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# Tracing enteric pathogen contamination in sub-Saharan African groundwater

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## HIGHLIGHTS

- Enteric bacteria markers reveal vulnerability of water supplies beneath city.
- Laterite/saprolite is not a sufficient barrier to microbiological contamination.
- First evidence for a perennial inland freshwater reservoir of *Vibrio cholera*.
- Tryptophan-like fluorescence is an indicator of enteric pathogenic bacteria markers.

## GRAPHICAL ABSTRACT


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## ABSTRACT

Quantitative PCR (qPCR) can rapidly screen for an array of faecally-derived bacteria, which can be employed as tracers to understand groundwater vulnerability to faecal contamination. A microbial DNA qPCR array was used to examine 45 bacterial targets, potentially relating to enteric pathogens, in 22 groundwater supplies beneath the city of Kabwe, Zambia in both the dry and subsequent wet season. Thermotolerant (faecal) coliforms, sanitary risks, and tryptophan-like fluorescence, an emerging real-time reagentless faecal indicator, were also concurrently investigated. There was evidence for the presence of enteric bacterial contamination, through the detection of species and group specific 16S rRNA gene fragments, in 72% of supplies where sufficient DNA was available for qPCR analysis. DNA from the opportunistic pathogen *Citrobacter freundii* was most prevalent (69% analysed samples), with *Vibrio cholerae* also perennially persistent in groundwater (41% analysed samples). DNA from other species such as *Bifidobacterium longum* and *Arcobacter butzleri* was more seasonally transient. Bacterial DNA markers were most common in shallow hand-dug wells in laterite/saprolite implicating rapid subsurface pathways and vulnerability to pollution at the surface. Boreholes into the underlying dolomites were also contaminated beneath the city highlighting that a laterite/saprolite overburden, as occurs across much of sub-Saharan aquifer, does not adequately protect underlying bedrock groundwater resources.

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Nevertheless, peri-urban boreholes all tested negative establishing there is limited subsurface lateral transport of enteric bacteria outside the city limits. Thermotolerant coliforms were present in 97% of sites contaminated with enteric bacterial DNA markers. Furthermore, tryptophan-like fluorescence was also demonstrated as an effective indicator and was in excess of 1.4 μg/L in all contaminated sites.



## 1. Introduction

Waterborne bacterial pathogens are a major threat to public health worldwide, although the greatest burden is in the developing world (Bain et al. 2014). Here, occurrences of enteric fevers, dysenteries and cholera are common, with diarrheal diseases a leading cause of child mortality (Liu et al. 2012). This is principally related to a lack of access to safe water and inadequate sanitation (Montgomery and Elimelech 2007; Sobsey et al. 2008). Globally, it is sub-Saharan Africa where most people (325 million) lack access to an improved drinking water source and only 30% of the population has access to improved sanitation (WHO/UNICEF 2014).

Monitoring for waterborne pathogens has traditionally been conducted by culture based methods that use surrogate organisms such as thermotolerant (faecal) coliforms (TTCs). However, many studies have questioned the link between TTCs and the presence of pathogenic species by reporting no significant relationship between the two (Ferguson et al. 2012; Savichtcheva and Okabe 2006; Wu et al. 2011). As a result, a range of candidate assays for identifying bacteria in drinking water sources are now being explored, including the use of microarrays, quantitative PCR (qPCR), and high throughput sequencing approaches (Aw and Rose 2012). While there is a growing body of research for tracking pathogens and faecal indicators in the environment using molecular techniques (e.g. Benami et al. 2013; Marti et al. 2013; Newton et al. 2013), there are very few studies that have employed these techniques as tracers to understand recharge pathways in the subsurface (Hunt et al. 2010; Hunt et al. 2014).

An emerging approach for detecting faecal contamination in drinking water is tryptophan-like fluorescence (TLF). TLF is a proteinaceous component of the fluorescent dissolved organic carbon spectrum and a well-established indicator of wastewater and biological oxygen demand (Baker 2001; Cohen et al. 2014; Henderson et al. 2009; Lapworth et al. 2008; Stedmon et al. 2011). Recently, it has been demonstrated as a precautionary real-time, reagentless indicator of TTCs in freshwater in Africa (Baker et al. 2015; Sorensen et al. 2015a). However, its effectiveness at inferring waterborne pathogen presence is unknown.

The main aim of this study is to characterise contaminant pathways to drinking water sources in sub-Saharan Africa using molecular techniques to detect a broad range of bacterial taxonomic DNA markers and virulence genes. Furthermore, this study contributes to filling important knowledge gaps through i) comparing TTC, TLF, and sanitary risk scores as indicators of waterborne enteric bacterial in groundwater sources, and ii) exploring the temporal changes in enteric bacterial occurrence in both shallow and deep groundwater sources.

## 2. Methods

### 2.1. Study site

Kabwe is the provincial capital of the Zambian Central Province with a rapidly expanding population of over 200,000. It is heavily groundwater dependent with boreholes exploiting the dolomitic bedrock, including the city's peri-urban wellfields, and unimproved shallow wells within the overlying laterite and saprolite superficials (5–20 m thick) frequently used for self-supply in informal settlements. The shallow water table is typically 5–10 m below the surface, with the superficials and bedrock usually in hydraulic connectivity. Such a shallow groundwater system comprising laterite and saprolite over fractured bedrock is common across sub-Saharan Africa (Bonsor et al. 2014; Taylor et al. 2010).

The climate is sub-tropical and 95% of precipitation occurs between mid-November and mid-April (Nkhuwa et al. 2006). There is an absence of natural surface waters due to the high infiltration capacity of the superficials. Inadequate sanitation is a significant issue in Kabwe. In established districts of the city there is an extensive reticulated sewerage system, but it is ageing, in need of investment, and leakage and overflow are widespread. Within the informal settlements this network is restricted to <11% of properties with pit latrines the predominant mechanism of human excreta disposal (LgWSC 2014). The combination of lack of access to safe water and poor sanitation has led to confirmed typhoid fever outbreaks as recent as 2012 and hospital admissions with cholera most years (Chipabika 2015; Kasoka 2014).

