Assessment of field fluorometers

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ABSTRACT

Two field fluorometers, devoted either to natural organic matter (NOM) or to tryptophan-like fluorescing substances, were tested for the characterization of a large set of water samples ($n = 263$) impacted to various degrees by untreated or poorly treated urban sewage. Both fluorometers yielded consistent results when testing discrete samples. A nonlinear correlation (coefficient of determination = 0.98) was found between the tryptophan concentration given by the tryptophan field fluorometer and the fluorescence intensity given by a bench-top fluorometer (excitation = 285 nm, emission = 335 nm), corresponding to tryptophan-like fluorescing substances. A linear correlation with a mediocre coefficient of determination (0.63) was found between the NOM concentration given by the NOM field fluorometer and the fluorescence intensity given by the bench-top fluorometer (excitation = 355 nm, emission = 405 nm). This could be related to the diversity of NOM present, as illustrated by the different shapes of synchronous fluorescence spectra collected for the same samples.

Key words | natural organic matter, sewage, synchronous fluorescence, tryptophan

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INTRODUCTION

In recent years, fluorescence has been receiving increasing interest for use in single-substance detection, mostly polycyclic aromatic hydrocarbons (Andrade *et al.* 2000; Sposito *et al.* 2009; Headley *et al.* 2013), or for spectral analysis in aquatic environments such as oceans (Coble 1996; Coble *et al.* 1998; Vignudelli & Santinelli 2004), estuaries (Parlanti *et al.* 2000; Hall *et al.* 2005) and rivers (Baker 2002a; Baker *et al.* 2003; Pons *et al.* 2011). In the latter case, research has mostly been focused on the characterization of dissolved organic matter (DOM) in order to track its origin (natural or anthropogenic). For such a purpose, excitation-emission matrices (EEM) are largely used in research laboratories. The fluorescence peaks that are most frequently detected with this technique have been attributed to groups of substances, often identified by letters (Table S1 in Supplementary Material, available online at http://www.iwaponline.com/wst/070/381.pdf). More details can be found in Henderson *et al.* (2009).

The use of EEM for continuous measurements is still not fully developed: the only example of this, to the best of our knowledge, is a 2-week experiment performed by Carstea *et al.* (2010) during which the response of a river to rainfall was monitored. In-situ fluorometers with a single pair of excitation (λ_{exc}) and emission (λ_{em}) wavelengths were deployed to measure substances such as hydrocarbons, chlorophyll, DOM or optical brighteners. These examples are mostly associated with the marine environment (sea, estuary) and less with continental waters. Field fluorometers, also fitted for single $\lambda_{exc}/\lambda_{em}$ combinations, can be installed in cabinets set along the river bank. The purpose of the present work is to compare the humic-like and tryptophan-like substances measured by two field fluorometers with the measurements provided by a bench-top spectrofluorometer operating in synchronous mode (constant wavelength difference). Although most natural organic matter (NOM) studies are conducted using EEM, synchronous fluorescence improves peak resolution (Patra & Mishra 2002), revealing the spectral components present in DOM (Sierra *et al.* 2005; Barker *et al.* 2009).

MATERIALS AND METHODS

Measurement of fluorescence

A 10AU fluorometer (Turner Designs, Sunnyvale, California) was fitted with an NOM kit (ref 10–303) with a 310–340 nm excitation filter and a 400–600 nm emission filter. A glass tube was used in the laboratory for the analysis of

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discrete samples. The fluorometer was calibrated according to the manufacturer's instructions with quinine sulfate (Sigma-Aldrich) in acidic conditions.

The SMF4 fluorometer (Safe Training Systems Ltd, Wokingham, UK) is equipped with a UV-LED emitting system. It is sensitive to tryptophan-like fluorescence. A quartz cuvette was used for all measurements. The fluorometer readings were calibrated against tryptophan (Sigma-Aldrich).

