Original article

Assessing the occurrence of waterborne pathogens in Lake Ziway and drinking water system of Batu (Ziway) Town, Ethiopia

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Abstract

Background: Surface and drinking water is routinely analyzed for physicochemical parameters and indicator bacteria. However, the presence of indicator bacteria may not necessarily be equally indicative of the presence of pathogens.

Objective: In this study, the physicochemical and bacterial indicator of water quality parameters were compared with the occurrences of waterborne bacterial pathogens from water and sediment samples of Lake Ziway, Meki and Qatar Rivers and drinking water system of Batu (Ziway) Town.

Methods: Seventy eight water and sediment samples were collected from April through November 2013 and analyzed for physicochemical parameters, indicator bacteria and pathogenic bacteria (*Salmonella, Shigella, Vibrio cholera*, *Vibrio* spp., *E. coli* O157:H7) using standard methods.

Results: The study showed that 97 to 100% of samples of sediment and surface water, 38% of the reservoir and 63% of tap water samples were contaminated with indicator bacteria. The highest proportion of pathogenic bacteria was detected from the lake sediment (52.7%), followed by Meki and Qatar Rivers sediment (50.0%), lake water (40.4%), Qatar (33.3%) and Meki (26.7%) River water, tap water (4.8%) and none from reservoir water samples. *Vibrio cholera*, *Vibrio* spp, *Salmonella* and *Shigella* were commonly detected from surface water and sediment samples (48.9%), whereas, *E. coli* O157:H7 was limited to a few sources (3.3%). With respect to the microbial loads, the highest count of 4.20 log CFU/100 g of indicator bacteria was detected from river sediments and the lowest count of 0.42 log CFU/100 ml from reservoir water. Differences in concentration of indicator bacteria were statistically significant (P<0.0001) between sample sources. Spearman rank correlations show some indicators and physicochemical parameters were significantly correlated with the presence of bacterial pathogens.

Conclusion: The present study showed that surface and sediment samples have high load of indicator bacteria and harbored different pathogenic bacteria. The detection of indicator bacteria in 38% of the reservoir and 63% of the tap water samples was indicative of the inadequacy of the treatment and post contamination of water in the distribution system. Fecal coliforms were significantly correlated with *Salmonella* and *Shigella*; *E. coli* with *Shigella,* and *Enterococci* with *Vibrio* spp. of surface and sediment samples. [*Ethiop. J. Health Dev.* 2014;28(2):116-125]

Introduction

Water is a vehicle of many diarrheal diseases caused by different pathogenic microorganisms (1). According to the World Health Organization (WHO), more than 88% of the burden of the diarrheal diseases is attributable to unsafe water, inadequate sanitation and poor hygiene (2). Liu *et al.* (3) reported that, in 2010, waterborne diseases accounted to annual deaths of 0.801 million (10.5% of total deaths), mainly children younger than five years. Most of the waterborne diarrheal morbidity and mortality occur in developing countries, of which the most vulnerable five countries were India, Nigeria, Afghanistan, Pakistan and Ethiopia (3).

It is established that water sources are polluted with wastes released from municipal domestic wastewater, agricultural run-offs and industrial discharges (4). Recent studies showed that bacterial pathogens such as *Salmonella* (5, 6), *Shigella* (7) *Vibrio* (8) and pathogenic *Escherichia coli* (*E. coli* O157:H7) (9) were detected from surface and drinking water, and sediment samples (9, 10). The increasing detection of pathogens in surface and drinking water is troubling and needs consideration to monitor the sources of these pathogens before imposing risks on human health.

Lake Ziway is one of the Rift Valley Lakes known as source of drinking water for Batu (Ziway) Town, site of high fishing and irrigation activities and habitat to bird population. The water quality has been increasingly deteriorated from time to time due to the ever increasing anthropogenic activities and discharge of municipal, industrial and agricultural wastes into the lake (11, 12). Previous studies regarding the quality problem of lake Ziway and the drinking water distribution system of Batu Town showed that the water samples had been found to be contaminated with fecal bacteria beyond the level recommended by WHO guideline values and Ethiopian standards (11, 13, 14).

Many of the hitherto conducted water quality studies were based on investigation of the surrogate indicator microorganisms and physicochemical parameters. However, the presence or absence of indicator bacteria

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may not necessarily corroborate the occurrence of some protozoan and viral pathogens that over-lived the indicators in environmental samples (15). Furthermore, indicator bacteria such as total coliforms and *Enterococci* are found in soil and sand that may not show the real fecal contamination of water sources (15, 16). It has also been shown that subtropical and tropical climates enhance indicator bacteria to multiply in the environment that may give false impression of increased microbial pollution and presence of pathogens (16, 17).