### 2.2. Groundwater sampling

Twenty-two potable groundwater supplies were sampled from a mixture of supplies distributed across the city during both the dry and subsequent wet seasons (Fig. 1). These encompassed nine deeper boreholes, including four from the city's peri-urban wellfields (K1, K4, K8, K12), and thirteen shallow wells. Private supplies were sampled from a mixture of higher and lower cost residential areas. Prior to sampling, interviews with the well owners, or other informed persons, were conducted to confirm that the supply had not been recently chlorinated. All samples were then obtained once field parameters (pH, specific electrical conductance, Eh, dissolved oxygen and temperature) had stabilised during pumping. Tryptophan-like fluorescence (TLF) was measured using a portable UviLux Fluorimeter ($\lambda_{ex} = 280 \pm 30$ nm, $\lambda_{em} = 360 \pm 50$ nm) (Chelsea Technologies Group Ltd., UK), with repeatability of ±0.12–0.29 μg/L up to a concentration of 50 μg/L (Sorensen et al. 2015a). Environmental factors that can affect TLF, such as temperature and turbidity (Khamis et al. 2015), were not considered to vary appreciably beneath the city (Sorensen et al. 2015a).

Samples for molecular analyses were collected by positive pressure filtration of 1–2 L of water onto 0.22 μm Sterivex polyethersulfone or PVDF filter cartridges (Millipore, UK). Preliminary tests (*unpublished*) showed no difference in DNA yield between the two filter types. Samples were pumped to remove water and approximately 2–3 mL of DNA/RNA Shield (Zymo Research, USA) was added to preserve the sample at room temperature. Nevertheless, the samples were still stored at −20 °C, except during shipment to the UK (24 h at room temperature), until DNA extraction.

Samples for thermotolerant coliforms (TTCs) were collected in sterile 60 mL brown-glass bottles. They were stored in a cool box and processed at the laboratory within seven hours of collection. TTCs were isolated and enumerated using the membrane filtration method and Membrane Lauryl Sulphate Broth (MLSB. Oxoid Ltd.) as the selective medium. An appropriate dilution of typically 50 mL was passed through a 0.45 μm nitrocellulose membrane (PALL Gelman, USA). This membrane was then placed onto an adsorbent pad saturated with MLSB within a plate, pre-incubated for an hour at ambient temperature (*c.* 25 °C), and then incubated (44 °C) for 18–24 h. All plates were examined within 15 min of removal from the incubator for cream to yellow colonies with a diameter of >1 mm. These were considered to be TTCs, counted, and reported as colony forming units (c.f.u) per 100 mL.

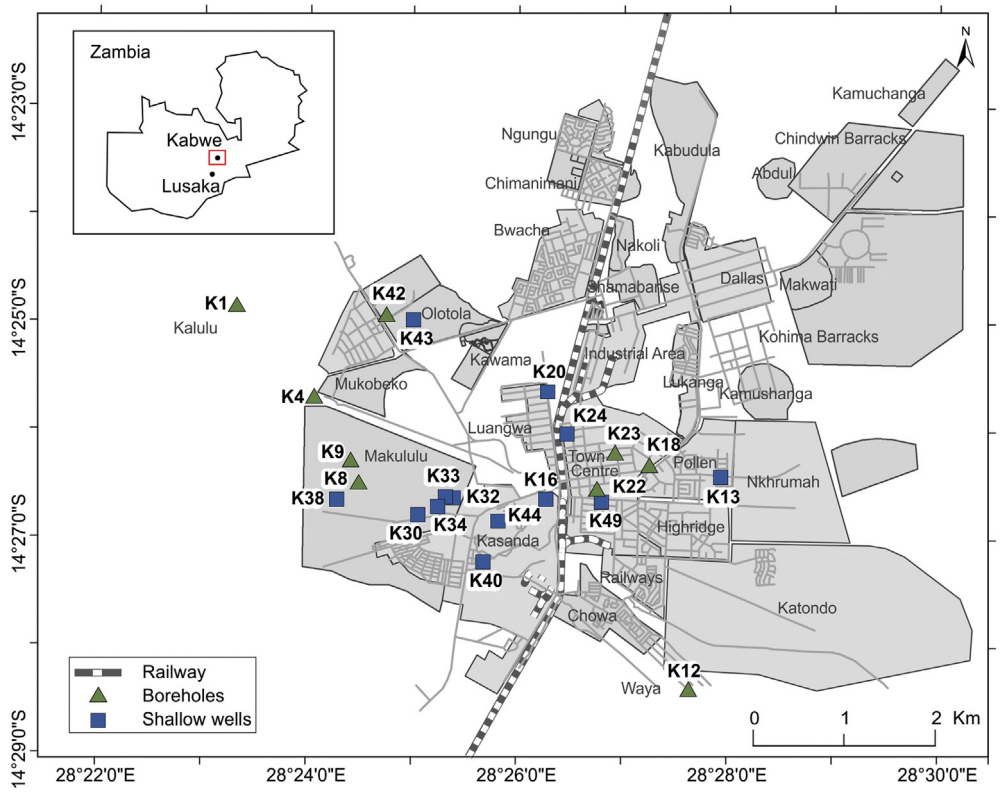


**Fig. 1.** Sampled groundwater supplies in Kabwe.

Repeated analysis indicated a Relative Standard Deviation of Reproducibility ($RSD_{RC}$) of 0.14 (Sorensen et al. 2015a). With a coverage factor of 2.06, determined from the number of duplicate analyses, the $RSD_{RC}$ 95% confidence intervals for a result of 100 c.f.u./100 mL would have upper and lower limits of 197 and 51 c.f.u./100 mL.

### 2.3. Molecular analyses

DNA was extracted from whole sterivex filters using the PowerWater DNA isolation kit (MO BIO Laboratories Inc., USA) following manufacturer's protocols. Extracted DNA was quantified and checked for quality on a Nanodrop (Thermo Scientific, UK) using a 260/280 ratio of >1.6 as a cut-off for rejecting samples for analysis, followed by visualisation on an ethidium bromide stained 1.2% agarose gel. Extracted DNA was stored at −20 °C in 1.5 ml micro centrifuge tubes for no longer that one month before analysis. For the detection of a range of potentially pathogenic and faecally-derived bacterial DNA markers, including virulence associated genes, we used the commercially available microbial DNA qPCR Array for water analysis (Qiagen Ltd., UK). The array is a pre-prepared 96-well format qPCR assay, with specific assays for 45 different predominantly pathogenic bacterial targets including *Actinomyces* spp., *Arcobacter* spp., *Bacteroides* spp., *Bifidobacterium* spp., *Campylobacter* spp., *Clostridium* spp., *Desulfovibrio* spp., *Enterococcus* spp., *Mycobacterium* spp., *Ruminococcus* spp., *Streptococcus* spp., *Vibrio* spp., and *Yersinia* spp.

