Physical Geography

Water, Climate, Environmental Change

UNIVERSITY OF BIRMINGHAM

Temperature and Turbidity Effects on the Fluorescence Intensity of Uranine, Resazurin and Resorufin

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Motivation

- Understanding groundwater – surface-water interactions and fluvial metabolic processes.
- Assessing those processes during variable environmental conditions (fig. 1).

Fig 1: Stream during high discharge with increased turbidity

Introduction

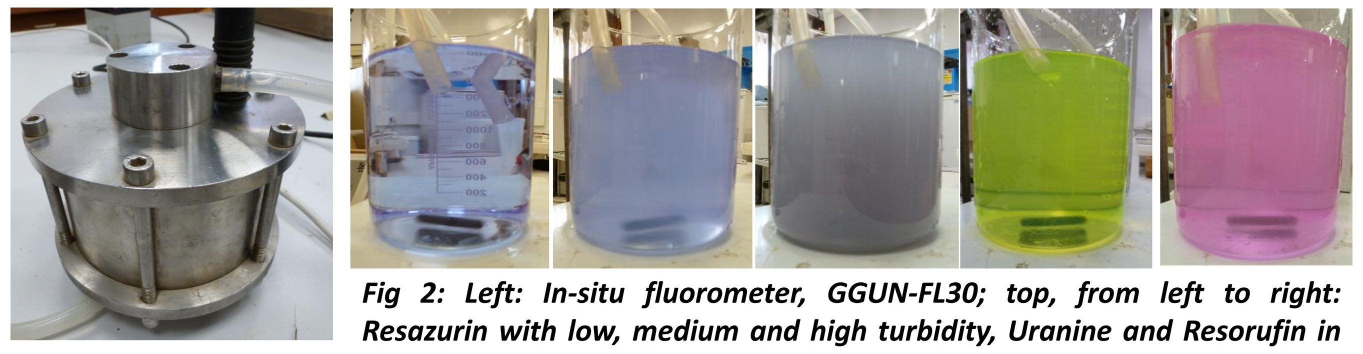

Fig 2: Left: In-situ fluorometer, GGUN-FL30; top, from left to right: Resazurin with low, medium and high turbidity, Uranine and Resorufin in solution.

- The use of reactive 'smart' tracers (Resazurin and Resorufin) has increased in recent years to study the metabolic activity in streams and groundwaters[1].
- Low-cost, high frequency in-situ measurements of the tracer concentrations can be achieved by GGUN-FL30 fluorometers (fig. 2)[2].
- However, the effect of turbidity on the tracer signals is not well understood, which might lead to misinterpretations (fig. 3)[3].

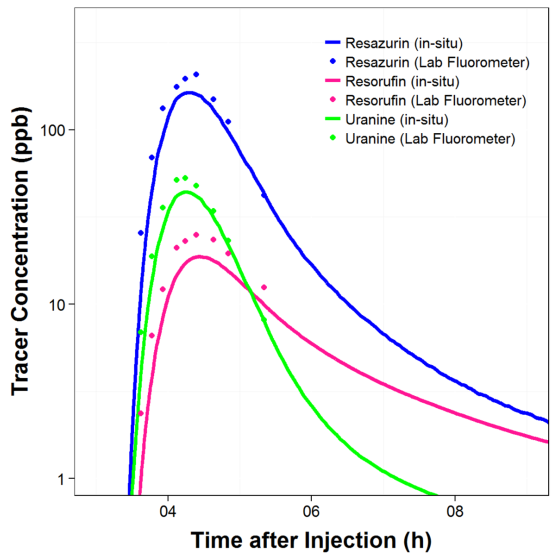

Fig 3: Breakthrough curves (in ppb) for three different tracers (Resazurin, Uranine and Resorufin) measured in-situ with a GGUN-FL30 (lines) and in the lab with a lab fluorometer (points).

Methodology

- Fluorescence intensities of different concentrations of Uranine, Resazurin and Resorufin in combination with different particle concentrations (Fuller's Earth) were analyzed with the GGUN-FL30 fluorometer in bench-top mode under controlled temperature (23°C) and pH (8.5) conditions.
- Fluorometers, filled with tracer solutions in the central chamber, were placed in a refrigerator and cooled from ≈23 °C to 5 °C in roughly 6 hours.

References

1. Haggerty, R., Argerich, A. & Martí, E. 2008. Development of a "smart" tracer for the assessment of microbiological activity and sediment-water interaction in natural waters: The resazurin-resorufin system. *Water Resources Research,* 44.
2. Lemke, D., Schnegg, P.-A., Schwientek, M., Osenbrück, K. & Cirpka, O. A. 2013b. On-line fluorometry of multiple reactive and conservative tracers in streams. *Environmental earth sciences,* 69, 349-358.
3. Schnegg, P.-A. & Flynn, R. 2002. Online field fluorometers for hydrogeological tracer tests. *Isotope und Tracer in der Wasserforschung,* 19.

