Occurrence of Waterborne Pathogens in Lake Zwai and Drinking Water System of Batu (Zwai) Town, Ethiopia: In Relation to Indicator Bacteria and Physicochemical Parameters

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This is to certify that the thesis prepared by Mekuria Mekonnen, entitled: *Occurrence of Waterborne Pathogens in Lake Zwai and Drinking Water System of Batu (Zwai) Town, Ethiopia: In Relation to Fecal Indicator Bacteria and Physicochemical Parameters* and submitted in partial fulfillment of the requirements for the Degree of Master of Science (Applied Microbiology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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ABSTRACT

Surface and drinking waters are routinely analyzed for physicochemical parameters and indicator bacteria. However, the presence/absence of indicator bacteria may not necessarily be equally indicative of the presence of pathogens. 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 Zwai, Meki and Qatar Rivers and drinking water system of Batu (Zwai) Town. Seventy eight water and sediment samples were collected from April through November 2013 and analyzed for the physicochemical parameters (pH, temperature, electrical conductivity, dissolved oxygen, nitrate, nitrite, ammonia and phosphate), indicator bacteria (*Escherichia coli, Enterococci*, *Clostridium perfringens,* total and fecal coliforms) and pathogenic bacteria (*Salmonella, Shigella, Vibrio cholera*, *Vibrio* spp.*, E. coli* O157:H7) using standard methods. All except temperature, the physicochemical parameters in reservoir and tap water samples met the maximum permissible value for drinking water. The highest proportion of pathogenic bacteria was detected from 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 in a few sources with low percentage (3.3%). With respect to the microbial load of the tested organisms, the highest count of 4.20 log CFU/100 g of indicator bacteria was detected from river sediment and the lowest count of 0.42 log CFU/100 ml recorded 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. *Shigella* was better significantly correlated with both indicator and pathogenic bacteria. Since surface and drinking water distribution system were contaminated with bacteria, the need to develop more effective monitoring and efficient treatment mechanism of the drinking water source with respect to microbial contamination is necessary.

Keywords/phrases
Enterococci, fecal coliforms, *Salmonella*, *Shigella*, waterborne diseases

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DEDICATION

This thesis is dedicated to my beloved mom, Yewbnesh Teklegiyorgis, who was deceased at the midst of my work. Mom, your motherly love dwells in my being throughout my life!!!

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

APHA	American Public Health Association
ARHD	Africa Regional Human Development
ASTM	American Society for Testing and Materials
BPW	Buffered Peptone Water
CFU	Colony Forming Unit
CSA	Central Statistical Agency
DO	Dissolved Oxygen
EC	Electrical Conductivity
EHEC	Enterohemorrhagic *E. coli* O157:H7
ES	Ethiopian Standard
ISO	International Standard Organization
LIA	Lysine Iron Agar
LTB	Lauryl Tryptose Broth
m-CP	membrane *Clostridium perfringens* Agar
MLSB	Membrane Lauryl Sulphate Broth
MOH	Ministry of Health
NHMRC	National Health and Medical Research Council
NRMMC	National Resource Management Ministerial Council
^{o}C	Degree Celsius
PACN	Pan Africa Chemistry Network
PFZ	Peptone Saline Solution
pH	Concentration of Hydrogen Ions
RADWQ	Rapid Assessment of Drinking-Water Quality
RVL	Rift Valley Lake
RVS	Rappaport Vassiliadis Soya Peptone Broth
SBA	Slanetz and Bartely Agar
SMAC	Sorbitol MacConkey Agar
TBA	Tryptone Bile Agar
TCBS	Thiosulphate Citrate Bile Sucrose agar

TSA	Tryptone Soy Agar
TSB	Tryptone Soya Broth
TSI	Triple Sugar Iron
UNDP	United Nation Development Program
UNGA	United Nations General Assembly
UNICEF	United Nations Children’s Fund
UV	Ultraviolet
WB	World Bank
WHO	World Health Organization
XLD	Xylose Lysine Desoxycholate agar

1. INTRODUCTION

1.1. Background of the study

Water is a vehicle of many diseases caused by different pathogenic microorganisms including bacteria, viruses and protozoa (WHO, 2008b; Woodall, 2009). Most of them cause diarrheal illness. 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 (Prüss-Üstün *et al.*, 2008). Liu *et al.* (2012) reported that waterborne diseases accounted were incriminated with annual deaths of 0.801 million (10.5% of total deaths) children younger than five years in 2010. 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 (Kosek *et al.*, 2003; Liu *et al.*, 2012).

Globally, many people lack access to improved drinking water sources and depend on poor sanitation services. According to 2012 report released by the World Health Organization (WHO) and the United Nations Children's Fund (UNICEF), about 780 million people lack access to safe drinking water and rely on unimproved water sources and 2.5 billion people lack adequate sanitation facilities (UNICEF/WHO, 2012). Across Africa, one third and two thirds of the populations have no access to clean water and clean sanitation, respectively (PACN, 2010).

Waterborne bacterial pathogens can be readily detected worldwide from different water sources. The human bacterial pathogens including *Salmonella* (Levantesi *et al.*, 2012; Momtaz *et al.*, 2013), *Shigella* (Faruque *et al.*, 2002), *Vibrio* (Shanan *et al.*, 2011; Cantet *et al.*, 2013) and pathogenic *Escherichia coli* (*E. coli* O157:H7) (Benjamin *et al.*, 2013) were detected from surface and drinking water, and sediment samples (Benjamin *et al.*, 2013; Maal-Bared *et al.*, 2013). The increasing identification of pathogens in surface and drinking water is troubling and needs consideration to monitor the sources of these pathogens before imposing risks on human and animal health.

In Ethiopia, waterborne and sanitation-related diseases, particularly diarrhea, pose a risk of morbidity and deaths of children under five years (WB/ARHD/MOH, 2005; Liu *et al.*, 2012). This is due to inadequate access to safe drinking water and sanitation. The recent data on household survey released by the Central Statistical Agency of Ethiopia (CSA) indicated that only 50.8% and 8.8% of the population of the country had access to improved source of drinking water and proper sanitation facilities, respectively (CSA, 2012). In addition, a report by the Rapid Assessment of Drinking-Water Quality (RADWQ) project indicated that only 68% of drinking water met the WHO guideline values and national drinking water quality standards (Dagnew Tadesse *et al.*, 2010; CSA, 2012).

1.2. Statement of the problem

Surface water that can be used for various purposes becomes grossly polluted under the growing burden of waterborne wastes released from municipal effluents, agricultural run-offs and industrial discharges. This, in turn, results in contamination of drinking-water sources and distribution systems through improper breach of pipelines, management of catchment areas, obsolete infrastructures, inadequate treatments and storage facilities (WHO, 2004; Corcoran *et al.*, 2010).

Lake Zwai is one of the Rift Valley Lakes (RVL) known as source of drinking water for Batu (Zwai) Town, site of high fishing and irrigation activities and habitat of bird population. However, the quality of the lake water has deteriorated from time to time due to the ever increasing activities and discharge of municipal, industrial and agricultural wastes into the lake (Malefia Tadele, 2009; Meshesha *et al.*, 2012). This prompted studies on ecological crisis surrounding the lake, in general, and that of the quality problem of drinking water distribution system of Batu Town, in particular (Kassahun Bedane, 2008; Brihanu Roba, 2008 ; Meshesha *et al.*, 2012). Based on the physicochemical and bacteriological quality indicators, Lake Zwai and drinking water distribution system of Batu Town were found to be contaminated with fecal bacteria beyond the level recommended by WHO guideline values and Ethiopian standards (ES, 2001; WHO, 2011a). Accordingly, waterborne and water related diseases become common in the area (preliminary assessment).

Many of the hitherto studies were based on investigation of the surrogate and associated physicochemical indicators. However, the presence or absence of indicator bacteria may not corroborate the occurrence of some protozoan and viral pathogens that over-lived the indicators in environmental samples. Furthermore, some 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 (Sherer *et al*., 1992; Whitman and Nevers, 2003). 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 (Solo-Gabriele *et al*., 2000; Whitman *et al*., 2003).

Consequently, interpretation of the presence of microbial indicators is sometimes misleading, for their source is not exclusively from fecal origin. Under the circumstances, direct measurement of pathogen microbes together with detection of indicator bacteria may warrant 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 Zwai, feed rivers and the drinking water system of Batu Town.

1.3. Objectives

1.3.1. General objective

The general objective of this study was to investigate the occurrences of selected waterborne microbial pathogens, indicator organisms and physicochemical parameters in the water and sediment samples of Lake Zwai and feed rivers and drinking water distribution system of Batu Town.

1.3.2. Specific objectives

1.3.2.1. To determine the physicochemical parameters and microbial loads of the indicator bacteria of water and sediment samples.

1.3.2.2. To examine the occurrence of selected waterborne bacterial pathogens of water and sediment samples.

2. REVIEW OF LITERATURE

2.1. Water pollution and diseases

Water is an essential constituent of life. Without it, life would not be possible. Improving access to safe water is the cornerstone for public health and sustainable development (WHO, 2008b). However, water can be polluted through directly or indirectly discharge of wastes into water bodies including rivers, lakes, wetlands and oceans without adequate treatments. These pollutants get into water bodies mainly by anthropogenic causes or factors and the water does not support human and animal uses (Xagoraraki and Kuo, 2008).

Water pollution source can generally be categorized as point and nonpoint source (Maa *et al.*, 2009). Point source water pollution is the contamination of water from a single, identifiable source such as sewage discharges from cities and industrial plants. Nonpoint source is a pollution that comes from many different contaminants such as agricultural runoff, urban storm water runoff and other area wide sources (Hogan, 2013).

Water can be polluted with chemical, physical and microbiological pollutants (WHO, 1996). Chemical contaminants may include organic substances such as detergents, food processing wastes, insecticides and herbicides, and inorganic substances such as fertilizer containing nitrate and phosphate, ammonium nitrate, acidity caused by industrial discharges, heavy metals (present in urban runoff and mine tailing area runoff). The physical contaminants include elevated temperature, discoloration and turbidity (Smith, 2010; Hogan, 2013).

Microbial contaminates are pathogens that cause diseases, which come from sewage treatment plants, septic systems, agricultural livestock operations and wildlife (Smith, 2010). They can occur in all types of water sources and particularly rampant areas where there are large amounts of untreated wastewater.

The deleterious causative agents of microbial water-related diseases are bacteria, virus and parasites (WHO, 2008b; Woodall, 2009). According to Gleick (2002), microbial water-related diseases are typically placed in four categories. These are waterborne disease, water-washed disease, water-based disease and water-related insect vector diseases of which the first three are most associated with the lack of improved domestic water. They causes diseases vary from mild gastroenteritis to sever and sometimes fatal diarrhea depending on the microbial loads individual exposed (Table 1).

Table 1 Categories of water-related diseases

Category	**Description of category**	**Example of diseases**
Waterborne diseases	Diseases related to consumption of water contaminated by human or animal feces or urine containing pathogenic microorganisms.	Cholera, typhoid fever, gastroenteritis, shigellosis, diarrheal disease, giardiasis, amoebiasis, infectious hepatitis, etc
Water-washed diseases	Diseases caused by poor personal hygiene and external body contact with contaminated water	Scabies, trachoma, conjunctivitis etc
Water-based diseases	Diseases caused by parasites found in intermediate organisms living in contaminated water/ pathogens go through the skin	Schistosomiasis, dracunculiasis, etc
Water –related insect vector disease	Diseases spread by vectors and insects that live in or close to water	Malaria (mosquitoes); filiariasis (mosquitoes), trypanosomiasis (tsetse flies) etc

Source: Gleick (2002)

2.2. Physicochemical parameters

Physicochemical parameters including pH, dissolved oxygen, nitrate, nitrite and phosphate are likely to affect the quality and operation aspect of water (APHA, 1998). Hydrogen ion concentration (pH) can determine the survival of microorganisms and the ionization of chemicals in the water. This, in turns, affects the treatment efficiency of the water. In addition, the greater acidity of the water can be increasing corrosiveness of the pipelines and contaminate the water. Hence, the taste of the water can be changed (Bitton, 2005). Thus, pH values of drinking water is expected to be between 6.5 and 8.5 as stipulated by WHO guideline and Ethiopian standard for drinking water (ES, 2001; WHO, 2011a).

