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Performance of faecal indicator bacteria, microbial source tracking, and pollution risk mapping in tropical water☆

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ABSTRACT

Faecal indicator bacteria (FIB) are used for the assessment of faecal pollution and possible water quality deterioration. There is growing evidence that FIB used in temperate regions are not adequate and reliable to detect faecal pollution in tropical regions. Hence, this study evaluated the adequacy of FIB, including total coliforms (TC), *Escherichia coli* (EC), Enterococci (IEC), and *Clostridium perfringens* (CP) in the high-altitude, tropical country of Ethiopia. In addition to FIB, for microbial source tracking (MST), a ruminant-associated molecular marker was applied at different water types and altitudes, and faecal pollution risk mapping was conducted based on consensus FIB. The performances of the indicators were evaluated at 22 sites from different water types. The results indicate that EC cell enumeration and CP spore determination perform well for faecal contamination monitoring. Most of the sub-basins of Lake Tana were found to be moderately to highly polluted, and the levels of pollution were demonstrated to be higher in the rainy season than in the post-rainy season. Markers associated with ruminants (BacR) were identified in more than three quarters of the sites. A bacterial pollution risk map was developed for sub-basins of Lake Tana, including the un-gauged sub-basins. We demonstrate how bacterial pollution risk mapping can aid in improvements to water quality testing and reduce risk to the general population from stream bacteria.

1. Introduction

Water pollution by pathogenic microorganisms from human and animal waste causes public and ecosystem health risks. Management of this problem is dependent on identifying the right indicators and knowing which sources of faecal matter are the cause. *Escherichia coli* (EC), *Clostridium perfringens* (CP), and Enterococci (IEC) are used as a proxy for detecting pathogenic microorganisms in soil and water samples. They are also used as faecal pollution indicators for the evaluation of faecal contamination and conceivable water quality decay in different freshwater sources (APHA-AWWA-WPCF, 1981; Toranzos et al., 1997; Byamukama et al., 2005; Rochelle-Newall et al., 2015). For several decades, faecal coliforms have been used as a standard indicator of recent faecal pollution. These microbial indicators have also been used in tropical countries (Byamukama et al., 2005). The maximum contaminant level (MCL) is the legal threshold for the amount of a substance that is permitted in public water systems. The MCLs set for temperate areas are accepted without question by tropical areas, although the source water quality in most tropical regions varies from that of temperate areas in three key ways: physico-chemical, biological and socioeconomic factors (Hazen, 1988; Hazen and Toranzos, 1990).

The performance of the traditional indicators was not adequately evaluated in tropical countries. It is well known that they work fine in temperate areas and such matrices are not always reliable and we should downscale to Ethiopian environment. Therefore, we should evaluate the performance of these traditional indicators in a highland tropical country with a different socio-economic and physical context.There is a growing body of evidence that the underlying assumptions of the assays being used are not automatically valid in tropical climates and should be evaluated (Desmarais et al., 2002; Byamukama et al., 2005; Espinosa et al.,

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2009; Sinigalliano et al., 2010; Reischer et al., 2013). Studies in tropical freshwater have shown that high proportions of faecal coliform-positive isolates may be of non-faecal origin (Scott et al., 2002). Moreover, some studies have reported that presumptive EC can become a normal inhabitant of tropical waters, as reported for pristine environments in some tropical waters (Rivera et al., 1988; Byappanahalli and Fujioka 1998; Ahmed et al., 2008). This apparent unreliability of traditional FIB in tropical conditions should lead to the development of alternative pollution indicators.

In addition to the developing alternative, easy-to-use, and inexpensive FIBs, it is important to monitor the source of faecal contamination for the proper management of drinking water supplies on a watershed scale. To this end, various genetic faecal markers have been developed for temperate waters. There are numerous and diverse assay tools that target faecal pollution from different animal sources to discriminate between pollution sources. These include BacR, a ruminant-associated faecal marker; Pig-2-Bac, a pig-associated faecal marker, and Bach HUM or HF 183 Taqman, a human-associated faecal marker (Reischer et al., 2013; Mayer et al., 2018). Recently developed quantitative polymerase chain reaction (qPCR) assays as well as standard faecal indicators have been widely applied in temperate regions. However, their performance has not been adequately evaluated in tropical regions, e.g. Ethiopia, where there is a high range of altitude that varies from −100 m above mean sea level (m.a.s.l.) to 4533 m.a.s.l. and varied water types and socioeconomic contexts. Moreover, neither standard FIB nor molecular markers (BacR) have been evaluated at different altitudes (low-, mid-, and highlands) and water sources (surface and groundwater) in tropical countries. The FIB with all its limitations generally indicate faecal pollution both from humans and animals, but they do not show source attribution. This research focuses on faecal pollution from cattle Microbial Source Tracking (MST) work because cattle are the dominant source of nitrogen pollution in the study area (Goshu et al., 2020). It is thus likely that the faecal input from cattle is also the dominant source of faecal pollution in the study area. Overall, there exists an urgent need to evaluate existing methods and parameters to devise affordable, easy-to-perform, and reliable techniques for faecal contamination monitoring in tropical regions.

Faecal pollution risk mapping is essential to manage the overall water quality of a lake basin. Benefits of pollution risk mapping include the easy identification of areas that need intervention, the management of potential sources of contamination, and the integration of drinking water protection activities with other environmental programs at the state, zone, district, and local levels. However, prior to this research, the faecal pollution risk map for the Lake Tana basin, both for gauged and un-gauged sub-basins, remained unknown. Therefore, this study aimed to provide the first evaluation of the performance of FIB, including total coliforms (TC), presumptive *E. coli* (EC), intestinal enterococci (IEC), and presumptive *Clostridium perfringens* spores (CP). Using a qPCR assay (BacR), this study also aimed to determine ruminant-associated faecal pollution at different water types located at different altitudes (1100−3835 m.a.s.l.) in a tropical highland country. Based on the results of the FIB evaluation and enumeration, a risk map was created showing the degree of pollution of sub-basins of the Lake Tana basin.

2. Materials and methods

2.1. A general overview of the study sites

This study includes two sets of sites. The first set, which consists of 22 sites, was used to evaluate the performance of FIB and then to select a consensus parameter (see section 2.6). The second set consists of 20 sub-basins (seven gauged and 13 un-gauged) in which a map showing the degree of pollution of various waters in the Lake Tana basin was established based on the results of the FIB enumeration (Fig. A1).

2.1.1. Study sites for performance evaluation and microbial source tracking

The performance study of FIB was conducted primarily in the Lake Tana watershed and also partly in the Chokie Mountains and Chifenchifit watersheds in the northern part of Ethiopia (Table 1). A total of 22 sampling sites − 12 groundwater and 10 surface water − were studied in the rainy and post-rainy seasons from June to December 2017. The examined locations were from different water types, pollution categories, and altitudes (Table 1). The rule for grading the degree of pollution or presumptive pollution was that sampling sites that have congested human settlements, pit latrines near or around the area, waste water pools, and cattle grazing areas were considered to be under high risk of being influenced; they were thus placed in the “high” presumptive pollution category (Table 1). Those that did not have any or had relatively less of these observable water source polluters were considered to be a lower risk and were categorized in the “low” presumptive pollution category, and those in between were categorized as “medium”.

2.1.2. Study sites for pollution risk mapping

Pollution risk maps were developed for the 20 sub-basins of Lake Tana for the rainy (July−September) and post-rainy (October−December) seasons. Six of the twenty sub-basins are gauged river sub-basins in the Lake Tana basin (please see the detailed description in Annex A.1 and Fig. A1).

