Monitoring organic loading to swimming pools by fluorescence excitation–emission matrix with parallel factor analysis (PARAFAC)

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1 **Monitoring organic loading to swimming pools by fluorescence**
2 **Excitation-Emission Matrix with parallel factor analysis**
3 **(PARAFAC)**

4

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14

15 **Abstract** Fluorescence Excitation –Emission Matrix spectroscopy combined with parallel
16 factor analysis was employed to monitor water quality and organic contamination in
17 swimming pools. The fluorescence signal of the swimming pool organic matter was low
18 but increased slightly through the day. The analysis revealed that the organic matter
19 fluorescence was characterised by five different components one of which was unique to
20 swimming pool organic matter and one which was specific to organic contamination. The
21 latter component had emission peaks at 420 nm and was found to be a sensitive indicator

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of organic loading in swimming pool water. The fluorescence at 420 nm gradually increased during opening hours and represented material accumulating through the day.

Keywords swimming pool, fluorescence, Excitation-Emission Matrix (EEM), wastewater, parallel factor analysis (PARAFAC)

1. INTRODUCTION

Monitoring of water quality in swimming pools is important in order to avoid health risk to swimmers and swimming pool staff. In general, there are three sources of organic matter: the water supplied to the pool, a passive loading of organics leached from the bodies of bathing guests, and a more direct loading of bodily wastes in the form of urine and faeces. The latter is most harmful, however, both contribute to the organic loading and hence the microbial quality in pools. Microbial safety of swimming pool water is required by law (Directive, 2006). Moreover, the organic matter concentration should be maintained low as it reacts with chlorine and produces a suite of chlorinated organic compounds (e.g. trihalomethanes THMs) which are known to be harmful.

To be effective, a water quality monitoring system needs to detect contamination at the initial stage. At present, for chemical and microbial water quality monitoring, a combination of sampling and subsequent analysis is usually applied and may not assure the health of the bathers. Therefore, there is a strong need for on-line sensors providing immediate information on water quality which enables a quick remedial action. Fluorescence might be a promising technique that fulfils the required criteria. Fluorescent properties of organic matter have been widely studied in various aquatic systems for many years (Coble, 1990; Muller et al., 2008; Henderson et al., 2009; Johnstone et al., 2009). However, limited studies have been carried out on the

fluorescence properties of chlorinated aquatic organic matter and these have mainly been focused on the chlorination of drinking (Fabbicino and Korshin, 2004; Johnstone and Miller, 2009; Roccaro et al., 2009) and recycled water (Hambly et al., 2010 a,b). To our knowledge, there is no information on fluorescent organic matter in swimming pools. Drinking water purification studies suggest that in chlorinated waters organic matter fluorescence will be low (Johnstone and Miller, 2009). In swimming pools, water has to be disinfected with chlorine and adequate free chlorine level has to be maintained to assure the microbial safety (Uhl and Hartmann, 2005). Chlorine dosages used for swimming pool disinfection are higher than those applied in drinking water treatment. Moreover, pool water is recycled and therefore chlorinated on continuous basis (Lee et al., 2009). Due to fluorescence quenching properties of chlorine and its reactivity (Henderson et al., 2009 and references therein), one can expect a very low background fluorescence of swimming pool organic matter, which may be ideal for using fluorescence to monitor for excessive organic loading, and indicate when further water quality treatment or other intervention are required.

Based on this assumption, a series of experiments employing both swimming pool and wastewater has been performed aiming at estimating the detection limit for anthropogenic contamination in swimming pool water. Wastewater fluorescence has been previously successfully investigated for detecting cross-connections between drinking and recycled water systems (Hambly et al., 2010a,b). The authors reported promising role of peak T_1 ($\lambda_{\text{ex/em}} = 300/350$ nm) in distinguishing recycled water samples from potable water samples (Hambly et al., 2010a). Moreover, combination of peak T_1 and C_1 ($\lambda_{\text{ex/em}} = 325/426$ nm) was able to further separate recycled water samples at various treatment

stages (Hambly et al., 2010b). In our study, the obtained fluorescence Excitation-Emission Matrices (EEM's) of swimming pool organic matter were evaluated by parallel factor analysis (PARAFAC) modeling which delivered information on both qualitative and quantitative aspects of the obtained fluorescence signal.