Consequently, interpretation of the presence of microbial indicators is sometimes misleading, for their source may not exclusively be from fecal origin. Under the circumstances, direct detection of pathogens together with measurement of indicator bacteria may guarantee establishing whether or not a water body is truly polluted by disease causing microorganisms. Hence, this study was initiated to investigate by coupling the occurrence of microbial pathogens and the presence of indicator bacteria together with some concurrent physicochemical parameters from water surface and sediment of the Lake Ziway, its feeder rivers and the drinking water system of Batu Town.

Methods

Study Area:

The study was carried out on drinking water distribution system of Batu Town and on shoreline of Lake Ziway and its feeder rivers located in the Ethiopian Rift Valley (Lat: 7°52′–8°8′N; Long: 38°04′–38°56′E) (Figure 1). Lake Ziway is a freshwater wetland found at a distance of about 160 km south of Addis Ababa. The lake is 31 km long and 20 km wide, with a surface area of 440 km^2. It has a maximum depth of 8.9 meters. Apart from seasonal runoffs and groundwater recharge, the lake is fed by two rivers, namely, Meki from the west and Qatar from the east, and is drained by River Bulbula which feeds Lake Abijata to the south. The catchment area of this lake is estimated to be 7025 km^2 and the lake lies at an altitude of 1,636 meters above sea-level. Lake Ziway water is the main valuable source for commercial fishing, irrigation and horticulture farming. The lake water has been increasingly deteriorated from time to time due to the ever increasing anthropogenic activities and discharge of municipal, industrial and agricultural wastes into the lake (11, 12).

The town of Batu lies on the western shore of the lake, extending along that shoreline and growing at a very fast rate in recent years. The present total projected population of Batu Town is estimated to be 59,746 of which 31, 388 are males and 28, 358 are females (18). Lake Ziway is valuable source of drinking water for Batu Town and its surrounding communities. The water treatment plant located at the outlet of the River Bulbula provides drinking water for the residents of the town.

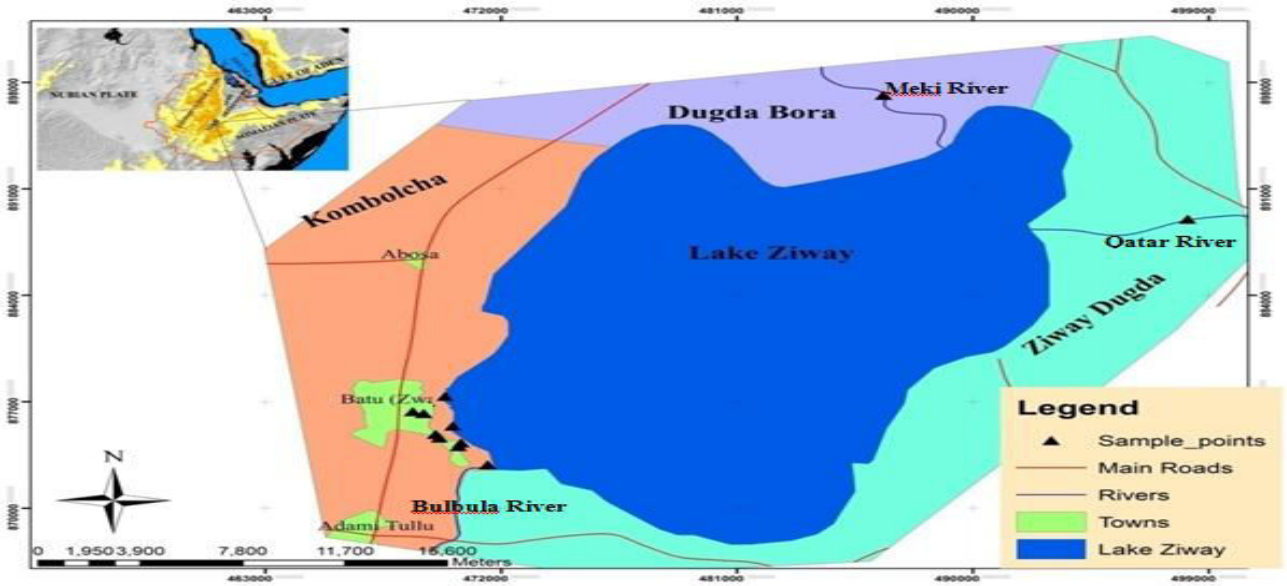

Figure 1: **Map of the study area with sampling sites**

***Sample Collection*:**

Surface water, drinking water and sediment samples were collected from different sampling sites around the town namely, the shoreline of Lake Ziway, Meki and Qatar Rivers and drinking water distribution system of Batu Town during April to November 2013. The lake water samples were collected from municipal drinking water collection site, around Share Ethiopia floriculture farming and fish landing site (near to Ziway Fisheries Research center). In addition, Meki and Qatar Rivers sites were selected so to compare the fecal pollution of the rivers with other parts of the lake. The tap water samples were collected from public tap, primary school, hotel and private house tap water (Figure 1). The

sampling sites of the lake and rivers were selected based on the anthropogenic impact on surface water and population size utilizing the drinking water. All the water samples (3 – 5, l from each site) were collected with pre-sterilized borosilicate bottles (1 l) using grab sampling methods and transported in ice-box to the microbiology and limnology laboratory of Addis Ababa University, for analysis within 24 h of collection in accordance with the procedures described in standard methods for the examination of water and wastewater (19).