The array also includes assays designed to detect enterohaemorrhagic *Escherichia coli* (EHEC), targeting the virulence factors eae, stx2A, and stxA. The bacterial species assays target the 16S rRNA gene, and use PCR amplification primers and hydrolysis-probe detection. As a positive control, each array contains two 'Pan Bacteria' assays that are designed to detect as broad a range of bacterial species as possible. A positive PCR Control assay is included to test for the presence of PCR inhibitors and the efficiency of the PCR. All laboratory procedures were carried out in UV and sodium hypochlorite sterilised PCR hoods with DNA free reagents and plasticware. The qPCR arrays were run on an Eppendorf Mastercycler® RealPlex 2 (Eppendorf, Stevenage, UK) using the Qiagen Microbial qPCR Mastermix (Qiagen, UK). The assay requires a minimum of 250 ng of DNA per sample, so DNA input was standardised to this level, and any samples with insufficient concentration (>2.5 ng/μL) were not analysed. PCR cycling conditions involved an initial PCR activation step of 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 15 s and annealing and extension at 60 °C for 2 min. FAM fluorescence was used for target detection. The full list of targeted species/groups, their habitat, route of transmission and potential as pathogens; potential mismatches in the procedure; and the list of groups the Pan Bacteria assays were designed for, are provided in Tables S1–S3.

### 2.4. Data analysis

Following the manufacturer's protocol (Qiagen, UK), the upper and lower bounds for negative, positive and inconclusive results were determined. For identification of microbial species or microbial genes, a positive call is based on the ΔCT method, where a No Template Control (NTC) is used to establish the lower CT (threshold cycle) value for positive calls and the upper CT value for negative calls by subtracting set values to determine the bounds. For example, if the NTC CT value is 39.5, then the lower CT value (positive call) is 39.5 − 6 = 33.5. The upper CT value (negative call) is 39.5 − 3 = 36.5. If the target species or virulence gene assay CT is between the lower CT value and the upper CT value, then the result is marked as inconclusive.

Non Metric Multidimensional Scaling (NMDS) was used to profile and visualise differences in the microbial communities across the different samples by using the raw CT values as inputs. An 'Envfit' analysis was used to examine the relationship between the ordination and significantly ($P < 0.001$) correlated qPCR targets. All analyses were carried out using R (v3.2.0) and the statistical package Vegan (Oksanen et al. 2013). Statistical differences between wells and boreholes in terms of

total bacterial DNA markers and faecal indicators were assessed by the Mann–Whitney rank sum tests. Correlations between numbers of bacterial DNA markers and faecal indicators were tested by Spearman's Rank. The non-parametric approaches do not require the data to conform to a particular statistical distribution and were performed using SigmaPlot (v11).

## 3. Results

### 3.1. Presence of bacterial DNA markers

Enteric bacterial taxonomic and virulence DNA markers were detected by qPCR in 72% of potable samples where sufficient DNA was available for analysis (Fig. 2). This included 92% of shallow well samples, but only two boreholes (K22 and K42). These contaminated boreholes were both private supplies in the urban area, with all peri-urban public supplies testing negative, or containing insufficient DNA. The median number of bacterial DNA markers was significantly higher in wells than boreholes (p-value = 0.004). The assay for *Aeromonas* spp. (including *Aeromonas enteropelogenes*, *Aeromonas hydrophila*, *Aeromonas punctata*, and *Aeromonas media*) was positive in all samples, including the negative controls, and was removed from the analysis as a likely PCR contaminant.

DNA from the opportunistic pathogen *Citrobacter freundii*, was the most commonly detected marker, and was identified in 69% of analysed samples where sufficient DNA were available (Fig. 2). Moreover, all other DNA markers were only noted where it was present, with the single exception of *Enterococcus faecium* in K22 in the wet season. DNA from the enteric bacterial species *Bifidobacterium longum* was present in 47% of analysed samples, and was concurrent with *C. freundii* in all contaminated sites in the dry season. Other prominent species of enteric bacteria included *Arcobacter butzleri*, *Desulfovibrio desulfuricans*, and *Enterococcus gallinarum/casseliflavus*, which were present in 34%, 31%, and up to 38%, of analysed samples, respectively. Significantly, DNA from the pathogens *Vibrio cholerae* and *Salmonella enterica* (cause of typhoid fever) were detected in 41% (including borehole K22) and 16% of analysed samples, respectively. The enterohaemorrhagic E. coli (EHEC) virulence factors eae, stx2A, and stxA were not detected in any of the samples.

### 3.2. Relationships between sites and bacterial DNA markers

Examination of the NMDS ordination for all supplies by semi-quantitative qPCR data indicates there are two clusters relating to boreholes and wells (p-value <0.001) (Fig. 3). Anomalies include the presence of boreholes K22 (dry season) and K42 (wet season) amongst the main cluster of wells. Moreover, wells K13, K24, K40, K44 in the wet season all reside in the borehole ordination. Three of these wells were within the main well cluster in the dry season. Overall seasonal differences were not significant (p-value = 0.05), with an inherent bias in the dataset anyway, due to the majority of boreholes having insignificant DNA in the dry season. There are apparent co-correlations within the DNA marker data: i) *Enterococcus spp.* and *V. cholerae*, and ii) *A. butzleri*, *B. vulgatus*, *and D. desulfuricans* (Fig. 3).

### 3.3. Seasonal trends

The onset of the wet season increased the amount of DNA within the groundwater system (Fig. 2). There was insufficient DNA for analysis at 45% of sites in the dry season but only 9% in the wet season. TTC counts and TLF generally increased within supplies in the wet season, but SRSs remained generally unchanged (Fig. 4). Overall, the total number of bacterial DNA markers actually tended to decline in the wet season, including K22 and K24 where detection of DNA markers dropped from 10 to 1 and 7 to 1, respectively.