The synchronous fluorescence spectra (SF50) ($\lambda_{em} - \lambda_{exc} =$ 50 nm) were collected on a Hitachi F-2500 apparatus, using a quartz cell. Ultra-pure water was used for the blank.

Water samples

A total of 263 water samples were collected in locations representing different anthropogenic pressures.

- Constructed wetlands downstream of rural wastewater treatment plants: 72 samples were collected at the inlet and outlet of the last stage for 3 consecutive days (1 sample/4 h) in three localities in Lorraine.
- Along the Meurthe River (111 samples): a 20 km stretch of river, where this sub-tributary of the Rhine River crosses Greater Nancy (population 350,000), was regularly sampled from bridges or with a boat. Several urban streams and combined sewers overflows (CSOs) discharge sewage that is occasionally untreated. The Greater Nancy wastewater treatment plant also discharges along this stretch.
- Along the Madon River (57 samples): 30 sampling stations were established along this sub-tributary of the Rhine River, between its source in the Vosges Mountains and its junction with the Moselle River. It can receive untreated or ill-treated domestic or farming sewage along its 100 km stretch.
- A periurban stream (the Chaudanne, in the western part of Greater Lyon) was sampled on a single day in April 2012 (23 samples). It can receive sewage from a CSO during rainfall events. Hyporheic water was sampled in permanent wells (30 cm below the river bottom), as well as surface water, along a 50 m stretch.

All samples were collected in dry weather conditions in clean polyethylene bottles, transported quickly in the dark to the laboratory and stored in a cold room in the dark at 4 °C until use (within 48 hours). Samples were tested with the field fluorometers with and without filtration (paper filter, ≈ 10 µm pore size). Only filtered samples were analyzed by the laboratory fluorometer. Dissolved organic carbon (DOC) was measured on a TOC-VCHS device (Shimadzu, Noisiel, France) (by combustion at 680 °C in an oxygen-rich environment over a platinum catalyst) and ammonia ($N\text{-}NH_4$) was analyzed by a miniaturized Nessler method (Hach method 8038 on a DR2400 spectrophotometer, Hach, Loveland, Colorado). Excel 2010 (Microsoft, Redmond, USA) was used for data treatment.

RESULTS

All measurements were performed in the laboratory.

Calibration

The SMF4 is not normally calibrated against tryptophan but against biological oxygen demand over 5 days. A calibration curve was built for the HIGH mode (which corresponds to the highest sensivity) of the device (Figure 1(a)). In SF50 mode, tryptophan exhibits maximum fluorescence at $\lambda_{max} =$ 285 nm. Its fluorescence is due to the indole group present in its chemical structure (Figure 1(b)). Other urine components such as indole acetic and propionic acids, as well as skatole, exhibit a maximum of fluorescence at the same λ_{max}, while others such as pterins, kynurenines or

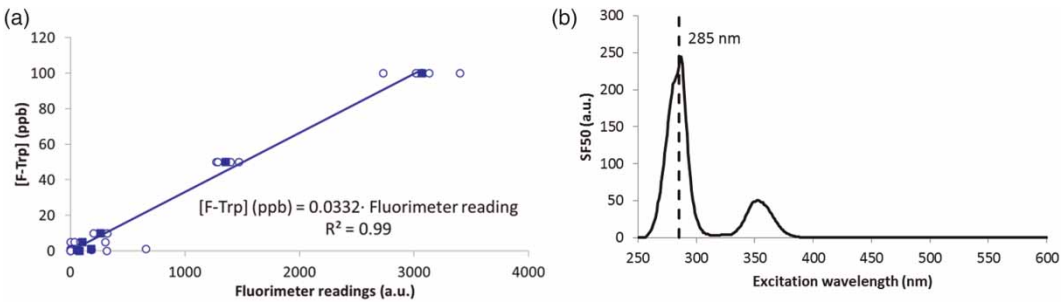

Figure 1 | (a) Calibration curve for tryptophan on the SMF4. Closed symbols = mean of four readings (open symbols) for each concentration; R^2 = coefficient of determination for the linear correlation based on the mean values; (b) SF50 spectrum of a solution of tryptophan in ultra-pure water. The band at $\lambda_{exc} \approx 350$ nm is due to water.