Lake and river sediment samples were collected from similar sampling stations with water samples from the bottom with Eckman Grab. The Eckman Grab captures the soft mud with thickness of about 20 cm from the center of which some 100 g of mud was collected using hand gloves and received in sterile plastic bottles and transported in ice-box to the laboratory for analysis within 24 h of collection.

***Water and Sediment Physicochemical Parameters Analyses*:**
Temperature (Temp.), electrical conductivity (EC) and dissolved oxygen (DO) of both water and sediment samples were processed and measured using salinity-conductivity-temperature meter (YSI model 33 S-C-T meter, USA) and (YSI Model 51B Dissolved Oxygen Meter, USA), respectively. pH of the samples was recorded using portable digital pH meter (Adwa AD111, Romania, Hungary). The nitrate nitrogen (NO_3-N) content of water samples was determined using sodium salicylate (20); nitrite nitrogen (NO_2-N) colorimetric method; ammonium nitrogen (NH_4-N) phenate method and phosphate phosphorus (PO_4-P) ascorbic acid method *as per* standard methods (19) using spectrophotometer (Jenway Ltd Flested, Dunmo, UK). Filtrates were also extracted from sediment samples and analyzed according to procedures described in environmental water and soil analysis manual (21).

***Tests for indicator bacteria*:**
The enumeration of indictor bacteria and the presence of pathogenic bacteria from water and sediment samples were processed by suspending them with peptone saline solution (0.85% (w/v) saline and 0.1% (w/v) peptone) and filtered through 47 mm diameter and 0.45µm pore size membrane filter(HAWG04756, Millipore, Cheshire, UK) according to standard methods (19, 22).

The enumeration of total coliforms and fecal coliforms in water and sediment samples was carried out with a membrane filtration *as per* standard methods for the examination of water and wastewater (19) and *E. coli* was enumerated according to ISO 9308-1 (23). The filtrates were placed on an absorbent pad saturated with Membrane Lauryl Sulphate Broth for total coliforms and fecal coliforms (AVONCHEM, Cheshire, UK), and incubated at 37^{o}C for total coliforms and at 44^{o}C for fecal coliforms for 14 – 18h. For *E. coli,* the filtrates were placed on Tryptone Soya Agar (CM131, Oxoid, England) incubated at 37^{o}C for 4 – 5h and transferred to Tryptone Bile Agar containing 20 g Tryptone (L42, Oxoid, England), 1.5 g bile salts no 3 (LP0055, Oxoid, England), 15 g agar-agar (Titan Biotech, India) and incubated at 44ºC for 18 – 20 h.

Entercocci were enumerated from samples according ISO 7899-2 (24). The water and sediment samples were placed on Slanetz and Bartely Agar (CM377, Oxoid, England) and the plates were incubated at 37º C for 44 h. *Clostridum perferingens* was enumerated from water and sediment samples as procedures described on ASTM (25). The filtered samples were transferred to *Clostridium perfringens* agar (m-CP) containing 30 g tryptone (L42, Oxoid, England), 20 gm yeast extract (64271, Merck KGaA, Germany), 5 g sucrose, 1 g L-cysteine hydrogen chloride, 0.1 $MgSO_4.7H_2O$ (Merck, Germany), 0.04 g bromcresol purple (Sigma, USA), 15 g agar-agar (Titan Biotech, India) and supplemented with 0.44 g D-cycloserine (SR0088E, Oxoid, UK).

***Tests for Pathogenic Bacteria*:**
The presence of *Salmonella* was qualitatively detected from samples according to ISO 19250 (26). Water and sediment samples, after being filtered using the same membrane filters (as before), were pre-enriched in 50 ml Buffered Peptone Water (M614, HiMedia, India); incubated at 37^{o}C for 16 - 20 h, and 0.1 ml enrichment liquid was introduced into 10 ml of Rappaport Vassiliadis Soya Peptone Broth (SRL-RM018, India), and incubated at 42^{o}C for 48 h. Thereafter, a loopful of enrichment broth was streaked on Xylose Lysine Desoxycholate agar (CM069, Oxoid, England), and incubated at 37^{o}C for 24 h. Four suspected discrete colonies from each plate were inoculated on Triple Sugar Iron (CM0277, Oxoid, England) and Lysine Iron Agar (CM0381, Oxoid, England) slant and incubated at 37^{o}C for 24 h.