2.2. Sampling

A total of 241water samples (192 for performance and 49 for mapping) were collected from wells, streams, and rivers. Surface water samples were collected using sterile Kimax Kimble glass bottles with butyl rubber stoppers from a 30 cm-deep, flowing section of a stream. The groundwater samples from wells equipped with a pump were collected after 2 min of water flushing. In the remaining wells provided with ropes and buckets, water samples were collected directly from the bucket after washing. The sample bottles were immediately placed in a dark cool box and transported to the Food and Chemical Engineering Laboratory of Bahir Dar University. The samples were then analysed within 6 h of the first sample being taken. Water samples for physicochemical analysis were collected in 500 ml polyethylene bottles (made by Thomas Scientific), kept in an icebox, and transported to the laboratory for immediate analysis within 6 h (APHA-AWWA-WPCF, 1981).

2.3. Physicochemical characteristics

In-situ measurements of electrical conductivity (Con), pH, dissolved oxygen (DO), temperature (T), and total dissolved solids (TDS) were taken with a YSI Pro Plus multi-parameter meter (Ohio, USA). Ammonium, nitrite, and nitrate were determined photometrically (Palintest Transmittance-display Photometer 8000; North East, England) at the water quality laboratory of the School of Civil and Water Resource Engineering, Bahir Dar University.

2.4. Determination of standard Faecal Indicator Bacteria

2.4.1. Presumptive detection, enumeration, and confirmatory tests

The presumptive simultaneous detection of TC and EC was undertaken by a membrane filtration technique using Chromo Cult Coliform Agar (CCA). *Clostridium perfringens* spores were detected

by solid Tryptose Sulfite Cycloserine Agar (TSC) agar, and IEC was detected by Slanetz and Bartley agar media. Chromo Cult Coliform Agar media were amended by the addition of Cefsulodin (5 mg/l; Sigma, Vienna, Austria), and CCP were amended by a *Clostridium perfringens* supplement (0.4 g/l). The pink and blue colonies on CCA agar were classified as TC and EC respectively. Black colonies growing on TSC plates were classified as CP. Pink to red colonies were classified as IEC. All agar media was from Merck.

2.4.2. Sampling and DNA extraction of water samples

Water samples were filtered over 0.2 μm polycarbonate filters (Millipore, Bedford, MA) and stored on dry ice at −80 °C during transportation to Technical University (TU) Vienna for further processing. DNA extraction was then performed using bead-beating and phenol/chloroform (Griffiths et al., 2000; Reischer et al., 2008; Mayer et al., 2018). In brief, cell lysis was achieved by the addition of Cetyltrimethylammonium bromide (CTAB) buffer and glass beads in a Fast Prep 24 bench-top homogeniser for cell lysis (MP Biomedical Inc., Irvine, CA) at a speed setting of 6 ms^{-1} for the 30 s. Polycarbonate filters were completely dissolved at this step, and the DNA was subsequently purified by washing procedures. Precipitation of the DNA was achieved by the addition of isopropanol. The extracted DNA was eluted in a 10 mM TRIS buffer (pH 8.0) and stored at −80 °C until further analysis. Before the qPCR assays, the DNA concentration of the sample DNA extracts was determined using the Quanti Fluor® dsDNA Kit (Promega, USA) according to the manufacturer's instructions. Fluorescence readings were taken on a Glomax Microplate Reader (Promega, USA).

2.5. Microbial source tracking MST genetic marker detection

In addition to the application of the ruminant-associated 16S-rRNA-gene marker BacR (Reischer et al., 2006), a general *Bacteroidetes* marker, AllBac (Layton et al., 2006), was used as quality control to assess the ability to amplify DNA in the samples. The assay was applied as a duplex qPCR, including an internal amplification control (IAC), non-competitive using the *ntb2* gene from *Tabaco nicotianum* L. (Anderson et al., 2011). All samples were measured in duplicate in at least two four-fold DNA dilution steps, and the results were compared. Samples with two matching AllBac concentrations (i.e., the ratio [concentration 1:16.4]/[concentration1:4] was between 0.5 and 2) in the 1:4 and 1:16 dilutions were judged free of PCR inhibiting substances in the 1:4 dilution. In addition to the dilution method to identify possible PCR inhibition, results from the IAC were evaluated.

The presence of PCR inhibitors was confirmed if the threshold cycle (Ct value) of the IAC assay in any sample was one cycle higher than that in negative control. Furthermore, controls included no-template controls as well as filtration and DNA extraction blanks.

All sample DNAs in the qPCR assay detecting ruminant-associated faecal pollution (i.e., BacR(Reischer et al., 2006); were measured in duplicate. The quality assessment of qPCR data was conducted as previously described (Reischer et al., 2006, 2011; Mayer et al., 2018). In brief, the reaction efficiency of all qPCR runs ranged from 95 to 105%. All negative controls and no-template controls were consistently negative (i.e., fluorescence never exceeded the threshold). We re-judged samples with replicates having standard deviations of a Ct-value > 1 in the four-fold DNA extract dilutions as not quantifiable, and they were thus not considered for further analysis. qPCR standard dilutions ranging from 10^0 to 10^6 targets per reaction were used in a linear regression model for calculation of the qPCR calibration curve. The results are reported as marker equivalents per filtered water volume (ME vol^{-1}), a method asserted by Reischer et al. (2006). Filtration volume, the use of 2.5 μl undiluted DNA extract in the qPCR, and the minimal theoretically detectable marker concentration per reaction (one copy) define the threshold of detection (Reischer et al., 2006). The threshold of detection of the herein presented data set was 107 copies.

All qPCR assays were run on a Rotor-Gene Q thermocycler (Qiagen Inc.) in a total reaction volume of 15 μl with 2.5 μl sample DNA dilution (1:4). The respective reaction mixtures were composed of 7.5 μl Rotor-Gene Multiplex PCR master mix (Qiagen Inc.), 2.5 μl sample DNA dilution, and 400 ng μl^{-1} bovine serum albumin, while the originally published primer and probe concentrations were maintained. Cycling parameters for AllBac, including the IAC, were 5 min at 95 °C for denaturation and 45 cycles of 30 s at 95 °C followed by 45 s at 60 °C. Cycling parameters for BacR were 5 min at 95 °C for denaturation and 45 cycles of 15 s at 95 °C followed by 60 s at 60 °C.

2.6. Evaluation of FIB and pollution risk mapping

Pollution risk maps were constructed for gauged and un-gauged sub-basins of the Lake Tana basin (see Fig. A1). Firstly, we evaluated different FIB at different altitudes and water types. We then selected the best FIB from among those that were tested, which we termed the consensus picture. Consensus FIB were selected based on their performance, which is defined as the discrimination efficacy of the tested FIB at different altitudes and water types of different levels of pollution. Better performing FIB have a higher discrimination ability. The discrimination ability is measured as a ratio of the number of pairs of compared sites of high and low pollution categories that have significant statistical test outcomes ($p \leq 0.05$) to the total number of pairs of compared sites. Those FIB that have better discrimination efficacy were selected a consensus FIB. Pollution classes were established between the highest achievable level in faecal material and raw sewage and the natural background level of the faecal pollution of surface water and groundwater habitats (expected levels < 1 Colony forming units (CFU) of the consensus indicator/100 ml (Kavka et al., 2006). As CP occurs at lower concentration levels in faecal material and raw sewage (approx. 10^4–10^5 CFU/100 ml) compared to EC, the pollution classes were adapted accordingly.

To classify the investigated sites into their respective pollution classes and develop a pollution map based on a statistical conservative estimate, we used 90 percentile log values of consensus FIB, as per the works of Kavka et al. (2006). To estimate the 90 percentile bacteria concentration for the un-gauged sub-basins, we used the approach of Yarahmadi (2003) and Hofstra and Vermeulen (2016) (see annex A.2 for details).

