BGS-UKRI Briefing Note

Tryptophan-like fluorescence (TLF) - a rapid *in-situ* screening tool for assessing faecal contamination risk in groundwater

Benchmark research evidence from a systematic programme of field testing in Africa and India

Keywords

Tryptophan, fluorescence, TLF, sensors, faecal contamination, groundwater, risk

Front cover

Photo of field team undertaking fieldwork in Malawi, 2016 (Lapworth DJ, BGS)

Bibliographical reference

Lapworth D.J & Sorensen J.P.R 2018. BGS-UKRI Briefing Note: Tryptophan-like fluorescence (TLF) as a rapid screening tool for assessing faecal contamination risk in groundwater. BGS Open Report OR/18/058, pp 3.

UK Research and Innovation
British Geological Survey
Expert | Impartial | Innovative

Highlights

- Tryptophan-like fluorescence (TLF) in groundwater is a novel *in-situ* approach to rapidly detect faecal contamination risk, which often leads to diarrheal disease, a leading cause of death for children under the age of 5 years.
- TLF has been tested against slower conventional plate counting methods in 5 countries (India, Kenya, Malawi, Uganda, and Zambia) and at >500 groundwater sources.
- TLF is most accurate in waters with low dissolved organic carbon and low-turbidity, so is particularly well suited for monitoring groundwater sources.
- TLF is strongly correlated with current methods for measuring thermotolerant coliforms.
- TLF technology is a commercially available, easy-to use, presents results in real-time and requires no chemical supplies.

Motivation

Faecally contaminated drinking water, a leading cause of diarrhoeal diseases, is currently consumed by an estimated 1.8 million people globally[1]. Diarrhoeal diseases are a leading cause of death for children under five years old[2,3]. The United Nations' Sustainable Development Goal (SDG) 6 calls for universal access to safe drinking water. Groundwater is a major source of drinking water globally[1] – in many regions it is the only source of drinking water in the dry season – and faecal contamination of groundwater remains a major concern.

There is now strong evidence for the suitability of tryptophan-like fluorescence (TLF) for assessing the risk of faecal contamination in groundwater. TLF provides a robust and rapid *in-situ* screening tool to enable more rapid monitoring of drinking water quality to help assess progress towards SDG 6.

Background

Faecal contamination of drinking-water sources is typically assessed using bacterial indicators such as *Escherichia coli* or thermotolerant coliforms (TTCs). These culture-based indicators are time-consuming, taking up to 24 hours to report results, and are impractical for rapid surveys.

The evidence base for using TLF to assess contamination risk in groundwater in the peer-reviewed literature has grown considerably in the last 3 years[4-10]. TLF has been systematically compared with a range of conventional technologies and tested in a range of climatic and hydrogeological settings to assess its ability to identify microbiological contamination, based on WHO risk categories of colony forming units (cfu) per 100 mL[11] (whereby 0 = very low risk; 1-9 = low risk; 10-99 = intermediate risk; 100-999 = high risk; and >1000 = very high risk).

TLF is most accurate in waters with low dissolved organic carbon (DOC) and low-turbidity, such as groundwater. It is strongly correlated with current methods for measuring TTCs and is effective to infer faecal contamination by TTCs above 10 cfu, i.e. for intermediate risk sources and above.

Results are generated real-time, with stable readings are usually obtained after 1 minute. Results can also be recorded and captured digitally with some sensors. A day of field-based training is generally adequate to understand the methodology and good sampling protocols are essential to obtain robust results[12]. TLF technology is commercially available and reagent-less. As such, it does not generate potentially hazardous waste, unlike many alternative techniques.

Evidence

Tryptophan is an amino acid associated with cellular activity and extracellular material, which fluoresces at low excitation and emission wavelengths. TLF sensors target the approximate excitation-emission wavelength pair, Ex280 nm/Em350 nm, in the region where the tryptophan signal is greatest.

Summary results presented here are from a standardised multi-country assessment including India, Kenya, Malawi, Uganda, and Zambia where TLF was tested against time-consuming culture-based methods for TTCs at >500 groundwater sources.

Correlation with TTCs:

There is a strong correlation between TLF and TTCs (ρ = 0.78, Spearman's rank correlation coefficient). Figure 1 shows a comparison of TLF with TTCs obtained using culture-based methods, with TLF data grouped based on WHO risk categories[11]. Significant differences exist between median TLF in different WHO risk categories.

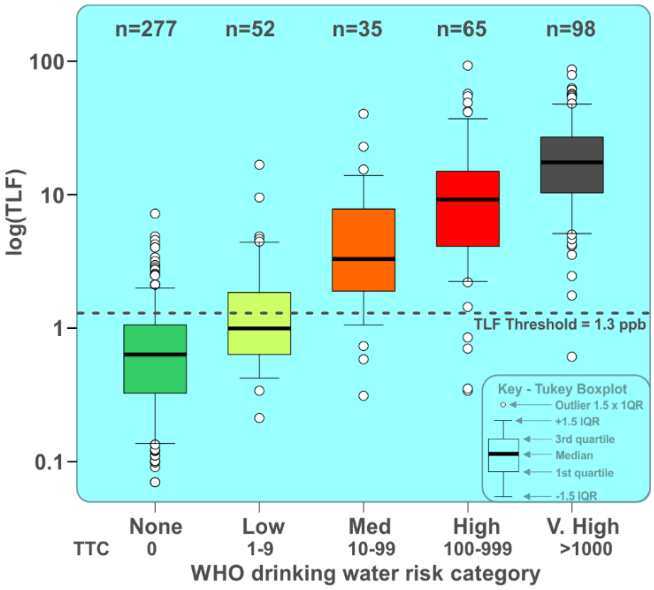

Figure 1. Comparison of groundwater TLF values with paired TTC results grouped by WHO risk categories

TLF is best applied for groundwater assessments:

Variables that can attenuate or enhance the TLF signal in water include dissolved organic carbon (DOC), turbidity, temperature and pH. In most groundwaters, these do not vary appreciably and have minimal interference with the TLF measurements. However, in some groundwater and many surface waters, these variables can vary substantially for which corrections to fluorescence data may be a possible solution[13].