Shigella was analyzed from water and sediment samples according to the standard methods for the examination of water and wastewater (19). The processed samples were placed on Xylose Lysine Desoxycholate agar (CM069, Oxoid, England), and incubated at 37^{o}C overnight. Colonies of confluent growth were transferred to pre-enrichment Selenite F Broth containing 19 g selentie base medium (CM0395, Oxoid, England), 4 g biselenite (BDH, England) and incubated for 6 h and streaked on Xylose Lysine Desoxycholate agar plates. The plates were then incubated overnight at 37^{o}C.

Vibrio was detected from water and sediment samples as described previously by (27). Filtered samples were spread plated on Thiosulphate Citrate Bile Sucrose agar containing 5 g yeast extract (64271, Merck KGaA, Germany), 10 g peptone (Uni-Chem, India), 10 g sodium thiosulfate, 10 g sodium citrate (SDFCL, Mumbai, India), 8 g ox-bile (L50, Oxoid, England), 20 g sucrose, 10 g sodium chloride, 1 g ferric citrate, 0.04 g bromothymol blue (845 YW160095, Merck, USA) and 14 g agar-agar (Titan Biotech, India). Subsequently, the plates were incubated at 37^{o}C for 24 - 48 h.

Enterohemorrhagic *E. coli* O157:H7 from water samples was detected according to standard method (19). Processed samples were inoculated into 50 ml 3x Lauryl Tryptose Broth (CM451, Oxoid, Hampshire, England) and incubated at 37°C for 24 h. The samples were serially diluted (10^{-3}, 10^{-4}) and spread plated (0.1 ml) onto Sorbitol MacConkey Agar containing MacConkey II agar (MD 21030, Becton Dickinson, USA) and D-sorbitol (MO 63178, Sigma, USA). Thereafter, the cultures were incubated at 37°C for 24 h.

***Data Analysis*:**
Statistical analyses were performed using IBM SPSS software version 20 (SPSS Inc, Chicago, USA). All statistical analyses of indicator bacteria were performed on log-transformed data, whereas, the physicochemical parameters with the normal data. The test for fecal indicator bacteria were expressed as colony forming unit, CFU per 100 ml (19) and of sediment sample as CFU per 100 g of wet sediment (22). The bacterial pathogens were qualitatively analyzed by their absence or presence in the samples. The mean concentration of different parameters in sediment and overlying water as well as in various sampling stations were compared using one way analysis of variance (ANOVA). A significance level of $P< 0.05$ was applied at all statistical tests. The correlation between the presence of pathogens, load of indicator bacteria and physicochemical parameters was determined through Spearman rank correlation. Simple linear regression was also used to develop the standard curve for physicochemical parameters of both water and sediment samples.

Results
Physicochemical Parameters:
The physicochemical parameters of water and sediment samples analyzed are shown in (**Error! Reference source not found.**). The pH values of all sample sources ranged from 7.45 to 8.33 and the electrical conductivity varied from 328.33 to 575.36 µS/cm. Similarly, the temperature values of all sample types found between 21.8°C to 24.0°C and of dissolved oxygen from 1.89 mg/l to 4.78 mg/l. Unlike, pH (except lake water), temperature and electrical conductivity, dissolved oxygen showed significant variations ($P<0.0001$) amongst sample sources during the study period.

The highest nitrate nitrogen (2.43 mg/l), nitrite nitrogen (0.15 mg/l) and ammonia nitrogen (18.21 mg/l) values were recorded from lake water and the lowest values of 0.04 mg/l, 0.03 mg/l and 0.47 mg/l from reservoir water samples, respectively. The phosphate phosphorus contents of all samples were within the range of 42.81 to 376.22 µg/l. The maximum (376.22 µg/l) and minimum (42.81 µg/l) mean values of phosphate phosphorus were detected from the lake sediment and Qatar River sediments, respectively. Statistical analysis shows that the nitrate nitrogen and nitrite nitrogen content of samples did not show significant variations ($P>0.05$) amongst sample sources, whereas, the ammonia nitrogen in water samples showed significant variations ($P=0.001$) between the sample sources.

Detection of Indicator Bacteria:
In this study, almost all of the lake and river water and sediment samples were contaminated with total coliforms, fecal coliforms, *Escherichia coli*, *Enterococci* and *Clostridium perfringens* (Table 1). But the degree of contamination among some water sources was variable. Accordingly, only 38.2% of finally treated (reservoir) water and 63.0% of the tap water samples were positive for all tested indicator organisms.

Of the lake and river water samples examined for the indicator organisms, the mean concentration range of total coliforms (4.02 – 4.11 log), fecal colifrms (3.73 – 3.99 log), *E. coli* (2.28 – 3.00 log), *Enterococci* (3.05 – 3.26 log) and *Clostridium perfringens* (3.18 – 3.59 log) CFU/ 100 ml was detected from water samples (Table 2). Similarly, the minimum - maximum values of both lake and river wet sediment samples recorded were 4.82 – 5-07 log for total coliforms, 4.27 - 4.65 log for fecal coliforms, 3.10 – 3.16 log for *E. coli*, 3.51 – 4.25 log for *Enterococci* and 4.10 – 4.16 log CFU/ 100 g for *C. perfringens*.