2.7. Data analysis

We compared the differences in bacterial mean counts of each indicator bacteria for a pair of sites in the surface water and site classes in groundwater sampling sites. We employed the software package IBM SPSS Statistics 25 for statistical analysis, and we carried out non-parametric tests, such as the Kruskal-Wallis H test and the Monte Carlo test. Our significance cut off was $p < 0.05$. The interquartile range divided by the median was used as a non-parametric indicator of variability. The bacteriological concentration ratios of medians (BCRMs) of various indicators were calculated by dividing the median concentration of an indicator bacteria of a high influence site by the median concentration of an indicator bacteria of a low influence site to further analyse the detected differences in discrimination ability of the indicators.

Table 1
Overview of sampling sites used for the evaluation of FIB, description and location, altitude category, water type, presumptive pollution category, and different uses of the source water.

Sampling Site code	Name of a sampling site	Geographic latitude and longitude		Altitude category	Water type	Presumptive pollution category	Uses of the water
Llu	Chifenchifit upstream	411064	1114600	Lowland	S	Low	D, W, C
Lld	Chifenchifit downstream	411234.9	1114309	"	S	High	–
Mld	Infranz downstream	313494	1285297	Midland	S	"	D, I, C
Mlu	Infranz upstream	311266	1282125	"	S	Low	D, I, C
St2u	GudoBahir upstream	322657.8	1281582	"	S	"	I, C
St2d	GudoBahir downstream	324958.4	1280174	"	S	High	–
Hlzu	Awhisha upstream	277356	1206429	Highland	S	Low	D, I, C, W
Hlzd	Awhisha downstream	276941	1206598	"	S	High	I, C, W
Hlu	Chokie upstream	372573	1176467	"	S	Low	D, C, W
Hld	Chokie downstream	372573	1176467	"	S	High	I, C, W
Gwm1	Bata Church	321614.4	1284519	Midland	G	Medium	I, C
Gwm2	"	321507	1284311	"	G	"	I, C
Gwm3	"	321516	1284300	"	G	"	I, C
Gwm4	"	321491.3	1284203	"	G	"	I, C
Gwi1	Kidanmihret Church	322450.6	1281903	"	G	High	C, W
Gwi2	"	322447.5	1281899	"	G	"	C, W
Gwi3	"	324311.3	1280930	"	G	"	C, W
Gwi4	"	324319.1	1282322	"	G	"	C, W
Gwo1	Robit area	332052.1	1292130	"	G	Low	D, I, C, W
Gwo2	"	332052.1	1292136	"	G	"	D, I, C, W
Gwo3	"	323985.5	1268804	"	G	"	D, I, C, W
Gwo4	"	324206.3	1268726	"	G	"	D, I, C, W

Abbreviations: Llu (lowland upstream), Lld (lowland downstream), Mld (midland downstream), Mlu (midland upstream), St2u (stream 2 upstream), St2d (stream 2 downstream), Hlzu (highland Zengena upstream), Hlzd (highland Zengena downstream), Hlu (highland upstream), Hld (highland downstream), Gwm (groundwater middle city), Gwi (groundwater inner city), and Gwo (groundwater outer city).S (stream), G (groundwater wells), I (irrigation), C (cattle watering), D (drinking), and W (washing).

3. Results

3.1. Physicochemical characteristics of the sites

Results of the physicochemical characteristics reveal significant differences among surfaces and groundwater habitats (Table 2). The exceptions are nitrite for surface water and ammonium for groundwater habitats ($p < 0.05$, $n = 63$ for surface water and $n = 60$ for groundwater sites, Kruskal-Wallis H test). This result confirmed the presence of a range of different habitats and pollution patterns in the investigated water systems, thus justifying the basic framework of having different categories of faecal pollution and testing the performance of indicators.

3.2. General levels of occurrence and abundance of FIB

Total coliforms (TC), presumptive EC , and CP were detected in 97%, 91%, and 93%, respectively, of all sampling sites throughout the sampling period (Fig. 1A and B)). TC, EC, and CP were detected in 100%, 96%, and 95% of the surface water sampling sites, respectively. TC was detected in 95%, CP in 90%, and EC was detected in 85% of all the groundwater sampling sites. Among the detected multiple microbial indicators, TC resulted in the highest value with a concentration of 3.39 log CFU/100 ml ($n = 123$; Fig. 1C) in the pooled data. It had also the highest concentration on all surface as well as groundwater sampling sites (Fig. 1A, 1B). The maximum concentrations of the other indicators were in the order of presumptive EC (3.21 log CFU/100 ml), IEC (3.28 log CFU/100 ml), and CP (2.38 log CFU/100 ml). In groundwater study sites, among the tested indicators, TC was detected in 95%, CP in 90%, and EC in 85% of all the sampling sites. Among the detected multiple microbial indicators, TC resulted in the highest value with a concentration of 3.39 log CFU/100 ml ($n = 123$; Fig. 1A, B, C) in the pooled data. It had also the highest concentration on all surface and groundwater sampling sites (Fig. 1A, B, C).

3.3. Performance of FIB

The performance of FIB (i.e., the discrimination efficacy) was evaluated based on eight corresponding low to very high influence sites and site classes for comparisons (Tables 3a and 3b). Based on these comparisons, the FIB showed varying levels of discrimination ability. Presumptive EC and CP showed equal performance and ability to significantly discriminate between paired sites: presumptive EC and CP = 63%, TC = 50%, and IEC = 20% (Table 3a). In surface water study sites, EC and CP were able to discriminate between 40% of the sites, and TC and IEC could significantly discriminate between 20% of the sites. In groundwater systems, EC, CP, and TC were able to significantly discriminate between 100% of the sites.

The BCRM indicates the discrimination ability of faecal indicators; a higher BCRM value correlates to a higher discrimination ability. The BCRM value of EC was 1.55–18.83, CP was 1.28–14.96, and TC was 0.66–11.88 in pooled samples. In surface water samples, the BCRM of EC was 1.55–3.69, CP was 1.28–3, and IEC was 1.12–47. In groundwater systems, the BCRM values were relatively higher compared to surface water systems, and the BCRM of EC was 2.33–18.83, CP was 3.34–14.96, and TC was 1.78–11.88 (Table 3b).

Correlation analysis of faecal indicators revealed that there is generally a significant correlation ($p \leq 0.01$) among most of the indicators (Table 4). Total coliforms strongly correlated with EC, CP, and IEC ($r = 0.356–0.528$, $n = 123$) in pooled samples. *Escherichia coli* correlated significantly with IEC and CP in pooled samples ($r = 0.601–0.647$, $n = 123$; see Table 4). *Clostridium perfringens* revealed a significant correlation with TC and EC only. The correlation among the indicators is more pronounced at highland than mid- and lowland located study sites ($r = 0.624–0,848$, $n = 28$). Total coliforms showed a very strong correlation with EC and CP at highland sites only ($r = 0.6–0.624$). IEC showed a significant correlation with EC in pooled samples only ($r = 0.848$). The correlation coefficient among indicators in mid- and lowland study sites is generally very low, with the exception of EC with TC and IEC correlations. All the evaluated faecal indicators showed a general trend