2. MATERIALS AND METHODS

2.1. Sampling and storage

Two swimming pools at the Gladsaxe Sport Centre (Søborg, Gladsaxe council, Denmark) were sampled during this study. One was a full length cold water pool (2700 m³) and the other a smaller warm water basin (50 m³). Each pool has a separate water treatment system. To minimize the adverse effect of chlorine on human skin, sodium chloride is normally added to the water. A sodium chloride concentration of about 0.4 % is maintained in both basins. Water temperature is maintained in range of 26-27 °C and 31-34 °C in the cold water and warm water basin, respectively. The pH was 7.4 in both pools. The warm water pool had a 13-time higher number of guests per m³ than the cold water pool, which corresponded to 4.6 and 0.35 persons/m³/day, respectively (Table 1).

A set-up of the water recirculation in the two systems is shown in Fig. 2. This set-up was similar for both systems; therefore it is shown as one. In this set-up, only elements of interest are shown. Both pool treatment systems contained coarse filtration and sand filtration. Moreover, the warm water pool system contained side stream activated carbon filtration and side stream UV treatment (these two stages were not in operation during the experiment). Chlorine is produced from electrolysis of sodium

chloride. This is an in-line and an on-site production and dosage in the warm water pool system and in the cold water system, respectively. In both systems, the water is recirculated and about 3 to 5 m³ of water is added per day. The filter beds had been working for in the cold and warm water pool, respectively, and both were backwashed a week before the study period.

During the experiment, samples were taken from a variety of sites to minimize sampling error and generate a representative set of samples. All sampling sites for both pool systems are marked in Fig. 1. For the cold water basin system, the sampling sites were as follows: directly in the pool (site 1), pipe collecting water from the basin, before the equalizing tank and sand filters (site 2), and a small pipe at the analytic board (site 3). For the warm water pool, samples were taken directly from the pool (site 4), the equalizing container collecting water from the basin (site 5) and a pipe at the analytic board (site 6).

The difference between sampling sites between the two systems (sites 2 and 4) was caused by their accessibility in terms of sampling and applying water quality sensors. In the first series of the experiment, water quality was monitored for 5 days (3 days site 2 only and 2 days sites 1 and 3-6) within the opening hours (7-21 on weekdays and 8-15 on weekend days) of the sport centre so the daily variability in fluorescence could be assessed. Conductivity was measured on site with Hach HQ 14d meter (Hach Co., USA). For fluorescence, absorbance, adsorbable organic halogens (AOX), and non-volatile organic carbon (NVOC) analyses, water samples were collected in acid washed and precombusted (550 °C) 40 ml glass vials with Teflon-lined silicone caps. 100 µl of Na₂S₂O₃ solution (concentration of 5 g/l) was added to every sample to bind free chlorine

and stop further reaction with organic matter during storage (Johnstone and Miller, 2009). Before sampling this preservation method had been evaluated for its effects on organic matter fluorescence. No adverse effects were seen (Supplementary Information, SI -1.). The collected samples were kept refrigerated at 4 °C, transported to the laboratory and analyzed within 3 days for NVOC and absorbance/fluorescence, and within 2 weeks for AOX.

2.2. Wastewater experiment

In addition to the pool sampling a laboratory experiment with wastewater additions was carried out. Raw municipal wastewater has been applied in the experiments as the source of domestic waste including fractions released directly from human bodies thus equivalent of anthropogenic organic matter in swimming pools (saliva, sweat, skin, hair, urine, faeces *etc.*). A 5 L swimming pool water sample was taken from the warm-water basin. The sample was kept refrigerated (4 °C) and used in the experiment on the following day. Before starting the experiment the pool water was spiked with sodium hypochlorite solution to re-establish in situ chlorine concentrations of 1.2 mg /L free Cl₂. The wastewater used in the experiment was raw sewage from a municipal wastewater treatment plant serving 135,000 persons (Lundtofte, Kgs. Lyngby, Denmark). The wastewater is mainly of residential origin, only 8-10% is industrial wastewater. It was filtered through a 1.6-µm pore size glass fiber filter before use. The characteristics of the water are shown in Table 2. The analyses were performed by a commercial laboratory (Miljoelaboratoriet I/S, Glostrup, Denmark) except the NVOC analyses which were carried out at DTU Environment (Kgs. Lyngby, Denmark). Total and combined chlorine

concentrations were measured by the DPD (*N,N*-diethyl-*p*-phenylenediamine) colorimetric method in a Allcon Test spectrophotometer (APHA, 2005).