Influence of turbidity on fluorescence intensity

- The impact of turbidity (quantified by particle concentration) on the fluorescence intensities of the three tracers depends on the specific tracer and its concentration (fig. 4).
- The quantitative effect of turbidity on the measured fluorescence intensity decreases linearly with increasing fluorescence intensity, independent of the tracer (fig. 5).
- The relative reduction (%) of the slope of the calibration measurements (mV/ppb) decreases linearly with increasing turbidity (particle concentration), independent of the tracers. This relative reduction can be used for the correction of the calculated tracer concentrations for turbidity.

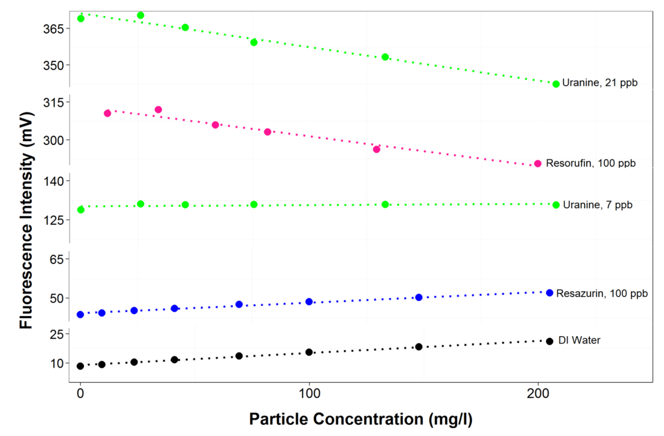

Fig 4: Impact of turbidity (particle concentration in mg/l) on the fluorescence intensities (mV) of three tracers and DI water, under different concentrations. Dotted lines are regression lines.

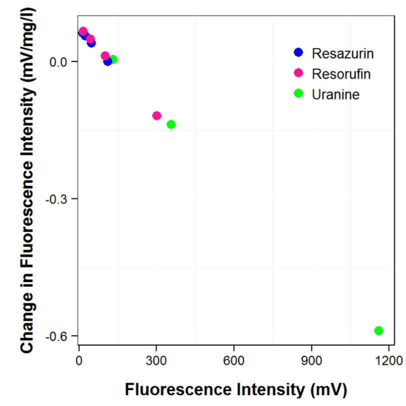

Fig 5: Change of the fluorescence intensity of three tracers due to increased particle concentrations (mV/mg/l) against the fluorescence intensity at one turbidity level.

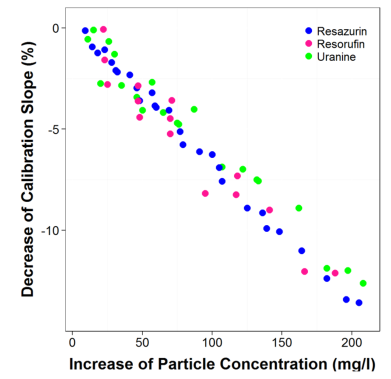

Fig 6: Change of the calibration slope (fluorescence intensity against tracer concentration) (%) for three tracers against the increased turbidity (particle concentration in mg/l) between the calibration period and the in-situ measurements.

Correction of fluorescence intensity for turbidity

- Resazurin has an impact on the turbidity measurements by detector 4, which is normally assumed to be independent of the tracer (fig. 7). The impact of Resazurin depends on the particle and tracer concentration.
- Tracer concentrations can be calculated based on Eq. 1, which includes temperature and turbidity correction coefficients. The latter are functions of the intensity measured at detector 4 and the Resazurin concentration.
- Calculated concentrations corrected for turbidity reproduce the known tracer concentrations better than the uncorrected concentrations (fig. 8). That can also be observed for lower or higher tracer concentrations.

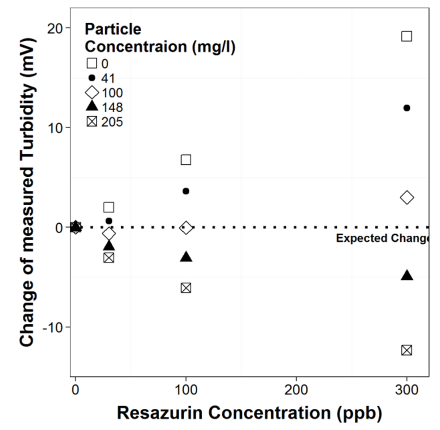

Fig 7: Change of measured turbidity (detector 4 of GGUN-FL30) (mV) against Resazurin concentration (ppb) for different particle concentrations (mg/l).