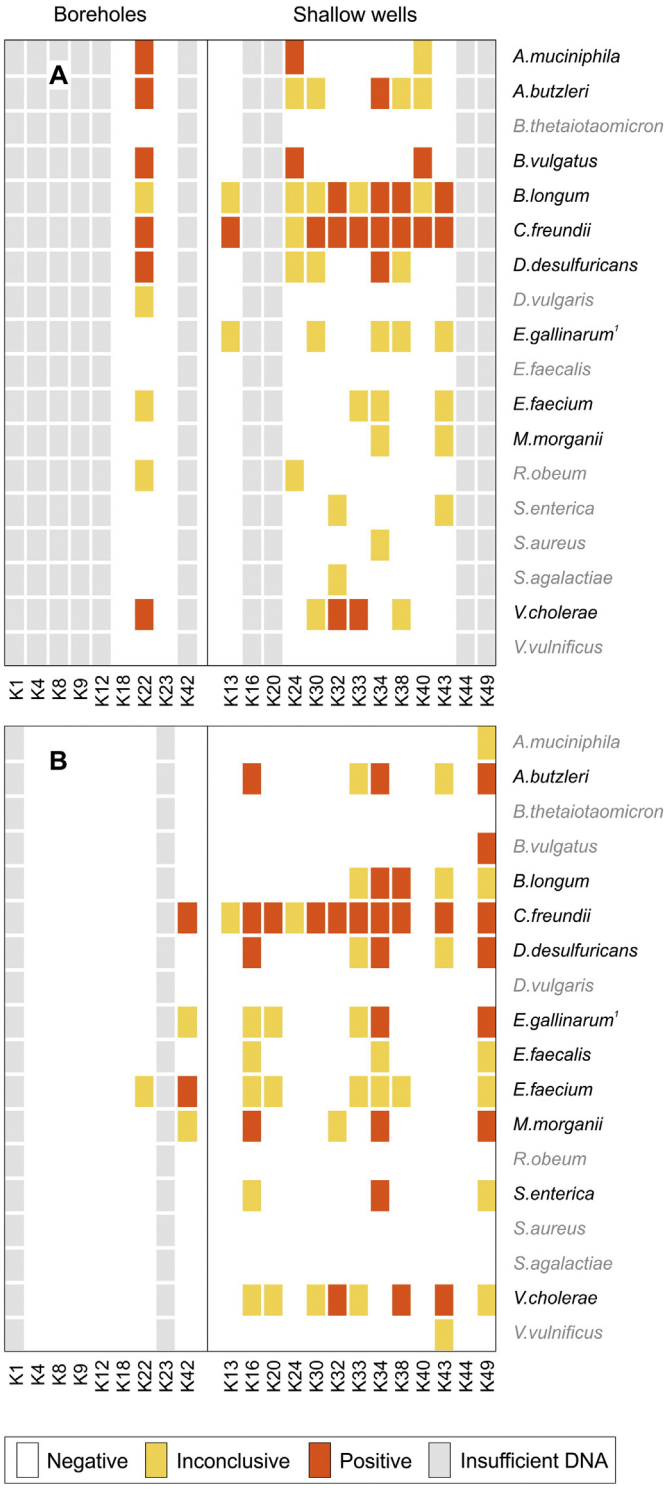


**Fig. 2.** Faecally-derived bacteria screening of groundwater supplies in the (A) dry and (B) wet seasons. Species names in grey denote occurrence in less than three sites. There was no evidence for *Actinomyces* spp., *A. skirrowii*, *Bifidobacterium* spp., *Campylobacter* spp., *C. marimammalium*, *Clostridium* spp., *H. pylori*, *L. gasseri*, *L. pneumophila*, *L. monocytogenes*, *Mycobacterium* spp., *R. bromii*, *S. dysenteriae*, *S. suis*, *V. parahaemolyticus*, *Yersinia* spp. and the *Escherichia* virulence genes stx2Am, stxA and eae. *E. gallinarum* includes *Enterococcus casseliflavus*.

Many bacterial DNA markers were transient within specific water supplies (Fig. 5). This included enteric species such as *B. longum* and *A. butzleri* which were more prevalent in the dry season, and several other bacterial taxa that completely disappeared in the wet season

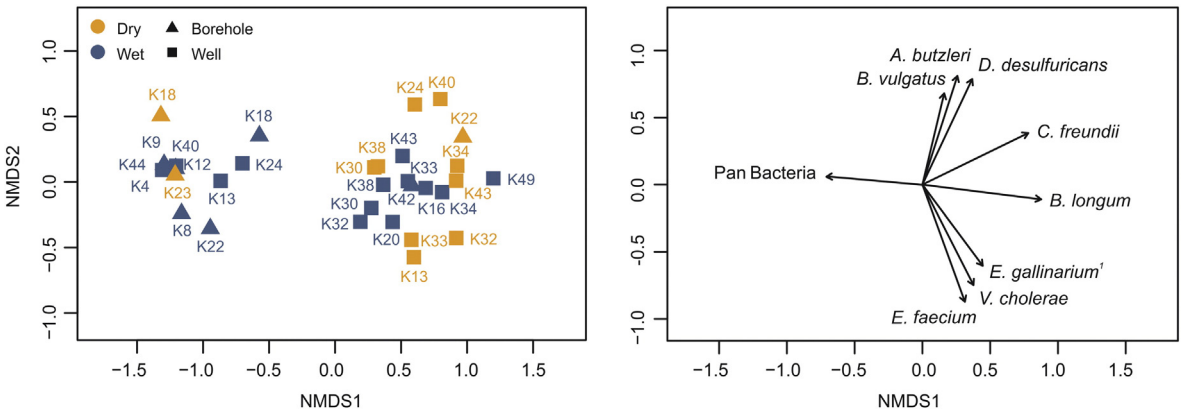


**Fig. 3.** NMDS plot showing the relationship between sites based on semi-quantitative detection of faecally-derived bacterial DNA markers and virulence genes in both seasons. *E. gallinarum* includes *Enterococcus casseliflavus*.

(Fig. 5). *C. freundii* was generally seasonally persistent at supplies, and those with evidence for *V. cholerae* in the dry season remained so in the wet season, excluding K22 (Fig. 2). The numerous sites with insufficient DNA in the dry season limit seasonal comparisons because these sites cannot be considered negatives for specific species.