2.3. Analyses

In total, 103 samples of swimming pool water have been analyzed for fluorescence EEM and NVOC to gain an understanding of how the fluorescence signal of pool organic matter varies in intensity and characteristics throughout the day.

2.3.1. NVOC

NVOC was measured using a Shimadzu TOC-V WP analyzer with ASI_V autosampler. The analyzer uses sodium persulfate solution, UV radiation and a temperature of 80°C to oxidize organic carbon. A 10-fold auto-dilution was used for analysing swimming pool water samples which had been previously found to show better reproducibility for samples containing chloride ions (data not shown). For wastewater analysis, a manual dilution of wastewater was prepared before analysis without further auto-dilution.

2.3.2. Fluorescence EEM with PARAFAC analysis

Fluorescence was measured in a 1- cm cuvette using a Varian Cary Eclipse Fluorescence Spectrophotometer. Wavelength range for excitation spectra was 240-450 nm while for emission 300-600 nm, with 5-nm and 2-nm steps, respectively. Excitation and emission slit widths were set to 5 nm and photomultiplier tube voltage to 1000v. The excitation and emission spectra measured from each sample were combined to create excitation emission matrices (EEMs). In such obtained EEMs both excitation and emission

wavelengths were corrected using Rhodamine B and a ground quartz diffuser, respectively. Sample inner filter effects were also corrected using absorption measurements. Absorption measurements were performed on Varian Cary 50 Bio UV-visible Spectrophotometer in a 1 cm quartz cuvette and UV-visible spectra recorded from 240 to 700 nm with 0.5 nm slit. The correction was followed by Raman calibration according to (Lawaetz and Stedmon, 2009). The calibrated and corrected fluorescence data were then modeled using the DOMFluor Toolbox in Matlab[®] according to the procedure recommended in (Stedmon and Bro, 2008). The number of fluorescence components was found by a validation method including split half and residual analysis. Short term changes in organic matter fluorescence immediately after addition to chlorinated water were monitored by measuring 20 successive EEMs within 160 minutes (repeated measurements without refilling the cuvette). A blank sample where swimming pool water was replaced by MilliQ water was also measured.

2.3.3. AOX

AOX concentration was measured using a rapid analysis test kit from Hach-Lange (LCK391) which is based on the same pre-treatment principle as the standard method (ISO Standard, 2004) but with wet-oxidation of the carbon disc and photometric determination of halogen ions (Cl⁻).

2.4. Detection limit

Detection limits were calculated using a method based on *t*-distribution test (Harris, 2003). This method generates a detection limit that has a 99 % chance to be greater than

the blank. Fluorescence EEMs of a blank sample which was swimming pool water collected the day before the experiment was performed. A sample close to the DL (0.75 % wastewater addition) was generated and measured 7 times. In addition a series of wastewater additions to swimming pool water were made in the concentration range of 0-2 % and their fluorescence measured. Fluorescence intensity of wastewater-like peaks, found during PARAFAC modeling were used as signal response for calculations. The signal detection limit was calculated according to equation 1. The concentration detection limit was calculated using the obtained calibration curve.

$$y_{DL} = y_{blank} + t * s \quad (1)$$

y_{DL} - signal detection limit

y_{blank} - fluorescence intensity for wastewater component in blank sample (average value)

t - a value from t -distribution test (equal to 3.14 for 7 measurements)

s - standard deviation of 0.75 % wastewater sample (fluorescence intensity of wastewater-like peak in the sample).

3. RESULTS AND DISCUSSION

3.1. PARAFAC components

Kinetics study of fluorescence in swimming pool water with wastewater addition showed that all components were quite stable within the measurement time (160 minutes)