Microorganisms are able to survive in various temperature ranges from below freezing point temperature to boiling point (100^{o}C) of water temperature. The increasing of water temperature can enhance the growth of microorganisms, the disinfection demand, nitrification processes, taste, odor, color and corrosion problems. Setting the optimum water temperature aids in selection of sources or the placement for water intake. Hence, the temperature of drinking water should not exceed 15^{o}C for the palatability of water is enhanced by its coolness (WHO, 2008b).

Oxygen is essential to all forms of aquatic life. The dissolved oxygen (DO) content of water depends on the temperature, salinity, turbulence and atmospheric pressure. DO variations can also occur in relation to biological activities such as photosynthesis and respiration, and high organic waste discharges into water body. Colder water can carry more dissolved oxygen than warmer water. Depletion of dissolved oxygen in water supplies can cause the microbial reduction of nitrate to nitrite, sulfate to sulfide and also cause an increase in the concentration of ferrous iron in solution (APHA, 1998; WHO, 2011a).

The DO concentration below 5 mg/l may adversely affect the functioning and survival of biological communities, while the concentration is below 2 mg/l may lead to death of most fishes. The DO may be reduced where there is high growth of microorganisms or prolonged period of high water temperature (NHMRC/NRMMC, 2011).

Electrical conductivity (EC) is the ability of a substance to conduct electricity. It is used to give an indication of the amount of inorganic materials in the water including, calcium, bicarbonate, nitrogen, phosphorus, iron, sulfur and others. The recommended EC value of drinking water is 1500 µṢ/cm (WHO, 1984).

Nitrate (NO_3^-), nitrite (NO_2^-) and ammonia (NH_3^+) are naturally occurring ions that are part of the nitrogen cycle. Nitrate can reach both surface and ground water as a consequence of agricultural activity, from wastewater treatment and from oxidation of nitrogenous waste product in human and animal excreta. This, in turns, it contaminates lakes, rivers, streams and ground water. Nitrite can be formed either due to the oxidation of ammonium compounds or chemically in distribution pipes by *Nitrosomonas* bacteria during stagnation of nitrate-containing and oxygen-poor drinking water in galvanized steel pipes (WHO, 2011b).

Nitrate has generally no significant health hazard in small quantity. However, when the concentration of nitrate is above 50 mg/l can cause methemoglobinemia or 'blue-baby syndrome' in infant and cancer in adult. This condition occurs due to the conversion of the consumed nitrate into nitrite and the nitrite involve in the oxidation of normal hemoglobin to methemoglobin. It harms the infants by reducing the ability of blood to transport oxygen (WHO, 1996).

Higher (> 1.5 mg/l) concentration of ammonia in drinking water can cause odor and taste problems. Moreover, a major concern with ammonia in drinking water is nitrification associated with the formation of nitrate and nitrites that can cause health problems at higher concentrations (WHO, 1996; WHO, 2011a).

Introduction of high concentration of phosphate along with nitrate into surface water can stimulate the growth of aquatic plants and algae. This, in turns, results in depletion of dissolved oxygen and death of aquatic organisms. Phosphates are generally not toxic to people or animals unless they occur at a very high level. If the level of phosphate greater than 1.0 mg/l, it may interfere with coagulation in water treatment plant. Consequently, organic particles harboring microorganisms may not be completely removed before distributing the drinking water to the users (Murphy, 2007).

Table 2 Maximum permissible concentration of parameters in drinking water

Parameters	**(WHO, 1984; WHO, 2011a)**	**(ES, 2001)[a]**
Total coliform in 100 ml sample		must not be detectable
***E. coli/* Fecal cliform in 100 ml sample**	must not be detectable	must not be detectable
Fecal streptococci/ Intestinal enterococci in 100 ml sample		must not be detectable
NO_3^- - N(mg/l)	11 (or 50 as NO_3^-)	50 (as NO_3^-)
NO_2^- - N(mg/l)	0.9 (or 3 as NO_2^-)	3(as NO_2^-)
Ammonium[b]	1.5	1.5
pH	6.5 - 8.0	6.5 - 8.5
Electric conductivity (µṢc/cm)	1500	
Temperature (°C)	< 15	

[a] Ethiopian drinking water quality standard

[b] The term ammonia includes the non-ionized (NH_3) and ionized (NH_4+) species

2.3. Indicator microorganisms

Indicator organisms are used for indicating the occurrence of fecal contamination, water treatment efficiency and the deterioration and post-contamination of drinking water in distribution systems (Bitton, 2005). According to Bitton (2005) indicator bacteria must be exclusively of fecal origin, present when pathogens are present and absent otherwise, occur in greater numbers than the associated pathogens, are more resistance to environmental stress and persist for a great length of time than the pathogens, do not multiply in the environment, can easily detectable and are non pathogenic. The following are the major indicator organisms.

Coliform bacteria

Coliform bacteria are Gram-negative, rod-shaped, non-spore-forming, aerobic and facultative anaerobic bacteria that are able to grow in the presence of bile salts (NHMRC/NRMMC, 2011). They are diverse groups of bacteria include *Escherichia coli*, *Enterobacter*, *Klebsiella* and *Citrobacter* and belong to the family *Enterobacteriaceae*. They are always present in both polluted and non-polluted waters, soils and plants, as well as from the feces of warm blooded animals (Bitton, 2005).

Total coliforms (TC) are coliforms which are common in the environment (soil or vegetables). They are generally harmless. They are able to ferment lactose to acid and gas within 24 h at 35±2°C. TC cannot be used as an indicator for the sanitary quality of untreated raw water, for they are naturally abundant in the environment. On the other hand, fecal (thermotolerant) coliforms (FC) are subgroup of TC bacteria. They exist in the intestines and feces of people and animals. The organisms produce acid and gas from lactose at 44 - 45°C. The presence of fecal coliform in drinking water often indicates recent fecal contaminations (WHO, 2011a).

Escherichia coli (E. coli) is a subgroup of fecal coliform. Most *E. coli* bacteria are harmless and natural inhabitant of the intestinal tract of humans and warm-blooded animals. Nonetheless, some strain can cause illness. They can be differentiated from the other thermotolerant coliforms by their ability to produce indole from tryptophan or by the production of the enzyme β-glucuronidase (ISO, 2000a). They are generally not found growing and reproducing in the environment. They are the only coliforms that satisfy criteria for an ideal fecal indicator. The presence of *E. coli* in a drinking water samples usually indicates recent fecal contamination. Hence, the presence in water samples is the best indicator of fecal pollution and the possible presence of enteric pathogens due to their prevalence in the guts of warm-blood animals. (NHMRC/NRMMC, 2011).

Some organisms such as *Enterovirus, Cryptosporidium, Giardia* and *Amoebae* however are more resistant than *E. coli.* The absence of *E. coli* does not necessarily indicate the removal of these pathogens. The spore producing sulfite-reducing clostridia, especially *Clostridium perfringens* can be used as an indicator for these organisms (WHO, 1996).

Enterococcus

Enterococci are a subset of fecal streptococci, comprising species of the genus *Streptococcus*. They are Gram-positive, facultatively anaerobic and relatively tolerant of sodium chloride and alkaline pH levels. Fecal streptococci, including intestinal enterococci all give a positive reaction with Lancefield's Group D antigen (ISO, 2000b).

Enterococci are relatively more specific for fecal pollution and good indicators of fecal pollution as compared to the rest of the fecal streptococci, because they tend to survive longer in water environment than *E. coli* and are more resistance to drying and chlorination, and the persistence patterns are similar to those of potential waterborne pathogenic bacteria. Most species do not multiply in the environments and are always present in feces of warm-blooded animals. The presence of intestinal enterococci give evidence for recent fecal contamination and useful in identification of potential sources such as inadequate treatment or breaches in distribution system (WHO, 2004).

Clostridium perfringens

Clostridum perfringens (CP) is an anaerobic, Gram-positive, rod-shaped bacillus which produces spores that are exceptionally resistant to extreme conditions in water environments, including UV irradiation, temperature, pH extremes, and chlorine disinfection processes. Like *E. coli*, CP does not multiply in most water environments and is a highly specific indicator of fecal pollution (WHO, 2011a).

C. perfringens is a protozoan and viral indicator (Payment and Franco, 1993) and as an indicator for past and present fecal contamination in fresh and marine waters and sediments (ASTM, 2002). Unlike other common fecal indicator bacteria such as *E. coli* and *Enterococci*, which are less resistant, *C. perferingens* is able to form spores that can withstand extreme environmental conditions in processing treatments and unfavorable growth conditions (Manafi and Siegrist, 2011).

2.4. Waterborne bacterial pathogens

Human and animal wastes are primary source of bacteria in water. Most bacterial pathogens are potentially transmitted through water infecting gastrointestinal tract and are excreted in feces of warm blooded animals. They gradually lose viability and the ability to causes infection, after being excreted in feces from the body of their host. The rate of decay varies with different bacteria. The most common waterborne bacterial pathogens are highly infectious or resistance to decay outside the body (Table 3). Pathogens with low persistence rapidly find a new host and spread through person-to-person contact or poor hygienic or sanitary conditions (WHO, 2004; WHO, 2008b).

Table 3 Bacterial waterborne pathogens and their significances in drinking water

Pathogens	**Health significance**[a]	**Persistence in water supplies**[b]	**Relative infectivity**[c]
***Salmonella* Typhi**	High	Moderate	Low
Other salmonellae	High	May multiply	Low
***Shigella* spp.**	High	Short	High
Vibrio cholera	High	Short to long	Low
***E. coli*- Enterohaemorrhagic**	High	Moderate	High

Source: (WHO, 2004)

[a] Health significance relate to the incidence and severity of disease, including association with outbreaks

[b] Detection period for infective stage in water at 20 °C: short, up to 1 week; moderate, 1 week to 1 month; long, over 1 month.

[c] High means infective doses can be $1–10^2$ organisms or particles, moderate $10^2–10^4$ and low $> 10^4$

The human important waterborne bacterial pathogens that can be transmitted by consuming contaminated water include *Salmonella, Shigella, Vibrio* and *E. coli* O157 (Woodall, 2009; NHMRC/NRMMC, 2011; WHO, 2011a).

Salmonella

Salmonella are Gram-negative, rod-shaped and facultative anaerobic bacilli that are predominately motile by peritichous flagella. They are members of the family *Enterobacteriaceae.* They are catalase-positive, oxidase-negative and ferment glucose and mannitol to produce acid or acid and gas. They do not ferment lactose, but most produce hydrogen sulfide on triple-sugar iron agar, and reduce nitrate to nitrite. The bacteria comprise about 2,000 serovars of somatic (O) or cell wall and flagellar (H) antigens (Percival *et al.*, 2004; WHO, 2011a).

The genus *Salmonella* is composed of two species, *Salmonella enterica* and *Salmonella bongori*. *S. enterica* is the species of most relevant for human infections and is further subdivided into six subspecies (*enetrica*, *salamae*, *arizonae*, *diarizonae*, *houtenae* and *indica*), of which the most important one is *S. enterica* subspecies *enterica* contains the majority of serotypes includes typhoid species/serovars (*S.* Typhi and *S.* Paratyphi) and the remaining non-typhoidal species/serovars (Percival *et al.*, 2004; WHO, 2008b).

Salmonella is one of the leading causes of intestinal illness all over the world (Levantesi *et al.*, 2012), with four clinical manifestation: typhoid fever/enteric fever, gastroenteritis, bacteremia/septicemia, and asyptomatic carrier state (Percival *et al.*, 2004; WHO, 2011a). Typhoidal fever is caused by *S.* Typhi and *S.* Paratyphi serovars are host adapted and can only infect human. Water contaminated with feces of human is the main vehicles of typhoidal fever infections. Similarly, non-typhoidal *Salmonella*, the ubiquitous subtypes found in a number of animal species, are more frequently associated with food borne than to water borne transmission (Levantesi *et al.*, 2012). *S.* Typhimurium are the commonly identified *Salmonella* in salmonellosis with the consumption of contaminated ground and surface water supplies. Generally, *Salmonella* spp. are relatively sensetive to disinfection and its exsitance is indicated by *E. coli* in drinking water supplies (WHO, 2011a).

Shigella

Shigella spp. is Gram-negative, facultative anaerobes and non-spore-forming bacteria that conform to the members of the family *Enterobacteriaceae*. They are catalase positive (with the exceptions in *Shigella dysenteriae*), oxidase negative and ferment sugar without gas production. The members do not produce H_2S or decarboxylate lysine. They reduce nitrates to nitrite. Members of the genus are classified based on their somatic O antigens and biochemical reaction, and comprise four pathogenic species namely: *S. dysenteriae*, *S. flexneri*, *S. boydi* and *S. sonnei* (Percival *et al.*, 2004; WHO, 2008b).

