reactor,  
988 membrane reactor, trickling filter, etc. Activated sludge is the  
989 most common process, involving the entrainment of air for  
990 microbial degradation of OM. In the final steps of the  
991 biological treatment, the sludge flocs are separated from the  
992 treated effluent, through sedimentation, before the effluent is  
993 discharged to a water body. In some WwTPs, additional  
994 treatment processes (tertiary and quaternary), such as filtration,  
995 chlorination, UV disinfection or reverse osmosis are adopted  
996 after the biological treatment and subsequent sedimentation  
997 (Yang et al. 2015b).

998 Few studies have focused, so far, on wastewater quality  
999 monitoring in treatment works, using fluorescence  
1000 spectroscopy, to understand the behavior of OM along the

1001 process train, the removal of components and the potential of  
1002 applying fluorescence as a control tool. Among these studies,  
1003 some looked into the treatment of specific domestic/industrial  
1004 wastewater (Janhom et al. 2009, Janhom et al. 2011, Zhu et al.  
1005 2011, Yu et al. 2013), the removal and behavior of refractory  
1006 OM in treatment works (Hur et al. 2011), characterization of  
1007 reverse osmosis permeates (Singh et al. 2009, Singh et al. 2012,  
1008 2015) or compared fluorescence EEM-PARAFAC and  
1009 HPLC/HPSEC techniques (Li et al. 2014). Fluorescence  
1010 monitoring of wastewater quality was performed at time frames  
1011 spanning from 1 month to 20 months, by collecting samples  
1012 from the inlet and outlet (Reynolds 2002, Riopel et al. 2014) or  
1013 along different treatment steps (Singh et al. 2009, Hambly et al.  
1014 2010, Murphy et al. 2011, Singh et al. 2012, Bridgeman et al.  
1015 2013, Cohen et al. 2014, Ou et al. 2014, Singh et al. 2015). The  
1016 longest monitoring study was undertaken by Cohen et al.  
1017 (2014), who analyzed the wastewater quality from municipal  
1018 treatment plants during 20 months. ENREF 23 ENREF 126Most  
1019 of the monitoring studies involved WwTPs that employed  
1020 activated sludge, as biological treatment process. Nevertheless,  
1021 a few long-term and short-term monitoring studies have proven  
1022 the capacity of fluorescence to evaluate the treatment  
1023 performance in plants that used trickling filters (Bridgeman et  
1024 al. 2013), anaerobic/anoxic/oxic (Yu et al. 2014), a novel  
1025 anoxic/aerobic/aerobic system (Ou et al. 2014) or other

1026 advanced biological treatments, such as phase isolated ditches,  
1027 bio-Deniphro process, sequencing batch reactors (Hur et al.  
1028 2011). Hur et al. (2011) found no difference in OM  
1029 fluorescence characteristics between conventional and  
1030 advanced biological treatment, while Bridgeman et al. (2013)  
1031 were able to show, using fluorescence spectroscopy, that  
1032 activated sludge was more effective than trickling filters, in  
1033 removing the organic fraction. Variations in the fluorescence  
1034 signal among WwTPs were also observed by Murphy et al.  
1035 (2011). Nevertheless, the general consensus is that the behavior  
1036 of certain fluorescence peaks can be followed along treatment  
1037 plants to test performance. Cohen et al. (2014) suggested using  
1038 both peak T and peak C components as indicators of total  
1039 microbial activity in wastewater. Therefore, varied  
1040 instrumentation available on market or under development  
1041 (Bridgeman et al. 2015) that measure both components may be  
1042 applied to monitor treatment efficiency.