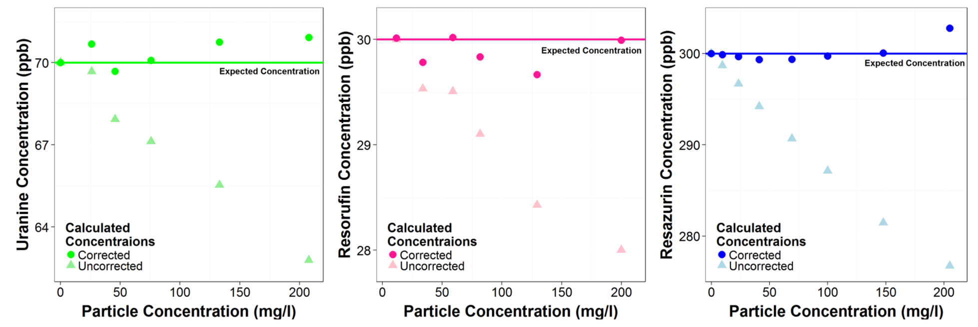

Fig 8:Uncorrected (triangles) and for turbidity corrected (circles) calculated tracer concentrations (ppb) against turbidity (particle concentration in mg/l) for three tracers (Uranine, Resorufin and Resazurin).

$$\left[\begin{pmatrix} k_1^1 & k_2^1 & k_3^1 \\ k_1^2 & k_2^2 & k_3^2 \\ k_1^3 & k_2^3 & k_3^3 \end{pmatrix} \circ \begin{pmatrix} p_1^1 & p_2^1 & p_3^1 \\ p_1^2 & p_2^2 & p_3^2 \\ p_1^3 & p_2^3 & p_3^3 \end{pmatrix} \circ \begin{pmatrix} q_1^1 & q_2^1 & q_3^1 \\ q_1^2 & q_2^2 & q_3^2 \\ q_1^3 & q_2^3 & q_3^3 \end{pmatrix}\right] \bullet \begin{pmatrix} C_1 \\ C_2 \\ C_3 \end{pmatrix} = \begin{pmatrix} U_1 \\ U_2 \\ U_3 \end{pmatrix}$$

Eq 1: Calculation matrix of the tracer concentrations (C_i, ppb), based on calibration coefficients (k^i_j, mV/ppb), temperature correction coefficients ($p^i_j(t)$, function of the temperature t), turbidity correction coefficients ($q^i_j(C_{Raz}, U_4)$, function of the concentration of Resazurin and the measured intensity at detector 4 (mV)) and the measured intensities (U_j, mV), where i = 1,2,3 indicate the three tracers, j = 1,2,3 the three detectors and ○ the element-wise Hadamard product.

Influence of temperature on fluorescence intensity

- The fluorescence intensities of all three tracers decrease with increasing temperature, with the strongest effect observed for Resorufin, followed by Resazurin and Uranine (fig. 9).
- The tracer concentrations tend to have an effect on the temperature dependent relative fluorescence intensities.

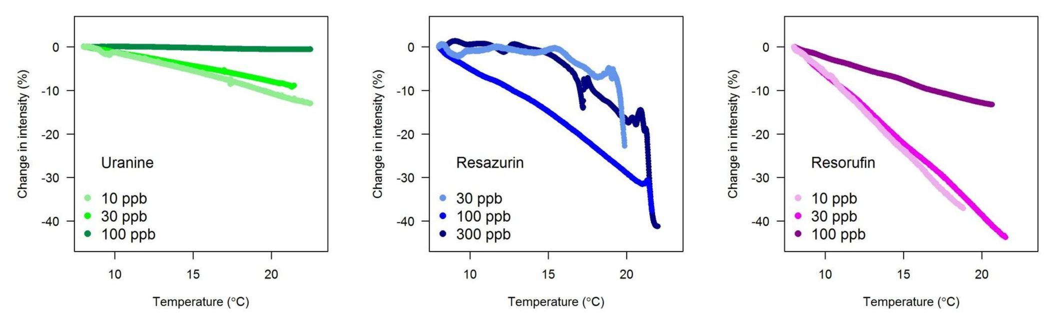

Fig 9: Change of the fluorescence intensity (%) of three tracers (left: Uranine, center: Resazurin, right: Resorufin) at different concentrations against the temperature (°C).

Conclusions

- **The effect of turbidity on the fluorescence intensities of Uranine, Resazurin and Resorufin depends on the tracer concentrations.**
- **The calculation of the tracer concentrations can be corrected for turbidity.**
- **The impact of temperature on the relative fluorescence intensities seems to be tracer concentration dependent.**

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