Shigella is the causative agent of bacillary dysentery or shigellosis, an infection of the large bowel that leads to cramps, diarrhea and fever. Shigellosis produces bloody stools as a result of inflammation and ulceration of intestinal mucosa. *S. dysenteriae* causes the most sever disease because of its potent Shiga toxin (exotoxin). *Shigella* spp. are mainly spread by direct fecal-oral route through person–to-person contact, contaminated food and water (Bitton, 2005).

Vibro

Vibrio spp. are Gram-negative, often curved or comma shaped rod bacteria and motile by means of a single polar flagellum. They are non-sporulating, catalase-positive and facultative anaerobes. Most species are oxidase-positive and reduce nitrates to nitrite (Champoux *et al.*, 2004). Different species tolerate a range of salinities and are commonly found in saltwater. *Vibrio cholera*, *V. parahaemolyticus* and *V. vulnificus* are the important human pathogenic species with pili (fimbriae) (Cantet *et al.*, 2013).

Cholera outbreaks continue to occur in many areas of the developing world and is one of the key indicators of the status of social development (WHO, 2008a). Cholera is acute diarrheal infection caused by ingestion of contaminated water or food with *Vibrio cholera*. *Vibrio cholera* O1 and *Vibrio cholera* O139 are distributed in natural water including drinking waters (Shanan *et al.*, 2011).

Enterohemorrhagic *E. coli*

Most *Escherichia coli* are non-pathogenic and normal inhabitant of the gut of human and some other animals. However, there are several types of pathogenic *E. coli* that are responsible for waterborne diseases outbreak. These includes: enterohemorrhagic *E. coli*, enterotoxigenic *E. coli*, enteropathogenic *E. coli*, enteroinvasive *E. coli* and enteroaggregative *E. coli* (WHO, 2004).

Enterohemorrhagic *E. coli* is a class of pathogenic *E. coli* which can cause diarrhea or hemorrhagic colitis in humans. Hemorrhagic colitis occasionally progresses into hemolytic uremic syndrome, which is characterized by acute renal failure in children and morbidity and mortality in adult (WHO, 2004). These are caused by a virulent Shiga-like toxin, potent toxins that are related to *Shigella dysenteriae* toxin. The commonest serotypes of Enterohemorrhagic *E. coli* is Enterohemorrhagic *E. coli* O157:H7 (EHEC) (Percival *et al.*, 2004).

The reservoirs for EHEC are ruminants, such as cattle and sheep and, to a lesser extent rabbits and pigs can also carry the organisms. According to Nwachuku and Gerba (2008) EHEC are also expected to be present in any waters in which *E. coli* is detected. The bacteria transmitted from person-to-person, via foodstuffs or water supplies contaminated with fecal matter from infected humans or animals (Percival *et al.*, 2004).

2.5. Bacteriological water qualities and waterborne diseases in Ethiopia

Increasing access to safe water is one of the Millennium Development Goals that Ethiopian and other nations of the world have adopted (UNGA, 2002). The country then has been wagging struggle to achieve these set goals. However, despite the multilateral efforts, previous research reports showed that most of the rural and urban drinking water sources had serious water quality problems.

A research conducted by Mengesha Admassu (2004) in North Gonder indicated that 50.0% of pipeline, 38.7% of spring and 28.6% of well water samples were contaminated with *E. coli*. A recent study carried out by Belay Bezabih *et al.* (2012) in west Amhara Region also showed that 19.5%, 20.0%, 100.0% 39.4% and 47.3% of pipe, reservoir, river, spring and well water analyzed were polluted with *E. coli*, respectively. Similarly, a report by Milkiyas Tabor *et al.* (2011) from Bahir Dar Town revealed that 45.8% and 40.0% of tap water and 88.4% and 88.5% of the household water samples were contaminated with total coliforms (TC) and fecal coliforms (FC), respectively.

Likewise, a study conducted by Mengestayhu Birhanu (2007 at Akaki Kalit sub city of Addis Ababa, central Ethiopia, showed that 100.0% of source water (boreholes and spring) were contaminated with FC and fecal streptococci (FS), whereas, 82.9% and 68.6% of pipeline water with TC and FS, respectively. Furthermore, high (100.0%) contamination of reservoir water with FC and FS was observed in the same study area. Similarly, the study conducted by Desta Kassa (2009) at Debreziet (Bishoftu) Town indicated that 17.0%, 83.0%, and 67.0% of public tap and 83.0%, 86.0%, and 76.0% of private tap water were contaminated with TC, FC and FS, respectively. On the other hand, a study conducted by Kassahun Bedane (2008) at Batu (Zwai) Town revealed that 68.0% of tap water was contaminated with TC.

Similarly, a recent study conducted on microbiological water quality of rural water sources (well, spring) from Wondo Genet (Southern Ethiopia) (Israel Deneke Haylamicheal and Awdenegest Moges, 2012), showed that the majority (85.7%) of the water points had detectable levels of TC bacteria. Other study in rural community of Dire Dewa Administrative Council (Eastern Ethiopia) (Desalegn Amenu *et al.*, 2013), and Jimma Town (Westren Ethiopia) (Divekulu Siyum and Delelegn Woyessa, 2013) showed that the majority of the water were contaminated with fecal coliforms above the recommended limit of WHO guidline values and ES standard.

In general, according to CSA (2012) of Ethiopia only 50.8% and 8.8% of the households of the country have access to improved source of drinking water and improved sanitation facilities, respectively. In addition, a report by the Rapid Assessment of Drinking-Water Quality (RADWQ) project indicated that only 68.0% of drinking water met the WHO guideline values and national (ES 261:2001) water quality standards (Dagnew Tadesse *et al.*, 2010; CSA, 2012). Hence, water quality issue is one of attention seeking problems in the country.

On the other hand, so far cross sectional studies conducted by different investigators at Tikur Anbessa, Jmma, Gonder, Bahir Dar university hospital and elsewhere indicated that various bacterial enteric pathogens were widely distributed in different parts of the country. *Salmonella*, particularly the multidrug resistant strain *Salmonella* Concord, is a major cause of salmonellosis in children in Ethiopia (Getenet Beyene *et al.*, 2011). The multidrug resistant *S.* Concord has been also isolated from children adopted from Ethiopia in Belgium and USA and parents nurturing them (Getenet Beyene *et al.*, 2011; Vanhoof *et al.*, 2012). Similarly, a study conducted by Gashaw Andargie *et al.* (2008) in Gonder Town food-handlers showed that *Salmonella* and *Shigella* strains were the commonly detectable enteric bacteria in the area.

Likewise, cholera, which is caused by the pathogen *V. cholera,* is one of the types of waterborne diseases affecting many in Ethiopia. According to WHO report the number of cases and deaths in 2007 were 24, 121 cases and 272 deaths (WHO, 2008a). However, the cases and deaths have declined from time to time and no case or death was reported in 2012 (WHO, 2013).

3. MATERIALS AND METHODS

3.1. Description of the study area

The study was carried out on drinking water distribution system of Batu (Zwai) Town and on Shoreline of Lake Zwai (and feed rivers) located at the Ethiopian Rift Valley (Lat: 7°52′–8°8′N; Long: 38°04′–38°56′E) (Figure 1) . Lake Zwai, (also referred to as Ziway or Zeway in the literature), is a freshwater wetland found at a distance of about 160 km south of Addis Ababa. It 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 again fed by two rivers, namely, Meki from the west and Qatar from the east, and is drained to the 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.

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 (CSA, 2007). Lake Zwai is source of drinking water for Batu Town and its surrounding communities. There is one water treatment sites on the lake to the outlet of Qatar River and provide drinking water for Batu Town. The lake water is also used for commercial fish farming, irrigation and horticulture farming.

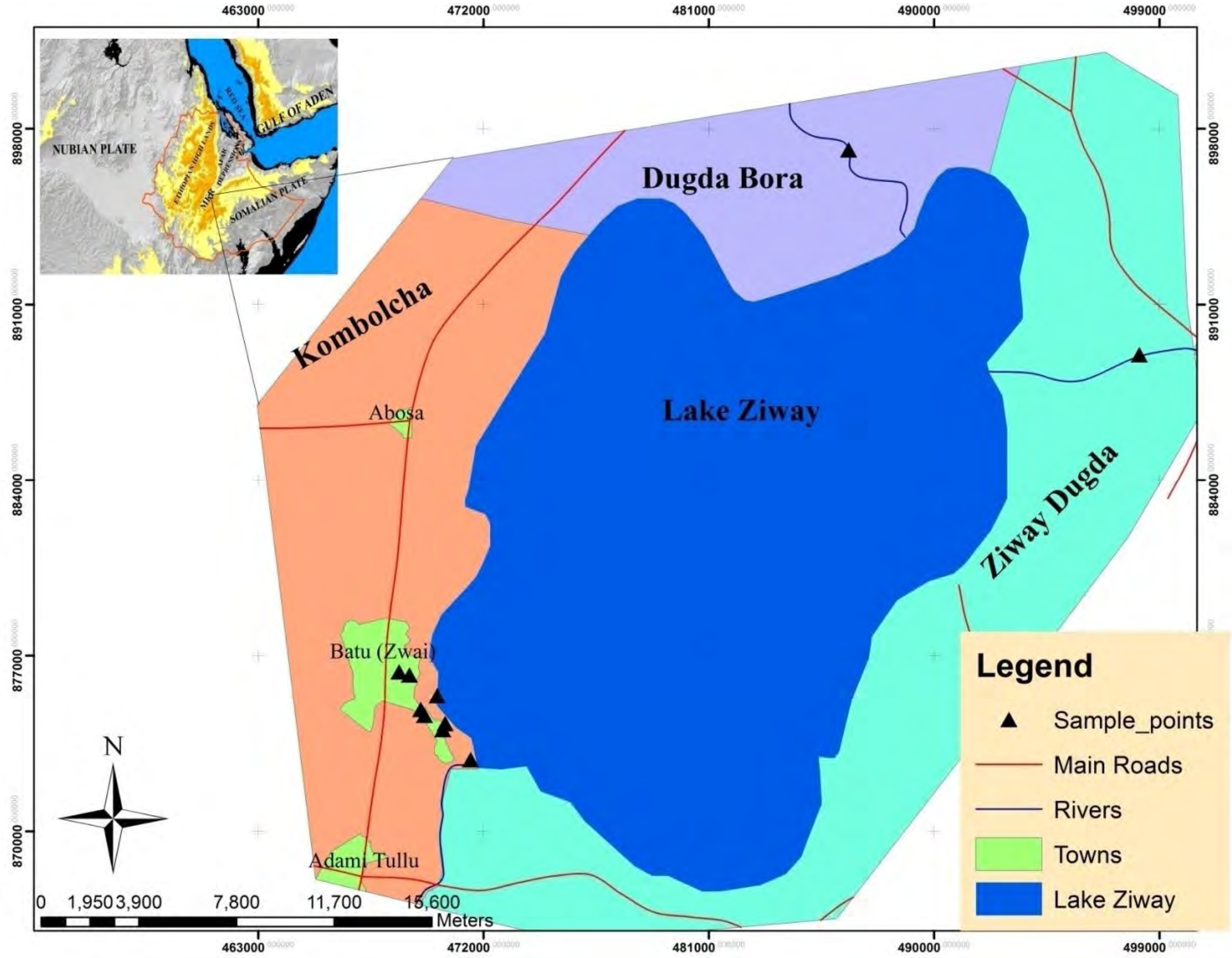

Figure 1 Site map of the study area

3.2. Study design

The study was designed to evaluate the quality of water sources (Lake Zwai and feed rivers) of Batu town drinking water system based on physicochemical parameters, indicator organisms and selected bacterial pathogens.

3.3. Sample collection

Surface water, drinking water and sediment samples were collected from different sampling sites around the town, Lake Zwai and Meki and Qatar Rivers and drinking water distribution system of Batu Town during April to November 2013 (Table 4). The sampling sites for this study were deliberately selected based on the anthropogenic impact on surface water and the number of population utilizing the water.

