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Real-time detection of faecally contaminated drinking water with tryptophan-like fluorescence: defining threshold values

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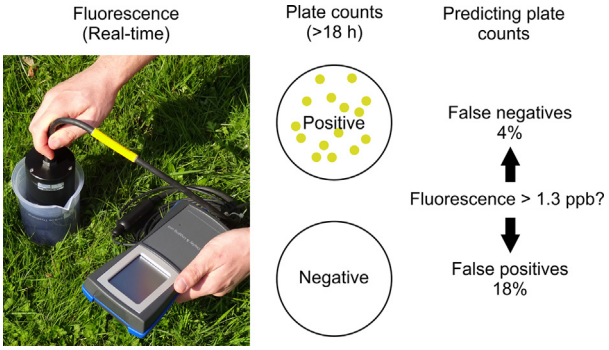
HIGHLIGHTS

- Urgent need to screen drinking water for faecal contamination rapidly.
- Multi-country assessment of TLF as an indicator of faecal contamination.
- A 1.3 ppb dissolved tryptophan threshold is effective to infer contamination.
- TLF is strongly correlated with thermotolerant coliform concentration.
- TLF is a commercially available, easy-to-use, reagentless, real-time option.

GRAPHICAL ABSTRACT



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ABSTRACT

We assess the use of fluorescent dissolved organic matter at excitation-emission wavelengths of 280 nm and 360 nm, termed tryptophan-like fluorescence (TLF), as an indicator of faecally contaminated drinking water. A significant logistic regression model was developed using TLF as a predictor of thermotolerant coliforms (TTCs) using data from groundwater- and surface water-derived drinking water sources in India, Malawi, South Africa and Zambia. A TLF threshold of 1.3 ppb dissolved tryptophan was selected to classify TTC contamination. Validation of the TLF threshold indicated a false-negative error rate of 15% and a false-positive error rate of 18%. The threshold was unsuccessful at classifying contaminated sources containing < 10 TTC cfu per 100 mL, which we consider the current limit of detection. If only sources above this limit were classified, the false-negative error rate was very low at 4%. TLF intensity was very strongly correlated with TTC concentration ($\rho_s = 0.80$). A higher threshold of 6.9 ppb dissolved tryptophan is proposed to indicate heavily contaminated sources (≥100 TTC cfu per 100 mL). Current commercially available fluorimeters are easy-to-use, suitable for use online and in remote environments, require neither reagents nor consumables, and crucially provide an instantaneous reading. TLF measurements are not appreciably impaired by common intereferents, such as pH, turbidity and temperature, within typical natural ranges. The technology is a viable option for the real-time screening of faecally contaminated drinking water globally.

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1. Introduction

Drinking water contaminated with faeces is consumed by an estimated 1.8 billion people globally (Bain et al., 2014a). This constitutes a major burden on public health due to the ingestion of enteric pathogens that cause infectious diseases (Bain et al., 2014b). Most significantly, it is considered to result in more than half a million deaths per annum from diarrhoea alone, with children under five particularly at risk (Prüss-Ustün et al., 2014). Accordingly, the United Nations have established Sustainable Development Goals (SDGs) for the universal access to safe drinking water for all and improvements in water quality by 2030.

Assessment of the faecal contamination of drinking water is typically inferred through the presence of surrogate indicator organisms, such as thermotolerant coliforms (TTCs), including *Escherichia coli* (WHO, 2011). Such bacteriological analyses requires well-trained personnel, working with sterile equipment and reagents, and entails >18 h work due to the necessity for culturing. In lower- and middle-income countries, these requirements can restrict: on-site bacteriological testing of water, the understanding and communication of risks to users, and subsequently behavioural change in communities (UNICEF, 2017), i.e. limiting progress towards meeting the SDGs. These methodological requirements even limit the ability of water companies in the most developed countries from assessing bacteriological water quality beyond, typically, a daily basis. Consequently, poor bacteriological quality drinking water can be released into a municipal supply network between testing and sporadic disease outbreaks still occur (e.g. Adler et al., 2017). Therefore, there is a global need for an easy-to-use technology that can detect faecal contamination in drinking water in real-time.

Numerous researchers have already proposed alternative methods (Berg and Fiksdal, 1988; Chen et al., 2015; Frahm and Obst, 2003; Guion et al., 2008; Gunda et al., 2014; Harwood et al., 2014; Lopez-Roldan et al., 2013; Maheux et al., 2011; Radke and Alocilja, 2005; Rinttilä et al., 2004; Rompré et al., 2002; Velasquez-Orta et al., 2017), yet this has not led to a single successful commercial product. Tryptophan-like fluorescence (TLF) is a component of UV-fluorescent dissolved organic matter at excitation-emission wavelengths of 280 nm and 360 nm and is a viable potential alternative. The term TLF is used to reflect the array of generally aromatic and proteinaceous compounds that share similar fluorescence properties to the amino acid tryptophan. It has long been considered an indicator of wastewater and biological activity in freshwater environments (Baker, 2001; Cammack et al., 2004; Carstea et al., 2016) and this has led to increased interest in its use as an early indicator of drinking water quality (Baker et al., 2015; Heibati et al., 2017; Sorensen et al., 2015a; Sorensen et al., 2015b; Sorensen et al., 2016; Stedmon et al., 2011). Crucially, TLF can now be quantified instantaneously in the field using commercially available LED UV-based fluorimeters that express TLF intensity as an equivalent concentration of dissolved tryptophan in parts per billion.