1043

### 1044 **5.3 Removal of fluorescence components along the** 1045 **treatment plant processes**

1046 Studies have shown that the OM, especially in the region  
1047  $E_m < 380$  nm is significantly removed after the biological  
1048 treatment process (Fig. 2). This is to be expected since the  
1049 biological treatment removes biodegradable material (Cohen et  
1050 al. 2014). Riopel et al. (2014) reported a 60% reduction in the

1051 peak T fluorescence. Within the  $E_m < 380$  nm region, peak T  
1052 component experiences a different degree of removal compared  
1053 to peak B component. Yu et al. (2013) found that peak T  
1054 fluorescence decreases with 60 % in the anaerobic/anoxic zone,  
1055 almost 40 % in the oxic zone and 5% in the final clarification  
1056 process, whilst peak B fluorescence is reduced by 55%, almost  
1057 100% and 0% in the respective zones. Yu et al. (2014) reported  
1058 slightly higher reduction percentages for peak B in the  
1059 anaerobic/anoxic/oxic system. They also observed that peak T  
1060 remained relatively consistent in the treatment process (41 - 48  
1061 %), but peak B decreased dramatically (33 - 7 %). However,  
1062 Murphy et al. (2011) and Janhom et al. (2009) found a poor  
1063 removal of peak B fluorescence. Janhom et al. (2009) stated  
1064 that peak B substances are not considered refractory and  
1065 suggested that these substances could be related to some  
1066 humic-bound proteinaceous constituents, which may be  
1067 biologically resistant. Nevertheless, Cohen et al. (2014) advises  
1068 caution when comparing the sensitivity of fluorescent  
1069 components to wastewater treatment due to possible multiple  
1070 differences in the treatment system. In addition to the  
1071 biological treatment, Cohen et al. (2014) found that soil-aquifer  
1072 treatment causes a further significant decrease in the  
1073 concentration of the OM fluorescing in the  $E_m < 380$  nm  
1074 region. Murphy et al. (2011) and Hambly et al. (2010) also  
1075 observed that chlorination generated a high removal rate of the

1076 peak T fraction at recycled treatment plants.

1077 Compared to peaks T and B components, peaks A and C

1078 are removed to a lower extent in the first stages of the treatment

1079 works (Fig. 2). Riopel et al. (2014) reported a reduction in the

1080 peak C component of 28 % and an increase in peak M with 4 %

1081 from influent to effluent. Cohen et al. (2014) found that one

1082 component in the  $E_m > 380$  nm region, sensitive to microbial

1083 activity, was removed, while other two components could not

1084 be removed by the biological treatment. Yu et al. (2013)

1085 observed a reduction in peak C - like component below 10 %.

1086 Later, Yu et al. (2014) showed that one component in the

1087 region  $E_m > 380$  nm increases from 6 % in the primary

1088 treatment to 19 % after the biological treatment. An increase in

1089 the fluorescence of this component was observed by Ou et al.

1090 (2014) in anoxic and aerobic treatments. Poor degradation of

1091 these components was also reported by Janhom et al. (2011) at

1092 an activated sludge treatment process. Yu et al. (2015b) found

1093 that with increasing retention times at sequencing batch reactor

1094 the peak C components increase in the soluble microbial

1095 products. These products are generated by substrate utilization

1096 or biomass decay and cell lysis, and are regarded as

1097 autochthonous matter. Cohen et al. (2014) and Riopel et al.

1098 (2014) suggest that these fluorescent components are either

1099 potentially produced during the process or are recalcitrant to

1100 decomposition. Riopel et al. (2014) mention that large

1101 molecules degrade into smaller molecules that have a fulvic-  
1102 like behavior, based on the polyphenol postulate of humic  
1103 substances formation. They explain that due to the high  
1104 microbial activity in WwTPs, the secreted exocellular enzymes  
1105 will oxidize the polyphenols into quinones. The quinones will  
1106 agglomerate with metabolites like amino acids or peptides,  
1107 leading to the formation of humic polymers, which could be  
1108 fulvic acids because they are smaller in size. Another  
1109 explanation for the poor removal of these components is  
1110 provided by Hur et al. (2011) who studied the fate of refractory  
1111 OM in WwTPs. Refractory OM is not easily removed by the  
1112 biological treatment process due to its recalcitrant nature.  
1113 Moreover, Hur et al. (2011) showed that in most WwTPs, the  
1114 percentage distribution of refractory OM increases in the  
1115 effluents.