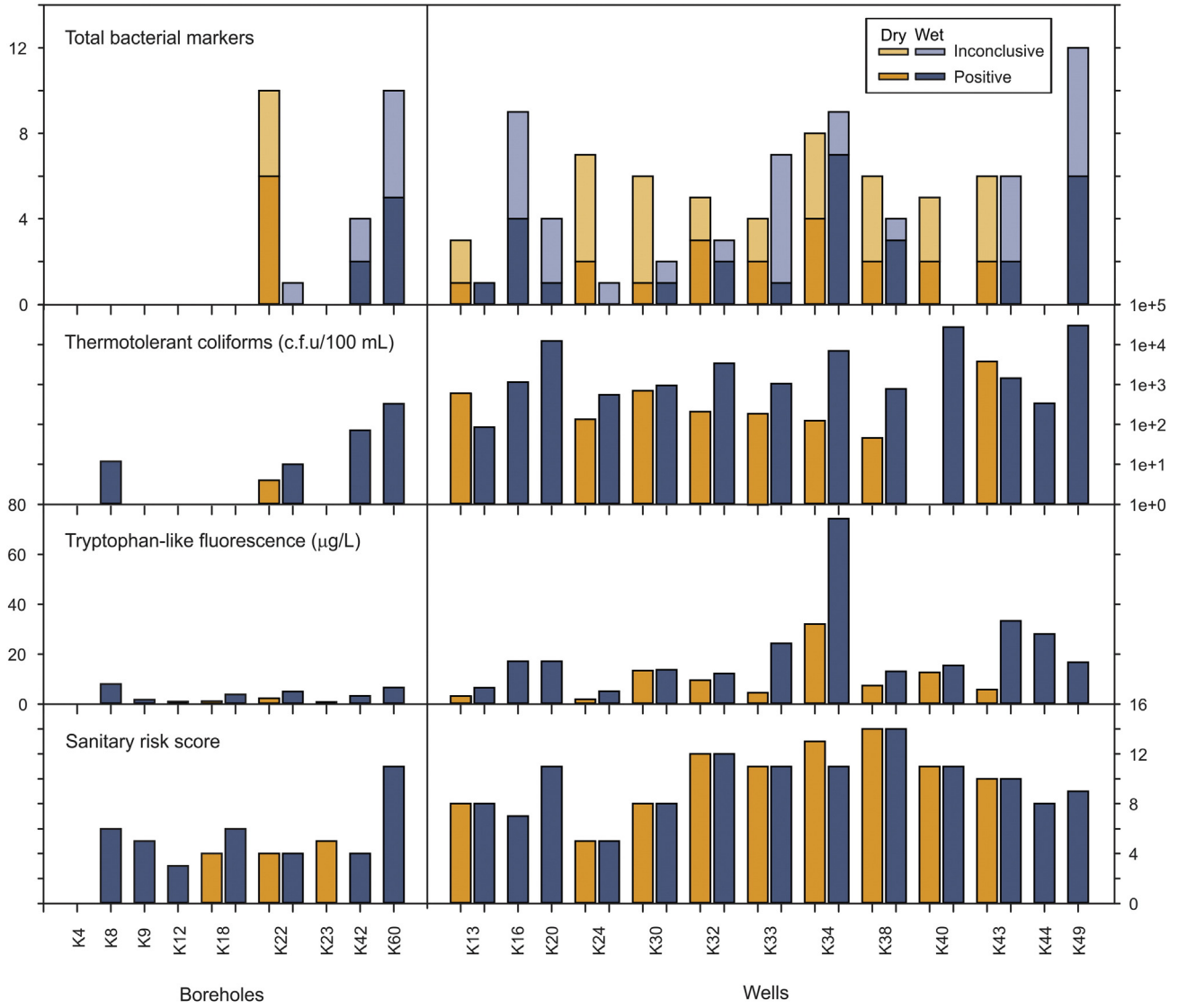


**Fig. 4.** Number of faecally-derived bacterial DNA markers, thermotolerant coliform counts, tryptophan-like fluorescence intensity, and sanitary risk scores for each groundwater supply where sufficient DNA were available for qPCR analysis in both seasons.

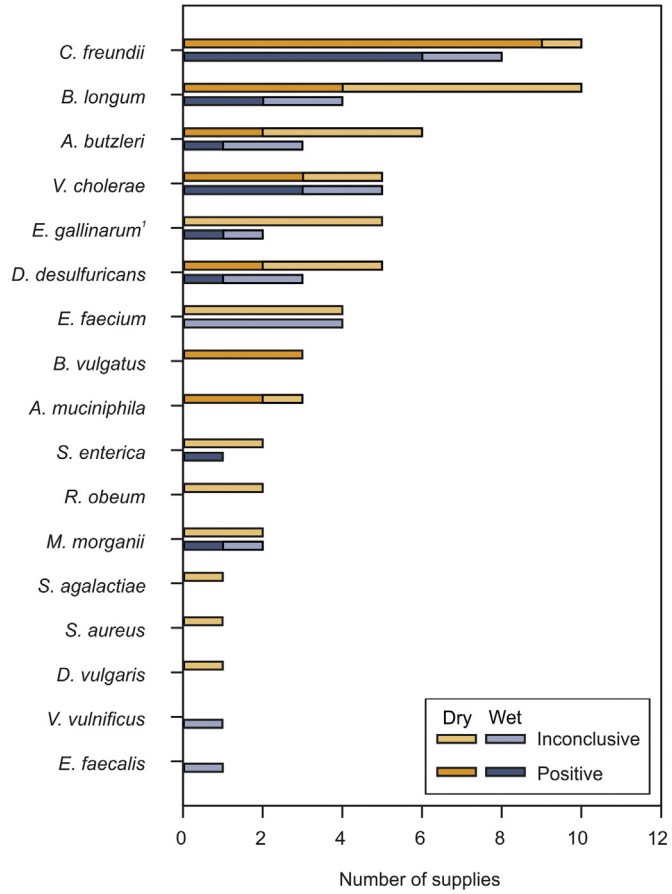


**Fig. 5.** Faecally-derived bacterial DNA markers detections in eleven groundwater supplies successfully analysed in both seasons. *E. gallinarum* includes *Enterococcus casseliflavus*.

