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Large-scale survey of seasonal drinking water quality in Malawi using in situ tryptophan-like fluorescence and conventional water quality indicators

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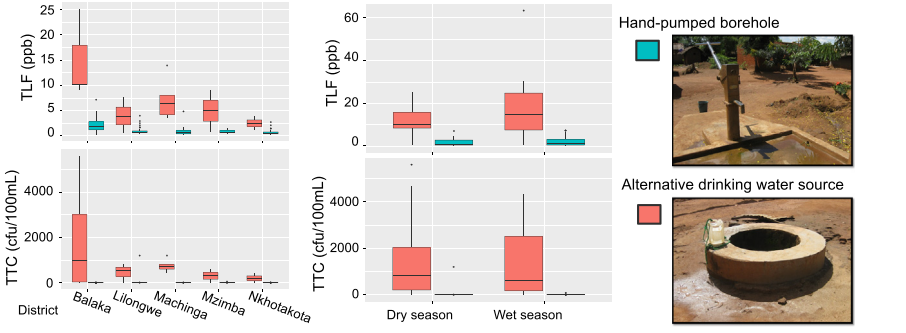
HIGHLIGHTS

- First large-scale study in a low resource setting
- HLF limits capability of TLF for detecting microbial contamination in drinking water
- Majority of hand-pump boreholes have good microbial water quality
- TLF suitable for rapid, high level screening of drinking water sources
- TLF not suitable as a direct alternative to culturing faecal indicator bacteria

GRAPHICAL ABSTRACT



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ABSTRACT

Faecally-contaminated drinking water is a risk to human health, with the greatest risks to those living in developing countries. UN Sustainable Development Goal 6 aims to address this issue. Tryptophan-like fluorescence (TLF) shows potential as a rapid method for detecting microbial contamination in drinking water, which could reduce the spread of waterborne diseases. This study is the first to investigate the effectiveness of TLF for a large-scale survey using a randomised, spot-sampling approach. The large-scale survey took place in Malawi, sub-Saharan Africa, in the dry season ($n = 183$). A subset of sources were revisited at the end of the following wet season ($n = 41$). The effectiveness of TLF was assessed by comparing TLF results to thermotolerant coliforms (TTC), humic-like fluorescence (HLF), inorganic hydrochemical data and sanitary risk scores. The most prominent differences in microbial water quality were observed between source types, with little variation between districts and seasons. TLF, TTCs, turbidity and sanitary risk scores were all elevated at alternative sources (shallow wells and tap stands) compared to hand-pumped boreholes. In the dry season, 18% of hand-pumped boreholes showed TTC contamination, which increase to 21% in the wet season. Groundwater recharge processes are likely responsible for seasonal variability of inorganic hydrochemistry at hand-pumped boreholes. TLF was able to distinguish no and low WHO risk classes (TTC 0–9 cfu/100 mL) from medium, high and very high risk classes (TTC 10 – >1000 cfu/100 mL). TLF failed to distinguish between no and low risk classes, which limits the use of TLF for assessing water quality to drinking water standards. This dataset indicates that HLF may raise baseline TLF for

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samples with low TLF values, increasing false positives. Therefore, TLF is better suited as a rapid high-level water quality screening tool to assess moderate and high levels of faecal contamination.

1. Introduction

At least 2 billion people worldwide drink from faecally-contaminated water sources (WHO, 2018). This resulted in approximately 1.31 million deaths from diarrhoeal diseases in 2015, which could have been broadly preventable with adequate water, sanitation, hygiene and healthcare facilities (Troeger et al., 2017; Pruss-Ustun et al., 2014). The majority of these deaths occurred in low and middle income countries (LMICs) and 38% were children under 5 years old (Troeger et al., 2017; Pruss-Ustun et al., 2014). In addition, where acute diarrhoeal infections do not result in death, they can lead to chronic gastrointestinal diseases (Verdu and Riddle, 2012). Sustainable Development Goal 6 (SDG 6) aims to address this issue by including an assessment of water quality in the Joint Monitoring Programme (JMP) method for measuring progress (WHO and UNICEF, 2018). The preceding Millennium Development Goal (MDG) assessment method focussed only on the provision of 'improved' water sources, defined by the JMP as those that are constructed to protect from contamination (WHO and UNICEF, 2012). However, 'improved' sources cannot guarantee an uncontaminated water supply and therefore it is likely that access to safe water has been overestimated (WHO and UNICEF, 2012; Bain et al., 2014). The JMP assessment method for SDG 6 states access to "improved water sources *that are free from contamination*" as a specific service level target (WHO and UNICEF, 2018).

Traditionally, culturing of bacterial coliforms, namely thermotolerant coliforms (TTCs) and *E. coli*, as proxy indicators for pathogens, has been used to infer microbial contamination in drinking water from faecal sources (WHO, 2017). The WHO (2017) guidelines for drinking water quality define risk categories based on the number of coliforms cultured from a 100 mL water sample. Methods for culturing bacteria are often difficult to carry out in LMICs due to lack of resources in terms of consumables, appropriate laboratory space and skilled technicians. However, it is in these countries where there is the greatest risk of death from contaminated drinking water (Troeger et al., 2017; Pruss-Ustun et al., 2014). There is a distinct lack of data regarding microbial contamination of water supplies in LMICs due to the complications of data collection; this has implications for measuring progress towards SDG 6 (WHO and UNICEF, 2018; Adelena and MacDonald, 2008). In addition, standard culture methods are unable to provide real-time data due to the required incubation period of at least 16 h (Bridgeman et al., 2015). This is not ideal when attempting to prevent disease outbreak if a contamination incident occurs.

Fluorescence spectrometry is used for monitoring environmental water quality (Baker, 2001; Baker, 2002; Baker et al., 2003; Hudson et al., 2007; Baker and Inverarity, 2004; Baker et al., 2015; Cumberland et al., 2012; Heibati et al., 2017). Different compounds fluoresce at different excitation and emission wavelengths and can therefore be used to identify different types of pollution (Baker, 2002; Carstea et al., 2020). Tryptophan-like fluorescence (TLF) refers to fluorescence peaks that occur at the same excitation-emission wavelength as that of the amino acid tryptophan. TLF shows potential as a rapid

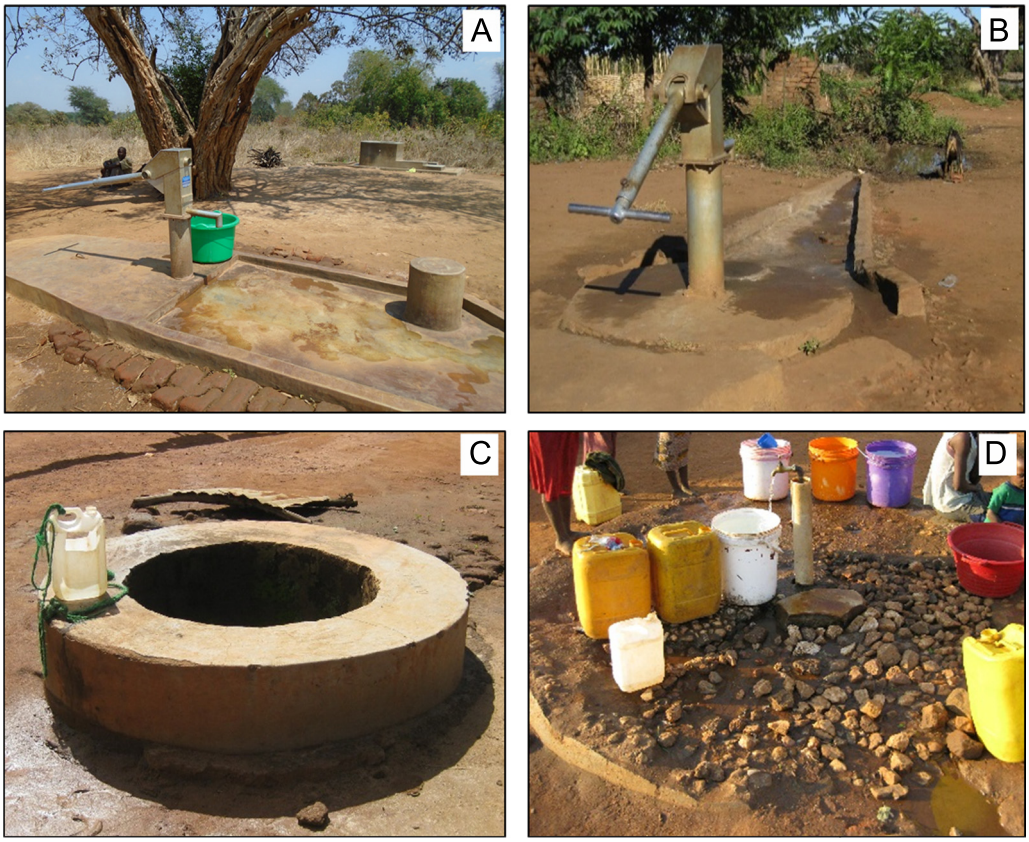

Fig. 1. Rural sources of drinking water studied: (A): Hand-pumped borehole; (B): Hand-pumped shallow well; (C): Open shallow well; (D): Tap stand.