4-pyridoxic acid, which do not have an indole group, present a λ_{max} close to 350 nm (Armstrong *et al.* 1958; Fukushima & Shiota 1972; Dubayová *et al.* 2003; Shi *et al.* 2010). Figure 2 shows the calibration results for the 10AU with quinine sulfate as a surrogate substance for NOM. Quinone-like fluorophores contribute largely to NOM fluorescence (Cory & McKnight 2005).

Analysis of discrete water samples

The SF50 spectra collected for the hyporheic and surface samples from the Chaudanne are plotted in Figure 3. Two bands are apparent: a wide band centred at $\lambda_{exc} = 350$ nm, corresponding to humic substances (Type C), and a smaller band, centred at $\lambda_{exc} = 290$ nm, which is slightly off the tryptophan band ($\lambda_{exc} = 285$ nm). In spite of this discrepancy, a linear correlation, with a coefficient of determination (R^2) of 0.66, was found between the apparent tryptophan concentration given by SMF4 (measured in filtered hyporheic samples) and the fluorescence intensity extracted from the SF50 spectra at $\lambda_{exc} = 290$ nm (Figure 4(a)). No correlation was found between the NOM concentrations given by the 10AU and the fluorescence intensities extracted from the SF50 spectra at $\lambda_{exc} = 350$ nm (Figure 4(b)).

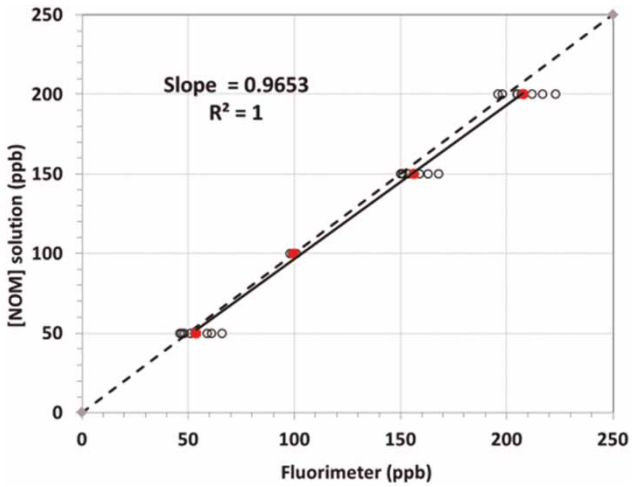

Figure 2 | Calibration of the 10AU against quinine sulfate, surrogate substance for NOM. Closed symbols = average of eight readings (open symbols) for each concentration; R^2 = coefficient of determination for the linear correlation based on the mean values.

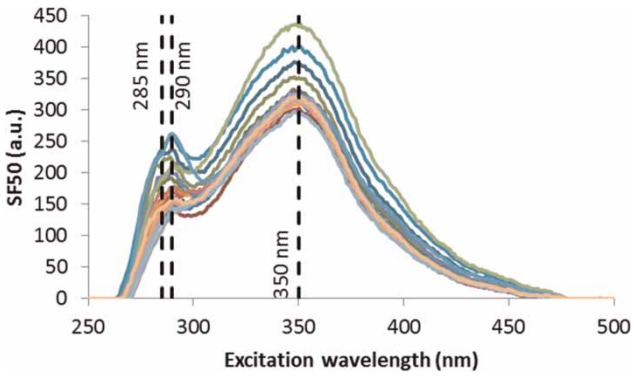

Figure 3 | Series of SF50 spectra collected for the Chaudanne samples.