TLF threshold to infer contamination risk:

Based on the current groundwater data (Figure 1), a threshold of 1.3 parts per billion tryptophan (corresponding to 10 cfu/100 mL) has been tested and validated using logistic regression and false positive and false negative rates to assess TTC contamination[8]. This threshold is equivalent to being able to differentiate between sites classed as medium risk or greater (using TTC results) based on WHO risk categories[11], but was not able to classify contaminated sources with <10 TTC cfu/100 mL. This groundwater threshold matches that reported by Sorensen *et al.* (2018), which also included surface water data and *E. coli* results[8].

Recommendations for future development

Commercial TLF sensors are already available on the market, yet, a key barrier to wider operational use is the relatively high capital cost of these sensors. Lower cost systems (c. £1000) are currently being developed and other improvements needed include better field calibration standards, improved stability of sensor standards, and employment of cuvette-based systems for sample analysis. Further case studies applying this technique in new settings are needed to test the suitability more widely, particularly in groundwater systems with high DOC (either due to anthropogenic pollution or natural sources). The method could also be further compared to other new techniques for microbiological risk assessment as they become available, such as field based real-time polymerase chain reaction (PCR) methods.

Conclusions

Relying exclusively on standard culture-based methods for assessing faecal contamination in groundwater sources limit the ability to monitor progress towards SDG6 for improved drinking water quality. TLF provides a rapid screening tool for groundwater quality, which could help monitor and assess water quality risks at drinking water sources, water point integrity and the impact of specific interventions.

For further details please contact:

Dan Lapworth, British Geological Survey, Wallingford, Oxfordshire, OX10 8BB djla@bgs.ac.uk

Acknowedgments:

BGS research was funded by UPGro (NE/L002078/1), REACH (trigr - GA/16F/057), FCFA (Hycristal – NE/M02452/1), Water for People and BGS research programmes. BGS partnered with many institutions to collect field observations between 2012-2017. UPGro, REACH (Aries code 201880) and FCFA research programmes are funded by UK Aid from the UK Department for International Development (DFID) for the benefit of developing countries. *AfriWatSan* partners funded through The Royal Society – DFID Africa Capacity Building Initiative. This work does not necessarily reflect the UK governments official policies.

References

[1] Bain et al. (2014) Global assessment of exposure to faecal contamination through drinking water based on a systematic review. *Tropical Medicine & International Health,* 19 (8), 917-927

[2] WHO Fact Sheet (updated May 2017) http://www.who.int/mediacentre/factsheets/fs330/en/

[3] Guerrant et al. (2002) Updating the DALYs for diarrhoeal disease. *Trends in Parasitology,* 18 (5), 191-193

[4] Lapworth et al. (2008). Tracing groundwater flow and sources of organic carbon in sandstone aquifers using fluorescence properties of dissolved organic matter (DOM). *Applied Geochemistry, 23*(12), 3384-3390. https://doi.org/10.1016/j.apgeochem.2008.07.011

[5] Stedmon et al. (2011). A potential approach for monitoring drinking water quality from groundwater systems using organic matter fluorescence as an early warning for contamination events. *Water Research,* 45(18), 6030-6038. https://doi.org/10.1016/j.watres.2011.08.066

[6] Sorensen et al. (2015) Are sanitation interventions a threat to drinking water supplies in rural India? An application of tryptophan-like fluorescence. *Water Research,* 88, 923-932. https://doi.org/10.1016/j.watres.2015.11.006

[7] Sorensen et al. (2015). In-situ tryptophan-like fluorescence: a real-time indicator of faecal contamination in drinking water supplies. *Water Research,* 81. 38-46. https://doi.org/10.1016/j.watres.2015.05.035

[8] Sorensen et al. (2018). Real-time detection of faecally contaminated drinking water with tryptophan-like fluorescence: defining threshold values. *Science of the Total Environment, 622*, 1250-1257. https://doi.org/10.1016/j.scitotenv.2017.11.162

[9] Sorensen et al. (2018). Online fluorescence spectroscopy for the real-time evaluation of the microbial quality of drinking water. *Water Research*, 137, 301-309. https://doi.org/10.1016/j.watres.2018.03.001

[10] Nowicki et al. (2018) Tryptophan-like fluorescence as a measure of microbial contamination risk in groundwater. *Science of the Total Environment*, 646, 782-791. https://doi.org/10.1016/j.scitotenv.2018.07.274

[11] WHO, 1997. Guidelines for Drinking-Water Quality (2nd Edition) WHO Press. Switzerland, Geneva.

[12] Ward et al. (2018). Assessing microbiological contamination in groundwater sources: Field note on using Tryptophan-like Fluorescence (TLF) probes. BGS Open Report OR/18/042, pp 12.

[13] Khamis et al. (2015) In situ tryptophan-like fluorometers: assessing turbidity and temperature effects for freshwater applications. *Environmental Science: Processes & Impacts*, 17, 740-752. https://pubs.rsc.org/en/Content/ArticleLanding/2015/EM/C5EM00030K#!divAbstract

[14] Innovate UK grant 12281: Development of low-cost and portable optical sensor for the instantaneous indication of pathogens in drinking water.