Table 4 Sampling sites and their abbreviations

	Sample source	**Sampling site ID**	**Sampling site description**
Water	Lake water	FLW	Fish landing site
		MCW	Municipal drinking water collection site
		SFW	Lake water around Share Ethiopia floriculture farming
	River water	MRW	Meki River water
		QRW	Qatar River water
	Reservoir water	WRB	Finally treated municipal reservoir water
	Tap water	PHW	Private house tap water
		PWC	Public tap water collection center
		BPW	Batu primary school tap water
		BHW	Batu town hotel tap water
Sediment	Lake sediment	FLS	Fish landing area sediment
		MCS	Municipal source water collection sediment
		SFS	Lake sediment around Share Ethiopia floriculture farming
	River sediment	MRS	Meki River sediemnt
		QRS	Qatar River sediment

The water samples were collected using pre-sterilized borosilicate bottle (1 l) in accordance with the procedures described in standard methods for the examination of water and wastewater (APHA, 1998). Lake and river water samples were collected manually in monthly basis, from a depth of approximately 30 cm from the surface, and tap water samples were collected directly from tap faucet discharging in pre-sterilized sample bottles containing sodium thiosulfate to neutralize free chlorine. The samples were transported in ice box and analyzed within 24 h of collection.

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. The sediment samples so collected were kept in sterile plastic bottles and transported in ice box to laboratory for analysis within 24 h of collection. Unless otherwise noted, all microbiological analyses were carried out in Applied Microbiology laboratory, and a nutrient analysis was undertaken in Limnology laboratory, Addis Ababa University.

3.4. Physicochemical parameters of water and sediments

Temperature (Temp.), electrical conductivity (EC) and dissolved oxygen (DO) of both water (surface water up to a depth of 30 cm, and reservoir and tap water from a bucket) and sediment samples were measured *in situ* 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).

3.4.1. Nutrient analysis of water

Aliquot of each water sample (100 ml) was filtered through 47 mm diameter and 0.45µm pore size membrane filter (HAWG04756, Millipore, Cheshire, UK), to analyze nitrate (NO_3-N), nitrite (NO_2-N), ammonium (NH_4 -N) and Phosphates (PO_4-P) according to standard methods.

The NO_3-N content of water samples was determined spectrophotometerically using sodium salicylate method (Monteiro *et al.*, 2003). Accordingly, 10 ml filtrate was transferred into 250 ml Erlenmeyer flask, and 1 ml sodium-salicylate solution (coloring reagent) was added to the same flask and oven dried at 95^oC to form nitronium ions (NO_2^+) from nitrate. Thereafter, 1 ml of concentrated H_2SO_4 was added to oven dried sodium salicylate and allowed to stand for 10 minutes by intermittent swirling to ensure the dissolution of the solids. When cold, 40 ml of distil water was added to each flask and swirled to mix up the solution. Finally, 7 ml of the solution containing potassium sodium tartarate and sodium hydroxide was added to the same flask. The developed yellow color was determined using spectrophotometer (Jenway Ltd Flested, Dunmo, UK) at 420 nm.

For nitrite determination, colorimetric method was utilized (APHA, 1998). Hence, 25 ml of filtered water samples was transferred to 250 ml Erlenmeyer flask, and 1 ml aminobenzenesulphoamid solution was mixed with the sample. After shaking the solution for 2 – 8 minutes, 1 ml of N-(1-Naphtyl) ethylenediamine dihydrochloride (NED dihydrochloride) solution was added to it. The solution was allowed to stand for 10 minute by swirling at irregular intervals. After 10 minutes, the solution was transferred to the cell of the spectrophotometer. The developed reddish purple color was determined using spectrophotometer (Jenway Ltd Flested, Dunmo, UK) at 543 nm.

Ammonia in water samples was analyzed through phenate method (APHA, 1998). A 25 ml filtrate was mixed with 1 ml phenol in 125 ml Erlenmeyer flask. After that, 1 ml sodium nitroprusside solution and 2.5 ml hypochlorite was added to the above flask. The solution was then covered with plastic wrap and allowed to stand at room temperature for 1 hour. The developed color was examined by spectrophotometer (Jenway Ltd Flested, Dunmo, UK) at a wave length 640 nm.

The phosphate content of water samples was determined spectrophotometerically using ascorbic acid method (APHA, 1998). Twenty five ml of filtrate was transferred into 250 ml Erlenmeyer flask. And 2.5 ml reagent solution (containing ammonium molybdate solution, sulphuric acid, ascorbic acid and potassium antimonyl tartrate solution that was mixed in the ratio of 2:5:2:1, respectively) was added. Thereafter, the solution was allowed to stand at room temperature for 20 minute to one hour. The developed blue color was determined using spectrophotometer (Jenway Ltd Flested, Dunmo, UK) at 885 nm wave lengths.

3.4.2. Nutrient analysis of sediment

Nitrate and phosphate contents of sediment samples were analyzed according to procedures described in environmental water and soil analysis manual by Trivedi and Raj (1992). Sediment samples free from stones and plant fragments were air dried and washed thoroughly with distilled water. Then, the samples were crushed with mortar and pestle to a fine powder and washed with distilled water.

For determination of nitrate, sediment sample (50 g) was added to 500 ml Erlenmeyer flask together with 250 ml extraction reagent solution containing 12.5 g copper sulphate and 0.6 g silver sulphate (w/v). The solution was shaken for 15 min and mixed with 0.4 g calcium hydroxide and 1 g magnesium carbonate in the same flask. The mixture was then filtered through Whattman No. 50 filter paper. The nitrate from filtrate (water) was analyzed by sodium salycilate method as before.

The phosphate content of sediment sample was analyzed by ascorbic acid as before according to APHA (1998). Accordingly, 1 g dry sediment was added to 200 ml sulphuric acid in 500 ml Erlenmeyer flask and shaken for 30 min. The suspension was then filtered through Whatman No. 50 filter paper (Trivedi and Raj, 1992)and measured for phosphate.

3.5. Procedures for microbiological analyses

Indicator and pathogenic microorganisms from water and sediment samples were analyzed either by membrane filtration or spread plate method (APHA, 1998). Water samples (0.1 ml – 2.0 l) were filtered through a vacuum pump (Edward High Vacuum Int., Crawley Sussex, England), as specified in standard method (APHA, 1998). The methods of Davies *et al.* (1995) and Ferguson *et al.* (2005) were used for preparation of sediment samples for analysis. Wet sediment samples (10 g) were suspended into appropriate diluents and vortexed for about 1 min to dislodge the bacteria from sediment particles. The sediment supernatant after settling for ten to thirty minutes and water samples were analyzed using membrane filtration method *as per* standard methods (APHA, 1998).

Test report of fecal indicator bacteria were expressed as CFU per 100 ml (APHA, 1998) and of sediment sample as CFU per 100 g of wet sediment (Ferguson *et al.*, 2005), whereas, of pathogenic bacteria were interpreted as present or absent (P/A) as described below with respective procedures.

3.5.1. Tests for indicator bacteria

Total and Fecal coliforms

The enumeration of total coiforms (TC) and fecal coliforms (FC) in samples was carried out with a membrane filtration *as per* standard methods for the examination of water and wastewater (APHA, 1998). Water samples (0.1-100 ml) and sediment supernatant samples (0.01-0.03 ml), after having suspended 10 g wet sediment in 90 ml Peptone Saline Solution (PFZ) (0.85% (w/v) saline and 0.1% (w/v) peptone) were filtered through 47 mm diameter and 0.45µm pore size membrane filters (HAWG04756, Millipore, Cheshire, UK). The filters were placed on an absorbent pad saturated with Membrane Lauryl Sulphate Broth (MLSB) (AVONCHEM, Cheshire, UK), and incubated at 37°C for TC and at 44°C for FC for 14 – 18h. The yellow colonies for both TC and FC were counted as CFU per 100 ml of water and as CFU per 100 g of wet sediments, respectively.

E. coli

E. coli was enumerated from samples according to ISO 9308-1 (ISO, 2000a). Water samples (0.5 – 100 ml) and 0.01 ml sediment supernatant samples, after having suspended 10 g wet sediment in 90 ml Tryptone Soya Broth (TSB) (CM0129, Oxoid, England) and vortexed were filtered through 47 mm diameter and 0.45µm pore size membrane filter (HAWG04756, Millipore, Cheshire, UK), and placed on Tryptone Soya Agar (TSA) (CM131, Oxoid, England). The plates were incubated at 37ºC for 4 – 5 h. Thereafter, the cultures were transferred to Tryptone Bile Agar (TBA) 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. The presence of *E. coli* was verified by transferring the membrane on Whattman filter paper placed on Petri dish soaked with indole reagent. The production of indole by *E. coli* was the positive test for *E. coli.*

Enterococcus

Entercocci (ENT) were enumerated from samples according ISO 7899-2 (ISO, 2000b). Water samples (1-100 ml) and 0.01 ml sediment supernatant, after having suspended 10 g wet sediment in 90 ml PFZ and vortexed was filtered through 47 mm diametere and 0.45µm pore size membrane filters (HAWG04756, Millipore, Cheshire, UK). The filters were then placed on Slanetz and Bartely Agar (SBA) (CM377, Oxoid, England). The plates were incubated at 37º C for 44 h. The red or maroon colonies on SBA were considerd as positive test for *Enterococci*.

Clostridium perfringens

Clostridum perferingens was enumerated from water and sediment samples by membrane filtration procedure described on ASTM (2002). Water samples were processed directly; sediment samples (10 g) were suspended in 90 ml PFZ. Water samples (0.2 -1.0 ml) and sediment supernatant samples (0.01 ml) after having vortexed were filtered through a membrane filter of 47 mm diameter and 0.45 µm pore membrane filter (HAWG04756, Millipore, England).

The membranes 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). Thereafter, the plates were then incubated anaerobically at 44^{o}C for 24h. The colonies on medium that showed color change from pale/straw to dark pink/magenta up on the exposure of ammonium hydroxide were considered as positive test for *C. perfringens* (ASTM, 2002).

3.5.2. Tests for pathogenic bacteria

Salmonella

Salmonella was qualitatively detected from samples according to ISO 19250 (ISO, 2010). Water samples (500 - 2000 ml) were filtered for one, or several times if water was turbid, using 47 mm diameter and 0.45 µm pore size membrane filters and pre-enriched in 50 ml Buffered Peptone Water (BPW) (M614, HiMedia, India). Likewise, sediment samples (10 g) were suspended in BPW (90 ml) and both filters and suspended sediments were incubated at 37^{o}C for 16 - 20 h. Thereafter, 0.1 ml pre-enrichment liquid was introduced into 10 ml of Rappaport Vassiliadis Soya Peptone Broth (RVS) (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 (XLD) (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 (TSI) (CM0277, Oxoid, England) and Lysine Iron Agar (LIA) (CM0381, Oxoid, England) slant and incubated at 37^{o}C for 24 h. The formation of alkaline (purple) slant and yellow and bubbles or cracks butt on TSI, and both an alkaline slant and butt on LIA with the release of hydrogen sulfide gas (blackening of the agar) on both media was an indication for the presence of *Salmonella* in the sample (ISO, 2010).

Shigella

Shigella was analyzed from water and sediment samples according to standard methods for the examination of water and wastewater (APHA 1998).Water samples (100 – 500 ml) were filtered through 47 mm diameter and 0.45 µm pore size membrane filter (HAWG04756, Millipore, England) and the filters were placed on XLD (CM069, Oxoid, England), and incubated at 37^{o}C overnight. Colonies of confluent growth were transferred to Selenite F Broth (SFB) (19 g selentie base medium [CM0395, Oxoid, England], 4 g biselenite [BDH, England]) and incubate for 6 h enrichment and streaked on XLD plates. The plates were then incubated overnight at 37^{o}C.

Likewise, sediment samples (10 g) were suspended in SFB (90 ml) and the plates were incubated overnight at 37^{o}C. A loopful of re-suspended sediment was streaked on XLD agar plates and incubated at 37^{o}C overnight. Colonies of water and sediment samples were then verified by streaking on TSI and LIA. The alkaline (purple) slant and yellow butt on TSI and alkaline (purple) and yellow butt on LIA, without the production of H_2S gas on both media was the positive test for the presence of *Shigella* (APHA, 1998).