### 3.4. Bacterial DNA marker relationships with faecal indicators and sanitary risk scores

Thermotolerant (faecal) coliforms (TTCs) were concurrent with the positive qPCR detection of bacterial markers in 97% of samples (Fig. 4). Nevertheless, there were several false-positives, including both shallow wells where specific bacterial DNA markers were absent which TTCs suggest were high (330 c.f.u/100 mL) and very high risk (27,600 c.f.u/100 mL). Overall, TTCs were present in 50% of all borehole samples. They were also cultured in 95% of shallow well samples and 40% were considered as very high risk (>1000 c.f.u/100 mL).

Tryptophan-like fluorescence (TLF) was elevated at supplies contaminated with bacterial DNA markers, with similar false-positives to TTC counts. The background TLF, at supplies uncontaminated with either bacteria or TTCs, was 1.4 μg/L ($n = 5$) on average. All supplies containing bacterial DNA markers, or only TTCs, exceeded this threshold. Furthermore, TLF in supplies containing enteric bacterial DNA markers was the most correlated indicator of total DNA markers, although this was not significant ($\rho = 0.35$, p-value $= 0.09$). Sanitary risk scores were reasonably consistent across the boreholes and were not elevated where DNA markers occur. Sanitary risk scores, TLF and TTCs were all significantly higher in wells than boreholes (p-value $< 0.001$).

## 4. Discussion

### 4.1. Understanding bacterial contamination

Faecally-derived bacterial DNA markers were only associated with supplies exploiting groundwater directly underlying the urban area, demonstrating their association with the human populous and limited lateral transport towards the peri-urban wellfields. Contamination was predominantly restricted to urban wells within the superficials. These are vulnerable as upper horizons can be highly permeable (e.g. 400 m/d, Bonsor et al. 2014), supplies typically contain no/poor sanitary seals, and water abstraction is via bucket and rope, stored on the ground, which can be a significant vector for microbial contamination (Cronin et al. 2006; Obasohan et al. 2010).

Bacterial DNA markers were also present within supplies exploiting the bedrock, which is inconsistent with the traditional view that lateritic/saprolite cover provides a significant barrier to contamination due to a high clay content towards it base (De Waele and Follesa 2003; Museteka 2013). Furthermore, CFC and $SF_6$ analysis of the dolomite aquifer has demonstrated a large fraction of modern water in the system (Sorensen et al. 2015b) and TTCs were in half of the borehole samples, which are considered evidence of recent contamination (Taylor et al. 2004). Therefore, near-surface polluted water must be able to bypass the laterite and saprolite. This could be because it is thinner than reported, contains preferential pathways, is drawn down into the bedrock through abstraction, or because of the widespread artificial pathways provided by the proliferation of wells. Alternatively, boreholes may be vulnerable due to incompetent casing or inadequate grouting around the casing providing a rapid pathway to the screen; nevertheless, those contaminated with enteric bacteria were satisfactorily protected at the surface. Irrespective of the reasoning, the rapid areal expansion of the city, and encroachment of informal settlements in the environs of the peri-urban wellfields poses a threat to the sustainability of the city's centralised water supply.

The greater prevalence of bacterial DNA markers before the seasonal rains may appear incompatible with fewer TTCs, less intense TLF, and less overall DNA in the system. The main transient species (*B. longum* and *A. butzleri*) are both considered to originate from the human gut (Prouzet-Mauleon et al. 2006; Schell et al. 2002) and TTCs and TLF suggest there was more wastewater within the supplies in the wet season. In these wells, greater contamination in the dry season could be a result of their focussed use, with many other local supplies running dry, as buckets and ropes could be a key pathway for supply contamination. Nevertheless, this would not explain the high numbers of enteric bacteria in borehole K22 in the dry season.

Despite the fact that from 2009 to 2011 three-quarters of all cholera cases in Africa occurred in inland regions, the pathogenic bacteria *Vibrio cholera* has scarcely been isolated from water samples in inland African settings (Rebaudet et al. 2013). Therefore, its widespread prevalence in groundwater supplies during both seasons here could be considered first evidence for a seasonally persistent reservoir of cholera vibrios in groundwater. These findings further substantiate the growing body of research indicating a significant entrenched cholera burden in sub-Saharan Africa (Deen et al. 2008; Gaffga et al. 2007). Nevertheless, there are inherent limitations to the molecular approach used in this study that need to be taken into account. The assay used is a commercial kit (Qiagen UK), and as such assurances of the specificity of each assay has to be taken at face value. In addition the qPCR assay detects the presence of DNA, not live viable cells, and therefore may not represent a recent faecal contamination event (Bae and Wuertz 2015).

### 4.2. Indicators of faecally-derived bacteria

*C. freundii*, the most frequently detected bacterial DNA marker, was seasonally persistent and was co-present in all supplies with one exception that contained other markers. *C. freundii* is generally considered to be of faecal origin, but can occur in the environment as a result of historic faecal contamination episodes (Table S1). It is part of the thermotolerant coliform (TTC) group, cultured in this study, and has been demonstrated to be the most abundant non-*E. coli* TTC in freshwater (Alonso et al. 1999). Therefore, it could be considered an excellent bacterial indicator organism in its own right, rather than the increasing

focus on solely *E. coli* (Edberg et al. 2000; Jalliffier-Verne et al. 2015). However, this species is also known to integrate into biofilms and cause persistent water supply contamination (Table S1). As a group, TTCs were effective in identifying the presence of other bacterial DNA markers.

Tryptophan-like fluorescence (TLF) was similarly successful at identifying bacterially contaminated sites to TTC, which is unsurprising given its significant relationships with TTCs in groundwater beneath the city (Sorensen et al. 2015a) and *E. coli* in surface waters elsewhere (Baker et al. 2015; Cumberland et al. 2012). TLF was actually elevated at K40 in the dry season, the only TTC false-negative. It was previously suggested that the absence of TTCs was due to the proximity to mine tailings resulting in slightly acidic groundwater (pH 5.1) loaded with toxic heavy metals (Sorensen et al. 2015a). This hydrochemical composition would have had less impact on TLF intensity. Therefore, TLF can be considered a valuable real-time indicator of waterborne enteric bacteria when intensity exceeds 1.4 μg/L. Sanitary risk scores highlight the obvious greater risks at wells over boreholes, but importantly do not discriminate between sites contaminated with enteric bacteria within each type of groundwater source.