1116 Tertiary and quaternary treatment stages are responsible  
1117 for removing most of the fraction that fluoresces in the region  
1118  $E_m > 380$  nm (Fig. 2). Hambly et al. (2010) observed that  
1119 chlorination generated a higher reduction in peak C compared  
1120 to previous treatment steps. Singh et al. (2012) found a  
1121 minimum of 97 % removal of peak C fluorophores after the  
1122 reverse osmosis process. Murphy et al. (2011) also reported  
1123 almost complete removal of components following reverse  
1124 osmosis treatment step.

1125 Removal of fluorescent compounds, like FWAs and  
1126 PAHs, was also analysed. Bridgeman et al. (2013) found  
1127 FWAs only in crude wastewater and not after other treatment  
1128 steps, concluding that this fluorescent fraction associates with  
1129 particulate matter, which is removed by the primary treatment  
1130 stage. In addition, Tavares et al. (2008) stated that subsequent  
1131 disinfection processes may further remove FWAs from  
1132 wastewater. According to Hayashi et al. (2002), up to 80 % of  
1133 FWAs are removed after the biological treatment, and thus  
1134 these compounds could be used as molecular markers of less  
1135 effective treatment processes. Ou et al. (2014) found that, for  
1136 coke wastewater, the novel anoxic/aerobic/aerobic system  
1137 successfully removed PAHs. While, Cohen et al. (2014)  
1138 observed no reduction in the pyrene-like component along the  
1139 treatment steps.

1140 In most monitoring studies, other changes in the  
1141 fluorescence spectra with regard to peak shape and position  
1142 were observed. However, the findings regarding peak position  
1143 are not consistent across studies, potentially due to differences  
1144 in the treatment process or source of wastewater. For example,  
1145 Zhu et al. (2011) observed that peak C presented a blue shift of  
1146 5 nm for the excitation wavelength and of 21 nm for the  
1147 emission wavelength, from influent to effluent, at membrane  
1148 bioreactor treated supermarket wastewater. Hur et al. (2011)  
1149 reported a 20 nm excitation wavelength red shift between



1150 influent and effluent, at refractory OM from municipal  
1151 wastewater. Yet, Riopel et al. (2014), using PARAFAC, found  
1152 no change in the peak C position or shape between sample  
1153 locations. Riopel et al. (2014) observed that the PARAFAC  
1154 component similar to peak T was elongated to longer  
1155 wavelengths at influent samples compared to effluent. They  
1156 attributed this elongation to the free or bound nature of the  
1157 components. In the study of Zhu et al. (2011), peak T  
1158 fluorescence displayed a red shift of 5 nm in the emission  
1159 wavelength, from influent to effluent (Zhu et al. 2011).  
1160 According to Zhu et al. (2011), the red shift is associated with  
1161 the presence of carbonyl containing substances, hydroxyl,  
1162 alkoxy, amino groups and carboxyl constituents, while a blue  
1163 shift is linked to a decomposition of condensed aromatic  
1164 moieties and the break-up of the large molecules into small  
1165 molecules.

1166

#### 1167 **5.4 Fluorescence control and optimisation of treatment** 1168 **processes**

1169 Increasingly stringent regulation has put major pressure  
1170 on water utilities to find new technologies and implement  
1171 control concepts that would improve the overall performance of  
1172 WwTPs (Rehman et al. 2015). As discussed in previous  
1173 sections, fluorescence spectroscopy has the potential to be used  
1174 as a highly effective monitoring technique of treatment quality.

1175 This could be achieved through the use of peak T fluorescence,  
1176 which could replace the out-dated and inaccurate BOD  
1177 (Bridgeman et al. 2013). Consequently, fluorescence  
1178 spectroscopy could provide the WwTPs with the optimum tool  
1179 for real-time control and remediation of plant performance  
1180 failures (Chong et al. 2013).