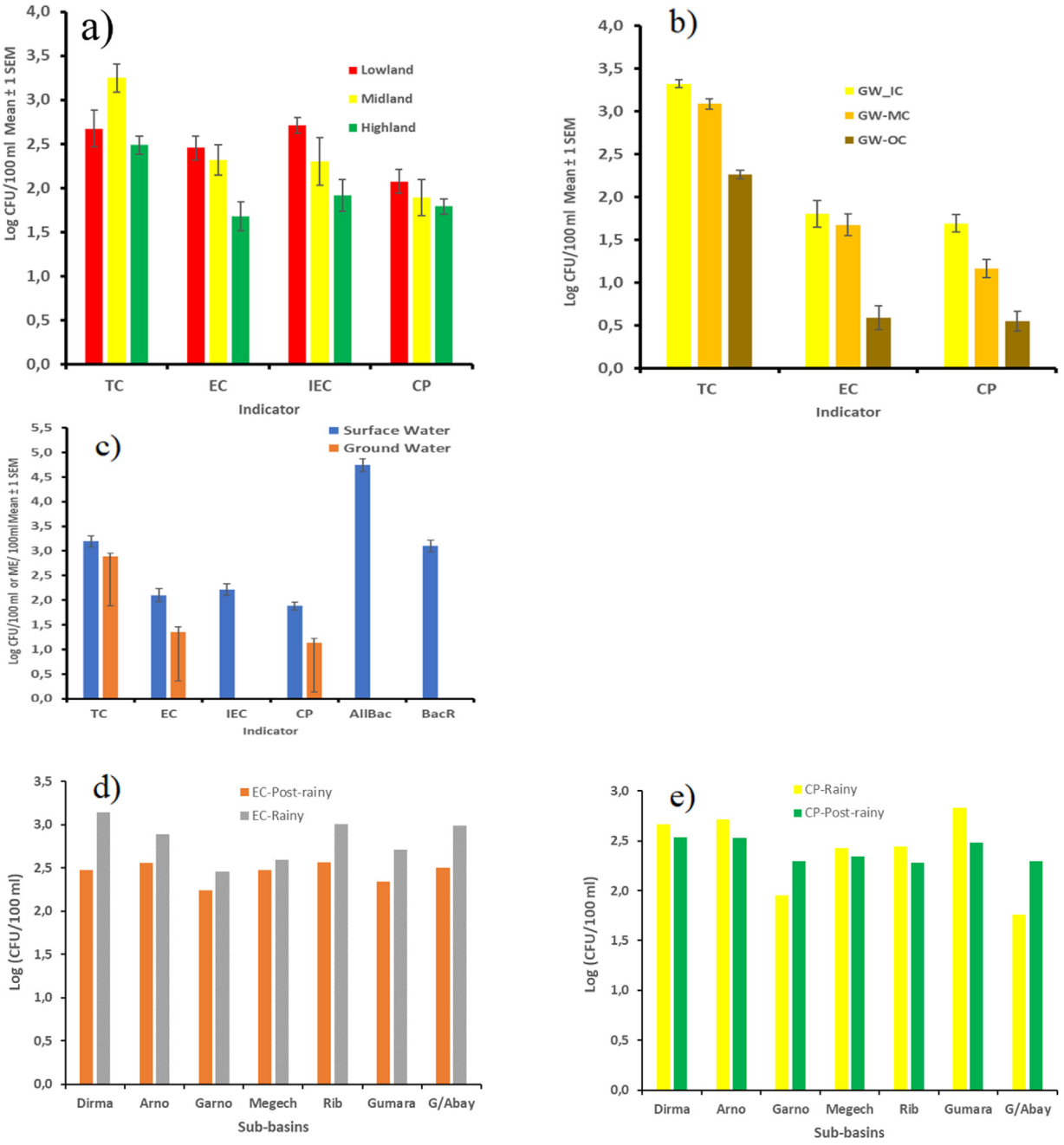

Fig. 1. The general level of occurrence of faecal indicators - a) in three altitude categories of Lake Tana Basin, Ethiopia; highland (≥2500m.a.s.l.); midland (>1100 and <2500m.a.s.l.) and lowland (≤1100 m.a.s.l. - b) in three presumptive pollution categories of the groundwater sampling sites-c) in surface and ground water -d) level of occurrence of FIB for average EC concentration in rainy and post rainy seasons of the year 2017-e) level of occurrence of average CP in rainy and post rainy seasons of the year 2017. The concentrations were in colony-forming units (CFU) per 100 ml expressed as Mean. The error bars indicate ±1 Standard Error of the mean (n = 14–20; 14 per altitude category of the surface water and 20 per pollution category of the groundwater). GW-IC-groundwater inner city, GW-MC-groundwater middle city, and GW-OC-groundwater outer city.

towards presumptive high-influenced sites that were typically more polluted than presumptive low influenced sites.

There is no significant difference in the non-parametric-based variation (NBV) values among the values of all faecal indicators in surface water sampling sites ($p > 0.05$, Kruskal-Wallis H test, $n = 7$ with eight sampling sites) (Table 5a and b). In groundwater systems, the NBV values of TC were significantly different from EC and CP NBV values. However, there remained no significant difference between the NBV values of EC and CP ($p > 0.05$, Mann-Whitney *U* test, $n = 7$ with 12 sampling sites).

3.4. Microbial source tracking

Markers associated with ruminants (i.e., BacR) were detected at all sites except Infranz upstream (Mlu) at least once during the study period from June to December 2017. The percentage occurrence of BacR ranged from 0% (Mlu) to 86% (Hld). The overall percentage occurrence of BacR in pooled sites was 37%. The BacR concentration ranged from 0 to 3.95 log ME/100 ml. The greatest concentration of the BacR marker was detected at Infranz downstream (Mld) (3.95 log ME/100 ml; Table 6).

Table 2
Physicochemical characteristics of the examined water habitats.

	Surface water							Groundwater						
	Ll		Ml		Hl			Gwi		Gwm		Gwo		
Parameter	x	SD	x	SD	x	SD	p value	x	SD	x	SD	x	SD	P value
T (°C)	20.6	2.2	19.6	2.3	14	5	$p < 0.05$	21.7	1.5	22.2	0.9	23.3	0.4	$p < 0.05$
DO (mg/l)	8.3	2.2	4.6	2.4	8.5	2.7	$p < 0.05$	1.6	0.5	2.5	0.7	3.1	1	$p < 0.05$
Cond. (μS/cm)	913	312	387	201	131	74	$p < 0.05$	999	259	186	99	424	377	$p < 0.05$
pH (−)	8.4	1	7.7	1	8.5	1	$p < 0.05$	6.8	0.3	6.3	0.2	6.7	0.4	$p < 0.05$
NO_3 (mgN/l)	2.52	2.41	0.88	0.86	0.48	0.21	$p < 0.05$	29.5	21.5	3	2.4	2.7	2.6	$p < 0.05$
NO_2 (mgN/l)	0.02	0.02	0.07	0.23	0	0.01	$p > 0.05$	0.4	0.6	0.1	0.1	0.1	0.2	$p < 0.05$
NH_4 (mgN/l)	0.06	0.04	4.72	5.65	0.08	0.07	$p < 0.05$	3.7	5.1	2.4	4.2	4.4	5.6	$p > 0.05$
TDS (g/l)	0.65	0.22	0.23	0.1	0.1	0.05	$p < 0.05$	0.5	0.1	0.1	0.1	0.2	0.1	$p < 0.05$

See Table 1 for groundwater abbreviations.Abbreviations: x (mean value), SD (standard deviation), Ll (surface water from lowland), Ml (surface water from midland), Hl (surface water from high land). T (temperature), DO (dissolved oxygen), Cond. (conductivity), NO_3 (nitrate), NO_2 (nitrite), NH_4 (ammonium), and TDS (total dissolved solids).

3.5. Pollution risk mapping

3.5.1. Sites used for performance study

About one third of the sites (Hlzu, Gwm-2, Gwm-3, Gwm-1, Gwi-2, Gwi-4, Gwo-1, Gwo-2, Gwo-3 Gwo-4, St-1, St-2) were classified into the same pollution class by the consensus FIB, presumptive EC, and CP approaches (Table 7a and b and Fig. 2A, B, and C). Sampling sites that had concentrations per 100 ml equal or greater than 5.5 log CFU for EC and 3.7 log CFU for CP would have been classified as having *excessive* pollution, but none of the sites qualified for this category. 3.5 log CFU −4.4 log CFU and 4.5 log CFU−5.4 log CFU concentrations/100 ml were classified as having *very high* and *high* levels of pollution, respectively, based on EC. None of our study sites fell into this pollution level except for the Gudo-Bahir stream sites. Sites with log 90% percentile presumptive EC values in the range of 2.5 log CFU and 3.4 log CFU/100 ml were deemed as having moderate levels of pollution, and one third of the study sites were in this pollution category. Forty percent of the sites were categorized under a *low* level of pollution, and only two sites, both groundwater, fell into the *very low* category of pollution (Table 7a and b).