*Bifidobacterium* have been suggested as a tracer of faecal contamination in the environment (Lamendella et al. 2008). Specifically, *B. longum* has been described as one of the main species detected in the human gut and wastewater (Ballesté and Blanch 2011; Mangin et al. 2006). Here, *B. longum* DNA was present in all contaminated sites in the dry season. However, it was seasonally transient and absent in many contaminated sites in the wet season.

## 5. Conclusions

A range of microbial DNA markers were only present in supplies exploiting groundwater directly underlying the city itself, with no evidence of lateral pathogen transport to the peri-urban wellfields. DNA markers were most prevalent in shallow wells in laterite/saprolite reflecting rapid subsurface pathways and increased sanitary vulnerability. There is the first recorded evidence for a perennial reservoir of cholera within inland African water and its prevalence in 41% of analysed samples highlights the great cholera burden in sub-Saharan Africa. Furthermore, tryptophan-like fluorescence was demonstrated as an effective indicator of enteric pathogenic bacteria markers for the first time, alongside the traditional culturing of surrogate organisms.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2015.08.119.

## References

Alonso, J.L., Soriano, A., Carbajo, O., Amoros, I., Garelick, H., 1999. Comparison and recovery of *Escherichia coli* and thermotolerant coliforms in water with a chromogenic medium incubated at 41 and 44.5 °C. Appl. Environ. Microbiol. 65 (no. 8), 3746–3749.

Aw, T.G., Rose, J.B., 2012. Detection of pathogens in water: from phylochips to qPCR to pyrosequencing. Curr. Opin. Biotechnol. 23 (no. 3), 422–430.

Bae, S., Wuertz, S., 2015. Decay of host-associated Bacteroidales cells and DNA in continuous-flow freshwater and seawater microcosms of identical experimental design and temperature as measured by PMA-qPCR and qPCR. Water Res. 70, 205–213.

Bain, R., Cronk, R., Wright, J., Yang, H., Slaymaker, T., Bartram, J., 2014. Fecal contamination of drinking-water in low-and middle-income countries: a systematic review and meta-analysis. PLoS Med. 11 (no. 5), e1001644.

Baker, A., 2001. Fluorescence excitation–emission matrix characterization of some sewage-impacted rivers. Environ. Sci. Technol. 35 (no. 5), 948–953.

Baker, A., Cumberland, S.A., Bradley, C., Buckley, C., Bridgeman, J., 2015. To what extent can portable fluorescence spectroscopy be used in the real-time assessment of microbial water quality? Sci. Total Environ. 532, 14–19.

Ballesté, E., Blanch, A.R., 2011. Bifidobacterial diversity and the development of new microbial source tracking indicators. Appl. Environ. Microbiol. 77 (no. 10), 3518–3525.

Benami, M., Gross, A., Herzberg, M., Orlofsky, E., Vonshak, A., Gillor, O., 2013. Assessment of pathogenic bacteria in treated graywater and irrigated soils. Sci. Total Environ. 458, 298–302.

Bonsor, H., MacDonald, A., Davies, J., 2014. Evidence for extreme variations in the permeability of laterite from a detailed analysis of well behaviour in Nigeria. Hydrol. Process. 28 (no. 10), 3563–3573.

Chipabika, E., 2015. An Assessment of Food Hygiene Practices Among Food Handlers in Restaurants in Kabwe Urban District MSc University of Zambia.

Cohen, E., Levy, G.J., Borisover, M., 2014. Fluorescent components of organic matter in wastewater: efficacy and selectivity of the water treatment. Water Res. 55, 323–334.

Cronin, A., Pedley, S., Breslin, N., Gibson, J., 2006. Monitoring source and domestic water quality in parallel with sanitary risk identification in Northern Mozambique to prioritise protection interventions. J. Water Health 4, 333–345.

Cumberland, S., Bridgeman, J., Baker, A., Sterling, M., Ward, D., 2012. Fluorescence spectroscopy as a tool for determining microbial quality in potable water applications. Environ. Technol. 33 (no. 6), 687–693.

De Waele, J., Follesa, R., 2003. Human impact on karst: the example of Lusaka (Zambia). Int. J. Speleol. 32 (no. 1), 5.

Deen, J.L., Von Seidlein, L., Sur, D., Agtini, M., Lucas, M., Lopez, A.L., Kim, D.R., Ali, M., Clemens, J.D., 2008. The high burden of cholera in children: comparison of incidence from endemic areas in Asia and Africa. PLoS Negl. Trop. Dis. 2 (no. 2), e173.

Edberg, S., Rice, E., Karlin, R., Allen, M., 2000. *Escherichia coli*: the best biological drinking water indicator for public health protection. J. Appl. Microbiol. 88 (no. S1), 106S–116S.

Ferguson, A.S., Layton, A.C., Mailloux, B.J., Culligan, P.J., Williams, D.E., Smartt, A.E., Sayler, G.S., Feighery, J., McKay, L.D., Knappett, P.S., 2012. Comparison of fecal indicators with pathogenic bacteria and rotavirus in groundwater. Sci. Total Environ. 431, 314–322.

Gaffga, N.H., Tauxe, R.V., Mintz, E.D., 2007. Cholera: a new homeland in Africa? Am. J. Trop. Med. Hyg. 77 (no. 4), 705–713.

Henderson, R., Baker, A., Murphy, K., Hambly, A., Stuetz, R., Khan, S., 2009. Fluorescence as a potential monitoring tool for recycled water systems: a review. Water Res. 43 (no. 4), 863–881.

Hunt, R.J., Borchardt, M.A., Bradbury, K.R., 2014. Viruses as groundwater tracers: using ecohydrology to characterize short travel times in aquifers. Groundwater 52 (no. 2), 187–193.

Hunt, R.J., Borchardt, M.A., Richards, K.D., Spencer, S.K., 2010. Assessment of sewer source contamination of drinking water wells using tracers and human enteric viruses. Environ. Sci. Technol. 44 (no. 20), 7956–7963.

Jalliffier-Verne, I., Leconte, R., Huaringa-Alvarez, U., Madoux-Humery, A.-S., Galarneau, M., Servais, P., Prévost, M., Dorner, S., 2015. Impacts of global change on the concentrations and dilution of combined sewer overflows in a drinking water source. Sci. Total Environ. 508, 462–476.

Kasoka, J.C., 2014. Study to determine factors contributing to recurrence of cholera in Kabwe urban. MSc, Medicine, University of Zambia, Lusaka.