1181 Additionally, Bridgeman et al. (2013) and Ahmad and  
1182 Reynolds (1995) suggested that fluorescence could improve the  
1183 process control in activated sludge process. The bacteria and  
1184 microorganisms that form the activated sludge are fed with  
1185 wastewater containing organic waste. In order to sustain the  
1186 biological activities into the activated sludge process for BOD  
1187 reduction, air is pumped into the tanks to provide sufficient  
1188 quantities of dissolved oxygen. Aeration is one of the most  
1189 energy intensive operations from the WwTPs, almost 65 % of  
1190 energy being consumed for the activated sludge process  
1191 (Rehman et al. 2015). Water utilities often over aerate to  
1192 ensure meeting discharge regulations (Bridgeman et al. 2013).  
1193 It is estimated that, by monitoring OM in WwTPs, 40 % of the  
1194 energy costs could be saved (Ahmad and Reynolds 1995).  
1195 Thus, fluorescence may be used to optimize process control in  
1196 treatment works and eliminate the unnecessary costs associated  
1197 with overtreatment (Bridgeman et al. 2013).

1198 Promising results regarding online monitoring and  
1199 process control were obtained by Singh et al. (2015), who

1200 published the first real-time study on two municipal recycled  
1201 treatment plants. The researchers used a peak C sensor to prove  
1202 the robustness of the technique in detecting reverse osmosis  
1203 membrane fouling and integrity. They showed that the sensor  
1204 was sufficiently sensitive to detect subtle differences between  
1205 membrane permeates and identify underperformance issues.  
1206 Also, no indication of fouling on probe and no deviation of  
1207 probe performance were observed, during the experimental  
1208 period. This study demonstrated the potential of using  
1209 fluorescence for treatment process assessment and control.

1210

## 1211 **6 Conclusions and future considerations**

1212 The use of real-time fluorescence could lead to a positive  
1213 change in the water industry, as operators would be able to start  
1214 immediate remedial actions in case of accidental pollution  
1215 events, cut costs associated with complex analytical approaches  
1216 and comply with discharge regulation. Wastewater treatment  
1217 processes reduce peak T fluorescence primarily by biological  
1218 treatment, and peak C through chlorination and reverse  
1219 osmosis. There are several simple probes or fluorometers  
1220 available on market that measure these two components or  
1221 more complex systems that convert the peak T fluorescence  
1222 signal into BOD values.

1223 However, in case of monitoring surface waters  
1224 contaminated with wastewater, the use of simple fluorometers

1225 may not be the best solution to identify the exact source and  
1226 take the appropriate remedial actions. Several fluorophores,  
1227 with varied origins, were shown to contribute to peaks T and C,  
1228 hindering the identification of the source of wastewater  
1229 pollution in natural water systems. Single or double wavelength  
1230 instruments could only be used as a time and cost effective first  
1231 measure for early warning.

1232 Implementation of fluorescence instrumentation for on-line  
1233 monitoring is relatively slow due to several factors, such as  
1234 high quantities of suspended solids, temperature, fouling etc. In  
1235 order to counteract these issues, dilution of samples is  
1236 recommended: to a factor of 10 or to an absorbance value of <  
1237  $0.05 \text{ cm}^{-1}$ , in the peak T absorbance region. However,  
1238 wastewaters are highly variable in concentration and  
1239 composition and therefore a general dilution factor may not be  
1240 recommended. In addition, post-measurement mathematical  
1241 correction could be applied to reduce the impact produced by  
1242 external factors.

1243

#### 1244 **Acknowledgements**

1245 The work of E.M. Carstea was supported by the  
1246 European Commission Framework Programme 7, through a  
1247 Marie Curie Intra-European Fellowship (PIEF-GA-2012-  
1248 329962).

1249

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Figure 1. Main techniques of processing fluorescence EEMs. Examples of a) peaks identified with the peak picking method, and b) components identified with PARAFAC, for samples of water systems impacted by domestic wastewater.