Vibrio

Vibrio was detected form water and sediment samples as described previously by Sudhanandh *et al*. (2012). Water samples (0.1 – 1 ml) and sediment supernatant (0.01 ml), after having suspended 10 g sediment into 90 ml PFZ were spread plated on selective medium Thiosulphate Citrate Bile Sucrose agar (TCBS) 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]. Thereafter, the plates were incubated at 37^{o}C for 24 - 48 h. The yellow colonies (*V.*

cholera) or green (*V. parahaemolyticus, or V. mimicus*) were an indication for the presence of these bacteria.

***Enterohemorrhagic E. coli* O157:H7**

Enterohemorrhagic *E. coli* O157:H7 (EHEC*)* from water samples was detected according to standard method (APHA, 1998). Water samples (100 ml) were inoculated into 50 ml 3x Lauryl Tryptose Broth (3xLTB) (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 (SMAC) containing MacConkey II agar (MD 21030, Becton Dickinson, USA) and D-sorbitol (MO 63178, Sigma, USA). The cultures were incubated at 37°C for 24 h. The colorless colonies were considered as presumptive test for EHEC.

3.6. Statistical 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 ordinary data. 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 to calculate the concentration of the variables both in water and sediment samples.

4. RESULTS AND DISCUSSION

For the present study, a total of seventy eight samples were analyzed for physicochemical, bacteriological and pathogenic parameters of source and distribution system to evaluate the water quality of Lake Zwai, feed rivers and tap water system of Batu Town.

4.1.Physicochemical parameters

The pH measures of all samples were within the mean range of 7.45 to 8.33 (Table 5). The data showed that the pH values of the various samples were not significantly different ($P>0.05$) amongst sources (lake, river water and sediment, reservoir and tap water) except the lake water with pH of 8.33. In general, the pH values of all water samples were slightly alkaline, and were within the range (6.5 to 8.5) of drinking water stipulated by WHO guideline values and Ethiopian drinking water standard ES 261:2001(ES, 2001; WHO, 2011a).

In this study, the pH (8.33) value of lake water was in close agreement with a recent report by Brihanu Roba (2008) and Girma Tilahun and Ahlgren (2010) from Lake Zwai water where the average pH was 8.39 and 8.65, respectively. The pH value of Meki (7.82) and Qatar (7.66) River water samples were similar to the previous report of Girum Tamire and Seyoum Mengistou (2012) from the same place with river water mean pH of 7.95 and 7.85, respectively. Similarly, the mean pH of tap water (7.49) was slightly lower than the previous report by Kassahun Bedane (2008) from Batu Town (8.3). However, the pH of tap water and reservoir water (7.52) was similar to Getnet Kasssa (2009) report from Bishoftu Town with 6.9 -7.4 of tap water and 7.0 -7.5 of reservoir water, respectively.

Table 5 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	$376.22 \pm 364.\ 71^{b}$
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

The highest (24.0^{o}C) and the lowest (21.8^{o}C) mean temperatures in this study were recorded from tap and reservoir water samples, respectively (Table 5). The data indicated that there was no statistical significant differences (P>0.05) between temperature of different sample sources (Table 5).

The mean temperatures of lake (22.98^{o}C) and Qatar River (24.00^{o}C) water samples were almost similar with previous work on lake water samples (22.40^{o}C) (Girma Tilahun and Ahlgren, 2010) and river water (23.87^{o}C) reported by Girum Tamire and Seyoum Mengistou (2012), however, the mean temperature of Meki River water (23.33^{o}C) was lower than a report (26.07^{o}C) by the same author. Similarly, the previous studies on drinking water quality conducted in Zwai town by Kassahun Bedane (2008) (23.2^{o}C), of Bahir Dar town by Getnet Kassahun (2008) (23.8^{o}C) and of Bishoftu Town by Desta Kassa (2009) (22.6^{o}C) reported similar temperature value with the present result (24.0^{o}C). All these studies were undertaken in hot area of the country where climate could contribute to high temperature records of water samples not meet the WHO guideline values where the average temperature expected to be less than 15^{o}C (WHO, 1996).

With regard to electrical conductivity (EC), the values of different samples were within the mean range of 328.33 - 575.36 µS/cm (Table 5). The measure of EC of various samples in this study did not show significant variations (P>0.05) amongst sample sources (Table 5).

In general, the mean range measurement of EC (328.33 - 575.36) µS/cm was comparable with previous report of Girma Tilahun and Ahlgren (2010) from Lake Zwai water (478 µS/cm), and river water of Meki River water (450.6 µS/cm) and of Qatar River water (424.5 µS/cm) reported by Girum Tamire and Seyoum Mengistou (2012). Similarly, the average mean value of EC of tap water (472.15 µS/cm) was slightly lower than the mean range measurement of EC (492 – 581) from Bishoftu Town reported by Desta Kassa (2009). For the present study, the EC values of different water sources were well below the WHO guideline values prescribed for drinking water purpose (1500 µS/cm) (WHO, 1984). Accordingly, the value of EC in different water samples could not be water quality problem of the study area.

The highest content of dissolved oxygen (DO) of 4.78 mg/l was measured from reservoir water, whereas, the lowest DO of 1.89 mg/l from lake sediments (Table 5). Unlike temperature, pH and EC, there was significant variations ($P<0.0001$) amongst sample sources in their DO contents. The DO pattern showed that river water (3.73 – 4.77 mg/l) and reservoir water (4.78 mg/l) samples contained higher DO than the lake and tap water indicative of the river water and treated water were a bit better than staid (lake) and contaminated tap water (Table 5).

In this study, the average concentration of DO (3.04 mg/l) in lake water samples was much lower than the one reported by Brihanu Roba (2008) where the average content of DO in the lake water was 8.72 mg/l. Similarly, the mean DO measurement of tap water samples (3.50 mg/l) was lower than previous report (7.9 – 9.3 mg/l) by Desta Kassa (2009) from Bishoftu Town.

For the present study, the nitrate nitrogen (NO_3-N) and nitrite nitrogen (NO_2-N) content of sample analyzed did not show significance variations ($P>0.05$) amongst sample sources, whereas, the ammonia nitrogen (NH_4-N) in water samples showed significant variations ($P=0.001$) between the sample sources (Table 5). The highest NO_3-N (2.43 mg/l), NO_2-N (0.15 mg/l) and NH_4-N (18.21 mg/l) measurements 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 (Table 5). In general, the data revealed that the concentration of both NO_3-N and NO_2-N in different water samples were much lower than the value recommended by WHO guideline values of 11.0 mg/l for nitrate nitrogen and 0.9 mg/l for nitrite nitrogen of drinking water, whereas, the same pattern held true for NH_4-N (1.5 mg/l) of reservoir and tap water samples (WHO, 2011a).

The mean nitrate nitrogen (2.43 mg/l) and ammonia nitrogen (18.21 mg/l) content of lake water samples were higher than the previous report of Girma Tilahun and Ahlgren (2010) from the same lake with the mean NO_3-N of 0.0032 mg/l and NH_4-N of 0.111 mg/l, respectively. This could be due to the present study data collections were carried out from the parts of the lake of which it was impacted by anthropogenic activities. Similarly, the mean nitrate nitrogen content of tap water (0.17 mg/l) of the present study was between the average range of nitrate nitrogen measurement of 0.07 – 1.54 mg/l of previous report of Kassahun Bedane (2008) from Batu Town and a little bit lower than the range of 0.2 – 2.8 mg/l reported by Desta Kassa (2009) from Bishoftu Town tap water.

The phosphate phosphorus (PO_4-P) contents of all analyzed samples were within the range of 42.81 - 376.22 µg/l (Table 5). The maximum (376.22 µg/l) and minimum (42.81 µg/l) mean contents of phosphate phosphorus were detected from the lake sediment and Qatar River sediments, respectively. The data showed that there was no significant variations ($P>0.05$) of the measurement of contents of PO_4-P amongst samples sources except the value of lake sediment (376.22 µg/l) (Table 5).

The mean phosphate (78.76 µg/l) content of the lake water was higher than the previous report from the same lake by Girma Tilahun and Ahlgren (2010) with mean values of 10.1 µg/l. This could be due to the present study was carried out on shoreline of the lake of which phosphate containing wastes can easily be introduced into the lake body. The phosphate measurement of tap water (114 .05 µg/l) in this study was lower than the previous work (220 – 480 µg/l) reported by Desta Kassa (2009) from Bishoftu Town tap water.

4.2.Indicator bacteria in water and sediments

Indicator bacteria were investigated from lake and river water, sediment and reservoir and tap water samples. The data showed that almost all of the lake and river water samples were contaminated with total coliforms (TC), fecal coliforms (FC), *Escherichia coli* (*E. coli*), *Enterococci* (ENT) and *Clostridium perfringens* (CP) (Figure 2). Most of the lake and river sediment samples were polluted with all types of indicator organisms. However, only 38.2% of the reservoir and 63.0% of the tap water samples were positive for all indicator organisms (Figure 2).

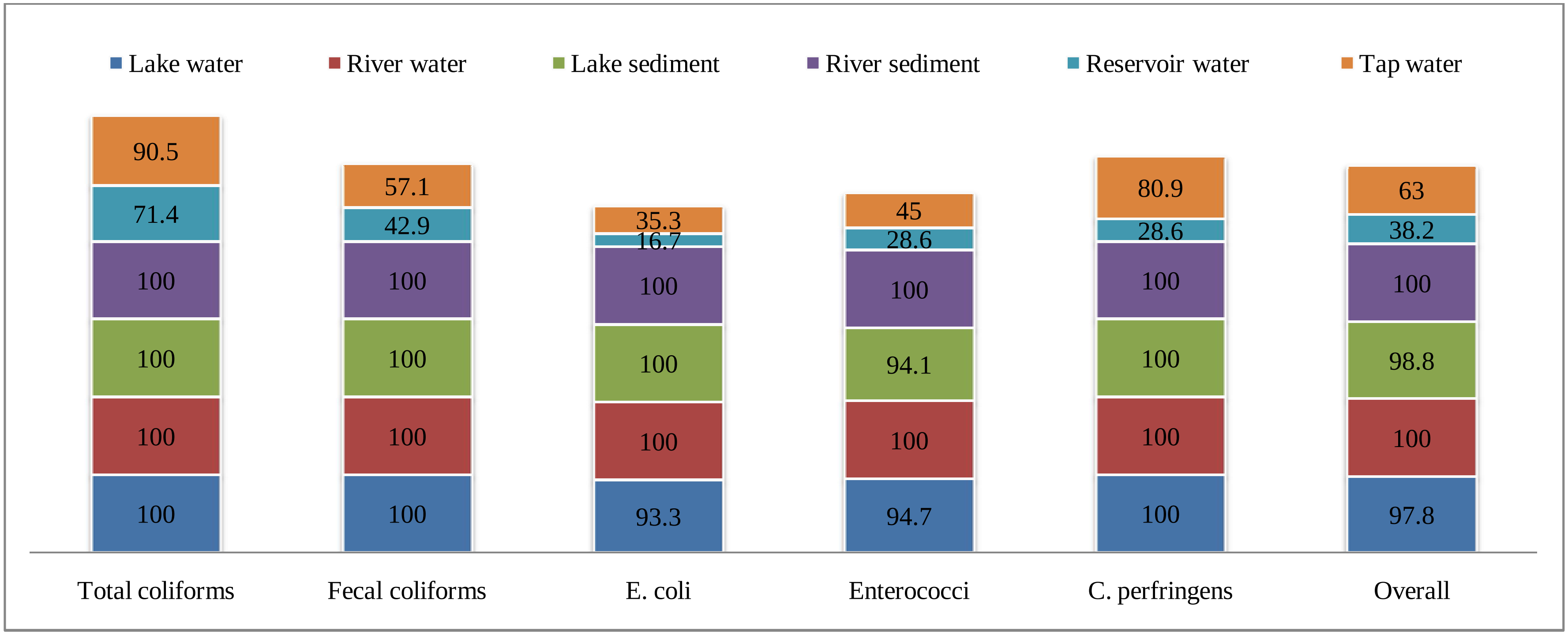

Figure 2 Percentage occurrences of indicator bacteria in water and sediment

The loads of indicator bacteria were determined from water and sediment samples. Of the lake and river water samples examined for the indicator organisms, the highest (4.11 log CFU/100 ml) of TC and the lowest (2.28 log CFU/100 ml) of *E. coli* was recorded from lake water samples and the same pattern of microbial load (4.12 log) of TC and (2.76 log CFU/100 ml) of *E. coli* was detected from Meki and Qatar River water samples, respectively (Table 6). Similarly, the maximum (5.07 log CFU/100 g) number of TC and minimum (3.16 log CFU/100 g) number of *E. coli* was detected from the lake wet sediment samples and the same pattern of microbial counts (4.98 log CFU/100 g) of TC and (3.10 log CFU/100 g) of *E. coli* was enumerated from Qatar and Meki River sediment samples, respectively (Table 6).