Khamis, K., Sorensen, J.P.R., Bradley, C., Hannah, D., Lapworth, D.J., Stevens, R., 2015. In situ tryptophan-like fluorometers: assessing turbidity and temperature effects for freshwater applications. Environ. Sci. Process. Impacts 17 (no. 4), 740–752.

Lamendella, R., Santo Domingo, J.W., Kelty, C., Oerther, D.B., 2008. Bifidobacteria in feces and environmental waters. Appl. Environ. Microbiol. 74 (no. 3), 575–584.

Lapworth, D.J., Gooddy, D., Butcher, A., Morris, B., 2008. Tracing groundwater flow and sources of organic carbon in sandstone aquifers using fluorescence properties of dissolved organic matter (DOM). Appl. Geochem. 23 (no. 12), 3384–3390.

LgWSC, 2014. Concept paper for Kabwe sanitation interventions 10 pp.

Liu, L., Johnson, H.L., Cousens, S., Perin, J., Scott, S., Lawn, J.E., Rudan, I., Campbell, H., Cibulskis, R., Li, M., 2012. Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. Lancet 379 (no. 9832), 2151–2161.

Mangin, I., Suau, A., Magne, F., Garrido, D., Gotteland, M., Neut, C., Pochart, P., 2006. Characterization of human intestinal bifidobacteria using competitive PCR and PCR-TTGE. FEMS Microbiol. Ecol. 55 (no. 1), 28–37.

Marti, R., Gannon, V.P.J., Jokinen, C., Lanthier, M., Lapen, D.R., Neumann, N.F., Ruecker, N.J., Scott, A., Wilkes, G., Zhang, Y., Topp, E., 2013. Quantitative multi-year elucidation of fecal sources of waterborne pathogen contamination in the South Nation River basin using Bacteroidales microbial source tracking markers. Water Res. 47 (no. 7), 2315–2324.

Montgomery, M.A., Elimelech, M., 2007. Water and sanitation in developing countries: including health in the equation. Environmental Sci. Technol. 41 (no. 1), 17–24.

Museteka, L., 2013. Assessment of the groundwater vulnerability and its application to the development of a protection strategy for groundwater in Kabwe. Faculty of Geoscience, George August University of Goettingen, Zambia.

Newton, R.J., Bootsma, M.J., Morrison, H.G., Sogin, M.L., McLellan, S.L., 2013. A microbial signature approach to identify fecal pollution in the waters off an urbanized coast of Lake Michigan. Microb. Ecol. 65 (no. 4), 1011–1023.

Nkhuwa, D., Xu, Y., Usher, B., 2006. Groundwater quality assessments in the John Laing and Misisi areas of Lusaka. Groundw. Pollut. Afr. 239–251.

Obasohan, E., Agbonlahor, D., Obano, E., 2010. Water pollution: a review of microbial quality and health concerns of water, sediment and fish in the aquatic ecosystem. Afr. J. Biotechnol. 9 (no. 4).

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Henry, M., Stevens, H., Wagner, H., 2013. vegan: Community Ecology Package.

Prouzet-Mauleon, V., Labadi, L., Bouges, N., Menard, A., Megraud, F., 2006. *Arcobacter butzleri*: underestimated enteropathogen. Emerg. Infect. Dis. 12 (no. 2), 307–309.

Rebaudet, S., Sudre, B., Faucher, B., Piarroux, R., 2013. Environmental determinants of cholera outbreaks in inland Africa: a systematic review of main transmission foci and propagation routes. J. Infect. Dis. 208 (no. suppl 1), S46–S54.

Savichtcheva, O., Okabe, S., 2006. Alternative indicators of fecal pollution: relations with pathogens and conventional indicators, current methodologies for direct pathogen monitoring and future application perspectives. Water Res. 40 (no. 13), 2463–2476.

Schell, M.A., Karmirantzou, M., Snel, B., Vilanova, D., Berger, B., Pessi, G., Zwahlen, M.C., Desiere, F., Bork, P., Delley, M., Pridmore, R.D., Arigoni, F., 2002. The genome sequence of *Bifidobacterium longum* reflects its adaptation to the human gastrointestinal tract. Proc. Natl. Acad. Sci. U.S.A. 99 (no. 22), 14422–14427.

Sobsey, M.D., Stauber, C.E., Casanova, L.M., Brown, J.M., Elliott, M.A., 2008. Point of use household drinking water filtration: a practical, effective solution for providing sustained access to safe drinking water in the developing world. Environ. Sci. Technol. 42 (no. 12), 4261–4267.

Sorensen, J.P.R., Lapworth, D.J., Marchant, B., Nkhuwa, D., Pedley, S., Stuart, M., Bell, R., Chirwa, M., Kabika, J., Liemisa, M., 2015a. In-situ tryptophan-like fluorescence: a real-time indicator of faecal contamination in drinking water supplies. Water Res. 81, 38–46.

Sorensen, J.P.R., Lapworth, D.J., Nkhuwa, D., Stuart, M., Gooddy, D., Bell, R., Chirwa, M., Kabika, J., Liemisa, M., Chibesa, M., 2015b. Emerging contaminants in urban groundwater sources in Africa. Water Res. 72, 51–63.

Stedmon, C.A., Seredyńska-Sobecka, B., Boe-Hansen, R., Le Tallec, N., Waul, C.K., Arvin, E., 2011. A potential approach for monitoring drinking water quality from groundwater systems using organic matter fluorescence as an early warning for contamination events. Water Res. 45 (no. 18), 6030–6038.

Taylor, R., Cronin, A., Pedley, S., Barker, J., Atkinson, T., 2004. The implications of groundwater velocity variations on microbial transport and wellhead protection–review of field evidence. FEMS Microbiol. Ecol. 49 (no. 1), 17–26.

Taylor, R., Tindimugaya, C., Barker, J., Macdonald, D., Kulabako, R., 2010. Convergent radial tracing of viral and solute transport in gneiss saprolite. Groundwater 48 (no. 2), 284–294.

WHO/UNICEF, 2014. Progress on Drinking Water and Sanitation: 2014 Update. World Health Organization, Geneva.

Wu, J., Long, S., Das, D., Dorner, S., 2011. Are microbial indicators and pathogens correlated? A statistical analysis of 40 years of research. J. Water Health 9 (no. 2), 265–278.