detection method for microbial contamination; recent studies investigating drinking water have shown a positive correlation between TLF, TTCs and *E. coli* (Sorensen et al., 2015b; Sorensen et al., 2015a; Sorensen et al., 2016; Sorensen et al., 2018a; Sorensen et al., 2018b; Nowicki et al., 2019). The majority of these published TLF datasets, focussed on groundwater-derived drinking water sources in LMICs, are relatively small and comprise short spot-sampling programmes in Africa and Asia. The *in situ* results obtained by the TLF probe indicate an advantage over traditional culturing methods, with no transport, storage, laboratory preparation and incubation of samples required. However, Bridgeman et al. (2015) found poor correlation for TLF with TTCs and *E. coli* in a study based in the United Kingdom. Therefore, further understanding is required regarding the nature of the TLF signal and how it relates to other water quality parameters (Carstea et al., 2020). In particular, the potential influence of humic-like fluorescence (HLF) has not been considered in previous studies of TLF in dLMICs. However, due to the close proximity of the HLF peak to the TLF peak on the excitation-emission matrix, it is possible that HLF could interfere with the TLF signal in certain conditions. There has only been one study related to groundwater-derived drinking water sources that considered both TLF and HLF and this was undertaken in the United Kingdom (Sorensen et al., 2018b). Both TLF and HLF relate to organic compounds but from different sources. TLF is commonly thought to be autochthonous and related to microbial sources, whereas HLF is allochthonous and truly dissolved (Baker et al., 2007). However, HLF has also been observed to be produced at certain stages of microbial activity, and whilst the TLF peak is most strongly associated with bacterial cells, HLF can also contribute to the TLF peak (Fox et al., 2017).

A potential benefit of TLF is the collection of large datasets over a large survey area within a short timeframe; this could be particularly advantageous in LMICs. This could have potential to reduce the spread of waterborne diseases, as public health interventions could be mobilised quicker in the event of a contamination outbreak. However, the technology currently has a high capital cost, which may prohibit wider use in this context.

This study took place in rural Malawi, a setting where simple, rapid water quality testing would be particularly advantageous. Malawi is a low income country (LIC) in sub-Sharan Africa and 84% of the population (approximately 14,800,000 people) live in rural areas (Government of Malawi, 2018). Hand-pumped boreholes are widely relied on for drinking water across sub-Saharan Africa, supplemented in some areas by shallow sources and surface water in the wet season. In 2017, 65% of the rural population in Malawi had access to at least basic drinking water sources but only 25% and 7% of the population had access to basic sanitation and handwashing facilities respectively (World Bank, 2019a).

This study is the first to investigate the effectiveness of TLF for a large-scale survey using a randomised, spot-sampling approach. The large-scale survey took place in the dry season, with a subset of sources revisited at the end of the following wet season. The effectiveness of TLF was assessed by comparing TLF results to TTC data, as well as inorganic hydrochemical data. In addition, this study is the first to measure HLF alongside TLF using a spot-sampling method in a low resource, large-scale, developing country setting.

2. Methods

2.1. Study location

Hand-pumped boreholes and alternative drinking water sources, including shallow wells and tap stands, were sampled in rural Malawi (Fig. 1). All sources in this study were located within the village boundaries. The large-scale dry season survey consisted of randomised sampling across five districts. A subset of sources in two districts were revisited at the end of the following wet season. The distribution of sampling across the country is shown in Fig. 2 and further details regarding sampling are provided in Section 2.2. District characteristics are listed in Table 1.

Groundwater is a crucial supply of drinking water in the rural areas. Poverty levels in rural areas vary, and higher poverty levels have been

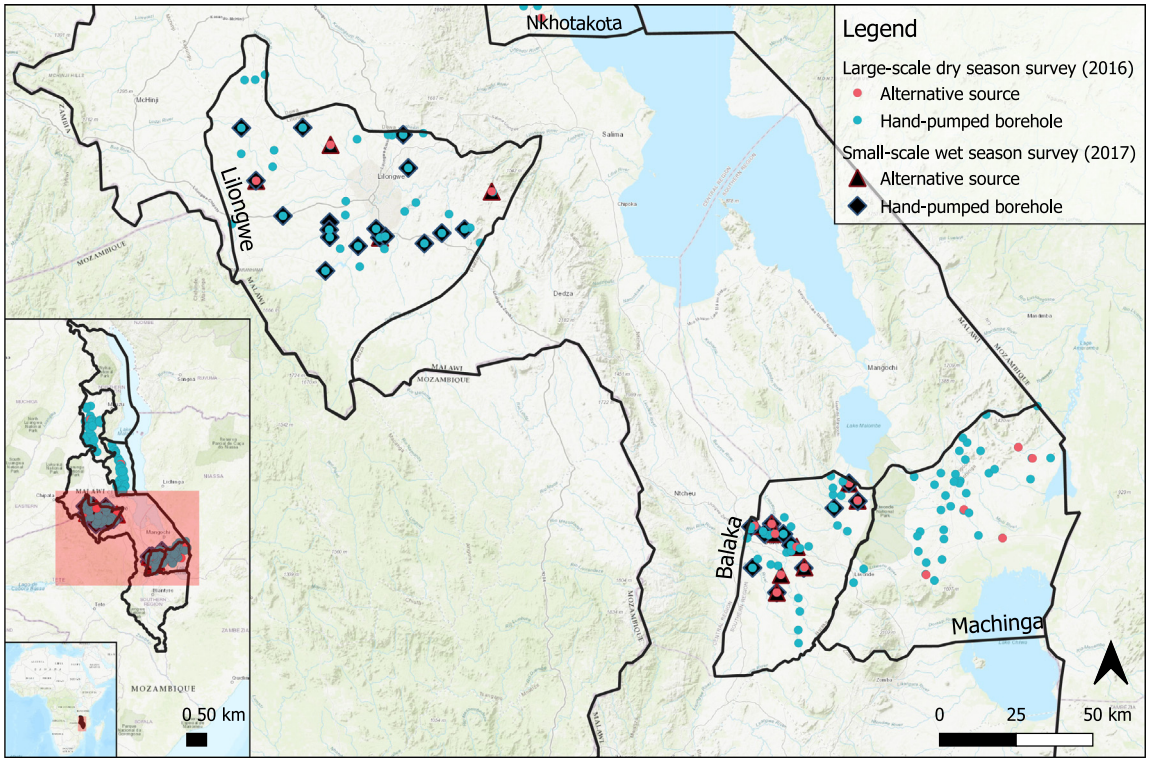

Fig. 2. Inset: Study area for large-scale dry season survey (2016) (red triangle defines the area shown in the main map) and main map: small-scale wet season survey in Balaka and Lilongwe (2017). Background map source: ESRI et al. (2020). For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.

Table 1
District characteristics and number of sources surveyed during the study (HPB = hand-pumped borehole).

