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Assessing microbiological contamination in groundwater sources: Field note on using Tryptophan-like Fluorescence (TLF) probes

Groundwater Programme

Open Report OR/18/042

BRITISH GEOLOGICAL SURVEY

GROUNDWATER PROGRAMME

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Assessing microbiological contamination in groundwater sources: Field note on using Tryptophan-like Fluorescence (TLF) probes

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Keywords

Tryptophan like fluorescence, water quality, field measurements

Front cover

Picture showing water quality sampling from a hand pump in Uganda (Dan Lapworth)

Bibliographical reference

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This BGS Open Report is an output from projects funded under the UPGro and REACH programmes focussed on the application of in-situ sensors for microbiological risk screening of groundwater sources in Africa (UPGro catalyst grant NE/L002078/1 and REACH catalyst grant GA/16F/057). These projects have been funded by UK aid from the UK Government, and led by the British Geological Survey, however the views expressed do not necessarily reflect the UK Governments official policies.

https://upgro.org/catalyst-projects/mapping-groundwater-quality/

https://reachwater.org.uk/funding/catalyst-projects-call-1/sensor-technology-for-rapidly-assessing-water-quality-risks-for-vulnerable-users/

1 Summary

This Field Note provides some practical guidance on the use of tryptophan-like fluorescence (TLF) sensors for assessing water quality in drinking water sources. The guidance is based on experience gained using TLF sensors to assess microbiological contamination risks in groundwater sources used for drinking water in Africa and the Indian Subcontinent. This note covers the use of TLF sensors to make measurements of grab samples from groundwater sources. A suggested reading list of selected research papers that have used TLF sensors in the field is provided at the end of the Note for those interested in further details related to the use of TLF sensors in the field.

2 Tryptophan-Like Fluorescence

Using fluorescence to measure water quality parameters is an established approach; dissolved organic matter (DOM), chlorophyll and algal biomass can all be detected in this way (Envirotech Online, 2017). A fluorimeter works by emitting light at a specified wavelength and detecting light emitted at a different wavelength from a target molecule or groups of molecules of similar chemical composition.

Tryptophan is an amino acid associated with cellular activity but is also associated with extracellular material and fluoresces at low excitation and emission wavelengths. TLF sensors target the approximate excitation-emission wavelength pair Ex280 nm/Em350 nm, where the TLF signal is greatest. A range of TLF probes/sensors are available on the market, but all aim to quantify the presence and intensity of fluorescence in the TLF region.

Recently, this technology has been applied to groundwater and early studies show that TLF has the potential to become a rapid screening tool for assessing levels of faecal contamination (Sorensen et al., 2015, 2018a). TLF data has been found to correlate with thermotolerant coliform (TTC) counts, which are used as an indicator of pathogens (Sorensen et al., 2018b, Nowicki et al., 2018., Sorensen et al., 2016, Baker et al., 2015).

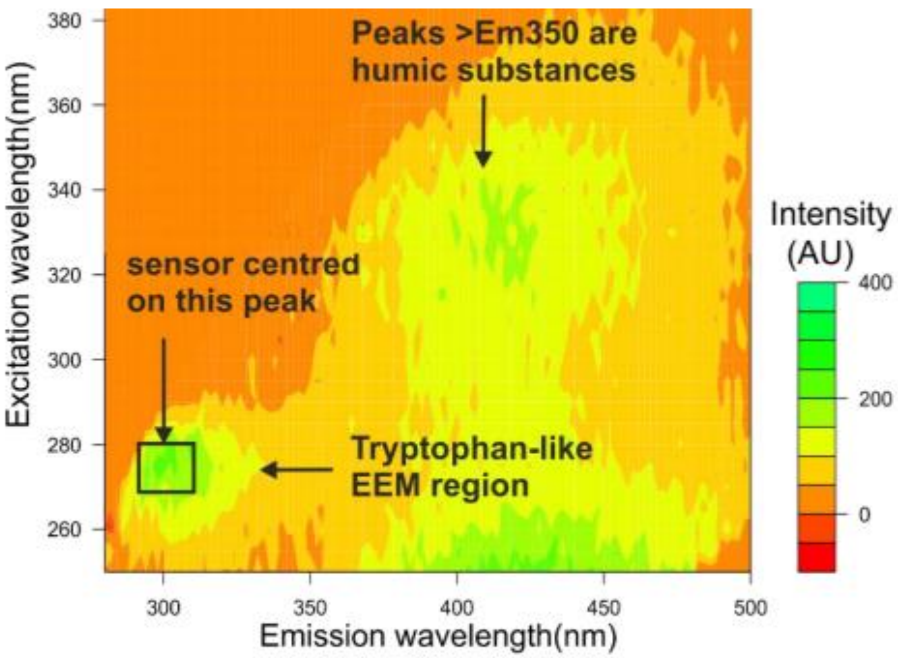


Figure 2.1 Left: Excitation-emission matrix (EEM) highlighting peaks and wavelength pairs used quantifying TLF and humic-like substances