Table1: **Concentration (mean ± SD) of physicochemical parameters of water and sediment samples**

Sample source	Physicochemical parameters*							
	pH (-)	Temp. (^{o}C)	EC (µS/cm)	DO (mg/l)	NO_3 – N (mg/l)	NO_2 - N (mg/l)	NH_4-N (mg/l)	PO_4 - P(µg/l)
Lake water	8.33 ± 0.30^{b}	22.98 ± 2.04^{a}	521.07 ± 244.11^{a}	3.04 ± 1.51^{ab}	2.43 ± 4.21^{a}	0.15 ± 0.21^{a}	18.21 ± 9.96^{b}	78.76 ± 35.22^{a}
Meki River water	7.82±0.12^{a}	23.33±3.05^{a}	456.67±25.17^{a}	4.77±0.45^{c}	0.22±0.22^{a}	0.09±0.05^{a}	10.79±4.71^{ab}	65.14±14.21^{a}
Qatar River water	7.66±0.22^{a}	24.00±3.00^{a}	358.33±163.27^{a}	3.73±0.11^{bc}	0.15±0.10^{a}	0.04±0.01^{a}	13.87±8.12^{b}	95.06±24.93^{a}
Lake sediment	7.57 ± 0.30^{a}	22.54 ± 1.90^{a}	575.36 ± 310.78^{a}	1.89 ± 0.72^{a}	1.63 ± 1.31^{a}	NA**	NA	376.22 ± 364. 71^{b}
Meki River sediment	7.53±0.06^{a}	23.33±1.53^{a}	420.00±10.00^{a}	3.00±1.00^{ab}	0.47±0.18^{a}	NA	NA	43.32±0.82^{a}
Qatar River sediment	7.45±0.10^{a}	22.00±4.00^{a}	328.33±187.64^{a}	2.50±0.50^{ab}	0.39±0.25^{a}	NA	NA	42.81±0.51^{a}
Reservoir water	7.52 ± 0.08^{a}	21.80 ± 1.64^{a}	441.25 ± 27.80^{a}	4.78 ± 1.05^{c}	0.04 ± 0.02^{a}	0.03^{a}	0.47 ± 0.23^{a}	73.83 ± 56.85^{a}
Tap water	7.49 ± 0.14^{a}	24.00 ± 1.15^{a}	472.15 ± 188.31^{a}	3.50 ± 0.52^{bc}	0.17 ± 0.17^{a}	0.03^{a}	0.67 ± 0.34^{a}	114.05 ±118.64^{a}
***P*-value**	<0.0001	0.480	0.648	<0.0001	0.313	0.178	0.001	0.026

*Values indicated with different letters are statistically significant; differences of concentration of physicochemical parameters were tested by Duncan – ANOVA
** Not analyzed

Table 1: **Occurrences (%) of indicator bacteria in different sample sources**

Sources	Number of samples	Indicator bacteria				
		Total coliforms	Faecal coliforms	*E. coli*	*Enterococci*	*Clostridium perfringens*
Lake water	19	100	100	93.3	94.7	100
Lake sediment	19	100	100	100	94.1	100
River water	6	100	100	100	100	100
River sediment	6	100	100	100	100	100
Reservoir water	7	71.4	42.9	16.7	28.6	28.6
Tap water	21	90.5	57.1	35.3	45	80.9

Table 2: **Concentration (mean ± SD) of indicator bacteria in water and sediment samples**

Sample source	**Fecal indicators in log CFU/ 100 ml for water and log CFU/ 100 g for wet sediment samples***					
	Total coliforms	**Fecal coliforms**	***E. coli***	***Enterococci***	***C. clostrdium***	Overall
Lake water	4.11 ± 0.41^{bc}	3.90 ± 0.56^{b}	2.28 ± 0.90^{b}	3.09 ± 0.97^{b}	3.18 ± 0.98^{c}	3.41±0.45
Meki River water	4.12±0.64^{bc}	3.99±0.66^{b}	3.00±0.30^{b}	3.05±0.39^{b}	3.56±0.25^{c}	3.54±0.44
Qatar River water	4.02±0.71^{b}	3.73±0.99^{b}	2.76±1.02^{b}	3.26±0.45^{b}	3.59±0.21^{c}	3.52±0.52
Lake sediment	5.07 ± 0.71^{d}	4.54 ± 0.66^{b}	3.16 ± 0.76^{b}	3.51 ± 1.10^{b}	4.16 ± 1.02^{c}	4.10±0.55
Meki River sediment	4.82±0.39^{cd}	4.27±0.81^{b}	3.10±0.32^{b}	3.98±1.10^{b}	4.14±0.66^{c}	4.13±0.85
Qatar River sediment	4.98±0.30^{d}	4.65±0.86^{b}	3.10±0.57^{b}	4.25±0.81^{b}	4.10±0.71^{c}	4.27±1.00
Reservoir water	0.82 ± 0.60^{a}	0.37 ± 0.49^{a}	0.10 ± 0.25^{a}	0.29 ± 0.50^{a}	0.45 ± 0.85^{a}	0.42±0.39
Tap water	1.42 ± 0.53^{a}	0.74 ± 0.68^{a}	0.25 ± 0.44^{a}	0.45 ± 0.64^{a}	1.80 ±1.06^{b}	0.96±0.35
***P*-value**	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	