(Supplementary Information, SI - 2). Comparison of the EEMs of swimming pool water with and without wastewater addition showed that swimming pool water exhibited very low fluorescence. Consequently, the fluorescence spectrum of a swimming pool with wastewater added is clearly dominated by the wastewater organic matter fluorescence (Fig. 2). PARAFAC modeling of the swimming pool samples, including samples with and without wastewater addition revealed that the fluorescence of organic matter in swimming pools could be characterized by five different fractions (Table 3 and Figure 3). There are no earlier studies of swimming pool water organic matter fluorescence to which to compare these data directly. However, the swimming pool water spectrum resembled the EEM of recycled water subjected to deep bed sand filtration, ultraviolet disinfection and super-chlorination which has been reported by Halby et al. (2010b). The swimming pool water organic matter fluorescence signals resembled those seen in natural waters. The broad and long wavelength peaks of component 1 have also been found in a variety of contrasting environments and it is thought to represent terrestrial material. Its presence in the wastewater is likely due to surface run off (drainage) being present. Component 2 had a protein-like fluorescence. This type of fluorescence signals is often associated with microbial activity found in many surface waters and associated with either biological productivity or sewage contamination and referred to as the T peak, or protein-like peak (Coble, 1996; Baker et al., 2004; Coble, 2007; Cumberland and Baker, 2007). Component 3 is a ubiquitous fluorescence signal known as C-peak, found in almost all types of waters, including surface, ground and marine waters (Coble 1996). However, additional horizontal peaks in this EEM region, originating from optical brighteners have been reported (Henderson et al., 2009 and references therein). These peaks are

characterized by excitation maxima at 375, 350 and 330 nm, and emission maxima at 410-450 nm. Therefore, they can overlap with the humic-like peak C. Beside their industrial applications (e.g. paper brightening), optical brightening agents are commonly used in household detergents and thus can be found worldwide in sewage and sewage-contaminated waters (Takahashi and Kawamura, 2007).

Component 4 resembles the previously identified M-peak. Originally, it was associated with surface water productivity (Coble, 1996) but then it was found to be more of a ubiquitous component (Coble, 2007). Component 5 exhibited a shape and form similar to the protein-like peak but both its emission and excitation maxima were shifted towards longer wavelengths.

3.2. Variation of fluorescence in swimming pool water samples

Among the fractions, component 5 was specific to swimming pool water and components 1 and 3 were specific to wastewater. Components 2 and 4 were present in both water types, however, at much higher concentrations than in wastewater (Fig.4).

3.3. Correlation of fluorescence components with wastewater addition

Components 1-4 showed strong correlation with wastewater concentration (R^2 0.985; 0.989, 0.987 and 0.995, respectively) (Fig. 5). The corresponding R^2 values for the fixed wavelength pairs (without PARAFAC) were close and equaled 0.991; 0.986; 0.989 and 0.993 (data not shown).

The best linear relationship between wastewater concentration and fluorescence was found for component 4. Components 1 and 3 have fluorescence maxima in excitation-

emission regions associated with humic substances content (Stedmon et al., 2003). As they were found to be correlated with the wastewater concentration, they were likely associated with wastewater humic matter. More specifically, component 1 represents terrestrial fraction of wastewater organic matter, and component 3 was associated with humic-like C-peak. Component 2 (protein-like) is characteristic for sewage contaminated waters and has been previously found to be correlated with sewage content in the water (Baker, 2001). Component 4 has been previously associated with phytoplankton or microbial productivity. In our experiment this peak was assumed to be associated with wastewater microbial activity. Within the five fluorophores only component 5 was not correlated with wastewater concentration, hence associated with swimming pool organic matter. Considering component 5's "position" in the EEM, which is between protein-like and humic-like regions, this component is most likely a combination of swimming pool microbial activity products and swimming pool humic-like substances. Fluorescence intensity of component 5 exhibited an average intensity of 0.047 R.U. with standard deviation of 0.002 R.U.

3.4. Daily variability of fluorescence in the swimming pool

The possibility of using components 1-4 for monitoring anthropogenic contamination in swimming pools was tested on fluorescence daily variation data. Compared to the cold water pool the warm water basin was supposed to contain more contamination due to smaller volume and high number of visitors. An example of daily variation of fluorescence in the warm water basin is shown in Fig. 6.

For the warm water swimming pool, among the four wastewater-like peaks component 3 shows the biggest increase through the day (almost doubled on both sampling days). Similarly, component 4 almost doubled through both sampling days. However, component 4 fluorescence exceeded the wastewater detection limit for two samples only, whereas component 3 was above the detection limit for the both entire days. Component 5, a swimming pool organic matter-like peak, showed some variation but no systematic trend during the day. Fluorescence of components 1 and 2 was below the wastewater detection limit through the whole sampling period.