District	Region	Poverty level (% of pop'n that is 'poor')[a]	Dominant regional geology[b]	Altitude (mAOD)	Topography	Average annual rainfall (mm/yr)[c]	Large-scale dry season survey		Small-scale wet season survey	
							HPBs	Alternative sources	HPBs	Alternative sources
Balaka	South	68 (Poor)	Sedimentary - Quaternary Alluvium	600	Rift valley plains	840	36	9	12	8
Machinga	South	75 (Poor)	Sedimentary - Quaternary Alluvium	750	Rift valley plains	844	32	5	–	–
Lilongwe Rural	Central	57 (Better off)	Crystalline Basement - Precambrian - Lower Paleozoic complex	1050	Plateau	734	30	3	17	4
Nkhotakota	Central	32 (Better off)	Crystalline Basement - Precambrian - Lower Paleozoic complex	470	Lake-shore	1214	30	2	–	–
Mzimba	North	62 (Better off)	Crystalline Basement - Precambrian - Lower Paleozoic complex	1380	Plateau	831	34	2	–	–
Total							162	21	29	12
Total number of sources sampled in large-scale dry season survey							183		–	
Total number of sources sampled in small-scale wet season survey							–		41	

[a] Poverty level defined by NSO Malawi (2011): 'poor' defined as population with total annual consumption of below MK37,002 (equivalent of just below USD1/day at time of report).
[b] Smith-Carrington and Chilton (1983).
[c] New et al. (1999).

found to increase barriers to safe drinking water in rural areas in Malawi (Mkondiwa et al., 2013). Hand-pumped boreholes (improved sources) and large diameter shallow wells (unimproved sources) are heavily relied on, particularly in the dry season, when few or no surface water alternatives are available. In some areas, tap stands were also available but were not as abundant. Boreholes are 30–50 m deep, with a narrow diameter (approximately 0.1 m). Crystalline basement aquifers are generally less productive than sedimentary aquifers, but are still able to supply hand pumps (yield requirement: 0.1–0.3 L/s) (Smith-Carrington and Chilton, 1983; MacDonald et al., 2012). Shallow wells are generally hand dug with a larger diameter (1–1.5 m) and draw from shallow aquifers, which means the limiting factor for yield is the dropping of groundwater levels in the dry season; abstraction is usually by rope and bucket. Tap stands are gravity-fed from surface water reservoirs. Risks to tap stand functionality include the reservoir running dry in the dry season and broken pipework; water quality risks are also relevant and include faecal contamination from surface runoff and animal (wild or domesticated) death in the reservoir leading to contamination of the source.

Malawi has a sub-tropical climate that is influenced locally by altitude; lake-shore and low lying districts have a semi-arid climate and are much hotter than those located on the higher altitude plateaus (Upton et al., 2018). The wet season occurs from November to April. For the 2016–2017 seasons, the dry season minimum average monthly rainfall was recorded in September 2016 (2 mm) and the wet season maximum average monthly rainfall occurred in January 2017 (243 mm) (World Bank, 2019b).

2.2. Experimental design

2.2.1. Large-scale dry season survey

The large-scale dry season survey took place from September–December 2016, at the end of the dry season. Districts in the Southern Region were surveyed first, followed by the Central and Northern Regions; this matches the pattern of the onset of the rains across the country. The rains commence in the Southern Region distinctly earlier than in the North.

Sources were selected using a two-stage stratified random sampling approach, this was to capture a representative sample across Malawi. The first stage of stratification related to district selection; districts were selected, based on accessibility. The second stage of stratification related to poverty level, classed as 'poor' or 'better off' (Table 1). District Water Office records were used to identify all villages with hand-pumped boreholes (both functional and non-functional) in each selected district.. Forty villages in each district were randomly selected without replacement, using the Rao-Hartley-Cohran (RHC) method (Cochran, 1977). If the village had more than one hand-pumped borehole, the source to be surveyed was selected at random on arrival. Sometimes, a non-functional borehole would be selected at random due to the sampling method and so no water quality analysis could be completed. Therefore the total number of hand-pumped boreholes sampled in the dry season is less than 200 (hand-pumped boreholes, dry season: $n = 162$). Further details are reported in Lapworth et al. (2020). The inclusion of non-functional boreholes in the selection process was required for a parallel project in which the functionality of hand-pumped boreholes was being assessed (Bonsor et al., 2018). Alternative sources were selected for sampling when located nearby to the selected hand-pumped borehole.

2.2.2. Small-scale wet season survey

In 2017, sampling took place in March–April at the end of the wet season. Forty sources were revisited in total, split between the districts of Balaka and Lilongwe. In addition, a shallow well in Lilongwe was sampled that had been dry during the dry season (2016) survey. Lilongwe and Balaka both have different poverty levels and dominant geology (Table 1). Sources were selected using the following criteria: 1) all alternative sources previously sampled; 2) all functional hand-pumped boreholes paired with an alternative source (i.e. in the same village); 3) functional hand-pumped boreholes with high sanitary risk scores and/or notable water quality records from the large-scale dry season survey (e.g. highest TTC count, lowest TLF reading).

2.3. Groundwater sampling

All sources in this study were regularly used by the communities. Therefore the sources were purged by frequent use and sampling is representative of water collected and used by communities. Nevertheless, more than 80 L was pumped immediately prior to sampling to make sure all equipment was thoroughly rinsed. Samples were collected directly from the hand-pump spout for boreholes, directly from the tap for tap stands and from the usual designated community sampling rope and bucket for shallow wells, to obtain a representative sample and avoid cross-contamination.

2.3.1. Tryptophan-like fluorescence (TLF)

A portable UviLux fluorimeter, calibrated by the manufacturer, was used to record TLF concentrations (Chelsea Technologies Group Limited, United Kingdom). The probe is set to measure fluorescence at the 280 ± 15 nm excitation 360 ± 27.5 nm emission wavelength and records fluorescence intensity in quinine sulphate units (QSU). The probe has a minimum detection limit of 0.17 ± 0.18 ppb dissolved tryptophan (Khamis et al., 2015). For each sample, the probe was first rinsed by fully immersing it in approximately 5 L of sample water. The probe was then transferred to another 5 L sample for the reading to be taken. The buckets used for rinsing and recording measurements were triple rinsed with sample water prior to use and were always stored appropriately to prevent sample contamination. All readings were taken in the shade, with a cover over the sampling bucket, to prevent interference from UV light. Readings were taken immediately after the sample was collected and readings had stabilised. The nature of the TLF probe and handset meant that readings were updated every few seconds to provide real-time data.

The fluorimeter was calibrated by the manufacturer by exposing it to known concentrations of tryptophan dissolved in deionised water (Chelsea Technologies Group Ltd, 2016a). The following formula was derived from these readings and a correction applied to relate instrument output to QSU: [QSU] = 1.1229E-04 x Signal - 0.305429. Where QSU is quinine sulphate units, where 1 QSU is a normalized fluorescence parameter which enables signals from different fluorimeters to be directly compared. TLF raw data was converted from QSU to ppb units using the equation: $T_{ppb} = 2.1130T_{QSU}$, for comparison with other published datasets (Chelsea Technologies Group Ltd, 2016a).

2.3.2. Humic-like fluorescence (HLF)

A portable UviLux fluorimeter, calibrated by the manufacturer, was used to record HLF concentrations (Chelsea Technologies Group Limited, United Kingdom). This probe is similar in design and function to the TLF probe described above, but has a slightly different set-up to cater for measuring HLF at the relevant emission wavelength. The probe is set to measure fluorescence at the 280 ± 15 nm excitation 450 ± 27.5 nm emission wavelength and records fluorescence intensity in quinine sulphate units (QSU). The probe is set to the same fluorescence excitation wavelength as the TLF fluorimeter instead of targeting the HLF peak, to capture any potential overlap in HLF fluorescence that could otherwise be mistaken for TLF intensity; there is a distinct overlap between the TLF and HLF regions. The sampling protocol was the same as described for the TLF fluorimeter. The HLF fluorimeter was calibrated by the manufacturer using known concentrations of pyrene tetrasulphonic acid (PTSA) dissolved in deionised water (Chelsea Technologies Group Ltd, 2016b). The following formula was derived from these readings and a correction applied to relate instrument output to QSU: [QSU] = 2.1320E-04 × Signal - 0.322399. Where QSU is quinine sulphate units, and 1 QSU is a normalized fluorescence parameter which enables signals from different fluorimeters to be directly compared. HLF raw data was converted from QSU to ppb units using the equation: $H_{ppb} = 1.3893H_{QSU}$, for comparison with other published datasets (Chelsea Technologies Group Ltd, 2016b).

