Monitoring dissolved organic matter using submersible tryptophan-like fluorometers

Kieran Khamis\textsuperscript{1,2}, J. Sorensen\textsuperscript{3}, C. Bradley\textsuperscript{2}, D. Hannah\textsuperscript{2}, R. Stevens\textsuperscript{1}

\textsuperscript{1} RS Hydro  
\textsuperscript{2} School of Geog. Earth & Env. Sci. University of Birmingham  
\textsuperscript{3} British Geological Survey
What is fluorescence?

**Fluorescence**: a form of luminescence which occurs over short time scales at the molecular/atomic level.

How does it work?

Energy levels diagram showing transitions between ground state $S_0$ and excited state $S_1$. Transitions include absorption and non-radiative transitions, leading to fluorescence.
Natural fluorescent compounds

• The fluorescent spectra of compounds important for water quality monitoring have been identified

‘Humic’ matter  Chlorophyll  Proteins
Excitation wavelength

Emission wavelength

Excitation Emission Matrix (EEM)

Bench top scanning fluorometer

Humic-like compounds (terrestrial origin)

Tryptophan-like peak related to microbial activity + correlated with BOD₅

However...Not suitable for remote field sites or if high resolution records are required.

Fellman et al. (2012) SOTE 39, 149-158

Hudson et al. (2008) Lim. & Oce. 55, 2452
Submersible fluorometers
Challenges to in-situ monitoring

- Quenching – e.g. temperature;
- Matrix interference – e.g. suspended particles in water column;
- Inner-filtering - concentration effect;
- Measurement repeatability - between/within sites and between sensors;
- To date no rigorous tests of submersible tryptophan fluorometers have been conducted.
The objectives of this study were to:

1. Test the performance of two commercially available tryptophan fluorometers in the lab;

2. Develop empirical correction factors to account for fluorescence quenching and matrix interference;

3. Undertake a field trial to assess sensor performance and test correction factors.
Minimum Detection Limit (MDL) and precision

<table>
<thead>
<tr>
<th></th>
<th>T1</th>
<th>T2</th>
<th>C1</th>
<th>C2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Calibrated relationship</strong></td>
<td>$y = 0.997x - 0.133$</td>
<td>$y = 1x + 0.0009$</td>
<td>$y = 1x - 0.00007$</td>
<td>$y = 1x + 0.00006$</td>
</tr>
<tr>
<td><strong>Relationship with Varian (ppb)</strong></td>
<td>$y = 0.99x - 0.1255$</td>
<td>$Y = 1x + 0.0022$</td>
<td>$y = 1x + 0.0076$</td>
<td>$y = 0.99x + 0.0129$</td>
</tr>
<tr>
<td><strong>Relationship with Varian (R.U)</strong></td>
<td>$y = 0.002x + 0.0041$</td>
<td>$y = 0.002x + 0.0044$</td>
<td>$y = 0.002x + 0.0044$</td>
<td>$y = 0.002x + 0.0044$</td>
</tr>
<tr>
<td><strong>MDL ± SD</strong></td>
<td>$1.99 ± 0.53$</td>
<td>$1.92 ± 0.57$</td>
<td>$0.17 ± 0.06$</td>
<td>$0.19 ± 0.15$</td>
</tr>
<tr>
<td><strong>Precision: CV (5ppb)</strong></td>
<td>3.03</td>
<td>2.49</td>
<td>0.45</td>
<td>0.22</td>
</tr>
<tr>
<td>(50ppb)</td>
<td>0.03</td>
<td>0.02</td>
<td>0.00</td>
<td>0.01</td>
</tr>
<tr>
<td>(400ppb)</td>
<td>3.79</td>
<td>4.86</td>
<td>4.63</td>
<td>6.27</td>
</tr>
<tr>
<td><strong>Accuracy (RMSE)</strong></td>
<td>0.63</td>
<td>0.62</td>
<td>0.57</td>
<td>0.58</td>
</tr>
</tbody>
</table>