3.6. General level of pollution and risk mapping

E. coli was detected in all sampled rivers, and the average EC concentration in pooled sites ranged from 2.34 log CFU to 3.14 log CFU/100 ml; this range represents the minimum average EC concentration in Gumara and the maximum EC concentration in Rib, respectively. In the rainy season, the average EC concentration ranged from 2.46 log CFU to 3.14 log CFU/100 ml; the minimum value was noted in Garno, and the maximum was noted in Dirma. In the post-rainy season, the average EC concentration ranged from

Table 4
Pearson correlation coefficients (r) between the various microbial parameters (i.e., TC, EC, IEC, and CP). The samples were collected from surface and groundwater sources distributed over three altitude categories: low-, mid-, and highlands. Asterisks indicate * significant ($p < 0.05$) and ** highly significant ($p < 0.01$) values.

Pooled	TC	EC	IEC	CP
TC		0.528**	0.356*	0.413**
EC	0.528**		0.647**	0.601**
IEC	0.356*	0.647**		0.167
CP	0.413**	0.601**	0.167	
Highland				
TC		0.624**	0.244	0.600**
EC	0.624**		0.848**	0.660**
IEC	0.244	0.848**		0.277
CP	0.600**	0.660**	0.277	
Midland				
TC		0.352	0.195	0.158
EC	0.352		0.227	0.124
IEC	0.195	0.227		0.266
CP	0.158	0.124	0.266	
Lowland				
TC		0.755*	0.238	0.450
EC	0.755*		0.594	0.384
IEC	0.238	0.594		0.133
CP	0.450	0.384	0.133	

Table 3
Pairwise habitat comparison based on the bacteriological parameters of presumptively high and low influence sites for surface water systems (i.e., streams [Table 3a]) and groundwater systems (i.e., wells [Table 3b]). The bacteriological parameters were EC, CP, IEC, and TC in log CFU/100 ml. See Table 1 for the location of the examined habitats =for the abbreviations of sampling sites. Abbreviations: level of significance (p) and bacterial concentration ratio of the medians (BCRM).

a)	Statistical test outcome of the examined surface water systems								
Comparison	Compared	EC		CP		IEC		TC	
type	pair	p	BCRM	p	BCRM	p	BCRM	p	BCRM
Site by site	Hlu-Hld	0.031	4	0.041	3	0.042	4.25	0.250	3.33
	Hlzu-Hlzd	0.569	1.8	0.470	3	0.982	1.12	0.361	0.4
	Mlu-Mld	0.179	3.69	0.476	1.8	0.506	47	0.482	0.66
	Llu-Lld	0.306	1.58	0.787	1.4	0.265	1.76	0.944	0.66
	St2u-St2d	0.047	1.55	0.049	1.28	0.113	–	0.029	1.32

b)		Statistical test outcome of the examined groundwater systems					
		EC		CP	TC		
Comparison type	Compared pair	P	BCRM	P	BCRM	P	BCRM
Site class by site class	Gwi-Gwm	0.05	2.33	0.005	4.47	0.001	1.78
	Gwi-Gwo	0.001	18.83	0.001	14.96	0.002	11.88
	Gwm-Gwo	0.000	14.12	0.000	3.34	0.000	6.68

Table 5
The bacteriological characteristics of faecal indicators for surface water (a) and groundwater (b). The concentrations of faecal indicator bacteria for TC, EC, IEC, and CP (log CFU/100 ml).

a) Sampling sites	TC			EC			IEC			CP		
	M	R	NBV	M	R	NBV	M	R	NBV	M	R	NBV
Hld	2.7	1.7–2.9	0.2	1.8	ND–2.5	0.6	2.2	ND–2.5	0.3	1.8	1.6–2.4	0.4
Hlu	2.2	1.6–2.6	0.3	1.3	ND–2.5	0.5	1.6	ND–2.3	1.0	1.3	1–2.4	0.6
Hlzd	2.6	2.6–3.4	0.3	2.0	1.5–2.6	0.3	2.3	1.0–3.3	0.8	2.2	1.3–2.3	0.4
Hlzu	2.8	2.7–2.9	–	1.7	1.5–3.3	0.5	2.2	1.0–3.3	0.8	1.7	1.3–2.4	0.4
Lld	2.9	2.0–3.3	0.7	2.6	2.1–3	0.4	2.9	2.6–3.1	0.2	2.1	1.3–2.7	0.5
Llu	2.8	1.6–3.2	0.5	2.4	1.6–3	0.3	2.7	2–3.1	0.4	2.2	1.6–2.7	0.4
Mld	2.7	2.6–2.9	0.1	2.4	2.1–2.5	0.2	2.7	2.2–3	0.2	2.1	1.3–2.8	0.4
Mlu	2.9	2.4–3.1	0.2	1.8	1.3–2.6	0.5	1.9	ND–3.1	1.1	1.0	ND–2.8	1.4

b) Sampling sites	TC			EC			CP		
	M	R	NBV	M	R	NBV	M	R	NBV
Gwm	3.1	2.6–3.6	0.1	1.8	ND–2.4	0.3	1.1	0.5–2.2	0.6
Gwi	3.3	3–3.7	0.02	1.75	ND–2.8	0.6	1.7	0.8–2.5	0.38
Gwo	2.2	2–2.7	0.19	0.5	ND–2.1	2.35	0.45	ND–1.6	1.78

Abbreviations: M (Median), R (Range), NBV (Non-parametric-based variation), and ND (non-detected). See Table 1 for the abbreviations of the sites.

Table 6
Distribution and detection frequency of microbial source tracking markers by PCR and range of concentrations by qPCR for host-associated markers. Comparisons of FIB abundance (%) and concentration (log CFU/100 ml) for different streams and the MST study (BacR) are also presented. See Table 1 for the abbreviations of the sites.

Number of positive samples (%) qPCR assay		FIB				BacR log ME/100 ml	FIB (Log CFU/100 ml)			
Site (Sampling events)	BacR	EC	CP	TC	IEC	BacR	EC	CP	TC	IEC
Llu (7)	2 (29%)	7 (100%)	6 (86%)	7 (100%)	7 (100%)	0–2.97	1.61–3.02	1.61–2.67	1.61–3.18	2–3.09
Lld (7)	3 (43%)	6 (86%)	7 (100%)	7 (100%)	7 (100%)	0–3.37	2.15–3.02	1.32–2.7	1.96–3.3	2.56–3.08
Mld (6)	3 (50%)	5 (83%)	6 (100%)	5 (83%)	6 (100%)	0–3.95	2.08–2.53	1.32–2.79	2.6–2.89	2.23–3
Mlu (6)	0	6 (100%)	5 (83%)	5 (83%)	6 (100%)	0	1.32–2.58	0–2.76	2.4–3.06	0–3.11
Hlzu (7)	2 (29%)	6 (86%)	7 (100%)	6 (86%)	7 (100%)	0–2.93	1.5–2.3	1.3–2.4	2.7–2.9	1–3.28
Hlzd (7)	3 (43%)	6 (86%)	7 (100%)	6 (86%)	7 (100%)	0–3.52	1.5–2.6	1.3–2.3	2.6–3.4	1–3.3
Hlu (7)	1 (15%)	6 (86%)	7 (100%)	6 (86%)	7 (100%)	0–3.48	0–2.55	1.04–2.4	1.61–2.57	0–2.3
Hld (7)	6 (86%)	7 (100%)	7 (100%)	7 (100%)	7 (100%)	0–3.82	0–2.52	1.61–2.45	1.71–2.9	0–2.9

Abbreviations: ruminant associated bacteroides (BacR) ,faecal indicator bacteria (FIB) and ND (non-detected).