2.3.3. TTCs

TTC counts were recorded using a plate counting method. Samples, collected in sterile 0.25 L polypropylene bottles, were transported to the laboratory in a cool box with ice packs. Sample preparation was undertaken within 8 h of collection. Samples were filtered through a 0.45 μm cellulose nitrate membrane (MF-Millipore). Membrane Lauryl Sulphate Broth (MLSB) was used to culture the TTCs, incubated at 44 °C for 18–24 h in a portable DelAgua kit (DelAgua, United Kingdom). Plate counts were recorded immediately after removal from the incubator, defined as the number of yellow colonies greater than 1 mm. The volume of sample filtered was 100 mL, unless a dilution was required due to high contamination levels. Due to time and space constraints in the incubator, not all sources could be tested as a series of dilutions. Therefore, an assessment of whether dilution was needed was based on the results of the sanitary risk score, TLF and turbidity results. For repeated samples, the average number of colonies was calculated. Where the average was between 0 and 1 cfu/100 mL, this was classed as low risk instead of no risk (WHO, 2011), as the more conservative approach. A blank was prepared at the beginning and end of each batch of samples for incubation, to confirm the medium was sterile and that there was no cross-contamination. In the large-scale dry season survey, one 0.25 L sample was collected for each source and for each batch of incubated samples one source was repeated, with two incubation plates prepared. Time and space constraints in the incubator prevented all samples from being repeated. In the small-scale wet season survey, three 0.25 L samples were collected from each source. For each source, one incubation plate was prepared from each sample bottle. Duplicate analysis was carried out for one source each day for each sample bottle. Where possible, repeats were performed for hand-pumped borehole sources as these typically have lower TTC counts. This ensured greatest replication at the sources with consistently low/no TTC counts because these are the risk classes with the smallest range of cfu/100 mL and have the greatest implications for drinking water quality assessment.

2.3.4. Hydrochemical sampling

Turbidity, temperature, pH and conductivity were measured at the water sources. Equipment was calibrated daily according to manufacturer protocols. Alkalinity was also measured in the field by titration with sulphuric acid using a bromocresol green indicator, but during the large-scale dry season survey this was only done at hand-pumped boreholes. Turbidity was recorded using a Hach 2100Q portable meter. The 10 mL vial was triple rinsed with sample water and then filled, all directly from the hand-pump spout, tap or rope and bucket. Three readings were taken for each sample and the median was used for analysis. Temperature readings were taken concurrently with TLF readings from the same sampling bucket, noting if any changes occurred during stabilisation of TLF readings. Mettler Toledo probes were used for measuring pH and conductivity. Three readings were taken for each sample and the median was used for analysis.

Samples for laboratory analysis were taken for hand-pumped boreholes only in the large-scale dry season survey and at all sources in the small-scale wet season survey. Chloride, nitrate, fluoride, sulphate and dissolved organic carbon (DOC) were measured. Samples for laboratory analysis were filtered (0.45 μm cellulose nitrate membrane; MF-Millipore) into a 30 mL Nalgene(™) bottle, un-acidified, leaving no air space. Samples were transported in a cool box with ice packs and stored at 4 °C, except during transportation to the United Kingdom (24 h at ambient room temperature). Anions were analysed by ion chromatography and cations by inductively coupled plasma mass spectroscopy. Cation samples were acidified and preserved with Aristar(™) grade concentrated nitric and hydrochloric acid (0.5% *v*/v) prior to analysis. DOC analysis was carried out on filtered samples (0.45 μm) using a gas analyser following acidification and sparging of samples. All DOC and inorganic analysis was undertaken in UKAS accredited laboratories in the United Kingdom.

2.4. Sanitary risk assessment

A sanitary risk assessment was undertaken at each source in both the wet and dry season. The risk assessment was based on the methodology detailed by WHO (1997), with the total number of positive responses indicating the level of risk. All aspects of the sanitary risk assessment were equally weighted in this study. The survey included an assessment of the condition of the source, features of construction and noting any nearby hazards that could be a source of contamination. Further details are provided in the Supplementary Information.

2.5. Data analysis

Spatial and temporal trends for hydrochemical parameters were determined visually from boxplot and scatter plot graphs and quantified using descriptive statistics. The results of the Shapiro-Wilk normality test indicated that the data are not normally distributed, therefore non-parametric tests were selected for analysis. The Kruskal-Wallis H-test was used to compare differences between districts and risk classes using mean ranks only, as the distribution of data varied between groups. Details of which specific groups were significantly different were identified using the post-hoc Dunn's test. No correction methods were used due to the small number of pair-wise comparisons and correction methods can be too conservative with a small number of groups. The Mann-Whitney U test was used to determine significant differences between source type and season. Significant differences were defined with $p \leq 0.05$. Statistical analysis was completed using R version 3.5.1.

3. Results

3.1. Potential hydrochemical interference with TLF

Temperature, turbidity, HLF and pH can potentially influence TLF in certain conditions (Reynolds, 2003; Baker et al., 2007; Khamis et al., 2015). In this dataset, temperature varied between districts, driven by differences in altitude affecting local climate, however, the values fall in a range considered to have negligible influence at the observed values of TLF (Table 2; temperature mean = 26 °C; range = 9 °C) (Khamis et al., 2015). Turbidity and pH values were also within ranges of negligible influence (Table 2; Reynolds, 2003, Baker et al., 2007, Khamis et al., 2015).

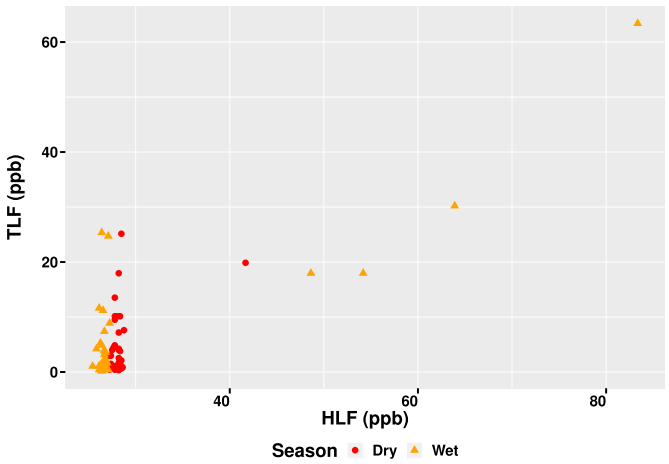

Fig. 3. Cross-plot and seasonal variation of TLF (ppb) and HLF (ppb) across Balaka and Lilongwe rural districts.

A small range of HLF is observed across the dataset, indicating TLF peaks were not influenced by HLF in this study, with the exception of two data points in the wet season (Fig. 3). However, it may be possible that HLF is influencing the TLF baseline. HLF was statistically lower in the wet season, despite four outliers ($p < 0.0001$). However, the difference in median values for the wet and dry season was small (wet season: median = 26.7 ppb; dry season: median = 28.1 ppb) (Fig. 3).

Table 2
Descriptive statistics for large-scale dry season survey. WHO exceedances highlighted in orange WHO (2017) guidelines: turbidity: 1 NTU; TTC: 0 cfu/100 mL; fluoride: 1.5 mg/L; nitrate: 50 mg/L.

