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In-situ tryptophan-like fluorescence: A real-time indicator of faecal contamination in drinking water supplies

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ABSTRACT

Enteric pathogens are typically inferred from the presence of surrogate indicator organisms such as thermotolerant (faecal) coliforms (TTCs). The analysis of TTCs requires time-consuming incubation in suitable laboratories, which can limit sampling resolution, particularly during critical pollution events. Here, we demonstrate the use of in-situ fluorimeters targeting tryptophan-like compounds as a rapid, reagentless indicator of TTCs in groundwater-derived potable water supplies in Africa. A range of other common indicators of TTCs were also determined including nitrate, turbidity, and sanitary risk survey scores. Sampling was conducted during both the dry and wet seasons to investigate seasonality. Tryptophan-like fluorescence was the most effective predictor of both presence/absence and number of TTCs during both seasons. Seasonal changes in tryptophan-like fluorescence in deeper supplies suggest it is transported more efficiently through the aquifer than TTCs. Moreover, the perennial elevated concentrations in some wells suggest it is more resilient than TTCs in groundwater. Therefore tryptophan-like fluorescence could also be a better indicator of some smaller, more easily transported, and long-lived, pathogenic enteric viruses. These sensors have the potential to be included in real-time pollution alert systems for drinking water supplies throughout the world, as well as for mapping enteric pathogen risks in developing regions.

1. Introduction

Many pathogens transmitted through drinking water are of faecal origin and these continue to pose a threat to human health globally (Ashbolt, 2004). In the USA, up to half of all groundwater supplies have had evidence of faecal contamination resulting in 750,000 to 5.9 million cases of waterborne illnesses per year (Macler and Merkle, 2000). However, these risks are most serious in the developing world where 99.8% of the annual 1.7 million deaths relating to unsafe water supply, sanitation, and hygiene occur (WHO, 2002).

The assessment of enteric pathogens in drinking water has traditionally been inferred using surrogate indicator organisms (Savichtcheva and Okabe, 2006). Currently, the WHO Guidelines for Drinking Water Quality, adopted as standards in many countries, use the indicator group thermotolerant (faecal) coliforms (TTC), or specifically *Escherichia coli*, as a measure of the safety of drinking water supplies. Analysis for these organisms requires well-trained operators working with sterile equipment and reagents in laboratory conditions, which are not always easily accessible. Furthermore, the procedure is time-consuming (>18 h), owing to the necessity for culturing, which can be critical during pollution events when timely intervention and consumer advice is essential.

Fluorescence spectrophotometry offers a potential alternative with multiple studies highlighting its use as a rapid reagentless wastewater indicator (Baker, 2001; Lapworth et al., 2008; Henderson et al., 2009). These observations are based on the positive relationship between tryptophan-like fluorescence (TLF) and labile organic carbon and microbial activity (Cammack et al., 2004; Hudson et al., 2007; Lapworth et al., 2007; Hudson et al., 2008).

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Technological advances now mean that portable fluorimeters targeting the TLF peak are commercially available as possible in-situ or roaming field sensors. Cumberland et al. (2012) demonstrated their potential in controlled laboratory conditions: observing tentative positive correlations with a range of indicator organisms, including *E. coli*, in river and effluent samples. Their use in groundwater is likely to be particularly promising given the general low turbidity and reasonably stable temperature, which can both significantly impact detectable fluorescent signals (Baker, 2005; Downing et al., 2012; Khamis et al., 2015).

This study is the first to pilot the use of in-situ TLF fluorimeters for the rapid assessment of the microbiological quality of drinking water supplies, with a focus on groundwater in the developing world. The aims of this work were to investigate the application of a portable fluorimeter to indicate i) whether a groundwater supply was faecally contaminated; ii) the extent of any contamination; and iii) compare TLF as an indicator of contamination against other common indicators such as NO_3, Cl, turbidity, and sanitary risk scores (SRS). Faecal contamination was determined through the presence of TTCs.

2. Materials and methods

2.1. Study site

Kabwe is located in Zambia's Central Province approximately 150 km north of the capital Lusaka. It has a population of over 200,000, with a high proportion residing within informal settlements on the outskirts of the city such as Makululu – regarded as one of the largest slums in southern Africa with an estimated 46,000 inhabitants (LgWSC, 2014). The city is predominantly underlain by several hundred metres of either the Lower Roan Group (quartzite, schist and pelite) or Upper Roan Group (dolomite). The bedrock is concealed beneath continuous saprolite and laterite superficial deposits that are typically 5–20 m thick (Houston, 1982). Groundwater is generally encountered 5–10 m below ground level, with the superficials typically in hydraulic connection with the deeper aquifer within the karstic bedrock. The local climate is sub-tropical with rainfall exhibiting strong seasonality: 95% falls between mid-November and mid-April (Nkhuwa et al., 2006). Natural surface waters are absent, as rainfall rapidly infiltrates into the subsurface.