A strong fluorescence peak centred at $\lambda_{exc} = 285$ nm is noticeable on almost all of the SF50 spectra collected from the constructed wetlands (Figure 5). Two other bands of much lower intensities can be observed at $\lambda_{exc} = 325$ nm and $\lambda_{exc} = 355$ nm: these bands are related to humic substances. A second-order polynomial ($R^2 = 0.98$) was found as the best fit between the tryptophan concentration given by the SMF4 and the fluorescence intensity extracted from the SF50 spectra at $\lambda_{exc} = 285$ nm for the inlet samples (Figure 6(a)). A higher dispersion of the data points can be observed for the outlet samples, leading to a second-order polynomial with a lower coefficient of determination ($R^2 = 0.84$). As the presence of tryptophan is generally associated with urine (Baker 2002b), it is interesting to compare the tryptophan concentrations to the N-NH_4 concentrations (Figure 6(b)). Two different behaviours can be observed. Some samples collected either at the inlet or at the outlet of the last stage of the constructed wetlands exhibit high N-NH_4 concentrations and high tryptophan concentrations: they are typical of badly treated domestic wastewater (Wu *et al.* 2006). Other samples had N-NH_4 concentrations close to zero. Those with a low tryptophan concentration, in the range of what was observed on the Chaudanne, could correspond to well-treated wastewater. For those with a high tryptophan concentration, it could be that the ammonification of organic nitrogen (i.e., tryptophan and related substances) was limited in the constructed wetland.

In Figure 7, the SF50 spectra collected along the Meurthe River (Figure 7(a)) and the Madon River (Figure 7(b)) are plotted. They exhibit a well-defined tryptophan-like fluorescence peak ($\lambda_{exc} = 285$ nm), two humic-like bands (a strong one at $\lambda_{exc} = 355$ nm and another one at $\lambda_{exc} = 325$ nm, which is more visible in the Meurthe River samples than in the Madon River samples) and a band centred at $\lambda_{exc} = 510$ nm, which corresponds to chlorophyll.

The tryptophan and NOM concentrations for these two rivers are included in the global plots (Figure 8) summarizing the data collected for all the filtered samples. A second-order polynomial correlation was obtained between tryptophan and the SF50 intensity at $\lambda_{exc} = 285$ nm, with a coefficient of determination slightly lower than for the

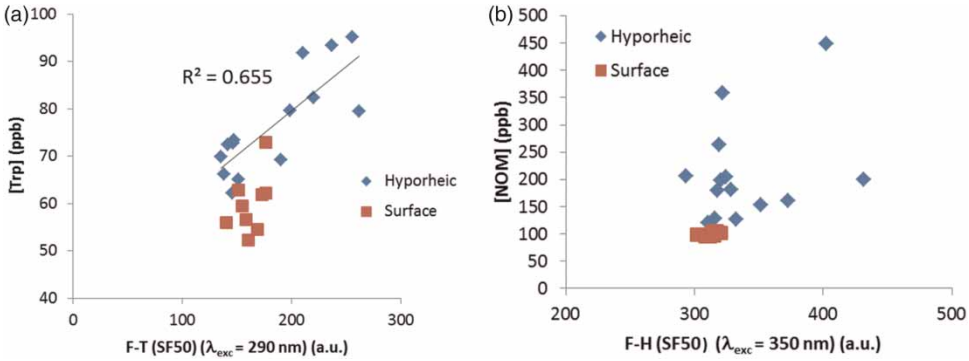

Figure 4 | Correlation between the concentrations ((a) tryptophan, (b) NOM) given by the field fluorometers and the SF50 fluorescence intensities.