*Values indicated with different letters are statistically significant; differences of densities of indicator organisms were tested by Duncan – ANOVA

Table 3: **Distribution of bacterial pathogens in water and sediment samples**

Sample source	**Percent (%) positive for water and sediment samples of pathogenic bacteria***					
	Salmonella	*Shigella*	*E. coli*O157:H7	*V. cholera*	*Vibrio* spp.	Overall %
Lake water	10.5	15.8	5.5	89.5	78.9	40.4
Meki River water	33.3	ND	ND	66.7	33.3	26.7
Qatar River water	ND	33.3	ND	33.3	33.3	33.3
Lake sediment	15.8	15.8	NA**	94.4	88.9	52.7
Meki River sediment	33.3	33.3	NA	66.7	33.3	50.0
Qatar River sediment	ND	33.3	NA	66.7	66.7	50.0
Reservoir water	ND	ND	ND	ND	ND	ND
Tap water	4.8	4.8	5.0	4.8	4.8	4.8

*Not detected
**Not analyzed

With regards to reservoir and tap water samples, the highest values (1.80 CFU/100 ml) of *C. perfringens* and the lowest (0.10 CFU/100 ml) of *E. coli* were detected from tap water and reservoir water samples, respectively (Table 2). The detection of total coliforms, fecal coliforms and *Enterococci* in reservoir and tap water samples was within the range of 0.82 – 1.42 log, 0.37 – 0.74 log and 0.29 – 0.45 log CFU/ 100 ml, respectively. In general, the overall microbial counts of indicator organisms in both water and sediment samples were declined in the order of Qatar River sediment (4.27 log), Meki River sediment (4.13 log), lake sediment (4.10 log), Meki River water (3.54 log), Qatar River water (3.52 log), lake water (3.41 log), tap water (0.96 log) and reservoir water (0.42 log) with significant variations (P<0.0001) amongst sources during the study period (Table 2).

Occurrences of Pathogenic Bacteria in Water and Sediments:

The occurrence of bacterial pathogens of the different water and sediment samples showed variations amongst the sample sources. Accordingly, 40.4% of the lake water samples were positive for *Salmonella*, *Shigella*, *E. coli* O157:H7, *Vibrio cholera* and *Vibrio* spp., whereas, 26.7% of Meki and 33.3% of Qatar River water samples were positive for all the pathogens, except *E. coli* O157:H7, *Shigella* ,and *Salmonella* in some of the river water samples. The highest percentage (89.5%) of *V. cholera* was detected from the lake water samples followed by 66.7% detection from Meki River water samples. Generally, the occurrence of *E. coli* O157:H7 was low in the river and lake water samples.

With respect to sediment samples, 50.9% of the lake and river sediment samples were positive for all pathogenic bacteria except *Salmonella* that was not detected from Qatar River sediment samples. Similarly, *V. cholera* was detected from 94.4% and 66.7% of the lake and river sediment samples, respectively. However, none of the reservoir water samples showed pathogens and only 4.8% of tap water samples were positive for all bacterial pathogens. It is generally estimated that 48.9% of the water and sediment samples harbored pathogens of the group *V. cholera*, *Vibrio* spp., *Salmonella* and *Shigella,* whereas, *E. coli* O157:H7 was detected from 3.3%, of the water samples (data not shown).

Correlation of Microbial Pathogens with Physicochemical Parameters and Indicator Microbes of Water and Sediments:

The Spearman rank correlation showed variations amongst pathogens, physicochemical parameters and indicator organisms. Accordingly, the presence of *Salmonella* in water samples was significantly positively correlated with *Shigella* (rho: 0.43, P<0.05) and the log-transformed concentrations of fecal coliforms (rho: 0.41, P<0.05) in the sediment samples. Similarly, there was a significant positive correlation between the occurrence of *Vibrio* spp. in the water and *Enterococci* (rho: 0.43, P<0.05) in the sediment and *V. cholera* (rho: 0.70, P<0.01) in the water samples. The negative correlation of *V. cholera* in the sediment and dissolved oxygen in the water samples (rho: -0.51, P<0.05) was significant during the period of the study.