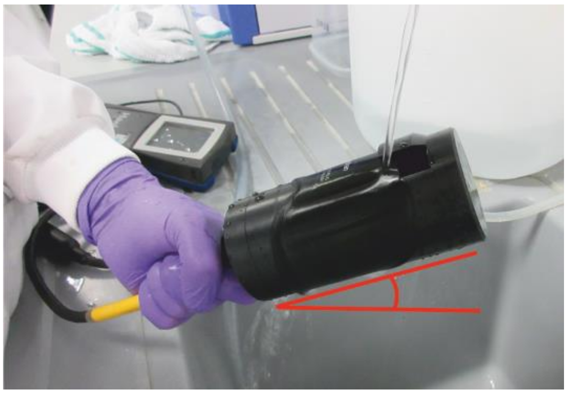
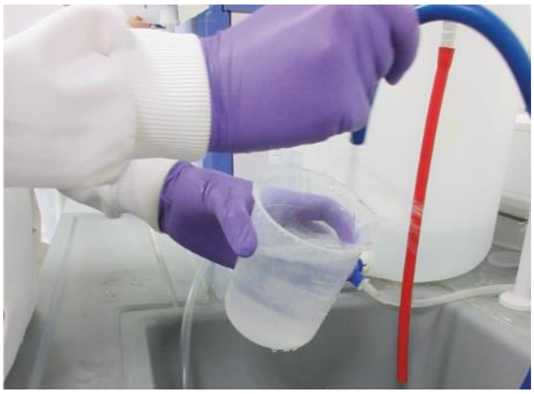
3 Using TLF probes in the field

3.1 EQUIPMENT NEEDED

- TLF probe
- Laptop and connection cable or handset to read sensor output
- Clear plastic beaker to make measurements in (optional)
- UV protective sampling bucket/container – *e.g.* new plastic or metal container with lid (this can also be used to carry sensors between sites)
- Sterile lab gloves for handling sensor in the field – change gloves between sites
- Alcohol wipes to clean the probe at the end of the day or after a highly contaminated site
- GPS and camera to record details of each site
- Data recording sheets (See example in Section 4)
- Equipment for measuring other parameters *e.g.* temperature, turbidity, pH, SEC, DOC etc.

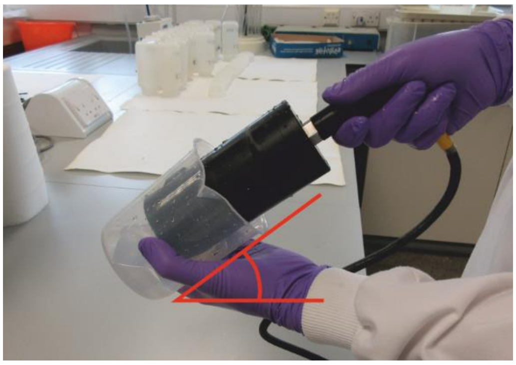
3.2 GROUNDWATER TLF MEASUREMENT PROCEDURE

1. Select an area to set up the equipment, preferably in the shade and sheltered from the wind to minimise the risk of UV interference or contamination from suspended particles in the air (Figure 3.1; Figure 3.3).
2. Before commencing water quality sampling, consider if the site has been purged adequately – if it is being used daily by several users from the community then it will normally be purged adequately, otherwise it is advised to pump 3 borehole volumes as a minimum.
3. Samples must be taken directly from the groundwater source, in the same manner as a regular user, i.e. direct from a hand pump spout or using a rope and bucket for a shallow well (Figure 3.2).
4. Put on gloves before handling the sensor and rinse it directly from the outflow of the pump spout or using in-situ equipment regularly used to obtain water from the well. Rinse the sensor (with particular attention to the sensor window) at an angle to reduce contamination transfer from hands and sensor cable to sensor window area.

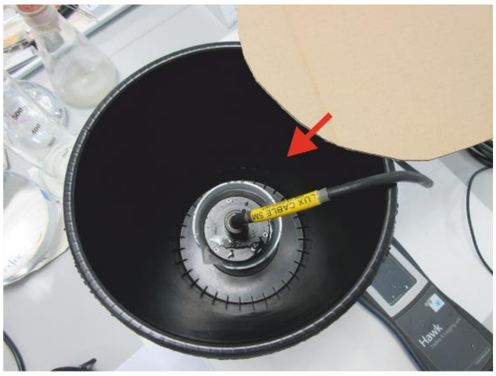



5. Rinse at least 3 times and fill the TLF measurement beaker directly at the outflow of the pump spout; take care not to contaminate the inside of the measurement beaker with hand contact.