2.34 log CFU to 2.57 log CFU/100 ml; the minimum value was noted in Gumara, and the maximum was noted in Rib River (Fig. 1D).

Likewise, CP was detected in all sampled rivers. The average CP concentration in pooled sites ranged from 1.76 log CFU to 2.83 log CFU/100 ml; the minimum average CP concentration was recorded in Gilgel Abay, and the maximum was recorded in Gumara. In the rainy season, the average CP concentration ranged from 1.76 log CFU/100 ml to 2.83 log CFU/100 ml; the minimum value was noted in G/Abay, and the maximum was noted in Gumara. In the post-rainy season, the average CP concentration ranged from 2.34 log CFU/100 ml to 2.57 log CFU/100 ml; the minimum value was noted in Gumara, and the maximum was noted in Rib River (Fig. 1E).

In the rainy season, nearly two thirds of the Lake Tana basin was moderately polluted. This includes major tributary rivers, such as Gilgel Abay, Gumara, Rib, Megech, and Dirma sub-basins. Twenty percent of the basin was highly polluted, and 20% of the basin had very low to low levels of pollution (Fig. 1D and E). In the post-rainy season, nearly half of the sites were within the low level of pollution, 35% were moderately polluted, and 10% were highly polluted, as indicated by the 90% EC concentration (Fig. 2).

In the rainy season, based on the 90 percentile CP concentration, 10% of the basin was classified as having low to very low levels of pollution, 35% as moderate, 35% as high, 15% as very high, and 5% was excessively polluted. In the post-rainy season, based on the 90 percentile CP concentration, 10% of the basin was classified as having low to very low levels of pollution, 30% as moderate, 40% as high, 10% as very high, and 5% was excessively polluted (Fig. 2).

4. Discussion

4.1. Physicochemical characteristics of the examined water habitats

The results of the physicochemical characterisation of the examined water habitats indicate the presence of a wide range of different aquatic habitats and pollution patterns. The physico-chemical characteristics of surface and groundwater sites support our presumptive pollution gradient approach: the pollution levels of the sampling sites follow the levels of anthropogenic activity in the surrounding areas. This provides a firm basis for site selection (Byamukama et al., 2005).

The characteristics of the groundwater sampling sites showed a chemical pollution gradient from the inner city, where there is a relatively high population density and more anthropogenic activities, to the middle of the city and then to the outskirts of the city, where population density is relatively less. However, groundwater sites also receive diffuse pollutants from livestock and humans in the watershed. The nonpoint source of pollution from humans is due to many points of open defecation in the watershed. The inner-

Table 7
a) Dual classification scheme of faecal pollution levels based on EC (Kavka et al. (2006) and CP spore concentrations (this work). b) Ninety 90 percentile of log EC and log CP for all sampling sites (n = 4–7 per sampling site) classified into the respective level of pollution based on the pollution classes of Table 7a. See Table 1 for the abbreviations of the sites.

a) Pollution class	EC (Log CFU/100 ml)	CP (Log CFU/100 ml)	Pollution class
Excessive	>5.5	>3.7	1
Very high	4.5–5.4	3.03–3.69	2
High	3.5–4.4	2.36–3.02	3
Moderate	2.5–3.4	1.69–2.35	4
Low	1.5–2.4	1.02–1.68	5
Very low	≤1.4	≤1.01	6

b) Site	EC Log CFU/ 100 ml	CP Log CFU/ 100 ml	Pollution class EC	Pollution class CP
Hld	2.3	2.4	5	3
Hlu	2.1	2.3	5	4
Hlzd	2.3	2.3	5	4
Hlzu	2.7	2.2	4	4
Lld	3.0	2.6	4	3
Llu	2.7	2.6	4	3
Mld	2.5	2.5	4	3
Mlu	2.5	2.7	4	3
Gwm-1	1.4	1.1	6	5
Gwm-2	2.1	1.1	5	5
Gwm-3	2.2	1.4	5	5
Gwm-4	2.1	2.2	5	4
Gwm-1	2.6	2.3	4	4
Gwi-2	2.1	1.5	5	5
Gwi-3	2.3	2.3	5	4
Gwi-4	2.6	2.2	4	4
Gwo-1	0.0	0.6	6	6
Gwo-2	0.5	0.3	6	6
Gwo-3	1.9	1.6	5	5
Gwo-4	0.5	0.8	6	6
St-1	3.7	2.4	3	3
St-2	3.5	2.7	3	3

city groundwater sites demonstrated significantly higher temperature, electrical conductivity, ammonium, and total dissolved solids than the middle and outer city stations. This can be explained by the high organic pollution of the sites. Accordingly, the dissolved oxygen concentration was significantly lower in the inner city than the middle and outer city groundwater sampling sites because organic waste pollution consumes oxygen as it degrades.

The surface water sampling sites also demonstrated significant variations across most physicochemical characteristics. This finding is in line with the concept that rivers and streams are highly heterogeneous on both spatial and temporal scales, and this heterogeneity has been reported by many researchers focusing on the physicochemical dynamics of rivers and streams (Raj and Azeez 2009). The higher variation of physicochemical characteristics in the surface water can be explained by the more dynamic and polluted nature of surface water than groundwater. Other studies have also followed the same general trend (Lari et al., 2014; Nwobodo et al., 2015; Moges et al., 2016). Lowland sampling sites had higher values of temperature, conductivity, nitrate, ammonium, and total dissolved solids than those sites at mid and high altitudes. A nonpoint source phosphate modelling experiment conducted in the Lake Tana basin by Moges et al. (2016) also illustrated a similar trend of increasing between highland upland and midland sites. Dissolved oxygen and pH values of lowland streams were lower than in streams at high altitude but higher than those at mid-altitude located sites. The highland streams had the lowest temperature and the highest dissolved oxygen and were slightly more alkaline than other streams.

In general, our physicochemical results for ground and surface water are in line with other studies (Zhang et al., 1996; Pawar et al., 1998; Goshu et al., 2010; Kebede et al., 2020) insofar as higher values of nutrients and organic pollution were noted in water bodies where there were high anthropogenic activities. Though we did not observe a clear concentration gradient for the physicochemical characteristics of the surface water as we did for the groundwater stations, downstream sites had relatively higher values than upstream sites of the same stream in most altitude categories. This could be because of intermittent pollution inputs within the watershed as a result of open defecation and open grazing magnified by watershed degradation, such as soil erosion (Simane et al., 2013; Sewnet 2015; Moges et al., 2016; Goshu and Aynalem 2017; Selassie 2017). This result confirms the presence of a range of different habitats and pollution patterns in the investigated water systems, thus constituting the need for a basic framework of different categories of faecal pollution and testing the performance of indicators.

4.2. General levels of bacteria occurrence and abundance

The general levels of occurrence and abundance of FIB show approximately the same gradient as that of the physicochemical characteristics of the examined water habitats. This further supports the need for the basic framework to test the performance of the indicators at different altitudes and source waters to produce affordable, easy-to-perform, and practicable indicators that help to map the pollution risks. The general levels of occurrence and abundance of TC, EC, and CP in most surface water sampling sites was higher than in groundwater sampling sites. In most cases, the sampling sites from the lowland areas had relatively higher levels of occurrence and abundance than mid- and high-altitude streams. *Escherichia coli*, IEC, and CP had a relatively higher pooled mean abundance in surface and groundwater habitats compared to other indicators. This was expected as it was observed in similar studies in Ethiopia (Goshu and Aynalem 2017; Kebede et al., 2020) and other East African countries (Byamukama et al., 2005). The higher observed TC levels at all sites were thus likely due to naturally occurring populations in the sampled habitats without being highly indicative of faecal pollution (Byamukama et al., 2005; Goshu et al., 2010).