**significant difference in precision at low concentration**
Thermal quenching

Raw data

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Tryptophan signal (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>200</td>
</tr>
<tr>
<td>10</td>
<td>150</td>
</tr>
<tr>
<td>15</td>
<td>100</td>
</tr>
<tr>
<td>20</td>
<td>50</td>
</tr>
<tr>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>30</td>
<td>50</td>
</tr>
<tr>
<td>35</td>
<td>100</td>
</tr>
</tbody>
</table>

Graphs showing the relationship between temperature and tryptophan signal.
Turbidity interference

100 NTU

200 NTU

900 NTU
Turbidity interference (Clay)

- Particle size influences response: attenuation greater with clay.
- Sensor specific responses: differences between and within manufacturers.
- Sensor specific calibrations may be required.
Turbidity interference (silt)

Out of range for 500ppb
Turbidity interference (clay)

Sensor C1
95% CI overlap
> 200 NTU

Sensor C2
95% CI overlap
> 200 NTU

Sensor T1
95% CI overlap
> 200 NTU
Turbidity interference (silt)

Sensor C1

Sensor C2

No 95% CI overlap

Sensor T1

95% CI overlap > 600 NTU

95% CI overlap > 800 NTU
Turbidity correction

Clay (Fullers Earth)

Silt (glacial outwash)
Urban field test site

Urban field test site

Chelsea fluorometer and Manta 2
- Stage
- Turbidity
- EC
- Tw
- Tryptophan

ISCO pump sampler
Field trial
Field trial: raw data
Field trial: corrected data

FE = Fullers Earth (clay)  GS = Glacial silt
Field trial: corrected data

- **T1 raw (ppb) vs. Lab tryptophan (ppb)**
  - $R^2 = 0.92$
  - $m = 0.69 \pm 0.04$
  - $c = 0.91 \pm 4.53$

- **T1 silt + Tw correction (ppb) vs. Lab tryptophan (ppb)**
  - $R^2 = 0.88$
  - $m = 0.95 \pm 0.07$
  - $c = -2.41 \pm 5.90$

- **T1 Tw correction (ppb) vs. Lab tryptophan (ppb)**
  - $R^2 = 0.92$
  - $m = 0.74 \pm 0.04$
  - $c = 1.17 \pm 4.55$

- **T1 clay + Tw correction (ppb) vs. Lab tryptophan (ppb)**
  - $R^2 = 0.91$
  - $m = 0.67 \pm 0.04$
  - $c = 7.95 \pm 4.38$
Field trial: corrected data

- For C1 raw (ppb): $R^2 = 0.77$, $m = 0.80 \pm 0.09$, $c = -23.0 \pm 10.84$
- For C1 silt + Tw correction (ppb): $R^2 = 0.56$, $m = 1.04 \pm 0.18$, $c = -14.71 \pm 15.58$
- For C1 Tw correction (ppb): $R^2 = 0.76$, $m = 0.86 \pm 0.10$, $c = -22.4 \pm 10.86$
- For C1 clay + Tw correction (ppb): $R^2 = 0.76$, $m = 0.96 \pm 0.11$, $c = -2.03 \pm 8.75$
Spatial survey (initial result)

All sites: $R^2 = 0.60$

River sites: $R^2 = 0.91$

Habitat type – clear residual pattern

Canal
Pond
River
Effluent

River samples: $R^2 = 0.67$

All samples: $R^2 = 0.72$

River samples: $R^2 = 0.64$
Conclusions

- Quenching of $T_1$ fluorescence was identified in the lab and varied between sensors (Turner & Chelsea)

- Temperature compensation appears relatively simple but evidence of \textit{hysteresis} requires further investigation

- Sediment particle size influenced sensor response to turbidity increases \textit{(implies site specific calibrations may be necessary)}

- Field tests highlight the potential to develop and apply correction factors to improve in-situ data output during both baseflow and event conditions

- Further work will improve calibration for $\text{BOD}_5 - T_1$ fluorescence
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