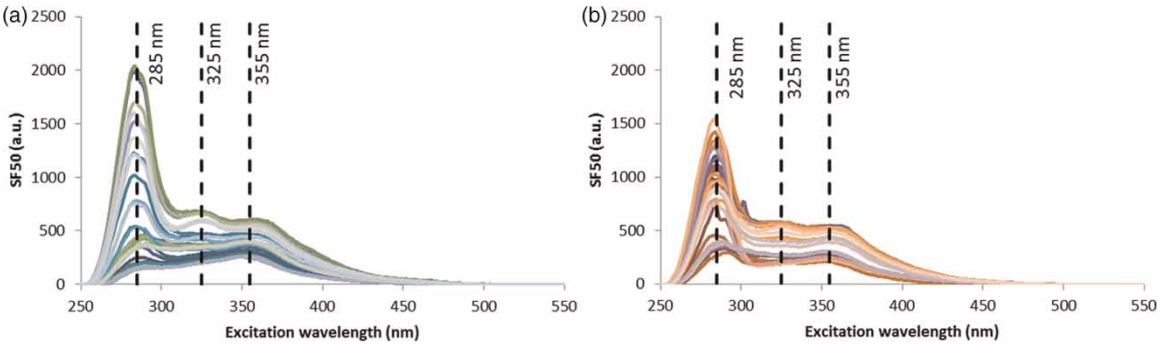

Figure 5 | SF50 spectra collected at the constructed wetlands ((a) inlet, (b) outlet).

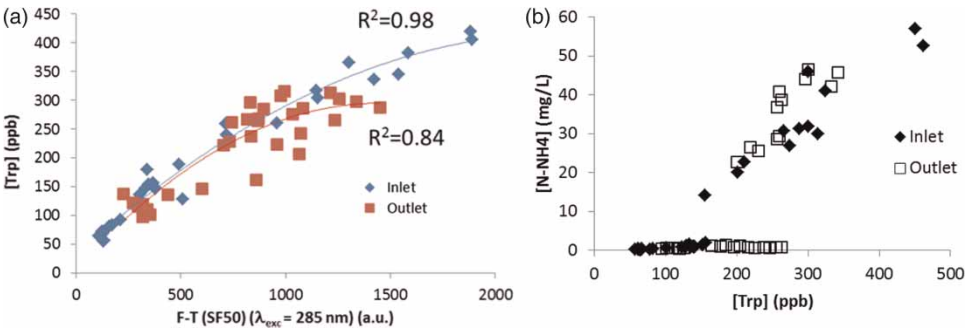

Figure 6 | Non-linear correlations (second-order polynomials) between tryptophan concentration (filtered samples) and (a) SF50 F-T intensity or (b) N-NH_4 in the constructed wetlands. R^2 = coefficient of determination.

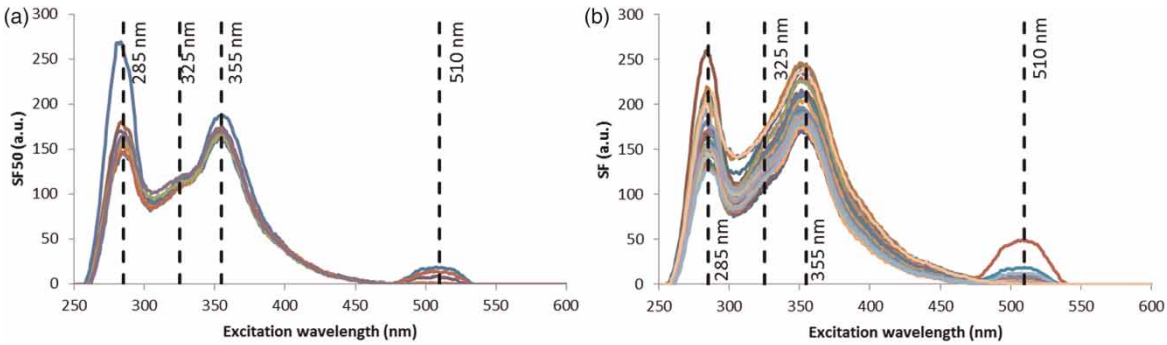

Figure 7 | SF50 spectra collected along (a) the Meurthe River (March 2012) and (b) the Madon River (March 2012).