In surface water sampling sites, the occurrence and abundance of most indicators followed an altitude gradient wherein lowland sampling sites had the highest occurrence and abundance of all FIB. Similarly, midland sampling sites showed a higher occurrence and abundance of FIB than highland sampling sites, with the exception of TC. Accordingly, highland sites showed the least occurrence and detection of all indicators (see Fig. 1A–C).

The general level of abundance of the evaluated indicators showed a comparable level to other studies in tropical areas. Goshu et al. (2010) conducted a study in mid-altitude areas of the Lake Tana basin; the authors reported a comparable level of abundance but slightly higher values (two logs) of TC (6.3 log CFU/100 ml), EC (6.1 log CFU/100 ml), and CP (4.1 log CFU/100 ml). Kebede et al. (2020) also reported a maximum EC concentration of 3.88 log CFU/100 ml and an IEC concentration of 4.04 log CFU/100 ml in the Awash River, a mid-altitude river in the central highland of Ethiopia. Moreover, Byamukama et al. (2005) studied several water bodies around Lake Victoria, Uganda, and reported comparable numbers but higher maximum median concentrations for TC (6.5 log CFU/100 ml) and EC (4.3 log CFU/100 ml).

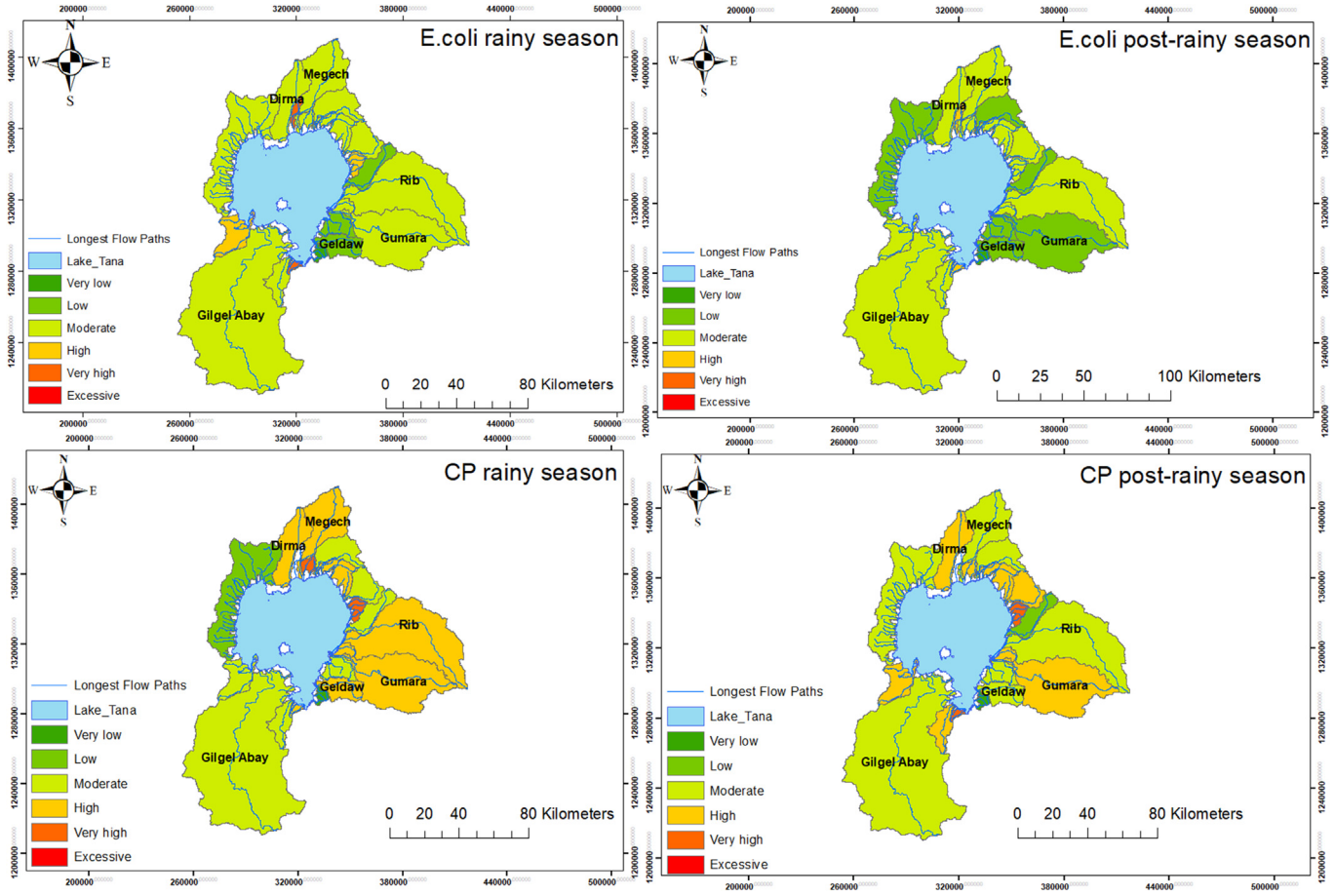

Fig. 2. Rainy and post-rainy seasons bacteria pollution risk maps of the Lake Tana basin that are classified into respective pollution levels based on the pollution classes of Kavka et al. (2006) for EC,And for CP the classification comes from this work.

4.3. Performance of FIB

The results of this study demonstrate that EC and CP spores showed better discrimination efficacy than other indicators. *Escherichia coli* and CP spores were able to significantly discriminate about two thirds of the sampling sites in pooled samples of surface and groundwater sources located at all altitudes. Moreover, EC and CP revealed higher values of BCRM compared to other indicators. This finding is in agreement with Byamukama et al. (2005), who tested the performance of EC and CP in tropical waters in Uganda (1180 m.a.s.l.). However, our study evaluated the performance of indicators at even higher altitudes, as high as 3850 m.a.s.l., and in different sources of water. Edberg et al. (2000) and Odonkor and Ampofo (2013) reported that EC was the best indicator of the bacteriological quality of water in most examined habitats. In the extant literature, CP seems to be the choice for measuring past or intermittent pollution, especially in circumstances where resistance to disinfectants and environmental stress was highest (Cabelli (1977).

Besides EC and CP, TC and IEC showed an acceptable efficacy at discriminating between water bodies in high and low pollution categories. The discrimination efficacy of EC and CP was even stronger in groundwater habitats than surface water habitats, presumably due to the strong pollution gradient in the groundwater habitat. This was further supported by comparatively higher values of BCRM in groundwater than in surface water. All the evaluated indicators in the groundwater that included EC, CP, and TC demonstrated strong discrimination efficacy.

Our results illustrate that the discrimination efficacy of indicators in surface water habitats was less significant as that of groundwater habitats, possibly because surface water habitats are prone to intermittent faecal pollution. Nonetheless, discrimination efficacy varied with altitude, and we noted that the efficacy increased with the increasing altitude. The lowland sites have larger watershed areas, are hotter, and have higher population densities of humans and animals than the mid- and highlands. Interestingly, the BCRM of EC and CP in surface water sites followed the altitude gradient with higher values for highlands and lower values for lowlands. In most cases, the discrimination ability of the indicators increased from lowland to highland.