	Hand-pumped boreholes														
	Balaka (n = 36)			Lilongwe (n = 30)			Machinga (n = 32)			Mzimba (n = 34)			Nkhotakota (n = 30)		
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
TLF (ppb)	0.3	7.2	2.2	0.4	4.0	1.0	0.2	4.9	0.9	0.4	1.5	0.8	0.2	2.7	0.7
HLF (ppb)	27.1	28.6	27.9	27.2	28.6	27.9	27.2	28.8	28.0	26.7	28.3	27.5	26.8	27.6	27.2
Turbidity (NTU)	0.1	**12.0**	0.9	0.3	**1.3**	0.4	0.1	**52.3**	**2.8**	0.1	**11.7**	**1.6**	0.4	6.6	1.0
TTC (cfu/100 mL)	0.0	**4.0**	**0.4**	0.0	**1200.0**	**40.5**	0.0	**8.5**	**0.4**	0.0	**50.0**	**1.7**	0.0	6.0	0.6
Sanitary Risk Score (%)	6.0	44.0	29.7	19.0	50.0	36.1	19.0	50.0	31.9	6.0	44.0	28.2	25.0	50.0	37.4
Temperature (°C)	25.0	28.6	27.1	23.8	25.6	24.5	25.2	28.7	26.8	22.4	25.8	24.4	27.2	29.9	28.2
pH	6.7	8.0	7.3	6.0	7.6	6.9	6.1	7.7	6.9	5.1	7.1	6.3	5.7	7.8	6.6
Conductivity (µS/cm)	330.0	2710.0	1030.6	194.7	1272.0	554.8	97.1	3951.0	587.6	59.2	1318.0	390.1	31.6	713.6	277.4
Iron (mg/L)	0.0	0.1	0.0	0.0	0.1	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Alkalinity (mg/L)	173.1	797.3	506.5	67.8	588.8	241.3	38.5	896.0	282.0	21.2	599.8	154.2	10.4	548.6	153.5
Fluoride (mg/L)	0.1	**2.4**	0.3	0.1	**3.9**	0.5	0.0	**8.4**	0.5	0.1	0.3	0.1	0.1	2.6	0.3
Chloride (mg/L)	3.3	596.6	68.1	0.3	34.4	6.1	2.2	395.5	31.4	1.6	190.5	16.3	0.6	21.9	4.0
Nitrate (mg/L)	0.1	27.5	5.5	0.1	**108.8**	14.8	0.1	41.7	4.6	0.4	28.3	3.4	0.1	13.2	2.0
Sulphate (mg/L)	3.9	454.0	93.7	0.1	485.5	86.5	0.1	103.1	14.2	0.1	33.2	1.1	0.1	42.6	4.2
	Alternative sources														
	Balaka (n = 9)			Lilongwe (n = 3)			Machinga (n = 5)			Mzimba (n = 2)			Nkhotakota (n = 2)		
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
TLF (ppb)	9.1	25.1	13.9	0.6	7.6	4.0	3.6	13.9	7.2	0.8	9.1	5.0	1.3	3.8	2.5
HLF (ppb)	27.8	41.7	29.6	27.8	28.8	28.3	27.4	28.5	28.0	27.5	28.1	27.8	27.4	27.4	27.4
Turbidity (NTU)	0.4	**44.5**	**13.9**	0.7	**21.3**	**7.7**	**3.1**	**518.0**	**130.1**	0.7	**78.8**	**39.8**	1.7	64.1	32.9
TTC (cfu/100 mL)	**5.0**	**5600.0**	**1750.4**	0.0	**816.7**	**455.6**	**450.0**	**1200.0**	**750.0**	0.0	**600.0**	**300.0**	0.0	400.0	200.0
Sanitary Risk Score (%)	56.0	69.0	63.7	38.0	56.0	46.0	56.0	63.0	59.5	31.0	63.0	47.0	50.0	63.0	56.5
Temperature (°C)	20.9	29.3	25.3	22.0	25.9	24.2	23.0	26.7	24.6	21.1	24.3	22.7	27.7	28.8	28.3
pH	6.7	8.5	7.7	5.8	6.8	6.2	5.9	7.4	6.5	5.2	6.6	5.9	6.7	6.9	6.8
Conductivity (µS/cm)	151.0	1718.0	734.5	98.7	363.3	234.2	23.3	335.1	158.6	55.2	549.8	302.5	133.0	670.7	401.9

3.2. Large-scale dry season survey: systematic difference in water quality between hand-pumped boreholes and alternative sources

Several hydrochemical parameters were significantly different between source type; TLF, TTCs, turbidity and sanitary risk scores were all significantly higher at alternative sources than hand-pumped boreholes (Mann-Whitney U: $p \leq 0.001$) (Fig. 4). Using TTCs and WHO (2017) risk classifications, the hand-pumped boreholes (which form the majority of the dataset) were all categorised as no risk or low risk, with the exception of only two sources. Both had adaptations to standard spillway design that increase contamination risk from surface water ingress (Fig. 5).

TLF was significantly higher in Balaka than all other districts, but no other significant differences were observed (Fig. 4, Table 2). TTCs however, only showed a significant difference between Balaka and Mzimba; for all other districts there was no significant difference and the alternative sources were highlighted as outliers in each district. HLF values across the whole dataset were generally stable (mean = 29.4 ppb, SD = 8.6), however, HLF concentrations at Mzimba and Nkhotakota were significantly different to each other and lower than all other districts. There was no significant difference in HLF concentrations between Lilongwe, Machinga and Balaka. Turbidity at Nkhotakota was significantly different to Balaka, Lilongwe and Mzimba. Conductivity showed a similar trend to TLF, with concentrations significantly higher in Balaka than any other district (Fig. 4, Table 2). In addition, conductivity in Lilongwe was significantly higher than that of Nkhotakota. Chloride, alkalinity, nitrate and sulphate were only measured at hand-pumped boreholes. Chloride and alkalinity again show a similar trend to TLF and were significantly higher in Balaka. Sulphate was elevated in Balaka and Lilongwe, significantly higher that all other districts, and in Lilongwe nitrate was also significantly higher than all other districts. Summary descriptive statistics are provided in Table 2.