Figure 2. Removal of fluorescent components during treatment; the removal percentages represent collective values from several studies (Tchobanoglous and Burton 1991, Reynolds 2002, Hambly et al. 2010, Janhom et al. 2011, Murphy et al. 2011, Singh et al. 2012, Cohen et al. 2014, Ou et al. 2014, Riopel et al. 2014, Yu et al. 2014) and unpublished data. Blue arrow – low decrease, Orange arrow – moderate removal, red arrow – high removal.

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Table 1. Fluorophores contributing to regions Em &lt; 380 nm &gt;.

Potential fluorophores	Component	Region	Peak position (nm)	Reference	Potential sources in Ww
Lignins	Lignin phenols	Em < 380 nm	~ 245 (295) / 302	<a href="#">Walker et al. (2009)</a>	Partially degraded food waste, undigested dietary fibre, toilet paper etc. Wastewater of paper and pulp industry ( <a href="#">Pokhrel and Viraraghavan 2004</a> ) fibres from food ( <a href="#">Huang et al. 2010</a> )
			270-290 / 300-350	( <a href="#">Hernes et al. 2009</a> )	
	Vanilic acid		/ 326	( <a href="#">Stedmon and Cory 2014</a> )	
	Syringic acid		/ 338	( <a href="#">Stedmon and Cory 2014</a> )	
	Breakdown products	Em > 380 nm	230-275 (300-390) / 400-520	( <a href="#">Baker 2002b</a> , <a href="#">Ciputra et al. 2010</a> , <a href="#">Osburn and Stedmon 2011</a> , <a href="#">Cawley et al. 2012</a> , <a href="#">Bassandeh et al. 2013</a> )	Paper mill effluents ( <a href="#">Baker 2002b</a> , <a href="#">Ciputra et al. 2010</a> , <a href="#">Cawley et al. 2012</a> , <a href="#">Bassandeh et al. 2013</a> )
Aromatic hydrocarbon	Toluene	Em < 380 nm	266 / 300 - 400	<a href="#">Persichetti et al. (2013)</a>	Municipal Ww ( <a href="#">Huang et al. 2010</a> , <a href="#">Mrowiec 2014</a> ); Ww with petrol derivatives ( <a href="#">Mehdizadeh et al. 2011</a> )
Phenols	Cresols		210-285 / 290-310	<a href="#">del Olmo et al. (1996)</a>	Pharmaceutical, fossil fuel or pesticide industries ( <a href="#">Tchaikovskaya et al. 2007</a> ); Domestic Ww from disinfectants ( <a href="#">Tertuliani et al. 2008</a> )
Aromatic amino acids	Tyrosine		275 / 304	<a href="#">Lakowicz (2006)</a>	Proteins and peptides ( <a href="#">Lakowicz 2006</a> ); Domestic Ww ( <a href="#">Burleson et al. 1980</a> , <a href="#">Dignac et al. 2000</a> , <a href="#">Huang et al. 2010</a> )
	Tryptophan		295 / 353	<a href="#">Lakowicz (2006)</a>	Proteins and peptides ( <a href="#">Lakowicz 2006</a> ); Livestock Ww ( <a href="#">Choi et al. 2013</a> )
Indole			230 / 330-350	<a href="#">Determann et al. (1998)</a>	Municipal Ww ( <a href="#">Dignac et al. 2000</a> , <a href="#">Tertuliani et al. 2008</a> , <a href="#">Huang et al. 2010</a> ); Coal tar, oil shale, personal care products, pesticides and pharmaceuticals ( <a href="#">Gu and Berry 1991</a> , <a href="#">Tertuliani et al. 2008</a> , <a href="#">Aiken 2014</a> )
DNA			267 / 327	<a href="#">Vayá et al. (2010)</a>	Proteins ( <a href="#">Lakowicz 2006</a> ); Municipal Ww ( <a href="#">Huang et al. 2010</a> )
Polyaromatic hydrocarbons			Em < 380 nm	Short UV	<a href="#">Baker et al. (2014)</a>
	Phenanthrene, anthracene, pyrene, fluoranthene, benzo[a]pyrene	Em > 380 nm	220-300 / 370-430	( <a href="#">Schwarz and Wasik 1976</a> , <a href="#">Patra and Mishra 2001</a> , <a href="#">Yang et al. 2016</a> )	Industrial Ww ( <a href="#">Cohen et al. 2014</a> , <a href="#">Ou et al. 2014</a> ); Municipal Ww ( <a href="#">Huang et al. 2010</a> )
Quinones		Em > 380 nm			Microbes, fungi, plants ( <a href="#">Aiken 2014</a> ); Activated sludge ( <a href="#">Hu et al. 2000</a> )
Flavonoids					Plants ( <a href="#">Aiken 2014</a> ); food ( <a href="#">Egert and Rimbach 2011</a> ); olive oil mill Ww ( <a href="#">Leouifoudi et al. 2014</a> )
Humic acids			220-320 (400-	<a href="#">IHSS (2015)</a>	