6. Place the TLF probe gently in the measurement beaker. Place it at an angle to reduce air trapping in the sensor cavity and check that no air is trapped.




7. Place the measurement beaker in a UV blocking container and swirl the sensor gently to remove any residual air bubbles before starting to take readings. Place a lid over the container before monitoring sensor output.

8. Monitor the fluctuation of readings until stable readings are obtained (values usually stabilise at one decimal place and ± 10%), this is usually obtained within 1-2 minutes unless, for example, there are fluorescing particles which are settling out or there is TLF leaching from the sensor housing or elsewhere due to contamination.
9. Once stable readings are obtained take a minimum of 4 manual readings and then record the average of these readings. Negative readings (close to zero) are possible for TLF, if using a factory calibration where the zero offset on the calibration can be slightly off, but make sure that you do not have bubbles in the sensor window as bubbles can cause negative readings.
10. If measuring other water quality parameters, take all samples at the same time before pumping is stopped and take readings – this should be done by someone else using a separate container while the TLF readings are being recorded.
11. Repeat the whole process (steps 3-10) until two repeatable TLF readings are obtained (i.e. TLF readings are within 10%).
12. Disconnect the TLF probe from the laptop/Switch off the handset to save batteries.
13. Place the TLF probe back in the storage container when finished, taking care not to place it on the ground or touch the sensor while emptying the sampling bucket.

Figure 3.1 Left: TLF probe and clean thermometer in rinsing bucket. Middle: TLF probe and thermometer covered by lid and placed in shade for TLF reading. Right: TLF probe used in a clean dedicated smaller container with integrated lid [Jade Ward and Dan Lapworth/BGS]

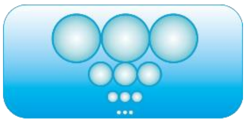
Figure 3.2 Left: Always fill the sampling bucket directly from the borehole spout or the bucket normally used for a shallow well. Right: for other sites such as for a dry river bed well use the sampling container which is normally used to draw water from the source [Jade Ward/BGS]

Figure 3.3 Left: arrangement of TLF probe with small beaker for taking measurements. Right: covering TLF probe with thick cloth to stop UV light interference [Saskia Nowicki/REACH]

3.3 POTENTIAL SOURCES OF ERROR WHEN MAKING TLF MEASUREMENTS

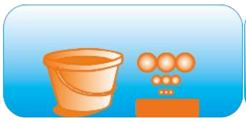
Some key issues we have identified through using TLF probes:

1. **Air bubbles** can lower TLF readings
2. **Contamination sources**:
    a. **Leaching of TLF material** from containers and the exterior of contaminated sensor units
    b. **Poor handling or inappropriate storage of the sensor**
3. **UV light interference** will affect reading level and stability in the field
4. **Temperature changes** can lead to changes in TLF measurements
5. **High turbidity** may influence TLF readings
6. **Recording measurements**:
    a. **Negative readings** can be real observations – don't ignore them
    b. **Readings must stabilise** before values are recorded, several measurements must be recorded. Small fluctuations are expected initially after the probe is switched on
7. **Equipment connections**: potential weak points include connections between sensors and readers



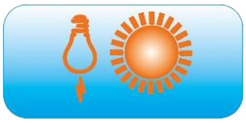
1. Air bubbles can get trapped in the sensor cavity/surface where the observations are made, these need to be removed or minimised prior to taking readings.

- These bubble effects can be minimised by placing the sensor in the sample container at an appropriate angle to minimise the likelihood of air being trapped and also by swirling the sensor in the container to remove any small rogue air bubbles that are on the sensor.
- Particular care and caution is required if the groundwater is degassing which can generate small air bubbles in-situ.



2. Contamination sources: It is critical not to contaminate your water sample, this can be done inadvertently in a number of ways: by handling the sensor; by leaching from the sensor casing if high TLF material has accumulated on the sensor; and by storing and using the sensor in a way that brings it in to contact with TLF material in the field.