3.5. Correlation between fluorescence, NVOC, AOX and combined chlorine

For the warm water pool, the NVOC content was in range of 2.2-2.8 mg/L on the first sampling day, and higher (range 2.5- 3.2 mg C/L) on the second sampling day, and increased through the day. The increase in NVOC was caused by input of two organic fractions, assigned as components 3 and 5 which were correlated with NVOC (Fig.7a). The R^2 values of the correlations indicate level of significance of almost 99.9 % and higher than 99.9 % for components 5 and 3, respectively.

The material released from bodies of bathers in swimming pools contains both organic matter and ammonia and can react with chlorine. The latter reaction forms chloramines. Both chlorinated and non-chlorinated organic matter can be detected as total or non-volatile organic carbon. Therefore, in this study the concentration of combined chlorine in the swimming pools was correlated with NVOC (R^2 of 0.626, data not shown). Consequently, a correlation between fluorescence component 3 and 5 and

combined chlorine concentration in the warm water pool was found (Fig.7b), with levels of significance higher than 95 % for both components.

High concentration of disinfection by-product in the swimming pool water, showed by chloramines, was also confirmed by the AOX content which was in range of 1.73-2.03 mg/L for the warm water pool. Negative correlations between AOX and NVOC, and AOX and fluorescence of components 3 and 4 were also observed (Fig. SI-5).

3.6. Cold water pool

For the cold water pool, almost all the wastewater-like components showed fluorescence below the wastewater detection limit. On the both sampling days, only one sample slightly exceeded the detection limit (data not shown). The NVOC values for the cold water pool were constant (1.8 – 1.9 mg C/L on both measurement days). Therefore, no correlation between fluorescence and NVOC, and fluorescence and combined chlorine was observed in the cold water pool. Combined chlorine concentration was in range of 0.4-0.6 mg/L, and AOX content varied from 1.0 to 1.26 mg/L, whereas THM concentration was 23 µg/L.

3.7. Monitoring organic matter loading and accumulation in swimming pool water

The trend in component 3 observed for the warm water pool represents organic loading to the pool which can be direct release of organic matter to the pool and/or a product of its initial oxidation. This component is stable and accumulates during the day but becomes oxidized during the night when the organic loading to the pool has stopped (i.e. there are no guests in the pool). No similar accumulation was observed in the cold water

swimming pool. Most probably, this is due to the high number of bathers in the warm water pool (Table 1). Moreover, a higher temperature in the warm water pool (31-34 and 27 °C in the warm and the cold water pool, respectively) could both promote release of organic substances from the bather's skin and stimulate the production rate of the oxidized fraction of component 3. The higher organic matter release and oxidation extent is confirmed by higher NVOC, THM and AOX concentration in the warm water pool (ref. sections 3.5. and 2.6., and Table 1). Similarly, Glauner et al. (2005) found that total organic carbon and THM concentration correlated well with the overall bather number in a pool.

Component 5 consists of substances permanently present in swimming pool water, which most likely can be associated with products of oxidation reactions in the swimming pool water. A relatively high concentration of chlorine in the investigated water excludes significant microbial production that had been previously related to this fluorescence region (Henderson et al., 2009). Therefore, component 5 is likely produced in chemical rather than microbial reactions proceeding in the swimming pool water.

3.8. Wastewater detection limit

The detection limits of wastewater in the swimming water were calculated for all the wastewater components (Table 4). It shows that the lowest detection limit was found for component 4 and equaled 0.2% v/v of wastewater. The highest detection limit has been obtained for component 1 and it was four times higher than for component 4. The detection limit for components 3 was 0.6 % v/v of wastewater, which for this experimental set up corresponded to 0.13 mg C /L. The five facts: i) specificity of

component 3 to wastewater, ii) its stability in chlorinated water, iii) the increase in fluorescence of this component through the opening hours, iv) its correlation with NVOC and combined chlorine, and v) its sensitivity to wastewater additions, confirms its usefulness for anthropogenic matter detection. This means that among the three wastewater components, component 3 was found to contain a fraction that might be released to water by the swimming pool guests. Therefore, the excitation and emission maxima of component 3 are recommended for monitoring of organic loading in swimming pool water. The fact that a humic-like fluorescence peak is a waste indicator in swimming pool water, is a novel finding. Previous works on detecting waste contamination in surface and potable waters showed that the protein-like peaks are the best waste indicators (Henderson et al., 2009 and references therein) and that peak C plays a supplementary role (Hambly et al., 2010a,b). However, in the highly oxidative environment of swimming pool water, tryptophan-like material does not persist. Most likely, the suite of reactions on the organic matter released in the pools does occur and one of the reaction products has a humic-like fluorescence signal, similar to what is found in many natural waters.