E. coli cells have been proven to directly emit TLF and also excrete compounds that fluoresce in the TLF region in controlled laboratory studies (Bronk and Reinisch, 1993; Dalterio et al., 1986; Dalterio et al., 1987; Dartnell et al., 2013; Fox et al., 2017; Seaver et al., 1998). For example, Fox et al. (2017) demonstrated a strong correlation ($r^2 = 0.98$) between TLF intensity and *E. coli* concentration during culturing, with the majority of TLF being intracellular in origin. Notably, *E. coli* is the preferred organism for the industrial production of tryptophan by fermentation of carbohydrates (Ikeda, 2006). Alternatively, if tryptophan is readily available in the environment, *E. coli* will import and hydrolyse tryptophan, almost quantitatively into indole that is then excreted (Li and Young, 2013). This would also enhance any TLF signal because pure indole fluoresces within the TLF region at 33% greater intensity than tryptophan (Fig. 1). It has also been observed that *E. coli* excrete tryptophan under nutrient limited conditions as they transit from a culturable to a dormant viable but non-culturable state (Arana et al., 2004). This will eventually occur when *E. coli* are released into many freshwater environments, and particularly groundwater where nutrients are typically limited.

In recent field studies of drinking water quality using commercially available portable fluorimeters, it has been demonstrated that TLF is significantly more intense in groundwater-derived sources contaminated with TTCs than those where TTCs were absent (Sorensen et al., 2015a; Sorensen et al., 2016). Moreover, significant positive correlations between TLF intensity and TTC concentration have also been observed in both groundwater- and surface water-derived sources (Baker et al., 2015; Sorensen et al., 2015a; Sorensen et al., 2016), in addition to TLF being elevated in the presence of enteric pathogens (Sorensen et al., 2015b). We now evaluate the ability of TLF to detect faecally contaminated drinking water against an assessment criteria that includes sensor performance, design, and interferences. To evaluate sensor performance a global dataset will be used to define optimal TLF thresholds to determine faecal contamination that could be universally applicable.

2. Methods

2.1. Available TLF-TTC data

Concurrent TLF and TTC data were collated from a mixture of unpublished and published studies of drinking water in four separate countries: India, Malawi, South Africa and Zambia (Table 1). The Indian study was conducted in Bihar State and 150 groundwater sources were selected to achieve spatial coverage across four villages with an approximate split between those near (<10 m) and those away (>10 m) from recently installed on-site sanitation (Sorensen et al., 2016). The Malawian study predominantly comprised sampling a randomly selected groundwater source in 40 randomly selected villages in each of five districts of the country (Balaka, Machinga, Lilongwe, Nkhotakota, Mzimba). In total, 39 of the 200 randomly selected sources were non-functional and an additional 21 sources were opportunistically sampled near the original randomly selected source. The South African study involved repeated sampling of 28 locations selected upstream, near to, and downstream of expected contributing sources of poor water quality in two surface water catchments in KwaZulu Natal (Baker et al., 2015). The Zambian study comprised sampling 65 groundwater sources across peri-urban, industrial, and lower and higher income residential land uses in the city of Kabwe, of which 46 sources were sampled during both wet and dry seasons (Sorensen et al., 2015a).

All studies used a portable fluorimeter targeting TLF on an unfiltered water sample and enumerated either TTCs by membrane filtration or *E. coli* by the Colilert® (IDEXX) method (Table 1). Although the studies

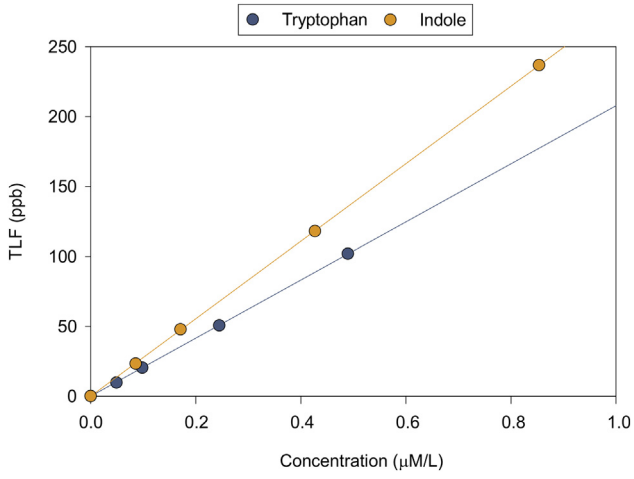

Fig. 1. Comparison of the TLF intensity emitted by dissolved tryptophan and indole. Analyses were performed on 0, 10, 20, 50, and 100 ppb solutions of both compounds with a UviLux fluorimeter (Chelsea Technologies Group Ltd., UK). The gradients of the regression lines are 278 and 209 for indole and tryptophan, respectively.