- Always rinse the TLF probe and sampling containers thoroughly with sample water or distilled water before each reading (Figure 3.1)
- Ensure any part of the probe or cable that may come into contact with the water sample is never placed on the ground or another potentially contaminated surface. It should be stored in a dedicated clean sampling container with a lid when not in use.
- Do not touch any part of the probe or sampling bucket that comes into contact with the groundwater sample. If this happens, carefully re-rinse the probe and bucket several times with sample water. Use clean lab gloves to handle the sensor and cable.
- Always use a clean plastic or metal container – test the container first with a low TLF groundwater (or distilled bottled water) to ensure that the container does not leach TLF compounds.
  Always sample the water source in the same manner that the local users would use to draw water (Figure 3.2)

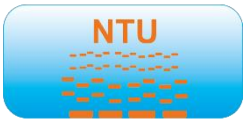


3. **Ambient UV light interference** also impacts the TLF result, therefore it must be eliminated whilst the TLF reading is being taken (Figure 3.1; Figure 3.3).

- Always cover the sampling container with a dedicated lid or cover whilst taking a TLF reading. Do not use a transparent outer container, a transparent inner container (see Figure 3.3) to put the sensor in is very helpful to check for bubbles and turbidity prior to taking measurements.

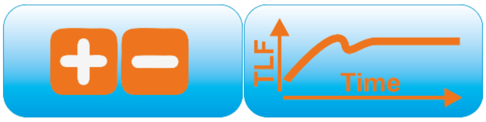
4. **Temperature** can influence the TLF reading due to alteration of the absorbance of fluorescence by the probe. This is known as thermal quenching. For the same concentration of tryptophan, a sample at a higher temperature will give a lower tryptophan reading.

- Always record the temperature of your water sample *at the time of TLF recording* – without contaminating it.



5. The influences of **turbidity, pH and specific electrical conductivity (SEC) and especially organic carbon** on TLF readings are important to consider, therefore it is recommended to also take reading of these water quality parameters.

- Always allow time for suspended particles to settle out and observe how this changes the TLF readings. Only take readings once the output has stabilised over a period of 3 minutes.
- If possible, note down pH and SEC, particularly if pH is >8 or <6 and SEC is >3000 µS/cm as these more extreme pH or high DOC (e.g. >10 mg/L) and high SEC smaples can potentially cause changes in TLF.



6. **Recording measurements:** Due to the differences between the quality of water used in the blank calibration standard and low TLF water samples small **negative readings are possible**. Air readings/air bubbles can also lead to negative readings so make sure this is not the reason.

- Once **stable readings are obtained, record several readings** (e.g. 10) every 5-10 seconds manually or use software to record automatically to obtain an average measurement.
- **Do not ignore negative values**, record these as usual once all other checks have been made to establish that the sensor is measuring in water not air (e.g. an air pocket in sensor cavity).

7. **Equipment connections:** Depending on the sensor that is being used, **the connection between the reader and the sensor may be a weak point**.

- Be careful when holding the sensor unit and do not put stress on the connection with the reader
- Take care to look after and protect sensor-reader connections, they can be vulnerable to damage if used incorrectly.

4 Example field sheet

General site information			
Village Name		Source type*:	
Easting		Northing	
Elevation		Date of completion	
Name of assessor		Date of assessment	

*Borehole-hand-pump, Well-hand-pump, Well, Spring, Dug river bed well, Other

Sample ID	**Sampling Time**	**TTC sample Y/N**

Trytophan Like Fluorescence (TLF) Measurment					
Notes:	Manual tryptophan readings taken after stable readings are obtained				
Water temperature (^{o}C): Turbidity (NTU): SEC (μS/cm):					
					Average

Water Point Sanitary Risk Survey *e.g.* for handpump borehole/well	**Y/N**
1. Cement floor extends less than 0.75 metre diameter?	
2. Ponding of water on the cement floor?	
3. Cracks in the cement floor which could permit water to enter the well?	
4. Pump loose where attached to the base allowing water to enter the casing?	
5. Ponding beyond the cement floor within 3 metres of the well?	
6. Drainage channel cracked, broken or in need of cleaning?	
7. Animals have access to within 10 m of the well?	
8. Latrines, ***including old latrines,*** within 10 m of the well?	
9. Additional latrines, ***including old latrines,*** within 30 m of the well?	
10. Open water sources within 20 m of the well?	
11. Uncapped wells within 30 m of the well?	
12. Scattered waste within 30 m of the well	
13. Waste dumps within 30 m of the well?	
Shallow wells only (for boreholes mark as N)	
14. Cover of the well insanitary?	
15. Walls of the well inadequately sealed at any point below ground level?	
16. Rope and bucket used?	
Field Observations	
Photos taken of the water point	
Depth to groundwater table (mbgl)	

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British Geological Survey holds most of the references listed below, and copies may be obtained via the library service subject to copyright legislation (contact libuser@bgs.ac.uk for details). The library catalogue is available at: http://geolib.bgs.ac.uk.

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