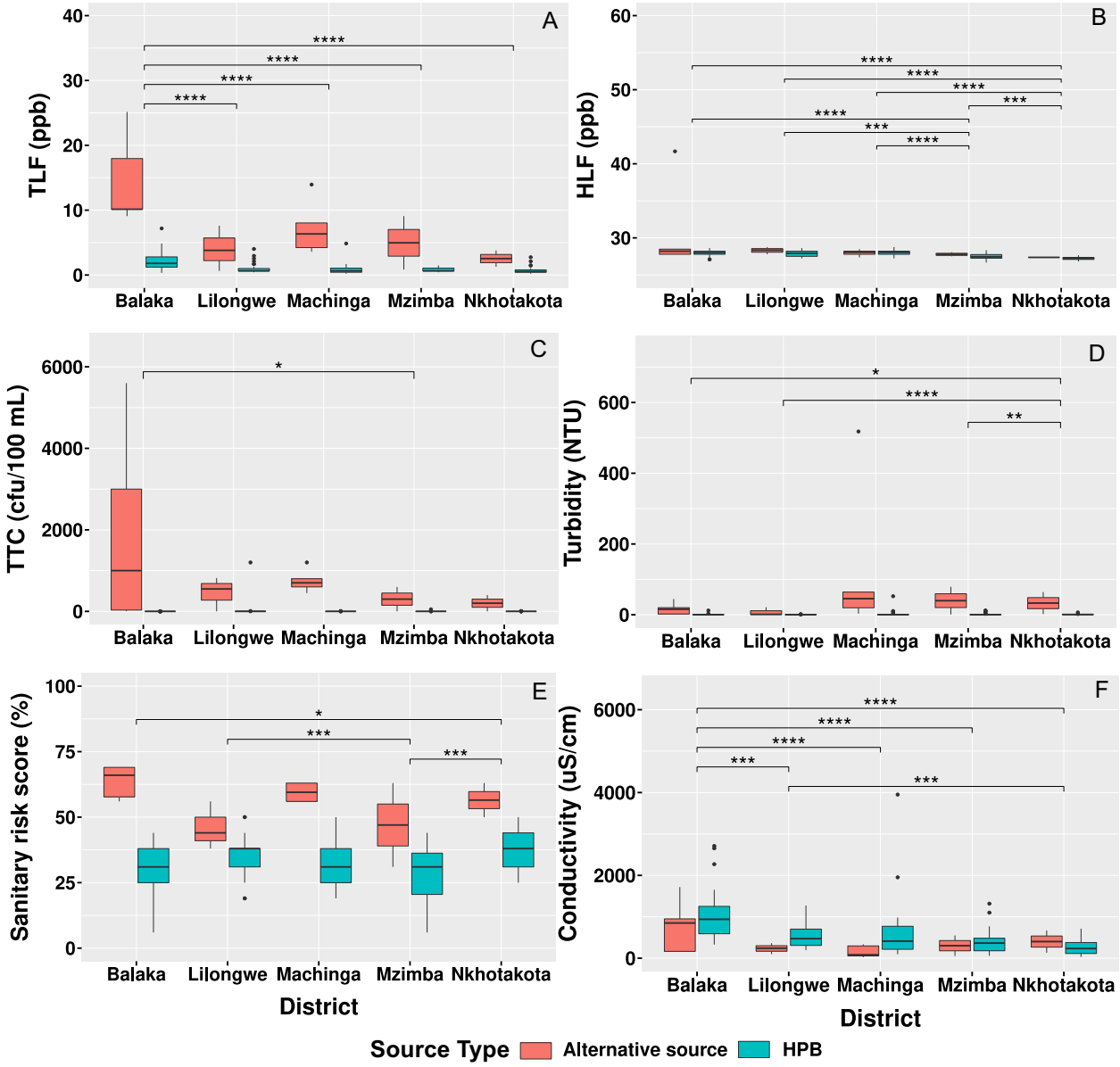

Fig. 4. Variation of selected hydrochemical parameters by district and source type in the large-scale dry season survey (2016). A: TLF (ppb); B: HLF (ppb); C: TTC (cfu/100 mL); D: Turbidity (NTU); E: Sanitary risk score (%); F: Conductivity (μS/cm). Boxes indicate the interquartile range and median, whiskers indicate maximum and minimum values except where outliers are indicated. Kruskal-Wallis and Dunns Test results: significant differences between districts (accounting for both source types together) are shown with the notation: **** = $p \leq 0.0001$; *** = $p \leq 0.001$; ** = $p \leq 0.01$; * = $p \leq 0.05$. HPB = hand-pumped borehole. Further information is provided in the Supplementary Information Table S3.

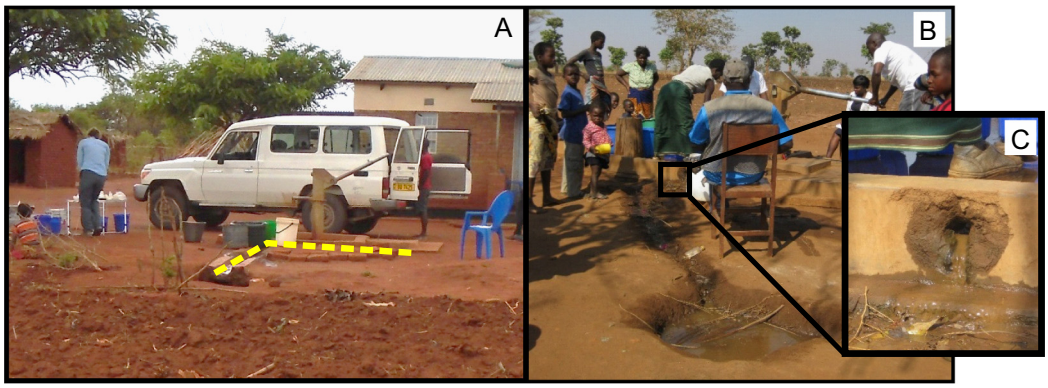

Fig. 5. Two hand-pumped boreholes had modifications to the standard drainage design. A: adaptation of the spillway - perpendicular to a normal arrangement and only about 5 m long B & C: Purpose-made hole in the cement apron resulting in an uncontrolled spillway and soakaway pond to form approximately 5 m from HPB. The original cement spillway is not used because the spillway does not align with the topographical gradient.

3.3. All dry and wet season data combined: TLF comparison with TTCs

Comparison of TLF to WHO risk classes using paired TTC data showed an overall trend of increasing TLF with risk WHO (2017) risk class (Fig. 6). There is a significant difference between combined no and low risk (TTC ≤ 9 cfu/100 mL) and medium, high and very high risk groups (TTC ≥ 10 cfu/100 mL) (Kruskal-Wallis: $\chi^2 = 60$, $p \leq 0.0001$; Dunn's test: $p \leq 0.006$). In general, for this dataset, the no and low risk classes represent hand-pumped boreholes and the high and very high risk classes represented alternative sources (Fig. 6).

3.4. Wet season vs dry season: temporal variation of water quality

Considering the data from the two districts (Balaka and Lilongwe) that were sampled in both seasons, there was no significant difference between seasons for TLF, TTCs, turbidity and sanitary risk score (Fig. 7). This was despite significant differences between source type in both seasons for these hydrochemical parameters (Mann-Whitney U: $p \leq 0.01$). Significant seasonal differences were observed for conductivity, pH, iron, fluoride and HLF ($p \leq 0.02$). In the wet season, pH and iron were lower, however, conductivity and fluoride concentrations were elevated (Table 3). In addition, the differences between hydrochemical parameters observed in the dry season in Balaka and Lilongwe were still present in the wet season. Conductivity and pH were the only inorganic hydrochemical parameters to be measured at all sources in both seasons and there was no significant difference between source type. Summary descriptive statistics are provided in Table 3, with WHO guideline exceedances occurring for turbidity, TTCs, fluoride and nitrate highlighted in orange.

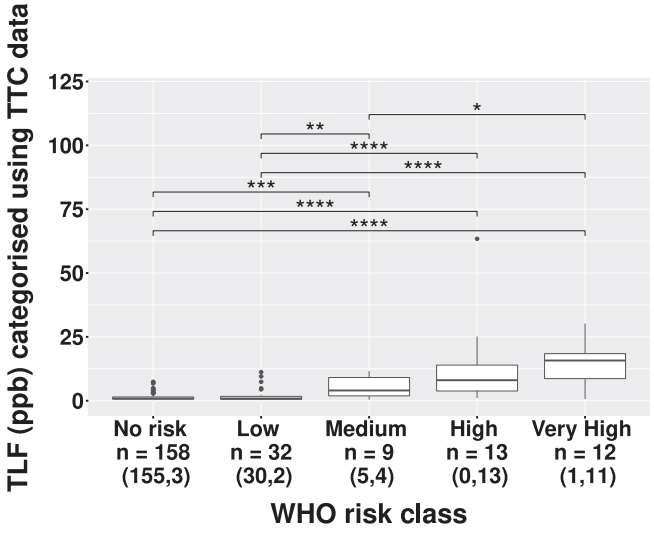

Fig. 6. TLF data categorised by WHO (2017) risk classes using TTC data: No risk (0 cfu/100 mL); Low risk (1–9 cfu/100 mL); Medium risk (10–99 cfu/100 mL); High risk (100–999 cfu/100 mL); Very High risk (≥1000 cfu/100 mL). Values in brackets indicates number of sources from each source type: (hand-pumped borehole, alternative source). Boxes indicate the interquartile range and median, whiskers indicate maximum and minimum values except where outliers are indicated. Significant differences between risk classes are shown with the notation: **** = $p \leq 0.0001$; *** = $p \leq 0.001$; ** = $p \leq 0.01$; * = $p \leq 0.05$.

4. Discussion

4.1. Source type is a key factor in determining microbial water quality

The most prominent differences in microbial water quality were observed between different source types (hand-pumped boreholes compared to alternative sources), with little variation between districts or seasons. Kanyerere et al. (2012) also studied microbial drinking water quality in Malawi and found similarly that hand-pumped boreholes were least contaminated. TLF, TTCs, turbidity and sanitary risk scores were all elevated at alternative sources, in agreement with other studies that shallow groundwater sources are more vulnerable to contamination and in particular, open shallow wells (Lavoie and Viens, 1983; Lloyd and Bartram, 1991; Parker et al., 2010; MacDonald et al., 2019). The highest median TLF and TTC concentrations were observed in Balaka but this was due to the larger number of alternative sources sampled in this district. This was not intentional in the experimental design, however, Balaka was the first district sampled and therefore was sampled earlier in the dry season when less alternative sources had dried up. In this study, only a small minority of samples taken from hand-pumped boreholes were found to be at risk of faecal contamination as defined by WHO (2017) assessment criteria. Therefore, this points to the design and construction of the alternative sources as the cause of vulnerability to faecal contamination. Ingress of contaminated surface water, either via direct surface run-off or infiltration into the shallow aquifer (or piped distribution system for tap stands) is likely to be the main source of microbial contamination at these alternative sources (Howard et al., 2003; Engstrom et al., 2015). This indicates that improving the construction of sources to meet the requirements for JMP 'improved' sources could greatly reduce contamination risk in rural areas. However, not all 'improved' sources were free from contamination, as found in other studies (Bain et al., 2014; Parker et al., 2010).