The discrimination ability of EC, CP, and TC in detecting low and high pollution categories follow the presumptive pollution gradient in groundwater sites that were located in the same altitude category. This was further confirmed by higher values of BCRM in the inner city than in the middle and outer parts of the city. We noted lower values of BCRM in the outer city groundwater sampling sites. In general, all the applied microbial indicators were highly correlated among surface water samples. Unlike other evaluated FIB, EC and CP also revealed a high correlation in the groundwater samples. For this reason, EC and CP were taken as a consensus picture in most of the investigated environments. A more reliable pollution risk map could be created using the dual faecal pollution indicators (EC and CP) for the investigated environment. These two independent parameters of faecal pollution have different ecological potential and persistence in the environment (Medema et al., 1997), yet both revealed high to very high correlation in ground and

surface water results. This strongly suggests that these two indicators function well in the studied area. Therefore, our results agree with the findings of Byamukama et al. (2005) for the Ugandan environment and support the use of EC and CP spores as a pair of complementary faecal indicator parameters in the high-altitude, tropical countries of Africa.

4.4. Microbial source tracking

This study indicates that the BacR marker can nearly always be detected in all study sites except midland upstream. Reischer et al. (2013) and Chase et al. (2012) also reported that BacR has strong host adaptation and broad distribution within the targeted bacterial subpopulation. The authors state that BacR represents obligatory symbionts in the ruminant digestive system, thus making it an ideal MST target.

In our study, we also detected FIB (i.e., TC, EC, CP, and IEC) in most of the sampling sites. The inability of BacR to be detected in the study sites where we have a clear detection of FIB suggests a need for more qPCR assays to be applied in higher spatial and temporal resolutions along with other MST assays.

4.5. Pollution risk mapping

Pollution risk mapping as applied by Kavka and Poetsch (2006) has often been based on one parameter and most frequently, this was presumptive EC quantification. However, as is demonstrated in the present work, the combined use of presumptive EC enumeration and CP spore determination proved a more effective means for faecal pollution monitoring in Ethiopia. For the creation of a faecal pollution map of the investigated study sites, a dual faecal mapping system was thus developed and applied. Some of the groundwater sampling sites, especially those located in the outskirts of the city, were closed wells. We thus expected remote contamination at these sites, and the environment was expected to be of low oxygen or close to an anaerobic environment. In such circumstances, the combined use of CP with EC is advisable as the CP method only detected spores from CP species, which are strictly anaerobic and exclusively of faecal origin (Ellis, 1989).

Based on this mapping approach, in pooled samples, more than 60% of the sites were classified into the same pollution class (Fig. 2). In surface water sampling sites, only 30% of the sites were in the same pollution category. However, in groundwater sampling sites, about 75% of the sites were classified into the same pollution class by the consensus faecal pollution indicators, namely EC and CP. The very clear pollution gradient in the groundwater sites is well captured by the consensus FIB. This can be explained by the more scattered location of the surface water sampling sites compared to the groundwater sampling sites and the more dynamic nature of the streams.

The investigated habitats were used for different purposes. Most of the surface water (i.e., streams) was used primarily for animal watering, irrigation, washing clothes, bathing, and cleaning utensils. In rural areas, it was not possible to imagine that the surface water could also be used for drinking purposes by the rural community. Furthermore, the groundwater found in the centre of the city in particular was rarely used for washing clothes; those groundwater sites in the city's inner suburbs were used for washing clothes and agricultural activities in their backyards but rarely for drinking. However, one third of the groundwater sampling sites (i.e., 4 out of 20) were located in the outskirts of Bahir Dar and were used for drinking by the local people. We suspect that pollution was infiltrating most of the sampling sites that we investigated, with the exception of wells in the outskirts of the city, which had non-detectable to very low levels of pollution. The level of pollution of sampling sites was considerably higher than the World health organization (WHO) drinking-water standard (Organization, 1993). According to WHO drinking-water standards, EC and CP should be absent in a water sample of 100 ml (Santé et al., 2004). In our study, coliforms were common in the environment and were generally not harmful. However, our results show that the presence of EC and CP bacteria in drinking water was usually a result of point and nonpoint source pollution, and this is a serious public health concern (Polo et al., 1998; Wright et al., 2004; Abdelzaher et al., 2010).

4.6. The general level of pollution and risk mapping of the Lake Tana Basin

The level of faecal pollution in tributary rivers and the pollution risk map of the associated sub-basins, including those that are ungauged, remain unknown. This is despite approximately 4.5 million people directly or indirectly depending on the surface and groundwater resources of the Lake Tana basin for agriculture, industry, recreation, ecosystem, and drinking water.

The present results indicate the remarkably high impact potential of anthropogenic faecal sources on the receiving rivers of the Lake Tana basin. More than 80% of the gauged rivers' sub-basins have a moderate to a very high level of pollution. The level of pollution is more pronounced in the rainy season than the post-rainy season; this is because the rainy season in the study area receives two thirds of the annual rainfall, and the runoff washes untreated agricultural and domestic waste into the receiving rivers. An estimation of the faecal pollution in the un-gauged catchments was proposed based on measurements of the level of faecal pollution in the adjacent rivers, the population density without latrine coverage, the livestock density, the watershed nitrogen export fraction, and the nitrogen river export fraction (Goshu et al., 2020). These factors indicate that more than three quarters of the un-gauged sub-basins similarly have moderate to very high levels of pollution. This estimation is in line with the findings of (Mengesha et al., 2004, Wondie 2009; Goshu et al., 2010, 2017; Abera et al., 2014, 2017, Ewnetu et al., 2014, Goshu and Aynalem 2017). The moderate to very high levels of pollution in the Lake Tana basin can be attributed to the low coverage of sanitation facilities and high livestock densities with open grazing systems (Mengesha et al., 2004; Alemayehu and Tassew 2017), aggravated by land degradation in the catchment (Yitaferu 2007; Selassie 2017).The results showed that faecal pollution has been a serious public and ecosystem health issue in the Lake Tana basin that needs an urgent intervention.

5. Conclusion

The results of this study demonstrate that the performance of FIB when used to differentiate between high and low pollution categories (i.e., its discrimination efficacy) varied with altitude and source water in highland tropical water. The FIB displayed better efficacy in groundwater systems than in surface water systems because of nonpoint intermittent pollution loads to surface water systems due to a high anthropogenic influence in the watersheds. Among the indicators, presumptive EC and CP spores performed better at differentiating high and low pollution categories in groundwater than in surface water. Most indicators showed a better discrimination efficacy when differentiating high and low pollution categories at highland sites (3850 m.a.s.l.), rather than at mid- (1800 m.a.s.l.) and lowland sites (1100 m.a.s.l.). From the applied faecal pollution indicators, only EC and CP showed a very high correlation in both surface and groundwater samples. In most

of the investigated samples, EC and CP thereby resulted in a consensus picture of faecal pollution. In the high-altitude, tropical country of Ethiopia, presumptive EC cell enumeration and CP spore determination seem to perform well for faecal contamination monitoring. A contamination risk map for the studied water bodies can be developed based on these consensus dual faecal indicators. Most of the sub-basins of Lake Tana were found to be moderate to highly polluted, and the level of pollution is higher in the rainy season than in the post-rainy season. Markers associated with ruminants (BacR) are identified in more than three quarters of the sites, indicating the presence of mainly ruminant-associated faecal pollution. Our research can aid in improvements to water quality testing and reduce the risk to the general population from stream bacteria. Further studies on the performance of molecular markers should be conducted at different source waters and altitudes with higher spatial and temporal resolutions as this study only represented a discrete region of the Ethiopian environment.

Credit author statment

Goraw Goshu: Conceptualization, Methodology, Validation, Original draft, Writing, Fund acquisition. **A.A. Koelmans**: Conceptualization, Methodology, Validation, Reviewing and Editing, Supervision, Project administration, Fund Acquisition. **J.J.M de Klein**: Conceptualization, Methodology, Validation, Reviewing and Editing, Supervision, Project administration, Fund Acquisition.

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.envpol.2021.116693.

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