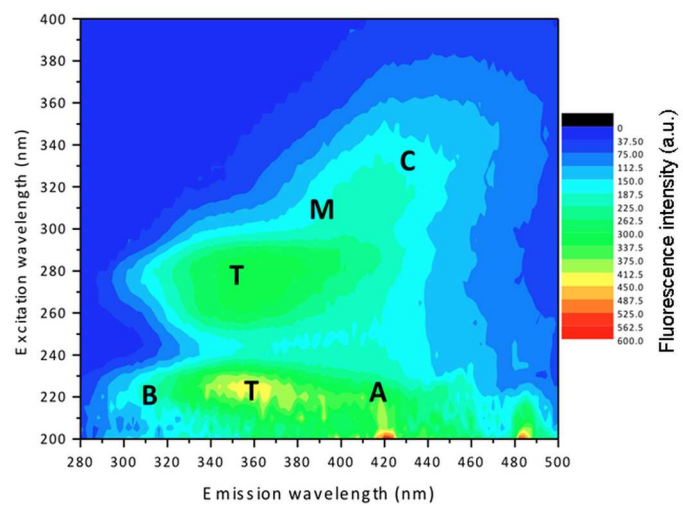
			500) / 400-550		Municipal Ww ( <a href="#">Huang et al. 2010</a> )
Pharmaceutical ly active compounds	Carbamazepine		308 / 410 (in 2 mol L <sup>-1</sup> HCl, and 20 min irradiation time)	<a href="#">Hurtado-Sanchez Mdel et al. (2015)</a>	Faeces, urine ( <a href="#">Zhang et al. 2008</a> )
	Fluorquinolone		290 / 500		
	Piroxican		294 / 372 (in media with pH < 2)		
Fluorescent whitening agents			360-365 / 400 - 440	<a href="#">(Takahashi and Kawamura 2006, Tavares et al. 2008)</a>	Laundry detergents, sanitary products, toilet paper and tissues; Papermaking industry ( <a href="#">Takahashi and Kawamura 2006</a> , <a href="#">Assaad et al. 2014</a> )