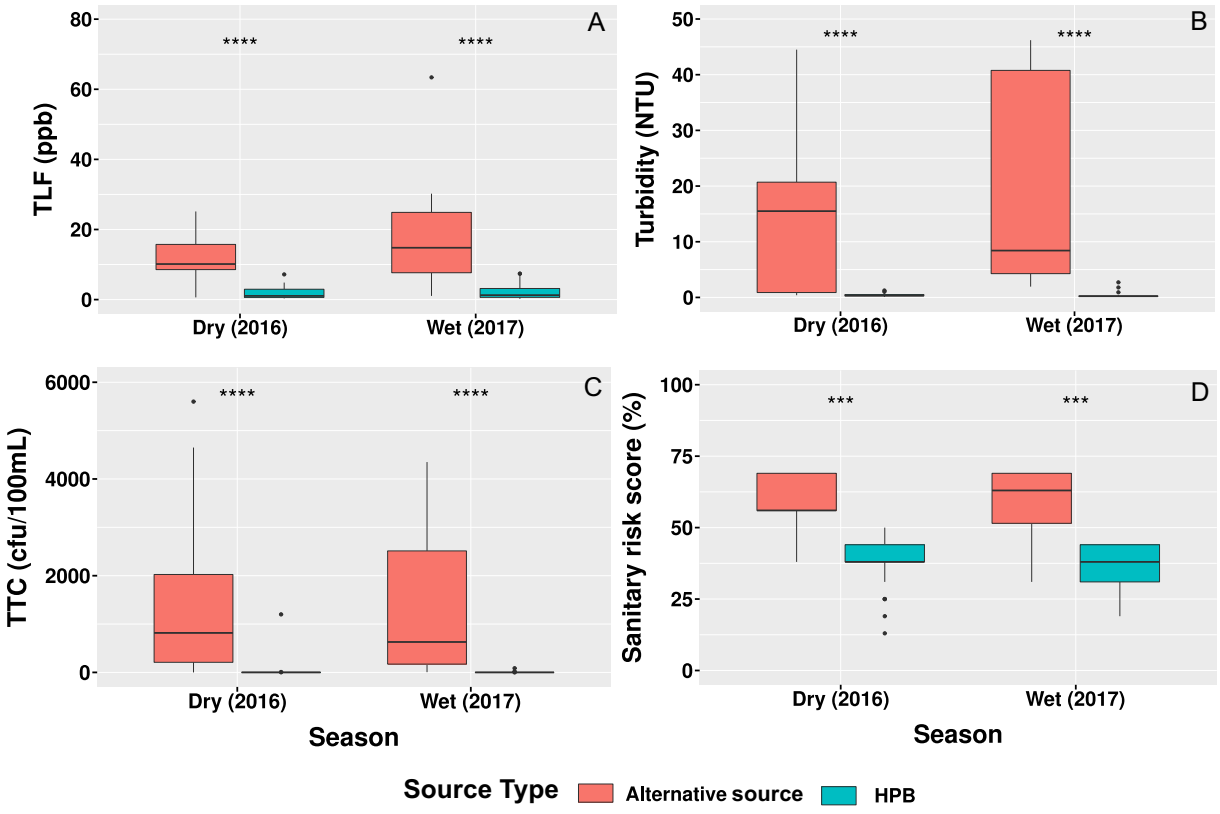

Fig. 7. Variation of selected hydrochemical parameters used as indicators of microbial contamination and sanitary risk scores for each season and source type. Boxes indicate the interquartile range and median, whiskers indicate maximum and minimum values except where outliers are indicated. Significant differences between source type within each season are shown with the notation: **** = $p \leq 0.0001$; *** = $p \leq 0.001$; ** = $p \leq 0.01$; * = $p \leq 0.05$. HPB = hand-pumped borehole.

4.2. Season is a key factor in determining inorganic water quality, with some evidence of anthropogenic influence

Conductivity, pH, iron and fluoride all showed seasonal differences which are likely to be related to groundwater recharge processes in the wet season. Conductivity and pH are the only inorganic hydrochemical parameters to be measured at all source types and there is no significant difference between source type. In contrast, the microbial indicators show no significant seasonal difference but there is greater variability of these parameters at alternative sources in the wet season (Fig. 7). Whilst only the inorganic parameters show seasonal differences, this still indicates groundwater recharge processes that can be attributed to the increased variability of microbial parameters observed at alternative sources. Elevated nitrate concentrations, an indicator of anthropogenic activity, are observed at hand-pumped boreholes in rural Lilongwe all year round with maximum values exceeding WHO (2017) guidelines (Table 3: max: dry season 65.4 mg/L; wet season 114.1 mg/L). Rural Lilongwe has a population of 1,600,000 people, the largest of all districts and sub-districts in the country (Government of Malawi, 2018). Subsistence farming is widespread; agricultural practices (application of fertiliser) and deforestation (to make way for farmland whilst enhancing the capacity for soil erosion and decreasing capacity for uptake of nitrogen from natural vegetation) are therefore suggested as the most likely sources of elevated nitrogen in this case. Waste dumps and pit latrines can also be sources of nitrate but these are often associated with elevated chloride and conductivity concentrations as well (Falliat and Rambaud, 1991; Cronin et al., 2007; Lapworth et al., 2017). However, chloride remains low in rural Lilongwe (Table 3: max: dry season 34.4 mg/L; wet season 85.2 mg/L). It is noteworthy that average conductivity increases in the wet season in all sources and both districts (Table 3; e.g. Lilongwe hand-pumped boreholes: dry season: mean = 639.4 μS/cm, max: 1272.0 μS/cm; wet season: mean = 1152.6 μS/cm, max = 2277.0 μS/cm). This could be due to enhanced shallow contamination sources, but there is a large range of values in the wet season and so a few sources with high values have a large influence.

4.3. TLF is not sensitive enough to be considered as an alternative to coliform culturing

In agreement with Nowicki et al. (2019), this TLF dataset fails to distinguish between WHO risk classes (based on TTC counts, Supplementary Information Table S1) for no and low risk. The WHO (2017) drinking water guidelines and the WHO-UNICEF JMP for measuring progress towards SDG 6 state 'no' risk as the required standard for compliance. TLF is unable to detect if this requirement has been met. This potentially limits the scope of TLF as a tool for detecting microbial contamination in drinking water. Sorensen et al. (2018a) combined four different TLF datasets from different countries and also concluded that a TLF threshold could not be defined between no and low risk. Currently, the lowest TLF threshold that can be defined marks the boundary between low and medium WHO risk classes. In addition, the error rates for the defined thresholds would require improvement if TLF was to be developed further for drinking water applications.

The findings of this study show that TLF is not sensitive enough to be considered as a direct replacement for established culturing methods, this was also concluded by Nowicki et al. (2019) in a smaller study in Kenya. Bridgeman et al. (2015) also found poor correlation for TLF with TTCs and *E. coli*. However, TLF may prove useful as a high-level screening tool to identify groundwater sources with moderate to high contamination risk. The significant differences in TLF, when data is grouped by either district or WHO risk class, are driven by underlying significant differences in TLF between source type, easily distinguished by construction design for this dataset. Markechova et al. (2013)

Table 3
Descriptive statistics for seasonal comparison. WHO exceedances highlighted in orange * = WHO (2017) guidelines: turbidity: 1 NTU; TTC: 0 cfu/100 mL; fluoride: 1.5 mg/L; nitrate: 50 mg/L.