The occurrence of *Shigella* in water was significantly correlated with the concentrations of fecal coliforms in water (rho: 0.47, P<0.05), *E. coli* (rho: 0.47, P<0.05) in sediment and *E. coli* O157:H7 in water (rho: 0.47, P<0.05). Moreover, there was also a significant positive correlation between the detection of *Shigella* in sediment and *Salmonella* in water.

Discussion

The present work, unlike the previous studies conducted in the same place, gave emphases to the direct assessment of the occurrences of diseases causing bacteria along with the quantification of the indicator bacteria and physicochemical parameters. Hence, with regard to the physicochemical parameters, almost all of physicochemical variables (pH, electrical conductivity, temperature) of the water source samples (Meki River, Quatar River, Lake Ziway) and the water samples from tap water distribution system (reservoir water, tap water) were within the same range reported before with pH (7.5 - 8.3), electrical conductivity (358.3 - 521.1 µS/cm) and temperature (21.8 - 24.0^{o}C) (14, 28, 29). The pH of water samples was slightly alkaline and within the acceptable standard (pH 6.5 - 8.5) of drinking water stipulated by WHO guideline values and Ethiopian drinking water standard ES 261:2001. However, the temperature range of the water samples in this study was not within the WHO standard of <15^{o}C. The higher values obtained in this and other studies in the country may be mainly due to the hot climate of the study areas that contribute to high temperature records of water samples.

The average concentration of dissolved oxygen (3.04 mg/l) of lake water samples was lower than the values (8.72 mg/l) of previous report of the same place (14). The lower values of oxygen in this study could be due to ever increasing introduction of the municipal wastes and agriculture runoff into the shore of the lake increasing the activities of microbes for utilizing the dissolved oxygen. In addition, the types of devices used to measure the dissolved oxygen might have also contributed to the difference in the results obtained. Similarly, dissolved oxygen content of tap water samples (3.50 mg/l) followed the same pattern compared to the dissolved oxygen of tap water samples (8.60 mg/l) reported from Bishoftu Town (30).

The mean nitrate nitrogen (2.43 mg/l) and ammonia nitrogen (18.21 mg/l) content of Lake Ziway water samples was higher than the previous report (0.0032 mg/l) and (0.111 mg/l) of the lake water, respectively

(28). Correspondingly, the mean nitrate nitrogen content of tap water (0.17 mg/l) of the present study was lower than the average value of previous reports (0.80 mg/l) from Batu (13) and Bishoftu towns' (1.5 mg/l) tap water (30). In general, the data revealed that the concentration of both nitrate nitrogen and nitrite nitrogen content of lake, river and pipeline water samples and ammonia nitrogen of reservoir and tap water samples were lower than the values recommended by WHO guideline (31).

Likewise, the mean phosphate phosphorus (78.76 µg/l) content of the lake water was higher than the previous report (10.1 µg/l) from the same lake water (28). Higher (376.22 µg/l) phosphate phosphorus concentration was found in the lake sediment compared to overlying lake water (78.76 µg/l) for the fact that phosphorus is settled in the sediment of the lake. The phosphate measurement of tap water (114 .05 µg/l) in this study was lower than the average values of previous work from Bishoftu Town (350.0 µg/l) (30).

In general, the higher amount of nutrient content (nitrate nitrogen, ammonia nitrogen and phosphate phosphorus) of lake water samples compared to the previous report could be due to the fact that data for the present study were collected from the parts of the lake which were impacted with anthropogenic activities; inconsiderate dumping of agricultural and municipal wastes in to the lake might have also played a part (11, 12).

With respect to indicator bacteria, the microbial load of total coliforms (4.11 log) and fecal coliforms (3.90 log) CFU/100 ml in lake water was greater than the average values of the previous report of total coliform (1.79 log) and fecal coliforms (1.58 log) CFU/100 ml from the same place (14). However, the results were similar with the average values of total coliforms (4.35 log) and fecal coliforms (3.10 log) CFU/ 100 ml reported by (32) from Lake Tana.

The mean counts of total coliforms (4.08 log) and fecal coliforms (3.86 log) CFU/100 ml of Meki and Qatar Rivers water samples were in a close agreement with a report from Lagabatu River (Central Highland of Ethiopia) with total coliforms (3.45 log) and fecal coliforms (3.40 log) CFU/100 ml (34).