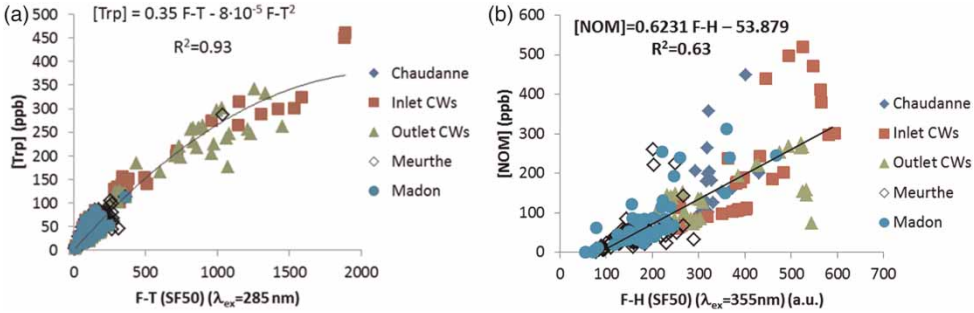

Figure 8 | Global relationship between (a) [Trp] and (b) [NOM] and the corresponding fluorescence intensities extracted from the SF50 samples. CW = constructed wetland.

reduced set of samples collected on the constructed wetland (Figure 8(a)). A linear correlation ($R^2 = 0.63$) could be established between the NOM concentration given by the 10AU and the SF50 intensity at $\lambda_{exc} = 355$ nm, again for all the filtered samples (Figure 8(b)). The offset (≈54 arbitrary units (a.u.)) is due to the fluorescence of water, as the SF50 spectra were not corrected for the water spectrum. No good correlation could be found between DOC and NOM concentration (Figure 9). The coefficient of determination for a linear correlation was only 0.48. This reflects the diversity of organic matter present in the samples. The SF50 spectra show the variability of the fluorescing substances present in the samples. Furthermore, part of DOC does not fluoresce, at least in the wavelength range taken into consideration by the 10AU. For the SMF4, the mean value of the ratio $[Trp]_{unfiltered}/[Trp]_{filtered}$ was 1.1 (standard deviation = 0.18), but no specific trend could be observed with respect to sample turbidity. The turbidity varied between 0 and 300 NFU for the whole set of samples. For the 10AU, the mean value of $[NOM]_{unfiltered}/[NOM]_{filtered}$ was 1.04 (standard deviation = 0.40). This ratio tends to decrease when turbidity increases. The dispersion of those ratios with respect to turbidity could be due to the different nature of the colloids and particles causing the turbidity.

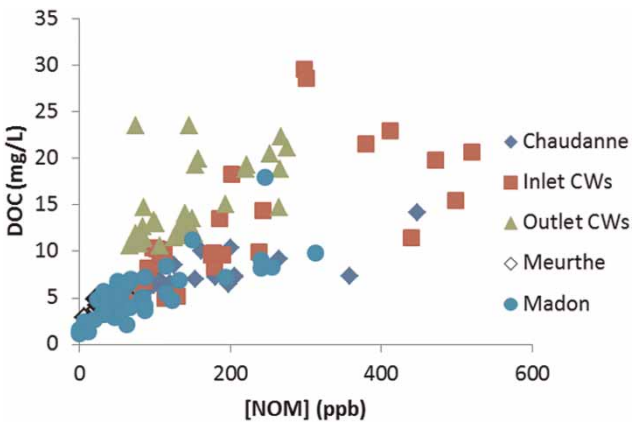

Figure 9 | DOC versus [NOM] for all samples.

CONCLUSIONS

Two field fluorometers were calibrated against quinine sulfate (for NOM) and tryptophan (for tryptophan-like fluorescing substances). They were tested in the laboratory successfully on discrete samples ($n = 263$) from different aquatic ecosystems and can cover a large range of concentrations of NOM and tryptophan. This study was a first step before the implementation of the devices for field monitoring. Considering the range of patterns found in synchronous spectra from the same water samples, the development of field fluorometers able to produce single-scan or EEM would be appreciated.

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