Groundwater is the major source of drinking water supply for the city. The centralised supply system abstracts groundwater from deep boreholes within peri-urban wellfields, which is then treated and piped directly to properties, or dispensed via communal taps and water kiosks within the informal settlements. Households frequently also self-supply groundwater to some extent as the centralised supply can be unreliable and is charged on a per volume basis. Within informal settlements this is generally restricted to vulnerable shallow hand-dug wells and illegal connections to pre-treated water within the centralised supply network. In more affluent areas, self-supply includes tapping the bedrock or superficials through deeper boreholes or shallow wells, respectively, with limited use of piped supplies.

Low levels of sanitation coverage are a major cause for concern within newer parts of the Kabwe. This includes the burgeoning informal settlements where coverage is estimated at less than 11% of properties and restricted to pit latrines (LgWSC, 2014). In established parts of the city, the sewerage network is more extensive, but is ageing, in need of investment, and is therefore prone to leakage and overflow. Furthermore, waste collection is limited to the larger businesses in the town centre. Typically, household waste is buried within gardens, burned, or illegally dumped. It should be noted that informal settlements are beginning to encroach into the wellfield areas, with concerns over the potential threats to the city's groundwater resources in the medium to long-term.

2.2. Groundwater sampling and analysis

A total of 117 groundwater samples were obtained from a mixture of supplies that were distributed across the city (Fig. 1). These were composed of 55 samples in the dry season (September 2013) and 62 in the subsequent wet season (January 2014), of which 45 were obtained from the same supplies. The dry season sampling included 25 boreholes and 30 shallow wells, whereas in the wet season 26 boreholes and 36 shallow wells were investigated. These supplies included the city wellfields (K1-12), a mixture of private supplies within both higher and lower cost residential areas, as well as those in the industrial zone (K26-28).

Groundwater samples were obtained once field measurements of pH, specific electrical conductance (SEC), Eh, dissolved oxygen (DO) and temperature had stabilised during pumping. Turbidity was also measured on an agitated pumped sample, as settling was rapid. In-situ TLF was undertaken by sampling 5 L of groundwater and immersing a portable UviLux Fluorimeter (λ_{ex} = 280 nm, λ_{em} = 360 nm) (Chelsea Technologies Group Ltd, UK) in the dark. The sensor utilises a UV light emitting diode (LED) light source and photomultiplier allowing a high level of sensitivity. The minimum detection limit for these sensors is 0.17–0.19 μg/L (Khamis et al., 2015) and repeatability is within ± 0.12–0.29 μg/L up to a concentration of 50 μg/L (Table S1).

The factory calibration was used which showed a good agreement with a range of ten synthetic standards and a bench Varian™ Cary Eclipse fluorescence spectrophotometer for the same wavelength pair at 20 °C (Fig. 2). Standards were produced by dissolving laboratory grade L-tryptophan (Acros Organics, USA) in ultrapure water. An excellent linear relationship between the portable (in μg/L) and bench (in Raman units) fluorimeters (R^2 0.9952) can be used to convert all concentrations on the portable device to Raman units by multiplication by 0.0024 at 20 °C.

Microbiological samples were collected in sterile 60 ml brown-glass bottles. Brief interviews with the well owners, or other informed persons, confirmed that the supplies sampled had not been recently chlorinated, or were chlorinated at a point further down the distribution system. Samples were stored in a cool box and transported back to the laboratory for analysis. All samples were processed within seven hours of collection.

Thermotolerant coliforms (TTCs) were isolated and enumerated using the membrane filtration method and Membrane Lauryl Sulphate Broth (MLSB. Oxoid Ltd) as the selective medium. Typically 50 mL (giving a limit of detection of 2 c.f.u/100 mL), or an appropriate dilution of the sample, were filtered through a 0.45 μm nitrocellulose membrane (PALL Gelman). The membrane was transferred on to an adsorbent pad saturated with MLSB, pre-incubated for approximately one hour at ambient temperature (around 25 °C), and then incubated at 44 °C for a total incubation time of between 18 and 24 h. Plates were examined within 15 min of being removed from the incubator. All cream to yellow colonies with a diameter greater than 1 mm were considered to be TTCs and were counted. No further tests were done to confirm the isolates. The results from 26 duplicate measurements were used to calculate the average Relative Standard Deviation of Reproducibility (RSD_{RC}) and the Uncertainty of Measurement of the microbiological analysis.

Hydrochemical samples were collected for Cl, NO_3, NH_4, SO_4, soluble reactive P (SRP), and total dissolved P (TDP) in 60 mL HDPE bottles after passing through a 0.45 μm nitrocellulose filter, with the bottle for SRP and TDP pre-treated with 0.45 g of potassium