4. CONCLUSIONS

- Organic matter fluorescence has a potential for monitoring swimming pool water quality.
- Among the two investigated pools, only the warm water pool exhibited waste contamination.

- In the swimming pool-wastewater samples, five fluorescence components have been found, one of which was unique to swimming pool organic matter (component 5, exhibiting excitation maximum at <240 and 310 nm and emission maximum at 360 nm), and four predominant in wastewater (components 1-4).
- Component 3 was a very good indicator for anthropogenic release to swimming pool water. It exhibited emission maximum at 420 and had two excitation peaks: one below 240 and the other at 330 nm.

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- 458

459 Figures

460

461 1. Set-up of the warm and cold water swimming pool systems. Sampling sites (described
462 in section 2.1.) are marked with numbers.

463 2. Example EEMs of the swimming pool water and wastewater samples – top row-
464 measured data, bottom row – PARAFAC model: (a) cold water pool sample, (b) warm
465 water pool sample with 0.75% addition of wastewater, (c) MQ water with 0.75% addition
466 of wastewater, (d) warm water pool sample.

467 3. EEMs of PARAFAC components (contour plots) found for the swimming pool water
468 (warm pool) and wastewater samples.

469 4. The fluorescence intensity of each of the five components (c1-c5): Milli Q water with
470 0.75% v/v wastewater, swimming pool water (stored overnight at 4°C without addition of
471 $\text{Na}_2\text{S}_2\text{O}_3$) and swimming pool water with 0.75% v/v wastewater. Values presented are
472 averages of seven replicate measurements and the error bars represent one standard
473 deviation.

474 5. Correlation between fluorescence of the PARAFAC components (c1-c5) and
475 wastewater concentration in the swimming pool - wastewater samples.

476 6. An example of daily variation of the PARAFAC fluorescence components 1 to 5 (c1-
477 c5) in the warm water pool.

478 7. a) NVOC, b) combined chlorine vs. fluorescence of PARAFAC components 3 and 5
479 (c3 and c5) in samples collected from the warm water pool.

480

481 Table 1 – Characteristics of the two investigated swimming pools.

Factor	Unit	WW, warm water pool. Experimental period (average from ref. period in Gladsaxe report in parenthesis)	CW, cold water pool Experimental period
Volume of pool	m ³	50	2700
Area of pool	m ²	39	1050
Temperature	deg C	31-34	27
Turn over time	h	0.5	5
Bathers per day	persons/d	228	900-1000
Person volumetric load	p/m ³ /d	4.6	0.35
Water addition/d	m ³ /d	3-5	3-5
Chlorine conc.	mg Cl ₂ /L	1.1	0.65
Filter area	m ²	7-8	21
Filter period (run time)	weeks	1	1
pH	-	7.4	7.4
Chlorine conc.	mg Cl ₂ /L	1.1	0.65
Combined chlorine	mg Cl ₂ /L	0.8-1.1	0.4-0.6
Pool THM	µg/L	32-50 (41)	23
Pool AOX	mg/L	1.7-2 (2.1)	1.0-1.3
Pool NVOC	mg/L	2.5-2.9 (3.5)	1.8-1.9

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486 Table 2 - Characteristics of filtered and unfiltered wastewater used in the experiment.

Parameter	Unfiltered WW	Filtered WW	Unit	Method/Standard
BOD	330	73	mg O ₂ /L	DS/EN 1899-1
COD	1.2E +03	180	mg O ₂ /L	DS/ISO 15705
Total nitrogen	60	42	mg N/L	DS/EN ISO 11905-1
Total phosphorus	12	6.5	mg P/L	DS 292
Coliform bacteria	1.3E +07		CFU/100 mL	DS 2255
Thermotol. coliform bacteria	1.3E +07		CFU/100 mL	DS 2255
HPC_{yeast}, 22°C, 72 h	1.5E +07		CFU/mL	DS/EN ISO 6222:2000
HPC_{yeast}, 37°C, 48 h	0.5E +07		CFU/mL	DS/EN ISO 6222:2000
NVOC		24.0	mg C/L	

487

488

489 Table 3 - Excitation and emission maxima of PARAFAC components found for
490 swimming pool – wastewater samples.

Component number	Excitation wavelength, nm	Emission wavelength, nm
1	260, <240, 370	520
2	280	330
3	<240, 330	420
4	<240	370
5	<240, 310	360

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493 Table 4 – Wastewater detection limits in the swimming pool water for the PARAFAC
494 components.