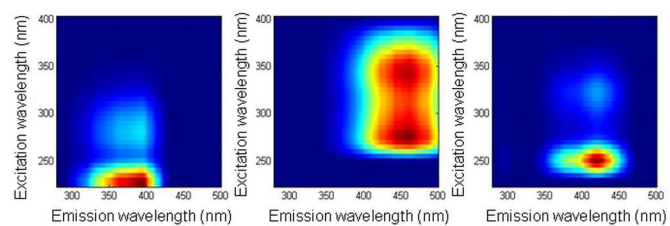
Ww – wastewater

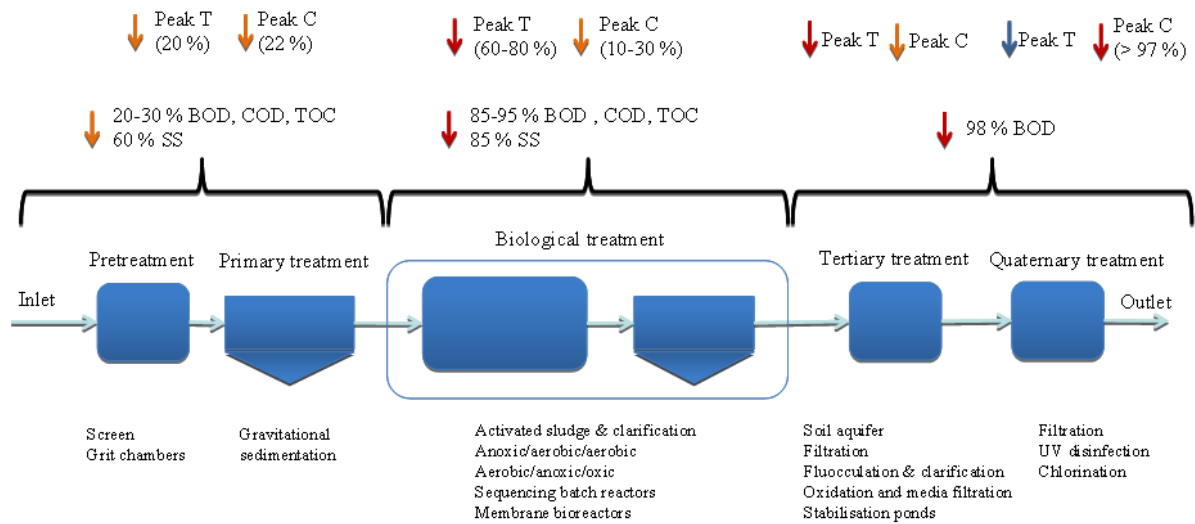
Table 2. Correlation coefficients for peaks T and C (or PARAFAC analogous components) with BOD, COD and TOC.

Reference	Samples	Sample size	Sample pH	Analysis temperature	Fluorescence Peak	BOD	COD	TOC
<a href="#">Reynolds and Ahmad (1997)</a>	Raw, settled and treated Ww	129	N/A	Room temperature	280 / 340	0.94-0.97	N/A	N/A
<a href="#">Ahmad and Reynolds (1999)</a>	Raw Ww	25	3 - 7	10-80 <sup>0</sup> C	248 / 350	0.97	N/A	N/A
<a href="#">Reynolds (2002)</a>	Raw Ww	56	6.8 ±0.4	26 ±10 <sup>0</sup> C	280 / 350	0.93	0.94	0.93
<a href="#">Baker and Inverarity (2004)</a>	Ww effluents and effluent impacted rivers	434	N/A	N/A	220 / 350	0.85	N/A	N/A
<a href="#">Wang et al. (2007)</a>	Ww impacted lake	26	N/A	Room temperature	294 / 320 360 / 425	0.54 0.65	0.16 0.03	N/A N/A
<a href="#">Hudson et al. (2008)</a>	Ww effluents	141	N/A	20 <sup>0</sup> C	280 / 350 300-370 / 400-500-	0.71 0.34	N/A N/A	0.77 0.75
<a href="#">Bridgeman et al. (2013)</a>	Domestic Ww, raw and treated	48	N/A	20 <sup>0</sup> C	275-285 / 340-360 320-355 / 410-470	0.92 0.88	0.56 0.78	N/A N/A
<a href="#">Cohen et al. (2014)</a>	Domestic and industrial Ww, raw and treated	25-34	7.8 – 8.5	Room temperature	<240 (275) / 346 <240 (305) / 422	0.82 0.72	0.82-0.99 0.91	0.85-0.99 0.99
<a href="#">Ou et al. (2014)</a>	Industrial Ww, raw and treated	120	7 - 9	Room temperature	280 / 320	N/A	0.92	N/A

Ww – wastewater; N/A – not available







- Several fluorophores contribute to common peaks hindering pollution source tracking
- Previous on-line studies may help build a strategy for wastewater analysis
- Dilution of samples, typically up to x10, useful to limit inner filter effect
- Calibration may not be needed for qualitative data
- Research gaps: online application of fluorescence and rapid data processing tools