	Hand-pumped boreholes											
District & Season	Balaka Dry (*n* = 12)			Balaka Wet (*n* = 12)			Lilongwe Dry (*n* = 17)			Lilongwe Wet (*n* = 17)		
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
TLF (ppb)	0.3	7.2	3.0	1.1	7.4	3.3	0.4	4.0	1.2	0.2	6.8	1.4
HLF (ppb)	27.2	28.5	28.0	25.4	27.0	26.4	27.2	28.6	28.0	26.1	27.1	26.6
Turbidity (NTU)*	0.1	0.8	0.3	0.1	**1.8**	0.4	0.3	**1.3**	0.5	0.1	**2.7**	0.4
TTC (cfu/100 mL)*	0.0	**4.0**	**0.6**	0.0	**86.2**	**8.7**	0.0	**1200.0**	**71.3**	0.0	**11.5**	**1.5**
Sanitary Risk Score (%)	13.0	44.0	31.9	19.0	44.0	35.0	38.0	50.0	40.8	31.0	44.0	37.4
Temperature (°C)	25.0	28.3	27.0	26.1	28.2	27.2	23.8	25.4	24.4	23.2	27.2	24.4
pH	6.8	7.7	7.2	6.5	7.4	6.9	6.3	7.6	6.9	5.4	7.2	6.4
Conductivity (μS/cm)	522.6	2654.0	1287.5	503.0	3443.0	1795.5	229.8	1272.0	639.4	264.5	2277.0	1152.6
Iron (mg/L)	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.1	0.0
Alkalinity (mg/L)	264.5	776.6	582.9	247.5	790.0	548.1	87.7	588.8	283.4	42.1	898.5	288.9
Fluoride (mg/L)*	0.1	**2.4**	0.4	0.0	**2.3**	0.7	0.1	**3.9**	0.6	0.0	**4.8**	0.9
Chloride (mg/L)	7.7	445.4	109.9	6.7	325.4	83.5	0.6	34.4	6.5	0.1	85.2	9.5
Nitrate (mg/L)*	0.1	27.4	8.2	0.0	25.7	7.7	0.4	**65.4**	14.3	0.1	**114.1**	17.2
Sulphate (mg/L)	14.9	386.1	136.3	10.3	330.9	117.7	4.1	485.5	120.1	0.8	319.5	92.4
DOC (mg/L)	-	-	-	0.9	2.3	1.4	-	-	-	0.5	2.1	1.2
	Alternative sources											
District & Season	Balaka Dry (*n* = 8)			Balaka Wet (*n* = 8)			Lilongwe Dry (*n* = 3)			Lilongwe Wet (*n* = 4)		
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
TLF (ppb)	9.5	25.1	14.6	11.2	63.4	25.3	0.6	7.6	4.0	1.1	8.9	4.4
HLF (ppb)	27.8	41.7	29.8	26.1	83.4	44.5	27.8	28.8	28.3	26.7	27.2	26.9
Turbidity (NTU)*	0.4	**44.5**	**15.4**	**3.6**	**46.2**	**28.1**	0.7	**21.3**	**7.7**	**1.9**	**10.3**	**5.4**
TTC (cfu/100 mL)*	**5.0**	**5600.0**	**1965.3**	**5.0**	**4350.0**	**1811.6**	0.0	**816.7**	**455.6**	**66.7**	**1216.7**	**431.7**
Sanitary Risk Score (%)	56.0	69.0	63.7	56.0	69.0	65.8	38.0	56.0	46.0	31.0	63.0	47.0
Temperature (°C)	20.9	29.3	25.1	23.3	28.1	26.0	22.0	25.9	24.2	23.8	25.3	24.4
pH	6.7	8.5	7.7	6.2	7.9	7.2	5.8	6.8	6.2	5.1	6.3	5.8
Conductivity (μS/cm)	151.0	1718.0	805.6	252.0	3326.0	1293.1	98.7	363.3	234.2	160.5	780.2	495.2
Iron (mg/L)	-	-	-	0.0	0.1	0.0	-	-	-	0.0	0.0	0.0
Alkalinity (mg/L)	-	-	-	73.3	719.2	374.0	-	-	-	25.4	186.5	100.5
Fluoride (mg/L)*	-	-	-	0.2	**2.7**	0.8	-	-	-	0.1	1.3	0.6
Chloride (mg/L)	-	-	-	1.6	515.0	78.3	-	-	-	2.1	22.7	9.5
Nitrate (mg/L)*	-	-	-	0.0	2.8	0.8	-	-	-	8.5	39.4	19.7
Sulphate (mg/L)	-	-	-	0.7	402.1	109.0	-	-	-	1.8	24.7	13.6
DOC (mg/L)	-	-	-	2.2	8.8	4.1	-	-	-	0.5	1.5	0.9

suggest the lack of selectivity of fluorescence makes it more suitable for identifying pollution plumes in environmental waters rather than trying to capture the detail required at low levels of contamination to determine compliance with drinking water standards; this is supported by the results from this study.

4.4. HLF limits the effectiveness of TLF for detecting microbial contamination in drinking water

It is unclear why TLF fails to distinguish between no risk and low risk classes, this could be partly due to the high uncertainty in plate counting methods at these low concentrations, however, HLF interference could provide one explanation. This is the first study to measure HLF alongside TLF in a spot-sampling survey, however Sorensen et al. (2018b) measured both parameters during an online water quality study in the United Kingdom. Sorensen et al. (2018b) found that TLF and HLF followed the same temporal trends in peaks and troughs and concluded that it was unnecessary to measure both parameters for water quality assurance. This dataset, however, shows a different result: HLF is relatively stable between districts and source type, especially in comparison to fluctuations in TLF (Fig. 3). It is clear that there is no large HLF interference with TLF peaks in this study with the exception of two outliers, however, the stable presence of HLF could instead be acting to raise the TLF baseline, which becomes apparent at lower concentrations of TLF and therefore masks any potential difference in TLF. In this case, the affected sources are most likely to be those classed as no and low risk sources (WHO, 2011).

Fluorescence peaks can vary in intensity depending on the source of organic matter (Baker, 2002). It is likely that the source of HLF in this study is derived from DOC leaching from the soil. All sources were in rural areas with predominantly agricultural land use, which, may explain HLF similarities between districts. However, HLF has also been associated with microbial activity (Fox et al., 2017). The source of TLF is likely to be

from microbial contamination from waste sources (Baker, 2002). This fits well with the highest TLF values being observed at the alternative sources, which are more vulnerable to faecal contamination due to their design. However, Fox et al. (2017) observed some extracellular fluorescent dissolved organic matter can also be associated with the TLF peak. In the wet season survey, the majority of sources show a small decrease in HLF and the general trend remains stable (Fig. 3), this could be due to dilution of dissolved organic carbon following seasonal recharge (McDonough et al., 2020). Conductivity, pH, iron and fluoride all vary seasonally, which also suggests that recharge processes are influencing groundwater chemistry. The few outliers with highly elevated HLF and TLF are associated with alternative sources as would be expected (Fig. 3).

5. Conclusion

The most prominent differences in microbial water quality are observed between different source types (hand-pumped boreholes and alternative sources i.e. shallow wells and tap stands), with little variation between districts or seasons. TLF, TTCs, turbidity and sanitary risk scores show no significant difference between seasons and are all elevated at alternative sources, which are inherently more vulnerable to contamination. Ingress of contaminated surface water, either via direct surface run-off or infiltration into the shallow aquifer (or piped distribution system for tap stands) is likely to be the main source of microbial contamination at these alternative sources. Hand-pumped boreholes showed overall good microbial water quality in comparison to alternative sources and when compared against WHO (2017) criteria, with the majority of sites sampled classed as no or low risk. Conductivity, pH, iron and fluoride all showed seasonal differences which are likely to be related to groundwater recharge in the wet season. Elevated nitrate concentrations in rural Lilongwe are likely to be associated with the high population density and widespread agricultural practices within the district.

TLF is currently unable to distinguish between the no and low WHO risk classes, which limits its use as a tool for detecting microbial contamination in drinking water. This dataset shows that HLF fluorescence may be masking TLF concentrations at low concentrations. TLF currently cannot provide the level of detail offered by traditional bacterial culturing methods. Instead, TLF is more suited to high-level screening of drinking water sources to identify sites with moderate to high levels of faecal contamination. In these circumstances, the benefits of TLF would be rapid results and, particularly in low resource settings, overcoming several data collection barriers to measuring progress towards SDG 6.

CRediT authorship contribution statement

Jade S.T. Ward: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Validation, Visualization, Writing - original draft, Writing - review & editing. **Daniel J. Lapworth:** Conceptualization, Formal analysis, Methodology, Project administration, Resources, Supervision, Validation, Writing - review & editing. **Daniel S. Read:** Conceptualization, Formal analysis, Methodology, Project administration, Supervision, Writing - review & editing. **Steve Pedley:** Conceptualization, Formal analysis, Methodology, Project administration, Resources, Supervision, Writing - review & editing. **Sembeyawo T. Banda:** Data curation, Investigation. **Maurice Monjerezi:** Project administration, Writing - review & editing. **Gloria Gwengweya:** Data curation, Investigation, Project administration, Validation. **Alan M. MacDonald:** Conceptualization, Methodology, Project administration, Resources, Supervision, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.140674.

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