Detection limit, DL	c1	c2	c3	c4
DL [% WW]	0.8	0.5	0.6	0.2

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Fig. 1.

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Fig. 2.

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Fig. 3.

Submitted to: Water Research

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513 Fig. 4.

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Fig. 5.

Fig. 6.

Fig. 7.

SI-1 - Tests on the effects of chlorination on organic matter fluorescence and the ability of sulfite addition to stop further loss of fluorescence during storage.

Introduction

As part of the preparations for this study and small experiment was carried out to resolve the effects on organic matter fluorescence of adding sulfite. As there was going to be a delay 1-7 days between sampling and analysis where in principle the chlorine in the pool water would continue to react with the organic matter in the samples during storage. To remove free chlorine and subsequently limit storage effects sulfite addition was tested.

Method

A large volume sample of tap water (Roskilde, Denmark) was taken for the experiment. The control samples consisted of unamended tap water and the organic matter fluorescence measured represents the natural organic matter in drinking water. Sodium hypochlorite (NaOCl) was added to a sub sample of tap water to create an analogy to swimming pool water with a free chlorine concentration of 1 mg/L Cl_2 . Tap water dispensed into three 1 l bottles. One left as control, the two others were filled with chlorinated water. After half an hour sodium sulfite was added (30:1 molar excess of sulfite to chlorine). A series of bottles were then filled and stored refrigerated. Measurements were made at the start ($t=0$), after 24 hours ($t=1$) and after 7 days ($t=3$).

Results

From the EEMs presented in Figure SI-1 it is clear that the sulfite addition had limited if any effects on the fluorescence. No substantial spectral changes are apparent. Comparing the start samples one can see that the addition of chlorine reduced the overall fluorescence intensity. The EEM of the start sample with sulfite has a slightly higher fluorescence intensity than the chlorinated only sample revealing that between the chlorine addition and the sulfite addition some fluorescence was lost. After one week's storage little change has occurred in the control and the sulfite amended sample whereas the chlorinated sample fluorescence has decreased. In order to see these changes better selected excitation and emission spectra are plotted in Figure SI-2 and SI-3. From the spectra in Figure SI-2 one can see that the control and sulfite amended samples are very stable. The fluorescence signal in the chlorinated sample continues to fall during storage as the chlorine reacts. Figure SI-3 compares the excitation spectra across treatments.

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Figure SI-1. Example EEMs of the three types of samples at the start (left) and after a weeks storage (right). Top row is the control (Tap water). Middle row is the chlorinated tap water. Bottom row is the chlorinated tap water amended with sulfite.

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Figure SI-2. Left: The excitation spectra with emission at 420 nm. Right Emission spectra with the excitation set to 240 nm.

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SI – 2. Kinetic study of fluorescence in swimming pool water with wastewater addition.

To test fluorescence stability of the swimming pool - wastewater samples, a simple experiment has been performed. A swimming pool water sample with a 0.75% wastewater addition was continuously analyzed within 160 minutes for fluorescence EEM, without refilling the cuvette. The results showed that all components were quite stable within the measurement time (Fig. SI-3.).

Fig. SI-3. Fluorescence intensity of each of the five components (c1-c5) of a swimming pool water sample with 0.75% wastewater addition, over 21 sequential measurements (approximately 160 min).

The standard deviation values were 2 and 3% for components 1 and 2, respectively, and 4% for components 3-5.

587 An analogous experiment has been performed for MQ water with 0.75% wastewater
588 addition (fig. SI-4).

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590

591 Fig. SI-4. Fluorescence intensity of each of the five components (c1-c5) of a MQ water
592 sample with 0.75% wastewater addition, over 20 sequential measurements
593 (approximately 160 min).

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595 For the MQ water, the standard deviation values were 1% for components 1 and 2, 3%
596 for component 3 and 4% for component 4, thus slightly higher than for the swimming
597 pool sample.

598

599 SI -3- AOX concentration in swimming pool water.

600

a)

b)

601 Fig. SI-5. (a) NVOC and (b) fluorescence intensity of PARAFAC components 3 and 4 vs.
602 AOX in the warm water swimming pool.