The data showed that relatively lower microbial load of TC (0.82 CFU/100 ml) and *E. coli* (0.10 CFU/100 ml) was detected from reservoir water samples. On the other hand, higher (1.80 CFU/100 ml) number of CP and lower (0.25 CFU/100 ml) number of *E. coli* was detected from tap water samples (Table 6). In general, the overall microbial counts of indicator organisms were declined in the order of Qatar River sediment, Meki River sediment, lake sediment, Meki River water, Qatar River water, lake water, tap water and reservoir water with a significant variations ($P<0.0001$) amongst sources during the study period (Table 6).

For the present study, higher counts of microbial loads were recorded from the sediment samples. Similar results were reported by Davies *et al*. (1995), from Whale Beach, Sydney and Craig et al. (2002) from Australia. This was due to sediment provides rich organic matters for the bacteria (Craige *et al*., 2004), decreasing sun light inactivation and providing protection against predators such as protozoan and bacteriophages (Lee *et al*., 2006; Friesa *et al*., 2008).

Table 6 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 mean
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
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
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
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
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
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
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
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
***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

For the present study, the microbial load of TC (4.11 log) and FC (3.90 log) CFU/100 ml in lake water was greater than the previous report by Brihanu Roba (2008) from the same place where the mean densities of TC and FC were 1.90 log and 1.72 log during dry season, and 1.68 log and 1.43 log CFU/100 ml during wet season. This could be due to ever increasing introduction of municipal wastes into the lake, in turns, allochthonous microbes introduced along with the discharged wastes. However, the result was similar with the previous report by Goraw Goshu (2010) from Lake Tana where the TC and FC counts ranged from 2.4 log to 6.3 log and non-detection to 6.2 log CFU/100 ml, respectively. Similarly, the *E. coli* counts (2.28 log) and *Enterocooci* (3.09 log) CFU/100 g in lake sediment was lower than a recent report by Haller *et al. (*2009*)* from Lake Geneva where the mean concentrations varied from 4 log to 5 log CFU/100 g.

The data showed that TC (4.08 log) CFU/100 ml and FC (3.86 log) CFU/100 ml of the combined mean counts of Meki and Qatar Rivers water samples were comparable with a recent report of Brihanu Million (2008) from Lagabatu River (Central Highland of Ethiopia) with TC and FC counts of 3.45 log and 3.40 log CFU/100 ml, respectively. However, the mean FC in river water was a little bit lower compared to FC counts of 5.17 log CFU/100 ml reported from Baynespruit River, South Africa (Gemmell and Schmidt, 2012). Similarly, the combined mean *E. coli* count of 2.90 log CFU/100 ml of Meki and Qatar Rivers was slightly lower than the *E. coli* count of 4.0 log CFU/100 ml from Athi and Nairobi Rivers in Kenya (Musyoki *et al.*, 2013).

With respective to tap water, the occurrence of TC (90.5%) and FC (57.10%) in tap water was higher than the previous report of Kassahun Bedane (2008) where TC (68.0%) and none of FC were detected from tap water. This could be due to improper breach off the pipelines whenever constructing the town, obsolete infrastructure and inadequate treatments of the source water (WHO, 2004; Corcoran *et al.*, 2010). The values of TC and FC in this study were almost similar to the previous report by Desta Kassa (2009) from Bishoftu Town where 100.0% and 86.0% of tap water were contaminated with TC and FC, respectively. Similarly, the present results of TC and FC were closely agree with the previous report from Bahr Dar town by Getnet Kassahun (2008) where the tap water was contaminated with TC (87.0%)

and FC (43.0%). The same pattern of the occurrence of TC (82.9%) and FC (68.6%) reported from water sources of Akaki Kality sub city of Addis Ababa by Mengistayehu Birhanu (2007). In addition, a study conducted by Mengesha Admassu *et al.* (2004) in Gonder showed that a closely similar occurrence of *E. coli* (50.0%) was observed as compared to the present report (35.3%).

In this study, the presences of indicator organisms in finally treated water (reservoir) could be an indicative for the inadequate treatment of the source water. This, in turns, the reservoir water may contain pathogenic microorganisms (WHO, 1996; WHO, 2004). Similarly, the tap water harbored twofold more bacterial loads as compared to the reservoir water samples. These higher bacterial loads could be associated with contamination of the distribution system through improper breach off pipelines, management of catchment areas, obsolete infrastructure and storage facilities (WHO, 2004; Corcoran *et al.*, 2010).

In general, 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* (Figure 3). Hence, the compliance value (42.7%) of tap water was lower compared to the national in compliance values (88.0%) for utility piped supplies tested for fecal coliforms reported by Dagnew Tadesse *et al.* (2010).

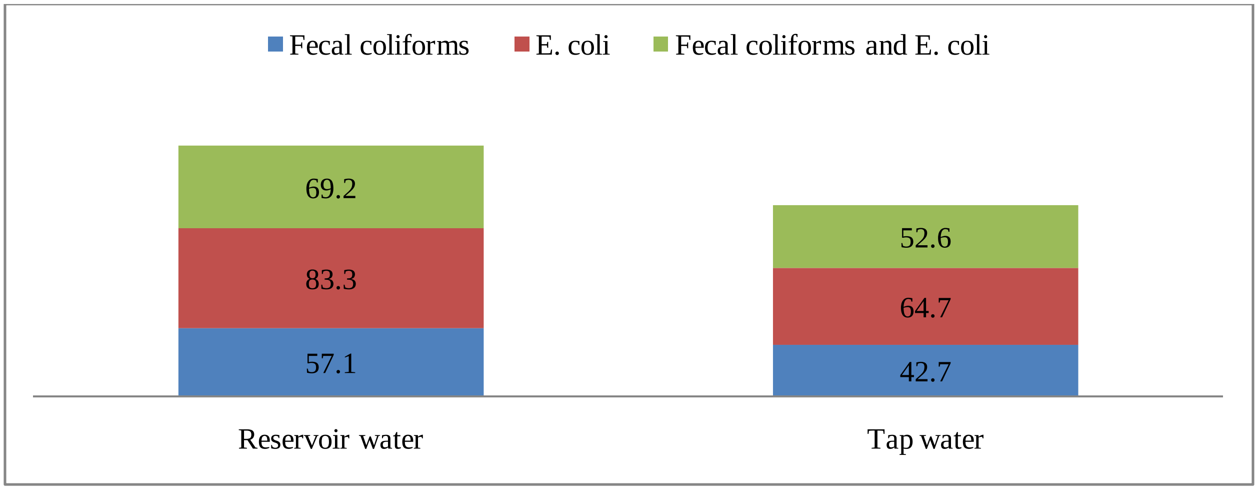

Figure 3 Compliance of water samples with WHO guideline values and national standards for fecal coliforms and *E. coli*

4.3.Occurrences of pathogenic bacteria in water and sediments

Microbial pathogens were analyzed from lake and river water and sediment, reservoir and tap water samples. Of the lake and river water samples tested for presence or absence of bacterial pathogens, 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 pathogens, except *E. coli* O157:H7 in both rivers, *Shigella* in Meki and *Salmonella* in Qatar River water samples. The highest percentage (89.5%) of *V. cholera* and the lowest percentage (5.5%) of *E. coli* O157:H7 were detected from the lake water samples. Similarly, the highest percentage (66.7%) of *V. cholera* was detected from Meki River water samples (Table 7).

Table 7 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 sediemnt	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 respect to sediment samples, 52.7% of the lake sediment samples were positive for all pathogenic bacteria. Similarly, 50.0% of Meki and Qatar River sediment samples were positive for all tested pathogenic bacteria except *Salmonella* for Qatar River sediment sample. The highest percentage (94.4%) of *V. cholera* was detected from the lake sediment and 66.7% of *V. cholera* from the sediment samples of both rivers. On the other hand, 4.8% of tap water samples were positive for all bacterial pathogens, whereas, none of the tested bacterial pathogens were detected from reservoir water samples. Generally, the data showed that *V. cholera*, *Vibrio* spp., *Salmonella* and *Shigella* were widely distributed (48.9% data not shown) in surface water and sediment samples, whereas, *E. coli* O157:H7 (3.3%, data not shown) were limited into a few sources (Table 7).

The data showed that no bacterial pathogens were detected from reservoir water indicating efficiency of the treatment. However, the existence of indicator bacteria (38%) in treated water samples can be an indicative for the existence of other types of pathogens including parasites and viruses of which over lived than bacterial pathogens. On the other hand, 4.8% tap water samples harbored all tested bacterial pathogens. This could be due to the contamination of distribution system through improper breach of pipelines and obsolete infrastructure of the system (WHO, 2004; Corcoran *et al*., 2010).

In general, in this research, there was higher pathogenic bacteria distribution in sediment samples than in water samples. The detection of higher bacterial population in sediment samples was in line with the previous reports as some enteric bacteria such as *Salmonella*, *Vibrio* spp. *E. coli* and *C. perfringens* can survive better in the sediment than the overlying water column (Burton *et al*., 1987; Chandrana *et al*., 2011).

In this study, the detection of *Salmonella* in lake water (10.5%) and sediment (15.8%), and the combined river 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 by Benjamin *et al*. (2013) from Central California coast. This could be due to differences in method utilized, number of samples and geographical location of which the study was conducted. 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. Similarly, the detection of *Shigella* (15.8%) in Lake Zwai water was in a close agreement with the values of 10.9% from surface water (lake and river) of Bangladesh using PCR method (Faruque *et al*., 2002).

Likewise, the detection of *Salmonella* (10.5%), *Shigella* (15.8%), *V. cholera* (89.5%) and *Vibrio* spp. (78.9%) in this study was lower than the previous report by Sudhanandh *et al.* (2012) form southern Kerala coast sea water, India where the detection of the above pathogens were 33.1%, 90.1%, 97.5% and 97.5% of samples examined, respectively. These differences could be due to the types of water samples analyzed, the place where the study conducted and sample size.

The *Salmonella* (4.8%) and *V. cholera* (4.8%) detection in tap water samples was in close agreement with a recent report of Momtaz *et al.* (2013) from southern Isfahan, Iran, where 5.5% and 8.3% were positive for *Salmonella* and *V. cholera*, respectively. On contrary, the occurrence of *Salmonella* in pipeline water was lower than the one reported by Akinyemi *et al.* (2011) from Nigeria of which 18.3% of the samples analyzed were positive for *Salmonella* isolates. Likewise, the prevalence of *E. coli* O157:H7 (5.0%) in tap water was comparable with the one reported by Heijnen and Medema (2006) from the Netherlands (7.4%). Similarly, the 4.8% presence of *Salmonella*, *Shigella* and *Vibrio* spp. in tap water was much lower than the report of Md. Shahidul *et al*. (2014) from Dhaka city, Bangladesh, where 35.0%, 60.0% and 50.0% of tap water samples were contaminated with these microbes, respectively.

4.4. Correlation of microbial pathogens with physicochemical parameters and indicator microbes of water and sediments

The data indicate some statistically significant Spearman rank correlation of pathogens with physicochemical parameters and indicator organisms. Accordingly, the presence of *Salmonella* in water was significantly positively correlated with fecal coliforms in sediment and *Shigella* in sediment, whereas, significantly negatively correlated with nitrite nitrogen in water (Table 8). The significant correlation of *Salmonella* and *Shigella* with each other could be an indicative for the close association of these parameters with each other (Santhiya *et al*., 2011).

Table 8 Spearman rank correlation of indicator bacteria and physicochemical parameters towards pathogenic bacteria of surface water and sediments

Pathogens *vs.* variables[a]	***Salmonella* (w)**	***Shigella* (w)**	***E. coli* O157:H7*(w)***	***V. cholera* (s)**	***Vibrio* spp. (w)**	***Vibrio* spp. (s)**
Fecal coliform (w)		0.47^{*}				
Fecal coliform (s)	0.41^{*}					
***E. coli* (s)**		0.47^{*}				
***Enterococcus* (s)**					0.43^{*}	
***Shigella* (w)**			0.47^{*}			
***Shigella* (s)**	0.43^{*}					
***V. cholera* (w)**					0.70^{**}	
NO_2-N (w)	-0.51^{*}					0.63^{**}
Dissolved Oxygen (w)				-0.51^{*}	-0.59^{*}	

[a] w, surface water; s, sediment

*Statistically significant correlation at the <0.05 levels

**Statistically significant correlation at the <0.01 level

The presence of *Vibrio* spp. in water was significantly positively correlated with the occurrence of *Enterococci* in sediment and *V. cholera* in water, whereas, negatively significantly correlated with dissolved oxygen in water. There was a significant positive correlations between the presence of *Vibrio* spp. in sediment with nitrite nitrogen in water, whereas, *V. cholera* in sediment significantly negatively correlated with dissolved oxygen in water (Table 8).