The tap water harbored high proportion of indicator bacteria. The occurrence of total coliforms (90.5%) and fecal coliforms (57.10%) in tap water samples was higher than the previously reported from Batu Town with total coliforms (68.0%) and non-fecal coliforms (13). These higher indicator bacterial loads could be associated with improper breach of pipeline for increasing construction in the town and obsolete infrastructure that led to the contamination of the distribution system (4). However, the values of total coliforms and fecal coliforms in this study were in close conformity with the previous report from Bishoftu Town where 100.0% and 86.0% of tap water samples were contaminated with total coliforms and fecal coliforms, respectively (30). Generally, the data showed that only 69.2% of reservoir water and 52.6% of tap water samples were in compliance with WHO guideline values and national drinking water standard for samples tested for fecal coliforms and *E. coli*. The presences of indicator organisms in the finally treated water (reservoir) samples could be indicative of inadequate treatment of the source water. Similarly, the tap water harbored twofold more bacterial loads than the reservoir water samples that may also indicate the dilapidation of pipelines, that led to leakage and inadequate treatment of the source water (4).

As regards pathogenic bacteria, the detection rate for *Salmonella* in the lake water (10.5%) and sediment (15.8%) samples, and the rivers water (16.7%) and sediment (16.7%) samples was higher than the values of 6.3% of surface water and 4.8% of sediment samples reported from Central California Coast (9). However, the occurrence of *E. coli* O157:H7 (5.5%) of lake water in this study was lower than the detection of 13.8% *E. coli* O157:H7 using Moore swabs and higher than 1.8% using grab sample method reported by the same author. These dissimilarities could be due to differences in methods utilized, size of samples and geographical locations in which the studies were conducted. The detection of *Shigella* (15.8%) in Lake Ziway water was in a close agreement with the values of 10.9% from surface water (lake and river) of Bangladesh using PCR method (7). The magnitude of *Salmonella* (10.5%), *Shigella* (15.8%), *V. cholera* (89.5%) and *Vibrio* spp. (78.9%) found in this study was lower than it was previously reported from southern Kerala coast water where the detection of the above pathogens for each kind was 33.1%, 90.1%, 97.5% and 97.5% of samples examined, respectively (27).

The discovery of *Salmonella* (4.8%) and *V. cholera* (4.8%) in tap water samples was in consistence with findings from southern Isfahan, Iran report where 5.5% and 8.3% of the samples scrutinized were positive for *Salmonella* and *V. cholera*, respectively (6). On the contrary, the occurrence of *Salmonella* in pipeline water was lower than the one reported from Nigeria (18.3%) (36). The prevalence of *E. coli* O157:H7 (5.0%) in tap water was comparable with the one reported from the Netherlands (7.4%) (37). Similarly, the 4.8% presence of *Salmonella*, *Shigella* and *Vibrio* spp. in tap water was much lower than the report from Dhaka city, Bangladesh where 35.0%, 60.0% and 50.0% of tap water samples were contaminated with these microbes, respectively (38).

In general, the data showed that the occurrence of the different pathogenic and indicator bacteria was higher in sediment samples than in the surface water samples. Previous reports also showed that some enteric bacteria such as *Salmonella, Vibrio* spp. *E. coli* and *C.*

perfringens can survive better in the sediment than in the overlying water column (39, 40).

The Spearman rank correlations indicate that the presences of bacterial pathogens were significantly correlated with some physicochemical parameters and log-transformed indicator bacteria. *Salmonella, Shigella* and *Vibrio* spp. of surface water and sediment samples were significantly correlated with some indicator organisms (fecal coliforms and *Enterococci*) and physicochemical parameters. However, the presences of *E. coli* O157 in water and *V. cholera* in surface water and sediment were not significantly correlated with any of the log-transformed indicator bacteria and physicochemical parameters. Similar results were reported from Central California Coast where generic *E. coli* were not significantly associated either with *E. coli* O157 or *Salmonella* (9). The data showed that Shigella built better association with both indicator organisms and pathogenic bacteria in water and sediment samples.

In conclusion, the present findings showed that 97 to 100% of surface water samples were contaminated with indicator bacteria. This indicates that the surface water in the study area face challenges due to the contaminations of fecal matter from the surrounding environment. Furthermore, 38% of the reservoir and 63% of tap water samples were also positive for these organisms indicating the inadequacy of the treatment and post-purification contamination of drinking water in the distribution system. The microbial load of indicator and pathogenic bacteria was more substantial in sediment than overlying water samples. The statistical correlation analysis revealed that fecal coliforms were significantly correlated with *Salmonella* and *Shigella*; *E. coli* with *Shigella,* and *Enterococci* with *Vibrio* spp. of surface and sediment samples. *Shigella* showed stronger association with both indicator and pathogenic bacteria and a few physicochemical parameters in water and sediment samples. Fecal coliforms, *Enterococci* and *Shigella* could be used to follow the bacterial pollution trend of Lake Ziway.

The detection of pathogenic bacteria in both water and sediment samples for this study was accomplished by a cultural method which may not show the exact type of the pathogen species. Hence, extensive studies with large numbers of samples concurrently collected samples from various water sources will be required using polyphasic approaches (cultural and molecular methods) to determine the actual pictures of surface and drinking water quality of the study area. Meanwhile, municipal effluents and floriculture runoffs that pollute the lake should be treated before discharge into the lake.

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