The occurrence of *Shigella* in water was significantly correlated with the concentrations of fecal coliforms in water, *E. coli* in sediment and *E. coli* O157:H7 in water (Table 8). Moreover, there was also a significant positive correlation between the detection of *Shigella* in sediment and *Salmonella* in water. In the present study, *Shigella* built better association with both indicator organisms and pathogenic bacteria in water and sediment samples.

In general, Spearman rank correlations indicate that the presences of bacterial pathogens were significantly correlated with some log-transformed indicator bacteria. *Salmonella* in water was significantly correlated with fecal coliforms in sediment, whereas, *Shigella* in water with fecal coliforms in water and *E. coli* in sediment and Vibrio spp. in water with *Enterococci* in sediment. However, the presences of *E. coli* O157 in water and *V. cholera* in surface water and sediment samples were not significantly correlated with any of the log-transformed indicator bacteria. Similar result was reported by Benjamin *et al*. (2013) from Central California coast, where concentration of generic *E. coli* was not significantly associated with the presence of either *E. coli* O157 or *Salmonella* in water or sediment samples.

For the present study, the data indicate that some bacteria from sediment samples were significantly correlated with the bacteria in the overlying water (Table 8). *Shigella* in sediment, for example, significantly correlated with, *Salmonella* in water and *Enterococci* in sediment with *Vibrio* spp in water. Accordingly, the significant correlations of sediment bacteria with overlying water were an indicative for recharging of the water from sediment organisms.

Likewise, the presence of pathogenic bacteria in drinking water samples showed no significant correlation with the log-transformed indicator bacteria and physiochemical parameters (Table 9). On the other hand, the occurrence of *V. cholera* in tap water showed strong (*rho*=1.0) significant correlation with *Vibrio* spp. in tap water. The positive correlation of *V. cholera* and *Vibrio* spp. could be due to the close association of both organisms (Santhiya *et al.*, 2011).

Table 9 Spearman rank correlation coefficient matrix among selected drinking water variables

	Salmonella	***Shigella***	***E. coli* O157**	***V. cholera***	***Vibrio* spp.**
Total coliform	-0.286	0.095	0.252	-0.060	-0.060
Fecal coliform	-0.188	0.163	0.294	0.126	0.126
E. coli	-0.138	0.256		0.395	0.395
Enterococcus	-0.156	-0.156	-0.163	0.339	0.339
C. perfringens	0.109	-0.230	0.282	-0.230	-0.230
Salmonella	1.000	-0.037	-0.038	-0.037	-0.037
Shigella	-0.037	1.000	-0.038	-0.037	-0.037
***E. coli* O157**	-0.038	-0.038	1.000	-0.038	-0.038
V. cholera	-0.037	-0.037	-0.038	1.000	1.000**
PO_4^{3-}	-0.220	0.044		-0.441	-0.441
NO_2-N	0.409	0.358	0.028	-0.281	-0.281
NO_{3-} N	0.258	-0.305	-0.375	-0.375	-0.375
NH_3	0.177	0.530		-0.177	-0.177
Temperature	-0.130	-0.130		0.286	0.286
pH	-0.281	-0.437			
Electric conductivity	0.307	0.358		0.230	0.230
Dissolved Oxygen	-0.103	-0.257		0.129	0.129

In general, in this study, although 4.8% of tap water samples were positive for bacterial pathogens, all log-transformed indicator organisms were not significantly correlated with the pathogenic bacteria (Table 9). Similar result was reported by Md. Shahidul *et al*. (2014) from Dhaka city, Bangladesh, where indicator bacteria were not significantly correlated with the presence of *Salmonella* and *Shigella* in the tap water samples. The work of Lemarchand and Lebaron (2003) from French coastal watershed of river water and treated effluents showed that the presence of fecal coliforms and streptococci were not significantly correlated with the occurrence of *Salmonella*. However, the lack of correlation of indicator organisms and pathogens in drinking water could be related to the samples size and the number of positive detection of pathogens in the samples analyzed (Wu *et al*., 2011).

5. CONCLUSION AND RECOMMENDATION

5.1.Conclusion

This study examined the occurrence of microbial pathogens, indicator bacteria and some physicochemical parameters of source water (Lake Zwai, Meki and Qatar Rivers) and Batu Town drinking water distribution system. The major findings of the study are the followings:

- For the present study, 97 to 100% of surface water samples were contaminated with indicator bacteria, whereas, 38% of the reservoir and 63% of tap water samples were also positive for these organisms indicating the inadequacy of the treatment and post contamination of drinking water in distribution system.
- The microbial load of indicator and pathogenic bacteria was more in sediment than overlaying water samples.
- Some indicator bacteria such as fecal coliforms were significantly correlated with *Salmonella* and *Shigella*; *E. coli* with *Shigella*, and *Enterococci* with *Vibrio* spp. of surface and sediment samples. However, the presences of *E. coli* O157:H7 of surface water and *V. cholera* in surface water and sediment samples were not significantly correlated with any of the indicator bacteria. Furthermore, the indicator bacteria were not significantly correlated with any tested bacterial pathogens in the tap water samples.
- *Shigella* built better association with both indicator organisms and pathogenic bacteria in water and sediment samples.
- With the exception of temperature in tested sample sources, all physicochemical parameters in reservoir and tap water samples were lower than the maximum permissible values for drinking water.

5.2.Recommendation

Based on the research findings, the following points are recommended:

- The detection of pathogenic bacteria from both water and sediment samples for this study was made by cultural method which may not show the exact type of the pathogens species. Hence, extensive studies with various types of 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.
- More than 40% of the reservoir and tap water samples were contaminated with indicator bacteria beyond the maximum permitted level recommended by the WHO guideline and ES. In addition, 4.8% of the tap water samples were positive for *Salmonella, Shigella, Vibrio cholera, Vibrio* spp. and *E. coli* O157: H7. Thus, establishing of efficient treatment mechanisms and up-to-date distribution system will be necessary to remove all pathogenic organisms and providing safe water for the communities.
- The direct detections of pathogenic microbes together with indicator bacteria are more necessitate for establishing whether or not a water body is truly polluted by pathogenic microorganisms.

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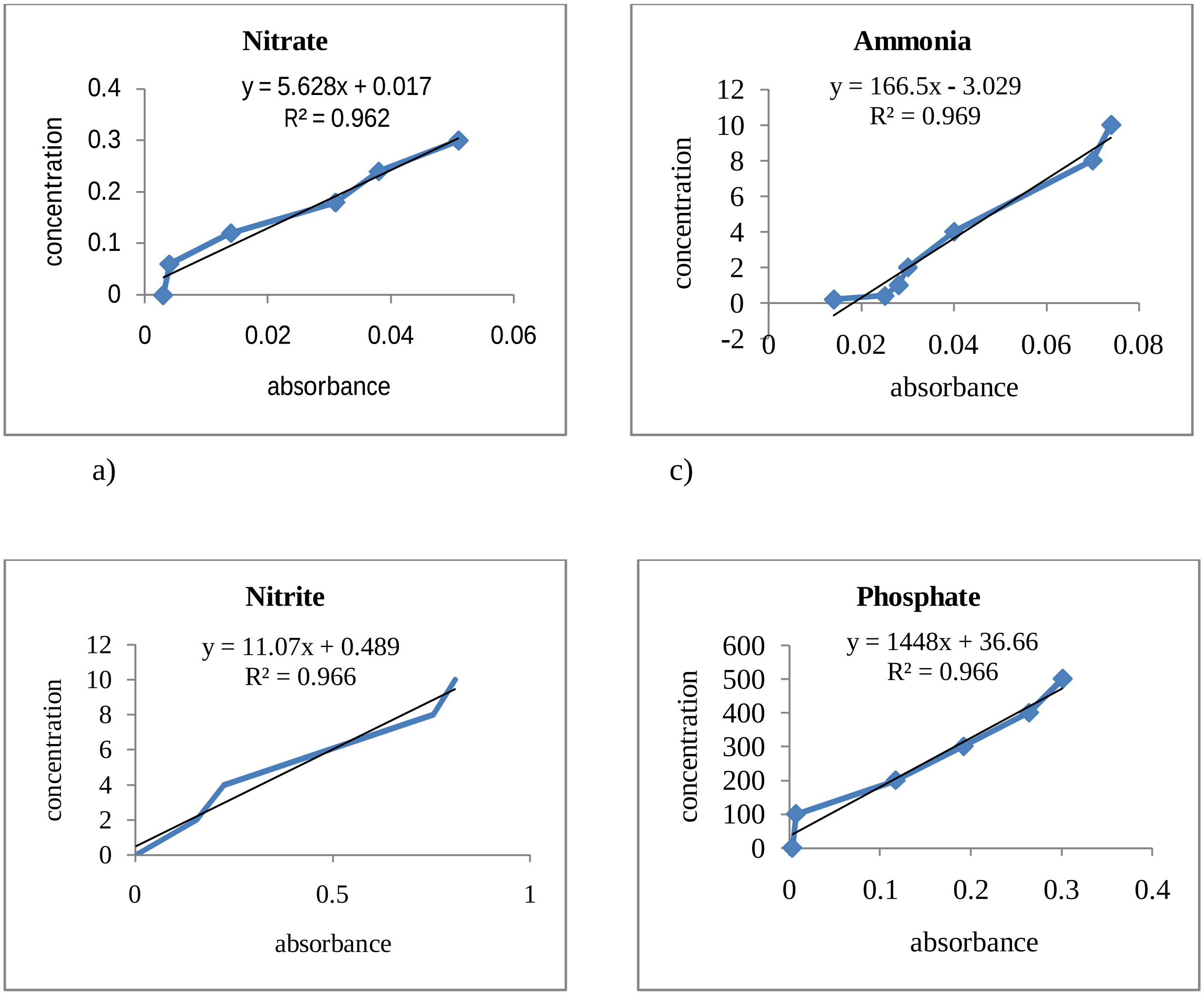
APPENDICES

Appendix I: Calibration curve for nitrate, nitrite, ammonia and phosphate



a) c)

b) d)

Appendix II: Concentration (mean) of physicochemical parameters at each sampling site

Sampling sites*	**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)**
FLW	8.30^{eg}	22.50^{a}	383.00^{ab}	2.20^{abc}	0.42^{a}	0.04^{a}	10.42^{ab}	75.03^{b}
MCW	8.52^{g}	24.16^{a}	390.00^{ab}	3.97^{ef}	0.61^{a}	0.04^{a}	13.18^{b}	63.59^{a}
SFW	8.12^{ef}	21.50^{a}	857.00^{d}	2.93^{bcde}	5.87^{b}	0.37^{b}	28.44^{c}	101.46^{a}
FLS	7.27^{a}	22.13^{a}	405.50^{ab}	1.43^{a}	0.86^{a}	NA***	NA	827.99^{a}
MCS	7.80^{cd}	22.87^{a}	628.75^{abcd}	2.25^{abcd}	1.23^{a}	NA	NA	112.68^{a}
SFS	7.67^{bcd}	22.67^{a}	730.67^{cd}	2.03^{ab}	2.79^{ab}	NA	NA	187.98^{a}
WRB	7.52^{abcd}	21.80^{a}	441.25^{abc}	4.78^{f}	0.04^{a}	0.03^{a}	0.47^{a}	73.83^{a}
PHW	7.67^{bcd}	24.25^{a}	377.00^{ab}	4.00^{ef}	0.07^{a}	0.03^{a}	0.63^{a}	124.99^{a}
PWC	7.39^{ab}	24.00^{a}	383.33^{ab}	2.97^{bcde}	0.21^{a}	0.03^{a}	0.97^{a}	82.99^{a}
BPS	7.47^{abcd}	23.33^{a}	490.00^{abc}	3.27^{bcde}	0.20^{a}	0.03^{a}	0.22^{a}	49.69^{a}
BHW	7.45^{abc}	24.33^{a}	670.00^{bcd}	3.60^{cdef}	0.25^{a}	0.04^{a}	0.88^{a}	193.04^{a}
***P*-value**	<0.0001	0.570	0.006	<0.0001	0.013	<0.0001	<0.0001	<0.0001

*Sites: FLW(S), lake water and sediment from fish landing site; MCW(S), lake water and sediment for municipal water treatment area; SFW(S), lake water or sediment around horticulture farming; WRB, reservoir water; PHW, private house tap water; PWB, public tap water; BPS, Batu primary school tap water; BHW, Hotel tap water

**Values indicated with different letters are statistically significant; differences of concentration of physicochemical parameters were tested by Duncan – ANOVA

***Not analyzed

Appendix III: Densities of indicator bacteria at each sampling site

Sampling sites	**Concentration (mean) of fecal indicator bacteria in log CFU/ 100 ml or g at each sampling site***					**Overall mean**
	TC	FC	*E .coli*	ENT	CP	
FLW	3.95^{c}	3.80^{cd}	2.58^{b}	3.00^{bc}	2.52^{bcde}	3.18
MCW	4.19^{cdef}	3.64^{c}	2.22^{b}	3.38^{bc}	3.41^{def}	3.37
SFW	4.22^{cdef}	4.40^{cde}	3.80^{cd}	2.78^{b}	3.95^{f}	3.83
FLS	5.01^{fg}	4.31^{cde}	2.74^{b}	3.39^{bc}	4.13^{f}	3.99
MCS	4.91^{efg}	4.62^{de}	2.90^{bc}	3.94^{bc}	4.09^{f}	4.13
SFS	5.36^{g}	4.76^{e}	4.07^{d}	2.75^{b}	4.32^{f}	4.40
WRB	0.82^{a}	0.37^{ab}	0.10^{a}	0.29^{a}	0.45^{a}	0.42
PHW	1.46^{ab}	1.10^{b}	0.61^{a}	0.98^{a}	1.19^{ab}	1.08
PWC	1.71^{b}	1.09^{b}	0.10^{a}	ND	2.12^{bcd}	1.08
BPS	1.08^{ab}	0.15^{a}	0.08^{a}	0.04^{a}	1.71^{abc}	0.72
BHW	1.43^{ab}	0.38^{ab}	ND	0.15^{a}	2.56^{cde}	0.91
***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

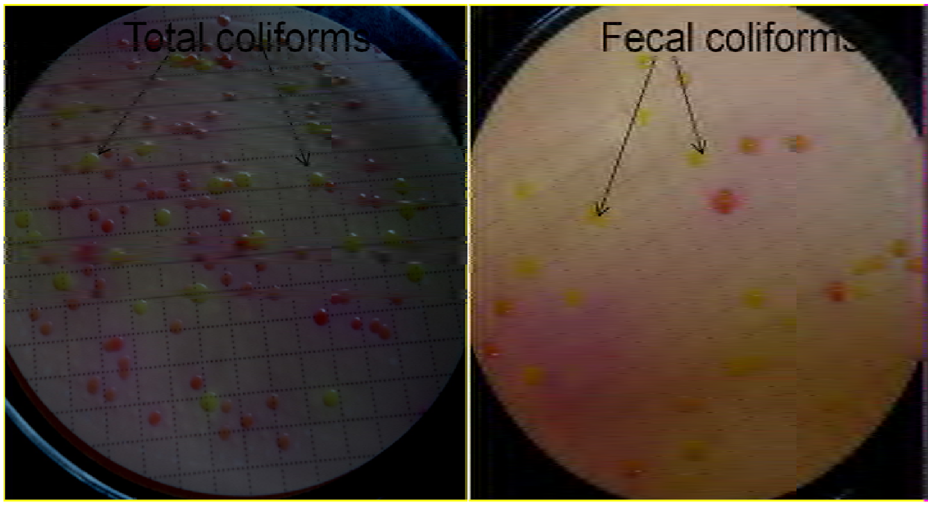
Appendix IV: Occurrences of pathogenic bacteria at each sampling site

Sampling sites	Percent (%) positive for pathogenic bacteria at each sampling site					Overall %
	SL	SH	EHEC	VC	VB	
FLW	14.3	14.3	ND	85.7	85.7	44.1
MCW	14.3	ND	ND	85.7	71.4	34.3
SFW	ND*	40.0	20.0	100.0	80.0	52.0
FLS	14.3	28.6	NA**	100.0	85.7	50.0
MCS	14.3	ND	NA	85.7	85.7	37.1
SFS	20.0	20.0	NA	100.0	100.0	43.5
WRB	ND	ND	ND	ND	ND	ND
PHW	ND	ND	ND	14.3	14.3	5.9
PWC	ND	ND	20.0	ND	ND	4.0
BPS	ND	20.0	ND	ND	ND	6.7
BHW	25.0	ND	ND	ND	ND	5.0

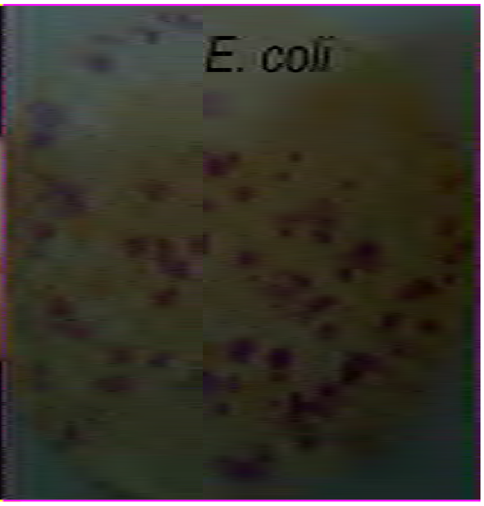
*Not detected

**Not analyzed

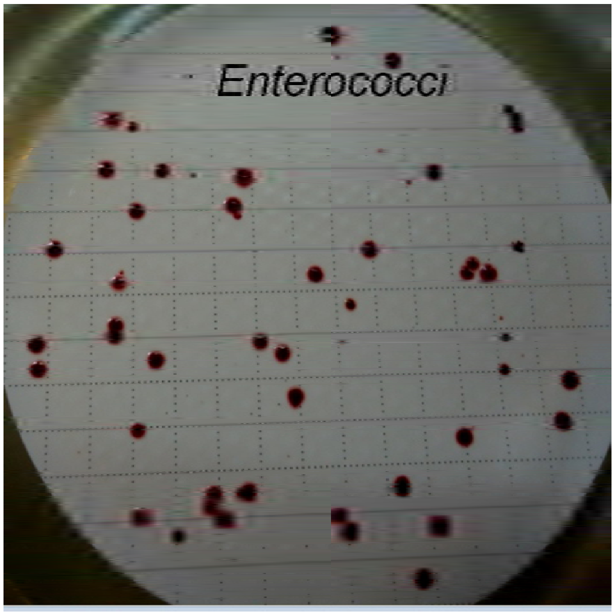
Appendix V: Indicator bacteria detected during analysis



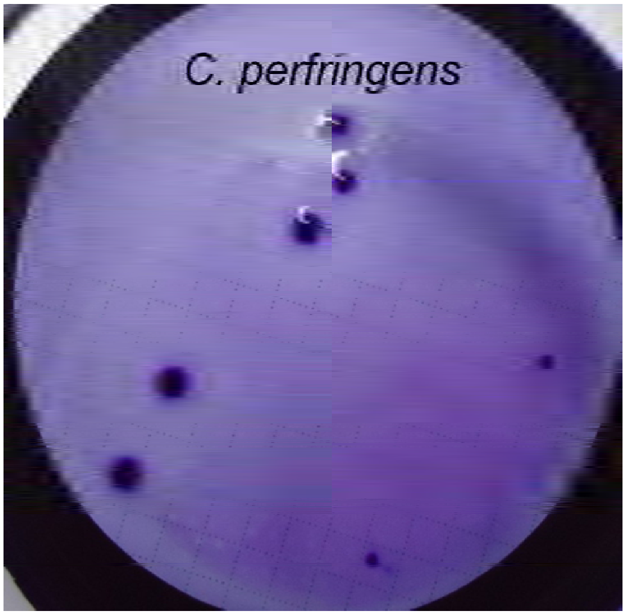
a. Growth of total and fecal coliforms on MLSB



b. Indole test for *E. coli*

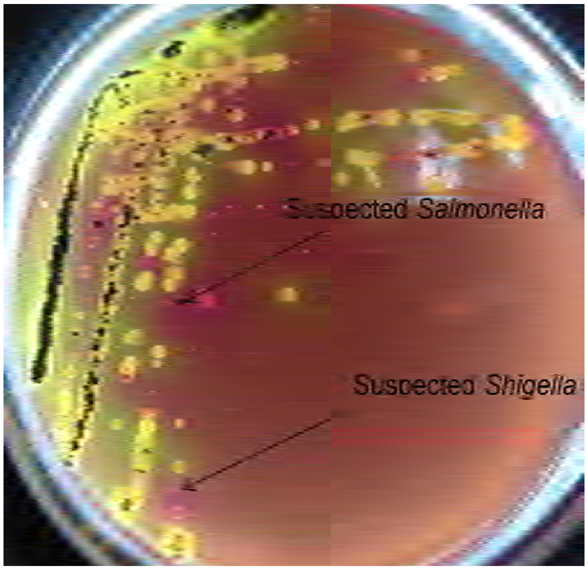


c. Growth of *Enteroccoci* on SBA

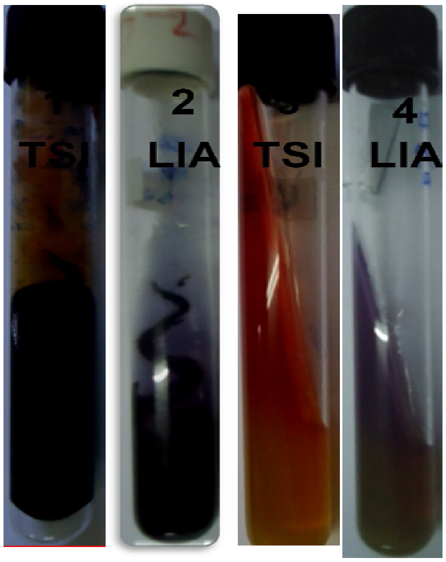


d. Growth of *Clostridium perfringens* on m-CP

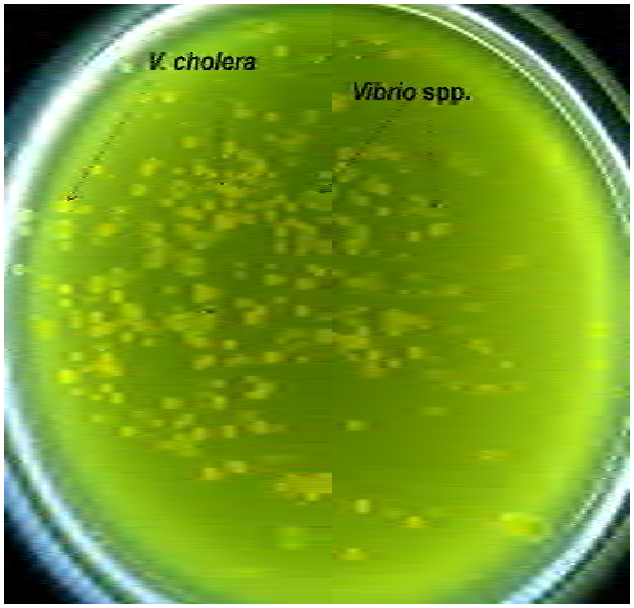
Appendix VI: Pathogenic bacteria detected during analysis



a. Growth of *Salmoenella* and *Shigella* on XLD agar



Confirmatory test for *Salmonella* (1 and 2) and *Shigella* (3 and 4)



b. Growth of *E. coli* O157:H7 on SMAC

c. Growth of *Vibrio* on TCBS

Appendix VII: Sampling and sample analysis

DECLARATION

I, the undersigned, declare that this is my work and that all sources of materials used for this thesis have been duly acknowledged.

Name: Mekuria Mekonnen Signature ____________ Date ___________

This thesis has been submitted for examination with our approval as the research advisors of the candidate.

Dr. Fassil Assefa Signature _______________ Date __________

Prof Brook Lemma Signature _____________ Date__________